Electronic Supplementary Information

for manuscript entitled

DNA-Mediated, Site-Specific Sorting of Gold Nanoparticles onto Silicon Wafers

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Contents

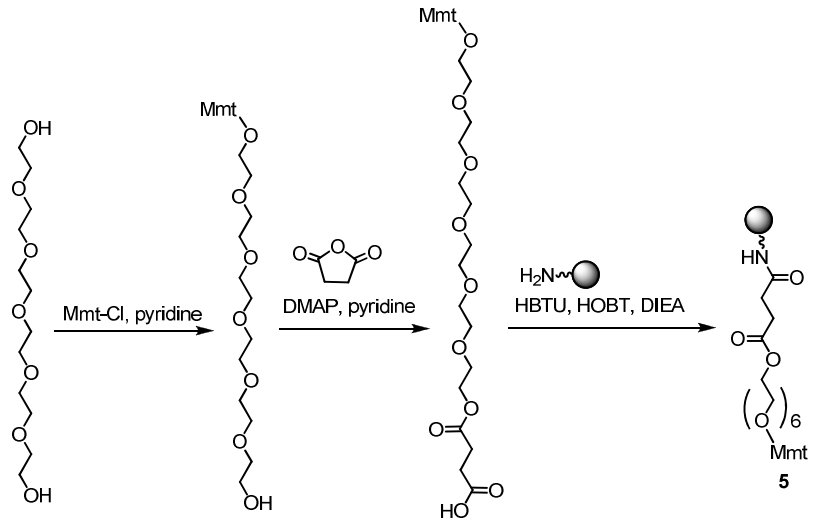
1. General Information

Anhydrous solvents were purchased and stored over molecular sieves and were used without further purification. Reagents were from Acros (Geel, Belgium), Aldrich/Sigma/Fluka (Deisenhofen, Germany), TCI (Zwijndrecht, Belgium) or ChemPur (Karlsruhe, Germany). Unmodified DNA oligomers were from Biomers (Ulm, Germany) in HPLC-purified form. Oxidized silicon wafers were from Crystec (Berlin, Deutschland). Reversed (i.e. 5'-) β-cyanoethyl phosphoramidites and DMT-Hexaethyloxy-Glycol phosphoramidite were from Chemgenes (Wilmington, MA, USA). NMR spectra were recorded on a Bruker AC250 or a Bruker Advance DRX 500 spectrometer. MALDI TOF mass spectra were acquired on Bruker REFLEX IV or BIFLEX IV spectrometers, using software packages XACQ 4.0.4 or XTof 5.1. Oligonucleotides were analyzed in linear negative mode. A mixture of 2,4,6-trihydroxyacetophenone (0.3 M in EtOH) and diammoniumcitrate (0.1 M in H_2O) in ratio (v:v = 2:1) was used as MALDI matrix.[1] Absorption spectra were recorded on a Perkin Elmer Lambda 750 spectrometer or on a NanoDrop ND-1000 spectrometer. Oligonucleotides were synthesized on a DNA-Synthesizer PE 8909 from Applied Biosystems (Foster City, CA, USA) via the phosphoramidite method. AFM (Atomic Force Microscopy)-was done on a NanoscopeII AFM with a Dimension™ 3100 detector head from Digital Instruments Veeco Metrology Group (Mannheim, Germany) with Si_3N_4 NCH-W cantilevers from Nanosensors (Neuchatel, Switzerland), or on a Level-AFM from Anfatec (Oelsnitz, Germany) with NSC 16/AIBS cantilevers from MikroMasch (Tallinn, Estland). Measurments were done in "non-contact" mode.

