

• SUPPORTING INFORMATION •

Preservation of DNA nanostructure carriers: Effects of freeze-thawing and ionic strength during lyophilization and storage

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Materials and Methods

Preparation of DNA nanostructures. All the oligonucleotides were obtained from Jie Li Biology and quantified with a U-3010 spectrophotometer (HITACHI). TDNs were constructed following a previously described method¹. Briefly, four oligonucleotides (sequences are listed in Table S1, Supporting Information) in equimolar quantities (100 nM each) were mixed in TM buffer (10 mM Tris, 5 mM MgCl₂, pH 8.0, all reagents were purchased from Sinopharm Chemical Reagent), heated to 95 °C for 2 min and then cooled to 4 °C in 30 sec with a Veriti™ 96-well thermal cycler (Applied Biosystems). DNA origamis were prepared following a protocol described by Rothmund *et al.*² Briefly, in a one-pot construction of DNA origami, 5 nM of M13mp18 DNA (New England Biolabs) was mixed with 50 nM of staple oligonucleotides (Supporting Information, Table S2 set for triangular origami and S3 set for rectangular origami) which were slowly annealed from 95 to 20 °C in a 1×TAE/Mg²⁺ buffer (Tris, 40 mM; acetic acid, 20 mM; EDTA, 2 mM; and magnesium acetate, 12.5 mM; pH 8.0). The prepared DNA origami was then filtered with 100 kDa (MWCO) centrifuge filters four times to remove unbound DNA strands and exchange buffer with different Mg²⁺ concentrations.

Lyophilization of DNA nanostructures. 1mL of DNA nanostructure solution was placed in a glass ampule and frozen with an ALPHA 2-4 freeze-drier (Christ) to -80 °C for 12 hours. The ampule containing the frozen sample was put into a vacuum freeze-drying machine, which was cooled to -50 °C beforehand, and kept vacuum at this temperature overnight, to lyophilize DNA nanostructures into dry powder. The lyophilized samples for accelerated aging tests were then incubated at 60 °C in a laboratory incubator (SHEL LAB).

Gel electrophoresis of DNA nanostructures. For TDNs, native polyacrylamide gel electrophoresis (native PAGE) was employed. TDNs (30 ng/well) mixed with loading buffer (TAKARA) were ~~pipet-~~ change into “pipeted to” ~~ted to~~ freshly prepared 8% native polyacrylamide gel. Then, the electrophoresis was performed with an ESP 100 electrophoresis apparatus (Tanon) at 100 V constant voltage for 60 min in 1×TAE buffer in ice bathe. 20 bp DNA ladder (TAKARA) was used as the marker. For DNA origamis, DNA origamis (30 ng/well) mixed with loading buffer were pipetted to the 0.5 % agarose gels and the electrophoresis was run at 100 V in 1×TAE buffer for 60 min in ice bathe. After electrophoresis, the gels were stained in Gel-Red solution and the electrophoresis images were taken with a G:BOX gel imaging system (Syngene). The yields of certain products were quantified from the intensities of corresponding bands on the gels using ImageJ.

AFM imaging of DNA origami structures. The AFM samples were prepared by depositing 10 µL of 2 nM DNA origami on a mica substrate. After 3 min, the remaining solution was removed from the substrate using a pipette. Subsequently, the substrate was washed with water to remove the impurities and blasted with an ear washing ball for drying the mica surface. AFM studies were conducted using a Nanoscope V Multimode 8 (Bruker), operated at Peak Force QNM mode in liquid.

HPLC analysis of TDNs. A SEC column (Phenomenex Yarra™ 5 µm SEC-4000, 300×21.2 mm) was used and all chromatograms were recorded at 260 nm. A typical SEC mobile phase was 30 mM Tris-HCl, pH 7.4, 450 mM NaCl. 100 µL of TDNs were loaded and analyzed at a flow rate of 1 mL/min with isocratic elution.

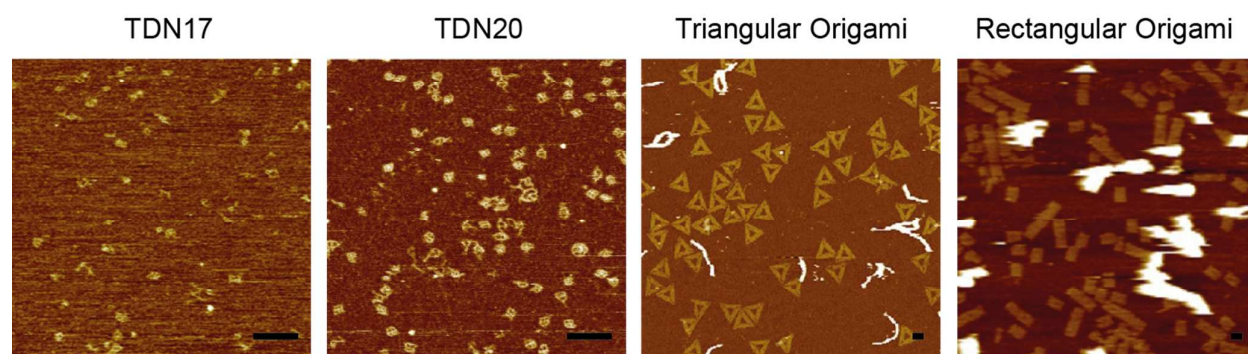


Figure S1. AFM images of lyophilized TDN17, TDN20, Triangular origami and Rectangular origami. Scale bars: 50 nm.

