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2D DNA lattice arrays assembled from DNA dumbbell tiles using poly(A-T)-rich stems

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1.Materials

All DNA strands were provided with denaturing PAGE purification from Shanghai Sangon Biotech Co., Ltd. Enzymes of T4 DNA ligase and Exonuclease-I were purchased from Takara Biotechnology Co., Ltd. (Dalian, China). Mica sheets for AFM imaging were from Nanjing Zhongjingkeyi Technology Co., Ltd. Water (18 MΩ cm) was from Milli-Q Ultrapure Water Purification System. Ethylenediaminetetraacetate (EDTA), urea, magnesium acetate tetrahydrate, boric acid, acetic acid, and tris(hydroxymethyl)aminomethane (Tris) were purchased from Sigma Aldrich Corporation. The TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), TBE buffer (89 mM Tris, 89 mM boric acid, and 2 mM EDTA, pH 8.3), and TAE-Mg buffer (40 mM Tris, 40 mM HAc, 2 mM EDTA and 12.5 mM $Mg(Ac)_2$, pH 6.0 ± 0.5) were freshly prepared.

2. Methods and Instruments

2.1 Gel electrophoresis was carried out on a polyacrylamide electrophoresis plate (length × width × depth= 150 mm × 100 mm × 1.5 mm) system from Beijing Liuyi Instrument Factory. The running buffer for the denaturing PAGE is TBE, and that for the native PAGE is TAE-Mg. After gel electrophoresis, the gel was dyed with GelRed™ about 30 min and analysed under UV light. The gel documentation and analysis were carried out with Image J.

2.2 UV-Vis measurement of DNA concentrations was performed with a Nanodrop 2000 (Thermo).

2.3 AFM imaging was carried out on a Fastscan AFM (Bruker Corp.) with ScanAsyst-Fluid+ tips under the PeakForce Tapping mode in fluid, the sample solution (2 µL) was deposited onto a freshly cleaved mica surface and left stay for 2 min. Then 80 µL TAE-Mg buffer was added on top of the sample and an extra 40 µL of the same buffer was added onto the AFM tip.

3. Experiments

3.1 Preparation of ligated DNA dumbbells

Equal amounts of a pair of two correspondent precursors of linear 5'-phosphorylated oligonucleotide hairpins (or a single 5'-phosphorylated D6 precursor, refer to Table S1) were mixed in 80 µL TE buffer (pH = 8.0) to a final concentration of 3.5 µM of each oligonucleotide. The mixture was annealed from 95 °C to room temperature over 3 h. Then 10×T4 buffer (660 mM Tris-HCl, 66 mM $MgCl_2$, 100 mM DTT, and 1 mM ATP, 10 µL) and T4 DNA ligase (350 U/µL, 10 µL) were added and the mixture was incubated at 16 °C for 16 h. The sample was heated at 65 °C for 10 min to inactivate T4 ligase, cooled via ice bath for 5 min, then added with 10 µL of exonuclease-I (5U/µL), and incubated at 37 °C for 30 min to digest unreacted linear oligonucleotides. Exonuclease-I was inactivated by heating at 80 °C for 5 min, then the sample was suddenly cooled via ice bath for 5 min.

DNA dumbbell molecules were finally purified by denaturing PAGE in TBE buffer at 4 °C and then by ethanol precipitation. The yields for D6, D11, D16, and D21 were calculated with UV-Vis measurements as 25%, 60%, 65%, and 65%.

