



NE-CAT Communications

A Biannual Newsletter of the Northeastern Collaborative Access Team Winter 2016



Message from the Director

Steve Ealick

The highly anticipated EIGER 16M has arrived at NE-CAT in December 2016. Preparations have been in progress for its installation since last year, but more needed to be completed after the EIGER's arrival for it to be fully experienced by our user community. Throughout January and February, we worked on minimizing disruptions while making it available to users at the fastest rate possible. We are cognizant of the reliance of our user community on RAPD and have built new computing infrastructure and software to seamlessly integrate the EIGER. You can read more about these preparations in this newsletter.

We have spent part of 2016 collaborating and communicating with the macromolecular crystallography community on current progress in the field. These communications are summarized later in the newsletter. Meetings and workshops are an excellent chance to learn new techniques and refine old ones. I encourage all our users to reach out to NE-CAT staff at meetings or at any time if you have a question related to crystallography or our beamlines.

You can find their contact information, and links to apply for beamtime on our website (<http://necat.chem.cornell.edu>).

Beamline Developments

1. EIGER 16M Preparation, Commissioning and Installation

The EIGER 16M was delivered in mid-December just before the end of the 2016-3 run cycle (Fig. 1). The detector was carefully uncrated and assembled in the engineering lab (Fig. 2).

An installation engineer from Dectris, Pascal Hofer, came to NE-CAT during the first week of January to assist in commissioning the EIGER. During commissioning, a hotspot was detected on one of the modules and this module was subsequently replaced.

Compared to the PILATUS, EIGER pixels are small ($\sim 1/4^{\text{th}}$ the area of a PILATUS pixel), at the same time, there are approximately three times more pixels in EIGER. The EIGER also has the ability for much higher frame rates than the PILATUS. A more extensive comparison of features between the two PADs is available in our Summer 2016 Newsletter.



Fig. 1 Malcolm Capel and Raj Rajashankar open the crate containing the EIGER.



Fig. 2 Ed Lynch and Malcolm Capel hook up the EIGER in the dry lab in preparation for commissioning.

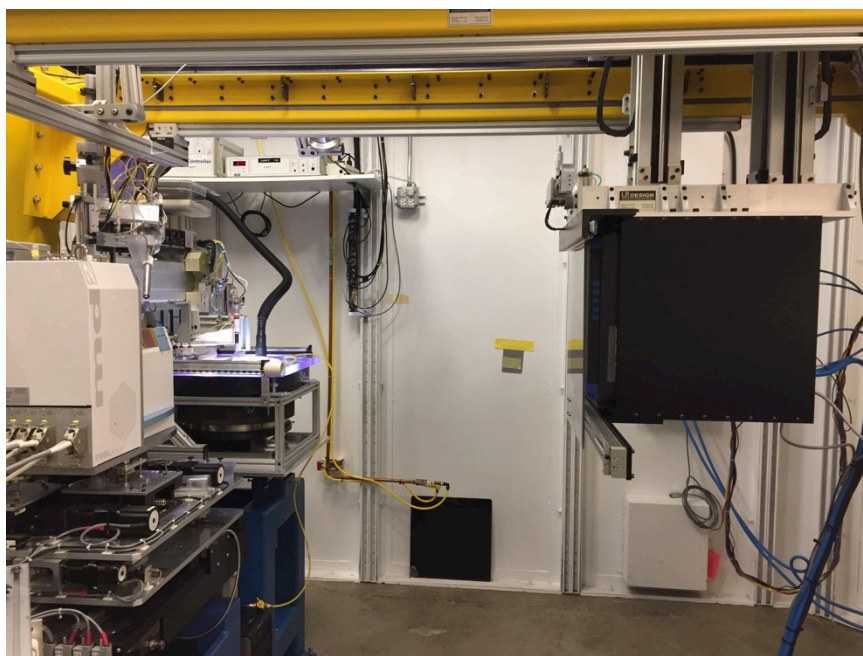
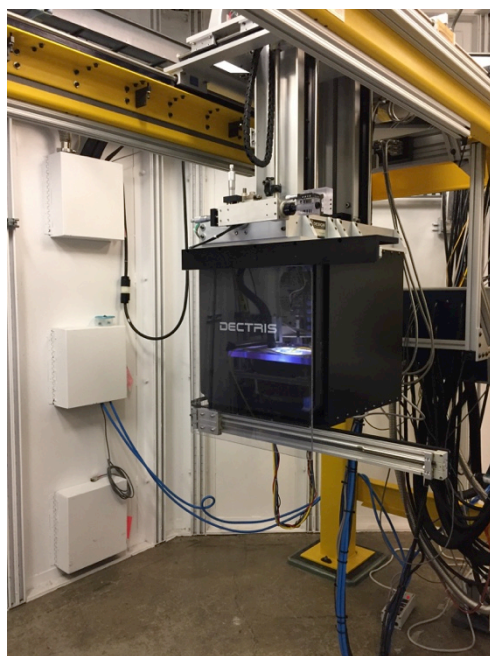


