



NE-CAT COMMUNICATIONS



National Center for
Research Resources

A Biannual Newsletter of the Northeastern Collaborative Access Team Winter 2009

A Message from the Director Steven Ealick



Welcome to the latest edition of NE-CAT Communications, our biannual newsletter. NE-CAT concludes 2009 very successfully with excellent scientific productivity and improvements in all areas. The installation of an improved ALS-style sample automounter on the 24-ID-E monochromatic beamline is complete and the improvements have received rave reviews from

users. We have also retrofit the 24-ID-C variable energy beamline with a second generation automounter in order to take advantage of the many improvements that will be detailed later in this newsletter.

In the last newsletter, I mentioned that based on the first half of the year, we expected to see an increase in overall publications in 2009. This is indeed true. We ended the year with 95 publications, an increase of 42% over last year. Out of 95 publications credited to NE-CAT resources, 16 publications named NE-CAT staffers as co-authors. I commend NE-CAT staffers for remaining active scientists and contributing extensively to current research. Also, NE-CAT now has over 500 PDB deposits with 186 PDB entries in 2009. I would like to thank all users for making the best use of NE-CAT facilities and keeping us highly productive.

NE-CAT applied to NCRP and received an administrative supplement in October 2009. This funding was made available through the stimulus bill approved by Congress earlier in the year. This funding will allow NE-CAT to enhance the support of the sample automounters and automated data processing. NE-CAT has advertised for two new positions, one a postdoctoral research associate and the other a computer programmer. The postdoctoral research associate will facilitate NE-CAT's beamline sample automounter program, including upgrades and maintenance of the existing systems. The computer programmer will aid in development of software for X-ray crystallographic data collection and processing. Current efforts in this area will be detailed later in this newsletter. We have interviewed candidates for the postdoctoral research associate position and the new hire will be joining NE-CAT in May. The search for a computer programmer remains

open. If you know any potential computer programmers with an interest in scientific programming, please direct them to the NE-CAT employment opportunities website at <http://necat.chem.cornell.edu/aboutus/Organization/Employment.htm>.

The administrative supplement will also allow NE-CAT to purchase a new 8-node computer cluster that will enable faster data processing and structure solution. The funding will also allow purchase of spares for critical components to the beamline infrastructure and the MD-2 microdiffractometers. Both instruments are in heavy use and this will eliminate down time in the case of hardware failure.

I would also like to congratulate Executive Committee member Tom Steitz on his Nobel Prize in Chemistry for studies of the structure and function of the ribosome. A detailed description of his seminal work in the field will be detailed later in this newsletter.

If you have not taken the opportunity to use NE-CAT's beamlines to date, I encourage you to do so in the future. For further information on our beamline capabilities and how to request time, please visit our website at <http://necat.chem.cornell.edu>.

Beamline Developments

1. Automounters

During the September shutdown, NE-CAT installed the advanced version of the cryogenic sample automounter on 24-ID-E and during the January shutdown, the old sample automounter on 24-ID-C was removed and replaced with a second generation automounter. Several modifications were made to the original ALS design of the cryogenic sample automounter to make it more robust and user friendly. These modifications include:

- a gripper heater that warms the entire gripper and not just the base, thereby reducing moisture condensation after mount,
- an angle change to the blower to prevent the need for an air shield between the sample and the automounter during gripper de-icing,
- an improved, transparent, monolithic Lexan Dewar lid providing better visibility of the pucks/samples and reduction of long-term ice build-up

inside the Dewar,

- better illumination of the puck storage Dewar for easier sample inspection and handling,
- more fans above and below the lid to reduce icing of the puck storage Dewar,
- a felt ring around the mouth of the Dewar to prevent moist air infiltration,
- taller posts inside the puck storage Dewar to aid puck loading,
- all electrical components have been moved from the back of automounter to an easy to access box (as seen in the photo) to enhance servicing
- and, most significantly, the addition of the compliance slide increases tolerance of gripper misalignment, negating gripper bending and allowing



Figure 1. The illuminated cryopuck storage Dewar with its transparent monolithic lid.

