NE-CAT Communications

A Biannual Newsletter of the Northeastern Collaborative Access Team Summer 2013

Message from the Director

Steve Ealick

On April 1, 2013, NE-CAT received word that the NIGMS Advisory Council had recommended that our grant be funded in full for the next five years. Currently, we have received our requested funding for the fiscal year. I want to thank all our PIs for their generous support either in the form of written material or a support letter for the grant. Also, I am proud of the NE-CAT staff who worked hard to write and assemble the grant. Congratulations to all.

I look forward to the hardware and software technologies that we will develop to address challenges with small crystals and low resolution crystallography as the "NE-CAT Center for Advanced Macromolecular Crystallography." We will also continue to adapt these frontier technologies for use by a broad spectrum of users.

As part of our funding agreement, all institutional users will be awarded beamtime through the General User program at the APS. This transition will occur over the next year. Interested users can read more about submitting a General User Proposal later in this newsletter.

For further information on our current beamline capabilities or to check out available beamtime, please visit our website at http://necat.chem.cornell.edu.

Beamline Developments

1. PMAC Controller Change

The motors of the MD2 microdiffractometer are controlled by a Programmable Multi-Axis Controller (PMAC), the Turbo PMAC2, made by Delta Tau Data Systems Inc and compact PCI boards that interface between the digital Turbo PMAC2 and the analog stepper motors. The MD2 microdiffractometer also comes with Windows-based control software developed by Maatel.

NE-CAT originally integrated the MD2 into our beamline control system by communicating with the Maatel software via a COM server. This method of communication has proven to be undesirable over the long term due to instability, lack of speed and, more importantly, control-flexibility. In order to improve integration of the MD2 with the beamline controls, during the January shutdown, NE-CAT replaced the Maatel software with a Linux-based PMAC controller developed in collaboration with Keith Brister from LS-CAT, APS.

This Linux-based controller integrates the PMAC directly into the beamline control system. This integration has resulted in increased data collection speed and enabled us to perform data collection procedures that could not be achieved with the COM server. One such example is provided in the following sub-section and more are under development.

2. Shutter-less Continuous Vector Scans

Development of the new PMAC controller is also a necessary step in implementing shutter-less vector scans (kinematic scans) and taking full advantage of the speed of the PILATUS-6MF detector. As a first step, during the May shutdown, shutter-less continuous vector scans became a reality. It is now possible to perform continuous vector scans on the 24-ID-C beamline without closing the shutter while the MD2 moves the crystal to a new position (Fig. 1). A quick test using 180 frames along the same vector (Fig. 1A), and identical oscillation angle and exposure time shows a five-fold decrease in time between shuttered collection and shutter-less collection (Fig. 1B). To perform a shutter-less data collection, select the 'On' button in the 'Shutterless Ops' control panel in the data collection window (Fig. 1C).

A

B

	shuttered	shutter-less
start	8:37:44 PM	8:31:37 PM
end	8:45:11 PM	8:33:07 PM
time elapsed	07:27.4	01:29.7

C

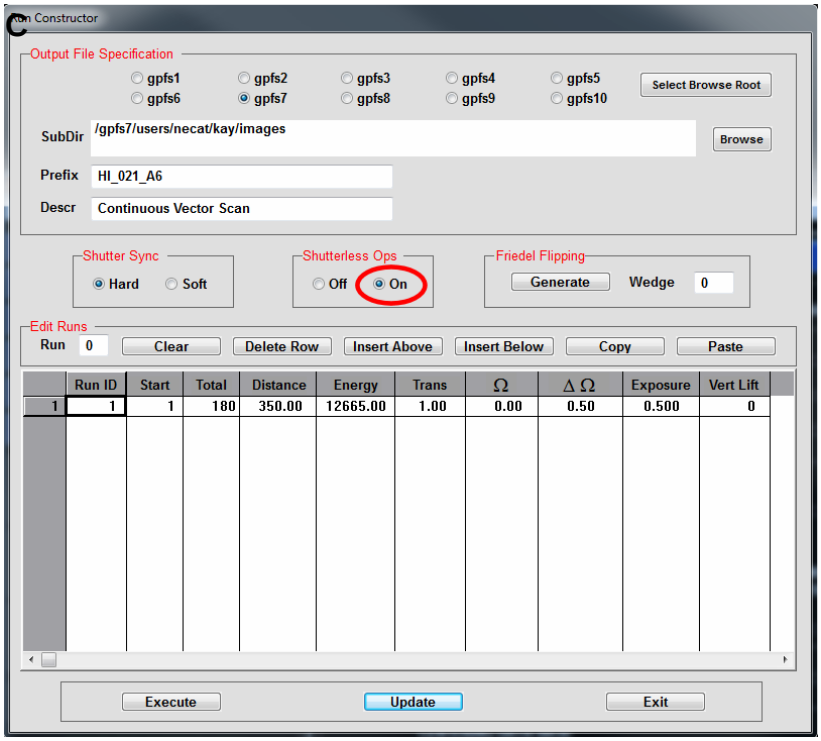

Fig 1. A) The vector used for the continuous vector scan shutter-less data collection test case. B) A chart comparing the time required to perform the same continuous scan using 0.5 degree oscillations and 0.5 second exposures over the vector in A. C) Highlighted in red is the button for enabling shutterless continuous vector scans.

