Influencing the Binding Selectivity of Self-Assembled Cyclodextrin Monolayers on Gold through Their Architecture

Menno R. de Jong, Jurriaan Huskens,* and David N. Reinhoudt*[a]

Abstract: Cyclodextrin derivatives modified with seven thioether moieties (**1**) or with one thiol moiety (**2**) bind to gold. Monolayers on gold of **1** or mixed monolayers of **2** and mercaptoundecanol were characterized by electrochemistry, wettability, and atomic force microscopy (AFM). Monolayers of **1** are well-ordered, but the order in the mixed monolayers depends on the ratio of **2** to mercaptoundecanol. With sufficient alkyl chains to fill the space under the cyclodextrin moiety of **2**, the monolayers are densely packed. Guest recognition at these monolayers in water was studied by surface plasmon resonance (SPR) spectroscopy. For simple organic guests, monolayers of **1** showed the same selectivity and binding strength as β-cyclodextrin in solution; however, the selectivity towards steroidal bile salts differs from solution. The mixed monolayers of **2**, in which the cyclodextrin is less substituted and has more flexibility, bind steroidal guests (**6a**–**6e**) with the same selectivity as β-cyclodextrin in solution.

Keywords: cyclodextrins · host–guest systems · monolayers · steroids · surface plasmon resonance

Introduction

Self-assembled monolayers (SAMs)[1] on gold are easily prepared and highly stable. When combined with the possibility for introducing functional groups, this makes them attractive for the modification of surface properties, for example, for sensing purposes. Our group has previously reported the self-assembly of various receptor molecules, such as resorcin[4]arenes[2] and crown ethers[3] on gold. We have monitored interactions of resorcin[4]arene monolayers with organic guests by quartz crystal microbalance (QCM)[2a] and surface plasmon resonance (SPR) spectroscopy, both in the gas phase[2e] and in aqueous solution.[4] The binding of metal ions from solution by SAMs of crown ethers was studied by electrochemical impedance spectroscopy.[3]

Cyclodextrins,[5] cyclic oligosaccharides that consist of six, seven (β-cyclodextrin, Figure 1), or eight glucose moieties, possess a hydrophobic cavity that enables the complexation of organic guests in aqueous solution. Sulfur-modified α-[6] and β-cyclodextrin[7] derivatives have been used by several groups for the preparation of SAMs on gold. Kaifer and co-workers used per-6-deoxy-(6-thio)-β-cyclodextrin with seven thiol moieties for binding to the gold surface.[7a] Their binding properties with metallocenes were studied on surfaces[7a] and on colloids.[8] Galla and co-workers reported cyclodextrins with one thiol moiety as the attachment point.[7e] Binding studies at SAMs of these adsorbates revealed that guest binding did not follow a Langmuir isotherm because of the disorder in the layers.[7f]

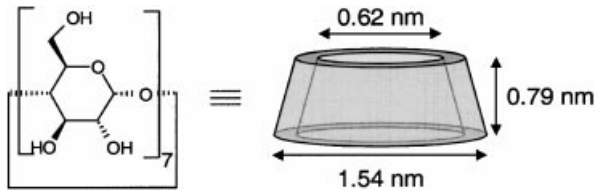

Figure 1. Structure and dimensions of β-cyclodextrin.

Our own strategy for obtaining dense, well-packed monolayers of receptor molecules involves filling the space underneath a head group with alkyl chains by using multiple attachment points (Figure 2a). For example, we substituted a resorcin[4]arene[2a] with four thioether units (4×40 Å^2) to match the size of the cavity head group (160 Å^2). We recently used the same approach for highly ordered monolayers of α-, β-, and γ-cyclodextrin.[9, 10] Persubstitution of cyclodextrins at their primary rim with thioethers yielded better organized monolayers than persubstitution with thiols.[9] Electrochemistry confirmed the formation of well-packed monolayers for the thioether-modified adsorbates, accomplished by a dense packing of the thioether moieties. Atomic force microscopy (AFM) showed hexagonal lattices with a lattice constant consistent with the size of the cyclodextrin head group for thioether-modified cyclodextrin adsorbates that are methy-

[a] Dr. Ir. J. Huskens, Prof. Dr. Ir. D. N. Reinhoudt, M. R. de Jong
Laboratory of Supramolecular Chemistry and Technology
MESA$^+$ Research Institute, University of Twente, PO Box 217
7500 AE Enschede (Netherlands)
Fax: (+31) 53-4894645
E-mail: smct@ct.utwente.nl

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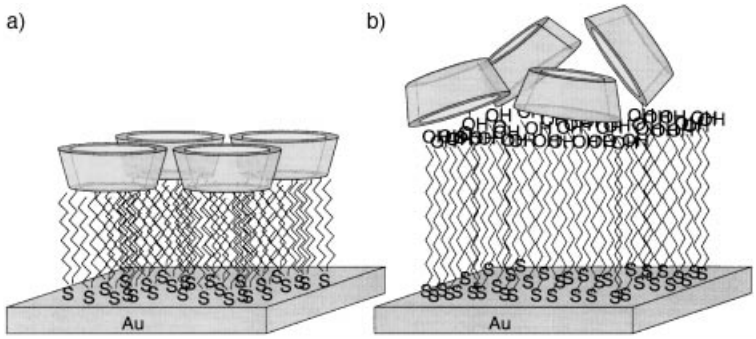


Figure 2. Architectures of cyclodextrin monolayers employed in this study.

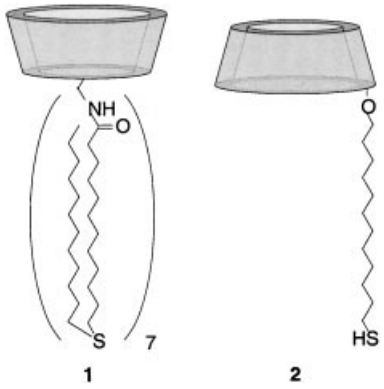
lated at the 2- and 3-positions, proving that the adsorbates are ordered and packed with their cavity pointing outward. The β-cyclodextrin adsorbates with free hydroxy groups at the 2- and 3-positions complex guests from aqueous solution. The binding of 1-anilinonaphthalene-8-sulfonate, a well-known guest for β-cyclodextrin, to these monolayers was best described by a Langmuir isotherm,[10a] indicating the presence of only one type of binding site.