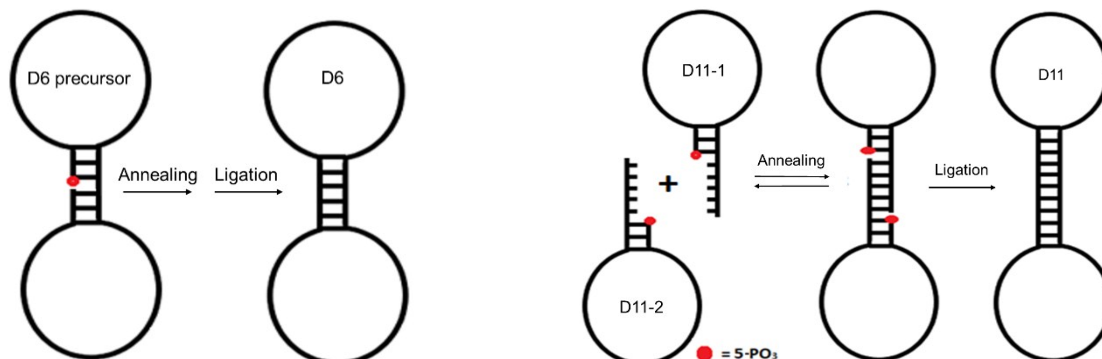


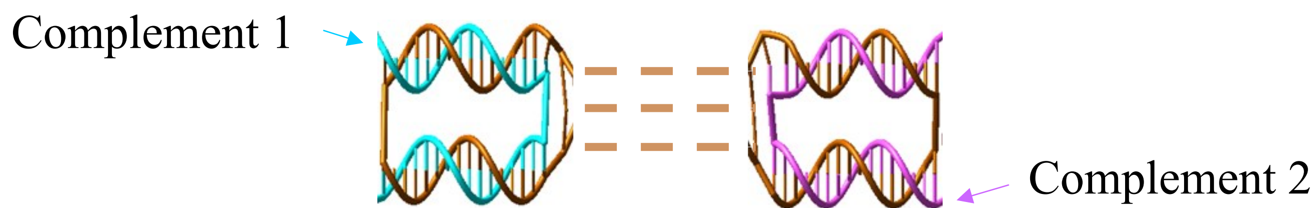
Fig. S1 Schematic illustrations of the preparation of DNA dumbbell molecules of D6 (left panel) and D11 (right panel). Left: The D6 precursor was first annealed to form a dumbbell with a nick at its stem and then ligased by T4 ligase to generate the dumbbell molecule of D6. Right: Two 5'-phosphorylated hairpins of D11-1 and D11-2 were annealed to generate an intermediate dumbbell molecule with a pair of two nicks in the central stem. Then by T4 ligase ligation, the two nicks were covalently connected to form the closed dumbbell molecule of D11. Preparation of D16 and D21 is similar to D11

Table S1: Sequences of D6 precursor, D11 precursors of D11-1 and D11-2, D16 precursors of D16-1 and D16-2, D21 precursors of D21-1 and D21-2.*

ID	Sequence (5' → 3')
D6 precursor:	5'P-AGATTCTCAACTCGTATTCTCAACTCGTCTCTGCCTCTCTTTGAAGATAGCGCACAATGGTCGGATTCTCAACAAG (5'P means 5'-phosphorylated)
D11-1:	5'P- CTTTGAAGATAGCGCACAATGGTCGGATTCTCAACAAGAAAGA
D11-2:	5'P-AGATTCTCAACTCGTATTCTCAACTCGTCTCTGCCTCTTCTTT
D16-1:	5'P-TTCTTTGAAGATAGCGCACAATGGTCGGATTCTCAACAAGAAAGAAGA
D16-2:	5'P-GAGAATTCTCAACTCGTATTCTCAACTCGTCTCTGCCTTCTCTCTTCT
D21-1:	5'P-CTTTCTTTGAAGATAGCGCACAATGGTCGGATTCTCAACAAGAAAGAAGAAA
D21-2:	5'P-GAGAAGAATTCTCAACTCGTATTCTCAACTCGTCTCTGCCTTCTTCTCTTTCTT

* In each group, the two deep gray shadowed codes will associate together and the two light gray shadowed codes will associate too to form the stem of a dumbbell molecule. For all the four dumbbell molecules, only the poly(A-T)-rich stems are specific, the two blue and green lettered head loops remain their same sequences.

