Electronic Supplementary Information

Synthesis of artificial chaperones in a novel type of Pickering emulsion for glycoprotein

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Characterization of materials

Scanning electron microscopy (SEM) images showing the morphologies of the poly (DVB-co-PBA) which synthesized by One-step solvothermal were obtained using a Hitachi SU1510 electron microscope (Japan). FT-IR spectras (4000-400 cm^{-1}) in KBr were recorded using a Vector 22 spectrometer (Bruker, brukeroptics. equips. cn/). Fluorescence (FL) measurements were carried out on a Thermo Scientific Lumina FL spectrometer (America) equipped with a 1×1 cm quartz cell. UV-vis spectra were measured on a Thermo Scientific Evolution 300 UV-vis spectrophotometer (America).

SEM characterization of the poly (DVB-co-PBA) that synthesized by one-step solvothermal

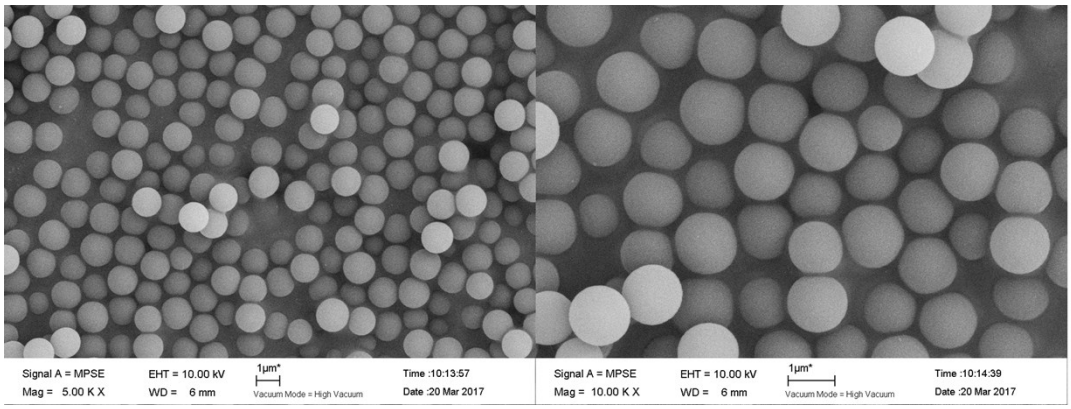


Fig. S1 The SEM of poly(DVB-co-PBA) that synthesized by one-step solvothermal

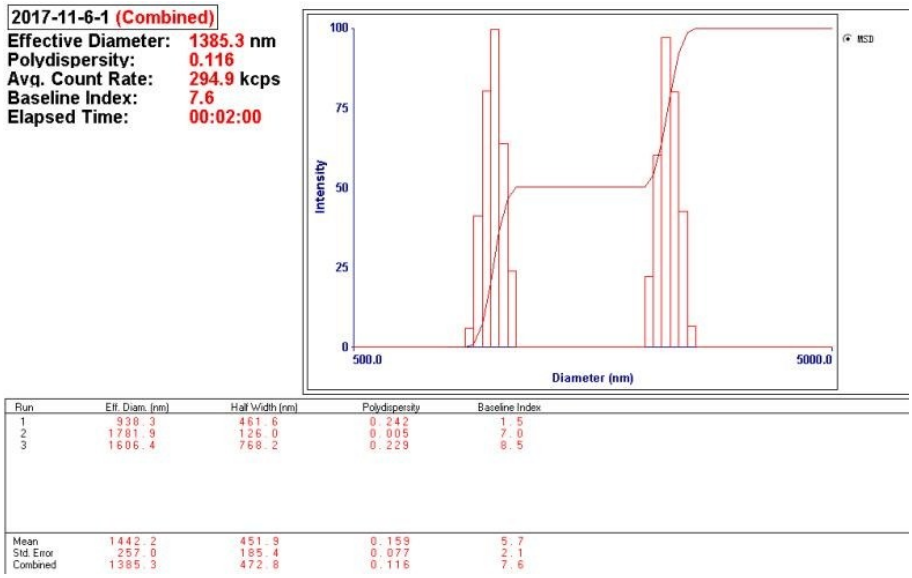


Fig. S2 The particle size distribution of poly(DVB-co-PBA)