Herein we report the binding of several structurally different guests to cyclodextrin monolayers, and compare the binding by the monolayer with binding by cyclodextrin in solution. We used a β-cyclodextrin heptasulfide and a β-cyclodextrin monoalkylthiol to prepare self-assembled monolayers on gold. The latter cyclodextrin adsorbate was used in an alternative strategy to obtain well-packed monolayers of receptor adsorbates. It consists of filling the space left under a head group that is attached to the gold only through a single thiol moiety with mercaptoalcohols (Figure 2b).[11] The mercaptoalcohols should prevent the formation of a quasi-two-layer system that is predicted by molecular dynamics calculations for pure monolayers of cyclodextrin adsorbates monosubstituted with a long alkyl chain.[12] These monolayers were characterized by a variety of techniques to verify that densely packed monolayers were obtained through both of the strategies employed. Surface plasmon resonance (SPR) spectroscopy was used to monitor the host–guest interactions of a variety of guests with the cyclodextrins in the monolayers. Guests were chosen that bind in the cavity or through the cavity, or require two cavities for strong binding. The effect of the architecture of the cyclodextrin monolayers on binding these types of guests was studied in detail.

Results and Discussion

Previously, we reported an amide-connected cyclodextrin heptathioether prepared from heptakis-6-deoxy-6-amino-β-cyclodextrin and a thioether carboxylic acid with a methyl-terminated chain that was one carbon atom shorter than the carboxylic acid terminated chain.[10a] The β-cyclodextrin heptathioether **1** described here was synthesized by the same procedure using a thioether carboxylic acid with alkyl chains of equal length.

A protected precursor of cyclodextrin monoalkylthiol **2** was synthesized by deprotonation of β-cyclodextrin, protected at the primary side with *tert*-butyldimethylsilyl groups, with lithium hydride, and subsequent reaction with 12-bromo-1-(*S*-trityl)mercaptododecane. All protecting groups were removed in one step with a solution of triethylsilane in trifluoroacetic acid to obtain **2**. All compounds were characterized satisfactorily by NMR and FAB-MS or matrix-assisted laser desorption/ionization (MALDI) MS.



The monolayers of the cyclodextrin adsorbates were characterized by electrochemistry and wettability studies (Table 1). The monolayers of **1** closely resemble those reported before.[9, 10a] The charge-transfer resistance (R_{CT}) towards the $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ external redox couple was higher than that reported before,[10a] reflecting the slightly better packing expected for thioethers with two alkyl chains of identical length.[2c] Although the contact angles are higher than those reported before, they are still indicative of a rather hydrophilic surface.

Table 1. Properties of self-assembled monolayers of **1** and of self-assembled monolayers containing varying ratios of mercaptoundecanol and **2**.

Monolayer	θ_a/θ_r [H_2O, °]	C_{ML} [$\mu F\,cm^{-2}$]	R_{CT} [$10^5\ \Omega$]
1	55/< 20	2.6	1.1
$HO-(CH_2)_{11}-SH$	< 20/< 20	2.7	4.4
2% **2**[a]	< 20/< 20	2.4	8.0
4.5% **2**[a]	< 20/< 20	2.4	8.1
9% **2**[a]	< 20/< 20	2.3	10.1
17% **2**[a]	< 20/< 20	2.6	6.1
2	< 20/< 20	7.1	2.0

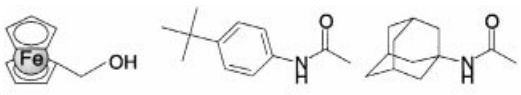
[a] Percentages given are molar percentages in solutions used for monolayer preparation.

Monolayers with a varying ratio of **2** to mercaptoundecanol were also prepared. The surface area occupied by the cyclodextrin head groups is smallest when they are oriented with the rims of the cavities perpendicular to the surface, rather than parallel. At 17% of **2**, we calculated that the cyclodextrin cavities are tightly packed even in this orientation. In the more dilute layers, the cyclodextrin cavities have

more orientational freedom. Wettability studies show that the outer surfaces of all mixed monolayers of **2** and mercaptoundecanol are hydrophilic. The R_{CT} and capacitance values of mixed monolayers of **2** and mercaptoundecanol are similar to those of monolayers of mercaptoundecanol. The slightly lower capacitance values compared with those of monolayers of mercaptoundecanol are indicative of a thicker monolayer in the case of the mixed monolayers, which is consistent with the cyclodextrin head groups resting on top of the closely packed alkyl part of the monolayer. We did not see phase-segregated domains in AFM images of the mixed monolayers, and this also suggests the formation of well-mixed, densely packed monolayers. At 17% of **2**, the packing properties of the layers start to deteriorate, as shown by increasing capacitance and decreasing R_{CT} values. Possibly, the very tight packing of the cyclodextrin head groups gives rise to the introduction of more defects. The monolayers of pure **2** have a considerably lower R_{CT} than the mixed monolayers, showing that the absence of the mercaptoundecanol causes more defects in the monolayer. In accordance, the capacitance value is much higher than that of the mixed monolayers, which means that the pure layer is thinner than the mixed monolayers. The capacitance is slightly lower than the previously reported values for monolayers of short-chain heptathioethers.[10a] This is tentatively attributed to the formation of two layers of cyclodextrins, as predicted by molecular dynamics.[12] Alternatively, it could be the result of the cavities being at a larger average distance from the surface, owing to the long S-alkyl spacer. The characterization of these cyclodextrin monolayers reveals that (except for pure **2**) they are densely packed with their cavities exposed to the outer surface of the monolayer.

