Electronic Supplementary Information

The Fabrication of Hybrid Micelles with Enhanced Permeability for Drug Delivery via a Diethoxymethylsilyl-Based Crosslinking Strategy

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1. Experimental Section

Materials.

N,*N*-dimethylacetamide (DMAc), triethylamine (TEA, 99%), Stannous (II) octanoate ($Sn(Oct)_2$, 92.5%-100%) and 2-hydroxyethyl disulfide were purchased from Sigma-Aldrich and used as received. 1,1'-Carbonyldiimidazole (CDI, 98%), 3-(diethoxymethylsilyl)propylamine (JH-M902, 97%), *N*,*N*′-dicyclohexylcarbodiimide (DCC, 98%) and 4-dimethylamino pyridine (DMAP, 99%) were purchased from J&K and used without further purification. ε-Caprolactone (ε-CL, J&K, 99%) was dried over CaH_2 and distilled under reduced pressure prior to use. 2,2′-Azobis(isobutyronitrile) (AIBN, J&K, 99%) was recrystallized twice from ethanol. Oligo (ethylene glycol) monomethyl ether methacrylate (OEGMA, average M_n = 300, Sigma-Aldrich) was purified by passing through a column filled with activated basic Al_2O_3 to remove the inhibitor. Doxorubicin (DOX) was obtained by neutralization of DOX·HCl according to the reported procedures.[1] 4-Cyanopentanoic acid dithiobenzoate (CPADB), 2-((2-hydroxyethyl)disulfanyl)ethyl 4-cyano-4-(phenylcarbonothioylthio)pentanoate (CPADB-SS-OH) 2-((2-hydroxyethyl)disulfanyl)ethyl methacrylate (HSEMA), 2-((2-(methacryloyloxy)ethyl)disulfanyl)ethyl 1H-imidazole-1-carboxylate (HSEMA-CDI) and 2-((2-(methacryloyloxy) ethyl)disulfanyl)ethyl (3-(triethoxysilyl)propyl)carbamate (TESSPMA) were synthesized according to the references.[2-5] HeLa and MRC-5 cells were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Fetal bovine serum (FBS) was obtained from ExCell Bio and used as received. Minimum Essential Medium, penicillin/streptomycin, and trypsin were obtained from Thermofisher and used as received.3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) was purchased from

Promega. All other chemicals were purchased from Tianjin Chemical Reagent Factory (China) and subjected to further purification following the standard protocols.

Synthesis of 2-((2-(methacryloyloxy) ethyl)disulfanyl)ethyl (3-(diethoxymethylsilyl)propyl)carbamate (DESSPMA)

HSEMA-CDI (10 g, 31.6 mmol) and 3-(diethoxymethylsilyl)propylamine (JH-M902, 5.4 g, 28.4 mmol) were dissolved in 100 ml of dry dichloromethane (DCM). 4-dimethylamino pyridine (DMAP, 0.77 g, 6.3 mmol) was later added and the mixture was stirred overnight at room temperature. After removal of the solvent, the product was purified by flash column chromatography (ethyl acetate:hexane = 1:5, v/v) to yield the product as a light yellow oil (12.4 g, yield: 89.3%). ^{1}H NMR (**Fig. S1**, $CDCl_3$, 400 MHz): δppm, 6.11 (s, 1H, C**H**$_2$=C), 5.56 (s, 1H, C**H**$_2$=C), 5.11 (m, 1H, CON**H**), 4.40 (t, J = 6.68 Hz, 2H, COOC**H**$_2$), 4.29 (t, J = 6.34 Hz, 2H, C**H**$_2$OOCNH), 3.75 (q, J = 7.0 Hz, 4H, OC**H**$_2$CH3), 3.17 (q, J = 6.56 Hz, 2H, NHC**H**$_2$), 2.95 (t, J = 6.70 Hz, 2H, $COOCH_2$C**H**$_2$S), 2.91 (t, J = 6.38 Hz, 2H, SC**H**$_2$$CH_2OOCNH$), 1.93 (s, 3H, C**H**$_3$C=$CH_2$), 1.64 - 1.53 (m, 2H, $SiCH_2$C**H**$_2$), 1.19 (t, J = 6.98 Hz, 6H, OCH_2C**H**$_3$), 0.59 (t, J = 8.30 Hz, 2H, SiC**H**$_2$), 0.10 (s, 3H, SiC**H**$_3$). ^{13}C NMR (**Fig. S2**, $CDCl_3$, 101 MHz): δppm, 167.1 (OC=O), 156.1 (OC=ONH), 136.0 (C=CH_2), 126.0 C=CH_2, 62.6 ($COOCH_2$), 62.2 (CH_2OOCNH), 58.1 (OCH_2CH_3), 43.6 ($NHCH_2$), 38.0 ($COOCH_2CH_2S$), 37.0 (SCH_2CH_2OOCNH), 23.3 ($NHCH_2CH_2$), 18.3 ($CH_3C=CH_2$), 18.2 (OCH_2CH_3), 11.1 ($SiCH_2$), -5.0 ($SiCH_3$). ESI-MS: M+Na^+ expected (observed): 462.1411 (462.1413).

Synthesis of PCL macro-chain transfer agents (PCL-SS-CPADB). PCL-SS-CPADB was synthesized according to the reported procedures.[6, 7] Briefly, CPADB-SS-OH (0.092 g, 0.22 mmol), ε-CL (0.653 g, 5.72 mmol), $Sn(Oct)_2$ (0.009 g, 0.022 mmol) and toluene (5.72 mL) were loaded in a thoroughly dried 25 mL of Schlenk tube with a magnetic stirring bar under nitrogen atmosphere. The reaction tube was degassed by three freeze-pump-thaw cycles and then immersed in an oil bath preheated to 90 °C to start the polymerization. After 24h, the mixture was precipitated in 10-fold ice-cold hexane three times. The purified product was finally dried under vacuum until constant weight.

Synthesis of PCL-SS-PDESSPMA diblock copolymer using PCL-SS-CPADB as a macro-CTA. Reversible addition-fragmentation chain transfer (RAFT) polymerization was employed for preparing PCL-SS-PDESSPMA diblock copolymer with PCL-SS-CPADB as a macro-CTA and AIBN as primary radical source. The typical procedure was as following, DESSPMA (0.35 g, 0.80 mmol), PCL-SS-CPADB (0.051 g, 0.016 mmol), AIBN (0.85 mg, 0.005 mmol) and DMAc (1.6 mL) were prepared in a thoroughly dried 10 mL of Schlenk tube with a magnetic stirring bar. The tube was degassed via three freeze-pump-thaw cycles and then immersed in a preheated oil bath at 70 °C to start the polymerization. After reaction for 120 min the polymerization was stopped by exposing the reaction mixture to air and diluting with 1 mL of THF. The product was collected by precipitation in 10-fold ice-cold hexane three times and dried under vacuum until constant weight.

Synthesis of PCL-SS-PDESSPMA-b-POEGMA amphiphilic triblock copolymer using PCL-SS-PDESSPMA-CPADB as a macro-CTA. PCL-SS-PDESSPMA-*b*-POEGMA amphiphilic triblock copolymer was synthesized by RAFT polymerization technique using PCL-SS-PDESSPMA-CPADB as a macro-CTA and AIBN as primary radical source. The procedure was as following, OEGMA (0.888 g, 2.96 mmol), PCL-SS-PDESSPMA-CPADB (0.097 g, 0.010 mmol), AIBN (0.54 mg, 0.0033 mmol) and DMAc (2.96 mL) were loaded in a thoroughly dried 10 mL of Schlenk tube with a magnetic stirring bar. After three freeze-pump-thaw cycles, the tube was immersed in a preheated oil bath at 70 °C for 60 min. The polymerization was stopped by exposing the reaction mixture to air and diluting with 1 mL of THF. The product was collected by precipitation in 10-fold ice-cold hexane three times and dried under vacuum until constant weight.

Synthesis of PCL-SS-PTESSPMA diblock copolymer using PCL-SS-CPADB as a macro-CTA. PCL-SS-PTESSPMA diblock copolymer was synthesized following similar procedure described above. Briefly, TESSPMA (0.388 g, 0.83 mmol), PCL-SS-CPADB (0.092 g, 0.027 mmol), AIBN (1.5 mg, 0.009 mmol) and DMAc (4.10 mL) were charged into a thoroughly dried 25 mL of Schlenk tube. The tube was degassed via three freeze-pump-thaw cycles and then immersed in a preheated oil bath at 70 °C to start the polymerization. After reaction for 120 min the polymerization was stopped by exposing the reaction mixture to air and diluting with 1 mL of THF. The product was collected by precipitation in 10-fold ice-cold hexane three times and dried under vacuum until constant weight.

