

Supporting information

DNA-assembled multilayer sliding nanosystems

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Materials and Methods

Design and self-assembly of the DNA origami structures

The DNA scaffold strands (p8064) were purchased from tilibit nanosystems. All other DNA strands were purchased from Sigma-Aldrich. The three-layer DNA origami structures were designed using caDNAno software¹. Five thymine bases were added to the respective staple strands at the edge of the origami to prevent non-specific aggregations. The DNA origami structures were prepared by mixing 15 nM scaffold strands with 10 times of the staple strands and capture strands in a buffer containing 0.5×TE (Tris, EDTA, pH = 8), 20 mM MgCl₂, and 5 mM NaCl. The mixture was then annealed as follows: 85°C for 5 min; from 65°C to 61°C, 1°C /5 min; from 60°C to 51°C, 1°C /90 min; from 51°C to 38°C, 1°C /20 min; from 37°C to 26°C, 1°C /10 min; held at 25°C. The annealed structures were purified using agarose gel to remove the excess staple and capture strands.

Preparation of the AuNPs

AuNPs (10 nm) were synthesized using a published method with minor modifications². A 1.25 mL HAuCl₄ solution (0.2%, w/v) was diluted in 25 mL double-distilled water and heated to boiling. A 1 mL sodium citrate solution (1%, w/v; containing 0.05% citric acid) was added to the flask under vigorous stirring. The solution in the flask was kept boiling for 5 min under stirring and then cooled down at room temperature. Bis(p-sulfonatophenyl)phenylphosphine dihydrate dipotassium salt (BSPP) (15 mg) was added to the AuNP solution (20 mL, OD ~ 1) and the mixture was shaken overnight at room temperature. The concentration of the AuNPs was estimated according to the optical absorption at 520 nm.

Functionalization of the AuNPs with DNA

The AuNP-DNA conjugation was carried out according to Ding *et al.* with minor modifications³. Thiol-modified DNA ([ThiolC6] TTTT GACTTACC) were reduced using tris(2-carboxyethyl)phosphine (TCEP) (100 mM, 1 h) in water. Thiol-modified

DNA and BSPP coated AuNPs were then incubated at a molar ratio of DNA to AuNPs of 300:1 in a 0.5×TBE buffer solution overnight at room temperature. The concentration of NaCl was slowly increased to 300 mM during the subsequent 20 h. The AuNP-DNA conjugates were then washed using a 0.5×TBE (tris-(hydroxymethyl)-aminomethan, borate, ethylenediaminetetraacetic acid) buffer solution with 300 mM NaCl in 100 kDa (MWCO) centrifuge filters to remove the free DNA. The concentration of the DNA modified AuNPs was measured by UV-vis absorption spectroscopy at 520 nm.

Self-assembly of the AuNPs with the DNA origami structures

For sliding system I, 10 times excess of the blocking strands 1,2,5,6 were added to the purified DNA origami and incubated at room temperature for 0.5 h to block the footholds 1,2,5, 6 and locking sites a, b (attachment of the AuNPs at positions 3 and 4). For sliding system II, 10 times excess of the blocking strands 3,4,5,6 were added to the purified DNA origami and incubated at room temperature for 0.5 h to block the footholds 3,4,5,6 (attachment of AuNPs at positions 1 and 2). The purified DNA-modified AuNPs were mixed with the DNA origami structures at a 10:1 molar ratio, and annealed by decreasing the temperature from 38 °C to 25 °C at a rate of 1 °C per 60 min. An agarose gel purification step (1% agarose gel in a 0.5×TBE buffer with 11 mM MgCl₂) was used to purify the successfully assembled product.

TEM characterization

The DNA origami structures were characterized using Philips CM 200 TEM operating at 200 kV. For imaging, the purified samples were deposited on freshly glow-discharged carbon/formvar TEM grids. Before depositing the sample solution, the grids were treated by negative glow discharge. After 10 min deposition, TEM grids were treated with a uranyl formate solution (2%) for negative staining of the DNA structures.

Fluorescence spectroscopy

Fluorescence spectra were measured using a Jasco-FP8500 Fluorescence Spectrometer with a quartz SUPRASIL ultra-micro cuvette (path length, 10 mm). All measurements were performed at room temperature in a buffer after agarose gel purification (0.5×TBE buffer with 11 mM MgCl₂, pH = 8). For the *in situ* fluorescence measurements, a 120 µL solution containing ~1 nM of the structures at the initial configuration was used. The fluorescence emissions at 578 nm and 663 nm were monitored using the dual-wavelength time-scan acquisition mode and a data pitch of 10 s. The excitation wavelengths were 550 nm and 647 nm for the ATTO 550 and ATTO 647N, respectively. Respective blocking and removal strands were added to enable the programmable sliding.

Numerical simulations

The finite-element simulations were performed using commercially available software COMSOL Multiphysics. The dielectric function of gold was interpolated from experimental data⁴. Water as the surrounding medium was taken into account with a refractive index of 1.332. The emission spectra of the fluorophores, as well as the

intrinsic quantum yields q_0 (0.8 for ATTO 550 and 0.65 for ATTO 647N) were considered as specified by the supplier of the molecules (available under <https://www.atto-tec.com>).

