Electronic Supplementary Material (ESI) for Nanoscale.

Supplemental Information

Single Molecule Contact Printing

Content

1. Supplemental results

1.1 Contact angle

For contact angle measurements 10 µL drops of distilled water were deposited on either a freshly stripped gold film, on a film that had been incubated 36 hours in air or for 36 hours in ethanolic thiol-solution, i.e. either 3,3'-dithiodipropionic acid (DTPA) or thioctic acid (TA). The angle was measured in triplicates with G10 measurement system (Krüss GmbH, Hamburg, Germany).

	Freshly stripped	36 hours air	36 hours DTPA	36 hours TA
I	30 °	67.0 °	23 °	20 °
II	29 °	63.5 °	23 °	20 °
III	31 °	65 °	23 °	18 °
Ø	30 °	65.1 °	23 °	19.5 °

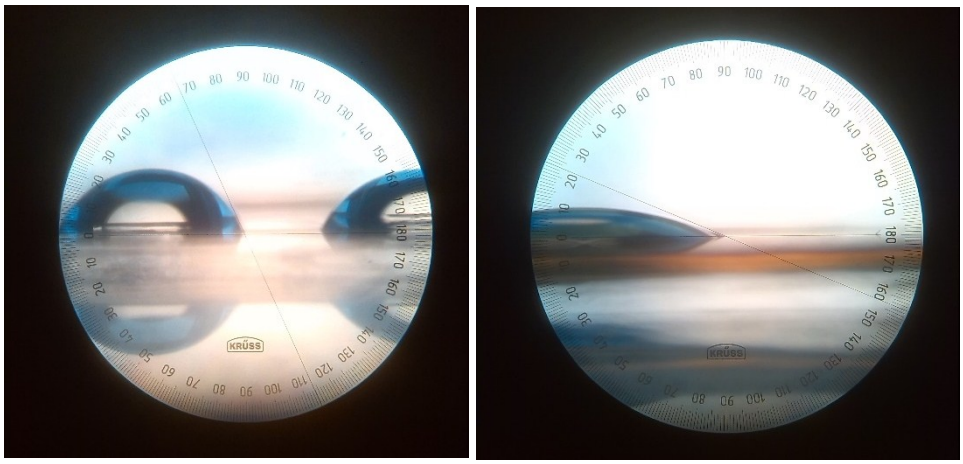


Fig S1: Exemplary photographs of contact angles of water droplets on gold films before (36 hours incubated at air, left) and after (right) DTPA coating.

1.2 Atomic Force microscopy

Typical sputtered Au films, as Xantec chips (Fig. S2B), exhibit a highly bumpy surface topology, with appearing on length scales comparable to the DNA origami structures used for printing and expected printed patterns. The printing process on such a surface is still possible due to the flexibility of the DNA origami. Nevertheless, since typical sputtered Au films exhibit a significant amount of surface features in the size regime of the studied objects, such Au films are not suitable to study the surface modification processes in the nanoscale by surface topology via AFM.

Therefore, Platypus template stripped Au films freshly stripped from a polished Si-wafer were applied as substrates. Fig S2. compares the surfaces of both Au films, imaged in contact mode by AFM with a Biolever mini cantilever. Fig. S2A demonstrates a very smooth gold surface consisting of large, flat terraces with lateral dimensions of several hundred nm separated by thin grooves. Small topological features onto the terraces lay within in the sub-nanometer height scale.

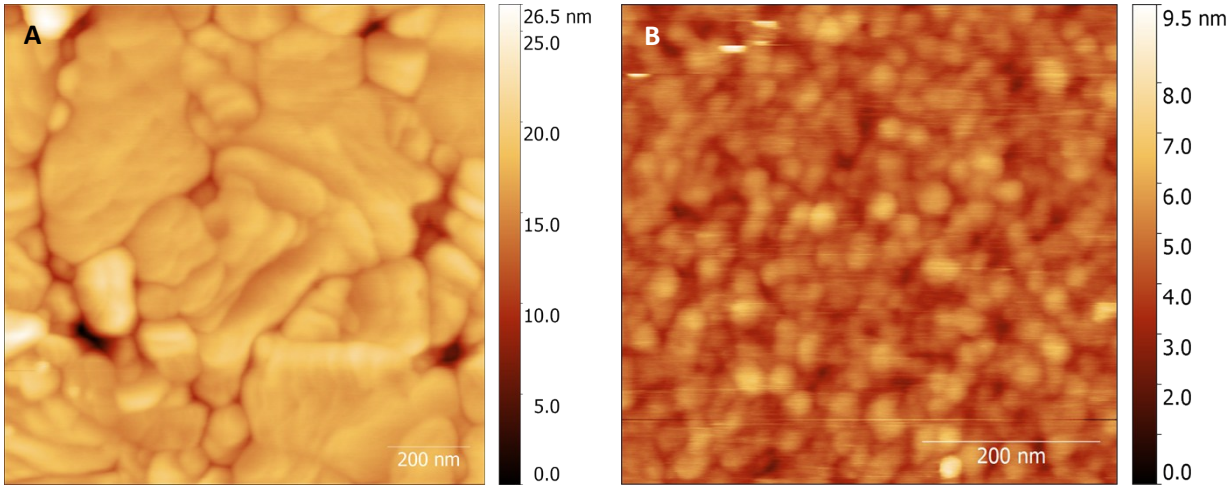


Fig. S2: Atomic force microscopy was applied to compare (A) Platypus template stripped and (B) Xantec gold surface. Scale bars 200 nm, height scale bar 0 to 3 nm.

1.3 X-Ray Photoelectron Spectroscopy (XPS)

After deposition of self-assembled monolayer onto Platypus gold chip with DTPA (see main text), carboxyl groups presented by DTPA molecules were activated by bioconjugation, through applying EDC and NHS (see main text method section and Fig 1 III). XPS spectra of chips containing non-activated and activated DTPA monolayers were obtained on a Kratos Analytical Ltd. (Manchester, UK) model Axis Ultra. Fig S2 demonstrates that the formation of active NHS-esters causes a noticeable increase of bound elements such as nitrogen (N 1s) as well as oxygen (O 1s) and carbon (C 1s) resulting from the covalent binding of NHS.

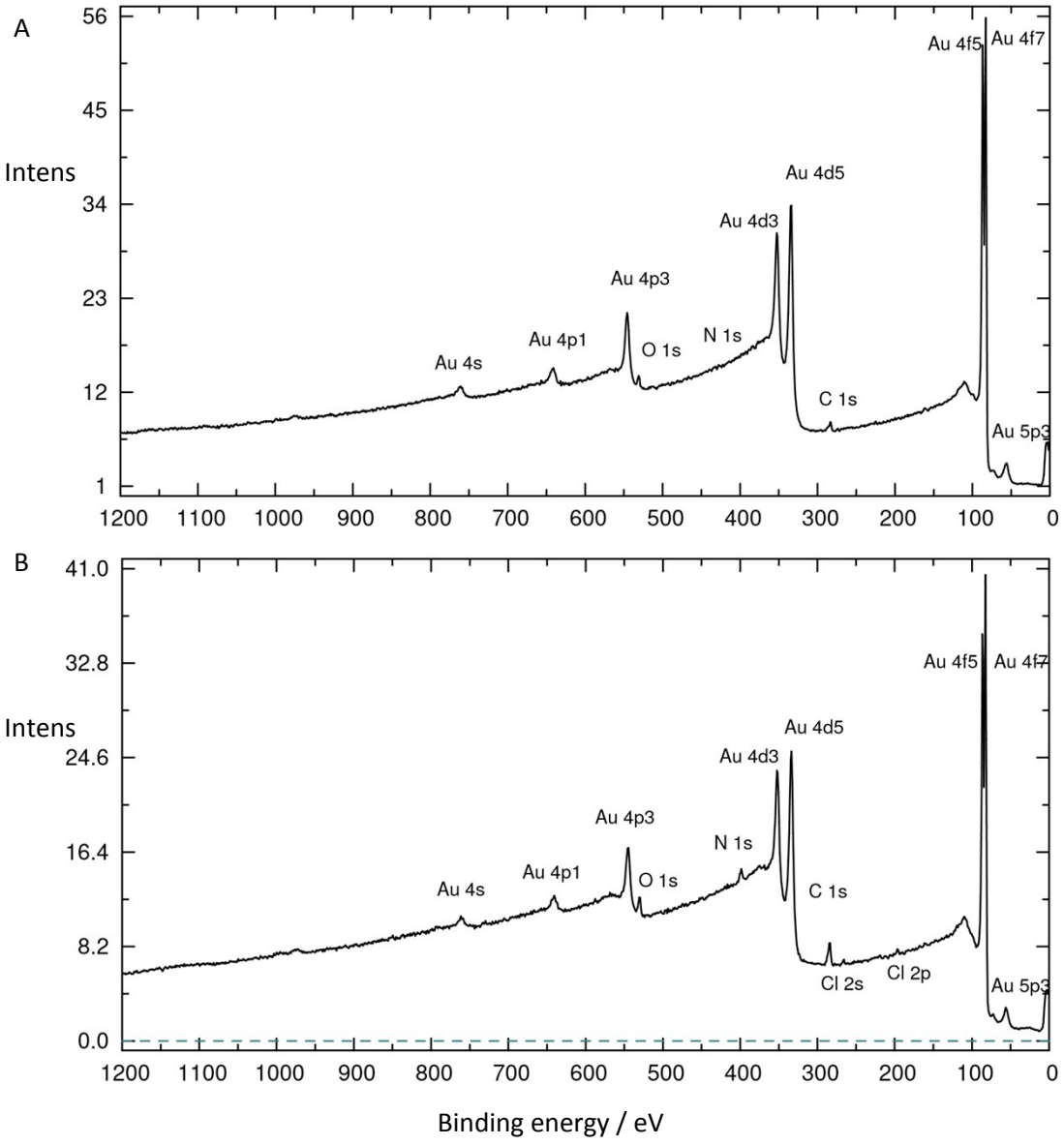


Fig S3: X-Ray Photoelectron spectra of (A) DTPA coated Au film and (B) EDC and NHS activated DTPA coated Au film.

1.4 Surface Plasmon Resonance

Surface Plasmon resonance was carried out in order to characterise films that were either printed with DNA origami stamps or with a randomly deposited control of dsDNA segments. The graphs below show the response to streptavidin (STV) application on surfaces containing biotinylated DNA strands applied either to printed patterns or randomly deposited dsDNA segments. For more information see main text.

