Supplementary Information

Redox-Responsive, Core Cross-linked Polyester Micelles

Zhonghai Zhang,[†,‡,§] Lichen Yin,[†,§] Chunlai Tu, [†] Ziyuan Song,[†] Yanfeng Zhang,[†] Yunxiang Xu,[†] Rong Tong,[†] Qin Zhou, [†,♯] Jie Ren*[,‡] and Jianjun Cheng*[,†]

[†] Department of Materials Science and Engineering, University of Illinois at Urbana–Champaign, 1304 W. Green Street, Urbana, IL, 61801, USA

[‡] Institute of Nano- and Bio-polymeric Materials, School of Material Science and Engineering, Tongji University, Shanghai, 200092, China

[♯] Department of Pharmaceutical Science, Guangdong Pharmaceutical University, Guangzhou, Guangdong, China

[§] Equal contribution.

*Corresponding Author: jianjunc@illinois.edu; renjie@tongji.edu.cn;

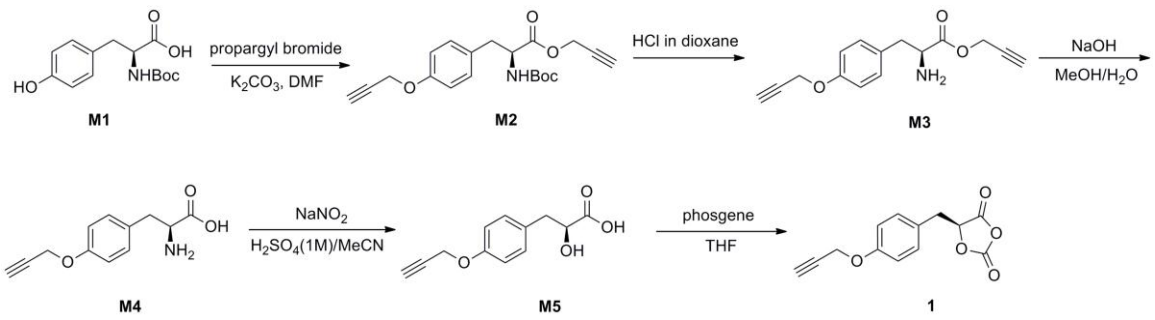
Experimental section

Materials. Boc-$_L$-tyrosine was purchased from Chem-Impex International (Des Plaines, IL, USA) and used as received. Anhydrous dichloromethane (DCM), hexane and tetrahydrofuran (THF) were dried by columns packed with alumina and stored in a glove box. Anhydrous dimethylformamide (DMF) was dried by passing the solvent through a column packed with 4Å molecular sieves. All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used as received unless otherwise specified.

Instrumentation. NMR spectra were recorded on a Varian U500 (500 MHz) or a VXR-500 (500 MHz) spectrometer. Gel permeation chromatography (GPC) experiments were performed on a system 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 (100 Å, 500 Å, 10^3 Å and 10^4 Å Phenogel columns, 5 μm, 300 × 7.8 mm, Phenomenex, Torrance, CA, USA) at 60 °C using DMF containing 0.1 M LiBr as the mobile phase. The MALLS detector was calibrated using pure toluene with no need for calibration using polymer standards and was used for the determination of the absolute molecular weights (MWs). The molecular weight of polymer was determined from the *d*n/*d*c value calculated offline by means of the internal calibration system processed by the ASTRA V software (Version 5.1.7.3, Wyatt Technology). Infrared spectra were recorded on a Perkin Elmer 100 serial FTIR

spectrophotometer calibrated with polystyrene film. Lyophilization was conducted on a Labconco FreeZone lyophilizer (Kansas City, MO, USA). Particle size and dispersity were measured on a Malvern Zetasizer Nano (Malvern, Worcestershire, UK). Fluorescence spectrum was recorded on a PekinElmer LS 55 fluorescence spectrometer (Santa Clara, CA, USA). Transmission electron microscopy (TEM) experiments were performed on a JEOL 2100 Cryo TEM at 80 kV. Samples were prepared by drop-casting micelle solutions onto 200 mesh carbon film supported copper grid (Electron Microscopy Sciences, Hatfield, PA, USA) and then air-drying at room temperature before measurement.

Scheme S1. Synthesis of Tyr(alkynyl)-OCA (**1**).



