Electronic Supplementary Information

Multi-Responsive Nitrobenzene-Based Amphiphilic Random Copolymer Assemblies

Hao Wu,[a] Jie Dong,[a] Cancan Li,[a] Yibiao Liu,[b] Ning Feng,[a] Liping Xu,[b] Xiaowei Zhan,[c] Huai Yang*[a] and Guojie Wang*[a]

[a]*School of Materials Science and Engineering, University of Science and Technology Beijing, Beijing 100083, China;* [b]*Research Center for Bioengineering & Sensing Technology, University of Science and Technology Beijing, Beijing 100083, China;* [c]*Department of Materials Science and Engineering, college of Engineering, Peking University, Beijing 100871, China*

AUTHOR EMAIL ADDRESS: *guojie.wang@mater.ustb.edu.cn; yanghuai@mater.ustb.edu.cn*

1. Materials

Triethylamine (99.5%) was purchased from Sinopharm Chemical Reagent Co. Ltd. Dimethylaminoethyl methacrylate (99%), 2-nitrobenzyl alcohol (98%), methacryloyl chloride (97%), copper bromide (98%), and 1,1,4,7,10,10-hexamethyltriethylenetrtramine (97%) were purchased from Aldrich. All the solvents were used without further purification.

2. Characterization

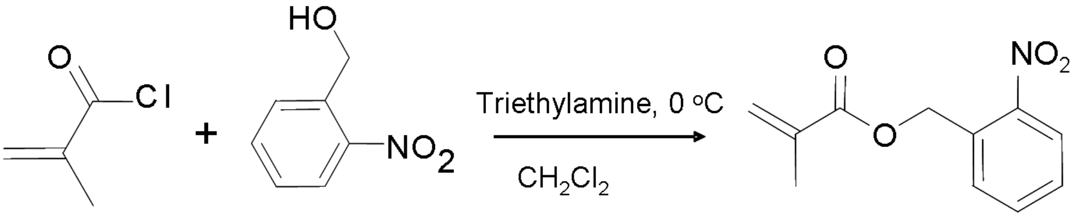
^{1}H-NMR spectra were recorded from $CDCl_3$ solution on a Bruker AM 400 spectrometer. The molecular weight and the molecular weight distribution (M_w/M_N) were obtained from gel permeation chromatography (GPC) (Waters 1515) with styragel columns relative to polystyrene standards using tertahydrofuran (THF) as eluent. The optical transmittances of micelle solutions at different temperatures were recorded at wavelength of 500 nm with a UV-vis spectrometer (JASCO V-570). The heating ramp was fixed at 1 °C/min. The fluorescence was performed on a Hitachi F-4500 fluorescence spectrophotometer, where the excitation wavelength was 560 nm for Nile Red. The morphologies of the polymeric micelle were characterized with a JEM-2010 EX/S Transmission Electron Microscope (TEM) and Bruke IIIa Atomic Force Microscope (AFM). Samples for TEM were prepared by depositing one droplet (3 μL) of aqueous polymer solution (0.25 mg/mL, sonicated for 10 min) on carbon-coated Nickel grids. The solvent was evaporated by freeze-drying procedure for 24 h. Samples for AFM were obtained by directly casting one droplet (3 μL) of aqueous polymer solution (0.25 mg/mL, sonicated for 10 min) on silicon chips and dried under room temperature. 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 $\lambda = 632.8$ nm) as the light source. All the 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 75 mW/cm^2.

3. Synthesis

3.1 Synthesis of 2-nitrobenzyl methacrylate.

2-Nitrobenzyl methacrylate was prepared according to reported procedures[s1]. Briefly, 2-nitrobenzyl alcohol (4.21 g, 27.5 mmol) and triethylamine (7.68 mL) were dissolved in dichloromethane (25 mL) in a 100 mL flask, which was then placed in an ice/water bath. A solution of methacryloyl chloride (4.84 g, 46.5 mmol) in CH_2Cl_2 (10 mL) was added drpowise into the flask under N_2 from an additional funnel over a period of 5 min. After 12 hours, the reaction mixture was poured into a separatory funnel and sequentially washed with an aqueous solution of NaOH (30mL, 1.0 mg/mL). The organic layer was separated and dried over anhydrous Na_2SO_4 for 12 hours. The crude mixture was purified by silica gel chromatography with 6:1 hexane/ethyl acetate. The pure product was obtained as a light yellow liquid (5.33 g, yield: 87.66%). ^{1}H-NMR (Bruker AM 400, $CDCl_3$): δ (ppm) 8.09 (d, H), δ 7.66–7.44

