Supporting Information for "Bioreducible Micelles and Hydrogels with Tunable Properties from Multi-Armed Biodegradable Copolymer"

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Experimental Section

Materials. Poly(ethylene glycol) methyl ether (PEO, M_n = 2000, Aldrich) was dried at 50 ^oC in vacuo overnight. ε-Caprolactone (CL, 99%, Aldrich) and dimethylformamide (DMF, analytical grade) were dried by calcium hydride, distilled under reduced pressure, and then stored over molecular sieves, respectively. α-Cyclodextrin hydrate (α-CD, 98%) was purchased from Acros and used without further purification. Dichloromethane (99.5%) and toluene (99.5%) were directly distilled from calcium hydride. Dicyclohexylcarbodiimide (DCC, 99.2%) and 4-(dimethylamino)-pyridine (DMAP, 99.1%) were purchased from GL Biochem (Shanghai, China) and used as received. 2,2-Dimethoxy-2-phenylacetophenone (DMPA, Aldrich, 99%), 3,3-dithiobis(propionic acid) (DTPA, 99%, Aldrich), 1,4-dithiothreitol (DTT, 99%, Aldrich) and stannous octoate ($SnOct_2$, Sigma, 95%) were used as received. Propargyl acrylate (98%) and Cystamine hydrochloride (98%) were purchased from Aldrich and

used as received. Triethylamine (98%) was distilled before use.

Methods. Fourier transform infrared (FT-IR) spectra were recorded on a Perkin Elmer Paragon 1000 spectrometer at frequencies ranging from 400 to 4000 cm^{-1}. Samples were thoroughly mixed with KBr and pressed into pellet form. ^{1}H NMR spectrum was performed at room temperature on a Varian Mercury-400 spectrometer. Molecular weight distribution (M_w/M_n) of polymer was determined on a gel permeation chromatograph (GPC, HLC-8320, Tosoh Corporation) equipped with a refractive index detector at 30 °C. The elution phase was DMF (0.01 $mol.L^{-1}$ LiBr, elution rate: 0.6 mL/min), and polymethylmethacrylate was used as the calibration standard. The mean size of nanoparticles was determined by dynamic light scattering (DLS) using a Malvern Nano_S instrument (Malvern, UK). The solution of nanoparticles was performed at a scattering angle of 90 ^{o}C and at 25 ^{o}C. All the measurements were repeated three times, and the average values reported are the mean diameter ± standard deviation. UV-vis spectra of samples were recorded at room temperature using a Spectrumlab54 UV-visible spectrophotometer. Transmission electron microscopy (TEM) was performed without negative staining using a JEM-2010/INCA OXFORD TEM (JEOL/OXFORD) at a 200 kV accelerating voltage. Samples were deposited onto the surface of 300 mesh Formvar-carbon film-coated copper grids, and excess solution was quickly wicked away with a filter paper. The rheological behavior of the hydrogels was investigated by a TA-ARG2 rheometer using a 40 mm parallel-plate geometry at 25 °C. The gap distance between the two plates was fixed at 0.3 mm.

Preparation of the Disulfide-Bond-Containing Tetra(alkyne). Cystamine

hydrochloride (225.2 mg, 1 mmol) and propargyl acrylate (884 μL, 8 mmol) were dissolved in 5 mL of acetonitrile, respectively. Triethylamine (0.31 mL, 2.23 mmol) was added to the cystamine hydrochloride solution, and the propargyl acrylate solution was then added dropwise to the cystamine hydrochloride solution in ice-water bath for 5 min, and then immersed into an oil bath at 25 °C with vigorous stirring for 24 h. The solution was concentrated under reduced pressure and then dissolved into 40 mL ether. The mixture was extracted sequentially with 10+10 mL distilled water, and then was extracted sequentially with 40+40 mL ether. The ether phase were combined, dried with anhydrous Na_2SO_4, concentrated under reduced pressure and then dried in vacuo to give the product tetra(alkyne) (601.7 mg, 90.4% yield). ^{1}H NMR ($CDCl_3$): δ (ppm) = 2.42-2.57 ((br overlapped, CH_2CH_2COO CH_2CCH, m), 2.70-2.85 ((br overlapped, $SSCH_2CH_2N$, NCH_2CH_2COO), 4.69 ($COOCH_2CCH$, s). TOF-MS, m/z: calcd for $C_{28}H_{36}N_2O_8S_2$, 592.1913; found, 593.2036 $[M + H]^+$.