2. DNA synthesis (General Protocol A)

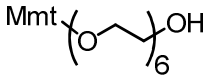
Glass bottles for reagents were dried at 130 °C for 4 h and were allowed to cool under vacuum. Phosphoramidites were dried at 0.1 mbar for 12 h and then dissolved in dry acetonitrile under an argon atmosphere. Molecular sieves were added to the solutions (3 Å, 10 beads). Syntheses were performed on 1 or 2 µmolar scale. The synthetic protocols were those of the manufacturer of the synthesizer (Perseptive Biosystems 8909 expedite DNA synthesizer). Cleavage of the DNA from controlled pore glass (cpg) and deprotection occurred in 2 mL polypropylene reaction vessel with ammonium hydroxide solution (1 mL, 30% in water) at 55 °C for 4 h. Thiol-terminated chains were deprotected with DTT in the deprotection solution (2 mL, 200 mM in NH_3, 30% in water). The supernatant was removed and the cpg washed with ammonium hydroxide solution (3 x 1 mL, 30% in water). The solutions were pooled und dried in a stream of compressed air.

3. Synthesis of solid support



Scheme S1. Synthetic route to support **5**.

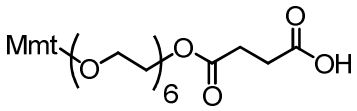
O-Monomethoxytritylhexaethyleneglycol



Hexaethyleneglycol (2.00 g, 7.00 mmol, 3.00 eq.) was dissolved in pyridine (3 mL). MMT-Cl (0.72 g, 2.33 mmol, 1.00 eq.) was added. The mixture was stirred 1 d at 20 °C. Then, dichloromethane (30 mL) was added and the solution was extracted with water (100 mL) and brine (100 mL). The organic layer was dried over $MgSO_4$ and concentrated. The crude was coevaporated with toluene (3x20 mL), followed by purification by column chromatography (acetone/MeOH 9:1). The title compound was isolated as a yellowish oil in a yield of 41% (0.92 mmol, 512 mg). TLC (Dichloromethane/methanol 9:1) R_f = 0.86. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm) = 7.40-7.38 (m, 4H, *Mmt*); 7.27-7.11 (m, 8H, *Mmt*); 6.71-6.74 (m, 2H, *Mmt*); 3.71 (s, 1H, CH_3); 3.67-3.50 (m, 22H, CH_2); 3.167-3.14 (m, 2H, CH_2). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm) 158.48, 144.62, 135.86, 128.47, 127.87, 127.76, 126.98, 113.20, 86.23, 72.57,

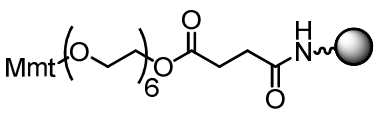
72.53, 70.75, 70.70, 70.69, 70.64, 70.57, 70.51, 70.33, 70.27, 63.23, 61.75, 55.22. FAB-MS: 553.3.

_O_-Monomethoxytrityl-_O'_-succinylhexaethyleneglycol



_O_-Monomethoxytritylhexaethyleneglycol (120 mg, 220 µmol, 1.00 eq.) dissolved in dichloromethane (2 mL) was treated with DIEA (115 µL, 660 µmol, 3.00 eq.) and succinic anhydride (28.6 mg, 286 µmol, 1.30 eq.). The mixture was stirred for 18 h at 20 °C, and then washed with citric acid solution (10 mL, 2% in water). The organic layer was dried over $MgSO_4$ and concentrated. The product was obtained as a slightly yellowish oil in a yield of 93% (205 µmol, 140 mg) and was used without further purification. TLC (dichloromethane/methanol 9:1) R_f = 0.66. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm) = 9.66 (brs, 1H, *COOH*); 7.40-7.38 (m, 4H, *Mmt*); 7.27-7.11 (m, 8H, *Mmt*); 6.76-6.74 (m, 2H, *Mmt*); 4.18-4.16 (m, 2H, CH_2); 3.71 (s, 1H, CH_3); 3.60-3.55 (m, 20H, CH_2); 3.15 (t, J = 6.8 Hz, 2H, CH_2); 2.56-2.54 (m, 4H, *Succinyl-*CH_2). ^{13}C-NMR (101 MHz, $CDCl_3$) δ (ppm) 175.77, 172.16, 158.48, 144.61, 135.85, 130.37, 128.46, 127.77, 126.80, 113.05, 86.24, 70.76, 70.70, 70.65, 70.61, 70.51, 70.44, 68.99, 63.81, 63.22, 55.22, 29.40. FAB-MS: 677.2.

