Electronic Supplementary Material (ESI) for Polymer Chemistry

Supplementary Information

GFP-Inspired Fluorescent Polymer

Hongping Deng,[ab] Qi Zhu,[a] Dali Wang,[a] Chunlai Tu,[a] Bangshang Zhu[b] and Xinyuan Zhu[ab]

[a] *School of Chemistry and Chemical Engineering, State Key Laboratory of Metal Matrix Composites, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, P.R. China.*

[b] *Instrumental Analysis Center, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, P.R. China.*

1. Experimental Section

1.1 Materials and methods

N-Acetylglycine (99%, Alfa), ethanolamine (98%, Alfa), 2-bromoisobutyryl bromide (97%, Alfa), were used as received. Dichloromethane (CH_2Cl_2), triethylamine and toluene were heated at reflux with CaH_2, and ethanol (EtOH) was heated at reflux with calcium oxide, and then distilled prior to use. Methyl methacrylate (MMA) was dried with CaH_2 and distilled before use. Succinic anhydride (Ac_2O) was directly distilled under reduced pressure. Cuprous bromide (CuBr) was washed with acetic acid and ethanol, and then dried at 50 °C for 24 h. Poly(ethylene glycol) monomethyl ether (PEG, Mn=2,000), 4-toluene sulfonyl chloride (Ts-Cl), 4-hydroxybenzaldehyde, and other reagents and solvents were purchased from Shanghai Sinopharm reagent Co. Ltd., Shanghai, and used without further purification unless otherwise mentioned.

The tests such as ^1H and ^{13}C nuclear magnetic resonance (^1H and ^{13}C NMR), Fourier transform infrared (FT-IR), gel permeation chromatography (GPC) were used to confirm the constituents, structure, molecular weight, and other properties. ^1H and ^{13}C NMR spectra were registered on a Varian MERCURY plus-400 spectrometer with dimethyl sulfoxide-d_6 (DMSO-d_6) or deuterated chloroform ($CDCl_3$) as the solvents at 298 K. FT-IR spectra were measured as KBr pellets on a Perkin Elmer Paragon 1000 spectrophotometer in the range of 4000–450 cm^{-1}. The relative molecular weights and molecular weight distributions were determined by GPC measurements on a Perkin Elmer Series 200 system (100 mL injection column, PL gel 10 mm 300×7.5 mm mixed-B columns, poly(methyl methacrylate) calibration. Dimethyl formamide (DMF) containing 0.05 mol/L LiBr was utilized as the mobile phase at a flow rate of 1.0 mL/min). High resolution mass spectrometer (HRMS) was performed on a Waters Micromass Q-TOF Premier Mass Spectrometer. HRMS data were acquired for each sample from 50 to 1000 Da with a 0.10 s scan time and a 0.01 s interscan delay over a 10 min analysis time. UV-Vis absorption spectra were performed on a Perkin Elmer Lambda 20 UV-Vis spectrometer in the range of 300–700 nm. The fluorescence emission measurements were carried out on a Perkin Elmer LS 50B fluorescence spectrometer in the range of 390–650 nm. Excitation wavelength was λ_{ex}=371 nm. Dynamic light scattering (DLS) measurements were performed with a Malvern Zetasizer Nano ZS90 apparatus equipped with a 4.0 mW He-Ne laser operating at λ= 633 nm. All samples were measured at room temperature and at a scattering angle of 90°. Transmission electron microscopy (TEM) studies were performed with a JEOL JEM-100CX-II instrument at a voltage of 200 kV. Sample was prepared by drop-casting micelle solutions onto carbon-coated copper grids and then air-drying at room temperature before measurement. For self-assembly experiments, a tetrahydrofuran (THF) solution of poly(ethylene glycol)-chromophore-poly(methyl methacrylate) (PEG-c-PMMA) with a concentration of 3 mg/mL was slowly added to 2.5 mL water under stirring with a magnetic bar. The solution was stirred for 1 h before measurements.

