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Surface Assisted Intermolecular Interactions in Self-Assembled Coumarin Submonolayers

Alexey Krasnoslobodtsev and Sergei Smirnov*

Department of Chemistry and Biochemistry, New Mexico State University, Las Cruces, New Mexico 88003

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Spectroscopic characterization of chemically immobilized coumarin molecules on a silica surface at different concentrations and in a variety of solvents is presented. The characterization involves surface concentration and orientation of coumarin molecules with different linkers. Due to high surface concentration (up to 10^{14} cm^{-2}), the immobilized molecules exhibit intermolecular interactions, which compete with solvation. The molecular orientation with respect to the surface and intermolecular interaction varies with the solvent, the linker, and the surface concentration of coumarin, but as a first approximation the data can be simplified as nearly random molecule orientation with respect to the surface.

Introduction

Thin molecular films on solid substrates have generated substantial interest in recent years, particularly the self-assembled monolayer (SAM) films. Low cost of production for such films and the possibility of controlled molecular architecture with unique physical and chemical properties and desired chemical functionality open a variety of new applications for SAMs in such areas as microelectronics[1] and photosensitive devices such as OLED,[2] electrochromics,[3] photovoltaics, and biosensors.[4,5] Such immobilization is also desirable for studying electrical properties of single molecules by scanning probe microscopy in liquids as well as in a vacuum because it prevents molecular motion. Different substrates including metals, metal oxides, and silicon have been successfully utilized.[1,6,8] The chemistry of SAMs differs for each substrate.[9−15] It is believed in many cases that self-assembled monolayers[16] form close-packed and aligned molecular films. Intermolecular interactions in such thin films can alter film properties in either desired or undesired directions and thus need detailed investigation.

In this paper, we intend to (a) optimize a protocol of molecule immobilization on flat silica surface and (b) investigate orientation of immobilized molecules with respect to the surface as a function of molecule surface concentration, solvent, and a linker. The latter will be of particular importance in photoinduced transient displacement current[7] study of surface immobilized molecules. These findings should help us in future development of DNA immobilization and its photoconductivity characterization on the surface.

Charge migration along the base-stack in DNA has been a center of long-standing controversy.[17−19] We plan to apply the transient displacement current technique to investigate this phenomenon for oriented DNA hairpins, which should allow us to resolve the controversy unambiguously. Because of required modification for the technique to study surface oriented molecules, preliminary calibrations using molecular systems accessible in solutions as well as on the surface were chosen.

The choice of coumarin derivative (7-diethylaminocoumarin-3-carboxylic acid, succinimidyl ester) was motivated by unique properties of this molecule: derivatives of coumarin are known to have high extinction coefficients and fluorescence yield, and they are very sensitive to changes in molecular environment.[10,20] Most importantly in this case, they possess dipole moments in both ground and excited states necessary for calibration of the transient displacement current. Moreover, derivatives of coumarin suitable for chemical binding to surfaces (such as D-1412) are commercially available.

Experimental Section

Materials. High transparency of quartz down to 200 nm is very useful in applications where optical studies of molecules absorbing in the UV region (such as DNA) are at interest. For that reason, we want to develop a protocol of covalent molecule

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* To whom correspondence should be addressed. E-mail: snsm@nmsu.edu.

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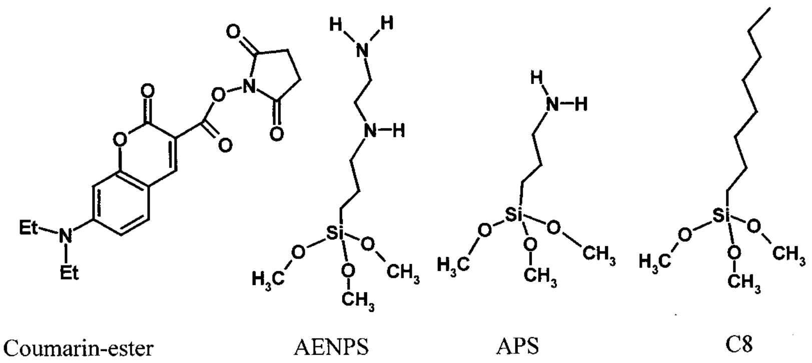
Chart 1. Structures of the Compounds Used in This Study and Their Abbreviations[a]



[a] 7-Diethylaminocoumarin-3-carboxylic acid succinimidyl ester (coumarin-ester), *N*-[3-(trimethoxysilyl)propyl]-ethylenediamine (AENPS), 3-amino-propyltrimethoxysilane (APS), and octyltrimethoxysilane (C8).

