Supporting Information

Modulation of cyclic topology toward enhanced drug delivery, from linear and tadpole-like to dumbbell-shaped copolymers

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1. Experimental section

Materials

Poly (ethylene glycol) (PEG_{45}, M_n=2000 g/mol) was obtained from Sigma-Aldrich, Epichlorohydrin (99.7%) and sodium azide (NaN_3, 98.0%) were bought from Aladdin and used as received. ε-Caprolactone (ε-CL, J&K, 99%) was dried over CaH_2 and distilled under reduced pressure prior to use. Stannous (II) octanoate ($Sn(Oct)_2$, Sigma-Aldrich, 92.5%-100%), *N*,*N*′-dicyclohexylcarbodiimide (DCC, J&K, 98%), 4-dimethylamino pyridine (DMAP, J&K, 99%), 4-Pentynoic acid (J&K, 95%), *N,N,N′,N″,N″*-pentamethyldiethylenetriamine (PMDETA, Aladdin, 99%) and copper(I) bromide (CuBr, Sigma-Aldrich, >99.999%) were used as received. Triethylamine (TEA) and doxorubicin hydrochloride (DOX·HCl, 98%) were provided by Sigma-Aldrich, and used as received. Toluene and dichloromethane (CH_2Cl_2) were supplied by Tianjin Chemical Reagent Factory (China) and dried by refluxing over sodium and distilled prior to use. All other reagents were of analytical grade and used as received.

Instruments and Measurements

The chemical structures of all polymers were characterized by ^1H NMR spectra, which recorded on a JNM-ECS 400 MHz spectrometer (JEOL, Tokyo, Japan) operated in the Fourier transform mode using deuterated chloroform ($CDCl_3$) and tetramethylsilane (TMS) as solvents and internal reference, respectively. The size exclusion chromatography and multiangle laser light scattering (SEC-MALLS) were used to determine the molecular weight (MW) and the polydispersity index (PDI=M_w/M_n) of prepared polymers. SEC was carried out 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) were connected in series to a Agilent 1260 series (Agilent Technologies), an interferometric refractometer (Optilab-rEX, Wyatt Technology), and a MALLS device (DAWN EOS, Wyatt Technology). The MALLS detector was operated at a laser wavelength of 690.0 nm. The FT-IR spectroscopic measurements were conducted on a NEXUS 670 FT-IR spectrometer (Nicolet, WI, USA) and solid samples were pressed into potassium

bromide (KBr) pellet prior to the measurements.

Synthesis of PCL-*b*-PEG$_{45}$-*b*-PCL (L)

The PCL-*b*-PEG$_{45}$-*b*-PCL (L) was synthesized via Sn(Oct)$_2$ catalyzed ring-opening polymerization of ε-CL using PEG$_{45}$ as a macroinitiator. Briefly, to a 25 mL Schlenk tube were added PEG$_{45}$ (0.4 g, 0.2 mmol), ε-CL (1.0273 g, 9 mmol), Sn(Oct)$_2$ (0.0073 g, 0.018 mmol) and toluene (0.18 mL). Then, the reaction mixture was degassed by three freeze-pump-thaw cycles and immersed in an oil bath preheated to 140 °C to start the polymerization. After 24 h, the mixture was precipitated into 10-fold ice-cold diethyl ether to remove any unreacted monomers. The white precipitate was collected and dried under vacuum.

^{1}H NMR (Figure S1, 400 MHz, $CDCl_3$): δ_{ppm}, 3.65 (m, -OC**H$_2$**C**H$_2$**O-), 2.31 (t, O=CC**H$_2$**CH$_2$CH$_2$CH$_2$CH$_2$O-), 1.65 (m, O=CCH$_2$C**H$_2$**CH$_2$C**H$_2$**CH$_2$O-), 1.38 (m, O=CCH$_2$CH$_2$C**H$_2$**CH$_2$CH$_2$O-), 4.06 (t, O=CCH$_2$CH$_2$CH$_2$CH$_2$C**H$_2$**O-).

Synthesis of Alkynyl-PCL(-OH)

Alkynyl-PCL(-OH) was prepared by ROP as previously reported[3]. Briefly, ε-CL (1.83 g, 16 mmol), propargyl alcohol (0.11 g, 2 mmol) acted as initiator and Sn(Oct)$_2$ (0.015 g, 0.04 mmol) served as catalyst were dissolved in 1.6 mL dried toluene and subsequently charged in a 20 mL Schlenk flask with a magnetic stirring bar. The reaction mixture was degassed through three pump-freeze-thaw cycles and then immersed in a thermo-stated oil bath at 120 ℃ for 5 h. The solution was precipitated in an excessive amount of cold methanol, and white solid product was obtained (yield 85%).

^{1}H NMR (Figure S3, 400 MHz, $CDCl_3$): δ_{ppm}, 1.38 (m, O=CCH$_2$CH$_2$C**H$_2$**CH$_2$CH$_2$O-), 1.65 (m, O=CCH$_2$C**H$_2$**CH$_2$C**H$_2$**CH$_2$O-), 2.31 (t, O=CC**H$_2$**CH$_2$CH$_2$CH$_2$CH$_2$O-), 2.48 (t, -C**H**), 4.06 (t, O=CCH$_2$CH$_2$CH$_2$CH$_2$C**H$_2$**O-), 4.68 (d, CHC**H$_2$**-)

Synthesis of 2,2-bis(2′-bromo-2′-methylpropionyloxymethyl)propionic acid (Bis(iBB)MPA)

The bis(hydroxymethyl) propionic acid (bis-MPA, 5 g, 37.28 mmol) and triethylamine (TEA, 9.43 g, 93.19 mmol) was dissolved in 50 mL of dried CH_2Cl_2. Next, 2-bromoisobutyryl bromide (20.57 g, 89.47 mmol) was added dropwise when the above solution cooled to 0 ℃. The reaction was continued for 12 h at room temperature, then water was added to quench the reaction and the reaction mixture was extracted with water for three times. The organic phase was dried over $MgSO_4$, and the solvent was evaporated. Finally, the white solid product was obtained by column chromatography using ethyl acetate/hexanes/1% formic acid mixtures as eluent (yield 53%).

^{1}H NMR (Figure S4, 400 MHz, $CDCl_3$): δ_{ppm}, 1.38 (s, 3H, -C**H$_3$**), 1.92 (s, 12H, -C(Br)-C**H$_3$**), 4.43-4.34 (q, 4H, -C**H$_2$**-).

Synthesis of Alkynyl-PCL(-2Br)

Alkynyl-PCL(-OH) (0.6 g, 0.26 mmol), DCC (0.25 g, 1.21 mmol) and DMAP (0.18 g, 1.51 mmol) were dissolved in 6 mL of dried toluene. After the solution cooled to 0 ℃, the Bis(iBB)MPA (0.39 g, 0.90 mmol) was added, and the reaction solution was stirred for 48 h at ordinary temperature. The insoluble solids *N*, *N*′-dicyclohexylurea (DCU) were removed by filtration, and then the organic solution was precipitated into cold methanol to obtain the target product (yield 83%).

^{1}H NMR (Figure S5, 400 MHz, $CDCl_3$): δ_{ppm}, 1.33 (s, -CC**H$_3$**), 1.39 (m, O=CCH$_2$CH$_2$C**H$_2$**CH$_2$CH$_2$O-), 1.65 (m, O=CCH$_2$C**H$_2$**CH$_2$C**H$_2$**CH$_2$O-), 1.91 (s, -C(Br)-C**H$_3$**), 2.31 (t, O=CC**H$_2$**CH$_2$CH$_2$CH$_2$CH$_2$O-), 2.48 (t, -C**H**), 4.06 (t, O=CCH$_2$CH$_2$CH$_2$CH$_2$C**H$_2$**O-), 4.42-4.31 (q, -CC**H$_2$**-, 4.68 (d, CHC**H$_2$**-).

Synthesis of Alkynyl-PCL(-2N$_3$)

First, Alkynyl-PCL(-2Br) (1 g, 0.3 mmol) was dissolved in DMF. Next, NaN_3 (0.42 g, 6.46 mmol) was added in the solution and the flask was placed in an oil bath thermo-stated at 50 ℃ for 48 h to react. The insoluble salt was eliminated by filtration and the target polymer was collected by precipitation of mixture from DMF to a large amount of glacial methanol (yield 89%).

^{1}H NMR (Figure S6, 400 MHz, $CDCl_3$): δ_{ppm}, 1.32 (s, -CC**H$_3$**), 1.39 (m, O=CCH$_2$CH$_2$C**H$_2$**CH$_2$CH$_2$O-), 1.46 (s, -C(N$_3$)-C**H$_3$**), 1.65 (m, O=CCH$_2$C**H$_2$**CH$_2$C**H$_2$**CH$_2$O-), 2.31 (t, O=CC**H$_2$**CH$_2$CH$_2$CH$_2$CH$_2$O-), 2.48 (t, -C**H**), 4.06 (t, O=CCH$_2$CH$_2$CH$_2$CH$_2$C**H$_2$**O-), 4.41-4.31 (q, -CC**H$_2$**-), 4.68 (d, CHC**H$_2$**-).

Synthesis of *c*PCL-N$_3$

The *c*PCL-N$_3$ was successfully synthesized by intrachain click cyclization of linear precursor as previously we reported[4]. Typically, 600 mL of DMF was charged in a 1000 mL three-neck flask and degassed by bubbling dry nitrogen gas for 1 h, and then 20-fold molar equivalents of PMDETA (0.76 mmol) and CuBr (0.76 mmol) were added into the flask under the protection of nitrogen flow. A solution of Alkynyl-PCL(-2N$_3$) linear precursor (0.1 g, 0.038 mmol) in degassed DMF (10 mL) was added to the copper catalyst solution via a syringe pump at the rate of 0.3 mL/h. The reaction was carried out at 100 °C in a nitrogen atmosphere for another 24 h after the polymer solution added completely. The DMF was removed under reduced pressure, and the concentrated residue was re-dissolved directly with CH_2Cl_2 and extracted with saturated EDTA solution to eliminate any excess copper. Organic phase was dried over anhydrous Mg_2SO_4, and the solution was concentrated and precipitated into cold methanol. The resulting polymer, *c*PCL-N$_3$, was harvested after dried under vacuum (yield 55%).

