

## Self-Assembly of Symmetric Finite-Size DNA Nanoarrays

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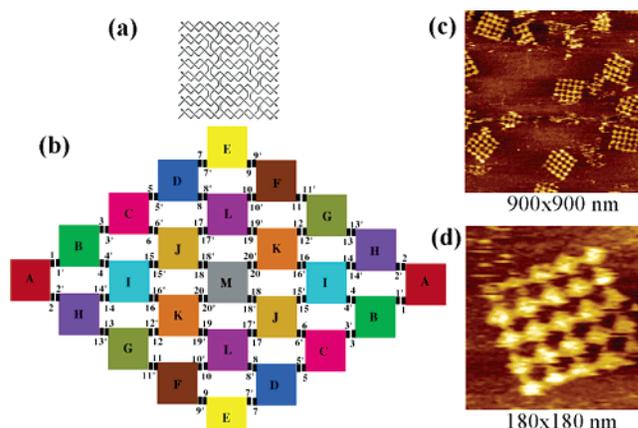
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Structural DNA nanotechnology aims at the construction of well-defined nano- to micrometer-scale structures from simple DNA building blocks.<sup>1</sup> In recent years, predictable self-assembly of DNA tiles composed of branched junctions to construct periodic 1-dimensional (1D) and 2-dimensional (2D) patterned lattices has been demonstrated.<sup>2</sup> DNA and RNA lattices of more complex patterns have also become possible through algorithmic self-assembly.<sup>2b,1</sup> The use of self-assembled DNA nanostructures as templates to organize metallic nanoparticles<sup>3</sup> or as molecular lithographic masks to produce well-ordered gold replicas<sup>4</sup> has made DNA self-assembly a promising tool for potential nanoelectronic applications. However, previous examples of self-assembled DNA lattices lack control of the final lattice size because terminating events are not programmed into the self-assembly. Such control is crucial since future nanoelectronic devices assembled on a DNA-based molecular print-board would require the DNA scaffolds to have defined boundaries; thus, self-assembly of finite-size DNA nanoarrays represents an immediate challenge for structural DNA nanotechnology.

One way to self-assemble a finite-size DNA nanoarray with  $N$  tiles is to synthesize  $N$  different tiles, each containing unique sticky-ends to connect to its neighboring tiles, so that each tile takes up a unique and well-defined position in the array. This requires a large number of DNA strands and therefore would be costly and time consuming if large arrays are desired. Further, a large set of tiles poses difficulties in designing unique sticky-end pairs with minimum similarity, thus making the assembly more error-prone when scaling up the lattice sizes.

Here we report a novel and more cost-effective strategy to produce finite-size DNA arrays. This strategy takes advantage of the geometric symmetry of the tile structure. In general, to use a total of  $N$  tiles to construct a fixed-size 2D arrays with  $C_m$  symmetry, where  $m = 2, 3, 4, \text{ or } 6$ , the number of unique tiles the fixed-size array requires is  $N/m$ , if  $N/m$  is an integral number, or  $\text{Int}(N/m)+1$ , if  $N/m$  is a nonintegral number. We herein demonstrate two examples of fixed-size arrays with  $C_2$  and  $C_4$  fold symmetry. Specifically, a  $5 \times 5$  array formed from DNA tiles with  $C_2$  symmetry requires 13 unique tiles instead of 25 (Figure 1a,b); while a  $5 \times 5$  array formed from DNA tiles with  $C_4$  symmetry requires 7 unique cross-shaped tiles instead of 25 (Figure 2a,b). Therefore, this strategy is cost-effective in material. Furthermore, within each self-assembled finite-size array, the unique tiles all share the same core strand sequences; therefore, only the individual sticky ends need to be different to result in a single way of connectivity between the tiles. This minimizes the design time and the sample preparation time dramatically. Thus, the finite-size DNA nanoarrays can be constructed efficiently.

