THE HITCHHIKER'S GUIDE TO THE BIOMOLECULAR GALAXY

MAY 2017

A PURDUE MINI-SYMPOSIUM ON INTEGRATING STRUCTURE, FUNCTION, AND INTERACTIONS OF THE BIOMOLECULAR GALAXY

MAY 10-11, 2017

ARMS 1010: PURDUE UNIV.

PURDUE UNIVERSITY

Welcome Letter

Welcome! We are excited to present **"The Hitchhiker's Guide to the Biomolecular Galaxy: A Purdue Mini-Symposium on Integrating Structure, Function, and Interactions of the Biomolecular Universe"**. We believe this event will provide an exceptional networking opportunity for faculty members, postdoctoral scholars, graduate and undergraduate students with diverse areas of expertise but a shared interest in structural biology and biophysics of macromolecules. We have organized this symposium with the hope of developing a network of scientists to foster new collaborations and strengthen existing ones.

The symposium will take place on May 10-11, 2017, on Purdue University's main campus in the Neil Armstrong Hall of Engineering.

Biomolecules-based research from multiple departments and disciplines will be highlighted in five scientific sessions:

Disease, Pathogenesis and Therapeutics

Biophysical Methods and Emerging Techniques

Disorder and Conformational Plasticity in Macromolecules

Macromolecular Complexes and Assemblies

Computational Biology

We are also happy to receive suggestions for additional/alternative scientific sessions. Ultimately, this symposium is organized to provide an enjoyable and enriching experience for the attendees. We would be grateful for your input to make this the best event it can possibly be.

Welcome to the symposium!

Organizer Acknowledgements

This symposium would not be possible without guidance and encouragement from our Purdue University community and the generous support of our sponsors.

We would like to offer a special thanks to the following persons for their advice, guidance and support during the planning of this year's symposium:

Dr. Angeline Lyon (Assistant Professor, Chemistry and Biological Sciences)
Dr. Nicholas Noinaj (Assistant Professor, Biological Sciences)
Dr. Carol Post (Professor, Biological Sciences and Medicinal Chemistry and Molecular Pharmacology)
Dr. Barb Golden (Professor, Ag-Biochemistry)
Dr. Cynthia Stauffacher (Professor, Biological Sciences)
Cathy Skidmore (Business Manager, Medicinal Chemistry and Molecular Pharmacology)
Brittany Vestal (Business Manager, Medicinal Chemistry and Molecular Pharmacology)
Satarupa Bhaduri (Graduate student, Biological Sciences)
Valentyn Stadnytskyi (Graduate student, Physics and Astronomy)
Joann Max (PULSe GSO president)
Heng Wu (PGSG Grant review & allocation officer)

We would like to acknowledge and thank those colleges, departments, and organizations that offered financial support:

College of Science (Dr. Elizabeth Taparowsky)
College of Pharmacy (Dr. Eric Barker)
College of Agriculture (Dr. Jay Akridge)
College of Veterinary Medicine (Dr. Willie Reed & Dr. Harm HogenEsch)
Purdue Drug Discovery Institute (Dr. Philip Low)
Department of Biological Sciences (Dr. Steve Konieczny)
Department of Biochemistry (Dr. Andrew Mesecar)
Department of Physics and Astronomy (Dr. John Finley)
Department of Chemistry (Dr. Timothy Zwier)
Department of Medicinal Chemistry and Molecular Pharmacology (Dr. Zhong-Yin Zhang)
The Purdue Center for Cancer Research (Dr. Cynthia Stauffacher & Douglas Cuttell)
Office of Interdisciplinary Graduate Programs
PULSe - Interdisciplinary Life Science (Dr. Colleen Gabauer)
PGSG - Purdue Graduate Student Government – Co-sponsorship Grant
PI4D – Purdue Institute of Inflammation, Immunology and Infectious Disease (Dr. Thomas Sors)

Last but not least, we would also like to thank our corporate donors for their generous sponsorship:

Anatrace (Ed Pryor)
Beckman Coulter Life Sciences (Jona Brown)
Fisher Scientific (Kevin Peterson)
Gold Biotechnology (Adam McChesney)
Hampton Research (Bob Cudney)
KinaSense, LLC (Dr. Steven Ouellette)
KPrimes Technologies (Nick Yonts)
MilliporeSigma (Dr. Jim Henderson)
MiTeGen (Benjamin Apker)
The Protein Society
Rayonix (Christine Muchmore)
Rigaku (Angela Criswell)
TTP Labtech (Dr. Issa Isaac)

Lorena Beese, Ph.D.

James B. Duke Professor of Biochemistry
Member of the Duke Cancer Institute

Education

B.A. Oberlin College, Mathematics and Biology
Ph.D. Brandeis University, Biophysics
Post-doctoral Fellow: Yale University Molecular Biophysics and Biochemistry (Advisor: Thomas A. Steitz)

Honors and Awards

1994 – 1997	Searle Scholar Award
2005	SER-CAT Outstanding Science Award
2005 – 2015	NIGMS (MERIT) Award
2009	Elected to the National Academy of Sciences

Research in the Beese laboratory is focused on using macromolecular X-ray crystallography, and biochemical, biophysical, genetic, and computational approaches to understand outstanding questions in cancer cell biology.

The Beese lab has made fundamental contributions to understanding the molecular basis of DNA replication and repair. DNA replication requires high fidelity to maintain genome integrity, as mutations introduced during this process can result in mutagenesis. Using X-ray crystallography and computational studies, the Beese group was able to capture high-resolution structures of DNA polymerase I in action. Using this approach, they were able to capture DNA polymerase I bound to mismatched bases and the structural basis of stalling and error repair.

A similar strategy was applied to elucidating the basis of human mismatch repair, which is essential for maintaining genomic stability. Mutations in mismatch repair are associated with increased mutation rates, and increase the likelihood of tumorigenesis. In collaboration with Professor Paul Modrich, structures of DNA repair enzymes were determined in complex with DNA, providing high-resolution insights into how the repair enzymes MSH2-MSH6 and MSH2-MSH3 recognize DNA lesions.

In addition to DNA repair, another area of interest are the enzymes responsible for post-translational lipid modification. Many signal transduction proteins, including the Ras GTPases, undergo this modification, which is essential for membrane localization and signal transduction. Thus, small molecules that block this lipidation from occurring are promising therapeutic strategies in the treatment of many cancers. Towards this goal, the Beese laboratory has determined the structure of two enzymes responsible for the addition of the lipid moiety, along with structures representing catalytic intermediates. More recently, a new avenue in the therapeutic potentials of these enzymes has been identified in human pathogens.

Official Program

All talks will be in ARMS 1010

Wednesday, May 10th

8:00 AM – 9:20 AM	**Breakfast, Check-in and Poster Setup**	
9:20 AM – 9:30 AM	**Opening Remarks *by Samantha Lee***	
Session 1: Disease, Pathogenesis and Therapeutics	***(Chair: Emma Lendy)***	
9:30 AM – 9:55 AM	**Esteban Orellana** ***(Graduate student in Department of Biological Sciences at Purdue University)***	**Enhancing miRNA therapeutic efficacy through vehicle free delivery**
9:55 AM – 10:20 AM	**Adriano Mendes** ***(Graduate student in Department of Biological Sciences at Purdue University)***	**Packaging of Sindbis virus genomic RNA in mosquito versus mammalian cells using next generation sequencing**
10:20 AM – 10:45 AM	**Lee Stunkard** ***(Graduate student in Department of Biochemistry at Purdue University)***	**Investigating the Catalytic Molecular Details of Malonyl-thioester Decarboxylating Enzymes**
10:45 AM – 11:10 AM	***Coffee Break***	
Session 2: Biophysical Methods and Emerging Techniques I	***(Chair: Scott Jensen)***	
11:10 AM – 11:35 AM	***Valentyn Stadnytskyi*** **(Graduate student in Department of Physics and Astronomy at Purdue University*)***	**Accessing chiral information in macromolecules via time-resolved circular dichroism spectroscopy**
11:35 AM – 12:00 AM	**Matthew Wohlever** **(Postdoctoral scholar in Department of Biochemistry and Molecular Biology at The University of Chicago*)***	**Msp1 is a membrane protein dislocase for tail-anchored proteins**
12:00 AM – 12:25 PM	**Rui Yan** ***(Graduate student in Department of Biological Sciences at Purdue University)***	**s2stigmator: real-time detection and single-pass minimization of TEM objective lens astigmatism**

12:25 PM – 1:20 PM	**Lunch & Talks**	
12:45 pm - 1:05 pm	**Edward Pryor - Anatrace** ***"An update on detergent usage in membrane protein structural biology"***	
1:05 pm - 1:20 pm	**Steve Ouellette - KinaSense *"A Startup Company's Journey Through the Biomolecular Galaxy"***	
Session 3: Disorder and Conformational Plasticity in Macromolecules ***(Chair: Kristina Kesely)***		
1:30 PM – 1:55 PM	**Lyman Monroe** ***(Graduate student in Department of Biological Sciences at Purdue University)***	**Variability of Protein Structure Models from Electron Microscopy**
1:55 PM – 2:20 PM	**Feifei Zhao** ***(Graduate student in Department of Chemistry at Purdue University)***	**Joule heating and thermal denaturation of proteins in nano-electronspray ionization mass spectrometry (nESI-MS) theta tips**
2:20 PM – 2:45 PM	**Ahmed Hassan** ***(Graduate student in Department of Comparative Pathology at Purdue University)***	**Repurposing approach identifies auranofin with novel antimicrobial activities against Clostridium difficile**
2:45 PM – 3:10 PM	***Coffee Break***	
Session 4: Macromolecular Complexes and Assemblies	***(Chair: Yvon Rugema)***	
3:10 PM – 3:35 PM	**Ignacio Mir-Sanchis** ***(Postdoctoral scholar in Department of Biochemistry and Molecular Biology at The University of Chicago)***	**Structural studies of the initiation of replication of two staphylococcal mobile elements linked to disease.**
3:35 PM – 4:00 PM	**Matthias Fellner** ***(Postdoctoral scholar in Department of Biochemistry and Molecular Biology at Michigan State University)***	**Structural Insights into the Catalytic Mechanism of LarE, a Sacrificial Sulfur Insertase of the N-type ATP Pyrophosphatase family**
4:00 PM – 4:25 PM	**Adam Yokom** ***(Graduate student in Department of Chemical Biology at University of Michigan)***	**Translocation mechanism of the AAA+ disaggregase Hsp104**
4:25 PM – 6:30 PM	**Poster Session and Reception:** ***Food and drinks while supplies last***	
7:00 PM	**Networking Event:** ***Stacked Pickle - 516 Northwestern. Ave.***	

Thursday, May 11th

8:30 AM – 9:30 AM	**Breakfast**	
Session 5: Biophysical Methods and Emerging Techniques II *(Chair: Vatsal Purohit)*		
9:30 AM – 9:55 AM	**Nathan Gardner** *(Postdoctoral scholar in Department of Biochemistry and Molecular Biology at The University of Chicago)*	**Plug-and-play: a calmodulin based fiducial marker to solve protein structures by Cryo-EM**
9:55 AM – 10:20 AM	**Amar Parvate** *(Graduate student in Department of Biological Sciences at Purdue University)*	**Cryo-electron tomography of New World Hantaviruses for morphological and structural analyses**
10:20 AM – 10:45 AM	**Erh-Ting Hsu** *(Graduate student in Department of Chemistry at Purdue University)*	**Monitoring Integral Membrane Proteases ZMPSTE24 and Ste24p Activities by a Peptide-Based FRET Assay**
10:45 AM – 11:10 AM	***Coffee Break***	
11:10 AM – 12:20 PM	**Keynote Presentation: Lorena Beese - Duke University**	
12:20 PM – 1:20 PM	**Lunch & Talks**	
12:35 PM - 12:55 PM	**Issa Isaac - TTP Labtech**	
12:55 PM - 1:10 PM	**Andy Mesecar - Purdue University *"StructureBio Facilities at Purdue"***	
Session 6: Computational Biology *(Chair: Aaron Krabill)*		
1:20 PM – 1:45 PM	**Jonathan Fine** *(Graduate student in Department of Chemistry at Purdue University)*	**Differential target profile based computational drug library design**
1:45 PM – 2:10 PM	**Aashish Jain** *(Graduate student in Department of Biological Sciences at Purdue University)*	**Phylo-PFP: Highly accurate phylogenomics-based protein function prediction method**
2:10 PM – 2:35 PM	**Matthew Pharris** *(Graduate student in Department of Biomedical Engineering at Purdue University)*	**A Rule-based Model of the Calcium/Calmodulin-dependent Protein Kinase II**

2:35 PM – 3:00 PM	**Ying Yang** ***(Graduate student in Department of Medicinal Chemistry and Molecular Pharmacology at Purdue University)***	**Addressing the Effects of Solvation in Protein-ligand Binding using Advanced GPU-enabled Computational Method WATsite**
3:00 PM – 3:30 PM	**Closing Remarks, Awards, and Acknowledgements**	

Oral Presentations

Session 1: Disease, Pathogenesis and Therapeutics

Enhancing miRNA therapeutic efficacy through vehicle free delivery

Esteban A. Orellana[2,4], Srinivasarao Tenneti[3], Loganathan Rangasamy[3], Philip S. Low[3], and Andrea L. Kasinski[1,2].
Purdue Cancer Center[1], Department of Biological Sciences[2], Department of Chemistry[3], and PULSe Graduate Program[4], Purdue University, West Lafayette, IN 47907

Efforts to search for better treatment options for cancer have been a priority and due to these efforts new alternative therapies have emerged. For instance, clinically relevant tumor suppressive microRNAs (miRNAs) that target key oncogenic drivers have been identified as potential therapeutics to treat cancer. MiRNAs are small non-coding RNAs that negatively regulate gene expression at the posttranscriptional level. It has been shown that aberrant mature miRNA levels, through misexpression of miRNA target genes and/or biogenesis of unprocessed miRNAs, can have profound cellular effects leading to a variety of diseases, including cancer. While altered miRNA levels contribute to a cancerous state, restoration of miRNA expression has therapeutic benefits. For example, ectopic expression of miRNA-34a (miR-34a), a tumor suppressor gene that is a direct transcriptional target of p53 and thus is reduced in p53 mutant tumors, has clear effects on reducing cell proliferation and increasing survival in murine models of cancer. It is expected that miRNA replacement therapies will have profound effects in the clinic; however, miRNA therapeutics are still in their infancy and there are critical challenges that need to be addressed for the advancement of miRNA based therapies. One of the biggest challenges for miRNA advancement into the clinic is efficient delivery of miRNA mimics due to problems such as delivery-associated toxicity, poor transfection, systemic clearance, degradation in circulation, immune response, and endosomal sequestration. Therefore, there is a critical need to identify strategies to overcome these obstacles and facilitate the advancement of miRNA therapies into clinical trials. In order to address these obstacles we hypothesized that a ligand targeted miRNA strategy could be used for successful delivery of the therapeutic miRNA into target tissues. For this purpose we employed folic acid (folate; FA) to deliver unprotected miR-34a specifically to cancer cells or tissues. Folic acid is essential for biosynthesis of nucleotide bases and as such many malignant cells overexpress high affinity folate receptors (FRs) in their cellular membrane, which renders FA as an attractive targeting ligand for tumor-specific miRNA delivery. In this present work, we have developed a protocol to conjugate folate to naked miR-34a and were able to show successful delivery of functional miR-34a both *in vitro* and *in vivo.* Collectively these studies will address an unmet need of therapeutically targeting miRNAs for the treatment of cancer. This work was supported by R00CA178091 (ALK) and P30CA023168 (Purdue University Cancer Center).