^{1}H NMR (Figure S7, 400 MHz, $CDCl_3$): δ_{ppm}, 1.10 (s, -CC**H$_3$**), 1.19 (s, -C(N$_3$)-C**H$_3$**), 1.32 (m, O=CCH$_2$CH$_2$C**H$_2$**CH$_2$CH$_2$O-), 1.58 (m, O=CCH$_2$C**H$_2$**CH$_2$C**H$_2$**CH$_2$O-), 1.88 (s, -C(N-CH=C)-C**H$_3$**), 2.25 (t, O=CC**H$_2$**CH$_2$CH$_2$CH$_2$CH$_2$O-), 4.00 (t, O=CCH$_2$CH$_2$CH$_2$CH$_2$C**H$_2$**O-), 4.34-4.08 (m, -CC**H$_2$**O-), 5.15 (d, CH=C-CC**H$_2$**O-), 7.69 (s, -NC**H**C-).

Synthesis of Alkynyl-PEG$_{45}$-Alkynyl

The terminal hydroxyl group of PEG$_{45}$ was absolutely converted to two alkynyl function by an esterification reaction. Simply, PEG$_{45}$ (1 g, 0.5 mmol), DCC (0.46 g, 2.25 mmol) and DMAP (0.27 g, 2.25 mmol) were dissolved with dried toluene and

charged in a round-bottom flask. 4-Pentynoic acid (0.17 g, 1.75 mmol) was added after the above solution cooled to 0 ℃, and the reaction mixture was stirred at room temperature for 48 h. After the reaction, the insoluble *N*, *N*′-dicyclohexylurea (DCU) solids were removed by filtration, and then the organic solution was precipitated into cold diethyl ether to obtain the final product (yield 80%).

^{1}H NMR (Figure S8, 400 MHz, $CDCl_3$): δ_{ppm}, 1.98 (C**H**-), 2.63-2.46 (m, CHCC**H$_2$**C**H$_2$**O-), 3.64 (m, -OC**H$_2$**C**H$_2$**O-), 4.26 (t, -OC**H$_2$**).

Synthesis of dumbbell-shaped *c*PCL-*b*-PEG$_{45}$-*b*-*c*PCL copolymer

The dumbbell-shaped *c*PCL-*b*-PEG$_{45}$-*b*-*c*PCL copolymer was prepared by click coupling. Briefly, *c*PCL-N_3 (0.055 g, 0.019 mmol) and Alkynyl-PEG$_{45}$-Alkynyl (0.02 g, 0.009 mmol) were dissolved with 1 mL DMF and subsequently charged in a 10 mL Schlenk flask equipped with a magnetic stirring bar. The reaction mixture was degassed through three pump-freeze-thaw cycles to remove any trace of oxygen in the system. Subsequently, PMDETA (0.016 g, 0.093 mmol) and CuBr (0.013 g, 0.093 mmol) catalyst were added quickly under a nitrogen flow. After another three freeze-pump-thaw cycles, the Schlenk flask was sealed and placed in a thermo-stated oil bath at 60 ℃ to start the polymerization. After 48 h, the reaction mixture was diluted with DMF, and transferred directly to a dialysis tube (MWCO: 10 kDa) and dialyzed against distilled water to remove the copper catalyst and any unreacted alkynyl-terminated PEG. The resulting copolymer, *c*PCL-*b*-PEG$_{45}$-*b*-*c*PCL, was obtained by freeze-drying (yield 48%).

^{1}H NMR (Figure S9, 400 MHz, $CDCl_3$): δ_{ppm}, 1.25 (s, -CC**H$_3$**), 1.38 (m, O=CCH$_2$CH$_2$C**H$_2$**CH$_2$CH$_2$O-), 1.65 (m, O=CCH$_2$C**H$_2$**CH$_2$C**H$_2$**CH$_2$O-), 1.91 (m, -C(N-CH=C)-C**H$_3$**), 2.31 (t, O=CC**H$_2$**CH$_2$CH$_2$CH$_2$CH$_2$O-), 2.77 (t, O=C-C**H$_2$**CH$_2$-), 3.04 (t, O=C-CH$_2$C**H$_2$**-), 3.64 (m, -OC**H$_2$**C**H$_2$**O-), 4.06 (t, O=CCH$_2$CH$_2$CH$_2$CH$_2$C**H$_2$**O-), 4.24 (t, -OC**H$_2$**C-), 5.22 (s, -CC**H$_2$**O-), 7.79 (s, -NC**H**C-).

Preparation and characterization of the self-assembled micelles

Micelles were prepared by dialysis method: 1.0 mg of copolymers was completely dissolved in 2.0 mL of DMF, the DMF solution of copolymers was then dialyzed against deionized water to eliminate the DMF using a dialysis bag with a molecular weight cutoff (MWCO) of 10000. Ultimately, micelle solution with a concentration of approximately 0.2 mg/mL was obtained after 24 h.

The average hydrodynamic diameter and polydispersity index of micelles were determined by dynamic light scattering (DLS) measurements using a Zeta sizer (Nano ZS, Malvern, Worcestershire, UK) at a fixed detection angle of 173°. Note that all measurements were conducted at 25 °C, and the data were gained from the average of three tests. The sample solution was passed through a Millipore 0.45 μm pore-sized syringe filter prior to measurements. The concentration of micelle solution used for the test was 0.2 mg/mL.

Critical micelle concentration (CMC) was measured using pyrene as a fluorescence probe and fluorescence spectra were recorded on a LS55 luminescence spectrometer (Perkin-Elmer). Firstly, 0.08 mL of pyrene solution (3×10^{-6} M in acetone) was added to containers, and the acetone was allowed to evaporate. Then 4 mL of polymer aqueous solution at different concentrations were added to the containers containing the pyrene residue and the combined solution of pyrene and copolymers was equilibrated at room temperature in dark for 24 h prior to measurements. The final concentration of pyrene was 6×10^{-8} M in water. Excitation was carried out at 340 nm, and emission spectra were recorded ranging from 350 to 600 nm. Excitation and emission bandwidths were set as 5 nm and 2.5 nm, respectively. From the pyrene emission spectra, the intensities (peak height) of I_{393nm} were recorded, and the CMC value was determined from the intersection of the tangent to the curve at the inflection with the horizontal tangent to the curve through the points at low concentration[5].

Transmission electron microscopy (TEM, with a TECNAI G^2 20 instrument operating at an acceleration voltage of 200 keV) were used to observe the morphology of micelles. TEM samples were prepared by adding one droplet (4 μL) of micelle solution onto a carbon-coated copper grid, and leaving it for 25 min before drawing off the excess solution. The phosphotungstic acid (1 % w/w) was used as negative

staining and stained the sample for 5 min. Followed, the sample was dried in air.

In vitro drug loading and drug release

In vitro drug loading and drug release study was performed according to our reported procedures[6]. Generally, free DOX was obtained from DOX·HCl. Concretely, DOX·HCl (2 mg) and TEA (96 μL) were dissolved in 4 mL of DMF and stirred overnight in dark at room temperature to remove hydrochloride. Next, the copolymers (20 mg) pre-dissolved in 2 mL of DMF was added to the above DMF solution of DOX and stirred at room temperature for 1 h. The mixture was later added dropwise into 6 ml of ultra-purified water under vigorous stirring. After stirring for another 1 h, the solution was placed in a dialysis bag (MWCO: 3.5 kDa) and dialyzed against 5 L of distilled water for 24 h, which was renewed every 3 h at initial 12 h to remove TEA, DMF and unloaded free DOX. Finally, the drug-loaded micelles were harvested by freeze-drying. The drug loading content (DLC) and entrapment efficiency (EE) were determined by measuring the concentration of DOX in drug-loaded micelle which dissolved in DMF, and the concentration was decided by testing the absorbance at 485 nm using a Lambda 35 UV-Vis spectrometer (Perkin-Elmer). The DLC and EE were calculated using the following equations,

$$\text{DLC (\%)} = W_{\text{drug loaded in particles}} / W_{\text{particles}} \times 100\% \quad (1)$$

$$\text{EE (\%)} = W_{\text{drug loaded in particles}} / W_{\text{drug fed for encapsulation}} \times 100\% \quad (2)$$

The in vitro drug release study was performed through a dialysis method by employing PBS (pH 7.4, 150 mM) and saline sodium citrate (SSC, pH 5.0, 150 mM) at 37 °C. Firstly, the PBS (pH 7.4) solution of DOX-loaded micelles with a concentration of 0.5 mg/mL was obtained by dialysis. Then, 1 mL of PBS solution was put into dialysis bag (MWCO: 3.5 kDa), which was immersed in a tube loaded with 25 mL of the release medium, and incubated in a shaking water-bath with 120 rpm at 37 °C. Next, 3 mL of release media in tube was taken out at 0.5, 1, 2, 4, 6, 8, 12, 24, 48, and 72 h, and the equivalent volume of fresh medium was supplemented each time. The drug concentration was calculated by measuring the absorbance at 499

nm according to a standard calibration curve obtained from free DOX·HCl in the corresponding release buffers. The experiment was performed in quadruplicate for each sample.

Confocal imaging

Confocal imaging was conducted following the reported procedures[4,6]. Briefly, HeLa cells were seeded in 12-well plates at a plating density of 1×10^3 cells per well in 1 mL of complete growth medium and incubated in a 37 °C, 5% CO_2 environment for 24 h. Solutions of DOX-loaded micelles were prepared in complete growth medium at concentrations equal to 50% of their IC_{50} value and then added to the wells and incubated for 4 h at 37 °C. Cells were later rinsed with PBS and fixed with 4% paraformaldehyde (PFA) solution for 20 min at room temperature. Finally, cells were counterstained with 2-(4-amidinophenyl)-6-indolecarbamidine (DAPI). Coverslips were mounted onto glass slides and imaged using Nikon A1R confocal microscope.

