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Sensors and Actuators B xxx (2010) xxx–xxx

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A short route of covalent biofunctionaliztion of silicon surfaces

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ARTICLE INFO

Article history:
Received 3 October 2010
Received in revised form
28 November 2010
Accepted 7 December 2010
Available online xxx

Keywords:
Silicon
Biofunctionalization
Biosensor
DNA
Non-specific
Adsorption

Agree on highlighted items

ABSTRACT

Covalently attached organic monolayers on etched Si(1 1 1) surfaces were prepared by heating solutions of 1-alkenes and 1-alkynes in a refluxing mesitylene. Surface modification was monitored by measurement of the static water contact angle, X-ray photoelectron spectroscopy (XPS), infrared reflection absorption spectroscopy (IRRAS), and atomic force microscopy (AFM). Flat and clean *N*-hydroxysuccinimide (NHS)-ester-terminated/1-decyl mixed monolayers were covalently attached in one step onto a silicon surface. This procedure allows a mild and rapid functionalization of the surface by substitution of the NHS-ester moieties with amines at room temperature. The NHS-ester groups were shown to be fully intact onto the surface. The surface reactivity of the NHS-ester moieties toward amines was qualitatively and quantitatively evaluated via the reaction with methoxytetraethyleneglycolamine (TEGamine), ~~biotin hydrazide~~ and finally functionalized with single strand and complete DNA molecules.

Moreover, domains of DNA were selectively immobilized, on silicon surface making use of TEGamine, which acts as protein repelling agent and therefore prevented non-specific DNA adsorption. The resulting DNA-modified surfaces have shown excellent specificity, and chemical and thermal stability under hybridization conditions.

1. Introduction

Designing and controlling the surface chemical properties of silicon and silicon-related surfaces through the immobilization of biomolecules receives an increasing attention regarding the development of advanced biochip, bioarray and biosensor technologies [1,2]. Extensive investigations have been devoted to the chemical functionalization of hydride-terminated silicon surfaces by covalent attachment of organic molecules and their subsequent transformations [3,4]. Such modifications enhance the stability of these surfaces and displays very good electronic properties compared to those formed on silicon oxide surfaces [5]. The ease and excellent reproducibility of the chemical modification protocol, and the possibility of photo-patterning of hydrogen-terminated silicon surfaces under laboratory conditions are a real asset for developing silicon surfaces as biosensor platforms [6–9].

Strother et al. have explored the chemical derivatization of hydrogen-terminated silicon surfaces for direct attachment of DNA. They found that the DNA-modified surfaces exhibited a high density of binding sites and a high specificity and stability to the hybridization conditions [10,11]. Although direct immobilization of DNA on chemically modified silicon surfaces was reported, other important criteria for using crystalline silicon in such applications still need to be met. These include: (i) organic functional groups should be made available and accessible on the semiconductor surface to facilitate the immobilization of chemical and biological species on these surfaces. (ii) Provide a specific interaction between the surface functional group and the target molecule to immobilize in order to avoid non-specific adsorption of the target on the surface, and (iii) provide a good stability of the surface monolayer in physiological environments to allow for reusability of such devices as well as to minimize the loss of material during chemical manipulations of the surface. Furthermore, utilizing crystalline silicon offers the possibility of using well-established microfabrication methods for the integration of diverse chemical and biochemical functionality into microelectronic platforms, and the use of intrinsic properties of silicon to detect molecular events occurring on the surface [12–14]. Electrical detection of molecular interactions on the surface, however, requires good electronic properties and a low density of surface states of the organic monolayer/silicon interface.

Recently, different strategies for the chemical functionalization and passivation of hydrogen-terminated silicon and porous silicon under various conditions were developed. Simple and functional 1-alkenes to form organic monolayers covalently attached to the semiconductor surface through Si–C bonds have been utilized. For example, reaction of ester-terminated alkenes with Si(1 1 1)–H led

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doi:10.1016/j.snb.2010.12.013

to the formation of an organic monolayer bearing a terminal ester group [15,16]. The ester functional group remained reactive and could be displaced with a variety of standard chemical reagents. For example, acidic hydrolysis gave a carboxylic acid terminal group that could be coupled to simple amino acids using approaches developed for solid phase synthesis.

The developments described above led to the investigations carried out in this study, which suggests a direct method for anchoring a terminal acid function and explores chemical pathways for attaching biomolecules on silicon surfaces (chemical adsorption of single and double-strand DNA and antibody–antigen coupling). This method is simple to carry out and requires fewer steps than those reported so far. It is based on the reaction of hydrogen-terminated silicon surfaces with undecylenic acid under chemical conditions to yield an organic monolayer covalently attached to the surface through silicon–carbon (Si–C) bonds and bearing a terminal acid group. The acid group is activated to a NHS ester group that is ready for nucleophilic substitution by an amino group. This versatile chemistry was applied to complex systems, i.e. proteins and DNA tethered to primary amino groups. The functionalized surfaces and the different steps leading to biomolecule immobilization were characterized using infrared, and X-ray photoelectron spectroscopy. Moreover, this relatively simple chemistry combined with patterning techniques was also conducted on hydrogen-terminated Si(1 1 1) surfaces to attach single and double strand DNA in a well-controlled fashion. This method proved to be reproducible and showed no detectable non-specific binding.

2. Experimental

2.1. Materials

Single-polished Si(1 1 1): of n-type, 475–550 μm thick, resistivity 1–5 Ω cm and p-type, 500–550 μm thick, resistivity 0.009–0.012 Ω cm (both by Addison Engineering, San Jose) were utilized in this study.