3. New Hardware for Automounter Servers

During the 2013-1 run, new computers were built to host the ALS sample automounter servers and beamline automounter control software. The purpose of the new hardware was to increase processing speed with new CPUs and facilitate sample centering inside the hutch. Originally, sample centering inside the hutch was managed through the Maatel software, which cannot run concurrently with the new PMAC controller.

4. Data Storage Update

Since the beginning of the sector 24 beamlines, two Hewlett-Packard EVA data storage arrays served all the needs of data collection, processing and storage at NE-CAT. Considering the aging technology, we recently upgraded our data storage system with three Nexan SATAbeasts, serving approximately 150 terabytes of disk space. After the 2013-1 run ended, it was decided to take the HP EVA data storage arrays offline. This reduces our current storage capacity by 28 terabytes, leaving NE-CAT with 150 terabytes of storage capacity. The EVAs remain functional and are available for future use should the need arise.

5. Cab-o-sil Contamination in LN_2

Many users have been asking about the presence of a white film or a white powder coating on their cryopucks. After some research, Ed Lynch has discovered that this substance is most likely Cab-o-sil, a fumed silica in unsaturated polyester resin. Cab-o-sil is used in MVE and may be in other dry shippers to absorb and hold the liquid nitrogen thus maintaining the cryogenic temperature during shipping. Therefore, the presence of a white coating on your cryopucks is an indication that the structural integrity of the dry shipper may be compromised.

Users are cautioned to check the holding temperature of the dry shipper over a multi-day time course and compare it to the listed working time of the shipper if white powder is being observed on cryopucks.

6. APS Upgrade

The Advanced Photon Source has been in operation since 1995. In 2006, planning and discussions began on a facility upgrade. In 2010, the Department of Energy (DOE) approved Critical Decision 0, authorizing development of a conceptual design. Next, in 2011, the Critical Decision 1 was approved by DOE, allowing for establishment of the preliminary technical