Synthesis of (*S*)-2-Amino-3-(4-(prop-2-yn-1-yloxy)phenyl)propionic acid propargyl ester (M3). Boc-$_L$-tyrosine (9.30 g, 33.1 mmol, 1 equiv.) and Potassium carbonate (13.75 g, 99.5 mmol, 3 equiv.) were suspended in anhydrous DMF (80 mL). Propargyl bromide (11 mL, 98.8 mmol, 3 equiv., 80 wt.% in toluene) was added dropwise at 0 ^{o}C. The reaction mixture was stirred for 24 h at room temperature. Water (300 mL) and ethyl ether (250 mL) were added. The aqueous phase was extracted with ethyl ether (3 × 150 mL). The combined organic phase was dried over anhydrous sodium sulfate and evaporated to give a yellow oil ((S)-prop-2-yn-1-yl 2-((tert-butoxycarbonyl)amino)-3-(4-

(prop-2-yn-1-yloxy)phenyl)propanoate, **M2**), which was used in the next step without further purification. The deprotection of **M2** was performed with HCl/dioxane (4 M, 150 mL) at room temperature for 24 h. After the solvent was removed under reduced pressure, a yellowish solid (**M3**, 9.36 g, 96 % for two steps) was obtained. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 7.21 (d, 2H, Ar*H*), 6.93 (d, 2H, Ar*H*), 4.80 (d, 2H, -COOCH_2C≡CH), 4.77 (d, 2H, -PhOCH_2C≡CH), 4.26 (t, 1H, -*CH*NH_2), 3.69 (t, 1H, -COOCH$_2$C≡C*H*), 3.58 (t, 1H, -PhOCH$_2$C≡C*H*), 3.07-3.18 (m, 2H, -CH_2PhOCH$_2$C≡CH). ^{13}C NMR (DMSO-d_6, 500 MHz): δ 168.3, 156.5, 130.7, 126.9, 114.8, 77.4-79.2, 55.3, 53.2, 34.8. ESI-MS (m/z): Calcd $C_{15}H_{15}NO_3$ 257.1 (M); found: 258.2 $(M+H)^+$.

Synthesis of 2-Amino-3-(4-(prop-2-yn-1-yloxy)phenyl)propionic Acid (M4). The compound **M3** (9.36 g, 31.9 mmol) from the previous step was dissolved in H_2O/methanol (1:1, v/v, 100 mL) containing NaOH (4.00 g, 100 mmol). The reaction solution was stirred for 16 h at room temperature and then neutralized by adding concentrated HCl. Water (50 mL) was added and the mixture was kept at 4 ^{o}C overnight. The precipitate was filtered, washed with ice-cold H_2O, and lyophilized. A white powder was obtained (**M4**, 5.09 g, 73 % yield). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 7.19 (d, 2H, Ar*H*), 6.91 (d, 2H, Ar*H*), 4.74 (d, 2H, -PhOCH_2C≡CH), 4.07 (t, 1H, -*CH*NH_2), 3.52 (t, 1H, -PhOCH$_2$C≡C*H*), 3.03-3.11 (m, 2H, -CH_2PhOCH$_2$C≡CH). ^{13}C NMR (DMSO-d_6, 500 MHz): δ 170.3, 156.7, 130.9, 127.7, 115.1, 79.6, 78.5, 55.6, 53.3, 34.9. ESI-MS (m/z): Calcd $C_{12}H_{13}NO_3$ 219.1 (M); found: 220.1 $(M+H)^+$.

Synthesis of 2-Hydroxy-3-(4-(prop-2-yn-1-yloxy)phenyl)propionic Acid (M5). In a 50-mL round-bottom flask, **M4** (4.38 g, 20.0 mmol) was dissolved in a mixture of

sulfuric acid (40 mL, 1 M) and acetonitrile (40 mL). Sodium nitrite (2.76 g, 40.0 mmol) dissolved in water (20 mL) was added slowly to the **M4** solution. The mixture was stirred under nitrogen for 16 h and then extracted with dichloromethane (200 mL × 3). The combined organic layers were dried, filtered, and concentrated under vacuum, giving the product as a solid (3.15 g, 72 %). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.18 (d, 2H, Ar*H*), 6.92 (d, 2H, Ar*H*), 4.66 (d, 2H, -PhOC*H*$_2$C≡CH), 4.45 (m, 1H, -*CH*OH), 2.90-3.14 (m, 2H, -C*H*$_2$PhOCH$_2$C≡CH), 2.52 (t, 1H, -PhOCH$_2$C≡C*H*), ^{13}C NMR ($CDCl_3$, 500 MHz): δ 178.3, 156.7, 130.7, 129.0, 115.1, 78.7, 75.8, 71.2, 55.9, 39.3. ESI-MS (m/z): Calcd $C_{12}H_{12}O_4$ 220.1 (M); found: 218.9 $(M-H)^-$.

