SUPPLEMENTARY INFORMATION

Materials

Polyallylamine hydrochloride (PAH, M_w 17500), poly(ethylene glycol) 2000 methyl ether (MPEG2000), succinic anhydride, N-hydroxysuccinimide, triethylamine, succinic acid bis(N-hydroxysuccinimide ester), boric acid, sodium tetraborate, Sephadex G-75, Trizma® base, sodium phosphate monobasic monohydrate, sodium phosphate dibasic, Dulbecco's modified Eagle's medium (DMEM), resazurin, bicinchoninic acid (BCA), tartaric acid, $CuSO_4$, 3,3',5,5'-tetramethylbenzidine, glucose, ethanol, uric acid, 30% hydrogen peroxide solution, glucose oxidase (GOX) from Aspergillus niger, Catalase (Cat) from bovine liver, horseradish peroxidase (HRP), alcohol oxidase (AOX) from Pichia Pastoris were purchased from Sigma-Aldrich and were used as received. Traut's reagent (2-thiolanimine hydrochloride), was purchased from MP Biomedicals™, fatal bovine serum (FBS) were purchased from Corning™. Active recombinant human Caspase 3 (Cas3) and FITC annexin V apoptosis detection kit were purchased from BD Biosciences. Urate oxidase (UOX) is a kind gift from Dr. Jianmin Li from Beijing Institute of Biotechnology.

Instruments

TEM images were obtained on a Philips EM-120 TEM instrument. Particle size and zeta potential were measured with Zetasizer Nano-ZS (Malvern Instruments Ltd., UK). NMR spectrum was conducted on AV400 NMR spectrometer from Bruke Corporation. UV-Visible absorption was acquired with a Beckman Coulter DU®730 UV/Vis spectrophotometer. Fluorescence intensities were measured with a Tecan GENios multifunction microplate reader. Fluorescence images were obtained on a Carl Zeiss Axio Observer inverted fluorescence microscope. Flow cytometry analysis was achieved using a BD LSRFortessa cell analyzer.

Experimental

Synthesis of the self-crosslinkable polymer vectors: Activation of Poly(ethylene glycol) 2000 methyl ether (MPEG-2000) was done by first converting it to PEG-succinate then to N-hydroxysuccinimide ester as described elsewhere[21]. The resulted PEG-succinate NHS ester was further reacted with polyallylamine hydrochloride (PAH, Sigma Aldrich, Mw 15000) at different PEG/PAH molar ratio in methanol using triethylamine as acid binding reagent. The successful synthesis of PEGylated PAH was verified by ^{1}HNMR spectrum (Fig. S1). The PEGylated PAH was dialyzed against deionized water to remove by products and then lyophilized. For the synthesis of the self-crosslinkable polymer vectors, PEGylated PAH was dissolved in borate buffer (pH 8.0) with 10 mM EDTA and reacted with 2-iminothiolane hydrochloride at 2-iminothiolane hydrochloride to PAH side chain amine molar ratio of 1 : 1.

Thiol groups quantification: Quantification of thiol groups on the self-crosslinkable polymer vector was achieved using Ellman's assay. Briefly, 10 µL of sample solutions were added to 990 µL of DTNB solution in Tris buffer (0.1M, pH 8.0) with a final DTNB concentration of 0.2 mM. The mixed solution was incubated at room temperature for 5 minutes in a quartz cuvette and the absorbance was measured at 412 nm under room temperature. The molarity of thiol groups in the assay was found by dividing the absorbance by 13600 M^{-1} cm^{-1}. The typical amount of thiol groups conjugated to the PAH was 17% of total amine groups in PAH, which was quantified with Ellman's assay using cysteine to make standard curve (See Fig. S5).

