The
SER-CAT SPECTRUM

Biannual Newsletter of the Southeast Regional Collaborative Access Team · Vol. 10, No. 1· Summer 2012

Director's Message

Bi-Cheng Wang

Dear Colleagues,

I hope you are having a great summer! I am happy to report that we had a great gathering during the 9th SER-CAT Symposium in Lexington, Kentucky on March 16, 2012, which was hosted and organized by Professors David Rodgers and Craig Vander Kooi of UKY. David and Craig, thanks for giving us such a great scientific program and an enjoyable evening at the Kentucky Horse Park. Thanks also to Dr. Denny Mills of APS for an overview of the APS upgrade and to the speakers for reporting on their advanced work at the symposium. Congratulations also to Dr. Peter Kwong and Dr. Jason Stagno for receiving the SER-CAT Scientific Awards (page 2), and Dr. Stefan Gajewski for receiving the Best Poster Award.

Plans for next year's Symposium are well underway. Professor Hong Li at the Florida State University will host the event on March 15, 2013. Please mark your calendars.

This year is SER-CAT's 14th year, but it is the 10th year of operations since commissioning in 2002. We are pleased to note that a most recent (7/12/2012) Nature paper by NIH PI Dr. Wei Yang marks the 1900th SER-CAT structure deposited in the PDB (page 4). Congratulations Wei!

Although the SER-CAT facility has been very successful (for example, in 2011, 105 structures from 22BM and 202 structures from 22ID were deposited in the PDB. We feel it is time to plan for a more capable facility with added new services to meet the expanding needs of our users for the next decade. A Forward-Looking-Task-Force has been established to study/implement appropriate upgrades. We value the inputs of our users and welcome your suggestions when you file your after-the-run report. Also, please forward your suggestions directly to my attention.

I hope that you will enjoy reading about these and other highlights in this issue of *The SER-CAT Spectrum*.

Best wishes, B.C.

10th SER-CAT Symposium and Board Meeting March 15-16, 2013

Florida State University TALLASSEE, FL

Hosted by Professor HONG LI

Details concerning registration and meeting information will be posted soon on SER-CAT's website.

Nominations, Please!

Nominations for the two annual SER-CAT Awards are requested. Please send your nominations for the 2012 SER-CAT Young Investigator Award and/or the Outstanding Science Award to **John Rose** before January 10, 2013. For detailed information, click on one of the links below:

2012 SER-CAT Young Investigator Award

2012 SER-CAT Outstanding Science Award

or

Send John Rose an email.

INSIDE THIS ISSUE:

Director's Message	1	Where Are They Now?	5
SER-CAT Award Nominations	1	SER-CAT Facility Update	6
9th SER-CAT SYMPOSIUM	2	SER-CAT Undergraduate Research	7
SER-CAT 1900th PDB Entry	4	SER-CAT Information	8

9th SER-CAT Symposium
by Prof. John Rose

The symposium began on the morning of March 6, 2012 with welcoming remarks from the University of Kentucky Associate Vice President for Research Dr. Martha Peterson followed by a welcome message from the meeting hosts Profs. David Rodgers and Peter Vander Kooi.

SESSION 1 – Chair Dr. Jonathan Wagner (University of Kentucky). The speakers in this session were Prof. Michael Wiener (University of Virginia), Prof. Christopher Davies (Medical University of South Carolina) and Dr. Peter Kwong (National Institute of Allergy and Infectious Diseases/NIH), 2012's SER-CAT Outstanding Science Award winner.

Prof. Wiener's title was "Going for baroque: TonB-dependent outer membrane active transport". He explained that in Gram-negative bacteria the outer membrane active transport system has three main components: a porin like outer membrane transport protein, an inner membrane TonB multiprotein complex which serves as the motor and the inner membrane itself which provides the protonmotive force that drives active transport. Dr. Wiener then presented structural and functional results on both the outer membrane transporter and the inner membrane TonB motor that provide intriguing insight into the manner by which this unique system functions.

