Supplementary Material (ESI) for Soft Matter

Supplementary Information:

Comparison of methods for orienting and aligning DNA origami

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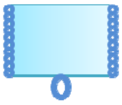
Contents

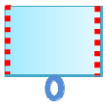
1. DNA origami designs and sequences

Design A

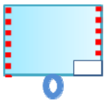
Design B



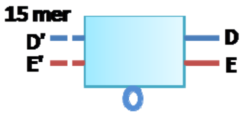
Design C



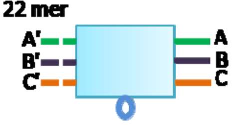
Design D



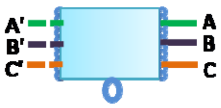
Design E1



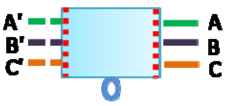
Design E2



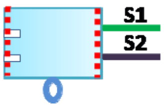
Design E3



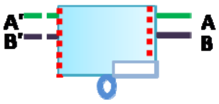
Design E4



Design E5



Design E6



Design A: Sequences for this rectangular DNA origami were adopted from Rothemund.[1]

Design B: Sequences for this rectangular DNA origami were adopted from Rothemund.[1]

Design C: The following oligos were omitted from the staple mix (names from ref [1]): r9t0f, r9t4f, r9t6f, r9t8f, r9t10f, r9t12f, r9t14f, r9t16f, r9t18f, r9t20f, r9t22j, r-9t2i, r-9t4e, r-9t6e, r09r8e, r-9t10e, r-9t12e, r-9t14e, r-9t16e, r-9t18e, r-9t20e, r-9t22e, r-9t24e

Design D: The following oligos were omitted from the staple mix (names from ref [1]): r9t0f, r9t4f, r9t6f, r9t8f, r9t10f, r-9t2i, r-9t4e, r-9t6e, r09r8e, r-9t10e, r-9t12e, r-9t14e, r-9t16e, r-9t18e, r-9t20e, r-9t22e, r-9t24e

Additional sequences that were eliminated for the notch on DNA origami D (names from ref [1])

r7t12f, r7t14f, r7t16f, r7t18f,r7t20f, r7t22f, r7t12e, r7t14e, r7t16e, r7t18e, r7t20e, r7t22e, r7t24h, r9t12f, r9t14f, r9t16f, r9t18f,r9t20f, r9t22j

Design E1:

15 mer linker strands that were used for design E1 (5' to 3')

SE7 CGATCGATAGATCGATAAAGGTGGCAACATAGTAGAAAA (in place of r9t6f)

SE8 GTGCATCATGACGATTTCATCAATATAATCCTATCAGAT (in place of r9t18f)

SE9 TCGATCTATCGATCGGGAAGAAAAATCTACGACCAGTCA (in place of r-9t8e)

SE10 ATCGTCATGATGCACGTAATGGGATAGGTCAAAACGGC (in place of r-9t18e)

Designs E2, E3, E4, and E6:

<u>22mer linker strands that were used for oligomer E2-E6</u>

SE1 GGATACCCGATCGATAGATCGAATAAATCCTCATTAAATGATATTC (in place of r9t2f)

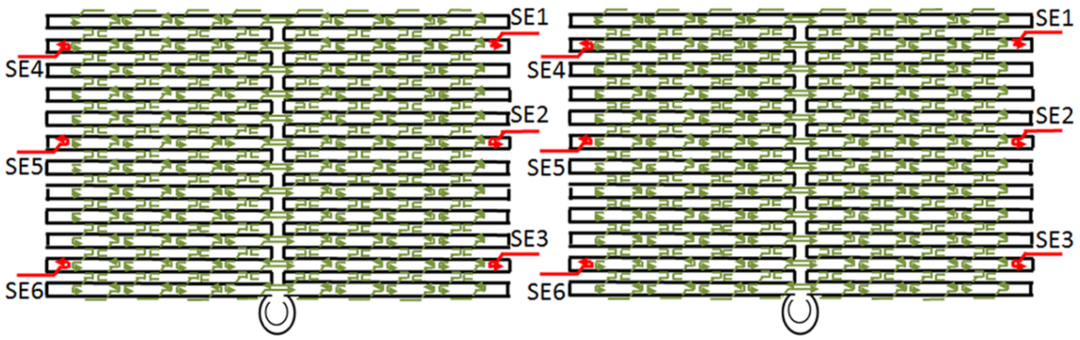
SE2 GCGATCCGTGCATCATGACGATGCTTATCCGGTATTCTAAATCAGA (in place of r9t10f)

SE3 AAGCTGAATAGAGAGTGATCAGAATCAATATCTGGTCACAAATATC (in place of r9t20f)

SE4 TGCATCTATCGTTCGGGTATCCGACAGCATCGGAACGAACCCTCAG (in place of r-9t4e)

SE5 ATCGTCATGATGCACGGATCGCCTGTAGCTCAACATGTATTGCTGA (in place of r9t12e)

SE6 CTGATCACTCTCTATTCAGCTTCGGTTTGCGTATTGGGAACGCGCG (in place of r9t22e)



Design E5:

43mer linker strands that were used for design E5

S1:TAAATATTGGAAGAAAAATCTACGACCAGTCATTTTTTTTTAGCGTACACG TAAAGGTGGCAACATAGTAGAAAA (in place of r9t6f and r-9t8e)

S2:CACGACGTGTAATGGGATAGGTCAAAACGGCGTTTTTTTTTAGCGTACACG ATTAATTACATTTAACACATCAAG (in place of r9t16f and r-9t18e)

Design ESTV:

Biotinylated oligonucleotides were used in place of oligos r5t20f, r7t4f, and r7t20f

B1 Biotin/TTTTCCCGGGTACTTTCCAGTCGGGAAACGGGCAAC (in place of r5t20f)

B2 Biotin/TTTTTGAGGCAGGCGTCAGACTGTAGCGTAGCAAGG (in place of r7t4f)

B3 Biotin/TTTTCGGAATTATTGAAAGGAATTGAGGTGAAAAAT (in place of r7t20f)

Sequences that were eliminated for the notch on DNA origami D and oligomer E6 (name of the sequence is directly adopted from Paul Rothemund's literature)[1]

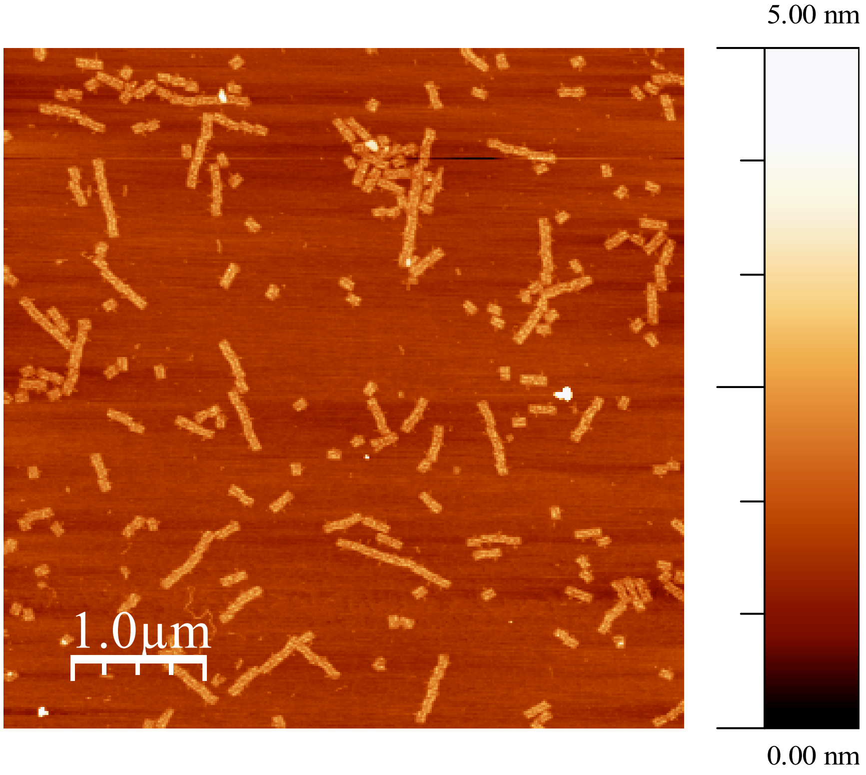
r7t12f, r7t14f, r7t16f, r7t18f,r7t20f, r7t22f, r7t12e, r7t14e, r7t16e, r7t18e, r7t20e, r7t22e, r7t24h, r9t12f, r9t14f, r9t16f, r9t18f,r9t20f, r9t22j

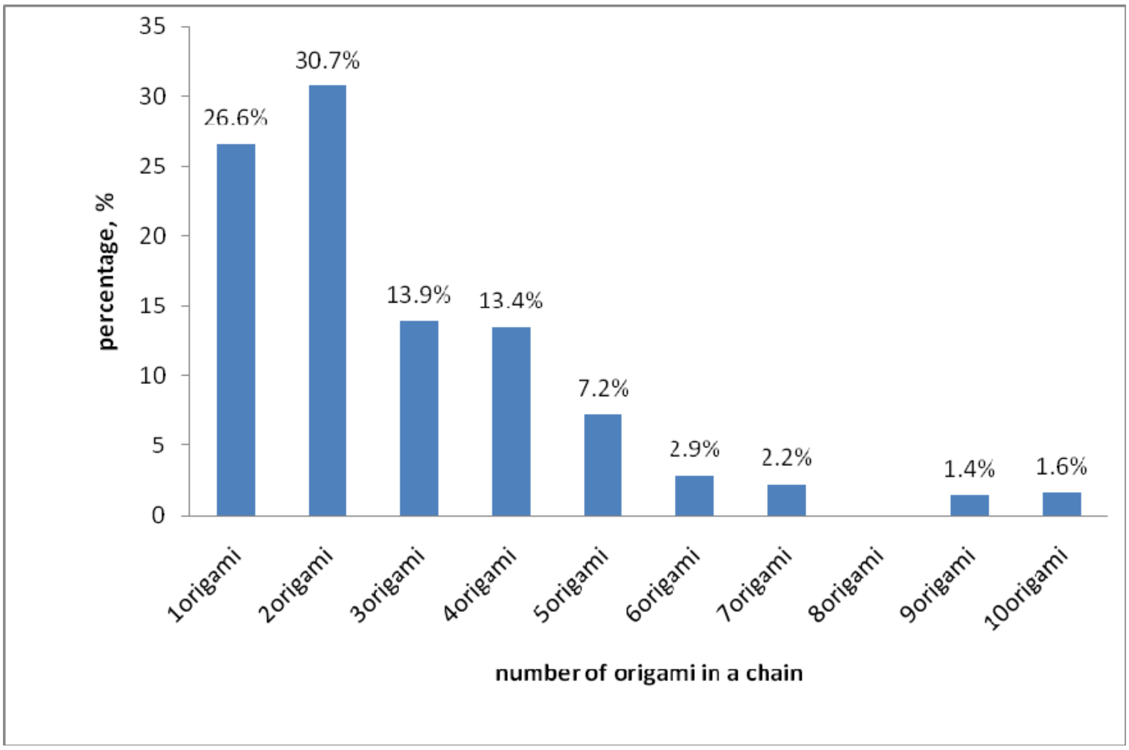
2. AFM images and statistics

General notes: Chain length was either counted directly (for designs and AFM images where loop regions and/or gaps between the origami were observable) or the origami chains were measured in nm and chain length was derived by dividing by the average length of an origami, which was about 97 nm. Poorly assembled DNA origami (those with rips or large un-structured regions) were not counted. If the distance between short edges of two single DNA origami was more than 20 nm, the origami were assumed to be unconnected.

