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Programmable DNA Hydrogels Assembled from Multi-Domain DNA Strands

Huiling Jiang, [a] Victor Pan, [a] Skanda Vivek,[b] Eric R. Weeks[b] and Yonggang Ke*[a]

Abstract: Hydrogels are important in biological and medical applications, such as drug delivery and tissue engineering. DNA hydrogels have attracted significant attention due to programmability and biocompatibility of the material. We developed a series of low-cost "one-strand" DNA hydrogels self-assembled from single-stranded DNA monomers containing multiple palindromic domains. This new hydrogel design is simple and programmable. Thermal stability, mechanical properties, and loading capacity of the one-strand DNA hydrogels can be readily regulated by simply adjusting the DNA domains.

Introduction

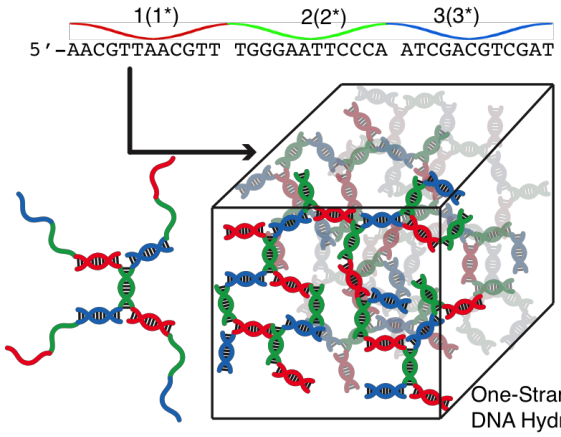
Hydrogels, formed by crosslinking molecules in aqueous solution, have attracted considerable attention as important biomedical materials because of their desired properties such as high water content, porosity, and tissue-like mechanical properties.[1] For example, hydrogels have been applied as three-dimensional carriers of mesenchymal stem cells due to their excellent biocompatibility and capability of retaining large amounts of water.[2] Another important and widely explored application of hydrogels is drug delivery, [3] where drugs can be incorporated into the interspace via chemical attachment or physical entrapment, and subsequently released at various release stimuli depending on the hydrogel properties. Both covalent and non-covalent interactions have been implemented for drug incorporation. Generally, multi-step reactions are necessary for covalent functionalization, which are stable and irreversible.[4] Meanwhile, reversible non-covalent interactions such as hydrogen bonding, ionic interactions and hydrophobic interactions have also been applied to load organic small drug molecules or inorganic nanoparticles into hydrogel systems.[5]

DNA has emerged as a important programmable material for biomaterial engineering.[6] In DNA hydrogels formed by Watson-Crick base pairing, researchers have realized a variety of desirable properties, such as self-healing, mechanical stability, minimal toxicity, and excellent biocompatibility.[7] However, previously reported DNA hydrogels typically utilize multi-strand designs, are relatively expensive, and often require labor-intensive multi-step synthesis.[5, 8]

Here we report a low-cost one-strand DNA hydrogel design. We show this simple system offers excellent programmability — where mechanical properties and cargo loading capacity can be easily tuned by changing the strand sequences and lengths. We expect this new DNA hydrogel system will provide a new enabling platform for hydrogel-based biomedical applications.

Results and Discussion

The formulation of multi-strand DNA hydrogel design typically involves two steps. First structurally well-defined motifs assemble from multiple DNA strands. Then the hydrogel is formed by joining the motifs together via base pairing. Our one-strand (OS) hydrogels use a different design strategy (Scheme 1). A DNA strand is designed to contain multiple domains, indicated by different colors. Each domain contains a self-complementary palindromic sequence. The hydrogel formation is a single-step process, in which individual strands are cross-linked together by the complimentary domains. For instance, a 36-base three-domain OS strand can hybridize with up to three neighboring OS strands. The crosslinking among the individual OS strands leads to the formation of a three-domain OS hydrogel.



Scheme 1. One-strand (OS) multi-domain DNA hydrogel. An OS strand consists of multiple domains, each containing a self-complementary palindromic sequence.