Prof. Davies' title was "Understanding antibiotic resistance at the structural level". He and his group are studying antibiotic resistance in *Neisseria gonorrhoeae*, which is a growing health concern. Dr. Davies reported on his studies on mutations of penicillin-binding protein 2 (PBP 2) in terms of kinetics and crystal structure.

SER-CAT Outstanding Science Award Lecture: Dr. Peter Kwong - Dr. Kwong's title was "HIV-1 vaccine design with HIV-1 Env structures, broadly neutralizing antibodies, and SER-CAT." Dr. Kwong heads a group targeting the HIV 1 glycoprotein gp120 as a possible candidate for vaccine design. Key to this undertaking is knowledge of the interaction of HIV-1 immunogen with antibody. Dr. Kwong then presented his groundbreaking work on characterizing the atomic-level interactions between HIV 1 gp120 and the potent neutralizing antibody PG9. For further details please see McLellan, J.S. et al. (2011) Nature, V480: 336-343.

SESSION 2 – Chair Dr. Matthew Parker (University of Kentucky). The speakers in this session were Dr. Jason Stagno (National Cancer Institute, NIH), the winner of the 2012 SER-CAT Young Investigator Award, Prof. Samuel Bouyian, (University of Missouri at Kansas City) and Dr. Guoxing Fu, (Georgia State University).

SER-CAT Young Investigator Award Lecture: Dr. Jason Stagno (National Cancer Institute, NIH). The title of Dr. Stagno's presentation was "Structural basis for RNA recognition by NusB and NusE in the initiation of transcription antitermination." Dr. Stagno reported the crystal structure of a NusB-NusE-dsRNA complex that is important for processive transcription antitermination. The structure revealed a new BoxB dsRNA-binding site consistent with the structural features of the classical lambda antitermination site. The data presented together with other known antitermination factor interactions suggests a specific model for antitermination complex assembly. For further discussion, see Nucleic Acids Res. (2011) V39: 7803–7815.

Prof. Bouyain's title was "Protein tyrosine phosphatases and contactins in nervous system development". Dr. Bouyain's group is investigating the biochemical and structural analyses of receptor protein tyrosine phosphatase zeta (PTPRZ) and its homolog receptor protein tyrosine phosphatase gamma (PTPRG). He reported the crystal structures of the carbonic anhydrase-like domains of PTPRZ and PTPRG in complex with fragments of contactin family members that suggest that PTPRG, PTPRZ and their interactions with contactins may play an important role in the adhesive interactions that underlie the construction of neural networks.

Dr. Fu's title was "Conformational changes and substrate recognition in *Pseudomonas aeruginosa* D-arginine dehydrogenase". Dr. Fu presented the first X-ray crystal structures of arginine dehydrogenase (DADH) at 1.06Å resolution and its complexes with iminoarginine and iminohistidine at 1.30 Å resolution. The crystal structures of the DADH complexes show two distinct ligand binding modes that are consistent with the 1000-fold difference in the kcat/Km values for d-arginine and d-histidine. The binding site plasticity, supported by observed kinetic data, suggests that the enzyme has relatively broad substrate specificity, being able to oxidize positively charged and large hydrophobic d-amino acids.

SESSION 3 – Chair: Prof. Bi-Cheng Wang (University of Georgia). The speakers in this session were Dr. Denny Mills (Advanced Photon Source, Argonne National Laboratory) and Prof. Peixuan Guo (University of Kentucky).

Dr. Mills' title was "APS-Update". Dr. Mills gave an update on the ongoing $350M APS Upgrade, which will continue over the next 5 years. He also told us that the APS has a new Director, Dr. Brian Stevenson and that Keith Moffatt has been appointed Senior Advisor for Life Sciences at the APS.