1.2 Cell culture and internalization

MCF-7 cells (a human breast adenocarcinoma cell line) were seeded in flexiPERM reusable cell culture chambers in combination of glass coverslips and cultured in DMEM (Dulbecco's modified Eagle's medium) supplied with 10% FBS (fetal bovine serum), and antibiotics (50 units/mL penicillin and 50 units/mL streptomycin) at 37 °C under a humidified atmosphere containing 5% CO_2. After 24 h of culture, the self-assembled micelles and control solution dissolved in DMEM

culture medium with a fluorescent chromophore concentration of about 3 μg/mL were added to culture wells respectively, and the cells were incubated at 37 °C for 6 h. Washed with PBS for 3 times, the cells were fixed with 4% formaldehyde for 10 min at room temperature, and then the slides were mounted and observed with a LSM510 META. The excitation wavelength of all samples was 405 nm.

2. Synthesis

2.1 Synthesis of 4-[(Z)-4-acetoxybenzylidene]-2-methyloxazol-5(4H)-one (1)

A mixture of p-hydroxybenzaldehyde (2.44 g, 20 mmol), N-acetylglycine (2.34 g, 20 mmol), NaOAc (1.64 g, 20 mmol) and Ac_2O (30 mL) was heated at 110 °C for 5 h under a nitrogen atmosphere. Upon completion, the mixture was cooled to room temperature. Then ice-cold water (40 mL) was added. Finally, the precipitate was filtered, washed with water and cold ethanol, and dried at 40 °C to give a yellow powder (2.01 g, 42%).

^{1}H NMR (400 MHz, DMSO-d_6, 298 K): δ (ppm): 8.24-8.20 (d, 2H, Ar), 7.29-7.24 (t, 3H, CH= and Ar), 2.40 (s, 3H, CH3), 2.30 (s, 3H, CH3).

^{13}C NMR (400 MHz, DMSO-d_6, 298 K): δ (ppm): 169.5, 168.0, 167.4, 153.0, 133.9, 133.1, 131.4, 130.0, 123.0, 21.5, 16.0.

HRMS: m/z calculated for $[C13H12NO4]^+$: 246.0766, found: 246.0762.

IR (KBr): 3043, 1798, 1760, 1658, 1607, 1592, 1506, 1422, 1375, 1314, 1298, 1264, 1233, 1216, 1198, 1173, 1160, 1038, 1018, 921, 893, 871, 856, 825, 764, 696, 673, 651, 631, 592, 541, 515, 484 cm^{-1}.

2.2 Synthesis of (Z)-1-(2-Hydroxyethyl)-4-(4-hydroxybenzylidene)-2-methyl-1H- imidazol -5(4H)-one (the chromophore)

Compound 1 (4.90 g, 20 mmol) and anhydrous potassium carbonate (K_2CO_3) (2.70 g, 20 mmol) were dissolved in 50 mL anhydrous ethanol. Then, an ethanol solution (50 mL) of ethanolamine (2.50 g, 42 mmol) was added dropwise and the mixture was refluxed for 4 h under N_2 protection. The mixture was cooled to room temperature and concentrated under reduced pressure, and the residue was purified by silica gel column chromatography using CH_2Cl_2 and CH_3OH (v/v=10:1) as the solvent. A crude yellow solid was obtained after rotary evaporation under reduced pressure, and further washed with CH_2Cl_2 to give the pure yellow solid. (2.31 g, 47%).

^{1}H NMR (400 MHz, DMSO-d_6, 298 K): δ (ppm): 10.07 (s, 1H, ArOH), 8.07-8.05 (d, 2H, Ar), 6.86 (s, 1H, CH=), 6.83-6.81(d, 2H, Ar), 4.95-4.92 (t, 1H, OH), 3.60-3.58 (t, 2H,CH2), 3.52-3.48 (m, 2H, CH2), 2.34 (s, 3H, CH3).

^{13}C NMR (400 MHz, DMSO-d_6, 298 K): δ (ppm): 170.6, 163.1, 160.2, 136.9, 134.8, 126.1, 116.4, 59.6, 43.5, 16.2.

