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## Covalently-bound organic monolayers for biosensor applications

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**Abstract:** In this paper, we present a detailed investigation on monolayer formation with thicknesses amounting 1 nm to 3 nm on silicon and silicon nitride surfaces. Thermal and photochemical methods are successfully used to form such stable and densely backed monolayers. Further, functionalisation of these surfaces with activated esters, amine groups are also presented. Static water contact angle measurements, atomic force microscopy (AFM), infrared reflection absorption spectroscopy (IRRAS) and x-ray photoelectron spectroscopy (XPS) are used to follow the progress of the reaction and final characterisation of the resulting surfaces. These monolayers can be further functionalised to link DNA and other biomolecules.

**Keywords:** silicon surfaces; organic monolayers; photochemical; biosensors; DNA; X-ray photoelectron spectroscopy; XPS; infrared reflection absorption spectroscopy; IRRAS; atomic force microscopy; AFM.

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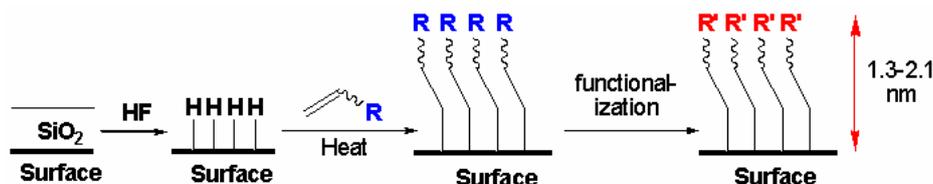
### 1 Introduction

Devices capable of continuously and selectively detect biomolecular interactions are of great importance in biotechnology and medical diagnostics. For example, nucleic acid microarrays are used for expression profiling, genotyping, DNA sequencing, diagnostics, immunology and drug discovery. Nonetheless, there are still technical hurdles to overcome before biomolecular-based diagnostic devices will become widely available and reusable. Definitely, the improvement of DNA- and protein-based biosensors and microchips relies on the reproducible and effective immobilisation of biomolecules onto solid substrates. In this context, functionalisation of inorganic surfaces (silicon, silicon nitride and silicon carbide) (Arafat et al., 2004, 2007; Rosso et al., 2008, 2006) by

covalent attachment of organic monolayers opens up new opportunities in a variety of these applications.

In this paper, we present a detailed investigation on monolayer formation on silicon and silicon containing surfaces based on the chemistry presented in Figure 1.

**Figure 1** Formation of covalently attached organic monolayers onto silicon and silicon containing surfaces (see online version for colours)



Surface = silicon, silicon nitride or silicon carbide

Thermal and photochemical methods are used to form such stable and densely backed monolayers and their functionalisation with esters, amine groups, and proteins will be shown. Static water contact angle measurements, atomic force microscopy (AFM), infrared reflection absorption spectroscopy IRRAS and X-ray photoelectron spectroscopy (XPS) are used to follow the progress of the reaction and final characterisation of the resulting surfaces.

## 2 Experimental

### *Wafers*

Single-polished Si(100): n-type, 500  $\mu\text{m}$  to 550  $\mu\text{m}$  thick, resistivity 1  $\Omega\text{cm}$  to 2  $\Omega\text{cm}$  (Seltec Silicon, Mitsubishi Silicon America) and single-polished Si(111): n-type, 475  $\mu\text{m}$  to 550  $\mu\text{m}$  thick, resistivity 1  $\Omega\text{cm}$  to 5  $\Omega\text{cm}$  (Addison Engineering, San Jose). Silicon nitride coated (thickness: 200 nm) silicon ( $10 \times 10 \times 0.5 \text{ mm}^3$ ) single side-polished wafers were supplied by Aquamarijn B.V., The Netherlands.

