Supporting Information

A robust DNA interface on silicon electrode

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1. EXPERIMENTAL DETAILS

1.1. Materials. All reagents were purchased in their highest grade of purity and were used without further purification unless otherwise stated. Undecylenic acid (≥95%), potassium ferricyanide (99%), *N,N,N′,N′*-tetramethylethane-1,2-diamine (99%), bovine serum albumin (96%) and *N*-hydroxysuccinimide (98%) were purchased from Sigma-Aldrich, whereas 1-(3-dimethylaminopropyl-3-ethylcarbodiimide hydrochloride (98%) and copper(I) bromide (99.99%) were obtained from Alfa Aesar. Ethanol, ethyl acetate, butanol, diethyl ether, glacial acetic acid, acetonitrile, and dichloromethane used for chemical reactions, purification, and surface cleaning were re-distilled prior to use. 1,3-Diazidopropane was synthesised from 1,3-dibromopropane according to the published procedure.[1] 1,8-Nonadiyne (97%, Alfa Aesar and 98%, Sigma) was redistilled from sodium borohydride (99+%, Sigma-Aldrich) under reduced pressure (60 °C, 25-30 Torr) and was collected over activated molecular sieves (3 Å pore diameter and 10-20 mesh beads) and was then stored under an argon atmosphere prior to use. Highly-doped single side polished p-type Si(111) wafers (< 0.001 Ω cm^{-1}) were obtained from Siltronix, SAS (Archapms, France). Ammonium fluoride (40% solution, Aldrich), hydrogen peroxide (30 wt.% solution in water, Sigma-Aldrich), and

sulfuric acid (Sigma-Aldrich) used for the etching and cleaning the electrode surface were of semiconductor grade. Hydrosilylation was carried out in a custom made UV reactor, comprising of 5 x 8W UVC lamps with a UV power output of 1.3 mW/cm^2.

All solutions, glassware, pipettes and pipette tips used for DNA handling were autoclaved using a Prestige Medical Century2 Autoclave. DNAs were purchased from BaseClick, Germany and Geneworks, Australia, and were stored at −20 °C. The sequences for DNA were 5'-GGG-GCA-GTG-CCT-CAC-AAC-CT-C_3-NH_2-3' (probe), 5'-GGG-GCA-GTG-CCT-CAC-AAC-CT-5-octadiynyl dU-3' (alkyne-DNA-probe), 5'-AGG-TTG-TGA-GGC-ACT-GCC-CC-3' (complementary), 5'-GGA-TGG-ACG-AAG-CGC-TCA-GG-3' (non-complementary), 5'-AGG-TTG-TGA-GGC-CCT-GCC-CC-3' (mismatched), 5'-AGG-TTG-TGA-GGC-GCT-GCC-CC-3' (mismatched).

1.2. Surface Modification. *1.2.1.UV hydrosilylation.* After cutting Si(111) wafer to a desired size, the wafer was sonicated in re-distilled ethanol for 5 minutes followed by rinsing with Milli-Q water (18 MΩ cm^{-1}, Millipore, Sydney). The wafer was further cleaned in Piranha solution (1 H_2O_2: 2 H_2SO_4) for 20 minutes. ***Caution: piranha is extremely powerful oxidiser and potentially explosive in contact with organic material and therefore should be handled with extreme care.*** The clean electrode was then rinsed with Milli-Q water and etched in degassed 40% NH_4F for 20 minutes. After drying, the sample was placed in a Petri dish containing either undecylenic acid (Scheme 1a) or 1,8-nonadiyne (Scheme 1b), which was then placed in a custom-made UV reactor, and the reaction was allowed to take place overnight under an inert atmosphere. Upon completion of the hydrosilylation reaction, the silicon sample was removed and rinsed with ethanol and boiling acetic acid (Scheme 1a,

SAM-1) or dichloromethane (Scheme 1b, **SAM-5**). The samples were stored in dichloromethane at 4 °C under an argon atmosphere until needed.

1.2.2. Immobilisation of DNA on Si(111) / undecylenic acid. Hydrosilylation of silicon wafers with undecylenic acid yielded surface **SAM-1** (Scheme 1a), which was activated with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) / *N*-hydroxysuccinimide (NHS) chemistry[2] (**SAM-2**) followed by overnight incubation in probe single stranded (ss)-DNA to yield **SAM-3.** Incubation with target DNA yielded **SAM-4**. EDC/NHS chemistry to form covalent bond between –COOH and $–NH_2$ terminated compounds is well-established in the literature,[2] hence is not discussed here in detail, however the relevant X-ray photoelectron spectroscopy (XPS) and X-ray reflectometry data confirming the successful fabrication in each step are given as Supporting Information and are in agreement with previous reports.

