Supporting Information

Engineering Proteinosomes with Renewable Predatory Behaviour Towards Living Organism

Chunyu Zhao, Mei Zhu, Ye Fang, Xiaoman Liu*, Lei Wang, Dafa Chen and Xin Huang*
MIIT Key Laboratory of Critical Materials Technology for New Energy Conversion and Storage, School of Chemistry and Chemical Engineering, Harbin Institute of Technology, Harbin, 150001 (China)
E-mails: liuxiaoman@hit.edu.cn; xinhuang@hit.edu.cn

Experimental Section

Materials*:* Bovine serum albumin (BSA, MW 66kDa, Sigma, purity>98%), 1, 6-Diaminohexane (Sigma, 98%), N, N-dimethylaminoethyl methacrylate (DMAEMA, Energy Chemical), Bromoethane (BE, Energy Chemical, 99%), N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride crystalline (EDAC, Energy Chemical, 99%), Hydrochloric acid (Sigma), Dextran (Dex, MW 70000, Pharmacia), 4-Formylbenzoic acid (Energy Chemical, 98%), N,N-dicyclohexylcarbodiimide (DCC, Energy Chemical), 4-(dimethylamino) pyridine (DMAP, Sigma), Chitosan Oligosaccharides (COS, MW 1kDa, TCI), L(+)-Arginine (Arg, Aladdin, 99%), MES monohydrate (Energy Chemical, 99%), N-hydroxysuccinimide ester (NHS, Sigma), 2-ethyl-1-hexanol (EH, Sigma), 4',6-diamidino-2-phenylindole (Sigma), Fluorescein isothiocyanate–Dextran (FITC-Dextran, MW~ 4 kDa, 70 kDa, Sigma), Fluorescein isothiocyanate isomer I (FITC, Sigma), Rhodamine B isothiocyanate (RBITC, Sigma), 2-(Dodecylthiocarbonothioylthio)-2-methylpropionic acid (Sigma), PEGylated bis(sulfosuccinimidyl)suberate (BS(PEG)5, Mw 532, Sigma, 98%), 2,4,6-trinitrobenzene sulfonic acid (TNBSA) solution (5% (w/v) in H2O, Sigma), fluorescein diacetate (FDA, Sigma), propidium iodide (PI, Sigma), N-Isopropyl acrylamide (NIPAAm, Energy Chemical) was recrystallized from hexane and toluene twice before use. 2,2'-Azobis(2-methylpropionitrile) (AIBN, Energy Chemical) was recrystallized from methanol. Gram-negative *Escherichia coli* (DH5α and ATCC 25922) and gram-positive bacteria (*Staphylococcus aureus,* ATCC6538) were obtained from Dingguo Biotechnology Co., Ltd. (China). In this study, phosphate-buffered saline (PBS: NaCl 9.0 g, Na_2HPO_4▪$12H_2O$ 3.401 g, NaH_2PO_4 4.86 g, DI water 800 mL) was used to obtain 50 mM, pH 7.4 solution.

Characterization methods: Optical and fluorescence microscopy was performed on a Leica DMI8 manual inverted fluorescence microscope at 20x, 40x and 63x magnification. Confocal images were obtained on a Leica SP5-Ⅱ confocal laser scanning microscope attached to a Leica DMI 6000 inverted epifluorescence microscope. Fluorescence activated cell sorter (FACS) analysis was undertaken on a Flow cytometry (Beckman Coulter, Inc.). Scanning electron microscopy (SEM) images were obtained on a SU8000 with the samples sputter-coated with 10 nm platinum. Transmission electron microscopy

(TEM) analysis was undertaken on a JEM-1400. Samples were prepared by adding one drop of a dispersion of proteinosomes onto a 200-mesh carbon film-coated copper grid followed by drying in a vacuum for one day. ^{1}H NMR spectra were recorded using a BRUKER ADVANCED spectrometer operating at 400 MHz at room temperature. Fourier Transform Infrared spectra (FTIR Spectrometer) were measured on a NICOLET IS5. The number of primary amine groups on the surface of BSA-NH_2 and COS-Arg was determined using a 2,4,6-trinitrobenzene sulfonic acid (TNBSA) assay. UV-vis spectra were measured on a PerkinElmer spectrophotometer (Lambda 750S, USA).

Synthesis of methacryloxyethyl dimethylethane ammonium bromide (MEDAB): MEDAB was synthesized according to the previously reported method[1]. N, N-dimethylaminoethyl methacrylate (DMAEMA) and bromoethane with mole ratio of 1:1.3 were dissolved in 5 mL of acetone in a round bottom flask. The reaction was carried out at 50°C for 5 h under the protection of N_2. After stopping the reaction, excess solvents were removed by vacuum distilled at 50 °C. Then the yellowish viscous coarse product was purified and precipitated by dehydrate ether for three times. After dried in vacuum at room temperature, final product was gained as white powder. ^{1}H-NMR (400 MHz, D_2O) δ 1.47 (m, 3H, -CH_3), 1.95 (s, 3H, CH_2=C-CH_3), 3.51 (m, 6H, -NCH_3CH_3), 3.85 (m, 2H, -CH_2CH_3), 4.16 (t, 3H, -O-CH_2-CH_2), 4.67 (t, 2H, C-O-CH_2) (Fig. S1, Supporting Information).

Synthesis of end-capped mercaptothiazoline-activated polymer (PNIPAAm-co-PMEDAB): The polymer was prepared according to our previous protocol[2]. Mercaptothiazoline-activated trithiol-RAFT agent (6.8 mg, 14.7 μmol), AIBN (0.7 mg, 4.2 μmol), NIPAAm (287 mg, 2.54 mmol), MEDAB (36.3 mg, 0.19 mmol) and acetonitrile (4 mL) were added to a 10 mL of round-bottom flask. The flask was then sealed and the solution was degassed via four freeze pump-thaw cycles. The polymerization was carried out at 65°C for 9 h (conversion 48%), and purified by three times precipitation in diethyl ether/hexane (1:2 volume ratio). The obtained polymer was characterized by ^{1}H-NMR spectroscopy in $CDCl_3$ (Fig. S2, Supporting Information). The molecular weight of the obtained polymer was determined by ^{1}H-NMR by comparing the integral of the proton of the CH_3 signal at δ=2.72 ppm in mercaptothiozalidine with that of the characteristic CH signal at δ=3.94 ppm in the repeat unit of NIPAAm and CH_2-O signal at δ=4.25 ppm in the repeat unit of MEDAB ($PNIPAAm_{52}$-*co*-$PMEDAB_8$, *Mn* 8040 $gmol^{-1}$).

Synthesis of the cationized bovine serum albumin (BSA-NH_2) and BSA-NH_2/PNIPAAm-co-PMEDAB conjugates: BSA-NH_2 was synthesized according to previous method[2]. Carbodiimide-activated conjugation of 1, 6-diaminohexane to aspartic and glutamic acid residues on the external surface of BSA was used to prepare the cationized protein BSA-NH_2. 1, 6-diaminohexane (1.5 g) was dissolved in deionized water and the solution was adjusted to pH 6.5 using 5 M HCl. The solution was added dropwise into a stirred solution of BSA (200 mg), and the coupling reaction was initiated by

adding N-(3-dimethylaminopropyl)-N/-ethylcarbodiimide 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 of 12–14 kDa MWCO) extensively against deionized water. The final product was obtained by freeze-drying.

The PNIPAAm-*co*-PMEDAB (20 mg in 5 mL of water) was added to a gently stirred solution of BSA-NH_2 (10 mg in 5 mL of PBS buffer pH 8.0) to with polymer: protein molar ratio of *ca*. 17: 1. The mixed solution was stirred for 12 h, and then purified by using a centrifugal filter (MWCO 50 kDa) to remove any unreacted PNIPAAm-*co*-PMEDAB and salts. The solution was then freeze-dried to give a white powder of BSA-NH_2/PNIPAAm-*co*-PMEDAB conjugates.