Detailed procedures for formation and analyses of the monolayers on silicon, silicon nitride and silicon carbide surfaces were previously reported (Arafat et al., 2004; De Smet et al., 2005; Scheres et al., 2007; Sieval et al., 2000a)

## 3 Results and discussions

### *3.1 Alkyl and alkenyl monolayers*

Formations of organic monolayers on Si(100) and Si(111) have been extensively investigated by thermal, photochemical and other methods. (Sun et al., 2005) Si – C attached alkyl or alkenyl monolayers can be formed by direct reaction of neat or solution of the corresponding alkene or alkyne in mesitylene with hydrogen-terminated crystalline silicon surfaces. Hydrogen-terminated silicon surfaces are produced by reaction of clean silicon samples with either dilute HF or ammonium fluoride solutions. Several methods

are employed to determine the quality of the formed monolayer, including water contact angle, infrared spectroscopy. High quality alkyl and akenyl monolayers are highly hydrophobic. The static water contact angle of the monolayers ranges from 108° to 110°. The crystallinity of the monolayers can be simply seen by recording the infrared spectra of the monolayers. Two possible infrared techniques are applied, attenuated reflection infrared spectroscopy (ATR) and infrared reflection absorption spectroscopy (IRRAS). Crystalline, densely packed monolayers have antisym/sym CH<sub>2</sub> stretching bands at 2920/2850 cm<sup>-1</sup>, respectively.

Table 1 lists the static water contact angle of the Si(100) and Si(111) surfaces modified by a variety of 1-alkenes, 1-alkynes and two esterified-1-alkenes (compounds I and II) using 447 nm light (15h) as compared to the same monolayers obtained under thermal conditions. The formation of the monolayer is almost complete after 10h. This is inline with previously published work (De Smet et al., 2005). The terminal polar groups of compounds I and II afford monolayers with lower contact angles.

**Table 1** Static water contact angles (°) of monolayers on silicon surfaces prepared by 447 nm irradiation (15h; r.t.) and thermal methods (heating in neat reactant or refluxing for 2h in 0.2M solution in mesitylene)<sup>a</sup>

<i>Reactants</i>	<i>447 nm</i>	<i>447 nm</i>	<i>Thermal</i> <sup>b</sup>
	<i>Si(100)</i>	<i>Si(111)</i>	<i>Si(100)</i>
CH ≡ C – C <sub>10</sub> H <sub>21</sub>	108	109	108
CH ≡ C – C <sub>12</sub> H <sub>25</sub>	110	110	110
CH ≡ C – C <sub>14</sub> H <sub>29</sub>	110	110	110
CH <sub>2</sub> = CH – C <sub>10</sub> H <sub>21</sub>	109	109	108
CH <sub>2</sub> = CH – C <sub>12</sub> H <sub>25</sub>	108	110	108
CH <sub>2</sub> = CH – C <sub>14</sub> H <sub>29</sub>	109	110	109
CH <sub>2</sub> = CH – C <sub>8</sub> H <sub>16</sub> COOCH <sub>3</sub> (I)	78	75	77
CH <sub>2</sub> = CH – C <sub>8</sub> H <sub>16</sub> COOCH <sub>2</sub> CF <sub>3</sub> (II)	85	86	88 <sup>c</sup>

Notes: <sup>a</sup>All experiments were performed at least twice; experimental error = ±1.0°.

<sup>b</sup>Apart from the last entry data taken from references (Sieval et al., 1998; Sieval et al., 2000b).

<sup>c</sup>Value obtained by using conditions as described in reference(Sieval et al., 2000b).