Fig. 3 The EIGER 16M mounted on the A-Frame in the 24-ID-E Hutch. Though it has more pixels, the EIGER is physically smaller than the PILATUS and it has approximately the same physical detector area as the Q315 it replaces. However, it is lighter and less deep than the Q315.

As implemented now, we are making use of basic features of the EIGER. To take full advantage of the EIGER, a high-performance computing and network environment will be required. We are in the process of making these upgrades, without impacting user operation. .

The EIGER is intended for the monowavelength beamline, 24-ID-E. Integration of the EIGER into the beamline controls and RAPD occurred with the detector in the dry lab during January and February while our users continued to use the Q315 during the first month of the run. Ed Lynch also constructed a custom carrier and protective cover. On the last Monday of February, during a scheduled 2-day machine studies period, the EIGER was mounted onto the A-frame in the 24-ID-E hutch (Fig. 3).

All preparations resulted in a speedy and seamless implementation of the EIGER. Three days were scheduled for integration of the EIGER, but after only two days, the detector was ready for use by our user community. In those two days, we carried out extensive testing and witnessed high quality data from the detector. Commissioning data analysis is still under progress, but our overall opinion is that the datasets from EIGER are, as expected, far superior to data collected on the Q315 and may even be better than PILATUS data. EIGER 16M data will be delivered in CBF format (like the PILATUS) and RAPD will process the data. Users wishing to process the data with HKL will need to obtain a license from HKL-XRAY.

XDS and iMOSFLM play well with the data. Data templates (e.g. XDS.INP or def.site) are available on our website.

2. ACA Session

During the 2016 American Crystallographic Association Annual Meeting, Surajit Banerjee organized and chaired a session entitled, "Crystal Sample Preparation: A Crystal is Just the Start!" As we all know, biological crystallization has many challenges. Despite obtaining a crystal, the process can be a long way from yielding informative structural knowledge. Multiple steps can aid the process, including the initial sample preparation (i.e. obtaining a pure and stable protein), techniques and biochemistry to optimize the crystal, and post-crystallization treatments.

The content of the session had various topics from: handling the crystal, finding suitable cryoprotectant, microseeding using automation, and dehydration. The session started with cation replacement and the dehydration effect on the diffraction of RNA by Adrian Ferré-D'Amaré of NIH. He gave a very nice example of how to introduce multiple variables in the transfer process (from mother liquor to dehydrating solution) of crystals. Patrick Shaw Stewart of Douglas Instruments discussed microseeding presented in the context of their new Random Microseed Matrix Screen. In addition, he has seen that the technique can be applied successfully to crystals grown in LCP and

believes that membrane crystallographers should consider microseeding more frequently than is currently done in the crystallographic community. Matthew Bowler from EMBL talked about integration of the HC1 humidity controller into a beamline control system and its automated use. He also provided a couple success stories, which should encourage more people to try it.

After the coffee break, Janet Newman, from CSIRO, Australia, gave a substantial background of the problem of protein degradation during crystallization trials. David Moreau from Robert Thorne's lab at Cornell University shared his experience with crystallization temperature and the temperature-dependence of various cryoprotectants. Miki Senda of KEK, Japan, discussed combining different kinds of cryoprotectant to obtain the best diffraction.

The most attractive part of the session was at the end. Elspeth Garman presented a short one-act play. Eddie Snell and Janet Newman both played the roles of different kind of crystals with several kinds of problems and 'Auntie E' guided them towards the best solution.

This session was most attended at the ACA 2017 meeting and the feedback was very positive.

