Supplementary data

Multi-Stimuli-Triggered Release of Charged Dye from Smart PEGylated Nanogels Containing Gold Nanoparticles to Regulate Fluorescence Signals

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\textbf{Materials and Instruments.} Ethylene glycol dimethacrylate (EGDMA; Kanto Chemical, Japan) and 2-(N,N-diethylamino)ethyl methacrylate (EAMA, Wako, Japan) were distilled over CaH\textsubscript{2} under reduced pressure. Potassium persulfate (KPS, Wako, Japan) was recrystallized from water, and then dried \textit{in vacuo}. Tetrachloroauric acid (HAuCl\textsubscript{4}, Wako, Japan) and 1,3,6,8-pyrenetetrasulfonic acid tetrasodium salt (PSA, Fluka) were used without further purification. Water was purified using the Milli-Q system (Millipore). Dynamic light scattering measurements were carried out using a Zetasizer Nano-ZS instrument (Malvern, UK) equipped with a 4.0mW He–Ne laser (633 nm). Elemental analysis was carried out using a Series II CHNS/O Analyzer 2400 (Perkin-Elmer, Waltham, MA). TEM analysis was carried out using a JEM-100CX microscope (JEOL, Japan) operating at 100 kV, and the average diameter and number of GNPs were determined from the
images using the Image J software (National Institutes of Health, US). The UV-vis spectra and fluorescence spectra were recorded using a UV-2400PC spectrometer (Shimadzu, Japan) and an F-7000 spectrometer (Hitachi, Japan), respectively. Ar ion (Ar⁺) laser irradiation was performed using the Stabilite 2017-AR system (beam diameter = 1.4 mm at the 1/e² points, Spectra Physics, US) with a 514.5nm filter.

**Preparation and Characterization of the PEGylated Nanogel.** α-Acetal-ω-vinylbenzyl-poly(ethylene glycol) macromonomer (acetal–PEG–Ph–CH=CH₂, \(M_n = 7,200\), \(M_w/M_n = 1.03\)) was synthesized as described in our previous report. The obtained acetal–PEG–Ph–CH=CH₂ (500 mg, 69 μmol) and KPS (29.3 mg, 108 μmol) were loaded into a one-necked round-bottom flask, which has been evacuated and purged with nitrogen three times. Then, a previously degassed mixture of deionized-distilled water (33 mL), EGDMA (20.5 μL, 0.11 mmol, 1.0 mol%), and EAMA (2.18 mL, 10.8 mmol) was added to the flask. Emulsion copolymerization was carried out at room temperature overnight. In this emulsion polymerization, the tertiary amino groups of the EAMA monomer and the KPS spontaneously formed a redox complex (initiator) through electron transfer from EAMA to KPS at room temperature. Purification was carried out by ultrafiltration (MWCO = 200,000) using methanol (MeOH), followed by the use of water to remove the unreacted PEG. The average diameter of the obtained nanogel as a function of temperature was measured by DLS (Figure S1). Elemental analysis of the PEGylated nanogel was carried out to determine the amine content (calcd.: C 62.8, H 10.1, N 6.0; found: C 61.7, H 9.5, N 5.9), and the resulting concentration of amine in the purified nanogel solution (30.4 mg/mL) was

![Figure S1](image.png)  
**Figure S1.** Temperature dependency of the diameter of PEGylated nanogel at pH6.
calculated to be 128 mM. To determine the pKₐ value of the PEGylated nanogel, a solution of PEGylated nanogel in distilled water (40 mL, 152 μg/mL) was adjusted to pH 3.5 by using 0.1 M HClaq, and the resulting solution was titrated with 0.01 M NaOHaq at various temperatures (5, 15, 25, 40, 50, and 60 ºC). An automatic titrator (DL-25, METTLER) was used for the titration. In this case, the titrant was added in quantities of 50 μL after the pH values were stabilized (minimal interval: 30 s). The α/pH curves were determined from the obtained titration curves (Figure S2).

Preparation and Characterization of the PEGylated GNG. Aqueous solution of HAuCl₄ (1.0 mL, 100 μg/mL, [Au] = 243 μM) at pH 6 was added to the PEGylated nanogel solution (1.0 mL, 115.4 μg/mL, [N] = 486 μM) at pH 6, and the resulting mixture was incubated at 60 ºC until the absorbance at the SPB reached a plateau. TEM samples were prepared by mounting 10-fold diluted drops of solutions on carbon-coated copper grids and allowing them to dry in the air. TEM analysis was carried out using a JEM-100CX microscope to measure the size of the GNPs. Both the average number of GNPs in a single PEGylated nanogel (n > 50 PEGylated GNGs) and the average diameter of the GNPs (n >200 GNPs) were determined from TEM images using the Image J software statistically. To determine the pKₐ value of the PEGylated GNGs, a solution of PEGylated GNGs in distilled water (40 mL, 216 μg/mL) was adjusted to pH 3.5 by using 0.1 M HClaq, and the resulting solution was titrated with 0.01 M NaOHaq at 25°C. An automatic titrator (DL-25, METTLER) was

![Figure S2. Degree of protonation (α) as a function of pH (α/pH curves) for PEGylated nanogel at 5 (purple), 15 (light blue), 25 (blue), 40 (red), 50 (orange) and 60°C (green).](image)
used for the titration. The titrant was added in quantities of 50 μL after the pH values were stabilized (minimal interval: 30 s). The α/pH curve was determined from the obtained titration curve (Figure S3).