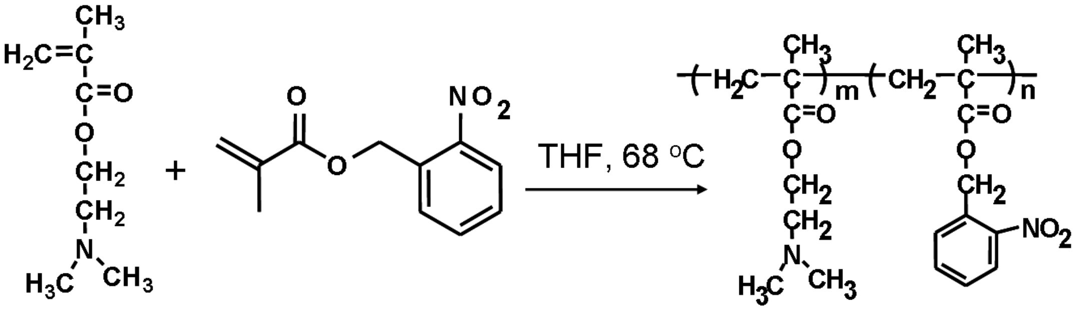
(m, 3H), δ 6.27 (s, H), δ 5.90 (s, H), δ 5.58 (s, 2H), δ 1.96 (s, 3H).



Scheme S1. Synthetic route of 2-nitrobenzyl methacrylate.

3.2 Synthesis of random copolymer PDMAEMA-co-PNBM.

In a typical experiment, a 5 mL ampule, flamed and dried under vaccum and filled with nitrogen, was charged with CuBr (0.15 g, 1 mmol), DMAEMA (4.71 g, 30 mmol), and monomer 2-nitrobenzyl methacrylate (2.21 g, 10 mmol), 3 mL THF. Then, PMDEA (35 mg, 0.2 mmol) was added through a syringe. Then the mixture was degassed three times using the freeze-pump-thaw procedure and sealed under vacuum. The ampule was placed in a preheated oil bath under the temperature of 68 °C for 18 hours. The reaction mixture was passed through a column filled with neutral Al_2O_3 and THF to remove the catalyst. The molar ratio between DMAEMA and NBM was 4:1, which was calculated from the integral values of the peak from 3.9 to 4.1 ppm ($-CH_2CH_2N-$ of DMAEMA units) and the peaks from 5.2 to 5.4 ppm ($-OCH_2-$ in the NMB units).



Scheme. S2. Synthetic route of the PDMAEMA-*co*-PNBM random copolymer.