We used SPR spectroscopy to monitor host–guest interactions between steroids and cyclodextrin monolayers.[13] Changes in the refractive index and thickness near an interface can readily be detected by SPR. Experimentally, the "plasmon resonance angle" is determined, which is the angle under which light, reflected at a prism/metal interface in the Kretschmann configuration, exhibits a minimum in the reflectance. The change of the plasmon angle during a surface binding experiment is proportional to the amount of material bound to the surface.[14]

The addition of ferrocenemethanol (**3**), 4-*tert*-butylphenylacetanilide (**4**), and 1-acetamidoadamantane (**5**), all of which contain known binding moieties for β-cyclodextrin in solution,



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to a monolayer of **1** gave rise to rapid and reversible changes in the SPR angle. The interaction of monolayers of **1** with small neutral organic guests was studied in detail by titration (Figure 3). The experimental data could be fitted to Langmuir isotherms (solid lines), confirming the previous finding that only one type of binding site is present on the monolayer and

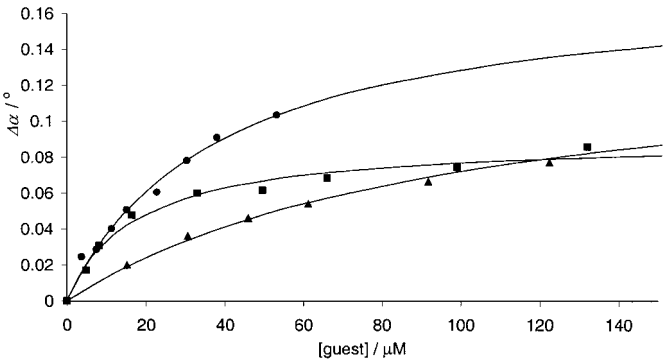

Figure 3. Change in SPR angle ($\Delta\alpha$) of a monolayer of **1** as a function of the concentration of ferrocenemethanol **3** (▲), 4-*tert*-butylphenylacetanilide **4** (●), and 1-acetamidoadamantane **5** (■).

that the cavities behave independently.[10a] When monolayers of mercaptoundecanol were put in contact with the same guest concentrations, no change in the SPR angle was observed, proving that the change in SPR angle is indeed the result of host–guest complexation at the monolayer of **1**.

To compare the binding of these guests by surface-confined cyclodextrins with the binding of these molecules by β-cyclodextrin in solution, the binding constants in solution were determined by microcalorimetry. The association constants for these small guests obtained at a monolayer and in solution are in surprisingly good agreement (Table 2). This indicates that the interior of the cavity is hardly affected by the perfunctionalization of the primary rim and that the

Table 2. The interaction of guests **3**–**5** with monolayers of **1** and with β-cyclodextrin in solution.

	1		Solution		
Guest	K [M^{-1}]	$\Delta\alpha_{sat}$ [°]	K [M^{-1}]	ΔH [kcal mol^{-1}]	$T\Delta S$ [kcal mol^{-1}]
3	9.9×10^3	0.145	1.0×10^4	−6.1	−0.7
4	2.6×10^4	0.179	3.0×10^4	−5.2	0.9
5	5.7×10^4	0.090	6.8×10^4	−5.9	0.7

microenvironment of a cavity in the monolayer is comparable to that of a cavity in solution. It shows that cyclodextrin heptathioether **1** is an excellent receptor adsorbate for the detection of small organic compounds, which bind in the cyclodextrin cavity.

Steroids are present in all eukaryotic organisms, where they play a role in numerous processes.[15] Their biological importance and hence their detection has attracted great scientific interest.[16] Our group has shown that resorcin[4]arene-based receptors complex steroids in chloroform solutions.[17] When we incorporated this class of receptors in monolayers, the interaction with steroids appeared to be largely governed by the hydrophobicity of the guest.[18] A class of steroids **6a**–**6e** whose interaction with cyclodextrins has been well studied is that of the bile salts. Their recognition by cyclodextrin derivatives has been studied both by our group[19] and by others.[20] NMR experiments have shown that these steroids are complexed through the cavity, with the aliphatic side chain of the steroid entering the cyclodextrin from the secondary

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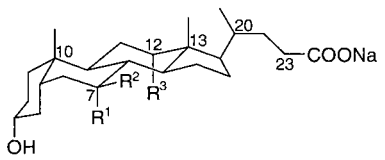

	Steroid	R^1	R^2	R^3
6a	Cholate	OH	H	OH
6b	Deoxycholate	H	H	OH
6c	Chenodeoxycholate	OH	H	H
6d	Ursodeoxycholate	H	OH	H
6e	Lithocholate	H	H	H

side.[21] Steroids **6a** and **6b** are not complexed as deeply as the others, because of the presence of the hydroxy group at C12 of the steroid skeleton.[22]

The interaction of monolayers of **1** with these steroids was also studied by SPR. The changes in the SPR angle ($\Delta\alpha$) as a function of the concentration of steroids for a monolayer of **1** are plotted in Figure 4. The experimental $\Delta\alpha$ data could be

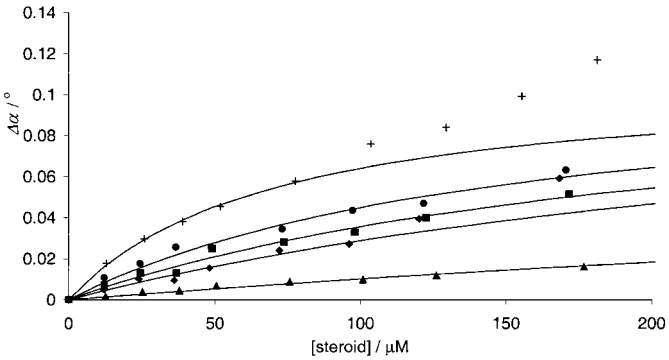

Figure 4. Change in SPR angle ($\Delta\alpha$) at a monolayer of **1** as a function of the concentration of **6a** (▲), **6b** (●), **6c** (■), **6d** (◆), **6e** (+). For **6e**, the last three points were not incorporated in the Langmuir fit.

fitted to Langmuir isotherms (solid lines). The titration data for steroid **6e** deviated from the fitted curve at higher concentrations, where $\Delta\alpha$ started to increase linearly. Titration of the steroids to a monolayer of mercaptoundecanol showed a linear increase in $\Delta\alpha$ for the higher concentrations of steroid **6e** and no change for the other steroids. Although the concentrations of the steroids were chosen to be below the critical micelle concentration (cmc), steroid **6e**, the most hydrophobic, apparently has some aspecific interaction with the layers. Thus, for **6e** the last points of the titration were not considered in the Langmuir fitting procedure.