1.2.3. Immobilisation of alkyne-terminated DNA on Si(111) / 1,8-nonadiyne. To a 1,8-nonadiyne modified Si(111) wafer (1 x 1 cm, **SAM-5**) was added 20 μL of (i) 1,3-diazidopropane (200 μM in 3:1:2 DMSO/H_2O/tBuOH), (ii) CuBr and *N*,*N*,*N*′,*N*′-tetramethyl-ethane-1,2-diamine in 3:1 DMSO/H_2O, and (iii) 200 μM DNA **(**alkyne-terminated). Reactions were carried out at room temperature for 1 hour in a humidity chamber to yield **SAM-6**.[1,3] For hybridisation ss-DNA modified surfaces was incubated overnight in a solution of 11.5 μM target DNA at 37 °C. The modified samples (**SAM-7**) were then rinsed thoroughly with redistilled ethanol and Milli-Q water. The procedure is illustrated in Scheme 1b.

1.3. Surface Characterization.

1.3.1. X-ray photoelectron spectroscopy. X-ray photoelectron spectroscopy data was acquired using an ESCALAB 220iXL spectrometer with a hemispherical analyser and multi-channel detector. Monochromatic Al Kα (1486.6 eV) was used as a source of radiation. The resolution of the spectrometer was ca. 0.6 eV. Spectra were recorded in normal emission, selecting a spot size of approximately 1 mm^2, and the analysing chamber was operated below 10^{-9} mbar. The incident angle was set to 58° to the analyser lens. High resolution spectra were recorded with 100 ms dwell time, 0.1 eV step size, and 20 eV pass energy of the analyser, whereas survey spectra were recorded over 1100 – 0 eV range with, 100 ms dwell time, 1.0 eV step size, and 100 eV pass energy of the analyser. Analysis of the XPS spectra was performed using XPSPEAK41 software. After background subtraction using the Shirley routine, spectra were fitted with a convolution of Lorentzian and Gaussian profiles as described previously.[4] Depending on the chemical composition of the modified surfaces, different weightings of these functions were used.

1.3.2. X-ray reflectometry. X-ray reflectivity measurements were obtained in air on a Panalytical Ltd X'Pert Pro reflectometer using Cu Kα X-ray radiation (λ = 1.54056 Å) produced from a 45 kV tube source. The X-ray beam was focused using a Göbel mirror and collimated with 0.2 mm pre- and post-sample slits. Reflectivity data was collected with a step size of 0.010° and a counting time of 15 s per step over the angular range of $0.05° \leq \theta \leq 4.00°$, where θ is the angle of incidence of the X-ray beam impinging on the surface. Samples were 20 x 40 mm in size. Structural parameters of the immobilised thin layers were refined using the MOTOFIT reflectivity analysis software[5] with reflectivity data as a function of $Q = 4\pi(\sin\theta)/\lambda$, the momentum transfer vector. Single or double layer model were used to fit the observed data.

1.3.3. Electrochemical measurements. Electrochemical experiments were performed in a conventional three electrodes polytetrafluoroethylene cell with the modified silicon surface as the working electrode, a platinum mesh (ca. 1200 mm^2) as the counter electrode, and silver/silver chloride in 3 M sodium chloride as the reference electrode. A rectilinear cross-section Viton gasket defined the geometric area of the working electrode to 24.6 mm^2 with a copper plate for electrical contact and gallium indium as a eutectic. Cyclic voltammetry measurements were performed using a BAS 100B electrochemical analyzer (Bioanalytical Systems, Inc., W. Lafayette, IN). Electrochemical impedance measurements were performed with a Solartron 1255B (Farnborough, U.K.) frequency response analyzer interfaced to a Solartron 1287 potentiostat/galvanostat module. Impedance data were measured in the frequency range of 0.1 MHz to 0.1 Hz. An ac potential with 15 mV peak to peak amplitude was superimposed on dc potential. All Faradaic impedance measurements were carried out in a solution of 5 mM $[Fe(CN)_6]^{-3/-4}$. Impedance data were recorded and analysed with ZPlot and ZView 3.1 software (Scribner Associates, Inc.). All electrochemical experiments were performed in dark in a Faradaic cage at room temperature 23 (±2) ^oC.