The multi-domain one-strand design is easy to be programmed to possess different numbers of domains and domain lengths, which are expected to affect the pore size and mechanical properties of OS hydrogels (Figure 1). We varied the number of domains from 3 to 4, 5, and 6 for the OS strands, while fixing the domain length (Figure 1A). In

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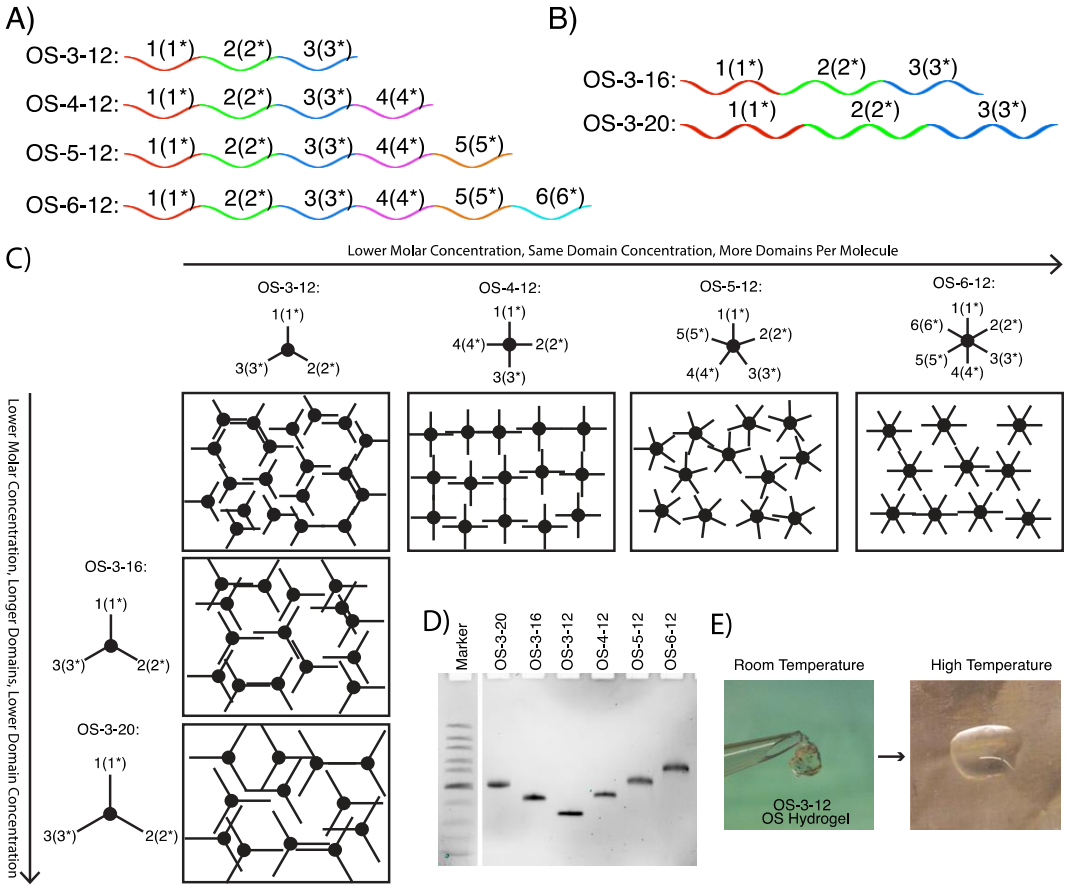

Figure 1. Designs and assembly of the multi-domain one-strand (OS) hydrogels. A) OS strands with different numbers of palindromic domains. Each domain contains 12 bases. B) Three-domain OS strands with different domain lengths (16-base and 20-base). C) Domain changes are expected to affect the physical properties or OS hydrogels. D) 10% Denaturing PAGE analysis of purified OS strands. E) The OS-3-12 DNA hydrogel melts at high temperature (left) reforms at room temperature (right).

addition, we designed and tested three-domain OS strands with different domain lengths of 12-, 16-, and 20-bases (Figure 1B). When OS hydrogels are prepared at same weight concentrations, these changes resulted in different molecular concentrations, crosslink strengths (domain lengths), and number of connections between individual molecules (Figure 1C). These changes in turn are expected to modulate the melting temperatures, mechanical properties, and pore sizes of OS hydrogels.

