DNA Nanotubes

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DNA Tube Structures Controlled by a Four-Way-Branched DNA Connector**

Masayuki Endo, Nadrian C. Seeman, and Tetsuro Majima**

The programmed self-assembly of molecular building blocks into desired structures is one of the most fascinating challenges in the field of supramolecular chemistry, and the basic methodology is also applicable for the creation of nanoscale materials.[1–3] Double-stranded DNA is a promising candidate for achieving the desired structural formation and arrangement, because of the reliable molecular assembly based on the base-pairing system and well-defined periodic structure of the double helix DNA. Structurally controlled crossover DNA motifs, called "DNA tiles", have been used as building blocks for creating one- and multi-dimensional nanostructures.[4–7] Recently, by utilizing these crossover DNA molecules, extended structures such as tube structures have been created.[8–10] Further extension of the design of desired DNA structures could be achieved by employing various chemically modified oligonucleotides.

Here we report a novel method for preparation of structurally controlled DNA tubes by using a DNA tile system[4–7] with the assistance of a four-way-branched DNA connector. Branched DNA can assemble multiple double helices by duplex formation.[11] In this study, we designed and synthesized a DNA–porphyrin connector, Porph-$(Tc)_4$ **1**, in which 10-mer DNA strands are connected to four spacers of a tetraphenylporphyrin derivative (Figure 1 a). We also employed the DNA tile system which can assemble two planar DNA tiles (tile A and tile B) into two-dimensional (2D) array structures by using the geometry of 2.5 helical turns between two DNA tiles (Figure 1 b and the upper part of

[*] Dr. M. Endo, Prof. Dr. T. Majima
The Institute of Scientific and Industrial Research
Osaka University
8-1 Mihogaoka, Ibaraki, Osaka 567-0047 (Japan)
Fax: (+81) 6-6879-8499
E-mail: endo@sanken.osaka-u.ac.jp
majima@sanken.osaka-u.ac.jp
Prof. Dr. N. C. Seeman
Department of Chemistry, New York University
New York, NY 10003 (USA)

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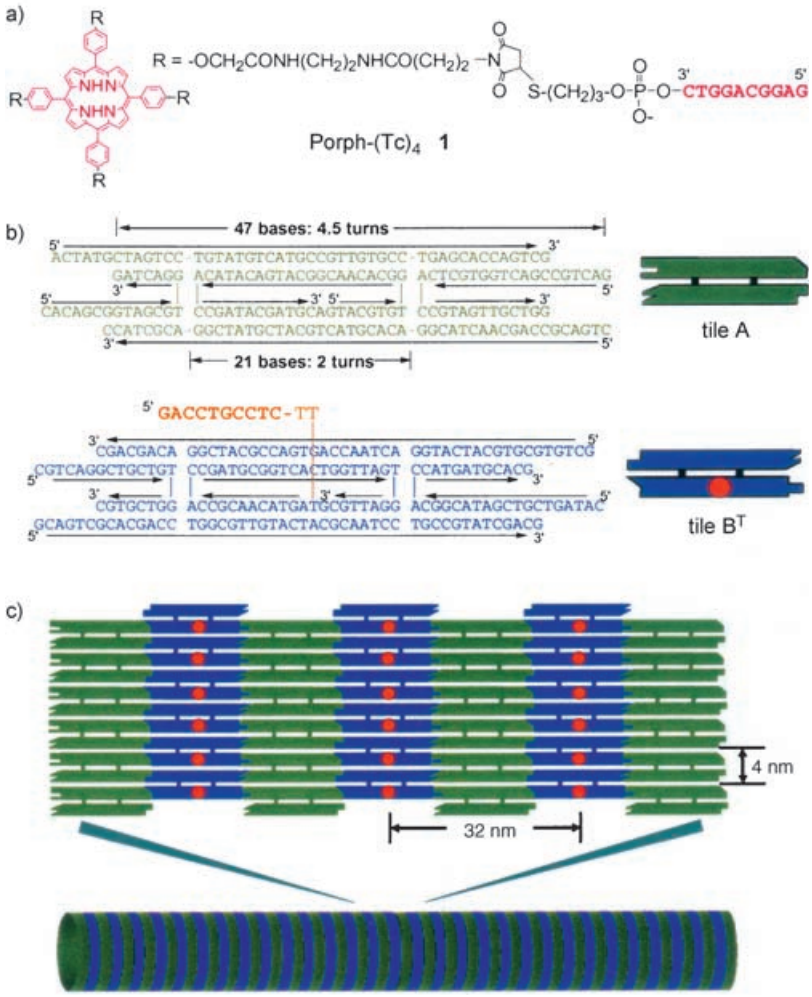

