



6th Annual

SER-CAT Symposium

March 20, 2009

**Shelby Center for Science and Technology
The University of Alabama in Huntsville**



Photo by
Dennis Keim

Every morning in a structural biology lab, a crystallographer wakes up and knows it must phase a protein structure faster than his/her most resourceful competitor or he/she will be scooped. Every morning in a structural biology lab, a competitor wakes up and must out phase a more complex structure or he/she will not be funded. It doesn't matter whether it is you or your competitor; when the sun comes up, you better start phasing.

--A **modified** African proverb

Welcome to the 6th Annual SER-CAT Symposium at the University of Alabama in Huntsville (UAH) to all SER-CAT users and others interested in X-ray crystallography. The goal of this symposium is to discuss new crystal structures, improved methods for structure determination and productive use of the SER-CAT facility. We hope you will enjoy your stay in Huntsville, known as the rocket city, home of Marshall Space Flight Center, NASA. The city holds the second largest research park in the U.S. and is the growing center for Biotechnology.

Organization Committee

Joseph D. Ng (Chair)
Diana Toh
Sheila Fore-Williams

The organization committee extends their gratitude to the following
for their support and assistance.

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Program

March 20, 2009

Shelby Science Center Auditorium Room 109
University of Alabama in Huntsville

- 7:45 a.m. Van pick up from Marriott Hotel to Shelby Science Center
- 8:00 a.m. Continental Breakfast
- 8:30 a.m. Welcome - *Joe Ng*
- 8:35 a.m. Welcome Remarks - *David Williams, UAH President*
- Session 1** Chair - *Joe Ng*
- 8:40 a.m. Recent Examples on Successful Sulfur Phasing - *Bi-Cheng Wang*
- 9:05 a.m. Sulfur SAD Phasing at SER-CAT 22ID Beamline: a Successful Case with Data Collected at 1.9 Å Wavelength - *Liqing Chen*
- 9:30 a.m. Structural Insights Toward an Understanding of Substrate Recognition by the FEN-1 Family of Enzymes - *Stephen Tomanicek*
- 9:55 a.m. HIV-1 Protease: Structural Perspectives on Drug Resistance - *Ying Zhang*
- 10:20 - 10:40 a.m. Coffee Break
- Session 2** Chair - *Leighton Coates*
- 10:45 a.m. **Keynote Speaker: Global genomic approaches to human biology and disease**
Richard Myers, Director and President of HudsonAlpha Institute for Biotechnology, Huntsville, Alabama
- 11:20 a.m. Methylation Assisted Crystallization of Protein Molecules - *Zhi-Jie (James) Liu*
- 11:45 a.m. Protein Crystallization Screening using Fluorescence - *Marc Pusey*
- 12:10 - 1:30 p.m. Lunch in Room 301 Lounge - Poster presentations and demonstrations
- Session 3** Chair - *Ronny Hughes*
- 1:35 p.m. SER-CAT Young Investigator Award Presentation: *Guoxing Fu*
Structural Basis for Substrate Specificity of Executioner Caspases
- 2:05 p.m. SER-CAT Outstanding Science Award Presentation: *James Hurley*
Midbody targeting of the ESCRT machinery by a noncanonical coiled coil in CEP55
- Talk will be presented by *Hyung Ho Lee*
- 2:35 p.m. **Challenges in the Structure Determination of Penicillin Binding Protein A from Mycobacterium Tuberculosis** - *Alena Fedarovich*
- 2:55 - 3:15 p.m. Coffee Break
- Session 4** Chair - *John Rose*
- 3:20 p.m. FAQ on χ^2 at ID - *Zheng-Qing (Albert) Fu*
- 3:45 p.m. Virtual Synchrotron - *John Chrzas*
- 4:10 p.m. Data Collection Automation at SER-CAT - *Richard Walter*
- 4:35 p.m. Opportunities for Macromolecular Research from the APS Renewal - *Mark Beno*
- 5:00 p.m. Group picture
- 5:15 p.m. Van ride return to the Marriott Hotel
- Evening Event**
- 6:30 - 10:00 p.m. Dinner reception at the Space and Science Rocket Center
Special Speaker: *Owen Garriott*

Recent Examples on Successful Sulfur Phasing

Bi-Cheng Wang

Department of Biochemistry and Molecular Biology and SER-CAT
University of Georgia, Athens, GA 30605

Over the past decade, interest from the crystallographic community worldwide in sulfur-SAD phase determination has grown. SER-CAT, for several years, has also embarked on a program of longer wavelength ($\lambda > 1.5\text{\AA}$) beamline optimization focused on identifying and correcting problems in the system. In addition, software development on collecting data with enhanced signal-to-noise ratio has been initiated. In this talk, I will present (1) a brief historical account on the practical aspects of SAS (SAD) and sulfur phasing, (2) 40+ structures solved by sulfur-SAD, and (3) recent successful cases of structures solved from medium resolution crystals or from large molecular weight proteins in low-symmetry space groups, and (4) future perspectives for routine S-SAD phasing.

