Six-Helix and Eight-Helix DNA Nanotubes Assembled from Half-Tubes

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SUPPLEMENTARY MATERIAL

This supplementary material contains four parts. The first part is the experimental methods used to conduct the experiments reported. The second part contains figures showing the sequences of the molecules used in this work. The third part show the sequence of the blunt-ended BTX molecule used in Figure 2c, 3a and in 3b. The fourth part illustrates schematically the structures of the 4HB molecules and their fusion into an 8HB molecule.

1. EXPERIMENTAL METHODS

Sequence Design

The sequences have been designed by applying the principles of sequence symmetry minimization, using the program SEQUIN.[1,2]

Synthesis and Purification

All strands were purchased from Integrated DNA Technologies, Inc. (Coralville, IO). The strands were purified by 20% denaturing PAGE, eluted in a solution containing 500 mM ammonium acetate, 10 mM magnesium acetate, and 2 mM EDTA from gel, followed by ethanol precipitation.

Formation of Hydrogen-Bonded Complexes and Nanotubes

Formation of 4HB and 8HB helix bundles were performed with 1 μM DNA (for each strand), 40 mM Tris, 20 mM acetic acid, 2 mM EDTA, and 12.5 mM magnesium acetate (total volume, 20 μL). This mixture was slowly cooled from 90˚C to room temperature in a 1L water bath stored in a Styrofoam box to anneal the DNA. BTX and 6HB species were prepared with 125 mM magnesium acetate. To prepare 6HB species, the two BTX molecules were annealed separately from 90 ˚C to room temperature. In a second step, the two BTX molecules were annealed together at 45 ˚C and then cooled slowly to room temperature.

Non-Denaturing Gel Electrophoresis

Annealed complexes, were run on non-denaturing gels to check for tile formation and stoichiometry. The systems were annealed at various DNA concentrations (0.1- 3 μM) in 40 mM Tris•HCl, 20 mM acetic acid, 125 mM Mg Acetate (6HB systems) or 12.5 mM Mg Acetate (8HB systems), 2 mM EDTA. Tracking dye containing buffer, 50% glycerol, and a trace amount of Bromphenol Blue and Xylene Cyanol FF was added to the annealed sample before loading them on 6-8% acrylamide gels, containing their respective buffer. Gels were run on a Hoefer SE-600 gel electrophoresis unit at room temperature, with the respective running buffer. After electrophoresis, the gels were stained with ethidium bromide. Mobilities are measured relative to Cyanol FF tracking dye. Logarithms for Ferguson analysis are taken to base 10.

Denaturing Gel Electrophoresis

Gels contain 5-20% acrylamide (19:1, acrylamide:bisacrylamide). These gels contain 8.3 M urea. The

running buffer consists of 89 mM Tris.HCl, pH 8.0, 89 mM Boric acid, 2 mM EDTA (TBE). The sample buffer consists of 10 mM NaOH, 90% formamide, 1 mM EDTA, containing trace amount of Xylene Cyanol FF and Bromophenol Blue tracking dyes. Gels are run on a Hoefer SE 600 electrophoresis unit at 55 ˚C (31 V/cm, constant voltage).

Atomic Force Microscopy

AFM images were obtained under the same buffer solution in a fluid cell on freshly cleaved mica on Nanoscope IV (Digital Instruments). Average lengths and heights of the arrays were calculated from 25 individual data for each sample.

Thermal Denaturation Profiles

DNA strands were dissolved to 1 μM concentration in 1mL of a solution containing 40mM sodium cacodylate, 10 mM magnesium acetate at PH7.4. The cacodylate buffer was used as a blank. Thermal denaturation was monitored at 260 nm on UV-visible spectrophotometers. Temperature was incremented at 0.1ºC/min.

