Supporting Information

Spatially Controlled DNA Nano-patterns by “Click” Chemistry Using Oligonucleotides with Different Anchoring Sites

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S1. Experimental details

A. General experimental

Silicon wafers were purchased from Silicon Materials Corporation (Germany). Triethoxyoctylsilane (Sigma-Aldrich) and 11-bromoundecyltrimethoxysilane (ABCR, Germany) were used as

received. Double distilled water (18.2 MΩ·cm, MilliQ system) was used. Solvents were of laboratory grade and used as supplied except where noted. The following solvents (toluene and DMF) were dried by molecular sieves for 24 hours before being used. Thin layer Chromatography (TLC): aluminium sheets, silica gel 60 F254 (0.2 mm; Merck, Darmstadt, Germany). Flash column chromatography (FC): silica gel 60 (VWR, Germany) at 0.4 bar; sample collection with an Ultra Rac II fractions collector (LKB Instruments, Sweden). Reversed-phase HPLC was carried out on a 250 × 4 mm RP-18 column (Merck) with a Merck-Hitachi HPLC pump (model L-7100) connected with a variable wavelength monitor (model L-7400). MALDI-TOF mass spectra were recorded with Applied Biosystems Voyager DE PRO spectrometer with 3-hydroxypicolinic acid (3-HPA) as a matrix. The melting temperature (T_m) curves were measured with a Cary-100 Bio UV-VIS spectrophotometer (Varian, Australia) equipped with a Cary thermoelectrical controller. The temperature was measured continuously in the reference cell with a Pt-100 resistor, and the thermodynamic data of duplex formation were calculated by the MeltWin (version 3.0) program using the curve fitting of the melting profiles according to a two-state model.¹ The dynamic light scattering experiment was performed on a DynaPro-Ms/X instrument (Protein-Solutions, Charlottesville, USA (now: Wyatt Technology Corporation, Santa Barbara, USA)). AFM characterization was made on Nanoscope Multimode IIIa (Veeco, USA) at the tapping mode.

B. The preparation of the azide-terminated silicon substrates

Silicon wafers cut into 1 × 1 cm² square pieces were soaked in chromosulfuric acid solution for 1 hour and then liberally rinsed with deionized H₂O and dried under a stream of N₂ gas. Cleaned wafers were immersed in aqueous NaOH (0.1 mol·L⁻¹) for 7 minutes and subsequently in HNO₃ (0.1 mol·L⁻¹) for 10 minutes to generate surface hydroxyl groups. After the silicon substrates had been washed with an excess of water and dried under a stream of N₂ flow, they were placed in a solution of 1.2% (V/V) 11-bromoundecyltrimethoxysilane (BUTS) and 2.8% (V/V) triethoxyoctylsilane (TEOS) in dry toluene (10 mL) at 20 °C for 24 hours. The wafers were then soaked for 5 min and

sonicated for 30 seconds in toluene, and then washed with ethanol and DMF. The bromo-terminated wafers were placed in saturated NaN_3 in DMF at 20 °C for 48 hours. Wafers were rinsed with DMF and then H_2O and dried under a stream of N_2 gas.² The wafers were stored in individual fluoroware containers and protected from heat and light until used or for up to 1 month. The proportion of BUTS/TEOS can be adjusted according to different requirements, while their total concentration remains the same. In order to prepare other mole-ratio of bromo-terminated silicon wafers, different concentration of BUTS and TEOS in dry toluene were introduced, such as 0.2% : 3.8%, 2.4% : 1.6%, 4% : 0.

C. Synthesis, purification, and characterization of the oligonucleotides 2-7

The syntheses of oligonucleotides were performed on a DNA synthesizer, model 392-08 (Applied Biosystems, Weiterstadt, Germany) at 1 μmol scale following the synthesis protocol for 3'-O-(2-cyanoethyl)phosphoramidites.³ After cleavage from the solid support, the oligonucleotides were deprotected in 25% aqueous ammonia solution for 12-16 h at 60 °C. The purification of the 'trityl-on' oligonucleotides was carried out on reversed-phase HPLC (Merck-Hitachi-HPLC; RP-18 column; gradient system (A = MeCN, B = 0.1 M $(\text{Et}_3\text{NH})\text{OAc}$ (pH 7.0)/MeCN, 95:5): 3 min 15% A in B, 12 min 15-50% A in B, and 5 min 50-10% A in B; flow rate 1.0 cm^3/min . The purified 'trityl-on' oligonucleotides were treated with 2.5% $\text{CHCl}_2\text{COOH}/\text{CH}_2\text{Cl}_2$ for 5 min at 0 °C to remove the 4,4'-dimethoxytrityl residues. The detritylated oligomers were purified again by reversed-phase HPLC (gradient: 0-25 min 0-20% A in B; flow rate 1.0 ml/min). The oligomers were desalted on a short column (RP-18) and lyophilized on a Speed-Vac evaporator to yield colorless solids which were frozen at -24 °C. The molecular masses of the oligonucleotides **2-7** were determined by MALDI-TOF mass spectrometry in the linear negative mode (see Table 1).

