A Message from the Director
Steven Ealick

Welcome to NE-CAT Communications. This is an exciting time in the history of NE-CAT. Our first insertion device beam line has been in operation for slightly over one year with its user base growing rapidly and the results of research conducted appearing in the journals. Construction of our second insertion device beam line is now complete and institutional users are in the final stages of crystallographically commissioning the beam line before opening usage up to the user community. Also, we are in the final stages of bringing into full operation a micro-diffraction capability which will provide users with many new opportunities for research.

As construction of NE-CAT’s x-ray beam lines transition into operation, we believe it is important to continually inform both current and future potential users of NE-CAT’s beam lines as to the capabilities of these beam lines, technology developments in progress, and the research being conducted. Current and potential users already have available to them the NE-CAT website (http://necat.chem.cornell.edu) which contains a wealth of detailed information on NE-CAT, the beam lines, the user program, and highlights. We also keep the general crystallographic community informed of our activities through presentations and posters at meetings, as well as convening topical workshops. However in parallel with these ongoing communications, we believe there is considerable value to be gained by reaching an even broader scientific community through publication of a bi-annual newsletter, this being the first in a continuing series. In this issue we provide the reader with the current status of the beam lines, examples of some of the technological developments being pursued, a snapshot of some of the research underway, and recent organizational changes.

This being our first issue, we would appreciate hearing from readers as to what additional information they would like to see included in future publications. To provide comments, learn more about NE-CAT’s user program, or if the reader is interested in becoming a user of NE-CAT’s beam lines, I encourage readers to directly contact Kanagalaghatta (Raj) Rajashankar by email at rajashankar@anl.gov.

General Background

NE-CAT’s basic mission is to design, construct, and operate synchrotron x-ray beam lines for a broad sector of the research community to address challenging problems in structural biology. Inherent in conduct of this mission, NE-CAT develops new instrumentation, software, experimental techniques, and disseminates these new developments openly to the general crystallographic community. NE-CAT is located at Sector 24 of the Advanced Photon Source (APS) which is, in turn, located within the Argonne National Laboratory, 30 miles southwest of Chicago. Currently two extremely high-brilliance x-ray beam lines are in operation, based on the use of canted insertion magnet devices. A third, bending magnet, beam line is scheduled to be in operation late this calendar year.

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NE-CAT Institutional Members

Columbia University
Cornell University
Harvard University
Massachusetts Institute of Technology
Memorial Sloan-Kettering Cancer Center
The Rockefeller University
Yale University

As the beam lines near full operation, 50% of the available beam time will be allocated equally to the institutional members and 50% allocated as a national research resource to the general structural biology research community. The latter beam time will be allocated on the basis of a proposal review system administered by the APS General User Program.

The Current Status of the Beam Lines

The optical trains of the NE-CAT beam lines located at Sector 24 of the APS are shown in Figure 1.
The Phase I beam line is a variable-energy insertion-device beam line covering the energy range from 5 to 25 KeV. This beam line has been in successful operation for slightly over one year, supporting the needs of institutional members as well as APS General Users. The Phase II beam line is a quasi fixed-energy beam line capable of providing x-rays at 12.66 and 14.78 KeV, by translating between a Si 220 crystal and a Si 311 first crystal in the monochromator, respectively. This beam line is now in the final phases of crystallographic commissioning by the institutional members and should be available to general users in the fall of 2007. Phase III is a bending magnet beam line. For a number of years in the past, during construction of NE-CAT’s insertion-device beam lines, NE-CAT has operated a bending magnet beam line at Sector 8 of the APS for use by its members and the APS General Users. Now that construction of the two insertion beam lines is completed, we are relocating the 8-BM beam line to Sector 24 as the Phase III construction project. In conjunction with this move many of the components will be upgraded and the optics changed to provide a higher demagnification, i.e., a smaller beam spot. Also, the move will consolidate all NE-CAT operations into a single location, as opposed to opposite sides of the storage ring, for improved efficiency and reduction of costs.

All the user end stations of the current three beam lines are equipped with state-of-the-art instrumentation. The insertion-device beam lines are capable of providing un-slitted beam spot sizes of approximately 20 vertical by 60 horizontal microns. In addition, microdiffraction capabilities are being introduced so that users can work with even smaller beam sizes, e.g., 5-20 microns (see the following section titled “Micro-Diffraction”). Data is taken with large-area CCD-based ADSC Quantum 315 detectors connected to a high-performance GPFS data storage system currently with a capacity of 30 TB (expandable to 75TB). The data acquisition systems have been optimized for very fast data acquisition, capable of exposures as short as 250 ms, with a dead time of 2 sec for un-binned images and 1 sec for binned images. In addition, NE-CAT also provides a fully equipped chemistry laboratory and cold room for the use of its users.