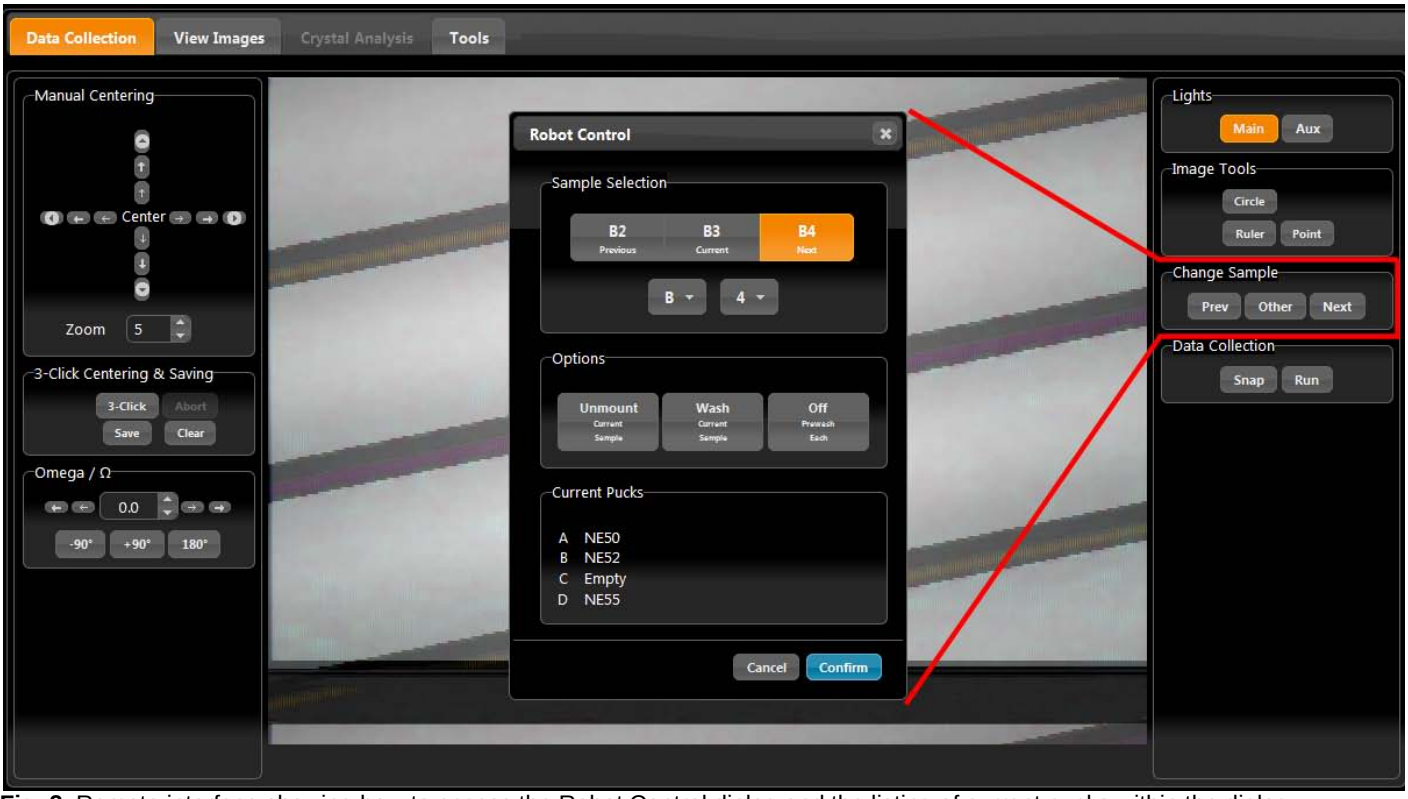

Fig. 2. Remote interface showing how to access the Robot Control dialog and the listing of current pucks within the dialog.

scope, authorizing a detailed preliminary design and initial research and development. The upgrade will increase the number of users and experiments that can be accommodated by the APS as well as the brightness of the X-ray beams. Obviously, normal operations are proceeding during the upgrade, making it non-disruptive to users.

When the upgrade is complete (currently projected for 2019), the storage ring current will increase from 100 mA to 150 mA. In preparation for this change, on the last day of the 2013-1 run, the APS performed high current studies. Similar to the studies in August 2012, participating beamlines, such as NE-CAT, were asked to provide information on the performance their high heat load (HHL) optical elements at the baseline 100 mA, as well as at 130 and 150 mA. The APS requested information such as rocking curves, first and third harmonic outputs, power loading on the monochromator including current, gap/energy, slit settings, coolant flow, and temperatures. The feedback will guide design HHL optics design during the upgrade.

7. Remote Data Collection

The web-based remote data collection interface has received some improvements during the 2013-1 run. It now shows the currently loaded pucks and their positions in the dewar. Users can see their currently loaded pucks using the 'Robot Control' dialog or by clicking the 'Other' button in the 'Change Sample' control panel (Fig. 2). Loaded pucks are listed at the bottom of the dialog with their position in the dewar. If a position does not have a puck, it will be denoted as 'Empty.' If the position has a puck that belongs to another group, then it will be denoted as 'Taken.'

By choosing the Dewars & Pucks tab, users can enter pucks (Add Puck), then load those pucks into a dewar by dragging the puck into the dewar. The names of more than one puck can be entered at the same time by simply using a comma-separated list.

8. General User Proposal Submission

As noted earlier in the newsletter, all Institutional users will be switching over the General User (GU) program over the next calendar year and each principal investigator (PI) will be required to submit a GU Proposal for his or her project. The APS GU program provides all PIs with access to beamtime. Requests for proposals are made three times a year, prior to each trimester. GU Proposals in Macromolecular Crystallography (MX) are submitted through the APS web site and sent to a pool of MX reviewers.

