Electronic Supplementary Material (ESI) for Nanoscale.

DNA Origami Frame Filled With Two Types of Single-Strand Tiles

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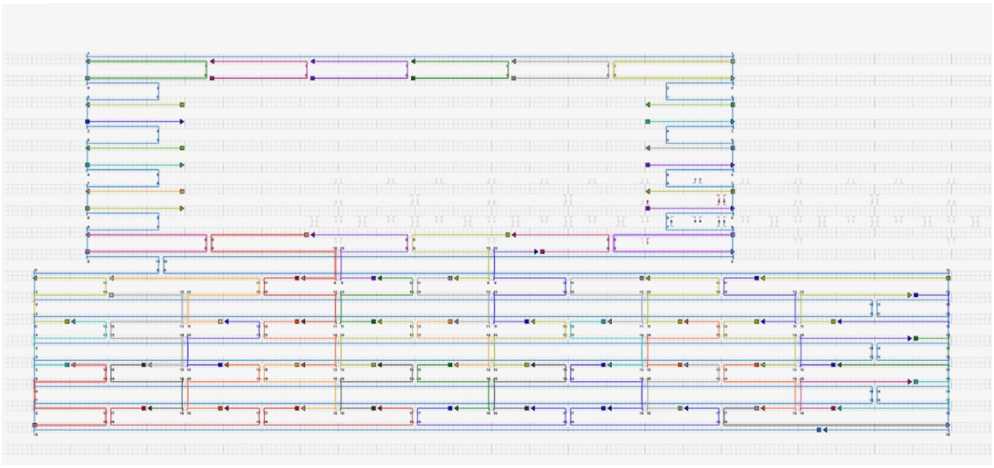
1. DNA origami design schematic

(A)



(B)

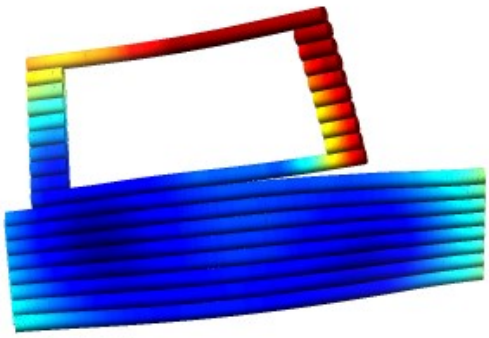
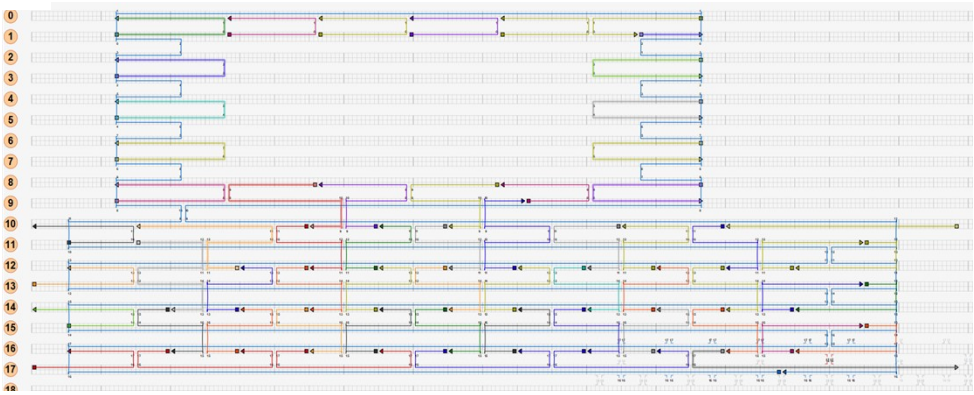


Fig. S1. (A) The design schematic of DNA origami structure (rectangle backbone and frame). (B) CanDo simulation result of (A).

2. Methods to prevent base stacking

(A)



(B)

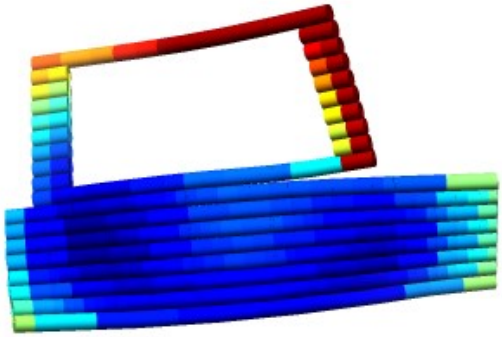


Fig. S2. (A) Staple stands with 14nt extended poly T at the end of the backbone. (B) CanDo simulation result of (A).

(A) (B)

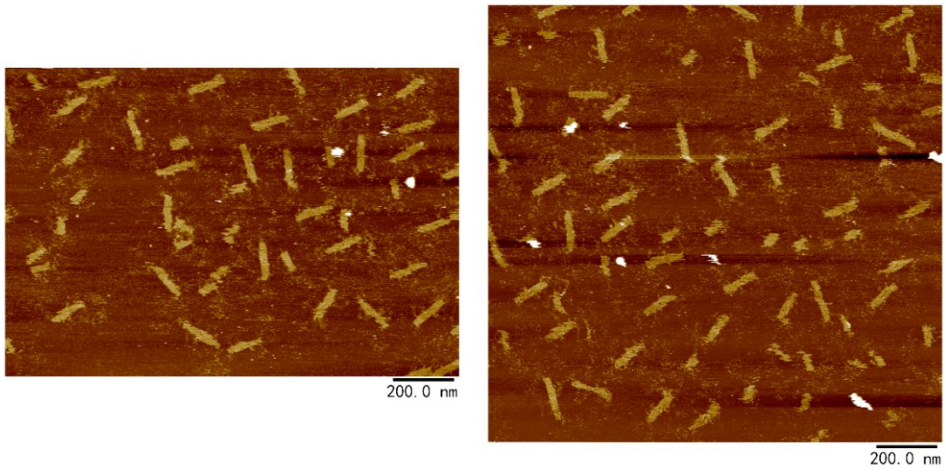
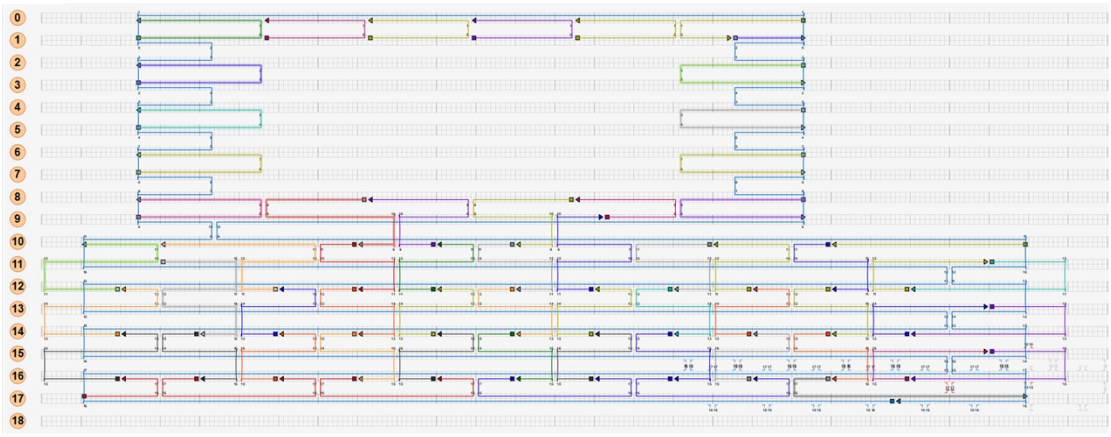


Fig. S3. The AFM results of the poly T extended method. (A) scale bar is 200nm. (B) Field of view is 1.5 μm.

Note that the extended staple strands at the left boundary of the backbone are 14nt long. Because of the design space, they showed only 8nt (We extended 6nt at the order form manually). Extensive changing of the boundary staple chains is avoided; therefore, two staples were extended at the right end (we also suspect that extending all the boundary chains will damage the stability of the boundary).

All the staples and M13 were linearly annealed from 95 to 4℃. Results are shown in Fig. 3. Most of the structures are still stacked together. From those results, we can conclude that poly T is not efficient in this experiment.

(A)



(B)

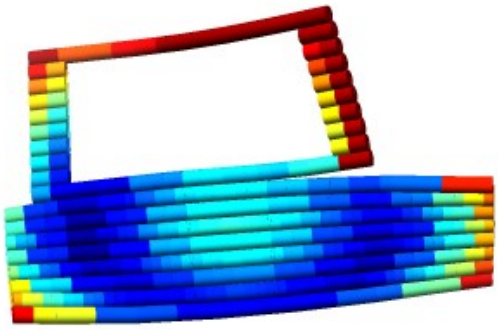


Fig. S4. (A) The boundary staple stands were extended via loop sequences as shown at the left and right end. (B) CanDo simulation result of (A).

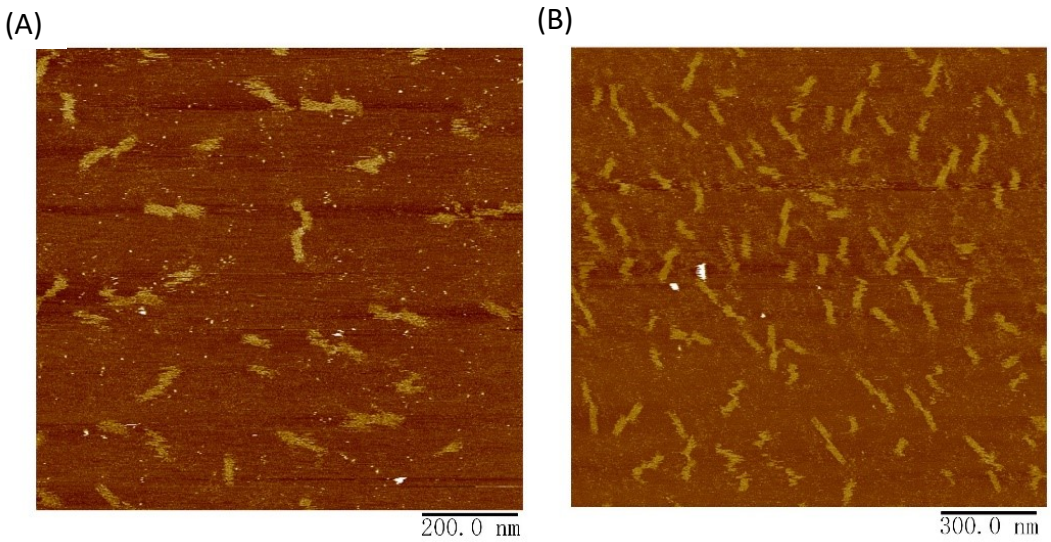


Fig. S5. The results of loop extended methods. (A) Field of view is 1 μm. (B) Field of view is 1.5 μm.