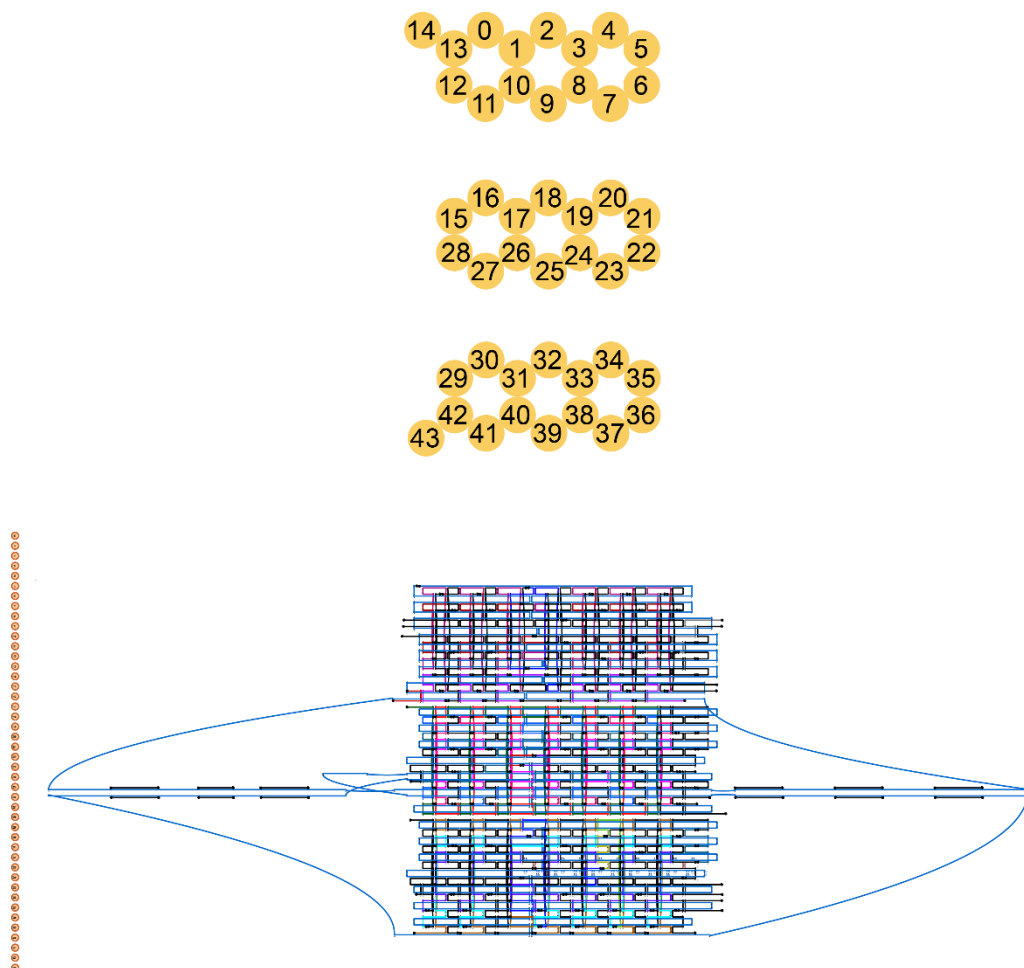


Figure S1. Routing schematic of the DNA origami.

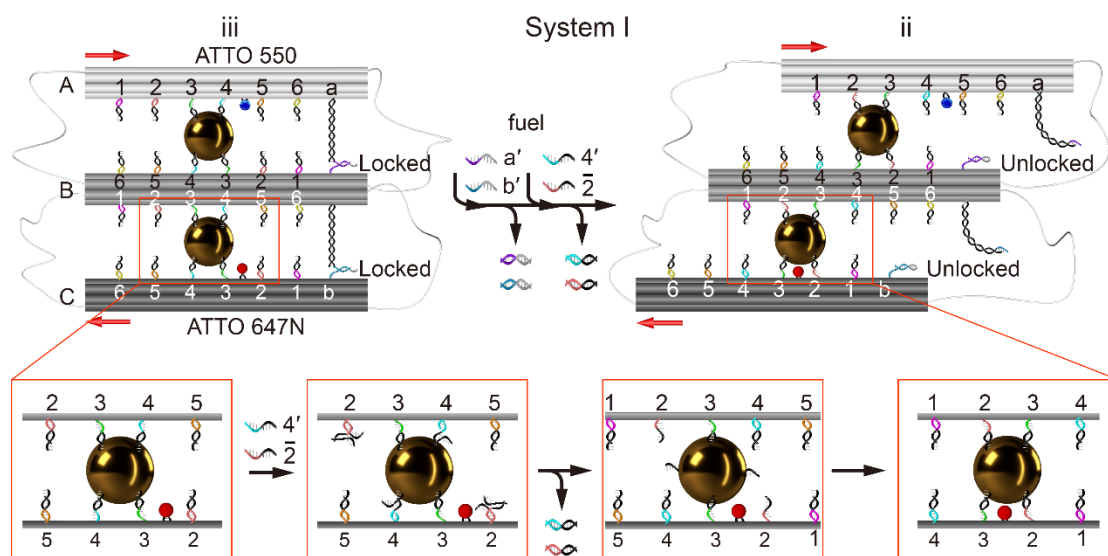
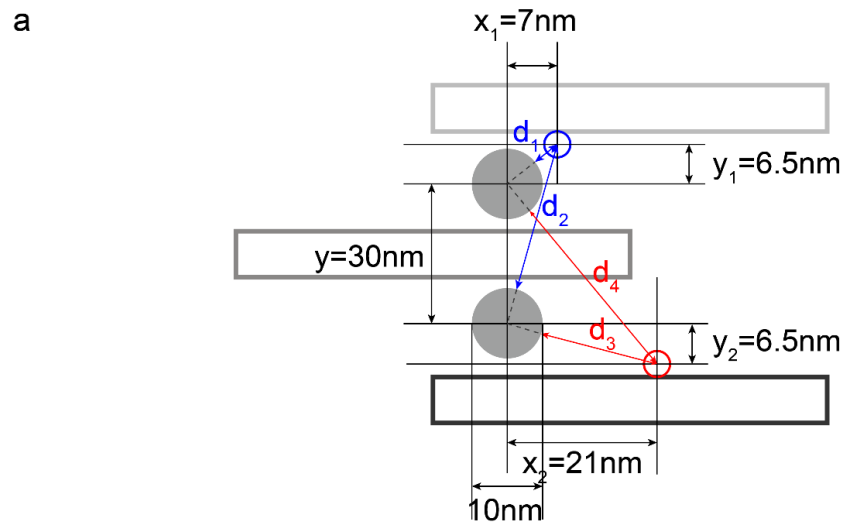


Figure S2. Schematic of the individual steps during sliding.



b

System I							System II				
		i	ii	iii	iv	v	i	ii	iii	iv	v
ATTO 550	d_1 (nm)	17	10.5	4.5	1.5	4.5	4.5	1.5	4.5	10.5	17
	d_2 (nm)	37	34	32	31.5	32	32	31.5	32	34	37
ATTO 647N	d_3 (nm)	4.5	1.5	4.5	10.5	17	17	10.5	4.5	1.5	4.5
	d_4 (nm)	32	31.5	32	34	37	37	34	32	31.5	32

Figure S3. (a) Schematic of the relative distances from the fluorophores to the AuNP surfaces. (b) Corresponding distances for systems I and II at different states used in the simulations.