The association constants obtained from the fitting procedure are shown in Table 3. Comparison with the previously reported solution data[19] for the complexation of these steroids by β-cyclodextrin reveals that the association constants in solution are higher than those at the surface. More interestingly, there is a difference in selectivity. In solution, **6a** and **6b** have far lower stability constants than the other steroids. In contrast, the monolayers of **1** complex steroid **6b** more strongly than its isomers **6c** and **6d**. The difference in selectivity for the steroids between solution and monolayers may be due to the fact that these relatively large guests are

Table 3. The interaction of bile salts with cyclodextrin monolayers and with β-cyclodextrin and a β-cyclodextrin dimer in solution.

	1		9% **2**			Solution[a]	
Guest	K [M^{-1}]	$\Delta\alpha_{sat}$ [°]	K [M^{-1}]	$\Delta\alpha_{sat}$ [°]	$K_{\beta\text{-CD}}$ [M^{-1}]	K_{dimer} [M^{-1}]	[M^{-1}]
6a	9.7×10^2	0.110	6.8×10^3	0.053		4.1×10^3	2.8×10^5
6b	6.4×10^3	0.114	1.1×10^4	0.072		3.6×10^3	2.4×10^6
6c	4.5×10^3	0.115	1.9×10^4	0.108		1.8×10^5	5.2×10^6
6d	4.2×10^3	0.124	4.8×10^4	0.107		7.8×10^5	3.6×10^6
6e	1.3×10^4	0.109	8.6×10^4	0.120		1.9×10^6	$8.9\cdot10^6$

[a] Taken from ref. [19].

complexed through the cavity instead of in the cavity. The persubstitution of the primary side blocks one side of the cyclodextrin and prevents protrusion through the cavity. Steroid **6b** is less affected by the blocking of the primary side, as in solution it is already less deeply included than **6c** and **6d**.[22]

The saturation values obtained by the fitting procedure were approximately the same ($\Delta\alpha_{max}\approx0.115°$) for all of the steroids. This was expected, as the amount of material bound at the surface for these similar steroids is nearly identical. The absolute value of the change in SPR angle can be related to a mass change.[23] Although this relationship is dependent on the type and thickness of the metal, the mass increase for the formation of a 1:1 host–guest complex should give rise to approximately this saturation value in the system used.[24]

Mixed monolayers of **2** and mercaptoundecanol containing 9% of **2** have close to the same surface concentration of cyclodextrins as monolayers of **1**. The binding of steroids to a monolayer with 9% of **2** was compared with the binding to monolayers of **1**. In the mixed monolayers, the cyclodextrins are spaced sufficiently far apart for the cavities to be readily accessible, and concentrated enough to ensure reasonable changes in the SPR angle upon complexation of guests in the cavities. The titration data for the addition of bile salts **6a**–**6e** to this layer are shown in Figure 5. Again, steroid **6e** showed some aspecific interaction at higher concentrations. It can easily be seen that these mixed monolayers interact more strongly with **6c**–**6e** than with **6a** and **6b**. This is in accordance with the complexation behavior known from solution and supports the notion that the different selectivity of monolayers of **1** towards the various bile salts is caused by

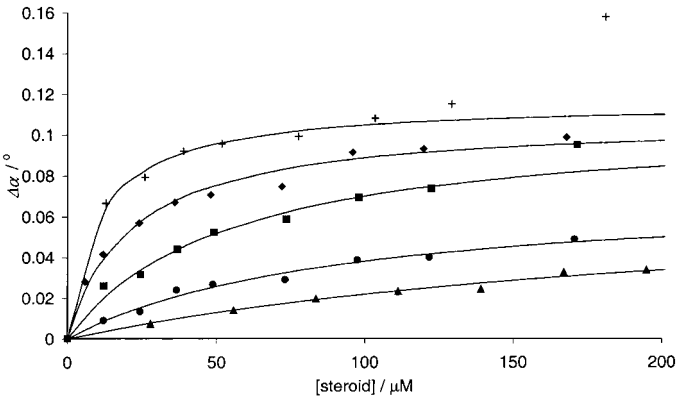

Figure 5. Change in SPR angle ($\Delta\alpha$) at a mixed monolayer containing 9% of **2** as a function of the concentration of **6a** (▲), **6b** (●), **6c** (■), **6d** (◆), **6e** (+). For **6e**, the last two points were not incorporated in the Langmuir fit.

the persubstitution on the primary rim and the resulting architecture of the monolayer.

When the titration data were fitted to Langmuir isotherms, the saturation values of the different steroids varied (Table 4). For **1a** and **1b**, far lower values were found than for the other

Table 4. Ratio of monomer to dimer complexation by mixed monolayers of **2**.

Guest	9% **2** θ_1:θ_2	4.5% **2** θ_1:θ_2
6a	0:100	0:100
6b	0:100	0:100
6c	48:52	67:33
6d	48:52	69:31
6e	59:41	85:15

steroids. This difference may be explained by looking at the binding behavior in solution. In solution, steroids **1a** and **1b** are known to require a β-cyclodextrin dimer for strong complexation,[19] whereas the other steroids are already strongly complexed by native β-cyclodextrin. At the 9% cyclodextrin surface, the cavities are fairly close to each other. Therefore, we assume that two processes can occur, as shown in Equations (1) and (2).

$$H + G \rightleftharpoons HG,\ K_1 = \frac{[HG]}{[H][G]} \quad (1)$$

$$H_2 + G \rightleftharpoons H_2G,\ K_2 = \frac{[H_2G]}{[H_2][G]} \quad (2)$$

In these equations, [H] and [H_2] are the surface concentrations of monomeric and dimeric binding sites, respectively, [G] is the guest concentration in solution, and [HG] and [H_2G] are the surface concentrations of the 1:1 complexes of a guest in a monomeric and dimeric binding site, respectively. Here, we assume that the binding behavior of all dimeric binding sites can be described by a single, average binding constant K_2. If we define surface coverages θ_1 and θ_2 as in Equation (3), Equation (4) follows .