Most of the structures still stacked together. The difference is that they are diagonally connected. Also, the structures seem a little loose.

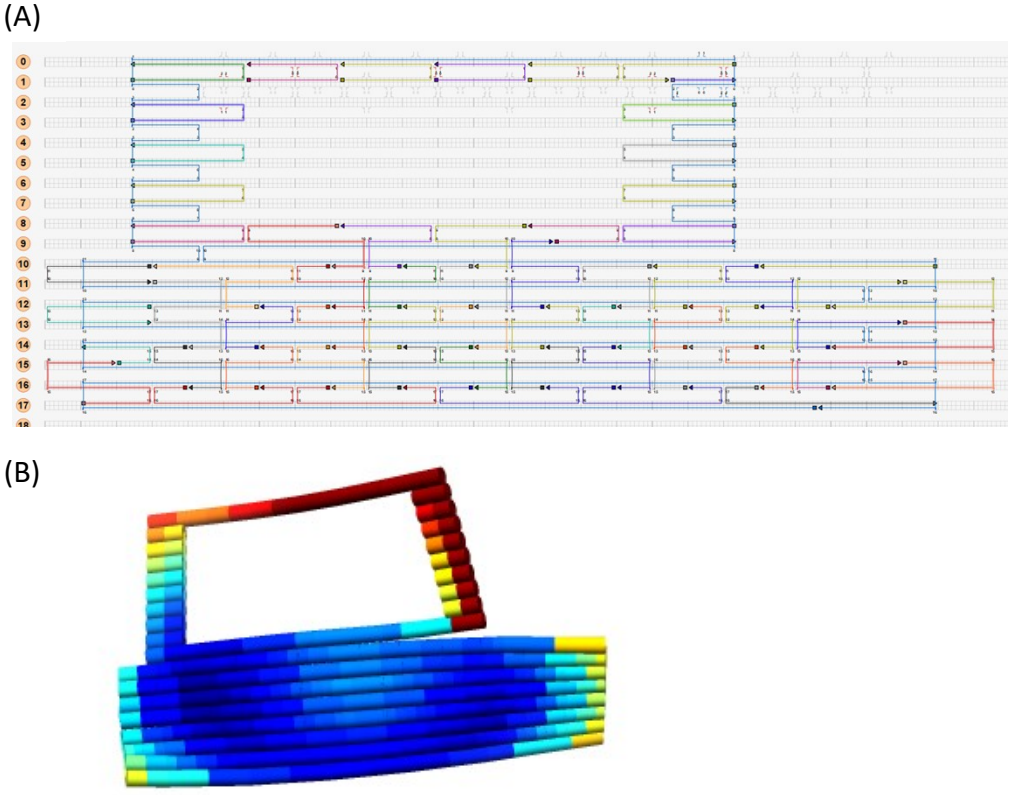


Fig. S6. (A)The boundary staple stands were extended via palindrome sequences: the left and right extended parts will self-connected. (B) CanDo simulation result of (A)

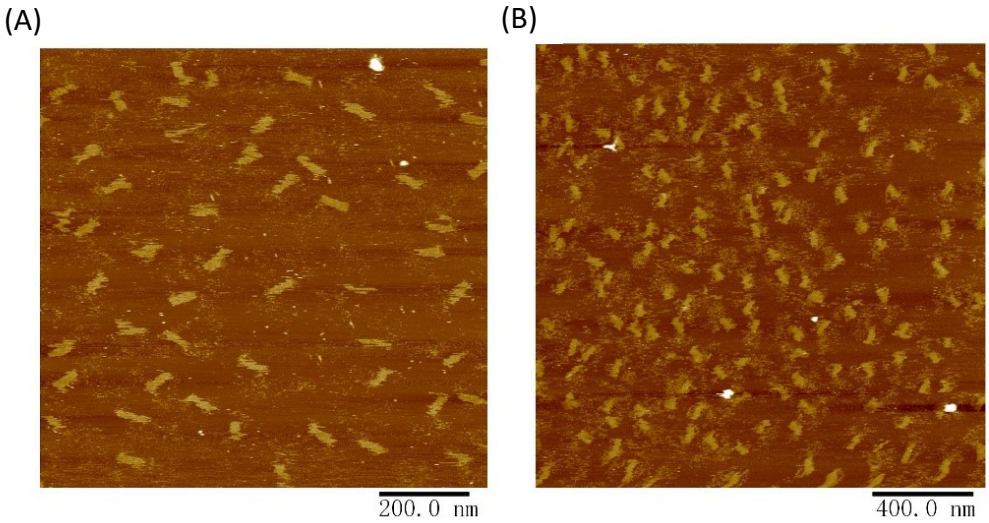


Fig. S7. The results of hairpin extended methods. (A) Field of view is 1 μm. (B) Field of view is 2 μm.

This method is efficient, structures are separated successfully. However, they are loosened. We suspect changing the boundary strands disrupts the edge to a certain degree. Boundaries were not compactly connected.

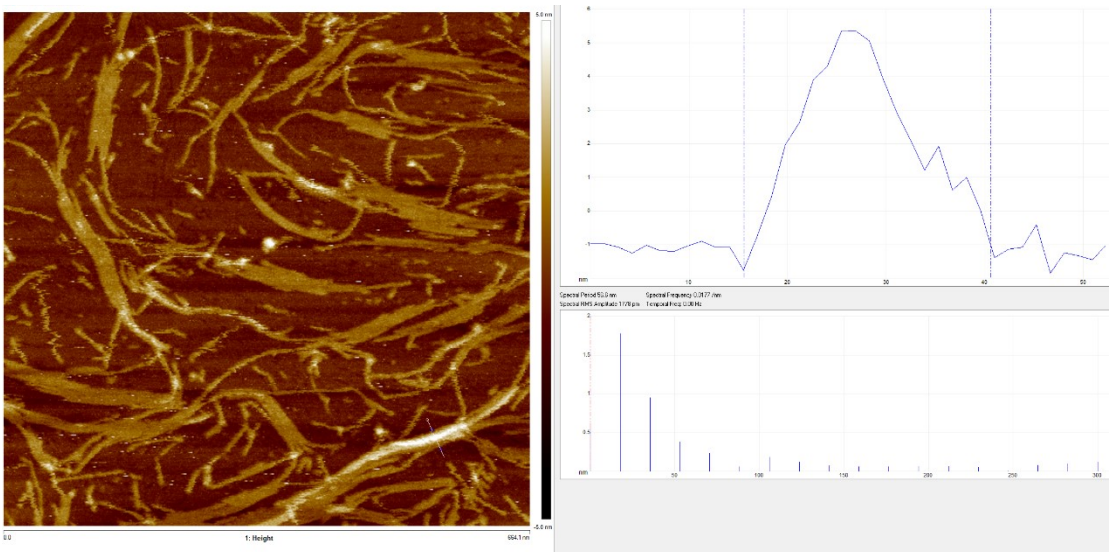


Fig. S8. The AFM results of 10μm SSTs samples after been stored in 4℃ for a week.

The same sample as in fig. 4(d), but the results are different. Results of the fresh sample, as shown in fig. 4(d) are compactly linked canvas structures. But be stored for a week, the canvas was

separated into many ribbons. The average width of these ribbons is 25nm. Some of the ribbons even self-assembled into the tubes, as the marked one at the bottom right corner. Considering that the tube was squashed, the hight (4-5 nm) is just right.

3. SSTs Filling Methods

3.1 One step annealing

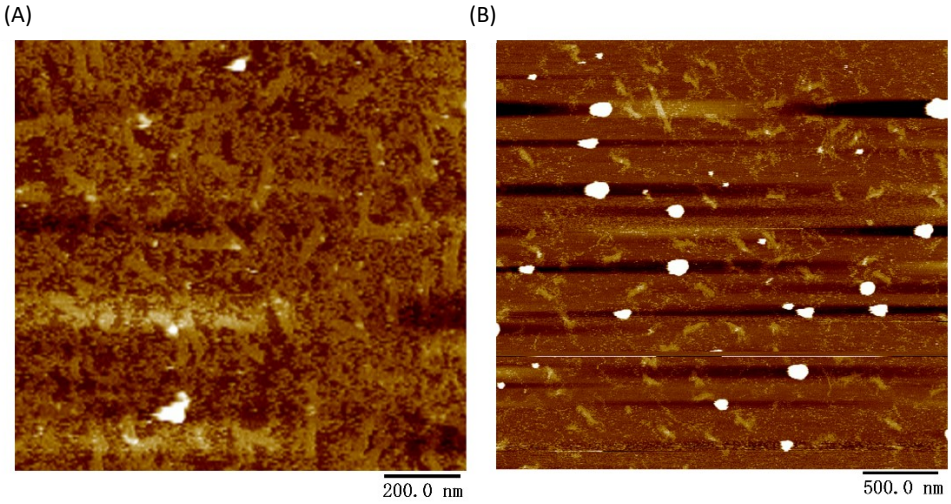


Fig. S9. The one step annealing results. (A) Final concentration of M13 is 2nM.Field of view: 1.2 μm.(B) Results after dilution from sample(A), concentration is 1nM. Field of view: 3 μm.

