Electronic Supplementary Information

Photo, pH, and thermo triple-responsive spiropyran-based copolymer nanoparticles for controlled release

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1. Materials

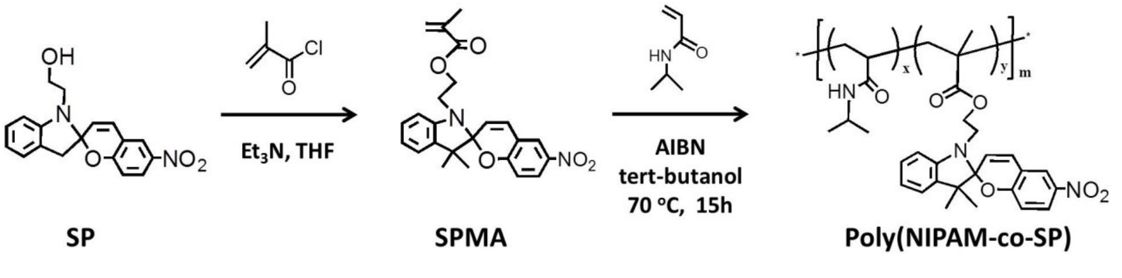
Triethylamine (Et_3N, 99.5%) and Tetrahydrofuran (THF, 98%) was purchased from Sinopharm Chemical Reagent Co. Ltd. *N*-isopropyl acrylamide (NIPAM, 99%), Azodiisobutyronitrile (AIBN, 99%) and Coumarin 102 were purchased from J&K Scientific Ltd. Methacryloyl chloride (98%) and Spiropyran (SP, 98%) were purchased from Sigma-Aldrich.

2. Instruments

^{1}HNMR spectra were obtained by Burker AM 400 spectrometer. The polymer molecular weight and molecular weight distribution (M_w/M_n) were detected by gel permeation chromatography (GPC, Waters 1515) with styragel columns relative to polystyrene standards. The lower critical solution temperature (LCST) and critical aggregation concentration (CAC) of the polymer were measured by UV-vis spectrometer (JASCO V-570). The morphologies of the copolymer nanoparticles were characterized with a JEM-2010 EX/S transmission electron microscope (TEM). 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. The fluorescence was obtained by a HitachiF-4500 fluorescence spectrophotometer. All the visible light and UV irradiation for the samples was carried out with a high-pressure mercury lamp (520 nm, 20 mW/cm^2; 365 nm, 20 mW/cm^2).

3. Synthesis of poly[(*N*-isopropyl acrylamide)-co-(1′-(2-methacryloxyethyl)-3′, 3′-dimethyl-6-nitro-spiro(2H-1-benzo-pyran-2,2′-indoline)]

The synthetic route of the copolymer poly(NIPAM-co-SP) is shown in Scheme 1.



Scheme S1. Synthetic route of poly(NIPAM-co-SP).

The monomer 1′-(2-methacryloxyethyl)-3’，3′-dimethyl-6-nitro- spiro(2H-1-benzo-pyran-2,2′-indoline) (SPMA) was prepared according to reported procedure.[s1] (SP 0.3 g, 8.5×10^{-3} mol), methacryloyl chloride (240 μL 2.5×10^{-3} mol) and Et_3N (230 μL) were added into 30 ml THF at 0 ^oC. Then the mixture was stirred for 28 hours. The resulting solution was filtered and extracted by saturated sodium bicarbonate solution and methylene chloride (1:1). The organic layer was washed three times by water, dried by $MgSO_4$, and then concentrated by evaporation. The product was re-crystalized by 95% ethyl alcohol (0.18 g, yield: 60%). ^1H NMR (300 MHz, $CDCl_3$) : 1.176 (s, 6H, $-C(CH_3)_2$), 1.897 (s, 3H, $=C-CH_3$), 3.393–3.552 (m, 2H, $-N-CH_2-$), 4.273 (t, 2H, $-O-CH_2-$), 5.545 and 6.053 (s, 2H, $=CH_2$), 6.642–6.748 (d, 2H, Ar-H and –CH=), 6.878–6.963 (m, 2H, Ar-H), 7.073–7.104 (m, 1H, Ar-H), 7.172–7.240 (m, 1H, Ar-H), 7.990–8.016 (m, 2H, Ar-H).

NIPAM (1.53 g, 1.35×10^{-2} mol), SPMA (0.094 g, 2.56×10^{-4} mol) and AIBN (0.037 g, 0.218 mol) were dissolved in 3 ml tert-butanol. After three times of freeze-pump-thaw cycle, the mixture was stirred under 70 ^oC for 15 hours. The copolymer was precipitated in diethyl ether (100 ml) and methyl alcohol (1 ml) mixture solution to remove the unreacted small molecule compounds. After filtered and dried at 40 ^oC under vacuum for 48 hours, the polymer poly(NIPAM-co-SP) was obtained (orange powder, 1.3 g, yield: 80%).

Fig. S1 shows the UV-vis absorbance spectra of poly($NIPAM_x$-co-SP_y) (0.4 mg ml^{-1}) and SPN-OH (0.03 mg ml^{-1}) in THF, from which the ratio between NIPAM and SP in the polymer could be determined (x:y=18:1)..