### *The mildest modification method ever*

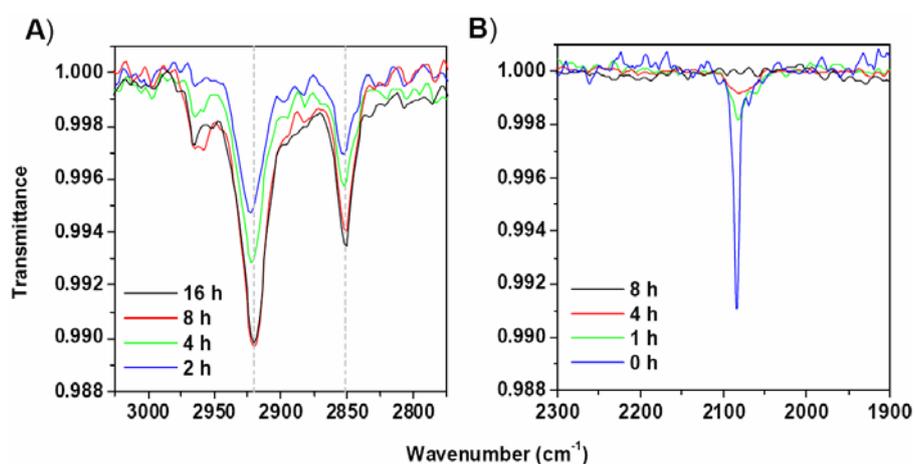
High-quality covalently bound organic monolayers on H-terminated Si(111) with unactivated 1-alkynes at r.t. in dark. Monolayers prepared in this manner are better than that of any other method we know of. These monolayers are prepared at 20°C to 80°C on freshly etched Si(111) under a reduced argon pressure ~10 mbar with neat 1-alkyne in ambient light and dark. A contact angle of 111° is obtained, indicative of high-quality densely packed organic monolayers that are at least as good as prepared by other methods (Sieval et al., 2000a). Therefore, the currently reported method, apart from being the mildest, also yields the best monolayers (Scheres et al., 2007).

*Modification of silicon nitride surfaces*

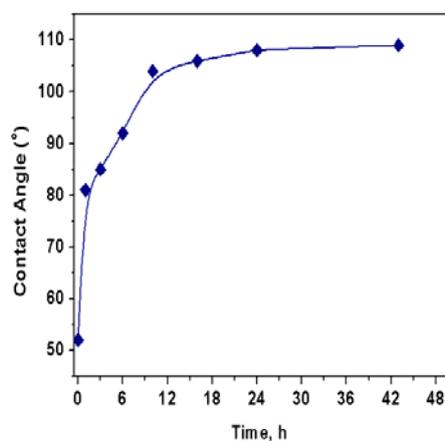
Thermal and photochemical methods have been applied to attach organic monolayers to silicon nitride surfaces. The chemistry of monolayer attachment is a bit different from that presented on silicon surfaces due to the presence of the active nitrogen and silicon atoms on the surface. Relatively high quality monolayers are obtained with maximum water contact angle of 108°. The slightly lower contact angle could be attributed to the presence of the electronegative nitrogen atoms on the nitride surface and also due to the presence of surface roughness (Arafat et al., 2007).

The mild visible light method could not be applied to the formation of monolayers on these surfaces, nevertheless, irradiation of surface with UV light in neat alkenes afforded a high quality monolayer after irradiation for 24h.

**Figure 2** IRRAS of the mildest conditions for monolayer formation (see online version for colours)

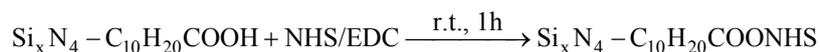


**Figure 3** Variation of the water contact angles vs. time of hexadecyl monolayers prepared by UV irradiation (see online version for colours)



