

Supporting Information

Supramolecular fluorescent nanoparticles functionalized with controllable physical properties and temperature-responsive release behavior

Chih-Chia Cheng,^{a*} Jyun-Jie Huang,^a Adem Ali Muhable,^b Zhi-Sheng Liao,^a Shan-You Huang,^a Shun-Chieh Lee,^c Chih-Wei Chiu,^b Duu-Jong Lee^{c,d,e*}

- a. Graduate Institute of Applied Science and Technology, National Taiwan University of Science and Technology, Taipei 10607, Taiwan. E-mail: cccheng@mail.ntust.edu.tw
- b. Department of Materials Science and Engineering, National Taiwan University of Science and Technology, Taipei 10607, Taiwan.
- c. Department of Chemical Engineering, National Taiwan University, Taipei 10617, Taiwan. E-mail: djlee@ntu.edu.tw
- d. Department of Chemical Engineering, National Taiwan University of Science and Technology, Taipei 10607, Taiwan.
- e. R&D Center for Membrane Technology, Chung Yuan Christian University, Chungli, Taoyuan 32043, Taiwan.

Experimental Section

Materials

Pyrene (98% pure) and adenine (> 99.5% purity) were purchased from Acros Organics (Geel, Belgium). All other chemicals and reagents were the highest purity available and were purchased from Sigma-Aldrich (St. Louis, MO, USA). All solvents were high performance liquid chromatography grade and obtained from TEDIA (Fairfield, OH, USA).

Characterization

Proton nuclear magnetic resonance (¹H-NMR). ¹H-NMR spectra were acquired using a Varian Inova-400 MHz magnetic resonance spectrometer (Palo Alto, CA, USA). Samples (at least 20 mg) were completely solubilized in approximately 0.7 mL of deuterated solvent and analyzed at various temperatures. *Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectroscopy (MALDI-TOF MS).* MALDI-TOF MS was performed using a Bruker Autoflex II instrument (Billerica, MA, USA) equipped with a nitrogen laser (337 nm, 3 ns pulse, > 20 Hz maximum firing rate). *Dynamic light scattering (DLS).* Particle size and particle size distribution were measured using a Nano Brook 90 Plus PALS (Brookhaven Instruments Corp., Holtsville, NY, USA). The DLS spectrometer was calibrated using standard monodisperse suspensions of polystyrene standard nanoparticles ranging from 60 nm to 500 nm (Polysciences, Warrington, PA, USA). *Cryogenic Transmission Electron Microscopy (cryo-TEM).* Cryo-TEM images were generated on a Philips Tecnai G2 20 S-TWIN scanning TEM (Philips, Eindhoven, The Netherlands) operated at 120 k using a single-tilt specimen holder. Samples were prepared using a FEI Vitrobot Mark IV vitrification system at 25 °C and 100% relative humidity, transferred onto carbon-coated copper grids, and a blotting process was applied to form a thin film. The grids were immediately plunged into liquid ethane and stored in liquid nitrogen before imaging. *Atomic Force Microscopy (AFM).* AFM images were scanned in tapping mode (Digital Instrument NS4/D3100CL/MultiMode) using silicon cantilevers in air at 25 °C and 40% relative humidity. AFM

samples were prepared by spin-coating onto a silicon substrate, then dried at ambient temperature for 1 d. *Ultraviolet-Visible (UV-Vis) and Photoluminescence (PL) Spectra.* UV-Vis and PL spectra were collected using a Hewlett-Packard 8453 diode-array spectrophotometer (HP, Waldbronn, Germany) and Hitachi F4500 luminescence spectrometer (Hitachi, Tokyo, Japan), respectively. Excitation wavelength (λ_{ex}) was 334 nm for PL measurements. *Experimental Measurement of Critical Micelle Concentration (CMC).* CMC values were determined via fluorescence methods using pyrene as a fluorescence probe following a typical procedure, as described previously.^{35,36} *UV-Vis Assessment of Lower Critical Solution Temperature (LCST).* The LCST behavior of the samples in aqueous solution was measured using an optical monitoring system. Transmittance of the aqueous solutions at 500 nm was measured over the temperature range from 5 to 70 °C at 1.0 °C/min by UV-Vis spectroscopy using a temperature controller (Jasco ETC-505T, Tokyo, Japan). The LCST values of sample solutions were determined as the temperature at which the solution exhibited 50% optical transmittance. *Small-Angle X-ray Scattering (SAXS).* SAXS experiments were carried out on the wiggler beamline BL17A1 of the National Synchrotron Radiation Research Center (NSRRC), Taiwan. An X-ray beam with a diameter of 0.5 mm and a wavelength (λ) of 1.1273 Å was used to record SAXS data in the 0.1-3.1 nm⁻¹ q range. Samples were prepared by lyophilization and stored at 4 °C prior to analysis. Lyophilized samples were sealed between two Kapton windows (thickness: 12 μm) and analyzed at 25 °C.