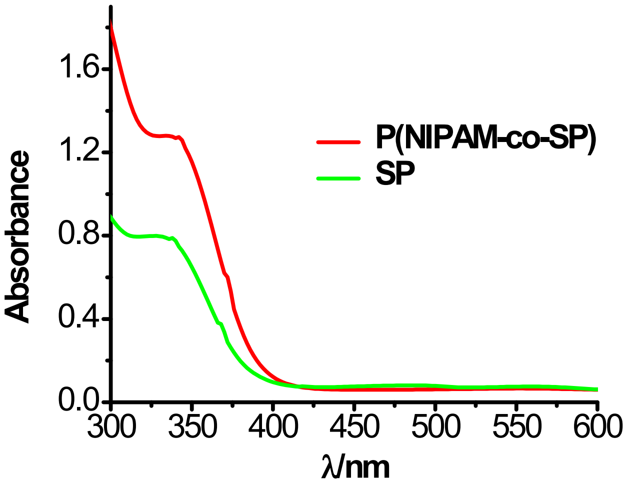


Fig. S1 UV-vis absorbance spectra of the synthesized polymer poly(NIPAM-co-SP) and the spiropyran SP in THF.

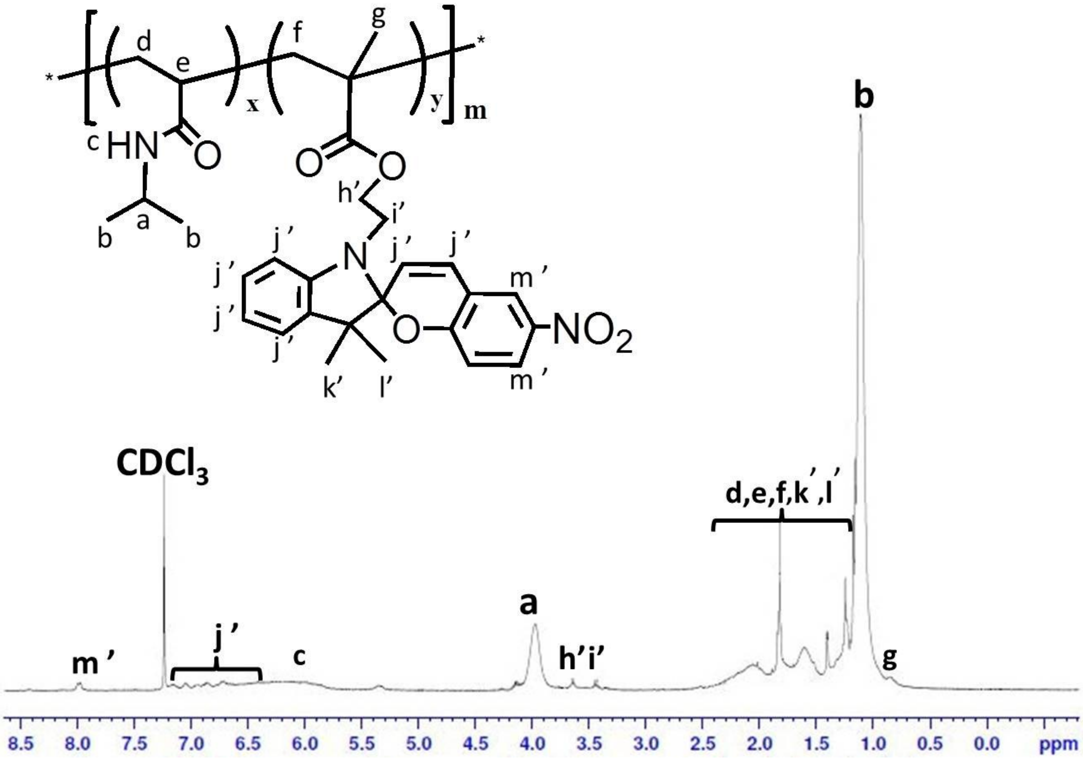
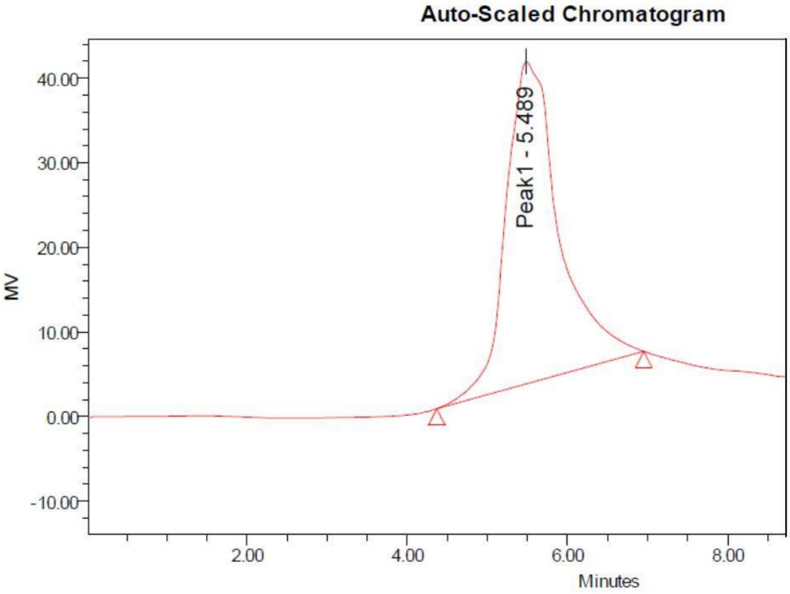
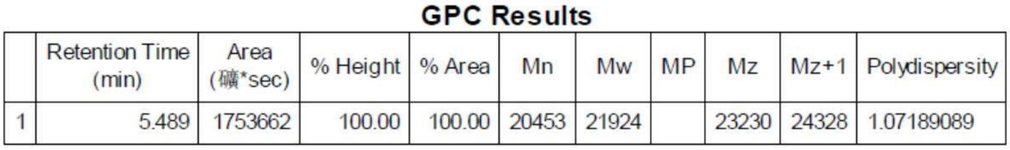