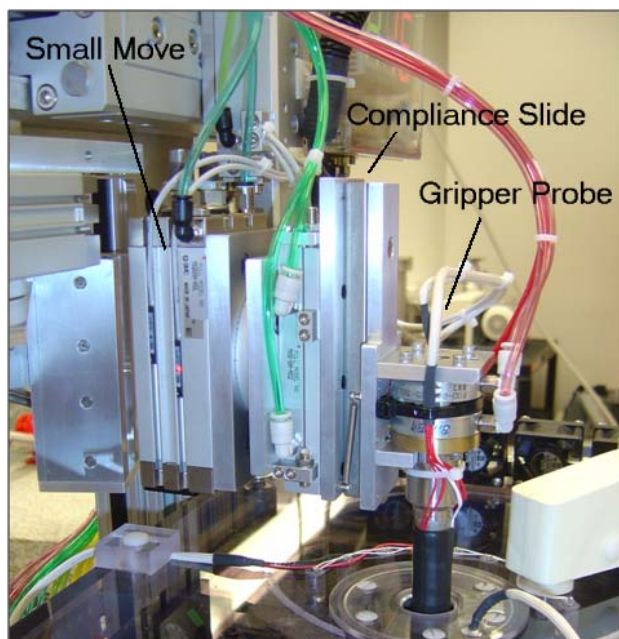


Figure 2. Location of the new compliance slide.

rapid adjustment of the gripper.

With the addition of the automounter to 24-ID-E, the beamline control software was also upgraded. From the user's perspective, a more streamlined, tabbed interface on 24-ID-E now matches the control interface found on 24-ID-C. Users familiar with either beamline should be able to easily switch from one beamline to the other with minimal training. The automounter was in use during the 2009-3 user run, but the sample cycling time on 24-ID-E was three times slower than on 24-ID-C due to a slow lift motor for the MD2 capillary and beamstop. This motor was replaced with a new, faster motor during the January 2010 maintenance shutdown.

2. Base Thawing Accessory

During the 2009-3 run, it was frequently observed that samples would move after sample alignment had been completed. This movement was attributed to the thawing of ice between the sample base and the goniometer. In order to speed up the rate of thaw, an accessory was built into the sample detector unit which blows air onto the frozen base. The time required for base thawing has been significantly reduced and bases are able to reach room temperature prior to initiation of sample alignment. As a side benefit, use of the accessory is accompanied by a decrease in temperature near the sample suggesting that samples are more cryogenically-protected when the base thawing accessory is in use.

3. Automatic Data Processing

During the 2009-2 run, NE-CAT introduced the EDNA pilot project. EDNA (<http://www.edna-site.org/>) is an environment for ODA (Online Data Analysis) geared to-

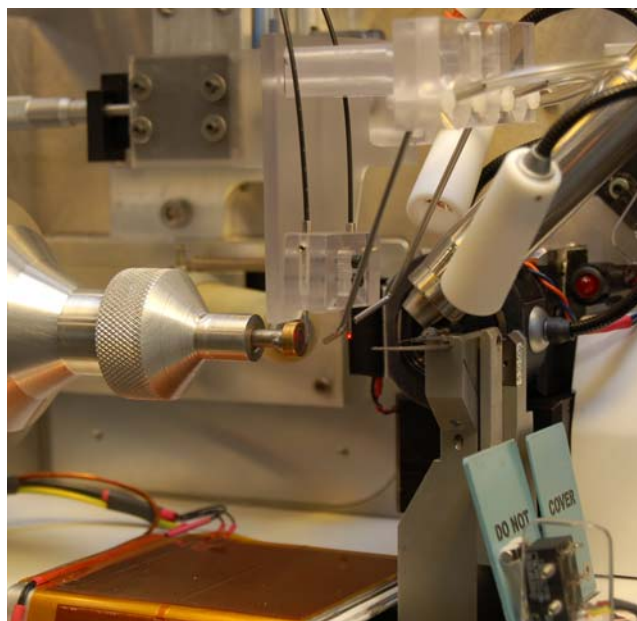


Figure 3. Base-thawing accessory warms the base and prevents sample movement during alignment.

ward the automation of the collection and processing of X-ray diffraction data from macromolecular crystals. EDNA is developed by a European collaboration supported by the BIOXHIT foundation (*J. Synchrotron Rad.* (2009), 16, 872-879). Malcolm Capel was introduced to EDNA in March 2009 when he travelled to the European Synchrotron Radiation Facility and the European Molecular Biology Laboratory (ESRF-EMBL) in March and gave a seminar on the "Installation & Development of the MD2 Microdiffractometer on NE-CAT Undulator Lines." It was decided to test EDNA at NE-CAT to determine if it was suitable as a starting point for optimal data collection, automated data analysis and prediction of data collection strategies minimizing radiation damage. Python-based software was written to interface EDNA with beamline software. A browser-based viewer for EDNA results was also developed.