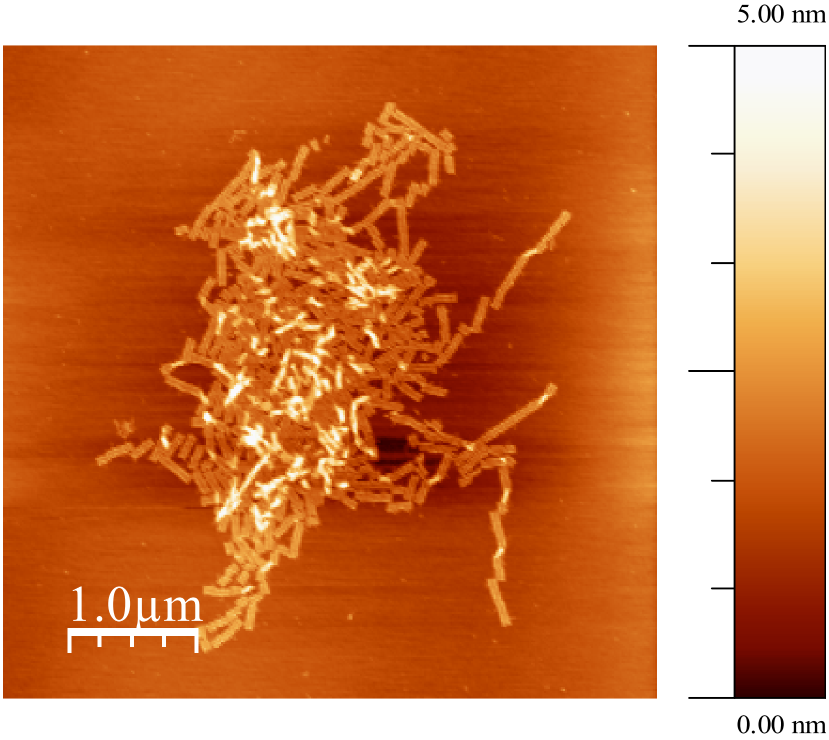
The histograms show % of origami found as singletons, in dimers, trimers, etc. Thus if there are three singletons, a pentamer, and a dimer present in an image, it would be recorded as 30% singleton, 20% dimeric, and 50% pentameric.

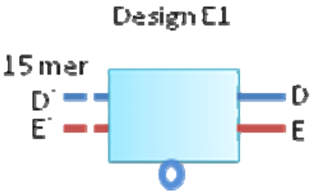
i) Chain length _ Design B (total number of origami counted = 625)

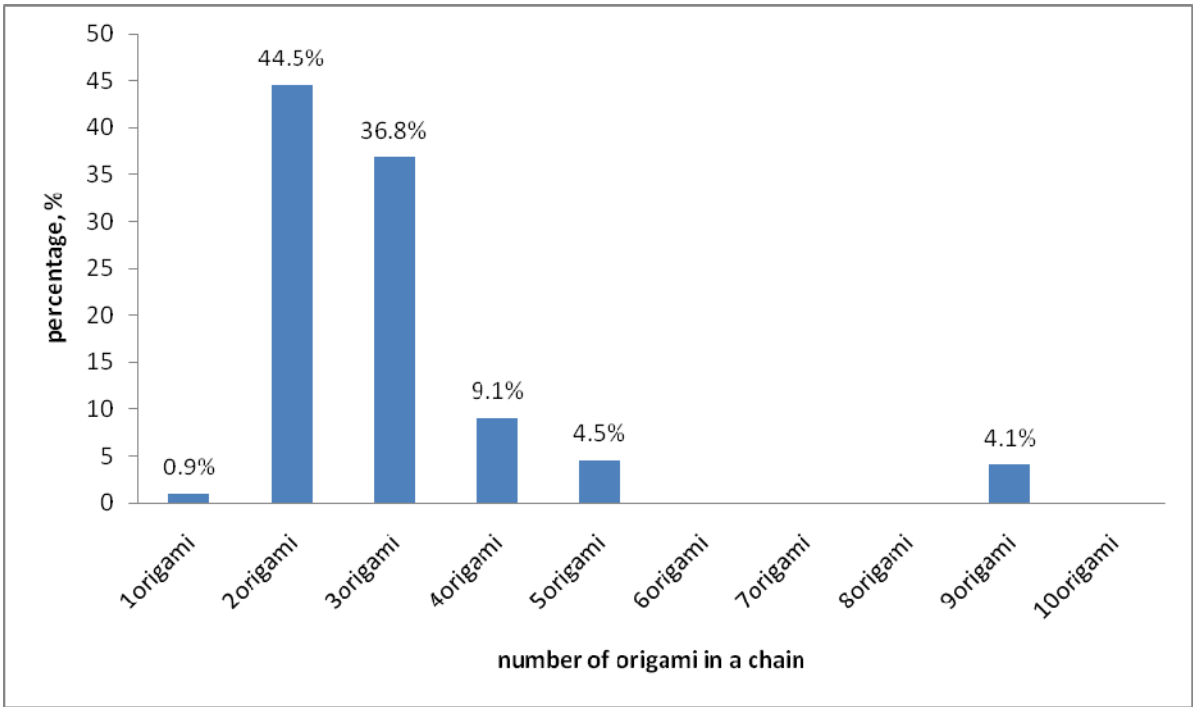




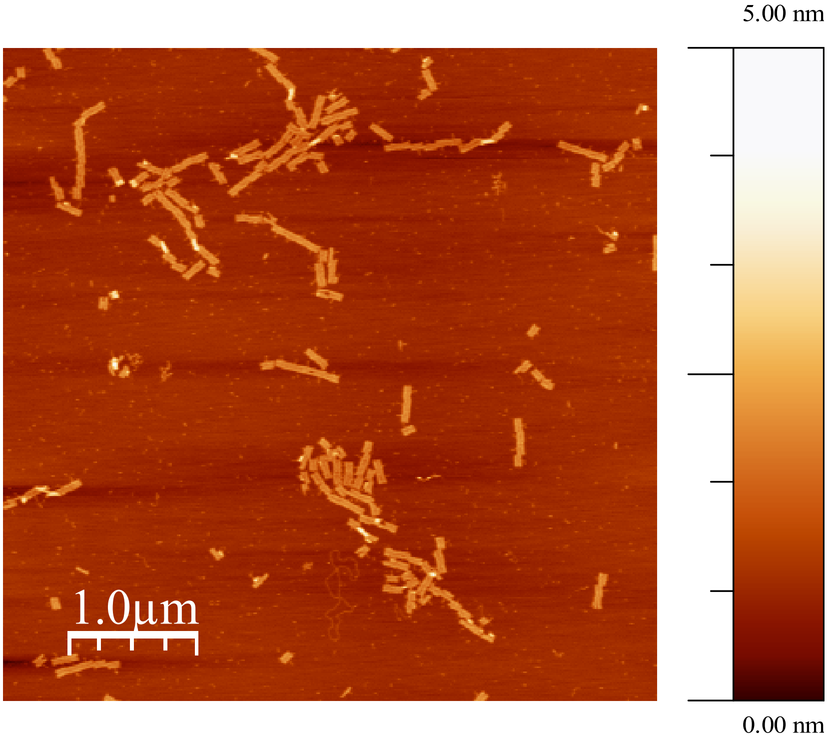
ii) Chain length _ Design E1 (total number of origami = 220)