immobilization on quartz substrates. Polished 25 × 13 mm^2 quartz slides of 0.3 mm thickness, from Quartz International, were used as substrates. Scanning AFM (using a microscope from Molecular Imaging) of the quartz surface in air showed its fairly high quality: the roughness measured with a fresh tip was within ±4 Å, mainly as grooves after polishing, which have a characteristic period of 0.1 nm. "Coumarin-ester", 7-diethylaminocoumarin-3-carboxylic acid succinimidyl ester, was purchased from Molecular Probes (D-1412). Its carboxamide analogue (we will call it "coumarin"), which is produced as a result of covalent immobilization, for solution measurements was prepared by reacting the 7-diethylaminocoumarin-3-carboxylic acid succinimidyl ester with propylamine (see Scheme 1 later for details). Two aminosilanes, 3-amino-propyltrimethoxysilane (APS) and *N*-[3-(trimethoxysilyl)propyl]-ethylenediamine (AENPS), were purchased from Aldrich, and octyltrimethoxysilane (C_8) was purchased from Fluka Chemika. The structures of these compounds are shown in Chart 1. The solvents (hexane, toluene, acetone, ethanol, methylene chloride, DMSO, and acetonitrile) were of HPLC grade from Aldrich and were used without further purification.

Procedure. *(a) Preparation of Substrates.* Initial cleaning of slides was done in 0.1 N solution of NaOH for 30 min. During this step, covalently bound organic impurities were eliminated. In the next step, the cleaning was extended in MeOH/HCl (1:1) solution for 30 min with subsequent rinsing in a copious amount of deionized water. After that, the slides were heated in concentrated H_2SO_4 for 2 h and then rinsed in deionized water and boiled in it immediately prior to silanization. This preparation procedure has proved to be effective; the slides were numerously recycled without noticeable deterioration, as confirmed by reproducible absorption spectra.

(b) Silanization. Trimethoxysilanes spontaneously react with surface hydroxyl groups so that the self-assembled layer forms.[16] The silanization of slides was performed using a 2% v/v acetone solution of a trimethoxysilane. If either of the aminotrimethoxysilanes (APS or AENPS) is used by itself, the surface after silanization becomes covered with a maximum concentration of amino groups. To vary the amino group concentration, a mixture with (trimethoxy)octylsilane (C_8) in an appropriate ratio was used instead of pure aminosilane. In all cases, the slides were treated in the solution by complete immersion for ca. 3 min. Longer exposures were found sometimes to be accompanied by silane polymerization and resulted in a higher concentration of immobilized coumarin. The silanized slides were washed in acetone, dried at ca. 100 °C in a stove for 5 min, and cooled for approximately 5 min.

(c) Staining. The staining of the silanized slides by coumarin-ester was performed only on one side. For that purpose, a drop of 0.6 mM DMSO solution of coumarin-ester was placed between the slide and the surface of a glass beaker. Reproducible results for the amount of immobilized coumarin were achieved when 2 h was provided for completing this step. The stained slides were washed in ethanol and kept in this solvent prior to further use. No noticeable deterioration of the surface concentration of coumarin within several days after staining was observed. Nevertheless, it was found that after 1 month of storage in ethanol the absorption declined by a factor of 6. The results discussed below are given for the measurements performed within the initial 48 h.

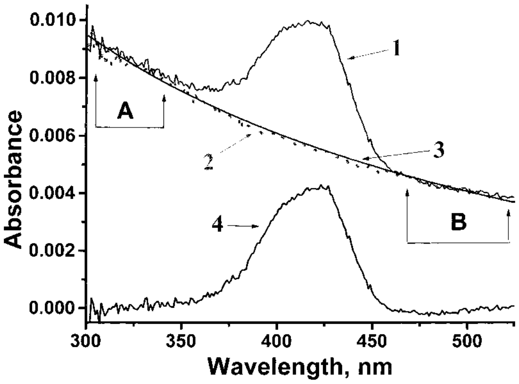


Figure 1. Illustration of the background subtraction procedure in absorption spectra: (1) original spectrum of a slide with immobilized coumarin; (2) the same slide naked; (3) the best fit to the background curve 1 using a function of wavelength λ, $f(\lambda) = [c_1/(c_2 + \lambda)] + c_3$, with three variables, c_1, c_2, and c_3, on the range identified by the two sections, A and B; (4) the "processed" spectrum is a result of subtracting curve 3 from curve 1.

(d) Measurements of Absorption Spectra. Absorption spectra were measured using a Perkin-Elmer Lambda 40 UV/vis spectrometer equipped with a homemade polarizer. A stained slide was placed vertically in a 1 cm quartz cuvette, filled with a solvent, at close to a 45° angle with respect to the incident light. The two linear polarizations of excitation light were used: vertical and horizontal.