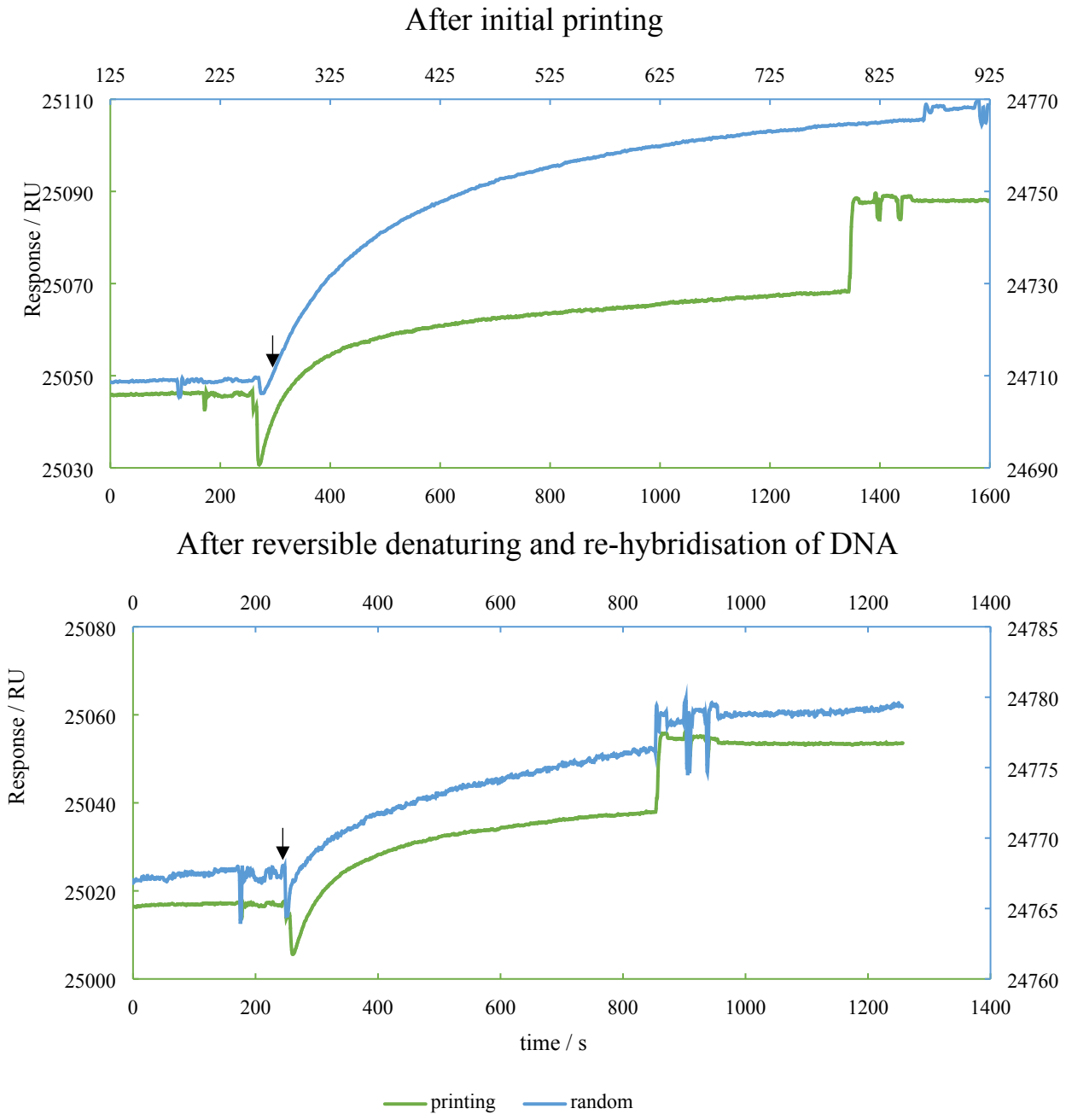


Fig. S4: Overlay of sensorgrams of initial and reversibility assays with STV for printed sample and a randomly deposited control of dsDNA segments. In the upper sensorgran, STV is applied immediately following the printing or random deposition process. In the lower, biotinylated DNA strands and associated

STV have been removed by NaOH treatment, and new biotinylated complements were added. We note that the sensorgrams were aligned in time to compensate system lags for clarity, but absolute response values are not adjusted. Black arrows indicate streptavidin injection. Sensorgram amplitude deflections directly before and after streptavidin injection correspond to microfluidic pump switches.

1.5 Single Molecule Contact Printing

In principle the experiment was repeated on coated template stripped gold films for reasons that were described in the main text. We hypothesised that a microfluidic system should not be necessary for the basic printing process. However, we assume that microfluidic systems such as that used in the instrument for the above described SPR analysis might be supportive for mixing and flowing fresh reagents across targeted surfaces, thus we increased incubation times and concentrations for printable samples.

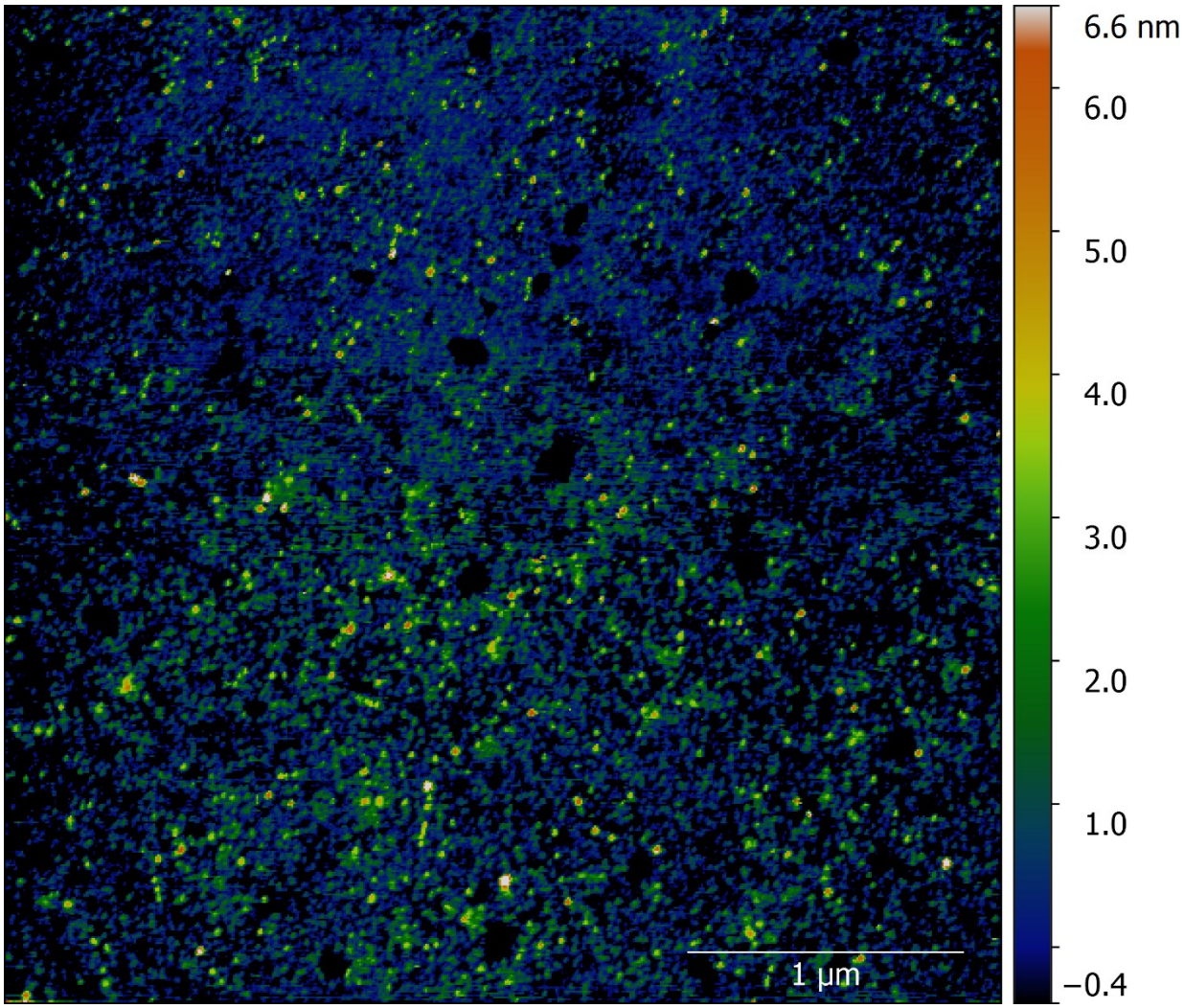


Fig. S5: Wide-view AFM image of DNA origami stamp printed and streptavidin probed patterns on DTPA-SAM coated gold film.

