A self-assembled monolayer of a fluorescent guest for the screening of host molecules

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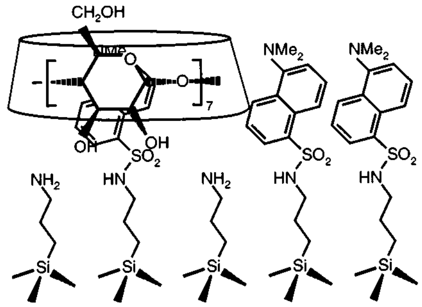
The polarity-dependent fluorescence of self-assembled monolayers of dansyl adsorbates on glass enables monitoring of its specific interactions with β-cyclodextrin.

Host–guest interactions are intensively studied phenomena in supramolecular chemistry.[1] The synthesis and functionalization of host molecules has led to a large variety of compounds with high affinities and selectivities for guests, varying from metal ions to (bio)organic molecules. Recent developments in combinatorial chemistry allow the preparation of synthetic receptor libraries.[2] The large number of host molecules that is generated in this way requires analytical techniques which enable the fast selection of suitable hosts for a specific guest. Self-assembled monolayers (SAMs) offer the possibility to immobilize guest derivatives on a variety of substrates, and monitor their interactions with compounds in the contacting solution. So far, several groups have used electrochemically active SAMs of ferrocene for the binding of macrocyclic hosts from aqueous solutions.[3] The complexation of the ferrocene headgroup resulted in a shift of its redox potential, which can be detected by cyclic voltammetry. Fluorescent self-assembled monolayers have rarely been used to monitor host–guest interactions. Hieftje *et al.* used a monolayer of fluorescently labeled single-stranded DNA to monitor interactions with proteins by fluorescence anisotropy.[4] Myles *et al.* reported the detection of barbituric acid derivatives by a self-assembled monolayer of fluorescent receptors on gold.[5] Here we describe the selective binding of β-cyclodextrin to self-assembled dansyl monolayers on quartz and oxidized silicon wafers, detected by fluorescence spectroscopy.



Self-assembled monolayers of dansyl adsorbates were prepared in a two-step procedure. First, a monolayer of 3-aminopropyltriethoxysilane was attached to the substrate.[6] This layer was converted into the desired dansyl SAM by reaction with dansyl chloride.[7] Ellipsometric measurements showed that the initially formed propylamine layer had a thickness of 6.7 ± 0.4 Å, which is close to the estimated length of the molecule (7.5 Å based on CPK models).[8] After reaction with dansyl chloride the layer thickness increased by 5.3 Å to a value of 12.0 ± 0.4 Å. Since the length of a dansyl group is 8.4 Å, the observed increase of only 5.3 Å was attributed to a relatively loose packing of the fluorescent groups. From these numbers a surface coverage of approximately 60% of the most densely packed monolayer was calculated. Wettability studies showed advancing contact angles with water of 67 ± 1 and 58 ± 1° for the 3-aminopropyltriethoxysilane SAM and the dansyl monolayer, respectively.[9] The hysteresis between advancing and receding contact angle for both surfaces was 30 to 35°, which indicates that monolayers were formed with a high degree of disorder. UV-VIS absorbance of the monolayer showed a strong absorption at 220 nm with a shoulder at 250 nm, and a third weak absorption at 330 nm (see Fig. 1).[10] Both position and relative intensity of these peaks are in good agreement with a spectrum of dansylamide taken in MeCN. The absorption at 220 nm was used to estimate the surface density of dansyl groups (ρ) from the Beer–Lambert law ($\rho = A\varepsilon^{-1}$), using the experimentally determined absorption coefficient of dansylamide in MeCN ($\varepsilon_{220} = 38900\ \mathrm{M}^{-1}\ \mathrm{cm}^{-1}$). The resulting density of 0.93 dansyl groups per 100 Å^2 indicates that only 25% of the amino groups had reacted with dansyl chloride.[11] Based on a CPK model, the adsorbate would cover an area of approximately 52 Å^2. This results in a surface coverage of 50%, which is in reasonable agreement with the 60% surface coverage calculated from the ellipsometric thickness.

Fluorescence measurements of the dansyl SAM in air showed the characteristic broad emission centered at 480 nm.[12] Exposure of the monolayer to water resulted in a shift of the emission maximum to 510 nm. Hence, the well-known polarity dependence of the emission maximum of 1-(dimethylamino)-naphthalene-5-sulfonate[13] was maintained upon its immobilization in a monolayer. Apparently, the packing density was not enough to prevent water from solvating the dansyl groups.

Titration of the layer with aliquots of a concentrated aqueous solution of β-cyclodextrin resulted in an increase of the monolayer fluorescence, accompanied by a shift of the fluorescence maximum from 510 nm (pure water) to 480 nm (11.3 mM of β-cyclodextrin), as shown in Fig. 2. This indicates that the host molecules are bound to the layer, which results in an effective shielding of the dansyl groups from the aqueous environment.[14] The dansyl SAM showed no significant changes of the position or the intensity of the emission maximum in

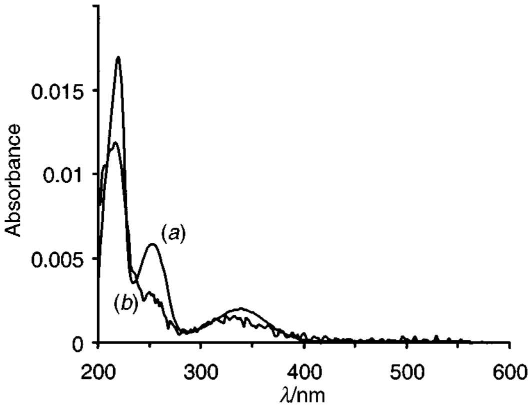


Fig. 1 UV–VIS absorption spectra of (*a*) 44 μM dansylamide in MeCN (shown absorbance was divided by a factor of 100) and (*b*) two dansyl monolayers (one on each side of the quartz slide).

