NE-CAT Communications

A Biannual Newsletter of the Northeastern Collaborative Access Team Summer 2016

Message from the Director

Steve Ealick

Big changes are coming to NE-CAT over the next couple of years. As detailed in the last newsletter, we are working on installing a secondary focus on 24-ID-E. In this newsletter, you will read about new Vertical Focusing Mirrors which increase the brilliance of our beamlines and a new kilohertz framing detector, the EIGER. Though we strive to make the changes and improvements seamless for our users, much preparatory work goes into laying the foundation for each change. NE-CAT beamline scientists are joining others in the crystallographic community to learn, discuss and drive the direction of change as NE-CAT moves toward integration of the new generation of ultra-fast detectors.

The goal of NE-CAT is to provide a state-of-the-art facility for our users so that they may solve the most difficult crystallographic problems while supported by scientific advice by our staff. With this goal in mind, we are forever moving forward with changes for the better. I encourage all our users to reach out to NE-CAT staff if you have a question about how to maximize your use of our two micro-crystallography beamlines. You can find their contact information, and links to apply for beamtime on our website (http://necat.chem.cornell.edu).

New NE-CAT Assistant Director

Frank Murphy has been promoted to the position of Assistant Director in recognition of his general participation as member of the NE-CAT management team, for his leadership in the development of RAPD, and for his participation in the preparation of grants. Frank joined NE-CAT in 2007 as a Staff Scientist. As part of our P41 grant, Frank has been the technical lead for TR&D 2, Beamline Automation Technologies for Structural Biology. Frank received his Ph.D in Biochemistry in 2000 from the University of Illinois Urbana-Champaign. Prior to joining NE-CAT, Frank was a postdoctoral research associate with Venki Ramakrishnan at the MRC Laboratory of Molecular Biology in Cambridge, England.

Beamline Developments

1. New Vertical Focusing Mirrors

As part of our continuing efforts to improve our beamlines, NE-CAT has purchased two new mechanically bent vertical focusing mirrors (VFMs). We re-purposed funds originally intended for the purchase of a bimorph VFM for 24-ID-C. In a bimorph mirror, piezoelectric blocks are imbedded within the mirror, and bending is effected by applying voltages to these independent piezos. This results in a mirror with high spatial frequencies and allows for correction of optical defects in the mirror from various sources. However, as mirror manufacturers can now routinely figure and polish mirrors of very high quality, we

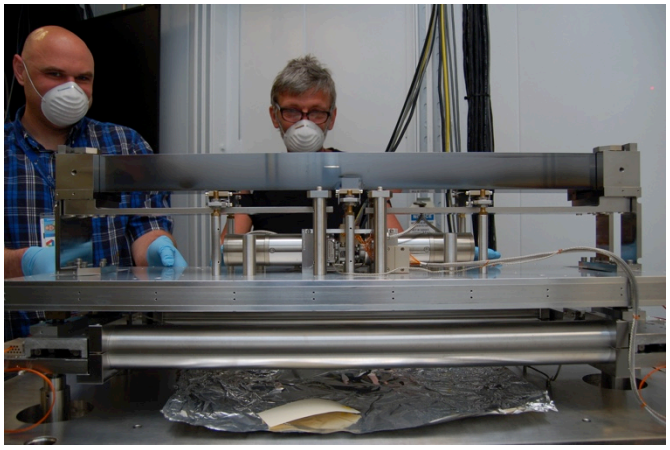

Fig. 1 Vertical Focusing Mirror. The mirrors on both beamlines now have the same design. The VFM for 24-ID-E is shown with Jim Withrow on the right and Ian Campton from FMB-Oxford in the center.

