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COMMUNICATION

Direct AFM observation of an opening event of a DNA cuboid constructed *via* a prism structure†

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A cuboid structure was constructed using a DNA origami design based on a square prism structure. The structure was characterized by atomic force microscopy (AFM) and dynamic light scattering. The real-time opening event of the cuboid was directly observed by high-speed AFM.

The precise creation of desired structures by controlled self-assembly is one of the ultimate goals of supramolecular chemistry and is a key approach in bottom-up nanotechnology.[1,2] In the field of DNA nanotechnology, various methods have been developed for preparing self-assembled structures and scaffolds for producing complicated patterns and for placing and arranging functional molecules and nanomaterials.[2–6] A novel DNA self-assembly system, the "DNA origami method", is an attractive technology for constructing a wide variety of 2D structures[6–7] and designing the programmed assembly of the components.[8] Selective positioning of various functional molecules and nanoparticles has been achieved using the DNA origami scaffold system.[9] In addition, box and multilayered structures constructed using the DNA origami method have been reported.[10] Therefore, the method is valuable for preparing designed 3D DNA structures with limited numbers of base pairs, which facilitates the defined assembly of medium-sized nanostructures. We recently described DNA prism structures constructed by folding multiple rectangular plates that were designed using the DNA origami method.[11]

In this study, we used the DNA origami method to prepare rectangular DNA origami plates that were folded using connection strands on the 2D structures into 3D box structures about 36 × 36 × 44 nm in size (Fig. 1). Our method was based on an extension of the method used to design prism structures.[11] We designed six rectangles, of which the four central rectangles formed the prism structure and the two square plates at the ends formed the top and bottom plates (Fig. 1b). Fig. 1a shows the six rectangular plates

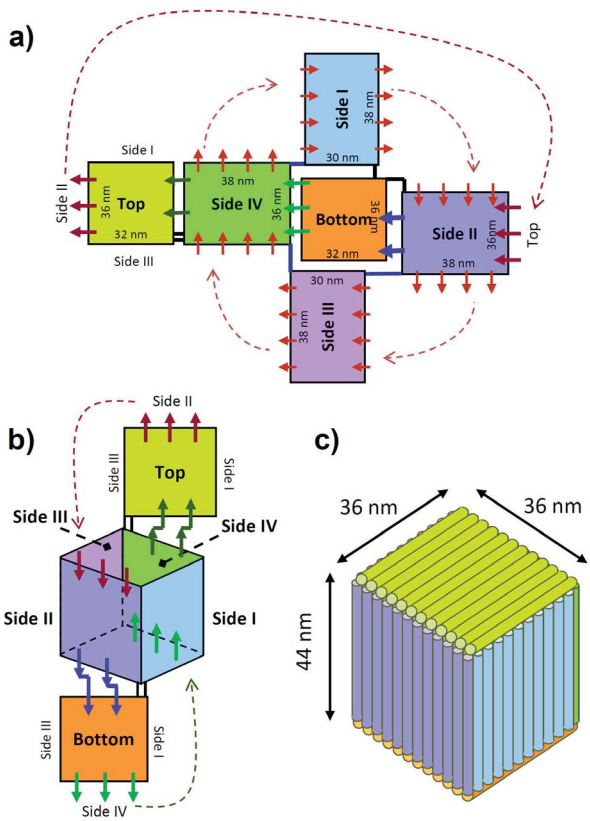

Fig. 1 DNA cuboid structure. a) The cuboid structure was constructed using six rectangular origami plates. b) The square prism structure and the top and bottom origami plates were folded, as shown, to construct the cuboid structure. c) Completed cuboid structure.

and the connection strands that were introduced at the sides of the top and bottom plates to enable the prism structure to be closed. The sizes of the rectangular plates were 32 × 36 nm for the top and bottom plates, 38 × 36 nm for the larger side plates, and 38 × 30 nm for the smaller side plates. The connecting strands were complementary to the strands on the adjacent rectangular side plates (sides I–IV). To connect adjacent plates, two thymidine (T_2) linkers were added to the connecting staple strands, constituting "flexible hinges" for their self-assembly.

