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One Step Growth of Protein Antifouling Surfaces: Monolayers of Poly(ethylene oxide) (PEO) Derivatives on Oxidized and Hydrogen-Passivated Silicon Surfaces

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We compare two routes for creating protein adsorption-resistant self-assembled monolayers (SAMs) by chemical modification of silicon surfaces with poly(ethylene oxide) (PEO) oligomeric derivatives. The first route involves the assembly of 2-methyl[(polyethyleneoxy)propyl]trichlorosilane (Cl$_3$SiMPEO) films onto oxidized silicon surfaces (OH−SiO$_x$) either by a liquid-phase process at room temperature or by a gas-phase process at 423 K, producing Si−O−Si bonds between the substrate and the organic layer. The second pathway makes use of the assembly of poly(ethylene glycol methyl ether) (MPEG) films onto hydrogen-passivated silicon surfaces (H−Si) using a liquid-phase process at 353 or 423 K, leading to the formation of Si−O−C bonds between the substrate and the organic layer. Structural investigation by X-ray reflectometry (XRR) reveals that the thickness and surface densities of the grafted PEO monolayers strongly depend on experimental conditions such as temperature and grafting time. Atomic force microscopy (AFM) shows that very smooth and homogeneous monolayers can be obtained with average roughnesses close to those measured on the corresponding bare substrates. Finally, the antifouling properties of the modified silicon surfaces were evaluated by X-ray photoelectron spectroscopy (XPS), using a membrane protein (P.69 antigen) as model protein. Both types of PEO monolayers exhibit excellent protein repellency, as soon as the grafting density is equal to or higher than 1.7 chains/nm^2.

Introduction

Biocompatible layers showing efficient antifouling properties toward nonspecific protein adsorption are a key element in the development of biosensing devices. Therefore, there is currently a strong interest in the investigation and the optimization of different strategies to produce such surfaces. In this context, poly(ethylene oxide) (PEO)-based compounds extensively proved their remarkable antifouling properties. Several routes have been explored to coat surfaces with PEO derivatives. Successful approaches use physical adsorption, covalent grafting, graft polymerization activated by UV, electrografting of PEO-modified acrylates, or spontaneous assembly of monolayers (SAMs) onto the surface.[1−16]

The latter strategy is particularly interesting when control of the layer morphology at the molecular level is required. So far, two strategies were mainly reported for the formation of self-assembled monolayers onto silicon surfaces. First, on oxidized silicon (OH−SiO$_x$), SAMs are formed via the reaction of silanes, chloro- or alkoxy-functionalized, leading to the formation of siloxane linkages (Si−O−Si).[17−21] When the silicon surface is hydrogen-passivated Si(H−Si), SAMs are formed by reaction of CH$_2$=CH−R[22−27] or HO−R and H(C=O)−R[28−32] compounds,

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(1) Lasseter, T. L.; Clare, B. H.; Nichols, B. M.; Abbott, N.; Hamers, R. J. *Langmuir* **2005,** *21,* 6344.
(2) Li, L.; Chen, S.; Zheng, J.; Ratner, B. D.; Jiang, S. *J. Phys. Chem. B* **2005,** *109,* 2934.
(3) Chen, H.; Zhang, Z.; Chen, Y.; Brook, M.; Sheardown, H. *Biomaterials* **2005,** *26,* 2391.
(4) Lasseter, T. M.; Clare, B. H.; Abbott, N.; Hamers, R. J. *J. Am. Chem. Soc.* **2004,** *126,* 10220.
(5) Xu, D.; Yu, W. H.; Kang, E. T.; Neoh, K. G. *J. Colloid Interface Sci.* **2004,** *279,* 78.
(6) Sharma, S.; Johnson, R. W.; Desai, T. A. *Biosens. Bioelectron.* **2004,** *20,* 227.
(7) Ostuni, E.; Grzybowski, B. A.; Mrksich, M.; Roberts, C. S.; Whitesides, G. M. *Langmuir* **2003,** *19,* 1861 and references therein.
(8) Herrwerth, S.; Rosendhal, T.; Feng, C.; Fick, J.; Eck, W.; Himmelhaus, M.; Dahint, R.; Gunze, M. *Langmuir* **2003,** *19,* 1880.
(9) Zhu, X.-T.; Jun, Y.; Staarup, D. R.; Major, R. C.; Danielson, S.; Boiadjiev, V.; Gladfelter, W. L.; Bunker, B. C.; Guo, A. *Langmuir* **2001,** *17,* 7798.
(10) Papra, A.; Gadegaard, N.; Larsen, N. B. *Langmuir* **2001,** *17,* 1457.
(11) Schwendel, D.; Dahint, R.; Herrwerth, S.; Schloerholz, M.; Eck, W.; Grunze, M. *Langmuir* **2001,** *17,* 5717.
(12) Chapman, R. G.; Ostuni, E.; Yan, L.; Whitesides, G. M. *Langmuir* **2000,** *16,* 6927.
(13) Alcantar, N. A.; Aydil, E. S.; Israelachvili, J. N. *J. Biomed. Mater. Res.* **2000,** *51,* 343.
(14) Ostuni, E.; Yan, L.; Whitesides, G. M. *Biointerfaces* **1999,** *15,* 3.
(15) Pale-Grosdemange, C.; Simon, E. S.; Prime, K. L.; Whitesides, G. M. *J. Am. Chem. Soc.* **1991,** *113,* 2.
(16) Gabriel, S.; Dubruel, P.; Schacht, E.; Jonas, A. M.; Gilbert, B.; Jérôme, R.; Jérôme, C. *Angew. Chem., Int. Ed.* **2005,** *44,* 2.
(17) Jung, G.-Y.; Li, Z.; Wu, W.; Chen, Y.; Olynick, D. L.; Wang, S.-Y.; Tong, W. M.; Williams, R. S. *Langmuir* **2005,** *21,* 1158.
(18) Sharma, S.; Desai, T. A. *J. Nanosci. Nanotech.* **2005,** *5,* 235.
(19) Popat, K. C.; Sharma, S.; Desai, T. A. *J. Phys. Chem. B* **2004,** *108,* 5185.
(20) Wang, M.; Liechti, K. M.; Wang, Q.; White, J. M. *Langmuir* **2005,** *21,* 1848.
(21) Tsukruk, V. V.; Lizinov, I.; Julthongpiput, D. *Langmuir* **1999,** *15,* 3029.
(22) Jin, H.; Kinser, R.; Bertin, P. A.; Kramer, D. E.; Libera, J. A.; Hersman, M. C.; Nguyen, S. T.; Bedzyk, M. J. *Langmuir* **2004,** *20,* 6252.
(23) Cicero, R. L.; Chidsey, Ch. E. D. *Langmuir* **2002,** *18,* 305.
(24) Sieval, A. B.; Linke, R.; Heij, G.; Meijer, G.; Zuilhof, H.; Sudholter, E. J. R. *Langmuir* **2001,** *17,* 7554.
(25) Cicero, L.; Lindford, M. R.; Chidsey, E. D. *Langmuir* **2000,** *16,* 5688.
(26) Buriak, J. M. *Chem. Commun.* **1999,** 1051.
(27) Boukherroub, R.; Morin, S.; Bensebaa, F.; Wayner, D. D. M. *Langmuir* **1999,** *15,* 3831.
(28) Hacker, Ch. A.; Anderson, K. A.; Richter, L. J.; Richter, C. A. *Langmuir* **2005,** *21,* 882.
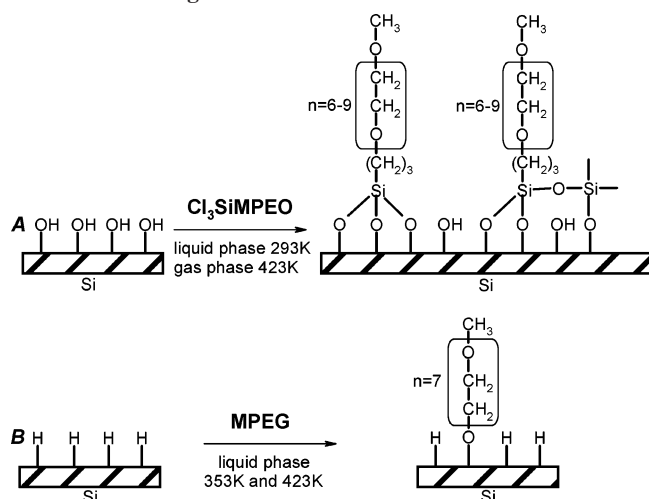(29) Major, R. C.; Zhu, X.-Y. *Langmuir* **2001,** *17,* 5576.
(30) Boukherroub, R.; Morin, S.; Sharpe, P.; Wayner, D. D. M.; Allongue, Ph. *Langmuir* **2000,** *16,* 7429.
(31) Kim, Y. Y.; Laibinis, P. E. *J. Am. Chem. Soc.* **1997,** *119,* 2297.
(32) Cleland, G.; Horrocks, B. R.; Houlton, A. *J. Chem. Soc., Faraday Trans.* **1995,** *91,* 4001.