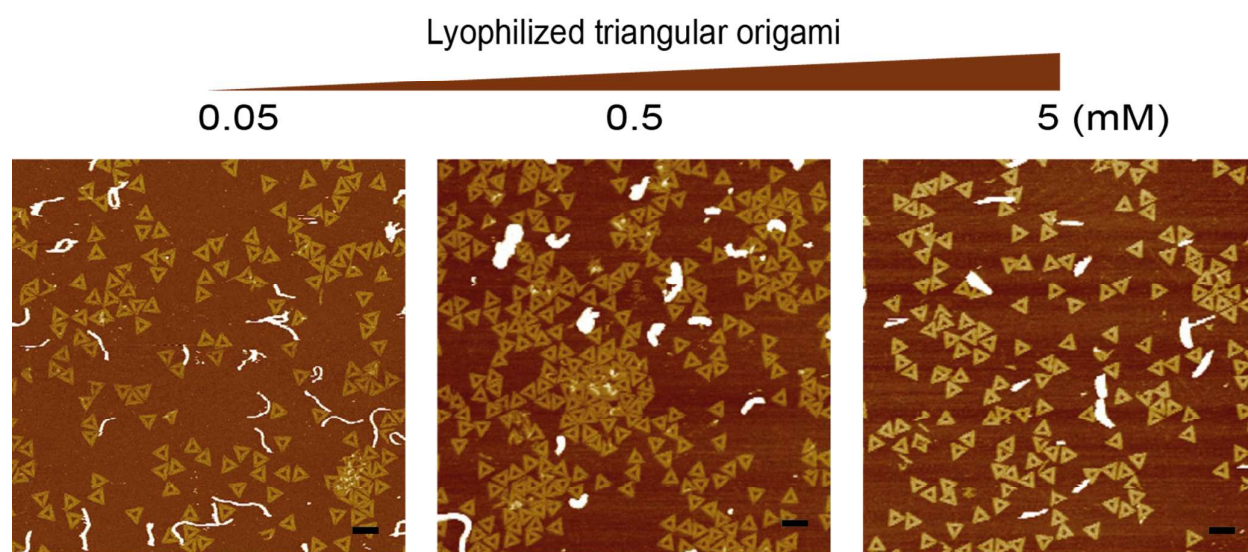


Figure S2. Large view AFM images of lyophilized **triangular** DNA origami structures from solutions within a range of Mg^{2+} concentrations (0.05 to 5 mM). Scale bars: 200 nm.

Change into "triangular"

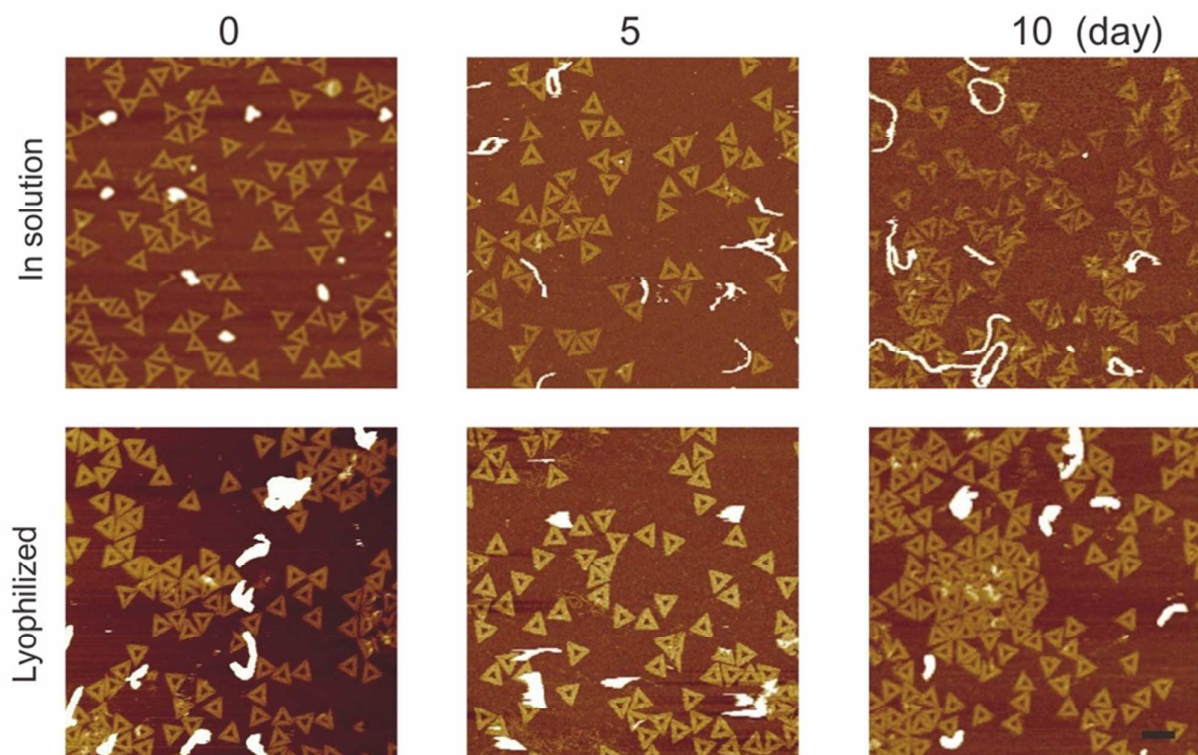


Figure S3. Large view AFM images of lyophilized triangular DNA origami structures treated with accelerated aging tests. Scale bars: 200 nm.

DNA Sequences used in this work:

Table S1. DNA sequences of TDNs

DNA		Sequence
TDN17	A	ACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGC CGCCATAGTA
	B	TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCGAG GGTCCAATAC
	C	TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCTACTATG GCGGCTCTTC
	D	TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCTGTTTGTATTGGA CCCTCGCAT
TDN20	S1	CAGTTGAGACGAACATTCCTAAGTCTGAAATTTATCACCCGCCAT AGTAGACGTATCA CCAGG
	S2	CTTGCTACACGATTCAGACTTAGGAATGTTTCGACATGCGAGGGTC CAATACCGACGATTACAG
	S3	GGTGATAAAACGTGTAGCAAGCTGTAATCGACGGGAAGAGCATG CCCATCCACTACTATGGCG
	S4	CCTCGCATGACTCAACTGCCTGGTGATACGAGGATGGGCATGCTC TTCCCGACGGTATTGGAC

Sequence of the M13mp18 Single-stranded DNA (from New England Biolabs) can be found at <https://www.neb.com/~media/NebUs/Page Images/Tools and Resources/Interactive Tools/DNA Sequences and Maps/Text Documents/m13mp18fsa.txt>.

