# Supporting Information

# Triple Stimuli-Responsive Crosslinked Polymeric Nanoparticles for Controlled Release

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#### 1. Materials

Triethylamine (99.5%) was purchased from Sinopharm Chemical Reagent Co. Ltd. 1,2-bis(2iodoethoxy)ethane (BIEE) was purchased from Tokyo Chemical Industry Co. LTD. Dimethylaminoethyl methacrylate (99%), 2-nitrobenzyl alcohol (98%), methacryloyl chloride (97%), copper bromide (98%), ethyl  $\alpha$ -bromoisobutyrate (98%) and 1,1,4,7,10,10hexamethyltriethylenetrtramine (97%) were purchased from Aldrich. All the solvents were used without further purification.

#### 2. Instruments

<sup>1</sup>H-NMR spectra were recorded from CDCl<sub>3</sub> solution on a Bruker AM 400 spectrometer. The molecular weight and the molecular weight distribution (M<sub>w</sub>/M<sub>N</sub>) 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 nanoparticle solution 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 uncrosslinked and crosslinked nanoparticles were characterized with a JEM-2010 EX/S Transmission Electron Microscope (TEM). Samples for TEM were prepared by depositing one droplet (3µL) 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. Dynamic light scattering (DLS) experiments were carried out both on Nano series Nano-ZS (Malvern Instrument) Zatasizer and 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. The dialyzed aqueous nanoparticle and nanogel solutions were filtered through 450 nm filter and the size distribution were measured at 25 °C. All the UV

irradiation for the samples was carried out with a high-pressure mercury lamp (365 nm, 500 W nominal powers) and UV light intensity was controlled at 75mW/cm<sup>2</sup>.

#### 3. Synthesis and characterization of 2-nitrobenzyl methacrylate (NBM) <sup>[1],[2],[3]</sup>

2-Nitrobenzyl methacrylate was prepared according to reported procedures. Briefly, 2nitrobenzyl 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<sub>2</sub>Cl<sub>2</sub> (10 mL) was added dropwise into the flask under N<sub>2</sub> 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<sub>2</sub>SO<sub>4</sub> 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 (5.33g, yield: 87.7%).<sup>1</sup>H-NMR (Bruker AM 400, CDCl<sub>3</sub>):  $\delta$  (ppm) 8.09 (d, H),  $\delta$ 7.66–7.44 (m, 3H),  $\delta$ 6.27 (s, H), $\delta$ 5.90 (s, H),  $\delta$ 5.58 (s, 2H),  $\delta$  1.96 (s, 3H).

### 4. Synthesis and characterization of random copolymer P(DMAEMA-co-NBM)

In a typical experiment, a 5mL ampule, flamed and dried under vacuum and filled with nitrogen, was charged with CuBr (0.15g, 1mmol), DMAEMA (4.71g, 30mmol), ethy 2-bromoisobutyrate (EBIB) (0.051 mL, 0.2 mmol) and monomer 2-nitrobenzyl methacrylate (2.21g, 10mmol), 3 mL THF. Then, PMDEA (35mg, 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 65°C for 18 hours. The reaction mixture was passed through a column filled with neutral Al<sub>2</sub>O<sub>3</sub> 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<sub>2</sub>CH<sub>2</sub>N- of DMAEMA units) and the peaks from 5.2 to 5.4 ppm (-OCH<sub>2</sub>- in the NMB units).

# 5. Preparation of micellar nanoparticles from the copolymer with and without Nile Red loaded

The copolymer (20 mg) was dissolved in THF (2 mL) and then deionized water (2 mL) was added at a rate of 1  $\mu$ L/s with quick stirring to induce the formation of the nanoparticles. After 4 hours, 18 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 (20 mg) and Nile Red (2 mg) were dissolved in THF (2 mL) and then deionized water (2 mL) was added at a rate of  $1\mu$ L/s with quick stirring. Then 9mL water was added to quench the micellar assemblies. THF was removed by evaporation at room temperature for 2 days.

# 6. CAC measurement of the copolymer

The critical aggregation concentration (CAC) of the P(DMAEMA-*co*-NBM) 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).

