Supporting Information

pH-Responsive and Switchable Triplex-Based DNA Hydrogels

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1. Materials and methods

Materials. HEPES sodium salt, magnesium chloride, acetic acid, ammonium hydroxide solution, acrylamide solution (40%), ammonium persulfate (APS) and N,N,N′,N′-Tetramethylethylenediamine (TEMED) and coralyne chloride were purchased from Sigma-Aldrich. The concentration of coralyne in stock solution was determined by using its extinction coefficient at 420 nm (ε_{420nm}), 14500 L/(mole·cm). DNA oligonucleotides (Table 1) were purchased from Integrated DNA Technologies (Coralville, IA, USA). All the solutions were prepared with ultrapure water purified by a NANOpure Diamond system (Barnstead International, Dubuque, IA, USA).

Polyacrylamide gel electrophoresis (PAGE). To confirm the stability of the duplex generated between (**5**) and (**7**) at pH=10.0, we performed the

electrophoretic experiments shown in Fig. S4.

NMR Measurements. The average molecular weight of the copolymers (**3**), (**4**) and (**6**) were determined by diffusion-ordered NMR spectroscopy (DOSY), which relates the chemical shifts of NMR resonances of a given molecule to the translational diffusion coefficient of the species. This method turned out to be a facile method for determining average molecular weights. As shown in Fig. S6, a calibration curve corresponding to the diffusion coefficients of poly(acrylic acid) of variable known molecular weights (30000, 450000 and 1250000) was deduced. The linear equation is y=-0.6774x-7.3027 (R^2=0.9827). Average molecular weights were calculated as 800000, 400000 and 360000 for copolymers (**3**), (**4**) and (**6**), respectively. The NMR spectra were recorded on a Bruker DRX 400 MHz or a Bruker Ultrashield Plus 500 MHz spectrometer. The NMR spectra were referenced to residual solvent signals and recorded at 298 K. ^{1}H NMR data are reported as follows: chemical shift in ppm on the δ scale, integration, and multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublets; bs, broad singlet; bm, broad multiplet; m, multiplet). Standard samples of 0.5 mg of commercially available poly(acrylic acid)s in 1 mL of D_2O were used for the calibration curve in the DOSY experiments.

2. Figures

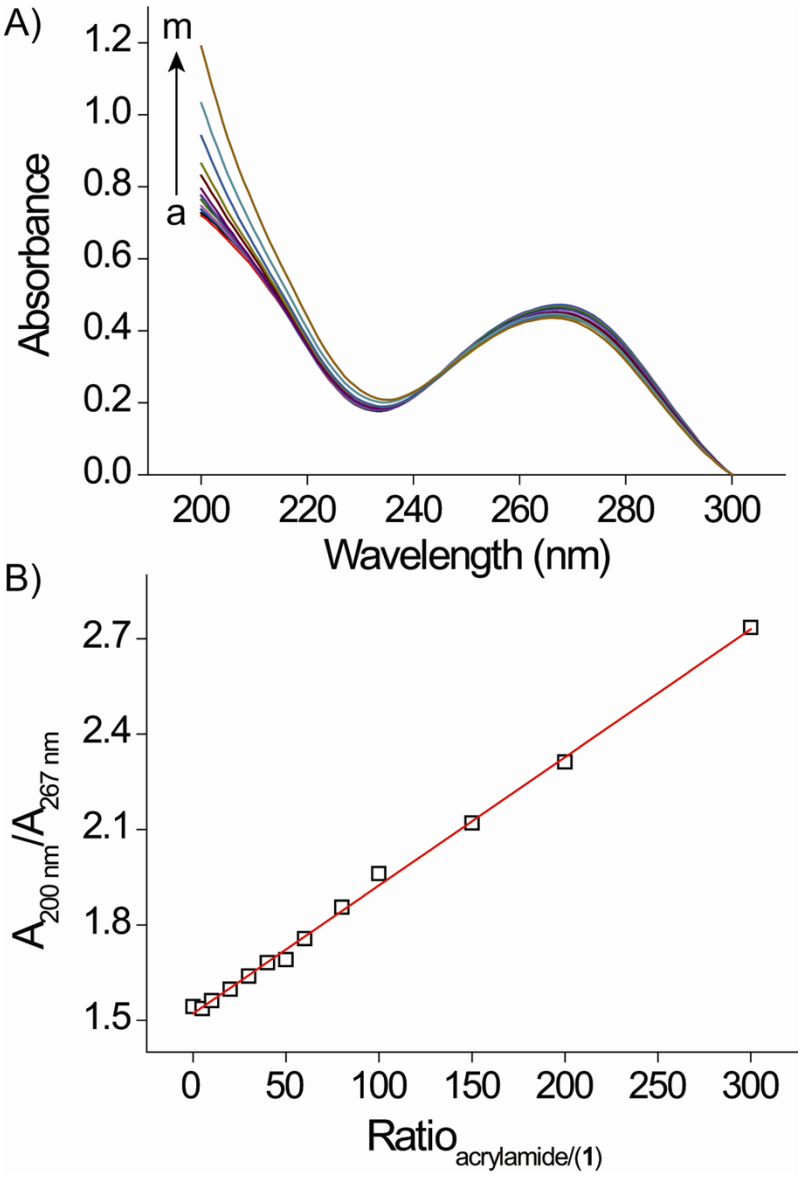


Fig. S1 (A) Absorption spectra of the polyacrylamide of different concentrations in the presence of constant centration of (**1**) (1 μM). The concentration of polyacrylamide from a to m corresponds to 0 μM, 5 μM, 10 μM, 20 μM, 30 μM, 40 μM, 50 μM, 60 μM, 80 μM, 100 μM, 150 μM, 200 μM, and 300 μM, respectively. (B) The calibration curve between the molar ratio of the acrylamide monomer/(**1**) and the ratio of absorbance at wavelength of 200 nm and 267 nm (A_{200nm}/A_{267nm}). The linear equation is $y=1.5215+0.00403x$ ($R^2=0.998$).

