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Highly Connected Two-Dimensional Crystals of DNA Six-Point-Stars

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This paper reports the design, construction, and self-assembly of a DNA six-point-star motif that contains a 6-fold rotational symmetry. This motif readily self-assembles into two-dimensional (2D), periodic arrays. The resulting arrays present the highest connectivity in all DNA 2D arrays reported so far; each six-point-star motif (tile) is connected with six neighboring tiles. The new arrays contain both 6- and 3-fold rotational symmetries and both triangular and hexagonal pores.

From mathematical consideration, periodic 2D arrays can only assume certain rotational symmetries perpendicular to the array planes: 2-, 3-, 4-, and 6-fold rotational symmetries.[1] We have previously developed symmetric DNA double-crossover motifs,[2] a symmetric three-point-star motif,[3] and a symmetric-cross motif.[4] These motifs contain 2-, 3-, and 4-fold rotational symmetries, respectively. A natural challenge is to develop a DNA motif that contains a 6-fold rotational symmetry. The six-point-star motif presented here satisfies this requirement.

The six-point-star motif is a new member of the star-motif family to which both the symmetric-cross motif (a four-point-star) and the three-point-star motif belong. The new motif contains six identical branches (Figure 1). Each is a four-arm junction. The branches are interconnected and related to each other by a 6-fold rotational symmetry. The six-point-star motif contains 13 DNA single strands, which fall into three groups: one central long strand (black), six identical blue strands, and six identical red strands. At the center, there are six single-stranded loops (T4), which connect the branches and prevent the helixes from stacking onto one another. The overall structure is expected to be flat. To further assemble into periodic two-dimensional (2D) arrays, the original, blunt-ended six-point-star motif has to be modified to contain single-stranded overhangs (sticky ends). We have engineered two complementary sticky-ends on each branch (which has two duplexes). Note that the 6-fold rotational symmetry is not compatible with any 2-fold rotational symmetry lying in the plane of the array. Thus, we cannot use the commonly used corrugation strategy[5] (any two neighboring tiles face-to-opposite sides of an array plane) to cancel potentially curvatures associated with the DNA motifs. Instead, all tiles in an array have to face to the same side of the array. To satisfy this requirement, the separation between any two neighboring tiles has to be an integral number of turns. Here, we use four turns (42 base pairs).

The tiles of three-point-star and six-point-star are complementary to each other in the sense of 2D crystal packing. For the six-point-star tile, there is a 6-fold rotational symmetry going through the center of the motif. In its 2D arrays, new crystallographic 3-fold rotational symmetries arise from 2D crystal packing. The 3-fold axes are parallel to the 6-fold axes of the component tiles. The symmetry for a three-point-star tile is in the opposite way. For the arrays of three-point-star tiles, the 3-fold rotational axes go through the center of the motif and pseudo-6-fold-rotational axes arise from the 2D crystal packing.[3]

Native polyacrylamide gel electrophoresis (PAGE) was used to initially characterize the individual, blunt-ended six-point-star motif

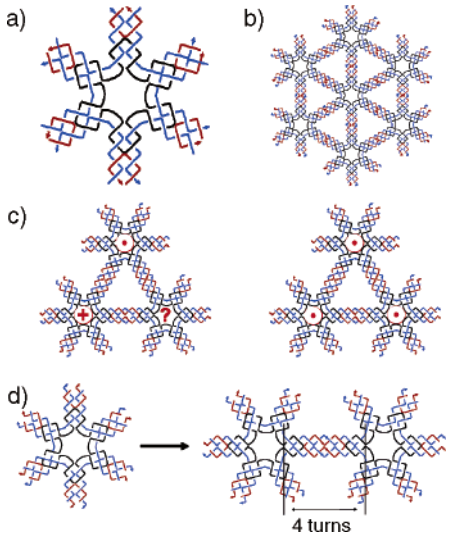

Figure 1. DNA six-pointed-star motif. (a) A blunt-ended six-point-star motif (tile) consists of six blue strands, six red strands, and one black strand. (b) An extended, periodic array assembled from sticky-ended six-point-star motifs. (c) Left: corrugated association will lead to conflict tile arrangement. Right: all tiles face to the same direction. In corrugated association, two adjacent tiles face to opposite directions. Dots and cross depict pointing in and out of the paper plane, respectively. (d) A detailed view of the association between two sticky-ended six-point-star motifs.