The final concentration of M13 scaffold strand is 2nM. The ratio of S1/S2: Staples: M13 is 100:10:1. All the strands were linearly annealing from 95 to 4°C for 14 hours. The structures were disorderly and chaotic. Additionally, significant flocculent structures are present in this sample. They were the SSTs assemblies, as the concentration of SSTs is 200nM.

3.2 SSTs with purified DNA origami frame

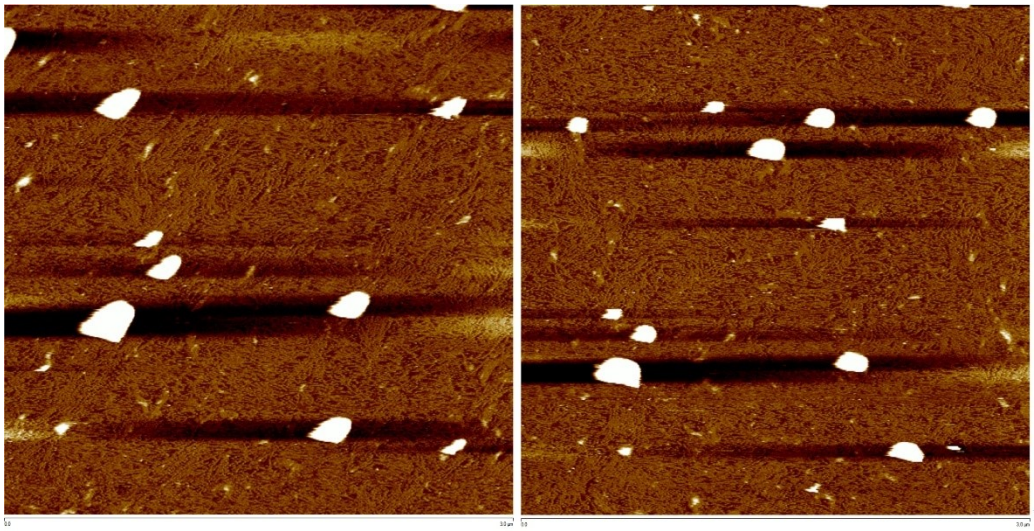


Fig. S10. SSTs added to purified DNA origami frame. The white dots are the impurities, could be salt particles.

30µL 2nM DNA origami frame was purified via Ultra-filtration 100k tube twice, 5K rcf (Millipore, Burlington, MA, USA). Then, SSTs were added to the purified sample in a ratio of 10:1, mixed solutions were linearly annealed from 50 to 4°C for 8 hours. There are rarely origamis, most of the structures were SSTs ribbons. We assessed these excess helper strands could be helpful in constraining the self-assembly of SSTs.

3.3 Ratio of SSTs to Staples, Annealing time, Annealing Temp.

5 times-6h-45°C

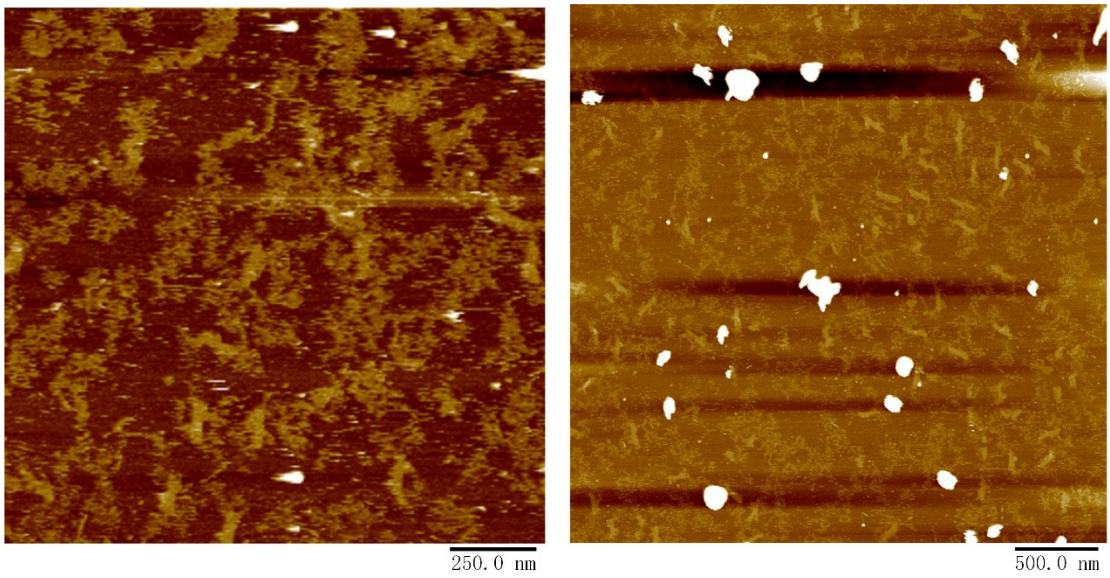


Fig. S11. SSTs 5times excess of staple strands. The mixed solution was linearly annealed from 45 to 4°C for 6 hours.

5 times-8h-45°C

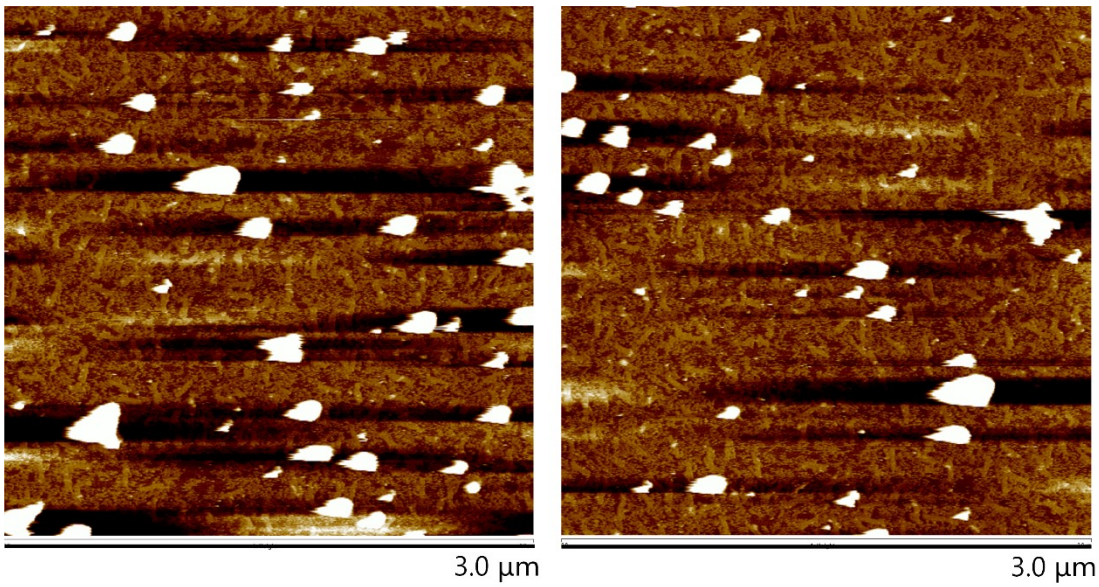


Fig. S12. SSTs 5times excess of staple strands. Mixed solution was linearly annealed from 45 to 4°C for 8 hours.

5 times-10h-45°C

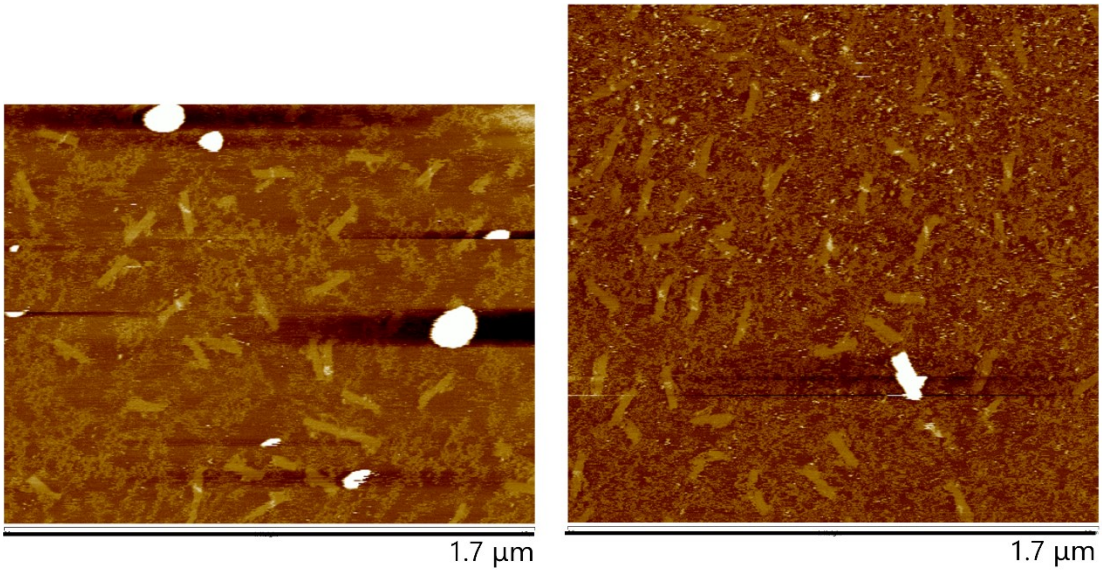


Fig. S13. SSTs 5times excess of staple strands. Mixed solution was linearly annealed from 45 to 4°C for 10 hours.

