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Functionalization of self-assembled monolayers on glass and oxidized silicon wafers by surface reactions

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ABSTRACT: Surface reactions were employed to introduce a variety of functional groups in self-assembled monolayers (SAMs) on hydroxylated surfaces such as glass and oxidized silicon wafers. The resulting layers were fully characterized by wettability studies, ellipsometry, Brewster angle infrared and x-ray photoelectron spectroscopy, UV–visible absorption and fluorescence measurements. Based on these measurements, it was concluded that the steric hindrance of the monolayer hampered the surface reactions, which generally led to sub-monolayer coverages. Detailed information concerning the interactions amongst adsorbates was obtained from fluorescence measurements. The characteristic excimer emission of pyrene-functionalized layers showed a large dependence on the solvent that was in contact with the SAM. Furthermore, efficient energy transfer could be observed in mixed monolayers that contained both fluorescein and lissamine groups. Copyright © 2001 John Wiley & Sons, Ltd.

KEYWORDS: self-assembled monolayers; surface modification; silicon oxide modification; glass modification

INTRODUCTION

The use of trichlorosilanes for the preparation of self-assembled monolayers (SAMs) on hydroxyl-terminated surfaces (e.g. glass and oxidized silicon wafers) was first reported by Sagiv in 1980.[1] Since then, the growth and structure of simple *n*-alkyltrichlorosilane SAMs have been investigated extensively, and have been reviewed by Ulman.[2] Similar to the monolayers on gold, an increasing effort is directed towards the potential application of trichlorosilane SAMs. One of the main interests is the formation of multilayers and their use in non-linear optical devices.[3] Other fields of interest are the use of SAMs on glass for biological and chemical sensing.[4] However, the high reactivity of trichloro- and trialkoxysilanes strongly limits the number of functional groups that can directly be introduced into SAMs on glass. As a consequence, the chemical diversity of these monolayers has been limited, especially when compared with SAMs on gold. To widen the range of functionalities, several groups have studied the completeness of surface reactions.[5] In most of these cases the surface reactions were carried out with small reactants, allowing almost complete functionalization of the primary formed monolayer. Also for the attachment of larger molecules, the reactivity of SAMs with terminal amine groups has frequently been used.[6] However, the yield of these surface reactions and the structure of the resulting layers have been given very little attention.

Recently, we have shown that the surface reaction of 3-aminopropyltriethoxysilane SAMs with dansyl chloride results in a sub-monolayer coverage of dansyl groups. We have found that fluorescence spectroscopy and UV–visible absorption measurements were very sensitive methods to analyze the monolayer monitor changes of the relatively open structure of the layer in the screening of suitable host molecules.[7]

In this paper, the use of a two-step procedure for the introduction of functional groups in self-assembled monolayers on hydroxylated surfaces is described (Scheme **1**). After the chemisorption of 3-aminopropyltriethoxysilane SAMs, the terminal amino groups were reacted with a variety of reagents to introduce the desired functionality. The yields of these surface reactions and the structure of the layers were studied by contact angle measurements, ellipsometry, Brewster angle IR and x-ray photoelectron spectroscopy (XPS), UV–visible absorption and fluorescence measurements. Furthermore, the

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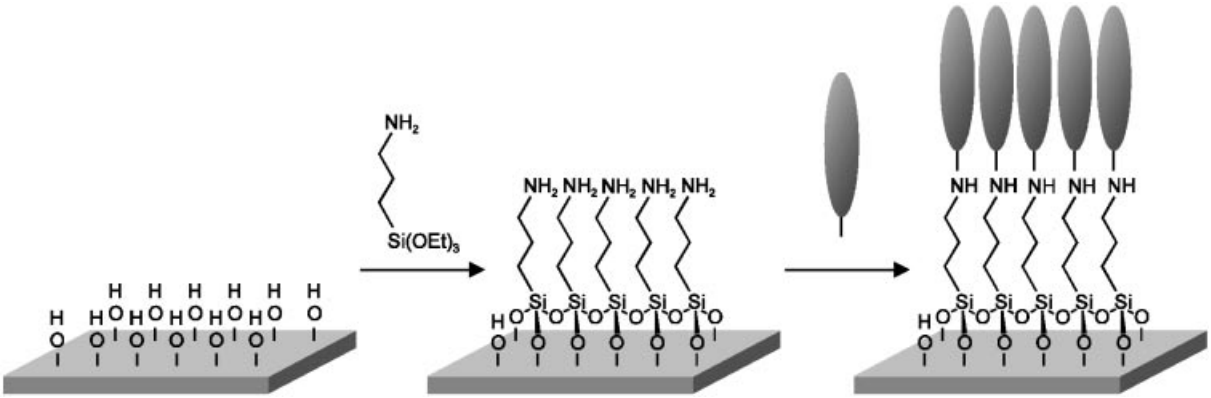


Scheme 1. Schematic representation of the preparation of functionalized SAMs by surface reactions

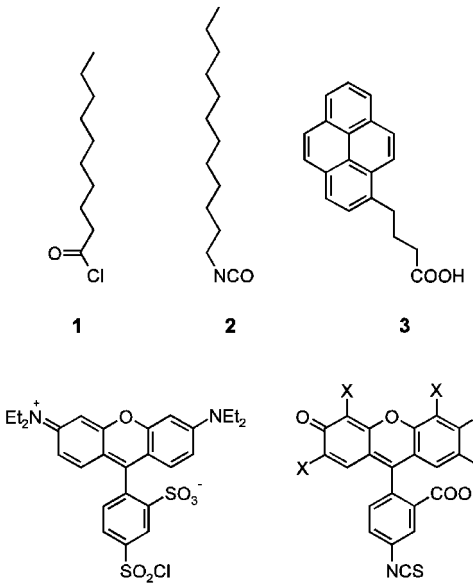
influence of interactions among the functional groups on the fluorescent properties was studied for pyrene-functionalized monolayers and for mixed monolayers of fluorescein and lissamine.