Table S2: Two complementary sequences to form tile cores for native PAGE



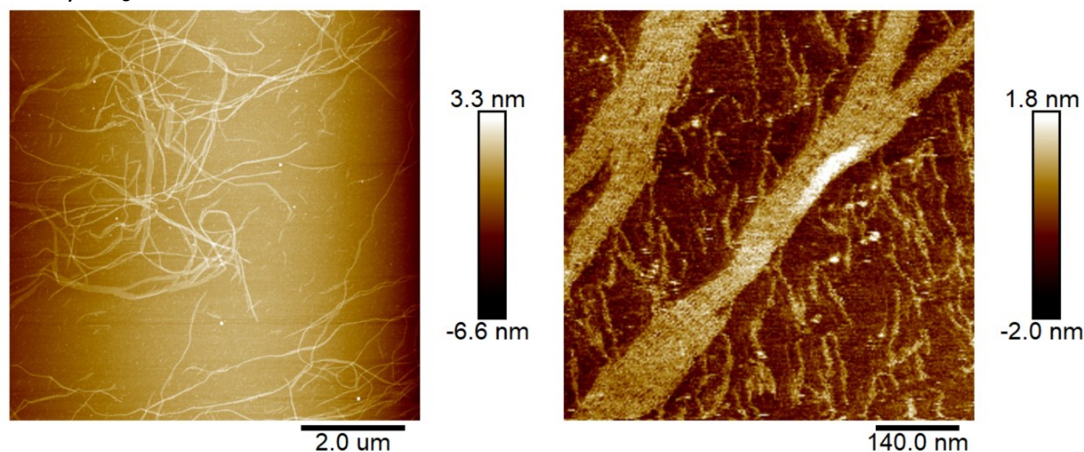
ID	Sequence (5' → 3')
Complement 1	TTGTGCGCTATCTTCAGTTGAGAATCCGACCA
Complement 2	GAATACGAGTTGAGAAGGCAGAGACGAGTTGA

3.2 Annealing ramps to create 2D arrays

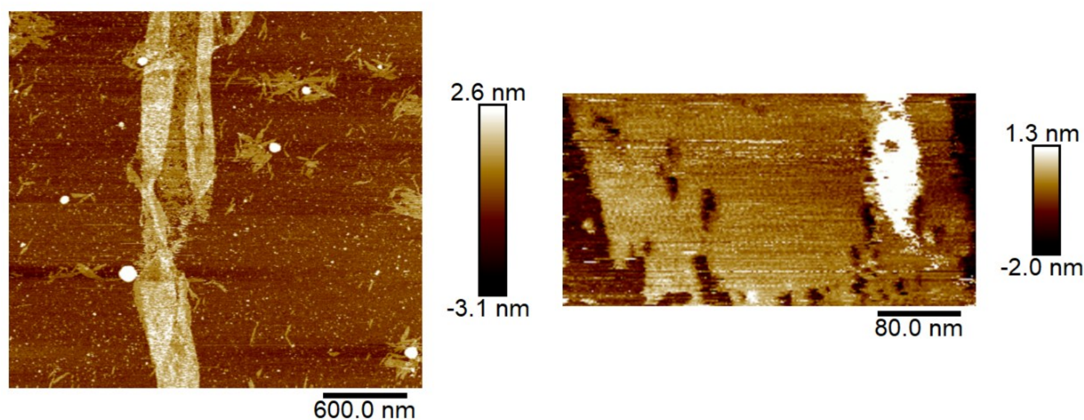
One pot annealing (applicable for the co-annealing): Each designed set of DNA strands were mixed in stoichiometric amounts with TAE-Mg buffer to a final volume of 20 μL and a final concentration of 0.2 μM for each strand, then the mixture was annealed in a thermocycler programmed from 95°C to 4°C for 18 to 37 h. Post annealing: The annealed 2D arrays were added with their corresponding X_n strands in equal molar ratios, then the mixture was annealed in a PCR thermo cycler using a cooling step of staying at 40 °C for 2 hours and cooling down at a rate of 0.1 °C per 5 minutes to 10 °C, about 24 hours in total.

