Electronic Supplementary Information for:

**Formation-dissociation of glucose, pH and redox triply responsive micelles and controlled release of insulin**

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1 Materials
S-dodecyl-S’-(α,α’-dimethyl-α”-acetic acid) trithiocarbonate (DDMAT) was prepared according to the literature.\(^1\) 2,2’-Azobisisobutyronitrile (AIBN, Aldrich, 98%) was recrystallized from ethanol. 3-Aminophenyl boronic acid (TCI), acryloyl chloride (TCI), sodium hydrogen carbonate (Acros Organics, 99.5%), D-glucose (Aldrich), N,N-dimethylformamide (DMF) (Aldrich 99.9%), tetrahydrofuran (THF) (Acros Organics, 99.9%), diethyl ether, dimethylsulfoxide-\(d_6\) (DMSO-\(d_6\), 99.9% D), CDCl\(_3\) (99% D), and methanol-\(d_4\) (99.8% D) were used as received. Methoxy poly(ethylene oxide) (\(M_n=1900\), Alfa Aesar) was dried by azeotropic distillation from toluene. \(p\)-Nitrophenyl chloroformate (\(p\)-NPC, 97%, Alfa Aesar), cystamine dihydrochloride (cystamine·2HCl, >98%, Alfa Aesar), pyridine (Py, 99.5%), triethylamine (Et\(_3\)N, 99%, Alfa Aesar), N-hydroxysuccinimide (NHS, 98%, Alfa Aesar), dicyclohexyl carbodiimide (DCC, 99%, Alfa Aesar), and glutathione (GSH, aldrich) were used as received.

2 Characterization
Nuclear magnetic resonance (NMR). \(^1\)H NMR spectra of samples were obtained from a Bruker DMX 500 NMR spectrometer with CDCl\(_3\), DMSO-\(d_6\) and methanol-\(d_4\) as solvents. The chemical shifts were relative to tetramethylsilane.

Gel permeation chromatography (GPC). GPC analysis was carried out with a HLC-8320 (Tosoh, Japan) analysis system with two columns (TSK gel super AWM-H×2, R0091+R0093), using DMF with 10 mM LiBr as eluents at a flow rate of 0.6 mL min\(^{-1}\) at 40 °C. PMMA calibration kit was used as the calibration standard.

Dynamic light scattering spectrophotometer (DLS). DLS studies were conducted using a Zetasizer Nano ZS90 instrument (Malvern Instruments) equipped with a multipurpose autotitrator (MPT-2) at a fixed scattering angle of 90°. The data were processed by cumulants analysis of the experimental correlation function. The micellar radiuses were calculated from the computed diffusion coefficient using the Stokes-Einstein equation.

Transmission electron microscopy (TEM). The morphology of copolymer micelles was observed with a JEOL JEM-2010 TEM at an accelerating voltage of 120 kV. The
samples for TEM observation were prepared by placing 10 μL of copolymer micelles solution on copper grids coated with thin films and carbon.

Optical transmittances. The optical transmittances of copolymer micelles aqueous solution (0.3 mg/mL) at various temperatures were measured at a wavelength of 500 nm on a UV-visible spectrophotometer (Lambda 35, PerkinElmer).

Critical micelle concentration (CMC) measurement. CMC was determined using pyrene as a fluorescence probe. 10 μL of pyrene (0.45 mg/mL) in acetone was added to a series of 10.0 mL volumetric flasks. After the acetone evaporated, a measured amount of PEO-SS-PAPBA solution was added to each flask, then added followed by doubly distilled water. The flasks were kept for 12 h to equilibrate the pyrene and the micelles. The fluorescence spectra were recorded using a Hitachi F2500 luminescence spectrometer (Hitachi, Ltd.) with an excitation wavelength of 393 nm. The emission wavelengths at 333 nm and 338 nm were monitored. The CMC value was chosen as the concentration when pyrene exhibited an apparent decrease in the $I_{338}/I_{333}$ ratio with an increasing concentration of the copolymer, indicating that the aggregation of the copolymer occurred.

3 Experimental procedures

3.1 Synthesis of APBA

3-Acrylamidophenylboronic acid (APBA) monomer was synthesized according to the literatures. 3-Aminophenylboronic acid (3.0 g, 0.022 mol) was dissolved in 80 mL of mixing solvents (THF/H$_2$O 1/1 v/v) in a round-bottom flask. Then, sodium hydrogen carbonate (3.7 g, 0.044 mol) and acryloyl chloride (4.0 g, 0.044 mol) were added to the flask at 0 °C. The reaction was carried out under vigorous stirring for 4 h. After removing THF and water by rotary evaporation, the obtained solid crude product was stirred in ethyl acetate for 2 h. After filtering the solid impurities, the ethyl acetate layer was washed with water (50 mL), saturated sodium bicarbonate solution (50 mL), water (50 mL) and brine (50 mL). The ethyl acetate was evaporated by rotary evaporation and 2.1 g of orange solid product (yield: 50.3%). Further, the purification of APBA monomer was carried out by the recrystallization from hot water three times.

