NE-CAT Communications

A Biannual Newsletter of the Northeastern Collaborative Access Team Spring 2015

Message from the Director

Steve Ealick

It has been a very busy time at NE-CAT since last fall. We are moving forward with the technical and software improvements detailed in our grant. In addition, with the shutdown of NSLS, demand for synchrotron beamtime in general and for advanced technologies at the NE-CAT beamlines in particular, has increased tremendously. We have taken this as an opportunity and have devised strategic plans to accommodate a greater number of experiments in a given amount of beamtime.

Happily, NE-CAT can accommodate greater demand as we were already in the process of installing a large capacity sample automounter on 24-ID-E prior to the closure of NSLS and put into motion actions for acquiring a high speed detector for 24-ID-E. Full implementation of remote data collection capabilities at both beamlines not only saved travel expenses for users, but also made it possible to allocate shorter, but more frequent beamtime trips. NE-CAT has also acquired additional storage space for data.

We are also making changes to the hardware at NE-CAT as we continue to push the frontiers of micro-crystallography and low resolution crystallography. Some of these improvements are detailed later in this newsletter. This is a very demanding, but exciting, time at NE-CAT as we move forward with the technical and software improvements proposed in our grant while handling the increased number of scientists needing synchrotron beamtime.

Beamline Developments

1. Large Capacity Sample Automounter on 24-ID-E Beamline

Over the summer of 2014, a second large capacity sample automounter was constructed by Ed Lynch and Jim Withrow. The dewar for this automounter also holds 14 cryopucks. This automounter incorporates modifications suggested by scientific staff and users who worked with the large capacity sample automounter installed on 24-ID-C in June 2014. During the September shutdown, the new sample automounter was installed on 24-ID-E (Fig. 1) and has been in continuous use since the 2014-3 run cycle.

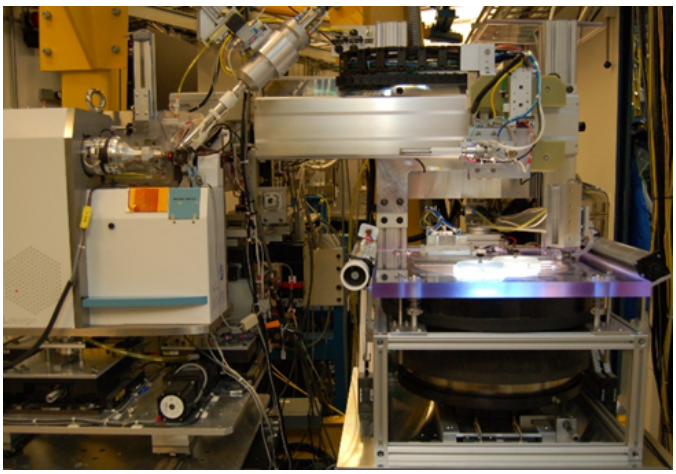

Fig. 1 Large capacity sample automounter installed on 24-ID-E.

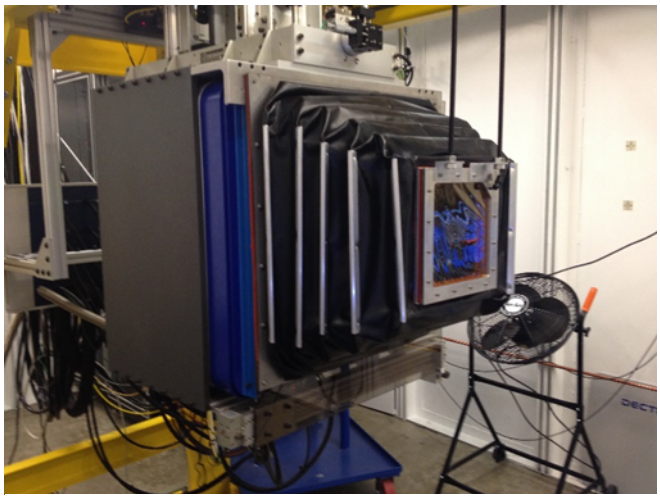

Fig. 2 Helium-filled cone supported from the A-frame in the 24-ID-C beamline hutch..

