



The SER-CAT SPECTRUM

Biannual Newsletter of the Southeast Regional Collaborative Access Team · Vol. 8, No. 2 · Winter 2010



Director's Message

Bi-Cheng Wang

Season's Greetings! Wishing you a Happy Holiday and a Prosperous New Year!

Looking ahead to 2011, I would like to invite each of you to Raleigh, NC for the 8th Annual SER-CAT Symposium on Friday, March 4th.

The continuing focus of the Symposium is "Interesting Structures, Interesting Methods and SER-CAT Advances". SER-CAT's annual board meeting, which is fully open to the membership, will be held on Saturday, March 5th. Both meetings are being hosted by Professors Robert Rose and Carla Mattos at North Carolina State University.

Nominations are being requested for the SER-CAT Young Investigator Award and the SER-CAT Outstanding Science Award. Please send your nominations to Professor John Rose in the SER-CAT Administrative Office before January 10th.

I am pleased to inform you that our staff members have had a very productive year in carrying out SER-CAT upgrades. The installation of the microdiffractometer MD2 was completed in October. Improvements have also been made in automated optimization of X-ray beams, automated sample alignment and command latency in remote data collection. (Please see Dr. John Chrzas' report on page 2.)

As reported in our last *SER-CAT Spectrum*, NCR/NIH awarded our detector upgrade application. A detector committee was formed to make a final evaluation of the type of detector most suitable for SER-CAT users. The committee members are Drs. Fred Dyda (Chair, NIH SER-CAT Board Member), William Furey (Pennsylvania SER-CAT Board Member), Nukri Sanishvili (GM/CA-CAT, external reviewer), John Chrzas (SER-CAT Operations), and John Rose (SER-CAT Administration). We anticipate that a final decision will be available soon after the New Year.

I hope you will enjoy reading this issue of the *SER-CAT Spectrum*, including the special research reports by Fred Dyda (page 3) and Peter Horanyi (Page 4) on some interesting findings in their recent publications.

Best wishes,
B.C.

8th SER-CAT Symposium and Board Meeting

March 4-5, 2011

North Carolina State University,
Raleigh, NC



Hosted by Professors Robert Rose and Carla Mattos

For current registration and meeting information, go to the meeting web site at:

<http://xrc3.bch.ncsu.edu/sercat>

Nominations, Please

Nominations for the two annual SER-CAT Awards are requested. Please send your nominations for the 2011 SER-CAT Young Investigator Award and/or the Outstanding Science Award to **John Rose** before January 10, 2011. For detailed information, click on one of the links below:

[2011 SER-CAT Young Investigator Award](#)

[2011 SER-CAT Outstanding Science Award](#)

or

Send [John Rose](#) an email.

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SER-CAT Advances

MD2 Update

John Chrzas

In the last *Spectrum*, we reported that the MD2 micro diffractometer installation on 22ID would occur in the August to October timeframe. I am happy to announce that the installation and commissioning of the MD2 was accomplished with minimal problems. The system has been fully integrated within the *SERGUI* control environment with little impact to the user community. The MD2 installation at SER-CAT is unique in that the instrument is controlled directly by *SERGUI* eliminating the Windows control software supplied by Maatel, which is used by other beamlines.

The MD2 provides for 2 types of beam shaping apertures: a set of single 5, 20 and 50 micron pinholes and a triple aperture system having 10, 20 and 50 micron pinholes. The triple aperture was installed during commissioning and is currently in use. We have not had the opportunity to verify the usefulness of the single 5 micron aperture. The user can select any of the three supplied apertures within *SERGUI* on either the Hutch or Sample pages. Fine positional adjustments of these apertures can be accomplished by using the **Pin Hole** pull down in the setup menu.

SERGUI's beam optimization protocols have been modified to allow for independent alignment of the aperture in both the horizontal and vertical directions. Users will see a new "**Table Button**" which will automatically align the MD2 table to the full beam. Table alignment is carried out by first moving the aperture and beam stop out of the beam path and placing a YAG crystal in the beam to visualize the beam's position. **NOTE: the user must remove their crystal from the goniometer before table alignment is initiated.** Images of the YAG crystal are then recorded using the MD2's on-axis camera at a zoom of 1x and 2x. Optical analysis software is used to position the table using the two images.

