NE-CAT Communications

A Biannual Newsletter of the Northeastern Collaborative Access Team Winter 2022

Message from the Director

Steve Ealick

In the last newsletter, we were waiting on the result of our P30 grant renewal. I am happy to report that the P30 grant renewal is a success and we anticipate that NE-CAT will be funded for the next five years! Look for our new grant number in a future newsletter to include in your publication acknowledgements.

This is the last run before the APS shuts down for the yearlong upgrade to install the multi-bend achromat lattice. With funding secured, NE-CAT is proceeding with upgrades to our facility during the shutdown while continuing to make our staff available to our user community. Expect not just an improved APS, but a bigger and better NE-CAT. Indeed, we are hiring and I'm sure that any of our staff would say that NE-CAT is a great place to work. Check out the job description in this newsletter. For the latest developments at NE-CAT, be sure to visit our website at: https://necat.chem.cornell.edu

New NE-CAT Co-Director

Dr. Frank Murphy has been named NE-CAT Co-Director. Frank was previously elevated to NE-CAT Assistant Director in 2016 and Associate Director in 2019. Frank received his Ph.D. in Biochemistry in 2000 from the University of Illinois Urbana-Champaign. Prior to joining NE-CAT in 2007, Frank was a postdoctoral research associate with Venki Ramakrishnan at the MRC Laboratory of Molecular Biology in Cambridge, England.

Beamline Developments

APS-U

The APS will go dark for the APS Storage Ring Installation period on April 17, 2023. The APS has been moving forward with component delivery and construction of the magnets for the new lattice despite supply chain issues and the estimate remains a return to user operations in Spring 2024 though the initial operations will be at reduced current of 25mA. There will also be reduced availability as the machine is tuned up. As detailed in our Summer 2022 Newsletter, NE-CAT plans to leave 24-ID-E unchanged for rapid commissioning and a return to user operations at 50mA stable beam. We anticipate 50mA stable beam in Fall 2024 but targets may be achieved earlier and stable beam may be available sooner than the estimated date; look for updates in future editions of this newsletter. We will be keeping our user community informed on the progress of the upgrade and the return to user operations through the continued issuance of these newsletters during the dark period.

Block Allocation Group

During the estimated year the APS will be down and unavailable for user operations, NE-CAT plans to offer full support from our highly trained crystallography staff for structure solution, and during data collection at other beamlines through a process known as Block Allocation Group (BAG) Time. BAGs are a mode of beam time access intended for groups of researchers who wish to combine their short beam time requests into a single proposal in order to permit greater flexibility in beam time allocation and scheduling. At this time, BAG Time is offered at SSRL and NSLS-II in the United States and at select synchrotrons in other countries. Currently, NE-CAT will be submitting BAG proposals at both SSRL and NSLS-II that will include interested members of our user community. We will be coordinating beamtime and providing our excellent staff support during data collection. Users may contact necat-bag@anl.gov in order to obtain more information or to join the BAG.

Table 1. Expected Flux, Spot Size and Energy Ranges for 24-ID-C and 24-ID-E after the APS-U compared to 2020

Beam Line	Total Flux Photon/sec @12.6 KeV	Flux through 5 µ Aperture @12.6 KeV	Focus Spot (v x h) µ 1 sigma	Spectral Range (KeV)
C-Line (2020)	1.4×10^{13}	10^{11}	3.5 x 17	6.5 - 19
C-Line (Post APS-U)	1×10^{14}	3×10^{13}	3 x 5	5 - 20
E-Line (2020)	1.0×10^{13}	10^{10}	3.5 x 30	12.662
E-Line (Post APS-U)	3×10^{14}	5×10^{13}	3 x 3	12.662

Practical Crystallography Class

The current session of the Practical Crystallography Class is February 2023. As always, we are attempting to improve the course with each iteration based on student feedback. The current session provides the class in a workshop format where students will be provided datasets if they do not have an existing dataset or do not have crystals for data collection. In addition, we are making it possible for students to use the NE-CAT computing resources during the workshop sessions. This is part of our greater goal of making our computational resources available to our users during the dark period. At this time, use of the NE-CAT computing resources will require both current registration as an APS user, an ANL account and VPN access. All current APS users who previously had an APS account to access the General User Proposal System and the ESAF system should have been converted to an ANL account during Fall 2022.

