Supporting Information for

Redox-Responsive, Core-Cross-Linked Micelles Capable of On-Demand, Concurrent Drug Release and Structure Disassembly

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Experimental section

Materials

Boc-$_L$-tyrosine was purchased from Chem-Impex International (Des Plaines, IL, USA) and used as received without further purification. Monomethoxy poly(ethylene glycol) (M_n = 2 K or 5 K) were purchased from Polyscience (Warrington, PA, USA) and used as received. Anhydrous dichloromethane (DCM), hexane, tetrahydrofuran (THF) and dimethylformamide (DMF) were purified by passing them through alumina columns and kept anhydrous by storing them in the presence of molecular sieves. Spectra/Por 6 dialysis tubing (MWCO 3500) was purchased from Spectrum Laboratories Inc (Rancho Dominguez, CA, USA). Phosphate-Buffered Saline (PBS) and Dulbecco's Modified Eagle Medium (DMEM) were obtained from Invitrogen (Carlsbad, CA, USA). MCF-7 breast cancer cell line was purchased from American Type Culture Collection (Manassas, VA, USA). Fetal Bovine Serum (FBS) was obtained from Lonza Walkersville Inc (Walkersville, MD, USA). 96 well BD Falcon culture plates were purchased from Fisher Scientific (Hampton, NH, USA). Camptothecin (CPT) and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used as received without further purification unless otherwise noted.

Measurements

Nuclear magnetic resonance (NMR) analyses were conducted on a Varian U500 (500 MHz) or a VXR500 (500 MHz) spectrometer. The molecular weights of prepared polyesters were determined by gel permeation chromatography (GPC) equipped with an isocratic pump (Model 1100, Agilent Technology, Santa Clara, CA, USA), a DAWN HELEOS multi-angle laser light scattering detector (MALLS detector, Wyatt Technology, Santa Barbara, CA, USA) and an

Optilab rEX refractive index detector (Wyatt Technology, Santa Barbara, CA, USA). The detection wavelength of HELEOS was set at 658 nm. Separations were performed using serially connected size exclusion columns (Phenogel columns 100 Å, 500 Å, 10^3 Å, 10^4 Å, 5 μm, 300 × 7.8 mm, Phenomenex, Torrance, CA, USA) at 60 ^oC using DMF containing 0.1 M LiBr as the mobile phase. Data processing was performed with ASTRA V software (Version 5.1.7.3, Wyatt Technology). HPLC analyses were performed on a Beckman Gold system (Beckman Coulter, Fullerton, CA, USA) equipped with a 126P solvent module, a System Gold 128 UV detector and an analytical C18 column (Luna C18, 250 × 4.6 mm, 5 μ, Phenomenex, Torrance, CA, USA). The size and size distribution of micelles were determined via the use of ZetaPlus dynamic light scattering (DLS) detector (15 mW laser, incident beam = 676 nm, Brookhaven Instruments, Holtsville, NY, USA). Infrared spectra were recorded on a PerkinElmer 100 serial FTIR spectrophotometer (PerkinElmer, Waltham, MA, USA). Lyophilization was conducted on a Labconco FreeZone lyophilizer (Kansas City, MO, USA). TEM results were collected on a JEOL 2100 cryo transmission electron microscope.

Synthesis of 1. Camptothecin (1.05 g, 3.0 mmol) and triphosgene (0.89 g, 3.0 mmol) were suspended in methylene chloride (100 mL) and purged with nitrogen for 10 min. 4-dimethylaminopyridine (0.98 g, 8.0 mmol) was added in one portion. After stirring for 20 min, 2-hydroxyethyl disulfide (1.23 g, 8.0 mmol) in tetrahydrofuran (10 mL) was added and the reaction mixture was stirred overnight at room temperature. The mixture was washed with DI water and HCl aqueous solution for 3 times respectively. Solvent was removed under vacuum and the crude product was purified by silica column chromatography using ethyl acetate as the eluent to get rid of side products including the dimer. A light yellow solid was obtained (0.81 g, 51% yield).

