

Three-dimensional DNA nanostructures constructed by folding of multiple rectangles

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ABSTRACT

The novel multi-arm DNA structures were designed using 2D-DNA Origami method, and these structures were folded into 3D hollow prism structures by introduction of connection strands into the arms. The opening of the prism structures were examined by a high-speed AFM, which showed the dissociation events of the connecting arms in the 3D-structures.

INTRODUCTION

Controlled self-assembly for making desired structures is one of the ultimate goals of the supramolecular chemistry and a key approach for the bottom-up nanotechnology. Oligodeoxyribonucleotide is one of the most promising molecules for preparing various self-assembled components and large scaffolds for the production of complicated patterns, and placing and arranging functional molecules and nanomaterials.¹ DNA nanotechnology has made it possible to construct not only 2D-structures but also 3D-structures.^{1,2} The novel DNA self-assembly system reported by Rothemund called “DNA Origami” is an attractive and fascinating strategy for the construction of fully addressable two-dimensional plate and also making a wide variety of designs for the 2D-structural patterns.³ The method is valuable for the reparation of the predesigned 3D-DNA structures with limited numbers of base pairs, which leads to the defined assembly of the middle size nanostructures. Herein, we combined the DNA Origami method for the preparation of 2D-DNA scaffolds and folding by connection strands on the 2D-structures into 3D-structures (Fig. 1).

RESULTS AND DISCUSSION

We designed the three novel DNA Origami scaffolds, whose multiple arms are branched from the center of the 2D-structures. Three-, four-, and six-arm DNA Origami structures having total 24 double helices were designed, and the staple strand sequences were assigned. These rectangle arms are connected by a template M13 single strand DNA (Fig. 1A). Fig. 1B shows the case of four-arm DNA Origami structure having connection strands for 3D-folding, in which nine connection strands were introduced to one side of each arm. The connection strands are complementary to the strands on the adjacent arm, which

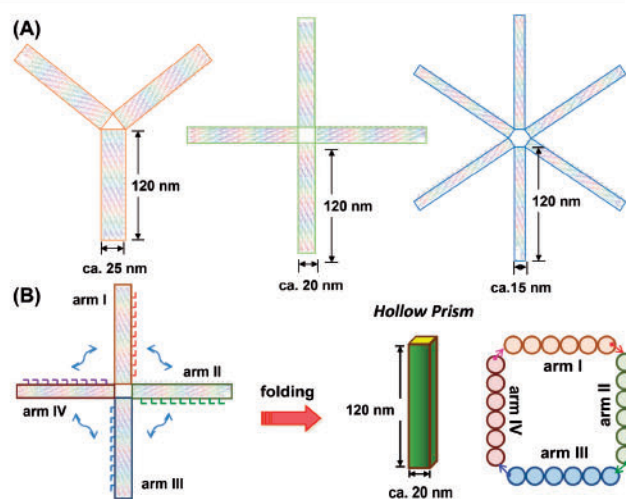


Fig. 1 Multiple-arm DNA structures and the preparation of DNA hollow prism structures. (A) Multiple-arm DNA structures: three-arm (Y-shape), four-arm (X-shape), and six-arm (asterisk-shape) structures. (B) The scheme for the folding of multi-arm DNA structures into a hollow prism structure by adding the connection strands (“ \rightarrow ” on the DNA arms).

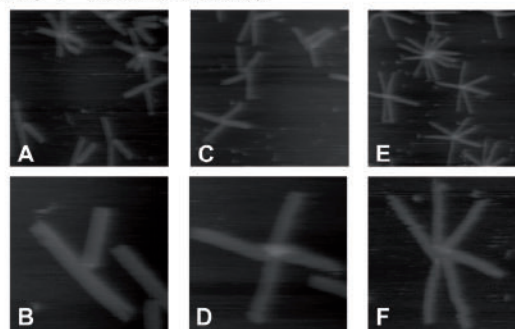


Fig. 2 AFM images of multi-arm DNA Origami structures. Three-arm (Y-shape) (A,B), four-arm (X-shape) (C,D), Six-arm structures (asterisk-shape) (E,F). The image size: A, C, E, 750 x 750 nm; B, D, F, 300 x 300 nm.

are located in the same distance from the center of the 2D-structures. For connection with a counterpart site on the adjacent arm, two thymidines were added to the connection strands as a linker for flexibility during hybridization of the connection strands. The designed hollow 3D-prism structures consists of corresponding number of double helices in the sides, which means that three- (8 helices), four- (6 helices), and six- (4 helices) rigid rectangles (total 24 helices) are connected by flexible hinges (Fig. 1).