5 times-14h-45°C

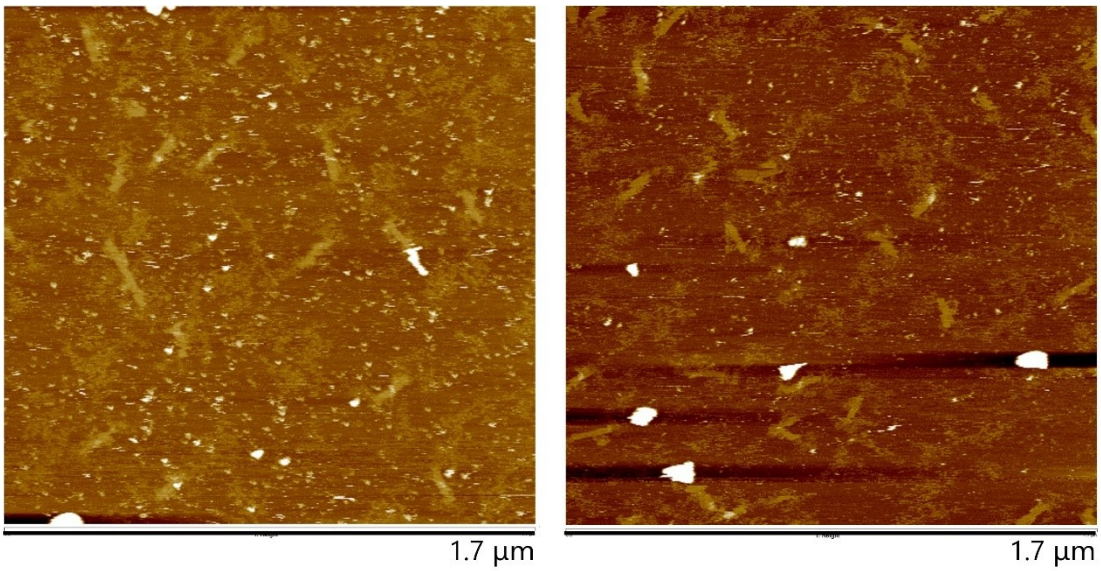


Fig. S14. SSTs 5times excess of staple strands. Mixed solution was linearly annealed from 45 to 4°C for 8 hours.

10 times-6h-45°C

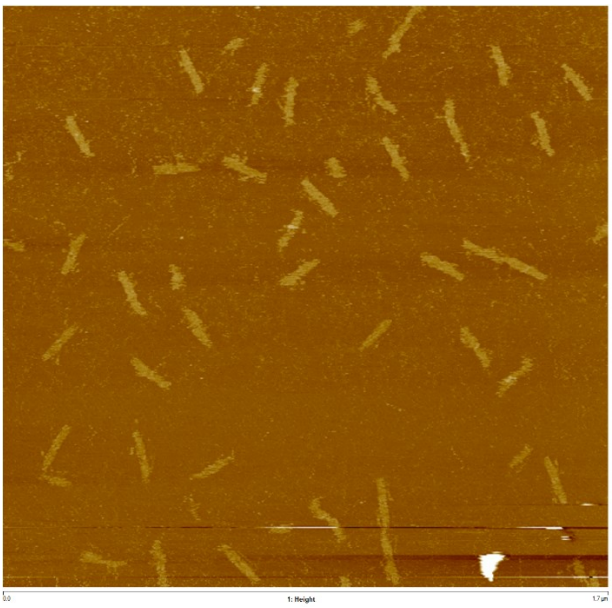


Fig. S15. SSTs 10times excess of staple strands. Mixed solution was linearly annealed from 45 to 4°C for 6 hours.

10 times-14h-45°C

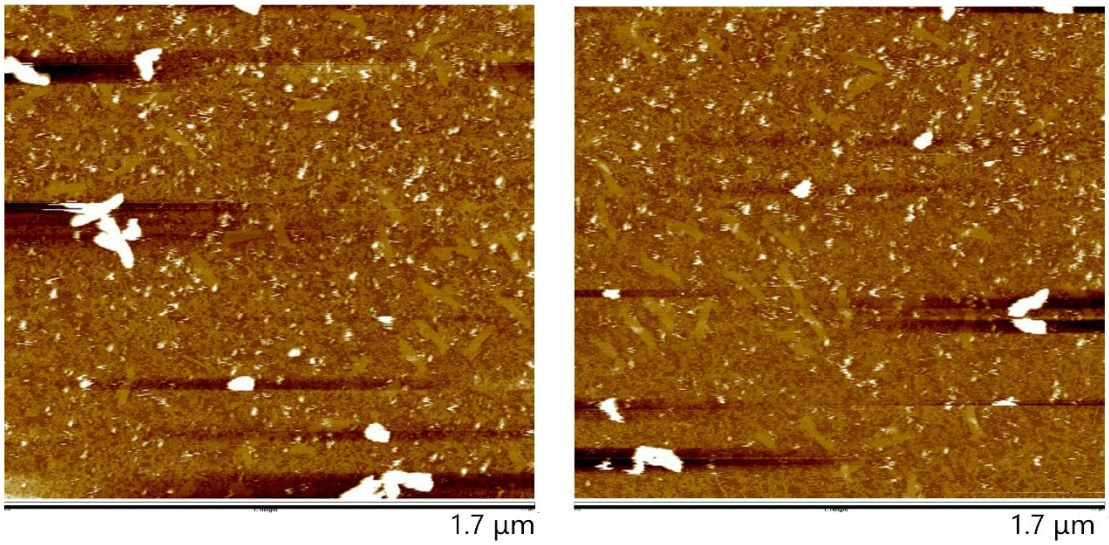


Fig. S16. SSTs 10times excess of staple strands. Mixed solution was linearly annealed from 45 to 4°C for 14 hours.

15 times-14h-45°C

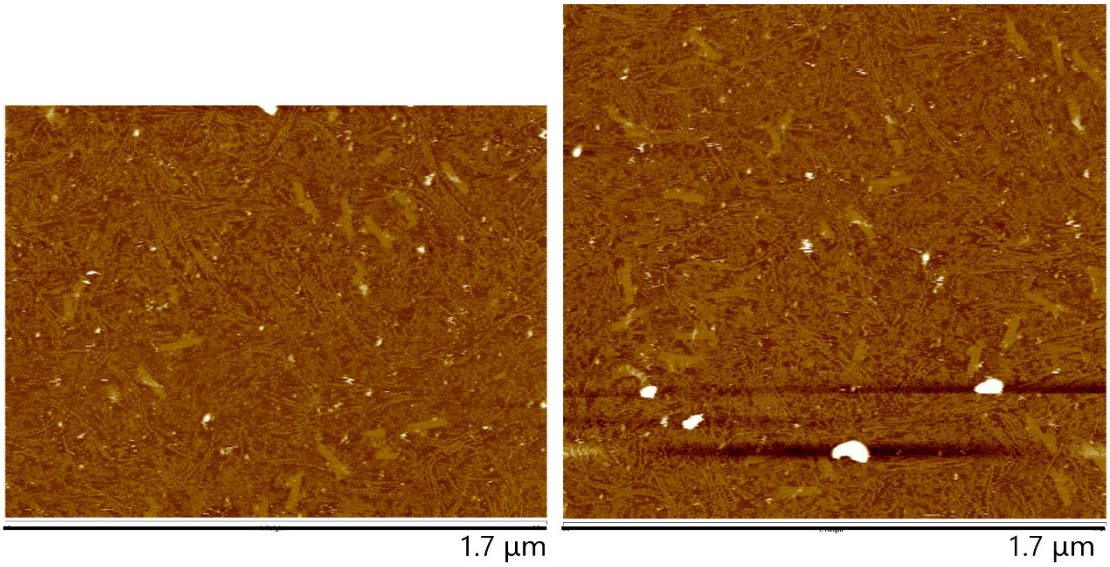


Fig. S17. SSTs 15times excess of staple strands. Mixed solution was linearly annealed from 45 to 4°C for 14 hours.

For the two factors, annealing time and ratio, the annealing time seems a bit more important. Longer time is better for filling efficiency, but it also produces a lot of reticulations.

At first, we thought 45 degrees Celsius was the right annealing temperature，then, we realized that the starting temperature of annealing also plays a vital role for SST assembly. We raised the annealing to 50 Celsius degrees and performed several control groups.

6 times-14h-50°C

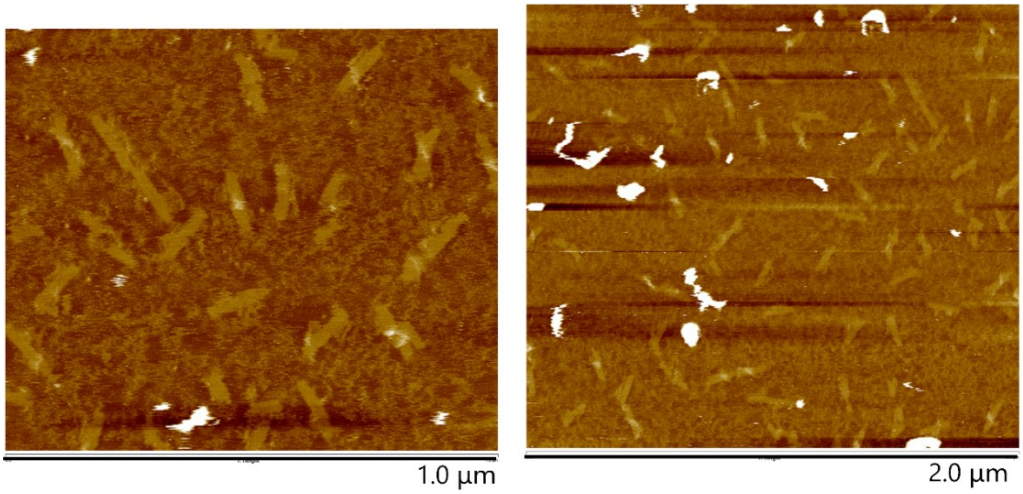


Fig. S18. SSTs 6times excess of staple strands. Mixed solution was linearly annealed from 50 to 4°C for 14 hours.

