NE-CAT Communications

A Biannual Newsletter of the Northeastern Collaborative Access Team Winter 2024

Message from the Director

Frank Murphy

Yes, faithful readers, we're back! New faces, new director, and of course, a new APS. First, I want to tell you how excited I am to be the new NE-CAT Director. Steve Ealick was the driving force behind NE-CAT: founder, Director, and PI for more than 20 years. Big shoes to fill, indeed. I have lots of goals for us over the coming years, but first and foremost is to be the beamlines you can count on for the highest quality data.

We have some new faces around the beamlines since you were last here. Anne Mulichak has joined us here at the beamlines, and you can see inside that she has all the experience needed to help you make the most of your projects. We also have two new principal investigators steering the ship, Drs. Nozomi Ando and Chris Fromme. They are both based in Ithaca, but they are integral to our planning and strategy as well as keeping the administrative gears well-oiled.

And finally, the upgrade and resumption of user operations. I have to admit that we have not returned to action as quickly as we had hoped, but we are finally set to return. Read on for the gory details, but suffice it to

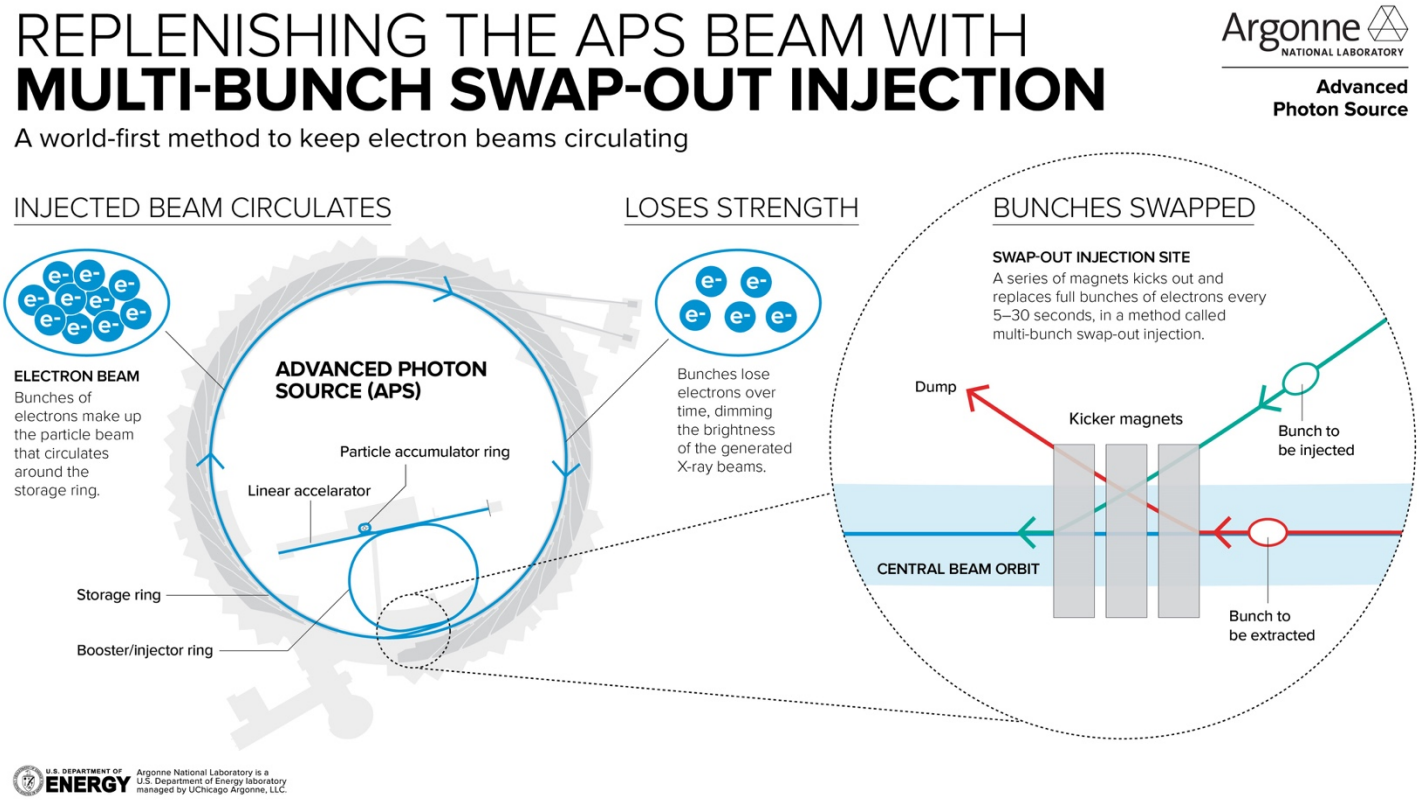

Figure 1 Multi-Bunch Swap-Out Injection Infographic

(Image by Argonne National Laboratory)

say that the new APS beam is as promised, and will bring faster and perhaps even better experiments for you here at NE-CAT. User operations on 24-ID-E are planned to fully resume in March, but keep in mind that the E beamline is essentially unchanged from before the upgrade. We did this so we could return as quickly as possible.That's it from me. I look forward to hearing from and seeing you in 2025. We'll be here in Chicago if you want to come collect your data the old-fashioned way, but we'll also be out at various meetings. And don't hesitate to drop me an email; I always enjoy hearing from you.

Beamline Developments

1. Return to Operations

The Advanced Photon Source began commissioning the new multi-bend achromat lattice in April 2024. Physicists were able to successfully store beam in the storage on April 20, 2024. Per the May 2024 APS Upgrade update, the APS became the first modern light source to demonstrate multi-bunch swap-out injection on April 29, 2024. This is a method of injecting electrons into the storage ring by kicking out low-energy bunches and replacing them with fresh ones. The new lattice will not allow top-up injection, the previous method of restoring depleted electrons that have been lost to scatter. The APS has an official infographic on the APS-U website which details the process of multi-bunch swap-out injection (Fig. 1).