Scheme 1.   Schematic Representation of the One-Step Investigated Functionalization Processes[a]

(A) Grafting of Cl₃SiMPEO molecules on an oxidized silicon surface. (B) Grafting of MPEG molecules on a hydrogen-passivated silicon surface.

leading to the formation of Si−C or Si−O−C covalent bonds, respectively. Both pathways have been used to grow PEO monolayers. The few examples of PEO−silane monolayers grown in the liquid phase were obtained after long reaction times and in the presence of a surface reaction activator in solution,[10,18] or, alternatively, were prepared starting from long-chain PEO compounds and from concentrated solutions[6] in order to get monolayers with high grafting densities and better antifouling properties. Examples of SAMs obtained by self-assembly of CH₂=CH−PEO or of OH−PEO derivatives demonstrated efficient antifouling properties; however, their growth required either a synthetic effort of the opportune PEO derivative,[1] or two-step procedures with a surface preactivation process and/or a catalytic activation in the reaction box.[9]

In the present study, we report on the growth of PEO monolayers obtained by one-step processes onto oxidized or onto hydrogen-passivated silicon surfaces. In the first investigated route, a trichloro-silane PEO derivative (Scheme 1A) was used to react with the oxidized silicon surface. The reaction was carried out either in liquid phase at room temperature or in gas phase at 423 K. Similar molecules were already used[6,10] to create PEO SAMs in solution; however, here we have selected a shorter PEO chain in order to reduce collapse effects occurring for long chain derivatives, which allows us to get very thin controlled antifouling layers,[1] and we focus on a trichlorosilane derivative instead of the more frequent trialkoxysilane derivative. We also show that gas-phase silanization of the PEO-silane represents an attractive route to create biocompatible films giving high quality monolayers, in the absence of solvents. In the second strategy, glycol-terminated PEO methyl ether (Scheme 1B) was grafted onto hydrogen-terminated silicon surfaces under thermal activation. This method was already successfully used to create hydrocarbon monolayers,[30−32] although there is not yet any detailed example of its application to PEO derivatives. The structural, morphological, and antifouling properties of the resulting monolayers are studied by X-ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM), and X-ray reflectometry (XRR).

Experimental Section

Materials. 2-Methyl(polyethyleneoxy)propyltrichlorosilane (Cl₃-SiMPEO, MW = 426−558 g/mol) (Scheme 1A) was purchased

from Gelest and poly(ethylene glycol methyl ether) (MPEG, M_W = 350 g/mol) (Scheme 1B) from Sigma Aldrich. Toluene (HPLC grade) and dichloromethane (HPLC grade) were obtained from Acros Organics, while sulfuric acid (98%) and hydrogen peroxide were from Merck VWR. One-side polished 475 μm-thick Si(100) wafers were obtained from ACM (Applications Couches Minces, France). P.69 pertactin was provided by GlaxoSmithKline Biologicals (Rixensart, Belgium) and was stored in phosphate buffer at pH 7.6 (50 mM of K₂HPO₄/KH₂PO₄ and 0.5 M of NaCl in 50/50 vol/vol water/glycerol mixtures), at the concentration of 211 μg/mL.

Preparation of Monolayers. *Oxidized Silicon Surfaces (OH-SiO$_x$).* The silicon wafers were cleaned for 20 min in freshly prepared hot piranha solutions (98% H₂SO₄/30% H₂O₂ 7:3 v/v) *(caution: piranha solution is a strong oxidant and reacts violently with organic substances)*, followed by thorough rinsing with 18 MΩ.cm water, and then were immediately used for the following chemical functionalization. The surface cleaning was confirmed by low water contact angle (lower than 10°) and by XPS, where the Si 2p photoemission region is composed of two separate peaks, at 99.4 and 103.3 eV, associated with the bulk silicon and with the surface oxidized silicon layer,[33] respectively (as will be discussed below).

Hydrogen-Passivated Silicon Surfaces (H-Si). Oxidized Si(100) surfaces were etched in a 1% HF solution during 1 min followed by rinsing in 18 MΩ.cm water, and then immediately transferred in a glovebox for direct functionalization. The surface hydrogen passivation was confirmed by the high contact angle value (θ > 110°) and by XPS, where no oxidized silicon signal was detected in the Si 2p photoemission spectrum (as will be discussed below).

Grafting of Cl₃SiMPEO. Liquid-Phase Silanization. 2-Methyl-(polyethyleneoxy)propyl]trichlorosilane monolayers were prepared in a glovebox ([H₂O] = 0.5 ppm) by immersion of the oxidized substrates in a 2 mM solution of Cl₃SiMPEO in toluene, for different reaction times ranging from 2 to 24 h.

Gas-Phase Silanization. Films were obtained using the gas-phase silanization procedure extensively described elsewhere,[34] for reaction times of 24 and 46 h and at 423 K. This relatively high temperature was selected on the basis of prior screening experiments, to raise the partial vapor pressure in PEO-silanes to levels compatible with a reaction occurring in a few hours. After reaction, all films were washed in a dichloromethane Soxhlet for 24 h before use or characterization.