Characterization of OS hydrogels

The OS hydrogels were prepared using crude DNA strands in 20 mM of Tris-HCl buffer (pH 7.5) containing 10 mM of $MgCl_2$ without purification. To clearly verify the lengths of DNA strands, we purified the crude DNA strands using 10% denaturing polyacrylamide gel electrophoresis and extracted the major product bands. The purified DNA strands were then loaded into another 10% denaturing polyacrylamide gel, which revealed that all DNA strands exhibited expected mobility (Figure 1D). In a typical experiment for OS hydrogel assembly, an OS strand (e.g. OS-3-12) was dissolved in 30-100 µl buffer. At room temperature, the solution quickly formed a transparent gel within minutes (Figure 1E). Raising temperature to above 70 °C breaks the hybridization between DNA domains and changes the gel to liquid formation. Cooling the solution to room temperature again will then bring it back to the gel formation. The liquid/gel switching process can be repeated multiple times without obvious change of the speed of gel formation.

The melting temperatures of OS strand hybridization are directly related to the domain lengths. We measured the melting temperatures of strand hybridization using real-time PCR (Figure S1 and Table S1). The measurements were done using 2 µM concentration OS solutions, which retained the liquid form. Results revealed a correlation between domain length and hybridization temperature between the OS strands. The OS-3-20, which has the longest domain length, exhibited highest melting temperature at 70 °C, followed by OS-3-16 at 66 °C. In comparison, the number of domains showed little effect on melting temperature. The OS-Y-12 (Y = 3, 4, 5 and 6) showed melting temperatures between 61 °C to 58 °C.

Rheology measurements were performed at 25 oC to study how the physical properties of OS DNA hydrogels were affected by the domain lengths and number of domains per strand (Figure 2). First, we screened the storage modulus (G') and loss storage (G'') of the DNA hydrogels with wt.% concentrations varying from 0.2 wt.% to 3.0 wt.% (Figure 2A). As expected, both G' and G'' increased with the rising total DNA wt.%. The G' was larger than G'' when the OS-3-12

concentration was higher than 0.5 wt.%, indicating that the OS-3-12 assembly above 0.5 wt.% results in materials with more gel-like properties. The G' of a 2 wt.% OS-3-12 hydrogel was around a few thousand Pa, slightly higher but comparable to previously reported 2 wt.% multi-strand Y-motif /Linker DNA hydrogel by Liu et al.[8a]

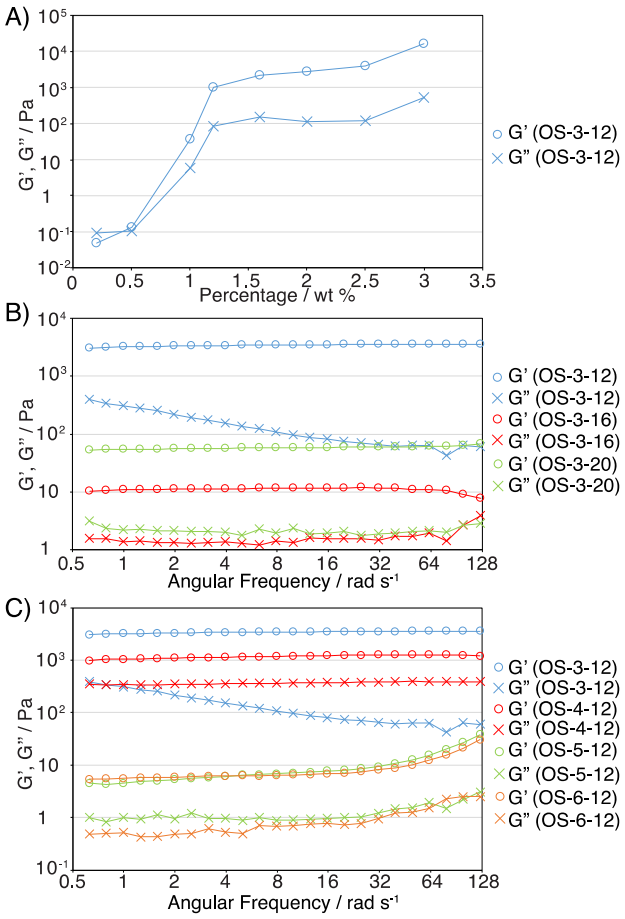

Figure 2. Rheological characterization of OS DNA hydrogels. A) Rheological measurement of the OS-3-12 DNA hydrogel with different weight percentages at a fixed 1 Hz frequency and a fixed 1% strain at 25 °C. B) Rheological angular frequency sweeps of 2 wt.% OS hydrogels with different domain lengths. D) Rheological angular frequency sweeps of 2 wt.% OS hydrogles with different numbers of domains. The angular frequency sweeps were performed between at 1% strain at 25 °C.