Figure 2 Dedication Ceremony of the Upgraded APS

Governor JB Pritzer speaks at the Dedication Ceremony on July 17, 2024 outside of the APS Auditorium.

Through May and June, the APS slowly increased the amount of current in the storage ring and was able to achieve up to 110mA of beam with the swap-out mode. The APS moved forward to 'first light,' which is the delivery of photons to a scientific beamline, on June 17, 2024 with an official dedication of the upgraded APS on July 17, 2024. The ring operated at 50mA after the dedication until the scheduled maintenance shutdown in August to allow for beamlines to begin re-commissioning.

NE-CAT began re-commissioning after the official dedication with shielding verification and survey by the APS. Survey needed to be performed because when the old storage ring was removed and the new storage ring was installed, it was discovered that the old ring was not circular. The new storage ring has been installed and aligned to the ideal storage ring design. This necessitates alignment of all beamlines to the new geometry of the storage ring. On August 9, 2024, x-rays were delivered by the APS through the Sector 24 front end. Using an ultrasensitive camera focused through a port of the monochromator, APS physicists detected the beam on the monochromator silicon crystals of both 24-ID-C and 24-ID-E. Though the focus was rough, NE-CAT was able to observe the beam making this our first major milestone: First Light! Initial observations showed a much more uniformly shaped beam. The beam produced by the old lattice was elongated in the horizontal direction. This elongation is not observed in the beam produced by the new lattice.

After the planned August shutdown, NE-CAT entered technical commissioning. The new beam produced by the upgraded APS is much smaller and with greater flux

Figure 3 First Light at NE-CAT

Dr. Malcolm Capel observes the new beam on the 24-ID-C monochromator through the ultrasensitive camera on August 9, 2024.

density. There is approximately 100 feet between the 24-ID frontend where the beam enters the sector from the storage ring and the end stations where the detectors and samples are located. This means that any deviation of the beam along its trajectory from source to destination can result in an angle change that can result in motion of the beam from the desired target. The initial beam was observed to display an excessive amount of horizontal motion which exceeded the width of the beam. As this degradation in data quality is unacceptable, the next two months were spent carefully steering the beam, eliminating mechanical sources of vibration from the NE-CAT beamlines and working with the APS on any causes perturbing the source.