$^1$H NMR (500 MHz, DMSO-$d_6$, δ, ppm): 10.05 (s, 1H, NH), 8.02 (s, 2H, B(OH)$_2$),
7.88, 7.82, 7.49, 7.30 (s, d, d, t, 1H each, ArH), 6.46-6.43, 6.27-6.21 (2d, dd, 1H each, vinyl CH2), 5.75-5.72 (dd, 1H, vinyl CH).

3.2 Synthesis of PEO-SS-DDMAT

PEO-SS-DDMAT was synthesized by three steps.

(i) Synthesis of p-NPC activated Methoxy poly(ethylene oxide) (PEO-NPC)

Under a nitrogen atmosphere and vigorously stirring, to a solution of PEO-OH (5.0 g, 2.63 mmol) and pyridine (1.07 mL, 13.2 mmol) in 50 mL of anhydrous DCM at 0 °C was added dropwise a solution of p-NPC (2.1 g, 10.5 mmol) in 25 mL of DCM. The reaction mixture was then warmed to 30 °C and reacted for 20 h. The activated PEO was isolated by precipitation in cold diethyl ether, filtering, and drying in vacuo. $M_n$NMR=2060 g/mol, $^1$H NMR (500 MHz, CDCl$_3$, δ, ppm): 3.38 (s, CH$_3$O), 3.64 (s, PEO OC$_2$H$_2$C$_2$O), 4.44 (t, PEO -C$_2$H$_2$OC(O)-), 7.39, 8.28 (d, phenyl).

(ii) Synthesis of PEO-Cys

To a solution of cystamine·2HCl (5.06 g, 22.5 mmol) and Et$_3$N (4.38 g, 44.9 mmol) in 30 mL of anhydrous DMSO at room temperature was added dropwise a solution of PEO-NPC (4.50 g, 2.25 mmol) in 20 mL of DMSO. The reaction mixture was stirred at 30 °C for 45 h. The resulting product, PEO-Cys, was isolated by twice precipitation in diethyl ether, filtration, and drying in vacuo. $M_n$NMR=2080 g/mol, $^1$H NMR (400 MHz, CDCl$_3$): δ 3.64 (s, PEO -OCH$_2$CH$_2$O-), 4.23 (m, PEO -CH$_2$OCONH-), 3.38 (s, CH$_3$O), 2.84 (m, -OCONHCH$_2$CH$_2$SSCH$_2$-), 3.07 (t, -SSCH$_2$CH$_2$NH$_2$).

(iii) Synthesis of PEO-SS-DDMAT

To a solution of DDMAT (1.36 g, 3.76 mmol) and NHS (0.44 g, 3.83 mmol) in 100 mL of anhydrous DCM was added DCC (1.86 g, 9.03 mmol). The reaction was carried out at room temperature for 24 h. Then, PEO-Cys (3.0 g, 1.44 mmol) was added to the above mixture and the system continued to react for 8 h. The resulting macro-RAFT agent, PEO-SS-DDMAT, was isolated by filtration, twice precipitation in diethyl ether, and drying in vacuo. $M_n$NMR=2430 g/mol, $M_n$GPC=2990 g/mol, $M_w/M_n$=1.23. $^1$H NMR (500 MHz, CDCl$_3$, δ, ppm): 3.64 (s, PEO -OCH$_2$CH$_2$O-), 4.21 (m, PEO -CH$_2$OCONH-), 3.47 (m, SCH$_2$C$_{11}$H$_{23}$), 3.37 (s, CH$_3$O), 2.81 (m, -CH$_2$SSCH$_2$CH$_2$-), 2.02 (s, -CH$_3$), 1.21 (m, SCH$_2$C$_{11}$H$_{23}$).
3.3 Synthesis of PEO-SS-PAPBA

