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Supporting Information

for

Engineering protein interfaces yields ferritin disassembly and reassembly under benign experimental conditions

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1. Production, expression and purification of mutant rHuHF-ΔDE.

To obtain recombinant human H-chain ferritin with a cleavage of the last 23 amino acids (including DE turn and E helix) at the carboxyl terminal, the mutagenesis was performed using Quick Change site-directed mutagenesis method according to the manufacturer's instruction. The mutated oligonucleotide of sequence 5'-AACTTGCGCAAGATGGGATAACCCGAATCTGGCTTGGCG-3' was used for introducing an amber mutation at position Ala-160, and then the mutant plasmid was transformed into *E. coli* strain BL21 (DE3).

Recombinant human H-chain ferritin (rHuHF) was purified as previously described [1,2] with some modifications. Briefly, the *E. coil* strain BL21 (DE3) which contained rHuHF-ΔDE expression plasmid was grown on LB medium supplemented with 50.0 mg/L of ampicillin sodium with shaking at 180 r.p.m. at 37 ˚C, and protein expression was induced with 1mM isopropyl β-D-1thiogalactopyranoside (IPTG) when an optical density reached an A_{600} of 0.6. The cells were harvested by centrifugation (12 000 × g for 10 min) after 8 h of induction and suspended in buffer A (50 mM Tris, pH 7.5, 0.15M NaCl). Next the cells were sonicated for 13 min on ice, and the lysate centrifuged for 10 min at 12 000 × g at 4 ˚C. The solution was then primarily purified by ammonium sulfate fractionation (60% saturated fraction). After centrifugation for 10 min at 12 000 × g at 4 ˚C, the precipitate was dissolved in minimal buffer B (50 mM Tris, pH 7.5), followed by dialysis (14.4 kD cut off) in buffer B three times to remove the ammonium sulfate. Finally, the protein was further purified by ion exchange chromatography and Sephacryl S-300 gel filtration chromatography.

2. Polyacrylamide gel electrophoresis.

According to the procedure as described by Zhang *et al*.[3] Electrophoreses were performed by using a 4-20 % polyacrylamide gradient gel and 15 % SDS-PAGE. Gels were stained with Coomassie Brilliant Blue R250.

3. Transmission electron microscopy (TEM)

TEM data was collected by a Hitachi S-5500 transmission electron microscope operating at 30 kV. Samples were diluted with appropriate buffer to get the required concentration prior to being placed on carbon-coated copper grids. After excess solution was removed with filter paper, the samples except ferritin loaded 200 Fe^{2+}/shell were stain using 2 % uranyl acetate for 10 min.

4. Fluorescence spectroscopy and thermal stability.

The fluorescence emission spectra were scanned at 25 ˚C using a Cary Eclipse spectrofluorimeter (Varian). By utilizing an excitation wavelength of 280 nm, the emission spectra ranging 290 to 450 nm was measured at the same concentration. All measurements were performed in triplicate. Thermal stability experiment was performed utilizing a Cary 50 UV-Vis spectrophotometer to record the changes in absorbance at 280 nm at temperatures ranging from 20 to 95 ˚C.[4] Samples in 50 mM Tris buffer (pH 7.5) were heated in a water bath to the indicated temperatures before measurement.

5. Dynamic Light Scattering (DLS) Analyses

DLS experiments were performed at 25 °C using a Wyatt model WDPN-08 dynamic light scattering instrument. The Dynamics software was used to calculate the size distribution of prepared proteins. Each

measurement was averaged over 30 runs. The protein concentration was 1.0 μM in mixing buffer solution containing Tris (50 mM).

6. Iron core formation.

Freshly prepared $FeSO_4$ solution dissolved in pH 2.0 ddH_2O was added to the apoferritin solution (100 mM Mops, 0.15 M NaCl, pH 7.0) in four increments at intervals of 20 minutes to make the final ratio of Fe^{2+}/protein as 200/1, and then the resulting solution kept at 4 ˚C for 18 h. After the incubation, iron core was determined by using TEM method without 2% uranyl acetate staining.

