Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Transfer of a Self-Assembled Protein Pattern from DNA Origami to a Functionalized Substrate

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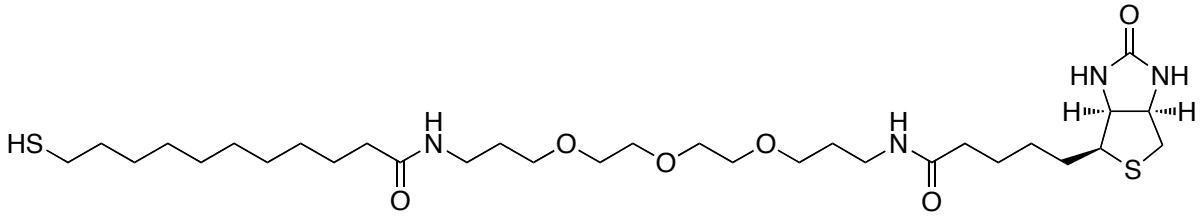
1) Materials

Au substrates Mica substrates with an epitaxial Au(111) layer 300 nm thick were purchased from Georg Albert PVD, Heidelberg, Germany and annealed in a flame prior to use. Biotinylated hexa(ethylene glycol) undecane thiol (**1**) (Nanoscience Instruments, Phoenix Az, US) was used as received. All unmodified DNA sequences were purchased from DNA Technology A/S in Risskov (Denmark). HPLC purified biotinylated sequences containing the disulfide linker were purchased from Metabion AG (Germany). M13mp18 viral DNA was purchased from New England Biolabs, Inc. Microcon centrifugal filter YM-100 devices (100,000 MWCO, catalogue number: 42413) were purchased from Millipore. The water used was purified on a MilliQ Biocell System.



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2) DNA Origami Design

A schematic representation of the triangle DNA origami structure is shown in Figure S1. The numbering of the staple strands is similar to the original paper.[1] Specific points were chosen on the DNA origami for functionalisation and, when reacted with SAMs, should produce clear, discernible patterns on the surfaces, visible in AFM images.

Supporting Information

Figure S1: Schematic illustration of the triangle DNA origami.

