

Self-Assembled DNA Hydrogels with Designable Thermal and Enzymatic Responsiveness

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DNA has received considerable attention as a promising building material owing to its ability to form predictable secondary structures through sequence-directed hybridization.^[1,2] It has been shown that DNA can be precisely designed with specific sequences and self-assemble into two- or three-dimensional nanostructures,^[3–9] fabricated to nanomachines or motors,^[10–13] or used as a programmable template to direct the assembly of nanoparticles.^[14–17] Recently, the concept of DNA assembly has been expanded to construct “DNA hydrogels”, which are crosslinked networks swollen in an aqueous phase.^[18–31] Though hydrogels have great potential in biological and medical applications,^[32–36] such as drug and gene delivery, biosensing, and tissue engineering, studying the preparation of DNA hydrogels with designable properties is still in its early stages. In the past, several methods have been reported to prepare DNA hydrogels, for example, DNA directly extracted from the nucleus in nature, behaves like a long linear polymer and forms a hydrogel via physical entanglement or by chemical crosslinking of small molecules.^[18–20] Similarly, DNA can be used as a negatively charged polymer and form a complex with cationic (poly)electrolytes through electrostatic interactions.^[21,22] However, both methods treated DNA as a polymer and did not take advantage of the self-assembly of DNA into ordered structures, therefore, the resulting hydrogels lacked precise structural control and specific responses. Instead of using physical interactions, DNA can be covalently grafted onto synthetic polymers and serve as a cross-linker, the recognition of complementary DNA strands leads to crosslinking of polymer chains and causes hydrogel formation.^[23–28] In general, the preparation of a DNA-polymer hybrid requires laborious modification steps, and an easy and fast strategy to build tailored DNA hydrogels is desired. Luo and his coworkers have developed a new approach to construct

pure DNA hydrogels: using well-designed DNA sequences, self-assembled DNA building blocks with more than two branches could be prepared and further enzymatic ligation between the building blocks led to DNA hydrogel formation.^[29] These DNA hydrogels have been demonstrated for potential applications in controllable drug release^[29] and cell-free protein-producing systems,^[30] however, the enzymatic ligation is rather slow and the preparation is time-consuming. More recently, we have reported that pure DNA hydrogels could be made based on duplex formation and intermolecular i-motif structures. The DNA hydrogels showed a fast sol-gel transition upon changes in the pH, that is, within minutes, and released cargoes in a pH-controlled manner.^[31] However, these DNA hydrogels were not stable under physiological conditions, which limited their in-vivo applications.

Herein, we propose a new and general platform to create pure DNA hydrogels through self-assembly. As illustrated in **Scheme 1**, we designed two kinds of building blocks: a Y-scaffold and a linker. The Y-scaffold is assembled from three single-stranded DNA (ssDNA) strands, whereby each strand has three segments: a “sticky end” segment to hybridize with its complementary part on the linker and two segments to hybridize with the two other strands. The linker is a linear duplex formed by two ssDNAs and contains two “sticky ends”. The “sticky ends” of the Y-scaffold and linker are complementary to each other, and we propose that this hybridization will lead to hydrogel formation. It is noted that the “sticky ends” are particularly designed to avoid palindromic sequences, therefore, prohibiting self-linking between the same type of DNA building blocks and ensuring the homogeneous property of DNA hydrogels.