The self-assembly of the 2D and 3D structures was carried out using M13mp18 single-stranded DNA and designed staple strands in a buffer containing Tris-HCl (pH 7.6), EDTA, and Mg^{2+}.[12] The

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mixtures were annealed from 85 °C to 50 °C by decreasing the temperature at a rate of 1.0 °C min^{-1}, and then by decreasing the temperature at a rate of 0.1 °C min^{-1} from 50 °C to 15 °C. After annealing, the DNA structures were observed in the same buffer solution using AFM.[13,14]

To confirm the formation of six rectangular origami plates, the self-assembly was done without connecting strands. AFM images of DNA structures after the annealing are shown in Fig. 2a,b. Six rectangular plates are clearly observed.

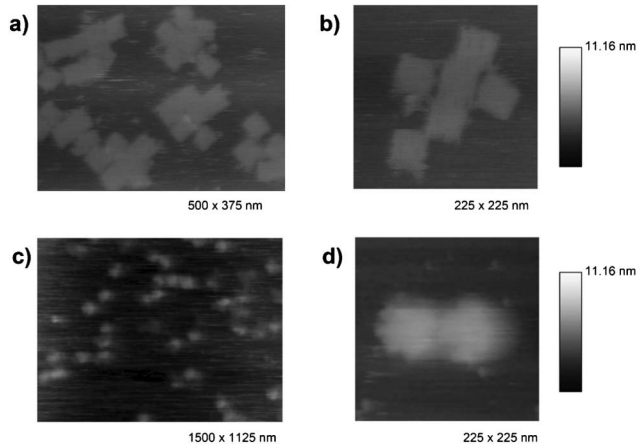

Fig. 2 AFM images of 2D and 3D structures. a), b) Six rectangular origami plates without connecting strands between the plates. c), d) Folded 3D structures with connecting strands between plates. Image sizes are indicated below the images.

To fold the 2D structures into 3D structures, we introduced connection staple strands into the rectangular plates (Fig. 1a,b), in which a T_2 linker was inserted at the side edges. After the annealing with connecting strands, the samples were analyzed using AFM (Fig. 2c,d). After the introduction of connecting strands on the plates, particle-like structures were observed, as reported in previous AFM studies of DNA boxes.[10b] Cross-sectional analyses of the assemblies are shown in Fig. S2.† The height of the assemblies varied around 4–8 nm. As the height of the box structures was not evident using AFM imaging because of its softness and tapping force, the sizes of the 3D assemblies were estimated using dynamic light scattering (DLS). DLS analysis showed that the structure was almost monodisperse (89%), and the diameter was about 68 nm (Fig. S3†). This size was similar to the designed size (67 nm). AFM and DLS strongly indicate that a 3D DNA structure with cuboid features corresponding to the desired structure (Fig. 1) has been formed.

We next observed the box opening event using a high-speed AFM imaging system that is capable of successively acquiring one AFM image per second.[14] Successive scanning of the sample forced the closed structures to open and converted them to 2D structures (Fig. 3 and Fig. S4†).[11] Although the opening of some structures was incomplete and some of the opened structures seemed damaged, we observed that many of the fully opened structures had six rectangular plates (Fig. S4†). Typical images captured during the opening of a box structure are shown in Fig. 3. The morphologic changes occurred over 10 s, which is a detectable time scale for our AFM instrument. Fig. 3 shows that the opening of the box began with the opening of one side of the box. Another side opened 3 s thereafter, and this structure was maintained until 9 s after the initial event. At 10 s, the third and fourth plates opened and all the six plates had opened 11–12 s after the initial event. AFM enabled us to identify the individual plates involved in the opening process. Because we designed sequential connections between the plates, the relative positions of the plates in the box structure could be identified. The four plates in the square prism opened to reveal plates II, III, I and IV and finally the top plate attached to the mica surface. This analysis shows that the positions of the six plates of this box structure are same as the design shown in Fig. 1. The number of scans for opening a single DNA box was