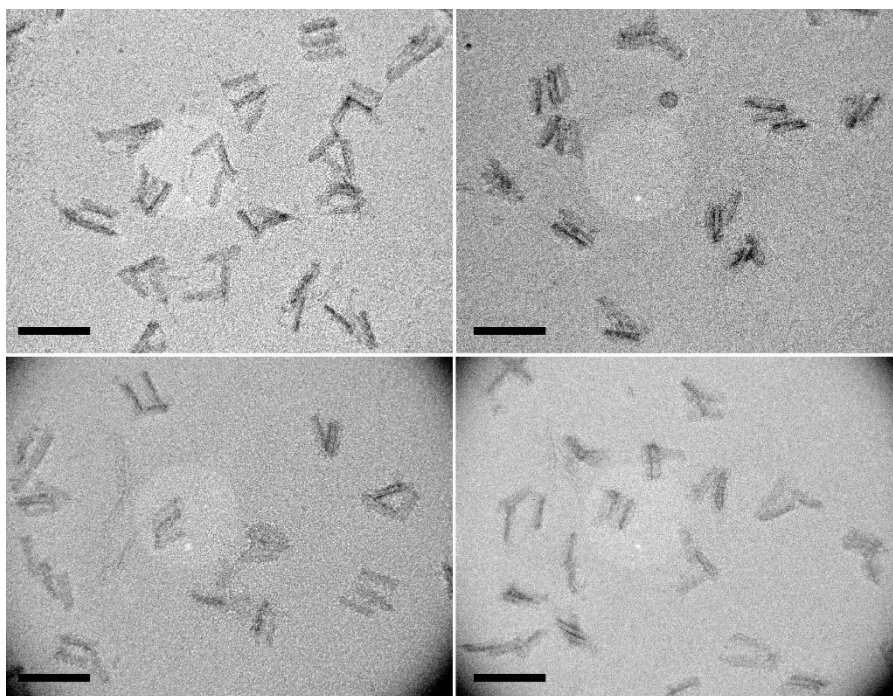


Figure S4. TEM images of the DNA origami filaments without AuNPs. Scale bar, 100 nm.

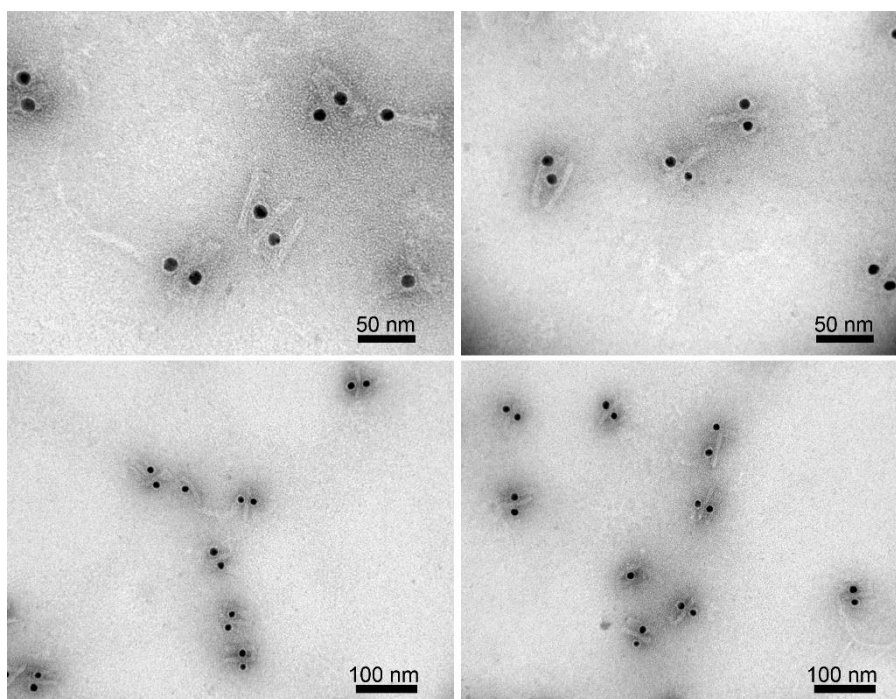


Figure S5. TEM images of the DNA origami-AuNP assemblies without the locking strands.

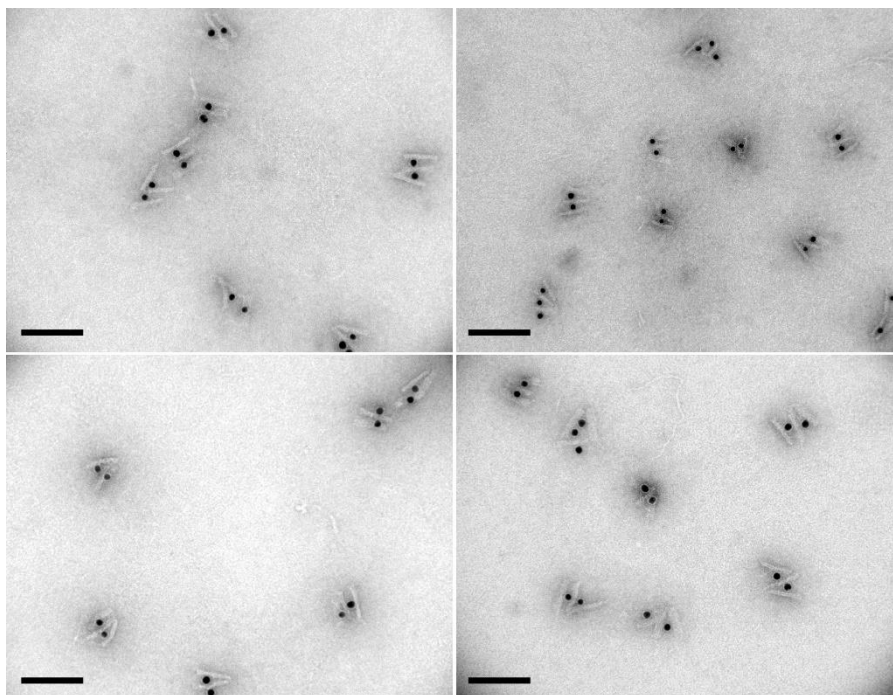


Figure S6. Additional TEM images of the DNA origami-AuNP assemblies with the locking strands. Scale bar, 100 nm.