Synthesis of PCL-SS-PTESSPMA-b-POEGMA amphiphilic triblock copolymer using PCL-SS-PTESSPMA-CPADB as a macro-CTA. The synthetic procedure of PCL-SS-PTESSPMA-*b*-POEGMA amphiphilic triblock copolymer was similar to the preparation of PCL-SS-PDESSPMA-*b*-POEGMA. In brief, OEGMA (0.774 g, 2.58 mmol), PCL-SS-PTESSPMA-CPADB (0.057 g, 0.009 mmol), AIBN (0.47 mg, 0.003 mmol) and DMAc (2.58 mL) were loaded in a thoroughly dried 10 mL of Schlenk tube with a magnetic stirring bar. After three freeze-pump-thaw cycles, the tube was immersed in a preheated oil bath at 70 °C for 63 min. The polymerization was stopped by exposing the reaction mixture to air and diluting with 1 mL of THF. The product was collected by precipitation in 10-fold ice-cold hexane three times and dried under vacuum until constant weight.

Micelle Preparation. The solution of polymeric micelles was prepared using the classical dialysis method. Taking PCL_{25}-SS-$PDESSPMA_{15}$-*b*-$POEGMA_{74}$ as an example, 10.0 mg polymer was dissolved in 1 mL DMF, the solution was next added dropwise into 8.0 ml of distilled water under vigorous stirring. The mixture solution was next subjected to dialysis (cellulose membrane, molecular-weight cut-off (MWCO), 3.5 kDa) against phosphate buffer solution (PB, pH 7.0, 5.0 mM) for 24 h, during which fresh PB buffer was replenished every 6h.

Determination of Critical Micelle Concentration (CMC). CMC of PCL-SS-PDESSPMA-*b*-POEGMA and PCL-SS-PTESSPMA-*b*-POEGMA was determined using pyrene as the fluorescence probe according to our previous.[5, 8] In brief, aliquots of pyrene solutions (3×10^{-6} mol/L in acetone, 120 μL) were added to containers, and the acetone was allowed to

evaporate. Six milliliter aqueous polymer solutions at various concentrations were subsequently added to the containers containing the pyrene residue. Note that all the aqueous sample solutions contained excess pyrene residue at an identical concentration of 6×10^{-8} mol/L. The solutions were kept at room temperature for 24 h to reach the solubilization equilibrium of pyrene in the aqueous phase. Excitation was carried at 340 nm, and emission spectra were recorded ranging from 350 to 400 nm. Both excitation and emission bandwidths were 5 nm. From the pyrene emission spectra, the intensity ratio of the third (I_{384}) and first band (I_{373}) were analyzed as a function of the polymer concentrations. The CMC value was determined from the intersection of the tangent to the curve at the inflection with the horizontal tangent through the points at low concentration.

Preparation of shell cross-linked (SCL) micelle solution. Taking PCL_{25}-SS-$PDESSPMA_{15}$-*b*-$POEGMA_{74}$ as an example, 10.0 mg polymer was dissolved in 1 mL DMF, and then added dropwise into 8.0 ml of distilled water under vigorous stirring. After stirring for 1 h, 20 μL TEA was added to trigger the sol-gel process, which proceeded for 4 h. DMF and TEA were removed by dialysis (cellulose membrane, MWCO, 3.5 kDa) against PB buffer solution (pH 7.0, 5.0 mM) for 24 h, during which fresh PB buffer was replenished every 6h.

NMR Characterization. ^{1}H and ^{13}C NMR spectra of the synthesized small organic molecules and polymers were analyzed on a JNM-ECS 400 MHz spectrometer (JEOL, Tokyo, Japan) using deuterated chloroform ($CDCl_3$) as the solvent.

Molecular Weight Analysis. The size-exclusion chromatography and multi-angle laser light

scattering (SEC-MALLS) analyses were used to determine the molecular weight (MW) and polydispersity ($Đ_M$) of the synthesized polymers. SEC using HPLC-grade DMF containing 0.1 wt% LiBr at 60 °C as the eluent at a flow rate of 1 mL/min, Tosoh TSK-GEL R-3000 and R-4000 columns (Tosoh Bioscience, Montgomeryville, PA, USA) were connected in series to a Agilent 1260 series (Agilent Technologies, Santa Clara, CA, USA), an interferometric refractometer (Optilab-rEX, Wyatt Technology, anta Barbara, CA, USA) and a MALLS device (DAWN EOS, Wyatt Technology, Santa Barbara, CA,USA). The MALLS detector was operated at a laser wavelength of 690.0 nm.

Transmission Electron Microscopy (TEM). TEM images were recorded on a JNM-2010 instrument operating at an acceleration voltage of 200 keV. The specimens for TEM observation were prepared by placing a drop of micelle solution onto a carbon-coated copper grid. After deposition, excess solution was removed using a strip of filter paper. The sample was further stained using phosphotungstic acid (1% w/w) and dried in air prior to visualization.

Size Distribution Measurements. Dynamic light scattering (DLS) was performed on a Zetasizer (Nano ZS, Malvern, Worcestershire, UK) at a fixed detection angle of 173°.

In Vitro Drug Loading and Drug Release Study. The procedure of DOX-loaded cross-linked micelles (SCL@DOX) were similar to the blank micelles. Briefly, DOX (2.0 mg, 0.007 mmol) and PCL_{25}-SS-$PDESSPMA_{15}$-*b*-$POEGMA_{74}$ (20 mg) were dissolved in 2.0 ml of DMSO. The mixture was next added dropwise into 16.0 ml of distilled water under vigorous stirring. After

stirring for 1 h, 40 μL TEA was added to trigger the sol-gel process, which proceeded for 4 h. The mixture solution was later transferred to a dialysis tube with a MWCO of 3.5 kDa and subjected to dialysis against 5.0 L of PB 7.0 buffer solution for 24 h to remove DMSO, TEA and unloaded free DOX. The PB buffer was renewed every 3 h during the course of initial 12 h. DOX-loaded non-crosslinked (NCL@DOX) micelles were prepared following an identical procedure described above without the addition of TEA.

For *in vitro* drug release study, the solution of drug-loaded micelles was split in equal volumes into four dialysis tubes with a MWCO of 3.5 kDa, which were then immersed in a Falcon tube containing 25 ml of PBS (pH 7.4, 10mM) buffer solution. The tube was kept in a horizontal laboratory shaker thermostated at a constant temperature of 37 °C and a stirring speed of 120 rpm.[9,10] At predetermined time intervals (0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72h), 3 ml of release medium was sampled and replenished with equal volume of fresh medium. The sample were analyzed by measuring the absorbance at 499 nm with UV-vis spectrometer. The concentration of released DOX from each cell was calculated based on the standard curves obtained from free DOX·HCl in the corresponding release buffers.

The encapsulation efficiency (EE) and the drug loading content (DLC) of the drug-loaded micelles were determined using the UV-vis spectrometer at 499 nm. The EE and DLC were calculated using the following equations,[11, 12]

$$EE\ (\%) = W_{DOX\ loaded\ in\ micelles} / W_{DOX\ fed\ for\ encapsulation} \times 100\% \qquad (1)$$

$$DLC\ (\%) = W_{DOX\ loaded\ in\ micelles} / W_{DOX\ loaded\ micelles} \times 100\% \qquad (2)$$

Cell Viability Assay. The cytotoxicity of various formulations was evaluated in vitro using the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl) -2H-tetr zolium

(MTS, Promega) assay. The cells were seeded in 96-well plates at a plating density of 2500 cells per well in 100 μl of complete growth medium and incubated in an incubator maintained at 37 °C and 5% CO_2 environment for 24 h. Free DOX·HCl, blank micelles, NCL@DOX and SCL@DOX micelles were prepared in serial dilutions in PB 7.0 buffer solution and then diluted in 5-fold in Opti-MEM medium (Invitrogen). The cells were then rinsed once with PBS and incubated with 100 μl of the sample solutions with different polymer concentrations at 37 °C for 48 h or 72 h. Cells were next rinsed with PBS and the medium was replaced with 100 μl of culture medium. After addition of 20 μl of MTS reagent to each well, cells were then incubated at 37 °C and 5% CO_2 for 3 h. The absorbance of each well was measured at a wavelength of 490 nm on a Tecan Safire2 plate reader (Männerdorf, Switzerland). Cell viability for each treatment condition was determined by normalizing to the cells only signal.

Cell Imaging. HeLa cells were seeded in 6-well plates at a plating density of 1×10^5 cells per well in 1 mL of complete growth medium and incubated in 5% CO_2, 37 ^oC environment for 24 h. The DOX concentration for free DOX•HCl, DOX-loaded P_2-based SCL micelles and DOX-loaded P_4-based SCL micelles in MEM was set at 20 μg/mL and were later added to the wells and incubated at 37 °C for 1 h. After incubation, cells were rinsed with PBS and fixed with 4% paraformaldehyde (PFA) solution at room temperature for 20 min. Finally, cells were counterstained with 2-(4-amidinophenyl)-6-indolecarbamidine(DAPI). Coverslips were mounted onto glass slides and imaged using Leica DM4000 biological microscope.

