Cryo-EM Imaging of DNA-PK Damage Repair Complexes
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Please briefly (16000 chars or less) summarize your most recent results to date:

Our major accomplishment in 2004 was demonstrating that cryo-electron micrographs collected on the FEI TF30 Polara microscope (300kV, FEG, liquid helium and nitrogen temperature) and Gatan UltraScan 4000 (4kx4k) CCD camera at Vanderbilt University are suitable for 3D reconstructions at subnanometer (<1 nm) resolution. This was demonstrated with an icosahedral virus (adenovirus) also studied in my lab. Achieving this goal required extensive work on the part of Dr. Mike Lewis, a senior research specialist, in developing new grid preparation techniques with the Vitrobot, an automated plunge freezing device, and in optimizing the alignments and data collection parameters on the Polara microscope. We found that in order to enhance the high resolution (<1 nm) information in digitally collected micrographs, it is important to collect images at high magnification (>200,000 X) and then to bin (or average adjacent pixels) in the images. Collecting images at high magnification partially overcomes the limitations of the CCD camera, which has a less than ideal detection quantum efficiency (DQE), at high spatial frequencies. In 2004 we also installed the Leginon software package, developed by the National Resource for Automated Molecular Microscopy at Scripps, for semi-automated data acquisition and we found that Leginon improves our data acquisition efficiency by a factor of two.

Our collaborator on the DNA-PKcs project, Dr. David Chen, moved from the Lawrence Berkeley National Laboratory to the University of Texas Southwestern Medical Center in 2004. During this transition his lab was unable to provide us with DNA-PKcs samples. Working with an old sample that we had stored in the freezer, we were able to work out cryo-grid preparation methods for this sample. In March 2005, Dr. Chen's new lab isolated a DNA-PKcs sample for us and we are currently collecting cryo-micrographs with the goal of achieving a subnanometer resolution structure by cryoEM single particle reconstruction methods.

Most recent products delivered:

Saban SD, Nepomuceno RR, Gritton LD, Nemerow GR, Stewart PL (2005) A cryoEM structure of adenovirus at 9 Angstrom resolution and semi-automated data acquisition with Leginon." Gordon Conference on Three-Dimensional Electron Microscopy, New London, New Hampshire.