Supporting Information

Stepwise Self-Assembly of DNA Tile Lattices Using dsDNA Bridges

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1. Materials and methods:

Complex design, sample preparation, and stepwise assembly. The design of cross-tiles, *A* and *B* was based on the structure of immobile 4-arm branched junctions. The subsequence used for all bulged loops was T_4. Sequences were designed to minimize the chance of undesired complementary association and sequence symmetry. Synthetic oligonucleotides were purchased from Integrated DNA Technologies (Coralville, IA) and purified by polyacrylamide gel electrophoresis (PAGE). Complexes were formed by mixing a stoichiometric quantity of each strand in physiological buffer, 1xTAE/Mg^{2+} (40 mM Tris acetate (pH 8.0), 2 mM EDTA, and 12.5 mM magnesium acetate). The final concentration of DNA was between 0.125 and 1.0 μM. For the first step high-temperature annealing, equimolar mixtures of strands were cooled slowly from 95 °C to 20 °C by placing the eppendorf-tubes in 2 L of boiled water in a styrofoam box for at least 40 hours to facilitate hybridization and then incubated overnight at 4 °C for structure stabilization. From the second to last step low-temperature annealing, DNA tiles' mixtures were cooled slowly from 42 °C (40 °C for 3rd step and 38 °C for 4th) to 20 °C by placing the eppendorf-tubes in 1 L of water at room temperature for ~4 hours. After each step of annealing, samples were incubated overnight at 4 °C before AFM imaging.

AFM imaging. AFM imaging was performed in tapping mode under 1xTAE/Mg^{2+} buffer. A 5 μL annealed-sample was dropped on freshly cleaved mica for 5 minutes. 30 μL of 1xTAE/Mg^{2+} buffer was then placed onto the mica and another 30 μL of TAE/Mg^{2+} buffer was placed onto the AFM tip. AFM images were obtained on a Digital Instruments Nanoscope IIIa with a multimode fluid cell head by tapping mode under buffer using a NP-S oxide-sharpened silicon nitride tips (Vecco).

Estimation of Assembly Yields. AFM images (2x2 μm) were examined; cross-tile assembly yields were estimated by dividing the number of observed cross-tiles participating in properly formed nanoarrays by the total number of cross-tiles observed. Raw numbers for tile counts are given below in Table S3.

