Electronic Supplementary Material (ESI) for Chemical Communications

Supporting information

Surface Modification of Mammalian Cells with Stimuli-Responsive Polymers

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MATERIALS AND METHOD

Materials

N-Methacryloylmannosamine (ManMA) was synthesized by a previously described method and obtained as a mixture of anomers. [1] *N*-isopropyl acrylamide (NIPAM) was purchased from Sigma-Aldrich. Irgacure 2959 was kindly provided by BASF Japan. α-Methyltrithiocarbonate-*S*-phenylaceticacid (MTPA) was synthesized as previously reported.[2] 4,4'-Azobis(4-cyanovaleric acid) (V-501, 98%) was obtained from Wako Pure Chemical. Pre-purified water was further purified using a Millipore Milli-Q system that includes reverse osmosis, ion exchange, and filtration steps (18.2 MΩ). Other reagents and solvents available in extra-pure grade were obtained commercially and used without further purification.

Synthesis of poly(N-isopropyl acrylamide) (PNIPAM)

NIPAM (3.0 g, 26.5 mmol), V-501 (3.3 mg, 0.012 mmol), and MTPA (14.8 mg, 0.057 mmol) were dissolved in ethanol (26.5 mL). The solution was degassed by purging with Ar gas for 30 min. Polymerization was carried out at 70°C for 19 h. After being cooled to room temperature, the polymer was purified by dialysis (MWCO 3500) in ethanol for 2 days and DI water for 1 day. The conversion of polymerization was 73.9%. GPC results showed that M_n(GPC) of PNIPAM was 1.2 x 10^4 with a polydispersity (M_w/M_n) of 1.16. The number-average degree of polymerization (DPn) of PNIPAM was estimated by NMR to be 141, and the number-average molecular weight (M_n(NMR)) was 1.6 x 10^4. PNIPAM (0.5 g) was dissolved in 7.14 mL of methanol.

After the polymer completely dissolved in the water, a trace of tris(2-carboxyethyl)phosphine (TCEP) was added to the solution, and then 64 μL of ethanolamine was added. The solution was stirred at ambient temperature for 1 h or until the polymer solution became colorless. The product (PNIPAM-SH) was purified by dialysis in methanol for 2 days and DI water for 1 day. Subsequently, PNIPAM-SH was obtained by lyophilization and characterized by ^{1}H NMR (Fig. S1), gel-permeation chromatography (GPC), and UV/Vis absorption spectroscopy. The UV spectra of PNIPAM and PNIPAM-SH are shown in Fig. S2.

Cell culture experiment

Human promyelocytic leukemia cells (HL-60) were purchased from the Health Science Research Resources Bank and maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum at 37°C in a humidified atmosphere of air containing 5% CO_2. The concentration of HL-60 was adjusted to 2 x 10^5 cells/mL. The cell suspension (1 mL) was poured into a 24-well tissue culture dish and 110 μL of 50 mM ManMA/PBS was added to the wells. After cell cultivation for 3 days, the cell suspension was centrifuged to remove free ManMA in the medium. The cells were then washed with fresh medium (serum-free) and centrifuged to remove any free ManMA from the medium. RPMI-1640 medium containing 2.5 wt% PNIPAM-SH and 0.05 wt% Irgacure 2959 was then added to the cells and the mixture was exposed to UV light for 15 min at 4°C. The cells were rinsed again three times with the culture medium containing 10% fetal bovine serum to remove any unreacted PNIPAM-SH.

Cell viability

The viability of the HL-60 cells immobilized with PNIPAM-SH was investigated by staining with calcein-AM. After the thiol-ene reaction of the ManMA-treated HL-60 cells with PNIPAM, the cells were rinsed twice with fresh medium to remove any non-reacted polymers. Subsequently, the cells were placed in contact with 1 μM calcein-AM dye and incubated for 15 min at room temperature. Then, the cells were observed by phase-contrast and fluorescence microscopy (Olympus IX-71).

Control of cell aggregation with temperature

The concentration of PNIPAM-immobilized HL-60 cells was adjusted to 2.0 x 10^5 in the culture medium containing 10% fetal bovine. The cell suspension (100 μL) was poured into glass bottom culture dishes. The dishes were kept at 25°C and 37°C for 25 min. Morphological analyses of the cells in the dishes were performed with an Olympus IX-71 phase-contrast microscope.