Packaging of Sindbis virus genomic RNA in mosquito versus mammalian cells using next generation sequencing

Adriano Mendes, Andrew L. Routh and Richard J. Kuhn
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Sindbis virus (SINV) is the prototype species of the genus Alphavirus in the family *Togaviridae*. As arboviruses, alphaviruses utilize predominantly mosquito vectors for transmission to vertebrate hosts. Infection between vector and host are fundamentally different owing to the difference in physiology between the two species. Insect tissue culture cells, such as the *Aedes albopictus* C6/36 cell line, have been employed as models for insect cell infection in the past and a variety of studies have shown that as in nature, alphaviruses infect these cells differently when compared to mammalian cells. The aim of this study was to examine the packaging of the genomic RNA of SINV particles purified from C6/36 (insect) or BHK (mammalian) cells. To do this we utilized a newly developed next generation sequencing approach known as ClickSeq. Using this approach, we have seen a substantial difference in the genomic RNA content between BHK and C6/36 derived particles. Significantly more cellular RNA was packaged into C6/36 SINV particles and this effect was time-dependent, as later time points showed more cellular RNA packaging. Our working model is that this may be related to whether or not the virus induces cell death as a result of infection. C6/36 cells are able to maintain growth while infected with SINV and we believe that this may affect the specificity of RNA packaging at later time points. Our future work seeks to explore this hypothesis as well as a possible link to persistent insect infection.

Investigating the Catalytic Molecular Details of Malonyl-thioester Decarboxylating Enzymes

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Decarboxylation reactions release the free energy necessary for carbon-carbon bond formation in the biosynthesis of polyketides and fatty acids. Ketosynthases from fatty acid and polyketide synthases perform a decarboxylative Claisen-condensation between an acyl-thioester intermediate and a malonyl-derived extender unit. However, the molecular details of decarboxylation and C-C bond formation is not well understood. We are synthesizing and characterizing non-hydrolyzable and non-decarboxylatable substrate mimics to capture intermediate catalytic states via X-ray crystallography. Capturing the substrate mimics in the active site of ketosynthases will yield insight into the mechanism of decarboxylation and polymerization of the two carbon units. Decarboxylation reactions are essentially non-reversible and have various possible chemical mechanisms, which makes them difficult to study. Ketosynthases are also challenging to study due to their multiple substrates and need for protein-protein interactions. Therefore, we have begun characterization of our substrate mimics with simpler systems, such as malonyl- and methylmalonyl-CoA decarboxylases (MCD and MMCD), as a proof of principle. We have successfully crystallized E. coli MMCD and the bifunctional acyltransferase/decarboxylase, LnmK, with our substrate mimics. Our substrate mimics preserve the thioester carbonyl as a thioester, ester or amide and have a nitro or sulfonate moiety in place of the carboxylate. Structures of MMCD and LnmK with multiple bound substrate mimics reveals mechanistic insight into decarboxylation. While MMCD and LnmK have no sequence homology to ketosynthases, this study reveals that our substrate mimics indeed inhibit decarboxylation reactions. Therefore, we are beginning to apply this concept to ketosynthases from type III polyketide synthases.

Session 2: Biophysical Methods and Emerging Techniques I

Accessing chiral information in macromolecules via time-resolved circular dichroism spectroscopy

Valentyn Stadnytskyi[1], Gregory Orf[2], Robert Blankenship[2], Sergei Savikhin[1]
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Circular dichroism (CD) spectroscopy is a quick and non-invasive tool for structural investigation of chiral molecules and systems. In photosynthetic proteins, chiral properties often originate from interaction between strongly coupled pigments, which leads to creation of excitonic states. Spectral properties of these states are sensitive to distance between chromophores and their relative orientation, hence they are structure sensitive. In addition, these states play crucial role in energy transfer, charge separation and electron transfer. Unfortunately, direct observation of these states is extremely difficult by transient absorption spectroscopy mainly because they are hidden by non-or weakly interacting chromophores. One of the many examples is Photosystem I where the special pair is spectrally hidden by other cofactors of the antenna but can be resolved using CD spectrum.

In this work, we report the concept and design of a shot-noise limited optical time-resolved CD spectrometer capable of measuring signals as low as 7 $x10^{-7}$ OD (20 deg), which is two orders of magnitude more sensitive than previously reported. The proposed spectrometer utilizes an ellipsometric method that gives enhancement in the detected levels of CD signals in comparison to conventional CD spectrometer. The extremely low sensitivity allowed us to successfully observe for the first time transient CD dynamics of triplet states in the Fenna-Matthews-Olson antenna protein complex. This new design allows accessing of the excitonic state and observe conformational changes on nanosecond/femtosecond time scale with a sensitivity significantly higher than previously reported. This technique is a great tool with the potential to measure changes in chiralities of different origin, such as secondary and tertiary structure of proteins or mutual orientation of chromophores in photosynthetic antenna complexes.

Msp1 is a membrane protein dislocase for tail-anchored proteins

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Mislocalized tail-anchored (TA) proteins of the outer mitochondrial membrane are cleared by a newly identified quality control pathway involving the conserved eukaryotic protein Msp1 (ATAD1 in humans). Msp1 is a transmembrane AAA-ATPase but its role in TA protein clearance is not known. Here, using purified components reconstituted into proteoliposomes we show that Msp1 is

both necessary and sufficient to drive the ATP-dependent extraction of TA proteins from the membrane. A crystal structure of the Msp1 cytosolic region modeled into a ring hexamer suggests that active Msp1 contains a conserved membrane-facing surface adjacent to a central pore. Structure-guided mutagenesis shows that the axial pore loops, but not the N-domain, are critical for TA protein extraction in vitro and for functional complementation of ΔMsp1 yeast in vivo. Together these data provide a molecular framework for Msp1-dependent extraction of mislocalized TA proteins from the outer mitochondrial membrane.

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*s²stigmator: r*eal-time detection and single-pass minimization of TEM objective lens astigmatism

Rui Yan[a], Kunpeng Li[a], Wen Jiang[a,b,c*]
[a]Markey Center for Structural Biology, Department of Biological Sciences, [b]Department of Chemistry, [c]Purdue Institute of Inflammation, Immunology and Infectious Disease, Purdue University, West Lafayette, IN 47907, USA

Transmission electron microscopy (TEM) has become a powerful technique for structural characterization of a wide range of materials including macromolecular complexes at near-atomic resolutions. In order to obtain high-quality images, minimization of the astigmatism of the objective lens is a critical task of the daily microscope alignment. The current method widely used by many microscopists is to visually examine the roundness of Thon rings from live images and simultaneously adjust objective lens stigmators to make the Thon rings as circular as possible. However, this subjective method is limited by poor sensitivity and potentially biased by the astigmatism of human eyes. Therefore, a quantitative method is desirable to rapidly and accurately estimate the astigmatism and provide real-time feedback for the adjustment of the stigmators, in order to efficiently correct astigmatism. In this work, an s2 power spectra based method, *s2stigmator*, was developed and implemented as a DigitalMicrograph script to allow fast and sensitive detection of the astigmatism in TEM live images. It can provide real-time feedback and user-friendly "radar"-style display to help guide the adjustment of objective lens stigmators and efficiently correct the astigmatism of the objective lens using a single-pass tuning strategy. With the help of *s2stigmator*, we have found that the large change of defocus/magnification between visual correction of astigmatism and subsequent data collection tasks, or even during data collection will inevitably result in undesirable residual astigmatism in the final images. These findings have essentially invalidated a basic assumption of current cryo-EM imaging strategies that assumes invariant astigmatism for different defocuses/magnifications used in the microscope alignment stage and the final data acquisition stage. Based on these findings, we recommend the same magnification and the median defocus of the intended defocus range for final data collection are used in the objective lens astigmatism correction task during microscope alignment and in the focus mode of the iterative low-dose imaging.

Session 3: Disorder and Conformational Plasticity in Macromolecules

Variability of Protein Structure Models from Electron Microscopy

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An ever increasing number of protein structures are solved each year by means of electron microscopy. One factor driving the popularity of electron microscopy for the determination of protein structures is its increasing resolution capability. However, the resolution and quality of electron microscopy maps range considerably which has a significant effect on the structural information that can reliably be extracted from this kind of data. As a result, the quality of atomic models derived from electron microscopy maps vary substantially. To understand the extent to which atomic structure models are supported by information in electron microscopy maps, we used two computational structure refinement methods to examine how much structures were modified using a dataset of 49 maps with their corresponding structure models. The extent of structure modification as well as the disagreement between refinement models produced by the two computational methods was found to scales inversely with the global and local map resolution. This works was published in Structure on-line (March, 2016)

Joule heating and thermal denaturation of proteins in nano-electronspray ionization mass spectrometry (nESI-MS) theta tips

Feifei Zhao, Sarah M. Matt, Jiexun Bu, Owen G. Rehrauer, Dor Ben-Amotz, Scott A. McLuckey
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Mass spectrometry (MS) is known as a fast and efficient method to identify molecular structures based on molecular mass and subsequent fragmentation pathways using a small sample amount. Development of soft ionization methods has made it possible to use mass spectrometry to analyze large biomolecular structures including proteins, lipids, carbohydrates, DNA *etc*. Nano-electrospray ionization (nESI), as one of the soft ionization methods, that allows the preservation of intact protein conformations, and is widely used to study protein tertiary and quaternary structures. A protein molecule is normally multiply charged through nESI, and the charge state distribution (CSD) is related to protein conformation. It is generally accepted that more unfolded structures have high charge state distribution (HCSD) while folded structures have low charge state distribution (LCSD).

Dual channel theta tips were recently developed as micromixers and nESI emitters. Different solutions can be loaded in each channel and, upon spraying, allow for a reaction to take place in the Taylor cone and subsequent droplet in sub-millisecond to seconds time scale. This technique has been used to study the kinetics of protein unfolding and folding by mixing protein solutions with acidic or buffered solutions. Short lived folding intermediates were observed. Applying differential voltages in the two channels before spraying can induce electroosmosis, which elongates the reaction time. Electroosmotically mixing protein with acid in a theta tip was shown to denature protein more efficiently compared to non-electroosmosis condition.

In this study, we found that electroosmosis of a protein in ammonium acetate buffer could induce Joule heating, which can denature a protein. Myoglobin, cytochrome *c* and carbonic anhydrase were denatured using this technique. The protein denaturation extent increases with electroosmotic voltage and buffer concentration. The temperature of the solution at the theta tip was measured via Raman thermometry and the temperature increases with electroosmotic voltage. Therefore, the Joule heating effect is proven via an independent measurement. Thermal denaturation of a myoglobin, cytochrome *c* and ubiquitin mixture is performed. The extent of denaturation, relative to each other, followed the trend of their previously published melting points. Joule heating in theta tip electroosmosis provides a fast and convenient way to thermally manipulate protein conformation, and also provides a temperature-controlled platform to study solution phase reactions analyzed via MS.

Repurposing approach identifies auranofin with novel antimicrobial activities against *Clostridium difficile*

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Clostridium difficile infection is the most common and costly healthcare-associated infection. C. difficile causes half a million cases and approximately 29,000 deaths annually in the United States alone. Only one new antibiotic, fidaxomicin, has been approved in the last 50 years for treatment of *C. difficile* infection.. However, recurrence rates for fidaxomicin were still high for infections involving the hypervirulent strains (24% recurrence rate). Thus, there is a pressing need to develop novel anticlostridial drugs with improved efficacy. Repurposing drugs, with well-characterized toxicology and pharmacology, to find new applications outside the scope of the original medical indication is a novel way to reduce both the time and cost associated with antimicrobial innovation. A library, containing 4,000 FDA approved drugs and small molecules, was screened against *C. difficile* toxigenic strain. The initial screening identified 18 non-antibiotic drugs and clinical molecules with potential activity against *C. difficile*. Among the active non-antibiotic drugs, auranofin (currently approved for treatment of rheumatoid arthritis) showed bactericidal activity, in an applicable clinical range, against clinical isolates of *C. difficile*. The minimum inhibitory concentrations at which 90% of clinical isolates of *C. difficile* were inhibited (MIC_{90}) were found to be 2 µg/ml. Auranofin was superior to the drugs of choice, vancomycin and metronidazole, in inhibiting toxin production by toxigenic *C. difficile* strains. In addition, auranofin suppressed spore formation which can potentially lower the transmission and recurrence of infection. Interestingly, auranofin has many additional advantageous qualities over drugs of choice, including neutralizing the *C. difficile* toxins in vitro suppressing the *C. difficile* toxin-mediated inflammation of the gut cells, and restoring their viability. Taken altogether, results garnered from this study indicate that auranofin is superior to the current anticlostridial drugs and warrants further investigation as novel antibacterial agent against *C. difficile*.

KinaSense: A Startup Company's Journey Through the Biomolecular Galaxy

Steve Ouellette
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KinaSense is a development stage company focused on bringing innovative assays to biomolecular researchers. The company's core technology was developed at Purdue University in the Department of Medicinal Chemistry and Molecular Pharmacology. KinaSense is integrating the technology into a pipeline to develop a suite of cell-based kinase activity assays for a wide range of kinase targets involved in cancer pathogenesis and driving progression of the disease. Our target products will enable researchers to probe endogenous kinase activity in the researcher's cellular model of choice, without the need for antibody-based methods or genetically engineered cell lines. In this talk, we will give an overview of the core technology and KinaSense's role in turning a Purdue University invention into an accessible tool for researchers studying kinase-related biology and novel kinase inhibitors.

Session 4: Macromolecular Complexes and Assemblies

Structural studies of the initiation of replication of two staphylococcal mobile elements linked to disease.

Ignacio Mir-Sanchis[1], Ying Pigli[1], Phoebe Rice[1].
[1]Department of Biochemistry and Molecular Biology, The University of Chicago.