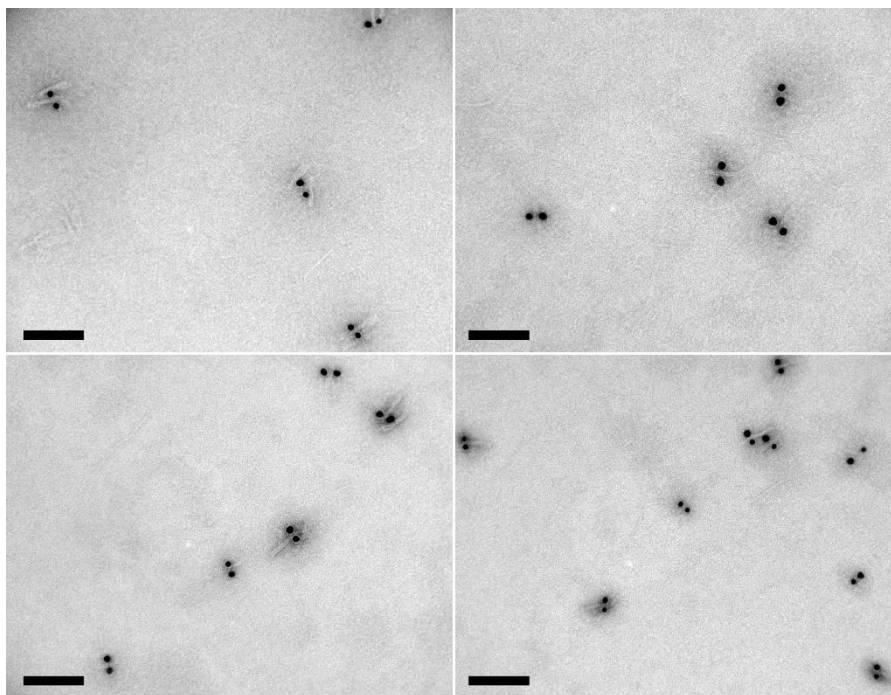


Figure S7. TEM images of system I before sliding. Scale bar, 100 nm.

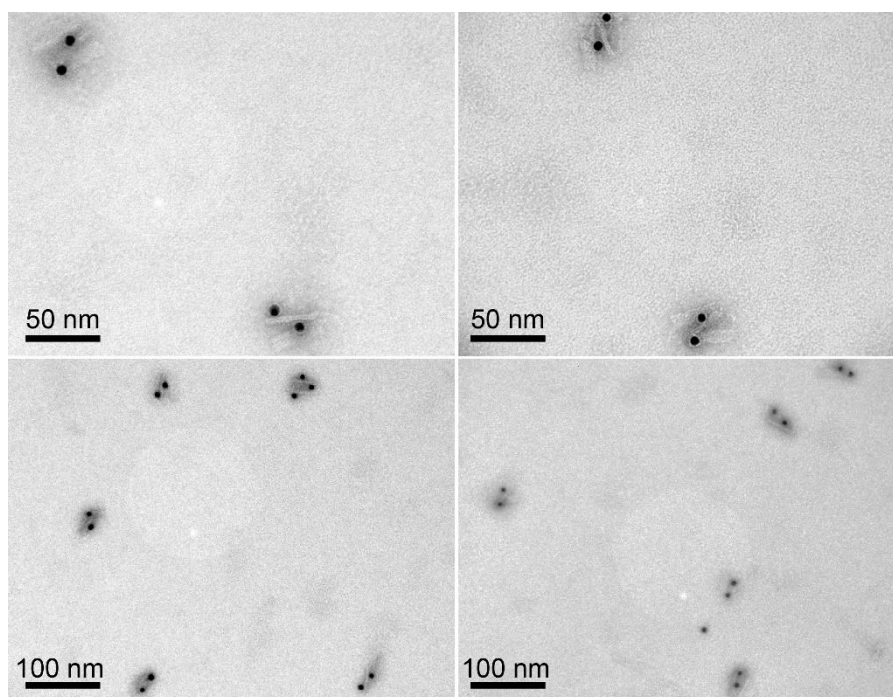


Figure S8. TEM images of system I after two sliding steps from state iii to i.

Table S1. Detailed sequences of the DNA origami sliding system.

Name	Sequence (5' - 3')
1[248]	CTGACCTTCTGGCCACGT
1[269]	ACGTGGCGACCAGTAAGT
1[332]	AAACAGCGTTCCGGCACC
1[353]	TTTCTGCAGTTAAATTGC
1[374]	CAGTTGGGCCTTTAGGTT
11[391]	CGGGTGCTGGACAGGCGGCGGTTGTGTACAT
12[315]	CCGATTGCAGGCAGCCTGGTCCGTAAAAAGACGTGGTG
13[248]	AATCAGGAAAGGGACATGAAAGCGCGAACCACCAGCAGACAC
13[269]	CAATACCTACGTCACACACAGACAATCGCCATTAAAAACGCT
13[290]	ACACTCGATTAAGTTGGAGAATGCGCGAACTGATAGCCGAAA
13[332]	CATGATTGCCGGATCAATTGTGAGAGATAGATCAC
13[353]	ACCAGACTTGTAGGGTAATCATTTCATGTTTACCAGTCGCCA
13[374]	TTTTTTTCTCACCGGAAACAATCGGCGAAACGTGTGG
13[403]	CAGTGTCTGTCGCCAGCACTGTTTGCGCCACGGGAACGGATAACTTTTTTT
14[221]	GAGGCGGTCGCAAATCAACAGTTGAA
14[252]	GAGCGGGGAACCATCACAAAGGGCCTGCAACCAATCAA
14[273]	CAAAGCCCCCTTGGGGTAAGAACGAGCCAGCGAACCTC
14[294]	AGGGGGATGTGCTGCAAGGAA
14[315]	ATTTAAAGCCGCCAGGGAGCTGATGGGTTGACAGGGTTTGGCGAA
14[336]	AGCGAGGCGGTGGCCCTTCAAAGAGTTGTAACTCTTC
14[357]	TTGGAATCGGAAGCGGTATCGGCACCAAGCTGGGAAGG
16[255]	AGATTTAGGAATACAAGGAATGCTT
16[276]	GTTGGGAAGAAGATTCAAGAGCAATAAA
16[318]	CCTGCCTGTTTGATACATTCCTTTTGAAG
16[339]	TGTTTGGGGCGTAGATTTTTCGGGGCTT
16[360]	AGATGGCATCGATTCCCAATTGCTAAAT
16[392]	CATAGCTCAAAGAGGAATGACTATTATAGTCAGAAGCACATCAAA
18[381]	CAATCATCCATCAAGGTAAAGATTCAAATAGCAAATAGTAGCGTTT
18[392]	AAGATTACATGTTTTAAATATTACGGTG
2[294]	CGCTCAATGCCTGAGGTCACGCTGCGCCACAAATC
2[308]	CTCACGGTTTTTCGGGGCAACTGGTTTT
20[255]	ATCATAAAAGAACCCTGCTCATTCAAGTGATCA
20[276]	CCAACCTTTTCATCACTTGCCCTGACGAGGATG
20[319]	CATTGCCGAGAGATACGCAAGGATAAAAGAC
20[339]	AAACAAGCCGGAGAGAACCCTCATATATCGGT
20[360]	GTAATCGGTTCTAGGCAATGCCTGAGTACCTC
20[392]	TCTGGAAATTAACATCCAATAGCAAGGC
21[300]	AGGCGCAAGAACGAGTGCCTT
22[294]	TATTTCACTACAAAGGAGACC
23[255]	AACTTTAAATAAGGAGAGTAATCTTGACGGGA
23[318]	ACATTATATTTTAGGGTAGCTATTTTTTGAG

