Supporting Information for "Stepwise and Reversible Nanopatterning of Proteins on a DNA Origami Scaffold"

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Detailed Experimental Procedures

Materials. Unmodified staple DNA strands were purchased from Integrated DNA Technologies (Coralville, IA) and used without further purification. Biotin-triethylene glycol (TEG)-modified strands were purchased from Japan Bio-Service (Japan) and purified by denaturing PAGE and reverse-phase HPLC. M13mp18 ssDNA (Takara, Japan) was used for the scaffold of DNA origami. Streptavidin was purchased from Sigma.

Formation of DNA origami and size-selective capture of a SA Tetramer in a well. The punched DNA origami motifs were formed with M13mp18 ssDNA as the scaffold (10 nM) and staple strands (100 nM for each strand including anchor strands) in a solution containing 40 mM Tris, 20 mM acetic acid, 2 mM EDTA, and 12.5 mM magnesium acetate (1x TAE/Mg buffer, 50 μL). This mixture was cooled from 90˚C to 25˚C at a rate of -1.0˚C/min using a PCR thermal cycler. Annealed mixture of the biotinylated origami (50 μL) was ultrafiltrated and washed twice with 500 μL of 1xTAE/Mg on a micro spin column (Amicon Ultra 100K) to remove excess staple strands. Then SA (3 eq. to the number of wells; for example, 240 nM for Fig. 2a in the manuscript) was added to the solution.

Removal of SA Tetramers from Selected Wells. To trigger strand displacement and remove SA from a selected well, 10 eq. of unset strands to the target SA were added to the solution. After being kept at r. t. overnight (usually the strand displacement is completed within 2 h), the mixture was treated on a micro spin column packed with GPC media (Microspin S-400HR, GE Healthcare, UK), which was equilibrated with 1x TAE/Mg buffer in advance, to remove SA/dsDNA complexes from the system.

Stepwise Introduction of SA Tetramers to Selected Wells. Punched origami was first annealed with staple strands in the absence of anchor strands. A combination of anchor strands for the target well (2 eq.) were added to the solution, and the mixture was kept at 37˚C for 5 min, and then cooled to 25˚C at a rate of -1.0˚C/min using a PCR thermal cycler. The mixture was ultrafiltrated to remove excess anchor strands, and then 2 eq. of SA was added. The mixture was again ultrafiltrated to remove excess SA from the system. This procedure was repeated three times with appropriate combination of anchor strands for stepwise introduction of SA tetramers to form a nanoarray with four SA tetramers.

Removal and Re-introduction of a SA Tetramer in a Well. To remove SA from a preformed SA nanoarray, unset strands (10 eq. to the target SA) for the 5th well were added to the solution. The mixture was kept at r. t. overnight, and then treated on a micro spin column packed with GPC media. After anchor strands without toehold were added, the mixture was kept at 37˚C for 5 min, and then cooled to 25˚C at a rate of -1.0˚C/min using a PCR thermal cycler. The mixture was ultrafiltrated to remove excess anchor strands, and SA (3 eq. to the well) was added to the solution again.

AFM imaging. AFM imaging of DNA origami was performed on a SPA-300HV system (SII, Japan). DNA origami solution (2 μL) was deposited on a freshly cleaved mica, additional 1x TAE/Mg buffer (200 μL) was added, and imaging was done using the fluid DFM scanning mode with a BL-AC40TS tip (Olympus, Japan).

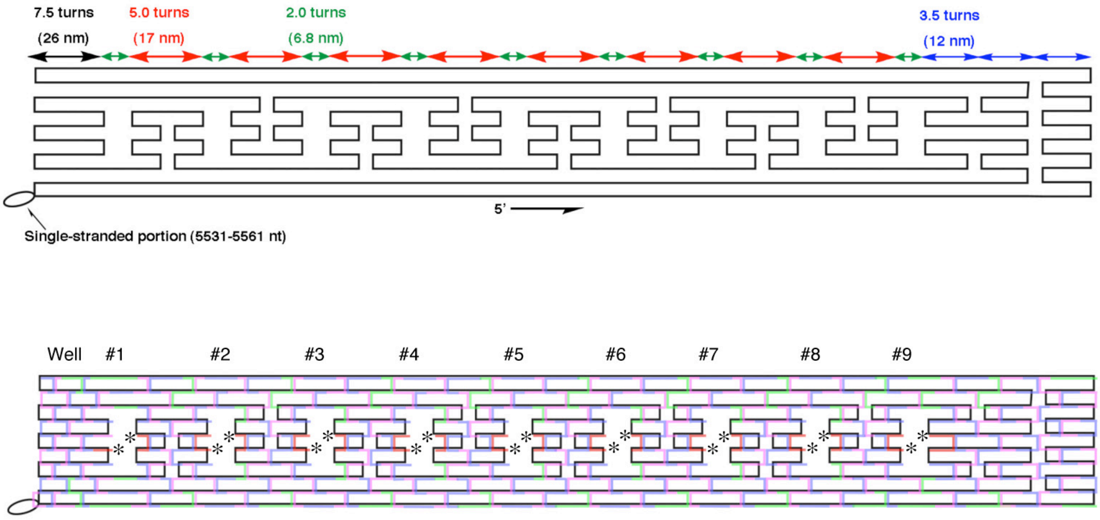

Figure S1. Folding pattern of the scaffold in the punched DNA origami.

Table S1. Sequences of the staple strands.