2. Supporting Figures:

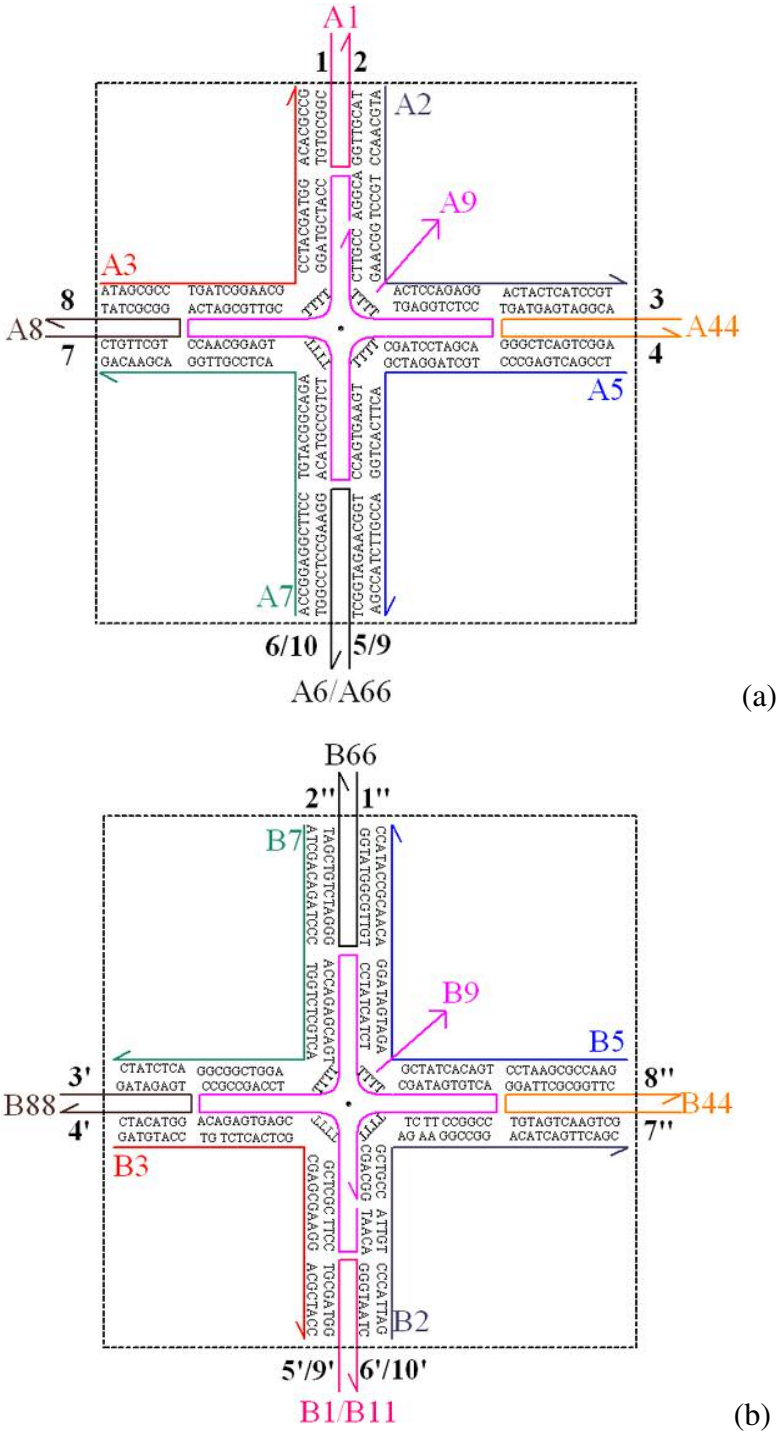


Figure S1. Detailed DNA strand structures and base sequences used in 2 × 2 formation in Figures 1 through 3. There are two tiles, (a) for tile A and (b) for tile B. Each tile consists of nine strands (A1 to A9 for A tile and B1 to B9 for B) indicated by different colors. Numbers n (n") and n' (n"') are complementary bases each other. All combinations of complementary bases are in table 1.

	Strand Name	5’ to 3’	3’ to 5’	Strand Name	
1(NS)	A1	CGCTA			
2(NS)	A1	AGGTC			
3(EW)	A44	TCACG	AGTGC	B88	3’(EW)
4(EW)	A44	TGAGC	ACTCG	B88	4’(EW)
5(NS)	A6	CTCGC	GAGCG	B1	5’(NS)
6(NS)	A6	GCATG	CGTAC	B1	6’(NS)
7(EW)	A8	GAGAG			
8(EW)	A8	CTGAC			
9(NS)	A66	TAGCA	ATCGT	B11	9’(NS)
10(NS)	A66	CCAGT	GGTCA	B11	10’(NS)
			GCAAC	B44	7”(EW)
			GTGAT	B44	8”(EW)
			AATAG	B66	1”(NS)
			TGAAT	B66	2”(NS)

Table1. Sets of the complementary sticky-ends for constructing stepwise assembly of NAs. The sticky-ends are designed such that the complementary sticky-end pairs are shown as (n and n’) and (n” is n’’’). NS and EW in the first and last columns indicate the directions of arm-strands, ‘north or south’ for NS and ‘east or west’ for EW. Note that n’’’, complementary of n”, will be found in Figure S2.

Figure S2. Double-stranded DNA bridges used in Figures 2 and 3. Schematic drawings of (a) horizontal, (x-direction), and (b) vertical (y-direction) bridges. Each bridge is composed of two strands with the two sticky-ends at the ends of dsDNA molecules. The complementary sticky-end of n is n' and of n" is n'''.