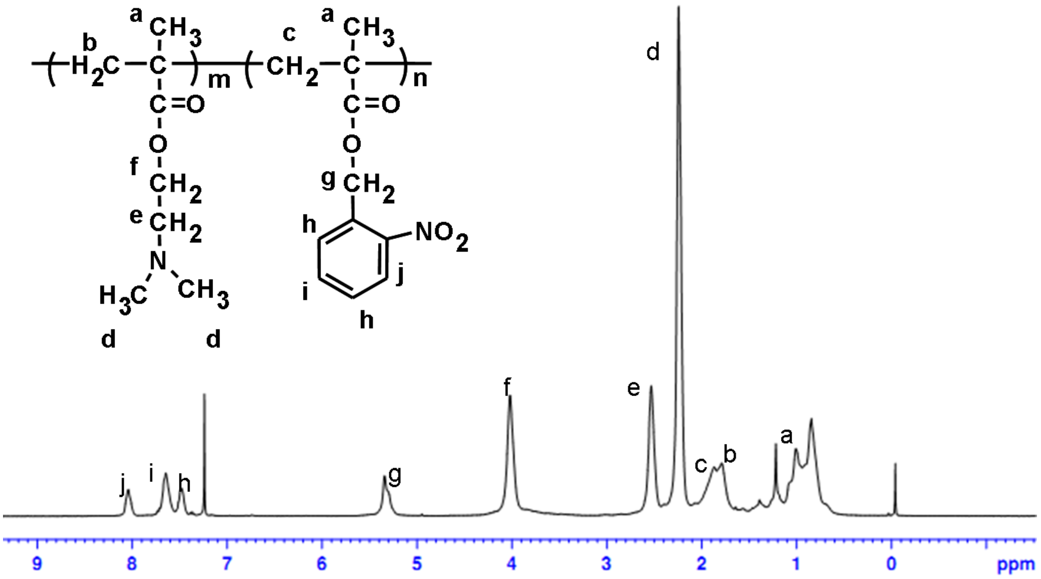


Fig. S1. ^{1}H NMR spectra of PDMAEMA-*co*-PNBM copolymer in $CDCl_3$

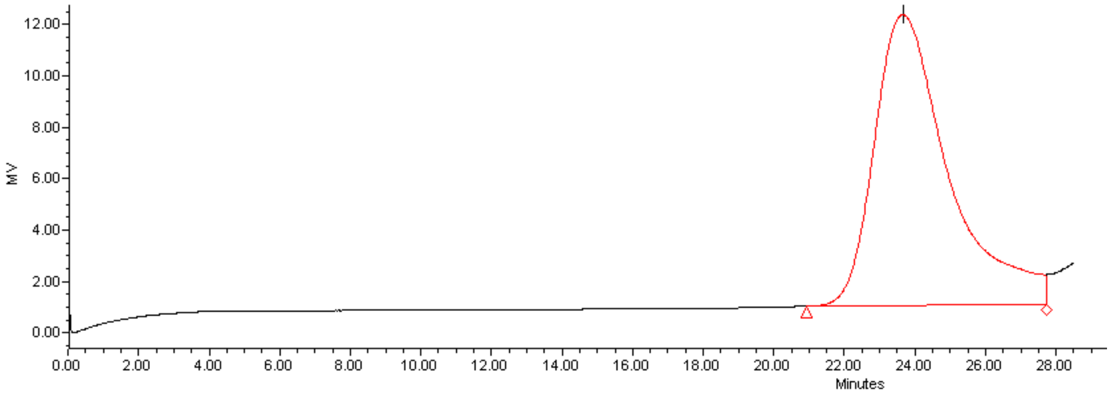


Fig. S2. GPC profile of PDMAEMA-co-PNBM random copolymer.

The number-average molecular weight (Mn) and polydispersity index (PDI) of the random copolymer were about 14600 g/mol and 1.66, respectively.

4. Preparation of the polymer micelles and the micelles loaded with Nile red

The copolymer (2.5 mg) was dissolved in THF (1 mL) and then deionized water (1 mL) was added at a rate of 1 μL/s with quick stirring to induce the formation of the micelle. After 4 hours, 9 mL water was added to quench the micellar assembly. Finally, THF was removed by evaporation at room temperature for 2 days.

As to the Nile red loaded micelle, first, the random copolymer (2.5 mg) and Nile red (0.25 mg) were dissolved in THF (1 mL) and then deionized water (1 mL) was added at a rate of 1 μL/s with quick stirring. 4 hours later 9 mL water was added to quench the micellar assemblies. THF was removed by evaporation at room temperature for 2 days.

5. The LCST of the copolymer

Typically, the lower critical solution temperature (LCST) is defined as the temperature at which the light transmission of the polymer solution decreases to 90% of the original value. Below LCST, the polymer is hydrophilic and soluble in water, while when the temperature is raised above LCST, the polymer undergoes a phase transition to a hydrophobic state, generating turbidity owing to aggregation.

The determination of its LCST was carried out by UV/vis spectroscopy at 500 nm. The turbidity of the polymer solution (0.5 mg/mL) was measured at different temperatures from 38 to 55 ^{o}C with 1 ^{o}C interval. To reduce as much as possible the margin of the error, the heating ramp was fixed at 1 ^{o}C/min and the experiment was performed in a short time. From Fig. S3 we can find, by incorporating 2-nitrobenzyl metharylate (NBM) into the polymer chain, the LCST of the synthesized copolymer PDMAEMA-*co*-PNBM is 44 ^{o}C.

