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Supporting Information

Disulfide-Mediated Reversible Two-Dimensional Self-Assembly of Protein Nanocages

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Experimental Section

1. Protein preparation

The mutant was named 4FC (E162C), and purified using the method previously reported with slightly modification[1]. Details are as follows: cDNA encoding the full-length amino acid sequence of rHuHF (recombinant human H-chain ferritin) was cloned into thepET-3a vector (Novagen) and verified by DNA sequencing. Mutagenesis (E162C) of the rHuHF cDNA was performed according to the fast site-directed mutagenesis kit (TIANGEN Biotech Co., Ltd.). Polymerasechain reaction was carried out using the pET-3a-rHuHF plasmid as template, and primers used here are N-terminal (5'-CGCAAGATGGGAGCGCCCTGTTCTGGCTTGGCGGAATAT-3') and C-terminal (3'-GCGTTCTACCCTCGCGGGACAAGACCGAACCGCCTTATA-5'). PCR amplification was as follows: denaturation at 94 °C for 3 min, followed by 18 cycles of 94 °Cfor 20 s, 55 °C (depending on the primers) for 30 s, and 68 °C for 2.5min, and a final extension cycle of 68 °C for 5 min. After the PCRreaction, the parental DNA template was digested with *Dpn* I enzyme for 1 h under 37 °C. Digestion product was transfered into *E. coli* competent cells (BL21). Ampicillin-resistant colonies were selected from which the plasmids were extracted, and the extracted plasmid was sequenced for confirmation of the site-directed mutation (Figure S2).

Recombinant human H-chain ferritin (rHuHF) was purified as previously described[1], and4FC was purified as follows. The *E. coli* cells of BL21 (DE3) containing the expression plasmid of pET-3a-4FC was grownat 37 °C in LB medium supplemented with 50 mg/L ampicillin, andprotein expression was induced with 1.0 mM isopropyl-β-D-thiogalactopyranoside (IPTG) when the cell density reached anabsorbance of OD_{600nm} = 0.6. The cells were harvested by centrifugation after 12 h of induction, and resuspended in buffer A (20 mM Tris−HCl, pH 7.0, 50 mM β-mercaptoethanol), followed by disruption by sonication. Thesupernatant of the resulting crude extract was collected bycentrifugation and fractionated by 20% saturation of ammonium sulfate. The pellet was collected by centrifugation after 4 h,

and resuspended in buffer A, further dialyzed against buffer A. The protein solution was applied to an anion-exchange column (DEAE-sepharose Fast Flow, GE Healthcare), followed by gradient elution with 0−0.5 M NaCl (ferritin waseluted at ~0.15 M NaCl). Protein purity was confirmed by SDS-PAGE (polyacrylamide gel electrophoresis). Protein concentration was determined according to the Bradford method with bovine serum album as standard.All the buffers in protein purification contained fresh 50 mM β-mercaptoethanol. The purification method of 4FCΔ3C was the same as that of 4FC.

2. Preparation of 2D 4FC nanocage arrays

Purified 4FC protein molecules were concentrated to ~10.0 μM through ultrafiltration centrifuge tube. The 2D protein arrays of 4FC formed gradually upon exposed to air with mildly shaking (80 rpm) at 4 °C.

3. TEM analysis

Protein samples (10 μL) were diluted with 20 mM Tris−HCl (pH 7.0, without β-mercaptoethanol) prior to placement on carbon-coated copper grids (Beijing Zhongxingbairui Technology Co., Ltd.), and excess solution was removed with filterpaper after 5 min. Then protein samples were stained with 2% uranyl acetate (Beijing Zhongxingbairui Technology Co., Ltd.) for 5 min. TEM micrographs were imaged at 80 kV through a Hitachi H-7650transmission electron microscope.

4. DLS analyses

DLS experiments were performed at 25 °C using a Viscotek model 802 dynamic light scattering instrument (Viscotek, Europe). The OmniSIZE 2.0 software was used to calculate the size distribution of samples. For all samples, protein concentration was 1.0 μM, and proteins were diluted with 20 mM Tris−HCl, pH 7.0, without β-mercaptoethanol.

5. SDS-PAGE of 2D 4FC nanocage arrays

2D arrays of 4FC were collected through centrifugation, and washed for 3 times with 20 mM Tris−HCl (pH 7.0, without β-mercaptoethanol). Resuspended precipitates of the 2D arrays were divided into two samples before loading for electrophoresis: one mixed with SDS loading buffer containing reducing agent (+ME), and the other one mixed with SDS loading buffer without reducing agent (-ME). The protocols of SDS-PAGE was followed the previously reported ones[2].

