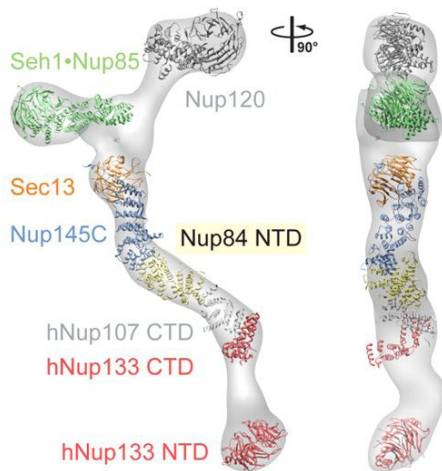


SMALL MATTERS

COLD NEWS FROM THE CRYOEM FACILITY @ NYSBC

USER HIGHLIGHT: MARTIN KAMPMANN



The Blobel group at Rockefeller published a pair of structural papers studying NUP84, a component of the nuclear pore complex. The nuclear pore complex is a ~ 50 MDa assembly responsible for nucleocytoplasmic transport in all eukaryotes. NUP84 is a membrane associated Y-shaped complex composed of seven proteins with a total mass of 587 kDa. The first paper (Kampmann and Blobel, Nat Struct Mol Biol 16, 782-8) described conformational analysis and 3D reconstruction of NUP84, while the second (Nagy et al, PNAS 106, 17693-8) described docking of the atomic coordinates of a subcomplex into the EM map. (Fig 1)

Following purification, NUP84 was deposited on carbon coated grids and negatively

stained with uranyl formate. The expertise of Ruben Diaz at NYSBC was essential for obtaining well stained complexes and the images were acquired at NYSBC on the JEOL 2100 microscope. SerialEM software was used for semi-automated image collection, as pairs of images were collected, for random conical tilt, one at 50 degree tilt and a second at 0 degree tilt.

The particles tended to land on the grid with a preferred orientation, and over 9000 pairs of particles were picked using the jweb program from the SPIDER suite. Particles were aligned and classified into 90 groups using reference-free techniques. Analysis of the class averages of the untilted particles revealed a large

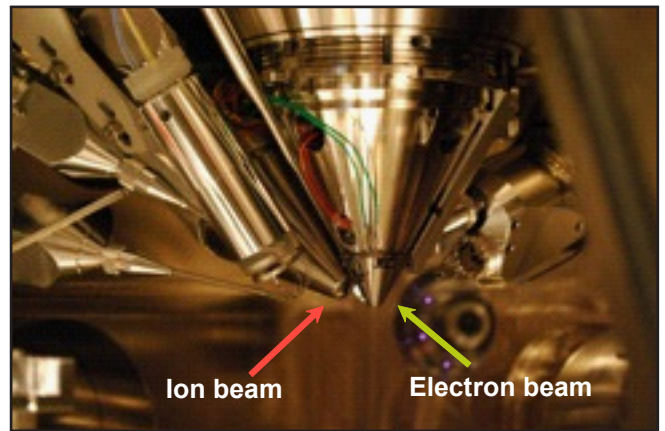
INSTRUMENTATION: DUAL BEAM SCANNING MICROSCOPE

NYSBC has been awarded an instrumentation grant to purchase a dual beam scanning electron microscope which will extend the capabilities currently offered to our affiliated researchers. To obtain 3D reconstructions of tissue, we currently employ electron tomography, which involves collecting images of the sample while systematically tilting it through a wide range of angles. However, section thickness is limited to be <500nm, due to the strong interaction between electrons and matter. In order to obtain larger scale reconstructions, tomograms from serial sections can be stacked up, but this process is tedious and prone to human error in collecting and handling the sections.

The new microscope will facilitate an alternative, which involves using a scanning electron microscope (SEM) to image the surface of a block of tissue and then to use a gallium ion beam to abrade this surface to reveal a

new surface for imaging. After numerous iterations of this cycle, the set of images can be aligned and stacked to produce a 3D data set that spans many microns in every dimension.

