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 for staple strands for srDO were designed using the Parabon inSêquoï™ program. 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 annealed by cooling as described in the previous section.

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. 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 N2 stream. The substrates were then exposed to a low pressure UV lamp at a distance of 2-3 cm (SEN light Co. UVL-20, Hg lamp, 20 watt with 254nm power = 50μW@1 meter) for 5 minutes and finally reacted in an O2 plasma (100 mtorr ~75% O2) 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 ~1 cm² of freshly cleaved mica for 5 minutes, then the surface was washed with 400 μl of MilliQ water and immediately blown dry with N2. 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 N2.

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. 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.
Figure S12: Rectangular origami structure and staple strand positions.

Staple strand sequences

seam-01  CAGTGCGTTGAGTAACAGTGCCGTATAAAACA
seam-02  TACCGTAATAGCAAGCCCAATAGGAGGACCGGAC
seam-03  CGCTC CCCGGAACAGGAGACCACTCATG
seam-04  GAGGTGAAGTATCGGTTTATCAGCAGGTAAAT
seam-05  ATTGACGGCCGATTGAGGGAGGGATTGCTTTC
seam-06  ACACTAAACTAATACGAAAAGAGCTACCGAAG
seam-07  CCCTTTTTATAGCAATAGCTATCTAAAAGAAT
seam-08  AAAAGGTGTATTTTCATTTGGGGCCTATTAAAT
seam-09  TAATTITTCCTCTGTAATCTCGCCGACGCTG
seam-10  TATGATATCGGAGACAGTCAAATCAATTGCGT
seam-11  AGATTTTCAAAACAGAAATAAAGAACCATCAA
seam-12  AACGCCATTCAGCTCATTTTTTAACAATAGAT
seam-13  AATACATTAATAGATTAGAGCCGTCCAATAGG
seam-14  CCATTCGCCATTCAGGTCTTTAATGCGCGAAC
x01-y01  TGATATAAGCGGATAAGTGCCGTTC
x01-y02  AACAACTTAATTTTCTGTATGGGAGAGGGT
x01-y03  AAAGAGTGTATTTTCTGTATTTGGCCTATTAAAT
x01-y04  CAATCATATAGCCGGAACGAGGCGCAGCAGC
x01-y05  ACITTTAAATGGCTTGGATGGTTCAGACCGT
x01-y06  CCAAGGCGTCTATCTAATAGCATAATTTAATGCAAA
x01-y07  AGAACATTTTTAACCTGCTATATCAATATGCAA
x01-y08  CTAAAGTAGCTACACGTTATTTTAAATGTC AGA
x01-y09  ATACTTTTACAAACAAACTATATGATAGC
x01-y10  CAATCTAAAGGCTATCAGTAACACCCCTGTA
x01-y11  GTGTTAGATGGGCCATGGGATAGTCACTGTGCAGTGT
x02-y01  TTTGCTCAGTACCAGGTATAGCC
x02-y02  CAGAATACGAGCGTTAAGTTGAATGTCAACAGT
x02-y03  TTACGGGGTTAAGGCGGTGTTTCATCGGAA
x02-y04  CGAGGGTACCTGCTCCATGTTACTAGGGAACC
x02-y05  GAACTGACAGAACGAGTAGTAAATCATTGTGA
x02-y06  ATTAACCTAAAGGAAATTACAGGGCTACACGAC
x02-y07  GACGATAAAATCCCAATCGTGAGGATTGCA
x02-y08  TCAAAAAGTGAATATAATGCTGTACGGTGTCT
x02-y09  GGAAGTTTAAAGCTAAATCGGTTGGCGGGAGA
x02-y10  AGCCTTTAAAAGAGAATCGATGATGGCCACCC
x14-y11 GTAACATTATCAAACCCTCAATCATAACACCGCCTGCAAC
x15-y01 CAGGTCAGTCTCCTATTAAGGCCG
x15-y02 AACGTCAGTACGCAGGAGAACGTTATGAGGGGTT
x15-y03 ACGCAGTACGCTAGCGACAGAATCAAGGTTGAGG
x15-y04 CAGGAGAGAGATTAACTGAACATCCTTATT
x15-y05 GTTTAGCTTTAATCTGAAGATTAGTGGCTTTA
x15-y06 ATACAAATGGGCTTGATGGAATAGATAAGT
x15-y07 TCCGGCTTATGCAAATCCGATGGCTATT
x15-y08 GAGGCGAAATGAGAAACACATCTTTAACC
x15-y09 GCCGAAATATATCTCTGATTATCAGGGA
x15-y10 AGTGCCACGCTGAGAGTTGCTGAACCTCAAATATT
x16-y01 TTCACAAACAAATAAAGTGATTG
x16-y02 CTTTGATAAGCAGCACCGTAATCACAATGAAA
x16-y03 CCATCGATAAATACATACATAAAGTGTTAGCA
x16-y04 AACGTAGAGAAGCGCATTAGACGGATAACATA
x16-y05 AAAACAGGGGGAGGTTTTGAAGCCGAACCTCC
x16-y06 CGACTTGCATCCTAATTTACGAGCAGAAAAAT
x16-y07 AATATCCCCCAACGCTCAACAGTATCTTACCA
x16-y08 GTATAAAGCTATATTGAAATGCTGAGGTTGG
x16-y09 TTAATACCTGAGCAAAAGAAGATTATTCAT
x16-y10 TTCACATAGGCGGAATTTTCTGTATG
x16-y11 ACCAGAAGAATCTAAAGCATCACCCCAGCAGCAAATGAAA