Vertical polarization in the described geometry corresponds to measurement of optical absorption with polarization parallel to the surface (||) and will be named as such from now on. Absorption polarized perpendicular (⊥) to the surface substrate was calculated as the difference between the double horizontal absorption and the vertical absorption spectra for a given stained slide.

$$A_{\|} = A_{\mathrm{v}} \qquad A_{\perp} = 2A_{\mathrm{h}} - A_{\mathrm{v}} \tag{1}$$

Naked slides showed some apparent absorption spectra, as a background, due to wavelength dependence of the refractive index mismatch between the solvent and quartz as well as due to minor impurities in quartz. The background signal was subtracted from each spectrum of a stained slide. Since there is a slight variation of the background with solvent, with light polarization, and from one slide to another, the background absorption was each time simulated (by fitting over the regions to the red, $\lambda > 470$ nm, and to the blue, $\lambda < 330$ nm, from the coumarin absorption) as a smooth function of wavelength, $f(\lambda) = c_1/(c_2 + \lambda) + c_3$. Figure 1 illustrates that the fittings of the background for the naked (curve

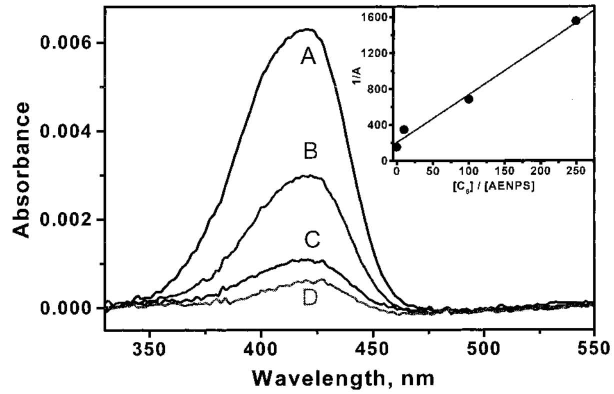


Figure 2. Absorption spectra of slides with different surface coumarin concentrations corresponding to different ratios of octylsilane/aminosilane ([C_8]/[AENPS] in the silanization step: (A) 0/1, (B) 10/1, (C) 100/1, and (D) 250/1. The inset shows that the reciprocal of the absorbance linearly depends on [C_8]/[AENPS]; see text for details.

2) and stained (curve 1) slides agree well. The result of background subtraction is given in a form of the processed spectrum (curve 4).

To eliminate an ambiguity in finding a maximum of absorption, spectra were approximated by a combination of two Gaussian peaks and the maximum of that approximation was assigned to be the absorption maximum.

Results

The two-step procedure described above produces immobilization of coumarin D-1412 on silica with good reproducibility. The maximum absorption for both types of aminosilanes was approximately 0.01. Dilution of aminosilanes with octylsilane in the silanization step decreases the immobilized coumarin concentration on the surface. Figure 2 shows the spectra variation for different dilution ratios: aminosilane/C_8 = 1/0, 1/10, 1/100, 1/250. Even though the coumarin concentration declines upon dilution, the absorption intensity reduction is not equal to the dilution ratio. A higher rate of immobilization for aminosilane, as compared to that of octylsilane, would account for this disproportion. Assigning the corresponding rate constants as k_{AM} and k_{C8}, one can calculate the surface concentration of coumarin, which is measured via absorbance A, to be equal:

$$\frac{1}{A} = \frac{1}{A_0}\left(1 + \frac{k_{C8}}{k_{AM}}\frac{[C_8]}{[AM]}\right) \tag{2}$$

where A_0 is the absorbance of a slide with maximum coumarin concentration (i.e., without C_8) and [C_8]/[AM] is the dilution ratio. Note that the coumarin concentration cannot exceed the concentration of surface amino groups. The inset in Figure 2 presents the graph of $1/A$ versus [C_8]/[AM], from which the ratio of the rate constants can be found, $k_{AM}/k_{C8} \approx 40$.

It is known that amines catalyze reaction between methoxysilanes and hydroxyl groups on silica[21] which suggests that aminomethoxysilanes would immobilize on silica more efficiently. The ability to form a pseudo-five-membered ring in γ-aminosilanes, as shown in Scheme 1, offers a possibility for self-catalytic reaction enhancement for the APS and AENPS aminosilanes.[22]

