

Multilayer Self-Assembly of Amphiphilic Cyclodextrin Hosts on Bare and Modified Gold Substrates: Controlling Aggregation via Surface Modification

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Self-assembled multilayers of the three modified cyclodextrins (hexakis(2,3-*O*-hexyl-6-deoxy-6-amino)- α -cyclodextrin (**1**), heptakis(2,3-*O*-hexyl-6-deoxy-6-amino)- β -cyclodextrin (**2**), and octakis(2,3-*O*-hexyl-6-deoxy-6-amino)- γ -cyclodextrin (**3**)) on bare gold, as well as on gold surfaces modified with mercaptopropionic (**4**) and mercaptooctanoic (**5**) acid, were investigated by cyclic voltammetric, contact angle, FT-IR, and quartz crystal microbalance measurements in neutral aqueous media. The level of organization of the aggregates formed by the three CD derivatives (**1**–**3**) on the negatively charged surface of the mercaptopropionic and mercaptooctanoic acid modified gold electrodes proved to be substantially better than that on bare gold electrodes. This finding suggests that although the amphiphilic character of compounds **1**–**3** can induce aggregation on a gold surface, the electrostatic interaction between the carboxylic acid groups and the positively charged cyclodextrins is the primary force leading to the formation of well-organized aggregates.

Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides made up of six, seven, or eight glucopyranose subunits (α -, β -, and γ -CD, respectively).¹ These natural receptors have toroidal shapes and are capable of forming inclusion complexes with a wide variety of hydrophobic guests.² The relatively rigid and well-defined cavities of these molecular hosts, combined with their solubility in water, have encouraged a substantial amount of research work for decades.³ Recently, we prepared a thiolated derivative of β -CD and demonstrated its chemisorption on gold surfaces to yield monolayers containing preformed binding sites.⁴ The CD cavities in these monolayers afford novel binding properties to the electrode–solution interface, a feature which is of obvious interest for the development

of new sensor technologies.⁵ The approach is not limited to CD receptors and our group has also reported two other examples^{6,7} of interfacial molecular recognition using entirely different host–guest pairs. However, the preparation of electrode surfaces capable of molecular recognition has proven rather difficult, typically requiring the assembly of mixed monolayers in several steps. The development of new methods for the easy preparation of monolayer and multilayer assemblies containing preformed binding sites is thus of substantial interest.

In aqueous media, the aggregation of amphiphilic molecules on the surface of working electrodes has been reported to occur at very low concentrations⁸ and often leads to highly organized, interfacial molecular assemblies. Therefore, we reasoned that the amphiphilic character of CD receptors **1**–**3** could be utilized to drive their self-assembly on electrode surfaces.

Among the numerous CD derivatives prepared so far, 2,3-*O*-hexyl-6-deoxy-6-amino- α -, β -, and γ -CD's (**1**–**3**) constitute a very interesting family of amphiphilic molecular hosts.⁹ The alkyl chains attached to the oxygen atoms in the C-2 and C-3 positions can be viewed as a nonrigid extension of the hydrophobic cavity that constitute the complexation site in unmodified CD receptors. Furthermore, the amino groups positioned on the C-6 carbon on the narrow opening of the CD cavity afford, in

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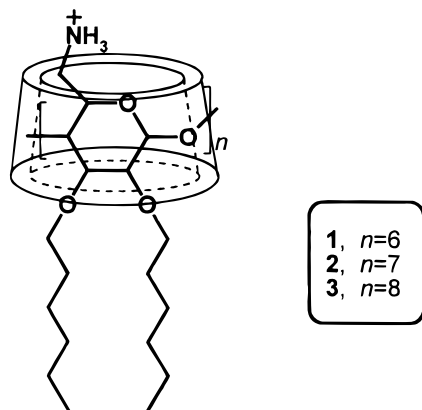
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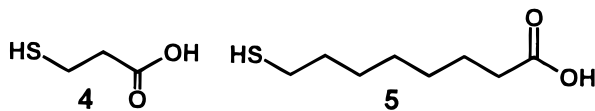
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neutral or acidic aqueous media, a dense crown of positive charges which defines a complexation site for negative-charged guests.^{10–13}

In this work, we report on the self-assembly of **1–3** on gold surfaces and the effect that the modification of the gold surface with the mercaptocarboxylic acids **4** and **5** exerts on the interfacial aggregation of these amphiphilic CD derivatives.



