SUPPORTING INFORMATION FOR

Bio-inspired Engineering Proteinosomes with Cell Wall Like Protective Shell by Self-assembly of Metal-chelated Complex

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1. Materials

Albumin from bovine serum (BSA, Sigma, 98%), nethyl-N'-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC, Sigma-Aldrich, 98%), DNA (Sigma, 98%), Tannic acid (TA, Sigma, 98%), iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$, Sigma, 98%), PEG-bis (N-succinimidyl succinate (Sigma, 98 %), Ethylenediaminetetraacetic acid (EDTA, Sigma, 98%), Alkaline phosphatase Orthophosphoric-monoester phosphohydrolase (ALP, Sigma, 10 DEA units/mg), p-Nitrophenyl butyrate (pNPB, Aladdin, 98%), 4-Nitrophenyl phosphate disodium salt hexahydrate (pNPP, Aladdin, 98%) and N',N'-dimethyl-N-[4-[(E)-(3-methyl-1,3-benzothiazol-2-ylidene)methyl] -1-phenylquinolin-1-ium-2-yl]-N-propylpropane-1,3-diamine (SYBR green I, Sigma, 10000x in DMSO) were used as received without further purification. Milli-Q water was used to prepare all the solutions in this study.

2. Characterization methods

Transmission electron microscopy (TEM) analysis was undertaken on a JEM21400, using a LaB6 filament at 120 kV in bright field mode. Samples were prepared by adding one drop of proteinosome solution (0.1 mg/mL) onto a 300 mesh, carbon film coated copper grid and the specimens were then dried in vaccum for one day. SEM

images were obtained on a HITACHI UHR FE2SEM SU8000 with the samples coated with 10 nm platinum. Atomic force microscopy (AFM) images were performed on a 3D manipulation force microscope.[1] Samples were prepared by adding one drop of proteinosome solution (0.1 mg/mL) onto a clean silica wafer and dried in vacuum for one day. Optical and fluorescence microscopy was performed on a Leica DMI8 manual inverted fluorescence microscope at 10x, 20x, 40x and 100x magnification. Fluorescence measurements were performed on a PerkinElmer spectrofluorometer (LS55, USA). UV-vis spectra were measured on a PerkinElmer spectrophotometer (Lambda 750S, USA). Zeta potential studies of sample solutions (0.2 mg/mL, pH 6.8, 5.0 mM PBS buffer) were carried out at 25°C using a ZETASIZER Nano series instrument (Malvern Instruments, UK). The pH measurements were made with a Seven Compact meter (METTLER TOLEDO, SUI). Oscillator was employed by a VORTEX instrument (IKA, GER). Assimilated solutions were executed by pipettors (GILSON, FRA).

3. Experimental section

The fabrication process of proteinosomes were conducted according to a previous report[2] with few modifications, and the details were shown in the following parts:

3.1 Synthesis of the cationized bovine serum albumin (BSA-NH_2)

Cationized bovine serum albumin (BSA-NH_2) was synthesized by carbodiimide activated conjugation of 1, 6-diaminohexane to aspartic and glutamic acid residues on the external surface of the protein. For this, a solution of 1, 6-diaminohexane (1.5 g, 12.9 mmol) was adjusted to pH 6.5 using 5 M HCl and added dropwise to a stirred solution of the protein (200 mg, 2.98 μmol). The coupling reaction was initiated by adding N'-ethyl-N'-(3-(dimethylamino) propyl) carbodiimide hydrochloride (EDAC, 100 mg) immediately and again (50 mg) after 5 h. The pH value was maintained at 6.5 using dilute HCl, and the solution was stirred for a further 6 h. The solution was then centrifuged to remove any precipitate, and the supernatant was dialyzed (dialysis tubing 12-14 kDa MWCO) extensively against Milli-Q water.

