

**7<sup>th</sup> Annual SER-CAT Symposium March 19, 2010**

*Room C156*

*Spallation Neutron Source, Building 8600, Oak Ridge National Laboratory, Oak Ridge,  
TN*

**7:45am                      Buses Leave Doubletree Hotel for ORNL**

**Session 1: SER-CAT Science**

*Chair: Leighton Coates (Oak Ridge National Laboratory)*

8:30 to 8:45              Welcome: Leighton Coates

8:45 to 9:30              **Keynote Speaker:** Biomass to Biofuels: overcoming Biomass  
Recalcitrance. (*Brian Davison, Chief Scientist for Systems Biology  
and Biotechnology, Oak Ridge National Laboratory*)

9:30 to 10:00            Allosteric modulation of Ras: a direct role for Q61 in catalysis  
(*Carla Mattos, North Carolina State University*)

10:00 to 10:30           The mechanism of Ippase catalyzed phosphoryl transfer  
(*Ronny Hughes, University of Alabama at Huntsville*)

10:30 to 11:00           Coffee Break

**Session 2 SER-CAT Awards and APS Status**

*Chair: John Rose (University of Georgia and SER-CAT)*

11:00 to 11:30           SER-CAT Outstanding Science Award Presentation and Talk  
Structure of ERA in Complex with the 3' End of 16S rRNA:  
Implications for Ribosome Biogenesis (*Xinhua Ji, Center for  
Cancer Research, National Cancer Institute*)

11:30 to 12:00           APS Update: Funding, Operations and Future Plans  
(*John Quintana, Advanced Photon Source, Argonne National  
Laboratory*)

12:00 to 12:10           Group Picture

12:10 to 1:30            Boxed Lunch and Tour of SNS Facility

**Session 3: Interesting Methods**

*Chair: Joe Ng (University of Alabama at Huntsville)*

- 1:30 to 2:00            An Introduction to Small-angle Neutron Scattering for Structural Biology (*William Heller, Oak Ridge National Laboratory*)
- 2:00 to 2:30            The MDS Strategy: Collecting Multiple Data Sets with Short Exposures Can Produce Better Data Than Traditional Long Exposures Within a Fixed X-ray Dose. (*B.C. Wang, University of Georgia and SER-CAT*)
- 2:30 to 3:00            Single Crystal Neutron Diffraction at ORNL  
(Christina Hoffmann, Oak Ridge National Laboratory)
- 3:00 to 3:30            Utilizing small-angle neutron scattering to investigate the polyglutamine aggregation pathway in Huntington's disease  
(Christopher Stanley, Oak Ridge National Laboratory)
- 3:30 to 4:00            Coffee Break

**Session 4: SER-CAT The Way Forward**

*Chair: B.C. Wang (University of Georgia and SER-CAT)*

- 4:00 to 4:30            Update on SER-CAT Upgrades and Others  
(*John Chrzas, University of Georgia and SER-CAT*)
- 4:30 to 5:00            Single-Line-Command Driven UI's for Data Processing at SER-CAT (*Zheng-Qing (Albert) Fu, University of Georgia and SER-CAT*)
- 5:00 to 6:30            Poster Session with cash bar
- 6:30 to 8:30pm        Working dinner with lecture Dean Myles by (ORNL)
- 8:30pm                Buses depart for the Doubletree

## Talk Abstracts

### **Biomass to Biofuels: overcoming Biomass Recalcitrance**

**Brian H. Davidson** (Chief Scientist for Systems Biology and Biotechnology) Oak Ridge National Laboratory

The conversion of lignocellulosic biomass into biofuels is a current challenge for a sustainable energy portfolio. First generation technologies are starting to be deployed. However, biomass is a complex molecular level laminate structure of primarily three major components: cellulose microfibrils, hemicellulose and lignin. A challenge of bioconversion is to understand how to access these monomers. This talk will include a brief status overview of the challenge and current efforts within the Bioenergy Science Center. There is a need to detailed analysis and analysis at the structural level of many aspects of these biological systems: ranging from the biomass structure itself, the critical plant formation mechanisms, the enzymes for deconstruction, and the impact of pretreatment.

