NE-CAT Communications

A Biannual Newsletter of the Northeastern Collaborative Access Team Winter 2025

Message from the Director

Frank Murphy

Hello from the frozen tundra here in Chicago! 2026 is shaping up to be an exciting year at NE-CAT, and this edition of the newsletter sets the tone for the coming year – new people, new technology development, and more chances to see us at workshops and meetings.

We had another new face in 2025 – Graeme Winter joined us from Diamond Light Source in April as Associate Director. Graeme's been working closely with the technical team and enjoying getting his hands a little dirty. Once we got over questioning his life choices, it's been a lot of fun getting to know him and experience his new and enthusiastic take on the technology trajectory of the beamlines.

You may have already experienced the new RAPD when you collected data in 2025. The team was aiming for radical improvements, and I think they accomplished that! One of the changes is that the project is much more flexible, so expect to see lots of changes, hopefully all for the better, as the project develops. And please, give some feedback – especially if you don't like something.

Be sure to look for us at the ASBMB in March and the IUCr/ACA in August. There's always candys and tchotchkes, and perhaps more importantly, staff to chat with and answer any burning questions on why the paperwork is such a pain. Also, if you or one of your colleagues needs training in MX, we will be having bootcamps this year; and we're always here to help!

Have a great 2026.

Beamline Developments

1. General User Program

In March 2025, 24-ID-E was the first MX beamline at the APS to return to general user operations after the APS-U. In 2025, NE-CAT welcomed back 70 user groups and added 20 new user groups to our user community.

As a reminder, the APS has implemented a new proposal system, the Universal Proposal System (UPS). Old proposals in the previous system do not carry over. 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.

To check your APS Registration, you must use your ANL domain account as your login. **ANL domain account passwords expire every 6 months**, or when your registration expires. You will receive an email from PasswordAdministrators@anl.gov letting you know the steps to follow to change your password before expiration. If it expires you need to reset it through the ANL helpdesk and will require a video/zoom meeting where you show a valid ID. More information can be found on the APS website: https://www.aps.anl.gov/Users-Information/User-Access-to-APS

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

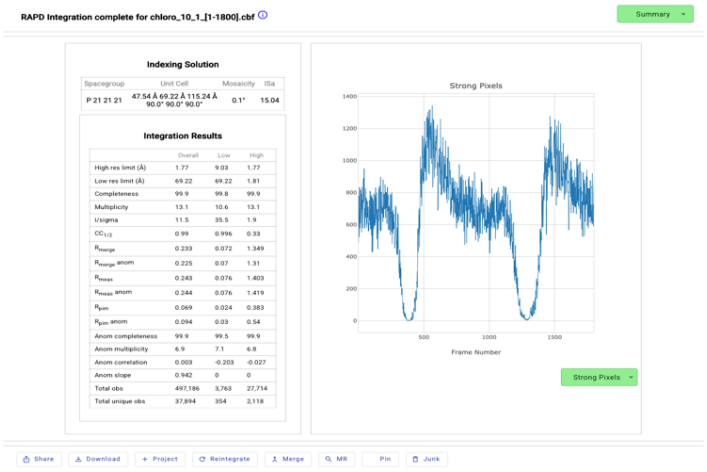

Figure 1 Data Integration in RAPDv2.

2. RAPDv2

RAPD (Rapid Automated Processing of Data), first released in 2010 as NE-CAT's automated data processing, analysis and structure solution suite was terminated during the APS-U. It was replaced with RAPDv2, a Python 3 compatible replacement which further extends RAPD with new data processing, data analysis and structure solution tools in a new AngularJS-based user interface. This update leverages the computing resources at NE-CAT to view processed data, data analysis and perform structure solution. The outward presentation of RAPDv2 simplifies the scientific and technical hardship of macromolecular X-ray crystallography. RAPDv2 automatically launches crystallographic programs with sensible defaults, scrapes the results from program output and presents the results to the users on a web-browser, eliminating the need for installation of programs.

By default, RAPDv2 will use XDS to integrate and scale data. New data integration pipelines in RAPDv2 include Xia2, DIALS, and autoPROC. After automated data integration, RAPDv2 offers data analysis through a combination of programs from CCP4 and Phenix. The space group of the dataset is verified, checked for pseudo-translation, twinning and non-crystallographic symmetry. Datasets can be assessed for isomorphism through either similarity in Bragg reflection intensities or unit cell variation and then merged to increase anomalous signal or to complete partial datasets. Molecular replacement using CCP4 and Phenix is available using a known structure or AlphaFold model. After molecular replacement, ligands can be detected through a search of a difference map or unmodelled density. Multi-dataset merging is also available through the click of a button. With designation of projects, in the future, data merging and structure solution can also be automated, providing users with a structure that only requires model building and refinement at the end of a synchrotron visit. Since 24-ID-E is not suitable for SAD phasing, the re-introduction of the SAD pipeline familiar to users of RAPD awaits the return of 24-ID-C.