3.2 Synthesis of BSA-NH_2/PNIPAAm conjugates

End-capped mercaptothiazoline-activated PNIPAAm (Mn = 8800 g mol^{-1}, 10 mg in 5 mL of water) was synthesized according to our previous reported method and added to a stirred solution of BSA-NH_2 (10 mg in 5 mL of PBS buffer at pH 8.0).[2] The mixed solution was stirred for 12 h, and then purified by using a centrifugal filter (MWCO 50 kDa) to remove any unreacted PNIPAAm and salts. After freeze-drying, the BSA-NH_2/PNIPAAm conjugate was obtained.

3.3 Preparation of proteinosomes

Proteinosomes were prepared by mixing an aqueous BSA-NH_2/PNIPAAm solution with 2-ethyl-1-hexanol followed by shaking the mixture by hand for 10 s. The samples were prepared at a constant aqueous/oil volume fraction (φ_w) of 0.06. Typically, 0.06 mL of aqueous BSA-NH_2/PNIPAAm (4.0 mg mL^{-1}, pH 8.5, sodium carbonate buffer) were mixed with 1.0 ml of the oil. The proteinosomes were then cross-linked in the continuous oil phase by addition of PEG-bis (N-succinimidyl succinate) (0.5 mg), which reacted with free primary amine groups of BSA-NH_2. Transfer of the cross-linked proteinosomes into water was achieved as follows. After 3 h sedimentation, the upper clear oil layer was discarded and 1 mL of 70% ethanol was added. The proteinosomes were washed three times by 70% ethanol via centrifugation-disperse process, then washed by Milli-Q water to complete the phase transfer process.

3.4 Generating TA-Fe^{3+} outer membrane around proteinosomes

Aliquots (5 μL) of $FeCl_3 \cdot 6H_2O$ (10 mg mL^{-1}) and TA (40 mg mL^{-1}) solutions were added to the aqueous proteinosomes suspension (490 μL) to yield the following final concentrations (proteinosomes: 10 mg mL^{-1}, $FeCl_3 \cdot 6H_2O$: 0.1 mg mL^{-1}, TA: 0.4 mg mL^{-1} in 0.5 mL of water). The suspension was vigorously mixed by a vortex mixer for 10 s. The pH of this suspension was subsequently raised by adding 0.5 ml of PBS buffer (20 mM, pH 7.4). The proteinosomes were washed with water three times to remove excess TA and $FeCl_3 \cdot 6H_2O$. In the washing step, the proteinosomes were spun down by centrifugation (2,000 g, 30 s) and the supernatant was removed.[3]

3.5 Measurement of antioxidant capacity of TA-Fe^{3+} protected proteinosomes

for scavenging ABTS radical

Stock solution, 7 mM of ABTS and 2.45 mM potassium persulfate ($K_2S_2O_8$) were prepared, and then by mixing the ABTS and $K_2S_2O_8$ aqueous solution, a blue green $ABTS^+$ was generated. The $ABTS^+$ solution was diluted using PBS buffer (0.1M, pH 7.4) and the concentration was determined from the UV absorbance at 734 nm. Afterwards, the reactant ratio of $ABTS^+$ to TA-Fe^{3+} protected proteinosome (15.6-250 μg/mL) was 100:1.[4] The percentage inhibition of absorbance at 734 nm was calculated for each concentration relative to a blank absorbance. The scavenging capability of $ABTS^+$ radical was calculated using the following equation:

$$ABTS^+ scavenging\ effect\ (\%) = \left(1 - \frac{As}{Ac}\right) \times 100 \quad (1)$$

where, A_C is the initial concentration of the $ABTS^+$ and A_S is absorbance of the remaining concentration of $ABTS^+$ in the presence of TA-Fe^{3+}-protected proteinosomes.