2stick1, GCTCAACATGTTTTAATGAATATA
2stick2, ATGCTGTACAAAGACAAAAGGGCGTATGGTTT
2stick3, ACCAGCGCAAAAGAACTGGCATGATTAATAACG
2stick4, GAATACCCGATAACCCACAAGAATGCGCTAAT
2stick5, ATCAGAGAAACCACCACACCCGCCGAGCGGGC
2stick6, GCTAGGGCGCTGGCGCGT
2stick7, GAAAGGAAGGGAAGAAAGCGAAAGGCGCTTAA
2stick8, TGCGCCGCGTCAGAGGGTAATTGATGAGTTAA
2stick9, GCCCAATAAACCGAGGAAACGCAATAAGACTC
2stick10, CTTATTACATCAATAGAAAATTCAACATTCAA
2stick11, CCGATTGACTTAGAGCTTAATTGCATATGCAA
2stick12, CTAAAGTACGGTGTCTGGAAGTTTTCATTTTT
2stick13, GCGGATGGGGGAGGGAAGGTAAATAGTTTATT
2stick14, TTGTCACAGCAGTATGTTAGCAAAAAAGTTAC
2stick15, CAGAAGGAATAAGAGCAAGAAACAGAACACCC
2stick16, TGAACAAATACAGGGCGCGTACTACGTGGCGA
2stick17, CCGGCGAATGGTTGCTTTGACGAGGACGGGAG
2stick18, AATTAACTATGAAATAGCAA
2stick19, AGATAGCCGAACCGTAGAAAATAC
2stick20, CACCACGGAATAATTGACGG
2stick21, AAATTATTTCCTTTTGATAAGAGGCATTCCAT
2stick22, ATAACAGTTGATTCCCAATTCTGCGAACGAGTAGATTTAGTTTGAC
2stick23, CATTAGATGGAAGCAAACTCCAAC
2stick24, AGGTCAGGATTAGAGAGTACCT
2stick25, TTAATTGCCATTAAAGGTGAATTA
2stick26, TCACCGTCACCGACTTGAGC
2stick27, CATTTGGGAAGCGTCTTTCC
2stick28, TATAAAAGAAACGCAAAGA
2stick29, ATACATAAAGGTGGCAACA
2stick30, AGAGCCTAATTATCCCAATC
2stick31, CTTTTTAAGAAAAGTAAGC
2stick32, TAGCTATCTTACCGAAGCC
2stick33, CAAATAAGAAAGCAGCCTTT
2stick34, ACAGAGAGAATAACATAAAA
2stick35, ACAGGGAAGCGCATTACACGTATA
2stick36, ACGTGCTTTCCTCGTTAGAATCAGAGCGGG
2stick37, AGCTAAACGCACTAAATCGGAACC
2stick38, CTAAAGGGAGCCCCCGATTTAGAGCTTGACGGGGAAAG
2stick39, GCCGTAAAAGGAGGCCGATTAAAGCGTCAAAA
2stick40, ATGAAAATACGATTTTTTGTTTAAGAACGCGA
2stick41, ATATTATTTTGCCAGTTACAAAATTTACCAAC
2stick42, GCTAACGATTAGAGCCAGCAAAATCAAAGCGA
2stick43, ACCAGACCACATTTCGCAAATGGTCAATAACC
2stick44, TGTTTAGCGTTTTAATTCGAGCTTCACCAGT
2stick45, AGCACCATTAATTTTATCCTGAATCTAAATCAA
2stick46, GATTAGTTGGAGGTTTTGAAGCCTAAACAGCC
2stick47, GGCGTTTTTTATCCGGTATTCTAAGGATTTTA
2stick48, GACAGGAAAATCAAGTTTTTTGGGGTCGAGGT
2stick49, ATCACCCACGGTACGCCAGAATCCATCAGATA
2stick50, TAGAAGGCAGCGAACCTC
2stick51, CCGACTTGCGGCTATTTTGC
2stick52, ACCCAGCTACACCATTAG
2stick53, CAAGGCCGAGACTTCAAATATCGCTATATTTT
2stick54, CATTTGGGGCGCGAGCTGAAAAGGT
2stick55, GGCATCAACAAAAAGATTAAGAGGA
2stick56, AGCCCGAAGAAACGTCACCAATG
2stick57, AAACCATCGAAGTTTGCCTT
2stick58, TAGCGTCAGATAGCCCCCTT
2stick59, ATTAGCGTTTCATTACCGCG
2stick60, CCCAATAGCAAGCAATGAGAAGT
2stick61, GTTTTTATAATCAGTGACGTCTATCAGGGCGA
2stick62, TGGCCCACTACGTGAACC
2stick63, CGAAAAACGGCCACCGAGTAAAAGTTTTCATC
2stick64, GTAGGAATGCCATCTTTTCATAATTCGGCATT
2stick65, TTCGGTCACTGTAGCGCGTTTTCAACCCTCAG
2stick66, CAGAATCATAGCAGCACCGTAATCGCAAAGCG
2stick67, GATTGCATTTCTACTAATAGTAGTAGCATTAA
2stick68, CATCCAATACTATTATAGTCAGAACCACCACC
2stick69, AGAGCCGCAGAGCCGCCACCAGAAAGTAGCGA
2stick70, AACCGCCACTCCCTCAGAGCCGCCCAAAATCA
2stick71, CCGGAACCAAGCAAGCCGTTTTTAAGTCTGTC
2stick72, CATCACGCAACGTGGACTCCAACGTCAAAGGG
2stick73, TATTAAAGAAATTAACCGTTGTAGCCCGCACTCA
2stick74, TCGAGAACAGAGCCACCA
2stick75, CCGGAACCGCCCCTCAGAGC
2stick76, CACCACCCTCCGCCAGCA
2stick77, TTGACAGGAATCAGGTCTTTACCCTGAAATCATA
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2stick79, TAAGCAATAAACGAGAATGACCATA
2stick80, AATCAAAAGGTTGAGGCAGGTCA
2stick81, GACGATTGGCTGTTTATCAA
2stick82, CAATAGATAAATTTACGAGC
2stick83, ATGTAGAAACCCAAGAACGG
2stick84, GTATTAAACCAAGTAAATACTTC
2stick85, TTTGATTAGTAATAACATTGAGTGTTGTTCCA
2stick86, GTTTGGAACAAGAGTCCAC
2stick87, AGATAGGGTCACTTGCCTGAGTAGGTCTTTCC
2stick88, TTATCATTCAATCAATAATCGGCTAATTTAGG
2stick89, CCATCCTAGTCCTGAACAAGAAAATAATGCAG
2stick90, AACGCGCCCTTGATATTCACAAACCTTTAAAC
2stick91, AGTTCAGAAAAGCCTCAGAGCATAAAGCTAAA
2stick92, TCGGTTGTGAATCCCCCTCAAATGAAATAAAT
2stick93, CCTCATTAAAACAACATGTTCAGCCAAAAGGT
2stick94, AAAGTAATGAATATAAAGTACCGAATAATATC
2stick95, CAGAGGCAAACAACGCCAACATGTAAGAACTC
2stick96, AAACTATCCTTATAAATCAAAAGAATAGCCCG
2stick97, CAAAATCCGGCCTTGCTGGTAATAGAGAATCG
2stick98, CCATATTTTTTTCGAGCC
2stick99, AGTAATAAGATCTGTCCAGA
2stick100, CGACGACAATAAGCCAGA