Determination of primary amine group on the surface of BSA-NH_2 by TNBSA measurement: The experiment was carried out according to previous method[3]. The chromogenic derivative formed by TNBSA and BSA-NH_2 was measured at 348 nm by UV-vis spectroscopy (LAMBDA 750 S).

Synthesis of Functionalized Dextran (Dex-CHO): The functionalized Dextran was prepared according to previous method[4]. Dextran (2 g, 0.0285 mmol, MW 70 000), 4-formylbenzoic acid (0.96 g, 6.4 mmol), and 4-(dimethylamino) pyridine (DMAP; 0.12 g, 0.984 mmol) were dissolved in 40 mL of dimethyl sulfoxide (DMSO), followed by the addition of N/,N-dicyclohexylcarbodiimide (DCC; 1.2 g, 5.83 mmol). The system was stirred at room temperature for 18 h and then the impurity was removed by filtration. The Dex-CHO product was obtained as a white solid after precipitation in a mixture of ethyl acetate and petroleum ether with a volume ratio of 1:9. The white solid was dissolved in the deionized water, and any insoluble impurities was removed by filtration, and then freeze-dried. The FTIR and ^{1}H-NMR study (Fig. S10, S11, Supporting Information) confirmed the successful synthesis of Dex-CHO.

Synthesis of L(+)-arginine grafted Chitosan Oligosaccharides (COS-Arg): *L*(+)-arginine was coupled to COS (COS-Arg) by amidation of the primary amine groups present in COS by using EDC/NHS as coupling agents as previously described with slight modifications[5-7]. Briefly, the COS (0.045 g) was dissolved in MES/HCl buffer (25 mmol L^{-1}, pH 5.0, 10 mL) in a 50 mL round-bottom flask equipped with magnetic stirring at room temperature. Subsequently, NHS (0.0357g) was dissolved in the COS solution, and followed by EDC (0.0594 g) was added to the reaction. *L*(+)-arginine (0.0015 g) was incorporated into the mixture and the coupling reaction proceeded for 48 h. The COS-Arg conjugate was dialyzed (dialysis tubing 500 Da MWCO) extensively against Milli-Q water to removed unreacted components. The purified COS-Arg was finally recovered by freeze-drying for 24 h. The final product was gained as white powder. The ^{1}H-NMR study in confirmed the successful synthesis of COS-Arg.

Synthesis of RBITC-labelled COS-Arg: RBITC-labelled COS-Arg was synthesized by adding 25 μL of RBITC DMSO solution (1.0 mg mL^{-1}) dropwise into a stirred COS-Arg aqueous solution (20 mg in 5 mL of PBS buffer pH 8.0) at room temperature. The mixed solution was stirred for 5 h, and purified by dialysis to remove DMSO and any unreacted RBITC. The solution was then freeze-dried to give a pink powder of RBITC-labelled COS-Arg.

In situ forming self-crosslinked Dex-CHO/COS-Arg hydrogel: Bulk hydrogel based on dextran was prepared *via* the Schiff base formation between Dex-CHO and COS-Arg at room temperature. Typically, 100 μL of COS-Arg aqueous solution (200 mg mL^{-1}) was rapidly mixed with 100 μL of Dex-CHO aqueous solution (200 mg mL^{-1}) and 50 μL of PBS buffer (0.05 M, pH = 7.5). The time required for gelation was determined by the test tube inversion method: typically no flow of the solution was observed for inverted samples within ≈4 min of vortexing the solutions at room temperature. Since the Schiff base groups are degradable via hydrolysis, and their stability decreases as the surrounding pH decreases, degradation of the Dex-CHO/COS-Arg Schiff base contained hydrogel in aqueous solution were preliminarily studied. Briefly, the as-prepared Dex-CHO/COS-Arg hydrogel was immersed into PBS buffer with pH value of 5.5. The time required for degradation was determined by the test tube inversion method: typically no flow of the all solution was observed for inverted samples within ≈90 min at room temperature. The morphology of the lyophilized hydrogel was characterized using scanning electron microscope.

Study of the permeability of proteinosomes: The permeability experiment of proteinosomes was carried out according to previous method[8]. We used FITC-dextran of different molecular weights ranging from 4 kDa to 150 kDa to assess the permeability of the generated proteinosomes. By mixing 0.1 mg mL^{-1} of different FITC-dextran with the measured proteinosomes and upon incubation for 30 min, the fluorescence microscopy images were captured from which the fluorescence intensity difference between the inside and outside of the proteinosomes was measured using the Image J software.

Preparation of bare proteinosomes and COS-Arg/Dex-CHO hydrogel-loaded proteinosomes: COS-Arg/Dex-CHO hydrogel-loaded proteinosomes were prepared as follows. Aqueous Dex-CHO (8 μL, 200 mg mL^{-1}) was added into a 1.5 mL of centrifuge tube containing BSA-NH_2/PNIPAAm-*co*-PMEDAB conjugates (20 μL, 15 mg mL^{-1}), COS-Arg (8 μL, 200 mg mL^{-1}), PBS (8 μL, 0.05 M, pH 8.0), and PEG-bis(N-succinimidyl succinate) (5 μL, 0.5 mg mL^{-1}) as the crosslinking agent to react with free remanent primary amine groups of BSA-NH_2. Then the 2-ethyl-1-hexanol (0.6 mL) was immediately added to the mixture, and the emulsion was vortexed for ≈ 50 s at room temperature to produce a dispersion of proteinosomes comprising a BSA-NH_2/PNIPAAm-*co*-PMEDAB conjugates

membrane and encapsulated COS-Arg/Dex-CHO aqueous solution. The water-in-oil proteinosomes were left unstirred for 12h and then the cross-linked proteinosomes were transferred into water. The upper clear oil layer was discarded and 1 ml of 75% ethanol was added and the emulsion was gently shaken. After three times centrifugation-dispersing cycles, the proteinosomes in aqueous solution was obtained. The bare proteinosomes without hydrogel was prepared according to the above steps: BSA-NH_2/PNIPAAm-*co*-PMEDAB conjugates (20 μL, 10 mg mL^{-1}), PBS (8 μL, 0.05 M, pH 8.0), and PEG-bis(N-succinimidyl succinate) (5 μL, 0.5 mg mL^{-1}), 2-ethyl-1-hexanol (0.4 mL).

Controlled release of COS-Arg from the COS-Arg/Dex-CHO hydrogel-loaded proteinosomes: To measure the release kinetics of COS-Arg, a dialysis tubing (MWCO 3000) containing the hydrogel-loaded proteinosomes suspension (the same amount of amino groups) was immersed in 10 mL of release medium (PBS buffer, pH 7.4 or pH 5.5) at the stirring rate of 160 rpm at 25 °C. At predetermined time intervals, 300 μL of the release medium was removed and replaced by fresh medium. The cumulative release amount of COS-Arg in the medium was determined by UV spectroscopy. The chromogenic derivative formed by TNBSA and amino of the COS-Arg was measured at 348 nm by UV-vis spectroscopy.

Adsorption and desorption of E.coli on the surface of the proteinosomes: *E.coli* was cultivated in an oscillating incubator at 37 °C overnight and subsequently the bacteria contained growth broth was collected by centrifugation and then suspended in PBS buffer and DI water to get a concentration of 1×10^8 cells mL^{-1}, respectively. Then the proteinosomes solution (20 μL) was placed in a 24-well plate and incubated with 1 mL of bacterial suspension (in PBS buffer and DI water) at 37 °C at the stirring of 160 rpm for bacteria adhesion tests, respectively. They were then gently low-speed centrifuged with sterile water to remove loosely attached cells and salts. For the bacterial detachment assessment, after incubation, the sample surfaces were immediately transferred into fresh PBS (pH 7.4) for 4 h incubation at 25 °C with stirring of 200 rpm, after which they were rinsed with low-speed centrifugation. Each sample was taken every hour to observe bacterial adhesion. The bacteria on the proteinosomes were stained by 5 μL of PI /FDA and subsequently were washed with sterile water.

