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Elucidation of Structure and Chemistry of Iron Core in Human Heart Ferritin *via* Graphene Liquid Cell

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Ferritin is a spherical protein complex that plays a major role in iron storage and metabolism. In the absence of ferritin, free-floating iron ions can form reactive oxygen species, thus interfering with the body's metabolism [1]. Despite ferritin's iron regulation, there are chronic cardiovascular diseases such as iron overload cardiomyopathy [2], and myocarditis associated with elevated iron levels in different organs [3]. In order to understand the cause of these diseases, investigating human heart apoferritin biomineralization is crucial. The evaluation of the biomineralization pathway from apoferritin to ferritin will provide insight into ferritin dysfunction. Ferritin's iron core is speculated to consist of several iron oxide nanoparticles, which are reported to be different minerals such as ferrihydrite, hematite and magnetite [4]. However, the iron core's exact mineral composition has not been yet reported. To that end, this work will investigate the biomineralization of iron oxides in apoferritin *via* advanced analytical Transmission Electron Microscopy (TEM) techniques. Figure 1 shows horse spleen ferritin imaged in dry condition acquired from 25kV LVEM25 electron microscope. The dark colored spots indicate horse spleen ferritin.

Ferritin is located throughout the human body. To simulate ferritin's natural environment, the biomineralization process of iron within apoferritin should be characterized in a liquid environment with the highest resolution available. This necessitates the use of graphene liquid microscopy (GLC) TEM. GLC has advantages over other techniques, such cryo TEM imaging because the sample does not need to undergo fast freezing or blotting processes [5]. GLC is also advantageous over *in-situ* fluid cell imaging because GLC can encapsulate the thin liquid sample without the extra thickness of silicon nitride surfaces and thick layer of liquid that reflects the electron beam, which makes HRTEM (High Resolution TEM) imaging and spatially resolved electron energy loss spectroscopy (EELS) difficult.

Human heart ferritin will be demineralized in presence of ferrizine, an organic chelator, to form apoferritins [5]. The pH of the solution will be maintained and the demineralization process will run for 60 minutes. After this process is complete, the apoferritins will be separated from the iron chelates by dialysis and the absorbance will be measured [6]. JEOL-ARM200CF Cs corrected Scanning / Transmission Electron Microscope (S/TEM) will be utilized to image the demineralization process Encapsulation of the iron into the apoferritin will be visualized in STEM mode, due to Z contrast of iron. After that, crystallinity information and chemical environment information for iron and oxygen will be continuously collected by selected area electron diffraction (SAED) and Fe L_3 edge and O K edge EELSfrom individual iron cores throughout the whole biomineralization process, respectively. Crystal structure will be verified *via* HRTEM, as shown in Figure 2. From the acquired HRTEM image as shown in Figure 2, the material might be polycrystalline in nature with grain boundaries. The interplanar distance was measured to be 0.25nm, which is similar to the (110) plane of ferrihydrite. This suggests that the nanoparticle might consist of ferrihydrite [7].

The knowledge gained from understanding the biomineralization process in apoferritins will give clues about the reasons for the presence of dysfunctional ferritins, causing diseases. As a long-term goal, the deviations of ferritin structures from the biopsies obtained from patients will be compared with the healthy human ferritin for the early diagnosis of cardiovascular diseases [8].

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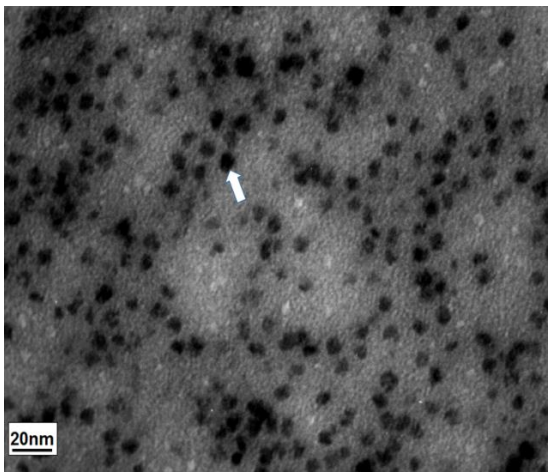

Figure 1. TEM image showing the distribution and morphology of horse spleen ferritin acquired from 25kV LVEM25 electron microscope. Arrow in the figure indicates the ferritin nanoparticle (Image Courtesy: Eva Coufalova, Delong Instruments)

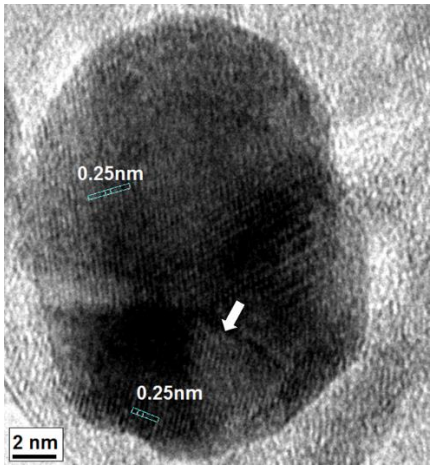

Figure 2. HR-TEM image of the heart ferritin acquired from JEOL JEM 3010 showing lattice fringes with interplanar spacing 0.25 nm (110) plane. Arrow in the figure indicates the grain boundary.