The EDNA pilot project monitored image snapshots and provided data collection strategies based on a pipeline of MOSFLM, RADDPOSE and BEST. Users were able to see individual snapshots with spot predictions, space group estimates and a data collection strategy. Predicted strategies allowed extremely efficient data collection, but did not take into account radiation damage. However, it became evident that the existing strategy was not sufficiently complex to deal with the difficult crystallographic problems common to users of NE-CAT. Incorrect determination of space group and inability to compute a data collection strategy occurred in a sufficient number of samples that NE-CAT has decided to implement a custom ODA. Still, the experience with EDNA has shown users and staff the many benefits automated data analysis can provide, especially in situations where data collection is faster than data processing.

4. Beamline Control Software

Due to the complexity of the beamline hardware and software control routines, it was often difficult for users to re-start software in the event of a server failure. Therefore, a routine for automatic reconnection of servers upon disconnect was established in November to simplify user and staff interventions in control network operations. All 16 Console client classes were modified to permit automatic reconnection to the appropriate Console server in event of server failures or restart. The scripting engine manages a data base containing parameters for regenerating client-server connections in event of transaction failure. The scripts will stall at the point of failure until reconnection succeeds. The point of failure or netid of the failed server will be provided in the diagnostic window. This greatly simplifies startup of beamline software.

Use of NE-CAT Beamlines

As can be seen in Figure 4, both NE-CAT beamlines were busy during the last run in 2009 with no un-scheduled time. General users received close to 50%

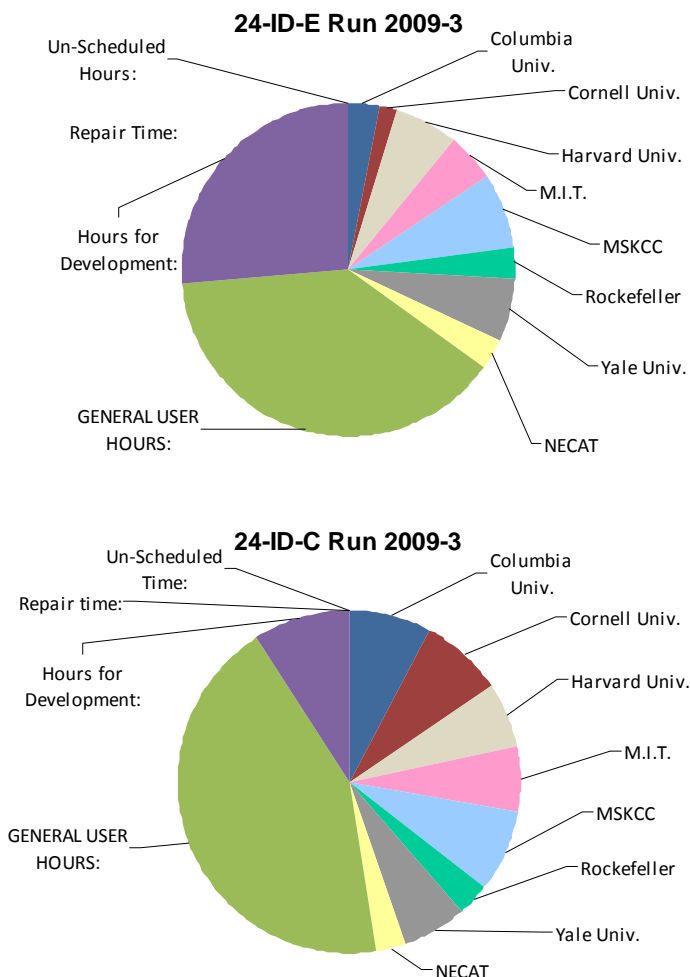


Figure 4. Pie charts showing distribution of scheduled time.