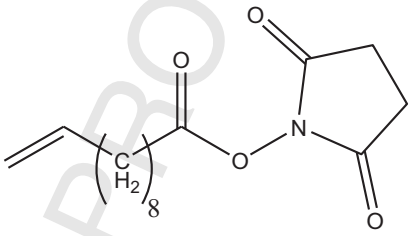
2.2. Chemicals

Petroleum ether (PE 40/60), methanol (MeOH), ethanol (EtOH), toluene, and dichloromethane (CH_2Cl_2) were distilled prior to use; acetone was used as obtained (Acros, 99+%). Mesitylene (Fluka, 99%) was distilled twice and stored over solid $CaCl_2$. The dried mesitylene was filtered through filter paper to remove any $CaCl_2$ particles before mixing with the 1-alkene or 1-alkyne solutions. 1-Decene (Fluka, 97%), 1-dodecene (Fluka, 99%), 1-tetradecene (Sigma, 99%), 1-hexadecene (Sigma, 99%), and 1-hexadecyne (Alpha Aesar, 98%) were distilled at least twice at reduced pressure (7 mbar). 1-Octadecene (Fluka, 95%) was distilled three times and further purified by column chromatography using petroleum ether 40/60 as eluent. 1-Octadecyne was synthesized and purified by recrystallization according to the procedure described by Sieval et al. [20]. 1,2,4-Trichlorobenzene was distilled twice and stored on $CaCl_2$. 1-Undecylenic acid (UA) (Acros, 99%) were distilled twice at reduced pressure; acetone (Acros, 99+%), 2-propanol (Fisher, p.a.), NH_4F (Sigma, 98+%), *N*-hydroxysuccinimide (NHS) (Sigma), *N*,*N*′-dicyclohexylcarbodiimide (DCC) (Sigma, 99%), ~~EZ-Link biotin hydrazide (Pierce), and *para*-trifluoromethyl benzylamine (TFBA) (Aldrich, 97%)~~ were used as received.

MilliQ water (18 MΩ) was used for all experiments. Undecylenic acid, ethanolamine, *N*-ethyl-*N*′-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), triethylamine (TEA), tetraethyleneglycol monomethyl ether, sodium dodecyl sulfate (SDS), ammonia (28%), phosphate-buffered saline (PBS) solutions (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.7 mM KH_2PO_4; pH 7.2) were obtained from Aldrich, and the pH was adjusted with a 0.1 M KOH solution. Tween 20 (polyoxyethylene sorbitan monolaurate) was obtained from Bio-Rad Laboratories.

2.3. Synthesis of N-succinimidyl undecyl-1-enate (NHS-UA)

The detailed preparation procedure of *N*-succinimidyl undecyl-1-enate (NHS-UA) is described by Macossay et al. [17]. In brief, UA (3.64 g, 19.8 mmol) and NHS (2.53 mg, 22.0 mmol) were dissolved in 100 mL of ethyl acetate. DCC (4.54 g, 22.0 mmol) was added to the solution on ice, and the mixture was stored at 4 °C overnight. The reaction mixture was filtered to remove depositions and evaporated in a low-pressure rotoevaporator to remove solvents. The resulting waxy solid was recrystallized from 20 mL of 2-propanol, collected by vacuum filtration with a Büchner funnel, and rinsed with a small amount of water. After drying in vacuum overnight, NHS-UA was obtained (3.37 g, 12.0 mmol, 61%) with purity >99% (GC).



^{1}H NMR (200 MHz, $CDCl_3$): δ 5.80 (m, 1H), 4.97 (m, 2H), 2.85 (s, 4H), 2.62 (t, 2H), 2.05 (q, 2H), 1.76 (m, 2H), 1.33–1.42 (m, 10H) [18,19]. ^{13}C NMR (400 MHz, $CDCl_3$, δ): 171.45, 170.11, 139.35, 114.15, 34.11, 32.32, 29.35, 29.38, 29.27, 29.12, 25.9, 25.00. MS calcd for m/z = 304.3, found 304.1. IR ν_{max} (cm^{-1}): 2925, 2854, 1819, 1787, 1725, 1640, 1380, 1208, 1070, 870.

2.4. Sample cleaning and etching

Samples of silicon were first wiped with a tissue saturated with chemically pure acetone. After that, the samples were placed in ultrasonic bath for at least 10 min in acetone. Then the samples were placed in an oxygen plasma cleaner (Harrick PDC-32G) for 10 min. Subsequently these samples were etched in an argon-saturated 40% aqueous NH_4F solution for 15 min under an argon atmosphere. To allow recycling of the relatively expensive ATR crystals, these were cleaned/recycled by oxidative removal of a previously formed monolayer in 'piranha solution' (30% H_2O_2:H_2SO_4 = 1:2 (v/v)) at 85 °C for 1 h (CAUTION: Piranha solutions should be handled with great care) [20], and subsequently etched with HF as described above (CAUTION: HF solution is very corrosive, it can affect the skin and the bones and should be handled with great care).

2.5. Monolayer preparation

A solution (8.5 mL, 0.2 M) of 1-alkene(s) in mesitylene was placed in a small three-necked flask fitted with a nitrogen inlet, a reflux condenser with a $CaCl_2$ tube, and a stopper. The solution was refluxed for at least 45 min under a flow of nitrogen. Subsequently, a cleaned and freshly etched sample was added to the refluxing solution, while maintaining a slow nitrogen flow. After 2 h the modified sample was removed from the solution and cleaned excessively rinsed with PE40/60, EtOH, and CH_2Cl_2, respectively.

2.6. Preparation of mixed monolayers

The detailed procedure of preparing mixed monolayers on Si(1 1 1) is described by Sun et al. [3]. In brief, cleaned Si(1 1 1)

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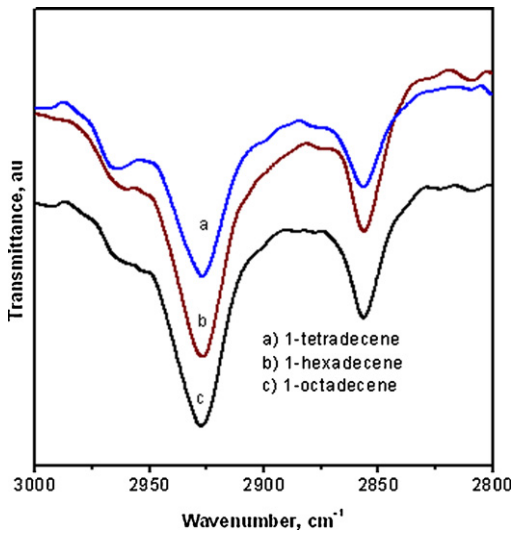


