Supporting Information for

Multiple Stimuli-Responsive Polymeric Micelles for Controlled Release

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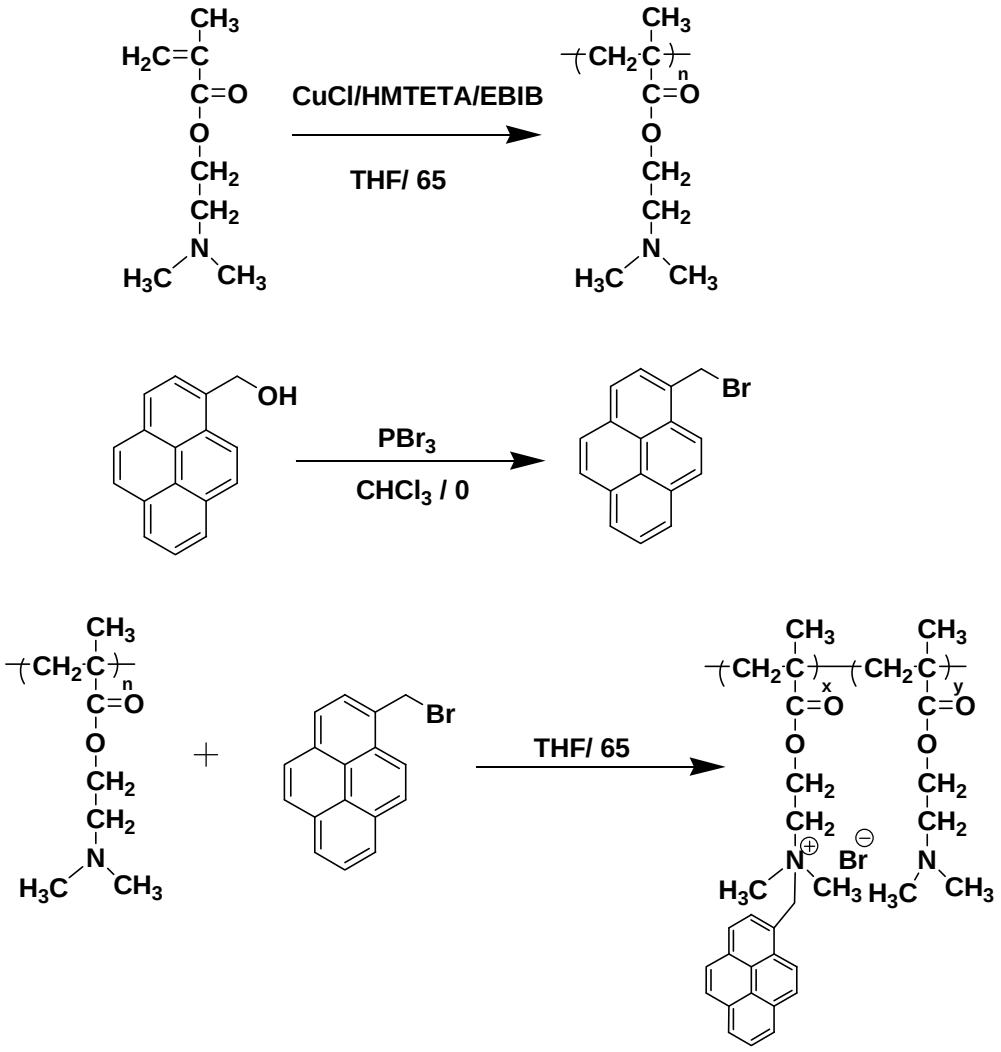
1. Materials

phosphorous tribromide (98.5%) from Sinopharm Chemical Reagent Co. Ltd, 1-pyrenemethanol (99%), 2-(Dimethylamino)ethyl methacrylate (99%) ethyl α-bromoisobutyrate (98%), copper bromide (98%), 1,1,4,7,10,10-hexamethyltriethylenetetramine (97%) from Aldrich and the conventional reagents were used as received. All atmosphere sensitive reactions were done under nitrogen. Deionized water with a conductance below 10^{-6} S cm^{-1} was used during all the experiments.

2. Synthesis of the pyrene-functionalized PDMAEMA



Scheme S1. Synthetic route to the pyrene-functionalized PDMAEMA.