### **Allosteric modulation of Ras positions Glutamine 61 for a direct role in catalysis**

Carla Mattos, Department of Molecular and Structural Biochemistry, 128 Polk Hall, North Carolina State University, Raleigh, NC 27695-7622

The Ras GTPase and its effector Raf are key mediators of the Ras/Raf/MEK/Erk signal transduction pathway, which plays a central role in cell growth and malignant transformation. Mutants of catalytic residue Q61 impair the GTPase activity of Ras and are found prominently in human cancers. Yet the mechanism through which Q61 contributes to catalysis has been somewhat elusive. We have found that Ras-GppNHp in the crystal binds calcium acetate from the crystallization mother liquor at a site remote from the active site and most likely near the membrane. This results in a shift in helix 3/loop 7 and a network of H-bonding interactions that propagates across the molecule, culminating in complete ordering of switch II and placement of Q61 in the active site in a previously unobserved conformation for catalysis. In this structure Q61 interacts with a water molecule that bridges one of the  $\gamma$ -phosphate oxygen atoms to the hydroxyl group of Y32. This active site arrangement suggests a novel role for Q61 where it interacts with the bridging water molecule to stabilize the transition state of the hydrolysis reaction. We propose that Raf together with the binding of  $\text{Ca}^{2+}$  and negatively charged group in the membrane at the allosteric site (mimicked in our structure by the acetate molecule), induce ordering of the switch I and switch II to complete the active site and bring intrinsic hydrolysis rates in Ras to biologically relevant levels. In order to determine the mechanism through which intrinsic hydrolysis occurs in Ras it is essential to know the position of hydrogen atoms in the catalytic residues as well as in the nucleophilic water and other water molecules in the active site. For this we are working on the neutron crystal structure of Ras-GppNHp. These data will be highly complementary to our X-ray crystal structures and will serve to fine-tune the mechanism through which hydrolysis of GTP occurs in Ras.

## The role of hydrogen in enzyme catalyzed phosphoryl transfer

Ronny C. Hughes<sup>1</sup>, Leighton Coates<sup>2</sup>, Joseph D. Ng<sup>1</sup>

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A model for the catalytic cycle of the Family I Inorganic Pyrophosphatase (IPPase) from the sulfur-reducing hyperthermophilic archaeon *Thermococcus thioreducens* (Tt-IPPase) is proposed in providing new insight into proton transfer mediated, metal-assisted enzyme catalysis. IPPase is a metal-dependant, multimeric enzyme that catalyzes the breakdown of pyrophosphate into two molecules of orthophosphate. Presently, many aspects of the catalytic process remain unclear or have been difficult to validate due to the lack of knowledge pertaining to hydrogen positions in the active site. Seventeen X-ray crystallographic structures of the enzyme have been determined at ultra-high resolution in complex with substrate, product, and in the presence of various metal cofactors and reactive species analogues. Large volume crystals (>6 mm<sup>3</sup>) of the enzyme suitable for neutron structural studies have been obtained in effort to determine the precise location of hydrogen atoms within the active site needed to complete and validate a mechanistic model for Tt-IPPase catalysis.

