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Supporting information for article:

Through-grid wicking enables high-speed cryoEM specimen preparation

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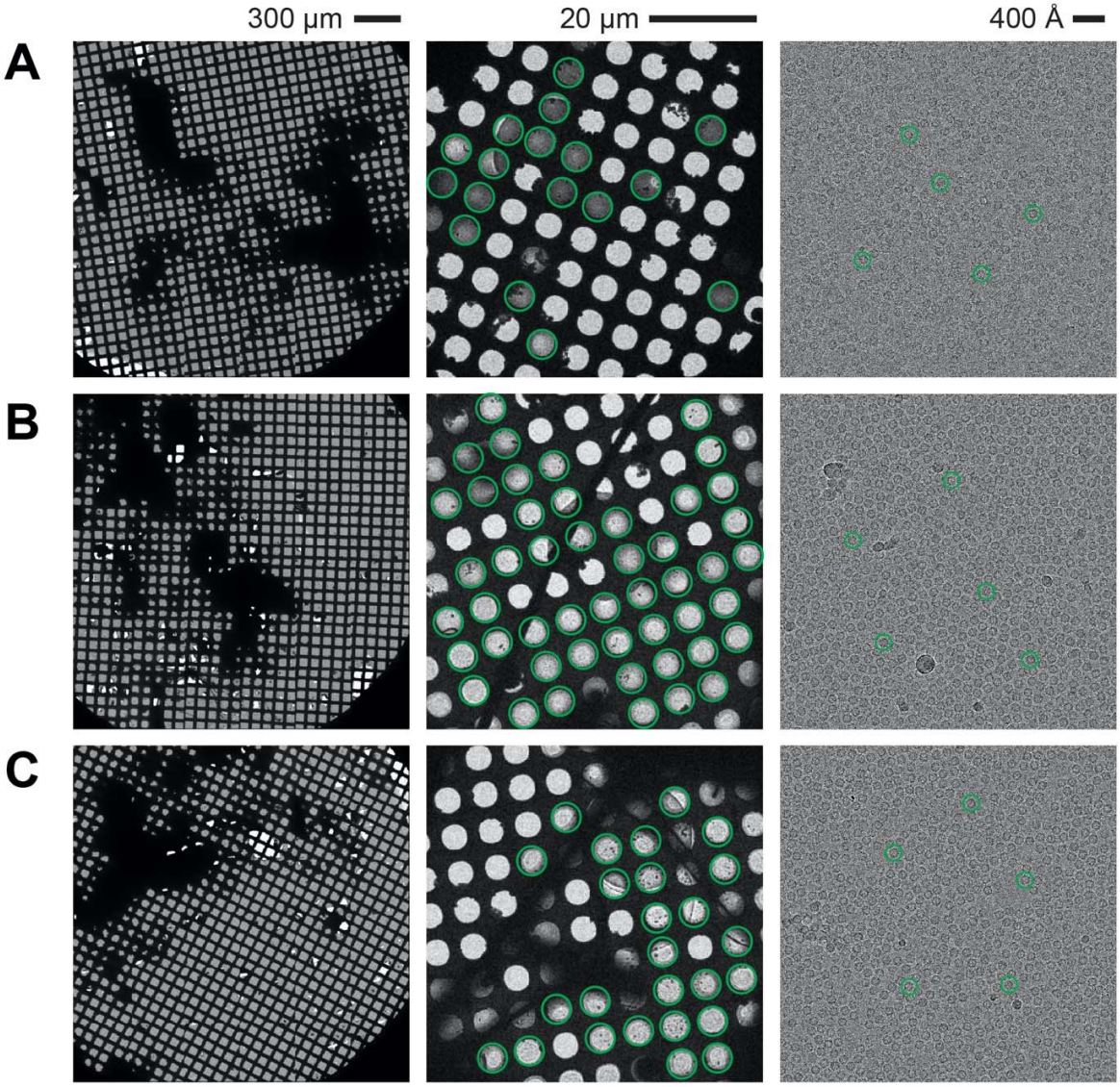

Figure S1 Reproducibility for human apoferritin specimen preparation. Grid atlases, grid squares, and high magnification images from three separate grids of human apoferritin prepared for cryoEM using BIU (*A*-*C*). Freezing conditions were identical to those used for the grids in Figure 4. Circular marks visible in the grid square images are due to low-exposure imaging used for eucentric height determination with the *EPU* software and fade over time. Green circles in the grid square show holes suitable for imaging. Green circles on the high magnification images show locations of five representative particles per image.

