



NE-CAT Communications

A Biannual Newsletter of the Northeastern Collaborative Access Team Winter 2015



Message from the Director

Steve Ealick

It is the nature of children to grow and change. The same could be said for NIH-funded facilities. We propose areas of research and development then proceed to fulfill them during the funded years of the grant. NE-CAT continuously strives for improvement: greater beam stability, faster data collection, and smaller beam size.

As a mature facility with a user base of over 150 labs, we try to make changes to our two beamlines in a way that impacts you the least. It has been part of our P41 grant to reduce the size of the beam on the 24-ID-E beamline as part of our R&D in microbeam technology. Now, after several years of planning and preparation, large-scale changes are coming to 24-ID-E. Soon, the time will come for installation of the secondary focus system. With the installation of the new single crystal on 24-ID-E last year, I am excited to see our long term plans finally come to fruition. I anticipate greater flux density and a smaller beam that will benefit all our users with microcrystals.

Communication between you and NE-CAT staff will be key during this year as we try to maintain our current level of user operations. As of the writing of this newsletter, the MX Beamlines at NSLS-II are still not available. As a result, demand for beamtime at NE-CAT has been strong. We have been allocating beamtime to all deserving proposals, though in smaller chunks of beamtime. We will make every possible effort to fulfill all beamtime requests while improving the facilities at NE-CAT. You can check for available beamtime on our website (<http://necat.chem.cornell.edu/index.htm>).

Beamline Developments

1. 24-ID-E Upgrade

Over the last few years, NE-CAT has been working on reducing the size of the beam on 24-ID-E while maintaining or increasing the flux density. This has been a multi-step project.

In 2015, NE-CAT replaced the crystals in the 24-ID-E monochromator, resulting in a 20% increase in flux and increased beam stability (see Spring 2015 Newsletter). In 2016, NE-CAT plans to install the new focusing system on 24-ID-E.

In collaboration with David Eisenberg at UCLA, NE-CAT acquired a KB focusing system that will allow a reduction of the beam size on 24-ID-E without loss of flux (see Winter 2013 Newsletter). The KB focusing system is composed of a support and bending mechanisms for 2 trapezoidal, rhodium-coated silicon mirrors and a vacuum chamber.

In addition, NE-CAT has been designing and constructing the support structures required to install the KB focusing system upstream of the MD2 microdiffractometer over the last year. The two-point bending system with pitch control and stripe selection will require a total of 5 control channels for each mirror (downstream and upstream) in the KB focusing

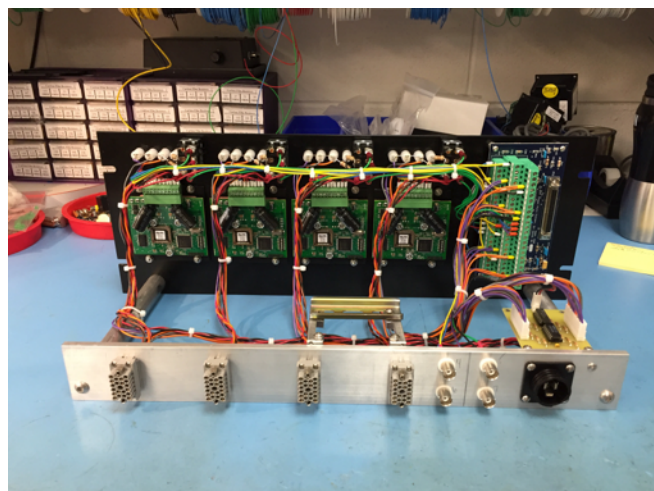


Fig. 1 One of the modules for the 15 channel motor controller system under construction. This module has 4 channels.

system. Five additional control channels are required for the alignment of the mirror vessel. The necessary motor controllers are being built in-house by Jim Withrow (**Fig. 1**).

Given the current configuration of 24-ID-E and the inability to extend the size of the hutch, a major renovation of the existing beamline architecture will be necessary to insert the KB focusing system. A new attenuator array will be needed to fit the smaller space. The beam position monitors and data collection shutter will need to move. Finally, the A-frame that holds the detector will eventually need to be reconstructed. This may necessitate a several week downtime during later part of 2016 during which 24-ID-E will be unavailable to users.

2. White-Beam BPM (Beam Position Monitor)

With a decrease in beam size, the stability of the delivered beam increases in importance. NE-CAT has taken a multi-pronged approach toward increasing beam stability on the beamlines and with the APS. Malcolm Capel is a member of the Beam Stability Working Group at the APS. This group is generating protocols and methods to allow real time reporting of delivered beam location from the operational sectors to the APS control room as well as facilitating communication between the beamlines and the APS control room.