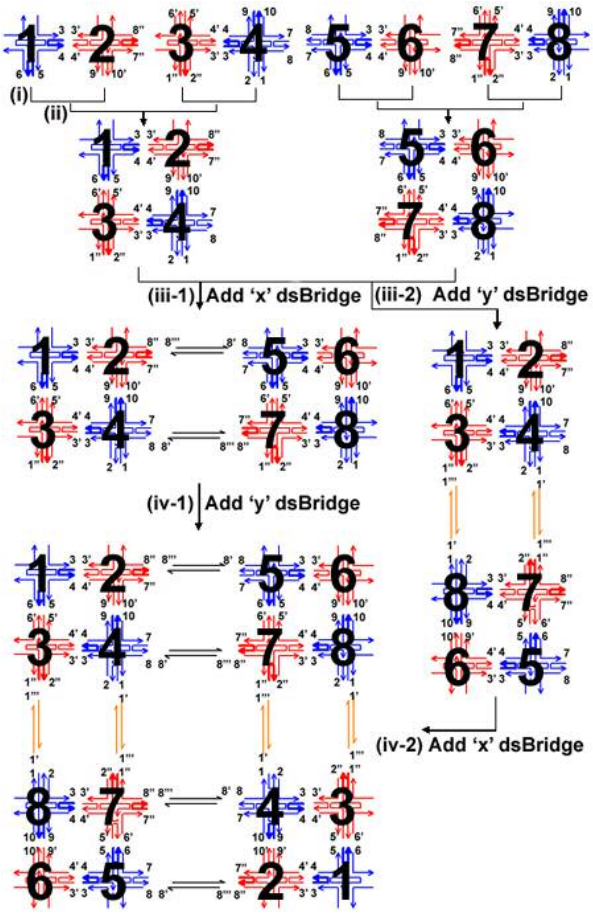


Figure S3. Four-step self-organization of the finite-size superstructures composed of 2 × 2 NAs and dsDNA nanobridges.