# 7. The LCST of the copolymer

Below the lower critical solution temperature (LCST) of PDMAEMA, 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 °C with 1 °C interval. To reduce as much as possible the margin of the error, the heating ramp was fixed at 1 °C/min and the experiment was performed in a short time. From

Figure S3 we can find, by incorporating 2-nitrobenzyl metharylate (NBM) into the polymer chain, the LCST of the synthesized copolymer P(DMAEMA-*co*-NBM) was 44°C.

#### 8. Preparation of crosslinked nanoparticles

First, a 10 mL of the above polymer micellar solution (1.0 mg/mL) was prepared. Then 1 mL of BIEE solution (12 mg/mL, dissolved in THF) was added into the micellar nanoparticle solution. Then the mixture was stirring for 24 h under 60 °C to allow for crosslinking. The prepared crosslinked nanoparticles (nanogels) were purified by dialysis using 20,000 g/mol membrane.

#### 9. FTIR measurement

FTIR spectroscopy was utilized to verify the crosslinking of the random copolymer. The sample of crosslinked polymer was prepared from 10 mL of nanogel solution (1.0 mg/mL). The solvent of the solution was evaporated by freeze-drying procedure for 24 h. Then the resulted solid was further dried at a higher temperature under vacuum before FTIR measurement.

## 10. Gel fraction measurement

In order to calculate the nanogel fraction, we first prepared a 1.0 mg/mL nanogel solution by the method stated above. UV-vis measurements were performed with samples of this solution diluted to 0.05 mg/mL, to determine the amount of2-nitrobenzyl moieties (at  $\sim$  300 nm absorption). Since the uncrosslinked polymer could not form aggregates at the concentration of 0.05 mg/mL (below CAC), the solution was dialyzed for 7 days using 50,000 g/mol membrane to remove the uncrosslinked polymer. By comparing the amount of 2-nitrobenzyl moieties before and after dialysis, we calculated the nanogel fraction to be about 64%.

# 11. Preparation of crosslinked nanoparticles encapsulated with guest molecules Nile Red

The polymer (20 mg) and Nile Red (2mg) were dissolved in 2 ml THF and then deionized water (2 mL) was added at a rate of 1  $\mu$ L/s with quick stirring to induce the formation of the nanoassemblies. After stirring for 4 hours, 18 mL deionized water was added and the mixture was open to the atmosphere to allow the organic solvent to evaporate. Then a BIEE organic solution (0.2ml, 60 mg/mL) was added to crosslink the nanoparticles at 60 °C. After 24 h, the mixture was dialyzed using a 10,000 g/mol membrane.

#### 12. Single stimulus triggered release experiments

**Photo-triggered:** The uncrosslinked nanoparticle solution (1.0 mg/mL) loaded with Nile Red and the nanogel solution (1.0 mg/mL) loaded with NR were irradiated under UV light (365 nm, 75 mW/cm<sup>2</sup>) separately. After UV irradiation for a certain time, the fluorescence of NR was measured.

**Acid-triggered:** The uncrosslinked nanoparticle solution (1.0 mg/mL) loaded with Nile Red and the nanogel solution (1.0 mg/mL) loaded with NR were stepwise adjusted to pH 6.0, 5.0, 4.0 over 1 hour, with dilute HCl. At each pH value, the fluorescence of NR was measured.

**Temperature-triggered:** The uncrosslinked nanoparticle solution (1.0 mg/mL) loaded with Nile Red and the nanogel solution (1.0 mg/mL) loaded with NR were stepwise heated to 40, 50, 60, 70 °C, followed by measuring the fluorescence of NR.

# 13. Multiple stimuli triggered release experiments

**PH& UV stimuli:** The nanogel solution (1.0 mg/mL) in a standard cuvette was first adjusted to pH 5.0, followed by fluorescence measurement; then the solution was irradiated under UV light (365 nm, 75 mW/cm<sup>2</sup>) for 5 min, followed by fluorescence measurement.

**UV & Heat stimuli:** The nanogel solution (1.0 mg/mL) in a standard cuvette was first irradiated under UV light for 5 min, followed by fluorescence measurement; then the solution was heated to 50 °C, followed by fluorescence measurement.

**PH& Heat stimuli:** The nanogel solution (1.0 mg/mL) in a standard cuvette was first adjusted to pH 5.0, followed by fluorescence measurement; then the solution was heated to 50 °C, followed by fluorescence measurement.

