Electronic supplementary information (ESI)

1. Materials

Acetic acid, glacial acetic acid, boric acid, EDTA, urea, tris(hydroxymethyl)-aminomethane (Tris), acrylamide and bis-acrylamide were purchased from Sinopharm Chemical Reagent Co., Ltd. All DNA strands were ordered from Shanghai Sangon Biotech Co., Ltd., purified either by the company or in our lab by PAGE purification. T4 DNA ligase and Exonuclease I were from Takara Biotechnology Co., Ltd. (Dalian, China). Mica sheets for AFM were from Nanjing Zhongjingkeyi Technology Co., Ltd. AFM cantilevers were from Bruker Corporation. Water (18 MΩ•cm) was from Milli-Q Ultrapure Water Purification System. The buffer of 1×TBE is composed of 89 mM Tris, 89 mM boric acid, and 2 mM EDTA at pH 8.3, and that of 1×TAE-Mg^{2+} is composed of 40 mM Tris, 2 mM EDTA and 12.5 mM $Mg(Ac)_2$ at pH 8.0.

2. Methods and Instruments

2.1 Gel electrophoresis was carried out on an acrylamide 150 mm × 100 mm × 1.5 mm (length×width×depth) electrophoresis plate (Beijing Liuyi Instrument Factory). The running buffer for the denaturing PAGE is 1×TBE, and that for the native PAGE is 1×TAE plus 2 mM $Mg(Ac)_2$. The gel documentation and analysis were carried out with Image J.

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

2.3 AFM imaging was carried out on a Fastscan AFM (Bruker Corp.) with Fastscan B or C tips in air at the ScanAsyst mode. The sample solution (5 μL) was deposited onto a freshly cleaved mica surface and left to stay adsorbed for 2 min. After the sample solution was dripped away with a filter paper, water (50 μL) was dropped onto the mica to wash the salts away twice. Then AFM imaging was performed on freshly prepared samples within 8 hours to minimize time-dependent degradation.

3. Experiment

3.1 Preparation of circular DNAs

A 100 μL solution of 42 (or 64, 84) nt linear 5'-phosphorylated oligonucleotide (3.5 μM) and its corresponding splint strand, always 20 nt (4.5 μM), in 66 mM Tris-HCl buffer (containing 6.6 mM $MgCl_2$, 10 mM DTT and 0.1 mM ATP, pH7.6) was heated to 95 °C for 5 min, then cooled down slowly to room temperature. T4 ligase (350 U/μL, 10 μL) and 10×ligase buffer (10 μL) were added. The mixture was incubated for 16 h at 16 °C. Then, the ligase was inactivated by heating at 65 °C for 10 min. After the ligation and inactivation step, 15 μL Exonuclease I (5U/μL) and 13 μL 10×Exonuclease I buffer were added to digest the remaining linear DNA templates and its corresponding splint strands by incubation at 37 °C for 30 min. The enzyme selectively digested the single-stranded DNA, and left the circularized DNA intact.

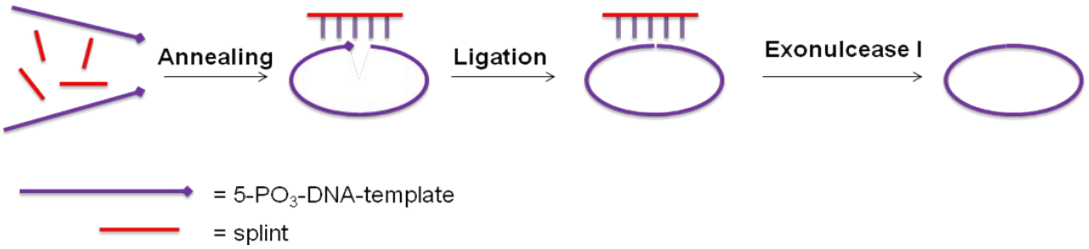

Fig. S1. Schematic illustration of preparation of circular DNA molecules. **(1) Annealing**: 5'-phosphorylated linear strand and its corresponding splint were mixed together to anneal from 95 °C to room temperature; **(2) Ligation**: T4 ligase and buffer were added and incubated at 16 °C for 16 h; **(3) Exonuclease I Treatment**: the residual linear DNAs were digested and removed by Exonuclease I treatment.

