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Imaging Graphene-enclosed Microtubules in their Polymerization Medium with Electron Microscopy

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Liquid phase electron microscopy has started a new era of observing biological material in their native liquid media, capturing their real-time dynamics and interactions with each other and inorganic materials [1]. Silicon based liquid enclosures have been offering a complete isolation of the sample from the vacuum of an electron microscope and keep it fully hydrated for sufficiently long time during the imaging; however, they typically include 50 nm silicon nitride (SiN) windows, which increase the overall sample thickness to a point, where TEM phase contrast is not applicable in low-Z materials [2, 3]. As an alternative approach, it was shown that the liquid samples can be encapsulated between graphene sheets [4] or samples on a continuous membrane can be covered by graphene [5]. Graphene coverage protects the sample from the vacuum, while offering a negligible substrate background for high resolution imaging.

We used this graphene approach to cover microtubule proteins (MTs) and performed scanning- and transmission electron microscopy (SEM/TEM) imaging (Fig. 1a). We used a simple method to transfer graphene onto the MTs, while keeping the MTs in their polymerization buffer in all the steps to maintain their structural integrity during the graphene transfer. We used sheets consisting of 3-5 layers of graphene (ACS Materials, USA) for coverage. Experiments were carried out with several different support substrates/membranes, such as 10-20 nm SiN, ultra-thin amorphous carbon (3 nm) and graphene attached TEM grids. The wetting properties and average number of MTs observed were similar in all the samples. Furthermore, we observed residues in various sizes originate from the general tubulin buffer. The samples typically did not contain liquid throughout the entire examined area but rather exhibited patches of wet regions (Fig. 1c), where we observed MTs. After considering their measured width (23 nm) and observed protofilaments (Fig. 1f), we can suggest that observed MTs have structurally intact regions [6].

Figure 1 shows the schematic representation of graphene-enclosed MTs for electron microscopy (a), a light microscopy image showing graphene floating on top of MTs in liquid with a 9-window SiN (10 nm) substrate (b), an overview SEM image showing the overall morphology of the samples (c), a higher magnification SEM image taken from a region with MTs (d) and a typical TEM image of a microtubule in a graphene enclosure (e). A plot profile was obtained from the red line in Fig. 1e and showed in Fig. 1f. It shows 2-striation region in the MT, which originates from the protofilaments around a hollow center [6]. A fast Fourier transform (FFT) image was obtained from the yellow box in Fig. 1e and showed in Fig. 1g. In the FFT image, we observed the spatial frequencies of 0.05, 0.09, 0.12 and 0.16 nm^{-1} in the equatorial line arising from the interference between protofilaments [6]

In summary, our study exhibits a graphene transfer method to keep MTs hydrated and intact up to some extent. It is applicable to various substrates for EM imaging. Wetting degree and interactions of MTs with substrate can affect their structure, which can change the finest feature resolved in the TEM images. According to our FFT analysis, we could resolve features down to 6 nm, which could be

attributed to our resolution for the current sample conditions.

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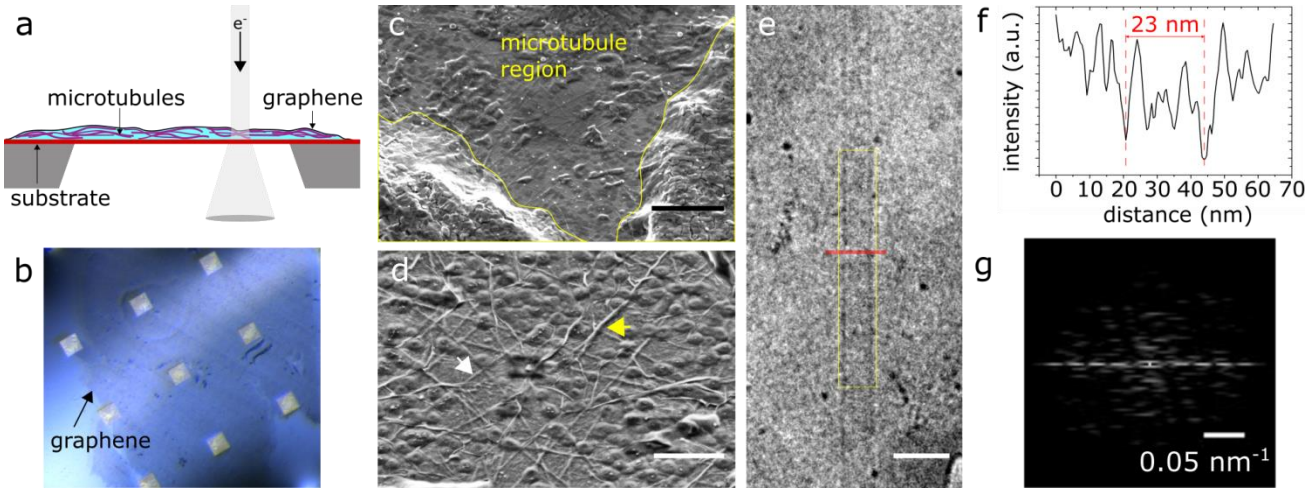

Figure 1. Electron microscopy imaging of graphene-enclosed microtubules. a) Schematic representation of the graphene-enclosed MT samples for EM. b) Light microscopy image of 9-window SiN substrate after drop-casting MT sample and transferring multilayer graphene. c) Overview SEM image showing the region, where MTs are observed. (d) Higher magnification SEM image from the microtubule region. In (d), the observed tube-shaped structures are the MTs lying under the graphene and the round bumps are the possible liquid pockets. The white arrow shows a single MT lying on the substrate and the yellow arrow shows MTs crossing over each other. e) TEM image of a microtubule in a graphene enclosure obtained from a fresh spot without pre-exposure. f) Plot profile of the red line in (e) showing the width of the MT and 2-striation inside originating from the protofilaments. g) FFT image obtained from the yellow box in (e) showing the four equatorial reflections. Scale bars in c, d and e are 3 µm, 1 µm and 50 nm, respectively. The TEM image in (e) was recorded at 200 kV and -7 µm defocus.

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