3. New Associate Director

In April 2025, NE-CAT welcomed Dr. Graeme Winter as our new Associate Director. Coming from Diamond Light Source, where he served as a Principal Scientist and Team Leader, Graeme brings a wealth of experience in synchrotron X-ray sources, crystallographic software development, and automation. During his time at Diamond Light Source, Graeme led key initiatives to automate crystallography by improving data processing workflows and developing unattended data collection. He holds a Master's in Mathematics from the University of Cambridge and a Ph.D. in Crystallography from the University of Manchester, earned while working part-time at Daresbury (the former home of the UK synchrotron). It was at Daresbury that Graeme developed xia2 (Crystallographic Infrastructure for Automation), a powerful data processing software that automates diffraction data reduction from images to scaled intensities and structure factor amplitudes, significantly increasing efficiency and minimizing user intervention. Furthermore, Graeme is a key contributor to the Diffraction Integration for Advanced Light Sources (DIALS) project, a collaborative effort focused on creating cutting-edge diffraction integration software. This project is vital for meeting the complex data analysis demands generated by advancements in high-performance pixel array detectors and new beamlines that deliver micron and sub-micron focus, allowing for more detailed and accurate data collection. With the completion of the Advanced Photon Source Upgrade (APS-U) and the installation of the new multi-bend achromat, Graeme's valuable experience will move NE-CAT in new directions as we take advantage of our enhanced light source.

4. Data Collection Bootcamp

In July 2025, NE-CAT held a 2-day Data Collection and Processing Bootcamp after the 75th Annual Meeting of the American Crystallographic Association. The

Figure 4 Students, NE-CAT Staff and SBGrid at the Data Collection and Processing Bootcamp.

Bootcamp was held at the Advanced Photon Source, Argonne National Laboratory. Unlike our previous Practical Crystallography Course, the Bootcamp focused on data collection and data processing. Students were trained to collect data using the NE-CAT REMOTE GUI, then collected data on their own crystals on 24-ID-E. They processed the data using XDS and DIALS under the tutelage of Graeme Winter and the NE-CAT staff.

Twenty students registered for the Bootcamp and 15 students attended. As with all our previous courses, it was free. Students were responsible for their own transportation and housing.

Our next data collection bootcamp will be April 8-9, 2026. Request a spot in the bootcamp at: https://forms.gle/Lr39kFrGP2bYbA9M8

5. CCP4/APS Summer School

If you are looking for a more intensive course on macromolecular crystallography, NE-CAT will be part of the 18th Annual CCP4/APS Crystallographic School, "From Data Collection to Structure Refinement and Beyond." The annual summer school will be held July 7 – 14, 2026.

The school comprises two parts: a data collection workshop and a crystallographic computing workshop. The data collection portion includes beamline training with at both GMCA and NE-CAT beamlines, and data processing, using only the participants' crystals. The crystallographic computation portion will feature many modern crystallographic software packages (including

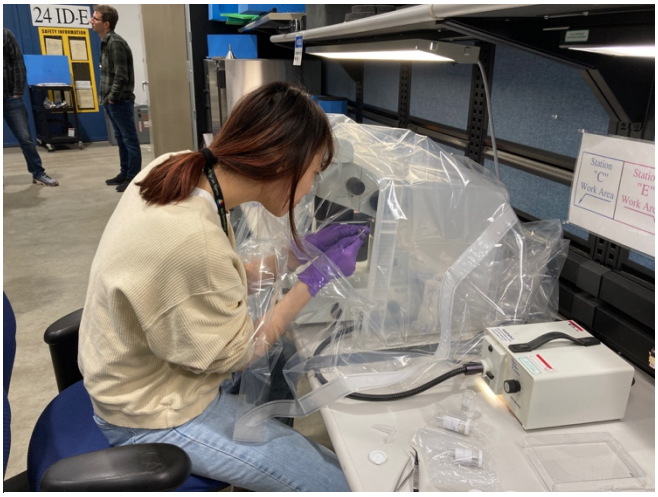

Figure 3 Harvesting crystals inside a plastic-enclosed tub.

