

**Supplementary Information**

**Polyethylene glycol derivatives polyelectrolyte-Protein  
nanoclusters for protein drug delivery**

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### **Determination the pKa of polymers.**

The copolymer (PEG-PAGE(R)) (20 mg) were dissolved in 20 mL of ultrapure water, and the initial pH was adjust to between 4 and 6. Then, the solution was titrated with NaOH solution (0.01 M) or HCl (0.012 M), 50  $\mu$ L per times, and recorded the pH of the solution.

### **Stability of Protein in PEG polyelectrolyte-hemoglobin nanoclusters.**

PEG polyelectrolyte-protein nanoclusters were re-suspended in 10 mL of phosphate buffer (pH 7.4, 20 mM) in test tubes, and then incubated in a 37 °C incubator under constant shaking at 80 rpm for 72 h. At certain intervals, the dispersions were centrifuged for 10 min at 11,000 rpm and 2 mL of the supernatant was withdrawn and replaced by fresh medium. The amount of the released protein in the supernatant was assayed via UV-vis spectrum.

The protein structure change was studied by CD spectroscopy. CD spectra of proteins obtained were smoothed by the adaptive smoothing method, and corrected by subtracting the proper baseline of solvent. Secondary structure analysis was performed using the CONTINLL algorithms in the CDPro software package. All the samples were filtered through a 0.45  $\mu$ m filter before measurement.

*In vitro* stability of PEG polyelectrolyte-protein nanoclusters was evaluated in PBS (10% FBS, pH 7.4,  $20 \times 10^{-3}$  M) at 37 °C. At various time points, the nanocluster sizes were analyzed by DLS. The morphology of PEG polyelectrolyte-protein nanoclusters was observed by TEM.

*In vitro* protein release of PEG polyelectrolyte-protein nanoclusters were assayed via UV-vis spectrum. PEG polyelectrolyte-protein nanoclusters were re-suspended in 10 mL of phosphate buffer (pH 7.4, 20 mM) in test tubes, and then incubated in a 37 °C incubator under constant shaking at 80 rpm for 72 h. At certain intervals, the dispersions were centrifuged for 10 min at 11,000 rpm and 2 mL of the supernatant was withdrawn and replaced by fresh medium. The amount of the released protein in the supernatant was assayed via UV-vis spectrum.

### **Gas-binding capability of protein in PEG polyelectrolyte-hemoglobin nanoclusters.**

The gas-binding capacity of Hb at different atmospheres could be monitored by UV-vis spectrophotometry. The oxygenated hemoglobin (OxyHb) state could be obtained by flowing O<sub>2</sub> gas over the PEG polyelectrolyte-hemoglobin

(carbon monoxide hemoglobin) nanoclusters solution for 0.5 h. Deoxygenated hemoglobin (DeoxyHb) could be obtained by flowing N<sub>2</sub> gas over the PEG polyelectrolyte-hemoglobin (OxyHb) nanoclusters solution for 1 h. The O<sub>2</sub> binding and release ability of Hb at different oxygen partial pressures was evaluated via oxygen half-saturation pressure (P<sub>50</sub>) and cooperativity (Hill coefficient). They could be obtained using an oxygen dissociation curve, which reflected the relationship between O<sub>2</sub> partial pressure (P) and O<sub>2</sub> saturation (Y) of oxygen carriers. First, the PEG polyelectrolyte-hemoglobin nanoclusters solution was converted to the complete deoxyHb state, and then different volumes of air were introduced into the airproof cuvette through a syringe. Oxygen saturation (Y): The percentage of oxyHb in (deoxyHb+oxyHb) at under certain oxygen partial pressure (P). The plot of Y vs. P was fitted as a sigmoidal curve by a Boltzmann function. P<sub>50</sub> was determined as the oxygen partial pressures when the O<sub>2</sub> saturation reached 0.5 in the sigmoidal curve. Furthermore, linear fitting of a Hill plot (Log (Y/(1 - Y)) versus Log P) was performed, and the Hill coefficient was obtained as the slope of the line.

