VIP DNA Nanotechnology

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Crystalline Two-Dimensional DNA-Origami Arrays**

*Wenyan Liu, Hong Zhong, Risheng Wang, and Nadrian C. Seeman**

Nanotechnology aims to organize matter with the highest possible accuracy and control. Such control will lead to nanoelectronics, nanorobotics, programmable chemical synthesis, scaffolded crystals, and nanoscale systems responsive to their environments. Structural DNA nanotechnology[1] is one of the most powerful routes to this goal. It combines robust branched DNA species with the control of affinity and structure[2] inherent in the programmability of sticky ends. The successes of structural DNA nanotechnology include the formation of objects,[3] 2D crystals,[4] 3D crystals,[5] nanomechanical devices,[6] and various combinations of these species.[7] DNA origami[8] is arguably the most effective way of producing a large addressable area on a 2D DNA surface. This method entails the combination of a long single strand (typically the single-stranded form of the filamentous bacteriophage M13, 7249 nucleotides) with about 250 staple strands to define the shape and patterning of the structure. With a pixelation estimated at about 6 nm,[8] it is possible to build patterns with about 100 addressable points within a definable shape in an area of about 10000 nm^2. Many investigators have sought unsuccessfully to increase the useful size of 2D origami units by forming crystals of individual origami tiles.[9] Herein, we report the 2D crystallization of origami tiles to yield a 2D array with edge dimensions of 2–3 μm. This size is likely to be large enough to connect bottom-up methods of patterning with top-down approaches.

Crystalline arrays are convenient for the propagation of patterns and other distributions of matter, so that multiple copies can self-organize into large periodic or aperiodic systems. DNA is a particularly powerful system for this type of organization, because it is possible to flank structural motifs with sticky ends, so that Watson–Crick complementary interactions can be programmed to establish the intermolecular contacts.[1] DNA double-crossover (DX) motifs are examples of small tiles (ca. $4 \times 16\ nm^2$) that have been programmed to produce 2D crystals;[4] often these tiles contain pattern-forming features when more than a single tile constitutes the crystallographic repeat. These motifs contain two parallel double helices held together by crossovers; the second dimension derives from the connection of one helix of a given tile to the other helix of an adjacent tile (Figure 1a). As well as the type of periodic pattern shown in Figure 1a, this form of intermolecular organization has been used to produce aperiodic 2D arrays.[10,11] The creation of 2D arrays of DNA origami tiles, at least on the micrometer scale, is a particularly appealing goal, since DNA origami tiles are

