

## Supporting Information

of

# Ferritin Light-Chain Subunits: Key Elements for the Electron Transfer Across the Protein Cage

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### Materials and Methods:

Horse spleen apoferritin (apo-hoSF) and all other chemicals were obtained from Sigma Aldrich (Spain). Recombinant human H-chain apoferritin and ferritin (apo-huFH and huFH), as well as recombinant human L-chain apoferritin and ferritin (apo-huFL and huFL) were purchased from MoLiRom (Italy). The iron amount is the same by the huFH and huFL (2500 iron atoms/ferritin molecule). UV-vis absorption was measured with a NanoDrop 2000c spectrometer (Thermo Scientific). The data presented in the work show representative results of at least triplicate experiments.

### Experimental section

**Preparation of H/L heteropolymeric human apoferritin.** Starting from recombinant human H and L apoferritins, three H/L heteropolymeric apoferritins were formed with 10, 50 and 90 % L-chain content. H and L apoferritins were mixed in precise proportions. For the disassembly 1 M HCl solution was added until pH 2 was reached. Once the pH was stabilized at pH 2, 1 M NaOH was added until pH 8 was reached for the reassembly of the apoferritin. After 30 min the reformed H/L heteropolymeric apoferritin was purified with spin columns (40kDa MWCO). The protein concentration was determined with the Pierce 660 nm Protein Assay Kit (Thermo Scientific Pierce).

**Analysis of the cytochrome c reduction activity.** The assay took place at room temperature (RT) in an inert environment (purged with 200 sccm/min 99.9999% N<sub>2</sub>) with a mixture of 0.5 mg/ml protein, 10 mM Tris-HCl buffer at pH7.0, 10  $\mu$ M oxidized cytochrome c (Cyt-c) and 50  $\mu$ M (NH<sub>4</sub>)<sub>2</sub>Fe(SO<sub>4</sub>)<sub>2</sub>. The Cyt-c reduction activity was determined by following the kinetics of the reaction. The UV-Vis spectra of the reaction were recorded every 30 s during the first 120 s and every 15 s thereafter. The reaction started with the addition of Cyt-c and the subsequent addition of Fe<sup>2+</sup> after 90 s. During the reaction the absorbance at several wavelengths was recorded: at 409 and 533 nm for the evolution of the oxidized form of Cyt-c and at 415 and 550 nm for tracking the reduced form. The aqueous solutions of (NH<sub>4</sub>)<sub>2</sub>Fe(SO<sub>4</sub>)<sub>2</sub> with 25 mM H<sub>2</sub>SO<sub>4</sub> were freshly prepared for each assay and purged with pure nitrogen. The concentration of Cyt-c in the reaction was determined photometrically at 410 nm for oxidized Cyt-c ( $\epsilon = 1.06 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ ) and at 550 nm for reduced Cyt-c ( $\Delta\epsilon = 21.1 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ ).<sup>10</sup> The SOD inhibition was measured by adding 10  $\mu$ g/ml recombinant bovine SOD ( $\geq 2500$  units/mg) to the reaction mixture.

**Demineralization assay.** The ferritin was purified with the desalting column (40 kDa MWCO, Thermo Scientific). The assay took place at room temperature (RT) in an inert environment with a mixture of 75  $\mu$ g/ml protein (ferritin) concentration, 10mM MES buffer pH7.0, 2.5 mM Ferene S, 200  $\mu$ M FMN and 2 mM NADH. Ferene S forms a complex specifically with Fe<sup>2+</sup>, which has characteristic absorption at 593 nm. The reaction kinetics was followed by measuring the UV-vis absorption at 593 nm every 30s. The base line was calibrated with measuring spectra of a mix of all the components before starting the reaction with NADH.