The study of TGA

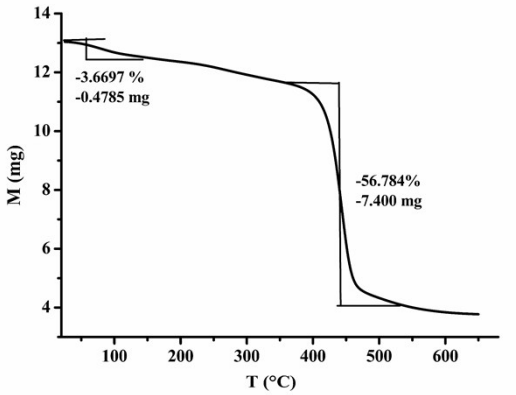


Fig. S3. TGA of poly(DVB-co-PBA) microspheres

In TGA, the temperature was slowly increased from 25 °C to 600 °C. Two weight losses were obtained for the tested samples. The first event, indicative of water loss, occurred at low temperature (50–150 °C). The second weight losses started at about 350 °C occurred from 450 °C, related to thermal processes involving the degradation of polymer chains.

The carbohydrate affinity of poly (DVB-co-PBA) microspheres

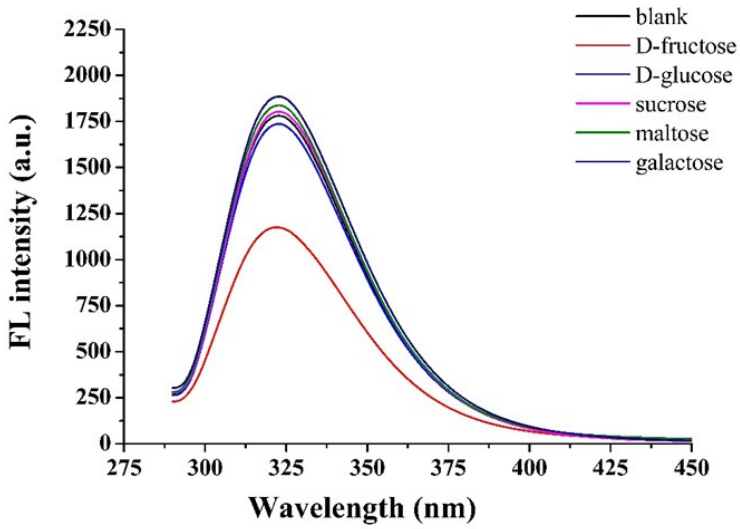


Fig. S4 Fluorescence response of poly (DVB-co-PBA) microspheres in presence of different types of monosaccharides (D-fructose, D-glucose, sucrose, maltose, galactose, the concentration of 10 mg mL^{-1})

The poly (DVB-co-PBA) microspheres combined with D-fructose to form a certain structure of the non-fluorescent complex of ground state through the intermolecular force, which led to the fluorescence intensity decrease. The application of fluorescence properties will appear in our follow-up study.

The contact angle of water droplet of the poly(DVB-co-PBA) particles

To illustrate the wetting properties of the poly(DVB-co-PBA) particles, the particles were deposited on a flat surface and pressed into a thin layer using a watch glass. The wetting properties of the polymers were estimated by direct image analysis of the contact angle of water droplet (50 μL) on the polymer layers (Fig. S3).

Assuming the roughness of the particle films are the same, the contact angle of water on poly(DVB-co-PBA) was 52.7º.

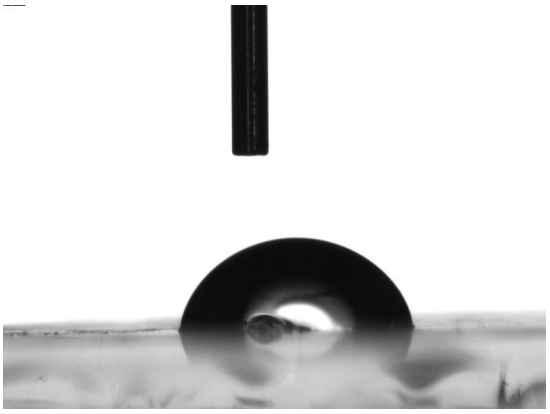


Fig. S5 Images of water droplets deposited on a thin layer of poly(DVB-co-PBA)