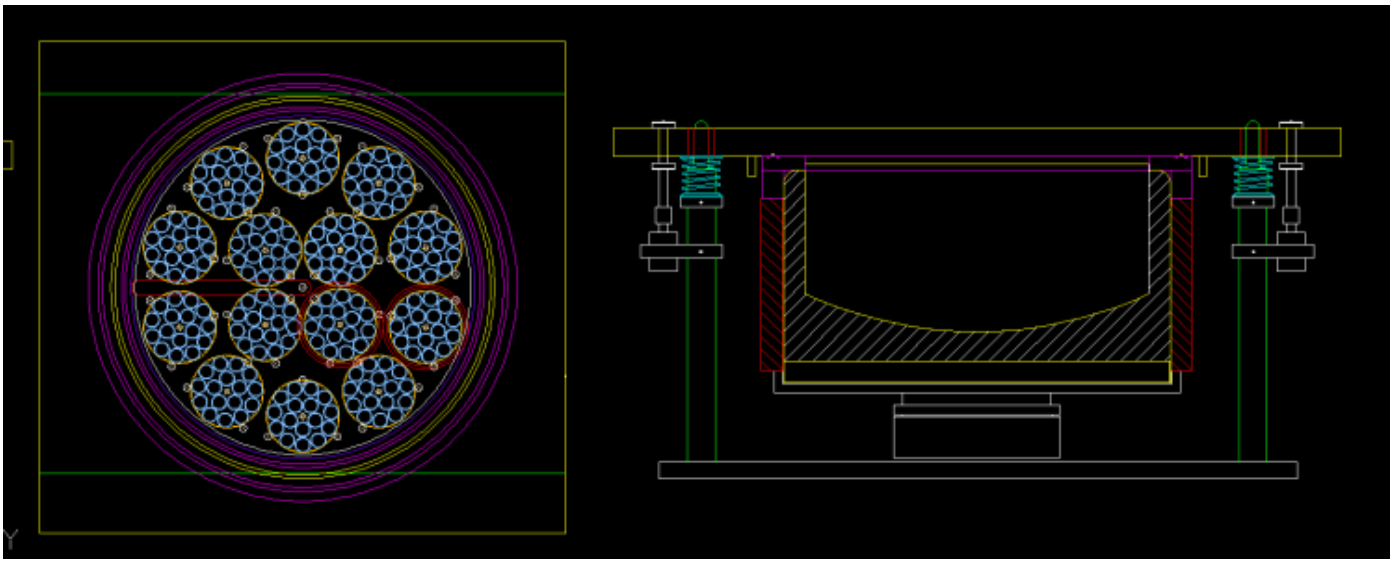

Fig. 3. CAD drawings of the new sample automounter cryopuck storage dewars.

The APS provides guidelines on submitting a GU Proposal at:

http://www.aps.anl.gov/Users/Help/Instructions/proposer_instructions.html

These are specific addendums to the APS instructions for applying for beamtime at NE-CAT.

- In the beamtime request tab, ask for a large number of shifts for life of the proposal. We recommend at least 30 or more.
- Techniques required should be general diffraction, single crystal and/or microdiffraction.
- For choice of beamline, select either 24-ID-C or 24-ID-E. Do not select any additional beamlines if you do not wish to be assigned time elsewhere at the APS.

We will send a notification to all members well in advance of this transition.

9. New Robot Dewars

Another change that is coming to NE-CAT is new, larger cryopuck storage dewars for the sample automounters (Fig. 3 & 4). Additionally, we are incorporating some design changes during the upgrade in order to improve the performance of the automounters and to allow for a higher puck capacity in a smaller footprint.

The long horizontal move from the dewar to the goniometer is currently performed by a pneumatic translator stage. This stage will be replaced by a very fast linear servo stage. The advantages of moving away from an open-loop pneumatic motor to a closed-loop servo motor include: continuous monitored feedback from speed or position sensors, adjustable distance of the actuator move, and adjustable speed and acceleration of the actuator move.

Currently, the samples are addressed in the dewar by a rotation and translation of the dewar. With the linear servo for the long horizontal move, this will be achieved by a rotation of the dewar and translation of the gripper. This will allow more efficient use of the current space and allow us to maintain the current minimum detector to sample distance.

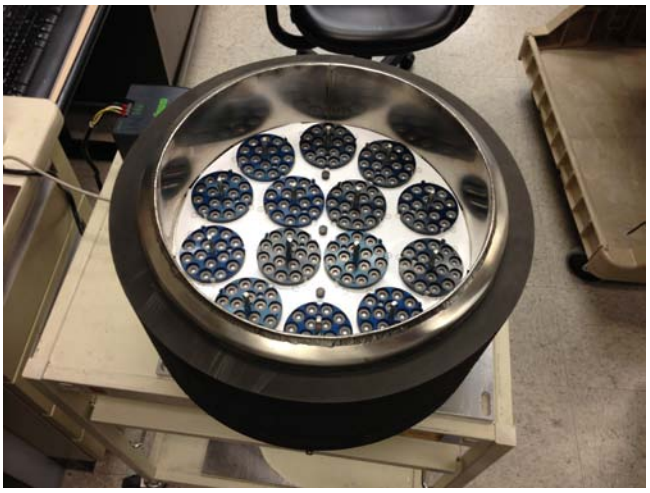

Fig. 4. New automounter storage dewar under construction in August 2013.

Research Highlights

Structural basis for how error-prone ribosomes function

Christine M. Dunham, Assistant Professor, Department of Biochemistry, Emory University School of Medicine

Crystal E. Fagan, graduate student

Jack Dunkle and Tatsuya Maehigashi, post-doctoral fellows

Biological fitness is critically dependent upon the accurate flow of genetic information from DNA to RNA to protein. Breakdown in the fidelity of translation by the ribosome is detrimental due its central role in the production of all proteins in every living organism. The bacterial ribosome is a major target for antibiotics that interfere with protein synthesis and that have critical applications in the clinic and the laboratory. Additionally, numerous human diseases are attributed to mutations in the accessory components of protein synthesis: protein translation factors, tRNAs or mRNAs. Therefore, understanding the molecular mechanisms at play in ribosome function is central to human health.