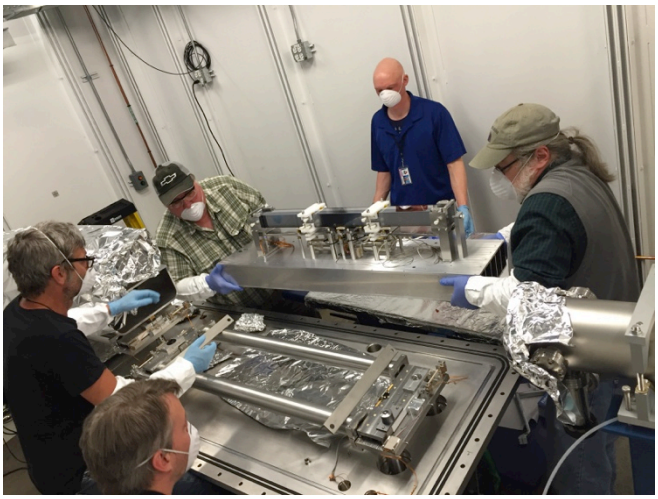

Fig. 4 Placement of the 24-ID-C VFM on the transition assembly. Shipping restraints hold the mirror in place over the support structure and cams. Clockwise from top left: Ed Lynch, Jon Schuermann, Malcolm Capel, Frank Murphy, Ian Campton.

decided that it was unnecessary to deal with the complexity, fragility and additional cost of a bimorph. Instead of a single bimorph mirror, NE-CAT obtained 2 VFMs from FMB-Oxford. Both mirrors arrived at NE-CAT in February 2016.

The goals of the VFM replacement are to install mirrors with lower surface slope errors and lower surface roughness such that the useful fraction of the mirror surface is increased and perturbations of the focus as a result of minor beam movements and energy changes are reduced. Better quality mirrors would improve the homogeneity of the beam profile in this scenario and result in increased brightness of the primary beam.

The original VFMs were a ULE of quartz while the new mirrors are pure silicon. In addition, the original VFMs were two different designs with the VFM on 24-ID-E being a 2-point U-bend and the VFM on 24-ID-C a 2-moment spring-driven 4-point bend. Both of the new VFMs are direct cam-driven elliptically bent mirrors (Fig. 1). The old designs resulted in a large amount of hysteresis in the VFMs. The hysteresis is substantially reduced by the new bender design. As a result, changing the focus of the beam is now rapid and reproducible. In the future, setting the focus of the beam at the sample or at the detector, which currently requires staff intervention, may become a simple automated task that can be executed by regular users.

The new mirrors were designed to be compatible with the existing mirror support and positioning systems. This simplified the extraction of the old VFMs and their replacement during the May 2016 shutdown (Fig. 2).

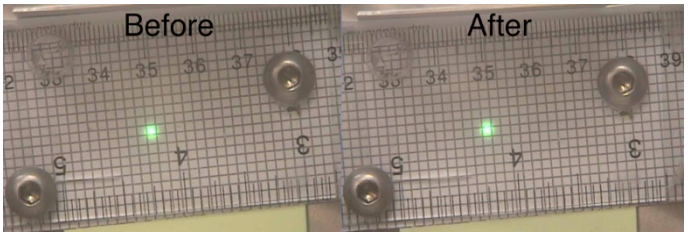

Fig. 3 Position of the beam on 24-ID-E prior to shutdown in April on the left and initial position of the beam on 24-ID-E after the return of the beam in June.

Ian Campton from FMB-Oxford came to assist in the installation of the VFMs, their connection to the motor controllers and additional beamline commissioning.

Prior to the May 2016 shutdown, location measurements were made during the last few days of operations in April. These measurements allowed for alignment of the mirrors using a laser. At the start of the new run cycle, the beam was easily located (Fig. 3) and this minimized the time required for beamline commissioning.