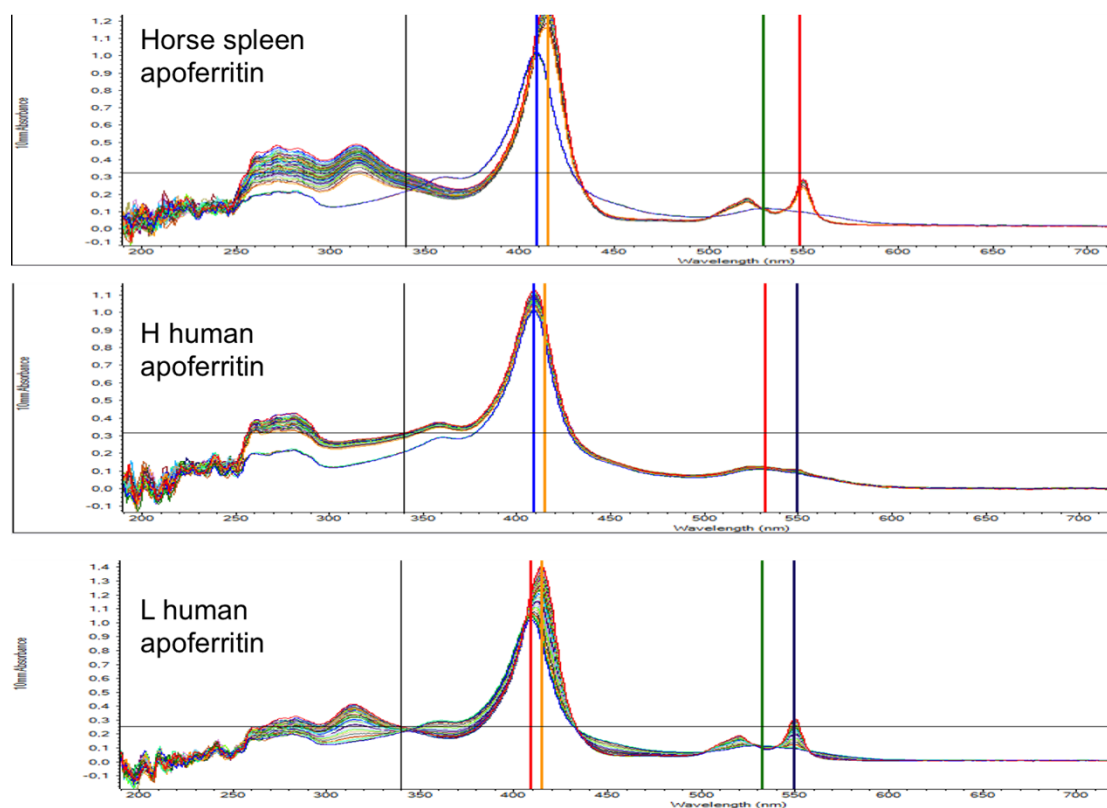
**Preparation of Pt-apoferritin.** The monomeric apoferritin was incubated with aqueous K<sub>2</sub>PtCl<sub>4</sub> in 1/15000 molar ratio at pH 7.4 with phosphate buffered saline (PBS). The solution was mixed in darkness at 30 °C for 2 hours and subsequently the excess salt was removed with the Zeba Desalting Column (40kDa MWCO, Thermo Scientific). This process is necessary in order to avoid the formation of metal particles outside the protein shell of apoferritin. Once desalted with spin columns the solution was immediately mixed with 1/20 (v/v) 1 M NaBH<sub>4</sub> and stirred for 20 min at 30 °C. After the reduction, the apoferritin-containing platinum nanoparticles (Pt-apo) were purified twice with spin columns (40kDa MWCO) for removal of unwanted byproducts and metal clusters outside the protein shell. Before use, the desalting columns were extensively washed with water in order to remove all NaN<sub>3</sub>, which was added as preservative (0.05%) in the original column liquid phase. After preparation, the

protein concentration was determined with the Pierce 660nm Protein Assay Kit (Thermo Scientific Pierce).