Preparation of CPT-S-S-poly(2). Synthesis of **2** was performed according to a previously reported method.[1] In a glovebox, **2** (123.1 mg, 0.500 mmol) was dissolved in dichloromethane (1mL), followed by the addition of mixture solution of **1** (13.2 mg, 0.025 mmol) and 4-Dimethylaminopyridine (3.1 mg, 0.025 mmol) in dichloromethane (1 mL). The mixture was stirred overnight at room temperature. Complete consumption of **2** was determined by the disappearance of peak at 1810 cm^{-1} in FTIR spectrum. Then the polymer was precipitated with ether, washed with ether for 3 times and dried under vacuum (100.5 mg, 90% yield).

Preparation of mPEG-poly(2). In a glovebox, **2** (123.1 mg, 0.500 mmol) was dissolved in dichloromethane (1mL), followed by the addition of mixture solution of $mPEG_{5k}$ (125.0 mg, 0.025 mmol) and 4-dimethylaminopyridine (3.1 mg, 0.025 mmol) in dichloromethane (1 mL). The complete consumption of **2** was determined by the disappearance of peak at 1810 cm^{-1} in FTIR spectrum. Then the polymer was precipitated with ether, washed with ether for 3 times and dried under vacuum (183.0 mg, 91% yield).

Synthesis of CPT-poly(2). $(BDI\text{-}EI)ZnN(TMS)_2$ was synthesized according to a previously reported method.[2] $(BDI\text{-}EI)ZnN(TMS)_2$ (9.3 mg, 12 μmol) was dissolved in anhydrous THF (500 μL). CPT (5.3 mg, 12 μmol) was added and the mixture was stirred for 20 min until the solution became clear. **2** (73.9 mg, 0.3 mmol) in THF (500 μL) was added in one portion, and the mixture was stirred at room temperature for another 16 h. Complete consumption of **2** was determined by the disappearance of peak at 1810 cm^{-1} in FTIR spectrum. Then the polymer was precipitated with ether, washed with ether for 3 times and dried under vacuum (60.3 mg, 93% yield).

Synthesis of bis(azidoethyl) disulfide. This reaction was performed according to a previously reported protocol.[3] ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 2.85 (t, 4H, (N_3CH_2-)$_2$), 3.57 (t, 4H, (-CH_2S-)$_2$). ^{13}C NMR ($CDCl_3$, 500 MHz): δ (ppm) 37.8, 50.1.

Synthesis of 1, 5-diazidopentane. NaN_3 (1.95 g, 30.0 mmol) was added to a solution of the 1, 5-dibromopentane (2.30 g, 10.0 mmol) in DMF (30 mL). The mixture was stirred at 60 °C for 10 h, at which point water (100 mL) was added. The product was extracted with ether (30 mL × 3) and washed with water (100 mL × 2). Solvent was removed under vacuum to get a yellow oil. Silica column chromatography was used to purify the crude product with hexane as the eluent (1.39 g, 90% yield). ^{1}H NMR ($CDCl_3$, 500 Hz): δ (ppm) 3.27 (t, 4H, ((N_3CH_2-)$_2$), δ 1.61 (m, 4H, ($N_3CH_2CH_2$-)$_2$), δ 1.46 (m, 2H, ($N_3CH_2CH_2$)$_2CH_2$). ^{13}C NMR ($CDCl_3$, 500 Hz): δ (ppm) 24.2, 28.7, 51.5.

Preparation of CCL micelles. CPT-S-S-Poly(**2**)$_{20}$ (5.0 mg, 0.025 mmol of alkynyl group), mPEG-PAHA (5.0 mg) and Bis-(azidoethyl) disulfide (3.2 mg, 0.015 mmol) were dissolved in anhydrous DMF (1 mL). Then the solution was added to nanopure water (20 mL) dropwise under vigorous stirring. After stirring for 15 min, Copper (II) chloride (1.0 mg, 7.5 μmol) in water (200 μL) and sodium ascorbate (1.5 mg, 7.5 μmol) in water (200 μL) was added in order. The mixture was stirred at room temperature for another 24 h. After that the micelle solution was transferred into a dialysis bag (MWCO 3500), and dialyzed against DI water for 48 h.

