Electronic Supplementary Information

Polymerization-Induced Proteinosome Formation

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1.Experimental Part.

1.1 Materials.

N-isopropylacrylamide (NIPAM, 97%) was purchased from Sigma-Aldrich. It was recrystallized from n-hexane and dried under reduced pressure. Bovine Serum Albumin (BSA, Dingguo, 96%), ovalbumin (OVA, Sigma, 98%), 2,2'-azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride (VA-O44, Beijing Ocean Co. Ltd., 98%), 2,2'-dithiodipyridine (Heowns, 99%), fluorescein isothiocyanate (FITC, Alfa Aesar, 95%), 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB, Alfa Aesar, 99%) and sodium sulforhodamine B (Dalian Meilun Biological Technology Co. Ltd., 98%) were used as received. *N,N*-Dimethylformamide (DMF) was distilled under reduced pressure before use. Chain transfer agent (CTA), 4-cyano-4-[(ethylsulfanylthiocarbonyl)sulfanyl]pentanoic acid, was synthesized in this laboratory.

1.2 Experimental procedures

Coupling of thiols on BSA.

Native BSA (600 mg, 9.0×10^{-6} mol) were dissolved in 60 mL of PBS (PH=8.0, 50 mM) in a 100 mL Schlenk flask, and 2,2'-dithiodipyridine (25.2 mg, 1.14×10^{-5} mol) dissolved in 1.5 mL of DMF was added dropwise to the protein solution. Thiol-disulfide exchange reaction between thiols on BSA and 2,2'-dithiodipyridine was conducted at 0 ˚C for 12 h. Low molecular weight compounds and organic solvents were removed after dialysis against PBS (PH=8.0, 50 mM) for one day in a dialysis tubing (MWCO = 7 WDa), and pyridyl disulfide coupled BSA was obtained.

Determination of thiol content of BSA by Ellman's method.

DTNB (4.7 mg, 1.2×10^{-5} mol) was dissolved in 2 mL of PBS (100 mM, PH=8.0), and 30 μL of the solution was added to 1 mL of BSA solution (10 mg, 4.2×10^{-7}mol). The mixture was vortexed for 30 minutes and subjected to UV-vis measurement.

Polymerization-induced formation of proteinosomes.

A typical procedure for the synthesis of PNIPAM/BSA proteinosomes was described as follows. BSA (7.5 mg, 1.1×10^{-7} mol) and NIPAM (12.5 mg, 1.1×10^{-4} mol) were dissolved in 4.75 mL of PBS (pH=7.0, 10 mM) in a 10 mL schlenk flask. RAFT CTA (0.0875 mg, 3.30×10^{-7} mol) dissolved in 0.125 mL of DMF was added to the above solution. After three pump-thaw cycles at 0 ˚C, VA-044 (0.5 mg, 1.5×10^{-6} mol) dissolved in 0.25 mL of PBS was added into the solution through a syringe. The RAFT polymerization was performed at 40 ˚C for 12 h. The polymerization was stopped by exposure of the solution to air.

The synthetic procedure for the synthesis of PNIPAM/OVA proteinosomes is similar to that of PNIPAM/BSA proteinosomes, where OVA（2.5 mg, 5.5×10^{-8} mol）instead of BSA was used.

Kinetics study of RAFT polymerization of NIPAM in the presence of BSA.

BSA (3.7 mg, 5.5×10^{-8} mol) and NIPAM (12.5 mg, 1.10×10^{-4} mol) were dissolved in 4.75 mL of PBS (pH=7.0, 10 mM) with deuterated water as solvent in a 10 mL Schlenk flask. RAFT CTA (0.0875 mg, 3.3×10^{-7} mol) dissolved in 0.125 mL of DMF and a trace amount of 1,3,5-trioxane used as internal standard were added to the protein solution. After three pump-thaw cycles at 0 ˚C, 0.25 mL PBS of VA-044 (0.5

mg, 1.55×10^{-6} mol) was added. Under an argon atmosphere, 1 mL of the above solution was transferred to a NMR tube and RAFT polymerization of NIPAM was performed on a NMR spectrometer at 40 ˚C. The ^{1}H NMR spectra were collected at every 10 mins.

Synthesis of FITC-Modified BSA

BSA (100 mg, 1.50×10^{-6} mol) was dissolved in 20 mL of PBS (pH=8.0, 50 mM), and 1 mL of FITC (1.0 mg, 2.5×10^{-6} mol) solution was added dropwise to the protein solution. The reaction was performed in an ice bath for 4 h in the dark. To remove the excess FITC, the solution was dialyzed against PBS (pH=8.0, 50 mM) for 2 days by using dialysis tubing (MWCO =7 WDa) in an ice bath in the dark. FITC-labeled BSA was obtained after freeze-drying.

2.Characterization.