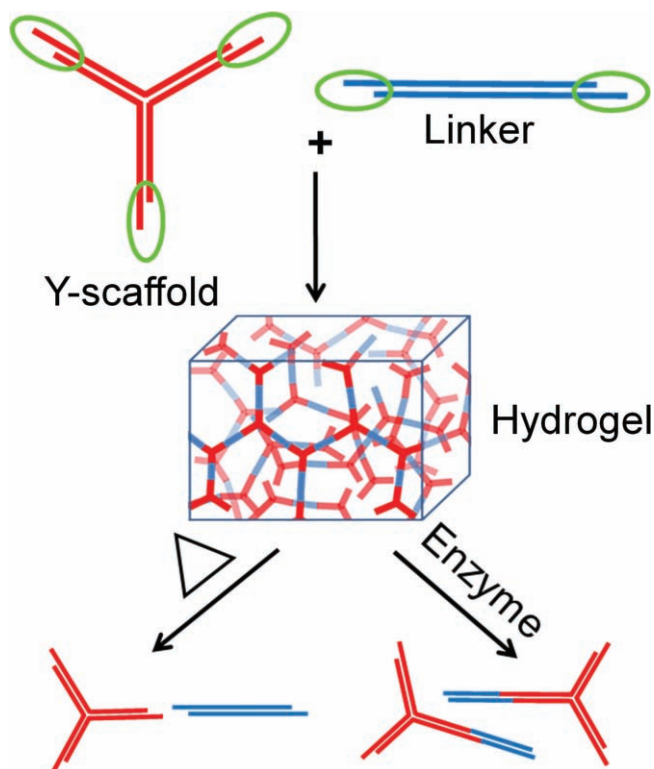
Our first experiment was to determine whether we could achieve the designed Y-scaffold and linker. In a typical experiment, stoichiometric amounts of DNA strands of the Y-scaffold (Y1, Y2, and Y3) and linker (R1 and R2, detailed sequences see Table S1) were separately added to 20 mM of Tris-HCl buffer (pH 7.5) containing 100 mM of NaCl to give a final concentration of 20 μ M for each DNA strand. The resulting mixtures were heated to 95 °C for 5 min, and subsequently cooled to room temperature in 2 h to let the designed structures form. Agarose gel electrophoresis was used to verify whether the Y-scaffold and linker had formed properly. ssDNA and partially assembled ssDNA were also loaded as controls. As shown in **Figure 1**, the assembled Y-scaffold (lane 7) migrated slower than each single strand, Y1, Y2, and Y3 (lane 1, 2, and 3). The three partially assembled structures, Y1 + Y2, Y1 + Y3, and Y2 + Y3 (lane 4, 5, and 6) were in between. Similarly, the assembled linker (lane 10) migrated slower than R1 and R2 alone (lane 8 and 9). These results demonstrate that the DNA

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Scheme 1. Schematic representation of DNA hydrogel formation. The Y-scaffold and linker are designed to crosslink by hybridization of their “sticky ends” (emphasized by green circles). By tailoring these “sticky ends” and inserting restriction sites in the linker sequences, the DNA hydrogels show thermal and enzymatic responsive properties.

building blocks, the Y-scaffold and linker, were indeed formed as designed and the clean single bands observed in lane 7 and 10 indicate that the assembling process was efficient. In order to detect the stability of both building blocks, we measured the melting temperature (T_m) by UV-vis spectroscopy. The T_m of the Y-scaffold and linker were 63 °C and 72 °C, respectively (Figure S1), therefore, all experiments hereafter were performed at temperatures below 60 °C in order to maintain the stability of these structures.

Next, we sought to determine if the Y-scaffold and linker could form DNA hydrogels. In our study, the Y-scaffold and linker contain 8-base long “sticky ends” (bp8). As calculated, the ideal molar ratio of the Y-scaffold and linker is 1:1.5, and the

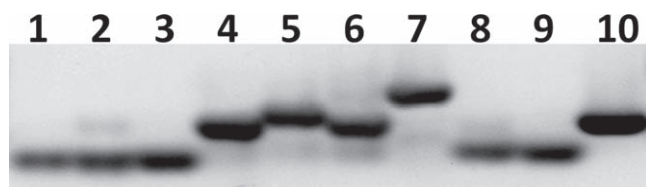


Figure 1. 3% Agarose gel electrophoresis analysis. Lane 1-3 are the ssDNA monomers for the Y-scaffold, Y1, Y2, and Y3, respectively; lane 4-6 are the partial assemblies containing only two of the three strands, Y1 + Y2, Y1 + Y3, and Y2 + Y3, respectively; lane 7 is the Y-scaffold; lane 8 and 9 are the ssDNA monomers for the linker, R1 and R2, respectively; lane 10 is the linker.

ratio of their “sticky ends” is 1:1, a ratio at which the starting materials are completely consumed and form the most dense network. We observed that if 500 μM of the Y-scaffold was mixed with 750 μM of the linker, the solution lost its fluidity within a minute and appeared to be gel-like (Figure 2A), suggesting a sol-to-gel transition occurred.