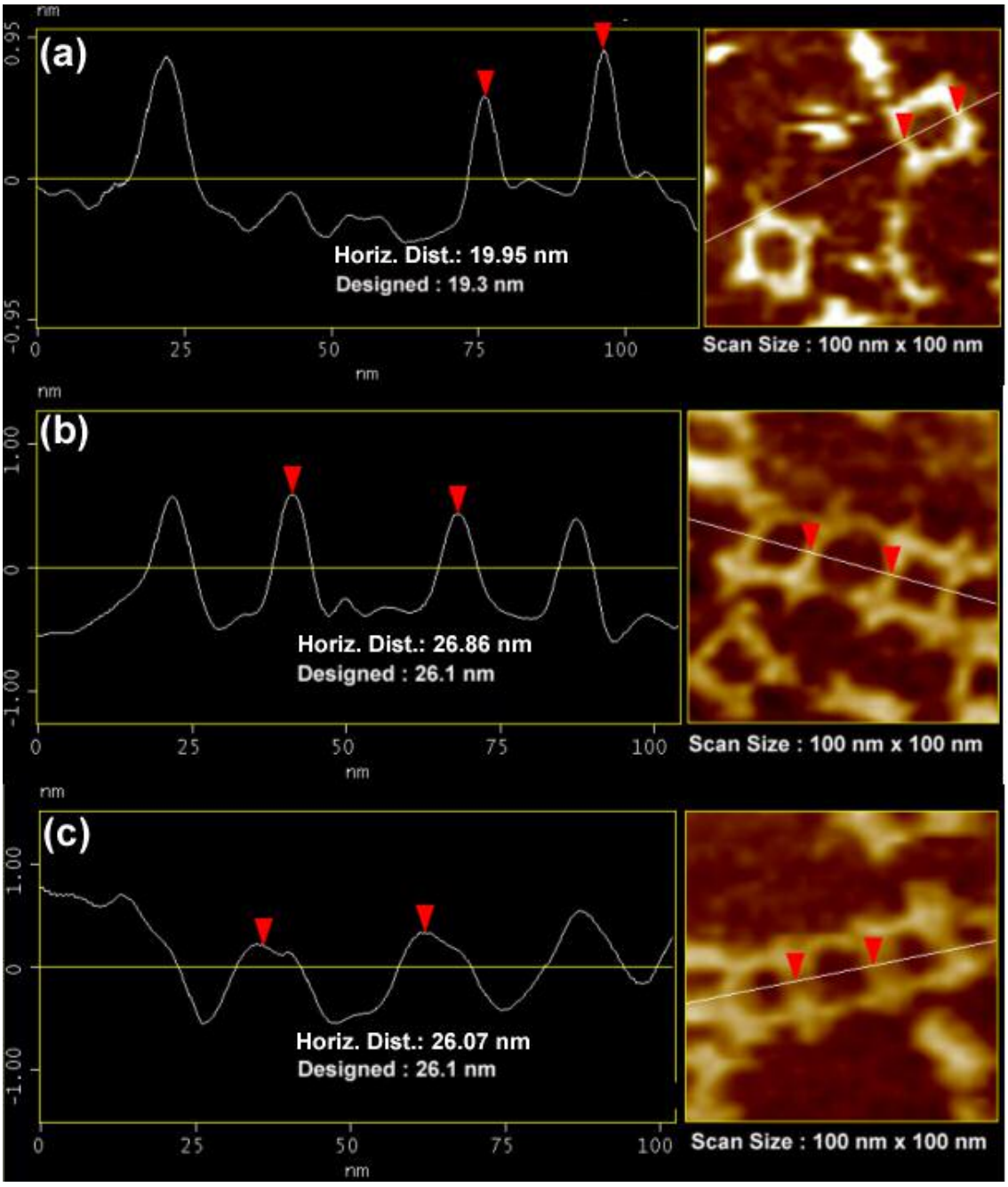
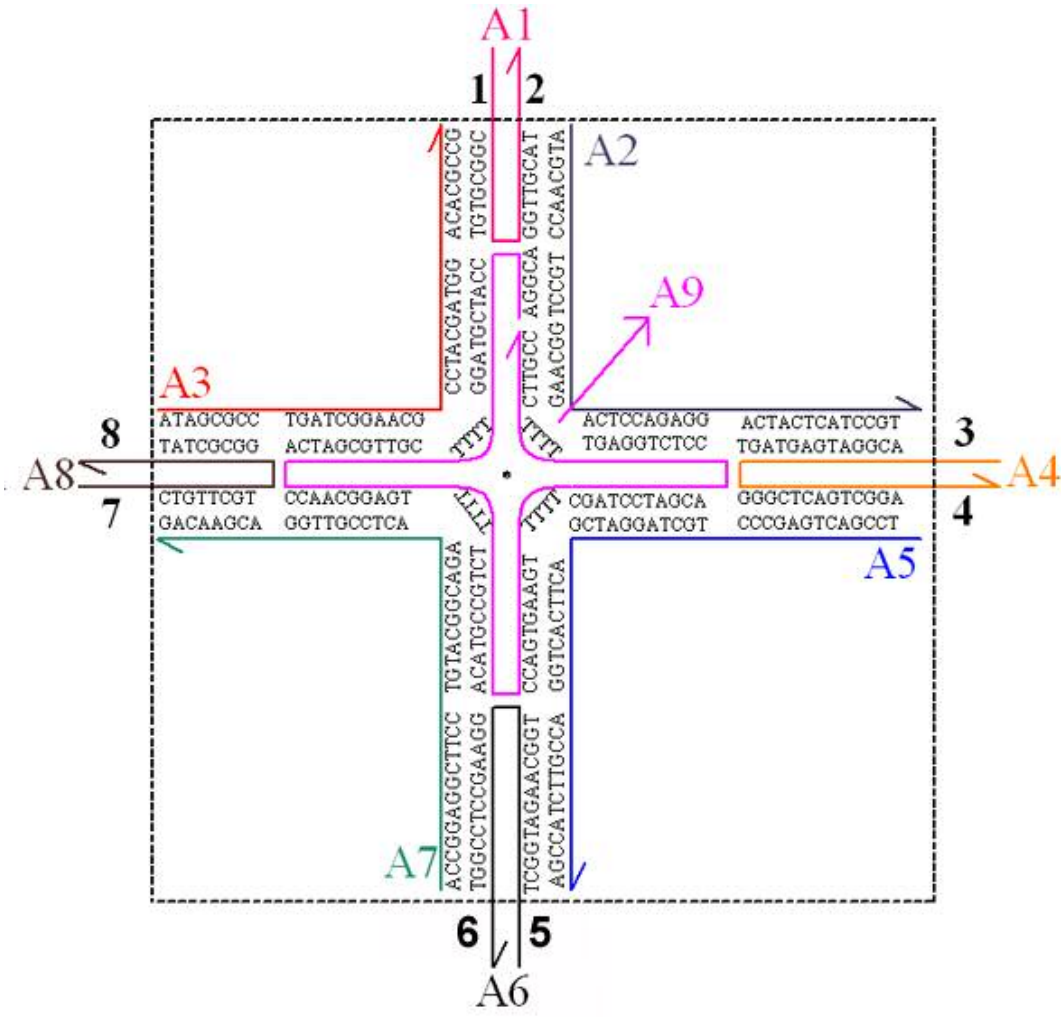
