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Investigation of In Situ Radiation Effects in Liquid Cell Electron Microscopy

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Electron-matter interactions play utmost importance during the examination of soft materials, including polymers, cells, etc. The amounts of electron dose rate, total electron dose and incident voltage have tremendous effects on the formation of radiation byproducts in liquid media. Over the past few years, in situ electron microscopy in liquid environment has experienced a surge of interest. Some of the recent applications have included the imaging of labeled structures within whole cells [3, 4] and solution-phase nanoparticles [5]. Fluid cell holders were introduced to allow live imaging in an electron microscope with a relatively thick Si_3N_4 window (usually 15 to 50 nm thickness), whereas the more recently introduced GLC-TEM only uses monolayers of graphene (0.2 to 1 nm thickness), improving the imaging resolution. Relative resistance of the two in situ techniques toward the radiation byproduct formation is evaluated in this work in terms of both monitoring morphology changes via electron imaging and via Monte Carlo simulation calculations of energy depositions per nm^3 voxels during electron imaging [9, 10]. Single scattering method was used with the assumption of the interaction of only the primary electrons with the organic matter [11]. Both computer simulation and electron microscopy experiments on soft materials including ferritins and filomicelles in liquid cell enclosures were carried out to understand the radiation effects of electron exposure.

In the Monte Carlo computer simulation research, ferritins were evaluated for their behavior under electron exposure in a 200 pixels X 200 pixels scanning field with 1000e- per pixel (1 pixel: 1nm X 1 nm) during STEM imaging [12]. Ferritins are considered as key molecules in iron homeostasis in an organism [12]. Characterization of these structures in an electron microscope requires utmost care to be applied to the imaging conditions due to the protein cage present in the shell of these structures. For the GLC-TEM case, ferritins are simulated to be encapsulated in between 1 nm of graphene (2-3 layers) and 2 nm of water (Fig.1A) and irradiated with both 80 (Fig. 2A) and 200kV (Fig. 2B) electrons. For the fluid cell case, ferritins are simulated to be encapsulated in both between 15 (Fig. 1B and Fig. 2C) and 50 nm (Fig.1C and Fig. 2D) Si_3N_4 windows and irradiated with 200kV electrons. The max energy deposited by electrons were reported as 23.3 and 9.6 eV/nm^3 for ferritins in GLC at 80 and 200kV, respectively, showing less electron beam induced damage to biological sample at 200kV, while the carbon-knock on damage for graphene occurs at 200kV and not at 80kV. 15 and 50 nm Si_3N_4 windows result in 42 and 68.8 eV/nm^3 energy deposition upon electron exposure, respectively, suggesting less electron damage in 15 nm window thickness, which will also result in formation of a lower number of secondary electrons.

In the electron microscopy imaging experiment to understand the electron damage on soft matter, filomicelles were investigated in GLC-TEM enclosures at 80kV for their degradation under electron overexposure. Filomicelles have been used as nanocarriers for long term drug release, which is an objective of considerable interest in medicine [13]. When composed of oxidation-sensitive polymers, they can undergo degradation via the cylinder-to-sphere transition under oxidative environments or in the proximity of inflammatory cells, resulting in the conversion of cylindrical filomicelles into spherical micelles. This oxidative environment can be simulated in electron microscope by mixing the nanoparticle medium with oxidative byproducts of radiolysis of water, specifically H_2O_2. This is achieved by encapsulating the samples

in GLC and bombarding them with electron-beam via TEM, which results in the formation of more oxidative by-products during radiolysis, namely, OH*, O_2 and H_2O_2 [14]. The presence of these species triggers oxidation-induced-degradation of the filomicelle-hydrogel into micelles [15] and observed in this work as well [16].

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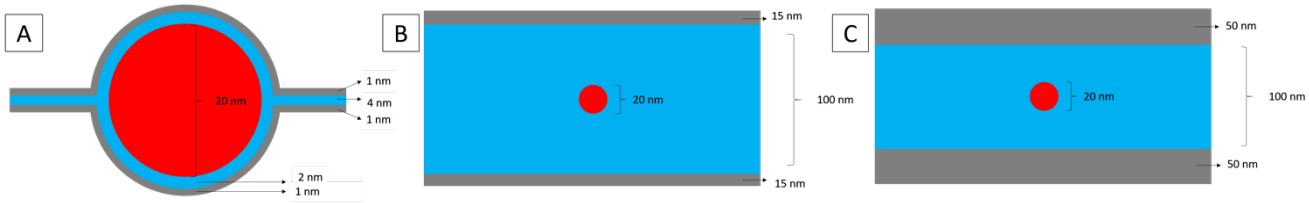

Figure 1. Experimental setup for A) GLC and B, C) Fluid cell STEM experimentation.

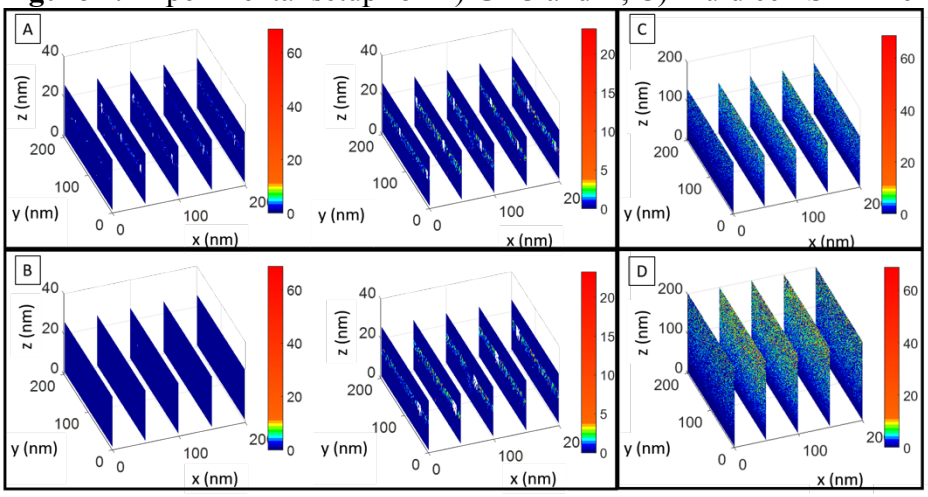

Figure 2. Monte Carlo simulation results for the energy deposition for 1) GLC-STEM at 80kV, 2) GLC-STEM at 200 kV, 3) Fluid cell-STEM with 15 nm thick Si_3N_4 window and 4) Fluid cell with 50 nm thick Si_3N_4 window (energy unit: eV/nm^3).