The synthesis of the core-shell amphiphilic polymer microspheres

The system of water-in-toluene

The poly(DVB-co-PBA) particles (200 mg) were dissolved in 15 mL toluene as an oil phase, and then ultrasound for 10 minutes. Acrylamide (AM, 150 mg), N,N′-methylenebis(acrylamide) (MBAA, 10 mg) and N-isopropylacrylamide (NIPAAm, 50 mg) were dissolved in 5 mL DDW as a water phase. After mixing the two phases, shaking vigorously for 5 min by hand. Initiator of ammonium persulfate (10 mg) and N,N,N′,N′-tetramethylethylenediamine (TEMED, 20 μL) were added to emulsion, the polymerization reaction was carried out at 30 °C for 24 h.

After polymerization, the solvent was removed by decantation[S1]. The resultant product was filtered with a sand core funnel of G5, and then the product was washed with methanol for three times to remove the residual oligomers and monomers, then vacuum drying.

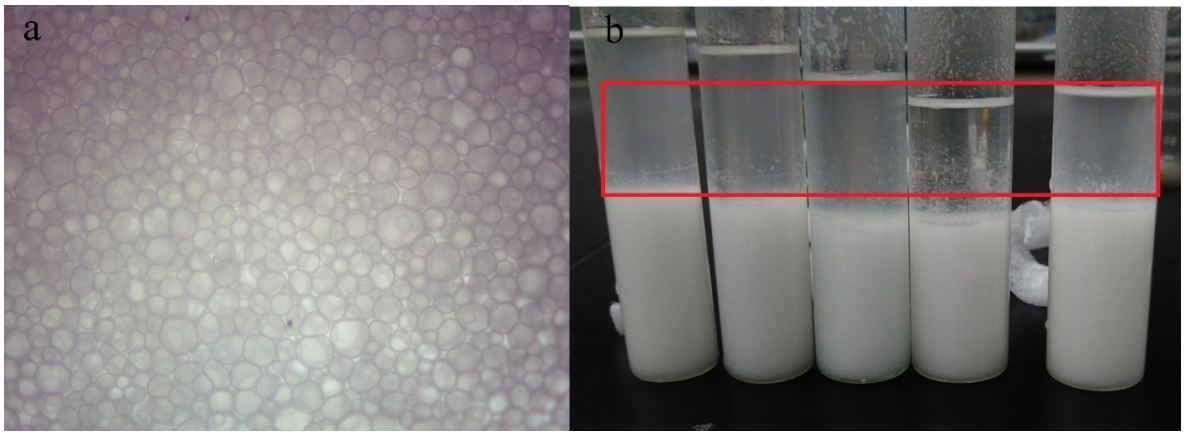
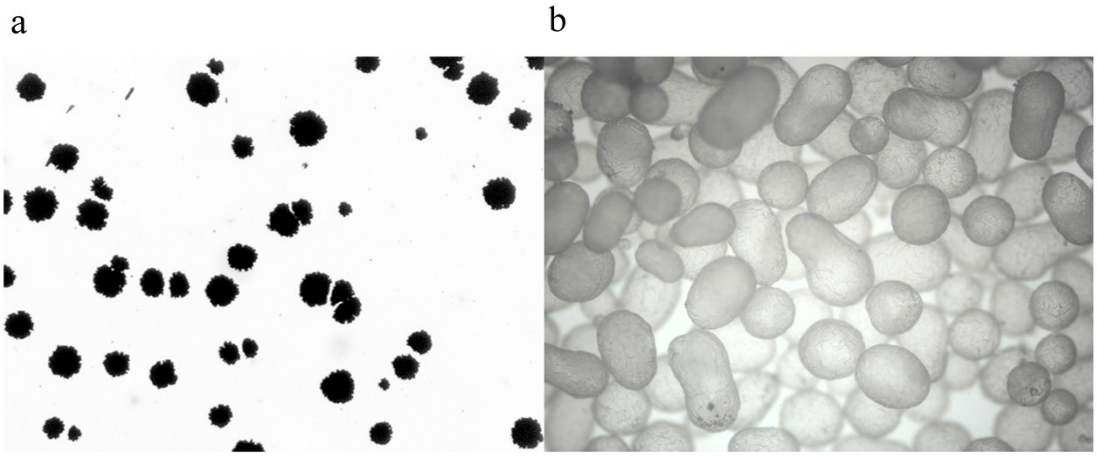


Fig. S6 Pickering emulsions of water-in-oil (a) Optical microscopy of Pickering emulsion (b) Picture of Pickering emulsion (water-in-toluene)

A tip for the synthesis of the core-shell amphiphilic polymer microspheres: To synthesize polymer microspheres of regular shape, the oil phase need excess (Fig. S5



(b)).