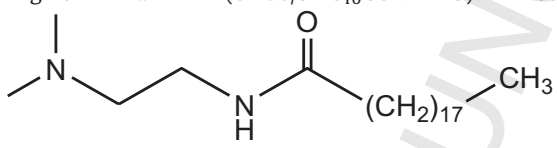
Fig. 1. FTIR spectra of (a) tetradecyl, (b) hexadecyl and (c) octadecyl monolayers prepared on Si(1 1 1) surfaces by reaction of the corresponding 1-alkenes in mesitylene at 200 °C.

chips were etched to a H-terminated surface by immersion in an argon-saturated 40% aqueous NH_4F solution for 15 min under argon atmosphere. Monolayers were prepared by immersing the freshly prepared H-terminated Si(1 1 1) chips into a deoxygenated solution of NHS-UA and/or 1-decene in 1,2,4-trichlorobenzene, followed by heating at 150 °C under argon atmosphere for 2 h. The total concentration of alkenes was 0.5 M, while the alkene composition was varied with different NHS-UA ratios of 0%, 50%, and 100%.

2.7. Generation of patterned-functionalized silicon surfaces

2.7.1. Monolayer formation on patterned surfaces

Spots of silicon oxide were formed on the surface of the hydrogen-terminated silicon sample surface in hot air by use of 10 μm open square masks by Adtek, made of chromium on quartz with a special antiscratch coating. These masks were separated by 50 μm vertical and horizontal distances. A 3:1 piranha solution (96% H_2SO_4:30% H_2O_2,) at 100 °C was carefully dropped on the open square area of the masks for 30 min using a fine tip Pasteur pipette (a glass pipette with very fine tip). This procedure led to the formation of 10 μm square spots of silicon oxide surfaces horizontally and vertically separated by 50 μm wide Si–H strips, Fig. 1. Thus, an alternating oxide/Si–H surface was created on these samples. The resulting surface was immersed in deoxygenated 0.5 M NHS-ester alkene in 1,2,4-trichlorobenzene as a solvent. This step allowed selective functionalization of the Si–H lines to yield a well-ordered pattern of oxide (squares) and NHS esteralkene-terminated (lines) (oxide/Si–C_{10}COONHS) surface. The NHS-terminated regions were then reacted with TEGamine (*N*-(3-(dimethylamino)propyl)octadecanamide) to give an (oxide/Si–C_{10}CONHTEG) surface.



2.7.1.1. TEGamine. The unreacted NHS groups were capped with ethanolamine. Finally, the oxide from the squares was converted to a new Si–H surface by dipping the silicon shard for 1.0 min in 2%HF (aqueous solution). This actually led to a reduction in the line width to approximately 20 μm. The chemical modification cycle was repeated by reaction of the surface with undecene to provide the final patterned surface, Si–$C_{10}CH_3$/Si–C_{10}CONHTEG, which was used for the immobilization of DNA.

2.8. Biomolecular immobilization on chemically modified silicon surfaces

2.8.1. DNA on NHS-terminated surfaces

(a) The single-strand DNA (*E. coli* probe 16S, 5′-H_2N-C_{12}-TT-CCT-GTT-ACC-GTT-CGA-CTT-G-3′, [DNA] = 20 μM) in PBS tethered to a primary amine group was reacted with an NHS-terminated surface. After 3 h reaction at room temperature the surface was rinsed with a detergent solution (2% Tween 20 in PBS) and water and then dried under a stream of nitrogen. The resultant surface was analyzed by FTIR.

(b) The grafted surface (Si–C_{10}CONHS/Si–C_{10}CONHTEG) was reacted overnight at room temperature with homopolymeric deoxythymidine (5′-/5-NH_2-C_6-TTT TTT TTT TTT TTT TTT TT-3′ (H_2N-dT20)) solution in PBS (37.4 μM) to give Si–C_{10}CONH-dT_{20}/Si–C_{10}CONHTEG surface. The remaining NHS groups were capped with ethanolamine (10–1 M solution for 2 h at RT).

The resulting surface was copiously rinsed with water and dried under a stream of nitrogen. The hybridization reaction was performed by immersing the above surface in 30.8 μM solution in PBS of the complementary labeled oligonucleotide with Cy3 in 5′-position: 5′-/5Cy3-AAA AAA AAA AAA AAA AA-3′ (Cy3-dA20). The hybridization reaction took place overnight at room temperature. Rinsing after the reactions with the oligonucleotides was carried out by shaking for 3–5 min in 0.2% SDS solution in PBS, followed by 2 min water rinsing.

2.9. Analysis of the monolayers

2.9.1. Contact angle measurements

Samples modified by all procedures discussed above were analyzed by different techniques. A small specimen (~ 5 mm × 10 mm) was cut of each sample directly after cleaning. Static water contact angles of two to three drops of MilliQ (3.5 μl) were obtained using an Erma Contact Angle Meter, G-1. The error of the contact angles is ±1°.

2.9.2. Infrared reflection–absorption spectroscopy (IRRAS)

FT infrared reflection–absorption spectra were recorded on a Bruker Tensor 27 equipped with a variable-angle reflection, Auto Seagull accessory. A Harrick grid polarizer was installed in front of the detector for measuring spectra with *p*-polarized (parallel) radiation with respect to the plane of incidence at the sample surface. Single channel transmittance spectra (4096 scans) were collected using a spectral resolution of 4 cm^{-1}. All spectra shown in this paper are the result of subtracting spectra of modified samples from those of as received cleaned samples, without any further data manipulation.

2.9.3. X-ray photoelectron spectroscopy (XPS)

XPS measurements were performed on a VG Ionex system equipped with a Clam II analyzer and a standard Al Kα X-ray source. Spectra were recorded at normal emission of 10^{-9} mbar within 10 min. All C_{1s} peaks corresponding to hydrocarbons were calibrated to a binding energy of 285.0 eV to correct for the energy shift caused by charging. The XPS measurements of Fig. 2, however, were performed using a Quantera SXM, equipped with monochromator and an Al Kα X-ray source, by Physical Electronics. The spot size was 100 μm in diameter.