The circularized DNA strands were purified by denaturing polyacrylamide gel electrophoresis. The protocol is as follows: (1) purify the circularized DNA by 10% denaturing polyacrylamide gel electrophoresis, using a constant voltage of 5 V/cm for 2 h, (2) cut the band out of the corresponding denaturing gel with a razor blade under a UV light, (3) after drying, chop and crush the gel band slice into fine pieces and transfer into a 1.5 ml microcentrifuge tube, (4) add the elution buffer (0.5 M ammonium acetate, 1 mM EDTA, 10 mM Tris-HCl, pH 7.6) at least twice of the gel volume into the tube and shake at 37 °C overnight, (5) centrifuge the tube to collect the supernatant, recover any residual DNA by rinsing with small volume of elution buffer and centrifuge again to combine the supernatants, (6) extract the eluent with n-butanol to 200 µl, (7) add 20 µl of 3M NaOAc (pH 5.2), then add 600 µl 100 % ethanol into the tube and store the tube at −20 °C overnight, (8) centrifuge at 10,000 rpm for 30 min at 4 °C and discard the supernatant, (9) wash the pellet with 1 ml 75 % ethanol (cold) and centrifuge again to collect the pellet, (10) dry and store circular DNAs at −20 °C. To use, circular DNAs were re-suspended in water and desalted with NAP-5 (GE) if needed.

3.2 Self-assembly to create nanostructures

Each designed set of DNA strands were mixed (final concentration of 0.5 µM for each strand) to a final volume of 20 µL 1×TAE-Mg^{2+} buffer (40 mM Tris, 12.5 mM $Mg(Ac)_2$ and 2 mM EDTA) in a 0.2 ml eppie. The eppie was floated on a thermo-insulated 2-liter water bath (95 °C) with a Styrofoam box and allowed to cool slowly to room temperature over 36 hours.

4. Additional AFM images of 2D DNA lattices in this research

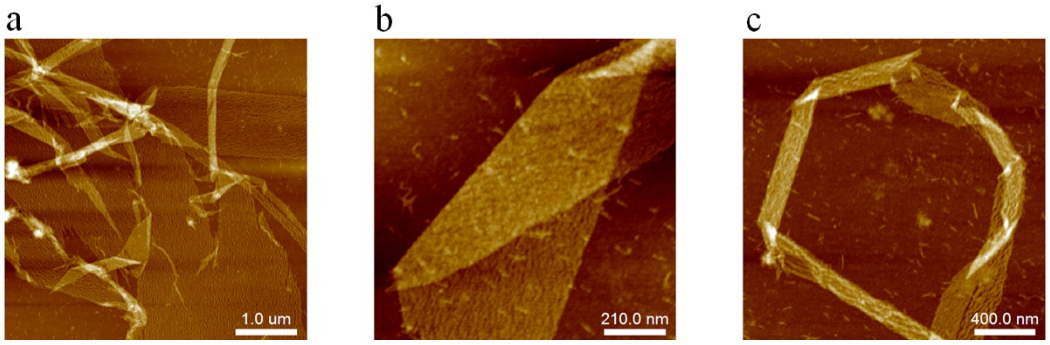

Fig. S2. More AFM images of $42A_C$-$42B_C$.

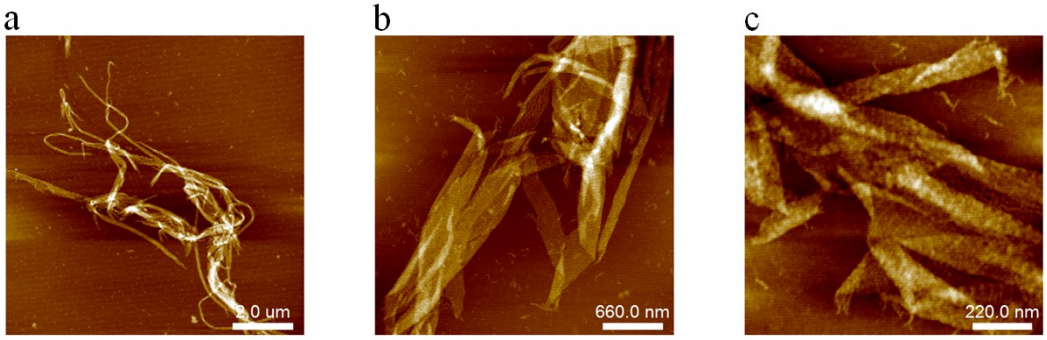

Fig. S3. More AFM images of $64A_C$-$64B_C$.

