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Rapid, High Yield, Directed Addition of Quantum Dots onto Surface Bound Linear DNA Origami Arrays

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Experimental Section

Materials: All staple strands and sticky-end strands were purchased from Integrated DNA Technologies Inc. Single-stranded M13mp18 DNA plasmid was purchased from Bayou Biolabs. Streptavidin (Thermo Scientific), Qdot® 525 Streptavidin Conjugate (Invitrogen) and chemicals were purchased from Aldrich.

AFM: All AFM imaging studies were performed with a Bruker Multimode8 and NanoscopeVI controller using SCANASYST-AIR mode. Circular grade V1 mica discs (10 mm, Ted Pella, Inc.) served as substrates.

Fluorescence microscope (FM): Fluorescence images were taken with a Rolera-MGi EMCCD camera (QIMAGING), with acquisition parameters: gain 2, EM gain 3500, exp. Time=100msec. A Sapphire (Coherent) 488nm laser (20mW collimated) was used for excitation and launched into a Nikon TE200 inverted epifluorescence microscope. The characteristics of the filters employed were excitation 470/40 nm and emission 515/30 nm.

Preparation of DNA Origami:

Single Rectangular DO (srDO): The sequences of staple strands for srDO were designed using the Parabon inSēquio™ program.[1] , Biotin-labeled staples were used to direct the local binding of, or address, SA or SA-Qdot species. The mixture of staple strands, biotin-labeled staples and M13mp18 ssDNA plasmid was brought to a volume of 50 µl using DO buffer (1×TAE buffer solution containing 40 mM Tris-HCl, pH 8.0, 20 mM Acetic acid, 2.5 mM EDTA, and 10.5 mM Magnesium chloride). The final concentration of M13mp18 ssDNA plasmid in the solution was 10 nM, and the molar ratio of the long viral ssDNA to the staple strands was 1:5. The sample was cooled from 90°C to 16°C over the course of 13h in a thermocyling machine (Primus96, MWG Biotech).[2] Staple sequences are listed at the end of the supporting information.

One Dimensional Rectangular DO (1DrDO): To prepare the one dimensional DO, designed sticky-ended strands were employed. The sequences are listed at the end of ESI. The mixture of staple strands, sticky-ended strands, biotin modified strands and M13mp18 ssDNA plasmid was brought to a volume of 50 µl using DO buffer. The final concentration of ssDNA plasmid in the solution was 10 nM, and the molar ratio of the ssDNA plasmid to all the other strands was 1:5. The sample was annealed by cooling as described in the previous procedure.

Purification of DO: To remove the excess staple strands, the DO solutions were dialyzed using the drop dialysis method.[3] 50 µl of DO solution was purified using 0.25 µm pore size membrane (Millipore Inc.) by dialysis for 30 min against 10 ml of DO buffer.

Silanization of glass coverslip (AP-glass):

Indexed cover glass (Eppendrof CELLocate) surfaces were cleaned with 5 minutes of sonication, first in ethanol and then in acetone then dried in a N_2 stream. The substrates were then exposed to a low pressure UV lamp at a distance of ~2-3cm (SEN light Co. UVL-20, Hg lamp, 20 watt with 254nm power = 50µW@1meter) for 5 minutes and finally reacted in an O_2 plasma (100 mtorr ~75% O_2) for 5 minutes. This cleaned coverslip was immersed in a freshly prepared 1% 3-aminopropyl-trimethoxysilane (APTES) in dry ethanol solution for 10 minutes at room temperature, rinsed by dipping and agitation in an ethanol (~10-20ml) bath, then annealed in an oven for 1hr at 120°C.

Assembly of SA and SA-Qdot on DO:

10 µl of 1 nM DO was used to cover ~ 1cm^2 of freshly cleaved mica for 5 minutes, then the surface was washed with 400 µl of MilliQ water and immediately blown dry with N_2. The number density and structural integrity of the immobilized origami were determined using AFM. Mica bound samples were then incubated at room temperature in 10 nM SA or SA-Qdot solutions and brought to a volume of 30 µl using DO buffer. After one minute, the sample was washed with 400µl of DO buffer and then with 400µl of MilliQ water and immediately blown dry with N_2.

sQD-1DrDO alignment using the combing method:

To align then immobilize the sQD-1DrDO complex in one direction a moving interface combing technique has been employed.[4] A small drop (typically 5 µl) of complex solution was deposited at the edge of an AP coated glass coverslip substrate. One flat edge of an untreated coverslip was then pressed/touched on top of the drop, forcing the drop to spread by capillary action along the untreated coverslip edge. This interface is slowly moved in one direction (as illustrated in Fig. 4a). After one minute incubation, the sample was wicked dry using an absorbent paper, then subjected to analysis by fluorescence microscopy and AFM analysis.