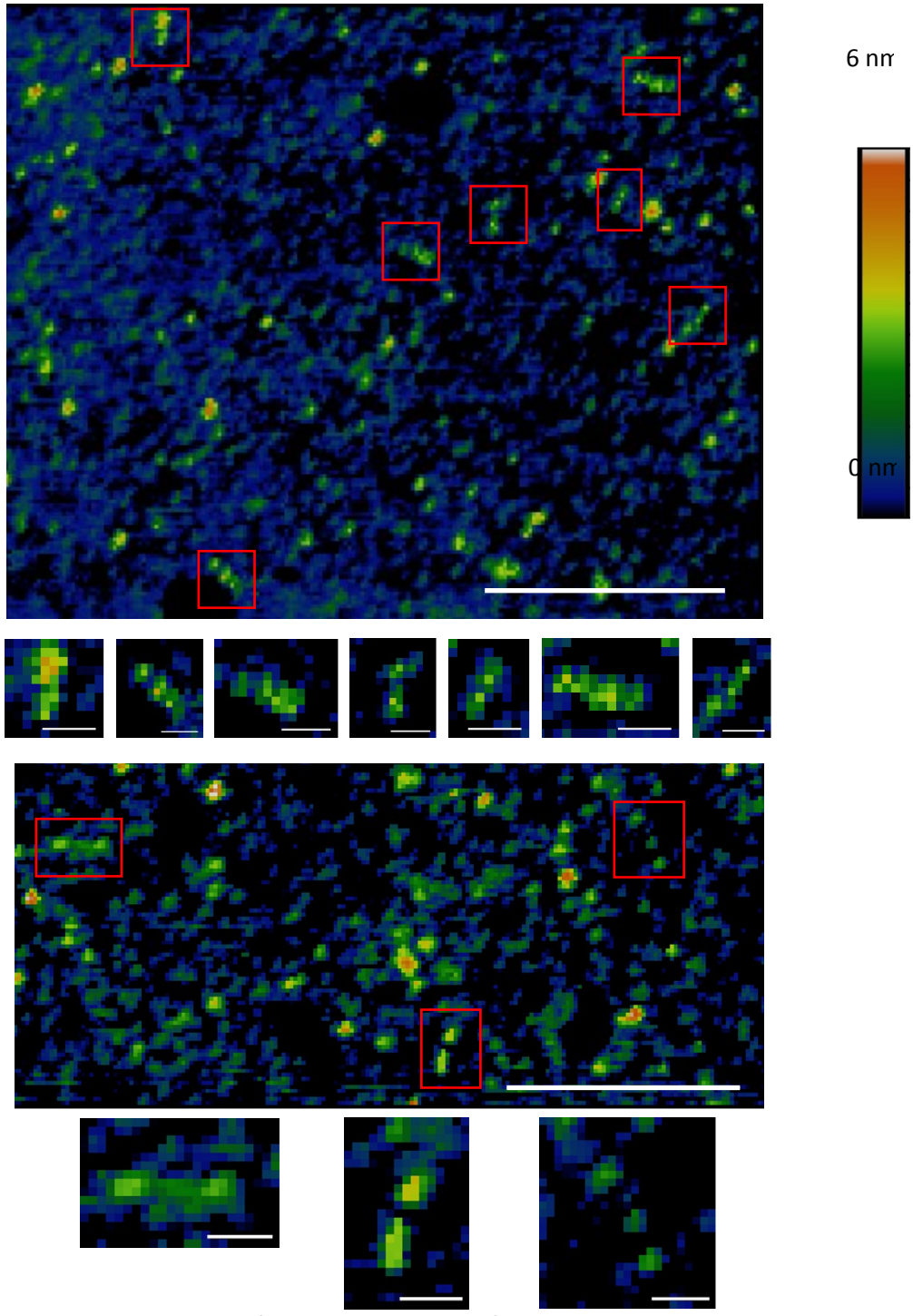


Fig. S6A: Additional sections of wide-view image of STV probed printed patterns. Exemplary patterns are highlighted by red boxes. Occurrence of magnified patterns in left to right order. Scale bar of sections 400 nm, scale bar of magnifications 40 nm, height scale bar 0 to 6 nm.

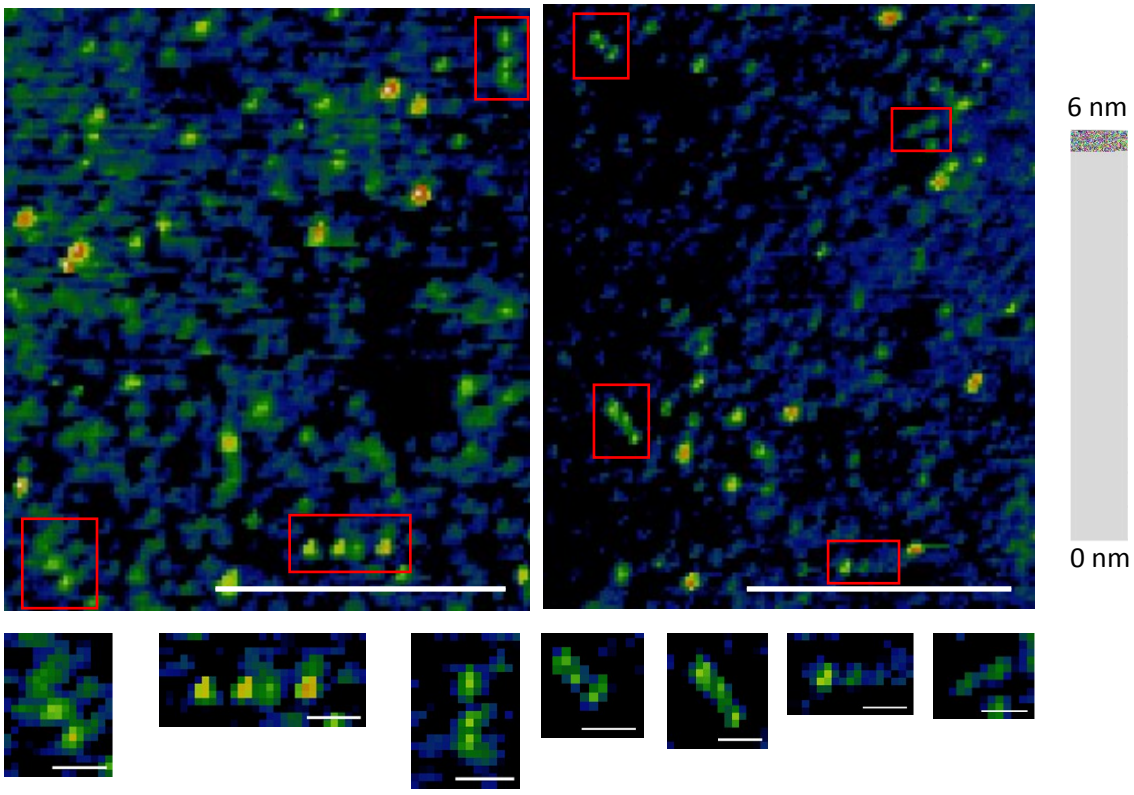


Fig. S6B: Additional sections of wide view image of STV probed printed patterns. Exemplary patterns are highlighted by red boxes. Occurrence of magnified patterns in left to right order. Scale bar of sections 400 nm, scale bar of magnifications 40 nm, height scale bar 0 to 6 nm.

1.5.1 Pattern analysis

AFM images were used to detect patterns and analyse their quality. The Z-Offset was increased to optimise contrast and simplify pattern recognition. Selected patterns had to match height and length criteria as described in the main text. For further analysis, height-profiles were extracted using Gwyddion 2.4 software and aligned profiles plotted in Fig. S7. In total 41 profiles were extracted.

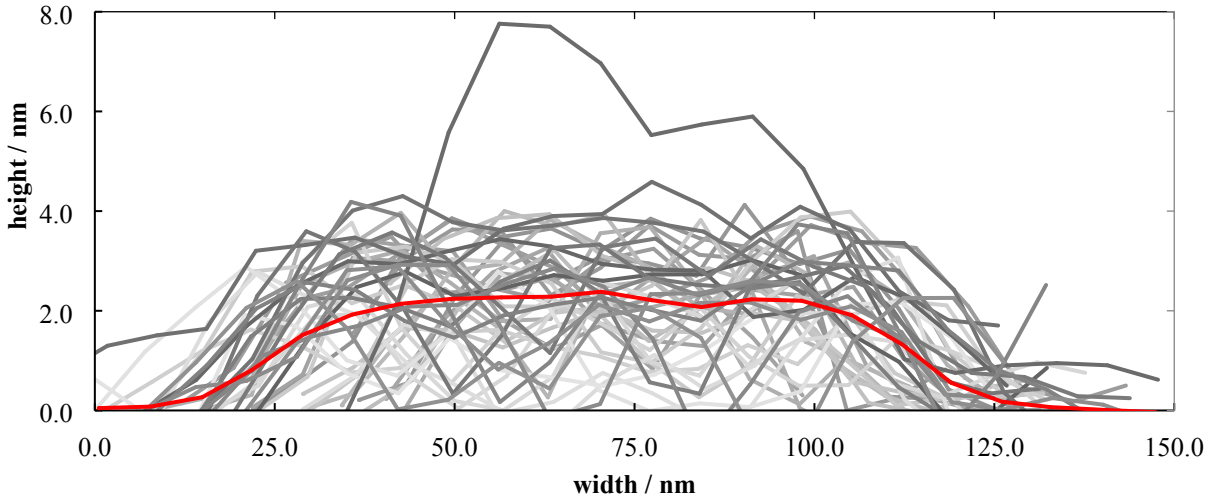


Fig. S7: Overlay of extracted height-profiles. For clarity, height-profiles from images below were aligned in baseline height and centre of width in the plot. The red curve represents mean profile of all 41 detected patterns. The length of the mean profile is ~90 nm, according to the method described in main text.

1.5.1 Negative control

Negative control was treated and AFM imaging was carried out as previously reported in the main text. No stripe patterns are observable.

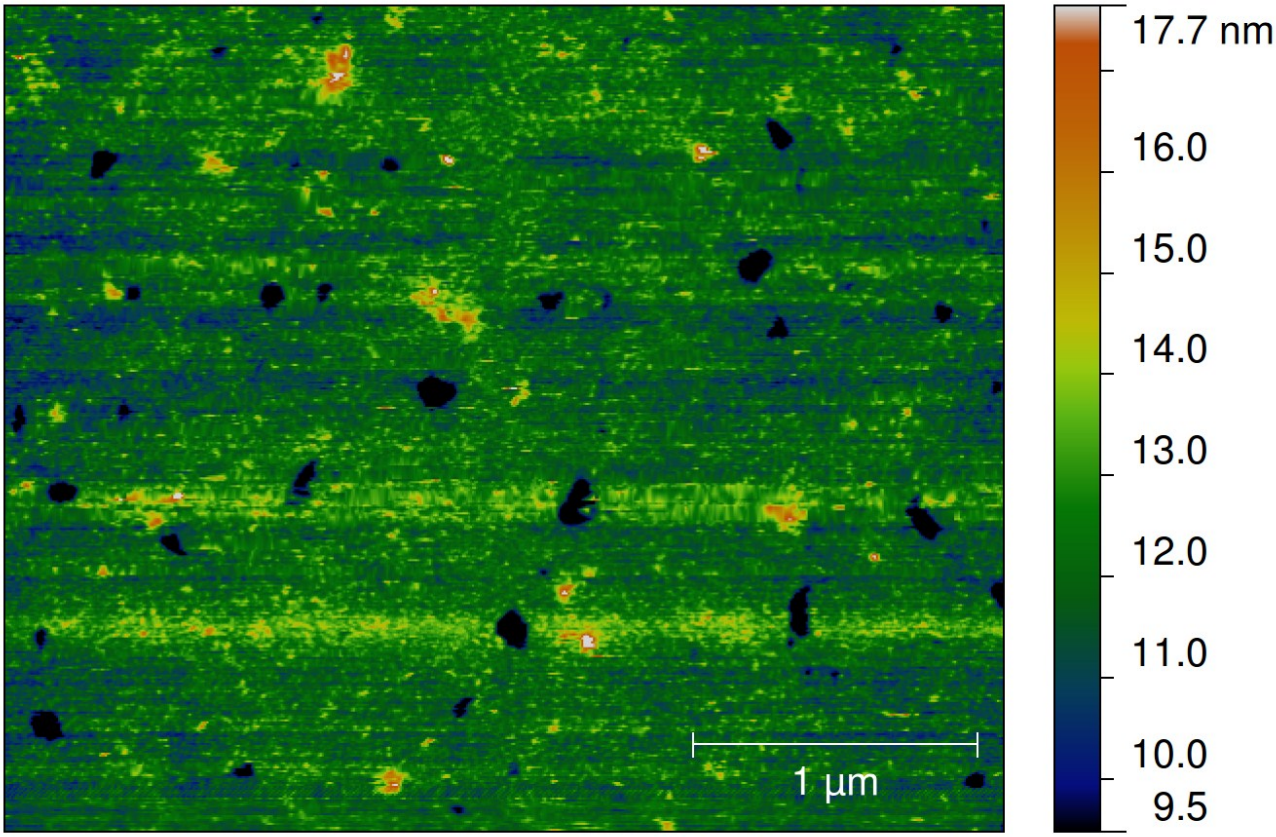
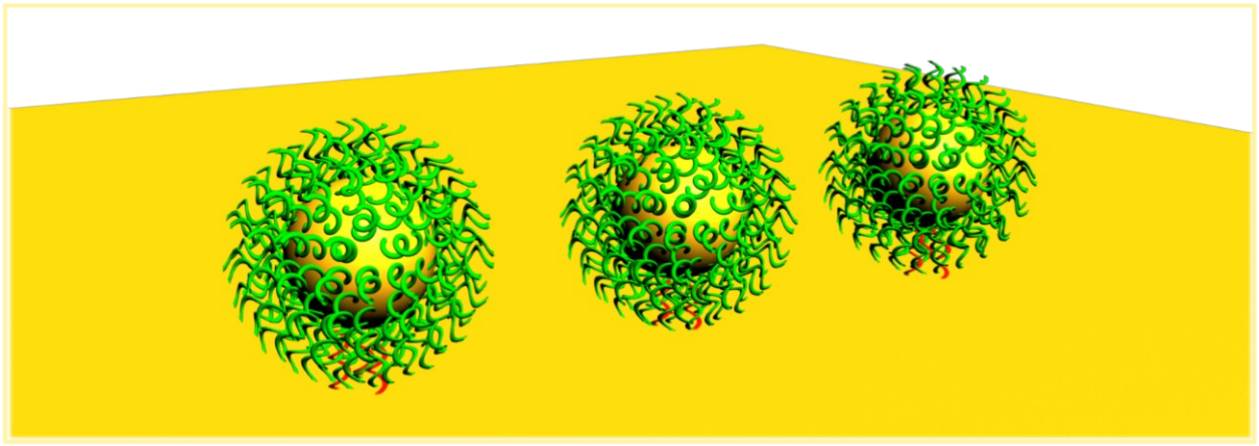