The bacterial ribosome has been extensively studied since the 1960s using genetic, biochemical and structural approaches. However, despite the wealth of information produced, many questions remain regarding ribosome selectivity, architectural arrangements, and regulation. One important outstanding question in the field is how the ribosome selects the correct (cognate) aminoacyl-transfer RNA (aa-tRNA) from the large available pool of incorrect (non-cognate and near-cognate) aa-tRNAs with both high speed (> 20 s^{-1}) and low error (≈ 10^{-4}). A delicate balance exists between speed and fidelity during the faithful decoding of messenger RNA (mRNA) by substrate tRNAs that is difficult to rationalize based solely on the small differences in stability that arise from the interaction of correct and incorrect codon-anticodon base pairs (which can differ by as little as a single hydrogen bond).

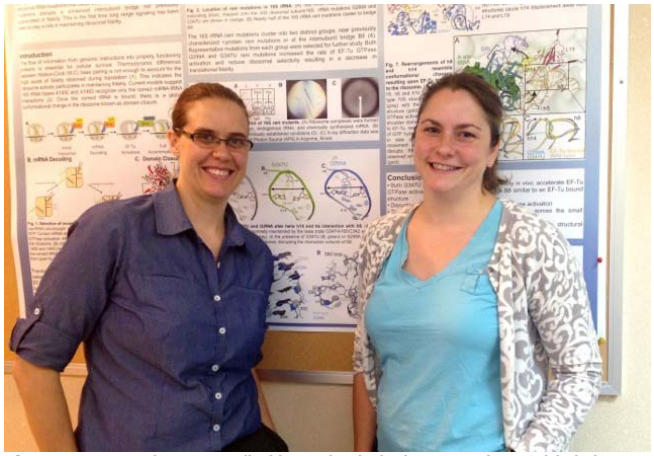

Fig. 5. Crystal Fagan (left) and Christine Dunham (right).

For each decoding event, selection of the cognate tRNA is enhanced by kinetic proofreading and induced fit mechanisms. Direct monitoring by ribosomal RNA (rRNA) of the base pairing between the codon of the mRNA and the anticodon of the tRNA preferentially allows selection of cognate interactions over non-cognate and near-cognate interactions. The accuracy of this decoding process depends in part on an induced-fit mechanism, as indicated by larger forward rate constants for the cognate substrate. For example, GTPase hydrolysis by EF-Tu and subsequent accommodation (movement) of aa-tRNA into the 50S A site are considerably faster for cognate aa-tRNA than for near-cognate aa-tRNA.

The fact that the ribosome could play a role in decoding became clear from studies with the antibiotic streptomycin. Streptomycin alters the fidelity of translation, rather than being purely a ribosome inhibitor. Streptomycin-resistant mutant bacteria were isolated that displayed increased fidelity while second site mutants that reverse the resistance were isolated that decrease the accuracy of decoding. The latter were termed ribosome ambiguity mutants (*ram*) and these mutations clustered to distinct regions, several of which lie far away from where the tRNA is decoded in the A site of the ribosome (**Fig. 6A**). The largest subset of mutations mapped to 16S rRNA helices 8 (h8) and 14 (h14), which interact with each other and with the 50S subunit to form intersubunit bridge B8, and at the shoulder domain of the small subunit (or 30S) near the decoding center. These 16S rRNA *ram* mutants increase miscoding *in vivo* and stimulate EF-Tu-dependent GTP hydrolysis *in vitro*, particularly in the near-cognate case. These data rule out a previous hypothesis that 16S h14 helps activate EF-Tu and instead, suggest that bridge B8 acts to negatively regulate GTP hydrolysis by the factor.

Structural basis of *ram* mutants

In collaboration with the Fredrick lab at The Ohio State University, we determined the structural basis of action for two disparate 16S rRNA *ram* mutants: one at the decoding center (G299A) and one at B8 (G347U) (**Fig. 6A**). Despite being located far away from each on the 70S ribosome, both mutations induce almost identical structural rearrangements in B8 bridge, that disrupt the interaction of h8 and h14 with large subunit ribosomal proteins L14 and L19. The finding that the 70S G347U mutation causes a disruption at h14 was not surprising given nucleotide G347 is located in h14. G347 is normally involved in a base triple interaction with C342 of h14 and A160 of h8, forming a Watson-Crick and Hoogsteen interaction, respectively. Our structure

reveals that the G347U mutation disrupts this base triple resulting in a widening of the entire h14 (**Fig. 6B**). This widening also causes movement away from the large ribosomal subunit, thereby preventing interactions that form B8.

