Supplementary Information

On the Structure and Chemistry of Iron Oxide Cores in Human Heart and Human Spleen Ferritins using Graphene Liquid Cell Electron Microscopy

Surya Narayanan^{a, &}, Emre Firlar^{a,b, &}, Md Golam Rasul^b, Tara Foroozan^b, Nasim Farajpour^c, Leigha Covnot^a, Reza Shahbazian-Yassar^{b*}, and Tolou Shokuhfar^{a*}

^a University of Illinois at Chicago, Department of Bioengineering, Chicago IL, 60607, USA

^b University of Illinois at Chicago, Department of Mechanical and Industrial Engineering, Chicago IL, 60607, USA

^c University of Illinois at Chicago, Department of Electrical and Computer Engineering, Chicago IL, 60607, USA

[&]Equal contribution; co-first authors

*Corresponding author(s): Email: tolou@uic.edu, rsyassar@uic.edu

Representation of all the d- spacings collected from different samples

D spacing of area 1	Matching iron oxide	D spacing	Matching iron oxide	D spacing of area 3	Matching iron oxide	D spacing of area 4	Matching iron oxide	D spacing of area	Matching iron oxide
(Ā)		of area 2 (Å)		(Å)		(Å)		5 (Å)	
		3.0	M(022)	3.2	FeOOH (020)	3.0	M(022)	3.0	M(022)
2.7	H(104)	2.7	H(104)	2.7	H(104)	2.7	H(104)	2.7	H(104)
2.2	H(113)/ Fh(112)	2.2	H(113)/ Fh(112)	2.1	Fh(112)	2.2	H(113)/ Fh(112)	2.2	H(113)/ Fh(112)
1.9	Fh(113)	1.9	Fh(113)	1.9	Fh(113)	1.9	Fh(113)	1.9	Fh(113)
1.6	H(116)	1.6	H(116)	1.6	H(116)	1.6	H(116)	1.6	H(116)
1.4	H(300)/ Fh(300)	1.4	H(300)	1.4	H(300)	1.4	H(300)	1.4	H(300)

Table S1: Diffraction from HSF in dry state

Table S2: Diffraction from HSF in wet state

D spacing	Matchin	D	Matching	D	Matching	D spacing	Matchin	D spacing	Matching iron
of area 1(Å)	g iron oxide	spacing of area 2 (Å)	iron oxide	spacing of area 3(Å)	iron oxide	of area 4 (Å)	g iron oxide	of area 5 (Å)	oxide
3.5	H(012)	3.5	H(012)			3.5	H(012)	3.7	H(012)
3.1	M(022)/ FeOOH (020)	3.1	M(022)/ FeOOH (020)	2.9	M(220)/- Fh(110)	3.0	M(022)/ FeOOH (020)	3.1	M(022)/ FeOOH (020)
1.9	Fh(113)	1.9	Fh(113)	1.9	Fh(113)	1.9	Fh(113)	1.9	Fh(113)
1.7	Fh(114)	1.7	Fh(114)	1.7	Fh(114)	1.7	Fh(114)	1.7	Fh(114)
1.5	Fh(115)	1.5	Fh(115)			1.5	Fh(115)	1.4	Fh(300)
1.1	H(128)	1.1	H(128)	1.1	H(128)	1.1	H(128)	1.1	H(128)

Table S3: Diffraction from HHF in dry state

D	Matchin	D	Matching	D	Matching	D	Matching	D spacing	Matching iron
spacing	g iron	spacing	iron oxide	spacing	iron oxide	spacing	iron	of area 5	oxide
of area	oxide	of area		of area		of area	oxide	(Å)	
1(Å)		2 (Å)		3(Å)		4 (Å)			
2.6	H(104)	2.7	H(104)	2.7	H(104)	2.7	H(104)	2.7	H(104)
1.9	Fh(113)	1.9	Fh(113)	1.9	Fh(113)	1.9	Fh(113)	1.9	Fh(113)
1.6	H(018)	1.5	H(018)	1.5	H(018)	1.6	H(018)	1.6	H(018)
	/M(511)								
	/MH(51								
	1)								
1.3	H(1010)	1.3	H(1010)	1.3	H(1010)	1.3	H(1010)	1.3	H(1010)
1.2	H(220)	1.2	H(220)	1.2	H(220)	1.2	H(220)	1.2	H(220)