2stick101, ATGGAAAGGTCATAAATATTCATTACCAAAAA
2stick102, CATTATGACCCTGTAATACTTTTGC
2stick103, GGGAGAAGGGATAGCGTCCAATACT
2stick104, GCGGAATCCGCAGTCTCTGAATT
2stick105, TACCGTTCCAGAGTGTACTG
2stick106, GTAATAAGTTCGTATAAACA
2stick107, GTTAATGCCCCAACGCTCAA
2stick108, CAGTAGGGCTTAATTTCCAGAAC
2stick109, AATATTACCGCCAGCCAGAAAATCCTGTTTGA
2stick110, TGGTGGTTCCGAAATCGG
2stick111, CAGCAGGCTTGCAACAGGAAAAACCTTACCAG
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2stick113, ACAGTGCCTTAACGGGGTCAGTGCGGATTAGG
2stick114, TGATACAGGTAAGCGTCATACATGGTAAAATG
2stick115, TTTAGACTCCTTTATTTCAACGCAAGGATAAA
2stick116, AATTTTTAGCCAGAGGGGGTAATAGAGGGTTG
2stick117, ATATAAGTGGATAAGTGCCGTCGAGCTTTTGA
2stick118, ATTAGCGGAGACTCCTCAAGAGAACCTATTAT
2stick119, TCTGAAACATGCGTTATACAAATTGCTCATGG
2stick120, AAATACCTCAAGCGGTCCACGCTGGTTTGCCC
2stick121, AGTTGCAGACATTTTGACGCTCAATAGCCTGTTT
2stick122, AGTATCATATGAAAGTAT
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2stick124, AGTACCAGGCATAGCCCG
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2stick126, ATATATTTTAAATGCAATGCCTGAGT
2stick127, AATGTGTAATAAAAACCAAAATAGC
2stick128, GAGAGGCTTATCACCGTACTCAG
2stick129, GAGGTTTAGTAGAAAACTTT
2stick130, TTCAAATATAAATGGTTTGA
2stick131, AATACCGACCCACCGGAATC
2stick132, ATAATTACTAGAAAACGTCTGAA
2stick133, ATGGATTATTTACATTGCAGCTGATTGCCCTT
2stick134, CACCGCCTGGCCCTGAGAG
2stick135, ACGGGCAAGCAGATTCACCAGTCAGTTAAATA
2stick136, AGAATAAAGTGTGATAAATAAGGCAATAGTGA
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2stick138, AGAACGCGACCGCCACCCTCAGAACGTTTACC
2stick139, AGACGACGGGTAAAGATTCAAAAGGGTGAGAA
2stick140, AGGCCGGAACACTATCATAACCCTCCGCCACC
2stick141, CTCAGAACGATGCAAATCCAATCGGGCTTAGG
2stick142, TTGGGTTATACCTTTTTAACCTCCCTTCTGAC
2stick143, ATTTATCAGACGCTGAGAAGAGTCCACGACCA
2stick144, GTAATAAAGTGGTTTTTCTTTTCACCAGTGAG
2stick145, GCGCCAGGAGGGACATTCTGGCCACGATAGCT
2stick146, TAGATTAAAAATCATAGG
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2stick148, TGTAAATGCTCGCCACCC
2stick149, TCAGAGCCAGGCATAGTAAGAGCAGACAGTCA
2stick150, AATCACCATCAATATGATATTCAAC
2stick151, CGTTCTAGATACATAACGCCAAAAG
2stick152, GAATTACGACCACCCTCATTTTC
2stick153, AGGGATAGCAAGTTTCGTCA
2stick154, CCAGTACAAATCATAGTTAG
2stick155, CGTAACGATCTCCCTTAGAA
2stick156, TCCTTGAAAACATAGACAGAGAT
2stick157, AGAACCCTTCTGACCTGCCAACGCGCGGGGAG
2stick158, AGGCGGTTTGCGTATTGG
2stick159, TGAATCGGAAAGCGTAAGAATACGCGCTATTA
2stick160, ATTAATTTTAAAGTTTTGTCGTCTATTCCACA
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2stick177, AGCCTTTAATCGAATTATTC
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2stick179, AATTAATTACATGGAAACAG
2stick180, TACATAAATCAATATCTTTAATG
2stick181, CGCGAACTGATAGCCCTACATTAATTGCGTTG
2stick182, CGCTCACTGCCCGCTTTCC
2stick183, GCTAACTCAAAACATCGCCATTAATGAATTAC
2stick184, CTTTTTTAATTTAACAATTTCATTGCGTAGAT
2stick185, CAAACATCCCTGAGCAAAAGAAGACAAAATCG
2stick186, CGCAGAGGTGTATCGGTTTATCAGCGTTAATA
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2stick188, AACGGTAATGGGAAGAAAAATCTACTTGCTTT
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2stick190, AGAAACAAAGTAACAGTACCTTTTTGATGAAA
2stick191, TTTCAGGTCAGAAATAAAGAAATTAAATACCG
2stick192, AACGAACCAAGCCTGGGGTGCCTAATGAGTGA
2stick193, AAAGTGTAACCAGCAGAAGATAAATTATTTGC
2stick194, ACGTAAAATTAACGTCAG
2stick195, ATGAATATACTAACGGATTC
2stick196, GCCTGATTGCATTTCTTA
2stick197, AACAGCTTATACCAGTCAGGACGTTCGTAAAA
2stick198, CTAGCATGTCAATCATATGTACCCC
2stick199, GGTTGATAATGCGATTTTAAGAACT
2stick200, GGCTCATTGATACCGATAGTTGC