$$\theta_1 = \frac{[HG]}{[H]_{tot}},\ \theta_2 = \frac{[H_2G]}{[H_2]_{tot}} = \frac{2[H_2G]}{[H]_{tot}} \quad (3)$$

$$K_1 = \frac{\theta_1}{(1-\theta_1-\theta_2)[G]},\ K_2 = \frac{\theta_2}{(1-\theta_1-\theta_2)[G]} \quad (4)$$

If the presence of guest molecules at the surface causes the same change in SPR angle independent of the number of cavities they are bound by, the total change is given by Equation (5).

$$\Delta\alpha = (\theta_1 + 1/2\theta_2)\Delta\alpha_{max} \quad (5)$$

Where $\Delta\alpha_{max}$ is the maximum possible SPR angle change, reached for purely monomeric complexation. Therefore, Langmuir binding curves are expected with saturation values between $1/2\Delta\alpha_{max}$ and $\Delta\alpha_{max}$. From comparison with the monolayer of **1**, $\Delta\alpha_{max}$ is estimated to be approximately 0.145° for a 9% layer of **2**.[25] Using this value, we calculated the ratio of monomer complexation to dimer complexation at the surface for steroids **6a**–**6e** (Table 4).[26] Steroids **6a** and **6b** are bound by two cyclodextrin cavities rather than one. This is in agreement with the strong preference of these guests for complexation by a dimer in solution.[19] It should be noted, however, that K values for a dimer cannot be directly compared with K_2 values obtained here, since the conformation a dimer adopts in order to bind a guest in solution may be entirely different from the orientation of and distance between the cavities in a monolayer. Moreover, the surface case merely represents an average situation. A mixture of monomer and dimer complexation is observed for **6c**–**6e**, also in qualitative agreement with the binding behavior in solution.[19]

Further evidence for 2:1 binding at the surface came from diluting the monolayer. The K_1 values are unaffected by this dilution, but the K_2 values should decrease because of the increased distance between the cyclodextrin cavities.[27] This should therefore be reflected in a change of the θ_1:θ_2 ratio [Eq. (4)] and thus to a change in the saturation value of $\Delta\alpha$ [Eq. (5)]. For steroids **6c**–**6e** dilution of the monolayer indeed led to a markedly increased ratio of monomer to dimer complexation (Table 4), supporting the model of both dimer and monomer complexation at the surface. Further dilution of the cyclodextrin adsorbate in the monolayer, which might have enabled the observation of monomer complexation even for **1a** and **1b**, reduced the SPR signal so that it was too small to obtain reproducible results.

Titrations of steroids to monolayers containing 17% of **2** were not described well by a Langmuir isotherm. Possibly, the lack of freedom of the cyclodextrin cavities in these layers causes the presence of different absorption sites as observed for pure cyclodextrin monothiol layers.[7f]

Conclusion

Densely packed monolayers can be prepared by filling the space under the cyclodextrin head group, either by persubstitution of the primary rim or by coadsorption of a monosubstituted cyclodextrin with a simple mercaptoalcohol. These monolayers have well-defined host-guest interactions with known guests for β-cyclodextrin. In all cases, the response to a certain concentration of an analyte is rapid and reversible, making these monolayers excellent candidates for online sensing applications. The selectivity of the monolayers depends on their architecture. Monolayers of the β-cyclodextrin heptathioether have excellent recognition properties for small organic guests that bind in the cavity, such as 1-acetamidoadamantane and ferrocenemethanol. For larger guests, like steroids, the selectivity of monolayers of monofunctionalized cyclodextrin more closely resembles the binding by native β-cyclodextrin in solution. Such cyclodextrin derivatives organized in a monolayer appear to be capable of cooperativity. The mode of incorporation of a cyclodextrin into a monolayer is a means of altering its selectivity. This offers the possibility may make it possible to screen sensor molecules for selectivity for a certain guest by assembling receptors on a monolayer, rather than by first synthesizing the optimal receptor and then incorporating it into a monolayer.

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Experimental Section

General: β-Cyclodextrin was dried prior to use. All other chemicals were used as received unless otherwise stated. Solvents were purified according to standard laboratory methods.[28] All reactions were carried out in an inert atmosphere. NMR spectra were taken on a 300 MHz NMR spectrometer, using residual solvent protons or tetramethylsilane as an internal standard. TLC was performed on aluminum sheets precoated with silica gel 60 F_{254} (Merck). The cyclodextrin spots were visualized by dipping the sheets in 5% sulfuric acid in ethanol followed by heating. Chromatographic separations were performed on silica gel 60 (Merck, 0.040–0.063 mm, 230–240 mesh). Matrix assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry was carried out using a Perseptive Biosystems Voyager-DE-RP MALDI-TOF mass spectrometer. FAB-mass spectra were obtained with a Finnigan MAT 90 spectrometer. For MALDI-TOF mass spectrometry α-cyano-4-hydroxycinnamic acid and for FAB-mass spectrometry *m*-nitrobenzylalcohol were used as the matrix. *tert*-Butyldimethylsilyl (TBDMS) protected β-cyclodextrin (TBDMSCD) was prepared according to a literature procedure.[29]

Heptakis-{6-deoxy-6-[12-(thiododecyl)dodecanamido]}-β-cyclodextrin (1): Compound **1** was synthesized with a procedure analogous to one that we have published before.[10a] Yield = 45%. $R_f = 0.56$ (CH_2Cl_2/MeOH 30% v/v); ^{1}H NMR ($CDCl_3$): $\delta = 7.06$ (s, 7H), 6.56 (s, 7H), 5.12 (s, 7H), 4.80 (s, 7H), 3.96–3.08 (m, 42H), 2.42 (t, ^3J(H,H) = 7.5 Hz, 28H), 2.22–2.05 (m, 14H), 1.52–1.45 (m, 42H), 1.29–1.19 (m, 238H), 0.83 (t, ^3J(H,H) = 6.5 Hz, 21H); ^{13}C NMR ($CDCl_3$): $\delta = 174.2$, 102.5, 84.3, 73.4, 71.1, 54.3, 43.1, 37.2, 36.4, 32.2, 31.9, 29.7, 29.6, 29.5, 29.3, 29.0, 26.0, 22.7, 14.2; MS (MALDI-TOF): calcd for $C_{210}H_{399}N_7O_{35}S_7$: 3807, found 3830 $[M + Na]^+$.