Thermal treatment of lysate from PNIPAM-immobilized HL-60 cells

A suspension of PNIPAM-immobilized HL-60 cells (2.0 x 10^7 cells/mL, 120 μL) was mixed with cell lysate buffer at 1:1 (vol). Free PNIPAM-SH was added to the lysate to a concentration of 50 mg/mL. Then, the lysate was incubated at 37°C to achieve precipitation and the turbid solution was centrifuged. After decantation, fresh lysis buffer (200 μL) was added and incubated at 4°C. After the unsolubilized substances were removed by centrifugation, the supernatant was used for western blot analysis.

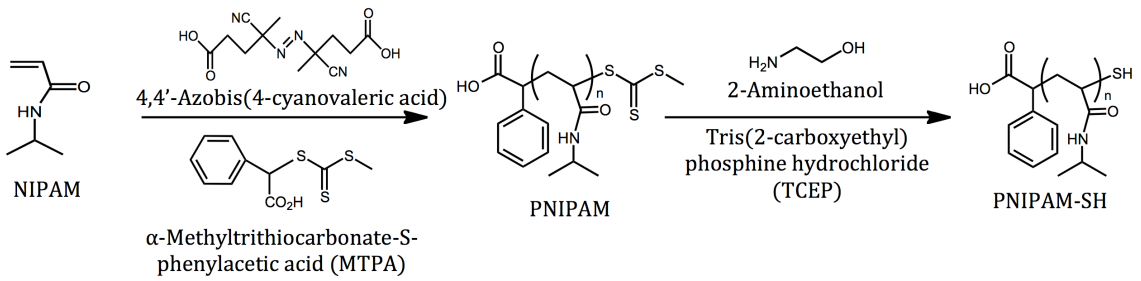
Western blotting

Lysates with and without thermal treatment were diluted 1:1 (vol) with loading buffer (50 mM Tris/HCl, pH 6.8, 100 mM dithiothreitol, 2% SDS, 0.1% bromophenol blue, 10% glycerol). After incubation at 100°C for 30 min, samples were separated by SDS / PAGE (7.5%). For western blot analysis, the gel-bound protein bands were electrotransferred to a poly(vinylidene difluoride) membrane. The membrane was loaded onto a SNAP i.d. Protein Detection System (Millipore, Billerica, MA, USA). The membrane was blocked by incubation for 10 min at room temperature in 0.5% skimmed milk in NaCl/Tris (Bio-Rad, Hercules, CA, USA) containing 0.1% (v/v) Tween-20 (NaCl/Tris-T). The blocked membrane was then incubated in NaCl/Tris-T and a monoclonal antibody against PSGL-1 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA); diluted 1:333 (v/v) with 0.5% skimmed milk in NaCl/Tris-T) for the detection of antigens for 10 min at room temperature. The membrane was washed three times with NaCl/Tris-T and subsequently incubated for 10 min at room temperature with biotin-labeled anti-mouse IgG (KPL, Gaithersburg, MD, USA; diluted 1:1000 (v/v) with 0.5% skimmed milk in NaCl/Tris-T). In addition, the membrane was incubated for 10 min with streptavidin alkaline phosphatase conjugate (Bio-Rad, Hercules, CA, USA; diluted 1:1000 (v/v) with NaCl/Tris-T) and biotinylated alkaline phosphatase conjugate (Bio-Rad, Hercules, CA, USA; diluted 1:1000 (v/v) with NaCl/Tris-T). Finally, color-developed buffer (25 × AP color development buffer (Bio-Rad, Hercules, CA, USA; 800 μL), AP color reagent A, B (Bio-Rad, Hercules, CA, USA; 200 μL each), and 18 mL of DI water) was placed in contact with the membrane for 30 min and rinsed with DI water.