Synthesis of BA-PPG

Adenine (42.3 g, 0.313 mol) and PPG diacrylate (molecular weight: 800 g/mol; 100 g, 0.125 mol) were dissolved in dimethyl sulfoxide (DMSO, 300 mL), then the solution was purged with dry nitrogen for 0.5 h. Sodium tert-butoxide (0.5 g, 0.005 mol) was added to the flask, and the mixture was magnetically stirred at 70 °C for 2 d. After evaporation of DMSO, the residue was dissolved in dichloromethane (1000 mL) and insoluble materials were removed by suction filtration using a Büchner funnel. Eventually, the solvent was evaporated using a rotary evaporator and the product was dried under

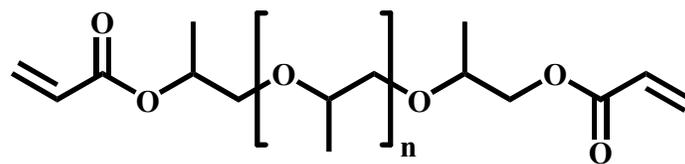
vacuum at 40 °C for 3 h; the product was a white solid. Yield: 93% (124.4 g). ¹H NMR (400 MHz, CDCl₃, 25 °C): δ = 8.35 (br; CH), 7.97 (br; CH), 5.92 (br; NH₂), 5.05 (br; CH), 4.49 (br; CH₂), 3.88–3.25 (br; CH₂ and CH), 2.93 (br; CH₂), 1.46–0.78 (br; CH₃) ppm; ¹³C NMR (100 MHz, CDCl₃, 25 °C): δ = 170.1, 155.4, 152.9, 149.7, 141.1, 119.5, 74.9, 73.1, 71.4, 70.6, 39.4, 34.4, 17.1, 16.4 ppm; MALDI-TOF MS analysis indicated the average molecular weight of BA-PPG was approximately 1,000 *m/z*.

Preparation of pyrene-loaded BA-PPG micelles

BA-PPG (2 mg) and pyrene (2 mg) were dissolved in 5 mL tetrahydrofuran (THF), stirred for 3 h at room temperature, transferred into dialysis tubing with a molecular weight cut off of 1000 Da and dialyzed for 12 h at 15 °C against a large volume of deionized water to remove un-encapsulated pyrene and the organic solvent. The amount of pyrene encapsulated in the micelles was directly assessed by UV-Vis spectroscopy at 346 nm for pyrene, and then the micelles were lyophilized to determine the total weight of the pyrene-loaded micelles. Pyrene-loading content was determined using:

$$\text{pyrene loading content (wt\%)} = W_{\text{pyrene}} / W_{\text{total}} \times 100\%$$

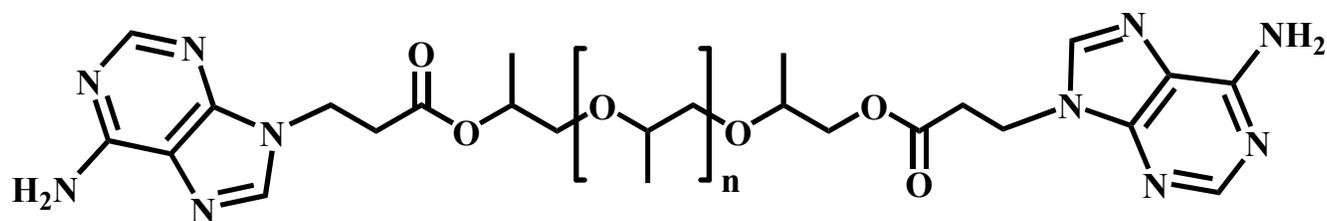
where W_{pyrene} is the weight of pyrene loaded into micelles and W_{total} , the weight of pyrene-loaded micelles after dialysis.