EXPERIMENTAL

Materials. All chemicals used for the preparation of SAMs and their modification by surface reactions were obtained from Aldrich or Molecular Probes. Toluene was freshly distilled from sodium and dichloromethane from K_2CO_3. For the preparation of SAMs of trichloro- and triethoxysilanes, different substrates were used: quartz slides (Hellma Quartz Suprasil) for their characterization by UV–visible absorption and fluorescence spectroscopy; n-type silicon wafers (both sides polished, Wacker) for Brewster angle infrared spectroscopy; and n-type silicon wafers (one side polished, Wacker) for wettability studies, ellipsometry and XPS. Prior to the monolayer deposition, the substrates were cleaned for 1 h in boiling 'piraña' (solution of 1:4 30% H_2O_2 and concentrated H_2SO_4), rinsed several times with high-purity water and dried in a stream of nitrogen. **Caution**: 'piraña' is a very strong oxidant and reacts violently with many organic materials.

Monolayer preparation. All glassware used to prepare monolayers was cleaned in boiling 'piraña' and rinsed several times with high-purity water. Formation of the self-assembled monolayers was achieved in a glove-box under an atmosphere of dry nitrogen. The freshly cleaned substrate was immersed in a 10 mM solution of the adsorbate in dry toluene for 4 h. After the substrate had been removed from the solution, it was rinsed with toluene (twice), dichloromethane (twice), and ethanol (twice) to remove any physisorbed material.

Surface reactions. SAMs of 3-aminopropyltriethoxysilane (APTES) were modified by surface reaction with reactants **1–6**. All surface reactions were performed in a glove-box under an atmosphere of dry nitrogen.



1 **2** **3**

4

5: X = H

6: X = Br

A substrate covered with a SAM of APTES was placed in a solution of 0.1 ml of triethylamine and 10–20 mg of one of the reactants [decanoyl chloride (**1**), dodecyl isocyanate (**2**), lissamine (**4**), fluorescein-5-isothiocyanate (**5**) or eosine-5-isothiocyanate (**6**)] in 20 ml of acetonitrile for 16 h (it was found that the final surface coverage of the surface reacted APTES monolayer after a 16 h reaction time did not critically depend on the amounts of reactant used during the modification). After the substrate had been removed from the solution it was rinsed with acetonitrile (twice), ethanol (twice) and dichloromethane (twice) and dried in a stream of nitrogen.

A substrate covered with a SAM of APTES was placed in a solution of 30 mg of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC), 30 mg of 4-(dimethylamino)pyridine and 20 mg of 1-pyrenebutyric acid (**3**) in 20 ml of DMSO for 16 h. After the substrate had been removed from the solution it was rinsed with DMSO (twice), ethanol (twice) and dichloromethane (twice) and dried in a stream of nitrogen.

Instrumentation. For XPS, a VG Escalab 220i-XL instrument with a monochromatic Al Kα x-ray source was used. Ellipsometric layer thickness measurements were performed on a Plasmos Ellipsometer ($\lambda = 633$ nm) assuming a refractive index of 1.46 for the monolayer and the underlaying oxide. The thickness of the silicon oxide layer was measured separately on an unmodified part of the same wafer and subtracted from the total layer thickness determined for the monolayer-covered silicon substrate. Wettability studies were carried out on a Krüss G10 contact angle measuring instrument, equipped with a charge coupled device (CCD) camera. Advancing and receding contact angles were determined automatically during growth and shrinkage of the droplet by the drop shape analysis routine. Brewster angle infrared spectra were obtained with a Bio-Rad FTS 60 A spectrophotometer at an angle of incidence of 73.7°. For each spectrum 512 scans were recorded with 2 cm^{-1} resolution. A background spectrum was recorded using a freshly cleaned silicon substrate. UV–visible absorption measurements were performed on a Hewlett-Packard 8452A diode-array spectrophotometer. The quartz slides were coated with a SAM on each side of the substrate, so that the presented absorption spectra are due to two monolayers. An unmodified quartz slide was used as a blank. Fluorescence emission and excitation spectra were measured with an SLM Instruments SPF-500C spectrofluorimeter, using excitation and emission bandwidths of 7.5 nm. The monolayer-covered quartz substrates were placed in a quartz cuvette, which allowed the recording of fluorescence spectra with the monolayers exposed to solvents. The emitted light was detected at an angle of 90° with respect to the excitation beam (see Fig. 1).