Experimental Section

Materials. 2,3-*O*-hexyl-6-deoxy-6-amino- α -, β -, and γ -CDs (**1–3**) were prepared according to published procedures.^{9a} Mercaptopropionic acid (**4**) was purchased from Aldrich and used without further purification. Mercaptooctanoic acid (**5**) was prepared as described by Whitesides and co-workers.¹⁴ $\text{Ru}(\text{NH}_3)_6\text{Cl}_3$, hexadecane, and Tris for buffer solutions were obtained from Kodak, Aldrich, and Fluka, respectively, and used without further purification. Gold wire 99.999% (0.5 mm diameter) was purchased from Johnson-Matthey and prepared for immediate use or further modification in electrochemical experiments as described elsewhere.^{8b} For Fourier transform infrared (FT-IR) and contact angle measurements, gold substrates were prepared in a clean room facility using a previously described sputtering method.¹⁵ All solutions for electrochemical measurements were freshly prepared using deionized water further purified by passage through a four-cartridge Barnsted Nanopure System ($\rho = 18 \text{ M}\Omega\cdot\text{cm}$).

Electrochemistry. The electrochemical equipment used for the cyclic voltammetry (CV) experiments has been described elsewhere.¹⁶ Gold beads were prepared as previously reported and used as working electrodes in all the electrochemical experiments.^{8b} The surface area of the gold bead electrodes was determined from the slope of the straight line obtained when the CV peak current of a standard 1.0 mM solution of $\text{Ru}(\text{NH}_3)_6^{3+}$ (in 0.1 M NaCl) was plotted as a function of the scan rate.^{8b} Further modification of the gold electrodes was carried out by

exposure to a 1.0 mM solution of the mercaptocarboxylic acid (**4** or **5**) in dry ethanol for a 12 h period. After being rinsed with dry ethanol and deionized water, the modified electrode was immersed in a single-compartment cell containing 10 mL of deoxygenated 1.0 mM $\text{Ru}(\text{NH}_3)_6\text{Cl}_3$ (pH = 7 Tris buffer, $I = 0.1$) solution. The cell was also fitted with a platinum counter electrode and a home-made NaCl saturated calomel electrode (SSCE) reference electrode.

Aggregation of the amphiphilic receptors on bare and modified gold surfaces was followed by the blocking of the electrochemical response (cyclic voltammetry, $\nu = 100 \text{ mV/s}$) of the $\text{Ru}(\text{III})/\text{Ru}(\text{II})$ couple following small aliquot additions (1–3 mL) of a 10 mM solution of the corresponding CD (**1–3**) in DMSO. In order to avoid kinetic effects on the aggregation process, a 4-min equilibration period was allowed before each measurement after each one of the CD additions.

Contact Angle Measurements. Contact angle measurements were carried out in a Ramé-Hart NRL contact angle goniometer (Model 100) equipped with an environmental chamber and a thermocouple that allowed controlled ambient conditions. Chamber humidification was carried out before performing the measurements by letting a small piece of wet paper equilibrate for 30 min inside the closed chamber.

Gold substrate modification to prepare Au-**4** and Au-**5** surfaces was carried out by exposing the freshly prepared sputtered surfaces to 1.0 mM ethanolic solutions of the corresponding thiol (**4** or **5**) for at least 12 h. Further aggregation of the amphiphilic CDs **1–3** on the different gold surfaces under study was performed by immersion of the substrate, during a minimum period of 2 h, into an aqueous dilute solution (40 μM) of the corresponding CD derivative. Contact angle readings were then carried out at 298 K after an equilibration period of at least 10 min and repeated at least three times.

FT-IR Spectroscopy. Grazing angle IR spectroscopy experiments were carried out on carefully cleaned bare and modified gold substrates (Au-**4**, Au-**5**) which were prepared as described above. Samples with adsorbed CD aggregates were also prepared by exposing for at least 2 h the bare or thiol-modified gold surfaces to a 40 μM aqueous solution of compound **2**. After equilibration with the CD aqueous solution, the samples were introduced into an FT-IR Perkin-Elmer 2000 spectrometer equipped with a MCT detector and a grazing reflectance accessory and scanned (400 repetitions) in the 700–5000 cm^{-1} region. Background subtraction was carried out by using spectra of bare or modified (Au-**4** or Au-**5**) gold substrates as references.

