Supporting Information

A strategy for high radioprotective activity by the assembly of PprI protein with a ROS-sensitive polymeric carrier

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1. Characterization methods

^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were taken by a Varian INVOA-400 instrument operated at 400 MHz. Fourier transform infrared (FT-IR) spectra were obtained on a Varian-1000 spectrometer; Scanning electron microscopy (SEM) images were obtained by a Hitachi S-4700 microscope working at an accelerating voltage (15 kV). Malvern Zetasizer with irradiation (He-Ne laser, 632.8 nm) was used to determine Zeta potential and Z-average size distribution of the nanoparticles. Absorbance in CCK-8 was measured at 570 nm with a Synergy 2 microplate reader (BioTek, USA). Blood samples were collected and analyzed by CELL-DYN 3700 blood cell analyzer (Abbott, Chicago).

2. Synthesis of PEG-CP_5K-NH_2

A typical procedure was described as follows[1]: A degassed solution of triethyl amine (Et_3N) (0.352 mM, 0.048 mL) in a methanol (10 mL) and acetonitrile (10 mL) were added to a previously dried flask containing $mPEG_{113}$-MAL (0.16 mM, 0.8 g) and peptide (0.24 mM, 186 mg), then stir at room temperature (RT) for 24 h. Purify the reaction mixture through dialysis against methanol using 2k MW cutoff tubing for 24 h to acquire purified PEG-CP_5K-NH_2.

3. Synthesis of PEG-CP_5K-NHS

A typical procedure was described as follows[2]: a previously degassed solution of Et_3N (16.19 μg/mL, 0.024 mL) in DMF and anhydrous methanol mixture (1 : 1 ratio, 20 mL) were added to a dried, degassed flask containing PEG_{113} -CP_5K-NH_2 (0.08 mM, 0.462 g) and NHS (0.2 mM, 0.0624 g). The contents were purged with argon for 20 min to eliminate the dissolved oxygen, and then stir at room temperature for 48 h. The reaction mixture was precipitated by cold diethyl ether for three times, and the product was dried under vacuum at room temperature. The structure of PEG-CP_5K-NHS was characterized by ^{1}H NMR spectrum (Figure S1).

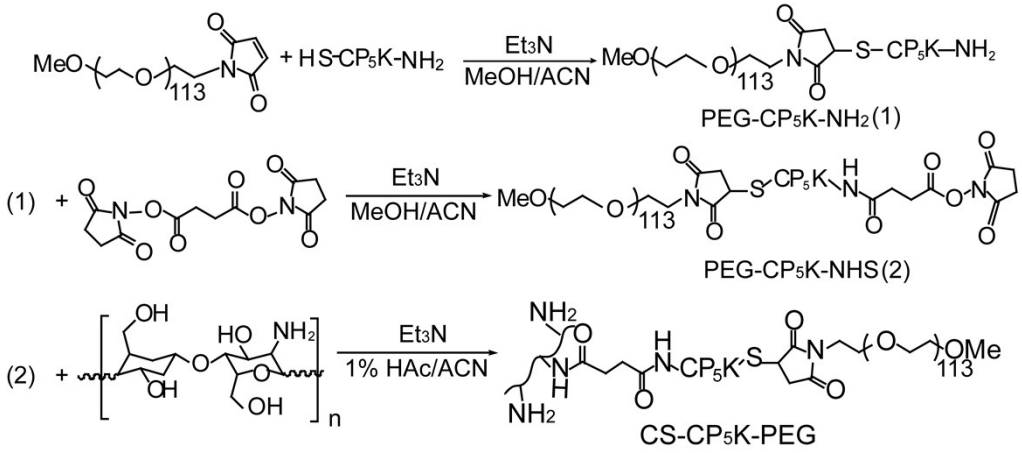
4. Entrapment efficiency of PprI protein

In order to evaluate the entrapment efficiency (EE) of PprI protein by CS-CP_5K-PEG, the Bradford assay was employed to quantify unpacked protein concentration after centrifugation using a 100kD ultrafiltration device (Millipore Centrifugal Filter Units). The Bradford Protein Assay Kit was purchased from Shanghai Yuanye Biotechnology Co. LTD., and the assay was conducted following the manufacturer's instructions[3, 4] (*https://www.thermofisher.com/order/catalog/product/23200?SID=srch-srp-23200*).