Evaluation of Cellular Uptake by Flow Cytometry (FCM) Analysis. HeLa cells were seeded in 24-well plates at a density of 50 000 cells per well in 1.0 mL of complete growth medium

and incubated for 24 h at 37 °C in 5% CO_2 environment. Then, fresh MEM containing free DOX, was added to replace the original medium, and the cells without drug treatment were set as a control. The DOX concentration for free DOX·HCl, DOX loaded NCL micelles and DOX loaded SCL micelles in MEM was set at 20 μg/mL. After incubation for 1 h, the polymer solutions were aspirated, and the cells were rinsed twice with PBS. Cells were then harvested by incubation with 200μL of Trypsin-EDTA, followed by resuspension with 1 mL of complete growth medium. Cells were transferred to 1.5 mL microcentrifuge tubes and pelleted at 300g for 5 min at 4 °C. The supernatant was aspirated, and the cell pellets were resuspended in 200 μL of PBS. Cells were analyzed for uptake of fluorescent polymer using a BD Accuri C6 Plus flow cytometer (BD Biosciences) with an excitation wavelength and emission wavelength of 488 nm and 595 nm. A minimum of 10 000 cells was analyzed each sample with the fluorescence intensity.

2. Characterization on polymer structure

PCL-SS-CPADB was first synthesized of by ring-opening polymerization of ε-CL. The typical ^{1}H NMR spectra of PCL-SS-CPADB are presented in **Fig**. **S3a.** The DP of CL was determined to be ~25 ($2I_{11}/(I_7+I_{10})$) by comparing the integrated ratio of two protons (peak 11) in CL units and four methylene protons (signal 7 & 10) adjacent to the disulfide bond in the double-head agent.

To incorporate the functional DESSPMA block, PCL_{25}-SS-CPADB was then used as a macro-chain transfer agent (macro-CTA_1) to carry out RAFT of DESSPMA. The DP of DESSPMA was determined by ^{1}H NMR (**Fig**. **S3b**). By comparing the integral ratio of NMR resonances assigned to DESSPMA block at 3.75 and 1.19 ppm (two characteristic peaks of

ethyl adjacent to the Si group, signal 14 and 15) and PCL block at 2.29 ppm (signal 16), the DP of DESSPMA block was determined to be ~15 ((DP of PCL)×I_{14}/(2I_{16})). The resulting block copolymer was thus denoted PCL_{25}-SS-$PDESSPMA_{15}$-CPADB. Similarly, the DP was determined to be 7 for the other PCL_{25}-*b*-PTESSPMA-CPADB homopolymer.

To further chain extension of hydrophilic OEGMA300 segments, PCL_{25}-SS-$PDESSPMA_{15}$-CPADB was then used as a macro-chain transfer agent (macro-$CTA_{2\text{-}1}$) to carry out RAFT polymerization of OEGMA. As shown in **Fig**. **S3c** the DP of OEGMA was determined by comparing the integral ratio of NMR resonances assigned to OEGMA block at 3.4 ppm (signal 8) with that of PCL block at 2.29 ppm (signal 19), the DP of OEGMA block was determined to be ~74 ((DP of PCL)×2I_8/3I_{19}).

Another amphiphilic triblock copolymer of PCL_{25}-SS-PTESSPMA-*b*-POEGMA was analyzed using the similar method (**Fig**. **S4**).

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Figure S1-S21

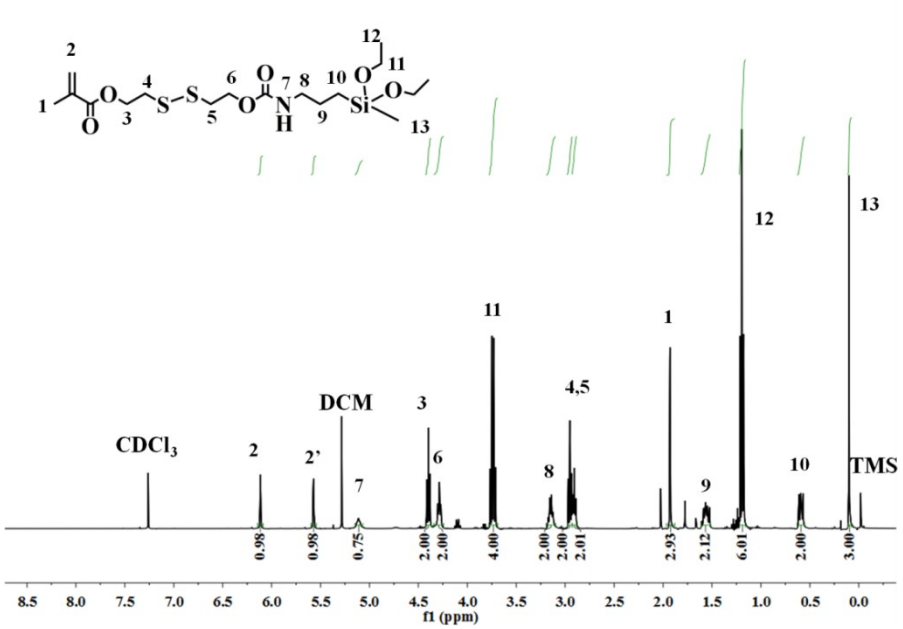

Figure S1. ^{1}H NMR spectrum of DESSPMA in $CDCl_3$.

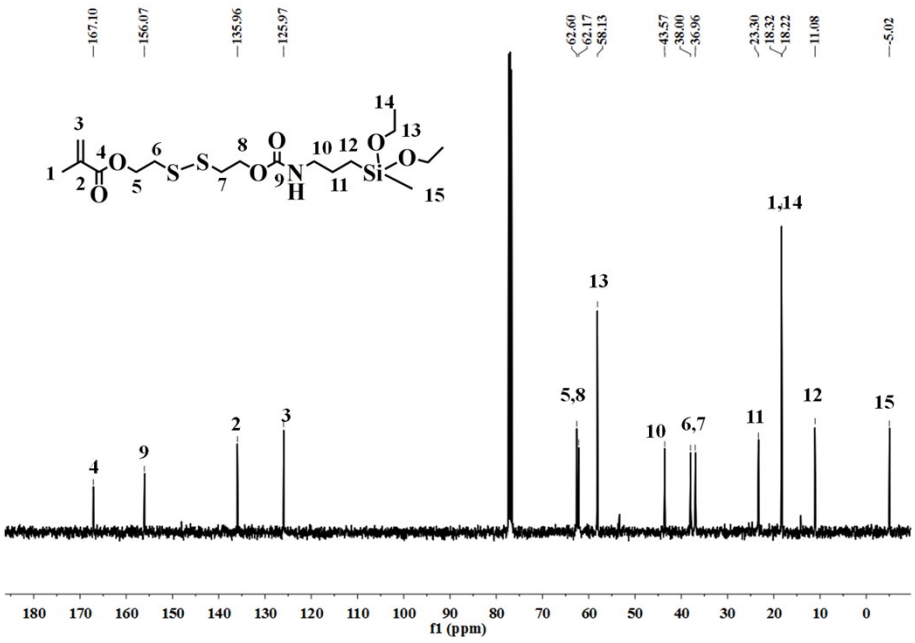

Figure S2. ^{13}C NMR spectrum of DESSPMA in $CDCl_3$.