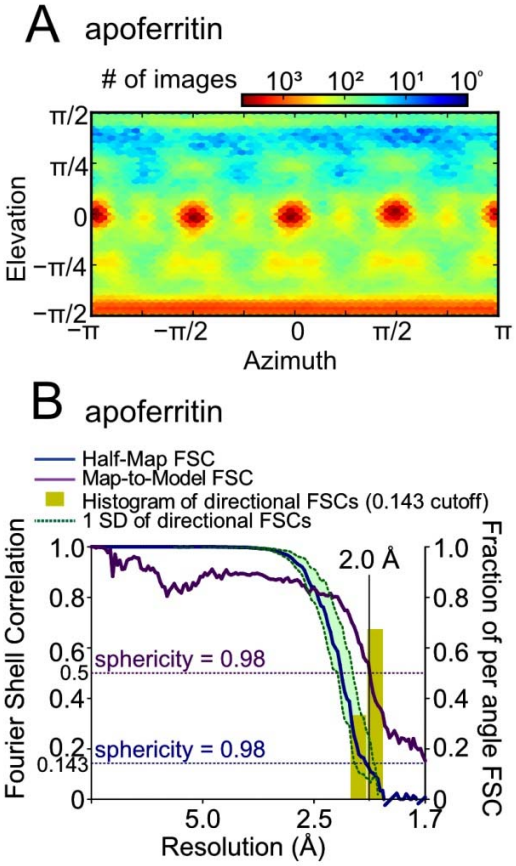

Figure S2 Apoferritin map validation. (*A*) Euler angle distribution for human light chain apoferritin. *(B)* Fourier shell correlation (FSC) curves for human light chain apoferritin show the map resolution from half-map (blue) and map-to-model (purple) correlation at FSC=0.143 and FSC=0.5, respectively. A histogram of directional resolutions sampled evenly over the 3DFSC (yellow) is also shown and the corresponding sphericity is indicated.