CCP4, PHENIX, XDS, HKL, and DIALS) taught by authors and other experts. This workshop will also introduce elements of CryoEM, such as model building and refinement. The daily schedule will be organized in three sections – lectures, tutorials, and hands-on (interactive troubleshooting of the technical difficulties participants face in their projects). An additional virtual day (June 22nd, 2026) will cover sample preparation (cryo-cooling), bioinformatics, and introductory crystallography lectures.

All details and a link to apply can be found on the school website: http://www.ccp4.ac.uk/schools/APS-2026/index.php

6. Humidity Chamber

For the members of our user community collecting data at room temperature to look at protein dynamics, it is

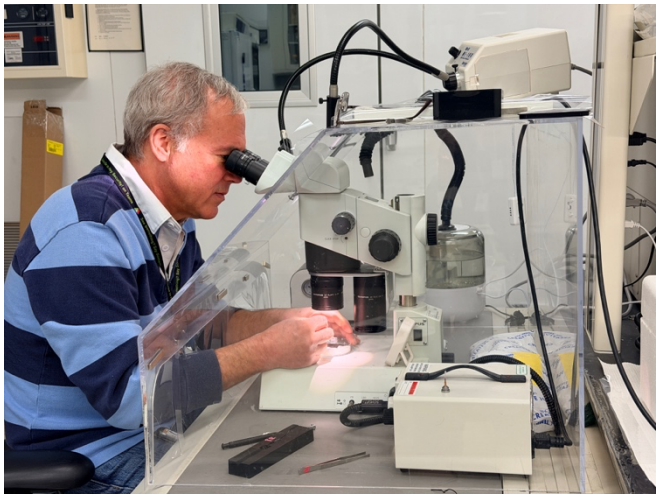

Figure 5 Harvesting crystals in a repurposed fume hood. The 'portable' fume hood was salvaged from cleanup efforts around the APS. It is still very heavy and not easy to move around.

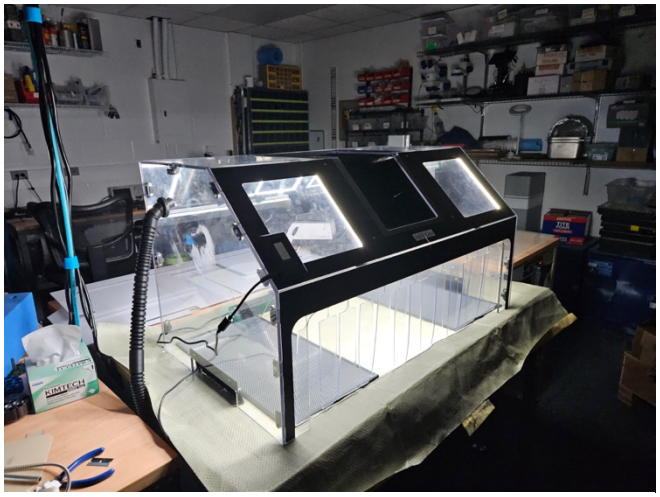

Figure 6 Initial build of a portable humidity chamber at MiTeGen.

necessary to maintain high humidity during crystal harvesting to prevent crystal desiccation. Crystal desiccation is rapid and can adversely affect the diffraction quality of crystals. Over the years, our efforts at providing a humid environment have evolved from a plastic tub for the lab of Nozomi Ando (Cornell University) in 2023 to a repurposed abandoned fume hood in 2025. The plastic tub in 2023 was a last-minute substitution when the Argonne Shipping and Receiving Department failed to deliver MiTeGen's humidity chamber to the beamline in time for the experiment. This launched initial discussions at NE-CAT for a humidity chamber at our facility to avoid future delivery issues.

In the fall of 2025, we began a collaboration with MiTeGen to develop sample hardware for room temperature data collection and serial crystallography. The collaboration with MiTeGen is two-fold: development of an easily portable humidity chamber that can be a moved and assembled at different locations at NE-CAT depending on a user's experimental requirements, and development of an easy-to-use serial crystallography mount that can quickly be loaded with crystals and is resistant to dehydration. The current MiTeGen humidity chamber is not easily portable, and their current serial crystallography mount has mylar windows to prevent sample desiccation which are not fast or easy to apply. NE-CAT is testing out new designs from MiTeGen and providing feedback so that they can improve their current models. The humidity chamber portion of the collaboration is close to becoming a final product as an intial build from MiTeGen brings to life an easily portable humidity chamber that is well-light, roomy, accepts many different stereo microscope models, and keeps samples at greater than 90% relative humidity.