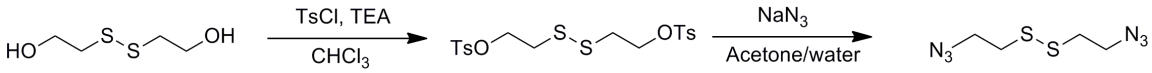
Synthesis of 5-[4-(Prop-2-yn-1-yloxy)benzyl]-1,3-dioxolane-2,4-dione (Tyr(alkynyl)-OCA, 1). To an anhydrous THF solution (40 mL) containing **M5** (3.15 g, 14.3 mmol), phosgene (23 mL, 32.2 mmol, 15 wt. % in toluene) was added dropwise at 0 ^{o}C. The mixture was stirred at room temperature for 16 h. The solvent was removed under vacuum. The residue was purified by silica gel chromatography in a glove box with DCM as the eluent (Note: The silica gel was pre-treated by drying it at 150 ^{o}C for 8 h under vacuum). The solution was collected and the DCM was evaporated under reduced pressure to give a yellow solid. The crude OCA was recrystallized in hexane/CH_2Cl_2 in glove box. A colorless OCA monomer **1** was obtained in crystalline form (2.02 g, yield: 57.4 %). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.17 (d, 2H, Ar*H*), 6.96 (d, 2H, Ar*H*), 5.27 (t, 1H, alpha-*H*), 4.68 (d, 2H, -PhOC*H*$_2$C≡CH), 3.19-3.35 (m, 2H, -C*H*$_2$PhOCH$_2$C≡CH), 2.52 (t, 1H, -PhOCH$_2$C≡C*H*), ^{13}C NMR ($CDCl_3$, 500 MHz): δ 166.5, 157.8, 147.9, 131.0, 124.5, 115.7, 80.1, 78.4, 75.9, 56.0, 35.8.

Scheme S2. Synthesis of 1,4-diazidobutane (**2**).

2

Synthesis of 1,4-diazidobutane (2). 1,4-dibromobutane (3.02 g, 14.0 mmol) and sodium azide (2.20 g, 33.8 mmol) were dissolved in DMF (20 mL) and stirred at room temperature for 24 h. DI water (30 mL) was added to quench the reaction, and the solution was extracted with ethyl ether (100 mL). The organic phase was combined, washed with saturated sodium bicarbonate solution (2 × 30 mL), and dried over $MgSO_4$. After filtration and solvent evaporation, a colorless oil (**2**, 1.67 g, 85 % yield) was obtained. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.26 (t, 4H, $N_3CH_2CH_2$-), 1.61 (m, 4H, $N_3CH_2CH_2$-), ^{13}C NMR ($CDCl_3$, 500 MHz): δ 51.1, 26.3.

Scheme S3. Synthesis of bis-(azidoethyl) disulfide (**3**).



3

Synthesis of bis-(tosylate ethyl) disulfide. 2-hydroxyethyl disulfide (3.08 g, 0.02 mol) and triethylamine (TEA, 5.06 g, 0.05 mol) were dissolved in chloroform (30 mL) and cooled to 0 °C in an ice bath. *p*-toluenesulfonyl chloride (TsCl, 11.45 g, 0.15 mol) was dissolved in chloroform (30 mL) and added dropwise to the above mixture via an addition funnel. The reaction was allowed to proceed under stirring at room temperature for 16 h, after which the reaction mixture was filtered to remove the TEA salts. The filtrate was washed with saturated sodium carbonate solution (3 × 75 mL) and then with

DI water (2 × 100 mL). The washed solution was dried over $MgSO_4$, filtered, and chloroform was evaporated under vacuum to give the tosylate intermediate (7.30 g, 79 % yield). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.44 (s, 6H, (-S-CH_2CH_2-Ph-C*H*$_3$)$_2$), 2.83 (t, 4H, (-S-C*H*$_2$$CH_2$-Ph-$CH_3$)$_2$), 4.19 (t, 4H, (-S-$CH_2$C*H*$_2$-Ph-$CH_3$)$_2$, 7.35 (d, 2H, Ar*H*), 7.78 (d, 2H, Ar*H*). ^{13}C NMR ($CDCl_3$, 500 MHz): δ 21.9, 37.2, 67.7, 128.2, 130.2, 132.8, 145.4.

