**Supplementary information for**

# Facile Fabrication of Reduction-Responsive Nanocarriers for Controlled Drug Release

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## Experimental Details

### Materials

Poly(ethylene glycol) [99.5 %; $M_n$ = 1000; Sinopharm Chemical Reagent Co. Ltd (China)] was dried by azeotropic distillation in the presence of dry toluene. Scandium trifluoromethanesulfonate [$Sc(OTf)_3$] was synthesized according to previous report.[1] 1,4-dithiothreitol (DTT; 99 %; Aladdin), mercaptosuccinic acid (98 %; Aladdin), paclitaxel [PTX; 99.5 %; Haoxuan Bio-technique Co. Ltd (China)], 1,4-butanediol [BD; 99.5 %; Wulian Chemical Engineering Co. Ltd (China)], buthionine sulfoximine (BSO; Sigma-Aldrich) were used as received. All other reagents were purchased from Sinopharm Chemical Reagent Co. Ltd (China) and used as received.

### Synthesis of PEG-PBMS multiblock copolymer

The PEG-PBMS multiblock copolymer was synthesized by one-pot melt polycondensation reaction in the presence of $Sc(OTf)_3$. The procedure was as follows: In a 100 mL Schlenk flask equipped with a mechanical stirrer, PEG (6.8 g, 6.8 mmol), 1,4-butanediol (2.7 g, 29.8 mmol), mercaptosuccinic acid (5.50 g, 36.6 mmol) and $Sc(OTf)_3$ (0.36 g, 0.73 mmol) were added and the flask was purged with argon and then put into an oil bath thermostated at 80℃. Vigorous stirring was carried out to ensure the uniformity after PEG was melted. After esterification for 4 h at 80 °C, polycondensation at 90 °C was carried out under high vacuum (< 100 Pa). After polycondensation for 8 h, the reaction was stopped and the product was dissolved in a small amount of $CH_2Cl_2$, precipitated in cold diethyl ether. Yield: 10.7 g (78 %).

### Preparation of micelles and CCL micelles from PEG-PBMS

50 mg of PEG-PBMS multiblock copolymer was dissolved in 50 mL of THF and 50 mL of

distilled water was added dropwise into the solution under vigorous stirring. The solution was dialyzed against distilled water for 24 h using a tubular dialysis membrane (MWCO = 14 kDa) to give aqueous solution of PEG-PBMS micelles. The CCL micelles in water were obtained by adding diluted $H_2O_2$ aqueous solution (1 mL, 3 wt-%) into the above micellar solution under vigorous stirring and dialyzed against distilled water for another 24 h. The final micellar concentrations were adjusted to 0.5 mg/mL. The *N,N*-dimethylformamide (DMF) solution of CCL micelles was prepared by dialyzing the aqueous solution of CCL micelles against DMF. The final concentration in DMF was also adjusted to 0.5 mg/mL.

**Preparation of PTX-loaded CCL micelles**

50 mg of PEG-PBMS and 5.0 mg of PTX were dissolved in 50 mL of THF, and then 50 mL of distilled water was added dropwise to the solution under vigorous stirring to induce the hydrophobic PTX incorporated into the hydrophobic micellar core. The solution was stirred for 1 h and dialyzed against distilled water for 24 h using a tubular dialysis membrane (MWCO = 14 kDa). Then 1 mL of 3.0 % $H_2O_2$ aqueous solution was added dropwise to the micelles solution under stirring. The micelles solution was stirred for 3 h and dialyzed against distilled water for another 24 h to remove extra $H_2O_2$. The final polymer concentration was adjusted to 0.5 mg/mL and the solid-state drug-loaded PEG-PBMS copolymer was recovered by lyophilization. The drug-loading capacity of the CCL micelles was investigated by reverse-phase high-performance liquid chromatography (RP-HPLC) analysis. A mobile phase of methanol/water (70/30, v/v) was used, and the flow rate was set at 1 mL/min (35 °C). The efficiencyefficiency and the drug encapsulation efficiency were calculated using eq. 1 and 2, respectively:

Drug loading content (%) = (weight of loaded drug/weight of polymer) × 100 (1)

Drug encapsulation efficiency (%) = (weight of loaded drug/weight of drug in feed) × 100 (2)

**PTX release from the PEG-PBMS CCL micelles**

*In vitro* release profiles of PTX from the CCL micelles were investigated by dialysis method (Fig. 2). Concretely, 4 mL of PTX-loaded CCL micelles solution was introduced into a dialysis bag (MWCO = 14 kDa) against 20 mL PBS buffer solution (pH 7.4, 20 mM) containing 10 mM DTT (mimicking intracellular reductive condition). Another group was conducted without DTT (non-reductive condition). Tween 80 (0.1 wt-%) was introduced into the PBS buffer solution to solubilize released PTX. At desired time intervals, 5 mL release mediums were taken out and replenished with an equal volume of corresponding fresh media. The amounts of released PTX were determined by RP-HPLC analysis. A mobile phase of methanol/water (70/30, v/v) was used, and the flow rate was set at 1 mL/min (35 °C). The release experiments were conducted in triplicate, and the results presented are the average data with standard deviations.

***In vitro* cytotoxicity studies**

The *in vitro* relative cytotoxicities of PEG-PBMS multiblock copolymer and PEG-PBMS CCL micelles against HeLa cells were evaluated by the Cell Counting Kit-8 (CCK-8) assay (Dojindo Laboratories, Japan). Appropriate amounts of non-crosslinked PEG-PBMS multiblock copolymer and PEG-PBMS CCL micelles were separately dissolved in PBS to obtain their extracts with concentration of 1000, 500, 100, 50, 10, 5, 1 and 0 μg/mL, respectively. HeLa cells were seeded into 96-well plates at a density of 10000 cells per well in 180 μL of culture medium. 24 h later, 20 μL extracts of various concentrations were added to the well. Cells were exposed to the gel precursor for 48 h. Before 100 μL fresh medium and 10 μL CCK-8 reagent were added, each well

was washed three times with PBS. After a further incubation of 1 h, the optical density (OD) of the wells was measured at 450 nm with a Microplate Spectrophotometer (SpectraMax M5). Non-treated cells were used as a negative control. Relative cell viability was calculated as follows:

Relative cell viability (%) = $[(OD450_{sample} - OD450_{blank})/(OD450_{control} - OD450_{blank})] \times 100$.

The cytotoxicities of PTX-loaded PEG-PBMS CCL micelles upon their reduction-response were also evaluated by CCK-8 assay. Micelles were diluted with final PTX concentrations of 50, 10, 5, 1 and 0.5 μg/mL, respectively. After HeLa cells were incubated for 24 h in 96-well plates, the cells were pretreated with or without 0.5 mM BSO for another 12 h, incubated with various concentrations of PTX-loaded CCL micelles for 48 h. As a comparison, another HeLa cell line was incubated with PTX stock solutions (free PTX) under the same conditions. PTX stock solutions were prepared according to a reference method.[2]

**Cell internalization studies**

Nile red (NR) was used as a fluorescence probe in cell internalization studies. 0.2 mL of NR's DMF solution (1.0 mg/mL) was added to 10 mL of PEG-PBMS CCL micellar solution (0.5 mg/mL) and then shaken the mixture violently. The micellar solution was finally dialysed against water to remove DMF and unloaded NR.

The internalization and intracellular distribution of NR-loaded CCL micelles in HeLa cells were monitored using confocal laser scanning microscopy (CLSM, BX61W1-FV1000, OLYMPUS, Japan). Autoclave sterilized coverslips were placed in 6-well plate. The HeLa cells were seeded in 6-well plates with a concentration of $2 \times 10^4$ cells per well in 1.8 mL of complete α-MEM and cultured for 24 h. Then the cells were pretreated with or without 0.5 mM BSO for another 12 h. Thereafter, 200 μL NR-loaded CCL micellar solution or saturated aqueous solution of free NR

were added, and cultured for another 1 h or 4 h. Then the culture media was removed, followed by washed the cells thrice with PBS and fixed with 4 % paraformaldehyde for 15 min. Cell nucleus were stained by Hochest 33324 (Sigma-Aldrich, blue fluorescence). After being mounted with neutral balsam, samples were observed with 60 × magnification microscope.

