SER-CAT

Light when YOU need it!

Southeast Regional Collaborative Access Team

Operated by the University of Georgia

2022 (19th) Annual SER-CAT Structural Biology Symposium
20th Anniversary Celebration Symposium of the SER-CAT Operation

Abstract Book

October 20, 2022

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2022 (19th) Annual SER-CAT Structural Biology Symposium October 20, 2022

Eastern Time	Virtual Events, Session Chairs and Speackers	
8:50 - 9:00 am (10m)	Log in	
9:00 - 9:05 am (5 m)	Welcome	B.C. Wang, UGA
9:05 - 9:15 am (10 m)	Opening Remarks	Karen JL Burg, UGA
9:15 - 9:25 am (10 m)	Opening Remarks	Susan Shows & Mike Cassidy, GRA
SESSION 1	Interesting Structures & Methods - 1	Session Chair: John Chrzas
9:25 - 9:50 am (25 m)	Towards an HIV-1 vaccine: 20+ years of SER-CAT!	Peter Kwong, NIH/VRC [E]
9:50 - 10:15 am (25 m)	The writer, reader and eraser of DNA methylation marks, a journey together with SERCAT	Xiaodong Cheng, MD Anderson
10:15 - 10:40 am (25 m)	The SER-CAT Virtual Beamline: Providing light when YOU need it in your home lab	Zhongmin Jin, SER-CAT/UGA
10:40 - 10:45 am (5m)	Break	
	Interesting Structures & Methods- 2	Session Chair: Zhongmin Jin
10:45- 11:10 am (25 m)	Insights into O antigen polysaccharide secretion - Substrate-bound conformations of the WzmWzt ABC transporter	Jochen Zimmer, UVA
11:10 - 11:35 am (25 m)	AlphaFold2 is Now Available at SER-CAT, Complementing Your Crystallographic Analyses and Function Research	Albert Fu, SER-CAT/UGA
11:35 - 12:00 pm (25 m)	Introduction to the NYX beamline at NSLS-II	Kevin Battaile, NYX/BNL
12:00 - 12:25 pm (25 m)	Introduction to the Southeastern Center for Microscopy of Macromolecular Machines at FSU	Scott Stagg, FSU
12:25 - 1:20 pm (55 M)	Lunch Break and Poster Session	Session Chair: Unmesh Chinte
1:20 -1:25 pm (5 m)	Log In	
SESSION 2	APS and SER-CAT	Session Chair: John Rose
1:25 - 1:30 pm (5 M)	Introducing APS Representative	B.C. Wang, UGA
1:30 - 2:00 pm (30 m)	The Upcoming APS Upgrade	Denny Mills, APS/ANL
2:00 - 2:30 pm (30 m)	Structural insights into immune recognition in cancer immunology and immunotherapy	Brian Baker, Notre Dame Outstanding Science Award Winner
2:30 - 2:55 pm (25 m)	Transcriptional repression of the hig toxin-antitoxin locus by the HigBA toxin-antitoxin complex	Ian Pavelich, Emory Young Investigator Award Winner
2:55 -3:20 pm (25 M)	SER-CAT Operations and Dark Period/Upgrade	John Rose & John Chrzas, UGA
3:20 - 3:30 pm (10 m)	Break	
SESSION 3	20 Years of Light When You Need It	Session Chair: B.C. Wang
3:30 - 3:55 pm (25 M)	Impact of the SER-CAT membership on structural biology research within the Intramural Research Program of the NIH	Alex Wlodawer, NIH/NCI [E]
3:55 - 4:20 pm (25 M)	Construction of SER-CAT Beamlines - My 7th Beamline Project	Gerold Rosenbaum, UGA
4:20 - 4:45 pm (25M)	SER-CAT's Beginning, Support and Impact - Thank You All	B.C. Wang, UGA
4:45 - 4:50 pm (5 m)	Break	
SESSION 4	Poster Session Highlights and Awards	Session Chair: Palani Kandavelu
4:50 - 5:20 pm (30 m)	Poster talks 5 mim each	Presenters of Selected Posters
5:20 -5:30 pm (10 m)	Presentation of Poster Awards	John Rose, UGA
5:30 - 5:35 pm (5 m)	Closing Remarks	John Rose, UGA

20 Years and Counting: Working with SER-CAT To Obtain an HIV-1 Vaccine

Peter D. Kwong, Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892.