References

1 Y. Iwasaki, H. Matsuno, *Macromol. Biosci.*, 2011, **11**, 1478.R.

2 S. Yusa, T. Endo, M. Ito, *J. Polym. Sci. A Polym. Chem.* 2009, **47**, 6827.



Scheme S1 Synthetic route of PNIPAM

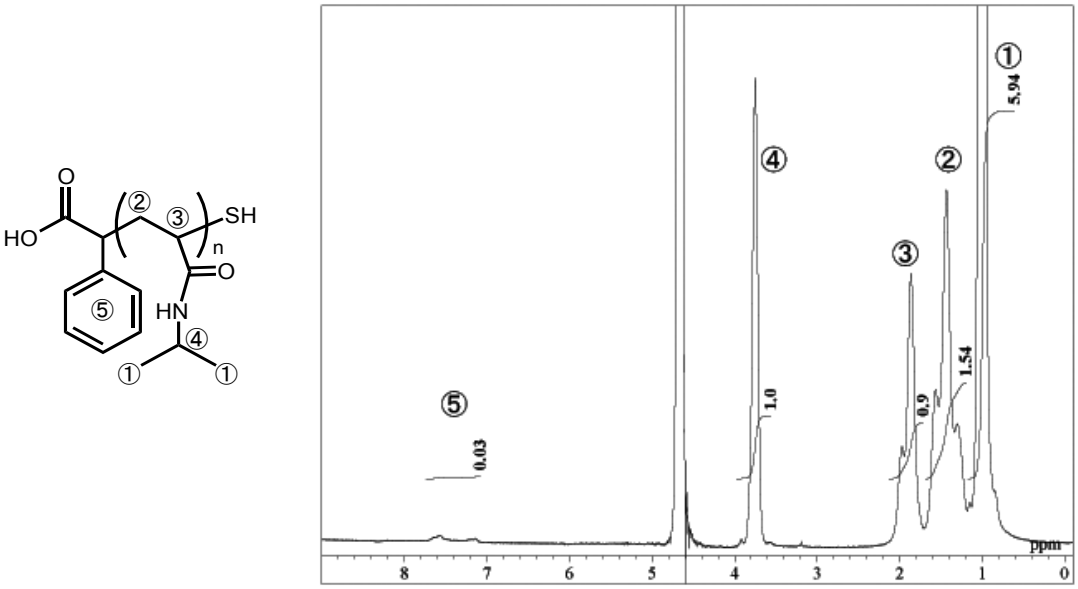


Fig. S1 Synthetic route of PNIPAM-SH

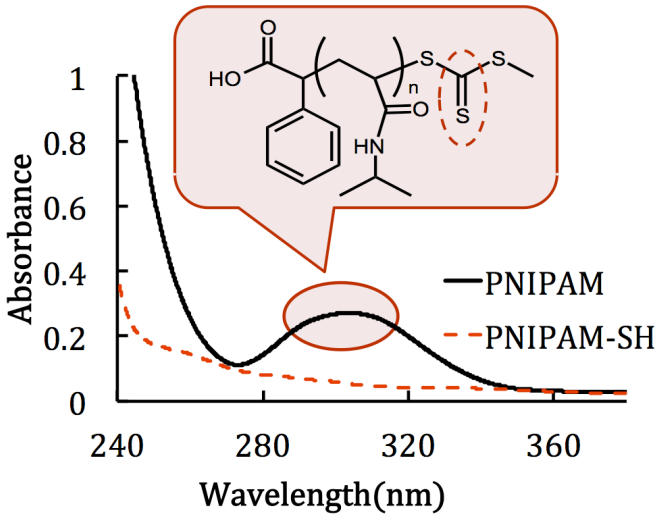


Fig. S2 UV spectra of PNIPAM and PNIPAM-SH.

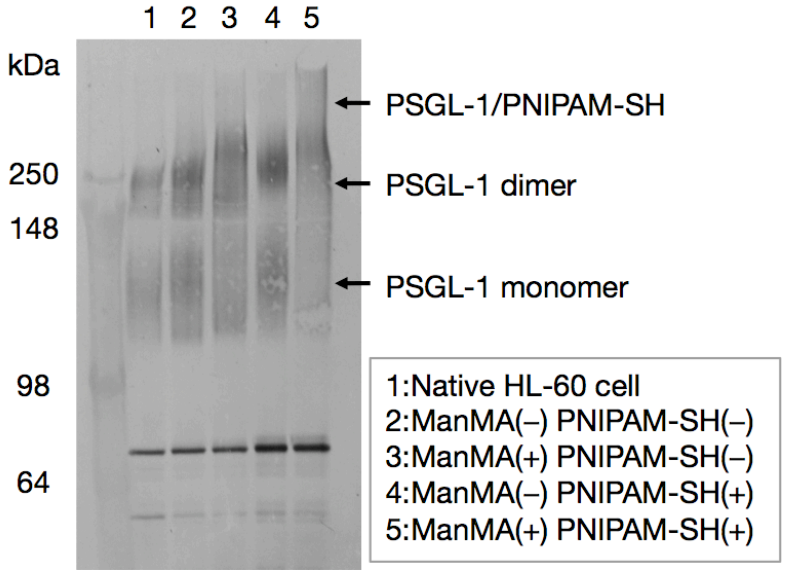


Fig. S3 Western blot analysis data showing PSGL-1 antibody reactive bands.