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Ultrastable Gold Substrates Improve the Resolution of 3D Reconstructed Density Maps from Electron Micrographs and Tomograms

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Ultrastable gold substrates (Fig. 1a), which are composed entirely of gold, reduce the average movement of cryogeninc specimen under electron irradiation by about 50-fold during a typical exposure in the bright field transmission electron microscope [1]. This reduces the motion-induced blurring of electron micrographs and improves the image quality. We demonstrate the use of these ultrastable gold substrates in high-resolution structure determination by single particle electron cryomicroscopy (cryo-EM). We show examples of high-resolution structure determination for several specimens. Apoferritin is a particularly challenging protein structure for single particle cryo-EM because sub-nanometer resolution is required in each particle image to successfully assign projection angles and thus calculate a 3D density [2]. We compare structures of apoferritin calculated from images collected under the same conditions on conventional and ultrastable gold substrates (Fig. 1b). These demonstrate that for small and challenging proteins, the improved image quality on ultrastable substrates can make the difference between success and failure in solving a structure. We also show 3D reconstructed density maps for the eukaryotic 80S ribosome (Fig. 1d) [3], and the hepatitis B viral capsid [4]. Each show improved resolution on gold substrates as compared to traditional amorphous carbon based supports. In particular, ribosome density maps, calculated from several hundred micrographs collected using ultrastable substrates, show sub-3Å features without the use of per-particle motion correction algorithms (Fig. 1d).

The vast majority of electron radiation induced motion on conventional amorphous carbon substrates is in the direction perpendicular to the plane of the specimen support. We demonstrate this by measuring the movement of substrates using movie collection with the current generation of high-speed direct electron detectors. This entails that when the specimen is tilted, the projected component of movement in the image plane increases with the sine of the tilt angle. This vertical movement accounts for much of the degradation of image quality at high tilt angles. 3D electron tomography uses a series of low-dose images to at known tilt angles to determine the 3D structure of an individual specimen. Knowledge of how the particles are displaced in three dimensions during irradiation allows revision of tomogram acquisition strategies to minimize the in-plane components of movement and thus improve image quality on all substrates. Further, using ultrastable gold substrates for the collection of electron tomograms reduces specimen movement, particularly at high tilt angles, and thus improves the resolution of density maps calculated from 3D tomograms. We use tomograms collected on both conventional and ultrastable gold supports under the same conditions to demonstrate a 20% improvement in the resolution of sub-tomogram averages collected on ultrastable substrates.

Specimens prepared for imaging on ultrastable gold substrates are made using standard cryo-prepration techniques and imaged using typical low-dose electron microscopy methods. Since the foils are made from a polycrystalline metal, there are some differences in imaging from traditional amorphous carbon foils. We discuss the practical aspects of preparing specimens and collecting optimal data using these substrates and point out common mistakes that can lead to degradation in image quality. The results herein demonstrate that reduced specimen movement improves the image quality in each micrograph and tomogram, and thus increases the resolution of 3D reconstructed density maps of biological specimen [5].

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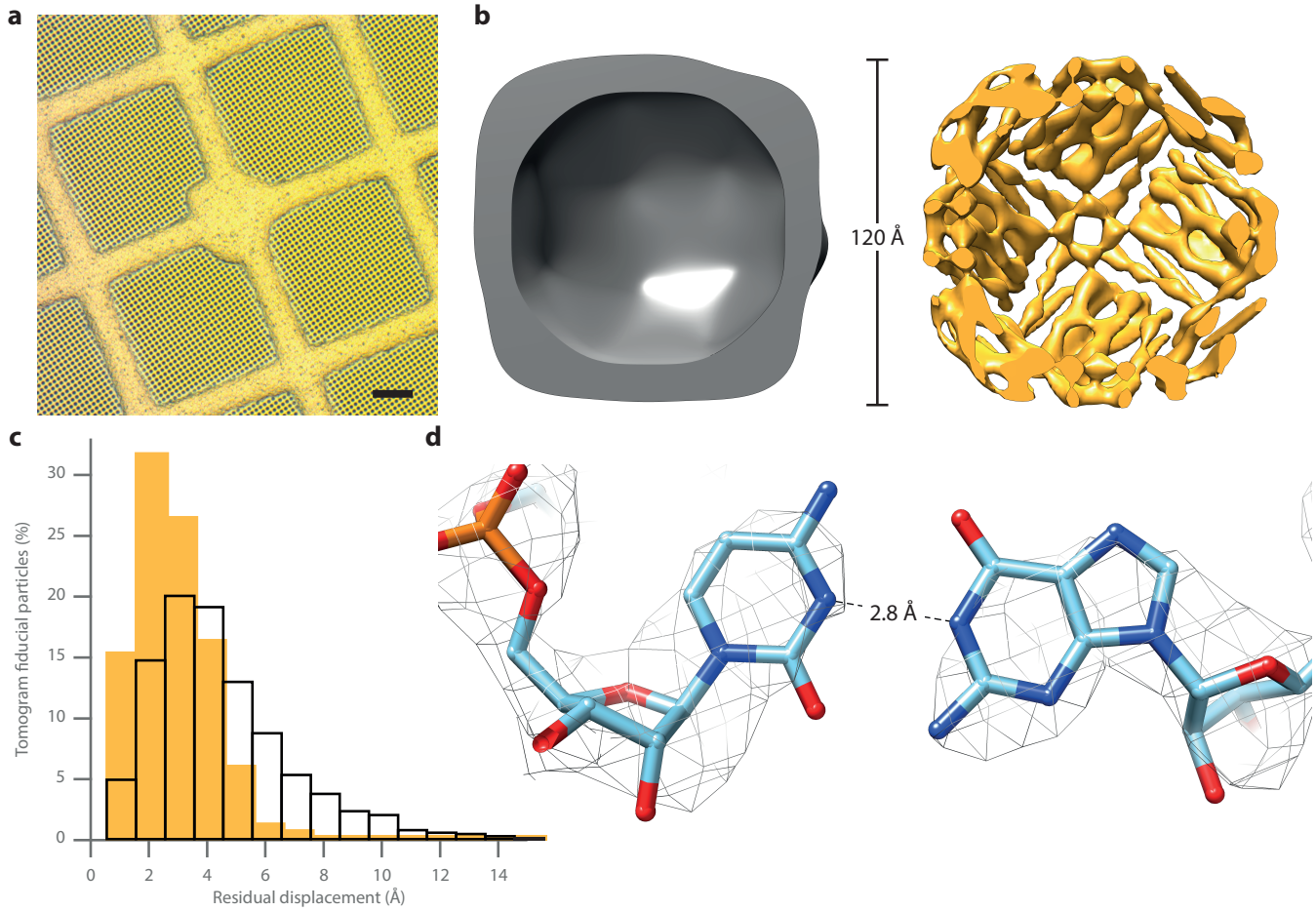

Figure 1. Optical micrograph (**a**) of an ultrastable gold support; bar is 20 µm. Apoferritin density maps (**b**) using conventional carbon (grey) and ultrastable (gold) supports. Nano-gold fiducial particles were tracked in electron tomograms; the residual in-plane displacement was measured for the fiducial particle at each tilt angle and subtracted from the position of a motionless particle. Panel **c** shows a histogram of these residual motions for tomograms on gold (gold bars) vs. carbon (black bars). The residual movement is halved on the gold substrates (5.3 vs. 2.6 Å RMS displacement). Panel **d** shows atomic model and density map for a G-C base pair within the 80S ribosome from data on a gold substrate.

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