Apart from visual observations, rheological tests, that is, frequency sweep tests, were also carried out on the samples described above to further confirm the DNA hydrogel formation. As shown in Figure 2B, the shear-storage modulus (G') was obviously higher than the shear-loss modulus (G'') over the entire frequency range, providing a clear signature of the gel-like state.^[37] In addition, the G' showed a slight frequency-dependent increase, suggesting a physical gel.^[37] This result, combined with previous visual observations, indicates that pure DNA hydrogels are indeed formed by self-assembly of DNA building blocks in a swift manner.

We further investigated the influence of the ratio of the DNA building blocks on the mechanical properties of the resulting DNA hydrogels. The Y-scaffold and linker were mixed at different molar ratios, namely 2:1, 1:1, 1:1.5, 1:2, and 1:3 with a total DNA content of 2 wt.% under the same experimental conditions as described above. As shown in Figure 2C, at ratios of 2:1 and 1:3, both G' and G'' were very low, around 0.1 Pa, indicating that the DNA mixtures behaved like liquids. We suppose that at these ratios the minority building block is saturated by the building block that is in excess, so that there are not enough DNA assemblies to create a continuous network. In contrast, at the ratios of 1:1, 1:1.5, and 1:2, G' was higher than G'' , suggesting that a hydrogel was formed; however, the mechanical strength of the DNA hydrogels at the ratios of 1:1 and 1:2 was lower than that formed at the ideal ratio of 1:1.5, because the former networks were partially crosslinked and not so dense as that formed at the ideal ratio. In addition, the concentration of the DNA building blocks can also influence the mechanical strength of the DNA hydrogels. We observed that a minimum concentration of 75 μM (with a total DNA content of 0.5 wt.%) of the Y-scaffold was required to form a gel-like state, and the strength of the DNA hydrogels increased with increasing concentration of the DNA building blocks (Figure S2).

In this study the DNA hydrogels were formed solely by self-assembly and there were no other interactions than base pairing/hybridization of the “sticky ends”. We propose that the formation of the DNA hydrogels is temperature dependent as is common for a DNA duplex, and is governed by the weakest link, that is, the base pairing of the “sticky ends”. The temperature-ramp rheological tests in Figure 2D show that the DNA hydrogels that were formed from 400 μM of Y-scaffold and 600 μM of linker can switch between the gel and the sol state when changing the temperature from 25 to 50 °C, and this process can be cycled many times, indicating that the DNA hydrogels respond to heat in a reversible way.

As the self-assembly occurs via hybridization of the “sticky ends”, we sought to determine how these “sticky ends” influence the mechanical and thermal properties of the DNA hydrogels. To do this, three more groups of Y-scaffold and linker with different lengths of “sticky ends” were designed as follows, bp4 (4-base long “sticky ends”), bp8M (a single-base mismatch within bp8), and bp12 (12-base long “sticky ends”),

