

Supplementary Information

**DNA molecular recognition of intercalators affects aggregation of
thermoresponsive polymer**

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Chemicals and Instruments

DNA was purchased from Life Technologies Corporation (Carlsbad, CA, USA). The base sequences of the DNA were as follows: conjugate DNA, 5′-HS-CTA AGT AAC TC-3′; complementary DNA, 5′-GAG TTA CTT AG-3′. NIPAM was kindly supplied by Kohjin Co. Ltd. (Tokyo, Japan). 3,6-Diaminoacridine hydrochloride (DAA) and 9-hydroxy-4-methoxyacridine (HMA) were purchased from Sigma-Aldrich Japan K.K. (Tokyo, Japan). Ammonium persulfate and *N,N,N′,N′*-tetramethylethylenediamine were purchased from Bio-Rad Laboratories K.K. (Tokyo, Japan). The purification of ssDNA–PNIPAM was performed using a filtration column containing Sephadex G-100 of DNA grade as a carrier and “NAP-5 columns” (GE Healthcare Japan Corporation, Hino, Japan). Ethidium bromide (EtBr) and all other reagents were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan).

Absorbance measurements were performed using a UV–vis spectrophotometer (U-3310; Hitachi, Ltd, Tokyo, Japan). Fluorescence anisotropy measurements were performed using a fluorescence spectrophotometer with accessory polarizers (FL-2500 and 650-0155; Hitachi, Ltd).

Preparation of ssDNA–PNIPAM

ssDNA–PNIPAM was synthesized using a modified scheme (Scheme S1) from our previous study,¹ as follows: the polymerizable pyridyl disulfide monomer *N*-[2-(2-pyridyl disulfide) ethyl]acrylamide (PDSAm) was first synthesized from 2,2-dipyridyl disulfide via a disulfide exchange reaction and introduction of a vinyl group. The monomer was then copolymerized with NIPAM and acrylamide (AAm) (Scheme S1(a)). The copolymer had a monomer composition (NIPAM:AAm:PDSAm) of 97:1.4:1.9, an average molecular weight (M_n) of 250 000, and a molecular weight distribution of 1.7. Next, ssDNA was conjugated to the copolymer (Scheme

S1(b)). The synthesized copolymer was dissolved in 50 mM glycine–NaOH buffer (pH 8.6), followed by mixing of the copolymer solution with conjugate DNA solution. The concentration of pyridyl disulfide in the copolymer was 0.10 mM, and the DNA concentration was 0.65 μ M. The conjugation was performed with mixing at 1400 rpm at room temperature for 3 h. Subsequently, propanethiol was added to degrade residual pyridyl disulfide groups and prevent the adsorption of pyridyl disulfide to intercalators. The amount of propanethiol added was adjusted to twofold mole of the initial pyridyl disulfide in the copolymer. The reaction was performed with mixing at 1400 rpm at room temperature for 2 h. The mixture was loaded onto a preparative gel filtration column to purify the ssDNA–PNIPAM from nonconjugated DNA, using 10 mM Tris–HCl (pH 7.4) as the eluting buffer. After freeze-drying overnight, the solid obtained was redissolved in water, desalted using NAP-5 columns, and freeze-dried overnight. The purified ssDNA–PNIPAM was redissolved in 100 mM, pH 7.4, Tris–HCl and was stored in a refrigerator.

Measurements of DNA–PNIPAM aggregation

ssDNA–PNIPAM was mixed with complementary DNA (tenfold the conjugated DNA) in 10 mM Tris–HCl (400 mM NaCl, pH 7.4) buffer, to prepare dsDNA–PNIPAM, and the hybridization was performed with mixing at 500 rpm at room temperature for 30 min. The binding of intercalators to dsDNA was performed by the addition of one of the intercalators to the dsDNA–PNIPAM solution, followed by mixing at 500 rpm at room temperature for 30 min. The final polymer concentrations of the solutions were adjusted to 0.025 or 0.050 w/v%, and the final intercalator concentrations were adjusted to 1.0 and 10 μ M for one- and tenfold amounts of DNA strand in the solution.