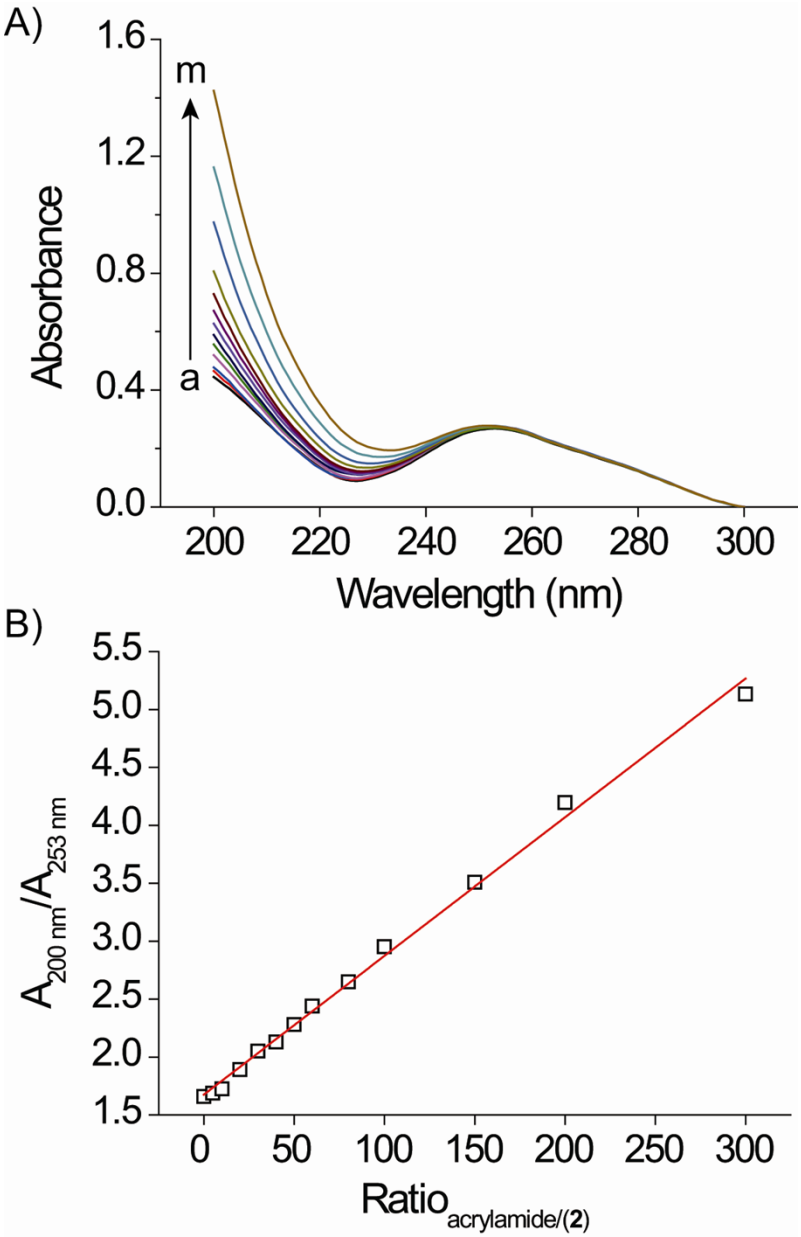


Fig. S2 (A) Absorption spectra of the polyacrylamide of different concentrations in the presence of constant centration of (**2**) (2 μM). The concentration of polyacrylamide from a to m corresponds to 0 μM, 10 μM, 20 μM, 40 μM, 60 μM, 80 μM, 100 μM, 120 μM, 160 μM, 200 μM, 300 μM, 400 μM, and 600 μM, respectively. (B) The calibration curve between the molar ratio of the acrylamide monomer/(**2**) and A_{200nm}/A_{253nm}. The

linear equation is y=1.676+0.01197x (R^2=0.996).