HRMS: m/z calculated for $[C13H13N2O3]^-$: 245.0926, found: 245.0921.

IR (KBr): 3193, 2971, 2815, 1708, 1641, 1600, 1556, 1516, 1453, 1416, 1366, 1302, 1286, 1256, 1235, 1172, 1144, 1105,1065, 1035, 1019, 974, 917, 852, 834, 765, 724, 664, 633, 600, 536, 514, 487 cm^{-1}.

2.3 Synthesis of PEG-Ts

PEG-OH (10.0 g, 5 mmol) was dissolved in 100 mL CH_2Cl_2 and cooled to 0 °C. Then, 4-toluene sulfonyl chloride (Ts-Cl) (5.76 g, 30 mmol) and KOH (1.70 g, 30 mmol) were added, respectively. After 3 h, another amount of KOH (0.56 g, 10 mmol) was added and the mixture was kept overnight. Upon completion, the mixture was filtered, washed with water, dried with anhydrous magnesium sulfate, then filtered, concentrated under reduced pressure, and precipitated into cold diethyl ether twice and filtered to obtain a white solid. (7.88 g, 75%).

^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ (ppm): 7.80-7.78 (d, 2H, phenyl), 7.35-7.32 (d, 2H, phenyl), 4.15-4.13 (t, 2H, -CH2-OSO2-), 3.82-3.44 (PEG, 2H per repeating unit, -O-CH2CH2-), 3.37 (s, 3H, CH3-O-), 2.44 (s, 3H, CH3-phenyl).

2.4 Synthesis of PEG-c

A mixture of PEG-Ts (2.10 g, 1 mmol), K_2CO_3 (2.72 g, 20 mmol) and chromophore (0.98 g, 4 mmol) was dissolved in 200 mL acetonitrile (CH_3CN), and refluxed at 110 °C for 32 h. After cooled to room temperature, acetonitrile was removed under reduced pressure and CH_2Cl_2 was added. The mixture was filtered, and then washed with water. After dried with anhydrous magnesium sulfate, the mixture was precipitated into cold diethyl ether twice to obtain a light yellow solid. (1.30 g, 60%).

^{1}H NMR (400 MHz, DMSO-d_6, 298 K): δ (ppm): 8.17-8.15 (d, 2H, phenyl), 7.02-7.00 (d, 2H, phenyl), 6.90 (s, 1H, -CH=C-), 4.94-4.92 (t, 1H, HOCH2-), 4.15-4.12 (t, 2H, -CH2-O-phenyl), 3.75-3.30 (PEG, 2H per repeating unit, -O-CH2CH2-), 3.22 (s, 3H, CH3-O-), 2.36 (s, 3H, -CH3).

^{13}C NMR (400 MHz, $CDCl_3$, 298 K): δ (ppm): 171.3, 162.1, 160.1, 136.7, 134.2, 127.6, 127.3, 115.0, 72.1, 70.7, 69.7, 67.6, 60.9, 59.2, 43.6, 16.1.

IR (KBr): 3423, 2888, 1704, 1645, 1602, 1511, 1466, 1410, 1360, 1344, 1281, 1257, 1243, 1147, 1114, 1061, 964, 843, 530 cm^{-1}.

2.5 Synthesis of PEG-c-Br

PEG-c (660 mg, 0.3 mmol) was dissolved in 15 mL CH_2Cl_2 and cooled to 0 °C. Triethylamine (0.36 mL, 2.6 mmol) and 2-bromoisobutyryl bromide (0.34 mL, 2.6 mmol) were added dropwise, respectively. The mixture was kept for 22 h and diluted with CH_2Cl_2, washed with water, concentrated under reduced pressure, and precipitated into cold diethyl ether twice to obtain a brown solid. (500 mg, 77%).

^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ (ppm): 8.16-8.13 (d, 2H, phenyl), 7.18 (s, 1H, -CH=C-), 7.00-6.98 (d, 2H, phenyl), 4.41-4.38 (t, 2H, -CH2-O-phenyl), 4.20-3.45 (PEG, 2H per repeating unit, -O-CH2CH2-), 3.37 (s, 3H, CH3-O-), 2.68 (s, 3H, -CH3), 1.90 (s, 6H, (CH3)2-).

