Electronic Supplementary Materials

Two-layer stacked multiple arm junction tiles and nanostructures assembled with small circular DNA molecules serving as scaffolds

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This file includes:

1 Experimental materials and methods

All DNA strands were provided with denaturing PAGE (polyacrylamide gel electrophoresis) purification by Sangon Biotech (Shanghai, China) without further purification. Detailed DNA sequences and structural designs are shown in the following sections. 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 (Nanjing, China). Pure water was from Milli-Q Ultrapure Water Purification System (Merck Millipore, Shanghai, China). The buffer of 1×TBE is composed of 89 mM Tris, 89 mM boric acid, and 2 mM EDTA at pH 8.0, and that of 1×TAE-Mg^{2+} is composed of 40 mM Tris, 40 mM HAc, 40 mM $Mg(Ac)_2$ and 2 mM EDTA at pH 8.0. (Sangon Biotech, Shanghai, China)

Preparation of circular DNA. The circular DNAs of 72 and 96 nt were circularised by T4 DNA ligase. Firstly, a 5′-phosphorylated DNA strand (3.5 μM) and its corresponding 20 nt splint strand (4.5 μM) were mixed in 80 μL TE buffer (pH = 8.0). The sample was heated to 95 °C for 5 minutes, then cooled down to room temperature within 4 hours. The T4 ligase (350 U/μL, 10 μL) and 10×T4 buffer (10 μL) were added to the sample, then the mixture was incubated for 16 hours at 16 °C. After reaction, the T4 ligase was inactivated at 95 °C for 5 minutes. Then, 10 μL 10×exonuclease I buffer and 10 μL exonuclease I (5 U/μL) were added to digest the remaining linear DNA residues of templates and splints by incubation at 37 °C for 30 minutes. Exonuclease I selectively digested single-stranded DNAs, and left circular DNAs intact. The circular DNA strands were purified by denaturing PAGE.

Assembly of DNA nanostructures via the one-pot protocol. $3AJ^2$-6E, $3AJ^2$-3E, and $4AJ^2$-4E were assembled via mixing DNA strands with an equimolar stoichiometric ratio at a final concentration of 0.2 μM (the concentration will be the same in the following contents unless otherwise noted) in 50 μL 1×TAE-Mg^{2+} buffer. The mixture was annealed in a thermocycler programmed to cool as follows: 95 °C to 60 °C at a rate of 1 °C per 5 minutes, then at a rate of 0.1°C per 10 minutes to 20 °C, and finally held at 20 °C at the end of the cycle.

Assembly of DNA nanostructures via the two-pot protocol. Two assemblies of ($3AJ^2$-3E + 3AJ-3E) and ($3AJ^2$-EOE + 3AJ-3O) were assembled via the two-pot protocol. In the

first step, each tile (either 3AJ^2-3E, 3AJ-3E, 3AJ^2-EOE, or 3AJ-3O) was annealed following the one-pot approach. In the second step, one third volume of a one-layer tile (3AJ-3E or 3AJ-3O) was added correspondingly to the annealed two-layer tile (3AJ^2-3E, or 3AJ^2-EOE) and gently mixed. Then, the mixture was annealed in a thermocycler programmed to cool from 50 °C to 4 °C in about 52 h: 50 °C to 20 °C at a rate of 0.1 °C per 10 minutes, then at a rate of 1°C per 10 minutes to 4°C, and finally held at 4 °C at the end of the cycle. The high concentration (0.5 μM) and off-ratio mixtures were prepared according to designs and annealed with the two-pot protocol.

Native PAGE of 3AJ^2 and 4AJ^2.

The annealed sample (circa 5 μL) of 3AJ^2 or 4AJ^2 with each arm carrying a 7 bp blunted overhang was mixed with the same amount of Glycerol Gel Loading Buffer (0.25% Bromophenol Blue; 0.25% Xylene Cyanole FF; 60% Glycerol), then subjected to native PAGE (8%) in an ice-water bath. A DNA Marker B (10 mM Tris-HCl (pH 7.6), 10 mM EDTA, 0.033% bromophenol blue, 0.008% xylene cyanol and 10% glycerol) was added in the first lane as the migration standard (bands of 100, 200, 300, 400, 500, 600 bp from bottom to top). Electrophoresis was carried out in 8% native polyacrylamide gel for 5 hours at 90 V in 1×TAE-Mg^{2+} buffer. Dyed by 4S GelRed for 1 hour, the gel was scanned using a Tanon 2500R laser scanner.

Native PAGE for the first-step products of 3AJ^2-3E and 3AJ^2-EOE.

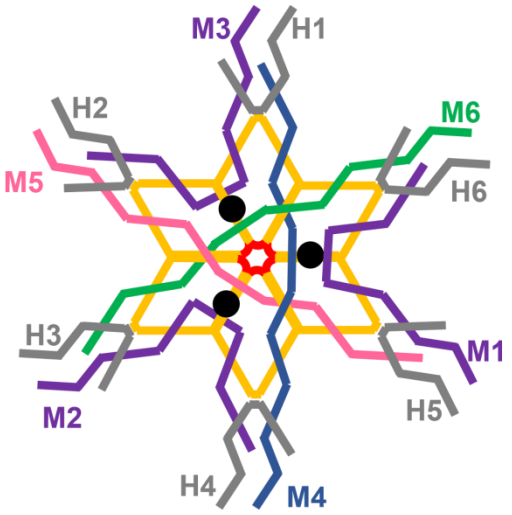
The first-step annealed samples of 3AJ^2-3E and 3AJ^2-EOE were mixed with the same amount of Glycerol Gel Loading Buffer VII (0.25% Bromophenol Blue; 0.25% Xylene Cyanole FF; 60% Glycerol) separately. A DNA Marker F plus (10 mM Tris-HCl (pH 7.6), 10 mM EDTA, 0.033% bromophenol blue, 0.008% xylene cyanol and 10% glycerol) was added in the first lane as the migration standard (bands of 200, 400, 600, 800, 1000, 1200, 1400, 1600, 1800, 2000 bp from bottom to top). Electrophoresis was carried out in 4% native polyacrylamide gel at 90 V in 1×TAE-Mg^{2+} buffer. Dyed by 4S GelRed for 1 hour, the gel was scanned using a Tanon 2500R laser scanner.

AFM imaging. A 2 μl annealed sample solution was spotted onto a freshly cleaved mica surface and stayed about 1 min for adsorption of DNA arrays to the mica surface. To get rid of debris, 70 μl 1× TAE-Mg^{2+} buffer was deposited onto the sample spot and moved away. Then 1× TAE-Mg^{2+} buffer was deposited on the spot and to the AFM tip

at 70 µl and 30 µl, separately. AFM imaging was carried out with the ScanAsyst mode in fluid (Dimension FastScan, Bruker) with Scanasyst-Fluid+ tips (Bruker).

2 DNA sequences used in this work

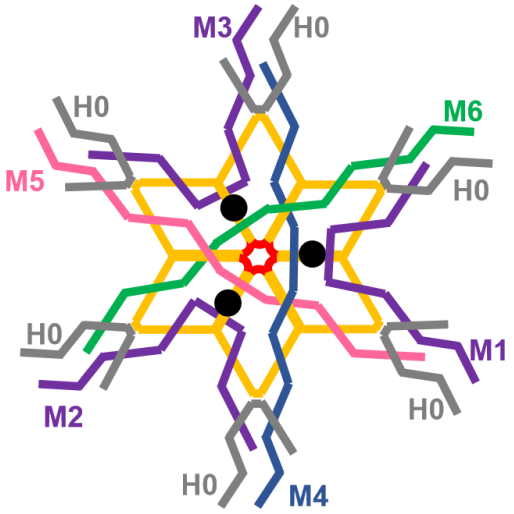
3AJ^2-6E (with three diagonally and specifically paired E-edges via upper-lower connection)



C1	TAAGATGAAGATAGCGCACAATTTCGGTCGGATTCCGTCTCTGTCTTTA ACTCGTCTATGCCAAGCCCGTTT
C2	GCGATAATCTCTGTGGACTGATTTGGGTACTCCACCAGGTAGGCGTTTA CGATCATCCTAAGCACCTTGTTT
H1	TCCTCAGGCATCGCGCACGTAG
H2	GCACCGCGCGCATGTCGAACTC
H3	CGAAGTACGATCCCACGACCGG
H4	CGATCCAACTGCGCGGACGTGC
H5	CTTCTTATATACGCTTCGCCCG
H6	ACCGGCCTCGCGATCGGCCGCC
M1	CGGTGCCGGGCGAAAGATTATCAGAGACGGATCGCGAG
M2	GCCGGTCCGGTCGTTGGAGTACTGCGCTATCATGCGCG
M3	GGATCGCTACGTGCGGATGATCGCTTGGCAGCGCAGTT
M4	TGAGGAGCACGTCCTAGACGAGTTGACGCCAAGGTGCTTAGCGATGCC
M5	ACTTCGGGCGGCCGAATCCGACCGTTGCCTCAGTCCACAGGCGTATAT
M6	AAGAAGGAGTTCGACTTCATCTTACGGGTCGCCTACCTGGGGGATCGT

