A Conformational Study of the 10-23 DNAzyme via Programmed DNA Self-Assembly

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Supporting Information

Oligonucleotides: All oligonucleotides were purchased from IDT Inc., purified by 10 – 15% denaturing PAGE, and their concentration were quantified by UV-vis absorption spectroscopy at 260 nm.

DNA strand sequences:

1A: 5'-TTC CTG ACG CGA TAA TCC CTC ATC TCG ATC CGT ACT GCC TGA CAG ACC TAA TGC ACG C-3'

1B: 5'-GGT CTG GCG TGC ATT ATC AGG CAG TAC GGA GGC TAG CTA CAA CGA CGA GAT GAG GGC AGG AAA TTA TCG CGT-3' (underlined bases are the catalytic core of 10-23 DNAzyme).

2B: 5'-GGT CTG GCG TGC ATT ATC AGG CAG TAC GGA TCG AGA TGA GGG CAG GAA ATT ATC GCG T-3'

3A: 5'-TTC CTG ACG CGA TAA TCC CTC ATC TCG CCT GAC GTT TTA CGT CCC GTA CTG CCT GAC AGA CCT AAT GCA CGC-3' (underlined bases are the Holliday Junction sequence)

3B: 5'-GGT CTG GCG TGC ATT ATC AGG CAG TAC GGA CAG CTT TTG CTG TGG CGA GAT GAG GGC AGG AAA TTA TCG CGT-3' (underlined bases are the Holliday Junction sequence)

Motif Composition:

M1: 1A + 1B

M2: 1A + 2B

M3: 3A + 3B

Surface-assisted slow annealing: 400 μL annealing solution was prepared by mixing ssDNAs in TA/Mg^{2+} buffer (40 mM tris base, 20 mM acetic acid, 20 mM magnesium

acetate for **M1** and **M2**, 15 mM magnesium acetate for **M3**, pH is adjusted to 8.0). Then, the fresh-peeled mica disc was immersed into the solution. The mica solution mixture was heated to 95 °C and slowly cooled down to 22 °C over 48 h in a water bath.

AFM images: AFM images in fluid mode were captured by MultiMode 8 (Bruker) using ScanAsyst-fluid mode with ScanAsyst-fluid+ probes (Bruker). After slow annealing, the original solution was removed, and then 20 μL TA/Mg^{2+} (40 mM tris base, 20 mM acetic acid, 20 mM magnesium acetate, pH is adjusted to 8.0) buffer was added onto the surface and scanned in fluid immediately. For 800 nm images, the line/sample ratio is 768. Images were clipped to 150 nm square with whole and single crystallized array from 800 nm images. FFT and inverse FFT were performed for 150 nm images by Spectrum 2D function in the software Nanoscope Analysis 1.5 (Bruker).

Structure analysis of 10-23 DNAzyme NMR structure: The pre-catalytic complex of 10-23 DNAzyme with RNA target (PDB id: 7PDU) was selected.[1] 3DNA was applied for structure analysis. The 10-23 DNAzyme hybrid was first tailored to two single A-form duplexes: from base -9 to -6, and +6 to +9 (Figure S7). The duplex vectors were deduced by 3DNA,[2] and the interhelical angle could be calculated.