PPG diacrylate

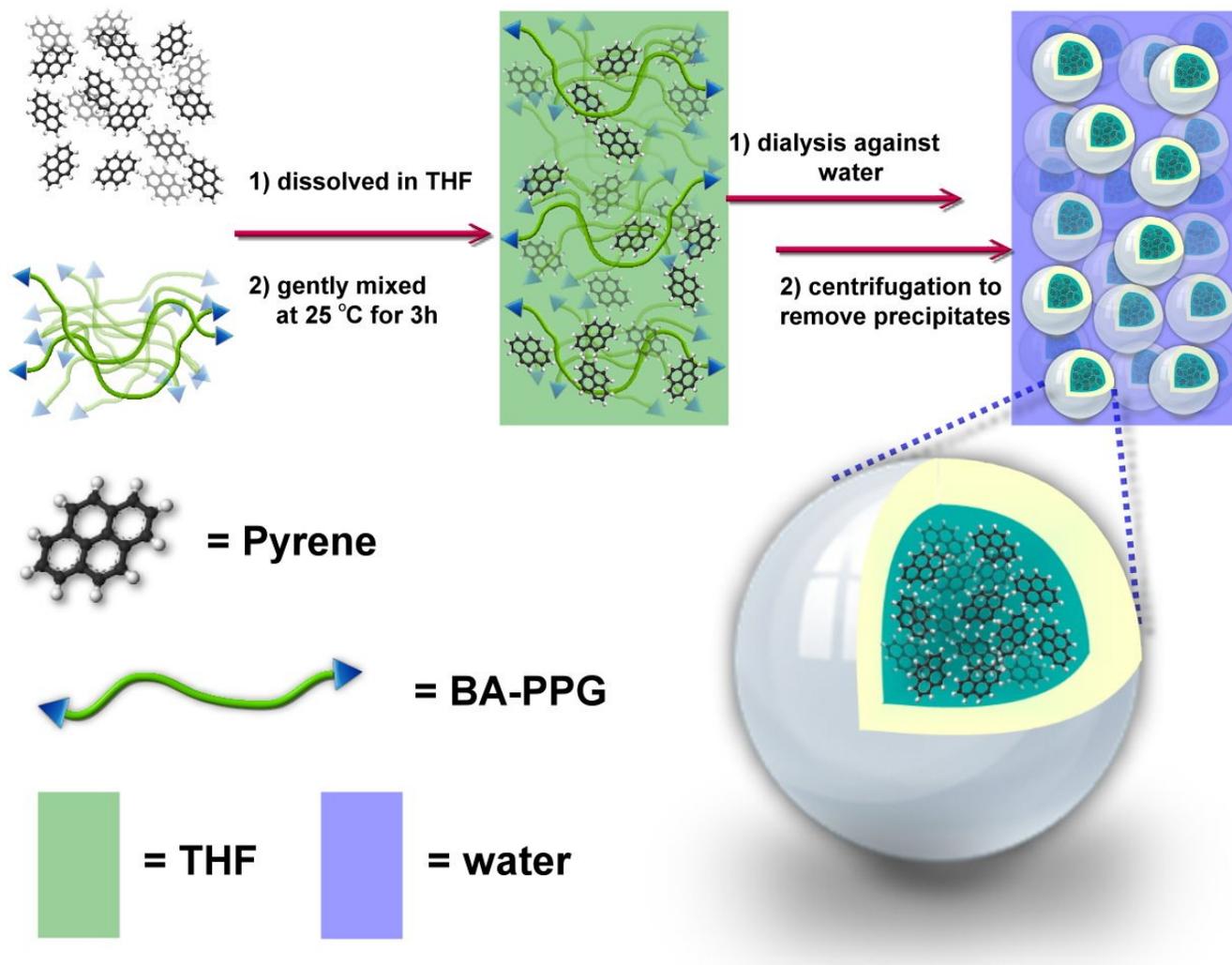
Adenine
t-BuOK
DMSO, 70 °C

Michael addition



BA-PPG

Scheme S1 Synthesis of BA-PPG.



Scheme S2 Schematic illustrating the formation of pyrene-loaded BA-PPG micelles.