7. Analysis of reversible dissociation/reassembly property.

The pH value of the rHuHF-ΔE solution was adjusted to 3.0 or 4.0 with 0.1 M HCl to disassemble protein into subunits. After 30 min, the solution pH was then adjusted back to 7.5, and kept for 12 h at 4 ˚C. Samples were analysed by TEM and native PAGE.

8. Encapsulation of curcumin within rHuHF-ΔDE.

Curcumin encapsulation experiment was carried out as previously described with some modification.[5] Curcumin was dissolved in DMSO to make a stock solution with a final concentration of 10 mM. The protein solution was prepared with a concentration of 2.0 μM in 20.0 mM Tris and with a volume of 5 mL. rHuHF-ΔE solution pH was adjusted slowly to pH 4.0 with HCl (1.0 M) to disassemble ferritin into subunits. After 30 min, 100 μL of cucumin stock solution was dropwise added to the protein solution with a rHuHF-ΔE/curcumin ratio of 1/100, and the solution pH was increased to 7.5 with NaOH (1.0 M). Then resultant mixture was allowed to stand at 4 ˚C for 24 h. Finally, the resultant solution was dialyzed (100 kDa cutoff) against 20.0 Mm Tris buffer (pH 7.5) three times at intervals of 6 h to remove free curcumin.

Supplementary figures (Figs. S1-8)

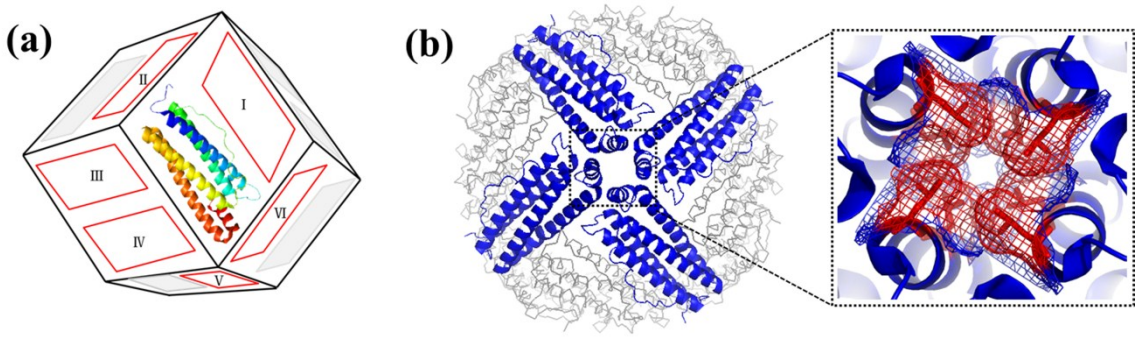

Fig. S1. (a) Schematic diagram illustrating the relation between symmetry-related subunits. (b) Human H chain ferritin viewed down the 4-fold axes and its apolar channel lined by 12 leucine residues (Leu 165, Leu169, Leu 173).