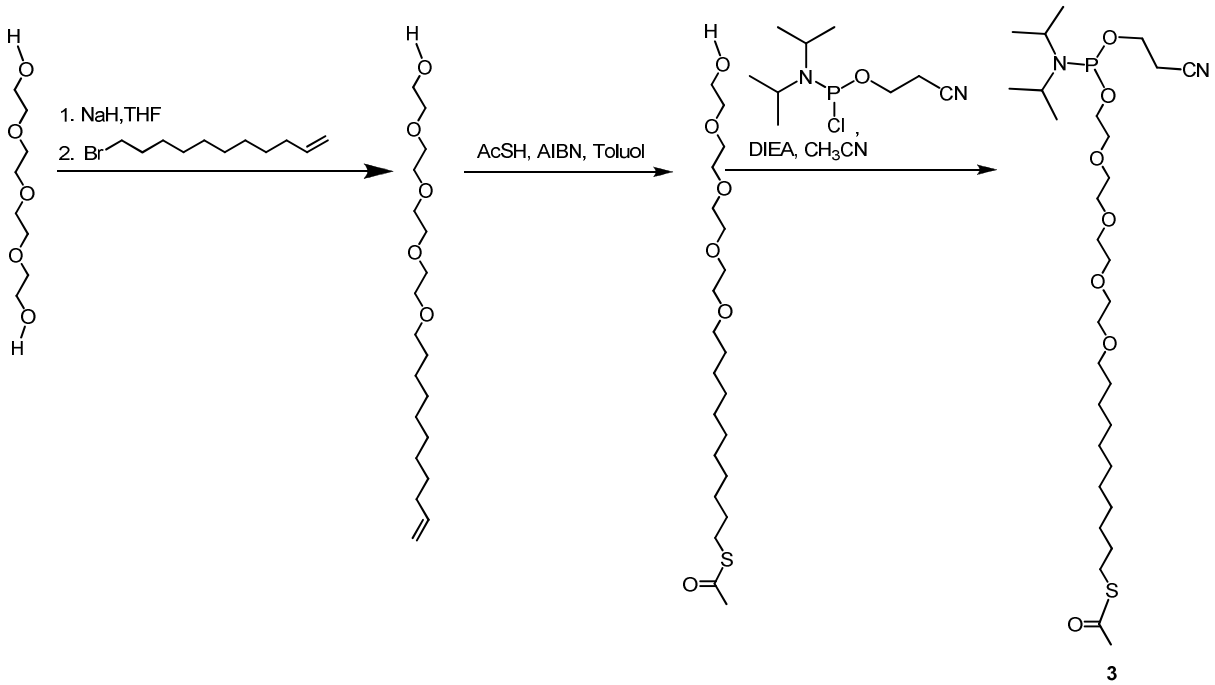
LCAA cpg loaded with _O_-monomethoxytrityl-_O'_-succinylhexaethyleneglycol (6)



Long-chain alkylamine (LCAA) cpg (533 mg, 51.0 µmol, 1.0 eq.) was mixed with _O_-monomethoxytrityl-_O'_-succinylhexaethyleneglycol (140 mg, 205 µmol, 4.00 eq.), HOBt

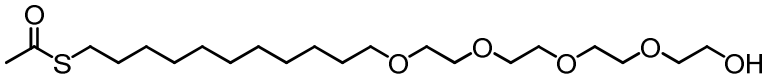
(32.0 mg, 205 µmol, 4.00 eq.), HBTU (74.0 mg, 196 µmol, 3.80 eq.), and DIEA (72.0 µL, 410 µmol, 8.00 eq.) in dry DMF (2 mL) for 18 h at 20 °C. Subsequently, the cpg was washed with dichloromethane (15 mL), ethyl acetate (15 mL), and acetonitrile (15 mL), and then dried at 0.1 mbar. The loading of the cpg was determined via absorption of the Mmt cation at 450 nm in a deblock solution. Loading: 12.0 µmol/g.