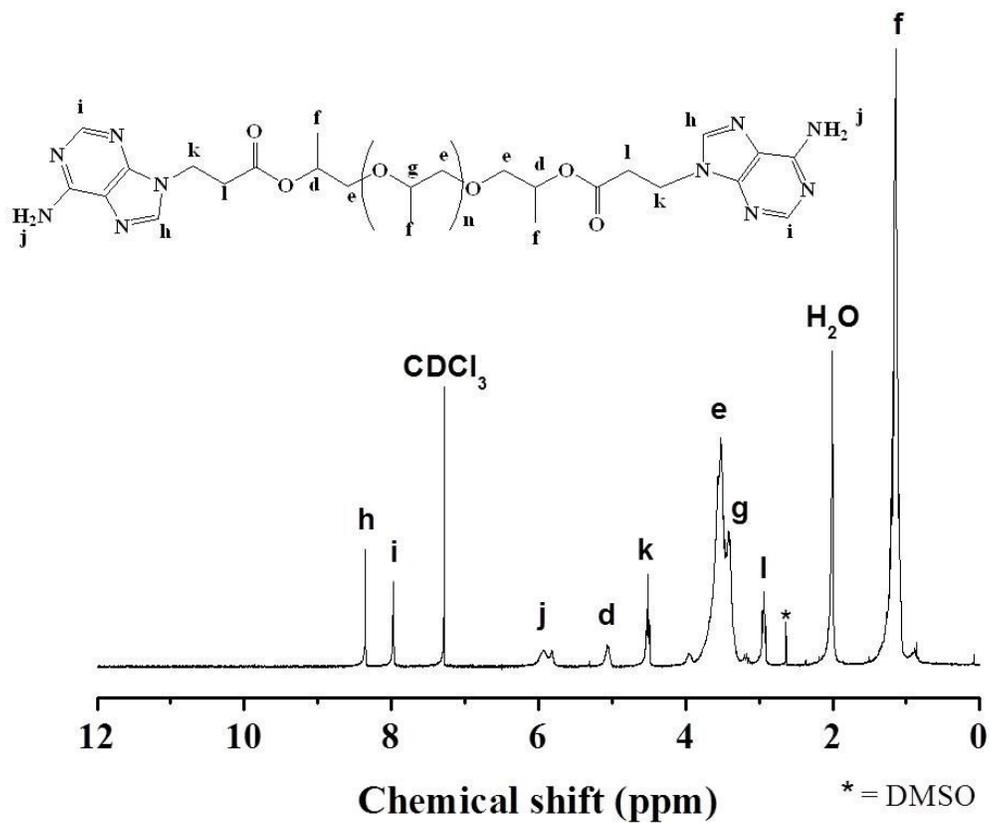


Fig. S1 ^1H NMR spectrum of BA-PPG in deuterated chloroform (CDCl_3).

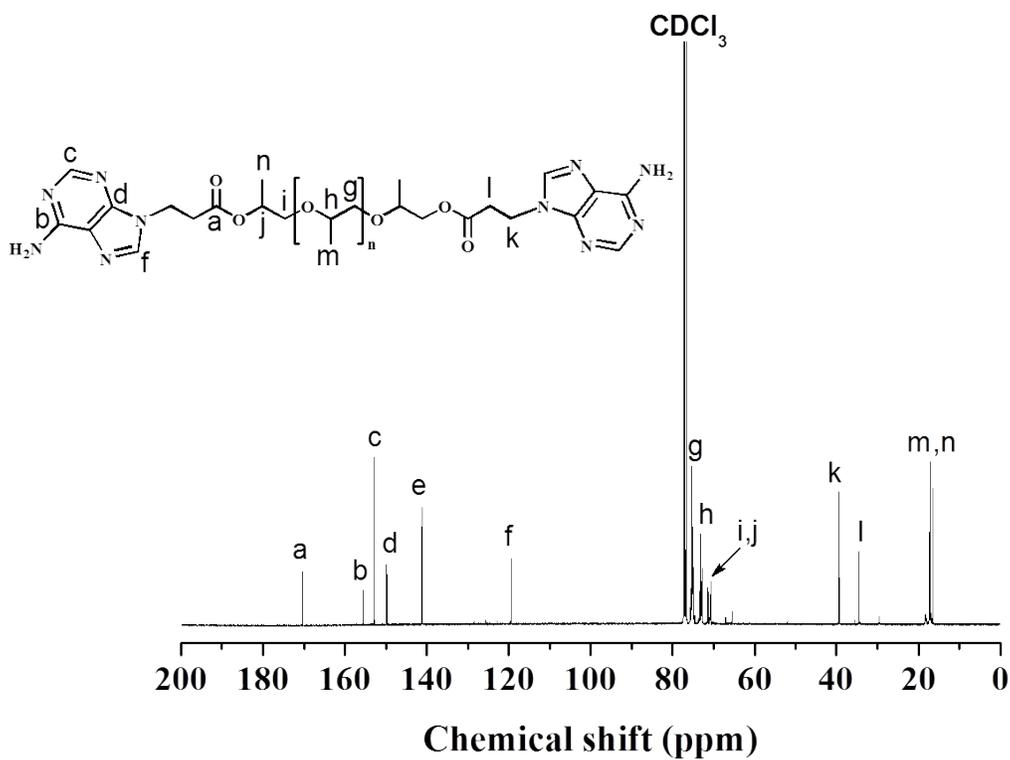


Fig. S2 ^{13}C NMR spectrum of BA-PPG in CDCl_3 .