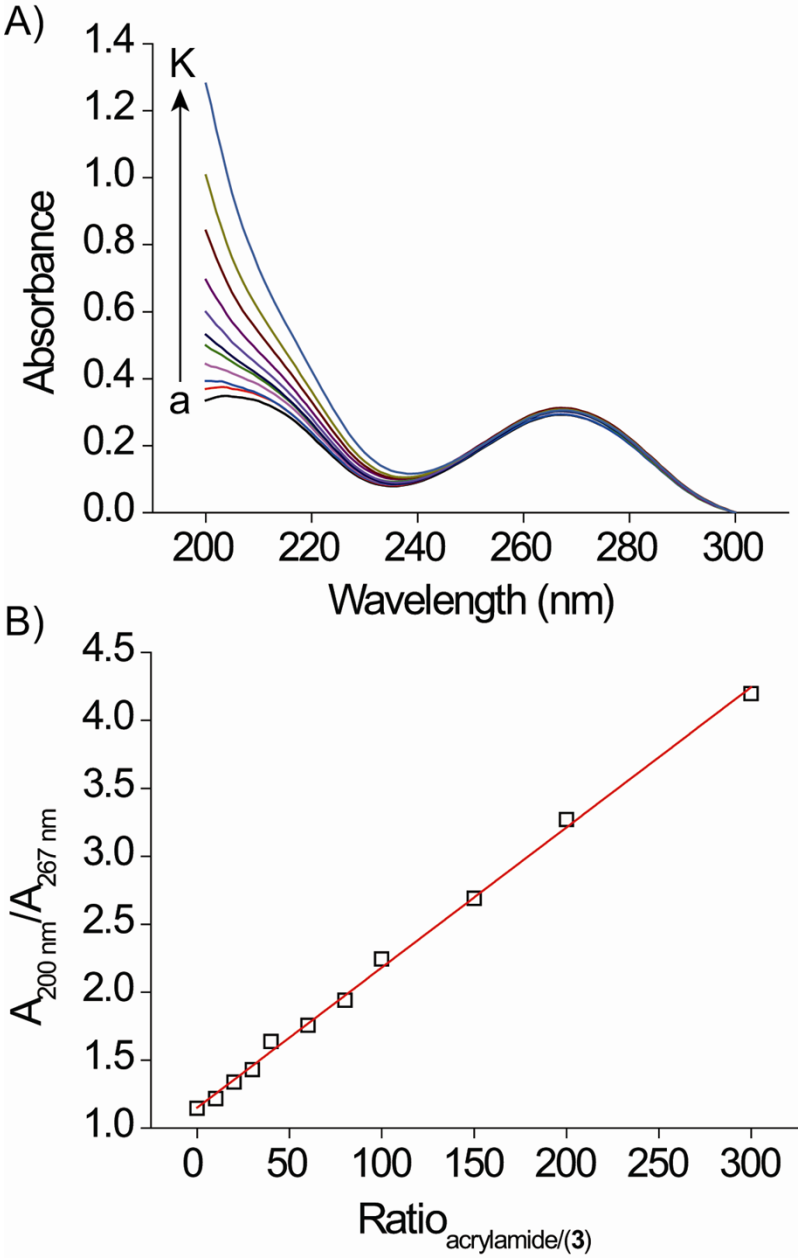


Fig. S3 (A) Absorption spectra of the polyacrylamide of different concentrations in the presence of constant centration of (**5**) (2 μM). The concentration of polyacrylamide from a to k corresponds to 0 μM, 20 μM, 40 μM, 60 μM, 80 μM, 120 μM, 160 μM, 200 μM, 300 μM, 400 μM, and 600 μM, respectively. (B) The calibration curve between the molar ratio of

the acrylamide monomer/(**5**) and A_{200nm}/A_{267nm}. The linear equation is y=1.1508+0.01031x (R^2=0.998).

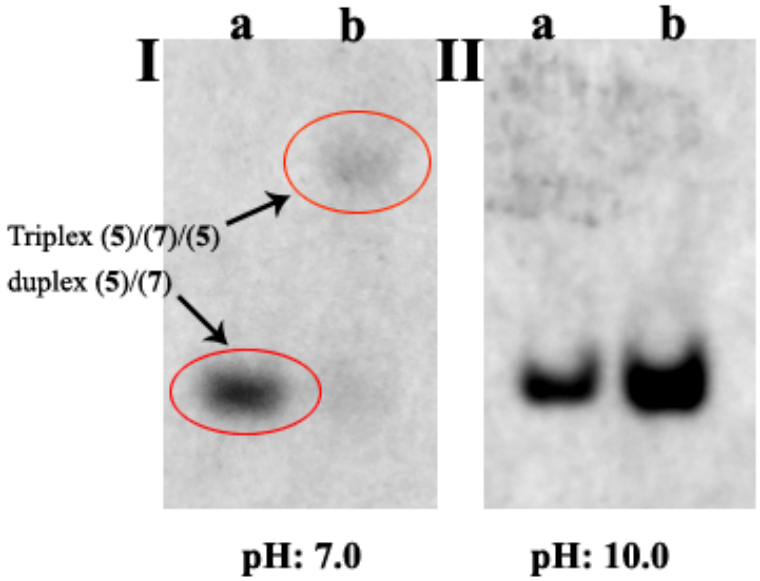


Fig. S4 PAGE confirmed formation of triplex (**5**)/(**7**)/(**5**) at pH=7.0 (Panel I) and its dissociation into duplex (**5**)/(**7**) at pH=10.0 (Panel II). Panel I shows the electrophoretic bands of the (**5**)(**7**) duplex and (**5**)/(**7**)/(**5**) triplex presented at pH=7.0. Panel II shows the as prepared (**5**)(**7**) duplex at pH=10.0 (lane a) and the triplex (**5**)/(**7**)/(**5**) subjected to pH=10.0 (lane b). The results demonstrate that the triplex (**5**)/(**7**)/(**5**) subjected to pH=10.0 yields a stable (**5**)(**7**) duplex structure.