SERGUI has also been modified to automatically optimize the beam if the user changes the energy by more than 500 eV using the following protocol:

1. Monochromator Tuning
2. Table Alignment
3. Vertical pin hole scan
4. Horizontal pin hole scan

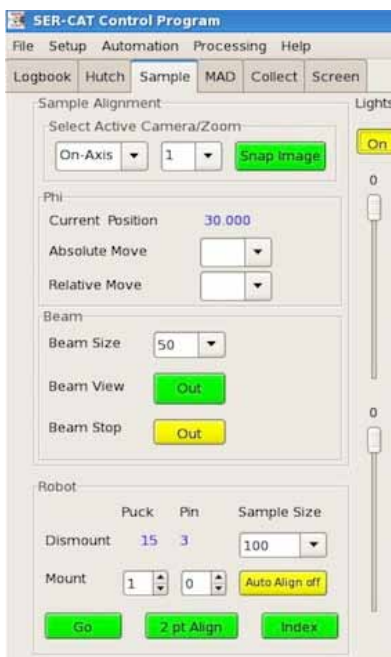
If any of these steps fail, the process will stop and the user will be informed about the source of the problem. If a crystal is mounted, then only the monochromator tuning will be carried out since the other steps would require the removal of the sample. Automatic beam optimization has been implemented on both 22ID and 22BM and, after some growing pains, has developed into a very useful feature.

Improved Sample Alignment

The most drastic *SERGUI* modifications were on the Sample Page. The input from a number of users was accumulated and their comments were the motivating force behind most of the following changes. The left hand section of this page now has two primary functions: Sample Alignment and Robot.

Sample Alignment

The "Select Active Camera/Zoom" section allows the user to easily change cameras as well as zoom value. *SERGUI* has been modified to allow for 1x, 2x, 4x, and 8x optical zooms of the MD2 on-axis camera. The optical zooms have been calibrated using an optical grating with 50 micron divisions. The 1x zoom has a 2 mm horizontal field of view while the 8x zoom has a 250 micron horizontal field of view. In addition, the light at each zoom value is now automatically adjusted for optimal contrast. The current camera digital zooms have been setup to provide 1x, 2x, 3x and 4x zoom.



The green **2 pt Align** button has also been moved to this section. Two-point alignment allows the user to align their sample using two clicks of the mouse as illustrated below.

1. Push the **2-pt Align** button
2. Left mouse click the crystal's center on the camera display
3. After phi rotates to the second phi position, left mouse click on the crystal's center on the camera display

The sample will move and then rotate back to the original phi position, which should give

the user some indication of the results of the alignment procedure.

The two-Point alignment procedure uses the fact that z (horizontal) is always uniquely defined with any camera orientation. Therefore, two different phi values provide enough information to solve the parametric equations for x and y (which are always the vertical). Any phi value can be used as the starting point for this process and under most circumstances the second phi value will be 90 degrees from the first. However, since the parametric equations used in the analysis have cosine and sine terms, if the second phi value saddles a symmetry point for the cosine term (in the denominator), then the second value will be 70 degrees from the first.

The "Phi" section now uses pull down options for selecting orthogonal orientations to the on-axis camera (+X, -X, +Y, -Y);

the previous version of *SERGUI* used independent buttons and allowed users relative moves of phi by ± 10 degrees. The new version provides options for up to 360 degrees of phi rotation.

Automated loop alignment has returned in two different incarnations: user initiated using the green **Go** button and automatically initiated during the crystal mounting process using the **Auto Align** toggle. The green **Go** button is located at the bottom of the Robot section next to the **2-ptAlign** button. When this button is pressed the software will perform two rounds of loop alignment using the 1x and 2x camera zoom settings. During the auto alignment process, the system will use the "Box Size" setting to limit the optical search path for the loop. Thus, it is important to provide the proper loop size if you are using automated loop alignment. For example, if you use a "Box Size" setting of 100 m and your loop is larger, you will end up aligning to the tip of the loop not the crystal! Also, if you carefully match the "Box Size" to the loop size your loop will be better centered.