4. Synthesis of the thioester-terminated phosphoramidite 3 and filler molecule 5



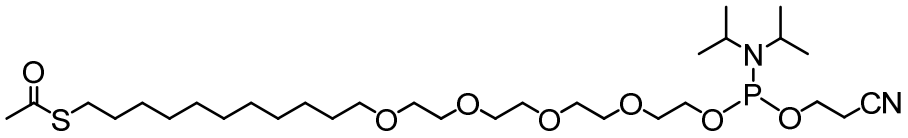
Scheme S2. Synthetic route to thioester-terminated phosphoramidite **3**.

13-*O*-[1-(Methylcarbonylthio)undec-11-yl]tetraethyleneglycol



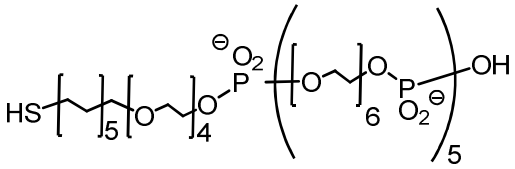
The thioester was synthesized as described in the literature[1,2] and was obtained in a yield of 85%.

13-*O*-[1-(Methylcarbonylthio)undec-11-yl]tetraethyleneglykol-1-*O*-[(2-cyanoethyl)-*N,N*-diisopropyl]phosphoramidite (3)



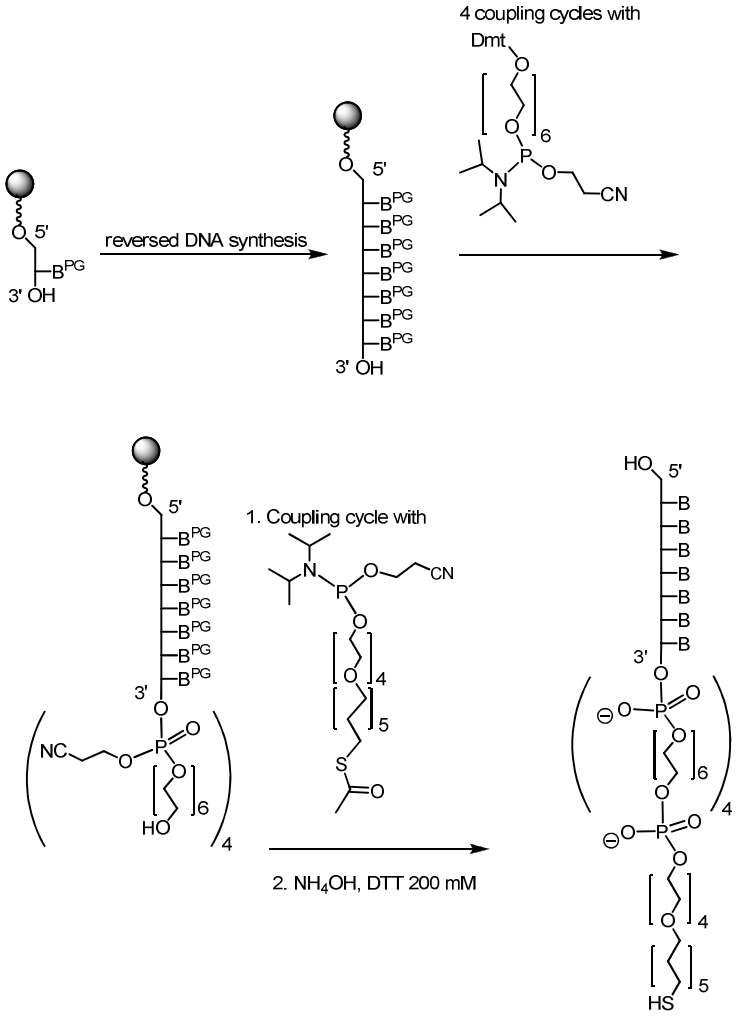
The thioester (1.04 g, 1.46 mmol, 1.00 eq.) was dissolved in acetonitrile (6 mL). After adding molecular sieves (3 Å, 10 beads) and DIEA (1.09 mL, 6.39 mmol, 2.60 eq.), the mixture was stirred for 30 min at 20° C. Then, cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (860 µL, 3.19 mmol, 1.30 eq.) was added, followed by stirring for 3 h at 20 °C. The mixture was added to dichloromethane (10 mL) and was washed with saturated aqueous $NaHCO_3$ solution (20 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The resulting crude was purified via column chromatography (ethyl acetate/toluene 1:1, 1% TEA), and the title compound was obtained as slightly yellow oil in 53% yield (1.30 mmol, 811 mg). TLC ethyl acetate /toluene, 1:1, 1% TEA) $R_f = 0.64$. ^{1}H-NMR (600 MHz, $CDCl_3$) δ (ppm) = 3.87-3.82 (m, 4H, *cyanoethyl*); 3.66-3.57 (m, 16H, CH_2); 3.44 (t, J = 6.8, 2H, CH_2); 2.86 (t, J = 7.3 Hz, 2H, CH_2); 2.67-2.65 (m, 2H, *isopropyl*); 2.32 (s, 3H, CH_3); 1.56-1.54 (m, 4H, CH_2); 1.31-1.26 (m, 14H, CH_2); 1.20-1.18 (m, 12H, *disopropyl*). ^{13}C-NMR (101 MHz, $CDCl_3$) δ (ppm) 195.91, 117.64, 71.42, 71.13, 71.08, 70.59, 69.95, 62.54, 58.49, 42.99, 42.89, 30.54, 29.53, 29.45, 29.40, 29.37, 29.04, 29.00, 28.70, 25.98, 24.50, 20.25. ^{31}P-NMR (243 MHz, $CDCl_3$, {^{1}H}) δ (ppm) 150.2.

