NE-CAT COMMUNICATIONS

National Center for Research Resources

A Biannual Newsletter of the Northeastern Collaborative Access Team Winter 2008

A Message from the Director Steven Ealick

Welcome to the Winter 2008 edition of NE-CAT Communications, our bi-annual newsletter. NE-CAT's programs continue to be successful. Both insertion beamlines are in full operation, operating with a high degree of reliability and continue to be heavily scheduled by the users. NE-CAT continues to add new capabilities to its beamlines and the science being pursued by our users and staff remains very strong. The following figure shows a "typical" day at the 24-ID-C user data taking area.

As examples of the type of research being conducted, we have included in this issue short summaries of research recently conducted by Zimmer, Nam, Erlandson, Miller, and Rapoport of the Harvard Medical School and Seranov, Haung and Patel from Memorial Sloan-Kettering Cancer Center.

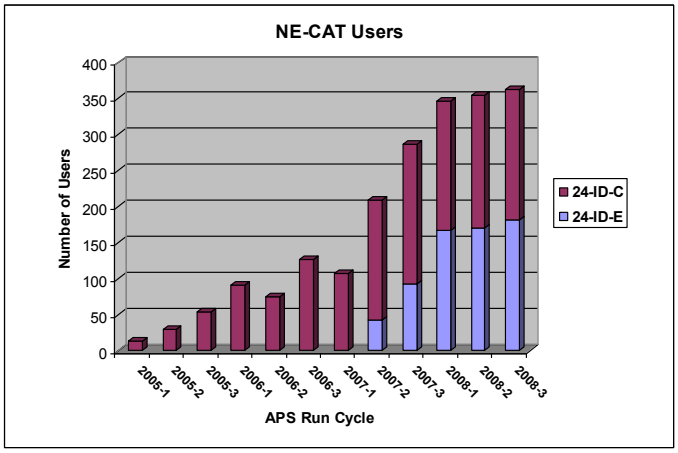
New capabilities for research are continually being added to the beamlines. After waiting 10 months since issue of the procurement, a second MD2 microdiffractometer has finally arrived and is being installed on the beamline. This second MD2 was purchased by the Howard Hughes Medical Institute through a grant to Professor Stephen Harrison, of Harvard. Professor Harrison has chosen to site the MD2 at NE-CAT for use by his research group and the entire NE-CAT user community. This acquisition will further strengthen our capabilities to address challenging crystallography studies, particularly the determination of macromolecular structures using very small, e.g. 5-10 micron, crystals and from crystals that are highly radiation sensitive.

The only potentially dark cloud on the horizon that may impact NE-CAT users during 2009 is the uncertainty of DOE's funding for the APS and, consequently, APS' ability to provide a full 5000 hours of beam time for the user program during 2009. Under the current DOE continuing resolution, APS has reassured its users that the full operating schedule for run 2009-1 will be maintained but APS has developed a contingency plan for a reduced operating schedule for the remainder of 2009 if its budget is reduced. Unfortunately with the change in administration coupled with the current economy problems, it is unclear as to how and when the DOE and APS budgets will finally be resolved.

If you have not taken the opportunity to use NE-CAT's beamlines to date, I encourage you to do so in the future. For further information, please visit our website at http://necat.chem.cornell.edu.

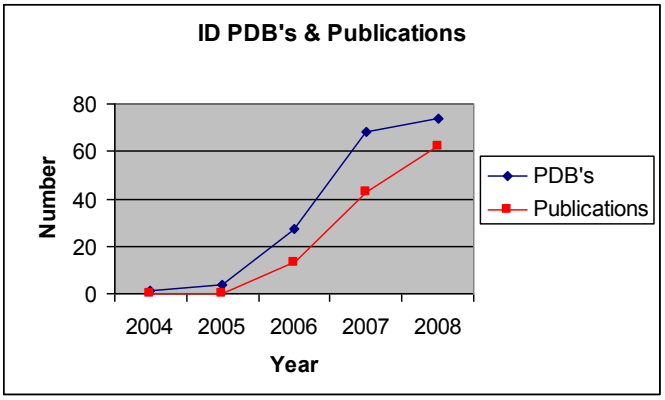
Use of NE-CAT beamlines

Both insertion device beamlines have consistently been fully scheduled for users since the last newsletter. During the 2008-3 run there were 182 users on the 24-ID-C beamline and 181 on the 24-ID-E beamline. The following figure shows the growth of users on the beamlines.