2stick201, GCCGACAATGTTCGGTCGCT
2stick202, GAGGCTTGCACCCTCAGCAG
2stick203, CGAAAGACAGAGGGTTAGAA
2stick204, CCTACCATATCAAAAACAGAGGT
2stick205, GAGGCGGTCAGTATTAACACAATTCCACACAA
2stick206, CATACGAGCCGGAAGCAT
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2stick209, GATCGTCAGGGAGTTAAAGGCCGCCATTAAAC
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2stick213, AAAAGAATGCACCAACCTAAAACGACGCATAA
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2stick215, TACAGAGGTGATTGTTTGGATTATGCCACGCT
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2stick228, TGCTCCATGTTACTTAGCCGGCCGAACGTTATTAATTTTAAA
2stick229, AGTTTGAGTAACATTATCATTAATTATCATCATATTC
2stick230, CTGATTATCACCTTGCTGAACCTCAA
2stick231, ATATCAAACCGGTCGACTCTAGAGGA
2stick232, TCCCCGGGTACCGAGCTCGAATTCGTAAT
2stick233, TGCCTGCACTCAATCAATATCTGGCCACCAGA
2stick234, AGGAGCGGTTGCGGAACAAAGAAAGTATTAAA
2stick235, TCCTTTGCAACGAGGCGCAGACGGTATCATCG
2stick236, CCTGATAAGTACAACGGAGATTTGCATTCAGT
2stick237, GAATAAGGTTTTTGTTAAATCAGCTCATTTTT
2stick238, TAACCAATACGTAACAAAGCTGCTTGGCTGAC
2stick239, CTTCATCAGACCAGGCGCATAGGCTCAATCAT
2stick240, AAGGGAACAACAATTCGACAACTCTAGATTAG
2stick241, AGCCGTCAGAGCACTAACAACTAATCAGTTGG
2stick242, CAAATCAAACGACGGCCAGTGCCAAGCTTGCA
2stick243, GTTGTAAACAGTTGAAAGGAA
2stick244, ATCTTTAGATAGATAATACAT
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2stick247, CCAAATCAAGGAACGCCATCA
2stick248, AAAATAATGGATATTCATTAC
2stick249, CAAGAACCAGGACAGATGAAC
2stick250,
2stick251, TTGAGGATGGTTATCTAAAAT
2stick252, TTGAGGAATCCCAGTCACGAC
2stick253, GGTAACGCCAGGGTTTCCTCTTCG
2stick254, CTATTACGGGGCGATCGGTGCGGGTGGTGCCG
2stick255, GAAACCAGTTTCCGGCACCGCTTCTTAGAAGTATTAG
2stick256, TGAGGGGAATCGTAACCGTGCATCTGGGAACA
2stick257, AACGGCGGCCCGTCGGATTCTCCGTCGCGTCT
2stick258, GGCCTTCCTGTAGCCAGCTTTCATCAACA
2stick259, GAGCGAGTAACAAATTGACCG
2stick260, TGTAGATGGGCGCCGACGACA
2stick261, CACTCCAGCCAGCGCAAAGCG
2stick262, CAACTGTTGGGAACCAGCTGG
2stick263, TGCTGCAAGGCGATTAAGTTG
2stick264, CGAAAGGGGGATGAGGCTGCG
2stick265, CCATTCGCCATTCGAAGATCG
2stick266, GTATCGGCCTCAGCACGTTGG
2stick267, TAATGGGATAGGTTTAAATGT

Table S2. Anchor and unset strands with toehold.

For the 4th well:

2stick99THbio	5′-Biotin-TEG-AGTAATAAGATCTGTCCAGA GACCGTCG-3′
2stick106THbio	5′-Biotin-TEG-GTAATAAGTTCGTATAAACA GACCGTCG-3′

For the 5th well:

2stick123THbio	5′-Biotin-TEG-TAAGAGGCTGGGTTTTGCTC GCACGCTG-3′
2stick130THbio	5′-Biotin-TEG-TTCAAATATAAATGGTTTGA GCACGCTG-3′

For the 6th well:

2stick147THbio	5′-Biotin-TEG-TCTGAGAGACTATAACTATA GCCAGCGT-3′
2stick154THbio	5′-Biotin-TEG-CCAGTACAAATCATAGTTAG GCCAGCGT-3′

Unset strands:

2stick99comp	CGACGGTC TCTGGACAGATCTTATTACT
2stick106comp	CGACGGTC TGTTTATACGAACTTATTAC
2stick123comp	CAGCGTGC GAGCAAAACCCAGCCTCTTA
2stick130comp	CAGCGTGC TCAAACCATTTATATTTGAA
2stick147comp	ACGCTGGC TATAGTTATAGTCTCTCAGA
2stick154comp	ACGCTGGC CTAACTATGATTTGTACTGG