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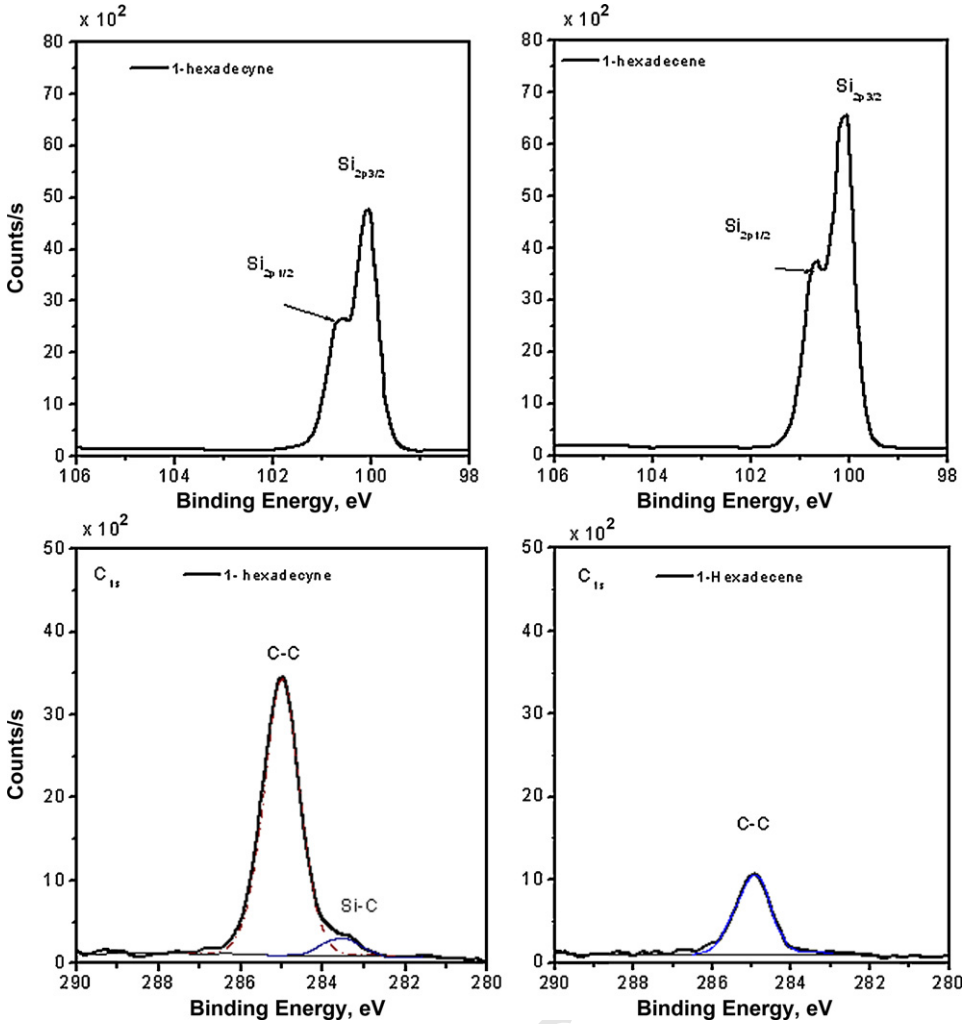

Fig. 2. XPS narrow scans of Si_{2p} region of 1-hexadecene (top, right) and 1-hexadecyne and C_{1s} region of 1-hexadecene (bottom, right) and 1-hexadecyne (bottom, left) monolayers on H–Si(1 1 1) after reflux of their 0.2 M solution for 2 h at 200 °C in mesitylene.

2.9.4. Atomic force microscopy (AFM)

Surface topography was imaged using a Nanoscope III atomic force microscope by Digital Instruments, Santa Barbara, CA. The contact mode (CM-AFM) was made with silicon nitride cantilevers with a spring constant of about 0.58 N/m.

3. Results and discussion

3.1. Formation of alkyl monolayers

Alkyl monolayers were analyzed by contact angle measurements. The water contact angles, as observed for the monolayers on silicon surfaces are listed in Table 1. This table also contains the infrared peak positions, as observed with *p*-polarized infrared beams, for the antisymmetric (ν_a) and symmetric (ν_s) CH_2 stretching vibrations.

The high values of the water contact angles (θ = 109–110°), measured for the unfunctionalized 1-alkenes, clearly indicate that the surface of the monolayer is completely terminated by methyl groups. The static water contact angles are comparable to those of thiols on gold [21,22], and to the monolayers on Si(1 1 1) prepared by Linford et al. [23]. This shows that a sufficiently high percentage of the hydrogenated silicon atoms have reacted with a 1-alkene to give a complete coverage of the surface. The surface properties of the monolayer are therefore not affected by residual Si–H and Si–OH groups that are present on the silicon surface [23].

The anti-symmetric and symmetric methylene stretching vibrations of the first three 1-alkenes appear near 2920 cm^{-1} and 2850 cm^{-1}, respectively (Table 1). These observed wavenumbers are indicative of densely packed monolayer of alkyl chains shown in Fig. 1 [24,25].

Fig. 2 depicts the C_{1s} and Si_{2p} regions of the XPS spectra of thermally prepared monolayers derived from 1-hexadecene and 1-hexadecyne on H–Si(1 1 1). A large difference in the intensity

Table 1
Static water contact angles (°) of alkyl and alkenyl monolayers on Si(1 1 1) surfaces prepared by thermal methods and the observed wave numbers of CH_2 antisymmetric, ν_a and symmetric, ν_s vibrations in the corresponding IRRA spectra.[a]

Reactants	Contact angle (°)	ν_a	ν_s
$CH{\equiv}C{-}C_{10}H_{21}$	109	2921	2851
$CH{\equiv}C{-}C_{12}H_{25}$	110	2921	2852
$CH{\equiv}C{-}C_{14}H_{29}$	110	2920	2849
$CH_2{=}CH{-}C_{10}H_{21}$	109	2921	2854
$CH_2{=}CH{-}C_{12}H_{25}$	110	2920	2850
$CH_2{=}CH{-}C_{14}H_{29}$	110	2919	2850
$CH_2{\equiv}CH{-}C_{14}H_{27}$	110	2920	2851
$CH_2{\equiv}CH{-}C_{16}H_{31}$	110	2919	2850

[a] All experiments were performed at least twice; experimental error = ±1.