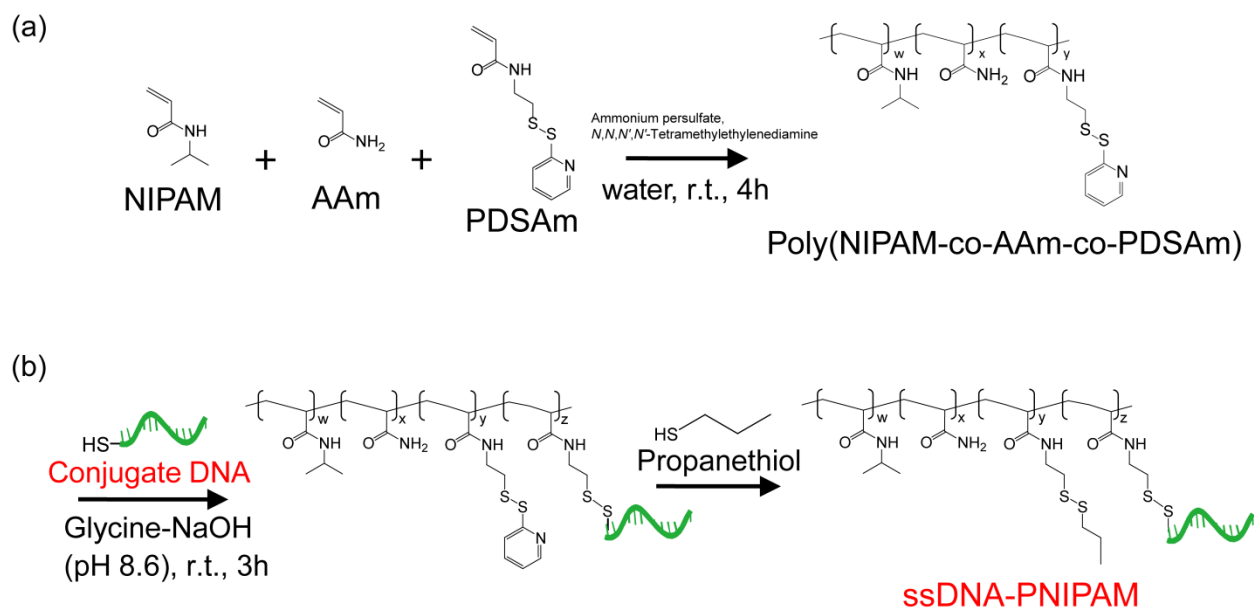
The observation of DNA–PNIPAM aggregation in the presence or absence of the intercalator with increasing temperature was observed by cloud-point measurements. When DNA–PNIPAM was not aggregated, light penetrated the solution. Conversely, when DNA–PNIPAM was aggregated, the solution became turbid and light transmission is inhibited; thus, the absorbance of the solution increases. The sample solution was placed in a plastic disposable cell, and the cell was sealed with Parafilm (Pechiney Plastic Packaging, Inc., Chicago, IL, USA). Aggregation was measured by observing the absorbance at 650 nm. The sample solutions were stored in a quartz cell at each of the predetermined temperatures for 3 min before increasing the temperature when a dramatic change in absorbance did not appear, whereas after absorbance had started increasing, the temperature was increased after a 10-min wait.

Fluorescence anisotropy measurements

Each intercalator (20 nM) was added to 0–400 μ M of free dsDNA and bound to it via mixing at 500 rpm at room temperature for 30 min. The sample solution was placed in a plastic disposable cell and set in the fluorescence spectrophotometer. A 10-nm slit width was used on both the excitation and the emission windows. The polarizers were fixed in front of the excitation and emission windows, in order that excitation and emission light can pass through the polarizers. The excitation and emission wavelengths were 444 and 507 nm and 387 and 455 nm for DAA and HMA, respectively. The intensity of the polarized emission light from the samples was measured at 25 °C as intercalators were excited by polarized excitation light. The anisotropy was calculated based on the following definition (1) from the literature,²

$$\text{Fluorescence anisotropy} = \frac{(I_{vv} I_{hh} / I_{hv} I_{vh}) - 1}{(I_{vv} I_{hh} / I_{hv} I_{vh}) + 2} \quad (1)$$

where I represents the intensity of the fluorescence, the subscripts designate the orientation of the polarizers at the excitation and emission slits, respectively, v means the vertical distance polarization, and h means the horizontal distance polarization.



Scheme S1 Synthetic schemes for (a) poly(NIPAM-co-AAm-co-PDSAm)¹ and (b) ssDNA-PNIPAM.

References

- 1 Y. Sugawara, T Tamaki, H Ohashi, T. Yamaguchi, *Soft Matter* **2013**, *9*, 3331–3340.
- 2 G. Gokulrangan, J. R. Unruh, D. F. Holub, B. Ingram, C. K. Johnson, G. S. Wilson, *Anal. Chem.* **2005**, *77*, 1963–1970.