**PH& UV & Heat stimuli:** The nanogel solution (1.0 mg/mL) in a standard cuvette was stepwise adjusted to pH 5.0, UV irradiated for 5 min and heated to 50 °C, followed by fluorescence measurement.



**Figure S1.** Plot of fluorescence intensity of Nile Red vs. concentration of random copolymer (left); fluorescence spectra of Nile Red in the polymer solution with different concentrations excited at 560 nm (right). The CAC is about 0.1 mg/mL.



**Figure S2.** Phase transitions of P(DMAEMA-*co*-PEGMA) in water, measured by UV/vis spectroscopy at 500 nm.



**Figure S3.** FTIR spectra of the random copolymer before (spectrum 1) and after (spectrum 2) crosslinking.

It is clearly shown that the carbon-nitrogen vibration peak (from the amino groups) at 2800 cm<sup>-1</sup> decreased and a large carbon-nitrogen vibration peak (from the ammonium groups) appeared at 3400 cm<sup>-1</sup> after crosslinking <sup>[4]</sup>. The decrement of the peak at 2800 cm<sup>-1</sup> and the appearance of the peak at 3400 cm<sup>-1</sup> proved the quaternization between the amine groups and the iodoethoxy groups. The band centered at 1730 cm<sup>-1</sup> is attributed to C=O vibration in esters. The degree of quaternization is estimated to be 42% by calculating the decrease of the carbon-nitrogen vibration band from the amino groups compared with the C=O vibration band.



**Figure S4.** UV-vis absorption spectra of nanogel solution at the concentration of 0.05 mg/mL before (black curve) and after (red curve) dialysis for 7 days.

The relative amounts of the 2-nitrobenzyl moieties (at  $\sim$  300 nm absorption) were determined before and after dialysis. Compared the reduction of the amount, we calculated the gel fraction was about 64%.



**Figure S5.** TEM images of uncrosslinked nanoparticles under triple stimuli: a) pristine nanoparicles; b) at pH 4.0, c) heated to50 °C and d) upon UV (365 nm, 75 mW/cm<sup>2</sup>) irradiation for 20 min. The concentration of the copolymer solution was 1.0 mg/mL.

The responsive behaviors of the uncrosslinked nanoparticles under different stimuli were investigated by TEM. Figure S6a shows the morphology of the pristine nanoassemblies (diameter,  $\sim 120$  nm). At pH 4.0, the PDMAEMA segments were protonated and extended in aqueous solution, leading to the swollen of the assemblies (diameter,  $300 \sim 400$  nm), shown in Figure S6b. When heated to 50 °C, above its LCST, the diameter decreased approximately to 50 nm (Figure S6c), since the PDMAEMA segments became more hydrophobic and the collapse of the polymeric nanoparticles occurred. After UV irradiation for 20 min, the

nanoassemblies were disrupted to small aggregates (diameter,  $30 \sim 50$  nm) due to the photolysis of the *o*-nitrobenzyl methacrylate, shown in Figure S6d.



**Figure S6.** DLS curves of the uncrosslinked nanoparticles before ( $\blacksquare$ ) and after different stimuli: pH 4.0 ( $\bullet$ ), 50 °C( $\blacktriangle$ ), and UV irradiation 20 min ( $\blacktriangledown$ ).

The average diameters of uncrosslinked nanoassemblies before stimulation, at pH 4.0, at 50 °C and upon UV irradiated for 20 min were 130, 360, 80 and 60 nm, respectively, determined by DLS.



**Figure S7.** Fluorescence spectra of Nile Red, indicating the acid-triggered release profiles from uncrosslinked nanoparticles (left) and shell-crosslinked nanoparticles (right).



**Figure S8.** Fluorescence spectra of Nile Red, indicating the temperature triggered release profiles from uncrosslinked nanoparticles (left) and shell-crosslinked nanoparticles (right).



**Figure S9.** Fluorescence spectra of Nile Red, indicating the photo-triggered release profiles from uncrosslinked nanoparticles (left) and shell-crosslinked nanoparticles (right). The UV light intensity was 75mW/cm<sup>2</sup>.

# References

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