Since its founding in 2001, the Structural Biology Section of the Vaccine Research Center has sought to apply structural biology to the development of antibody-based vaccines – especially a vaccine against HIV-1. As demonstrated by the structure-based design of effective therapeutics against HIV-1, atomic-level structures allow organic chemistry to be applied directly to the manipulation of biological interactions. But is atomic-level "design" of vaccines possible?

We have used SER-CAT collected data almost exclusively over the past 20 years to provide the requite structural data. First, we have sought to understand how HIV-1 is able to evade the immune response – obtaining structures of the critical vaccine antigen, the HIV-1 envelope glycoprotein trimer. Second, we have pursued an antibody-to-vaccine approach, embedded within an informatics-based philosophy, to obtain insight into sites of vulnerability that might be most amendable to vaccine design. Third, we have combined HIV structural insights with neutralizing antibody insights to create immunogens capable of inducing broadly neutralizing antibodies.

In this talk, I will highlight specific SER-CAT collected structures, which have spurred vaccine development.

Support for this work was provided by the Intramural Research Program of the Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health.

The writer, reader and eraser of DNA methylation marks, a journey together with SERCAT

Xiaodong Cheng and John R. Horton

Department of Epigenetics and Molecular Carcinogenesis, University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA

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The establishment (by writers), detection (by readers), and alteration or elimination (by erasers) of epigenetic DNA modifications are essential for controlling gene expression ranging from bacteria to mammals. The DNA methylations occurring at cytosine and adenine are carried out by *S*-adenosyl-L-methionine-dependent methyltransferases.

We will summarize our work, resulting from the use of SERCAT for the last 20 years, and present our recent data on DNA methylation and its associated ageing somatic mutations.

Work supported currently by the U.S. National Institutes of Health (R35GM134744), the Cancer Prevention and Research Institute of Texas (RR160029), and funds from the Texas Tobacco Settlement – Molecular Mechanisms of Tobacco Carcinogenesis. X.C. is a CPRIT Scholar in Cancer Research.

The SER-CAT Virtual Beamline: Providing light when YOU need it in your home lab

Zhongmin Jin, John Chrzas, John Gonczy, James Fait, Zheng-Qing "Albert" Fu, Palani Kandavelu, Unmesh Chinte, Michael Molitsky, Roderick Salazar, Norma Duke, John Rose and B.C. Wang.
Southeast Regional Collaborative Access Team, Advanced Photon Source and the Department of Biochemistry and Molecular Biology University of Georgia, Athens, GA 30602.

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". After 20 years of beamline operation and continuous development and upgrade, SER-CAT currently provides full services to users including remote access, automatic beamline motion control, sample mounting and automatic alignment, automatic sample screening and data collection, automatic parallel data processing, data archiving and transferring, mail-in crystallography and 16-hour per day staff support.

SER-CAT beamlines 22ID and 22BM were designed and built for remote operation. The experiment control interface SERGUI had been 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 and 22BM. Today, SER-CAT members have flexibility to schedule for remote beamline access as needed and virtual beamline concept has become a reality and highly productive. For the past five years, SER-CAT's 22-ID beamline has been the top producer of Protein Data Bank depositions from the APS, according to the BioSync report.

An overview of SER-CAT's "Virtual Beamline" infrastructure and development including remote access, robotics, beamline motion control, software integration, automated sample screening and data processing will be described and discussed.

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.