Sulfur SAD Phasing at SER-CAT 22ID Beamline: a Successful Case with Data Collected at 1.9 Å Wavelength

Liqing Chen,^a Li-Rong Chen,^b Zheng-Qing Fu,^c Mingdong Huang,^d Chuanbin Bian,^d Cai Yuan,^d Lin Lin,^e Bi-Cheng Wang,^b and Edward J. Meehan^a

^aLaboratory for Structural Biology, Department of Chemistry, Graduate Program of Biotechnology, University of Alabama in Huntsville, Huntsville, Alabama 35899

^bDepartment of Biochemistry and Molecular Biology, University of Georgia, Athens, GA 30602

^cSoutheast Regional Collaborative Access Team (SER-CAT), Advanced Photon Source, Argonne National Laboratory, Argonne, IL 60439

^dState Key Lab of Structural Chemistry, Fujian Institute of Research on the Structure of Matter, Chinese Academy of Sciences, 155 Yang Qiao Xi Lu, Fuzhou, Fujian, 350002, China ^eFujian Normal University, Fuzhou, Fujian, China

A successful sulfur SAD phasing experiment has been carried out using synchrotron radiation of 1.9 Å wavelength at SER-CAT 22ID beamline, Advanced Photon Source, Argonne National Laboratory, Chicago, USA. Seven sets of S-SAD data from five crystals of *Penicillium expansum* lipase were collected with or without using the helium beam paths. The effects of helium path use and data redundancy on data quality/accuracy are discussed. The results demonstrated the feasibility of collecting excellent S-SAD data at SER-CAT 22ID beamline.

Structural Insights Toward an Understanding of Substrate Recognition by the FEN-1 Family of Enzymes

Stephen J. Tomanicek^{1#}, Juliette M. Devos¹, Charles E. Jones², Nancy G. Nossal², and Timothy C. Mueser¹

¹Department of Chemistry, The University of Toledo, Toledo, Ohio, 43606,

²Laboratory of Molecular and Cellular Biology, NIDDK, National Institutes of Health, Bethesda, Maryland 20892

[#] Currently at Neutron Scattering Sciences Division, Oak Ridge National Laboratory, 1 Bethel Valley Road, Oak Ridge, TN 37830

The duplication of genomic information is central to the survival of organisms in all kingdoms of life. DNA replication and repair processes are essential for maintaining the fidelity and genomic stability required for life. Specifically, the fidelity of DNA replication is highly dependent on the function of the flap endonuclease (FEN-1) family of enzymes. The FEN-1 family of DNA replication associated DNA repair enzymes are structure specific 5' to 3' endonucleases that are members of the RAD2/RAD27 family of eukaryotic nucleases.

Bacteriophage T4 RNase H, a FEN-1 family nuclease, removes RNA pentamer primers and adjacent DNA of Okazaki fragments on the lagging strand during T4 phage DNA replication. The structure of native T4 RNase H revealed an active site composed of highly conserved Asp residues and two bound fully hydrated magnesium ions. Our crystal structure of T4 RNase H in complex with a fork DNA substrate bound in its active site (see figure below) is the first structure of a FEN-1 family protein with its complete branched substrate. This structure revealed how these enzymes bind branched DNA substrates through HhH2 motif recognition of duplex DNA and an active site recognition of the single stranded arm of 5' flap DNA. Recent metal-free structures of the enzyme have shown that metal binding to T4 RNase H is accompanied by both an increase in protein mobility and stability, while additional structures of active site mutants in the presence and absence of catalytic metal ions have helped to further characterize the role of divalent metal ions in the catalytic mechanism of these enzymes. Together, these structural studies have provided a more complete understanding of how catalysis is facilitated by the structure-specific substrate recognition of these essential enzymes in both DNA replication and repair.

HIV-1 Protease: Structural Perspectives on Drug Resistance

Ying Zhang, Xiaxia Yu, Chen-Hsiang Shen, Yuan-Fang Wang, Johnson Agniswamy, Robert W. Harrison & Irene T. Weber
Departments of Biology, Chemistry, and Computer Science, Molecular Basis of Disease Program,
Georgia State University, Atlanta, GA, USA.

John M. Louis
Laboratory of Chemical Physics, National Institute of Diabetes, Digestive and Kidney Diseases,
National Institutes of Health, Bethesda, MD, USA.

Arun K. Ghosh
Departments of Chemistry and Medicinal Chemistry, Purdue University, West Lafayette, IN, USA.