^{13}C NMR (400 MHz, $CDCl_3$, 298 K): δ (ppm): 171.6, 135.2, 115.5, 72.1, 70.7, 69.6, 67.8, 63.1, 59.2, 55.2, 40.1, 30.9, 15.2.

IR (KBr): 2880, 1738, 1708, 1644, 1601, 1511, 1467, 1359, 1344, 1281, 1255, 1113, 963, 949, 843, 531 cm^{-1}.

2.6 Synthesis of PEG-c-PMMA

PEG-c-PMMA was synthesized by atom transfer radical polymerization (ATRP) using PEG-c-Br as the macroinitiator. CuBr (29 mg, 0.2 mmol) and 2,2'-bipyridine (Bpy) (78 mg, 0.5 mmol) were added into a reaction flask under N_2 protection, and then 2.2 mL of toluene was added. Subsequently, 230 mg (0.1 mmol) of the macroinitiator was added and degassed under nitrogen purge. At last, methyl methacrylate (2.2 mL, 20 mmol) was added into the flask and polymerization was performed at 80 °C for 8 h. The product was solubilized in THF and purified through an aluminum column to remove the copper complex. The solution was concentrated, precipitated into cold diethyl ether twice, filtered and dried at 40 °C for 24 h. (800 mg, 36%).

^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ (ppm): 3.66-3.64 (PEG, 2H per repeating unit, -O-CH2CH2-), 3.63-3.56 (methoxy groups of PMMA, -OCH3), 2.06-1.42 (methylene groups of PMMA, -CH2-), 1.01-0.84 (methyl groups of PMMA, -CH3).

^{13}C NMR (400 MHz, $CDCl_3$, 298 K): δ (ppm): 178.6, 178.3, 178.0, 177.3, 177.2, 70.7, 54.6, 54.4, 53.6, 53.3, 52.8, 50.0, 45.0, 44.7, 19.1, 18.9, 16.6.

IR (KBr): 2998, 2953, 1732, 1634, 1486, 1450, 1386, 1272, 1243, 1194, 1149, 1065, 989, 966, 913, 842, 751, 483 cm^{-1}.