Synthesis of PDMAEMA

PDMAEMA was synthesized via atom transfer radical polymerization (ATRP). In a typical experiment, a round-bottom flask dried under vacuum were charged with 0.038 g (2.65×10^{-4} mol) of CuBr, 0.072 g (2.65×10^{-4} mol) of 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTETA), 4 mL of anhydrous THF, 0.039 mL (2.65×10^{-4} mol) of ethy 2-bromoisobutyrate (EBIB) and 4.5 mL (2.65×10^{-2} mol) of dimethylaminoethyl methacrylate under a N_2 atmosphere. The reaction mixture was deoxygenated with nitrogen. After polymerization for 8 hours at 65°C, the reaction mixture was cooled with liquid nitrogen, and diluted with CH_2Cl_2. The reaction mixture was then passed through a column of neutral alumina to remove the copper salts. The polymer was precipitated twice from an excess of hexane, filtered, and dried at 40 °C under vacuum for 48 h. (3.20 g, yield: 80%). 1H NMR ($CDCl_3$) δ (ppm): 4.1 (t, 2H, $OCH_2CH_2N(CH_3)_2$), 2.6 (t, 2H, $OCH_2CH_2N(CH_3)_2$), 2.3 (s, 6H, $OCH_2CH_2N(CH_3)_2$), 2.1-1.6 (broad, 2H, aliphatic main chain), 1.3-0.7 (broad, 3H CH_3 main chain). GPC: Mn = 1.18×104 g mol-1, PDI = 1.27.

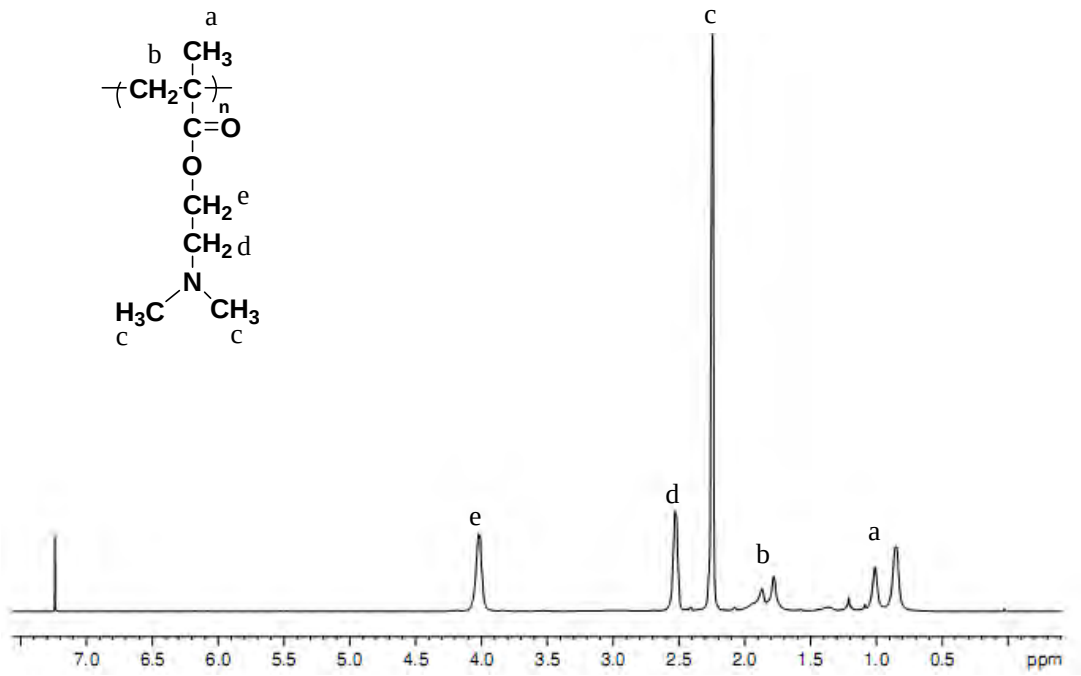


Figure S1. ^{1}H NMR spectra of PDMAEMA in $CDCl_3$.

Synthesis of 1-(bromomethyl)pyrene

1-(Bromomethyl) pyrene was synthesized as follows: To an ice cold solution of 1-(hydroxymethyl) pyrene (0.139 g, 0.60×10^{-3} mol) in 35 mL of dry chloroform, phosphorus tribromide (0.0568 g, 0.21×10^{-3} mol) was added, and the resulting solution was stirred for 12h. The reaction mixture was neutralized with saturated sodium bicarbonate solution. The organic layer was separated and the solvent was evaporated under reduced pressure. 1-(Bromomethyl) pyrene was obtained as green crystal after recrystallization from chloroform (0.127 g, yield: 65%). 1H NMR ($CDCl_3$) δ (ppm): 5.25 (s, 2H), 8.06-8.13 (m, 5H), 8.20-8.26 (m, 3H), 8.37-8.40 (d, 1H).