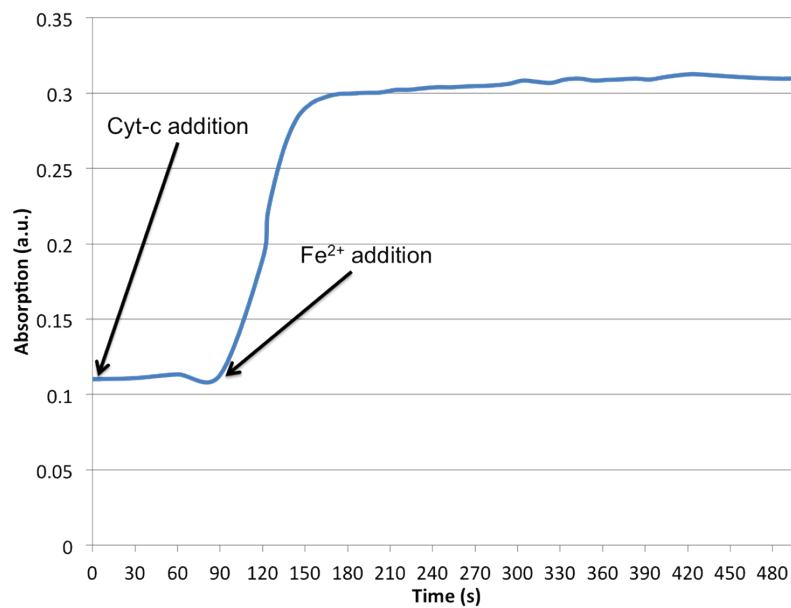
**Ferroxidase assay.** The assay took place at ambient conditions with a mixture of 0.5 mg/ml protein, 10 mM Tris-HCl buffer pH 7.0 and 50  $\mu\text{M}$   $(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2$ . The reaction kinetics was followed by UV-vis spectroscopy recording the absorption spectra every 30s. The base line was calibrated with measuring spectra of a mix of all the components before starting the reaction with  $\text{Fe}^{2+}$ .

**Crosslinking.** 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) was used as a zero-length crosslinking agent to detect the interactions of Cyt c with L- or H-chains. For this purpose 0.5 mg/ml apoferritin and 10  $\mu\text{M}$  oxidized cytochrome c (Cyt-c) were mixed in 10 mM MES buffer of pH 5.5 and EDC was added directly to the mixture to a final concentration about 200  $\mu\text{M}$ . The reaction was incubated for 2h at RT before standard SDS-PAGE.

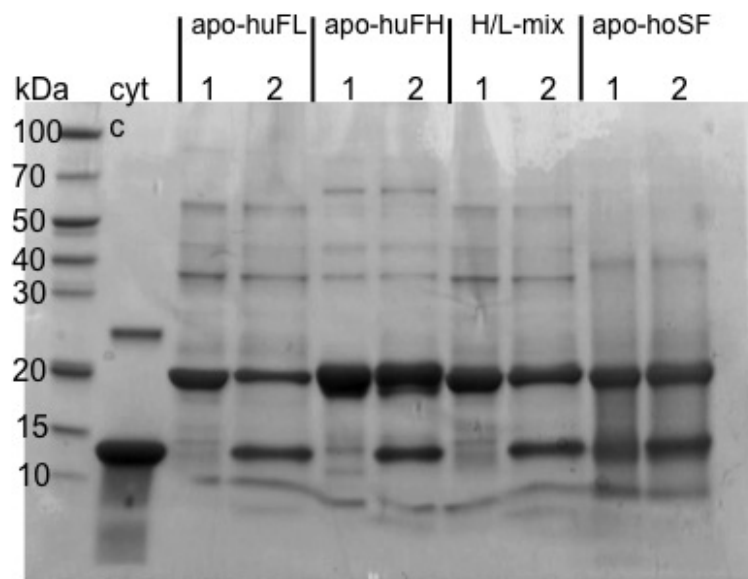
Formaldehyde was used as an unspecific protein crosslinking agent. For this purpose, formaldehyde was added to the mixture with the final concentration of 0.4% (w/v). The reaction was carried out at RT for 30 min.