Grafting of MPEG. The H-Si(100) substrates were immersed in neat MPEG. The reaction was carried out in a glovebox at room temperature (RT), 353 K, or 423K, for times ranging from 2 to 24 h. After removal from the solution, the samples were washed in a dichloromethane Soxhlet for 24 h before characterization.

Adsorption of P.69. To check the antifouling properties of the above modified surfaces, a droplet of P.69 antigen solution was placed for 1 h at room temperature onto the poly(ethylene oxide) modified surfaces. The samples were then gently rinsed in ultrapure water before characterization.

Characterization Techniques. *X-ray Photoelectron Spectroscopy (XPS).* XPS measurements were performed using a SSX-ESCA 300 photoelectron spectrometer (Surface Science Instruments) with a monochromatic Al Kα X-ray source ($h\nu$ = 1486.6 eV). The energy resolution was set to 1.4 eV, and the photoelectron takeoff angle (TOA) was 55°. All binding energies were referenced to the bulk Si 2p core level at 99.4 eV.[35] The base pressure in the spectrometer was in the low 10⁻¹⁰ Torr range. Quantitative information was obtained by the photoemission peak areas of each element normalized by the sensitivity factors of the element tabulated for the used spectrometer.

Atomic Force Microscopy (AFM). AFM images were recorded in air in intermittent-contact mode (IC-AFM) with a Nanoscope IV from Veeco Instruments (Santa Barbara, CA). The cantilevers

(33) Raider, S. I.; Flitsch, R. *IBM J. Res. Dev.* **1978**, *22*, 294.

(34) Pallandre, A.; Glinel, K.; Jonas, A. M.; Nysten, B. *Nano Lett.* **2004**, *4*, 365.

(35) Moulder, J. F.; Stickle, W. F.; Sobol, P. E.; Bomben, K. D. *Handbook of Photoelectron Spectroscopy*; Perkin-Elmer Corporation, Physical Electronics Division: Eden Prairie, MN, 1992.
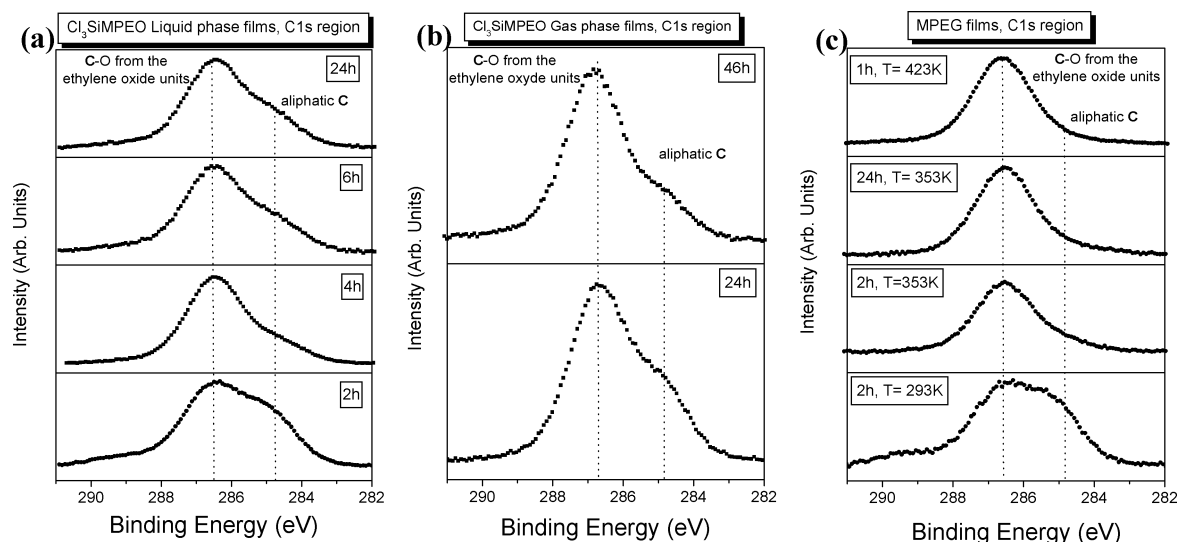
Figure 1. C 1s photoemission spectra of (a,b) Cl₃SiMPEO films obtained after different functionalization times, (a) in liquid phase at room temperature and (b) in gas phase at 423 K (b), and (c) of MPEG films obtained for different reaction times at different temperatures (from bottom to top: RT, 353 K, and 423 K).

(Pointbrobe Tapping-mode cantilevers from Nanosensors) were silicon cantilever with a resonance frequency around 160−165 kHz and a typical spring constant of around 40 N/m and an integrated silicon tip with an apex radius of curvature around 10 nm. So-called soft-tapping conditions were used; i.e., the ratio between the set-point amplitude, and the free amplitude of the cantilever vibration was always kept above 0.8.

X-ray Reflectometry (XRR). The experimental setup is a Siemens D5000 2-circles goniometer of 30 cm radius and 0.002° positioning accuracy. The incident beam (Cu Kα radiation, $\lambda = 0.15418$ nm) was obtained from a Siemens or Rigaku rotating anode operated at 40 kV and 300 mA, fitted with a graphite secondary monochromator and a scintillation counter. The beam was defined by a 40 μm-wide slit placed at 17.5 cm from the focal spot. Parasitic scattering was decreased by a 200-μm-wide slit placed after the 40 μm slit. The sample was fixed at the center of the goniometer with an automated procedure, using a vertical stage of 1 μm resolution. The intensity was scaled to unit incident intensity, and corrected for spill-over at very low angles of incidence. Data are reported as a function of K_{Z0}, the vertical component of the wavevector of the incident photons in a vacuum.

XRR data were analyzed in two ways. First, an estimation for the average thickness of the monolayer, d, was obtained from the value of K_{Zmin} in the reflectogram, according to $d = \pi/(2K_{Zmin})$. Here, K_{Zmin} is the K_{Z0} value corresponding to the first minimum in the reflectivity curve, arising from destructive interference. Second, a model of electron density was fitted to the data as described previously.[34,36] Quantitative parameters characteristic for the grafted layers could then be obtained from the electron density profiles $\rho(z)$ in the following way. First, the electron density profile obtained for a clean silicon wafer, $\rho_{Si}(z)$, was subtracted from $\rho(z)$. Then the area between the differential profile $(\rho(z) - \rho_{Si}(z))$ was computed, giving access to the total number of electrons belonging to grafted molecules, per unit surface. By dividing this area by the average thickness of the layer, d, its average electron density was obtained. The grafting density was defined as the ratio between the average electron density of the layer, and the electron density of the PEO crystals computed from published crystallographic data.[38] Furthermore, by dividing the area of the differential profile by the number of electrons contained in each MPEO molecule, an estimate was obtained for the number of grafted MPEO molecules per unit area (or for the area per molecule). Due to experimental errors and uncertainties in the

processing of the data, errors of about 10% are to be expected on the values obtained from the density profiles.