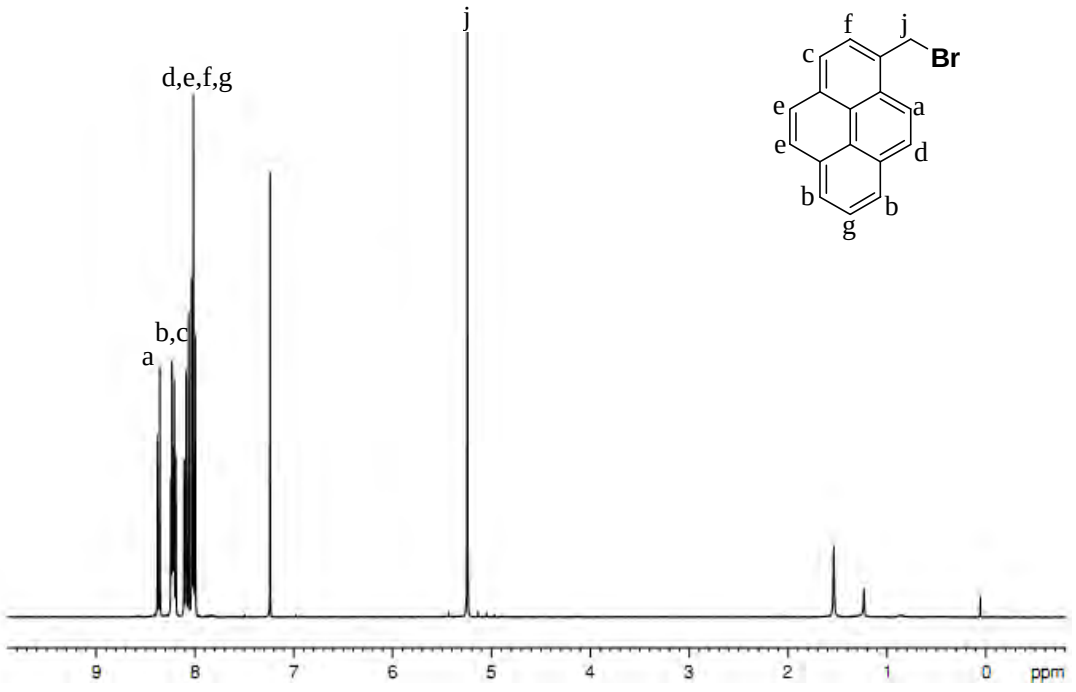


Figure S2. ^{1}H NMR spectra of 1-(bromomethyl) pyrene in $CDCl_3$.

Quaternization of PDMAEMA

Quaternization of the polymer PDMAEMA (0.31 g, 2×10^{-3} mol) with 1-(bromomethyl) pyrene (0.118 g, 0.4×10^{-3} mol) with molar ratio of 5:1 was carried out at 65°C in THF, stirred for 24 h. The polymer was precipitated twice from an excess of hexane, filtered, and dried at 40°C under vacuum for 48 h (0.325 g, yield: 76%). 1H NMR ($CDCl_3$) δ (ppm): 7.9-8.4 (broad, pyrene), 3.7 (s, 2H,NCH_2pyrene), 4.0 (t, 2H, $OCH_2CH_2N(CH_3)_2$), 2.5 (t, 2H, $OCH_2CH_2N(CH_3)_2$), 2.3 (s, 6H, $OCH_2CH_2N(CH_3)_2$), 2.1-1.6 (broad, 2H, aliphatic main chain), 1.3-0.7 (broad, 3H，CH_3 main chain).