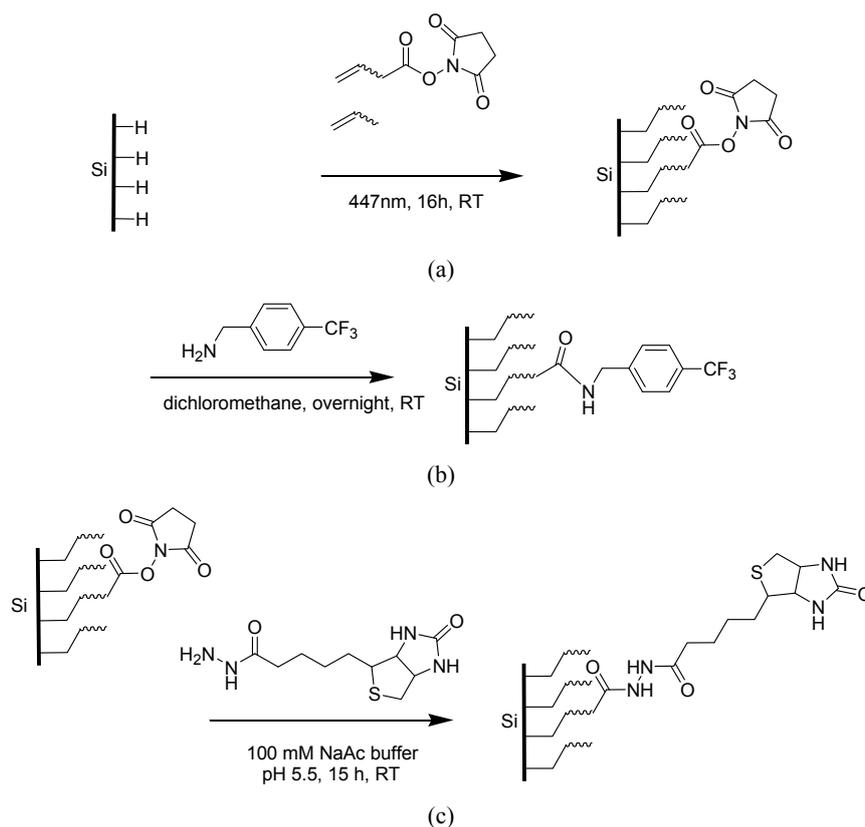
### 3.2 Attachment of biolinkers

Conventionally, a monolayer of carboxylic acid ester is attached to the surface. Hydrolysis in acid or alkaline medium affords a carboxylic acid-terminated monolayer. Chemical activation of the acid function with *N*-hydroxy succinimide (NHS) was used to prepare NHS-activated esters (Voicu et al., 2004). The terminal NHS group is the key linker to the attachment of biomolecules in order to prepare bionanosensor. EDC = *N,N*-dicyclohexylcarbodiimide



Performing the above two steps is always hampered by some difficulties such as the degradation of the formed monolayer in alkaline medium and incomplete reaction in acid medium. A mild one-step attachment of NHS-groups onto silicon surfaces can be done via the irradiation with visible light. This provides a mild, room-temperature alternative for the binding of covalently attached, Si-C high quality linked monolayers (Yang et al., 2008).

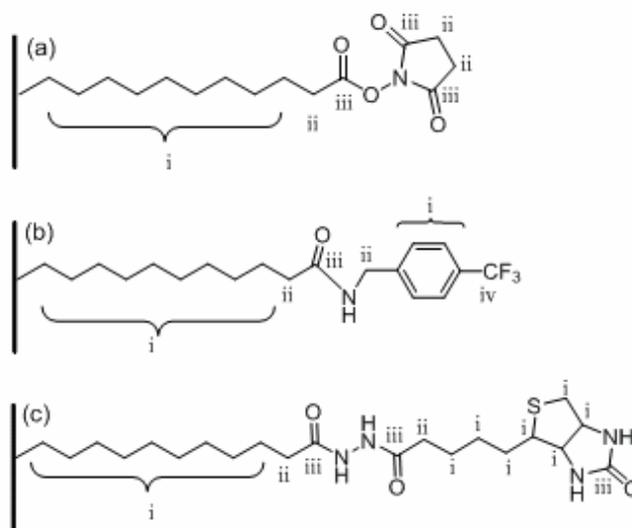
**Figure 4** Reaction scheme



Mixed monolayers were obtained from photochemical reaction ( $\lambda = 447$  nm) of a hydrogen-terminated silicon Si(111) surface with a mixture of N-succinimidyl undecylenate (NHS-ester-alkene) and 1-decene in different ratios. The mole fraction of the NHS ester groups on the resulting mixed monolayers was expected to approximate the mole fraction of NHS-ester-alkene in the reaction mixture (Liu et al., 2004). IRRAS reveals a clear increase of NHS-ester functionalities in the mixed monolayers upon increasing the fraction of NHS-ester-alkene from 0 to 100% in alkene mixture, as evidenced by the characteristic C = O stretching vibrations at  $1819\text{ cm}^{-1}$ ,  $1788\text{ cm}^{-1}$  and  $1745\text{ cm}^{-1}$  (Figure 5). AFM analysis shows that the resulting surfaces are clean and very flat, e.g., with easily recognisable Si edge steps and an average surface roughness of the 100% NHS-modified surface of  $\sim 0.1$  nm.

