Communication

Pseudohexagonal 2D DNA Crystals from Double Crossover Cohesion

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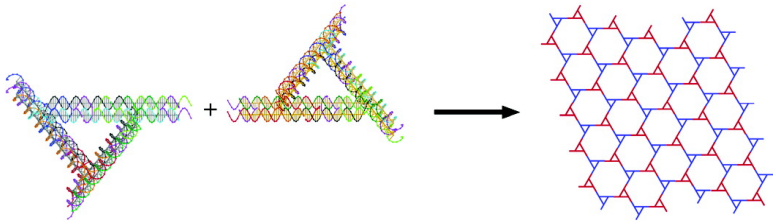

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Pseudohexagonal 2D DNA Crystals from Double Crossover Cohesion

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The control of the structure of matter on the finest possible scale requires the successful design of both stiff intramolecular motifs and robust intermolecular interactions. Previous motifs used to design 2D crystalline arrays have included the double crossover (DX),[1,2] the triple crossover (TX),[3] the DNA parallelogram,[4] and the four-by-four structure.[5] These motifs have been used to produce 2D crystalline arrays lacking symmetry or with twofold symmetry.[6] By contrast, all previous attempts to produce trigonal or hexagonal arrays have met with failure. Given the inherent rigidity of triangles and the importance of trigonal motifs in nature,[7] it is key to solve this problem. The flexibility of three-arm junctions was discovered in the first attempt to assemble a hexagonal lattice.[8] Triangles built from bulged three-arm junctions[9] demonstrated cyclic closure with trimers and above, not just from the hexamers one would have expected.[10] Triangles whose edges were flanked by coplanar helices derived from DX molecules behaved in a similar fashion.[11]

We have overcome these problems by the development of a new motif, the DX triangle. This motif is derived by combining the DX motif (Figure 1a) with the bulged triangle motif (Figure 1b). The resulting motif is illustrated in Figure 1c. The DX molecule has been shown to be about twice as stiff as conventional linear duplex DNA.[12,13] Thus, one might expect that this doubly thick triangle would be more rigid than the simple bulged junction triangle. In addition, the DX triangle is capable of a double intermolecular interaction that may be more robust than the single helical interactions used previously, because it is less sensitive to errors in twist. Here, we report the self-assembly of a trigonal array from this motif. We demonstrate that improving the intermolecular contacts is the key feature of the DX triangle motif that enables formation of trigonal arrays.

Two triangles were designed to produce a pseudohexagonal trigonal lattice arrangement when combined. The edges of the triangles contain 65 nucleotide pairs in each of their DX helices, and they terminate in 5′ sticky ends six nucleotides in length. There are four turns per edge within each triangle. The sequences of the triangles are contained in the Supporting Information. Only the sticky ends differ between the two triangles. The triangles are designed to cohere with each other to produce a continuous DX structure 13 double helical turns (estimated ∼46 nm) in length. Figure 1d illustrates a group of six triangles, three of each species, flanking a hexagon. The edge of the hexagon, lacking one triangle, is 9 turns (∼30 nm) in length; the center-to-center distance should be ∼34 nm. Figure 1e shows the way that the two DX triangles are designed to associate into pseudohexagonal trigonal 2D arrays. The red and blue triangles show an elaboration of the six-triangle complex illustrated in Figure 1d.

The strands were synthesized by conventional phosphoramidite procedures[14] and were purified by denaturing polyacrylamide gel electrophoresis. Stoichiometric mixtures of the strands (estimated by OD_{260}) for each triangle were prepared separately to a concentration of 0.5 μM in a solution containing 40 mM Tris-HCl, pH 8.0, 20 mM acetic acid, 2 mM EDTA, and 12.5 mM magnesium acetate.

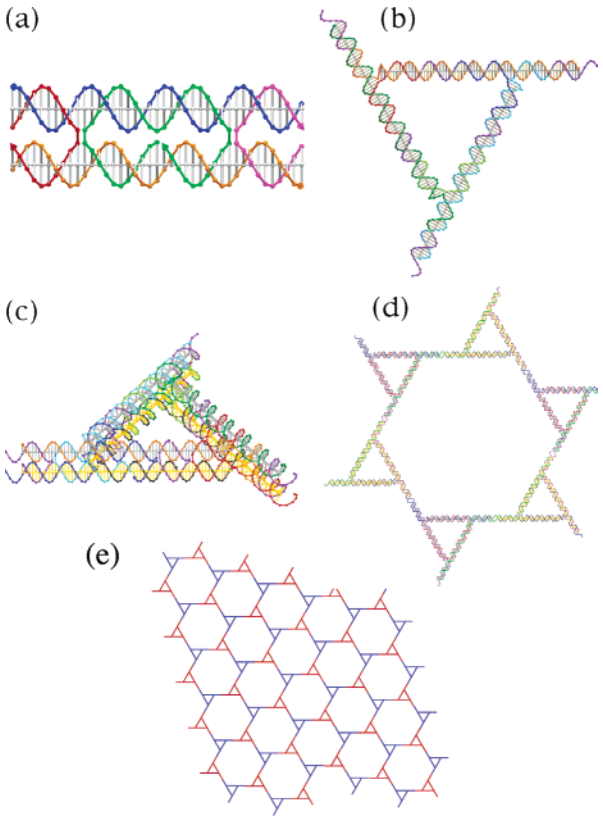

Figure 1. Motifs discussed here. (a) DX motif. (b) Bulged junction triangle. (c) DX triangle. (d) Trigonal arrangement of six DX triangles of two different species. (e) Schematic pseudohexagonal trigonal lattice of the two triangles.