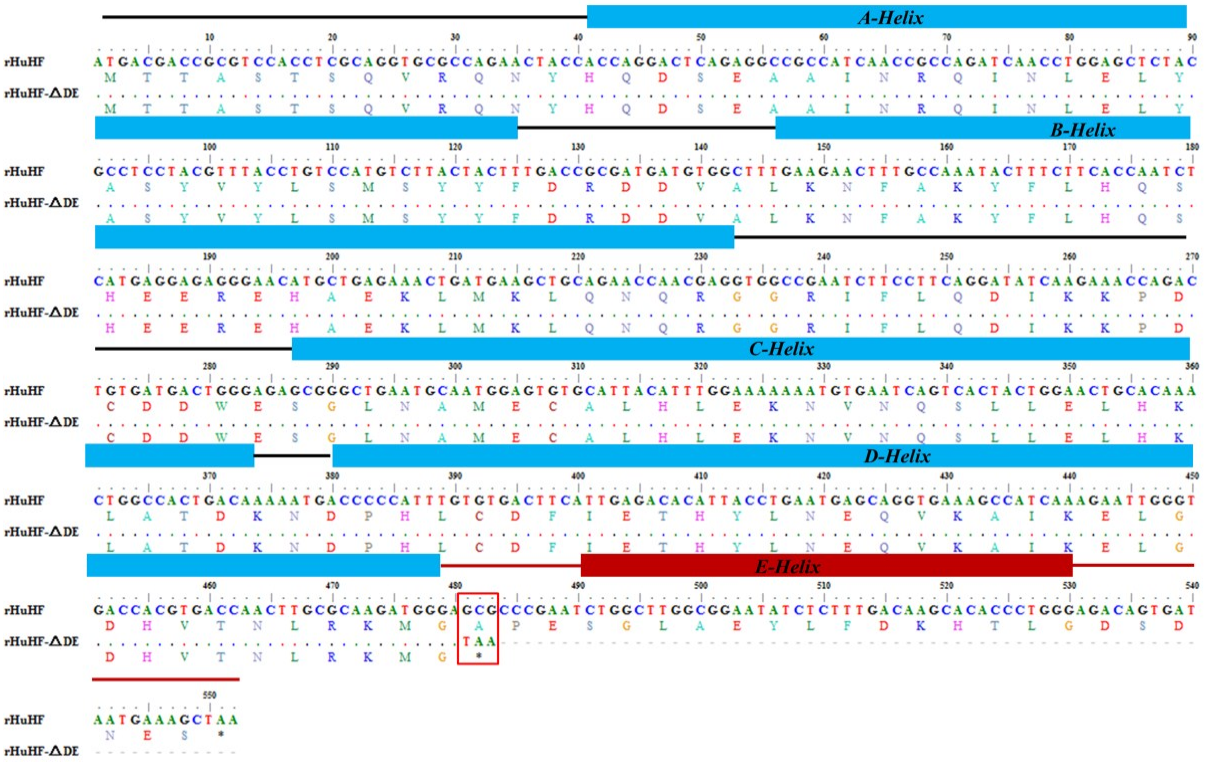

Figure S2. Complete nucleotide sequences of rHuHF and rHuHF-ΔDE subunits. The chains of rHuHF-ΔDE mutants were prepared by inserting an amber mutation at position Ala-160, followed by expression in *E. coli*. The position of an amber mutation is highlighted within a red box.

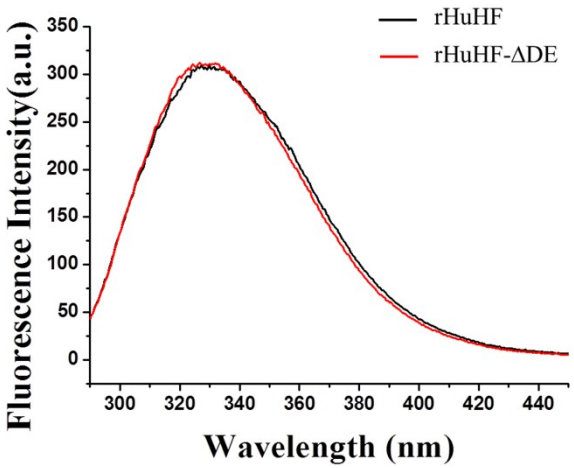

Fig. S3. The flurescence spectra of rHuHF and rHuHF-ΔDE. Conditions: 1.0 μM proteins in 100 mM Mops , 0.15 M NaCl, pH 7.0, 25 ˚C, $\lambda_{Ex.}$ = 280 nm, $\lambda_{Em.}$= 327 nm.

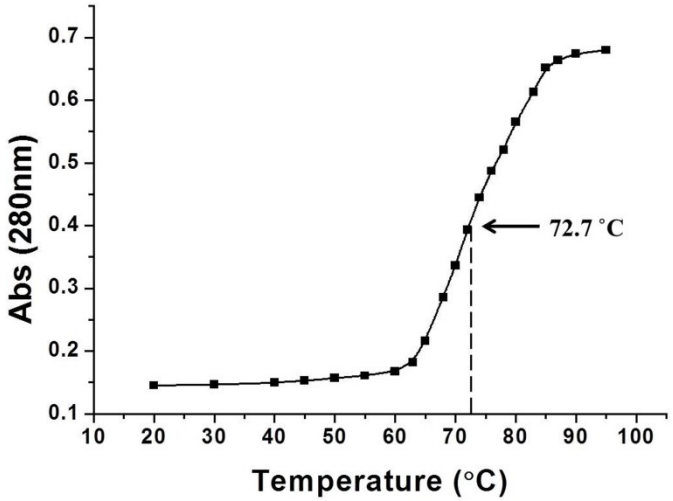

Fig. S4. Thermal denaturation curve of rHuHF-ΔDE in temperature range from 20 ˚C to 95 ˚C measured by UV absorbance at 280 nm. Conditions: 1.0 μM proteins in 50 mM Tris pH 7.0.

