18th Annual SER-CAT Structural Biology Symposium

March 18–19, 2021

HOSTED JOINTLY BY:

St. Jude Children's Research Hospital®

Structural Biology

Southeast Regional Collaborative Access Team (SER-CAT)

St. Jude Children's Research Hospital

President and CEO: James R. Downing, MD

St. Jude Children's Research Hospital is leading the way the world understands, treats and cures childhood cancer and other life-threatening diseases. It is the only National Cancer Institute-designated Comprehensive Cancer Center devoted solely to children. Treatments developed at St. Jude have helped push the overall childhood cancer survival rate from 20 percent to 80 percent since the hospital opened more than 50 years ago. In addition to children with cancer, St. Jude also accepts patients with blood disorders and related life-threatening diseases, such as sickle cell anemia and primary immunodeficiency disease.

St. Jude freely shares the breakthroughs it makes, and every child saved at St. Jude means doctors and scientists worldwide can use that knowledge to save thousands more children. St. Jude investigators have the freedom to focus on making big discoveries, backed by extraordinary resources and support teams. Our culture and campus foster the free exchange of ideas among scientists and clinicians for creative, collaborative science.

One of the ways St. Jude shares its discoveries is through St. Jude Cloud. This data-sharing resource is available for the global research community and offers one of the world's largest repositories of next-generation sequencing data and analysis tools for pediatric cancer. Through St. Jude Global, the hospital launched an ambitious initiative to improve access to care to children with cancer and life-threatening blood diseases in every corner of the world, and to develop plans to enhance its quality and provide better options for cures.

St. Jude Graduate School of Biomedical Sciences

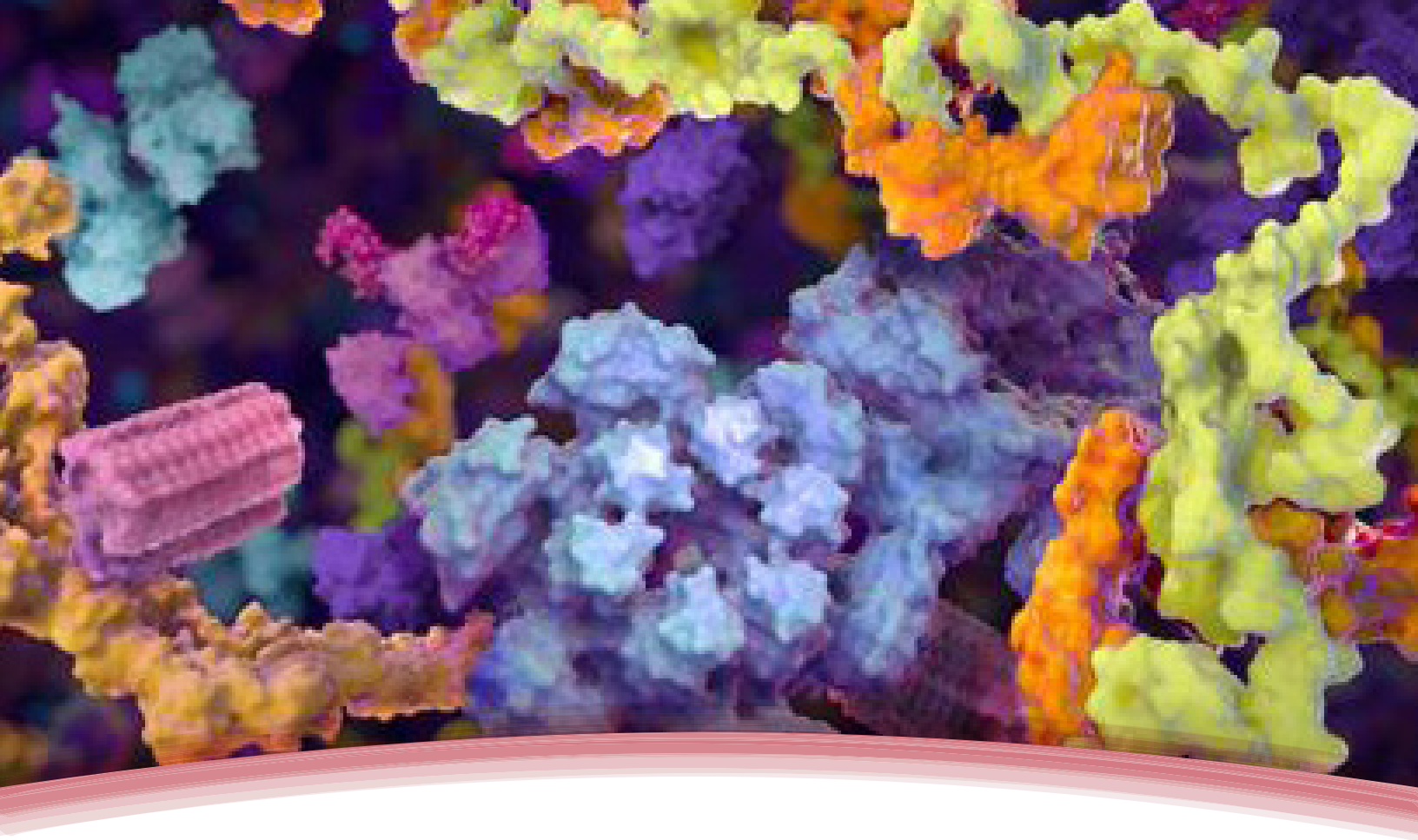
President and Dean: Stephen White, DPhil

The St. Jude Children's Research Hospital Graduate School of Biomedical Sciences works to fundamentally advance global health and to find cures for pediatric catastrophic diseases. The graduate school promotes education and research across disciplines, and trains future leaders and innovators, including researchers probing the molecular basis of disease and therapy, medical practitioners conducting clinical and translational research, and health professionals improving health care systems worldwide.

Doctoral research can be pursued in diverse areas with top basic and translational research faculty. In addition to the PhD program in biomedical sciences, the graduate school also offers masters programs in Global Child Health and Clinical Investigation. These programs' innovative curricula are designed for independent and fearless students who thrive in an interactive, challenging and creative research and clinical environment.

St. Jude Graduate School of Biomedical Sciences

St. Jude Children's Research Hospital
Graduate School of Biomedical Sciences



St. Jude Department of Structural Biology

Chair: Charalampos Kalodimos, PhD

The Department of Structural Biology at St. Jude aims to understand life and disease at atomic detail. Our researchers work to decipher the molecular details of key biological processes such as cell signaling, cell death, DNA repair, protein folding and misfolding and cell transport. The detailed structures of proteins involved in these processes can be leveraged to help design new medicines, materials and diagnostic procedures. Availability of atomic-level structures of proteins and their complexes enables the rational design of small molecules that manipulate their function and can thus have powerful therapeutic potential.

Through our technology core centers, we use sophisticated biophysical techniques such as nuclear magnetic resonance spectroscopy, x-ray crystallography, cryo-electron microscopy and tomography, single-molecule spectroscopy and mass spectrometry to determine the three-dimensional structures of biological molecules. Such studies provide a precious glimpse into the cellular activity and increase our understanding of life's most fundamental processes and the diseases that result when these processes malfunction. Because proteins are very dynamic molecules, we are also using experimental and computational tools to investigate how proteins adjust rapidly their structures to carry out their biological function.