Robot Use at 24-ID Beamlines 2009-2 & 3

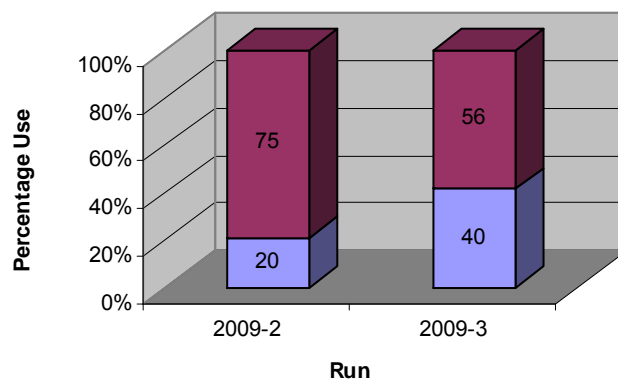


Figure 5. Comparison of Automounter Use During 2009-2 and 2009-3 runs.

of the available non-development time on 24-ID-C with a division of 47% of time going to general users and 53% going to institutional users. The reverse is true on 24-ID-E. After taking into account the hefty amount of developmental time (26%) required for installation and commissioning of the second generation sample automounter on 24-ID-E, 53% of the remaining available time was given to general users, while 47% was allotted to institutional users. Overall, the goal of splitting time between general users and CAT members was achieved for 2009-3.

With the full subscription of both beamlines, maximal use of allotted time increases in importance. As a result, an increasing number of users are also using automation to allow them to test more samples. A comparison of the 2009-2 and 2009-3 runs (Fig. 5) shows an overall increase from 21% use of the sample automounter to 42%. Though the 24-ID-E beamline had a shortened run during 2009-3, 43% of its users chose to use the second generation automounter for at least part of their visit to NE-CAT. Robot use on 24-ID-C only increased 2% between the 2009-2 and 2009-3, rising from 39% to 41%.

NE-CAT currently maintains two complete sets (14) ALS-style cryo-pucks on-site as well as appropriate tools for loading cryo-pucks. Users without cryo-pucks who wish to explore use of the sample automounter can contact a staff member about loading cryo-pucks on-site prior to the start of their beamtime. Currently, NE-CAT does not loan tools and cryo-pucks to users for use at home or other locations. However, funds from the administrative supplement will be used to acquire new cryo-pucks and tools for a loan program. The new post-doctoral research associate will be in charge of this program.

Research Highlights



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NE-CAT Executive Committee Member, Thomas Steitz Wins Nobel Prize

In 2009, the Nobel Committee awarded the prize in Chemistry to Thomas A. Steitz, a Howard Hughes Medical Institute investigator at Yale University, Venkatraman Ramakrishnan of the Medical Research Council Laboratory of Molecular Biology, and Ada E. Yonath of the Weizmann Institute of Science for crystallographic determination of ribosome structures at high-resolution and the use of these structures for atomic level clarification of how the ribosome

links amino acids to proteins and of the working principles of antibiotics attacking the ribosomes of bacterial pathogens. NE-CAT Executive Committee member, Thomas Steitz was awarded his third of the prize for two pieces of seminal work in the field of ribosomal structure and function. First, his 1998 publication in *Cell*, "A 9 Å resolution X-ray crystallographic map of the large ribosomal subunit," which solved the phase problem for the large ribosomal subunit. It is also the first ribosomal crystal structure. This research paved the way for crystallographic studies of large and small ribosomal subunits at atomic resolution. Second, his pair of publications in *Science* in 2000, "The complete atomic structure of the large ribosomal subunit at 2.4 Å resolution," and "The Structural Basis of Ribosome Activity in Peptide Bond Synthesis," which led to atomic level understanding of how formation of peptide bonds between amino acids is catalyzed on the ribosome. The structure showed the surprising interpenetration of the RNA tertiary structure by long strands of polypeptide emanating from globular proteins bound to the ribosome exterior. Moreover, with the precept that structure alone cannot illuminate catalytic function, the structure of the ribosome was determined in complex with substrate and inhibitors to demonstrate that the catalytic site of the ribosome is devoid of protein and thus peptidyl transfer occurs solely via RNA. Thus, the ribosome acts as a ribozyme.



Molecular Architecture of the Nuclear Pore Complex

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Exchange of molecules between the nucleus and the cytoplasm occurs exclusively through the nuclear pore complex (NPC), a gigantic protein assembly of 40-60 MDa that resides in circular openings in the double-layered nuclear envelope of the eukaryotic cell. The NPC is built in a highly modular fashion from its ~ 500 proteins (also called nucleoporins or nups), with stable components forming an architectural scaffold to which more dynamic, accessory factors are recruited. The central cavity of the NPC is filled with a gel-like matrix composed of fibrous extensions characteristic of a subset of nucleoporins. This matrix forms the main permeability barrier, and transport across it is facilitated by an ensemble of soluble nuclear transport factors. Transport against a protein concentration gradient is fueled and coordinated by the highly conserved small G protein Ran.