Dilution assay. Aqueous solution of micelles (200 μL) was placed in a vial, followed by the addition of pure DMF (2 mL). The solution was shaken for 1 min to fully mix DMF and water. Then the size and size distribution of micelles were measured by DLS.

***In vitro* release study.** Micelles were dispersed in PBS (pH = 7.4, 5 mL), and then transferred into a dialysis bag. The dialysis bag was immersed in PBS (30 mL) with or without dithiothreitol (DTT) and incubated at 37 ^{o}C (100 r/min). At selected time intervals, 1 mL aliquot of the dialysis medium was withdrawn and brought to pH = 2 with phosphoric acid (85%, 30 μL) prior to HPLC measurement and the same amount of fresh medium was added. A mixture of acetonitrile and water (containing 0.1% TFA) at a volume ratio of 1:3 was used as the mobile phase for HPLC measurements. The flow rate was set at 1 mL/min. Released CPT was quantified by the standard curve of free CPT.

Time-dependent stability of micelles. CCL and UCL micelles were dispersed in PBS and incubated at 37 ^{o}C (100 r/min) simultaneously. At selected time intervals, size and size distribution of the micelles were measured by DLS.

Degradation study of micelles. First, the size and size distribution of micelles were measured by DLS. Then DTT was added in one portion with a final concentration of 10 mM. After incubated at 37 ^{o}C (100 r/min) for 6 h, the micelles were evaluated by DLS. Afterwards, micelle solution was diluted with 10-fold volume of DMF and DLS measurement was conducted again. Second, micelles were lyophilized and dispersed in DMF (1 mL), followed by the addition of DTT (10 mM). After incubated at 37 ^{o}C (100 r/min) for 6 h, the mixture became clear. Photographs of micelle solution before and after incubation with DTT were taken.

MTT study. *In vitro* cytotoxicity of micelles and free CPT was measured by MTT assay. MCF-7 cells were seeded in a 96-well plate at an initial density of 5×10^3 cells/well, allowed to attach for

24 h and treated with free CPT or CCL micelles at various CPT concentrations for 48 h at 37 ℃.

Cells without micelle or free CPT treatment were used as control. Free CPT of various concentrations were first dissolved in DMSO and then diluted with a 10-fold volume of PBS prior to adding to the cells. The MTT assay was performed by following the standard procedure.

Influence of GSH-OEt on the cytotoxicity of micelles and free CPT against MCF-7 cells. MCF-7 cells were seeded in a 96-well plate with an initial density of 5×10^3 cells/well and allowed to attach for 24 h. GSH-OEt with a final concentration of 10 mM was added and incubated with cells for 4 h. Then the medium was replaced with fresh medium, followed by the addition of CCL micelles or free CPT at various CPT concentrations. Cells were further incubated for 48 h. Cell viability was assessed by the MTT assay.

TEM measurements. TEM samples were prepared on 200 mesh carbon film supported copper grids. One drop of the micelle solution (~ 10 μL) (0.25-0.5 mg/mL) was placed on the grid and allowed to stand for 10 min. Filter paper was then used to remove the residual solution. The resulting sample was imaged using JEOL 2100 Cryo TEM at 80 kV.

