Electronic Supplementary Information

Reversibly tuning mechanical properties of DNA hydrogel by DNA nanomotor

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1. Material

All oligonucleotides (see **Table S1**) were synthesized using BioAutomation MerMade-12 DNA synthesizer with a standard phosphoramidite DNA synthesis protocol and purified by HPLC using water/acetonitrile/TEAA (triethylamine acetate buffer, 100 mM, pH 7.0) as eluent. Water used in all experiments was Millipore Milli-Q deionized (18.2 MΩ cm^{-1}). All other chemical reagents were purchased from Sigma-Aldrich or Alfa Aesar and used directly after received.

Table S1 ssDNA sequences for preparation of DNA hydrogel

Sample	Sequence
Y1	5'- CGA TTG ACT CTC CAC GCT GTC CTA ACC ATG ACC GTC GAA G -3'
Y2	5'- CGA TTG ACT CTC CTT CGA CGG TCA TGT ACT AGA TCA GAG G -3'
Y3	5'- CGA TTG ACT CTC CCT CTG ATC TAG TAG TTA GGA CAG CGT G -3'
L1	5'- GAG AGT CAA TCG CCC TAA CCC TAA CCC TAA CCC ACG TCG TCT GTC TGA -3'
L2	5'- GAG AGT CAA TCG CCC TAA CCC TAA CCC TAA CCC TCA GAC AGA CGA CGT -3'
L1t	5'- GAG AGT CAA TCG TTT TTT TTT TTT TTT TTT TTT ACG TCG TCT GTC TGA -3'
L2t	5'- GAG AGT CAA TCG TTT TTT TTT TTT TTT TTT TTT TCA GAC AGA CGA CGT -3'

2. Experimental Section

2.1 Preparation and characterization of DNA assemblies

For preparation of Y scaffold, three oligonucleotide strands, Y1, Y2 and Y3 were mixed stoichiometrically in phosphate buffer (100 mM phosphate, pH 8.0; 100 mM NaCl) to give a finial concentration of 60 μM for each strand. And then the mixture was heated to 95 °C and cooled to room temperature slowly. Similarly, the linker was formed by mixing of L1 and L2 stoichiometrically following the same annealing procedure. All DNA assemblies were characterized by native polyacrylamide gel electrophoresis (8%, Acr/Bis=19:1) in 1×TBE buffer with 300 V for 3 hours at 4 °C.

2.2 Measurements of melting temperature

Temperature ramp test was carried out from 4 °C to 95 °C at a rate of 1 ℃/min on a Cary100 UV-vis spectrometer (Agilent Technologies) equipped with a temperature controller.

2.3 Characterization of i-motif structure and its melting temperature

Three single-stranded DNAs (ssDNAs) Y1, L1 and L2 were mixed at a molar ratio of 2:1:1 in 100 mM, pH 8.0 or pH 5.0 phosphate buffer containing 100 mM NaCl to give a finial concentration of 40 μM for L1. And then the mixture was heated to 95 °C and cooled to room temperature slowly to form Y1L1L2 assembly. The samples were diluted with buffer to give a final concentration of Y1L1L2 of 4 μM. The scanning was carried out at the range of 220-350 nm at room temperature in a quartz cuvette of 1 mm optical length with bandwidth of 1 nm, and the scanning speed was 60 nm/min. Meanwhile, to measure the melting temperature of i-motif structure, the temperature ramp test was carried out from 5 °C to 90 °C at a rate of 1 °C/min in a quartz cuvette of 1 cm optical length with bandwidth of 2 nm.

2.4 DNA hydrogel preparation

To form a 4 wt% hydrogel (40 μL), three ssDNAs Y1, Y2, and Y3 (500 μM, 40 μL) were mixed as an equal molar ratio, and the solution was lyophilized to get a white solid. After adding 20 μL phosphate buffer (100 mM phosphate; 100 mM NaCl), the Y scaffold solution (1 mM, 20μL) was prepared well. And the linker solution (1.5 mM, 20μL) was prepared by mixing of L1 and L2 (750 μM, 40 μL) stoichiometrically following the similar protocol. Then two solutions were mixed at room temperature with a vigorous string, and the mixture changed from a liquid state to a transparent gel state with several seconds.