Table S1. Single-strand oligonucleotides used in this study.

Entry	Sequences	[M-1] ⁻	[M-1] ⁻
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	G* = modified G with single terminal triple bonds	(calc.)	(found)
ODN-2	5'-d(A G* TATTGACCTA)-3'	3747.6	3746.3
ODN-3	5'-d(A G* TATT G* ACCTA)-3'	3851.7	3851.8
ODN-4	5'-d(AGTATT G* ACCTA)-3'	3747.6	3746.9
ODN-5	5'-d(TTTTTT G* TTTTT)-3'	3716.5	3716.7
ODN-6	5'-d(TTTTTT G* AAAAA)-3'	3761.6	3762.1
ODN-7	5'-d(TTTTTTAGTATT G* ACCTAAAAAAA)-3'	7452.0	7452.4

D. Immobilization of various single-stranded oligonucleotides on the azide-terminated silicon wafer surfaces

To the single-stranded oligonucleotide (22 μ M) in H₂O, a 1:1 CuSO₄-TBTA ligand complex (0.03 cm³ of a 20 mM stock solution in H₂O/DMSO/*t*-BuOH 4 : 3 : 1), tris(carboxyethyl)phosphine (TCEP; 0.03 cm³ of a 20 mM stock solution in water), 0.04 cm³ of H₂O and 0.08 cm³ DMSO were added. Then the azide-terminated silicon wafer was immersed in this solution, which was continuously shaken gently for several hours at 20 °C (for **ODN-2** to **ODN-5**: 20 hours; for **ODN-6** and **ODN-7**: 2 hours to accomplish the DNA immobilization and assembly.) The DNA-immobilized surface was successively washed with deionized H₂O, ethanol, n-hexane, and finally by deionized H₂O to ensure that all unbound DNA and other reactants, catalyst were removed thoroughly from the surface. Then the surface was substantially dried under a flow of N₂ gas and investigated by AFM.

Scheme S1. Surface modification of silicon substrates conjugated with single-stranded DNA incorporated 8-aza-7-deaza-7-(octa-1,7-diynyl)-2'-deoxyguanosine by “click” chemistry.

E. Hybridization of oligonucleotide probes to complementary targets

To a 1:1 CuSO_4 -TBTA ligand complex (0.03 cm^3 of a 20 mM stock solution in $\text{H}_2\text{O}/\text{DMSO}/t\text{-BuOH}$ 4 : 3 : 1), tris(carboxyethyl)phosphine (TCEP; 0.03 cm^3 of a 20 mM stock solution in water), 0.04 cm^3 of H_2O and 0.08 cm^3 DMSO were added. The single-stranded oligonucleotide (22 μM) **ODN-7** in $250 \mu\text{L}$ of a 1 M NaCl solution was annealed by heating the solution at 80°C for 5 min followed by slow cooling. This oligonucleotide solution was added to the above solution. Then the azide-terminated silicon wafer was immersed in this solution and continued to shake slightly for several hours at 20°C . The DNA-immobilized surface was washed with deionized H_2O , ethanol, n-hexane, and finally deionized H_2O to ensure that all unbound DNA and unreacted catalyst were removed from the surface. Then the surface was substantially dried under a flow of N_2 gas and investigated by AFM.

Figure S1. T_m curves of ODN-7 strand was measured (75 °C - 15 °C) by UV spectroscopy at 260 nm in 1 M NaCl, 100 mM MgCl₂, and 60 mM Na-cacodylate (pH 7.0) (a) with 0.5 μM of single-strand concentration and (b) 2.0 μM of single-strand concentration; (c) and (d) was measured at 260 nm in 100 mM NaCl, 10 mM MgCl₂ and 10 mM Na-cacodylate (pH 7.0) with 0.5 μM and 2.0 μM of single-strand concentration, respectively.

Table S2. T_m -values of hairpin ODN-7 with different single-strand concentration.^a

Single-strand (ODN-7)	Concentration [μM]	T_m [°C]	ΔG°_{310} ^b [kcal/mol]
5'-d(TTTTTTAGTATT G *ACCTAAAAAAA)	0.5	47 (41)	-8.7 -7.8
	2.0	47.5 (41)	-9.0 -7.7

^a Measured at 260 nm in 1 M NaCl, 100 mM MgCl₂, and 60 mM Na-cacodylate (pH 7.0) with different single-strand concentration. Data in parentheses were measured in 0.1 M NaCl, 10 mM MgCl₂ and 10 mM Na-cacodylate (pH 7.0) at the same concentration. ^b ΔG°_{310} values are given with 15% error.