This imaging process is automated and there is no limit, in principle, to the size of the volume that can be produced. Although, the resolution of the SEM images is lower than the corresponding TEM images, they are suitable for tracking membranes and organelles within the tissue, thus opening up many new possibilities to study tissue organization and intercellular interactions. This brings a new dimension to our efforts to



Ion beam

Electron beam

span the information gap between X-ray and NMR and the various light microscopies.

Neurobiology will likely represent a major application, as investigators seek to map the neural connections within the brain. The long bodies of axons with frequent branching of

SERVICES: TOMOGRAMS ON DEMAND

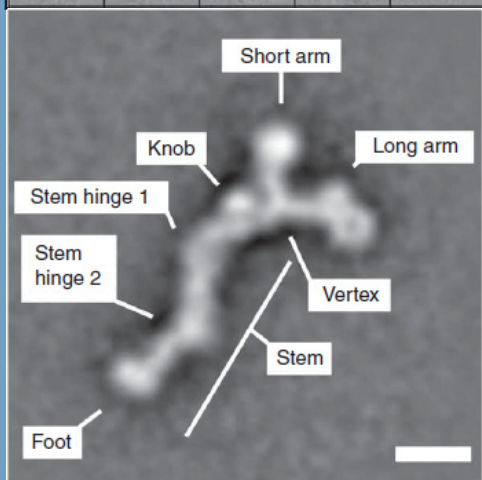
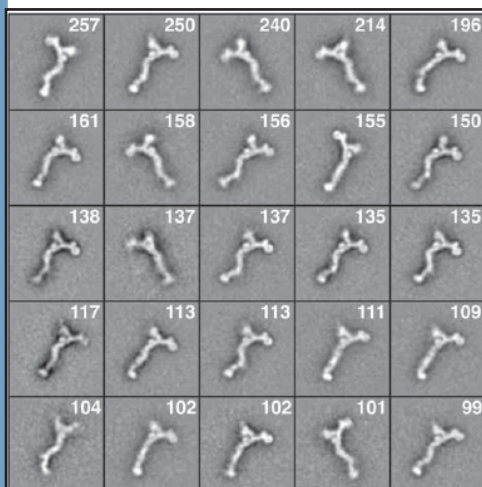
Software for calculating tomographic reconstructions from tilt series has been installed and running on the computing cluster at NYSBC for quite some time. One of the packages in use is Protomo by Hanspeter Winkler. This software is unique in that it does a very good alignment and reconstruction without the need for fiducial markers. We have a set of scripts that automate nearly every step, so we are now able to offer reconstruction of data sets collected at your home institution. If you are interested in using this service, please contact Bill Rice (rice@nysbc.org). Of course, we are also happy for you to come here to collect the data on one of our microscopes.

UPCOMING JOURNAL CLUBS

6 MAY - 4-6PM Fabiana Renzi (Mount Sinai) presents "FREALIGN: High resolution refinement of single particle structures" Grigorieff (J Struct Biol 2007)

20 MAY 2010 - 4-6PM - Ruben Diaz (NYSBC) Comparison of Available Dual Beam Technologies

NUCLEAR PORE COMPLEX (cont.)



number of different conformations. Figure 2a shows a montage of several class averages, and figure 2b shows a detailed view of one of the well defined classes. This analysis demonstrates how calculation 2D averages can yield insights into protein flexibility, even before calculating a 3D reconstruction.

For random conical tilt untilted particles are aligned and classified into groups, and a 3D reconstruction is made from each group. Because the particles have a random orientation on the grid, tilting produces a set of many different views which permits 3D reconstruction. Scripts for calculating structures using this technique are available at NYSBC and are documented on our website. Following refinement, final maps were calculated at 35Å resolution. This resolution allowed the overall shape of the complex to be seen, and also allowed docking of high-resolution structures of the components.