12-Bromo-1-(*S*-trityl)mercaptododecane:[30] A mixture of 1,12-dibromododecane (5.00 g, 3.68 mmol), triphenylmethyl mercaptane (17.8 g, 0.111 mmol), and potassium carbonate (3 g) in acetonitrile (300 mL) was refluxed overnight. After evaporation of the solvent, the residue was dissolved in dichloromethane and washed with HCl (1M), NaOH (1M), and brine, and dried over $MgSO_4$. After removal of the solvent the crude product was purified by repeated crystallization from hexane to give 12-bromo-1-(*S*-trityl)mercaptododecane as a colorless solid in 45% yield. ^{1}H NMR ($CDCl_3$): $\delta = 7.45$ (d, $J = 7.8$ Hz, 6H), 7.35–7.09 (m, 9H), 3.33 (t, $J = 7.5$ Hz, 2H), 2.06 (t, $J = 7.5$ Hz, 2H), 1.82–1.72 (m, 2H), 1.45–1.10 (m, 18H); ^{13}C NMR ($CDCl_3$): $\delta = 174.2$, 102.5, 84.3, 73.4, 71.1, 54.3, 43.1, 37.2, 36.4, 32.2, 31.9, 29.7, 29.6, 29.5, 29.3, 29.0, 26.0, 22.7, 14.2; FAB-MS: calcd for $C_{31}H_{39}BrS$: 522.2, found 523.3 $[M + H]^+$.

Heptakis(6-O-*tert*-butyldimethylsilyl)mono-2-O-(12-thiotrityl-dodecyl)-β-cyclodextrin (2a): LiH (18 mg, 2.3 mmol) was added to a solution of dried (100 °C, 0.1 mbar, 5 h) TBDMSCD[29] (2.0 g, 1.03 mmol) in dry THF (30 mL). The mixture was refluxed for 2 h. 1-Bromo-12-thiotrityldodecane (0.87 g, 1.7 mmol) was added and reflux was continued for 16 h. The solvent was removed in vacuo and the residue was dissolved in dichloromethane. The solution was washed with HCl (1M), water, and brine, and dried over $MgSO_4$. After removal of the solvent and purification by column chromatography (ethyl acetate/ethanol/water 100:2:1), the product was obtained as a white powder in 30% yield. ^{1}H NMR ($CDCl_3$): $\delta = 7.40–7.14$ (m, 15H), 4.88–4.84 (m, 7H), 4.12–3.13 (m, 44H), 2.07 (t, $J = 8$ Hz, 2H), 1.58–1.03 (m, 18H), 0.86–0.79 (m, 63H), 0.02 - -0.04 (m, 42H); FAB-MS: calcd for $C_{115}H_{206}O_{35}SSi_7$: 2373.2, found 2374.2 $[M - H]^-$.

Mono-2-O-(12-thiododecyl)-β-cyclodextrin (2): A solution of triethylsilane in trifluoroacetic acid was added to a solution of heptakis(6-O-*tert*-butyldimethylsilyl)mono-2-O-(12-thiotrityl-dodecyl)-β-cyclodextrin (0.40 g, 0.17 mmol) in trifluoroacetic acid until it became colorless. The solvent was removed in vacuo and methanol was added and evaporated three times to remove residual acid. The residue was dissolved in water and washed three times with diethyl ether. After lyophilization the product was obtained as a white powder in 76% yield. ^{1}H NMR (D_2O): $\delta = 5.06–4.88$ (m, 7H), 3.84–3.39 (m, 44H), 2.38 (t, $J = 9$ Hz, 2H), 1.42–1.12 (m, 18H); FAB-MS: calcd for $C_{54}H_{94}O_{35}S$: 1334.5, found 1333.8 $[M - H]^-$.

Calorimetry: Titrations were performed at 25 °C using a Microcal VP-ITC titration microcalorimeter. Sample solutions were prepared using pure water (Millipore Q2). Titrations were performed by adding aliquots of a β-cyclodextrin solution to the guest solution. The titrations were analyzed using a least squares curve fitting procedure. Control experiments were performed to correct for the heats of dilution of host and guests.

Monolayers, gold substrates: Gold substrates were prepared by vapor deposition of 200 nm gold on a glass slide of 25 mm diameter with a 2 nm chromium layer for adhesion. Before use, the gold substrates were cleaned in an oxygen plasma for 5 min. The resulting oxide layer was removed by leaving the substrates in EtOH for 10 min.[31] For SPR measurements 47.5 nm thick gold-coated glass substrates were used. For AFM measurements, gold substrates were purchased from Metallhandel Schröer GmbH, Lienen, Germany (200 nm gold on 5 nm chromium on glass substrates [11×11 mm^2]). These samples were stored under nitrogen. Prior to use, substrates were flame annealed with a H_2 flame (quality 6). The annealing yielded reproducibly large Au(111) terraces of a few square micrometers in size. After annealing, the substrates were allowed to cool to room temperature and transferred with minimal delay into the adsorption solution.

Monolayer preparation: All glassware used to prepare monolayers was immersed in *piraña* at 70 °C for 1 h. **Warning**! *piraña* solution should be handled with caution; it has detonated unexpectedly. Next, the glassware was rinsed with large amounts of high purity water (Millipore). Cleaned gold substrates were immersed with minimal delay into a 0.1 mM adsorbate solution in EtOH and H_2O (2:1, v/v) for 16 h. The sulfide monolayers were prepared at 60 °C in EtOH and $CHCl_3$ (1:2, v/v) for 16 h. Subsequently, the substrates were removed from the solution and rinsed repeatedly with chloroform, ethanol, and water to remove any physisorbed material.