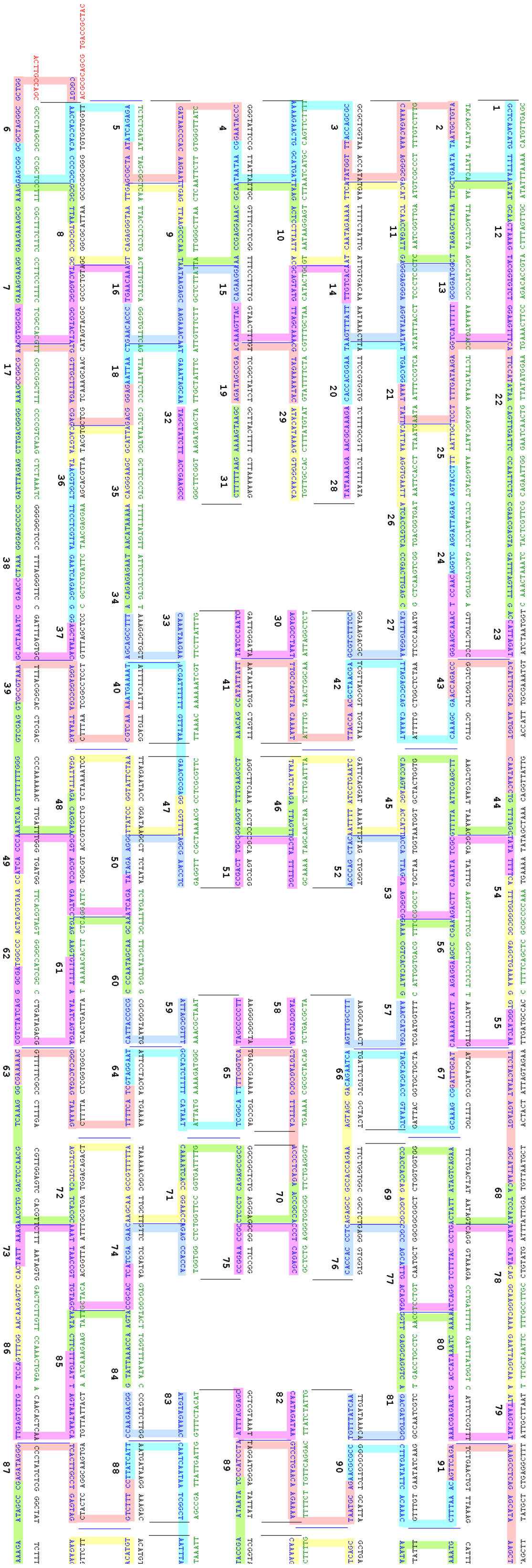


Figure S2. Detailed structure of the punched DNA origami with toehold anchor strands (1/3).

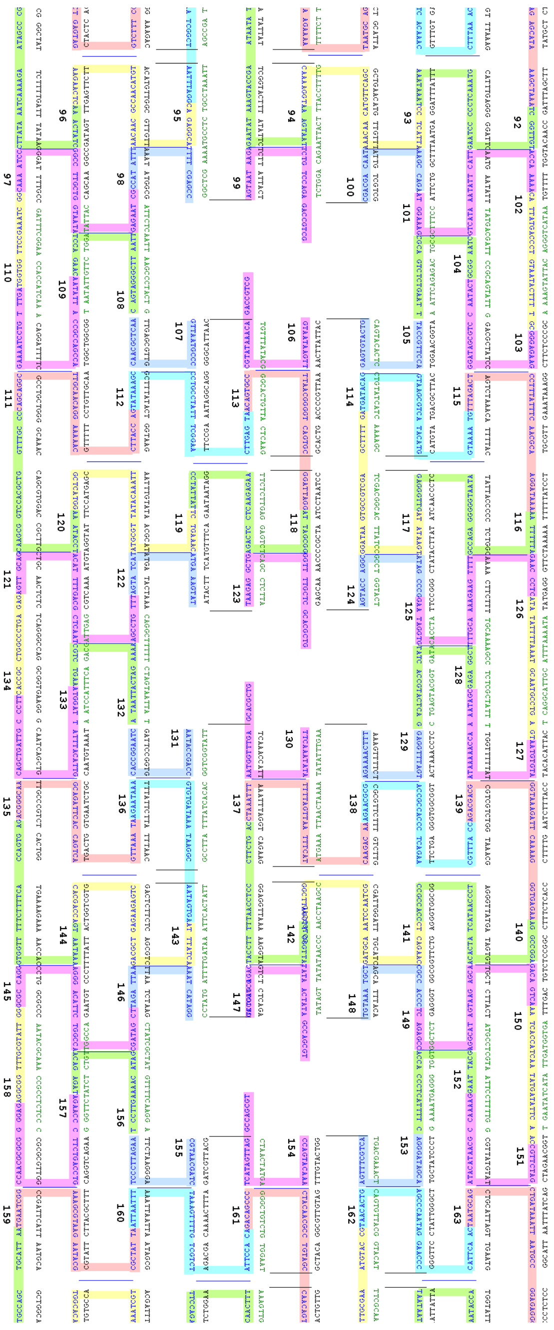


Figure S3. Detailed structure of the punched DNA origami with toehold anchor strands (2/3).

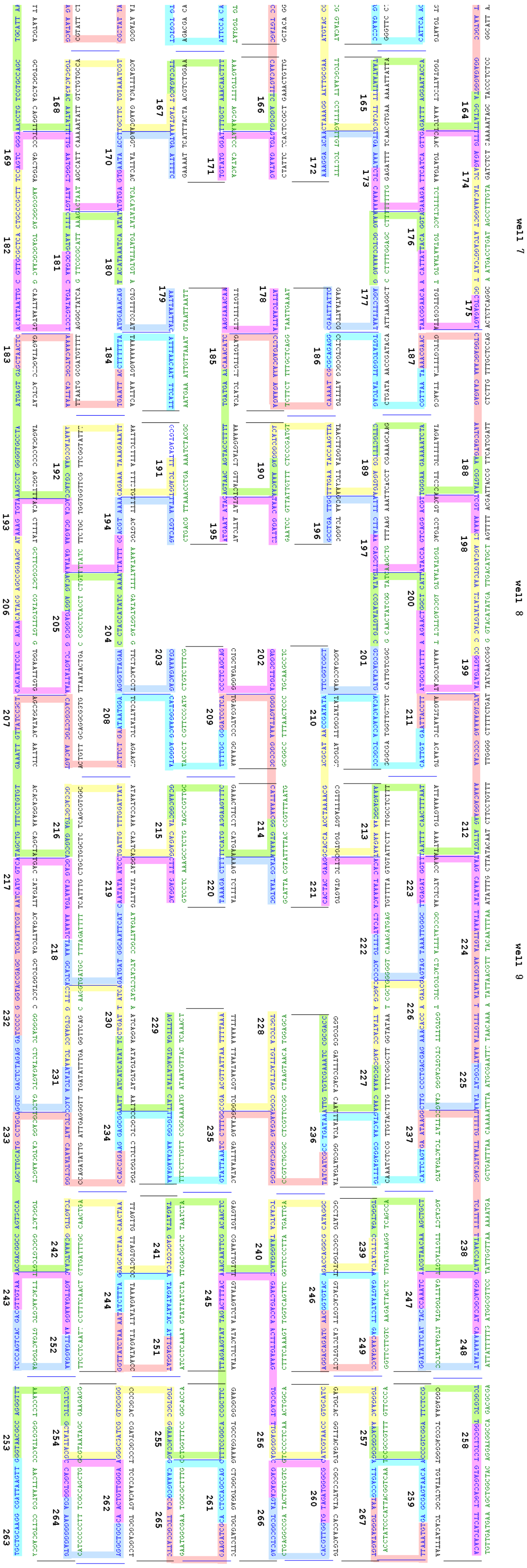
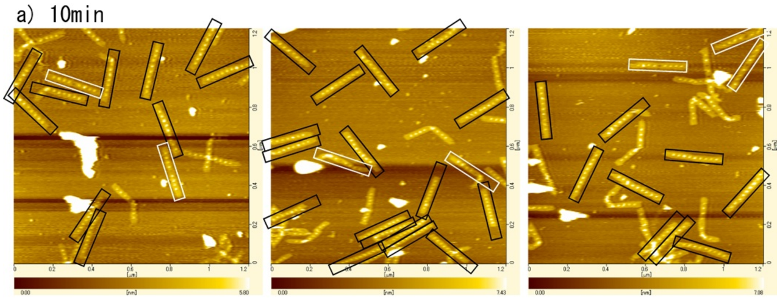
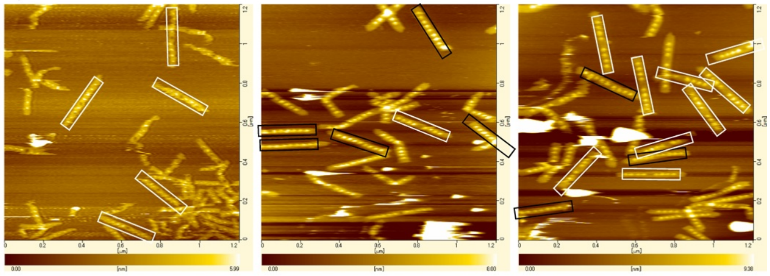