Filler Molecule (5)



The synthesis of **5** was performed starting from **6** via automated DNA synthesis on a Perseptive Biosystems 8909 expedite DNA synthesizer using the protocol provided by the manufacturer, as detailed in General Protocol A. Four chain extension cycles were performed with a commercial phosphoramidite building block for hexaethyleneglycol (18-*O*-dimethoxytritylhexaethylene glycol-1-(2-cyanoethyl)-(*N,N*-diisopropyl)-phosphoramidite; marketed as 18-spacer phosphoramidite by Chemgenes, Ashland, MA). followed by an extension cycle with thioester-terminated phosphoramidite **3**. Yield: 25% HPLC (C_{18} column): CH_3CN gradient 0% to 90%, elution at 40-45% B, MALDI-TOF-MS: 2099.9.

5. Synthesis of 3'-thiol-terminated DNA strands 1 and 2



Scheme S3. Synthetic route to thiol-terminated oligonucleotides **1** and **2**.

The syntheses of **1** and **2** were performed on an Perseptive Biosystems 8909 expedite DNA synthesizer using the protocol provided by the manufacturer, as detailed in General Protocol A, starting from controlled pore glass loaded with the first nucleoside via the 5'-position. Chain extension cycles with 5'-phosphoramidites were used to build the DNA chain, followed by an extension cycle with **3**. The deprotection solution contained DTT, as described in General Protocol A.