Fig. S2 ^{1}H NMR spectrum of poly(NIPAM-co-SP) in $CDCl_3$ (300 MHz).

The number-average molecular weight (Mn) and polydispersity index (PDI) of the random copolymer were about 2.05×10^4 g mol^{-1} and 1.07, respectively. The number of repeat unit (m) was calculated to be 8 according to the ratio between NIPAM and SP (x:y=18:1) and the polymer molecular weight.





GPC Results

	Retention Time (min)	Area (礦*sec)	% Height	% Area	Mn	Mw	MP	Mz	Mz+1	Polydispersity
1	5.489	1753662	100.00	100.00	20453	21924		23230	24328	1.07189089

Fig. S3 GPC trace of the random copolymer poly(NIPAM-co-SP).

Fig. S4 shows the FTIR spectrum of poly(NIPAM-co-SP). The strong band centered at 1394 cm^{-1} was ascribed to stretching of nitro groups in poly(NIPAM-co-SP). The strong band centered at 1532 cm^{-1} was ascribed to -C-C- stretching of the benzene ring of spiropyran.[s2] The characteristic band of amide carbonyl groups in poly(NIPAM-co-SP) appeared at 1656 cm^{-1}.

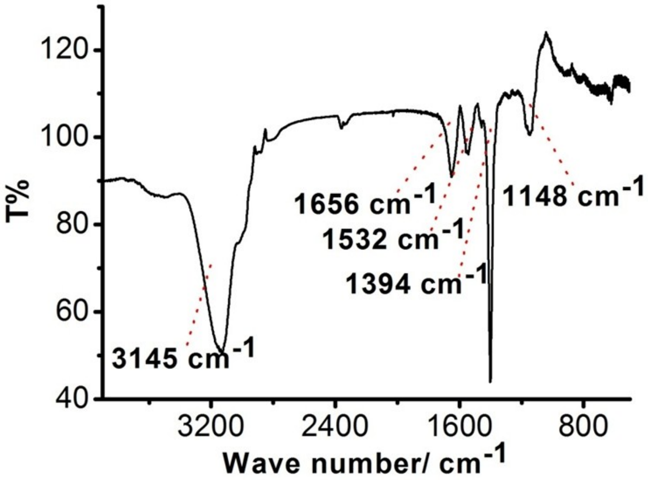


Fig. S4 FTIR spectrum of poly(NIPAM-co-SP).

4. The LCST of the copolymer

The lower critical solution temperature (LCST) of poly(NIPAM-co-SP) was determined to be about 35 ºC. The determination of its LCST was carried out by measuring the transmittance at 500 nm of the nanoparticle solution (0.2 mg ml^{-1}) from 15 °C to 60 °C.

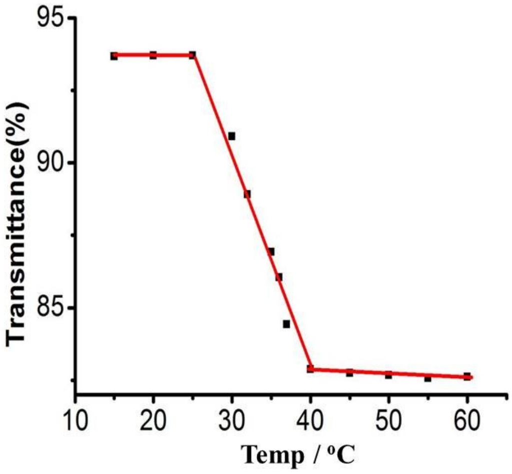


Fig. S5 Transmittance at 500 nm of poly(NIPAM-co-SP) aqueous solution (0.2 mg mL^{-1}) as a function of temperature with a heating rate of 1 °C/min.

5. The CAC of the copolymer

The CAC of the copolymer poly(NIPAM-co-SP) was determined using Nile Red (NR) as a hydrophobic probe. Fig. S6 shows the plot of the absorption intensity of NR at 550 nm versus the log of concentration of the copolymer, from which it can be seen that the CAC of the copolymer was about 0.2 mg ml^{-1}.

