

Electronic supplementary information for

Turbidimetric detection of ATP using polymeric micelles and DNA aptamers

Daisuke Miyamoto,[‡] Zhonglan Tang, Tohru Takarada* and Mizuo Maeda

*Bioengineering Laboratory, Discovery Research Institute, RIKEN, Wako, Saitama 351-0198, Japan.
E-mail: ttkrd@riken.jp; Fax: +81-48-462-4658; Tel: +81-48-467-5489*

[‡] *Present address: Tsukuba Research Center for Interdisciplinary Materials Science (TIMS),
University of Tsukuba, Tsukuba, Ibaraki 305-8573, Japan.*

1. Materials

All of the oligodeoxyribonucleotides were purchased from Genome Science Laboratory (Fukushima, Japan) with HPLC purification grade. *N*-Isopropylacrylamide (NIPAAm), ammonium persulfate (APS), *N,N,N',N'*-tetramethylenediamine (TMEDA), ATP, GTP, CTP and UTP were obtained from Wako Pure Chemical Industries (Osaka, Japan). All of the chemicals were used as received unless otherwise noted; NIPAAm was recrystallized from hexane, and TMEDA was distilled under reduced pressure before use.

2. Synthesis of poly(*N*-isopropylacrylamide) grafted with single-stranded DNA (PNIPAAm-*g*-DNA)

NIPAAm (65.9 μ mol) and 12-base DNA modified with a methacryloyl group at the 5'-end (DNA macromonomer; 198 nmol) were dissolved in 378 μ l of 10 mM Tris-HCl buffer (pH 7.4). After the monomer solutions were purged with Ar gas for 3 min, APS (571 nmol) and TMEDA (38.1 μ mol) were added. Polymerization was carried out at 30°C for 14 hours. The remaining initiators and monomers were removed by gel filtration chromatography (Sephadex G-100) using the 10 mM Tris-HCl buffer (pH 7.4) as an eluent. To remove the buffer ions, the solution was dialyzed (MWCO = 1.0×10^3) against water for 24 hours. Finally, PNIPAAm-*g*-DNA was obtained by freeze-drying.

3. Characterization of PNIPAAm-*g*-DNA

The weight average molecular weight (M_w), the number average molecular weight (M_n) and the polydispersity (M_w/M_n) of PNIPAAm-*g*-DNA were measured by gel permeation chromatography (GPC) using mono-dispersed polystyrene as a standard: flow rate, 0.5 ml/min; detection, RI; eluent,

N,N-dimethylformamide containing 0.1% trifluoroacetic acid; temperature, 40°C; column, TOSOH TSKgel α -3000. The compositions of the PNIPAAm-g-DNA were determined by gravimetry and based on the UV absorption at 260 nm of the DNA macromonomer units. The cloud point of PNIPAAm-g-DNA was determined as the temperature at which the transmittance at 500 nm begins to decrease slightly from 100%. The transmittance reached a plateau value of about 99.7%. The transmittance curve of PNIPAAm-g-DNA solution was monitored at a heating rate of 0.5 °C/min.

4. Preparation and characterization of DNA-linked polymeric micelles

PNIPAAm-g-DNA was dissolved in 500 μ l of 20 mM Tris-HCl buffer (pH 8.3) containing 300 mM NaCl and 5 mM MgCl₂ to give a final concentration of 0.12 mg/ml. The solutions were then heated at 40°C for 30 min to give a DNA-linked polymeric micelle through the self-assembly of dehydrated PNIPAAm-g-DNA. The hydrodynamic radius of DNA-linked polymeric micelle in the identical buffer was determined to be 46.0 ± 0.3 nm by dynamic light scattering measurement (Zetasizer 3000HS, Malvern Instruments Ltd., Malvern, UK) at 40°C.