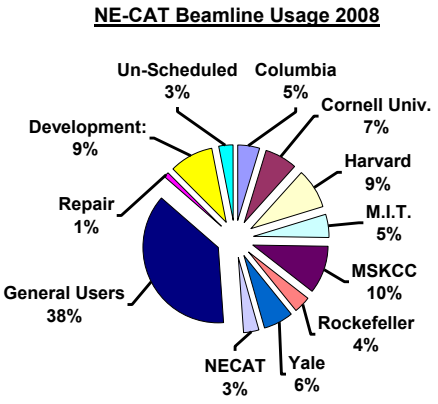
As can be seen from this figure, as the two insertion device beamlines become fully scheduled, the number of users able to use the beamlines reaches an asymptotic limit. To accommodate more users in the future, we are developing productivity enhancement tools such as sample placement robotics systems and new software as well as installing a bending magnet beamline.

As the number of users has increased, the numbers of structures solved and the number of publications have increased commensurately, as evidenced in the following figure.



The blue line shows the number of new structures determined and entered into the Protein Deposition Data Base as a function of year, based on research conducted on both insertion-device beamlines. The red line shows the number of publications.

The following pie chart shows the distribution of users for entire 2008 using both insertion device beamlines.



When deducting the Development" and "Repair" times to obtain the 'available time" for users , the time used by the General User Program is 42%, very close to our target of providing 50% of "available" time to users from the general crystallography community.

Arrival of Second MD2 Microdiffractometer

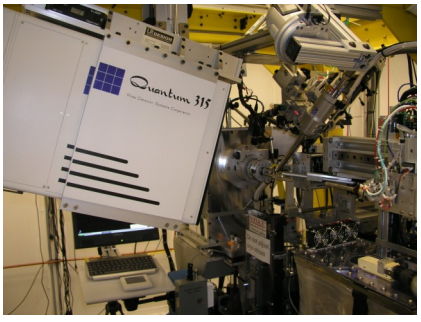
NE-CAT's second MD2 microdiffractometer arrived in early November. This MD2 is nearly identical to the original but also comes with a mini-Kappa goniometer attachment needed by a number of NE-CAT users. The following picture shows the MD2 being mounted on its alignment support stand.

The MD2 was shipped without the proper packing materials to protect it from shock and vibration during the rough handling it experienced during shipment. So a considerable amount of NE-CAT staff time as well as Maatel effort (the manufacturer) was required to test all the critical components for damage and "retune" the air-bearing spindle. In addition to the physical tests that could be performed in the laboratory, the MD2 was mounted on the beamline to conduct early crystallographic tests. To date, within the uncertainties of the measurements that could be made, the MD2 seems to have not incurred any serious damage. We expect that this MD2 will be fully functional and available to users of the 24-ID-C beamline at the restart of APS user operations beginning in early February.

Beamline Developments

A number of new capabilities have been added to the beamlines since the last newsletter.

Researchers desiring to obtain data at the highest resolution possible have requested that we implement the "detector tilt" capability on both beamlines. This capability was engineered into the detector support designs but until recently never fully implemented. The photograph below shows the ADSC Q315 detector tilted to 17 degrees approximately 220mm from the sample position. The extreme limits of tilt are 19