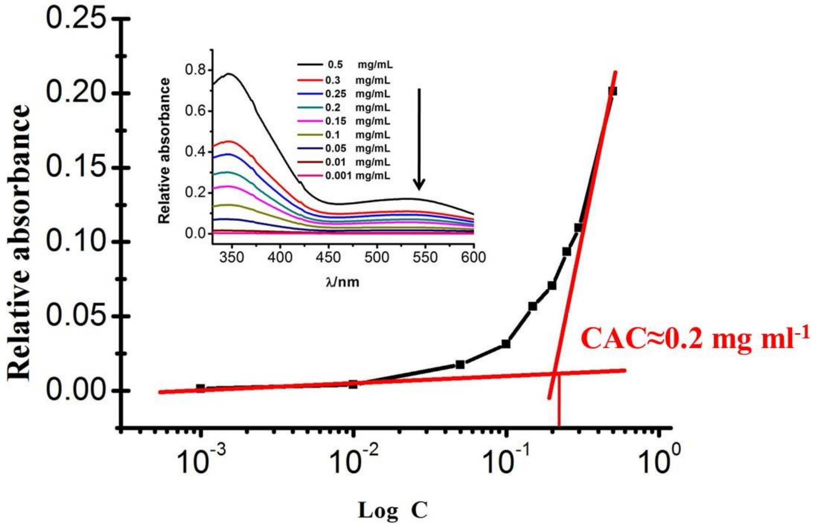


Fig. S6 Plot of the absorption intensity of NR at 550 nm versus the log of concentration of the copolymer. Inset: absorption spectra of NR loaded in the polymer solution with different polymer concentrations.

6. Preparation of nanoparticles and nanoparticles loaded with coumarin 102

The copolymer (2 mg) was dissolved in THF (1ml). Then 1 ml of deionized water was added at a rate of 1 μL s^{-1} into the solution. After that, 9 ml of deionized water was added to the above solution to quench the formation of the copolymer nanoparticles. THF was removed by evaporating at room temperature. As to the polymer nanoparticles loaded with coumain 102, the random copolymer (2 mg) and coumain 102 (0.2 mg) were dissolved in THF (1 mL) and then deionized water (1 mL) was added at a rate of 1μL s^{-1} with stirring. More deionized water (9 ml) was added to quench the nanoparticles assembles. THF was removed by evaporating at room temperature.

Samples for TEM were prepared by depositing one droplet (3 μL) of aqueous copolymer solution (0.2 mg ml^{-1}) on carbon-coated nickel grids, and the solvent was evaporated by freeze-drying procedure for 24 h. Fig. S7a and S7b show the diameters of the nanoparticles were about 80-110 nm (Fig. S7a). The diameters of the nanoparticles loaded with coumarin 102 were about 120-130 nm (Fig. S7b).

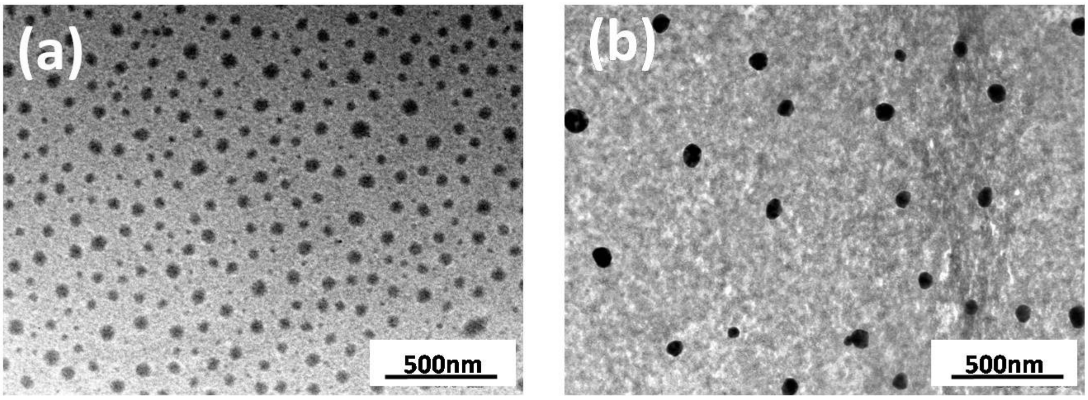


Fig. S7 TEM images of blank nanoparticles (a) and nanoparticles loaded with coumarin 102 (b).

