Electronic Supplementary Information (ESI)

One-pot aqueous synthesis of sub-10 nm responsive nanogels

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SI 1. Experimental Procedures

1.1. Materials

Poly(ethylene glycol) dimethacrylate (PEGDMA, $M_n \approx 550$ g/mol) was purchased from Sigma-Aldrich, and was purified with neutral Al_2O_3. Divinylbenzene (DVB), ammonium persulfate (APS), sodium dodecyl sulfate (SDS), 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH) were purchased from Alfa Aesa, and were used as received without further purification. The water used in all experiments was of Millipore Milli-Q grade.

1.2. Synthesis of the Nanogels

PEGDMA, DVB (2.8×10^{-3} mol/L), and SDS (7.1×10^{-3} mol/L) was dissolved in water (50.0 mL) in a 150 mL round-bottom flask equipped with a stirrer, a N_2 gas inlet, and a condenser. After 30 min N_2 purge, the temperature was raised to 70.0 °C and the polymerization was initiated by adding APS (4.5×10^{-3} mol/L). The polymerization was allowed to proceed for 7h. The product was purified by centrifugation (Thermo Electron Co. SORVALL® RC-6 PLUS superspeed centrifuge), redispersion in water, and 3 days' dialysis (Spectra/Por® molecularporous membrane tubing, cutoff 12000-14000) against water. To investigate the effect of the feeding mol ratio $f_{P\text{-}D}$ of PEGDMA to DVB on the size of the nanogels, nanogels were prepared by changing the feeding amount of PEGDMA. The nanogels synthesized with 4.2×10^{-3} mol/L of PEGDMA were denoted as SPN-1.

The contents of sulfur in the SPN-1 were determined to be 2.636%. We speculate that the sulfur element is due to the unique enriched of ionic sulfate groups on the surface of the nanogels by using an extraordinary quantity of initiator APS in the synthesis (also see zeta-potential tests in Fig. S6 below), rather than the residual SDS. To prove the clearance of SDS after 3-days' dialysis, we have synthesized

similar nanogels (as a control, and referenced as "the controlled nanogels") by using AAPH (4.5×10^{-3} mol/L) as the initiator under otherwise the same conditions for the synthesis of SPN-1. It turned out that nearly no sulfur signal was detected on the controlled nanogels. While the elemental analysis on the controlled nanogels indeed prove the clearance of SDS after 3-days' dialysis, the control experiments also provide additional proof that the synthesis approach presented in this paper can readily lead to sub-10 nm nanogels even if the initiator was changed (see Fig. S6).

1.3. Drug Loading and Release Tests

5-FU was loaded into the nanogels by a method reported in our previous work.[S-1] The nanogel dispersion (5.0 mL) was stirred in an ice water bath for 30 min. 5-FU solution (2.0 mL, 1.0 mg/mL, pH = 12.0) was then added dropwisely to the vial. After being stirred for 4h, the pH of the mixture was adjusted to 7.4 by an addition of HCl solution and the suspension was continuously stirred overnight in an ice-water bath. The suspension was then centrifuged at 37.0 °C. To remove free 5-FU, the precipitate was redispersed in PBS (5.0 mL, 5.0×10^{-3} mol/L, pH = 7.4), and further purified at 37.0 ^{o}C by repeated centrifugation and washing for at least six times. All the upper clear solutions were collected, and the concentration of free 5-FU was determined by UV-vis spectrometry at 270 nm, based on the linear calibration curve ($R^2 > 0.99$) measured using 5-FU solutions with known concentrations under the same conditions. The amount of 5-FU loaded into the nanogels was calculated from the decrease in 5-FU concentration. The loading content is expressed as the mass of loaded drug per unit weight of dried nanogels.