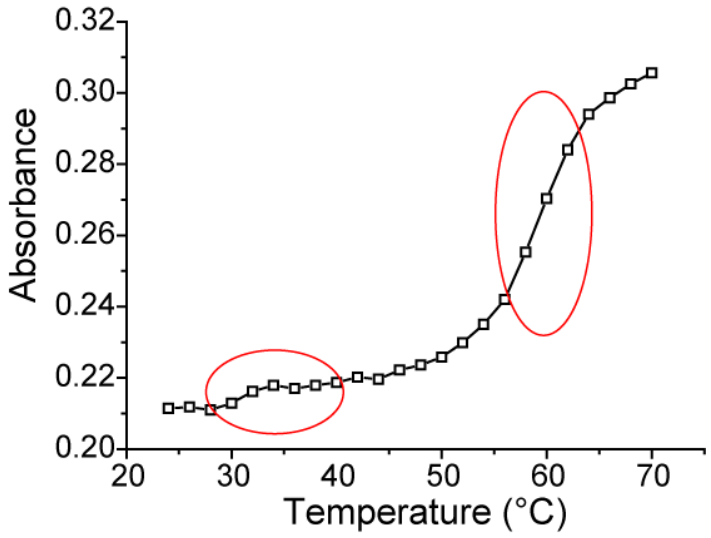


Fig. S5 UV-melting profile of (**6**)/(**7**) hydrogel at 260 nm. Two melting processes indicated by red circles correspond to the dissociations of triplex (**5**)/(**7**)/(**5**) and duplex (**5**)/(**7**), and T_m values were 32°C and 60°C.

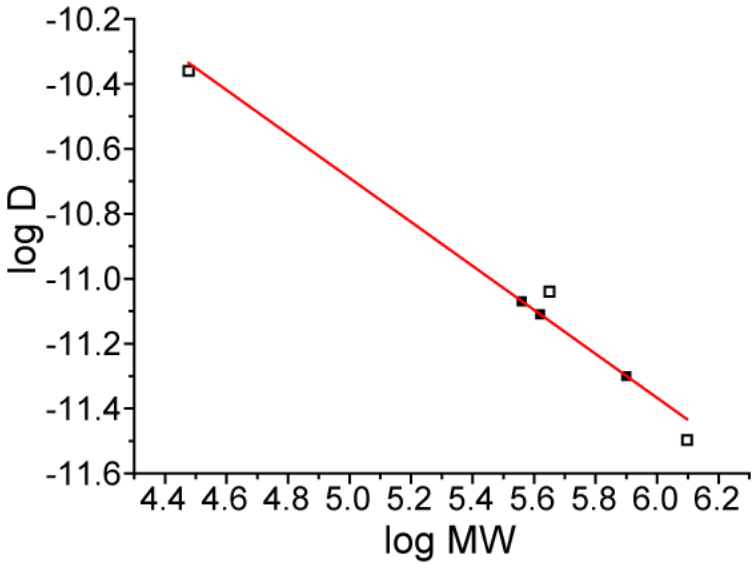


Fig. S6 A calibration curve corresponding to the diffusion coefficients of a series of poly(acrylic acid) of variable known molecular weights (30000, 450000 and 1250000). The linear equation is y=-0.6774x-7.3027 (R^2=0.9827). The diffusion coefficient derived from the DOSY spectrum

of copolymers (**3**), (**4**) and (**6**) were then introduced into the calibration curve, resulting in an estimated average molecular weight of 800000, 400000 and 360000 for copolymers (**3**), (**4**) and (**6**), respectively.

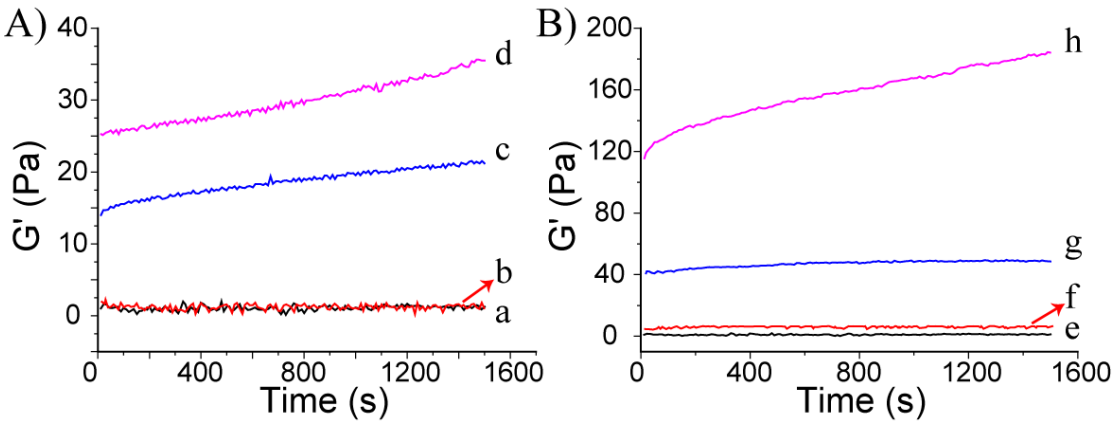


Fig. S7 (A) Rheological optimization of DNA loading of polyacrylamides to prepare (**3**)/(**4**) hydrogel. Curve a corresponds to the pure polyacrylamide. The ratios of (**1**):acrylamide monomer/(**2**):acrylamide monomer from curve b to d are 1:174/1:175, 1:110/1:97 and 1:81/1:80, respectively. (B) Optimization of DNA loading of polyacrylamides to prepare (**6**)/(**7**) hydrogel. Curve e corresponds to the pure polyacrylamide. The ratios of (**5**):acrylamide monomer from curve f to h are 1:125, 1:79 and 1:51, respectively.