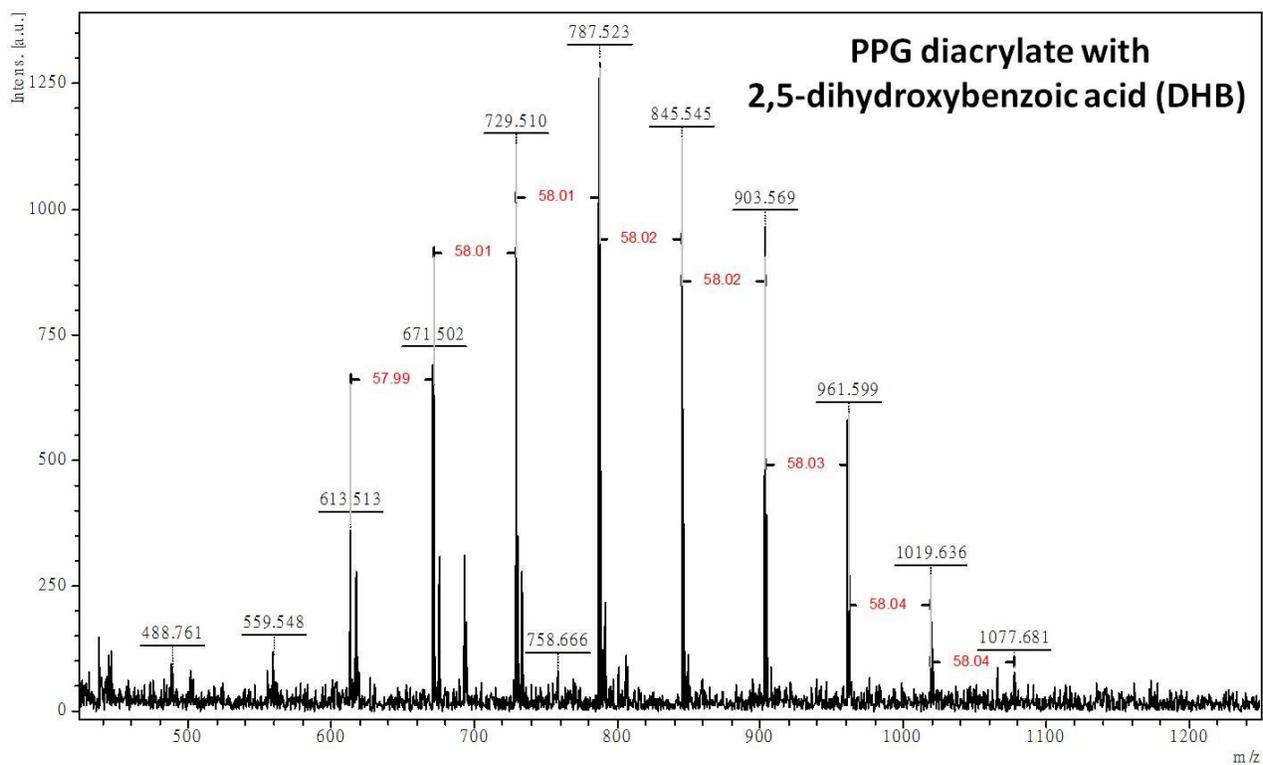


Fig. S3 MALDI-TOF mass spectrum of PPG diacrylate.