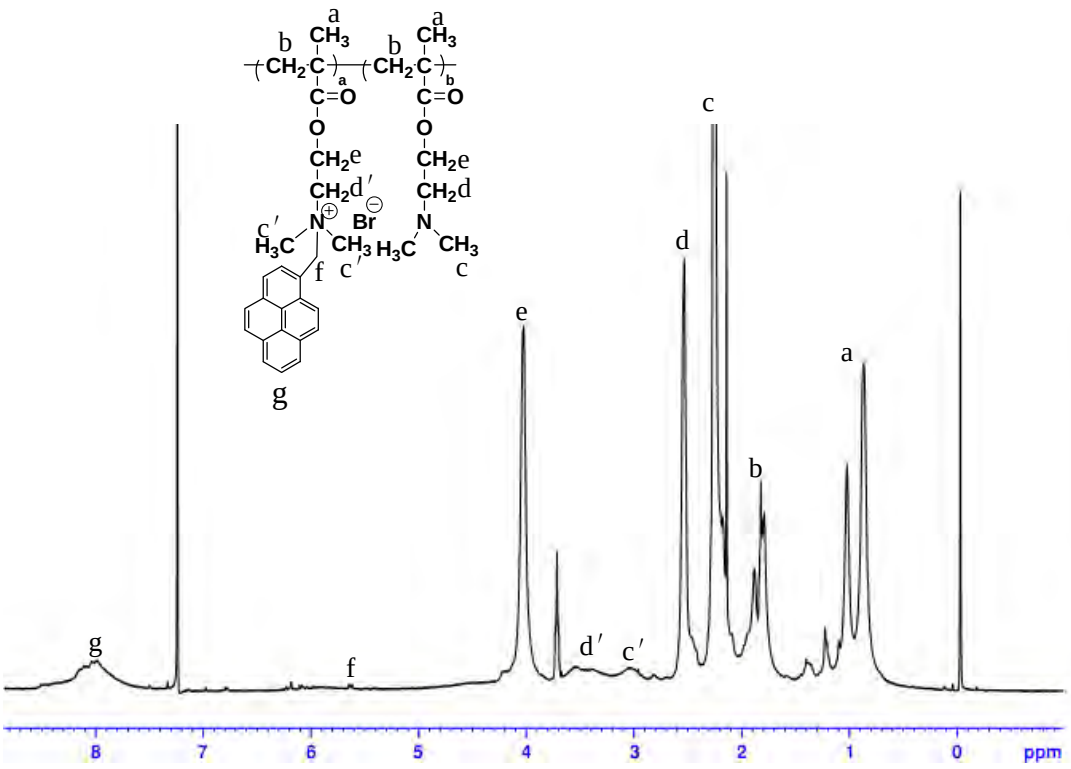


Figure S3. ^{1}H NMR spectrum of the pyrene-functionalized PDMAEMA in $CDCl_3$.

3. Characterization

^{1}H-NMR spectra were recorded from $CDCl_3$ solution on a Bruker AM 400 spectrometer. The molecular weight of the polymer was determined by gel permeation chromatography (GPC) (Waters 1515) with styragel columns relative to polystyrene standards using tertahydrofuran (THF) as eluent. TEM experiments were performed at 200 kV using JEM 2010. Dynamic light scattering (DLS) experiments were carried out on the ALV/SP-150 spectrometer equipped with an ALV-5000 multi-digital time correlator and a solid-state laser (ADLS DPY 425II, output power ca. 400 MW at λ = 632.8 nm) as the light source. All measurements were carried out at the scattering angle of 90°. All solution concentrations for DLS were 1 mg/mL. The hydrodynamic radius ($<R_h>$) was obtained by fitting the correlation function with the CONTIN program. The fluorescence spectra were obtained from a Hitachi F-4500 fluorescence spectrophotometer. UV irradiation for the samples was carried out with a high-pressure mercury lamp (365 nm, 500 W nominal power) and UV light intensity was controlled at 80 $mWcm^{-2}$. For the DLS experiments, the micellar solution was irradiated by the UV light for 40 min.

The degree of functionalization was determined according to the following relation by elemental analysis:

$$12[8(1-X)+25X]／14[(1-X)+X] = C/N$$

Where X is the degree of functionalization, C and N is the content of carbon and nitrogen in pyrene-functionalized PDMAEMA measured by elemental analysis, respectively. According to the above relation, the degree of functionalization was measured to be 13.9%.

4. LCST of the PDMAEMA and the pyrene-functionalized PDMAEMA

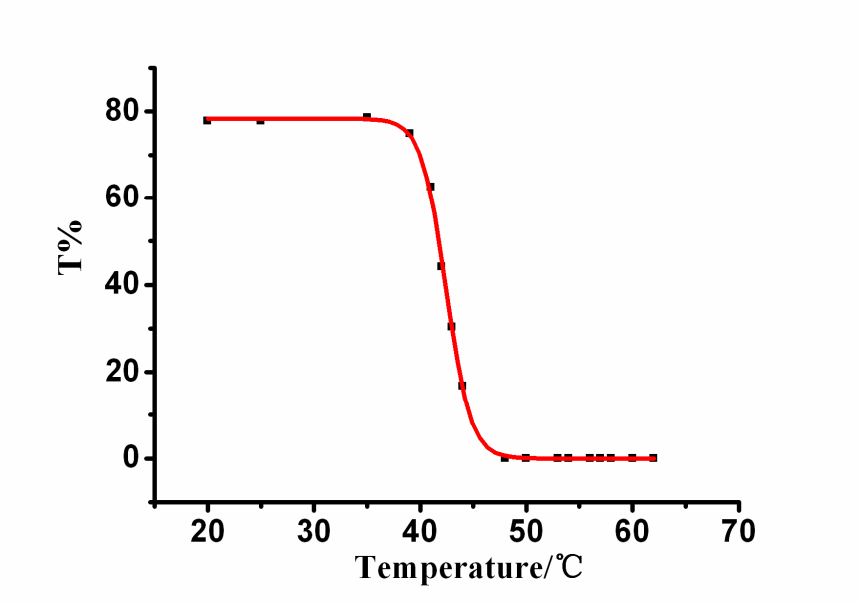


Figure S4. Transmittance at 500 nm of PDMAEMA aqueous solution (1.0 mg/mL) as a function of temperature with a heating rate of 0.1°C/min.

The LCST of the PDMAEMA is about 42°C**,** shown in Figure S4.