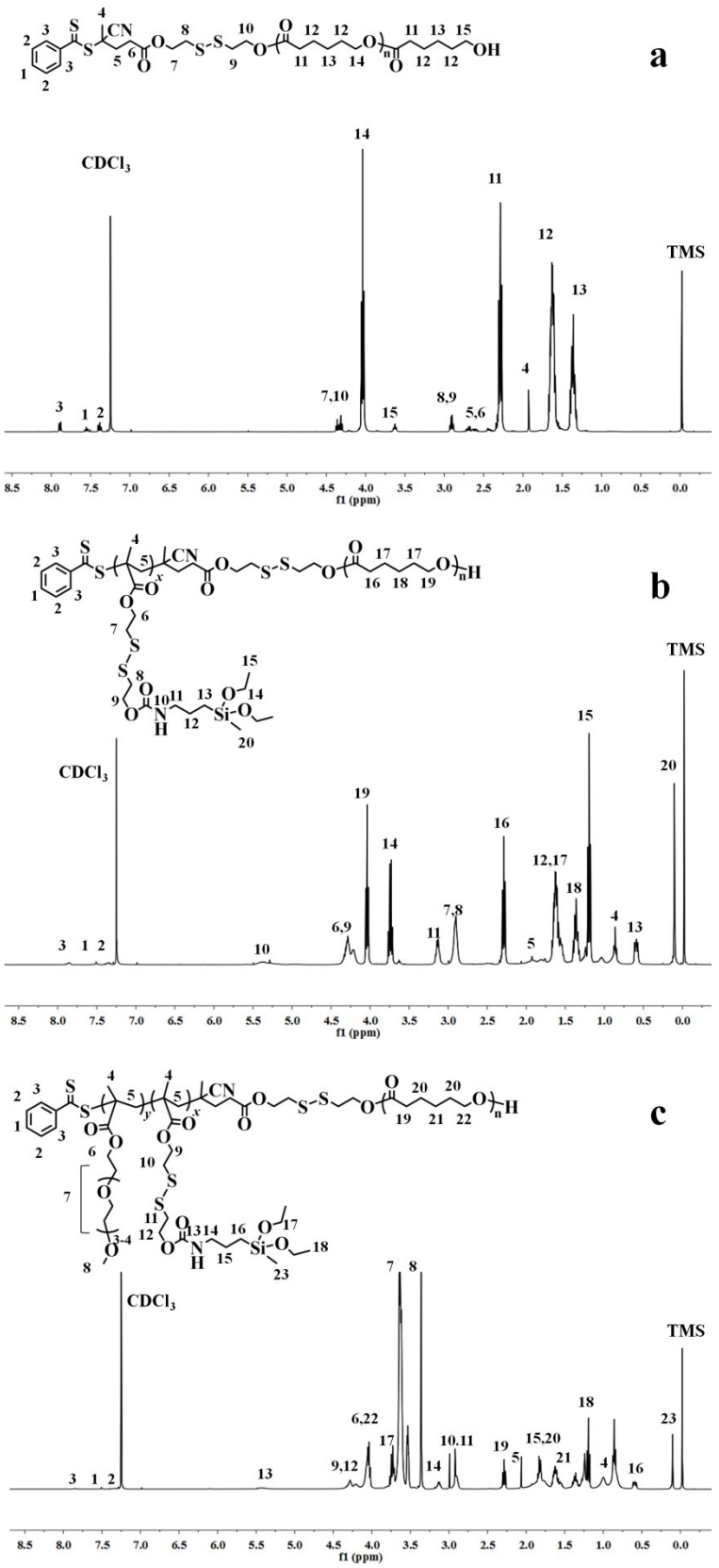

Figure S3. ^{1}H NMR spectra of (a) PCL_{25}-SS-CPADB, (b) PCL_{25}-SS-PDESSPMA-CPADB, (c) PCL_{25}-SS-PDESSPMA-*b*-POEGMA in $CDCl_3$.

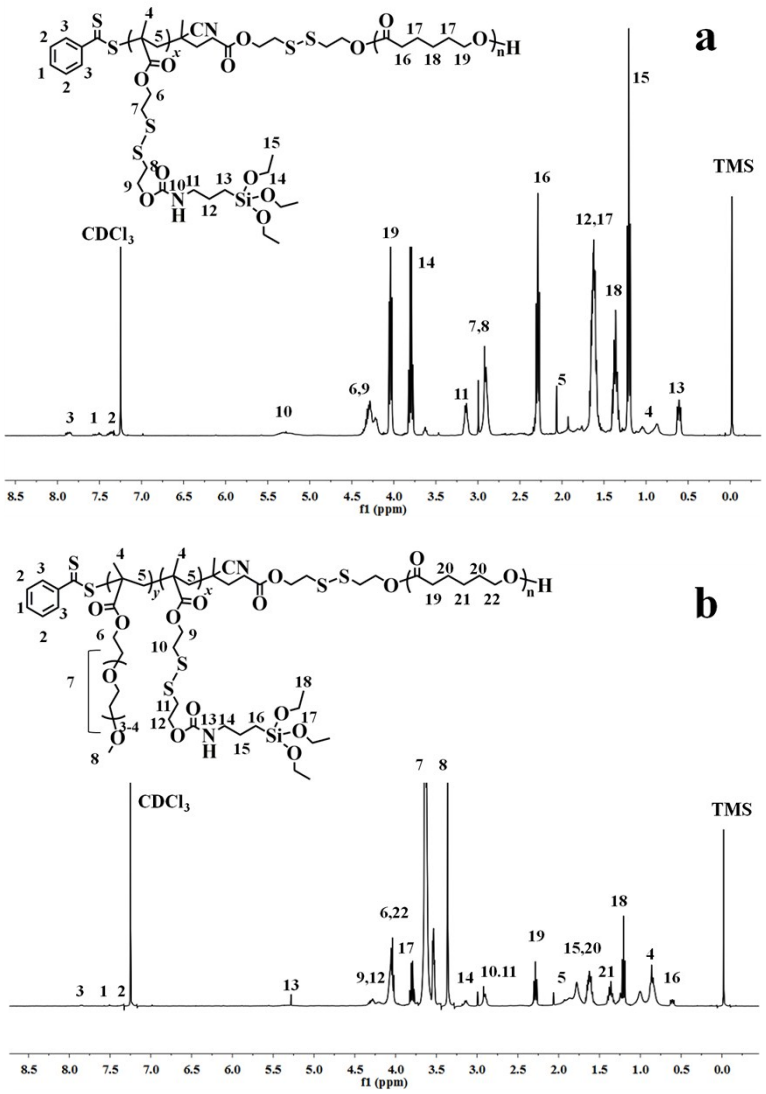

Figure S4. ^{1}H NMR spectra of (a) PCL_{25}-SS-PTESSPMA-CPADB and (b) PCL_{25}-SS-PTESSPMA-*b*-POEGMA in $CDCl_3$.

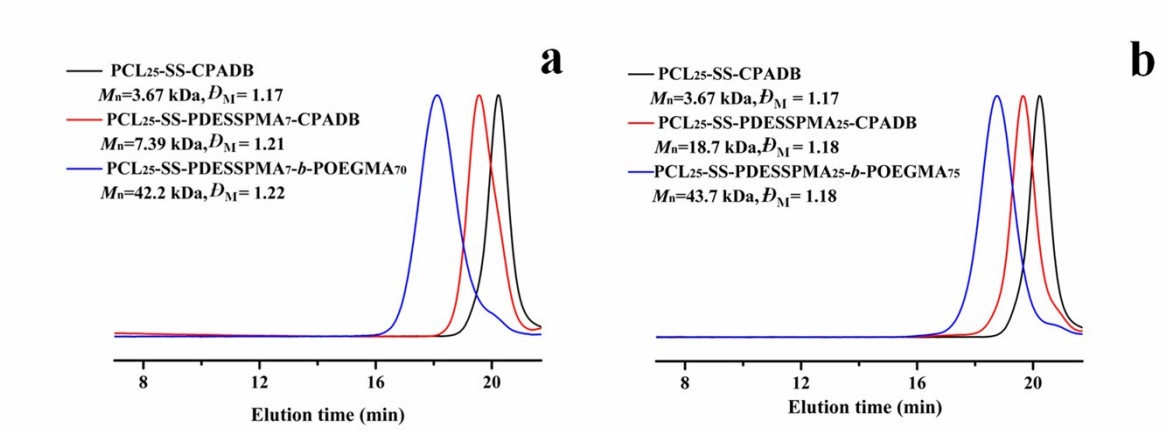

Figure S5. SEC elution traces of (a) PCL_{25}-SS-CPADB, PCL_{25}-SS-$PDESSPMA_7$-CPADB, PCL_{25}-SS-$PDESSPMA_7$-*b*-$POEGMA_{70}$, (b) PCL_{25}-SS-CPADB, PCL_{25}-SS-$PDESSPMA_{25}$-CPADB, PCL_{25}-SS-$PDESSPMA_{25}$-*b*-$POEGMA_{75}$.

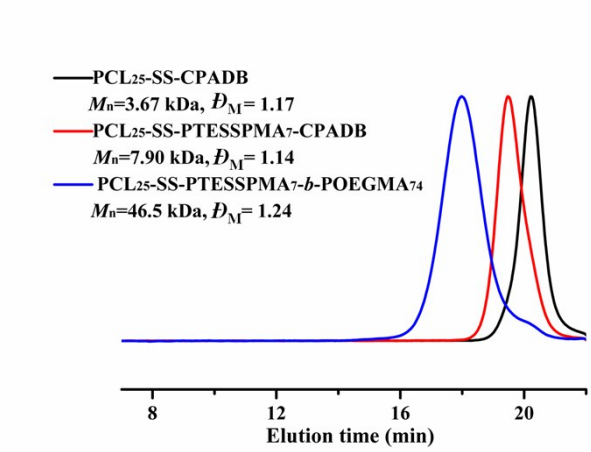

Figure S6. SEC elution traces of PCL_{25}-SS-CPADB, PCL_{25}-SS-$PTESSPMA_7$-CPADB, PCL_{25}-SS-$PTESSPMA_7$-*b*-$POEGMA_{74}$.