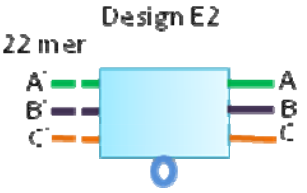


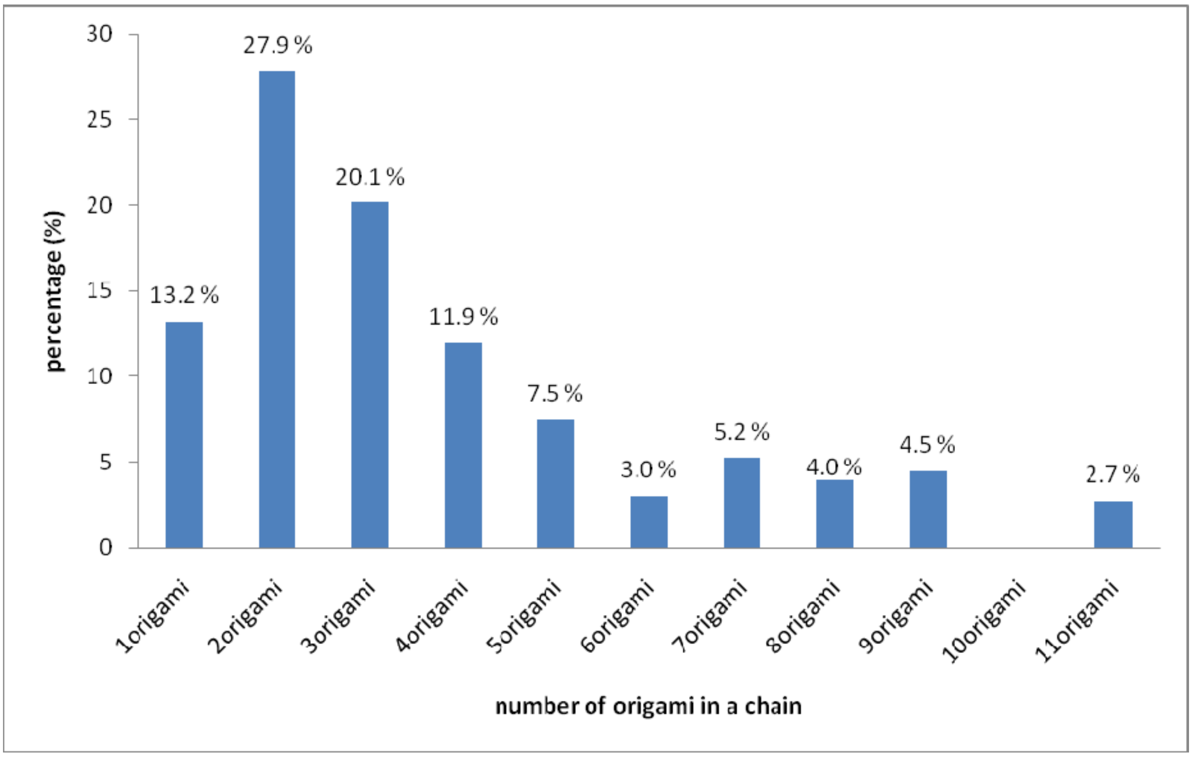




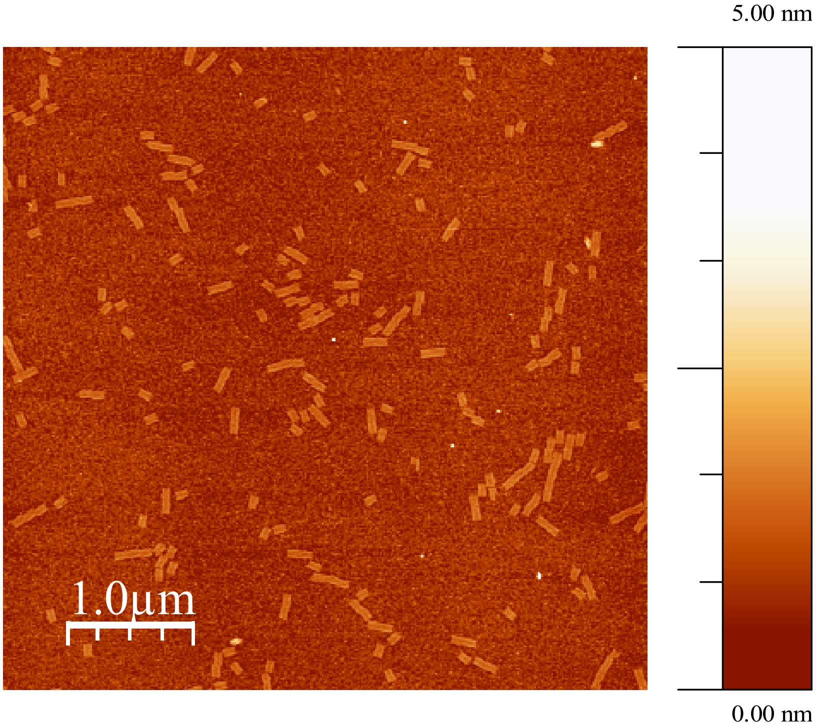
iii) Chain length_Design E2 (total number of origami = 402)

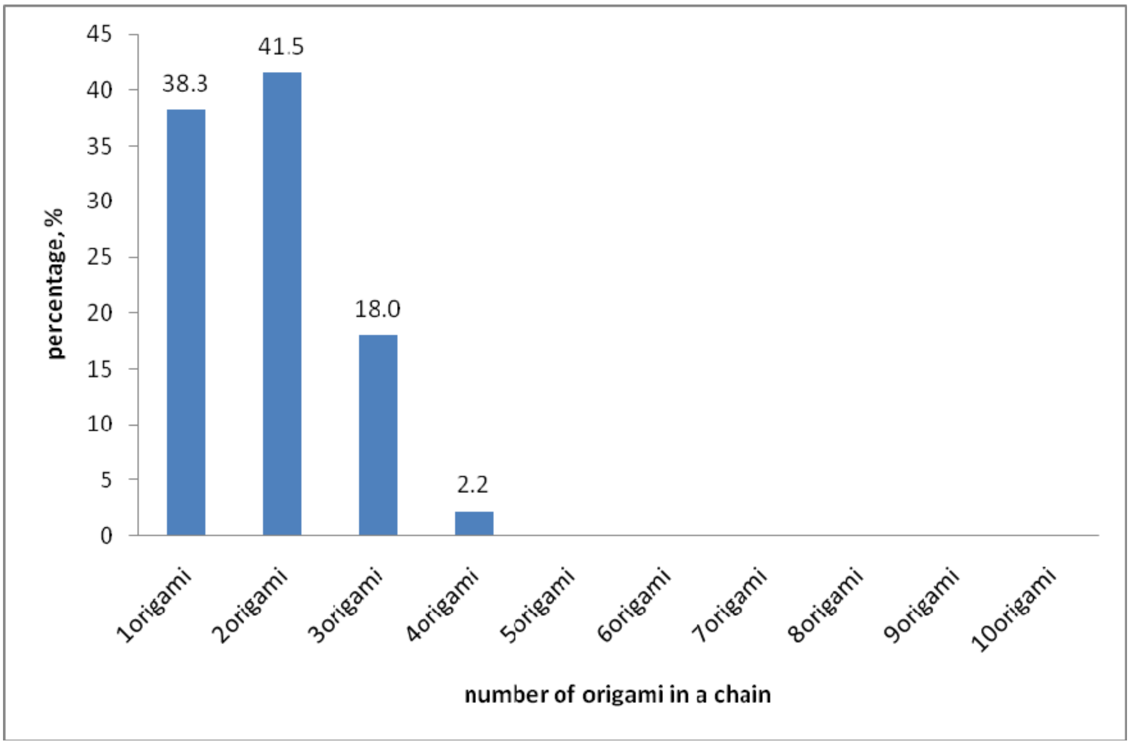






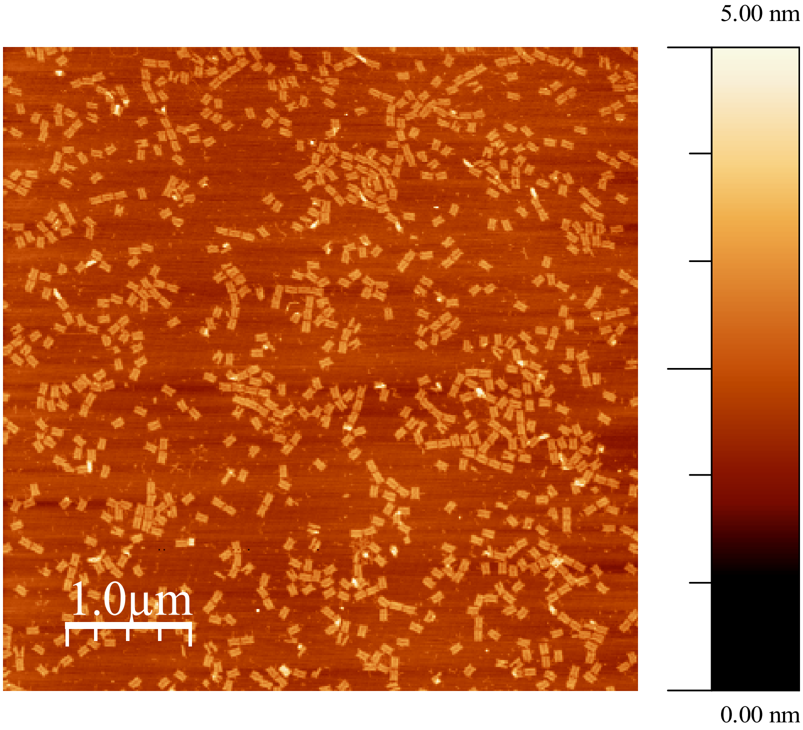
iv) Chain length _ Design E3 (total number of origami = 183)

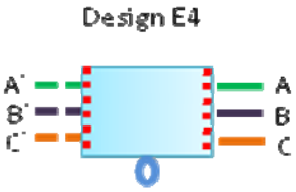


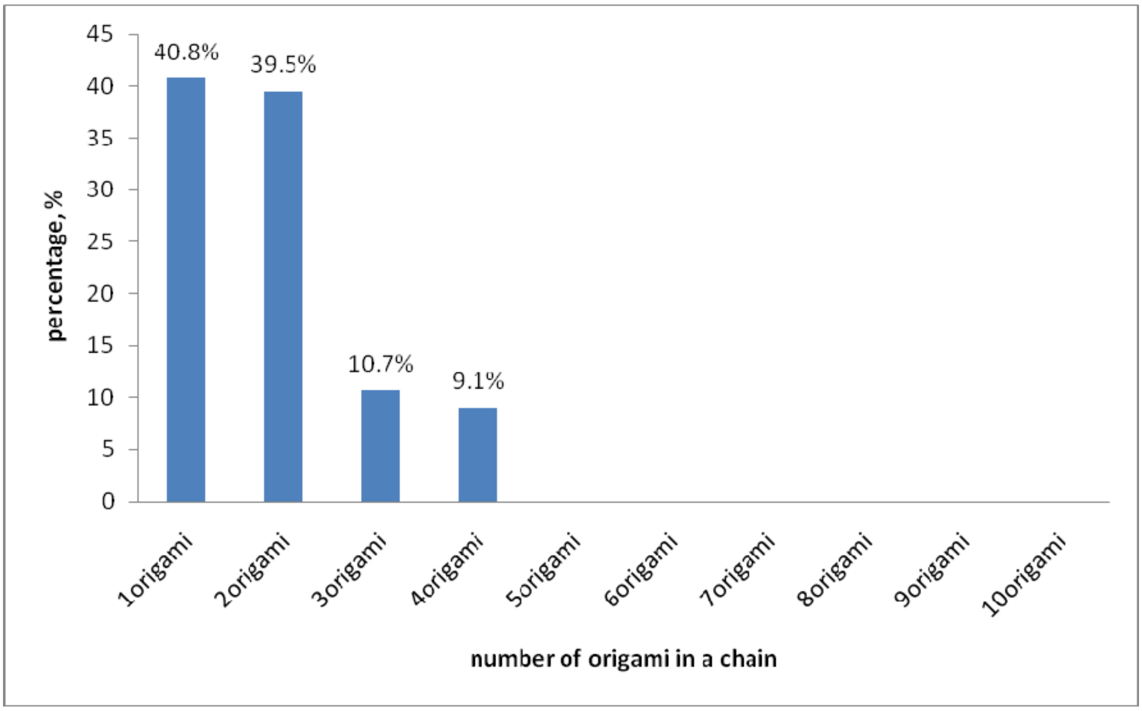


v) Chain length_Design E4

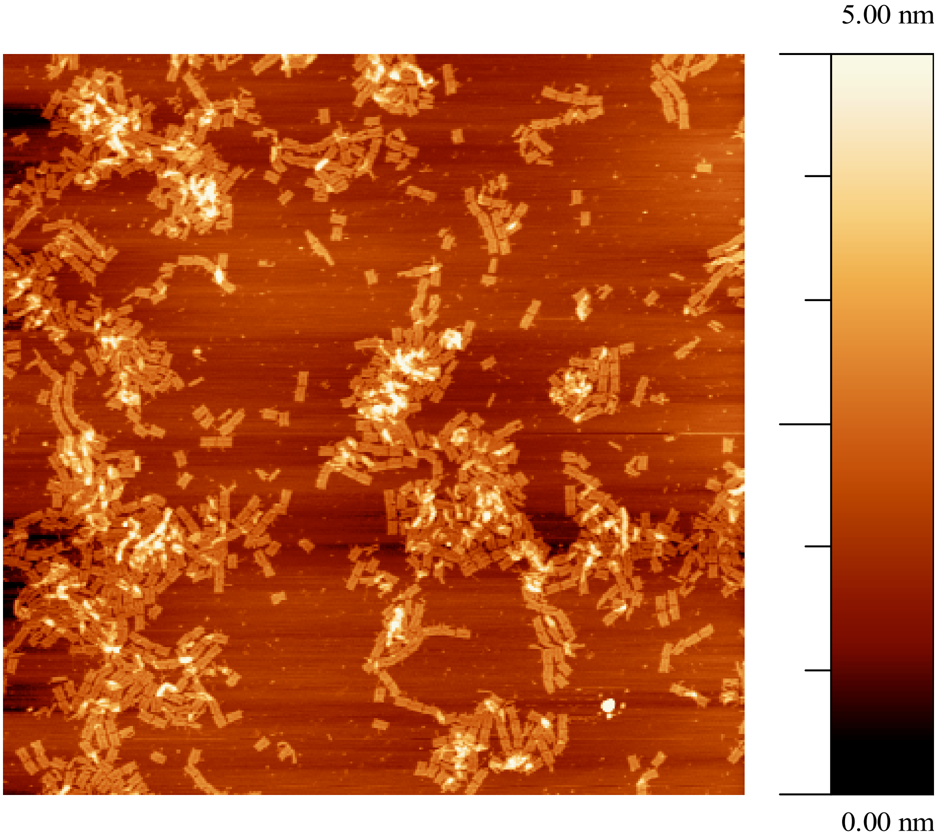
a. 1eq linker strands at 70 min. annealing time (total number of origami = 309)