Fig. 1 Beamline Technician Ed Lynch inspecting the monochromator in its shipping crate after arrival at NE-CAT.

Due to high demand, the class was full within 24 hours of registration being made available. As a result, we are currently planning on offering our practical crystallography class in select locations around the United States during the APS-U down period. The first in-person session of the course is tentatively scheduled for June 21-23, 2023 in Boston, Massachusetts. Harvard Medical School, in the person of Steve Harrison, will be our host. The course will be free, but students who wish to attend will be responsible for their own travel and housing. The course will be limited to 20 students. Stay tuned for more information.

Dual Channel-Cut Monochromator

Part of NE-CAT's preparation for the APS-U has been purchases of capital equipment. Already mentioned in the Summer 2022 newsletter are the two new MD3 microdiffractometers. NE-CAT has also purchased a new monochromator for 24-ID-C. FMB Oxford completed manufacturing the dual channel-cut monochromator and shared it plus attendant controls,

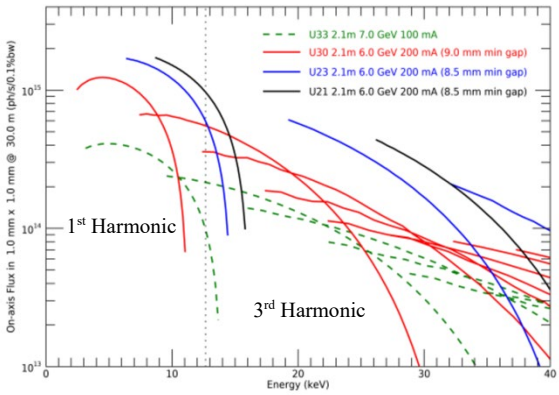

Fig. 2 Flux estimates for different undulators. Tuning curves calculated at 30 meters for a 1mm x 1mm aperture for 3.3-cm undulator (green dashed), 3.0-cm undulator (solid red), 2.3-cm undulator (solid blue), and 2.1-cm undulator (solid black). All odd harmonics to the 15th harmonic are shown. The first two lines for each set are for the 1st harmonic and the 3rd harmonic.

cooling and support structures via video conference on October 20 prior to packaging and shipping from the United Kingdom. Given the size and weight, such items travel by boat; it was delivered in January 2023.

As part of the APS-U, the undulators for the two insertion device beamlines at NE-CAT will be upgraded. 24-ID-C will be moving from our current 3.3-cm period undulator to a 3.0-cm period undulator. 24-ID-E will be moving from a 3.3-cm period undulator to a 2.1 cm period undulator. The current peak of the power spectrum on the 3.3-cm period undulator is 2 sigma below 12.6 keV. The 2.1-cm period undulator is expected to move the peak much closer to 12.6 keV (See Fig. 1). As a result, we expect to see a 2-fold gain in flux at the Selenium K-edge on 24-ID-E (See Table 1).

Staff Scientist Position Available

NE-CAT is seeking applicants for a Staff Scientist at the Research Associate and Sr. Research Associate levels. Primary responsibilities will be to provide training and support to users of NE-CAT's two state-of-the-art X-ray beamlines, provide crystallographic expertise to ensure the highest quality data and structures, and contribute to the continued development of beamline software tools.

The prospective applicant will collaborate with other NE-CAT team members to enhance and extend the capabilities of the NE-CAT beamlines – ideally contributing to the continued development of software tools that improve the user experience at NE-CAT and will be expected to take initiative and contribute to the team effort in an inclusive and respectful environment. To be considered for the Senior Research Associate level, a candidate should have significant experience managing synchrotron beamlines as well as advanced experimental and programming capabilities.