4. Additional AFM images of 2D DNA arrays of this research

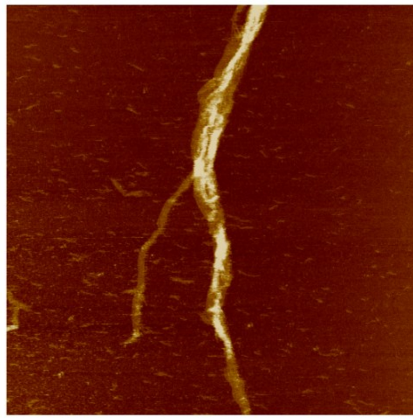
Array-$D6_O$

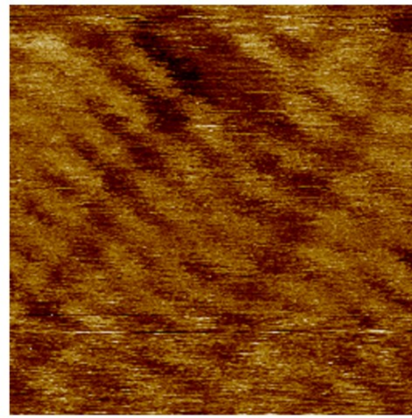


Array-$D6_E$

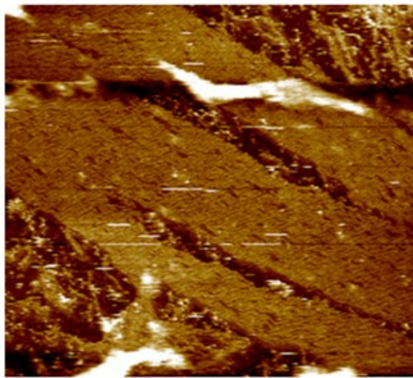


Array-D11$_O$

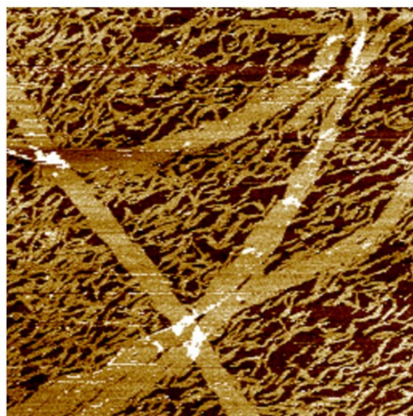


