A Message from the Director
Steven Ealick

Welcome to the second issue of NE-CAT Communications. Since issuing the first newsletter during summer 2007, there is a great deal of very good news to report. The best news is that the National Center for Research Resources (NCRR) will provide funding for another five years of NE-CAT’s operations. In addition to continuing our existing program, this award makes possible the initiation of a number of important new collaborative research programs (“core collaborations”) and the hiring of additional beamline support staff needed as our second and third beamlines come into full operation. This award was based on the outcome of the NCRR review of our proposal, held July 2007, in Washington D.C. A major focus of this proposal is to develop new X-ray microdiffraction capabilities and apply these capabilities to challenging problems in macromolecular structural biology. The proposal approved provides 50% of the available beam time on NE-CAT’s beamlines as a national resource to the general community of users with the remaining 50% to be shared among NE-CAT’s institutional members. The 50% of beam time reserved for national resource users will be awarded on the basis of review and approval of proposal submissions, half by NE-CAT and half by the Advanced Photon Source (APS) General User Program.

Core collaborations will consist of collaborative research between specific members of the research community and NE-CAT staff members. A major goal of these collaborative programs is to have the research drive future NE-CAT beamline developments. Core 1 collaborations will be pursued with James Berger and Stephen Quake (Univ. California-Berkeley), David Eisenberg (Univ. California-Los Angeles), Stephen Harrison (Children’s Hospital-Boston and Harvard Medical School), and Roderick MacKinnon (Rockefeller Univ.) and will be primarily devoted to the development of new capabilities in microdiffraction crystallography. In Core 2, Jamie Cate (Univ. California-Berkeley), Wayne Hendrickson (Columbia Univ.), and Hao Wu (Cornell Univ.) will collaborate with NE-CAT staff members to develop new beamline instrumentation, hardware, and methods. Core 3 research will be devoted to developing new computational tools with Steven Ealick (Cornell Univ.), Nikola Pavletich (Memorial Sloan-Kettering), Brenda Schulman (St. Jude Children’s Research Hospital), and Thomas Steitz (Yale Univ.).

Other important news to report is that our second insertion-device beamline, 24-ID-E, has been successfully commissioned and will be open to the general community of users upon resumption of APS accelerator operations in February 2008. This beamline has been optimized for microdiffraction research by providing extremely stable beams and is equipped with an MD2 microdiffractometer providing well collimated beams with adjustable diameters ranging from 5-100 microns.

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.

Current Status and Usage of the NE-CAT Beamlines

24-ID-C Variable-Energy ID Beamline

The 24-ID-C beamline has now been in various stages of operation since February 2005. During the period from February 2005 to August 2006, the beamline was being commissioned by the staff and users, both institutional members as well as nonmembers. In October 2006 the beamline was officially declared operational for all users (General Users, institutional members, and core collaborators). As can be seen in the following figure, the number of users during this time period has
grown continually and over 49 papers were published in quality journals in 2007.

It should be noted that as the beamline reaches 100% utilization, the number of users will asymptotically reach ~140 users per run cycle.

Also, as can be seen in the next figure, the amount of time allocated to the general users has grown steadily to 36%, rapidly approaching our eventual goal and commitment to NCRR of 50%.

Since the start of full operations in October 2006, the beamline has operated at a very high level of reliability. There has been only one outage of the beamline (December 13, 2007) in which a user group lost a complete 8 hour shift (previously the few and very infrequent outages lasted only a few hours). Also during this period, the beamline capabilities have undergone continuous improvement to meet the increasing needs of the users. (See the section “Beamline Upgrades and Enhancements.”)

**24-ID-E Microdiffraction Beamline**

After a very successful commissioning program, the 24-ID-E beamline equipped with a MD2 micro-diffractometer was declared to be fully operational and available to the general user community for APS run 2008-1 starting in February 2008. As with 24-ID-C, this beamline exhibits a high degree of X-ray beam position stability (within a few microns) over a long period of time and a high degree of reliability. During the commissioning period of 2007-3, in addition to usage by our staff, we have had more than 40 users try the microdiffraction beamline. Many of these experiments were novel in nature, attempting to exploit new research opportunities with beam sizes in the range of 5-20 microns. Several preliminary results are reported under the section titled “Microdiffraction”.