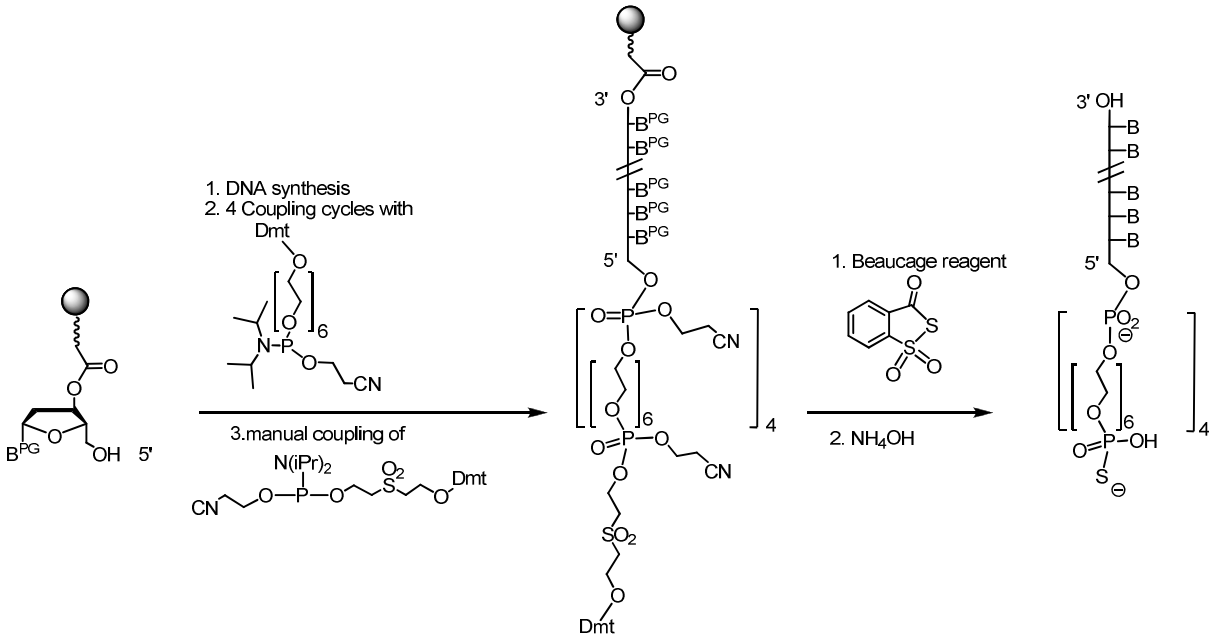
3'-[Mercaptoundecantetrakis(ethylene glycolphosphoryl)]-GATGACATCCGA-5' (1)

Yield: 20%, HPLC: CH_3CN gradient 0% to 90%, eluation a 20% B, MALDI-TOF-MS: 5468.8.

3'-[Mercaptoundecanyltetrakis(ethylene glycolphosphoryl)]-ATGCTCAACTCT-5' (2)

Yield: 14%, HPLC (C_{18} column): CH_3CN gradient 0% to 90%, elution at 30% B, MALDI-TOF-MS: 5399.9.

6. Syntheses of phosphorothioate-terminated DNA strands 11-13



Scheme S4. Synthetic route to phosphorothioate-terminated DNA sequences **11-13**.

Syntheses were performed on a Perseptive Biosystems 8909 expedite DNA synthesizer via the protocol provided by the manufacturer (General Protocol A), starting from conventional controlled pore glass loaded with the first nucleoside via its 3'-position. After the last coupling step, the phosphoramidite building block of chemical phosphorylation reagent (Glen Research Inc., Sterling, VA, USA) was coupled manually. For this, the cpg (20 µmol loading) was dried in a polypropyene reaction vessel with the phosphoramidite (197 mg, 0.30 mmol, 15.0 eq.) for 12 h at 0.1 mbar. The solids were treated with activator solution (4,5-dicyanoimidazole, 0.25 M in abs. CH_3CN, 100 µL), and the resulting slurry shaken for 5 h at 20 °C. Then, the cpg was washed with acetonitrile (3 x 100 µL) and dichloromethane (3 x 100 µL), and dried at 0.1 mbar for 30 min. Sulfurization was induced by treatment with Beaucage reagent (3*H*-1,2-benzodithiol-3-one 1,1 dioxide, 0.05 g in 5 mL abs. acetonitrile) for 20 min, followed by washing with acetonitrile. The oligonucleotide was cleaved from the solid support and fully deprotected with ammonia (30% in water).

