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New cryoEM Methods for Studying Native Biological Complexes, *in situ* and in Action

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The last decade has witnessed advances in high-resolution cryoEM "revolutionizing" structural biology; as such, cryoEM has become a highly sought-after means of biochemical and biomedical investigations. Results from cryoEM are beginning to significantly enhance our understanding of the cellular processes responsible for maintenance, transmission and expression of genetic information at the molecular level. At the heart of these processes lie macromolecular complexes, within and outside the cell, which can now be studied by cryoEM. Understanding how and why these complexes function relies on visualizing their three-dimensional (3D) structures with atomic details and the molecular interactions among their constituent molecules, preferentially *in situ* if possible or otherwise close to their native state from endogenous sources.

By elevating the fundamental limitation of cryoEM—radiation damage to biological molecues that limits signal/noise ratio, imaging with electron-counting cameras has opened a floodgate of high-resolution cryoEM structures of purified complexes and opens the door for structural studies of functional complexes in situ and in action. Towards this end, we have developed an integrative proteomics cryoEM methods to determine atomic structures of native cellular complexes, sub-particle refinement and nucleic acid modeling methods to model genomic RNA and DNA in action. Of particular note, our cryoID method [1] (Figure 1) allows determination of atomic structures of native complexes in cellular milieu, capturing their multiple states, including those in act of carrying out their functions.

While the single-particle cryoEM methods have made it routine to determine structures of isolated macromolecular complexes at atomic resolution by averaging hundreds of thousands of particles. The biological functions of these complexes, however, are carried out through their interactions and often depend on their spatial arrangements within cells or sub-cellular organelles. Such molecular sociology information requires the use of cryogenic electron tomography (cryoET), which has another limitation, known as the "missing-wedge" problem. Anisotropic resolution arising from the intrinsic "missing-wedge" problem has presented major challenges in visualization and interpretation of tomograms. We have developed *IsoNet*, a deep learning-based software package that iteratively reconstructs the missing-wedge information and increases signal-to-noise ratio, using the knowledge learned from raw tomograms (Figure 2) [2].

Applications of *cryoID* and *IsoNet* have enabled atomic structure determination of previously intractable biological systems from cellular milieu [3] and direct interpretation of molecular sociology native complexes in cells [2], respectively. Integration of these new computational methods should open the door to *in situ* atomic resolution structures of cellular complexes in their native cellular environment [4].

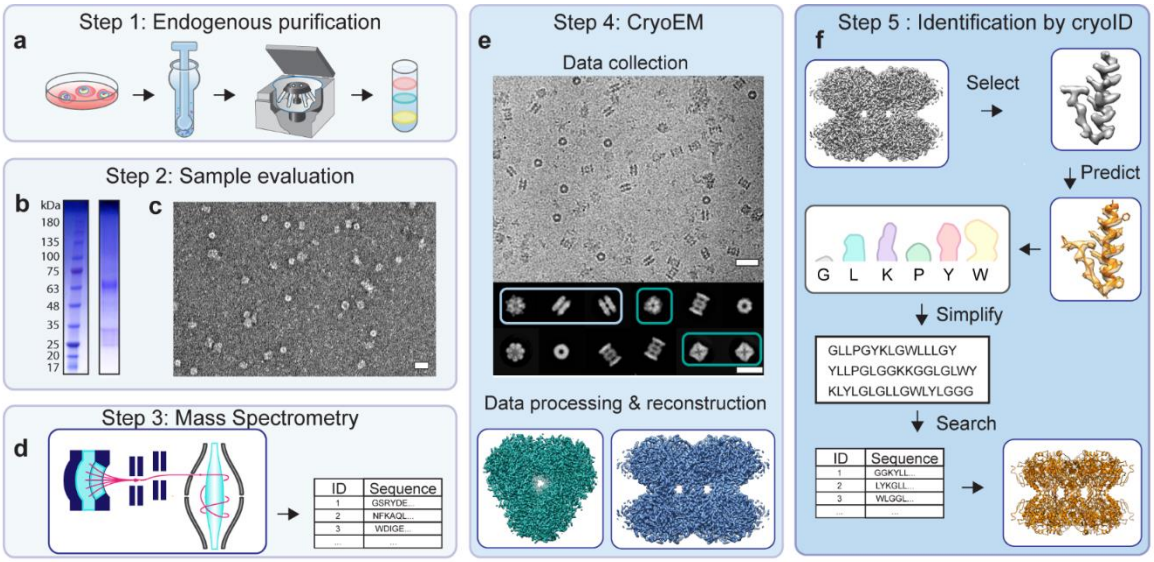

Figure 1. Endogenous structural proteomics workflow with *cryoID* [1]. **(a)** Protein complexes are enriched by sucrose gradient fractionation. **(b,c)** Fractions are evaluated by SDS-PAGE (**b**) and negative stain electron microscopy (**c**). **(d)** Mass spectrometry identifies a list of all proteins in each fraction. **(e)** cryoEM analysis yields near-atomic resolution cryoEM maps. **(f)** The proteins in the cryoEM maps are identified using *cryoID* [1].

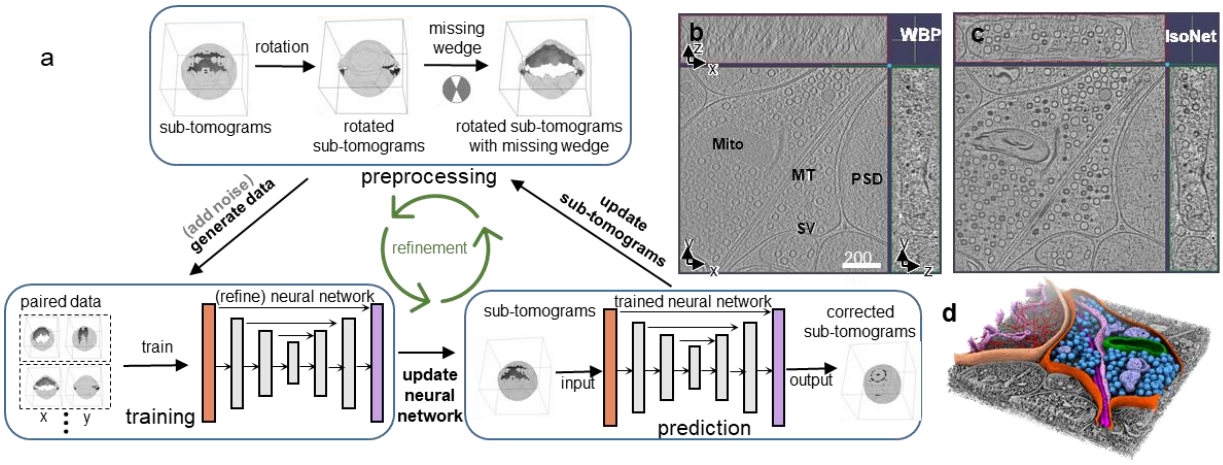

Figure 2. *IsoNet* [2] with neuron network for missing-wedge compensation in cellular cryoET. (**a**) Workflow of IsoNet. (**b,c**) Comparison of cellular cryoET from weighted back-project (WBP) (**b**) and from *IsoNet* (**c**). (**d**) 3D rendering of a synapse of cultured neurons.

References:

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[4] I thank CM Ho and Y Liu for figures and NIH for support (GM071940).

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