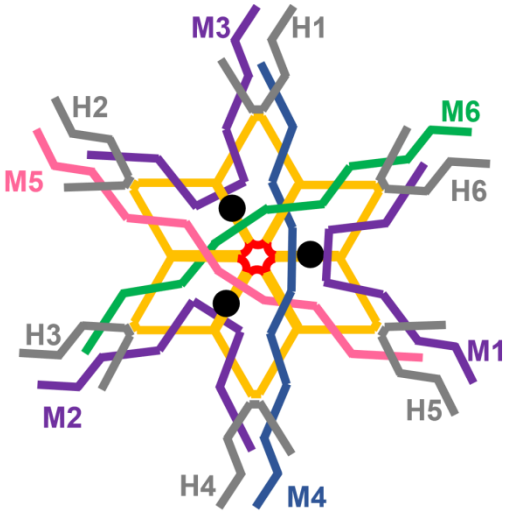
3AJ^2-6E (with the same 8 nt sticky end of a palindromic sequence

GTAATTAC for self-adjusting connection)



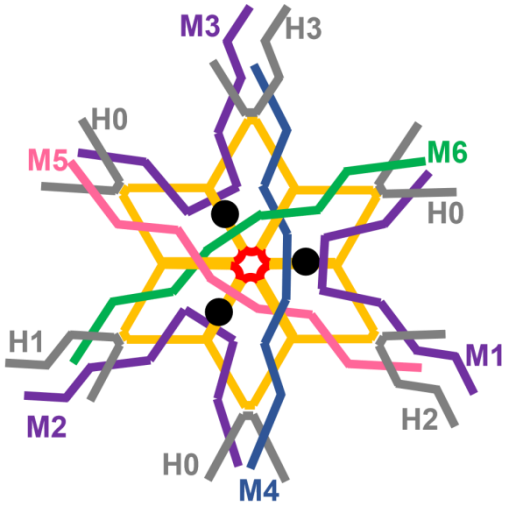
C1	TAAGATGAAGATAGCGCACAATTTCGGTCGGATTCCGTCTCTG TCTTTAACTCGTCTATGCCAAGCCCGTTT
C2	GCGATAATCTCTGTGGACTGATTTGGGTACTCCACCAGGTAGG CGTTTACGATCATCCTAAGCACCTTGTTT
H0	TATGTTCACTCTCGGTAATTAC
M1	CGAGAGTAGATTATCTGCGCTATGAACATAGTAATTAC
M2	CGAGAGTTGGAGTACGCTTGGCAGAACATAGTAATTAC
M3	CGAGAGTGGATGATCAGAGACGGGAACATAGTAATTAC
M4	CGAGAGTTAGACGAGTTGACGTCGCCTACCTGGGAACATAGT AATTAC
M5	CGAGAGTCTTCATCTTACGGCCTCAGTCCACAGGAACATAGTA ATTAC
M6	CGAGAGTAATCCGACCGTTGGCCAAGGTGCTTAGAACATAGT AATTAC

3AJ^2-6E (with an asymmetric design using all six arms for connection in Figure 2)



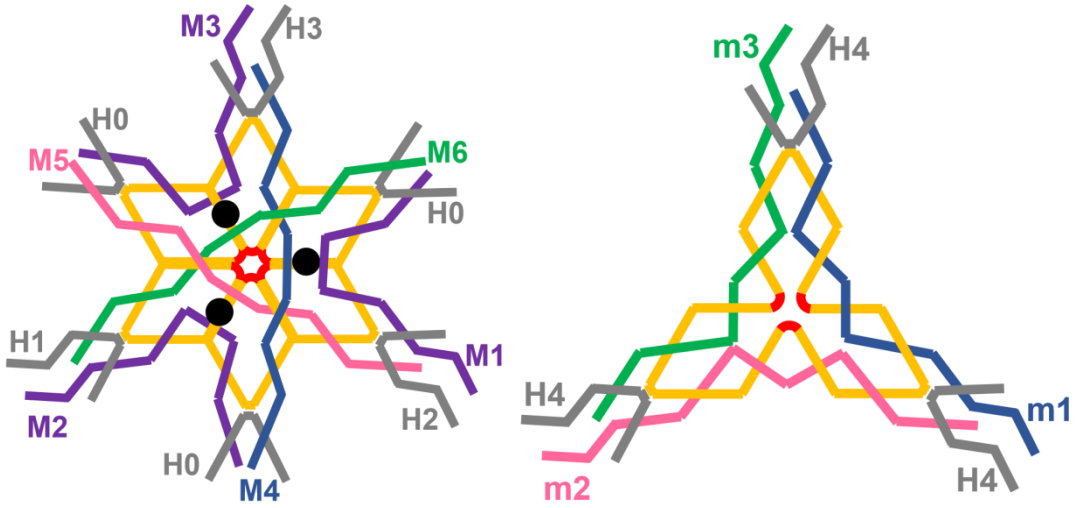
C1	TAAGATGAAGATAGCGCACAATTTCGGTCGGATTCCGTCTCTG TCTTTAACTCGTCTATGCCAAGCCCGTTT
C2	GCGATAATCTCTGTGGACTGATTTGGGTACTCCACCAGGTAGG CGTTTACGATCATCCTAAGCACCTTGTTT
H1	TCCTCAGGCATCGCGCACGTAG
H2	GCACCGCGCGCATGTCGAACTC
H3	CGAAGTACGATCCCACGACCGG
H4	CGATCCAACTGCGCGGACGTGC
H5	CTTCTTATATACGCTTCGCCCG
H6	ACCGGCCTCGCGATCGGCCGCC
M1	ACTTCGCGGGCGAAAGATTATCAGAGACGGATCGCGAG
M2	AAGAAGCCGGTCGTTGGAGTACTGCGCTATCATGCGCG
M3	GGATCGCTACGTGCGGATGATCGCTTGGCAGCGCAGTT
M4	TGAGGAGCACGTCCTAGACGAGTTGACGCCAAGGTGCTTAGC GATGCC
M5	GCCGGTGAGTTCGACTTCATCTTACGGGTCGCCTACCTGGGGG ATCGT
M6	CGGTGCGGCGGCCGAATCCGACCGTTGCCTCAGTCCACAGGC GTATAT

3AJ^2-3E



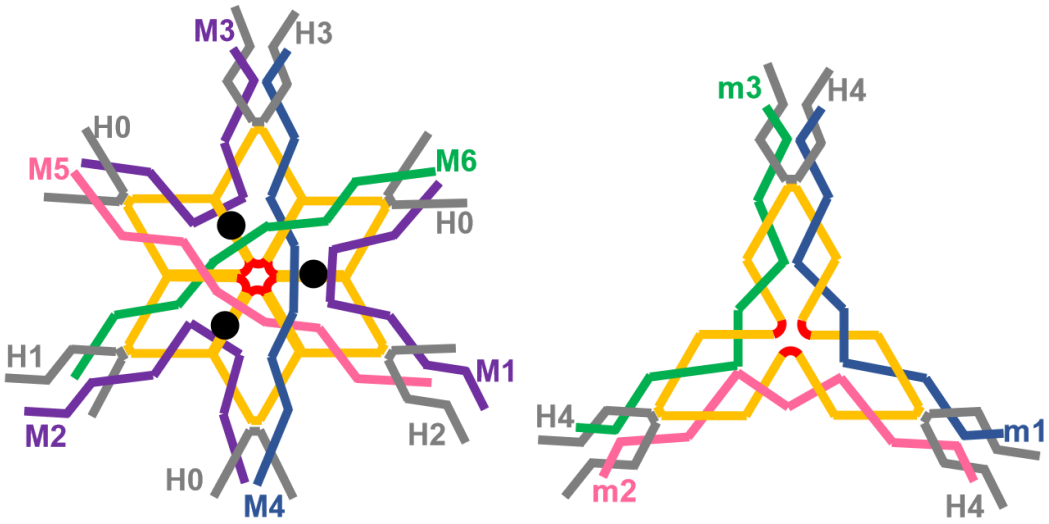
C1	TAAGATGAAGATAGCGCACAATTTCGGTCGGATTCCGTCTCTGT CTTTAACTCGTCTATGCCAAGCCCGTTT
C2	GCGATAATCTCTGTGGACTGATTTGGGTACTCCACCAGGTAGG CGTTTACGATCATCCTAAGCACCTTGTTT
H0	TCCCTCTGGGCCTT
H1	TCCTCAGGCATCGCGCACGTAG
H2	GCACCGCGCGCATGTCGAACTC
H3	ACGCGTACGATCCCACGACCGG
M1	TGAGGAGAGTTCGAAGATTATCAGAGACGGAGAGGGA
M2	CGGTGCCTACGTGCTGGAGTACTGCGCTATAGAGGGA
M3	ACGCGTCCGGTCGTGGATGATCGCTTGGCAAGAGGGA
M4	AAGGCCCTAGACGAGTTGACGCCAAGGTGCTTAGGGATCGT
M5	AAGGCCCAATCCGACCGTTGCCTCAGTCCACAGCATGCGCG
M6	AAGGCCCCTTCATCTTACGGGTCGCCTACCTGGGCGATGCC