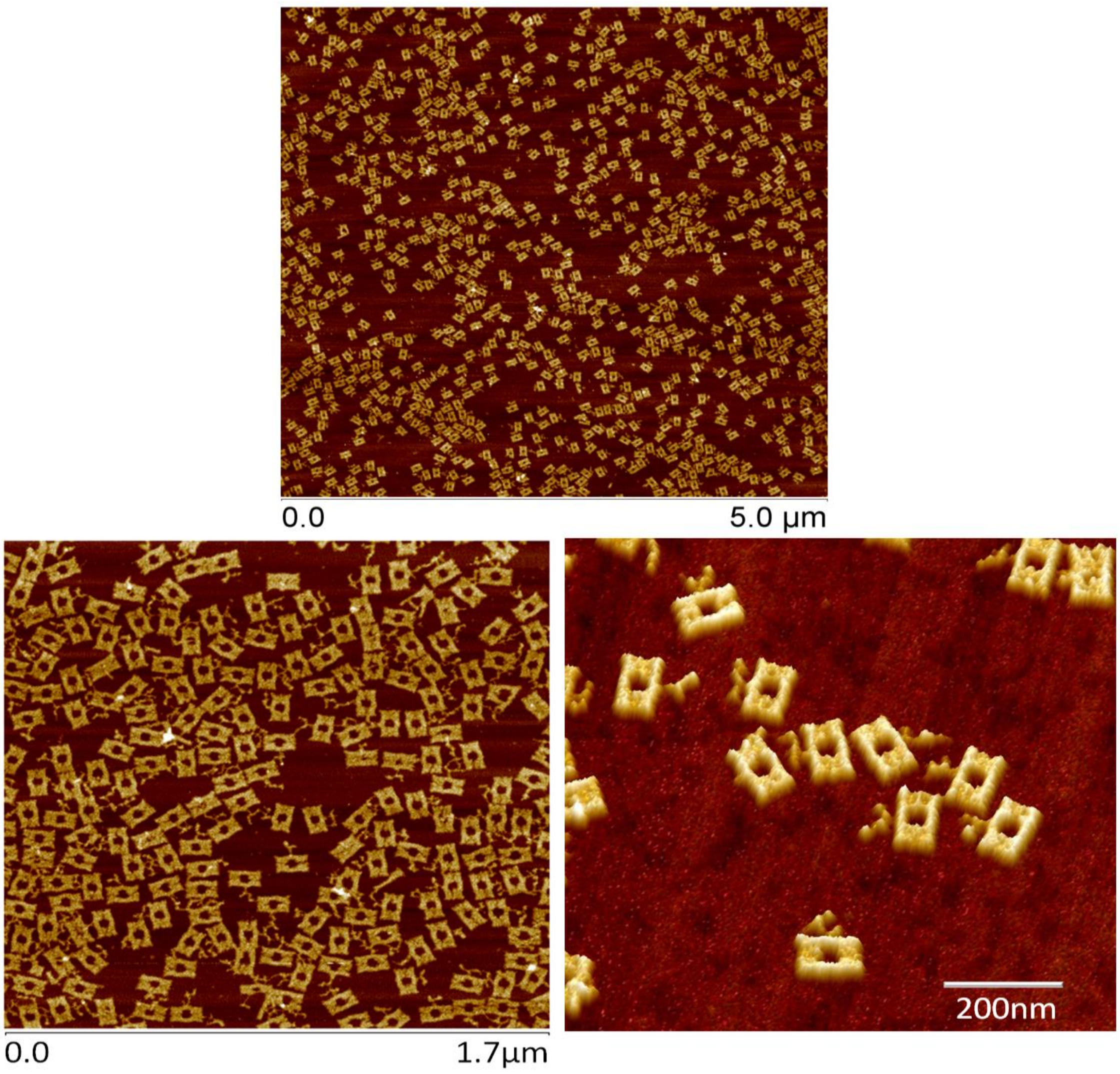


Figure S1: AFM images of single rectangular origami (srDO) platforms on mica

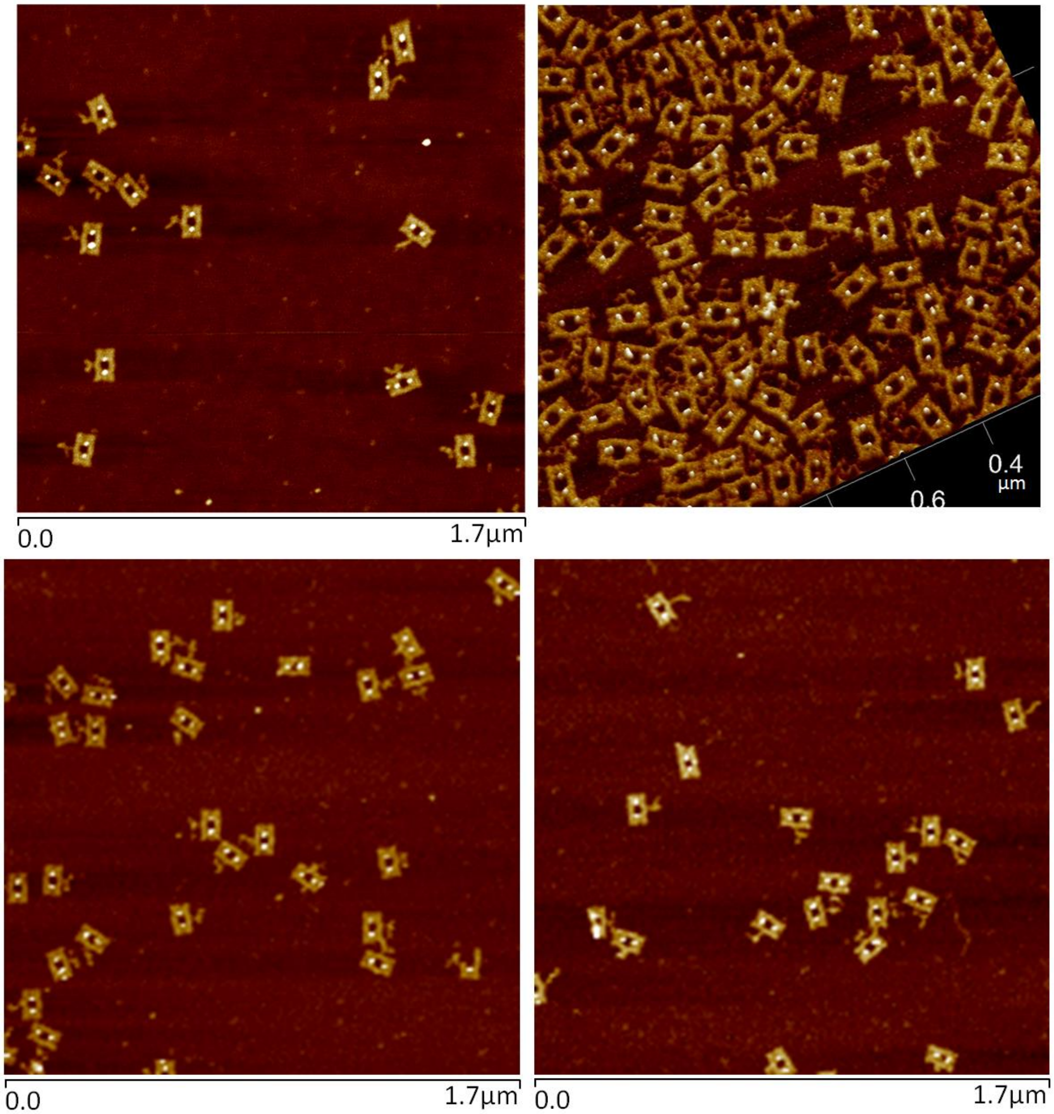


Figure S2: Directed assembly of streptavidin (SA) to addresses on srDO