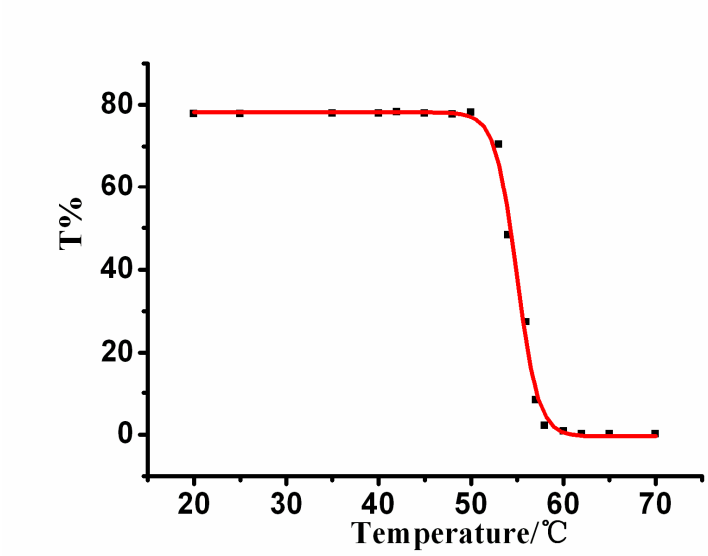


Figure S5. Transmittance at 500 nm of pyrene-functionalized PDMAEMA aqueous solution (1.0 mg/mL) as a function of temperature with a heating rate of 0.1 °C/min.

The LCST of the pyrene-functionalized PDMAEMA is about 54 °C, shown in Figure S5.

5. CMC of the pyrene-functionalized PDMAEMA

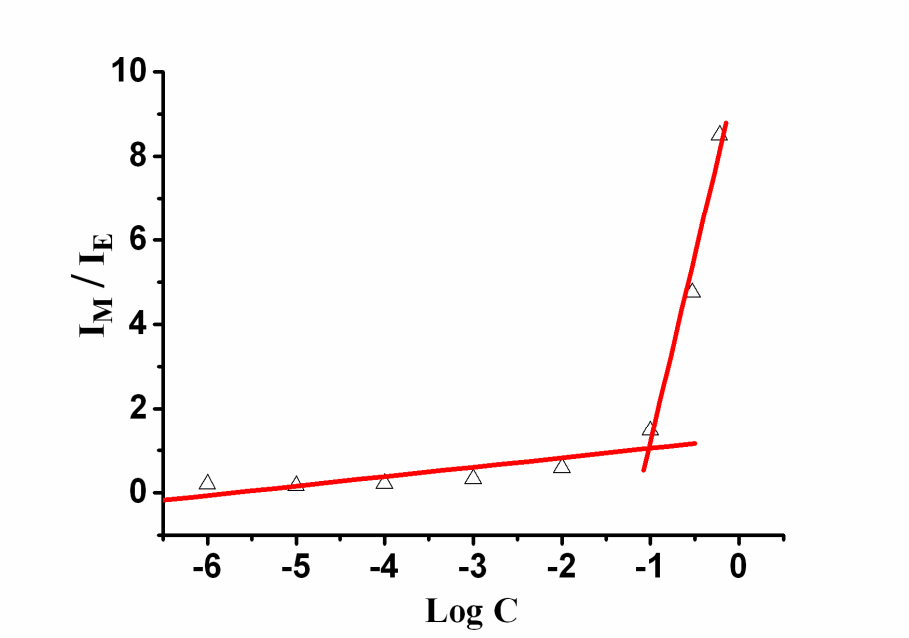


Figure S6. Intensity ratio I_M/I_E as a function of concentration of pyrene-functionalized PDMAEMA.

The intensity ratio I_M/I_E versus log C (C is the concentration of pyrene-functionalized PDMAEMA aqueous solution) is shown in Figure S6, where I_M and I_E represent the intensities of monomer and excimer emissions of pyrene, respectively. The critical micelle concentration (CMC) value of pyrene-functionalized PDMAEMA in aqueous solution was determined to be about 0.1 mg/mL.