Fig. S8: Wide-view AFM image of the randomly deposited "negative" control of biotinylated dsDNA segments for random immobilisation and probing with streptavidin. No patterns can be observed.



2. Printing onto bare gold films using thiolated DNA ink strands

A second protocol was applied to test whether the described printing technology is able to accurately separate large (~10 nm diameter) gold-nanoparticles via printing. The protocol is very similar to the work of (Gallego *et al.* 2017) and described in S2.2. The direct printing method was also confirmed for streptavidin molecules similar to what is described in the main text. This is briefly described in 2.1.

The ink strands were labelled with thioctic acid linkers instead of amine (main text). The intramolecular disulphide in thioctic acids is thought to bind more strongly to gold surfaces than single thiols.

2.1 Printing patterns for attachment of STV

For printing patterns directly onto gold for protein (STV) attachment, DNA origami stamps were treated as described in the main text, but hybridized with ssDNA "handles" carrying a 3'-end thioctic acid modification instead of primary amine groups.

A freshly purified origami sample was diluted 1 to 10 with origami buffer. Additionally, the origami treated gold film was incubated at 4 °C for 16 hours. Afterwards the surface was blocked with 1 mM ethanolic mercaptohexanol for 1 hour.

Samples were rinsed in distilled water and the surface-bound DNA origamis were denatured with 25 mM NaOH for 20 s. Again, the samples were rinsed with distilled water and ethanol, then finally dried with a stream of nitrogen gas.

To confirm the printing process with thioctic acid-labels binding directly to bare gold, we first probed the cross pattern also with biotinylated DNA strands and streptavidin, as previously described in main text and analysed via AFM-based imaging. As shown in Fig. S9 the designed cross pattern from the DNA origami was transferred to the surface observable in streptavidin cross patterns with nearly the same dimensions. These results demonstrate, that a printing procedure can be used to arrange proteins in distinct and complex nanopatterns directly on surfaces too. Again it is noteworthy to mention that probably insufficient blocking of unspecific bindings sites, working in non-clean room environment as well as insufficient sample cleaning (contamination of stamp solution with unbound ink strands) results in high background signal.

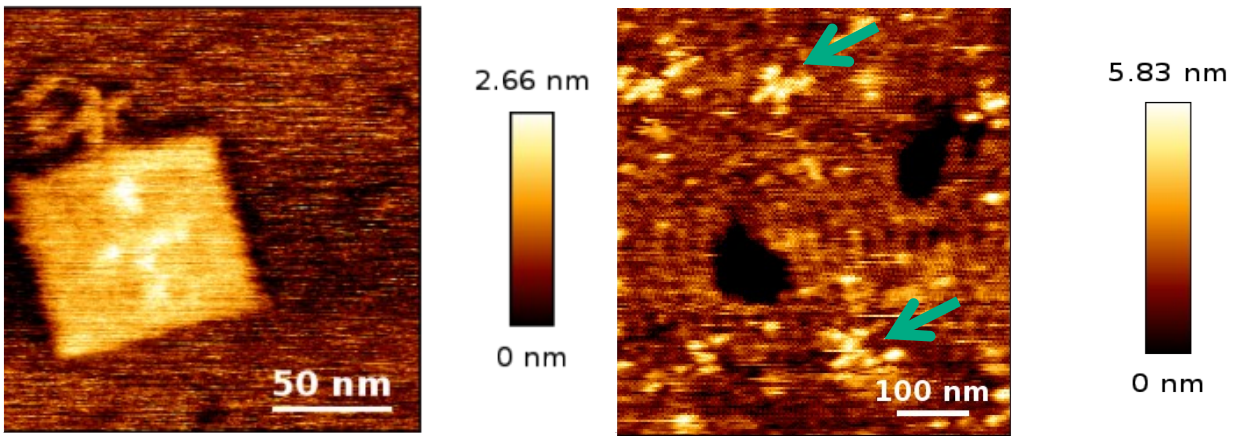


Fig. S9: Exemplary AFM images of a cross pattern origami (left) and its corresponding printed pattern probed with streptavidin (right). Patterns were highlighted by green arrows.

2.2 DNA Origami Design

To whether the printing technology could be used to precisely separate large nanoparticles from each other, we designed a new pattern on DNA origami stamps consisting of 3 printing areas, which were programmed to have a distance of 40 nm between adjacent gold nanoparticles (80 nm total pattern length) and comprise 3 anchor points as shown below.

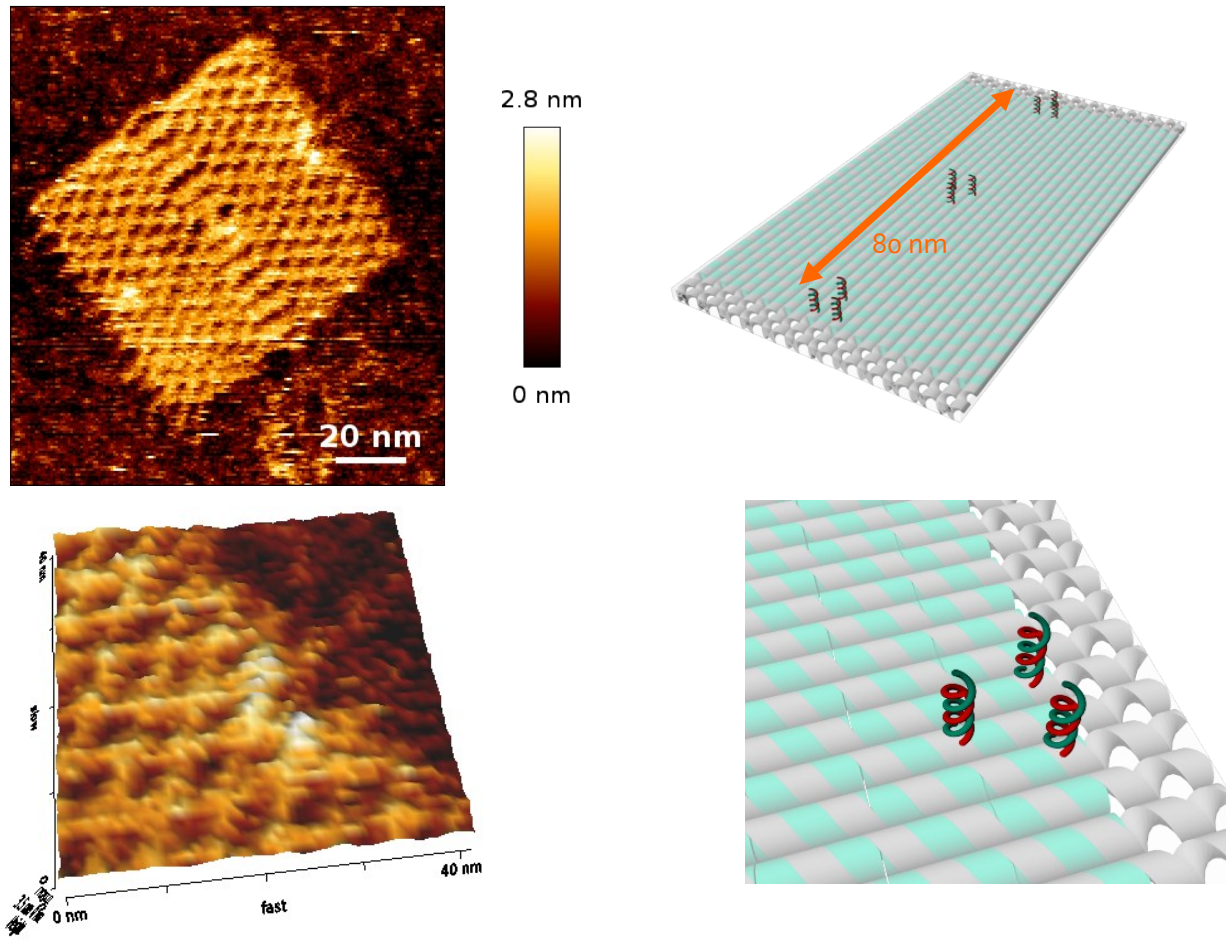


Fig. S10: Design of DNA origami stamp for separation of gold-nanoparticles onto pre-printed patterns. Images on the left show atomic force micrographs of (upper) the DNA origami stamp carrying the binding areas and (lower) a zoom in on binding area consisting of 3 identical anchor points. The corresponding schematic representations are shown on the right side.