Results and Discussion

Film Grafting. The successful grafting of 2-methyl(poly-ethyleneoxy)propyl]trichlorosilane (Cl₃SiMPEO) onto oxidized Si(100) substrate was first confirmed by XPS. Figure 1a shows the C 1s core level photoemission spectra of Cl₃SiMPEO films obtained after different reaction times. The raw spectra show a main component centered at 286.4 eV, which is the fingerprint of aliphatic carbon atoms bound to oxygen originating from the ethylene oxide units of Cl₃SiMPEO.[5,6,30,37] A second minor component appearing around 284.9 eV is characteristic for pure aliphatic carbon atoms[5,6,30,37] and originates from the presence of a short hydrocarbon chain in the Cl₃SiMPEO molecule (Scheme 1A). For the film obtained after 2 h of reaction, the intensity of the latter component is higher than predicted on the basis of the elemental composition of Cl₃SiMPEO. This is probably due to a weak atmospheric hydrocarbon contamination physisorbed onto or within the monolayer, as the grafting density achieved after 2 h of functionalization is rather low (see below). Indeed, bare silicon surfaces are well-known to be prone to adsorption of organic contaminants.

The C 1s core level photoemission spectra of Cl₃SiMPEO monolayers prepared in the gas phase are shown in Figure 1b. The experimental curves consist also of a main contribution at 286.4 eV from the ethylene oxide carbon atoms and a minor contribution at 284.9 eV from the pure aliphatic carbon atoms. The intensity of the latter contribution is higher after 24 h of reaction time than after 46 h, suggesting a progressive increase of the grafting density with time. Moreover, in all Cl₃SiMPEO film spectra, no chlorine was detected. We can thus assume that the 3 Cl atoms of Cl₃SiMPEO molecule have reacted, with water, or with hydroxyl groups on the Si surface, or with neighboring silane molecules leading to partial lateral reticulation.[17]

The C 1s photoemission spectra recorded for MPEG films linked to the hydrogen-passivated silicon surface are shown in Figure 1c. The spectrum of the film obtained after 2 h reaction at room temperature (bottom panel) presents two contributions of nearly equivalent intensity. As already mentioned, the first component appearing at 286.4 eV is due to carbon atoms bound to oxygen from the ethylene oxide units, whereas the second

(36) Arys, X.; Laschewsky, A.; Jonas, A. M. *Macromolecules* **2001**, *34*, 3318.

(37) Beamson, G.; Briggs, D. *High-Resolution XPS of Organic Polymers*— *The Scienta ESCA Database*; John Wiley & Sons Ltd.: Chichester, U.K., 1992.

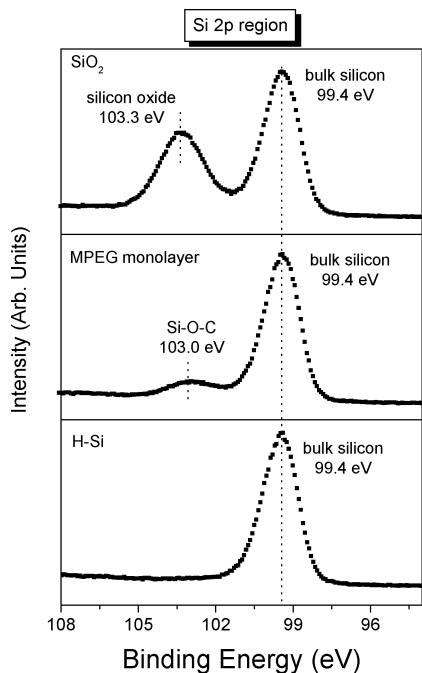(38) Takahashi, Y.; Tadokoro, H. *Macromolecules* **1973**, *6*, 672.

Figure 2. Si 2p photoemission spectra of a hydrogen-passivated silicon surface (bottom panel), a MPEG film grown onto a hydrogen-passivated silicon surface (central panel), and a native silicon oxide surface (top panel), respectively.

component at 284.8 eV is again attributed to aliphatic carbon atoms from a hydrocarbon contamination. However, MPEG films obtained after 2 and 24 h reaction at 353 K, or after 1 h reaction at 423 K, show C 1s photoemission spectra (upper panels) characterized by a single peak centered at 286.4 eV only due to the ethylene oxide carbon atoms. No evidence for hydrocarbon contamination was detected by XPS, suggesting that a higher grafting density was reached by thermally activating the process.

The analysis of the Si 2p core level photoemission spectra recorded before and after the functionalization of the H−Si surface by the MPEG films indicates that during the process a thin oxide layer is formed at the interface between the substrate and the monolayer. Indeed, whereas the Si 2p photoemission spectrum of an unmodified H−Si surface (Figure 2, bottom panel) shows a single component centered at 99.4 eV, corresponding to bulk silicon,[33,35] a new broad signal at 103.0 eV characteristic for

Si−O−C bonds appears in the Si 2p photoemission spectrum of MPEG monolayers (Figure 2, central panel). The Si 2p photoemission spectrum of a native silicon oxide layer on silicon is also shown in Figure 2, top panel. The peak due to oxidized silicon atoms shows up at 103.3 eV.

Film Morphology. Atomic force microscopy (AFM) images were recorded in order to get information on the homogeneity of the functionalized surfaces, since ungrafted patches in the layers would be extremely detrimental to antifouling properties. In addition, blobs of gels (3D cross-linked silanes) would also be deleterious, since these blobs could expose SiO_x groups at their outer surface, which would again favor protein adsorption. The topographical images of $Cl_3SiMPEO$ films prepared by liquid-phase silanization (Figure 3a,b) or by gas-phase silanization (Figure 3c) indicate that both experimental procedures lead to homogeneous monolayers with surface roughness in the sub-nanometer range. We did not observe the appearance of islands of grafted molecules, even for short grafting times, suggesting that homogeneous random grafting of the molecules occurs both in the liquid and gas phases. The root-mean-square roughnesses measured on $Cl_3SMiPEO$ surfaces prepared by liquid- and gas-phase silanization are 0.28−0.31 and 0.10−0.30 nm, respectively. These values are equivalent to the root-mean-square roughness measured for an unmodified HO−SiO_x surface, corroborating the high uniformity of the modified surfaces. A small number of clusters appear from time to time in the images, especially for the liquid-phase process, due to limited polymerization of the silane during grafting.

