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Novel sample delivery for small nanoparticles and biomolecules for x-ray diffractive imaging and cry-electron microscopy

Cryo-electron microscopy (Cryo-EM) is one of the key techniques in the field of structural biology. Recent years brought considerable improvements both on the software and hardware of the microscopes, and resolving high-resolution structures of proteins has become a standard procedure. However, most cryo-EM grids are still prepared by plunge freezing, a technique developed about ~40 years ago. The sample is pipetted into grid holes to form thin liquid layers that are subsequently plunged into liquid ethane. During this process, proteins are exposed to the air-water interface, causing a preferential orientation or damaging their structure. Here, we present the novel freeze-and-deposit sample-delivery approach to deposit particles for cryo-EM using cryogenic-gas shockfreezing technology. The cooling process produces cold high-density beams of nanoparticles, including biological specimens such as viruses or proteins. The concept was originally designed for XFEL single-particle-imaging experiments [1, 2]. In this process, nanoparticles and macromolecules are aerosolized, for instance, by electrospray ionization, and then rapidly cooled in the gas phase using a cryogenic buffer-gas cell [3, 4]. Adopting this with cryo-EM-grid handling allows for the controlled deposition of already shockfrozen samples on the grid, completely bypassing the need for blotting and the exposure of particles to the air-water interface. This approach will ensure the deposition of particles in a near-native state and overcome the issue of deposition of preferentially oriented proteins.

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