Drug resistance is a major challenge in treatment of the HIV/AIDS pandemic. We are investigating the molecular mechanisms of drug resistance to inhibitors of HIV protease. This knowledge is applied in structure-guided designs for novel antiviral inhibitors with enhanced interactions with conserved regions of the protease structure. New antiviral inhibitors are being analyzed. Crystal structures at high to atomic resolutions (0.84-1.5Å) demonstrate structural changes due to drug resistance mutations and diverse inhibitors or peptides. We have analyzed HIV-1 protease with ~20 different single mutations and selected combinations to reveal the molecular mechanisms of drug resistance. Drug resistance mutations show distinct effects: 1) mutations in the inhibitor binding cavity can directly alter inhibitor binding; 2) mutations at the dimer interface can alter protease stability; 3) other mutations can have indirect effects on protease activity and inhibition by altering the unliganded protease or the interactions with reaction intermediates. In order to design new drugs we have analyzed many protease complexes with clinical and investigational inhibitors. Our drug design strategy of introducing new polar interactions with inhibitors is firmly based on early studies of the conserved pattern of protease-inhibitor hydrogen bonds. Darunavir, which was approved in 2006 for AIDS therapy, and a series of novel antiviral inhibitors have demonstrated the success of this structure-guided strategy to combat resistance. The new insights into the mechanisms of drug resistance and strategies for drug design have wide impact in many diseases. Moreover, the data provide a uniquely valuable resource for analysis of structural variation due to mutations or ligands.

Acknowledgements:

The research was supported in part by the Georgia State University Molecular Basis of Disease Program, the Georgia Research Alliance, the Georgia Cancer Coalition, the National Institute of Health grants GM062920 and GM053386.

Global genomic approaches to human biology and disease

SPECIAL KEYNOTE SPEAKER

Richard Myers

Director and President of Hudson-Alpha Institute for Biotechnology, Huntsville Alabama

Methylation Assisted Crystallization of Protein MoleculesZhi-Jie LiuNational Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences,
Beijing, 100101, China

A case study on the successful crystallization of a nuclease employing a reductive methylation. The key to crystallization was the successful introduction of 44 new cohesive (NZ) CH...O contacts (3.2 - 3.7 Å) by the addition of 2 methyl groups to the side chain amine nitrogen (NZ) of 9 lysine residues of the nuclease. The new contacts dramatically altered the crystallization properties of the protein, resulting in crystals that diffracted to 1.2 Å resolution. Analytical ultracentrifugation analysis and thermodynamics results revealed a more compact protein structure with better solvent exclusion of buried Trp residues in the folded state of the methylated protein, assisting crystallization. Several recent successful crystallization of protein molecules will also be presented.

Protein Crystallization Screening using FluorescenceMarc L. Pusey

Extremozyme Inc., 301 Sparkman Drive, Huntsville, AL 35899

Current crystallization screening practice is essentially a yes/no, crystal/no crystal, method. Non-crystalline precipitate or clear solutions are routinely dropped from further consideration when in fact they may actually be close to crystallization conditions. We propose that crystallization screening can be better and more rapidly carried out using fluorescence anisotropy. Fluorescence anisotropy is a measure of the rotational rate (size) of the analyte. Data is obtained as a curve of anisotropy vs. protein concentration. For crystallization conditions increasing concentrations result in a gradual increase in the anisotropy. Clear solution conditions give flat lines, while hard precipitation conditions give high anisotropy values, indicative of solute aggregation, even at low concentrations.

A Phase I instrument has been assembled to test the FACTs (Fluorescence-based Analytical Crystallization Technologies) approach. In preliminary experiments the data indicated hits for a number of conditions scored as clear solutions or precipitate in plate screening trials. Optimization screening resulted in crystals in ~70% of those conditions, approximately a doubling of the crystallization conditions. We are now testing with a larger group of model proteins to better define the optimal assay parameters.

The benefits of FACTs are: a) Higher success rate – Many discarded outcomes are in fact close to crystallization conditions; b) Reduced costs - The method can potentially be implemented using only microgram quantities of protein for a 96 condition screen; c) Faster – Screening can be completed and lead conditions identified within 2 days of having a pure protein in hand; d) Easier results analysis – Evaluation of the data is based on quantitative measurements and is amenable to automation.

Using manual pipetting, the current system can measure assay volumes down to 1.0 μL . Assuming an assay solution composition of 80% precipitant solution + 20% of 10 mg/mL protein solution, this translates to ~ 0.7 mg of protein for a 96 condition screen. Our goal is to reduce the assay volume to 1-10 nL ($<10 \mu\text{g}/96$ conditions) with improved dispensing and detection technologies.

This work was supported by NIH/NIGMS SBIR Grant 1R43GM084488.

Structural Basis for Substrate Specificity of Executioner Caspases

Guoxing Fu¹, Alexander A. Chumanevich¹, Johnson Agniswamy¹, Bin Fang¹, Robert W. Harrison² and Irene T. Weber¹

¹Department of Biology and ²Department of Computer Science, Molecular Basis of Disease Program, Georgia State University, Atlanta, Georgia 30303