5. Experimental procedure for turbidimetric detection of ATP: The first system

DNA3, DNA4 and NTP (N = A, G, C or U) were dissolved in 100 μ l of 20 mM Tris-HCl buffer (pH 8.3) containing 300 mM NaCl and 5 mM MgCl₂. The solution was heated at 90°C for 10 min and was cooled to 40°C on a temperature ramp of 3 °C/min. The solution after annealing was added to the micellar dispersion and was stirred for 1 min. The transmittance of the micellar dispersion at 500 nm was measured at 40°C with a UV-visible spectrophotometer (Shimadzu UV-2500, Shimadzu Co., Kyoto, Japan).

6. Experimental procedure for turbidimetric detection of ATP: The second system

DNA3 and NTP (N = A, G, C or U) were dissolved in 100 μ l of 20 mM Tris-HCl buffer (pH 8.3) containing 300 mM NaCl and 5 mM MgCl₂. The solution was added to the micellar dispersion and then annealed in a manner similar to that described above. Subsequently, DNA2 was added to the micellar dispersion and its transmittance at 500 nm was measured at 40°C with the UV-visible spectrophotometer.

7. Melting temperature measurements

The melting curve of the double-stranded DNA (dsDNA) in 20 mM Tris-HCl buffer (pH 8.3) containing 300 mM NaCl and 5 mM MgCl₂ was obtained by measuring the change of absorbance at 260 nm versus temperature with the UV-visible spectrophotometer. The melting temperature (T_m) was determined from the maximum in the first derivative of the melting curve. Both the heating and cooling curves were measured, and the T_m values obtained from them coincided with each other to within 2.0°C. The T_m values presented in Table S2 are an average of at least two independent

experiments. The error of T_m values is $\pm 1.0^\circ\text{C}$.

Table S1 Melting temperature (T_m) values of dsDNA used in the present study

Strands ^a	T_m ($^\circ\text{C}$)
DNA1 / DNA2	58.6
DNA1 / DNA3	57.4
DNA3 / DNA4	58.2

^a Each concentration of DNA is $2.33\ \mu\text{M}$.

The melting curve of the duplex between the DNA (5'-GCCACCAGC-3') tethered to the surface of the polymeric micelle and its complementary DNA is shown in Fig. S1. The melting curve of the duplex between the unmodified DNA with the identical sequence and its complementary one is also depicted. Almost the same T_m values (51.8°C for dsDNA on the surface and 52.5°C for that in solutions) and a similar degree of hypochromic effect were observed, indicating that the stability of the duplex on the surface of the polymeric micelles was almost as high as that in solutions.

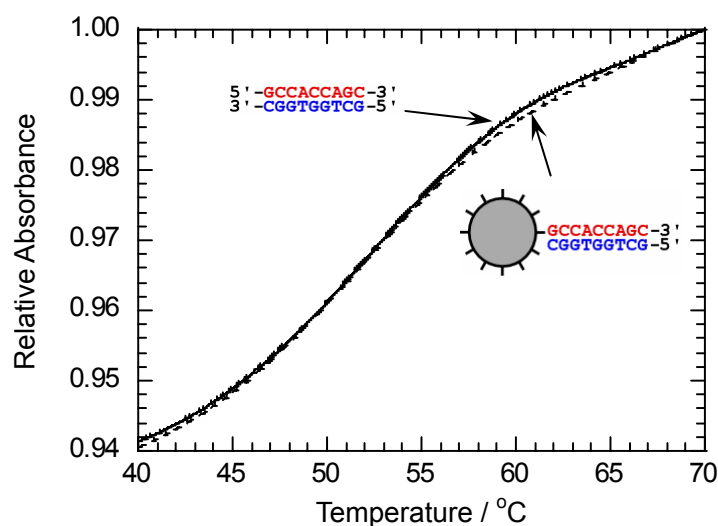


Fig. S1 The melting curve of duplex between the DNA (5'-GCCACCAGC-3') tethered to the surface of polymeric micelle and its complementary DNA (3'-CGGTGGTCG-5') in 10 mM Tris-HCl buffer (pH 7.4) containing 300 mM NaCl (broken line) and that of duplex between free DNA and its complementary one (solid line). The concentration of each DNA was $3.0\ \mu\text{M}$.