DNA Origami formation was carried out by using M13 viral single strand DNA and DNA strands (staple strands) in a buffer containing HEPES (pH 7.5), EDTA, and Mg^{2+} . The mixtures were annealed from 95 °C to 15 °C (-1.0 °C/min). After annealing, the DNA structures were observed by AFM in a same buffer solution.

AFM images of multi-arm DNA structures after annealing are shown in Fig. 2. The rectangle arms were observed from three kinds of DNA Origami. Self-assembly by annealing, Y-shape, X-shape, and asterisk-shape structures were observed, and almost all DNA assemblies formed these shaped structures. The rectangle arms maintained their planar structures and the structures had the corresponding numbers of the arms, whereas all the imaged structures were difficult to keep their designed shape indicated in the Fig. 1A because of the flexibility of the single strand linkage of the center connection. The sizes of the rectangle arms in the three-, four-, and six-arm structures are $127 \pm 4 \times 32 \pm 3$ nm, $125 \pm 3 \times 27 \pm 3$ nm, and $129 \pm 5 \times 20 \pm 2$ nm, respectively, which agree to the original design.

These structures were further folded into 3D structures. We introduced the connection strands which consist of 24mer and 8mer DNA parts and a T_2 linker inserted between these DNA parts. After the annealing process, the samples were analyzed by AFM in the solution (Fig. 3). By the introduction of the connection strands on the arms and annealing, no multi-arm structures were observed, and linear structures appeared. All the three types of the structures showed the extended structures, in which multiple units connected to the long axis. The typical cross sectional analyses of the assemblies are shown in the bottom lines of Fig. 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 is almost two layers of the double helices and the width is longer than a half of the total length of the short edges of rectangles (ca. 70 nm) in the 2D structures. The detailed analyses of the surface of the 3D structure showed that there is groove along the long axis. These analysis results indicate that the observed structures are similar to the cracked tubular structures reported in the DNA tube formation.⁴ The length of three kinds of tubular structures are 115 ± 8 nm/unit, which corresponds to the length of the

long edge of the rectangles of the arms (ca. 120 nm). The widths of the 3D-assemblies from the three-, four-, and six-arm structures are 45 ± 2 nm, 44 ± 2 nm, and 49 ± 2 nm, respectively. The numbers of aligned DNA units into extended structures increased in the order of assemblies from three-, four-, and six-arm structures, indicating that the overlapping of the ends of the assemblies is better for the hexagonal end than for the triangular and square ends. From these observations, the DNA structures observed here exhibited the features of DNA tubular structures. The sides of the 3D-structures are rigid and planar Origami rectangles, therefore, the 3D-assemblies constructed here are defined size of hollow prism structures having the corresponding number of side walls.

Tube opening process was observed by high-speed AFM imaging, which can successively obtain one AFM image for one second.⁵ Successive scanning of the sample forced to open the closed structures to the 2D-structure (Figure 4). The prism structures completely opened with different scanning numbers from one time to 30 times depending on the individual prisms. The prism was torn in the middle of the surface, and the sides of prism adsorbed onto the mica surface. Interestingly, the opened prism never went back to their original multi-arm structures. After opening, the 2D-structure formed single rectangle with ca. 130×90 nm. DNA strand can be observed on the opened 2D structure. These opening events may depend on the way of attachment of the prisms on the mica surface and other physical factors such as the ion strength and the force from the AFM tip.

CONCLUSION

A novel method for the preparation of hollow DNA prisms has been demonstrated using the multi-arm DNA Origami system and the connecting strands which converts the 2D DNA structures into prism forms. The introduction of the connection strands to the 2D structures can be a straightforward method to fold the designed 2D structures into the various 3D-forms.

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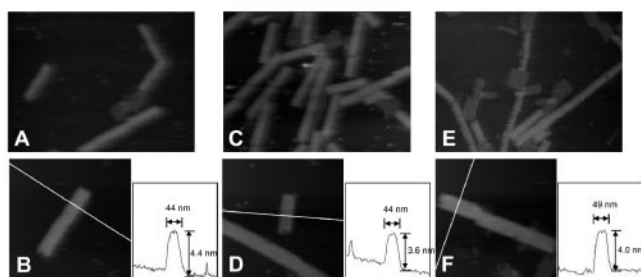


Fig. 3. AFM images of the folded DNA structures. AFM images of (A,B), (C,D), and (E,F) show the 3D-structures assembled from three-arm, four-arm, six-arm 2D structures possessing connection strands. Image size: (A)(C)(E) 1000 x 750 nm; (B)(D)(F) 375 x 375 nm.