2. RESULTS

Undecylenic Acid. *2.1. X-ray photoelectron spectroscopy (XPS).* UV-light induced hydrosilylation of undecylenic acid onto freshly etched Si(111) produced **SAM-1** (Scheme 1), which was analysed by XPS (Fig. SI-1 to 3) and XRR (data not given here).

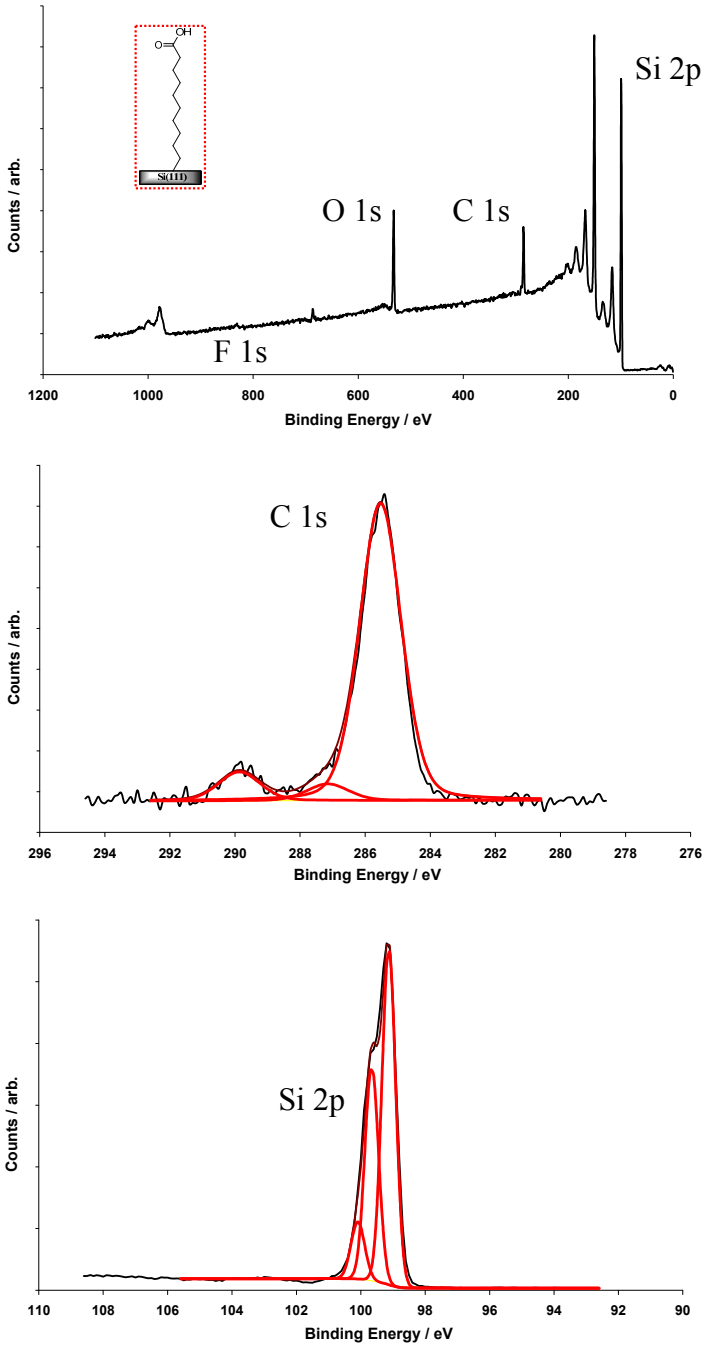


Fig. SI-1 XPS spectra of undecylenic acid modified Si(111) surface after UV-hydrosilylation (**SAM-1**). (a) Survey spectrum (b) narrow scan of the C 1s region, and (c) narrow scan of the Si 2p region. No peak was observed in the 102-105 eV region indicating the absence of SiO_x in the freshly prepared sample.

The C 1s narrow scan in Fig. SI-1 shows two distinct peaks at 285.26 and 289.76 eV. The asymmetric peak is deconvoluted into two peaks. The signal at the lowest binding energy (285.26 eV) is attributed to the aliphatic C-C carbons of the main undecylenic acid chain, and the second peak at 286.90 eV is assigned to carbon adjacent to the carboxyl moiety. The smaller, higher binding energy peak at 289.76 eV, is attributed to the carboxyl carbon of undecylenic acid.[6] This peak appears at a higher binding energy as the carbon is bonded to highly electron withdrawing oxygen group. The approximate peak ratio of 9:1:1 is consistent with those expected for undecylenic acid.