Each mixture was cooled from 90 °C to room temperature in a 500-mL water bath over the course of 48 h. To form the array, the two complexes were mixed in stoichiometric quantities, warmed to 45 °C, and cooled slowly to room temperature in a thermos containing a 500-mL water bath over 24 h; sometimes the sample was cooled another 24 h to 16 °C. AFM imaging was performed by spotting a 5−7 μL sample drop on freshly cleaved mica, which was left to adsorb to the surface for 3 min. To remove buffer salts, 5−10 drops of doubly distilled water were placed on the mica, the drop was shaken off, and the sample was dried with compressed air. Imaging was performed in contact mode under 2-propanol in a fluid cell on a NanoScope IV (Digital Instruments) instrument, using commercial cantilevers with Si_3N_4 tips (DI).

The triangles migrate as single bands on non-denaturing gels (Supporting Information). Figure 2 shows atomic force micrographs of arrays produced by the self-assembly of the triangles.

The honeycomb structure of arrangements is evident from the images shown in Figure 2. The quality of the lattice is evident in the images shown in Figure 2a−c. The lattices have a certain tendency to stack on each other, as shown in Figure 2d; the array

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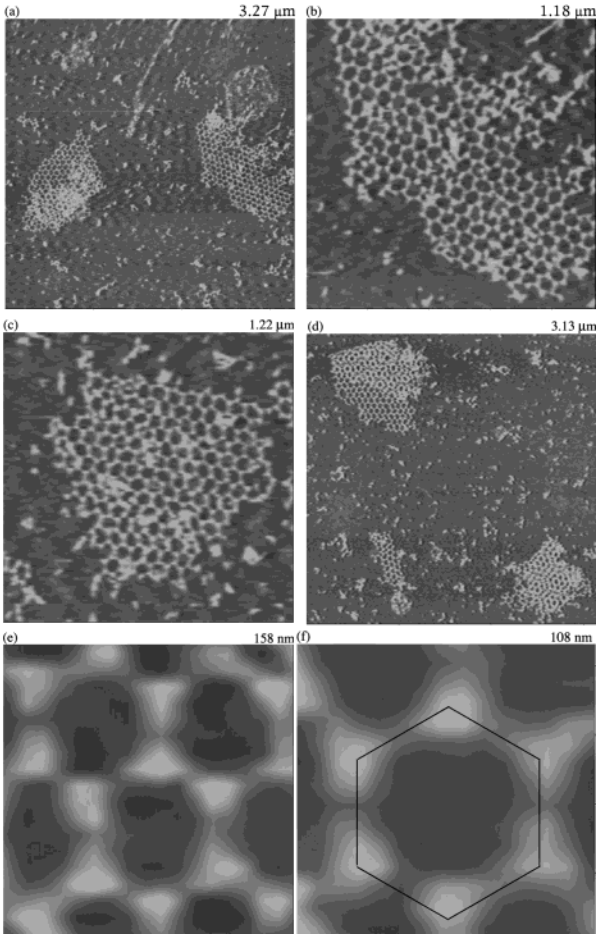

Figure 2. AFM images of pseudohexagonal trigonal arrays. Field sizes are indicated in the upper right corners. (a) A pair of 2D arrays. The honeycomb nature of the arrays are evident. (b) Zoom of the array on the right in (a). (c) Zoom of another array. (d) Image containing two stacked arrays, virtually complete on the lower right, partial on the upper left. (e) Zoomed image containing 15 DX triangles. (f) Further zoom of (e) showing six complete triangles, similar to the arrangement in 1d, and with a center−center hexagon superimposed.

in the upper left illustrates this point clearly, because the array on top is only about half the size of the array below it. Note that the arrays seem to stack over each other so that the cavities appear to be continuous between layers. The zoomed images shown in Figure 2e,f demonstrate clearly the hexagonal nature of the array; the center-to-center hexagon in Figure 2f has an edge of ∼38 nm, in good agreement with the expected length.

Given the previous failures to form hexagonal arrays, it is of central importance to establish which of the differences between the current system and previous systems has proved to be the key change, the greater stiffness of the DX or the cohesion of the double sticky ends. To resolve this issue, we have repeated these experiments by removing the sticky ends from one of the helices on each of the triangles. When we put these modified molecules through the same protocols that we did for the doubly sticky-ended triangles, we were unable to produce lattices of the sort shown in Figure 2. Thus, the key difference is the use of double sticky ends.

We suspect that the previous failures were due to differences between ideal and actual twists along a single helix; two helices apparently are able to bind successfully while maintaining the orientation of the plane defined by the two helix axes of the DX edges. Nevertheless, we cannot exclude the possibility that the flexibility of the single-helical connection contributes to the failure of those molecules to form honeycomb arrays; indeed, the recent work of Brun et al.[15] shows that single-helical triangles combined by DX cohesion yields polygons in addition to hexagons.

Thus, the substitution of DX arms for double helical arms leads to robust self-assembly in 2D. If this conclusion is correct, we ought to be able to use this approach in other motifs that have proved ineffective or difficult when used as components of 2D arrays connected by single helical sticky ends. We have tested this notion in a number of systems and found that it is correct. We have successfully built robust 2D arrays using DX versions of a small 3D triangle,[16] a six-helix bundle,[17] a large and unwieldy DNA parallelogram,[4] and a previously unreported 3D TX motif (P. Constantinou, T. Wong, J. Kopatsch, L. B. Israel, C. Mao, B. Ding, R. Sha, X. Zhang, X. Yang, and N. C. Seeman, in preparation). We expect that the use of this form of cohesion will prove of value in the future, in both two-dimensional applications and possibly in three-dimensional assemblies as well.

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Supporting Information Available: The sequences of the molecules used and a non-denaturing gel showing migration as a single band. This material is available free of charge via the Internet at http://pubs.acs.org.

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