Zeta potentials of the proteinosomes: Zeta potentials of the samples dispersed in deionized water and PBS buffer (pH 7.4) were determined with a NanoZSP Zeta apparatus, respectively. Results are reported as the mean of three separate measurements on three different batches standard deviation.

Live/dead staining assays: A standard live/dead staining assay was performed using the fluorescein diacetate (FDA, yielding green fluorescence in live organisms) and propidium iodide (PI, yielding red fluorescence in cell membrane-damaged, dead organisms) to examine the biocidal activity of sample surfaces. Upon completion of the experimental treatments described above, the sample surfaces were

stained with PI (5 μg mL^{-1}) for 3 min and FDA (0.5μg mL^{-1}) for 6 min in the dark, respectively. The surfaces were rinsed by low-speed centrifugation and observed by CLSM.

Cytotoxicity assay: ATCC 3T3 murine fibroblasts were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10 vol % fetal bovine serum, 300 mg mL^{-1} L-glutamine, 100 units mL^{-1} penicillin, and 100 μg mL^{-1} streptomycin. The cells were detached from the culture flask by addition of 0.25 % trypsin-EDTA solution, and re-suspended in fresh medium for subsequent experiments. ATCC 3T3 fibroblasts in culture medium (1 mL) at a density of 10^4 cells mL^{-1} were seeded in each well of a 24-well plate, and incubated in a humidified atmosphere of 5 % CO_2 at 37 °C for 24 h. After replacing the medium with a fresh one, the hydrogel-loaded proteinosomes (equivalent amount used in bacteria tests) were placed in the cell layer. The parallel experiment without the samples was conducted as a blank control. After 24 h of incubation at 37 °C, the culture medium was removed, followed by the addition of 900 μL of culture medium and 100 μL of MTT solution (5 mg mL^{-1} in PBS) into the wells. After 4 h of incubation, the MTT solution and medium were removed. The obtained formazan crystals were dissolved by 1 mL of dimethyl sulfoxide (DMSO) to measure their optical absorbance at a test wavelength of 490 nm using a PerkinElmer spectrophotometer (Lambda 750S, USA). The results were expressed as percentages relative to the control experiment. According to the following formula: Cell viability (%) = Mean OD/Control OD x 100%, the cell viability was 91.1%, suggesting the low cytotoxicity of proteinosomes. Results are given as the mean of triplicate experiments and standard deviation.

Hemolysis test: The interactions between red blood cells (RBCs) and the proteinosomes were assessed according to a traditional hemolysis test procedure (*Colloids Surf., B* 2013, **111**, 333). The hemolysis ratio was calculated based on the absorbance value at 541 nm using a PerkinElmer spectrophotometer (Lambda 750S, USA). Fresh blood extracted from a healthy white rabbit was immediately mixed with 3.8 wt% sodium citrate solution at a dilution ratio of 9:1. We mixed the anticoagulated whole blood (0.1 mL) with the hydrogel-loaded proteinosomes in normal saline at 37 °C. After 120 min of incubation, 2 mL of normal saline was added to the samples, and then further incubated for 60 min at 37 °C to stop the hemolysis. Positive and negative controls were produced by adding 0.1 mL of blood to 2 mL of distilled water and normal saline, respectively. After incubation, the blood cells were removed by centrifugation (3000 rpm, 3 min) and the optical density (OD) of the supernatant was measured at 541 nm. The hemolysis ratio was calculated according to the following formula:

$$\mathrm{HR}(\%) = \frac{\mathrm{OD}_{test} - \mathrm{OD}_{neg}}{\mathrm{OD}_{pos} - \mathrm{OD}_{neg}} \times 100\%$$

Where OD_{test} is test sample absorbance value, OD_{pos}, and OD_{neg} are the positive (water) and negative (saline) control, respectively. The obtained data showed that the designed proteinosomes had low

hemolysis ratio (1.56 %). Results are given as the mean of triplicate experiments and standard deviation.

Statistical Analysis: Each result was an average of at least three parallel experiments. All data were presented as mean ± standard error. The statistical significance was assessed by analysis of variance (ANOVA): *, $p < 0.05$; **, $p < 0.01$

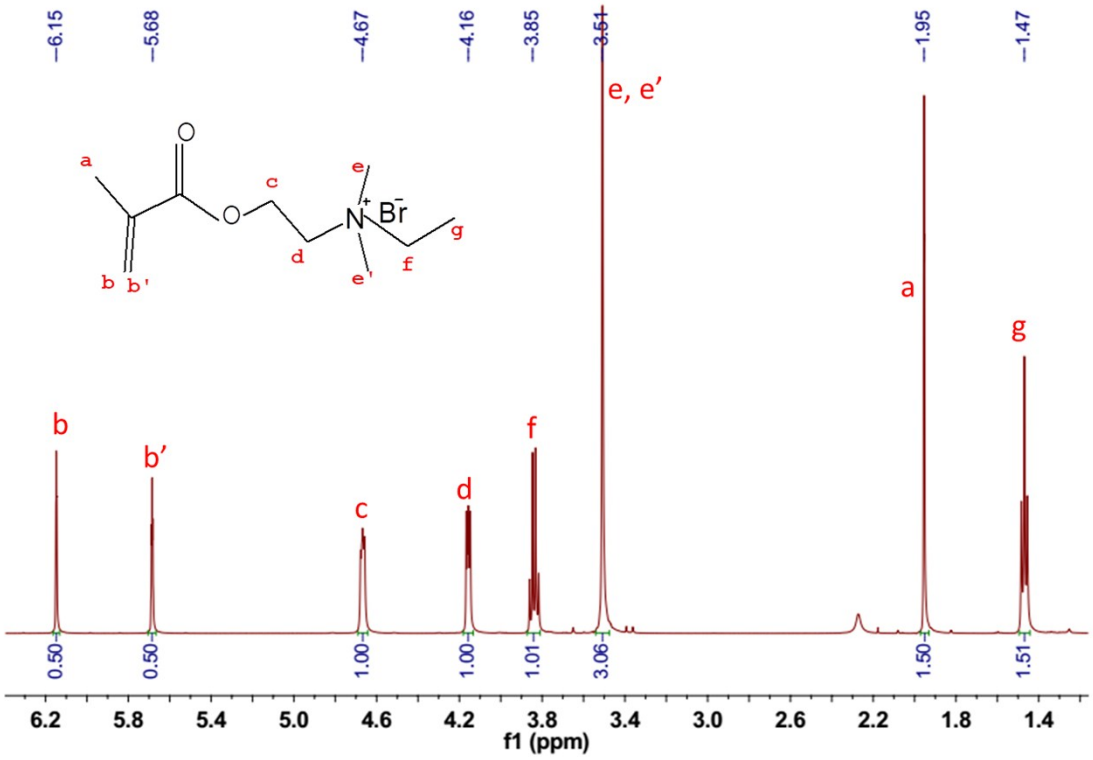

Figure S1. [1]H-NMR spectrum of methacryloxyethyl dimethylethane ammonium bromide (MEDAB) in $CDCl_3$.