3AJ^2-3E + 3AJ-3E



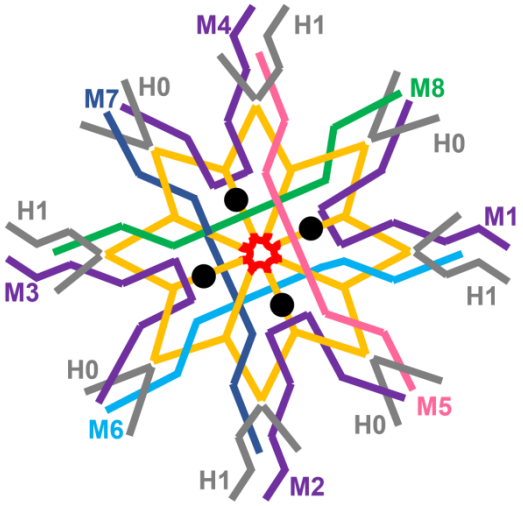
C1	TAAGATGAAGATAGCGCACAATTTCGGTCGGATTCCGTCTCTGT CTTTAACTCGTCTATGCCAAGCCCGTTT
C2	GCGATAATCTCTGTGGACTGATTTGGGTACTCCACCAGGTAGG CGTTTACGATCATCCTAAGCACCTTGTTT
H0	TCCCTCTGGGCCTT
H1	TCCTCAGGCATCGCGCACGTAG
H2	GCACCGCGCGCATGTCGAACTC
H3	CGAAGTACGATCCCACGACCGG
M1	TGAGGAGAGTTCGAAGATTATCAGAGACGGAGAGGGA
M2	CGGTGCCTACGTGCTGGAGTACTGCGCTATAGAGGGA
M3	GGATCGCCGGTCGTGGATGATCGCTTGGCAAGAGGGA
M4	AAGGCCCTAGACGAGTTGACGCCAAGGTGCTTAGGGATCGT
M5	AAGGCCCAATCCGACCGTTGCCTCAGTCCACAGCATGCGCG
M6	AAGGCCCCTTCATCTTACGGGTCGCCTACCTGGGCGATGCC
C1	TAAGATGAAGATAGCGCACAATTTCGGTCGGATTCCGTCTCTGT CTTTAACTCGTCTATGCCAAGCCCGTTT
H4	CGATCCAACTGCGCGGACGTGC
m1	ACTTCGGCACGTCCCTTCATCTTACGGGCTTGGCAGCGCAGTT
m2	ACTTCGGCACGTCCAATCCGACCGTTGTGCGCTATGCGCAGTT
m3	ACTTCGGCACGTCCTAGACGAGTTGACAGAGACGGGCGCAGTT

3AJ^2-EOE + 3AJ-3O



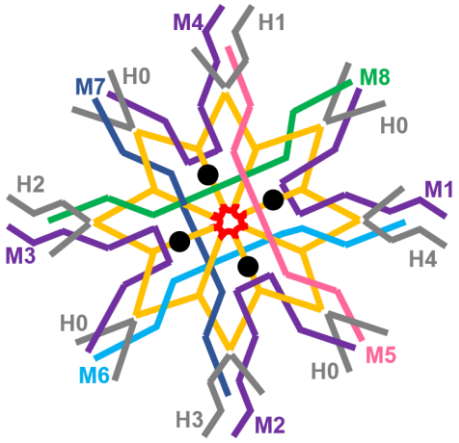
C1	TAAGATGAAGATAGCGCACAATTTCGGTCGGATTCCGTCTCTGT CTTTAACTCGTCTATGCCAAGCCCGTTT
C2	GCGATAATCTCTGTGGACTGATTTGGGTACTCCACCAGGTAGG CGTTTACGATCATCCTAAGCACCTTGTTT
H0	TCCCTCTGGGCCTT
H1	TCCTCAGGCATCGCGCACGTAG
H2	CGAAGTACGATCCCACGACCGG
H3	CGAGTCCAAGTCCGTTAACCATTGGTCCTTCT
M1	TGAGGACCGGTCGTAGATTATCAGAGACGGAGAGGGA
M2	ACTTCGCTACGTGCTGGAGTACTGCGCTATAGAGGGA
M3	ACCAATGGTTGGATGATCGCTTGGCAAGAGGGA
M4	AAGGCCCTAGACGAGTTGACGCCAAGGTGCTTAAACGGACTTG
M5	AAGGCCCAATCCGACCGTTGCCTCAGTCCACAGGGGATCGT
M6	AAGGCCCCTTCATCTTACGGGTCGCCTACCTGGGCGATGCC
C1	TAAGATGAAGATAGCGCACAATTTCGGTCGGATTCCGTCTCTGT CTTTAACTCGTCTATGCCAAGCCCGTTT
H4	GACTCGCAAGTCCGTTAACCATTGGTAGAAGG
m1	ACCAATGGTTCTTCATCTTACGGGCTTGGCAAACGGACTTG
m2	ACCAATGGTTAATCCGACCGTTGTGCGCTATAACGGACTTG
m3	ACCAATGGTTTAGACGAGTTGACAGAGACGGAACGGACTTG

4AJ^2-4E (sticky end cohesion with the palindromic sequence ACGCGT)



C1	GGTAGCTAACTATGTTGCCTGTTTCCACTACAACAGTATCTAAG CTTTCGTGTGTGGGGAACATCCACATTTCTTTAGTGAATCGAAG CGCGGTTT
C2	CGGAATTAGTGGCGAAGTACGTTTATTCCAAGACCGACGTACG AATTTACAACTATGCGGATGTGACGATTTGCTTCTACGATATGC TTCGTCTTT
H0	TCCCTCTGGGCCTT
H1	ACGCGTCTCGCGATCGGCCGCC
M1	ACGCGTGGCGGCCGAGTTAGCTGTCACATCAGAGGGA
M2	ACGCGTGGCGGCCGATTCACTACGAAGCATAGAGGGA
M3	ACGCGTGGCGGCCGCCCCACACTACTTCGCAGAGGGA
M4	ACGCGTGGCGGCCGTGTTGTAGCGTACGTCAGAGGGA
M5	AAGGCCCATCGTAGAAGCTCACCCCGCGCTTCGATCGCGAG
M6	AAGGCCCCGCATAGTTGTTTTGGCAGGCAACATATCGCGAG
M7	AAGGCCCGGTCTTGGAATCGACGGCTTAGATACATCGCGAG
M8	AAGGCCCCACTAATTCCGGAAAGTGTGGATGTTATCGCGAG

4AJ^2-4E （adjacent connection with specific base pairing for the Archimedean tiling 3·6·3·6）



C1	GGTAGCTAACTATGTTGCCTGTTTCCACTACAACAGTATCTAAGCTTTCGTGTGTGGGGAACATCCACATTTCTTTAGTGAATCGAAGCGCGGTTT
C2	CGGAATTAGTGGCGAAGTACGTTTATTCCAAGACCGACGTACGAATTTACAACTATGCGGATGTGACGATTTGCTTCTACGATATGCTTCGTCTTT
H0	TCCCTCTGGGCCTT
H1	TCCTCAGGCATCGCGCACGTAG
H2	GCACCGCGCGCATGTCGAACTC
H3	CGAAGTACGATCCCACGACCGG
H4	CGATCCAACTGCGCGGACGTGC
M1	ACTTCGCTACGTGCAGTTAGCTGTCACATCAGAGGGA
M2	GGATCGGAGTTCGAATTCACTACGAAGCATAGAGGGA
M3	TGAGGAGCCGGTCGTCCCCACACTACTTCGCAGAGGGA
M4	CGGTGCGCACGTCCTGTTGTAGCGTACGTCAGAGGGA
M5	AAGGCCCATCGTAGAAGCTCACCCCGCGCTTCGCATGCGCG
M6	AAGGCCCCGCATAGTTGTTTTGGCAGGCAACATGCGATGCC
M7	AAGGCCCGGTCTTGGAATCGACGGCTTAGATACGCGCAGTT
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3 Additional figures

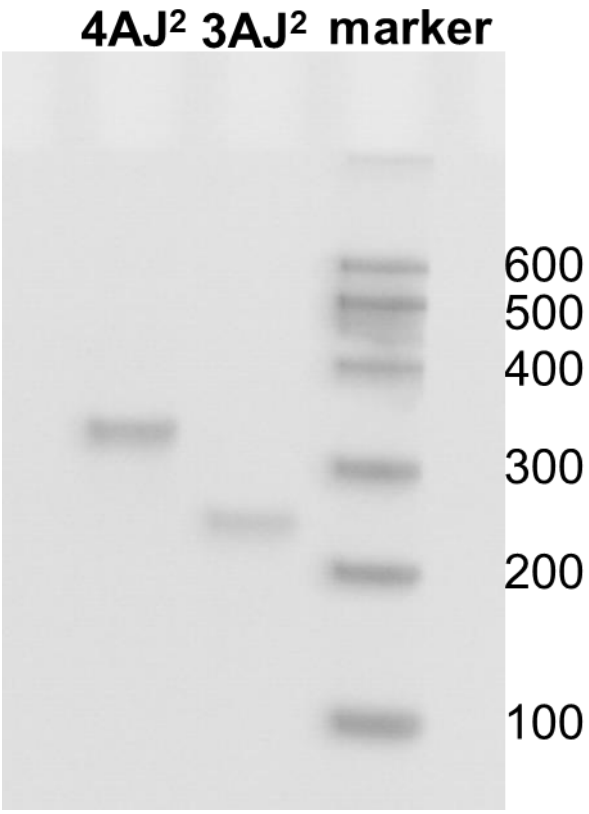


Fig. S1. Native PAGE photo of two-layer tiles of $3AJ^2$ and $4AJ^2$, with each arm carrying a 7 bp blunted overhang. PAGE analysis was carried out at 4 °C. The structure name for each sample is indicated at the top of the gel lane, and the right lane is a DNA ladder (DNA Marker B, Sangon, Shanghai, China).