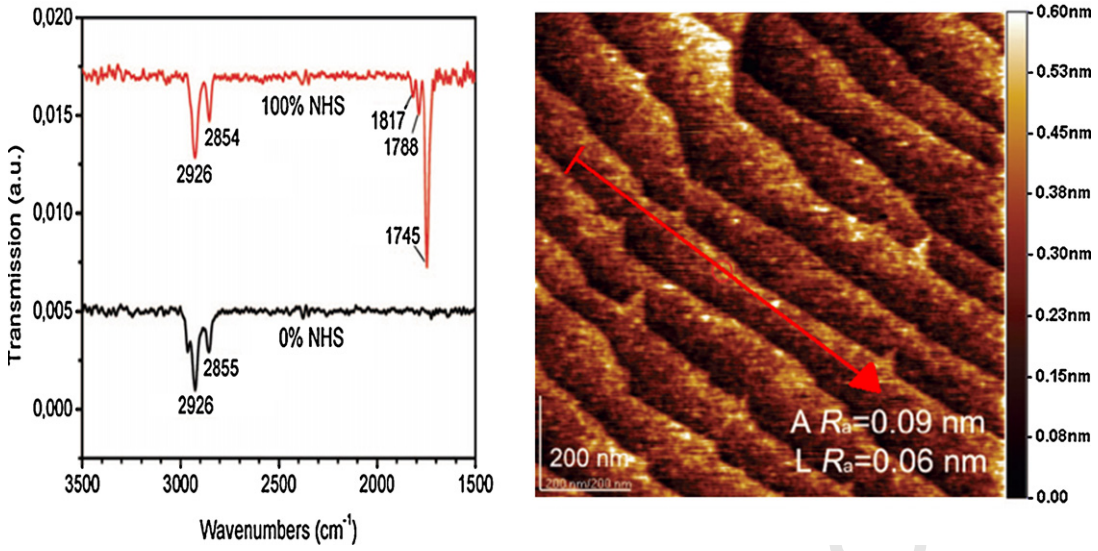

Fig. 3. IRRA spectra of the pure NHS-UA monolayer (100% NHS, with the characteristic triple-peak carbonyl pattern of NHS-esters) and the pure 1-decene monolayer (i.e. 0% NHS) attached on Si(1 1 1) (left). AC mode AFM topographic image of 100% NHS-ester-terminated surface ($1 \times 1\ \mu m^2$; color scale from 0 to 0.60 nm) (right). Average area roughness (A R_a) and average line roughness (L R_a) (along the arrow) are marked in the image.

of C_{1s} emission is observed, clearly displaying the minimal reactivity of the 1-hexadecene and the relatively high reactivity of 1-hexadecyne at the reaction conditions. Consequently, the 1-hexadecene-modified Si surface yields a large Si_{2p} peak and the more reactive 1-hexadecyne results in a modified Si surface with a relatively smaller Si_{2p} emission due to the increased coverage of the Si substrate by the hexadecenyl monolayer [26]. We note that for both monolayers, and in particular the 1-hexadecene-treated Si surface, the Si_{2p} narrow scan has a completely flat baseline around 103–104 eV, which is consistent with the absence of even trace amounts of silicon oxide (SiO_2). For the incomplete 1-hexadecyl monolayer [27], the degree of oxidation of the 1-alkyne-derived monolayer is slightly lower than that observed for the 1-alkene-derived monolayer. This is a result of the fact that 1-alkynes can in principle react with two silicon atom [20]. Such a tandem reaction would reduce the number of unreacted sites at the silicon surface. As a result, a smaller number of these unreacted sites can yield oxidized surface sites at later stages, which explains the displayed observation. However, further investigations are required to firmly ascertain this interpretation.

3.2. Formation and characterization of mixed monolayers

Mixed monolayers were obtained by boiling mixtures of 1-decene and the synthesized NHS-UA with a hydrogen-terminated Si(1 1 1) surface in different proportions. The mole fraction of the terminal NHS-ester groups on the resulting mixed monolayers was expected to be approximately similar to the mole fraction of NHS-UA in the reaction mixture [28]. As expected, the water contact angle of the resulting mixed monolayers varied from 110° for 0% NHS-esteralkene to 52° for 100% NHS-ester-alkene (Table 2).

IRRA spectroscopy depicted in Fig. 3 (left) clearly reveals the NHS-ester functionalities in the pure NHS-UA monolayer (i.e. 100% NHS) by the appearance of the characteristic C=O stretching vibrations at 1817, 1788, and 1745 cm^{-1} belonging to the ester carbonyl stretch and the symmetric and asymmetric carbonyl stretches in the succinimidyl end groups, respectively [29].

This technique also reveals that the NHS-ester-terminated monolayers are not well-ordered. This is reflected by the values of the antisymmetric and symmetric CH_2 stretching vibrations showing up at 2926 and ~2854 cm^{-1}, respectively [18]. Again, these

Table 2
Static water contact angles (°) of mixed monolayers prepared with different proportions of 1-decene to NHS-UA by thermal reactions.

Reactants	Contact angle (°)
100% CH≡C–$C_{10}H_{21}$	110
75% CH≡C–$C_{10}H_{21}$:25% NHS-UA	92
50% CH≡C–$C_{10}H_{21}$:50% NHS-UA	81
25% CH≡C–$C_{10}H_{21}$:75% NHS-UA	67
100% NHS-UA	52

results further confirm that a hydrosilylation reaction is taking place mainly at the carbon–carbon double bond, since no residual absorption at 1715 cm^{-1} was observed, which would appear if more than 10% of the monolayer was in the form of the silyl ester.

AFM analysis presented in Fig. 3 (right) shows that the resulting surfaces were clean and flat with easily recognizable Si(1 1 1) edge steps and with an average line roughness of 0.06 nm on the terrace surfaces and an average area roughness of 0.09 nm over the completely measured surface of a 100% NHS-ester-terminated monolayer.

Moreover, X-ray photoelectron spectroscopy was performed on the Si(1 1 1) samples modified with mixed monolayers to check for the terminal functional groups of these layers. The structural formula of the NHS-UA shows that there are three types of carbon atoms that can be distinguished by XPS measurements.