The *in vitro* release of 5-FU from the nanogels was evaluated by the dialysis method. A dialysis bag filled with fresh 5-FU-loaded nanogel dispersion (5.0 mL) was immersed in PBS (50.0 mL, 5.0×10^{-3} mol/L, pH = 7.4) at different temperatures. To demonstrate drug release by a stepwise treatment, the *in vitro* release profile of 5-FU-loaded nanogels was measured with the releasing medium alternately changed between 37.0 ^{o}C and 42.0 ^{o}C. The released 5-FU outside of the dialysis bag was sampled at defined time period and assayed by UV-vis absorption at 270 nm. Cumulative release is expressed as the total percentage of drug released through the dialysis membrane over time.

1.4. Cytotoxicity Tests

B16F10 cells (6×10^3 cell/well) were cultured in DMEM containing 10% FBS and 1% penicillin-streptomycin in a 96-well plate, and exposed to free 5-FU, empty nanogels, and 5-FU-loaded nanogels, respectively. The plate was incubated at 37.0 °C for 24h. The medium was then aspirated, and these wells were washed three times using fresh serum-free DMEM. After that, 25 μL of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) solution (5.0 mg/mL in PBS) were added to the wells. After incubation for 2h, the solution was aspirated and 100.0 μL of DMSO was added to each well to dissolve the formazan crystal, and the plate was sealed and incubated overnight at 37.0 °C with gentle mixing. Three portions of the solution obtained from each well were transferred to three respective wells of a 96-well plate. Cell viability was measured using a microplate reader at 570 nm. Positive controls contained no drug or nanogels, and negative controls contained MTT. Parallel wells (in triplicate) also contained only medium (no cells) and the same concentrations of nanogels.

In temperature-dependent cell viability assays, the plates were incubated at different temperatures for different times. Positive controls contained no drug or nanogels, and negative controls contained MTT. Parallel wells (in triplicate) also contained only medium (no cells) and the same concentrations of nanogels.

1.5. Characterization

The contents of sulfur in the nanogels were determined by using a Dionex ICS-1500 ion chromatography system. TEM images were taken on a JEOL JEM-2100 transmission electron microscope at an accelerating voltage of 200 kV. The nanogels were air-dried on a carbon-coated copper grid for TEM measurements. The pH value was measured on a EUTECH PH 700 instruments.

Dynamic light scattering (DLS) was performed on a 90Plus multi angle particle sizing analyzer equipped with a BI-9000AT digital autocorrelator (Brookhaven Instruments, Inc.). A He-Ne laser (35 mW, 659 nm) was used as the light source. In dynamic LLS (DLS), the Laplace inversion (here the CONTIN method was used) of each measured intensity-intensity time correlation function in a dilute dispersion can lead to a line-width distribution $G(\Gamma)$. For a purely diffusive relaxation, Γ is related to the

translational diffusion coefficient D by $(\Gamma/q^2)_{C\rightarrow 0,q\rightarrow 0} = D$, so that $G(\Gamma)$ can be converted to a transitional diffusion coefficient distribution and hydrodynamic diameter ($<D_h>$) distribution by using the Stokes-Einstein equation, $<D_h> = 2(k_B T/6\pi\eta)/D$, where k_B, T, and η are the Boltzmann constant, the absolute temperature, and the solvent viscosity, respectively.[S-2] All samples were passed through Millipore Millex-HV filters with a pore size of 0.80 μm to remove dust before the DLS measurements.

Reference

[S-1] W.T. Wu, J. Shen, Z. Gai, K.L. Hong, P. Banerjee and S.Q. Zhou, *Biomaterials*, 2011, **32**, 9876.

[S-2] B. Chu, *Laser Light Scattering*. 2nd ed. New York: Academic Press; 1991.

SI2. Figures.