The surface concentration of coumarin, n_s, can be calculated from the absorbance, A, using Lambert–Beer's Law:

$$n_s\ (\mathrm{cm}^{-2}) = 6 \times 10^{20}\frac{A}{\epsilon} \tag{3}$$

where n_s is given in units of cm^{-2} and the extinction coefficient of coumarin, ϵ, is given in $\mathrm{M}^{-1}\,\mathrm{cm}^{-1}$. As a result of coumarin-ester reaction with surface attached amine, the resulting amide bonding of the (now carboxyamide) coumarin slightly changes its optical properties (see Scheme 1). One can mimic these changes in solution by reacting coumarin-ester with propylamine. The absorption spectra in ethanol and toluene of both coumarin-ester and coumarin are shown in Figure 3. Upon amination, the λ_{max} of coumarin shifts to the blue and the maximum extinction coefficient drops from 56 000 to 47 000 $\mathrm{M}^{-1}\,\mathrm{cm}^{-1}$. Using the latter value, the maximum concentration of immobilized coumarin is calculated to be $10^{14}\ \mathrm{cm}^{-2}$ which is lower than previously reported for the surface concentration of hydroxyl groups.[23–26] At such high concentrations, the interactions between coumarin molecules are expected to be significant and comparable to the interaction with solvent. Thus, the results presented further will be discussed with consideration of the effects of a solvent, the coumarin concentration, and a linker.

Effect of Solvent. Both coumarin-ester and coumarin possess substantial ground-state dipole moments. The solvation energy, E_{solv}, of a molecule with the dipole μ_g and the radius a can be estimated:[29]

$$E_{solv} = -\frac{{\mu_g}^2}{a^3}\frac{\epsilon - 1}{2\epsilon + 1} \tag{4}$$

For the ground-state dipole moment, $\mu_g \sim 7$ D,[20,27] and with a conservative estimate for the molecular radius $a \sim 4.5$ Å, the difference in solvation energy of coumarin in two solvents, hexane and acetonitrile, exceeds k_BT by a factor of 4. As a result, the coumarin molecule is more soluble in acetonitrile than in hexane. Accordingly, its absorption spectrum is more red shifted in acetonitrile than in hexane (see Table 1). Actually, the total shift in the absorption spectrum includes also dispersion interaction of the transition moment μ with the solvent:

$$E_{disp} = -\frac{\mu^2}{a^3}\frac{n^2 - 1}{2n^2 + 1} \tag{5}$$

where n is the solvent refractive index. The transition moment μ can be estimated using[28]

$$\mu^2 = \frac{9.2 \times 10^{-3}}{\tilde{\nu}}\int \epsilon(\tilde{\nu})\,\mathrm{d}\tilde{\nu} \tag{6}$$

and equals ca. 7 D for coumarin. Combination of eqs 4 and 5 produces a reasonable description of the spectral shift of either coumarin in solutions. On the other hand, the

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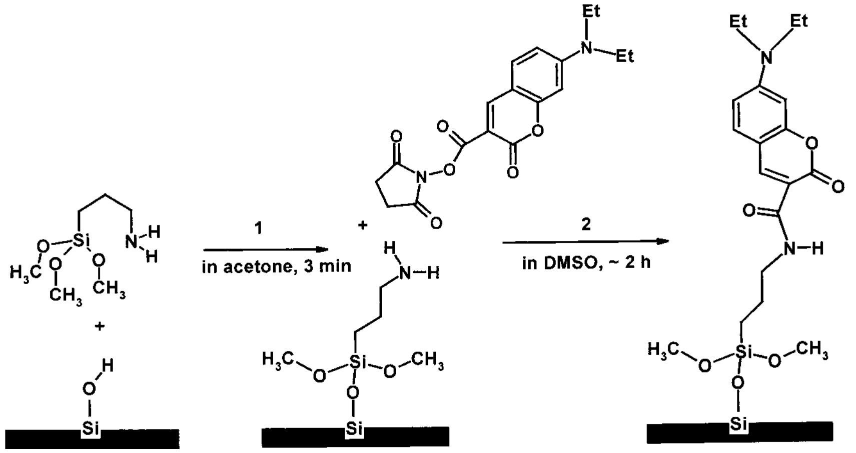
Scheme 1. Two-Step Coumarin Self-Assembly Procedure[a]



[a] (1) Silanization of silica surface by trimethoxyaminosilanes; (2) functionalization of amines by coumarin-ester to yield coumarin anchored to the surface.

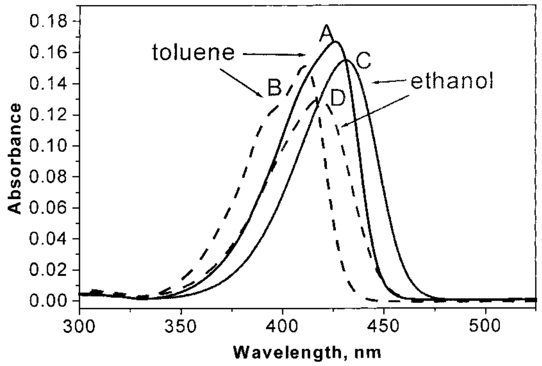


Figure 3. Absorption spectra in solution for both coumarin-ester (solid) and (aminated) coumarin (dashed), in two solvents, toluene and ethanol.

