Tri-Institutional PhD Program Chemical Biology

13TH ANNUAL TRI-I CHEMICAL BIOLOGY SYMPOSIUM

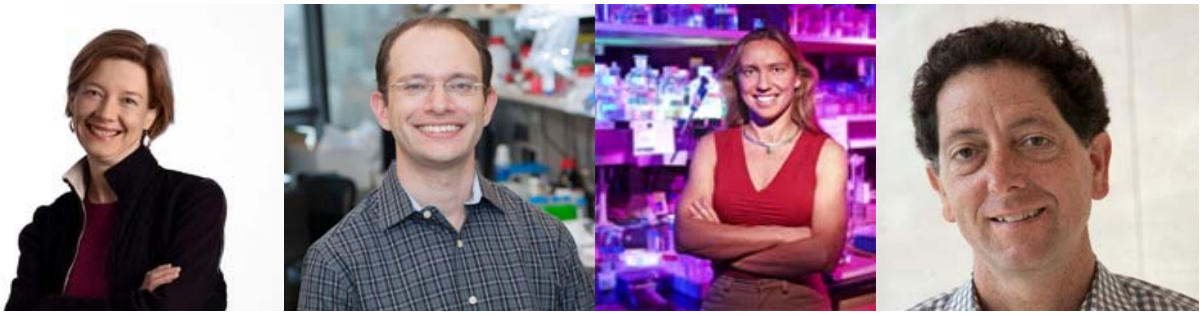
Thursday, September 7th, 2017

9:00 am – 6:00 pm

Memorial Sloan Kettering Cancer Center
Rockefeller Research Labs (RRL 116 & RRL 104)
430 East 67th Street, New York City

KEYNOTE SPEAKERS

Professor Helen Blackwell (University of Wisconsin-Madison)
Professor Daniel Heller (Memorial Sloan Kettering Cancer Center)
Professor Dorothee Kern (Brandeis University)
Professor Kevan Shokat (University of California, San Francisco)



RESEARCH TALKS & POSTER SESSION

Presentations by Tri-Institutional Students and Postdoctoral Fellows

REGISTRATION

Please visit http://chembio.triiprograms.org/symposium to register.

CONTACT

chembio.triiprograms.org T: 212-746-6049 tpcb@triiprograms.org

PROMOTIONAL PARTNERS

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Memorial Sloan Kettering Cancer Center

Tri-Institutional PhD Program
Chemical Biology

Welcome from the 2017 Symposium Planning Committee!

We are excited that you are joining us for the 13th Annual Symposium of the Tri-Institutional PhD Program in Chemical Biology (TPCB), where we bring together the chemical biology community from our Tri-Institutions as well as attendees from other NY area campuses. We hope the symposium gives both TPCB and non-TPCB students the opportunity to exchange ideas, discuss science and socialize and will foster fruitful interactions among scientists.

We are very pleased to welcome our keynote speakers this year: Professors Helen Blackwell from University of Wisconsin-Madison, Daniel Heller from Sloan Kettering Institute, Dorothee Kern from Brandeis University, and Kevan Shokat from University of California San Francisco. In addition, three TPCB students will present their research: John Zinder from the Lima Laboratory at Sloan Kettering Institute, Shi Chen from the Luo Laboratory also at Sloan Kettering Institute, and Zhen Chen from the Kapoor Laboratory at The Rockefeller University. A poster session will be held during lunch, featuring the work of students and postdocs in the Tri-institutional Chemical Biology community.

We would like to thank our Program Coordinator, Margie Mendoza, for her tremendous input and help throughout the planning of the program. We would also like to thank Dr. Daniel Heller and Program Director, Dr. Derek Tan for their assistance with organizing the symposium schedule. Finally, we want to acknowledge Executive Director Kathleen Pickering and Associate Directors Dr. Tarun Kapoor and Dr. Scott Blanchard for their contributions and commitment to TPCB.

We would like to extend a special thanks to our promotional partners: Organic and Biomolecular Chemistry, Chemical Society Review, Chemical Science, Cell Chemical Biology, Biochemistry, ACS Chemical Biology, Nature Chemical Biology and The New York Academy of Sciences. Thank you to all of our speakers, poster presenters, student volunteers and others for their contributions to the success of our Symposium.

We hope that you enjoy the day!

Rachel Leicher (Shixin Liu Laboratory, The Rockefeller University)
Linamarie Miller (Sebastian Klinge Laboratory, The Rockefeller University)
Ashley Chui (Daniel Bachovchin Laboratory, Memorial Sloan Kettering Cancer Center)
2017 Symposium Planning Committee

About TPCB:

Established in 2001, TPCB is a leading PhD graduate program in chemical biology, offered jointly by three premier institutions in New York City. We provide an unparalleled combination of world-class faculty, state-of-the-art facilities, and collaborative research opportunities to train the next generation of scientific leaders working at the interface of chemistry, biology, and medicine.

For more information, please visit our website (chembio.triiprograms.org) or contact us:
Tel: 212-746-5267; Email: tpcb@triiprograms.org

13th ANNUAL CHEMICAL BIOLOGY SYMPOSIUM SCHEDULE
Sponsored by the Tri-Institutional PhD Program in Chemical Biology (TPCB)

9:00 am – 6:00 pm Thursday, September 7, 2017
Memorial Sloan Kettering Cancer Center
Rockefeller Research Labs (RRL 116 & RRL 104)
430 East 67th Street, New York City

8:30 am **Breakfast**

9:15 am **Welcome Remarks**
Ashley Chui, Rachel Leicher, Linamarie Miller (TPCB Symposium Planning Committee)

9:30 am **Professor Helen Blackwell, University of Wisconsin-Madison**
Synthetic ligands for the interception of bacterial communication: New languages, new outcomes

10:20 am **John Zinder, TPCB Student, Lima Lab, Memorial Sloan Kettering Cancer Center**
Structural and biochemical characterization of the nuclear 3' to 5' RNA degradation machinery of S. cerevisiae

10:40 am **Coffee Break**

11:10 am **Professor Daniel Heller, Memorial Sloan Kettering Cancer Center**
Nanochemical biology

12:00 pm **Shi Chen, TPCB Student, Luo Lab, Memorial Sloan Kettering Cancer Center**
Dissecting the conformational dynamics of protein lysine methyltransferase SET8

12:30 pm **Lunch, In front of RRL 104**

1:00 pm **Poster Session A and B, RRL 104**

3:00 pm **Professor Dorothee Kern, Brandeis University**
Evolution of catalysis and regulation over 3.5 billion years – exploitation for novel cancer drugs