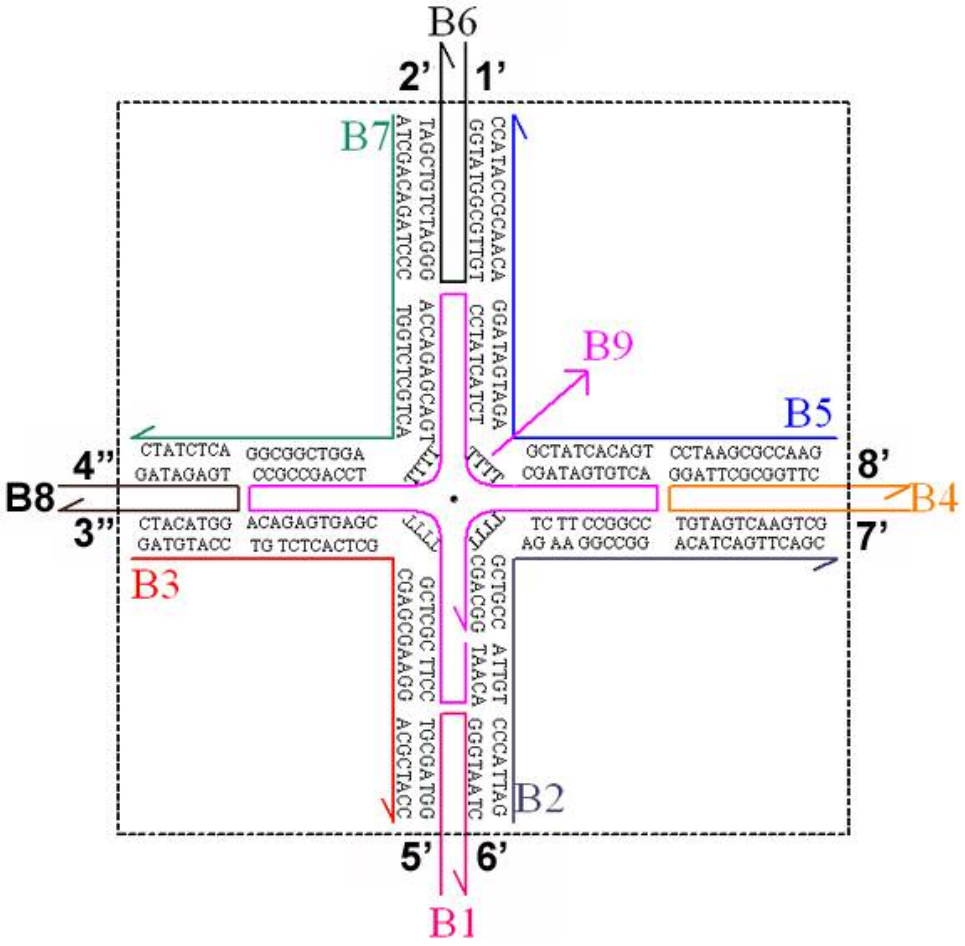