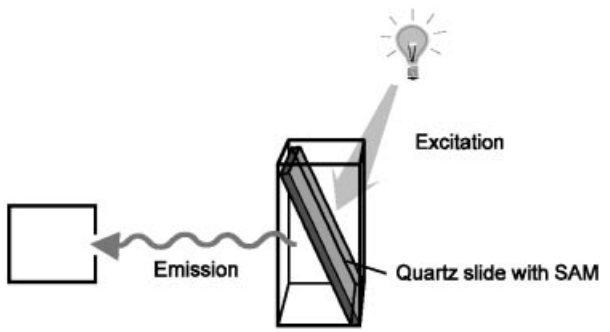


Figure 1. Schematic representation of the fluorescence measurement set-up

RESULTS AND DISCUSSION

SAMs of APTES and dodecyltrichlorosilane ($C_{12}H_{25}SiCl_3$) were prepared by exposing glass substrates or oxidized silicon wafers for 4 h to a 10 mM solution of the adsorbate in dry toluene. The APTES monolayers were further functionalized exploiting the reactivity of primary amines in subsequent surface reactions with isothiocyanates, isocyanates, sulfonyl chlorides and activated carboxylic acids. Although in solution these reactions go to completion, it is known that the immobilization of a reactant in a SAM inhibits its reactivity.[8] Here, different compounds (**1–6**) were used for the reaction with APTES monolayers.

A first indication of the quality of the formed layers can be obtained from wettability studies and ellipsometric thickness measurements (see Table 1). SAMs of $C_{12}H_{25}SiCl_3$ and APTES had thicknesses that were close to the length of the adsorbates in an all-*trans* conformation. In agreement with a dense packing of the adsorbate molecules, the dodecyl SAM had advancing and receding contact angles of 110° and 100°, respectively. This combination of high hydrophobicity and a very small hysteresis is only observed for highly ordered alkyl-terminated monolayers.[2a] The amino-terminated APTES monolayer had a significantly lower hydrophobicity, as was expressed by the advancing contact angle of 67° (it was recently shown that impurities from air adsorb quickly on amino-terminated monolayers, which leads to an increase in the advancing contact angle from 35° directly after preparation to 70° after several days in air[9]). Moreover, the weak van der Waals interactions between the short propyl chains of the adsorbates were not sufficient to induce a highly ordered packing. Consequently, a large hysteresis of 35° between the advancing and receding contact angle was found.

The surface reaction of APTES monolayers with reactants **1–6** generally led to a significant increase in the ellipsometric thickness of the organic film (Table 1). However, in most cases the observed layer thickness was less than the length of the adsorbate in the all-*trans* conformation, indicating sub-monolayer coverages due to the incomplete modification of the amino-terminated SAM. Only the surface reactions with fluorescein- and eosin-5-isothiocyanate (**5** and **6**, respectively) resulted in layer thicknesses that were in accordance with full monolayer coverage. Wettability studies of the surface-reacted SAMs showed that the introduction of bulky substituents (such as compounds **3–6**) prevents an ordered packing of the adsorbates as expressed in the large hysteresis. Linear alkyl substituents (**1** and **2**) allow a better stacking of the adsorbates, which results in a relatively small hysteresis (<20°). However, to accomplish stacking of the alkyl chains, the sub-monolayer coverage requires a relatively large tilt angle, resulting in a smaller layer thickness.

Brewster angle infrared spectroscopy showed absorp-

Table 1. Wettability and ellipsometric thickness of self-assembled monolayers on oxidized silicon wafers

Monolayer	Contact angle (°)[a]		Thickness (Å)[b]	Adsorbate length (Å) (±0.5 Å)[c]
	Adv. ±1°	Rec. ±1°		
$C_{12}H_{25}SiCl_3$	110	100	15.8 ± 0.5	18
APTES	67	32	6.7 ± 0.5	7.5
Reaction of APTES SAM with:				
$C_9H_{19}COCl$ (**1**)	95	82	14 ± 1	21
$C_{12}H_{25}NCO$ (**2**)	95	75	13 ± 1	24.5
1-Pyrenebutyric acid (**3**)[d]	76	40	17 ± 1	21
Lissamine (**4**)	52	23	14 ± 1	21
Fluorescein-5-isothiocyanate (**5**)	69	<15	20 ± 1	19
Eosin-5-isothiocyanate (**6**)	70	<15	21 ± 1	20.5

[a] Advancing and receding contact angles determined with water as the probing liquid.
[b] Layer thickness determined with ellipsometry.
[c] Length of the adsorbate in the most extended conformation (including APTES for surface-reacted SAMs) based on CPK model.
[d] 1-Pyrenebutyric acid was reacted with the APTES monolayer via EDC coupling.

tions corresponding to the stretching modes of the methyl and methylene groups for SAMs of dodecyltrichlorosilane, and for APTES monolayers after surface reaction with $C_9H_{19}COCl$ (**1**) and $C_{12}H_{25}NCO$ (**2**), respectively (see Fig. 2). The methylene stretching modes, $\nu_a(CH_2)$ and $\nu_s(CH_2)$, are sensitive to the degree of order in SAMs, since both absorptions shift to lower wavenumbers with increasing crystallinity.[10] For SAMs of $C_{12}H_{25}SiCl_3$ the symmetric and asymmetric methylene stretching vibrations were found at 2853 and 2923 cm^{-1}, respectively. A more liquid-like packing of the surface-reacted APTES monolayers with compounds **1** and **2** shifted both methylene absorptions to 2856 and 2928 cm^{-1}. In accordance with the ellipsometric measurements, the CH absorptions of the $C_{12}H_{25}SiCl_3$ SAMs were roughly twice as intense as those of the surface-reacted APTES SAMs. Unfortunately, the intensities of the amine and the carbonyl absorptions were not sufficient to allow an unambiguous identification of these groups.

