Electronic Supplementary Material (ESI) for ChemComm.
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Supporting Information

Reversible conformational changes in parallel type G-quadruplex structure inside a thermoresponsive hydrogel

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1. Materials

All reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan) unless otherwise noted. *N*-Isopropylacrylamide (NIPAAm) was kindly provided by KJ Chemicals (Tokyo, Japan) and purified by recrystallization in toluene/hexane. Ammonium persulfate (APS) was purchased from Kanto Chemical (Tokyo, Japan). ssDNA (5′-ACC TGG GGG AGT ATT GCG GAG GAA GGT-3′) that was aminoalkyl modified either at both 5′- and 3′-ends or only at the 5′-end was purchased from GeneDesign, Inc. (Osaka, Japan). SUNBRIGHT DE-100PA (α-aminopropyl-ω-aminopropoxy polyoxyethylene) was purchased from NOF (Tokyo, Japan). DNAse and RNAse free ultra-pure distilled water was purchased from Invitrogen (Carlsbad, USA).

2. Preparation of ssDNA-crosslinked hydrogels

The ssDNA-crosslinked thermoresponsive PNIPAAm hydrogels and poly(acrylamide) (PAAm) hydrogels can be synthesized by free radical polymerization. Terminal methacryloyl-modified ssDNA and DE-100PA were prepared following previously reported method [S1]. (Methacryloyl succinimide (22.5 μmol) was mixed with dimethylsulfoxide and commercially available aminoalkyl-modified ssDNA or DE-100PA (0.44 μmol) in 50 mM Na_2CO_3/$NaHCO_3$ buffer solution (pH 9.0) for 12 h.) ssDNA-crosslinked PNIPAAm gel, referred to as DNA-PNI gel, was synthesized by polymerizing NIPAAm (2.0 M) or AAm (2.0 M) with 5′- and 3′-methacryloyl- modified ssDNA (4.0 mM) as crosslinkers in 20 mM Tris-HCl buffer solution (pH 7.5) containing APS (8.0 mM) and TEMED (80 mM). PNIPAAm gel with ssDNA pendants, referred to as DNA pendant-PNI, was synthesized by polymerizing NIPAAm (2.0 M) and 5′-methacryloyl-modified ssDNA (4.0 mM) with both chain end methacryloyl- modified DE-100PA (4.0 mM) as the crosslinkers in 20 mM Tris-HCl buffer solution (pH 7.5) containing APS (8.0 mM) and TEMED (80 mM).

The synthesis was carried out at 4˚C for 24 h inside glass capillaries (0.63 mm ϕ) to obtain cylinder-shaped gels or between two glass slides brought together by two magnets and with a 0.1 mm spacer between them to obtain sheet shaped gels (Figure S1). The hydrogels were immersed in 10 mM Tris-HCl buffer solution (pH 7.5) for 3 days.

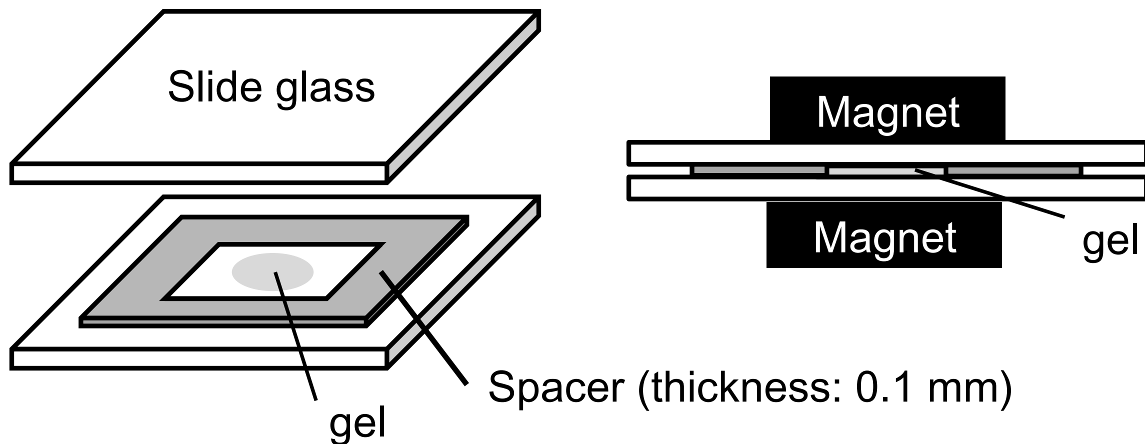

Fig.S1 Schematic illustrations of sheet gel preparation.