$$1.8814 \times \text{oxyHb}\% + 1.4773 \times \text{deoxyHb}\% + 0.8419 \times \text{metHb}\% = A540/A523 \quad (1)$$

$$2.0201 \times \text{oxyHb}\% + 1.3345 \times \text{deoxyHb}\% + 0.5473 \times \text{metHb}\% = A576/A523 \quad (2)$$

$$1.2539 \times \text{oxyHb}\% + 1.8095 \times \text{deoxyHb}\% + 0.6161 \times \text{metHb}\% = A554/A523 \quad (3)$$

$$Y = (\text{oxyHb}\%) / (\text{oxyHb}\% + \text{deoxyHb}\%) \quad (4)$$

#### **Antibacterial Experiments of protein in PEG polyelectrolyte-lysozyme nanoclusters.**

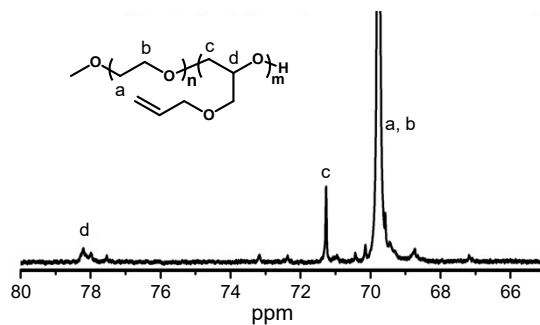
A 100 μL portion of bacterial suspensions (pre-treated with saccharides, OD<sub>600</sub> = 1.0) was mixed with PBS, PEG polyelectrolyte-lysozyme nanoclusters solution (final concentration of 50 μM) or lysozyme solution (final concentration of 50 μM), and then the resulting bacteria suspensions (bacteria + drug group) were diluted 100 000-fold with PBS and 100 μL of the diluted solution was spread onto solid agar plates. All the plates were incubated at 37 °C for 12 h.

#### **Pharmacokinetics of PEG polyelectrolyte-hemoglobin nanoclusters**

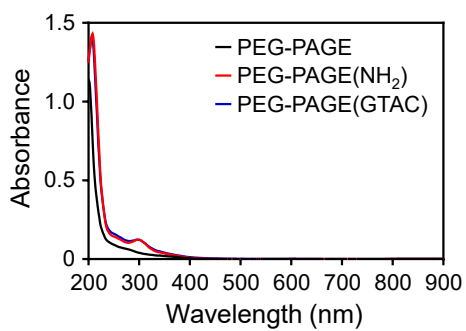
In order to quantify the carrier materials *in vivo*, PEG-PAGE-R polymer were further labelled with RhB. RhB was conjugated to polymers by reacting of the carboxylic groups of RhB with the hydroxyl groups of polymer in the presence of DCC and DMAP in DMF.

The *in vivo* pharmacokinetics was measured with male wistar rats (100-150 g and 4-6 weeks old). Three mouse were injected in the tail vein with

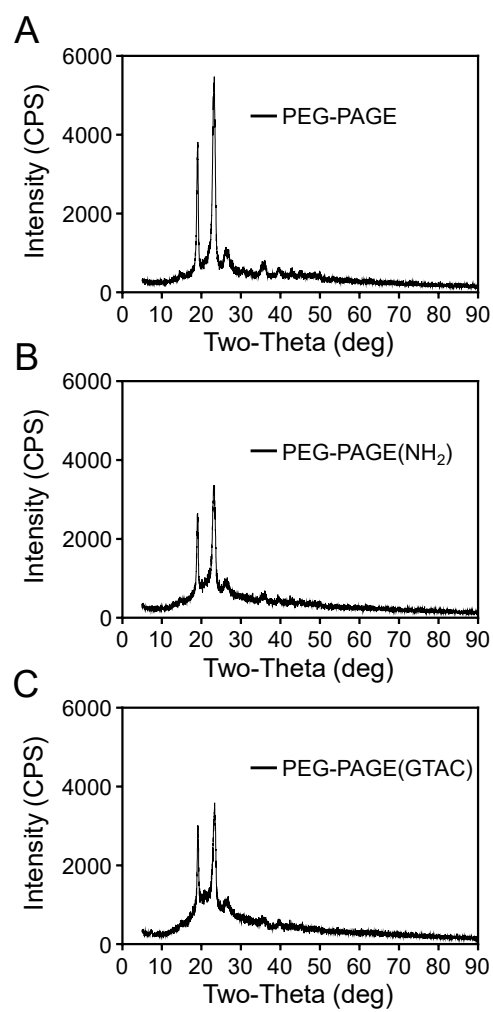
nanoclusters (1 mg RhB/kg), respectively. At predetermined time intervals, blood samples (0.2 mL) were collected and mixed with Triton X-100 solution (20  $\mu$ L). Then the polymer was extracted with  $\text{CH}_2\text{Cl}_2$  solution for three times. After evaporated the solution, the samples were redissolve with 0.3 mL acetonitrile and determined the RhB content by UV spectra. The concentration of nanoclusters in the blood was calculated by assuming that the total blood volume in the mouse is 7% of its body weight.