Table 1. Absorption Maxima of the Two Coumarins in Solutions Measured at ca. 3×10^{-5} M

	solvent					
solute	hexane	toluene	aceto-nitrile	methylene chloride	ethanol	water
coumarin-ester	*a*	426	431	435	431	444
coumarin[b]	401[c]	411	414	417	417	427

[a] Not soluble. [b] Obtained by adding ca. 50 μL of propylamine to 4 mL of solution. [c] Prepared in CH_2Cl_2 and dissolved in hexane.

surface immobilized coumarin demonstrates much weaker solvent dependence of its maximum wavelength (see Table 2 and Figure 4). Moreover, the λ_{max} is strongly dependent on coumarin concentration (see below) and varies with light polarization. The latter needs some explanation.

The lowest energy transition in coumarin is a charge transfer (CT) transition, with a transition moment parallel to the molecule's ground and excited-state dipole moments. It is oriented along the long axis of the coumarin molecule.[7,20,30,31] By varying the light polarization, one can monitor molecular distribution with respect to the surface. The perpendicular polarization (⊥) selects molecules mostly oriented perpendicular to the surface, while the parallel polarization (||) favors molecules along the surface. The difference in the absorption intensity at the two polarizations reveals the anisotropy of the molecules' *orientation*, while the λ_{max} reflects primarily the anisotropy of molecular *environment* caused by different interactions with the solvent and other coumarin molecules.

Figure 4 illustrates that solvent effect on coumarin orientation on the surface is measurable but not dramatic because absorption intensities at parallel and perpendicular polarizations are comparable. However, the less polar toluene does not provide as much advantage in solvation as ethanol does, and as a result there is slightly more of the perpendicular absorption in ethanol as compared to that in toluene. This trend for solvation-assisted favoring of perpendicular coumarin orientation with solvent polarity is presented in Table 2 for four solvents used. Note that in hexane the anisotropy in absorption intensity could be as high as 50% in favor of parallel polarization while in methylene chloride the very same slide shows more than 25% anisotropy in favor of perpendicular polarization.

Effect of a Linker. Out of the two aminosilanes used for immobilization of coumarin, the longer one, AENPS, provides more flexibility for coumarin orientation. The change in molecular orientation with solvent presented above for that linker shows that the anisotropy in intensity, though it exists, is not dramatic and corresponds to almost random orientation, as it should be for a flexible chain. Another linker, APS, does not provide as much freedom in chain conformation, forcing coumarin to occupy a limited number of most favorable conformations and thus more certain orientations. Table 2 shows that, indeed, with the APS linker coumarin in all solvents has a biased preference to be oriented "parallel" to the surface.

Effect of Concentration. A high concentration of molecules on the surface causes them to interact. These interactions are primarily of a dipole–dipole nature and can be classified into two categories: the ground-state dipole–dipole interaction and the transition dipole–dipole moment interaction upon optical excitation, or so-called intermolecular excitonic coupling.

The dipole–dipole interaction between two dipole moments, μ_1 and μ_2, in a vacuum is described by

$$E_{\text{dip-dip}} = \frac{(\vec{\mu}_1\vec{\mu}_2)}{R^3} - \frac{3(\vec{\mu}_1\vec{R})(\vec{\mu}_2\vec{R})}{R^5} \qquad (7)$$

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(31) According to the AM1 optimized structure of 7-diethylamino-coumarin, its transition moment is oriented along the axis approximately parallel to a line connecting C2 carbon and nitrogen of amine (almost exactly the same results in AM1 and ZINDO/s); the value of the transition moment as well as the dipole moment in the ground state (almost parallel to the transition moment) varies from the coumarin-3-carboxylic acid succinimidyl ester to its carboxamide analogue. These data are available as Supporting Information.

Table 2. Solvent Dependence of the Optical Absorption Parameters of Coumarin Immobilized on Silica by Means of the Two Different Linkers

			solvent				
			hexane ($\epsilon = 1.9$)	toluene ($\epsilon = 2.4$)	CH_2Cl_2 ($\epsilon = 8.9$)	ethanol ($\epsilon = 24.6$)	acetonitrile ($\epsilon = 37.5$)
APS[a] linker	λ_{max} (nm)	$\perp$	405 ± 1[c]	408	411	408	406
		$\parallel$	414	415	419	417	417
	$A_{max} \times 10^3$	$\perp$	4.7 ± 0.1[d]	6.1	6.8	6.2	6.3
		$\parallel$	7.9	8.4	7.7	6.9	6.9
AENPS[b] linker	λ_{max} (nm)	$\perp$	408	410	408	407	407
		$\parallel$	416	420	420	419	420
	$A_{max} \times 10^3$	$\perp$	5.0	5.6	6.5	6.0	5.6
		$\parallel$	6.2	5.8	6.1	6.1	5.5

[a] 3-Amino-propyltrimethoxysilane. [b] *N*-[3-(Trimethoxysilyl)propyl]-ethylenediamine. [c] 1 nm is a typical accuracy for all wavelength maxima. [d] 10^{-4} is a typical accuracy for all absorbances.