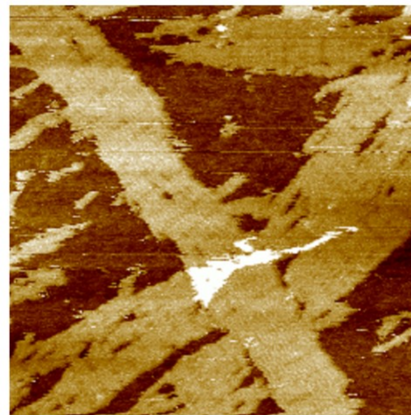


Array-D11$_E$

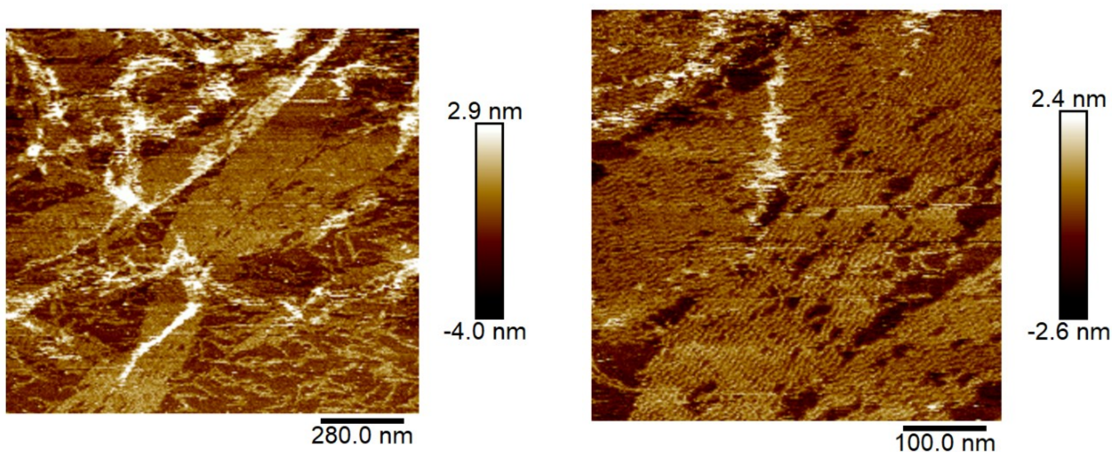


Array-D16$_O$

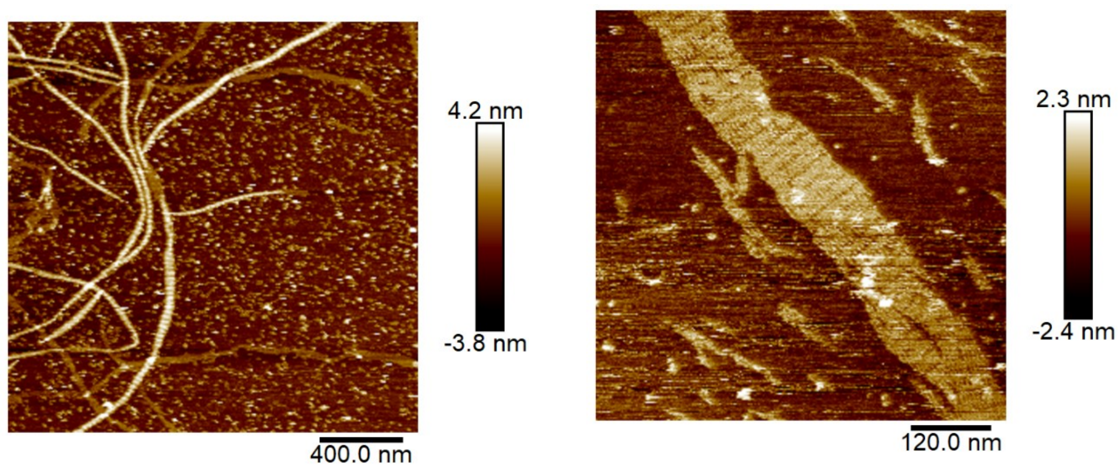




Array-D16$_E$



Array-D21$_O$



Array-D21$_E$

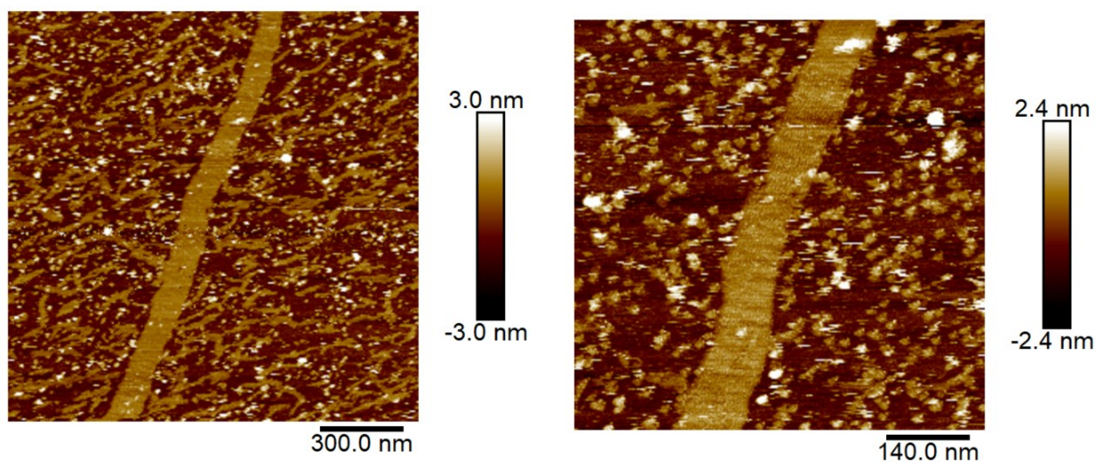
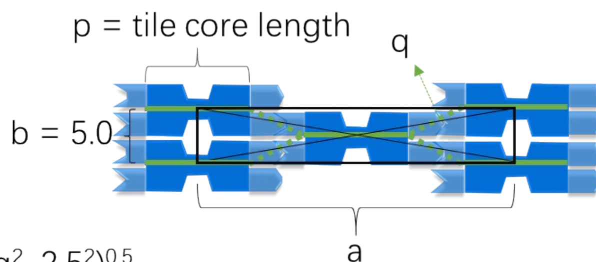