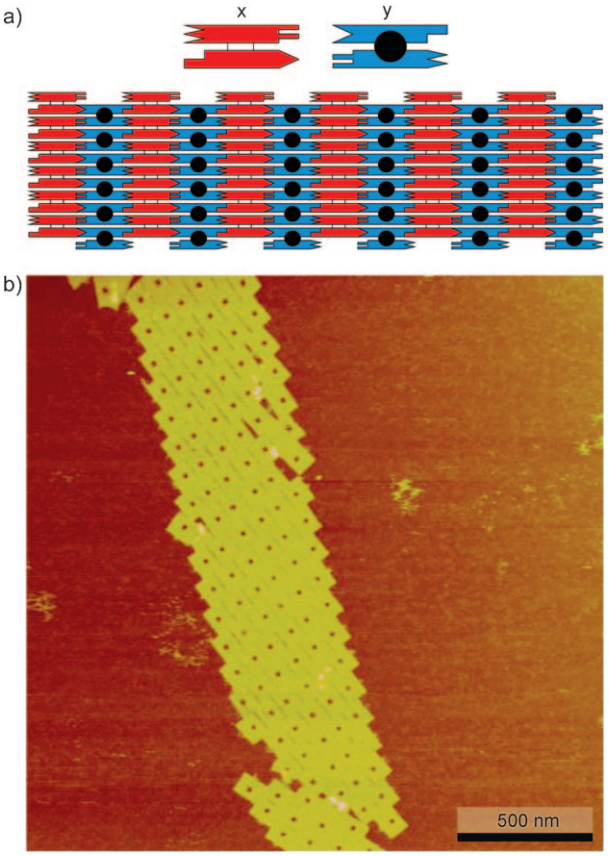

Figure 1. Formation of 2D arrays from molecules with parallel double-helical domains. a) A double-crossover (DX) molecule. The schematic structure contains two molecules, X (red) and Y (blue); Y is a DX+J molecule containing an extra double-helical domain (the black filled circle) perpendicular to the plane of the helix axes. The sticky ends are represented as complementary geometrical features. The key to producing a second dimension in the array with the DX tiles is the binding of the "top" domain of the red tile to the "bottom" domain of the blue tile. This system readily forms a 2D crystalline array in which the extra domain produces a series of stripes separated by the sum of the lengths of the two double helices; the stripes are typically separated by 32 nm. b) An attempt to form a 2D array from rectangular DNA origami tiles by using the strategy described in (a). The tiles all have a small cavity at their center. The helix axes are parallel to the long axes of the origami tiles. No more than about six tiles (equivalent to, say, the red tiles in (a)) repeat in the long direction, and no more than about six tiles are joined horizontally.

[*] W. Liu, Dr. H. Zhong, Dr. R. Wang, Prof. N. C. Seeman
Department of Chemistry, New York University
New York, NY 10003 (USA)
Fax: (+1) 212-260-7905
E-mail: ned.seeman@nyu.edu

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basically large versions of the DX motif that typically contain many parallel double helices, rather than two. However, this approach has proved to be unsuccessful. Figure 1b shows the product of a failed attempt to produce a 2D origami tile through the self-assembly of a rectangular origami tile with a cavity at its center. There are rarely more than half-a-dozen tiles in the second dimension, so the result is scarcely better than a 1D array.

The key to solving this problem lies in recognizing that all the helix axes in Figure 1b lie parallel to the direction in which the tiles actually cohere. Thus, a possible alternative method for the creation of a 2D origami array would be to use an origami tile whose helix axes propagate in two independent directions. A schematic representation of two such tiles (A and B) is shown in Figure 2a (see Figures S1 and S2 in the Supporting Information for the strand structures of these molecules). The use of two independent tiles enables AFM analysis of individual tiles without intertile cohesion. An AFM image of the A tile, with dimensions of 100×100 nm^2, is shown in Figure 2b. The key feature of this system is that there are two domains to the origami tile, one in a plane above the other. The two domains clearly have orthogonal directions of propagation, which solves the problem that arose when we tried to use the DX-tile approach with DNA origami. There is a small vacant horizontal boxlike feature on the bottom plane of the A tile; this feature is vertical in the B tile. In both cases, it leads to a visible bifurcation in the central part of the origami tile. This feature is emphasized in the inset in Figure 2b. Height analysis of the tile is shown in Figure S3 of the Supporting Information.

Yan et al.[12] showed that small crosslike DNA motifs may have a certain amount of curvature. To overcome this problem and produce flat 2D arrays, they used a corrugation strategy: by rotating the orientations of alternate members of the array within the plane, they were able to cancel out errors. We also found this type of tactic necessary to avoid tube formation; an alternating array is shown on the right of Figure 2a. However, our corrugation strategy differs from that used by Yan et al.: As the two layers of each origami tile have opposite orientations relative to the tile plane, we alternated the origami tiles with the same tiles rotated by 90°, so that the top layer of one tile was bonded to the bottom layer of the next. The result of this approach was the same, which indicates that the two domains distort from planarity in opposite relative directions (for example, one curves up, and one curves down). Thus, the designed alternating structure looks like a braided origami pattern (Figure 2a), wherein the top layer of one tile is bonded to the bottom layer of the adjacent tile. We implemented this approach by using two different tiles, A and B, to build a 2D crystalline array of origami tiles. An AFM image of the resulting array shows that the origami tiles form a regular rectilinear array resembling a latticework of roughly 3×2 μm^2 in size (Figure 3a); the edges contain steplike features. The zoomed image of a 2D array in Figure 3b shows the quality of the crystalline arrangement and the alternating orientation of the bifurcation feature.