Preparation of the Thiol-Terminated PEO-b-PCL. The thiol-terminated linear PEO-b-PCL precursor (PEO-b-PCL-SH) was synthesized by ring-opening polymerization of ε-caprolactone with mono-hydroxyl-terminated PEO initiator and stannous octoate catalyst, followed by both the sequential esterification and 1,4-dithio-threitol (DTT) reduction reactions. First, PEO (200.0 mg, 0.1 mmol), CL (64.05 μL, 0.6 mmol), and a dry stirring bar were put into a tube where the exhausting-refilling process was carried out for three times using a Schlenk line. After adding $SnOct_2$ catalyst (70.2 μL, 6.92 mg, diluted by 1mL toluene), the tube was immersed into an oil bath at 135 °C with vigorous stirring for 24 h. The resulting

product was dissolved in 5 mL CH_2Cl_2, poured dropwise into an excess of ether. The purified block copolymer was dried in vacuo at 40 °C to give 210.8 mg of the copolymer PEO-b-PCL_5 (78.5% yield). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.31-1.42 ($COCH_2CH_2\mathit{CH_2}CH_2CH_2O$, m), 1.53-1.70 ($COCH_2\mathit{CH_2}CH_2\mathit{CH_2}CH_2O$, m), 2.25-2.33 ($CO\mathit{CH_2}CH_2CH_2CH_2CH_2O$, t), 3.37 ($\mathit{CH_3}$, t), 3.60-3.67 ($O\mathit{CH_2CH_2}O$, s), 3.67-3.69 ($\mathit{CH_2}OH$, t), 4.02-4.10 ($COCH_2CH_2CH_2CH_2\mathit{CH_2}O$, m), 4.22 ($\mathit{CH_2}$, t).

Then, PEO-b-PCL_5 (M_n = 2570, 200.0 mg, 0.08 mmol), DTPA (33.6mg, 0.16 mmol), DCC (66.0mg, 0.32 mmol) and DMAP (9.8mg, 0.08 mmol) were put in a 25 mL flask with 3 mL of THF as solvent under a N_2 atmosphere. The flask was immersed into an oil bath at 25 °C with vigorous stirring for 48 h. The resulting product was added 3~5 drops of acetone, filtered, and poured dropwise into an excess of cold ether. The precipitate was washed three times by using cold ether and then dried in vacuo at 40 ^{o}C to give 177.7 mg of the copolymer PEO-b-PCL_5-DTPA (84.4 % yield). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.31-1.43 ($COCH_2CH_2\mathit{CH_2}CH_2CH_2O$, m), 1.56-1.70 ($COCH_2\mathit{CH_2}CH_2\mathit{CH_2}CH_2O$, m), 2.27-2.33 ($CO\mathit{CH_2}CH_2CH_2CH_2CH_2O$, t),2.70-2.81($CO\mathit{CH_2}CH_2\mathbf{SS}CH_2\mathit{CH_2}COOH$),2.90-2.98($COCH_2\mathit{CH_2}\mathbf{SS}\mathit{CH_2}CH_2COOH$), 3.37 ($\mathit{CH_3}$, t), 3.61-3.67 ($O\mathit{CH_2CH_2}O$, s), 4.03-4.09 ($COCH_2CH_2CH_2CH_2\mathit{CH_2}O$, m), 4.22 ($\mathit{CH_2}$, t).