Prof. Guo's title was "Application of nanotechnology in macromolecule crystallization". Dr. Guo presented an interesting talk on the application of nanotechnology to RNA crystallization. Topics covered included: bottom-up self-assembly; the use of scaffold motifs; ribozyme processing; use of sticky ends and palindrome sequences; formation of unique oligomer complexes; formation of patterned arrays to permute crystallization; methods to control of twisting angle; and the formation of 1D and 2D sheets.

SESSION 4 – Chair: Mr. David Meekins (University of Kentucky). The speakers in this session were Prof. Bi-Cheng Wang (SER-CAT & University of Georgia) and Dr. John Chrzas (SER-CAT & University of Georgia).

Prof. Wang's presentation was entitled "Forward-looking possibilities for X-ray sources having optimized extended wavelength capabilities." His presentation outlined the opportunities

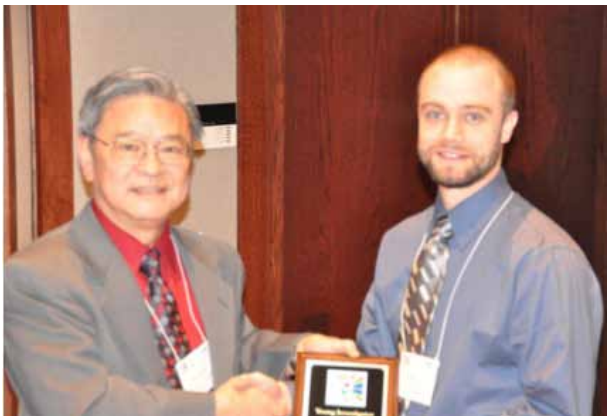
and community interest in having a dedicated and optimized extended X-ray beamline in the US. Several potential applications of such a beamline were presented including light element-SAD phasing (e.g. S-SAD), heavy element-SAD (e.g. Xe-SAD/MAD phasing) and the positive identification of bound metals or surface ions based on the wavelength dependency of their anomalous scattering profile.

Dr. Chrzas' title was "SER-CAT facility update." Dr. Chrzas' presentation focused on recent upgrades to SER-CAT hardware and software. Topics covered included the delivery status of the new Rayonix MX300HS detector (January 2013), improvements to the SER-CAT network and computing infrastructure (10 GB internet connections, new 1.1 Tflop computer cluster and a 100 Tbyte data storage system), a kappa upgrade to the MD2 (available upon request), expanded capacity (430 crystals) of the BAM-S crystal storage dewars. Dr. Chrzas also demonstrated, via remote access to SER-CAT, two new features of SER-GUI: diffraction based crystal centering and helical data collection.

POSTER SESSION – Chair Peter Vander Kooi (University of Kentucky). The poster session was buzzing with people presenting their work. As part of this year's SERCAT Symposium activities, a prize was awarded for the best poster presentation. This years prize was awarded to Stefan Gajewski, St. Jude Children's Research Hospital, for his poster entitled, "Structural transitions within an intrinsically flexible protein captured at low resolution", which was deemed the best of the 23 posters presented by the selection committee.

EVENING SESSION – The evening dinner session was hosted by the Kentucky Horse Park, where guests were invited to wander the Museum of the Horse and take in a very informative lecture in the Hall of Champions on Kentucky's prizewinning horses. The local band "Love, Peace and Chicken Grease" also entertained guests during the evening program.

Dr. Peter Kwong (r) receives the SER-CAT Outstanding Science Award from Prof. B. C. Wang



Dr. Jason Stagno (r) receives the SER-CAT Young Investigator Award from Prof. B. C. Wang

Mr. Stefan Gajewski (l) receives the Outstanding Poster Prize from Dr. Peter Vander Kooi