Preparation of the protein nanocapsules: Negatively charged protein, including BSA, GOX, HRP, EGFP, AOX, UOX and Cas3 were mixed with the polymer vector at polymer/protein molar ratio of 1:20 to form the protein/polymer assemblies, followed by air-bubbling into the solution to crosslink the polymer vectors. The resulted nanocapsules were dialyzed against 10 mM phosphate buffer (pH 7.0) and further purified with Sephadex G-75 to remove excess polymer vectors and proteins. To

create nondegradable nCas3, Cas 3 was mixed with PEG conjugated PAH without thiolation. The Cas3/polymer assembly was further crosslinked by succinic acid bis(N-hydroxysuccinimide ester) at succinic acid bis(N-hydroxysuccinimide ester) to PAH side chain amine molar ratio of 0.1 : 1, which forms more stable amide bonds.

BCA protein content quantification: All the protein content in solution was determined by bicinchoninic acid (BCA) colorimetric protein assay. Briefly, a tartrate buffer (pH 11.25) containing 25 mM BCA, 3.2 nM $CuSO_4$, and appropriately diluted protein or nanocapsules was incubated at 60 °C for 30min. After the solution was cooled to room temperature, absorbance reading at 562 nm was determined with a UV-Vis spectrometer. GOX, HRP, Cat, UOX and AOX solutions with known concentration were used as standards.

HRP activity assay: H_2O_2 and 3,3',5,5'-tetramethylbenzidine were dissolved in sodium acetate buffer (50mM, pH 5.1) at a final concentration of 10 mM and 0.5 mM, respectively. Native HRP and nHRP were then added to the assay solutions, respectively. The mixture was placed in a quartz cuvette and the absorbance change at 655 nm was monitored with a UV-Vis spectrometer under room temperature.

GOX activity assay: Glucose, 3,3',5,5'-tetramethylbenzidine and HRP were dissolved in sodium acetate buffer (50mM, pH 5.1) at a final concentration of 90 mM, 0.5 mM and 0.01mg/ml, respectively. Native GOX and nGOX were added to the assay solutions. The mixture was placed in a quartz cuvette and the absorbance change at 655 nm was monitored with a UV-Vis spectrometer under room temperature.

Cat activity assay: H_2O_2 was dissolved in phosphate buffer (20mM, pH 7.0) at a final concentration of 10 mM. The native Cat and nCat were added to the assay solution, respectively. The mixture was placed in a quartz cuvette and the absorbance change at 240 nm was monitored with a UV-Vis spectrometer under room temperature.

UOX activity assay: Uric acid was dissolved in borate buffer (100mM, pH 8.5) at a final concentration of 0.12 mM. Native UOX and nUOX were added to the assay solutions, respectively. The mixture was placed in a quartz cuvette and the absorbance change at 290 nm was monitored with a UV-Vis spectrometer under room temperature.

AOX activity assay: Ethanol, 3,3',5,5'-tetramethylbenzidine and HRP were dissolved in phosphate buffer (20mM, pH 6.0) at a final concentration of 20 mM, 0.5 mM and 0.01mg/ml, respectively. Native AOX and nAOX were added to the assay solutions, respectively. The mixture was placed in a quartz cuvette and the absorbance change at 655 nm was monitored with a UV-Vis spectrometer under room temperature.

Stability evaluation of nCat: The stability of native Cat and nCat against proteolysis were evaluated by challenging with 0.5 mg/mL trypsin and incubating in Tris-HCl buffer with 10 mM $CaCl_2$ at 37°C. The activity of native Cat and nCat were measured every 20 min with the Cat activity assay described above.

Stability evaluation of nGOX: The stability of native GOX and nGOX against proteolysis were evaluated by challenging with 0.5 mg/mL pepsin and incubating in pH 2.7 glycine-HCl buffer at 37°C. The activity of native GOX and nGOX were measured every 30 min with the GOX activity assay described above.