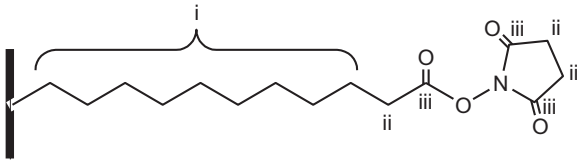

Fig. 4 shows the XPS narrow scans of C_{1s} of 100% NHS-ester-terminated monolayers on Si(1 1 1). The C_{1s} signal of the NHS-ester-terminated monolayer can be resolved into three peaks, ranging from low to high binding energy (BE), as follows: (i) a peak at 285.1 eV (with a full width at half-maximum (FWHM) of 1.4 eV) for carbons in the alkyl chain. (ii) A peak at 286.4 eV for R-carbons adjacent to the carbonyl carbon atoms. These three R-carbons, with general shift of 0.4–0.7 eV [30], shift much more than an ordinary R-CH_2 (peak ii) in an alkyl chain because of the strong electron-withdrawing effect from the imide group in the NHS moiety. (iii) A

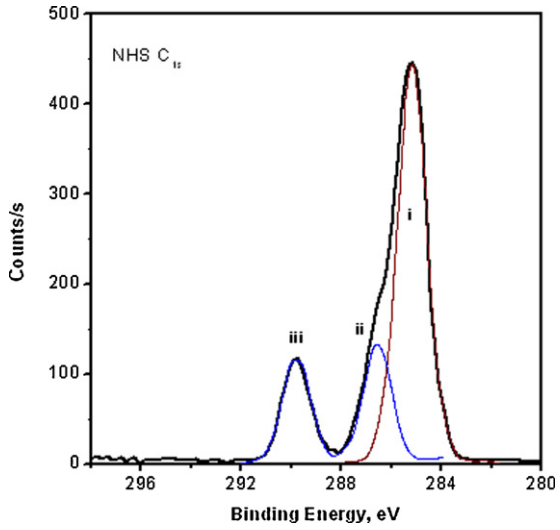

Fig. 4. XPS narrow scans of C_{1s} of a 100% NHS-ester-terminated.

peak at 289.7 eV for the carbonyl carbon atoms. The ratio between these three peaks for a 100% NHS-ester-terminated monolayer is 9.4:2.7:2.4, which, taking into consideration experimental error, is very close to the theoretical ratio of 9:3:3.

Fig. 5 presents a comparison of scans of N_{1s}, C_{1s}, F_{1s}, and O_{1s} of 100% and 50% NHS-ester-terminated monolayers. Approximately the same BEs and chemical shifts are observed in N_{1s}, C_{1s}, F_{1s}, and O_{1s} narrow XPS spectra of both the 50% and the 100% NHS-ester-terminated monolayers, albeit at different atomic ratios.

3.3. Immobilization of DNA on NHS-modified surfaces

The availability of the NHS groups on the surface for further reaction was demonstrated by the aminolysis with TEGamine, which is known to inhibit nonspecific adsorption of proteins:

$$\mathrm{Si(1\ 1\ 1)\text{–}C_{10}H_{20}COONHS + R\text{-}NH_2 \xrightarrow[2\ h]{RT} Si(1\ 1\ 1)\text{–}C_{20}H_{20}CONH\text{–}R,}$$

$$\text{where } R = C_{23}H_{48} \quad (1)$$

The choice of TEGamine is based on the fact that short oligoethylene glycol are efficient protein repelling, and therefore, can prevent the nonspecific adsorption of biomolecules on solid substrates [31]. Fig. 6 illustrates the IR spectrum of the NHS-UA modified Si(1 1 1) surface after reaction with TEGamine. Evidently, the NHS characteristic IR band disappeared and is replaced by peaks at 1650 and 1550 cm^{-1} assigned to the carbonyl function and the C–N–H vibration, which includes both N–H bending and C–N-stretching of the amide. Peaks at 3300 and 3100 cm^{-1} are observed as well and are attributed to the NH stretch and an overtone of the 1550 cm^{-1} peak, respectively.