7. DLS data of the copolymer nanoparticles

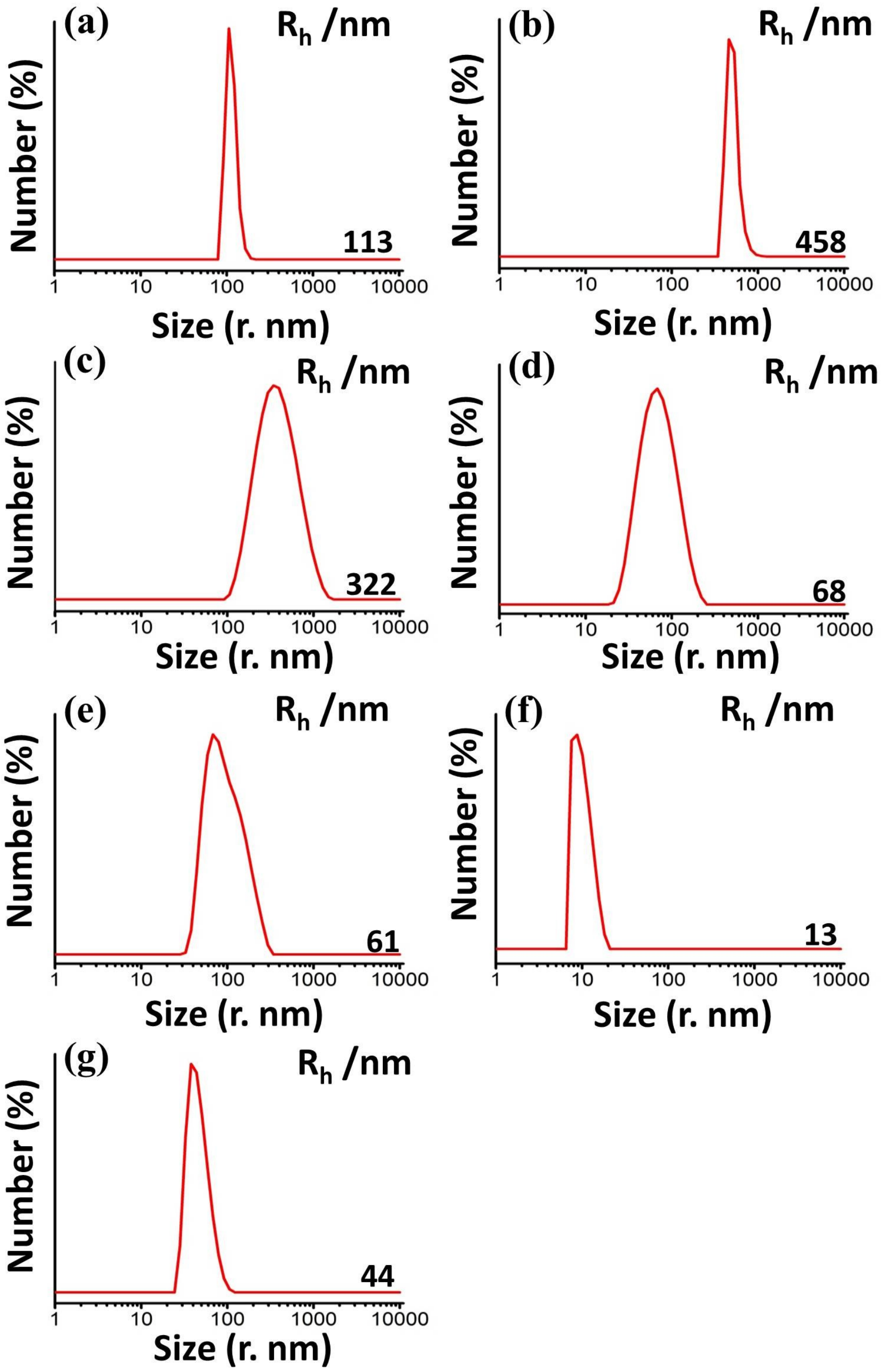


Fig. S8 Size distribution for poly(NIPAM-co-SP) assemblies in water obtained from DLS analysis undert stimuli: (a) before stimuli (primary nanoparticles), (b) after UV irradiation, (c) at pH 5, (d) at pH 9, (e) at 45 ºC, (f) combined stimulation of pH 5 and UV light irradiation, (g) combined stimulation of pH 9 and UV light irradiation. (UV light: 365 nm, 20 mW/cm^2, 5 min)

Fig. S8 shows the change in volume averaged size distribution before and after the multiple stimuli, determined by dynamic light scattering (DLS). The hydrodynamic diameter (R_h) of the primary nanoparticles was about 113 nm (Fig. S8a). After UV light irradiation, the R_h increased to 458 nm due to the photo-isomerization which could increase the hydrophilicity greatly (Fig. S8b). At pH 5, the R_h increased to 322 nm, owing to the swelling of the nanoparticles (Fig. S8c). At pH 9, the R_h decreased to 68 nm, due to the shrinkage of the nanoparticles (Fig. S8d). At 45 ^{o}C, the hydrophilicity of NIPAM segments decreased and the R_h of nanoparticles were shrunk to 61 nm (Fig. S8e). Fig. S8f and Fig. S8g show the R_h of the nanoparticles under combined stimulation of H^+/UV and OH^-/UV were 13 nm and 44 nm, respectively. The morphological changes determined by DLS are in accordance with those revealed by TEM.

8. UV-vis absorbance spectra of the copolymer nanoparticles under pH and temperature stimulation.

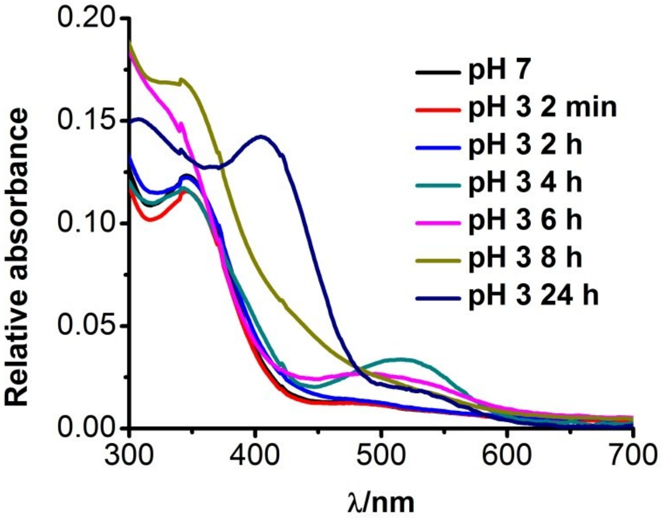


Fig. S9 UV-vis absorbance spectra of the polymer nanoparticles in aqueous solution under pH stimulation.