Staphylococcal Cassette Chromosome elements (SCC) are a family of genomic islands that when encoding *mec*A gene (then called SCC*mec*) turns *Staphylococcus aureus* into methicillin resistant strain (MRSA). These islands are classified as non-replicative mobile elements. SCC elements encode the recombinases that catalyze their integration/excision reactions, but the molecular mechanisms by which SCCs are horizontally transferred remain unclear. We discovered that the genes surrounding the recombinases are highly conserved and related to replication. Among these conserved genes, I will present our current studies of two of them: *cch*, *lp*1413. The first protein, Cch, is a large ATPase protein always located upstream of the recombinases. It shows low sequence homology but nearly identical predicted secondary structure with the replication initiator protein (Rep) from another family of SCC-unrelated Gram positive genomic islands called SaPIs. The second gene present in the *cch*-containing operon is called *lp*1413 and we have characterized it as single stranded DNA binding protein. Here we present the 2.9Å X-Ray crystal structures of Cch and the 2.2Å crystal structure of LP1413, both from SCC*mec* type IV (present in the epidemic staphylococcal strain USA300). Our Cch model shows that forms a 420KDa ring shaped hexamer, the common architecture of the replicative helicases. Surprisingly, the closest structural homologs to Cch's AAA+ domain were the archaeal MCM replicative helicases. We show that Cch binds dsDNA and has ATP-dependent helicase activity *in vitro*, with 3' to 5' polarity indicating that is not only structural but also functional MCM homolog. In addition, I will present our initial cryo-EM data regarding SaPI-encoded Rep, which is not only the SaPI's initiator of replication but also has helicase activity (ring shaped hexamer while helicase mode). Using structural biology techniques, our model system will allow us to study helicase loading and translocation, a pivotal step in DNA replication.

Structural Insights into the Catalytic Mechanism of LarE, a Sacrificial Sulfur Insertase of the N-type ATP Pyrophosphatase family

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The *lar* operon in *Lactobacillus plantarum* encodes five Lar proteins (LarA/B/C/D/E) which collaboratively synthesize and incorporate a niacin-derived nickel-containing cofactor in LarA, a nickel-dependent lactate racemase. Previous studies have established that two molecules of LarE catalyze successive thiolation reactions by donating the sulfur atom of their exclusive cysteine residues to the substrate. However, the catalytic mechanism of this very unusual sulfur sacrificing reaction remains elusive. In this work, we present the crystal structures of LarE in ligand-free and several ligand-bound forms, demonstrating that LarE is a new member of the N-type ATP pyrophosphatase family with a conserved *N*-terminal ATP pyrophosphatase domain and a unique *C*-terminal domain harboring the putative catalytic site. Structural analysis combined with a structure-guided mutagenesis study lead us to propose a catalytic mechanism that establishes LarE as a new paradigm for sulfur transfer through sacrificing the catalytic cysteine residue.

Translocation mechanism of the AAA+ disaggregase Hsp104

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The Heat Shock Protein (Hsp) 100s class of AAA+ disaggregases form hexameric ring complexes and translocate substrates through the central channel to re-solubilize, re-fold or degrade misfolded proteins. Additionally, the yeast Hsp104 complex is used for prion propagation and promotes thermotolerance. Hsp104 has been shown to disassemble neurodegenerative aggregates such as amyloid beta and alpha synuclein. Our recently published work has established an ATP-bound Hsp104 spiral hexamer state using cryo EM. This study revealed a spiral staircase arrangement of the conserved tyrosine loops, a unique heteromeric AAA+ interaction and a distinct Middle Domain conformation. In order to better understand the mechanism of translocation for AAA+ proteins, our current work has focused on a Hsp104 substrate-bound complex. We have performed cryo EM analysis on this complex to identify the substrate engaged state to near atomic resolution, revealing a conformational ratchet arrangement of the Hsp104 hexamer. This analysis demonstrates how Hsp100s and other AAA+ proteins might translocate polypeptide through the central channel.

Session 5: Biophysical Methods and Emerging Techniques II

Plug-and-play: a calmodulin based fiducial marker to solve protein structures by Cryo-EM

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Membrane proteins are promising drug targets, but their structures are notoriously difficult to obtain. Cryo-EM now offers an alternative to crystallography for solving the structure of membrane proteins and complexes. However, cryo-EM generally provides lower resolution as protein size and symmetry are limiting factors. We are developing a plug-and-play system consisting of calmodulin and antigen binding fragments (Fab) to increase the mass of a target protein and provide a clear fiducial marker to aid in orienting the target for cryo-EM structures. Using phage display of a diverse Fab library, we selected Fab that bind to a calmodulin binding peptide (CBP) bound conformation of calmodulin with high affinity. We obtained Fabs for two separate epitopes on calmodulin, which allows two Fabs to bind calmoduin simultaneously for a total mass of 105 kDa. We have verified the system by fusing CBP to maltose binding protein (MBP) and observing the MBP:calmodulin:Fab complex by negative stain EM. For use as a fiducial marker, the CBP fusion must be rigid to maintain a constant orientation. CBP is a helical peptide and we fused it as an extension of the C-terminal MBP helix to form one continuous helix. To tailor our calmodulin system to different proteins, only the calmodulin binding peptide must be fused to a helix of the protein of interest. We will next extend the system for use with membrane proteins; GPCRs are only about 45 kDa, but we expect our calmodulin system will bring the mass of a complex with a GPCR up to 150 kDa while providing a fiducial marker and allowing us to solve the cryo-EM structure of the small receptor.

Cryo-electron tomography of New World Hantaviruses for morphological and structural analyses

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Hantaviruses belong to the *Bunyaviridae* family and constitute a group of human pathogens causing hemorrhagic fevers with 15-40% mortality. In the absence of any anti-viral drugs or vaccines, they are a human health threat. Hantaviruses are classified as BSL3. Most electron microscopy facilities do not allow BSL3 samples to be loaded on the microscope. Hence a method to purify and inactivate *Orthobunyavirus* (a BSL2 virus) using 1% glutaraldehyde was optimized. The inactivated virus sample was used to perform cryo-electron tomography (cryo-ET). This method of virus purification was replicated in a BSL3 facility to prepare and inactivate Andes virus (a Hantavirus) samples for cryo-ET. It was observed that Andes virus displays 3 morphologies - round, elongated and irregular morphologies. In contrast, a predominantly round morphology has been reported for other Hantaviruses. It is not known whether the diverse morphologies have any bearing on the lethality of the virus and what are the structural characteristics of these viruses. The Gn-Gc glycoprotein complex on the virus surface is involved in binding to host cell receptors and fusion. Tomographic data showed glycoprotein spikes were clearly visible on the surface of both the glutaraldehyde fixed viruses. It was initially hypothesized that the Andes virus has a tetrameric 4-fold spike complex like Hantaan and Tula viruses. Further analyses showed that the Andes virus spike had a different symmetry and a smaller size than the other Hantavirus spikes reported in literature.

Monitoring Integral Membrane Proteases ZMPSTE24 and Ste24p Activities by a Peptide-Based FRET Assay

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Human ZMPSTE24 is a unique intramembrane zinc metalloprotease that is localized to both the endoplasmic reticulum and inner nuclear membranes. The enzyme catalyzes two distinct cleavage steps in the maturation of lamin A, including the endoproteolytic removal of the C-terminal -aaX residues adjacent to an isoprenylated cysteine residue, and a second discrete site-specific upstream cleavage.

Improper prelamin A processing caused by mutations in the gene encoding ZMPSTE24 results in progeroid diseases. Our preliminary data showed that both ZMPSTE24 and another novel CaaX protease, RCE1, could efficiently cleave the CaaX motif from prelamin A. This suggests that the ability of ZMPSTE24 to perform the upstream cleavage determines the level of accumulated uncleaved prelamin A in progeroid diseases.

Therefore, it is important to develop assays for monitoring the upstream proteolytic activity of ZMPSTE24. We measured the enzymatic activity using a newly established fluorescence-based assay with full-length **a**-factor containing a 2-aminobenzoic acid (Abz) fluorophore at the N-terminus and a dinitophenol (DNP) quencher at the C-terminus. After cleavage, quantification of the fluorescence from the dequenched cleaved peptides enabled us to directly monitor the cleavage activity of ZMPSTE24 and its yeast homolog, Ste24p. Our data revealed that the disease mutants demonstrated reduced upstream cleavage activity.

We will utilize this fluorescence-based assay and other biochemical methods to study precise catalytic mechanisms and structural bases of ZMPSTE24 and Ste24p, by which further insights into progeroid diseases will be provided.

Session 6: Computational Biology

Differential target profile based computational drug library design

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Modern drug discovery uses large libraries to screen against one or few disease targets using high-throughput screening with iterative design endeavors costing millions of dollars. This paradigm is optimized to develop drugs against a single target which was thought necessary to minimize side effects. However, it has an intrinsic high failure rate due to efficacy of wrong target selection, resistance mechanisms of singular targeting or toxicity due to unknown off-target effects. Our work indicates that most human ingestible drugs function as a differential interaction with multiple proteins from different druggable protein classes. Here, we introduce computational methods for compound library development. Instead of screening millions of compounds that are randomly synthesized and have intrinsic high failure rate, one uses virtual screening with multiple proteins combined with target and antitarget based lead optimization to make libraries focused on signaling pathways. We evaluate proteome wide effects of a single compound to translate the utility of computational methods to medicine. We will introduce our new fragment-based hierarchical and flexible interaction design program (candock) to predict the affinity of a molecule with targets and off-targets at the proteome scale to develop pathway specific libraries. Candock: (i) incorporates protein side and main chain flexibility during the design and interaction calculations, (ii) samples the torsional conformational space effectively by effectively implementing atoms to fragments linking, (iii) uses a statistical potential to identify the best atom type for a given chemical environment for pocket specific design, and (iv) optimizes compound designs based on a given differential interactions profile. Our approach has led to potent, non-toxic, pathway specific molecules verified experimentally for anticancer and immunomodulatory effects. This approach of pathway specific compounds will have a broader impact leading to chemical control of biological pathways in diseases with overlapping mechanisms hopefully reducing failure rates in drug discovery.

Phylo-PFP: Highly accurate phylogenomics-based protein function prediction method

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In this era of genomics, new sequences are being determined at a much faster rate than they can be experimentally annotated, creating a gap between known protein sequences and annotated proteins. Thus, computational algorithms which can accurately predict protein functions have gained a great deal of attention. A conventional way of annotating unknown proteins is to transfer function from the top hits of homology search, but this has been shown to have a low coverage in genome annotation and often

cause erroneous annotations. Here we have developed Phylo-PFP, a sequence based function prediction software, which utilizes sequence similarity and phylogenomics to predict function, and is capable of mining functional information from PSI-BLAST hits with very large E-values (low sequence similarity). We compared Phylo-PFP with our previous method, PFP, which was ranked among top predictors in Critical Assessment of Functional Annotation (CAFA2), a world-wide function prediction competition. We found that it significantly increased the accuracy on the benchmark dataset. To compare it with other methods available, we replicated the analysis of CAFA2, and showed that Phylo-PFP outperforms the best prediction programs to date.

A Rule-based Model of the Calcium/Calmodulin-dependent Protein Kinase II

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In the brain, learning and memory formation depend on a process called synaptic plasticity. In synaptic plasticity connections between neurons dynamically shift in size and cation conductivity. These shifts are delicately controlled within neurons by calcium-dependent protein signaling mechanisms. Because dysregulation of these mechanisms can lead to neurological disorders, researchers have long sought understanding through experimental techniques. However, common experimental techniques such as fluorescent microscopy and genetic knockdown lack the spatiotemporal resolution necessary to fully describe the protein interactions behind synaptic plasticity. Therefore, computational models are increasingly used to complement experimentation. To recapitulate experimental results, models often require detailed spatial and kinetic parameters, rapidly accruing computational expense. Computational expense is especially problematic in models of calcium/calmodulin-dependent protein kinase II (CaMKII), the so-called “memory molecule”.

To rigorously model CaMKII is to fully account for its complex structure. The CaMKII structure features twelve subunits, and each can exhibit a variety of binary states: for example, open or closed, ligand bound or unbound, and active or inactive. Importantly, a CaMKII subunit’s ability to transition states depends on its neighboring subunits. This subunit inter-dependence would typically implicate a system of 10^20 differential equations, which is computationally intractable. To reduce this system, we apply rules, based on experimental observations, to inform the conditions by which a subunit may transition in state. With rule-based modeling, we present the first spatial-stochastic model of CaMKII as a complete twelve-subunit enzyme.

Our rule-based CaMKII model is consistent with experimental results and exhibits increased parameter robustness compared to non-ruled model variants. As ruled models are less computationally expensive, future work will incorporate our ruled CaMKII into larger network models of calcium-dependent neuronal signaling. Ultimately, we expect to use this model to more effectively characterize molecular mechanisms of memory formation and eventually predict therapeutic targets for neurological disorders.

Addressing the Effects of Solvation in Protein-ligand Binding using Advanced GPU-enabled Computational Method WATsite

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Computer-aided drug design (CADD) plays a vital role in drug discovery which has also become an indispensable tool in the pharmaceutical industry. With the emergence of new hardware and increasing computational power, five times increase in speed of molecular dynamic (MD) simulation has been achieved.

The most fundamental goal in CADD is to accurately and efficiently predict whether a given ligand will bind to the target protein, and if so, how strongly it will bind. Solvation effects, such as solvent re-organization, or the release of tightly bound water upon ligand binding can contribute significantly to the entropic term of the binding free energy. Thus, how to efficiently and accurately account for the solvation effects caused by the water molecules still remains one of the most significant challenges in CADD.

Here, we present the development and application of GPU-accelerated computational program WATsite. First, the localized positions of water molecules, i.e. hydration sites, are identified and their thermodynamic profiles, i.e. enthalpy and entropy of desolvation, are then computed. In addition, the influence of protein flexibility on water locations and thermodynamic profiles will be discussed.