S2. Large scale AFM images

The AFM investigation was conducted on a flat silicon substrate (with DNA nanostructures on it) using a NanoScope IIIa instrument (DI, USA) in the tapping mode at room temperature (~ 20 °C).

Figure S2. Large scale AFM images of silicon substrates modified with single-stranded DNA by “click” chemistry. (size: $1 \times 1 \mu\text{m}$, height: 5 nm) (a) undecylazide / octyl pre-modified silicon wafer; (b) **ODN-2** (5'-d(AG*TATTGACCTA)-3'); (c) **ODN-3** (5'-d(AG*TATTG*ACCTA)-3'); (d) **ODN-4** (5'-d(AGTATTG*ACCTA)-3'); (e) large scale image of **ODN-4** on the substrate (size: $2 \times 2 \mu\text{m}$).

Figure S3. AFM images of silicon substrate after immersing in a solution containing **ODN-4** (5'-d(AGTATTG*ACCTA)-3') (CuSO_4 -TBTA ligand, TCEP, 20 h, r.t). From the above images, we could conclude that nearly no DNA subsides on the blank silicon surface.

Figure S4. AFM images of azide-modified silicon substrate after immersing in the solution containing **ODN-4** (5'-d(AGTATTG*ACCTA)-3') for 20 hours. In this controlled experiment, the catalysts were not added, so the “click” reaction could not occur under this condition. Thereafter, the substrate was washed by distilled water for three times. We observed that, but not much, DNA residues remained on the azide-modified surface, but DNA nanostructure could not be found compared with the result when the “click” reaction was involved.

Figure S5. Large-scale AFM image obtained from **ODN-4** (5'-d(AGTATTG*ACCTA)-3') using ethanol as the last solvent before the drying process and AFM imaging, which is almost identical to the morphology obtained by the water or hexane treatment.

Figure S6. Large scale AFM images of silicon substrates modified with **ODN-4** (5'-d(AGTAT TG*ACCTA)-3') by "click" chemistry on pre-modified substrate with different mole ratios of BUTS and TEOS. (a) the ratio of BUTS : TEOS is 1 : 9; (b) the ratio is 6 : 4; (c) the ratio is 10 : 0, the size of images (a-c) is 1 × 1 μm, height: 5 nm. (d) ~ (f) large scaled images of **ODN-5** (5'-d(TTTTTT G*TTTTT)-3') coated silicon substrate, the scales range from 1 μm to 4 μm.

Figure S7. Large-scale AFM images of silicon substrates modified with **ODN-6** (left) (5'-d(TTTTTTG*A AAAA)-3') and **ODN-7** (right) (5'-d(TTTTTTAGTATTG*ACCTAAAAAAA)-3') by “click” chemistry, the size is $2 \times 2 \mu\text{m}$.

Figure S8. AFM images of silicon substrates modified with self-complementary **ODN-7** (5'-d(TTTTTTAGTATTG*ACCTAAAAAAA)-3') by “click” chemistry. The hairpin structure of **ODN-7** was formed in the 0.2 M NaCl solution before surface immobilization. The size of the image is $1 \times 1 \mu\text{m}$.

References

- (1) Petersheim, M.; Turner, D. H. *Biochemistry*, **1983**, 22, 256–263.
- (2) Lummerstorfer, T.; Hoffmann, H. *J. Phys. Chem. B* **2004**, 108, 3963–3966.
- (3) Seela, F.; Xiong, H.; Leonard, P.; Budow, S. *Org. Biomol. Chem.*, **2009**, 7, 1374–1387.

S3. MALDI-mass spectra of oligonucleotides

Figure S8. MALDI-mass spectra of ODN-2

Figure S10. MALDI-mass spectra of ODN-3

Applied Biosystems Voyager System 6327

Mode of operation: Linear
Extraction mode: Delayed
Polarity: Negative
Acquisition control: Manual
Accelerating voltage: 25000 V
Grid voltage: 91%
Guide wire 0: 0.2%
Extraction delay time: 150 nsec
Acquisition mass range: 1500 – 15000 Da
Number of laser shots: 300/spectrum
Laser intensity: 2337
Laser Rep Rate: 20.0 Hz
Calibration type: Default
Calibration matrix: 3-Hydroxypicolinic acid
Low mass gate: 1000 Da
Digitizer start time: 23.58
Bin size: 4 nsec
Number of data points: 12668
Vertical scale: 500 mV
Vertical offset: 0%
Input bandwidth: 500 MHz
Sample well: 14
Plate ID: PLATE 1
Serial number: 6327
Instrument name: Voyager-DE PRO
Plate type filename: C:\VOYAGER\100 well plate.plt
Lab name: PE Biosystems
Absolute x-position: 17476.9
Absolute y-position: 42014.7
Relative x-position: 649.447
Relative y-position: -212.838
Shots in spectrum: 300
Source pressure: 1.54e-008
Mirror pressure: 2.065e-007
TC2 pressure: 0.002763
TIS gate width: 8
TIS flight length: 690