In the Si 2p narrow scan, no peak was detected in the 102-105 eV region, a region that is attributed to SiO_x. This absence of any significant oxides on the surface is indicative of a well-formed monolayer. Due to the presence of advantageous oxygen, the O 1s emission at 533 eV cannot unambiguously be assigned to the C=O and –OH moieties of the carboxylic acid of the undecylenic acid. The survey spectrum also displays a small peak at 686.5 eV, which is attributed to residual fluorine from the etching process.[6,7]

2.2. XPS characterisation of EDC/NHS activated Si(111) surfaces.

The XPS spectra of the EDC/NHS activated surface (**SAM-2**) are shown in Fig. SI-2. The high-resolution N 1s spectrum indicates the presence of several different chemical species.

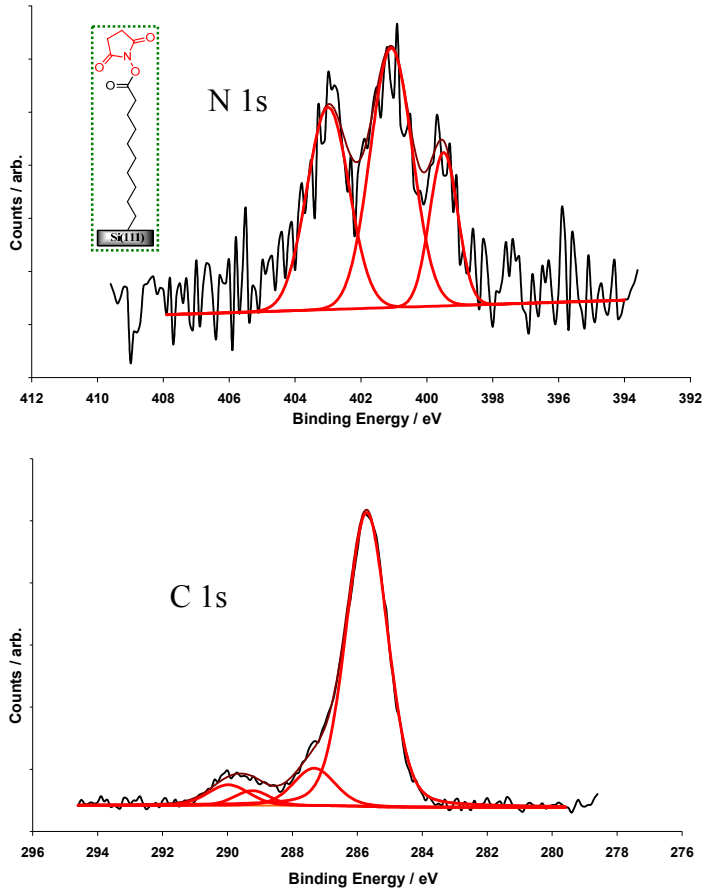


Fig. SI-2: XPS spectra of EDC/NHS activated Si(111)/undecylenic acid surface (**SAM-2**). N1s and C1s peaks corresponding to the formation of succinimide ester confirms the successful activation of undecylenic acid.

Succinimide ester forms as an end product of the activation process. The peak at 402.9 eV is attributed to the N 1s of the succinimide ester, which appears at higher binding energy due to its attachment with the electronegative oxygen in the ester.[8] The peak at 401 eV is attributed to the protonated tertiary amine of EDC, while the peak at 399.4 eV is assigned to the secondary imine and amine of EDC.[8] The peak at 289.6 eV in the C 1s spectra can be deconvoluted into two peaks, i.e., peaks at 289.9 and 289.2 eV correspond to the C=O and C-N moieties, respectively.[9] The presence of the succinimide ester in both the carbon and nitrogen narrow scans indicates that the undecylenic acid was successfully activated.