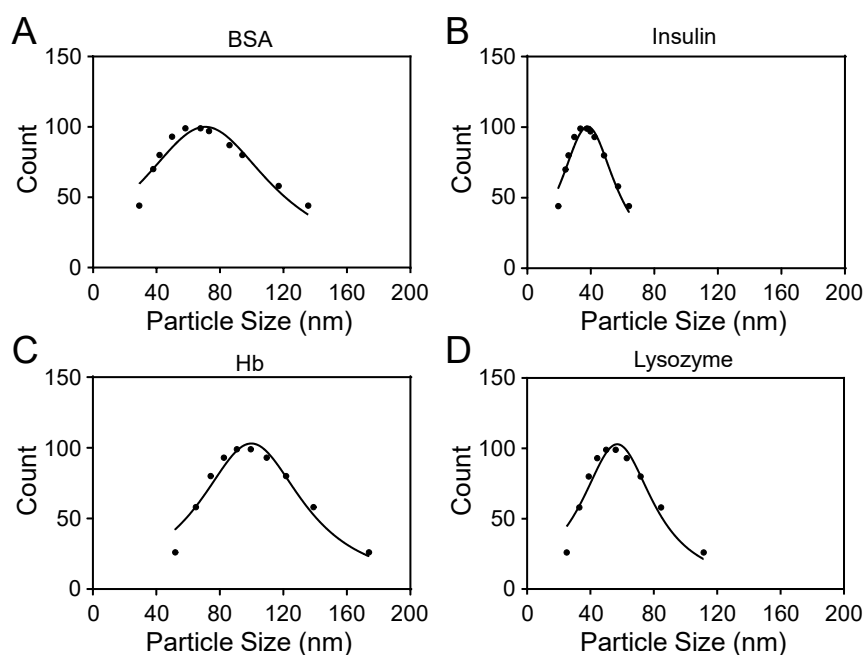
**Figure S1.** <sup>13</sup>C NMR spectra (100 MHz, DMSO-d<sub>6</sub>) of PEG<sub>5k</sub>-PAGE<sub>18</sub>.



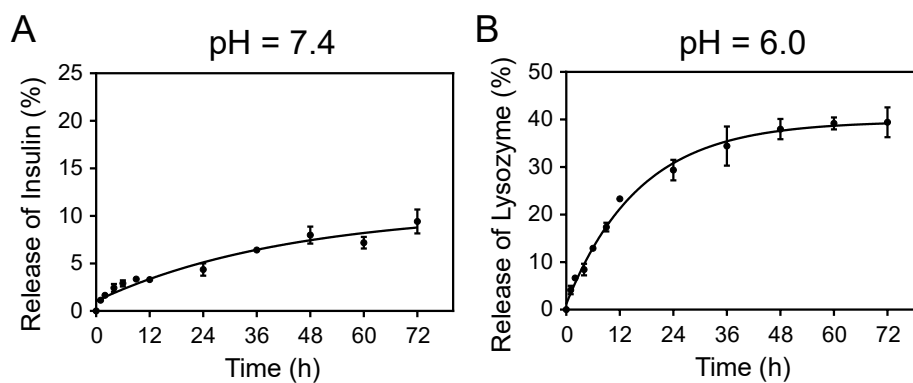
**Figure S2.** UV-vis spectrometry of PEG-PAGE, PEG-PAGE(NH<sub>2</sub>) and PEG-PAGE(GTAC) in aqueous solution.