Poster Presentations

First Name	Last Name	Title	#
Kedar	Puvar	Biochemical studies of Legionella effectors involved in E1/E2-independent ubiquitination	1
Mark	McLean	Mechanisms in Cancer Signaling: The Role of the Membrane in the Recruitment of the Oncogene KRas4b	2
Ben	Watson	Regulation of cell signaling by Fic-mediated post-translational modifications	3
Jamie	VanPelt	Optimization of Novel Small Molecule Inhibitors Through Structural Characterization of Beta-Lactamases	4
Doungkamol	Alongkronrusmee	Identification of a novel interaction site of the mu-delta opioid receptor heterodimers	5
Sandeep	Singh	Complexities of Super-Complexes in Energy-Transducing Membranes	6
Reena	Blade	Strategic Modifications to a Novel Class of Antibacterial Peptides, Cationic Amphiphilic Polyproline Helices (CAPHs), for Superior Cellular Uptake and Enhanced Antibacterial Activity	7
Ahmadreza	Ghanbarpour	FibPredictor 2: A computational approach for the prediction of amyloid fibril classes and key residue interactions	8
Jozlyn	Clasman	Crystal Structure and Kinetic Profile of a Core Papain-like Protease of MERS Coronavirus with Utility for Structure-based Drug Design	9
Mengmeng	Zhu	Efficient deep learning famework for biological 3D image data retrieval: an application with protein binding ligand prediction	10
Phillip	Rushton	Modeling the Molecular Structure of a Cellulose Synthase Plant Conserved Region with X-ray Crystallography and Small Angle X-ray Scattering Illustrates Potential Functions	11
Yingyuan	Sun	Structural changes of bacteriophage ΦX174 during DNA ejection induced by lipopolysaccharides	12
Ziyun	Ding	Computational Methods for Predicting Protein-Protein Interactions in Plants	13
Kai	Zhang	Inhibition of MCP-1/CCR2 Signaling Offers Protection Against Ethanol-Induced Damage to the Developing Brain	14
Elisabeth	Garland-Kuntz	Structural Insights into Phospholipase Ce: A Mulit-Method Biophysical Approach	15
Matthew	Therkelsen	Structural studies of flavivirus rearrangements using cryo-EM	16
Yongchao	Wang	Simplify Your Transfection and Improve Your Transfection Efficiency	17
Candi	Esquina	Electrostatic Regulation of Phospholipase C β	18
Neha	Agrawal	Targeting HIV Reservoirs in Brain by Inhibition of P-Glycoprotein at the Blood-Brain Barrier	19
Mitchell	Moseng	Mortalin caught in action: structures of the nucleotide binding domain in the apo, ADP, AMP, and N6P-ADP bound states	20
Peng	Lin	Volumetric Chemical Imaging by Stimulated Raman Projection Microscopy and Tomography	21
Huaqun	Zhang	Structural insights into regulatory interactions between CHIP and the molecular chaperone Hsp70	22
Yuri	Efremov	Integrated spinning disk confocal and atomic force microscopy for mechanical and structural studies on living cells	23
Brianna	Hudson	Mechanisms and Regulation of PLCβ and its Effects on Membrane Adsorption	24
Ellen	Kolb	Structural insight into small molecule inhibitors of the Notch transcription complex	25

Jason	McCoy	Inhibition of Growth Differentiation Factor 8 (GDF8) via Growth Differentiation Factor-associated Serum Protein (GASP)	26
Stephanie	Gates	Molecular snapshots of a powerful AAA+ machine: Hsp104	27
Monita	Sieng	Characterization of Rap1A-dependent activation of PLCε	28
Evan	Billings	The Crystal Structure of the Transferrin Binding Complex in *N. gonorrhoeae*	29
Xusi	Han	Fast global surface search for electron density maps	30
Austin	Dixon	Structural Analysis of LigB from Plants to Determine the Mechanism of Substrate Specificity	31
Allison	Norvil	Dnmt3b Methylates DNA by a Noncooperative Mechanism, and Its Activity is Unaffected by Manipulations at the Predicted Dimer Interface	32
Anna	Ratliff	Evaluation of Substrate Binding Dynamics of Ste14p using Electron Paramagnetic Resonance (EPR) Spectroscopy	33
Jeremy	Bakelar	Structural and functional studies of the β-barrel assembly machinery	34
Renata	Everett	Structural Bases for the Binding of SARS Plpro and ISG15	35
Robert	Cassell	Development of Biased Delta Opioid Agonists for the Treatment of Alcohol Use Disorder	36
Marianne	Maresh	Rescuing Proteasome Inhibition in Response to HIV Tat	37
Brandon	Anson	Kinetic Characterization and Effect on Inhibition of Two Gain of Function Mutants in MERS CoV 3CL Protease	38
John	Hausman	Structural and Function Characterization of the Deubiquitinating Bacterial Effector ChlaDub2	39
Himesh	Makala	Identification of novel scaffolds to inhibit Human mitotic kinesin Eg5 by binding to the second allosteric binding site using computational methods	40
Genki	Terashi	MAINMAST: Building main-chain models for medium resolution electron microscopy maps	41
Nicklaus	Steussy	Structure Based Inhibitor Design in Bacterial HMG-CoA Reductase	42
Sai	Maddhuri	Protein Secondary Structure Detection in cryo-EM maps using Deep Learning	43

Poster Abstracts

[1] Biochemical studies of Legionella effectors involved in E1/E2-independent ubiquitination

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Proteins of the SidE family of Legionella pneumophila effectors function as a unique group of ubiquitin-modifying enzymes. Along with catalyzing NAD-dependent ubiquitination of host proteins independent of the canonical E1/E2/E3 pathway, they have been also shown to produce phosphoribosylated free ubiquitin. This modified ubiquitin product is incompatible with conventional E1/E2/E3 ubiquitination processes, leading to a lockdown of various cellular functions that are dependent on ubiquitin signaling. We have shown that in addition to free ubiquitin, ubiquitin chains are also modified by SdeA in a similar fashion. This renders the ubiquitin chains unable to be processed by a variety of deubiquitinating enzymes. These observations broaden the scope of SdeA's modulatory functions during Legionella infection.

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[2] Mechanisms in Cancer Signaling: The Role of the Membrane in the Recruitment of the Oncogene KRas4b

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Ras proteins constitute an integral element of signal transduction where extracellular growth factors control various nuclear transcription events involved in cell division, proliferation, and apoptosis. The isoform KRas4b is mutated in 90% of pancreatic and 45% of colorectal adenocarcinomas, and mutants that activate KRas4b are considered "drivers" of the cancerous state. Intervention that either prevents KRas4b activation or returns the signaling cascade to its normal form could significantly improve cancer outcomes. Ras activity is controlled, and signaling mediated by, critical protein-protein interactions. GTPase activating proteins (GAPs), such as p120, bind to activated Ras, dramatically increasing the rate of GTP hydrolysis thus returning the system to the inactive GDP bound state. Guanine exchange factors (GEFs), such as Son of Sevenless (SOS), bind and effect the exchange of GDP for GTP, thus turning "on" KRas4b. Most importantly, these multi-protein complexes all operate on a membrane surface, which is a critical partner in signaling. Despite this critical role of the membrane, there is incomplete knowledge as to the role of the bilayer composition in anchoring the protein to the membrane and the importance of specific lipid type in dictating the final orientation of KRas4b on the surface. Using a fully post-translationally modified KRAS4b, we investigated the role of lipid identity in the recruitment of KRas4b to a Nanodisc membrane surface of defined composition. Application of a newly developed single frequency fluorescence anisotropy decay experiment to this system revealed that KRas4b has a significant binding preference for Nanodisc bilayers containing PIP2. We conducted molecular dynamics simulations to look for an origin of this specificity. In the case of membranes containing PIP2 the protein formed long-lived salt bridges with PIP2 head groups but not the monovalent DMPS, explaining the experimentally observed lipid specificity. Additionally, we report that PIP2 forms key contacts with Helix-4 on the catalytic domain of KRas4b that orient the protein in a manner expected to facilitate association with upstream and downstream signaling partners. Supported by NIH MIRA Award GM118145 and partnership with the Frederick National Laboratory for Cancer Research.

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[3] Regulation of cell signaling by Fic-mediated post-translational modifications

Ben Watson
Purdue University

We recently reported a previously unknown mechanism of regulating ER (endoplasmic reticulum) stress by a novel post-translational modification (PTM) called Adenylylation/AMPylation1. Specifically, we showed that the human Fic protein, HYPE, catalyzes the additional of an AMP (adenosine monophosphate) to the ER chaperone, BiP, to alter the cell's unfolded protein response (UPR) pathway. HYPE levels are upregulated in response to ER stress. Accordingly, aberrant expression of HYPE leads to cell death due to their inability to adequately cope with ER stress. Thus, HYPE functions as a key determinant of cell fate by regulating ER homeostasis. Here, we determine the structural and kinetic parameters for the HYPE-BiP interaction, with the ultimate goal of designing therapeutics to manipulate this interaction during disease.

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[4] Optimization of Novel Small Molecule Inhibitors Through Structural Characterization of Beta-Lactamases

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Antibiotic resistance is rapidly becoming a serious threat to public health. Bacteria have been evolving in ways that have greatly decreased the efficiency of many commonly used antibiotics. One of the ways that bacteria have become resistant to antibiotics is through the expression of a class of proteins called β-lactamases. β-lactamases target antibiotics that contain a β-lactam ring and deactivate them through hydrolysis. A possible method for combating antibiotic resistance would be to develop inhibitors that target these enzymes, allowing current antibiotics to still be viable. Potential inhibitors have been developed, however, structural data of β-lactamases in complex with these drugs would provide valuable insight into the optimization needed to make these inhibitors clinically applicable. The proteins of interest in this study are the β-lactamases NDM and KPC-2. Many different variants of NDM

have been discovered and they typically differ by only a few amino acids. The ultimate goal is to elucidate the structure of the NDM variants in complex with various inhibitors using X-ray crystallography. KPC-2, like other Class A β-lactamases, contains a conserved loop region referred to as the Ω-loop and it has been proposed that the Ω-loop may play a key role in antibiotic resistance. Using various NMR techniques, the structure and dynamics of both apo and inhibitor bound KPC-2 will be studied to better understand the role of the Ω-loop in antibiotic resistance.

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[5] Identification of a novel interaction site of the mu-delta opioid receptor heterodimers

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Mu opioid receptor (MOR) agonists remain highly effective for the treatment of pain, and would represent ideal analgesics if their side effects could be prevented. Evidence suggests that MORs can interact with delta opioid receptors (DORs) to form MOR-DOR heterodimers which may contribute to adverse effects of mu opioids. Our goal is to develop drug-like compounds that disrupt the heterodimers to lessen these side effects. Yet, a lack of tools to investigate the role of MOR-DOR heterodimers in preclinical and clinical models is stifling our ability to target the heterodimers with drugs. To aid in the development of such tools, it is important to understand the MOR-DOR interaction: determining which amino acids in the heterodimer interface play major roles in the MOR-DOR formation. We constructed DOR mutants to probe for destabilization of the MOR-DOR heterodimers. We used a multipronged approach including calcium signaling assay, bimolecular fluorescence complementation and co-immunoprecipitation to investigate the impact of mutations on the stability and function of the heterodimers. We confirmed that amino acids 242, 243 and 244 located in the intracellular loop, as previously proposed by others, were crucial in stabilizing the heterodimers. Intriguingly, we identified novel amino acids 208, 209, 222, 279 and 288 to be necessary for the heterodimer formation. These amino acids are located on the extracellular regions, thereby providing a promising new target for the heterodimer disruption. These findings will move us closer to our goal to develop small-molecule drugs that can be used with opioids to prevent their side effects.

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[6] Complexities of Super-Complexes in Energy-Transducing Membranes

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A unique ‘super complex’ in energy-transducing membranes was obtained from thylakoid membranes that function in oxygenic photosynthesis. Quantitative characterization of the subunit composition by Orbitrap mass spectrometry showed a dominant contribution of major subunits of the ATPase and the photosystem I reaction centers to isolated high molecular weight super-complexes. The content of the other major protein complexes of the photosynthetic energy-transducing membrane, the cytochrome b6f and photosystem II complexes, was minimal. Transient formation of ‘super complexes’ in the non-appressed regions of the thylakoid membrane, dependent upon environmental parameters, is proposed to result from (i) lateral diffusion of the intra-membrane complexes and (ii) interaction of the ATPase complex with PSI reaction centers enriched in the non-appressed compartment. A modification is suggested of the classical Singer-Nicolson model that emphasizes the role of lateral diffusion of membrane proteins and complexes, and random ‘elastic’ collisions between complexes as the major determinant of interactions between membrane proteins. It is suggested that the interaction of intra-membrane protein complexes is characterized not only by diffusional mixing isolated multi-component complexes, but also the existence of a subset of ‘tight’ complexes that can have a significant role in intra-and trans-membrane chemistry. The isolation of a relatively abundant subset of PSI–ATPase complexes expands the repertoire of known ‘super-complexes’ in chloroplast membranes, implies a sub-domain of localized energy coupling, and suggests an updated perspective of the ‘fluid mosaic model’ [Science 175, 720-731, 1972; Nature, 438, 578-580, 2005].

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[7] Strategic Modifications to a Novel Class of Antibacterial Peptides, Cationic Amphiphilic Polyproline Helices (CAPHs), for Superior Cellular Uptake and Enhanced Antibacterial Activity

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Purdue University

Serious health threats posed by intracellular bacterial infection emphasize the need to establish new antimicrobial agents. Antimicrobial peptides (AMPs) are naturally found within the body and act as antibiotics, but lack the ability to penetrate the cell to clear intracellular pathogens. However, cell penetrating peptides (CPPs) have shown promise as effective means of delivering therapeutic cargo intracellularly. In an effort to address the necessity for new antibacterial therapies, features of CPPs and AMPs were combined to develop a dual action antimicrobial peptide, cationic amphiphilic polyproline helices (CAPHs). CAPHs have proven to be an effective antimicrobial agent to combat an array of both Gram negative and Gram positive intracellular bacteria, including Brucella, Listeria monocytogenes, and Salmonella enterica. To further enhance the antimicrobial efficiency of CAPHs against intracellular pathogens we have investigated different strategies to increase cell uptake and alter subcellular localization. By increasing both the cationic nature and exploring different hydrophobic motifs we have improved the therapeutic potential of CAPHs.

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[8] FibPredictor 2: A computational approach for the prediction of amyloid fibril classes and key residue interactions

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Fibril formation has a marked role in many diseases such as Alzheimer's and Parkinson's and can cause drug inactivation in many protein-based products such as Glucagon. Due to crystallization difficulties, available 3D structures of fibrils are few. However, computational methods can be a way to predict the fibril structures and identify the key interactions between residues that promote amyloid formation. Previously, Fibpredictor 1, a program for fibril structure prediction, was developed in our lab which generates models of beta-sheet chains in different rotations and positions relative to each other and ranks them using statistical scoring functions. Following this approach, we have attempted to improve the scoring and prediction by modifying our approach in development of Fibpredictor 2. In this approach, larger fibril systems with multiple beta sheets are generated and water interactions with are also considered. To describe the method briefly, peptide backbones are generated and side chains are predicted by Scwrl4 program. Then short Molecular Dynamics (MD) simulations are run. The simulations are done using OpenMM package which supports running MD simulations on GPU architectures. We use the AMOEBA force field, a recently developed force field which has shown to improve our predictions in some cases. After the simulation, the method is validated by comparing the residue interactions in the snapshots of crystal structure simulation trajectory and those of the models. Our method could predict the parallel or anti-parallel orientations in all tested fibril structures correctly, and could rank models with the closest interaction score to the crystal structure as top models in some of the tested cases.