10 times-8h-50°C

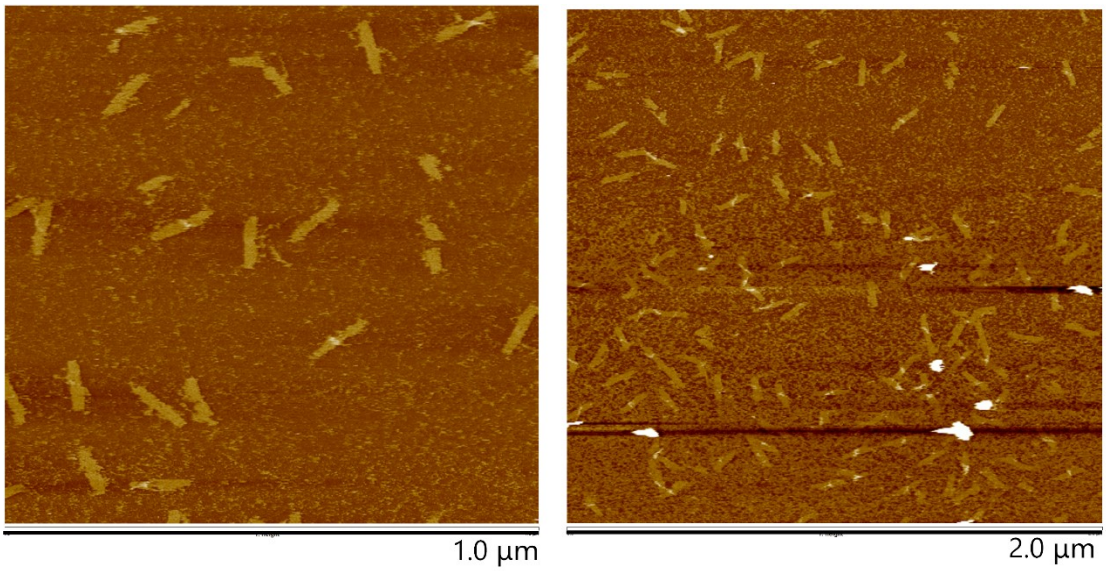


Fig. S19. SSTs 10times excess of staple strands. Mixed solution was linearly annealed from 50 to 4°C for 8 hours.

10 times-14h-50°C

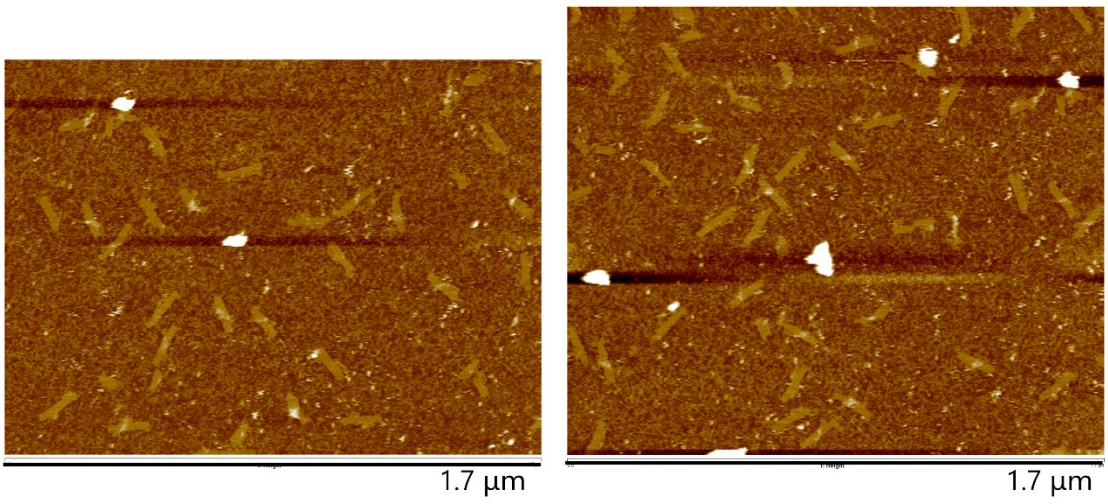


Fig. S20. SSTs 10times excess of staple strands. Mixed solution was linearly annealed from 50 to 4°C for 14 hours.

15 times-14h-50°C

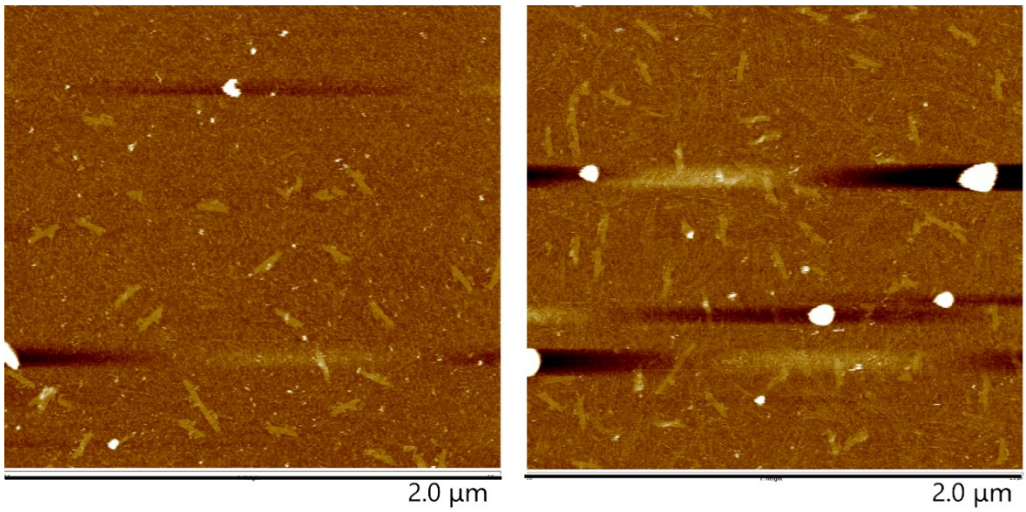


Fig. S21. SSTs 15times excess of staple strands. Mixed solution was linearly annealed from 50 to 4°C for 14 hours.

20 times-22h-50°C

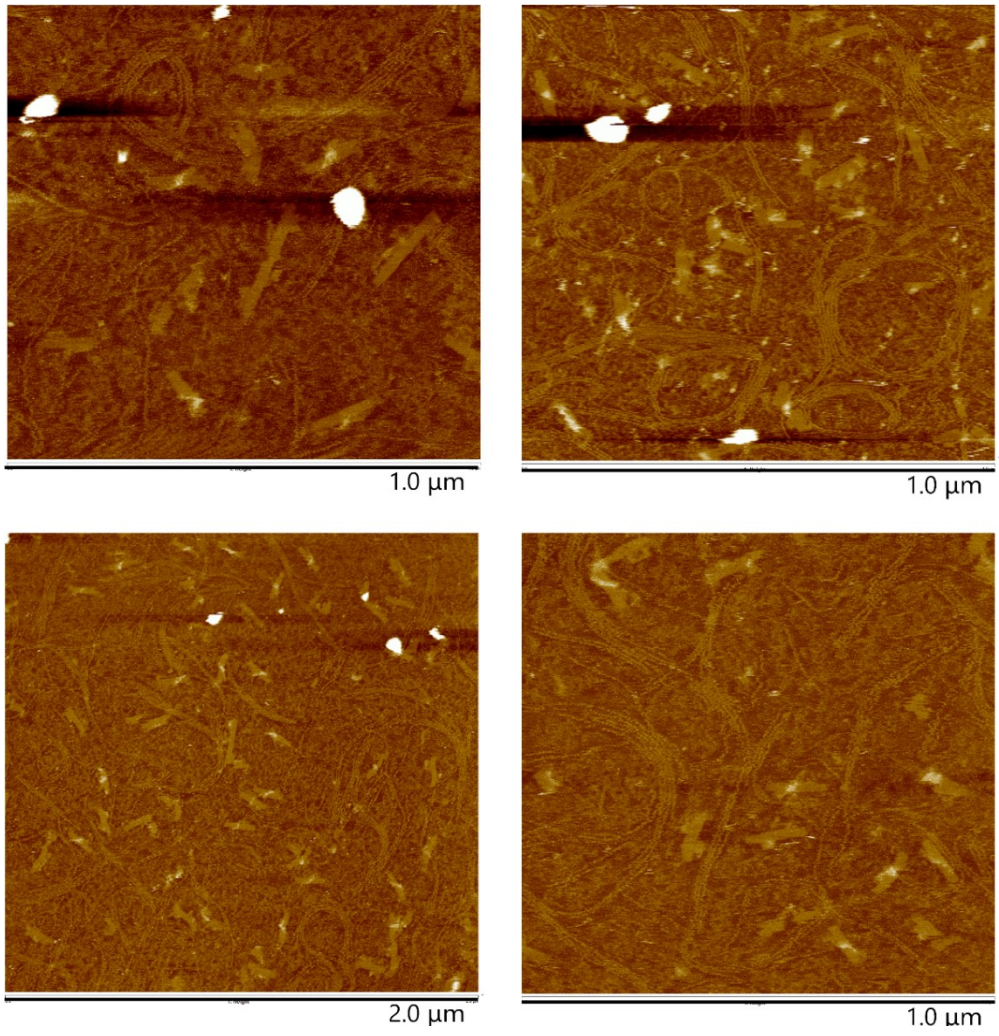


Fig. S22. SSTs 20times excess of staple strands. Mixed solution was linearly annealed from 50 to 4°C for 22 hours.

20 times-30h-50°C

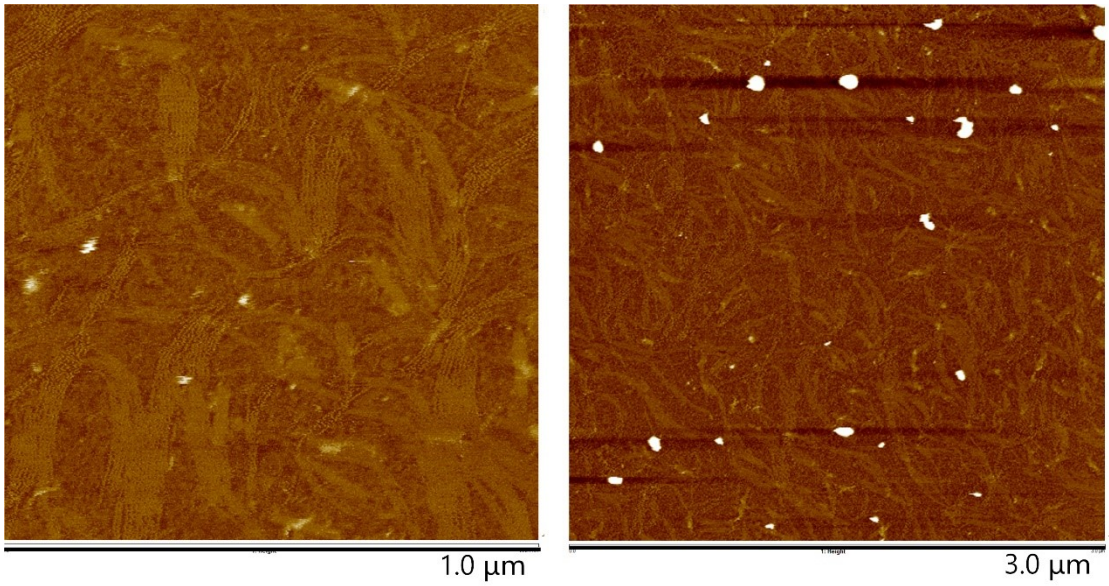


Fig. S23. SSTs 20times excess of staple strands. Mixed solution was linearly annealed from 50 to 4°C for 30 hours.

