Supplementary information

Bioinspired structural transition of synthetic polymers through biomolecular ligand binding

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Experimental details

Materials. Amine-terminated PNIPAM (M_n = 5000, M_w/M_n = 1.16, m : r = 48 : 52) was purchased from Sigma-Aldrich. A monomer NIPAM was kindly supplied from KOHJIN and was purified by recrystallization with hexane. ANM was purchased from Wako. NovaSynTGR resin and 9-fluorenylmethyloxycarbonyl (Fmoc) amino acid derivatives were purchased from Novabiochem. All other reagents were purchased from Nacalai Tesque. Ultrapure water with more than 18.2 MΩ·cm was supplied by a Milli-Q system (Merck Millipore) and used in all of the experiments.

Peptide synthesis. Peptides with a free *N*-terminus and an amidated *C*-terminus were synthesized by standard Fmoc-based solid-phase procedures following an established protocol.[1] Peptide chains were assembled on a NovaSynTGR resin (amino group 0.25 mmol g^{-1}) using Fmoc amino acid derivatives. To remove protecting groups from the side chains and cleave the peptide chains from the resin, resins were treated with trifluoroacetic acid (TFA)/thioanisole/*m*-cresol (10/0.75/0.25, v/v/v) for 3 h. The peptides were purified by reverse-phase high-performance liquid chromatography (HPLC; ELITE LaChrom, HITACHI High-Technologies) using a COSMOSIL 5C18-AR-300 packed column (20 × 150 mm, Nacalai Tesque) with a linear gradient from 99.9% H_2O/0.1% TFA to 99.9% acetonitrile/0.1% TFA at a flow rate of 6 mL min^{-1}. The peptides were identified by liquid chromatography-electron spray ionization mass spectrometry (Prominence UFLC system, MS-2020, Shimadzu).

Synthesis and characterizations of PNIPAM. Free radical polymerization of NIPAM (1.0 g, 8.8 mmol) was performed with 2,2'-azobis(isobutyronitrile) (29.6 mg, 0.18 mmol) in degassed ethanol at 60 °C for 12 h. Polymerization was terminated by cooling and was poured into diethyl ether to precipitate the polymer. The resulting polymer was dissolved in toluene (50 mL) and purified by hexane precipitation. After drying under reduced pressure, PNIPAM was collected as white solids (0.48 g, 48.0%). The molecular weight of PNIPAM was determined by SEC (HLC-8120 Gel Permeation Chromatography System, TOSOH) equipped with TSKgel GMH_{XL} and $G2000H_{XL}$ (TOSOH) and ultraviolet and refractive index detection using *N*,*N*-dimethylformamide containing 10 mM LiBr as an eluent at a flow rate of 1.0 mL min^{-1} at 40 °C (Fig. S6). Purified PNIPAM was characterized by ^{1}H NMR spectroscopy (400 MHz for 1H; JEOL AL-400, JEOL) in dimethyl sulfoxide (DMSO)-d_6 solution at 120 °C. The chemical shifts were reported in ppm downfield relative to DMSO (d 2.46) for ^{1}H NMR as a standard (Fig. S7). The meso diad contents of PNIPAM were calculated by the ^{1}H NMR integral ratio of three split methylene signals at 1.15–1.78 ppm, assignable as meso (1.15–1.39 ppm), raceme (1.39–1.57 ppm), and meso (1.57–1.78 ppm) diads.[2]