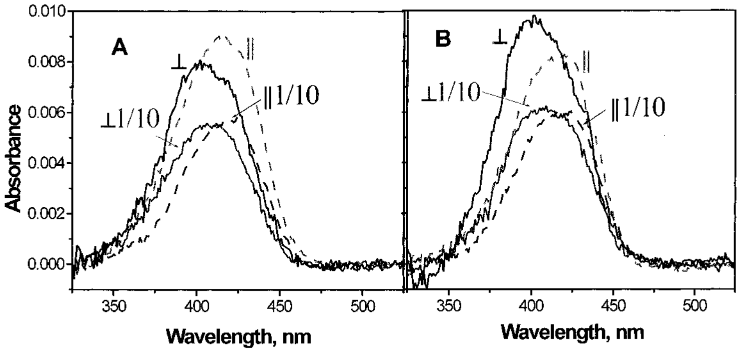

Figure 4. Absorption spectra of coumarin-stained slides in toluene (A) and in ethanol (B) at different light polarizations with respect to the surface. Two concentrations are given for each solvent: without dilution (($\perp$) perpendicular and ($\parallel$) parallel polarizations) and with the dilution ratio of aminosilane/octylsilane equal to 1:10 ($\perp$1/10 and $\parallel$1/10, respectively). See text for details.

where R is the distance between the two dipoles.

Obviously, the dipole–dipole interaction competes with solvation (see eq 4) and thus affects the orientation of molecules with respect to each other and the surface. Comparing eq 7 with eq 4 suggests that $E_{dip-dip}$ should be smaller than E_s in solvents of moderate polarity because $R > a$. Solvation affects molecules' orientation on the surface by thermodynamic "weighting out" of their energetic preferences to be solvated by either "remote" solvent or near-surface tails of other immobilized molecules. As is clear from eq 7, orientation perpendicular to the surface should be unfavorable for molecules with nonzero ground-state dipole moment, particularly in nonpolar solvents. Indeed, $E_{dip-dip}$ is positive for collinear dipoles equally distant from the surface. A tilted orientation would be more advantageous and is likely realized when high surface densities are achieved.

The excitonic coupling in general is not affected by the ground-state dipole–dipole interaction and is observed for molecules with zero ground-state dipole moments as well. Nevertheless, because of its dependence on the molecules' mutual orientation, which is defined by the molecules' interaction in the ground state, the two effects are often difficult to separate. The expression for the energy of the excitonic interaction looks almost identical to eq 7:

$$E_{exc} = \frac{(\vec{M}_1\vec{M}_2)}{R^3} - \frac{3(\vec{M}_1\vec{R})(\vec{M}_2\vec{R})}{R^5} \qquad (8)$$

where now the interaction takes place between the transition dipole moments, M_1 and M_2, rather than the ground-state dipole moments. Again, the molecules' orientation perpendicular to the surface increases the energy of transition due to the repulsion between M_1 and M_2. For that reason, the absorption maximum for perpendicular polarization is always blue shifted with respect to that of parallel polarization as well as absorption maximum in the solution.

Both types of intermolecular interaction can be reduced by diluting aminosilanes with dull octylsilane during the silanization step. Dilution causes a decrease of coumarin surface concentration but not linearly, as explained above. The concentration decrease should be accompanied by diminishing intermolecular interaction, that is, smaller spectral anisotropy, but the anisotropy of intensity should not change as much. Figure 4 illustrates, on the examples of toluene and ethanol solvents, that the anisotropy of intensity remains almost the same upon dilution. The absorption signal in toluene is greater at parallel ($\parallel$) polarization for both concentrated and diluted samples, while in ethanol the signal at perpendicular ($\perp$) polarization is more intense in both cases.

The wavelengths of absorption maxima, λ_{max}, on the other hand, do shift upon dilution, and the difference between maxima for the two polarizations becomes smaller in less concentrated samples. This trend is expected for weakening interaction between molecules upon dilution. As Figure 5 shows, such behavior is observed in all solvents. The less obvious spectral variation of Figures 4 and 5 is that the absorption maximum at parallel polarization progresses away from the λ_{max} value of coumarin in the corresponding homogeneous solution. Unfortunately, we were not able to investigate that phenomenon at even lower surface concentrations of coumarin because of weak signals.