The concentration of protein was determined according to the standard curve at 595 nm (Figure S2, Electronic Supplementary Information). On the basis of the assessment of concentration of unpacked PprI protein (C_1) and initial PprI protein (C_0), PprI protein entrapment efficiency (EE) was estimated as EE = (C_0 - C_1)/C_0 × 100%.

5. Statistical analysis

Results were expressed as means ± standard deviations (SD). The data was analyzed using paired analysis of variance on SPSS 16.0 and the group means were compared by LSD Test. $P < 0.05$ was considered significant.



Scheme S1. Synthesis pathway of CS-CP_5K-PEG.

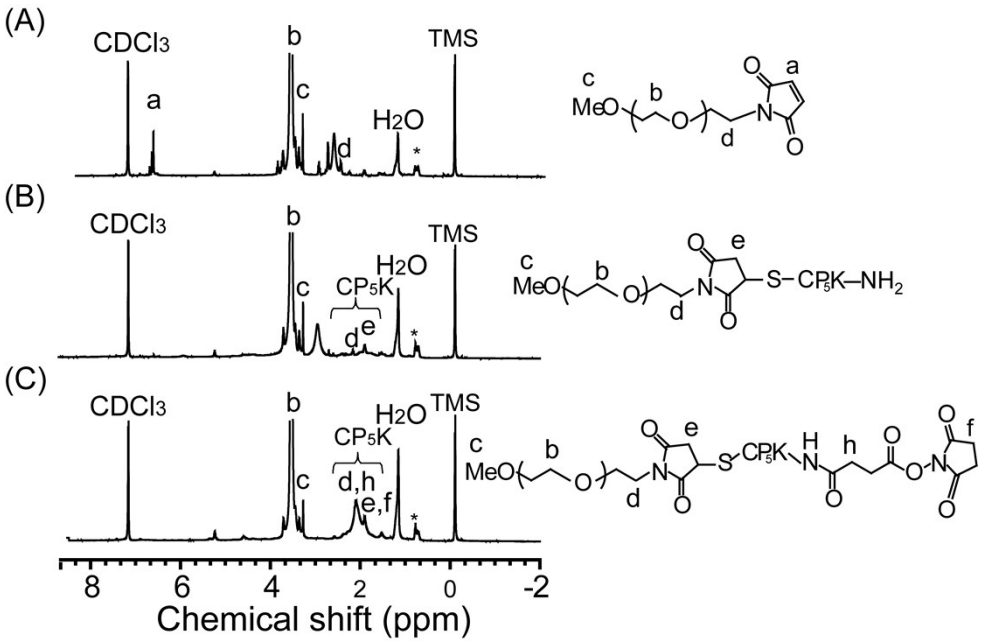


Figure S1. ^{1}H NMR spectra of (A) $mPEG_{113}$-MAL, (B) PEG-CP_5K-NH_2, and (C) PEG-CP_5K-NHS (400 M, $CDCl_3$). * indicates the signals from reactive hydrogen or grease. (In comparison with $mPEG_{113}$-MAL, the disappearance of maleimide peak at 6.75 ppm and the characteristic peaks appears at 2.11 ppm (CP_5K) for PEG-CP_5K-NH_2, indicating that peptide CP_5K was conjugated to mPEG-MAL[1, 5]. The characteristic peak to NHS at 2.31 ppm suggested the conjugation of NHS to PEG-CP_5K-NH_2.[6])