6. Reversible control of 2D nanocage arrays of 4FC

The disassociation of 2D 4FC arrays into monomers was performed upon adding 5 mM TCEP (Tris (2-carboxyethyl) phosphine) into the resuspended precipitates. And the suspension was placed at room temperature under stirring (180 rpm). Samples at different time points were analyzed by TEM and DLS.

References

1 T. Masuda, F. Goto, T. Yoshihara and B. Mikami, *Biochem. Biophys. Res. Commun.*, 2010, **400**, 94-99.

2 U. K. Laemmli, *Nature*, 1970, **227**, 680.

Supplementary figures

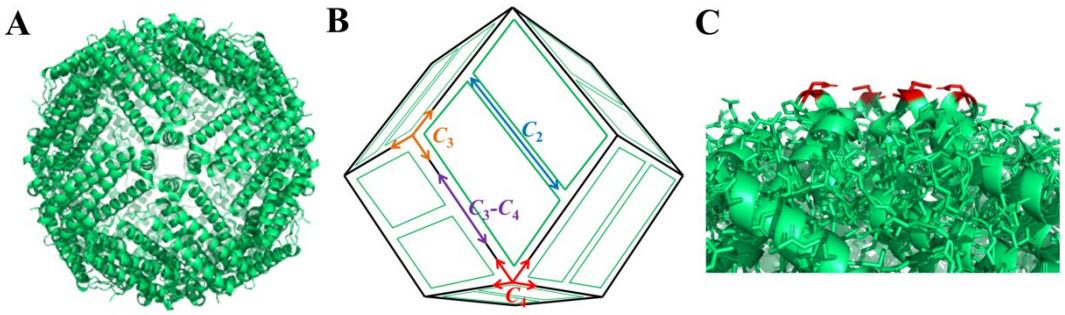

Fig. S1 (A) Overall structure displayed in cartoon of shell-like ferritin. (B) Ferritin has the approximate geometry of a rhombic dodecahedron (bond black line), each face of which consists of two subunits (green rhombus). All the subunits are involved in C_2 (blue), C_3 (orange), C_4 (red) and C_3–C_4 (purple) interface interactions. (C) Side chains displayed in stick of residues in C_4 interface of rHuHF. Residues highlighted in red are hot spots at the C_4 interface.

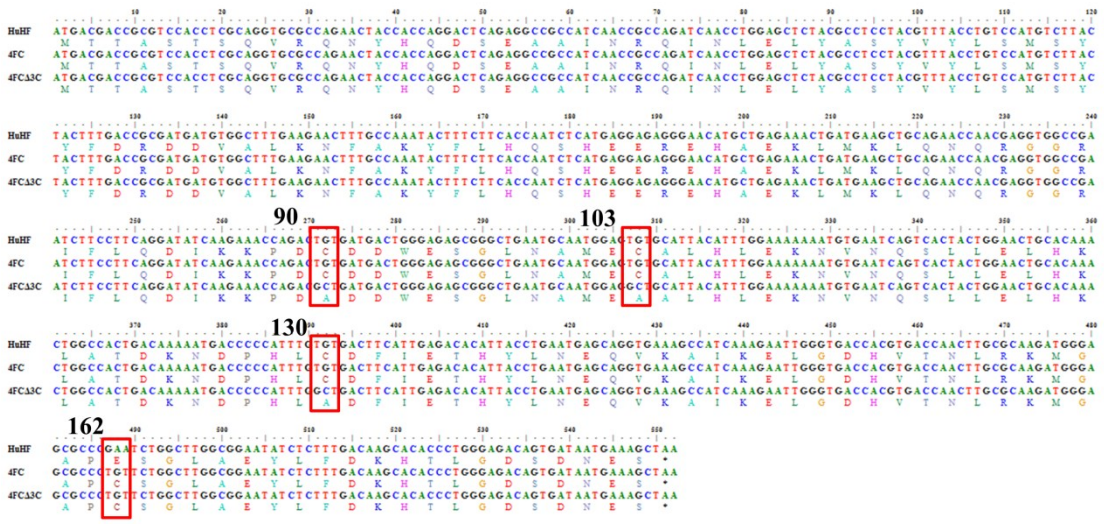

Fig. S2 Complete nucleotide sequences of rHuHF, 4FC, and 4FCΔ3C. The base pair labeled with red square is mutation sites. The mutation site of 4FC was E162C, and the mutation sites of 4FCΔ3C were E162C/C90A/C103A/C130A.