Despite high surface concentration, no excimer fluorescence was observed (see Figure 6). In cases deviating from the described silanization procedure where polymerization of silanes in solution was allowed, much higher apparent concentrations of coumarin were realized and

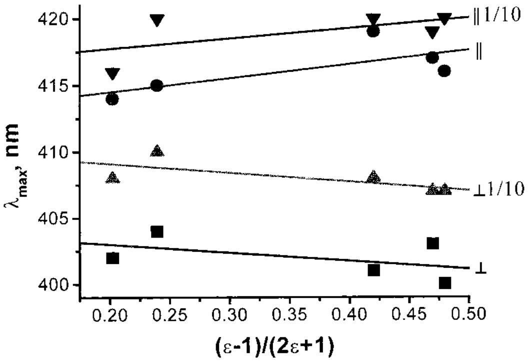

Figure 5. Variation of the maximum absorption wavelength, λ_{max}, with the solvent polarity factor, $(\epsilon - 1)/(2\epsilon + 1)$, for two light polarizations in the two slides with immobilized coumarin. The concentrations are identified as follows: without dilution ((⊥) perpendicular and (||) parallel polarizations) and with the dilution ratio of aminosilane/octylsilane equal to 1:10 (⊥1/10 and ||1/10, respectively). The solid lines are given only for guidance.

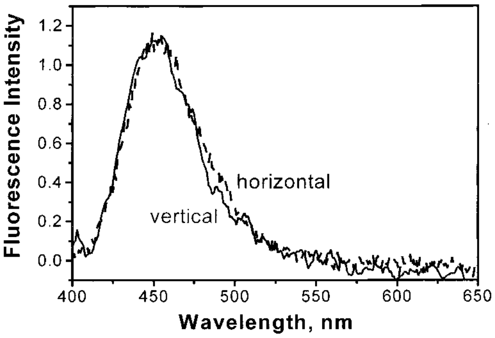

Figure 6. Fluorescence spectra of immobilized coumarin from a slide oriented vertically at 45° with respect to a detector and placed in acetonitrile. Two light polarizations of the detector are shown: vertical (solid line) and horizontal (dashed line). Excitation at 390 nm was not polarized and was also oriented at 45° with respect to the slide surface.

excimer fluorescence around 530 nm was often observed. Such erroneous occasions will not be discussed because they correspond to more than monolayer attachment. Note also that Figure 6 indicates no anisotropy in luminescence for immobilized coumarin.

Effect of a Substrate. We observed approximately the same surface densities of coumarin on other oxide surfaces such as ITO, glass, and mica. None of these substrates possesses desired transparency in the UV which would match that of quartz, and thus they will not be as useful for studying photoinduced electron transfer along DNA, where excitation in the UV is desired.[6,17] Nevertheless, mica and ITO will be valuable substrates in studying single DNA molecules by scanning microscopy (STM and AFM).

Discussion

Ability to control surface density and orientation of immobilized molecules is one of the crucial features for future applications of self-assembled molecular architectures. Despite extensive studies, the detailed mechanism of silanization is a matter of controversy.[12,14−16,23−26,32−37] Different deposition conditions, including the nature of silanes, their concentration, solvent, duration and temperature of the reaction, water content, and the temperature and time of postcuring, affect the density and quality of the resultant film.[23,25,26,35−37] The number of Si−O−Si bonds between the silanes and the silica surface (mono-, di-, or tri-) and the extent of the silanes' lateral polymerization are not identified unambiguously.

The highest density of coumarin that we observe, ca. 10^{14} cm^{-2}, is noticeably less than what is considered to be the density of hydroxyl groups,[23−26,37] ca. $n_{OH} = 5 \times 10^{14}$ cm^{-2}. Our lower density of coumarin also correlates with weak anisotropy in molecular orientation upon excitation and with lack of anisotropy in luminescence. All these facts suggest that molecular packing is far from being a dense ordered arrangement such as for linear alkanethiols on gold or linear alkane-silanes on oxide surfaces.[16] It reflects the specific conditions of formation of these films and provides information about the mechanism of siloxane self-assembly.