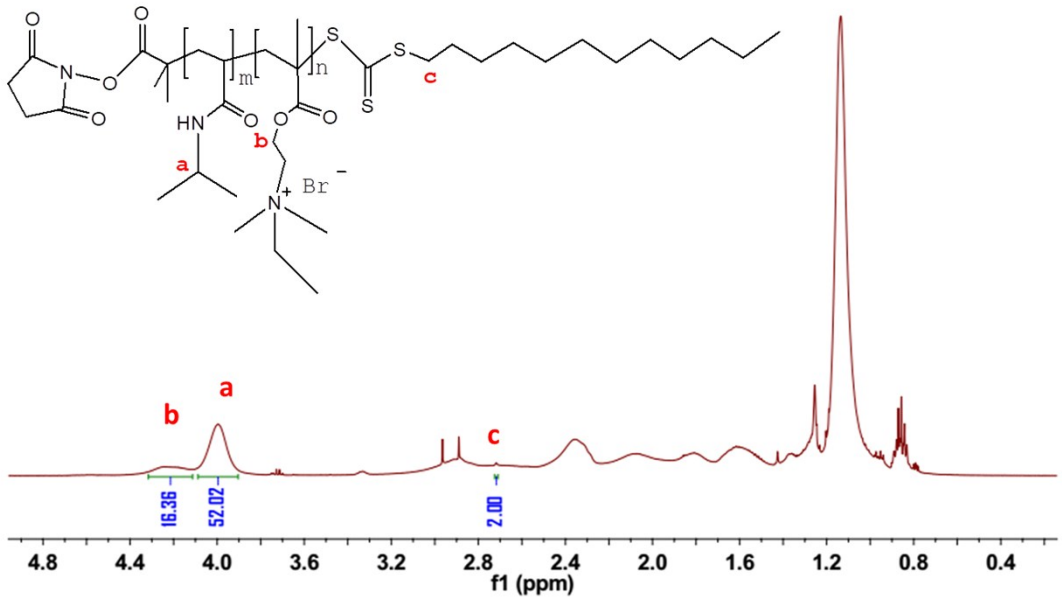

Figure S2. [1]H-NMR spectrum of mercaptothiazoline-activated polymer (PNIPAAm-*co*-PMEDAB) in $CDCl_3$.

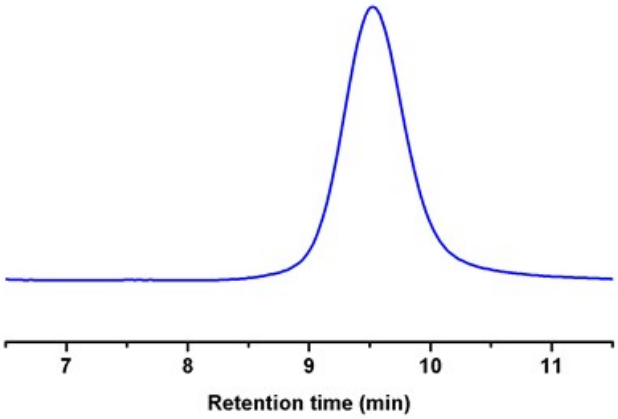

Figure S3. GPC profile of (PNIPAAm-*co*-PMEDAB). N,N-Dimethylformamide (DMF) was used as the eluent with a PNIPAAm-*co*-PMEDAB concentration of 1.0 mg mL^{-1}. The PDI value was 1.21

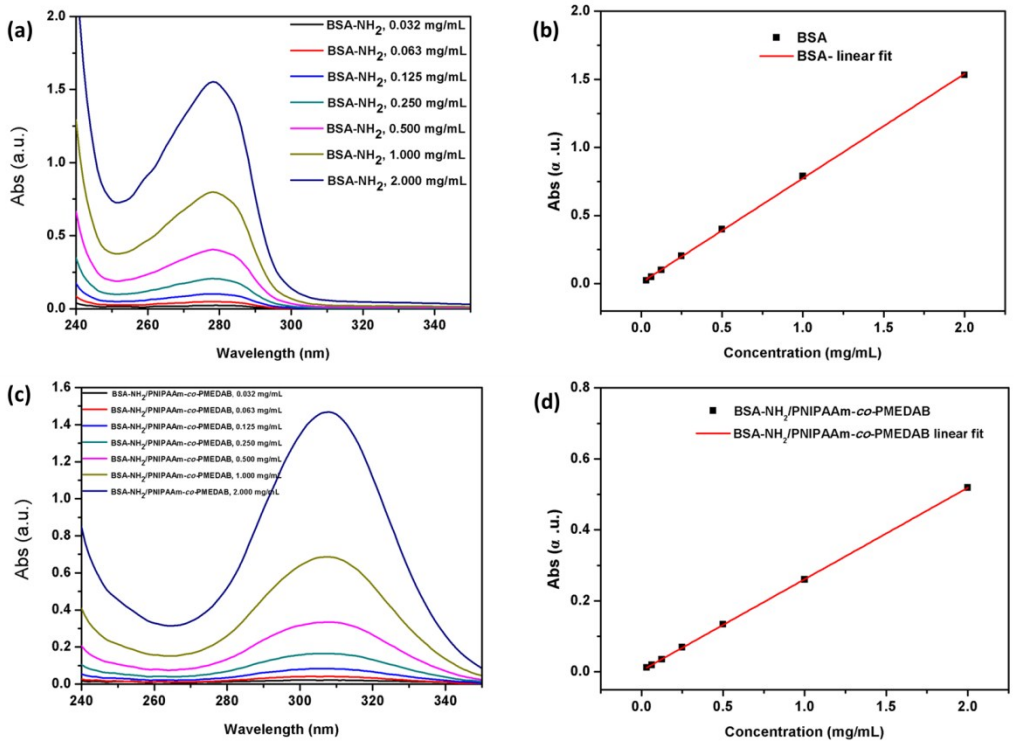

Figure S4. (a) UV-vis spectra of BSA-NH_2 at different concentrations in aqueous solution, (b) Calibration curve for BSA-NH_2 determined by plotting the UV-vis absorbance at 278 nm against concentration, (c) UV-vis spectra of BSA-NH_2/ PNIPAAm-*co*-PMEDAB at different concentrations in aqueous solution, (d) Calibration curve for BSA-NH_2/ PNIPAAm-*co*-PMEDAB determined by plotting the UV-vis absorbance at 278 nm against concentration.

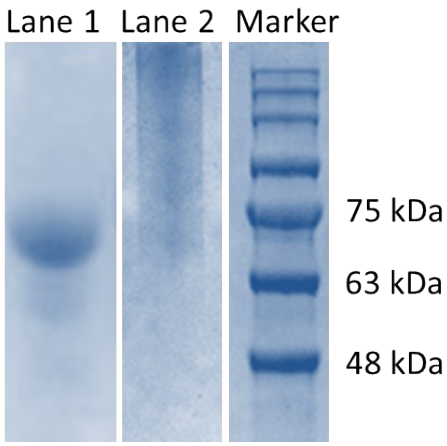

Figure S5. SDS–PAGE profiles; lane 1, BSA-NH_2; lane 2, BSA-NH_2/ PNIPAAm-*co*-PMEDAB; and marker lane.