Table S2. Staple sequences for the triangular DNA origami:

A01	CGGGGTTTCCTCAAGAGAAGGATTTTGAATTA
A02	AGCGTCATGTCTCTGAATTTACCGACTACCTT
A03	TTCATAATCCCCTTATTAGCGTTTTTCTTACC
A04	ATGGTTTATGTCACAATCAATAGATATTAAAC
A05	TTTGATGATTAAGAGGCTGAGACTTGCTCAGTACCAGGCG
A06	CCGGAACCCAGAATGGAAAGCGCAACATGGCT
A07	AAAGACAACATTTTCGGTCATAGCCAAAATCA
A08	GACGGGAGAATTAACTCGGAATAAGTTTATTTCCAGCGCC
A09	GATAAGTGCCGTCGAGCTGAAACATGAAAGTATACAGGAG
A10	TGTACTGGAAATCCTCATTAAGCAGAGCCAC
A11	CACCGGAAAGCGCGTTTTTCATCGGAAGGGCGA
A12	CATTCAACAAACGCAAAGACACCAGAACACCCTGAACAAA
A13	TTTAACGGTTCGGAACCTATTATTAGGGTTGATATAAGTA
A14	CTCAGAGCATATTCACAAACAAATTAATAAGT
A15	GGAGGGAATTTAGCGTCAGACTGTCCGCCTCC
A16	GTCAGAGGGTAATTGATGGCAACATATAAAAGCGATTGAG
A17	TAGCCCGGAATAGGTGAATGCCCCCTGCCTATGGTCAGTG

A18	CCTTGAGTCAGACGATTGGCCTTGCGCCACCC
A19	TCAGAACCCAGAATCAAGTTTGCCGGTAAATA
A20	TTGACGGAAATACATACATAAAGGGCGCTAATATCAGAGA
A21	CAGAGCCAGGAGGTTGAGGCAGGTAACAGTGCCCG
A22	ATTAAAGGCCGTAATCAGTAGCGAGCCACCCT
A23	GATAACCCACAAGAATGTTAGCAAACGTAGAAAATTATTC
A24	GCCGCCAGCATTGACACCACCCTC
A25	AGAGCCGCACCATCGATAGCAGCATGAATTAT
A26	CACCGTCACCTTATTACGCAGTATTGAGTTAAGCCCAATA
A27	AGCCATTTAAACGTCACCAATGAACACCAGAACCA
A28	ATAAGAGCAAGAAACATGGCATGATTAAGACTCCGACTTG
A29	CCATTAGCAAGGCCGGGGGAATTA
A30	GAGCCAGCGAATACCCAAAAGAACATGAAATAGCAATAGC
A33	CCTTTTTTCATTTAACAATTTTCATAGGATTAG
A34	TTTAACCTATCATAGGTCTGAGAGTTCCAGTA
A35	AGTATAAAATATGCGTTATACAAAGCCATCTT
A36	CAAGTACCTCATTCCAAGAACGGGAAATTCAT
A37	AGAGAATAACATAAAAAACAGGGAAGCGCATT
A38	AAAACAAAATTAATTAAATGGAAACAGTACATTAGTGAAT
A39	TTATCAAACCGGCTTAGGTTGGGTAAGCCTGT
A40	TTAGTATCGCCAACGCTCAACAGTCGGCTGTC
A41	TTTCCTTAGCACTCATCGAGAACATAGCAGCCTTTACAG
A42	AGAGTCAAAAATCAATATATGTGATGAAACAAACATCAAG
A43	ACTAGAAATATATAACTATATGTACGCTGAGA
A44	TCAATAATAGGGCTTAATTGAGAATCATAATT
A45	AACGTCAAAAATGAAAAGCAAGCCGTTTTTATGAAACCAA
A46	GAGCAAAAGAAGATGAGTGAATAACCTTGCTTATAGCTTA
A47	GATTAAGAAATGCTGATGCAAATCAGAATAAA
A48	CACCGGAATCGCCATATTTAACAAAATTTACG
A49	AGCATGTATTTTCATCGTAGGAATCAAACGATTTTTTGT
A50	ACATAGCGCTGTAAATCGTCGCTATTCATTTCAATTACCT
A51	GTAAATACAATCGCAAGACAAAGCCTTGAAA
A52	CCCATCCTCGCCAACATGTAATTTAATAAGGC
A53	TCCCAATCCAAATAAGATTACCGCGCCCAATAAATAATAT
A54	TCCCTTAGAATAACGCGAGAAAACCTTTTACCGACC
A55	GTGTGATAAGGCAGAGGCATTTTCAGTCCTGA
A56	ACAAGAAAGCAAGCAAATCAGATAACAGCCATATTATTTA
A57	GTTTGAAATTCAAATATATTTTAG
A58	AATAGATAGAGCCAGTAATAAGAGATTTAATG
A59	GCCAGTTACAAAATAATAGAAGGCTTATCCGGTTATCAAC
A60	TTCTGACCTAAAATATAAAGTACCGACTGCAGAAC