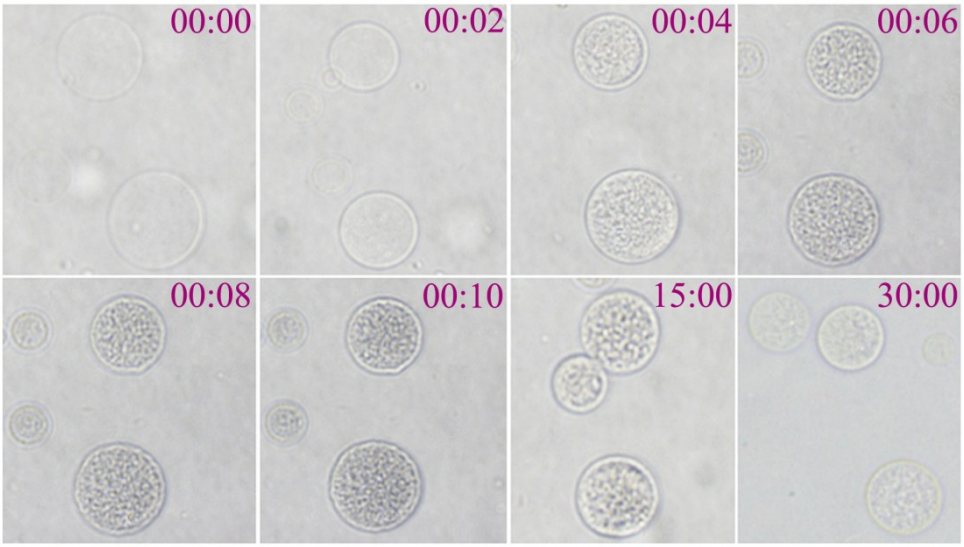

Figure S1. Optical microscopy images of formation procedure for the TA-Fe^{3+} shell around proteinosomes with increased contrast, and degradation procedure for the TA-Fe^{3+} shell in the presence of EDTA.

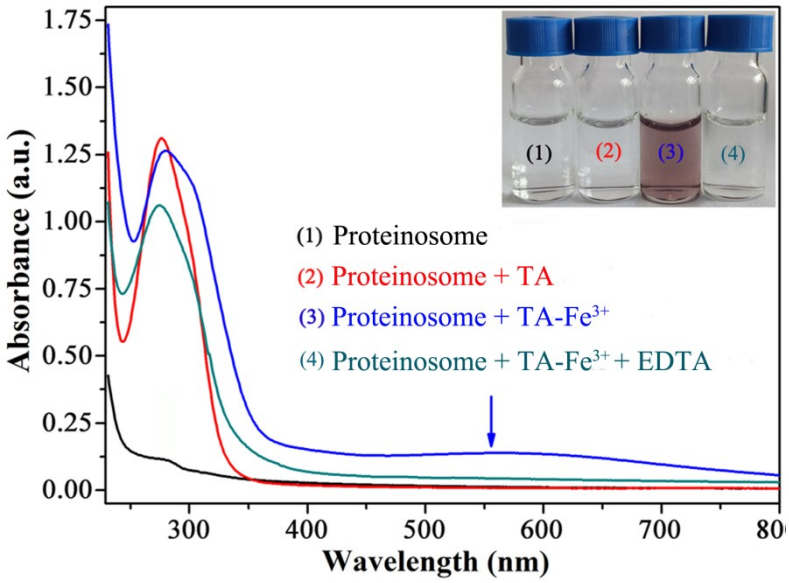

Figure S2. UV-vis absorption spectra of bared proteinosome, TA protected proteinosomes, TA-Fe^{3+} protected proteinosomes and EDTA added TA-Fe^{3+} protected proteinosome in aqueous solution, respectively.

