CryoET of Single Particle CryoEM Grids Reveals Widespread Particle Adsorption to the Air-Water Interface, Which May be Reduced with New Plunging Techniques

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Single particle cryo-electron microscopy (cryoEM) has commonly been performed with the assumption that the protein particles were suspended safely between the air-water interfaces at the time of vitrification. Studies of dozens of single particle cryoEM samples on grids by cryo-electron tomography (cryoET) has revealed that the vast majority of particles are adsorbed to the air-water interfaces at the time of vitrification (Figure 1) [1].

Particle adsorption to the air-water interfaces may potentially cause particle preferred orientation, conformational changes, and particle degradation [1]. While particle adsorption to the air-water interfaces may be physically avoided using substrates [2, 3], an alternative method for reducing or possibly eliminating air-water interface interactions is to decrease the time between sample-to-grid application and plunge freezing sufficiently so as to limit the extent of particle diffusion to the air-water interfaces and reduce the amount of equilibration of particles adsorbed to the air-water interfaces.

Here we present three single particle specimen (apo ferritin, hemagglutinin, and insulin receptor) prepared using Spotiton with varying sample application to plunge freezing times (spot-to-plunge times) (Figure 2). With a spot-to-plunge time of 400 ms, apo ferritin, a protein complex with high symmetry, preferentially adsorbs to the air-water interfaces (Figure 2a), with few particles remaining non-adsorbed. This preferential adsorption is reduced significantly with a spot-to-plunge time of 100 ms (Figure 2b).

Both hemagglutinin and insulin receptor, at both long and short spot-to-plunge times, remain preferentially adsorbed to the air-water interfaces prior to vitrification. When hemagglutinin and insulin receptor are prepared with long spot-to-plunge times (800 and 600 ms, respectively), they both present a limited number of preferred orientations (Figure 3c). However, with spot-to-plunge times of 100 and 200 ms, respectively, a significant reduction in preferred orientations was seen (Figure 3d). With these three samples we show the potential for reducing and potentially eliminating air-water interactions.
References:

[4] This work was supported by grants from the Simons Foundation (SF349247), NIH (GM103310, OD019994-01, R01-MH1148175, R01 GM084162, VRC intramural funding), NIMHD (5G12MD007603-30), A*STAR and the Agouron Institute [F00316].

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**Figure 1.** Single particle cryoEM grids plunged with a sample application to freeze time on the order of 1 second. The vast majority of particles are adsorbed to an air-water interface.

**Figure 2.** Effects of longer and shorter spot-to-plunge times using Spotiton. Cross-sections of tomograms of apoferritin grids prepared with a spot-to-plunge time of 400 ms (a) and 100 ms (b). The preparation in (b) resulted in more than an order of magnitude greater number of non-adsorbed particles than in (a). Long spot-to-plunge times of hemagglutinin and insulin receptor (c) resulted in limited number of preferred orientations, while shorter times (d) resulted in many more orientations (red boxes).