

## **Incorporating Electrospray Ionization in Cryo-EM Specimen Preparation**

**Hongwei Wang**

**School of Life Sciences, Tsinghua University, P. R. China**

**Email: [hongweiwang@tsinghua.edu.cn](mailto:hongweiwang@tsinghua.edu.cn)**

The current method of specimen preparation is not keeping pace with innovations in other major steps of cryo-EM structural biology. We have developed a new specimen preparation method using electrospray ionization equipment, which is an important technique for liquid separations in mass spectrometry. This method provides an alternative solution to the problem of macromolecules absorption to the air-water and solid-water interfaces. By refining the spraying parameters, the method produces an optimal ice thickness for the target macromolecules, due to the effective wetting of charged liquid droplets on the grid surface. We have used this method to prepare cryo-specimens and achieved 3D reconstructions of the 70S ribosome, 20S proteasome, apo-ferritin, and ACE2, at resolutions of 2.8 Å, 2.1 Å, 2.2 Å, and 3.5 Å, respectively. Further analysis of the specimens demonstrated that the macromolecules stay mostly in the middle of the amorphous ice layer, away from the air-water and solid-water interfaces.