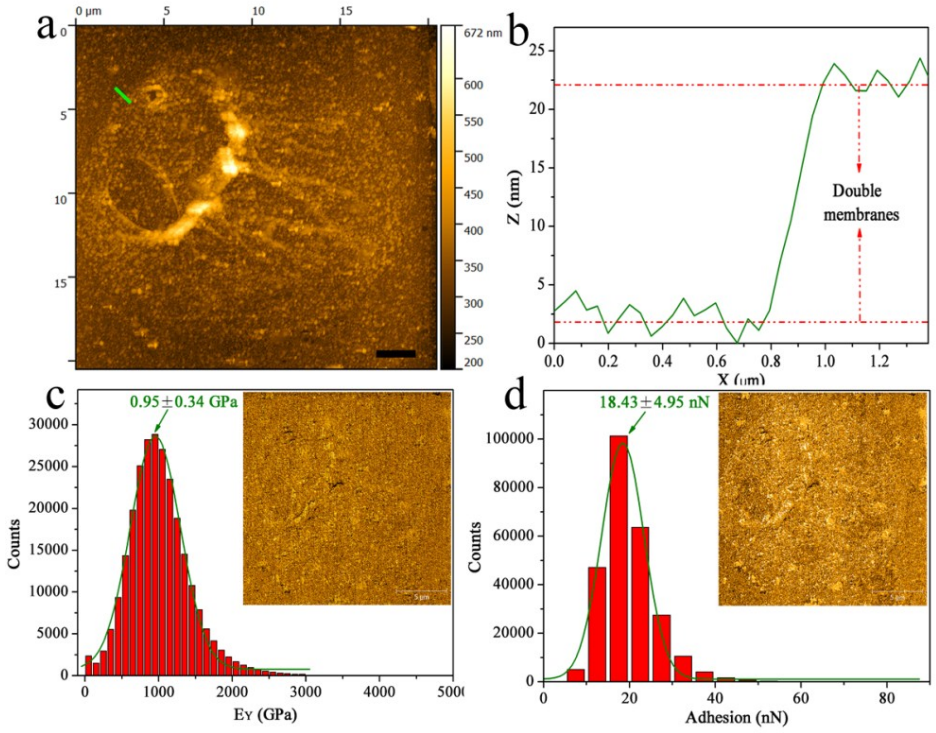

Figure S3. (a) Atomic force microscopy (AFM) and (b) corresponding height profile along the green trace that traverses double membrane thicknesses associated with the collapsed/folded sample images of a dried bared proteinosome; (c) The Young's modulus and(d) adhesion images of bared proteinosomes as well as the corresponding statistical histograms. The single peak fitting is performed by Gauss curve in statistical histogram.

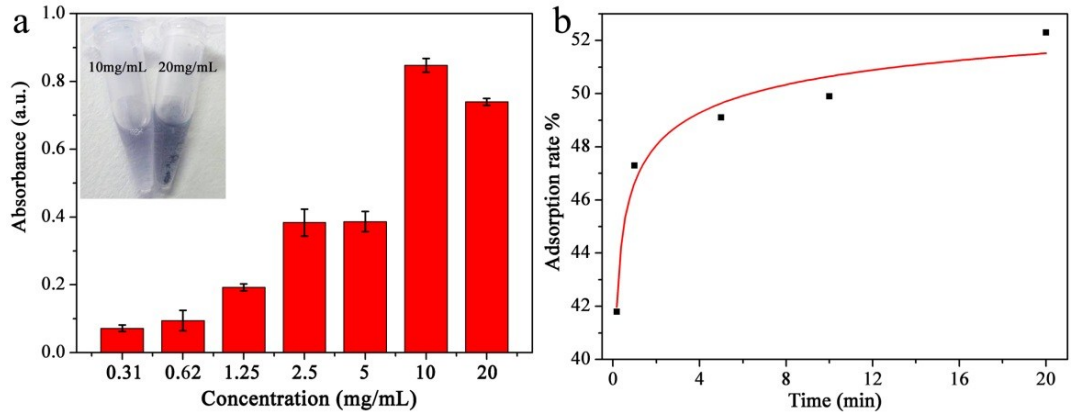

Figure S4. (a) UV-vis absorption histogram at 556 nm of the proteinosomes prepared at the different concentrations of $FeCl_3 \cdot 6H_2O$. (b) Adsorption rate dynamic monitoring of $FeCl_3 \cdot 6H_2O$ (10 mg/mL) and TA (40 mg/mL) onto proteinosomes.