Both sector 24 beamlines served as beta test sites for a new web-based system for requesting sector-specific steering adjustments for the last nine months (or two run cycles). The new system is designed to speed up the implementation of steering requests and to eliminate errors. At the beginning of the 2016-1 run cycle, the new steering request system has been



Fig. 2 Compton scattering hard X-ray BPM prototype.

released for general use at the APS by all beamlines.

As part of the APS upgrade to an MBA lattice, Malcolm Capel is collaborating with Mohan Ramanathan and Bingxin Yang to develop a Compton scattering hard X-ray BPM (XBPM). These BPMs would be placed upstream of the monochromator and allow the APS controllers and the down-stream beamline/s to monitor the location of the beam delivered to the beamline.

A prototype of the Compton white-beam BPM (**Fig. 2**) was installed in the A hutch of Sector 24 in May 2015. Measurements on the performance of the Compton XBPM are taken on the machine study days, which occurred every Tuesday during the run cycle, and did not impact user operations at NE-CAT.

3. Studies of Delivered Beam Stability

Given the impending decrease in size, beam stability studies are necessary to determine the current stability of the beam coming from the storage ring and at the sample. This will pinpoint areas of weakness as well as document changes in beam stability when hardware or software is changed. White-beam stability studies are also being conducted in our station A hutch during machine studies as part of Malcolm Capel's collaboration to develop the white-beam BPM. In addition, NE-CAT has continued conducting beam stability studies using the sCMOS camera (as detailed in the Spring 2015 Newsletter). Both sets of studies have identified areas of weakness in maintaining current beam stability as well as shown areas in which

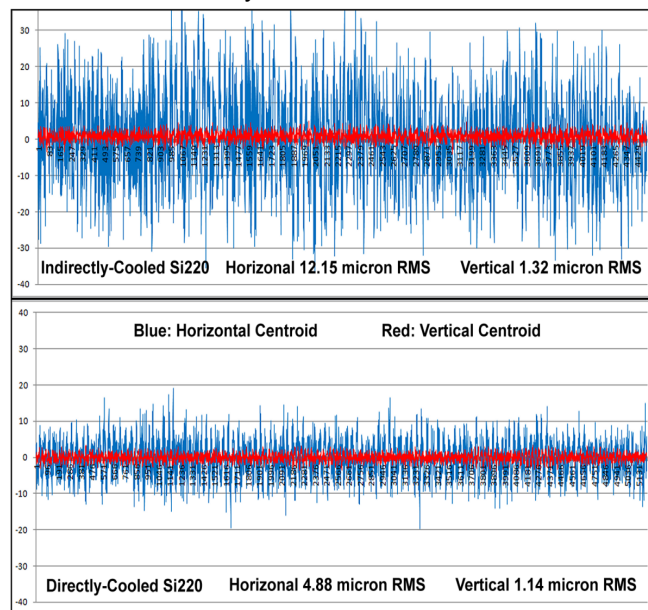


Fig. 3 Traces of the beam motion using the sCMOS camera and a scintillator. This measures beam motion at the sample location. The two traces compare the beam motion on 24-ID-E between the old crystal (top) and the new crystal (bottom).

changes, such as the replacement of the 24-ID-E monochromator crystals last year, have resulted in improvement (**Fig. 3**).

Research Highlight

How lipid A is enzymatically modified in gram-negative bacteria to counter polymyxin antibiotics

Filippo Mancia, Assistant Professor of Physiology & Cellular Biophysics, Columbia University, New York, NY

Vasileios I. Petrou, Post-Doctoral Research Scientist, Department of Physiology & Cellular Biophysics, Columbia University, New York, NY

The World Health Organization has identified antibiotic resistance as one of the three greatest threats to human health. The Centers for Disease Control and Prevention estimates that antibiotic-resistant bacteria already cause serious infections in 2 million Americans each year, killing 23,000 as a result. The situation is particularly critical with the emergence of many multi-drug resistant (MDR) Gram-negative (GN) strains of pathogens, including *Klebsiella*, *Acinetobacter*, *Pseudomonas aeruginosa*, *Enterobacter* (notably

Salmonella), and *E. coli*, which are responsible for increasingly resistant nosocomial and non-nosocomial infections. Increasingly, clinicians worldwide are confronted with the reality of infections by GN pathogens that are resistant to all available antibiotics except polymyxins. Two polymyxins have become available for clinical use in the 1960s: polymyxin B and polymyxin E (i.e., colistin). They have a narrow antibacterial spectrum, mainly against GN bacteria, and are efficient against most current MDR GN pathogens. However, the clinical use of polymyxins waned in the 1970s due to reports of toxicity. The rapid increase of resistance against all other classes of antibiotics has necessitated their resurgence, and they are currently used as a last resort treatment for MDR GN infections. Resistance to polymyxin-class antibiotics is a growing concern, as a result, one that can critically impair our ability to combat MDR infections.