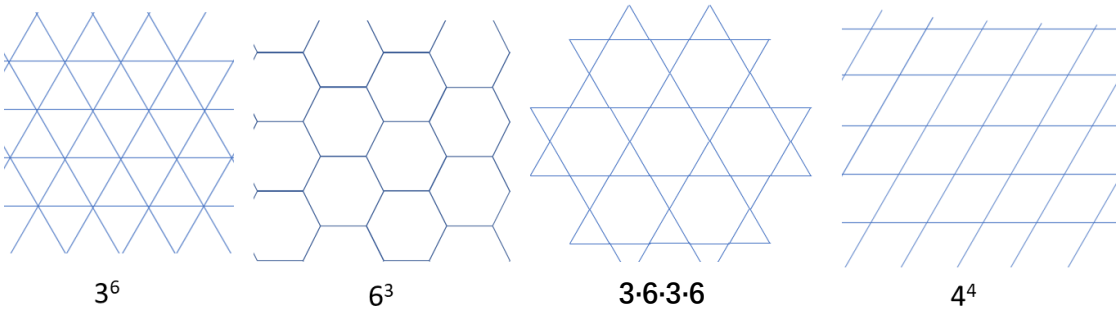


Fig. S2 Four typical types of Archimedean tiling patterns.

We will prove that using all 6 arms of a $3AJ^2$ tile (either $3AJ^2$-6E or $3AJ^2$-6O) to construct the 3^6 tiling pattern, at least an upper-lower connection is a must. First, the tile core of $3AJ^2$ is sitting at every pivot point, and every edge (or the connection arm between every two tile cores) must be equal, which means either $3AJ^2$-6E or $3AJ^2$-6O, but not combination of them, can be used for tiling 3^6. Then we take the basic unit of the smallest regular triangle for analysis. In the smallest regular triangle, every tile at vertices must use two adjacent arms for connection, one upper and one lower arm. There are only two connection modes: 1) every edge is joined with the upper-lower connection (the three diagonal connection mode described in the main text body); 2) one edge is joined with the upper-upper (or lower-lower) connection between two adjacent tiles (vertices), then the rest of two edges from the two adjacent tiles (vertices) connected to the third tile (or the third vertex) must have one edge with the upper-lower connection because the adjacent arms of the third tile must be at the upper and lower positions, separately.

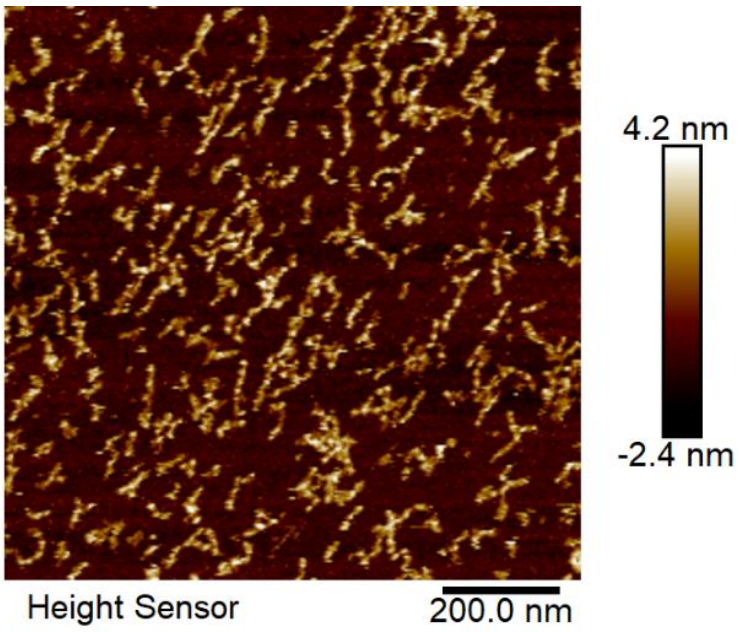


Fig. S3. AFM images of 3AJ^2-6E of three diagonally and specifically paired E-edges with upper-lower connection.

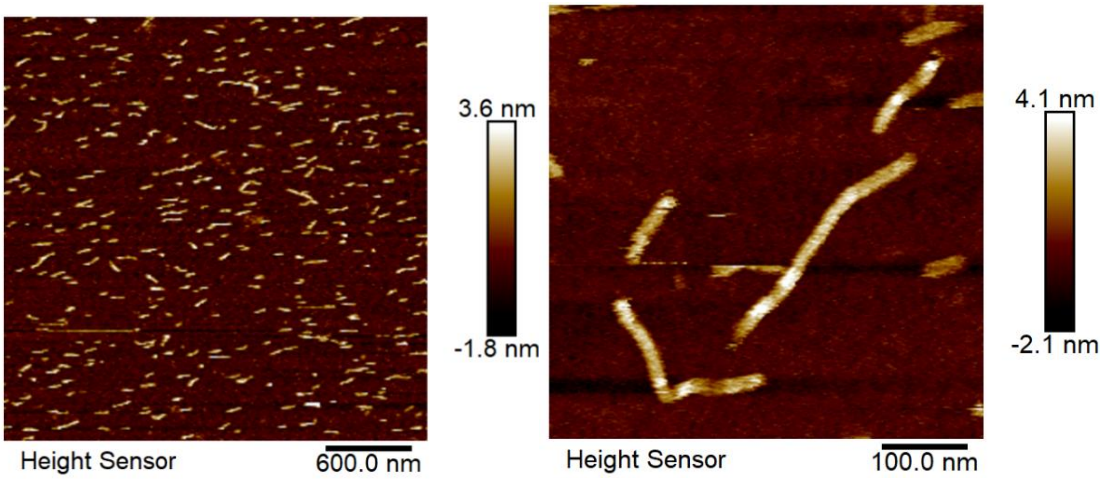


Fig. S4. AFM images of 3AJ^2-6E with the same 8 nt sticky end of a palindromic sequence GTAATTAC for self-adjusting connection.