As hoped, the new beams on 24-ID-C and 24-ID-E are more brilliant and more uniform. The new beams are also 3-4-fold smaller (7 µm and 5 µm vertical, 24-ID-C and 24-ID-E, respectively) than the old beams. The secondary beamlets are now completely absent and the non-Gaussian distribution has been eliminated on 24-ID-E. The beam on 24-ID-C is also more uniform (Fig. 4). Due to the beam size reduction in the vertical, NE-CAT now offers two modes of focus: best focus and fill aperture. At best focus, the beam is sharply focused on the sample and very small in the vertical. In fill aperture mode, the beam is slightly under-focused to broaden the beam direction in the vertical but the beam distribution is remains Gaussian.

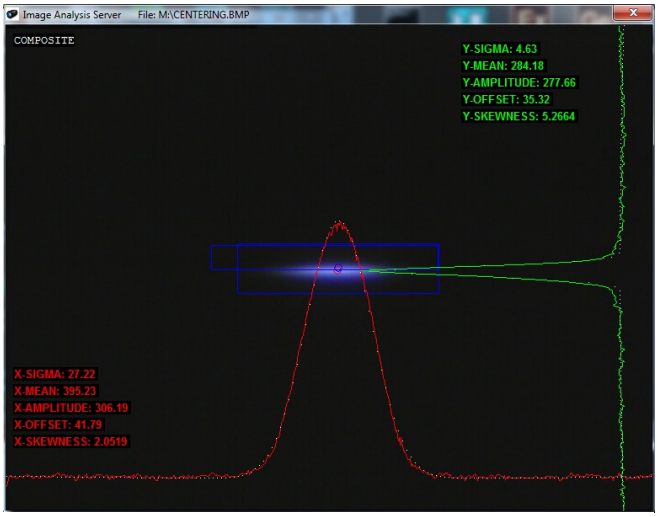

Fig. 2 Size and shape of the focused beam on 24-ID-C after the installation of the new VFMs.

2. EIGER Detector

To take full advantage of a smaller, more stable beam, 24-ID-E will need a better detector. Currently, we have an ADSC Q315 CCD Detector available for data collection on 24-ID-E. Having acquired a PILATUS for 24-ID-C, we are favorably impressed with the speed and sensitivity of a photon counting detector. NE-CAT applied through the High End Shared Instrumentation (HEI) Grant for an EIGER X 16M detector. The EIGER is a hybrid pixel array detector built by DECTRIS. Like the PILATUS, it is a photon counter but with multiple design improvements.

The EIGER 16M is composed of 32 modules laid out in a 4 x 8 grid while the PILATUS 6M has 60 modules in a 5 x 12 grid. However, compared to the PILATUS, the EIGER has smaller individual pixels. The pixels on the EIGER are 75 µm x 75 µm whereas the PILATUS has larger 172 µm x 172 µm pixels. The overall active area of the EIGER is smaller, but contains more pixels (See table). This means that the EIGER will provide better spot separation.

Property /Characteristic	PILATUS-6MF	EIGER X 16M
Detector Type	Photon Counter	Photon Counter
Active Area (mm x mm)	423.6 x 434.6	311.2 x 327.8
Edge Pixel Count	2463 x 2527	4150 x 4371
Pixel Size (microns)	172 x 172	75 x 75
Detector Module Tiling	5 x 12	4 x 8
Tiling Gap (mm)	1.77 x 2.72	0.75 x 2.78
Pixel Counting Depth	1048576 (20 bit)	4096 (12 bit)
Image Counting Depth	1048576 (20 bit)	65536 (16 bit) 4294967296 (32 bit)
Read Out Time	950 µs	Continuous, 3 µs deadtime
Image Size (Mbytes)	24	72
Maximum Frame Rate (Hz)	25	133
Max Counting Rate /Pixel/sec	1.5 MHz	
Quantum Efficiency (@12.4 keV)	~84%	~84%
Approximate Cost (millions $)	1.5	2.1

The EIGER is a faster detector than the PILATUS with a higher dynamic range and a new continuous readout. This is the result of each pixel having a readout buffer. After the acquisition of a frame, the information is transferred to the readout buffer. As this transfer is occurring, acquisition of the next frame can begin after only 3 microseconds. In addition, frames can be added together in auto-summation mode to extend the data depth. Each EIGER pixel is 12 bits, but auto-summation extends the data depth to 32 bits, whereas, the PILATUS has a set bit depth of 20 bits per pixel. This provides greater dynamic range and avoids overflows (See comparison table for additional statistics).

