DNA Nanostructures

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Sequence Symmetry as a Tool for Designing DNA Nanostructures**

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DNA sequence symmetry brings at least three advantages to any given DNA structure: 1) minimization of the required sequence space and simplification of sequence design; 2) reduction of the number of different DNA strands; and 3) cancellation of some unpredictable distortions in DNA nanostructures, thus allowing DNA two-dimensional (2D) arrays to grow to large sizes. Herein, DNA sequence symmetry is introduced into the design of DNA nanostructures, to give DNA 2D arrays that reach a width of 1 mm.

DNA is an excellent system for studying self-assembly and constructing nanoscale patterns[1,2] because of its extraordinary capability of molecular recognition, well-predictable double-helix structures, huge diversity of unique sequences, and various preexisting toolkits. It is generally believed that DNA sequence symmetry should be eliminated when designing a DNA motif. Each DNA segment has to be unique and can only base pair with its sole, complementary segment, which promotes the formation of designed structures. This rule has been established for 20 years[3] and strictly followed in the field of DNA nanotechnology.[1,4,5] The success of DNA nanotechnology proves the effectiveness of this rule. As DNA nanotechnology has matured, more knowledge and experience have accumulated. We would like to ask: can sequence symmetry provide any advantage? Herein, we will address this question and show a successful application of sequence symmetry.

We studied sequence symmetry with a cross motif (Figure 1)[4d] in which nine different strands (**1**–**9**) associate with each other and fold into a nearly planar structure (Figure 1a). There is a pseudo-fourfold rotational symmetry of the DNA backbones, but no symmetry when taking the bases into account. If a true fourfold symmetry exists, strands **2**–**5** will be related to each other by the symmetry and be identical, strands **6**–**9** will be identical, and strand **1** will become a circular repetitive tetramer. The symmetric cross structure still consists of nine strands, but only three different sequences (Figure 1b). The unique DNA sequences are reduced by four times as all four arms of the corss become identical to each other. Note that sequence symmetry is still avoided when designing each individual DNA strand. Symmetry is only applied to the construction of the cross motif out of the three different strands.

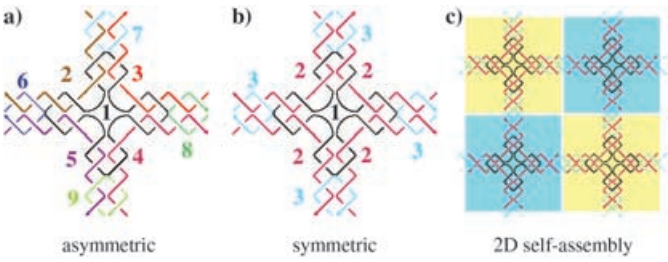

Figure 1. Introduction of sequence symmetry. a) An asymmetric cross motif consisting of nine unique strands. The arrows indicate the 3′ ends of the DNA strands b) A symmetric cross motif containing only three unique strands. c) Corrugated self-assembly in two dimensions. Any two neighboring units face in opposite directions; yellow and turquoise squares indicate units facing up and down, respectively. The dimensions of a unit cell of the 2D array are 4.5×4.5 turns.

Sequence symmetry substantially influences the self-assembly behavior of the cross motif. The cross structures with sticky ends can be programmed to self-assemble into 2D arrays. Some unpredictable curvatures are associated with the cross motif, because of our limited understanding of branched DNA structures and oversimplification by the DNA duplex model. If the curvatures accumulate, small aggregates will be generated instead of large 2D arrays. A corrugation strategy has been used[4d] to prevent curvature from accumulating during 2D assembly. Adjacent building blocks face up and down alternately in each growing direction, such that the curvatures cancel each other out (Figure 1c). This strategy has been proved to be effective.