3:50 pm **Zhen Chen, TPCB Student, Kapoor Lab, The Rockefeller University**
Potent and specific chemical inhibitors for ribosome biogenesis and the mechanistic studies of their physiological target, Mdn1

4:10 pm **Professor Kevan Shokat, University of California, San Francisco**
Chemical tricks for drugging the undruggable

5:00 pm **Poster Prize Awards & Closing Remarks**
Professor Derek Tan (TPCB Director) and TPCB Symposium Planning Committee

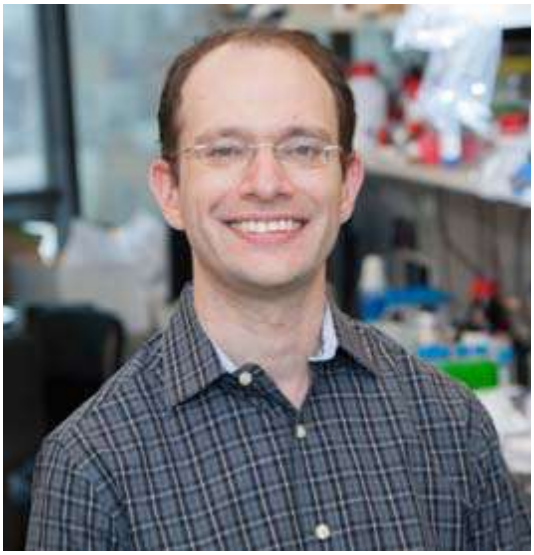
5:20 pm **Reception**
(Posters will still be on display)

Keynote Speakers

Helen Blackwell, PhD
Professor of Chemistry
University of Wisconsin-Madison

Helen E. Blackwell completed her undergraduate studies at Oberlin College, and moved on to graduate work in organic chemistry in the lab of Robert Grubbs at Caltech. Her postdoctoral work was in Stuart Schreiber's lab at Harvard. In 2002, Blackwell started as a professor of chemistry at the University of Wisconsin-Madison, where she continues to work today. Her lab works at the interface of organic chemistry and bacteriology in order to understand the role of chemical signals in host-bacterial interactions and infectious disease. The Blackwell lab has developed a range of synthetic compounds that allow for the study of the cell-signaling pathway in bacteria called "quorum sensing." Helen and her research team's interdisciplinary work have earned her numerous awards including a Dupont Young Professor Award (2007), a Camille-Dreyfus Teacher-Scholar Award (2007), and an ACS Arthur C. Cope Scholar Award (2010). She was also selected as a "Top 35 Innovator under the Age of 35 in the US" by the MIT Technology Review (2005), as an Alfred P. Sloan Foundation Fellow (2006), and as a fellow of the American Association for the Advancement of Science (AAAS, 2010).

Daniel Heller, PhD
Assistant Member of Molecular Pharmacology
Memorial Sloan Kettering Cancer Center



Dr. Daniel Heller is an Assistant Member in the Molecular Pharmacology Program at Memorial Sloan Kettering Cancer Center and an Assistant Professor in the Department of Pharmacology at Weill Cornell Medical College of Cornell University. Dr. Heller obtained his BA in history from Rice University and PhD in chemistry from the University of Illinois at Urbana-Champaign in 2010. He completed a Damon Runyon Cancer Research Foundation Postdoctoral Fellowship in the laboratory of Robert Langer at the David H. Koch Institute for Integrative Cancer Research at MIT in 2012. Dr. Heller's laboratory focuses on the development of nanoscale technologies for the treatment, diagnosis, and research of cancer. He has published over 50 peer-reviewed papers and is an inventor on 14 issued or pending patents. He is a 2012 recipient of the National Institutes of Health Director's New Innovator Award, a 2015 Kavli Fellow, and a 2017 recipient of the Pershing Square Sohn Prize for Young Investigators in Cancer Research.

Keynote Speakers

Dorothee Kern, PhD
Professor of Biochemistry
Brandeis University

Dorothee Kern obtained her B.S., M.S., and Ph.D. in Biochemistry from Martin Luther University of Halle in Germany under the mentorship of Dr. G. Fischer. She was a postdoctoral fellow in David Wemmer's lab at University of California, Berkeley, and is currently a Professor of Biochemistry and HHMI Investigator at Brandeis University. She is the recipient of numerous awards including the Pfizer Award in Enzyme Chemistry from the ACS, the Dayhoff Award and the National Lecturer from the Biophysical Society. Recently she was elected to the National Academy of Science of Germany Leopoldina. In 2015, she founded Relay Therapeutics, a Biotech company aimed at creating breakthrough medicine based on protein dynamics. Her lab is focused on using biophysical analytical techniques to visualize proteins during function, hence time-resolved at atomic resolution. Specifically, they are interested in the evolution of the catalytic power of enzymes by visualizing proteins and interacting partners at the atomic level using techniques such as NMR, x-ray crystallography, FRET and computation. By using these approaches, her lab was a pioneer in revealing the molecular mechanism of Gleevec, a key cancer therapeutic.

Kevan Shokat, PhD
Professor of Cellular and Molecular Pharmacology
University of California, San Francisco

Kevan Shokat obtained his B.A. in Chemistry from Reed College and his Ph.D. in Organic Chemistry at UC Berkeley with Dr. Peter Schultz. He was a postdoctoral fellow in immunology at Stanford University with Dr. Chris Goodnow and began his independent research career at Princeton University. He is now a Professor in the Department of Cellular and Molecular Pharmacology and an HHMI investigator at UC San Francisco. He is also a Professor of Chemistry in the Department of Chemistry at UC Berkeley. He has received numerous awards including the Eli Lilly Award, and is a member of the National Academy of Sciences, the Institute of Medicine, and the American Academy of Arts and Sciences. Additionally, he founded Intellikine, Inc., a company focused on commercializing a series of PI3K and mTOR small molecule inhibitors for cancer and inflammatory disease, and is a co-founder of Araxes Pharmaceuticals and eFFECTOR Pharmaceuticals. He is a pioneer in the development of chemical methods for investigating cellular signal transduction pathways, focusing on protein and lipid kinases. His lab uses a combination of chemical synthesis and protein engineering to create uniquely traceable and regulatable kinases, allowing the function of over 100 different kinases to be uncovered across disease areas including oncology, metabolism, and infectious disease.