Cell proliferation assay: The toxicity of the BSA nanocapsules was assessed by the resazurin assay using native BSA as control. HeLa cells (10000 cells/well) were seeded on a 96-well plate in 100 μL Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fatal bovine serum (FBS) and 1% penicillin/streptomycin (P/S) the day before exposure to BSA nanocapsules. After incubation with nanocapsules at different concentrations for 24 hours, the cells were washed with PBS and incubated with 100 μL fresh medium containing resazurin (10 μL 0.1mg/mL in

PBS) for 3 h. The cell viability was then determined by measuring the fluorescence intensity of each well (E_x = 550 nm, E_m = 595 nm) by using a microplate reader. Untreated cells and fresh medium were used as controls for 100% and 0% cell proliferation, respectively.

Cell internalization studies: Studies of intracellular delivery with the protein nanocapsules were achieved using fluorescence microscopic technique and fluorescence-activated cell sorting (FACS). HeLa cells were cultured in DMEM supplemented with 10% FBS and 1% P/S. Cells (50000 cells/well, 24-well plate) were seeded the day before exposing to samples. Nanocapsules or native proteins with different concentrations were added into the cell cultures. After incubation at 37 °C for 4 hours, the cells were washed three times with PBS and either visualized with a fluorescent microscope or analyzed via FACS.

Cell Apoptosis Assay: HeLa cells (50000 cells/well, 24-well plate) were seeded the day before adding samples. Native Cas3, nCas3, and nBSA were incubated with HeLa cells at 37°C for 48 hours, respectively. Cas3 delivery were analyzed by measuring the percentage of cells that underwent apoptosis by staining cells with FITC-annexin V apoptosis detection kit and analyzing using FACS.

