

**NE-CAT COMMUNICATIONS** 



#### A Biannual Newsletter of the Northeastern Collaborative Access Team Summer 2009

# A Message from the Director Steven Ealick



Welcome to the latest edition of NE-CAT Communications, NE-CAT's biannual newsletter. Since our last newsletter, NE-CAT's insertion device beamlines have continued to be heavily booked by users and the scientific productivity continues to grow, as evidenced by the number of PDB depositions and publications issued, as shown in the following figure.



The data shown for 2009 represent only part of the current year. If the PDB depositions and publications continue to be published at the same rate, the final 2009 numbers will again show continuing growth. In addition to the number of publications, the quality and impact of publications remains high. As examples, we have included in this issue two summaries of recently conducted research using the beamlines.

In the last newsletter we reported that the 2009 APS run cycle may be shortened due to budget constraints. Fortunately, APS received additional funds from the Department of Energy sufficient to operate the accelerator for the full run schedule and avoid personnel reductions. The current budget forecasts for 2010 look very promising for APS. However, in light of the current economic environment, we and APS will have to wait for final congressional action. To keep pace with the science pursued by our users, we are continually adding new and important capabilities to the beamlines, as described in the following sections of this newsletter. Of particular importance, our new MD2 microdiffractometer has been installed and is fully operational on the 24-ID-C variable energy beamline. The productivity of this microdiffractometer beamline has been substantially increased by interfacing the MD2 with an ALS-type sample placement robotics system. This combination of a microdiffractometer interfaced with a sample mounting robot has been so well received by our users that we are now in the process of building an identical robot to be installed on the 24-ID-E beamline.

Our planning for introducing new research capabilities on the beamlines benefits substantially from the advice and recommendations we receive from our Resource Advisory Committee. This Committee, the members of which are shown in the following photograph, met at NE-CAT on January 23.



Pictured (from left to right are Steve Ealick (NE-CAT Director), Ashley Deacon\* (SSRL), Amy Swain (NCRR), Janet Smith\* (Univ. Michigan), Alfonso Mondragon\* (Northwestern Univ), Keith Hodgson\* (SSRL), and John Chrzas\* (SER-CAT). Resource Advisory Committee Members are denoted by asterisks..

The Committee reviewed and provided recommendations on a number of our near-term and longer-term planned beamline upgrade projects. Their recommendations were well appreciated and a number of them have already been implemented. We thank the committee members and Amy Swain from NCRR for taking time away from their busy schedules to assist us in planning our future upgrades.

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 on our beamline capabilities and how to request time, please visit our website at http://necat.chem.cornell.edu.

#### **Beamline Developments**

Since the last newsletter, the NE-CAT staff have been very busy adding new capabilities to the beamlines. The major new addition is the replacement of the original goniometry on the 24-ID-C variable energy beamline with an ACCEL/Maatel MD2 microdiffractometer coupled with an ALS-type automatic crystal mounting robotic system. The MD2 provides users with a precise goniometer and a Kappa attachment, both with "spheres -of-confusion" during sample rotation of a few microns. The MD2 is also equipped with a X-ray beam defining system capable of providing well collimated beams down to 5 microns in size, with minimal scattering background. The MD2 also provides a superb sample imaging system capable of visualizing crystals of a few microns in size.

Many of our users are now coming to the beamline with a hundred or more crystals to study. Formerly researching such a large number of crystals was very time consuming and exhausting, requiring mounting each crystal manually and having to go through the time consuming safety requirements needed to enter and leave the radiation enclosures. To eliminate these delays and manual effort, we have provided users with a highly reliable automated sample placement robotics system controlled from the user beamline control and data taking area. Users have found that the time needed now to research their many crystals has decreased by a factor of 2-3 using the robotic system. As part of the effort to decease the time needed for the robot to mount a sample and take data, the interface software supplied with the MD2 microdiffractometer was replaced with a newly designed interface to our control system.

A great deal of effort was needed to engineer this new MD2-robot integrated system to be highly reliable as well as to introduce both hardware and software protective features to protect the delicate microdiffractometer from damage while the robot is mounting and dismounting samples. During APS run cycles 2009-1 and 2, users have found this system to be highly reliable and of such benefit to their research that we are now building an improved version of the original robotic system to be installed on the 24-ID-E beamline. A recent photograph of the 24-ID-C end station follows.