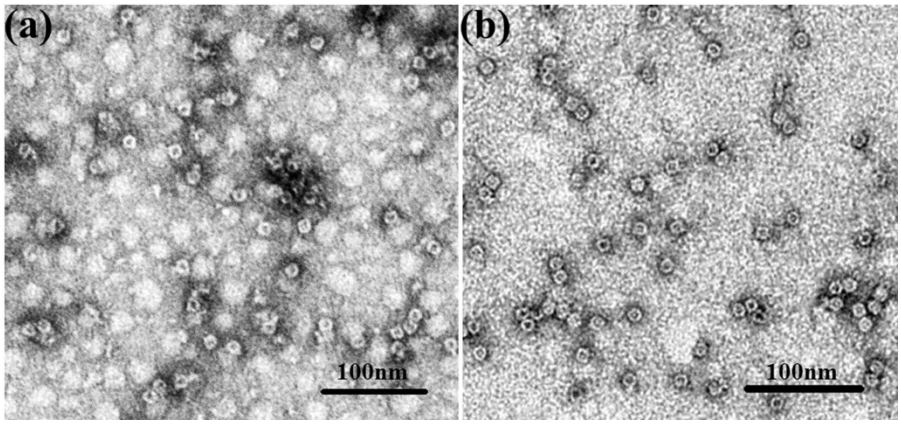

Fig. S5 TEM images of (a) rHuHF at pH 3.0 and (b) rHuHF at pH 7.5. Scale bars represent 100 nm.

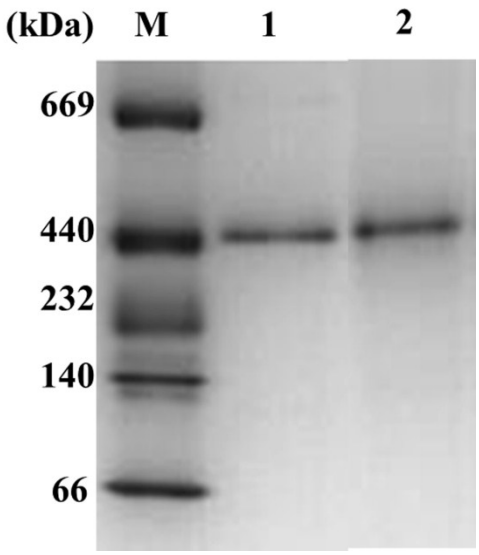

Fig. S6 Native PAGE analyses of the reassembly properties of rHuHF- ΔDE at pH 4.0. Land 1, rHuHF-ΔDE at pH 7.5; Land 2, reassembled rHuHF-ΔDE with adjusting pH 4.0 back to 7.5.

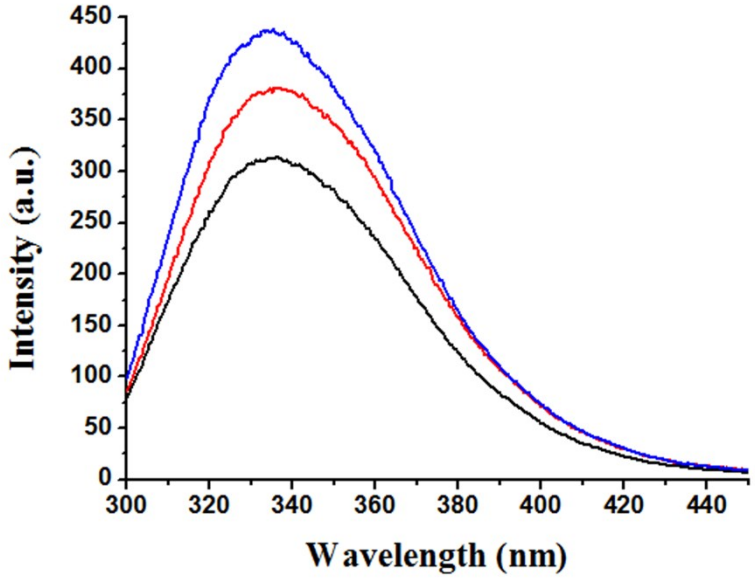

Fig. S7 Fluorescence spectra of rHuHF-ΔDE at pH 7.5 (blue) and 4.0 (black), and reassembled form at 7.5 (red). Conditions: proteins with the same concentration in 50 mM Mops.