Microplate reader was used for quantitative determination of cellular uptake of NR-loaded PEG-PBMS micelles. 180 μL suspension of the HeLa cells were seeded into each well of 96-well plate ($5\times 10^3$ cells) and incubated for 24 h. The cells were pretreated with or without 0.5 mM BSO for another 12 h. Then 20 μL PBS containing NR-loaded PEG-PBMS micelles or free NR solutions were added. After incubated for 1 h or 4 h, cells were washed with PBS twice, and finally 100 μL PBS was added. Cells incubated with equivalent amount PBS was used as negative control. Fluorescence intensity was measured with a microplate reader (SpectraMax, molecular devices, AmericaX) with excitation wavelength 550 nm and emission wavelength of 605 nm. Each group was tested in quintuplicate.

**Characterization**

Proton Nuclear Magnetic Resonance Spectroscopy ($^1$H NMR, $^{13}$C NMR) spectrum was recorded on a Bruker Avance DMX500 spectrometer in $CDCl_3$ with tetramethylsilane as internal standard. Fourier transform infrared (FT-IR) spectra were recorded using a PE Paragon 1000 spectrometer (KBr disk). The molecular weight and molecular weight distribution were determined by size exclusion chromatography (SEC) at 60 °C. The SEC system consisted of a Waters degasser, a Waters 1515 Isocratic HPLC pump, and columns: Styragel, HT 3, HT 4. DMF was used as the mobile phase at a flow rate of 1 mL/min and standard poly(methyl methacrylate) was used for calibration. The hydrodynamic diameter and size distribution of micelles were determined by

dynamic light scattering (DLS) at 90 ° angle to the incident beam and at 25 °C on a Brookhaven 90 Plus particle size analyzer. All micelles solutions had a final polymer concentration of 0.5 mg/mL and were filtered through a 0.45 $\mu$m filter. Transmission electron microscopy (TEM) images were obtained using JEM-1230 operating at an acceleration voltage of 60 kV. A drop of 0.5 mg/mL micelles solution was dropped onto the surface of Formvar-carbon film-coated copper grids. Excess solution was quickly wicked away with a filter paper. All grids were finally stained by 2 wt % phosphotungstic acid. The TEM result of inverted micelles solution was obtained by operating as below. A drop of 0.5 mg/mL inverted micelles solution was dropped onto the surface of Formvar-carbon film-coated copper grid. Excess solution was quickly wicked away with a filter paper. The grid was finally stained by $OsO_4$. All the ultraviolet spectrophotometric (UV) measurements were performed on an ultraviolet-visible spectrophotometer (UV-2550, Shimadzu Ltd., Japan).