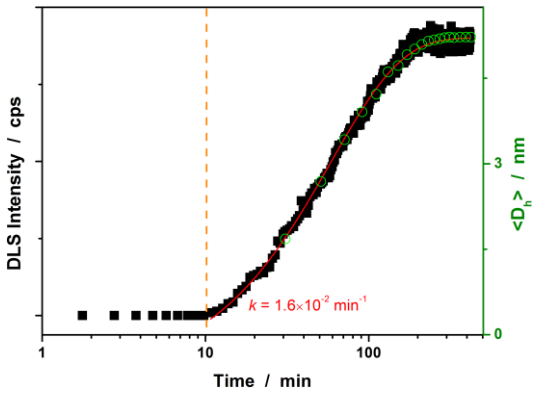

Fig. S1 DLS intensity (■) and DLS size (○) evolution of the nanogels' formation at the reaction temperature of 70.0 ^{o}C. Solid lines: 1st-order kinetic fits.

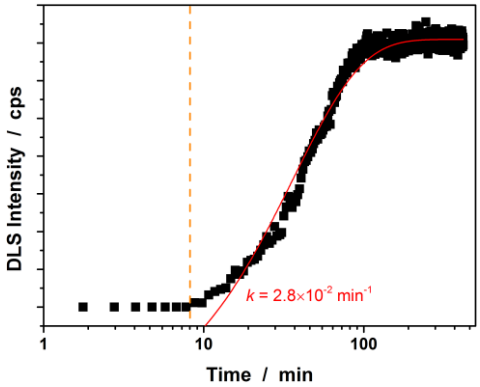

Fig. S2 Kinetic curves of the nanogels' formation at the reaction temperature of 76.5 ^{o}C. Solid lines: 1st-order kinetic fits.

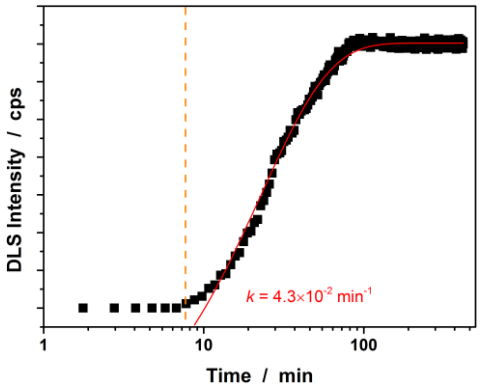

Fig. S3 Kinetic curves of the nanogels' formation at the reaction temperature of 81.9 ^{o}C. Solid lines: 1st-order kinetic fits.

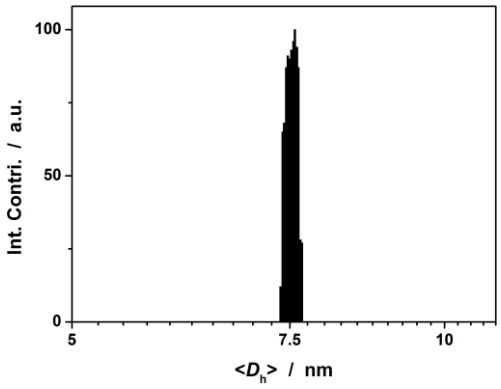

Fig. S4 DLS size distribution of the nanogels synthesized at the reaction temperature of 76.5 ^{o}C, measured at 25.0 ^{o}C.

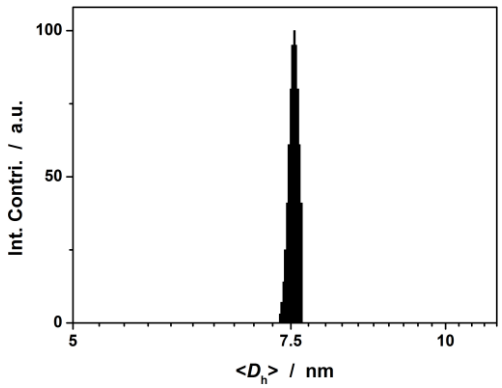

Fig. S5 DLS size distribution of the nanogels synthesized at the reaction temperature of 81.9 ^{o}C, measured at 25.0 ^{o}C.

