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Development of nanostructured electron microscopy grids for time resolved single particle reconstruction for transmission electron microscopy

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Transient structural states of macromolecules such as ribosomes, ion channels, etc., can be viewed with electron microscopy by rapid quenching to liquid nitrogen temperatures. A key challenge of time-resolved cryo-EM is to rapidly mix reactants and macromolecules and at the same time deposit them in a thin aqueous film (<200 nm) on carbon-coated electron microscope grids before plunging into liquid ethane. A standard method to achieve this thin layer is by blotting a grid onto which a few microliter drop of one reactant has been deposited, and then spraying a fine mist containing the other reactant onto the wet grid as it is being plunged [1]. We are now exploring an alternative method that involves mixing before spraying, and depositing droplets onto a novel, dry, hydrophilic carbon film on the grid.

Carbon surface is typically not hydrophilic. Using fresh, plasma-cleaned carbon film produces a sufficiently hydrophilic surface to spread small (1 to 5) micron droplets (Fig. 4). We are exploring methods to produce a structured, hydrophilic carbon film that allows larger droplets (5 to 20+ microns) to spread quickly enough and thin enough (200 nm) for TEM.

A water droplet (3 microliters) sitting on an untreated amorphous carbon surface exhibits a contact angle of 66.7^o (Fig.1a). If the surface is covered with nanostructures (Fig. 1c) the contact angle is reduced to a much smaller value (11.8^o, see Fig. 1b). This structured surface (Fig. 1c) is made out of silicon nanorods grown by the oblique angle deposition technique [2]. The nanorods serve as absorbers and can spread the aqueous film very quickly.

Figure 2a shows a water droplet located next to a patterned region containing a nanostructured surface. The wicking region is several hundred microns in width. Figure 2b shows a schematic representation of the hemi-wicking nature [3] of such a nanostructured surface and defines the apparent contact angle for the case where water invades the nanostructured region. These wicking regions can take the form of any high surface area structure. We investigate nanopillars, nanosprings, carbon nanotubes (CNT), and e-beam patterned silicon/silicon dioxide surfaces.

Nanostructures are too dense to image with TEM, so it is necessary to develop a patterned structure. The use of commercially available Quantifoil (carbon film with holes) as a shadow works very well as a fine pattern (Fig. 3). Effort is underway to examine the liquid-spreading characteristics and wicking property of the structure.

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[2] T. Karabacak and T.-M. Lu, in *Handbook of Theoretical and Computational Nanotechnology*, edited by M. Rieth and W. Schommers (American Scientific Publishers, 2005), chap. 69.
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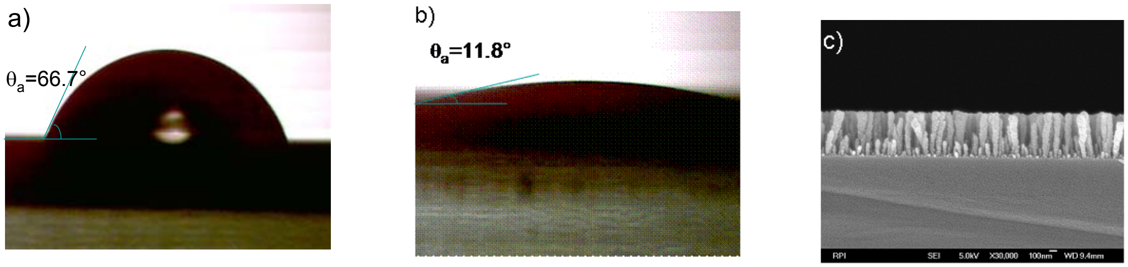

Fig. 1. a) An optical micrograph showing a water droplet sitting on an amorphous carbon surface with a contact angle of 66.7°. b) The contact angle is reduced dramatically to 11.8° when the water droplet is sitting on a nanotructured surface such as the one shown in c). c) Cross section view of Si nanorods grown by the oblique angloe deposition technique. The scale bar is 100nm.

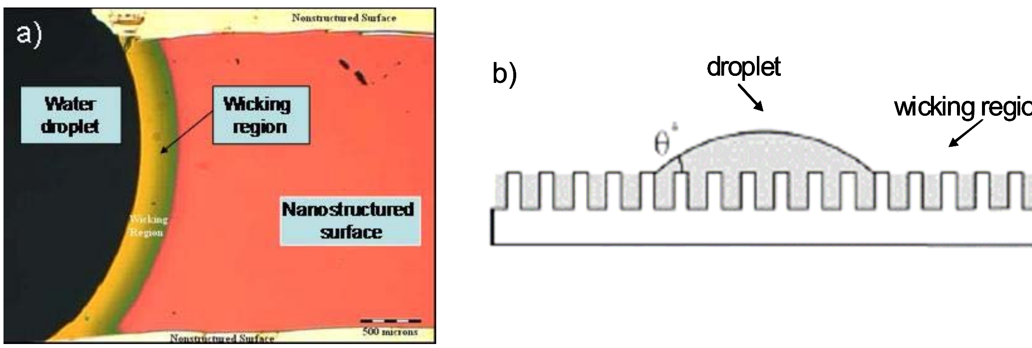

Fig. 2. a) Millimeter scale water droplet situated near patterned region of a nanostructured surface. The wicking region is several hundred microns in width. b) A schematic from reference [2] showing the hemi-wicking nature of nanostructured surface.

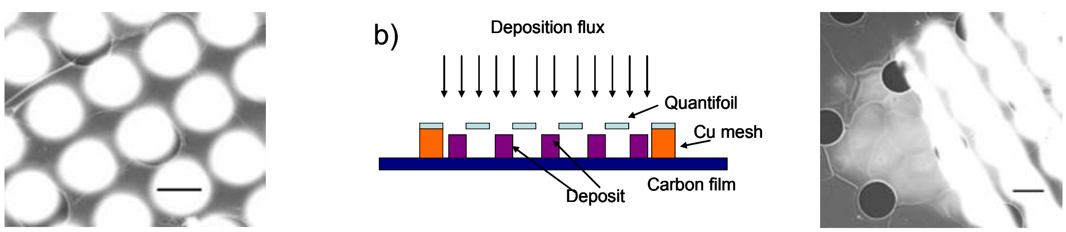

Fig. 3. a) TEM of pillars deposited on a Quantifoil grid. Scale bar is 2000nm. b) A schematic showing the process of depositing pillars through a Quantifoil grid as the mask. c) Large droplet infiltrating pillars. Scale bar is 2000nm.

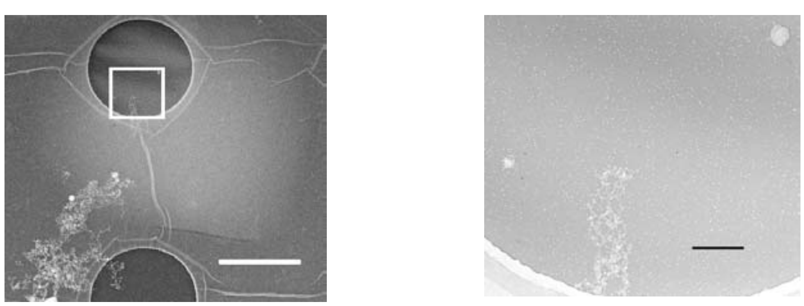

Fig. 4. a) A droplet lying over a Quantifoil hole. Scale bar is 2000nm. b) Inset blown up. Small, bright dots are ferritin. Larger, more diffuse dots are 50S ribosome subunits. Scale bar is 200nm.

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