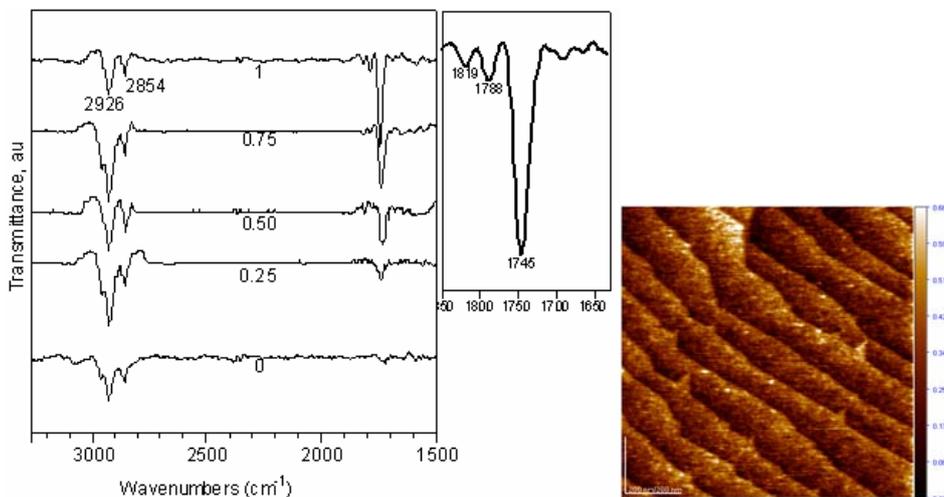
A schematic depiction of the types of atoms that are distinguishable by XPS is given in Figure 5. The  $C_{1s}$  signal of NHS ester-terminated monolayer (a) can be disassembled into three peaks, from low to high BE, as which clearly discriminate the different carbon atoms presented in Scheme 3a. The ratio between these three peaks for a 100% NHS ester-terminated monolayer was 9.4:2.7:2.4, which is within experimental error equal to the theoretical ratio of 9:3:3 (see Table 2).

**Figure 5** The carbon atoms that can be distinguished by XPS in the monolayers with (a) NHS (b) TFBA (c) biotin hydrazide



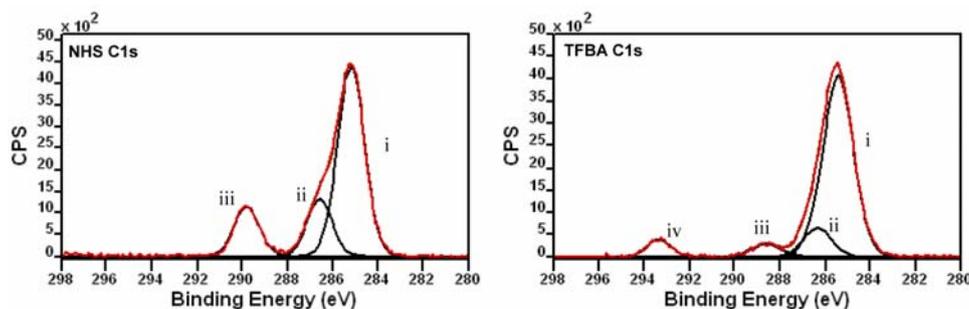
**Table 2** Atomic ratios of 100% and 50% NHS-ester-terminated monolayer by XPS analyses

	<i>F/C</i>		<i>C<sub>i</sub>:C<sub>ii</sub>:C<sub>iii</sub>:C<sub>iv</sub></i>	
	<i>Theor.</i>	<i>Exp.</i>	<i>Theor.</i>	<i>Exp.</i>
100% NHS	0	0.2:14.8	9:3:3	9.4:2.7:2.4
100% NHS + TFBA	3:19	3.3:18.6	15:2:1:1	15.0:2.1:1.1:1.1
50% NHS	0	0.1:24.8	19:3:3	19.3:3.3:2.4
50% NHS + TFBA	3:29	3.2:28.8	25:2:1:1	25.0:2.0:1.1:1.2