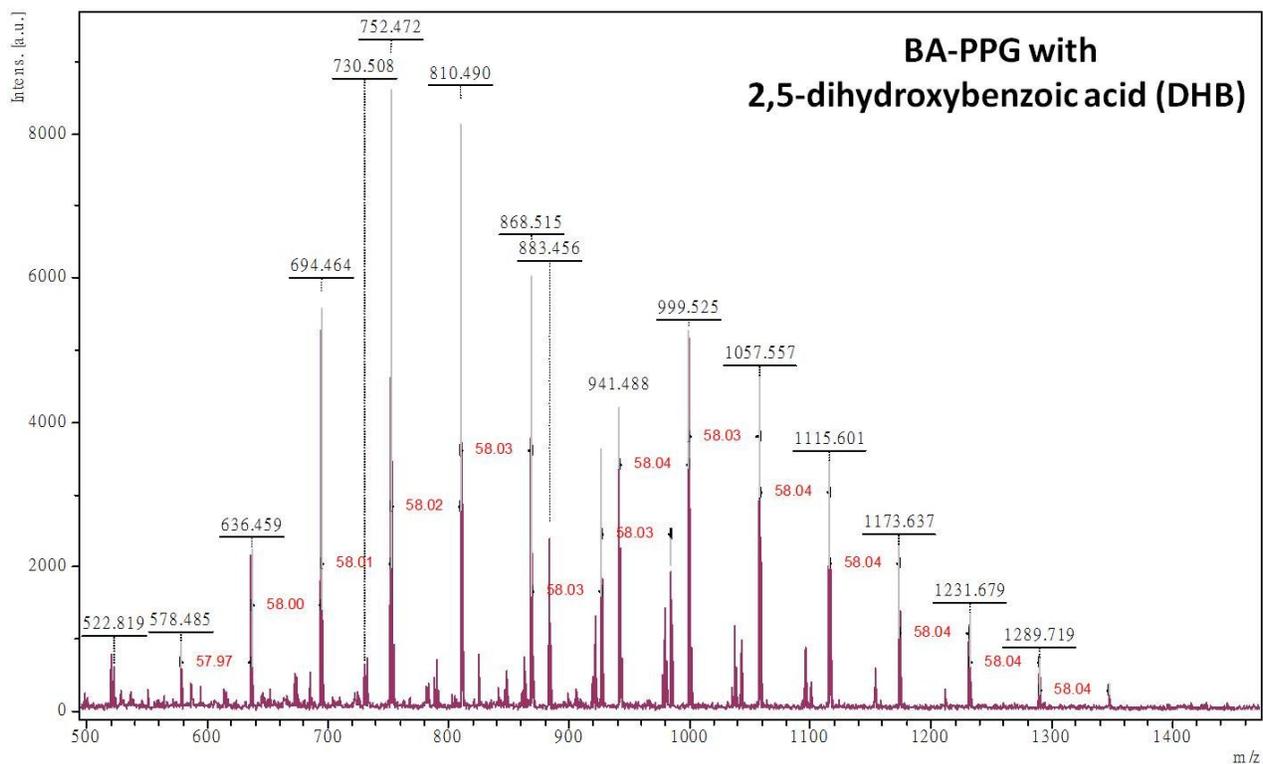


Fig. S4 MALDI-TOF mass spectrum of BA-PPG.

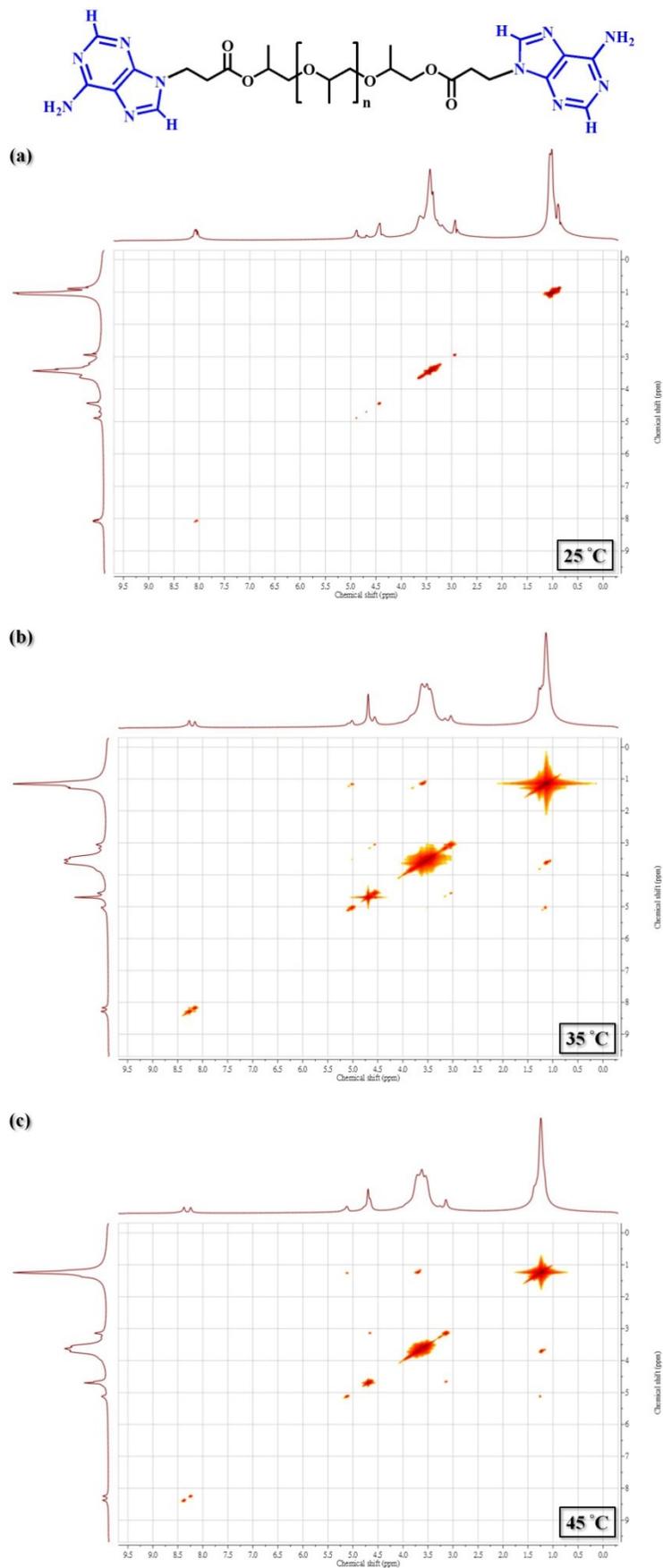


Fig. S5 Two-dimensional ^1H - ^1H NMR spectra of BA-PPG in deuterium oxide (D_2O) at (a) 25 °C, (b) 35 °C, and (c) 45 °C.