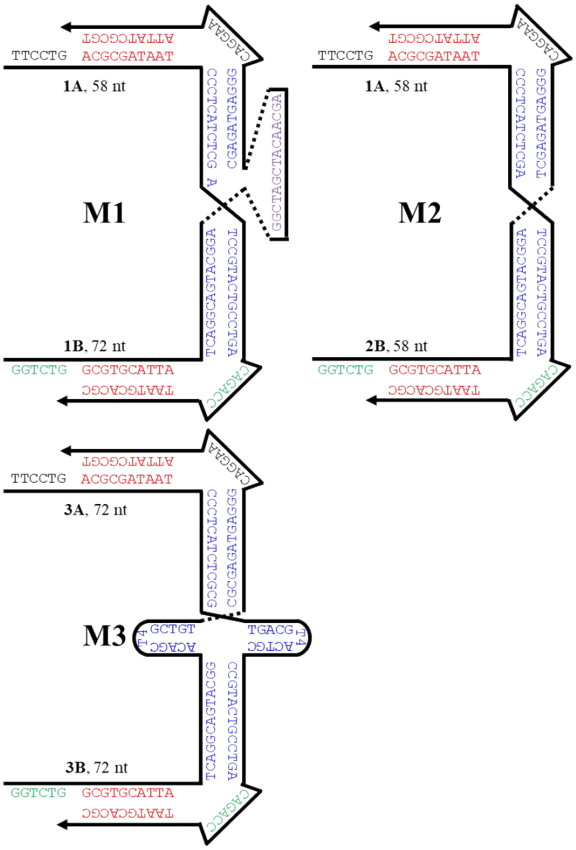

Figure S1. Schematic designs with sequences for the C-shaped motif for DNA 2D array assembly. **M1**: DNAzyme loop, **M2**: duplex, **M3**: Holliday Junction.

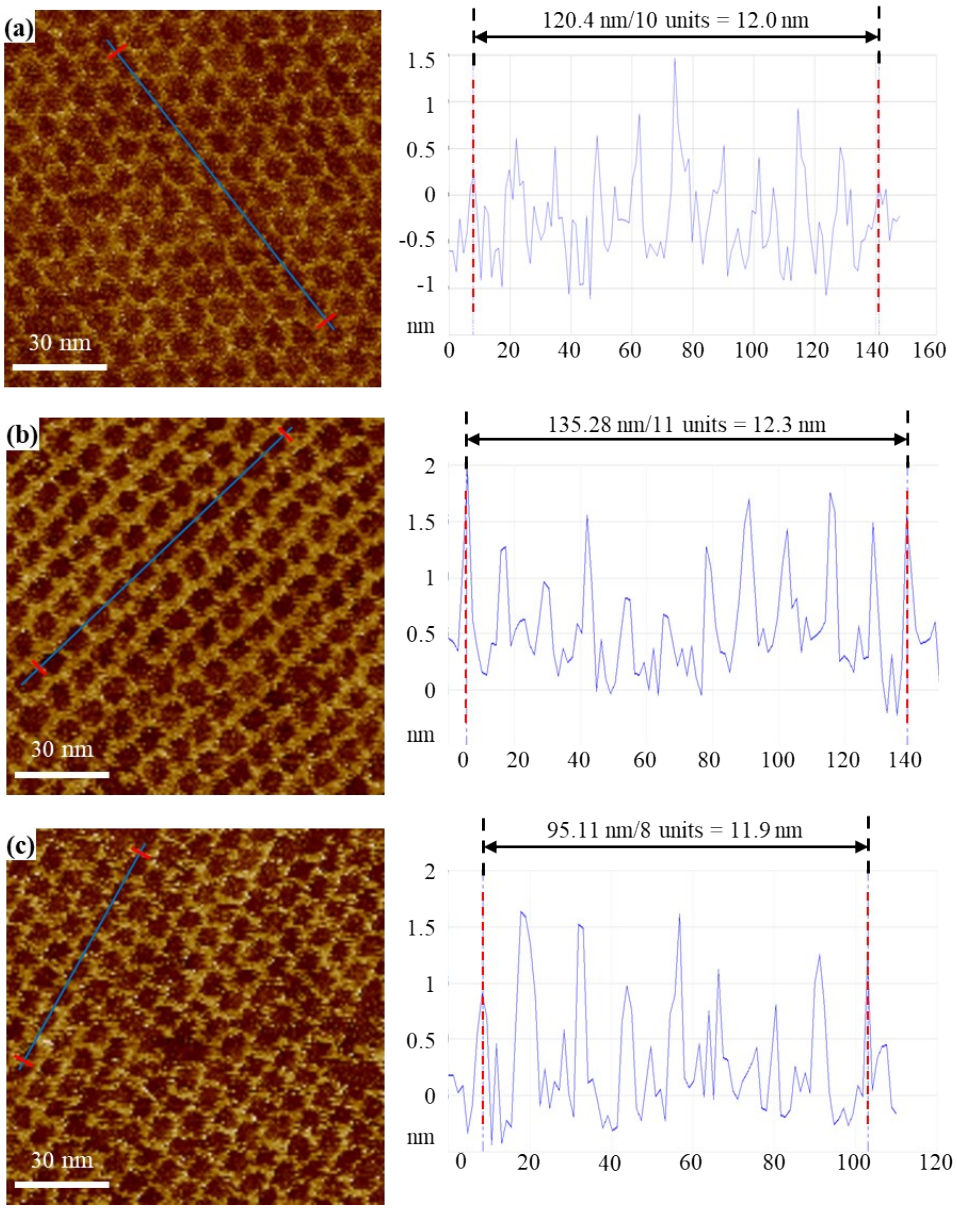

Figure S2. AFM analysis of 2D array. A cropped single crystallized DNA 2D array AFM image (Left) and a section profile to determine the repeating distance of the latitudinal duplexes (right), the section line (blue) and analyzed region (red) are indicated on AFM images. (a) **M1** with DNAzyme (b) **M2** with duplex (c) **M3** with Holliday Junction.