Figure S4. Section measurement of 2 × 2 NA + dsDNA bridges. Section profile distance measurements of (a) 2 × 2 NA, (b) 2 × 2 NA with horizontal, and (c) 2 × 2 NM with vertical dsDNA bridges. All experimental distance measurements were in excellent agreement with designed structures with less than 5% (± 1.0 nm) deviation.


A1
1
2
A2
A3
A9
8
A8
7
3
A4
4
A5
A7
6
5
A6
ATAGCGCC
TATCGCGG
TGATCGGAACG
ACTAGCGTTGC
CTGTTCGT
GACAAGCA
CCAACGGAGT
GGTTGCCTCA
ACTCCAGAGG
TGAGGTCTCC
ACTACTCATCCGT
TGATGAGTAGGCA
CGATCCTAGCA
GCTAGGATCGT
GGGCTCAGTCGGA
CCCGAGTCAGCCT
CCTACGATGG
GGATGCTACC
ACACGCCG
TGTGCGGC
CTTGCC AGGCA
GAACGG TCCGT
GGTTGCAT
CCAACGTA
TGTACGGCAGA
ACATGCCGTCT
ACCGGAGGCTTCC
TGGCCTCCGAAGG
CCAGTGAAGT
GGTCACTTCA
TCGGTAGAACGGT
AGCCATCTTGCCA

(a)



(b)

Figure S5. DNA strand structures and base sequences used in Nanotrack (NT) construction in Figure 4. There are two tiles, (a) for tile A and (b) for tile B. Each tile consists of nine strands (A1 to A9 for A tile and B1 to B9 for B) indicated by different colors. Numbers from 1 to 8 indicate eight different combinations of 5-bases stick ends and n and n' are complementary each other. All combinations of complementary bases can be found in table 2.

	Strand Name	5’ to 3’	3’ to 5’	Strand Name	
1(NS)	A1	CGCTA	GCGAT	B6	1’(NS)
2(NS)	A1	AGGTC	TCCAG	B6	2’(NS)
3(EW)	A4	TCACG			
4(EW)	A4	TGAGC			
5(NS)	A6	CTCGC	GAGCG	B1	5’(NS)
6(NS)	A6	GCATG	CGTAC	B1	6’(NS)
7(EW)	A8	GAGAG	CTCTC	B4	7’(EW)
8(EW)	A8	CTGAC	GTCAG	B4	8’(EW)
			CTATT	B8	3’’(NS)
			ATTGA	B8	4’’(NS)

Table2. Sets of the complementary sticky-ends for constructing assembly of NT.