The IR spectrum of the crystalline sample of **2** was recorded in a KBr pellet (2% w/w of **2**) prepared by a standard pressing procedure and scanned in the absorbance mode.

QCM Measurements. Quartz crystal microbalance (QCM) measurements were carried out using a Maxtek Model PM-710 frequency monitor equipped with a fully covered Teflon probe designed for experiments in which immersion in the deposition medium exposes only one face of the oscillating quartz crystal to the solution. Since a fraction of the exposed crystal surface is not covered with gold, and there is a possibility of aggregation of the amphiphilic CDs on quartz, the deposition experiments described in this work were carried out using a home-made glass cell attached to the center of the exposed face of the crystal by means of a FETFE O-ring and a metal clamp. In this configuration, the deposition solution contained in the glass cell could only interact with the gold layer covering the center of the quartz crystal.

In order to maintain a constant temperature throughout the experiments, the probe and the attached glass cell, filled with 5 mL of a supporting electrolyte solution (pH = 7 Tris buffer, $I = 0.1$), were immersed in a 4-L water bath and equilibrated for at least 3 h. After this equilibration period, the frequency of the crystal was found to be stable to $\pm 2 \text{ Hz}$ for at least 5 h.

Once the system was stable, aggregation of the amphiphilic CD **2** on the surface of the bare and modified gold electrodes under study was monitored as the CD concentration was gradually increased. The frequency of vibration of the crystal in the neutral supporting electrolyte solution (pH = 7 Tris buffer, $I = 0.1$) was taken as zero mass and the frequency difference before and after exposure of the gold electrode ($A = 0.95 \text{ cm}^2$) to a solution containing increasing concentrations of the modified

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CD **2** (in the 0–75 μM range) provided a direct way to determine the surface coverage as described below. Frequency readings were taken 60 min after the addition of the corresponding aliquot of a concentrated solution of the amphiphilic CD (13 mM in DMSO).

As expected, frequency changes in the instrument as a consequence of mass loading on the quartz crystal oscillator were found to obey the Sauerbrey eq.¹⁷

$$\Delta f = \frac{-2f_0^2 \Delta m}{[\rho\mu]^{1/2} A} \quad (1)$$

where Δf is the change in frequency (Hz) upon mass deposition, f_0 is the resonating frequency (Hz) of the crystal before deposition, ρ and μ are the density (2.648 g/cm³) and shear modulus of quartz (2.947×10^{11} dyn/cm² for an AT cut crystal), respectively, and $\Delta m/A$ is the deposited mass per unit area (g/cm²). This was confirmed by correlating the reported surface coverage of a monolayer of dodecanethiol on gold deposited from a 5 mM ethanolic solution (1.8×10^{-7} g/cm²) with the observed frequency change as described by Turro and co-workers.^{17b}

Density changes induced by the presence of organic solvents in aqueous solution are known to affect the resonance frequency of the QCM crystals.^{17b} In order to correct the deposition data for the presence of DMSO (the solvent in the stock solution), control experiments were carried out to record the frequency changes associated with the corresponding additions (0–30 mL) of DMSO to the neutral aqueous solution. In this way, subtraction of the DMSO-induced frequency changes from the total differences observed in the deposition experiments led to corrected values that only reflect the deposition of **2** on the bare and modified gold surfaces.

Before any of the deposition experiments are set up, the quartz crystal (AT-cut, 5 MHz frequency, polished gold evaporated on a Ti underlayer) was cleaned by treatment with a mixture of H₂O₂/H₂SO₄ (30/70 v/v) at room temperature for 5–10 min. Chemical modification of the evaporated gold electrodes on quartz was carried out by exposing the substrates (already mounted in the glass cell) to the corresponding 1.0 mM ethanolic solution of the mercaptocarboxylic acid for a period of at least 12 h. After being rinsed with ethanol, pure water, and the neutral buffer solution (pH = 7 Tris, $I = 0.1$), the cell was filled with the latter and set for the aggregation experiments.

In the calculations of the surface coverage and thickness of the aggregates, the solvent and supporting electrolyte molecules were not considered and only chloride counterions were assumed to compensate for the cationic CD derivatives adsorbed on the different gold surfaces.