Caspase-3, -6 and -7 are executioner caspases and cleave many proteins at specific sites to induce apoptosis, which is disrupted in many diseases including cancer, heart disease, and neurodegenerative diseases. Their recognition of the P5 position in substrates has been investigated by kinetics, modeling and crystallography. Caspase-3 and -6 recognize P5 in pentapeptides as shown by enzyme activity data and interactions observed in the crystal structure of caspase-3/LDESD and in a model for caspase-6/LDESD. In caspase-3 the P5 main-chain was anchored by interactions with Ser209 in loop-3 and the P5 Leu side-chain interacted with Phe250 and Phe252 in loop-4 consistent with 50% increased hydrolysis of LDEVD relative to DEVD. Caspase-6 formed similar interactions and showed a preference for polar P5 in QDEVD likely due to interactions with polar Lys265 and hydrophobic Phe263 in loop-4. Caspase-7 exhibited no preference for P5 residue in agreement with the absence of P5 interactions in the caspase-7/LDESD crystal structure. Initiator caspase-8, with Pro in the P5-anchoring position and no loop-4, had only 20% activity on tested pentapeptides relative to DEVD. Therefore, caspases-3 and -6 bind P5 using critical loop-3 anchoring Ser/Thr and loop-4 sidechain interactions, while caspase-7 and -8 lack the P5-binding residues. These discoveries will be valuable for the future design of novel inhibitors that are more specific for target caspase members. The distinct preferences observed for P5 residue in substrates will help define the particular cellular signaling pathways associated with each executioner caspase.

Acknowledgements

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Midbody targeting of the ESCRT machinery by a noncanonical coiled coil in CEP55

Hyung Ho Lee,¹ Natalie Elia,² Rodolfo Ghirlando,¹ Jennifer Lippincott-Schwartz,² James H. Hurley¹
¹Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases,
²Cell Biology and Metabolism Branch, National Institute of Child Health and Human Development,
Bethesda, MD 20892, USA.

The ESCRT (endosomal sorting complex required for transport) machinery is required for the scission of membrane necks in processes including the budding of HIV-1 and cytokinesis. An essential step in cytokinesis is recruitment of the ESCRT-I complex and the ESCRT-associated protein ALIX to the midbody (the structure that tethers two daughter cells) by the protein CEP55. Biochemical experiments show that peptides from ALIX and the ESCRT-I subunit TSG101 compete for binding to the ESCRT and ALIX-binding region (EABR) of CEP55. We solved the crystal structure of EABR bound to an ALIX peptide at a resolution of 2.0 angstroms. The structure shows that EABR forms an aberrant dimeric parallel coiled coil. Bulky and charged residues at the interface of the two central heptad repeats create asymmetry and a single binding site for an ALIX or TSG101 peptide. Both ALIX and ESCRT-I are required for cytokinesis, which suggests that multiple CEP55 dimers are required for function.

Challenges in the Structure Determination of Penicillin Binding Protein A from *Mycobacterium Tuberculosis*

¹Alena Fedarovich, ²Robert A. Nicholas and ¹Christopher Davies
¹ Dept. of Biochemistry and Molecular Biology, Medical University of South Carolina, Charleston, SC, 29425 USA; ² Dept of Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC, 27599 USA.

Penicillin-binding proteins (PBPs) are bacterial enzymes responsible for the final stages of cell-wall peptidoglycan synthesis and are the targets for β -lactam antibiotics. *Mycobacterium tuberculosis* (*Mtb*) H37Rv, expresses four major PBPs, three of which are associated with the antibacterial activity of several β -lactams (Chambers H, 1995). The smallest of these and hence the most amenable for structural studies is PBPA, a Class B PBP of molecular weight 51.6 kDa. The determination of its three dimensional structure would provide the basis for rational drug design against *Mtb*.

The structure of *Mtb* PBPA was solved at 2.05 Å resolution by multiwavelength anomalous dispersion (MAD). MAD data were collected from crystals of selenomethionine-substituted protein containing an additional methionine (in place of leucine) to supplement the five existing methionines. Phasing was complicated by the presence of pseudo-translational symmetry that appeared to mask twinning. The way this problem was overcome will be described. Challenges with refolding, purification and crystallization of the native and selenomethionine proteins will also be discussed.

Chambers H.F. et al., Antimicrob Agents Chemother (1995), 39: 2620-2624

FAQ on χ^2 at ID

Zheng-Qing Fu

SER-CAT, APS, Argonne National Laboratory, Department of Biochemistry & Molecular Biology,
Athens, GA 30602. email: fuzq@anl.gov

In the past a few years, some users experienced and reported bumpy spatial χ^2 when using HKL2000/DENZO to process their data collected at 22ID. To address this concern, control tests were performed at randomly selected times during the last run of 2008 to diagnose the potential problems. The tests results will be presented and discussed.

Virtual Synchrotron

John Chrzas, James Fait, John Gonczy, Zhongmin Jin, Albert Fu, John Rose, and BiCheng Wang
SER-CAT University of Georgia, Argonne National Laboratory

From its inception, SER-CAT has been working towards the concept of “Providing Light When YOU Need It!”. The ultimate goal of this project has been to provide the SER-CAT membership with their own virtual synchrotron, which could be integrated into their daily work much like the x-ray lab down the hall. Over the last year or two the remote access program has been developed into a good reliable attended capability. Recent developments on automating the entire data collection experience from mounting – alignment – data collection – data processing will be presented. SER-CAT is very close to achieving its goal of “Providing Light When YOU Need it!”.