Phenix Workshop

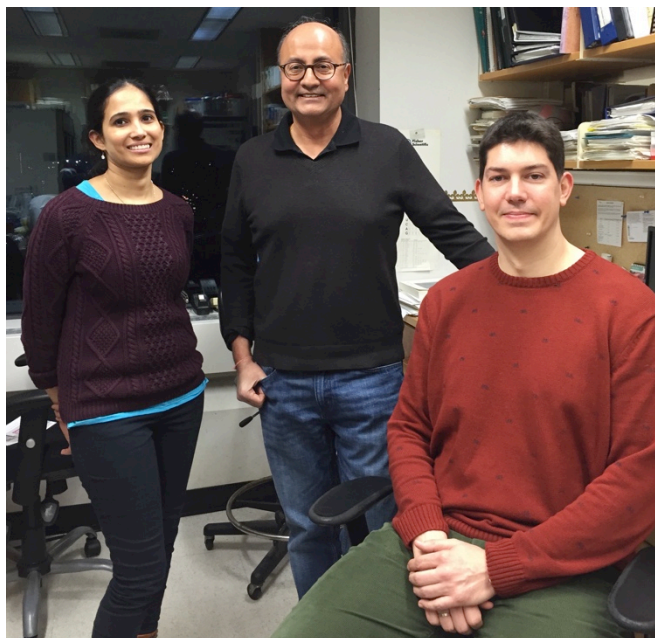


Fig. 4 From left to right: Rinku Jain, Aneel Aggarwal, and Javier Coloma.

Together with SBCGrid, NE-CAT hosted a Phenix Workshop at Harvard Medical School from November 10-11, 2016. The 1.5 day workshop covered topics in experimental phasing, automated model building, and real space refinement. The first day of the workshop was composed of talks by Paul Adams, Tom Terrwilliger, and Pavel Afonine. On the second day, participants of the workshop brought their own data or datasets from X-ray crystallography and electron microscopy to an interactive session. In this session, tutors, including Jon Schuermann from NE-CAT, assisted participants at different stages of the structure solution process by providing advice on how to use the Phenix software or troubleshooting problems with datasets.

Research Highlights

A path to drug discovery against Zika virus

Aneel Aggarwal, Professor, Icahn School of Medicine at Mount Sinai, New York, NY

Rinku Jain, Research Assistant Professor, Icahn School of Medicine at Mount Sinai, New York, NY

Javier Coloma, Instructor, Icahn School of Medicine at Mount Sinai, New York, NY

The Zika virus (ZIKV) has emerged as a major health concern over the past year. Its link to microcephaly in newborn infants and the Guillan-Barré syndrome in adults led the World Health Organization (WHO) to declare ZIKV infections a Public Health Emergency of International Concern. ZIKV was first isolated in Uganda in 1947. The strains causing the current outbreak in the Americas are most closely related to the strain that caused an epidemic in the French Polynesia in 2007. ZIKV belongs to the same *Flavivirus* genus as other mosquito-borne human pathogens, such as dengue virus, yellow fever virus, and West Nile virus, among others. The public health emergency posed by ZIKV has invigorated efforts to develop a vaccine, eradicate the *Aedes* mosquito vectors, and to develop antivirals based on the targeting of enzymatic activities central to the life cycle and survival of ZIKV.

To help guide the discovery of antiviral compounds against ZIKV, Drs. Rinku Jain, Javier Coloma, and Aneel Aggarwal at the Icahn School of Medicine at Mount Sinai (Fig. 4), in collaboration with the NE-CAT staff, have determined the high-resolution structures of two key enzymes from the French Polynesia strain of the ZIKV, namely NS3-Hel and NS5-MTase. NS3-Hel unwinds RNA and is essential for virus's replication, while NS5-MTase methylates or "caps" the 5'-end of the viral RNA for stability and evasion of the host immune response. The structures were recently published in the journals *Nature Structural & Molecular Biology* (Jain *et al.*, 2016) and *Cell Reports* (Coloma *et al.*, 2016).

As expected, the structure of ZIKV NS3-Hel is similar to that of RNA helicase from Dengue and other flaviviruses. It is composed of three domains, where domains 1 and 2 comprise the tandem a/b RecA-like folds characteristic of SF1 and SF2 helicases. Most of the variability is in the conformation of loops typically involved in binding ATP and RNA. Importantly, the structure allows the identification of potential druggable "hotspots" between domains 1 and 2 (site 1) and at the confluence of domains 2 and 3 (site 2), which can now be exploited for *in silico* drug discovery.