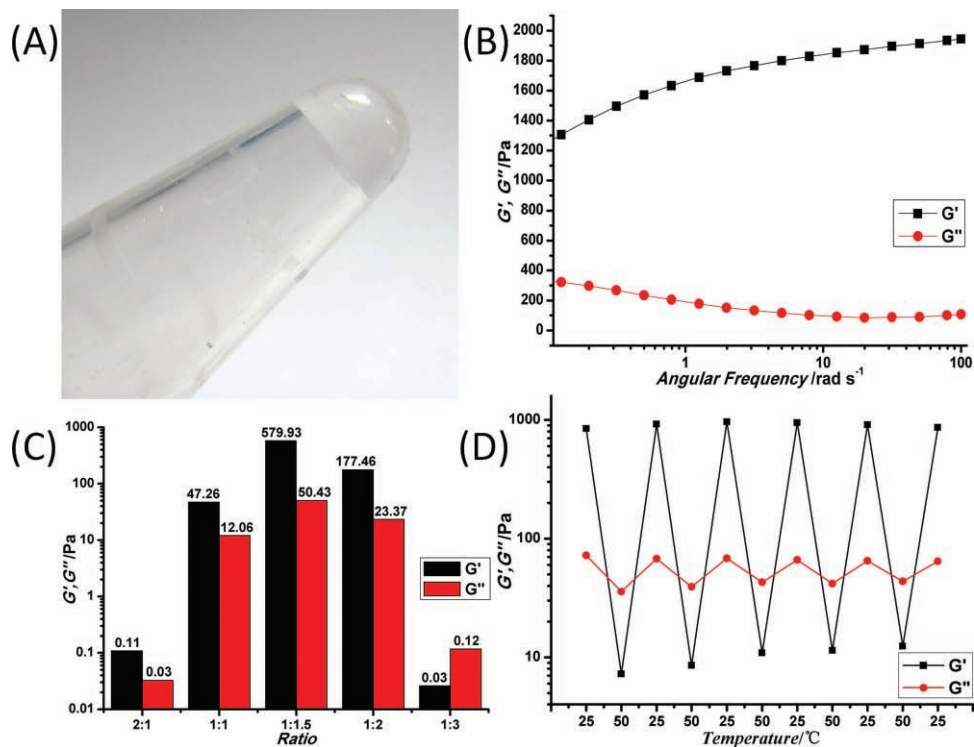


Figure 2. A) DNA hydrogels prepared from 500- μm of Y-scaffold and 750 μm of linker with 8-base long “sticky ends”. B) Frequency sweep test carried out between 0.05 and 100 rad s^{-1} at a fixed strain of 1% at 25 °C. C) DNA hydrogels with different molar ratios (Y-scaffold/linker = 2:1, 1:1, 1:1.5, 1:2, and 1:3) with a total DNA content of 2% (w/v) were tested by time-scan rheological tests performed at a fixed frequency (1 Hz) and strain (1%) at 25 °C for 3 min. D) Reversible thermal response of DNA hydrogels formed from 400 μm of Y-scaffold and 600 μm of linker with 8-base long “sticky ends”. The rheological test was performed on the gel at a fixed frequency (1 Hz) and strain (1%) at 25 °C and 50 °C, respectively, for 5 cycles.

for detailed sequences see Table S1. As shown in **Figure 3A**: at 25 °C, the G'' of the bp4 group is higher than the G' at frequencies below 20 rad s^{-1} , however, when the sweep frequency is increased to above 20 rad s^{-1} the relation between G' and G'' reverses, which is typical behavior of concentrated solutions.^[37] The reason for this is that the linkage between the Y-scaffold and linker via only four base-pairs is not strong enough to

form a gel (see Figure S3). This result also explains why the system reported previously by Luo and co-workers needed covalent crosslinking through enzymatic ligation to stabilize their DNA hydrogels with 4-base long “sticky ends”.^[29,30] In contrast, as the length of the “sticky ends” increased from 4 to 8 and 12, inspection of Figure 3A reveals that the G' of the bp8, bp8M, and bp12 group is higher than their G'' for the