NE-CAT has also automated several routine beamline operations to minimize data collection time and be more



user friendly. Three areas which have received ease-ofuse improvements are beam location determination, EXAFS scans, and beam size adjustments.

Regular users of 24-ID-C will remember performing scans of a phosphor-coated needle in order to determine the beam location. These scans required a great deal of time and user interaction in order to mount the needle, center the needle, manually adjust the beam in the horizontal direction and mechanically scan the needle in the vertical direction. The arrival of the MD2 microdiffractometer eliminated the need for needle scans and replaced it with easy on-axis visualization of the beam center. However, manual beam location centering still required the user to attenuate the beam, change the MD2 into beam location mode, and adjust the MD2 table to properly align the beam. To minimize the manual steps needed, NE-CAT has implemented automatic beam location centering on both beamlines. Now, with the single click of the mouse, software will automatically perform all the actions necessary to place the beam in the correct position in approximately 30 seconds.

Automatic beam centering has provided the foundation for development of additional time-saving features, such as automated EXAFS scans. As a microfocus beamline, it is necessary to carefully align the beam after changing energy. Users with SAD or MAD experiments on 24-ID-C were formerly burdened with multiple steps in order to successfully obtain an EXAFS scan of their crystal: moving to the new wavelength, centering the determining the baseline, adjusting the beam. attenuations, adjusting aperture size, sliding the fluorescence detector in. and, finally, running the scan. In addition, the scans were extremely time-consuming. All these steps have now been fully automated. The simple selection of an edge from the periodic table will perform all these functions and the scans now occur in less than half the time it would take a person to go through the operations manually.

Finally, to maximize data quality in microdiffraction, it is often advantageous to change the beam aperture size to match the size of the crystals. In the past, aperture change was a procedure to be carried out only by NE-CAT staff because of the fragility of the MD2 aperturebeamstop assemblies. This often forced a user to use the same aperture size during overnight data collection when support staff were not available. Both MD2 microdiffractometers are now fitted with triple apertures assemblies. Each assembly contains three apertures; this allows a choice of 10, 30 and 70 micron-size beams on 24-ID-C and 5, 20 and 50 micron-size beams on 24-ID-E. More importantly, the changing of aperture size is software-driven, allowing users to execute aperture size selection from the control console without the need to enter the radiation enclosure or for a NE-CAT staff member to be present

#### **Use of NE-CAT Beamlines**

As can be seen from the following graph, the total number of users visiting NE-CAT's beamlines has grown dramatically since startup of the first beamline operation in 2005. The number of users visiting has now reached an asymptotic limit, i.e., the number of users has saturated the current time available on the two operating beamlines. The slight dip for 2009-1 and 2 is most likely due to two factors. On 24-ID-C in 2009-1,



10% of the time was unavailable to users due primarily to the need to commission the new MD2 microdiffractometer. A second contributing factor for the slight decrease in both 2009-1 and 2 may be due to tight research budgets in this recessionary period forcing university groups to send fewer personnel to the beamlines. In order to accommodate additional users in the future, we plan to increase productivity and capacity. To dramatically increase productivity, a sample placement robotic system will shortly be installed on the 24-ID-E beamline. To increase capacity, installation of the third beamline, a bending magnet beamline, will begin shortly.

During 2009-1 and 2, 664 users (some repeat users) of NE-CAT's beamlines researched some 5300 crystals and obtained more than 1150 data sets. Use of the sample placement robotics system on 24-ID-C has Increased dramatically, from 23% during 2009-1 to 48% in 2009-2. Also with easy selection of different sized apertures, 50% of the users now use multiple apertures during their runs.

As can be seen in the following pie chart, NE-CAT's beamlines were used by a broad spectrum of users,



from our seven member institutions and General Users distributed coast to coast as well as Canada and Australia. 42% of the available time (available time is defined as total time available minus development time) was used by General Users, close to the 50% target we have established. Development time at 15% was abnormally high during this period due to the need to set aside time to commission the new MD2 microdiffractometer on the 24-ID-C beamline. Also of note the NE-CAT beamlines had a high level of utilization, with only 3% of the beam time left unscheduled.

A particular strength of the NE-CAT program is the level of crystallographic support provided to users. In recognition of this level of support, an increasing number of users are including NE-CAT staff as coauthors on their publications, as can be seen in the section titled "Publications" under "Staff Activities" which appears later in this newsletter. Of the 68 publications to date based on work conducted on NE-CAT's beamlines in 2009, 10 represent collaborations between users and NE-CAT staff.