Evaluation of cellular uptake by flow cytometry

The procedures for flow cytometry are same with our previously reported paper[7]. HeLa cells were seeded in 24-well plates at a plating density of 1×10^6 cells per well in 1.0 mL of complete growth medium and incubated for 24 h at 37 °C in 5% CO_2 environment. Next, fresh MEM containing different samples, 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, C@DOX and L@DOX micelles in MEM was set at 25 μg/mL. After incubation for 4 h, the sample solution was aspirated, and the cells were rinsed twice with PBS. Cells were then harvested by incubation with 200 μL of Trypsin-EDTA, followed by resuspension with 1 mL of complete growth medium. Subsequently, cells were transferred to 1.5 mL of 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 samples using a BD Accuri C6 Plus flow cytometer (BD Biosciences) with an excitation wavelength and emission wavelength of 488 nm and 595 nm, respectively. A

minimum of 10,000 cells was analyzed for each sample with the fluorescence intensity.

Cell viability assay

Depending on the method our group previously reported[4-7], the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) (MTS) assay was used to evaluate the *in vitro* cytotoxicity of drug-loaded micelles. The HeLa cells were seeded in 96-well plates at a density of 2,500 cells per well in 100 μL of complete growth medium and incubated in an incubator maintained at 37 ℃ and 5% CO_2 environment for 24 h. Free DOX·HCl, blank micelles, DOX-loaded micelles were prepared in serial dilutions in water and then diluted 10-fold in OptiMEM medium (Invitrogen). The cells were then rinsed once with PBS and incubated with 40 μL of the sample solutions at 37 °C for 24 h. Cells were then rinsed with PBS, and the medium was replaced with 100 μL of culture medium. Subsequently, 20 μL of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carbox-ymethoxyphenyl)-2-(4-sulfophenyl)-2Htetrazolium (MTS, Promega) reagent was added to each well, which were further incubated at 37 °C, 5 % CO_2 for 3 h. The absorbance of each well was measured at 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.

Degradation study

The degradation study was carried out based on the previously reported procedures.[4] Specifically, the copolymer C or L (0.016 g, 4.6×10^{-6} mol) was dissolved in a mixed solution of 10.13 mL of dichloromethane and 2.25 mL of methanol in a 50 mL Falcon tube. After the addition of sulfonic acid (0.017 g, 1.173×10^{-4} mol) to the reaction mixture, the reaction was sealed and stirred at room temperature. At the predetermined time points (3, 9, 12, 24, 48 and 72 h), 1 mL of degradation solution was retracted and further quenched by extraction with saturated aqueous $NaHCO_3$. After removal of the organic layer under reduced pressure and drying in vacuo, the

degraded products were dissolved in DMF for SEC analysis.

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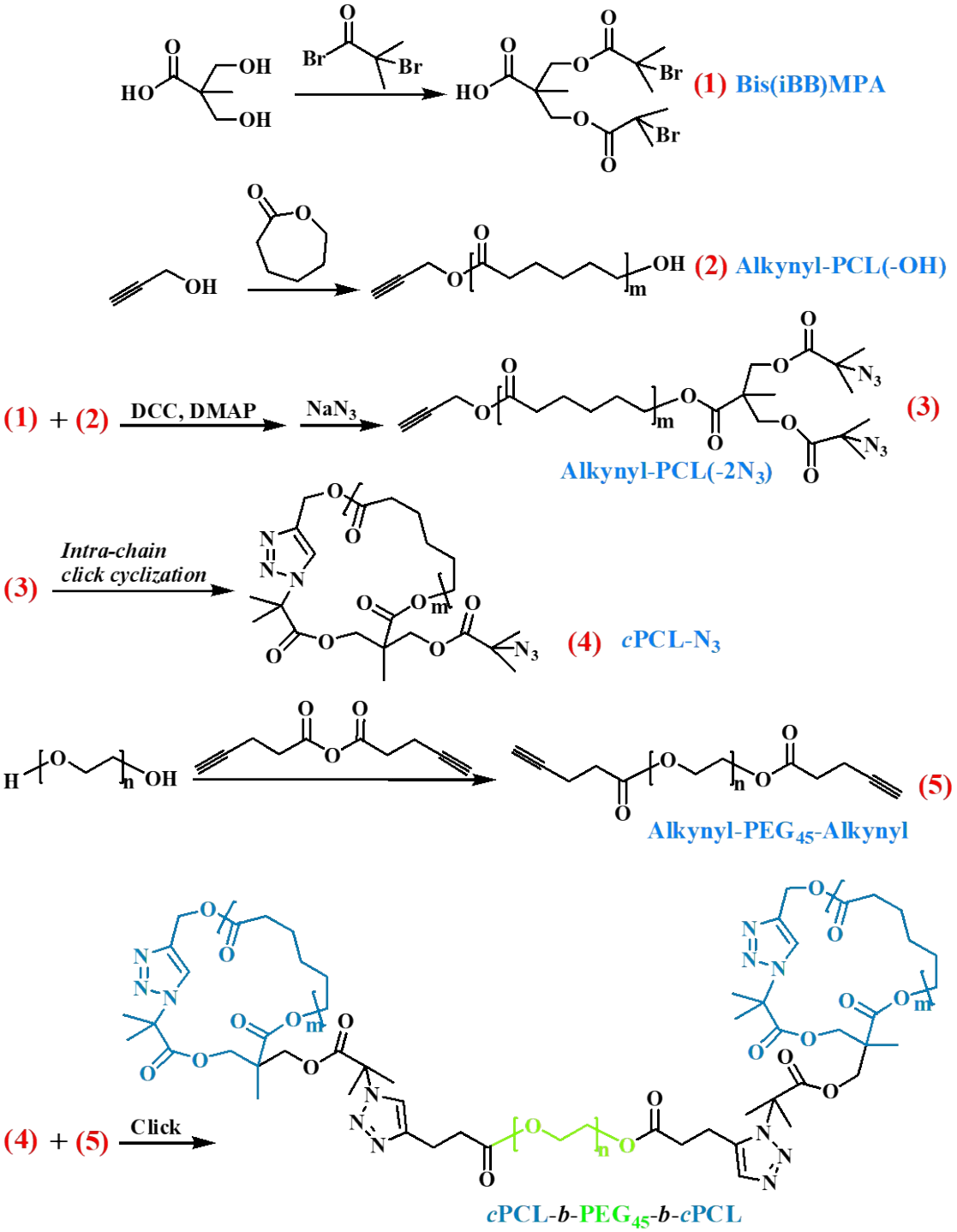
2. **The detailed discussion on the effect of biodegradation property of PCL on DOX release from the micelles**.

The biodegradation of aliphatic polyesters occurs via hydrolysis of labile ester bonds and its biodegradation rates ultimately depend on access of water to the esters bonds. Since PEG segments are highly hydrated, water can cross the PEG shell freely and contact the surface region of PCL core, resulting in the swelling of micelles. On the other hand, water cannot freely penetrate the inner part of the PCL core due to the strong hydrophobic and crystallizable character of the PCL block. Therefore, the degradation of *c*PCL-*b*-PEG-*b*-*c*PCL or PCL-*b*-PEG-*b*-PCL micelles in aqueous solution can be divided into two stages[1] including (i) the interfacial erosion stage, during which the degradation mainly occurs at the interface region between the PEG shell and PCL core (takes 60 days), and (ii) the core erosion stage, where the degradation mainly occurs in the PCL core (takes 150 days). According to the results of the above studies, we believe that the biodegradation property of PCL moiety exerts insignificant effect on the release of DOX from DOX-loaded micelles.

At present, there are still some drawbacks for PCL-*b*-PEG-*b*-PCL copolymers for controlled drug release, such as poor drug loading capability, insufficient drug release, and low cellular uptake efficiency due to the stealth properties of PEG moiety. Therefore more efforts should be focused on tuning the properties of copolymers and modulating the chemical, physical, and biological properties of the resultant self-assembled micelles by introducing functional groups and regulating the topological structure of copolymers in the future.



Scheme S1 Synthesis steps of dumbbell-shaped *c*PCL-*b*-PEG$_{45}$-*b*-*c*PCL copolymer.

Table S1. Summary of MW and PDI of the synthesized polymers.

Samples	*M*n [a] (KDa)	*M*n [b] (KDa)	PDI [b]
Alkynyl-PCL(-OH)	2.338	2.755	1.168
Alkynyl-PCL(-2Br)	2.753	3.007	1.083
Alkynyl-PCL(-2N_3)	2.621	2.791	1.148
*c*PCL-N_3	2.621	2.379	1.280
Alkynyl-PEG_{45}-Alkynyl	2.160	2.334	1.082
*c*PCL-*b*-PEG_{45}-*b*-*c*PCL	7.670	7.979	1.201
PCL-*b*-PEG_{45}-*b*-PCL	7.448	8.031	1.249

[a] Determined by ^{1}H NMR. [b] Determined by SEC-MALLS.

Table S2. Drug-loading content (DLC) and entrapment efficacy (EE) for DOX with *c*PCL-PEG_{45}-*b*-*c*PCL and PCL-*b*-PEG_{45}-*b*-PCL micelles.

Sample	D_h[a] (nm)	PDI[a]	Theoretical drug loading content (wt%)	Drug loading content[b] (%)	Drug loading Efficiency[b] (wt%)
*c*PCL-PEG_{45}-*b*-*c*PCL	102.1	0.258	10	8.89	72.04
PCL-*b*-PEG_{45}-*b*-PCL	89.1	0.179	10	4.20	38.84

[a] D_h and PDI of DOX-loaded micelles were determined by DLS.