Automated loop alignment can also be included in the crystal mounting process. This is achieved by toggling the normally yellow **Auto Align off** button in the Robot section of the Sample page. If this button is toggled, then the color of the button will change to green and display **Auto Align On** and the system will attempt to auto align your loop after a successful robot mount. This capability can be a time saver if your remote connection is experiencing a latency problem.

Improving Remote Latency

While this may not be the right place to talk about latency issues during remote data collection, it is a problem for some users and there may be an easy fix. The compression used by NX is determined during the setup process (before you log in). If you are experiencing a latency problem, logoff and go into your NX setup menu. At the bottom of the setup menu will be a slider bar for the type of connection you have. This slider controls the compression setting for NX, so if you select Modem, the system will apply maximum compression, which may help your latency problem.

The latency problem is due to the camera displays within the python code for *SERGUI*. We are currently working on a number of projects in an attempt to improve this process by:

1. Improved python programming
2. Replacing the python code with C code
3. Looking into a web based interface for sample alignment

We will keep you updated on the status of these projects.

SER-CAT Spotlight

G-Domain Dimerization is the Key to Dynamin's Stimulated GTPase activity **Fred Dyda, Ph. D.**



Dynamin is a large, atypical GTPase that plays an important role in clathryn-mediated endocytosis. Dynamin assembles to form collars at the necks of clathryn-coated invaginated pits and catalyzes the fission of these membrane necks as it hydrolyses GTP. Neck fission is essential so that the vesicles can move into the cell. While isolated dynamin tetramers have an appreciable basal

GTPase activity, this is stimulated several hundred-fold upon oligomerization into larger structures; in EM images of dynamin tetramers assembled on lipid tubes, these look like rungs of a ladder.

We have been interested in establishing the structural basis of dynamin's assembly stimulated GTPase activity using X-ray crystallography. Unfortunately, full-length dynamin has thus far been recalcitrant to crystallization. The key to our recent success was the design of a dynamin construct in which its oligomerization, membrane binding, and proline-rich domains were deleted, while keeping those parts of the molecule known to be important for GTPase activity. The resulting protein was monomeric but formed stable dimers when complexed with the transition state analog GDP.ALF4 and crystallized readily. The structure was solved with diffraction data collected at the ID22 beamline of SER-CAT (also using some in-house data), and was refined with the ID22 data at 2.0Å resolution.

GTPases often use an arginine residue (the so-called "arginine finger", sometimes provided by another effector molecule) to speed up the GTP hydrolysis reaction. The basis of this effect is that arginine provides a positive charge close to the scissile phosphate thereby stabilizing the developing negative charge at the transition state. It had long been assumed that dynamin would also use one of its own basic amino acids to achieve the stimulated level of GTP hydrolysis, but the identity of the residue remained obscure. Surprisingly, it appears from our structure that dynamin solves the problem of stabilizing a negative charge buildup by dimerization as this creates a monovalent cation binding site next to the scissile phosphate. Superposition of our structure with other GTPases revealed that the monovalent cation bound to dynamin (a Na⁺ ion) occupies a very similar position to that of the guanidinium head groups seen for arginine fingers.

Another interesting feature of the dynamin structure is the location and orientation of the Bundle Signaling Element, or BSE. This module is formed by 3 alpha helices contributed from different regions of the molecule: one from the N-terminus of the G domain (the domain that binds and hydrolyzes GTP), one from its C-terminus, and the third provided by the so-called GTPase effector domain (or GED). The GED is encoded by a sequence far downstream in the primary sequence from the G domain. In the crystallized construct, the intervening sequences were deleted and replaced by a short linker. We observed that the BSE forms a well-defined structure and explains why the GED is needed for the structural integrity of the G domain. Furthermore, the orientations of the two BSEs of the individual G domain monomers define the relative orientation of the membrane and the G domains. It is possible that changes in BSE orientation as a result of G domain dimerization is an important mechanism for transmitting conformational information toward the membrane.