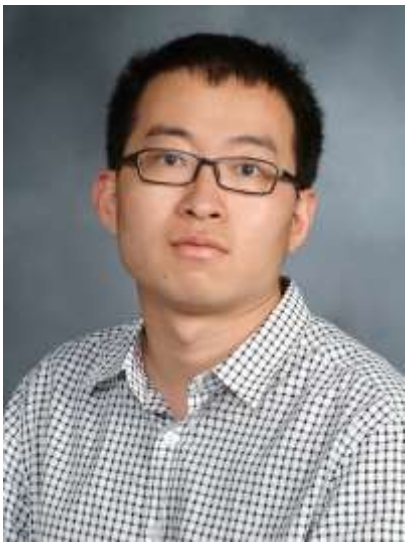
Student Speakers

John Zinder
Christopher Lima Laboratory
Memorial Sloan Kettering Cancer Center

John Zinder completed a B.A. in Chemistry at Cornell University working on store-operated calcium entry in mast cells under Barbara Baird. He then went on to complete his M.S. in Biochemistry at University of Colorado, Boulder where he performed *in vivo* characterization of SAM sensitive riboswitches and the enzymology of DNA replication in *B. subtilis* and phage SPP1. John then joined the Tri-Institutional PhD program in Chemical Biology and now works in the laboratory of Christopher Lima at Memorial Sloan Kettering Cancer Center where he uses X-ray crystallography to study nuclear 3' to 5' RNA decay.

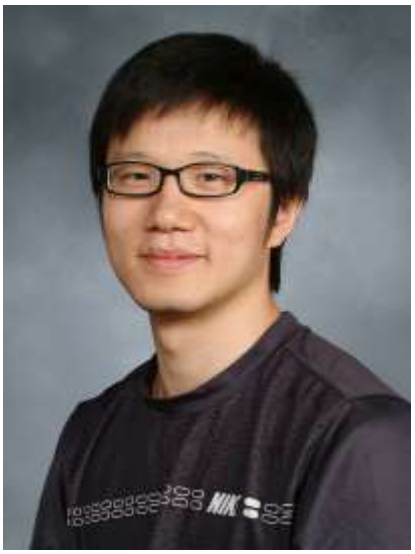
Shi Chen
Minkui Luo Laboratory
Memorial Sloan Kettering Cancer Center

Shi Chen completed his B.S. in Chemical Biology at Tsinghua University in Beijing, China. There, he worked under Yu Rao on the synthesis of multi-substituted pyrazoles via transition metal-catalyzed C-H activations. He also worked under Jinying Yuan on the synthesis and characterization of functional magnetic nanoparticles for maltose binding protein-fused enzyme immobilization. He then joined the Tri-Institutional Program in Chemical Biology and joined Minkui Luo's laboratory at Memorial Sloan Kettering Cancer Center where he currently works on interrogating the conformational dynamics of protein lysine methyltransferase SET8.



Student Speakers

Zhen Chen
Tarun Kapoor Laboratory
The Rockefeller University



Zhen Chen completed his B.S. in Chemistry at Peking University, where he developed methods to build patterns of close-packed nanoparticle monolayers using microfluidic channels under Wei Wang. Zhen then moved to Texas to complete his M.S. in Chemistry under Zachary Ball at Rice University, where he developed site-specific protein modifications using an enzyme-like metallopeptide catalyst based on the combination of transitional metal catalyst and molecular recognition motifs. In 2012, Zhen joined the Tri-Institutional PhD Program in Chemical Biology and is currently in Tarun Kapoor's lab at The Rockefeller University characterizing and developing the biochemical purification of Mdn1, a dynein-like AAA ATPase. Zhen has won notable awards such as the Harry B. Weiser Excellence in Teaching Award as well as the Stephen C. Hofmann Fellowship for outstanding research achievement.

13th ANNUAL CHEMICAL BIOLOGY SYMPOSIUM

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Poster Session Schedule

1:00-3:00 pm Thursday, September 7, 2017
Memorial Sloan Kettering Cancer Center
Rockefeller Research Labs (RRL 104)
430 East 67th Street, New York City

Session A (odd numbers) – 1:00-2:00 PM

A1: Amanda Acosta Ruiz
A3: Malik Chaker-Margot*
A5: Shi Chen*
A7: Didar Ciftci*
A9: Emma Garst*
A11: Darren Johnson*
A13: Rachel Leicher*
A15: Fangyu Liu*
A17: Linamarie Miller*
A19: Cristina Santarossa*
A21: Chaya Stern*
A23: Ryan Williams

Session B (even numbers) – 2:00-3:00 PM

B2: Alexis Jaramillo Cartagena*
B4: Neha Chauhan
B6: Ashley Chui*
B8: Tapojyoti Das
B10: Roozbeh Eskandari
B12: Mehtap Isik*
B14: Jacob Litke*
B16: Michaelyn Lux*
B18: Rudolf Pisa*
B20: Zheng Ser*
B22: Nathan Westcott

*Indicates a TPCB Student

TPCB Symposium Poster Abstracts

A1. Optical dissection of G protein-coupled receptor signaling

Amanda Acosta Ruiz, Vanessa Gutzeit, Deo Singh, Victor Vivcharuk, Joshua Levitz
Joshua Levitz Laboratory, Weill Cornell Medicine

G protein-coupled receptors (GPCRs) form the largest class of membrane receptors and bridge the extracellular and intracellular spaces by sensing stimuli, such as light, hormones, or neurotransmitters, and converting them into intracellular signals. The class C GPCR family includes the metabotropic glutamate (mGluRs) and GABA (GABABRs) receptors, which modulate neuronal excitability and synaptic strength and serve as drug targets for schizophrenia, depression, addiction, fragile X syndrome, and Alzheimer's disease despite a limited mechanistic. The limitations of pharmacology and genetic knockout in terms of spatiotemporal precision and subtype specificity, has made it difficult to gain a picture of how specific receptors modulate neuronal function and, ultimately, behavior. Furthermore, the activation mechanism of class C GPCRs remains elusive because, unlike class A rhodopsin-like GPCRs, they contain large, extracellular ligand binding domains (LBDs) that couple, via a poorly understood mechanism, to a transmembrane domain (TMD). My research aims to use optical methods to develop a complete biophysical mechanism of how class C GPCRs activate and signal, and to determine how these receptors modulate neural activity in physiology and disease. We have recently developed a family of chemical optogenetic tools for the manipulation of specific mGluRs in native systems, including in vivo, to study the physiological roles of mGluRs and other related signaling proteins with high precision. As a complement to neurophysiological studies, we are also using a combination of ensemble and single molecule fluorescence assays to gain biophysical insight into the cooperative activation process of homomeric and heteromeric mGluR2-containing receptors.