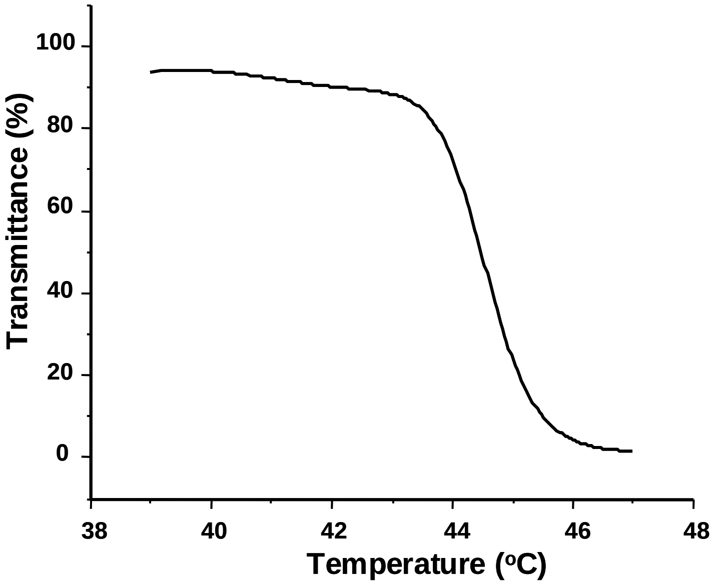


Fig. S3 Phase transitions of PDMAEMA-*co*-PEGMA in water, measured by UV/vis spectroscopy at 500 nm.

6. The CMC of the copolymer

The critical micelle concentration (CMC) of the PDMAEMA-*co*-PNBM random copolymer was measured by the fluorescent probe, Nile Red. 0.05 Mg of Nile Red was added into polymeric micelle aqueous solutions with different concentrations which were sonicated for 10 min before fluorescent emission measurements (excitation at 560 nm).

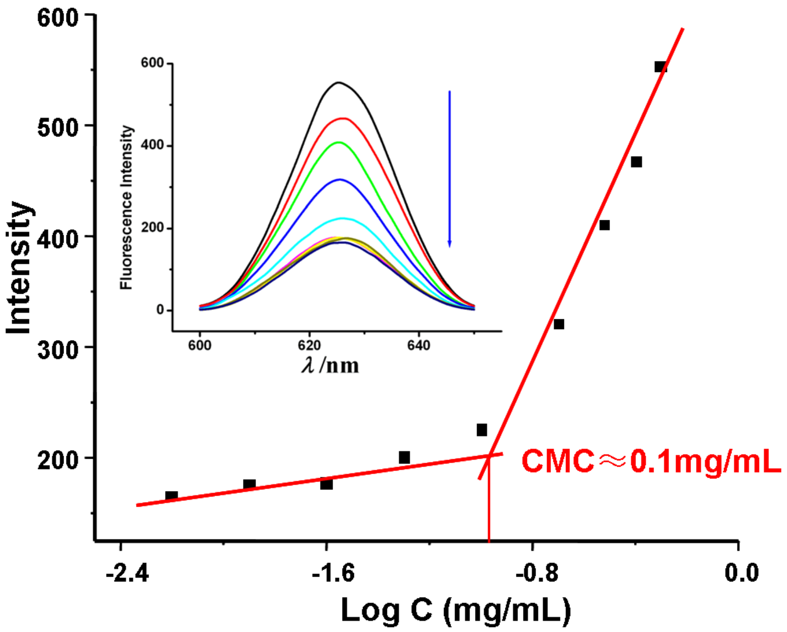


Fig. S4 Determination of CMC for the PDMAEMA-*co*-PNBM random copolymer by using the fluorescent method with Nile red as a probe excitated at 56o nm, the CMC is ca. 0.1 mg/mL.

7. Stimuli-responsive experiments

Photo-responsive experiment: The copolymer micelles assembled in water at room temperature, where the pH was 7.4, were irradiated under UV light (365 nm, 75 mW/cm^2). Then the photo-responsive properties were investigated.

Acid-responsive experiment: The copolymer micelles assembled in water at room temperature, where the pH was 7.4, were adjusted to proper pH with diluted hydrochloric acid. Then the acid-responsive properties were investigated.

Temperature-responsive experiment: The copolymer micelles assembled in water at room temperature, where the pH was 7.4, were adjusted to proper temperature. Then the temperature-responsive properties were investigated.