Figure S4. Detailed structure of the punched DNA origami with toehold anchor strands (3/3).

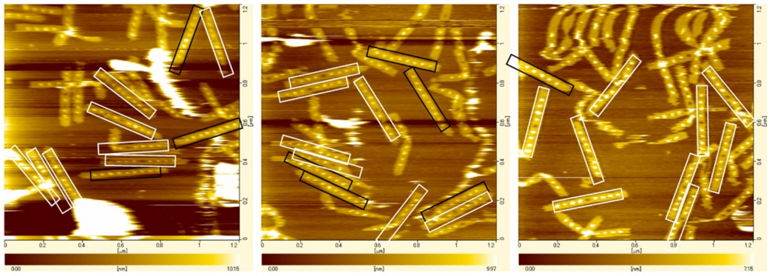
a) 10min



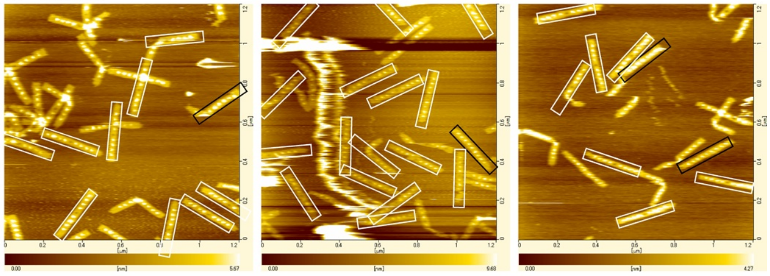
b) 30min



c) 60min



d) 120min



e) 2days

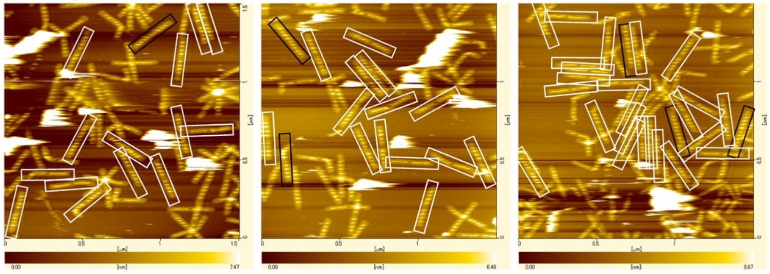


Figure S5. Time course of the removal of SA from the 5th well. White boxes indicate DNA origami with no SA in the 5th well, where as black boxes does one with SA. (a) 83.7% yield (36/43), (b) 34.8% (8/23), (c) 28.6% (10/35), (d) 11.1% (4/36), (e) 10.3% (6/58).