Finally, PEO-b-PCL_5-DTPA (M_n = 2706.8, 162.4 mg, 0.06 mmol) and DTT (37.0 mg, 0.24 mmol) were completely dissolved in 2 mL of DMF under a N_2 atmosphere. The reaction mixture was stirred vigorously at room temperature for 24 h. The solution was concentrated under reduced pressure and then precipitated into a large excess of

cold ether. The precipitate was washed three times by using cold ether and then dried in vacuo to give the targeted product PEO-b-PCL_5-SH (132.7 mg, yield 83.2%). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.32-1.43 ($COCH_2CH_2\mathit{CH_2}CH_2CH_2O$, m), 1.58-1.70 ($COCH_2\mathit{CH_2}CH_2\mathit{CH_2}CH_2O$, m), 2.26-2.33 ($CO\mathit{CH_2}CH_2CH_2CH_2CH_2O$, t), 2.61-2.67 ($CO\mathit{CH_2}CH_2$**SH**), 2.72-2.81 ($COCH_2\mathit{CH_2}$**SH**), 3.38 ($\mathit{CH_3}$, t), 3.62-3.68 ($O\mathit{CH_2CH_2}O$, s), 4.03-4.08 ($COCH_2CH_2CH_2CH_2\mathit{CH_2}O$, m), 4.22 ($\mathit{CH_2}$, t). FT-IR (KBr, cm^{-1}): 2940 ($\nu_{C\text{-}H}$ for PCL), 2882 ($\nu_{C\text{-}H}$ for PEO), 1734 ($\nu_{C=O}$ for PCL). $M_{n,GPC}$ = 2660, M_w/M_n = 1.33.

Preparation of Copolymer 1 and 2. Under 2,2-dimethoxy-2-phenylacetophenone (DMPA) catalyst and 365 nm irradiation for 30 min, the thiol-terminated PCL-b-PEO was finally click conjugated with tetra(alkyne) to produce the multi-armed mPCL-b-PEO. A representative example is as follows. PEO-b-PCL_5-SH (50.0 mg, 0.02 mmol), tetra(alkyne) (1.2 mg, 1.96 μmol), DMPA (4.5 mg, 0.9 wt.%) and a dry stirring bar were put into a tube where the exhausting-refilling process was carried out for three times using a Schlenk line. The solution tube was then irradiated under a 365 nm high-pressure mercury lamp (150 W) for 30 min, where the distance between the lamp and tube was 15 cm. The solution was concentrated under reduced pressure and then precipitated into a large excess of ether. The precipitate was washed three times by using ether and then dried in vacuo to give the product copolymer **1** mPCL_5-b-PEO (20.7 mg, 48.3% yield). ^{1}H NMR: δ (ppm) = 1.32-1.43 ($COCH_2CH_2\mathit{CH_2}CH_2CH_2O$, m), 1.56-1.70 ($COCH_2\mathit{CH_2}CH_2\mathit{CH_2}CH_2O$, m), 2.25-2.34 ($CO\mathit{CH_2}CH_2CH_2CH_2CH_2O$, t), 2.56-2.68 (br overlapped, H-4,8, m), 2.69-2.77 (br overlapped, *H-2,3*, m), 2.79-2.88 (br overlapped, *H-6,7,* m), 2.89-2.95 (br overlapped, *H-1,5*, m), 3.38 ($\mathit{CH_3}$, t), 3.60-3.78

(O*CH*$_2$*CH*$_2$O, s), 4.01-4.13 (COCH$_2$CH$_2$CH$_2$CH$_2$*CH*$_2$O, m), 4.19-4.26 ((br overlapped, *H-g,h*, m). FT-IR (KBr, cm^{-1}): 2946 ($\nu_{C\text{-}H}$ for PCL), 2882($\nu_{C\text{-}H}$ for PEO), 1738 ($\nu_{C=O}$ for PCL). $M_{n,GPC}$ = 13170, M_w/M_n = 1.48.

Measurement of the Critical Aggregation Concentration of Copolymers. The critical aggregation concentration (cac) of copolymers was determined employing the hydrophobic dye solubilization method using 1,6-diphenyl-1,3,5-hexatriene (DPH) as a probe molecule. UV-vis spectra of samples were recorded in the range of 200-500 nm at room temperature.