8. Atomic force microscopy (AFM) characterization

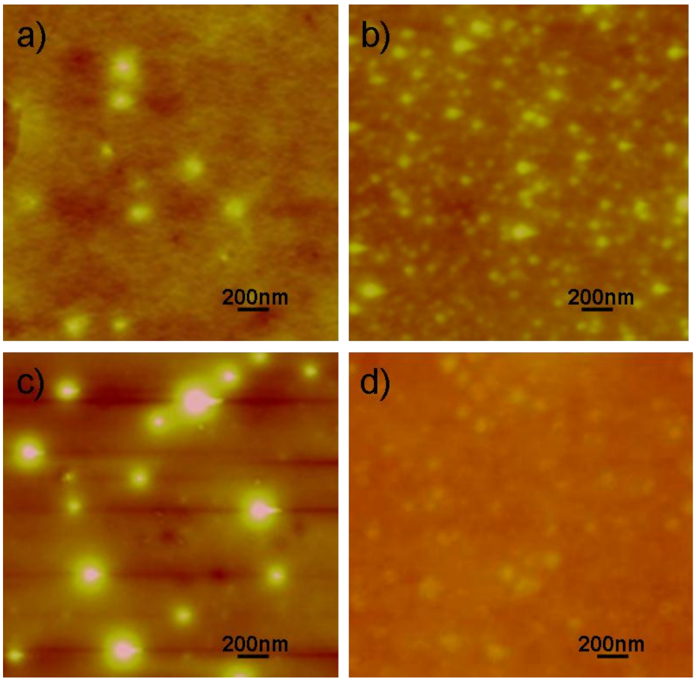


Fig. S5 AFM images of the micellar assemblies under different stimulus: a) before stimulus; b) after UV (365nm, 75 mW/cm^2) irradiation for 20 min; c) in pH 3.0; d) heated to 60 ^{o}C. The concentration of the copolymer solution was 0.5 mg/mL.

The responsive behaviors of the polymeric micelles under the stimuli were investigated by AFM. Fig. S5 a) shows the morphology of the micelles (diameter 70 ~ 120nm) before any stimulus. After UV irradiation for 20 min, the micelles disrupted due to the photolysis of the o-nitrobenzyl methacrylate. The size of the assemblies decreased to 30 ~ 50 nm. In pH 3.0 ambience, the PDMAEMA segments were protonated and extended in aqueous solution, leading to the swollen of the micelles (diameter 100 ~ 300 nm, Fig. S5 c). When heated above its LCST (60 ^{o}C), the diameter decreased approximately to 50 nm, since the PDMAEMA segments became more hydrophobic and the collapse of the polymeric micelles occurred.

9. UV-vis absorbance spectrum of polymeric micelle without NR in aqueous solution

Fig. S6 shows the UV-vis spectrum of the polymer micelle (0.5 mg/mL) without NR loaded. The peak around 280 nm is attributed to the absorbance of 2-nitrobenzyl groups.

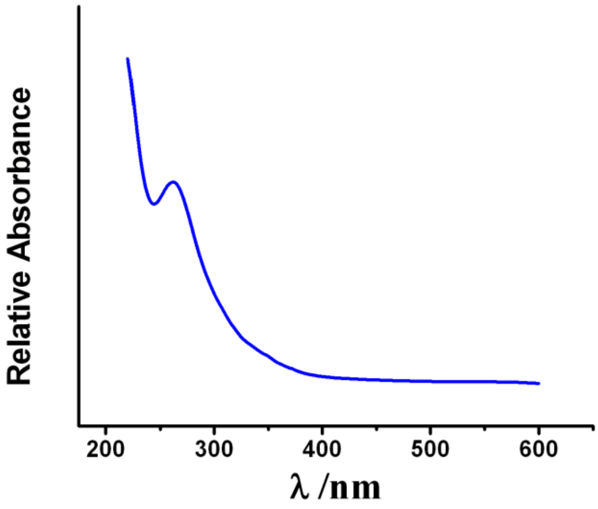


Fig. S6 UV-vis absorbance spectrum of the polymer micelle without NR loaded in aqueous solution (0.5 mg/mL).

10. Fluorescence emission spectra

Fluorescence emission spectra (excitation at 560 nm) were employed to study the releasing behaviors of encapsulated NR molecules. Fig. S7a shows the fluorescence of NR in the polymeric micelles decreased with increasing UV irradiation time. Fig. S7b shows the temperature-triggered release behavior of the polymeric micelles. With the increase of the temperature, the fluorescence emission intensity decreases, indicating the release of NR molecules. When the micelle solution was treated at different pH, the loaded NR molecules were released from the swelled micelles, as shown in Fig. S7c.