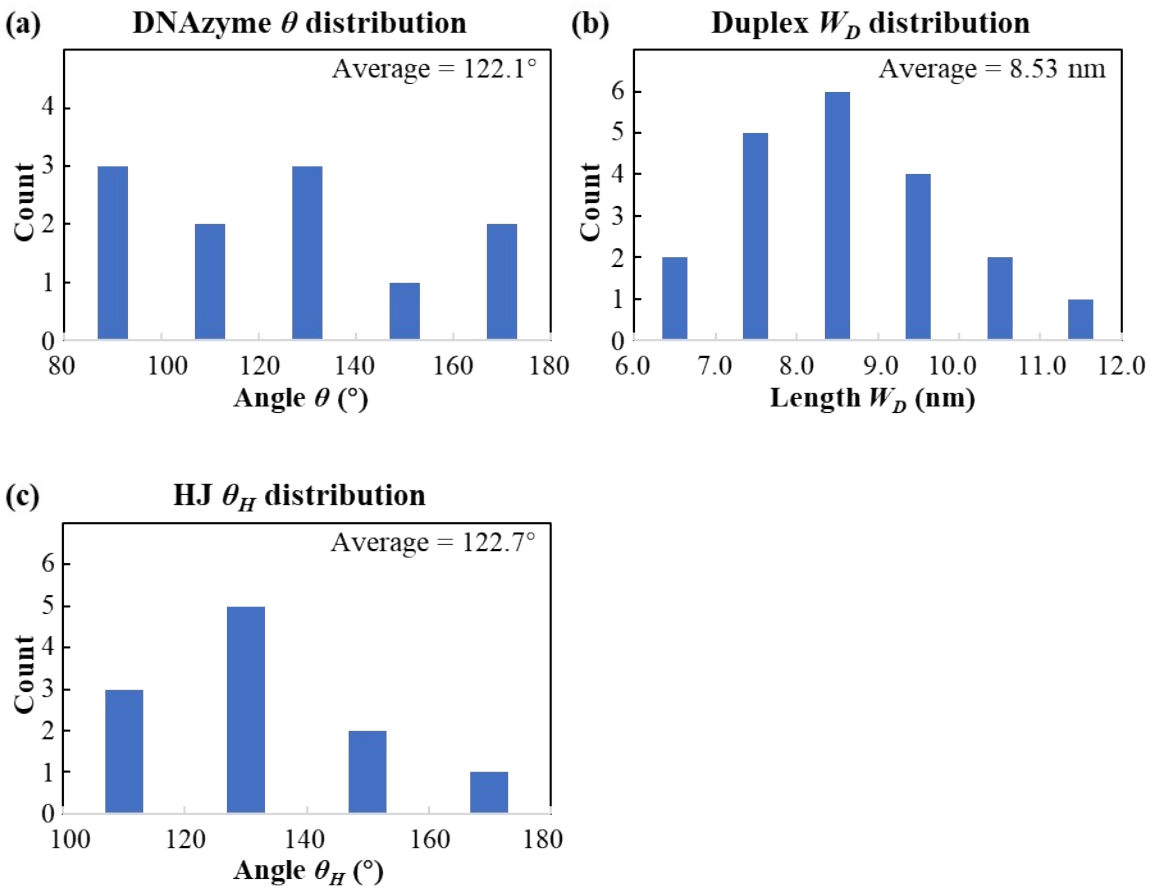

Figure S3. Distribution of the interhelical angle and length. Where X-axis is the calculated angle or length, and Y-axis is the total number of measurements with angles falling within the designated range on the X-axis. (a) Interhelical angle θ distribution of **M1** with DNAzyme (b) Length W_D distribution of **M2** with duplex (c) Interhelical angle θ_H distribution of **M3** with Holliday Junction.