Acquired: 17:13:00, February 01, 2008

Printed: 14:54, February 04, 2008

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Figure S9. MALDI-mass spectra of ODN-4

Applied Biosystems Voyager System 6327

Mode of operation: Linear
Extraction mode: Delayed
Polarity: Negative
Acquisition control: Manual
Accelerating voltage: 25000 V
Grid voltage: 90%
Guide wire 0: 0.2%
Extraction delay time: 150 nsec
Acquisition mass range: 1500 – 10000 Da
Number of laser shots: 300/spectrum
Laser intensity: 2281
Laser Rep Rate: 20.0 Hz
Calibration type: Default
Calibration matrix: 3-Hydroxypicolinic acid
Low mass gate: 1000 Da
Digitizer start time: 23.82
Bin size: 4 nsec
Number of data points: 9286
Vertical scale: 500 mV
Vertical offset: 0%
Input bandwidth: 500 MHz
Sample well: 86
Plate ID: PLATE 1
Serial number: 6327
Instrument name: Voyager-DE PRO
Plate type filename: C:\VOYAGER\100 well plate.plt
Lab name: PE Biosystems
Absolute x-position: 27385.9
Absolute y-position: 6579.16
Relative x-position: 408.428
Relative y-position: -88.3428
Shots in spectrum: 300
Source pressure: 2.004e-007
Mirror pressure: 9.495e-008
TC2 pressure: 0.001
TIS gate width: 8
TIS flight length: 688

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Figure S11. MALDI-mass spectra of ODN-5

Applied Biosystems Voyager System 6327

Mode of operation: Linear
Extraction mode: Delayed
Polarity: Negative
Acquisition control: Manual

Accelerating voltage: 25000 V
Grid voltage: 93%
Guide wire 0: 0.2%
Extraction delay time: 150 nsec

Acquisition mass range: 1500 – 15000 Da
Number of laser shots: 300/spectrum
Laser intensity: 2211
Laser Rep Rate: 23.0 Hz
Calibration type: Default
Calibration matrix: 3-Hydroxypicolinic acid
Low mass gate: 1000 Da

Digitizer start time: 23.62
Bin size: 4 nsec
Number of data points: 12684
Vertical scale: 500 mV
Vertical offset: 0%
Input bandwidth: 500 MHz

Sample well: 18
Plate ID: PLATE 1
Serial number: 6327
Instrument name: Voyager-DE PRO
Plate type filename: C:\VOYAGER\100 well plate.plt
Lab name: PE Biosystems

Absolute x-position: 36923.1
Absolute y-position: 42530
Relative x-position: -224.376
Relative y-position: 302.521
Shots in spectrum: 300
Source pressure: 3.123e-007
Mirror pressure: 7.516e-008
TC2 pressure: 0.001
TIS gate width: 8
TIS flight length: 688

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Printed: 13:01, March 09, 2010

Figure S12. MALDI-mass spectra of ODN-6

Applied Biosystems Voyager System 6327

Mode of operation: Linear
Extraction mode: Delayed
Polarity: Negative
Acquisition control: Manual

Accelerating voltage: 25000 V
Grid voltage: 91%
Guide wire 0: 0.2%
Extraction delay time: 150 nsec

Acquisition mass range: 1500 – 15000 Da
Number of laser shots: 300/spectrum
Laser intensity: 2261
Laser Rep Rate: 20.0 Hz
Calibration type: Default
Calibration matrix: 3-Hydroxypicolinic acid
Low mass gate: 1000 Da

Digitizer start time: 23.576
Bin size: 4 nsec
Number of data points: 12665
Vertical scale: 500 mV
Vertical offset: 0%
Input bandwidth: 500 MHz

Sample well: 26
Plate ID: PLATE 1
Serial number: 6327
Instrument name: Voyager-DE PRO
Plate type filename: C:\VOYAGER\100 well plate.plt
Lab name: PE Biosystems

Absolute x-position: 26495.6
Absolute y-position: 36968.8
Relative x-position: -491.882
Relative y-position: -178.712
Shots in spectrum: 300
Source pressure: 2.304e-007
Mirror pressure: 7.738e-008
TC2 pressure: 0.001
TIS gate width: 8
TIS flight length: 688

Figure S13. MALDI-mass spectra of ODN-7