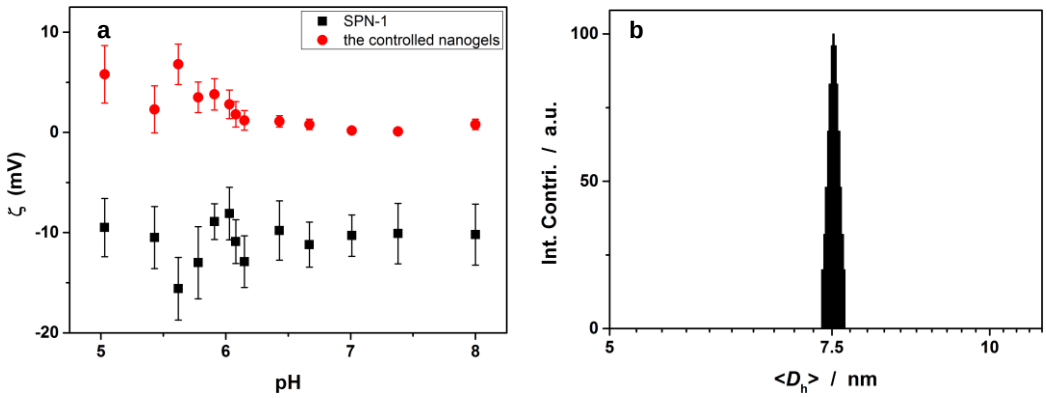

Fig. S6 (a) Zeta-potential of SPN-1 and the controlled nanogels. (b) DLS size distribution of the controlled nanogels. All measurements were made at 25.0 ^{o}C.