After the formation of the individual origami tiles, they were mixed together. The annealing temperature in this annealing step is crucial for the formation of the 2D DNA origami array. Since the tiles used to create DNA-origami 2D arrays contain multiple sticky ends at each connection site, the annealing temperature is different from that of normal DNA tiles that contain only one or two sticky ends at each connection site. Therefore, the temperature had to be carefully optimized: If the temperature is too high, the origami tiles that are formed in the first annealing step are damaged and cannot associate with each other to form 2D arrays. On the other hand, if the temperature is too low, a great number of crystal nuclei are created, and those tiny array pieces randomly aggregate with each other, so that no large 2D crystals are formed. We attempted the annealing of the

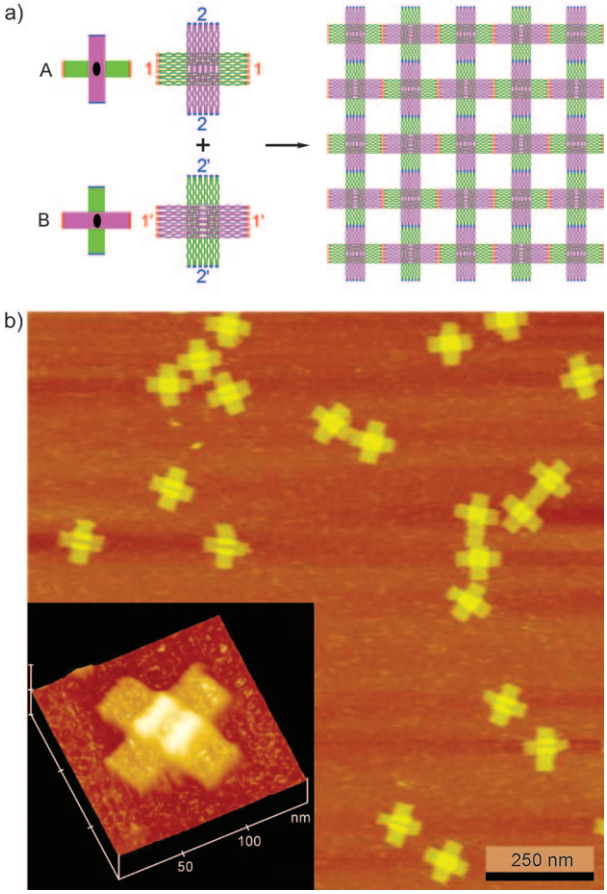

Figure 2. Origami tiles with orthogonal directions of propagation. a) Schematic structure of the tiles. Two different tiles, A and B, are shown. On the left are simplified drawings showing the orthogonal nature of the propagation directions of the tiles; their twofold axes have color-coded sticky ends, red and blue. In a more realistic representation to the right, the sticky-end sets in A are labeled with the numbers 1 and 2; the complementary sticky ends are labeled 1′ and 2′ in B; these images were generated by the program NanoEngineer (www.nanoengineer-1.net). The purple rectangular domain lies above the green rectangular domain in both tiles. This arrangement leads to the woven pattern shown on the right when the two tiles are combined. In the green domain, there is a cavity drawn horizontally in the A tile and vertically in the B tile. Except for the sticky ends, the two tiles are the same. b) Atomic force micrographs of the A tile. Individual tiles are seen to form the crosslike structures shown in (a). The inset emphasizes the overlap of the two domains either side of the cavity: the cavity is visible and flanked clearly by the white region, which represents a thicker system (see the Supporting Information).