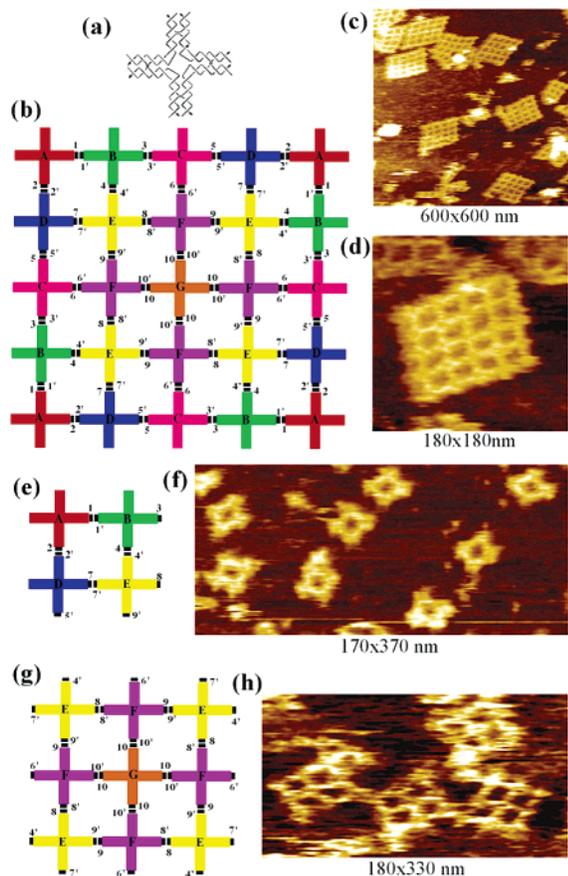
In Figure 1, a and b show an example of a  $5 \times 5$  fixed-size array self-assembled from a DNA tile containing  $C_2$  symmetry. This is a new tile structure we recently constructed,<sup>2n</sup> which has 8 DNA helices joined together in a plane with two crossovers running from one helix to its neighboring helices. The dimension of a single 8-helix bundle tile is  $\sim 17$  nm along the helix axis, and  $\sim 14$  nm



**Figure 1.** (a) 8-helix bundle tile, blunt ended. (b) Design of a  $5 \times 5$  fixed-size array based on the tile shown in (a). To form the 25-tile finite-size array, a total of 13 unique tiles is required. Each unique tile is of a different color. The numbers represent the corresponding sticky ends. A total of 20 pairs of sticky ends are involved. (c,d) AFM images showing the formation of the  $5 \times 5$  array as designed.

perpendicular to the helix axis in the plane. The sticky ends can only point along the direction of the helix axis. The structure has  $C_2$  symmetry with the symmetry axis perpendicular to the tile plane. The reason that we chose the 8-helix structure to demonstrate the fixed-size array is because the large cavity resulting from this tile assembly can easily be visualized by atomic force microscopy (AFM). The 13 unique tiles are different only in the sticky ends pointing out from the 5' ends of the outmost helix in the tiles and are each represented by a different color in Figure 1b. The sticky-end associations are labeled by the corresponding numbers, e.g.  $n$  pairs with  $n'$ . To form the array, a two-step annealing procedure was used. We first formed each individual tile separately by combining their component DNA strands stoichiometrically and cooling from 90 to 40 °C and then combined all the 13 tiles in the correct ratios together into one solution at 40 °C and followed by further cooling to 10 °C. Figure 1c shows an AFM image of the sample deposited onto a mica surface. The magnified image shown in Figure 1d reveals a well-defined fixed-size array with 25 tiles. The dimension of each individual tile measures 16.9 nm  $\times$  14.2 nm, consistent with our design parameters. The dimension of the  $5 \times 5$  array measures 110 nm on each side. No 2D arrays larger than the designed dimensions are observed, and the overall geometry of the  $5 \times 5$  array evidences a  $C_2$  symmetry.