Fig. S7 Representation of the W-type microspheres undergo a conformational switching by water absorbing swelling

The system of water-in-chloroform

Fig.S8 The picture of Water Ⅱ-type amphiphilic polymer microspheres in the water after two months

The Water Ⅱ-type amphiphilic polymer microspheres showed better stability which was not easy to be damaged due to the absorption swelling of the hydrogel core. The reason may be that the corrosion of chloroform strengthened the cross-linking between the core and the shell.

The synthesis of the core-shell strong hydrophobic polymer microspheres

The poly(DVB-co-PBA)@L-Cys (20 mg) was dissolved in 3 mL DDW as a water phase, initiator of benzoylperoxide (4.0 mg) was dissolved in 1.5 mL divinylbexzene as an oil phase. After mixing the two phases, shaking vigorously for 5 min by hand, polymerization was carried out for 14 hours at room temperature. After polymerization, the resultant product was filtered with a sand core funnel of G5, and then the product was washed with methanol for three times to remove the residual oligomers and monomers, then vacuum drying.

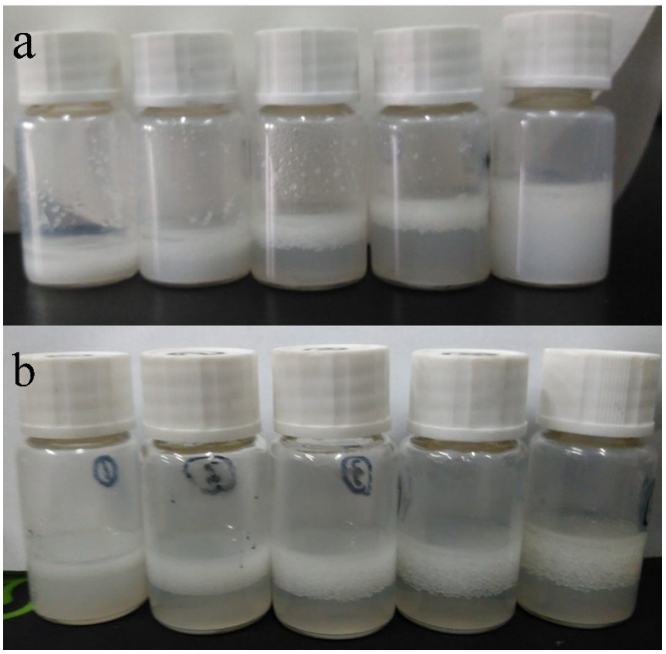


Fig. S9 Pickering emulsions of oil-in-water (a) optimization of the addition of water (b) optimization of the addition of DVB

The optimal ratio of water phase and oil phase was 2: 1 (v/v) and the optimal polymerization time was 14 hours.

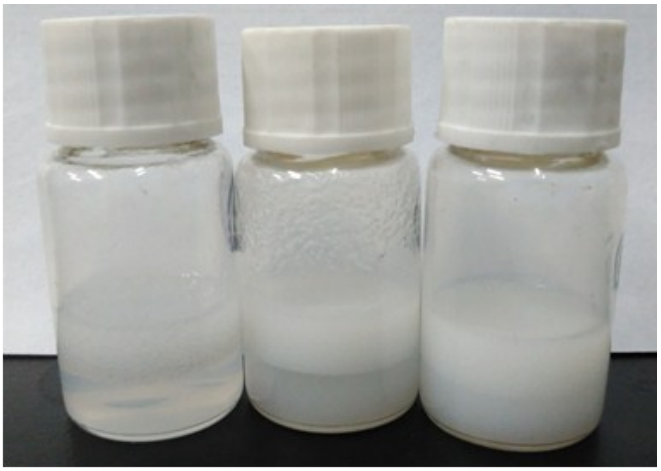


Fig. S10 Optimization of the addition of stabilizer particles

Too small additions of the stabilizers may result in the inability to form a stable Pickering emulsion and excessive additions may lead to poor appearance of the synthetic materials and difficulty in purification. The optimal addition of the poly(DVB-co-PBA)@L-Cys particles was 20 mg.