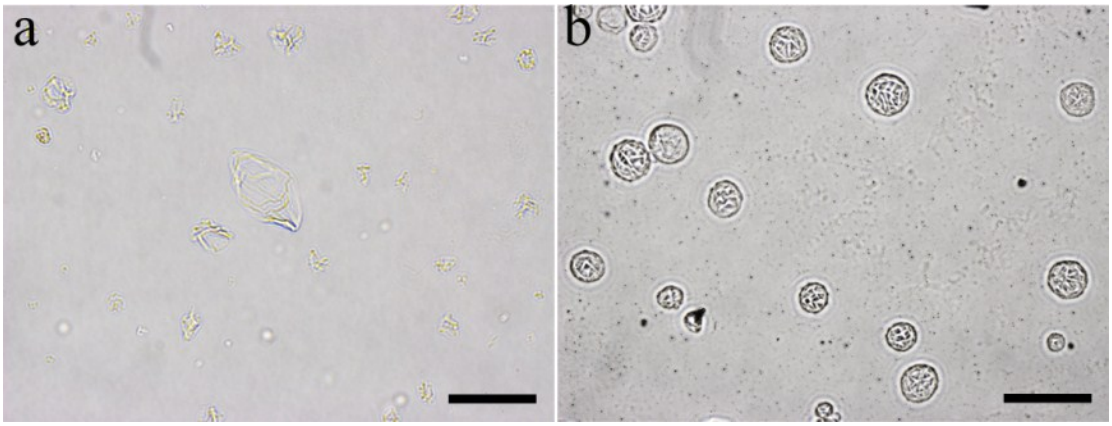

Figure S5. Optical microscopy images of air-dried (a) bared proteinosome, (b) TA-Fe^{3+} protected proteinosomes dispersed in water, showing the maintained spherical structure after forming the protected shell. Scale bars in (a) and (b) are 50 μm.

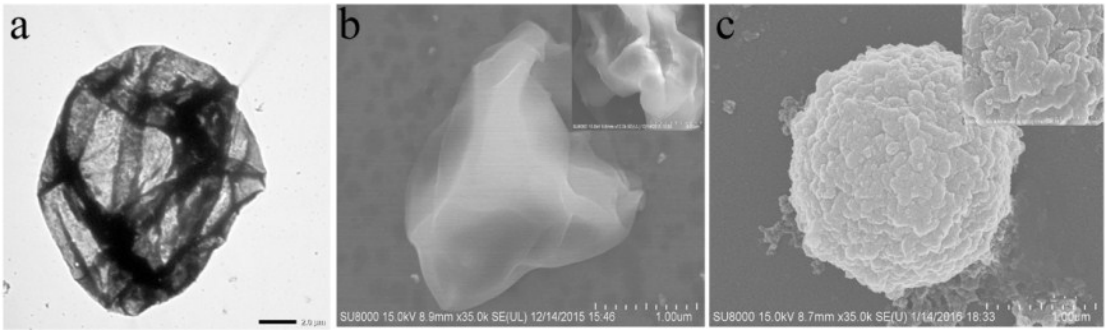

Figure S6. Structural characterization of bared proteinosome and TA-Fe^{3+} protected proteinosomes, respectively. (a) Transmission electron microscope (TEM) image of a dried bared proteinosome, and scanning electron microscope (SEM) images of (b) a dried bared proteinosome and (c) TA-Fe^{3+} protected proteinosomes.

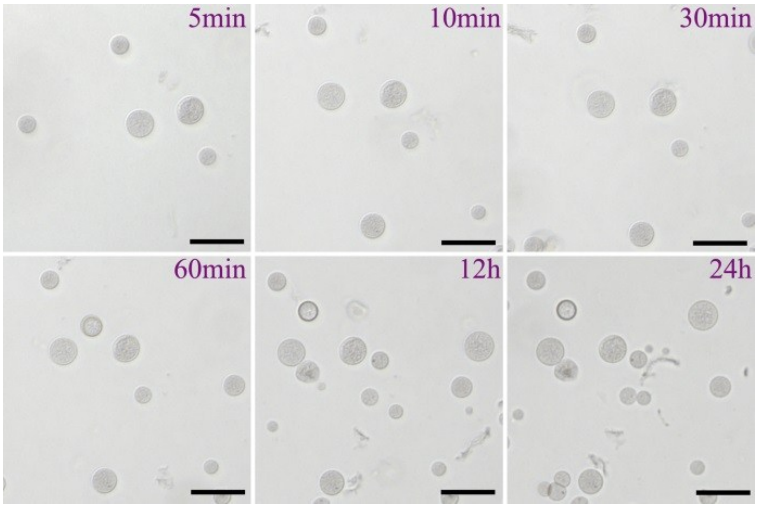

Figure S7. Optical microscopy images of TA-Fe^{3+} protected proteinosomes dispersed in serum medium, monitored by 24 hours. Scale bars are 25 μm.