23[339]	GCTAAATTTTAAATCTGATAAATTAATGAGAA
23[360]	AATAAAGATGTGTATATGATATTCAACCTAAA
23[381]	AAAGAATAGGGTGAAGACAGTCAAATCAATGT
23[391]	CGGATTGAGCCCCAAAAACAGCCGGTTG
24[231]	CCTTATGAACAAAGGGATATTCATTACCGGCGCAGGAACCTA
25[132]	GATTTAGAAGTATTAGACTTT
25[174]	AACTCGTATTAAATCCTTTGCCCGAACG
25[391]	GAATAGAAAGGAACAAACCCGT
25[440]	CGTGGGAACAAACGGCGGATTGACCGTA
25[496]	GCGCATCGTAACCGTGCATCTGCCAGTT
25[552]	GGAAGATCGCACTCCAGCCAGCTTTCCG
25[90]	AGCACTAACAATAATAGATTAGAGCCG
26[112]	TCAGATGAATATACAGTAACA
26[147]	AAAACAGAAATAAAGAAATTGCGTAGAT
26[413]	AACAACCTTTCAACAGTTTCAGCGGAGTG
26[469]	AGTTTTGTCTGCTTTCCAGACGTTAGTA
26[525]	ACTACAACGCCTGTAGCATTCCACAGAC
26[63]	ACAATAACGGATTGCGCTGATTGCTTTG
27[255]	ACCTTCTGAACCACCAGTCGCCTGGAGG
27[276]	GGTAGAAAAAATCTAATATAATCCTGATTCATCATGTACAACACAC
27[318]	AGTTTTTTGTCCATCAAGGAGCCTGGTG
27[339]	TCGCGTCTGGCCTTTCCAAAAGATA
27[360]	ACTTTAAATTTTCATCAAAATTTTAAATG
27[381]	ACCGAAGATTGAGTAACACTAAAGCGCA
27[392]	TTTTTTTCTGAGGCTTGCAGGGACCGATATA
3[255]	TCACGCAGCCACCGTGCTTTTCCTCGTTAGGCGCGTAAAG
3[276]	GCAATACAGTGTTTAGCGGGAGCTAAACGCGCTTACTGG
3[339]	GTTGCCCCCAGCGCGCCGTTTTCACGGTTGTGTGAAGTG
3[360]	GGTAAAGGTGCCCCGGGGTTTCTGCCAGATCATGGTGCG
3[381]	CATAAACTACCTGCGCCTGTTCTTCGCGGGTACCGTTTC
30[392]	AAGAAAATAGTCCCAATCCAAATAAGAAACGTAACGTC
31[381]	GTTTATTTTGTACAGCAACATTTTAAAGGAGC
32[256]	CGTGTGATAAAGCCCCATATTTAACAACCAATAAATTATCAACCA
32[276]	AATAAGATTATACATGTAATTTAGGCAGGTAATTCTGAACAACGGC
32[318]	GTAGCGCTCAAGTTGGTAAATATTGACGACCGATTTCATGATTATAA
32[392]	AAAAATGAAACAATGAAATAGCTTACCG
33[339]	TTTCGGTCCGTAATTTTCATTAAAGGTGAAAGACAACGCA
33[360]	TAGCGTTATGAAACCCGTCACCGACTTGTCATATGAAAA
34[392]	AAGCCCTATAAAAGAAACGCAGGAATAA
35[300]	GCCTGTTTTTCGAGCCAGTAAT
36[294]	AAGAGAATGCCTTTAGAAAAA
36[385]	AATCAAAGCAAGGCTGGGAATTAGAGCCAGCAAAACACCATT
37[244]	TAAACCACAGAACGAATTTAATACCGAC

37[265]	TATCATTCAATAGATTAATTTGGCGTTA
37[286]	CAATAATGAAAAATACTTTTTCCGGAAT
37[307]	GGAATATAAAGTACCATCCTAATTTAAACGCCAGCTCAGACT
37[328]	CGCAATAAAGACTCCAGAACCTCGGCAT
37[349]	ACCAGAAATGTTAGCCACCACCCCTTAT
37[370]	AGCAGATCATAATCAGAACCTTTTCAT
37[406]	TTTTTTTCGGAACCGCCTCCCTCGAACCAGAGCCACCCTTTTTT
38[234]	TTCAGCTAGAATCGAACGCTCAACAGTATTTGAAATGGT
38[255]	ACGACGAGCCAACAAATTCTTACCAGTATAAATAACATC
38[276]	AGGTAAAAGGCATTTAGTATCATATGCGATAAACACAAA
38[318]	ACATTCAGAAATTACAGTAGCGACAGAAGTTTCAACCA
38[339]	AGCGCCAATTATCACATCGATAGCAGCACATAGCCCTCA
38[360]	AGAAAATAGCCATTCGGAAACGTCACCATGCCATCGCCA
39[286]	GGCTTATTAGGAATAAGAGTCAATCCAA
39[406]	TTTTTTTTCCAGTAAGCGTCATATCTCTGAATTTACCGTTTTTTTT
4[227]	ATTGCAAATCCAGAGAAAGCGACTATGGTTGCTTTGACGATTTTTTT
4[266]	ATGGAAACTATCGTAGGGCGATGCGCC
4[287]	ATAACATCGGTACGCGATTAAAGGGATTAGCGTAACCACCAC
4[308]	GTCGCTGGCGCTTTTAAAGTGCCACACA
4[318]	ACATACGTTGTGGTGCTGCGGCCAGAATCACA
4[329]	GAGCACAGCATCAGCCTAATGAATTGTT
4[350]	CGTGCCGGCTTACGCATTAATTCATAGC
4[371]	CAGCGTGTGGTGCCCTGCCCCGAGCTCGA
40[241]	CTCCCGACCGCACTCTAC
40[262]	ACGCGAGAAGCCGTCAA
40[304]	AAAAGCAATAGCCAAAAC
40[325]	GAGAATTTTGAGCGCTTG
40[346]	ACAGGGAATAACCCAAGT
40[367]	TACAGAGAAGCCCAATAC
40[381]	ACCATTAATCACCGAGAGCCGAGCGCAGCATG
41[286]	TGTTTCATCGCCGGTATCTATTTGCACCCACTTTTTTACATAGC
42[241]	TTTTTTTTGCGGGAGGAATA
42[262]	AGAAAACAAGCGCGTTTTGCCTTAAATCAAGAATAA
42[325]	CGGAGGGTAAACTGAACGCTAACGAGCGTCATAG
42[346]	GTAGGAAACCCAGAGAGAGCGCATAGCCTAATTTGCCATTGA
42[367]	ATAAGCCGAATTGAGTTAGAATAAAATAAACAGCCATAGGAT
43[258]	TTCTATATAAATAGGTCAATTAATAATATATGATGAAA
43[279]	TATTGATGCAAATAGTGCTTGAAAAATGGAAACAAAAT
43[300]	TAATTACATTTAACAATTTGT
43[321]	CCACAGGTCAAACAGTGTCGGAACCTATACCAACCGCC
43[342]	GAGATATTCAAACGGGGCATGAAAAAGTATACACCCCTC
43[363]	CCCTCATTAAGTGTACAGACTCCTGCCGTCCATTTTC
43[391]	GCTAGGATTAGCTCAGTCAATAGGAACCCAT