XPS measurements of monolayers of $C_{12}H_{25}SiCl_3$, APTES and its functionalized layers (APTES reacted with **1, 3, 4, 5** and **6**) showed the presence of Si, O, C, N and for **6** also Br (see Fig. 3). The presence of sulfur could not be confirmed owing the small amounts present in the monolayers of **4–6**, in addition to its low atomic sensitivity factor (since the S_{2p} signal overlapped with signals from the silicon wafer, the even weaker S_{2s} signal had to be used for the detection of sulfur; atomic sensitivity factors were obtained from Ref. 11). Since also for freshly cleaned silicon wafers signals for Si, O and traces of C were detected, it was impossible to determine the exact elemental composition of the SAMs. However, from the relative intensities of the bromine and nitrogen signals we were able to estimate that 10–15% of the propylamines were modified by the surface reaction with eosin-5-isothiocyanate (**6**). Combined with an area of 20 $Å^2$ per amino group, as was determined by Durfor *et*

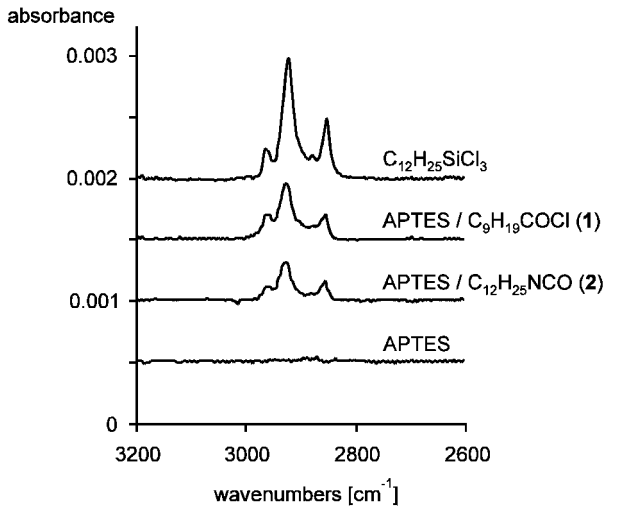


Figure 2. Brewster angle infrared spectra of SAMs on oxidized silicon wafers

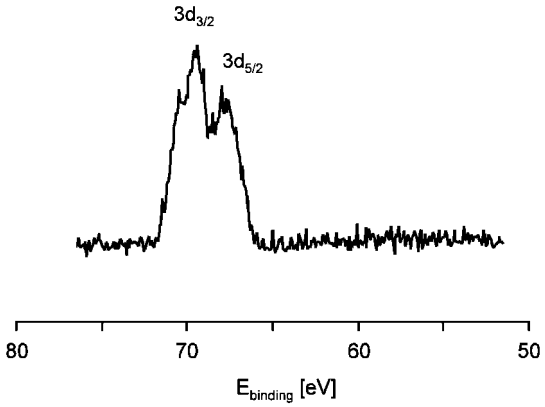


Figure 3. XPS Br_{3d} spectrum for an APTES monolayer after reaction with eosin-5-isothiocyanate (**6**)

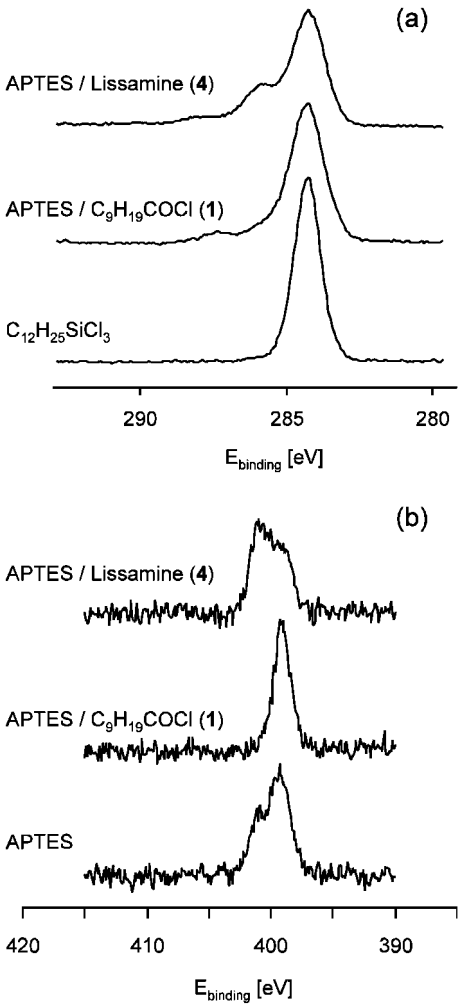


Figure 4. (a) XPS C_{1s} spectra of SAMs on oxidized silicon wafers. (b) N_{1s} spectra of SAMs on oxidized silicon wafers

al.[12a] [one referee correctly remarked that compared with the procedure reported in Ref. 12a, no curing step was performed; according to the thickness (Table 1), this did not affect the surface coverage] a surface density of one eosine group per 166 ± 33 Å^2 was calculated.