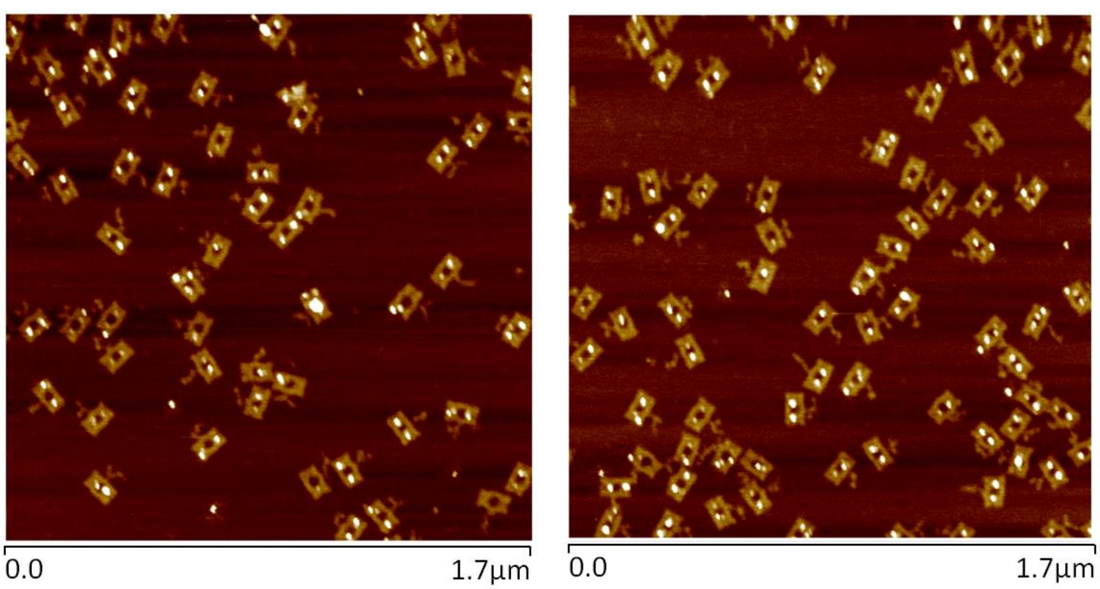


Figure S3: Directed assembly of SA-QDs to addresses on srDO

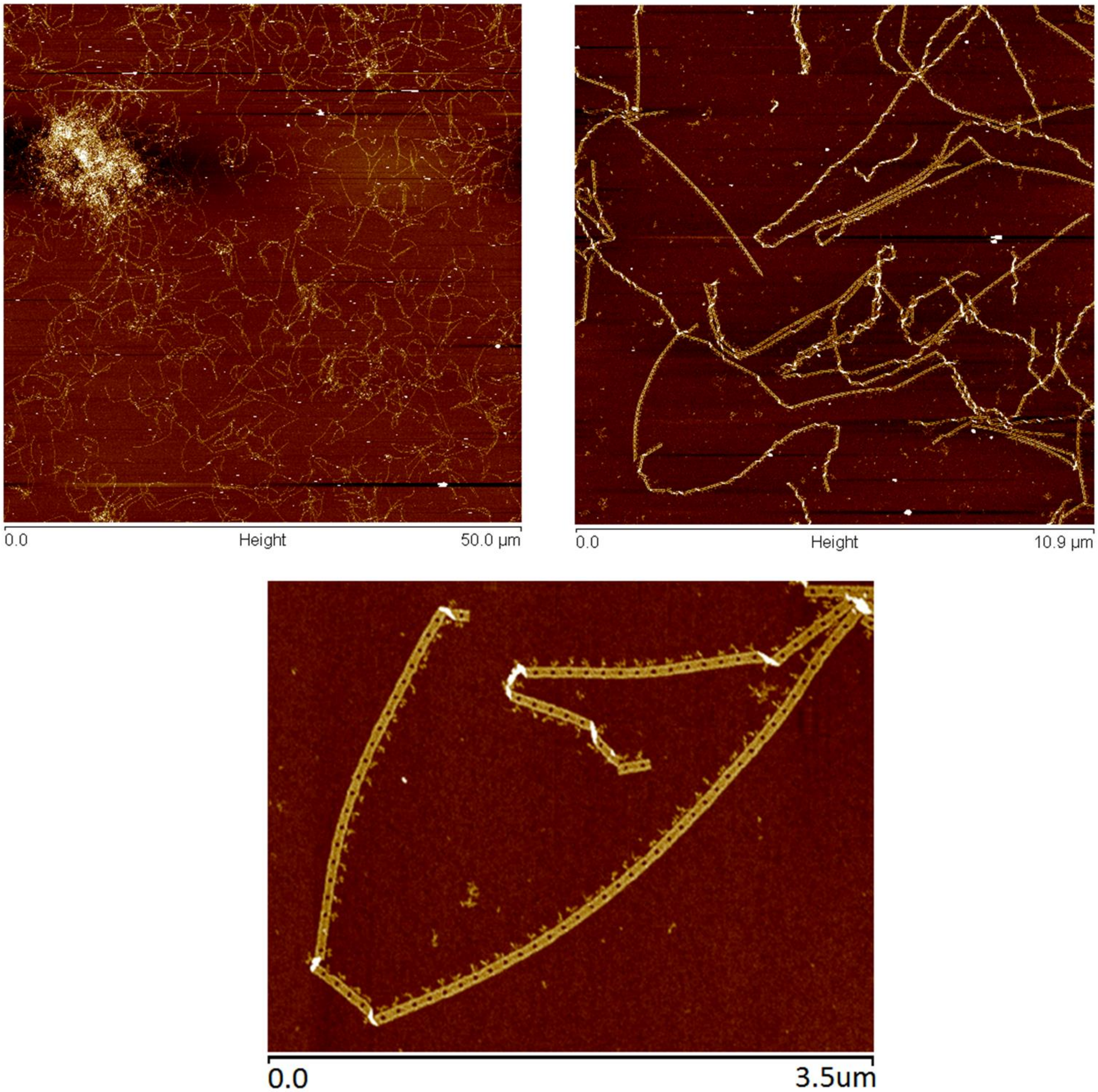
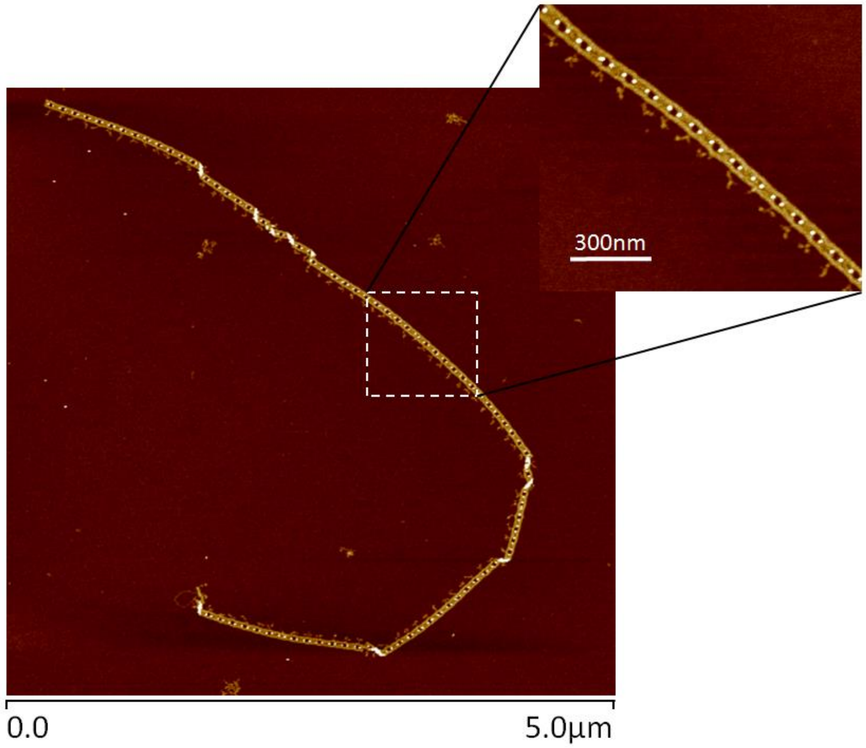