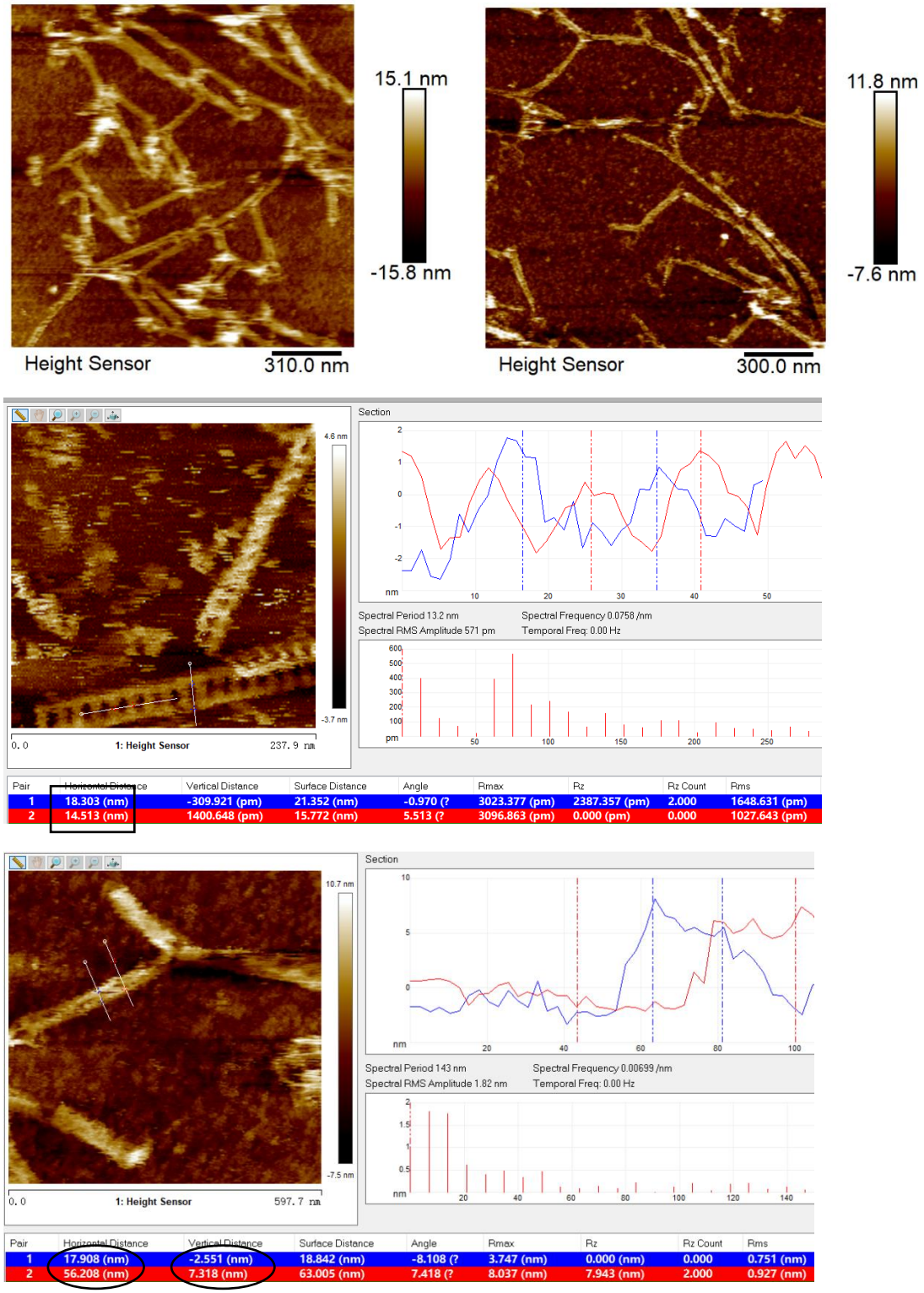


Fig. S5. More AFM images and parts of ladders and nanotubes with section profiles for 3AJ[2]-6E with asymmetric connection in Figure 2.

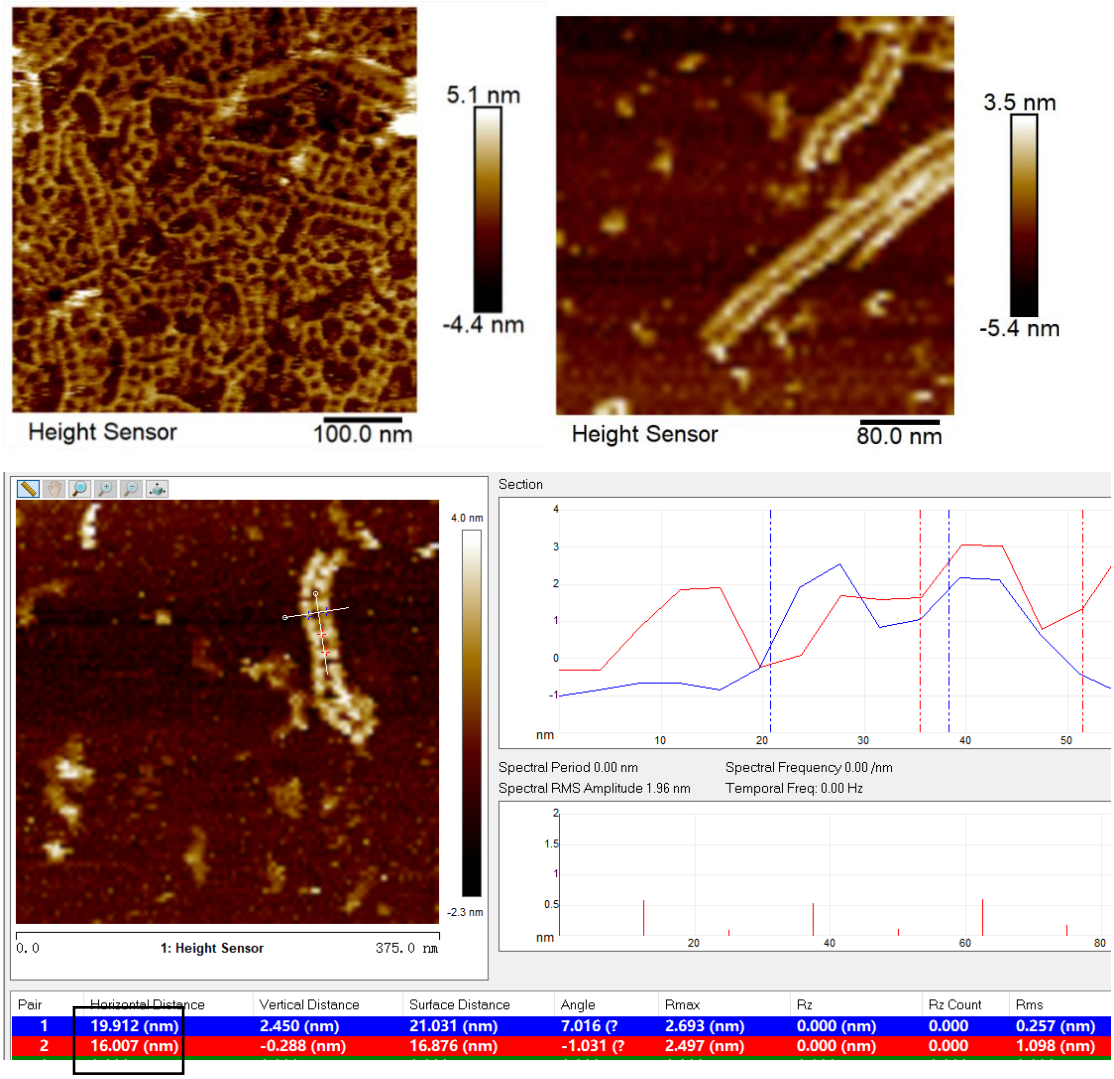


Fig. S6. AFM images of two-layer 3AJ[2]-3E assemblies and a selected ladder structure with section profiles.

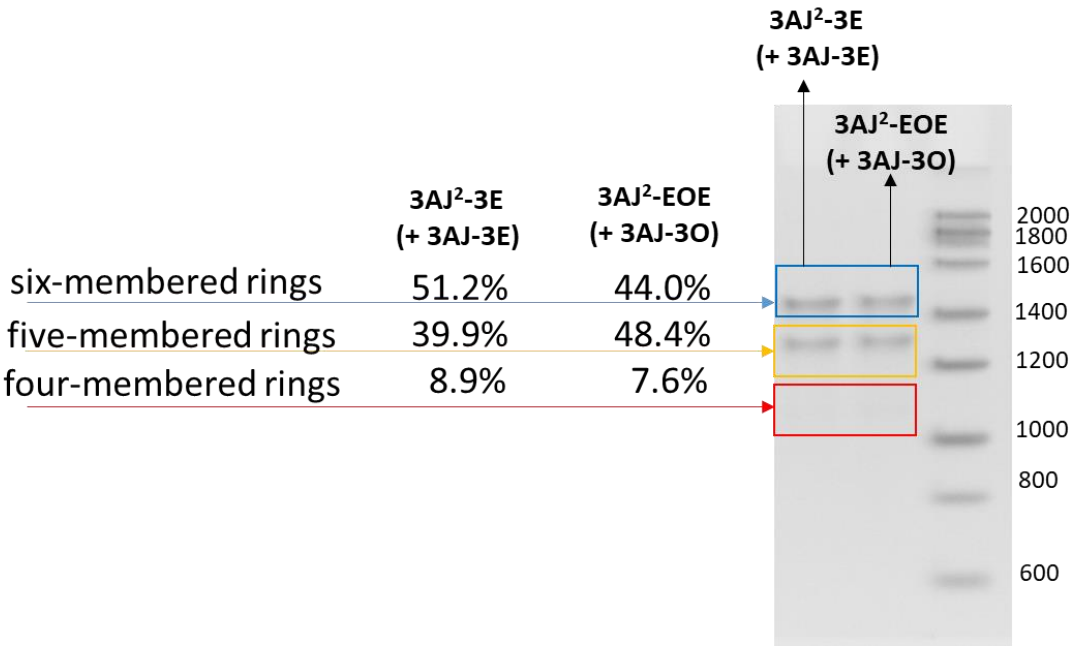


Fig. S7. Native PAGE photo of the first-step products of 3AJ^2-3E with E4-E4' connection and 3AJ^2-EOE with E6-E6' connection. Native PAGE was carried out at 4 °C. The structure symbol for each sample is indicated at the top of the gel lane and the product identities are directed by arrow lines. The proportion of each component is analysed with image J and listed at the left side.