2.3. XPS characterisation of DNA-modified Si(111) surfaces.

It is difficult to deconvolute the XPS spectra (Fig. SI-3) obtained from the complex DNA-modified surfaces, which contains functional groups in a wide variety of chemical environments. Despite this, it reveals basic information about the success of conjugating DNA on activated silicon surfaces. Firstly, a peak at 401.7 eV in the N 1s narrow scan is attributed to the nucleotide nitrogen signal.[10] Furthermore, the peak at 402.9 eV due to the succinimide ester is reduced by 78% compared to the EDC/NHS spectra (Fig. SI-2), indicating the successful attachment between the terminal amine group of the DNA and the succinimide ester. The peak at 192.1 eV in the P 2s narrow scan, which is slightly higher than the usual peak of 188 eV obtained for native phosphorus, is therefore attributed to the phosphorus within the electronegative environment, i.e., the phosphate backbone of the DNA. The peaks from the C 1s narrow scan could not be correlated confidently due to the complexity of the modified surface. XRR data also support the formation of undecylenic acid monolayer on Si(111) with a thickness 9.5 Å (data not given here).

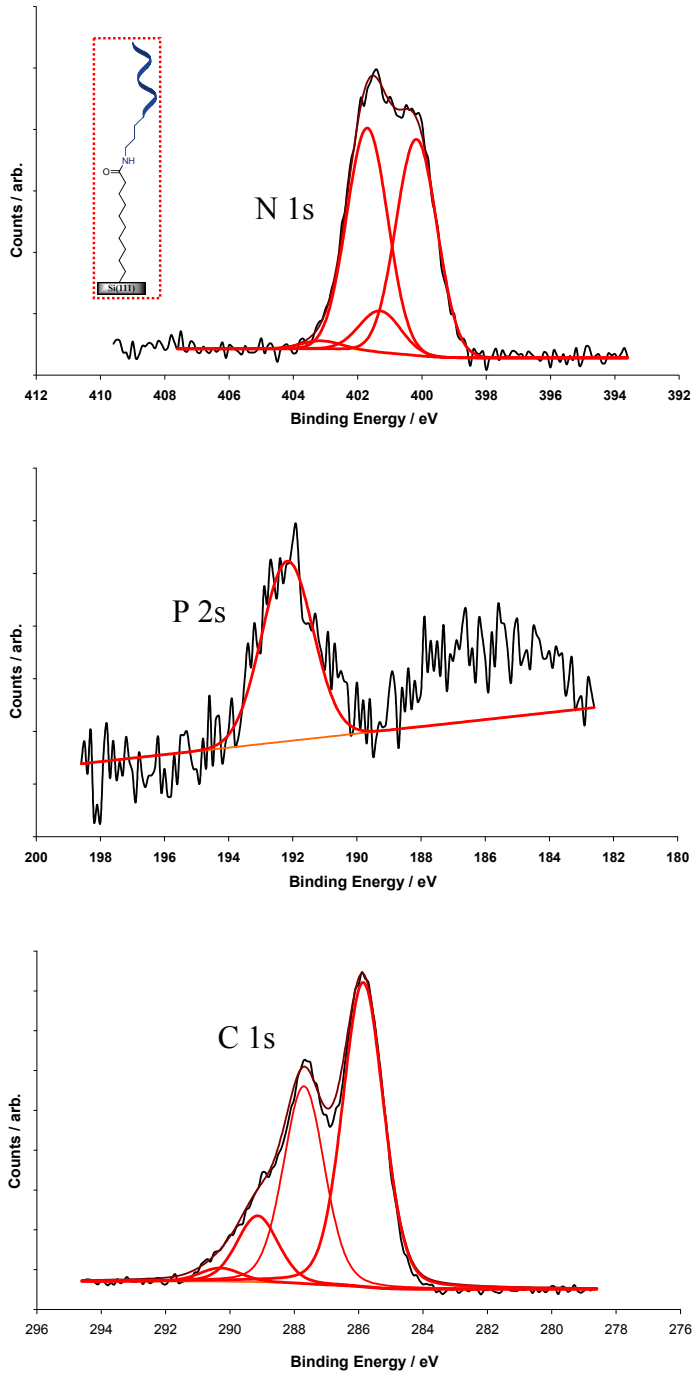
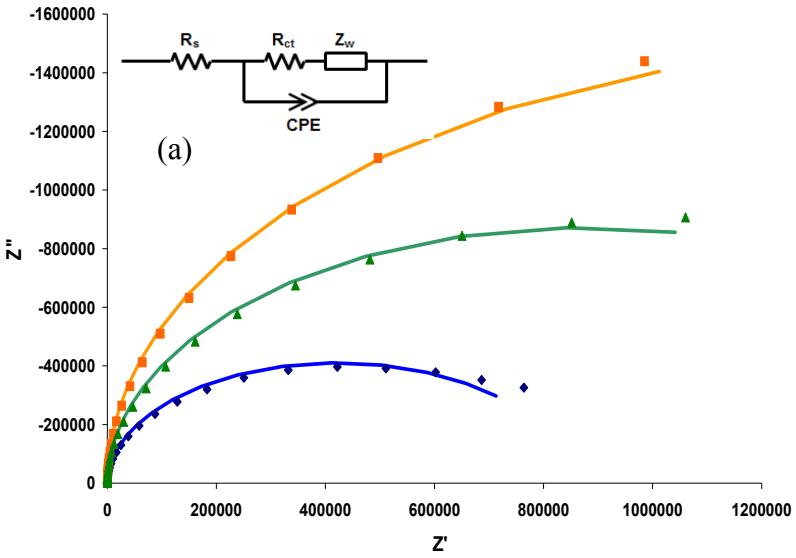
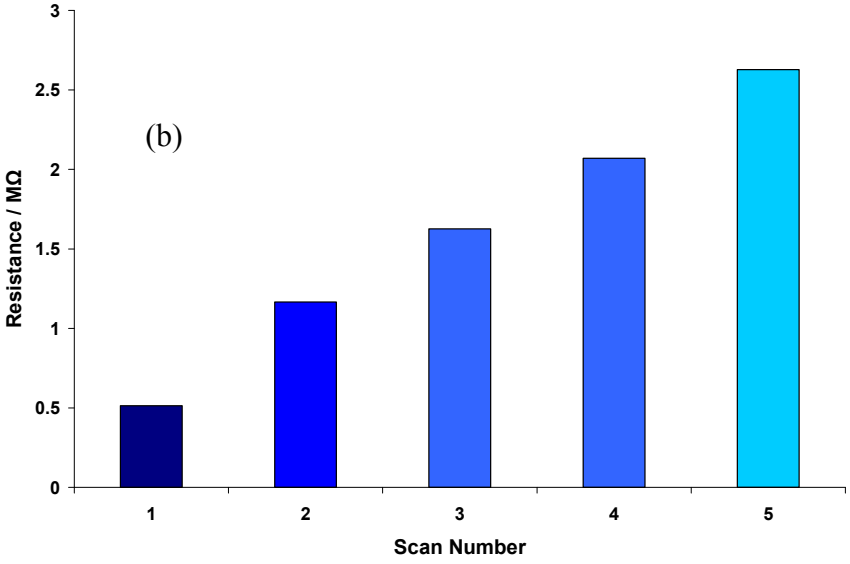