5[406]	TGTGTTCTAACGGCGCCTCCTCATTTTTTTT
6[226]	TTTTTTTGCACGTATAACGAGTAAAAGATTTTTTTT
6[301]	TGCGGTACCTGTGCACTCTAG
7[318]	ATCCGCTGCGGGCGGAGTGTCAGTGCGCGTGAG
7[391]	AGCAGCATGCCAACGGCAGCAACACTGG
8[255]	GCTACAGGAATCAGTTATAATCAGTGAGAATTAAGTGGT
8[276]	ACCCGCCAGGAGGCCAGAAATCCTGAGATTCTTTGAACT
8[361]	ATTCGTACACGCGTAGCCAGCGGTGCCGGTTTCTTGCA
8[381]	TTTTTTTCAGTTGAGGATCCCCGTCCGTGAATCAGATGCCGGGTATCCCTTCCG T
9[339]	TGTTTCCCATAACCGCTGCATCAGACGATTGCGGCTGTGTCCATCCT
9[391]	CGACATAGTAAAAAAGCCGCTCTGGTC
42[304]	TAAGCTATCCCAAATCAGATTTTATCCTGAATCGGAG
0[297]	TAAGCTATCCATTGGCAGACCGTAAAGCACTCATGGA

Foothold strands

Name	Sequence (5' - 3')
7[244]	GAAAGCCGGCGAACGTGAAGGGAAACAATATCTGTCCATTGTGAACTGGT AAGTCT
7[265]	TTTGATTAGAGCTTGACGGGCGCGCCTTGCCGTTGTACAATCACAGGGTA AGTCT
7[286]	GGAACCTAAAGGGGTGTAGCGTAGAAGATTAGTATCTCTAGGTGGTAAG TCT
7[307]	ACAGGAACAGCCGGGCGCGGTAGGTAGCATGGTAAGTCT
7[328]	GCCTTTGCGTATTGGGCTGGGGTGCGGGGTCGGTCACTTGTGTCATGGGT AAGTCT
7[349]	AGCCCAACGCGCGGGGATAACTCAGCTGGAGGGTAATGACTAGCTTAGGT AAGTCT
7[370]	TGCCCAGCTGCATTAATCGCTCACATCCCACTGCTCGT
9[244]	CGCGAAAAACCGTCTATGCCCACTAACAGAGGCCAGCCTTGTGAACTGGT AAGTCT
9[265]	GAGTGGACTCCAACGTCCCAAATCAATAAAAAACGCTCCAATCACAGGGT AAGTCT
9[286]	AATAGAGTCCACTATTACGAGGTGTTACCAATTTTGATCTCTAGGTGGTA AGTCT
9[307]	TCTTTTCGGAAGCACGCACTCAATCCCTTCGTCTGAAATGTCAGGTAGCAT GGTAAGTCT
9[328]	GACAATAGCCCGAGATATGCCCTTCAAACGCCCCGGCCATGTGTCATGGGTA AGTCT
9[349]	GTGAAATCCCTTATAAAGAGAGAGCGATGCTAACGGAACTAGCTTAGGT AAGTCT
9[370]	AGCATGGTGGTTCCGAACCACGCTGTGATGAAGAACGT

11[244]	TATCTGGTCAGTTGAGTATTAAAGATAAAACCCTTTTGTGAACTGGTAAGTCT
11[265]	AAATATCAAACCCTAGTGCCATACCGAATAAGAATCAATCACAGGGTAAGTCT
11[286]	AGCATCACCTTGCTAGCAAATCTAAAACATATTTTTCTCTAGGTGGTAAGTCT
11[307]	TGAATGGCTATTAGTCTTTCTAGGTAGCATGGTAAGTCT
11[328]	GCTATTACGCCAGCTTCCCAGCTTCTCGACGCAGTGTGTCATGGGTAAGTCT
11[349]	GCGATCGGTGCGGGAACGACGCCGGAATACTTAAAACTAGCTTAGGTAAGTCT
11[370]	CCATTCAGGCTGCGCAACTGTTTTTCAGAGACAGCGCCCGCCAG
16[245]	CAAGTAAAATACGTAATAATAGTACAAAAGAAGTCTAGCTTAGGTAAGTCT
16[266]	TCAGAGGAAGTTTCCATGATAGCGCAAAATATGTGTCATGGGTAAGTCT
16[287]	CCCCAGCGATTATGAGGACTAAAGACTATCGTCATTACCAGAGGTAGCATGGTAAGTCT
16[308]	AATAGCAACGGCTACAGATCCCCCACTCCAATCTCTAGGTGGTAAGTCT
16[329]	CCGAGACAGCATCGGAACAGTTCAAGCGAACCAATCACAGGGTAAGTCT
16[350]	ACAATCGTCACCCTCAGCCATAAAGCGTTTTTTGTGAACTGGTAAGTCT
16[371]	TAAGTTAAAGGCCGCTTTTTACCCGCCCGAA
18[245]	AACGAAAATAAATTACGCCAACACATTCCTAGCTTAGGTAAGTCT
18[266]	GCGAGAGTACGAGGCATAGTATCAGTTGTGTGTCATGGGTAAGTCT
18[287]	ATTATCAGATGATTTTATACCAAGCGCGATAACCCTATTACAAGGTAGCATGTAAAGTCT
18[308]	CTTTCGATTAATTGTAATTGCTTCGCAATCTCTAGGTGGTAAGTCT
18[329]	ACAGCTTAAAAGGCGGTCATTTAGTTTGCAATCACAGGGTAAGTCT
18[350]	AATTCGAATGGCTTAGAGCTTAATTCTGTTGTGAACTGGTAAGTCT
18[371]	TCGCCCAGAATTGCATGCTGTTCCATAT
20[245]	AAAGAAATAATGGAAGTGGCTTGAATTAAGTCTTAGGTAAGTCT
20[266]	GGAATTATGTTTGGGGACGTTTAATTTCTGTGTCATGGGTAAGTCT
20[287]	TGAACGGTGACCTTTTGGCAATTCATCACGTTAATAAATTGAGGTAGCATGGTAAGTCT
20[308]	AGGAACGTAAATCATCAATAATAACTTCTCTAGGTGGTAAGTCT
20[329]	TCGTTTGTAAATTCGTTTTTCATACCAAAACAATCACAGGGTAAGTCT
20[350]	CCAGCTTGTAACGGAAAAGGGCATAAATTGTGAACTGGTAAGTCT
20[371]	TGTGAGCGTATAAGCTAATAGATTAAGC
23[244]	AACTAATTTTAAGAAGGGTTAACGGTCAACTAGCTTAGGTAAGTCT
23[265]	TTGCATTATACCAGTCAATTATACGAACTGATGTGTCATGGGTAAGTCT
23[286]	GGCTTGAAAACACCTAGGCTGGCTGACCTGAAAGAGGACAGAAGGTAGC