[b] Determined by UV-vis measurement.

Figure S1-S17

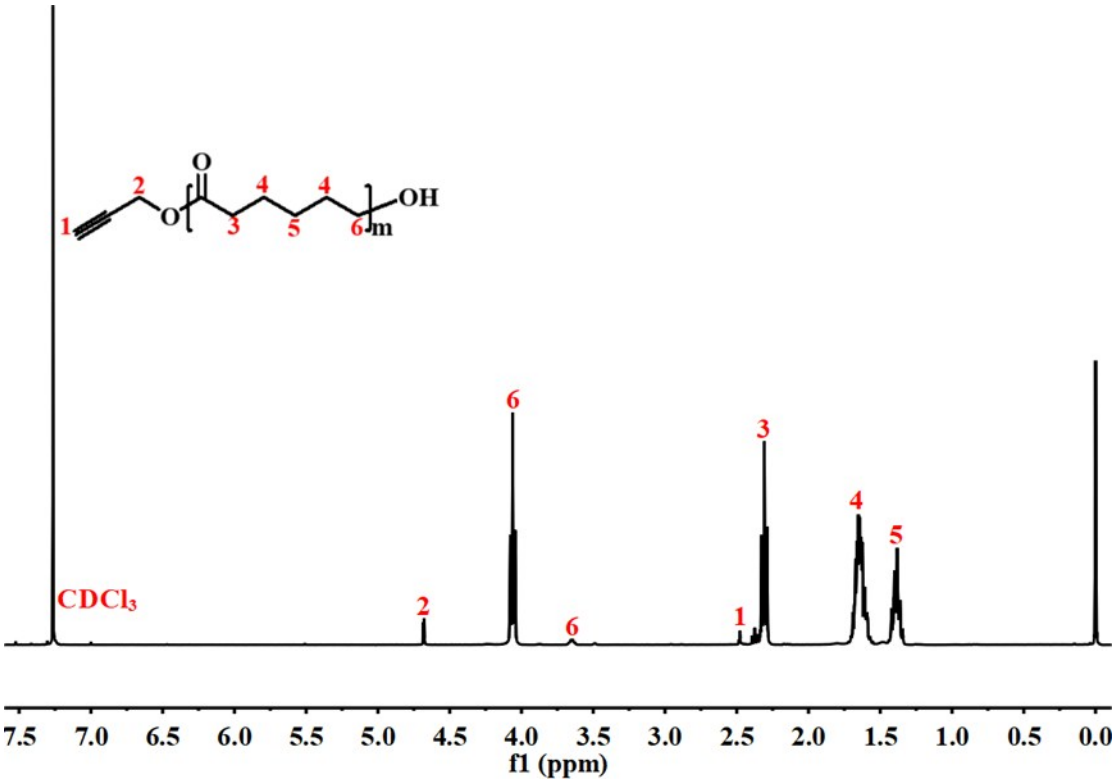


Figure S1. ^{1}H NMR spectrum of Alkynyl-PCL(-OH) in $CDCl_3$.

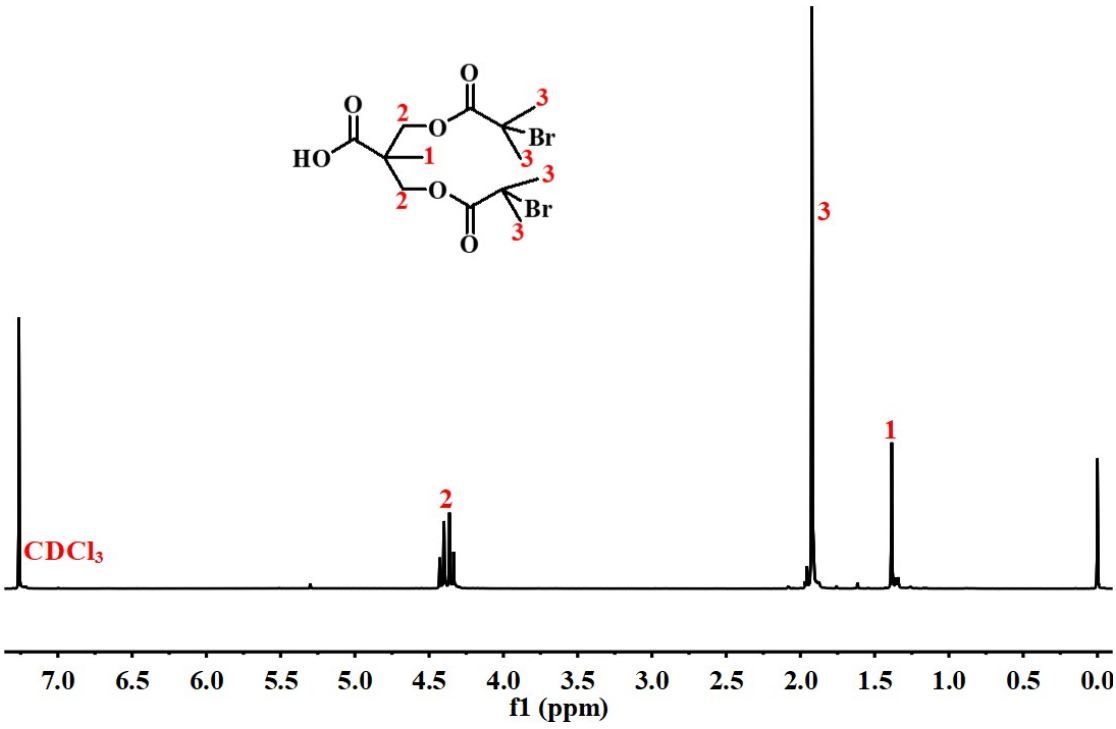


Figure S2. ^{1}H NMR spectrum of Bis(iBB)MPA in $CDCl_3$.

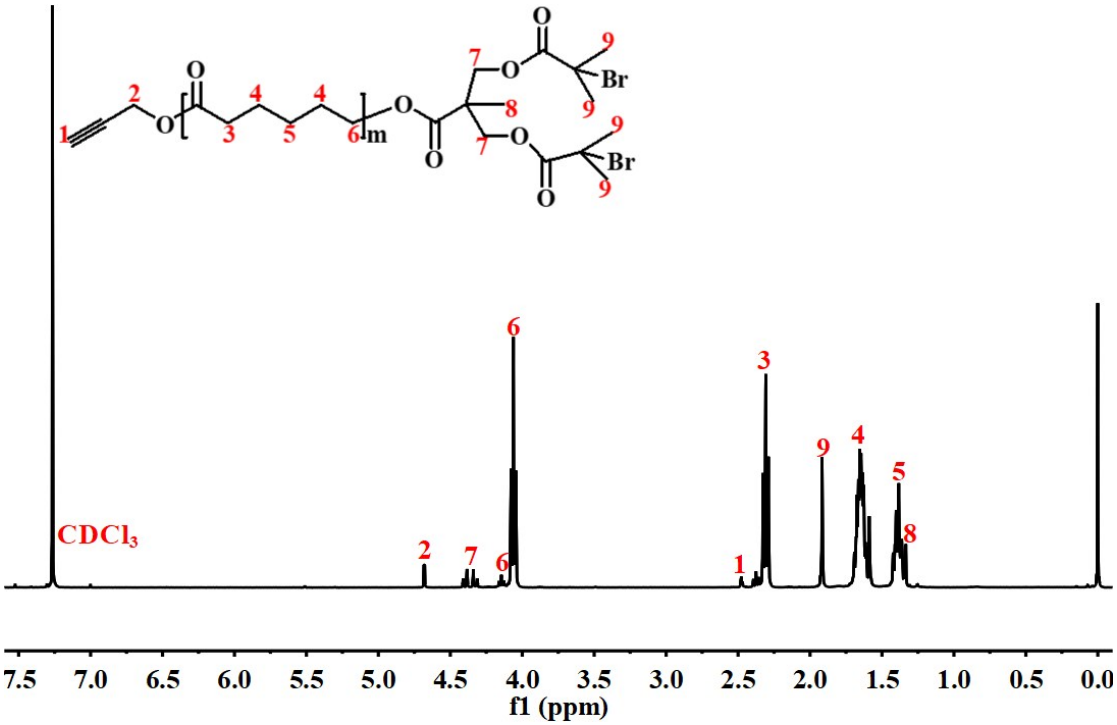


Figure S3. ^{1}H NMR spectrum of Alkynyl-PCL(-2Br) in $CDCl_3$.