3. Analytical and Spectral Characterization Data

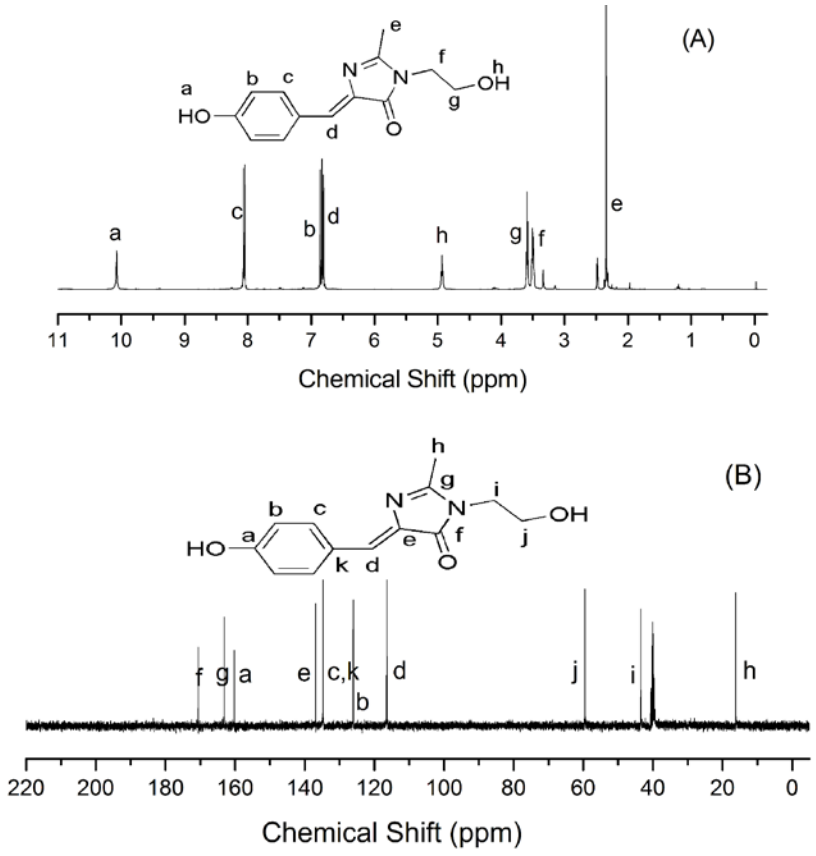

Fig. S1 ^{1}H NMR (A) and ^{13}C NMR (B) spectra of the chromophore in DMSO-d_6.