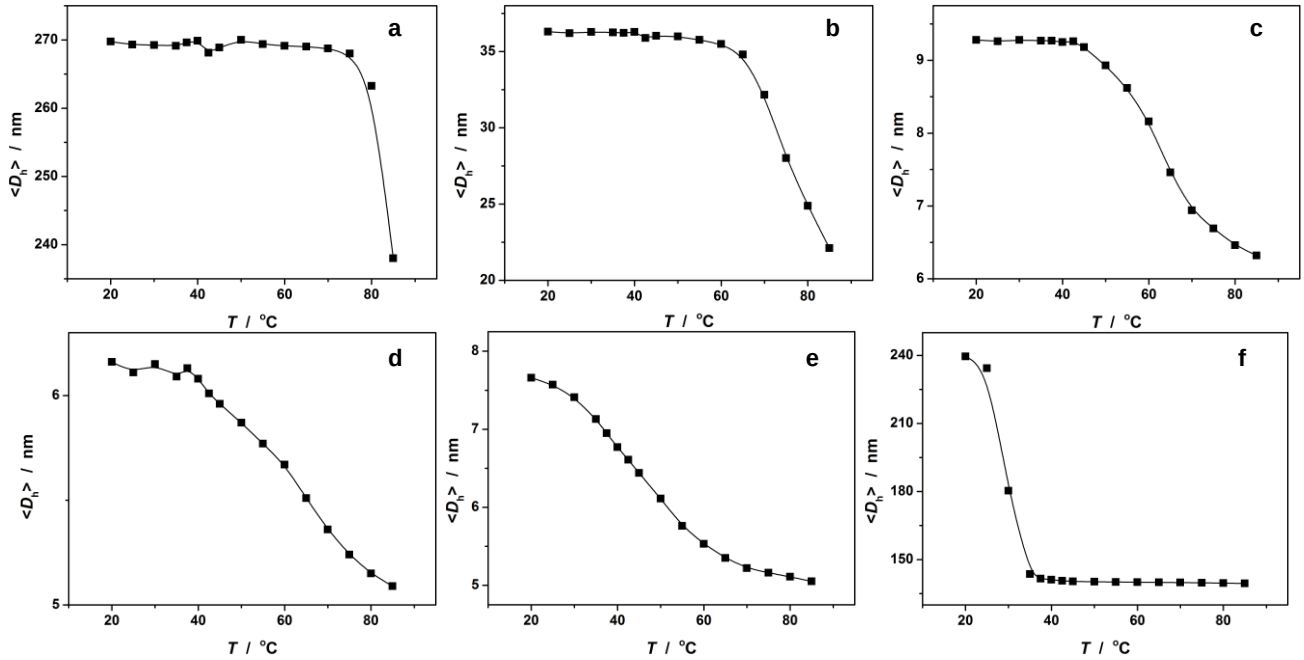

Fig. S7 Temperature-dependent <D_h> of the nanogels synthesized with $f_{P\text{-}D}$ of (a) 19.0, (b) 9.0, (c) 6.2, (d) 3.0, (e) 1.5, and (f) 0.1.

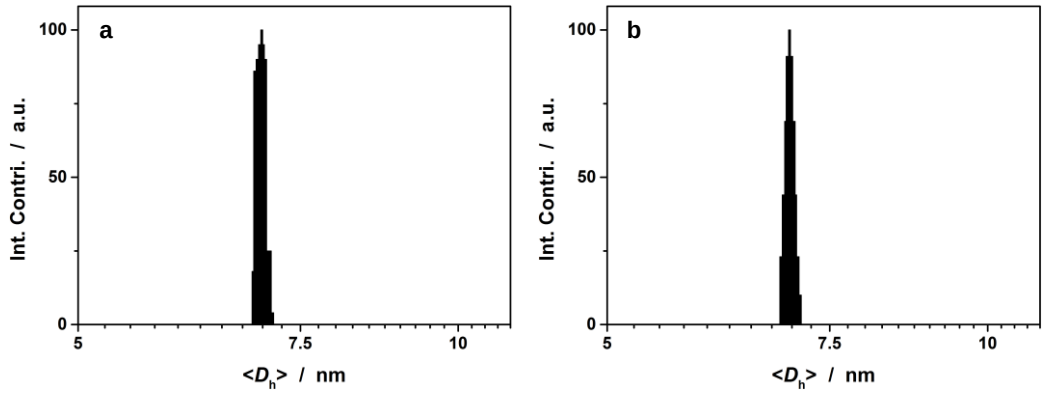

Fig. S8 The 5-FU-loaded nanogels (a) kept a narrow size distribution with neglectable change in size, in comparison with that of the empty nanogels (b).

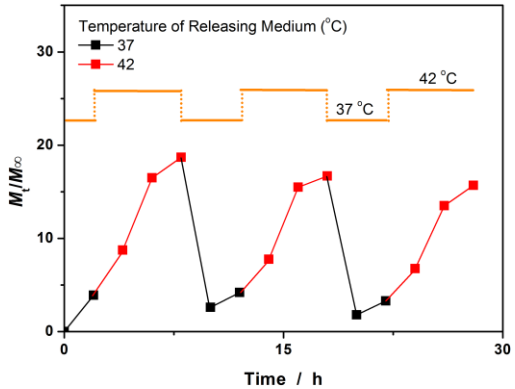

Fig. S9 *In vitro* release profile of 5-FU-loaded SPN-1 in PBS (pH 7.4) with the releasing medium alternately changed between 37.0 °C and 42.0 °C.

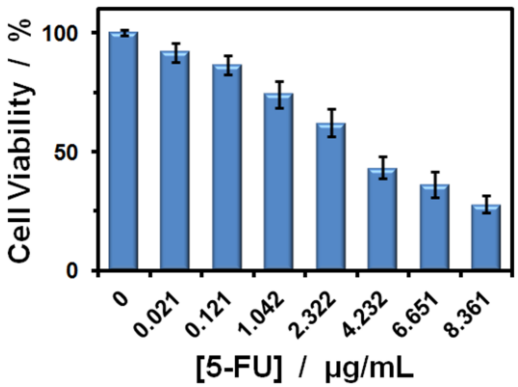

Fig. S10 B16F10 cell viability following treatments with 5-FU-loaded SPN-1, where [5-FU] is the concentration of total 5-FU.