Polymyxins are nonribosomal lipopeptides that are polycationic at physiological pH. They act primarily by permeabilizing GN bacterial membranes. In *E. coli* and *Salmonella*, it has been shown that the most effective modification for reduction of negative membrane charge and development of polymyxin resistance is the attachment of the 4-amino-4-deoxy-L-arabinose sugar moiety (L-Ara4N) to phosphate groups of lipid A – the lipid component of bacterial lipopolysaccharide (LPS) – at the 1 and 4' positions. The addition of the arabinose moiety causes a charge modification of the bacterial outer membrane, limiting its interactions with cationic peptides, and enabling bacteria to develop resistance to polymyxin-class antibiotics and natural antimicrobial peptides. The donor of L-Ara4N is the lipid carrier undecaprenyl phosphate (UndP). The attachment of L-Ara4N to lipid A is catalyzed on the periplasmic side of the inner membrane by ArnT (a.k.a. PmrK), an integral membrane lipid-to-lipid glycosyltransferase (GT), and the last enzyme to act in the aminoarabinose biosynthetic pathway common to GN bacteria (**Fig. 5A**).

Researchers from Assistant Professor Filippo Mancia's lab in the Department of Physiology and Cellular Biophysics at Columbia University, in collaboration with NE-CAT staff have determined the structure of ArnT from a GN bacterium. Results of this research were published in the journal *Science* on February 5, 2015 – Petrou, V.I., *et al.*, Structures of aminoarabinose transferase ArnT suggest a molecular basis for lipid A glycosylation. (2016). *Science*, 351: 608-612.

Two structures of ArnT from *Cupriavidus metallidurans* (*Cm*), one in the apo conformation and the other bound to UndP, at 2.8 and 3.2 Å resolution respectively (**Fig. 5B**), were determined from crystals grown in lipidic

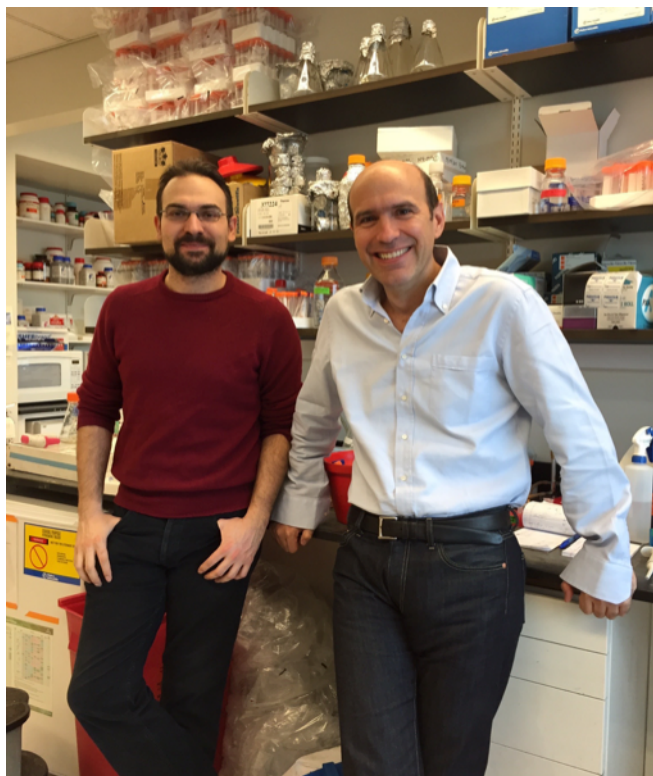


Fig. 4 Vasileios Petrou (first author of the *Science* paper) on the left and Filippo Mancia on the right in their lab at Columbia University.

cubic phase, with diffraction data collected at APS on 24-ID-C and 24-ID-E beamlines. These structures provide the first molecular insights into the transmembrane (TM) architecture and the catalytic machinery of ArnT family enzymes. ArnT consists of a TM domain with thirteen TM helices and a periplasmic soluble domain. The overall fold is reminiscent of protein glycosyltransferases from bacteria and archaea, but ArnT possesses unique features that are related to its function as a lipid-to-lipid glycosyltransferase.