3'-ACCAACTGACGCTA-(18-Spacer)$_4$-PO$_2$SH-5' (11)

Yield: 16% HPLC (C_{18} column): CH_3CN gradient 0% to 90%, elution at 20%, MALDI-TOF-MS: 5689.5.

3'-TTCTTTCTTTTT-(18-Spacer)$_4$-PO$_2$SH-5' (12)

Yield: 17% HPLC (C_{18} column): CH_3CN gradient 0% to 90%, elution at 15%, MALDI-TOF-MS: 5023.5.

3'-AACTACCATAGAAAAT-(18-Spacer)$_4$-PO$_2$SH-5' (13)

Yield: 15%, HPLC (C_{18} column): CH_3CN gradient 0% to 90%, elution at 20%, MALDI-TOF-MS: 6338.7.

7. Preparation of surfaces and nanoparticles, and sorting assays

DNA-Bearing Gold Nanoparticles 7 and 8

For the immobilization of DNA on gold nanoparticles, purified 3'-thiol-terminated oligonucleotides **1** or **2** (2.4 nmol, solution in water) were added to a suspension of citrate-capped nanoparticles (1.2 mL) in a polypropylene vial (for 15 nm diameter nanoparticles 1.4 x 10^{12} particles per mL; for 60 nm diameter particles 2.6 x 10^{10} particles per mL) at 20 °C, followed by incubation for 18 h. Afterwards, filler molecule **5** (2.4 nmol, solution in water) was added to the suspension, followed by incubation for another 18 h. To buffer the suspension it was brought to 10 mM KH_2PO_4 by adding 18 µL of a 1 M stock solution of KH_2PO_4 (pH 7). In the subsequent salt aging process, the nanoparticle suspension was first brought to 0.05 M NaCl concentration by adding aliquots from a 2 M stock solution. The suspension was allowed to stand for 24 h. Then it was brought to 0.1 M NaCl concentration by addition of another aliquot. After 24 h, the suspension was brought to 0.2 M NaCl, and after another 24 h it was brought to 0.3 M NaCl concentration. To remove the excess thiols the suspension was centrifuged and the pellet was resuspended in buffer (1.5 mL, 0.3 M NaCl, 10 mM KH_2PO_4, 0.01% NaN_3). Precipitates were harvested by centrifugation two times for either 25 min at 14 000 rpm (15 nm particles) or for 15 min at 3 000 rpm (60 nm particles). The precipitates were resuspended in buffer (100 µL, 0.3 M NaCl, 10 mM KH_2PO_4, 0.01% NaN_3) to obtain a final concentration of 4 nM nanoparticles (15 nm diameter) or 0.2 nM nanoparticles (60 nm diameter).

Cleaning the wafer surface

Silicon oxide wafers (Crystec, Berlin) were first stored in acetone for 12 h to remove the protective film. Then, wafers were added to a solution of H_2O_2 / H_2SO_4 (1:2, 80 °C) for 30 min. After rinsing with doubly distilled water, wafers were stored for 30 min in NaOH

(2.5 M), washed with water and stored for 15 min in HCl (0.1 M). Finally, the surfaces were rinsed with water and methanol, dried in a stream of argon and stored in polypropylene vials.