My lab has pioneered studies toward solving the structure of the NPC at high resolution using X-ray crystallography as the main tool. We exploit the fact that the NPC

is organized into subcomplexes of only a few nucleoporins and less than 1 MDa each, which are then amenable to detailed structural studies. For reasons of sheer size and dynamics, the native NPC appears to be out of reach for crystallography for the foreseeable future. However, reconstructing a composite NPC structure from its subcomplexes emerges now as a promising approach. We have used the NE-CAT beamlines extensively over the past five years, and have enjoyed the excellent user support and beam characteristics, including the microfocus setup. Installation of robotic sample mounting significantly accelerated the process of structure determination, for us especially since we entirely rely on crystal screening at the beamline.

One of the two main architectural building blocks of the NPC is the 575 kDa heteroheptameric Nup84 complex¹. It forms a branched, Y-shaped structure ('Y-complex'), as determined by electron micrographs of the complex reconstituted from recombinant proteins. Over the past five years, we have solved structures of the majority of the Y-complex²⁻⁶. These structures reveal that the arms of the Y are essentially constructed from stacked helical domains that interact with very high affinity, decorated with four β -propeller domains. The β -propellers likely form protein-protein interaction sites to assemble the Y-complex into a higher-order structure, but it is yet unclear how this is accomplished. We were able to establish that three of the five stacked helical domains share a common architecture with the scaffold protein Sec31 and Nup93/Nic96⁷ – surprisingly, since the sequence identity between the domains is exceedingly low. Sec31 together with Sec13, which is also part of the Y-complex, forms the outer coat of the COPII vesicle. We termed this helical domain the ancestral coatomer element 1, ACE1 (ref. 3). In our most recent study published in *Nature Structural and Molecular Biology* we were able to show that the neighboring ACE1 proteins Nup145C and Nup84 heterodimerize in a similar fashion as Sec31 homodimerizes in the COPII coat⁶. Thus, we established that the NPC and the COPII coat not only share similar architectural elements, but that they also share similar joints. Even though it is still unclear how the entire NPC will come together, it is evident that it is constructed in a lattice-like fashion with elements that also occur in other membrane coats, notably COPII.

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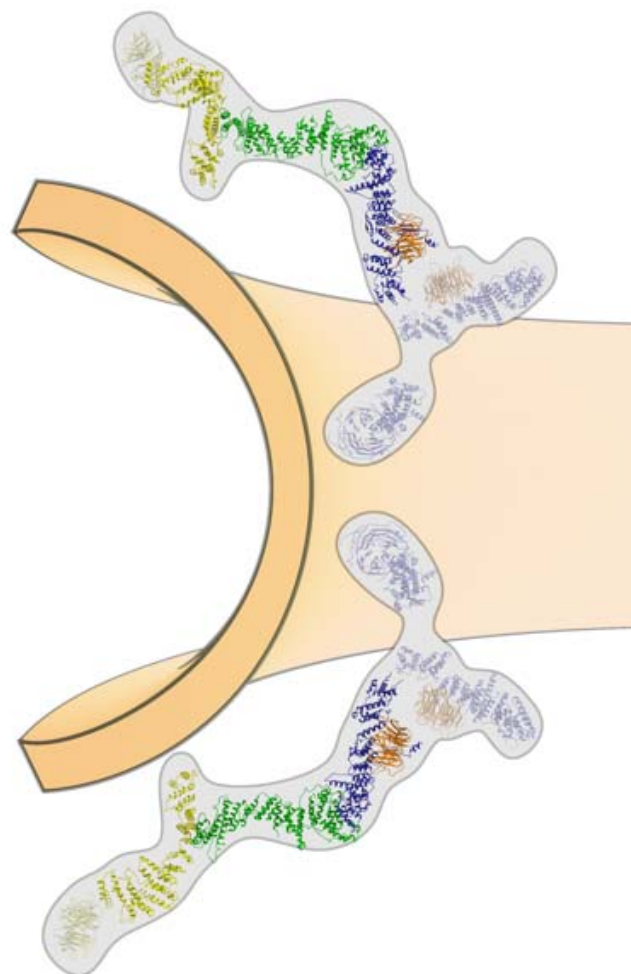


Fig. Tentative placement of the Y-complex with respect to the circular opening in the nuclear envelope⁶, the site where the nuclear pore complex is located.