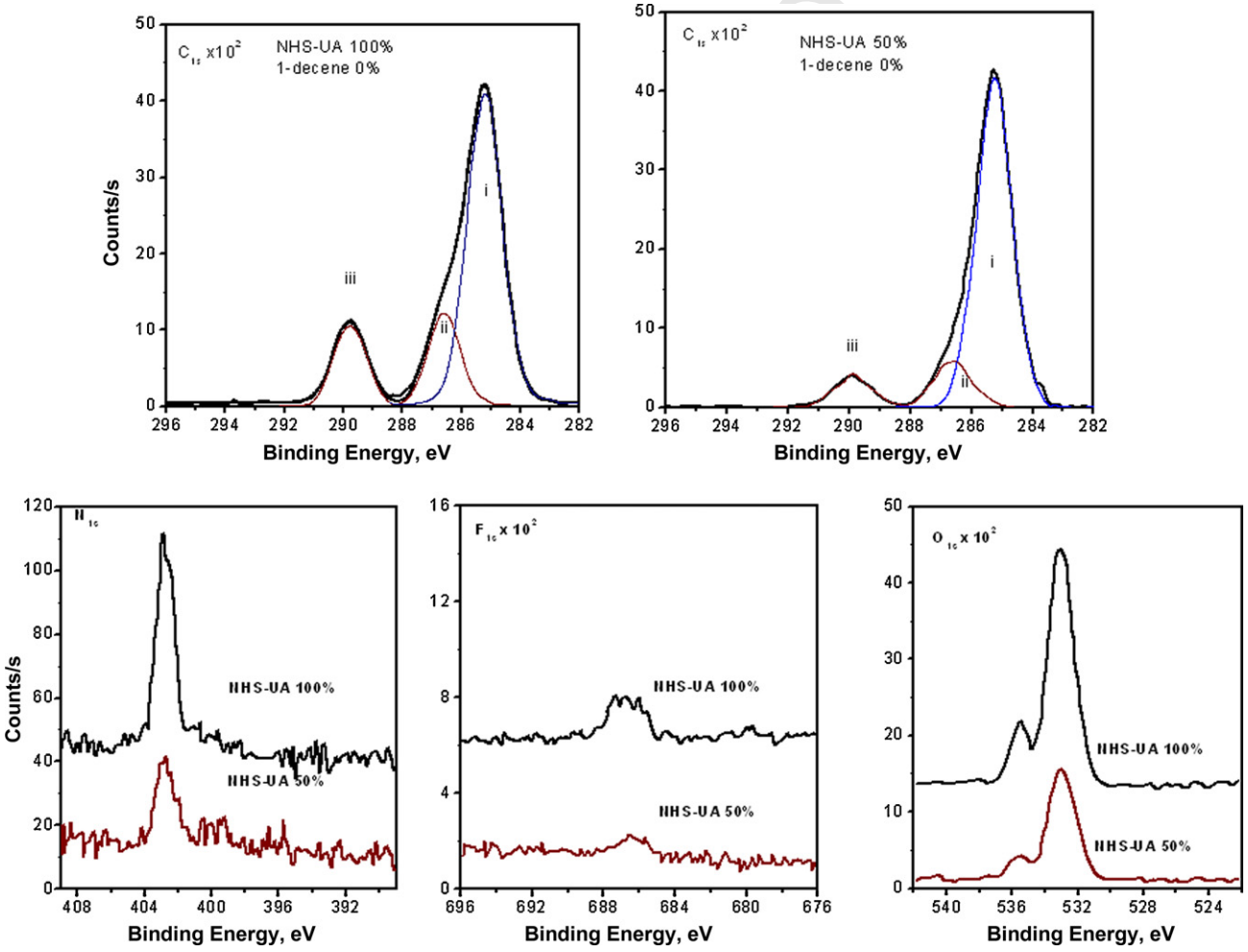

Fig. 5. XPS narrow scans of 100% and 50% NHS-UA-terminated monolayers on Si(1 1 1). Upper row scans are for C_{1s} regions and lower row scans are for N_{1s}, O_{1s} and F_{1s} regions.

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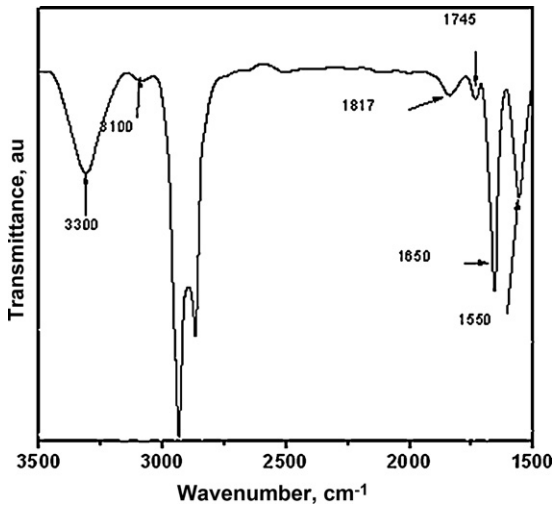

Fig. 6. ATR-FTIR of Si(1 1 1) surface functionalized NHS-UA ester after reaction with TEGamine. The background used is the spectrum of clean ATR Si(1 1 1). The IR spectra is baseline-corrected.

The above aminolysis reaction should in principle be amenable to any accessible primary amine, including those of biologically active molecules such as proteins and DNA. We have demonstrated the utility of this approach by the immobilization of a single-strand DNA (*E. coli* probe 16S, [DNA] 20 μM in PBS) tethered to a primary amine linker at the 5′-position. After 3 h of reaction at RT, characteristic peaks of the amide function at 1650 and 1550 cm^{-1} are observed along with the NHS ester peaks at 1817, 1788, and 1745 cm^{-1} in the IR spectrum, suggesting that almost all NHS groups are reacted. The resulting surface was then exposed to a capping agent (ethanolamine) to deactivate the remaining unreacted NHS groups on the surface. Characteristic peaks for C=O at 1705 cm^{-1} and for C–N at 1610 cm^{-1} associated with the DNA became evident as can be seen in Fig. 7. Other DNA peaks below 1500 cm^{-1}, such as the phosphate diester bands, cannot be observed because of strong absorptions from bulk silicon.

Elements present at the DNA-modified surface were identified by a wide-scan XPS measurement. The chemical state and the atomic concentrations of the elements present were determined from accurate narrow-scan measurements. Standard sensitivity

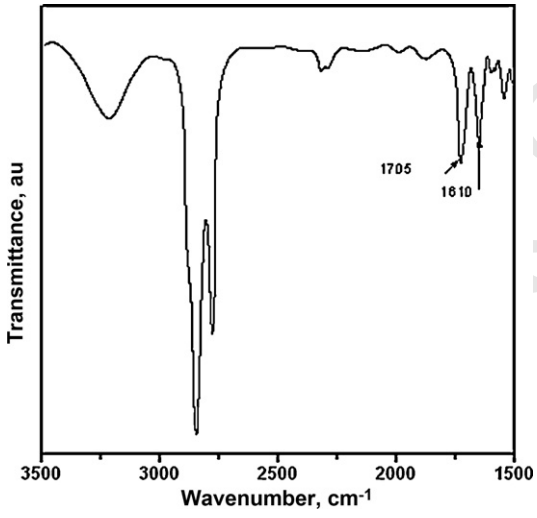

Fig. 7. ATR-FTIR of Si(1 1 1) surface functionalized with single-strand DNA immobilized on the activated ester surface.

Table 3
Apparent concentrations (at.%) measured at the surfaces of DNA modified Si(1 1 1) as evaluated from the narrow scan XPS measurements for C_{1s}, N_{1s}, O_{1s} and Si_{2p}.

Sample	C_{1s}				N_{1s}	O_{1s}	Si_{2p}
	C_xH_y	C–O/C–N	C=O/OCN	O–C=O			
25%NHS-DNA	12.6	6.2	2.7	0.4	4.6	50.4	0.32
50%NHS-DNA	11.5	6.1	2.3	0.4	4.3	51.8	0.24
75%NHS-DNA	10.3	5.8	2.5	0.5	4.6	52.5	0.20
100%NHS-DNA	9.3	5.8	2.5	0.5	4.1	53.7	0.19

factors were used to convert peak areas to atomic concentrations. These results are shown in Table 3.

