Supplementary Material (ESI) for Analyst
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Supporting Information

Linker immobilization of protein and oligonucleotide on indium tin oxide for detection of probe-target interactions by Kelvin physics

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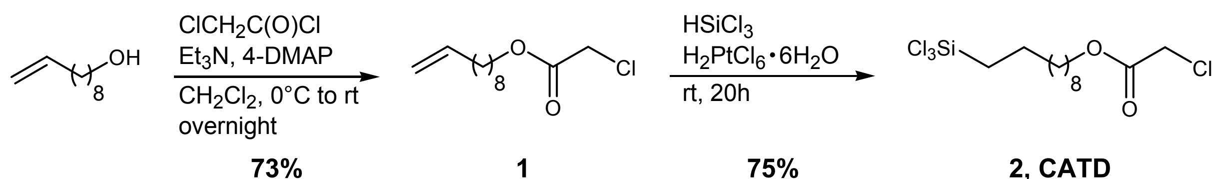
I. General remarks

The following includes detailed experimental procedures, surface characterization data and spectroscopic data for the synthesized compounds. Anhydrous solvents (DMF, PhMe, $CHCl_3$) were systematically used. Freshly distilled anhydrous DMF (from CaH_2, under high vacuum) was systematically used for the immobilization of biotinthiol. $H_2PtCl_6.6H_2O$ was purchased from Strem®. Biotin, octyltrichlorosilane (OTS), avidin, BSA and Dulbecco's phosphate buffered saline (PBS) were purchased from Sigma-Aldrich®. Other reagents were also purchased from Sigma-Aldrich® and used as received unless otherwise noted. Thiolated oligonucleotide probe, non-thiolated oligonucleotide target and non-thiolated oligonucleotide mismatch were purchased as 50 μM solutions from the DNA synthesis faculty of the Sick Kids Hospital (Toronto, Ontario, Canada). ITO slides (10 mm x 10 mm x 1.1 mm) were purchased from Delta Technologies Ltd®. The preparation of the mixed CATD/OTS SAMs and the biotinthiol immobilization step were performed in a glove box maintained under an inert (N_2) and anhydrous (P_2O_5) atmosphere. Microarray maps were printed using a Virtek Chipwriter Professional arrayer (Waterloo, Ontario, Canada). The microarrayer tip was programmed to pick protein or oligonucleotide samples (10 nL) from a pin reservoir via capillary action, and to deposit the spots (diameter ~ 100 μm) onto the desired surface in a micro-scale uniform fashion. The spacing between the spots was 250 μm. The printing was performed at 25 °C in a chamber maintained at a humidity of 65-70%. ^1H and ^{13}C NMR spectra were recorded at room temperature on Varian 300 and 400 MHz spectrometers using $CDCl_3$ as the NMR solvent. ^1H and ^{13}C NMR spectra are referenced to the residual solvent peak (7.27 ppm and 77.23 ppm, respectively). Chemical shifts are given in ppm. The abbreviations s, t and m respectively stand for singlet, triplet and multiplet.

II. CATD linker and biotinthiol syntheses

II. A. CATD synthesis

CATD[i] was synthesized in two steps from commercially available dec-9-en-1-ol with a 55% overall yield (***Scheme S1***).



Scheme S1: 2 steps CATD synthesis

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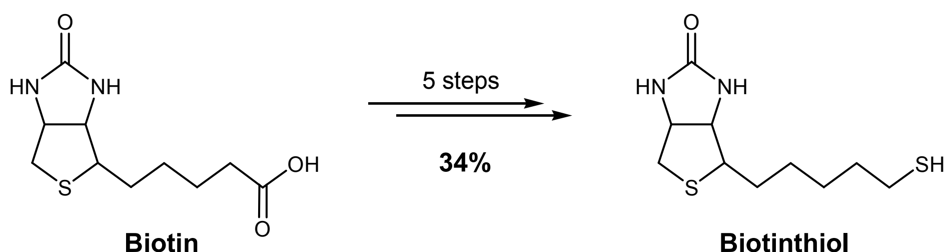
O-(α-chloroacetyl)-dec-9-en-1-ol 1. To a stirred solution of dec-9-en-1-ol (3.68 mL, 20.0 mmol, 1.0 equiv.), Et_3N (5.60 mL, 40.0 mmol, 2.0 equiv.) and 4-DMAP (0.25 g, 2.0 mmol, 0.1 equiv.) in CH_2Cl_2 (50 mL) was added dropwise chloroacetyl chloride (3.90 mL, 48.0 mmol, 2.4 equiv.) at 0°C. After addition, the resulting solution was allowed to warm to room temperature then stirred overnight. The reaction was quenched with a saturated NH_4Cl aqueous solution and the resulting aqueous layer was extracted with CH_2Cl_2. The combined organic layers were then dried over anhydrous $MgSO_4$, filtered and finally evaporated in *vacuo*. Purification was achieved by flash column chromatography on silica gel (Hexanes/EtOAc gradient). **O-(α-chloroacetyl)-dec-9-en-1-ol 1**: yellow oil (3.38 g, 73%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.81 (m, 1H), 4.99 (m, 1H), 4.93 (m, 1H), 4.18 (t, *J* = 6.6 Hz, 2H), 4.06 (s, 2H), 2.04 (m, 2H), 1.65 (m, 2H), 1.4 to 1.2 (m, 10 H).