Data Collection Automation at SER-CAT

Richard L. Walter & Gina M. Ranieri
Shamrock Structures LLC, Woodridge, IL

Improved efficiency in data collection is a major focus of synchrotron beam line development throughout the world. In its ultimate distillation, multi-million dollar detectors that purport the collection of every sample placed on the spindle as “efficiency” are gaining acceptance. However, for several reasons, this approach seems less than efficient (to be kind). Alternatively, methods to identify and improve upon specific areas of known inefficiency in data collection seem to be a more cost effective means of truly improving data collection efficiency.

The efficiency of any diffraction experiment can be altered at numerous points, from those under beam line to control to those dependent upon the practices and preferences of individual experimenters. Perhaps the most obvious area for improvement is limiting the duty cycle required to get each frame of

collected data “in the bank”. Improved beam line flux and detector read out times have superbly optimized this part of the experiment to the point where it is almost hard to imagine that any other efficiencies remain to be gained. Another area of major time loss, the mounting of fresh samples, has been wonderfully overcome by facilities like SER-CAT where highly efficient and almost flawless sample automounters have become a part of the standard data collection protocol of most experimenters.

The SER-CAT staff has now turned its attention to the next obvious area of improved efficiency upon which it has some control: sample-to-sample screening. Over the past year as we have evaluated the slowdowns on these otherwise efficient lines, our conclusions about limited efficiency continued to come down to one area: the time needed to mount, center, and check each and every sample for quality using diffraction. In our experience, despite great advances in sample changing and point-and-click centering, the greatest proportion of “lost” time was still coming from this screening portion of the experiment.

To address this outage, the SER-CAT staff has implemented a new automated screening protocol that will also, ultimately, serve as a platform for fully automated data collection. This presentation will highlight the operation of this promising new technology on the SER-CAT beam lines, pointing out both the strengths and weaknesses of the current implementation. We have been utilizing the system for several months and believe that the tools provided can be very useful to experimenters who require high efficiency in data collection either due to high sample volume or simply because they do not wish to spend the 24-48 hour grinding shifts that are the historical hallmark of the synchrotron trip.

Opportunities for Macromolecular Research from the APS Renewal

Mark A. Beno

X-ray Science Division, Advanced Photon Source, Argonne National Laboratory,
9700 S. Cass Ave, Argonne, IL 60439

Soon after the Advanced Photon Source (APS) began operations in 1996, two macromolecular beamlines, the Structural Biology Center (SBC) and BioCARS, were among the first operational facilities and contributed greatly to the early success of the facility. The number of sectors devoted to macromolecular crystallography (MX) has continued to grow since then. With the addition of the Life Sciences Collaborative Access Team to this community, there are currently 8 sectors with a total of 21 beamlines (17 operational) devoted to MX at the APS. Each year approximately 3500 users come to the APS facility for x-ray experiments that result in the publication of more than 1000 papers annually, more than 45% from MX beamlines. The APS MX community has deposited more than 6300 structures in the Protein Data Bank and has maintained a rate of more than 1000 structures deposited/year for the last three years.

The core of the APS is a high-energy (7-GeV) electron accelerator that feeds a 1.1- km-circumference storage ring where undulators and bending magnets deliver x-rays to more than 60 independently operating beamlines. Although the APS accelerator has delivered unprecedented reliability (x-ray

availability >97% and mean time between faults > 85 hours for the past five years), recent operational experience shows that the accelerator as well as some of the original beamlines are facing serious obsolescence and maintenance issues. To continue to operate reliably the APS requires a major renewal of both the accelerator and many beamlines that would bring the facility to the state-of-the-art. Innovations in instrumentation, x-ray optics, and x-ray sources could generate orders-of-magnitude improvements in sensitivity and precision, and deliver new capabilities, thereby enabling experiments that could not be accomplished today. Greatly enhanced flux and beam stability are examples of the possible benefits for the MX beamlines.

A Tale of Two PCNAs: Structure Determination of DNA Replicative Proteins from 'Hot' and 'Cold' Archaea

Miranda L. Byrne-Steele, Ronny C. Hughes, Damien Marsic, Edward Meehan, and Joseph D. Ng
Laboratory for Structural Biology, University of Alabama in Huntsville, 301 Sparkman Dr. Huntsville,
AL 35899

The process of DNA replication in all organisms is complex and requires the concerted action of multiple proteins in order to achieve faithful replication of the genomic template. Since DNA replication is essential for the reproduction of an organism at any given temperature, proteins involved in this process represent ideal targets for comparative structural analysis in order to gain insight into the molecular basis of thermal stability. To this end, we have determined the crystallographic structure of two classes of proteins central to the DNA replication machinery from a hyperthermophilic marine archaeon *Thermococcus thio-reducens* and a psychrophilic archaeon *Methanococcoides burtonii* DSM 6242. To-date, the crystal structure of a family B DNA polymerase from *T. thio-reducens* (TtpolB) has been solved to 2.0 Å. In addition, the structure of the homotrimeric proliferating cell nuclear antigen (PCNA) homolog or DNA sliding clamp from this organism (TtPCNA) has also been solved to 1.86 Å. In order to provide a comparison between proteins from two differing environments, the structure of the PCNA from the psychrophilic archaeon *M. burtonii* DSM 6242 (MbPCNA) has been determined to 2.4 Å. Structural analysis among the selected targets relative to their counter temperature homologs reveals high topological equivalence with variable sequence identity. Molecular regions that may be important for thermal stability are identified and discussed.