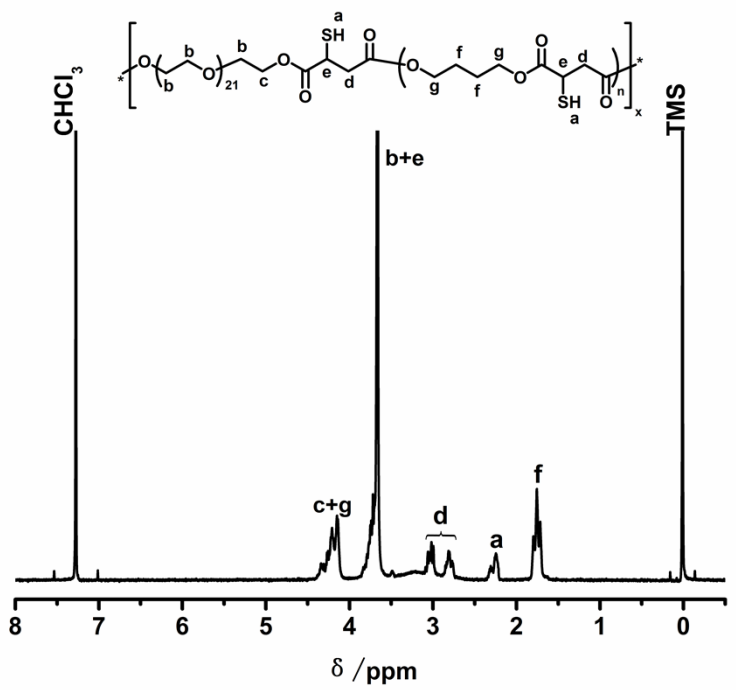


**Fig. S1.** $^1$H NMR spectrum of PEG-PBMS multiblock copolymer.

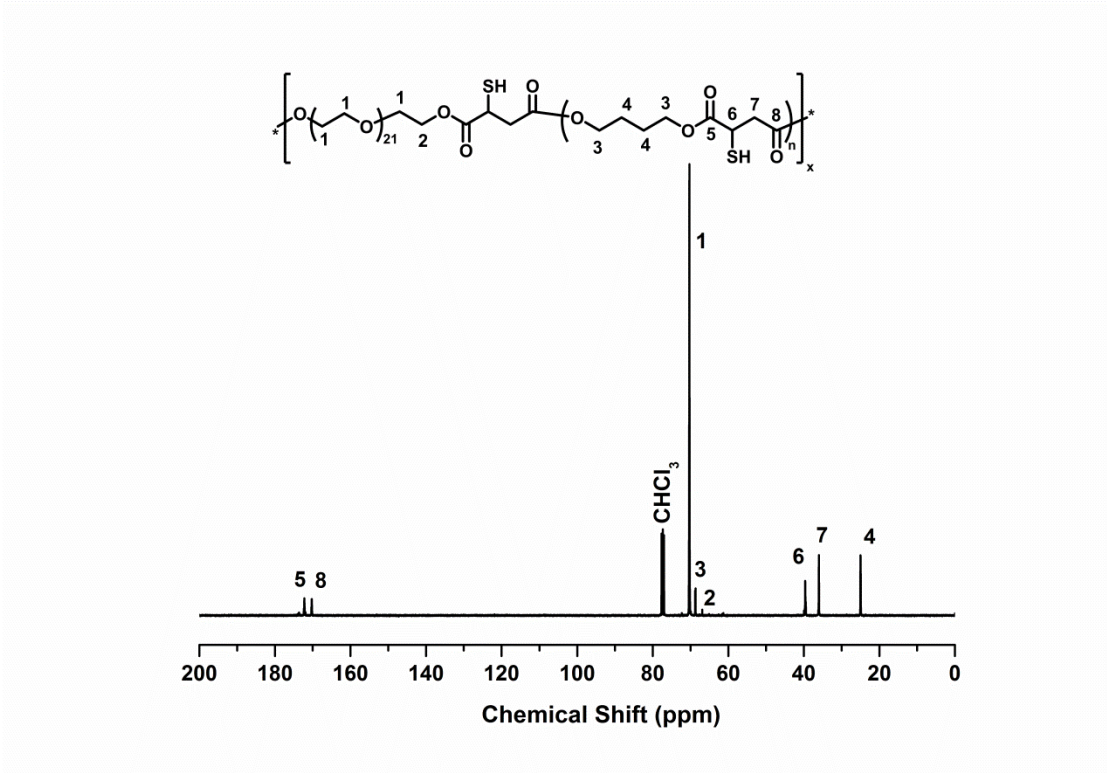


**Fig. S2.** $^{13}C$ NMR spectrum of PEG-PBMS multiblock copolymer.

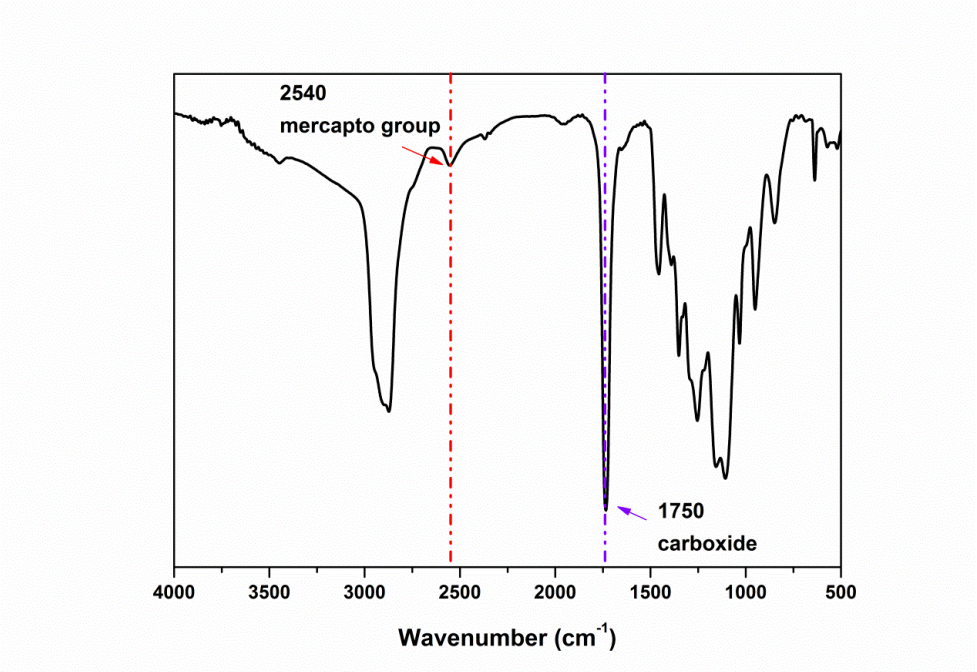

**Fig. S3.** FT-IR spectroscopy of PEG-PBMS multiblock copolymer.

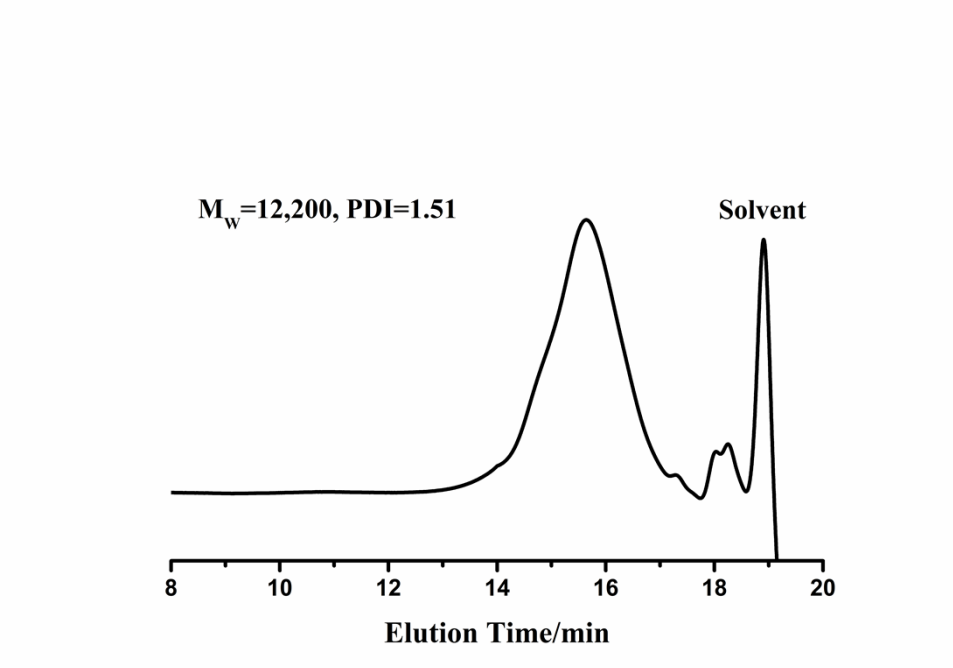