Despite the presence of traces of carbon in the silicon substrate, all monolayer-coated substrates showed a significant increase of the C_{1s} signal. A useful feature in the XPS C_{1s} spectra is the presence of up to three different peaks [see Fig. 4(a)]. This splitting of the carbon signal is caused by the different oxidation states of the carbon atoms, which is reflected in their binding energies.[13] Consequently, for monolayers of $C_{12}H_{25}SiCl_3$ only one C_{1s} signal was observed at 284.5 eV, whereas the surface-reacted monolayer of APTES with $C_9H_{19}COCl$ showed three signals (C— alkyl 284.5 eV, C—N 286 eV, N—C═O 287.8 eV). Also the introduction of the other functional groups (**2–6**) resulted in a broad C_{1s} signal with its maximum at 284.5 eV and shoulders at 286 and 288 eV, corresponding to the different oxidation states of the carbon atoms.

Similarly to the carbon signal, the XPS N_{1s} signal of the APTES monolayer is split into two peaks. Here two signals can be distinguished at 399 and 401 eV that are attributed to the presence of uncharged and positively charged nitrogens, respectively.[11] Similar observations have been made by Bierbaum *et al.*,[14] who observed by XPS the partial protonation of 3-aminopropyl- and 17-aminoheptadecyltrimethoxysilane SAMs.

The relative amount of protonated nitrogens was found to vary strongly with the nature of the functional group that was introduced by the surface reaction [see Fig. 4(b)]. For the apolar substituents **1** and **3** the signal at 401 eV was virtually absent, so less than 5% of the nitrogens were protonated. More polar substituents, such as fluorescein (**5**) and eosin (**6**), showed very similar XPS N_{1s} signals to the unmodified APTES layer with 20–30% of the nitrogens protonated. This indicates that the protonation of unreacted amines is disfavored in an apolar environment compared with the more polar layers of fluorescein and eosin. An exceptionally high amount of positively charged nitrogen (60%) was found for the layers that were functionalized with lissamine (**4**). Two reasons account for this. One is that the lissamine has positively charged nitrogens and the other is that only a fraction of the amino groups had reacted with **4** (see below). The low reactivity might be due to the zwitterionic character of **4**.

UV–visible absorption spectroscopy is a very valuable technique for the characterization of functionalized SAMs.[12,15,16] The use of quartz substrates that are transparent down to 200 nm permits the detection of chromophoric groups in sub-monolayer quantities using standard diode-array spectrophotometers. In addition to its use for confirmation of the surface attachment of a chromophore by its characteristic absorption bands, UV–visible absorption spectroscopy can also be used in a more quantitative manner. The Beer–Lambert law relates the absorption (A) to the surface density of the chromophores (ρ): $A = \varepsilon\rho$.[12] The values for the molar absorption coefficients (ε) of the chromophores can be determined from solution experiments. Although the restricted rotational freedom of the molecules in well-packed monolayers might influence their absorption coefficient, we have experienced that the obtained surface densities of the relatively disordered surface-reacted SAMs are in good agreement with the results obtained from the other analytical techniques, such as ellipsometry, Brewster angle IR and XPS.

The UV–visible absorption spectra of the APTES monolayers after the reaction with chromophores **3–6**, clearly confirm the successful surface attachment of the chromophoric groups (see Figs 5 and 6). The monolayer absorption maxima were used to estimate the surface densities of the corresponding chromophores and are given in Table 2. Comparison of the determined surface densities with the molecular areas (the molecular areas of the chromophores were determined from CPK models

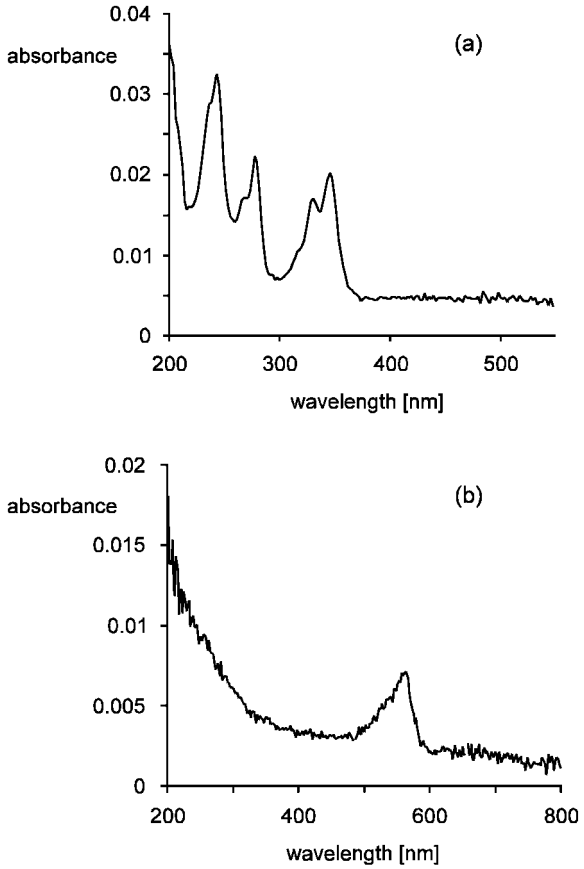


Figure 5. UV–visible absorption spectra of surface-reacted APTES monolayers with (a) 1-pyrenebutyric acid (**3**) and (b) lissamine (**4**). The absorption spectra were measured with a monolayer on both sides of a quartz slide