2. Helium-Filled Beam Path to Reduce Air-Absorption

The goal of one of the core research and development projects at NE-CAT is to develop the facilities for optimal data recording and processing from crystals that diffract to 4 Å or lower. Data at this low resolution have inherently low signal-to-noise but there is high value in including diffraction spots at very small Bragg angles. The helium cone can also be useful for data collection at long wavelengths, where air absorption is non-negligible.

The scattering cross-section of helium is much lower than air; therefore, the design and construction of a helium-filled beam path to reduce air absorption of Bragg diffraction spots was proposed. The original design for a helium-filled beam path at NE-CAT called for a construct that could expand to different distances and be supported from the A-Frame holding the detector. This design was composed of three neoprene cones with accordion pleats. The pleats would allow for expansion and contraction of the helium-filled beam path as the detector moved. Kapton windows at the front and back would allow containment of the helium gas. When filled with helium gas, the cone would provide a lower absorption beam path from the beamstop to the detector. The only regions still exposed to air would be from the egress of the primary beam from the MD2 to the beamstop and a sliver of air between the second Kapton window to the front of the detector. The design also allowed the use of the MD2 and the existing cryostream.

A preliminary design of the helium-filled beam path was constructed in August 2014. A single neoprene cone was hung from the 24-ID-C A-Frame (Fig. 2). This cone was used by Wayne Hendrickson's group to collect highly redundant data from multiple crystals for use in sulfur-SAD experiments.

3. Grid Scanning on Remote Data Collection Interface

Remote data collection is available on both NE-CAT beamlines through our unique web interface. Incrementally, the web interface has gained new capabilities. Simple vector scanned data collection for taking snapshots and datasets along long rod-like crystals has been available since nearly the beginning. Most recently, grid scanning techniques have also been implemented (Fig. 3). Grid scanning is useful for locating small samples in large loops, isolating a crystal or crystals in lipidic cubic phase or finding a crystal in

Fig. 3 New grid scanning technique in remote GUI. Highlighting an individual spot will show its location in the scan and in the histogram of peak heights. The central portion of the diffraction image for that spot will also be displayed.

opaque or icy cryoprotectant.

For more information on remote data collection, watch our new video tutorial (http://youtu.be/jWJMoMjx_vE). It is available on our YouTube Channel.

4. Membrane Protein Structures 2015 Meeting

From April 9 – 12, 2015, the Membrane Protein Structures 2015 Meeting was held at the APS. NE-CAT joined groups from UCSF, USC, AECOM, ANL and NIGMS to organize the meeting. This two and a half day symposium featured workshops, talks and posters focused on the technological developments in structure determination of membrane proteins and highlighted recent results. A full schedule of the events and workshops can be found at the meeting's website (http://aps.anl.gov/mps2015).

Surajit Banerjee and Cyndi Salbego were members of the local planning committee and helped organize the meeting. In addition, as part of the symposium's workshops, Surajit hosted beamline tours of NE-CAT for multiple groups of attendees on April 9th (Fig. 4) and presented a poster describing the technology development at NE-CAT. Cyndi provided logistical support for local organization.

5. New Crystal for the 24-ID-E Monochromator

In order to meet both challenges in micro-crystallography: microbeam creation and long term stability, the monochromator on 24-ID-E was re-designed. The original monochromator on 24-ID-E had two single crystals, one providing X-rays of 12662 eV (selenium edge) and another at 13474 eV (bromine edge). A translation stage allowed the monochromator to switch between the two fixed energies (Fig. 5A). However, the stage had proven to be a source of instability and, in daily operations at NE-CAT, the

Fig. 4 Surajit Banerjee shows Membrane Protein Structure attendees the HC1 dehydration device during a tour of the NE-CAT beamlines.

Si(311) crystal was never used. These crystals were also indirectly cooled, making them unsuitable for high heat loads.

As part of improvements to 24-ID-E detailed in our grant, NE-CAT planned to deliver stable X-ray micro beams of approximately 5 microns in size. As a result, a new single Si(220) crystal was designed by NE-CAT for the 24-ID-E monochromator. The new crystal is directly cooled to allow for a higher heat load. This will not only accommodate the increased current from the APS upgrade, projected to be at least 150 mA and eventually 200 mA, but will also allow access to the third harmonic. Access to the third harmonic will triple the brilliance of 24-ID-E and increase heat load accordingly.