PEO-SS-PAPBA was synthesized by RAFT with PEG-SS-DDMAT as the macro-RAFT agent (Scheme S1). APBA (0.4 g, 2.1 mmol) and PEO-SS-DDMAT (0.73 g, 0.30 mmol) were dissolved in 5 mL of DMF/H$_2$O (95/5, v/v), and then AIBN (4.92 mg, 0.03 mmol) was added. The flask was degassed with three freeze-evacuate-thaw cycles. The polymerization reaction was performed at 70 °C for 24 h. PEO-SS-PAPBA copolymer was obtained after precipitation in diethyl ether twice. $M_{n \text{NMR}} = 3870$ g/mol, $M_{n \text{GPC}} = 4480$ g/mol, $M_w/M_n = 1.34$. $^1$H NMR (500 MHz, Methanol- $d_4$, $\delta$, ppm): 6.83-7.31 (ArH), 4.19 (m, PEO -CH$_2$OCONH-), 3.64 (PEO -OCH$_2$CH$_2$O-), 3.37 (CH$_3$O), 1.56-2.06 (vinyl CH$_2$, CH and C$_{11}$H$_{23}$)

![Scheme S1 Synthesis of PEO-SS-PAPBA copolymer](image)

3.4 Self-assembly of PEO-SS-PAPBA copolymer

40 mg of PEO-SS-PAPBA copolymer was dissolved in 3 mL of NaOH solution (pH 12.0). Then, the hydrochloric acid solution (pH 2.0) was added to above solution dropwise until the solution was changed from transparent to translucent. After stirring vigorously at room temperature for 12 h, the stable micelles formed. Subsequently, the micelle solution was dialyzed against water with dialysis membrane (MW cut-off:
3500 Da) for 48 h to remove Na\(^+\) and Cl\(^-\). After dialysis, the micelle solution was adjusted to 0.3 mg/mL.

3.5 Preparation of micelles loading insulin

30 mg of PEO-SS-PAPBA copolymer was dissolved in 10 mL of NaOH solution (pH 12.0). Then, the hydrochloric acid solution (pH 2.0) containing 8 mg of insulin was added to above solution dropwise until the solution was changed from transparent to translucent. The micelle solution was dialyzed against water with dialysis membrane (MW cut-off: 3500 Da) for 24 h and replaced with fresh water at the interval of 6 h. At last, the dialyzed micelle solution was adjusted to 1.0 mg/mL.

3.6 DLE and DLC measurements of the micelles

Measurement of the standard curve of insulin: preparing a series of insulin aqueous solutions with different concentrations and measuring the UV absorption intensity at the wavelength of 232 nm.

To take 3 mL of micelle solution loading insulin (1.0 mg/mL) and freeze-dried the solution, the dried micelles were obtained. Then, 6 mL of DMF was added to dissolve the micelles, and the micelles were destroyed which led to the complete release of insulin. Then, the drug loading efficiency (DLE) and drug loading content (DLC) could be calculated according to the following formulae:

\[
\text{DLC (wt\%)} = \left(\frac{\text{weight of loading insulin}}{\text{weight of copolymer}}\right) \times 100\%
\]

\[
\text{DLE (wt\%)} = \left(\frac{\text{weight of loading insulin}}{\text{weight of insulin in feed}}\right) \times 100\%
\]

3.7 In vitro release of insulin loaded in micelles

To take 3 mL of micelle solution loading insulin (1.0 mg/mL) into the dialysis membrane (MW cut-off: 3500 Da) and immerse it into 25 mL of PBS (pH 11.3). At the set interval, 2 mL of release solution was withdrawn and replaced by 2 mL of fresh PBS. The cumulative release amount (E) was calculated according to the following formulae:

\[
E = \frac{V_e \sum_{i=1}^{n-1} C_i + V_0 C_n}{m_{\text{insulin}}}
\]

Here, E was the cumulative release amount. \(V_e\) was the replaced PBS volume (2 mL).
\( V_0 \) was 25 mL. \( C_n \) was the concentration of insulin after \( n \) times replacements of PBS. \( m_{\text{insulin}} \) was the content of insulin loading in micelles.

**Fig. S1** \(^1\text{H} \) NMR spectrum of APBA monomer

**Fig. S2** \(^1\text{H} \) NMR spectra of (a) PEO-NPC, (b) PEO-Cys and (c) PEO-SS-DDMAT
**Fig. S3** $^1$H NMR spectrum of PEO-SS-PAPBA

**Fig. S4** GPC traces of PEO-SS-DDMAT and PEO-SS-PAPBA
**Fig. S5** Critical micelle concentration (CMC) of PEO-SS-PAPBA copolymer

**Fig. S6** The photographs of micelle solution at different pH values (9.5, 11.3 and 12.5)

**Fig. S7** The photograph of precipitation of micelle solution (5 mg/mL) after adding GSH (10 mM)
Fig. S8 The standard curve of insulin in aqueous solution

References