### Structure of ERA in Complex with the 3' End of 16S rRNA: Implications for Ribosome Biogenesis

Xinhua Ji, National Cancer Institute, Frederick, MD 21702 USA

ERA is essential for bacterial viability. Our first structure of apo-ERA shows that the protein is composed of an N-terminal GTPase domain followed by an RNA-binding KH domain (Chen, Court & Ji, *Proc. Natl. Acad. Sci. USA* **96**:8396-8410, 1999). However, the functional relationship between the two domains remained unclear. Ten years later, we determined another two crystal structures of the protein: a binary complex with GDP and a ternary complex with a non-hydrolysable GTP-analog (GNP) and 12 nucleotides at the 3' end of 16S rRNA (Chen, Court & Ji, *Proc. Natl. Acad. Sci. USA* **106**:14843-14848, 2009). The structures reveal that the KH domain recognizes a total of nine nucleotides, including the <sub>1531</sub>AUCA<sub>1534</sub> sequence, which is highly conserved in all forms of life, and the anti-Shine-Dalgarno <sub>1535</sub>CCUCC<sub>1539</sub> sequence, which is conserved in bacteria only. We also show that GTP binding is a prerequisite for sequence-specific RNA recognition by ERA and that its GTP-hydrolyzing activity is stimulated upon RNA recognition. Our data, together with the structure of ERA in complex with GNP (RIKEN Structural Genomics/Proteomics Initiative, 2004), has established a functional cycle of the protein, suggesting that ERA serves as a chaperone for the maturation of 16S rRNA and a checkpoint for the assembly of the 30S ribosome subunit.

"An Introduction to Small-angle Neutron Scattering for Structural Biology"

**William T. Heller**, Center for Structural Molecular Biology; Oak Ridge National Laboratory

Small-angle scattering methods have attracted the attention of members of the life science community as they seek to understand the structure and function of increasingly complex and dynamic macromolecular systems. Many of the applications of the techniques involve the use of x-rays, primarily owing to their greater availability, but the use of neutrons provides unique opportunities when studying biological systems. Small-angle neutron scattering (SANS) shares fundamental concepts with small-angle X-ray scattering (SAXS), but the properties of the neutron and the ability to label biological macromolecules with deuterium, an isotope of hydrogen that has a dramatically different interaction with neutrons, opens additional avenues for understanding the structure of biological macromolecular systems. In addition to providing an introduction to SANS that will include the underlying theory, practical experimental considerations, as well as basic data analysis and modeling, an overview of the facilities available in the Center for Structural Molecular Biology (CSMB) at Oak Ridge National Laboratory will be provided. The talk will conclude with specific examples of the use of SANS in the study of biological structures that highlight the unique capabilities of SANS, including protein-protein complexes, protein-DNA complexes, and other examples.

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**The MDS Strategy: Collecting Multiple Data Sets With Short Exposures Can Produce Better Data Than Traditional Long Exposures Within a Fixed X-ray Dose.**

B.C. Wang<sup>1</sup>, Z.J. Liu<sup>1\*</sup>, L. Chen<sup>1</sup>, W. Zhou<sup>1†</sup>, H. Xu<sup>1</sup>, H. Zhang<sup>1</sup>, J.T. Swindell II<sup>1</sup>, J.P. Rose<sup>1</sup>, Z.-Q. Fu<sup>1</sup>, J. Chrzas<sup>1</sup>, G. Rosenbaum<sup>1</sup> and M. Benning<sup>2</sup>. <sup>1</sup>Department of Biochemistry and Molecular Biology and SER-CAT, University of Georgia, Athens, GA 30602, <sup>2</sup>Bruker AXS, Madison, WI 53711.

A fundamental question facing the crystallographer is "For a fixed X-ray dose, what is the best strategy for collecting a data set from a given crystal that will increase structure solvability?" A common answer to this question is to do longer exposures for each diffraction image, so that we may better "visualize" the diffraction spots to get better data?

Interestingly, we have found that a significantly better data set may be produced for a given crystal and X-ray dose by collecting the complete data set multiple times using a shorter exposure time, such that the total X-ray dose remains the same.

This means that a better data set may be obtained within an equivalent total exposure time from merging multiple data sets of shorter exposures, even if the images from each data set give somewhat weaker "visible" spots!

This is the basis of the proposed Multiple-Data-Set Data Collection Strategy. Both the theoretical and practical aspects of this strategy will be discussed. In addition, some recent examples of the Multiple-Data-Set Data Collection Strategy will be presented.