**Figure 6** IRRAS, AFM of NHS activated esters (see online version for colours)

#### *Modification with biotin trifluoromethyl benzyl amide (TFBA)*

Confirmation that the NHS moieties were attached fully intact comes from a quantitative XPS analysis of surfaces in which the NHS-monolayer was allowed to react with TBFA. TFBA quantitatively replaces the NHS moiety, and yields for both the 100% and 50% NHS monolayers ratios between the expected carbon atoms that are in excellent agreement with theoretical predictions (see Table 2).

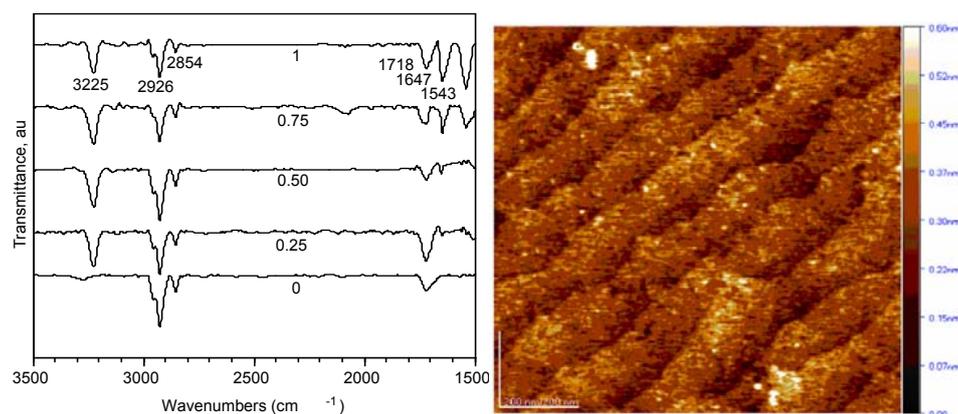
**Figure 7** XPS narrow scans of C1s of a 100% NHS-terminated monolayer (left) and a TFBA-modified monolayer (right) (see online version for colours)

#### *Modification with biotin hydrazide*

As a second example, illustrated in Figure 8, the functionalisation of NHS-containing monolayers on Si(111) for bio-applications was demonstrated by modifying such monolayers with biotin hydrazide, to obtain a biotinylated semiconductor surface. After incubation overnight in a  $\sim 5$  mM solution of biotin hydrazide in 100 mM sodium acetate buffer pH 5.5, the static contact angle of the monolayers was measured again. In comparison to NHS-terminated monolayers with the same fraction of NHS, the contact

angle decreases by  $10 \pm 3^\circ$ , as biotin is more hydrophilic than NHS ester. In addition, AFM measurements display that the resulting surface is really clean and flat, as after sonication of the sample in water – to remove any physically adsorbed hydrazide – the surface roughness is again only 0.06 nm, and AFM clearly displays the terrace structure of Si(111) substrate. Such a flat and bioactive surface does indeed match the requirements of bio-SPM research and biochip fabrication.

**Figure 8** IRRA spectra of mixed monolayers with different NHS mole fractions after modification with biotin hydrazide (see online version for colours)



## 4 Conclusions

It is easily possible to prepare covalently attached, well defined non- and functionalised monolayers on silicon surfaces using extremely mild techniques at r.t. using visible light or reduced argon pressures. For the chemically inert silicon nitride surface UV light can be used to prepare such monolayers. For linking of biomolecules, *N*-hydroxysuccinimidyl (NHS-) terminated mixed monolayers using visible light at room temperature could also be formed on silicon surfaces. These NHS moieties could be quantitatively substituted by small primary amines under mild conditions. In addition, functionalisation with larger amines. Finally, in combination with lithographic techniques, the possibility of light-induced attachment widens the possibilities for the application of biofunctionalised silicon surfaces in biomolecule arrays and biosensors.

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