# **Research Highlights**

# Structural Basis for Protein Synthesis by the Ribosome



Jamie Cate, Departments of Chemistry and Molecular and Cell Biology, University of California, Berkeley, CA

Protein synthesis, or translation, directly couples genotype to phenotype in the cell. Translation occurs on the ribosome, a complex of many proteins and RNAs that are highly conserved in all forms of life. The ribosome is divided into two

parts, or subunits, that carry out distinct roles in the process of making a protein. Although they have distinct functions, the two ribosomal subunits work together during protein synthesis. Ribosomes are incredibly large molecular machines in biological terms, 21 nm in diameter, and a complete understanding of protein synthesis will require an atomic-resolution "movie" of the ribosom in action. In this regard, the eribosome is no different from other molecular machines, such as RNA and DNA polymerases, helicases, myosins and kinesins. All of these molecular machines use conformational changes as a basis for function. And in each case, many X-ray crystal structures at atomic resolution have been necessary to obtain clear mechanistic insights into how they work. Cryo-EM reconstructions and X-ray crystal structures of the ribosome have identified many moving parts within the ribosome. However, critical aspects of ribosomal dynamics remain to be probed at atomic resolution due to a lack of high-resolution structural information.

We have been working with ribosomes from the common bacterium Escherichia coli to gain an understanding of how proteins are made. We have been able to grow crystals of the E. coli ribosome in a number of interesting conformational states that shed light on how the ribosome works. Most recently, we have focused on a key series of events that occurs after each amino acid is added to a growing protein chain. After the amino acid is added, the substrates of the ribosomemessenger RNA (mRNA) and transfer RNAs (tRNAs)have to be moved by one genetic code-word, or codon, so that the process can be repeated. This process is termed "translocation" and involves many large-scale rearrangements in the ribosome. These rearrangements involve a "ratcheting" of the two ribosomal subunits with respect to each other in a complicated set of orthogonal rotations. It has been unclear, however, how the large-scale rearrangements identified in lowresolution images taken by cryo-EM relate to molecular events within the ribosome.

In a recent trip to the NE-CAT beamlines, we made a breakthrough in our understanding of translocation using new crystals of the E. coli ribosome that we had obtained. By using the high-brilliance X-rays generated at the APS, we were able to measure the X-ray diffraction data necessary to determine three high-resolution structures of the ribosome. One of the structures was at 3.45 Å resolution, a resolution sufficient to see and refine all of the molecular details of how the two ribosomal subunit move with respect to each other as part of translocation. We were surprised to see that the ribosome, in fact, makes those rearrangements in a stepwise manner. The ribosome hands off some of the contacts between the two ribosomal subunits in one step, and once those are in place rearranges the remaining contacts. This new insight into the movement of mRNA and tRNAs on the ribosome during translocation was recently published in Science (Zhang, W., Dunkle, J.A. and Cate, J.H.D. (2009) 325, 1014-7). The atomic-resolution "movie" of protein synthesis is far from complete, and my group and others will continue to need X-ray crystallography to make the necessary "still frames" to fill in the gaps in our knowledge of how the ribosome works.

The figure below shows the structural rearrangements in the ribosome during translocation. The ribosome adopts at least four ratcheted states during the process of translocation ( $R_0$  through  $R_F$ ). Difference vectors between corresponding phosphorous or C-alpha atoms are shown, color coded by the extent of the movement in each transition.



0Å 20Å

# Molecular Architecture of Acid Sensing Ion-Channels and P2X Receptors



Eric Gouaux, Vollum Institute, Oregon Health and Science University and Howard Hughes Medical Institute

Chemical synapses are the primary site of communication between neurons in the peripheral and central nervous systems. At these specialized junctions, a presynaptic neuron releases a burst of neurotransmitter following activation of voltage-dependent calcium channels. The rise of neurotransmitter concentration in the synaptic cleft, in turn, leads to binding of transmitter to receptors located on postsynaptic cells, activating these receptors. Many of the postsynaptic receptors are ligand-gated ion channels, integral membrane proteins that harbor one or more binding sites for a specific transmitter and a membrane-spanning, ion channel domain. Binding of transmitter induces opening of the ion channel and, thus, conversion of the extracellular, chemical gradient into an electrical signal.