Results and Discussion

Aggregation of Compounds 1–3 on Bare Gold Electrodes. Aggregation of the amphiphilic CDs (**1–3**) on the surface of bare gold electrodes was initially detected by the blocking of the electrochemical response of a 1.0 mM solution of Ru(NH₃)₆Cl₃ in pH = 7 Tris buffer ($I = 0.1$).¹⁸ As can be seen in Figure 1, the presence of the modified CD **2** in concentrations as low as 20 μM is enough to block substantially the reversible voltammetric response of the Ru(III)/Ru(II) couple. A marked decrease in the electron transfer rate is evidenced by the shift of the cathodic peak potential (until its final disappearance when the concentration of **2** reaches 30 μM) and the literal suppression of the anodic peak.

Due to the poor definition of the cathodic peak in the presence of the modified CD receptors, we monitored the effects of increasing concentrations of receptor **2** by plotting the maximum cathodic current observed at a given

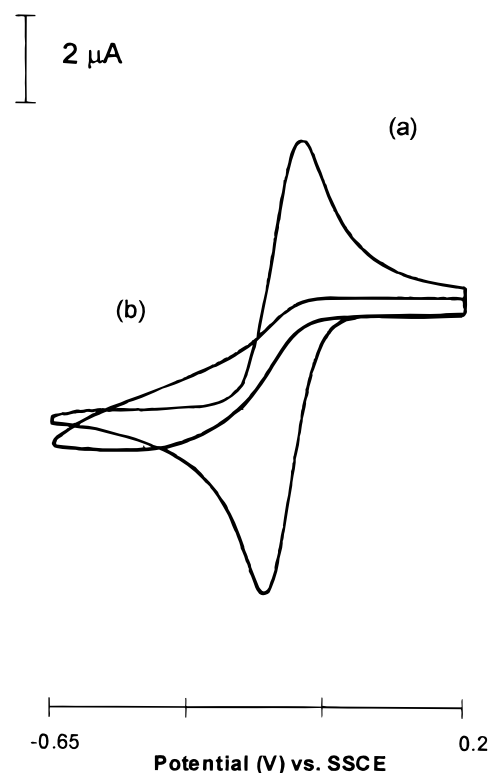


Figure 1. CV response on bare gold of a 1.0 mM Ru(NH₃)₆Cl₃ solution (pH = 7 Tris buffer) (a) in the absence and (b) in the presence of 20 μM **2**. Scan rate was 0.1 V s⁻¹.

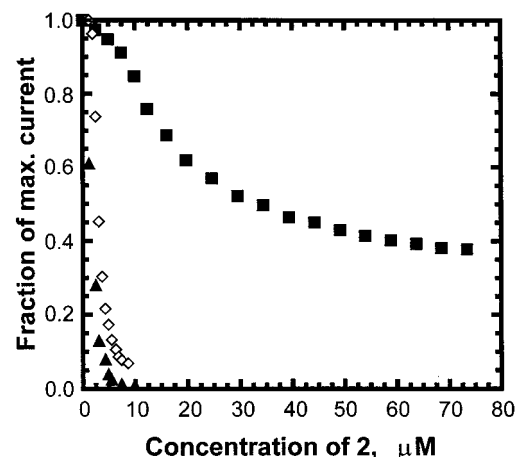


Figure 2. Fraction of the cathodic peak current observed for 1.0 mM Ru(NH₃)₆Cl₃ (ipc) in the presence/absence of the modified CD) using (■) bare gold, (◇) Au-4, and (▲) Au-5, modified electrodes as a function of the concentration of **2** in the contacting solution. Scan rate was 0.1 V s⁻¹.

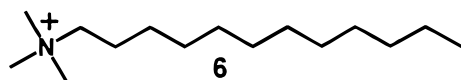
concentration of **2** normalized to the cathodic peak current observed in the absence of **2**. As can be seen from the resulting curve (Figure 2), a reduction of the maximum cathodic current density of about 60% at concentrations of **2** of 75 μM clearly reveals the aggregation of the amphiphilic receptor on the gold electrode surface.

In order to investigate the influence of the CD unit on the aggregation of the amphiphilic receptor, we performed control electrochemical blocking experiments with the simple surfactant **6** replacing the modified CD **2**. The length of this surfactant is comparable to the total length of receptors **1–3** (from the crown of positive charges to the end of the alkyl tails).

Absolutely no blocking of the Ru(III) voltammetric response was observed when **6** was present in the 2–500

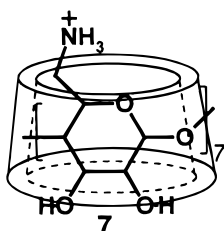
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(18) Tris was selected instead of phosphate for the buffer system because the phosphate anions interact strongly with amino-CD derivatives. See, for instance, refs 10 and 13.