Preparation of Doxorubicin-Loaded Nanoparticles in Aqueous Solution. Copolymers (10 mg) and doxorubicin hydrochloride (DOX, 5 mg, 8.6 μmoL) were dissolved in 6.5 mL of DMF, in which 1.5 fold of Et_3N (12.9 μmoL) was added to neutralize HCl in solution. Distilled water (1.5 mL) was then added gradually at a speed of 30 μL/min using a microsyringe untill the formation of nanoparticles. The resulting nanoparticles solution was then put into a dialysis bag (3500 MWCO) and subjected to dialysis against 4×1 L of distilled water for 24 h. The drug-loaded nanoparticles solution was lyophilized and stored at 4 ^oC. The drug-loaded nanoparticles (1 mg) was dissolved in 5 mL of DMF and then analyzed by UV absorbance at 500 nm. The drug loading capacity of nanoparticles is calculated as the weight ratio of actual drug to drug-loaded nanoparticles, and the drug loading efficiency of nanoparticles is calculated as the weight ratio of actual and added drug content. The blank nanoparticles were fabricated similarly.

In Vitro Doxorubicin Release from Drug-Loaded Nanoparticles. The lyophilized

drug-loaded nanoparticles (4 mg) were directly immersed into 1 mL of buffer solution (pH = 7.4) and then put into a dialysis bag (3500 MWCO). The dialysis bag was put in 15 mL of buffer solution at 37 ^{o}C. The drug-released solution was changed periodically (2, 6, 12, 24, 48 h…), and the amount of doxorubicin released from nanoparticles was measured by UV-vis at 500 nm at room temperature. All release experiments were carried out in duplicate, and all data were averages of six determinations used for drawing figures. The calibration curve of DOX in aqueous solution is y (abs) = -0.0132 + 23.8758 c (c: mg/mL).

Preparation of Hydrogel. Copolymer **1** (15 mg) were dissolved in 1 mL of distilled water, in which α-CD (30 mg) was added in solution under vigorous stirring for 30 min followed by sonication for 5 min. The mixed solution was then incubated at 25 ^{o}C, allowing the mixture to form the hydrogel.

Preparation and In Vitro Doxorubicin Release of Drug-Loaded Hydrogel. Copolymer **1** (15 mg) and doxorubicin hydrochloride (6 mg) were dissolved in 1 mL of distilled water, in which α-CD (30 mg) was added in solution under vigorous stirring for 30 min followed by sonication for 5 min. The mixed solution was then incubated at 25 ^{o}C, allowing the mixture to form the DOX-Loaded hydrogel. The DOX-loaded hydrogel was placed upside-down in a dialysis bag with a molecular weight cutoff of 3500. The dialysis bag was put in 15 mL of distilled water at 37 °C. The drug-release solution was changed periodically, and the amount of DOX released from the hydrogel was measured by UV-vis spectroscopy at 500nm at room temperature.

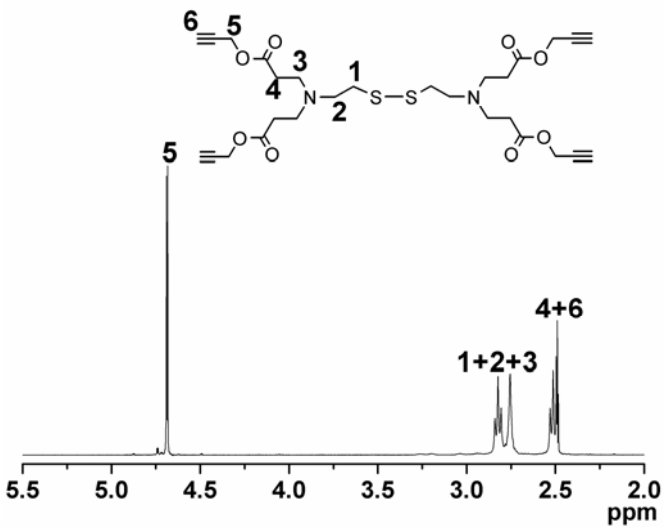

Fig. S1. ^{1}H NMR spectrum ($CDCl_3$) of tetra(alkyne)