We have further demonstrated the symmetric assembly strategy using another tile structure that has  $C_4$  symmetry. We recently constructed a family of DNA tiles<sup>2d</sup> which resemble a crosslike structure composed of four 4-arm DNA branch junctions. Self-assembly from a single unit of the crosslike structure resulted in 2D nanogrids, which display periodic square cavities. The tile structure contains a four-fold symmetry perpendicular to the tile plane (Figure 2a). Figure 2b illustrates the formation of a  $5 \times 5$



**Figure 2.** (a)  $C_4$  symmetric DNA tile, blunt ended. (b) Design of a  $5 \times 5$  fixed-size array based on the tile shown in (a). To form the 25-tile finite-size array, a total of 7 unique tiles is required in all 10 pairs of double sticky ends. (c,d) AFM images showing the formation of the  $5 \times 5$  array as designed. (e,f) When only the four corner tiles are used, a  $2 \times 2$  array is formed. (g,h) When only the three center tiles are used, a  $3 \times 3$  array is formed.

fixed-size array from the crosslike structure requiring only 7 unique tiles, each tile represented by a different color. AFM images in Figure 2, c and d clearly demonstrate the correct formation of the  $5 \times 5$  fixed-size array from the cross-shaped tile structure. The dimension of the cavity is about  $17.2 \text{ nm} \times 16.8 \text{ nm}$ , which matches the design parameters. It is notable that the  $5 \times 5$  arrays observed by AFM most of the time do not show a perfect square, but rather a diamond shape. This is due to the flexibility of the cross-shaped tile, in which the acute angle of the cross may range from  $90^\circ$  to as little as  $45^\circ$  under a stress. However, this does not affect the integrity of the tile nor the connectivity of the sticky ends.

It should also be noted that within the same design, instead of using all different unique tiles, one can use a smaller number of tiles to form smaller finite-size arrays. For example, in Figure 2b, if one uses the four corner tiles of red (A), green (B), blue (D), and yellow (E), a finite-size  $2 \times 2$  4-tile array can be produced (Figure 2e,f). On the other hand, if one only uses the three center tiles of orange (G), purple (F), and yellow (E), a  $3 \times 3$  9-tile array can be produced (Figure 2g, h). In principle, if one used the four side tiles of red (A), green (B), pink (C), and blue (D), a 16-tile square with a large cavity space should be formed, but due to the flexibility of the individual tiles and less cooperativity of the assembly, a perfect square of this size has not been observed.

For the fixed-size array formed by these crosslike tiles, when only one or two tiles on the outside are missing, the overall shape of the array does not change. However, if one or two tiles in the center are missing, some other array shapes can be formed, such as a triangle or a five-point star shape (see Supporting Information). Again, this is due to the flexibility of the cross-shaped tile and the occasions of such arrays are rare although it is statistically possible in a molecular self-assembly. Because the 8-helix bundle tile in Figure 1 is a very rigid motif, other shaped fixed-size arrays based on this tile were not observed.

In summary, we have defined a novel strategy to produce fixed-size DNA nanoarrays. We have proved the working principle of this strategy by demonstrating the formation of fixed-size arrays with two different symmetries. By adding sticky-ends to the outside frame of the fixed-size arrays, individual fixed-size arrays could be further used to form larger arrays with defined dimensions in a hierarchical way. The strategy reported here provides a powerful means to produce molecular lithographic masks for nanoelectronic device constructions or templates for small-scale protein nanoarrays. The next obvious challenge is to purify the fixed-size array and localize the array on a solid substrate. It is also worth noting that Winfree has recently proposed another interesting route to increase the complexity of self-assembled shapes with minimal numbers of tiles.<sup>5</sup> The high parallelism and accurate control at nanometer-scale precision offered by DNA self-assembly, when combined with top-down methods, may lead to nanofabrication with complex molecular architectures.