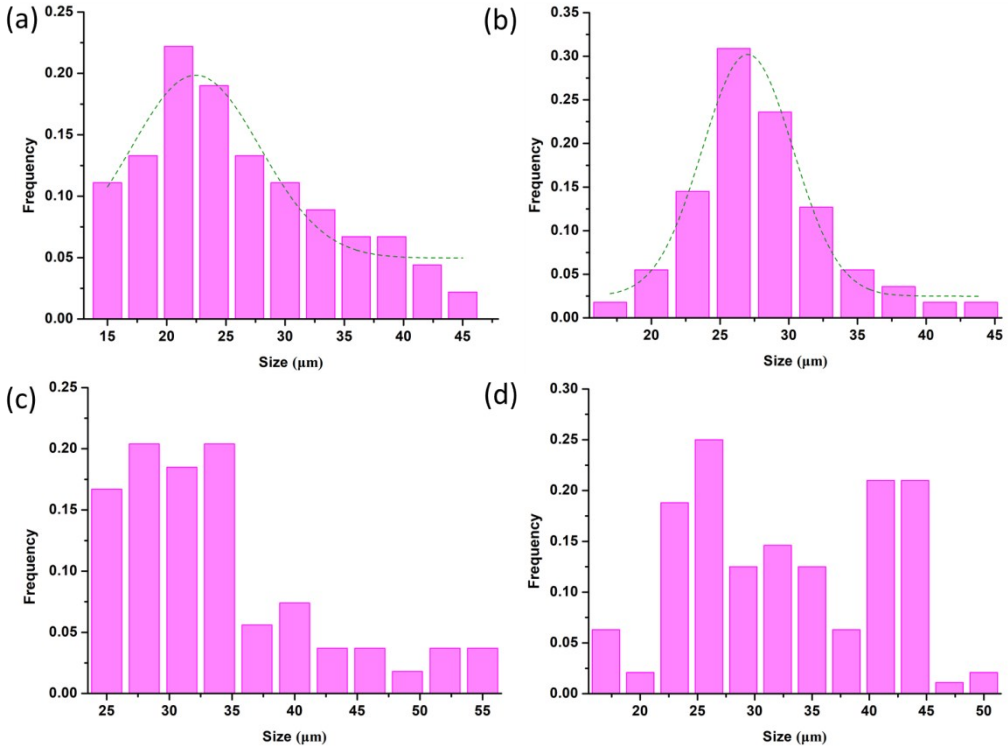

Figure S6. (a-d) Particle size distribution of proteinosomes prepared at different concentrations of BSA-NH_2/ PNIPAAm-*co*-PMEDAB (8.0, 6.0, 4.0 and 2.0 mg/mL, respectively).

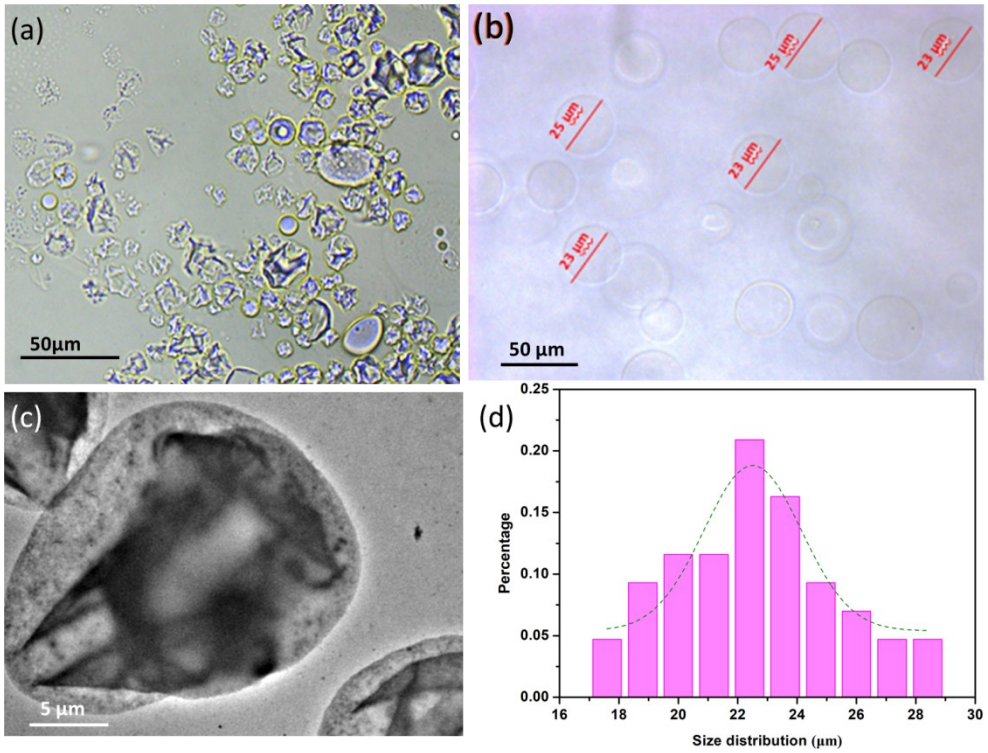

Figure S7. (a) Optical microscopy images of proteinosomes dispersed in oil after partial drying in air, (b) optical microscopy images of proteinosomes dispersed in water, (c) transmission electron microscope (TEM) of proteinosomes, (d) particle size distribution of hydrogel-loaded proteinosomes.

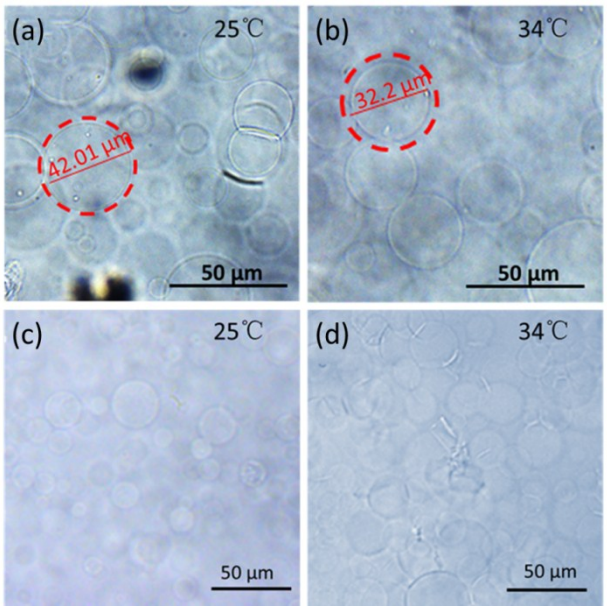

Figure S8. (a, b, c, d) Optical microscopy images of the hydrophobicity transition of BSA-NH_2/ PNIPAAm-*co*-PMEDAB proteinosomes, the shrinkage behavior (a, b) and the aggregation behavior due to the hydrophobic interaction (c, d).

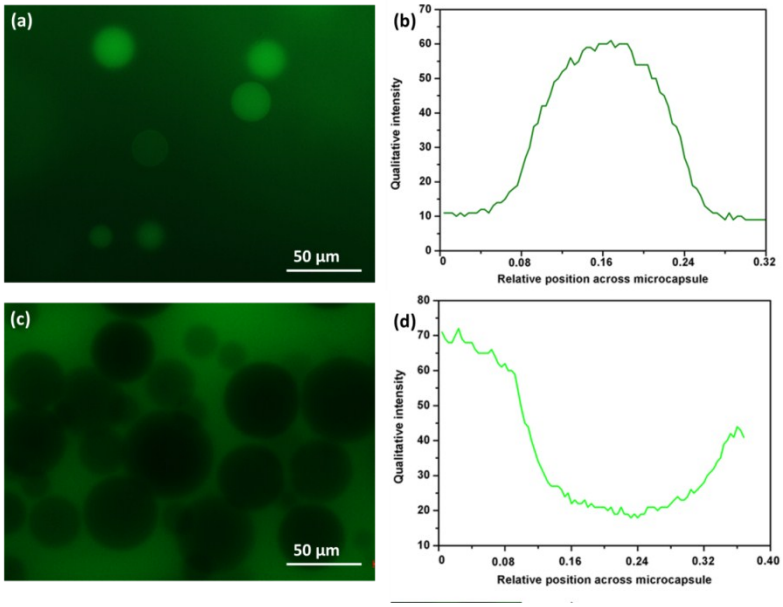

Figure S9. Study on the permeability of the constructed proteinosomes based on the diffusion of the fluorescent-labeled dextrans (FITC-dextran) and the corresponding fluorescence intensity line profiles of selected microcapsule are shown in the fluorescence images. (a, b) The fluorescent-labeled dextrans (FITC-dextran) with molecular weight of 4 kDa; (c, d) the fluorescent-labeled dextrans (FITC-dextran) with molecular weight of 70 kDa.