Duties and Responsibilities:

- Provide user training and support, both onsite and via remote conferencing. This involves work nights and weekends, approximately nine months of the year, when the APS has run cycles and is open for data acquisition. During these months, the staff scientist will rotate assignments with other staff members to provide support 24 hours a day, seven days a week.
- Use existing crystallographic software: CCP4, PHENIX, XDS, etc. to assist users in data collection, processing, and structure solution. Test and develop modifications as necessary. This requires familiarity with Linux and programming.
- Test and debug new beamline hardware and software. Evaluate beamline performance to produce optimum data.
- Use knowledge of synchrotron beamline optics and experiment-specific methods to develop data collection and structure determination procedures for current projects and new techniques including microcrystals, room temperature data collection, and serial crystallography.
- Contribute to the continued development of remote data collection and real time data analysis software for the beamline data acquisition systems.
- Collect data on macromolecular crystals for structure solution, model building, and refinement.
- Participate in cooperative research in which the scientist's expertise is combined with that of members of the NE-CAT user community to improve the processes of protein purification, crystallization, and determination of macromolecular structures.
- Publish results and help prepare presentations and documentation to disseminate information about advances to the macromolecular crystallography community.

Qualifications:

- Doctorate in Biochemistry, Structural Biology, Biophysics, Chemistry, or related field.

- At least three years doctoral or post-doctoral experience involving macromolecular crystallography. Record of publications of research in the scientific literature.
- Fluency in the English language, written and oral.
- Experience operating and managing synchrotron beamlines.

For more information and to apply, visit AcademicJobsOnline.org

Research Highlights

Hideki Aihara, Associate Professor, Department of Biochemistry, Molecular Biology, and Biophysics, University of Minnesota Twin Cities

Hydrolytic deamination of cytosine (C) into uracil (U) in DNA is one of the most common sources of genetic mutation, and it is a major driver of evolution in biology. In addition to spontaneous deamination of cytosines, which is estimated to occur over 100 times per mammalian cell every day, enzymatic deamination of cytosines in DNA plays key roles in various important biological processes, including innate immune responses against viruses and transposons, antibody diversification in adaptive immunity, and the accumulation of somatic mutations to drive cancer evolution[1]. The activity of APOBEC family single-stranded (ss) DNA cytosine deaminases has also been harnessed in base editing technologies, where an engineered Cas9-guide RNA complex directs APOBECs for site-specific C-to-T base substitutions in genomic DNA without making double-strand breaks[2]. Furthermore, recent studies have identified a family of double-stranded (ds) DNA deaminase (DddA) toxins involved in antagonism between Gram-negative bacteria, which has been adapted to develop CRISPR-free DddA-derived cytosine base editors (DdCBEs) capable of base editing in mitochondrial DNA[3]. Given their biological importance and utility in genome editing applications, our laboratory is interested in better understanding the diverse DNA cytosine deaminase enzymes through structural studies.

The human APOBEC deaminases catalyze cytosine deamination in ssDNA in a sequence context dependent fashion. For instance, APOBEC3A (A3A) and APOBEC3B (A3B) deaminate cytosines in 5'-T**C** motif, which is responsible for the characteristic "APOBEC signature" mutations found widely in cancer genomes[4]. Our crystal structures of A3A and A3B bound to ssDNA showed a sharply bent 'U'-shaped

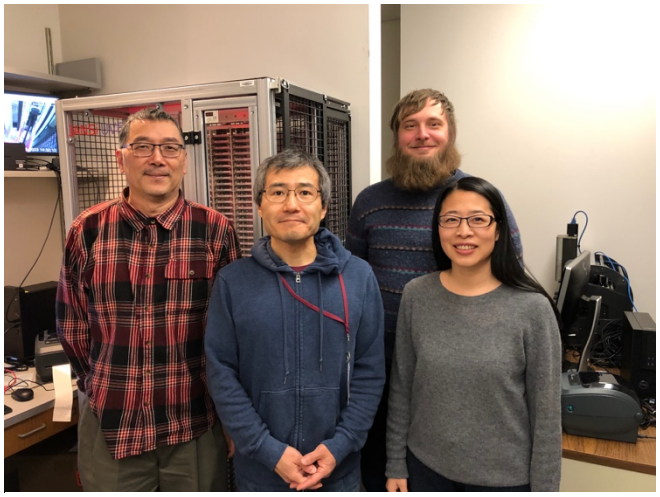

Fig. 3 Members of the Aihara lab contributed to the work on structural studies of DNA cytosine deaminase enzymes. From left to right: Ke Shi, Hideki Aihara, Hicholas Moeller, Lulu Yin.