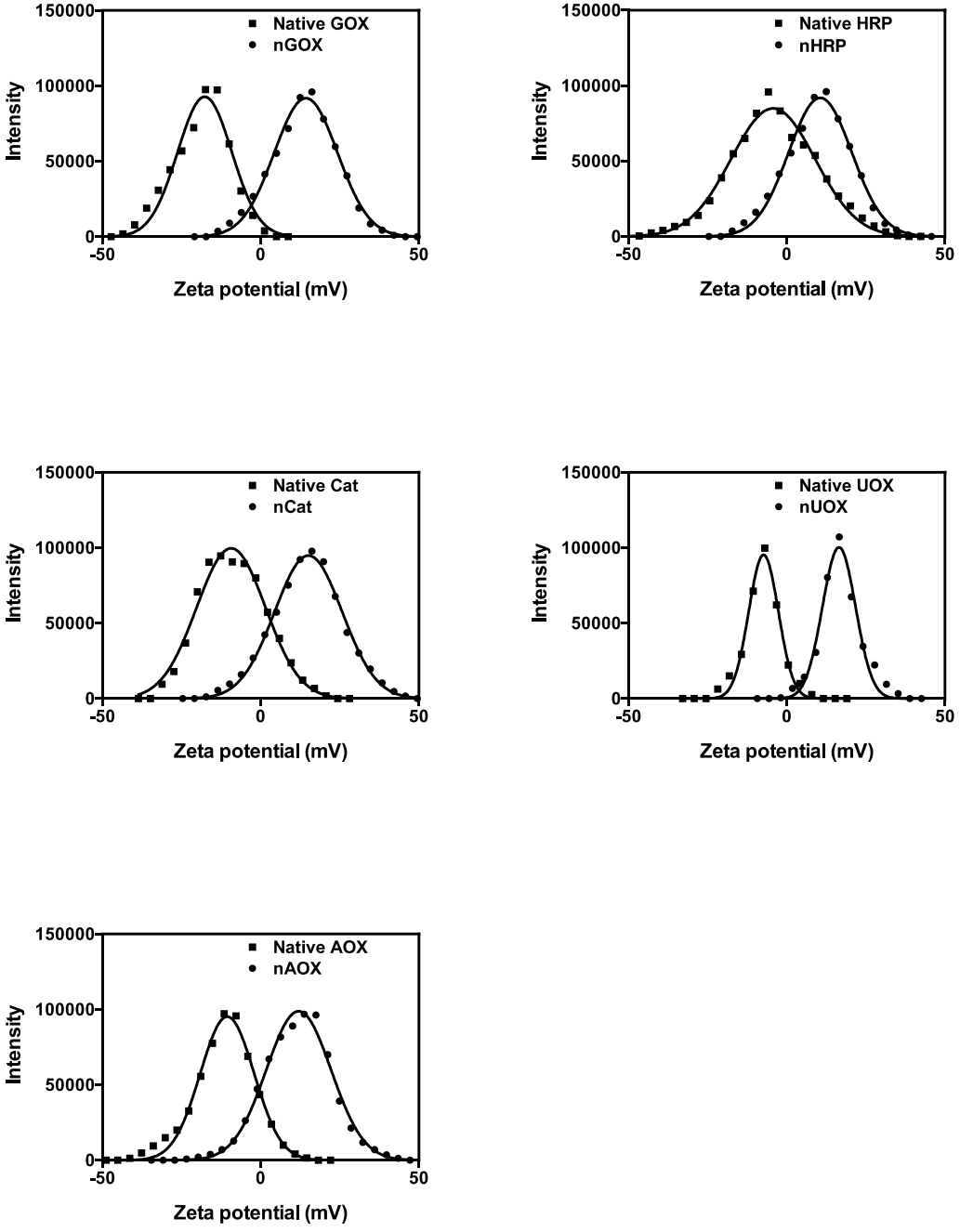

Fig. S1 Zeta potential of GOX, HRP, Cat, UOX, AOX and their nanocapsules counterparts.