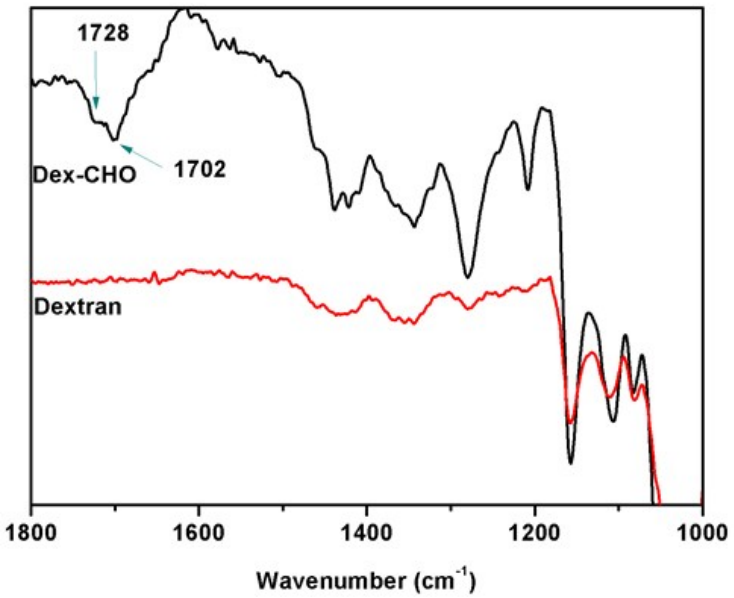

Figure S10. FTIR spectra of dextran and Dex-CHO confirming successful conjugation between –OH groups in dextran and carboxyl groups of formyl benzoic acid. Absorption peaks are observed at 1702 cm^{-1} (Dex-CHO, formyl benzoic acid, C=O stretch) and 1728 cm^{-1} (Dex-CHO, ester C=O stretch).

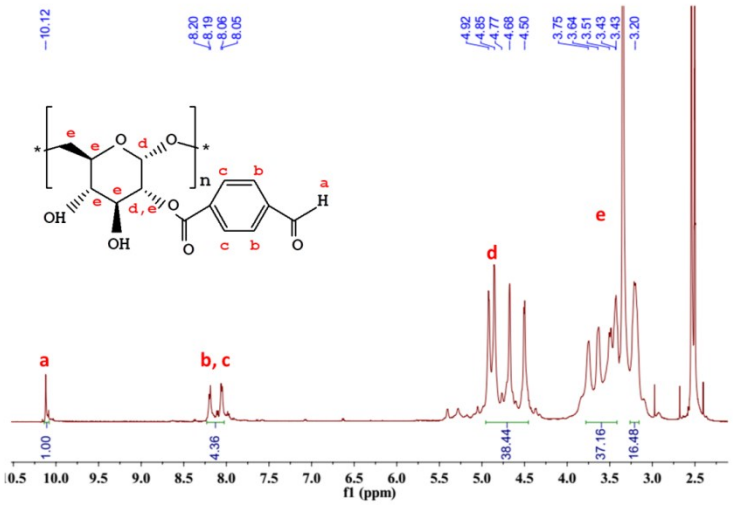

Figure S11. ^{1}H-NMR spectrum of Dex-CHO in $(CD_3)_2SO$. Based on the integrated intensity of the aldehyde resonance at 10.12 ppm, ca. 46 benzyl aldehyde groups were linked per dextran chain.

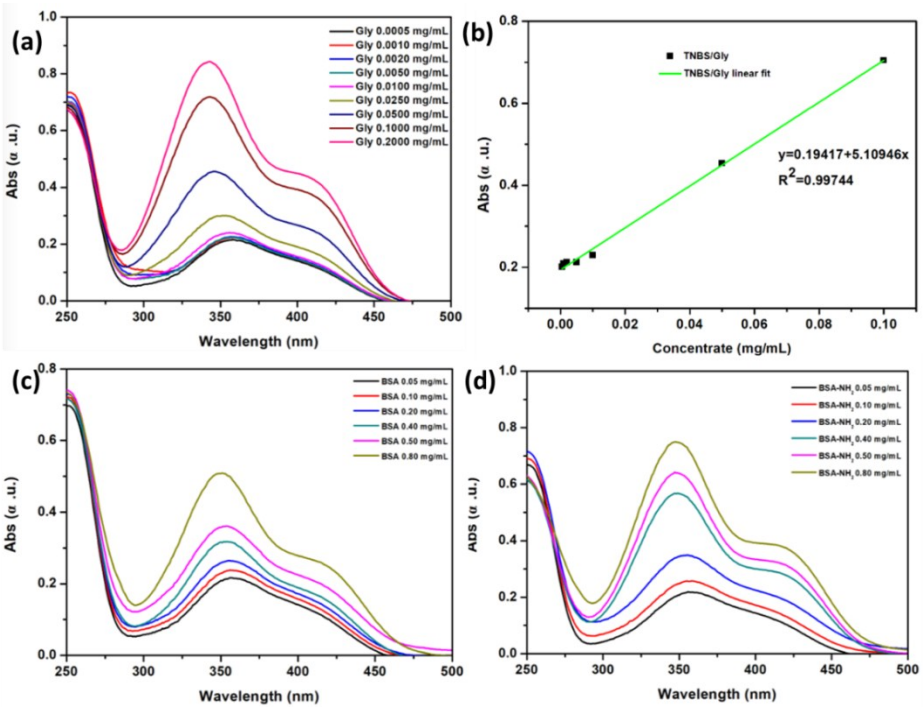

Figure S12. Control experiments associated with the 2,4,6-trinitrobenzene sulfonic acid (TNBSA) assay for the determination of free primary amino groups in BSA-NH_2. A highly chromogenic derivative with an absorption band at 348 nm is produced on reaction of primary amines with TNBSA. (a) UV-Vis spectra obtained for TNBSA/glycine control assay, (b) corresponding calibration curve based on plotting the absorbance at 348 nm against the concentration of glycine, (c) UV-Vis spectra obtained for TNBSA/BSA assay, (d) UV-Vis spectra obtained for TNBSA/ BSA-NH_2 assay. Using the standard curve, the number of primary amine groups per BSA-NH_2 was determined to be 78.

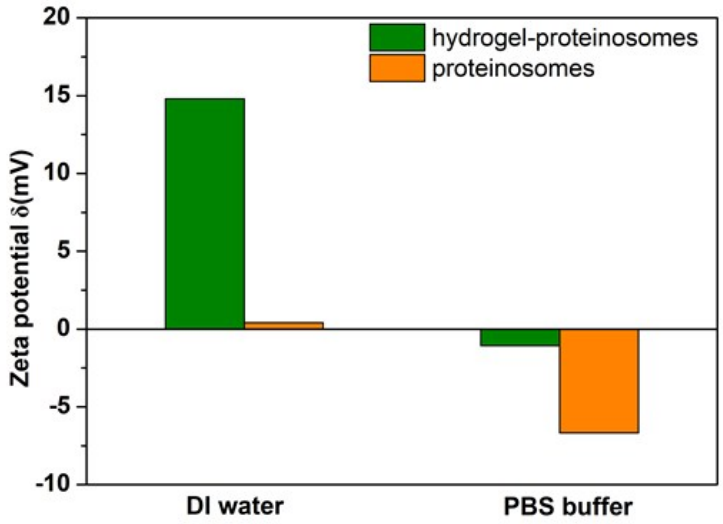

Figure S13. Zeta potentials of the hydrogel-loaded proteinosomes dispersed in deionized water and PBS buffer (pH 7.4) were determined with a Nano ZSP, respectively.