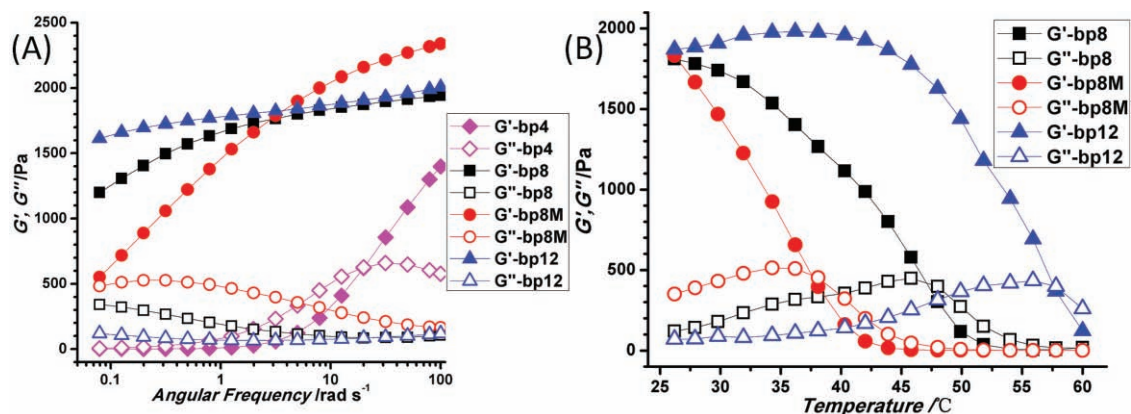


Figure 3. Rheological properties of DNA hydrogels with different “sticky ends”. A) Frequency sweep tests were carried out between 0.05 and 100 rad s^{-1} at a fixed strain of 1% at 25 °C. The “sticky ends” are: bp4 (4-base long), bp8 (8-base long), bp8M (single-mismatch within “sticky ends” of bp8), and bp12 (12-base long). B) Temperature-ramp rheological tests were carried out from 25 °C to 60 °C at a rate of 2 °C min^{-1} at a fixed frequency (1 Hz) and strain (1%).

entire swap frequency range, which is a typical mechanical property of hydrogels. In addition, the mechanical strength of the DNA hydrogels can be compared by using the ratio G'/G'' ; for example, a higher G'/G'' indicates a better mechanical strength. The G'/G'' ratios of the samples containing the bp4, bp8, bp8M, and bp12 group were 0.5, 13, 5.7, and 21, respectively (Figure S4), providing additional evidence that the “sticky ends” play an important role in the mechanical strength of the DNA hydrogels: i) the longer the “sticky ends”, the stronger the DNA hydrogels; ii) at the same length of “sticky ends”, the presence of mismatched sites decreases the mechanical strength of the DNA hydrogels because of the lower stability of the “sticky ends”.

To provide additional insight into the thermal responsive behavior of DNA hydrogels, we studied the temperature dependence of G' and G'' of the DNA hydrogels. As shown in Figure 3B, when the temperature was increased from 25 °C to 60 °C at a rate of 2 °C min⁻¹, the G' of all three gel samples decreased and had an intersection with G'' at different temperature points, suggesting these gels indeed respond to temperature and the intersection points are the transition temperatures where the samples convert from the gel to the sol state. The transition points of the DNA hydrogels containing bp8, bp8M, and bp12, as determined from Figure 3B, were 47.0 °C, 37.5 °C, and 58.0 °C, respectively, suggesting that the formation of a hydrogel is governed by the stability of the “sticky ends” (see Supporting Information), and the responsive temperature or the sol-gel transition temperature of the prepared DNA hydrogel is tunable by tailoring the “sticky ends”.

Next, we studied the enzymatic responsiveness of the designed DNA hydrogels. It is known that restriction enzymes (restriction endonucleases) can cut double-stranded DNA at specific recognition sequences (known as restriction sites).^[38,39] We propose that a linker containing a restriction

site would be cleaved by its corresponding restriction enzyme, and as a result, the continuous network composed of this linker would be broken, so that, on a macroscopic scale, the DNA hydrogels would switch to the sol state. To support our proposition, two types of linker (with 8-base long “sticky ends”) were designed with different restriction sites inserted into their duplex domain: linker H contained the BamH I restriction site (5'-GGATCC-3') and linker R contained the EcoR I restriction site (5'-GAATTC-3'). Following our established procedure, both linkers assembled with the Y-scaffold to form hydrogel H (Figure 4a) and hydrogel R (Figure 4d), respectively. In order to test the responsiveness of the DNA hydrogels to enzymes, 30 U BamH I and EcoR I, each in their corresponding reaction buffer, were added to hydrogel H and hydrogel R. After incubation at room temperature for 48 h, hydrogel H remained its gel state in the presence of the EcoR I restriction enzyme (Figure 4b), however, it turned into a solution when digested by the BamH I restriction enzyme (Figure 4c). Similarly, hydrogel R did not undergo a gel-to-sol transition upon adding the BamH I restriction enzyme (Figure 4e), but it was dissolved in the presence of the EcoR I restriction enzyme (Figure 4f). These results suggest that DNA hydrogels that contain restriction sites respond to the respective restriction enzyme and undergo a gel-to-sol transition as designed. As the DNA hydrogels modified with restriction sites only respond to their corresponding restriction enzyme this high specificity may be useful to trigger the controlled release of substances.