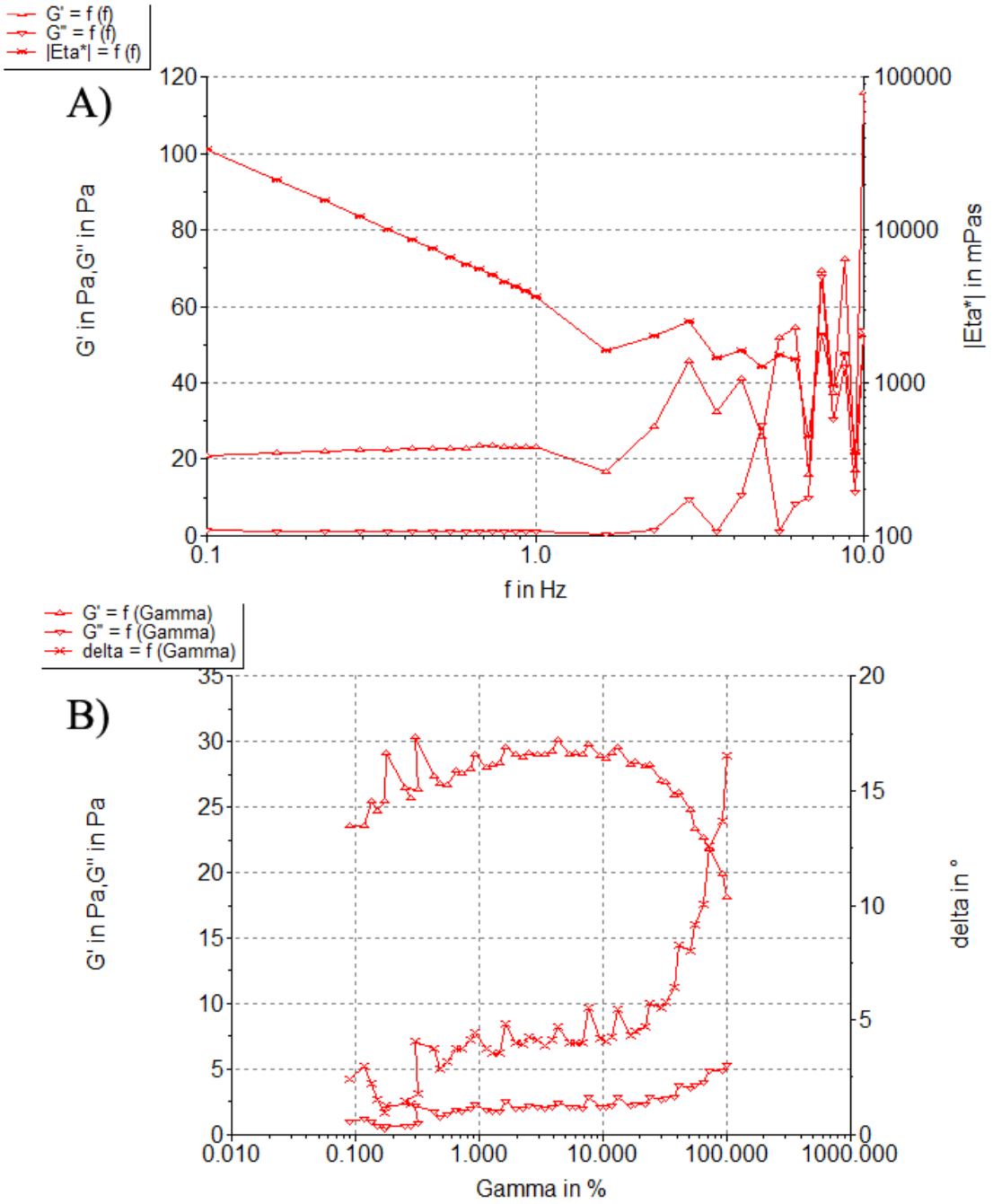
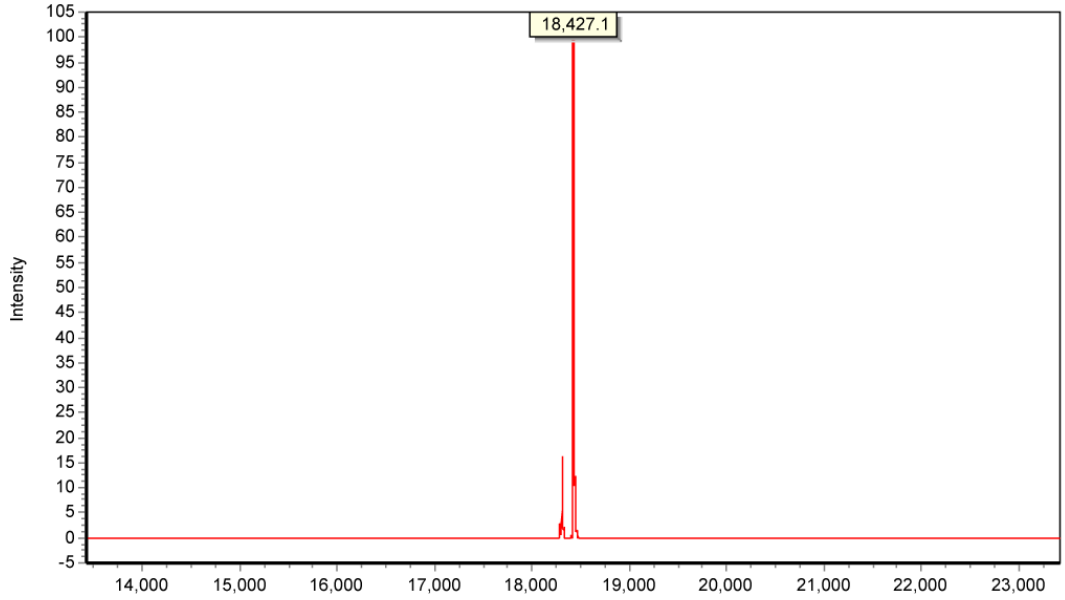


Fig. S8 Oscillatory frequency (0.1-10 Hz) (A) and strain (0.01-100%) (B) sweep tests for the (**3**)/(**4**) hydrogel.