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Single Crystal Diffraction at ORNL

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Neutron single crystal diffraction (NSCD) is a method complementary to X-ray single crystal diffraction (XSCD). NSCD is extraordinarily sensitive to light elements in the presence of heavy metals, which is due to interacting with the nucleus rather than with the electron shell in the diffraction process. This makes it an outstanding probe to determine the location of hydrogen in many materials from inorganic framework structures to organic molecular and macro-molecular arrangements. Recent studies have shown that hydrogen is fundamental in determining function and stability of structures in many molecular compounds. Neutron and X-ray single crystal diffraction are most effectively used as complementary methods, where the X-ray data provides the backbone or framework, and the neutron data provides the hydrogen and light element positions. Moreover, neutrons are carrying a magnetic moment, which allows interaction with unpaired electrons in the structure. This feature allows investigation of magnetic structures and spin density distribution, for example. A number of neutron single crystal diffractometers, optimized for different scientific emphases are currently available in the user program. The suite of neutron diffractometers is being expanded continuously to provide a comprehensive range of instrumentation for SCD investigations. At the HFIR a four axis monochromatic SCD at the thermal beam-port HB-3A is currently in the user program, and a Quasi-Laue SCD "Imagine" in the HFIR guide hall, on CG-4D, is scheduled for commissioning in 2011. At the SNS a high pressure diffractometer "SNAP" on beam line 3, a general purpose scd "TOPAZ" on beamline 12 are in the user program. A macromolecular diffractometer "MaNDi", is scheduled for commissioning in 2012, and an elastic diffuse scattering spectrometer "Corelli" is scheduled for commissioning in 2013. More information on available neutron scattering instruments, proposal submission, meetings and deadlines is available on the web at <<http://neutrons.ornl.gov/>>.

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## **Utilizing small-angle neutron scattering to investigate the polyglutamine aggregation pathway in Huntington's disease**

Christopher Stanley<sup>1</sup> Tatiana Perevozchikova<sup>2,3</sup>, and Valerie Berthelier<sup>3</sup>

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<sup>2</sup>*Genome Science and Technology, University of Tennessee – ORNL, Knoxville, TN;*

<sup>3</sup>*Graduate School of Medicine, University of Tennessee Medical Center, Knoxville, TN*

The presence of an abnormally expanded polyglutamine (polyGln) sequence in huntingtin protein ultimately results in  $\beta$ -sheet-rich fibrillar aggregates, a hallmark of Huntington's disease. Current challenges are to map out the polyGln aggregation pathway by identifying the various precursor structures and establish their pathological roles. We are using time-resolved small-angle neutron scattering (SANS) to probe the aggregates formed by peptides having the protein context of huntingtin exon 1 (HD protein) and with varying polyGln lengths. SANS is a particularly useful technique for following structural changes on the nanometer length-scale in solution. From the time-resolved scattering data, we obtain snapshots of the polyGln structures as the kinetics reaction ensues, which yields quantitative information on the size and shape of precursors and the internal structure of the resulting fibrils. This research is providing new insights into the pathway of polyGln aggregation and should later assist in determining the role that precursors play in neuronal toxicity.

## **Update on SER-CAT Upgrades and Other**

John Chrzas, Zheng-Qing Fu, Zhongmin Jin, Andy Howard, Jim Fait, John Gonczy, Rod Salazar, Unmesh Chinte, John Rose, Bi-Cheng Wang, SERCAT, APS, Argonne National Lab, Argonne, IL 60439. Email: chrzas@anl.gov

The last year has seen a large increase in the SER-CAT “Virtual Home Synchrotron” program. As the remote usage increases, we learn what the user wants and needs to improve their beam time efficiency. This is even more critical as we adopt 12 hour shift allocations. Recent software upgrades will be presented as well as future hardware/software upgrades in support of mini-beam operations on 22ID.