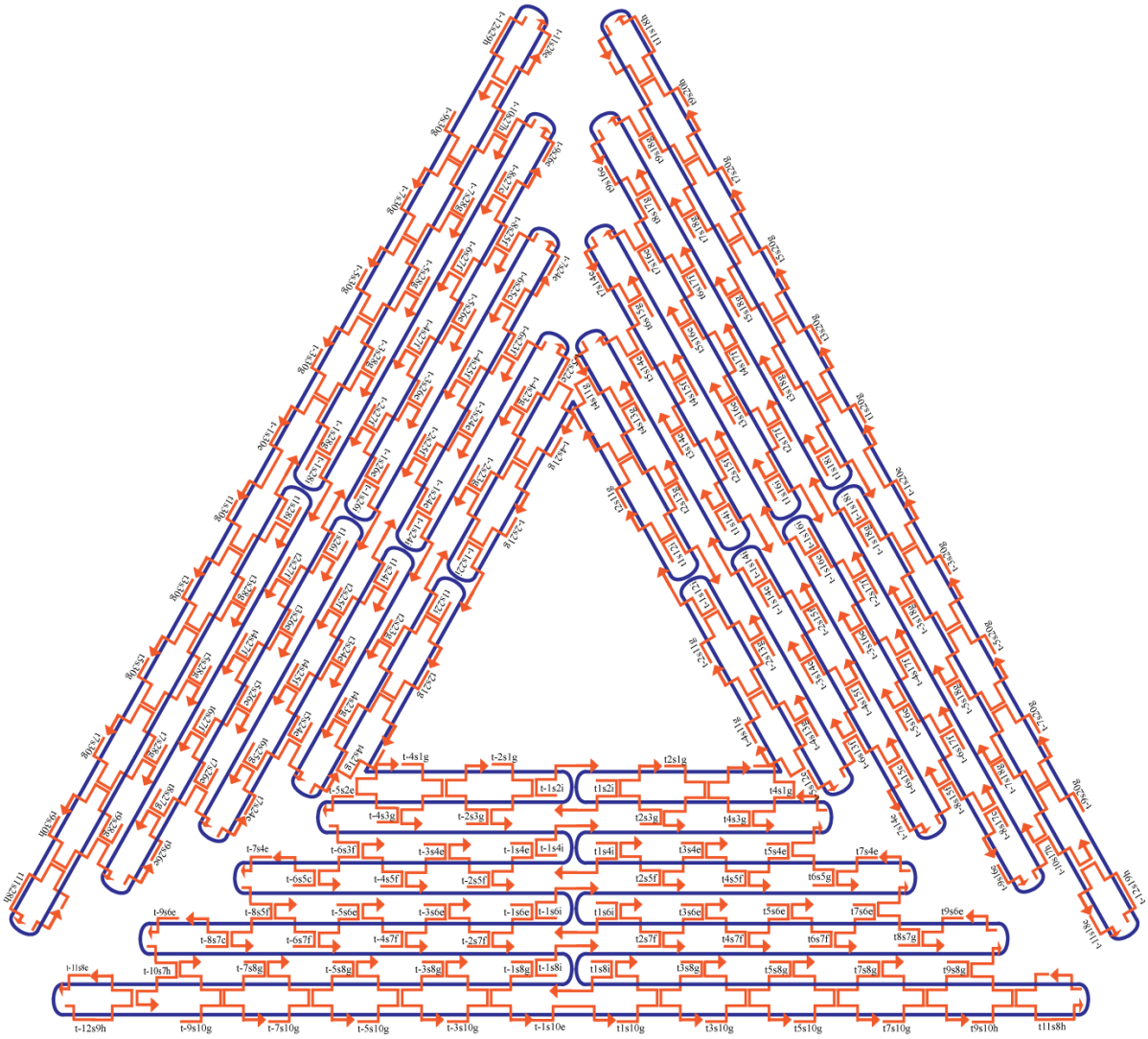
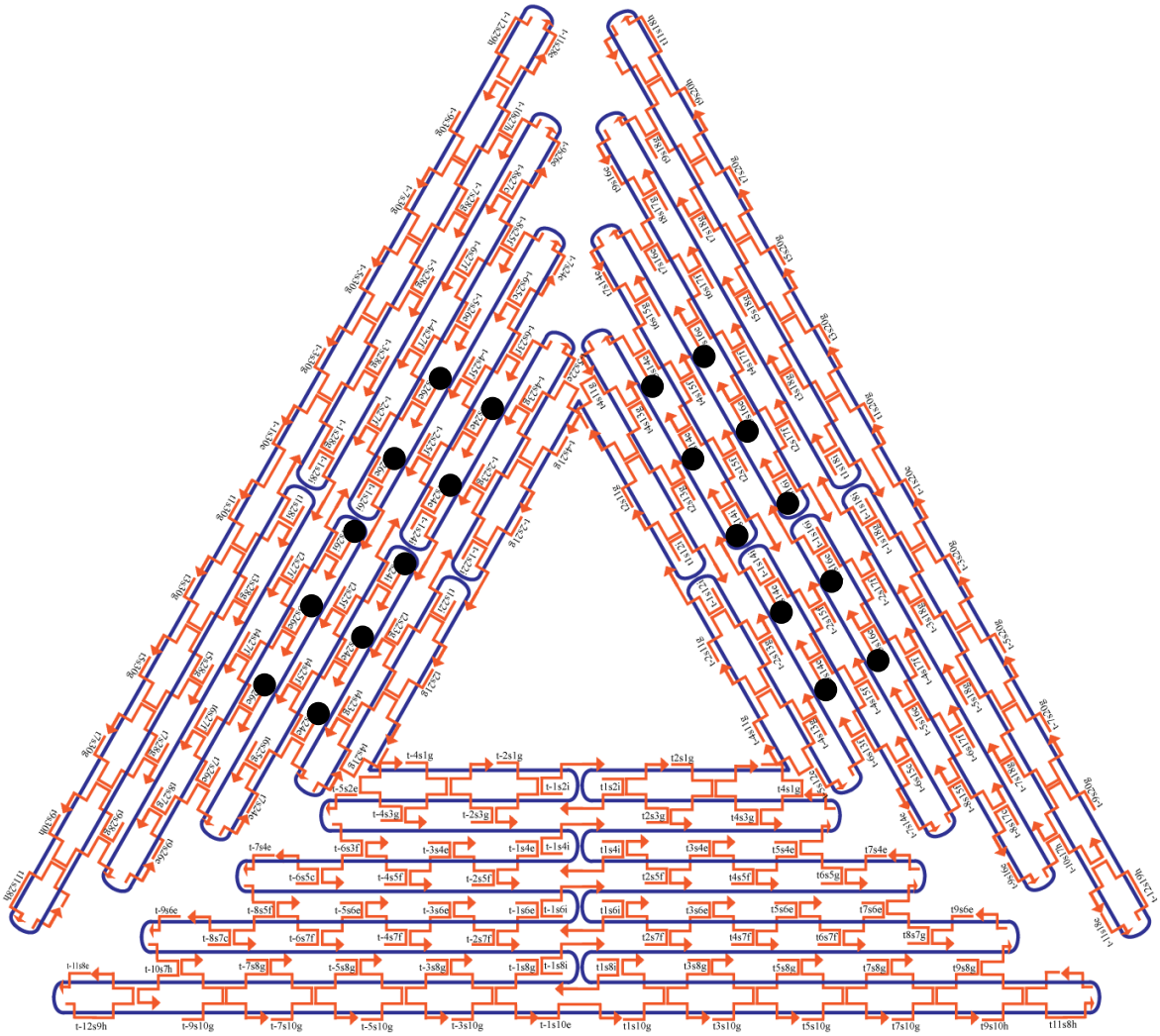