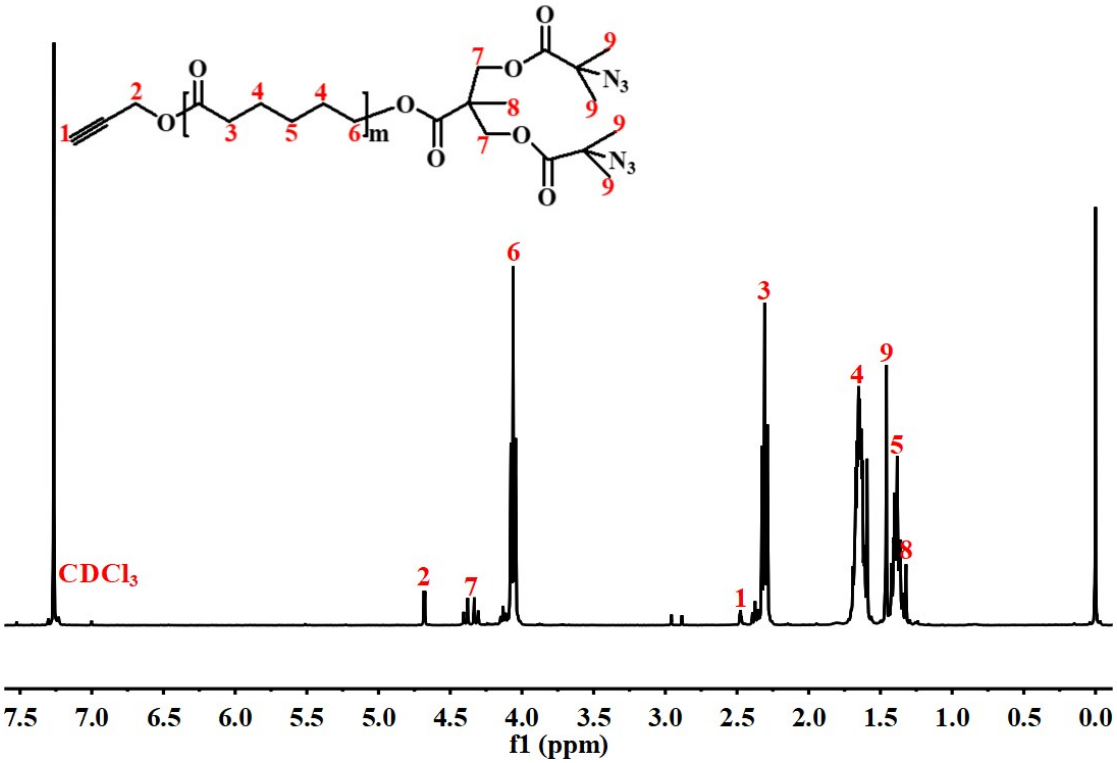


Figure S4. ^{1}H NMR spectrum of Alkynyl-PCL(-2N_3) in $CDCl_3$.

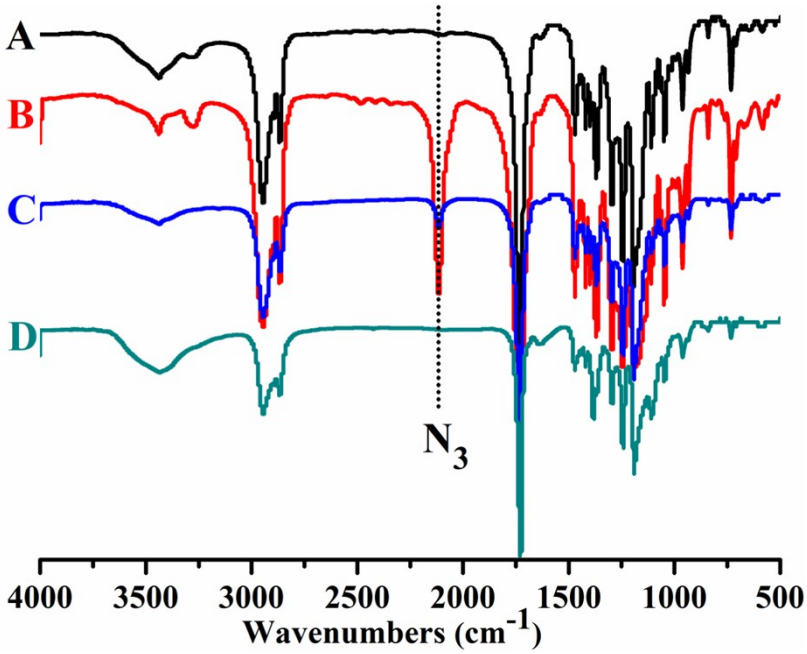


Figure S5. FT-IR spectra of the Alkynyl-PCL(-2Br) (A), Alkynyl-PCL(-2N_3) (B), *c*PCL-N_3 (C) and *c*PCL-*b*-PEG_{45}-*b*-*c*PCL (D).

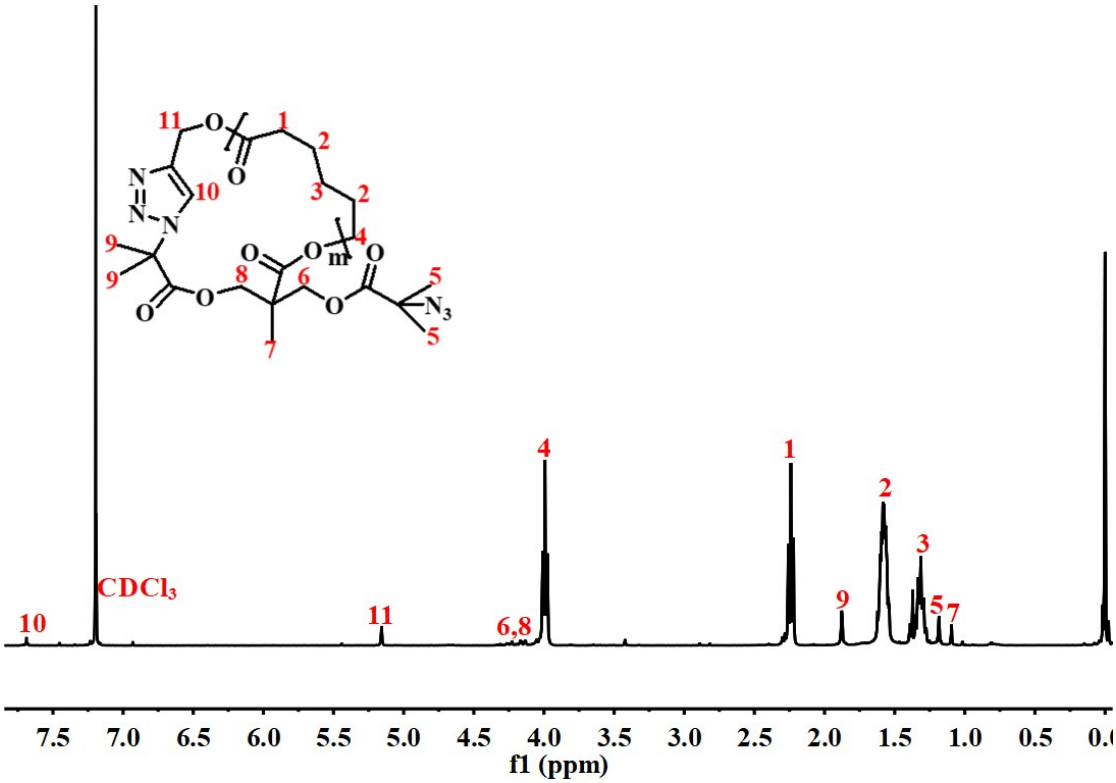


Figure S6. ^{1}H NMR spectrum of *c*PCL-N_3 in $CDCl_3$.

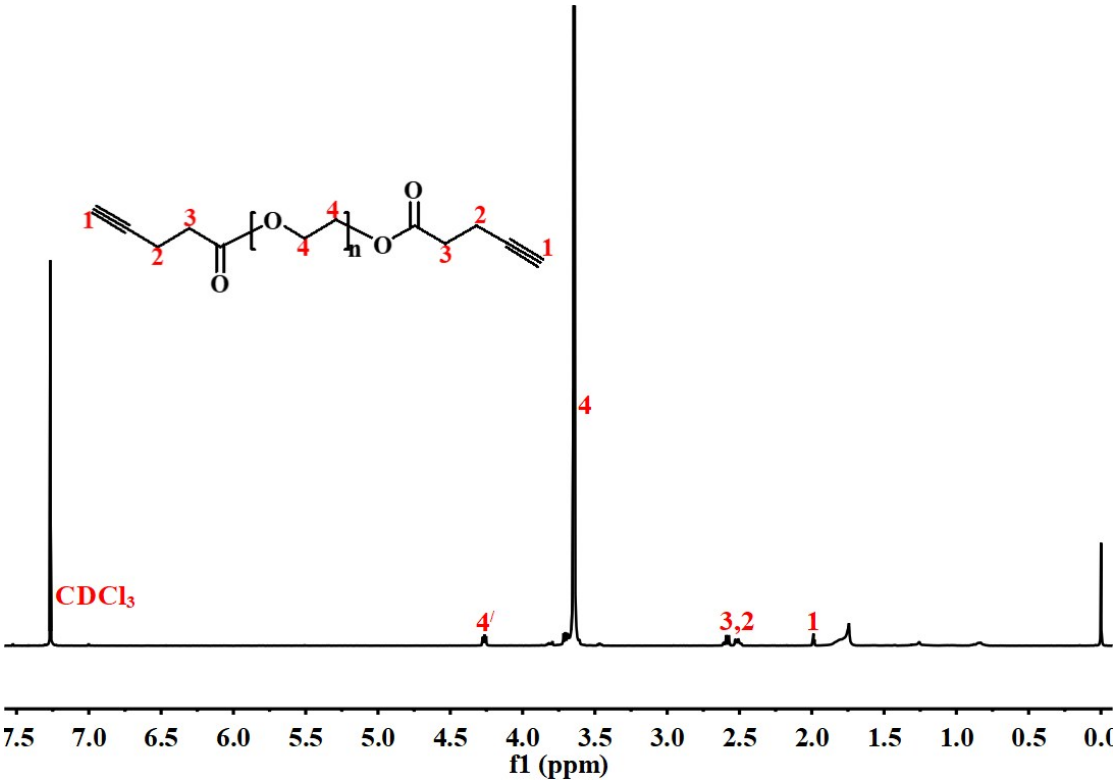


Figure S7. ^{1}H NMR spectrum of Alkynyl-PEG$_{45}$-Alkynyl in $CDCl_3$.