St. Jude Department of Structural Biology

Agenda

9:00–9:10 am

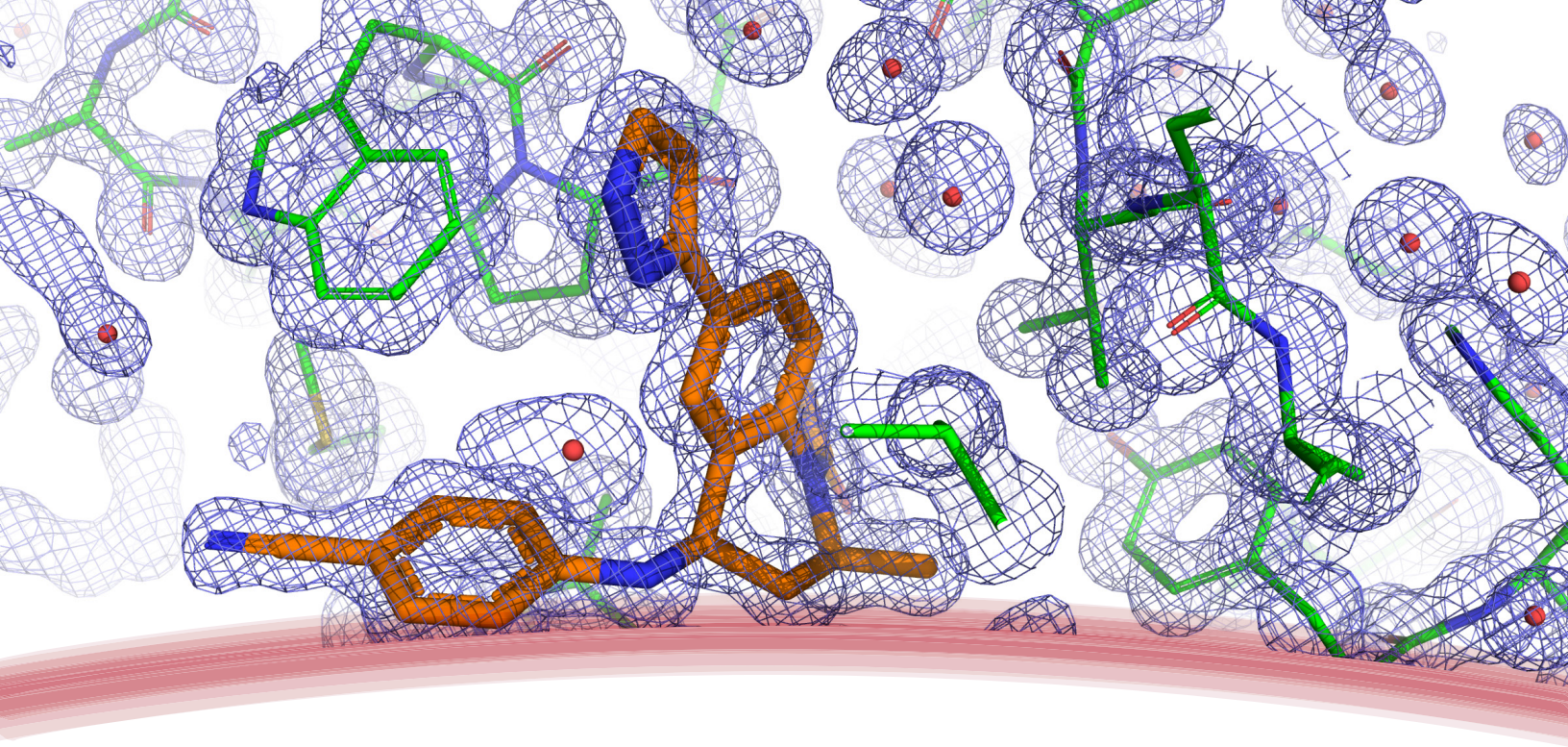
Welcome
Darcie Miller, PhD
Director, Biomolecular X-ray Crystallography Center
Department of Structural Biology
St. Jude Children's Research Hospital

9:10–9:15 am

Opening remarks
Scott Blanchard, PhD
Member, Department of Structural Biology
St. Jude Children's Research Hospital

9:15 am–4:25 pm — **Sessions 1, 2, and 3 Presentations**

4:25–4:30 pm — **Closing remarks**
Darcie Miller, PhD

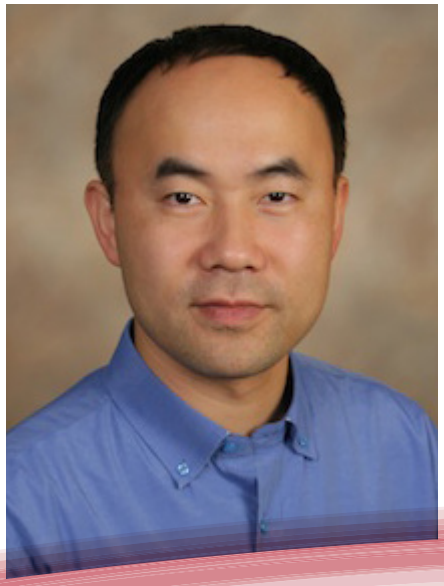


Schedule of Events

Zoom event. All times are in Central Time, beginning at 8:45 am CT / 9:45 am ET.

TIME	EVENT
8:45 - 9:00 am	Log in
9:00 - 9:10 am	**Dr. Darcie Miller, St. Jude Children's Research Hospital** Welcome
9:10 - 9:15 am	**Dr. Scott Blanchard, St. Jude Children's Research Hospital** Opening Remarks
SESSION 1	**General Scientific Talks and Keynote Speaker; Session Chair: Dr. Stephen White**
9:15 - 9:40 am	**Dr. Pei Zhou, Duke University** Enhancing Chemotherapy with Small Molecule Translesion Synthesis Inhibitors
9:40 - 10:05 am	**Dr. Fred Dyda, NIDDK, National Institutes of Health** The mechanistic origins of spacer acquisition in CRISPR-Cas: Repurposing DNA Transposition
10:05 - 10:30 am	**Dr. Eric Ortlund, Emory University** Disrupting a key H-bond network drives glucocorticoid receptor gene suppression without off-target gene activation
10:30 - 10:45 am	**Break**