Table S4: Diffraction from HHF in wet state

D spacing of area 1(Å) 3.8	Matching iron oxide γ- Fe ₂ O ₃ (D spacin g of area 2(Å)	Matchin g iron oxide	D spaci ng of area 3(Å)	Matchin g iron oxide	D spacin g of area 4 (Å) 4.6	Matchin g iron oxide Fh(002)	D spaci ng of area 5 (Å)	Matching iron oxide	D spacing of area 6 (Å)	Matching iron oxide
2.8	220) ¹ Fh(110)/ M(220)/ MH(220) /ɛ Fe ₂ O ₃ (41 1)/ M(220)/ MH(220) /ɛ Fe ₂ O ₃ (41 1)	3.0	M(220)/ MH(220)/ Fh(110)	3.0	M(220) / MH(220)/	2.7	H(104)	2.7	H(104)	2.7	H(104)
		2.2	Fh(112) /H(113)	2.2	Fh(112)/ H(113)						
						2.4	Fh(110)	2.4	Fh(110)	2.4	Fh(110)
		2.0	Fh(113)	2.0	Fh(113)					2.3	Fh(112)
1.8	H(024)			1.8	H(024)	1.8	H(024)	1.8	H(024)	1.8	H(024)
1.5	Fh(115)			1.6	H(116)/ MH(513)	1.6	H(116)/ MH(513)	1.6	H(116)/ MH(513)	1.6	H(116)/ MH(513)
		1.3	H(1010)			1.3	H(10 <u>10</u>)	1.3	H(10 <u>10</u>)	1.3	H(10 <u>10</u>)
1.2	H(220)	1.2	H(220)					1.2	H(220)	1.2	H(220)



Figure S1. Structural Characterization of HSF and HHF in dry state: **A**. Bright field TEM (BF-TEM) image and the corresponding SAED inset of HSF: SAED pattern of HSF collected from the represented area indicates different d spacings of 3.1, 2.7, 2.2, 1.9, 1.5 and 1.4 Å representing crystal structures with different family of planes: G(020)/M(220), H(104), Fh(112) or H(113), Fh(113), H(108), and H(300), respectively.⁸ **B**. BF-TEM image and the corresponding SAED inset of HHF: SAED pattern of HHF collected from the represented area indicates different d spacings of 2.7, 1.9, 1.5, 1.3 and 1.2 Å representing crystal structures with different family of planes: H(104), Fh(113), H(1010) and H(220), respectively. Red, Yellow and Blue color represents FeOOH, hematite (H) and ferrihydrite (Fh), respectively (Scale bars of the TEM image in Figure A and B: 10nm)

SAED collected from the represented area of HSF and HHF in dry state



EELS collected from wet and dry HSF :

Figure S2. Chemical Characterization of HSF in wet and dry state: A. STEM-EELS shows the comparison of Fe $L_{2,3}$ edges in HSF iron cores in wet and dry condition. **B.** Relative area integral ratio of $L_3:L_2$ Fe edges in both wet and dry HSFs. The lower ratios in HSF (4.14 ± 0.06) in dry state as compared to HSF (4.24± 0.06) in wet state indicates the presence of Fe⁺³ in higher quantities in wet state as compared to dry ferritins (n=3 for both the kind of analysis)

The ratio of O/Fe or Fe^{3+}/Fe^{2+} was reported in case of HSF in dry and wet state to draw a comparison of iron oxide minerals in dry and wet state. The mechanism by which iron oxides convert from iron(III) oxide to iron(II) oxide during the degradation of the protein structure is not well explored.

Integrity of GLCs after electron beam exposure

Earlier studies have shown that GLC fabrication methods have implications in preserving the liquid state of the protein. It is reported that the imperfections in the graphene contributes to cracks during fabrication, resulting in water leakage. However, the GLCs used in this work has multiple layers and resistant to damage.² Figure S3 shows the time series images of one such GLC pockets in the Ronchigram mode (See Video S1). The higher electron dose in Ronchigram mode induces the bubble formation and increasing the image magnification results in the increased size of the bubble. To control the formation of bubble in Ronchigram mode, the videos were also recorded at lower magnification. In the HAADF-STEM image, the electron dose rate was controlled experimentally to prevent the bubble formation.