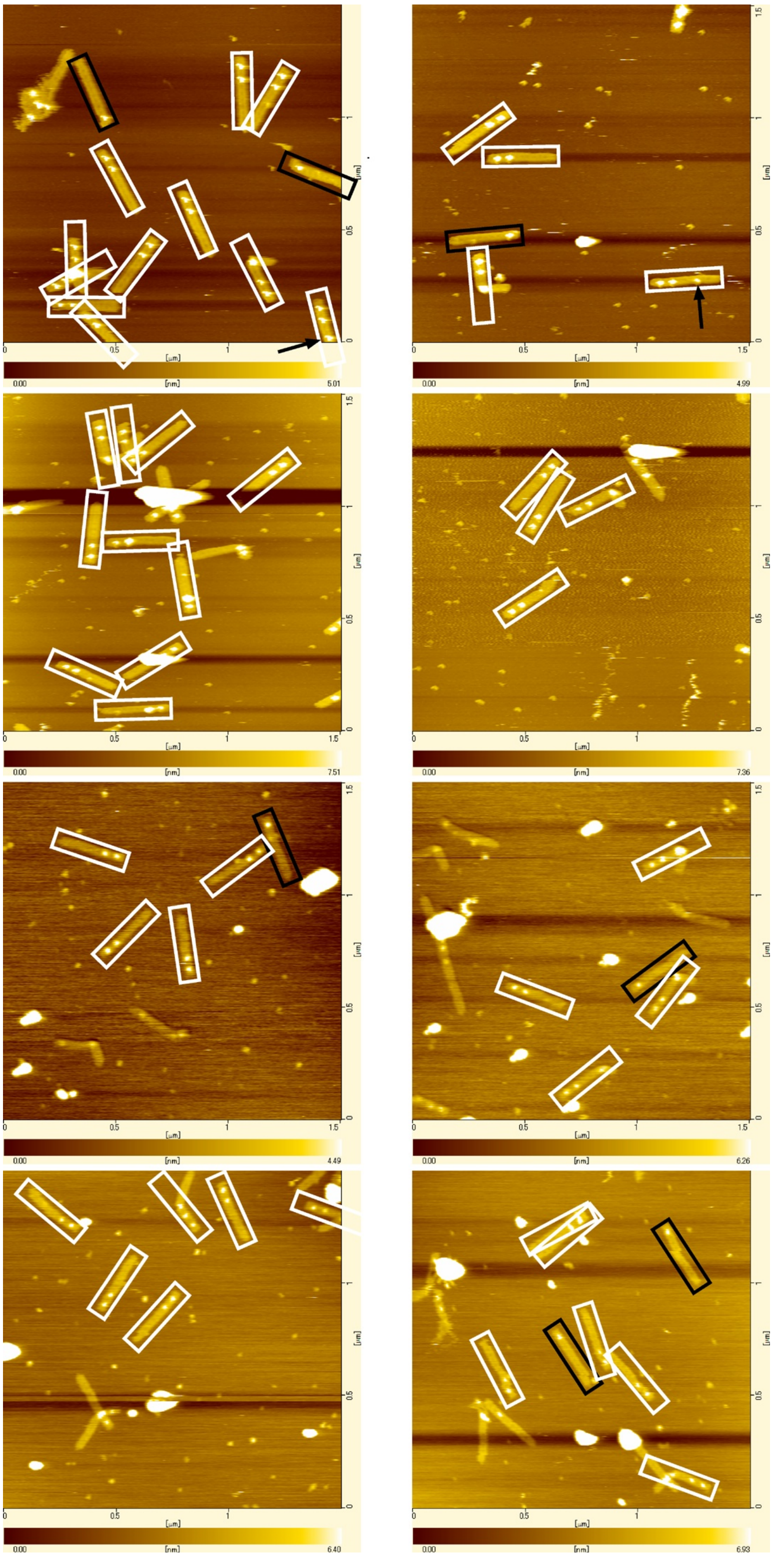


Figure S6. Quantification of the efficiency in the stepwise introduction. Totally 56 motifs were examined and 49 motifs hold SA in the 3rd well (indicated with white boxes). Black boxes indicate motifs with no SA in the targeted 3rd well. Two motifs were found with additional SA in the 6th well (indicated by the arrows).

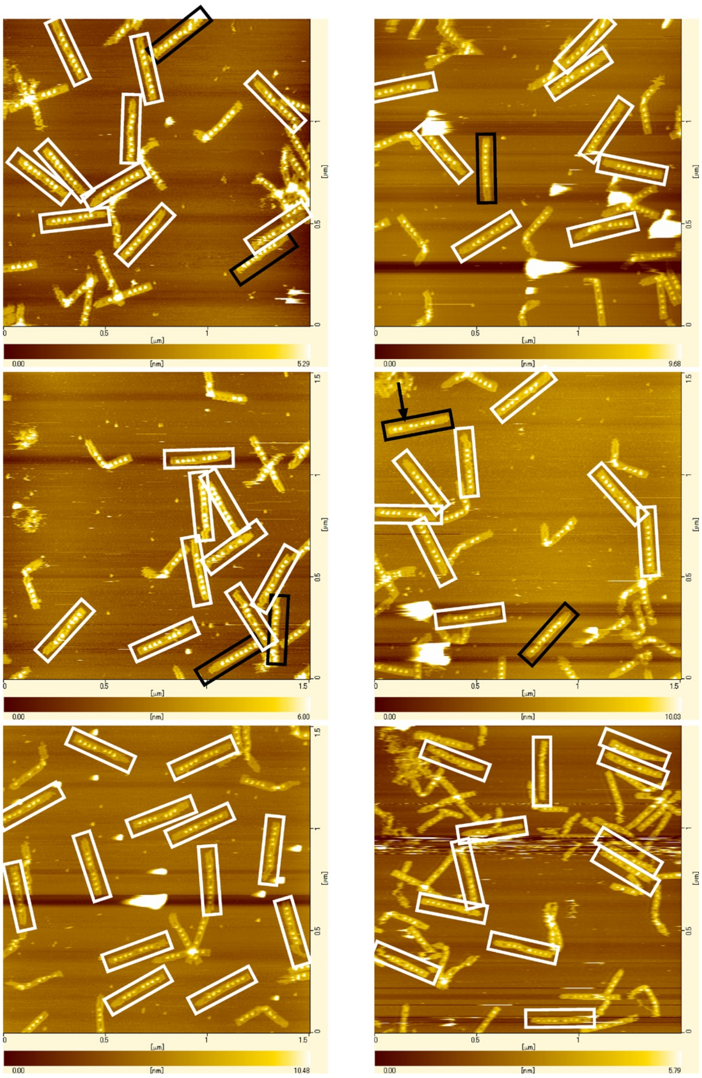


Figure S7. Quantification of the removal efficiency in the combination of removal and introduction. Totally 67 motifs were examined and only 7 motifs contained SA in the 5th well (indicated with white boxes). Black boxes indicate motifs with SA in the 5th well. Lack of SA in an unintended well was found in one motif (indicated by the arrow).