A61	GCGCCTGTTATTCTAAGAACGCGATTCCAGAGCCTAATTT
A62	TCAGCTAAAAAAGGTAAAGTAATT
A63	ACGCTAACGAGCGTCTGGCGTTTTAGCGAACCCAACATGT
A65	TGCTATTTTGCACCCAGCTACAATTTTGTGTTTGAAGCCTTAAA
B01	TCATATGTGTAATCGTAAAACTAGTCATTTTC
B02	GTGAGAAAATGTGTAGGTAAAGATACAACCTT
B03	GGCATCAAATTTGGGGCGCGAGCTAGTTAAAG
B04	TTCGAGCTAAGACTTCAAATATCGGGAACGAG
B05	ACAGTCAAAGAGAATCGATGAACGACCCCGGTTGATAATC
B06	ATAGTAGTATGCAATGCCTGAGTAGGCCGGAG
B07	AACCAGACGTTTAGCTATATTTTCTTCTACTA
B08	GAATACCACATTCAACTTAAGAGGAAGCCCGATCAAAGCG
B09	AGAAAAGCCCCAAAAAGAGTCTGGAGCAAACAATCACCAT
B10	CAATATGACCCTCATATATTTTAAAGCATTA
B11	CATCCAATAAATGGTCAATAACCTCGGAAGCA
B12	AACTCCAAGATTGCATCAAAAAGATAATGCAGATACATAA
B13	CGTTCTAGTCAGGTCATTGCCTGACAGGAAGATTGTATAA
B14	CAGGCAAGATAAAAAATTTTAGAATATTCAAC
B15	GATTAGAGATTAGATACATTTTCGCAAATCATA
B16	CGCCAAAAGGAATTACAGTCAGAAGCAAAGCGCAGGTCAG
B17	GCAAATATTTAAATTGAGATCTACAAAGGCTACTGATAAA
B18	TTAATGCCTTATTTCAACGCAAGGGCAAAGAA
B19	TTAGCAAATAGATTTAGTTTGACCAGTACCTT
B20	TAATTGCTTTACCCTGACTATTATGAGGCATAGTAAGAGC
B21	ATAAAGCCTTTGCGGGAGAAGCCTGGAGAGGGTAG
B22	TAAGAGGTCAATTCTGCGAACGAGATTAAGCA
B23	AACACTATCATAACCCATCAAAAATCAGGTCTCCTTTTGA
B24	ATGACCCTGTAATACTTCAGAGCA
B25	TAAAGCTATATAACAGTTGATTCCCATTTTGT
B26	CGGATGGCACGAGAATGACCATAATCGTTTACCAGACGAC
B27	TAATTGCTTGGAAGTTTCATTCCAAATCGGTTGTA
B28	GATAAAAACCAAAATATTAAACAGTTCAGAAATTAGAGCT
B29	ACTAAAGTACGGTGTCGAATATAA
B30	TGCTGTAGATCCCCCTCAAATGCTGCGAGAGGCTTTTGCA
B31	AAAGAAGTTTTGCCAGCATAAATATTCATTGACTCAACATGTT
B32	AATACTGCGGAATCGTAGGGGGTAATAGTAAAATGTTTAGACT
B33	AGGGATAGCTCAGAGCCACCACCCCATGTCAA
B34	CAACAGTTTATGGGATTTTGCTAATCAAAAGG
B35	GCCGCTTTGCTGAGGCTTGCAGGGGAAAAGGT
B36	GCGCAGACTCCATGTTACTTAGCCCGTTTTAA
B37	ACAGGTAGAAAGATTCATCAGTTGAGATTTAG

B38	CCTCAGAACCGCCACCCAAGCCCAATAGGAACGTAAATGA
B39	ATTTTCTGTCAGCGGAGTGAGAATACCGATAT
B40	ATTCGGTCTGCGGGATCGTCACCCGAAATCCG
B41	CGACCTGCGGTCAATCATAAGGGAACGGAACAACATTATT
B42	AGACGTTACCATGTACCGTAACACCCCTCAGAACCGCCAC
B43	CACGCATAAGAAAGGAACAACCTAAGTCTTTCC
B44	ATTGTGTCTCAGCAGCGAAAGACACCATCGCC
B45	TTAATAAAACGAACCTAACCGAACTGACCAACTCCTGATAA
B46	AGGTTTAGTACCGCCATGAGTTTCGTCACCAGGATCTAAA
B47	GTTTTGTGAGGAATTGCGAATAATCCGACAAT
B48	GACAACAAGCATCGGAACGAGGGTGAGATTG
B49	TATCATCGTTGAAAGAGGACAGATGGAAGAAAAATCTACG
B50	AGCGTAACTACAACTACAACGCCTATCACCGTACTCAGG
B51	TAGTTGCGAATTTTTTCACGTTGATCATAGTT
B52	GTACAACGAGCAACGGCTACAGAGGATACCGA
B53	ACCAGTCAGGACGTTGGAACGGTGTACAGACCGAAACAAA
B54	ACAGACAGCCCAAATCTCCAAAAAAAATTTCTTA
B55	AACAGCTTGCTTTGAGGACTAAAGCGATTATA
B56	CCAAGCGCAGGCGCATAGGCTGGCAGAACTGGCTCATTAT
B57	CGAGGTGAGGCTCCAAAAGGAGCC
B58	ACCCCCAGACTTTTTTCATGAGGAACTTGCTTT
B59	ACCTTATGCGATTTTATGACCTTCATCAAGAGCATCTTTG
B60	CGGTTTATCAGGTTTCCATTAAACGGGAATACACT
B61	AAAACACTTAATCTTGACAAGAAGCTTAATCATTGTGAATT
B62	GGCAAAAGTAAAATACGTAATGCC
B63	TGGTTTAATTTCAACTCGGATATTCATTACCCACGAAAGA
C01	TCGGGAGATATACAGTAACAGTACAAATAATT
C02	CCTGATTAAAGGAGCGGAATTATCTCGGCCTC
C03	GCAAATCACCTCAATCAATATCTGCAGGTCGA
C04	CGACCAGTACATTGGCAGATTCACCTGATTGC
C05	TGGCAATTTTAAACGTCAGATGAAAACAATAACGGATTTCG
C06	AAGGAATTACAAAGAAACCACCAGTCAGATGA
C07	GGACATTCACCTCAAATATCAAACACAGTTGA
C08	TTGACGAGCACGTATACTGAAATGGATTATTTAATAAAAG
C09	CCTGATTGCTTTGAATTGCGTAGATTTTCAGGCATCAATA
C10	TAATCCTGATTATCATTTTGCGGAGAGGAAGG
C11	TTATCTAAAGCATCACCTTGCTGATGGCCAAC
C12	AGAGATAGTTTGACGCTCAATCGTACGTGCTTTCCTCGTT
C13	GATTATACACAGAAATAAAGAAATACCAAGTTACAAAATC
C14	TAGGAGCATAAAAGTTTGAGTAACATTGTTTG
C15	TGACCTGACAAATGAAAAATCTAAATATCTT

