DNA Nanostructures

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# A pH-Triggered, Fast-Responding DNA Hydrogel**

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Over the past two decades, DNA has been demonstrated as an excellent building material for nanotechnology, especially in the fabrication of novel functional DNA nanostructures[1–3] and nanomotors.[4] In addition to planar two-dimensional grids[2] and isolated three-dimensional nanostructures,[3] the concept of DNA assembly has recently been expanded to realize unconstrained three-dimensional DNA structures, such as "DNA dendrimers"[5] and "DNA hydrogels".[6] The reported gelling processes, which are based on double-helix formation followed by enzyme-catalyzed ligation, are rather slow and require overnight ligation at room temperature.[6a] Furthermore, the release of the entrapped materials and drugs from the gel is very slow; for example, it takes over 10 days to release entrapped insulin.[6a] This may limit the potential applications of these gels if fast trapping and releasing processes are required. In this study, we report the preparation of a fast, pH-responsive DNA hydrogel with three-armed DNA nanostructures (Y units) assembled together through the formation of intermolecular i-motif structures. This DNA hydrogel can be switched to the non-gel state in one minute by simply using environmental pH changes.

Our strategy is illustrated in Figure 1. A Y-shaped DNA nanostructure (the Y unit) is formed from three single-stranded DNAs (ssDNAs), with each 37-mer ssDNA containing two functional domains: an 11-mer interlocking i-motif domain containing two cytosine-rich stretches (marked in black in Figure 1) and a 26-mer domain for formation of the double-stranded Y shape (marked in red, green, and blue in Figure 1). The 26-mer domains have been carefully designed to have two half-complimentary sequences, so that equal amounts of the three DNA strands will hybridize to each other to produce the Y-unit with the three interlocking domains sticking out. At high pH values, the interlocking (i-motif-formation) domains are in random coils, so the Y units are isolated because of electrostatic repulsion. When the pH value is lowered and becomes slightly acidic, the cytosines in the C-rich domains become partially protonated, which leads to the formation of a C···$CH^+$ triple hydrogen bond between protonated ($CH^+$) and unprotonated cytosines.[7] In our design, the i-motif domains within the same Y unit are linked to the rigid double-stranded central domains and point in different directions, which prevents them from forming intra-Y-unit i motifs. Therefore, only the inter-Y-unit i-motif structures can be formed. Accumulation of this process leads to an extended three-dimensional network in solution, namely the DNA hydrogel.

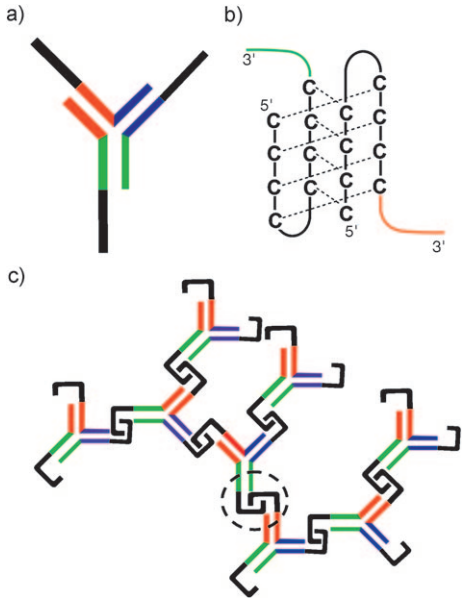


***Figure 1.*** Principle structure of the pH-responsive DNA gel. a) A Y-shaped DNA nanostructure with three free interlocking domains (Y unit); b) enlargement of the circled region in (c) to show the formation of inter-Y-unit i motif: two black domains from neighboring DNA Y units form an i motif to cross-link adjacent Y units; c) DNA hydrogel made from the three-dimensional assembly of DNA Y units. The sequences (with different domains in different colors) of the three DNA strands used in the assembly are: a: 5′-CCCCTAACCCCTG-GATCCGCATGACATTCGCCGTAAG-3′; b: 5′-CCCCTAACCCCCTTACGGC-GAATGACCGAATCAGCCT-3′; c: 5′-CCCCTAACCCCAGGCTGATTCGGTT-CATGCGGATCCA-3′.