Synthesis of cross-linker bis-(azidoethyl) disulfide (3). Bis-(tosylate ethyl) disulfide (6.90 g, 15.0 mmol) obtained from the previous step was dissolved in acetone (50 mL); sodium azide (5.85 g, 90.0 nmol) was dissolved in DI water (30 mL) and added to the acetone solution. The stirred mixture was refluxed at 80 ^{o}C overnight followed by removal of acetone under reduced pressure. The product was extracted with dichloromethane (DCM, 4 × 50 mL), and the organic layers were combined, dried over $MgSO_4$, filtered, and concentrated under vacuum, giving an orange-yellow oil (2.50 g, 82 % yield). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.85 (t, 4H, (N_3C*H*$_2$$CH_2$S-)$_2$), δ 3.57 (t, 4H, ($N_3CH_2$C*H*$_2$S-)$_2$). ^{13}C NMR ($CDCl_3$, 500 MHz): δ 37.8, 50.1.

Synthesis of monomethoxy poly(ethylene glycol)-*b*-Poly(Tyr(alkynyl)-OCA) (mPEG-*b*-poly(1)). Polymerization was proceeded in the glove box. **1** (49.2 mg, 0.2 mmol, 20 equiv.) was dissolved in DCM (1 mL) followed by addition of monomethoxy poly(ethylene glycol) (50 mg, 0.01 mmol, MWs = 5,000 mg/mol, 1 equiv.) and DMAP (100 μL, 0.1 M, 1 equiv.). The complete monomer consumption was confirmed by measuring the intensity of the anhydride peak of OCA at 1810 cm^{-1} by FTIR (Figure S1). After the polymerization was completed, mPEG$_{5k}$-*b*-poly(**1**)$_{20}$ was precipitated with ethyl

ether, collected by centrifugation, and dried under vacuum (87.5 mg, 96.8 % yield).

Preparation of uncross-linked (UCL) micelles and determination of critical micelle concentration (CMC). $mPEG_{5k}$-*b*-poly(**1**)$_{20}$ (10.0 mg) was dissolved in DMF (2 mL) into which DI water (20 mL) was slowly added under vigorous stirring. After vigorous stirring for another 2 h at room temperature, the UCL micelles were obtained and further dialyzed against DI water for 24 h to remove DMF (MWCO of 3500 Da).

The CMC of the micelle was determined using Nile Red (NR) as a fluorescence probe.[1] NR in THF (0.1 mg/mL, 10 μL) was added to a glass vial via a microsyringe. After THF was evaporated, a micellar solution (2 mL) was added. The concentration of the micellar solution was varied from 3×10^{-2} to 5×10^{-4} mg/mL. Then the solution was racked for more than 1 h. Finally, fluorescence spectra were recorded with the excitation wavelength at 557 nm.

Preparation of core-cross-linked (CCL) micelles. CCL micelles were prepared according to described procedures.[2] $mPEG_{5k}$-*b*-poly(**1**)$_{20}$ (10.0 mg, 1.1×10^{-3} mmol) and cross-linker **2** (1.4 mg, 1.3×10^{-2} mmol, 0.6 mol equiv. of alkynyl group) were dissolved in DMF (2 mL), into which DI water (20 mL) was slowly added under stirring by using a microsyringe. After stirring overnight, Copper(II) chloride ($CuCl_2$, 112 μL, 5 mg/mL, 4.1×10^{-3} mmol, 0.25 mol equiv. of alkynyl group) and ascorbic acid sodium salt (164 μL, 5 mg/mL, 4.1×10^{-3} mmol, 0.25 mol equiv. of alkynyl group) were added to the above mixture and crosslinking was allowed to proceed for 24 h at room temperature. The CCL

micelles were thus achieved and subsequently purified by dialysis against DI water for 24 h (MWCO of 3500 Da).