While the new crystal and mount were designed by NE-CAT, the actual components were built by FMB-Oxford. The new monochromator crystal and mount arrived in December 2014. During the May shutdown, the old two crystal assembly was removed and the new single Si(220) crystal was installed (Fig. 5B). As 24-ID-E is a side-bounce, this required careful alignment of the crystal (Fig. 5C).

The installation was successful. Increased stability of the beam in both the horizontal and vertical directions was observed with the

return to operations in June 2015. The horizontal and vertical focus of the beam at the sample is also much improved. We see a vertical reduction in the beam size by a third and a horizontal reduction of almost a factor of 2 (Fig. 5D).

6. Crystal Shipping for Dehydration Tests

As part of our low resolution crystallography focus, NE-CAT acquired the HC1 humidity control device. Use of the HC1 to study the effect of humidity on diffraction resolution requires crystals at ambient conditions, i.e. not frozen. Now, we are beta testing a method for users to send unfrozen crystals to the beamline for testing. We have purchased a portable incubator from MicroQ (Fig. 6A). This controlled refrigeration device can maintain temperature between 0 ^{o}C and 42 ^{o}C during shipping.

Once commissioned, we can send the portable incubator to the users. At the home institution, users can harvest crystals into dialysis buttons filled with mother liquor. The buttons are racked into a custom holder and placed inside the controlled refrigeration device (Fig. 6B). The buttons are then shipped at a controlled temperature to NE-CAT where the users or staff can subject the crystals to dehydration by the HC1.