Figure S4: AFM images of one-dimensional rectangular origami (1DrDO) on mica



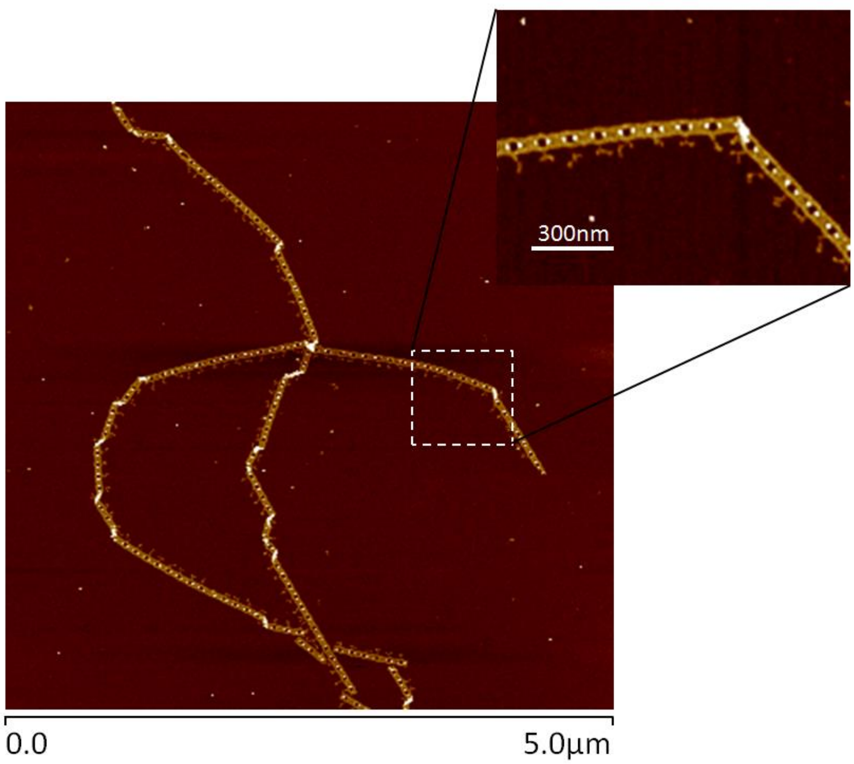


Figure S5: Directed assembly of SA to addresses on 1DrDO

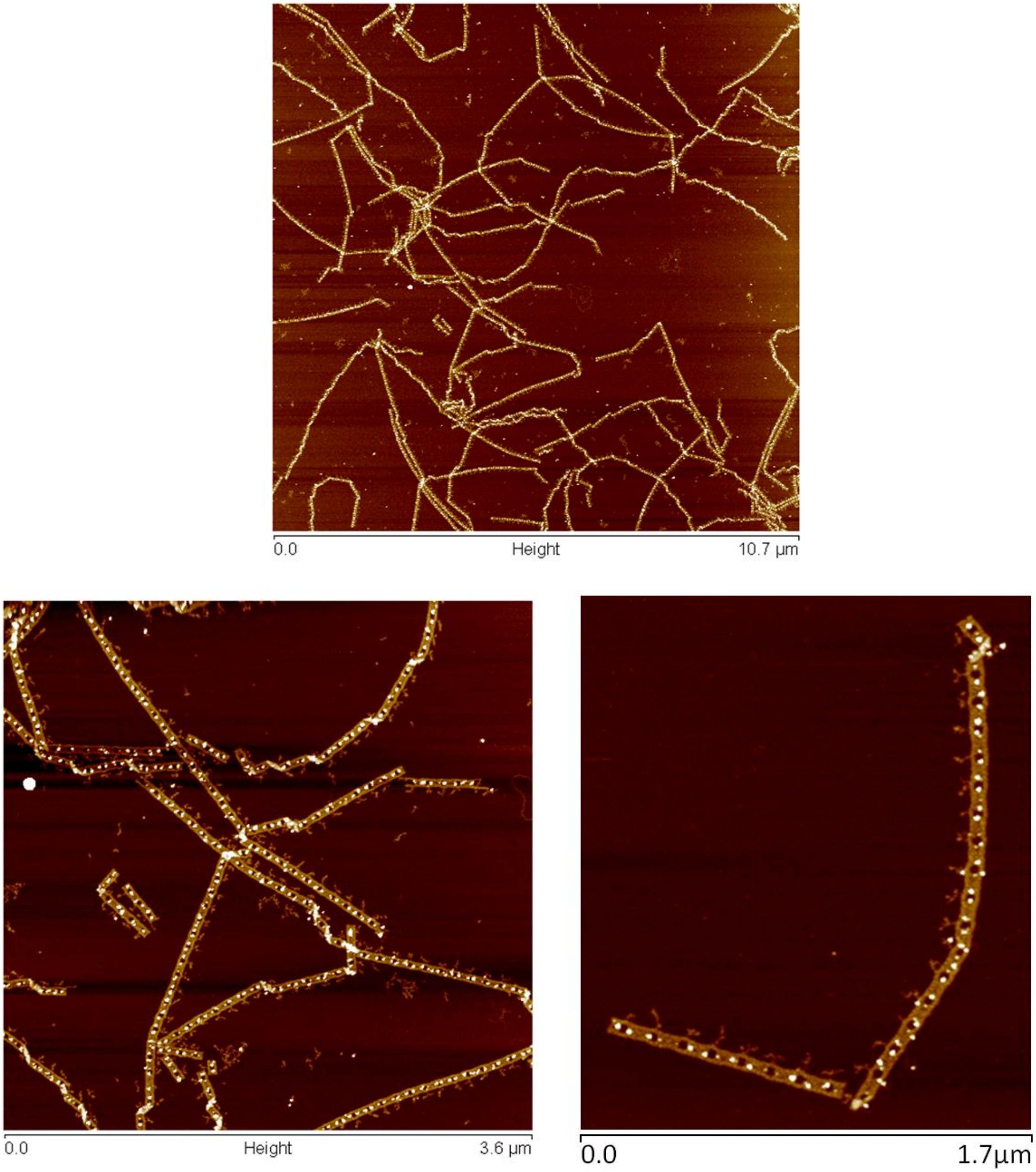


Figure S6: Directed assembly of SA-QDs to addresses on 1DrDO

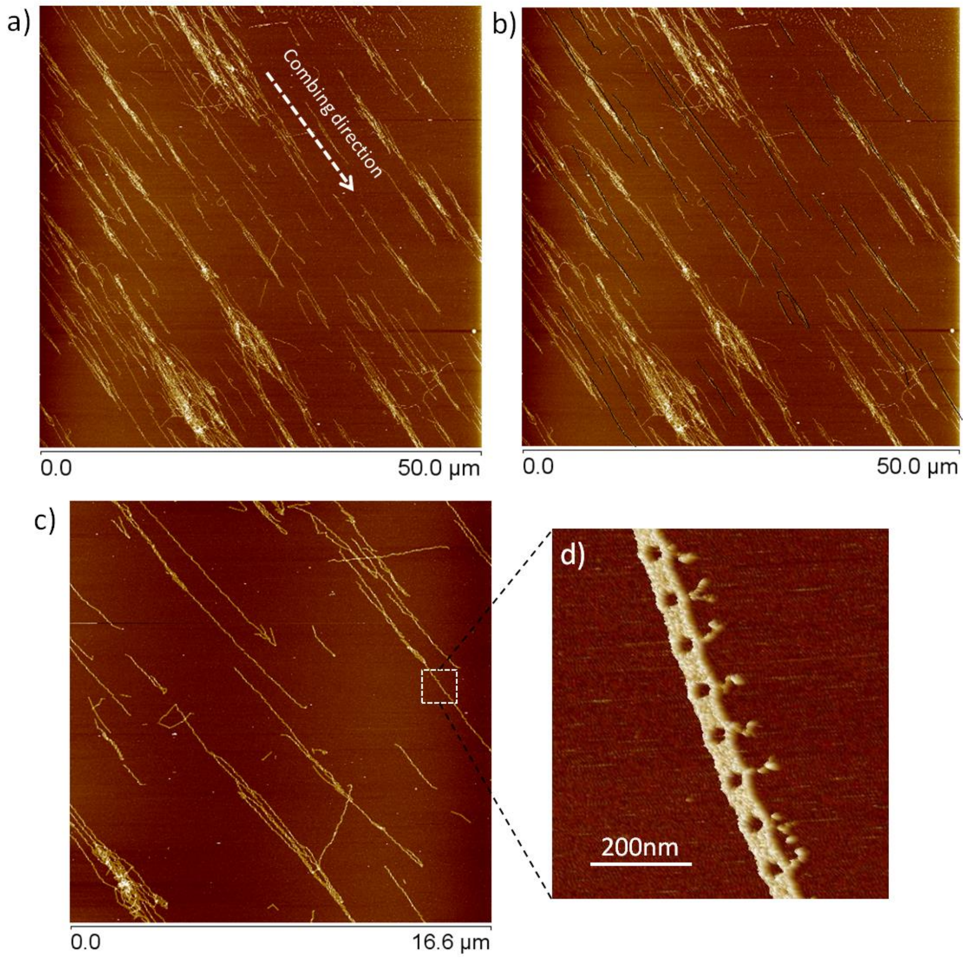


Figure S7: a) Large area AFM image of combed 1DrDO on mica; arrow indicates the combing direction; b) NIH ImageJ software was used to analyze 1DrDOs to determine length distribution (mean and standard deviation value) for apparently single constructs. Black overlay lines indicate which 1DrDO were analyzed; c) different region of combed 1DrDO; d) inset shows high resolution AFM image of the combed 1DrDO construct.

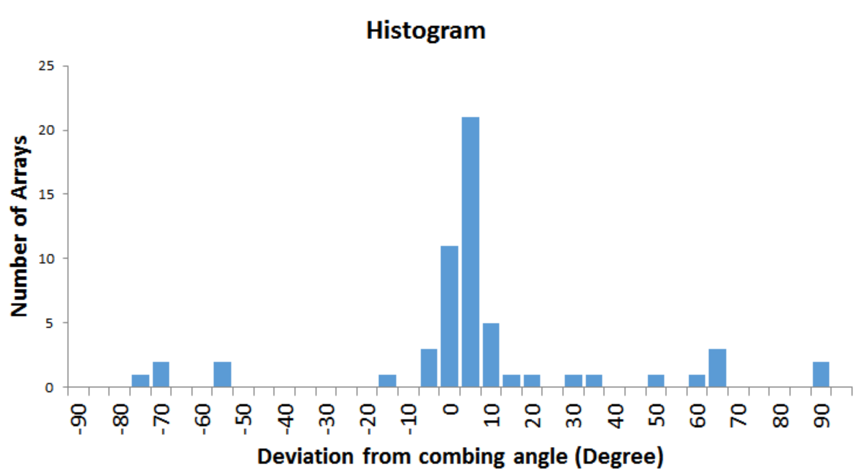


Figure S8: The bins at the center of this histogram show apparent normal distribution of the origami chain alignment dominated by combing while outliers at both extremes likely indicate other forces at work. N=58 number of origami chains were analyzed. If we take ±10 degree as successful chain alignment than we see that 77% (40/58) are aligned along flow direction.