6. Fluorescence emission spectra of the polymeric micelle under the stimuli

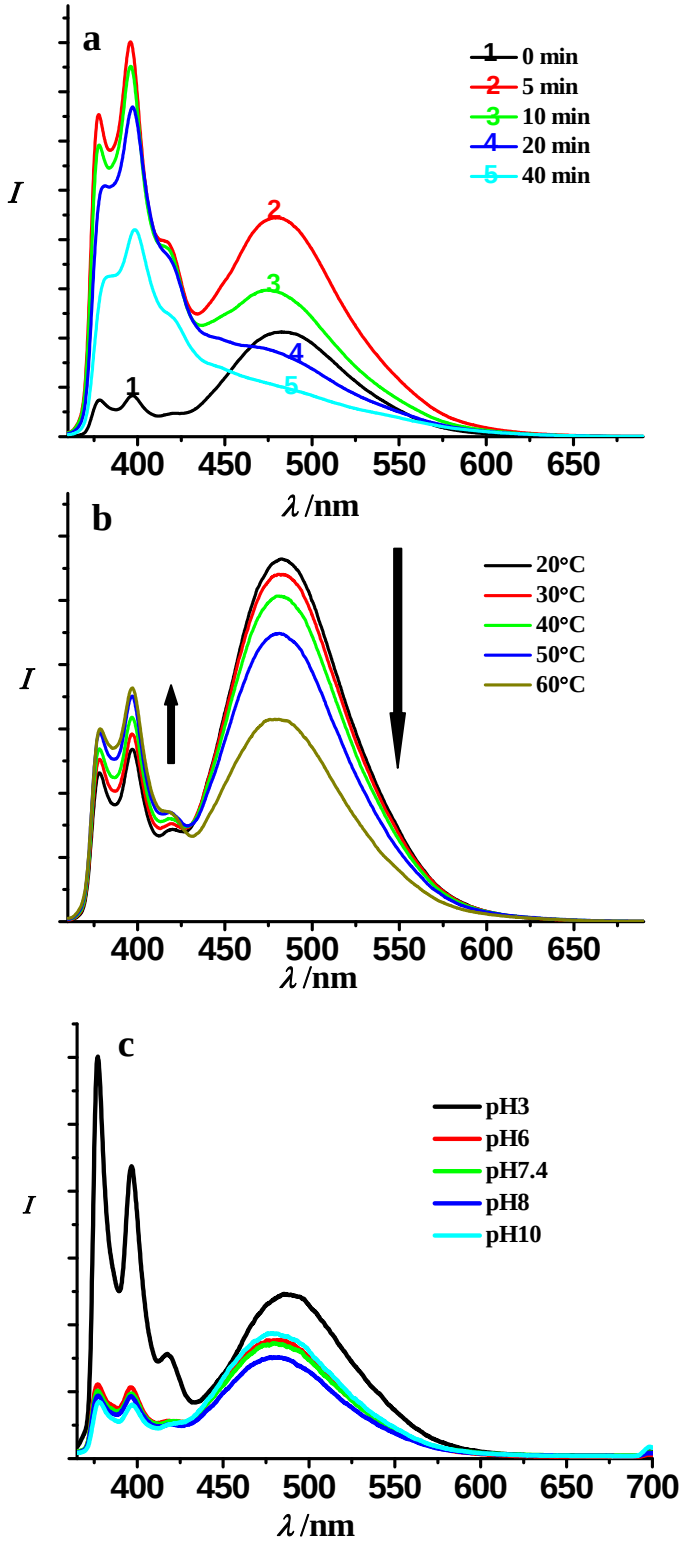


Figure S7. Fluorescence emission spectra of the micellar solution (λ_{ex} = 350 nm): a) under UV irradiation for different time; d) at different temperature; c) at different pH.

The fluorescence of pyrene incorporated into the polymer was measured, shown in Figure S7. The emission bands located at 375 nm ~ 395 nm and around 480 nm are attributed to pyrene monomer and excimer emission, respectively.

For the light stimulus, both the monomer emission and the excimer emission first increased and then decreased with increasing the UV exposure time, shown in Figure S7a. Before UV exposure, the fluorescence emission intensity of pyrene was weak due to the micellar structure, where the pyrene groups were aggregated in the shell of micelle and suffered self-quenched. The fact that I_E/I_M (I_E for the emission intensity of excimer, IM for the emission intensity of monomer) was high reflected that the pyrene groups were in close spatial proximity due to the micelle structure. After UV light irradiation for 5 min, both the monomer and excimer emission increased magnificently, as implied that the photolysis of the pyrene-grafted polymer occurred and the produced acid led to the dissociation of the micelle. With increasing the irradiation time further, the monomer emission decreased and the excimer band disappeared at last, where the micelle could be completely dissociated.

For the temperature stimulus, the pyrene monomer emission increased at the expense of pyrene excimer emission when the micellar solution was heated from 20 to 60°C, shown in Figure S7b. At 20°C, the high excimer emission resulted from the aggregation of pyrene in the shell of micelle. At 60 °C, where the PDMAEMA segments became more hydrophobic, the high monomer emission resulted from the pyrene groups which were separated by the collapsed PDMAEMA chains.

For the pH stimulus, both the monomer and excimer emission increased at pH 3 compared with those at neutral and basic pH, shown in Figure S7c, which implies that the pyrene groups aggregated and suffered self-quenched when they were located in the shell of the micelles at neutral and basic pH. It should be also noted that the monomer emission was stronger than the excimer emission at pH 3, while the former was weaker than the latter at neutral and basic pH. At pH 3, all of the PDMAEMA segments were protonated and extended in the solution, which led to the micelles swelled or dissociated, thus the dissipated pyrene exhibited a strong monomer

emission. At pH 6, pH 8 and pH 10 the fluorescence changed little comparing with that at pH 7.4, which implies that the micro environment of pyrene chromophore changed little although the micelles aggregated to large ones at pH 10.