At the same weight concentration, OS DNA hydrogels' physical properties are affected by both the lengths of domains and the number of domains. We tested 12-base, 16-base, and 20-base three-domain OS DNA hydrogels at 2 wt % (Figure 2B). The results revealed that the rigidity of OS DNA hydrogels is inversely proportional to the domain lengths. Among the three samples, the OS-3-12 DNA hydrogel showed strongest elastic behavior while the OS-3-20 DNA hydrogel appeared softest. For three-domain OS DNA hydrogels at equal weight percentage, a longer-domain OS DNA hydrogel has lower molar concentration, which is expected to lead to lower levels of crosslinking among the DNA strand units. As a result, the longer domain and the fewer crosslinks may be responsible for the softer DNA hydrogel formation.

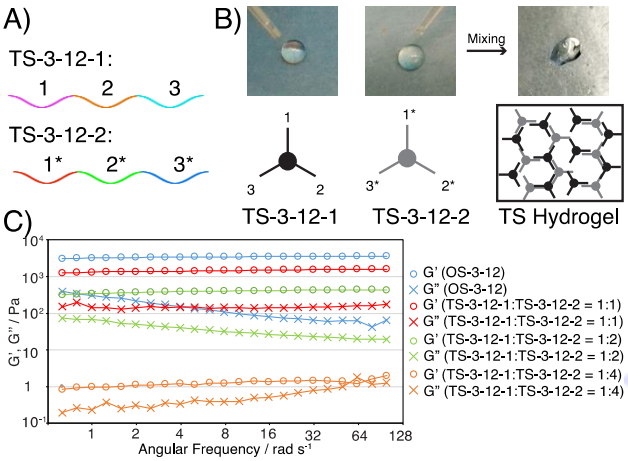

Figure 3. Two-strand (TS) hydrogels. A) The TS-3-12-1 strand contains three 12-base domains complementary to the three 12-base domains on the TS-3-12-2 strand. B) Solutions of the TS-3-12-1 and TS-3-12-2 (left) only forms hydrogel when the two solutions were mixed together at room temperature (right). C) Rheological angular frequency sweeps of 2 wt.% OS-3-12 and 2 wt.% TS-3-12 DNA hydrogels. The angular frequency sweeps were performed between at 1% strain at 25 °C.

The numbers of domains of OS strands also affects the DNA hydrogel properties. We performed rheology measurements on OS-3-12, OS-4-12, OS-5-12, and OS-6-12 DNA hydrogels at 2 wt.% (Figure 2C). These hydrogels all utilize the 12-base domain design, but with differing numbers of domains. The experimental results showed that the OS DNA hydrogels with more domains exhibited lower rigidity. At the same weight concentration, the OS-3-12, OS-4-12, OS-5-12, and OS-6-12 DNA hydrogel should all have the same concentration of 12-base domains. However, the molar concentrations of these DNA hydrogels are inversely proportional to the number of domains of the OS strands. The relatively lower molar concentration of longer OS strand may cause lower crosslinking efficiency, resulting in reduced hydrogel rigidity.

Two-strand multi-domain hydrogels

The OS hydrogels can be easily modified to two-strand designs, which may be more desirable in certain applications (Figure 3). As an example, we demonstrated a three-domain, two-strand (TS) design by using non-palindromic sequences (Figure 3A). The design includes two DNA strands: a TS-3-12-1 strand with three 12-base domains, each complementary to a 12-base domain on a TS-3-12-2 strand. Different to OS DNA hydrogel, a 2 wt.% solution containing only one TS strand (TS-3-12-1 or TS-3-12-2) retained its liquid formation at room temperature (Figure 3B; Left). On the other hand, transparent DNA hydrogel formed within minutes when equal amount of 2 wt.% TS-3-12-1 solution and 2 wt.% TS-3-12-2 solution were mixed together at room temperature (Figure 3B: Right). Therefore, the TS hydrogel system offers