The topographical images of MPEG films obtained by thermally activated grafting onto the H−Si(100) surfaces are presented in Figure 4. AFM images of MPEG films prepared at 353 K (Figure 4a−c) reveal the high homogeneity and flatness of the monolayers and show that no aggregates are present on the surface. Although films prepared at 423 K (Figure 4d) also show good uniformity, some MPEG clusters, of 6 nm height and 25 nm diameter, appear on the surface. The root-mean-square roughness for the MPEG films obtained at 353K (0.1 nm) is equivalent to the root-mean-square roughness characteristic of a hydrogen-passivated Si(100) substrate, reflecting the high quality of the monolayers obtained by activation at 353 K. The root-mean-square roughness of the MPEG films obtained at 423 K is ∼0.32 nm, mirroring the presence of few aggregates onto the surface.
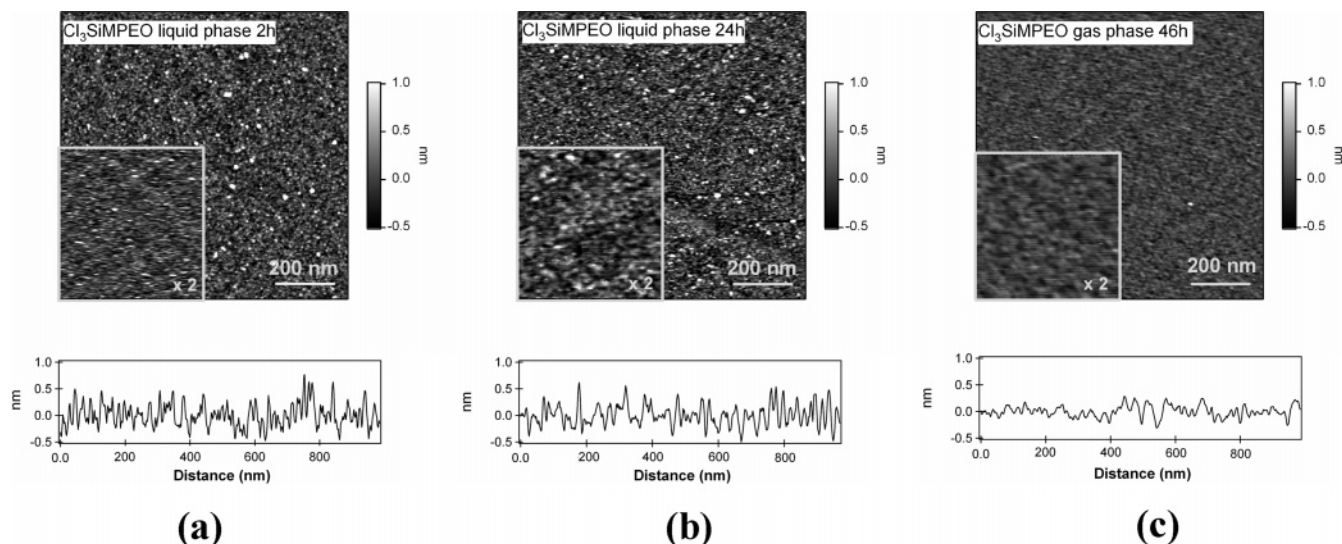

(a)

(b)

(c)

Figure 3. AFM topographical images (100 × 100 nm²) and corresponding section profiles of $Cl_3SiMPEO$ films prepared (a, b) in liquid phase after 2 and 24 h of reaction, and (c) in gas phase after 46 h of reaction.
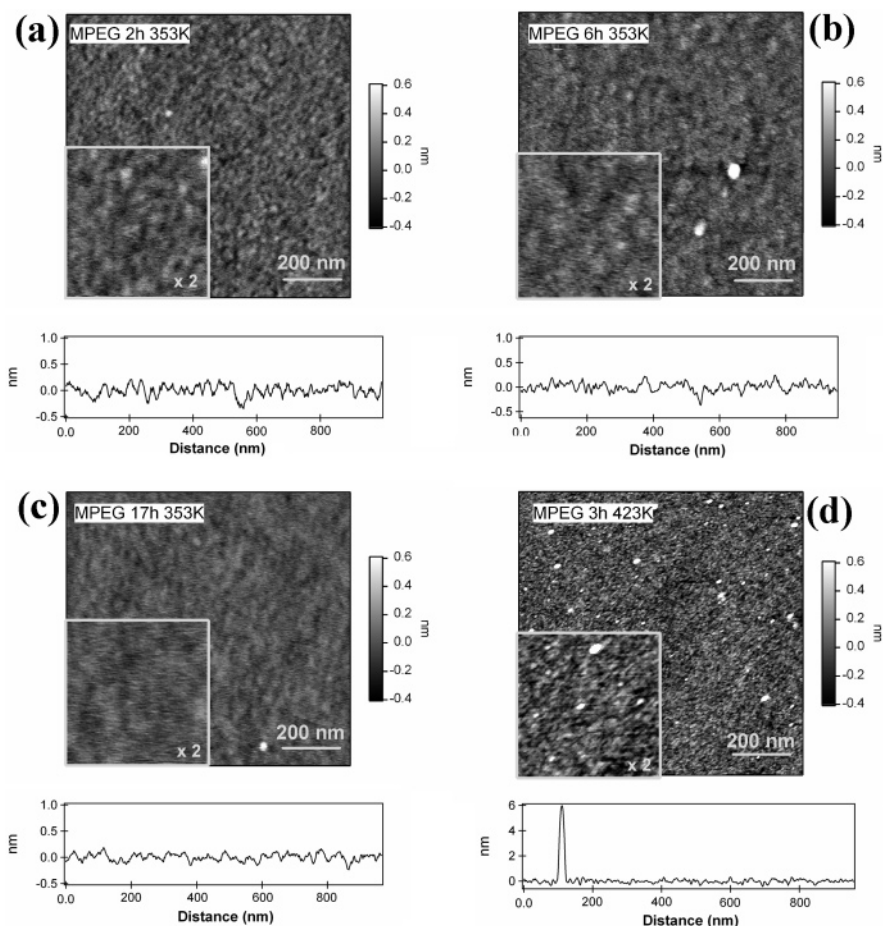
Figure 4. AFM topographical images (100×100 nm²) and corresponding section profiles of MPEG prepared in liquid phase at (a−c) 353 K after 2, 6, and 17 h, and (d) at 423 K after 3 h of reaction.

Film Thickness, Density, and Structure. To get further insight on the structure of the prepared surfaces, we investigated the films by X-ray reflectometry (XRR). This technique provides accurate information on the thickness and on the electron density profile of the monolayers, and therefore may lead to an estimation for the grafting density. Figure 5 shows the X-ray reflectograms (Figure 5a) and the related electron density profiles (Figure 5b) for the Cl₃SiMPEO films obtained either by liquid-phase or gas-phase silanization. Film parameters obtained from the XRR study (film thickness, electron density, unit surface area per grafted molecule, and estimated grafting density) are summarized in Table 1. The comparison of these experimental data with the theoretical values expected for a dense and well-ordered monolayer demonstrates the quality of the films and provides details on the structure of the monolayers. Crystalline poly-(ethylene oxide) (PEO) in its helical conformation (its more stable conformation) has an electron density of 400 e/nm³; the average length of each ethylene oxide unit is 0.27 nm when projected along the helical chain axis.[38] If we consider the crystalline structure of pure PEO as the reference for an ideal, ordered, and well-packed monolayer of Cl₃SiMPEO of 100% grafting density, the comparison with the experimental structural data of Table 1 suggests that our Cl₃SiMPEO films are amorphous, as indicated by their lower electron density values. Accordingly, the molecules occupy a larger surface area than in the crystalline form, and the film thickness is lower than the theoretical length of the molecule in its crystalline conformation. In addition, the evolution of the structural characteristics of the films with reaction time indicates that a significant structural change occurs between 2 and 4 h of reaction in the liquid phase. For longer reaction

times (up to 24 h), no significant changes of the structural properties of the monolayers are observed. The grafting densities, estimated from the ratio between the unit surface area per molecule of PEO in its crystalline structure and the experimental unit surface area per molecule computed from the XRR data, range from 30% after 2 h of functionalization up to 40% for longer functionalization times. Concerning the monolayers prepared in the gas phase, the highest grafting density achieved is 35% after 46 h of reaction, while the thickness of these monolayers is the lowest measured. This can be explained by the fact that, in a solution containing Cl₃SiMPEO molecules with different PEO chain length ($n = 6-9$), the shorter molecules have a lower vapor pressure than the longer ones. Therefore, films prepared in the gas phase will be mainly composed of the shorter Cl₃-SiMPEO molecules.