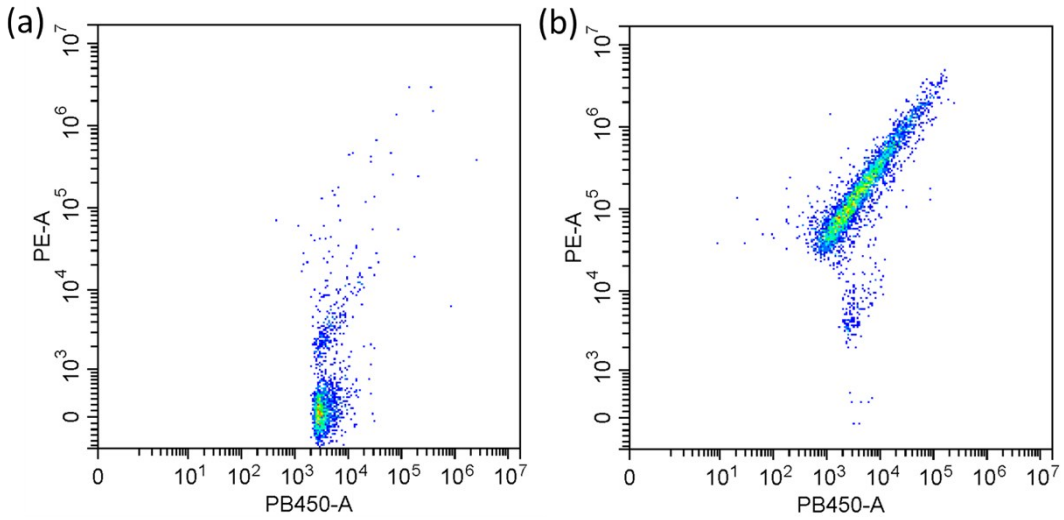

Figure S14. Control experiment: the single populations of *E.coli* (a) or the proteinosomes (b) un-mixed were observed in control experiments that pseudo-color plots were basically the same as before after 8 hours.

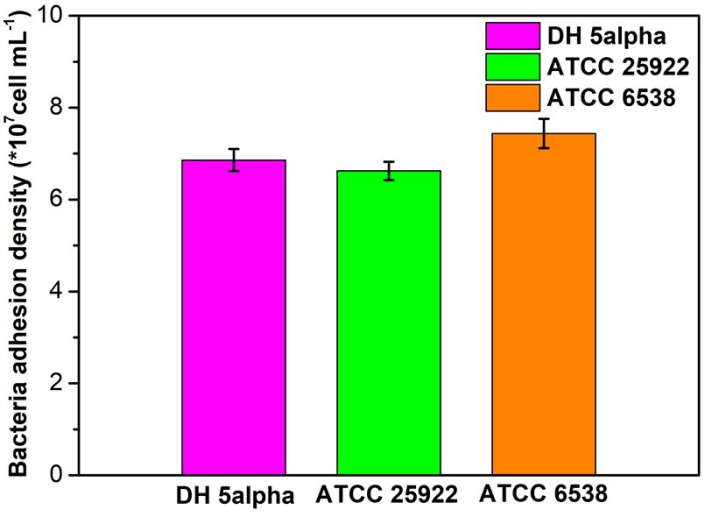

Figure S15. The number of attached bacteria on the surface of the hydrogel-loaded proteinosome. Error bars indicate the standard deviation of three replicating measurements.

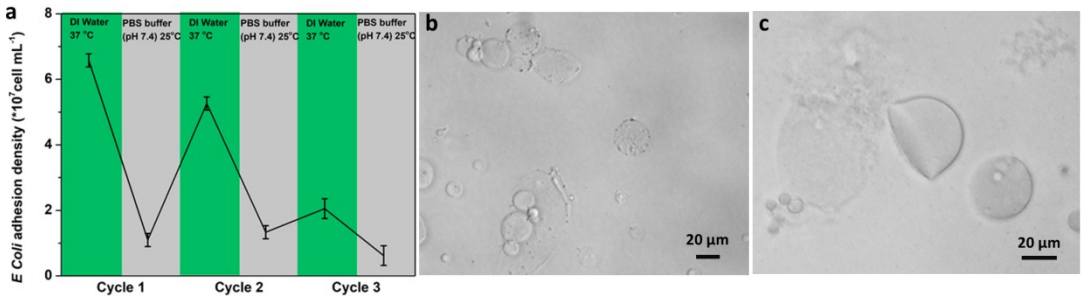

Figure S16. (a) Reversible binding and release of attachment and de-attachment of *E. coli* from the surface of the proteinosomes at DI water 37 °C or PBS buffer (pH 7.4) 25 °C, respectively. (b) Optical microscopy images of the attached *E. coli* on the surface of the proteinosomes at 37 °C in water in the third cycle test. (c) Optical microscopy images of the attached *E. coli* on the surface of the proteinosomes at 25 °C in PBS in the third cycle test.

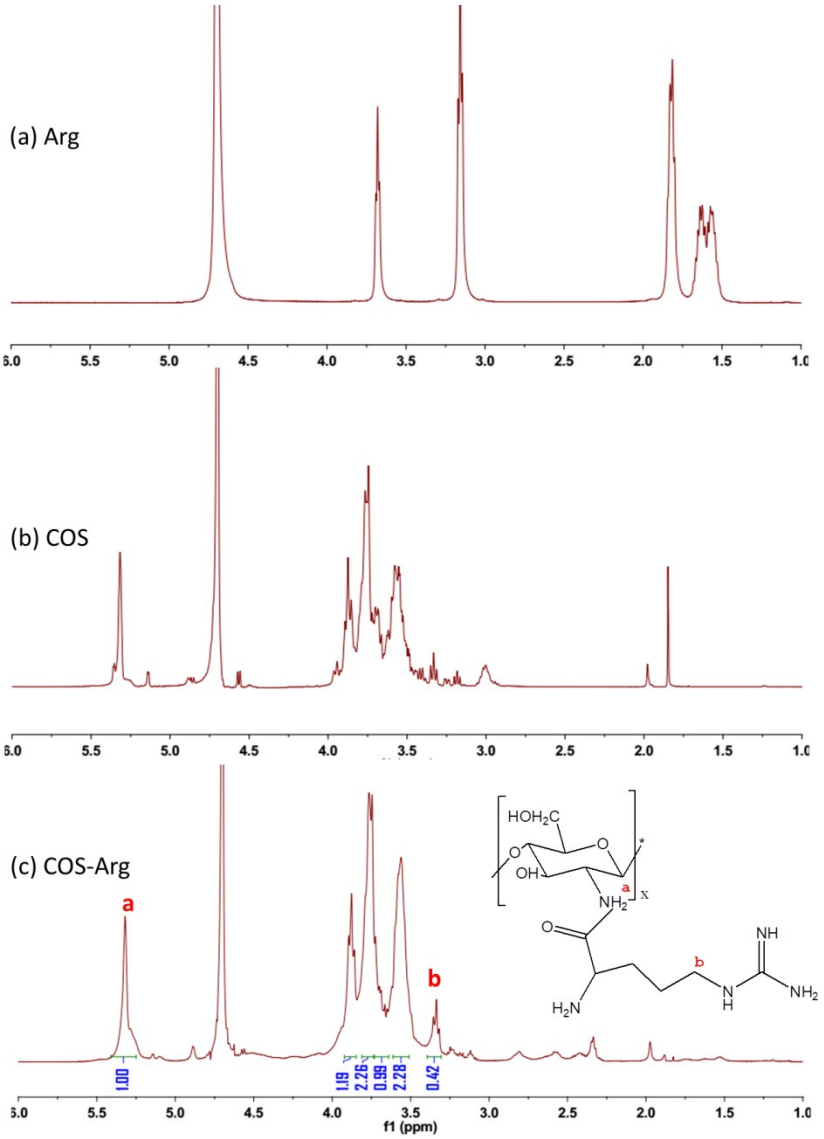

Figure S17. ^{1}H-NMR spectrum of COS-Arg in D_2O. The arginine substitution degree was ca. 0.21.