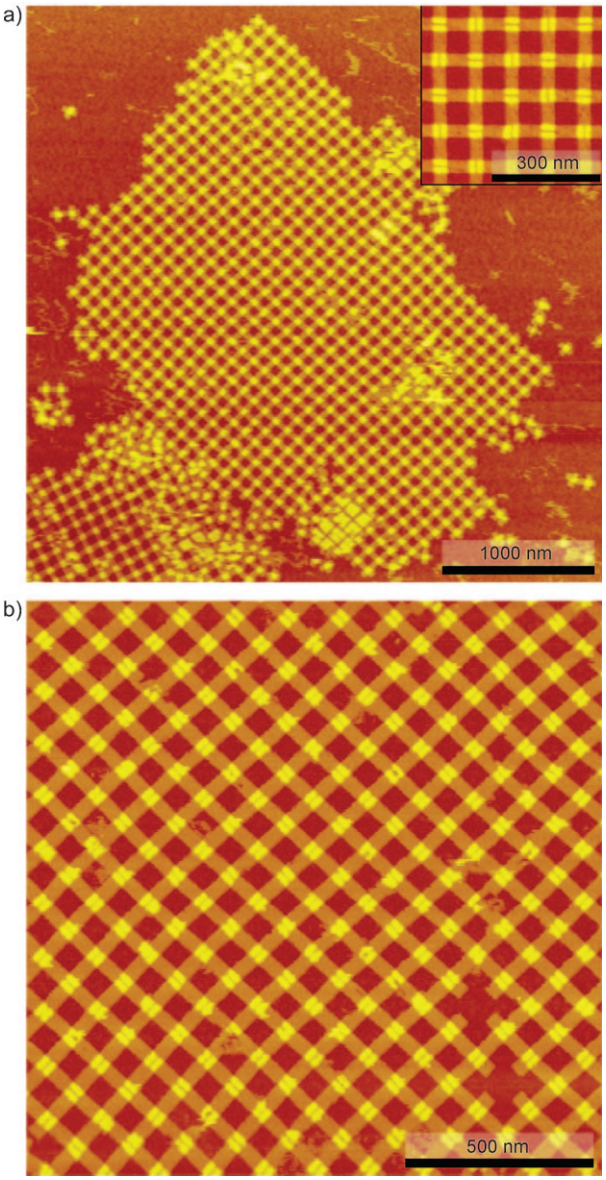

Figure 3. AFM image of a two-dimensional origami array. a) View of an array with dimensions of about $2\times3\ \mu m^2$. This array is one of the cleanest that we have observed, but it is certainly not the largest: the array dimensions can reach nearly 10 μm (see Figure S4 in the Supporting Information). The edges of this array are often straight, but they do demonstrate the steplike features typical of a growing lattice. b) Magnified view of the array in (a) showing the high quality of the array and the alternating cavity-flanking features (see also the magnified image in the inset in (a)).

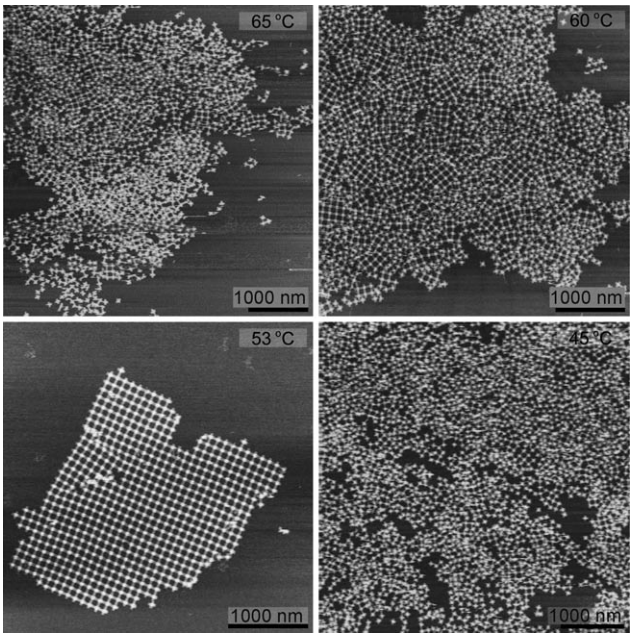

Figure 4. Optimization of the temperature for the formation of 2D arrays. It is evident from these AFM images that only annealing of the tiles with a starting temperature of 53 °C was successful.

origami tiles with each other at 45, 53, 60, and 65 °C; only annealing at 53 °C was successful (Figure 4).

