NE-CAT Communications

A Biannual Newsletter of the Northeastern Collaborative Access Team Winter 2011

National Center for Research Resources

Message from the Director

Steve Ealick

The PILATUS-6MF has arrived! If you noticed the extra 'F' in the name, then you will be happy to know that NE-CAT purchased an upgrade, making our PILATUS one of the new Fast models that can frame at 25 Hz. This makes our PILATUS twice as fast as the original model. NE-CAT is excitedly and busily preparing the hardware and software to support the PILATUS-6MF. Users should expect to see the PILATUS-6MF on 24-ID-C during the first run cycle of 2012.

As per the NCRR website, "[o]n December 23, 2011, President Barack Obama signed the Fiscal Year 2012 Omnibus Appropriations bill. As part of this legislation, the National Center for Research Resources (NCRR) is dissolved and the National Center for Advancing Translational Sciences (NCATS) is established." Under NCRR, NE-CAT was a Biomedical Technology Research Center in the division of Biomedical Technology, specifically an Undulator Resource for Structural Biology. With some other Biomedical Technology programs, we have been transferred to the National Institute of General Medical Sciences (NIGMS). The NIGMS has established two new divisions that will bring together existing the NIGMS programs with programs transferred to the NIGMS from the NCRR. NE-CAT will be a part of the Division of Biomedical Technology, Bioinformatics, and Computational Biology (BBCB). Also, we will continue to prepare for our grant renewal. We have had two meetings with our Resource Advisory Committee in 2011 and intend to have one more prior to the submission of our proposal to the NIGMS in May 2012.

Beamline Developments

1. PILATUS-6MF Preparation, Commissioning and Installation

With the PILATUS-6MF, short, shutterless exposures can be expected to be the norm for data collection and NE-CAT intends to take full advantage of the 25 Hz framing rate. This will require a very stable beam. Currently, residual beam motion is compensated by performing three sweeps through the desired oscillation angle during a single exposure. With shutterless operation, multi-sweep exposures cannot

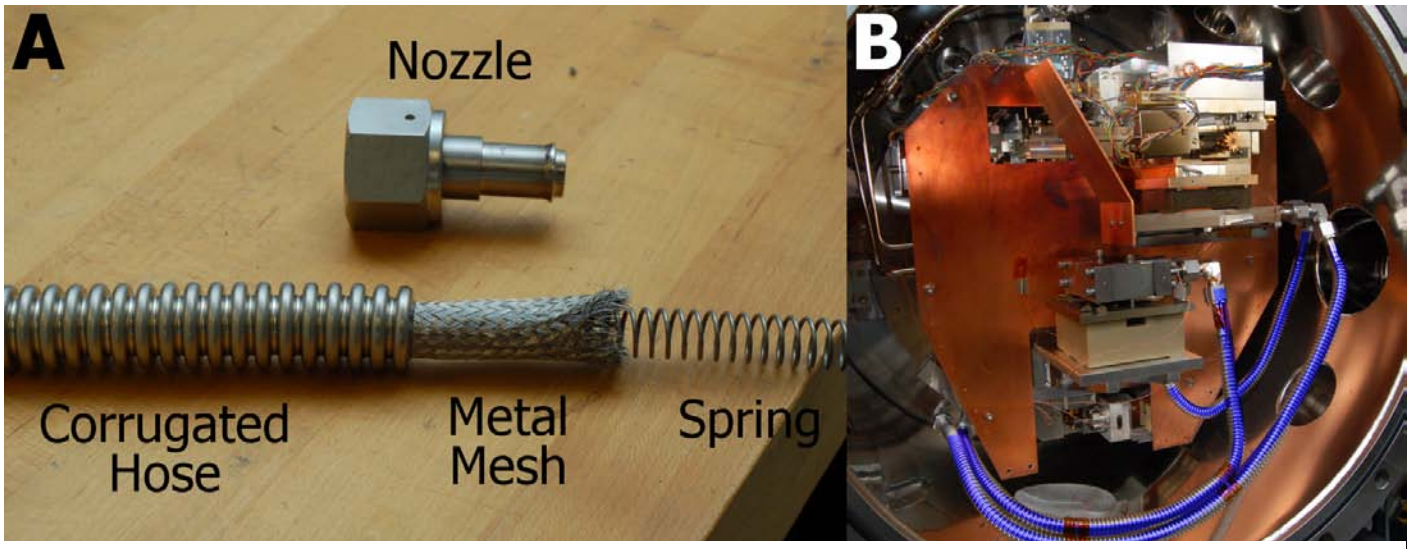

Fig. 1 (A) The various parts of the new, modified LN_2 delivery hoses, showing the sandwiching of the metal mesh between the corrugated hose and the spring. (B) The location of the LN_2 delivery hoses after installation in the monochromator.

be used to compensate for residual beam motion.