Figure 1. The DNA–porphyrin connector and the DNA tiles system employed in the experiment. a) Structure of the DNA–porphyrin conjugate, Porph-$(Tc)_4$ **1**. b) The sequences of DNA tile A (green) and DNA tile B^T (blue), which has an extra single strand (orange sequence). The orange dot on tile B represents the extra single strand. c) Two-dimensional DNA array prepared from the tiles A and B^T (top) and the three-dimensional DNA tube structure formed in the presence of Porph-$(Tc)_4$ **1** (bottom).

Figure 1c).[4–7] The center strand of tile B^T has an extra 12-mer single strand (T = tag strand) that has 10 bases as a recognition sequence for hybridization with a complementary DNA strand (Tc = complementary to the tag strand) and two additional thymidines as a linker. We planned to assemble the four B^T tiles by using the DNA–porphyrin connector, which captures and brings multiple B^T tiles together by hybridizing with the extra tag strands of the B^T tiles. The four neighboring tiles constrained by the DNA–porphyrin connector could then induce tube formation during assembly with the A tiles. The length between the center of the porphyrin and the 5′-end of the DNA strand in the DNA–porphyrin connector is 7–8 nm (Figure 1a), which could allow the alignment of the four short axes of the B^T tiles side by side (total 16 nm) for A–B^T array formation.

DNA–porphyrin conjugate **1** was synthesized by coupling the tetramaleimide-linked tetraphenylporphyrin with a 3′-thiol-modified 10-mer DNA strand (Tc) that is complementary to the tag strand of the tile B^T (see Supporting Information). Ten DNA strands and Porph-$(Tc)_4$ **1** were mixed together and annealed from 95 °C to room temperature for 36 h in a buffer containing 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES; pH 7.5), ethylenediaminetetraacetate (EDTA), and Mg^{2+}.[12]

After complex formation, we observed the DNA nanoscale structures by using atomic force microscopy (AFM) in solution.[12] In the case of annealing with tiles A and B^T only, 2D DNA arrays were obtained which were similar to those previously described with the A–B array system (Figure 2a).[6,7] By contrast, with addition of 1/4th of an equivalent of Porph-$(Tc)_4$ **1** connector and annealing with tiles A and B^T, the large 2D structures were not observed and fiber-like structures appeared (Figure 2b), the lengths of which reaching over 20 μm. When 1/16th of an equivalent of **1** was annealed with tiles A and B^T, we obtained a mixture of fibers and the usual 2D arrays (see Supporting Information). Thus, the formation of fiber structures depended on the stoichiometry between the tiles and Porph-$(Tc)_4$ **1**.

To characterize the detailed nanoscale structures, we analyzed the surface of the DNA fibers. A cross-section analysis of the long axis of the DNA fiber structure reveals