**24-BM Bending Magnet Beamline**

Movement of NE-CAT's 8-BM bending magnet beamline to Sector 24 has begun and all the 8-BM end station equipment has been moved to Sector 24. Movement of all the beamline optics is awaiting final approval from APS on the 24-BM Final Design Document (FDR).

**Beamline Upgrades and Enhancements**

The key to a successful microdiffraction beamline is maintaining the micron-sized beam position on the crystal during the course of an experiment. In addition to compensating for instabilities in the accelerator source position, attention must also be directed to minimizing other sources of instabilities such as floor vibrations, vibrations of the monochromator crystal due to liquid nitrogen flow, cryo fluid pressure bumps due to filling of the cryo-cooler Dewar, etc. All of these sources have now been successfully minimized at 24-ID-E, resulting in excellent long-term beam position stability. To maintain good beam stability, a high-precision quadrant diode beam position monitor (bpm) is used to measure the beam position and is coupled with an automated beam steering feedback system. Horizontal adjustments to the beam position are made by adjusting the monochromator Bragg axis and vertical adjustments are made by pitching the vertical focusing mirror. Also to minimize steering parallax errors, the bpm has been moved 1.6 meters downstream and directly mounted onto the rotating photon shutter, within 0.25 m of the crystal position as shown in the following figure.
Another important addition to the variable-energy 24-ID-C beamline has been the introduction of a “beam steering table”. With the introduction of this table, users are assured that the beam position automatically remains constant when changing energies.

More and more users of 24-ID-C have been requesting a sample placement robotic system to greatly speed up the screening of large numbers of crystals. To meet this request, NE-CAT has been modifying and upgrading the ALS-type sample placement robotics system (shown below), which was previously used at 8-BM, for installation on 24-ID-C. The major modification needed was to reverse the “handedness” of the robot, i.e., looking downstream from the source, the robot must be mounted to the left side of the goniometer rather than the right side as was the case on 8-BM. The modifications and upgrades have now been completed and the robot tested. The robot has been installed on the beamline during the January 2008 accelerator shutdown and is currently being commissioned. The robot is expected to be available for general usage in the March-April 2008 time frame.

Microdiffraction

An increasing number of the crystals brought to NE-CAT’s beamlines tend to be very small (<20 microns) and extremely sensitive to radiation damage. To best study these crystals, NE-CAT has developed a microdiffraction beamline, 24-ID-E, employing a MD2 microdiffractometer as the heart of its operation. The MD2 provides a high-resolution goniometer, the ability to easily change beam spot sizes from 5-100 microns, and a superb visualization system to visualize these very small crystals and the X-ray beam itself. During commissioning, now successfully concluded, NE-CAT staff, core collaborators, and general users performed a number of preliminary experiments to explore the potential of this beamline to provide new research capabilities. These early experiments have been highly successful, resulting in the initiation of a number of new collaborative research programs.

One of the important and obvious advantages provided by a microdiffraction beamline is the ability to match the X-ray beam size to the size of the crystal, dramatically reducing the extraneous scattering background when searching for weak diffractions. Early users of the 24-ID-E beamline have taken advantage of this feature in analyzing weakly diffracting crystals.

Another important capability of these small beams is the ability to irradiate selected portions of a single crystal e.g., selecting the best areas of imperfect crystals to study or moving to a “fresh” spot on the crystal after significant radiation damage has occurred. As an example, the picture on the left shows a microcrystal of thiaminase II, measuring 16x16x300 microns which was very sensitive to radiation damage, from Steven Ealick’s laboratory at Cornell University. By rastering a 20 micron beam across this needle-like crystal, a complete data set was obtained to 2.3 Å. The black spots on the crystal show the local radiation damage suffered by the crystal after it was irradiated at five different positions with the 20 micron beam.

As another example, some large thin plate-like crystals as (A) shown in the following figure are bent. Diffraction spots are considerably broadened when using a large
100 micron beam (B) in contrast to using a smaller 20 micron beam (D). By using a small beam rastered across the crystal higher quality data can be obtained.

In another type of application conducted with James Berger’s group from the University of California-Berkeley, the next figure shows that good diffraction images can be obtained by irradiating small crystals contained in a section of a microfluid chip.