	ATGGTAAGTCT
23[307]	TTTGCGGGAGAAAGTAAAACGAACTAGGGCTCATTATCAGGTTCTCTAGG TGGTAAGTCT
23[328]	ACCATTAAGCTATACATTAAACTGGAGCCAATCACAGGGTAAGTCT
23[349]	CGAACGAGCGAGCTTTAATATATGAACGTTGTGAACTGGTAAGTCT
23[370]	AACAGTTAATTCTACAAATATAGCATGT
25[244]	AGTTTTGATACATAGTGTGCGAGCGGAACACTAGCTTAGGTAAGTCT
25[265]	CACTAAAGGAGATTTGTATCAAAGGAGCTGTGTCATGGGTAAGTCT
25[286]	ACGACGACACTATCAAACAAAATTCCTGAGGTAGCATGGTAAGTCT
25[307]	ATACGGAACAACATTCAGAGAGTACCTTTATCGGTAACCAATTCTCTAGGT GGTAAGTCT
25[328]	CAGACCGGATAAGATCCAAAAAATAATCAATCACAGGGTAAGTCT
25[349]	CGCCGACTCACGTTGAAAATCCCTGTAGTTGTGAACTGGTAAGTCT
25[370]	AGACTTCGAATATAGAATAATCATTA
27[244]	TTTTTTTAGAGGGGGTGCCACTAAACCTAACTAGCTTAGGTAAGTCT
27[265]	TTGAAATGTTTAGACTGTAAACGGAAGAATATGTGTCATGGGTAAGTCT
27[286]	AACTCCAATACTGCGGATTTTCATTCTTTGAAGGTAGCATGGTAAGTCT
27[307]	CAGGTCAGGATTGTAAATATTCATTGAAGGCTTTCAGCTTGTCTCTAGGT GGTAAGTCT
27[328]	CAATCAAATGCTTTAAACGAGGGTTTCTTAACAATCACAGGGTAAGTCT
27[349]	CAAGAAAACGAGAATGACAGCGAAATAGTTGTTGTGAACTGGTAAGTCT
27[370]	ATCTCAAAAATCAGGTCTTGCGGGACAACCA
30[245]	TACCTGAGCAAAAGAAGATGTGAGTGTTTTGAAAGCGAACTTGTGAACT GGTAAGTCT
30[266]	CAAACATCAAGAAAACAGTACTTAGTTGTCTAAGACAATCACAGGGTAAG TCT
30[287]	GATAGCTTAGATATTACATTTGAATTACGCTACAATATAGAATCTCTAGGTG GTAAGTCT
30[308]	ACCGCCACCCTCAGGTACTCATTACCAACACCCTGAGGTAGCATGGTAAG TCT
30[329]	ACCCTCAGAACCGCGCCCGGATTTCCAGTAGACGGTGTGTCATGGGTAAG TCT
30[350]	AGAGCCACCACCCTGAGAGGGGTTACAACATAAAAACTAGCTTAGGTAAG TCT
30[371]	AGGGATAGCAAGCCACCAGGCTTATTTACAGCCTT
32[245]	ACCAAATCGTCGCTATTTGAGAGACATCGAGCGGGTATTTGTGAACTGGTA AGTCT
32[266]	ATCTTTCCCTTAGAATCAATTTATTTTATTCTTTCCTCAATCACAGGGTAAG TCT
32[287]	AACGAGCATGTAGAAACCAATTCTCTAGGTGGTAAGTCT
32[308]	GTTGCCCCCTGCCTATTCCCGTATAAGTCAGAATACCCAGGTAGCATGGTA

	AGTCT
32[329]	GTGCTATTATTCTGAAATCAGTGCCTAATATGAGGAAATGTGTCATGGGTA AGTCT
32[350]	TATGTATTAAGAGGCTGTGGTAATACAAGAACAAAGTTACTAGCTTAGGTA AGTCT
32[371]	AAGTCAAGAGAAGGATTTTTGATGATAATAAAAAAGTA
34[245]	CTTGGCTTAGGTTGGGTTGACCTACGCCTGTCAACATGTTGTGAACTGGTA AGTCT
34[266]	ATCCTATATGTAAATGCATTTTAGTAAGTCCTGTCCAGCAATCACAGGGTAA GTCT
34[287]	CATAATTACTAGCGATGAACGCGAGAAAAATATCCCGACAAATCTCTAGGT GGTAAGTCT
34[308]	AGTACAGGAGGTTGAGGGAGCCGCGAACTGGGAGGGAGAGGTAGCATGG TAAGTCT
34[329]	AGTGACGATTGGCCTTGCCGCCACCTTATTAAAGGGCGTGTGTCATGGGTA AGTCT
34[350]	TTTCAAACAAATAAATCTCAGAGCAAACGTAGTTTACCACTAGCTTAGGTA AGTCT
34[371]	AGGAAGCCAGAATGGAACCACCCTAAAGGTGAATCAAT