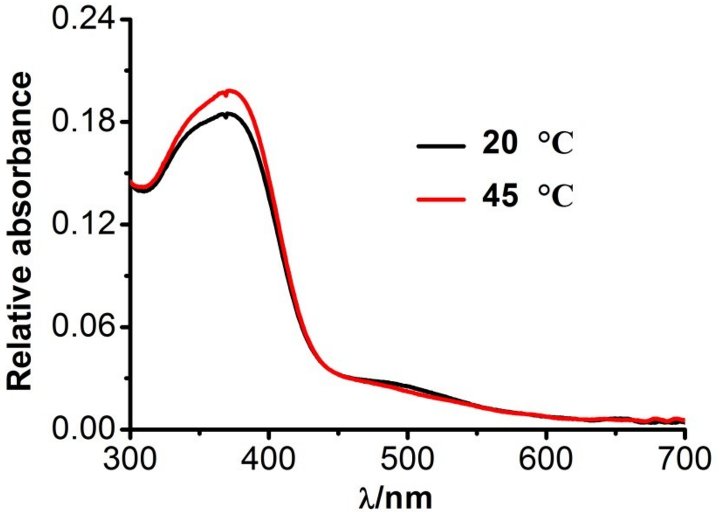


Fig. S10 UV-vis absorbance spectra of the polymer nanoparticles in aqueous solution at different temperature.

9. Fluorescence emission spectra of coumarin 102 in THF under UV light, pH and temperature stimulation

Fig. S11, S12 and S13 show the effects of UV light irradiation, pH and temperature change on the fluorescence of coumarin 102, respectively, from which it can be seen that the fluorescence intensity of coumarin 102 changed little under different stimulations.

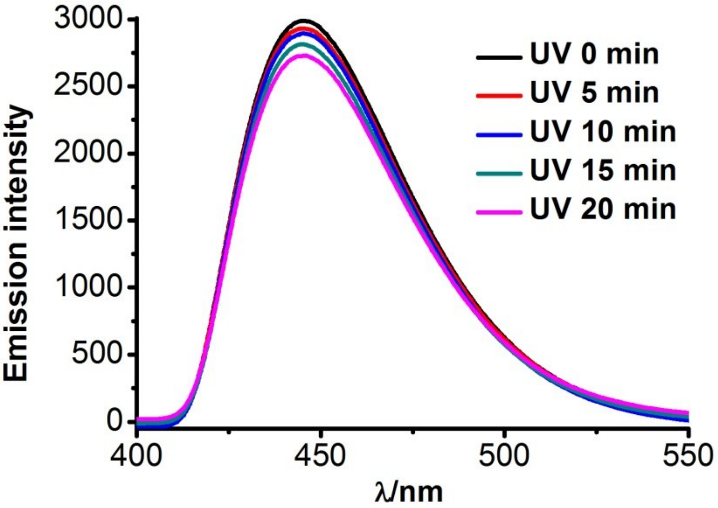


Fig. S11 Fluorescence emission spectra of coumarin 102 in THF solutions (0.045 mg ml^{-1}, λ_{ex}=395 nm) under UV light irradiation for different time. (365 nm, 20 mW/cm^2)

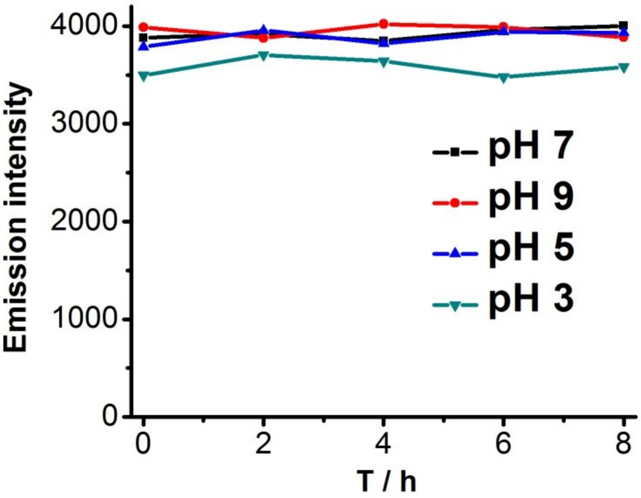


Fig. S12 Fluorescence emission intensity of coumarin 102 in THF solutions (0.045 mg ml^{-1}, λ_{ex}=395 nm) at different pH values in 8 hours.

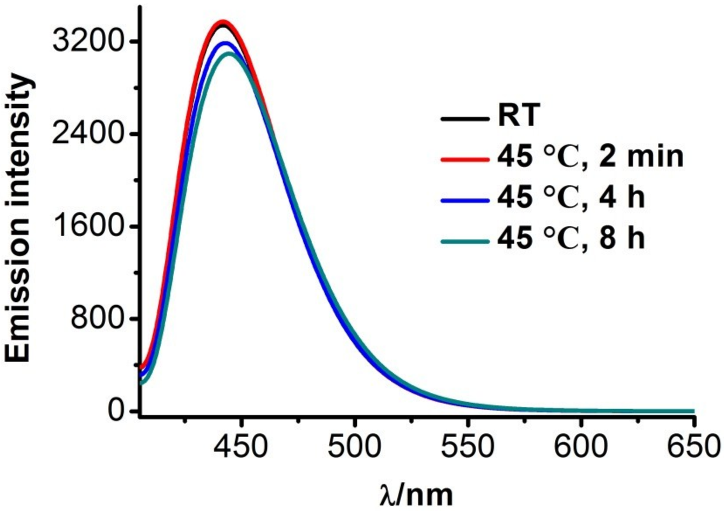


Fig. S13 Fluorescence emission spectra of coumarin 102 in THF solutions (0.045 mg ml^{-1}, λ_{ex}=395 nm) at 45 ºC for different time.

