Mirror Image DNA Nanostructures for Chiral Supramolecular Assemblies

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1. Experimental Section:

Preparation of the DNA nanostructures: DNA oligonucleotides were purchased from Integrated DNA Technology (Coralville, IA) and IBA GmbH (Germany), and purified by denaturing PAGE or HPLC. To assemble the nano-junction, equal molar amount of DNA strands was mixed at the final concentration of 1 μ M in 1xTAE-Mg buffer (40 mM Tris-acetic acid buffer, pH 8, 2 mM EDTA, 12.5 mM magnesium acetate). To assemble the nanotube or 2D array, the involving DNA strand was brought to the concentration of 5 μ M or 100 μ M in 1xTAE-Mg solution, respectively. These pre-mixed samples were heated to 90 °C and cooled down to room temperature over 48 hrs.

AFM imaging: 2 μ L of the annealed sample was deposited on a piece of freshly cleaved mica (Ted Pella, Inc.), and left to adsorb on the surface for 2 min. 30 μ L 1xTAE-Mg buffer was added on the mica to cover the sample. Imaging was performed on a diMultiMode V AFM (Veeco Inc.) in tapping mode, using the tip on the thinner and shorter cantilever of the NP-S tips (Veeco Inc.)

Exonuclease digestion: Exonuclease I and Exonuclease III (Epicentre biotechnology) were added to DNA nanostructure samples to reach a final enzyme concentration of 2 U/ μ L (Exo I) or 20 U/ μ L (Exo III). The DNA/enzyme mixture was incubated at 37 °C for 45 min.

Fluorescence microscope imaging: The exonuclease treated DNA was stained by YOYO-1 (Invitrogen), an intercalating fluorescent dye, at a dye/base-pair ratio of 1:5. 3 μ L of the fluorescently labelled sample was deposited on a glass slide and covered immediately by a cover-slip. Fluorescence images were acquired using a Zeiss LSM 5 Duo microscope at excitation/emission wavelengths of 470/505.

CD measurement: CD spectra were collected between 320 to 200 nm using a JASCO J-815 CD spectrophotometer. To obtain the thermal transition curves, the annealed DNA nano-junction solution was heated from 20 to 85 °C in 1 °C/min increments, and the CD signal was monitored at 280 nm.

Non-denaturing PAGE: 10 μ L of 1 μ M desired DNA sample was loaded into each well of a pre-casted 14% polyacrylamide (19:1 acrylamide:bis) gel. The electrophoresis was performed in 1xTAE-Mg buffer at constant voltage of 10 V/cm for 5 hrs. The gel was stained with Sybr Gold (Invitrogen) and imaged with the Epichem3 Darkroom gel documentation system (UVP Bioimaging Systems).

2. DNA sequences:

D- and L-DNA were purchased from IDT (<u>www.idtdna.com</u>) and IBA GmbH (Göttingen, Germany), respectively.

DNA sequences used to assemble nano-junction J1:

J1-1: (16-nt) 5'-CGCAATCCTGAGCACG-3'

J1-2: (16-nt) 5'-CGTGCTCACCGAATGC-3'

J1-3: (16-nt) 5'-GCATTCGGACTATGGC-3'

J1-4: (16-nt) 5'-GCCATAGTGGATTGCG-3'

DNA used to assemble one-strand 2D array (for both D-DNA and L-DNA): (32-nt)

5'-CCATGGACTGGCCAGTGGTTCGAACCTGATCA-3'

DNA used to assemble one-strand nanotube (for both D-DNA and L-DNA): (52-nt)

5'-CCAAGCTTGGACTTCAGGCCTGAAGTGGTCATTCGAATGACCTGAGCGCTC A-3'

3. Supplemental figures:



Figure S1: Temperature dependent circular dichroism monitoring the thermal denaturing process of L-DNA J1 (left panel) and D-DNA J1 (right panel).



Figure S2: Zoom-out AFM images of L-DNA nanotubes. Tubes tend to tether randomly on the surface, consistent with the fluorescence image present in Figure 2c.



Figure S3: More zoom-in AFM images showing the generality of the right-handed chirality of the L-DNA nanotubes. Images are $1 \times 1 \mu m^2$.



Figure S4: More zoom-in AFM images showing the generality of the left-handed chirality of the D-DNA nanotubes. Images are $1 \times 1 \mu m^2$.



Figure S5: More AFM images of the L-DNA 2D array. The inserted panel shows an area where three pieces of array meet together.