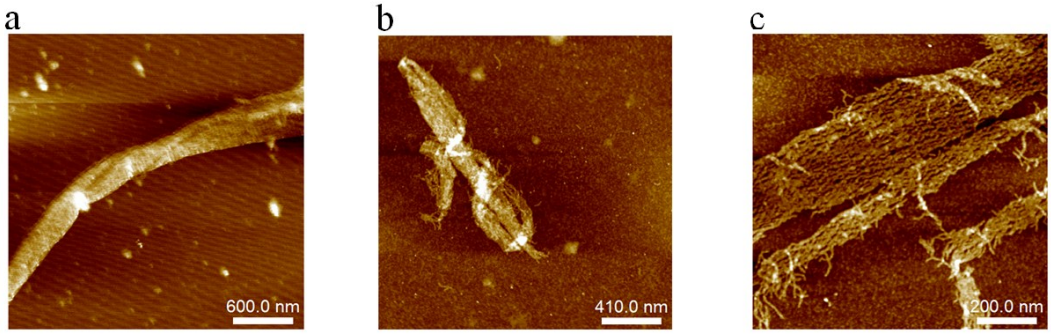

Fig. S4. More AFM images of $84A_C$-$84B_C$.

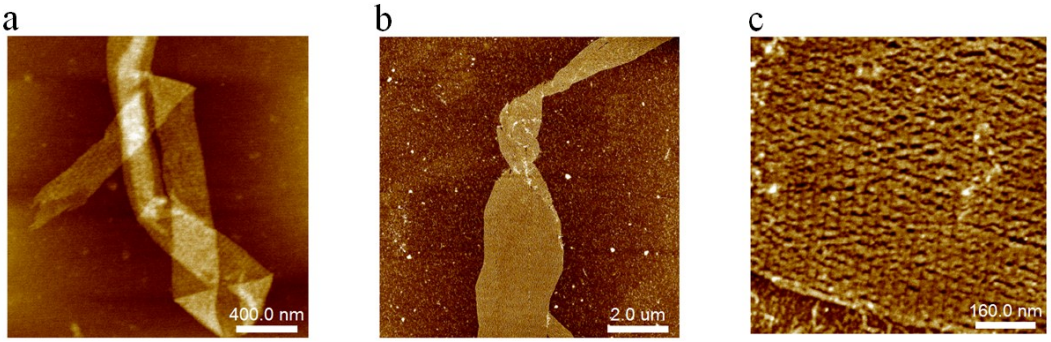

Fig. S5. More AFM images of $64A_C$-$42B_C$.

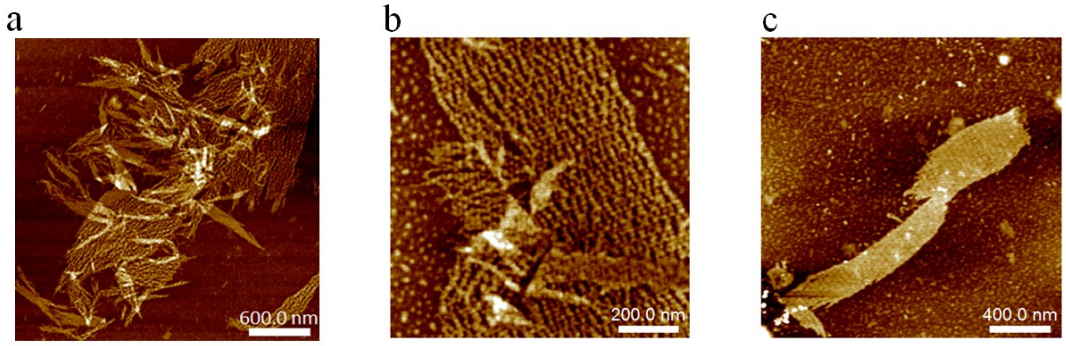

Fig. S6. More AFM images of the $42A_C$-$84B_C$.