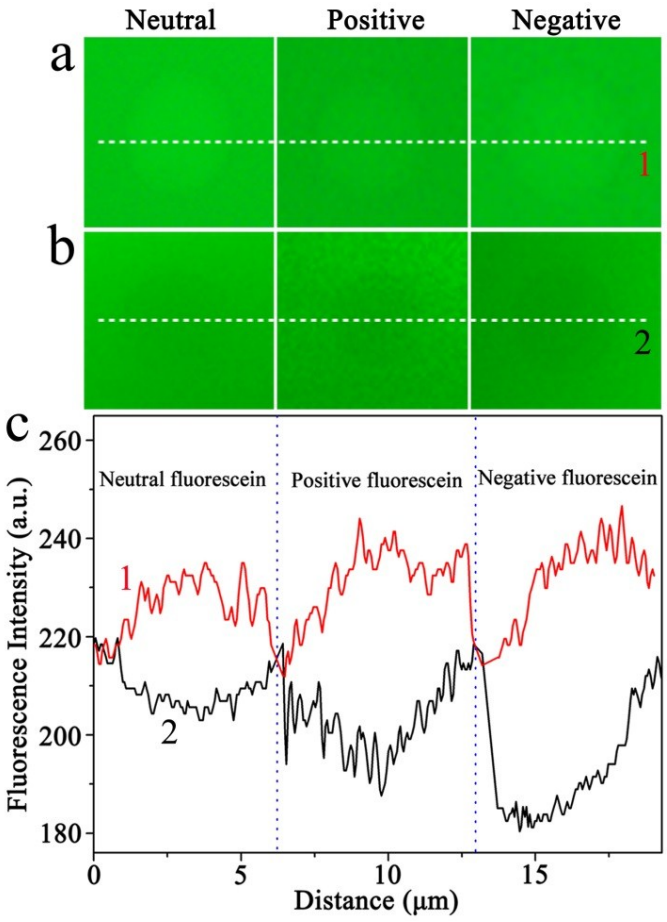

Figure S8. Fluorescence microscopy images of bared proteinosome (a) and TA-Fe^{3+} protected proteinosomes (b) dispersed in water with adding neutral fluorescein, positive fluorescein and negative fluorescein, respectively. (c) The corresponding fluorescence intensity of bared proteinosomes (red line) and TA-Fe^{3+} protected proteinosomes (black line), respectively.

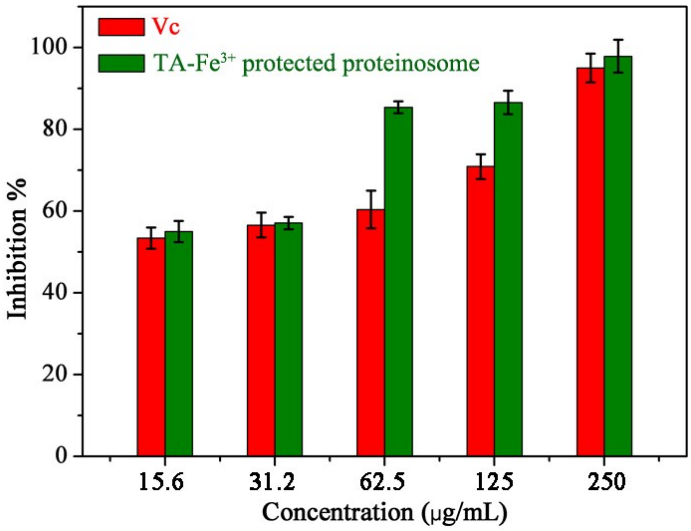

Figure S9. Scavenging effect of TA-Fe^{3+} protected proteinosomes and Vc for $ABTS^{+}$ at different concentrations.

References

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