Preparation of NR-containing CCL micelles. NR-containing CCL micelles were prepared by the same procedure as for blank CCL micelles. $mPEG_{5k}$-*b*-poly(**1**)$_{20}$ (10.0 mg, 1.1×10^{-3} mmol) and cross-linker **3** (2.7 mg, 1.3×10^{-2} mmol, 0.6 mol equiv. of alkynyl group) were dissolved in DMF (2 mL). NR in DMF (10 mg/mL, 100 μL) was added to the above solution. DI water (20 mL) was then added dropwise under stirring. After stirring overnight, $CuCl_2$ (112 μL, 5 mg/mL, 4.1×10^{-3} mmol, 0.25 mol equiv. of alkynyl group) and ascorbic acid sodium salt (164 μL, 5 mg/mL, 4.1×10^{-3} mmol, 0.25 mol equiv. of alkynyl group) were added and crosslinking was allowed to for 24 h at room temperature to prepare the CCL micelles. Then the cross-linked micelles obtained after purified by dialysis against DI water for 24 h (MWCO of 3500 Da).

Redox-triggered NR release from CCL micelles. The study of the redox-triggered release of NR in particles is similar as described procedures.[3] The different NR-containing CCL micelles (CCL2 to 5, the mole ratio of cross-linker **2** and **3** is 2/8, 5/5, 8/2 and 10/0, respectively) were prepared by the same procedure as the above NR-containing CCL process. The concentration of the CCL micelles was diluted to 6×10^{-3} mg/mL which is below its CMC (15.9×10^{-3} mg/mL). Then the NR-containing CCL micelles (2 mL, 6×10^{-3} mg/mL) were gently shocked with adding D,L-dithiothreitol (DTT, 10 mM) or without DTT. The release properties of the CCL micelles were analyzed by measuring the fluorenscence intensity of the solution at selected time points.

Supplementary Figures

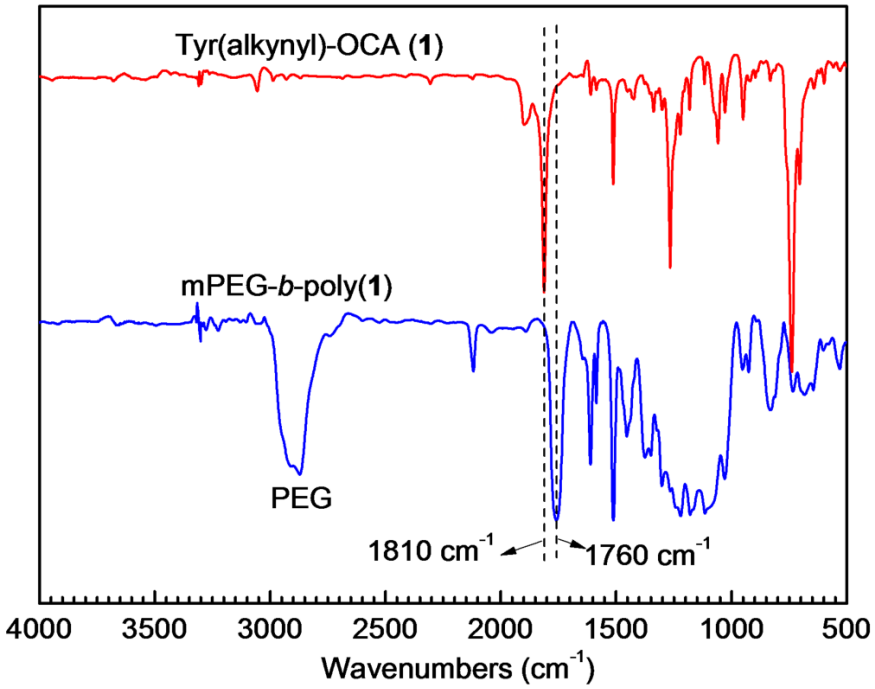


Figure S1. FTIR spectra of Tyr(alkynyl)-OCA (**1**) and mPEG-*b*-poly(**1**). After the polymerization was completed, the anhydride band of **1** at 1810 cm^{-1} disappeared while an ester band of poly(**1**) at 1760 cm^{-1} was observed.