Sequence Name: (**1**)

Oligo Sequence: 5'- /5Acryd/GGA GGG GAG GGG AGG TTT ACC TCC CCT CCC CTC CCT TTG CCT CCC CTC CCC TCC GTA CTC -3'



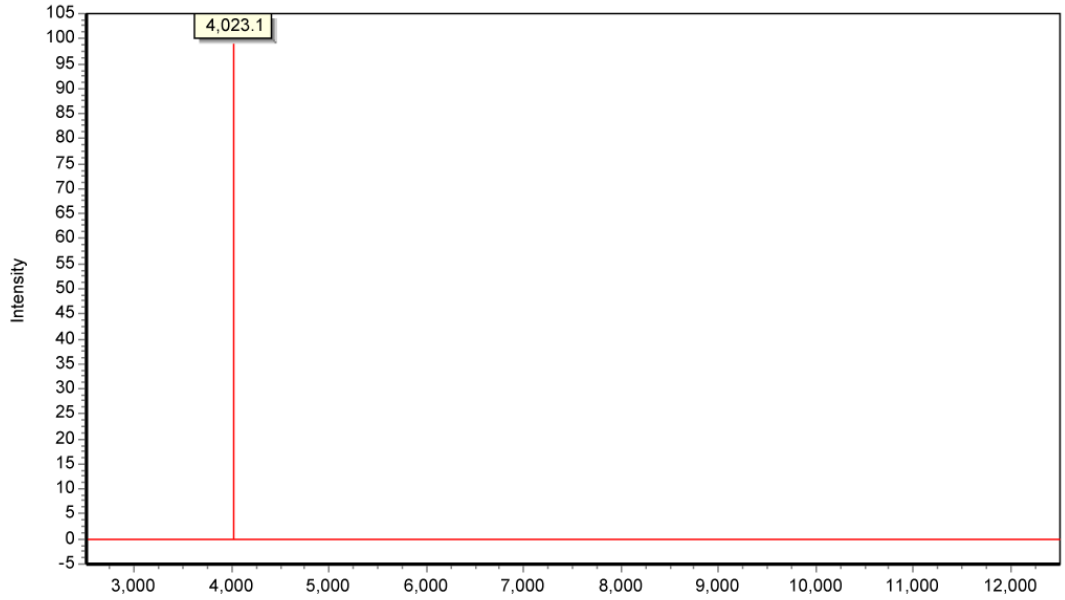
Calculated Molecular Weight: 18,426.9

Measured Molecular Weight: 18,427.1

Fig. S9 ESI mass spectrometry confirmed purity of sequence (**1**).

Sequence Name: (**2**)

Oligo Sequence: 5'- /5Acryd/GAG TAC GGA GGG -3'



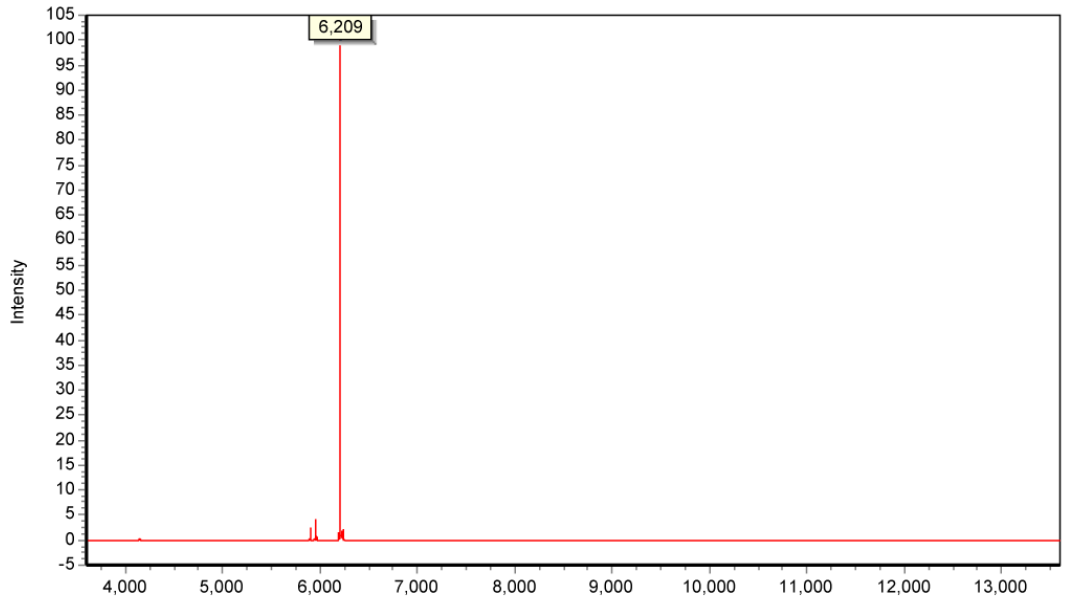
Calculated Molecular Weight: 4,022.7

Measured Molecular Weight: 4,023.1

Fig. S10 ESI mass spectrometry confirmed purity of sequence (**2**).