3. Measurement of the swelling ratio of DNA-PNI gel

The gel samples were immersed in 10 mM Tris-HCl buffer solution (pH 7.4) for 1 h at 12, 15, 20, 25, 27, 30, 32, 35, 40, 45, 55 ˚C . Diameters of the cylindrical gel samples were measured by using a digital microscope (VHX-900, VH-Z100W, Keyence, Osaka, Japan). The swelling ratio is defined as the diameter of the gel normalized by the inner diameter of the glass capillary (d_0 = 0.63 mm) used in the gelation process.

4. Measurement of CD spectra of free ssDNA

The G-quadruplex structure of free ssDNA was determined by circular dichroism spectroscopy (J-720W, Jasco, Tokyo, Japan). The ssDNA (100 μM) was dissolved in 10 mM Tris-HCl buffer solution (pH 7.5) and kept at 25˚C or 45˚C for 1 h prior to measurements. The spectra were recorded over wavelengths of 220-300 nm at each temperature with a 1-s time constant, 1-nm step resolution, and 1-nm bandwidth.

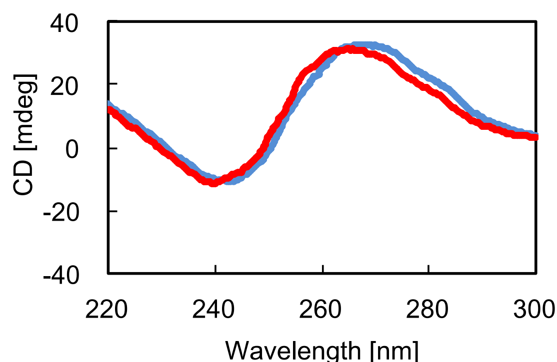

Fig.S2 CD spectra of free ssDNA in 10 mM Tris-HCl buffer solution (pH7.5) at 25 (Blue line) and 45˚C (Red line). The DNA samples were kept at each temperature for 1 h prior to measurement.

5. Measurement of CD spectra of ssDNA inside hydrogel by UCS

The G-quadruplex structure of ssDNA inside the hydrogel was determined by universal chiroptical spectrophotometer 1 (UCS-1: J-800KCM), which is capable of measuring all polarization phenomena in solid materials as well as in solutions [S2]. For CD measurement, sheet-shaped gel was placed between two 1.5-cm diameter cylindrical quartz glasses and fixed by a holder to suppress any change in volume. Most of the gel surface was physically attached to these glasses (Figure S3). The holder was immersed in water baths set at 25, 35, 40, 45, 55 ˚C. Spectra were recorded over wavelengths of 220-300 nm with a 1-s time constant, 1-nm step resolution, and 1-nm bandwidth.

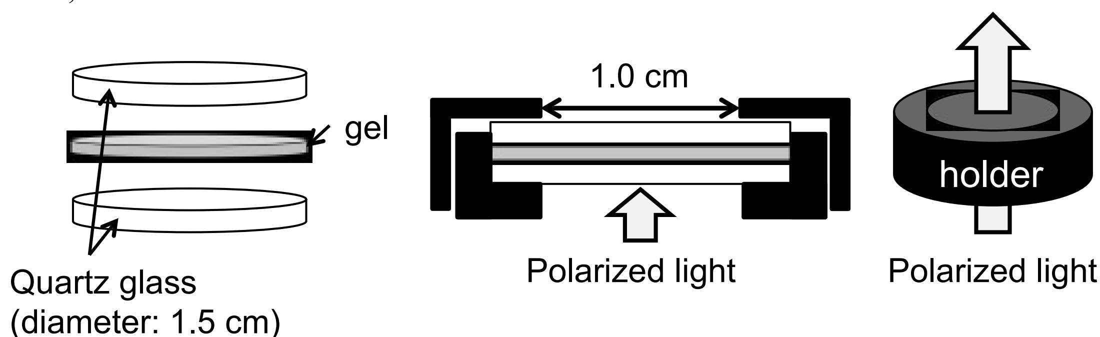

Fig.S3 Schematic illustration of the measurement of CD spectra by UCS-1.