![α/pH curve](image)

**Figure S3.** Degree of protonation (α) as a function of pH (α/pH curve) for PEGylated GNG at 25°C.

**Photothermal Properties of the PEGylated GNG.** Aqueous solutions (3.0 mL) of the PEGylated GNG (81 μg/mL, [Au] = 24 μg/mL) and PEGylated nanogel (57 μg/mL, [Au] = 0 μg/mL) at 25 °C were stirred and irradiated with the Ar⁺ laser at 514.5 nm and a fluence of 39 W/cm² for 10 min (23.4 kJ/cm²) using the Stabilite 2017-AR system and a FieldMaster-GS Power and Energy Meter. The temperature of the samples was monitored using a 9669-10D microelectrode.

**Formation of Polyion Complexes from PSA and PEGylated GNG.** A PEGylated GNG aqueous solution (456 μg/mL, [N] = 1.92 mM) and a PSA aqueous solution (625 μg/mL, 100 μM, [SO₃⁻] = 400 μM) were prepared. The aqueous solution (0 ~ 233.1 μL) of PEGylated GNG was added to the PSA aqueous solution (160 μL) at various N/SO₃⁻ ratios (N/S = 0, 1, 2, 3, 4, 5, 6, and 7) at 25 °C, followed by the addition of distilled water and 0.1 M HCl to adjust the total volume to 1.6 mL ([PSA] = 10 μM) and the pH to 3.5. The fluorescence of the PSA was measured by exciting the solution at 355 nm while monitoring the emission at 385 nm. For the pH-triggered release experiment, a solution of PIC (N/SO₃⁻ = 5) at pH 3.5 was adjusted to pH 9.5 with 0.1 M NaOH. Then, the fluorescence spectrum of the solution at pH 9.5 (ex. = 355 nm) was measured.
Thermal Release of the PSA from PSA-Loaded PEGylated GNG. To prepare the PIC at N/\text{SO}_3^- = 6, a PEGylated GNG solution (50 µL, 1.14 mg/mL [N] = 4.8 mM) was added to a PSA solution (100 µL, [PSA] = 100 µM, [\text{SO}_3^-] = 400 µM), followed by the addition of 200 mM phosphate buffer (PB) at pH 8 (50 µL) and distilled water (800 µL) to adjust the total volume to 1.0 mL ([PSA] = 10 µM) and the pH to 8. The cell containing the PIC solution (1.0 mL) was sealed with a plastic plug and placed in a fluorescence spectrometer.
equipped with a temperature control system. After the solution was allowed to stand in the cell at a given temperature, the fluorescence spectra of the solution (ex. = 355 nm) were measured. The cumulative release of PSA from the PEGylated GNG was determined based on the standard curve corresponding to the fluorescence intensity of the PSA alone in 10 mM PB solution at pH 8.

**Photothermal Release of the PSA from PSA-Loaded PEGylated GNG.** Figure S5 shows a schematic representation of the experimental set-up for the photothermally-triggered release of PSA from the PEGylated GNG. A PEGylated GNG solution (50 µL, 1.14 mg/mL [N] = 4.8 mM) was added to a PSA solution (100 µL, [PSA] = 100 µM, [SO$_3^-$] = 400 µM) to prepare the PIC at N/SO$_3^-$ = 6, followed by the addition of 200 mM phosphate buffer (PB) at pH 8 (50 µL), 500 mM NaClaq (300 µL) and distilled water (500 µL) to adjust the total volume to 1.0 mL ([PSA] = 10 µM, [NaCl] = 0.15 M) and the pH to 8. As a control sample, a PEGylated nanogel (without GNP) solution (750 µL, 76 µg/mL [N] = 4.8 mM) was added to a PSA solution (100 µL, [PSA] = 100 µM, [SO$_3^-$] = 400 µM) to prepare the PIC at N/SO$_3^-$ = 6, followed by the addition of 200 mM PB at pH 8 (50 µL) and 1.5 M NaClaq (100 µL) to adjust the total volume to 1.0 mL ([PSA] = 10 µM, [NaCl] = 0.15 M) and the pH to 8. The cell containing the PIC solution (100 µL) was sealed with a plastic plug and irradiated with a 600mW Ar$^+$ laser ($\lambda$ = 514.5 nm) at a fluence of 39 W/cm$^2$ for 8 min. The fluorescence spectra of the solution (ex. = 355 nm) were measured at appropriate time intervals (0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 min).

**Figure S5.** Schematic representation of the experimental set-up used for photothermally-triggered release of PSA from PSA-loaded PEGylated GNG.
The cumulative release of PSA from the PEGylated GNG or the PEGylated nanogel was determined based on the standard curve corresponding to the fluorescence intensity of the PSA alone in 10 mM PB containing 0.15 M NaCl at pH 8.

References