Insights into O antigen polysaccharide secretion - substrate bound conformations of the WzmWzt ABC transporter

Jochen Zimmer, Department of Molecular Physics and Biological Physics, University of Virginia School of Medicine, Charlottesville, VA 22903

O antigens are ubiquitous protective extensions of lipopolysaccharides in the extracellular leaflet of the Gram-negative outer membrane. Following biosynthesis in the cytosol, the lipid-linked polysaccharide is transported to the periplasm by the WzmWzt ABC transporter. Often, O antigen secretion requires the chemical modification of its elongating terminus, which the transporter recognizes via a carbohydrate-binding domain (CBD). We identified that methylated mannose or rhamnose caps the O antigen of *Aquifex aeolicu*s. Crystal and cryo electron microscopy structures reveal how WzmWzt recognizes this cap between its carbohydrate and nucleotide-binding domains in a nucleotide-free state. ATP binding induces drastic conformational changes of its CBD, terminating interactions with the O antigen. Combined with mutagenesis and functional analyses, our results elucidate critical steps in the recognition and translocation of polysaccharides by ABC transporters.

The AlphaFold2 is Now Available at SER-CAT, Complementing Your Crystallographic Analyses and Function Research

Zheng-Qing Fu, Zhongmin Jin, John Chrzas, Michael Molitsky, James Fait, John Rose, Bi-Cheng Wang, Department of Biochemistry & Molecular Biology, University of Georgia, Athens, GA 30602 and SER-CAT, APS, Argonne National Lab, Argonne, IL 60439.

Advances in structure prediction by Alphafold2 improve model accuracy significantly to such a level (Jumper *et al.*: Nature **596**:583-589, July 15, 2021; Evans *et al.*: Protein complex prediction with AlphaFold-Multimer. bioRxiv 2021, https://doi.org/10.1101/2021.10.04.463034) that it could potentially provide a simple way to phase X-ray diffraction data via molecular replacement in cases where a homologous X-ray structure is unavailable. Since the Alphafold2 model is based on the target protein's sequence, the approach addresses model bias error associated with the molecular replacement method. The approach would also significantly increase the efficiency of structure production since it is not dependent on isomorphous heavy atom derivatives or anomalous scatterers (*e.g.,* selenomethionine labeling). More recently, an AI (Artificial Intelligence)-based protein interaction screening and identification method has been developed (Fu *et al.*: Int. J. Mol. Sci. 2022, *23*, 11685) which could significantly speed up the searching process for unknown specific protein-protein bindings by prioritizing a long list of potential binding partners, extending the application of AlphaFold2 far beyond structure predictions.

In this talk, we 1) announce that a client-server program developed at SER-CAT will be open to all SER-CAT users and 2) discuss the use of Alphafold2 for screening potential specific protein-protein bindings.

Work supported by the SER-CAT Member Institutions (see www.ser-cat.org), University of Georgia Research Foundation and the Georgia Research Alliance.

Introduction to the NYX beamline at NSLS-II

Kevin Battaile
New York Structural Biology Center

NYX is a protein crystallography beamline operated by the New York Structural Biology Center which is the intellectual offspring of the X4 beamlines at the former NSLS. NYX is one of five structural biology beamlines at NSLS-II encompassing solution scattering and x-ray footprinting in addition to protein crystallography. The design goal of NYX is high energy resolution to optimize anomalous scattering experiments with a small beam focus. In 2022 we have installed an Eiger2 XE 9M detector which has demonstrated excellent performance. In collaboration with SER-CAT staff, we have successfully performed *de novo* phasing experiments using trypsin and lysozyme on data collected at the selenium absorption edge. In August we commissioned an Irelec ISARA2 sample mounting robot which we expect to be highly reliable and give very fast sample exchanges. In early 2023 we are planning on installing an Arinax MD2s diffractometer. Data from NYX is stored on a 3.5 PB file system and can be downloaded via Globus. The combination of the equipment upgrades we have performed and are planning should make NYX a high-reliability, high-throughput beamline while still being capable of collecting excellent quality data.