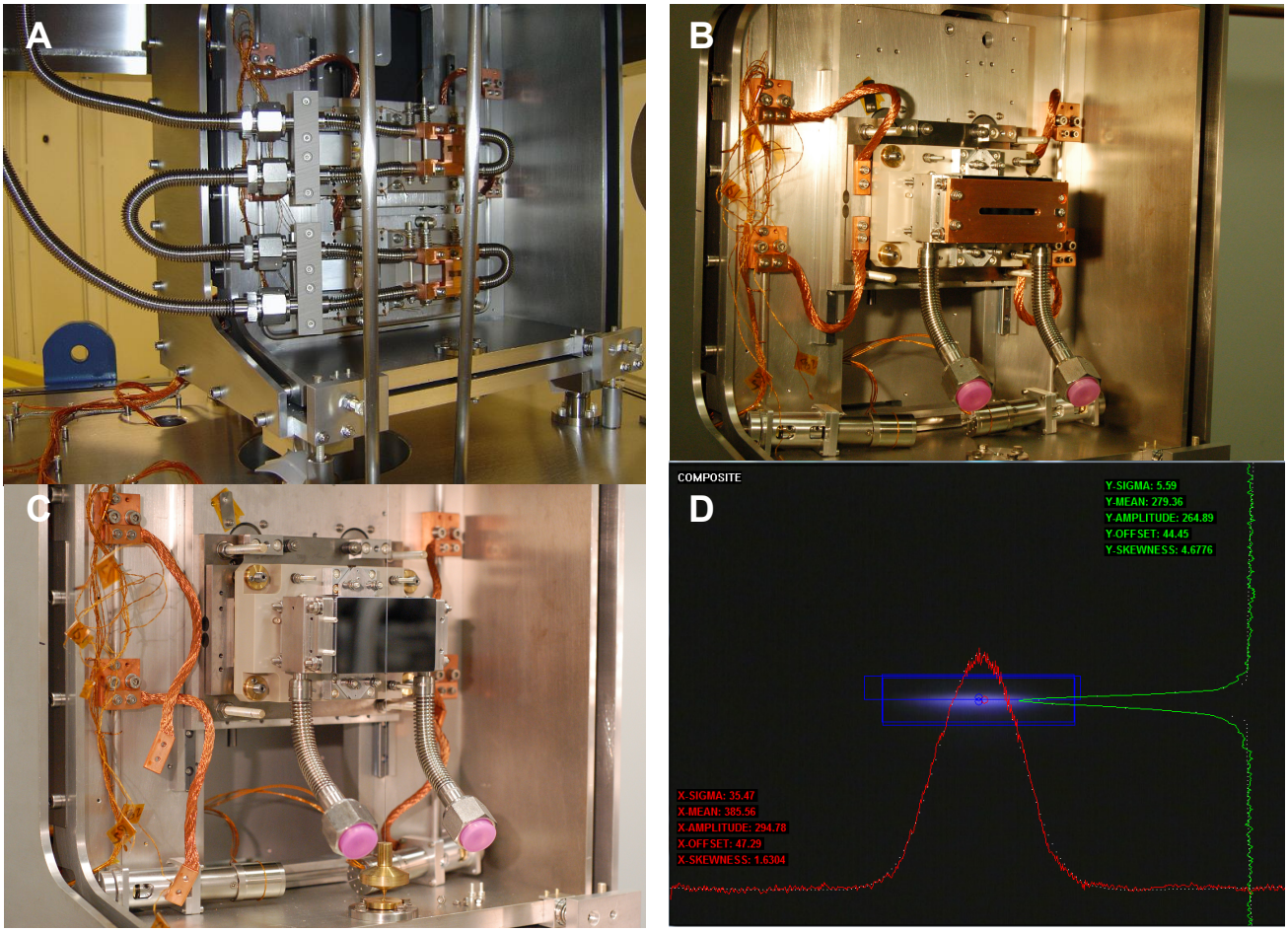

Fig. 5 **(A)** Two crystal monochromator installed on 24-ID-E in 2006. **(B)** New Si(220) crystal and mount installed in the original housing for the 24-ID-E monochromator. The design includes a new direct cooling system. **(C)** The custom pendulum and fishing line apparatus designed to align the new Si(220) crystal. **(D)** Image of the new, more tightly focused beam on 24-ID-E in June 2015 after installation of the new Si(220) crystal.

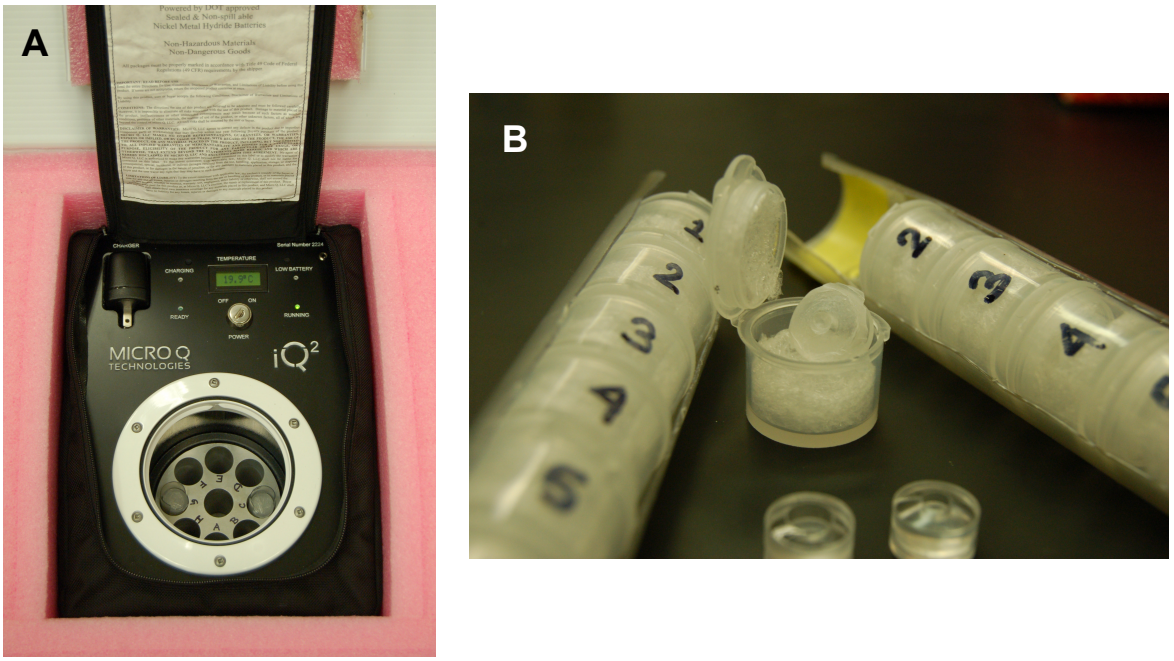

Fig. 6 (A) IQ2 portable incubator from MicroQ with two sleeves of harvested crystals inside. **(B)** Dialysis button, button container and custom holder or sleeve for the harvested crystals.