In summary, we have successfully built a new and versatile strategy to prepare DNA hydrogels with designable responsive properties completely based on DNA self-assembly. Most significantly, by properly tailoring the “sticky ends” of the DNA building blocks (Y-scaffold and linker), the DNA hydrogels formed rapidly without any chemical treatment. The formation mechanism and properties of the DNA hydrogels were systematically studied. We also demonstrated that the DNA hydrogels reversibly respond to thermal stimulus, by switching between the gel and sol state across a transition temperature, as well as respond to enzymes when restriction sites are inserted into one of the building blocks (i.e., the linker). The preparation principle and responsive mechanisms reported in this paper provide a basic and prospective platform to build functional biomaterials using biomolecules with well-defined compositions and structures,^[40,41] therefore, providing a new class of intelligent materials for a diverse range of biological and biomedical applications.^[32–36]

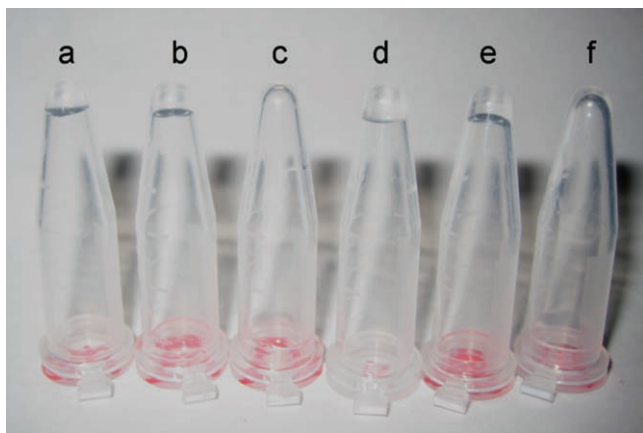


Figure 4. Enzymatic responsive behavior of DNA hydrogels. Hydrogel H (a–c) and hydrogel R (d–f) contain restriction sequences of BamH I and EcoR I on their linkers, respectively. In each tube, 10 μ L of DNA hydrogel was incubated in 10 μ L of buffer a,d) without enzymes, b,f) with 30 U EcoR I, and c,e) with 30 U BamH I at room temperature for 48 h. For BamH I, the reaction buffer contained 20 mM of Tris-HCl (pH 8.5), 100 mM of KCl, 10 mM of MgCl₂, and 1 mM of dithiothreitol; for EcoR I, the reaction buffer contained 50 mM of Tris-HCl (pH 7.5), 100 mM of NaCl, 10 mM of MgCl₂, and 1 mM of dithiothreitol.

Experimental Section

Materials: All oligonucleotides (see Table S1) were purchased from Invitrogen Biotech (Beijing, China) and PAGE purified. The BamH I and EcoR I restriction enzymes were purchased from the TaKaRa Biotech company (Dalian, China). All chemicals were of reagent grade or better and used as received.

Preparation of Y-scaffold and Linker: Stoichiometric amounts of the DNA strands for the Y-scaffold and linker were separately added in two Eppendorf (EP) tubes with a buffer solution containing 20 mM of Tris-HCl (pH 7.5) and 100 mM of NaCl to obtain a final concentration of 20 μ M, after which the mixtures were heated to 95 °C for 5 min and cooled to room temperature in 2 h to form the desired DNA nanostructures.

Preparation of DNA Hydrogels: In a typical experiment, a stock solution of the building blocks was prepared, whereby stoichiometric amounts of the Y-scaffold and linker DNA strands were added in a buffer solution containing 20 mM of Tris-HCl (pH 7.5) and 100 mM of NaCl to obtain a final concentration of 1 mM and 1.5 mM, respectively, after which the mixtures were heated to 95 °C for 5 min and cooled to room temperature in 2 h. Calculated volumes of the Y-scaffold and linker stock solutions were added in 1.5 mL EP tubes and mixed quickly.