Participants of the Ninth Annual SER-CAT Symposium

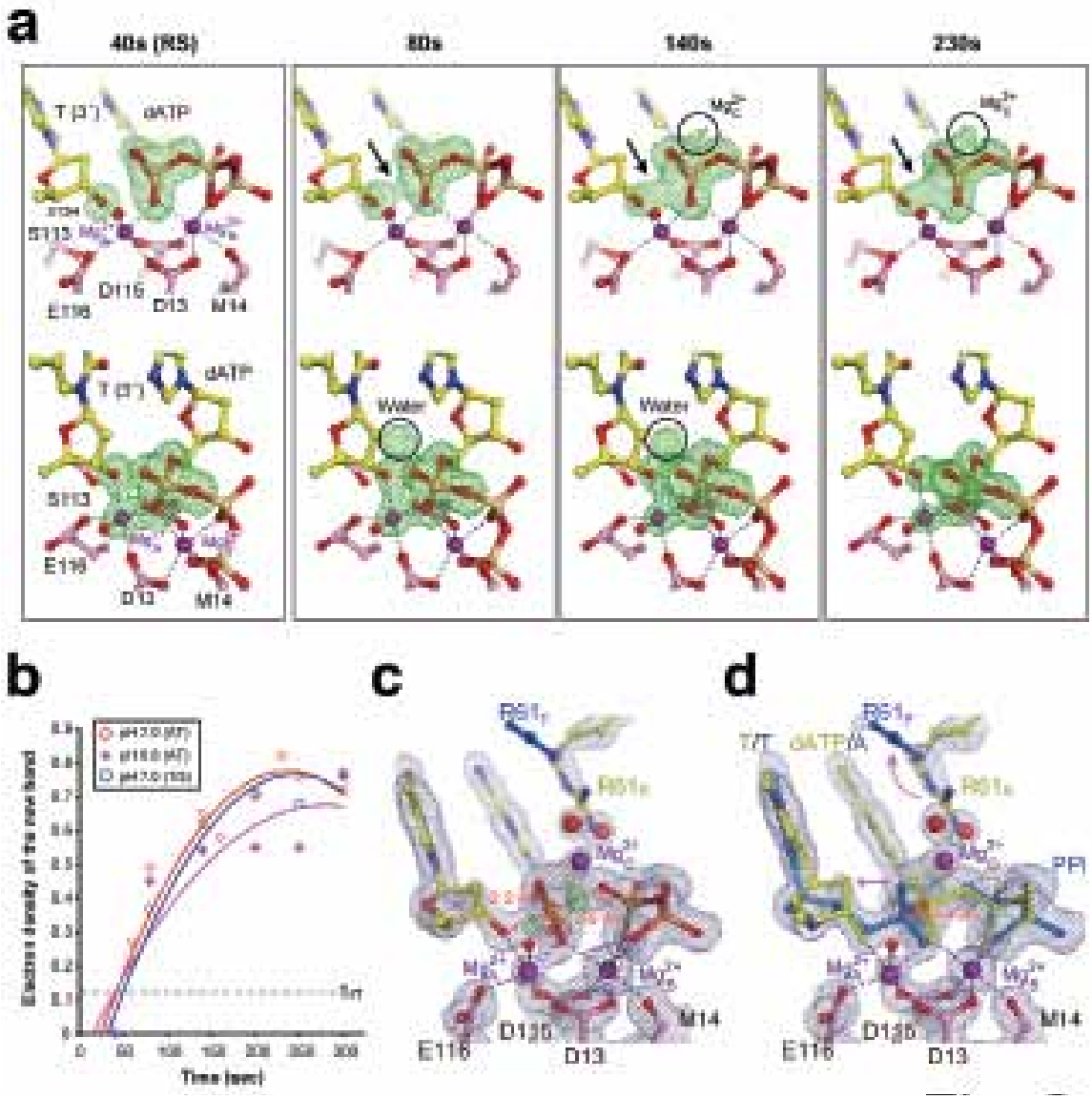
SER-CAT Passes Another Milestone: 1900th PDB Entry

Reported by Prof. John Rose

On July 11, 2012, the 1900th SER-CAT structure was released by the Protein Data Bank. The structure (PDB entry 4ED3) was one of a series of structures determined by Wei Yang's group at NIDDK that were used to visualize how DNA polymerase makes a phosphodiester bond by time-resolved crystallography....**and ON 22 BM!**!!