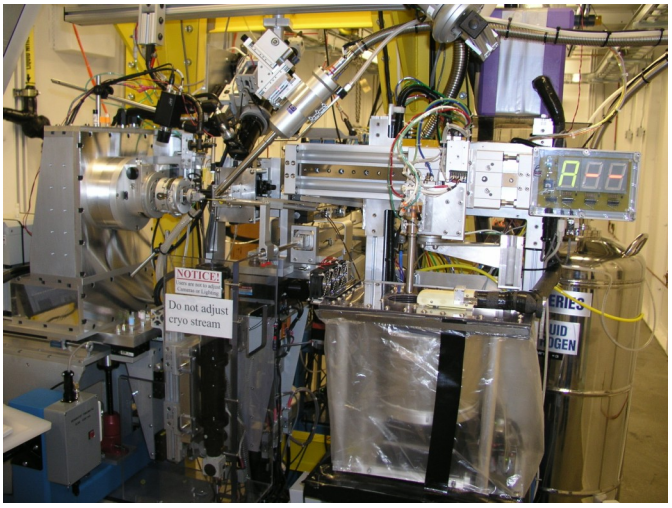
degrees at 200mm and 10 degrees at 900mm from the source. Researchers doing ribosome work generally work at a sample to detector distance of 400mm, where a maximum tilt angle of 16 degrees is now routinely available. For all tilt positions the ray from the sample to the center of the detector is always perpendicular to the detector face. The detector parameters, e.g., tilt angle and distance from crystal, are automatically provided to

the HKL software.

Earlier we had reported that the ALS pneumatic sample placement robot initially used at 8-BM had been rebuilt and installed on the 24-ID-C beamline. Compatibility with 24-ID-C mandated a complete redesign and replacement of all structural elements of the robot except for the support weldment, its table, the sample Dewar, and sample Dewar rotary stage and translator. The following developments were completed in order to insure that the robotic system was fully functional and ready to be used routinely by users.

1. A gravity feed liquid nitrogen sump for filling the sample Dewar at atmospheric pressure was installed to eliminate splashing or convection in the liquid nitrogen sample Dewar.
2. An internally illuminated transparent Dewar lid so that the sample Dewar contents are easily observed, while retaining the requisite insulating properties.
3. A sample emplacement sensor for objective determination of the mount / dismount state of the robot.
4. Console scripting for sequencing interactions between the robot, the Galil goniometer controller and the data collection sequence.

The robot has now been in continual use during the 2008-3 run and found to be highly reliable. Based on the demonstration that its operation is reliable, more and more users are using the robot and beginning to acquire their own sets of pucks and hardware. Users of the robot system have found that the robot approximately doubles their overall logistical efficiency of 24-ID-C, mainly due to the elimination of the time penalties of the hutch door interlock cycle and translation of the detector. Based on the success of this heavily modified ALS designed robot, we plan to shortly build a second robot system for the 24-ID-E beamline. A photograph of the robotic system now in use at 24-ID-C is shown below.



Since these robots will have to interface with the very delicate air bearings of the MD2 microdiffractometers, the "small" two-state pneumatic stage of the robots will be replaced with a proportionally controlled pneumatic stage so that the robot's sample gripper will engage the MD2's magnetic sample mount at near zero velocity and low impact.

We were able to take advantage of "a fire sale" to acquire additional capacity for our HP EVA-5000 data storage system because a large user returned several of these units to the vendor. Acquisition of the second data store adds 23TB more of storage to the original 30TB. Each unit is powered by separate UPS power supplies providing total redundancy. The GPFS operating software for both units has also been updated to the most recent release.

Alloy_Complete Ready for Alpha Testing

Frank Murphy of NE-CAT is developing a suite of software to facilitate optimal data collection from very small and/or radiation sensitive crystals and involving multiple crystal and multiple sweep data collection. The first step in this development was development of a software package titled Alloy_Complete. Alloy_Complete creates a simulation of diffraction data created by a full 360 degree rotation of the crystal in the beam. Information from an x file from HKL2000 (a.k.a. Denzo) is used to simulate the full 360 degree rotation of the crystal, which is utilized to determine the shortest phi rotations needed to achieve specified completeness targets. If one has already collected a partial data set, Alloy_Complete will take a scale file from HKL2000 (a.k.a. Scalepack) and calculate a strategy for completing the data set which includes the data input (see Figure 1A below).