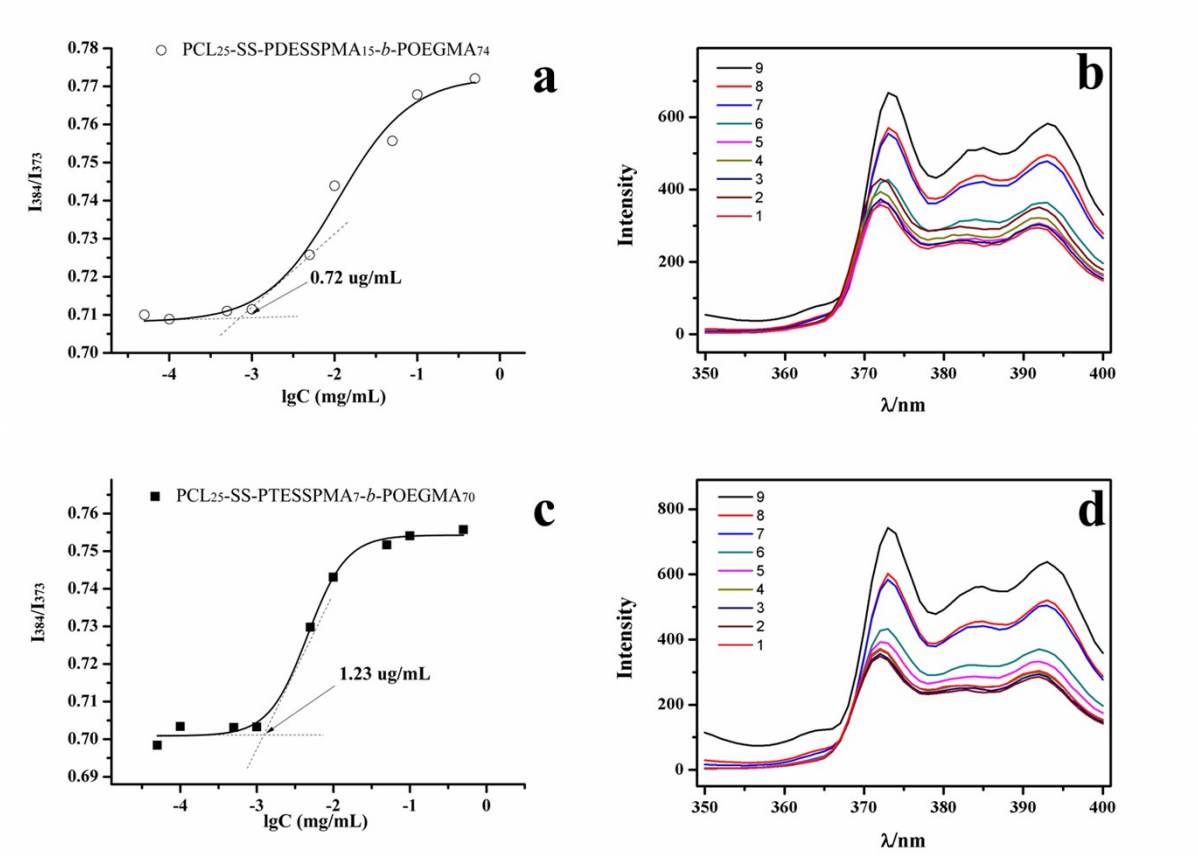

Figure S7. Plots of the ratio (I_{384}/I_{373}) of the fluorescence intensities as a function of logarithm of the polymer concentration and fluorescence spectra of P_2 (a & b) and P_4 (c & d).

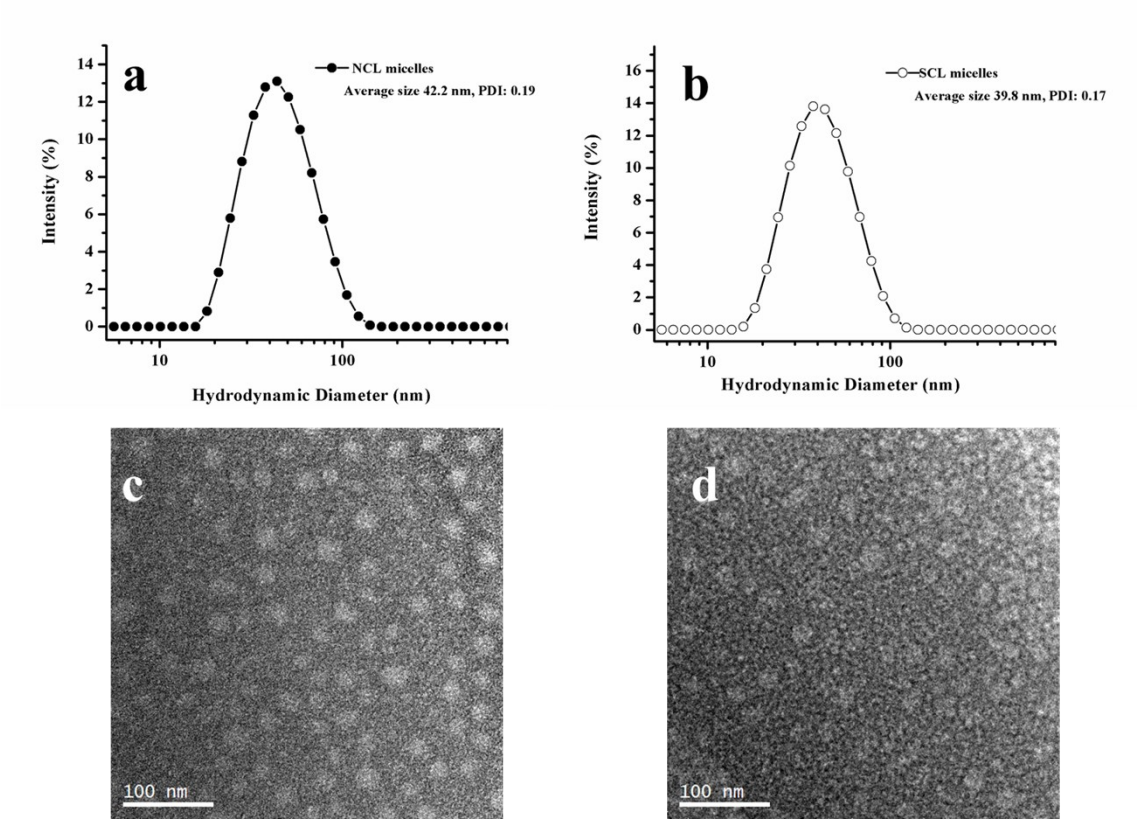

Figure S8. Size distributions and TEM images of (a & c) NCL and (b & d) SCL blank micelles of PCL_{25}-SS-$PDESSPMA_{25}$-*b*-$POEGMA_{75}$ in an aqueous phase.

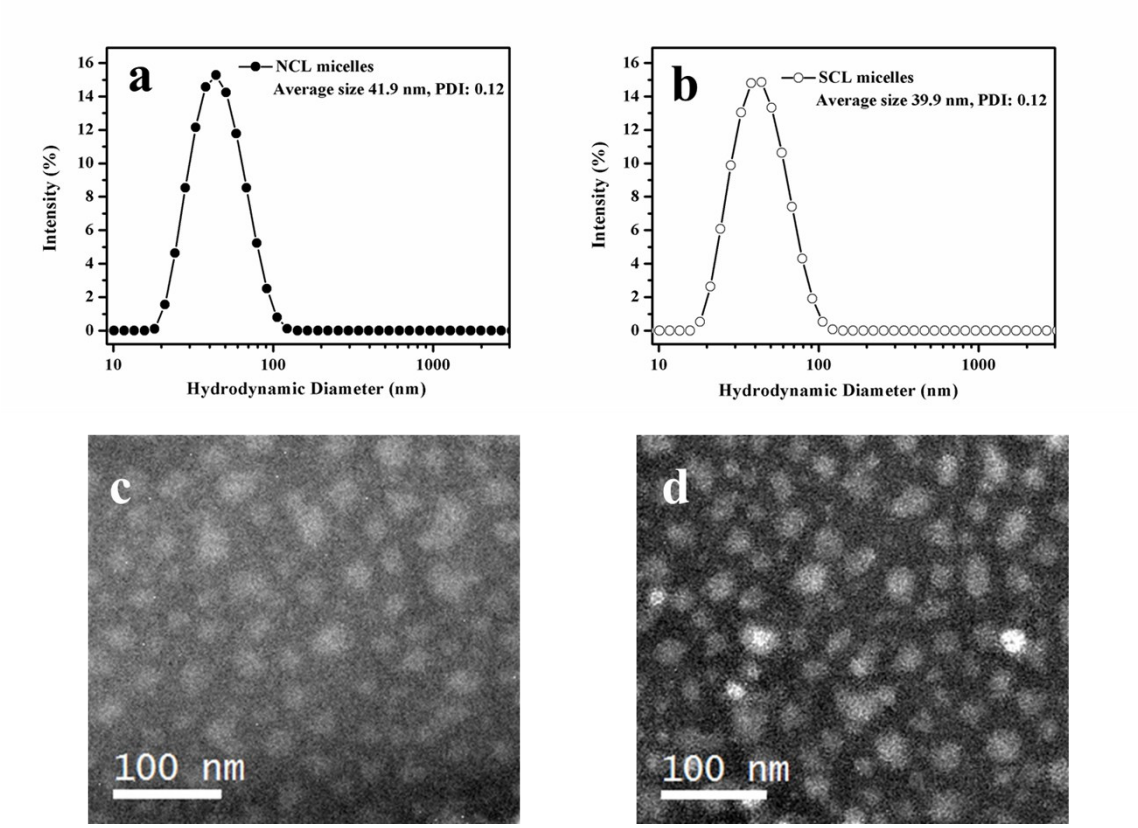

Figure S9. Size distributions and TEM images of (a & c) NCL and (b & d) SCL blank micelles of PCL_{25}-SS-$PTESSPMA_{7}$-*b*-$POEGMA_{74}$ in an aqueous phase.