10:45 - 10:50 am	**Dr. Stephen White,** **St. Jude Graduate School of Biomedical Sciences** Introduction of Keynote Speaker
10:50 - 11:50 am	**Keynote Speaker: Dr. Ian A. Wilson, Scripps Research** Structural insights into antibody responses to SARS-CoV-2 and escape mutations
11:50 - 1:00 pm	**Break for lunch following brief announcement by Dr. Darcie Miller**
SESSION 2	**SER-CAT Award Winners, SER-CAT & APS Updates; Session Chair: Dr. Raquel Lieberman**
1:00 - 1:05 pm	**Return**
1:05 - 1:15 pm	**Prof. Bi-Cheng Wang, Director of SER-CAT, SER-CAT & UGA** SER-CAT Remarks and Introduction of APS Speaker
1:15 - 1:45 pm	**Dr. Dennis Mills, APS, Argonne National Laboratory** The Advanced Photon Source Upgrade Project – an update
1:45 - 2:10 pm	**Dr. R. Scott Williams, NIEHS, National Institutes of Health** Endogenous DNA 3' blocks are vulnerabilities for BRCA1 and BRCA2 deficiency and are reversed by the APE2 family nucleases
2:10 - 2:35 pm	**Ha An Nguyen, Emory University** The regulatory role of tRNAs in translation
2:35 - 2:45 pm	**Prof. Bi-Cheng Wang** SER-CAT Award Presentations
2:45 - 3:00 pm	**Break**
3:00 - 3:25 pm	**Dr. John Chrzas, SER-CAT & UGA** SER-CAT Upgrade update
SESSION 3	**SER-CAT Member Talks; Session Chair: Dr. Tudor Moldoveanu**
3:25 - 3:45 pm	**Dr. Raquel Lieberman, Georgia Institute of Technology** Molecular architecture glaucoma-associated myocilin
3:45 - 4:05 pm	**Dr. Bo Liang, Emory University** Crystal structure of the HRSV M2-1 protein in complex with a short positive-sense gene-end RNA
4:05 - 4:25 pm	**Dr. Christopher Radka, St. Jude Children's Research Hospital** Structure and mechanism of Staphylococcus aureus oleate hydratase (OhyA)
4:25 - 4:30 pm	**Dr. Darcie Miller** Closing Remarks



Pei Zhou

9:15 - 9:40 am

Enhancing Chemotherapy with Small Molecule Translesion Synthesis Inhibitors

ABSTRACT:

Chemotherapy remains an effective treatment option for many cancer patients. Despite a high rate of initial treatment success, the majority of the patients eventually relapse with resistant tumors that render subsequent rounds of treatment ineffective. It is increasingly recognized that mutagenic translesion synthesis (TLS), a DNA damage tolerance pathway, plays an important role in cancer cell survival and development of resistance after chemotherapy, suggesting that targeting TLS is an attractive avenue for improving chemotherapeutics. However, development of small molecules with high specificity and *in vivo* efficacy for mutagenic TLS has been challenging. Employing a high-throughput screening campaign, we discovered a small molecule inhibitor, JH-RE-06, that disrupts mutagenic TLS by preventing the recruitment of mutagenic POL ζ. Remarkably, our structural analysis reveals that JH-RE-06 targets a nearly featureless surface of REV1 that interacts with the REV7 subunit of POL ζ. Binding of JH-RE-06 induces REV1 dimerization and blocks the REV1-REV7 interaction and POL ζ recruitment. JH-RE-06 inhibits mutagenic TLS and enhances cisplatin-induced-toxicity *in vitro*; furthermore, co-administration of JH-RE-06 with cisplatin suppresses the growth of xenograft human melanomas in mice. Taken together, these results establish the feasibility of developing TLS inhibitors as a novel class of chemotherapy adjuvants.

ABOUT THE SPEAKER:

Dr. Zhou obtained his PhD training in the area of chemical biology at Harvard University from 1993-1998 and postdoctoral training in the area of structural biology at Harvard Medical School from 1998-2001. In 2001, Dr. Zhou established an active research program at Duke University School of Medicine to probe the structure and dynamics of macromolecular assembly and inhibition. His main research interest centers on the role of structure and dynamics on macromolecular complex assembly and function, enzyme inhibition, and structure and dynamics-based drug development.

Fred Dyda

9:40 - 10:05 am

The mechanistic origins of spacer acquisition in CRISPR-Cas: Repurposing DNA Transposition

ABSTRACT:

DNA transposition and spacer acquisition in CRISPR-Cas are similar processes, yet their logic appears inverted: while DNA transposition integrates well-defined sequences at random sites, spacer acquisition integrates random, invading DNA fragments at a well-defined site, called CRISPR array. We show based on biochemical and structural data that the resolution of the apparent contradiction lies with Casposons that are mobile genetic elements inhabiting the twilight world between transposition and spacer acquisition with properties that are reminiscent of both forms of DNA transactions.

ABOUT THE SPEAKER:

Dr. Dyda is a senior investigator at the Laboratory of Molecular Biology of the National Institute of Diabetes and Digestive and Kidney Diseases at the National Institutes of Health in Bethesda, Maryland. His scientific area of focus is the structural biochemistry of mobile genetic elements. Currently, his research group is investigating a number of prokaryotic and eukaryotic DNA transposases and their assemblies. Dr. Dyda received his undergraduate education at Eötvös Lóránd University and his PhD at the University of Pittsburgh in 1992.

Eric Ortlund

10:05 - 10:30 am

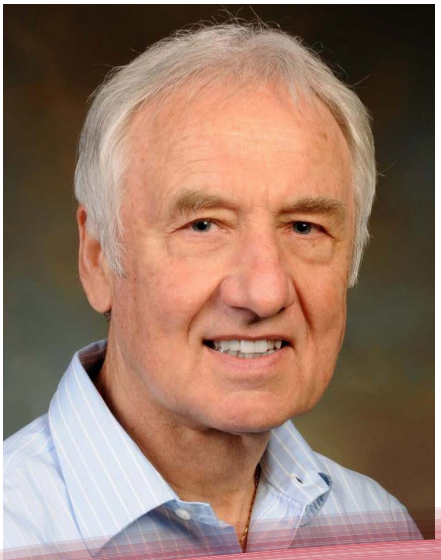
Disrupting a key H-bond network drives glucocorticoid receptor gene suppression without off-target gene activation

ABSTRACT:

Duchenne muscular dystrophy is a genetic disorder that shows chronic and progressive damage to skeletal and cardiac muscle leading to premature death. Antiinflammatory corticosteroids targeting the glucocorticoid receptor (GR) are the current standard of care but drive adverse side effects such as deleterious bone loss. Through subtle modification to a steroidal backbone, a recently developed drug, vamorolone, appears to preserve beneficial efficacy but with significantly reduced side effects. We use combined structural, biophysical, and biochemical approaches to show that loss of a receptor-ligand hydrogen bond drives these remarkable therapeutic effects. Moreover, vamorolone uniformly weakens coactivator associations but not corepressor associations, implicating partial agonism as the main driver of its dissociative properties. Additionally, we identify a critical and evolutionarily conserved intramolecular network connecting the ligand to the coregulator binding surface. Interruption of this allosteric network by vamorolone selectively reduces GR-driven transactivation while leaving transrepression intact. Our results establish a mechanistic understanding of how vamorolone reduces side effects, guiding the future design of partial agonists as selective GR modulators with an improved therapeutic index.