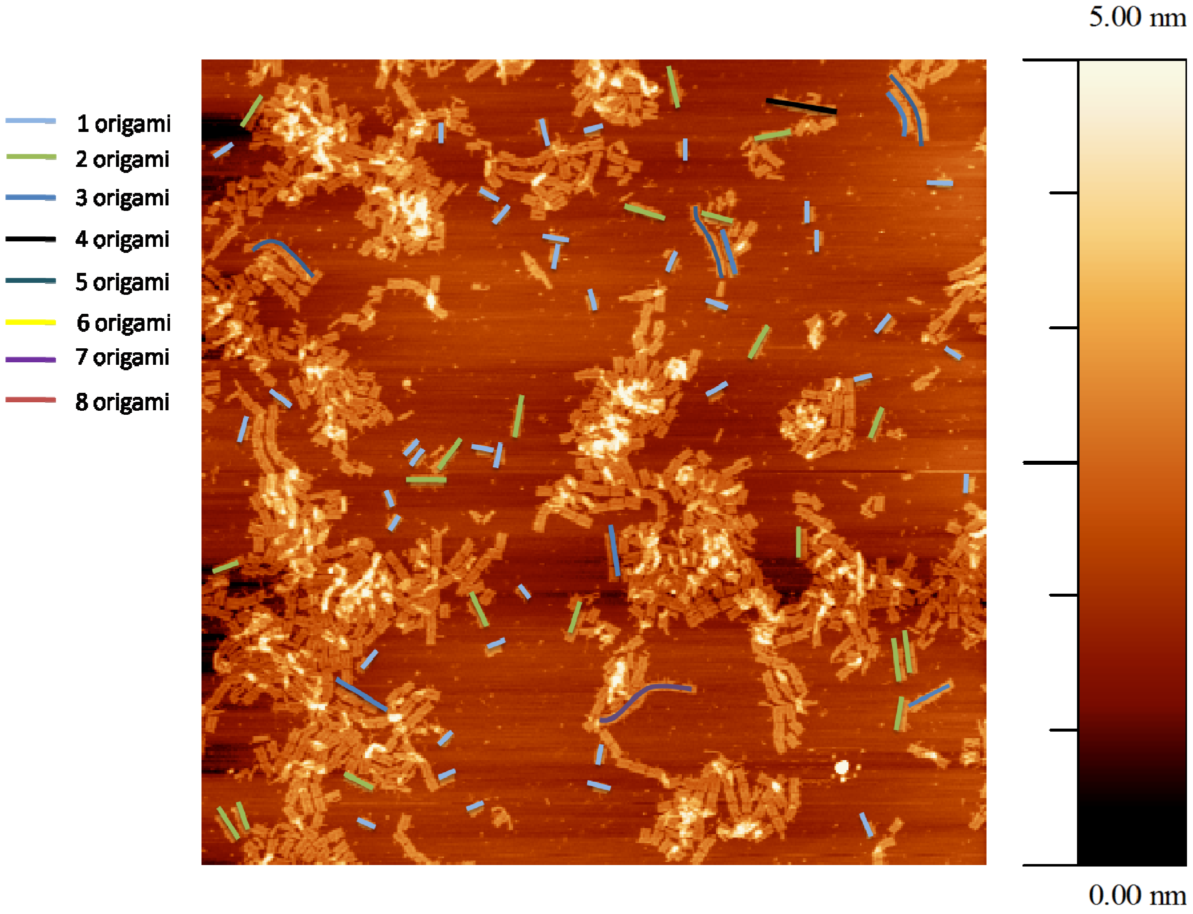


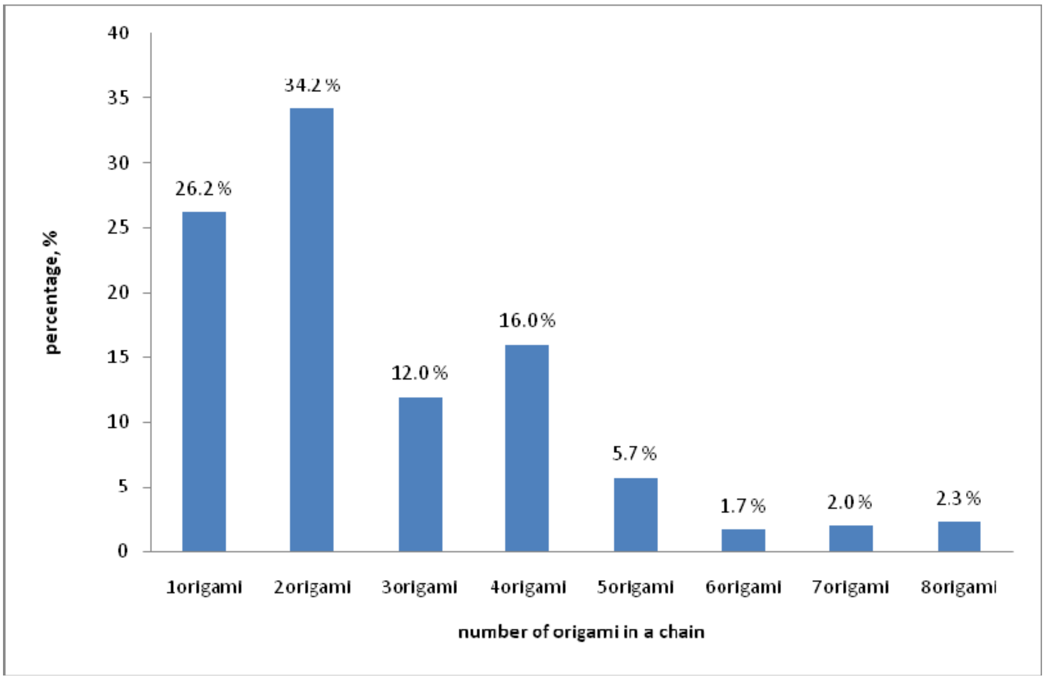




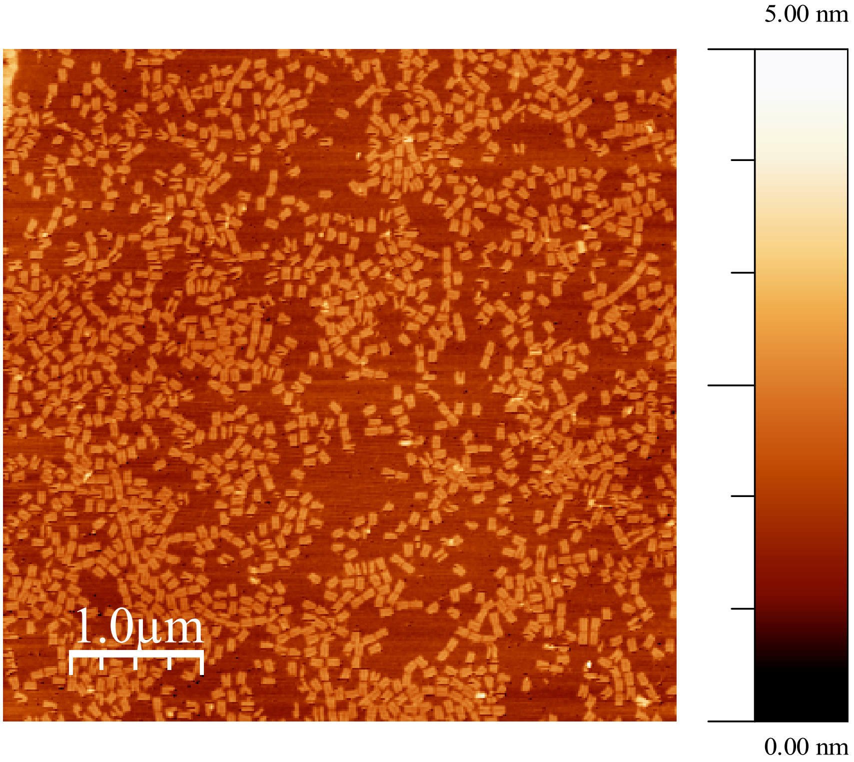
b. 1eq linker strands at 7hr. annealing time (total number of origami = 351) showing the original image and illustration of how chain length was measured.