Monolayer characterization: The advancing and receding contact angles with water were measured on a Krüss G10 Contact Angle Measuring Instrument equipped with a CCD camera. The contact angle measurements were measured during the growth and shrinkage of a droplet. Electrochemical measurements (cyclic voltammetry and impedance spectroscopy) were performed on a Autolab PGSTAT10 (ECOCHEMIE, Utrecht, The Netherlands) in a three electrode system consisting of a gold working electrode (clamped to the bottom of the cell, exposing a geometric area of 0.44 cm^2 to the electrolyte solution), a platinum counter electrode, and a mercurous sulfate reference electrode (+0.61 V_{NHE}). Cyclic voltammetric capacitance measurements were conducted in 0.1M K_2SO_4 between −0.35 V_{MSE} and −0.25 V_{MSE} at scan rates ranging from 0.1 V s^{-1} to 2.0 V s^{-1}. Impedance spectroscopy measurements were performed in 1mM $[K_3Fe(CN)_6]$/$[K_4Fe(CN)_6]$ and 0.1M K_2SO_4 at −0.2 V_{MSE} with an amplitude of 5 mV using a frequency range from 50 kHz to 0.1 Hz. The charge-transfer resistance of the monolayer was obtained by fitting the experimental data to an equivalent circuit consisting of the monolayer resistance parallel with the monolayer capacitance, in series with the solution resistance.[32] The AFM measurements were carried out with a Nanoscope III AFM (Digital Instruments, Santa Barbara, California, USA) in tapping mode. AFM scans were performed in water using a liquid cell. Silicon nitride cantilevers with nominal spring constants of 0.38 N m^{-1} and 0.06 N m^{-1} were used. SPR measurements were performed in a two-channel vibrating mirror angle scan setup based on the Kretschmann configuration, described by Kooyman and co-workers.[33] Light from a 2 mW HeNe Laser is directed onto a prism surface by means of a vibrating mirror. The intensity of the light is measured by means of a large-area photodiode. This set-up allows determination of changes in plasmon angle with an accuracy of 0.002°. The gold substrate with the monolayer was optically matched to the prism using an index matching oil. A cell placed on the monolayer was filled with 800 µL of a 1mM KOH solution. After stabilization of the SPR signal, titrations were performed by removing an amount of KOH solution and adding the same amount of stock solutions of the bile salts in KOH. Between additions, the cell was cleaned by repeated washings with KOH solution (700 µL, three or four times). SPR measurements were repeated three times for each monolayer guest system.