Sequence Name: (**5**)

Oligo Sequence: 5'- /5Acryd/TTC TTT TCT TTT CTT TTC TT -3'



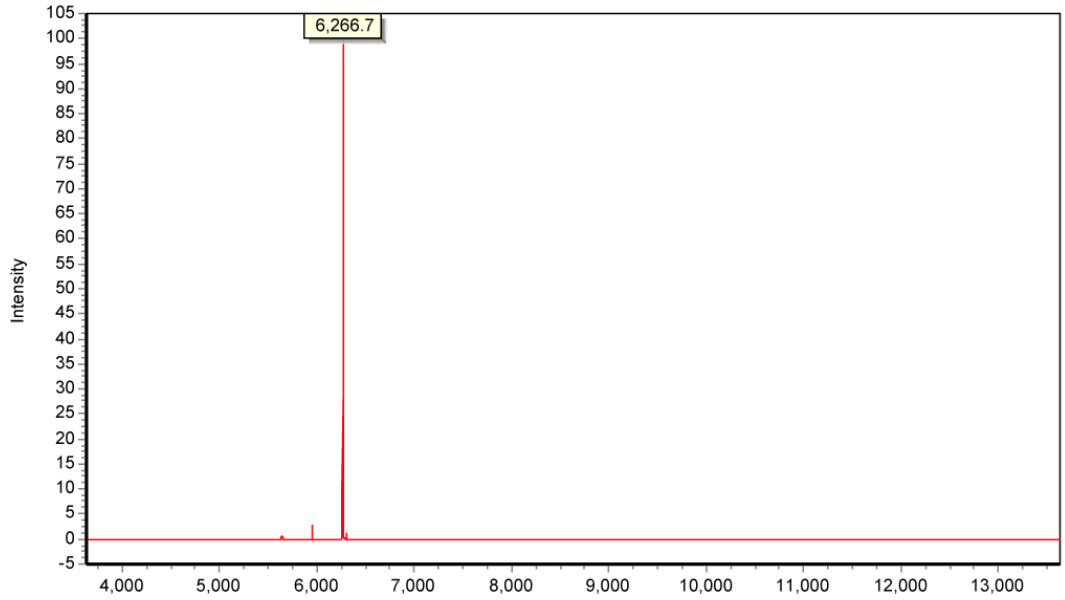
Calculated Molecular Weight: 6,209.1

Measured Molecular Weight: 6,209.0

Fig. S11 ESI mass spectrometry confirmed purity of sequence (**5**).

Sequence Name: (**7**)

Oligo Sequence: 5'- AAG AAA AGA AAA GAA AAG AA -3'



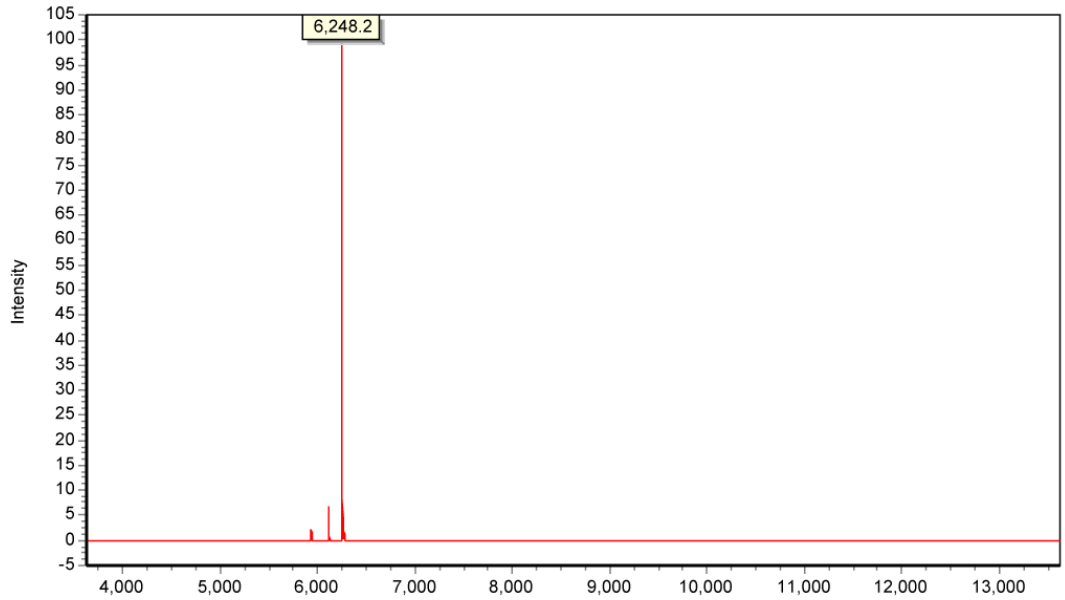
Calculated Molecular Weight: 6,266.2

Measured Molecular Weight: 6,266.7

Fig. S12 ESI mass spectrometry confirmed purity of sequence (**7**).