References

1. Seeman, N.C., *J. Theor. Biol.* **1982** *99*, 237-247.

2. Seeman, N.C., *J. Biomol. Str. & Dyns.* **1990** *8*, 573-581.

2. SEQUENCES OF STRANDS OF 4HB AND BTX MOLECULES THAT CAN BY COMBINED TO YIELD NANOTUBES.

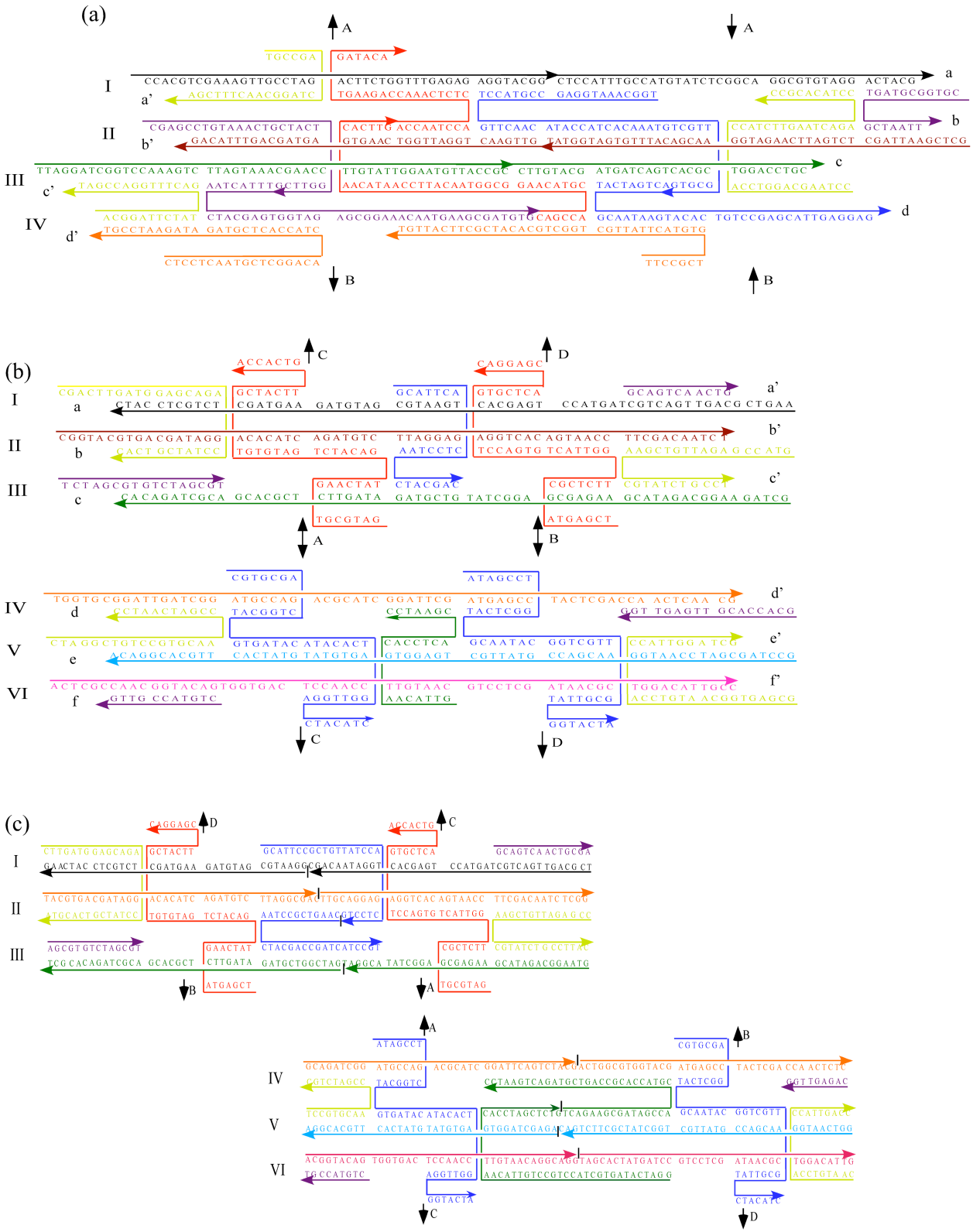

Figure S1. Sequences of strands of nanotube-forming molecules used in this Work. (a) The sequence of the 4HB molecule. (b) The sequences of the BTX molecules phased opposed each other. (c) The sequences of the BTX molecule phased oppositely.

3. SEQUENCE OF BTX MOLECULES USED IN FIGURE 2C, 3A AND 3B.

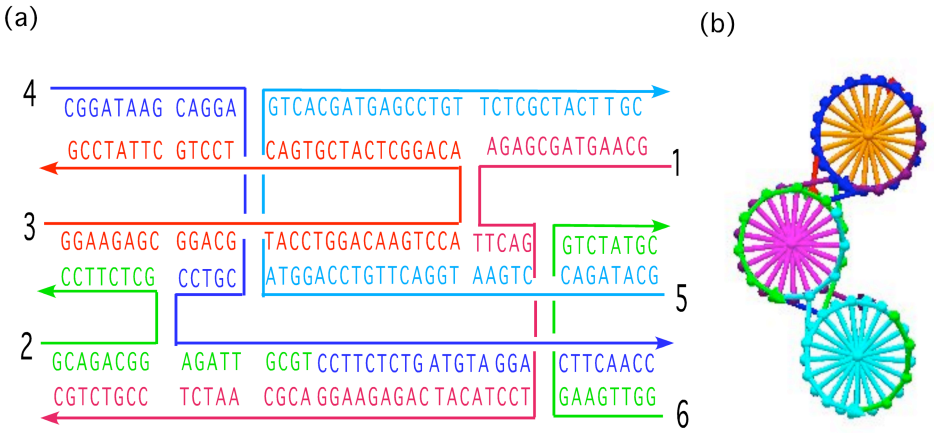

Figure S2. (a) The sequences of the six strands are shown. (b) A cross-section is illustrated.