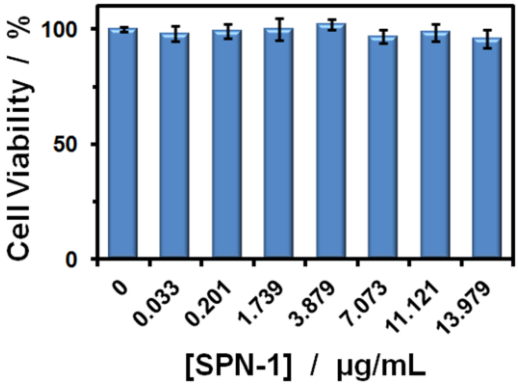

Fig. S11 B16F10 cell viability following treatments with empty SPN-1.

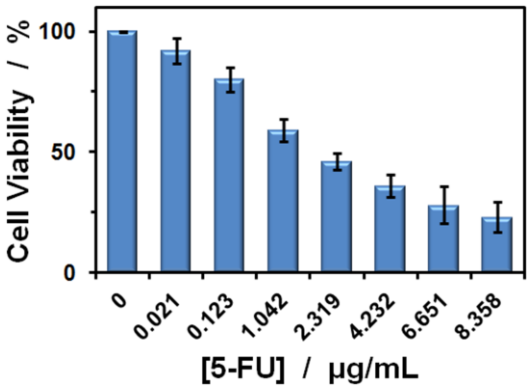

Fig. S12 B16F10 cell viability following treatments with free 5-FU.

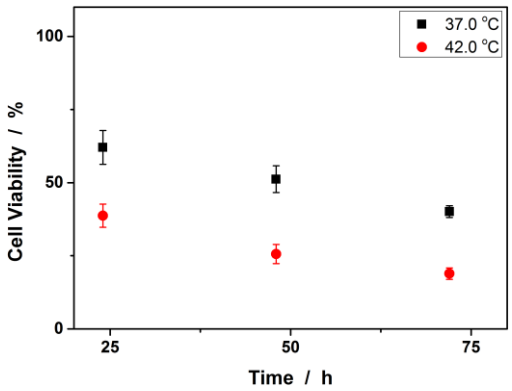

Fig. S13 B16F10 cell viability following treatments with 5-FU-loaded SPN-1 (with a total 5-FU concentration of [5-FU] = 2.322 μg/mL) for different times at different temperatures.

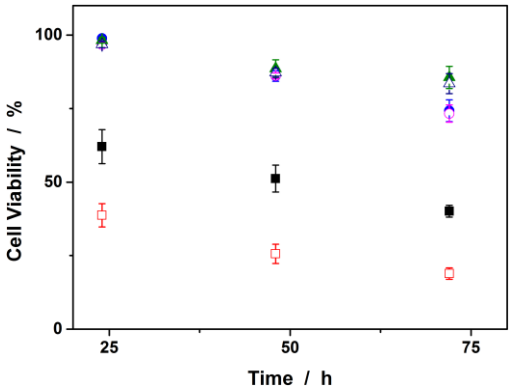

Fig. S14 A comparison of the IC_{50} of bigger nanogels (●,○: 269.3 nm, synthesized with $f_{P\text{-}D}$ = 19.0; ▲,Δ: 36.2 nm, synthesized with $f_{P\text{-}D}$ = 9.0) with that of sub-10nm nanogels (■, SPN-1). B16F10 cell viability was measured following treatments with 5-FU-loaded nanogels for different times at different temperatures (solid symbols: 37.0 °C; hollow symbols: 42.0 °C). The total 5-FU concentration was set to [5-FU] = 2.322 μg/mL.