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**Supporting Information Available:** DNA sequences, experimental methods, designs, additional AFM images. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## References

- (1) Seeman, N. C. *Nature* **2003**, *421*, 427–431.
- (2) (a) Winfree, E.; Liu, F.; Wenzler, L. A.; Seeman, N. C. *Nature* **1998**, *394*, 539–544. (b) LaBean, T. H.; Yan, H.; Kopatsch, J.; Liu, F.; Winfree, E.; Reif, J. H.; Seeman, N. C. *J. Am. Chem. Soc.* **2000**, *122*, 1848–1860. (c) Mao, C.; Sun, W.; Seeman, N. C. *J. Am. Chem. Soc.* **1999**, *121*, 5437–5443. (d) Yan, H.; Park, S. H.; Finkelstein, G.; Reif, J. H.; LaBean, T. H. *Science* **2003**, *301*, 1882–1884. (e) Liu, D.; Wang, M.; Deng, Z.; Walulu, R.; Mao, C. *J. Am. Chem. Soc.* **2004**, *126*, 2324–2325. (f) Ding, B.; Sha, R.; Seeman, N. C. *J. Am. Chem. Soc.* **2004**, *126*, 10230–10231. (g) Rothmund, P. W. K.; Papadakis, N.; Winfree, E. *PLoS Biol.* **2004**, *2*, 2041–2053. (h) Shih, W. M.; Quispe, J. D.; Joyce, G. F. *Nature* **2004**, *427*, 618–621. (i) Malo, J.; Mitchell, J. C.; Venien-Bryan, C.; Harris, J. R.; Wille, H.; Sherratt, D. J.; Turberfield, A. J. *Angew. Chem., Int. Ed.* **2005**, *44*, 3057–3061. (j) Mathieu, F.; Liao, S.; Kopatsch, J.; Wang, T.; Mao, C.; Seeman, N. C. *Nano Lett.* **2005**, *5*, 661–665. (k) Park, S. H.; Barish, R.; Li, H.; Reif, J. H.; Finkelstein, G.; Yan, H.; LaBean, T. H. *Nano Lett.* **2005**, *5*, 693–696. (l) Chworos, A.; Severcan, I.; Koyfman, A. Y.; Weinkam, P.; Oroudjev, E.; Hansma, H. G.; Jaeger, L. *Science* **2004**, *306*, 2068–2072. (m) Chelyapov, N.; Brun, Y.; Gopalkrishnan, M.; Reishus, D.; Shaw, B.; Adleman, L. *J. Am. Chem. Soc.* **2004**, *126*, 13924–13925. (n) Ke, Y.; Liu, Y.; Zhang, J.; Yan, H. Manuscript in preparation.
- (3) (a) Loweth, C. J.; et al. *Angew. Chem., Int. Ed.* **1999**, *38*, 1808–1812. (b) Xiao, S.; et al. *J. Nanopart. Res.* **2002**, *4*, 313. (c) Li, H.; Park, S. H.; Reif, J. H.; LaBean, T. H.; Yan, H. *J. Am. Chem. Soc.* **2004**, *126*, 418–419. (d) Le, J. D.; Pinto, Y.; Seeman, N. C.; Musier-Forsyth, K.; Taton, T. A.; Kiehl, R. A. *Nano Lett.* **2004**, *4*, 2343. (e) Niemeyer, C. M.; Koehler, J.; Wuerdemann, C. *ChemBioChem* **2002**, *3*, 242. (f) Deng, Z.; Tian, Y.; Lee, S.; Ribbe, A. E.; Mao, C. *Angew. Chem., Int. Ed.* **2005**, *44*, 3582–3585.
- (4) Deng, Z.; Mao, C. *Angew. Chem., Int. Ed.* **2004**, *43*, 4068–4070.
- (5) Soloveichik, D.; Winfree, E. *DNA Computing, Lect. Notes Comput. Sci.* **2005**, *3384*, 344–354.

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