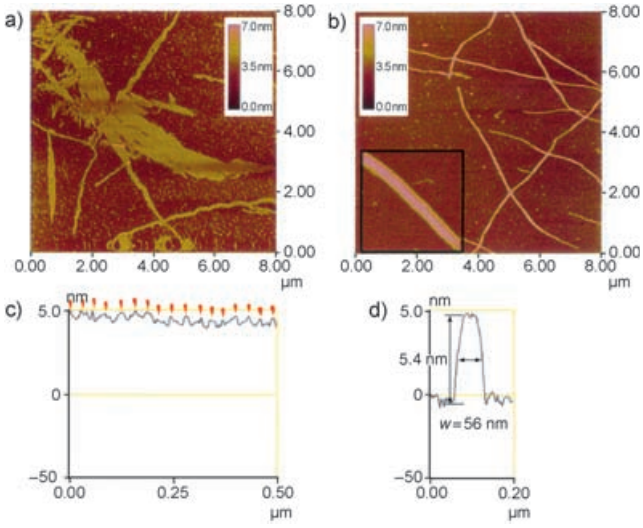

Figure 2. AFM images of the DNA structures. a) Annealing with tiles A and B^T. Image size: 8×8 μm². b) Annealing with tiles A and B^T in the presence of Porph-$(Tc)_4$ **1**. Image size: 8×8 μm². Inset: Expanded image of the DNA structure prepared from tiles A and B^T with **1**. Image size: 500×500 nm². c) Cross-section analysis of the long axis of the DNA structure shown in the inset of (b). Orange arrows represent peaks of the periodical stripes. d) Cross-section analysis of the short axis of the DNA structure shown in the inset of (b).

that periodic stripes are observed on the surface of the fiber (Figure 2c). The distance between two stripes was 29–34 nm, which corresponds to the total length of the long axis of the A and B tiles (32 nm; Figure 1c). Therefore, these stripes originated from the extra strand of tile B^T. In the case of the initial A–B^T 2D array without the addition of the connector **1**, stripes were not clearly observed, because the extra single strand (tag strand T) attached to the B^T tile was flexible (see Supporting Information). In contrast, the A–B* 2D array, which has hairpins on the 2D tile that work as topological markers, showed clear stripes, because the stable hairpins are oriented out of the plane of the 2D array as described previously (see Supporting Information).[6,7] These suggest that the strong stripe formation on the DNA fiber is induced by duplex formation between the tag strand of the tile B^T and its complementary strand (Tc) of the connector **1**. The individual DNA fibers showed uniform width (approximately 55 nm) and height (5.2–5.6 nm; Figure 2d). The height of the stripes was 0.3–0.8 nm. The analysis indicates that the height of the DNA fiber structures is larger than the two layers of the double helices. According to the cross-section analysis of the DNA fiber (Figure 2b, inset), the center of the top surface is slightly squashed by 0.2–0.3 nm as compared to both edges (Figure 2d).[13] From these observations, the DNA structures observed here exhibited the features of DNA tube structures, similar to those described in previous reports.[8,12,13] We conclude that the DNA structures obtained here are tube structures. In the present study, the locations of the DNA–porphyrin connectors in the DNA tube structures remain unclear. Casual inspection of the AFM images gives the impression that the DNA–porphyrin connectors are on the outside of the tube structures. However, from thickness measurements, we cannot exclude the possibility that they are on the inside, the circumstance that is most likely to lead to tube formation.

We also obtained DNA structures with a height lower than the tubes, and these two structures were located on the same DNA fibers (Figure 3a). We noted that in the detailed images of these lower-height structures (Figure 3b), each stripe on the DNA surface has two or three blocks of dotlike structures, which were characterized by a cross-section analysis of the stripes (red arrows in Figure 3d). Cross-section analysis for the long axis revealed that the stripes were separated by 29–34 nm (Figure 3c), which corresponds to the total length of the long axis of the A–B^T tiles as described previously. The height of the surface of the lower-height structures was 2.7 nm (Figure 3d). The height of the stripes was 0.3–1.0 nm, which is comparable to the stripes of the tube structures shown in Figure 2. A high-resolution AFM image of the DNA surface in the lower-height structures in a different area is shown in Figure 3e. Each stripe is separated and the individual A tiles can be observed to be the same as those of the A–B* arrays. In this lower-height section, the visible part of the array contains seven A tiles and a similar number of less well resolved B^T tiles as repeating units.