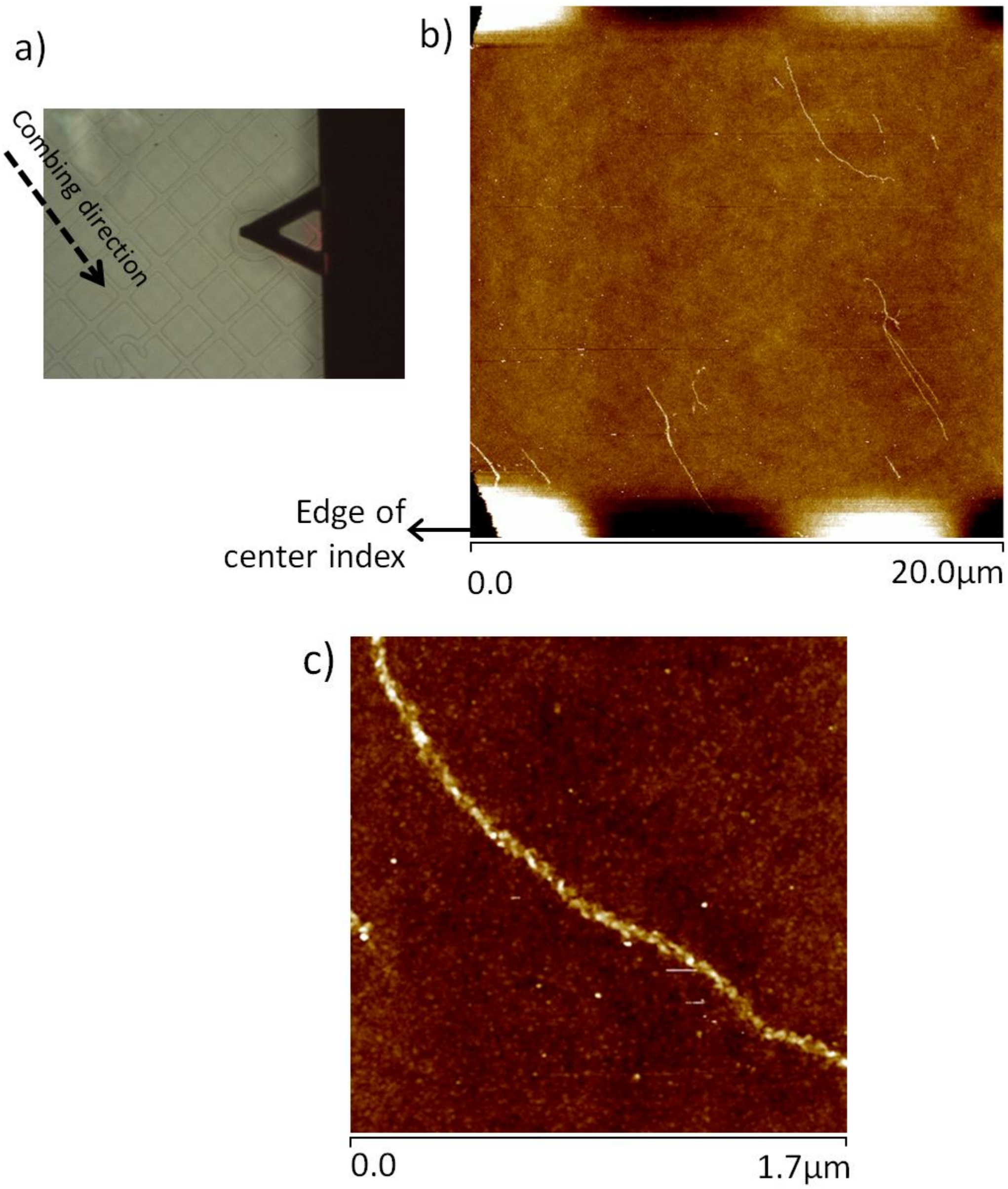


Figure S9: a) Arrow indicates the combing direction on indexed AP-glass; Light microscope image was taken during AFM imaging in air; b) low magnification AFM image of combed 1DrDO; c) high resolution AFM image showing a single 1DrDO construct.

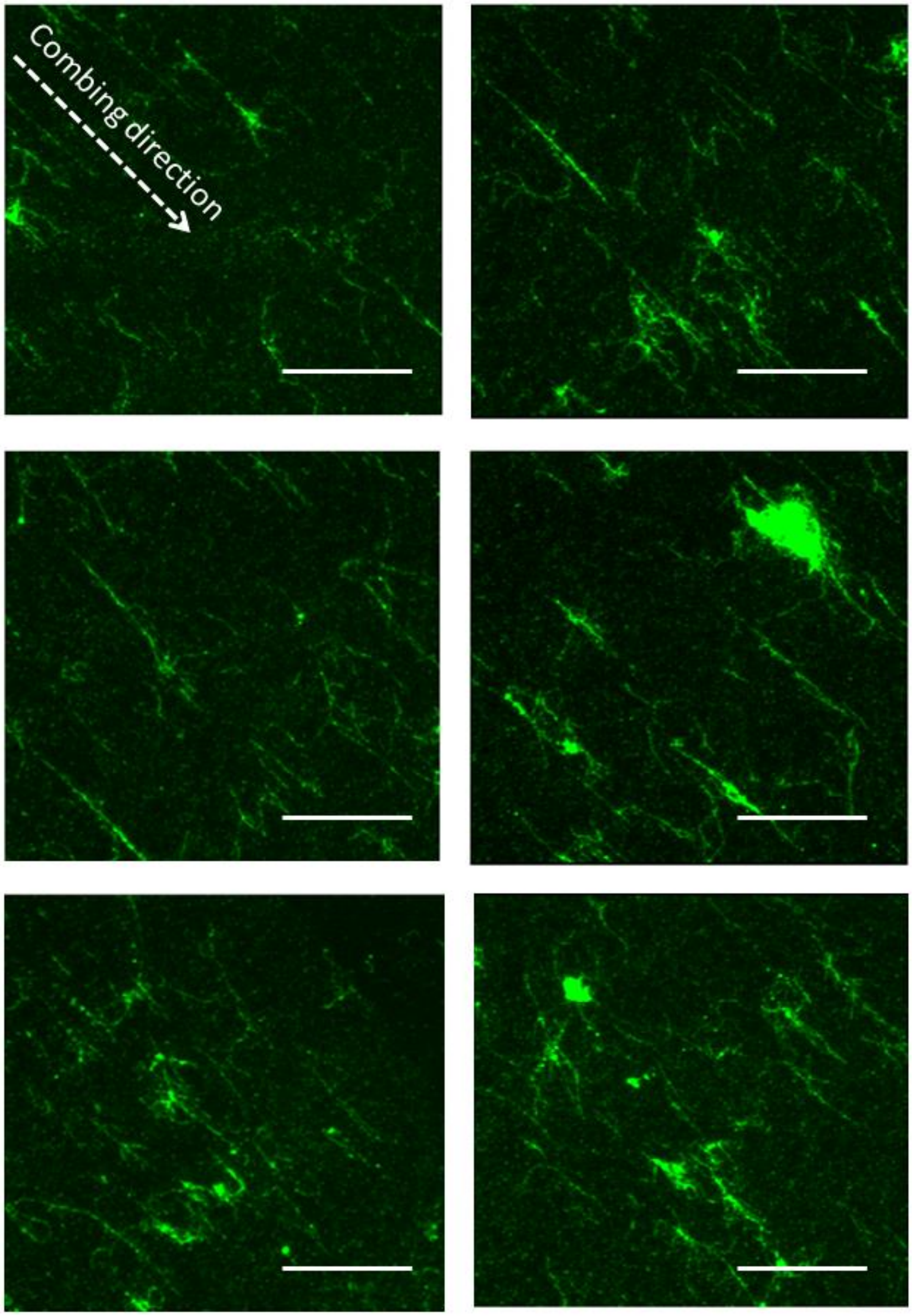


Figure S10: Fluorescence microscopy images of combed sQD-1DrDO construct on AP-glass; Scale bar 20μm