Structural Studies of the Hepatitis B Virus Surface Protein (HBsAg) Aimed at Developing Next Generation Vaccines: a Progress Report

Quentin Florence¹, Hao Xu¹, Jonny Yokosawa², James Lara² and Yury Khudyakov², and John P. Rose¹.

¹Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA 30602 and

²Epidemiology & Bioinformatics, Laboratory, Division of Viral Hepatitis, Centers for Disease Control and Prevention, Atlanta, GA 30333.

The purpose of this study is to derive a 3-dimensional structure by X-ray diffraction of the HBsAg protein, which is the antigenic component of the currently licensed vaccine for hepatitis B virus (HBV). Despite its worldwide usage and its application as a carrier for epitope presentation, the tertiary structure of this important protein is still unknown. This lack of information has impeded the development of bivalent vaccines where HBsAg is used as a carrier for foreign antigenic epitopes. Additionally, the lack of this conformational information does not allow for understanding the effects of mutations, especially polymerase drug-resistance mutations in the region of the HBV genome overlapping with the HBsAg open reading frame, on antigenic properties of the HBsAg major neutralizing antigenic epitope.

The first step in the X-ray structure determination process is the production of crystals of the protein under study. Initial crystallization trials on the HBsAg protein provided by the CDC have produced some promising hits. Results from the initial crystallization screen together with plans on crystal optimization will be presented.

Work supported by a GRA/CDC Collaboration Planning Grant GRA.VAC09.G.

Active-Site Interactions and the proposed catalytic cycle of *Thermococcus Thioreducens'* Inorganic Pyrophosphatase

Ronny C. Hughes, Miranda Byrne, Ernie Curto, Steven Tomanicek, Leighton Coates, and Joseph D. Ng

Laboratory for Structural Biology, *University of Alabama in Huntsville*, MSB 203C, Huntsville, AL 35899, USA

Inorganic pyrophosphatase catalyzes what would seem to be a simple phosphoryl transfer reaction, the hydrolysis of the pyrophosphate bond. However, the active site of this enzyme displays an elaborate network of non-covalent interactions consisting of hydrogen bonds and ionic interaction between 3-4 divalent metal cations, water molecules, protein groups, and the substrate. Furthermore, the enzyme functions as a homohexamer which acts in a highly coordinated manner. For these reasons, deciphering the structural mechanism of the catalysis has been quite challenging and remains unclear despite much

effort and the availability of a large body of biochemical and structural information. Here we report the most extensive collection of X-ray crystal structures thus far obtained for the enzyme. Using high throughput techniques adapted from structural genomics, a total of 4992 initial crystallization trials were conducted using a combinatorial approach consisting of purified protein, pyrophosphate, six different divalent metal cations, substrate analogs, and several known inhibitors. To date, crystal structures have been determined for sixteen unique complexes including a calcium inhibited enzyme-substrate complex and several reaction intermediates determined at near atomic resolution as high as 0.9Å resolution and are being used in effort to decipher the molecular reaction mechanism of the enzyme. Crystals as large as 8mm³ of the protein have also been obtained and deemed usable for neutron crystallography. The neutron structure will provide unambiguous hydrogen positions useful in determining the protonation states of the active site water molecules and the surrounding protein groups needed to decipher the complete catalytic cycle of the enzyme.

Crystal Structure and Biochemical Characterization of *Mycobacterium tuberculosis* Ribokinase (Rv2436)

Eugene Masters, Yanjie Sun, Yimin Wang, William Parker, Rongbao Li
Drug Discovery Division, Southern Research Institute, Birmingham, Alabama

Ribokinase (RK) catalyzes the phosphorylation of ribose to ribose-5-phosphate using ATP and functions as a key enzyme in ribose metabolism. RK belongs to the PfkB family of carbohydrate kinases. The *Mycobacterium tuberculosis* (Mtb) gene Rv2436 has been tentatively annotated as a ribokinase based on its sequence similarity with other ribokinases. In order to confirm the identity of Rv2436 as a ribokinase and to better understand ribose metabolism within the bacterial cell we have expressed, purified and characterized the protein biochemically and structurally. The crystal structure of Rv2436 in complex with ATP analog (AMP-PNP) and ribose has been determined to 2.0 Å resolution. The structure contains two molecules within the asymmetric unit. Each monomer in turn forms a dimer around a crystallographic axis through the interaction of lid-like domains. This lid domain can cover the substrate binding cleft of the catalytic body of the enzyme. The Mtb RK structure exhibits high overall structural overlap with other previously determined ribokinases and also conservation of catalytically important residues within the binding cleft. Additionally, biochemical studies confirm its preference for ribose as a substrate; consist with other organisms. One notable difference between previously solved RK structures and the Mtb RK structure has to do with the conformation of the lid domains with respect to the catalytic body. Previously solved RK structures consist of either Apo forms in which both lids of the dimer are in an open arrangement or substrate bound complexes in which both lids of the dimer cover the substrate binding cleft in a closed conformation. Within the Mtb RK structure, ribose is bound into one monomer with the lid domain tightly closed over the substrate binding cleft while in the other monomer the lid is in an open conformation with AMP-PNP bound into the binding cleft.