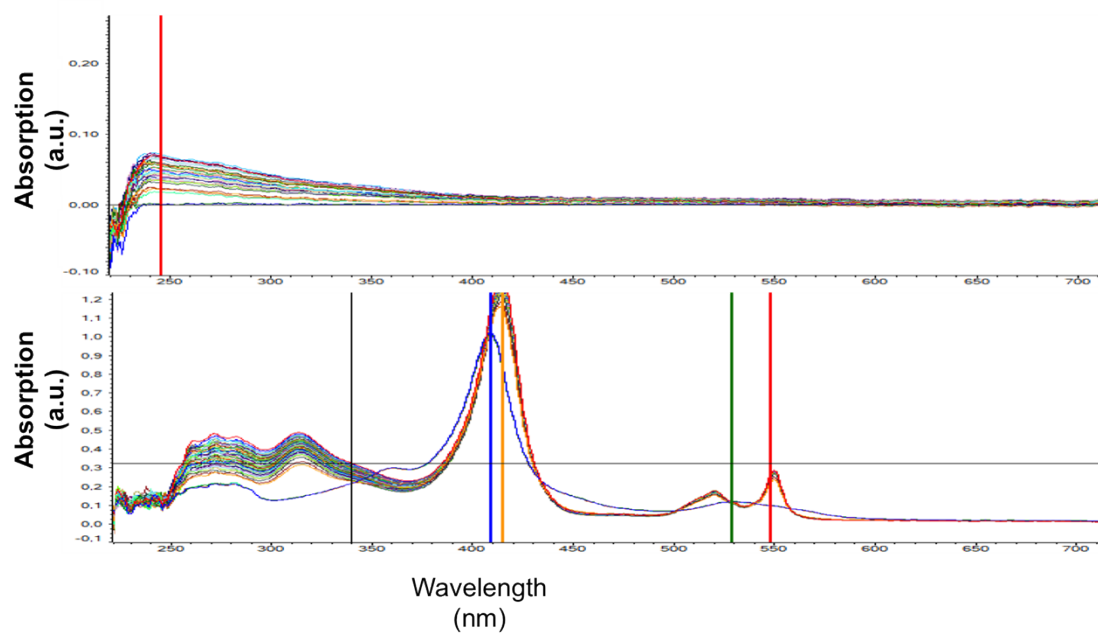
**Figure S1.** Cyt-c reduction activity measured with UV-Vis spectroscopy. The solution contained 0.15 mg/ml protein (apo-hoSF: horse spleen apoferritin; apo-huFH: human H-chain apoferritin and apo-huFL: human L-chain apoferritin), 10  $\mu\text{M}$  oxidized Cyt-c, 10 mM Tris-HCl buffer at pH 7.0 and 50  $\mu\text{M}$   $(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2$ .



**Figure S2.** Reaction kinetics of the Cyt-c reduction. Formation of the reduced Cyt-c was followed by measuring the absorbance at 550 nm. The solution contained 0.30 mg/ml protein, 10 mM Tris-HCl buffer at pH 7.0, 10  $\mu$ M Cyt-c and 50  $\mu$ M  $(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2$ .



**Figure S3.** The EDC-crosslinking of Cyt c with L- or H-chains of apoferritin. After the crosslink the proteins were separated in a 4-20% gradient SDS-polyacrylamide gel. The proteins on the gel were visualized by staining with the Imperial Protein Stain (Thermo Scientific). Lines 1: apoferritin as indicated; Line 2: apoferritin as indicated and Cyt c crosslinked with EDC.



**Figure S4.** Upper spectra: Ferroxidase activity characterized with UV-Vis spectroscopy. The solution contained 0.15 mg/ml protein (apo-hoSF: horse spleen apoferritin;), 10 mM Tris-HCl buffer at pH7.0 and 50  $\mu\text{M}$   $(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2$ . Lower spectra: Cyt-c reduction activity characterized with UV-Vis spectroscopy. The solution contained 0.15 mg/ml protein (apo-hoSF: horse spleen apoferritin;), 10  $\mu\text{M}$  Cyt-c, 10 mM Tris-HCl buffer at pH7.0 and 50  $\mu\text{M}$   $(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2$ .