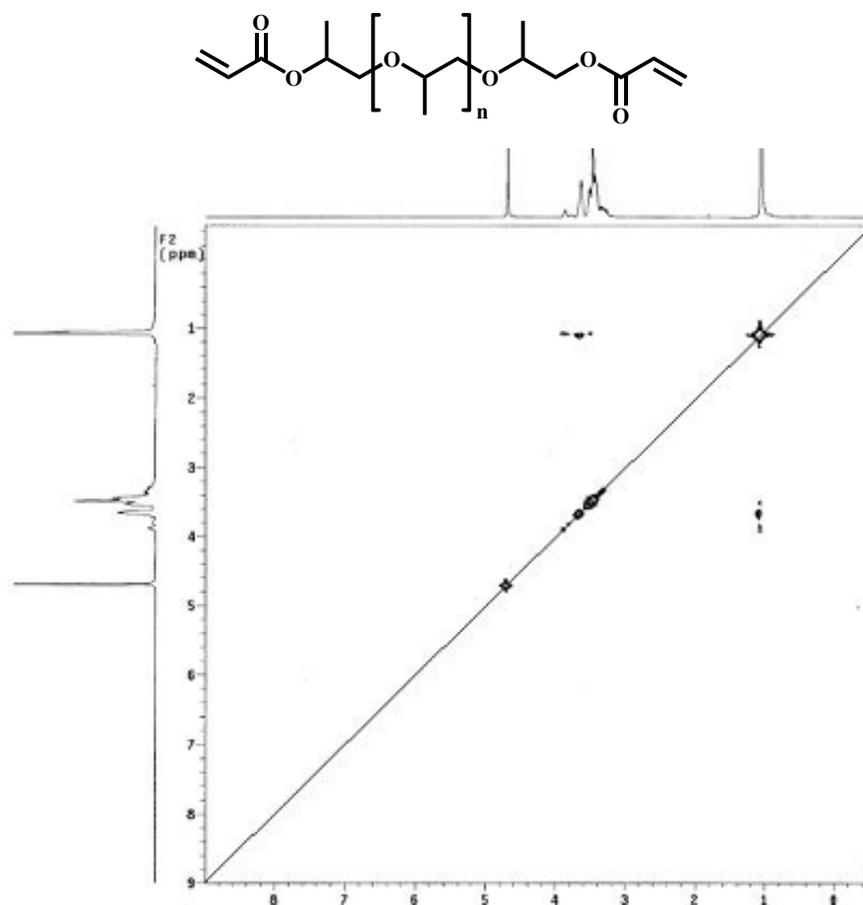


Fig. S6 Two-dimensional ^1H - ^1H NMR spectrum of PPG diacrylate in D_2O at 25°C .

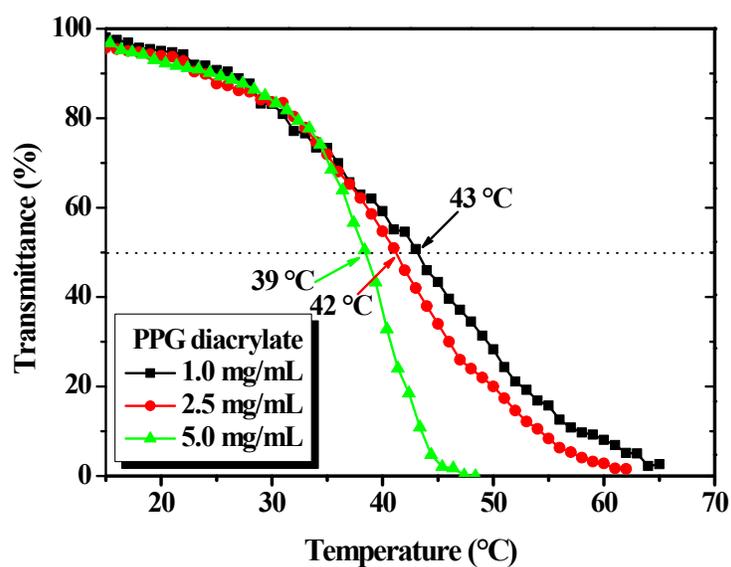


Fig. S7 Transmittance curves of various concentrations of PPG diacrylate in water.