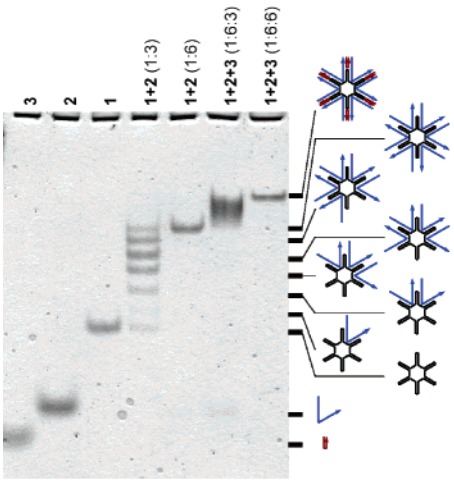

Figure 2. Native polyacrylamide gel electrophoretic (PAGE) analysis of the blunt-ended six-point-star motif. The identities of all the bands are shown at the right side. DNA strands and their ratios are indicated on the top (**1**, **2**, **3** are strands black, blue, and red, respectively).

(see Figure 2). Despite being a complicated structure, the desired DNA tile migrates as a single sharp band, suggesting that the tile was properly formed and was stable under the experimental conditions. When nonstoichiometric ratios were used, a series of incomplete structures formed, which allowed a clear assignment of the identity of each band in the gel.

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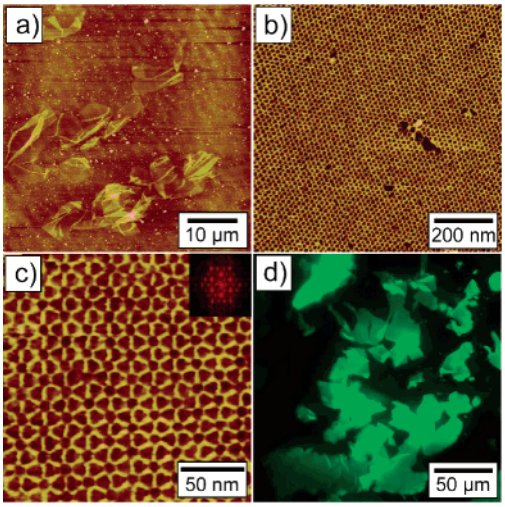

Figure 3. AFM and fluorescence images of DNA 2D arrays assembled from sticky-ended six-point-star motifs. (a) An AFM image and (b and c) its zoom-in views. The inset shows a corresponding Fourier transform pattern. (d) A fluorescence image shows the large array domains.

For the sticky-ended tiles, DNA strands would assemble into individual tiles and further assemble into arrays. After assembly, the arrays were examined by both atomic force microscopy (AFM) and fluorescence microscopy (FM). From AFM images, we observed regular, periodic structures, as we expected. They contained two types of pores: larger equilateral triangles and smaller hexagons. The overall morphologies of the arrays were isotropic and each dimension of the arrays reached 40 μm long. The DNA arrays, after staining with a fluorescence dye (YOYO-1), could be visualized by fluorescence microscope, too (Figure 3d). The fluorescence microscopy was a quicker and more convenient means than AFM for analysis of the DNA self-assembly. However, the overview provided by fluorescence microscopy did not give structural information at the nanoscale.

Self-assembled DNA arrays can template nanofabrication[6] and organize nanoparticles[7] and biomacromolecules.[5] One limitation of the currently available DNA arrays is that they are not as stable as needed. To overcome this problem, partially ligating self-assembled DNA nanostructures have been proven to be effective.[8] However, the enzyme activity on the non-natural DNA structures is of concern. A potentially more effective and less expensive way is to increase the association strength among the tiles. An intuitive approach is to elongate the sticky-ends to strengthen the sticky-end association, but it contradicts the knowledge we have learned from folding and self-assembly of biomacromolecules. Instead of relying on small number of strong interactions, those molecules use a large number of weak interactions to ensure specific molecular recognition and reversible dynamics. Indeed, it has been shown that strong sticky-end association forces DNA 2D arrays to curl up and form tubular structures.[9] Inspired by biological self-assembly, we have chosen to increase the connectivity among the tiles in the arrays but retain the strength of each individual sticky-end association. In the current assembly system, each tile is connected with six neighbor tiles through 12 sticky-end association. In contrast, each tile is connected with only three[3] or four tiles[4,5,10,11] in previously reported DNA arrays. It is clear that the new DNA motif can readily assemble into well ordered 2D arrays. However, it remains to be tested whether this strategy has improved the stability of self-assembled DNA arrays. There is no convenient way to quantitatively measure the robustness of assembled DNA 2D arrays.

In summary, we have designed and constructed a novel DNA six-point-star motif and have demonstrated that this motif can self-assemble into 2D periodic arrays. This motif is one of the most complicated motifs that have been reported till now. It contains 13 DNA strands, 12 DNA duplexes, 6 four-arm junctions, 6 single-stranded loops, and 12 sticky ends. The motif is also one of the easiest motifs to assemble. It needs only three different, single-stranded species because of a 6-fold rotational symmetry. More importantly, associations from 12 sticky-ends of each motif could potentially make the 2D arrays stable and robust. We are currently developing methods to test this hypothesis.

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Supporting Information Available: Experimental method and DNA sequences. This material is available free of charge via the Internet at http://pubs.acs.org.

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