Sticky End staples

MR-X1-Y1 ATAGAGAGGGTTGGTATATAAGCGGATAAGTGCC
MR-X1-Y2 GATTTTTGCTAAACAACTTAATTTTCTGTATG
MR-X1-Y3 AGACAGCAGCGGAAAGAGACGGGCTCGTAC
MR-X1-Y4 AGGCAGACGGTCAATCATATAGCCGGAACGAG
MR-X1-Y5 TGCTAATTTCAACTTTAATTGGCTTGAGATG
MR-X1-Y6 CCCATGATACCTCGTATATGGAAGAGCAGAA
MR-X1-Y7 AAGGAGTCAGAAGCAAAGCTTTACCCTGACTA
MR-X1-Y8 TTAATGGTATATATTATGCAACTAAAGTGCAACATGTT
MR-X1-Y9 TTAACCCCTGTAATATTTTTACCAAAACATTT
MR-X1-Y10 AAGTAGACATGTCATACATATAACGCTGATGA
MR-X1-Y11 AAAGTAGATGGCGCATGGAATAGCTACAG
MR-X1-Y12 GTCTTCACAAACAAATAAAACGATTGCGCTT
MR-X1-Y13 GGAAGCAGCACCGTAATCACAATGAAAACATC
MR-X1-Y14 CCTTCACAAACAAATAAAACGATTGCGCTT
MR-X1-Y15 GCGGAAGCGCATTAGACGGATAACAT
MR-X1-Y16 TACACTATGTAATGCTGAGGTGGTTTTA
MR-X1-Y17 ATGCGCTAGGCAAAAGAAGATTATTCAT
MR-X1-Y18 ACCAGGCAATTATATCAAGAAAGACCACAG
MR-X1-Y19 TTGAATCTAAAGCATACCCCAGCAGCAAATGAAA

Biotin labeled staple strands

Biotin-X6-y5 CTTCCATCTACCCCAATCAGTAAAAAATCTTTT/3BioTEG/
Biotin-X6-y6 AGCGTTAATTAGAAAGATTTTCATCAGTTATAGT TT/3BioTEG/
Biotin-X7-y6 ATAGCGTCACACATTTATCTTTTT/3BioTEG/
Short-X7-y6 TTACAGGAAAAACGAA
Biotin-X12-y5 AAGAAACGGAATCTTACCCCAACGCTTACATTTAGCA TT/3BioTEG/
Biotin-X12-y6 AGCGAAATCACGGATTTAAACCAAATTAAACAA TT/3BioTEG/
Biotin-X11-y6 CGCACAGGTACCCGAC CTTTTTT/3BioTEG/
Short-X11-y6 TACAGGAAAAACGAA

S14
References:

(1) Parabon Computation Inc.