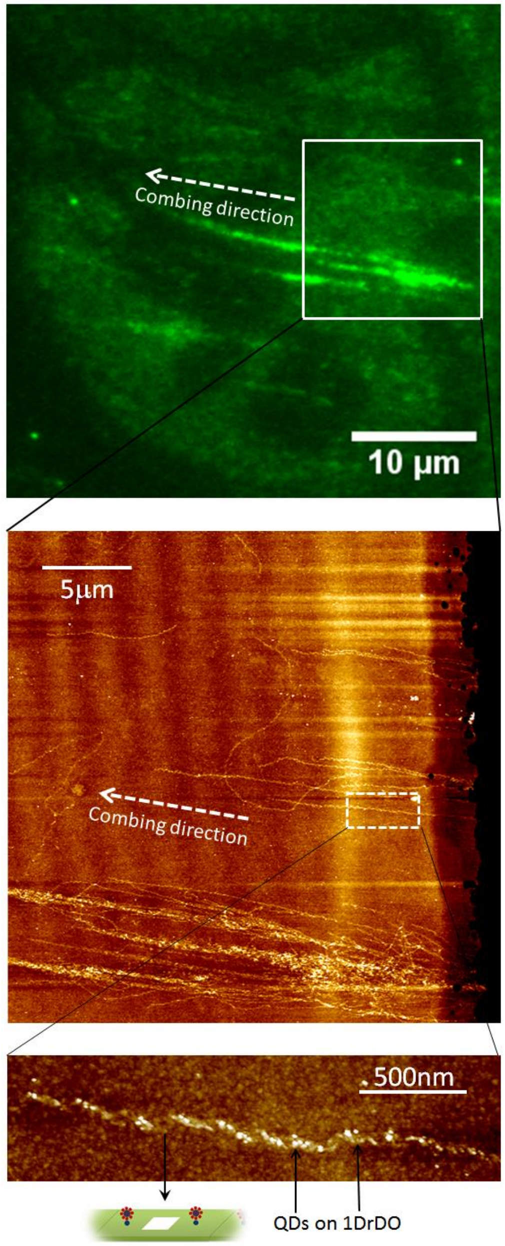


Figure S10. Wide range of Fluorescence microscopy and high resolution of AFM and of the same region of combed sQD-1DrDO construct on AP-glass.

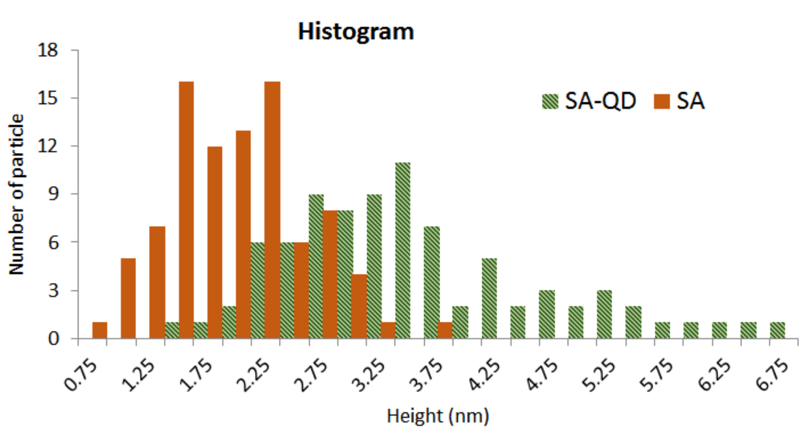


Figure S11: Histogram chart of SA and SA-QDtd height (excluding origami height) on origami chain.

Rectangular origami sequence (Black = m13, Ä=5′ end; A̤ =3′ end)

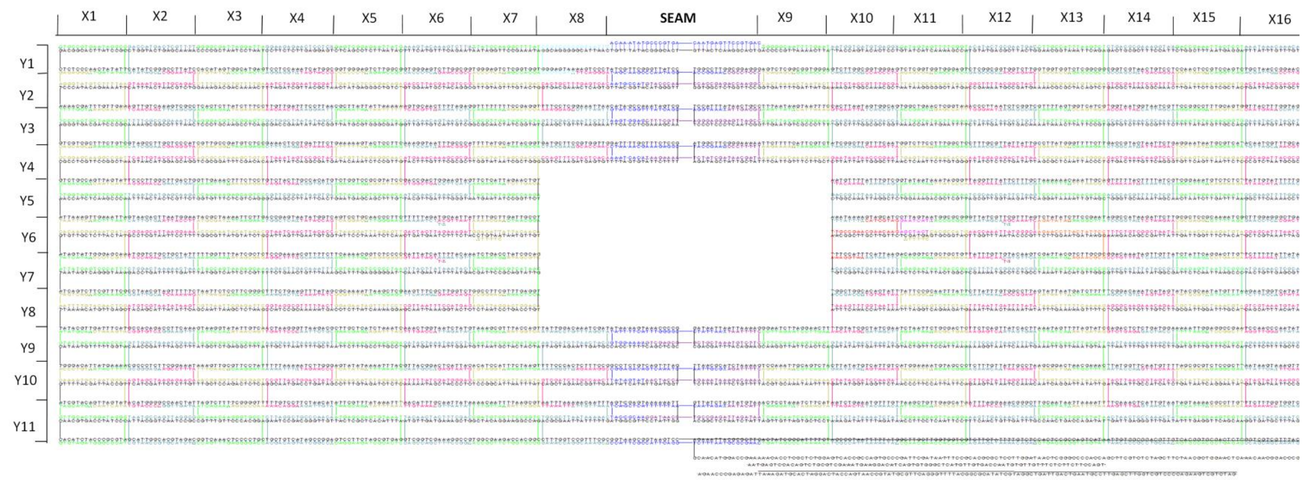


Figure S12: Rectangular origami structure and staple strand positions.

Staple strand sequences

seam-01	CAGTGCCTTGAGTAACAGTGCCCGTATAAACA
seam-02	TACCGTAATAGCAAGCCCAATAGGACCGGAAC
seam-03	CGCCTCCCCGGAACCAGAGCCACCAACCCATG
seam-04	GAGGTGAAGTATCGGTTTATCAGCAGGTAAAT
seam-05	ATTGACGGCCGATTGAGGGAGGGATTGCTTTC
seam-06	ACACTAAACTAAAACGAAAGAGGCTACCGAAG
seam-07	CCCTTTTTATAGCAATAGCTATCTAAAAGAAT
seam-08	AAAAGGTGTATTTTCATTTGGGGCCTATTAAT
seam-09	TAATTTTCCTTCTGTAAATCGTCGGCGAGCTG
seam-10	TATGATATCGGAGACAGTCAAATCAATTGCGT
seam-11	AGATTTTCAAAACAGAAATAAAGAACCATCAA
seam-12	AACGCCATTCAGCTCATTTTTTAACAATAGAT
seam-13	AATACATTAATAGATTAGAGCCGTCCAATAGG
seam-14	CCATTCGCCATTCAGGTCTTTAATGCGCGAAC
x01-y01	TGATATAAGCGGATAAGTGCCGTC
x01-y02	AACAACTTAATTTTCTGTATGGGAGAGAGGGT
x01-y03	AAAGACAGGCGGGATCGTCACCCTTTTTGCTA
x01-y04	CAATCATATAGCCGGAACGAGGCGCAGCAGCG
x01-y05	ACTTTAATTGGGCTTGAGATGGTTCAGACGGT
x01-y06	CCCTCGTTATAGTAAGAGCAACACTAATTTCA
x01-y07	AGCAAAGCTTTACCCTGACTATTATATCATAA
x01-y08	CTAAAGTAGCTCAACATGTTTTAATAGTCAGA
x01-y09	ATACTTTTTACCAAAAACATTATGATATGCAA
x01-y10	CAATCATAACGGTAATCGTAAAACACCCTGTA
x01-y11	GTGTAGATGGGCGCATGGGATAGGTCACGTTGTAGCATGT
x02-y01	TTTTGCTCAGTACCAGGTATAGCC
x02-y02	CGGAATAGCAGACGTTAGTAAATGTCAACAGT
x02-y03	TTCAGCGGGTTAAAGGCCGCTTTTCATCGGAA
x02-y04	CGAGGGTACCTGCTCCATGTTACTAGGGAACC
x02-y05	GAACTGACAGAACGAGTAGTAAATCATTGTGA
x02-y06	ATTACCTTAAAGGAATTACGAGGCTACCAGAC
x02-y07	GACGATAAAATCAAAAATCAGGTCGGATTGCA
x02-y08	TCAAAAAGTGAATATAATGCTGTACGGTGTCT
x02-y09	GGAAGTTTAAAGCTAAATCGGTTGGCGGGAGA
x02-y10	AGCCTTTAACAAGAGAATCGATGATGTACCCC