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[1] a) A. Ulman, *An Introduction to Ultrathin Organic Films*, Academic Press, San Diego, CA, **1991**; b) H. O. Finklea in *Electroanalytical Chemistry*, *Vol. 19* (Eds.: A. J. Bard, I. Rubinstein), Marcel Dekker, New York, **1996**, pp. 109 – 335.
[2] a) K.-D. Schierbaum, T. Weiss, E. U. Thoden van Velzen, J. F. J. Engbersen, D. N. Reinhoudt, W. Göpel, *Science* **1994**, *265*, 1413 – 1415 b) E. U. Thoden van Velzen, J. F. J. Engbersen, D. N. Reinhoudt, *Synthesis* **1995**, *8*, 989 – 1008; c) E. U. Thoden van Velzen, J. F. J. Engbersen, P. J. de Lange, J. W. Mahy, D. N. Reinhoudt, *J. Am. Chem. Soc.* **1995**, *117*, 6853 – 6862; d) E. U. Thoden van Velzen, J. F. J. Engbersen, D. N. Reinhoudt, *J. Am. Chem. Soc.* **1994**, *116*, 3597 – 3598; e) B.-H. Huisman, R. P. H. Kooyman, F. C. J. M. van Veggel, D. N. Reinhoudt, *Adv. Mater.* **1996**, *8*, 561 – 564.
[3] a) S. Flink, B. A. Boukamp, A. van den Berg, F. C. J. M. van Veggel, D. N. Reinhoudt, *J. Am. Chem. Soc.* **1998**, *120*, 4652 – 4657; b) S. Flink, F. C. J. M. van Veggel, D. N. Reinhoudt, *J. Phys. Chem. B* **1999**, *103*, 6515 – 6520.
[4] A. Friggeri, F. C. J. M. van Veggel, D. N. Reinhoudt, R. P. H. Kooyman, *Langmuir* **1998**, *14*, 5457 – 5463.
[5] a) G. Wenz, *Angew. Chem.* **1994**, *106*, 851 – 870; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 803 – 822; b) *Chem. Rev.* **1998**, *98*, Issue 5; c) J. Szejtli, T. Osa, *Comprehensive Supramolecular Chemistry, Vol. 3* (Eds.: J. L. Atwood, J. E. D. Davies, D. D. MacNicol, F. Vögtle, J.-M. Lehn), Pergamon, Oxford, **1996**.
[6] a) Y. Maeda, T. Fukuda, H. Yamamoto, H. Kitano, *Langmuir* **1997**, *13*, 4187 – 4189; b) H. Yamamoto, Y. Maeda, H. Kitano, *J. Phys. Chem. B* **1997**, *101*, 6855 – 6860; c) T. Fukuda, Y. Maeda, H. Kitano, *Langmuir* **1999**, *15*, 1887 – 1890; d) H. Kitano, Y. Taira, H. Yamamoto, *Anal. Chem.* **2000**, *72*, 2976 – 2980.
[7] a) M. T. Rojas, R. Königer, J. F. Stoddart, A. E. Kaifer, *J. Am. Chem. Soc.* **1995**, *117*, 336 – 343; b) G. Nelles, M. Weisser, R. Back, P. Wohlfahrt, G. Wenz, S. Mittler-Neher, *J. Am. Chem. Soc.* **1996**, *118*, 5039 – 5046; c) M. Weisser, G. Nelles, P. Wohlfahrt, G. Wenz, S. Mittler-Neher, *J. Phys. Chem.* **1996**, *100*, 17893 – 17900; d) M. Weisser, G. Nelles, G. Wenz, S. Mittler-Neher, *Sens. Actuat. B* **1997**, *100*, 58 – 67; e) C. Henke, C. Steinem, A. Janshoff, G. Steffan, H. Luftmann, M. Sieber, H.-J. Galla, *Anal. Chem.* **1996**, *68*, 3158 – 3165; f) A. Michalke, A. Janshoff, C. Steinem, C. Henke, M. Sieber, H.-J. Galla, *Anal. Chem.* **1999**, *71*, 2528 – 2533; g) P. He, J. Ye, Y. Fang, L. Suzuki, T. Osa, *Anal. Chim. Acta* **1997**, *337*, 217 – 233; h) M. Lahav, K. T. Ranjit, E. Katz, I. Willner, *Chem. Commun.* **1997**, 259 – 260.
[8] a) J. Liu, S. Mendoza, E. Roman, M. J. Lynn, R. L. Xu, A. E. Kaifer, *J. Am. Chem. Soc.* **1999**, *121*, 4304 – 4305; b) J. Liu, J. Alvarez, A. E. Kaifer, *Adv. Mater.* **2000**, *12*, 1381 – 1383.
[9] M. W. J. Beulen, J. Bügler, B. Lammerink, F. A. J. Geurts, E. M. E. F. Biemond, K. G. C. van Leerdam, F. C. J. M. van Veggel, J. F. J. Engbersen, D. N. Reinhoudt, *Langmuir* **1998**, *14*, 6424 – 6429.
[10] a) M. W. J. Beulen, J. Bügler, M. R. de Jong, B. Lammerink, J. Huskens, H. Schönherr, G. J. Vancso, B. A. Boukamp, H. Wieder, A. Offenhäuser, W. Knoll, F. C. J. M. van Veggel, D. N. Reinhoudt, *Chem. Eur. J.* **2000**, *6*, 1176 – 1183; b) H. Schönherr, M. W. J. Beulen, J. Bügler, J. Huskens, F. C. J. M. van Veggel, D. N. Reinhoudt, *J. Am. Chem. Soc.* **2000**, *122*, 5039 – 5046.
[11] a) K. Motesharei, D. C. Myles, *J. Am. Chem. Soc.* **1994**, *116*, 7413 – 7414; b) K. Motesharei, D. C. Myles, *J. Am. Chem. Soc.* **1998**, *120*, 7328 – 7336; c) Wenz suggested coadsorbing hexanethiol and cyclodextrin monothiols as a method to improve the binding properties of monolayers of cyclodextrin (see ref [7d]). However, no binding studies were reported to date.
[12] X. Qian, R. Hentschke, W. Knoll, *Langmuir* **1997**, *13*, 7092 – 7098.
[13] Previously, we measured the complexation of 1-anilinonaphthalene-8-sulfonate in monolayer-confined β-cyclodextrin by electrochemical impedance spectroscopy.[10a] Addition of less highly charged organic guests to monolayers of heptathioether **1** did not lead to a change in the charge transfer resistance. Interactions of guests with the cavities in the monolayer may require a large electrostatic interaction between the reporter redox couple and the guest to give a reponse in an impedance spectroscopy experiment. This may in part be caused by the very high charge transfer resistance of the monolayers.
[14] H. Raether in *Physics of Thin Films*, *Vol. 9* (Eds.: G. Has, M. Francombe, R. Hoffman), Academic Press, New York, **1977**.
[15] a) L. F. Fieser, M. Fieser, *Steroids*, Reihold, New York, **1959**; b) W. Templeton, *An Introduction to the Chemistry of the Terpenoids and Steroids*, Buttworth, London, **1969**.
[16] H. Danielsson, J. Sjövall, *Sterols and Bile Acids*, Elsevier, Amsterdam, The Netherlands, **1985**.
[17] I. Higler, P. Timmerman, W. Verboom, D. N. Reinhoudt, *J. Org. Chem.* **1996**, *61*, 5920 – 5931.
[18] A. Friggeri, F. C. J. M. van Veggel, D. N. Reinhoudt, *Chem. Eur. J.* **1999**, *5*, 3595 – 3602.
[19] M. R. de Jong, J. F. J. Engbersen, J. Huskens, D. N. Reinhoudt, *Chem. Eur. J.* **2000**, *6*, 4034 – 4040.
[20] A. Ueno, *Supramol. Sci.* **1996**, *3*, 31 and references therein.
[21] a) A. Mucci, L. Schenetti, M. A. Vandelli, F. Forni, P. Ventura, G. Salvioli, *J. Chem. Soc. Perkin Trans. 2* **1996**, 2347 – 2349; b) P. Ramos Cabrer, E. Alvarez-Parrilla, F. Meijide, J. A. Seijas, E. Rodríguez Núñez, J. Vázquez Tato, *Langmuir* **1999**, *15*, 5489 – 5495.
[22] Z. J. Tan, X. X. Zhu, G. R. Brown, *Langmuir* **1994**, *10*, 1034 – 1039.
[23] R. P. H. Kooyman, H. Kolkman, J. van Gent, J. Greve, *Anal. Chim. Acta* **1988**, *213*, 35 – 45.
[24] By using the relationship derived for silver in ref. [23], SPR sensitivity is calculated to be approximately 6° for every ng mm^{-2}.
[25] Monolayers of **1** that give $\Delta\alpha_{max} = 0.115°$ have 14 alkyl chains per cavity, giving them the same cyclodextrin concentration as mixed monolayers of 7% of **2** and mercaptoundecanol.
[26] Using $K_1{:}K_2 = \theta_1{:}\theta_2$, which follows from Equation (4).
[27] We have previously shown that dilution of a monolayer of crown ethers prohibits the formation of a sandwich complex between two crown ethers and potassium ions, resulting in a loss of potassium over sodium selectivity for these monolayers. See ref. [3a].
[28] D. D. Perrin, W. F. L. Armarego, *Purification of Laboratory Chemicals*, 3rd ed., Pergamon, Oxford, **1989**.
[29] E. van Dienst, B. H. M. Snellink, I. von Piekartz, M. H. B. Grote Gansey, F. Venema, M. C. Feiters, R. J. M. Nolte, J. F. J. Engbersen, D. N. Reinhoudt, *J. Org. Chem.* **1995**, *60*, 6537 – 6545.
[30] This compound has been synthesized before by a different procedure: S.-W. Tam-Chang, J. C. Mason, *Tetrahedron* **1999**, *55*, 13333 – 13344. The spectral data we present closely resemble those described previously.
[31] H. Ron, I. Rubinstein, *Langmuir* **1994**, *10*, 4566 – 4573.
[32] a) Boukamp, B. A. *Solid State Ionics* **1986**, *18 – 19*, 136; b) Boukamp, B. A. *Solid State Ionics* **1986**, *20*, 31.
[33] A. T. M. Lenferink, R. P. H. Kooyman, J. Greve, *Sens. Act. B* **1991**, *3*, 261 – 265.

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