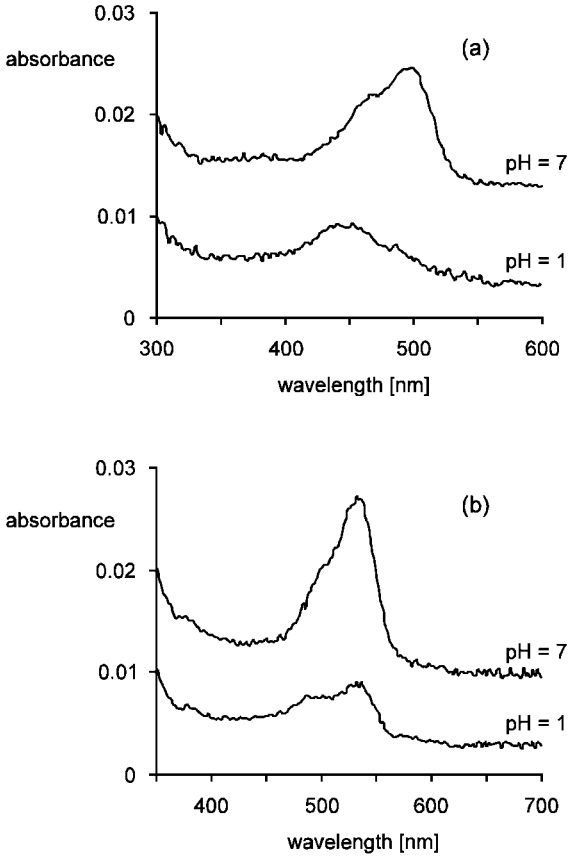


Figure 6. pH-Dependent UV–visible absorption spectra of surface-reacted APTES monolayers with (a) fluorescein-5-isothiocyanate (**5**) and (b) eosin-5-isothiocyanate (**6**). The absorption spectra were measured with a monolayer on both sides of a quartz slide

with the adsorbates in an all-*trans* conformation and oriented perpendicular to the glass surface) shows that the surface reaction with lissamine proceeded with exceptionally low conversions, which might be caused by repulsive interactions between the charged groups.

Surface reactions of the uncharged reactants **3, 5** and **6** with APTES monolayers resulted in much better surface coverages. However, based on the ellipsometric and XPS measurements, even higher densities of chromophoric groups would have been expected for the fluorescein **5** and the eosin **6** layers. The discrepancy between the UV–visible measurements and the ellipsometric and XPS measurements might be caused by the relatively high density of chromophores that forces the molecules into a preferred orientation perpendicular to the surface. The resulting alignment of the transition dipole with the surface normal results in a smaller value for ε.[15] Moreover, the pH dependence of the absorbance of fluorescein and eosin is maintained upon their immobilization on a glass surface (Fig. 6), which adds another variable to the exact value of the absorption coefficient.

Table 2. Surface coverages of functionalized SAMs on glass

Surface-reacted APTES SAM	ε ($l\,mol^{-1}\,cm^{-1}$)[a]	Coverage ($Å^2$ molecule^{-1})[b]	Molecular area ($Å^2$ molecule^{-1})[c]
1-Pyrenebutyric acid (**3**)	30000 (341 nm)	66 ± 4	40
Lissamine (**4**)	88000 (568 nm)	585 ± 117	150
Fluorescein-5-isothiocyanate (**5**)	73000 (494 nm)[d]	202 ± 17	115
Eosin-5-isothiocyanate (**6**)	95000 (521 nm)[d]	186 ± 11	130

[a] Molar absorption coefficients were obtained from Molecular Probes; the absorption maximum is given in parentheses.
[b] Coverages were determined from the UV–visible absorption spectra using the Beer–Lambert law.
[c] The molecular area was estimated from CPK models, for the most-extended conformation of the adsorbates, perpendicularly oriented to the surface.
[d] The absorption coefficient corresponds to the dianion of the chromophore, which is the predominant species at pH 7.

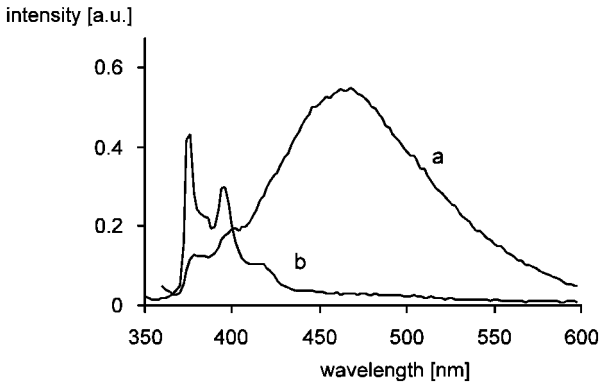


Figure 7. Emission spectra of a surface-reacted APTES monolayer with 1-pyrenebutyric acid (**3**) on glass (excitation at 340 nm). (a) Dry monolayer; (b) monolayer in contact with water

The extremely high intrinsic sensitivity of fluorescence spectroscopy (the use of near-field scanning optical microscopy allows the detection of single fluorescent molecules as was first shown by Betzig and Chichester[17]) makes it a valuable tool for the study of SAMs. Apart from the identification of fluorophores by their characteristic emission and excitation spectra, intermolecular interactions can influence their fluorescent properties, and contain information about the monolayer structure. Moreover, fluorescence spectroscopy can be used for the detection of host–guest interactions at monolayers.[4e,7,18]