In conclusion, the annealing starting temperature is important. SST formed massive reticular structures at 45°C. While under the same conditions, SSTs were more inclined to form the striped structure at 50°C. Also, the annealing time is important, the short time is not adequate enough for SSTs filling, but longer time results in forming reticulations. Finally, we determined the filling conditions as: 20 times-22h-50°C.

4. Purification Results

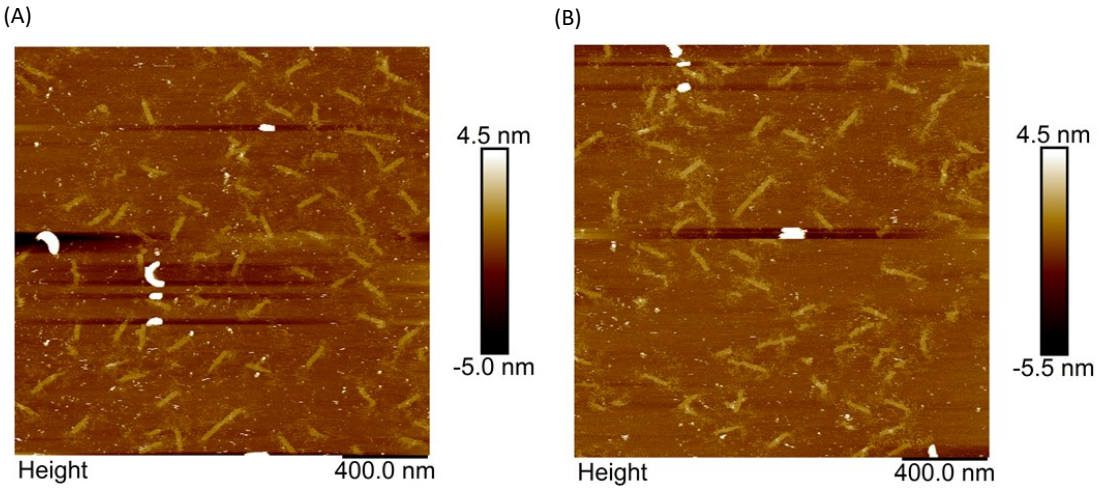


Fig. S24. Lane A sample was concentrated results via ultrafilter tube with a centrifugal velocity of 3K rcf. (A) Concentration result of up band of Lane A. (B) Concentration result of down band of Lane A.

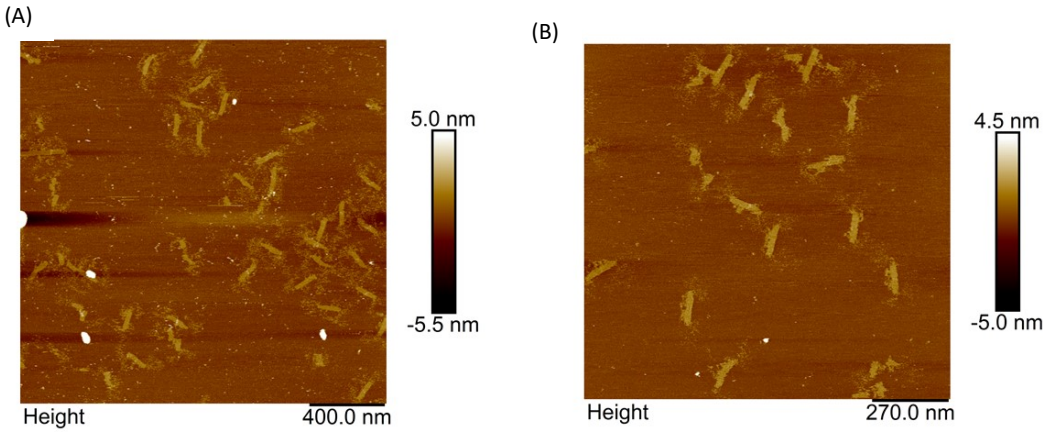


Fig. S25. Lane B concentrated results via ultrafilter tube with a centrifugal velocity of 3K rcf. (A) Concentration result of down band of Lane B. (B) Concentration result of up band of Lane B.

We found that the results of up and down band samples are similar. We believe the monomer (down band) stacked together during the gel extraction process. Therefore, monomer stacked into dimer, and the structures in up and down band look alike (S24, S25).

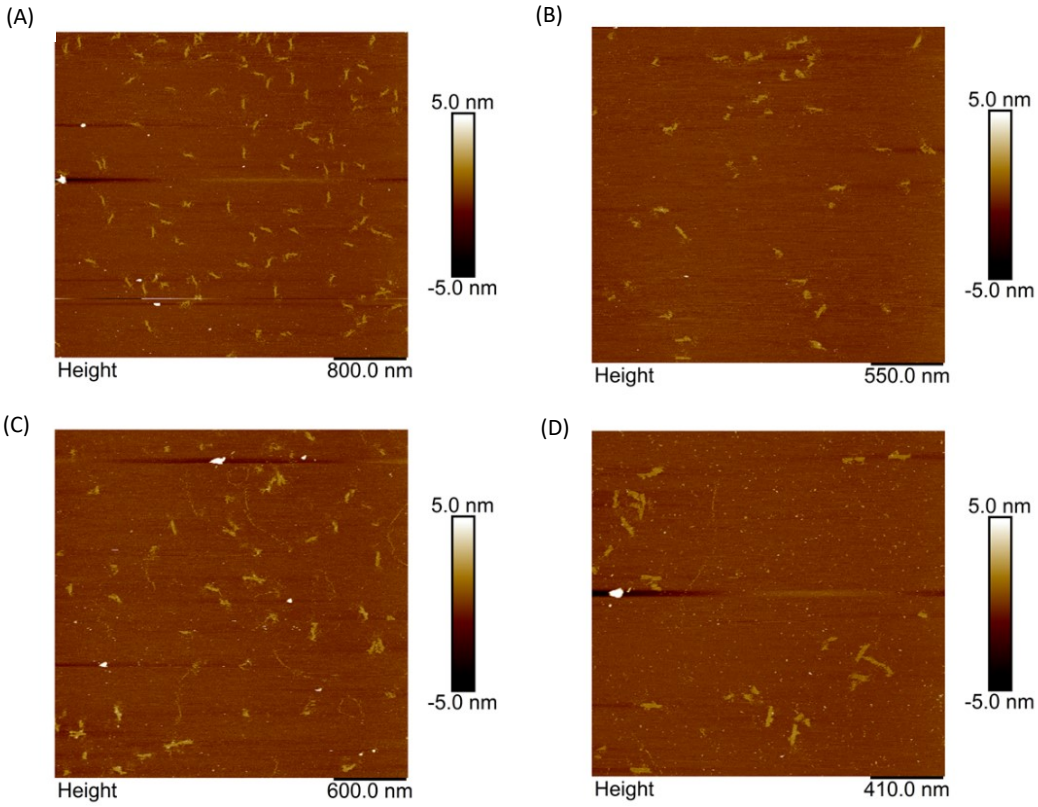


Fig. S26. Gel sample concentrated results (A) Concentration result of up band of Lane B, centrifuge velocity is 3.5K rcf. (B) Concentration result of down band of Lane B, centrifuge velocity is 3.5K rcf. (C) Concentration result of up band of Lane B, centrifuge velocity is 2.5K rcf. (D) Concentration result of down band of Lane B, centrifuge velocity is 3K rcf.

5. Filling Analysis

We estimated the filling areas in each marked origami frame. The designed DNA origami frame was illustrated as the green wireframe (a long and a short rectangle). To estimate the filling areas, we set four groups for different filling areas. Group one: the filling area is 50% smaller than the designed area. Group two: filling area is 50%-90% of the designed area. Group three: filling area is 90%~100% of the designed area. Group four: filling area larger than designed area.

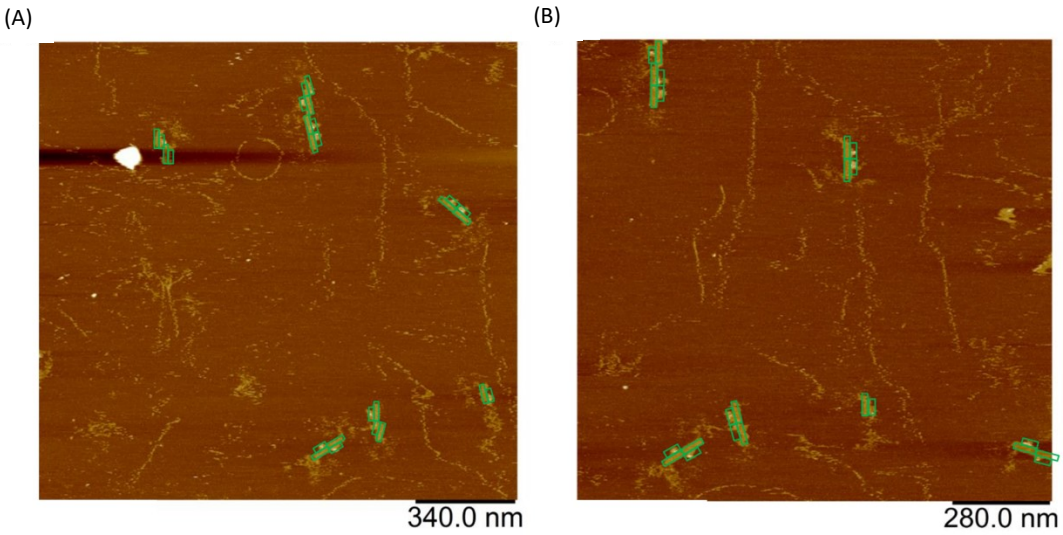


Fig. S27. Filling analysis. (A) There are 13 filling origamis. 5 of them are classified into group one (<50%). 7 of them are classified into group two (50%-90%). One of them is classified into group three (100%). (B) There are 12 filling origamis, 6 of them classified into group one, 5 of them belong to group two. One of them belongs to group three.

