

## Opportunities Linking Omics and Structural Biology at PNNL: Excelling at Cryo-EM

Trevor Moser,<sup>1</sup> Irina Novikova,<sup>1</sup> Amar Parvate,<sup>1</sup> Samantha Powell,<sup>1</sup> and **James E. Evans<sup>1\*</sup>**

<sup>1</sup>Pacific Northwest National Laboratory, Richland, WA

**Project Goals:** This project is focused on the purchase, installation and operation of a new state-of-the-art cryogenic Transmission Electron Microscope (Krios G3i) at EMSL to advance DOE-BER user research in protein/small molecule structural biology and whole cell ultrastructure. The microscope is now fully operational and has started imaging samples from EMSL and DOE-BER users.

**Abstract:** The acquisition, commissioning and operation of the new Krios G3i instrument at EMSL was a 3-year joint funding venture between EMSL and DOE-BER. This project was designed to rejuvenate cryo-EM research at EMSL by replacing an outdated 25-year old instrument that was limited by analyzing single samples in a manual format to now allowing 12 samples to be loaded and imaged in a semi-automated fashion. Due to the joint funding approach, the new microscope is now available to the general EMSL user community and DOE/BER researchers in a 50/50 split allocation. EMSL users continue to access this new instrument via the normal EMSL user proposal calls which permit combining cryo-EM with other capabilities at EMSL - such as mass spectrometry or super-resolution fluorescence microscopy. Access for DOE-BER users is free of charge to the users as it is funded by this current project which also allows for an expedited submission and review process for cryo-EM only projects.

The KriosG3i microscope has complete screening, data collection and image processing workflows for: 1) micro-electron diffraction of small molecule or protein crystals, 2) single particle analysis of soluble and membrane protein complexes and 3) electron tomography of whole cells or isolated organelles. It is equipped with a K3 direct electron detector, Ceta-D camera, phase plate and Bioquantum energy filter. In addition to semi-automated data collection, we have installed automated image processing workflows for real-time monitoring feedback of session quality and full 3D reconstruction of all workflows. To date we have demonstrated sub-2 angstrom 3D reconstructions for single particle and micro-electron diffraction workflows and sub-nanometer resolution for whole cell tomography. While we can provide very rapid access for samples that arrive pre-frozen on clipped and pre-screened grids, we can also begin with samples that arrive in buffer and require all steps of the cryo-EM workflow. In a subset of cases, we can also start from a provided gene of interest and employ or cell-free expression system to produce enough protein for structural characterization. If users are interested in access to the Krios cryo-EM capability at EMSL, please contact the team listed above or join the poster session to discuss the various mechanisms of access.

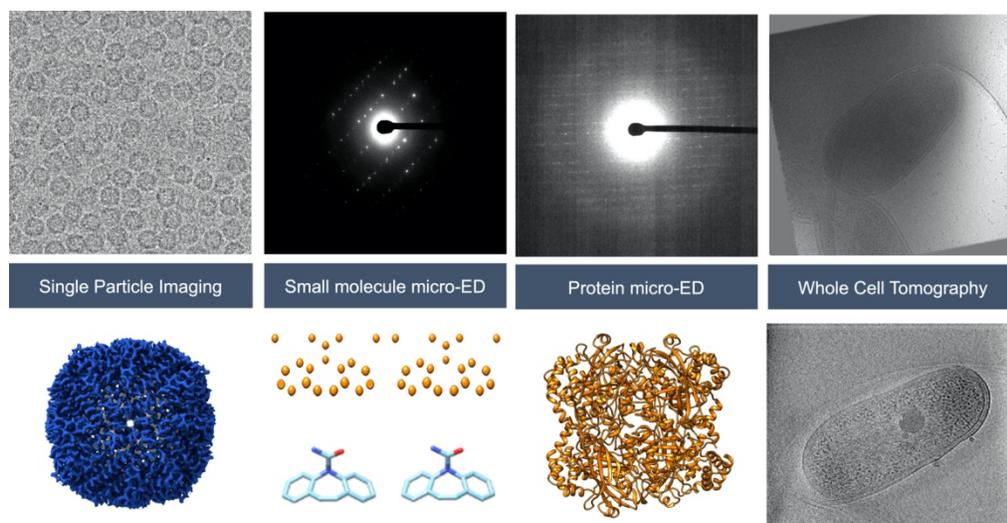


Figure Caption: Benchmarking examples of each of the cryo-EM sample workflows available to users on the new Krios G3i microscope at EMSL. Top row shows raw data while bottom row shows reconstructed volumes.

**Funding statement:** Pacific Northwest National Laboratory is operated by Battelle for the U.S. Department of Energy under Contract DE-AC05-76RL01830. This program is supported by the U. S. Department of Energy, Office of Science, through the Genomic Science Program, Office of Biological and Environmental Research, under FWPs 74194 and 74195. The work was performed at EMSL (grid.436923.9), a DOE Office of Science User Facility sponsored by the Biological and Environmental Research program.