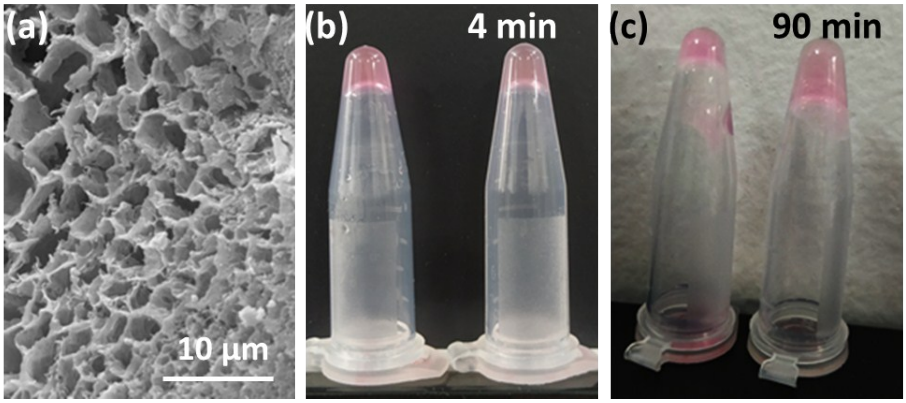

Figure S18. (a) SEM image of a hydrogel prepared from Dex-CHO (200 mg/mL) and COS-Arg (200 mg/mL) in pH 7.5 buffer showing continuous 3D network. (b) Photography showing formation of hydrogels in pH 7.5 buffer. (c) Photography showing gel to sol transition against pH where the left sample is with the addition of 20 µL PBS buffer (pH 5.5).

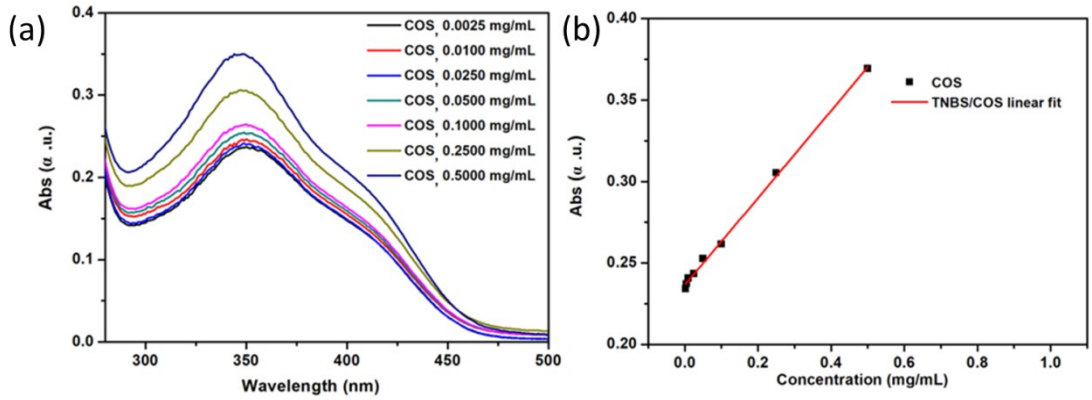

Figure S19. Control experiments associated with the 2,4,6-trinitrobenzene sulfonic acid (TNBSA) assay for the determination of free primary amino groups in COS. A highly chromogenic derivative with an absorption band at 348 nm is produced on reaction of primary amines with TNBSA. (a) UV-Vis spectra obtained for TNBSA/COS control assay, (b) corresponding calibration curve based on plotting the absorbance at 348 nm against the concentration of COS.

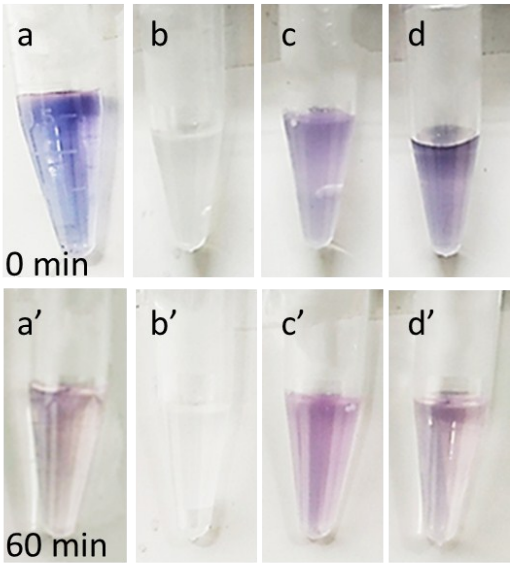

Figure S20. Litmus as a pH indicator to observe the metabolized acid by *E.coli* decreased the pH of the system. Sample a, Sample b, Sample c and Sample d represented litmus in PBS buffer (50 mM, pH 5.5), *E.coli*, *E.coli* with litmus, and *E.coli* with litmus in proteinosomes solution, as well as changes in color of the Sample a', Sample b', Sample c' and Sample d' after one hour, respectively.

Table S1. BSA stoichiometry in BSA-NH_2/ PNIPAAm-*co*-PMEDAB conjugates based on UV-vis spectroscopic analysis of three different sample concentrations.

A (278 nm)	BSA-NH_2 (mg/mL)	BSA-NH_2/PNIPAAm-*co*-PMEDAB (mg/mL)	Number of polymer
0.03477	0.0368	0.125	10.2
0.06925	0.0812	0.250	10.2
0.13396	0.1659	0.50	9.8

Table S2. Number of linked primary amine groups in BSA-NH_2.

A (348 nm)	Glycine (mg/mL)	BSA (mg/mL)	BSA-NH_2 (mg/mL)	Number of primary amine groups in BSA-NH_2
0.22494	0.009832	0.1	0.05	78
0.25283	0.01148	0.2	0.1	78.5
0.31116	0.02290	0.4	0.2	78.3

References

1 B. Dizman, M. O. Elasri, L. J. Mathias, *J. Polym. Sci., Part A: Polym. Chem.* 2006, **44,** 5965.

2 X. Huang, M. Li, D. C. Green, D. S. Williams, A. J. Patil, S. Mann, *Nat. Commun.* 2013, **4,** 2239.

3 X. Huang, A. J. Patil, M. Li, S. Mann, *J. Am. Chem. Soc.* 2014, **136**, 9225.

4 P. Wen, X. Liu, L. Wang, M. Li, Y. Huang, X. Huang, S. Mann, *Small* 2017, **13***, 1700467*.

5 V. M. Gaspar, J. G. Marques, F. Sousa, R. O. Louro, J. A. Queiroz, I. J. Correia, *Nanotechnology* 2013, **24,** 275101.

6 B. P. Antunes, A. F. Moreira, V. M. Gaspar, I. J. Correia, *Carbohydr. Polym.* 2015, **130**, 104.

7 O. Cota-Arriola, M. O. Cortez-Rocha, A. Burgos-Hernandez, J. M. Ezquerra-Brauer, M. Plascencia-Jatomea, *J. Sci. Food Agric.* 2013, **93,** 1525.

8 L. Liu, D. Su, X. Liu, L. Wang, J. Zhan, H. Xie, X. Meng, H. Zhang, J. Liu, X. Huang, *Chem. Commun.* 2017, **53**, 11678.