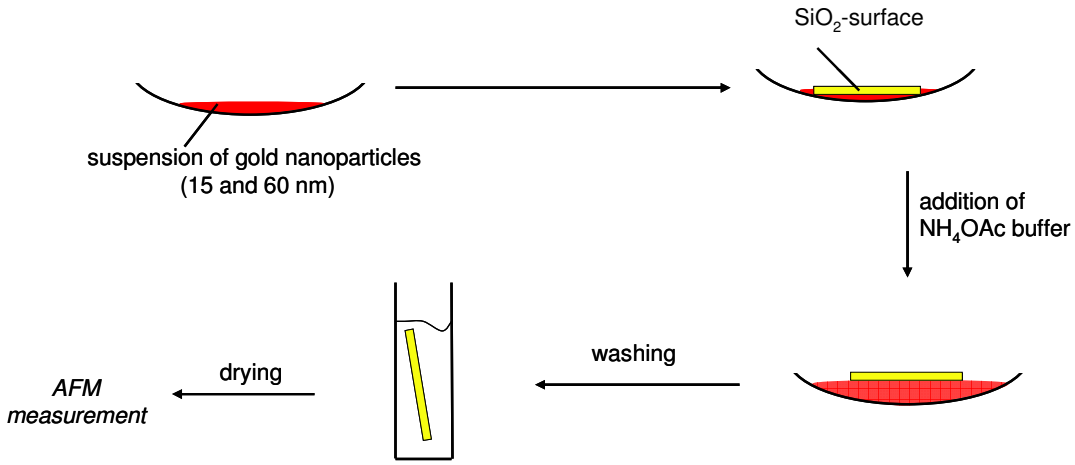
Silanization of the surface and coupling of bifunctional linker

The cleaned wafers were added to a solution of 3-aminopropyldiisopropylethoxysilane (10 µL) in water (25 µL), ethanol (465 µL) and H_2SO_4 (2.5 µL, 5%) for 2 h. Then, the surfaces were rinsed with ethanol and dried in an argon stream. The coupling of the bifunctional linker molecule α-bromoacetic acid (6 mg, 44 nmol) was induced in DMF (2 mL) with DMAP (0.5 mg, 4 nmol) and EDC (9 mg, 42 nmol) for 2 h in a polypropylene vial. After washing the surface with ethanol, the wafer was dried in an argon stream.

Immobilization of capture oligonucleotides 11-13 and hybridization of the splint strands 14 and 15.

Spots were generated by spotting of solutions of 5'-phosphothioate-DNA strands **11**, **12** or **13** (0.5 µL, 0.25 mM) in phosphate buffer (pH 7.4) onto the surface for 2 h at 20 °C. Surfaces were then washed with double-distilled water and dried in a stream of argon. Hybridization of splint strands was achieved by adding solutions of DNA **14** or **15** (1 µL of 1.5 µM solution in 0.25 M NH_4OAc buffer) to the respective spots for 12 h at 4 °C, followed by rinsing the surfaces with aqueous NH_4OAc buffer (2 mL of 0.25 M).

Hybridization of Nanoparticles to Surfaces (Sorting Assay)



Scheme S5. Cartoon of the sorting assays leading to site-specific binding of nanoparticles to the DNA-labeled areas of the silicon wafer.

Sorting of nanoparticles onto spots with different DNA-strands was induced in the hollow side of a watch glass (diameter 2.5 cm). The wafer was placed upside down in a drop of the suspension (50 µL) of the mixture 15 nm nanoparticles (2 nM) and 60 nm nanoparticles (0.1 nM) in buffer (0.3 M NaCl, 10 mM KH_2PO_4, 0.01% NaN_3). Sorting was allowed to occur for 10 min at 40 °C and 10 min at 20 °C. Then, NH_4OAc buffer (1 mL, 1 M) was added to the watch glass. The wafer was transferred to a separate vessel and incubated for 2 min in fresh NH_4OAc buffer (1 mL), while shaking at 400 rpm on a rotating platform. Finally the slides were dried in a stream of argon and analyzed by AFM.

References for Supporting Information

1. C. Pale-Grosdemange, E. S. Simon, L. K. Prime, G. M. Whitesides, *J. Am. Chem. Soc.* **1991**, *113*, 12-20.
2. S. Svedhelm, L. Öhberg, S. Borelli, *Langmuir* **2002**, *18*, 2848-2858