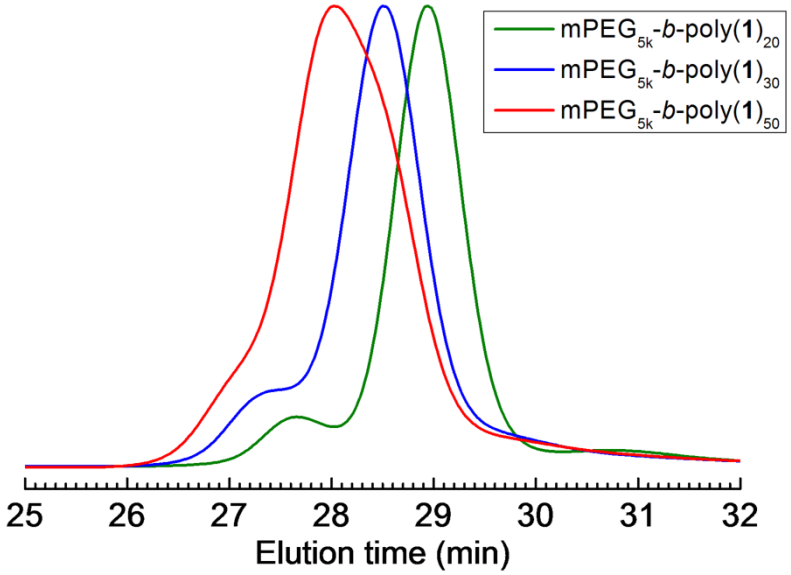


Figure S2. Overlay of GPC curves of mPEG$_{5k}$-*b*-poly(**1**)$_{20}$ (green), mPEG$_{5k}$-*b*-poly(**1**)$_{30}$ (blue), and mPEG$_{5k}$-*b*-poly(**1**)$_{50}$ (red).

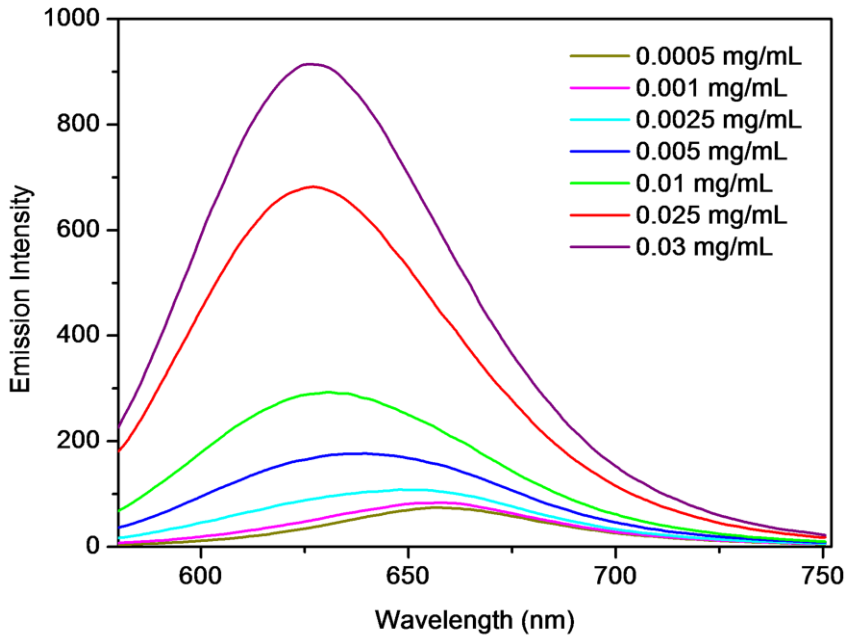


Figure S3. The emission spectra of mPEG$_{5k}$-*b*-poly(**1**)$_{20}$ solution at different polymer concentrations with excitation of 557 nm using NR as the probe.