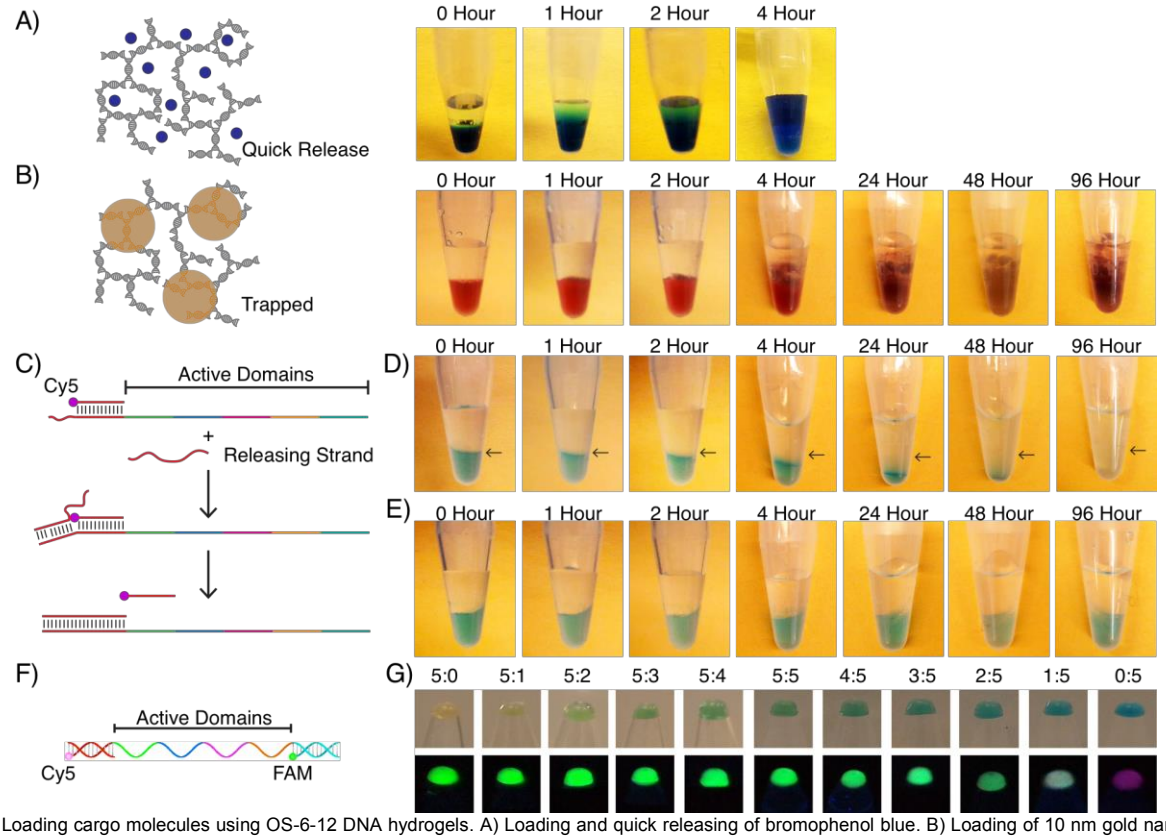

Figure 4. Loading cargo molecules using OS-6-12 DNA hydrogels. A) Loading and quick releasing of bromophenol blue. B) Loading of 10 nm gold nanoparticles in OS-6-12 hydrogel. The particles were trapped and cannot be released after 96-hour incubation in buffer. C) Design of loading and releasing of a Cy5-DNA. The releasing was achieved by using a strand displacement reaction. D) The Cy5-DNA was fully released after 96 hours, while the hydrogel retained its original shape. Black Arrows indicate the top edge of the hydrogel. E) No Cy5-DNA release in the absence of the releasing DNA strand. F) Two domains of the OS-6-12 was modified to bind to the Cy5-DNA strands and an FAM-DNA, respectively. G) White-light images and UV images of FOS-6-12 DNA hydrogels with the two fluorescence dyes at different loading ratio from 5:0 to 0:5.

easy handling at room temperature while pocessing same programmability as the OS hydrogel system. The melting temperature of 1μM TS-3-12-1 and 1μM TS-3-12-2 hybridization is 58 °C, slightly lower that 2 μM OS-3-12 strand. This is likely due to non-perfect stoichiometry between the two TS strands.