Photograph (A) at low magnification and (B) at high magnification shows crystals contained in a well of a microfluidic chip and (C) shows that high quality data frames can be obtained by irradiating the chip directly in the X-ray beam.

In yet another collaborative program, with Robert Thorne’s group from Cornell University, we are exploring the use of specially designed matrix mounts for crystallographic research. In the following figure, (A) shows a solution at first examination that looks like it contains a precipitate. However under high magnification (B) the solution is seen to actually contain many ~5 micron crystals. Using a matrix mount (C) with 25 micron openings the crystals can be scooped out of solution as shown in (D). By irradiating crystals fixed on the matrix mount, high quality data can be obtained. In this specific example, by merging together ~5-8 images from each of 16 different crystals a near complete 3.3Å data set was obtained.

It is quite evident that in future microdiffraction experiments, there is a need to merge data taken from separate crystals or multiple spots on a single crystal to obtain a complete data set. Currently merging such data sets together manually is a very laborious activity. Consequently, we are now finalizing the development of a software package that will provide fully automated merging of partial data sets. This software package will be in beta testing during early spring 2008.

Research Highlights

Crystallographic Trapping in the Rebeccamycin Biosynthetic Enzyme RebC

Katherine S. Ryan and Catherine L. Drennan

Katherine Ryan (left) is a Howard Hughes Medical Institute Predoctoral Fellow in the Biology Department of MIT and Catherine Drennan (right) is an Associate Professor in the Chemistry and Biology Departments of MIT and a Howard Hughes Medical Institute Professor.

Microorganisms can be rich sources of novel compounds, including anti-cancer molecules. The bacterium Lechevalieria aerocolonigenes is one such example; it contains a gene cluster that encodes the enzymes to biosynthesize rebeccamycin, which is a human DNA-topoisomerase I inhibitor. Rebeccamycin analogs are currently in clinical trials against a variety of human cancers. Rebeccamycin, an L-tryptophan derived alkaloid, is made naturally by an unprecedented series of reactions.

Biochemical studies carried out in the laboratory of our collaborator Christopher T. Walsh of Harvard Medical School established the biochemistry of the initial steps of rebeccamycin construction, starting from L-tryptophan to the intermediate molecule chromopyrrolic acid. The next step, a net eight-electron oxidation of chromopyrrolic acid to the rebeccamycin aglycone, was the most mysterious. Annaleise Howard-Jones, a postdoctoral researcher in the Walsh laboratory, established that two enzymes were responsible for this dramatic oxidation, with a cytochrome P450 apparently responsible for the
full chemistry, and a second enzyme – RebC – acting to accelerate product formation and to eliminate side-reactions. We decided to pursue the structure of RebC in parallel with Dr. Howard-Jones’s biochemical studies, with hopes of understanding how RebC might play the role of promoting the correct final product and eliminating spurious side products.

Our approach was straight-forward: gather native and heavy-metal data sets to solve the structure, and subsequently gather data of RebC with various bound molecules. But with RebC there was a twist: while we suspected that RebC was an enzyme (after all, it bound the cofactor FAD), we had no idea what molecule it might in fact bind or react with. Further, we were perplexed that the cytochrome P450 “partner” enzyme seemed to produce a small amount of the correct product without RebC. Why would RebC need be an enzyme if its partner enzyme was capable of carrying out the full chemistry all on its own (albeit at low levels)?

Because of these issues, we did not know what would be best to soak into RebC, and we took the na"ive approach of carrying out soaking experiments using all the compounds that were available to us. Among these were chromopyrrolic acid, and we were excited upon collecting a home data set on a crystal soaked for one week with chromopyrrolic acid to see that there was strong density in what appeared to be the binding pocket, among other dramatic changes. Yet attempts to repeat this soak over shorter periods of time (on (more beautiful crystals!) all failed; only with outrageously long one-week soaks was it possible to obtain density in the binding pocket.