O-(α-ChloroAcetyl)-10-Trichlorosilyl Decan-1-ol 2 (CATD). In a heavy-walled tube equipped with a magnetic stirring bar, **O-(α-chloroacetyl)-dec-9-en-1-ol 1** (2.19 g, 10.0 mmol, 1.0 equiv.) and $H_2PtCl_6.6H_2O$ (52 mg, 0.10 mmol, 1.0 mol. %) were placed. The tube was transferred into a glove-box and $HSiCl_3$ (2.10 mL, 20.6 mmol, 2.0 equiv.) was added to the solution. The tube was tightly fastened then removed from the glove-box. The resulting solution was stirred at room temperature for 20 hours behind a protecting shield. Purification was achieved by Kugelrohr distillation under high vacuum. **CATD 2**: colourless oil (2.59 g, 75%); bp = 155-175 ºC (0.40 Torr); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.20 (t, *J* = 6.6 Hz, 2H), 4.08 (s, 2H), 1.8 to 1.2 (m, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.6, 66.6, 41.1, 32.0, 29.6, 29.4, 29.3, 29.2, 28.6, 25.9, 24.5, 22.5.

II. B. Biotinthiol synthesis

Biotinthiol was synthesized in five steps from commercially available biotin with a 34% overall yield (***Scheme S2***). Each of the intermediate steps have already been reported in different publications.[ii,iii,iv]



Scheme S2: Biotinthiol synthesis

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III. Preparation of clean ITO slides

Prior to cleaning our ITO slides, their conductive side was detected using a voltmeter. ITO slides were then separately sonicated in dishwashing soap for 30 minutes then thoroughly rinsed with distilled water and methanol. After 20 minutes of sonication in methanol, the slides were dried under a gentle stream of N_2. Subsequently, the slides were plasma cleaned for 5 minutes under nitrogen gas plasma with the conductive side facing upward. Finally, ITO slides were transferred into a 60%-maintained ($MgNO_3.6H_2O$) humidity chamber in individual open vials for 24 hours.

IV. Formation of mixed CATD/OTS SAMs onto ITO slides

Neat CATD (10 μL) and neat OTS were separately diluted with anhydrous toluene (10 mL). The resulting solutions were portioned (500 μL of each) and mixed into separate test tubes to which clean ITO slides were soaked. The test tubes were tightly capped with rubber stoppers and set aside for 2 hours. Afterwards, the crystals were rinsed three times with toluene then sonicated with another portion of toluene for 5 minutes. This rinsing procedure was repeated with chloroform. Finally, the slides were dried under a gentle stream of N_2 and individually stored in closed vials for further biotinthiol and thiolated oligonucleotide immobilization.

V. Biotin and oligonucleotide microarray preparation

V. A. Biotin microarray

In a first step, biotinthiol (1 mg) was dissolved in freshly distilled DMF (1 mL). The resulting 1 mg/mL biotinthiol solution was then portioned (1000 μL) into separate test tubes to which mixed CATD/OTS SAM-coated ITO slides were soaked. The test tubes were tightly capped with rubber stoppers then placed in a dark compartment overnight. The following day, the slides were rinsed three times with methanol, dried under a gentle stream of N_2 then individually stored in vials prior to the proteins (avidin and BSA) microarray printing.

In a second step, 1 mg/mL solutions of avidin (biotin specific) and BSA (biotin non-specific) were separately prepared in PBS (1 mL). 8 x 8 microarray maps were then printed with these solutions onto biotin-functionalized mixed CATD/OTS SAM-coated ITO slides. The microarray maps were printed in an alternating fashion such that 4 lines (8 spots each) of printed avidin would alternate with 4 lines of printed BSA. After printing, the slides were kept inside the humidity chamber for 1 hour. Finally, the slides were thoroughly washed with PBS followed by ultra pure water, dried under a gentle stream of N_2 then individually stored in closed vials for SKN analysis.

V. B. Oligonucleotide microarray

For our oligonucleotide microarrays, three different types of single stranded oligonucleotide molecules were used (***Figure S1***): a thiolated oligonucleotide probe (A), a non-thiolated oligonucleotide target (B), and a non-thiolated oligonucleotide mismatch (C).