10. Fluorescence emission spectra of coumarin 102 loaded in the nanoparticles under pH and temperature stimulation

Fig. S14a, S14b and S14c show the fluorescence emission spectra of coumarin 102 loaded in the polymer nanoparticles at pH 3, pH 5 and pH 9, respectively. At pH 3, 50% of loaded coumarin 102 could be released in 8 hours; at pH 5, 25% of the loaded molecules could be released in 8 hours; at pH 9, little of the loaded coumarin 102 could be released.

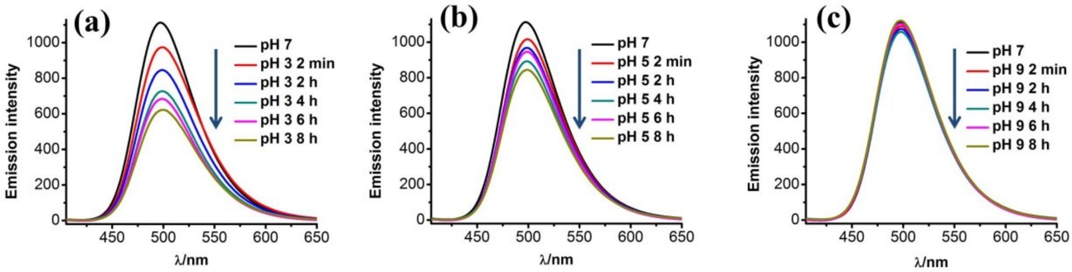


Fig. S14 Fluorescence emission spectra of coumarin 102 loaded in the copolymer nanoparticles (λ_{ex}=395 nm): (a) at pH 3**;** (b) at pH 5; (c) at pH 9.

Fig. S15 shows the fluorescence spectra of coumarin 102 loaded in the copolymer nanoparticles under 45 ºC for different time, from which it can be seen that no significant release of coumarin 102 could be observed at high temperature.

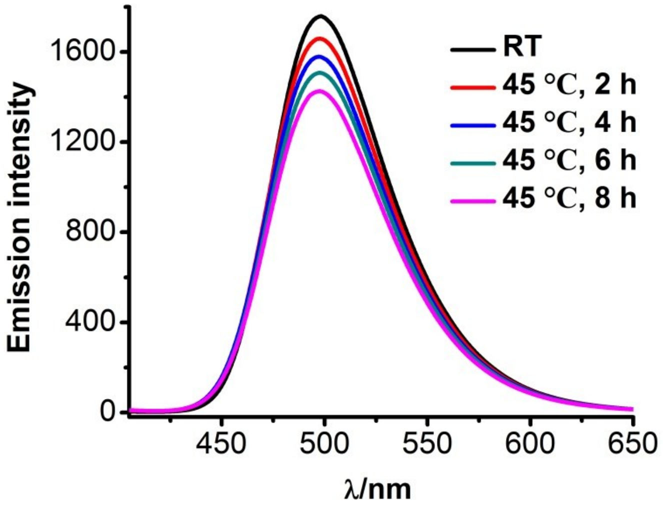


Fig. S15 Fluorescence emission spectra of coumarin 102 loaded in the polymer nanoparticles at 45 ºC for different time. (λ_{ex}=395 nm)

11. Fluorescence emission spectra of coumarin 102 loaded in the nanoparticles under combined stimulation

Fig. S16a, S16b and S16c show the fluorescence emission spectra of coumarin 102 loaded in the copolymer nanoparticles under pH 3, 5 and 9 combined with UV light irradiation, respectively. At pH 3, the loaded coumarin 102 could be completely released under UV light irradiation for 12 min; at pH 5, the loaded molecules could be completely released under UV light irradiation for 15 min; while at pH 9, about 40% of the loaded molecules could be released under UV light irradiation for 15 min.

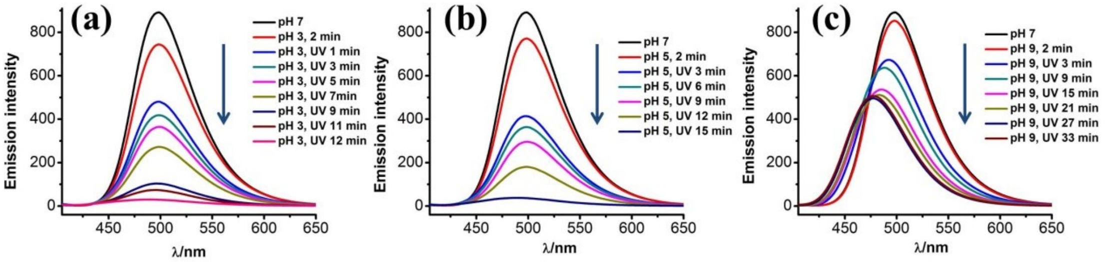


Fig. S16 Fluorescence emission spectra of coumarin 102 loaded in the polymer nanoparticles under combined stimulation of pH 3 (a), pH 5 (b) and pH 9 (c) with UV light irradiation. (365 nm, 20 mW/cm^2, λ_{ex}=395 nm)