Pyrene is a very prominent example of a fluorophore that exhibits changes of its fluorescence spectrum due to intermolecular interactions. With increasing concentrations the structured emission of pyrene around 390 nm is replaced by a very broad emission centered at 480 nm. This phenomenon has been attributed to the formation of an *exci*ted state di*mer* (excimer) that is formed by two pyrene molecules after the excitation of one of the pyrenes:[19]

$$\text{pyrene} + \text{pyrene}^* \rightarrow (\text{pyrene})_2^*$$

Also the pyrene-functionalized SAMs showed the characteristic excimer emission when the layers were not exposed to a solvent (see Fig. 7, spectrum a). Upon exposure of the SAM to any of the tested solvents, the excimer emission lost intensity and the structured emission below 400 nm increased. For most of the solvents tested (DMSO, methanol and dichloromethane) the excimer emission decreased to 10% of the initial value but was still clearly visible. Only in water was the excimer emission of the pyrene-functionalized monolayer completely absent (see Fig. 7, spectrum b).

These observations indicate that the pyrene groups are closely stacked when the monolayer is dry. To minimize the empty space in the monolayer, the adsorbate molecules are tilted with respect to the surface normal, which is reflected in the ellipsometric thickness. Solvation of the monolayer increases the distance between the pyrene groups and this prevents the formation of excimers. The especially effective prevention of excimer formation by water might be caused by the efficient breakage of hydrogen bonds of the amide network, which increases the rotational flexibility of the monolayer.

Although ellipsometry, XPS and UV–visible absorption measurements clearly proved the presence of eosin in the surface-reacted APTES monolayers with eosin-5-isothiocyanate (**6**), no fluorescence could be detected. The main reason for the undetected emission is probably the low quantum yield, known for eosin derivatives ($\Phi_{flu} = 0.2$).[20] Moreover, for many fluorophores concentration quenching has been observed, which results in a decrease of the fluorescence intensity with increasing concentration of fluorophores.[21] Nevertheless, for fluorescein- and lissamine-functionalized monolayers the expected emission bands were detected at 520 and 590 nm, respectively (see Fig. 8). The emission band of the fluorescein has a large overlap with the excitation band of lissamine. Therefore, it can be expected that in mixed monolayers of both fluorophores the excitation of fluorescein will lead to energy transfer from fluorescein to lissamine, which emits at 590 nm.

Mixed monolayers of lissamine and fluorescein were

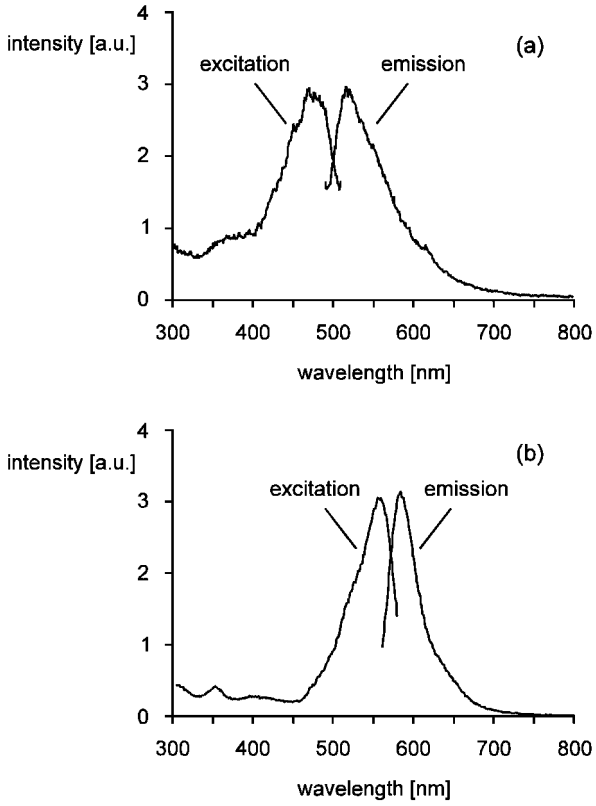


Figure 8. (a) Excitation (emission at 530 nm) and emission (excitation at 460 nm) spectrum of fluorescein-functionalized SAM. (b) Excitation (emission at 600 nm) and emission (excitation at 560 nm) spectrum of lissamine-functionalized SAM

