Geometric self-sorting in DNA self-assembly†

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Two types of DNA star motifs (tiles) can recognize and associate with like tiles to form 2D arrays but exclude unlike tiles even though the local interactions between any two tiles are exactly the same.

This communication reports an atomic force microscopy (AFM) study of a self-sorting behavior in DNA self-assembly. There are two closely related component motifs (tiles) in the reported system. Between any two tiles, the local association is exactly the same; however, each tile associates only with like tiles but excludes unlike tiles. The discrimination originates from the differences in geometry and valence between the component DNA tiles.

Self-sorting, sorting self from others, is commonly observed in natural systems. One example is crystallization. Many related molecules are present in the same solution, but one set of molecules recognizes and interacts with themselves to form well-ordered three-dimensional aggregates (crystals), and ignores all other molecules existing in the mixture. Similar processes have been explored for supramolecular assembly,[1] chemical reactions,[2] and the preparation of dynamic combinatorial libraries.[3] They are realized through engineering different local chemical interactions. For example, different hydrogen-bonding patterns, different coordination pairs, or different reaction partners. As self-sorting is a useful tool in chemistry, it would be desirable to expand the mechanisms of self-sorting. The self-sorting system in this work relies on a geometric mismatch between the global shapes of components instead of any mismatch of local interactions. Self-sorting assembly facilitates and maximizes local interactions, while mixed assembly will prevent some local interactions.

The studied system contains two closely related DNA motifs (tiles): a four-point-star motif[4] (**I**), also named as a cross motif, and a three-point-star motif[5] (**II**) (Fig. 1). Both motifs are flat, geometric structures and contain either 3- or 4-fold rotational symmetry. All branches from the two motifs, including the sticky ends (single-stranded overhangs) at the outside, are identical. The only difference between the two motifs is their number of component branches: three and four, respectively. Both motifs contain three groups of strands: blue/black, red, and green. The two motifs share the same blue and red strands, but use a different central strand (either blue or black). In separate solutions, each motif self-assembles into periodic two-dimensional (2D) arrays (tetragonal or hexagonal).[4,5] When the two motifs are co-present in a solution, two types of assemblies would be possible. One is a hetero-assembly, where the assembly process is dominated by the individual, local, sticky-end association between branches of any tiles. DNA tiles randomly associate together, regardless of **I** or **II** motifs, and no regular patterns will be generated. The other is self-sorting assembly, or homoassembly. Random networks are not thermodynamically stable because such networks contain many unpaired sticky-ends and/or geometric distortions in DNA tiles. In stable assemblies, all sticky-ends should associate with one another, and all tiles should remain in their geometric shapes without distortions. To satisfy these requirements, the four-point-star tiles (**I**) and three-point-star tiles (**II**) should associate into tetragonal and hexagonal lattices, respectively.

We followed previously reported experimental procedure.[6] Briefly, DNA sequences have been designed by a computer program "SEQUIN".[7] Individual DNA strands were purified from denaturing polyacrylamide gel electrophoresis (PAGE) and combined in a Mg^{2+}-containing buffer at designated molar ratio. Assembly was performed by slowly cooling the mixture solution from 90 °C to room temperature over two days and the resultant DNA aggregates were characterized by AFM.

The DNA tiles exhibit a strong self-sorting behavior under our experimental conditions (Fig. 2 and Table 1). We have not observed random networks, instead, we have observed well-ordered, regular, periodic 2D arrays, either tetragonal or hexagonal, which are assembled from 4-point-star (**I**) or 3-point-star motif (**II**), respectively. When the content of **II** is high, there are only hexagonal arrays; when the content of **I** is high, there are only tetragonal arrays. In a narrow ratio window (**I** : **II** = 20 : 80–30 : 70), the two different types of arrays co-exist (Fig. 3). We have

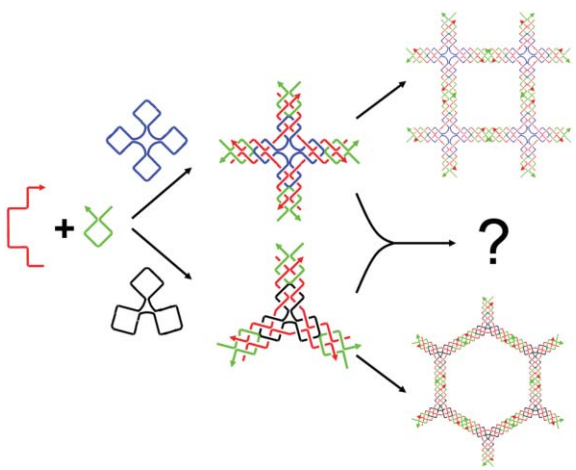

Fig. 1 The self-sorting system contains two DNA motifs: a cross motif (**I**) and a three-point-star motif (**II**). Lines with the same color represent DNA strands with the same sequence.