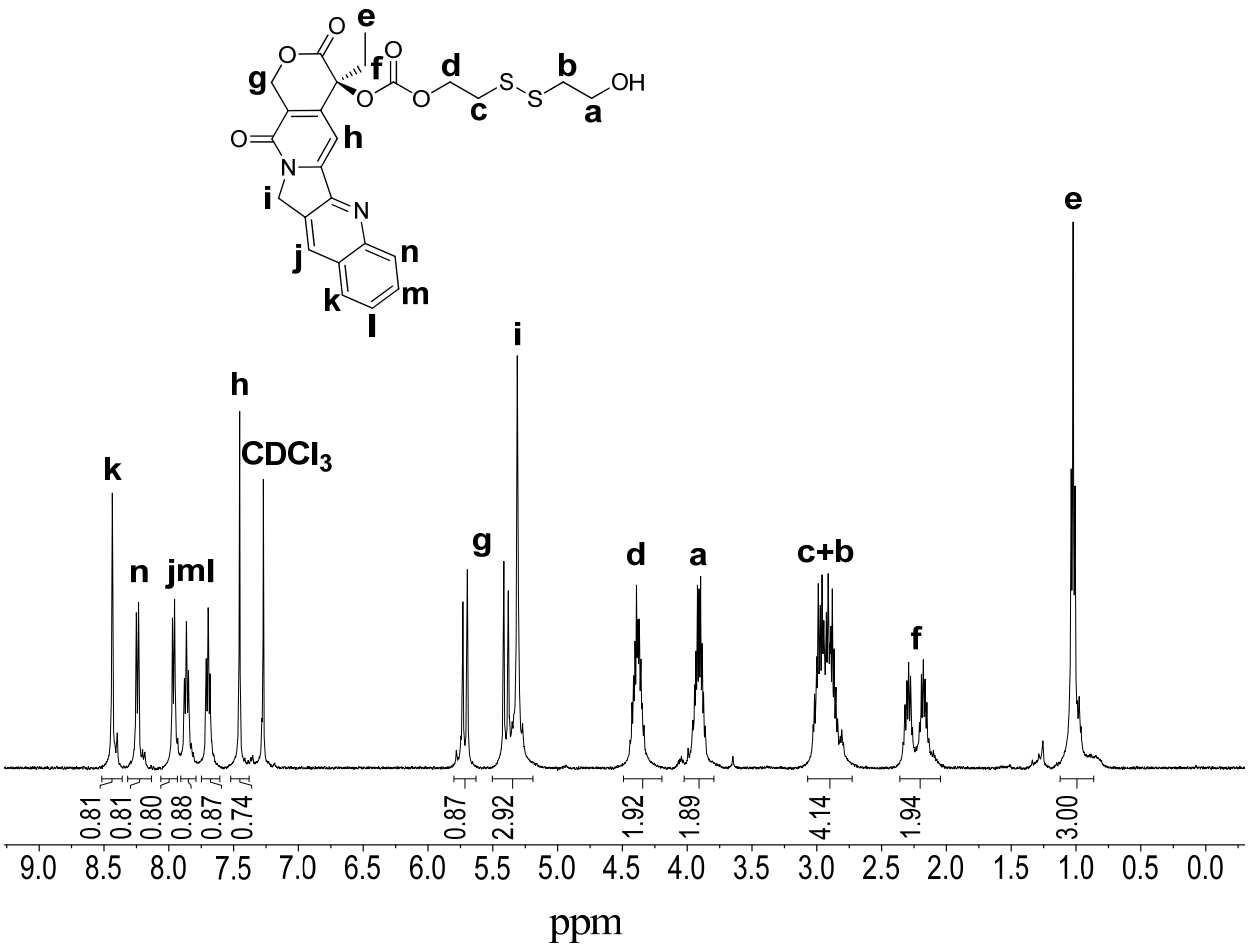

Figure S1. ^{1}H NMR spectrum of CPT-S-S-OH in $CDCl_3$.