Narrow C_{1s} peaks observed at four different binding energies were used to identify the chemical state as follows: C_xH_y appear at a binding energy of 284.8 eV, aliphatic C, (C–O/C–N), including C in C–O–C, C–N–C, C–OH, C–NH_2 appear at 286.4 eV. Carbon in C=O/OCN including C in N–C=O–N, amides, N=C^N–N, . . . , C=O appear at 288.0 eV. While C in O–C=O appear at 289.0 eV. The Si_{2p} peak of SiO_2 appears at a binding energy of 103.0 eV. The N_{1s} peak of organically bound N is present at a binding energy of 399.8 eV, and The O_{1s} peak is present at a binding energy of 532.3 eV.

3.4. Immobilization of DNA on patterned silicon surfaces

The patterning technique, which is described earlier, takes advantage of the selective oxidation of the silicon surface when reacted with piranha solution through a mask in hot air at 100 °C. In this case, a mask composed of 10.0 μm circles with a 50.0 μm pitch was used. Upon oxidation, the surface, with a pattern consisting of oxide circles with hydrogen-terminated lines, was reacted with activated NHS ester alkene, and then reacted with TEGamine to produce a surface in which the lines were resistant to nonspecific adsorption or other chemical reactions. Removal of the oxide from the circles is achieved by immersing the surface in 2.0% (v/v) HF aqueous solution followed by reaction of the newly formed hydrogen-terminated silicon with NHS ester alkene. The resulting substrate (Si–C_{10}COONHS/Si–C_{10}CONHTEG) was then reacted with H_2N-dT20 (overnight at room temperature with 37.4 μM H_2N-dT20 solution in PBS) followed by a final capping with ethanolamine of the remaining NHS groups. The hybridization reaction of the immobilized DNA target was performed with its complementary oligonucleotide bearing a Cy_3 fluorescent label, Cy_3-dA20. After an overnight reaction at room temperature with 30.8 μM Cy3-dA20 solution in PBS, the surface was imaged using fluoresce confocal microscopy (Fig. 8a). The fluorescence signal was observed in the expected regions (squares), implying efficient inhibition of the nonspecific adsorption. The following control experiment was carried out to determine the extent of nonspecific binding of Cy3-dA20. The derivatized Si–C10CONH-dT20/Si–C10CONHTEG surface was dipped overnight in the PBS solution of the Cy3-dA20 and Cy5-dC20. Any nonspecifically bound DNA would be demonstrated by the presence of emission from Cy5. We found that, while there was some nonspecifically bound DNA, the amount was close to the detection limit of the instrument and, as such, was difficult to quantify. Given that there is significant room to optimize further the hybridization conditions, we are satisfied that these surfaces exhibit satisfactory binding specificity. The denaturation and the reversibility of these surfaces were explored as well. Denaturation was accomplished by heating the surface bearing the double-strand DNA at 65 °C for 1 h in 0.2% SDS (in PBS) + 0.1 M NaCl mixture (followed by 3–5 min shaking in 0.2% SDS solution in PBS and 2 min of water rinsing). Imaging of the resulting surface showed that the pattern was no longer observed (Fig. 8b). Subsequent hybridization was performed by re-immersing the resulting surface in the Cy3-dA20 solution overnight

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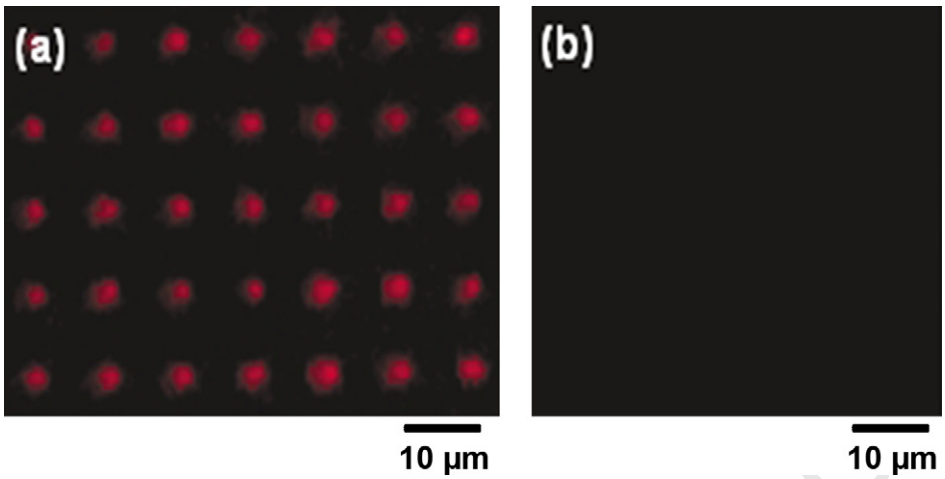

Fig. 8. Fluorescence confocal microscope image of: (a) patterned silicon surface bearing hybridized oligonucleotides (dT20 and Cy3-dA20) on the squares and (b) after de-hybridization.

at room temperature. The hybridization/denaturation cycle was repeated three times without further changes to the fluorescence image.

4. Conclusion

We have shown that the reaction of hydrogen-terminated silicon surfaces with 1-alkens and 1-alkynes takes place under thermal conditions primarily at the carbon–carbon double bond to provide a 1-alkyl and 1-alkenyl monolayers on silicon surfaces. The *N*-hydroxysuccinimide-terminated surface can be easily used to attach simple amines and single-strand DNA tethered to a primary amine immobilized to the surface by amide bond formation at room temperature in a relatively shortt process. Site-directed immobilization of DNA on Si(1 1 1)-modified patterned surfaces with limited nonspecific adsorption was achieved by suitable surface chemistry manipulation. Moreover, this surface chemistry provides excellent stability under hybridization/denaturation conditions. This reasonably simple method provides a potential platform for immobilization of complex structures on silicon surfaces for applications in biosensing and the fabrication of new hybrid materials and devices.

Acknowledgement

This research was supported by the Deanship of Scientific Research at King Abdulaziz University under Grant No. 114/28.

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