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Molecular monolayers on silicon as substrates for biosensors

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ABSTRACT

(111) silicon surfaces can be controlled down to atomic level and offer a remarkable starting point for elaborating nanostructures. Hydrogenated surfaces are obtained by oxide dissolution in hydrofluoric acid or ammonium fluoride solution. Organic species are grafted onto the hydrogenated surface by a hydrosilylation reaction, providing a robust covalent Si–C bonding. Finally, probe molecules can be anchored to the organic end group, paving the way to the elaboration of sensors. Fluorescence detection is hampered by the high refractive index of silicon. However, improved sensitivity is obtained by replacing the bulk silicon substrate by a thin layer of amorphous silicon deposited on a reflector. The development of a novel hybrid SPR interface by the deposition of an amorphous silicon–carbon alloy is also presented. Such an interface allows the subsequent linking of stable organic monolayers through Si–C bonds for a plasmonic detection. On the other hand, the semiconducting properties of silicon can be used to implement field-effect label-free detection. However, the electrostatic interaction between adsorbed species may lead to a spreading of the adsorption isotherms, which should not be overlooked in practical operating conditions of the sensor. Atomically flat silicon surfaces may allow for measuring recognition interactions with local-probe microscopy.

1. Introduction

Attaching molecules to surfaces in order to act as probes is a key issue for biological and chemical sensors. The performance of the sensors in terms of reliability, efficiency and sensitivity generally depends on the control of the immobilization chemistry of the probes [1–3]. Covalent anchoring appears as an attractive route, since it offers the best performances in terms of stability and can be made reproducible and with a high yield. In this framework, the advances performed in the last decade in silicon surface chemistry open the way to the use of silicon as a substrate and possibly as a transducer in (bio) sensors built using an efficient and well controlled surface chemistry [4–6]. Especially, anchoring the probes through a direct silicon–carbon bond appears as a promising route to provide a more robust and reliable chemistry than the more conventional anchoring to a silica layer through silanization [7–11].

Silicon surfaces can be hydrogenated by oxide dissolution in a hydrofluoric acid solution [12]. Furthermore, (111)-oriented surfaces can be obtained atomically flat if oxygen-free ammonium fluoride solution is used instead [13]. These surfaces provide a convenient platform for the grafting of organic species, especially through the hydrosilylation of alkene precursors [5]. By using this chemical route, carboxyl-terminated monolayers can be anchored to silicon through a direct covalent Si–C bond [14]. Carboxyl-terminated monolayers covalently anchored to silicon surfaces are really appealing as they can easily be activated by reaction with N-hydroxysuccinimide (NHS) in the presence of a peptide coupling carbodiimide such as N-ethyl-N′-(3-dimethylaminopropyl)-carbodiimide (EDC). Biomolecules can then be attached to the activated ester through a classical amidation reaction with the amino end of a probe molecule tethered with an aminolinker, allowing for the covalent attachment of the probe through the formation of an amide bond [15]. However, the conditions of this reaction are more stringent here than when the reaction is carried out in solution, because, at a surface, the reaction products cannot be purified, and unwanted reactions can lead to irreversible surface contamination. In the present work, the various possible reaction paths have been studied and the reaction conditions have been subsequently optimized, allowing us to anchor DNA oligomers or proteins to the surface. The molecular recognition

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event between the anchored probe biomolecule and the target molecule in solution can be detected either by fluorescence [16], surface plasmon resonance [17] or by field effect [18]. The problems specific to each of these detection schemes will be discussed.

2. Experimental

The (111), 10 Ω cm n-Si samples were shaped as $15 \times 15 \times 0.5$ mm^3 platelets, then two opposite sides were 45° bevelled for characterisation by multiple-reflection, internal reflectance (ATR) infrared spectroscopy. This characterisation was made either ex-situ (comparison of the spectra taken before and after a chemical treatment) or in-situ at the contact with the solution used for the chemical treatment, which allowed for an investigation of the reaction kinetics. For the in-situ experiments, a temperature-regulated cell was used, where a liquid could be flown against the silicon surface, the silicon prism being pressed with an O-ring seal against an aperture in the cell wall (Fig. 1) [19,20].

The initial surfaces were prepared by cleaning in a "piranha" mixture (H_2SO_4:H_2O_2, 3:1 by vol.), then etching either in oxygen-free concentrated NH_4F solution (with silicon dissolution, leading to an atomically flat hydrogenated surface, termed SiH) [13] or in concentrated HF solution (etching limited to oxide dissolution, leaving an atomically rough hydrogenated surface, termed SiH_x) [12]. In some experiments, the modifications were carried out on a hydrogenated amorphous silicon layer a-Si:H (or silicon–carbon alloy a-$Si_{1-x}C_x$:H) deposited onto the silicon prism. Prior to the modification, the a-Si:H surface was (re)hydrogenated by exposing it to HF vapours for a few seconds. In addition to the infrared measurements, the atomically flat silicon surfaces (obtained by modification of SiH surfaces) were characterized by atomic force microscopy (AFM), which provided a convenient monitoring of surface morphology and cleanliness.