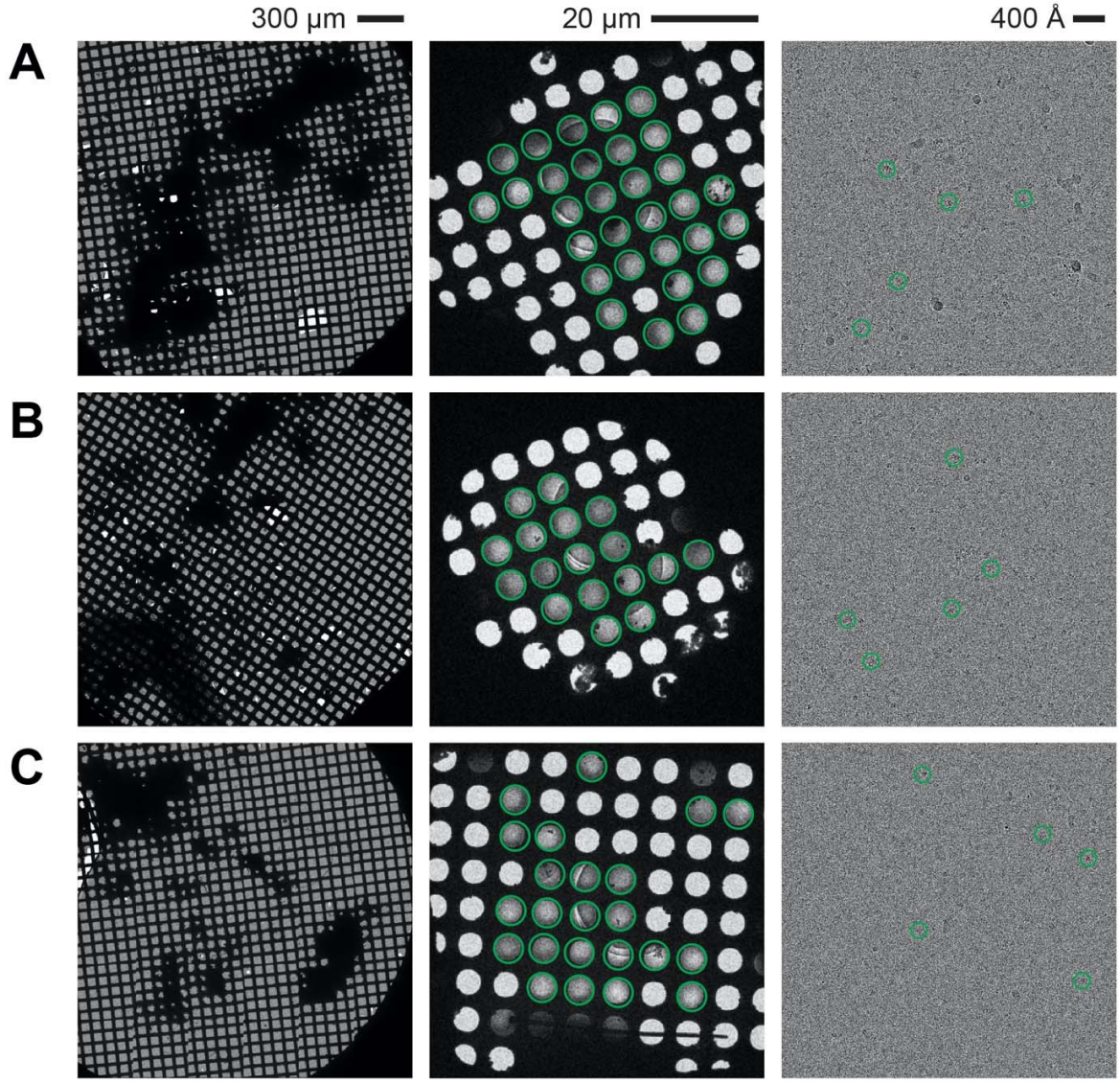

Figure S3 Reproducibility for hemagglutinin trimer vitrified using BIU. Grid atlases, grid squares, and high magnification images for three separate grids of hemagglutinin trimer prepared for cryoEM with BIU (*A*-*C*). Freezing conditions were identical to those used for the grids in Figure 6. Circular marks visible in the grid square images are due to low-exposure imaging used for eucentric height determination with the *EPU* software and fade over time. Green circles on the grid square show holes suitable for imaging. Green circles on the high magnification images show locations of five representative particles per image.

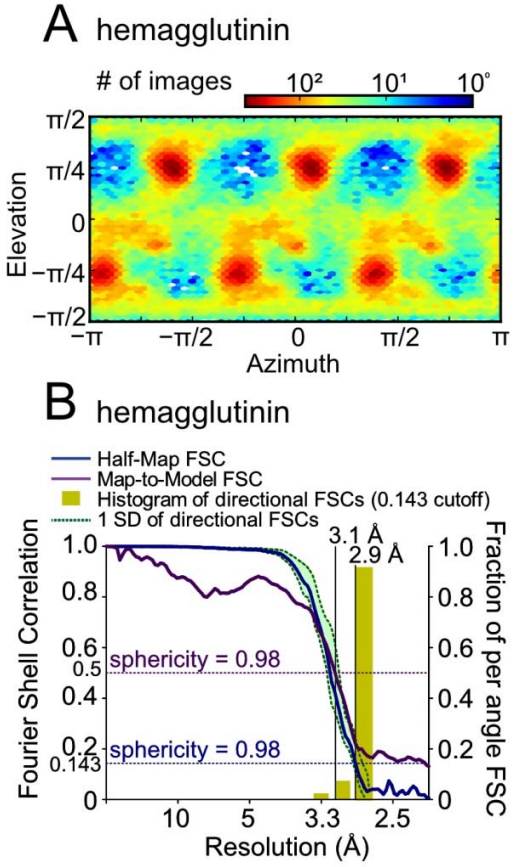

Figure S4 Hemagglutinin map validation. (*A*) Euler angle distribution for influenza A H3N2 hemagglutinin trimer. *(B)* Fourier shell correlation (FSC) curves for influenza A H3N2 hemagglutinin trimer show the map resolution from half-map (blue) and map-to-model (purple) correlation at FSC=0.143 and FSC=0.5, respectively. A histogram of directional resolutions sampled evenly over the 3DFSC (yellow) is also shown and the corresponding sphericity is indicated.

Movie S1. Comparison of filter paper and glass fiber filter for through-grid wicking. Water (5 μL) applied to a hydrophilic holey gold grid does not consistently wick through the grid when it is placed on filter paper (top) but quickly wicks through the grid when it is placed on a glass fiber filter (bottom). The frame number is indicated in the top left corner of the image, with each frame corresponding to 1/480 sec.

Movie S2. High speed video of the BIU process. A video recorded at 480 frames per second shows the high-frequency generating circuit of the piezoelectric transducer fully energized for ~45 msec. A further 30 msec elapses where the glass fiber filter remains in contact with the grid. The grid is plunged into cryogen ~60 msec after it breaks contact with the glass fiber filter. The frame number is indicated in the top left corner of the image, with each frame corresponding to 1/480 sec.