We compared 2 wt.% TS-3-12 hydrogel with 2 wt.% OS-3-12 hydrogel (Figure 3C). At 25 °C, the G's of OS and TS gels are both higher than their respective G''s at the entire swap range, a typical mechanical property for hydrogels. However, the TS-3-12 DNA hydrogel showed lower G' and G'' of OS-3-12. These results suggest that the OS DNA in comparison to OS-3-12 DNA hydrogel. In addition, the gap between G' and G'' of TS-3-12 is narrower than the G'/G'' gap hydrogels exhibit more rigidity than TS DNA hydrogels of the same concentration. Lower rigidity of TS DNA hydrogels may be partially due to the imperfect stoichiometry between the two TS DNA strands. We then tested mechanical properties of 2 wt.% TS hydrogels with different ratios between TS-3-12-1 and TS-3-12-2 (Figure 3C). When TS-3-12-1 to TS-3-12-2 ratio was changed from 1:1 to 1:2 and 1:4, we observed significantly reduced stiffness, especially at 1:4 ratio, likely due relatively larger numbers of unhybridized single-stranded domains. At the 1:4 ratio, the G' of TS-3-12 gel dropped to the low value of ~1 Pa. This result is consistent with the study on Y-motif /Linker hydrogel [8a]. Liu et al showed that at ratios of 2:1 and 1:3, the Y-motif /Linker gel had low G' around 0.1 Pa, likely also due to the large amount of unbounded motifs in excess.

Cargo loading and releasing

The multi-domain, one-strand DNA hydrogels provide a simple system for carrying single-component or multi-component cargos. We demonstrated cargo loading and release using OS-6-12 hydrogel (Figure 4). First, we demonstrated quick release of small-molecule cargos. Bromophenol blue was loaded to 30 μl 2 wt.% hydrogel and then the hydrogel was immersed into 60 μl buffer (Figure 1A). Because bromophenol blue is much smaller then the expected pore size of the hydrogel, the bromophenol blue quickly diffused out to the buffer while DNA hydrogel volume remained unchanged within 4 hours. Unlike small-size molecules, large carges can be trapped inside of the DNA hydrogel for a long period of time. We prepared 50 μl 2 wt.% DNA hydrogel with 10 nm gold nanoparticles and then added 100 μl buffer on top of the red-colored hydrogel (Figure 1B). Over a period of 96 hours, no obvious release of gold nanoparticles was observed. It is worth noting that the hydrogel started to expand and broke apart after 4 hours. This is likely due to the solution exchange between the buffer and hydrogel loaded with gold nanoparticles.

We then demonstrated the domains of the OS-6-12 strand can be modified for specific binding and releasing of DNA strands (Figures 4C and 4D). Unlike physically trapping

cargos during DNA hydrogel formation, the hybridization-loaded DNA cargos can be engineered to be released in response to specific stimuli, such as complementary DNA strands. The modified strand is named (Cy5)-OS-6-12. The Cy5-DNA binds to (Cy5)-OS-6-12 during hydrogel formation (Figure 4C). The other five active palindromic domains crosslink the (Cy5)-OS-6-12 to form hydrogel. During releasing step, DNA strand (Cy5)-Release complementary to both the poly-T segment and the first domain was added to replace the Cy5-DNA. We added the Cy5-DNA strand with the (Cy5)-OS-6-12 at a 1:7 molar ratio to form a DNA hydrogel and studied the Cy5-DNA release over 96 hours (Figure 4D). The 30 µl 2 wt.% hydrogel submerged in 100 µl buffer retained its volume and shape during the study. Excessive amount of (Cy5)-Release strand was added at the 0 hour. A gradual release of Cy5-DNA was observed and completed after 96 hours. In contrast, no release was observed in the absence of (Cy5)-Release strand at the same conditions (Figure 4E).