Notably, we identify cavities for both lipidic substrates, lipid A and the UndP carrier, accessible from the membrane environment (**Fig. 5B**). The structures also reveal the atomic-level details of the active site where L-Ara4N is actually transferred and where the two cavities converge (**Fig. 5B**). We observe a significant coil-to-helix structural transition upon binding of UndP that stabilizes the carrier lipid near the active site and rearranges a flexible loop covering the active site, likely enabling subsequent binding of lipid A. These structures have allowed us to identify, map and test key functional residues inside the UndP cavity and within the active site, utilizing a polymyxin growth assay, and to propose a catalytic mechanism for ArnT enzymes in which two highly conserved Asp residues “activate” the acceptor Lipid A phosphate group for a nucleophilic attack. Importantly, while the overall pairwise sequence identity between ArnT from *Cm* and ArnT from *E. coli* or *Salmonella* is ~25%, conservation is substantially higher for residues lining the cavities or the active site, and all the key residues identified are conserved across the three species.

Elucidation of the molecular determinants of ArnT function and catalytic mechanism could enable the development of drugs to combat resistance of GN bacteria to polymyxin-class antibiotics and antimicrobial peptides.

Staff Activities

Poster

S. Banerjee, M. Capel, L. Kinsland, I. Kourinov, F. Murphy, D. Neau, K. Perry, A. Lynch, K. Rajashankar, C. Salbego, J. Schuermann, N. Sukumar, J. Withrow, And S. Ealick “NE-CAT: Crystallography Beamlines for Challenging Structural Biology Research”, 2014 Annual Meeting of the American Crystallographic Association, Philadelphia, PA, July 25-29, 2015.

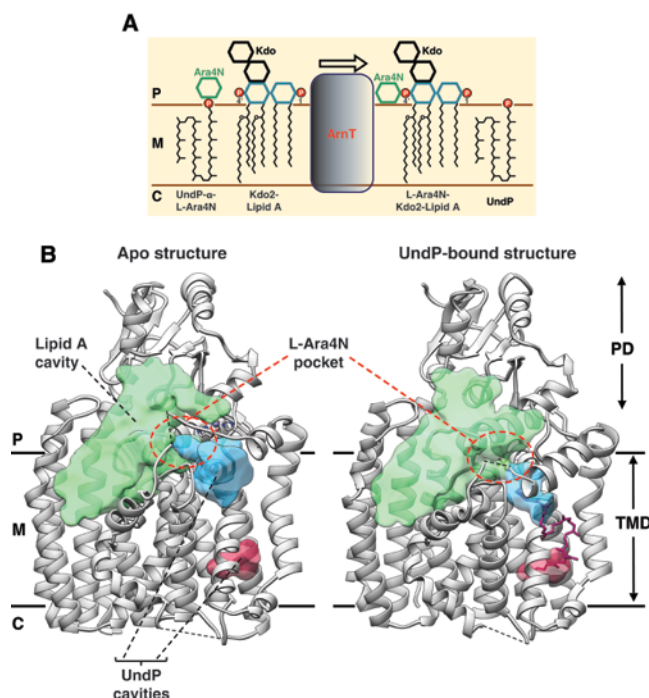


Fig. 5 ArnT function and structures from *Cupravidus metallidurans* (ArnT_{Cm}). (A) Schematic representation of the reaction catalyzed by ArnT. The 4-Amino-4-deoxy-L-arabinose (L-Ara4N) sugar is transferred from the undecaprenyl phosphate (UndP) carrier to lipid A. 1 and 4' acceptor phosphate positions on lipid A are marked. (B) Overview of the ArnT_{Cm} structure in the apo conformation (left) and bound to UndP (right). Cavities for the binding of lipid A (in green) and the UndP carrier (in blue and red) are indicated. The L-Ara4N pocket located at the junction of the two cavities is circled by a dashed line. P: Periplasm, M: Membrane (inner), C: Cytoplasm; PD: Periplasmic domain; TMD: TM domain. Dashed lines represent missing segments in the structure.

Publications

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Meeting Attendance

Jon Schuermann, DIALS-6, Lawrence Berkeley National Laboratory, Berkeley, CA, May 26 - May 29, 2015.

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