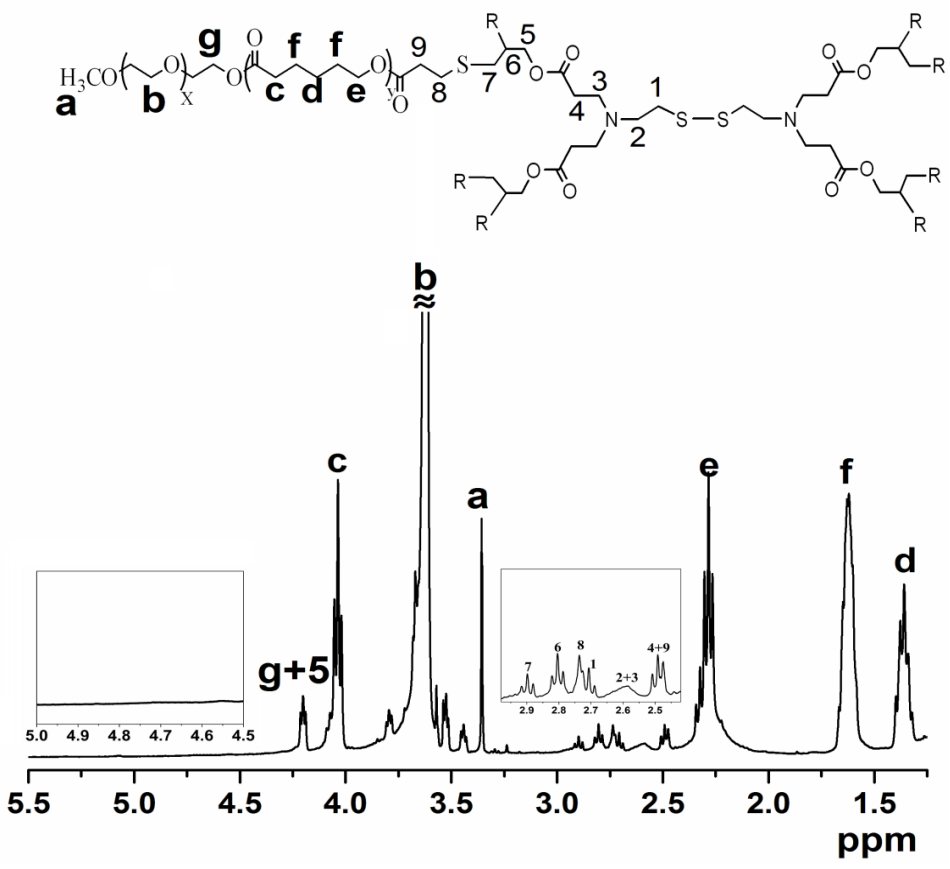

Fig. S2. ^{1}H NMR spectrum ($CDCl_3$) of copolymer **1** $mPCL_5$-b-PEO.

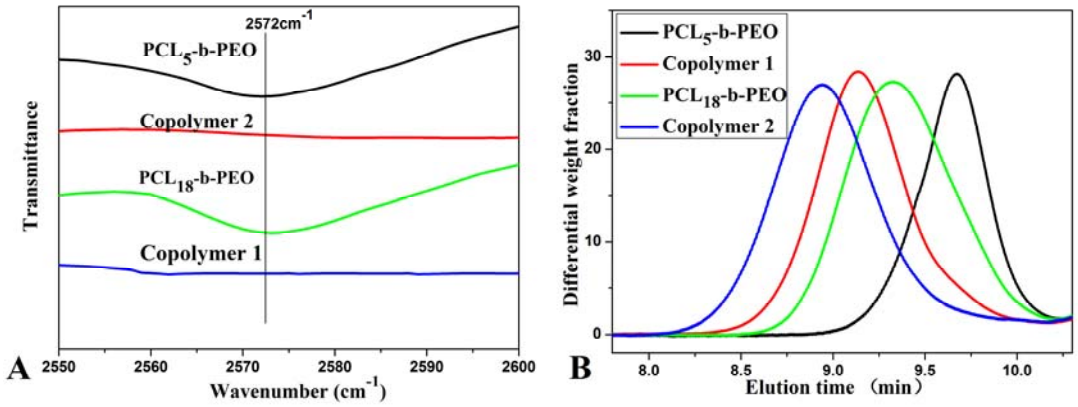

Fig. S3. The expanded FT-IR spectra (A) and GPC traces (B) of block copolymers **1** and **2**, and the precursors PCL_5-b-PEO and PCL_{18}-b-PEO.