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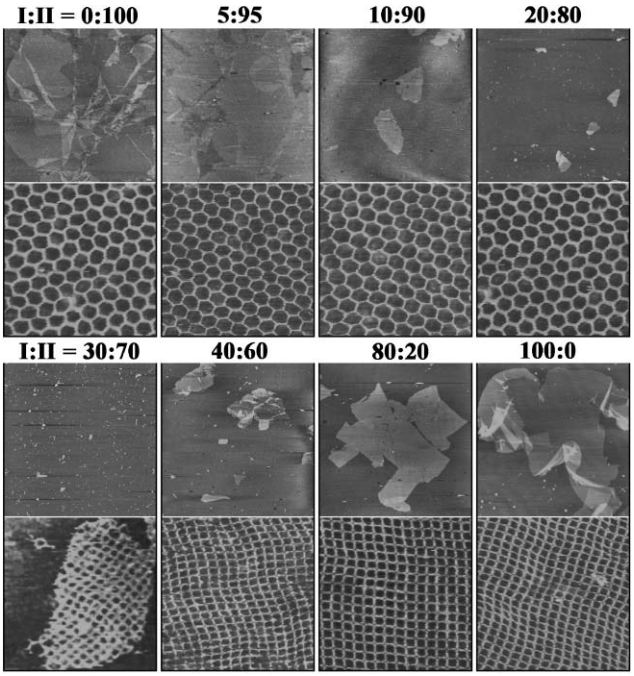

Fig. 2 Typical AFM images of the aggregates assembled from mixture of tiles **I** and **II** at different ratios. Each set contains a 30 μm image (top) and a 300 nm image (bottom).

found that even at **I** : **II** = 30 : 70, **I** still predominates the assembly process to form tetragonal arrays, and prevents **II** from assembling. It is probably because tetragonal arrays are energetically more stable than hexagonal arrays. Each **I** tile associates with four neighboring tiles in tetragonal arrays, but each **II** tile only associates with three neighboring tiles in hexagonal arrays.

The array size changes as the **I** : **II** ratio changes. When the ratio is close to either 100 : 0 or 0 : 100, the arrays are large, 30 μm or larger. When the ratio changes away from these values, the array size decreases as observed by AFM imaging. At **I** : **II** = 30 : 70, interestingly, double-layered tetragonal lattices have been observed, whose sizes ranges from 100 nm to several micrometers (Fig. 2). The decrease of the array size is likely due to the low **I** concentration (0.18 μM), much lower than the concentration of (**I** + **II**). This hypothesis has been confirmed by our control experiments with pure **I** motif at concentrations lower than 0.20 μM (Fig. S1 in ESI†).

Occasionally we have observed doping phenomena: one type of tile is incorporated into 2D arrays of another type of tile (Fig. 4). We suspect that it is due to the statistical nature of the assembly process. In theory, introduction of foreign tiles into arrays is thermodynamically unfavorable. However, during the crystal growth process, a large number of DNA tiles associate with each other during a relatively short period. Once foreign tiles are buried inside the arrays, the defects are relatively stable. At the array edges, the foreign tiles would inhibit future growth. Indeed, we have often observed foreign tiles only at peripherals of DNA arrays (Fig. S2 in ESI†). How to control the kinetic process is also an important issue in semiconductor nanocrystal doping. It is worth pointing out that we have only observed that motif **I** could dope into the hexagonal arrays of **II**, but not the other way. This observation suggests that the hexagonal lattice, which has a larger pore size and fewer connecting branches, can tolerate more impurities. This observation shows that self-sorting of this DNA system is not complete. A small extent of mixing happens, which could be regarded as errors or defects in DNA self-assembly.

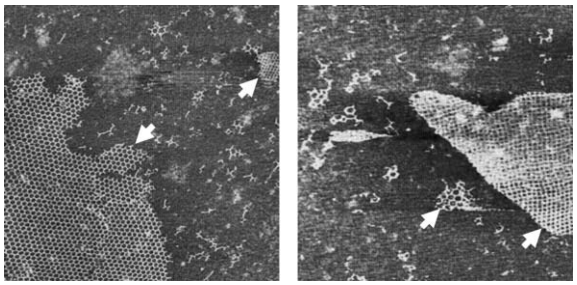

Fig. 3 Co-existence of two types of DNA arrays (indicated by arrows) in samples with a **I** : **II** ratio of 20 : 80 (left; image size: 1.85 × 1.85 μm.) and 30 : 70 (right; image size: 1.5 × 1.5 μm).

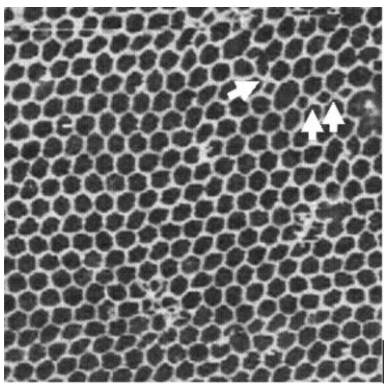

Fig. 4 AFM image of a sample with **I** : **II** = 20 : 80. The arrows indicate cross motifs in a hexagonal lattice. Image size: 500 × 500 nm.

A mixture system with rhombus motifs has been examined before.[8] It does not exhibit a self-sorting behavior, instead, the component tiles are well mixed and incorporated into the final structures. We speculate that the different behaviors between these two systems originate from the flexibility difference. The previous rhombus motifs might be relatively flexible and can accommodate significant structural deformation, while the current star motifs are less flexible and less likely to tolerate geometric defects.

In summary, we have discovered a geometric self-sorting behavior in DNA self-assembly. To some extent, this behavior is

Table 1 The distribution of the two types of DNA array at different tile ratios based on AFM imaging

Tile ratio (**I** : **II**)	0 : 100	5 : 95	10 : 90	20 : 80	30 : 70	40 : 60	80 : 20	100 : 0
No. of tetragonal arrays	0	0	0	3	29	30	30	30
No. of hexagonal arrays	30	30	30	27	1	0	0	0
No. of total arrays	30	30	30	30	30	30	30	30

similar to the effect of crystal packing. The currently studied system provides an excellent tool to study errors/defects in molecular self-assembly. Reduction of errors has fundamental importance for molecular computation and algorithmic self-assembly. Previous efforts focus on amplification of local mismatches and the outcomes remain elusive.[9] In contrast, the current system deals with global interactions and has a very low error rate, thus, presenting an alternative strategy for error resistance in self-assembly.

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