To examine the difference between the tube and lower-height structures, we analyzed the boundaries of these structures on the same fiber (Figure 3a), and two interesting features were observed: 1) The stripes in the tube and lower-height areas are successive without any gap. 2) The height of the lower-height DNA structures is clearly changed to almost half the height of the tube ones, and the width of the short axis of the lower-height structures (ca. 65 nm) is always larger than that of the tubes (ca. 55 nm) on the successive DNA structures. From these observations, we conclude that the lower-height areas on the DNA fibers are incomplete tubes with the height of single-layer duplexes, as described previously.[8,13]

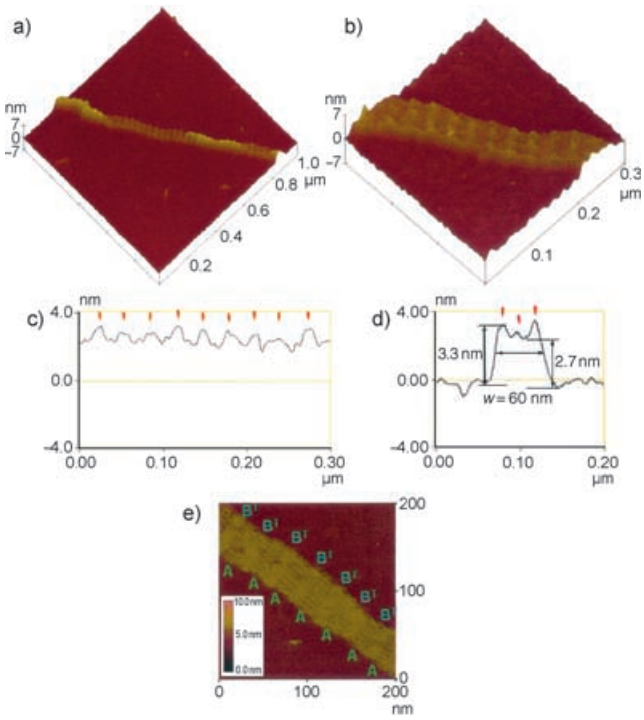

Figure 3. AFM images of the DNA structures. a) Mixed area of normal and lower-height DNA structures. Image size: $1\times1\ \mu m^2$. b) Expanded image of the lower-height section. Image size: $300\times300\ nm^2$. c) and d) Cross-section analysis of the lower-height area in Figure 3b for the long (c) and short (d) axes. Orange arrows in (c) represent peaks of the periodical stripes and red ones in (d) represent dotlike structures on the stripe of the lower-height area. e) High-resolution AFM image of the DNA nanostructure of the lower-height area. Image size: $200\times200\ nm^2$.

We estimated the complex-formation process by using the UV absorption change at 260 nm versus temperature. The duplex formation of Porph-$(Tc)_4$ **1** with its complementary strand occurs at 46 °C, which is slightly higher than the initial temperature of the A–B array formation (40–45 °C). This indicates that the complex formation between **1** and four B^T tiles precedes assembly of the A–B tiles, and then the constrained B^T tiles with **1** and the A tiles form some constrained array leading to the tube formation.

We have demonstrated a novel method for the preparation of DNA tubes, by using the A–B tile system and the four-way-branched DNA connector which converts DNA arrays into DNA tube structures. The DNA–porphyrin connector clearly restricted the extension to the short axis of the tile, while the long axis did not change as compared to that of the usual A–B tile system. We expect that the DNA tubes

prepared by using this method can be employed as nanoscale scaffolds for the preparation of structurally defined materials and devices.[13,14]

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[12] Complex formation was carried out in a solution (100 μL) containing 0.5 μM oligonucleotides (total 10 strands), 0.125 μM Porph-$(Tc)_4$ **1**, 10 mM HEPES (pH 7.5), 1 mM EDTA, and 5 mM $Mg(OAc)_2$. Samples were annealed from 95 °C to room temperature over 36 h in a 2-L water bath kept in a styrol box.[7] A sample (4 μL) was deposited on a freshly cleaved mica plate and left for 1 min to adsorb onto the surface. After addition of a solution (30 μL) containing 10 mM HEPES (pH 7.5), 1 mM EDTA, and 5 mM $Mg(OAc)_2$ in a fluid cell and another 30 μL of the same solution onto the AFM tip, images were acquired on a Digital Instruments NanoScope IV atomic force microscope by using the tapping mode.
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