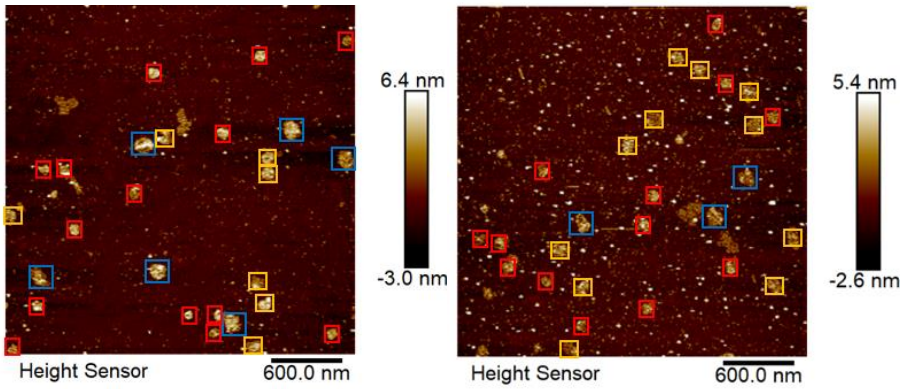
Sample 1

$Number_{total}$ = 54

$Number_{red}$ = 14 + 13= 27; $Number_{red}$ / $Number_{total}$ = 50.0%

$Number_{yellow}$ = 7 + 11= 18; $Number_{yellow}$ / $Number_{total}$ = 33.3%

$Number_{blue}$ = 6 + 3= 9; $Number_{blue}$ / $Number_{total}$ = 16.7%



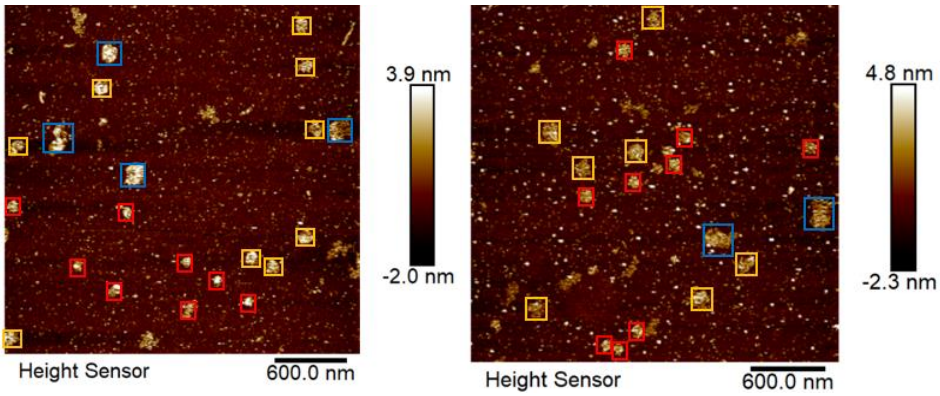
Sample 2

$Number_{total}$ = 38

$Number_{red}$ = 8 + 9= 17; $Number_{red}$ / $Number_{total}$ = 44.7%

$Number_{yellow}$ = 8 + 7= 15; $Number_{yellow}$ / $Number_{total}$ = 39.5%

$Number_{blue}$ = 4 + 2= 6; $Number_{blue}$ / $Number_{total}$ = 15.8%



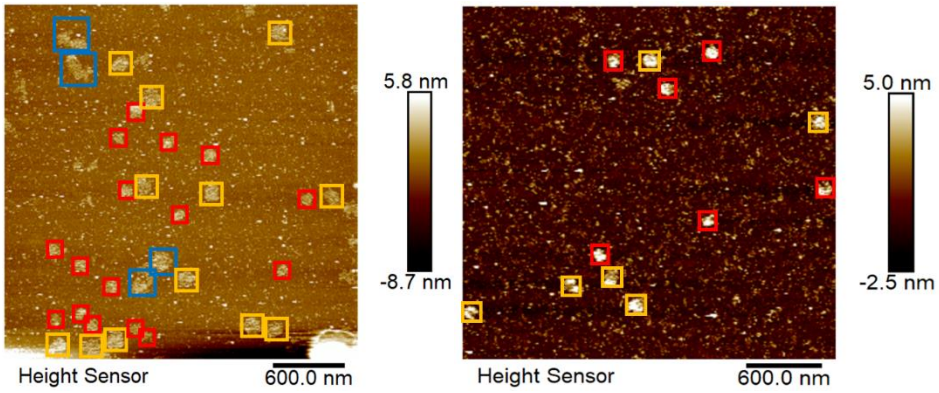
Sample 3

$Number_{total}$ = 44

$Number_{red}$ = 16 + 6= 22; $Number_{red}$ / $Number_{total}$ = 50.0%

$Number_{yellow}$ =12+ 6= 18; $Number_{yellow}$ / $Number_{total}$ = 40.9%

$Number_{blue}$ = 4 + 0= 4; $Number_{blue}$ / $Number_{total}$ = 9.1%



Average Value and Standard Deviation (S)

$$S=\sqrt{\frac{\sum_{i=1}^{n}(x_i-\bar{x})^2}{n}}$$

$Number_{red}$ / $Number_{total}$ = (50.0% + 44.7% + 50.0%) / 3 = 48.2 (±2.5)%
$Number_{yellow}$ / $Number_{total}$ = (33.3% +39.5% + 40.9%) / 3 = 37.9 (±3.3)%
$Number_{blue}$ / $Number_{total}$ = (16.7% +15.8% + 9.1%) / 3 = 13.9 (±3.4)%

Fig. S8. AFM images of ($3AJ^2$-3E + 3AJ-3E) at 0.2 μM $3AJ^2$-3E. The particles framed in red, yellow and blue are nanocage I, nanocage II, and nanocage III, respectively. Assuming that the nanocage number proportions deposited on mica are the same as in solution, we counted the numbers of three types of nanocages in three batches of typical samples and calculated their number proportions of 48.2(±2.5)% for nanocage I, 37.9(±3.3)% for nanocage II, and 13.9(±3.4)% for nanocage III.