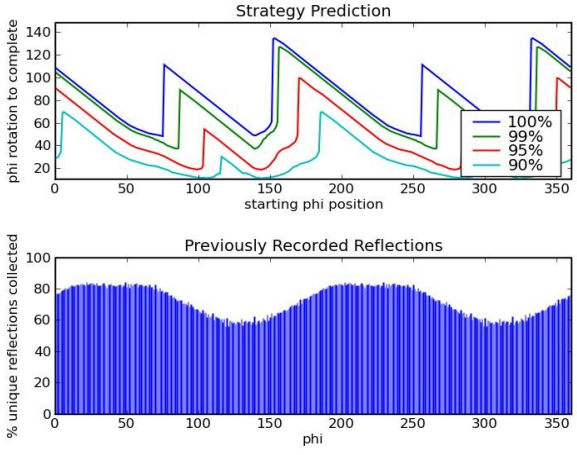


Figure 1a

Alloy_Complete outputs its strategy as a graph which is interactive: by clicking on a given point in the phi rotation to complete vs. starting phi position plot (Figure 1A), a new plot is generated which displays the completeness, redundancy and the type of reflection collected for the selected collection (Figure 1B on next page). Alloy_Complete is currently in alpha testing, to be released for general use shortly.

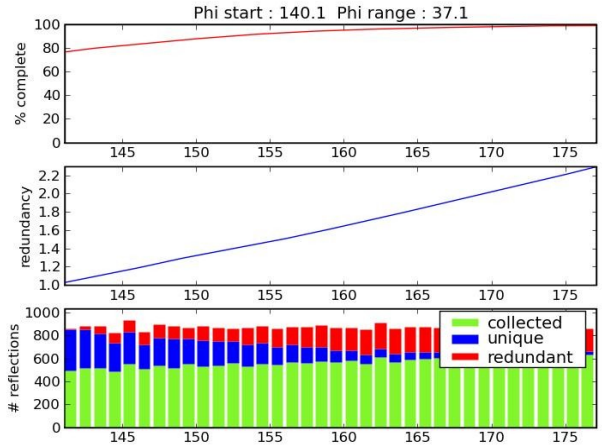
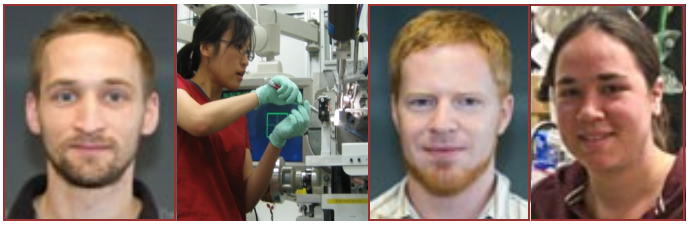


Figure 1b

Research Highlights

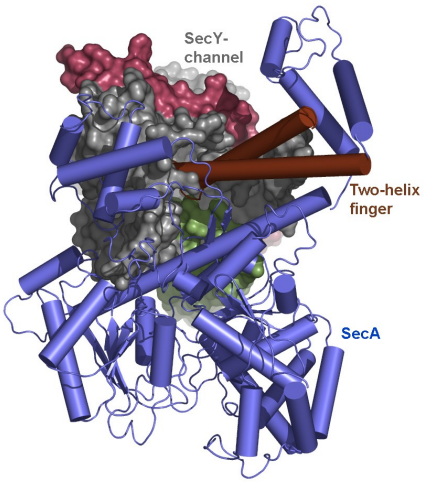
Crystal Structure of the SecA ATPase in Complex with the Protein Translocation Channel

Jochen Zimmer, Yunsun Nam, Karl Erlandson, Stefanie Miller, Tom Rapoport
Department of Cell Biology, Harvard Medical School, Boston



Shown here (front row) are Jochen Zimmer, Yunsun Nam, Karl Erlandson, and Stefanie Miller. Tom Rapoport is shown on the second line.

