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Imaging Graphene-Encapsulated Microtubules at Room Temperature with Electron Microscopy

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High resolution electron microscopy of biological materials in their native state and at room temperature is a great challenge due to the difficulties in sample preparation and the limitation of radiation damage. Membrane based liquid cells have been used for this purpose that contain two silicon nitride (SiN) membranes of typically 50 nm in thickness and a spacer between them ideally defining the desired sample thickness [1]. Even though this design principle has been successful for studies involving metallic particles and labelled proteins in liquid over the last fifteen years, there are only a few examples of imaging label-free biological molecules in fully hydrated conditions [2]. The key limiting factor arises from low amplitude contrast of biological materials in water so that electron-dose efficient phase contrast is needed to achieve nanometer resolution. Attaining phase contrast is possible for up to a few hundreds of nanometer liquid (or glassy ice) thickness. However, such thin liquid cell is difficult to prepare due to bulging of the SiN membrane and since standard liquid cells use a SiN thickness of 50 nm for each window [2, 3]. It was shown that single- or multi-layer graphene can be used to encapsulate liquid pockets in high vacuum recently. Van der Waals interactions between graphene sheets are believed to provide stable liquid pockets with an estimated thickness of 100 nm [4] and the window thickness is negligible thus obtaining the thinnest possible liquid cell window for high resolution imaging. It can also be used for enclosing large biological structures such as eukaryotic cells fixed on thin-film substrates [5].

In our study, we imaged microtubule proteins, immobilized on multi-layer graphene coated TEM grids (Plano GmbH, Germany) and covered these by a sheet of multi-layer graphene (ACS Materials, USA). We followed a simple graphene transfer method onto microtubules [6]. In a typical sample, we observed patches of wet regions throughout the entire examined area in which microtubules were located. In dry regions, we observed residues originating from the polymerization buffer of microtubules. Besides graphene coated transmission electron microscopy (TEM) grids, different sample support substrates/membranes such as 10-20 nm SiN and ultra-thin amorphous carbon (3 nm) coated TEM grids were also tested by following the same sample preparation procedure for a comparison of their wetting properties. We obtained the highest number of intact microtubules with the graphene coated sample supports. It was recently reported that the number of stable liquid pockets obtained in a graphene liquid cell strongly depends of the solvent conditions [7].

Figure 1a shows the schematic representation of graphene-encapsulated microtubules for electron microscopy. In our graphene transfer protocol, the commercially obtained graphene-PMMA (Polymethyl methacrylate) stack was transferred on salt crystals, and subsequently PMMA was removed in acetone. Then graphene-salt stack is floated on deionized water and free-floating graphene was fished out with a metallic loop (Figure 1b). In parallel, a 2 µL of microtubule solution was casted onto a graphene coated TEM grid and the loop with graphene was lowered onto the grid (Figure 1c). Upon drying of the excess liquid, liquid pockets formed in between the graphene sheets (Figure 1f). As seen in Figure 1f, graphene layer nicely follows the contour of the microtubules and isolate them from vacuum. Figure 1d and 1e show the TEM images obtained from graphene-encapsulated microtubules. In Figure 1e, it is possible to observe interior details of the microtubules.

In summary, our study demonstrates a graphene transfer method to keep microtubule proteins partially hydrated and intact up to some extent. It is applicable to various substrates for electron mi-

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croscopy. But more experiments are needed to optimize the buffer specifications and graphene transfer protocol to increase the wetting of the sample. [9]

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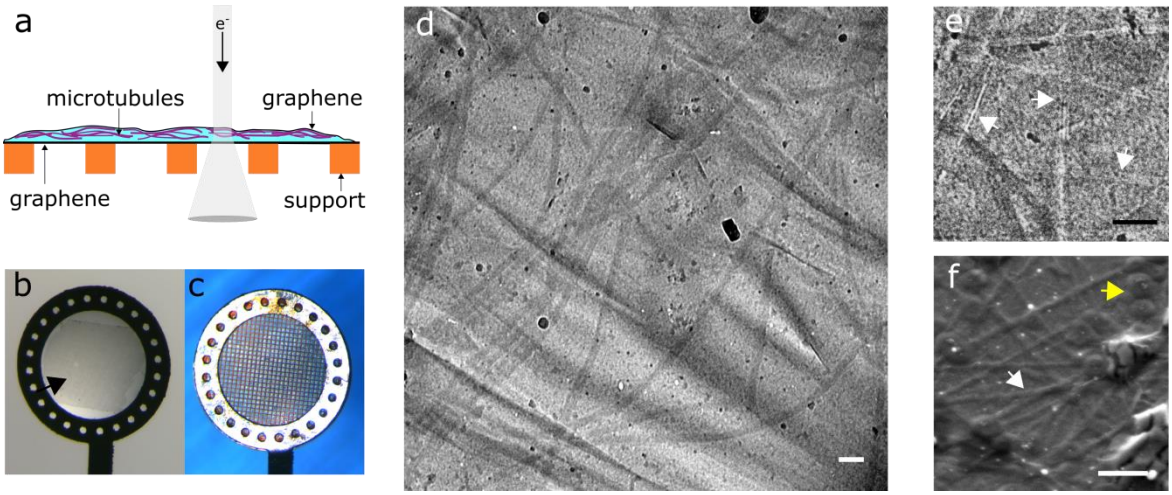

Figure 1. Electron microscopy (EM) of graphene-encapsulated microtubules. a) Schematic representation of a graphene-encapsulated microtubule sample for EM. b) Light microscopy image of a graphene sheet on a droplet surface held by a metal loop. c) Graphene in the loop brought in contact with a graphene coated TEM grid to trap the liquid sample in between. d) Overview TEM image showing a region with microtubules. (e) Higher magnification TEM image obtained from the microtubule region. White arrows point the observed microtubules. e) Overview SEM image. White arrow points a single microtubule lying under a graphene sheet and yellow arrow points possible liquid pockets observed.

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