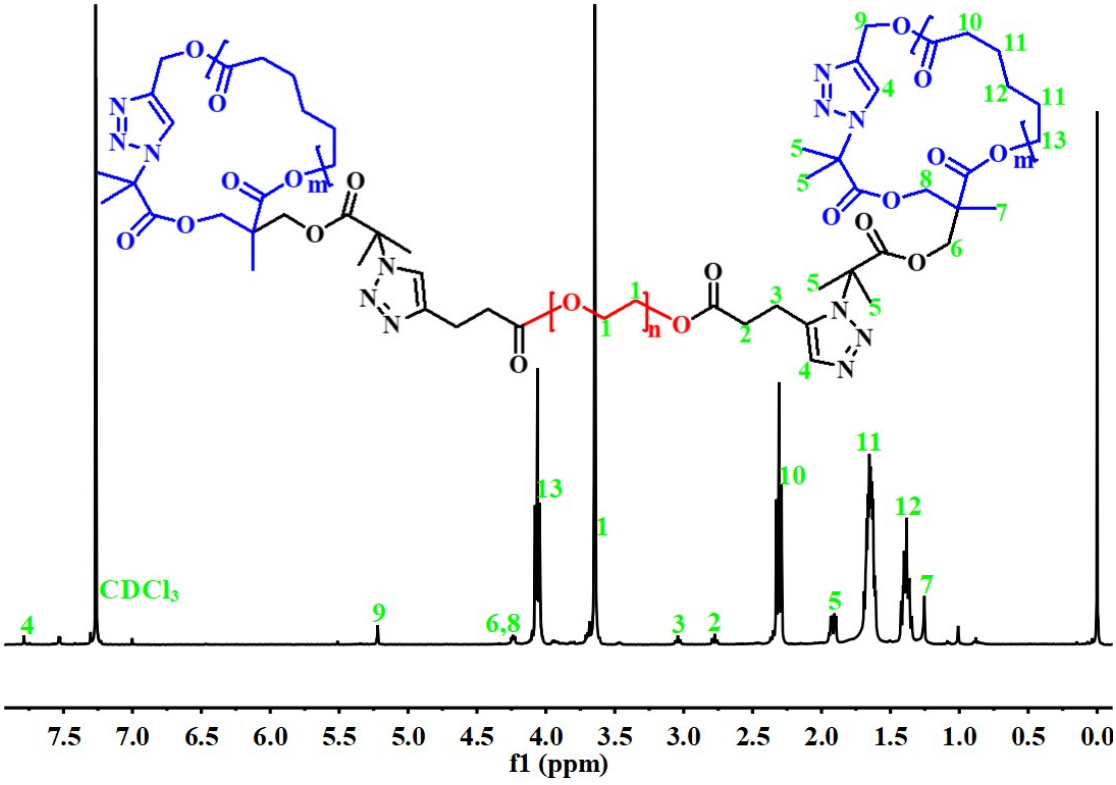


Figure S8. ^{1}H NMR spectrum of *c*PCL-*b*-PEG$_{45}$-*b*-*c*PCL in $CDCl_3$.

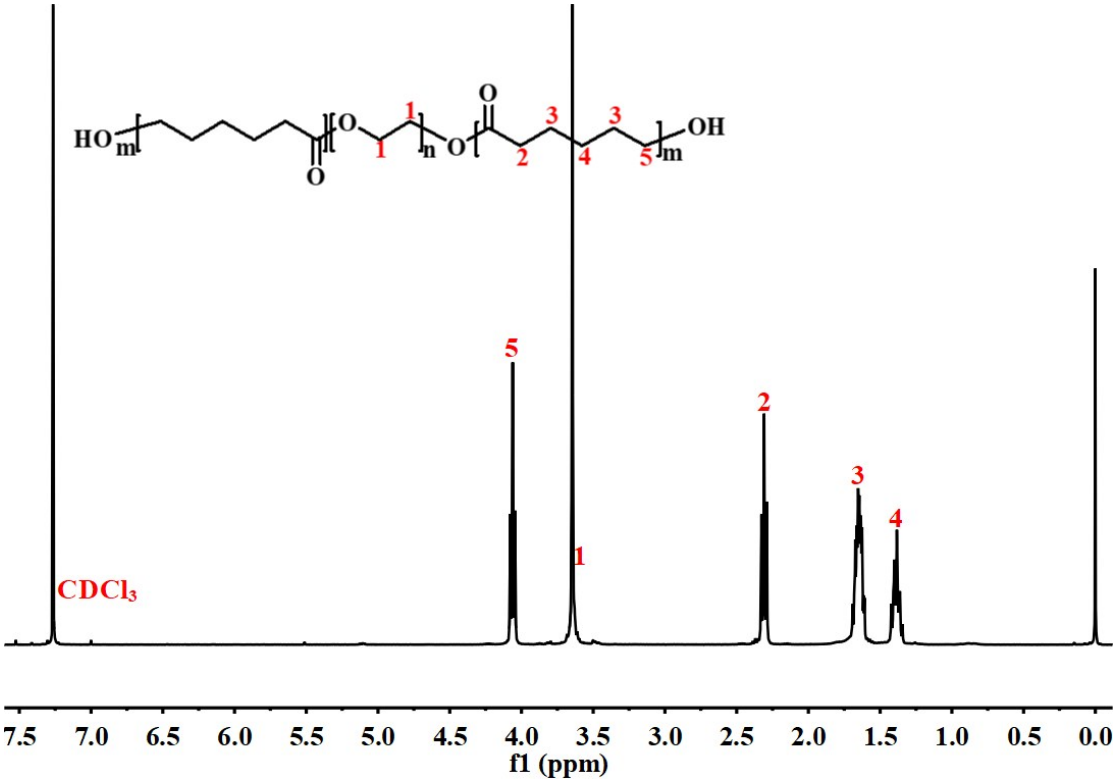


Figure S9. ^{1}H NMR spectrum of PCL-*b*-PEG$_{45}$-*b*-PCL in $CDCl_3$.

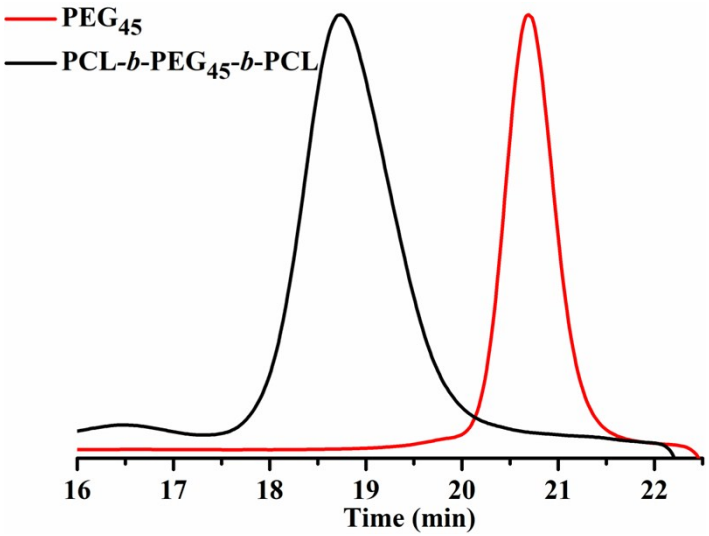


Figure S10. SEC elution traces of linear copolymer using DMF as an eluent.

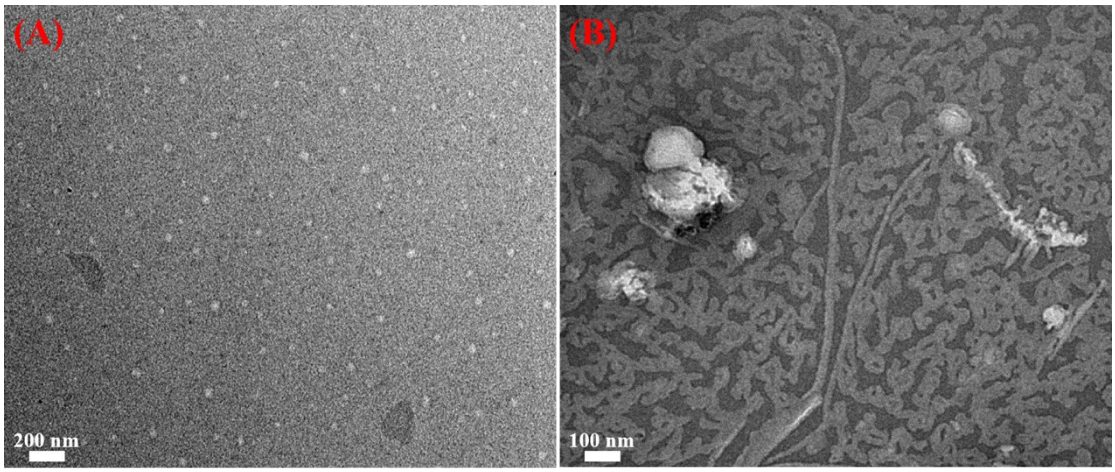


Figure S11. TEM images of C (A) and L (B) micelles in an aqueous phase at a polymer concentration of 0.2 mg/mL.

