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Ultrastable gold substrates: Properties of a support for high-resolution electron cryomicroscopy of biological specimens

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ABSTRACT

Electron cryomicroscopy (cryo-EM) allows structure determination of a wide range of biological molecules and specimens. All-gold supports improve cryo-EM images by reducing radiation-induced motion and image blurring. Here we compare the mechanical and electrical properties of all-gold supports to amorphous carbon foils. Gold supports are more conductive, and have suspended foils that are not compressed by differential contraction when cooled to liquid nitrogen temperatures. These measurements show how the choice of support material and geometry can reduce specimen movement by more than an order of magnitude during low-dose imaging. We provide methods for fabrication of all-gold supports and preparation of vitrified specimens. We also analyse illumination geometry for optimal collection of high resolution, low-dose data. Together, the support structures and methods herein can improve the resolution and quality of images from any electron cryomicroscope.

1. Introduction

Structure determination of biological molecules using cryo-EM has undergone a revolution recently, such that near-atomic resolutions are now possible (Kühlbrandt, 2014). Improved, more stable electron microscopes and new computational methods have contributed to these advances (Henderson, 2015a). But a dramatic improvement has been afforded by the increased quantum efficiency of new high-speed direct electron detectors, resulting in improved images (Cheng, 2015; Henderson, 2015a). It has long been known that cryo-specimens move when irradiated with the electron beam (Henderson and Glaeser, 1985; Dubochet et al., 1988). This results in image blurring and loss of high-resolution information. Direct electron detectors permit tracking of specimen movement during irradiation, allowing realignment of movie frames to reduce (but not eliminate) image blurring (Brilot et al., 2012; Bai et al., 2013; Li et al., 2013). Importantly, these detectors also allow investigation of the origin of specimen movement and the development of new methods to reduce it (Russo and Passmore, 2014a).

There have been several recent attempts to design supports that reduce the movement of the specimen during irradiation, by changing either the geometry or the material composition of the suspended foil (Vonck, 2000; Rhinow and Kühlbrandt, 2008; Yoshioka et al., 2010; Glaeser et al., 2011; Russo and Passmore, 2014a). We recently showed that much of the particle motion in cryo-EM is due to movement of the support: upon irradiation, supports with a perforated carbon foil over a metal mesh grid move by a surprising amount: 200–400 Å in the direction parallel to the electron beam (Russo and Passmore, 2014b). By carefully analysing the origins of this we designed a support intended to nearly eliminate it, and improve many of the practical aspects of specimen preparation for electron cryomicroscopy. The design (Fig. 1) comprises a circular disk of gold, 3 mm in diameter, having a mesh pattern on which is suspended a thin, polycrystalline gold foil with a regular array of micrometer-sized holes. The design is essentially the same as standard Quantifoils (Ermantraut et al., 1998) except the mesh grid and thin foil are entirely made from gold.

Building upon our previous work (Russo and Passmore, 2014b), here we explain the physical origins of the vast reduction in movement on all-gold supports. We further provide: (1) a practical framework for manufacturing these supports in the laboratory (2) procedures for using them to prepare vitreous specimens and (3) carefully tested methods for collecting optimal data using low-dose techniques specifically optimised for these ultrastable supports. Together these comprise a way to reduce the movement of vitrified biological specimens to less than two ångströms in a typical low-dose micrograph, and thus directly improve the images from any high-resolution electron cryomicroscope.

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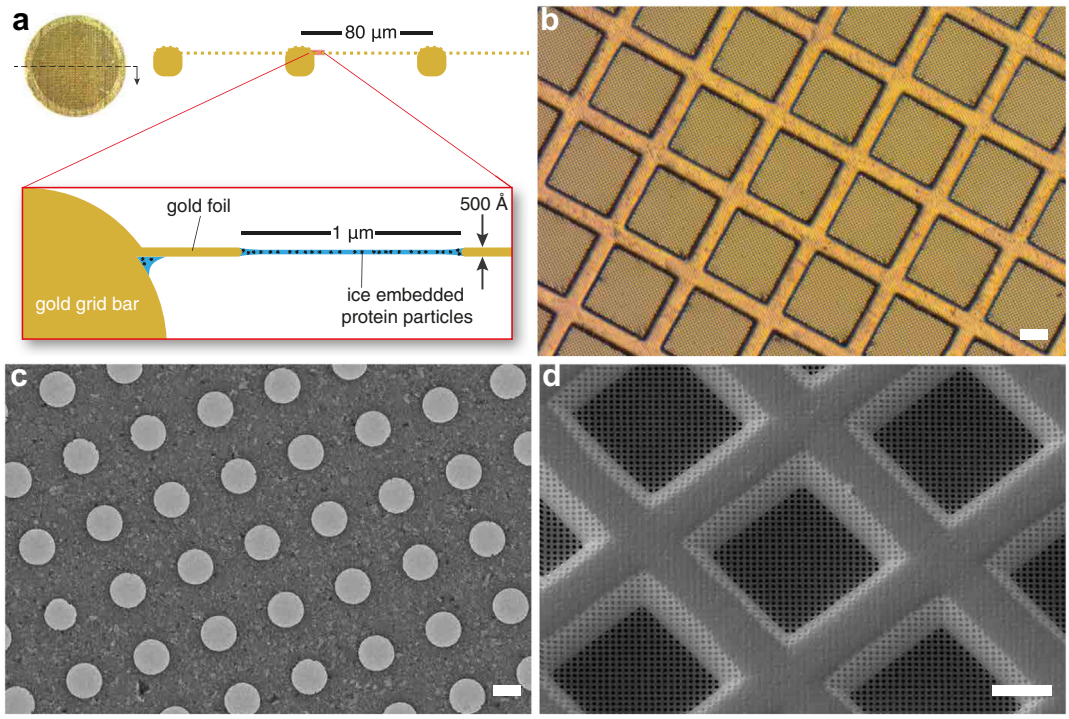