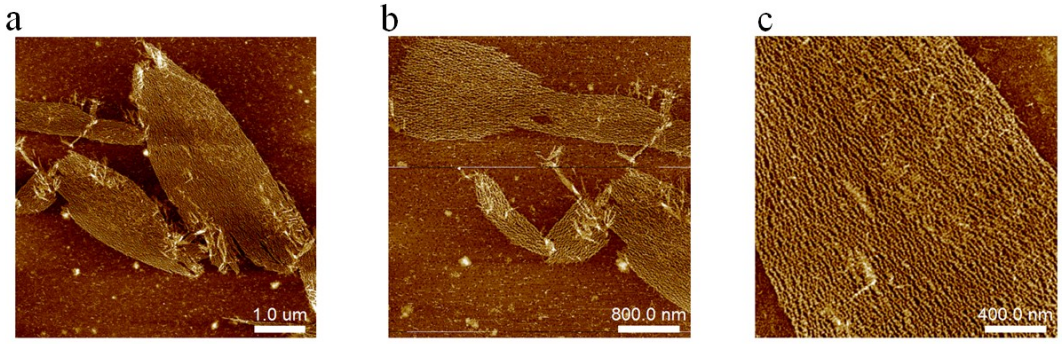

Fig. S7. More AFM images of $84A_C$-$64B_C$.

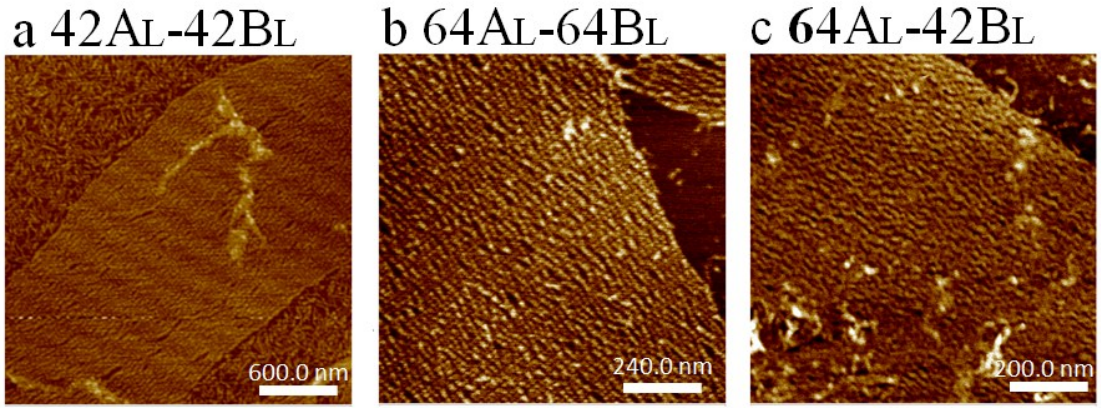

Fig. S8. AFM images of $42A_L$-$42B_L$, $64A_L$-$64B_L$, and $64A_L$-$42B_L$.

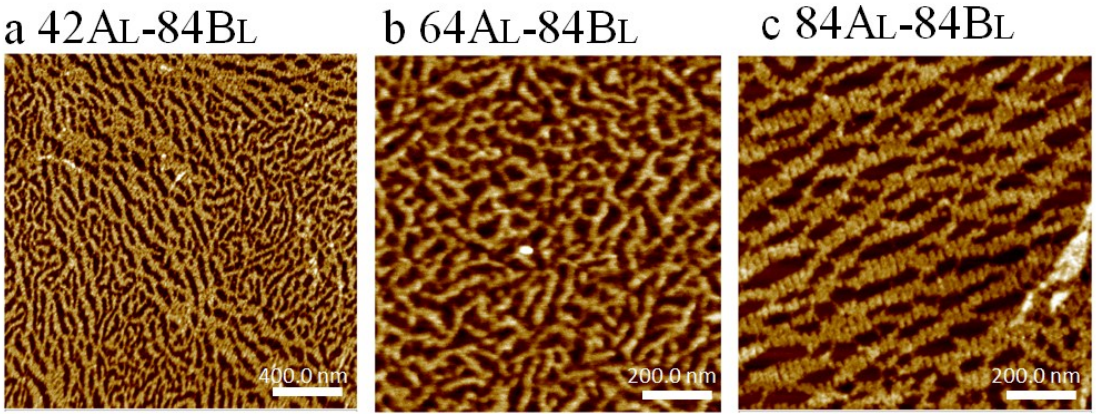

Fig. S9. More AFM images of $42A_L$-$84B_L$, $64A_L$-$84B_L$, and $84A_L$-$84B_L$.