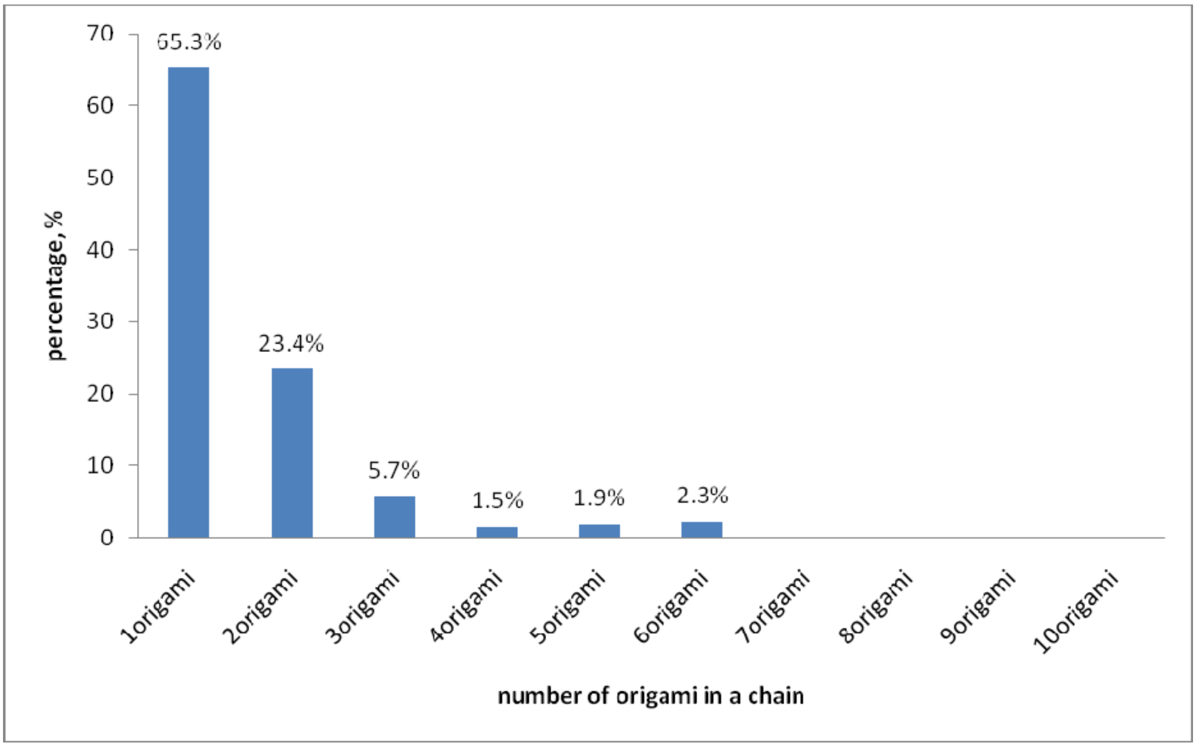



1 origami
2 origami
3 origami
4 origami
5 origami
6 origami
7 origami
8 origami
5.00 nm
0.00 nm

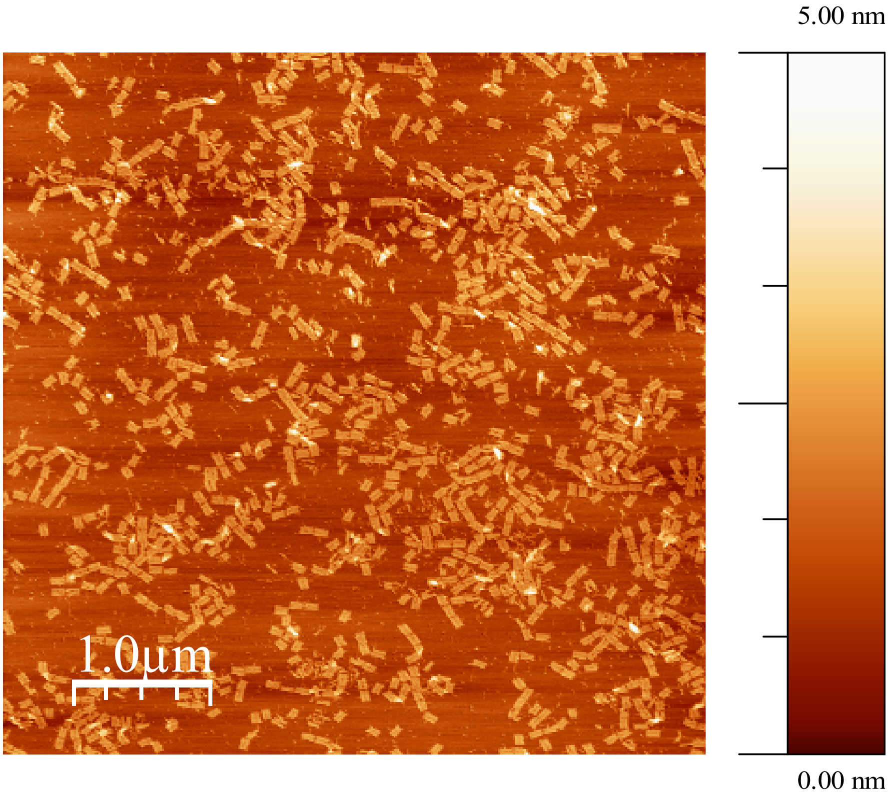

percentage, %
40
35
30
25
20
15
10
5
0
26.2 %
34.2 %
12.0 %
16.0 %
5.7 %
1.7 %
2.0 %
2.3 %
1origami
2origami
3origami
4origami
5origami
6origami
7origami
8origami
number of origami in a chain

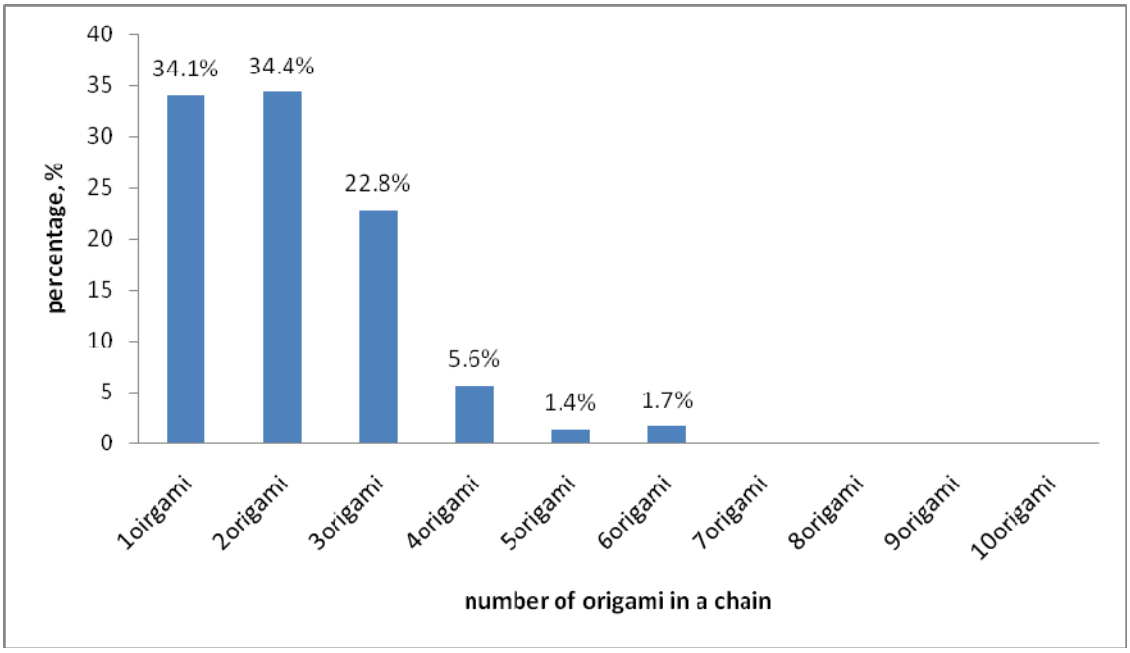
c. 2eq linker strands at 70 min. annealing time (number of origami in a chain = 265)





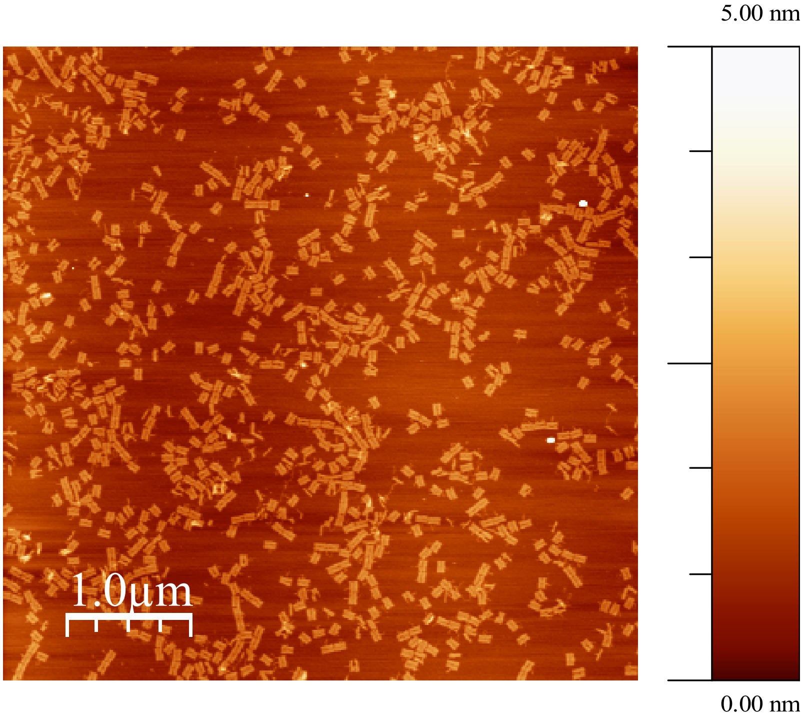
d. 2eq linker strands at 7hr. annealing time (total number of origami = 355)

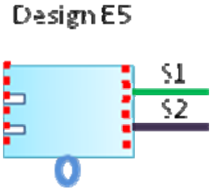

5.00 nm
1.0µm
0.00 nm

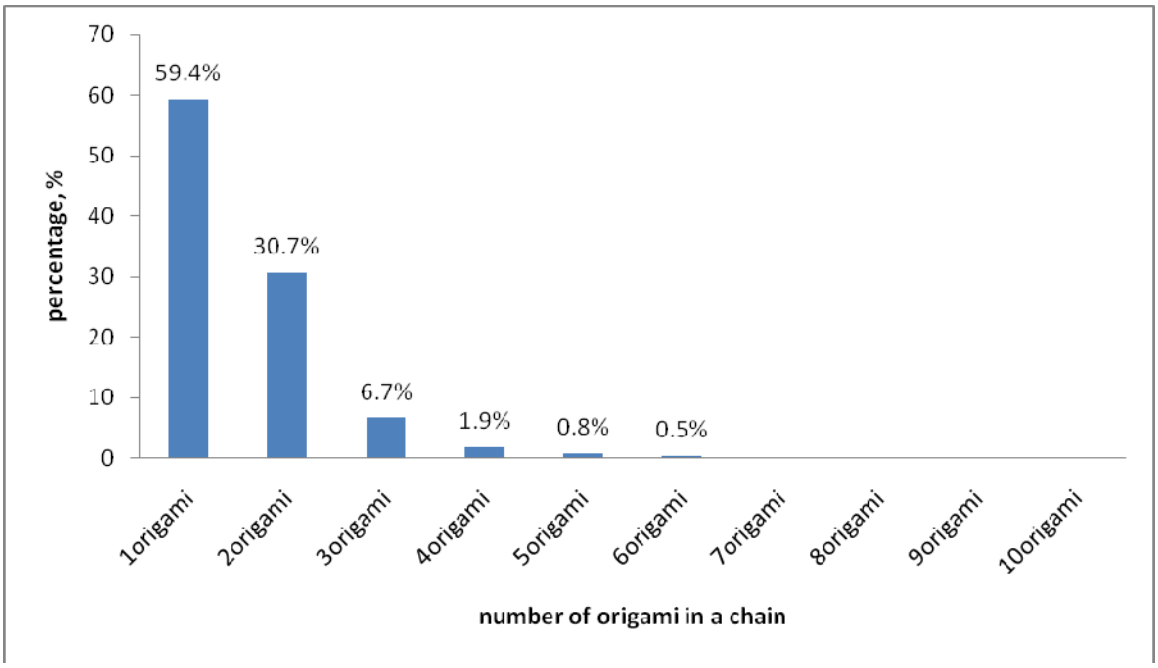

percentage, %
40
35
30
25
20
15
10
5
0
34.1%
34.4%
22.8%
5.6%
1.4%
1.7%
1oirgami
2origami
3origami
4origami
5origami
6origami
7origami
8origami
9origami
10origami
number of origami in a chain