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To verify our design, stoichiometric amounts of DNA strands a–c were mixed in 50 mM β-morpholinoethanesulfonic acid (MES) buffer (pH 8.0; with 50 mM NaCl) to give a final concentration of 40 μM for each strand. The mixture was heated to 95 °C for 5 min and then cooled to room temperature over 2 h to form the designed Y unit. The DNA assembly was characterized by 10% native polyacrylamide gel electrophoresis (Acr = acrylamide, Bis = *N*,*N*′-methylenebisacrylamide; Acr/Bis 29:1). As illustrated by Figure 2, all of the DNA strands can assemble with their complementary strands to form the required DNA structural units with the expected gel mobility. The assembled DNA Y unit (a combination of all three DNA strands; Figure 2, lane 7) has the slowest gel-shift mobility, whereas the three partially assembled structures (containing two of the three strands: a + b, b + c, or a + c) have identical gel mobilities that fall between those of the unassembled strands and the fully assembled Y units. A single dominant band is observed in lane 7 in Figure 2, which confirms the efficient assembly of the designed DNA Y unit.

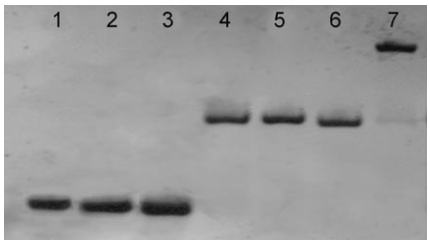


***Figure 2.*** Native polyacrylamide gel electrophoresis (10%, Acr/Bis = 29:1) analysis of the hierarchical self-assembly. Lanes 1–3: DNA strands a–c, respectively; lanes 4–6: partly complementary structures formed by two of the three strands (lane 4: a + b; lane 5: a + c; lane 6: b + c); lane 7: fully assembled DNA Y unit (a + b + c).

Addition of 6 M HCl (0.5 μL) into a 0.75 mM solution of assembled DNA Y units in 50 mM MES buffer (40 μL; pH 8.0; with 50 mM NaCl) will change the pH value of the system to 5.0. This should induce the formation of inter-Y-unit i-motif structures and consequently transform the fluidic solution into a transparent gel. Addition of 6 M NaOH (0.5 μL) to the system should reverse this process because of the deformation of the interlocking inter-Y-unit i motifs. To help visualize the gelling transition, we employed water-soluble citrate-modified 13 nm gold nanoparticles (GNPs) as "tracer agents" (see Figure S6 in the Supporting Information). The GNPs (7 nM concentration) were introduced into the DNA solution before the gelling process was started. Upon formation of the cross-linked DNA gel, the GNPs were trapped within the gel and could not be dispersed into the upper layer of the MES buffer (pH 5.0; 50 mM MES, with 50 mM NaCl; Figure 3a). The resulting GNP-containing gel is very stable; no GNP release was observed, even after incubation for several days without stirring. However, upon addition of NaOH to render the upper solution slightly basic (pH 8), the DNA gel quickly disassembled, such that the trapped GNPs were released and dispersed into the whole solution in one minute (Figure 3b).

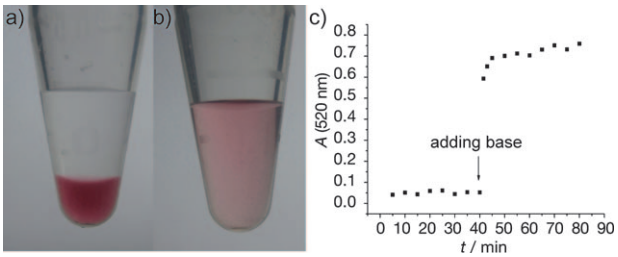


***Figure 3.*** Gel transition switched by pH change (visualized with GNPs. a) 7 nM GNPs were trapped in DNA hydrogel (40 μL) with a covering layer of 50 mM MES buffer (pH 5.0, 50 mM NaCl). b) After the pH value of the buffer had been changed to pH 8.0, the GNPs were released from the DNA hydrogel to form a uniform solution. c) Time trace of the absorption at 520 nm for the upper part of the solution before and after addition of NaOH.