Dr. Fred Dyda is senior investigator at the Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892

Recent SER-CAT Structure

PDB# 3LWO

J. Zhou, C. Lv, B. Liang, M. Chen, W. Yang,
H. Li
J. Mol. Biol., 401, 690 (2010)



RESEARCH FOCUS

The Lattice Effect

Dr. Peter Horanyi



Well-refined and validated X-ray crystal structures are considered accurate representations of the atoms in the crystal. But does a protein trapped in the lattice of the crystal accurately represent the structure of the protein, free from the lattice, in solution?

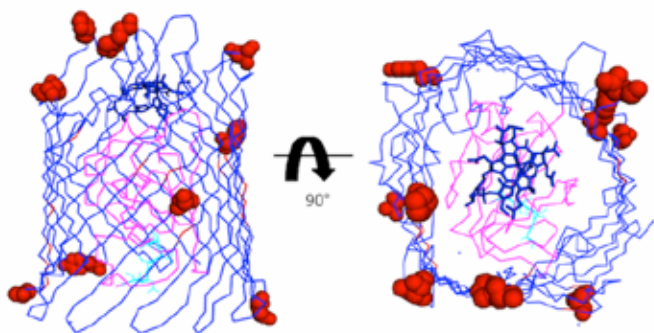
A macromolecule in a crystal is in a very different environment than it is in solution. The lattice, that manifests itself most obviously in intermolecular

crystal contacts, imposes a force upon the contents of the unit cell which differs from that in free solution. This force, in turn, gives rise to lattice energy. What is the magnitude of this lattice energy? And, what effect, if any, does it have on the crystal structures that we determine?

Our paper [Freed *et al.*, *Biophysical Journal* 2010 Sep 8;99(5):1604-10.] presents the experimental determination of the lattice energy for a crystal of an integral membrane protein, the bacterial outer membrane corrinoid transporter BtuB, and describes the effects of this lattice energy on the conformation of the protein. Naturally, a stabilizing force originating from the crystal lattice is expected at the crystal contacts. In addition to this, however, residues greater than 17Å away from the lattice contacts, on another domain that bears no such contacts, are influenced by the lattice. X-ray crystallography and Site Directed Spin Labeling Electron Paramagnetic Resonance (SDSL-EPR) spectroscopy were used to measure the lattice effect. EPR is exquisitely sensitive to nanosecond-scale dynamics of amino acids labeled with paramagnetic spin labels, introduced by reaction with cysteine point mutants individually placed at strategic positions in the protein (using the existing crystal structure as a guide). In one method of spectral analysis, the line shape is modeled as two dynamic species in equilibrium: one at the rigid (near-stationary) limit, and the other completely mobile. The ratio of these yields an equilibrium constant, and the variation of equilibrium constants in different samples (crystalline versus solution) yields the free energy difference $\Delta\Delta G$ between two environments. Using SDSL EPR we analyzed crystals of spin labeled BtuB, along with protein in the crystallization solution or in buffer, stabilizing lattice energy of 1.5 Kcal/mole was determined.

The impetus for this study came from experiments performed during the last ten years and from discrepancies between experiments performed with different experimental methods. Outer membrane transporters, such as BtuB and others involved in the uptake of various organometallic substrates

Crystal contacts of BtuB



BtuB is made up of 594 amino acids, the amino terminal 135 residues, or the luminal domain, is depicted in pink. The carboxy terminal 459 residues form a 22 stranded beta barrel colored blue. Residues depicted as red spheres are the crystal contacts. The amino terminal portion of BtuB that was spin labeled is colored cyan. The substrate, Cyano-Cobalamin is dark-blue sticks. Figure was made using PyMol.