Fig. SI-3. XPS spectra of ss-DNA modified Si(111) surface. Reduction of N 1s peak (corresponding to the succinimide ester) and appearance of P 2s peak as compared with the EDC/NHS activated Si(111) (**SAM-2**) confirm the successful attachment of DNA.

2.4. Electrochemical study of the Si(111)/undecylenic acid/ss-DNA modified surface.





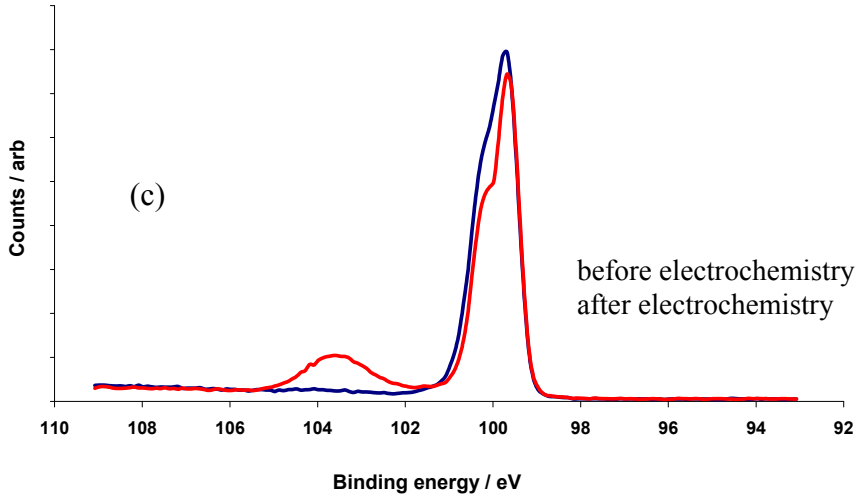
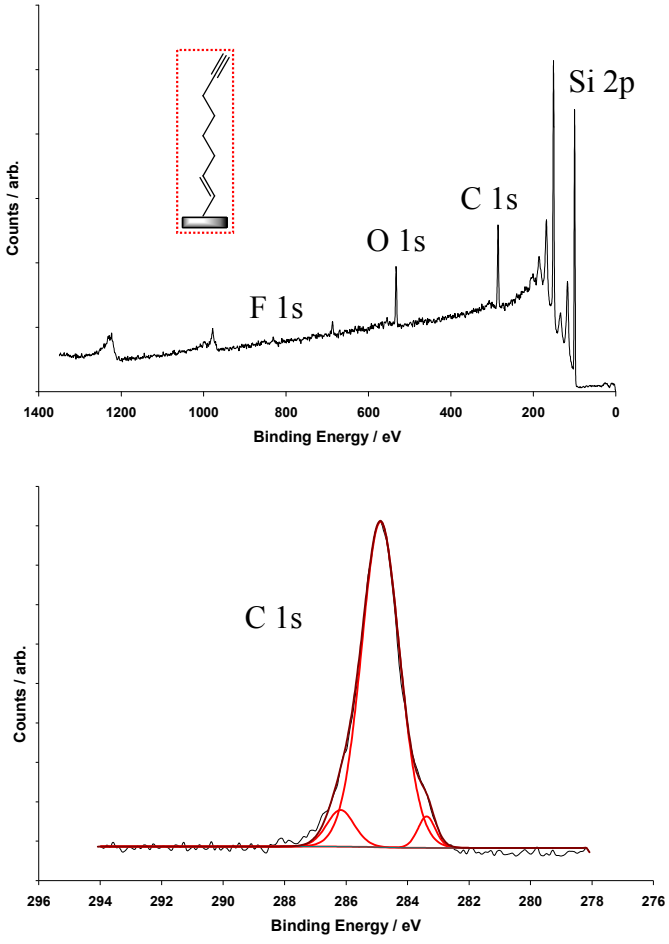


Fig. SI-4 (a) Nyquist plots for (♦) single-stranded, (■) complementary, and (▲) non-complementary DNA. Plot (b) shows the increase in resistance with consecutive scans for the ss-DNA modified surface indicating the formation of insulating SiO_x, whereas the peak at 102-105 eV [before (—) and after (—) electrochemistry] in plot (c) confirms the formation of oxide under potential. Impedance was measured at 0.2 V in a solution of 5 mM $[Fe(CN)_6]^{-3/-4}$ + 0.05 M phosphate buffer + 0.3 M NaCl at pH 7. ss-DNA was immobilized according to the strategy (a) in Scheme 1. Inset shows the equivalent Randles circuit.

1,8-Nonadiyne. *2.5. XPS and XRR characterisation of 1,8-nonadiyne modified Si(111) surfaces.*

The UV-hydrosilylation of 1,8-nonadiyne on freshly etched Si(111) produced a well-defined acetylene monolayer (**SAM-5**) as determined by XPS and XRR measurements.



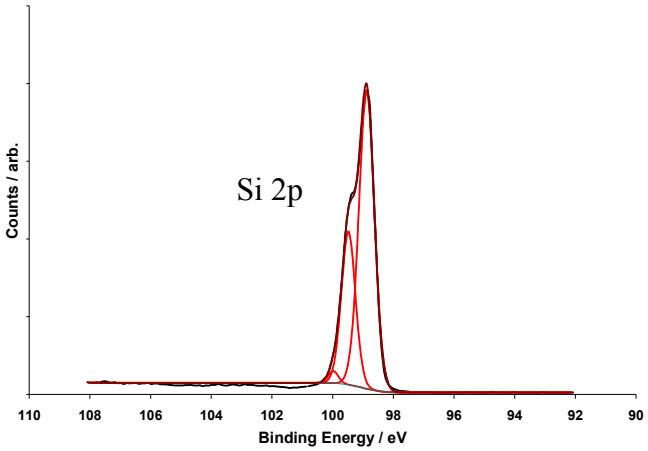


Fig. SI-5 XPS spectra of 1,8-nonadiyne modified Si(111) surface. Absence of peak at 102-105 eV for Si 2p indicates that the surface is free from insulating SiO_x.