Research Highlights

Crystal structure of a eukaryotic group II intron lariat

Navtej Toor, Assistant Professor of Chemistry and Biochemistry, Department of Chemistry, University of California San Diego, San Diego, CA.

When film editors throw away bad takes and stitch together their final cut, they unknowingly mimic one of the most fundamental processes of life, splicing. Within our cells illegible sections of genes, known as introns, are removed by splicing to produce coherent messages used as directions for making proteins. These excised introns have a unique branched circular shape known as lariats, reminiscent of a cowboy's lasso.

Researchers in Assistant Professor Navtej Toor's lab in the Department of Chemistry and Biochemistry at UC San Diego, in collaboration with NE-CAT staff, have observed at atomic resolution how these ubiquitous lariat molecules are formed. Their research article was published in the journal *Nature* on October 9, 2014.

"It's all about the knot," said lead author Aaron Robart, a postdoc in the Toor lab (Fig. 7). "How the knot in the intron RNA is tied and how it's handled by the ribozyme is what splicing is really all about." In our cells introns are removed by one of the most complex molecular machines ever discovered: the spliceosome. To understand the atomic details of gene splicing the Toor lab looked to an unlikely source, the brown algae *Pylaiella littoralis*, which harbors highly active group II introns. Group II introns are the molecular ancestors of our splicing machinery but perform the same reaction using only one catalytic RNA molecule; providing a tractable model system to tackle the complexity of splicing.

This structure of the lariat also provides exciting new details of how metal ions fuel the splicing machine and provide specificity. "When it comes to splicing, accuracy is everything," said Jessica Peters (Fig. 7), coauthor and graduate student in the Toor lab. "It's almost hard to imagine but our cells perform this reaction constantly and if it's off even a little bit the results are disastrous." In humans the way a gene is pieced together by splicing allows one gene to make many different products and mistakes have severe penalties in the form of disease.

The new findings also provide a glimpse into

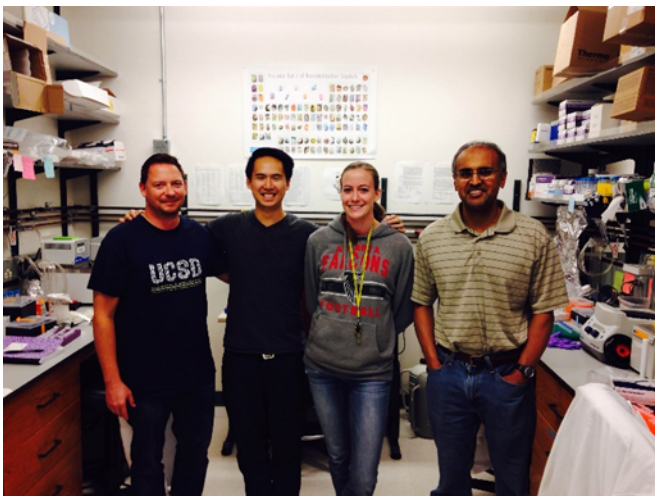

Fig. 7 The members of the Navtej Toor lab who participated in this research. From left to right: Aaron Robart, Russel Chan, Jessica Peters and Navtej Toor.

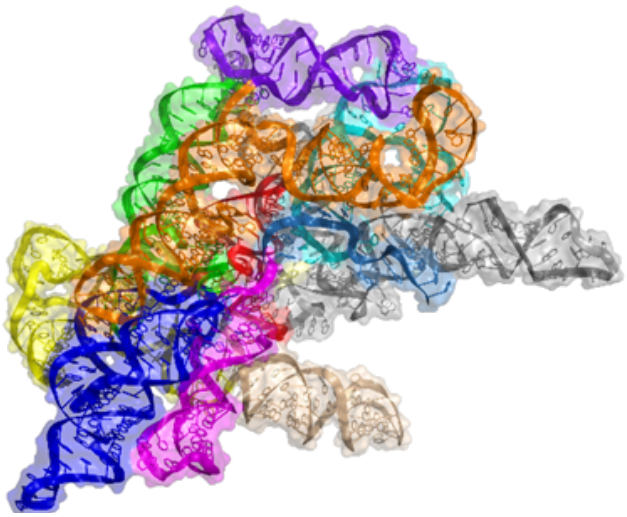

Fig. 8 Crystal structure of the group II intron lariat. Domain 6 is shown in purple and contains the bulged adenosine responsible for lariat formation.

our past. "What excites me is the role lariats have played in shaping our DNA landscape," said Russell Chan (Fig. 7), coauthor and senior graduate student in the Toor lab. "The unusual nature of the lariat bond is what allowed introns to lasso DNA and colonize our genomes." This intron invasion has indeed been prolific with at least half of our DNA being comprised of these non-coding elements.