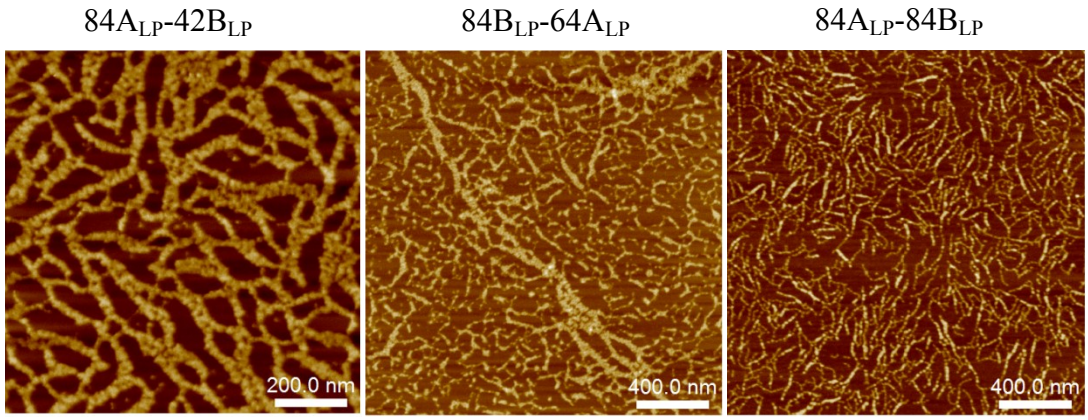

Fig. S10. AFM images of linear tile assemblies with 5'-phosphorylated oligonucleotides as the central strands. The subscript $_{LP}$ in the assembly labels of 84A$_{LP}$-42B$_{LP}$, 84B$_{LP}$-64A$_{LP}$, and 84A$_{LP}$-84B$_{LP}$ represents that the central strand in a tile is a linear 5'-phosphorylated strand. The three assemblies including a DAE tile carrying a linear 5'-phosphorylated 84-nt central strand grew polycrystalline lattices, similar to 42A$_L$-84B$_L$, 64A$_L$-84B$_L$, and 84A$_L$-84B$_L$, in Fig. 5 of the main text.

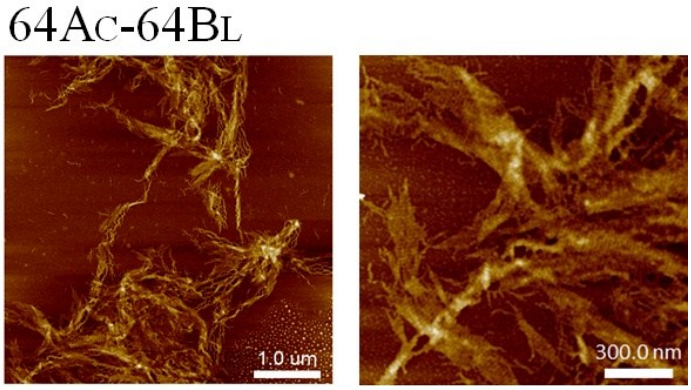

Fig. S11. AFM images of a cross-combination assembly of 64A$_C$-64B$_L$, with one circular tile and one linear tile. Its crystalline form is between single crystalline and polycrystalline lattices.