XPS spectra of the 1,8-nonadiyne modified surface are summarised in Fig. SI-5. The peak of the C 1s region is deconvoluted to three peaks, with the largest peak corresponding to the main alkyl chain at 284.88 eV. Two smaller peaks at 286.18 and 283.38 eV are attributed to C≡C and the silylated olefin (Si-C=C) bond, respectively.[11,12] In the Si 2p narrow scan, no peak at 102-105 eV, corresponding to oxides or suboxides of silicon, is detected. The absence of any significant oxides is indicative of a well-formed monolayer. The O 1s emission, visible in the wide scan, is attributed to adventitiously adsorbed oxygen. The survey spectra also display a small peak at 686.5 eV. This signal is attributed to residual fluorine from the etching process, which is not uncommon.[13]

2.6. X-ray reflectivity spectra of the 1,8-nonadiyne modified silicon.

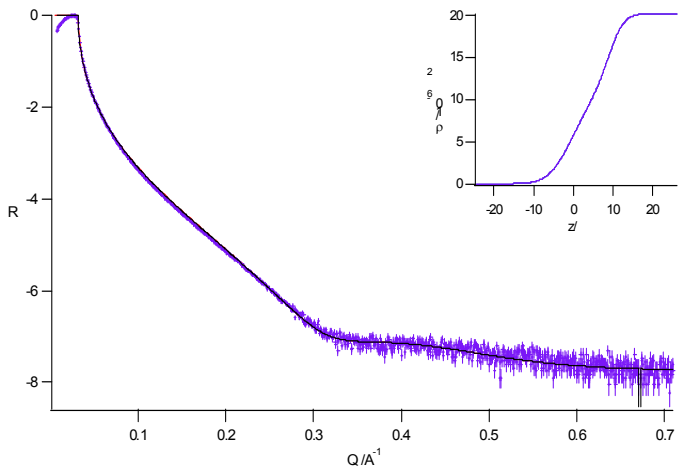


Fig. SI-6 X-ray reflectivity spectrum acquired on a Si(111)/1,8-nonadiyne surface. The inset shows the depth-profile view of the refined scattering-length densities.

Only one Keissig fringe is observed in the XRR spectrum (Fig. SI-6) suggesting the formation of a monolayer with a thickness of 9.2 Å. The electron density, calculated from scattered light density of the material[14] of 0.40 $e^-/Å^3$, is close to that reported by Sieval *et al.*[15] (i.e., 0.31-0.32 $e^-/Å^3$) for an analogous surface suggesting the formation of a densely packed organic layer.

2.8. Detection of DNA

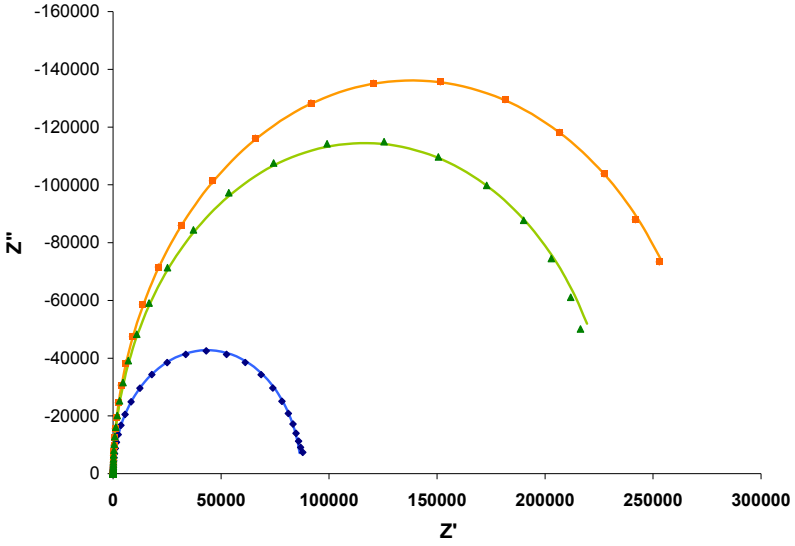


Fig. SI-7 Nyquist plots for (♦) single stranded, (■) complementary, and (▲) non-complementary DNA without BSA. Plots were measured in a solution of 5 mM $[Fe(CN)_6]^{-3/-4}$ + 0.1 M KCl at 0.2 V. ss-DNA was immobilized according to the strategy (b) in Scheme 1. Comparison of this figure with Figure 1b of the article reveals that with BSA as antifouling agent, the modified surface can be used successfully to detect DNA.

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