Figure S2: Modifications on triangle DNA origami.



List of modified staple sequences for forming one line:

1. **Biotin-SS-t1s16i:** **BIOTIN-TT-S-S-** GGCATCAAATTTGGGGCGCGAGCTAGTTAAAG
2. **Biotin-SS-t1s14i:** **BIOTIN-TT-S-S-**GTGAGAAAATGTGTAGGTAAAGATACAACTTT
3. **Biotin-SS-t3s14e:** **BIOTIN-TT-S-S-** CAATATGACCCTCATATATTTTAAAGCATTAA
4. **Biotin-SS-t3s16e:** **BIOTIN-TT-S-S-** CATCCAATAAATGGTCAATAACCTCGGAAGCA
5. **Biotin-SS-t5s16e:** **BIOTIN-TT-S-S-** TTAGCAAATAGATTTAGTTTGACCAGTACCTT
6. **Biotin-SS-t5s14e:** **BIOTIN-TT-S-S-**TTAATGCCTTATTTCAACGCAAGGGCAAAGAA
7. **Biotin-SS-t-1s14e:** **BIOTIN-TT-S-S-** ATTTTCTGTCAGCGGAGTGAGAATACCGATAT
8. **Biotin-SS-t-1s16e:** **BIOTIN-TT-S-S-** ATTCGGTCTGCGGGATCGTCACCCGAAATCCG
9. **Biotin-SS-t-3s16e:** **BIOTIN-TT-S-S-** GACAACAAGCATCGGAACGAGGGTGAGATTTG
10. **Biotin-SS-t-3s14e:** **BIOTIN-TT-S-S-**GTTTTGTCAGGAATTGCGAATAATCCGACAAT

Additional list of modified staple sequences to form the V-shape:

11. **Biotin-SS-t1s24i:** **BIOTIN-TT-S-S-** CCTGATTAAAGGAGCGGAATTATCTCGGCCTC
12. **Biotin-SS-t1s26i:** **BIOTIN-TT-S-S-** GCAAATCACCTCAATCAATATCTGCAGGTCGA
13. **Biotin-SS-t3s24e:** **BIOTIN-TT-S-S-** TAATCCTGATTATCATTTTGCGGAGAGGAAGG
14. **Biotin-SS-t3s26e:** **BIOTIN-TT-S-S-** TTATCTAAAGCATCACCTTGCTGATGGCCAAC
15. **Biotin-SS-t5s24e:** **BIOTIN-TT-S-S-** AATGGAAGCGAACGTTATTAATTTCTAACAAC
16. **Biotin-SS-t5s26e:** **BIOTIN-TT-S-S-** TAATAGATCGCTGAGAGCCAGCAGAAGCGTAA
17. **Biotin-SS-t-1s24e:** **BIOTIN-TT-S-S-** CAGTTTGACGCACTCCAGCCAGCTAAACGACG
18. **Biotin-SS-t-1s26e:** **BIOTIN-TT-S-S-** GCCAGTGCGATCCCCGGGTACCGAGTTTTTCT
19. **Biotin-SS-t-3s24e:** **BIOTIN-TT-S-S-** TGTAGATGGGTGCCGGAAACCAGGAACGCCAG
20. **Biotin-SS-t-3s26e:** **BIOTIN-TT-S-S-** GGTTTTCCATGGTCATAGCTGTTTGAGAGGCG

3) Experimental procedures

Assembly of the DNA Origami

Triangular shaped DNA origami were formed according to a modified version of Rothemund's method with a molar ratio of 1:30 between the viral DNA (5 nM) and each of the staple strands and biotin modified strands in the 1 x TAE-Mg^{2+} (tris, 40 mM; EDTA, 2 mM; magnesium acetate, 12.5 mM; adjusted to pH 8.0 with acetic acid) in a total volume of 100 µl. The solution annealing was performed by gradually decreasing the temperature from 65 °C to 4 °C in 2 h on an Eppendorf Mastercycler Personal machine. After annealing the sample was purified using a Microcon centrifugal filter (100,000 Da MWCO, 300 x g speed, 15 min) followed by washing with the 1 x TAE-Mg^{2+} to remove excess staple strands. 5 nM solutions of biotin-modified origami solutions were incubated with 2 µl of 50 µM streptavidin for 1 h, mixing gently at 500 rpm and 4 °C and filtered using 100kDa filters to remove excess streptavidin.

Preparation of self-assembled monolayers

All glassware was cleaned with piranha solution, H_2SO_4/H_2O_2, 70:30 (v/v) (Caution! Piranha solution is a strong oxidizing agent and should be handled with care.), rinsed with deionised water (18.2 MΩ·cm) and dried in an oven at 150 °C. Thiolate monolayers were prepared by immersion of the freshly annealed Au substrates in a 1 mM solution of thiol **1** in degassed ethanol for 18 h at room temperature. Modified substrates were rinsed in copious amounts of ethanol and dried in a stream of N_2 gas.

Self-assembled monolayer modification

2µl of the streptavidin–bound DNA origami were deposited onto the biotinylated SAMs' surface together with 8 µl of 1 x TAE-$Mg^{2+}$and allowed to react for 24 hrs. The samples were then rinsed with 10 ml of 1 x TAE, 12.5 mM Mg^{2+} buffer. A 50 mM solution of TCEP dissolved in 1 x TAE, 12.5 mM Mg^{2+} buffer was added to the sample surface and left for 5 h to cleave the disulfide bond between the DNA origami and the SAM. The samples were finally rinsed with C_2H_5OH/H_2O, 50:50 (v/v) and dried in a stream of N_2 gas.

Scanning Force Microscopy

Olympus micro cantilever tapping mode probes with a 26.1 N m^{-1} nominal spring constant and 300 kHz frequency were used as received on a Multimode Nanoscope (III) scanning force microscope (Veeco Instruments, Santa Barbara, CA), operated in air, for the images on Au substrates. Olympus OMCL-TR400 PS scanning force microscopy probes with a 0.08 N m^{-1} nominal spring constant and a frequency of 34 kHz were used as received on a Multimode Nanoscope (III) scanning force microscope (Veeco Instruments, Santa Barbara, CA), operating under fluid for the images on mica.