vi) Chain length _ Design E5

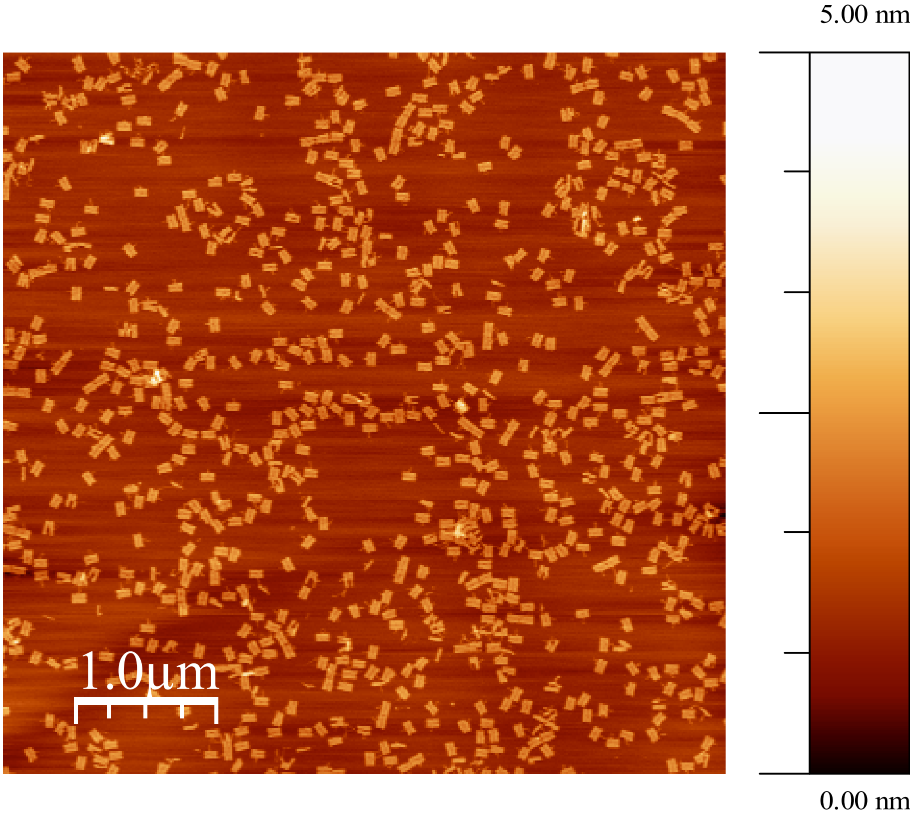
a. 1eq linker strands at 7h annealing time (number of origami in a chain = 1251)

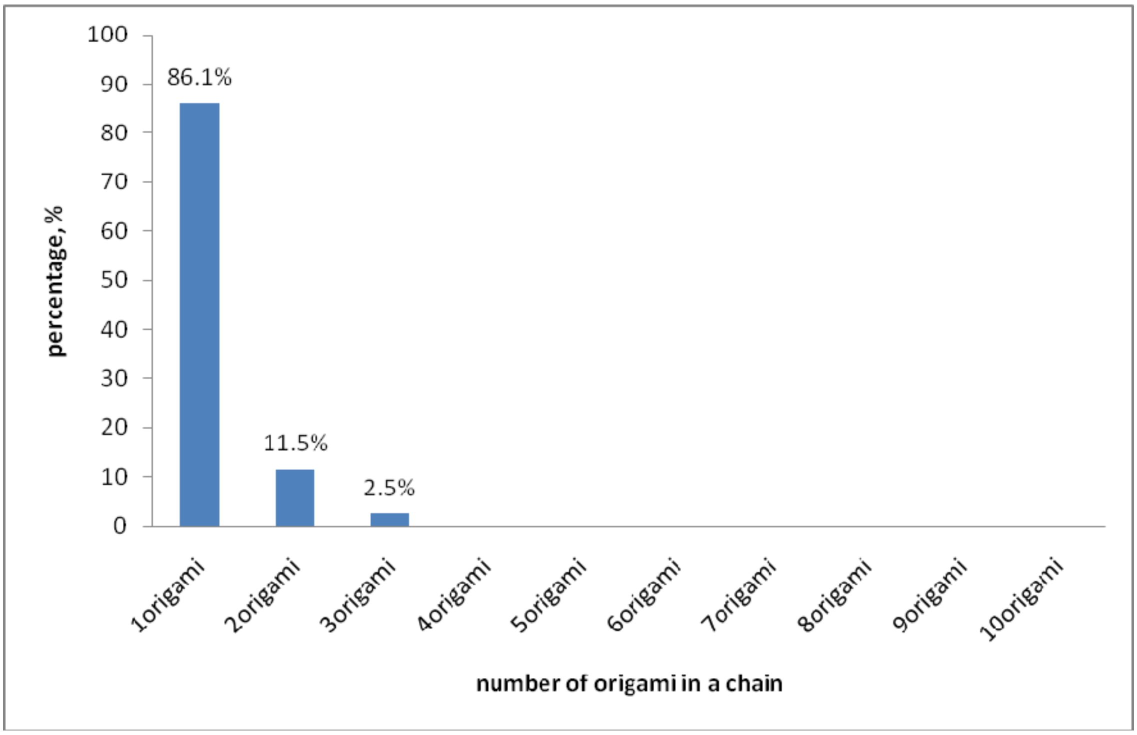




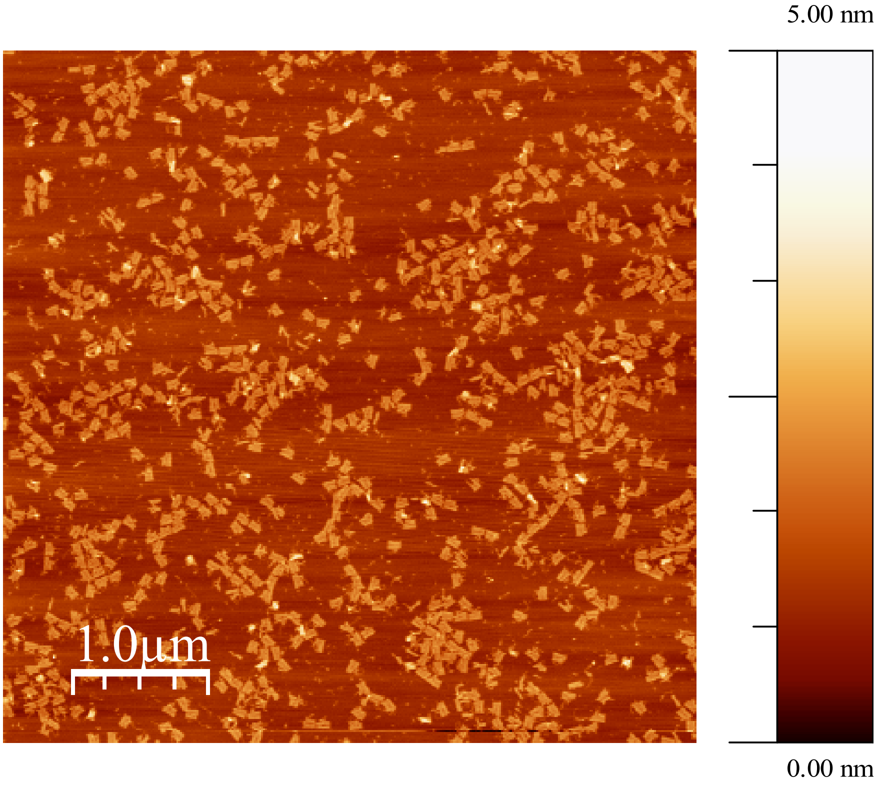


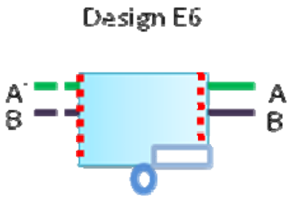
b. 10eq linker strands at 7h annealing time (number of origami in a chain = 1327)