C16	AGAATCAGAGCGGGAGATGGAAATACCTACATAACCCCTC
C17	GCGCAGAGGCCGAATTAATTATTTGCACGTAAATTCTGAAT
C18	AATGGAAGCGAACGTTATTAATTTCTAACAAC
C19	TAATAGATCGCTGAGAGCCAGCAGAAGCGTAA
C20	GAATACGTAAACAGGAAAAACGCTCCTAAACAGGAGGCCGA
C21	TCAATAGATATTAAATCCTTTGCCGGTTAGAACCT
C22	CAATATTTGCCTGCAACAGTGCCATAGAGCCG
C23	TTAAAGGGATTTTAGATACCGCCAGCCATTGCGGCACAGA
C24	ACAATTCGACAACCTCGTAATACAT
C25	TTGAGGATGGTCAGTATTAACACCTTGAATGG
C26	CTATTAGTATATCCAGAACAATATCAGGAACGGTACGCCA
C27	CGCGAACTAAAACAGAGGTGAGGCTTAGAAGTATT
C28	GAATCCTGAGAAGTGTATCGGCCTTGCTGGTACTTTAATG
C29	ACCACCAGCAGAAGATGATAGCCC
C30	TAAAACATTAGAAGAAGTCAAACCTTTTATAATCAGTGAG
C32	TCTTTGATTAGTAATAGTCTGTCCATCACGCAAATTAACCGTT
C33	CGCGTCTGATAGGAACGCCATCAACTTTTACA
C34	AGGAAGATGGGGACGACGACAGTAATCATATT
C35	CTCTAGAGCAAGCTTGCATGCCTGGTCAGTTG
C36	CCTTCACCGTGAGACGGGCAACAGCAGTCACA
C37	CGAGAAAGGAAGGGAAGCGTACTATGGTTGCT
C38	GCTCATTTTTTAACCAGCCTTCCTGTAGCCAGGCATCTGC
C39	CAGTTTGACGCACTCCAGCCAGCTAAACGACG
C40	GCCAGTGCGATCCCCGGGTACCGAGTTTTTCT
C41	TTTCACCAGCCTGGCCCTGAGAGAAAGCCGGCGAACGTGG
C42	GTAACCGTCTTTCATCAACATTAAAATTTTTGTAAATCA
C43	ACGTTGTATTCCGGCACCGCTTCTGGCGCATC
C44	CCAGGGTGGCTCGAATTCGTAATCCAGTCACG
C45	TAGAGCTTGACGGGGAGTTGCAGCAAGCGGTCATTGGGGC
C46	GTTAAAATTCGCATTAATGTGAGCGAGTAACACACGTTGG
C47	TGTAGATGGGTGCCGGAACACAGGAACGCCAG
C48	GGTTTTCCATGGTCATAGCTGTTTGAGAGGCG
C49	GTTTGCGTCACGCTGGTTTGCCCCAAGGGAGCCCCCGATT
C50	GGATAGGTACCCGTCGGATTCTCCTAAACGTTAATATTTT
C51	AGTTGGGTCAAAGCGCCATTGCCCCGTAATG
C52	CGCGCGGGCCTGTGTGAAATTGTTGGCGATT
C53	CTAAATCGGAACCCTAAGCAGGCGAAAATCCTTCGGCCAA
C54	CGGCGGATTGAATTCAGGCTGCGCAACGGGGGATG
C55	TGCTGCAAATCCGCTCACAATCCCAGCTGCA
C56	TTAATGAAGTTTGATGGTGGTTCCGAGGTGCCGTAAAGCA
C57	TGGCGAAATGTTGGGAAGGGCGAT

C58	TGTCGTGCACACAACATACGAGCCACGCCAGC
C59	CAAGTTTTTTGGGGTCGAAATCGGCAAAATCCGGGAAACC
C60	TCTTCGCTATTGGAAGCATAAAGTGTATGCCCCGCT
C61	TTCCAGTCCTTATAAATCAAAAGAGAACCATCACCCAAAT
C62	GCGCTCACAAGCCTGGGGTGCCTA
C63	CGATGGCCCCACTACGTATAGCCCGAGATAGGGATTGCGTT
C64	AACTCACATTATTGAGTGTTGTTCCAGAAACCGTCTATCAGGG
Link-A1C	TTAATTAATTTTTTACCATATCAAA
Link-A2C	TTAATTTTCATCTTAGACTTTACAA
Link-A3C	CTGTCCAGACGTATACCGAACGA
Link-A4C	TCAAGATTAGTGTAGCAATACT
Link-B1A	TGTAGCATTCTTTTTATAAACAGTT
Link-B2A	TTTAATTGTATTTCCACCAGAGCC
Link-B3A	ACTACGAAGGCTTAGCACCATTA
Link-B4A	ATAAGGCTTGCAACAAAGTTAC
Link-C1B	GTGGGAACAAATTTCTATTTTTGAG
Link-C2B	CGGTGCGGGCCTTCCAAAAACATT
Link-C3B	ATGAGTGAGCTTTTAAATATGCA
Link-C4B	ACTATTAAAGAGGATAGCGTCC
Loop	GCGCTTAATGCGCCGCTACAGGGC

Table S3. Staple sequences for the rectangular DNA origami:

1	CAAGCCCAATAGGAAC CCATGTACAAACAGTT
2	AATGCCCCGTAACAGT GCCCGTATCTCCCTCA
3	TGCCTTGACTGCCTAT TTCGGAACAGGGATAG
4	GAGCCGCCCCACCACC GGAACCGCGACGGAAA
5	AACCAGAGACCCTCAG AACCGCCAGGGGTCAG
6	TTATTCATAGGGAAGG TAAATATT CATTCACT
7	CATAACCCGAGGCATA GTAAGAGC TTTTAAAG
8	ATTGAGGGTAAAGGTG AATTATCAATCACCGG
9	AAAAGTAATATCTTAC CGAAGCCCTTCCAGAG
10	GCAATAGCGCAGATAG CCGAACAATTCAACCG
11	CCTAATTTACGCTAAC GAGCGTCTAATCAATA
12	TCTTACCAGCCAGTTA CAAAATAAATGAAATA
13	ATCGGCTGCGAGCATG TAGAAACCTATCATAT
14	CTAATTTATCTTTCCT TATCATTCATCCTGAA
15	GCGTTATAGAAAAAGC CTGTTTAG AAGGCCGG
16	GCTCATTTTCGCATTA AATTTTTG AGCTTAGA
17	AATTACTACAAATTCT TACCAGTAATCCCATC
18	TTAAGACGTTGAAAAC ATAGCGATAACAGTAC
19	TAGAATCCCTGAGAAG AGTCAATAGGAATCAT

20	CTTTTACACAGATGAA TATACAGTAAACAATT
21	TTTAACGTTTCGGGAGA AACAATAATTTCCCT
22	CGACAACCTAAGTATTA GACTTTACAATACCGA
23	GGATTTAGCGTATTAA ATCCTTTGTTTTTCAGG
24	ACGAACCAAAACATCG CCATTAAA TGGTGGTT
25	GAACGTGGCGAGAAAG GAAGGGAA CAAACTAT
26	TAGCCCTACCAGCAGA AGATAAAAACATTTGA
27	CGGCCTTGCTGGTAAT ATCCAGAACGAACTGA
28	CTCAGAGCCACCACCC TCATTTTCCTATTATT
29	CTGAAACAGGTAATAA GTTTTAACCCCTCAGA
30	AGTGTACTTGAAAGTA TTAAGAGGCCGCCACC
31	GCCACCACTCTTTTCA TAATCAAACCGTCACC
32	GTTTGCCACCTCAGAG CCGCCACCGATACAGG
33	GACTTGAGAGACAAAA GGGCGACAAGTTACCA
34	AGCGCCAACCATTTGG GAATTAGATTATTAGC
35	GAAGGAAAATAAGAGC AAGAAACAACAGCCAT
36	GCCCAATACCGAGGAA ACGCAATAGGTTTACC
37	ATTATTTAACCCAGCT ACAATTTTCAAGAACG
38	TATTTTGCTCCCAATC CAAATAAGTGAGTTAA
39	GGTATTAAGAACAAGA AAAATAATTAAAGCCA
40	TAAGTCCTACCAAGTA CCGCACTCTTAGTTGC
41	ACGCTCAAAATAAGAA TAAACACCGTGAATTT
42	AGGCGTTACAGTAGGG CTTAATTGACAATAGA
43	ATCAAAATCGTCGCTA TTAATTAACGGATTCG
44	CTGTAAATCATAGGTC TGAGAGACGATAAATA
45	CCTGATTGAAAGAAAT TGCGTAGACCCGAACG
46	ACAGAAATCTTTGAAT ACCAAGTTCCTTGCTT
47	TTATTAATGCCGTCAA TAGATAATCAGAGGTG
48	AGATTAGATTTAAAAG TTTGAGTACACGTAAA
49	AGGCGGTCATTAGTCT TTAATGCGCAATATTA
50	GAATGGCTAGTATTAA CACCGCCTCAACTAAT
51	CCGCCAGCCATTGCAA CAGGAAAAATATTTT
52	CCCTCAGAACCGCCAC CCTCAGAAGTGGAGACT
53	CCTCAAGAATACATGG CTTTGTGATAGAACCAC
54	TAAGCGTCGAAGGATT AGGATTAGTACCGCCA
55	CACCAGAGTTCGGTCA TAGCCCCCGCCAGCAA
56	TCGGCATTCCGCCGCC AGCATTGACGTTCCAG
57	AATCACCAAATAGAAA ATTCATATATAACGGA
58	TCACAATCGTAGCACC ATTACCATCGTTTCA
59	ATACCCAAGATAACCC ACAAGAATAAACGATT
60	ATCAGAGAAAGAACTG GCATGATTTTATTTTG
61	TTTTGTTTAAGCCTTA AATCAAGAATCGAGAA