Synthesis of ANM-labeled PNIPAM. 3-Mercaptopropionic acid (1.27 mg, 12 μmol) and ANM (7.5 mg, 24 μmol) were dissolved and stirred in DMSO for 2 h to obtain carboxylated ANM. 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (6.9 mg, 36 μmol) and *N*-hydroxysuccinimide (4.1 mg, 36 μmol) were added to the carboxylated ANM solution, and the mixture was stirred at room temperature for 1 h to activate the carboxyl groups. Amine-terminated PNIPAM (20.0 mg, 4.0 μmol) and triethylamine (1.2 mg, 12 μmol) dissolved in DMSO were added to the carboxyl-activated ANM solution, and the mixture was incubated for 24 h with stirring. All of the reactions were performed at ambient temperature. The resultant solution was dialyzed against DMSO, methanol and water in that sequence (molecular weight cut off: 3,500 Da). The solution was lyophilized to obtain white powdered ANM-labeled PNIPAM (16.7 mg, 83.5%). Conjugation of ANM onto the PNIPAM terminus was quantitatively characterized using an ultraviolet-visible (UV-vis) spectrophotometer (V-550, Jasco) (Introduction efficiency ~ 100%).

CPT measurements. PNIPAM (M_n = 9400, M_w/M_n = 1.9, m : r = 49 : 51; 1 mg mL^{-1}) dissolved in BR buffer (pH 7.5) prepared from H_3PO_4 (40 mM), CH_3COOH (40 mM), H_3BO_3 (40 mM), and NaOH (200 mM), and the peptides were incubated for 1 h at 10 °C. Solutions were heated from 10 °C to 40 °C at a heating rate 0.2 °C min^{-1}, and their transmittance at 500 nm was monitored using a UV-vis spectrophotometer. The CPTs were defined as the temperature of 50% transmittance.

DSC measurements. PNIPAM (10 mg mL^{-1}) dissolved in BR buffer (pH 7.5) in the presence or absence of the peptide (6.0 mM) were sealed in a stainless steel pan (PerkinElmer) and incubated for 20 min at 10 °C. The DSC (DSC 8000, PerkinElmer) measurements were performed from 10 °C to 40 °C at a heating rate of 2.0 °C min^{-1}. The enthalpy change during the structural transition was calculated by integration of the resultant peak area.

IR absorption measurements. PNIPAM/peptide mixture, PNIPAM and peptide solutions at PNIPAM and peptide concentration of 10 mg mL^{-1} and 3.0 mM, respectively, were dissolved in PBS prepared from D_2O. The mixture solution was incubated for 20 min at 20 °C after mixing PNIPAM and peptide solutions. A 15 μL of each solution was sealed with CaF_2 windows (Jasco) and 0.012 mm spacer (Jasco). IR absorption spectra were obtained using a Fourier transform IR spectrometer (FT/IR-4100, Jasco) with a cumulative number of 100 and a resolution of 2.0 cm^{-1} at ambient temperature (below the CPT). The background spectra for each solution were obtained using PBS.

Peptide binding-triggered structural transition of PNIPAM. The peptide (final concentration: 3.0 mM) dissolved in PBS (pH 7.4) was added to aqueous PNIPAM solutions (final concentration: 1 mg mL^{-1}) at 25 °C, and the mixtures were visually observed under visible light.

Fluorescence measurements of ANM-labeled PNIPAM. The fluorescence spectra were measured using a fluorescent spectrophotometer (FP-6500, Jasco). ANM-labeled PNIPAM (50 μg mL^{-1}) dissolved in PBS (pH 7.4) in the presence or absence of peptides (2.0 mM) were incubated at 20 °C for 20 min. The solutions were heated to 45 °C at a heating rate of 1.0 °C min^{-1}, and fluorescence spectra excited at 350 nm were recorded.

DLS measurements. An aliquot (50 μL) of aggregates for globular PNIPAM with and without ANM-labeled PNIPAM dissolved in PBS (pH 7.4) was applied to DLS measurements (Zetasizer Nano ZSP, Malvern) with a micro cell (ZEN2112, Malvern) at 34 °C and 45 °C (above the CPT), respectively. The solutions were heated to the temperatures at a heating rate of 1.0 °C min^{-1}, and were incubated for 5 min before DLS measurements.