24-ID-C remains under redevelopment as we upgrade it with a new dual crystal monochromator, new MD3 microdiffractometer, new 30-puck dewar and new sample automounter. However, on November 21, 2024, we manually mounted a thaumatin crystal and collected a dataset on the re-commissioned 24-ID-E beamline, achieving our second major milestone on the way to welcoming back users.

The new beam on 24-ID-E has a maximum width and height of 6 microns. Currently, we estimate a flux density at least 10X greater than prior to the upgrade. Reasonable statistics for thaumatin can be obtained with exposures as low as 10msec and using as little as 0.1% of the available beam.

On November 23, 2024, after collecting 1440 degrees of data for over 50-fold redundancy, Dr. Igor Kourinov was able to perform sulfur-SAD phasing on thaumatin. The electron density from the sulfur-SAD phasing clearly shows disulfide linkages in the structure. 24-ID-E is calibrated to above the Selenium K edge of 12659eV with only 0.23 electrons available at this energy for anomalous phasing from sulfur. In contrast, selenium has 3.84 electrons available at this energy. This shows that the beam is stable enough for high-quality data collection.

NE-CAT begins scientific commissioning of 24-ID-E in February 2025. 24-ID-E is our monowavelength beamline. It is suitable for collecting native datasets which can be phased using molecular replacement. It is currently equipped with an MD2 microdiffractometer, a 14-puck storage dewar, a sample automounter and an Dectris Eiger detector. It runs NE-CAT's web-based remote data collection GUI. During the dark period, the original version of RAPD, our automated data processing suite, originally put into production in 2010, was replaced with RAPD version 2 (RAPDv2) which utilizes Python 3+ and a new SLURM-based compute cluster. An additional 150 Tbytes of NVMe (Non-Volatile Memory Express) storage has been added to our existing storage. However, the NVMe replaces the old gpfs1 and gpfs2 file systems which were used for data collection. The new storage runs under BeeGFS. The new storage is designed to accommodate the faster data collection times and higher throughput made possible by the upgraded APS. Backups of data collected during a run will be on the slower storage arrays for long-term availability.

2. How to Apply for Beamtime

During the upgrade, the APS implemented a new proposal system, the Universal Proposal System (UPS). Old proposals in the previous system do not carry over. In order to submit a proposal in UPS, all users who intend to collect data must link their Open Researcher and Contributor ID (ORCID) to both their APS User Registration and the Universal Proposal System.

All users who wish to submit a proposal or who intend to collect data should check their APS Registration to make sure it is valid. User registrations expire after 2 years or when a visa expires. Processing of user registrations and site access is a complex process. We recommend providing at least two weeks for processing, but it's never too early to update.

In order to collect data on 24-ID-E, a Macromolecular Crystallography (MX) General User proposal must be submitted in UPS. Full instructions and helpful tips for submitting a proposal using the new Universal Proposal System are available on the NE-CAT website: https://necat.chem.cornell.edu/getbeam

3. New Staff Scientist

Last September, Dr. Anne Mulichak joined NE-CAT as a new staff scientist. Anne received her Ph.D. in Physical Chemistry from Michigan State University in 1991 under crystallographer Dr. Alexander Tulinsky. She has extensive experience in protein crystallography in both academic and pharmaceutical industry settings, as a research assistant professor in the Biochemistry Department at MSU and as a post-doctoral and research scientist supporting drug discovery efforts at The Upjohn Co. and Merck & Co., respectively. While these positions provided synchrotron experience from a user perspective, Anne also has experience on the beamline side as a

crystallographer at APS sector 17 for 18 years. As staff scientist and later Manager of Operations at Sector 17, her work was particularly focused on developing and optimizing high-throughput and fully automated data collection methods. Most recently, Anne was Director of X-ray Sciences at Helix Biostructures, providing hands-on x-ray data collection services for pharmaceutical and biotech companies at synchrotron sources worldwide. Anne is looking forward to helping users at the beamline once again at NE-CAT and the newly upgraded APS.