Sequence Name: (**7a**)

Oligo Sequence: 5'- AAG AAA AGT AAT GAA AAG AA -3'



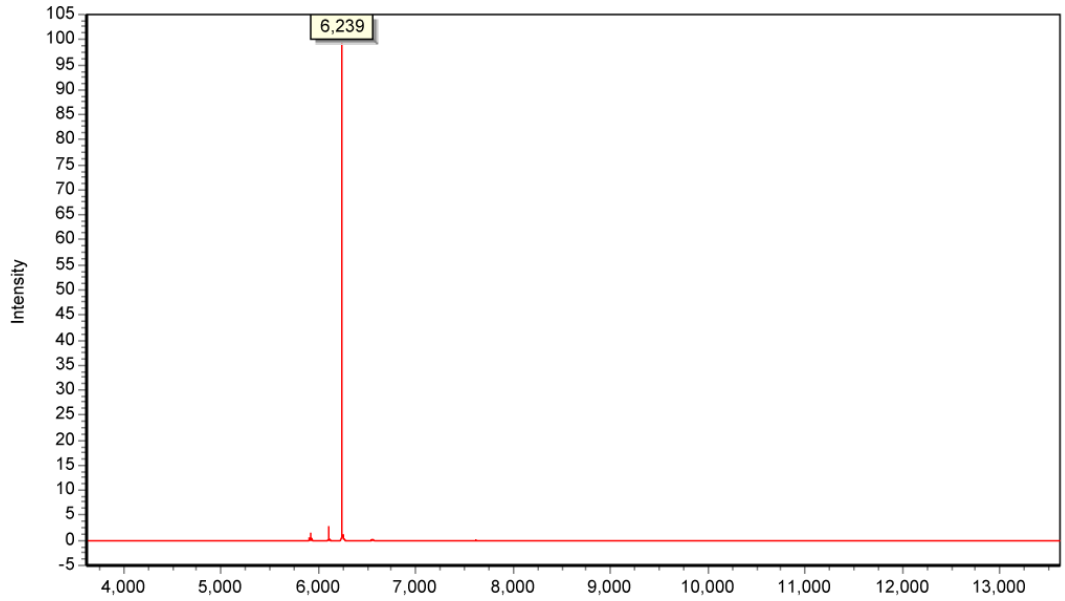
Calculated Molecular Weight: 6,248.2

Measured Molecular Weight: 6,248.2

Fig. S13 ESI mass spectrometry confirmed purity of sequence (**7a**).

Sequence Name: (**7b**)

Oligo Sequence: 5'- AAG ATA AGA TAA GAT AAG AA -3'



Calculated Molecular Weight: 6,239.2

Measured Molecular Weight: 6,239.0

Fig. S14 ESI mass spectrometry confirmed purity of sequence (**7b**).

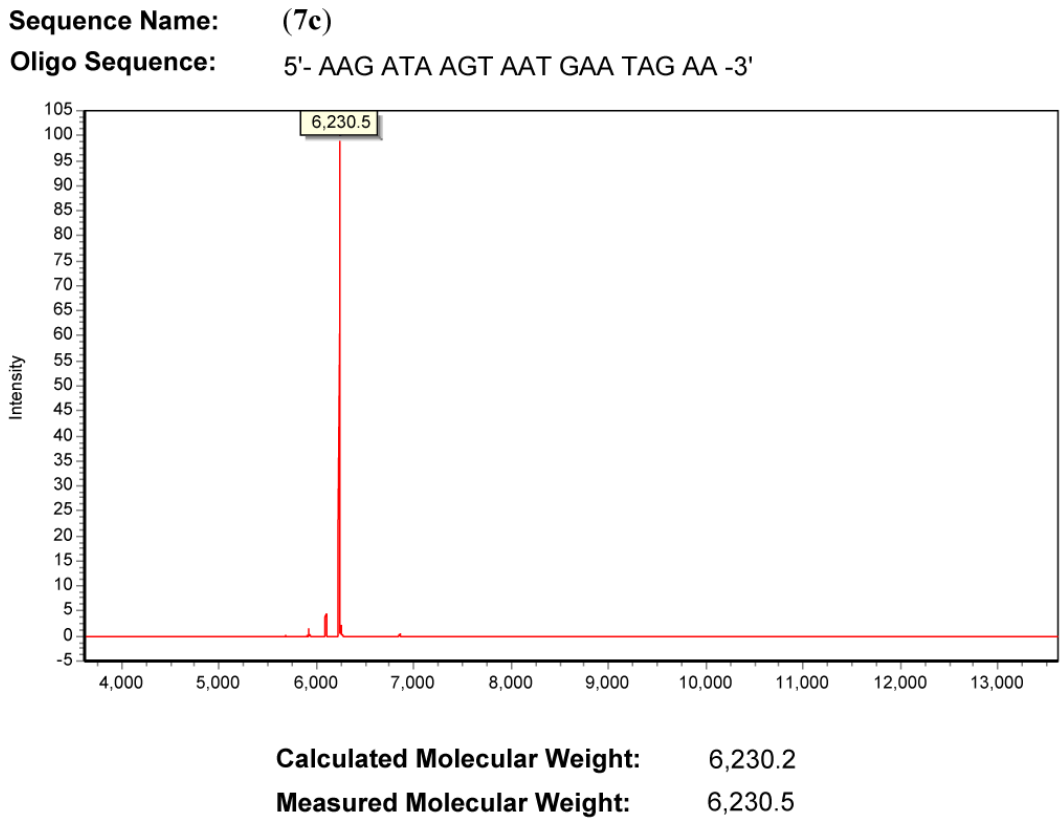


Fig. S15 ESI mass spectrometry confirmed purity of sequence (**7c**).