The Southeastern Center for Microscope of Macromolecular Machines at FSU

Scott Stagg, Department of Biological Sciences, Florida State University, Tallahassee, FL 32306

The SECM4 at Florida State University (FSU) will be a service center that will enable sample preparation and cryogenic electron microscopy (cryo-EM) imaging of specimens for high-resolution biomolecular structure determination. The center will be led by Dr. Scott Stagg and Dr. Kenneth Taylor and will feature two staff members with complimentary expertise. This will be the second generation of the SECM4. The first generation provided high-resolution cryo-EM data collection for cryo-EM experts. In the new generation of the SECM4, we are expanding the scope of the resource by offering a large number of new services including cryo-EM specimen optimization, specimen preparation, specimen screening, high-resolution data collection, routine single particle data analysis, and training for all aspects of cryo-EM from specimen preparation to processing. We will target users in the greater Southeast to be clients for the center, specifically focusing expanding into the underserved IDeA states that have not benefitted from the current explosion in cryo-EM due to the extraordinary costs that serve as an entry barrier.

The center will: 1) enable users at universities across the Southeast to gain entry the cryo-EM field without making multi-million dollar investments in instrumentation, 2) offer screening and specimen optimization services, that will address the biggest bottleneck in cryo-EM right now, which is preparation of cryo-EM samples that will reconstruct to high-resolution, and 3) by offering in person training that will enable interested users to gain entry into the field of cryo-EM without having to seek out a collaboration with an already saturated cryo-EM expert.

The Upcoming APS Upgrade

Denny Mills

APS/ANL

Structural insights into immune recognition in cancer immunology and immunotherapy

Brian M. Baker
Department of Chemistry and Biochemistry
University of Notre Dame

T cells of the immune system recognize peptide antigens bound and presented by major histocompatibility complex (MHC) proteins. Recognition occurs via the T cell receptor (TCR), a specialized immune receptor with structural features similar to antibody Fab fragments. TCR recognition of peptide/MHC complexes is at the heart of the cellular immune system. While the role of cellular immunity in immunity against viruses and other pathogens has been studied for decades, only recently has the importance of cellular immunity in cancer become well appreciated. With this appreciation, considerable effort has been directed towards new, immune-based cancer immunotherapies. Our laboratory has participated in this work through our basic studies of TCR specificity and cross-reactivity; studies of how TCRs recognize tumor antigens, including neoantigens generated by cancer-associated mutations; and efforts to identify and engineer more effective TCRs for cancer immunotherapy.

Transcriptional repression of the hig toxin-antitoxin locus by the HigBA toxin-antitoxin complex

Ian Pavelich, Department of Biochemistry, Emory University, Atlanta, GA 30322

Regulation of ubiquitous bacterial type II toxin-antitoxin (TA) gene pairs occurs via a negative feedback loop whereby their expression is typically responsive to changing levels of toxins at the transcriptional level. While this mechanism can explain how certain TA complexes are regulated, accumulating evidence suggests diversity in this regulation. One system for which the negative feedback loop is not well defined is the plasmid-encoded HigBHigA TA pair originally identified in a post-operative infection with antibiotic resistant *Proteus vulgaris*. In contrast to other type II TA modules, each *hig* operator functions independently and excess toxin does not contribute to increased transcription *in vivo*. Structures of two different oligomeric complexes of HigBHigA bound to its operator DNA reveal similar interactions are maintained suggesting plasticity in how *hig* is repressed. Consistent with this result, molecular dynamic simulations reveal both oligomeric states exhibit similar dynamics. Further, engineering a dedicated trimeric HigBHigA complex does not regulate transcriptional repression. We propose that HigBHigA functions via a simple on/off transcriptional switch regulated by antitoxin proteolysis rather than traditional methods. The present studies thus expand the known diversity of how these abundant bacterial protein pairs are regulated.

The SER-CAT Upgrade Plan

John Chrzas, Michael Molitsky**,** Zhongmin Jin**,** Zheng-Qing Fu, James Fait, John Rose, Bi-Cheng Wang, Department of Biochemistry & Molecular Biology, University of Georgia, Athens, GA 30602 and SER-CAT, APS, Argonne National Lab, Argonne, IL 60439.