4. Block Allocation Group Ending

Run Cycle	2023-2	2023-3	2024-1	2024-2
(8-Hour) Shifts*	26	42	38	33
Group Trips	40	79	70	50

During the year the APS was undergoing the upgrade, NE-CAT offered full support from our highly trained crystallography staff for structure solution, and during data collection through Block Allocation Group (BAG) Time at NSLS-II. Thirty-eight groups were part of the BAG program and users were able to use AMX, FMX and NYX beamlines at NSLS-II through the NE-CAT BAG proposal # 311950. If you collected data through the NE-CAT BAG, then please add the NE-CAT BAG proposal number to your beamline acknowledgement. For example, if you collected data at AMX or FMX, we suggest:

> The Center for Bio-Molecular Structure (CBMS) is primarily supported by the NIH-NIGMS through a Center Core P30 Grant (P30GM133893), and by the DOE Office of Biological and Environmental Research (KP1607011). NSLS-II is a U.S.DOE Office of Science User Facility operated under Contract No. DE-
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> SC0012704. This publication resulted from the data collected using the beamtime obtained through NECAT BAG proposal # 311950.

Eventually, the NSLS-II BAG will come to an end, but NE-CAT will be supporting users concurrently on both 24-ID-E and the BAG for our first run cycle. Therefore, there will be user support provided for both 24-ID-E and at NSLS-II during 2025-1 and 2025-2.

5. Email Change

Everyone at NE-CAT now has cornell.edu email addresses. Our anl.gov email addresses were discontinued on September 30, 2024. The newsletter list has also moved to NECAT-NEWS-L@cornell.edu.

Contact us through our cornell.edu email addresses.

- Frank Murphy - frankvmurphy@cornell.edu
- Malcolm Capel - mc329@cornell.edu
- Ali Kaya - ali.kaya@cornell.edu
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- Jim Withrow - jwithrow@cornell.edu
- Cyndi Salbego - csalbego@cornell.edu

6. New Co-Principal Investigators

The NE-CAT P30 grant has transition from a single PI grant to one with three Co-PIs. The new PIs on the grant are: Dr. Frank Murphy, Dr. Nozomi Ando, and Dr. Chris Fromme. Dr. Frank Murphy is the new Director of NE-CAT. Dr. Nozomi Ando and Dr. Chris Fromme are both faculty at Cornell University. Dr. Ando is in the Department of Chemistry and Chemical Biology. The Ando lab studies the motions that proteins undergo during catalysis and regulation. Focused on complex protein systems, the Ando lab develops new techniques that allow for novel solutions. In particular, the Ando lab studies regulation of and catalysis by metalloenzymes, proteins that use metal-containing

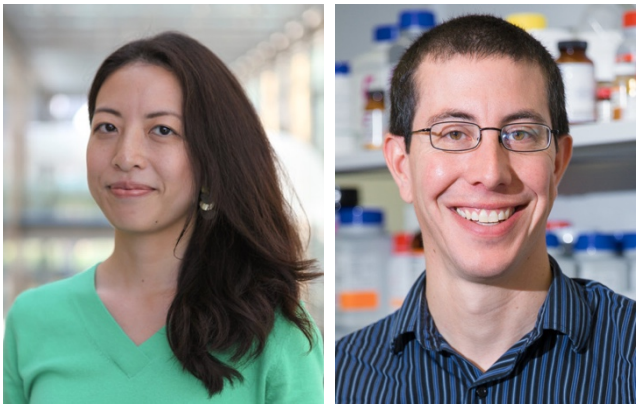

Figure 4 – Dr. Nozomi Ando (left) and Dr. Chris Fromme (right)

cofactors to perform chemistry. Dr. Fromme is in the Weill Institute for Cell and Molecular Biology and the Department of Molecular Biology and Genetics. The Fromme lab is focused on understanding the biochemical and structural basis for the activation of GTPase proteins at the Golgi, the "Grand Central Station" within our cells, as it serves as the primary sorting organelle at the nexus of the secretory and endocytic trafficking pathways. The regulators for all incoming and outgoing Golgi traffic are GTPases of the Rab and Arf families. These GTPases serve many critical functions and are themselves tightly regulated. The Fromme lab attempts to understand the molecular logic governing intracellular trafficking at the organelle-level. Both Dr. Ando and Dr. Fromme use macromolecular x-ray crystallography as one of their tools.