62	AGGTTTTGAACGTCAA AAATGAAAGCGCTAAT
63	CAAGCAAGACGCGCCT GTTTATCAAGAATCGC
64	AATGCAGACCGTTTTT ATTTTCATCTTGCGGG
65	CATATTTAGAAATACC GACCGTGTTACCTTTT
66	AATGGTTTACAACGCC AACATGTAGTTCAGCT
67	TAACCTCCATATGTGA GTGAATAAACAAAATC
68	AAATCAATGGCTTAGG TTGGGTACTAAATTT
69	GCGCAGAGATATCAAA ATTATTTGACATTATC
70	AACCTACCGCGAATTA TTCATTTCCAGTACAT
71	ATTTTGCGTCTTTAGG AGCACTAAGCAACAGT
72	CTAAAATAGAACAAAG AAACCACCAGGGTTAG
73	GCCACGCTATACGTGG CACAGACAACGCTCAT
74	GCGTAAGAGAGAGCCA GCAGCAAAAAGGTTAT
75	GGAAATACCTACATTT TGACGCTCACCTGAAA
76	TATCACCGTACTCAGG AGGTTTAGCGGGGTTT
77	TGCTCAGTCAGTCTCT GAATTTACCAGGAGGT
78	GGAAAGCGACCAGGCG GATAAGTGAATAGGTG
79	TGAGGCAGGCGTCAGA CTGTAGCGTAGCAAGG
80	TGCCTTTAGTCAGACG ATTGGCCTGCCAGAAT
81	CCGGAAACACACCACG GAATAAGTAAGACTCC
82	ACGCAAAGGTCACCAA TGAAACCAATCAAGTT
83	TTATTACGGTCAGAGG GTAATTGAATAGCAGC
84	TGAACAAACAGTATGT TAGCAAATAAAAGAA
85	CTTTACAGTTAGCGAA CCTCCCGACGTAGGAA
86	GAGGCGTTAGAGAATA ACATAAAAGAACACCC
87	TCATTACCCGACAATA AACAAACATATTTAGGC
88	CCAGACGAGCGCCCAA TAGCAAGCAAGAACGC
89	AGAGGCATAATTTTCAT CTTCTGACTATAACTA
90	TTTTAGTTTTTCGAGC CAGTAATAAATTCTGT
91	TATGTAAACCTTTTTT AATGGAAAAATTACCT
92	TTGAATTATGCTGATG CAAATCCACAAATATA
93	GAGCAAAAACCTTCTGA ATAATGGAAGAAGGAG
94	TGGATTATGAAGATGA TGAAACAAAATTTTCAT
95	CGGAATTATTGAAAGG AATTGAGGTGAAAAAT
96	ATCAACAGTCATCATA TTCCTGATTGATTGTT
97	CTAAAGCAAGATAGAA CCCTTCTGAATCGTCT
98	GCCAACAGTCACCTTG CTGAACCTGTTGGCAA
99	GAAATGGATTATTTAC ATTGGCAGACATTCTG
100	TTTT TATAAGTA TAGCCCGGCCGTCGAG
101	AGGGTTGA TTTT ATAAATCC TCATTAAATGATATTC
102	ACAAACAA TTTT AATCAGTA GCGACAGATCGATAGC
103	AGCACCGT TTTT TAAAGGTG GCAACATAGTAGAAAA

104	TACATACA TTTT GACGGGAG AATTAAC TACAGGGAA
105	GCGCATT A TTTT GCTTATCC GGTATTCTAAATCAGA
106	TATAGAAG TTTT CGACAAAA GGTAAGTAGAGAATA
107	TAAAGTAC TTTT CGCGAGAA AACTTTTTATCGCAAG
108	ACAAAGAA TTTT ATTAATTA CATTTAACACATCAAG
109	AAAACAAA TTTT TTCATCAA TATAATCCTATCAGAT
110	GATGGCAA TTTT AATCAATA TCTGGTCACAAATATC
111	AAACCCTC TTTT ACCAGTAA TAAAGGGATTACCA GTCACACG TTTT
112	CCGAAATCCGAAAATC CTGTTTGAAGCCGGAA
113	CCAGCAGGGGCAAAAT CCCTTATAAAGCCGGC
114	GCATAAAGTTCCACAC AACATACGAAGCGCCA
115	GCTCACAATGTAAAGC CTGGGGTGGGTTTGCC
116	TTCGCCATTGCCGGAA ACCAGGCATTAAATCA
117	GCTTCTGGTCAGGCTG CGCAACTGTGTTATCC
118	GTTAAAATTTTAACCA ATAGGAACCCGGCACC
119	AGACAGTCATTCAAAA GGGTGAGAAGCTATAT
120	AGGTAAAGAAATCACC ATCAATATAATATTTT
121	TTTCATTTGGTCAATA ACCTGTTTATATCGCG
122	TCGCAAATGGGGCGCG AGCTGAAATAATGTGT
123	TTTTAATTGCCCCGAAA GACTTCAAAACACTAT
124	AAGAGGAACGAGCTTC AAAGCGAAGATACATT
125	GGAATTACTCGTTTAC CAGACGACAAAAGATT
126	GAATAAGGACGTAACA AAGCTGCTCTAAAACA
127	CCAAATCACTTGCCCT GACGAGAACGCCAAAA
128	CTCATCTTGAGGCAAA AGAATACAGTGAATTT
129	AAACGAAATGACCCCC AGCGATTATTCATTAC
130	CTTAAACATCAGCTTG CTTTCGAGCGTAACAC
131	TCGGTTTAGCTTGATA CCGATAGTCCAACCTA
132	TGAGTTTCGTCACCAG TACAACTTAATTGTA
133	CCCCGATTTAGAGCTT GACGGGGAAATCAAAA
134	GAATAGCCGCAAGCGG TCCACGCTCCTAATGA
135	GAGTTGCACGAGATAG GGTTGAGTAAGGGAGC
136	GTGAGCTAGTTTCCTG TGTGAAATTTGGGAAG
137	TCATAGCTACTCACAT TAATTGCGCCCTGAGA
138	GGCGATCGCACTCCAG CCAGCTTTGCCATCAA
139	GAAGATCGGTGCGGGC CTCTTCGCAATCATGG
140	AAATAATTTTAAATTG TAAACGTTGATATTCA
141	GCAAATATCGCGTCTG GCCTTCCTGGCCTCAG
142	ACCGTTCTAAATGCAA TGCCTGAGAGGTGGCA
143	TATATTTTAGCTGATA AATTAATGTTGTATAA
144	TCAATTCCTTTAGTTT GACCATTACCAGACCG