G299A indirectly alters h14, also disrupting bridge B8.

The 16S rRNA G299A mutation is located in h12, near previously identified *ram* mutations at the interface of ribosomal proteins S4 and S5. G299 normally interacts with the conserved 16S rRNA 560 loop, which contains several sharp backbone turns and links the 5' and central domains of 16S rRNA. The G299A mutation causes the disruption of a single hydrogen bond with G558 but otherwise, only small changes are observed at the site of the mutation. Remarkably, however, significant structural changes in the 70S G299A structure occur ~80 Å away at B8. As in the G347U structure, contacts between h14 and L14 are abrogated as h8 and h14 shift away from the 50S subunit (**Fig. 6B**). This indicates an important role for RNA tertiary interactions between h12 and the 560 loop, which have long-range implications for h14 positioning. These data provide direct evidence that 16S rRNA helices are capable of transmitting conformational signals across the ribosome subunits. Moreover, the similar structural effects of G299A and G347U imply that they influence decoding via a common mechanism.

A unified model for *ram* action and implications for the decoding process.

A comparison of our two 70S *ram* structures with other ribosome structures revealed that our structures most closely resemble that seen in the ternary complex (EF-Tu•GTP•aminoacyl-tRNA) bound state of the 70S ribosome. This is surprising given we do not have ternary complex present in either structure and yet our structures resemble a 70S-EF-Tu 'primed' complex (**Fig. 6C**). Our biochemical and structural studies together provide evidence that disruption and/or distortion of B8 is an important aspect of GTPase activation by EF-Tu during normal decoding. We propose that, by destabilizing B8, G299A and G347U reduce the energetic cost of attaining the GTPase-activated state and thereby decrease the stringency of decoding. This previously unappreciated role for B8 in controlling the decoding process may have relevance for many other ribosomal mutations known to influence translational fidelity. These data also provide evidence that GTPase activation involves B8 disruption and reveal, for the first time, long-distance conformational signaling or allostery across the 30S.