NE-CAT is now beginning preparations for delivery of the EIGER detector in approximately late 2016 or early 2017.

3. High Data-Rate MX Meeting

The EIGER X 16M detector is capable of collecting crystallographic data at kilohertz frame rates. Since the previous convention of writing diffraction images as separate files is not suitable for this detector, it uses an image format called HDF5. The HDF5 format was designed by the HDF group many years ago and is similar to a filesystem in structure. Dectris and the HDF group have been working together to make the HDF5 format work for the crystallographic community.

The purpose of the High Data-Rate MX (HDRMX) Meeting at Brookhaven National Laboratory (BNL) in May 2016 was to discuss the changes software developers are making to data processing and beamline specific programs to handle the HDF5 format, as well as network and data storage infrastructure required to handle the high data rate.

Due to limited attendance space available at BNL, Jon Schuermann (NE-CAT) decided to host a satellite video conference of the meeting at APS to allow other beamline personnel to contribute to the discussions. As a result, personnel from NE-CAT, GMCA-CAT, IMCA-CAT, LS-CAT, SBC-CAT and SER-CAT all participated in the HDRMX meeting and were able to contribute to the future development of the HDF5 format within the crystallographic community.

Research Highlights

The structural basis of inhibitor binding in essential peptidoglycan biosynthetic enzyme MraY

Seok-Yong Lee, Associate Professor of Biochemistry, Duke University School of Medicine, Durham, NC

Ellene H. Mashalidis, Postdoctoral Associate, Duke University School of Medicine, Durham, NC

Ben C. Chung, former Postdoctoral Associate, Duke University School of Medicine, Durham, NC

Due to rampant antibiotic resistance, serious bacterial infections have emerged worldwide that are difficult to treat with commonly used antibiotics and traditional regimens. This has led some researchers to suggest that global healthcare is on the brink of a "post-antibiotic" era. Several classes of existing antibiotics target the formation of the bacterial cell wall. The most widely used antibiotics of this type, the penicillins, act by inhibiting the biosynthesis of peptidoglycan, a major cell wall component. While the penicillins have been important in treating life-threatening bacterial infections, they are no longer effective against many resistant strains. The peptidoglycan biosynthetic pathway is particularly vulnerable to chemotherapeutic intervention and is rich in alternative antibiotic targets. New compounds that inhibit this pathway are urgently needed.

Phospho-MurNAc-pentapeptide translocase (MraY) catalyzes an essential step of peptidoglycan formation and has long been considered a promising target for antibacterial chemotherapy. MraY is an integral membrane protein that transfers hydrophilic phospho-MurNAc-pentapeptide from UDP-phospho-MurNAc-pentapeptide (UM5A) to the lipid carrier undecaprenyl phosphate (C_{55}-P), yielding Lipid I (Fig. 6). This is the first membrane-associated step of peptidoglycan biosynthesis and inhibition of MraY is bactericidal. MraY is a very attractive target for antibiotic development. It is the target of five different classes of natural product antibiotics with high *in vitro* and *in vivo* efficacy against a wide variety of bacterial strains. One such class, the muraymycins, is particularly active against MRSA and *Pseudomonas aeruginosa,* and muraymycin analogs are able to protect mice against *S. aureus* infection. While each class of MraY inhibitor is structurally distinct, they share a common structural feature, a nucleoside core, which is also present in the natural substrate UM5A. The substructures of MD2 are the 5'-amino ribosyl, the uridine, and the peptidic moieties (Fig. 6b).