Monomer conversions of NIPAM were determined on a Varian UNITY-plus 400 M nuclear magnetic resonance spectrometer at 40 ˚C. Thiol content of BSA was determined on a Shimadzu UV-2450 spectrometer by using a quartz cell of 1 cm path length. Z-average sizes ($D_{h,z}$) of proteinosomes were determined on a Malvern Zetasizer Nano-S90 equipped with a 10 mW He−Ne laser (633 nm) at an angle of 90°. Apparent number-average molecular weights of PNIPAM obtained at different monomer conversions were determined on two size exclusion chromatographs, one is equipped with a Hitachi L-2130 HPLC pump, three Shodex columns (5000−5K, 400−0.5K, and 5−0.15K), and a Hitachi L-2490 refractive index detector with DMF

as the mobile phase; the other equipped with a CoMetre 6000 LDI pump, Shodex SB-802.5, 803, and 804 HQ columns, and a Schambeck SFD GmbH RI2000 refractive index detector with PBS (pH=7.0, 10 mM) as the mobile phase. Lower critical solution temperatures (LCSTs) of PNIPAM at different monomer conversions were determined on a SETARAM micro-differential scanning calorimeter (μ-DSC) at a scanning rate of 0.3 K/min. Transmission Electron Microscopy (TEM) images of proteinosomes were collected on a Tecnai G2 F20 S-TWIN electron microscope operated at a voltage of 200 kV. The proteinosomes were stained with $Pb(Ac)_2$ and protein molecules were stained. The TEM specimens were prepared by depositing aqueous solutions of the stained proteinosomes on copper grids at 40 ˚C in an oven and water was evaporated at this temperature. Confocal Laser Scanning Microscopy (CLSM) images of the proteinosomes were collected on a Zeiss LSM710 confocal laser scanning microscope. Atomic force microscopy (AFM) images were recorded on a Nanoscope IV atomic force microscope (Digital Instruments Inc.) operated in the tapping mode using Si cantilevers with a scan rate of 1.0 Hz and a resonance frequency of 320 kHz. The AFM sample was prepared by depositing aqueous solution of the proteinosomes on the surface of mica at 40 ˚C in an oven and water was evaporated at this temperature. Far-UV circular dichroism (CD) spectra were collected on a Jasco J-715 spectropolarimeter at a scanning speed of 100 nm/min. Wavelength scans in the range of 190 to 250 nm were collected with a spectral resolution of 0.5 nm.

3.Supplementary Figures

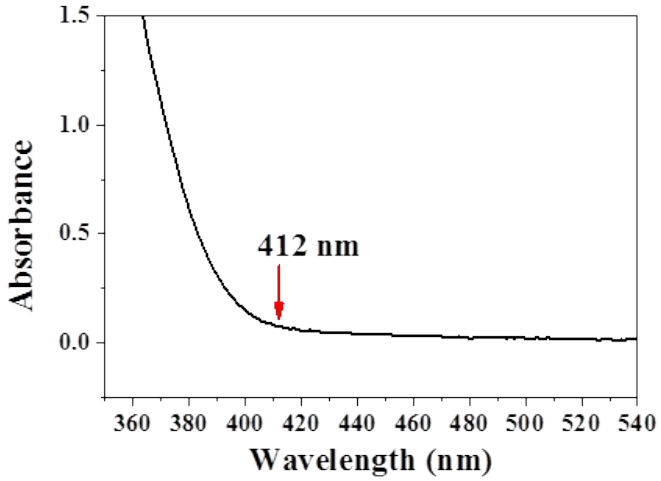


Figure S1. Ellman's analysis of BSA after coupling with 2,2′-dipyridyl disulfide.

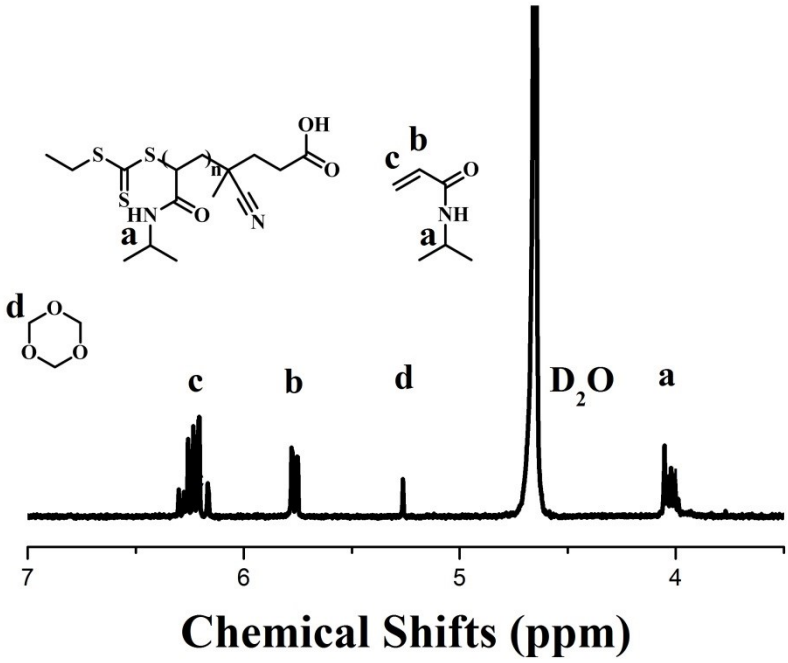


Figure S2. ^{1}H NMR spectrum of a polymerization system with PNIPAM, NIPAM monomer and a trace of trioxane.