B2. Mechanistic investigations of Crl: an unconventional transcription activator

Alexis Jaramillo, Elizabeth Campbell, Seth Darst
Seth Darst Laboratory, The Rockefeller University

Sigma factors orchestrate global transcription programs in bacteria by assembling with the catalytic core of the RNA polymerase enzyme (RNAP), forming a holoenzyme and recruiting it to specific DNA promoter sequences. The stress response transcription program in Salmonella typhimurium (S. typhimurium) is coordinated by the sigma factor σS. Crl is an unconventional transcription factor that interacts directly with σS to promote transcription of σS-dependent genes; this presents a new paradigm for transcription activation.

In this study, in vitro abortive transcription assays demonstrated the σS factor from S. Typhimurium (Sty) forms a holoenzyme with the Escherichia coli (E. coli) RNAP to transcribe σS-dependent genes and that Sty Crl enhances the activity of the σS-holoenzyme complex. Single particle cryo-electron microscopy was employed to obtain the three-dimensional structure of a σS-holoenzyme in the presence of Crl. This structure reveals novel interactions between Crl and the β' subunit of RNAP polymerase, suggesting Crl helps tether σS to the core enzyme thereby enhancing the stability of the σS-holoenzyme complex.

A3. The complete structure of the small subunit processome

Jonas Barandun, **Malik Chaker-Margot**, Mirjam Hunziker, Kelly R. Molloy, Brian T. Chait and Sebastian Klinge
Sebastian Klinge Laboratory, The Rockefeller University

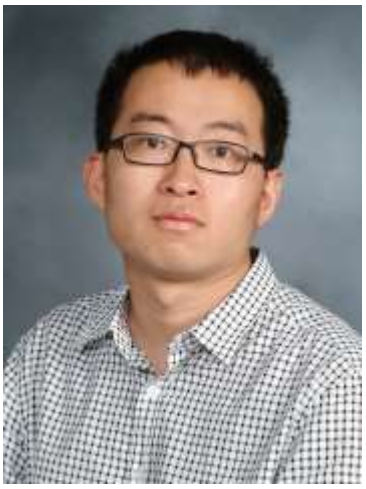
The small subunit processome represents the earliest stable precursor of the eukaryotic small ribosomal subunit. The cryo-EM structure of the Saccharomyces cerevisiae small subunit processome at an overall resolution of 3.8 Å provides an essentially complete atomic model of this assembly. In this nucleolar superstructure, 51 ribosome assembly factors and two RNAs encapsulate the 18S rRNA precursor and 15 ribosomal proteins in a state that precedes pre-rRNA cleavage at site A1. Extended flexible proteins are employed to connect distant sites in this particle. Molecular mimicry, steric hindrance as well as protein- and RNA-mediated RNA remodeling are used in a concerted fashion to prevent the premature formation of the central pseudoknot and its surrounding elements within the small ribosomal subunit.

Mechanisms of intracellular sterol transport

B4. Neha Chauhan, Yves Sere, Johannes Graumann, Anant K. Menon
Anant Menon Laboratory, Weill Cornell Medicine

Cellular cholesterol homeostasis requires rapid transport from its point of import (lysosomes) or synthesis (ER) to the PM and other organelles. This homeostatic regulation occurs by non-vesicular pathways and requires soluble transport proteins (STPs). None of the reported STPs (Oxysterol binding protein and its homologs and StART proteins) are physiologically necessary in transporting cholesterol between the ER and PM (biosynthetic route of transport), indicative of functional redundancy or specialized roles. Thus, bona fide STPs remain to be identified. We employed an unbiased chemo-proteomic strategy to identify STPs in yeast, Saccharomyces cerevisiae. Cytosolic sterol binding proteins (SBPs) from yeast were captured with a photo-reactive clickable cholesterol analog and subsequently identified by quantitative proteomics. The ~300 putative SBPs obtained were prioritized by considering their annotated function, reported phenotypes (amphotericin/nystatin resistance/sensitivity), whether they represent uncharacterized ORFs and also according to their reported essentiality for growth. The ~85 proteins prioritized by these criteria were subjected to additional filters: reported interactions with known lipid metabolism proteins, gene ontology (sterol/lipid/kinase function), information on pathways (sterol and trafficking) and mammalian orthologs. We obtained a working list of ~60 proteins. By virtue of their ability to bind cholesterol, these proteins are candidate STPs. We are currently validating the uncharacterized ORFs from our candidate list using in vitro and in vivo tests. Because most intracellular trafficking mechanisms are conserved between yeast and mammals, this study in yeast is expected eventually to yield the identity of mammalian STPs and provide novel insights into aspects of intracellular sterol trafficking.

A5. Dissecting the conformational dynamics of protein lysine methyltransferase SET8



Shi Chen, Rafal Wiewiora, Fanwang Meng, Wenyu Yu, Hao Hu, Anqi Ma, Nicolas Babault, Hua Zou, Junyi Wang, Gil Blum, Robert Skene, Jian Jin, Y. George Zheng, Peter J. Brown, Cheng Luo, John Chodera, Minkui Luo
Minkui Luo Laboratory, Memorial Sloan Kettering Cancer Center

Protein lysine methyltransferases (PKMTs) are the family of enzymes responsible for catalyzing the methylation of lysine residues in both histone and non-histone proteins, involved in diverse physiological and pathological pathways. During the past decade, the knowledge of PKMTs was largely extended from enzymology, structural, and functional perspectives, which laid the foundation for developing potential therapeutics against multiple diseases. However, limited knowledge is available about how individual PKMTs undergo dynamic conformational changes in the process of the reaction. In this research, multiple approaches were used to explore the conformational dynamics of SET8, which is the sole enzyme catalyzing monomethylation of H4K20 and essential for several key biological functions, including maintenance of genome integrity, initiation of DNA double-strand-break repair, and regulation of cell cycle, etc. A series of new structures of SET8 were solved with various ligands. Molecular dynamics simulations were conducted in a millisecond time scale and analyzed by Markov State Model to reveal the hidden conformations and kinetic correlations between different conformations. Steady state and pre-steady state kinetics of SET8-mediated methylation were characterized to dissect the stepwise details of binding event. Numbers of molecular switches were identified and validated to characterize the conformational change pathways between different conformations. Taken together, a model describing the conformational dynamics of SET8 in the process of assembling the Michaelis complex is derived. Additionally, structure-function relationship of SET8 cancer mutations were also predicted and validated based on the proposed model.