Peptide binding-triggered fluorescence enhancement of ANM-labeled PNIPAM. The peptide (final concentration: 2.0 mM) dissolved in PBS (pH 7.4) was added to aqueous ANM-labeled PNIPAM solutions (final concentration: 50 μg mL^{-1}) at 30 °C, and the mixtures were visually assessed under UV light at 350 nm.

Peptide concentration and temperature-dependent fluorescence observation. ANM-labeled PNIPAM (200 μg mL^{-1}) dissolved in PBS (pH 7.4) with various concentrations of peptide (0, 2.5, 5.0, and 7.5 mM) were incubated in a 384-square-well plate (Porvair) at 0 °C for 20 min. The fluorescence of the mixtures was observed using transilluminator (Benchtop 2UV, Analytic Jena, US) at 350 nm while the mixtures were heated to 40 °C at a heating rate of 1 °C min^{-1}.

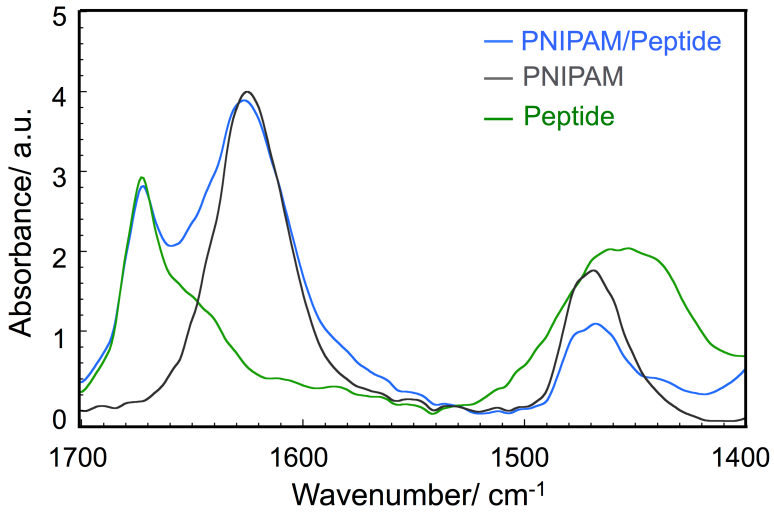

Fig. S1 IR absorption spectra of PNIPAM/peptide mixture, PNIPAM, and peptide solutions.

