

Pushing the limits of sample vitrification

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Cryogenic Electron Microscopy (cryo-EM) is a powerful imaging technique for visualizing molecular structures with unprecedented detail. To prepare samples for cryo-EM a process called vitrification is typically used which involves rapidly freezing a thin layer of sample to create a non-crystalline solid. Plunge freezing is currently the most commonly used method for preparing samples for Single particle analysis and Cryo Electron Tomography (cryo-ET) workflows. This method works with isolated proteins but is not ideal for thicker samples like whole cells which is necessary for Cryo-ET. To address this challenge, a Vitrojet for cells (VitroJet4Cells) was developed which utilizes ethane jet to vitrify samples. The power of jet has already been proven effective with VitroJet for single particles. A functional prototype of the machine was successfully developed and is currently being used for testing various samples. The cooling potential of the jet is primarily examined using a micro loop set up, which can accommodate a wide range of samples, frozen samples are then examined using an X-ray beam to determine their vitrification quality. The machine is also compatible with standard EM grids. Clipped EM grids with adherent cells are, blotted, vitrified and subsequently utilized for making lamellas to be used in cryo-ET.

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