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DNA Prism Structures Constructed by Folding of Multiple Rectangular Arms

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Predesigned and controlled self-assembly to make desired structures is one of the ultimate goals of supramolecular chemistry and a key approach for bottom-up nanotechnology.[1,2] Oligodeoxyribonucleotides are among the most promising molecules for preparing various self-assembled components and large scaffolds for the production of complicated patterns as well as for placing and arranging functional molecules and nanomaterials.[2−5] DNA nanotechnology has made it possible to construct not only two-dimensional (2D) structures but also three-dimensional (3D) structures.[3,6] The novel DNA self-assembly system reported by Rothemund, so-called "DNA origami", is an attractive strategy for constructing fully addressable 2D plates and making a wide variety of designs for 2D structural patterns.[7] Selective positioning of functional molecules and nanoparticles on the DNA origami scaffold system has been achieved.[8] In addition, six-helix bundled tube and box structures formed using the DNA origami method have been reported.[9] Therefore, the method is valuable for the preparation of predesigned 3D DNA structures with limited numbers of base pairs, which leads to the defined assembly of the middle-sized nanostructures.

In this work, we have combined the DNA origami method for the preparation of 2D DNA scaffolds with folding into 3D structures through connection strands on the 2D structures (Figure 1). We designed three novel DNA origami scaffolds whose multiple arms branched from the center of the 2D structures. Three-, four-, and six-arm DNA origami structures having a total of 24 double helices were designed, and staple strand sequences were assigned. These rectangular arms were connected by a template M13mp18 single-stranded DNA (ssDNA) (Figure 1A). Figure 1B shows the case of the four-arm DNA origami structure having connection strands for 3D folding, in which nine strands were introduced to one side of each arm to connect to the adjacent arm. For connection with a counterpart site on the adjacent arm, two thymidines (T_2) were added to the connection strands as linkers for flexibility during hybridization of the connection strands. The hollow 3D prism structures were designed to consist of a corresponding number of double helices on their sides, which means that three (eight helices), four (six helices), and six (four helices) rigid rectangles (24 helices total) were connected by flexible hinges (Figure 1).

DNA origami formation was carried out using M13mp18 viral ssDNA and 226 DNA strands (staple strands) in a buffer containing HEPES (pH 7.5), EDTA, and Mg^{2+}. The mixtures were annealed from 95 to 15 °C by decreasing the temperature at a rate of 1.0 °C/min, after which the DNA structures were observed in the same buffer solution using atomic force microscopy (AFM).

AFM images of multiarm DNA structures after annealing are shown in Figure 2. The rectangular arms were observed from three kinds of DNA origami. Y-shaped, X-shaped, and asterisk-shaped structures formed via self-assembly by annealing were observed, and almost all

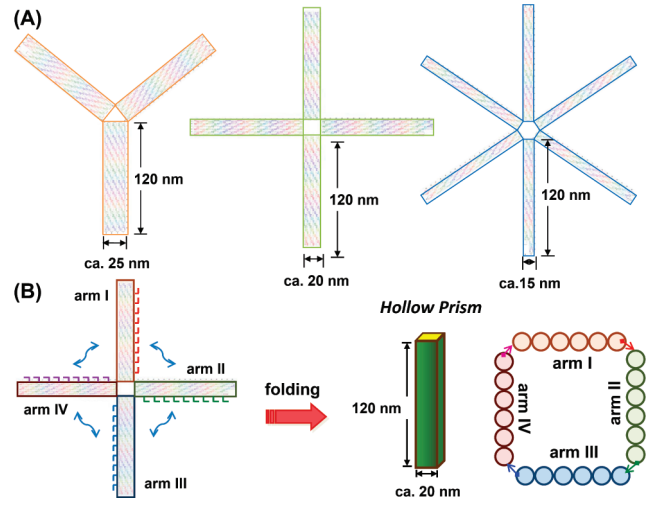

Figure 1. Multiple-arm DNA structures and the preparation of DNA hollow prism structures. (A) Multiple-arm DNA structures: three-arm (Y-shaped), four-arm (X-shaped), and six-arm (asterisk-shaped) structures. (B) Scheme for the folding of multiple-arm DNA structures into hollow prism structures by introduction of connection strands (indicated by "¬" on the DNA arms).

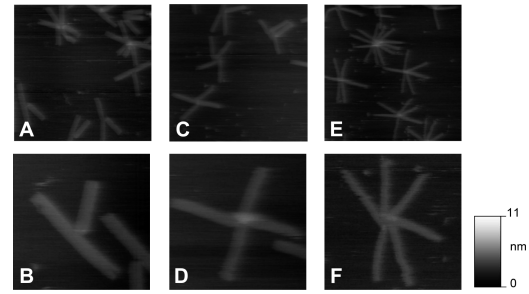

Figure 2. AFM images of multiple-arm DNA origami structures: (A, B) three-arm (Y-shaped) structure; (C, D) four-arm (X-shaped) structure; (E, F) six-arm (asterisk-shaped) structure. Image sizes: (A, C, E) 750 × 750 nm; (B, D, F) 300 × 300 nm.

of the DNA assemblies formed these shaped structures. The rectangular arms maintained their planar structures, and the structures had the corresponding numbers of arms, but it was difficult for all of the imaged structures to keep their designed shapes indicated in Figure 1A because of the flexibility of the single-stranded linkage of the center connection. The sizes of the rectangular arms in the three-, four-, and six-arm structures were (127 ± 4) × (32 ± 3) nm, (125 ± 3) × (27 ± 3) nm, and (129 ± 5) × (20 ± 2) nm, respectively, which are consistent with the original design.