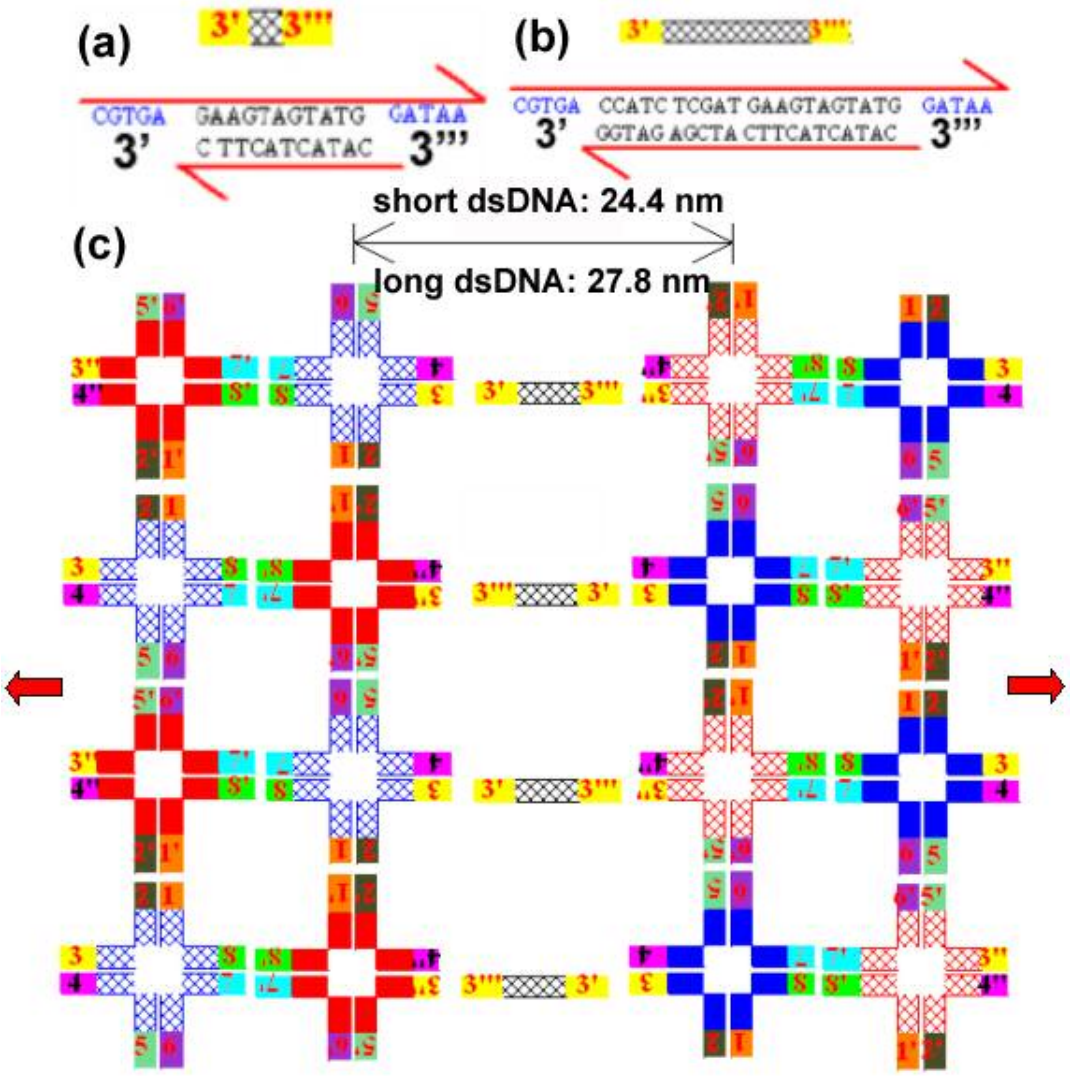


Figure S6. Cartoons of nanotrack + double stranded DNA bridges. Schematic drawings of (a) the short- (16 bases, 1.5 full-turns) and (b) the long- (26 bases, 2.5 full-turns) duplex DNA bridges. Each bridge consists of two strands with two sticky-ends at the ends of dsDNA molecules. The complementary sticky-end of n is n' and of n" is n'''. (c) Cartoon of nanotracks with the bridges. Here the solid and hatched cross-tiles indicate upward and downward facings, respectively. Designed distances between the nanotracks are 24.4 nm for the short-dsDNA bridge and 27.8 nm for the long.