(primarily those containing iron), possess a conserved amino acid terminal sequence on the periplasmic-facing surface of the transporter. In 2000 and 2003 [Merianos et al., *Nat Struct Biol.* 2000 Mar;7(3):205-9, Fanucci et al., *Biochemistry.* 2003 Feb 18;42(6):1391-400.], colleagues at the University of Virginia used SDSL-EPR to characterize the mobility of this conserved region in the absence and presence of bound substrate. Their data clearly indicated a disordering of the region upon substrate binding. The addition of a spin-label, or any other mutation and/or labeling for that matter, can perturb a structure from its native conformation and can introduce artifacts and some of the mutants in these EPR studies yielded artifactual data. However, following the careful correction for these effects, the order to disorder transition was still quite apparent. Within the same year (2003), the Wiener laboratory solved their first set of BtuB structures [Chimento et al., *Nat Struct Biol.* 2003 May;10(5):394-401.]. The conserved periplasmic region was different in the apo- and holo- structures; however, it was relatively well-ordered in both cases unlike the EPR data previously suggested. Subsequent EPR experiments [Kim et al., *Biochemistry.* 2008 Jan 15;47(2):670-9., Flores Jiménez et al. *Protein Sci.* 2010 Feb;19(2):269-78.] clearly demonstrated the effects of osmolytes (such as those used in crystallization) on the dynamics of this region. In aqueous conditions, order to disorder occurs; in the presence of the PEG used for crystallization, the order to disorder transition is blunted. It was then assumed that the difference in mobility arose solely from osmolyte effects; however, these recent EPR experiments on slurries of crystals showed the additional effect of the lattice, completing the analysis. In our analysis, we were able to determine the structure of the spin labeled outer-membrane protein at 2.5Å resolution

for both the apo and holo forms. We used the SER-CAT BM beamline to robotically screen 80 crystals (stored in pucks), and then collected highest-resolution data on the ID line from the best and largest (50µm) crystals that we had screened on BM. The weekday 12-hour remote access shifts have emerged as the collective favorite for the eight crystallography labs using SER-CAT at the University of Virginia. We have just started to use the newly-installed MD2 microdiffractometer at the ID line, and we can already see a great improvement in the visualization of our smaller crystals.

So, what, if anything, do these results on lattice energy mean to macromolecular crystallographers? Perhaps it is best to combine the lattice energy result with those obtained with different osmolytes. The environment of the crystal is not equivalent to that of a dilute aqueous solution. A protein may often possess solvent-exposed domains or regions that can undergo conformational change during function, and the equilibrium between multiple conformations will be affected by the crystal lattice, osmolytes and any other factors that affect the chemical potential of water in the vicinity of the protein. An interesting question is: Exactly what in vitro milieu best approximates the in vivo environment? For example, the cytosol is very densely packed with protein “solutes”, so perhaps the high osmolytes conditions frequently needed for crystallization best represent the interior of the cell. Future studies on lattice energies of macromolecular crystals, as well as careful comparisons of structures obtained with different experimental methods (for example, crystallography vs. NMR) or structures obtained from different crystal forms, will provide more information on this topic.

Dr. Peter Horanyi is a Research Associate in Prof. Michael Wiener’s laboratory at the University of Virginia Medical School. Daniel Freed is a Graduate Student in Prof. David Cafiso’s laboratory in the Chemistry Department at the University of Virginia. This work was supported by the National Institutes of Health grants Nos. NIGMS 035215 to D.C. and NIGMS 079800 to M. W.



In 2011, may all your structures be very high resolution!

The SER-CAT Spectrum

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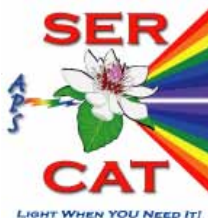
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SER-CAT is supported by the Member Institutions (shown above in the right-hand column), including the Department of Health and Human Services under Contract Numbers N01-CO-56000 and 2515145M and the Georgia Research Alliance. The contents of this publication do not reflect the views or policies of any of the supporting entities, nor does mention of trade names, commercial products or organization imply endorsement by Member Institutions, the U. S. Government or any commercial entities.