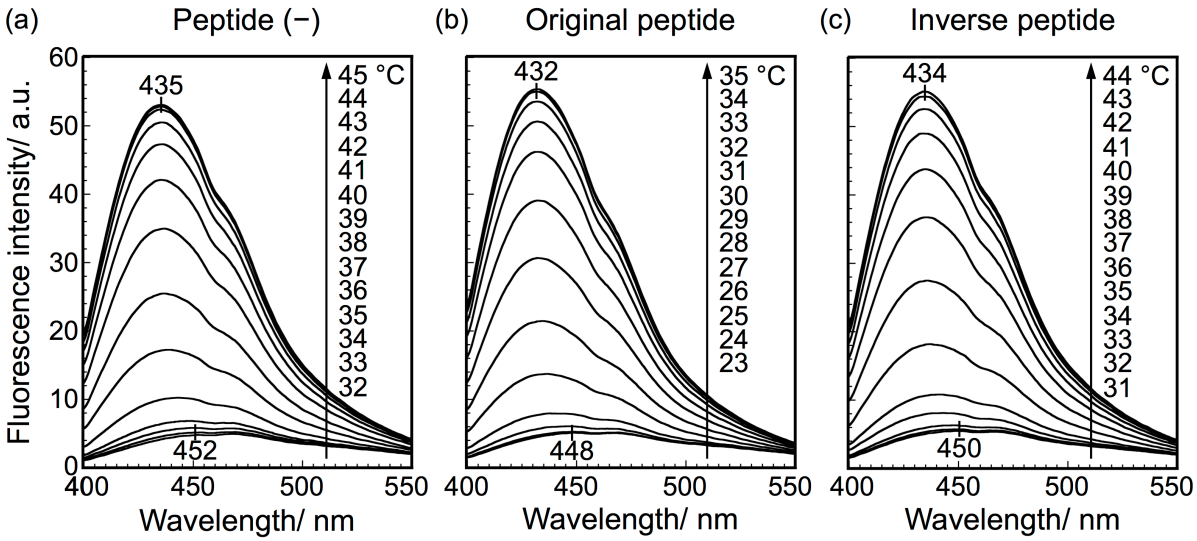

Fig. S2 Fluorescence spectra of ANM-labeled PNIPAM solutions with or without PNIPAM-binding or inverse peptide.

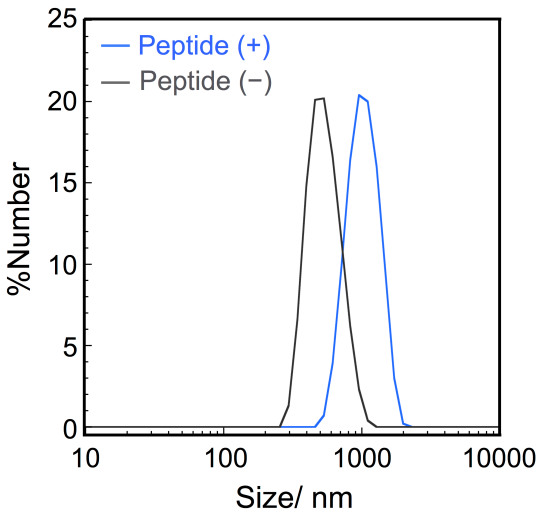

Fig. S3 DLS profiles for aggregates of globular PNIPAM with and without PNIPAM-binding peptide at 34 °C and 45 °C, respectively.

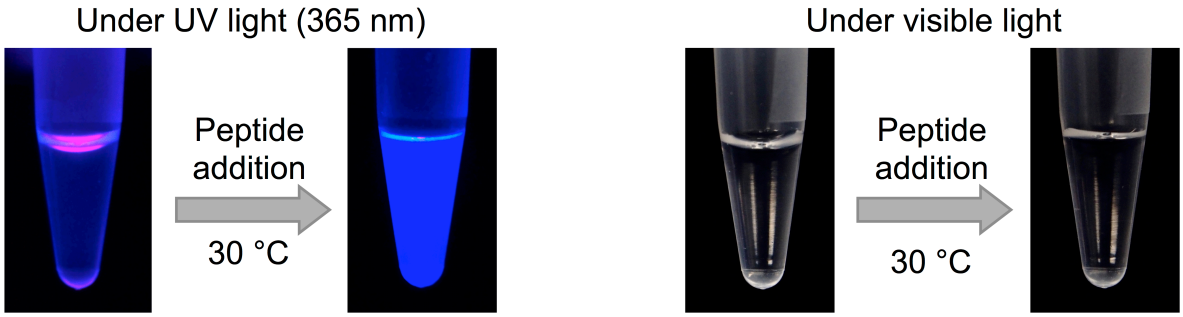

Fig. S4 Photos of ANM-labeled PNIPAM solutions before (left) and after (right) peptide addition under UV light (365 nm; left) or visible light (right) at 30 °C.

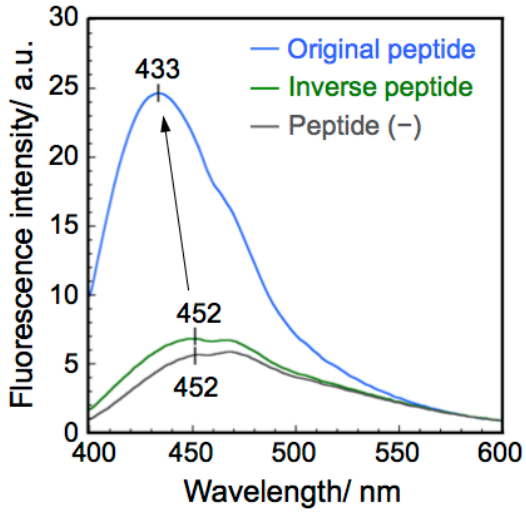

Fig. S5 Fluorescence spectra of ANM-labeled PNIPAM before and after peptide addition at 30 °C.