2.3 Probing with gold-nanoparticles

10 nm (diameter) gold nanoparticles labeled with a 19-base poly-T handle were kindly provided from Robert Schreiber, former fellow in A. Turberfields Lab (Oxford, UK). They were purified by ultrafiltration as previously described for DNA Origami (section 2.3) in a Tris-EDTA buffer. 1 nM of purified nanoparticles were added to samples and incubated for 1 hour. Afterwards the samples were dipped into 50 mL distilled water for 10 seconds, rinsed with ethanol and dried with a stream of nitrogen gas.

2.3.1 AFM

AFM was carried out in the "Quantitative imaging" mode on the JPK Nanowizard 3 described in the main text, in air using SNL-10 cantilever purchased from Bruker Corporation (Billerica, Massachusetts, USA).

An exemplary image is shown below (Fig. S11). From this image nearly 30 height-profiles were extracted (Fig. S12 and S13) as described before for streptavidin with Gwyddion 2.4 Software.

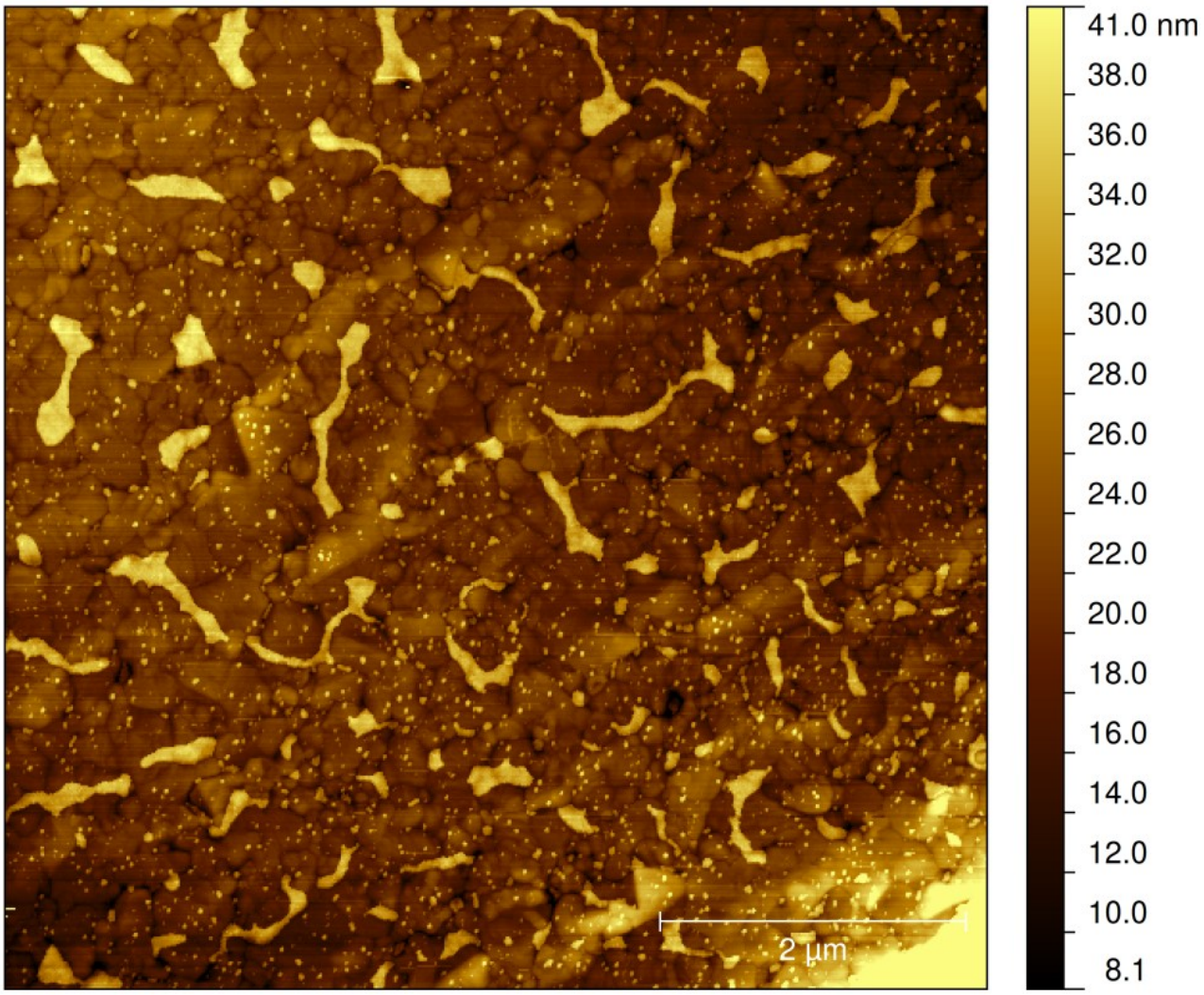


Fig. S11: Atomic force micrograph with immobilized gold nanoparticles arranged in predefined patterns on gold surface.

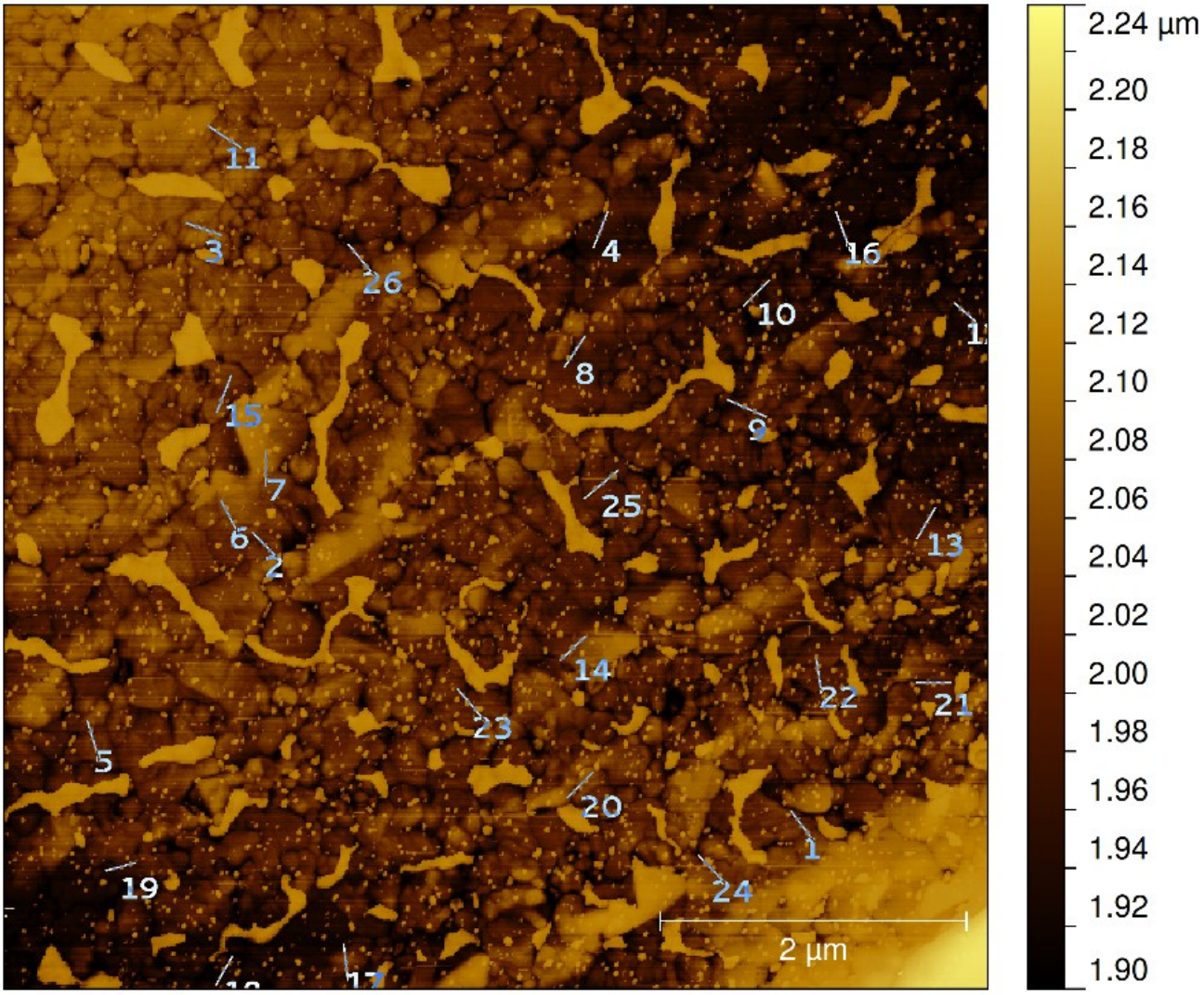


Fig. S12: The height profiles of several patterns are highlighted by numbered light-blue bars.

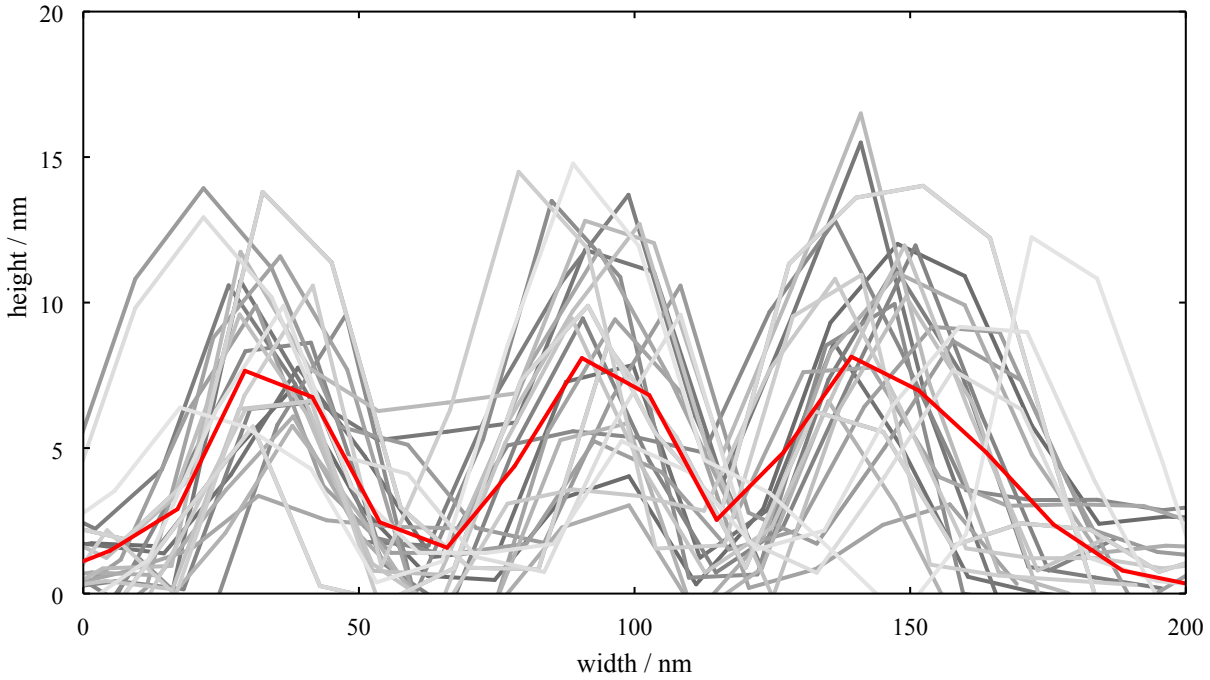


Fig. S13: Overlay of extracted height-profiles and a representation of a mean curve (red) after rough manual alignment.