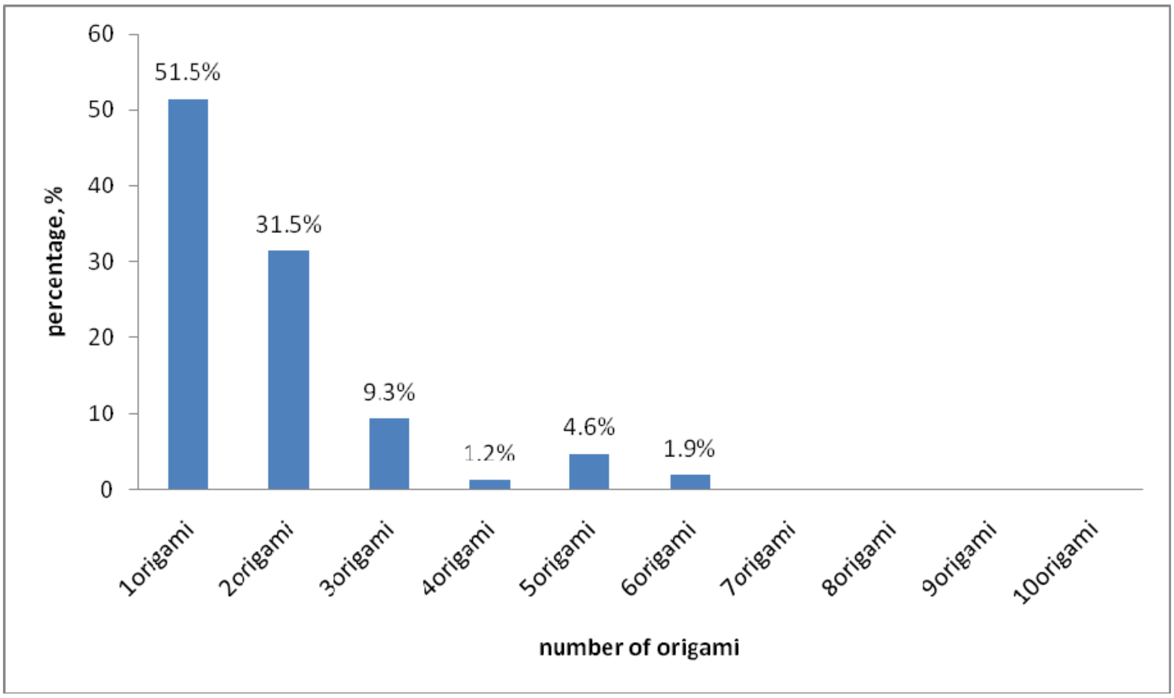




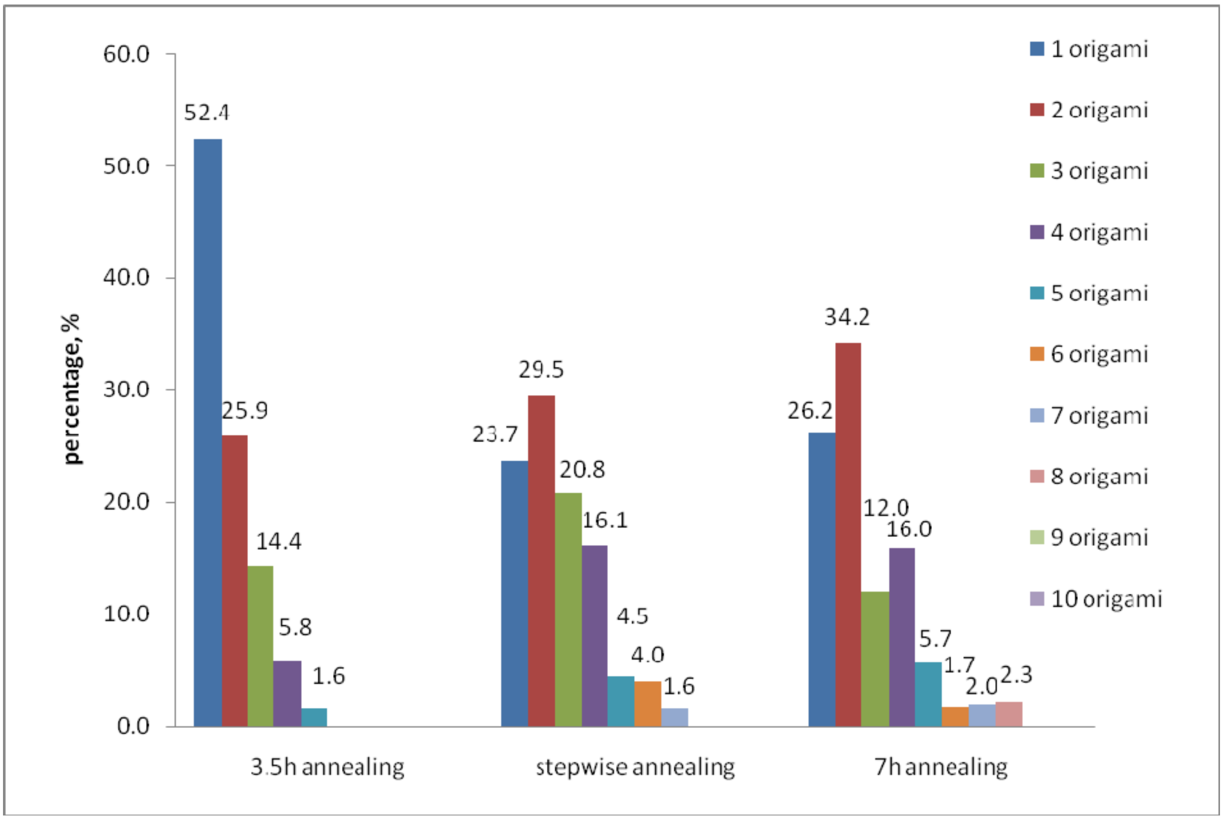
vii) Chain length_Design E6 (total number of origami = 324)