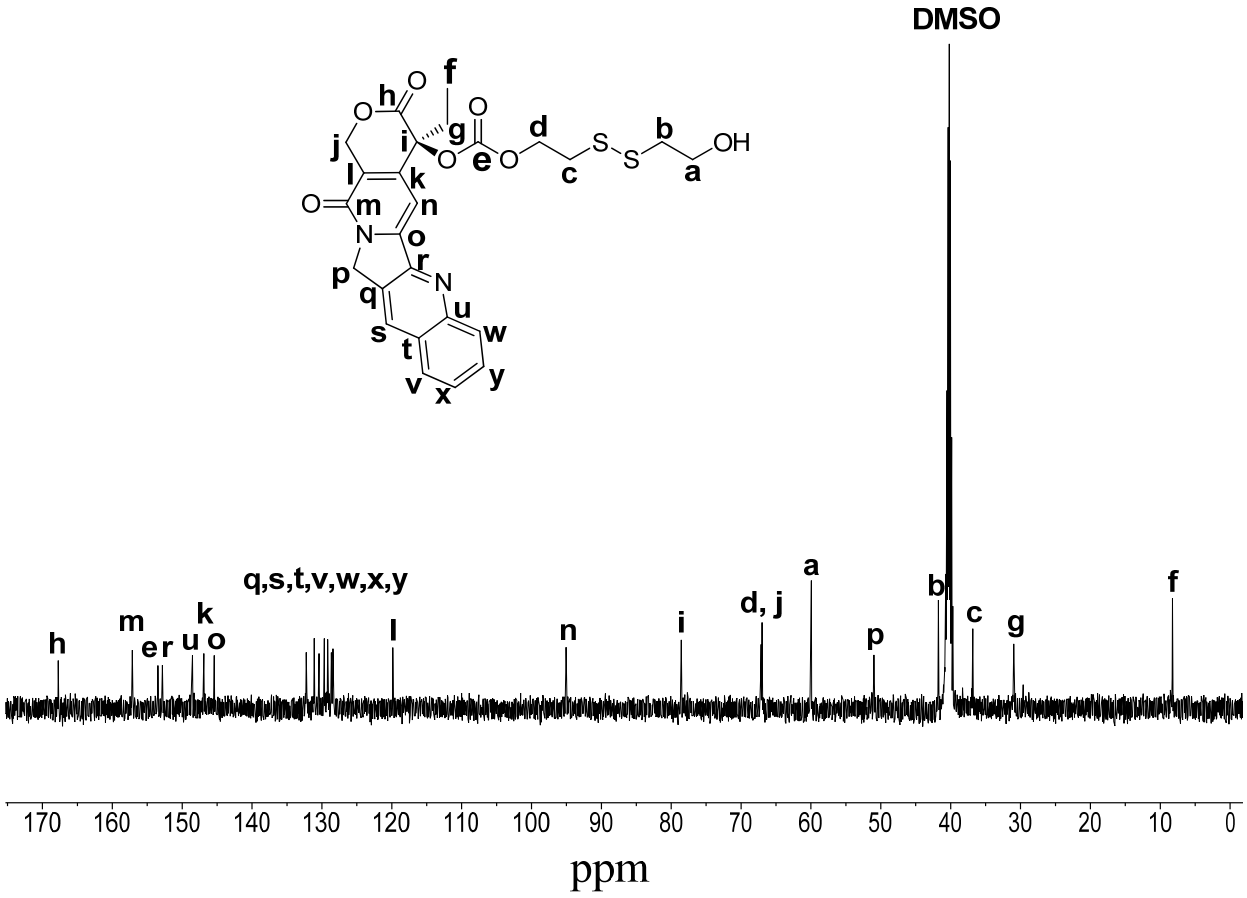

Figure S2. ^{13}C NMR spectrum of CPT-S-S-OH in DMSO-d_6.