x02-y11	GGTTGATAGCGGATTGACCGTAATCGTAACCGTGCATCTG
x03-y01	CCGTACTCATTAGGATTAGCGGGG
x03-y02	TAGAAAGGAGTTTTGTCGTCTTTCGTGTATCA
x03-y03	TACAGAGGCTGAGGCTTGCAGGGAAGTGAGAA
x03-y04	AAAGAGGAGTGTCGAAATCCGCGAGCAACGGC
x03-y05	TTAAGAACCCTGACGAGAAACACCCAACTTTG
x03-y06	ATAGCGAGCAGATACATAACGCCAATGCGATT
x03-y07	GAAGCCCGAACGAGAATGACCATAAAACCAAA
x03-y08	ATAACAGTCTTAGAGCTTAATTGCATTAAGAG
x03-y09	CAAGGATATAAAGCCTCAGAGCATCATTCCAT
x03-y10	AGCCCCAATGAGAGTCTGGAGCAATTTCAACG
x03-y11	CCAGTTTGAGGGGACGTCCGTGGGAACAAACGATCAGAAA
x04-y01	ACTCCTCAAGAGAAGGAGGAGGTT
x04-y02	TAGTACCGTAGCGTAACGATCTAAAACAACTA
x04-y03	AAGGAATTCCGATATATTCGGTCGCTTTGAGG
x04-y04	ACTAAAGACATCGCCTGATAAATTCAGATGAA
x04-y05	CGGTGTACAGTGAATAAGGCTTGCTGGCTCAT
x04-y06	TATACCAGCCACATTCAACTAATGAGGCTTTT
x04-y07	GCAAAAGATTTAAACAGTTCAGAAAAAGACTT
x04-y08	CAAATATCTCATTTTTGCGGATGGTGATTCCC
x04-y09	AATTCTGCTAGCAAAATTAAGCAAAAAATTTT
x04-y10	TAGAACCCCTATCAGGTCATTGCCAAACAGGA
x04-y11	AGATTGTAACAACCCGTCGGATTCACGACAGTATCGGCCT
x05-y01	AGAACCGCGTATTAAGAGGCTGAG
x05-y02	TAATTTTTAGACAGCCCTCATAGTCCACCCTC
x05-y03	TGAGGAAGCATCGCCCACGCATAAGCGAATAA
x05-y04	CGCATAGGCAACGGAGATTTGTATCTTTTTCA
x05-y05	TTGGGAAGACAAAGCTGCTCATTCAGACCAGG
x05-y06	CAGAGGGGTTGAGATTTAGGAATATCAGGACG
x05-y07	ATTCGAGCAATCCCCCTCAAATGCAGTTTTGC
x05-y08	AGATTTAGTCCTTTTGATAAGAGGGCGTTTTA
x05-y09	TTTAAATGAGGCAAGGCAAAGAATGAACGAGT
x05-y10	TATTTAAAGAGAGATCTACAAAGGTCATATAT
x05-y11	CAGGAAGATCGCACTCTAAATGTGAGCGAGTATAAGCAAA
x06-y01	ATTCTGAAACATGAAACACCCTCA
x06-y02	GAACCGCCGCCTGTAGCATTCCACTCACGTTG
x06-y03	AAAATCTCCGACAATGACAACAACTTTCCATT
x06-y04	AAACGGGTAGCGCGAAACAAAGTACTGGCTGA
x06-y07	AAAATGTTTCATAAATATTCATTGTTCAAAGC
x06-y08	GAACCAGAGAGTACCTTTAATTGCTTTGACCA
x06-y09	TTAGATACATCCAATAAATCATACCAATGCCT
x06-y10	GAGTAATGGAGGGTAGCTATTTTTTTGTAAAC
x06-y11	GTTAATATCAGCTTTCATCAACATCAGCCAGCTTTCCGGC
x07-y01	AGCCACCATATTTCGGAACCTATT
x07-y02	AGGCTCCACAGTACAAACTACAACACCCTCAG
x07-y03	TAATGCCAATACCGATAGTTGCGCCAAAAAAA
x07-y04	TCTTGACACCCAGCGATTATACCAAAAATACG
x07-y05	CTAACGGAAGAACCGGATATTCATAAGAGTAA
x07-y07	AAACTCCACAATACTGCGGAATCGTAGACTGG
x07-y08	AATGGTCAACAGGTCAGGATTAGACCGGAAGC
x07-y09	AAGATTCATAGTAGTAGCATTAACATTTCGCA
x07-y10	AATTCGCAATAAATTAATGCCGGATGTAGGTA
x07-y11	ACCGCTTCTGGTGCCGCTGGCCTTCCTGTAGCTTTGTTAA
x08-y01	GTTAATGCCCCCTGCCCCCTCATT
x08-y02	TTCAGGGACACTGAGTTTCGTCACAAAGGAGC
x08-y03	CTTTAATTTTTCTTAAACAGCTTGCTACGAAG
x08-y04	GCACCAACACACTCATCTTTGACC
x08-y09	ATAACCTGTTTAGCTAGCATCAATTCTACTAAAAAGGGTG
x08-y10	AGAAAGGCTCAACCGTTCTAGCTGTTAAATTT