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[9] Crystal Structure and Kinetic Profile of a Core Papain-like Protease of MERS Coronavirus with Utility for Structure-based Drug Design

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The Ubiquitin-like domain 2 (Ubl2) is located in nonstructural protein 3 (nsp3) immediately adjacent to the N-terminus of the papain-like protease (PLpro) domain in all coronaviruses, including the deadly Middle East Respiratory Syndrome (MERS) coronavirus. The Ubl2 domain has been proposed to play a critical role in protease regulation and stability as well as in viral infection. However, our recent cellular studies reveal that truncations of the Ubl2 domain (MERS PLpro-ΔUbl2) had no effect on MERS PLpro multifunctional activities in cells. Here, we test the hypothesis that the Ubl2 domain is not required for the catalytic function of MERS PLpro in vitro. First, the X-ray crystal structure of MERS PLpro-ΔUbl2 was determined to 1.9 Å and then compared to the crystal structure of MERS PLpro containing the N-terminal Ubl2 domain. While the structures were found to be nearly identical, the strong electron density surrounding the active site of MERS PLpro-ΔUbl2 reveals for the first time the intact structure of the substrate-binding loop. Moreover, steady-state kinetic studies of PLpro-ΔUbl2 and PLpro-Ubl2 catalysis against three different ubiquitin-like substrates, ubiquitin chain substrates, and a purported MERS PLpro inhibitor revealed no differences in substrate specificity, catalytic efficiency, and inhibition. Finally, the thermal stability of each enzyme was evaluated via circular dichroism melting studies, and once again essentially no differences between enzymes were observed. Our conclusion is that the core domain of MERS PLpro is sufficient for catalysis and stability in vitro, and this core MERS PLpro enzyme can be used to evaluate potential inhibitors.

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[10] Efficient deep learning framework for biological 3D image data retrieval: an application with protein binding ligand prediction

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Proteins are 3D structures and can therefore be represented as 3D images. Most proteins function by binding specific ligands, at binding sites called protein pockets. This underlies numerous biological processes as well as crucial application fields such as drug discovery. Therefore, it would be of great impact if, that given a protein pocket, we could predict its binding ligands. Yet this has long been a challenging task due to the huge varieties of proteins and ligands.

Deep learning is class of machine learning algorithms and can automatically learn hidden feature representations of data without human prior knowledge. Inspired by its huge success in 2D image recognition, we developed a 3D deep convolutional neural network framework for protein structural data retrieval to predict protein binding ligands. Our method performs considerably better than previous non-deep learning methods. Given a protein pocket, we can identify its potential binding ligands among over 150 ligand candidates with overall top-1 accuracy of over 68%, and with top-3, top-10 accuracies of over 80%, 90% respectively. The top-1 accuracies for over 20 ligand species are over 80%, and over 80 ligand species have top-10 accuracies of over 80%. Our method demonstrates the considerable strength of employing deep learning in protein 3D structure data analysis, and could be applied to many computational research that use biomolecular structure data, such as protein-protein binding, protein-DNA/RNA binding, EM map analysis, etc.

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[11] Modeling the Molecular Structure of a Cellulose Synthase Plant Conserved Region with X-ray Crystallography and Small Angle X-ray Scattering Illustrates Potential Functions

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The processive plant cellulose synthase (CesA) synthesizes (1→4)-β-D-glucans. Between 18-36 CesAs assemble into a complex that forms a cellulose microfibril as the fundamental scaffolding unit of the plant cell wall. Within the CesA catalytic domain (CatD) is a 125-amino acid insertion known as the plant-conserved region (P-CR), whose function and molecular structure are unknown. Recombinantly expressed rice secondary cell wall OsCesA8 P-CR domain purifies as a monomer and shows distinct α-helical secondary structure by circular dichroism analysis. A molecular envelope of the P-CR was derived by small angle X-ray scattering (SAXS). The P-CR was crystallized and structure solved to 2.4Å resolution revealing an anti-parallel coiled-coiled domain that has an uncommonly high proportion of aromatic residues intercalated in 'knob-in-hole' heptad positions. Connecting the coiled-coil α-helices is an ordered loop that bends back towards the coiled-coils and forms several hydrophobic interactions. The P-CR crystal structure fits the molecular envelope derived by SAXS, which in turn fits into the CatD molecular envelope as predicted in previous work (Olek et al., 2014, Plant Cell 26:2996)2. This places the P-CR between the membrane and substrate entry portal with the connecting loop facing the catalytic core, where it is likely making a hydrophobic contact. This positioning indicates that the P-CR could function in protein-protein interactions in the cellulose synthase complex (CSC) through its coiled-coil and/or substrate entry through its N- and/or C-terminus. Understanding the molecular structure of CesAs, CesA protein-protein interactions and substrate/product pathways is critical to designing novel CesA mutants and chimeras that produce modified cellulose microfibrils for improved catalytic conversion of biomass to biofuels.

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[12] Structural changes of bacteriophage ΦX174 during DNA ejection induced by lipopolysaccharides

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Unlike dsDNA bacteriophages such as T4 and HK97, which use a preformed tail for transporting their genomes into a host bacterium, the ssDNA bacteriophage ΦX174 is tailless. However, a tail-like structure assembles after contact with the host cell. Using cryo-electron microscopy (Cryo-EM) and time-resolved small angle x-ray scattering (SAXS), we show that lipopolysaccharides (LPS) form bilayers that interact with ΦX174 at one of its twelve icosahedral 5-fold vertices and induce the ejection of the ssDNA genome. The cryo-EM structures of ΦX174 complexed with LPS have been determined for the pre- and post ssDNA ejection states. The ssDNA ejection is initiated by the loss of the G protein pentamer from the vertex that encounters the LPS, followed by the conformational change of two polypeptide loops on the major capsid F proteins surrounding the 5-fold channel at the vertex contacting the LPS.

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[13] Computational Methods for Predicting Protein-Protein Interactions in Plants

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Protein-protein interactions (PPIs) play an essential role in biological processes and functions. Previously, the identification of PPIs was limited to experimental techniques which is time and labor consuming. As a result, current PPIs derived from experiments only covered a small fraction of whole PPI networks. We developed a complementary computational approach to aid this large-scale PPI prediction in Arabidopsis, soybean, and maize. Here, we considered sequence information, co-expression, functional association, and phylogenetic profile to predict PPIs. Based on six-cross validation results on golden standard dataset, our prediction method achieved 85% accuracy using sequence feature by support vector machine, and 85.4% accuracy using other combined features by random forest. We predicted 91,362 PPIs in Arabidopsis, 126,216 PPIs in soybean, and 6,062 PPIs in maize.

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[14] Inhibition of MCP-1/CCR2 Signaling Offers Protection Against Ethanol-Induced Damage to the Developing Brain

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Fetal ethanol exposure may result in fetal alcohol spectrum disorder (FASD) and one of the most devastating effects of developmental exposure to ethanol is the loss of CNS neurons. The underlying molecular mechanisms, however, are unclear. Ethanol-induced neuronal death is accompanied by neuroinflammation. Monocyte Chemoattractant Protein 1 (MCP-1), is a chemokine which is involved in neuroinflammation. Itsr eceptor CCR2 is also mainly expressed by microglia. MCP-1 recruits monocytes, memory T cells, and dendritic cells to sites of inflammation produced by either tissue injury or infection. Although Microglia represents the first line of defence in brain pathologies, prolonged and sustained activation of microglia may have cytotoxic effects. There is also research showed that either chronic alcohol exposure during adulthood or developmental alcohol exposure prenatally may sensitize the immune system to subsequent insult and lead to excess neuroinflammation, neurotoxicity, and the development of alcohol-related disorders such as adult AUD or FASD. Elevated expression of MCP-1 has been observed in multiple sclerosis, stroke and Alzheimer's disease patients. And several mice studies showed the detrimental effects caused by

overexpression of MCP-1 in those diseases could be abolished (or partially abolished) by CCR-2 antagonist. We hypothesize that the inhibition of MCP-1/CCR2 signaling offers protection against ethanol-induced damage to the developing CNS by reducing microglia activation. In this study, we used a third trimester equivalent mouse model as well as an immortalized microglia cell line (SIM-A9) of ethanol exposure to determine the role of MCP- 1 and its receptor CCR2 in ethanol neurotoxicity in the developing brain. We found MCP-1 synthesis inhibitor Bindarit or CCR2 antagonist RS504393 decreased ethanol-induced apoptosis and microglial activation in the in the brain of postnatal 4 day-old mice. We also found that EtOH induces MCP-1 in SIM-A9 cells and blocking MCP-1/CCR2 signaling decreases Ethanol/MCP-1 induced SIM-A9 cell activation. In summary, our data suggest that inhibition of MCP-1/CCR2 signaling offers protection against ethanol-induced damage to the developing brain, the protection may be offered by reducing microglia activation.

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[15] Structural Insights into Phospholipase Cε: A Multi-Method Biophysical Approach

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Phospholipase C (PLC) enzymes hydrolyze phosphatidylinositol lipids to produce second messengers, including inositol-1,4,5-triphosphate (IP3) and diacylgycerol, which increase intracellular calcium and activate protein kinase C, respectively. PLC epsilon (PLCe) contributes to cardiac hypertrophy, where it is chronically activated by the small G protein Rap1A, as well as to oncogenic Ras signaling pathways downstream of receptor tyrosine kinases. Specifically, Rap1A and Ras proteins are proposed to bind to the C-terminal Ras-association (RA) domain of PLCe, promoting conformational changes that increase lipase activity and/or membrane association. However, the role of the RA domains in PLCe activity is poorly understood. We are using, electron microscopy (EM), including negative stain and cryo-EM, as well as small-angle X-ray scattering and biochemical approaches to determine the roles of the RA domains in basal regulation and activity upon G protein activation. These results provide the first structural insights into this essential enzyme and improve understanding of its roles in cardiovascular disease and cancer.

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[16] Structural studies of flavivirus rearrangements using cryo-EM

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Flaviviruses such as dengue virus, West Nile virus and Zika virus are transmitted by mosquitoes and cause severe disease in humans. During an infection, nascent virus assembles at the ER and moves through the secretory pathway. When the virus reaches the trans-Golgi network, it encounters a low pH environment and undergoes a structural rearrangement. This conformational change is the first step in viral maturation, and results in the viral glycoproteins converting from trimers to dimers. Previous work has elucidated the structures of immature and mature flaviviruses, but the intermediate steps remain unclear. We are using a combination of tools including the antibody E16 and heavy metal labels to trap and identify transition states of Kunjin virus, a strain of West Nile virus. We examined these labeled states with cryo-electron microscopy, and determined the differences between these structures and those of the immature and mature virus. From these results, we propose intermediate states of the structural rearrangements that occur during flavivirus maturation.

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[17] Simplify Your Transfection and Improve Your Transfection Efficiency

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In biomedical research, scientists perform experiments to enhance or diminish the function of genes and observe characteristics of a model organism. Such gain- and loss-of-function studies are a cornerstone of many fields of experimental biology in understanding genetic pathways, inferring the function of genes and proteins, and more. The central concern that determines the success of these experiments is how to improve transfection efficiency and in the meantime, minimize cytotoxicity. The best-rated transfection reagents such as Lipofectamine 3000 and FuGENE confer excellent transfection efficiency on most easy-to-transfect cell lines. However, these two reagents prove to underperform on difficult-to-transfect cell lines such as neuronal cell lines and primary cells. Moreover, transfection with these two reagents requires the application of serum free or optimal medium, which complicates the process and is time consuming. To address these shortcomings, we have developed a transfection reagent, TRANSFECTIN X that is nanoparticle made of biodegradable material. In our studies, TRANSFECTIN X displays superior high transfection efficiency on a broad spectrum of cell types including continuous cancer cell lines, stem cells, immortalized cells, and primary cells. More impressively, it shows an extreme advantage over Lipofectamine 3000 with respect to transfection efficiency on difficult-to-transect cell lines or primary cells. In addition, due to its biodegradable characteristic, TRANSFECTIN X shows little-to-no cytotoxicity. Last but not the least, TRANSFECTIN X is serum and antibiotics compatible, and requires no need to change medium due to its no cytotoxicity. With solid experimental evidence, we believe our product, TRANSFECTIN X, can be your first choice in attaining gain- or loss-of-function studies by performing DNA or RNA transfection.

[18] Electrostatic Regulation of Phospholipase C β

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Phospholipase C (PLC) is an enzyme class that cleaves phospholipids directly before the phosphate group, and it is involved in signal transduction. PLC splits the phospholipid, phosphatidylinositol-4,5-bisphosphate (PIP2), into the second messengers inositol-1,4,5-triphosphate (IP3) and diacylglycerol (DAG). When there is an increase in IP3, there is an increase in intracellular calcium release. An increase in intracellular calcium release, along with DAG, leads to the activation of protein kinase C (PKC) thus activating other pathways. The PLC class of enzymes has six isoforms that include PLC β, -γ, -δ, -ε, -ζ and –η. The PLCβ isoform has been extensively studied out of the six isoforms. It has a role in the inflammatory pathway, and it is crucial for normal cardiovascular function. Deletion of the X-Y linker of PLCβ has been shown to increase basal activity. The C-terminus of the X-Y linker forms a helix, which blocks the active site of PLCβ. To further understand the autoinhibition of PLCβ via the X-Y linker, charge reversal of the acidic residue stretch thought to cause this autoinhibition will be conducted. Charge reversal of basic residues located in the TIM barrel, which are thought to form electrostatic interactions with the acidic residues of the X-Y linker, will be conducted as well. Preliminary experiments showed increased basal activity from the charge reversal of the acidic residue stretch located in the X-Y linker. This observation was reserved by the charge reversal of the TIM barrel basic residue stretch. There is more to be known about the electrostatic regulation of PLCβ and how signal transduction is affected. The more insight we have from the electrostatic regulation of PLCβ, the closer we get to designing new chemical probes that can potentially treat cardiovascular disease. Through mutagenesis of acidic residues within the X-Y linker and basic residues in the TIM barrel, the use of differential scanning fluorimetry (DSF) to determine thermal stability, and liposome based activity assays, we will understand more about how these interactions play a role in the electrostatic regulation of PLCβ.