In order to take advantage of the PILATUS-6MF's shutterless operation mode, NE-CAT must eliminate as many sources of potential beam instability as possible. Beam stability can be compromised by monochromator vibration caused by liquid nitrogen (LN_2) flow for cooling and floor vibrations.

In the Summer 2011 newsletter, the modification and replacement of monochromator crystal standoffs was described in detail. To further reduce residual beam motion, additional modifications were made to the monochromator cryo-cooling loop during the September 2011 shutdown. The monochromator on 24-ID-C was opened for replacement of the liquid nitrogen delivery hoses. The original corrugated hoses are believed to create turbulence that is transferred to the monochromator crystals. New hoses, composed of a smooth metal mesh sandwiched to the corrugated hose by a spring, were made over the summer. The metal mesh eliminates the corrugation while the spring prevents the mesh from collapsing (Fig. 1).

The first modification reduced a significant amount of beam motion and the second modification provided an additional modest improvement. Currently, it is possible to take single sweep data at speeds better than 50 msec/exposure.

The PILATUS-6MF was delivered in mid-October. After delivery, it was assembled in the engineering lab for commissioning. The detector was commissioned during the final week of October. An installation engineer from Dectris visited NE-CAT for this purpose. During commissioning, a weakly radioactive Fe-source was used to ensure that the detector was fully operational (Fig. 2).

Fig. 2 Malcolm Capel passes a weakly radioactive Fe-source over the front of the PILATUS-6MF to test sensitivity during commissioning.

The surface of the detector is covered with PET, a mylar foil with a thin film of aluminum on both sides. It makes a beautiful reflective surface that cannot be touched without damage to the silicon sensors located directly below. For regular use on the beamline, NE-CAT plans to construct an automated cover to protect the surface of the detector from contact when people are inside the hutch.

During November, hardware was built and software was written to integrate the PILATUS-6MF with the C beamline. Over the Thanksgiving weekend, the PILATUS-6MF was temporarily installed on 24-ID-C to test the hardware and software (Fig. 3A). The PILATUS-6MF was tested for 2 days. Data collection was performed in both shutterless and shuttered modes. Initial tests show almost no background, excellent statistics, and the possibility of detecting crystal diffraction at higher resolution with lower X-ray dose (Fig. 3B).

With the arrival of the PILATUS-6MF, NE-CAT also increased local data storage with the purchase and

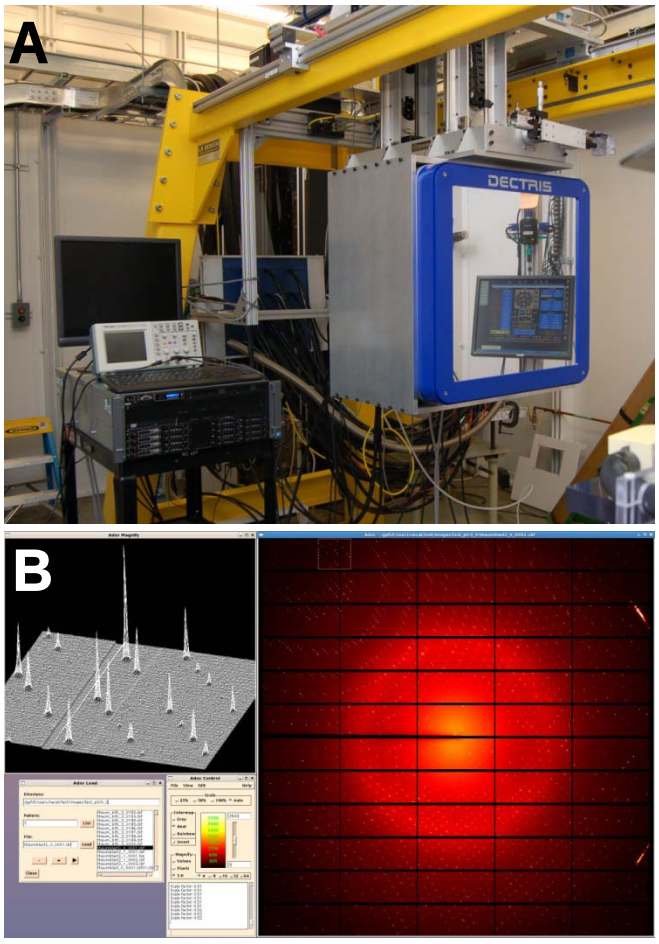

Fig. 3 (A) PILATUS-6MF installed on 24-ID-C during Thanksgiving weekend. (B) Diffraction of thaumatin using the PILATUS-6MF.

delivery of 2 new SATABeasts. The SATABeasts are 84 terabytes each and will be made available to users during the 2012-1 run cycle.