Fig. S2 More AFM images of all the eight 2D arrays. For each 2D array, a panoramic image and a high resolution image are provided. In array-D11$_E$, we chose two images having the same bright mark but presenting different texture details: the left panoramic image presents the dim stem stripes perpendicular to the array longitude, whereas the right high resolution image presents the tile helix strands parallel to the array longitude. In Array-D21$_O$, the left panoramic image presents both two-layered brighter nanotubes and monolayered nanoribbons, and the right high resolution image presents both stem stripes perpendicular to and the tile helix strands parallel to the array longitude.

5. Theoretical model for estimation of lattice parameters of a 2D centered rectangular unit cell formed by 5 tiles (data were provided in Table 1 of the main body)



$a = 2*p + 2*(q^2-2.5^2)^{0.5}$

where p is the theoretical tile core length (length of solid green segments), q is the theoretical joint distance (length of dashed green segments), and both can be estimated as 0.34*(number of bps) nm.

b is assigned to 5.0 nm of two closely packed duplexes.

$c = d = 0.5*(a^2 + b^2)^{0.5}$

$\varphi = 2*\text{arctg}\,(b/a)$

Fig. S3 Theoretical model for estimation of lattice parameters.

6. Oligonucleotide sequences used in this work

6.1 Sequences of four helper strands for even number of 4 half turns connection of $D6_E$, $D11_E$, $D16_E$, and $D21_E$

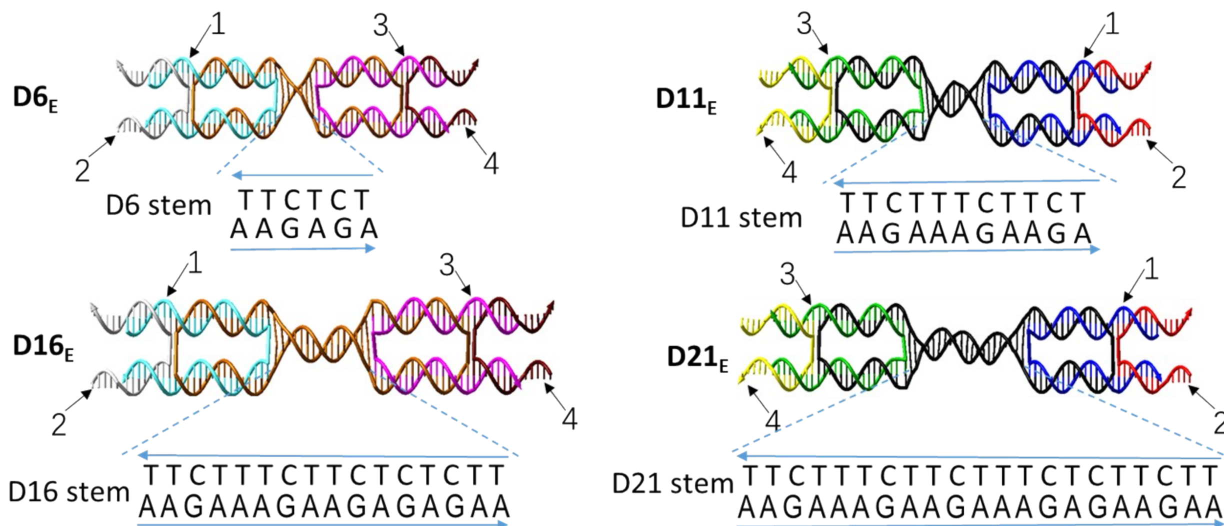


Fig. S4. Schematic illustrations of four helper strands of 1-4 located in $D6_E$, $D11_E$, $D16_E$, $D21_E$, and of four stem sequences under $D6_E$, $D11_E$, $D16_E$, and $D21_E$.