**Fig. S4.** SEC curve of PEG-PBMS multiblock copolymer.

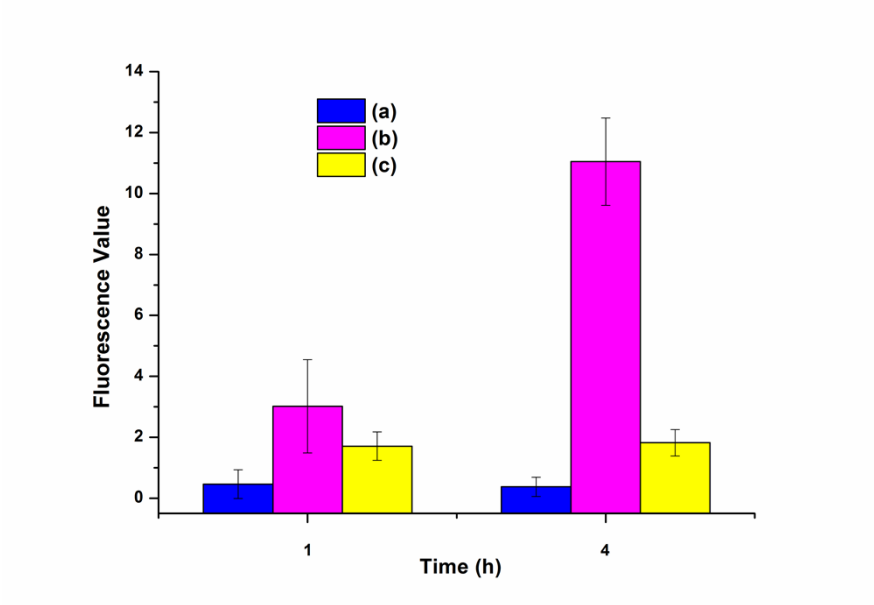


**Fig. S5.** Quantitative determination of cellular uptake of NR-loaded micelles by microplate reader. (a) free NR with non-pretreated HeLa cells; (b) NR-loaded PEG-PBMS CCL micelles with non-

pretreated HeLa cells; (c) NR-loaded PEG-PBMS CCL