Determining the Function of the N-terminal Domain of AmiC, an Essential Cell Division Protein in *Neisseria gonorrhoeae*

Rosanna Robertson,¹ Robert Nicholas,² and Christopher Davies¹

¹Department of Biochemistry and Molecular Biology, Medical University of South Carolina, Charleston, SC, ² Department of Pharmacology, University of North Carolina, Chapel Hill, NC

Gonorrhea is the sexually transmitted infection caused by the Gram-negative bacterium *Neisseria gonorrhoeae*. Due to widespread emergence of antibiotic resistance, only a single class of antimicrobials, the cephalosporins, remains to treat gonorrhea. Cephalosporins disrupt bacterial cell wall biosynthesis, a complicated process requiring concomitant breakdown and synthesis of cell wall peptidoglycan. One enzyme involved is the peptidoglycan hydrolase AmiC, an *N*-acetylmuramyl-L-alanine amidase. Mutation or deletion of AmiC results in improper cell separation during division and increases susceptibility to a number of antimicrobial agents. The overall goal of this project is to evaluate AmiC as a prospective antimicrobial target through biochemical and structural studies and to identify inhibitors of AmiC through chemical compound library screening. Previously, we have expressed and purified full-length AmiC, as well as the catalytic C-terminal domain. Sequence alignments, site-directed mutagenesis, and ICP-MS have revealed that AmiC is a zinc-dependent amidase. Lytic activity of the constructs has been confirmed by turbidity, zymogen, and fluorescent peptidoglycan assays. Recently, a small N-terminal domain construct has been expressed and purified. Although the exact function of the N-terminal domain is unknown, it might target AmiC to the peptidoglycan during cell division and, possibly, bind directly to the peptidoglycan. GST-pulldown and peptidoglycan binding assays have yet to reveal any direct interaction between the N-terminal domain construct and other division proteins or with peptidoglycan. Surprisingly, preliminary activity assays reveal that the N-terminal domain, alone, is active against peptidoglycan substrate. We have obtained crystals diffracting to 2Å and belonging to the P2₁2₁2 space group. Efforts to phase the structure by heavy atom methods are underway. Future studies will include continued crystallization trials and high-throughput assay development.

Signal-Based Data Collection: An Approach to Automated Data Collection Aimed at Increasing Structure Solution Success

John P. Rose, John Chrzas, Zheng-Qing Fu, James Fait and Bi-Cheng Wang.
SER-CAT, Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA 30602.

Aided by technological advances in molecular biology, synchrotron X-rays, crystal cooling, X-ray detectors, phasing methods and structural genomics (notably high-throughput methods related to all aspects of the structure determination process) there has been an explosive growth in protein structure determination over the last decade. It is estimated that fully 80% of new structures are determined using

synchrotron X-rays and many facilities are planning beamline upgrades or completely new beamlines (at considerable cost) to meet increasing demands for beam time. We believe there is another and more cost-effective approach - increase beamline structure determination efficiency.

To do this we are developing an innovative system for automatic and cost-effective data collection and structure determination we term Signal-Based Data Collection (SBDC). SBDC represents a new paradigm in that data collection is driven and directed by feedback from the data reduction and structure determination processes. The goal of SBDC is to automatically (without user intervention) collect enough data from one or more crystals to ensure that the anomalous scattering signal in the final scaled data is sufficient to solve the structure. Details of the SBDC approach and current status will be presented.

Preparation of the Ebola Virus Glycoprotein for Structural Characterization Related to Development of a Multiepitope Vaccine

Dayong Zhou¹, Hao Xu¹, Frank Michel², Jeff Hogan², and John Rose¹. ¹Department of Biochemistry and Molecular Biology, Franklin College and ²Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia, Athens, GA 30602 USA.

Ebola virus is a filovirus containing a single stranded 19 kb, negative sense RNA. The genome encodes 7 structural proteins and one non-structural protein.

NP - VP35 - VP40 - GP/sGP - VP30 - VP24 - L

The (GP/sGP) glycoprotein, a target for vaccine development, is an integral membrane protein, which is processed to form the mature Gp₁/Gp₂ heterotrimeric spikes observed on the virus surface.

We have currently expressed and purified the intact 644-residue Ebola virus glycoprotein (GP) and the 295-residue soluble glycoprotein variant (sGP) in *E. coli*, insect and mammalian cell lines in preparation for crystallization trials. Initial results will be presented.

Developing a Low Cost Puck Based Crystal Loading and Shipping Systems for SER-CAT

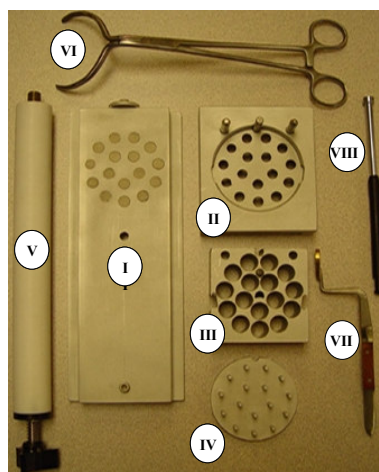
James Tucker Swindell II, John Rose, and Bi-Cheng Wang.
 SER-CAT, Department of Biochemistry and Molecular Biology,
 University of Georgia, Athens, GA 30602.