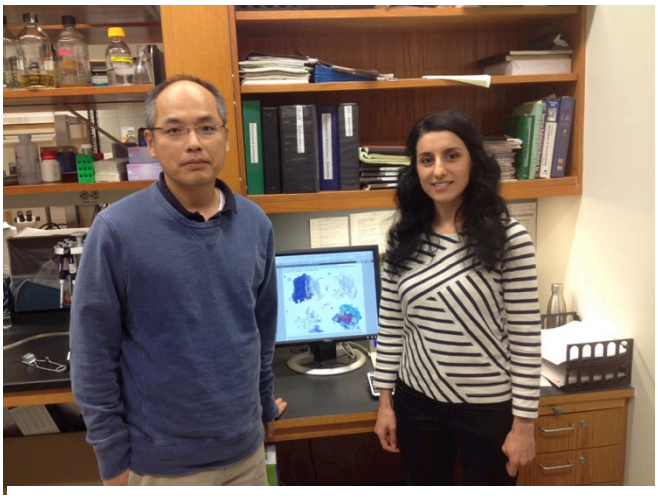

Fig. 5 Seok-Yong Lee (left) and Ellene Mashalidis (right) in their lab at the Duke University School of Medicine.

Ellene Mashalidis and Ben Chung, postdoctoral associates in the laboratory of Professor Seok-Yong Lee at Duke University School of Medicine have determined the structure of MraY bound to a muraymycin analog (MD2) with the support of the NE-CAT staff. Their findings were recently published in *Nature* (Nature. 2016 Apr 18;533(7604):557-60.). The Lee Lab had previously published the structure of MraY in the absence of inhibitors in *Science* (Science. 2013 Aug 30;341(6149):1012-6.). Crystals of MraY bound to MD2 were generated in detergent micelles and diffracted to 2.95 Å. The structure of the MraY-MD2 complex was solved with molecular replacement, using the unliganded MraY structure (PDB ID: 4J72) as a search model.

Comparison of the structures of MraY bound to MD2 and its unliganded form reveals that the enzyme undergoes significant conformational changes upon inhibitor binding (Figure 6). In the presence of MD2, two new binding sites are formed on the cytoplasmic side of MraY to accommodate the nucleoside and peptidic moieties of the inhibitor. The strongest interaction between MD2 and the protein occur in the nucleoside binding pocket, where the 5'-amino ribosyl and uracil moieties of MD2 bind to MraY like a two-pronged plug inserting into a socket. The peptidic tail of MD2 forms additional hydrogen bonding interactions with the protein, which likely further impart selectivity for MraY. Unexpectedly, MD2 does not interact with the three aspartate residues or the Mg^{2+} cofactor required for MraY activity, meaning that this competitive inhibitor likely binds to its target in a manner that is in some ways distinct from that of the natural substrate UM5A. Upon MD2 binding, the surface electrostatic potential of MraY rearranges and