micelles with HeLa cells pretreated by 0.5 mM BSO. Each group was tested in quintuplicate.

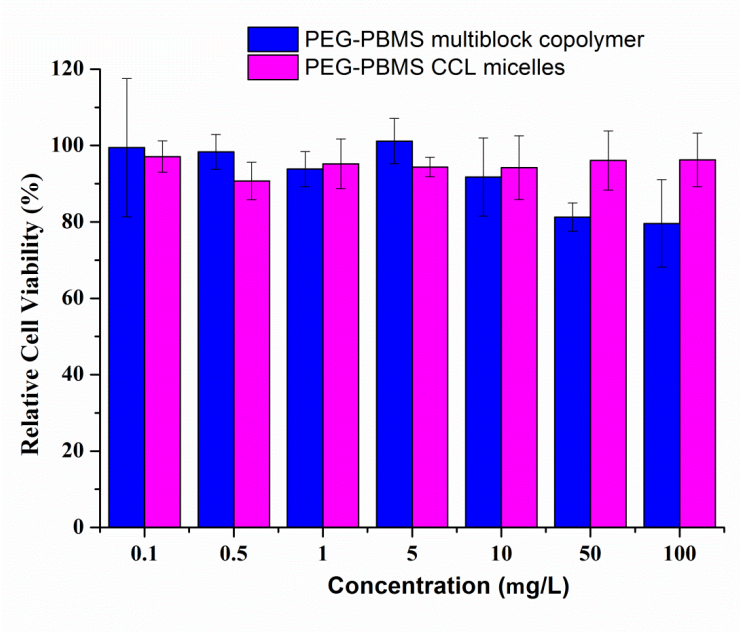


**Fig. S6.** Relative cell viability of PEG-PBMS multiblock copolymer and PEG-PBMS CCL micelles against HeLa cells. Each group was tested in quintuplicate.

## References


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