Films of MPEG self-assembled on H-Si(100) surfaces were also investigated by XRR. Figure 6 shows the X-ray reflectograms and the related electron density profiles of MPEG films prepared at 353 K (Figure 6a,b, respectively) and MPEG films prepared at 423 K (Figure 6c,d, respectively). Each series of data is compared to XRR data collected on a MPEG film obtained at room temperature after 5 h of reaction. Film characteristics extracted from the above curves are summarized in Table 2. The structural data extracted from the analysis of the X-ray reflectograms are compared to the data of the crystalline poly(ethylene oxide) (PEO) ($n = 7$) in its helical conformation. For films prepared at 353 K, we observed a progressive increase of the thickness and of the grafting density with reaction time, and a decrease of the corresponding surface area per grafted molecule, while the mean electron density of the monolayers remains almost
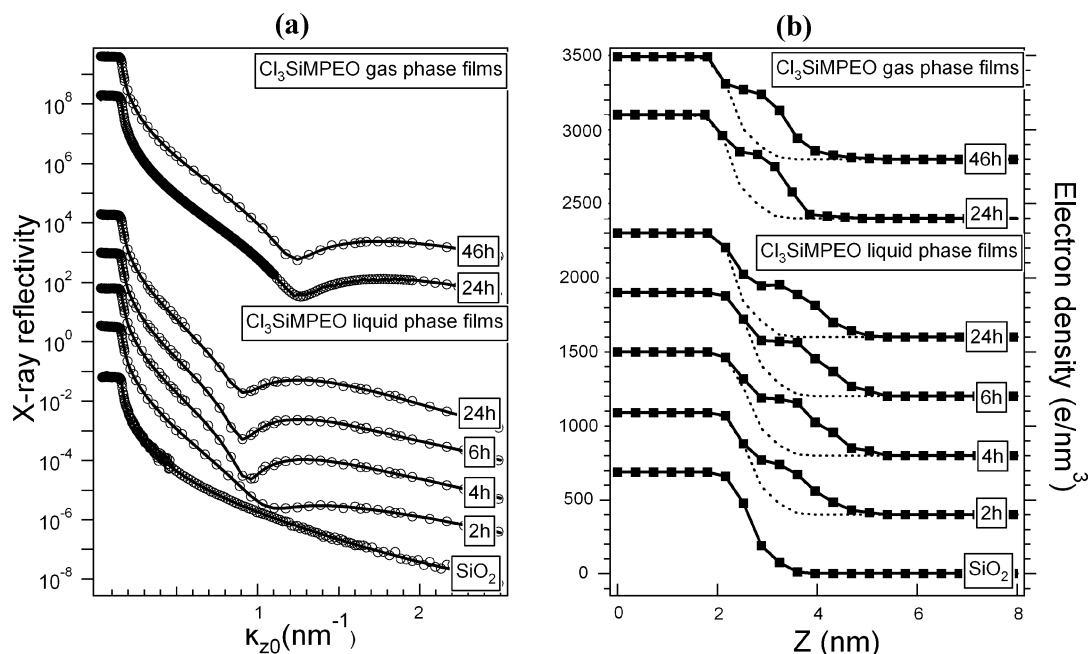
Figure 5. (a) X-ray reflectograms of $Cl_3SiMPEO$ films obtained after different reaction times, in liquid phase at room temperature (lower part) or in gas phase at 423 K (upper part). The bottom reflectogram was recorded on a bare silicon wafer. Circles are experimental data, lines are fits using the electron density profiles shown on the right part. (b) Corresponding electron density profiles. Squares correspond to computed densities, and continuous lines were obtained by cubic spline interpolation. The dotted lines are the electron density profile of the bare silicon wafer.

Table 1. Parameters Obtained from the Fitting of the XRR Data of $Cl_3SiMPEO$ Films and from the Comparison with the Theoretical Values of a PEO Chain Either in Its Crystalline or Amorphous Phase[a]

	film thickness, d^b (nm)	film electron density[c] (e/ nm^3)	area per grafted molecule[d] (nm^2)	grafting density[d] (chains/nm^2)	grafting density[e] (%)
$Cl_3SiMPEO$ $n = 6-9$					
in helical conformation	$2.1-2.9^f$				
crystalline PEO ($n = 6-9$)	$1.6-2.4^f$	400^f	0.21 (unit cell)^f		
amorphous PEO		360^f			
2 h liq	1.3	270	0.72	1.4	30
4 h liq	1.6	310	0.51	2.0	42
6 h liq	1.7	280	0.54	1.9	40
24 h liq	1.7	290	0.51	2.0	42
24 h gas	1.2	270	0.65	1.5	33
46 h gas	1.2	290	0.61	1.7	35

[a] The error on the thickness has been estimated to be ± 0.03 nm, and the error on the film electron densities has been estimated to be $\pm 10\%$. [b] Average thickness obtained from the first minimum in the reflectivity curve. [c] Average electron density obtained from the electron density profiles resulting from the fitting of the reflectivity data. [d] Computed from the two first columns of the table. [e] The grafting density is expressed relative to packing of chains in bulk crystalline PEO. [f] Theoretical values, from ref 38.

constant. Such a behavior suggests that conformational changes of PEO chains occur with the increase of the grafting density, PEO chains being progressively arranged with a standing-up orientation. A less marked trend is observed for the films prepared at 423 K for which a relatively high thickness is already reached after 1 h reaction time, and for which only a minor increase is observed with reaction time. In addition, for the latter films, the XRR data do not reveal a well-defined structural evolution, possibly because of the presence of few clusters into the films, as previously evidenced by the AFM investigation.

The comparison between the XRR data obtained for all the films grown at high temperature with those collected for the film prepared after 5 h of reaction at room temperature, which shows the lowest grafting density, clearly testifies for the activation role of temperature for the grafting process of hydroxyl-ended MPEG on the hydrogen-terminated silicon.