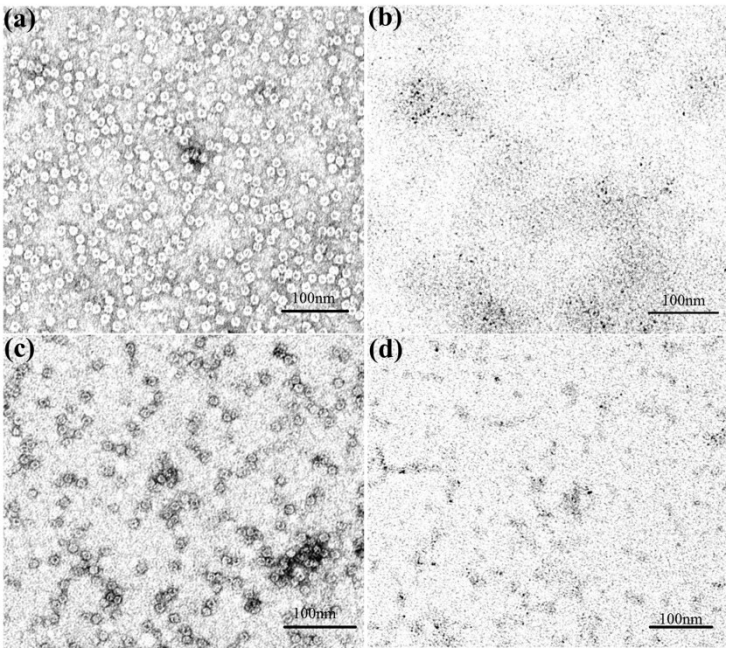

Fig. S8 TEM images of (a) rHuHF and (b) rHuHF- ΔDE with 2% uranyl acetate staining, and ferric iron cores formed within (c) rHuHF and (d) rHuHF-ΔDE without stanining. Scale bars represent 100 nm.

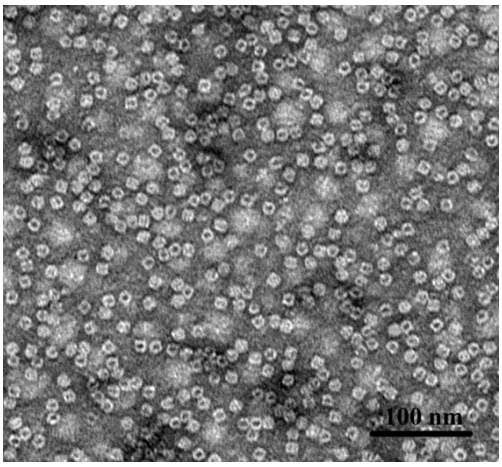

Fig. S9 Ferritin shell dispersed well in 10 mM PBS. After stored at 4 ˚C for 24 h, rHuHF- ΔDE was observed with 2% uranyl acetate staining, Scale bars represent 100 nm.

References

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2. L. Chen, G. Bai, S. Yang, R. Yang, G Zhao, C. Xu, and W Leung, *Food Research International*, 2014, **62**, 1147-1153.
3. T. Zhang, C. Lv, S. Yun, X. Liao, G. Zhao and X. Leng, *Food Chemistry*, 2012, **130**, 273‑278.
4. X. Liao, C. Lv, X. Zhang, T. Masuda, M. Li and G. Zhao, *Free Radical Biology and Medicine*, 2012, **53**, 375-382.
5. L. Chen, G. Bai, R. Yang, J. Zang, T. Zhou, and G. Zhao, *Food Chem*, 2014, **149**, 307-312.