2. Easier Positioning of Thin Crystals Using the Occluded View Alignment

With very thin crystals, it is often difficult to visually discern the location of the crystal when in the plane of the loop. It becomes even more difficult when the sample is small and the lensing effect of excess cryo solution in the loop distorts the true position. To compensate for this issue, a new scanning method was added to the beamline software during August.

Known as the Occluded View Alignment, the method requires the user to center at any point in the loop. Then choosing the view corresponding to the plane of the loop (or the Occluded View), the user can define a box for scanning. The software calculates a series of points for scanning perpendicular to the plane of view based on user-entered criteria, then takes snapshots at each point. Similar to the Raster Snap vector-based scanning method, the DISTL component of RAPD is used to calculate a series of diffraction quality metrics. A summary of the diffraction metrics is displayed and the user can use this dialog to plot a selected subset of the metrics as a function of the scan position index. The diffraction maximum occurs when beam position coincides with the crystal, which otherwise cannot be determined by visual alignment. The user can select a data record corresponding to this maximum in the scan point dialog that causes the MD2 x,y,z centering stages to step to the selected scan point coordinates. This point can then be entered into the vector database for use in other vector-based scanning methods.

Fig. 4 Visual of the Occluded View Alignment with the edge of loop aligned with the plane of view. Note the inability to visually detect the location of the thin crystal. Red ellipses show areas of impending X-ray diffraction.

For ease of visualiziation, clicking on each point in the image also brings up the corresponding diffraction image in the ADXV image viewer. This enhancement is now available in diffraction-based alignment protocols and Raster Snap.

3. Enhanced Beamline Software Speed and Stablity using Redis

Redis is an open source memory-resident key-value store. It is a non-SQL data structure server which can be used to store strings, hashes, lists, sets and sorted sets. Since it is memory-resident, Redis is extremely fast and capable of performing up to 180,000 transactions/second. Its performance is also independent of the size of the database. Due to these advantages, the beamline software (CONSOLE) was adapted to use Redis during the month of August 2011. This reduced the client load on the MD2 comwrapper as well as provided greater stability and speed for the RAC (Remote Access Channel).

After several months of testing, Redis has been proven capable of handling the needs of CONSOLE. The beamline software now relies solely on Redis.

4. Rapid Identification of Metals in Samples using New Fluorescence Detector

NE-CAT has acquired a new X-ray Fluorescence detector that is capable of collecting Energy Dispersive X-ray emission spectrum. Manufactured by Amptek, the X-123SDD is composed of: (1) the XR-100SDD silicon drift X-ray detector and preamplifier, (2) the DP5 digital pulse processor and multi-channel analyzer (MCA), and (3) the PC5 power supply.

The X-123SDD uses a silicon drift detector (SDD). A SDD is a special type of photodiode, functionally similar to a planar Si-PIN photodiode but with a unique electrode structure. It has much lower capacitance than a planar diode of the same area, and since electronic noise at short shaping times is proportional to capacitance, the SDD yields much lower noise at high count rates. Lower noise implies better resolution, particularly at low energies. X-123SDD is suitable any energy between 1 – 25 keV and has an energy resolution of ~130eV.

NE-CAT currently uses older Cyberstar Scintillation Detectors fitted with yttrium aluminum perovskit (YAP) crystals by Oxford Danfysik. The YAP crystal allows for a high count rate, but has poorer energy resolution. Currently, the detectors are not connected to an MCA and, hence, are not capable of discerning the difference between fluorescence emission, incident X-ray or Compton scattered photons. Instead the counts