Antifouling Properties. The ability of all PEO grafted films to resist protein adsorption was investigated by XPS. Since nitrogen originates only from the adsorbed proteins, this was evaluated by monitoring the nitrogen-to-silicon ratio, computed from the photoemission peak areas measured after immersion of the different films in a P.69 antigen solution for 1 h. The nitrogen-to-silicon ratios for all PEO grafted films are summarized in Table 3. The N/Si ratios measured on the unmodified silicon surfaces (oxidized and hydrogen-passivated, respectively) are also reported as reference to establish the efficiency of the protein-repulsive layer. For an unmodified oxidized silicon surface, the N/Si ratio reaches 2.8%, while the N/Si ratio measured on an unmodified hydrogen-terminated silicon surface is close to 88%. The difference originates from the preferential interaction of the hydrophobic membrane protein with the hydrophopic hydrogen-passivated silicon surface. The dramatic decrease of the N/Si ratio on grafted PEO layers indicates that both $Cl_3SiMPEO$ films (Table 3, entries 1−6) and MPEG films (Table 3, entries 7−14) exhibit efficient antifouling properties. The amount of adsorbed proteins is progressively reduced to zero as the PEO monolayer grafting density increases. Indeed, the films showing the poorest antifouling properties are characterized by the lowest grafting density, and by a significant amount of hydrocarbon contamination, as evidenced by the analysis of the C 1s photoemission
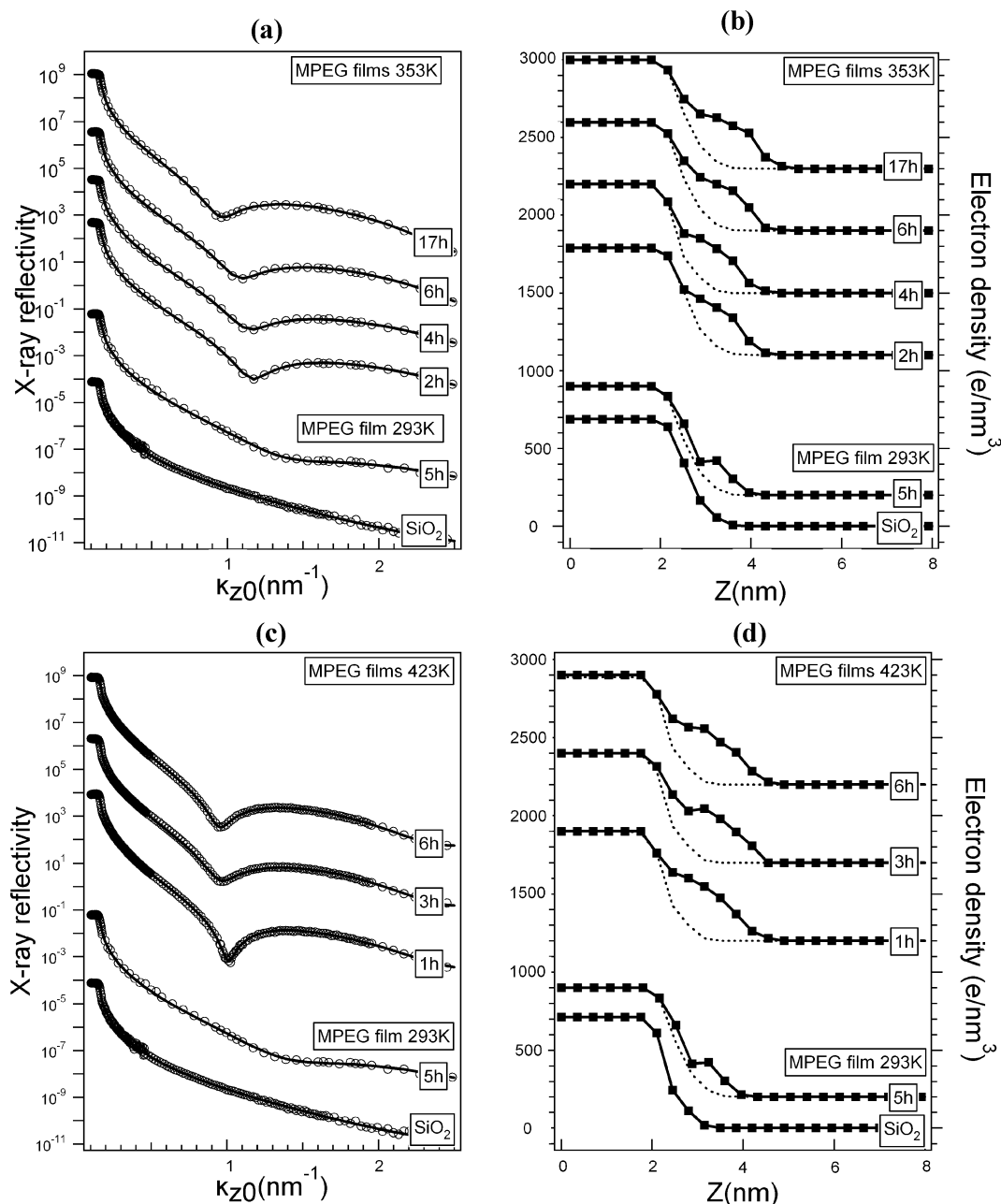
Figure 6. X-ray reflectograms of MPEG films obtained after different reaction times (a) in the liquid phase at 353 K, or (c) at 423 K. For each series, the two lowest reflectograms are those a bare silicon wafer and of a MPEG film prepared at RT. Circles are experimental data, lines are fits using the electron density profiles shown on the right part. (b, d) Corresponding electron density profiles. Squares correspond to computed densities, and continuous lines were obtained by cubic spline interpolation. The dotted lines are the electron density profile of the bare silicon wafer.

spectra (Figure 1). The higher adsorption observed on the incompletely grafted monolayer (sample 1 in Table 3) results either from adsorption on unprotected parts of the silicon wafer, or on patches of hydrophobic organic contaminants which are expected to favor protein adsorption. The N/Si ratio measured on the MPEG film obtained after 5 h of reaction at room temperature (Table 3, entry 7) is 11.9% which represents the poorest antifouling efficiency of this class of films, corresponding to a very low grafting density.

A qualitative evidence for the antifouling properties of the above layers is also given by the comparison of the AFM topographical images recorded for a silicon surface immersed in the P.69 antigen solution, before (Figure 7a) and after (Figure 7b) functionalization with a Cl$_3$SiMPEO layer. The topographical image recorded on the bare silicon indicates the presence of

randomly adsorbed proteins, with aggregates of different size and of dimensions reaching 6×10 nm^2, whereas the image recorded on the PEO-modified surface submitted to the same treatment exhibits a highly uniform topography with roughness in the sub-nanometer range, similar to the PEO films before immersion in the protein solution. Accordingly, the root-mean-square roughness measured on the unmodified surface, where proteins adsorb, is 0.9 nm, whereas for the PEO film it is 0.3 nm, corroborating the protein repellency of the PEO layer.
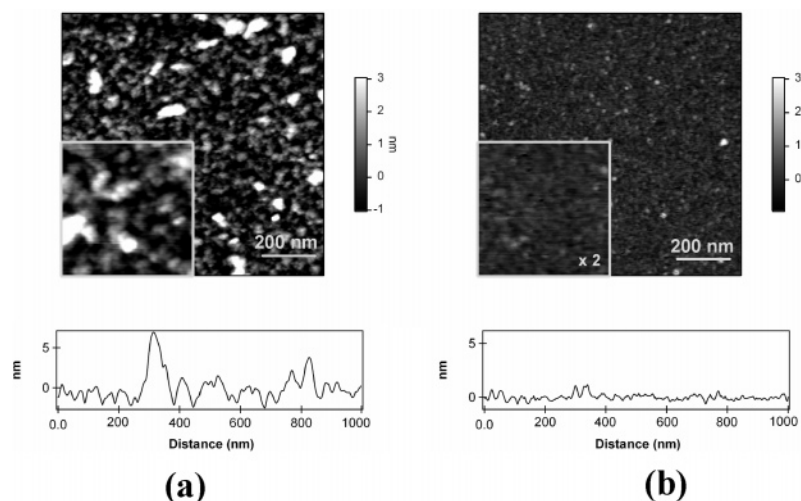
Stability of the Films. As Si−O−Si linkages are stable at neutral pH, Cl$_3$SiMPEO monolayers are not susceptible to degrade even for a long storage period in usual conditions.[39] Si−O−C linkages are, however, known to undergo hydrolysis in the

(39) Sharma, S.; Johnson, R. W.; Desai, T. A. *Langmuir* **2004**, *20*, 348.