B6. Advancing aminopeptidase inhibitors as anti-AML agents

Ashley J. Chui, Darren C. Johnson, Marian C. Okondo, Daniel A. Bachovchin
Daniel Bachovchin Laboratory, Memorial Sloan Kettering Cancer Center

Aminopeptidases perform essential functions in a wide array of biological processes, including cell maintenance, growth and development, and immune system signaling. Among the variety of aminopeptidases, our lab has recently discovered that inhibitors of dipeptidyl aminopeptidases 8 and 9 (DPP8/9) – including Val-boroPro – induces pyroptosis, a lytic form of cell death, selectively in monocytes and macrophages. We further showed that DPP8/9-inhibitor induced pyroptosis is mediated by the proprotein form of caspase-1. As DPP8/9 inhibitors selectively kill monocytes and macrophages, we hypothesized that DPP8/9 might be therapeutic targets for cancer cells (i.e. acute myeloid leukemia (AML) cells) that arise from the monocyte/macrophage lineage. Indeed, we have found that Val-boroPro has selective cytotoxic activity against the vast majority of AML cell lines and inhibits AML progression in vivo. Based on these data, we propose that pyroptosis induction is a potential new therapeutic strategy for AML. This strategy, however, is limited in that DPP8/9 inhibitors do not kill 100% of cells in sensitive AML lines, meaning DPP8/9 inhibitors are unlikely to completely eliminate cancers in vivo. Encouragingly, we have found evidence of synergistic killing with Val-boroPro in combination with aminopeptidase inhibitors. Therefore, the central goal of this study is to investigate the synergistic killing from the inhibition of both DPP8/9 and aminopeptidases and to explore the potential therapeutics of this synergy for AML.

A7. Single molecule resolution dynamics in eukaryotic glutamate transporters (EAATs)

H. Didar Ciftci, Olga Boudker
Olga Boudker Laboratory, Weill Cornell Medicine

Excitatory amino acid transporters (EAATs) pump neurotransmitter from the synaptic cleft into neuronal and glial cells powered by the electrochemical gradients of Na+, H+ and K+ ions across the membrane. They function in a trimeric form where each protomer can move independent of each other. A protomer's transport cycle starts in its extracellular side facing conformation where it binds glutamate and changes its conformation to release the substrate into cytoplasm. Once the cargo is released the transport domain reorients itself back to extracellular side facing conformation through a K+ dependent step. In our studies we focus on identifying the rate-limiting step of the transport cycle where we analyze the rate of transport dynamics using single molecule fluorescence resonance energy transfer (smFRET) imaging. For smFRET imaging, we introduced single cysteine mutations at sites, which could report on transport domain movements. Single cysteine mutants are purified and tested for their labeling efficiency and functionality. Functional mutants are further analyzed in sm-FRET imaging platform to observe transport domain movements in real time when the transporter is loaded with either substrate, Na+ ions and protons or with K+ ions.

B8. Structural characterization of lipid-binding domain of alpha synuclein in context of its functional role at the synapse

Tapojyoti Das, Meraj Ramezani, David Holowka, Marcus Wilkes, Cristian Follmer, David Snead, Igor Dikiy, Trudy Ramlall, Barbara Baird and David Eliezer
David Eliezer Laboratory, Weill Cornell Medicine

The normal function of alpha synuclein, a protein causally linked to Parkinson's disease and enriched in the presynaptic neuronal termini, is dependent on its lipid membrane interactions. The N-terminal lipid-binding domain of alpha synuclein interconverts between extended helix and broken helix conformations under a dynamic equilibrium, the conformational exchange being of unknown significance. In view of that, a structural model is tested where the membrane-apposed vesicle serves as a unique topological scaffold for the broken helix state of the lipid-binding domain, acting as a bridge between the two membranes. In a study using targeted mutagenesis, NMR-based in-vitro lipid vesicle affinity measurements and cell-based vesicle release assays we show that the A30P mutant, which disrupts the first helix and decreases membrane affinity of the entire domain, remains functional to prevent calcium-triggered vesicle release. In contrast, V70P mutant that disrupts the second helix and decreases membrane affinity only beyond the mutation site, by itself or in combination with A30P, becomes nonfunctional. This indicates an important role of the second helix for the normal function of alpha synuclein. In addition, our findings indicate that rather than the overall lipid membrane affinity, a balanced ratio of membrane affinities of both the helices is important for the normal function of alpha synuclein.

A9. Reconstitution of S-palmitoylated IFITM3 for antiviral mechanism studies

Emma Garst, Avital Percher and Howard C. Hang
Howard Hang Laboratory, The Rockefeller University

Protein fatty-acylation controls many key cellular pathways in eukaryotes and also regulates host immunity to pathogens. For example, proteomic studies from our laboratory revealed S-palmitoylation of interferon-induced membrane proteins (IFITMs) and other immune-associated proteins. In particular, site-specific S-palmitoylation of IFITM3 regulates the antiviral activity of this key IFN-effector against viruses such as influenza virus and dengue virus. The discovery of S-palmitoylated IFITM3 antiviral activity in mammalian cell lines and in humans highlights new host factors involved in pathogen resistance; however, the precise mechanism(s) of action for these immune effectors is still unclear. To address the potential biochemical mechanisms by which IFITMs inhibit virus entry, we report progress towards the expression, purification and site-specific lipidation of recombinant IFITM3 for in vitro reconstitution and structural studies. These studies should reveal how site-specific S-palmitoylation affects IFITM3-lipid interactions and structure and may also elucidate how this family of IFN-effectors prevents virus translocation across cellular membranes.

B10. Treatment assessment in PDX model of clear cell renal cell carcinoma (ccRCC) with hyperpolarized [1-^{13}C] pyruvate

Roozbeh Eskandari, Yiyu Dong, Lidia S. Cunha, Kristin Granlund, Vessellin Miloushev, Valentina Di Gialleonardo, Sui Seng Tee, Sangmoo Jeong, James J. Hsieh, Kayvan R. Keshari
Kayvan Keshari Laboratory, Memorial Sloan Kettering Cancer Center

Cancer cells shift their metabolism to aerobic glycolysis known as the Warburg effect. In targeted gene sequencing studies of patient derived RCC cell lines (MSK IMPACT), we identified cells with differential activation of the PI3KCA pathway via an activating point mutation (JHRCC228) as compared to another patient derived cell line (JHRCC12). It has been shown that the PI3K/mTOR axis directly controls glycolytic enzymes. Studying this pathway and corresponding metabolism can be achieved by hyperpolarized magnetic resonance spectroscopy (HP-MRS). In this work, we accurately recapitulate RCC response to FDA-approved therapeutics in vivo by modulating the PI3K/mTOR pathway and quantitatively assessing metabolic flux.