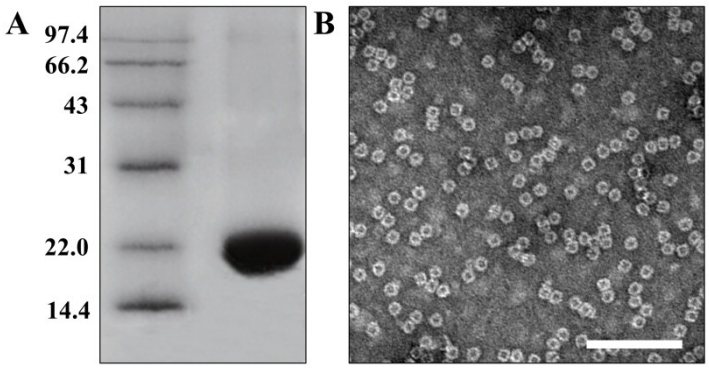

Fig. S3 SDS-PAGE (A) and TEM image (B) of purified 4FC mutant. Scale bar represents 100 nm.

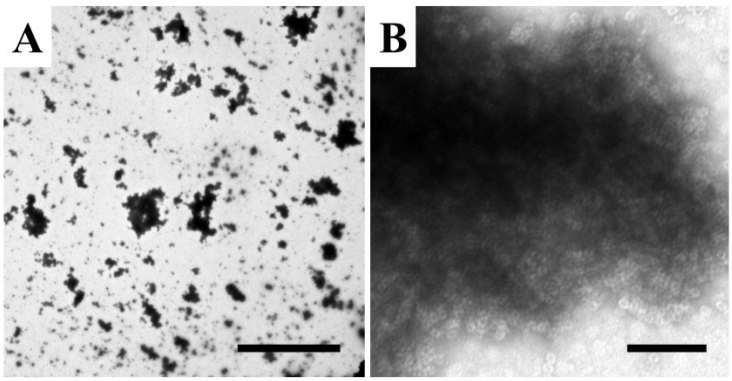

Fig. S4 TEM images of 4FC aggregates formed due to fast oxidation in 12 h. (B) is the enlargement view of (A). Scale bars in (A) and (B) are 10 μm and 100 nm, respectively.

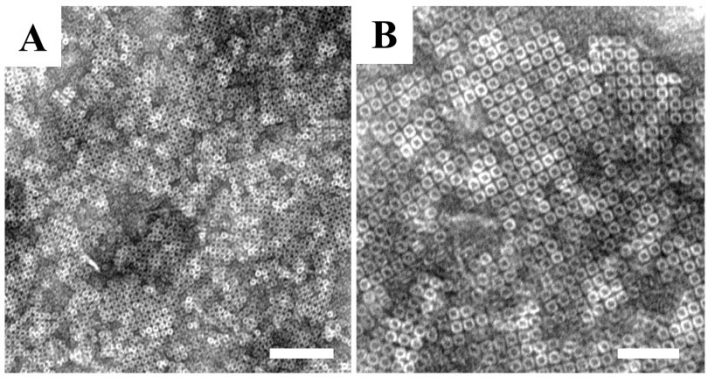

Fig. S5 TEM images of 4FCΔ3C 2D arrays formed under the same condition as 4FC 2D arrays. (A) The overall view of the 4FCΔ3C 2D arrays. (B) The enlargement view. Scale bar is 100 nm in (A), and 50 nm in (B).

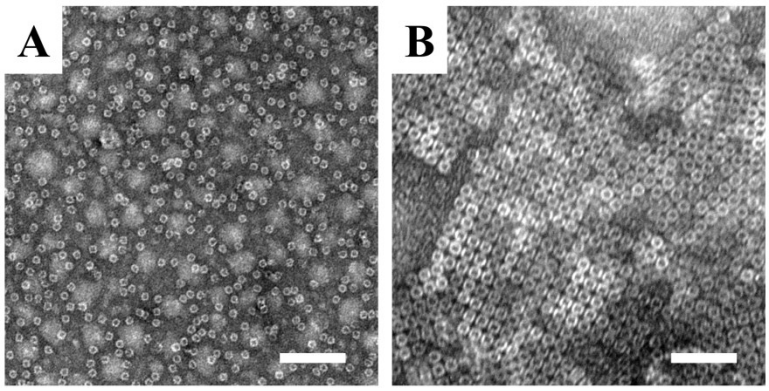

Fig. S6 Effect of urea on 4FC self-assembly. (A) TEM image showed that the presence of 10 mM urea cannot facilitate 4FC association into 2D array. (B) Addition of urea (10 mM) has no effect on 4FC 2D array induced by the formation of disulfide bonds in air. Scale bars in (A) and (B) are 100 nm and 50 nm, respectively.