Figure 2 shows a photograph of the user end station for the Phase I beam line. The x-ray beam enters through the shielding wall on the far right of the photograph, passes through a vertically focusing mirror, and irradiates a sample located in the goniometry shown in the center of the photograph. On the far left can be seen the ADSC Quantum 315 detector. A photograph of the Phase II beam line user end station is shown as Figure 3 in the following section titled “Micro-Diffraction”.

Micro-Diffraction

During the past year an increasing number of our users have been bringing smaller and smaller crystals to the beam line for structural determinations. This is most probably a consequence of the difficulties encountered in growing high-quality large crystals and a realization on the part of our users that they have routinely available to them at NE-CAT a high-brilliance x-ray beam of dimensions down to 20 microns. Also, we are observing that more researchers are requesting even smaller beam sizes in order to minimize backgrounds and to take data rastering across crystals in an attempt to
circumvent the radiation damage problem. The trend is quite clear, researchers will increasingly be researching smaller and smaller crystals. In order to be able to routinely analyze crystals down to the 5 micron scale, improved beam stability, goniometry with smaller spheres of confusion, better sample visualization, reduced scattering backgrounds, more automated techniques to center such small crystals in the beam will all have to be provided. To meet this need, we have embarked on a two phase program. First, to implement micro-diffraction capabilities as quickly as possible, we now are in the final phases of commissioning a MD2 micro-diffractometer purchased from ACCEL. The MD2 is manufactured by Maatel and is based on the micro-diffractometers developed by EMBL and successfully used at ESRF. The MD2 is a complete integrated system with a goniometer having a sphere of confusion of less than 2 microns, a co-axial sample visualization system, beam size defining aperture and capillary, and a sophisticated suite of control software. A photograph of our current installation, now located on the Phase II beam line, is shown in Figure 3.

The MD2 is mounted on a unique alignment support system developed by NE-CAT which provides all the degrees of motion necessary to reproducibly align the MD2 to the x-ray beam to within a micron. Other new features unique to our installation are:

1. A high-precision beam position monitor and a fast rotary shutter mounted directly onto the upstream surface of the MD2.
2. Vacuum is maintained downstream to the rotary shutter and a helium filled flight path is then maintained from the shutter through the sample visualizer of the MD2 to minimize background scattering.

As an illustration of the power of the MD2 to produce a small beam with minimal scattering, the top photograph of Figure 4 shows the un-slitted, focused beam at the MD2. The bottom photograph shows the 20 micron beam through the MD2, shaped by the defining aperture and capillary.

Figure 5 illustrates how the MD2 is used to collect structural data on a crystal that is extremely sensitive to radiation damage. A full data set was obtained on a 16x16x300 micron crystal using a 20 micron beam by moving the beam to different portions of the crystal when the degree of radiation damage begins to deteriorate the quality of data. In all, 5 different portions of the crystal were irradiated and the resulting data sets successfully merged.

**Beam Line Enhancements**

In order to move toward more rapid data acquisition and analysis, NE-CAT continually introduces improved capabilities on its beam lines. Here we will present two examples of past important upgrades in capabilities.

NE-CAT has replaced its pneumatically operated photon shutter assemblies on all its beam lines with rotary shutters in order to achieve faster and more reliable beam open and closed times and improve beam position stability by removing a source of vibration. A photograph of the rotary shutter components is shown in Figure 6. The heart of this new shutter is a miniature, high-performance stepping motor-driver combination capable of acceleration rates up to 8x10^5 steps /sec^2 with no measurable loss of steps. The stepping motor rocks a 1 cm diameter shielding plug with a 1 mm open slot to block and unblock the beam using only 26 motor steps. Introduction of this rotary shutter has reduced the variability of beam transit times from 4 ms with a pneumatic shutter to less than 0.2 ms and provides the capability of reliable data taking with exposures as short as 200 ms.
The heart of NE-CAT’s data flow system is a Hewlett-Packard EVA 5000 Storage Area Network System (SAN), capable of write speeds up to 75 MB/sec and with a current storage capacity of 30 TB (expandable to 75 TB). We have re-configured the storage network to support the IBM Global Parallel File System (GPFS). This revision allows all member nodes of the data network to directly access the SAN via Fibre Channel, in a parallel fashion. GPFS mediates concurrent read and write access to the virtual drives and their file systems to any node running GPFS. The end result is a speedup of a factor of up to five fold, relative to the former use of NFS, retrieving files from the SAN for analysis, as shown in Figure 7.