2.5 Rheological characterization

An AR-G2 rheometer (TA Instruments) was used to characterize of the mechanical properties of the formed hydrogels. Three types of rheological experiments were performed in 8 mm parallel-plate geometry using 40 μL hydrogels (with gap size of 0.15 mm): (i) Time sweep test was carried out at a fixed strain of 1% and frequency of 1 Hz at 25 ℃ for 3 min; (ii) Strain sweep test was carried out from 0.1% to 5000% with a fixed frequency of 1 Hz at 25 °C; (iii) Temperature ramp test was carried out from 5 ℃ to 60 °C at a fix strain of 1% and frequency of 1 Hz with rate of 2 °C/min.

2.6 Reversibly tuning mechanical properties of hydrogel

We firstly prepared a 4 wt% hydrogel at pH 5.0 of 180 μL and then cut about 30 μL hydrogel as sample to measure its mechanical strength by rheological time sweep test. The remained gel was weighted and calculated its volume to obtain the required volume of alkali solution. After adding a certain volume of 3 M NaOH solution to adjust pH value of hydrogel to 8.0, we cut about 30 μL hydrogel to measure its mechanical strength. And we weighted the remained gel, added a certain volume of 3 M HCl solution to vary pH value back to 5.0 and sampled 30 μL gel to take

rheological test. This process repeated three times.

3. Supplementary Data

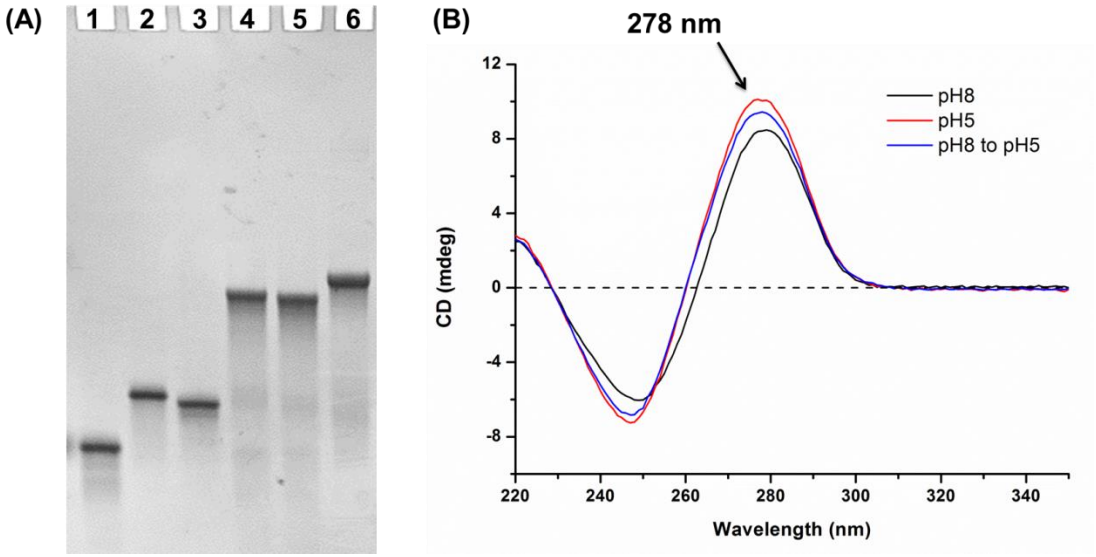

Figure S1 Characterization of DNA assemblies. **(A)** Characterization of different DNA assemblies by 8% native PAGE. Lane 1: Y1, Lane 2: L1t, Lane 3: L2t, Lane 4: Y1L1t, Lane 5: Y1L2t and Lane 6: L1tL2t. **(B)** CD spectra of DNA assembly Y1L1tL2t at different pH conditions.