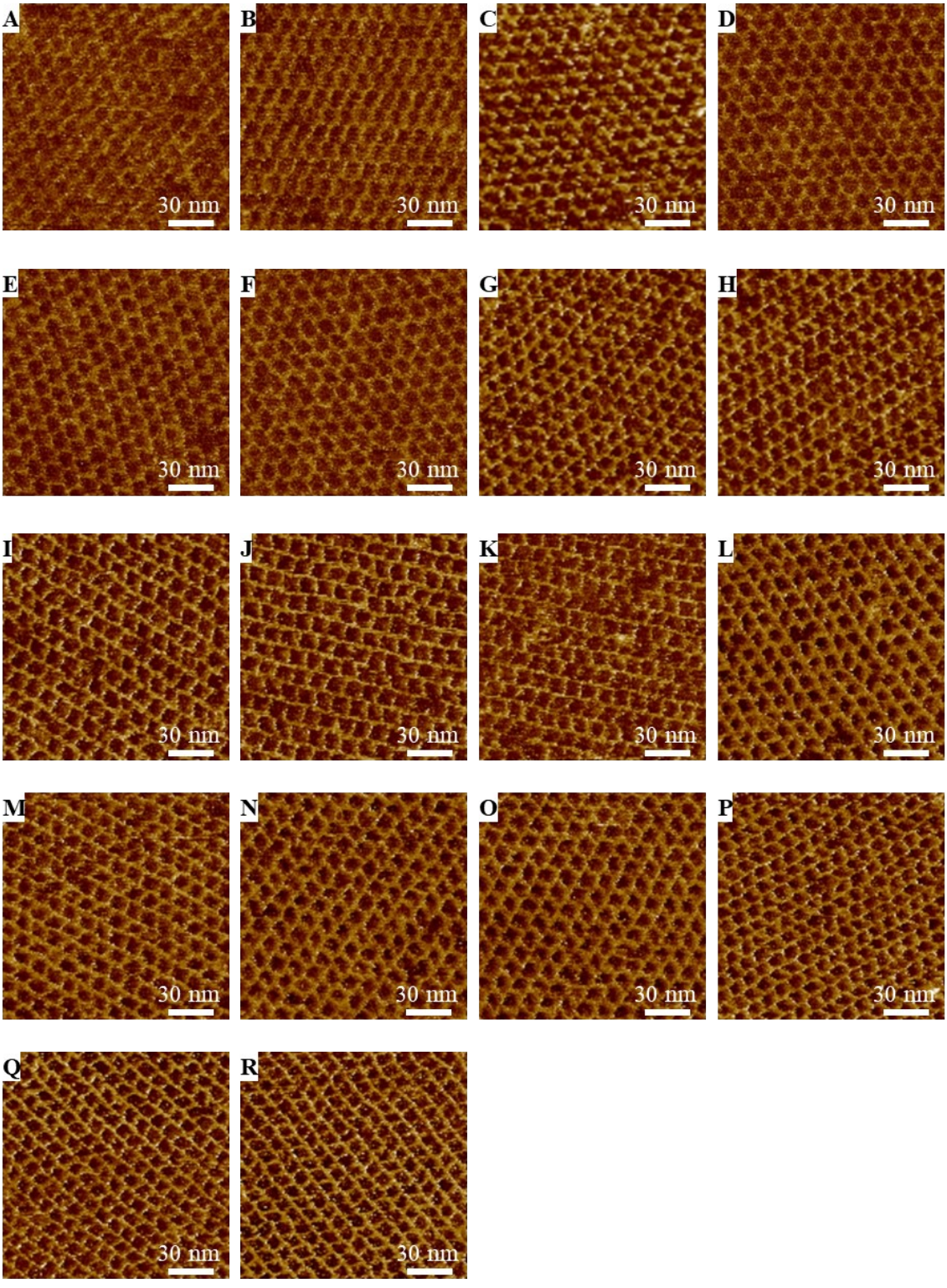

Figure S4. AFM images of 2D arrays of **M1** motif (containing DNAzyme) used for FFT analysis. Sample numbers are at the upper left of each image.