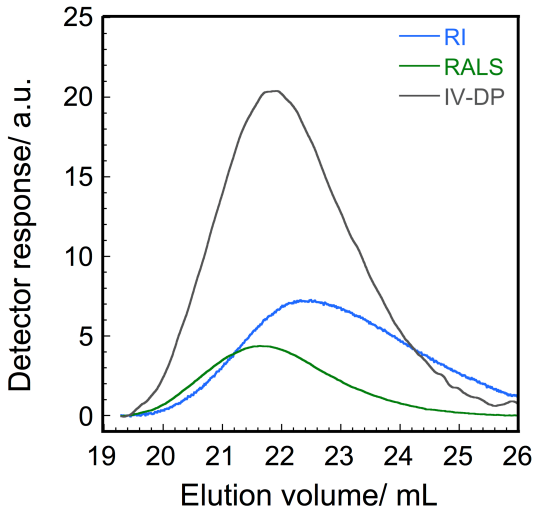

Fig. S6 SEC curves of PNIPAM (M_n: 9400) obtained by using the refractive index (RI) and right angle laser light scattering (RALS) detectors; and the viscometer (IV-DP).

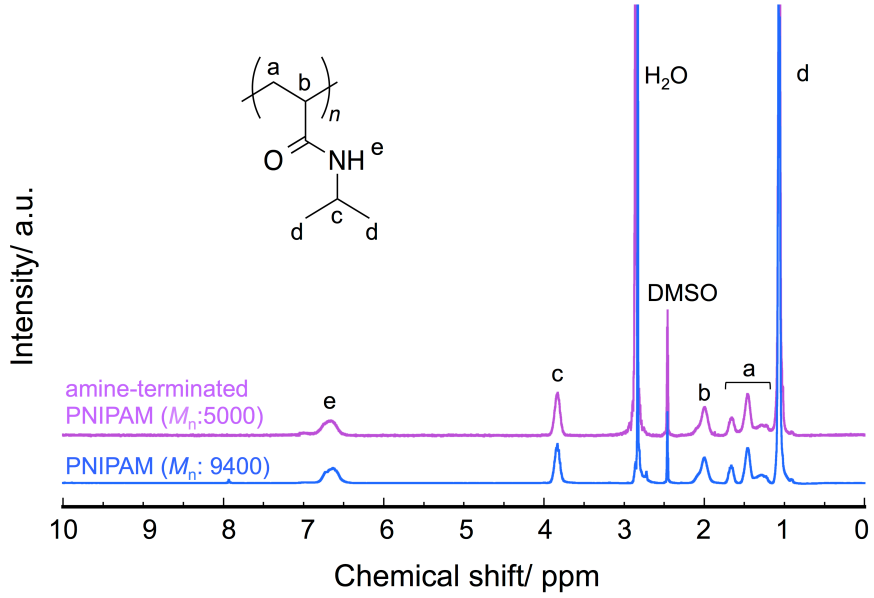

Fig. S7 ^{1}H NMR spectra of PNIPAMs measured in DMSO-d_6 at 120 °C.

References

1 W. C. Chan and P. D. White, in *Fmoc Solid Phase Peptide Synthesis* eds. W. C. Chan and P. D. White, Oxford University: New York, 1st edn., 2000, pp. 41-76.

2 M. Ito and T. Ishizone, *J. Polym. Sci., Part A: Polym. Chem.*, 2006, **44**, 4832-4845.