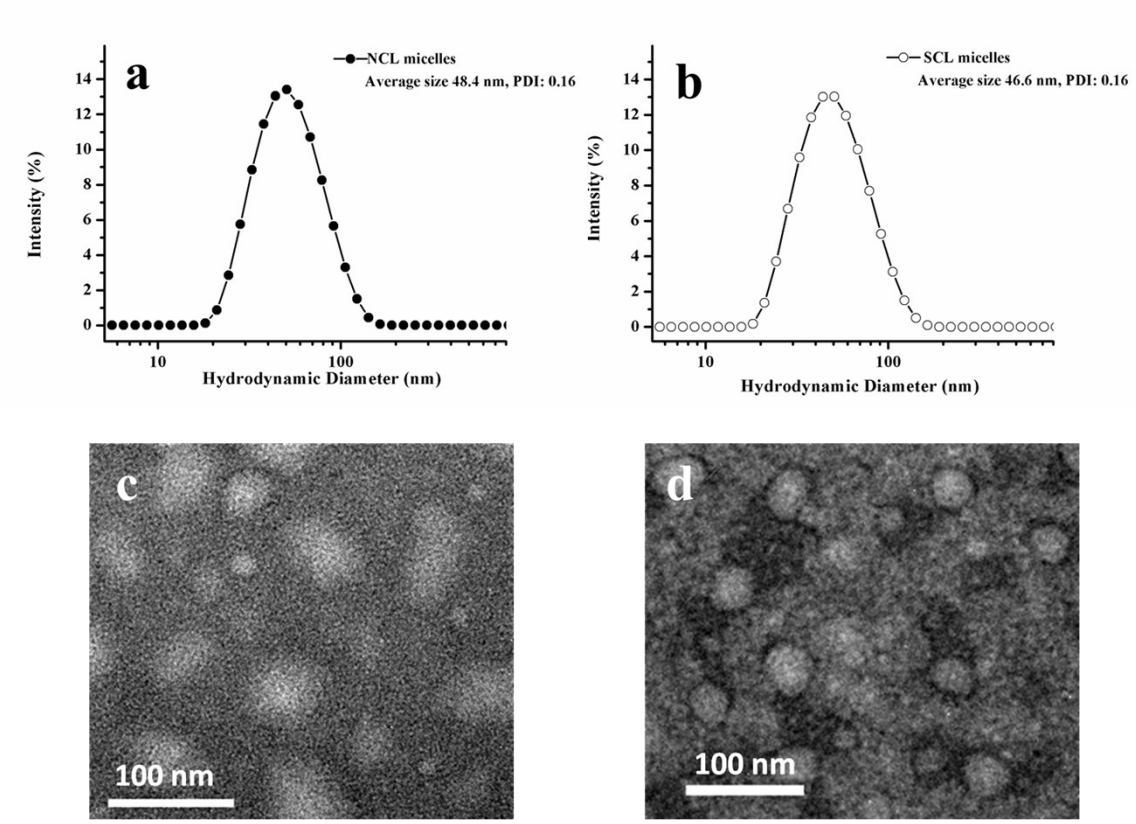

Figure S10. Size distributions and TEM images of (a & c) NCL and (b & d) SCL blank micelles of PCL_{25}-SS-$PTESSPMA_{14}$-*b*-$POEGMA_{77}$ in an aqueous phase.

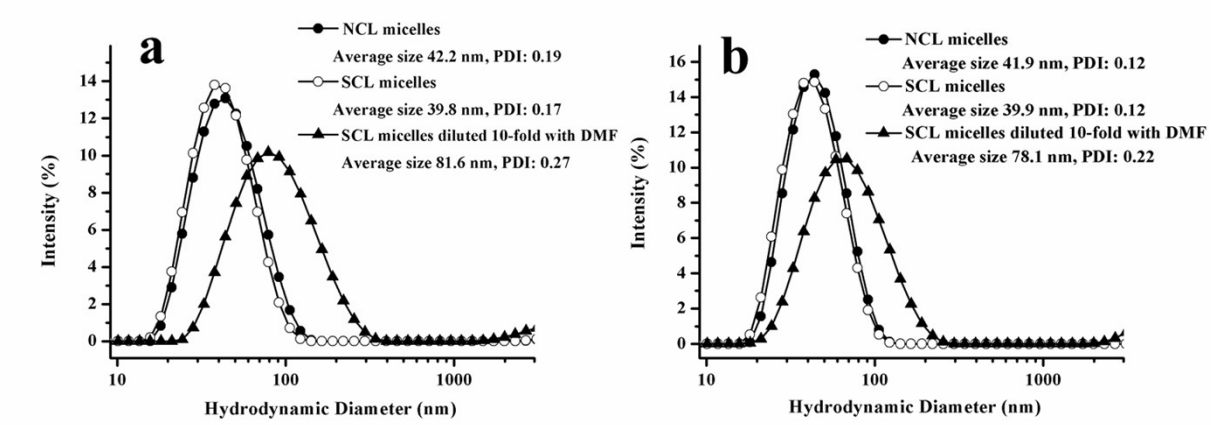

Figure S11. Size distributions of (a) $\mathbf{P_3}$ and (b) $\mathbf{P_4}$-based (●) NCL and (○) SCL micelles in water and (▲) SCL micelles diluted 10-fold with DMF.

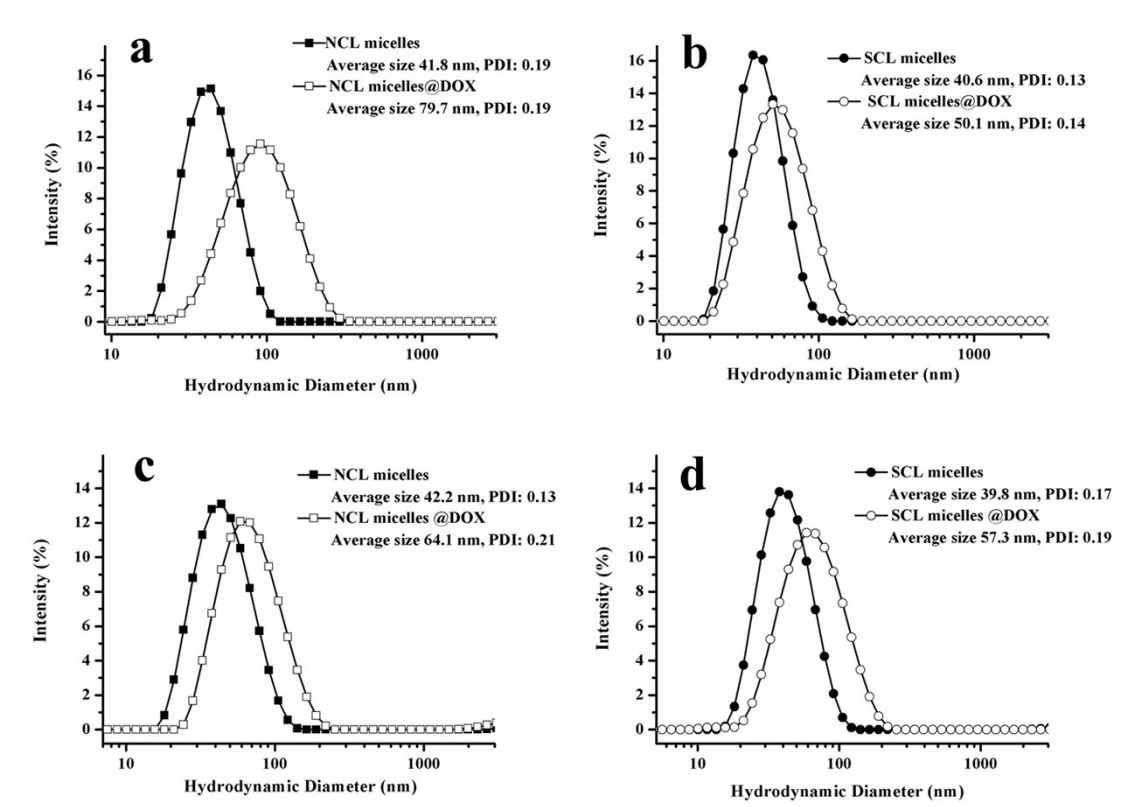

Figure S12. Size distributions of $\mathbf{P_2}$ (a& b) and $\mathbf{P_3}$ (c& d) based micelles in water: (■) NCL (□) NCL@DOX (●) SCL and (○) SCL@DOX.