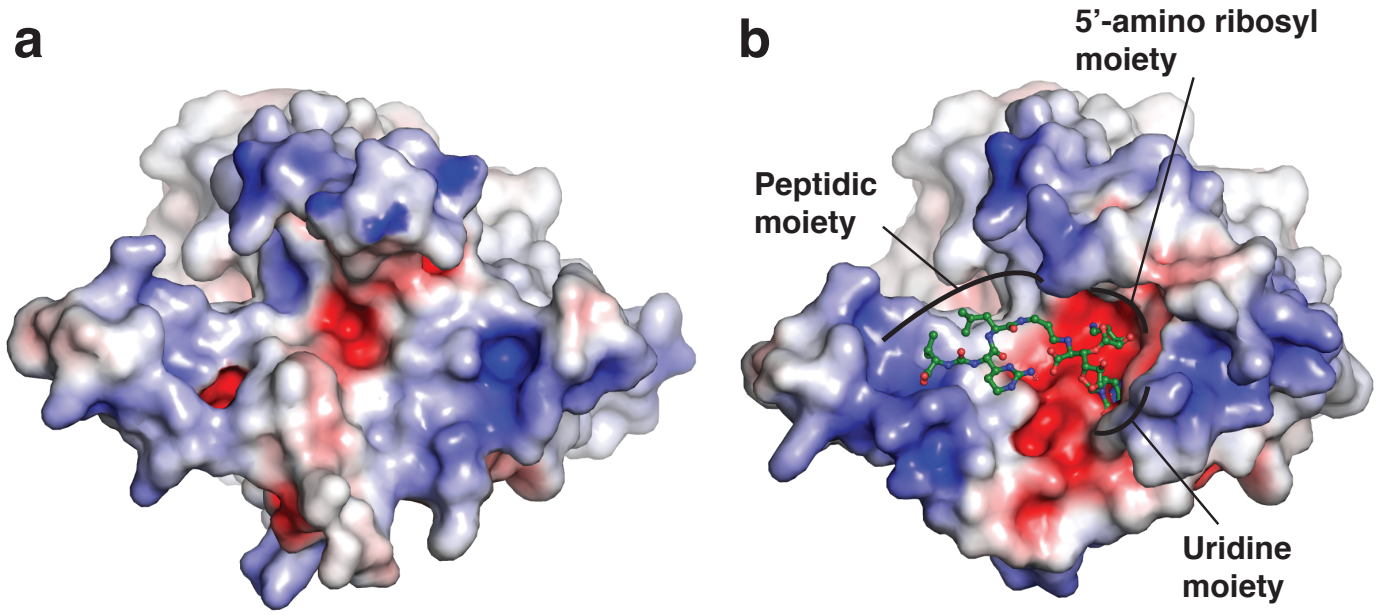

Fig. 6 a, Electrostatic surface representation of apoMraY$_{AA}$, viewed from the cytoplasm. **b,** Electrostatic surface representation of MraY$_{AA}$ in complex with MD2. MD2 is green and shown in ball-and-stick representation.

a large acidic region becomes available to interact with the inhibitor.

Using the structure as a guide, Drs. Mashalidis and Chung conducted enzymatic and biophysical experiments to elucidate the key residues involved in the protein-inhibitor interaction. They showed that the crucial interactions MD2 makes with MraY occur in the nucleoside pocket. A phenylalanine residue engaging in a π-stacking interaction with the uracil moiety and a hydrogen bond between an aspartic acid residue and the 5'-amino ribosyl moiety are crucial for MD2 binding. This study demonstrates the large conformational changes MraY can undergo in the presence of an inhibitor. The authors postulate that this structural plasticity could explain how MraY is inhibited by many structurally distinct compounds. They believe that their findings can inform the design of new inhibitors targeting MraY and thereby lead to novel antibiotics that act on bacterial cell wall formation.

Staff Activities

Poster

S. Banerjee, M. Capel, L. Kinsland, I. Kourinov, A. Lynch, F. Murphy, D. Neau, K. Perry, K. Rajashankar, C. Salbego, J. Schuermann, N. Sukumar, J. Withrow, And S. Ealick "NE-CAT: Crystallography Beamlines for Challenging Structural Biology Research", 2016 Annual Meeting of the American Crystallographic Association, Denver, CO, July 22-26, 2016.

Publications

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Meeting Attendance

Steve Ealick, K. Raj Rajshankar, NIH P41 Principal Director's Meeting, Rockville, MD, March 14-15, 2016.

Frank Murphy, David Neau, K. Raj Rajshankar, Jon Schuermann, Jim Withrow, Consortium for Management of Experimental Data in Structural Biology High Data-Rate MX Meeting, Brookhaven National Laboratory, NY, May 26-28, 2016.

Session Chair

Surajit Banerjee, "Crystal Sample Preparation: A Crystal is Just the Start," 2016 Annual Meeting of the American Crystallographic Association, Denver, CO, July 22-26, 2016.

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Rockefeller University
Yale University