Different types of the core-shell amphiphilic polymer microspheres

Table S1 Different types of W-type microspheres

Material type	AM (mg)	NIPAAm (mg)	MBAA (mg)
All AM	142.16	0	10
4：1	142.16	56.58	10
1：1	71.08	113.16	10
All NIPAMM	0	113.16	10

The study of UV-VIS spectroscopy

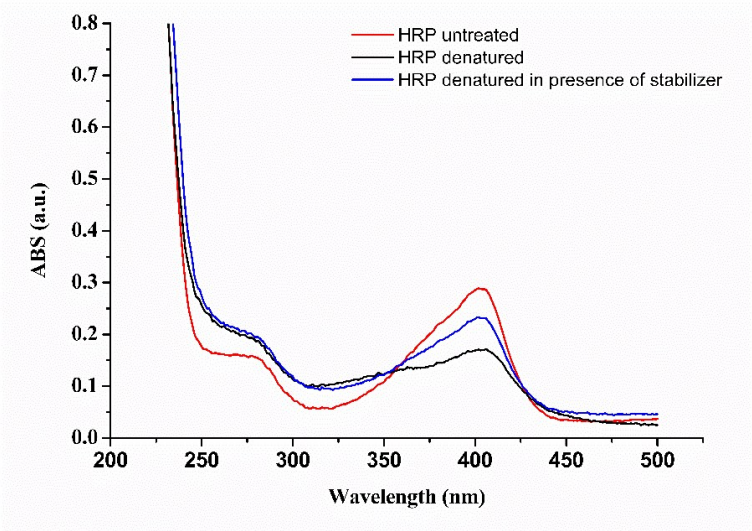


Fig.S11 UV-VIS absorption spectrum of HRP of different groups

The study of ANS probe

The ANS probe has a maximum excitation wavelength at 350 nm and a maximum emission wavelength at 514 nm when present alone. However, the maximum emission wavelength becomes 460 nm when combined into the hydrophobic region of the protein, corresponding to a significant enhancement of the fluorescence intensity[S2-S3].

To evaluate the capability of the synthetic materials for maintaining the natural conformation of protein, Horseradish peroxidase (HRP) solution (1 mg $mL^{-1,}$ pH=7.4, PBS buffer) with different types of materials were heated at 60°C for 30 min. The solution was filtered with 0.22 μm water filters and the ANS probe was added to the solution, and then determined by using fluorescent instrument.

The study of FITC-HRP

Labeling of HRP with FITC was performed as described previously[S4]. Briefly, 20 mg HRP was dissolved in 3 mL 0.1 M sodium carbonate buffer (pH 9.0) and 1 mg FITC was dissolved in 1 mL DMF. The FITC solution was added dropwise into the

solution of HRP. Finally, the mixture was incubated overnight at 4 °C and then dialyzed against PBS (10 mM, pH =7.6) and acetate buffer (10 mM, pH=5.5) to remove unreacted FITC. In the following text, FITC labeled HRP will be referred as FITC-HRP.

The different types of the synthetic materials were added to FITC-HRP solution. After heating denaturation treatment, the solution was removed by centrifugation. The materials were washed by water for three times and then observed with a confocal fluorescence microscope.

The changes of catalytic activity of HRP under heat stress

In order to investigate the catalysis of HRP in presence of the W-type polymer microspheres which were used as thermal stabilizers under heat stress. The assay procedure as follows.

The different types of material (1mg) were added into the 2 mL Horseradish peroxidase (HRP) solution (1 mg mL^{-1}, pH=7.4, PBS buffer), and then vortex for 1 minute to mix well. The above solution was placed in a temperature-controlled water bath and set the heating conditions for 1 ° C min^{-1}.

The enzyme activity test was performed every 10 min. 120 μL of the newly configured guaiacol solution (250 μM) and 10 μL of the newly prepared H_2O_2 solution (75 μM) was added into the 1 mL centrifuge tube, and then the heat treated HRP was added into the above solution with a total volume of 400 μL. The solution was mixed and detected at 700 nm by using an UV-VIS spectrophotometer.