The structures ZIKV NS5-MTase were determined bound to S-adenosylmethionine (SAM), and to SAM and 7-methyl guanosine diphosphate (7-MeGpp; a mimic of the 5' cap structure). The SAM and 7-MeGpp binding sites are separated by > 15 Å (Fig. 5). A positively charged tunnel spans the two sites where, based on the structure of Dengue NS5 with RNA (Zhao *et al.* (2015) *Proc. Natl. Acad. Sci.*), the ZIKA viral RNA likely binds. This RNA binding tunnel may provide a basis for inhibitors that are selective for ZIKV and other flavivirus methyltransferases. In particular, SAM analogs with functionalities that co-occupy the RNA binding tunnel may provide better specificity relative to human RNA methyltransferases.

According to Dr. Aggarwal, "...for any drug discovery project, a key requirement is an accurate structural model. I am particularly pleased that we were able to determine all of these structures to high resolution (better than 1.5Å) by taking advantage of the high flux and the PILATUS detector at NE-CAT. We've now teamed-up with medicinal chemists and are encouraged by some of the new compounds designed on the basis of these high- resolution structures".

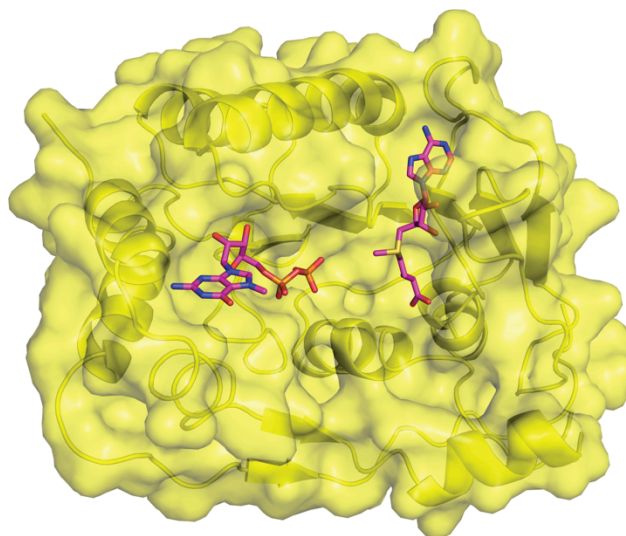


Fig. 5 Crystal structure of the ZIKV NS5 methyltransferase bound to S-adenosylmethionine and 7-methyl guanosine diphosphate.

Staff Activities

Posters

J. Schuermann, D. Neau, K. Rajashankar, I. Kourinov, F. Murphy, "Solving Structures by Experimental Phasing at NE-CAT", SBGrid/NECAT Phenix Workshop, Boston, MA, November 10-11, 2016.

Publications

Campbell, E. A., Kamath, S., **Rajashankar, K. R.**, Wu, M., and Darst, S. A. (2017) Crystal structure of Aquifex aeolicus sigmaN bound to promoter DNA and the structure of sigmaN-holoenzyme, *Proc Natl Acad Sci U S A*.

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of Heart 6-Phosphofructo-2-Kinase/Fructose-2,6-Bisphosphatase (PFKFB2) and the Inhibitory Influence of Citrate on Substrate Binding, *Proteins* 85, 117-124.

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Coloma, J., Jain, R., **Rajashankar, Kanagalaghatta R.**, Garcia-Sastre, A., and Aggarwal, Aneel K. (2016) Structures of NS5 Methyltransferase from Zika Virus, *Cell Rep* 16, 3097-3102.

Schormann, N., Zhukovskaya, N., Bedwell, G., Nuth, M., Gillilan, R., Prevelige, P. E., Ricciardi, R. P., **Banerjee, S.**, and Chattopadhyay, D. (2016) Poxvirus Uracil-DNA Glycosylase - an Unusual Member of the Family I Uracil-DNA Glycosylases, *Protein Sci* 25, 2113-2131.

Rechko, O., Gupta, Y. K., Malik, R., **Rajashankar, K. R.**, Johnson, R. E., Prakash, L., Prakash, S., and Aggarwal, A. K. (2016) Structure and mechanism of human PrimPol, a DNA polymerase with primase activity, *Sci Adv* 2, e1601317.

Hofmann, L., Tsybovsky, Y., Alexander, N. S., Babino, D., Leung, N. Y., Montell, C., **Banerjee, S.**, von Lintig, J., and Palczewski, K. (2016) Structural insights into the *Drosophila melanogaster* retinol dehydrogenase, a member of the short-chain dehydrogenase/reductase family, *Biochemistry* 55, 6545-6557.

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