ABOUT THE SPEAKER:

Dr. Eric Ortlund is a Professor of Biochemistry at Emory University SOM who studies ligand-mediated signaling in nuclear receptors (NRs). His lab uses structural and biochemical studies of human NRs in order to assess their roles in metabolism, stress, homeostasis and disease. His research on steroid receptors (SRs) reveals novel modes of binding (to DNA, RNA & ligands) as well as insights into the evolution of SR – hormone relationships. Further research in his lab focuses on lipid-regulated NRs, particularly liver receptor homolog-1 (LRH-1). His lab has extensively characterized LRH-1, including solving the first crystal structures of a ligand-bound receptor, characterizing functional regions, and decoding the transcriptional & structural effects of ligand binding. Recent breakthroughs in the lab have enabled the generation, evaluation and improvement of LRH-1 modulators using a structure-guided approach. In addition to his own lab, Dr. Ortlund serves as the Scientific Director for the Emory Integrated Lipidomics & Metabolomics Core, which provides quantitative measurements as well as integration of ‘omics data to signaling pathways in disease. He also serves as an mPI of a multisite consortium to pair ‘omics data to determine the molecular changes of exercise.



Ian A. Wilson, Keynote

10:50 - 11:50 am

Structural insights into antibody responses to SARS-CoV-2 and escape mutations

ABSTRACT:

SARS-CoV-2 emerged as a global pandemic in 2020 with devastating health and socioeconomic consequences. Many human neutralizing monoclonal antibodies (nAbs) have now been isolated from COVID-19 convalescent patients by research groups worldwide. Many of these nAbs target the receptor binding domain (RBD) of the spike protein, which engages the ACE2 receptor on human cells for viral entry. We have been determining crystal structures of many nAbs bound to the spike protein RBD to determine their binding sites (epitopes) and possible mechanisms of neutralization to reveal key sites of vulnerability on the virus. Recurring motifs as well as novel binding modes used by antibodies for RBD recognition have been delineated, including which antibody germline genes in the human immune repertoire are preferentially used to target the RBD. We have also been analyzing the effects of recent mutations in the different lineages originally isolated in the UK, South Africa and Brazil on antibody binding and neutralization. Such structural and functional information can be utilized to assess the vaccine responses against SARS CoV-2, and aid in design of next-generation-vaccine immunogens and novel therapeutics that inhibit entry of SARS CoV-2 into host cells.

ABOUT THE SPEAKER:

Dr. Wilson obtained his BSc in Biochemistry from Edinburgh University (1971), DPhil (1976) and DSc (2000) in Molecular Biophysics from Oxford University, and was awarded a DSc (hon.) from University of St. Andrews (2007). He was a postdoctoral fellow at Harvard University (1977-1982) and joined The Scripps Research Institute as a faculty member in 1982. He is currently Hansen Professor of Structural Biology and Chair of the Department of Integrative Structural and Computational Biology at Scripps Research. His laboratory focuses on the structural basis of immune recognition of microbial pathogens. His lab has worked extensively on the structure and function of the surface glycoprotein antigens on enveloped viruses. His lab's current focus is on how SARS-CoV-2, influenza virus, HIV-1, HCV and *P. falciparum* are recognized by broadly neutralizing and protective antibodies to inform on design of novel vaccines and therapeutics. Dr. Wilson is a Fellow of the Royal Society, Corresponding Fellow of the Royal Society of Edinburgh, Member of the American Academy of Arts and Sciences, Foreign Associate of the National Academy of Sciences, Fellow of the American Academy of Microbiology, and an Honorary Fellow of Corpus Christi College, Oxford. He has authored more than 800 papers and is on the Statistical Board of Reviewing Editors of Science and the Editorial Board of Immunity.

Bi-Cheng Wang

1:05 - 1:15 pm

SER-CAT Remarks and Introduction of APS Speaker

ABOUT THE SPEAKER:

Bi-Cheng (B.C.) Wang, PhD, is SER-CAT PI and Director, Georgia Research Alliance Eminent Scholar in Structure Biology, and Professor of Biochemistry and Molecular Biology at the University of Georgia. Dr. Wang's research work has been focused on the application of structural biology tools for the structure-function studies of relevant biological macromolecules, which include T7 RNA polymerase, neurophysins and their hormone complexes, glutathione transferases, glutamine binding protein and complex, aldehyde dehydrogenase, augmenter of liver regeneration, green fluorescent protein, hemerythrin, myohemerythrin, ferrochelatase and plant cell wall polysaccharides.

His interests also include the application of multidisciplinary methodologies for structural genomics and structural proteomics, the development of advanced instrumentation for diffraction studies including SER-CAT synchrotron beamline at the APS, remote access for beamline operations, robotic sample mounting technologies, and some special usages of in-house X-ray sources for macromolecular crystallography. In the 1980s he developed a novel software package for single isomorphous replacement and single wavelength anomalous scattering phasing methods, including the use of unlabeled native protein crystals. His publication list includes more than 210 peer-reviewed manuscripts and book chapters. He has listed more than 200 oral and 310 poster presentations at the national and international conferences.

He has been recognized by numerous awards including the UGA Lamar Dodd Creative Research Award, the ACA Distinguished Service Award, the ACA Patterson Award, the SER-CAT Golden Magnolia Award, the Shanghai Tech University's iHuman Structure of Life Award, and been elected as an ACA Inaugural Fellow.

Dennis Mills

1:15 - 1:45 pm

The Advanced Photon Source Upgrade Project – an update

ABSTRACT:

X-rays are a critical tool for determining the structure and dynamics of matter. However, limitations of current x-ray sources prevent researchers from fully investigating complex systems. The APS upgrade (APS-U) will overcome these limitations by improving APS photon coherence and brightness by up to three orders of magnitude over the existing source, particularly at high energies. When the project is completed, the upgraded Advanced Photon Source (APS) will be the world's brightest hard x-ray storage-ring light source and will revolutionize researchers' ability to use hard x-rays to explore physical, chemical, and biological systems in multiple dimensions, from the atomic scale to the macroscopic scale. A brief overview of the upgrade project will be given along with the current schedule for the project.

ABOUT THE SPEAKER:

Dennis Mills is the Deputy Associate Laboratory Director for Photon Sciences at Argonne and Deputy Director of the Advanced Photon Source. His main scientific interests are X-ray optics, diffraction physics and the user of the unique properties of synchrotron radiation, such as polarization and modulated time structure, for studying materials properties.

Dennis Mills received his BS degree from Rensselaer Polytechnic Institute and his PhD at Cornell University in Applied Physics in 1979. He worked as a Staff Scientist at the Cornell High Energy Synchrotron Source from 1979 until 1988 when he joined the staff of the Advanced Photon Source at Argonne as group leader for X-ray Optics and Beamlines. In 1998 he was promoted to Senior Scientist at Argonne and was selected for an APS Division Director position in 1999. In 2002 he was appointed to his current position as Deputy Associate Laboratory Director for Photon Sciences.

Awards include a Guggenheim Fellowship (1987), University of Chicago Medal for Distinguished Performance at Argonne (1997), the Advanced Photon Source's Arthur H. Compton Award (1998) for work related to x-ray optics, and the Argonne Board of Governors Pinnacle of Education Award (2008) for his contributions to the development of the National Neutron and X-ray Scattering School. He served as the Main Editor for the Journal of Synchrotron Radiation from 2000-2008 and has been invited to contribute articles/chapters in 9 books on the properties and uses of synchrotron radiation. He is a Fellow of the American Physical Society.