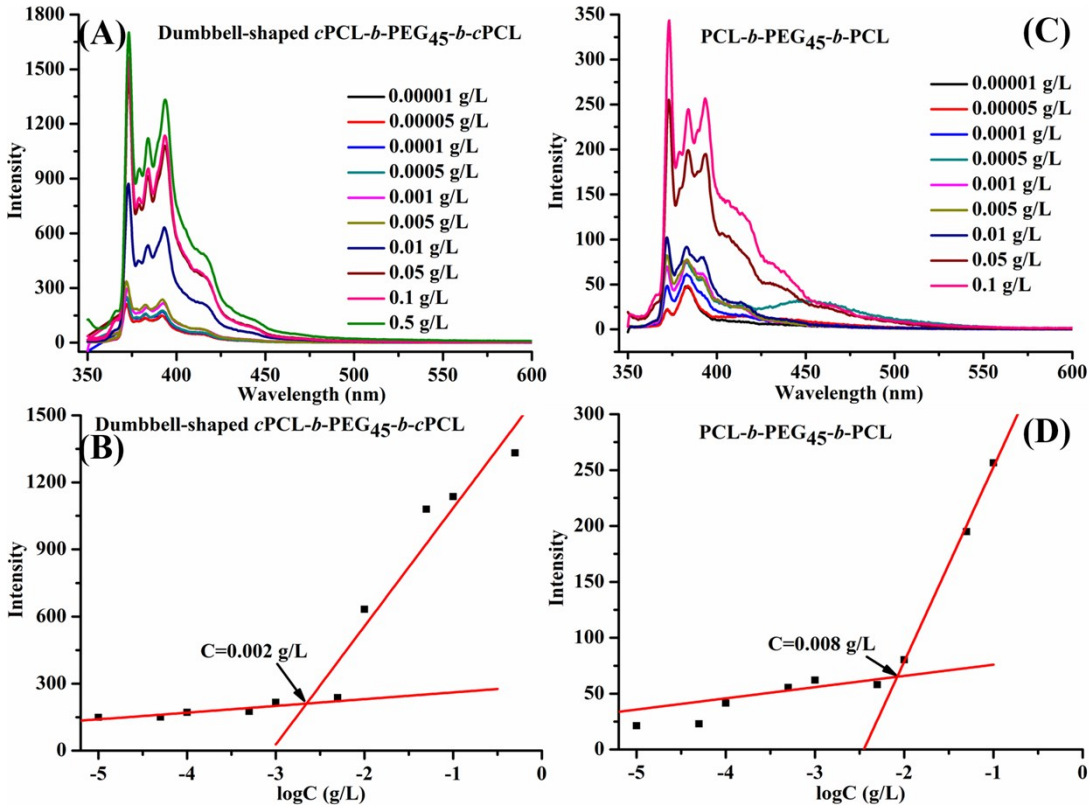


Figure S12. (A, C) Fluorescence emission spectra of pyrene with increasing concentration of C and L micelles, and (B, D) the intensity in the emission spectra as a function of logarithm of C and L concentration. λex=340 nm. [Pyrene]= 6×10^{-8} M;

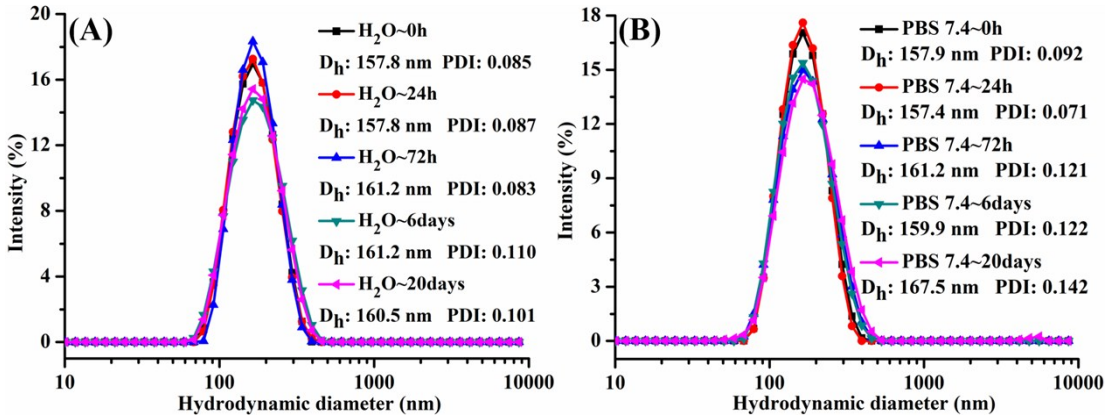


Figure S13. D_h and PDI of C micelles in (A) H_2O and (B) PBS (pH 7.4, 150 mM) (polymer concentration = 0.2 mg/mL) in 20 days monitored by DLS.

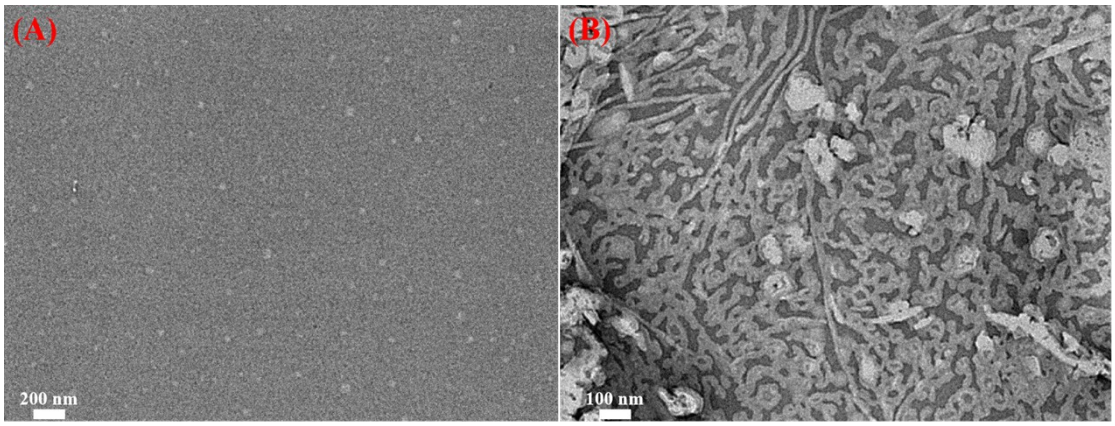


Figure S14. TEM images of micelles self-assembled by (A) DOX-loaded *c*PCL-PEG_{45}-*b*-*c*PCL and (B) DOX-loaded PCL-*b*-PEG_{45}-*b*-PCL in an aqueous phase.

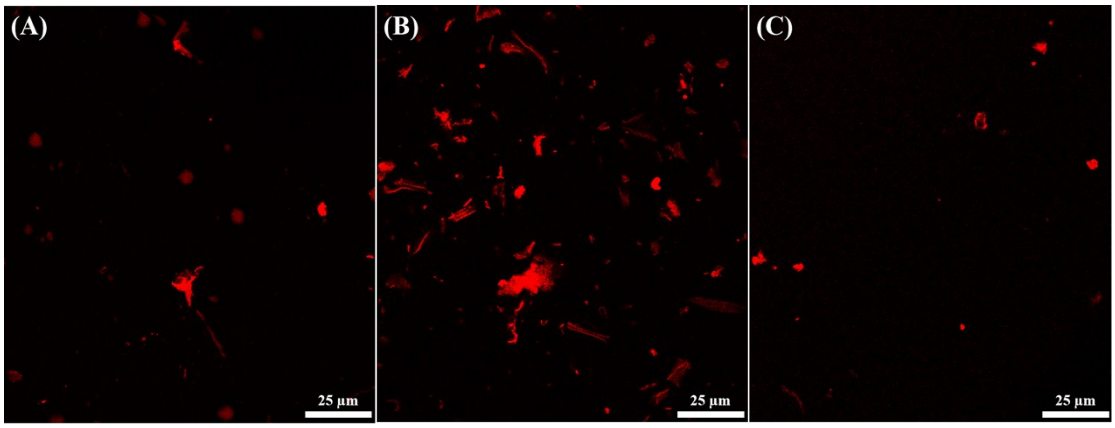


Figure S15. CLSM images of DOX-loaded micelles based on *c*PCL-PEG$_{45}$-*b*-*c*PCL (A), PCL-*b*-PEG$_{45}$-*b*-PCL (B) and mPEG$_{45}$-*b*-*c*PCL (C) in an aqueous phase.

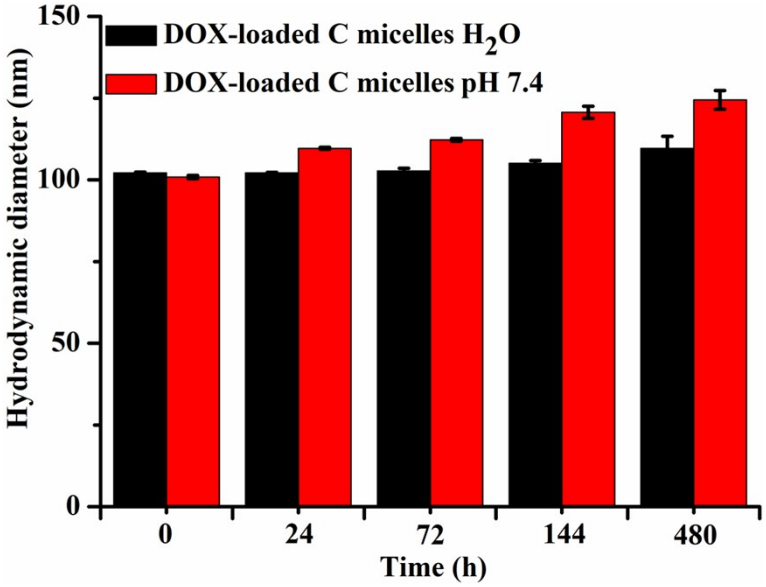


Figure S16. Average size of DOX-loaded C micelles in H_2O and PBS (pH 7.4, 150 mM) (polymer concentration = 0.2 mg/mL) in 20 days monitored by DLS