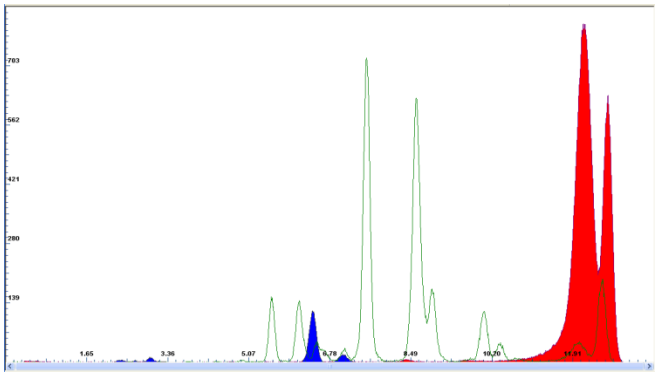

Fig. 5 Fluorescence scan of a myoglobin crystal on 24-ID-E using the X-123SDD. Excitation occurs at 12662 eV. Incident and Compton scattered photons are displayed as two red peaks. Peaks outlined in green are from a calibration standard containing many different metals. The blue peak at 6.42 keV reflects the Kα emission line of the Fe found in myoglobin.

from the detector reflect a combination of all three types.

As a single unit, the X-123SDD, along with its built-in MCA, can both detect and differentiate between emission lines, incident X-ray scatter and incident Compton scatter and output counts in any region of interest.

During the 2011-3 run, the X-123SDD was installed on 24-ID-E and could be used to scan for metals in crystals without changing energy. This is a very fast procedure and only takes a couple of minutes per sample. On 24-ID-E, the fluorescence detector is capable of detecting metals with emission lines less than ~12.2 keV. Compton scattering at 12662 eV, the wavelength of 24-ID-E, blocks the signal from any peaks above 12200 eV. As excitation occurs at 12662 eV, metals with emission lines above this energy cannot be detected on 24-ID-E.

NE-CAT plans to move the X-123SDD to 24-ID-C so that it can be used to detect metals with emission lines up to 22 keV. In addition, the higher resolution and lower noise should result in cleaner EXAFS scans.

5. RAPD License

Interest in the ability of RAPD to rapidly and robustly auto-index, integrate data and allow easy use of mini-kappa goniometers has been expressed in the crystallography community including LS-CAT and groups at Diamond Light Source. In response, the full source code for RAPD has been made available at the open source repository, github (https://github.com/RAPD). RAPD is available under the BSD 3-Clause License.

Research Highlights

Molecular Structure of Full-Length *Drosophila* Cryptochrome

Brian Crane, Cornell University, Department of Chemistry and Chemical Biology, Ithaca, NY

Most higher organisms, including human beings, rely on circadian clocks to pace their metabolism to the day-night (or diurnal) cycle. Circadian clocks control the timing of numerous biological processes including those related to metabolic rate, sleep, damage repair, and cell growth. “Jet lag” is a common circadian disturbance caused by travel across time zones, while more serious problems, including sleep syndromes, psychological disorders, and cancer, are associated with chronic clock malfunction[1]. While the clock machinery in humans has many layers of complexity, its key components and mechanisms are shared with lower animals, including the fruit fly *Drosophila,* which has served an important experimental model for understanding the biochemistry of circadian rhythms[2]. At the molecular level, circadian clocks are negative feedback loops composed of interacting proteins, whose levels oscillate over the diurnal cycle to time gene expression.

Light signals set circadian clocks through the reception of photons by specific light sensitive proteins. Work on biological photosensors goes back to Charles Darwin,

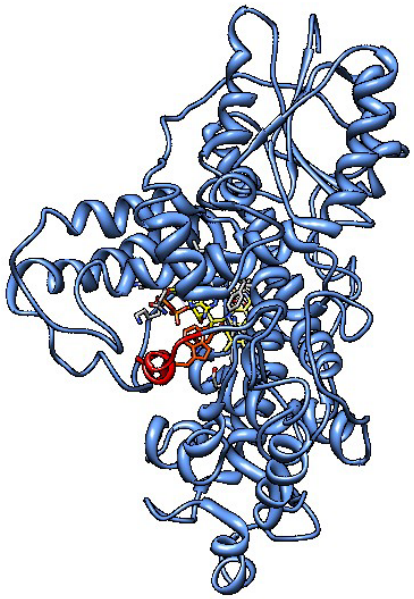

Fig. 6 The structure of full-length *Drosophila* cryptochrome. The tail helix (red) binds along the flavin (yellow) and juts invariant Trp536 (orange) into what would be the active center of a photolyase.

who first documented the ability of plants to move in response to light patterns[3]. In *Drosophila*, the circadian clock light sensor is known as cryptochrome, named for its cryptic ability to translate light signals[4; 5]. Recently, cryptochromes have also been implicated in the ability of organisms to respond to magnetic fields[6]. Another intriguing aspect of cryptochromes is that they are evolutionarily related to photolyases, which are enzymes that use light to repair damage to DNA caused by UV radiation. Given the similarities between cryptochromes and photolyases their very different DNA repair and light-sensing functions have been a puzzle to reconcile. However, there are differences between the protein families. Compared to photolyases, cryptochromes have a variable C-terminal peptide extension that is pivotal for their function.