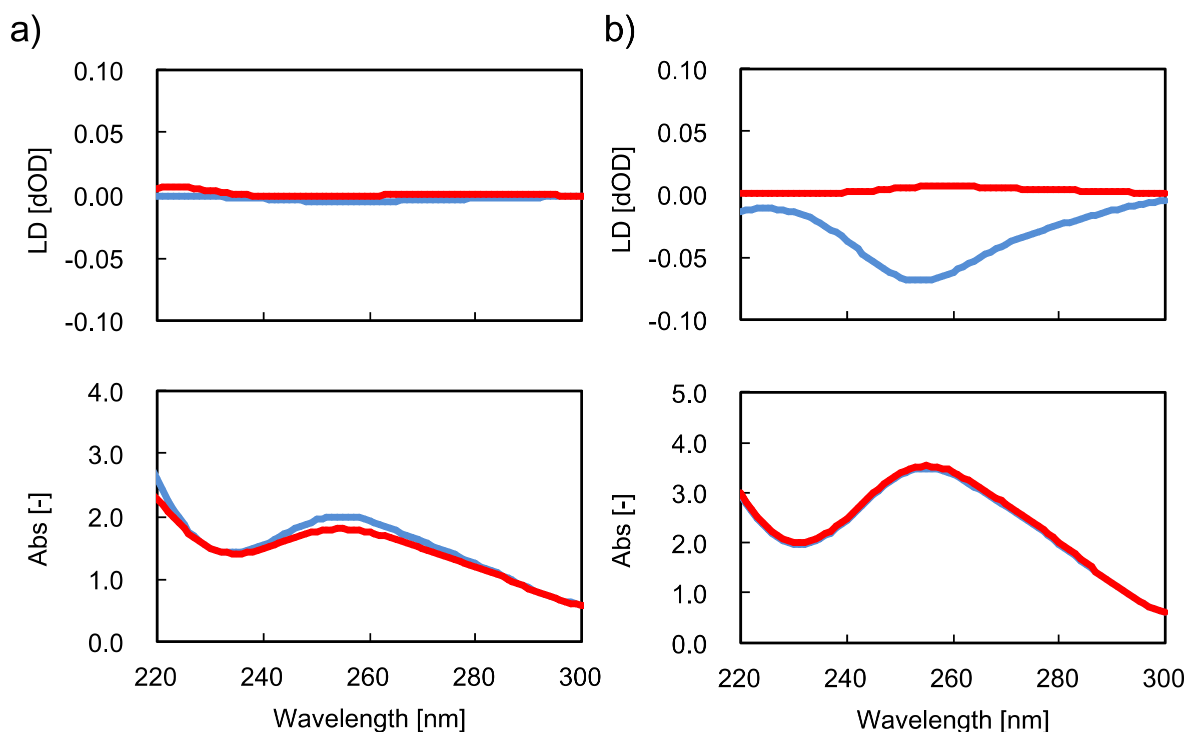

Fig.S4 LD and UV spectra of the a) DNA-PNI gel and b) DNA-PAA gel at 25 (gray line) and 45˚C (black line). These samples were kept in 10 mM Tris-HCl buffer solution (pH 7.5) at each temperature for 1 h prior to measurements.