5. Oligonucleotide sequences and designs used in this work

5.1 Sequences of the linear templates and their corresponding splints for synthesis of circular molecules

Table S1: Sequences of the linear templates and their corresponding splints for synthesis of circular molecules

ID*	Sequence (5'→3')
42A	TGTAGTATCGTGGCTGTGTAATCATAGCGGCACCAACTGGCA
42B	AGTACAACGCCACCGATGCGGTCACTGGTTAGTGGATTGCGT
42A -splint	CGATACTACATGCCAGTTGG
42B -splint	GCGTTGTACTACGCAATCCA
64A	TAAGATGAAGATAGCGCACAATGGTCGGATTCCGTCTCTGTC

	AACTCGTCTATGCCAAGCCCTG
64B	CTCAGCTGTGATCATACTATGCTAGTCCTGTAGGTCGCACGA CCTGGCGTTCGCATGGCCTATC
64 A -splint	CTTCATCTTACAGGGCTTGG
64 B -splint	CACAGCTGAGGATAGGCCAT
84A	TAAGATGAAGATAGCGCACAATGGTCGGATTCTCAACTCGTA TTCTCAACTCGTATTCTCAACTCGTCTCTGCCCTGACTTCTA
84B	AGGTAGCCTGGAGCATAGAGGCATTGGCTGGCCCAGCCCTTG AAGATGAAGATCGTTTGATGTTCCTAACGTACCAACGCACGG
84A-splint	CTTCATCTTATAGAAGTCAG
84B-splint	CAGGCTACCTCCGTGCGTTG

*IDs of 42A, 42B, 64A, 64B, 84A, and 84B represent the strand codes, but not any modifications, corresponding to the central strands of DAE tiles of $42A_{C\ (or\ L,\ or\ LP)}$, $42B_{C\ (or\ L,\ or\ LP)}$, $64A_{C\ (or\ L,\ or\ LP)}$, $64B_{C\ (or\ L,\ or\ LP)}$, $84A_{C\ (or\ L,\ or\ LP)}$, and $84B_{C\ (or\ L,\ or\ LP)}$ respectively.

5.2 Sequences for the DAE-O of $42A_C$-$42B_C$ (or $42A_L$-$42B_L$)

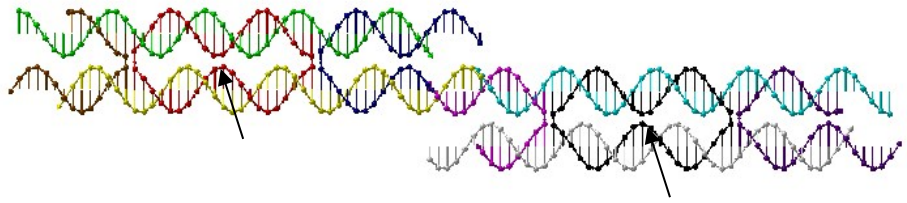

Fig. S12. Schematic illustration of $42A_C$-$42B_C$ (or $42A_L$-$42B_L$ with nicks pointed by arrows) in this research, different color lines mean different DNA strands.

Table S2: Sequences of the DNA strands.

ID	Sequence (5'→3')
Red line (42A)	TGTAGTATCGTGGCTGTGTAATCATAGCGGCACCAACTG GCA
Black line (42B)	AGTACAACGCCACCGATGCGGTCACTGGTTAGTGGATTG CGT
Green line	GATGGCGACATCCTGCCGCTATGATTACACAGCCTGAGC ATTGACAC
Yellow line	CTGACGCTGGTTGATCGGACGATACTACATGCCAGTTGG ACTAACGG
Cyan line	CGCTACCGTGCATCATGGACTAACCAGTGACCGCATCGG ACAGCAGC
Grey line	GCAGTCGCACGACCTGGCGTTGTACTACGCAATCCTGCC GTATCGACG
Dark orange line	GTAGCGCCGTTAGTGGATGTC
Dark blue line	GACTGCGTGTCAATGCTCACCGATCAACCAG
Pink line	CGTCAGGCTGCTGTGGTCGTGC
Purple line	GCCATCCGTCGATACGGCACCATGATGCACG

5.2 Sequences for the DAE-O of $64A_C$-$64B_C$ (or $64A_L$-$64B_L$, $64A_C$-$64B_L$)

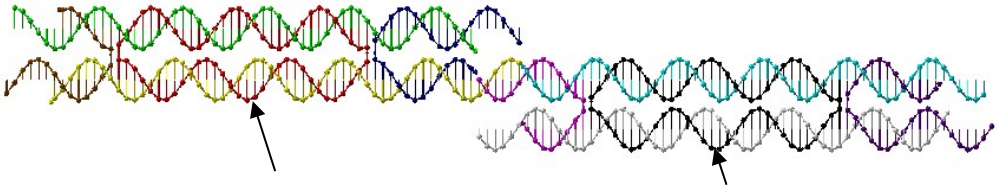

Fig. S13. Schematic illustration of nanostructural design of $64A_C$-$64B_C$ (or $64A_L$-$64B_L$ with nicks pointed by arrows, or $64A_C$-$64B_L$) in this research, different color lines mean different DNA strands.