[19] Targeting HIV Reservoirs in Brain by Inhibition of P-Glycoprotein at the Blood-Brain Barrier

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The complete eradication of HIV is hindered by virus reservoir formation in the host. Locations of these reservoirs include the central nervous system (CNS), resting memory CD4+ T-cells and macrophages. Sanctuaries of HIV, such as those in the CNS, are believed to occur in part as a result of the limited accessibility of antiretroviral therapy (ARTs) across the blood-brain barrier (BBB). The BBB is a system of endothelial capillary cells, characterized by tight junctions between cells and membrane transporter proteins at the surface. Therefore, improving the penetration of HIV therapeutics across the BBB is a significant challenge in the eradication of HIV that would prevent the formation of virus reservoirs. P-glycoprotein (P-gp), an ATP Binding Cassette (ABC) family transporter expressed at the BBB, has been shown to contribute to the limited penetration by ARTs. Many in vitro and in vivo studies have confirmed currently used HIV therapeutics to be substrates of P-gp. The inhibition of P-gp efflux activity is a promising strategy for improving the penetration of ARTs across the BBB and thereby, leading to the prevention of HIV reservoir formation. Here we present an innovative approach towards the development of Trojan Horse Inhibitors of P-gp based on the HIV-1 nucleoside reverse transcriptase inhibitor (NRTI) –Abacavir and protease inhibitors (PIs)-Atazanavir, Lopinavir, Nelfinavir, Ritonavir, Saquinavir, and Indinavir. These heterodimers have been designed to perform a dual function as: (1) P-gp inhibitors at blood brain barrier and (2) prodrug heterodimers which revert back to release Abacavir (NRTI) and the corresponding protease inhibitor in response to the reducing cell environment. As a result, the bioavailability of the antiretroviral therapy across the blood-brain barrier will be increased resulting in the successful elimination of the HIV reservoirs in central nervous system.

[20] Mortalin caught in action: structures of the nucleotide binding domain in the apo, ADP, AMP, and N6P-ADP bound states

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Mortalin, a member of the Hsp70-family of molecular chaperones, functions in a variety of processes including mitochondrial protein import and quality control, Fe-S cluster biogenesis, mitochondrial homeostasis, and regulation of p53. Mortalin has been implicated in regulation of apoptosis, cell stress response, neurodegeneration and cancer, and was identified as a target of the antitumor compound MKT-077. Like other Hsp70-family members, Mortalin consists of a nucleotide binding domain and a substrate binding domain. We now present crystal structures of the nucleotide binding domain of human Mortalin, in the apo, ADP, AMP, and N6P-ADP bound states at 2.8 Å, 2.78 Å, 1.75 Å, and 1.49 Å resolutions, respectively. The overall structures and active site organizations are similar, with a few key differences in side chain positions. Specific residues in the nucleotide binding pocket, which are not conserved in other Hsp70-family members, lead to lower nucleotide affinity and slower turnover relative to cytosolic Hsp70. Our structures may also contribute to the understanding of disease associated Mortalin mutations and to improved Mortalin-targeting antitumor compounds.

[21] Volumetric Chemical Imaging by Stimulated Raman Projection Microscopy and Tomography

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Volumetric imaging allows global understanding of three-dimensional (3D) complex systems. Light-sheet fluorescence microscopy and optical projection tomography have been reported to image 3D volumes with high resolutions and at high speeds. Such methods, however, usually rely on fluorescent labels for chemical targeting, which could perturb the biological functionality in living systems. We demonstrate Bessel-beam-based stimulated Raman projection (SRP) microscopy and tomography for label-free volumetric chemical imaging. Our SRP microscope enables fast quantitation of chemicals in a 3D volume through a two-dimensional lateral scan. Furthermore, combining SRP and sample rotation, we demonstrate the SRP tomography that can reconstruct the 3D distribution of chemical compositions with optical spatial resolution at a higher speed than the Gaussian-beam-based stimulated Raman scattering sectioning imaging can. We explore the potential of our SRP technology by mapping polymer particles in 3D volumes and lipid droplets in adipose cells.

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[22] Structural insights into regulatory interactions between CHIP and the molecular chaperone Hsp70

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The ubiquitin ligase CHIP plays an important role in protein quality control by ubiquitinating proteins chaperoned by Hsp70/Hsc70 and Hsp90, targeting such chaperoned proteins for degradation. CHIP dimers associate with Hsp70/Hsc70 via TPR-binding EEVD motifs of Hsp70/Hsc70 that lie at the extreme C-termini of the proteins, at the end of 25-30 residue unstructured tail segments. The CHIP-mediated chaperoned-ubiquitination pathway thereby promotes the proteasomal degradation of clients, mitigates accumulation of misfolded proteins and regulates cellular levels of chaperoned proteins. We present a 2.91Å resolution structure of the TPR domain of human CHIP in complex with alpha-helical “lid” subdomain and the flexible tail of human Hsc70. We also present structures of human CHIP-TPR in complex with Hsc70-tail (GPTIEEVD) and in complex with a threonine phosphorylated Hsc70-tail (GP(pT)IEEVD) at 1.89Å and 1.39Å resolutions, respectively. We find that the CHIP-TPR interacts simultaneously with determinants within the lid domain and with the C-terminal PTIEEVD motif of the tail. We show that the previously undiscovered interaction between CHIP and the Hsc70 lid is required for proper CHIP-mediated ubiquitination of Hsp70/Hsc70-bound client proteins.

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[23] Integrated spinning disk confocal and atomic force microscopy for mechanical and structural studies on living cells

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We used the AFM in conjunction with a spinning disk confocal (SDC) microscope to directly visualize AFM indentation of living cells (3T3 fibroblasts and MDA-MB-231 breast cancer cells) with high spatial and temporal resolution. The main objective of this work is to better correlate nanomechanical information such as Young’s modulus with cellular structures such as the cytoskeleton in living cells. With novel live cell imaging probes to fluorescently label F-actin, microtubules, and membrane, we were able to directly observe structural changes during the indentation process of a living cell with a spherical indenter. We used three protocols to observe the AFM indentation process with the SDC microscope, allowing the capture of either a quasi-static or dynamic picture of the indentation. The presence of apical stress fibers correlated with cell stiffness. Stiff 3T3 cells had dense and thick apical stress fibers; this caused an anisotropic indentation geometry. Soft MDA-MB-231 cells lack apical stress fibers and isotropic indentation geometry was observed. Application of a jasplakinolide derivative-based actin probe led to significant increase in cell stiffness, indicating that this probe should be used with caution in studies where mechanical properties of cells are measured. We expect that this integrated imaging and biophysical approach will contribute to a more comprehensive understanding of both normal and altered cell mechanical behavior.

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[24] Mechanisms and Regulation of PLCb and its Effects on Membrane Adsorption

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Phospholipase C (PLC) enzymes are peripheral membrane proteins that are activated by the G_q subfamily of G-protein coupled receptors. The enzymes hydrolyze the membrane component phosphatidylinositol-4, 5-bisphosphate (PIP_2) into two potent second messengers, inositol-1, 4,5- triphosphate (IP_3) and diacylglycerol (DAG), in response to diverse stimuli. PLCb enzymes are required for normal cardiovascular function and increased expression is associated with arrhythmias, hypertrophy, and heart failure. Previous studies have shown the C-terminal domains of PLCb contribute to its regulation and membrane association. However, there is no structural information on how the membrane and C-terminal domains contribute to PLCb regulation. We seek to understand how the membrane enhances association and promotes conformational changes within PLCb that increase catalytic activity. Beginning with model membrane systems, we are applying atomic force microscopy and complementary biochemical assays to evaluate the role of the C-terminal regulatory domains in membrane adsorption and activity. We are also investigating how the physical and chemical properties of the membrane itself, e.g. surface charge, fluidity, and composition, regulate PLCb adsorption and activation. These studies provide the first structure-based approach to understanding how the cell membrane itself regulates the activity of this essential effector enzyme.

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[25] Structural insight into small molecule inhibitors of the Notch transcription complex

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Notch signaling is a highly conserved pathway in metazoans that is crucial for regulating embryonic development and tissue homeostasis. Adjacent cells expressing membrane bound Notch receptors and ligands interact, leading to a signaling cascade that ultimately results in transcription of Notch-target genes. During transcriptional activation, the Notch transcription factor, CSL (CBF1/RBP-J, Su(H), Lag1), forms a core Notch ternary complex (NTC) with NICD (Notch Intracellular Domain) and Maml (Mastermind-like), and then recruits several coactivators. Mutations in Notch signaling proteins have been implicated in certain cancers, cardiovascular diseases, and neurodegeneration. Gamma-secretase inhibitors (GSIs), dominant negative peptides, and monoclonal antibody therapeutics have been used with some success to downregulate Notch activity, but there is a push in the field to identify small molecule inhibitors of Notch-mediated tumorigenesis that could be used to complement existing therapies. Our collaborators have developed small molecule inhibitors that specifically target two critical components of Notch-mediated transcription and tumorigenesis: 1.) the Ankyrin repeats of NICD and 2.) the recently identified transcriptional coactivator NACK (Notch Activation Complex Kinase). The long-term goal of this research project is to use structural and biophysical approaches to characterize the specific interactions made between these Notch proteins and small molecule inhibitors to facilitate the optimization of highly specific and potent therapeutics.

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[26] Inhibition of Growth Differentiation Factor 8 (GDF8) via Growth Differentiation Factor-associated Serum Protein (GASP)

Jason McCoy
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Growth Differentiation Factor 8 (GDF8) is a member of the TGF-β superfamily of ligands, and the mature dimer of GDF8 is a well-established muscle growth inhibitor. Previous studies have demonstrated that inhibition of GDF8 via extracellular antagonists results in a dramatic increase in muscle mass in mice. Additionally, knockout mice lacking GDF8 show a very similar hyper-muscular phenotype while over expression of GDF8 leads to muscle wasting. GDF8's ability to control muscle growth and atrophy make it a valuable therapeutic target for muscle wasting disease, such as muscular dystrophy and cancer cachexia. Although there are many extracellular antagonists of GDF8, the TGF-β superfamily has over 33 distinct, structurally similar, ligands and many antagonists are able to inhibit a variety of family members. However, two related proteins, GASP1 and GASP2, are highly specific inhibitors of GDF8 and its close relative, GDF11. GASP1 is of particular interest as it binds GDF8 in with a 1:1 stoichiometry, 1 GASP1 molecule to 1 GDF8 dimer, and is 100x more potent than GASP2. The potency and specificity of GASP1 relies heavily on the Follistatin (Fs) domain within the protein. Studies have shown the Fs domain is able to bind without the aid of other domains and retains specificity. Our lab is focusing on new structural data and preliminary inhibition studies to elucidate residues within the GASP1 Fs domain that contribute to specificity and inhibition of GDF8. Insight into this highly specific interaction will lead to new therapeutic targets for the specific inhibition of GDF8, eliminating off target effects that current treatments face.

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[27] Molecular snapshots of a powerful AAA+ machine: Hsp104

Stephanie Gates
University of Michigan

Hsp104 is a yeast AAA+ protein capable of disaggregating prions and fibrils associated with neurodegenerative diseases, such as Parkinson's Disease and Dementia. Similar to other AAA+ proteins, Hsp104 forms a hexameric ring and threads substrate through the central channel, a process that is coupled to ATP hydrolysis. Hsp104 contains 12 nucleotide-binding domains (NBDs), with two per monomer, which also contain conserved Tyrosine pore loops that bind substrate for translocation through the channel. AAA+ family members are thought to be highly dynamic and conformational changes are likely connected to translocation. To better understand this conserved mechanism, our lab has used cryo-EM to determine the hexameric structure of Hsp104 in a variety of nucleotide and substrate binding states at high resolution. Analysis of these states has revealed snapshots of both a non-processive and processive mechanism of binding and translocation of polypeptides, helping us understand how hydrolysis, substrate binding, and conformational changes drive the powerful disaggregation ability of Hsp104 and other similar AAA+ proteins.

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[28] Characterization of Rap1A-dependent activation of PLCε

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Phospholipase C (PLC) enzymes hydrolyze the lipid phosphatidylinositol 4,5-bisphosphate (PIP_2) to produce diacylglycerol (DAG) and inositol 1,4,5-triphosphate), which activate protein kinase C and promote intracellular release, respectively. These second messengers are critical for many physiological processes, especially within the cardiovascular system where they regulate cell growth and survival. The PLCe subfamily has emerged as key players in cardiovascular health, including increased contractility and regulation of hypertrophic gene expression. PLCe is activated downstream of G protein coupled receptors, including the b adrenergic receptors, through direct interactions with small G proteins. The best characterized PLCe activator is the small G protein Rap1A. In contrast to canonical PLC activation, Rap1AŸGTP binds to PLCe and translocates the activation complex to the perinuclear membrane, where phosphatidylinositol-4-phosphate (PI_4P) is the preferred substrate. Activation by Rap1A results is associated with the onset of cardiac hypertrophy. However, while Rap1A is known to interact with the C-terminal Ras association (RA) domain of PLCe, little is known about the molecular basis of activation. We seek to characterize the interactions between Rap1 and PLCe using structural and functional studies to map the entire Rap1A binding site on PLCe, and determine whether activation results in conformational changes that release autoinhibition and/or increase membrane association. These studies provide the first molecular details of the Rap1A-depedent activation of PLCe.

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[29] The Crystal Structure of the Transferrin Binding Complex in N. gonorrhoeae

Evan Billings
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Within the Neiserria family of bacteria, there are two pathogenic species, N. gonorrhoeae and N. meningitidis that are responsible for causing the diseases gonorrhea and meningitis, respectively. To survive, these bacteria need to import iron for metabolic use and, interestingly, have evolved a way to import iron from their host. In humans, iron is transported throughout the body by proteins such as serum transferrin in the blood. Neisseria have evolved specialized proteins on the surface of their membranes that specifically bind to transferrin to import the iron for their own metabolism. The surface proteins that are responsible for this are called transferrin binding protein A (TbpA) and its coreceptor transferrin binding protein B (TbpB). Together they form the transferrin binding complex. In 2012, the structure of this complex in N. meningitidis was determined by our research group. My project focuses on the structural determination of this complex in N. gonorrhoeae; which is not known. I am expressing these proteins in E. coli and will then purify them by affinity chromatography, crystallize them using high-output screening methods and determine the crystal structures using the molecular replacement method. I will subsequently compare the N. gonorrhoeae structures to the N. meningitis strain to determine the similarities in the two iron piracy mechanisms between the two strains. TbpA and TbpB are both targets for antibiotic and vaccine discovery, giving this research potential for the development of treatments. Blocking this binding system to prevent the importation of iron from transferrin, would prevent the pathogenic bacteria to thrive within hosts.

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[30] Fast global surface search for electron density maps.