Research Highlights

The 2024 Nobel Prize in Chemistry was awarded half to Dr. David Baker for computation protein design and half jointly to Demis Hassabis and John Jumper of Google DeepMind for protein structure prediction. However, Dr. Baker's methods for computational protein design, most famously ROSETTA, are also termed '*ab initio*' structure prediction as the method starts without knowledge of structurally similar folds then calculates the lowest potential energy based on physiochemical interactions. ROSETTA uses a statistics-based energy function to calculate the lowest potential as seen in similar interactions in structural databases. The Google DeepMind project produced AlphaFold, which uses a neural network to incorporate previously determined structures in the Protein Data Bank and multiple sequence alignment to predict a protein structure. Models from both *ab initio* structure prediction and AlphaFold can be used successfully in Molecular Replacement to solve the phase problem. That makes this year's Nobel Prize in Chemistry one which touches anyone who uses x-ray crystallography as a technique for investigating the mechanisms of macromolecules.

NE-CAT is also proud to be one of the beamlines which supports the research of Dr. David Baker. The Baker lab and the Institute of Protein Design at the University of Washington, iterates between computation and laboratory experiments while improving their protein design methods. In many instances, after designing a protein, the lab will recombinantly express the protein, crystallize it and then solve the structure to verify that their method produces accurate predictions. At least once a month prior to the APS-U, the Institute of Protein Design under the auspices of Dr. Asim Bera, collected data at NE-CAT, using 24-ID-C to collect high resolution data up to 0.65 Å. NE-CAT has supported Dr. Baker's research since 2006 with 38 publications where NE-CAT beamlines are acknowledged.

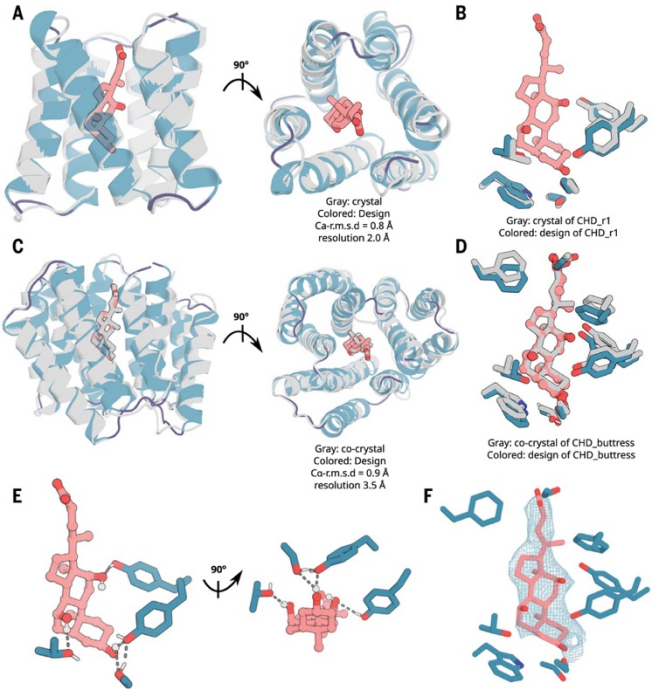

Figure 5 - X-ray crystallography demonstrates accuracy of design approach

Demonstrating the accuracy of protein structure predicted by the Baker lab after the proteins were crystallized and the 3D structure was determined. Image from Science. 2024 Jul 18;385(6706):276–282. doi: 10.1126/science.adn3780

Staff Activities

Meetings

If you are attending a meeting in 2025 such as the Biophysical Society, ASBMB, ACA or the Protein Society, look for NE-CAT scientists either at a poster, in a scientific session or at a booth.

Publications

Richardson, B. C., Turlington, Z. R., Vaz Ferreira de Macedo, S., Phillips, S. K., Perry, K., Brancato, S. G., Cooke, E. W., Gwilt, J. R., Dasovich, M. A., Roering, A. J., Rossi, F. M., Snider, M. J., French, J. B., and Hicks, K. A. (2024) Structural and Functional Characterization of a Novel Class A Flavin Monooxygenase from Bacillus niacini, *Biochemistry 63*, 2506-2516. PMID: 39265075.

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Acknowledgements

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When publishing work resulting from data collected at

NE-CAT, we ask our users to acknowledge us, by mentioning our grant number, in your funding or acknowledgements section. For suggested text and a complete list of grants, see our Acknowledgement Request on our website: https://necat.chem.cornell.edu/acknowledgement