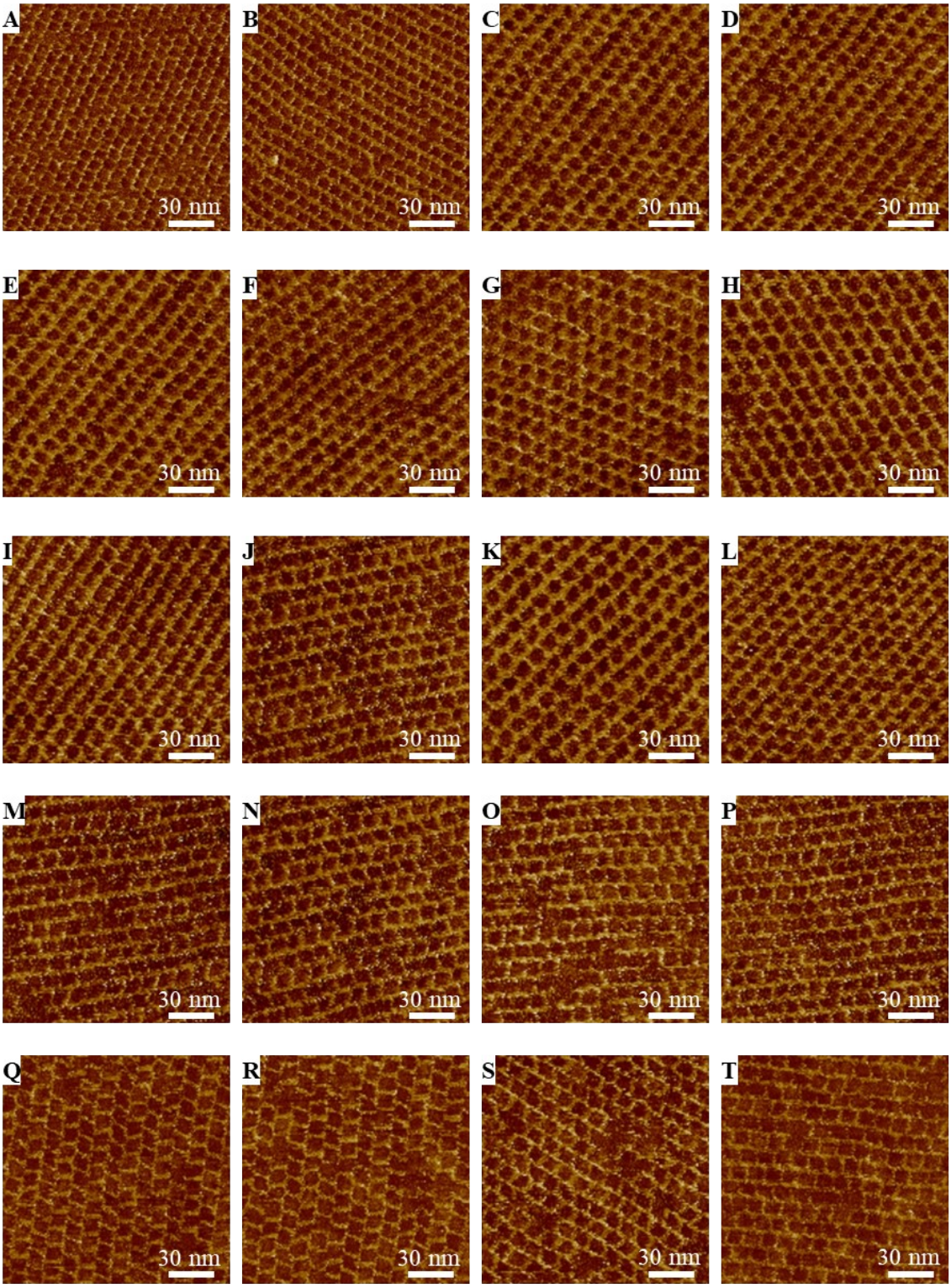

Figure S5. AFM images of 2D arrays of **M2** motif (containing duplex) used for FFT analysis. Sample numbers are at the upper left of each image.