The group II intron lariat structure revealed a host of new interactions between the intron RNA domains that explained roles for conserved secondary structure elements in structural stability and lariat formation (Fig. 8). Domain III was found to form a large external brace stabilizing a large segment of the structure through two long distance interactions: μ-μ′ and the newly discovered τ-τ′ tertiary interactions. Furthermore domain II, long thought to be unnecessary for splicing activity, was revealed to serve as a central organizational node by coordinating a confluence of four tetraloop-receptor interactions. Of these interactions the newly discovered π-π′ tertiary contact was particularly exciting due to its position directly adjacent to the bulged adenosine that forms the lariat bond. This new interaction plays a pivotal role in directing how the knot within the lariat RNA rope is handled in the splicing catalytic mechanism. The π-π′ interaction is predicted to pull the first splicing step lariat product out of the active site, simultaneously clearing the active site and allowing the second step reactants to enter. The structure also expanded the number of highly coordinated Mg^{2+} metal ions that fuel the splicing machine and provide specificity to the reaction. Two new metals were shown to position the 5' splice site by binding around the GUGYG sequence universally conserved in splice site recognition from group II to mammalian introns.

Looking to the future, the Toor lab aims to expand upon this snap-shot of splicing by obtaining high resolution structures along each step of the splicing path. This will ultimately provide a complete molecular understanding of the intricate details required for proper processing of our genes by splicing.

Staff Activities

Presentations

Frank Murphy, "Synchrotron Beamlines - It's Not Uphill Both Ways Anymore", Ribosome Alumni Meeting during LMB Alumni Symposium, Cambridge University, Cambridge, England, July 10-12, 2014.

David Neau, "Recent Developments at NE-CAT, a Macromolecular Crystallography Synchrotron Facility", Indo-US International Conference/Workshop on Recent Advances in Structural Biology and Drug Discovery, Indian Institute of Technology, Roorkee, India, October 9-11, 2014.

Surajit Banerjee, "NE-CAT: Crystallography Beamlines for Challenging Structural Biology Research" Vanderbilt Institute of Chemical Biology, Vanderbilt University, Nashville, Tennessee, August 1, 2014.

Posters

N. Sukumar. "A comparative analysis on X-ray structure of cobalamin binding proteins", 23rd International Union of Crystallography (IuCr) and General Assembly, Montreal, Quebec, Canada, August 5–12, 2014.

I. Kourinov, M. Capel, S. Banerjee, F. Murphy, D. Neau, K. Perry, K. Rajashankar, J. Schuermann, N. Sukumar, S. Ealick, "NE-CAT Crystallography Beamlines for Challenging Structural Biology Research", 23rd International Union of Crystallography (IuCr) and General Assembly, Montreal, Quebec, Canada, August 5–12, 2014.

S. P. Kearns, P. D. Swartz, **K. Perry**, T. A. Roy, J. D'Antonio, and E. L. D'Antonio "Exploration of Monosaccharide Inhibitors for Trypanosoma cruzi Glucokinase and Hexokinase", 66th Southeastern Regional Meeting of the American Chemical Society, Nashville, TN, October 16-19, 2015.

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", Membrane Protein Structures 2015 Meeting, Advanced Photon Source, Lemont, IL, April 9-12, 2015.

Publications

Broussard, T., Pakhomova, S., **Neau, D. B.**, Bonnot, R., and Waldrop, G. L. (2015) Structural Analysis of Substrate, Reaction Intermediate and Product Binding in *Haemophilus influenzae* Biotin Carboxylase, *Biochemistry* [Epub ahead of print].

Schormann, N., **Banerjee, S.**, Ricciardi, R., and Chattopadhyay, D. (2015) Binding of undamaged double stranded DNA to vaccinia virus uracil-DNA Glycosylase, *BMC Struct. Biol.* 15, 10.