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The three dimensional (3D) structure of proteins and other biomolecules provides the molecular basis for understanding mechanisms of biological functions, interactions, pathways, and serves as foundation for numerous areas in biotechnology. Cryo-electron microscopy (cryo-EM) is an important technique in structural biology used to solve large protein complex structures. The electron microscopy databank (EMDB) is a fundamental resource of the tertiary macromolecule structures obtained from cryo-EM. To take full advantage of this resource, the ability to perform a real-time structure-based search is essential. However, different from atomic structures solved from X-ray crystallography or NMR, there is no established method for efficient comparison of low-resolution electron density maps. Here we have developed a web-server named EM-SURFER, which allows users to search against the entire EMDB in few seconds. The method uses a compact representation of electron density maps based on the 3D Zernike

descriptors, which is a rotation-invariant series expansion for representing global surface of EM maps. The unique ability of EM-SURFER to detect global surface similarity in EM maps should prove invaluable in structural biology.

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[31] Structural Analysis of LigB from Plants to Determine the Mechanism of Substrate Specificity

Austin Dixon, Nitya Josyula, Clint Chapple and Jeremy Lohman
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LigB and its homologs comprise a family of enzymes found in both the eukaryotic and prokaryotic domains of life. Specifically, LigB belongs to the family of class III extradiol-type catecholic dioxygenases and catalyzes the ring-opening reaction of catechols. The enzyme utilizes an iron-II ion in its active site for the insertion of molecular oxygen into catechols. Interestingly, the biological function of LigB homologs varies widely across species. We aligned the sequences of four LigB homologs, Arabidopsis thaliana, Beta vulgaris, Portulaca, Escherichia coli, all produce different compounds, and found that sequence conservation is incredibly high; the highest being 73% conservation between Arabidopsis thaliana and Beta vulgaris. Using the structure of Escherichia coli as a guide, we found that the catalytic residues in the active sites of all four enzymes are completely conserved in sequence alignments. The goal of this project is to solve the structure of LigB from four different species bound to their physiological substrate and cofactor, Arabidopsis thaliana, Beta vulgaris, and Portulaca; using Escherichia coli as a guide. Solving the structures of these various LigB's bound to their physiological substrates will allow the first ever glimpse into their catalytic mechanism; shedding light on their various catalytic intermediates and ultimately answering the question of their respective substrate specificities and regioselectivities.

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[32] Dnmt3b Methylates DNA by a Noncooperative Mechanism, and Its Activity Is Unaffected by Manipulations at the Predicted Dimer Interface.

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The catalytic domains of the de novo DNA methyltransferases Dnmt3a-C and Dnmt3b-C are highly homologous. However, their unique biochemical properties could potentially contribute to differences in the substrate preferences or biological functions of these enzymes. Dnmt3a-C forms tetramers through interactions at the dimer interface, which also promote multimerization on DNA and cooperativity. Similar to the case for processive enzymes, cooperativity allows Dnmt3a-C to methylate multiple sites on the same DNA molecule; however, it is unclear whether Dnmt3b-C methylates DNA by a cooperative or processive mechanism. The importance of the tetramer structure and cooperative mechanism is emphasized by the observation that the R882H mutation in the dimer interface of DNMT3A is highly prevalent in acute myeloid leukemia and leads to a substantial loss of its activity. Under conditions that distinguish between cooperativity and processivity, we show that in contrast to that of Dnmt3a-C, the activity of Dnmt3b-C is not cooperative and confirm the processivity of Dnmt3b-C and the full length Dnmt3b enzyme. Whereas the R878H mutation (mouse homologue of R882H) led to the loss of cooperativity of Dnmt3a-C, the activity and processivity of the analogous Dnmt3b-C R829H variant were comparable to those of the wild-type enzyme. Additionally, buffer acidification that attenuates the dimer interface interactions of Dnmt3a-C had no effect on Dnmt3b-C activity. Taken together, these results demonstrate an important mechanistic difference between Dnmt3b and Dnmt3a and suggest that interactions at the dimer interface may play a limited role in regulating Dnmt3b-C activity. These new insights have potential implications for the distinct biological roles of Dnmt3a and Dnmt3b.

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[33] Evaluation of Substrate Binding Dynamics of Ste14p using Electron Paramagnetic Resonance (EPR) Spectroscopy

Anna Ratliff, Christine Hrycyna
Purdue University

Many proteins involved in carcinogenesis undergo CaaX processing, which leads to their proper localization and function. Over 100 CaaX proteins are currently known to be involved in many key cellular processes, such as proliferation, differentiation, trafficking, and gene expression. The C-terminal CaaX motif is comprised of a cysteine residue (C), two aliphatic amino acids (a), and terminates with one of several amino acids (X). These proteins undergo three sequential post-translational modifications: 1) isoprenylation of the cysteine residue via the addition of a farnesyl or geranylgeranyl isoprenoid 2) endoproteolyis of the three terminal aaX residues 3) methyl esterification of the newly exposed cysteine residue. We seek to determine the mechanism of the final step performed by the enzyme isoprenylcysteine carboxyl methyltransferase (Icmt), an integral membrane protein that resides in the endoplasmic reticulum. Two chemically different substrates are necessary for methyl esterification including the hydrophilic methyl donor, S-adenosyl-L-methionine (SAM), and the methyl acceptor, a lipophilic isoprenylated CaaX protein. Elucidating the mechanism of Icmt has been challenging because of the difficulty to purifying the membrane protein and that it does not have any homology with soluble methyltransferases. We use the yeast Icmt, Ste14p, as a model to the human enzyme as the human variant is not functional when purified. Furthermore, the only available crystal structure is of a related Icmt from M. acetivorans, which has minimal sequence homology and only offers a static view of the C-terminal half of the protein.
To elucidate the SAM binding mechanism, a combinatorial method of site-directed spin labeling and electron paramagnetic resonance (EPR) spectroscopy analysis is being used to determine the conformational dynamics of Icmt. We first optimized expression and purification of Ste14p in order to generate large enough quantities for further analysis. Utilizing Ste14p-baculovirus infected Sf9 cells has increased protein expression four-fold from our yeast model, which allows for efficient, large scale protein

production. Purification of Ste14p followed by FPLC has been optimized to reduce protein aggregation and loss of enzymatic activity, both of which are necessary for EPR spectroscopy. A library of single cysteine mutants was generated by introducing single mutations into cysteine-less Ste14p, Ste14p-TA. We selected mutants with activity levels ≥50% of Ste14p-TA as candidates for EPR characterization. We have selected H141C-TA and S157C-TA as the first mutants to be studied. With our optimized protein expression and purification system, we are now poised to introduce a nitroxide spin label to our library of single cysteine mutants. Previous work with EPR in our laboratory efficiently introduced a nitroxide spin label to Ste14p-TA and two mutant forms of Ste14p-TA with single cysteine point mutations. Additional EPR spectra will be collected with samples purified with optimal culturing and purification protocols previously described and we will expand our library of Ste14p-TA cysteine mutants. Overall, our analysis will be used to better understand the structural dynamics of SAM binding unique to the methyltransferase Icmt.

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[34] Structural and functional studies of the β-barrel assembly machinery

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β-barrel outer membrane proteins (OMPs) are found within the outer membranes (OM) of Gram-negative bacteria and serve critical functions including nutrient import, cell signaling, and protection. In the final stage of their biogenesis, nascent OMPs are folded and inserted into the OM by a conserved multi-protein complex called the β-barrel assembly machinery (BAM) complex. Although essential, the mechanistic details of BAM complex function have remained elusive. Recently, we and others reported structures of fully assembled BAM in differing conformational states. These studies have provided the molecular blueprint detailing protein-protein interactions within the complex and have revealed new details about BAM, suggesting a dynamic mechanism that may use conformational changes to assist in the biogenesis of new OMPs. Building on this recent progress we have performed a robust structure guided mutagenesis and functional investigation of the central component BamA, which has identified protein binding interfaces and conformational changes important for BAM function, including the large conformational switch of the BamA β-barrel domain.

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[35] Structural Basis for the Binding of SARS PLpro and ISG15

Renata K. Everett, Jozlyn R. Clasman, Andrew D. Mesecar*
Purdue University

Severe acute respiratory syndrome (SARS) is a respiratory illness caused by the SARS coronavirus (SARS-CoV). SARS initiated a global pandemic in 2003, and despite the fact that its mortality rate is estimated at 10%, no treatment or vaccines are currently available. SARS-CoV is a positive-sense, single-stranded RNA virus, and in order for the virus to replicate, the polyprotein must be processed by two viral proteases: the papain-like protease (PLpro) and the 3C-like protease (3CLpro). Unlike SARS 3CLpro, PLpro possesses deubiquitinating and deISGylating activities in addition to protease activity. These additional functions are believed to aid in viral replication within the host and to help the virus evade the host's innate immune response. Ubiquitination and ISGylation are both post-translational protein modifications. Ubiquitin is employed in several signaling processes within the cell, notably as a signal for a protein's proteosomal degradation. ISG15, however, is released after infection as an anti-viral response. Though ISG15 is composed of two ubiquitin-like domains, it remains distinct from ubiquitin in both structure and function. Here, we investigate the differences in binding and activity between SARS PLpro and ISG15 compared to ubiquitin. Better understanding of the individual functions of SARS PLpro will help elucidate the complex mechanism of viral survival within a host. We report the synthesis and X-ray crystal structure determination of SARS PLpro–ISG15 covalent complexes. Analysis of this data highlights important structural features for SARS PLpro/ISG15 binding and helps explain substrate preferences of SARS PLpro compared to those of other viral proteases. Additionally, the structure of full-length human ISG15 in complex with a protein has not been reported to date, so this data may provide useful information toward the study of ISG15 and other deISGYlating enzymes.

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[36] Development of Biased Delta Opioid Agonists for the Treatment of Alcohol Use Disorder

Robert Cassell
Purdue University

Alcohol use disorder (AUD) is a chronic, relapsing psychological disorder which is characterized by the problematic consumption of alcohol, and is a disorder which is often associated with comorbid anxiety and mood disorders. Framing the issue, AUD was estimated to have afflicted over 15 million Americans during the year 2015 with the financial cost to society being placed at an estimated $249 billion for the year 2010, thus necessitating the development of new and effective treatments. As a target in the possible treatment of AUD and comorbid anxiety and depression, the delta opioid receptor (DOR) has been extensively validated in animal models but has thus far failed to translate effectively in to human medicine. Based on our previous findings, we hypothesize that negative outcomes of DOR agonist treatment are likely to result from signaling through the β-arrestin 2 recruitment pathway as opposed to the canonical G-protein pathway with which this receptor is traditionally associated. Strongly G-protein biased small molecule ligands are not known commercially or in the literature. Here we present our efforts in developing novel DOR-specific G-protein biased ligands based on a variety of scaffolds in order to further test our hypothesis through animal studies as an extension of our previous work, as well as to serve as a springboard for further clinical evaluation.

[37] Rescuing Proteasome Inhibition in Response to HIV Tat

Marianne Maresh
Purdue University

The proteasome is an enzyme complex responsible for degrading damaged proteins. During certain viral infections, the proteasome undergoes structural changes to become the immunoproteasome (IP). The IP processes foreign proteins into small peptides to be paired with major histocompatibility complex, class I (MHC-I) for antigen presentation. This initiates a proper immune response. One of the mechanisms by which the human immunodeficiency virus (HIV) evades the immune system is by producing the Tat protein that limits the activity of the IP. This results in reduced MHC-I expression and diminishes the immune response. The goal of this work is to rescue the function of the IP by blocking its inhibition by Tat. Preliminary control experiments are being conducted in which human embryonic kidney cells (HEK) are transfected with HIV Tat DNA, then dosed with OVA, a peptide from the IP-mediated degradation of ovalbumin, that is known to form an MHC-I. The extent OVA-MHC-1 complex expression both in the presence of Tat and without will be measured using flow cytometry and an antibody that recognizes the OVA-MHC-I complex and fluoresces. It is expected that fluorescence will remain unchanged between non-transfected cells and HIV Tat transfected cells in response to the OVA peptide because the antigen already exists in peptide form. Cells will then be transfected with ovalbumin DNA to produce ovalbumin and HIV Tat protein. It is expected the signal corresponding to the OVA-MHC-I complex will be diminished, as Tat will prevent the IP from processing ovalbumin into peptides.

[38] Kinetic Characterization and Effect on Inhibition of Two Gain of Function Mutants in MERS CoV 3CL Protease

Brandon J. Anson, Kevin Song, Sofia Gonzalez, Andrew D. Mesecar
Purdue University

The importance of long-range interactions in the function of Coronaviral 3CL proteases has been speculated for several years. This is exemplified by the three members of the 2c β-Coronavirus subclade: MERS (Middle East Respiratory Syndrome), HKU4, and HKU5. Despite maintaining over 80% sequence identity between them, HKU4 and HKU5 $3CL^{pro}$ are more kinetically similar to the rest of the Coronaviral family, than to MERS. MERS $3CL^{pro}$ exhibits 100-fold weaker dimerization affinity when compared to other Coronaviral $3CL^{pro}$'s. Dimerization is known to be necessary for catalysis and is induced by substrate binding. Yet, the precise interactions that communicate substrate binding across the dimeric interface remain to be elucidated. This presents an opportunity to examine these networks and how they couple substrate binding with dimerization. Additionally, these same long-range interactions are believed to modulate substrate specificity despite the 2c β-CoV $3CL^{pro}$ maintaining 100% sequence conservation in the active site, within this subclade. This study examines two gain of function mutations, distal to the active site, that have extraordinary impacts on the kinetics of this enzyme.

[39] Structural and Functional Characterization of the Deubiquitinating Bacterial Effector ChlaDub2

John M. Hausman[1], Aditya Babar[1], Chittaranjan Das[1]
[1]Purdue University

Chlamydia trachomatis is an obligate intracellular pathogen known for causing several human diseases upon infection. The bacterium causes sexually transmitted disease upon urogenital infection and blindness upon ocular infection. During infection, C. trachomatis expresses a myriad of bacterial effectors to manipulate the host cell into facilitating its own metabolic and reproductive requirements. As part of this process, the reticulate body of C. trachomatis expresses two deubiquitinating enzymes (DUBs), ChlaDub1 and ChlaDub2, that are predicted to be responsible for removing polyubiquitin chains from the chlamydial inclusion membrane in order to evade lysosomal targeting. We present the structure of the bacterial effector, ChlaDub2 (93-339) at 2.6 Å resolution as well as biochemical studies to characterize the deubiquitinating properties of ChlaDub2. The structural analysis showed a potential binding interface for ubiquitin and also key residues that may be responsible for substrate recognition. Different constructs and mutations will be generated to determine the catalytically active domain. These studies will provide further insight into the mechanism of the deubiquitination of the polyubiquitin chains ligated to the inclusion membrane of C. trachomatis.