This outstanding work, based on over 120 data sets collected on SER-CAT's 22BM, is described in the recent paper by Nakamura, T, Zhao, Y, Yamagata, Y, Hua, JY and Yang, W, "*Watching DNA polymerase h make a phosphodiester bond*" (*Nature*, 487: 196-201, 2012).

Congratulations to Wei Yang and Colleagues !



Reaction time course

Where Are They Now? The 2007 SER-CAT 'Young Investigator' Award Winner

Dr. Ping Liu

Currently located
at Life Technologies in
Frederick, Maryland

"Development of Protein-Based Therepeutics"

I will always remember that day when I first saw crystal structures in my Bioinformatics class during my first year in graduate school at Georgia State University. I was so amazed by the beauty of these structures and intrigued about how to determine them. That class was taught by Drs. Irene Weber and Robert Harrison. Later, I learned that Dr. Weber's research focused on understanding the molecular basis of diseases by means of crystallographic and biochemical studies. Fascinated by protein crystallography, I decided to join her lab for graduate studies. During my PhD research, we collaborated with Dr. Phang C. Tai and Dr. Chung-Dar Lu in the Biology Department at Georgia State University to determine the structures of two bacterial carboxylesterases and explore their potential biochemical applications in industry. We found that these esterases can activate certain pro-drugs used in cancer gene therapy. One of these drugs, CPT-11 (or irinotecan), specifically targets colon cancer cells. These esterases have several appealing advantages over their counterparts of mammalian origin including high thermostability, the ability to be mass produced, and straightforward purification. The fruitful results of this work led us to file a provisional patent application for their use in cancer gene therapy, and I was awarded the SER-CAT young investigator prize in 2007.

After obtaining my PhD degree in 2007, I joined Dr. Daniel Leahy's laboratory in the Department of Biophysics and Biophysical Chemistry at Johns Hopkins University School of Medicine for post-doctoral training. My research was to study the ligand activation mechanism of epidermal growth factor receptor (EGFR) family members. Human epidermal growth factor receptor (EGFR/ErbB1/HER1) and its human homologues including ErbB2 (HER2/c-neu), ErbB3 (HER 3) and ErbB4 (HER4), are receptor tyrosine kinases that regulate cell growth and development and are associated with various cancers. The EGFR activation mechanism was proposed nearly 10 years ago based on two structures of the EGFR extracellular region complexed with ligand. These structures showed that ligand binding to EGFR induces a large conformational change in EGFRs, leading two receptors to form a symmetric dimer. However, this change failed to explain the negative cooperativity of ligand binding to receptors observed in biochemical experiments, as well as the absence of ErbB2 homodimerization in the absence of ligand. Recently, the crystal structure of an asymmetric, singly-ligated form of a *Drosophila* EGFR dimer was reported, which provided a molecular rationale for negative cooperativity in this receptor and raised the question of its mechanistic relationship to human EGFR homologs. To address this issue, I determined the crystal structure of the extracellular region of HER4/ErbB4 complexed with its ligand Neuregulin-1β. Interestingly, this 3 Å crystal structure, combined with cell-based assays, suggested that two types of ErbB dimer exist when compared to EGFR:ligand structures, one of which resembles the *Drosophila* EGFR dimer and that a single ligand is sufficient to activate EGFR dimers. This new model provides molecular basis for both negative cooperativity of human EGFR and the absence of ErbB2 homodimerization in the absence of ligand.

With a background in Medicine, I was also interested in research with direct biomedical applications. The JM-a isoform of ErbB4 was found to be elevated in breast cancer suggesting it as a potential cancer target. During my postdoc, I participated in a collaboration project with a Finnish group to characterize the interaction between the JM-a isoform of ErbB4 and an anti-ErbB4 monoclonal antibody being developed by Genentech. The 3.4 Å structure of the ErbB4:Fab complex revealed the mAb targeted the JM-a specific region and could be a potential therapeutic to block ErbB4 cleavage.