My research group studies, in part, the atomic structure and molecular mechanism of transmitter-gated ion channels. Over the past several years we have studied two separate classes of ligand-gated ion channels: acid sensing ion channels (ASICs) and ATP-gated ion channels known as P2X receptors. To accomplish this work, we relied upon the extensive use of synchrotron radiation at the NE-CAT beamline (APS) and beamline X-29 (NSLS). In 2007 we reported the structure of acid sensing ion channel 1a (ASIC1a) from chicken<sup>1</sup>. This structure provided the first view of an ASIC and it also provided a number of surprises, the first of which was that ASICs are trimeric in subunit stoichiometry, a fundamental issue that had been unresolved since the cloning of the genes. Second, we were able to identify a number of acidic residues in such close proximity that one or both must bear a proton. These sites are thus implicated in proton binding. This ASIC structure, however, relied on a protein construct that was inactive in electrophysiological assays. We then proceeded to define the boundaries of a chicken ASIC1a construct that was functional and amenable to crystallization. In August of this year, we reported the structure of a functional chicken ASIC1a channel, thus faithfully describing the closed-state structure of the ion channel and ion binding sites<sup>2</sup>. This, for the first time, illustrated how monovalent cations interact with the pore of a trimeric ion channel protein, being coordinated in a trigonal antiprism geometry by carbonyl oxygen atoms from main chain carbonyl groups or the carboxyl group of an aspartic acid residue.

Contemporaneous with our studies on ASICs were experiments directed at the structure determination of an ATP-gated P2X receptor—also a trimeric, ligand-gated ion channel protein. Upon solution of the structure of the zebrafish P2X4 receptor, we not only elaborated a novel fold and architecture of an ATP-gated ion channel protein, but we also demonstrated that the likely ATP binding site is far from the transmembrane ion channel, thus suggesting that ATP-dependent activation must involve large scale conformational changes in the receptor<sup>3</sup>. A major surprise, however, was that ASICs and P2X share a common transmembrane, ion channel architecture (as shown in the following figure) and possess remarkably similar negatively charged, extracellu-

lar vestibules, thus implying that elements of gating and ion conduction may share unexpected and common features in these two otherwise unrelated classes of ligand -gated ion channel proteins.



### References

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### **NE-CAT Staff Changes**

John Unik, NE-CAT's Project Administrator since the beginning of NE-CAT construction, is leaving at the end of September. John has a long association with building beamlines at APS. As a Division Director at Argonne, he and his division were involved in the construction of the Structural Biology beamlines. After retiring from Argonne he served as Project Administrator for construction of the Southeast Regional CAT beamlines. When construction finished there, he joined NE-CAT to work with Malcolm Capel in building NE-CAT's beamlines and participating in establishing the user support activities.

#### Staff Activities

#### **Publications**

Choi, M., Sukumar, N., Liu, A., and Davidson, V.L. Defining the Role of the Axial Ligand of the Type 1 Copper Site in Amicyanin by Replacement of Methionine with Leucine, Biochemistry (in press) (2009)

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Ostrander, E. L., Larson, J. D., Schuermann, J. P., and Tanner, J. J. A Conserved Active Site Tyrosine Residue of Proline Dehydrogenase Helps Enforce the Preference for Proline over Hydroxyproline as the Substrate Bio-Chemistry *48*, 951-959 (2009).

# Presentations

Use of Micro-focused Beamline in Solving the Crystal Structure of the Avian Thymic Hormone (ATH) in Calcium Bound Form, Jon Schuermann, 2009 ACA Meeting, Toronto CA, July 25-30, 2009.

Malcolm Capel, "Installation and development of the MD2 Microdiffractometer on NE-CAT's Undulator Beamlines", APS Technical Working Group (TWG) Meeting, April 16, 2009.

#### **Poster Sessions**

Microdiffraction-What Can be Done?, K. Rajashankar, M. Capel, S. Harrison, and S. Ealick, 2009 ACA Meeting, Toronto CA, July 25-30, 2009

Northeastern Collaborative Access Team (NE-CAT) Beamlines at the Advanced Photon Source, S. Ealick, M. Capel, F. Murphy, D. Neau, K. Perry, K. Rajashankar, J. Schuermann, and N. Sukumar, 2009 ACA Meeting, Toronto CA, July 25-30, 2009

### **Committee Memberships**

K. Rajashankar, APS Beam time Allocation Committee

M. Capel, BioCAT Resource Advisory Committee

M. Capel, APS Partner User Council (PUG)

Malcolm Capel, Member Committee on the "Proteins to Organisms" project for Renewal of the Advanced Photon Source Project

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