7. ^{1}H NMR spectra of the pyrene-functionalized PDMAEMA in water and THF (deuterated solvents) before and after UV light irradiation

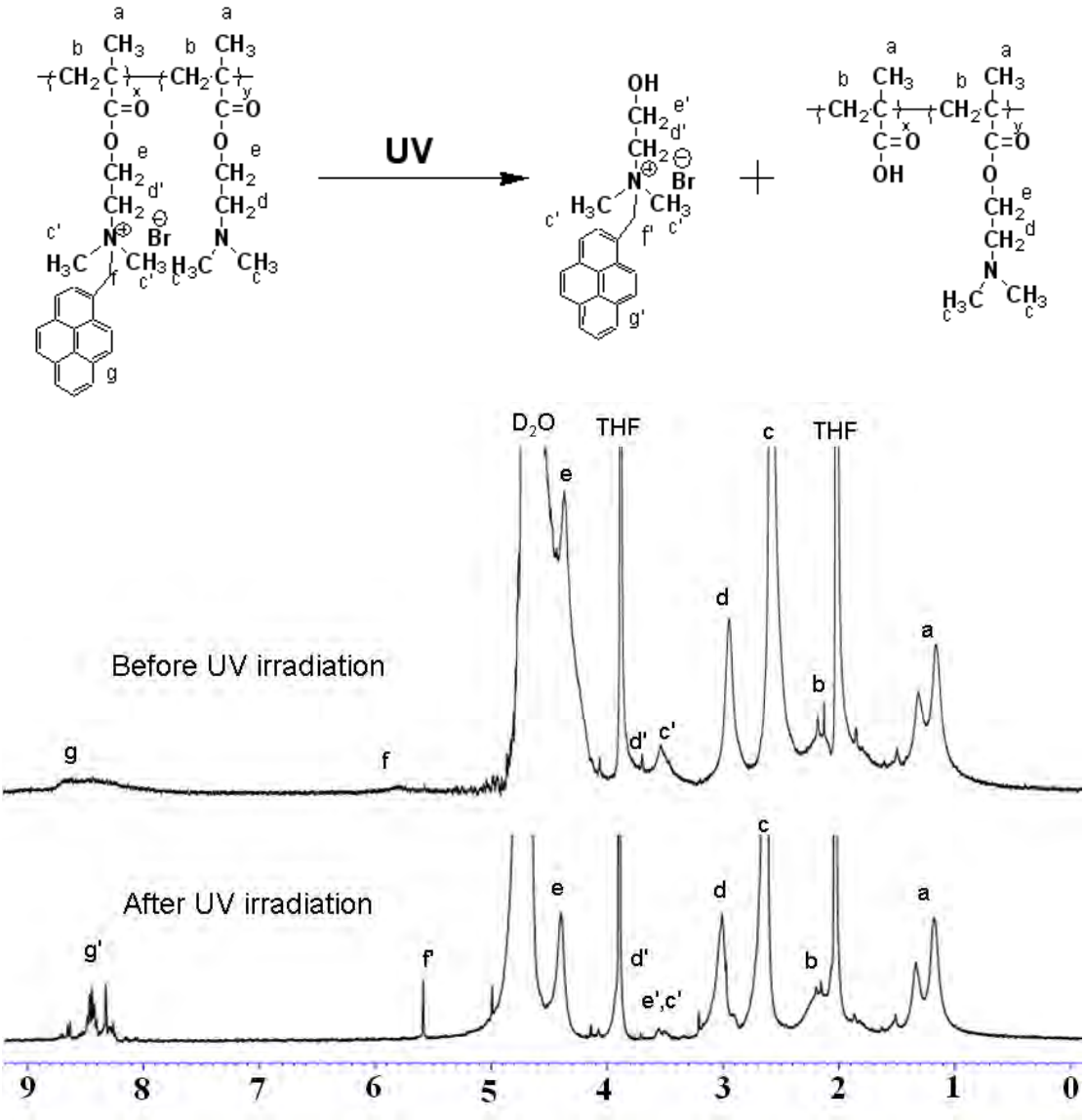


Figure S8. ^{1}H NMR spectra of the pyrene-functionalized PDMAEMA in water and THF (deuterated solvents) before and after UV light irradiation.

As shown in Figure S8, the detachment of pyrene from the polymer under UV irradiation was confirmed by ^{1}H NMR. In this experiment, in order to better observe the spectral changes, the micellar aggregate solution was prepared using a higher polymer concentration of 1.0 mg/mL in THF followed by adding about 10% of water (deuterated solvents). The used UV irradiation intensity was 10 mW/cm^2. Before UV irradiation, the absorption band of protons of pyrene is broad ($\delta \approx 8.5$ ppm), which indicates that the chromophore pyrene is attached to the polymer; while after UV irradiation, the broad absorption of protons of pyrene is gone and multi-peaks of protons of monomer pyrene appear. The change in the absorption of protons of pyrene before and after UV irradiation demonstrates the photoinduced cleavage reaction.