DNA conformation, with the target cytosine and the 5' (–1) thymine bases flipped out and making specific contacts with the protein[5] (**Figure 2**). The studies not only revealed structural basis of the APOBEC mutation signature but also explained why hairpin-forming sequences are mutational hotspots targeted by A3A[6]. The structural information also enabled us to generate a A3A mutant that preferentially deaminates a 5'-C**C** motif[5]. Such APOBEC variants with altered sequence selectivity could be useful in base editing applications. Interestingly, the bacterial DddA toxin shares the strong 5'-T**C** preference with A3A/B, despite being a dsDNA-specific deaminase[3]. We have recently determined crystal structures of DddA-dsDNA complexes, which show a distinct mechanism of DNA substrate engagement underlying the convergent sequence selectivity (https://doi.org/10.21203/rs.3.rs-2031914/v1).

As premutagenic lesions, deaminated DNA bases are repaired by multiple cellular mechanisms including the highly conserved base excision repair (BER) pathway featuring uracil DNA glycosylase. Recent studies have also identified a novel lesion-specific DNA repair endonuclease from archaea and bacteria, designated EndoQ, which exhibits a unique dual specificity for U and hypoxanthine (Hx: deaminated adenine) bases in DNA. Our crystal structures EndoQ-DNA complexes[7] revealed how EndoQ achieves high selectivity towards these chemically divergent substrates to initiate the repair of deaminated lesions, without cleaving undamaged DNA. We also demonstrated that EndoQ, as the only known nuclease that cleaves DNA at deoxyuridine nucleotide, is useful for studying DNA deamination and repair in mammalian systems[7].

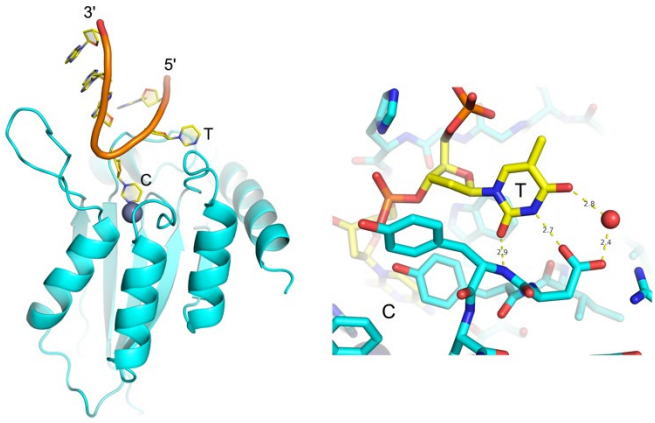

Fig. 4 A3A-ssDNA crystal structure shows why A3A has a strong preference to deaminate cytosines in the 5'-TC motif. Gray spheres represent the zinc ion in the active site, whereas the small red sphere (scaled at 1/3 of van der Waals radius) represents a water molecule that mediates the –1 T recognition.

We hope that a better understanding of DNA deamination processes will help develop therapeutic strategies to slow tumor evolution or aid in protein engineering for genome editing applications. We thank NE-CAT for generous beamtime and excellent support.

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Staff Activities

Upcoming Meetings

You will be able to find NE-CAT Staff at the following meetings in 2023:

The Protein Society 37th Annual Symposium in Boston, Massachusetts, July 13-16, 2023.

ACA 73rd Annual Meeting in Baltimore, Maryland, July 7-11, 2023.

Publications

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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