The OS-6-12 strand was modified to further demonstrate loading of multiple DNA strands with controlled stoichiometry (Figures 4F and 4G). In the modified strand design, the first and the last domains of the OS-6-12 were changed to non-palindromic sequences to hybridize with the Cy5-DNA and a FAM-labeled DNA (FAM-DNA), respectively (Figure 4F). The modified strand ((Cy5/FAM)-OS-6-12) has four remaining palindromic domains available for crosslinking. We mixed the two fluorophore-labeled DNA strands with the OS-6-12 at a 1:7 ([Cy5-DNA+FAM-DNA]:[(Cy5/FAM)-OS-6-12]) molar ratio to form a two-fluorophore-loaded DNA hydrogel. The ratio between the FAM-DNA and Cy5-DNA strands was varied from 5:0 to 0:5, resulting in a series of DNA hydrogels with different color (Figure 4G).

Conclusions

In summary, we have demonstrated a group of low-cost, programmable DNA hydrogels using a simple one-strand multi-palindromic-domain design. By modifying the domain length and the number of domains, we programmed the one-strand DNA hydrogels to exhibit variable chemical and physical characteristics, such as melting temperature and rheological properties. In addition, the domains of a one-strand DNA hydrogel can be easily modified for loading molecular cargos, while the unmodified palindromic domains are still capable of crosslinking with each other to form the hydrogel. We also showed that the multi-domain design could be implemented for making multi-strands non- palindromic-sequence DNA hydrogels. By applying our new design strategy to longer DNA strands and/or more DNA strands, we believe increasingly complex and programmable DNA hydrogels can be readily constructed. We expect these new DNA hydrogels will provide a low-cost, highly programmable platform for many potential biological and biomedical applications.

Experimental Section

Materials:

All oligonucleotides were either synthesized on an Expedite 8900 Nucleic Acid Synthesis system, or purchased from IDT. The synthesis and de-protection processes were carried out according to the instructions provided by the reagent manufactures. Subsequently, the deprotected DNA was precipitated by adding 1/10 volume of 3M NaOAc (pH = 5.2) and 3X volume of cold ethanol. After placing in a freezer at -20 ^{o}C for 30 min, the DNA products were collected by centrifugation at 14,000 rpm for 30 min. DNA products were further purified by 10% denaturing PAGE. Two fluorophore-labelled DNA were purchased from IDT. 10 nm gold nanoparticle was purchased from BBI Solutions. All chemicals were of reagent grade or better, and were used as received.

DNA Sequences.
OS-3-12:
AACGTTAACGTTTGGGAATTCCCAATCGACGTCGAT
OS-4-12:
AACGTTAACGTTTGGGAATTCCCAATCGACGTCGATCGTGGATC
CACG
OS-5-12:
AACGTTAACGTTTGGGAATTCCCAATCGACGTCGATCGTGGATC
CACGGAGGCGCGCCTC
OS-6-12:
AACGTTAACGTTTGGGAATTCCCAATCGACGTCGATCGTGGATC
CACGGAGGCGCGCCTCAGCGGTACCGCT
OS-3-16:
ACAACGTTAACGTTGTAGTGGGAATTCCCACTTAATCGACGTCGA
TTA
OS-3-20:
ATACAACGTTAACGTTGTATTTAGTGGGAATTCCCACTAAAATAAT
CGACGTCGATTATT
TS-3-12-1:
TATCAGATTCGATGCAAGTGAAATGTCGGTAACCAG
TS-3-12-2:
TCGAATCTGATAATTTCACTTGCACTGGTTACCGAC
(Cy5/FAM)-OS-6-12:
TGTGTGTGTGTGTTGGGAATTCCCAATCGACGTCGATCGTGGAT
CCACGGAGGCGCGCCTCTCCTTCCTTCCTT
(Cy5)-OS-6-12:
TTTTTTTTTGTGTGTGTGTGTTGGGAATTCCCAATCGACGTCGAT
CGTGGATCCACGGAGGCGCGCCTCAGCGGTACCGCT
(Cy5)-Release:
ACACACACACACAAAAAAAAA
Cy5-DNA:
ACACACACACACA-Cy5
FAM-DNA:
AAGGAAGGAAGGA-FAM

DNA Hydrogel Preparation:

OS DNA hydrogel: DNA strands were dissolved with a buffer solution containing 20 mM Tris-HCl buffer (pH 7.5) and 10 mM $MgCl_2$ to obtain a final desired concentration, followed by heating to 95 ^{o}C for 5 min and cooling at room temperature for 2 h to form the final one-strand DNA hydrogels.