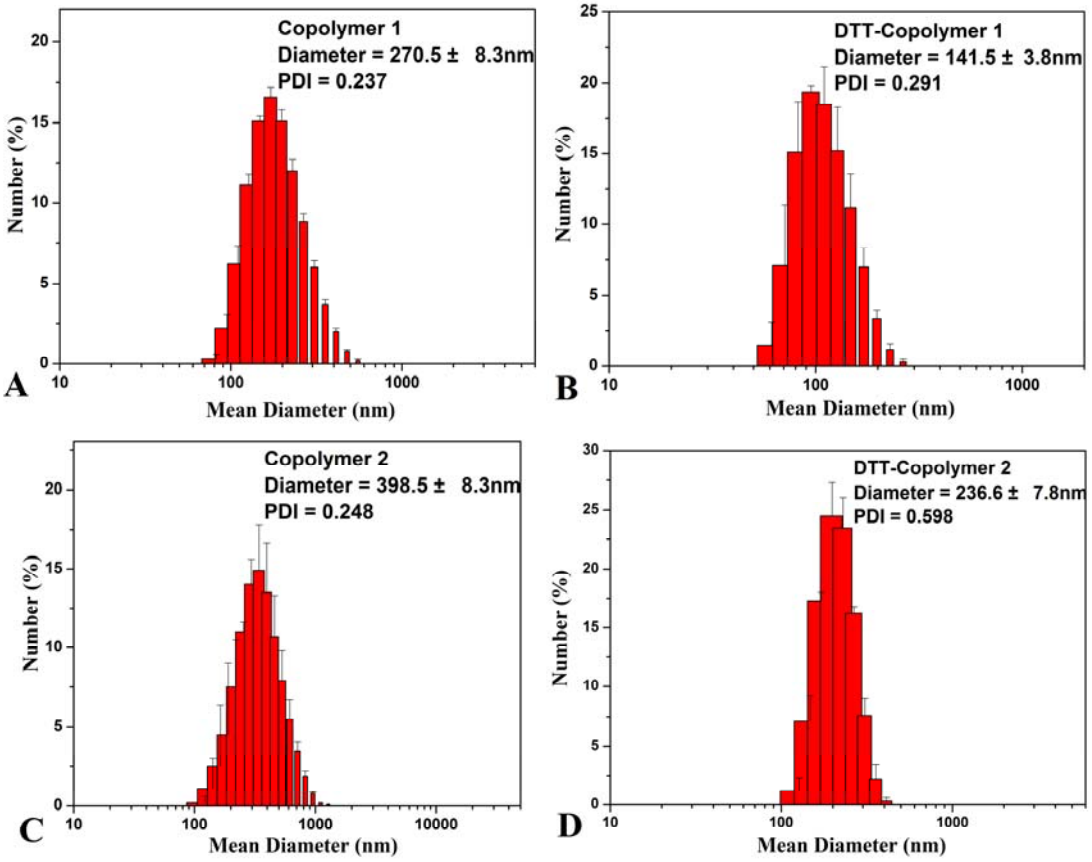

Fig. S4. The average diameter and polydispersity index (PDI) of copolymer **1** micelles

(A) and the micelles after DTT addition (B), and copolymer **2** micelles (C) and the micelles after DTT addition (D) determined by DLS.