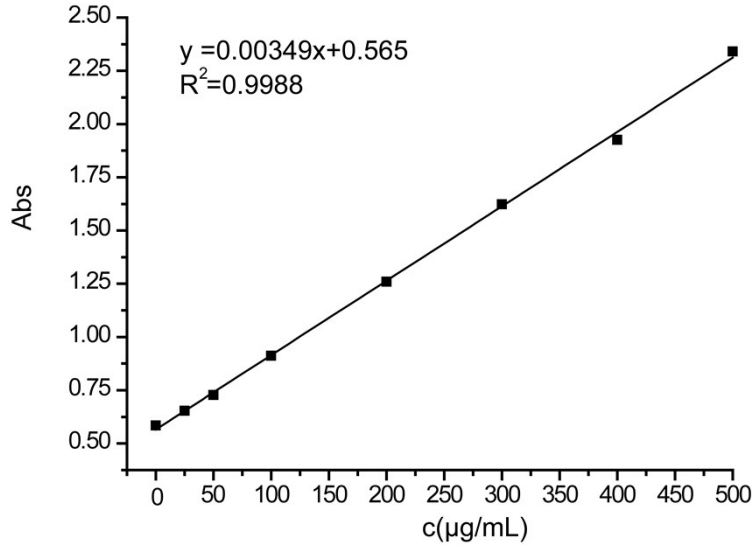


Figure S2. The Bradford protein assay standard curve (λ_{max} = 595 nm).

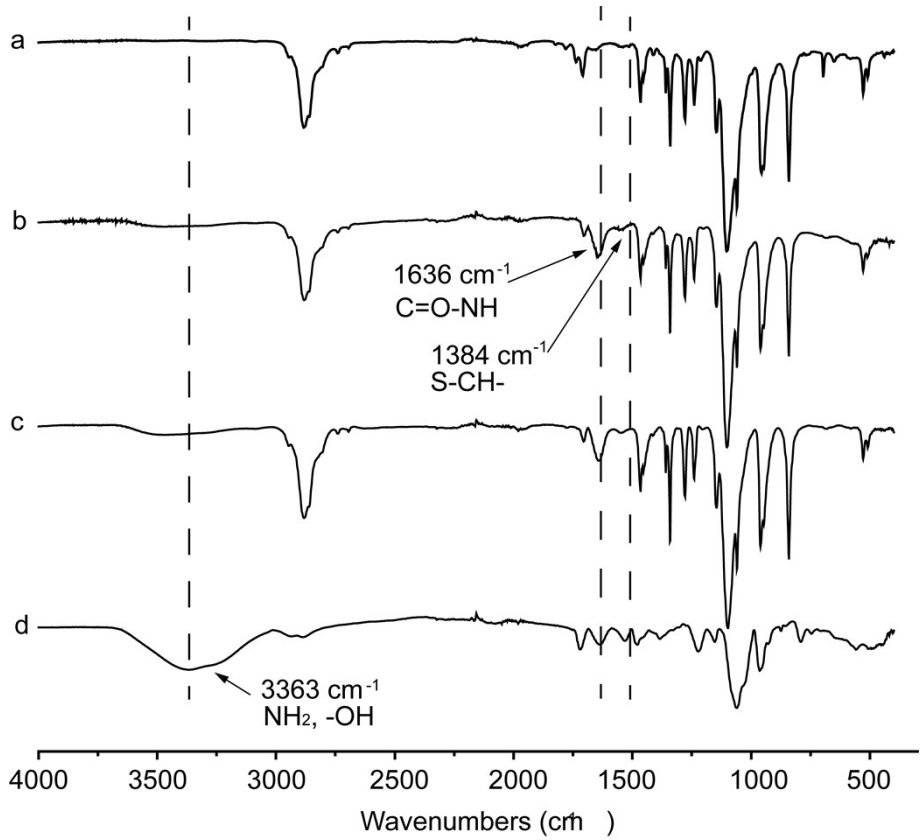


Figure S3. FT-IR spectra of (a) $mPEG_{113}$-MAL, (b) PEG-CP_5K-NH_2, (c) PEG-CP_5K-NHS and (d) CS-CP_5K-PEG.

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