The APS will be undergoing a major facility upgrade beginning April 17, 2023 (https://www.aps.anl.gov/APS-Upgrade). The APS will not be providing x-rays for the following year. During this 1-year "Dark Period", the SER-CAT beamlines will also be undergoing their own upgrade (SER-CATU). In this talk, we will present the upgrade plans for the current undulator beamline (22ID-D) and the plans for a new fixed energy undulator beamline (22ID-E).

Work supported by the SER-CAT Member Institutions (see www.ser-cat.org), University of Georgia Research Foundation, and the Georgia Research Alliance.

The SER-CAT Program: Supporting our users during the APS Dark Period

John P. Rose, John Chrzas, Zhongmin Jin, Zheng-Qing Fu, Palani Kandavelu, Unmesh Chinte, and Bi-Cheng Wang, University of Georgia, Athens, GA 30602 and SER-CAT Sector 22, Advanced Photon Source, Argonne National Laboratory, Argonne, IL 60439

SER-CAT, Sector 22 is a member-funded research resource for scientists in the southeastern and other regions of the U.S., including ~170 NIH-funded research groups. SER-CAT's virtual beamline 22ID ranks as the top U.S. beamline in terms of PDB deposits (biosync.sbkb.org) over the past five years, with over 98% of all SER-CAT data currently being collected from the researcher's home institution. This throughput is in part based on APS undulators.

The undulator X-rays provided by the Advanced Photon Source (APS) and its extensive user base currently account for over 55% of all Protein Data Bank (PDB) publications coming from the United States (data taken from biosync.sbkb.org). Thus, the APS "dark period" scheduled for April 2023 will have a significant impact on the nation's structural biology community. This will also be the first time that a light source producing a majority of the nation's PDB entries has gone dark, leaving hundreds of APS user groups looking for alternate beamlines to support their research needs. Finding beam time during the "dark period" will be challenging since few facilities in the U.S. can provide undulator A intensity.

This presentation will provide an overview of SER-CAT's plans for user support during the APS Dark Period. At last year's Symposium, we announced a partnership with the ALS for data collection on beamlines 5.0.1 and 5.0.2 and invited Marc Allaire to give an overview of the beamlines and the b4 data collection GUI. This year we are pleased to add a partnership with the NYSBC for data collection on the NYX beamline 19-ID at NSLS II and have invited Kevin Battaile to introduce the beamline and its operation.

An accompanying presentation by John Chrzas will present SER-CAT facility upgrade plans.

Work supported by the SER-CAT Member Institutions (see www.ser-cat.org), University of Georgia Research Foundation, and the Georgia Research Alliance.

Impact of the SER-CAT membership on structural biology research within the Intramural Research Program of the NIIH

Alex Wlodawer

NIH/NCI

Construction of SER-CAT Beamlines – My 7th and 8th Beamlines

Gerd Rosenbaum, University of Georgia (retired), Sterling Engineering, Inc., Westchester, IL 60154

The proposal for construction of SER-CAT's insertion device and bending magnet beamlines submitted to the Proposal Selection Board was based on essentially duplicating the beamlines of the Structural Biology Center (SBC) I had just built and successfully commissioned since the targeted user community was the same. Since the SBC was funded by government money, the SBC design drawings were available to UGA for no charge with the proviso that improvements to designs we planned for SER-CAT out of experience at the SBC would be freely available to ANL. This arrangement saved SER-CAT at least $500,000 in design costs and six months of time.

All construction projects face a man-power problem: you need highly qualified engineers and technicians with special skills and training, but only for 1 or 2 years – you don't find any. The solution: either one is part of a large organization and can tap into its resources or one contracts third party services. We retained the services of Larry Rock and his team with whom I had worked closely on the design of the NSLS X9 beamline and the X-ray optics and endstation design of the SBC. They would help with design upgrades for SER-CAT and recast hundreds of drawings from ANL drawing into SER-CAT drawings. They also handled the fabrication of components: finding shops and parts vendors, got quotes, sent out orders and did initial quality inspection of fabricated parts. For assembly of components on the APS floor, we planned to lease experienced technicians from the APS which needs a large pool of highly trained technicians for the intense demands during shut-downs but can farm out technicians during the lesser demands during runs. Hutches and shielding was APS's domain. We provided the drawings, APS took control of the rest.