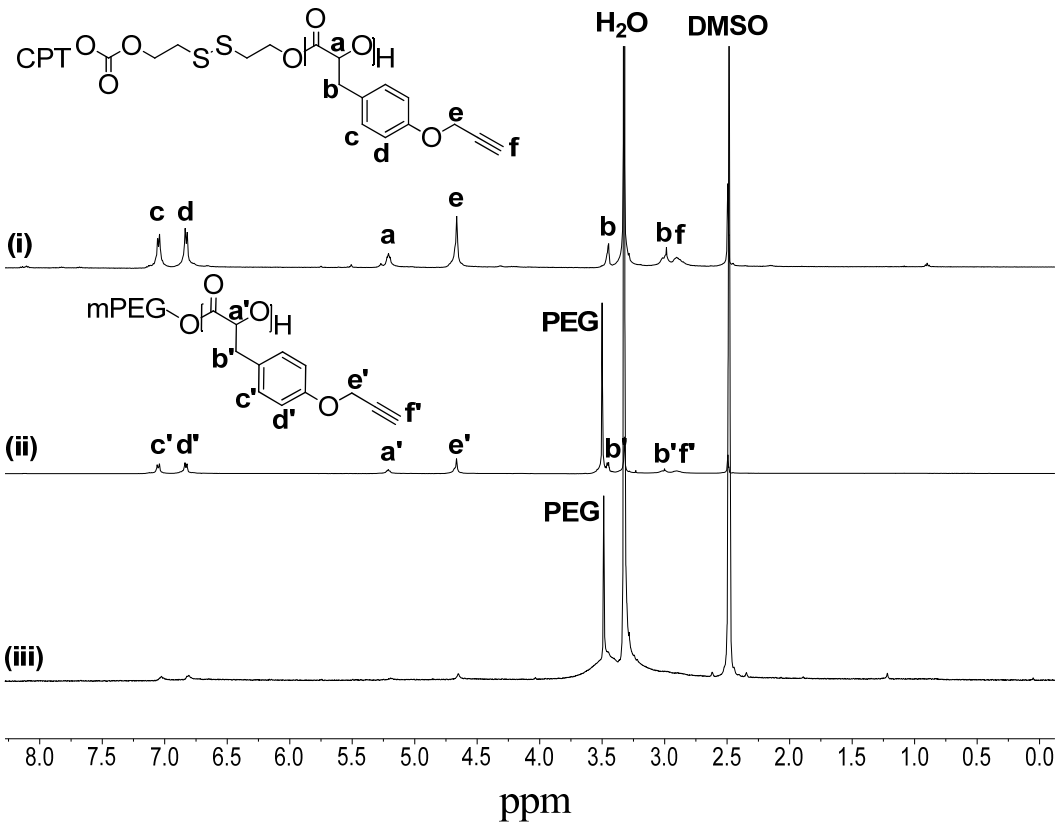

Figure S3. ^{1}H NMR spectra of (i) CPT-S-S-poly(**2**)$_{20}$, (ii) mPEG$_{2k}$-poly(**2**)$_{20}$ and (iii) CCL1 (CPT-S-S-poly(**2**)$_{20}$ and mPEG$_{2k}$-poly(**2**)$_{20}$ were used for the preparation of CCL1).

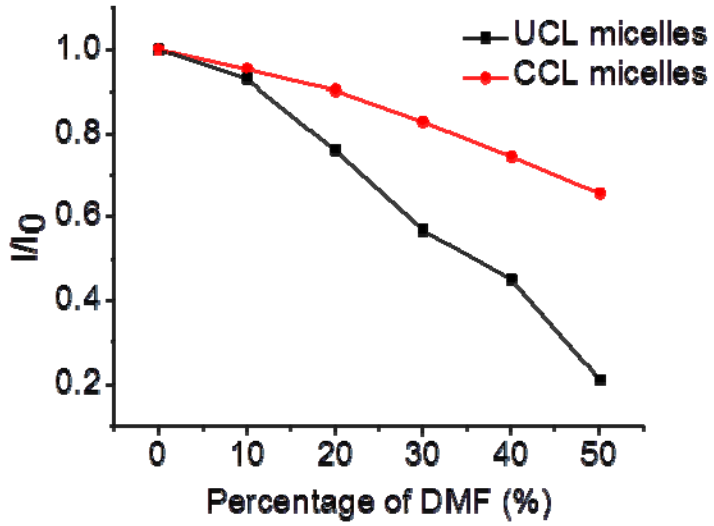

Figure S4. Change of normalized light scattering intensity with the percentage of DMF (in terms of volume) added to the micelle solution. I_0 indicates the scattering intensity in water.

