Supporting Information

A Supramolecular Hydrogel with Identical Cross-linking Point Density but Distinctive

Rheological Property

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1. Materials: All oligonucleotides (see Table S1) were synthesized using ABI 394 DNA synthesizer with a standard phosphoramidite DNA synthesis protocol and purified by HPLC. Water used in all experiments was Millipore Milli-Q deionized (18.2 M Ω cm⁻¹).

Table S1 DNA sequences for the preparation of polypeptide-DNA hydrogels

(A) Azido-DNA L₁: 5'-azido-AAGTGTCCAGTT-3'

(B) SE12 (12-base long sticky ends)
12a: 5'-ACTATGCGTGAATCCGTTAGAACTGGACACTT-3'
12b: 5'-CTAACGGATTCACGCATAGTAACTGGACACTT-3'

(C) SE8 (8-base long sticky ends)8a: 5'-ACTATGCGTGAATCCGTTAGAACTGGAC-3'8b: 5'-CTAACGGATTCACGCATAGTAACTGGAC-3'

(D) SE8M (single mismatch in 8-base long sticky ends) 8M-a: 5'-ACTATGCGTGAATCCGTTAGAACAGGAC-3' 8M-b: 5'-CTAACGGATTCACGCATAGTAACAGGAC-3'

Red sequences are "sticky ends"; Blue base is single-base mismatch site.

2. Synthesis of polypeptide-DNA conjugate.



Figure S1. Characterization of polypeptide-DNA conjugate by 10% de-native PAGE. Lane 1, azido-DNA; lane 2, polypeptide-DNA conjugate after purification.

3. Preparation of DNA linkers.

Stoichiometric amounts of ssDNA were mixed together in $1 \times \text{TBE}$ buffer containing 100 mM NaCl and then heated to 95 °C for 3 min, and subsequently cooled to room temperature in 2 h to form the designed double stranded DNA linker that was assessed by 10% native PAGE.



Figure S2. DNA linkers are characterized by 10% native PAGE. (a) Linker of 12bp "sticky ends". Lane 1, azido-DNA L_1 ; Lane 2 and 4, ssDNA 12a and 12b; Lane 3, 5 and 6, dsDNA 12a L_1 , 12b L_1 and 12a12b; Lane 7,12a12b L_1 . (b) Linker of 8bp "sticky ends". Lane 1, azido-DNA L_1 ; Lane 2 and 4, ssDNA 8a and 8b; Lane 3, 5 and 6, dsDNA 8a L_1 , 8b L_1 and 8a8b; Lane 7,8a8b L_1 . (c) Linker of 8bpM "sticky ends". Lane 1-2, ssDNA 8Ma and 8Mb, Lane 3, dsDNA 8Ma8Mb.

4. T_m measurement of the DNA linkers.



DNA linkers with different lengths of "sticky ends" assembled with azido-DNA into more advanced structures, namely assembled structures. The structures with "sticky ends" of SE12 (12-base long), SE8 (8-base long), and SE8M (single mismatch within SE8) are measured by UV melting experiments, respectively, which was carried out from 4 °C to 90 °C at a rate of 1 °C min⁻¹. Two melting points are obtained for each assembled structures, one is of the double strand region (20 bp) of DNA linker, and the other one is of the double strand region formed

of azido-DNA and "sticky ends" of DNA linkers. The melting points are 68.5 °C (double strand region of DNA linker), 53.5 °C (SE12), 39.6 °C (SE8), and 20.8 °C (SE8M), respectively.

5. Rheological tests.



Figure S3. The mechanical strength of hydrogel samples (5 wt%) with different DNA linkers were characterized by time scan rheological test performed at a fixed frequency of 1 Hz and strain of 1 % at 25 °C.

The hydrogel could be also mechanically tuned by varying relative molar ratio of two components. In our study, DNA linker (SE12) and polypeptide-DNA were mixed together with different molar ratios of "sticky ends" at 0.6:1, 0.8:1, 1.0:1 and 1.2:1, respectively, with a fixed total mass content of 5 wt%. Theoretically, the mechanical property of the hydrogels should be the highest when the "sticky ends" from DNA linker and polypeptide-DNA are at the ratio of 1:1, i.e., the networks are fully cross-linked. In our experiment, shown in Fig. S5, the hydrogel at the ratio of 0.8:1 and 1.0:1 exhibited the highest mechanical strength, and beyond this range, when the system had either polypeptide-DNA or DNA-linker in excess, the hydrogel exhibited lower mechanical strength.



Figure S4. Rheological strain sweep of polypeptide-DNA hydrogels (3 wt%) which was performed between 0.04% and 100% at 25 °C with a fixed frequency of 1 Hz.



Figure S5. Rheological properties of polypeptide-DNA hydrogels with different pHs. Frequency sweep rheological tests were carried out between 0.05 Hz and 100 Hz at a fixed strain of 1% at 25 °C.



Figure S6. Rheological properties of polypeptide-DNA hydrogels with different DNA linkers. Frequency sweep rheological tests were carried out between 0.05 Hz and 100 Hz at a fixed strain of 1% at 25 °C.



Figure S7. Rheological properties of polypeptide-DNA hydrogels with different molar ratios of linkers (sticky ends of DNA linker/polypeptide-DNA = 0.6:1, 0.8:1, 1.0:1, and 1.2:1). The total polypeptide and DNA content was 5 wt.% and the frequency sweep rheological tests were carried out between 0.05 Hz and 100 Hz at a fixed strain of 1% at 25 °C.

6. Circular dichroism spectroscopy.

The circular dichroism (CD) spectra were collected on an Applied Photophysics Chirascan spectropolarimeter which was carried out at the range of 180-350 nm at room temperature in a quartz cell of 1 mm optical length with a scanning speed of 60 nm·min⁻¹.



Figure S8. CD Spectroscopy of polypeptide in phosphate buffer (100 mM) with different pHs. Red line, CD curve of polypeptide in phosphate buffer of pH 5.0 (red line) and pH 8.0 (black line).