The Crane group at Cornell University determined the molecular structure of *Drosophila* cryptochrome (dCRY) with an intact C-terminus and carried out biochemical experiments to probe its mechanism of action[7]. The structure determination was particularly challenging due to an unusual case of non-merohedral twinning. The microfocused beam line of NE-CAT 24-ID-E enabled the structure solution by allowing data collection from small minimally twinned crystals. The unique C-terminal "tail" helix of dCRY docks in a groove near the photoactive pigment bound by the protein (a flavin adenine dinucleotide cofactor or FAD). Remarkably, the tail serves as a structural mimic for the DNA substrate of photolyases. The dCRY structure, combined with biochemical and cellular studies indicate that light causes the photoreduction of the flavin cofactor, which then restructures the tail, changes the conformation of the protein, and thereby allows reaction with down-stream partners in the circadian clock feedback network. Thus, photolyases harness light to repair DNA damage, whereas cryptochromes use related chemistry to drive conformational changes that gate signaling events.

Fig. 7 Picture of Anand Vaidya, Brian Crane and Joanne Widom.

The work was done in collaboration with the laboratory of Michael Young at the Rockefeller University through an interaction that grew out of Cornell's tri-institutional program in Chemical Biology.

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Workshop

The NE-CAT workshop on "Advances in Low to Moderate Resolution Phasing and Refinement" was held at Rockefeller University on September 19, 2011. The workshop was organized by Dr. Seth Darst of Rockefeller University, Dr. Igor Kourinov of NE-CAT, Dr. Kay Perry of NE-CAT and Dr. K. Raj Rajashankar of NE-CAT. The workshop was organized into four sections with an introduction and discussion led by Dr. Stephen C. Harrison of Harvard University. The sections were chaired by Dr. Steven E. Ealick of Cornell University, Dr. Darst, Dr. Rajashankar and Dr. Perry.

The workshop started with an introduction by Dr. Stephen C. Harrison. He challenged speakers and participants to consider the following questions:

1. When and why, in determining low resolution structures, are low resolution data important or necessary?
2. When can low resolution data contribute to phase determination, even when high resolution data can be gathered?

Fig. 8 Workshop speakers (from left to right): John Tainer, Yong Xiong, Silvia Russi, Randy Read, Axel Brunger, Frank Dimaio, Malcolm Capel, Tom Terwilliger.

To see if these questions were answered, non-attendees can view abstracts, Powerpoint presentations, video and audio from the workshop at the workshop website (http://necat.chem.cornell.edu/workshops/lowresolutionworkshop.php).

Staff Activities

Talks

Jon Schuermann, "APS Status Report," Kappa Workgroup Meeting 2011, BESSY II, Berlin, Germany, November 28-29, 2011.

Jon Schuermann, "RAPD and STAC," Kappa Workgroup Meeting 2011, BESSY II, Berlin, Germany, November 28-29, 2011.

Jon Schuermann, "RAPD GUI," Kappa Workgroup Meeting 2011, BESSY II, Berlin, Germany, November 28-29, 2011.

Posters

Surajit Banerjee, Roberto Vanacore, M. Sundaramoorthy, Ariel Boutaud, Billy G. Hudson, "Crystal Structure of Type IV Collagen α-2 NC1 Domain Monomer," Collagen Gordon Research Conference, Colby-Sawyer College, New London, New Hampshire, July 17-22, 2011.

Igor Kourinov, Malcolm Capel, Surajit Banerjee, Amit Belani, Leslie Kinsland, Ed Lynch, Frank Murphy, David Neau, Kay Perry, Kanagalaghatta Rajashankar, Cynthia Salbego, Jonathan Schuermann, Narayanasami Sukumar, James Withrow & Steven E. Ealick, "NE-CAT Crystallography Beamlines for Challenging Structural Biology Research," XXII Congress and General Assembly, International Union of Crystallography, Madrid, Spain, August 22-30, 2011.

Publications

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Workshop Attendance

Jon Schuermann, “New methods in Macromolecular Crystallography using synchrotron radiation,” Satellite Workshop of the Third Joint BER II and BESSY II Users' Meeting, BESSY II, Berlin, Germany, November 30, 2011.

Committee Meetings

Malcolm Capel - ID 23A/B and ID 29 Beamline Review Panel for ESRF Upgrade Programme, November 7-9, 2011, Grenoble, France.

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