Fig. S13 shows an analysis of height-profiles of some of the observed patterns. The mean peak distance is approximately 50 nm and matches well with our expectations of anchor points separated by approximately 40 nm.

These results demonstrate that the printing onto bare gold surfaces can be easily adapted. Furthermore, the results show that the technology can be used to separate gold-nanoparticles by defined distances, which can be utilised in the generation of plasmonically active surfaces. It is worthwhile to mention that gold-nanoparticles tend to form large scaled clusters on surfaces as seen in Figure S8. We assume that immobilization and washing conditions have to be optimised.

Furthermore, printing with thiolated DNA on gold seems to exhibit only short term stability. The samples were stored at 4 °C, but after 1 week of storage patterns were no longer detectable. We assume that low density SAMs such as this blocking layer, which form during short deposition times (Love *et al.* 2005), allow further rearrangement of SAMs (Wang et al. 2014, Bürgi 2015) and patterns. Therefore, printing on SAMs, as described in the main text, increases overall stability of the deposited pattern.

Printing on pre-deposited SAM bears the advantages of being able to adjust important SAM-enabled properties, such as long term stability or chemical resistance in environments or solvent conditions (e.g. organic phases) that may be hostile to DNA origami stability. Furthermore, the subsequent blocking step in direct printing onto gold may lead to the unwanted substitution of already printed strands, thus further reducing yield (Love et al. 2005).

2.3.2 Scanning Electron Microscopy

Furthermore, SEM was carried out with ULTRA 55 (Carl Zeiss SMT, Oberkochen, Germany). Electron high tension (EHT) of 1.0 kV was applied. Further settings were noted below corresponding image. SEM analysis yielded large, clustered agglomerations of gold nanoparticles on the surface, potentially arising due to drying-induced shear forces, ageing or other artifacts during preparation and storage for imaging.

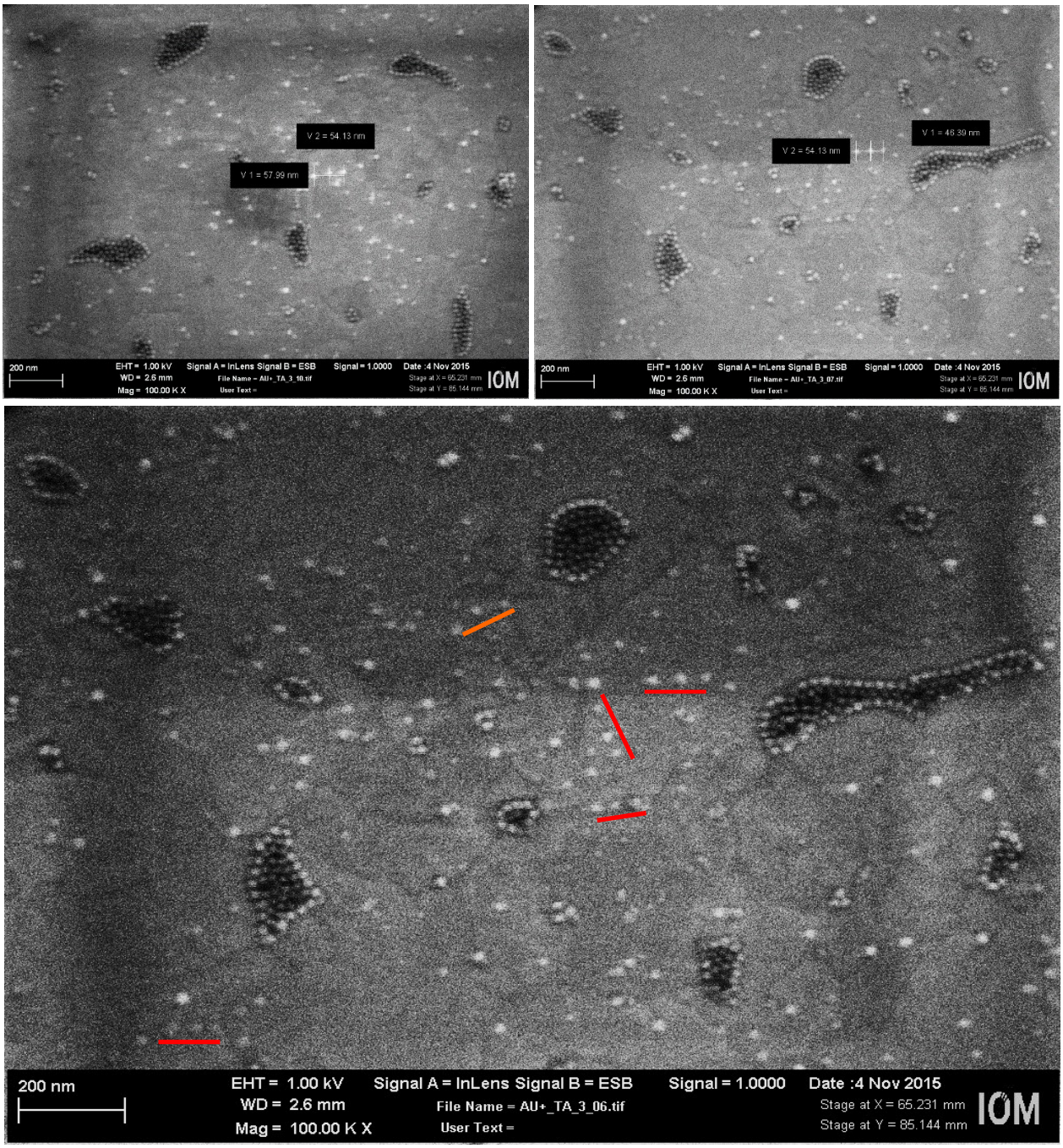


Fig. S14: Scanning electrode micrographs of printed 3 nanoparticle patterns. The patterns were programmed to be around 50 nm apart. Potential patterns are highlighted by red bars.

3. References

Gallego, Isaac; Manning, Brendan; Prades, Joan Daniel; Mir, Monica; Samitier, Josep; Eritja, Ramon (2017). DNA-Origami-Driven Lithography for Patterning on Gold Surfaces with Sub-10 nm Resolution. Advanced materials.
DOI: 10.1002/adma.201603233

Love, J. Christopher; Estroff,Lara A.; Kriebel, Jennah K.; Nuzzo, Ralph G. and Whitesides, George M. (2005). Chem. Rev, 105, 1103-1169.
DOI: 10.1021/cr0300789

Xiaoyu Wang, Yongsuo Liu, Zhenling Chen, Yong Li, Kang Sun, Xingyu Jiang (2014). Diffusion of self-assembled monolayers of thiols on the gold surfaces covered with polydimethylsiloxane stamps. J. Mater SCi., 49, 4394-4398.
DOI:10.1007/s10853-014-8148-9

Bürgi Th. (2015) Properties of the gold–sulphur interface: from self-assembled monolayers to clusters. Nanoscale, 7, 15553-15567.
DOI: 10.1039/C5NR03497C

4. DNA sequences

Section 4.1 contains all sequences to form a proper rectangular DNA origami structure. All other sections summarize sequences that were replaced in in 4.1.

Unmodified DNA-oligonucleotides were purchased from Eurofins Genomics GmbH, Ebersberg, Germany. All modified DNA-oligonucleotides were purchased from biomers.net GmbH, Ulm, Germany.

4.1 DNA Origami stamp

Oligo name	sequence
s1-oligo1	CTGGCCCTGAGAGAGTTGCAGCAAATAGGGTT
s1-oligo2	AGACGGGCAACAGCTGATTGCCCTAGAGTCCA
s1-oligo3	GGCGCCAGGGTGGTTTTTCTTTTGTCAAAG
s1-oligo4	GAGTGTTGAAGGGAGCCCCCGATTCACGTATA
s1-oligo5	CTATTAAAAGGTGCCGTAAAGCACGAGCGGGA
s1-oligo6	GGCGAAAAACCATCACCCAAATCGATTTTA
s1-oligo7	GACGGGGAAAAAGAATAGCCCGAGGCGGTCCA
s1-oligo8	GAACCCTATTCCAGTTTGGAACATCACCGC
s1-oligo9	TGGGGTCGGAACGTGGACTCCAACCACCAGTG
s1-oligo10	ACGTGCTTCTTCTTTGATTAGTAATCATGGAA
s1-oligo11	GCTAAACATCCATCACGCAAATTACGTCTGAA
s1-oligo12	GACAGGAAAATCAGTGAGGCCACCACCAGT
s1-oligo13	CTTGCCTGTGGTTGCTTTGACGAGTAGAGCTT
s1-oligo14	TAGCAATATCCTCGTTAGAATCATAAATCG
s1-oligo15	AGAGTCTGGGAGGCCGATTAAAGGAAGTTTTT
s1-oligo16	ATACCTACATTAGTCTTTAATGCGGCAACAGT
s1-oligo17	ATGGATTATACGTGGCACAGACAAATGAAAAA
s1-oligo18	CACACGACATAGAACCCTTCTGATCAAATA
s1-oligo19	TAGCCCTAGCAACAGGAAAAACGCTAACATCA
s1-oligo20	GAATGGCTATTTTGACGCTCAATACCGTTG
s1-oligo21	CGTAAGAATTTACATTGGCAGATTCGAGTAAA
s1-oligo22	GCCACGCTTAGAGCCGTCAATAGAAGTAACAT
s1-oligo23	TCTAAAGCATATCTTTAGGAGCACCCACCAGA
s1-oligo24	TCAAACCCAGTTGAAAGGAATTGCTGATTA
s1-oligo25	TTGAGGATAGTATTAACACCGCCTCGAACTGA
s1-oligo26	TAATAGATGAGAGCCAGCAGCAATATTTTT
s1-oligo27	TATCTAAAATCACCTTGCTGAACCCCTGAAAG
s1-oligo28	TATCATTTACAGAAATAAAGAAATACCAAGTT
s1-oligo29	AGGAGCGGACCTACCATATCAAAAATTCATTT
s1-oligo30	TCAGATGAGATTATACTTCTGAAATGAAAC
s1-oligo31	TTTTCAGGTAATTTTAAAAGTTTGTAATACAT
s1-oligo32	CACGTAAATGCGGAACAAAGAAATAACAAC
s1-oligo33	GGGTTAGAAATTATCATCATATTCAGGAAGGT
s1-oligo34	ACAAAATCGCTTCTGTAAATCGTCGGTCTGAG