Fig. 1. Ultrastable support design. (a) Ultrastable supports, made entirely of gold, comprise a 3 mm disc with a square mesh that supports a ≃500 Å thick foil containing a regular array of micrometer-sized holes. An aqueous protein solution is suspended across the holes and plunge frozen at ≃80 K. (b) Optical micrograph of an all-gold support, showing the square mesh and perforated foil. Scale bar is 25 µm. (c) Transmission electron micrograph taken with 300 keV electrons, showing the perforated polycrystalline gold foil. The holes are 1.2 µm in diameter. Scale bar is 1 µm. (d) Scanning electron micrograph of an ultrastable support taken at 30 degrees tilt from the horizontal axis. Scale bar is 25 µm. Diagram in (a) is reproduced from Russo and Passmore (2014b).

2. Results

2.1. Support fabrication

Although we explored different methods to produce an all-gold support, the simplest and most reproducible was to start with a standard carbon foil suspended on a gold grid such as a Quantifoil, C-flat or lab-made perforated carbon support (Ermantraut et al., 1998; Quispe et al., 2007; Adrian et al., 1984). Gold is deposited on the carbon foil by vacuum evaporation, and the support carbon is removed using a low-energy argon–oxygen plasma. A detailed protocol describing the process is provided in Supplementary file 1. After fabrication, the support has the geometry shown in Fig. 1 and has a mass of 0.99 ± 0.03 mg. This means that although the grid is made of a precious metal, the raw material cost is negligible compared to the cost of manufacture or the cost of microscope time and other consumables used in the process.

2.1.1. Mechanical stability

We previously showed that on widely used Quantifoil supports, much of the specimen movement induced by an electron beam is due to motion of the support itself (Russo and Passmore, 2014b). Compared to amorphous carbon foils on gold grids of the same geometry, all-gold supports have as much as a 50-fold reduction in movement parallel to the beam. This results in a twofold reduction in the movement of particles perpendicular to the beam, which is in the plane of the image. But why does gold move less than carbon? One might expect that the gold moves less than carbon because it is thicker and therefore stronger. Previously, we measured the strength of the gold and carbon foils by atomic force microscopy (AFM) and found that exactly the opposite is true; the gold is actually 40 times less rigid at room temperature (10.5 N/cm vs. 412 N/cm) (Russo and Passmore, 2014b). Since the difference in modulus between these two materials will not change by orders of magnitude with cooling (Haynes, 2015), this cannot explain the reduction in movement on the gold foils. To understand this perplexing difference further, we performed a series of experiments on carbon foils to try to pinpoint the origin of the movement.

It has long been known that amorphous carbon, as manufactured, undergoes chemical and physical changes when it is first irradiated by high energy electrons (Miyazawa et al., 1999). Based on this observation, some pre-irradiate the carbon before use with a dose that is large enough to saturate any of the radiation-induced chemical and physical changes in the specimen ($\sim 100\, e^-/\text{Å}^2$ at 100 keV (Miyazawa et al., 1999)). This is thought to strengthen, clean and improve the conductivity of the carbon films. Our previous measurements of the vertical motion of carbon foils also show a saturation behaviour to the movement, such that after about $50\, e^-/\text{Å}^2$ it nearly stops (Russo and Passmore, 2014b). We tested the effect of pre-irradiation on the vertical motion of the carbon foils in cryogenic conditions, and the results are shown in Fig. 2a. Pre-irradiation at 300 keV using $100\, e^-/\text{Å}^2$ makes the trajectories of vertical movement more uniform across the foil, indicating that it does have an effect on either the generation of force by the electron beam or the mechanical response of the foil to this force. Still, it fails to reduce the movement and therefore pre-irradiation is not sufficient to eliminate the motion of the carbon foil.

To further explore the effects of pre-irradiation on the carbon films, we repeated the experiment in a slightly different way: irradiate the carbon around specific individual holes, remove the grid from the microscope, warm it to room temperature, cool it again to 77 K and re-image the same holes. The resulting vertical motion trajectories are shown in Fig. 2a. Again, irradiation makes the holes move more uniformly in the vertical direction, but fails to eliminate the movement. This experiment illustrates the high degree