Our initial Western blots for P-S6, downstream of the PI3K/mTOR pathway, demonstrates subtle difference in expression with rapamycin in both cell lines. Our isotopic tracing studies demonstrate reduced glycolytic metabolism to lactate in PI3KCA mutant cells after treatment with rapamycin. Using [1,6-13C] glucose, rapid accumulation of [3-13C] lactate levels were measured in JHRCC228 cells, which decreased nearly 70% after rapamycin treatment (P-value=0.023). Extracellular total lactate was also significantly decreased in JHRCC228 cells (17.4% and 31.6% for JHRCC12 and JHRCC228).

Hyperpolarized experiments were preformed on a SPINlab hyperpolarizer (GE Healthcare). The polarized solution of 100mM [1-13C] pyruvate was injected intravenously in the tail vein of a catheterized animal and HP data were acquired. With treatment of a single dose of rapamycin 15mg/Kg, 24 hours before the HP MRS study, JHRCC228 xenografts demonstrate a significant decrease in metabolism (1.25±0.053 to 0.939±0.042, P-value=0.0016) as compared to non-responding JHRCC12 xenografts. This data supports our long-term treatment studies on tumor growth that shows higher effect on JHRCC228 over JHRCC12 comparing to vehicle groups.
In conclusion, while in vitro studies of downstream proteins in the PI3K/mTOR axis in RCC cell lines didn’t show a significant difference, tracing studies demonstrates a drop in glycolytic metabolism with treatment. Utilizing HP-MRS, we were able to accurately reflect the metabolic response to rapamycin at an early time point non-invasively, providing the potential for use of this approach in future clinical studies.

A11. DPP8 and DPP9 are therapeutic targets for acute myeloid leukemia

Darren C. Johnson, Marian C. Okondo, Ashley J. Chui, Fiona C. Brown, Casie Reed, Elizabeth Peguero, Elisa de Stanchina, Alex Kentsis & Daniel A. Bachovchin
Daniel Bachovchin Laboratory, Memorial Sloan Kettering Cancer Center

New therapeutic strategies are needed for acute myeloid leukemia (AML), which has an overall 5-year survival rate of ~25%. We recently discovered that small molecule inhibitors of the serine peptidases DPP8 and DPP9 (DPP8/9) trigger a lytic form of cell death called pyroptosis selectively in monocytes and macrophages. Here we show that DPP8/9 inhibitors induce pyroptosis in the vast majority of AML cell lines and primary AML samples, and that the expression of caspase-1 predicts sensitivity to these inhibitors. All cells from other lineages were completely resistant to DPP8/9 inhibition. We further demonstrate that DPP8/9 inhibitors effectively inhibit AML progression in mice.

B12. Model binding systems to advance affinity predictions of free energy methods

Mehtap Isik, Sonya M. Hanson, Andrea Rizzi, John D. Chodera
John Chodera Laboratory, Memorial Sloan Kettering Cancer Center

Establishing reliable physical models to predict the affinity of small molecules to biomolecular targets and anti-targets can accelerate the structure-based design of drugs and chemical biology probes. Widely used computational methods such as docking are not accurate enough to guide molecular design. To bridge this gap, alchemical free energy calculations are developed to provide rigorous binding free energy estimates using atomistic simulations. In well-behaved protein systems, free energy calculations were shown to achieve accurate enough predictions to assist the lead optimization stage of drug discovery. But beyond initial promising results, the robustness of free energy methods in general target classes is unknown. It is crucial to test the current methods with technically challenging proteins to push the boundary of application areas.

I dissect the performance of alchemical free energy methods by comparing computational predictions to highly accurate experimental measurements by taking advantage of model systems with isolated challenges. How accurately we predict the correct or major binding site in a promiscuous binder protein? Human Serum Albumin model system represents challenge of multiple binding sites. HSA is the most abundant protein in blood plasma and has exceptional ability to bind a variety of small molecule drugs and it has at least 8 known binding sites. Predicting HSA affinity accurately has promising pharmacological impact because of how drastically it can modulate drug pharmacokinetics.

Capturing the sensitivity of current methods with model systems with isolated challenges and establishing protocols capable of depicting correct chemical state of the system will improve computational affinity estimates.

A13. Investigating the mechanism of PRC2-mediated H3K27me3 spreading using single-molecule techniques

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Epigenetic marks play a key role in regulating gene expression. These post-translational modifications contribute to heterochromatin and euchromatin states, which control gene expression profiles that can determine cellular differentiation and development. Failure to maintain the proper epigenetic landscape during cell differentiation and division can result in disease states such as cancer. The players that facilitate the maintenance of these reversible chromatin marks are often essential proteins in the cell. One key player in heterochromatin maintenance is PRC2, a histone methyltransferase responsible for the establishment of the H3K27me3 repressive mark, maintaining transcriptionally silent states throughout development. Dysregulation of PRC2 levels and point mutations in its subunits have been found in various cancers. Therefore, we are investigating the specific spatiotemporal dynamics of PRC2-mediated H3K27me3 spreading, its kinetic mechanism, how it affects mechanical transitions of chromatin. Understanding the fundamental mechanism of PRC2 will give insights to identify its role in cancer and potentially provide therapeutic strategies. To this end, we are interrogating the consequences of oncogenic histone mutation H3K27M on the PRC2 mechanism. We will harness the power of single-molecule techniques, including optical trapping force microscopy and total internal reflection fluorescence (TIRF) microscopy, to visualize H3K27me3 spreading and PRC2 dynamics.