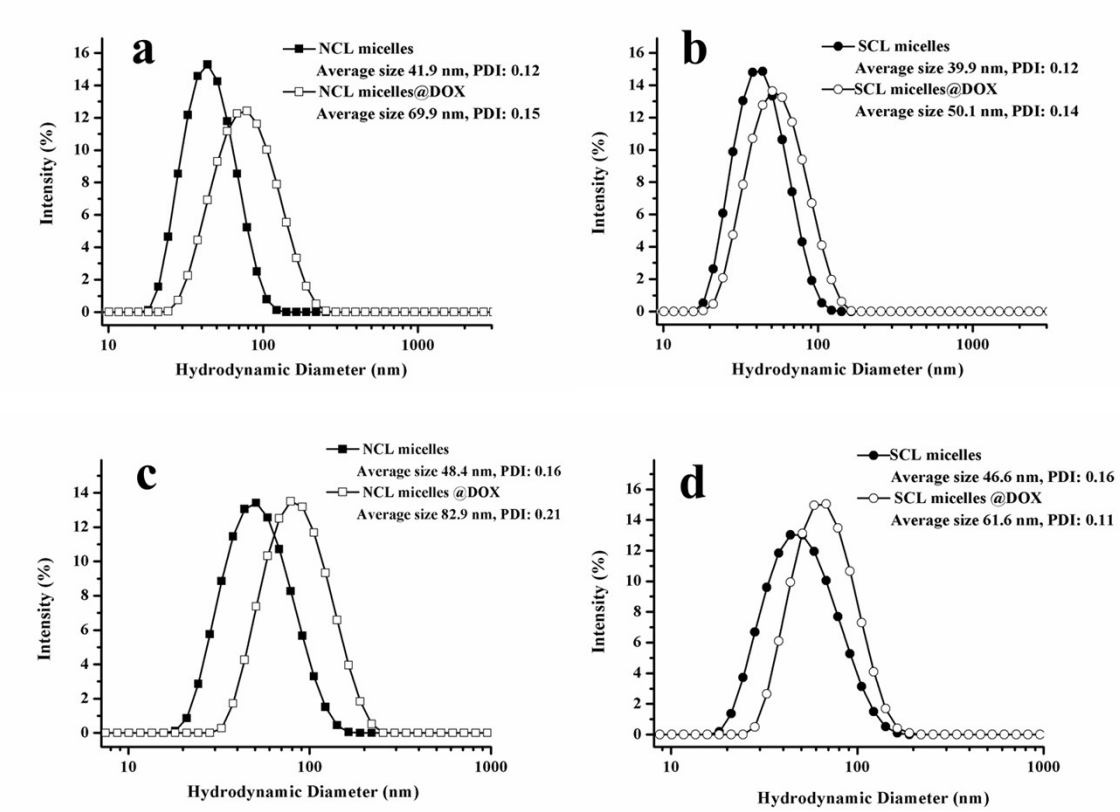

Figure S13. Size distributions of $\mathbf{P_4}$ (a& b) and $\mathbf{P_5}$ (c& d) based micelles in water: (■) NCL (□) NCL@DOX (●) SCL and (○) SCL@DOX.

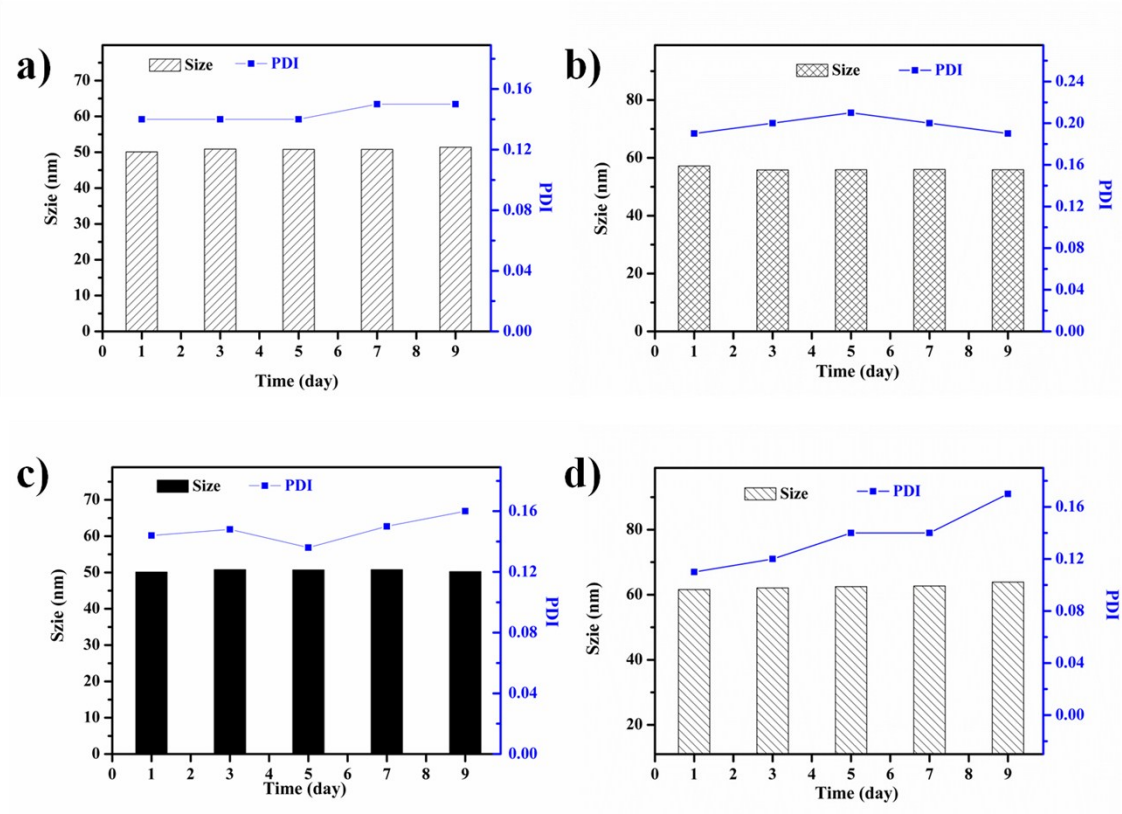

Figure S14. Size distribution of $\mathbf{P_2}$ (a), $\mathbf{P_3}$ (b), $\mathbf{P_4}$ (c) and $\mathbf{P_5}$ (d) based DOX loaded SCL micelles in PBS (pH 7.4, 150 mM) buffer solution for 9 days.

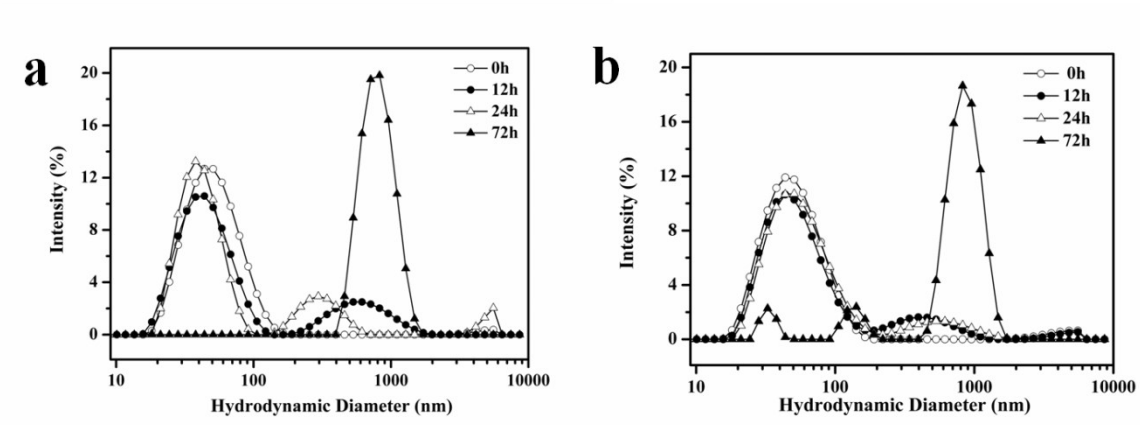

Figure S15. DLS-monitored size changes of P2 (a) and P4 (b) -based SCL micelles incubated with 10 mM GSH for various periods.

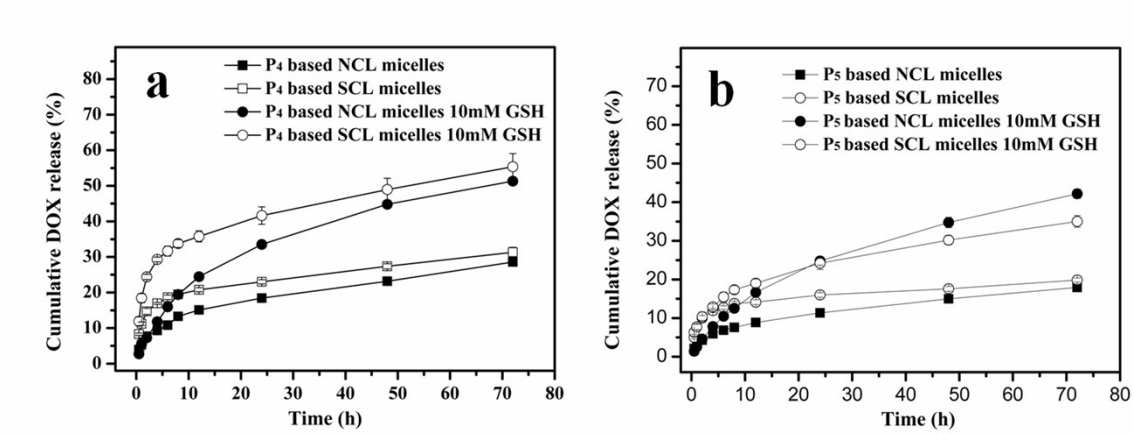

Figure S16. *In vitro* drug release profiles of NCL and SCL micelles in the physiological condition (PBS, pH 7.4, 150 mM) with and without 10 mM GSH at 37 ^{o}C: (a) $\mathbf{P_4}$-based micelles (b) $\mathbf{P_5}$-based micelles. The data were expressed as mean ±SD, n=3.