x08-y11	TTGTTAAACAAAAATAATTCGCGTGAAACCAGGCAAAGCG
x09-y01	GCCACCCTTAAGTTTTAACGGGGT
x09-y02	CATTAAAGTCATAATCAAAATCACTCAGAGCC
x09-y03	TAAGCAGAAAAGGGCGACATTCAAAAATTATT
x09-y04	AGCAAGAAACAATGAAAAGAAAAG
x09-y09	CGTCAGATTGAGTGAATAACCTTGCCTTAGAATCCTTGAA
x09-y10	TAGAAGTAAAAATTATTTGCACGTAGGTTTAA
x09-y11	TGATAGCCCTAAAACAAGGAGCACTAACAACTTGAGGATT
x10-y01	AGGAGTGTACTGGTAACAGAACCG
x10-y02	CCACCCTCAGCGTTTGCCATCTTTGTGAATTA
x10-y03	TCACCGTCACCAGCGCCAAAGACATAGCCGAA
x10-y04	CAAAGTTATAAGCCCAATAATAAGTTACAAAA
x10-y05	TAAACAGCGAGCCTAATTTGCCAGTTTATTTT
x10-y06	CATCGTAGGAACAAGCAAGCCGTTAAAGGTAA
x10-y07	AGTAATTCATATAAAGTACCGACAACCGACCG
x10-y08	TGTGATAATTTAATGGTTTGAAATAACATAGC
x10-y09	GATAGCTTCATAAATCAATATATGGAATATAC
x10-y10	AGTAACAGTAGAACCTACCATATCTTAGACTT
x10-y11	TACAAACATATCTAAAATATCTTTTCGCCATTAAAAATAC
x11-y01	CACCCTCATGGCTTTTGATGATAC
x11-y02	GAGCCATTTCATAGCCCCCTTATTAGAGCCAC
x11-y03	AAACCGAGAAAATTCATATGGTTTACCGACTT
x11-y04	TTATCCCACCCACAAGAATTGAGTCCAGAAGG
x11-y05	ACCGCGCCAACGAGCGTCTTTCCACATATTAT
x11-y07	TTAAATAAAGCCAGTAATAAGAGATGTCCAGA
x11-y08	ACGCTGAGCATCTTCTGACCTAAAATAAGGCG
x11-y09	ACATCGGGTTTAATGGAAACAGTAAGATTAAG
x11-y10	ACTCGTATTGAATAATGGAAGGGTTACCTTTT
x11-y11	CGAACGAACCACCAGCAGGAATTGAGGAAGGTATTCGACA
x12-y01	CAGTAAGCGTCATACAGAGCCGCC
x12-y02	ACCAGAACTCATCGGCATTTTCGGTGGGAATT
x12-y03	AGAGCCAGTTGTCACAATCAATAGGAAACGCA
x12-y04	ATAATAACAATATCAGAGAGATAAATCCAAAT
x12-y07	CATGTTCAGGCAGAGGCATTTTCGGAATAAAC
x12-y08	ACCGGAATATATTTTAGTTAATTTAAGAGTCA
x12-y09	ATAGTGAACATTTGAATTACCTTTAGAAACAA
x12-y10	TAACGGATGTTTGGATTATACTTCTAAATCCT
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x13-y02	CCAGTAGCAGACTGTAGCGCGTTTCACCACCA
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x13-y04	TTTAACGTAGGGTAATTGAGCGCTGGAATACC
x13-y05	AAGGCTTAGCTACAATTTTATCCTATTTTTTG
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x13-y07	CTAGAAAAGCCAACATGTAATTTAGCTAATGC
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x13-y10	AATTTTAACAATATAATCCTGATTTCGCCTGA
x13-y11	GTGAGGCGGTCAGTATATATCTGGTCAGTTGGACGTTATT
x14-y01	AATGGAAAGCGCAGTCGCCAGCAT
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x14-y03	CATTAGCAGAAACGCAAAGACACCCTGGCATG
x14-y04	ATTAAGACCCCTGAACAAAGTCAGCAAAAATG
x14-y05	AAAATAGCTGCTATTTTGCACCCATCCGGTAT
x14-y06	TCTAAGAAATAATCGGCTGTCTTTCCTGTTTA
x14-y07	TCAACAATCGCCATATTTAACAACAGCCTGTT
x14-y08	TAGTATCAAAGACAAAGAACGCGAGTCTGAGA
x14-y09	GACTACCTAAGAAAACAAAATTAAAATACCAA
x14-y10	GTTACAAAGATGATGGCAATTCATAAGTTTGA

x14-y11	GTAACATTATCAAACCCTCAATCATAACACCGCCTGCAAC
x15-y01	CAGGTCAGTCCTCATTAAAGCCAG
x15-y02	AACGTCACGTAGCGACAGAATCAAGGTTGAGG
x15-y03	ACGCAGTAGTGGCAACATATAAAAAGGCCGGA
x15-y04	CAGAGAGAGAGAATTAACTGAACATCCTTATT
x15-y05	GTTTTAGCTTAAATCAAGATTAGTAGCCTTTA
x15-y06	CCTGAACAATGTAGAAACCAATCACGCGAGGC
x15-y07	ATACAAATGGGCTTAATTGAGAATAGATAAGT
x15-y08	TCCGGCTTATGCAAATCCAATCGCTATGCGTT
x15-y09	GAGGCGAATGATGAAACAAACATCTTTTAACC
x15-y10	GCGGAACAATATTCCTGATTATCAATCGCGCA
x15-y11	AGTGCCACGCTGAGAGTTGCTGAACCTCAAATATCATTTT
x16-y01	TTCACAAACAAATAAAACGATTGG
x16-y02	CCTTGATAAGCAGCACCGTAATCACAATGAAA
x16-y03	CCATCGATAAATACATACATAAAGTGTTAGCA
x16-y04	AACGTAGAGAAGCGCATTAGACGGATAACATA
x16-y05	AAAACAGGGGGAGGTTTTGAAGCCGAACCTCC
x16-y06	CGACTTGCATCCTAATTTACGAGCAGAAAAAT
x16-y07	AATATCCCCCAACGCTCAACAGTATCTTACCA
x16-y08	GTATAAAGCTATATGTAAATGCTGAGGTTGGG
x16-y09	TTATATAACCTGAGCAAAAGAAGATTATTCAT
x16-y10	TTCAATTAGAGCGGAATTATCATCAAGAAACC
x16-y11	ACCAGAAGAATCTAAAGCATCACCCCAGCAGCAAATGAAA

Sticky End staples

MR-X1-Y1	ATAGAGAGGGTTGATATAAGCGGATAAGTGCC
MR-X1-Y2	GATTTTTGCTAAACAACTTAATTTTCTGTATG
MR-X1-Y3	AGACAGCAGCGAAAGACAGGCGGGATCGTCAC
MR-X1-Y4	AGGCAGACGGTCAATCATATAGCCGGAACGAG
MR-X1-Y5	TGCTAATTTCAACTTTAATTGGGCTTGAGATG
MR-X1-Y6	CCCTATCATAACCCTCGTTATAGTAAGAGCAA
MR-X1-Y7	AAGGAGTCAGAAGCAAAGCTTTACCCTGACTA
MR-X1-Y8	TAAATATGCAACTAAAGTAGCTCAACATGTTT
MR-X1-Y9	TTAACCCTGTAATACTTTTTACCAAAAACATT
MR-X1-Y10	AAGTAGCATGTCAATCATAACGGTAATCGTAA
MR-X1-Y11	AAAGTGTAGATGGGCGCATGGGATAGGTCACG
MR-X16-Y1	GTCTTCACAAACAAATAAAACGATTGGCCTTG
MR-X16-Y2	GGAAGCAGCACCGTAATCACAATGAAACCATC
MR-X16-Y3	CCTAAATACATACATAAAGTGTTAGCAAACGT
MR-X16-Y4	GCGGAAGCGCATTAGACGGATAACATAAAAAC
MR-X16-Y5	GTTGGGAGGTTTTGAAGCCGAACCTCCCGACT
MR-X16-Y6	CACATCCTAATTTACGAGCAGAAAAATAATAT
MR-X16-Y7	TTACCAACGCTCAACAGTATCTTACCAGTATA
MR-X16-Y8	TAACTATATGTAAATGCTGAGGTTGGGTTATA
MR-X16-Y9	ATGCCTGAGCAAAAGAAGATTATTCATTTCAA
MR-X16-Y10	AACGAGCGGAATTATCATCAAGAAACCACCAG
MR-X16-Y11	TTGAATCTAAAGCATCACCCCAGCAGCAAATG

Biotin labeled staple strands

Biotin-X6-y5	CCTTCATCTACCCAAATCAACGTAAAAAATCT TT/3BioTEG/
Biotin-X6-y6	ACGTTAATTAGAAAGATTCATCAGGTAATAGT TT/3BioTEG/
Biotin-X7-y6	ATAGCGTCACAACATTA CTTTTT/3BioTEG/
Short-X7-y6	TTACAGGAAAACGAA
Biotin-X12-y5	AAGAAACGGAATCTTACCAACGCTCAATAGCA TT/3BioTEG/
Biotin-X12-y6	AGCAAATCACGGGTATTAAACCAAATAAACAA TT/3BioTEG/
Biotin-X11-y6	CGACGACAGTACCGCAC CTTTTT/3BioTEG/
Short-X11-y6	TCATCGAGAATCATT

References:

(1) Parabon Computation Inc.
(2) Liu, W.; Zhong, H.; Wang, R.; Seeman, N. C. *Angew Chem Int Ed Engl* **2011**, *50*, 264.
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