Oligo name	sequence
s2-oligo1	GCGCAACTGTTGGGAAGGGCGATCATTCTCCG
s2-oligo2	ACCAGGCAAAGCGCCATTCGCCATAATGGG
s2-oligo3	CCAGCTTTCCGGCACCGCTTCTGGCGCATCGT
s2-oligo4	TGGGAACAATCAAAAATAATTCGCTGAGAGTC
s2-oligo5	ATAGGTCAATCAGCTCATTTTTTACGGTAA
s2-oligo6	AACCGTGCTTTTGTTAAAATTCGCATGTACCC
s2-oligo7	CTTCCTGTGTAACAACCCGTCGGGGTGCGG
s2-oligo8	GGAACGCCAACGGCGGATTGACCGTTCAGGCT
s2-oligo9	TTTGTTAACGTTGGTGTAGATGGGTGCCGGAA
s2-oligo10	TGGAGCAAAACCGTTCTAGCTGATGCCTCAGA
s2-oligo11	TCGTAAAAAGACAGTCAAATCACAAAAACA
s2-oligo12	CGGTTGATAGGTAAAGATTCAAAAGGAGAAGC
s2-oligo13	TGCCGGAGTATCAGGTCATTGCCGTCTGGC
s2-oligo14	TGATATTCACAAGAGAATCGATGAAACCAATA
s2-oligo15	AAGGCCGGCTAGCATGTCAATCATATTAAATT
s2-oligo16	GCATAAAGATTCTACTAATAGTAGTTAATTGC
s2-oligo17	TTATGACCATTTGGGGCGCGAGCTGTTTTA
s2-oligo18	CTTTATTTAAATGGTCAATAACCTTGGAAGTT
s2-oligo19	AACATCCAAAATTAAGCAATAAAAAATTAA
s2-oligo20	TGGCATCACTAAATCGGTTGTACCCATCAATA
s2-oligo21	ATATTTTCCTGTAATACTTTTGCGGGGTGAGA
s2-oligo22	TGAATATAACTCCAACAGGTCAGGATCGTCAT
s2-oligo23	AATATGCACGAGCTTCAAAGCGAATGCTTT
s2-oligo24	TCATTCCAGCCCGAAAGACTTCAACCATAAAT
s2-oligo25	AGTACCTTCGGATGGCTTAGAGCTAGCATT
s2-oligo26	GGAAGCAAATGCTGTAGCTCAACATGAAAAGG
s2-oligo27	TTTTAATTACTAAAGTACGGTGTCGTTTAGCT
s2-oligo28	AAATATTCAGAGGCTTTTGCAAAATCAGGACG
s2-oligo29	AAACAGTTTTACCAGACGACGATAAAACGA
s2-oligo30	CAAAAATCCATAGTAAGAGCAACAGTAGAAAG
s2-oligo31	TGCCAGAGGTCCAATACTGCGGAATTAGAG
s2-oligo32	AAATAGCGATTGAATCCCCCTCAAACCAGACC
s2-oligo33	ACCCTCGTCAGAAAACGAGAATGAATATCGCG
s2-oligo34	TTGGGAAGGGCTTGAGATGGTTTACGGTCAAT

Oligo name	sequence		Oligo name	sequence
s1-oligo35	CAATTACCCATAAATCAATATATGTTAGGTTG		s2-oligo35	ACTAACGGGACGAGAAACACCAGTTTGAAA
s1-oligo36	AAACATCAATTTGAATTACCTTTTGATGCA		s2-oligo36	ATTCATCAAAGCTGCTCATTCAGTCCAGGCGC
s1-oligo37	TTAATTTTCCTGATTGCTTTGAATTGCGTAGA		s2-oligo37	CTTTAATCGGCTCATTATACCAGGAAGTTT
s1-oligo38	ATAACCTTGCGCAGAGGCGAATTTTATTTG		s2-oligo38	GTAAATTGAAAAATCTACGTTAATAAAAACCA
s1-oligo39	AAACAGTATGAGCAAAAGAAGATGTAATGGAA		s2-oligo39	CTTGCCCTAACAACATTATTACAGCTATCATA
s1-oligo40	AGACTACCGATAAATAAGGCGTTACAGTAGGG		s2-oligo40	CATAAGGGAGATTTGTATCATCGCAAAGACAG
s1-oligo41	GGTTATATTAAATTTAATGGTTTGAACAACGC		s2-oligo41	GAGGACAGATTATACCAAGCGCGTACAGAG
s1-oligo42	AATCCAATAATATATTTTAGTTATTTTCGA		s2-oligo42	ATAGGCTGTACACTAAAACACTCAATGAGGAA
s1-oligo43	TAAACACCATTTATCAAAATCATAGCTATTAA		s2-oligo43	ATTGTGTCGGAACGAGGCGCAGAATTTCAA
s1-oligo44	GACCGTGTTTTTTAACCTCCGGCTGAGTGA		s2-oligo44	TACAACGGAACCGAACTGACCAACAACGAGTA
s1-oligo45	TTCTGACCAACTATATGTAAATGCTTTAATGG		s2-oligo45	CCCCAGCGATGAACGGTGTACAGAGAATAAGG
s1-oligo46	CTTAATTGATCAACAATAGATAAGTTAAACCA		s2-oligo46	CATCGGAAGCATAACCGATATATTACTTTCAA
s1-oligo47	CAACATGTCATGTTCAGCTAATGCGCAAGCCG		s2-oligo47	GCTTTGAGTGCGCCGACAATGACAAGGAAC
s1-oligo48	GCCAGTAAGTAATTCTGTCCAGATTACCGC		s2-oligo48	GTTTCCATGTGAATTTCTTAAACATTTTTTCA
s1-oligo49	AAGAAAAATAAAGCCAACGCTCAAAATAAGAA		s2-oligo49	TGAGGCTTTCACCCTCAGCAGCGCTGATAA
s1-oligo50	GCCTGTTTAGAATCGCCATATTTAAATACC		s2-oligo50	TCGCCCACCGAGGGTAGCAACGGCAAACAAAG
s1-oligo51	ATAAACAAAATTTAGGCAGAGGCAATTTCATC		s2-oligo51	CCGATAGTGACTAAAGACTTTTTCTCTTTGAC
s1-oligo52	AGTACCGCAATCAAGATTAGTTGCATTATTTA		s2-oligo52	CAGTTTCAGTTAGCGTAACGATCTGTATAGCC
s1-oligo53	TTTTTATTCTCCCGACTTGCGGGATTTTTGTT		s2-oligo53	AACTAAAGCGCCTGTAGCATTCCAGGAGGT
s1-oligo54	GCCCAATATATTCTAAGAACGCGCCTTTAC		s2-oligo54	CGTTGAAAACACTGAGTTTCGTCACCACCCTC
s1-oligo55	ACCCAGCTATTCCAAGAACGGGTATCCTGAAC		s2-oligo55	TGTCGTCTGGATTTTGCTAAACACGGTCGC
s1-oligo56	AAGCCTTAACTCATCGAGAACAAAGAACGC		s2-oligo56	CCCTCATAGCGGAGTGAGAATAGAAACAACCA
s1-oligo57	TAGCGAACTTCATCGTAGGAATCACGACGACA		s2-oligo57	AACTACAAGAATTGCGAATAATAAGCTTGATA
s1-oligo58	TCCCAATCACCCACAAGAATTGAGACCAGAAG		s2-oligo58	CGGAATAGTCCTCAAGAGAAGGATCATTAAAG
s1-oligo59	TAACGTCAAGGGTAATTGAGCGCTACGGAATA		s2-oligo59	TTAGTACCCTGAAACATGAAAGTAATTTAC
s1-oligo60	AGAGAGAAAGAATTAACTGAACAACTCCTT		s2-oligo60	AGAACCGCAATGCCCCCTGCCTATCTTTTGAT
s1-oligo61	AATAATAACAAAATAAACAGCCATTATTTTGC		s2-oligo61	AGCGGGGTAGAGGGTTGATATAAAAAGTTT
s1-oligo62	GAGAGATACAAATAAGAAACGATGGTTTTG		s2-oligo62	GCTGAGACGTGTATCACCGTACTCACAGACAG
s1-oligo63	AAAGTCAGAAAATGAAAATAGCAGAGGCGTTT		s2-oligo63	CTATTATTGCCACCCTCAGAACCGCCAGTACA
s1-oligo64	GAAACCGAAATAGAAAATTCATATCCGTCACC		s2-oligo64	CCAGAATGCAGAGCCGCCGCCAGCAGAATCAA
s1-oligo65	CCCAAAAGCACCACGGAATAAGTTAGCCAGCA		s2-oligo65	CGTTCCAGCCACCCTCAGAGCCGGCGCGTT
s1-oligo66	ATTACGCAAAGGTGGCAACATATTTAGCAA		s2-oligo66	GATACAGGCGCCACCCTCAGAACCCCCCTTAT
s1-oligo67	AGCGCCAAATAGCCGAACAAAGTTTTAAGCCC		s2-oligo67	GGAGGTTGAAACAAATAAATCCTTAGGATT
s1-oligo68	TCACAATCGGAAACGCAATAATAAATATCA		s2-oligo68	ACCACCACGAAAGCGCAGTCTCTGATTAAGAG
s1-oligo69	CGCAAAGAAACTGGCATGATTAAGCCCTGAAC		s2-oligo69	CAGAGCCATAAGCGTCATACATGGTTCGGAAC
s1-oligo70	TTATTCATTAAAGGTGAATTATCAGGTTTACC		s2-oligo70	GCAGCACCGTAATCAGTAGCGACATTGACA
s1-oligo71	GACTTGAGCCATTTGGGAATTAGTATTTTG		s2-oligo71	GTTTGCCTTTAGCGTCAGACTGTACCACCAGA
s1-oligo72	AAATCACCAGTAGCACCATTACCAAAAAGAAA		s2-oligo72	TTCATCGGCATTTTCGGTCATAGCGCCACCCT
con-oligo1	GCCTCTTCGCTATTACGCCAGCTGGCGTATTG		end-oligo1	CGCTGGTTTGCCCCAGCAGGCGAAAAATCCCT
con-oligo2	GTGAGCGAAGCCAGCTTTCATCAAATGGCCCA		end-oligo2	TATAAATCAAGCCGGCGAACGTGGTACAGGGC
con-oligo3	CTACGTGAACCGTCTATCAGGGCGCATTAAAT		end-oligo3	CGTTAATAATCTGCCAGTTTGAGGCACTCCAG
con-oligo4	ACAAAGGCAGGGTAGCTATTTTTGTGAGAAGT		end-oligo4	GCGTACTAAGTAGAAGAACTCAAAATTACCGC