Murphy, M. W., Lee, J. K., Rojo, S., Gearhart, M. D., Kurahashi, K., **Banerjee, S.**, Loeuille, G. A., Bashamboo, A., McElreavey, K., Zarkower, D., Aihara, H., and Bardwell, V. J. (2015) An ancient protein-DNA interaction underlying metazoan sex determination, *Nat. Struct. Mol. Biol.* 22, 442-451.

Patra, A., **Banerjee, S.**, Johnson Salyard, T. L., Malik, C. K., Christov, P. P., Rizzo, C. J., Stone, M. P., and Egli, M. (2015) Structural Basis for Error-Free Bypass of the N7-Methyl-Formamidopyrimidine-dG Lesion by Human DNA Polymerase eta and Sulfolobus solfataricus P2 Polymerase IV (Dpo4), **J. Am. Chem. Soc.** [Epub ahead of print].

Jensen, J. L., Indurthi, V. S., **Neau, D. B.**, Vetter, S. W., and Colbert, C. L. (2015) Structural insights into the binding of the human receptor for advanced glycation end products (RAGE) by S100B, as revealed by an S100B-RAGE-derived peptide complex, *Acta Crystallogr. D,* 1176-1183.

Bolla, J. R., Su, C. C., Delmar, J. A., Radhakrishnan, A., Kumar, N., Chou, T. H., Long, F., **Rajashankar, K. R.**, and Yu, E. W. (2015) Crystal structure of the Alcanivorax borkumensis YdaH transporter reveals an unusual topology, *Nat. Commun.* 6, 6874.

Ren, A., Wang, Xin C., Kellenberger, Colleen A., **Rajashankar, Kanagalaghatta R.**, Jones, Roger A., Hammond, Ming C., and Patel, Dinshaw J. (2015) Structural Basis for Molecular Discrimination by a 3′,3′-cGAMP Sensing Riboswitch, *Cell Reports 11*, 1-12.

Su, C.-C., Bolla, Jani R., Kumar, N., Radhakrishnan, A., Long, F., Delmar, Jared A., Chou, T.-H., **Rajashankar, Kanagalaghatta R**., Shafer, William M., and Yu, Edward W. (2015) Structure and Function of *Neisseria gonorrhoeae* MtrF Illuminates a Class of Antimetabolite Efflux Pumps, *Cell Reports 11*, 61-70.

Blobaum, A. L., Xu, S., Rowlinson, S. W., Duggan, K. C., **Banerjee, S.**, Kudalkar, S. N., Birmingham, W. R., Ghebreselasie, K., and Marnett, L. J. (2015) Action at a Distance: Mutations of Peripheral Residues Transform Rapid Reversible Inhibitors to Slow, Tight Binders of Cyclooxygenase-2, *J. Biol. Chem.* 290, 12793–12803.

Kudalkar, S. N., Nikas, S. P., Kingsley, P. J., Xu, S., Galligan, J. J., Rouzer, C. A., **Banerjee, S.**, Ji, L., Eno, M. R., Makriyannis, A., and Marnett, L. J. (2015) 13-

methylarachidonic acid is a positive allosteric modulator of endocannabinoid oxygenation by cyclooxygenase, *J. Biol. Chem. 290*, 7897–7909.

Szulik, M. W., Pallan, P. S., Nocek, B., Voehler, M., **Banerjee, S.**, Brooks, S., Joachimiak, A., Egli, M., Eichman, B. F., and Stone, M. P. (2015) Differential stabilities and sequence-dependent base pair opening dynamics of watson-crick base pairs with 5-hydroxymethylcytosine, 5-formylcytosine, or 5-carboxylcytosine, *Biochemistry 54*, 1294-1305.

Ren, A., Kosutic, M., **Rajashankar, K. R.**, Frener, M., Santner, T., Westhof, E., Micura, R., and Patel, D. J. (2014) In-line alignment and Mg^{2+} coordination at the cleavage site of the env22 twister ribozyme, *Nat. Commun. 5*, 5534.

Brown, N. G., Watson, E. R., Weissmann, F., Jarvis, M. A., VanderLinden, R., Grace, C. R., Frye, J. J., Qiao, R., Dube, P., Petzold, G., Cho, S. E., Alsharif, O., Bao, J., Davidson, I. F., Zheng, J. J., Nourse, A., **Kurinov, I.**, Peters, J. M., Stark, H., and Schulman, B. A. (2014) Mechanism of Polyubiquitination by Human Anaphase-Promoting Complex: RING Repurposing for Ubiquitin Chain Assembly, *Mol. Cell 56*, 246-260.

