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Meeting-report

Towards Correlative Electron Microscopy Imaging for Proteins and Cells

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Emergent application of liquid-phase transmission electron microscopy (TEM) to biological systems such as DNA molecules [1], proteins [2], viruses [3], and full cells [4, 5] has revealed the dynamic motions, hydrated structures, and spatial distributions of nanoparticulate carriers in cells, all of which are not accessible to traditional dried or cryogenic TEM. Meanwhile, these experiments still experience multifold challenges in terms of debatable electron beam effects on the samples, loss of spatial resolution due to motion blurring and the presence of a liquid environment, and a lack of three-dimensional (3D) molecular-level information. Our previous work [2] showed that the membrane protein dynamics observed in graphene liquid cells (GLCs) are validated as the generic transformations occurred when immersed in a liquid environment, through comparisons with molecular dynamics (MD) simulation. Biophysics-based analysis was performed to extract the biomechanical properties such as the bending modulus and effective interfacial tension of the protein-liquid assemblies.

Building upon our previous work on imaging biological systems, here we introduce advancements in two directions for protein and cell imaging respectively. In the first case, we examine in detail the morphology difference for the cytochrome P450 2J2 nanodiscs in equilibrium exhibiting seemingly intact relaxation fluctuations, and those that are damaged over extended periods of observation time due to accumulated doses. In equilibrium, the nanodiscs are largely of a circular disk shape with fluctuations due to lipid diffusion and bending flexibility of the membrane scaffold proteins. When the imaging time is more than ~5 min under a dose rate of 100 e^{-} $Å^{-2}$ s^{-1}, visible extension along the disk direction (and thinning of the disk) was consistently observed before the disintegration of the nanodiscs. This result shows clear visible differences for the nanodiscs undergoing equilibrium fluctuations from those being damaged. Particularly, in the presence of liquids, beam-induced structural damage manifests as visible disappearance of proteins, likely due to the diffusions of protein fragments in liquids. The second advancement is to push from single molecule imaging to the imaging of cells. For cell imaging, SiN_x-based liquid chambers were used given the large size of cells. As shown in Figure 1A, neuron cells with neurites were cultured on the chip surface (the features under optical microscope). The nanoscale morphology of the neuron cells is hard to directly observe under the current liquid-phase TEM setup due to the large sample thickness, where we expect that the observations of thin regions of cell periphery are still possible. Meanwhile, as a comparison and potential correlative observations, neurons were cultured by similar processes on the polyurethane acrylate (PUA) substrate, and imaged with epoxy infiltration. The anticipated neuronal elements such as synaptic vesicles, synaptic mitochondria and neurofilaments were clearly shown in TEM imaging (Figure 1B). We foresee culturing of neuron cells onto liquid-phase TEM chips as the first step towards imaging their hydrated structures and synaptic transmission process at the resolution not accessible before, and ex-situ TEM imaging can serve as correlative comparison to highlight morphology details [6].

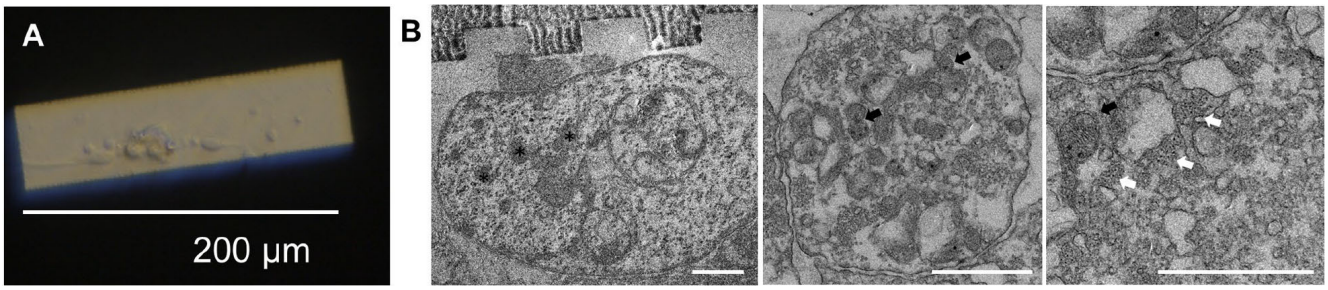

Fig. 1. (A) Optical microscopy image of neuron cells on the SiN_x window of liquid-phase TEM chamber. (B) Epoxy infiltered neurons cultured on PUA substrate. Asterisk: synaptic vesicle; black arrow: mitochondria; white arrow: neurofilament. Scale bars: 1 µm (B).

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