Preparation of Enzymatic Responsive DNA Hydrogels: The method of preparation was identical as described above except that two types of linker were prepared here: linker H (containing a BamH I restriction site), and linker R (containing an EcoR I restriction site). For this preparation different buffer solutions for the corresponding enzymes were used: for tests using EcoR I the buffer contained 50 mM of Tris-HCl (pH 7.5), 100 mM of NaCl, 10 mM of MgCl₂, and 1 mM of dithiothreitol, whereas for tests using BamH I the buffer contained 20 mM of Tris-HCl (pH 8.5), 100 mM of KCl, 10 mM of MgCl₂, and 1 mM of dithiothreitol. 5 µL of the Y-scaffold and 5 µL of the linker were added in 1.5 mL EP tubes and mixed quickly, after which the DNA hydrogel formed within one minute.

Gel Electrophoresis: 3% Agarose gel (wt.%) was prepared in 1×TBE (Tris/Boric acid/EDTA) buffer with Gel Red stain (Biotium, CA).

Measurements of Melting Temperature (T_m): Temperature-ramp tests were carried out from 4 °C to 95 °C at a rate of 1 °C min⁻¹ on a Cary 100 UV-vis spectrometer (Varian company) equipped with a temperature controller.

Rheological Tests: Rheological tests were carried out on an AR2000ex rheometer (TA Instruments) equipped with a temperature controller. Three types of rheological experiments were performed: i) frequency-sweep tests were carried out on mixtures between 0.05 and 100 rad s⁻¹ at 25 °C at a fixed strain of 1%; ii) time-scan tests were done at a fixed frequency and strain of 1 Hz and 1%, respectively, at 25 °C for 3 min; iii) temperature-ramp tests were performed at a fixed frequency and strain of 1 Hz and 1%, respectively, and the changes in the shear-storage modulus (G') and shear-loss modulus (G'') were measured from 25 °C to 60 °C at a rate of 2 °C min⁻¹.

Restriction Enzymatic Cleavage of DNA Hydrogels: 10 µL of DNA hydrogel was incubated with 30 U of EcoR I or 30 U of BamH I in 10 µL of the corresponding reaction buffer at room temperature for 48 h.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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- [1] N. C. Seeman, *Angew. Chem.* **1998**, *110*, 3408; *Angew. Chem. Int. Ed.* **1998**, *37*, 3220.
 [2] N. C. Seeman, *Nature* **2003**, *421*, 427.
 [3] E. Winfree, F. Liu, L. A. Wenzler, N. C. Seeman, *Nature* **1998**, *394*, 539.
 [4] H. Yan, S. H. Park, G. Finkelstein, J. H. Reif, T. H. LaBean, *Science* **2003**, *301*, 1882.