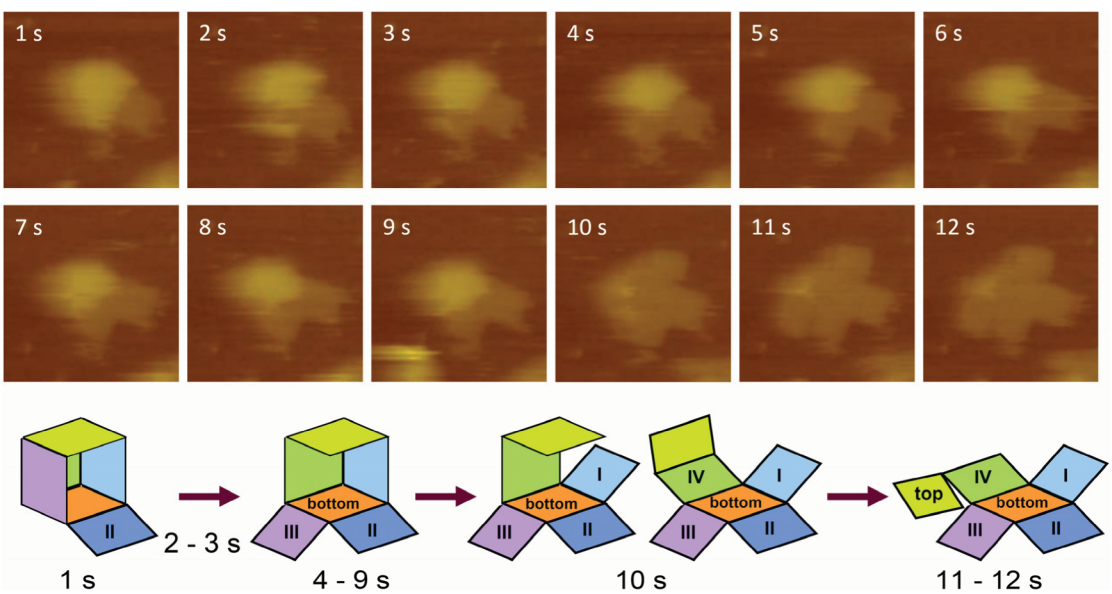

Fig. 3 Stepwise changes in the morphology of a cuboid structure. a) High-speed AFM images of the structural changes in a single box. b) Putative sequence of the unfolding of the box. The scanning speed was one image per second. The scale of the images is 200 × 200 nm.

varied depending on the experiment, and it is unclear what the driving force for the opening events of the box structure is. The opening events may depend on the mode of attachment of the box structures on the mica surface and physical factors such as the tapping force from the AFM tip (Fig. S5†).

The approach for the preparation of the 3D structure here is conceptually similar to the former studies.[10b,10d,11] The introduction of connection strands into 2D structures is an easy method of folding designed 2D structures into various 3D forms. Methods of controlling the closing and opening processes of DNA cuboid structures would enable manipulation of nanostructures.

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12 The sample solution (50 μL) containing 0.01 μM M13mp18 single stranded DNA (New England Biolabs), 0.05 μM staple DNA strands (5 eq), 20 mM Tris-HCl (pH 7.6), 1 mM EDTA, 10 mM $MgCl_2$ was annealed from 85 °C to 50 °C at a rate of –1.0 °C min^{-1}, and then from 50 °C to 15 °C at a rate of –0.1 °C min^{-1} using a thermal cycler.
13 AFM images were obtained on an AFM system (Nano Live Vision, RIBM, Tsukuba, Japan) using a silicon nitride cantilever (resonant frequency = 1.0–2.0 MHz, spring constant = 0.1–0.3 N m^{-1}, EBDTip radius < 15 nm, Olympus BL-AC10EGS-A2). The sample (2 μL) was adsorbed on a freshly cleaved mica plate for 5 min at rt, and then washed three times with the same buffer solution. Scanning was performed in the same buffer solution using a tapping mode.
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