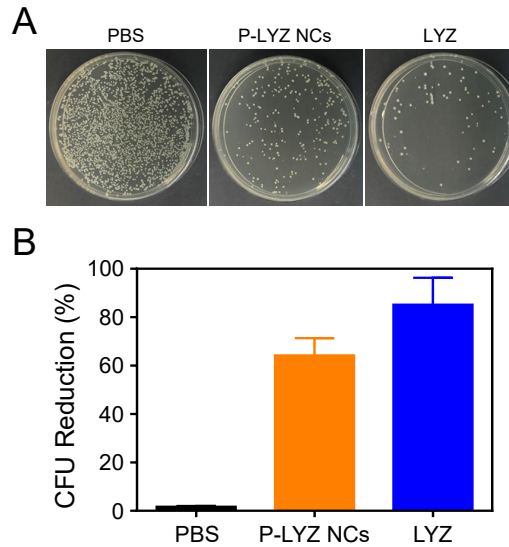
**Figure S3.** The XRD diffraction pattern of (A) PEG-PAGE, (B) PEG-PAGE(NH<sub>2</sub>) and (C) PEG-PAGE(GTAC).



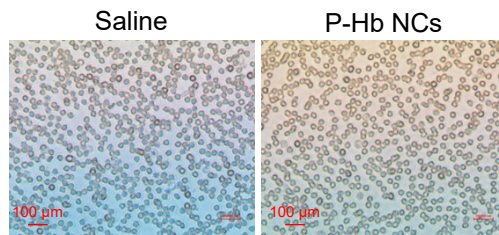
**Figure S4.** Histograms of the average particle size of bovine serum albumin nanoclusters, (B) insulin nanoclusters, (C) hemoglobin nanoclusters and (D) lysozyme nanoclusters.



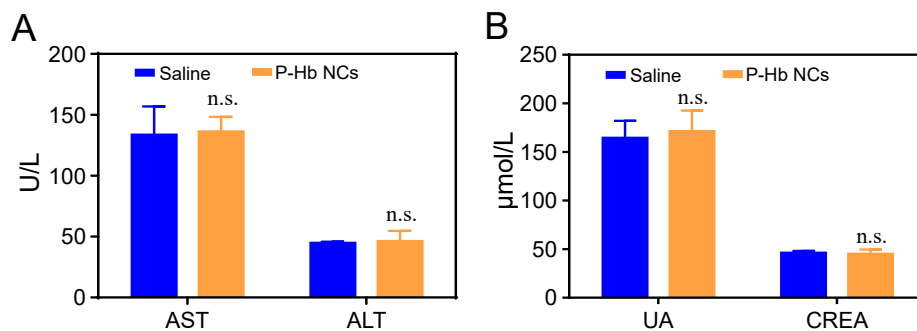
**Figure S5.** *In vitro* protein release of (A) PEG polyelectrolyte-insulin nanoclusters and (B) PEG polyelectrolyte-Lysozyme nanoclusters in PBS (pH 7.4, 0.01 M) at 37 °C over 72 h.



**Figure S6.** (A) CFU for staphylococcus xylosus treated with PBS, staphylococcus xylosus treated with PEG polyelectrolyte-lysozyme nanoclusters and staphylococcus xylosus treated with free lysozyme. (B) Bactericidal activity of PEG polyelectrolyte-lysozyme nanoclusters and free lysozyme towards staphylococcus xylosus.



**Figure S7.** Morphology of RBC after incubation with saline or PEG polyelectrolyte-hemoglobin nanoclusters at 37 °C for 6 h.



**Figure S8.** Alterations of (E) C3, (F) PLT, (G) AST and ALT, (H) UA and CREA levels of mice after *i.v.* injection of HbNGs and saline. Statistical P-values: No significance: n.s., \*P < 0.05, \*\*P < 0.01.