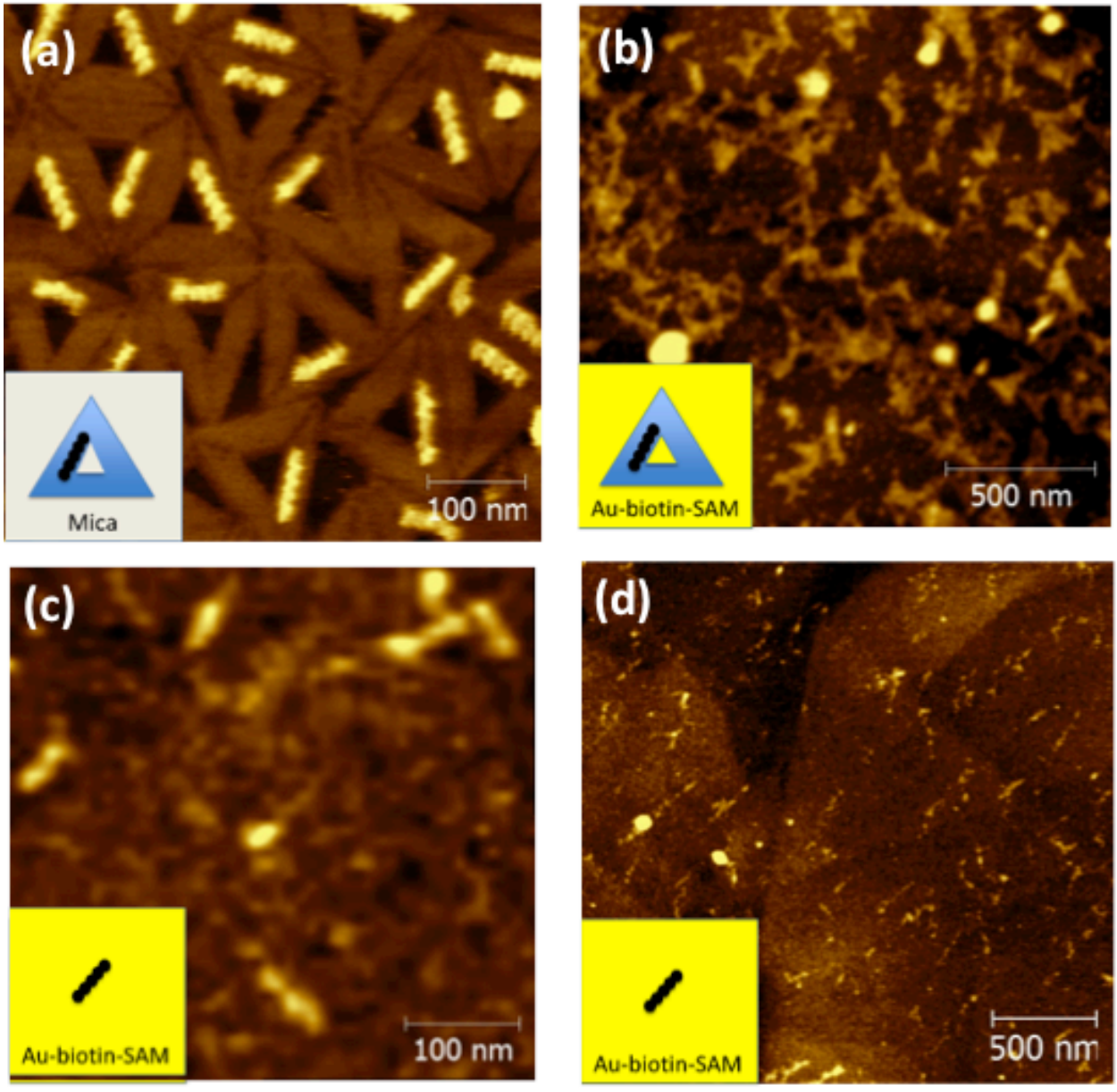


Figure S3 Triangle DNA origami with 10 streptavidins in a line pattern. a) AFM imaging on mica, b) AFM imaging on a biotinylated Au-SAM, c) and d) AFM imaging on a biotinylated Au-SAM after cleavage of the origami with TCEP.