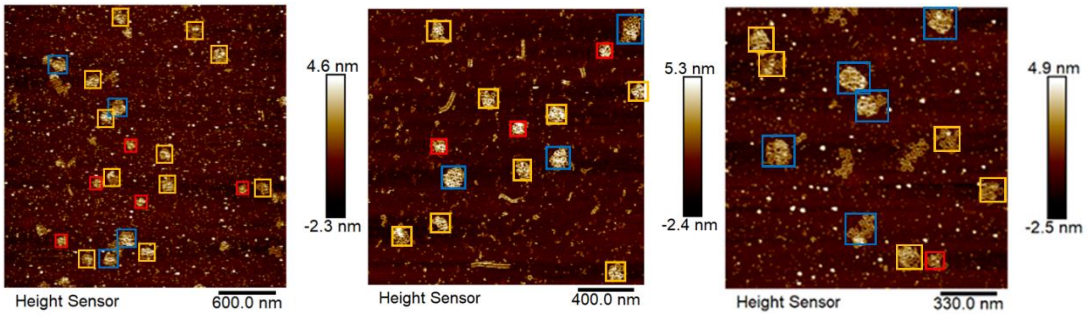
Sample 1

$Number_{total}$ = 45

$Number_{red}$ = 5 + 3 + 1= 9; $Number_{red}$ / $Number_{total}$ = 20.0%

$Number_{yellow}$ = 11 + 8 + 5= 24; $Number_{yellow}$ / $Number_{total}$ = 53.3%

$Number_{blue}$ = 4 + 3 +5= 12; $Number_{blue}$ / $Number_{total}$ = 26.7%



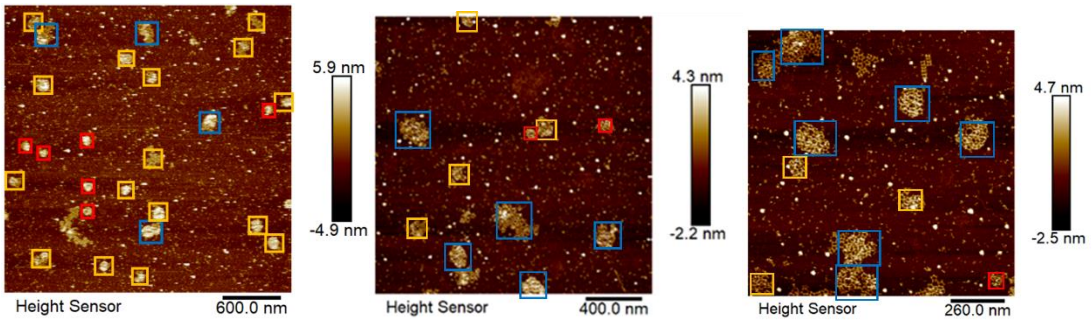
Sample 2

$Number_{total}$ = 47

$Number_{red}$ = 6 + 2 + 1= 9; $Number_{red}$ / $Number_{total}$ = 19.1%

$Number_{yellow}$ = 15 + 4 + 3= 22; $Number_{yellow}$ / $Number_{total}$ = 46.9%

$Number_{blue}$ = 4 + 5 +7= 16; $Number_{blue}$ / $Number_{total}$ = 34.0%



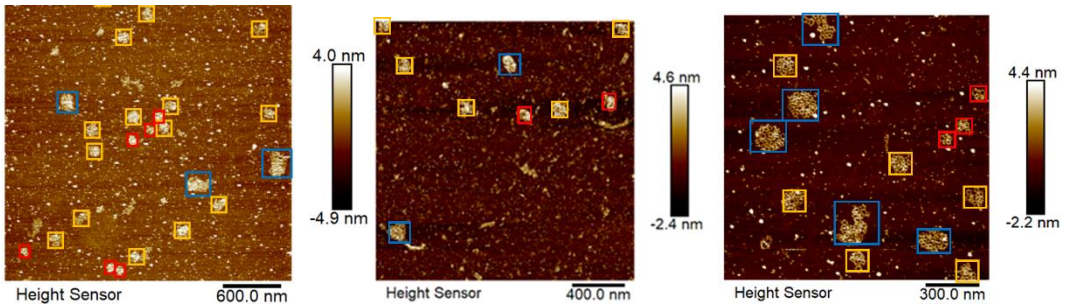
Sample 3

$Number_{total}$ = 46

$Number_{red}$ = 6 + 2 + 3= 11; $Number_{red}$ / $Number_{total}$ = 23.9%

$Number_{yellow}$ = 14 + 5 + 6= 25; $Number_{yellow}$ / $Number_{total}$ = 54.3%

$Number_{blue}$ = 3 + 2 + 5= 10; $Number_{blue}$ / $Number_{total}$ = 21.8%

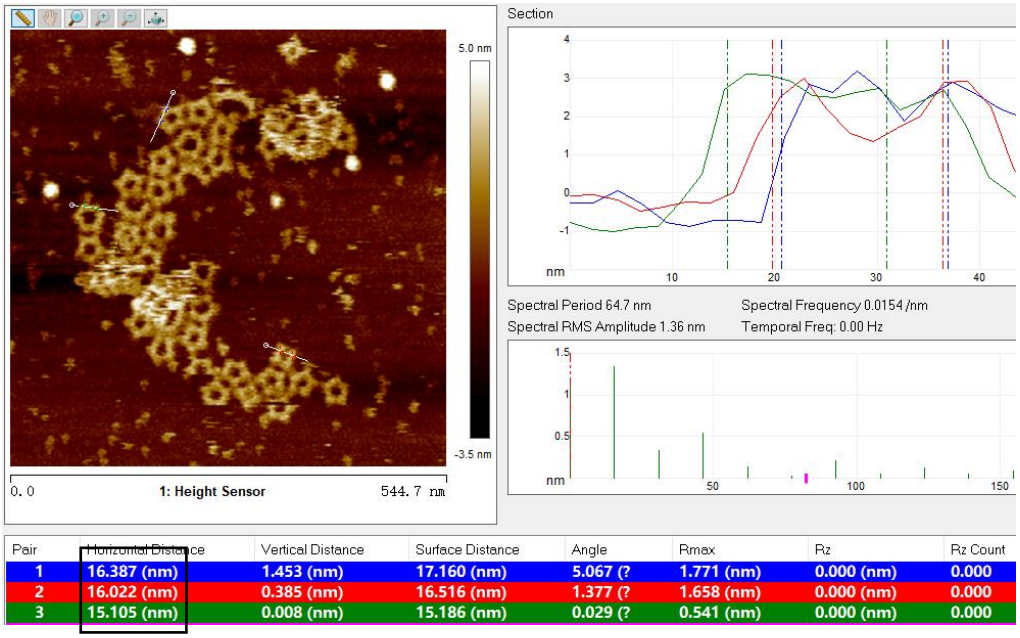


Average Value and Standard Deviation (S)

$Number_{red}$ / $Number_{total}$ = (20% + 19.1% + 23.9%) / 3 = 21.0 (±2.1)%

$Number_{yellow}$ / $Number_{total}$ = (53.3% +46.9% + 54.3%) / 3 = 51.5 (±3.3)%

$Number_{blue}$ / $Number_{total}$ = (26.7% +34.0% + 21.8%) / 3 = 27.5 (±5.0)%



Pair	Horizontal Distance	Vertical Distance	Surface Distance	Angle	Rmax	Rz	Rz Count
1	16.387 (nm)	1.453 (nm)	17.160 (nm)	5.067 (?	1.771 (nm)	0.000 (nm)	0.000
2	16.022 (nm)	0.385 (nm)	16.516 (nm)	1.377 (?	1.658 (nm)	0.000 (nm)	0.000
3	15.105 (nm)	0.008 (nm)	15.186 (nm)	0.029 (?	0.541 (nm)	0.000 (nm)	0.000

Fig. S9. AFM images of ($3AJ^2$-3E + 3AJ-3E) at 0.5 μM $3AJ^2$-3E. The particles framed in red, yellow and blue are nanocage I, nanocage II and nanocage III, respectively. Assuming that the nanocage number proportions deposited on mica are the same as in solution, we counted the numbers of three types of nanocages in three batches of typical samples and calculated their number proportions of 21(±2.1)% for nanocage I, 51.5(±3.3)% for nanocage II, and 27.5(±5.0)% for nanocage III. The side lengths of 4-, 5- and 6-membered rings are indicated as 16.022, 16.387, and 15.105 nm from their section profiles.

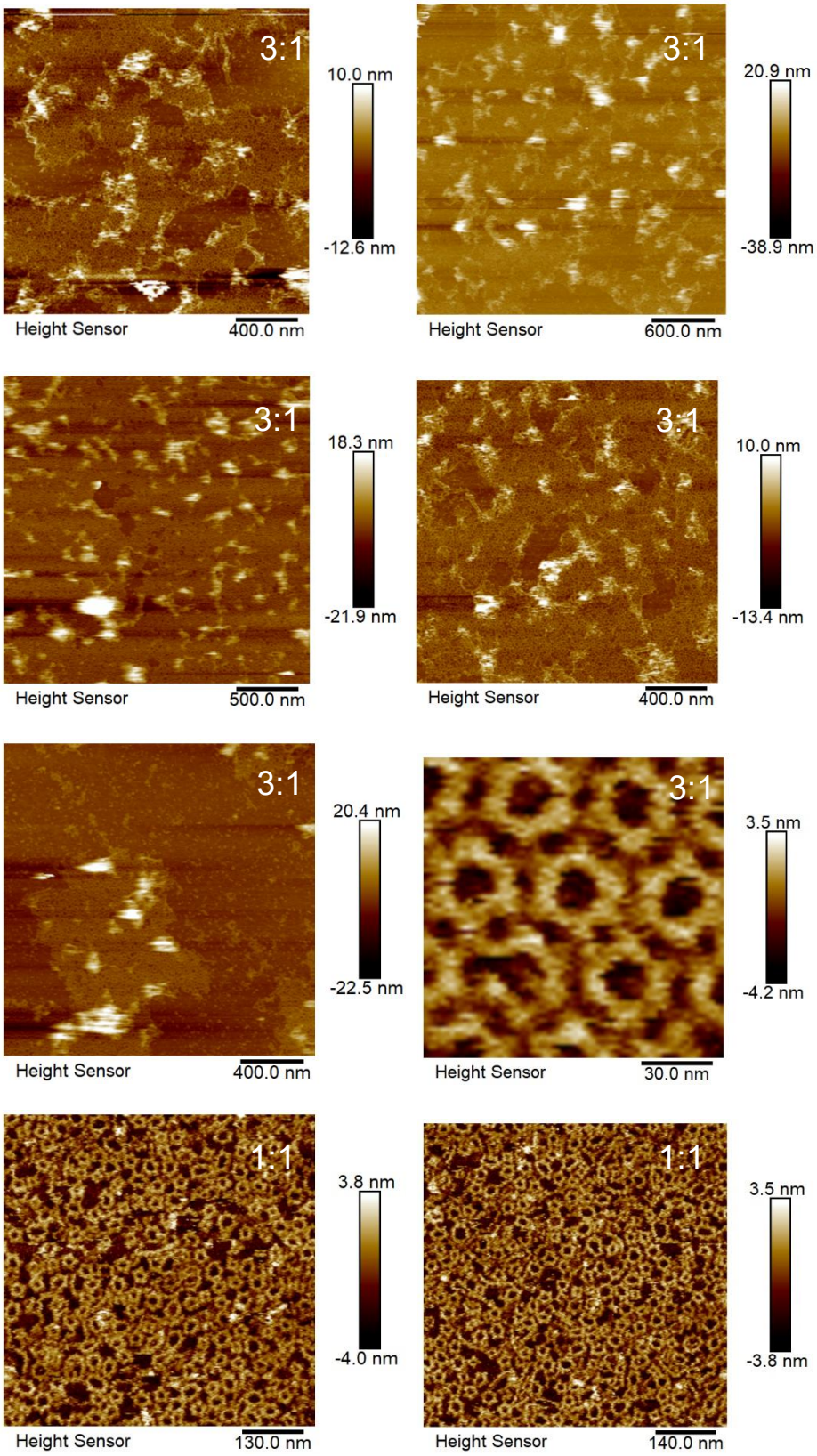
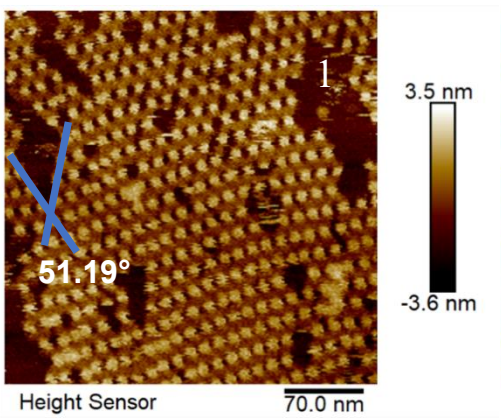
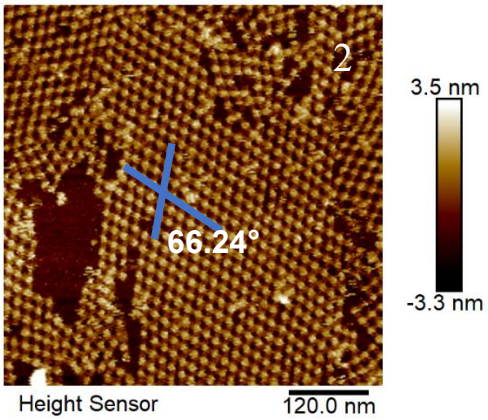
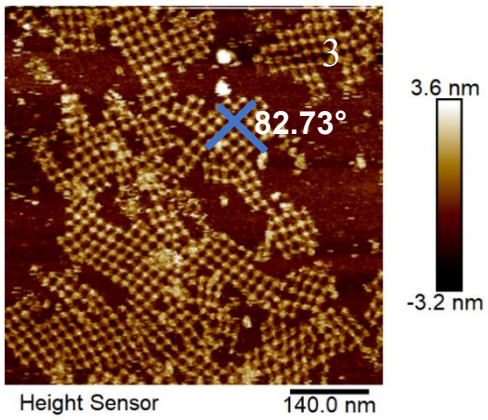
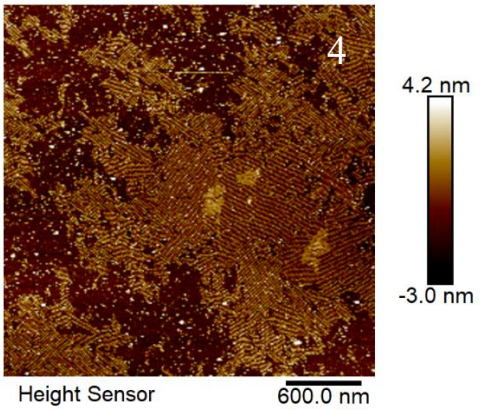