8. TEM images of the polymeric micelle under the stimuli

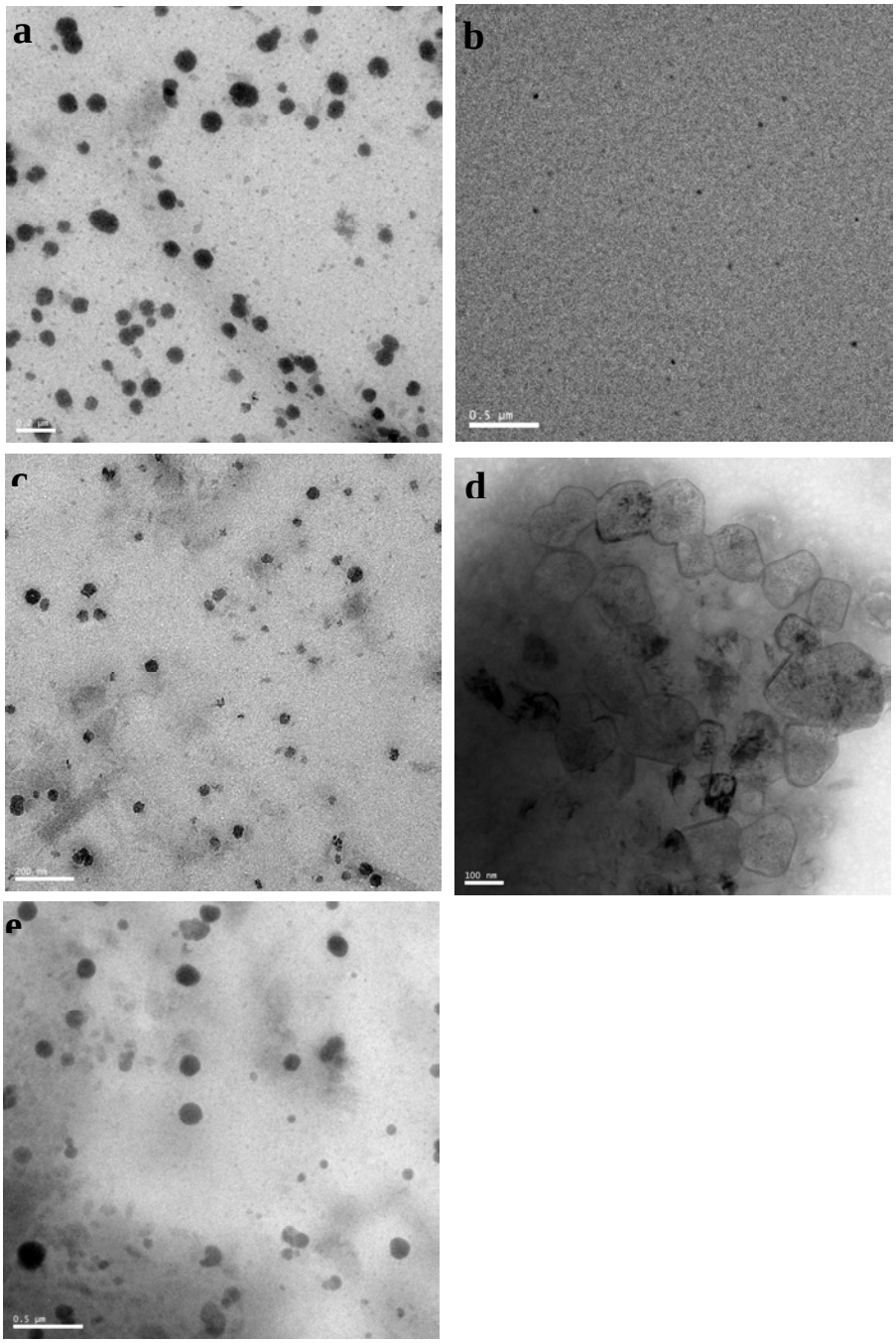


Figure S9. TEM images of the micelles under the stimuli: a) before stimulus presentation (the scale bar is 200 nm); b) after UV light irradiation (the scale bar is 500 nm); c) at 60°C (the scale bar is 200 nm); d) at pH 3 (the scale bar is 100nm); e) at pH 10 (the scale bar is 500 nm).

The diameter of the micelles were approximately 60-100 nm before stimulus presentation, as shown in Figure S9a . After UV light irradiation, where the micelles were dissociated because of the photoinduced cleavage reaction (Figure S9b). When the micelles were heated to 60°C (above the LCST), the diameter decreased to 40-80 nm shown in Figure S9c, as is attributed to that the PDMAEMA segments became more hydrophobic and the core of the micelle would be shrunk and then the micelle would become smaller. At pH 3, the diameter increased to about 200 nm which is corresponding to the swelled micelles (Figure S9d). At pH 10, the large micelles with diameter of 300 nm correspond to the complex aggregates for the deprotonation of the PDMAEMA segments, while the small ones with diameter of 30 nm correspond to the single shrunk micelle, shown in Figure S9e.