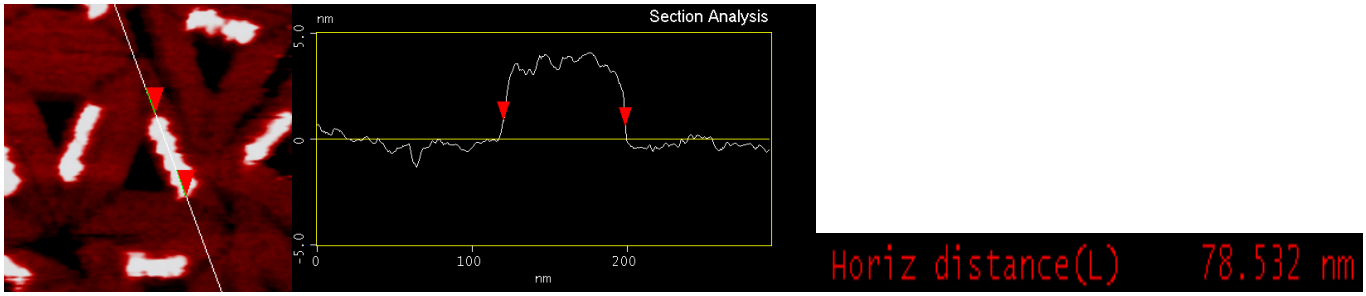


Figure S4 Profile measurement of a streptavidin pattern on the DNA origami nanostructures deposited on a mica surface.

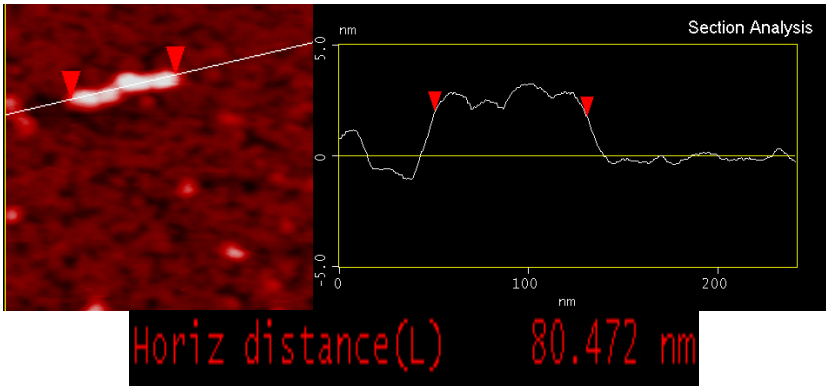


Figure S5 Profile measurement of a streptavidin pattern after transfer to the SAM biotinylated surface. The profile height and the horizontal distance is comparable to the streptavidins pattern measured in Figure S4**.**