SER-CAT has developed two sets of low cost tools allowing more of its members access the beamlines remotely for crystal screening and data collection.

The SER-CAT Puck Loading Kit:

- 1 magnetic slide base (I)
- 1 puck mount (II)
- 1 guide puck (III)
- 1 recovery wafer (IV)
- 1 screw down separator (V)
- 1 bent hemostat (VI)
- 1 bent cryovial holder (VII)
- 1 Hampton crystal wand (VIII)
- 1 instructional CD

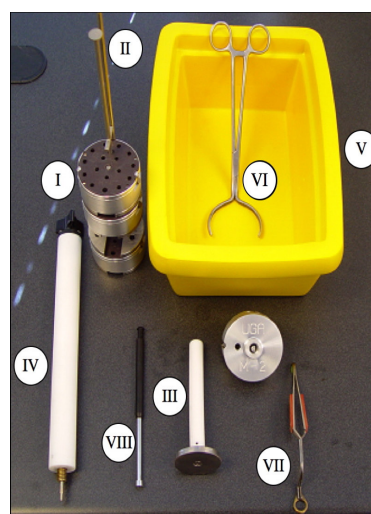
The SER-CAT Crystal Loading Kit.



The SER-CAT Crystal Shipping Kit:

- 4 ALS style pucks (I)
- 1 shipping cradle (II)
- 1 magnetic puck holder (III)
- 1 puck separator (IV)
- 1 foam dewar (V)
- 1 bent hemostat (VI)
- 1 bent cryovial holder (VII)
- 1 Hampton crystal wand (VIII)
- 1 instructional CD

The SER-CAT Crystal Shipping Kit.



A price for the SER-CAT Puck Loading Kit has not been determined yet but will be available for purchase within the month. The price of The SER-CAT Crystal Shipping Kit including shipping and handling is \$1500. If you are interested in purchasing a kit please contact John Rose (rose@bcl4.bmb.uga.edu).

Participants

Name	Email
Byrne-Steele, Miranda	byrneaml@email.uah.edu
Beno, Mark	beno@anl.gov
Chen, Liqing	chenlq@uah.edu
Chen, Lirong	lchen@secsg.uga.edu
Chrzas, John	chrzas@anl.gov
Coates, Leighton	coatesl@ornl.gov
Davies, Christopher	davies@musc.edu
DeLucas, Larry	duke2@uab.edu
Elliot, Amicia	adelliott@gmail.com
Enemark, Eric	eric.enemark@stjude.org
Fedarovich, Alena	fedorove@musc.edu
Florence, Quentin	qflo@secsg.uga.edu
Fu, Guoxing	gfu1@student.gsu.edu
Fu, Zheng-Qing (Albert)	fuzq@anl.gov
Furey, William	fureyw@pitt.edu
Hughes, Ronny	ron618_331@yahoo.com
Lee, Hyung Ho	leehyung@nidk.nih.gov
Liang, Wenguang	wgliang@yahoo.com
Liu, James	zjliu@ibp.ac.cn
Masters, Eugene	masters@sri.org
Medina, Catherine	kmorris@bcl4.uga.edu
Meehan, Edward	meehane@uah.edu
Newton, M. Gary	newton@secsg.uga.edu
Ng, Joseph	ngj@email.uah.edu
Pemble, Charles	Charles.Pemble@stjude.org
Pusey, Marc	marc@extremozyme.com
Qiu, Li	lq0001@uah.edu
Ranieri, Gina	gina.ranieri@gmail.com
Robertson, Rosanna	robertro@musc.edu
Rodgers, David	david.rodgers@uky.edu
Rose, John P.	rose@BCL4.bmb.uga.edu
Rose, Robert	bob_rose@ncsu.edu
Ruble, John	johnruble@knology.net
Rydel, Timothy	timothy.j.rydel@monsanto.com
Sugitani, Norie	sugitan@uah.edu
Swindell, II James Tucker	jtswinde@secsg.uga.edu
Toh, Diana	tohd@email.uah.edu
Tomanicek, Stephen	tomaniceksj@ornl.gov
Volz, Karl	kvolz@uic.edu
Walter, Richard	rwalter@shamrockstructures.com
Wang, Bi-Cheng	wang@BCL1.bmb.uga.edu
Weber, Irene	iweber@gsu.edu
White, Stephen	stephen.white@stjude.org
Wilson, Randall	wilsonrc@uah.edu
Xu, Hao	xhao@secsg.uga.edu
Yen, Sandra	sandrayen103@yahoo.com
Yu, Xi Xia	xyu3@student.gsu.edu
Zhang, Ying	y Zhang20@student.gsu.edu
Zhou, Dayong	dzyzhou@uga.edu