Table S3: Sequences of four helper strands of 1-4 for even number of 4 half-turns connection

ID	Sequence (5' → 3')
1	GAGCTGACTTGTGCGCTATCTTCAGTTGAGAATCCGACCACGTGATGC
2	TGACGGCATCACGGTCAGCTCACATC
3	CTAACGGCGAATACGAGTTGAGAAGGCAGAGACGAGTTGAGCTACCGA
4	CGTCAGGTAGCTCGCCGTTAGGATGT

6.2 Sequences of four helper strands of 5-8 for odd number of 5 half-turns connection of $D6_O$ $D11_O$, $D16_O$, and $D21_O$

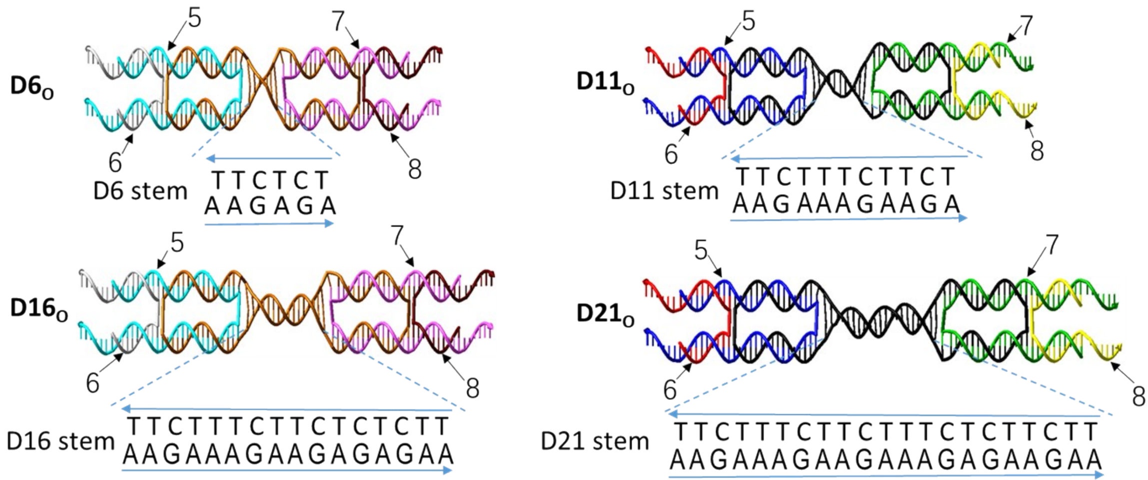


Fig. S5. Schematic illustrations of four helper strands of 5-8 located in $D6_O$, $D11_O$, $D16_O$, $D21_O$, and of four stem sequences under $D6_O$, $D11_O$, $D16_O$, and $D21_O$.

Table S4: Sequences of four helper strands of 5-8 for odd number of 5 half-turns connection

ID	Sequence (5' → 3')
5	CACTAACGGCGAATACGAGTTGAGAAGGCAGAGACGAGTTGAGCTACCGATGGCAGTC
6	CATCGGTAGCGCCGTTAGTGGATGTC
7	GAGAGCTGACTTGTGCGCTATCTTCAGTTGAGAATCCGACCACGTGATGCTAGAC
8	TAGCATCACGGTCAGCTCTCGACTGC

6.3 Sequences of four X_n strands

Table S5: Sequences of four four X_n strands

ID	Sequence (5' → 3')
X_6	TTCTCT
X_{11}	TTCTTTCTTCT
X_{16}	TTCTTTCTTCTCTCTT
X_{21}	TTCTTTCTTCTTTCTCTTCTT