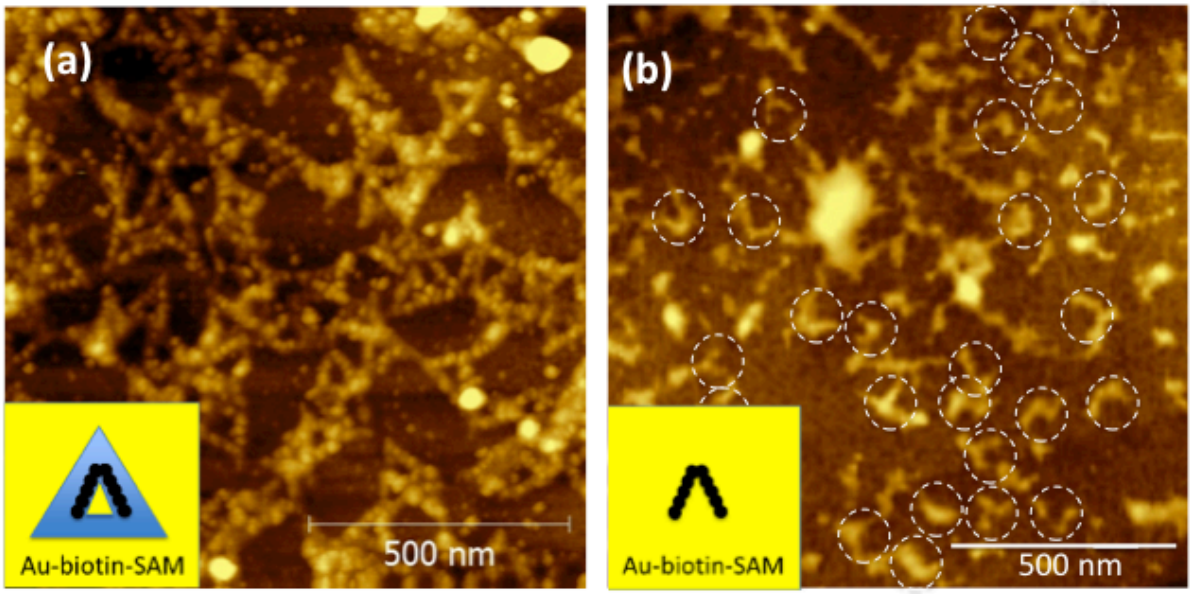


Figure S6 Triangle DNA origami with 20 streptavidins in a V pattern. a) AFM imaging on a biotinylated Au-SAM, b) AFM imaging on a biotinylated Au-SAM after cleavage of the origami by TCEP. The white circles indicate V shaped patterns.

SAM reactivity

Tests were carried out to determine the reactivity of the biotin immobilised on the SAMs. 2 µl of 50 mM streptavidin solution were placed on the SAM surface and left to react for 1 h before rinsing and imaging. Streptavidin molecules were observed on the SAM surface (Figure S7c) however the coverage was incomplete.

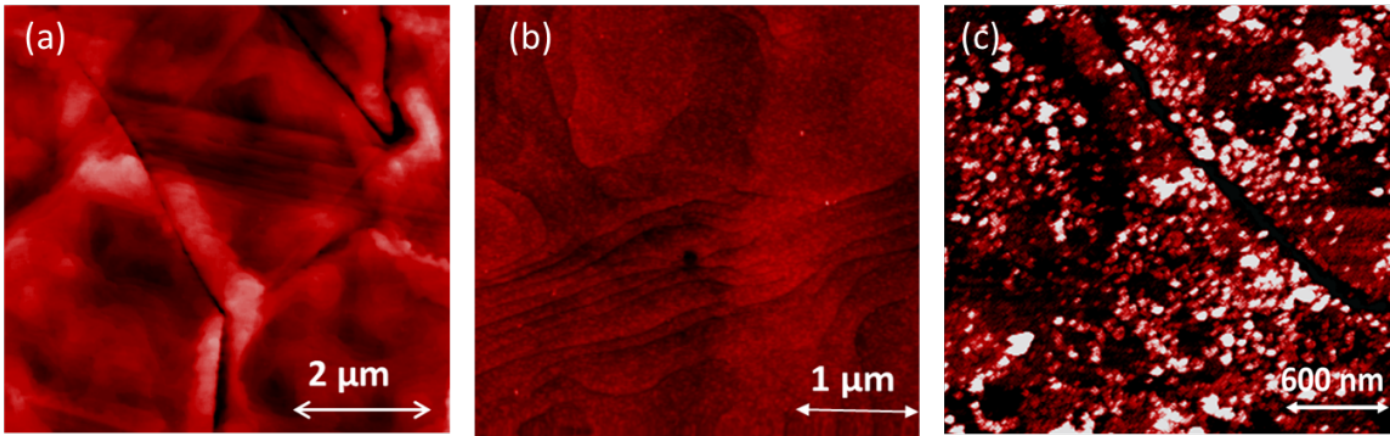


Figure S7. (a) Annealed Au surface, (b), biotinylated SAM on Au, (c) streptavidin modified SAM on Au.

References

(1) Rothemund, P. W. K. *Nature* **2006**, *440*, 297.