## **Single-Line-Command Driven UIs for Data Processing at SERCAT**

Zheng-Qing Fu, Zhongmin Jin, Andy Howard, John Chrzas, Jim Fait, John Gonczy, Rod Salazar, Unmesh Chinte, John Rose, Bi-Cheng Wang, SERCAT, APS, Argonne National Lab, Argonne, IL 60439. Email: fuzq@anl.gov

As part of our efforts to monitor the data quality on-the-fly at SERCAT, command-driven UIs (user interfaces) for some of the widely-used programs have been developed, which include CMDDENZO, CMDXDS, CMDDTREK.PY and XGENPROC.PY. They exploit functions in different packages such as DENZO/SCALEPACK, D\*TREK, SPGR4D, SGXPRO, XDS, X-GEN for X-ray single crystal diffraction data deduction. These non-graphics UIs are not intended to match the expert use of the original programs, but to provide a quick way to automatically process and characterize a data set, which includes determining Space group, Rmerge, Completeness, Redundancy, I/SigI etc. They also provide a set of handy diagnostic tools to find problems before too late, which would be more helpful for remote data collection.

## Poster Abstracts

### **The Bio-Deuteration Lab at Oak Ridge National Laboratory**

Kevin L. Weiss<sup>1</sup>, Qiu Zhang<sup>1</sup>, Dean A.A. Myles<sup>2</sup>

<sup>1</sup>*Oak Ridge National Laboratory, Chemical Sciences Division, Center for Structural Molecular Biology*

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The Bio-Deuteration Laboratory (BDL) has been established at Oak Ridge National Laboratory (ORNL) for the production of <sup>1</sup>H/<sup>2</sup>H-labeled biological macromolecules to support development of the neutron structural biology research and user programs at ORNL's neutron scattering facilities. Studies at these facilities are enhanced by the design and production of selectively, partially, and fully-deuterated biological macromolecules. Deuterium-labeling provides contrast and allows portions of macromolecular structures to be highlighted and analyzed *in situ*. The development of the High Flux Isotope Reactor (HFIR) and Spallation Neutron Source (SNS) facilities at ORNL offers unprecedented opportunities to develop a world-leading program of neutron structural biology research in the life sciences. The BDL will serve as a central training/user facility that will be accessible to the broader scientific community.

### **Neutron Diffraction Studies on the Toho-1 $\beta$ -lactamase**

Steve Tomanicek, Leighton Coates

Oak Ridge National Laboratory, Neutron Scattering Science Division

$\beta$ -lactam antibiotics have been used effectively over several decades against many types of bacterial infectious diseases. The most common cause of resistance to the  $\beta$ -lactam antibiotics is the production of  $\beta$ -lactamase enzymes that inactivate these antibiotics by rapidly hydrolyzing the amide group of the  $\beta$ -lactam ring. The expanded-spectrum third-generation cephalosporins and monobactams were developed to be stable and effective against the growing number of resistant class A  $\beta$ -lactamases. However, due to abusive clinical use of these antibiotics, class A extended-spectrum  $\beta$ -lactamase (ESBLs) enzymes arose in resistant bacteria that were capable of hydrolyzing the expanded-spectrum antibiotics which lead to treatment problems in many clinical settings. Thus, a more complete understanding of the mechanism of catalysis of these ESBL enzymes will impact current antibiotic drug discovery efforts. We have used neutron crystallography to more fully characterize the location of the hydrogen atoms and the resulting hydrogen bonding interactions in the active site region of the Toho-1 CTX-M-type ESBL. The neutron structure of the Toho-1 E166A/R274N/R276N triple mutant in its apo form, which is the first reported neutron structure of a  $\beta$ -lactamase enzyme, clearly reveals the active site protonation states and hydrogen-bonding network of the Toho-1 ESBL prior to substrate binding and subsequent acylation. This neutron structure is in agreement with a proposed mechanism for acylation that identifies Glu166 as the general base for catalysis. In addition, the protonation states of the active site residues in this neutron structure are

consistent with the prediction of a proton transfer pathway from Lys73 to Ser130 that is likely dependent on the conformation of Lys73 during the acylation reaction in the catalytic mechanism.