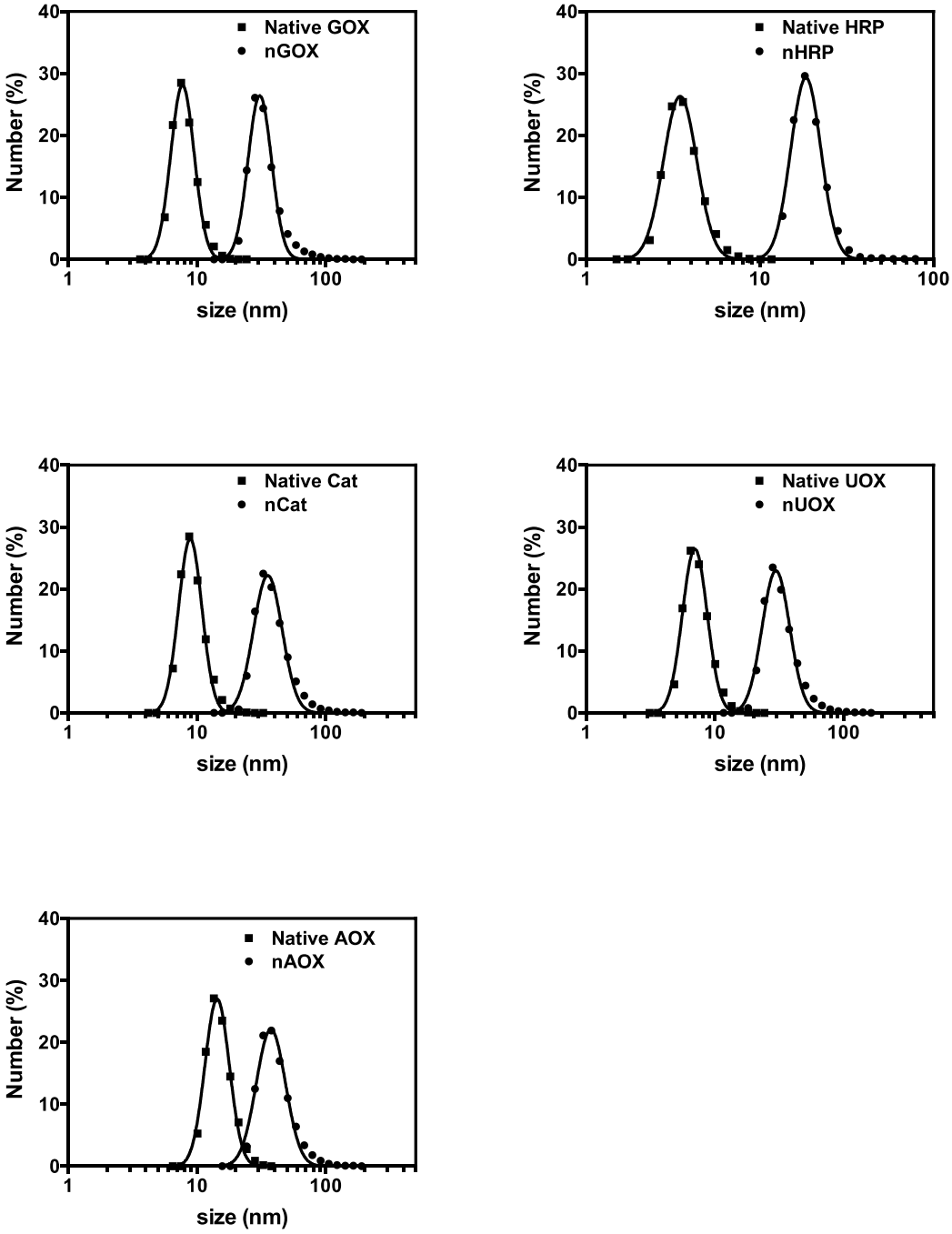

Fig. S2 Size distribution of GOX, HRP, Cat, UOX, AOX and their nanocapsules counterparts.

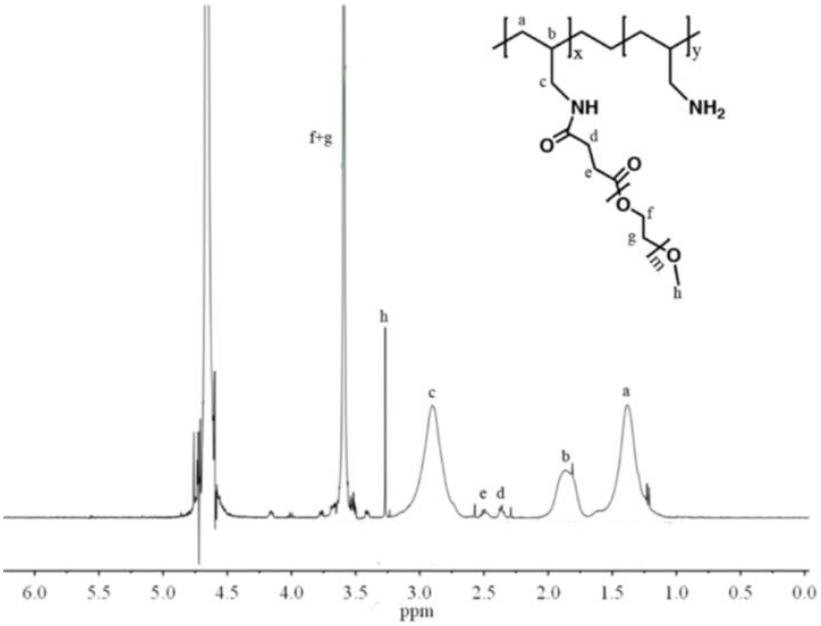

Fig. S3 [1]HNMR spectrum of PEGylated PAH at PEG/PAH molar ratio of 4.

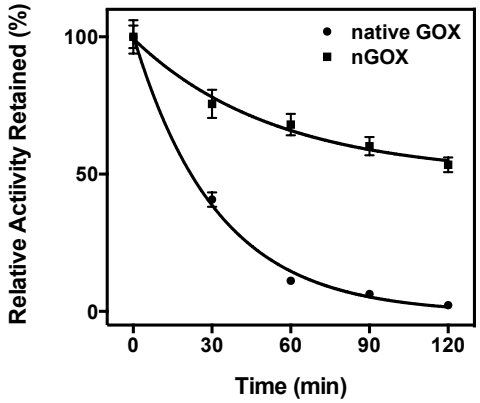

Fig. S4 Relative activities of native GOX and nGOX after exposure to 0.5 mg/ml pepsin in Glycine-HCl buffer at pH 2.7 at 37°C.