This allowed to keep the Construction team very small: myself, John Gonczy (project engineer) and John Unik (Project Administrator). About 1 year into the project, Jim Fait (programmer) joined the team.

John Gonczy had worked with me assembling the SBC beamlines. Then and now he was responsible for the layout and details of the hutches and utilities, and he supervised the assembly of components on the floor be the leased technicians. He also worked with me on the design of upgrades and new components. John Unik handled all the communications with APS and ANL and also with SER-CAT headquarters at the UGA. Having been an ANL division director before his retirement he knew the inner workings of ANL and how to move things forward. He was the shield that allowed the rest of the team to concentrate fully on building the beamlines. Jim Fait took on programming the beamline control and data acquisition software and also selecting and installing computers and beamline control hardware.

Building the SER-CAT beamlines was the smoothest construction I ever had. No weekly meetings (as John Gonczy liked to point out); because of the small size and working in one office, communication was a daily natural. Since the SER-CAT Director was far away, we established weekly report going to B.C. John Unik would collect weekly reports from each team member, added administrative issues and composed the weekly report that should give B.C. the progress and status of the project.

At the Dedication of SER-CAT in 2002, I handed the beamline over to the newly hired beamline manager John Chrzas.

SER-CAT's Beginning, Support, and Impact - Thank You All

Bi-Cheng (B.C.) Wang, Department of Biochemistry and Molecular Biology
University of Georgia, Athens, GA, 30602

The Advanced Photon Source (APS) at Argonne National Laboratory (in Lemont, Illinois) is a storage-ring-based high-energy X-ray light source facility. It is one of five X-ray light sources owned and funded by the US Department of Energy Office of Science. The APS saw its first light on March 26, 1995 - Wikipedia

The idea of forming a Collaborative Access Team (CAT) at the APS was conceived during the last day of the Siemens Area Detector Users Group Meeting, hosted at UGA on April 17-19, 1997. In June, we held a meeting at UGA discussing submitting a proposal to NSF for building the first of the two synchrotron beamlines beamline (an ID and an MB). More than 30 macromolecular crystallographers in the Southeast attended. By September 1997, Georgia Research Alliance (GRA) committed a matching fund of $1.5M for the Georgia institutions. By the end of that year, we raised $4.5M, including those from the participating institutions, and submitted a Letter of Intent for the beamline project to the APS in November.

From 11/97 to 02/99, two proposals were submitted to NSF but not funded for various reasons. But during that period, we continued the fundraising, and over $16M was raised during the first three years. At the same time, we announced a call for proposals for the construction of SER-CAT beamline with certain specifications. Four proposals were received in 1998 and reviewed by an Advisory Committee of eminent beamline scientists in the US and Europe. On August 2, 1998, the Committee recommended Gerold Rosenbaum and his colleagues to be the SER-CAT Construction Team. On August 31, a full construction proposal by Rosenbaum Team was submitted to APS and approved in early 1999.

On March 12, 1999, SER-CAT institutions signed a MOU with the APS. The construction of the 22-ID beamline officially began on June 15, 1999. The 22-ID beamline took more than three years to complete. We held a dedication ceremony of 22-ID on the morning of October 18, 2002, and a half-day symposium in the afternoon titled: Data Collection: Current and Future. We also announced that John Chrzas would be the SER-CAT Beamline Manager in future operations.

With the continued efforts by the then newly recruited, dedicated management team SER-CAT started the practice of mail-in crystallographic services and shortly added the remote access with automounting of samples (GRA provided an initial grant). Today the SER-CAT's virtual beamline 22ID is the top US ID beamline in terms of the total number of PDB deposits (biosync.sbkb.org) in the last five years.

In this presentation, I will share some slides on the earlier days, beamtime construction, and other SER-CAT milestones that have brought us to this celebration. Thank You All.