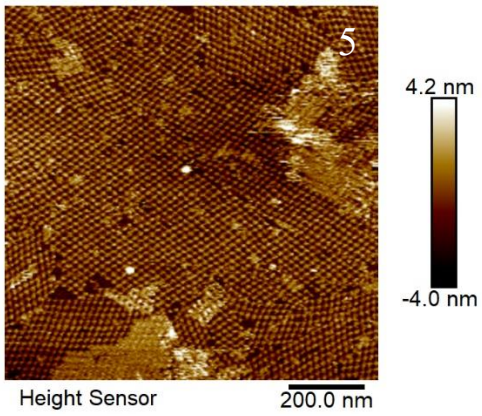
Fig. S10. More AFM images of (3AJ^2-EOE + 3AJ-3O) with the stoichiometric ratio of 3AJ^2-EOE/3AJ-3O at 3:1 and 1:1, labelled at the upper right corner.

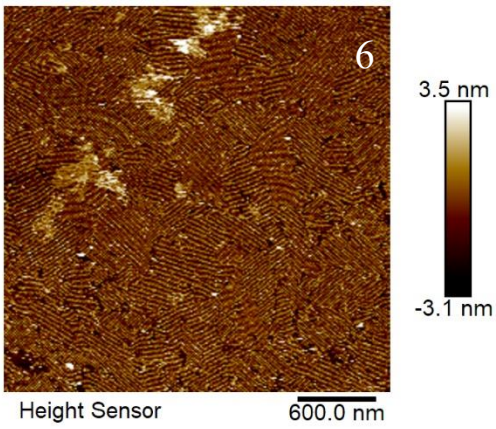

1
51.19°
3.5 nm
-3.6 nm
Height Sensor
70.0 nm

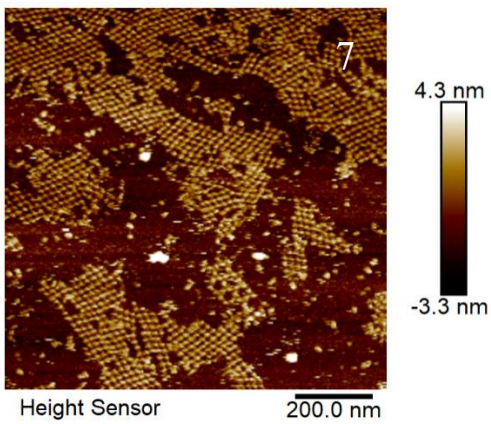

2
66.24°
3.5 nm
-3.3 nm
Height Sensor
120.0 nm

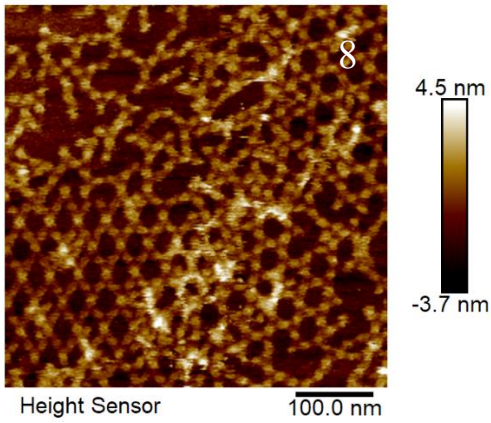

3
82.73°
3.6 nm
-3.2 nm
Height Sensor
140.0 nm

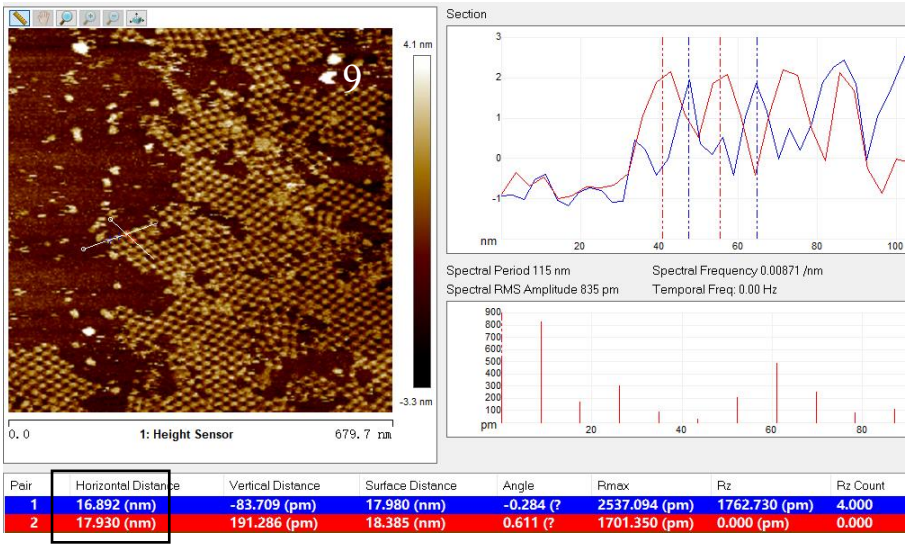

4
4.2 nm
-3.0 nm
Height Sensor
600.0 nm


5
4.2 nm
-4.0 nm
Height Sensor
200.0 nm


6
3.5 nm
-3.1 nm
Height Sensor
600.0 nm


7
4.3 nm
-3.3 nm
Height Sensor
200.0 nm


8
4.5 nm
-3.7 nm
Height Sensor
100.0 nm



Pair	Horizontal Distance	Vertical Distance	Surface Distance	Angle	Rmax	Rz	Rz Count
1	16.892 (nm)	-83.709 (pm)	17.980 (nm)	-0.284 (?	2537.094 (pm)	1762.730 (pm)	4.000
2	17.930 (nm)	191.286 (pm)	18.385 (nm)	0.611 (?	1701.350 (pm)	0.000 (pm)	0.000

Fig. S11. More AFM images of 4AJ^2-4E in fluid. The angle of rhombus lattice from different singly crystalline domains can vary at 51°, 66°, and 83°, which are labelled images of 1, 2, and 3. From the 4th to 6th images, polycrystalline 2D arrays composed of several to many single crystalline domains are shown. In the 7th image, a very small 3•6•3•6 tiling pattern can be found at the central bottom region. The 8th image is a zoom-in image of the 3•6•3•6 tiling pattern assembled via adjacent connection of specific base pairing. In the 9th image, the rhombus lattice linear constant is measured as 17.5 nm from the section profiles.