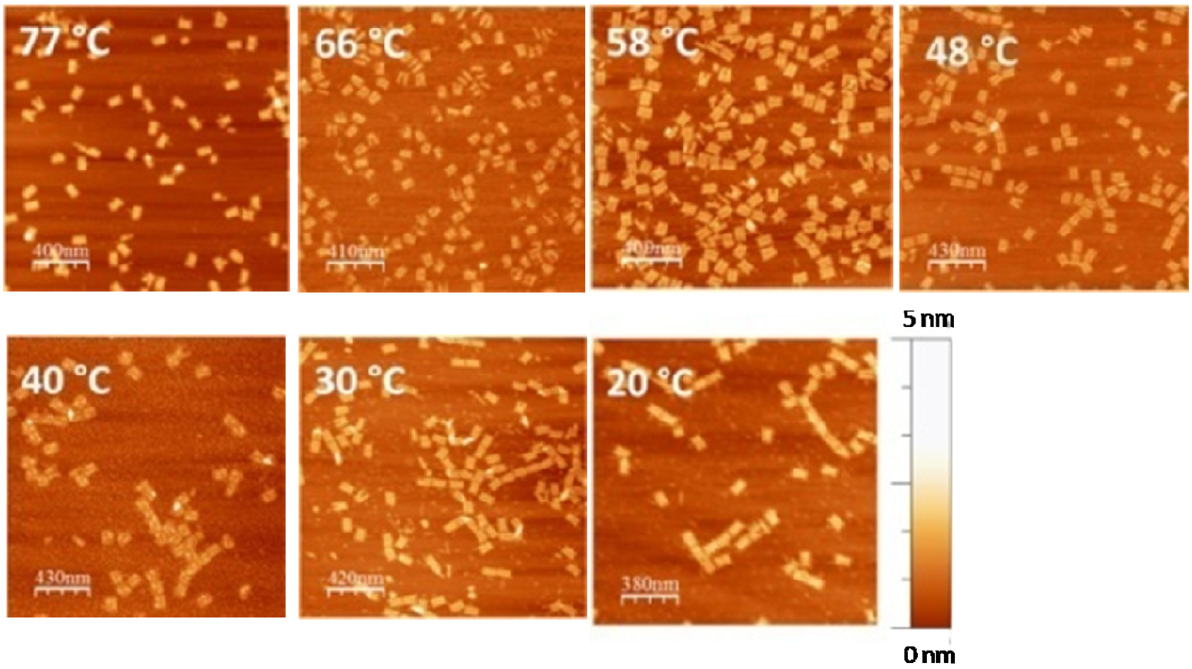


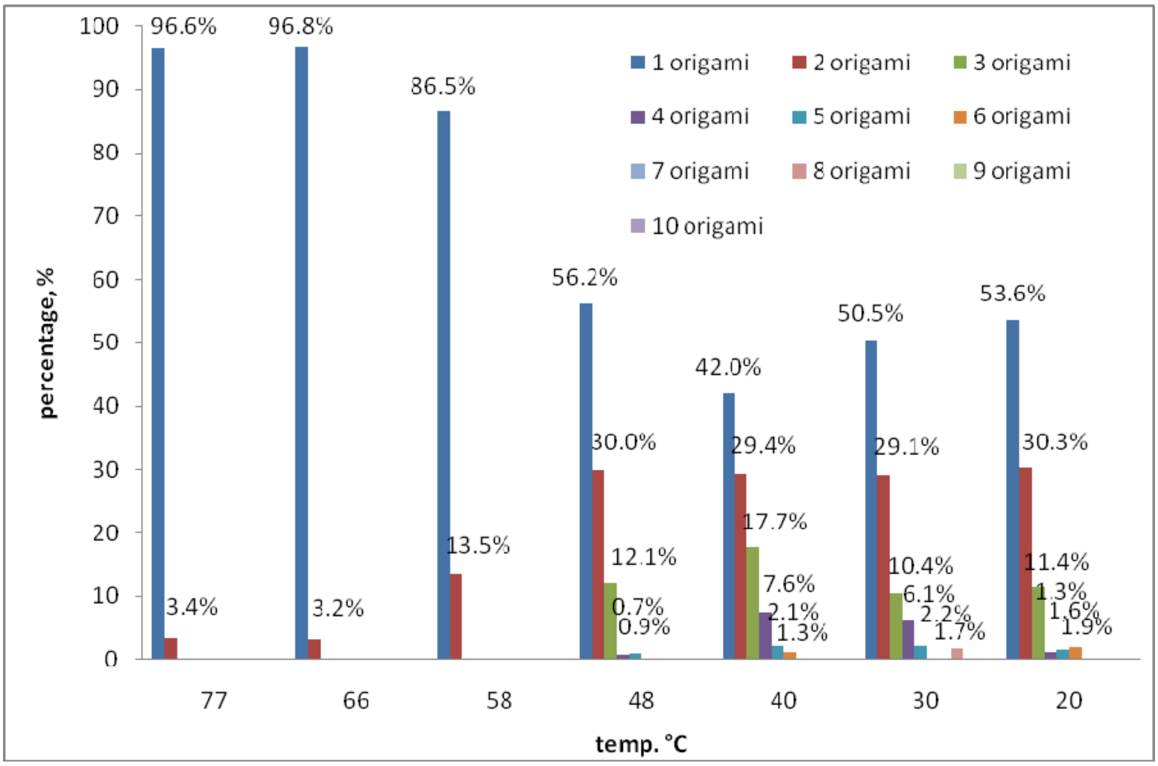


viii) Chain length vs annealing time _ 70 min. vs. stepwise vs. 7h annealing

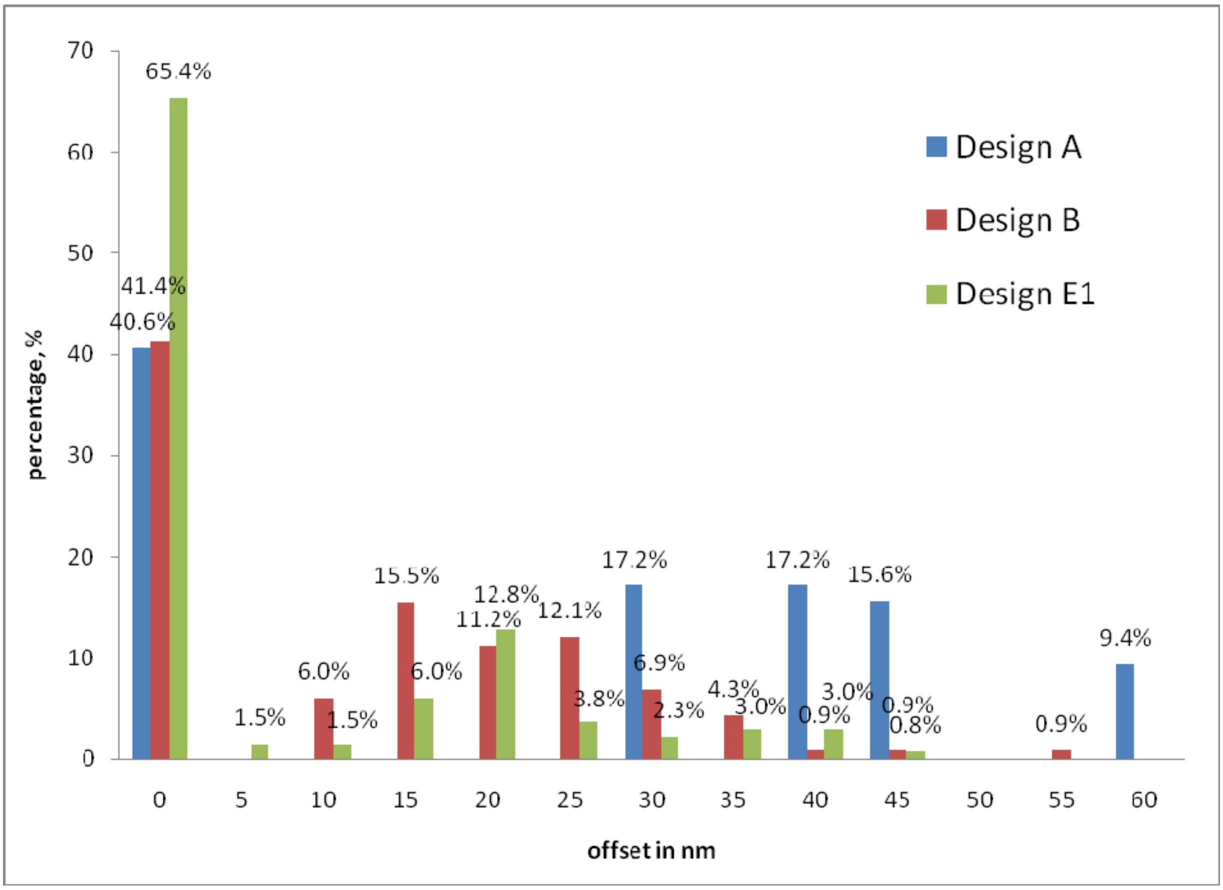


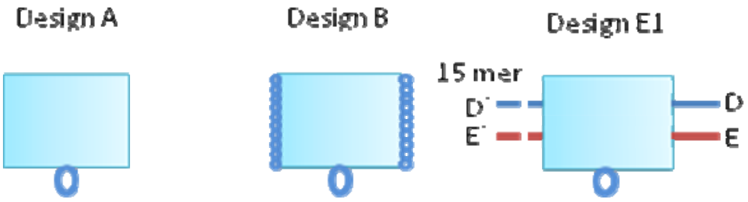
3. Self-assembly of Design E4 (3.5h annealing at -1°C/3min)



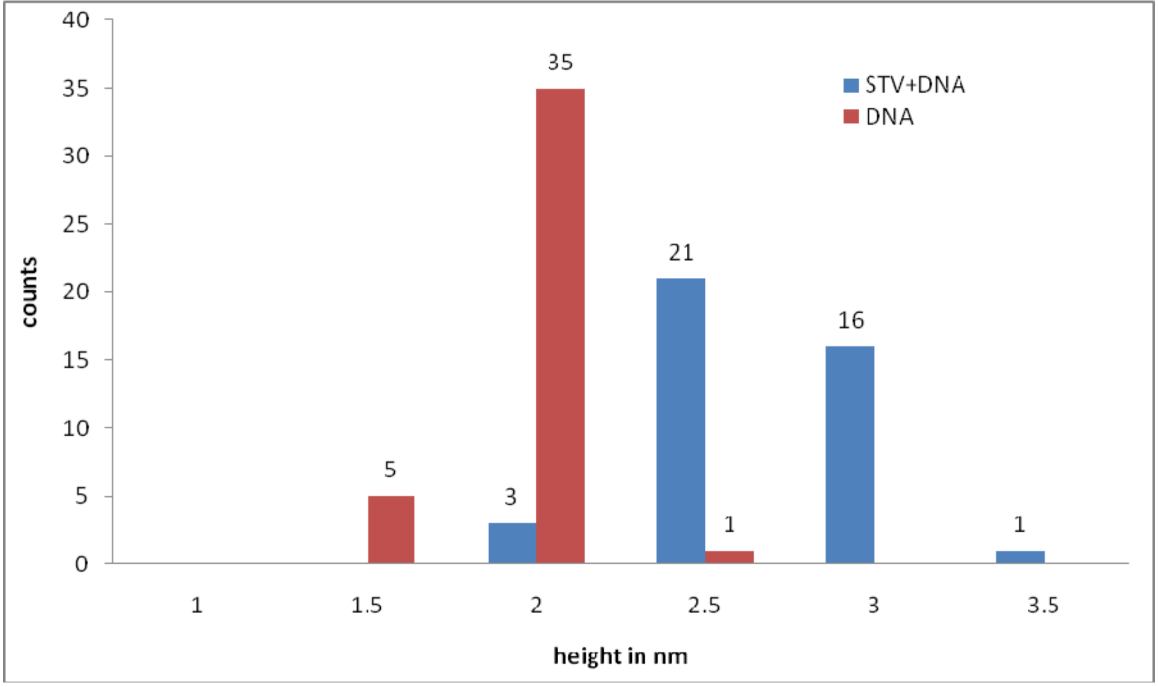


4. Offset measurements

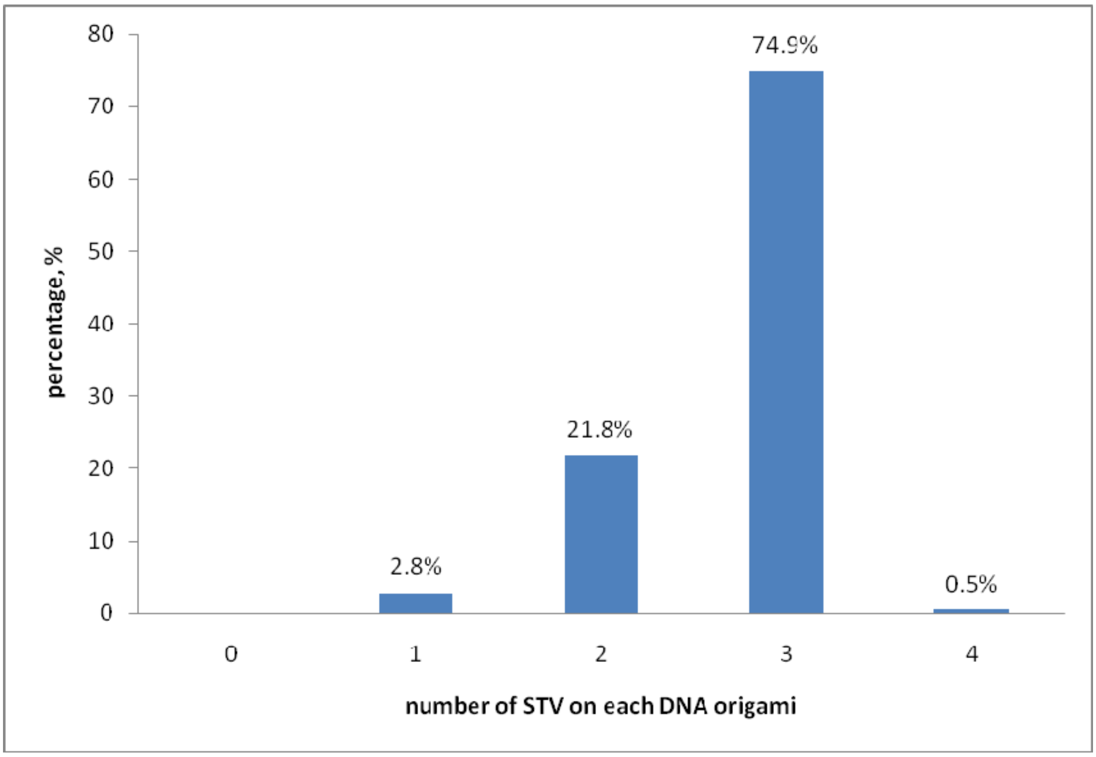




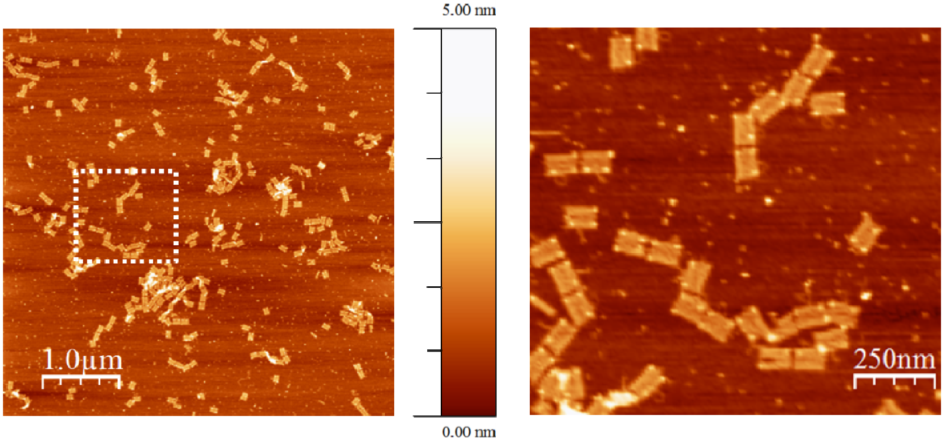
5. Height of STV on DNA origami vs. height of DNA origami



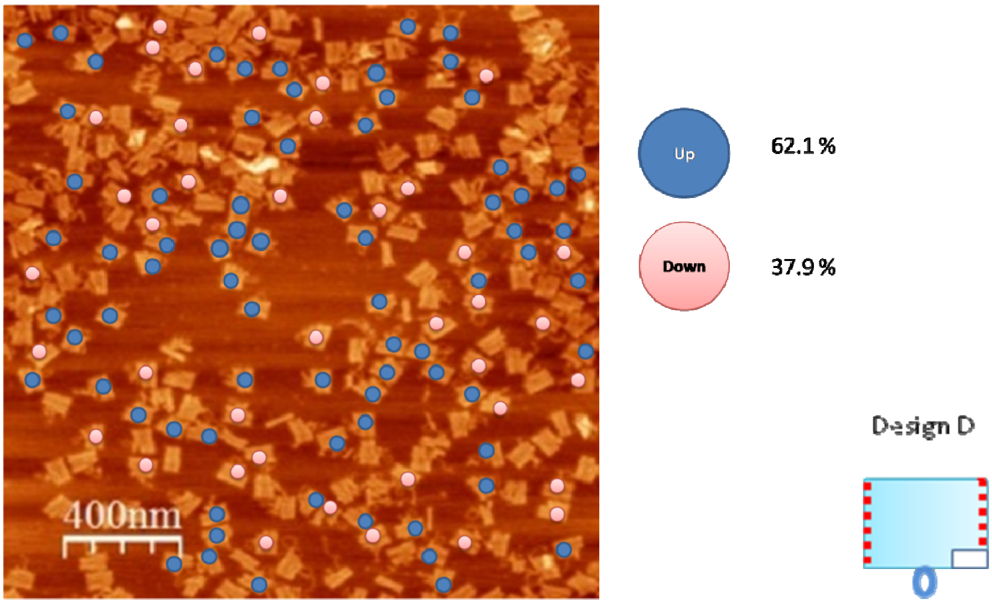
6. Streptavidin occupancy on Design ESTV



7. AFM images of streptavidin modified Design E4; low magnification image is on the left and boxed area is zoomed in on the right.



8. Design D _ up and down configuration



9. References

1. P. W. K. Rothemund, *Nature*, 2006, **440**, 297-302.