As part of this collaboration, NE-CAT intends to hold a serial crystallography workshop where users will be trained on sample loading and data collection using the improved MiTeGen products. Feedback will be delivered to MiTeGen on the effectiveness of their new designs.

Research Highlights

Matthew Schellenberg, Assistant Professor of Biochemistry and Molecular Biology, Mayo Foundation for Medical Education and Research, Rochester, MN

Essential cellular functions rely on the timely processing of genetic information stored within DNA. This task requires the function of Topoisomerases, a small family of enzymes that manipulate DNA topology to allow smooth movement of large nuclear complexes. Within the family of human Topoisomerases, Topoisomerase II (TOP2) relaxes and untangle DNA by creating double-strand breaks by a mechanism which is shared between the two highly conserved TOP2 isoforms TOP2A and TOP2B. DNA replication requires TOP2A function, thus TOP2A is often upregulated in proliferating cells including cancer cells. The rapid formation and resolution of a large amount of double-strand breaks is an Achilles heel for cancer, as any unresolved DNA double-strand breaks can cause cell death. Cancer chemotherapy regimens often use anthracyclines, which target this vulnerability by binding to TOP2-DNA cleavage complexes and prevent re-ligation of the DNA strands. This mechanism is very effective against solid and metastatic tumors, but carries a significant risk of developing cardiomyopathy and ultimately heart failure.

Figure 7 From left to right, Jan Kubeš, Matthew Schellenberg and Anh Cong

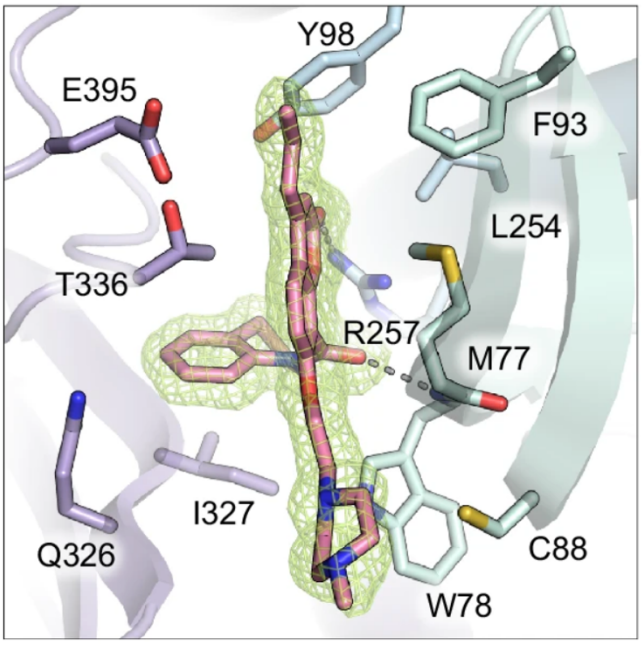

Figure 8 Molecular architecture of the obex pocket binding topobexin (pink) in TOP2B. Electron density corresponding to topobexin from a composite omit map (green mesh, contoured at 1σ) reveals the location and conformation of topobexin (pink) within this pocket.

Cardiac damage from anthracycline treatment is a detrimental consequence for cancer survivors, and a limiting factor to the efficacy of cancer treatment. Cardiac cells lack TOP2A, and only express the non-essential TOP2B isoform. The high structural similarity between TOP2A and TOP2B means both are targeted by anthracyclines, and undesirable off-targeting of TOP2B is responsible for cardiotoxicity. Efforts to develop a TOP2A-selective anthracycline for safer cancer treatments have not been successful due to the high structural similarity between TOP2A and TOP2B.