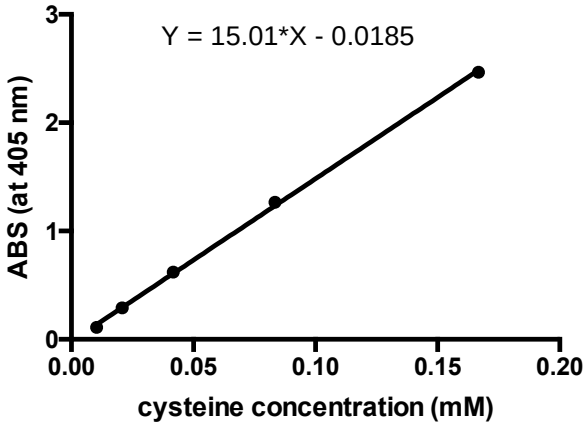

Fig. S5 Standard curve for Ellman's assay using cysteine as standard.

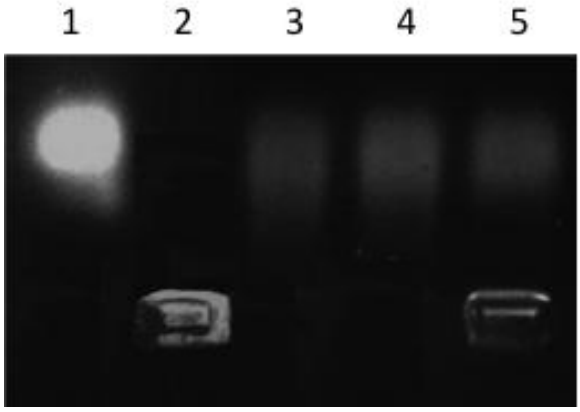

Fig. S6 Agarose gel analysis of nBSA under different GSH concentration 1) native BSA, 2) nBSA with 10 mg/ml heparin only, 3) nBSA with 10 mg/ml heparin and 20 mM GSH, 4) nBSA with 10 mg/ml heparin and 10 mM GSH, 5) nBSA with 10 mg/ml heparin and 5 mM GSH.

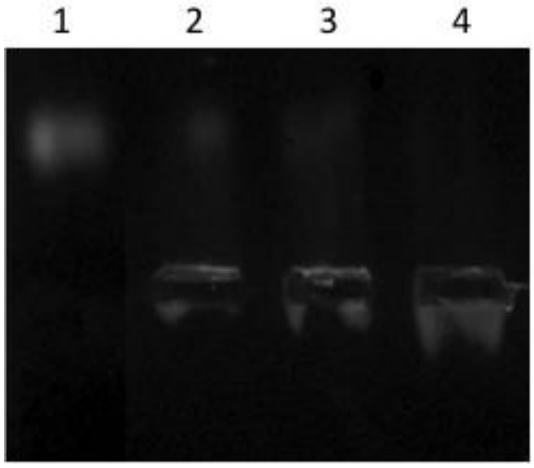

Fig. S7 Agarose gel analysis of nBSA with different PAH to BSA ratios 1) native BSA, 2) PAH/BSA molar ratio is 2, 3) PAH/BSA molar ratio is 4, 4) PAH/BSA molar ratio is 8.

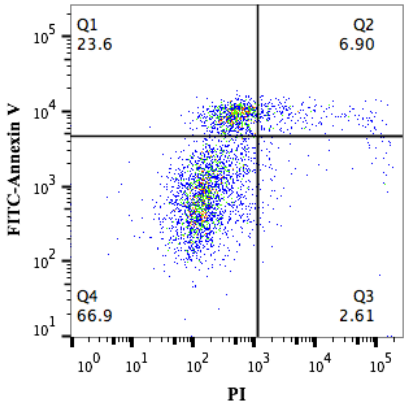

Fig. S8 Flow cytometry analysis of cell apoptosis of cells treated with 5 μg/ml nondegradable nCas3.