Oligo name	sequence
con-oligo5	GTTTTTATCGGTACGCCAGAATCCAGAGATCT
con-oligo6	AATTAGCAATAAATCATACAGGCAATTCTGGC
con-oligo7	CAACAGAGCAGTAATAAAAGGGACAGGCAAAG
con-oligo8	CATTTTTGTAATTGCTCCTTTTGACAGTTGGC
con-oligo9	AAATCAACTCAATCAATATCTGGTTAAGAGGT
con-oligo10	TGGATAGCGGGGTAATAGTAAAATTAATCCTG
con-oligo11	ATTGTTTGTGGCAATTCATCAATAGTTTAGAC
con-oligo12	TAAGAACTATTGTGAATTACCTTATACATTTA
con-oligo13	ACAATTTCAGAAAACAAAATTAATTGCGATTT
con-oligo14	ACTTAGCCGAAATCCGCGACCTGCCGAGAAAA
con-oligo15	CTTTTTCACGCAAGACAAAGAACGTCCATGTT
con-oligo16	CGGGATCGGCAGGGAGTTAAAGGCACCGACAA
con-oligo17	AAGGTAAATAAGAGAATATAAAGTCGCTTTTG
con-oligo18	TCTGTATGTTCCAGACGTTAGTAATAGAAGGC
con-oligo19	TTATCCGGGCAAGCAAATCAGATAATGAATTT
con-oligo20	TGCCGTCGTTTGCTCAGTACCAGGAAGCGCAT
con-oligo21	TAGACGGGTAACATAAAAACAGGGCGGATAAG
con-oligo22	ATATTCACAGGCAGGTCAGACGATAGAAAATA
con-oligo23	CATACATAGTATGTTAGCAAACGTTGGCCTTG
con-oligo24	GGCCGGAAACGTCACCAATGAAACCATCGATA

Oligo name	sequence
end-oligo5	TAATGTGTAATCAGAAAAGCCCCAATTGTAAA
end-oligo6	CAGCCATTAAACATCGCCATTAAACAGAGGTG
end-oligo7	CATTTCGCCAACGCAAGGATAAAATGCCTGAG
end-oligo8	AGGCGGTCTTAGAAGTATTAGACTTTTGCCCG
end-oligo9	AAGAGGAATATAACAGTTGATTCCATTAGATA
end-oligo10	AACGTTATTTTAACGTCAGATGAAAACAATAA
end-oligo11	TTACGAGGAGGTCTTTACCCTGACAAAAGATT
end-oligo12	CGGATTCGCCCTTAGAATCCTTGAGAAGAGTC
end-oligo13	ACGTAACAGTTGAGATTTAGGAATAAAAGGAA
end-oligo14	AATAGTGAGGAATCATAATTACTACAAATTCT
end-oligo15	CAAAAGAAGCTGACCTTCATCAAGCCAAATCA
end-oligo16	TACCAGTATAATATCCCATCCTAAGCTGTCTT
end-oligo17	CTTTCGAGTAAACGGGTAAAATACGAAAGAGG
end-oligo18	TCCTTATCACAATTTTATCCTGAACCTAATTT
end-oligo19	GTACCGTAATCTCCAAAAAAAAGGTCAGCTTG
end-oligo20	GCCAGTTAGAGCAAGAAACAATGATAAGAAAA
end-oligo21	AAACAGTTCACCCTCAGAGCCACCGAACCCAT
end-oligo22	GTAAGCAGAGACAAAAGGGCGACAGACGGAAA
end-oligo23	CTCAGAGCAGTGTACTGGTAATAAGCCCGTAT
end-oligo24	TAGCGTTTGCCATCTTTTCATAATCCGCCTCC

4.2 Stripe pattern overhangs and handles

Oligo name	sequence
s2-o34_hand	GCAAGACCTCGCAACTTTTGGGAAGGGCTTGAGATGGTTTACGGTCAAT
s2-o35_hand	GCAAGACCTCGCAACTTACTAACGGGACGAGAAACACCAGTTTGAAA
s2-o36_hand	GCAAGACCTCGCAACTTATTCATCAAAGCTGCTCATTCAGTCCAGGCGC
s1-o34_hand	GCAAGACCTCGCAACTTACAAAATCGCTTCTGTAAATCGTCGGTCTGAG
s1-o35_hand	GCAAGACCTCGCAACTTCAATTACCCATAAATCAATATATGTTAGGTTG
s1-o36_hand	GCAAGACCTCGCAACTTAAACATCAATTTGAATTACCTTTTGATGCA
con-o12_hand	GCAAGACCTCGCAACTTTAAGAACTATTGTGAATTACCTTATACATTTA
end-o12_hand	GCAAGACCTCGCAACTTCGGATTCGCCCTTAGAATCCTTGAGAAGAGTC
INK	GTTGCGAGGTCTTGCTT-amine

Oligo name	sequence
s2-o37_hand	GCAAGACCTCGCAACTTCTTTAATCGGCTCATTATACCAGGAAGTTT
s2-o38_hand	GCAAGACCTCGCAACTTGTAAATTGAAAAATCTACGTTAATAAAAACCA
s2-o39_hand	GCAAGACCTCGCAACTTCTTGCCCTAACAACATTATTACAGCTATCATA
s1-o37_hand	GCAAGACCTCGCAACTTTTAATTTTCCTGATTGCTTTGAATTGCGTAGA
s1-o38_hand	GCAAGACCTCGCAACTTATAACCTTGCGCAGAGGCGAATTTTATTTG
s1-o39_hand	GCAAGACCTCGCAACTTAAACAGTATGAGCAAAAGAAGATGTAATGGAA
con-o13_hand	GCAAGACCTCGCAACTTACAATTTCAGAAAACAAAATTAATTGCGATTT
end-o13_hand	GCAAGACCTCGCAACTTACGTAACAGTTGAGATTTAGGAATAAAAGGAA
BIO	Biotin-TEG-AAGCAAGACCTCGCAAC

4.3 Cross pattern overhangs and handles

Oligo name	sequence
s2-o37_hand	GCAAGACCTCGCAACTTCTTTAATCGGCTCATTATACCAGGAAGTTT
s2-o38_hand	GCAAGACCTCGCAACTTGTAAATTGAAAAATCTACGTTAATAAAAACCA
s2-o39_hand	GCAAGACCTCGCAACTTCTTGCCCTAACAACATTATTACAGCTATCATA
s1-o37_hand	GCAAGACCTCGCAACTTTTAATTTTCCTGATTGCTTTGAATTGCGTAGA
s1-o38_hand	GCAAGACCTCGCAACTTATAACCTTGCGCAGAGGCGAATTTTATTTG
s1-o39_hand	GCAAGACCTCGCAACTTAAACAGTATGAGCAAAAGAAGATGTAATGGAA
con-o13_hand	GCAAGACCTCGCAACTTACAATTTCAGAAAACAAAATTAATTGCGATTT
end-o13_hand	GCAAGACCTCGCAACTTACGTAACAGTTGAGATTTAGGAATAAAAGGAA
con-oligo2_cr_1	GCAAGACCTCGCAACTTGTGAGCGAAGCCAGCTTTCATCAAATGGCCCA
INK_th	GTTGCGAGGTCTTGCTT-thioctic acid

Oligo name	sequence
con-oligo4_cr_1	GCAAGACCTCGCAACTTACAAAGGCAGGGTAGCTATTTTTGTGAGAAGT
con-oligo6_cr_1	GCAAGACCTCGCAACTTAATTAGCAATAAATCATACAGGCAATTCTGGC
con-oligo8_cr_1	GCAAGACCTCGCAACTTCATTTTTGTAATTGCTCCTTTTGACAGTTGGC
con-oligo10_cr_1	GCAAGACCTCGCAACTTTGGATAGCGGGGTAATAGTAAAATTAATCCTG
con-oligo14_cr_1	GCAAGACCTCGCAACTTACTTAGCCGAAATCCGCGACCTGCCGAGAAAA
con-oligo16_cr_1	GCAAGACCTCGCAACTTCGGGATCGGCAGGGAGTTAAAGGCACCGACAA
con-oligo18_cr_1	GCAAGACCTCGCAACTTTCTGTATGTTCCAGACGTTAGTAATAGAAGGC
con-oligo20_cr_1	GCAAGACCTCGCAACTTTGCCGTCGTTTGCTCAGTACCAGGAAGCGCAT
con-oligo22_cr_1	GCAAGACCTCGCAACTTATATTCACAGGCAGGTCAGACGATAGAAAATA
BIO	Biotin-TEG-AAGCAAGACCTCGCAAC

4.4 '3 spot' patterns for gold nanoparticles

Oligo name	sequence
3'pT-s1-oligo43	TAAACACCATTTATCAAAATCATAGCTATTAATTTTTTTTTTTTTTT
3'pT-con-oligo12	TAAGAACTATTGTGAATTACCTTATACATTTATTTTTTTTTTTTTTT
3'pT-con-oligo13	ACAATTTCAGAAAACAAAATTAATTGCGATTTTTTTTTTTTTTTTT
3'pT-con-oligo15	CTTTTTCACGCAAGACAAAGAACGTCCATGTTTTTTTTTTTTTTTT
3'pT-s2-oligo30	CAAAAATCCATAGTAAGAGCAACAGTAGAAAGTTTTTTTTTTTTTT

Oligo name	sequence
3'pT-s2-oligo36	ATTCATCAAAGCTGCTCATTCAGTCCAGGCGCTTTTTTTTTTTTTT
3'pT-end-oligo10	AACGTTATTTTAACGTCAGATGAAAACAATAATTTTTTTTTTTTTT
3'pT-end-oligo12	CGGATTCGCCCTTAGAATCCTTGAGAAGAGTCTTTTTTTTTTTTTT
3'pT-end-oligo15	CAAAAGAAGCTGACCTTCATCAAGCCAAATCATTTTTTTTTTTTTT
INK_th	AAAAAAAAAAAAAAAA-Thioctic acid