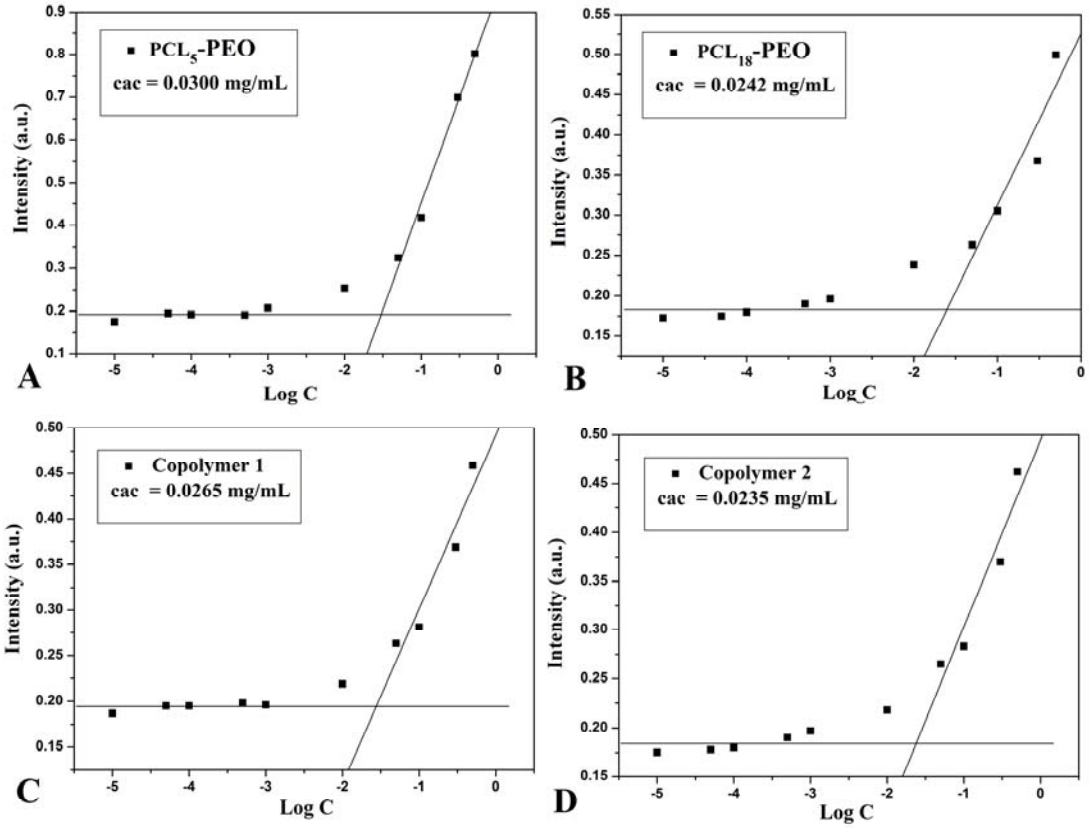

Fig. S5. Relationship of the absorbance intensity of DPH as a function of the copolymers concentration at room temperature.

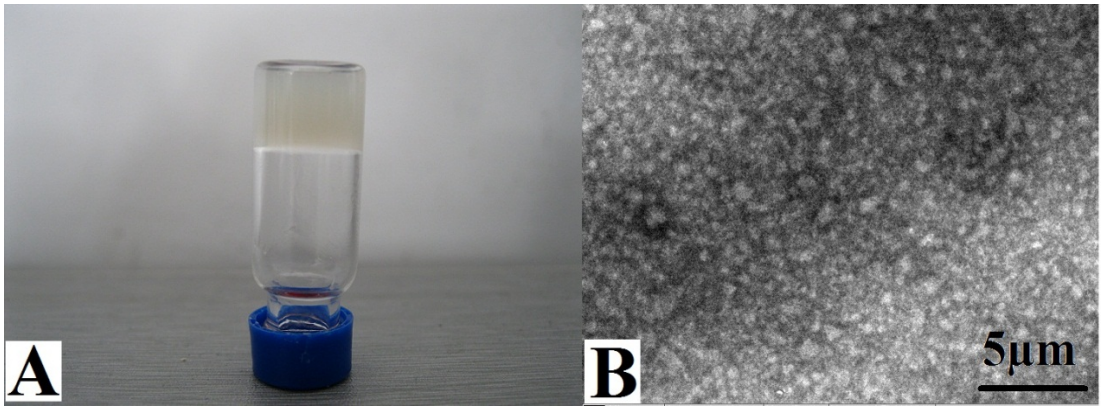

Fig. S6. Digital image (A) and TEM image (B) of 3 wt% Copolymer **1**/6 wt% α-CD hydrogels.