However, each arm of the asymmetric cross motif has a different base composition and a curvature of different direction and extent. The corrugation strategy is not enough to cancel all curvatures. To further remove any curvature and promote growth of planar 2D arrays, new strategies are needed. The introduction of sequence symmetry is promising for this purpose. If a true fourfold symmetry exists, the four arms are symmetrically related and have exactly the same curvatures no matter what they are. When blocks of the symmetric cross assemble in two dimensions, all curvatures are cancelled, and large 2D arrays would be expected. In previous reports, DNA 2D arrays often showed anisotropic shapes,[4] presumably resulting from unequal cohesion strengths between DNA blocks in different directions or from curvature accumulation, or both. Sequence symmetry will remove this problem. The block containing the symmetric cross has exactly the same pair of sticky ends in each direction. Thus, the cohesion strength between the blocks in any direction is exactly the same, which allows equal growth of the 2D arrays in each direction.

The strategy of sequence symmetry has been experimentally proved to be very successful. We followed previously reported experimental protocols (see the Supporting Information).[4] First, the symmetric cross motif was characterized

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by native polyacrylamide gel electrophoresis (PAGE). The symmetric cross complex migrated as a single sharp band with an expected mobility in the gel (Figure 2, the second lane from the right), which indicates that the cross complex is

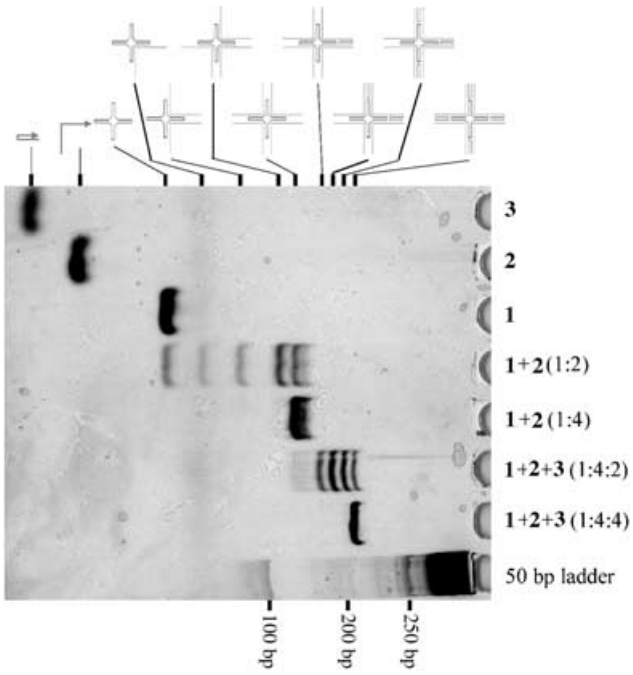

Figure 2. Native PAGE (6%) analysis of the symmetric cross motif. The DNA content of each lane is indicated at the top of the gel image and the identity of each band is indicated on the left. The molar ratios of the strands are given in parentheses. The lane on the far right (50 bp ladder) contains a series of DNA duplex size markers.

stable. Then we modified strand **3** to get complementary sticky ends, and used atomic force microscopy (AFM) to examine whether the cross motif could self-assemble into 2D arrays (Figure 1c and Figure 3). In previous reports[4] and in our own studies,[6] DNA 2D arrays with asymmetric motifs were normally less than 1 μm wide. In sharp contrast, DNA 2D arrays assembled from the symmetric motif are normally in a size range of 20 to 40 μm. The arrays are well-ordered, as judged by direct observation of the images and by Fourier-transform analysis. The observed repeating distance is (18.9 ± 0.3) nm, in good agreement with the value calculated from the

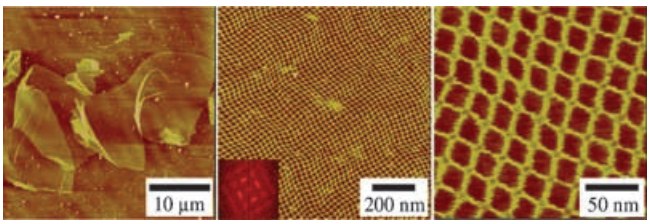

Figure 3. AFM analysis of DNA 2D arrays self-assembled from a symmetric cross motif. Image scanning areas decrease from left to right, thus showing the entire 2D array and the detailed structure. A Fourier-transform pattern (inset, center) shows the regularity of the DNA array.

model ($(46 \times 0.33) + (2 \times 2) = 19.2$ nm) by assuming that the pitch of the DNA duplex is 0.33 nm per base pair and the diameter of a DNA duplex is 2 nm.