R. Scott Williams

SER-CAT Science Award Winner

1:45 - 2:10 pm

Endogenous DNA 3' blocks are vulnerabilities for BRCA1 and BRCA2 deficiency and are reversed by the APE2 family nucleases

ABSTRACT:

Put here Loss of APE2 nuclease is lethal in cells with mutated BRCA1 or BRCA2, making APE2 a prime target for homologous recombination-defective cancers. However, since the function of APE2 in DNA repair is poorly understood, it is unclear why BRCA-deficient cells require APE2 for viability. Based on genetic interaction profiles of APE2 coupled to a biochemical and structural dissection of APE2 a role for APE2 is established in resolving diverse blocked 3' DNA ends, problematic lesions that preclude DNA synthesis. These blocks include complex DNA damage generated by topoisomerase1 (TOP1) conversion of genome embedded ribonucleotides into 3' blocked adducts. The sensitivity of BRCA-deficient cells to 3' blocks indicates that they represent a tractable vulnerability in homologous recombination-deficient tumor cells. Biochemical and structural characterization of recombinant human and *Xenopus laevis* APE2 demonstrates that these enzymes harbor broad 3'-end processing activities capable of Top1 DNA protein crosslinks and 2'-3'-cyclic-phosphate modified DNA ends. Our X-ray structural analysis of APE2 homologs highlights that compared to the structurally related EEP (endonuclease exonuclease phosphatase) fold enzymes APE1 and TDP2, APE2 has divergent substrate interaction regions appropriate accommodating diverse 3' adducts via a wedge and cut mechanism.

ABOUT THE SPEAKER:

Dr. Williams heads the Structural Cell biology Group within the Genome Integrity and Structural Biology Laboratory at NIEHS/NIH in Research Triangle Park, NC. He earned his PhD in Biochemistry (2003) from the University of Alberta, Canada. He then completed his postdoctoral training at the Scripps Research Institute in La Jolla, California with John Tainer, before joining NIH/NIEHS as tenure track Principal investigator in November 2009. Williams was tenured and named Deputy Chief of the Genome Integrity and Structural Biology Laboratory in 2016. As a graduate student studying structure and function of the BRCA1 tumor suppressor, Dr. Williams became interested in cellular responses to DNA damage, a subject area which remains the primary focus of his research.

Ha An Nguyen

SER-CAT Young Investigator Award Winner

2:10 - 2:35 pm

The regulatory role of tRNAs in translation

ABSTRACT:

The accuracy of information transfer through the steps of the Central Dogma is vital for all cellular life. The ribosome, a giant macromolecular machine made up of both proteins and RNA, is responsible for the last step where it decodes the nucleotide sequence of mRNA to synthesize polypeptides using tRNAs as the adaptor molecule. While the correct base pairing between the tRNA anticodon and mRNA codon is the fundamental driving force behind the fidelity of protein synthesis, it is also known that other elements of the tRNA as well as the ribosome acts as regulatory mechanisms during protein synthesis. Here, we uncovered a previously unrecognized mechanism with which the ribosome can distinguish correct tRNAs from incorrect ones. By comparing structures of cognate and mismatched tRNA-mRNA pairs bound to the bacterial ribosome, we found that a specific ribosomal RNA nucleotide act as a recognition sensor with a particular tRNA base pair. This tRNA base pair at positions 32 and 38 is critical for accurate decoding by ensuring the uniform binding of all tRNAs to the ribosome. When this base pair is disrupted, translation fidelity is lost, and the ribosome is no longer able to recognize the incorrect tRNA-mRNA base pairing. Collectively, these structures revealed a previously unappreciated way in which the ribosome senses the integrity of tRNAs and their interaction with mRNA to ensure accurate gene expression.

ABOUT THE SPEAKER:

Ha An Nguyen obtained her undergraduate degree from University of Richmond. Now she is currently a 5th year Chemistry graduate student in Dr. Christine Dunham's laboratory at Emory University. Ha An has been studying the mechanisms of bacterial translation regulation using biochemical and structural biology techniques. She has 2 first-author publications so far highlighting the role of tRNAs in maintaining translation fidelity. Next, Ha An is excited to expand her structural expertise to further investigate interesting biological questions.

John Chrzas

3:00 - 3:25 pm

SER-CAT Upgrade update

ABSTRACT:

An update of the current status and recent upgrades of the SER-CAT beamlines will be presented. The SER-CAT upgrade plans for the upcoming APS upgrade period will also be discussed.

ABOUT THE SPEAKER:

John Chrzas is a Senior Research Scientist in the Department of BioChemistry and Molecular Biology at the University of Georgia and the Associate Director for Facilities for SER-CAT. His main scientific interests include x-ray optics, software development and beamline automation.

John Chrzas received his BS (1986), MS (1988), and PhD (1992) in Physics from the Illinois Institute of Technology (IIT). He worked as a Postdoc at the Advanced Photon Source in 1993 and then joined the staff of the Center for Synchrotron Radiation Research and Instrumentation at the Illinois Institute of Technology in 1994. While working for IIT he held position of beamline scientist for both MR-CAT and IMCA-CAT and was promoted to Executive Director of IMCA-CAT in 2001. In 2002 he joined the University of Georgia as the Operations Manager for SER-CAT. He was promoted to his current position as Associate Director of Operations for SER-CAT in 2019.

Raquel Lieberman

3:25 - 3:45 pm

Molecular architecture glaucoma-associated myocilin

ABSTRACT:

Myocilin, a modular multidomain extracellular protein, is best known for its association with glaucoma, an eye disease that affects over 60 million individuals worldwide. We have elaborated molecular details of myocilin structure and misfolding relevant to glaucoma pathogenesis. Namely, mutations in the myocilin olfactomedin domain (mOLF) are causative for the eye disease glaucoma due to a toxic gain of function, intracellular aggregation. Until recently our structural studies were limited to a dissect-and-rebuild approach involving the isolated mOLF domain and the N-terminal coiled-coil regions, but we have recently overcome the hurdle of expression and purification of full-length myocilin and have obtained our first images of full-length myocilin by negative stain transmission electron microscopy. Our new data expand beyond our inferences from the smaller recombinant myocilin constructs, enable comparison of myocilin to other OLF-family proteins, and lay the foundation for a better molecular understanding of myocilin structure and function.

ABOUT THE SPEAKER:

Dr. Lieberman obtained her PhD in Chemistry from Northwestern University in 2005. She did postdoctoral training in the area of structural biology and misfolding of neurodegenerative diseases at Brigham & Women's Hospital/Harvard Medical School and Brandeis University from 2005-2007. In 2008, Dr. Lieberman moved to Georgia Tech's School of Chemistry & Biochemistry where she established a research program focused on biophysical and structural characterization of proteins involved in misfolding disorders and ameliorating the misfolding phenotype by using chemical biology approaches. Rooted in basic research, the long-term goal of her research program is to convert mechanistic discoveries into disease-modifying therapies.