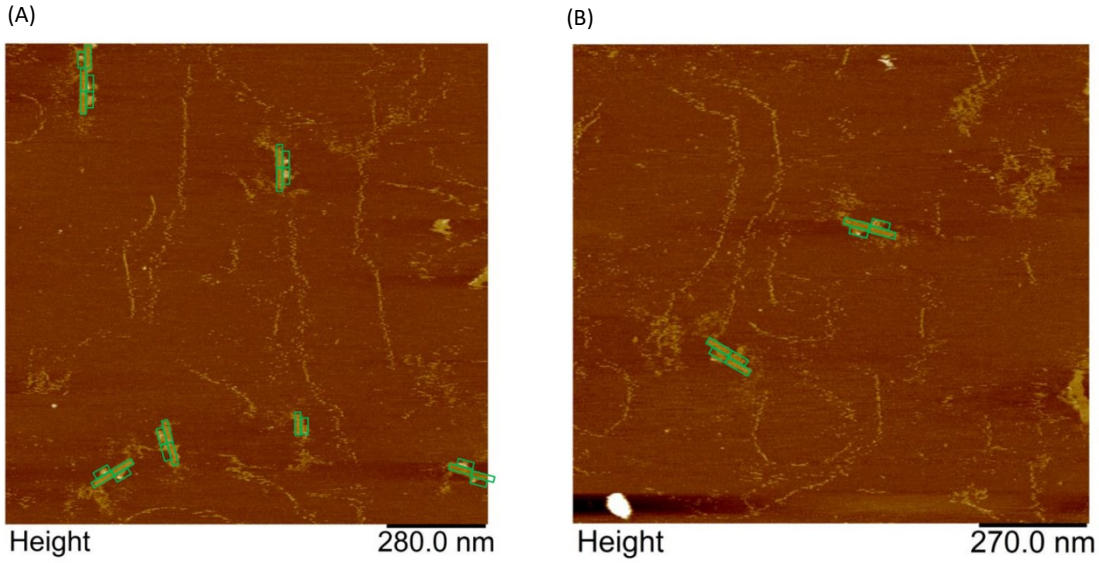


Fig. S28. Filling analysis. (A) There are 12 filling origamis. 3 of them were classified into group one, 8 of them were in group two. One of them belongs to group three. (B) There are 4 filling origamis. 3 of them belong to group two. One of them belongs to group three.

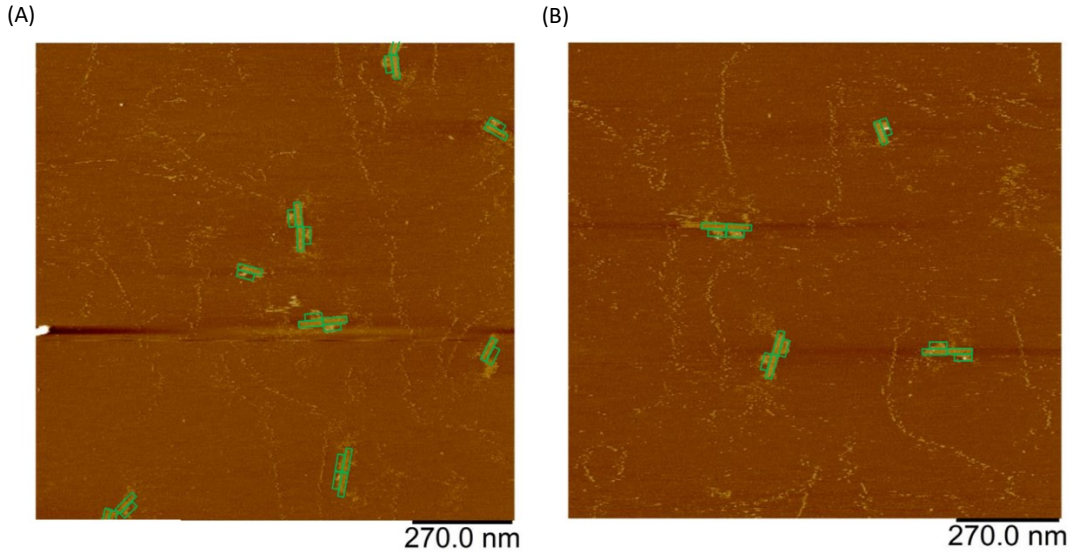


Fig. S29. Filling analysis. (A) There are 9 filling origamis. 5 of them were classified into group one, 4 of them belong to group two. (B) There are 7 filling origamis. 5 of them belong to group two. Two of them belong to group three.

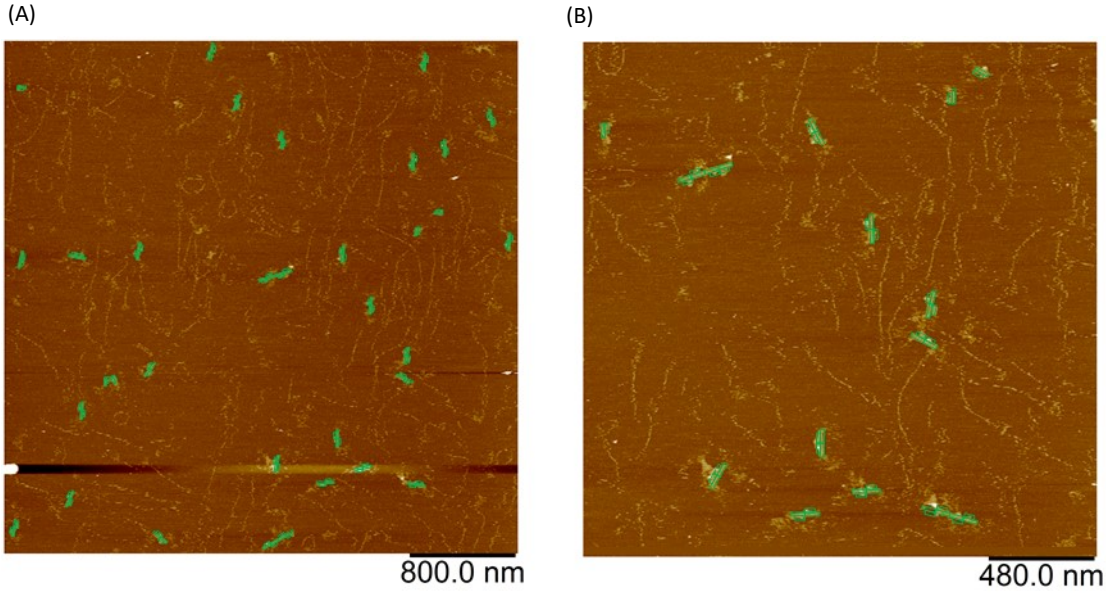


Fig. S30. Filling analysis. (A) There are 52 filling origamis. 17 of them were classified into group one, 18 of them belong to group two, 10 belong to group three, 7 of them belong to group four. (B) There are 24 filling origamis. 7 belong to group one, 9 of them belong to group two, 2 of them belong to group three, 6 of them belong to group four.

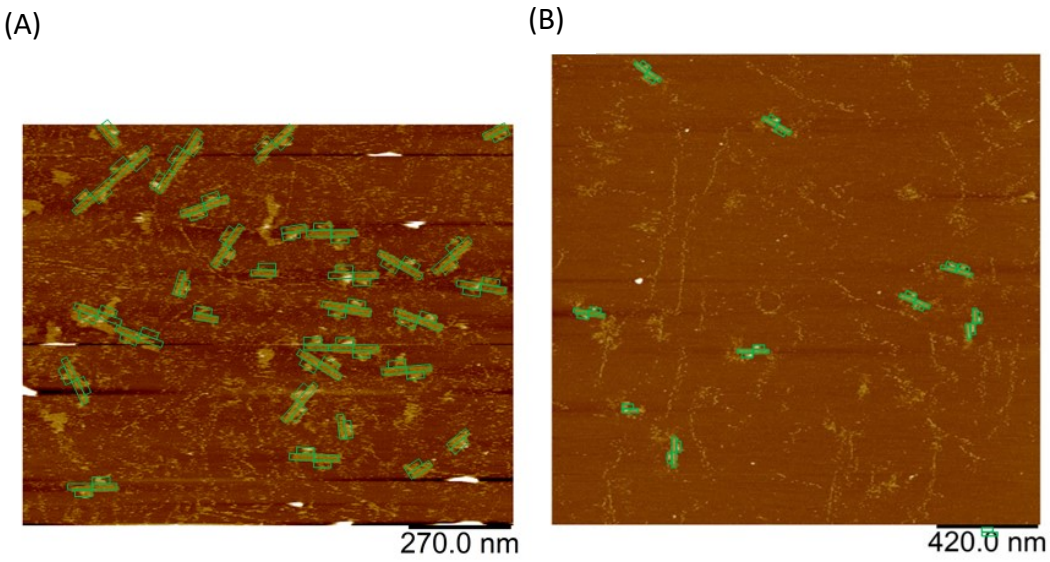


Fig. S31. Filling analysis. (A) There are 54 filling origamis. 10 of them are classified into group one, 42 of them belong to group two, 2 of them belong to group three. (B) There are 17 filling origamis. 8 of them belong to group one, 8 belong to group two, 1 of them belong to group three.