In a number of studies, it was shown that the surface concentration of amines achieved in analogous procedures amounted to ca. $n_{NH_2} = 2 \times 10^{14}$ cm^{-2}.[12,26] The difficulty of making an ordered compact array from functional tails, different from linear aliphatics, is viewed as the main reason for the surface density to be less than that of surface hydroxyl concentration n_{OH}. Siloxanes that are bi- and tridentate hydrogen bonded to surface hydroxyls are energetically favored geometries for adsorption[34] and could thus explain a factor of 2 difference between n_{OH} and n_{NH_2}. But covalent Si−O−Si bond formation with the surface is possible with no more than two bonds per siloxane,[32,34] with one bond per siloxane being the most favorable. If only one Si−O−Si bond with the surface is formed, the remaining two methoxy groups sterically hinder covalent bonding of new siloxanes upon neighboring hydroxyl groups. Most, if not all, nearest neighbor hydroxyls become inaccessible, and as a result less surface density of amines is achieved. This description also agrees with kinetics study of surface modifications[16,24] and the role of water. Usually, ca. 20% of surface hydroxyls are modified into Si−O−Si bonds within a few minutes and the remaining portion very gradually builds up.[16,24,32] On this last slow step, lateral polymerization is likely to proceed because the gaps between already attached siloxanes can be filled only by a single Si−O−Si bond formed from two Si−OCH$_3$ bonds of the neighboring siloxanes. The latter can barely proceed without hydrolysis yielding one Si−O−Si bond and elimination of two methanols per two methoxy groups, thus the importance of (sometimes traces) of water. Note that the water is necessary only for lateral polymerization of the last step but the first step proceeds without it. If water is present throughout the whole process, polymerization can spread out away from the surface thus building more than a monolayer, and that is what was occasionally observed when moisture was not well controlled. Without water, the (unused for reaction with hydroxyls) methoxy groups of siloxanes remain intact. In our preliminary experiments, this hypothesis was confirmed by silanization in two steps: the regular first step (3 min of silanization plus washing and drying) was followed by washing in deionized water, a regular drying, and another regular step of silanization the same as the first one. Coumarin staining was done in a regular way only after the second step. The resulting two-step silanized

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film had much higher surface concentration of coumarin and a more dramatic change in the spectra. Neither the films exposed to silane for the same total time period without intermediate washing in water nor the films only washed in water without the second step of silanization showed any changes in surface concentration as compared to the films treated by the standard procedure.[38]

It has been noted before that amines catalyze reaction of siloxanes with the surface, but there is no agreement on whether it is an autocatalysis, that is, when products accelerate the reaction, or a self-catalysis, that is, when the amino group from the molecule accelerates only reaction of the molecule itself.[21] All four bases on aminosiloxane participate in hydrogen bonding with surface hydroxyls, and a preference in choice of hydrogen bonded group(s) depends on coverage.[34] On the basis of our studies with mixtures of dull alkyl silanes and aminosilanes, it is unambiguous that autocatalysis plays an insignificant role in silanization with aminosilanes; the self-catalysis makes methoxysilanes with amino groups approximately 40 times more reactive. Indeed, if the amine terminus of already immobilized silane were capable of accelerating reaction of oncoming silanes, then no discrimination in reactivity between aminosilane and the dull silane should be observed. It is not clear at the moment whether the existence of the amine base (at any position) aiding the initial physisorption or the amine-specific γ-position in aminosilane is vital to the self-catalytic effect, but the fact of self-catalysis seems to be definite.

Even though a very little difference is observed between the two silane linkers we used for coumarin immobilization and anisotropy in intensity is small, the difference is reproducible suggesting that the orientation of coumarin is somewhat restrained by the linker and thus can be used to tag the linker orientation and the way it attaches to the surface. A simplified conformational search[39] on coumarin with the two linkers was performed by MM+ and AM1 using HyperChem Pro 6. It shows that the angle between the molecular transition moment[31] and the Si−O bond at the surface is larger for the short APS linker than for the AENPS. In the “all-trans” conformation, the angles are 55° (70° and 39° for the two different Si−O bonds) and 24° (9° and 38° for the two different Si−O bonds), respectively. Averaged between all these conformations, angles become 69° and 47° for APS and AENPS, respectively. These agree with the experimental observation that the APS linker forces the orientation of coumarin to be preferentially more parallel to the surface and thus support the above conclusion that the attachment takes place by a single Si−O−Si bond.

Conclusions

We have demonstrated that surface modification of hydrophilic oxides (and silica in particular) by dry solutions of aminomethoxysilanes leads to a typical concentration of surface amines of ca. 10^{14} cm^{-2}, as quantified by UV absorption of coumarin molecules immobilized on amines. The amino group on silanes self-catalyzes the reaction of aminosilanes with the surface approximately by a factor of 40, as compared to that for dull methoxysilanes; to the best of our knowledge, it is the first quantitative evaluation of the self-catalytic effect in aminomethoxysilanes. Immobilization of silanes most likely proceeds via formation of a single Si−O−Si bond per silane which is reflected in anisotropy of immobilized coumarins. The remaining two methoxy groups of silanes prevent further immobilization of silanes. The resulting self-assembled film is partially exposed to solvent and allows orientational flexibility of immobilized coumarins making their angular distribution almost random.

The surface density in the monolayer can be increased to an excess of 2×10^{14} cm^{-2} by the second silanization step preceded by brief hydrolysis of the initial layer.

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Supporting Information Available: AM1 and ZINDO/S calculations of the UV spectra of the 7-diethylaminocoumarin-3-carboxylic acid succinimidyl ester molecule and its carboxamide analogue; energies and geometries of different conformations of coumarin. This material is available free of charge via the Internet at http://pubs.acs.org.

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