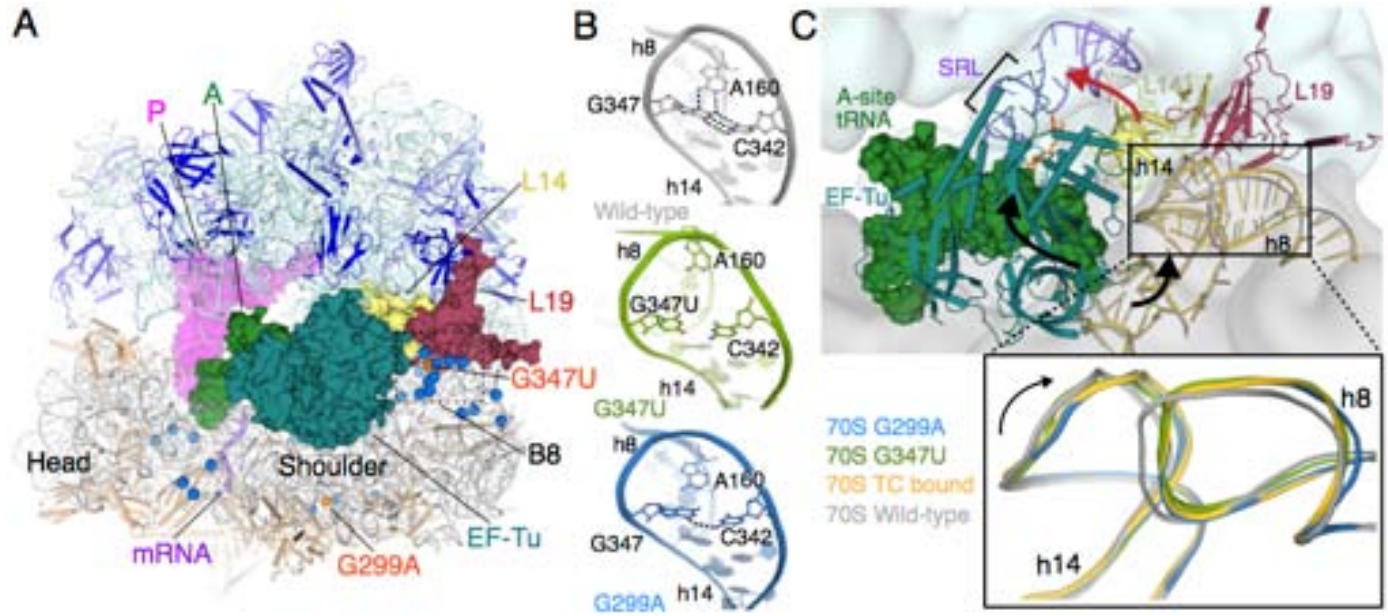

Fig. 6. Structural basis for *ram* mutations. (A) Positions of 16S rRNA mutations G299A and G347U (orange) that increase miscoding mapped onto the 70S structure (PDB 2WDG). Most of the 16S rRNA *ram* mutations isolated cluster to regions distant from the tRNA binding sites, nearly half of which map to h8/14, which contacts L14 (yellow) and L19 (maroon) to form intersubunit bridge B8 (boxed). Although mutation G347U is proximal to B8, G299A resides ~80 Å away. 16S rRNA, mRNA and r-proteins are shown as cartoon and other 30S domains are indicated. (B) Intersubunit bridge B8 is formed by contacts between h14 of the 30S subunit and L14 / L19 of the 50S subunit (top, wild-type; PDB 2WDG), many of which are lost in the mutant structures. 70S G347U (middle; green) and G299A (bottom; blue) mutations cause h14 displacement away from L14 and L19, disrupting the hydrogen bonding network between the subunits. The rRNA backbone of the wild-type 70S structure is shown for comparison (top; gray).
(C) Model for how *ram* mutations affect EF-Tu mediated GTP hydrolysis. Intersubunit B8 is shown (h8, h14, L14 and L19) with black arrows indicating previously identified structural changes that occur upon EF-Tu binding. As indicated with a red arrow, is where a new route for how B8 possibly affects GTP hydrolysis via the sarcin-ricin loop (SRL; purple). Inset shows a zoomed in view of how both 16S rRNA mutations, G299A (blue) and G347U (green) result in the movement of h8 and h14, disrupting the intersubunit B8 interaction in a similar manner as observed when TC binds to the ribosome (PDB accession code: 2XQD, gold).

Staff Activities

Meet Our Technical Support and Administrative Staff

With the funding of our grant, we have decided to take a look at the NE-CAT staffers who have been here the longest and who have proven to be indispensable to not only to our past and future goals but our day to day operations.

Leslie Kinsland, based in Ithaca, helped with submission of the initial successful P41 proposal and was the first official NE-CAT staff member. She coordinates all our P41 grant related activities. These include compiling the sections and hundreds of pages that comprise our grant proposals, yearly progress reports to NIH, and publication monitoring, reporting and compliance. Without her, NE-CAT would be overwhelmed by all the administrative duties required to keep our very large P41 grant compliant with NIH rules. Leslie and Cyndi work together as a team to coordinate various committee meetings, to keep the webpage updated and to manage all of NE-CAT's purchasing requirements.

Ed Lynch has been with NE-CAT since the beginning, over 11 years, and has been essential to the construction and operation of the beamlines. During this time, he has helped build hardware, such as the sample automounters, and customized commercial pieces to suit the unique needs of cutting edge crystallography. A skilled machinist, Ed regularly makes custom pieces for users with special requirements. He also serves as our Technical Safety Captain.

Cyndi Salbego recently celebrated 10 years of service as NE-CAT's administrator. NE-CAT users will surely recognize the vital role that Cyndi plays in the success of NE-CAT as she is their liaison not only to NE-CAT but also with APS and Argonne during the process of preparing for a trip to NE-CAT. In addition to her valuable support to users, Cyndi is busy working behind the scenes maintaining the NE-CAT website and providing administrative support to the NE-CAT staff.

Jim Withrow will celebrate 10 years with NE-CAT this fall. Jim builds and maintains all beamline electronics, computer workstation, network and electrical infrastructure. His electronic skills help us automatically monitor our incubators, cold room and safety interlocks. From failed hard drives to dead switches, Jim is on-call to fix all our electrical devices. He is also developing the controlling program for the new sample automounters. In addition, Jim is our Electrical Safety Captain.

Posters

Kay Perry, Malcolm Capel, Surajit Banerjee, Igor Kourinov, Ed Lynch, Frank Murphy, David Neau, Kanagalaghatta Rajashankar, Cynthia Salbego, Jonathan Schuermann, Narayanasami Sukumar, James Withrow & Steven E. Ealick, “NorthEastern Collaborative Access Team (NE-CAT) Beam Lines at the Advanced Photon Source” 2013 Annual Meeting of the American Crystallographic Association, Honolulu, Hawaii, July 20 – July 24, 2013.

Presentations

Kanagalaghatta Rajashankar, “Better Detector; Better Data,” Annual Meeting of the P41 Principal Investigator Meeting, “Tackling More Challenging Biomedical Research Needs With New Consortia of P41 Centers,” Rockville, MD, March 18 and 19, 2013.