The DNA arrays were often larger than the AFM imaging fields. AFM imaging is well-suited to the study of nanoscale structures. Its drawbacks are that the imaging area is small (practically limited to less than 50 μm for our DNA samples), and that the imaging process is very slow, which prevented us from surveying the overall situation of the DNA samples. To overcome these limitations, we used fluorescence microscopy, which allows the imaging of arrays with areas up to the millimeter scale. This technique provides complementary information to that from the AFM images.

Figure 4 shows fluorescence images of DNA arrays stained with YOYO-1 immediately before imaging. Sample preparation for fluorescence imaging is much simpler than for

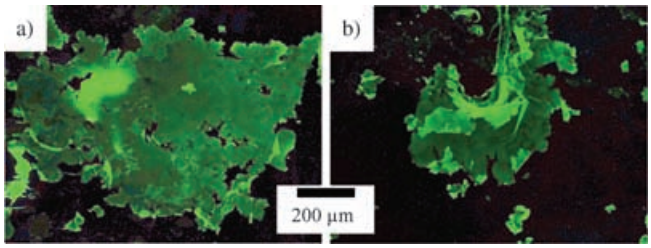

Figure 4. a) and b) Fluorescence images (taken from different areas within the same sample) of DNA 2D arrays self-assembled from the symmetric cross motif with a) the largest DNA array found with width 1 mm.

AFM imaging and avoids destroying the DNA arrays. DNA arrays can be easily found under a fluorescence microscope. The overall morphologies of the arrays are very similar to each other when observed by both fluorescence and AFM imaging. The largest array that we have found reaches a size of 1 mm width (Figure 4a), which is hundreds of times larger than previously reported DNA 2D arrays self-assembled from asymmetric motifs.[4]

The DNA arrays can be used as templates to fabricate nanostructures of other materials. We have previously reported a strategy to transfer DNA nanostructures to gold structures by vapor deposition of gold against flat substrate-supported DNA structures.[6] One major concern of this strategy is that the areas of the gold structures are too small to be useful for technological applications. With the new DNA arrays developed here, we can easily address this concern. The gold structures extend up to 40 μm^2 (Figure 5).

In conclusion, we have demonstrated that sequence symmetry is a powerful tool for designing DNA nanostructures. We are currently testing the symmetry strategy in various DNA nanostructures and hope to determine how general sequence symmetry can be applied. Sequence symmetry dramatically reduces the size of unique sequences and decreases the number of DNA strands. This reduction is important for the fabrication of complicated structures because they need large pools of unique sequences, which, in turn, dramatically increase the difficulty of sequence design. We believe that sequence symmetry will be appreci-

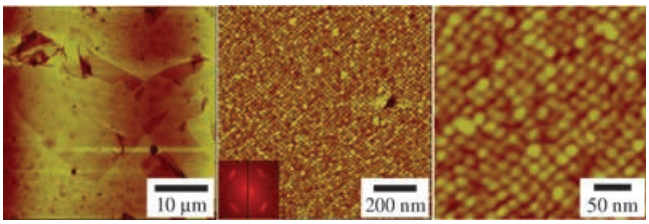

Figure 5. AFM imaging analysis of gold nanostructures replicated from DNA 2D arrays. Scanning areas decrease from left to right, thus showing the entire domain and the detailed structure. A Fourier-transform pattern (inset, center) shows the regularity of the metal structure.

ated as a new tool for the design in these complicated cases. Sequence symmetry is also a useful tool for fine-tuning the structures of DNA motifs, as in the example herein. It is a common concern for molecular self-assembly as to whether self-assembled 2D arrays can be grown to sizes large enough to satisfy the requirements of many technological applications. Herein, we have clearly demonstrated that large 2D arrays are feasible.