My passion to work on biomedical applications and the experience of collaboration with pharmaceutical company inspired me to pursue a career path in industry after my post-doctoral training. In 2011, I took a Staff Scientist position with Life Technologies working as a Technical Lead to oversee development of therapeutic proteins expressed in mammalian cell lines. Although I am currently away from determining crystal structures, I have to say that ten years of training in Structural Biology and Protein Biochemistry bestowed on me a solid foundation in the field of Protein Science that will serve me all my life during my career.

SER-CAT Facility Update

by John Rose & John Chrzas

Since the last News Letter, several updates to SER-CAT beamline hardware and software have been made as highlighted below.

DETECTOR: The new Rayonix MX300HS detector scheduled for delivery in January 2013 has improved readout and noise characteristics compared to the current MAR MX300 detector in use on 22ID. The readout specifications for the Rayonix detector are 10 images per second in standard 2x2 binning (pixel size ~ 78 microns) and a read noise of less than one 12 keV photon (or 0.5 12 keV photons in the slower 2 Hz readout mode).

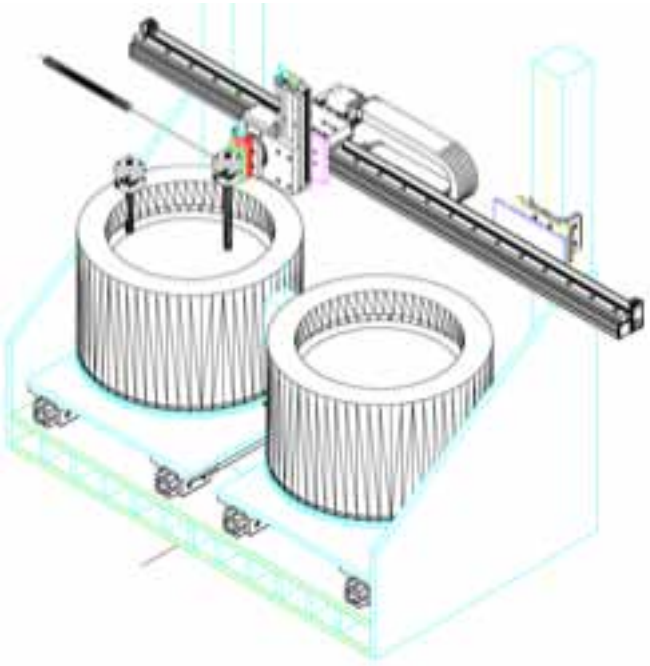
Unlike the Pilatus photon counting detector, the Rayonix detector is an integrating detector. Thus, users will have the option of collecting either wide slice (≥ 0.5° phi steps/image) data with HKL2000 processing, as currently done using the MAR detector or fine slice (< 0.5° phi steps/image) data in shutterless (continuous rotation) mode with XDS processing. In either mode, data collection speed will significantly increase. The current MAR detector can collect 6 (180 image) data sets per hour. The new Rayonix detector will collect 20 data sets per hour using the current shuttered mode of data collection to over 40 data sets per hour in shutterless mode.

Once the Rayonix detector is installed on 22ID, the current MAR detector on 22ID will be moved to 22BM to increase its experimental capabilities.

SAMPLE ROBOTICS: The SER-CAT sample mounting robots are highly modified versions of the Berkeley/ALS Auto-Mounter (BAM) systems. The SER-CAT modifications have made the BAM-S systems both highly reliable and fast. The BAM-S can change samples in under 25 seconds, making it the fastest sample changer at APS. Considered state of the art by many facilities, the BAM-S systems been adopted by NE-CAT and GM/CA CAT at the APS. A BAM-S system is currently being built for installation at SBC-CAT and a system is under consideration by MX beamlines at the Canadian Light Source.