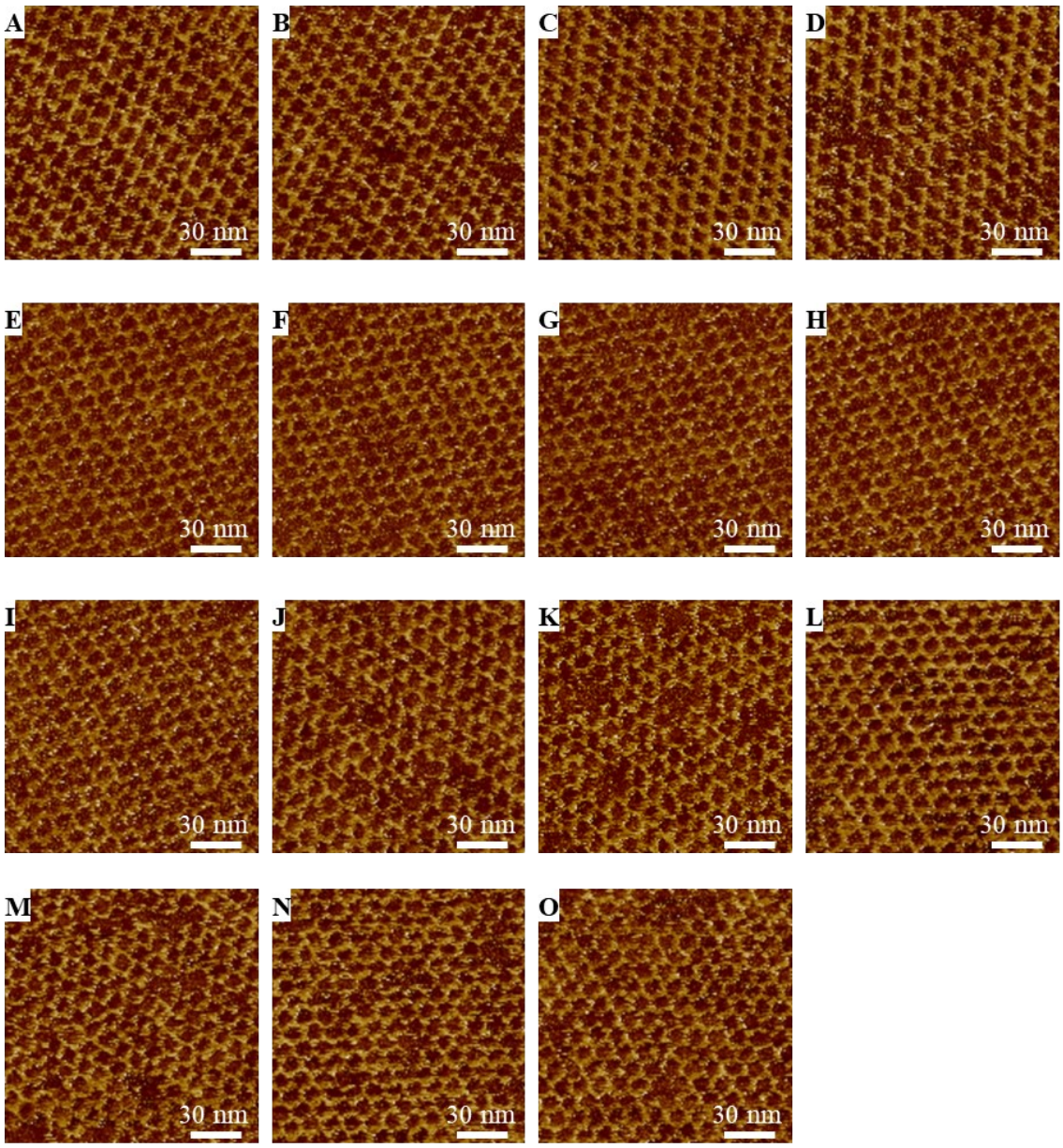

Figure S6. AFM images of 2D arrays of **M3** motif (containing Holliday junction) used for FFT analysis. Sample numbers are at the upper left of each image.