Bo Liang

3:45 - 4:05 pm

Crystal structure of the HRSV M2-1 protein in complex with a short positive-sense gene-end RNA

ABSTRACT:

The M2-1 protein of the human respiratory syncytial virus (HRSV) is a transcription anti-terminator that regulates the processivity of the HRSV RNA-dependent RNA polymerase (RdRP). Here, we reported a crystal structure of HRSV M2-1 bound to a short positive-sense gene-end RNA (SH7) at 2.7 Å resolution. We identified multiple critical residues of M2-1 involved in RNA interaction and examined their roles using mutagenesis and MicroScale Thermophoresis (MST) assay. We found that hydrophobic residue Phe23 is indispensable for M2-1 to recognize the base of RNA. We also captured spontaneous binding of RNA (SH7) to M2-1 in all-atom simulations using a robust Gaussian accelerated molecular dynamics (GaMD) method. Both experiments and simulations revealed that the interactions of RNA with two separate domains of M2-1, the zinc-binding domain (ZBD) and the core domain (CD), are independent of each other. Collectively, our results provided a structural basis for RNA recognition by HRSV M2-1.

ABOUT THE SPEAKER:

Dr. Liang is an Assistant Professor in the Department of Biochemistry at Emory University School of Medicine and the Co-Scientific Director of the Robert P. Apkarian Integrated Electron Microscopy Core (IEMC) at Emory University. Before joining as a faculty at Emory in 2016, Dr. Liang earned dual bachelor's degrees in Biology and Computer Science at the University of Science and Technology of China (2004). He received his PhD in Molecular Biophysics from Florida State University (2009). He then completed a postdoctoral fellowship in Biological Chemistry and Molecular Pharmacology (BCMP) and Microbiology and Immunobiology (MBIB) at Harvard Medical School (2016). Dr. Liang's primary scholarly focus is on scrutinizing high-resolution structural details of large assemblies, including ribonucleoprotein complexes and membrane proteins, using integrated cryo-electron microscopy (cryo-EM) and x-ray crystallography.

Christopher Radka

4:05 - 4:25 pm

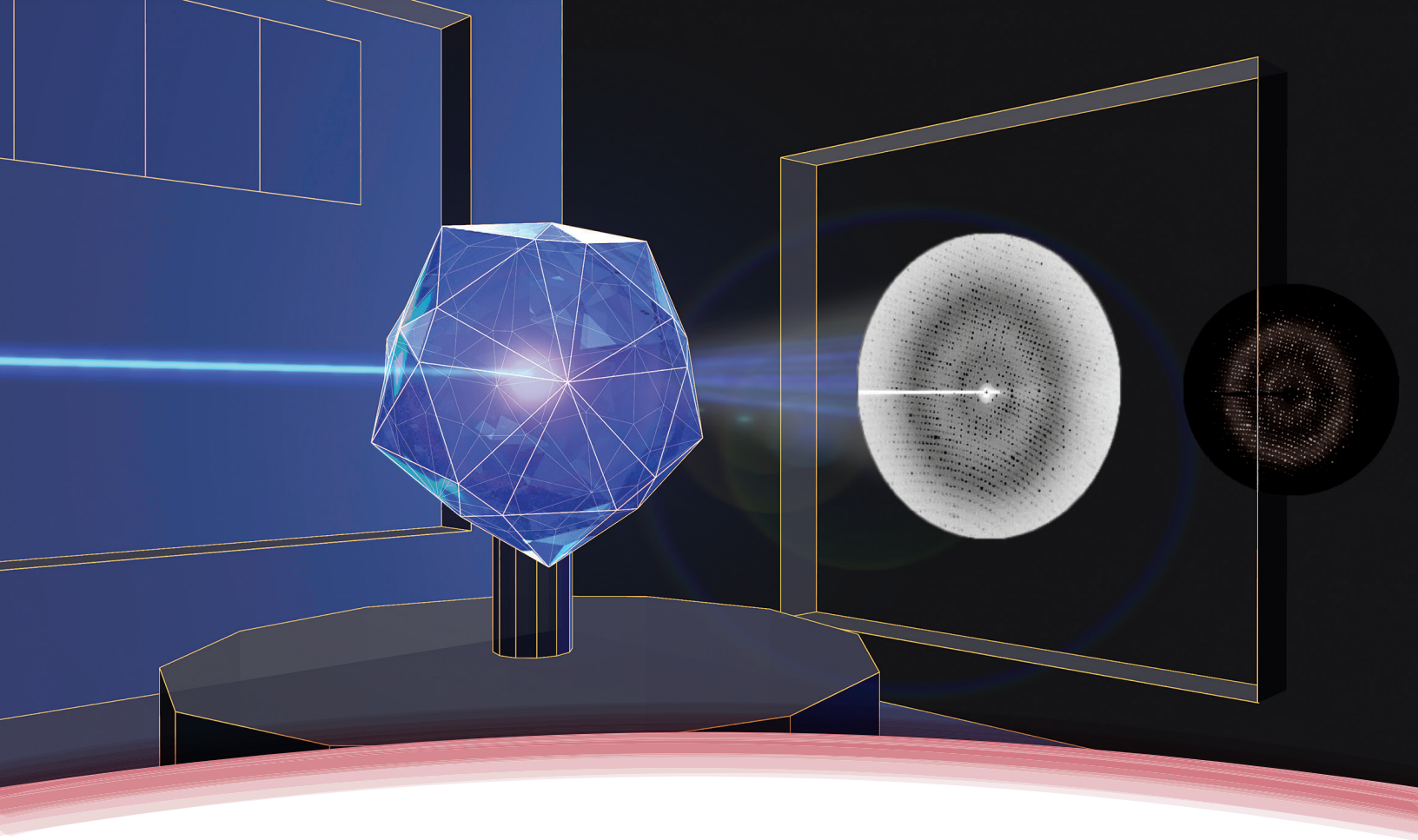
Structure and mechanism of Staphylococcus aureus oleate hydratase (OhyA)

ABSTRACT:

FAD-dependent bacterial oleate hydratases (OhyA) catalyze the addition of water to isolated fatty acid carbon-carbon double bonds. *Staphylococcus aureus* **uses** OhyA to counteract the host innate immune response by inactivating antimicrobial unsaturated fatty acids. Mechanistic information explaining how OhyAs catalyze regio- and stereospecific hydration is required to understand their biological functions and the potential for engineering new products. In this study, we deduced the catalytic mechanism of OhyA from multiple structures of *S. aureus* OhyA in binary and ternary complexes with combinations of ligands along with biochemical analyses of relevant mutants. The substrate-free state shows Arg81 is the gatekeeper that controls fatty acid entrance to the active site. FAD binding engages the catalytic loop to simultaneously rotate Glu82 into its active conformation and Arg81 out of the hydrophobic substrate tunnel, allowing the fatty acid to rotate into the active site. FAD binding also dehydrates the active site, leaving a single water molecule connected to Glu82. This active site water is a hydronium ion based on the analysis of its hydrogen bond network in the OhyA•PEG400•FAD complex. We conclude that OhyA accelerates acid-catalyzed alkene hydration by positioning the fatty acid double bond to attack the active site hydronium ion, followed by the addition of water to the transient carbocation intermediate. Structural transitions within *S. aureus* OhyA channel oleate to the active site, curl oleate around the substrate water, and stabilize the hydroxylated product to inactivate antimicrobial fatty acids.