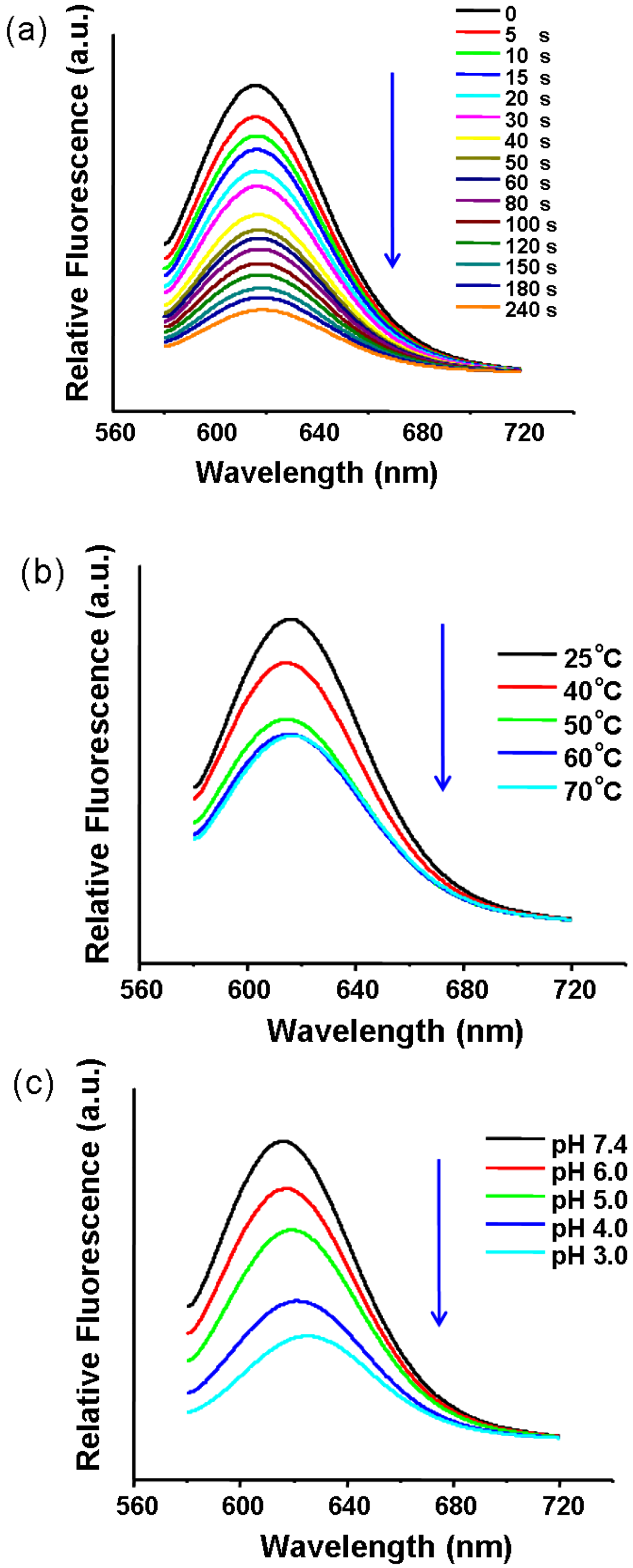


Fig. S7 Fluorescence spectra of NR-loaded micelle in aqueous solution (0.5mg/mL), excitated at

560 nm: (a) upon UV irradiation (pH 7.4, room temperature) for different time, (b) under different temperature (pH 7.4, before UV irradiation) and (c) at different pH (room temperature, before UV irradiation).

11. FTIR Measurements

Infrared spectroscopy was used to prove the reaction between the amine groups and the carboxylic acid groups generated after UV irradiation. 10 Mg random copolymer and 1 mL chloroform were placed in a standard cuvette, while the solution was exposed to UV light (365 nm, 75 mW/cm^2) under stirring for 40 min. Then the solvent was evaporated and the aggregates were dried at 70 ^{o}C before FTIR measurements.