Fig. S17a shows the color change from bright yellow to colorless of the copolymer nanoparticles loaded with coumarin 102 under the combined stimulation of temperature and UV light irradiation. Fig. S17b shows the TEM image of the nanoparticles loaded with coumarin 102 under combined stimulation of temperature and UV light irradiation, from which it can be seen the primary micellar nanoparticles were dissociated. Fig. S17c shows the fluorescence spectra of coumarin 102 loaded in the copolymer nanoparticles under combined stimulation of temperature and UV light irradiation, from which it can be seen that the fluorescence decreased with the increase of irradiation time and the fluorescence of coumarin 102 almost disappeared after UV light irradiation for 9 min at 45 ºC, indicating most of the loaded molecules were released. Fig. S17d shows the release profiles of coumarin 102 under temperature and UV light stimulation, from which it can be seen that no significant release of coumarin 102 occurred at high temperature (45 ºC), while more molecules could be released upon the combined stimulation compared with those under single stimulation of temperature or UV light irradiation. At 45 ºC, the loaded coumarin 102 could be released completely under UV light irradiation for 9 min. While at room temperature, about 60% of coumarin 102 could be released under UV light irradiation for the same time.

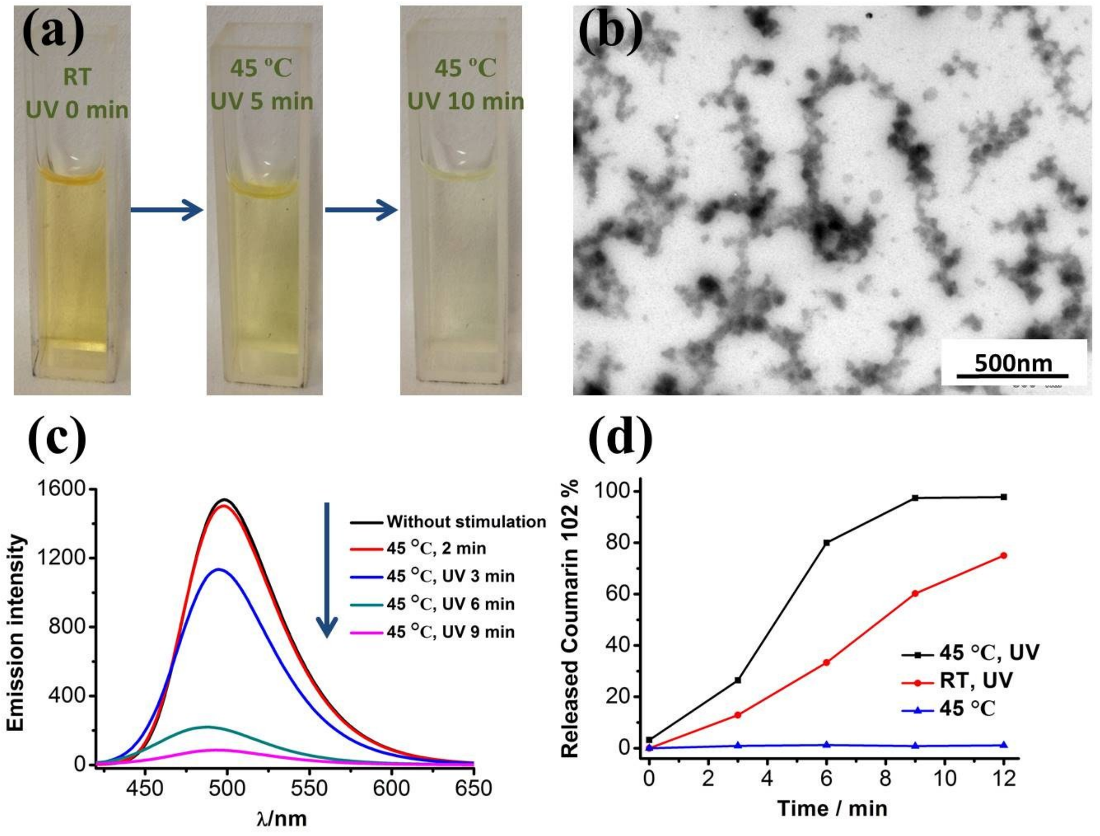


Fig. S17 (a) Color change of the polymer nanoparticles loaded with coumarin 102 under combined stimulation of temperature and UV light irradiation; (b) The TEM image of the nanoparticles loaded with coumarin 102 under combined stimulation of temperature (45 ºC) and UV light irradiation for 10 min; (c) Emission spectra of coumarin 102 loaded in the polymer nanoparticles under combined stimulation of temperature (45 ºC) and UV light irradiation; (d) Release profiles of coumarin 102 loaded in the polymer nanoparticles under temperature and UV light stimulation. (365 nm, 20 mW/cm^2, λ_{ex}=395 nm)

References

[S1] Y. Shiraishi, R. Miyamoto and T. Hirai, *Org. Lett.,* 2009, **11**, 1571-1574.

[S2] W. G. Tian and J. T. Tian, *Langmuir,* 2014, **30**, 3223-3227.