- [5] P. W. K. Rothmund, *Nature* **2006**, *440*, 297.
 [6] Y. G. Ke, Y. Liu, J. P. Zhang, H. Yan, *J. Am. Chem. Soc.* **2006**, *128*, 4414.
 [7] Y. He, T. Ye, M. Su, C. Zhang, A. E. Ribbe, W. Jiang, C. D. Mao, *Nature* **2008**, *452*, 198.
 [8] E. S. Andersen, M. Dong, M. M. Nielsen, K. Jahn, R. Subramani, W. Mamdouh, M. M. Golas, B. Sander, H. Stark, C. L. P. Oliveira, *Nature* **2009**, *459*, 73.
 [9] T. Liedl, B. Högberg, J. Tytell, D. E. Ingber, W. M. Shih, *Nat. Nanotechnol.* **2010**, *5*, 520.
 [10] B. Yurke, A. J. Tuberfield, A. P. Mills Jr., F. C. Simmel, J. L. Neunmann, *Nature* **2000**, *406*, 605.
 [11] D. S. Liu, S. Balasubramanian, *Angew. Chem.* **2003**, *115*, 4912; *Angew. Chem. Int. Ed.* **2003**, *42*, 5734.
 [12] H. J. Liu, D. S. Liu, *Chem. Commun.* **2009**, 2625.
 [13] C. Y. Wang, Z. Z. Huang, Y. H. Lin, J. S. Ren, X. G. Qu, *Adv. Mater.* **2010**, *22*, 2792.
 [14] C. A. Mirkin, R. L. Letsinger, R. C. Mucic, J. J. Storhoff, *Nature* **1996**, *382*, 607.
 [15] A. P. Alivisatos, K. P. Johnsson, X. Peng, T. E. Wilson, C. J. Loweth, M. P. J. Bruchez, P. G. Schultz, *Nature* **1996**, *382*, 609.
 [16] A. A. Zinchenko, K. Yoshikawa, D. Baigl, *Adv. Mater.* **2005**, *17*, 2820.
 [17] F. W. Huo, A. K. R. Lytton-Jean, C. A. Mirkin, *Adv. Mater.* **2006**, *18*, 2304.
 [18] T. Amiya, T. Tanaka, *Macromolecules* **1987**, *20*, 1162.
 [19] F. Topuz, O. Okay, *Macromolecules* **2008**, *41*, 8847.
 [20] F. Topuz, O. Okay, *Biomacromolecules* **2009**, *10*, 2652.
 [21] H. W. Tang, X. R. Duan, X. L. Feng, L. B. Liu, S. Wang, Y. L. Li, D. B. Zhu, *Chem. Commun.* **2009**, 641.
 [22] M. C. Morán, M. G. Miguel, B. Lindman, *Soft Matter* **2010**, *6*, 3143.
 [23] D. C. Lin, B. Yurke, N. A. Langrana, *J. Biomech. Eng.* **2004**, *126*, 104.
 [24] Y. Murakami, M. Maeda, *Macromolecules* **2005**, *38*, 1535.
 [25] T. Liedl, H. Dietz, B. Yurke, F. Simmel, *Small* **2007**, *3*, 1688.
 [26] H. Yang, H. Liu, H. Kang, W. Tan, *J. Am. Chem. Soc.* **2008**, *130*, 6320.
 [27] B. Wei, I. Cheng, K. Q. Luo, Y. Mi, *Angew. Chem.* **2008**, *120*, 337; *Angew. Chem. Int. Ed.* **2008**, *47*, 331.
 [28] Z. Zhu, C. C. Wu, H. P. Liu, Y. Zou, X. L. Zhang, H. Z. Kang, C. J. Yang, W. H. Tan, *Angew. Chem.* **2010**, *122*, 1070; *Angew. Chem. Int. Ed.* **2010**, *49*, 1052.
 [29] S. H. Um, J. B. Lee, N. Park, S. Y. Kwon, C. C. Umbach, D. Luo, *Nat. Mater.* **2006**, *5*, 797.
 [30] N. Park, S. H. Um, H. Funabashi, J. F. Xu, D. Luo, *Nat. Mater.* **2009**, *8*, 432.
 [31] E. J. Cheng, Y. Z. Xing, P. Chen, Y. Yang, Y. W. Sun, D. J. Zhou, L. J. Xu, Q. H. Fan, D. S. Liu, *Angew. Chem.* **2009**, *121*, 7796; *Angew. Chem. Int. Ed.* **2009**, *48*, 7660.
 [32] A. S. Hoffman, *Adv. Drug. Deliv. Rev.* **2002**, *43*, 3.
 [33] R. V. Ulijn, N. Bibi, V. Jayawarna, P. D. Thornton, S. J. Todd, R. J. Mart, A. M. Smith, J. E. Gough, *Mater. Today* **2007**, *10*, 40.
 [34] P. Calvert, *Adv. Mater.* **2009**, *21*, 743.
 [35] B. V. Slaughter, S. S. Khurshid, O. Z. Fisher, A. Khademhosseini, N. A. Peppas, *Adv. Mater.* **2009**, *21*, 3307.
 [36] B. Xu, *Langmuir* **2009**, *25*, 8375.
 [37] A. Borzacchiello, L. Ambrosio, in *Hydrogels. Biological Properties and Applications* (Ed: R. Barbucci), Springer, Milan **2009**, pp. 9–20.
 [38] A. Pingoud, J. Alves, R. Geiger, in *Methods of Molecular Biology*, Vol 16. (Ed: M. M. Burrell), Humana Press, Totowa, NJ **1993**, Ch. 8.
 [39] R. J. Roberts, T. Vincze, J. Posfai, D. Macelis, *Nucleic Acids Res.* **2007**, *35*, D269.
 [40] S. Zhang, *Nat. Biotechnol.* **2003**, *21*, 1171.
 [41] E. Ruiz-Hitzky, M. Darder, P. Aranda, K. Ariga, *Adv. Mater.* **2010**, *22*, 323.