This result demonstrates that 1) the stability of the DNA gel is strongly dependent on the pH value, which suggests that the formation of inter-Y-unit i-motif structures is responsible for the gelling process; 2) the DNA gel can be swiftly switched by environmental pH changes; and 3) the DNA gel may have potential applications as a pH-sensitive drug-delivery system.

Figure S1 in the Supporting Information shows photographs of the transition from a fluid state into a gel state upon the environmental pH value being changed from pH 8.0 to pH 5.0. The transition happens quite quickly, and the gel state is achieved in less than one minute following the pH change. Figure S2 in the Supporting Information shows an optical photograph and an FE-SEM image of the DNA hydrogel prepared at a DNA Y unit concentration of 2.25 mM. This photograph suggests that the hydrogel can be easily molded. The FE-SEM image shows extended fine lamellar structures, which matches our expectation that our DNA building blocks (the DNA Y units) are extended in three directions.

The shear-storage modulus ($G'$) and shear-loss modulus ($G''$) are two important rheological characters to indicate the states of materials. If the $G'$ value is much greater than the $G''$ value, the material behaves like a solid; if the opposite is true, the material behaves like a liquid. The hydrogel lies in between these two states, with comparable $G'$ and $G''$ values. As shown in Figure 4a, at pH 5.0, if the concentration of the DNA Y unit is lower than 0.45 mM, both the $G'$ and $G''$ values are very small and the $G'$ value is smaller than the $G''$ value. This result shows that, under such conditions, the system is more like a liquid, without any obvious gel character. If the concentration of the DNA Y unit is increased to 0.75 mM, both the $G'$ and $G''$ values increase dramatically and the $G'$ value becomes greater than the $G''$ value; the changing trends of the $G'$ and $G''$ values cross at around 0.5 mM DNA Y-unit concentration. This result indicates that the system is a hydrogel under these conditions and the gelling-transition concentration is 0.5 mM DNA Y unit. From Figure 4a, we can also determine that, with an increase in the concentration of the DNA Y unit, the $G'$ value increases from 300 Pa (0.75 mM) to 1100 Pa (2.25 mM), which indicates that the gel becomes stronger. These facts demonstrate that the gel-forming ability is strongly dependent on the concentration of the initial DNA

  