Many proteins are transported across or are integrated into lipid membranes to reach their final destination. Transport across the membrane occurs through an evolutionary conserved membrane-embedded protein complex, the SecY channel. This channel has the amazing ability to form either a pore across the bilayer or to open laterally to release transmembrane (TM) segments of a polypeptide chain into the membrane. The engine that moves the polypeptide chain into the channel can either be the SecA ATPase, as in the case of bacterial post-translational translocation, or the ribosome, if translocation occurs co-translationally. With the help and support of NECAT's ID 24-C and E beam lines, we were able to solve several crystal structures of the SecA ATPase in complex with the SecY channel to a maximum resolution of 4.5Å[1].



Our crystal structure shown here reveals that SecA and SecY undergo major conformational changes upon complex formation. Although a translocation substrate is not present in our complex, the structure allows us to speculate on how SecY is prepared for the arrival of a translocating polypeptide chain and how SecA pushes a translocating substrate into the channel.

The insertion of an α-helical domain of SecA, termed the "two-helix finger", into the cytoplasmic opening of the SecY channel results in an outward movement of some of SecY's TM-helices by up to 12Å. This rearrangement opens a window in the channel wall that is about 5Å wide and located almost exactly at the centre of the membrane. This window would allow the intercalation of the signal sequence, which in turn opens the channel. The window might also allow a translocating polypeptide chain to be continuously exposed to the apolar membrane core; if energetically favorable, a passing hydrophobic TM region could exit laterally into the lipid phase.

The structure of the SecA–SecY complex immediately suggests that SecA might use its two-helix finger to move polypeptide chains into the channel. This model is supported by crosslinking experiments in which a translocation substrate was linked to SecA and SecY. With this approach we were able to show that during translocation the polypeptide chain spans the distance from the tip of SecA's two-helix finger to the centre of the SecY channel[2]. Similar to protein-translocating hexameric ATPases, such as ClpX and p97[3], it seems that SecA critically relies on a conserved Tyr residue found at the tip of its two-helix finger. Mutagenesis of this Tyr residue to polar or charged residues almost completely abolishes translocation, whereas bulky, hydrophobic residues are tolerated.

The next major step is to determine the structure of a SecA-SecY complex in the presence of a translocation substrate. The continuing support by the NECAT beam lines will be invaluable to reach this ambitious goal.

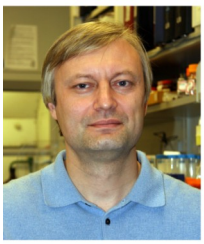
1. Zimmer, J., Nam, Y. & Rapoport, T. A. Structure of a complex of the ATPase SecA and the protein-translocation channel. *Nature* **455**, 936-43 (2008).

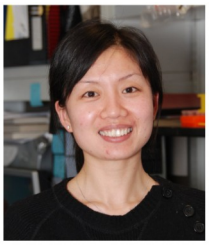
2. Erlandson, K. J. et al. A role for the two-helix finger of the SecA ATPase in protein translocation. *Nature* **455**, 984-7 (2008).

3. Martin, A., Baker, T. A. & Sauer, R. T. Pore loops of the AAA+ ClpX machine grip substrates to drive translocation and unfolding. *Nat Struct Mol Biol* **15**, 1147-51 (2008).