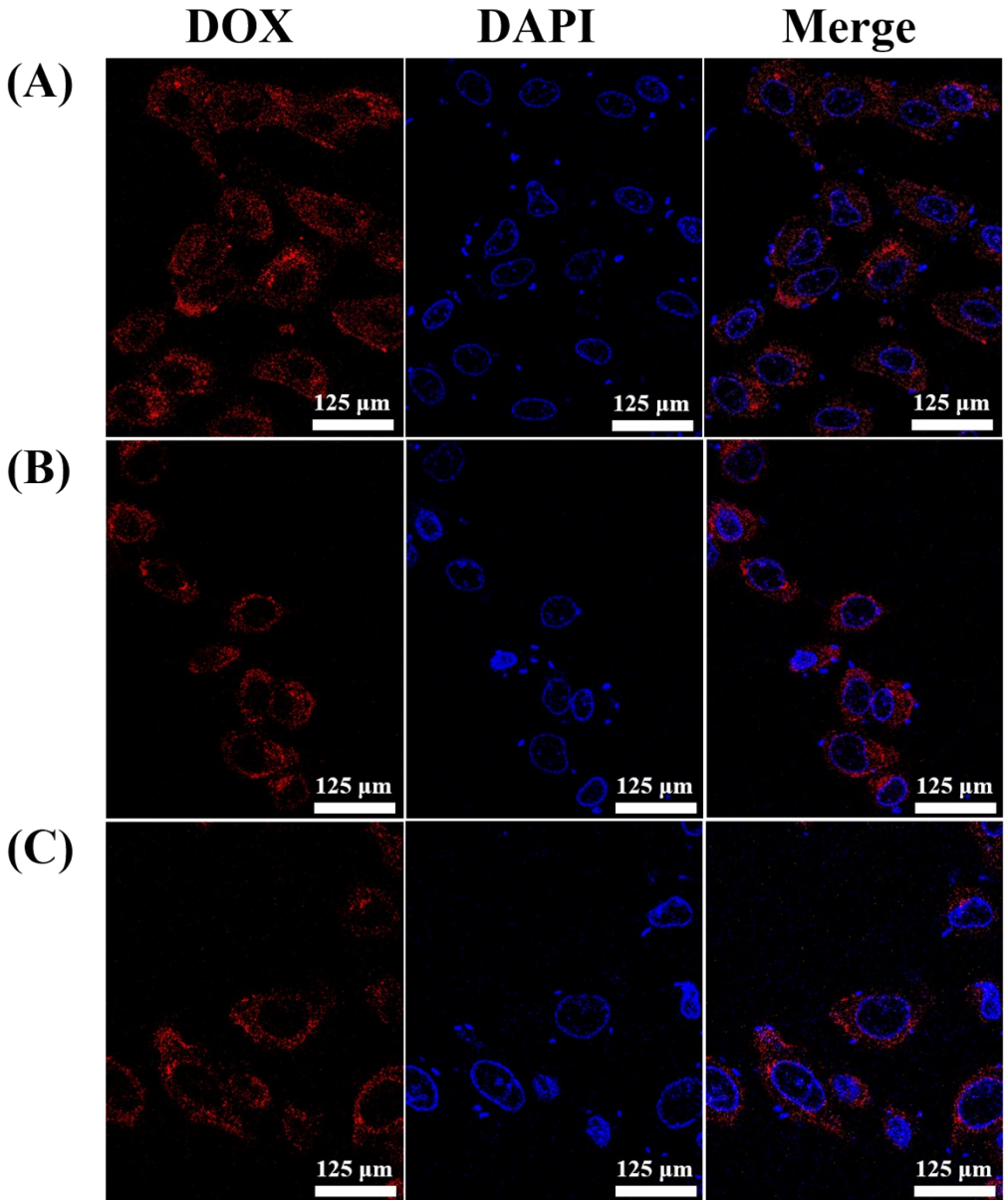


Figure S17. Confocal imaging of free DOX (A), micelles of C@DOX (B) and L@DOX (C) uptake in HeLa cells (nuclei stained blue with DAPI). Note that cells were treated with polymer or free drug at 50% of their respective IC_{50} values to minimize cell death.

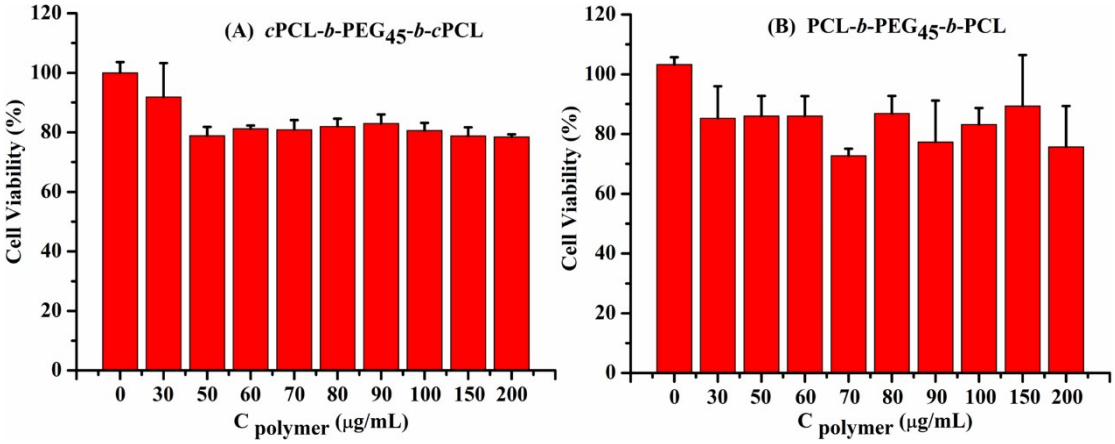


Figure S18. *In vitro* cytotoxicity of blank C (A) and L micelles (B) in HeLa cells. Cell viability was determined by MTS assay and expressed as % viability compared to the untreated cells control. The data were expressed as mean ± SD, n = 3.

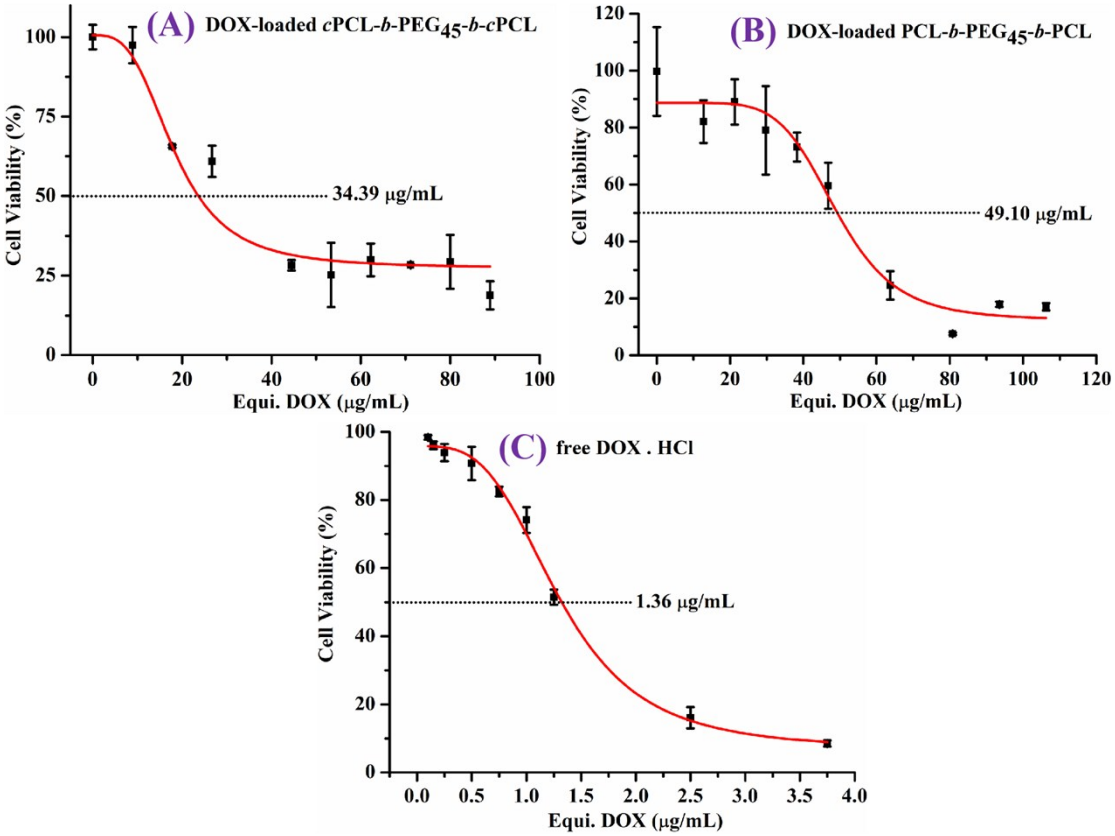


Figure S19. *In vitro* cytotoxicity of C@DOX (A), L@DOX (B) and free DOX (C) in HeLa cells for 24 h of incubation. The data were expressed as mean ±SD, n = 3.

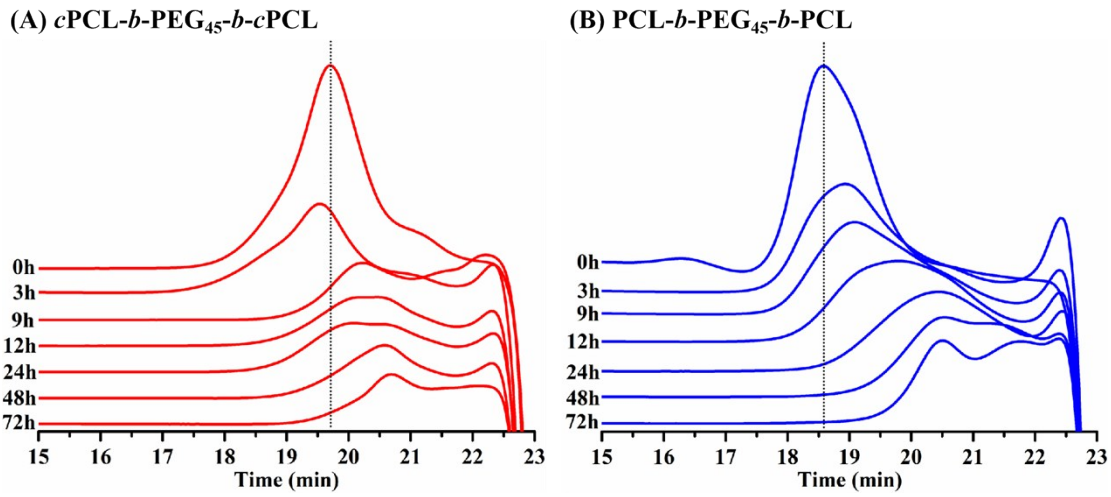


Figure S20. SEC analyses of the degraded products of *c*PCL-*b*-PEG$_{45}$-*b*-*c*PCL (A) and PCL-*b*-PEG$_{45}$-*b*-PCL (B) at various degradation time points.