ABOUT THE SPEAKER:

Dr. Radka obtained his PhD training at the University of Alabama at Birmingham from 2012-2017 characterizing structure-function relationships of metallochaperones using X-ray crystallography and biochemical techniques. Since 2018, he has trained in lipid biochemistry as a postdoctoral research fellow at St. Jude Children's Research Hospital. Dr. Radka's main research interests center on investigating the mechanistic enzymology and functional roles of metabolites in lipid signaling.



Special Thanks to Session Chairs

Stephen W. White, DPhil
President and Dean, St. Jude Graduate School of Biomedical Sciences
Member, Structural Biology
St. Jude Children's Research Hospital

Raquel Lieberman, PhD
Professor, Chemistry and Biochemistry
Georgia Institute of Technology

Tudor Moldoveanu, PhD
Assistant Member, Structural Biology
St. Jude Children's Research Hospital

Symposium Organizing Committee

Darcie Miller, PhD

Director, X-Ray Crystallography Center

St. Jude Children's Research Hospital, Structural Biology

Lynette Nelson, PhD

Administrative Director

St. Jude Children's Research Hospital, Structural Biology

Anna Acerra, MS

Manager of Digital Business

St. Jude Children's Research Hospital

John P. Rose, PhD

SER-CAT Associate Director of Program

Associate Professor, UGA

Zheng-Qing (Albert) Fu

Senior Protein Crystallographer, SER-CAT

Senior Research Scientist, UGA

Brady O’Boyle

Structural Insights into human acetylcholinesterase active site accommodation of MMB-4 and the latest generation of reactivators

O’Boyle, Brady[1]; McGuire, Jack[1]; Bester, Stephanie[1]; McDonough, John[2]; Myslinski, James[3]; Height, Jude[3]; Pegan, Scott[1,2]

[1]Department of Pharmaceutical and Biomedical Sciences, University of Georgia, [2]U.S. Army Medical Research Institute of Chemical Defense, [3]U.S. Army Futures Command, Combat Capabilities Development Command, Chemical Biological Center, Chemical Sciences Division, Agent Chemistry Branch

ABSTRACT

Acetylcholinesterase (AChE) inhibiting organophosphate based pesticides and nerve agents, are threats to the public due to the rapid onset of severe and often fatal health complications. Recently, the devastating effects and on-going use of nerve agents have been underscored with the death of the brother of Kim Jong Un as well as assassination attempts on Russian nationals. Highlighted by these events and wide use of organophosphate based pesticides worldwide, the need to development more effective therapeutics is salient. Recent efforts to develop new reactivators that can reverse the covalent modification of AChE by organophosphate have been develop. Here we provide new structural insight into the reactivator MMB-4 and others via X-ray crystallographic structures using human AChE. Additionally, we biochemical examine how this new breed of reactivators differ from their predecessors like pralidoxime, HI-6, and HLo-7. These data help to not only evaluate the effectiveness of the next generation of oxime reactivators, but also yields a better understanding of them that paves the way for future reactivator design.

Justin Shepard

The development of betacoronavirus subgroup 2b protease inhibitors through structure-based drug design

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ABSTRACT

For nearly two decades, betacoronaviruses have emerged as formidable pathogens of human consequence as frequent outbreaks have occurred including the 2002 severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1) outbreak, the 2012 Middle East respiratory syndrome (MERS) outbreak, and the ongoing pandemic caused by SARS-CoV-2, or COVID-19. If these spillover events are any indication, these will not be the last coronaviruses to jump from animals to humans. Upon attachment, entry, and uncoating, coronaviruses initially translate two viral polyproteins, which are required to be cleaved into mature proteins to allow viral replication. This cleavage event is mediated by two viral encoded proteases. One of these, papain-like protease (PLpro), also holds a secondary function as an immune suppressant. In short, PLpro deconjugates host immune proteins like ubiquitin and ubiquitin-like interferon stimulated gene product 15 (ISG15) from viral and host proteins. These activities of PLpros assist in allowing the maturation of virions to proceed without host interference. Here, novel drug compounds shown to inhibit PLpros through inhibition assays were crystallized in complex with PLpro from a bat subgroup 2b betacoronavirus, which has a sequence similarity of 82.2% to SARS-2 PLpro, and an even greater similarity to SARS-CoV-1 PLpro. Using this structural information, greater insights into the potency of PLpro inhibitors are revealed providing a foundation for future drug discovery against current and future coronavirus subgroup 2b health threats.

Dayong Zhou

The Native-SAD structure of PA5083 – a 116 residue protein with 1 ordered sulfur was determined to 1.9Å using in-house data.

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ABSTRACT

PA5083 from *Pseudomonas aeruginosa,* a Rid2 (Reactive Intermediate Deaminase) protein known to have broad imine deaminase activity against iminoarginine has been determined to 1.9Å resolution using Native-SAD. The Rid family enzymes are of interest since they play important roles in nutrition, amino acids biosynthesis, mitochondrial maintenance and other biological processes by reducing the accumulation of toxic metabolite intermediates.

The structure determination is noteworthy in that (1) the data were collected in-house (λ = 1.5418) and consisted of a single set of 1440 quarter degree images (total rotation 360°, multiplicity 16.6), (2) the 116 residue enzyme has only two sulfur containing residues (Met 1 and Cys 17) giving a Bijvoet ratio 0.374 assuming Met 1 is disordered (it was), (3) the initial results from *phenix. autosol* gave a “very low” (*autosol*) FOM of 0.16 with R and Rfree values of 0.5049 and 0.5505 respectively and (4) the structure was built from the initial *autosol* phases using several rounds of model building with *phenix.autobuild*. The R and Rfree values for the refined model are 0.200 and 0.233 respectively.

Details of the Native (sulfur atom)-SAD analysis and the PA5083 crystal structure will be presented.

Work supported in part by funds from the University of Georgia Foundation, and the National Institutes of Health (1S10OD021762-01).

John Rose

The crystal structure of selenomethione labeled recombinant (S195A) bovine trypsin reveals no electron density for three surface loops that includes the C191 - C220 disulfide.

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ABSTRACT

Crystals of bovine trypsin (UniProt ID P00760) are used by SER-CAT as a quality control standard for determining beamline optimization and performance. However, a selenomethionine labeled standard for MAD/SAD energy optimization and other studies was also needed. To provide this MAD/SAD standard a S195A mutant of bovine trypsin with selenomethione labeling was expressed, purified and crystalized. Crystals of the recombinant protein had a similar habit and diffraction quality compared to native crystals. An 18 sec data set to 1.5 Å resolution was collected on beamline 22ID at the selenium absorption edge using a Rayonix MX300HS 10 Hz CCD detector.

The data set (99.1% complete) consisting of 180 one-degree images each exposed for 0.1 seconds was collected. Following SER-CAT protocols the data were auto processed using *cmdKylin*. Phases were then generated using *phenix.autosol* and *phenix.autobuild* placed 190 of the 223 amino acids giving R and Rfree values of 0.2122 and 0.2332, respectively. Refinement (3 rounds) of the *auobuilt* model was carried out using *phenix.refine* and converged to give R and Rfree values of 0.1994 and 0.2187, respectively with good stereochemistry.