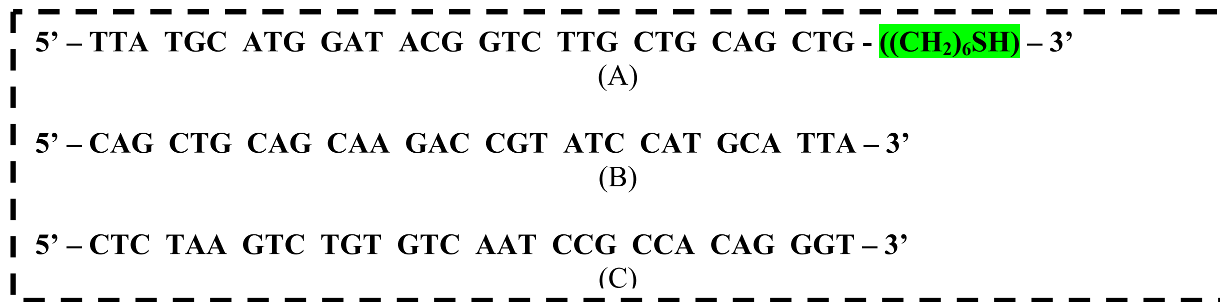

Figure S1: Nucleotide sequence of the (A) thiolated oligonucleotide probe, (B) non-thiolated oligonucleotide target and (C) the non-thiolated oligonucleotide mismatch.

For the purpose of our study, three different types of oligonucleotide microarrays were printed. The first type was a "one sample" microarray map, which consisted in printing thiolated oligonucleotide probe spots (8 x 8) onto mixed CATD/OTS SAM-coated ITO slides. The second type was a "two sample" microarray map, which consisted in printing alternating lines (8 spots each) of thiolated and non-thiolated oligonucleotides. The third type was a "complementary hybridization" microarray map. The latter consisted in printing first a 8 x 8 "one sample" microarray map of thiolated oligonucleotide probe then in subsequently printing a 8 x 8 alternating "two sample" microarray map of non-thiolated oligonucleotide target and mismatch on top of the former "one sample" microarray map. After printing, the slides were kept inside the humidity chamber for 1 hour then individually stored in closed vials. The following day, the slides were thoroughly washed with PBS followed by ultra pure water, dried under a gentle stream of N_2 then analyzed with SKN.

VI. Surface characterization and analysis

VI. A. SAMs characterization

VI. A. 1. Contact angle measurements

A drop of distilled water was gently deposited onto SAM-coated ITO slides. A digital photograph was then taken laterally. Using a protractor and visual approximation, the contact angle between the water droplet surface and the slide surface was measured (see ***Table S1*** in section VI. A. 2.).

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VI. A. 2. XPS analysis

In order to determine the optimal reaction time for the formation of our SAMs onto ITO slides, we performed a time trial experiment. SAM-coated ITO slides were analyzed using a Leybold (specs) Max 200 X-ray photoelectron spectrometer. The analysis was performed at an angle of 90°. The following atoms were analyzed: C, O, Cl, Si, In, and Sn, as they were the primary atoms composing the molecular structure of our SAMs and the ITO surfaces (***Table S1***). The respective relative ratios of chlorine to carbon, which are a reflection of our surfaces nature and coverage, were also calculated (***Table S2***). They allowed us to determine the optimal reaction time for the formation of our SAMs, which is 2 hours.

Sample	**Time** (min)	**Contact Angle** (°)	**XPS** (atomic %)					
			C (1s)	**O (2s)**	**Cl (2p)**	**Si (2p)**	**In (3d5)**	**Sn (3d5)**
Control[a]	0	9	--	61.4	--	12.9	13.0	4.9
1	30	41	43.5	37.2	0.70	8.90	6.90	2.2
2	60	50	47.3	34.1	0.70	10.1	5.70	1.7
3	**120**	**64**	**48.7**	**32.2**	**1.10**	**10.9**	**5.20**	**1.6**
4	180	58	52.3	30.8	0.90	9.90	4.50	1.0
5	240	51	55.0	28.9	0.97	10.2	3.70	0.80

Table S1: Contact angle values and XPS atomic percentages (determined at 90° angle of analysis) of SAM-coated ITO slides prepared after various reaction times. [a] The control is a clean bare ITO slide.

Sample	**Time** (min)	**Cl/C atomic % ratios**
Control[a]	0	--
1	30	0.016
2	60	0.015
3	**120**	**0.021**
4	180	0.017
5	240	0.017

Table S2: Calculated chlorine/carbon atomic percentages ratios. [a] The control is a clean bare ITO slide.

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VI. B. SKN Measurements

The microarray maps prepared in section V. were analyzed under ambient conditions using our SKN instrument. Only a 1000 μm x 1000 μm portion of the microarray grids were scanned, as it was enough to reveal relevant information about the microarray surfaces. Our SKN measurements allowed us to not only determine that biotinthiol and our thiolated oligonucleotide probe actually immobilized onto our SAMs, but also to reveal the specific biotin-avidin interactions and the complementary oligonucleotide probe-target hybridization.

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