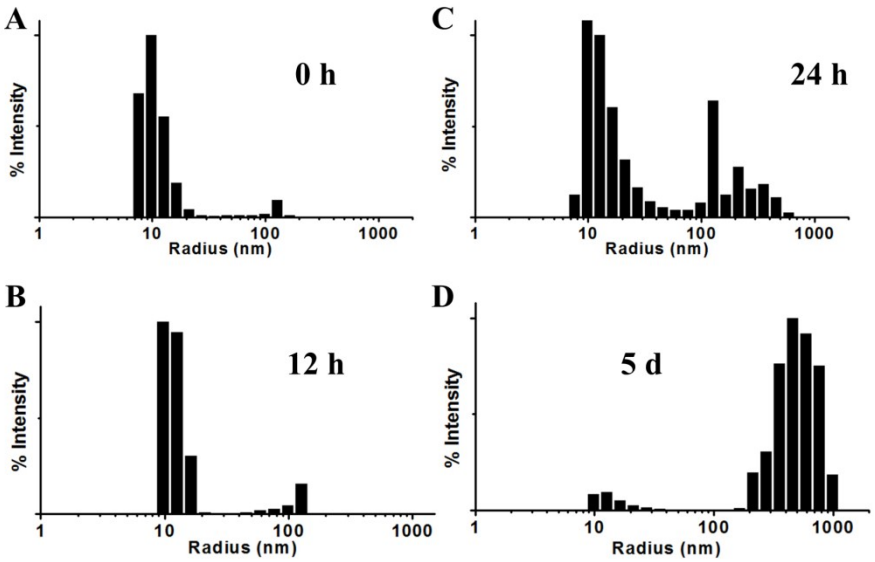

Fig. S7 Dynamic light scattering (DLS) analyses of 4FC oxidative assemblies upon exposed in air for different times. (A) – (D) Represent the size distribution of 4FC in solution upon exposed in air for different times 0 h, 12 h, 24 h, and 5 d, respectively. Proteins are buffered in 20 mM Tris-HCl, pH 7.0 with the existence of 50 mM of β-mercaptoethanol.

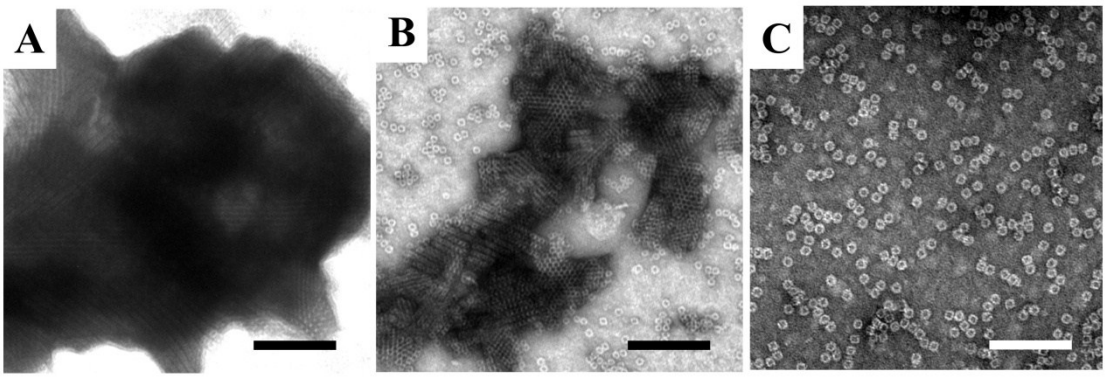

Fig. S8 Kinetics of the depolymerization of 2D 4FC nanocage arrays. TEM images of 2D 4FC arrays upon treatment with fresh reducing agent TCEP (5 mM) for 0 h (A), 1 h (B), and 5 h (C), respectively. Scale bars are all 100 nm.

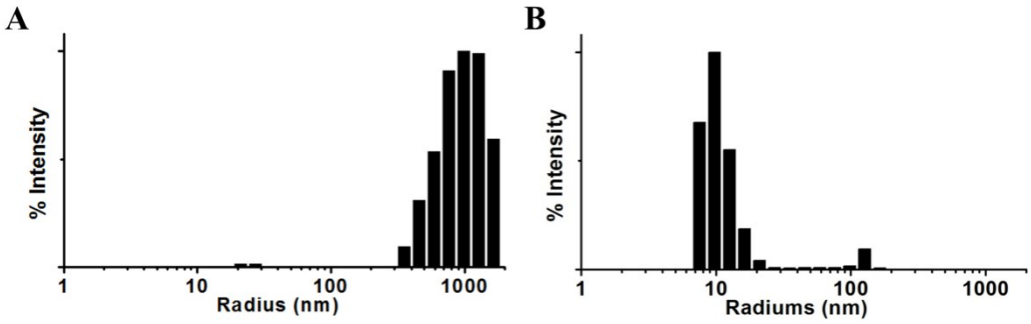

Fig. S9 DLS analyses of the disassembly of 2D 4FC arrays before (A) and after (B) treatment with TCEP for 5 h.