**Beam Line Utilization**

Since October, 2006, with the addition of APS General Users to the Phase I beam line, more than 230 users (institutional members and APS General Users) have conducted research on the Phase I ID beam line. No users reported any significant loss of beam time during this entire period due to malfunctions of the beam line and in the “User Summary Forms” filed at the end of each run all generally reported successful experiments. The major problems reported by users were poor or weakly diffracting crystals. The pie chart shown in Figure 8 shows the distribution of Phase I beam line usage by user category for the period October 2006 through April 2007. The usage reported for “Development” is higher than expected for a mature beam line due to the need to bring into operation Phase II which necessitated days of access to the optics enclosure housing both the Phase I and Phase II monochromators, resulting in Phase I being taken off line for substantial periods of time.

**Highlights of Research Using NE-CAT Beam Lines**

**Christopher Lima**
Memorial Sloan-Kettering Research Cancer Center

RNA is the intermediary used to translate our genome into functional molecules such as proteins, microRNA, and structured or catalytic RNA complexes such as the ribosome and spliceosome. During and after transcription, RNA quality is maintained by nuclear and cytoplasmic RNA surveillance, pathways that protect the cell from aberrant RNA that contains mistakes, deletions, or unreadable codes. In addition, RNA decay balances transcription and is used to regulate the lifetime of a particular RNA.

In eukaryotes, mRNA degradation can proceed in both directions, either 5’ to 3’ or 3’ to 5’. During 3’ to 5’ decay, RNA degradation is catalyzed by an essential multi-subunit 3’ to 5’ exoribonuclease termed the RNA exosome. In the cytoplasm, the exosome is recruited to ribosomes to facilitate translation-dependent mRNA decay, and in the nucleus, RNA exosome activities are required for processing and/or degradation of nuclear RNA such as mRNA, rRNA, snRNA, snoRNA, and tRNA. Due to its architectural complexity, the structure and activities of the eukaryotic exosome remained shrouded in mystery.

The recent highlight from the Lima lab was the determination of the atomic structure of the 300 kDa...
nine-subunit human RNA exosome, a 3'-5' exoribonuclease complex composed of nine distinct protein subunits that comprise the eukaryotic exosome core (Figure 9); Rrp41, Rrp42, Rrp43, Rrp45, Rrp46, Rrp4, Rrp40, Cs14, and Mtr4. In conjunction with the structure, the Lima lab utilized reconstituted exosomes from human and yeast to define the biochemical activities of the exosome. These studies, have provided a framework for understanding the diverse functions of the exosome in biology by elucidating the first structure for any eukaryotic exosome and establishing a rationale for its activities in the cell.

Viruses with lipid-bilayer membranes initiate infection by fusion of viral and host-cell membranes. One or more proteins on the surface of the virus particle facilitate the fusion event. These proteins are often the targets of neutralizing antibodies, and in the case of the HIV envelope protein, the target of an approved antiviral drug. Herpesviruses have a number of distinct surface proteins, four of which are essential for viral entry. One of these, known as gD (for “glycoprotein D”), binds cellular receptors and therefore mediates attachment to a host cell; until recently, the functions of the other three (gB, gH, and gL) had not been assigned.

The crystal structure of the ectodomain of gB from herpes simplex virus type 1 (HSV-1), the cause of cold sores and the prototype member of the herpesvirus family (which includes a number of human pathogens), has now shown that gB is the viral fusion protein. Moreover, gB turns out to resemble the fusion protein of a completely unrelated virus, vesicular stomatitis virus (VSV), a cousin of rabies virus. The gB structure determination used diffraction data collected at NE-CAT beamline 24-ID-C. The ectodomain (that is, the large part of the protein that projects outside the viral membrane) was prepared as a recombinant protein (700 amino-acid residues) expressed in insect cells. It forms a stable trimer, as does intact gB on the surface of a virus particle. After some fine-tuning of the expressed fragment, we obtained well-ordered crystals in space group P1 with a=83 Å, b=99 Å, c=100 Å, α=67°, β=71°, γ=78°. Initial experimental phases were obtained using single-wavelength anomalous dispersion, with crystals grown from SeMet substituted protein. Using the NE-CAT beam line was critical for the success of this project as only a few small, rapidly decaying crystals of SeMet protein were obtained.