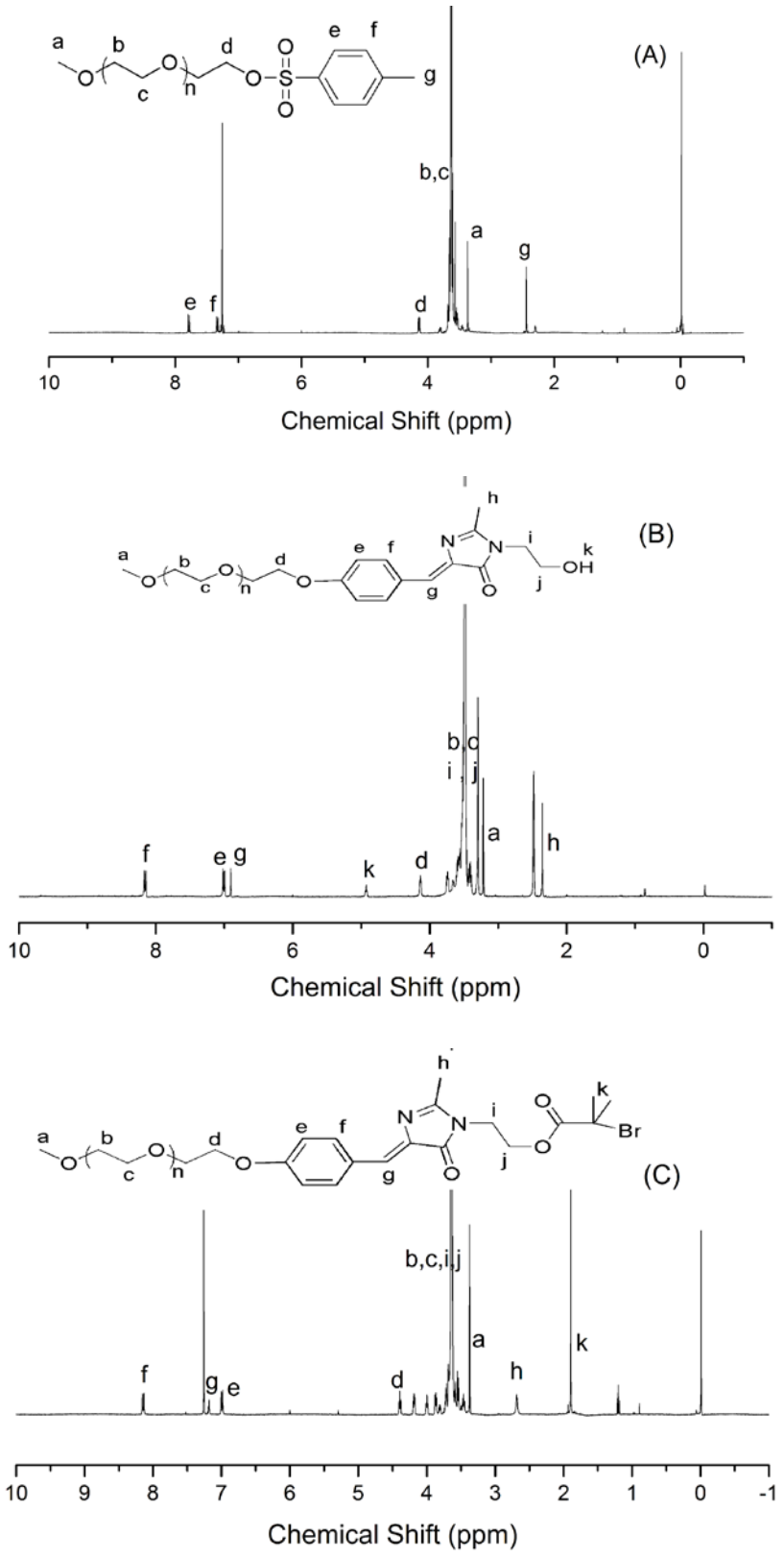

Fig. S2 ^{1}H NMR spectra of PEG-Ts (A), PEG-c (B) and PEG-c-Br (C) in $CDCl_3$.

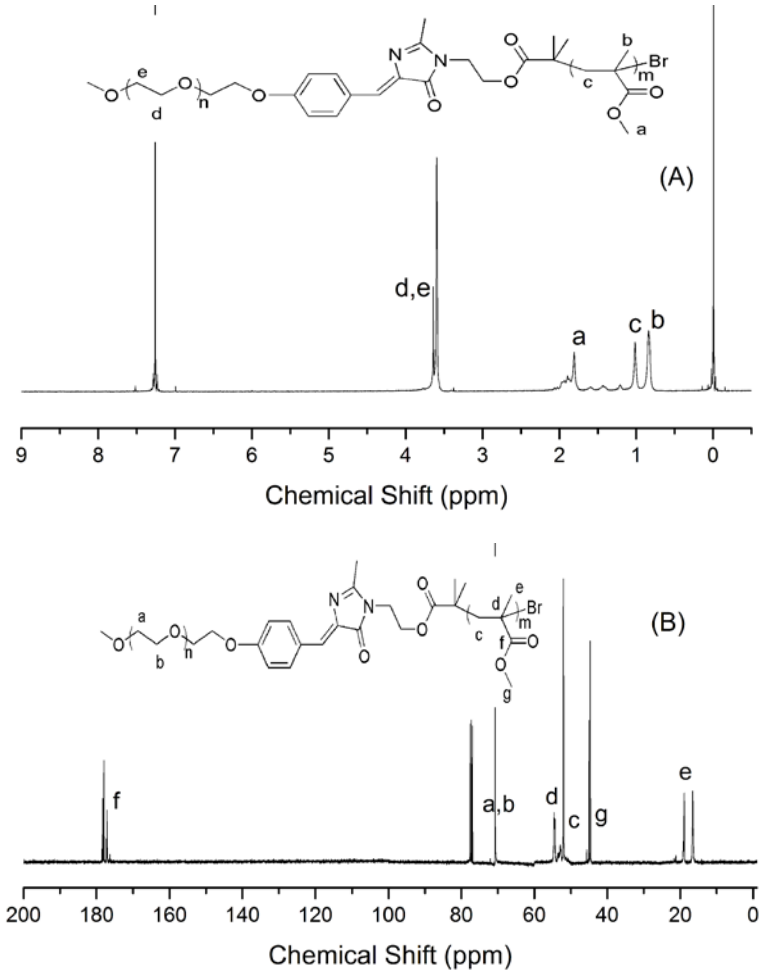

Fig. S3 ^{1}H NMR (A) and ^{13}C NMR (B) spectra of PEG-c-PMMA in $CDCl_3$.