Grafting of carboxyl-terminated alkyl chains was achieved by immersing the freshly hydrogenated silicon sample into outgassed undecylenic acid in a Schlenk tube, which was kept at 180 °C overnight (thermal activation) or under UV illumination (312 nm, 6 mW/cm^2) for 3 h (photochemical activation). These protocols are known to lead to dense layers of 10-carboxydecyl groups covalently anchored to the silicon surface [4,6,8,15,21,22]. After this treatment, the sample was thoroughly rinsed in hot acetic acid, which has been shown to remove the acid overlayer physisorbed onto the carboxyl end groups of the grafted layer. [21]The obtained monolayers have been shown to be dense (surface concentration 2.5 10^{13} cm^{-2}, limited by steric hindrance between the carboxyl groups [21], orientation of the carboxyl groups in the direction normal to the surface [23], high electronic passivation and chemical stabilization of the silicon surface [24]). When a lower density of terminal carboxyl groups was desired, the undecylenic acid was replaced by an undecylenic acid/decene mixture. Previous studies enable us to know the stoichiometry of the grafted layer, which is slightly different from that of the grafting liquid [21].

The carboxyl-termination was converted to an activated ester, then an amine-terminated molecule was anchored to the activated surface, according to procedures detailed in the next section. The amine-terminated molecule was either a simple amine for assessing the procedure, or a biomolecule (DNA oligomer or biotin). The chemical state of the surface was monitored at each step using ex-situ or in-situ infrared spectroscopy. Finally, the activity of the surfaces modified with biomolecules was assessed by fluorescence measurements on surfaces exposed to target molecules labelled with Cy3 or Cy5 or by surface plasmon resonance (SPR).

3. Results

3.1. Anchoring through an optimized activation–amidation procedure

The acid-terminated surface (Si–$(CH_2)_{10}$–COOH) was activated by the classical EDC–NHS activation route (EDC = N-ethyl-N′-(3-dimethylaminopropyl)carbodiimide, NHS = N-hydroxysuccinimide), leading to the "activated" ester (Si$(CH_2)_{10}$–COO–N[CO–CH_2–CH_2–CO]) [15]. The reaction was carried out at temperatures ranging from 5 to 20 °C, and followed by in-situ infrared spectroscopy (Fig. 2). A too high temperature may lead to spontaneous transformation of EDC to its urea derivative by reaction with water, while a too low temperature may decrease the reaction rate. In practice, a temperature of 15 °C was found to be a good compromise. Under typical conditions (5 mM EDC, 5 mM NHS, 15 °C), the disappearance of the νCO band associated with the acid group COOH (1715 cm^{-1}) and the appearance of the three bands characteristic of the activated ester occur with similar biexponential relaxation laws, plausibly due to the presence of two reaction paths. The longer characteristic time is around 50 min (see Fig. 2), so that a reaction time of 1 h 30 min nearly leads to a stable state. One can note that the NHS ester bands appear at 1735, 1780 and 1815 cm^{-1} during in-situ measurements (see Fig. 2), whereas they appear at 1745, 1790 and 1820 cm^{-1} during ex-situ measurements (see Fig. 3).

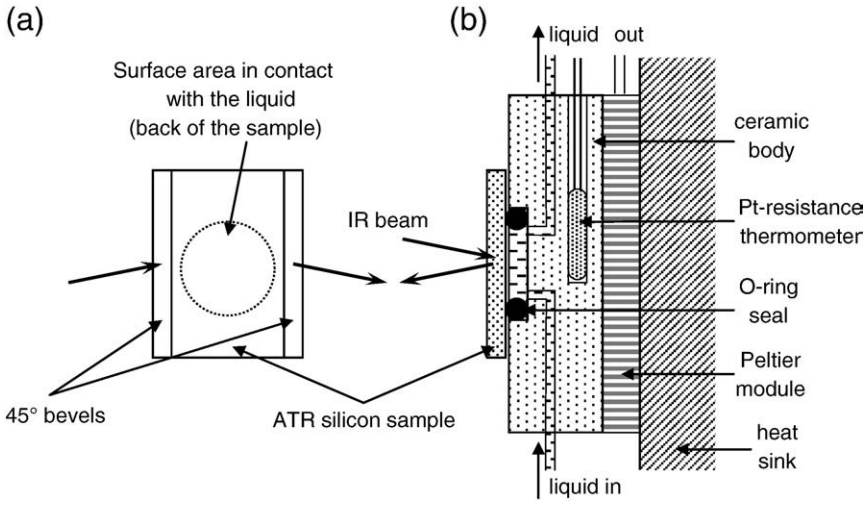

Fig. 1. Scheme of the in-situ IR arrangement. (a) Front view of the ATR silicon sample and (b) side view of the sample mounted on the circulation cell.