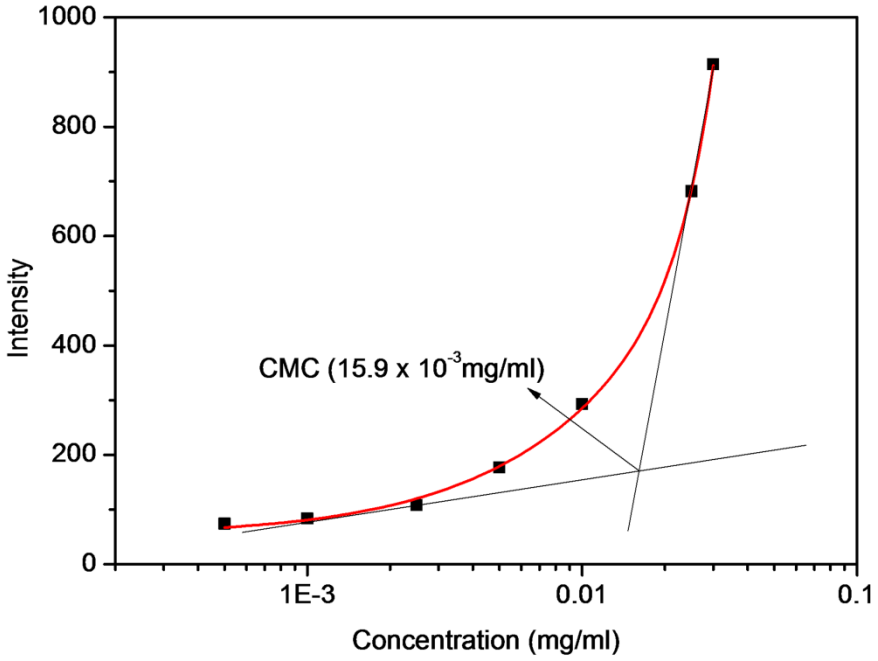


Figure S4. The fluorescence intensity at the maximum emission wavelength as a function of mPEG$_{5k}$-*b*-poly(**1**)$_{20}$ concentration when using NR as the probe.

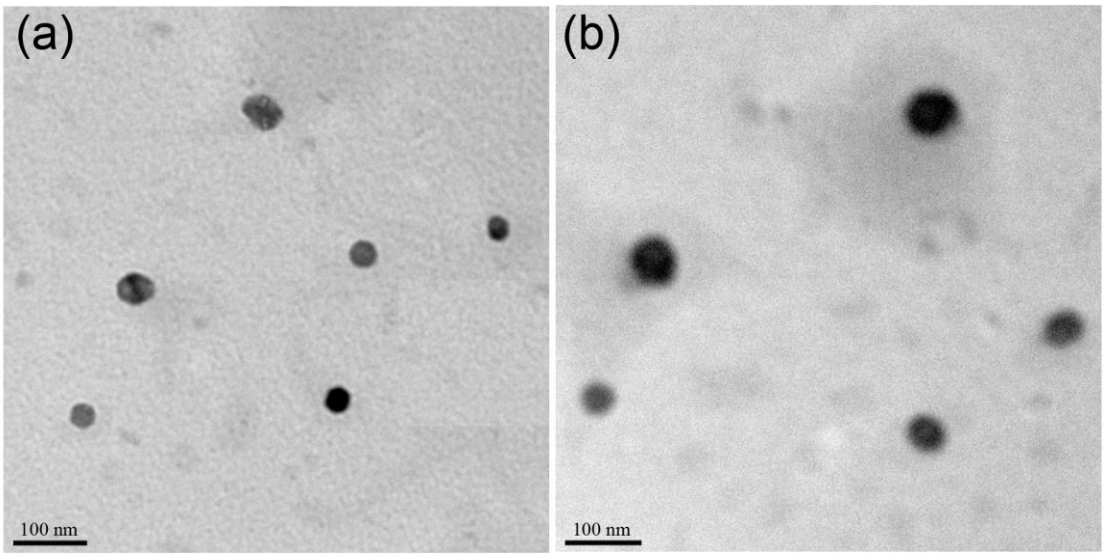


Figure S5. TEM image of UCL (a) and CCL micelles (b). CCL micelles were prepared with 1,4-diazidobutane (**2**) as the cross-linker.

References

(1) Chen, C. J.; Liu, G. Y.; Liu, X. S.; Pang, S. P.; Zhu, C. S.; Lv, L. P.; Ji, J. *Polym. Chem.* **2011**, *2*, 1389-1397.

(2) Cajot, S.; Lautram, N.; Passirani, C.; Jerome, C. *J. Control. Release* **2011**, *152*, 30-36.

(3) Lv, C.; Wang, Z.; Wang, P.; Tang, X. *Langmuir* **2012**, *28*, 9387-94.