Jon Schuermann, “APS Status Report,” Kappa Meeting, 2013, Lund, Sweden, April 16 and 17, 2013.

Jon Schuermann, “RAPD,” Kappa Meeting, 2013, Lund, Sweden, April 16 and 17, 2013.

Malcolm Capel, “Shutterless Vector Scanned Data Collection Methods for the Pilatus 6M,” InterCAT Technical Workgroup Meeting, APS, IL, July 18, 2013.

Publications

Apostol, M. I., **Perry, K**., and Surewicz, W. K. (2013) Crystal structure of human prion protein fragment reveals a motif for oligomer formation, *J. Am. Chem. Soc. 135*, 10202−10205.

Cantara, W. A., **Murphy, F. V. t.**, Demirci, H., and Agris, P. F. (2013) Expanded use of sense codons is regulated by modified cytidines in tRNA, *Proc. Natl. Acad. Sci. U. S. A. 110*, 10964–10969.

Galaleldeen, A., Taylor, A. B., Chen, D., **Schuermann, J. P.**, Holloway, S. P., Hou, S., Gong, S., Zhong, G., and Hart, P. J. (2013) Structure of the *Chlamydia trachomatis* Immunodominant Antigen Pgp3, *J. Biol. Chem. 288*, 22068–22079.

Duda, D. M., Olszewski, J. L., **Schuermann, J. P., Kurinov, I.**, Miller, D. J., Nourse, A., Alpi, A. F., and Schulman, B. A. (2013) Structure of HHARI, a RING-IBR-RING Ubiquitin Ligase: Autoinhibition of an Ariadne-Family E3 and Insights into Ligation Mechanism, *Structure 21*, 1030-1041.

Wan, L. C., Mao, D. Y., Neculai, D., Strecker, J., Chiovitti, D., **Kurinov, I.,** Poda, G., Thevakumaran, N., Yuan, F., Szilard, R. K., Lissina, E., Nislow, C., Caudy, A. A., Durocher, D., and Sicheri, F. (2013) Reconstitution and characterization of eukaryotic N6-threonylcarbamoylation of tRNA using a minimal enzyme system, *Nucleic Acids Res*. *41*, 6332-6346.

Yu, X., Seegar, T. C., Dalton, A. C., Tzvetkova-Robev, D., Goldgur, Y., **Rajashankar, K. R.**, Nikolov, D. B., and Barton, W. A. (2013) Structural basis for angiopoietin-1-mediated signaling initiation, *Proc. Natl. Acad. Sci. U. S. A.* 110, 7205-7210.

Sartmatova, D., Nash, T., Schormann, N., Nuth, M., Ricciardi, R., **Banerjee, S.**, and Chattopadhyay, D. (2013) Crystallization and preliminary X-ray diffraction analysis of three recombinant mutants of Vaccinia virus uracil DNA glycosylase, *Acta Crystallogr. F 69*, 295-301.

Broussard, T. C., Kobe, M. J., Pakhomova, S., **Neau, D. B.**, Price, A. E., Champion, T. S., and Waldrop, G. L. (2013) The Three-Dimensional Structure of the Biotin Carboxylase-Biotin Carboxyl Carrier Protein Complex of E. coli Acetyl-CoA Carboxylase, *Structure 21*, 650-657.

Sukumar, N. (2013) Crystallographic studies on B12 binding proteins in eukaryotes and prokaryotes, *Biochimie 95*, 976-988.

Demirci, H., **Murphy IV, F.**, Murphy, E., Gregory, S. T., Dahlberg, A. E., and Jogl, G. (2013) A structural basis for streptomycin-induced misreading of the genetic code, *Nat. Commun. 4*, 1355.

Ketkar, A., Zafar, M. K., Maddukuri, L., Yamanaka, K., **Banerjee, S.**, Egli, M., Choi, J. Y., Lloyd, R. S., and Eoff, R. L. (2013) Leukotriene Biosynthesis Inhibitor MK886 Impedes DNA Polymerase Activity, *Chem. Res. Toxicol. 26*, 221-232.

Chae, P. S., Rana, R. R., Gotfryd, K., Rasmussen, S. G., Kruse, A. C., Cho, K. H., Capaldi, S., Carlsson, E., Kobilka, B., Loland, C. J., Gether, U., **Banerjee, S.**, Byrne, B., Lee, J. K., and Gellman, S. H. (2013) Glucose-Neopentyl Glycol (GNG) amphiphiles for membrane protein study, *Chem. Commun. (Camb.) 49,* 2261-2372.

Committee Participation

Malcolm Capel, ABBIX Preliminary Beamline Design Review, Upton, New York, March 22, 2013.

Malcolm Capel, NSLS-II Lab Director’s Beamline Review, Upton, New York, April 4, 2013.

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