We estimated the filling area for all the 205 structures. 70% is the average filling value of the 205 structures imaged from figure S27 to S31. More figures without marked label are attached at fig. S32.

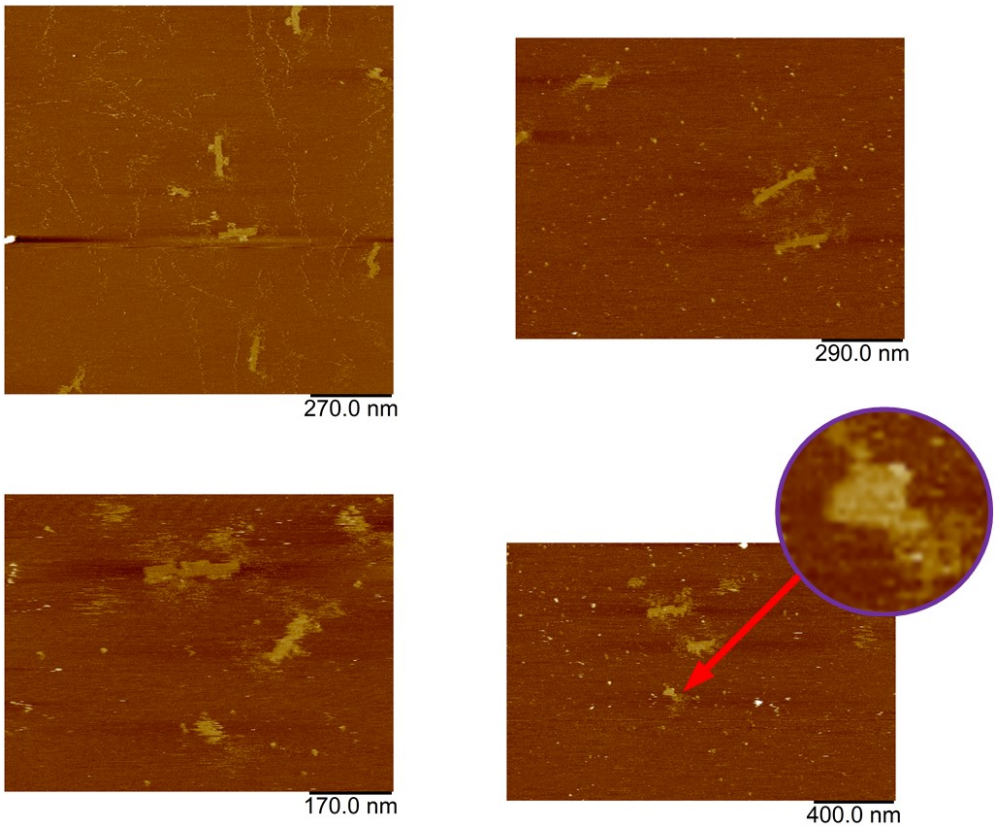


Fig. S32. More filling results. Raw data without marked with green frame.

6. DNA Sequences

Table. S1 The DNA sequences of S1 &S2

S1	CGCTTGCATT	CAGCACTCGTC	ATTTCCGATT	GATAAGAATGG
S2	AATCGGAAAT	CCATTCTTATC	AATGCAAGCG	GACGAGTGCTG

Table. S2 DNA sequences of origami wireframe.

	Sequence
14[31]16[32]	CCTGCAGGGAATTCGTAATCATGGGAGTTGCA
12[15]10[8]	AAACGGCGGGTAGCTATTTTTGAGAATTAATGCCGGAGAG
10[95]12[96]	GAAGCAAAGTAATCGTAAAACTAGAAATAATT
12[143]10[136]	TTAAATCAAGAAAAGCCCCAAAAAGCCCGAAAGACTTCAA
14[79]12[80]	TTCCCAGTGTTTGAGGGGACGACGGCCTTCCT
12[79]10[80]	GTAGCCAGAGAGAATCGATGAACGCTCCAACA

14[63]16[64]	TGTAAAACTGTGAAATTGTTATCCAGCTGATT
15[192]16[176]	CCCCCTCAAATCGTCATAAATATTTCGGGAAA
16[63]16[80]	GCCCTTCACCCTTATAAATCAAAAGAATAGCCCGAGATAGCAGTGAGA
14[95]16[96]	GGGTAACGAACATACGAGCCGGAATGGTTTTT
14[15]12[16]	AGAGGATCGATTGACCGTAATGGGGTGGGAAC
16[159]17[199]	CATTAATGAGGGCGAAAAACCGTCTATCATAGACTGGATAGCGTCCAATACTGCGG
14[127]16[128]	GAAAGGGGCTGGGGTGCCTAATGAGGGAGAGG
12[63]14[64]	AACATTAAAACCGTGCATCTGCCACACGACGT
11[24]14[32]	AGATCTACAAAGGCTAACCCGTCGGATTCTCCATAGGTCACGTTGGTGCTTGCATG
11[192]12[176]	ACCCTGACCCATAAATCAAAAATCTAAACGTT
16[31]16[48]	GCAAGCGGCTGTTTGATGGTGGTTCCGAAATCGGCAAAATCCGCCTGG
14[143]12[144]	TATTACGCGCTTCTGGTGCCGGAAAATTTTTG
16[47]14[48]	CCCTGAGATCATAGCTGTTTCCTGGACGGCCA
10[135]12[128]	ATATCGCGTTTTAATTACCCCGGTTGATAATCGCTCATTT
14[111]12[112]	GCAAGGCGGAAGATCGCACTCCAGATAGGAAC
12[175]11[191]	AATATTTTGCAAATATTTAAATTGAGGTCTTT
12[95]14[96]	CGCGTCTGACAGTATCGGCCTCAGATTAAGTT
13[192]14[176]	GAGAATGAAATGCTTTAAACAGTTCGCAACTG
14[47]12[48]	GTGCCAAGTAGATGGGCGCATCGTATGTGAGC
10[63]12[64]	TACCTTTAGAGTCTGGAGCAAACACTTTCATC
16[79]14[80]	CGGGCAACGCTCACAATTCCACACCCAGGGTT
16[127]16[144]	CGGTTTGCCCACTATTAAAGAACGTGGACTCCAACGTCAAAATCGGCC
14[159]16[160]	GTGCGGGCTAATTGCGTTGCGCTCGCCAGCTG
16[95]16[112]	CTTTTCACGGTTGAGTGTTGTTCCAGTTTGGAACAAGAGTGTATTGGG
16[111]14[112]	CGCCAGGGGCATAAAGTGTAAAGCGATGTGCT
16[175]15[191]	CCTGTCGTACTGCCCGCTTTCCAGCATTGAAT
10[159]12[160]	AAGAGGAACAGGAAGATTGTATAAGTTAAAAT
12[111]9[113]	GCCATCAACATGTCAATCATATGTCGAGCTTCAAAGCGAACAACTAAAGT
16[143]14[144]	AACGCGCGGTGAGCTAACTCACATCTCTTCGC
14[175]13[191]	TTGGGAAGTTCGCCATTCAGGCTGCAGAAAAC
12[47]10[24]	GAGTAACATCAGGTCATTGCCTGAATTGCTCCTTTTGATAAGAGGTCTAGCTGATA
12[127]14[128]	TTTAACCACCAGCTTTCCGGCACCCAGCTGGC
10[199]10[160]	TATTATAGTCAGAAGCAAAGCGGATTGCATCAAAAAGATT
17[8]14[16]	CCAGCAGGCGAAAATCTCCACGCTGGTTTGCCCCCGGGTACCGAGCTCTCGACTCT
12[159]14[160]	TCGCATTAACCAGGCAAAGCGCCAGGCGATCG

Table. S3 Helper strands

1[19]0[19]	AGCCTTTATTTCAACGCGCTTGCATTTGACCCTGTAATACTTTTGCGGGAGA
1[45]0[45]	CAGCACTCGTCCGCTTGCATTTCGGTTGTACCAAAAACATTA
1[66]0[66]	CAGCACTCGTCCGCTTGCATTGCCTCAGAGCATAAAGCTAAA
1[87]0[87]	CAGCACTCGTCCGCTTGCATTTTAGCAAAATTAAGCAATAAA
1[108]0[108]	CAGCACTCGTCCGCTTGCATTTCATACAGGCAAGGCAAAGAA
0[154]1[154]	TAGTAGTAGCATTAACATCCAATAAACAGCACTCGTCCATCAATTCTACTAA

9[19]8[19]	TATGATATTCAACCGTTCATTTTTGCGCGAACGTAAGTCAAATCACCATCAA
8[65]10[64]	GTCGTGAGCAGGCGAACGTAAGGATGGCTTAGAGCTTAATTGCTGAATTTAGAGAG
10[79]8[66]	GGTCAGGAATAATGCTGTAGCTCGTCGTGAGCAGGCGAACGTAA
8[107]10[96]	GTCGTGAGCAGGCGAACGTAAAACATGTTTTAAATATGCCAGACCG
9[114]8[108]	ACGGTGTCTGGAAGTGTCGTGAGCAGGCGAACGTAA
8[154]9[154]	AATTCTGCGAACGAGGTCGTGAGCAGTTCATTCCATATAACAGTTGATTCCC

2[39]2[19]	CCATTCAAGGATAAAAATTTT
3[19]3[39]	TAGAACCCTCATATATAAGCG
4[39]4[19]	CCATTTTTAAATGCAATGCCT
5[19]5[39]	GAGTAATGTGTAGGTAAAGCG
6[39]6[19]	CCATTAAGATTCAAAAGGGTG
7[19]7[39]	AGAAAGGCCGGAGACAAAGCG

2[154]2[136]	GAGCTGAAAAGGTGGGAAAT
3[136]3[154]	GACGATTTCATTTGGGGCGC
4[154]4[136]	CCTGTTTAGCTATATGAAAT
5[136]5[154]	GACGAGCAAATGGTCAATAA
6[154]6[136]	CATTAGATACATTTCGAAAT
7[136]7[154]	GACGATAGATTTAGTTTGAC