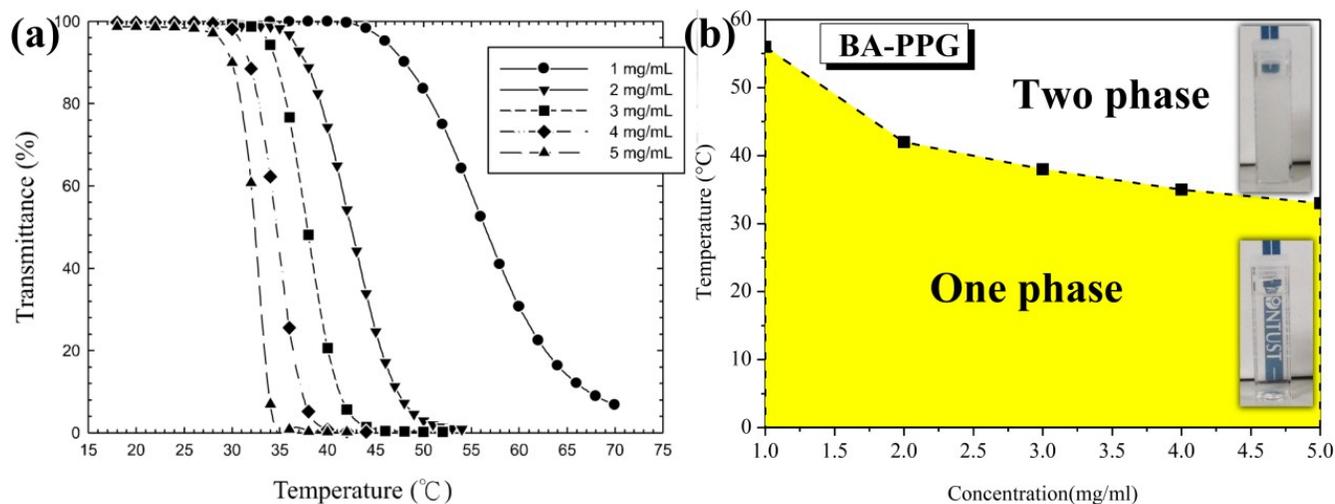


Fig. S8 (a) Transmittance (measured at 500 nm) as a function of temperature and sample concentration for BA-PPG in water. **(b)** Cloud point of various concentrations of BA-PPG in water. The dashed lines show the assumed boundaries between different types of thermoresponsivity. **(b)** The inset photographs display the phase-transition behavior of BA-PPG micelles in water at temperatures below and above the LCST.

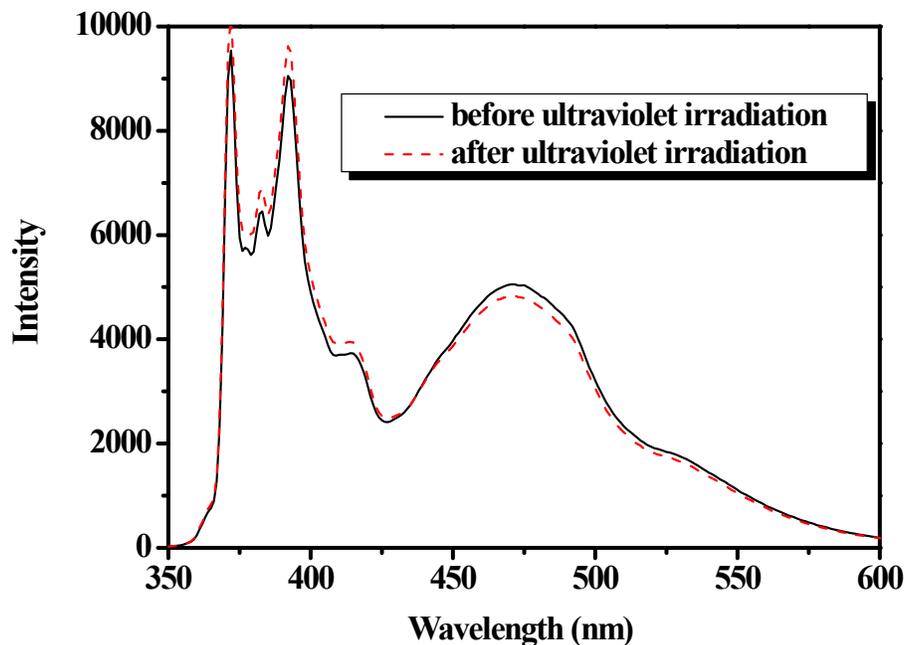


Fig. 9 PL spectra of pyrene-loaded BA-PPG micelles in aqueous solution before and after ultraviolet irradiation for 2 h (at 365 nm, using a hand-held ultraviolet light source in the dark, ~ 0.8 mW/cm²).