The biochemical mechanism of TOP2 allows for the use of a different class of TOP2 inhibitors termed “catalytic inhibitors” that prevent the formation of TOP2-DNA cleavage complexes. The catalytic inhibitor dexrazoxane targets both TOP2A and TOP2B, and is effective at preventing off-target cardiotoxicity of anthracyclines. However, TOP2B-selectivity is required to also avoid interference with cancer killing effects. Since all known inhibitor and ligand binding sites are identical between TOP2A and TOP2B, the development of TOP2B-selective inhibitors requires a new inhibitor binding pocket that is not conserved between the two isoforms. To achieve this goal, our collaborative team including research groups at Charles University in the Czech Republic discovered a new inhibitor binding site on the TOP2 ATPase domain. The mechanism of inhibition at this binding site was defined using structural studies of the ATPase domains of TOP2A and TOP2B bound to inhibitors and various ligands representing different ATP-hydrolysis states. Our structures defined a new allosteric mechanism of inhibition of TOP2, where inhibitors bind and act as a physical obstacle to block the conformational change triggered by ATP-hydrolysis. We named the class of inhibitors that act via this mechanism *obex*, meaning obstacle in Latin, to differentiate them from other known classes of inhibitors. Additionally, this pocket contains residue differences between TOP2A and TOP2B isoforms which can be leveraged for isoform-selective inhibition. Based on structural information collected thus far, we worked with medicinal chemists within our team to carry out structure-guided drug design, which has resulted in the development of a new inhibitor family of TOP2 catalytic inhibitors. Our lead molecule topobexin has both high potency and TOP2B-selectivity, and is highly effective at preventing anthracycline cardiotoxicity *in* vivo. X-ray crystallography and the NE-CAT beamlines (24-C and 24-E) at the APS have been vital in the development process, as the technique allows visualization of protein-inhibitor interactions accurately to the atomic level. Additionally, the molecular perspective provided by our structural studies is complemented by cell and animal models, creating a comprehensive story of drug development that connects atomic interactions to phenotypes in whole organisms. We are working on developing *obex* derivatives with enhanced pharmacological properties in hope to translate this new therapy to the clinic to provide robust cardioprotection to cancer patients receiving anthracycline chemotherapy. We thank the NE-CAT staff and facility for their generous support throughout this project.

Staff Activities

NE-CAT joined with three other NIH P30 resources at the APS to present an informational booth at the

Figure 9 Cyndi Salbego and other representatives from NIH-funded CATs at the APS speaking to attendees at ASBMB.

American Society for Biochemistry and Molecular Biology (ASBMB) Annual Meeting and the 75th Annual Meeting of the American Crystallographic Association. The booth provided informational pamphlets describing all four resources and NE-CAT distributed fidget toys branded with the NE-CAT name to remind visitors that we were accepting users to the resource.

Look for our booth again in 2026 at the ASBMB Annual Meeting in Oxon Hill, Maryland and at the International Union of Crystallography (IUCR) 27th Congress in Calgary, Canada.

It was fun and exciting to meet up with our users in 2025 at the 26th West Coast Structural Biology Workshop, Industrial Biostructures America Conference, the Protein Society Annual Meeting, 12th International Workshop on Radiation Damage to Biological Samples, ACA, the LCLS/SSRL User's Meeting, and the Pittsburgh Diffraction Conference.

If you want to touch base with your favorite NE-CAT scientist, we will be at several meetings this coming year, including: ASBMB Annual Meeting, the Protein Society Annual Symposium, IUCR 27th Congress, the Biophysical Society Annual Meeting, and Diffraction Methods in Structural Biology 2026.

Publications

Pallapati, A. R., Korkmaz, F., Rojekar, S., Sims, S., Misra, A., Gimenez-Roig, J., Gangadhar, A., Laurencin, V., Gumerova, A., Cheliadinova, U., Sultana, F., Vasilyeva, D., Cullen, L., **Schuermann, J.**, Munitz, J., Kannangara, H., Parte, S., Pevnev, G., Burganova, G., Tumoglu, Z., Witztum, R., Wizman, S., Kramskiy, N., Igel, L., Sen, F., Ranzenigo, A., Macdonald, A., Hutchison, S., Teunissen, A. J., Burkart, H., Saxena, M., Ginsburg, Y., Goosens, K., Zhou, W., Ryu, V., Moldavski, O., Barak, O., Pazianas, M., Caminis, J., Bhasin, S., Fitzgerald, R., Kim, S. M., Quinn, M., Haider, S., Appt, S., Frolinger, T., Rosen, C. J., Lizneva, D., Gupta, Y. K., Yuen, T., and Zaidi, M. (2025) Efficacy and safety of a therapeutic humanized FSH-blocking antibody in obesity and Alzheimer's disease models, *J Clin Invest 135*. PMID: 40663423. **PMC12404759**.

Lam, K. H., Gao, L., Przykopanski, A., Chen, B., Huang, T., Kruger, M., Bartels, A. M., Dorner, M. B., **Perry, K.,** Dorner, B. G., Rummel, A., and Jin, R. (2025) A nut-and-bolt assembly of the bimodular large progenitor botulinum neurotoxin complex, *Sci Adv 11*, eadx5831. PMID: 40864694. **PMC12383247**.

Acknowledgements

NE-CAT is supported by a grant from the National Institute of General Medical Sciences (P30 GM124165) and contributions from the following NE-CAT institutional members: Columbia University, Cornell University, Genentech, Harvard University, Massachusetts Institute of Technology, Memorial Sloan-Kettering Cancer Center, Rockefeller University, and Yale University.

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