Experimental Section

Oligonucleotides: DNA sequences were designed by the computer program SEQUIN.[3b] All oligonucleotides were purchased from IDT, Inc. and purified by 20% denaturing PAGE. Strand **1**: 5′-AggCAC-CATCgTAggTTTTCTTgCCAggCACCATCg-TAggTTTTCTTgCCAggCACCATCgTAggTTTTCTTgCCAgg-CACCATCgTAggTTTTCTTgCC-3′ (circularized); strand **2**: 5′-ACTATgCAACCTgCCTggCAAgCCTACgATggACACgg-TAACg-3′; strand **3**: 5′-CgTTACCgTgTggTTgCATAgT-3′; strand **3′**: 5′-CgCgCgTTACCgTgTggTTgCATAgTCATg-3′.

Formation of DNA complexes and 2D arrays: Strands **1** (0.6 μM), **2** (2.4 μM), and **3** or **3′** (2.4 μM) were combined in Tris/acetate/EDTA/Mg^{2+} (TAE-Mg^{2+}) buffer, which consisted of Tris (40 mM, pH 8.0), acetic acid (20 mM), EDTA (2 mM), and $Mg(CH_3COO)_2$ (12.5 mM). Individual DNA complexes for native PAGE analysis were formed by cooling solutions of the mixture as follows: 95 K/1 min, 65 K/30 min, 50 K/30 min, 37 K/30 min, and 22 K/30 min. DNA 2D arrays were formed by slowly cooling the DNA solution from 95 °C to room temperature over 48 h. For individual cross molecules, we used strands **1**, **2**, and **3**; for 2D arrays, we used strands **1**, **2**, and **3′**.

Denaturing PAGE: Gels contained 20% polyacrylamide (19:1 acrylamide/bisacrylamide) and 8.3 M urea. The running buffer was Tris/borate/EDTA (TBE), which consisted of Tris buffer (89 mM, pH 8.0), boric acid (89 mM), and EDTA (2 mM). Electrophoresis runs were performed on a Hoefer SE 600 unit at 55 °C and 600 V (constant voltage). After electrophoresis, the gels were stained with Stains-All dye (Sigma) and scanned.

Native PAGE: Gels contained 6% polyacrylamide (19:1 acrylamide/bisacrylamide), and electrophoresis runs were performed on an FB-VE10-1 electrophoresis unit (FisherBiotech) at 4 °C and 100 V (constant voltage). The running buffer was TAE-Mg^{2+}. After electrophoresis, the gels were stained with Stains-All dye (Sigma) and scanned.

Replication of DNA patterns: All the metal evaporations were carried out with a thermal evaporator (Turbo Vacuum Evaporator EFFA). The evaporation speed was adjusted to 0.2 nm s^{-1}. After metal evaporation (to a thickness of about 20 nm), a drop of premixed epoxy adhesive was placed on the gold film and immediately covered by a glass slide. The epoxy was allowed to solidify completely (normally left overnight), and the metal replicas were mechanically lifted off the mica surfaces.

AFM imaging: A drop of DNA sample solution (2 μL) was spotted onto a freshly cleaved mica surface and left there for 10 s to allow for strong adsorption. The sample drop was then washed off with $Mg(CH_3COO)_2$ solution (10 mM, 30 μL) and the surface was dried by compressed air. DNA samples and their metal replicas were imaged by tapping-mode AFM on a Nanoscope IIIa microscope (Digital Instruments) with NSC15 tips (silicon cantilever, Mikro-Masch). The tip–surface interaction was minimized by optimizing the scan set-point.

Fluorescence microscopy imaging: A drop of DNA sample solution (3 μL, in TAE-Mg^{2+} buffer) was spotted onto a clean glass slide and left for 5 min to allow DNA arrays to adsorb onto the glass surface. Then a YOYO-1 (Molecular Probes) solution (1.7 μM in TAE-Mg^{2+} buffer, 1 μL) was spotted onto the DNA sample, which was immediately protected with a coverslip and imaged with a fluorescence microscope.

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