OS3-12 DNA was observed to dissolve into clear aqueous solution after less than 1 min of heating at about 55-60 ^{o}C. Following removal from heat, the gel reformed after cooling to room temperature. The liquid-gel switching process can be repeated multiple times without obvious change of gel formation speed.

TS DNA hydrogel: TS-3-12-1 and TS-3-12-2 were dissolved with a buffer solution containing 20 mM Tris-HCl buffer (pH 7.5) and 10 mM $MgCl_2$ to obtain a final desired concentration. The two solution then mixed together at 1:1 ratio and then subjected to a 2-hour annealing process (from 95 ^{o}C to room temperature) to form the two-strand DNA hydrogels.

Measurements of Melting Temperature:

Melting points were measured on a Step One Plus real-time PCR system (Applied Biosystem, CA). All DNA strands were dissolved with Tris-HCl buffer (20 mM, pH 7.5) containing 10 mM of $MgCl_2$ to reach final concentration 2 μM. SYBR Green was added as the fluorescence source. All samples were heated to 95 °C for 30 seconds, followed by cooling to 25 °C. Temperature was then increased to 85 °C with a speed of 0.3 °C /step.

Rheology Measurement:

Rheological tests were carried out on an AR2000ex rheometer equipped with a temperature controller. Frequency sweep tests were carried out on mixtures between 0.64 and 64 rad/s at 25 °C at a fixed strain of 1%. The rheological experiments were performed on 25mm parallel plates using 20 μl of hydrogels (resulting in a gap size of 0.05 mm) angular frequency sweep (0.64-64 rad/s) was carried out with a fixed strain sweep of 1% at 25 °C.

Cargo Loading and Releasing:

Bromophenol-blue- and gold-nanoparticle-loaded hydrogels: 1 μl 1% bromophenol blue was added to 30 μl of 2 wt.% OS-6-12 hydrogel, heated to 95 °C, mixed with vortex mixer, then allowed to cool to room temperature. 10 nm gold-nanoparticle solution was concentrated to 400 nM via centrifugation before addition into the DNA hydrogels. 2 μl concentrated 10nm gold-nanoparticle (400 nM) was added to 30 μl of 2 wt.% OS-6-12 hydrogel, heated to 95 °C, mixed with vortex mixer, then allowed to cool to room temperature.

Cy5-DNA hydrogel and release: 100 μM Cy5-DNA solution containing 20 mM of Tris-HCl buffer (pH 7.5) and 10 mM of $MgCl_2$ were added to 30 μl 2 wt.% (Cy5)-OS-6-12 hydrogel, followed by heating to 95°C for 5 min and cooling at room temperature for 2 h to form the DNA hydrogels. The final ratio of (Cy5)-OS-6-12:Cy5-DNA was around 7:1. 100 μl of Tris buffer containing 300 μM Release DNA was added for the strand displacement study.

Cy5 and FAM DNA hydrogel: Variable amount of 100 μM FAM-DNA and 100 μM Cy5-DNA solution containing 20 mM of Tris-HCl buffer (pH 7.5) and 10 mM of $MgCl_2$ were added to 10 μl 2 wt.% (Cy5)-OS-6-12 or (Cy5/FAM)-OS-6-12 hydrogel, followed by heating to 95°C for 5 min and cooling at room temperature for 2 h to form the final DNA hydrogels. The final ratio of (Cy5/FAM)-OS-6-12:(FAM-DNA+Cy5-DNA) was 7:1.

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Keywords: DNA nanotechnology, hydrogel, programmable nanomaterials

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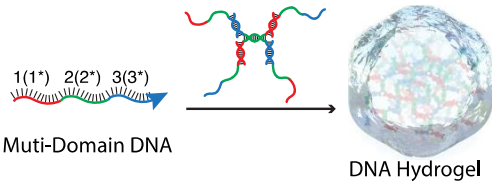

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Title

One-strand DNA hydrogels: a new family of simple DNA hydrogels assembled from one-strand multi-domain DNA. Altering the total numbers of domains and the lengths and sequences of individual domains provides a facile way for modulating one-strand hydrogel's physical properties, loading capacity, and releasing mechanism.