CUNY CONSTRUCTION UPDATE

We are operating on our normal schedule of 9am-6pm, Monday -Friday. We continue to run monthly resolution tests to monitor for potential disruptions. A brief period of disruption is anticipated after the removal of the pile of dirt behind the center. The pile was scheduled to be cleared in October 2009. I think it is safe to say they are behind schedule.

PREVIOUS JOURNAL CLUBS

* *22 Apr 2010 - SachaDeCarlo presented "The resolution dependence of optimal exposures in liquid nitrogen temperature electron cryomicroscopy of catalase crystals" by Baker et.al. *J Struct Biol* *169* 431-37 (2010)

* *08 Apr 2010 - KdDerr Presented "High-pressure cooling of protein crystals without cryoprotectants" by Kim et. al. *Acta Cryst* (2005) D61, 881-890

* *25 Mar 2010 - PaulGottlieb presented "Cryo-EM Model of the Bullet-Shaped Vesicular Stomatitis Virus" by Peng Ge, et al. *Science* *327*, 689 (2010)

* *04 Mar 2010 Bill Rice Presented "Optimal Determination of Particle Orientation, Absolute Hand, and Contrast Loss in Single-particle Electron Cryomicroscopy" by Peter Rosenthal and Richard Henderson

NEW DEVELOPMENT: AFFINITY GRIDS

Affinity Grids were developed in Tom Waltz's lab at the Harvard Medical School (Kelly et. al., *J Mol Biol* 382, 423-433). These grids are based on a carbon film which is coated with a monolayer of Ni-NTA lipids, thus providing a rapid technique to produce purified specimens for single-particle electron microscopy.

The allure of these grids is that they combine protein purification with EM sample preparation is a single step. Faster sample preparation reduces the opportunity for labile macromolecular complexes to

degrade. Furthermore, binding to the substrate keeps the complexes away from the air/water interface where they are likely to denature. Because these grids also concentrate His-tagged samples, they are ideal for macromolecular complexes with limited expression levels or low concentrations.

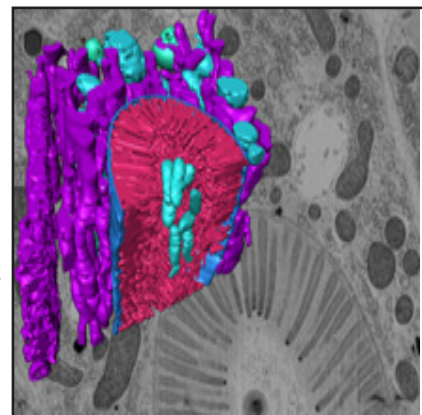
At the NYSBC, we have started experimenting with Affinity grids. We are interested in testing them on a variety of samples. Please Contact KD Derr (kderr@nysbc.org) if you are interested in trying Affinity grids with your samples.

DUAL BEAM (cont.)

spines make neural connections particularly challenging to characterize. In fact, this new instrument will be useful in characterizing any intercellular interface, the topology of membrane systems or the distribution of organelles.

We also plan to use this instrument to prepare frozen, unstained cells for imaging by cryo-tomography. Although ultramicrotomy is routine for fixed, embedded tissue, cryo-ultramicrotomy produces a range of artifacts and is notoriously difficult to apply. This dual beam microscope will enable preparation of thin, frozen-hydrated samples that are free from distortions and that preserve the native molecular structures within the cell.

to quantitate electron scattering from freeze-dried samples, which has been shown to be proportional to the mass of the complexes. This information will help us assess the homogeneity of preparations meant for single-particle analysis and help in the interpretation of their 3D structures.



Finally, the instrument will be equipped with a detector that will allow us to determine the molecular mass of macromolecular complexes. Specifically, a STEM detector will allow us

Needless to say, we are very excited about all these new opportunities and hope to take delivery of the instrument during the fall of 2010.

NEXT ISSUE

Stay tuned for next issue when we introduce you to JOHN HENRY, the sample insertion robot on the JEOL 1230. John Henry was developed in conjunction with JKD Instruments. He is one part of our high throughput membrane protein crystallization lab.

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