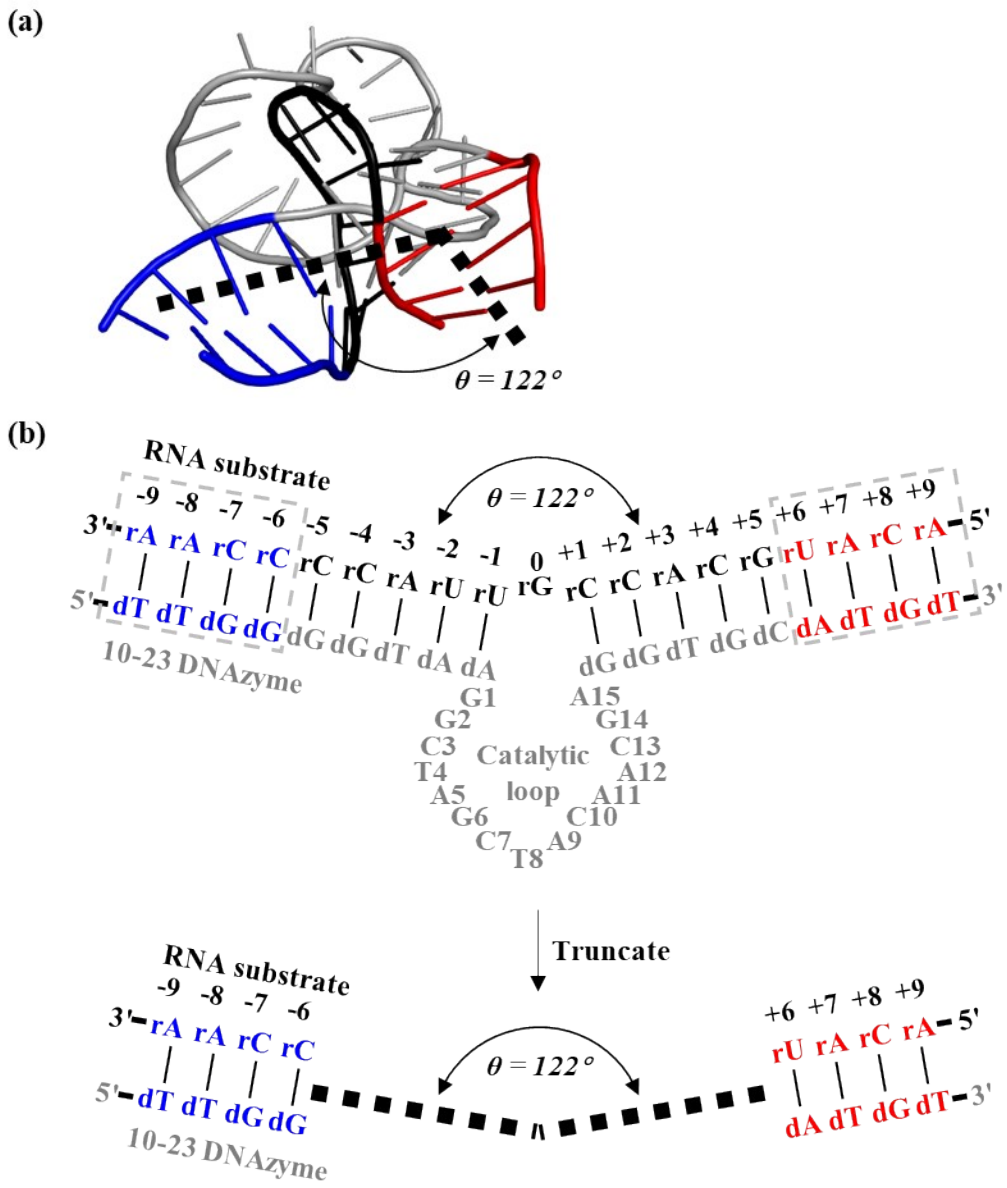

Figure S7. Measuring the interhelical angle of 10-23 DNAzyme from its NMR structure. (a) The molecular structure of NMR-solved 10-23 DNAzyme, helical segments applied in angle measurement were shown in red and blue color (b) Schematic drawing of truncating in dash-line box and measuring of the interhelical angle, colors are corresponding with the molecular structure. Two helical arms were first truncated to two short duplexes in terminal, and 3DNA was applied to obtain duplex axis vectors for further angle determination.