Table S2 The melting temperatures of DNA assemblies at different pH conditions

Sample	pH 5.0	pH 8.0
Y1Y2Y3	56 °C	61 ℃
Y1L1	44 ℃	49 ℃
L1L2	42 °C, 56 °C	62 ℃
Y1tL1t	44 ℃	49 ℃
L1tL2t	58 ℃	63 ℃

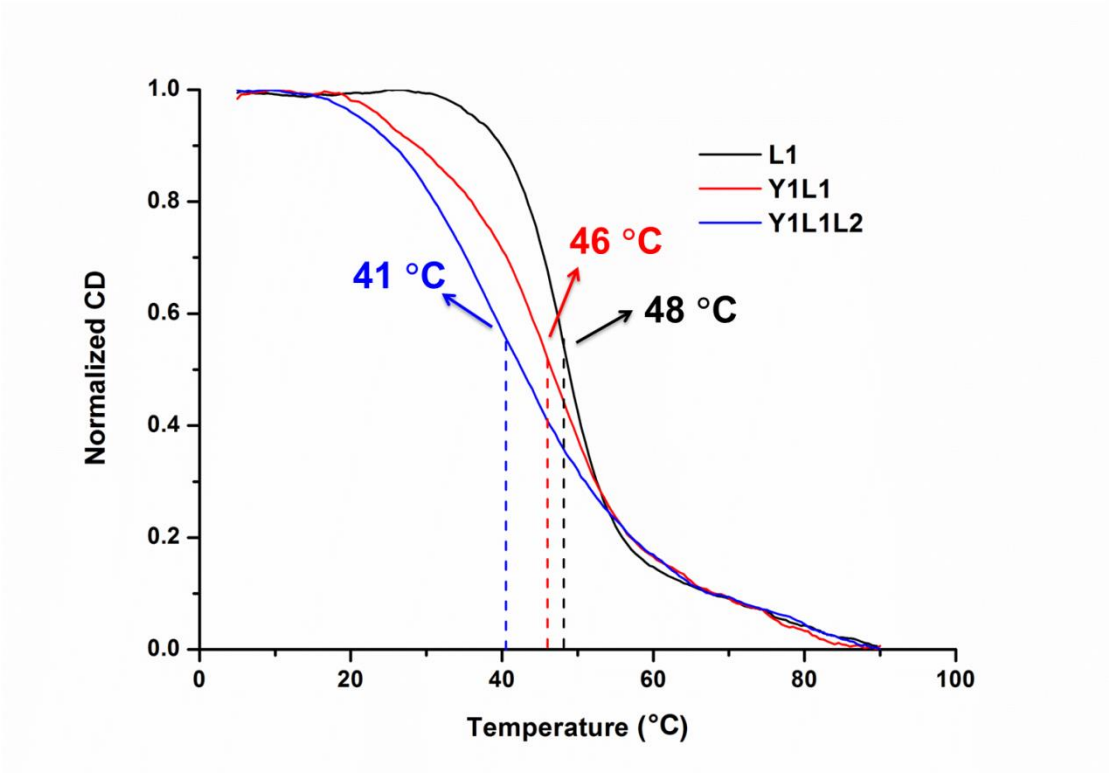

Figure S2 The melting temperatures of i-motif structure in different molecular environments at pH 5.0.