Ayres, C. A., Schormann, N., Senkovich, O., Fry, A., **Banerjee, S.**, Ulett, G. C., and Chattopadhyay, D. (2014) Structure of *Streptococcus agalactiae* glyceraldehyde-3-phosphate dehydrogenase holoenzyme reveals a novel surface, *Acta Crystallogr. F 70,* 1333-1339.

Cavalier, M. C., Pierce, A. D., Wilder, P. T., Alasady, M. J., Hartman, K. G., **Neau, D. B.**, Foley, T. L., Jadhav, A., Maloney, D. J., Simeonov, A., Toth, E. A., and Weber, D. J. (2014) Covalent small molecule inhibitors of Ca-bound S100B, *Biochemistry 53,* 6628-6640.

Robart, A. R., Chan, R. T., Peters, J. K., **Rajashankar, K. R.**, and Toor, N. (2014) Crystal structure of a eukaryotic group II intron lariat, *Nature 514*, 193-197.

Neau, D. B., Bender, G., Boeglin, W. E., Bartlett, S. G., Brash, A. R., and Newcomer, M. E. (2014) Crystal Structure of a Lipoxygenase in Complex with Substrate: the Arachidonic Acid Binding Site of 8R-Lipoxygenase, *J. Biol. Chem. 289,* 31905-31913.

Xu, Y., Tao, Y., Cheung, L. S., Fan, C., Chen, L.-Q., Xu, S., **Perry, K.**, Frommer, W. B., and Feng, L. (2014) Structures of bacterial homologues of SWEET transporters in two distinct conformations, *Nature 515*, 448-452.

Sanches, M., Duffy, N. M., Talukdar, M., Thevakumaran, N., Chiovitti, D., Canny, M. D., Lee, K**., Kurinov, I.**, Uehling, D., Al-Awar, R., Poda, G., Prakesch, M., Wilson, B., Tam, V., Schweitzer, C., Toro, A., Lucas, J. L., Vuga, D., Lehmann, L., Durocher, D., Zeng, Q., Patterson, J. B., and Sicheri, F. (2014) Structure and mechanism of action of the hydroxy-aryl-aldehyde class of IRE1 endoribonuclease inhibitors, *Nat. Commun. 5*, 4202.

Montemayor, E. J., Katolik, A., Clark, N. E., Taylor, A. B., **Schuermann, J. P.**, Combs, D. J., Johnsson, R., Holloway, S. P., Stevens, S. W., Damha, M. J., and Hart, P. J. (2014) Structural basis of lariat RNA recognition by the intron debranching enzyme Dbr1, *Nucleic Acids Res. 42*, 10845–10855.

Althoff, T., Hibbs, R. E., **Banerjee, S.**, and Gouaux, E. (2014) X-ray structures of GluCl in apo states reveal a gating mechanism of Cys-loop receptors, *Nature 512*, 333-337.

Huo, Y., Nam, K. H., Ding, F., Lee, H., Wu, L., Xiao, Y., Farchione, M. D., Jr., Zhou, S., **Rajashankar, K.**, **Kurinov, I.**, Zhang, R., and Ke, A. (2014) Structures of CRISPR Cas3

offer mechanistic insights into Cascade-activated DNA unwinding and degradation, *Nat. Struct. Mol. Biol. 21*, 771-777.

Yang, G., Fu, Y., Malakhova, M., **Kurinov, I.**, Zhu, F., Yao, K., Li, H., Chen, H., Li, W., Lim, D. Y., Sheng, Y., Bode, A. M., Dong, Z., and Dong, Z. (2014) Caffeic acid directly targets ERK1/2 to attenuate solar UV-induced skin carcinogenesis, *Cancer Prev. Res. (Phila) 7*, 1056-1066.

Committee Participation

Malcolm Capel, NYX Beamline Review, Brookhaven National Laboratory, NY, February 25-27, 2015.

K. Rajashankar, APS Macromolecular Crystallography Beamtime Allocation Committee, 2008-2015.

Meeting Attendance

Steve Ealick, K. Raj Rajshankar, NIH P41 Principal Director's Meeting, Rockville, MD, March 16-17, 2015.

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

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