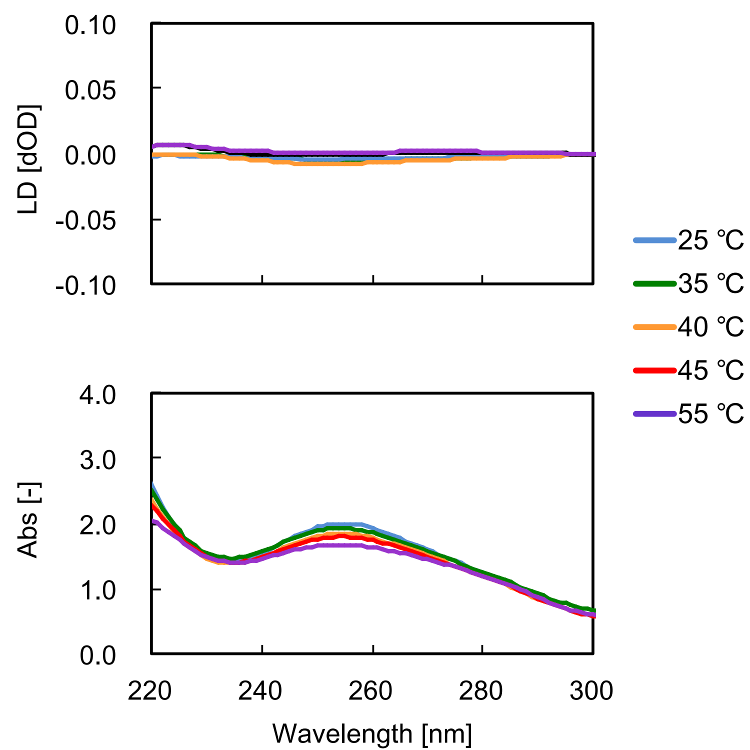

Fig.S5 Temperature-dependent LD and UV spectra of the DNA-PNI gel. This gel was kept for 30 min at 25 and 55˚C and for 1 h at 35 and 40˚C before measurements.

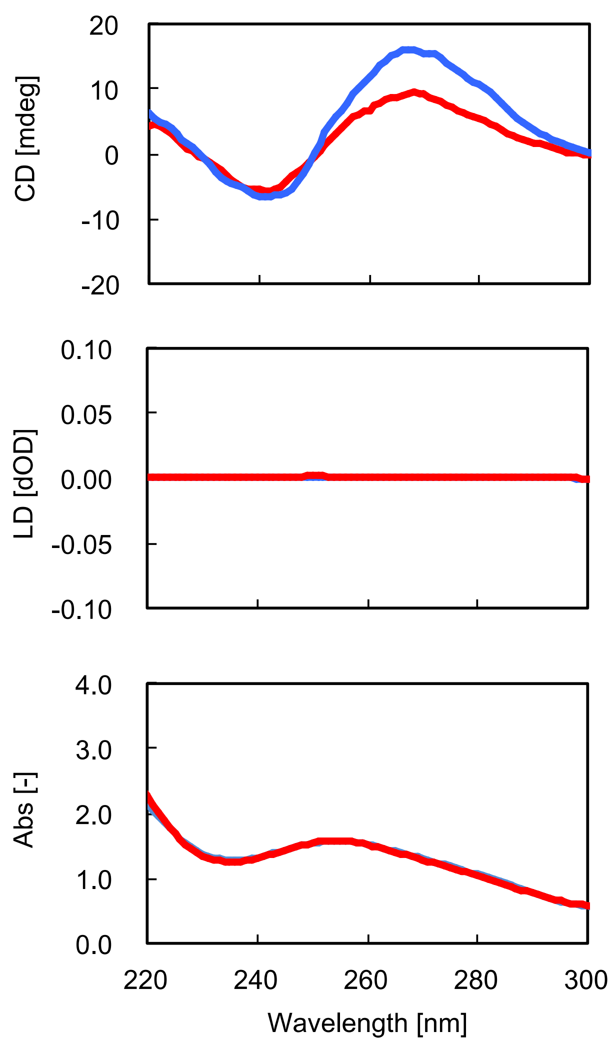

Fig.S6 CD, LD, and UV spectra of the DNA pendant-PNI gel a) at 25˚C (gray line), 45˚C (black line). These samples were kept in 10 mM Tris-HCl buffer solution (pH 7.5) at each temperature for 1 h prior to measurements.

References

[S1] Y. Murakami and M. Maeda, *Biomacromolecules*, **2005**, *6*, 2927-2929.

[S2] R. Kuroda, T. Harada and Y. Shindo, *Rev. Sci. Instrum.*, **2001**, *72*, 3802-3810.