B14. Imaging metabolite dynamics in living cells using a spinach-based riboswitch

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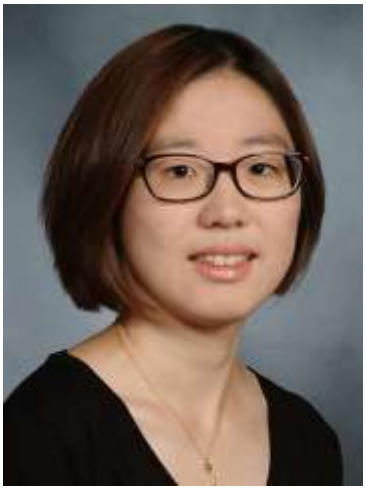
Riboswitches are natural ligand-sensing RNAs that are typically found in the 5'-untranslated regions (UTRs) of mRNA. Numerous classes of riboswitches have been discovered, enabling mRNA to be regulated by diverse and physiologically important cellular metabolites and small molecules. Here we describe Spinach riboswitches, a new class of genetically encoded metabolite sensor derived from naturally occurring riboswitches. Drawing upon the structural switching mechanism of natural riboswitches, we show that Spinach can be swapped for the expression platform of various riboswitches, allowing metabolite binding to directly induce Spinach fluorescence. In the case of the thiM thiamine pyrophosphate (TPP) riboswitch, we show that insertion of Spinach results in an RNA sensor that exhibits fluorescence upon binding TPP. This TPP Spinach riboswitch binds TPP with similar affinity and selectivity as the endogenous riboswitch and enables discovery of agonists and antagonists of the TPP riboswitch using simple fluorescence readouts. Furthermore, expression of the TPP Spinach riboswitch in E. coli enables imaging of dynamic changes in intracellular TPP concentrations that occur in response to extracellular thiamine. Additionally, we show that other riboswitches that use a similar structural mechanism as the TPP riboswitch, including the xpt guanine, the xpt adenine, and the yitJ SAM riboswitches, can be converted into Spinach riboswitches. Thus, Spinach riboswitches constitute a novel class of RNA-based fluorescent metabolite sensors that take advantage of a diverse population of naturally occurring ligand-binding riboswitches.

A15. Structural basis of the gating mechanism of cystic fibrosis transmembrane conductance regulator



Zhe Zhang, **Fangyu Liu**, Jue Chen
Jue Chen Laboratory, The Rockefeller University

Cystic fibrosis transmembrane conductance regulator (CFTR) is an ATP-gated chloride channel which belongs to the ATP-binding cassette (ABC) transporter family. Here, we present the structure of CFTR in its phosphorylated, ATP-bound conformation. Compared with its no ATP-bound conformation, phosphorylation and ATP binding disengage the R domain from its inhibitory position. The cytoplasmic pathway, found closed off in other ABC transporters, is cracked open in CFTR, consistent with its unique channel function. Unexpectedly, the extracellular mouth of the ion pore remains closed, indicating that local movements of the transmembrane helices can control ion access to the pore even in the NBD-dimerized conformation.

B16. Diastereoselective palladium-catalyzed cascade to form pyranobenzofurans and furobenzofurans

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The pyranobenzofuran and furobenzofuran architecture is a structural motif found in many bioactive natural products, including chafuroside A, and (-)-panacene. Synthesizing this scaffold diastereoselectively is challenging in a single synthetic step, as it requires the concurrent formation of two rings with regio- and stereocontrol. To access this architecture, we have developed a unique cascade reaction involving oxypalladation of a phenol, a β-hydride elimination/migratory insertion sequence and π-allyl cyclization of a pendant alcohol nucleophile. Ultimately, this methodology allows facile access to complex architectures from simple linear precursors with high diastereoselectivity. The cyclized products can potentially be used in the synthesis of natural products or can serve as scaffolds for library design.

A17. Structural studies of the LSU processome from the nucleolus: the Nsa1 particle

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Eukaryotic ribosome biogenesis is a highly regulated and energetically costly process. In addition to the ribosomal proteins and ribosomal RNA (rRNA) that make up the mature ribosome, biogenesis requires a large collection of assembly factors and small nucleolar RNAs. In the yeast Saccharomyces cerevisiae, ribosome biogenesis begins with transcription of the 35S pre-ribosomal RNA in the nucleolus. Processing of this rRNA leads to distinct maturation pathways for the small and large subunits, which then progress through the nucleus and finish maturation in the cytoplasm. Years of biochemical data and technological improvements in cryo-electron microscopy (cryo-EM) have allowed for the structural characterization of intermediates in the ribosome assembly pathway. These structures have provided insight into the overall progression of ribosome assembly, and into the function of many assembly factors that would be difficult to fully characterize otherwise. Currently, structures of large subunit (LSU) biogenesis intermediates from the nucleolus, and therefore from the earliest stages of LSU biogenesis, have not been characterized. These intermediates must be better studied in order to gain insight into the nucleolar stage of LSU assembly. Here, we present a preliminary cryo-EM model of a late nucleolar LSU particle, with density for assembly factors that have not previously been structurally characterized.

B18. A rational strategy to design probes for the chemical genetic analysis of AAA+ proteins

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AAA+ (ATPases associated with diverse cellular activities) proteins are required for a wide range of cellular processes, such as cytoskeletal organization and protein degradation. As many of the biochemical steps involving these proteins occur within seconds to minutes, fast- acting chemical inhibitors can be powerful tools to dissect their functions. However, we lack strategies to rationally design chemical inhibitors for any one of the 100s of proteins in the AAA+ superfamily. Here we devise simple rules to design inhibitors with selectivity profiles that match sequence variation across the nucleotide-binding sites of AAA+ proteins. Our approach yields spastazoline, the only known chemical inhibitor of a microtubule-severing enzyme. In addition, we introduce a precise single-carbon substitution in a promiscuous chemical inhibitor to engineer selectivity for VCP/p97, a AAA+ protein required for proteostasis. Our findings open systematic chemical genetic analyses of AAA+ proteins, and suggest how conserved active sites in native proteins can be selectively targeted.

