In-Situ Liquid Electron Microscopy for Imaging Proteins and Polymer Assemblies in their Native State

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Electron microscopy (EM) is one of the most powerful techniques for structural determination at the nanoscale, with the ability to image matter down to the atomic level. The short wavelength associated with electrons is pivotal for achieving high resolution. Electrons interact strongly with matter hence electron microscopy is only possible by keeping the beam under high vacuum in order to avoid undesired scattering events in the beam path. High vacuum comes at the expense that the EM samples conventionally must be in the solid-state. This means that samples in liquid form or containing liquids- especially water- require special preparation techniques involving either controlled drying or cryogenic treatments, which often alters the microstructure and chemical nature of the sample. Such alterations become particularly critical for biological samples and soft materials where meso- and nano-scale structure are controlled by the presence of water (and/or other liquids). While some of these limitations can be overcome using fast vitrification processes to solidify liquid samples, vitrified water is not liquid water and its structure and hydrogen bond network is very different. Even so, cryogenic TEM is still limited to providing static snapshots, and does not allow **dynamic observation**. An efficient approach for TEM imaging of materials in liquid form is achieved by encapsulating the liquid sample within electron-transparent materials such as graphite foils , silicon nitride films (Si_3N_4) , ionic liquids or graphene sheets which form a barrier between the liquid medium and the high vacuum of the microscope column.[1] We now have the possibility of using **Liquid TEM (LTEM)** to study nanoscopic structures within liquids and monitor dynamic processes. This approach offers a step-change in our ability to study matter in its “virgin-state” on the nano and micron scale, removing the artifacts induced by drying or cryogenic treatments.

We investigate two different soft organic systems in liquid state *in-situ* (i) a polymer assembly with a localised asymmetry , and (ii) the well known ferritin protein. Both systems were imaged over time with a high frame-rate- acquisition direct detection camera, the K2-IS from Gatan. The ultra-high sensitivity of the K2 allows low-dose imaging modes limiting considerably electron dose damage and facilitating high spatial and temporal resolution.[2] Particles were imaged displaying brownian translational and rotational motion withour the need for staining agents. A high variety of particle profiles were then acquired over time for both systems (i) the polymer assembly and (ii) the protein ferritin hence making time a fundamental element of the particle 3D reconstruction process. These findings set the foundation for future time-resolved 3D structure reconstruction of unlabeled soft organic materials in their native environment. Moreover our results open a new avenue for next generation biological *in-situ* TEM.
This study was accomplished using a Jeol JEM 2200FS equipped with an in-column omega filter in combination with the *in-situ* K2-IS camera from Gatan. The *in-situ* liquid TEM holder used was the Ocean holder from DENS solution.

References

1. Williamson et al. *Nature Mater*, 2003,2,552.
2. Milazzo et al. *Ultramicroscopy,* 2010,110,741