Rothemund[8] created a map of the Western Hemisphere with DNA origami based on M13. The image of the structure clearly showed about 85 patterned pixels, which occupied about 40 % of the visible area. Periodic repeats containing 8 DNA small tile units have already been constructed.[7] Algorithmic self-assembly[10,11] can lead to even greater diversity and pattern complexity. The ability to form 2D crystalline arrays of DNA origami tiles expands greatly the current capacity to generate bottom-up pattern complexity. Furthermore, without increasing the size of the scaffold strands, the successful assembly of the array reported herein suggests that it will be possible to make oligoorigami tiles in two dimensions, so that applications such as capturing[13] and assembly lines[14] are not frustrated by the limited sizes of single M13-based origami tiles. This study brings us to the point where the bottom-up organization of matter can meet the limits of the top-down organization of matter.[15] Thus, complexity generated by bottom-up construction might well be replicable on a large scale by routine methods used industrially.

Experimental Section

Design of the origami tiles: A circular single-stranded M13mp18 DNA genome (7249 nucleotides (nt) in length) is held together by 201 short staple strands to form the DNA origami tiles. These origami tiles contain two identically sized rectangular domains (one green and one purple in Figure 2, 288 nt long and 12 helices wide) that sit on top of one another at an angle of 90°; consequently, the tile has an aspect ratio of 1:1 (ca. $95\times95\ nm^2$). The two rectangular domains have opposite orientations relative to the tile plane (one domain is face-up, and the other is face-down), and they are connected to each other by the M13 scaffold strand; they are also connected by 18 joint staple strands in the middle of the tile to enforce a 90° angle between the two domains. The M13 scaffold strand is designed to contain two-nucleotide single-stranded spacers to maintain flexibility at joints between the two domains. We also inserted one or two thymidine spacers into the joint staple strands for the same purpose.

For the assembly of the origami tiles into 2D arrays, the basic tile was modified by adding sticky ends to each branch of the tile to form two complementary tiles, A and B. Thus, these two tiles have an axis

of two-fold rotational symmetry perpendicular to the tile plane. Each tile domain contains one set of sticky ends, and each set is composed of eight different 5-nt sticky ends that were designed by using the program SEQUIN.[16] The sticky-end associations are indicated by different colors and complementary numbers in Figure 2a.

Formation and assembly of the tiles: Individual tiles were assembled by thermal annealing of the mixture of the M13 scaffold DNA and all staple strands, including the purified sticky-end strands, in 1 × Tris/acetate/EDTA/Mg^{2+} buffer (TAE–Mg^{2+}, pH 8.0; Tris = 2-amino-2-hydroxymethylpropane-1,3-diol, EDTA = ethylenediaminetetraacetic acid) from 90 to 16 °C over the course of 13 h in a thermocycling device. The origami tiles were formed as designed in high yield with a highly homogenous shape. The surface plot of one origami tile (Figure 2b, inset) clearly shows a double-layer structure with one domain lying at 90° to the other. The AFM height profile of the origami tiles (see Figure S3 in the Supporting Information) revealed that the average tile size is around 100×100 nm^2, and the height of the central part of the tile is roughly twice the height of the surrounding part of the tile. These dimensions are in good agreement with the design.

Two-dimensional array formation: The formation of the DNA origami 2D array was carried out through a two-step annealing process. In the first step, the two tile types were formed separately by mixing M13 scaffold strands with their component staple strands, including purified sticky-end strands, and annealing from 90 to 16 °C in a chilling/heating incubator (ECHOthermTM IN35) over 178.5 h (see the Supporting Information).

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