A19. The design of improved cell-permeable inhibitors of cytoplasmic dynein

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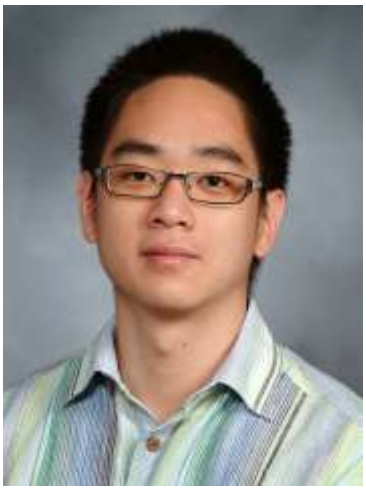
Cytoplasmic dyneins are AAA+ ATPase motor proteins responsible for transporting cargo towards the microtubule minus-ends. However, dissecting the precise functions of dynein is difficult due to its rapid timescale of action (>1 µm/s in cells). Ciliobrevins were the first cell-permeable inhibitors of cytoplasmic dynein. However, complete inhibition of dynein by ciliobrevins requires high doses (50-100 µM), and thus their use as chemical probes has been limited by their low potency. Ciliobrevins also suffer from suboptimal chemical properties, such as the potential to isomerize. To overcome these limitations, we report the design and characterization of conformationally constrained isosteres of ciliobrevin, termed dynapyrazoles. Dynapyrazoles inhibit dynein-dependent cellular processes, such as intraflagellar transport and lysosome motility, at single-digit micromolar concentrations. Furthermore, we report that while ciliobrevins inhibit both the basal and microtubule-stimulated ATPase activity, dynapyrazoles only inhibit the microtubule-stimulated activity. Thus, our studies suggest that the design of alternative scaffolds can lead to inhibitors with improved potencies and unique modes of action, resulting in potentially useful tools to study dynein in cells.

B20. Cross-linking mass spectrometry methods for optimized large-scale interaction proteomics



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Protein-protein interactions and assembly of protein complexes is central to cell regulation and signaling. The BAF complex is a chromatin remodeling complex that is mutated in approximately 20% of cancers. In rhabdoid tumors, biallelic inactivation of the tumor supressor SMARCB1 gene results in altered protein-protein interactions within the BAF complex, affecting its activity and changing transcriptional regulation. Cross-linking mass spectrometry enables identification of protein-protein interactions and the exact interaction sites. Furthermore, the cross-link information can be leveraged to improve protein structural models. Recent development of commercially available mass spectrometer cleavable cross-linkers improves sensitivity and efficiency of cross-linked peptide identification. In our study, we report an optimized strategy for cross-linker identification using the mass spectrometer cleavable cross-linker discuccinimidyl sulfoxide (DSSO). To evaluate specificity of cross-linking, we used both simple and complex protein matrices as background proteome. We sought to improve the sensitivity and accuracy of MS identification of cross-linked peptides by using different proteases, ion fragmentation methods, MS acquisition strategies and protein target database for peptide-spectra matching. Our results suggest that tandem collision induced dissociation and high energy collision induced dissociation returns the largest number of cross-link identifications. These optimized parameters can be applied to cross-linking mass spectrometry experiments to identify more protein-protein interactions sites and to build more comprehensive protein-protein interaction networks. Extending this work to studying disrupted protein-protein interactions in rhabdoid tumors and the exact composition of the mutated BAF complex will provide insights into the mechanisms of cancer transformation and therapy.

A21. Towards quantifying forcefield error in binding free energies

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Alchemical binding free energy calculations hold great promise for predicting protein-drug binding affinities in drug discovery, although several sources of uncertainty continue to limit the application of these techniques. When quantum mechanics (QM) data is used to parameterize molecular mechanic (MM) force fields, the level of QM theory contributes to a systematic bias in the calculated binding free energies. However, the statistical uncertainty that arises due to fitting the MM parameters is seldom considered[1]. We cast the parameterization of force fields as a statistical inference problem and adopt a Bayesian probabilistic framework to automate the parameterization of torsion parameters from QM data. Given that the result of Bayesian inference is a probability distribution (the posterior), we get a distribution of parameters that are consistent with the QM data that can then be used to estimate the uncertainty in computed properties due to parameter error. To propagate the parameter uncertainty, we compute properties, such as hydration or binding free energy with a reference forcefield set from the posterior and estimate the property for other sets from the collection by reweighting. The result is a distribution of the computed property where the width represents the error due to parameter uncertainty. This approach also provides an automated way to select the most probable Fourier terms for the torsions. In addition, it can allow us to reconsider the suitability of current molecular mechanics functional forms to reproduce the properties we are interested in.

B22. Chemical proteomics reveals ADP-ribosylation of small GTPases during oxidative stress

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ADP-ribosylation is a posttranslational modification involved in cellular homeostasis and stress, but has been challenging to analyze biochemically. To facilitate the detection of ADP-ribosylated proteins, we show that an alkyne-adenosine analog, N6-propargyladenosine (N6pA), is metabolically incorporated in mammalian cells and enables fluorescent detection and proteomic analysis of ADP-ribosylated proteins. Notably, our analysis of N6pA-labeled proteins up-regulated by oxidative stress revealed differential ADP-ribosylation of small GTPases. We discovered that oxidative stress induced ADP-ribosylation of Hras on Cys181 and 184 in the C-terminal hypervariable region, which are normally S-fatty-acylated. Downstream Hras signaling is impaired by ADP-ribosylation during oxidative stress, but is rescued by ADP-ribosyltransferase inhibitors. Our study demonstrates that ADP-ribosylation of small GTPases is not only mediated by bacterial toxins but is endogenously regulated in mammalian cells. N6pA provides a useful tool to characterize ADP-ribosylated proteins and their regulatory mechanisms in cells.

A23. Non-invasive ovarian cancer biomarker detection via an optical nanosensor implant

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Ovarian cancer is the fifth-leading cause of cancer-related deaths among females in the United States. Diagnosis after metastasis occurs in 61% of patients, higher than any other form of cancer. Late detection hinders prognosis, though 91% five-year survival rates are seen with earlier detection. Detection of ovarian cancer in high-risk populations, patient response to treatment, and disease relapse is necessary to increase survival rates.

Fluorescent single-walled carbon nanotubes exhibit ideal properties for in vivo biosensing, including photostability, tissue penetration, and environmental sensitivity. Nanotube emission can be modulated by analyte binding, causing a shift in emission wavelength. These characteristics potentiate the use of SWCNT as optical transducers of analyte concentration in vitro, ex vivo, and in vivo.

To address the necessity of early detection for ovarian cancer, we developed a specific sensor for the protein biomarker HE4 nanotubes. The biosensor exhibits low-nanomolar sensitivity for HE4 and strong specificity, characterized by a shift in its fluorescence center wavelength in vitro. We also detected HE4 in ovarian cancer patient serum and ascites ex vivo. We further demonstrated that this sensor can quantify HE4 concentrations in the peritoneal cavity of mice. Finally, the sensor was implanted in ovarian cancer-bearing mice and could quantify HE4 in the peritoneal cavity of these mouse models.

We expect this optical biomarker sensor to aid in early ovarian cancer detection, both in ex vivo samples and in vivo in certain patient populations, thereby reducing patient burden and mortality.