The current 22ID BAM-S system was installed in 2008 and has recently been reconfigured to hold 15 full pucks (ALS-, UGA- or UNI-pucks) or 225 crystals. The BAM-S system on 22ID has been heavily used with over 20,000 crystal mounts in the one-year period ending June 1, 2012. However, to meet the increased sample storage demands of the faster Rayonix detector, a new two-Dewar system has been designed which will hold 30 pucks (450 crystals) while retaining the fast 25 sec sample change rate. The new BMM-S2 system, which will be installed in mid-2013, will also accommodate Rigaku pucks.

SAMPLE GONIOMETRY: The Bruker/Maatel MD2 is considered state of the art in beamline sample goniometry. It has been in service for five APS runs and over 7,100 data sets have been collected in the one-year period ending June 1, 2012. An MK3 kappa axis attachment was installed in January 2012 to allow the user a means of optimally orienting the crystal in the beam. However, a problem with the electromagnet, which secures the pin to the goniometer, has limited its use, due to mismounted or dropped crystals. Until the problem with the electromagnet has been solved, the MK3 will be made available for use by request only.



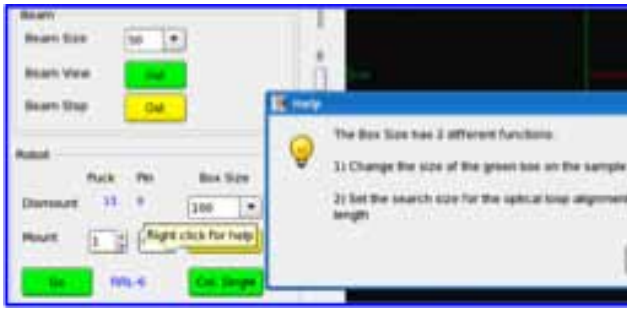
COMPUTING: A 10 Hz detector such as the Rayonix MX300HS will place increased demands on computing and network resources. As part of the detector upgrade SER-CAT improved both its networking and data storage infrastructure. The entire Sector network has been upgraded to 10 GB (including the detector interconnect). The improved network speed should improve SERGUI performance by reducing network bottlenecks. On-site data storage has been increased to 100 Tbytes with the installation of a new PANSAS file system. The 100 Tbyte file system has capacity to store over 6 million compressed images, or over 34,000 (180 image) data sets. The file system can be expanded to 500 Tbytes in the future if needed.

The new detector, with its increased data rate, will also impact data processing, especially when running in shutterless data collection mode. A new 96 processor Penguin cluster capable of running at 1.1 Tflops has been installed to meet the increased data processing needs. A multiprocessor version of XDS has been installed on the cluster, which can provide near real time data processing of data collected in shutterless data collection mode.

SERGUI: Several updates have been made to SERGUI to improve reliability and to add functionality. A common complaint from users is that SERGUI sometimes freezes. During this run, we have begun to log every time SERGUI is started and exits cleanly. From our initial review of the logs, it appears that network bottlenecks are the main culprit and we are in the process of recoding the SERGUI data flow to address this issue. We are also developing a tool that users can use to determine if there are bandwidth limitations from APS to the home facility, which may need to be addressed. Remember copying data from APS to the home lab during your SERGUI session will slow down the data collection if external band-

width is an issue.

A couple of useful functions have been recently added to SERGUI: Beamline Updates and Tool-Tip Help. Users will notice that when SERGUI starts, it asks for PI name, institution and telephone number and that the first thing the user sees is an editable text file called the "Beamline Update". The purpose of this file is to let the user know what is currently happening at either the APS or SER-CAT that may effect data collection or data quality. It is like a blog with both SER-CAT staff and beamline users contributing. It is a good idea to scroll through the Update to see any problems previous users may have had. Importantly, if you are having any problems report them in the Update and call user support. The user can also use the Update to offer suggestions or ideas for new tools. The Beamline Updates are reviewed both during data collection by user support staff and at the weekly staff meeting.