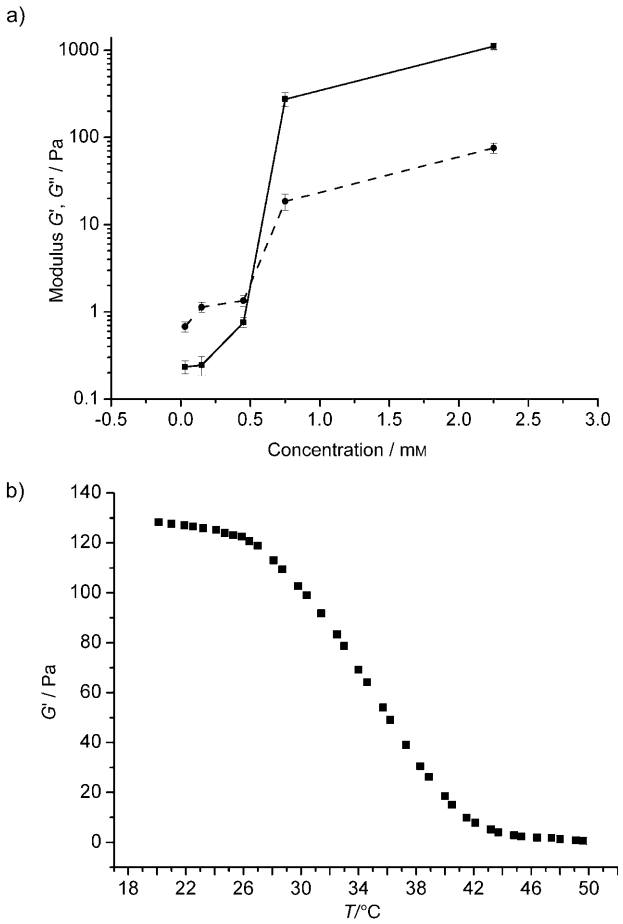


***Figure 4.*** Rheology tests for DNA hydrogels prepared at pH 5.0. a) Storage modulus ($G'$, solid line) and loss modulus ($G''$, dashed line) versus the Y-unit concentration (2.25, 0.75, 0.45, 0.15, and 0.03 mM) at 25 °C. b) Variation of the $G'$ value of the hydrogel as a function of temperature at a fixed Y-unit concentration of 0.60 mM.

Y unit, which supports the idea that the DNA gel is indeed formed by the interlocking of different DNA Y units.

At a fixed DNA Y-unit concentration (0.75 mM), the pH-dependent hydrogel formation has been verified by determination of the rheological properties: as the pH value decreases from 8.0 to 6.0, no change in the value of $G'$ is observed; the $G'$ value is always lower than 0.1 Pa (Figure S3 in the Supporting Information). However, as the pH value decreases to below 6.0, a dramatic increase in the $G'$ value is observed, which suggests that the system is now a hydrogel (see Figure S3 in the Supporting Information). The pH-dependent non-gel-to-gel transition, which happens at a pH value just below 6.0, agrees well with the earlier report that the i-motif structures have a sharp transition at a p$K_a$ value of around 6.3. A stable i motif can only form at pH values below the p$K_a$ value and, hence, our results strongly support the theory that the gelation process is due to the formation of inter-Y-unit i-motif structures. Furthermore, pH-dependent CD spectra of the DNA Y units (2 μM) clearly confirm the formation of the inter-Y-unit i motif at pH 5.0, whereas only duplex structures were observed at pH 8.0 (see Figure S5 in the Supporting Information).

As the DNA hydrogel is based on the interlocking of assembled DNA secondary structures, which are only stable under their melting point, such DNA assemblies should be temperature dependent. As shown in Figure 4b, the strength of the hydrogel decreases with an increase in temperature and the gelling-transition point is about 37 °C at 0.60 mM Y-unit concentration; this value is close to the melting point of the Y-shaped DNA assemblies (see Figure S4 in the Supporting Information). Therefore, the thermal stability of this type of DNA hydrogel should be precisely adjustable through the DNA sequences used in the building of the DNA Y unit; this is a unique advantage of this system, especially for future in vivo applications such as controllable drug delivery, for which it may be possible to exploit local temperature variations between normal tissues and tumor regions as a potential release trigger.

In conclusion, we have demonstrated a novel strategy to prepare pH-responsive hydrogels made entirely of DNAs. These hydrogels are formed by cross-linking a single-type DNA Y-unit building block through the formation of inter-Y-unit i-motif structures. The fast transformation of the i motifs[4e] enables such DNA gels to respond quickly to environmental pH changes to controllably trap and release cargos (such as the GNPs used here) in a pH-dependent manner. In principle, other materials, such as nanoparticles,[8] therapeutic proteins,[9] polymers,[10] and even protein-producing systems,[11] could be incorporated into such DNA-gel systems, to allow the development of functional, responsive biomaterials that have applications in a wide range of disciplines, such as biosensing, tissue engineering, nanomechanical devices, and drug delivery. The fact that this system is pH responsive makes it particularly attractive for targeting pathological conditions in biological samples, such as tumors and sites of infection, because of their relatively high extracellular acidity.[12]

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