In-situ TEM observation of liposomes in liquid cells using graphene sandwiched holy silicon nitride grids

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Commercial liquid TEM holders and chips for *in-situ* TEM observation have made great innovation, especially, for samples composed of high contrast heavy elements distributed in a solution. Recent days, the method is improving to be more powerful and more common for observation of dynamic reactions or motions in liquid phase. [1] Nevertheless, for soft matter samples composed of low contrast lighter elements (such as H, C, N, O), we sometime require better signal to noise ratio and enhance contrasts. The improvements may further activate not only soft matter materials sciences, including polymers or gels, but also medical, pharmaceutical, or biological sciences.

Two main reasons of difficulties of in-situ observation of soft matter samples using commercial systems are the thickness of silicon nitride membrane and expansion of the thickness of liquid layer due to high vacuum of TEM column. To obtain better signal to noise ratio and enhance contrasts, we used graphene as an ideal ultra-thin membrane of tough carbon sheets for packing liquid with thickness of only few carbon atoms and good electric and thermal conductivity.

Graphene has already been used as liquid cells membrane. [2, 3, 4] Conventional graphene liquid cells packed sample solution between graphene layers, which directly adhered due to van der Waals force. Therefore, variety of their thickness difference of liquid layer was utilized and high-resolution images have been observed successfully. Here, we designed graphene liquid cells with a spacer of commercial silicon nitride electron microscope (EM) holy grids with certain thickness. The advantage of silicon nitride EM grids is designable of hole diameters and pitch by MEMS technique in addition to cleanness, hardness and not deformed property. Silicon nitride membrane were sandwiched by graphene sheets deposited on both top and bottom surfaces. The thickness of liquid layer and cell volumes can be easily and precisely controllable. Moreover, due to regularly aligned many holes with much wider field of view, high throughput of data acquisition can be easily achieved using conventional automatic data acquisition cryo-EM software.

In this experiment, we observed liposomes as a reference sample of soft matter using the graphene liquid cells. Liposomes consist of lipid bilayers, which is one of the typical soft matter samples. This sample is promising candidate material as drug or gene delivery carrier, or thermal therapy with encapsulation of such as anticancer drugs, si-RNA, or nano-sized gold particles. [5, 6] For these applications, fine structural analysis, especially in liquid phase, is essential.

We got clear images of liposomes using our graphene liquid cells. We will discuss the analysis of the images or movies, compared to images of liposomes using cryo or conventional silicon nitride based liquid cells.

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