However, inspection of the refined model *(COOT)* showed that the electron density of three surface loops (totaling 27 residues) was missing with the loss of the C191 - C220 disulfide. The presentation will provide details of the production of selenomethione labeled protein, its crystallization, data collection and structure solution. It will also explore possible causes for the missing loop density.

Work supported in part by funds from the SER-CAT Member Institutions, the University of Georgia Foundation, and the National Institutes of Health (S10 RR25528 & 1S10 RR028976).

Zhongmin Jin

SER-CAT 'Light When You Need It'!

JIN, ZHONGMIN[1,2]; Gonczy, John[1,2]; Fait, James[1,2]; Fu, Zheng-Qing[1,2]; Chrzas, John[1,2]; Rose, John[1,2]; Wang, Bi-Cheng[1,2]

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ABSTRACT

Since 1999, SER-CAT has been working towards the concept of providing its members with a "*Virtual Beamline"*, which could be integrated into their daily workflow much like your X-ray lab down the hall.

We began investigating robotic crystal mounting automation in 2000 with Oceaneering Space Systems. In 2003, a highly modified Berkeley ALS Automounter was installed on 22BM. Using 22BM as a user-based testbed, the beamline and experiment control interface *SERGUI* was continually modified until a reliable, robust and user-friendly system was achieved. In 2006, the SER-CAT *Virtual Beamline* came online providing remote crystal screening and data collection capability on both 22ID (capacity 450 crystals) and 22BM (capacity 96 crystals).

The *SERGUI* beam line control interface allows the direct remote access of SER-CAT beamline from their home labs including: 1) beamline/goniometer optimization, 2) wavelength selection, 3) fluorescence scans, 4) automatic crystal centering and rastering, 5) automated crystal screening, 6) MAD/SAD/Helical data collection and others. Today over 95% of SER-CAT members routinely collect data remotely. SER-CAT has also implemented 12-hour shifts with 16-hours/day and 7-days/week of on-site user support to assist users for their remote accesses from home labs. Multiple Access Time (MAT) shifts have also been made available to SER-CAT members for fast turn-around and flexible data collection capabilities.

SER-CAT also provides its members the on-the-fly automated data processing using XDS, KYLIN and DIALS. HKL2000, XDS, MOSFLM, and DIALS for manual data processing. All user data can be remotely, quickly, reliably and securely downloaded from SER-CAT's Globus/GridFTP archive server to the users' home systems anywhere and anytime.

Work supported by the SER-CAT Member Institutions, University of Georgia Research Foundation, The National Institutes of Health (S10_RR25528 and S10_RR028976) and the Georgia Research Alliance.

Joseph D. Ferrara

Joseph D. Ferrara[1], Amanda Cochran[1], Mark Del Campo[1], Christian R. Göb[2], Pierre Le Maguerès[1], Mathias Meyer[3], Horst Puschmann[4], Christian Schürmann[2], Alexandra Stanley[2], Paul N. Swepston[1], Akhilesh Tripathi[1], Fraser White[2], Jakub Wojciechowski[2]

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ABSTRACT

In order to address the loss of crystallographic training opportunities resulting from the cancellation of conventional schools around the world due to the COVID-19 pandemic we have started an online crystallography school with live lectures and live Q&A using Zoom Webinar. Since we were trying to reach a large audience in a relatively short period, we have limited the school to ten 1 hour lectures covering practical aspects of small molecule crystallography including data collection, data processing and structure solution. In the school, we also covered some advanced topics that students commonly see in their work: absolute structure determination, twinning and disorder. To round out the education, we provided lectures on macromolecular crystallography and powder diffraction. So that students might practice on their own, we used freely available data reduction and structure solution software, as well as data sets with which to practice. To give students credit for course completion, we provided an online exam and an electronic certificate of completion.

At this poster, we will provide some insight into the issues of holding lectures with up to 750 students of very diverse backgrounds and review the efficacy of the school in teaching crystallography for the two cohorts of students.

Pierre Le Maguerès

New Tools for Structural Biology from Rigaku

Pierre Le Maguerès[1], Mark Del Campo[1], Christian R. Göb[2], Christian Schürmann[2], Jakub Wojciechowski[2], Joseph D. Ferrara[1] ,Fraser White[2]

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ABSTRACT

At Rigaku Oxford Diffraction we are constantly striving to improve our products. In the past year, we have introduced a new HPC detector, the HyPix-Arc 100°, a new laboratory automation system, Flow, and a new system for solution scattering, the BioSAXS nano. We have also introduced a new DW optic for enhanced performance for both protein and small molecule crystallography. We will explore these new products in this poster.

Benjamin Apker

From Art to Science: Advanced Cryocooling Technology for Biomolecular Cryocrystallography

Benjamin Apker[1a], Joyce Frank[1b], David Closs[1c], and Robert Thorne[1d]

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ABSTRACT

In biomolecular structure determination by X-ray crystallography, crystals are cooled to cryogenic temperatures, typically by manual plunge cooling in liquid nitrogen or by manual insertion in a cold nitrogen gas stream. Internal ice formation can destroy or severely degrade protein lattice diffraction. Ice forms rapidly in surface solvent, from moisture in ambient air that condenses on crystals during handling, and in the liquid nitrogen used for cooling and storage. Roughly 20% of PDB deposited data sets – and presumably a much larger fraction of all data sets - show structure factor errors consistent with ice contamination. Even when ice does not form, crystal mosaicities always worsen and diffraction resolution sometimes degrades.

Modest and highly variable cooling rates achieved in hand plunging can lead to substantial crystal-to-crystal nonisomorphism and substantial changes in the protein's conformation from its biologically relevant form. To prevent ice formation, crystals are grown or soaked in solutions containing cryoprotectants. Cryoprotectants can displace weakly bound ligands and complicate interpretation of weak density in electron density maps that may be associated with functionally relevant minor conformers, and reduce overall electron density contrast between solvent and protein. These problems are especially acute for crystals of membrane proteins and large complexes, which tend to have high solvent contents, large solvent cavities and more labile structures, and which often require larger cryoprotectant concentrations to prevent ice formation.

To address these problems, MiTeGen has developed NANUQ™, the world's most advanced instrument for crystal cryocooling. This instrument delivers the largest cooling rates possible using liquid nitrogen – roughly 50,000 K/s for 50 micrometer crystals, or roughly 100 times larger than obtained in gas stream cooling – and also allows variable rate cooling at rates as low as 10 K/s. Ultrafast cooling reduces or eliminates the need for cryoprotectants, and more accurately captures the biologically relevant structure. Smaller cooling rates can facilitate internal solvent redistribution and biomolecular relaxations that can in some cases improve crystal order, especially when using large crystals. Cooling reproducibility is dramatically improved. Automated sample handling and storage eliminates crystal damage and loss and ice formation that occur during manual handling. Get the most out of your crystals, get everyone in your lab using the same reliable and reproducible protocols, and maximize the throughput of your crystallography pipeline using NANUQ's proven technology.

Thank you.

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Structural Biology