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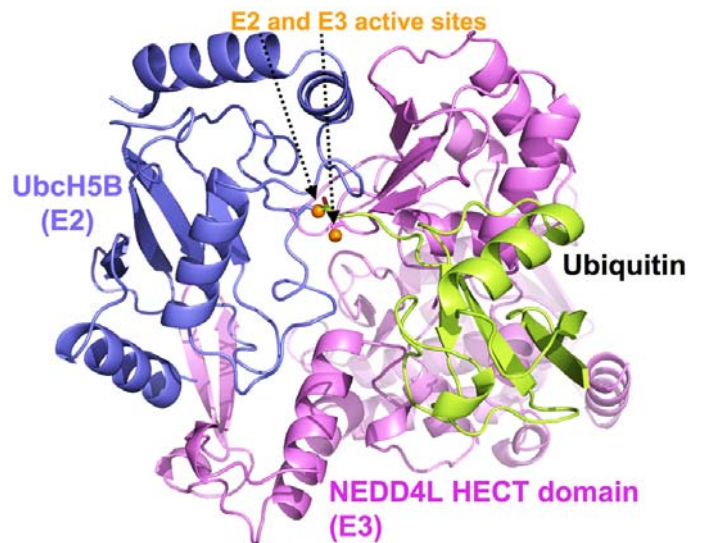
Insights into ubiquitin transfer cascades from a structure of a Ubch5B~Ubiquitin-HECT^{NEDD4L} complex

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Posttranslational modifications by ubiquitin (Ub) serve as important mechanisms of modulating stability, localization, and function of cellular proteins. Ubiquitin is covalently attached to target proteins through a hierarchical enzymatic cascade typically involving E1, E2, and E3 enzymes. During this cascade, the Ub C-terminus becomes covalently attached to a catalytic Cys of E2 through a thioester bond resulting in a labile E2~Ub intermediate. In cascades involving HECT-class E3s, a catalytic Cys in HECT E3 attacks the thioester linkage in E2~Ub. Ub is transferred from the E2 Cys to the HECT Cys resulting in a thioester-linked E3~Ub intermediate. Ultimately, Ub is transferred from the HECT E3 Cys to a target protein.

Despite a wealth of structural data on E2s and the catalytic domains of HECT E3s, a fundamental question remained: How do E2 and HECT E3 active sites approach each other to facilitate Ub transfer between them? To gain insight into this process, we crystallized an E2~Ub (Ubch5B~Ub) covalent intermediate bound to the catalytic domain of a HECT E3 family member, NEDD4L. We were able to trap this complex by 1) by generating a stable serine-linked Ubch5B~Ub oxyester mimic of the natural thioester intermediate, and 2) by replacing NEDD4L's catalytic cysteine with serine or alanine, which negates the HECT E3 attacking the E2~Ub intermediate. Using the beamlines at NECAT, we collected diffraction data on crystals of a complex to 3.3 Å resolution.

The crystal structure reveals combinatorial interactions between individual components of the Ubch5B~Ub intermediate (i.e. Ubch5B and Ub) and two flexibly-tethered lobes of the NEDD4L HECT domain. Most importantly, the complex adopts a compact architecture that brings the E2 and E3 catalytic centers into close



proximity, with the Ub C-terminus in between them. This architecture is facilitated by HECT domain interactions with both the E2 and Ub. Indeed, mutational analyses confirm the broad importance of the structurally-observed interactions for Ub transfer from Ubch5B to members of the NEDD4L family of HECT E3s. Thus, through critical support from NECAT, we were able to gain insights into an important step in the ubiquitination cascade. Continuing support by the NECAT beamlines will be critical for achieving the next ambitious goal: a structure of a full-length HECT E3~Ub complex caught in the act of transferring Ub to a substrate.

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Contributors to Ubch5B~Ubiquitin-HECTNEDD4L complex structure shown left to right: Hari Kamadurai, Judith Souphron, Danny Scott, David Duda, Brenda Schulman

Staff Activities

Publications

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