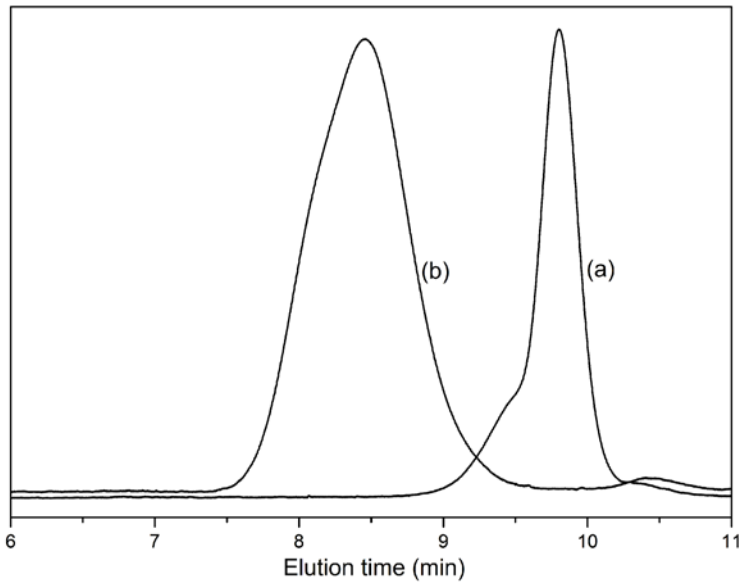

Fig. S4 Gel permeation chromatography (GPC) curves of PEG-c-Br (a) and PEG-c-PMMA (b).

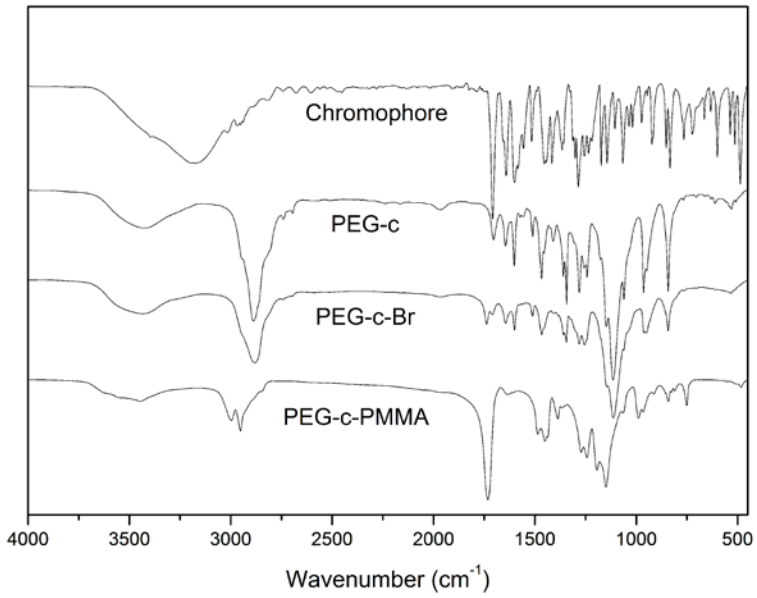

Fig. S5 The infrared spectra of the chromophore, PEG-c, PEG-c-Br and PEG-c-PMMA.

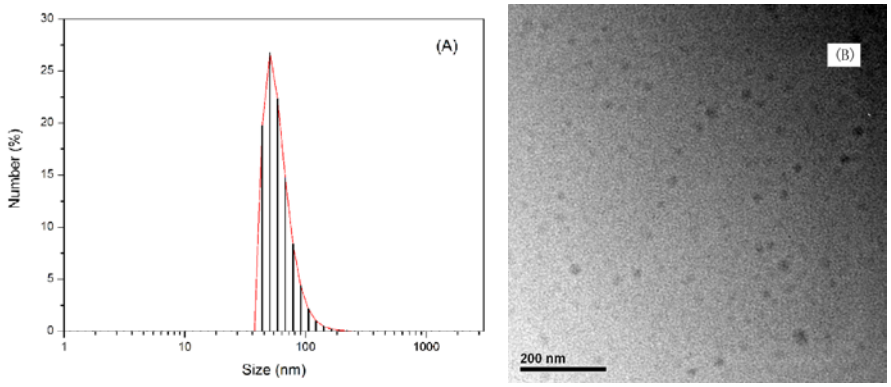

Fig. S6 The size distribution of PEG-c-PMMA micelles determined by DLS (A) and TEM (B).