Table 2. Parameters Obtained from the Fitting of the XRR Data of MPEG Films and from the Comparison with the Theoretical Values of a PEO Chain Either in Its Crystalline or Amorphous Phase[a]

	film thickness, d^b (nm)	film electron density[c] (e/ nm³)	area per grafted molecule[d] (nm²)	grafting density[d] (chains/nm²)	graftin g density[e] (%)
MPEG $n = 7$					
in helical conformation	2.2[f]				
crystalline PEO ($n = 7$)	1.9[f]	400[f]	0.21 (unit cell)[f]		
amorphous PEO		360[f]			
5 h 293 K	1.1	150	0.11	1.0	20
2 h 353 K	1.3	220	0.64	1.6	33
4 h 353 K	1.4	260	0.54	1.9	40
6 h 353 K	1.4	250	0.51	2.0	42
17 h 353 K	1.6	260	0.44	2.3	48
1 h 423 K	1.5	310	0.39	2.6	55
3 h 423 K	1.6	270	0.43	2.3	49
6 h 423 K	1.6	300	0.38	2.7	57

[a] The error on the thickness has been estimated to be ±0.03 nm, and the error on the film electron densities has been estimated to be ±10%. [b] Average thickness obtained from the first minimum in the reflectivity curve. [c] Average electron density obtained from the electron density profiles resulting from the fitting of the reflectivity data. [d] Computed from the two first columns of the table. [e] The grafting density is expressed relative to packing of chains in bulk crystalline PEO. [f] Theoretical values, from ref 38.

Figure 7. AFM topographical images (1×1 μm²) and corresponding section profiles of (a) a bare silicon wafer and (b) a Cl₃SiMPEO film (Table 3, entry 4), both immersed in the P.69 antigen solution for 1 h and then rinsed in water.

Table 3. Nitrogen to Silicon Ratios Measured from the Photoemission Peak Area on the Unmodified Silicon Substrates (Oxidized or Hydrogen-Passivated), on Cl₃SiMPEO Films Obtained in Liquid Phase or in Gas Phase after Different Reaction Times, and on MPEG Films Prepared at 353 K and 423 K and after Different Reaction Times[a]

entry	sample	film preparation conditions	% N/Si
a	OH-Si(100)		2.8
1	Cl₃SiMPEO	liquid phase 2 h of reaction	1.2
2	Cl₃SiMPEO	liquid phase 4 h of reaction	0
3	Cl₃SiMPEO	liquid phase 6 h of reaction	0
4	Cl₃SiMPEO	liquid phase 24 h of reaction	0
5	Cl₃SiMPEO	gas phase 24 h of reaction	0.8
6	Cl₃SiMPEO	gas phase 46 h of reaction	0
b	H-Si(100)		88.3
7	MPEG	5 h of reaction 293 K	11.9
8	MPEG	2 h of reaction 353 K	1.3
9	MPEG	4 h of reaction 353 K	0
10	MPEG	6 h of reaction 353 K	0
11	MPEG	24 h of reaction 353 K	0.9
12	MPEG	2 h of reaction 423 K	0
13	MPEG	4 h of reaction 423 K	0
14	MPEG	6 h of reaction 423 K	0

[a] All surfaces were immersed in a P.69 antigen solution for 1 h, followed by gentle rinsing in water before measurement.

Table 4. Nitrogen to Silicon Ratios Measured from the Photoemission Peak Area on MPEG Films Obtained at 423 K after Different Reaction Times[a]

sample	film preparation conditions	6 days in CH₂Cl₂ %N/Si	30 days in CH₂Cl₂ %N/Si	6 days in air %N/Si	6 days in PBS %N/Si
MPEG	2 h of reaction at 423 K	0	0.5	1.3	2.7
MPEG	4 h of reaction at 423 K	0	0	0.1	2.3
MPEG	6 h of reaction at 423 K	0	0	0.7	2.2

[a] Stored under different conditions: in a CH₂Cl₂ Soxhlet for 6 days and for 30 days, in air for 6 days and in a phosphate buffer solution (pH = 7.5) for 6 days, and then immersed in a P.69 antigen solution for 1 h.

presence of water molecules,[28,30] inducing film desorption and consequent degradation of their antifouling activity. We therefore carried out preliminary investigations in order to find the best storage conditions of MPEG monolayers allowing to avoid hydrolysis. The efficiency of the antifouling properties of MPEG films obtained at 423 K and stored under different conditions, in air, in a CH₂Cl₂ Soxhlet, or in a phosphate buffer solution, was checked. Table 4 presents the N/Si ratios of the different films, after 1 h of immersion in a P.69 protein solution. These tests demonstrate that the films stored in the CH₂Cl₂ Soxhlet for 6 or 30 days retain most of their antifouling activity, whereas films stored in buffer solution present a rather strong degradation and consequently a poor antifouling activity. The films stored in air present a slight degradation that could result from the environmental humidity.

Conclusions

In this article, we have compared different one-step strategies to build antifouling monolayers onto oxidized or hydrogen-terminated silicon surfaces. For most investigated surfaces, PEO layers of grafting density equal or higher than 1.7 chains/nm^2 appear to ensure proper protein repellency properties to the surface. This result will obviously be modulated depending on the nature of the protein used to probe repellency, but it provides a good starting point for a more systematic investigation targeted toward more diverse proteins.

Onto oxidized silicon surfaces, the silanization in liquid phase using short Cl$_3$SiMPEO molecules and gentle experimental conditions, such as low concentration solution and short reaction times, is an easy and efficient method to produce protein antifouling monolayers. Besides, when the use of solvents has to be avoided, silanization in the gas phase proved to be a new alternative one-step method for the creation PEO-silanes monolayers with successful antifouling properties.

Onto hydrogen-passivated silicon surfaces, the thermally activated self-assembly of hydroxyl-ended MPEG molecules appears to be an efficient new method to grow biocompatible monolayers, and it has the advantage of being easy, quick, and efficient. We investigated the stability of the antifouling properties of such MPEG films and found that their storage in a CH$_2$Cl$_2$ Soxhlet maintains their antifouling properties for at least 30 days.