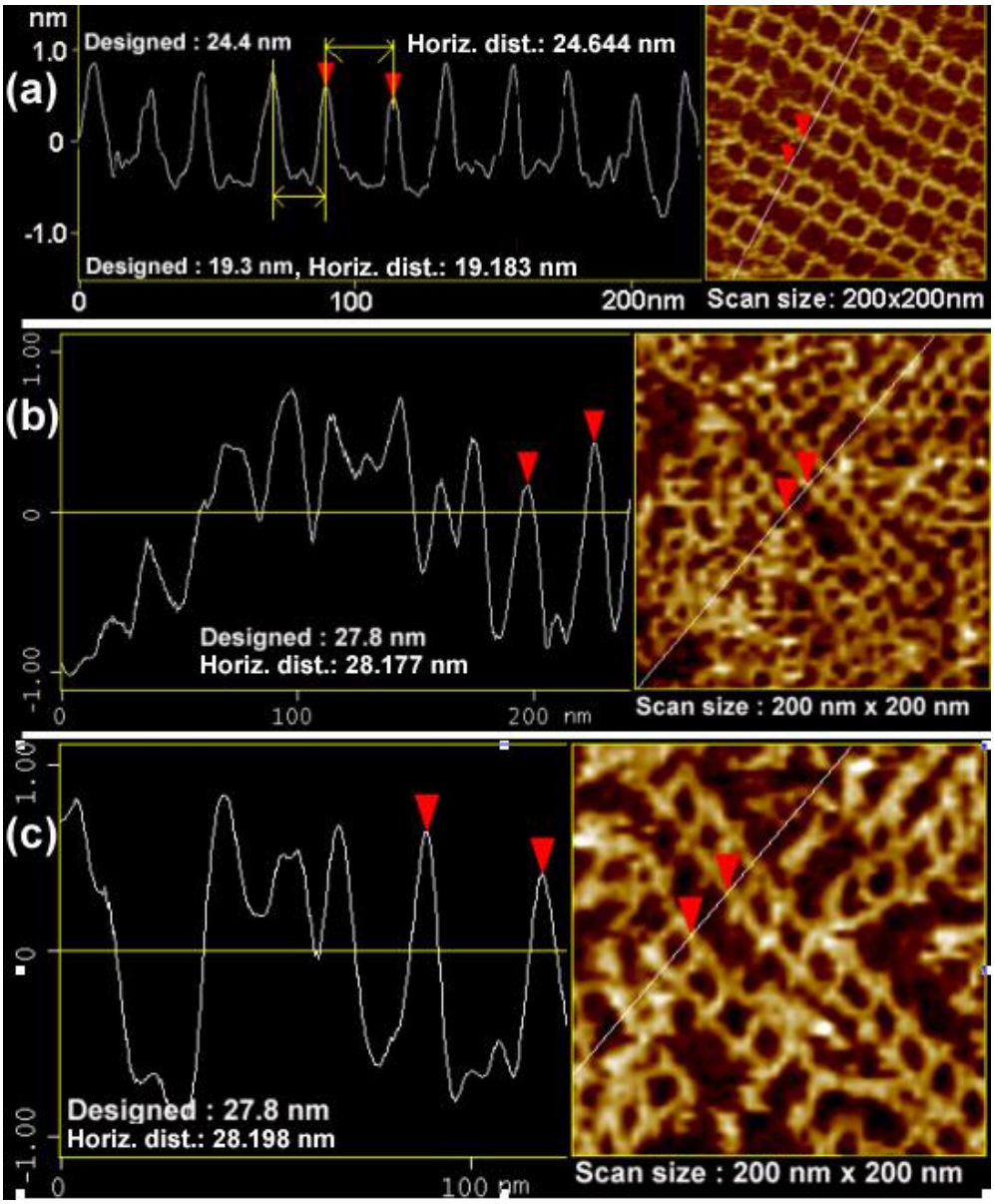


Fig. S7. Section measurements of Nanotrack + double stranded DNA bridges. Section profile AFM images of (a) nanotracks with the short dsDNA bridges. The measured distance between nanotracks is ~24.6 nm, matching the designed distance, 24.4 nm. (b) and (c) are two examples of section profiles of nanotracks with the long dsDNA bridges. Both data are in good agreement with designed structures with less than ± 1 nm differences.

Nanoarray	A representative AFM image in main figure	N_{NA} (per 1 μm^2)	N_T (per 1 μm^2)	Final yield (%)
(1 × 2)	Figure 1c	288	355	81
(2 × 2) without arm strands	Figure 1d	1184	1321	90
(2 × 2) with arm strands	Figure 1e	510	676	75
(2 × 2) with a single bridge	Figure 2c	180	550	33
(2 × 2) with both bridges	Figure 2d	122	720	17

Table S3. Yield analysis for nanoarray production; The assembly yield is defined by N_{NA}/N_T where N_{NA} stands for number of observed cross-tiles participating in properly formed nanoarrays, and N_T is the total number of cross-tiles observed. We have analyzed 2 μm × 2 μm scan size for each nanoarray and summarize yields in this table.