Figure S3: Time series TEM images liquid encapsulation within the GLCs and bubble formation in liquid. The formation of bubbles is controlled by the electron dose rate. The bubbles grow with higher electron dose rate as observed in Figure S3-B and C

The presence of liquid in HAADF-STEM mode was evident by the observation of bright contrast region in the image (Figure S4–A). The dark circular areas in the image indicates the water bubbles formed because of electron beam irradiation during EELS acquisition (Figure S4-B). The 9 eV plasmon peak in the low-loss spectrum further confirmed the presence of liquid.



Figure S4: Evidence of water presence in the liquid pockets of GLC. A. HAADF-STEM image of water in GLC. The dark circular areas indicated by the red arrows are the bubbles formed because of electron beam irradiation during EELS acquisition. The White color square indicates the region of interest from which the EELS low loss spectrum was acquired. Scale bar is 50 nm. **B.**

Low-loss EELS spectrum of water in GLC. The first peak at 5eV indicates the $\Pi \rightarrow \Pi^*$ transition of the graphene layer.³ The second peak at 9 eV indicates the presence of water.⁴ The third peak at 33 eV indicates the $\Pi + \sigma$ Plasmon of graphene.⁵

The integrity of GLCs after electron beam exposure was also evaluated in this study (Figure S5). Figure S5- A and C shows the HAADF-STEM image before and after the EELS acquisition. During EELS acquisition, the liquid cells were exposed to high electron dose of 10^6 e/Å^2 (Figure S5-B). Although significant damage, and drying out of water were expected, it was observed that the liquid cells remained intact (Figure S5–C). While the formation of bubbles was evident during EELS acquisition, the bright region in the HAADF-STEM image indicates the presence of liquid.



Figure S5: The integrity of GLC after chemical characterization. A. The HAADF-STEM image acquired from the GLC area before the EELS acquisition. The contrast in the image is due to the bubble formation and the liquid area contributes to the brightness in the image. **B.** The HAADF-STEM image with the selected area (white color box) from where the EELS spectrum was acquired. Inset in figure B shows the average low loss signal collected from the selected area. The arrows at 5, 9 and 33 eV indicate the presence of graphene ($\Pi \rightarrow \Pi^*$) transition, the hydrogen gas bubble formed as a radiolysis product, and ($\Pi \rightarrow \sigma$) plasmon of graphene, respectively (as also shown in Figure S4). **C.** The HAADF-STEM image collected from the same area after EELS acquisition indicates that the integrity of graphene liquid cell is maintained even after the chemical characterization.

Thickness measurement of graphene layers and its effect on electron dose

Raman Spectroscopy studies were carried out to determine the number of layers of graphene present in GLCs. Figure S6 indicates the peaks referring to G and 2D bands of graphene 1580 cm⁻¹ and 2700 cm⁻¹, respectively. By calculating the ratios of the intensity of the G and 2D peaks, as well as observing the near-edge structure of 2D peaks, it is possible to identify the thickness of the graphene layers⁶. For a single layer graphene, the intensity of the 2D peaks will be greater than the intensity of G peak. Also, the 2D peak would exhibit a sharper and systematic fit at higher intensities. However, with increase in the number of layers, the intensity of the 2D peak declines. Also, the peaks become broader, with certain distinct features⁶. Based on this analysis, the number of layers in GLCs are approximately 3-4, as observed from Figure S6.



Figure S6: Evidence of Raman Spectroscopy indicating the thickness of graphene in GLCs. The ratio of $I_{2D/G} \le 2$ indicates the presence of multiple layers of graphene.

There is a pronounced change in the behavior of electron induced radiolysis when there are single layer vs multiple layers of graphene. This was also demonstrated recently in presence of MoS_2^7 . While the conductive single layer of graphene can help dissipate the heat and the charge from the electrons, multiple layers of graphene can provide structural stability⁷. Having multiple layers can also prevent the displacement of ionized ions from the graphene layers, thus protecting the integrity⁷.

Video S1:

Bubble formation in GLC confirms the presence of liquid: This video demonstrates the presence of liquid in GLCs via the Ronchigram mode. The GLCs are identified at lower magnification based on the contrast that appears as a result of the presence of thick liquid. Further, the bubble formation is induced as a result of higher electron dose. When the electron dose is lowered, the bubbles that are already formed condense to form the liquid.

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