In unraveling this story, we learned much about the chemistry of indole alkaloids. Chromopyrrolic acid, a bis-indole compound, is not stable at room temperature in aerobic conditions for an entire week. In fact, at very low levels, it can spontaneously form the rebeccamycin aglycone (among other products) – although the spontaneous reaction is slow enough that an enzyme is clearly needed in a physiological setting. A week-long soak was needed not because chromopyrrolic acid takes a long time to bind in the binding site, but because a degradation product of chromopyrrolic acid was being slowly produced over time, and being ‘captured’ by RebC. It took a week for enough of this low-level degradation product to be bound in RebC to see suitable density. Based on the shape of the electron density, electrostatic arguments, and HPLC analysis, we determined that we had not bound chromopyrrolic acid itself in our structure, but instead captured 7-carboxy-K252c. This compound is an intermediate in the conversion of chromopyrrolic acid to the rebeccamycin aglycone, and we believe (but do not know) that this molecule is likely to be a substrate of RebC, and hence it has a unique affinity for the binding site of RebC. Our collaborator Dr. Howard-Jones went on to purify 7-carboxy-K252c synthetically, and she found that it decomposed quickly in ambient conditions. The instability of the compound precluded directly testing if it was a substrate for RebC. Collectively, this work gave us a simple model for the two-enzyme system (see figure to left). The cytochrome P450 enzyme produced 7-carboxy-K252c (or a related intermediate). With RebC present, it would be converted by RebC to the rebeccamycin aglycone. However, without RebC present, the intermediate decomposed to a number of compounds, which included (at low levels) the rebeccamycin aglycone.

Our crystallographic ‘trap’ – crystalline RebC – was amazingly able to bind and stabilize 7-carboxy-K252c over a week-long period, enabling us to use macro-molecular crystallography to identify a small molecule. Because of the instability of the molecule, other techniques are less useful in characterizing this molecule, and we are excited to have been able to use crystallography at NE-CAT’s beamline to reveal the likely substrate of RebC. We hope that our studies will be one small step in enabling us to understand fully how rebeccamycin is made, which might lead to protein engineering or other studies that will result in improved drug molecules based on the structure of rebeccamycin.


Figure 1. One possible scheme for the production of the rebeccamycin aglycone, based on our structural work.

Resource Advisory Committee Meeting

The NE-CAT Resource Advisory Committee met at the NE-CAT facility on Friday, November 16, 2007. This committee advises NE-CAT on matters including establishment of priorities, research and development activities, collaborative research, resource utilization, the user program, training, and dissemination of information. Members in attendance were John Chrzas (University of Georgia), Ashley Deacon (Chairman, Stanford Synchrotron Radiation Laboratory), Alfonso Mondragon (Northwestern University), and Janet Smith (University of Michigan Medical School). Absent was Keith Hodgson (Stanford Synchrotron). Representing NCCR at this meeting was Amy Swain, Program Director. The following picture shows Steve Ealick pre-
senting an overview of NE-CAT’s activities to the committee.

Meet the NE-CAT Staff

In the previous issue of “Communications” we introduced you to our most recent staff hire, Frank Murphy. In this issue we would like to introduce you to the two leaders of NE-CAT’s activities at the Advanced Photon Source. In the following photograph are shown Malcolm Capel (right) and Kanagalaghatta Rajashankar (left).

Malcolm is the Deputy Director of NE-CAT, responsible for all the day-to-day activities at the beamlines and responsible for all technical developments, construction, upgrades, maintenance, etc. Malcolm received his Ph.D. in Biochemistry in 1981 and was employed by the National Synchrotron Light Source (NSLS) at Brookhaven for nearly thirteen years where he constructed and operated several successful macromolecular beamlines. Malcolm came to the APS in 1999 to finish development of the 8-BM beamline for the Whitehead Institute. When this beamline was transferred to NE-CAT in 2001, Malcolm then joined NE-CAT. Since joining NE-CAT he has been responsible for the construction of both NE-CAT insertion device beamlines and is currently relocating the 8-BM beamline to Sector 24.

Kanagalaghatta Rajashankar, “Raj”, joined NE-CAT in 2005 and is currently Operations Team Leader responsible for all beamline user activities. These duties include all beamline scheduling activities, development of the user facilities and providing leadership for all the beamline user support staff. Raj received his Ph.D. in Biophysics in 1997 and since then has established himself as an eminent crystallographer, having published some 47 papers. Before joining NE-CAT he was a Senior Research Scientist at Memorial Sloan-Kettering Cancer Center.

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