145	CGAGTAGAACTAATAG TAGTAGCAAACCCTCA
146	GAAGCAAAAAAGCGGA TTGCATCAGATAAAAA
147	TCAGAAGCCTCCAACA GGTCAGGATCTGCGAA
148	CCAAAATATAATGCAG ATACATAAACACCAGA
149	CATTCAACGCGAGAGG CTTTTGCATATTATAG
150	ACGAGTAGTGACAAGA ACCGGATATACCAAGC
151	AGTAATCTTAAATTGG GCTTGAGAGAATACCA
152	GCGAAACATGCCACTA CGAAGGCATGCGCCGA
153	ATACGTAAAAGTACAA CGGAGATTTTCATCAAG
154	CAATGACACTCCAAAA GGAGCCTTACAACGCC
155	AAAAAAGGACAACCAT CGCCACGCGGGTAAA
156	TGTAGCATTCCACAGA CAGCCCTCATCTCCAA
157	GTAAAGCACTAAATCG GAACCCTAGTTGTTC
158	AGTTTGGAGCCCTTCA CCGCCTGGTTGCGCTC
159	AGCTGATTACAAGAGT CCACTATTGAGGTGCC
160	ACTGCCCCGCCGAGCTC GAATTCGTTATTACGC
161	CCCGGGTACTTTCCAG TCGGGAAACGGGCAAC
162	CAGCTGGCGGACGACG ACAGTATCGTAGCCAG
163	GTTTGAGGGAAAGGGG GATGTGCTAGAGGATC
164	CTTTCATCCCCAAAAA CAGGAAGACCGGAGAG
165	AGAAAAGCAACATTAA ATGTGAGCATCTGCCA
166	GGTAGCTAGGATAAAA ATTTTGTAGTTAACATC
167	CAACGCAATTTTGTAG AGATCTACTGATAATC
168	CAATAAATACAGTTGA TTCCCAATTTAGAGAG
169	TCCATATACATACAGG CAAGGCAACTTTATTT
170	TACCTTTAAGGTCTTT ACCCTGACAAAGAAGT
171	CAAAAATCATTGCTCC TTTTGATAAGTTTCAT
172	TTTGCCAGATCAGTTG AGATTTAGTGGTTTAA
173	AAAGATTCAGGGGGTA ATAGTAAACCATAAAT
174	TTTCAACTATAGGCTG GCTGACCTTGTATCAT
175	CCAGGCGCTTAATCAT TGTGAATTACAGGTAG
176	CGCCTGATGGAAGTTT CCATTAAACATAACCG
177	TTTCATGAAAATTGTG TCGAAATCTGTACAGA
178	ATATATTCTTTTTTCA CGTTGAAAATAGTTAG
179	AATAATAAGGTCGCTG AGGCTTGCAAAGACTT
180	CGTAACGATCTAAAGT TTTGTCGTGAATTGCG
181	ACCCAAATCAAGTTTT TTGGGGTCAAAGAACG
182	TGGACTCCCTTTTCAC CAGTGAGACCTGTCGT
183	TGGTTTTTAACGTCAA AGGGCGAAGAACCATC
184	GCCAGCTGCCTGCAGG TCGACTCTGCAAGGCG
185	CTTGCAATGATTAATG AATCGGCCCCGCCAGGG
186	ATTAAGTTCGCATCGT AACCGTGCGAGTAACA

187	TAGATGGGGGGTAACG CCAGGGTTGTGCCAAG
188	ACCCGTCGTCATATGT ACCCCGGTAAAGGCTA
189	CATGTCAAGATTCTCC GTGGGAACCGTTGGTG
190	TCAGGTCACTTTTGCG GGAGAAGCAGAATTAG
191	CTGTAATATTGCCTGA GAGTCTGGAAAAGTAG
192	CAAAATTAAAGTACGG TGTCTGGAAGAGGTCA
193	TGCAACTAAGCAATAA AGCCTCAGTTATGACC
194	TTTTTGCGCAGAAAAC GAGAATGAATGTTTAG
195	AAACAGTTGATGGCTT AGAGCTTATTTAAATA
196	ACTGGATAACGGAACA ACATTATTACCTTATG
197	ACGAACTAGCGTCCAA TACTGCGGAATGCTTT
198	CGATTTTAGAGGACAG ATGAACGGCGCGACCT
199	CTTTGAAAAGAACTGG CTCATTATTTAATAAA
200	GCTCCATGAGAGGCTT TGAGGACTAGGGAGTT
201	ACGGCTACTTACTTAG CCGGAACGCTGACCAA
202	AAAGGCCGAAAGGAAC AACTAAAGCTTTCCAG
203	GAGAATAGCTTTTGCG GGATCGTCGGGTAGCA
204	ACGTTAGTAAATGAAT TTTCTGTAAGCGGAGT
205	TTTT CGATGGCC CACTACGTAAACCGTC
206	TATCAGGG TTTT CGGTTTGC GTATTGGGAACGCGCG
207	GGGAGAGG TTTT TGTA AAC GACGGCCATTCCCAGT
208	CACGACGT TTTT GTAATGGG ATAGGTCAAACGCGCG
209	GATTGACC TTTT GATGAACG GTAATCGTAGCAAACA
210	AGAGAATC TTTT GGTTGTAC CAAAACAAGCATAAA
211	GCTAAATC TTTT CTGTAGCT CAACATGTATTGCTGA
212	ATATAATG TTTT CATTGAAT CCCCCTCAAATCGTCA
213	TAAATATT TTTT GGAAGAAA AATCTACGACCAGTCA
214	GGACGTTG TTTT TCATAAGG GAACCGAAAGGCGCAG
215	ACGGTCAA TTTT GACAGCAT CGGAACGAACCCTCAG
216	CAGCGAAAA TTTT ACTTTCA ACAGTTTCTGGGATTT
Loop1	AACATCACTTGCCTGAGTAGAAGAACT
Loop2	TGTAGCAATACTTCTTTGATTAGTAAT
Loop3	AGTCTGTCCATCACGCAAATTAACCGT
Loop4	ATAATCAGTGAGGCCACCGAGTAAAG
Loop5	ACGCCAGAATCCTGAGAAGTGTTTTT
Loop6	TTAAAGGGATTTTAGACAGGAACGGT
Loop7	AGAGCGGGAGCTAAACAGGAGGCCGA
Loop8	TATAACGTGCTTTCCTCGTTAGAATC
Loop9	GTACTATGGTTGCTTTGACGAGCACG
Loop10	GCGCTTAATGCGCCGCTACAGGGCGC

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