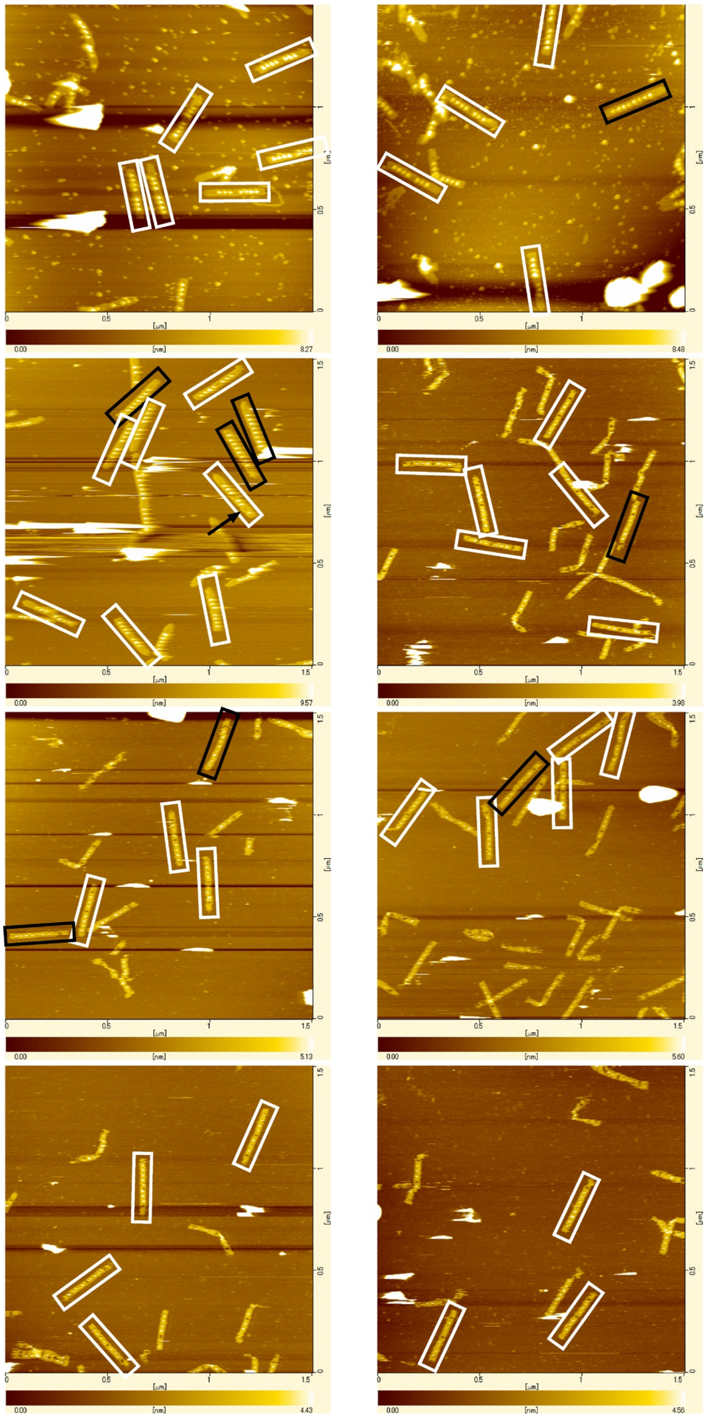


Figure S8. Quantification of the introduction efficiency in the combination of removal and introduction after the addition of anchor strands. To confirm that the 5th well was still empty after anchor strands were added, totally 46 motifs were examined. Only 8 motifs contained SA in the 5th well (indicated with white boxes). Black boxes indicate motifs with SA in the 5th well. Lack of SA in an unintended well was found in one motif (indicated by the arrow).

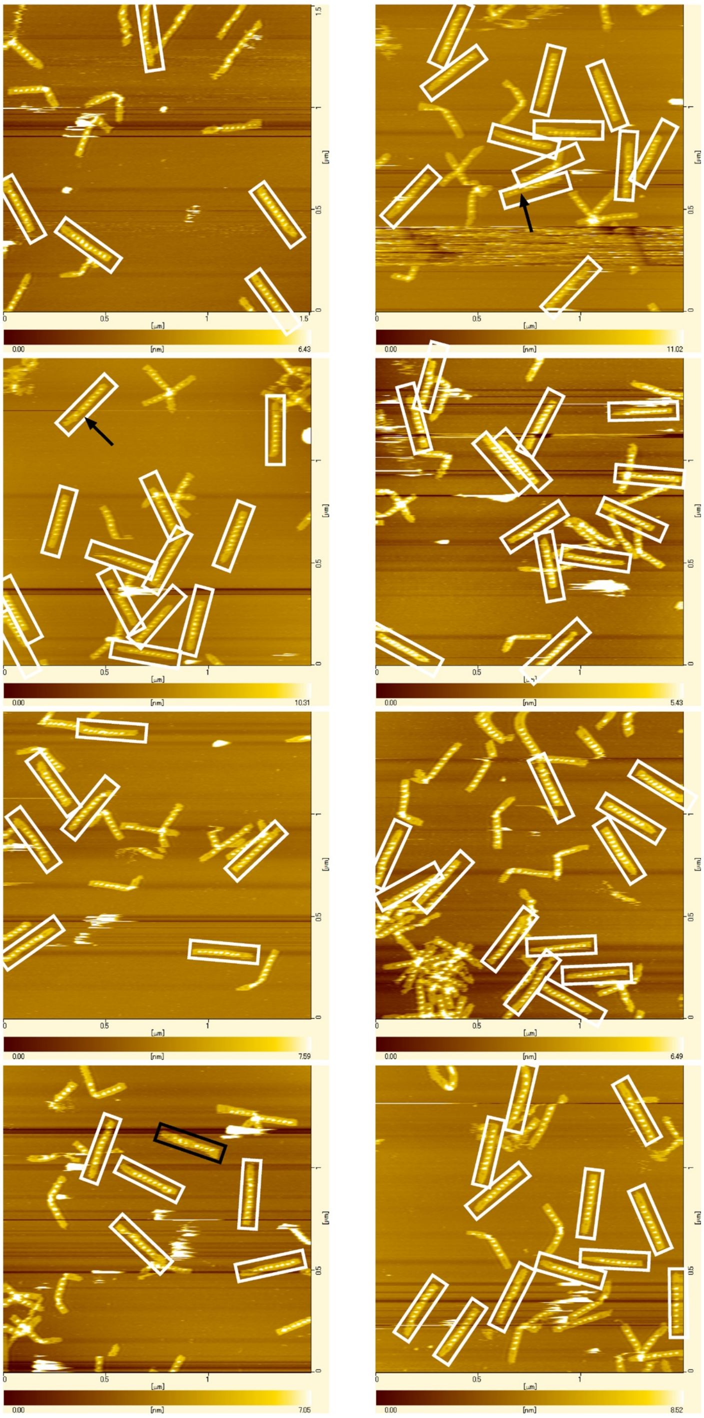


Figure S9. Quantification of introduction efficiency at the final step in the combination of removal and introduction. Totally 70 motifs were examined and 69 motifs contained SA in the 5th well (indicated with white boxes). Black box indicates a motif with no SA in the 5th well. Lack of SA in an unintended well was found in two motifs (indicated by the arrows).