To subsequently fold these 2D structures into 3D structures, we introduced connection strands into the multiarm structures (Figure 1B). These consisted of 24-mer and 8-mer DNA moieties and a T_2 linker inserted between these DNA moieties. After the annealing process,

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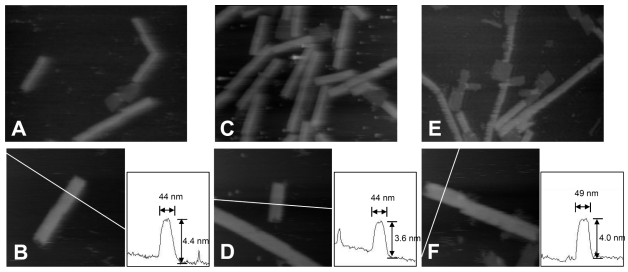

Figure 3. AFM images of the folded DNA structures assembled from (A, B) three-arm, (C, D) four-arm, and (E, F) six-arm 2D structures possessing connection strands. Image sizes: (A, C, E) 1000 × 750 nm; (B, D, F) 375 × 375 nm.

the samples were analyzed by AFM in the solution (Figure 3). After the introduction of the connection strands on the arms and annealing, no multiarm structures were observed, and linear structures appeared. All three types showed extended structures in which multiple units connected and extended to the long axis. The typical cross-sectional analyses of the assemblies are shown in the bottom lines of Figure 3. The DNA assemblies from different numbers of arms showed uniform length, width, and height. The dimensions of the height (3.6−4.4 nm) and width (43−51 nm) of the individual units show that the height was almost two layers of the double helices and the width was longer than half the total length of the short edges of the rectangles (∼70 nm) in the 2D structures. Detailed analysis of the surface of the 3D structure showed that there is a groove along the long axis. The analysis results indicate that the observed structures are similar to the crushed tubular structures reported in DNA tube formation.[10] The lengths of the three kinds of tubular structures were 115 ± 8 nm/unit, corresponding to the length of the long edge of the rectangles of the arms (∼120 nm). The widths of the 3D assemblies from the three-, four-, and six-arm structures were 45 ± 2, 44 ± 2, and 49 ± 2 nm, respectively. The numbers of aligned DNA units combining to form extended structures increased in going from the three- to the four- to the six-arm structure assemblies, indicating that the overlapping of the ends of the assemblies was better for the hexagonal end than for the triangular and square ends. From these observations, the DNA structures observed here exhibited features of DNA tubular structures. Cryo-EM analysis of the 3D structure of the assembly from the four-arm structure revealed the relatively flexible hollow tubular structure (Figure S1 in the Supporting Information). The sides of the 3D structures consist of rigid planar origami rectangles, and therefore, the 3D assemblies constructed here should have the defined sizes of hollow prism structures with the corresponding number of side walls.

The prism-opening process was observed by high-speed AFM imaging, which can successively acquire one AFM image per second.[11] Successive scanning of the sample forced the closed structures to open, forming the 2D structures (Figure 4). The prism structures opened with different numbers of scans, depending on the individual prisms. Images of a typical opening event of a single triangular prism are shown in Figure 4A. Morphological changes occurred in 2−30 s, which is a detectable time scale for our AFM instrument. The prism was torn in the middle of the surface, and then the sides of opened prism adsorbed onto the mica surface. Interestingly, the opened prisms never reverted to their original multiarm structures. After opening, the 2D structure formed a single rectangle with dimensions of ∼130 × 90 nm. A DNA strand can be observed on the opened 2D structure (orange arrows). We propose that this DNA strand is the M13 single strand located near the center of the three rectangles that dissociated from the complementary staple strands to form a relaxed planar rectangle. This means that if one connection edge between arms on the surface of the prism is torn by physical force, the multiple connection strands maintain adhesion of other arms without returning to the original multiarm 2D

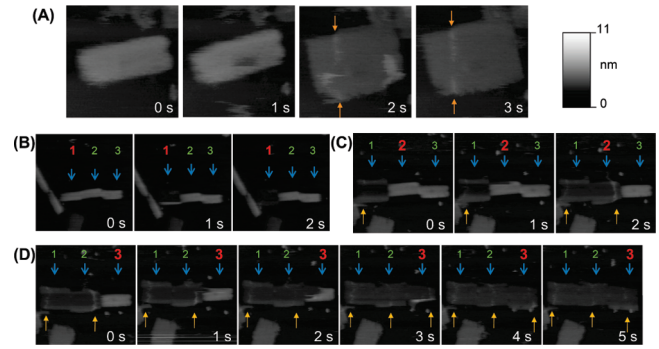

Figure 4. Process of prism structure opening. (A) AFM images of the opening of a single triangular prism. (B−D) Sequential opening of three successive square-prism structures; (B), (C), and (D) correspond to the tube-opening events for the first, second, and third prism units. The zero of time (0 s) is defined as one image (1 s) before the obvious morphology change. Image sizes: (A) 150 × 150 nm; (B) 500 × 500 nm; (C, D) 300 × 300 nm.

structure. The same opening events were observed in the case of three successive square prisms (Figure 4B−D). The first and second prisms opened within 2 s; on the other hand, the third opened in 5 s. These opening events may depend on the mode of attachment of the prisms on the mica surface as well as on other physical factors such as the ion strength and the force from the AFM tip. In fact, increasing the tapping force led to much easier prism opening.

A novel method for the preparation of hollow DNA prisms using the multiarm DNA origami system and connecting strands that convert 2D DNA structures into prism forms has been demonstrated. The introduction of the connection strands into the 2D structures may be a straightforward method for causing designed 2D structures to fold into various 3D forms.

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

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