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High Resolution In-situ Study of Reactions in Graphene Liquid Cells

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Nanoparticle growth, chemical reactions or biochemical activity 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, all suffer from a few key limitations that do not allow for ultra high-resolution imaging or spectroscopy [1]: 1) two Si_3N_4 layers (50-500 nm thick) used as electron transparent windows and 2) the thickness of the liquid surrounding the sample. In these liquid cells, the imaging resolution is usually limited to nanometers. Electron energy-loss spectroscopy (EELS) is degraded by multiple scattering events in the thick window layers, and the strong core-loss signals associated with the presence of Si and N [2]. In addition to the increased sample thickness, radiation damage is a fundamentally limiting factor when examining beam sensitive materials and /or hydrous samples in TEM. It has been shown that coating the specimen with carbon, metal or graphene [3-7], or lowering the temperature[6] have positive effects against radiation damage by reducing electrostatic charging [3], mass loss [4], loss of crystallinity [5], or defect formation rate [6,7]. These studies 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.

In this research, we develop 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 reactions in a aqueous environment (see Figure 1) [8]. We show that the energy deposited by the incoming electrons is dissipated by graphene from the area irradiated at a rate equivalent to the beam current of several electrons per $Å^2$ per second. This would therefore provide a reduction of radiation damage, allowing for high resolution imaging and spectroscopy of beam sensitive materials. Details, such as individual Fe atoms or polypeptide 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 show that using selective small area scanning, beam induced local physical or chemical reactions can be initiated in-situ and monitored in real time at nm precision inside graphene liquid cells (GLC) (see Figure 2). By carefully controlling the induced electron dose rate, we show that reactions, such as liquid/ gas phase transition (bubble formation and condensation), or nanoparticle/ nanowire growth can be initiated at selected locations in GLC, and recorded at nm resolution. This technique also allows us to perform a quantitative study of radiation damage effect on different encapsulated samples, such as water or protein, by observing local reaction processes. We find that different types of chemical bonds can be selectively broken in a controlled fashion by adjusting the electron dose rate. This further allows us to limit radiation damage of beam sensitive materials to be within the hydrogen bond breakage level. Therefore, it is possible to conserve the interactions between amino acid residues and preserve the functionality of hydrous biological samples while maintaining the imaging and EELS resolution.

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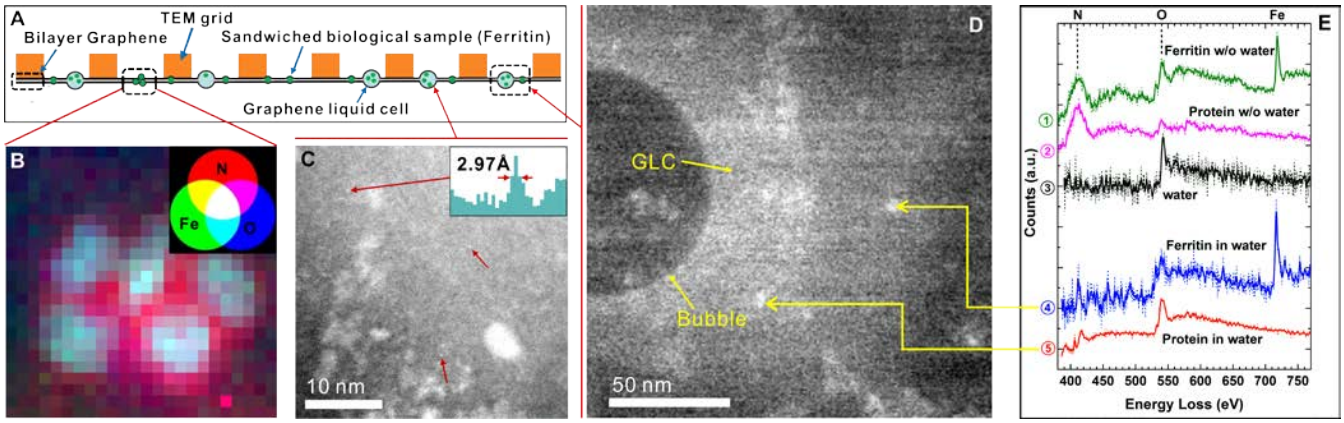

Figure 1. Schematic diagram (A), as well as STEM images (C and D) of ferritin molecules in GLCs and graphene sandwiches. (B) is a EELS map of ferritin molecules sandwiched between graphene sheets with 1 nm resolution, showing both the protein shell and the iron core. (C) shows individual Fe atoms in a liquid environment. (E) shows spectrums 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) is taken from water inside a GLC, (4) (5) were acquired through repeat scanning of a $(1.3\ nm)^2$ area located at the position shown in **(D)** with a below bubble formation threshold area averaged dose rate and a total scanning time of 1s. (4) is taken at the center of the iron core. (5) is taken at the edge of a ferritin molecule.

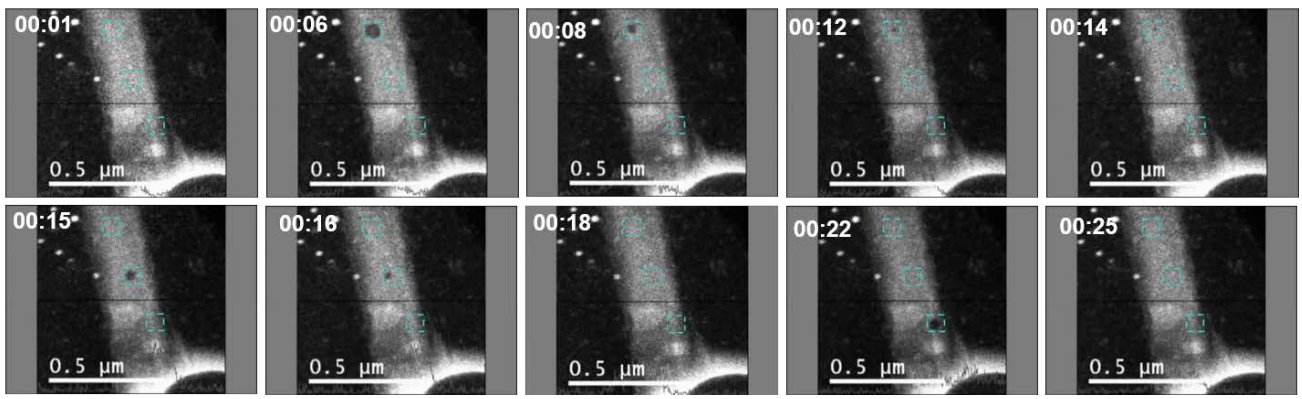

Figure 2. Video clips showing controlled beam induced local bubble formation and condensation process in GLC. The video is recorded via repeat scanning in HAADF mode. In selected areas a bubble is formed at 00:06s, 00:15s, and 00:22s respectively by temporary switching to small area scanning.

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