Table S1. Measurements and angle calculations for **M1** (DNAzyme) array

Sample	L (nm)	L' (nm)	W (nm)	W' (nm)	d_1 (nm)	d_2 (nm)	θ (°)
A	10.56	11.62	10.24	7.30	3.63	4.62	116.0
B	10.56	10.12	10.65	9.11	3.63	4.62	N/A
C	10.56	10.89	11.10	8.76	3.63	4.62	N/A
D	10.56	13.97	10.09	5.62	3.63	4.62	83.8
E	10.56	13.36	10.46	6.26	3.63	4.62	96.2
F	10.56	13.64	10.09	5.81	3.63	4.62	88.9
G	10.56	11.08	11.25	8.72	3.63	4.62	N/A
H	10.56	11.11	11.80	9.22	3.63	4.62	N/A
I	10.56	11.78	11.50	8.31	3.63	4.62	162.7
J	10.56	11.48	11.20	8.30	3.63	4.62	162.1
K	10.56	11.04	10.45	8.00	3.63	4.62	144.1
L	10.56	12.08	10.55	7.22	3.63	4.62	118.4
M	10.56	11.98	11.00	7.70	3.63	4.62	132.6
N	10.56	11.15	11.55	8.94	3.63	4.62	N/A
O	10.56	11.38	11.25	8.44	3.63	4.62	N/A
P	10.56	10.22	9.49	7.81	3.63	4.62	136.4
Q	10.56	10.33	9.61	7.82	3.63	4.62	136.9
R	10.56	9.56	10.10	9.16	3.63	4.62	N/A

Table S2. Measurements and length calculations for **M2** (Duplex) array

Sample	L (nm)	L' (nm)	W (nm)	W' (nm)	d_1 (nm)	d_2 (nm)	θ (°)
A	10.56	10.43	10.95	9.09	4.29	4.29	
B	10.56	11.94	11.6	8.26	4.29	4.29	
C	10.56	9.65	11.75	10.86	4.29	4.29	
D	10.56	9.65	11.75	10.86	4.29	4.29	
E	10.56	9.92	12.35	11.15	4.29	4.29	
F	10.56	11.79	11.15	7.99	4.29	4.29	
G	10.56	11.64	10.45	7.48	4.29	4.29	
H	10.56	14.02	10.75	6.10	4.29	4.29	
I	10.56	11.18	10.85	8.25	4.29	4.29	
J	10.56	12.25	10.8	7.31	4.29	4.29	

K	10.56	11.91	12.9	9.44	4.29	4.29
L	10.56	11.36	10.55	7.81	4.29	4.29
M	10.56	11.18	11.35	8.72	4.29	4.29
N	10.56	11.2	12.1	9.41	4.29	4.29
O	10.56	11.34	11.9	9.08	4.29	4.29
P	10.56	11.25	11.35	8.65	4.29	4.29
Q	10.56	10.98	10.74	8.33	4.29	4.29
R	10.56	11.04	10.74	8.27	4.29	4.29
S	10.56	11.91	9.535	6.45	4.29	4.29
T	10.56	11.48	9.945	7.15	4.29	4.29

Table S3. Measurements and angle calculations for **M3** (Holliday Junction) array

Sample	L (nm)	L' (nm)	W (nm)	W' (nm)	d_1 (m)	d_2 (nm)	θ (°)
A	10.56	12.32	10.15	6.70	4.29	4.29	102.59
B	10.56	10.84	9.89	7.63	4.29	4.29	125.70
C	10.56	10.41	10.74	8.89	4.29	4.29	N/A
D	10.56	11.99	10.75	7.47	4.29	4.29	121.01
E	10.56	10.56	11.75	9.75	4.29	4.29	N/A
F	10.56	10.49	11.05	9.12	4.29	4.29	N/A
G	10.56	10.71	10.55	8.40	4.29	4.29	156.63
H	10.56	10.59	10.55	8.52	4.29	4.29	166.45
I	10.56	10.59	11.40	9.37	4.29	4.29	N/A
J	10.56	12.16	10.55	7.16	4.29	4.29	113.17
K	10.56	11.97	11.05	7.75	4.29	4.29	129.13
L	10.56	10.80	10.42	8.18	4.29	4.29	145.03
M	10.56	10.79	9.49	7.29	4.29	4.29	116.29
N	10.56	10.80	10.05	7.82	4.29	4.29	131.46
O	10.56	11.00	10.12	7.71	4.29	4.29	127.96

References

1. J. Borggräfe, J. Victor, H. Rosenbach, A. Viegas, C. G. W. Gertzen, C. Wuebben, H. Kovacs, M. Gopalswamy, D. Riesner, G. Steger, O. Schiemann, H. Gohlke, I. Span and M. Etzkorn, *Nature.,* 2022, **601**, 144-149.
2. X. Lu and W. K. Olson, *Nucleic Acids Res.*, 2003, **31**, 5108-5121.