Chemical reactions or biochemical activities often occur in the presence of a liquid. To study liquid sample in an electron microscope, several liquid cell designs have become commercially available in recent years that enable materials to be imaged in a carefully controlled liquid environment within the vacuum of a TEM. However, the Si3N4 layers (50-500 nm thick) used as electron transparent windows in these liquid cells have limited the imaging resolution to nanometers [1], also degraded the electron energy-loss spectroscopy (EELS) signal [2]. On the other hand, radiation damage is a fundamentally limiting factor when examining beam sensitive materials and/or hydrous samples in TEM. Traditional coating and cryo techniques [3, 4] have shown positive effects against radiation damage and the results suggest that it is possible to reduce radiation damage to below breakage of covalent bonds. However, further reduction of radiation damage is needed for characterization of biological samples, since many biological structures and functions are related to the much weaker hydrogen bonds. We introduce a biocompatible approach of encapsulating liquid-containing samples in monolayers of graphene. This not only allows biological samples to be directly imaged at atomic resolution in a native liquid state without limitations from the window thickness, but also enables nm-scale analysis using EELS to quantify biochemical reactions in an aqueous environment (Fig. 1 and 2) [5]. We show that the graphene encapsulation provides a radiation damage reduction mechanism, allowing for high resolution imaging and spectroscopy of beam sensitive materials. Details, such as individual Fe atoms or polypeptides of unstained protein, are resolved in a liquid environment. EELS elemental identification of ferritin molecules with 1 nm resolution is achieved showing both the iron core and the protein shell. We also show that beam induced reactions can be initiated in-situ and monitored in real time at nm precision inside graphene liquid cells (GLC) (Fig. 3). By carefully controlling the induced electron dose rate, we show that radiation damage can be limited to be within hydrogen bond breakage level, preserving the functionality of the ferritin protein while observing the valence change of the iron core showing initial stages of iron release by ferritin.

References:

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Fig. 1: Schematic diagram, as well as HAADF images (A and B) of ferritin molecules in graphene sandwiches (A) and GLCs (B). (B) shows individual Fe atoms in a liquid environment. (C) is a EELS map of ferritin molecules sandwiched between graphene sheets with 1 nm resolution, showing both the protein shell and the iron core inside a graphene liquid cell.

Fig. 2: EEL spectra of ferritin (1, 2, 4, 5) and water (3). (1) and (2) are taken from the iron core (1) and protein shell of ferritin (2) in graphene sandwiches where the liquid has dried. (3, 4, 5) are taken from water (3), at the center of the iron core (4), and at the edge of a ferritin molecule (5).

Fig. 3: Stills from a video showing controlled beam induced local bubble formation and condensation process in GLC. The video is recorded via repeat scanning in HAADF mode. In selected areas a bubble is formed at 00:06s, 00:15s, and 00:22s respectively by temporary switching to small area scanning.