Table S3: Sequences of the DNA strands.

ID	Sequence (5'→3')
Red line (64A)	TAAGATGAAGATAGCGCACAATGGTCGGATTCCGTCT CTGTCAACTCGTCTATGCCAAGCCCTG
Black line (64B)	CTCAGCTGTGATCATACTATGCTAGTCCTGTAGGTCGC ACGACCTGGCGTTCGCATGGCCTATC
Green line	GATGGCGACATCCCGAGTTGACAGAGACGGAATCCGA CCATTGTGTGAGCATTGACAC
Yellow line	CTGACGCTGGTTGATCGGCGCTATCTTCATCTTACAGG GCTTGGCATAGAACTAACGG
Cyan line	CGCTACCGTGCATCATGGGCCAGGTCGTGCGACCTAC AGGACTAGCATAGACAGCAGC
Grey line	GCAGTCGCACGACCTATGATCACAGCTGAGGATAGGC CATGCGAACTGCCGTATCGACG
Dark orange line	GTAGCGCCGTTAGTGGATGTC
Dark blue line	GACTGCGTGTCAATGCTCACCGATCAACCAG
Pink line	CGTCAGGCTGCTGTGGTCGTGC
Purple line	GCCATCCGTCGATACGGCACCATGATGCACG

5.3 Sequences for the DAE-O of $84A_C$-$84B_C$ (or $84A_L$-$84B_L$)

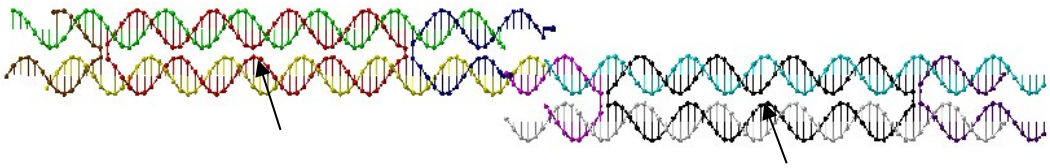

Fig. S14. Schematic illustration of nanostructural design of $84A_C$-$84B_C$ (or $84A_L$-$84B_L$ with nicks pointed by arrows) in this research, different color lines mean different DNA strands.

Table S4: Sequences of the DNA strands.

ID	Sequence (5'→3')
Red line (84A)	TAAGATGAAGATAGCGCACAATGGTCGGATTCTCAAC TCGTATTCTCAACTCGTATTCTCAACTCGTCTCTGCCCT GACTTCTA
Black line (84B)	AGGTAGCCTGGAGCATAGAGGCATTGGCTGGCCCAGC CCTTGAAGATGAAGATCGTTTGATGTTCCTAACGTACC AACGCACGG
Green line	GATGGCGACATCCGTTGAGAATACGAGTTGAGAATAC GAGTTGAGAATCCGACCATGAGCATTGACAC
Yellow line	TCATCTTATAGAAGTCAGGGCAGAGACGAACTAACGG CTGACGCTGGTTGCATCGGTTGTGCGCTATCT
Cyan line	CGCTACCGTGCATCATGGACATCAAACGATCTTCATCT TCAATGGGCTGGGCCAGCCAATGACAGCAGC
Grey line	CCTCCGTGCGTTGGTACGTTAGGATGCCGTATCGACGG CAGTCGCACGACCCCTCTATGCTCCAGGCTA
Dark orange line	GTAGCGCCGTTAGTGGATGTC
Dark blue line	GACTGCGTGTCAATGCTCACCGATCAACCAG
Pink line	CGTCAGGCTGCTGTGGTCGTGC
Purple line	GCCATCCGTCGATACGGCACCATGATGCACG