The study of TEM

To evaluate the capability of the material for maintaining protein conformation, Horseradish peroxidase (HRP) solution (1 mg mL^{-1}, pH=7.4, PBS buffer) with different types of material were heated at 60°C for 30 min, then the solution was filtered with 0.22 μm water filters and observed by using Transmission Electron Microscope (TEM).

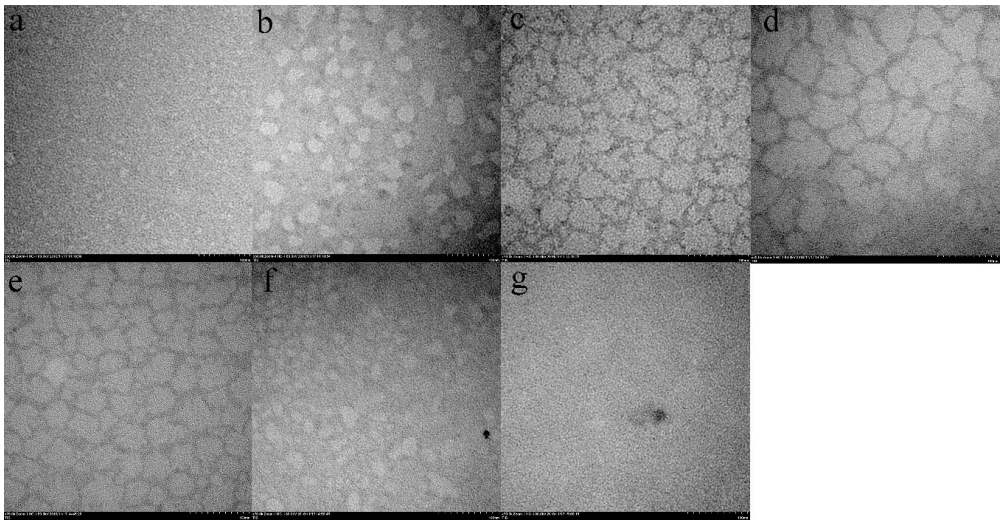
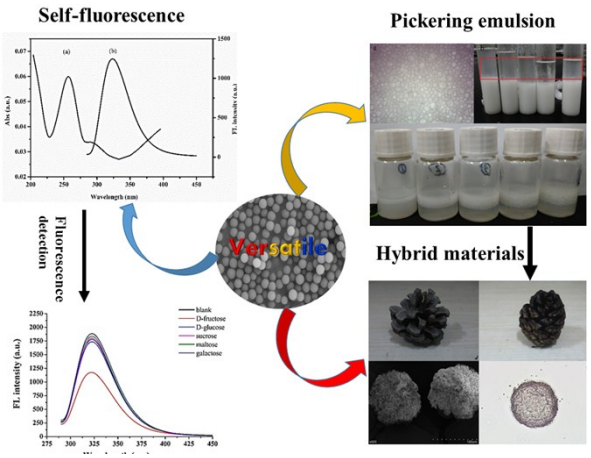


Fig. S12 The TEM of heat-denatured HRP with addition of different materials, a: untreated group, b: heat-denatured in absence of thermal stabilizers, c-g: all AM, 4:1, 2:1, all NIPAAm (the molar ratio of AM/NIPAAm of the W-type microspheres), poly(DVB-co-PBA))

Graphical Abstract



References

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S2 Semisotnov G V, Rodionova N A, Razgulyaev O I, et al. Study of the “molten globule” intermediate state in protein folding by a hydrophobic fluorescent probe[J]. Biopolymers, 1991, 31(1): 119-128.

S3 Haskard C A, Li-Chan E C Y. Hydrophobicity of bovine serum albumin and ovalbumin determined using uncharged (PRODAN) and anionic (ANS-) fluorescent probes[J]. Journal of Agricultural and Food Chemistry, 1998, 46(7): 2671-2677.

S4 Cohen S, Yoshioka T, Lucarelli M, et al. Controlled delivery systems for proteins based on poly (lactic/glycolic acid) microspheres[J]. Pharmaceutical research, 1991, 8(6): 713-720.