[40] Identification of novel scaffolds to inhibit Human mitotic kinesin Eg5 by binding to the second allosteric binding site using computational methods

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Human mitotic kinesins are potential anticancer drug targets because of their essential role in mitotic cell division. Eg5 motor protein promotes spindle separation which has gained much attention pharmaceutically and has many inhibitors in different phases of clinical trials. All the drug candidates that have been considered so far bind to the site 1 formed by loop L5, helices α2 & α3 of Eg5, Where these drug candidates bind uncompetitively with ATP/ADP. Intrestingly, Eg5 also has a site 2 formed by helices α4 & α6 where inhibitors bind competitivly to ATP/ADP. In this study, we performed in-silico screening of diversity set-III from National cancer institute (NCI) and Zinc database to identify potential specific inhibitors for Human Eg5 (Kinesin-5) specially targeting the site 2. The compounds were ranked based on the glide extra precision docking scores and the top ranked compounds were found to have pyridazine scaffold. The top five compounds were further evaluated for other durg like properties. The most favourable sites of the ligands were mapped using E-pharmacophore. Stability of protein-ligand complexes were analyzed using

molecular dynamic simulations. The results show that pyridazine analogues have good MDCK, permeability properties and high binding affinity to the human Eg5. This suggests that pyridazine could be a new scaffold for designing inhibitors against Human mitotic kinesin Eg5.

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[41] MAINMAST: Building main-chain models for medium resolution electron microscopy maps

Genki Terashi and Daisuke Kihara,
Department of Biological Sciences, Department of Computer Science

An increasing number of electron microscopy (EM) maps were determined at near-atomic resolution in recent years. The significant progress of the cryo-EM poses a pressing need for software for structural interpretation of EM maps, such as identifying protein main-chains, building all-atom protein models, and model validation. Particularly, tools are needed for maps determined around a 4 Å resolution, where finding correct connection of main-chain and assigning the amino acid sequence into EM density map are still challenging problems.

Here we report a novel de novo protein structure modeling method for EM maps of near atomic resolution. The method, named MAINMAST (MAINchin Model trAcing from Spanning Tree), consists of mainly four steps. The first step is to identify locally dense points (LDPs) in an EM map, where protein atoms are more likely to exist. We applied the mean shift algorithm, a non-parametric clustering algorithm. The second step is to connect LDPs into a Minimum Spanning Tree. The third step is to refine tree structures. The next step is to determine the direction of the protein by threading the amino acid sequence of the protein to the longest path in the tree. Finally, full atom models are constructed from the main-chain models, which undergo structure refinement.

We evaluated the performance of MAINMAST on two benchmark sets, a set of simulated density maps at a 5.0 Å resolution and a set of actual EM maps. Compared with two existing de novo modeling programs, Pathwalking in the EMAN2 package and Rosetta, MAINMAST achieved substantially better performances than the two methods in terms of root mean square deviation of generated models and structure coverage.

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[42] Structure Based Inhibitor Design in Bacterial HMG-CoA Reductase

C Nicklaus Steussy, Mohamed Seleem, Mark A Lipton, Cynthia V. Stauffacher
Purdue University, West Lafayette IN, USA

The emergence of multi-drug resistant pathogenic bacteria is one of the great challenges to modern medicine. The gram positive cocci Methicillin Resistant Staphylococcus aureus (MRSA) and Vancomycin Resistant Enterococcus faecalis (VRE) are two particularly virulent examples. In vivo studies have shown that the eukaryotic like 'mevalonate' isoprenoid pathway used by these pathogenic cocci is essential to their growth and virulence [1]. Our structures of HMG-CoA reductase (HMGR) from P. mevalonii demonstrated that the bacterial enzymes are structurally distinct from the human enzymes allowing for specific antibacterial activity [2]. High throughput in vitro screening against bacterial HMGR at the Southern Research Center, Birmingham, AL uncovered a lead compound with a competitive mode of action. Our x-ray crystal structures of HMGR from E. faecalis complexed with the lead compound and its variations have informed the synthesis of new inhibitors with improved effectiveness.

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[43] Protein Secondary Structure Detection in cryo-EM maps using Deep Learning

Sai Raghavendra Maddhuri, Genki Terashi and Daisuke Kihara
Kihara Lab, Purdue University

With the advancements of cryo-EM in the field of structural biology, determination and validation of 3D structures of bio molecules such as proteins has improved over the past few years. For EM maps of medium range resolution (6 – 10 Angstroms), extracting structure information from a map and building a structure model is a challenge. In this work, we aim to make use of deep learning to accurately identify protein secondary structures such as alpha helix, beta sheet, coil/turn from maps of medium range resolution. We developed a deep convolutional neural network architecture (called DeepSSPred) which is trained using simulated EM maps. We use this architecture to then identify secondary structures in the experimental EM maps taken from the EMDataBank. The deep learning architecture makes classification predictions, voxel-wise, on those volumetric EM maps.

List of Attendees

First name	Last name	University/Institution	Department
Neha	Agrawal	Purdue University	Chemistry
Intekhab	Alam	University of Michigan	Life sciences institute
Ahmad	Alimadadi	Purdue University	Agronomy
Sarah	Allen	KinaSense	
Doungkamol	Alongkronrusmee	Purdue University	Medicinal Chemistry and Molecular Pharmacology
Kathleen	Anderson	Purdue University	Biological Sciences
Stephanie	Angel	Purdue University	Biological Sciences
Brandon	Anson	Purdue University	Biological Sciences
Taylor	Bailey	Purdue University	Comparative Pathobiology
Jeremy	Bakelar	Purdue University	Biological Sciences
Gillian	Barth	Purdue University	Biology
Lorena	Beese	Duke University	Biochemistry
Richard	Bekeris	Purdue University	Biological Sciences
Aaron	Benjamin	Purdue University	Biochemistry
Satarupa	Bhaduri	Purdue University	Biological Sciences
Jason	Bice	Purdue University	MSE
Evan	Billings	Purdue University	Biological Science
Patricia	Bishop	Purdue University	Chemistry
Reena	Blade	Purdue University	Chemistry
Valorie	Bowman	Purdue University	Biology
Adrian	Buganza Tepole	Purdue University	ME, BME
Austin	Campbell	Purdue University	Biological Sciences
Ariana	Cardillo	Purdue University	Chemistry
Robert	Cassell	Purdue University	Medicinal Chemistry and Molecular Pharmacology
Jessica	Christian	Purdue University	Physics
Jozlyn	Clasman	Purdue University	Biological Sciences
Salomé	Colorado	Purdue University	Biology
William A.	Cramer	Purdue University	Biological Sciences
Voichita	Dadarlat	Purdue University	EVPRP
Chitta	Das	Purdue University	Chemistry
Ziyun	Ding	Purdue University	PULSe
Austin	Dixon	Purdue University	Biochemistry
Trevor	Doyle	Purdue University	MCMP
Aida	Ebrahimi	Purdue University	Electrical Engineering
Yuri	Efremov	Purdue University	Mechanical Engineering
Darren	Erdman	Purdue University	Physics and Astronomy
Satchal	Erramilli	University of Chicago	Biochemistry and Molecular Biosciences

Candi	Esquina	Purdue University	Chemistry
Renata	Everett	Purdue University	Biological Sciences
Matthias	Fellner	Michigan State University	Biochemistry and Molecular Biology
Jonathan	Fine	Purdue University	Chemistry
Alan	Friedman	Purdue University	Biological Sciences
Sarah	Gafter	Purdue University	Food Science
Rui	Gan	Purdue University	Biochemistry
Sanniv	Ganguly	Purdue University	Biological Sciences
Nathan	Gardner	University of Chicago	Biochemistry and Molecular Biology
Elisabeth	Garland-Kuntz	Purdue University	Chemistry
Stephanie	Gates	University of Michigan	Biological Chemistry
Ahmadreza	Ghanbarpour	Purdue University	MCMP
Barb	Golden	Purdue University	Biochemistry
Brenda	Gonzalez	Purdue University	Biological Sciences
Michael	Gregory	University of Illinois	Biochemistry
Dan	Gurnon	DePauw University	Chemistry and Biochemistry
Lisha	Ha	Purdue pharmacy	MCMP
Mark	Hall	Purdue University	Biochemistry
Xusi	Han	Purdue University	Biology
S. Saif	Hasan	Purdue University	Biological Sciences
Ahmed	Hassan	Purdue University	Comparative Pathobiology
John	Hausman	Purdue University	Chemistry
Chad	Hewitt	Purdue University	Medicinal Chemistry and Molecular Pharmacology
Jerry	Hirschinger	Purdue University	Chemistry
Nicole	Hjortland	Purdue University	Biochemistry
Erh-Ting	Hsu	Purdue University	Chemistry/PULSe
Brianna	Hudson	Purdue University	Chemistry
Seth	Huff	Anatrace Products/Molecular Dimensions	Sales
Issa	Isaac	TTP Labtech	
Aashish	Jain	Purdue University	Biological Sciences
Bolin	Jeff	Purdue University	Biological Sciences
Scott	Jensen	Purdue University	Physics
Wen	Jiang	Purdue University	Biological Sciences
Alireza	Karbakhsh Ravari	Purdue University	Physics
Joseph	Kasper	Purdue Research Foundation	Office of Technology Commercialization
Ahmad	Kazem	Purdue University	Food Science
Kristina	Kesely	Purdue University	BCHM
Katie	Kitamura	Purdue University	ECE

Nicole	Klinkner	Purdue University	Biochemistry
Ellen	Kolb	University of Cincinnati College of Medicine	Molecular Genetics, Biochemistry, and Microbiology
Aaron	Krabill	Purdue University	MCMP
Charles	Kuntz	Purdue University	MCMP
Travis	Lantz	Purdue University	Chemistry
Samantha	Lee	Purdue University	Biochemistry
Emma	Lendy	Purdue University	PULSe
Zhe	Li	Purdue University	Physics
Peng	Lin	Purdue University	Biomedical Engineering
Yue	Liu	Purdue University	Biology
Jeremy	Lohman	Purdue University	Biochemistry
Sue Heidi	Loperena-Medina	Purdue University	PULSe
Angeline	Lyon	Purdue University	Chemistry
Sai Raghavendra	Maddhuri	Purdue University	Computer Science
Himesh	Makala	SASTRA University	Biotechnology
Eileen	Mallery	Purdue University	Botany
Marianne	Maresh	Purdue University	Medicinal Chemistry and Molecular Pharmacology
Islam	Matar	Purdue University	Comparative Pathobiology
Joann	Max	Purdue University	Chemistry
Adam	McChesney	Gold Bio	Chemicals/Reagents
Jason	McCoy	University of Cincinnati	Molecular Genetics, Biochemistry, and Microbiology
Mark	McLean	University of Illinois	Biochemistry
Adriano	Mendes	Purdue Univeristy	Biology
Andy	Mesecar	Purdue University	Biochemistry
Saki	Mihori	Purdue University	Biology
Oleg	Mikhailovskii	Purdue University	Chemistry
Ignacio	Mir-Sanchis	The University of Chicago	Biochemistry and Molecular Biology
Lyman	Monroe	Purdue University	Biology
Corey	Moore	Purdue University	Biological Sciences
Jasmine	Moore	Purdue University	MCMP
Mitchell	Moseng	Miami University of Ohio	Chemistry
Ryan	Murphy	Purdue University	Food Science
Monessha	Nambiar	Purdue University	Chemistry
Jillian	Ness	Purdue University	Biology
Nicholas	Noinaj	Purdue University	Biological Sciences
Allison	Norvil	Purdue University	Biochemistry
Esteban	Orellana	Purdue University	Biological Sciences

Steve	Ouellette	KinaSense	
Allison	Page	Purdue University	Chemistry/Physics
Tejasvi	Parupudi	Purdue University	ECE
Amar	Parvate	Purdue University	Biological Sciences
Dhabaleswar	Patra	University of Michigan	Life Sciences Institute
Matthew	Pharris	Purdue University	Biomedical Engineering
Carol	Post	Purdue University	MCMP, Biology
Edward	Pryor	Anatrace	
Vatsal	Purohit	Purdue University	Biology
Kedar	Puvar	Purdue University	Chemistry
Swetha	Ramadesikan	Purdue University	Biological Sciences
Anna	Ratliff	Purdue University	Chemistry
Logan	Richards	Purdue University	Biochemistry
Ngango Yvon	Rugema	Purdue University	Chemistry
Phillip	Rushton	Purdue University	Biological Sciences
Manalee	Samaddar	Purdue University	Food Science
Tim	Schmidt	Purdue University	Biology
Thomas	Shriver	Purdue University	Chemistry
Monita	Sieng	Purdue University	Chemistry
Sandeep	Singh	Purdue University	Biological Sciences
Cathy	Skidmore	Purdue University	Medicinal Chem/Molecular Pharm
Steve	Sligar	University of Illinois	Biochemistry
Madison	Smith	Purdue University	Biochemistry
David	Sohutskay	Purdue University	Biomedical Engineering
Valentyn	Stadnytskyi	Purdue University	Physics and Astronomy
Cynthia	Stauffacher	Purdue University	Biological Sciences
Nic	Steussy	Purdue University	Biology
Lee	Stunkard	Purdue University	Biochemistry
Brendan	Sullivan	Purdue University	Physics
Chen	Sun	Purdue University	Biology
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Matthew	Therkelsen	Purdue University	Biological Sciences
Elisabeth	Van Niekerk	Michigan State	Biochemistry
Michelle	Van Camp	Purdue University	Chemistry
Jamie	VanPelt	Miami University	Chemistry and Biochemistry
Julien	Varennes	Purdue University	Physics and Astronomy
Yongchao	Wang	University of Kentucky	Pharmacology
Ben	Watson	Purdue University	Biology
Mike	Wendt	Purdue University	MCMP

Michael	White	Purdue University	Biology
Matthew	Wohlever	University of Chicago	Biochemistry & Molecular Biology
Hongwei	Wu	Indiana University	Chemistry
Xiaoyu	Xu	Purdue University	Biomedical Engineering
Ravi	Yadav	Purdue University	Interdisciplinary Life Sciences
Rui	Yan	Purdue University	Biological Sciences
Ying	Yang	Purdue University	Medicinal Chemistry and Molecular Pharmacology
Adam	Yokom	University of Michigan	Chemical Biology
Nick	Yonts	Sartorius Corp.	Sartorius Corp.
Erica	Zbornik	Purdue University	Biology
Matthias	Zeller	Purdue University	Chemistry
Lu	Zhang	Purdue University	Animal Sciences
Huaqun	Zhang	Miami University	Chemistry and Biochemistry
Kai	Zhang	University of Kentucky	Pharmacology
Feifei	Zhao	Purdue University	Chemistry
Mengmeng	Zhu	Purdue University	Biological Sciences