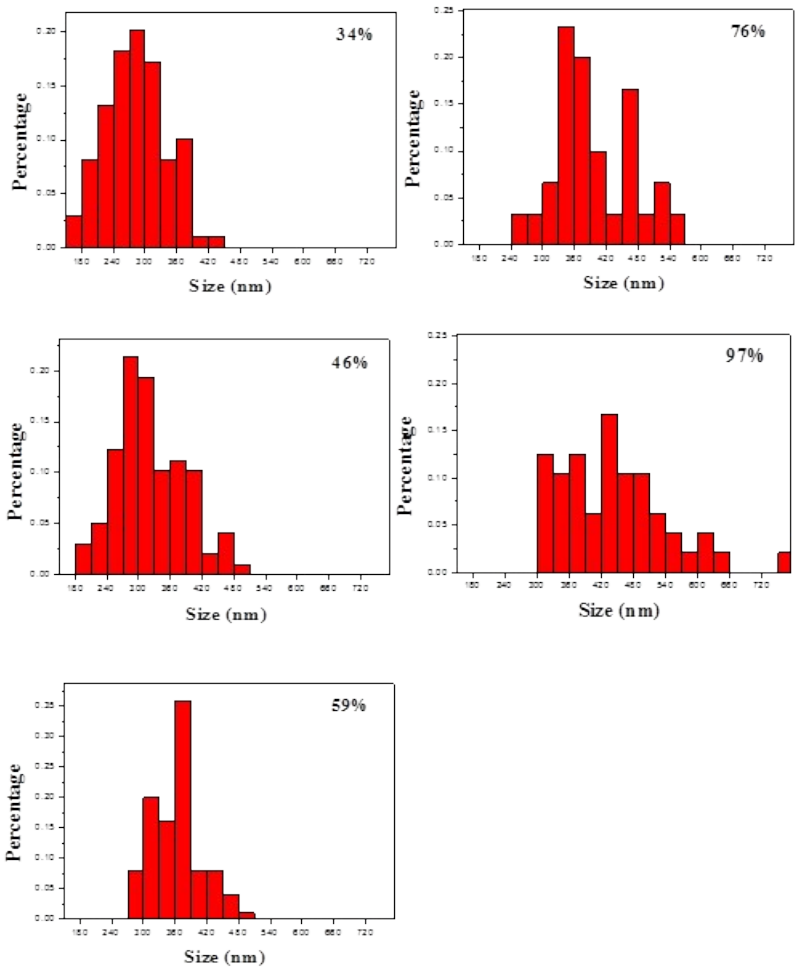
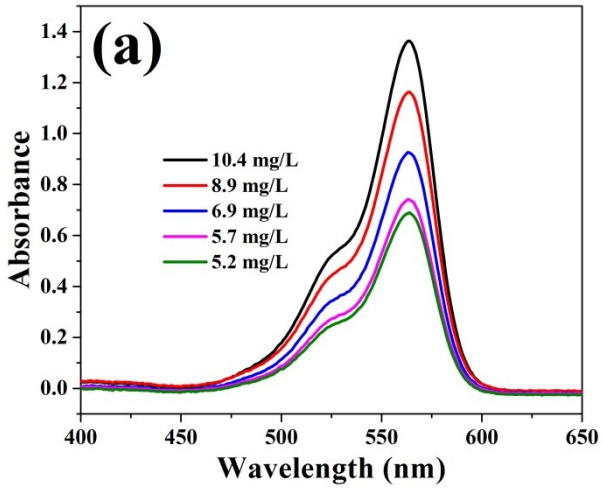


Figure S3. Size distributions of the proteinosomes prepared at 34, 46, 59, 76, and 97% monomer conversions.



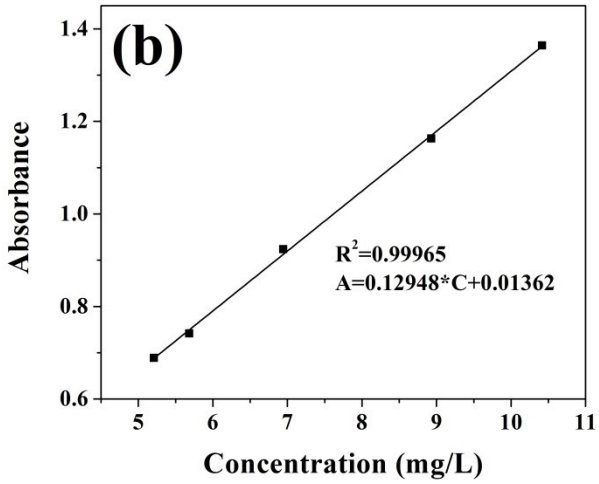


Figure S4. (a) UV-vis spectra of sodium sulforhodamine B (SRB) in PBS (pH=7.0, 10 mM) at different concentrations, (b) a standard curve of SRB in PBS.