Locking strands for TEM imaging

3[255]-7-1-C9-staple	GCT AAC GAT TCA ATT CTG ATC TAC TGT GTG tttt TCACGCAGCCACCGTGCTTTCCTCGTTAGGCGCGTAAAG
5[406]-7-2-H4-staple	CAT TGA GAT TCA TCG AAT GAT TCT GTA TGG tttt TGTGTTCTAACGGCGCCTCCTCATTTTTTT
1[248]-11-1-A1-staple	CAC ACA GTA GAT CAG AAT TGA ATC GTT AGC tttt CTGACCTTCTGGCCACGT
9[391]-11-2-B6-staple	CCA TAC AGA ATC ATT CGA TGA ATC TCA ATG tttt CGACATAGTAAAAAAGCCGCTCTGGTC
25[244]-16-1-A8-staple	GCT AAC GAT TCA ATT CTG ATC TAC TGT GTG tttt AGTTTTGATACATAGTGTGCGAGCGGAACACTAGCTTAGGT AAGTCT
18[392]-16-2-E4-staple	CAT TGA GAT TCA TCG AAT GAT TCT GTA TGG tttt AAGATTACATGTTTTAAATATTACGGTG
24[231]-20-1-F6-staple	GCT AAC GAT TCA ATT CTG ATC TAC TGT GTG tttt CCTTATGAACAAAGGGATATTCATTACCGGCGCAGGAACC TA
23[381]-20-2-D6-staple	CAT TGA GAT TCA TCG AAT GAT TCT GTA TGG tttt AAAGAATAGGGTGAAGACAGTCAAATCAATGT
20[245]-27-1-A6-staple	ACG CAA CAC TAT AGC AAA CTT TAT CTA ACG tttt AAAGAAATAATGGAAGTGGCTTGAATTAAGTCTTAGGT AAGTCT
16[371]-27-2-G4-	CGT TAG ATA AAG TTT GCT ATA GTG TTG CGT tttt

footholds	TAAGTTAAAGGCCGCTTTTTACCCGCCCGAA
20[255]-23-1-H4-staple	ACG CAA CAC TAT AGC AAA CTT TAT CTA ACG tttt ATCATAAAAGAACCCTGCTCATTCAAGTGATCA
27[255]-23-2-D8-staple	CGT TAG ATA AAG TTT GCT ATA GTG TTG CGT tttt ACCTTCTGAACCACCAGTCGCCTGGAGG
40[241]-30-1-D2-staple	ACG CAA CAC TAT AGC AAA CTT TAT CTA ACG tttt CTCCCGACCGCACTCTAC
32[392]-30-2-E10-staple	CGT TAG ATA AAG TTT GCT ATA GTG TTG CGT tttt AAAAATGAAACAATGAAATAGCTTACCG
38[234]-34-1-D12-staple	ACG CAA CAC TAT AGC AAA CTT TAT CTA ACG tttt TTCAGCTAGAATCGAACGCTCAACAGTATTTGAAATGGT
31[381]-34-2-A10-staple	CGT TAG ATA AAG TTT GCT ATA GTG TTG CGT tttt GTTTATTTTGTACGCAACATTTTAAAGGAGC

Locking strands for system I

5[406]-7-2-H4-staple2	AGGTGAGTAAGT tttt TGTGTTCTAACGGCGCCTCCTCATTTTTTT
9[391]-11-2-B6-staple2	AGGTGAGTAAGT tttt CGACATAGTAAAAAAGCCGCTCTGGTC
18[392]-16-2-E4-staple	ACTTACTCACCT AAACACACATCA tttt AAGATTACATGTTTTAAATATTACGGTG
23[381]-20-2-D6-staple	ACTTACTCACCT AAACACACATCA tttt AAAGAATAGGGTGAAGACAGTCAAATCAATGT
16[371]-27-2-G4-footholds	TGTAGAGGATGA tttt TAAGTTAAAGGCCGCTTTTTACCCGCCCGAA
27[255]-23-2-D8-staple	TGTAGAGGATGA tttt ACCTTCTGAACCACCAGTCGCCTGGAGG
32[392]-30-2-E10-staple	TCATCCTCTACAACTATCTACTC tttt AAAAATGAAACAATGAAATAGCTTACCG
31[381]-34-2-A10-staple	TCATCCTCTACAACTATCTACTC tttt GTTTATTTTGTACGCAACATTTTAAAGGAGC

Blocking strands and removal strands

B-1	ACTTTCACCTCCTTACCTAAGCTAGT
R-1	ACTAGCTTAGGTAAGGAAGTGAAAGT
B-2	TGTATCCATCCTTACCCATGACACA
R-2	TGTGTCATGGGTAAGGATGGATACA
B-3	GCTATTCCTTCCTTACCATGCTACCT
R-3	AGGTAGCATGGTAAGGAAGGAATAGC
B-4	CAACCATGTTTCTTACCACCTAGAGA
R-4	TCTCTAGGTGGTAAGAAACATGGTTG
B-5	CCTTATATCCTCTTACCCTGTGATTG
R-5	CAATCACAGGGTAAGAGGATATAAGG

B-6	ATTCTTCAATCCTTACCAGTTCACAA
R-6	TTGTGAACTGGTAAGGATTGAAGAAT
Open lock a	A GGT TAG TAT TGA TGT GTG TTT AGG TGA GT
Open lock b	T ATG ATA GAG GAG ATA GAT AGT TGT AGA GG

Table S2. Sample volumes added to drive system I for *in situ* fluorescence detection in Fig. 2c (main text). Small volumes with high concentration (200 μM) of the blocking and removal strands were used to reduce the dilution effect. The raw data were corrected by calculation of the signal reduction through dilution and addition of these values.

Step	Strands added	Volume added per stand
State iii to ii	$4', \bar{2}$	0.5 μL , 0.5 μL
State ii to i	$3', \bar{1}$	0.5 μL , 0.5 μL
State i to ii	$1', \bar{3}$	1 μL , 1 μL
State ii to iii	$2', \bar{4}$	1 μL , 1 μL
State iii to iv	$3', \bar{5}$	2 μL , 1 μL
State iv to v	$4', \bar{6}$	2 μL , 1 μL

Table S3. Sample volumes added to drive system II for *in situ* fluorescence detection in Fig. 4c (main text).

Step	Strands added	Volume added per stand
State i to ii	1', $\overline{3}$	0.5 μ L, 0.5 μ L
State ii to iii	2', $\overline{4}$	0.5 μ L, 0.5 μ L
State iii to iv	3', $\overline{5}$	1 μ L, 0.5 μ L
State iv to v	4', $\overline{6}$	1 μ L, 0.5 μ L
State v to iv	6', $\overline{4}$	1 μ L, 2 μ L

Supporting References

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