Crystal Structure of a Lysine Riboswitch

A. Serganov, L. Huang, D.J. Patel
Structural Biology Program, Memorial Sloan-Kettering Cancer Center

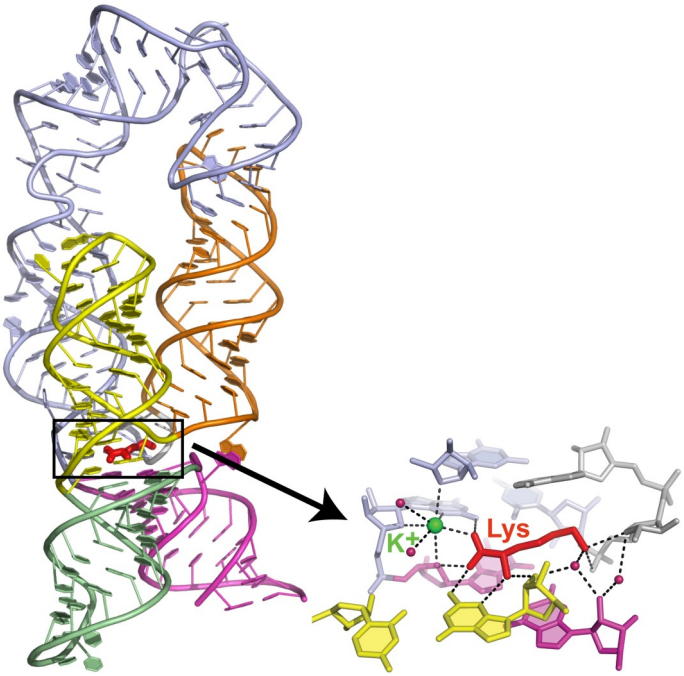

Alexander Serganov


Lili Huang


Dinshaw Patel

All living beings usually express those genes whose products are needed most at the given moment. In order to save resources and provide high adaptability to environmental conditions, organisms developed different systems to sense various physical and chemical cues and transmit them to the gene expression machinery that allows turning on or off a particular set of genes. Recently discovered gene-controlling circuits involve regions on messenger RNAs called riboswitches. Riboswitches can directly bind cellular metabolites present at an elevated concentration and effectively modulate expression of adjacent genes involved in metabolism and transport of these compounds. Riboswitches are highly abundant in bacteria, where they direct expression of many genes in response to various metabolites, including coenzymes, amino acids, sugars, and nucleobases. Each riboswitch is folded into an evolutionary conserved three-dimensional structure capable of specific recognition of the corresponding metabolite.

Amongst the most puzzling riboswitches is a lysine riboswitch, a ~170 nt long multi-helical RNA that can selectively bind amino acid lysine in a free form, but not incorporated into the protein, where this amino acid is often found at the interface between protein and RNA. This riboswitch also interacts with lysine-like antibiotics, and mutations in the riboswitch sequence provide antibiotic resistance in bacteria. Through experiments conducted primarily at NE-CAT, we have determined the structures of the metabolite-sensing domain of the bacterial lysine riboswitch in the complex with lysine and in the unbound state. The structure of the lysine-bound riboswitch, recently published in Nature[1] and shown in the next column, displays an intricate RNA conformation



composed of five helical stems which radiate from the junctional region. The lysine riboswitch crystals diffracted to 2.1 Å resolution that is higher than typically found for large RNA structures.

To obtain even better resolution, the crystal was overexposed at the microfocus beamline 24-ID-E and a complete 1.9 Å resolution data set was collected from several spots after translation of the beam along the crystal. Such high resolution allowed us to get a detailed view on how lysine binding locks the riboswitch in the conformation that turns off gene expression. Lysine is located within a tight cleft in the RNA junctional region and uses shape complementarity and multiple hydrogen bonds for specific binding. Some lysine-RNA interactions are mediated by a potassium cation found in the lysine binding pocket. The identity of this critical cation was revealed by collecting anomalous data at the 24-ID-C using 1.84 Å wavelength. At this energy, the anomalous signal of potassium is stronger than the signal from magnesium and sodium cations also present in the crystal.

The structure of the lysine riboswitch, the first structure of an amino acid-specific riboswitch, has provided insights into a mechanism of lysine riboswitch-dependent gene control and antibiotic resistance to lysine-like antibiotics at the molecular level. The data collected from the crystals of the riboswitch bound to lysine analogs have shown that the lysine-binding pocket has a potential to accommodate a novel generation of the lysine-like compounds which could be designed on the basis of the riboswitch structure.

[1]*Nature* **455**, 1263-1267 (2008)

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