Fig. S8 clearly shows that the carbon-nitrogen vibration peak (from the amino groups) at 2800 cm^{-1} decreases and a large carbon-nitrogen vibration peak (from the ammonium groups)[s2] appears at 3400 cm^{-1} after UV irradiation. The decrement of the peak at 2800 cm^{-1} and the appearance of the peak at 3400 cm^{-1} proved the reaction between the amine groups and the carboxylic acid groups after UV irradiation.

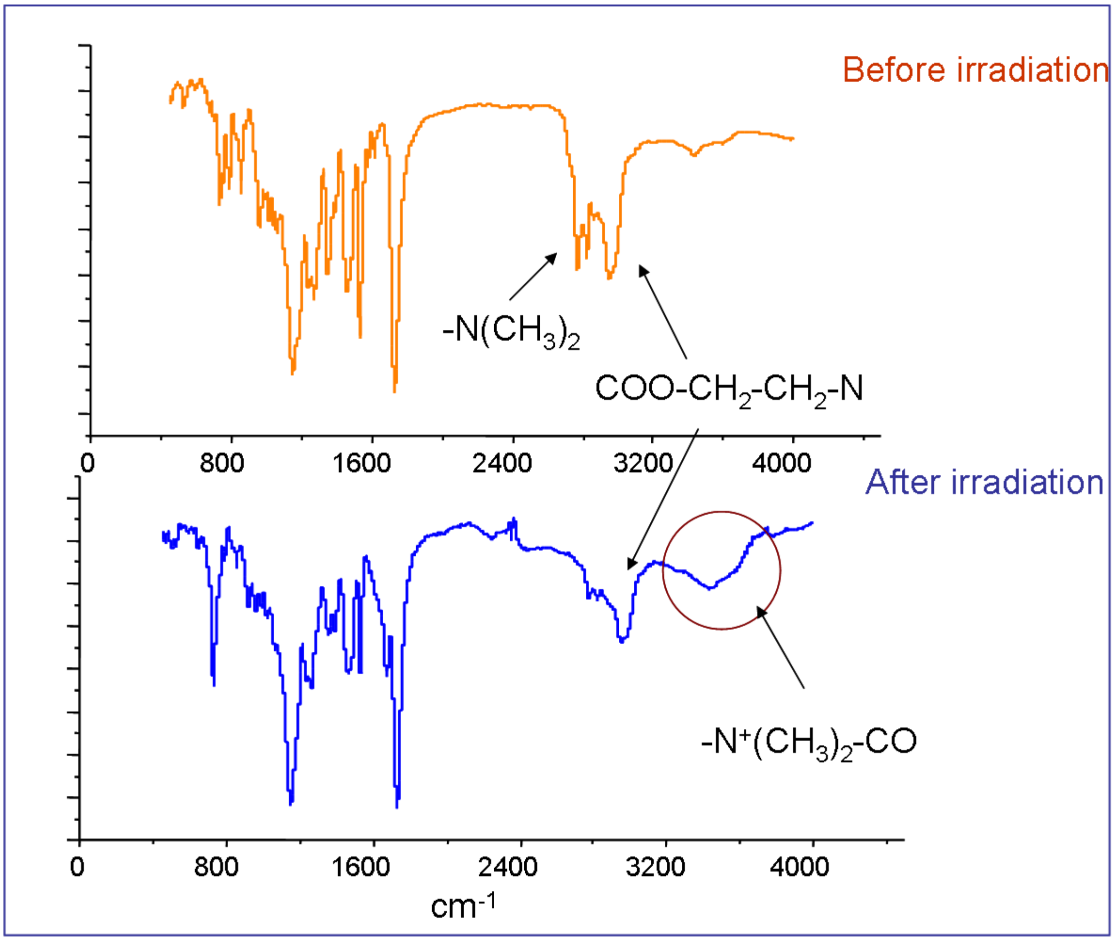


Fig. S8 FTIR spectra of the random copolymer before and after UV irradiation.

References

[S1] (a) J. S. Doh and D. J. Irvine. *J. Am. Chem. Soc.*, 2004, **126**, 9170-9171. (b) X. G. Jiang, C. A. Lavender, J. W. Woodcock and B. Zhao. *Macromolecules*, 2008, **41**, 2632-2643.

[S2] L. Zhou, C. Gao and W. J. Xu, *J. Mater. Chem.*, 2009, **19**, 5655-5664.