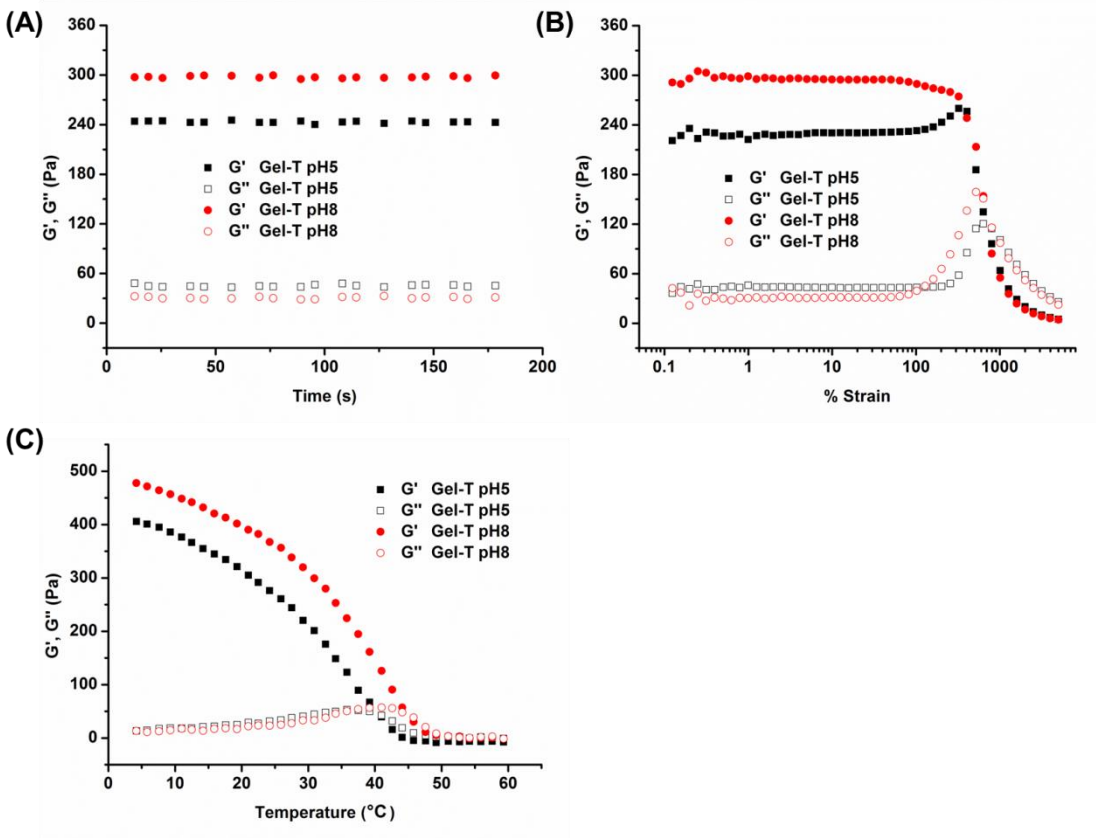

Figure S3 The mechanical properties of Gel-T at different pH conditions. **(A)** The mechanical strength of hydrogels at different environmental pH was characterized by time sweep test with a

fixed strain of 1% and frequency of 1 Hz at 25 ℃. **(B)** The shear-thinning property of hydrogels with different pH values was measured by strain sweep test from 0.1% to 5000% with a fix frequency of 1 Hz at 25 ℃. **(C)** The thermal responsiveness of hydrogels at different pH was characterized by temperature ramp test from 4 ℃ to 60 ℃ with a fixed strain of 1% and frequency of 1 Hz.

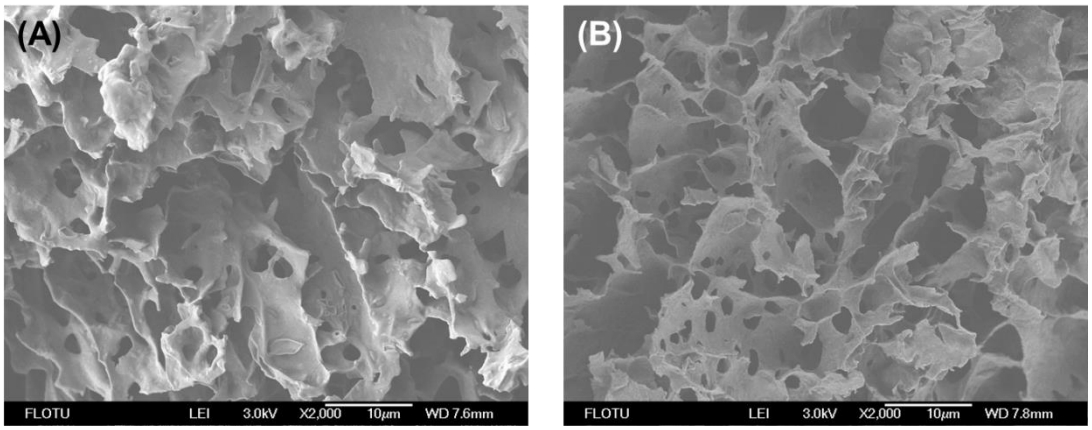

Figure S4 The scanning electron microscopy (SEM) images of lyophilized hydrogel samples at (A) pH 5.0 and (B) pH 8.0.