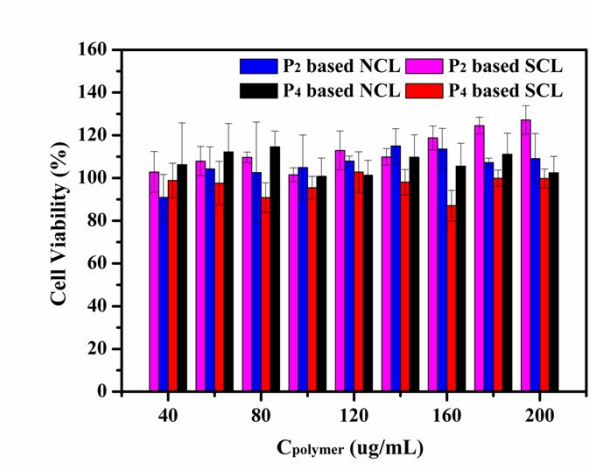

Figure S17. *In vitro* cytotoxicity of $\mathbf{P_2}$ and $\mathbf{P_4}$-based blank micelles in HeLa cells for 48 h incubation. Cell viability was determined by MTS assay and expressed as % viability compared to the untreated cells control. The data were expressed as mean ±SD, n=3.

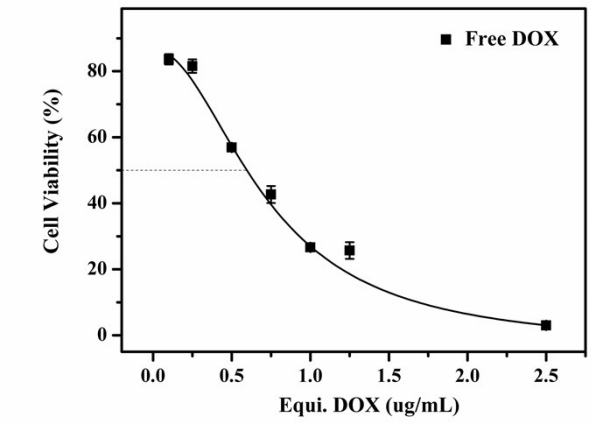

Figure S18. *In vitro* cytotoxicity of free DOX in HeLa cells for 48 h incubation. Cell viability was determined by MTS assay and expressed as % viability compared to control untreated cells. The data were expressed as mean ±SD, n = 3.

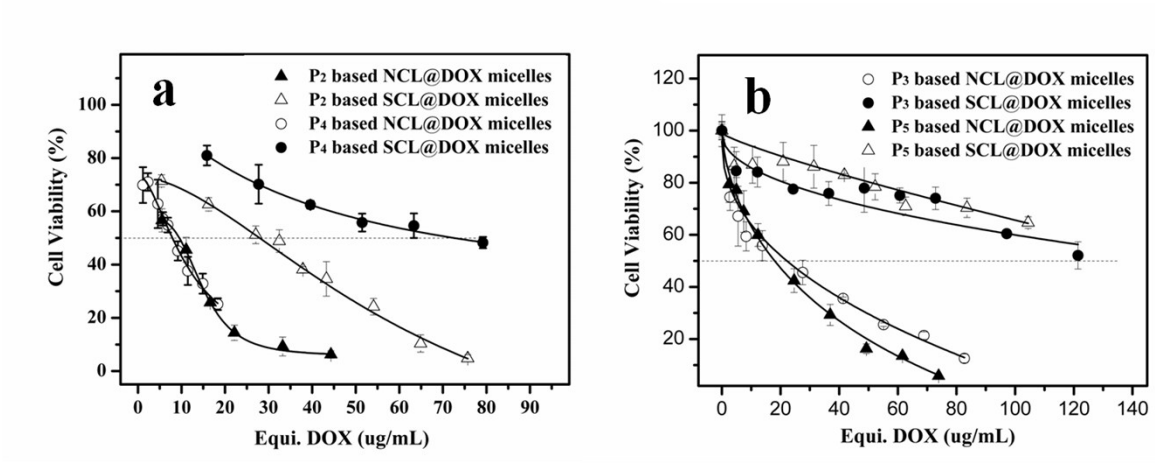

Figure S19. *In vitro* cytotoxicity of (a) $\mathbf{P_2}$ and $\mathbf{P_4}$-based NCL@DOX and SCL@DOX micelles (b) $\mathbf{P_3}$ and $\mathbf{P_5}$-based NCL@DOX and SCL@DOX micelles in HeLa cells for 48 h incubation, Cell viability was determined by MTS assay and expressed as % viability compared to control untreated cells. The data were expressed as mean ±SD, n = 3.

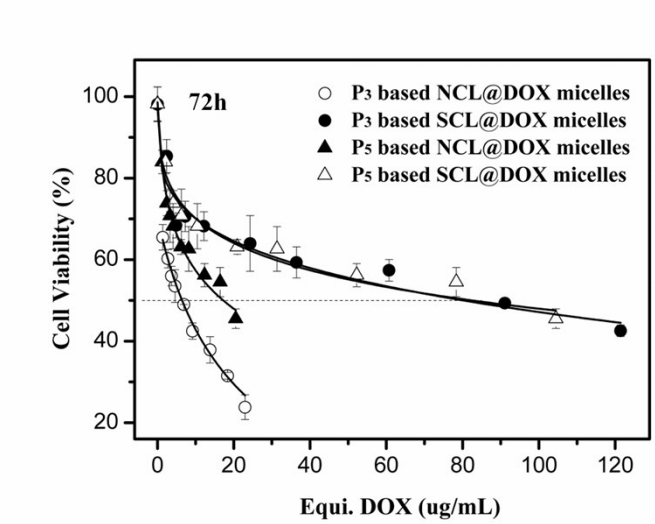

Figure S20. *In vitro* cytotoxicity of $\mathbf{P_3}$ and $\mathbf{P_5}$-based NCL@DOX and SCL@DOX micelles in HeLa cells for 72 h incubation. Cell viability was determined by MTS assay and expressed as % viability compared to control untreated cells. The data were expressed as mean ±SD, n = 3.

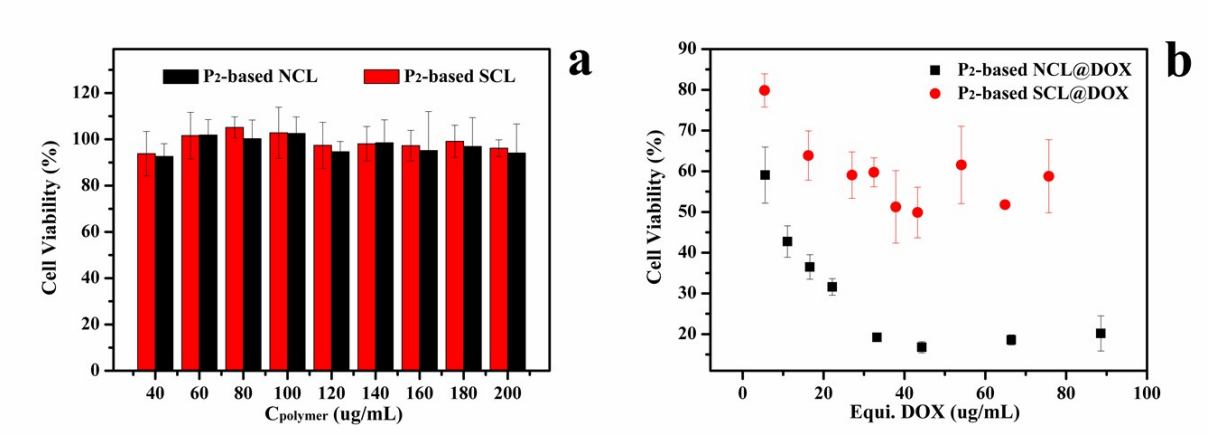

Figure S21. *In vitro* cytotoxicity of (a) $\mathbf{P_2}$ and $\mathbf{P_4}$-based blank micelles, (b) $\mathbf{P_2}$ and $\mathbf{P_4}$-based NCL@DOX and SCL@DOX micelles in MRC-5 cells for an incubation of 48 h, Cell viability was determined by MTS assay and expressed as % viability compared to control untreated cells. The data were expressed as mean ±SD, n = 3.