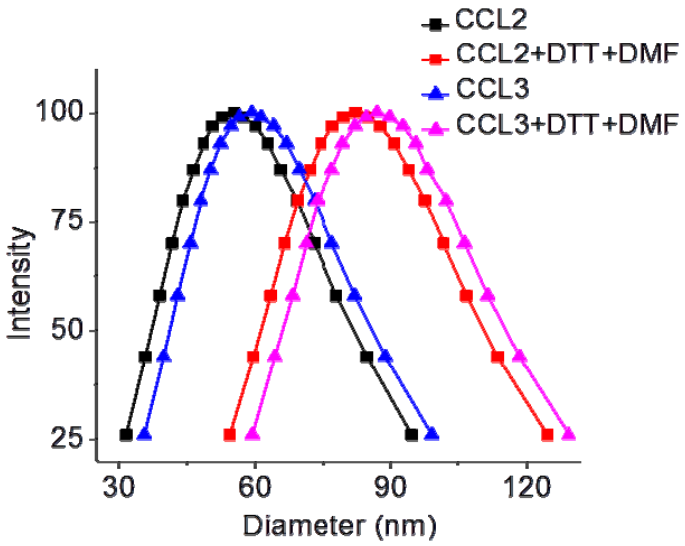

Figure S5. Size changes of CCL2 and CCL3 in response to DTT treatment followed by 10-fold dilution with DMF.

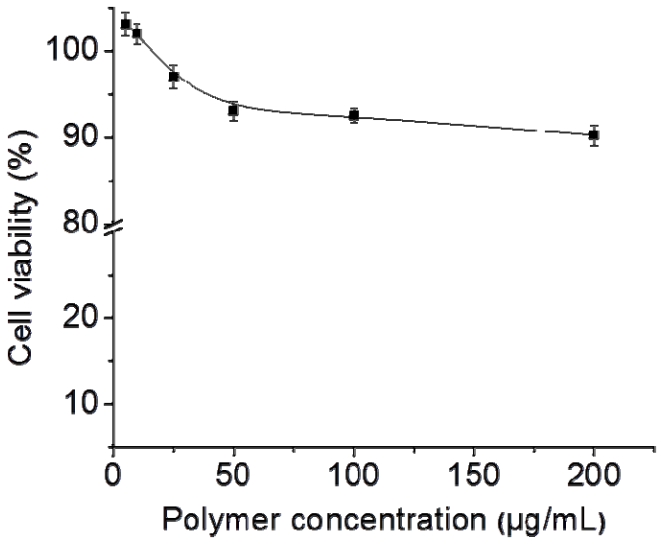

Figure S6. Cytotoxicity of $mPEG_{2k}$-poly(**2**)$_{20}$ against MCF-7 breast cancer cells.

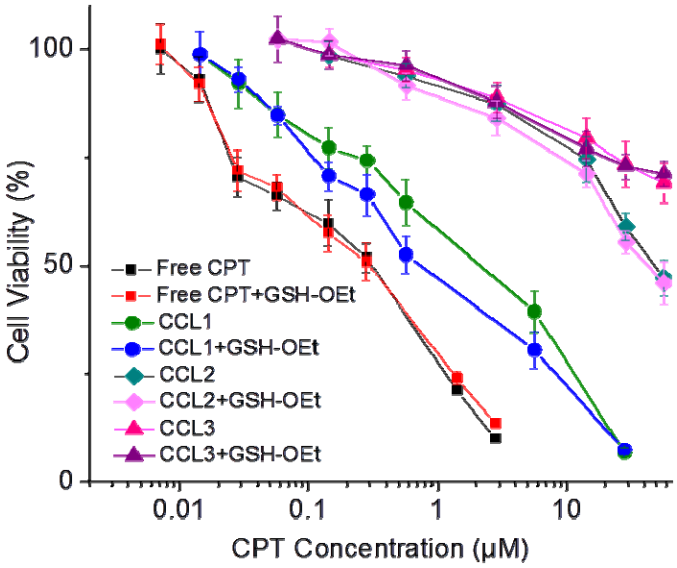

Figure S7. Influence of GSH-OEt on the cytotoxicity of free CPT, CCL1, CCL2 and CCL3 against MCF-7 cells.

References

(1) Zhang, Z. H.; Yin, L. C.; Xu, Y. X.; Tong, R.; Lu, Y. B.; Ren, J.; Cheng, J. J. *Biomacromolecules* **2012**, *13*, 3456-3462.

(2) Tong, R.; Cheng, J. J. *Bioconjugate Chem.* **2010**, *21*, 111-121.

(3) Zhang, Z.; Yin, L.; Tu, C.; Song, Z.; Zhang, Y.; Xu, Y.; Tong, R.; Zhou, Q.; Ren, J.; Cheng, J. *ACS Macro Lett.* **2012**, *2*, 40-44.