4. MOLECULAR STRUCTURE OF THE 4HB MOLECULES.

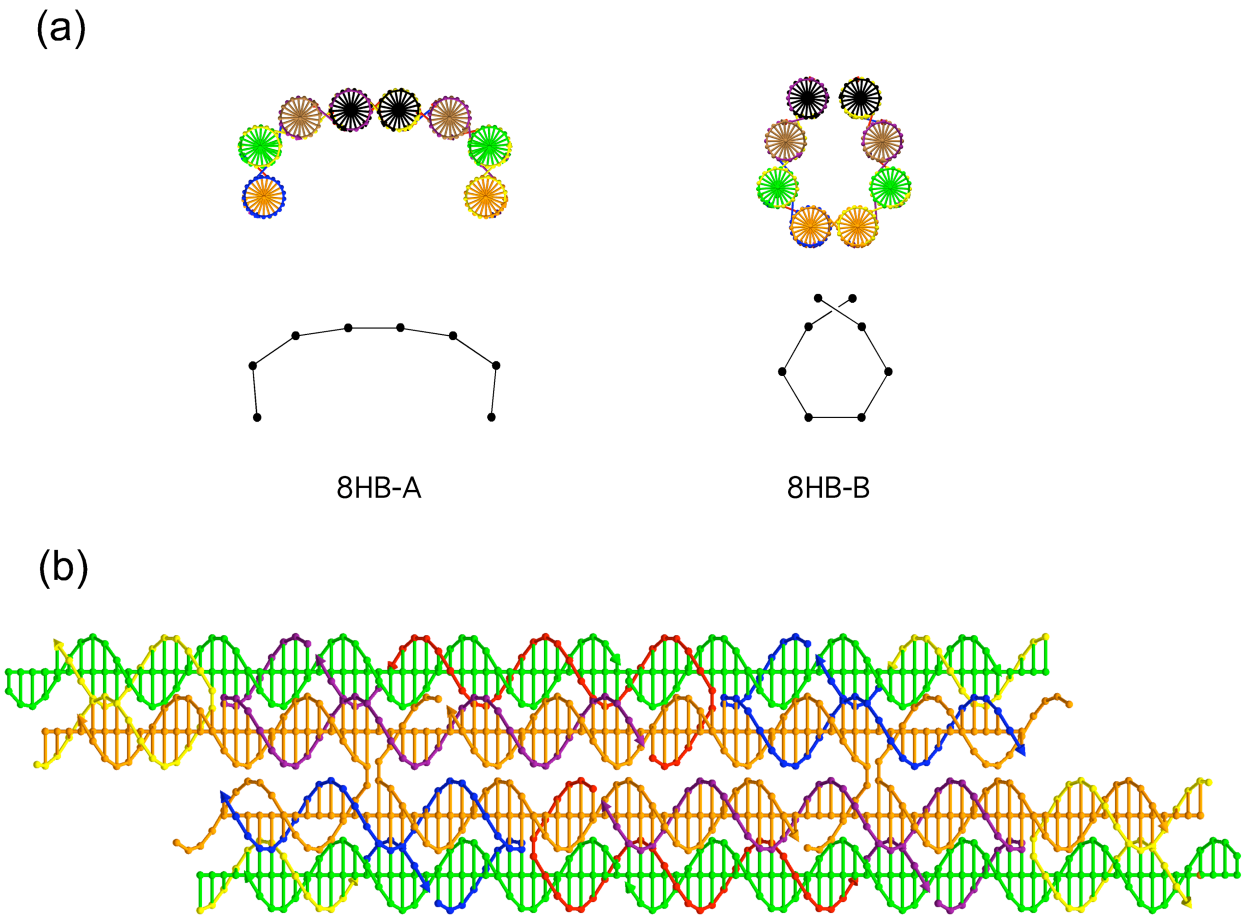

Figure S3. (a) A cross-section view of proposed structures of open 8HB-A (Figure 2d, lane 2) and 8HB-B (lane 3). All helices in 8HB-A are placed according to each theoretical dihedral angles (diagrams below), whereas positions of helices in 8HB-B are idealized not to overlap, regardless of the estimated dihedral angles. (b) Geometrical view of four helices of the circular 8HB molecule from the bottom, showing the attachment point of helices IV. The crossover strands from helix III never come close to each other (the yellow, purple, red and blue strands). Thus, the helices IV are connected to each other by the other strand (orange).