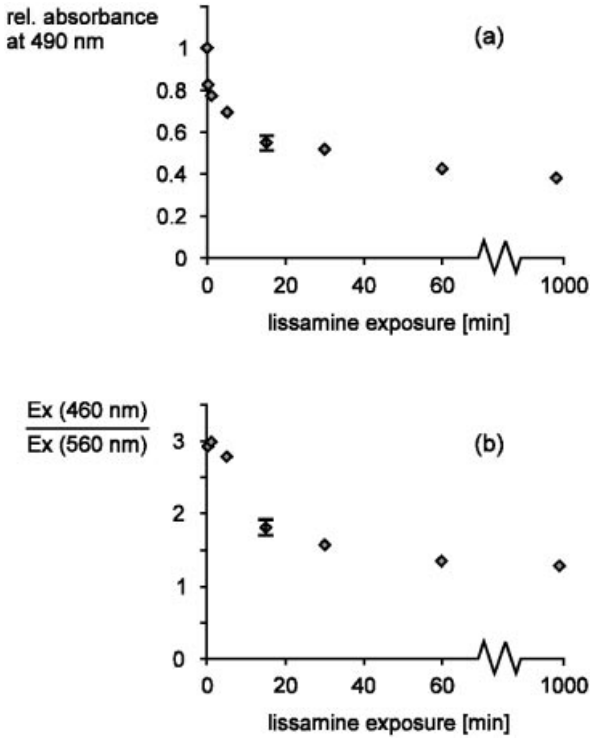


Figure 9. (a) Fluorescein absorbance at 490 nm of mixed monolayers of fluorescein **5** and lissamine **4** as a function of the surface reaction time of an APTES SAM with lissamine and subsequent modification of remaining amines with fluorescein-5-isothiocyanate. (b) Intensity of the lissamine emission via fluorescein excitation (Ex_{460}) divided by the intensity of the lissamine emission by direct excitation (Ex_{560}) as a function of the surface reaction time of an APTES SAM with lissamine and subsequent modification of remaining amines with fluorescein-5-isothiocyanate

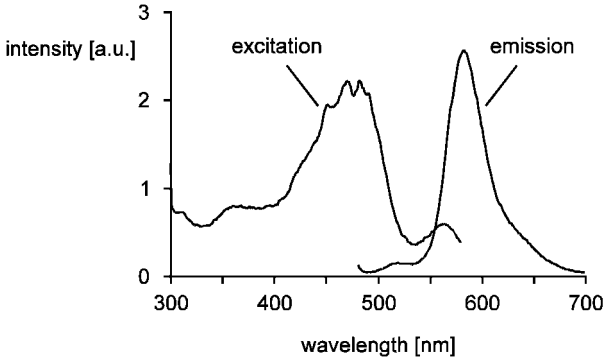


Figure 10. Excitation (emission at 600 nm) and emission (excitation at 460 nm) spectra of a mixed monolayer of fluorescein and lissamine. The mixed SAM was prepared by surface reaction of an APTES SAM with lissamine (**4**) for 10 s and subsequently for 16 h with fluorescein-5-isothiocyanate (**5**)

prepared by short exposures of APTES SAMs to a solution of lissamine (**4**) and triethylamine (varying from 10 s to 16 h), after which the partially reacted amino-terminated layer was exposed for 16 h to a solution of fluorescein-5-isothiocyanate (**5**) to complete the surface reaction. To estimate the relative amount of both fluorophores on the glass surface, UV absorption measurements were conducted. Since, especially at short exposure times, the lissamine absorbance at 560 nm was too low for an accurate measurement, the much higher fluorescein absorbance at 490 nm was used to monitor the surface reaction in time [see Fig. 9(a)]. From these measurements, it was evident that the relative amount of lissamine increased rapidly over the first 20 min of the surface reaction. Consequently, the completion of the surface reaction by fluorescein-5-isothiocyanate resulted in a decrease in the fluorescein absorbance.

The maximum loading of lissamine, which was obtained within 1 h, did not prevent the remaining amines from reacting with fluorescein-5-isothiocyanate. These observations are in accordance with incomplete surface coverages that were obtained for the surface reaction of lissamine **4** with APTES monolayers, as was found by ellipsometry and UV–visible absorption measurements. Furthermore, it shows that the surface reaction of lissamine with the APTES SAM is not terminated owing to the lack of reactive amino groups.

The fluorescent properties of the mixed monolayers clearly prove that energy transfer from fluorescein to lissamine occurs. The excitation of the fluorescein molecules at 460 nm led to a very weak fluorescein emission at 520 nm and a much stronger lissamine emission at 590 nm (see Fig. 10). Furthermore, direct excitation of the lissamine at 560 nm resulted in an emission at 590 nm that was less intense than the sensitized emission via the fluorescein. The relative heights of both excitation bands at 460 and 560 nm are given in Fig. 9(b) as a function of the surface reaction time. The fluorescence spectra show that increased surface concentrations of lissamine makes its sensitized emission via the fluorescein less intense. Simultaneously, the higher lissamine absorbance at 560 nm increases its emission intensity by direct excitation. These observations are in accordance with a strong distance dependence of energy transfer processes.[19]

CONCLUSIONS

SAMs of APTES on glass and oxidized silicon wafers can be used for the immobilization of a variety of functional groups via surface reactions. The well-known reactivity of amines has been exploited for the introduction of chromophoric and fluorophoric groups in SAMs. Extensive characterization of the resulting monolayers has shown that only part of the amino groups of the APTES SAM are able to undergo surface reactions. The incomplete conversion of the amino groups is attributed to the steric hindrance of the surface-reacted layer, which prevents the approach of reactants to the shielded amines. The conversion efficiency falls grossly into two cate-

gories: if the reactant is not bulky, the conversion is efficient; if the reactant is (very) bulky and/or charged, the conversion is inefficient.

Especially fluorescence spectroscopy has proven to be a valuable technique for the detection of interactions among the fluorophoric groups in the SAM. Moreover, fluorescence spectroscopy showed that the exposure of the pyrene modified layer to solvents affected the structure of the SAM. Consequently, interactions between fluorescent monolayers and the contacting solution can also be detected, which makes them attractive for sensing applications.

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