Another feature recently added to SERGUI is Tool-Tip (or Hover) Help. If the user is unsure of the input parameter's function (or expected input), he/she can simply place the cursor over the input box and a help callout will appear. The callout will have a brief description of the parameter and its use, and in most cases, a link to a more detailed PDF help file. Thus the user has help at his/her fingertips.

Undergraduate Research at SER-CAT

by Richard Michael Walsh

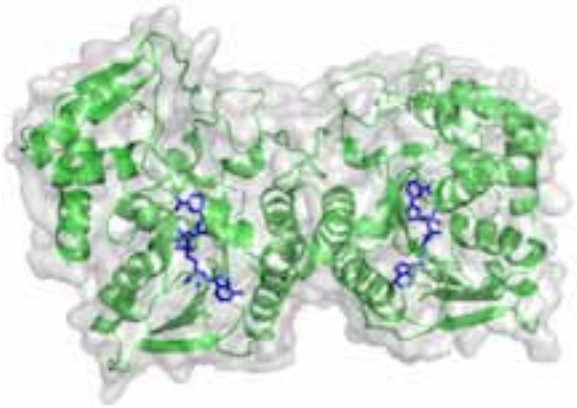
UGA student

At the University of Georgia, two semesters of undergraduate research are required for all Biochemistry & Molecular Biology majors. Undergraduates are thus presented with the unique opportunity to participate in real scientific research; an experience that goes far beyond the typical lecture oriented learning that most undergraduates encounter. Using remote access to state of the art facilities at SER-CAT undergraduates at UGA are granted an unparalleled introduction to structural biology. For example, in the laboratory of Prof. Zachary Wood, undergraduate students are introduced to the world of structural biology and the process of crystal structural determination. Undergraduates typically begin their education in the Wood lab by learning the important skills behind protein purification and crystallization. Those who show initiative are guided through the process of data collection, structure determination, and refinement. Below the undergraduate research experience of several Wood lab undergraduates who have collected data at SER-CAT are presented.

Gregory Custer was an Engineering major who worked on structure of human UDP-glucose dehydrogenase (hUGDH). Using data he collected at SERCAT in 2011, he solved and refined the structure. His structure captured a never-before observed conformational change in the enzyme structure that produced a model that better matched the biochemical data. A manuscript describing Greg's work is in preparation. He will be beginning his graduate studies at the University of Maryland this fall.

William Peeples was a Biochemistry & Molecular Biology major. Using data he collected at SERCAT in 2011 he solved two crystal structures: a mutant hUGDH and a mutant ketopantoate reductase from *Staphylococcus aureus* (sKPR). Both mutants exhibit altered kinetic and allosteric properties. A manuscript describing Williams's work is in preparation. He will begin his graduate studies at the University of Texas Southwestern this fall.

Richard Walsh was a Biochemistry & Molecular Biology major who collected data at SERCAT in 2011 and solved the structure of a mutant human UDP-xylose synthase (hUXS). (See structure below). The structure was used in combination with kinetic and biochemical data to elucidate the enzyme's allosteric mechanism. A manuscript describing Richard's work is in preparation. He is currently working as a technician in the Wood lab refining four new crystal structures and plans on attending graduate school in the fall of 2013.



Mutant Human UDP-xylose synthase

Woolim Kwon is a Junior Biochemistry & Molecular Biology major and a fairly new addition to the Wood lab. She assisted in the model building and refinement of two hUXS mutants that illustrate how a single point mutation results in boneless fish. She has begun purification of glutathione reductase from *Bartonella henselae* and is looking forward to going through the whole structure determination process from growing crystals to the refined crystal structure. Woolim plans to go to medical school after graduation.

Nicholas Dunn is a Junior Biochemistry & Molecular Biology major who is one of the newest additions to the Wood lab. He has been attempting to crystallize the ternary complex of a mutant sKPR that shows an alternate catalytic mechanism to wild type sKPR. Once crystals are obtained, he plans to collect data at SER-CAT and solve his first crystal structure. Nick plans to go on to graduate school after graduation.

The SER-CAT Spectrum

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