The gB protein (Figure 10) has an elongated, spike-like aspect. Those viral fusion proteins for which data are available work by undergoing a series of conformational rearrangements, coupled to interaction with each of the two fusing bilayers. Thus, the “prefusion” conformation may be very different from the “postfusion” conformation. By coincidence, the structure of the VSV fusion glycoprotein, G, was determined in the laboratories of Felix Rey (Institut Pasteur) and Yves Gaudin (CNRS, Gif-sur-Yvette) at the same time as our work on gB, and a chance correspondence led to the discovery of the similarity of the two proteins. The VSV G protein was known to be in a postfusion conformation, and we could therefore conclude that the same is probably true of gB in our crystals. Information from the extensive earlier studies on VSV-G further enhanced interpretation of the gB results. Conservation of gB among the various herpesviruses allows us to extend our conclusions to members of the entire family.
Electron transfer between biological macromolecules usually occurs within a complex in which the participating redox centers maintain a specific geometrical relationship and the intervening protein matrix can promote and regulate electron flow between centers. In most cases, the complexes are only transiently stable and must first form before the electron transfer events can occur. Methylamine dehydrogenase (MADH), a quinoenzyme, and amicyanin, a cupredoxin, both isolated from Paracoccus denitrificans, form one of the best characterized physiological electron transfer complexes. Past work in the Mathews lab has included studies of the wild type proteins as well as of site directed mutants containing alterations in the MADH quinoenzyme and in amicyanin, mostly focused on the complex interface and the copper binding region.

A recent highlight from the Mathews lab is the study of a new electron transfer complex, one between a related quinoprotein, aromatic amine dehydrogenase (AADH) and the cupredoxin, azurin, both isolated from Alcaligenes faecalis. MADH and AADH are both αβ2 heterotetramers, each containing the redox cofactor tryptophan tryptophylquinone (TTQ) within the β-subunit, although they are only about 30% identical in sequence. However, azurin differs substantially from amicyanin, having only 21% sequence identity and a different copper coordination geometry involving five coordinating ligands instead of four. In addition, the electron transfer activity of either quinoenzyme with the complementary cupredoxin partner in solution is negligible.

When the MADH and AADH portions of the two complexes are aligned, the amicyanin and azurin molecules are located approximately in the same position, with their copper positions about 5 Å apart, but their orientations differ by approximately 90° (Figure 11). The size of the AADH/azurin interface (~500 Å²) is about 3/4 as large as that of MADH/amicyanin interface and the fraction of hydrophobic residues is larger within the former (~70%) than within the latter (~60%); also, MADH and amicyanin are linked by 2 salt bridges and three bridging waters while AADH and azurin are linked by only one hydrogen bond and no water linkages. These differences account for the substantially weaker binding in solution of azurin to AADH than of amicyanin to MADH. The distance between the copper and TTQ cofactor is also about 3.5 Å greater in the AADH/azurin complex than in the MADH/amicyanin complex. This difference is also consistent with the observed electronic coupling between copper and TTQ in the two complexes (0.13 cm⁻¹ vs 12 cm⁻¹, respectively) as observed in solution.

Recent Staff Activities

The NE-CAT resident staff members continue to be active disseminating information on beam line activities. Three posters were presented at the APS Users Meeting held at APS from May 7-12, 2007. Malcolm Capel has been invited to make a presentation titled "Implementation of Microdiffraction Techniques at Northeastern Collaborative Access Team Beamlines" at the Micro-Crystals, Micro-Beams, and Multiple Crystals Workshop at the 2007 ACA Meeting to be held in Salt Lake City, July 21-26, 2007. NE-CAT will also present a poster at this meeting. Malcolm Capel, has also been
extremely active within APS, currently serving on the Bio-CAT Executive Committee, IMCA-CAT Beam Line Design Review Board, the APS Sector 12 Large-Offset Monochromator Design Review Committee, and the LS-CAT Optics Design Committee.

**Personnel Changes**

NE-CAT has experienced a number of staff changes in 2007. Craig Ogata, Jun Wang, and Xiaochung Yang have all left NE-CAT. With Craig, the former Associate Director for User Operations, leaving there was a need to re-organize NE-CAT’s management structure. As part of the new management structure, Malcolm Capel has been promoted from Associate Director of Technical Development to Deputy Director. Malcolm is now in charge of all of NE-CAT’s technical developments as well as the user program at the APS site. With the second beam line, Phase II, coming into full operation and several support personnel leaving, additional beam line support staff will be needed. To partially fill this need, Dr. Frank Murphy shown here was hired in May, 2007 as Beam Line Scientist. Frank received his Ph.D in Bio-Chemistry in 2000 from the University of Illinois. Prior to his hire by NE-CAT he held a Postdoctoral Appointment at the Medical Research Council- Laboratory of Molecular Biology, Cambridge, UK.

**References**