In-situ Observation of Individual Ferritin Molecules in Graphene Sandwiched Structure

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It is important to reveal three dimensional structures of proteins with atomic resolution for drug design and development and for understanding how drugs work. Transmission electron microscopy (TEM) is one of the powerful tools to determine structures of nanomaterials [1, 2]. However, we cannot introduce proteins directly into the TEM chamber because TEM samples need to be put into vacuum. Therefore, we usually embedded proteins inside amorphous ice film [3]. Although the amorphous ice can keep structures of proteins even under vacuum and electron beam irradiation conditions, the ice supporting layer with a thickness of a few hundred nanometers inhibits high-resolution observations of relatively small proteins having a diameter of 5 ~ 10 nm. To consistently realize high resolution, supporting layers should be thinner rather than several tens of nanometers.

In this work, we have applied a new technique to support proteins in a very thin water layer encapsulated inside two graphene sheets [4], and successfully observed a cage structure of a ferritin molecule, which is more difficult to be visualized so far comparing to a capsulated iron core.

Figure 1 shows a schematic illustration of the graphene sandwiched structure. Water and sample molecules were packed between two layers of the monolayer graphene sheets. The inside liquid layer is not formed uniformly but dappled with various sizes. At the other region with no puddles, two graphene sheets contact with each other and they seal liquid molecules. Thickness of the liquid layer can be reduced to less than several tens of nanometers, depending on size of the puddles, which can provide atomic-resolution imaging.

Figure 2(a) shows a TEM image of a monolayer graphene. This represents almost no contrast indicating a single crystal with no defects in the wide area. By using such uniform graphene sheets, ultrapure water was sandwiched between them, as shown in Fig. 2(b). This image shows dark contrast area where water molecules are unevenly enclosed. In contrary, brighter area corresponds to bilayer graphene for sealing water region. When protein-dispersed water is utilized, the protein molecules can be encapsulated with very thin water layer inside the graphene package.

Figures 3(a) shows a structural model of a ferritin molecule. Ferritin is a cage-shaped protein which has a role to store the iron(Ⅲ) inside. This illustration shows an iron oxide core in yellow and red at the center of rainbow colored proteins. Approximately 2500 iron atoms are stored in the ferritin
molecule and a diameter of the core is approximately 7 nm. Figure 3(b) shows a TEM image of the ferritin molecule in solution with the graphene sandwiched structure. Lattice fringes of the iron oxide can be clearly seen at the center of the image. It is also found that weak contrast due to the proteins is wrapped around the iron crystal in the image, if carefully observed. Detailed procedures and results will be presented at the poster session.

**References**


![Figure 1](image1.png)

**Figure 1.** An illustration of water encapsulated inside graphene sandwiched structure.

![Figure 2](image2.png)

**Figure 2.** TEM images of (a) mono-layer graphene and (b) ultrapure water encapsulated inside of graphene sandwiched structure.

![Figure 3](image3.png)

**Figure 3.** (a) Illustration of ferritin molecule designed by PDB file based on the X-ray crystal structure analysis result and (b) TEM image of ferritin in solution sandwiched by graphene layers.