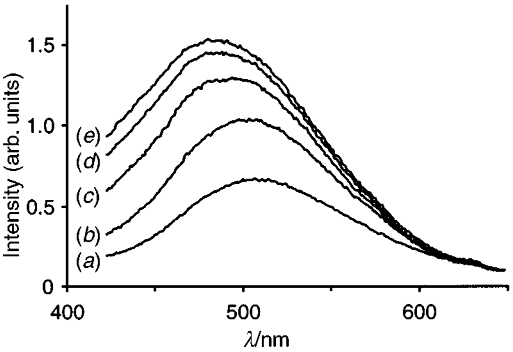


Fig. 2 Emission spectra of a dansyl monolayer (excitation at 350 nm) at different concentrations of β-cyclodextrin in the contacting aqueous solution: (*a*) 0, (*b*) 2.4, (*c*) 5.3, (*d*) 8.8 and (*e*) 11.3 mM.

titration experiments with solutions of α- and γ-cyclodextrin. Hence, the observed changes of the emission spectra were the result of molecular recognition rather than aspecific binding of cyclic oligosaccharides to the dansyl monolayer. Moreover, the addition of aliquots of a competitive guest (50 mM cyclohexanol) to the 10 mM β-cyclodextrin solution resulted in decomplexation from the dansyl layer and changed the wavelength of the emission maximum and its intensity to the values observed in pure water. Due to the covalent binding of the dansyl group to the propylamine monolayer, we exclude the possibility of the complexation of one dansyl group by two β-cyclodextrin molecules as was reported by Dunbar *et al.* for solution experiments.[15] Consequently, the observed phenomena are attributed to the 1 to 1 complexation of the immobilized dansyl guests by β-cyclodextrin hosts.

To provide enough space for every dansyl group to bind a β-cyclodextrin molecule, the average area per dansyl group should be 180 Å^2, which would result in a layer thickness well below the determined 12 Å. This means that not every dansyl is capable of forming an inclusion complex. The determined surface density of fluorophores would allow 60% of the dansyl groups to be complexed by a cyclodextrin molecule. Consequently, the observed emission spectrum is the sum of the two fluorescent species present at the surface (free dansyl, and the complex of β-cyclodextrin and dansyl). The fluorescence intensity (I) at a given wavelength is therefore given by eqn (1),

$$I = k_{\mathrm{dans}}[\mathrm{dans}] + k_{\mathrm{compl}}[\mathrm{comp}] \tag{1}$$

where k_{dans} and k_{compl} represent the proportionality constants that connect the intensities and the surface concentration of the species.[16] Combined with the equation for the association constant (K = [compl]/[dans][β-CD]) this results in eqn. (2),

$$\frac{I}{I_0} = \frac{1 + (k_{\mathrm{compl}}/k_{\mathrm{dans}})K[\beta - \mathrm{CD}]}{1 + K[\beta - CD]} \tag{2}$$

where I_0 is the emission intensity in the absence of β-cyclodextrin. From the fit of eqn. (2) to the experimental values, an association constant of 221 ± 19 M^{-1} was obtained (see Fig. 3), which is of the same order of magnitude as the reported values of 100–200 M^{-1} for complexes in aqueous solutions.[14]

In summary, self-assembled monolayers of 3-aminopropyltriethoxysilane can be effectively modified by reaction with sulfonyl chlorides. In this way, different functions like dyes can be introduced. Efficient solvation of the surface-bound dansyl groups maintains their polarity dependent emission, which has been used to study the interactions with several hosts in aqueous solutions.

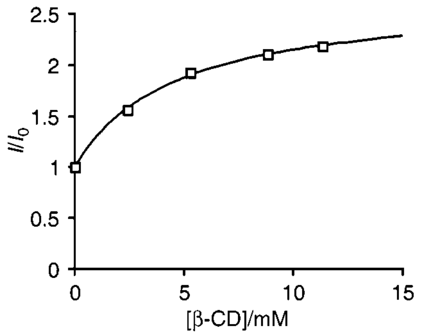


Fig. 3 Relative fluorescence intensity at 505 nm in dependence of the β-cyclodextrin concentration. The solid line shows eqn. (2) with an association constant of K = 221 M^{-1} and $k_{\mathrm{compl}}/k_{\mathrm{dans}}$ = 2.67.

Notes and references

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7 Modification of the 3-aminopropyltriethoxysilane SAMs was achieved by reaction with 10 mg dansyl chloride and 0.1 ml triethylamine in 25 ml of dry MeCN for 16 h in a glovebox. After the reaction the substrates were extensively rinsed with MeCN, EtOH and $CHCl_3$.

8 Ellipsometry was performed on a Plasmos Ellipsometer (λ = 633 nm) assuming a refractive index of 1.46 for the monolayer and the underlying 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.

9 Experimental details of the contact angle measurements are described in S. Flink, B. A. Boukamp, A. van den Berg, F. C. J. M. van Veggel and D. N. Reinhoudt, *J. Am. Chem. Soc.*, 1998, **120**, 4652.

10 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.

11 For self−assembled propylamine monolayers a surface density of 3.9 amino groups per 100 Å^2 was determined by J. H. Moon, J. H. Kim, K. Kim, T.-H. Kang, B. Kim, C.-H. Kim, J. H. Hahn and J. W. Park, *Langmuir*, 1997, **13**, 4305.

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