μM concentration range revealing that the amphiphilic cyclodextrins have a much larger tendency to aggregate at the gold–solution interface than individual surfactant molecules. Therefore, this finding suggests that the grouping of amphiphilic functionalities afforded by the cyclic oligomeric structure of the CD derivatives is an important factor that fosters their aggregation on the gold surface.

No blocking of the Ru(III) cathodic voltammetric response was observed when the nonamphiphilic CD **7** was used in place of receptor **2**. In this case, the modified heptakis(6-deoxy-6-amino)- β -cyclodextrin features the same CD glucopyranose units as compound **2**, but lacks the hydrophobic alkyl chains at positions C-2 and C-3. The inability of this CD to block the Ru(III) voltammetric response strongly indicates that the combination of the CD molecular pocket and the covalently attached alkyl chains on the C-2 and C-3 positions in compound **2** are responsible for the effective aggregation of this compound on the surface of bare gold electrodes.



Moreover, the number of sugar units in the CD receptor seems to have little or no effect on the aggregation properties of the amphiphilic CDs on bare gold surfaces since voltammetric experiments with compounds **1** and **3** at comparable concentration levels resulted in similar blocking behavior of the electrochemical response of the Ru(III) probe.

Aggregation of **1–3** on Modified Gold Electrodes.

Self-assembled monolayers provide an ideal methodology to design and control the chemical functionality of surfaces.¹⁹ We anticipated that the aggregation of the amphiphilic CDs **1–3** should therefore be markedly affected by the chemical modification of the gold surface. From previous studies it is known that at neutral pH most of the amino groups of the modified CD receptor⁷ are protonated.^{12c} This positively charged side of the amphiphilic CD receptors can be used to template electrostatically the aggregation of the CD amphiphiles if we position an array of negatively charged functional groups ($-\text{COO}^-$) on the surface of the gold electrode.²⁰

In order to investigate how the interfacial aggregation of compounds **1–3** was affected by the presence of negatively charged functional groups at the gold surface, we repeated the Ru(III) blocking experiments using gold electrodes modified with the two mercaptocarboxylic acids (**4** and **5**). Figure 3 shows the CV response of a 1 mM

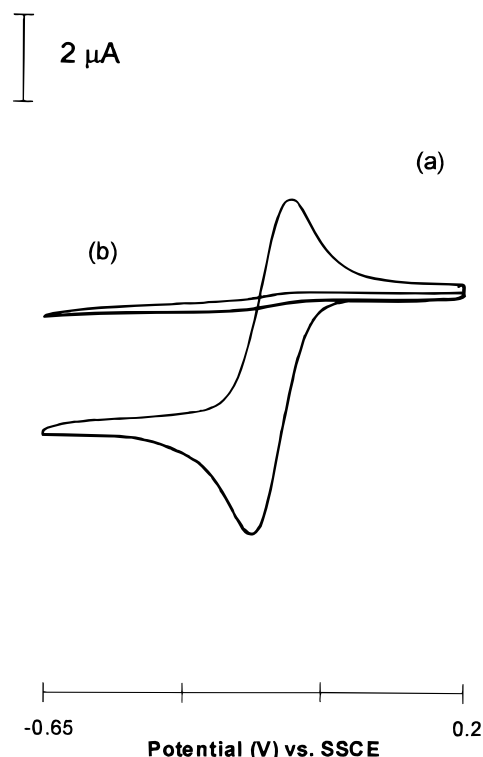


Figure 3. CV response on a Au–**4** electrode of a 1.0 mM Ru(NH₃)₆Cl₃ solution (pH = 7 Tris buffer) (a) in the absence and (b) in the presence of 5 μM **2**. Scan rate was 0.1 V s^{−1}.

Ru(III) solution on a Au–**4** modified electrode before and after the addition of compound **2** to a final concentration of 5 μM . Comparison of Figures 1 and 3 shows a greatly improved blocking effect of the Ru(III) voltammetric response by CD **2** on the Au–**4** electrode surface. On this modified surface a 5 μM concentration of the amphiphilic receptor is enough to reduce the maximum current density by roughly 80%. As can be seen in Figure 2, the improved blocking efficiency of the interfacial aggregates of **2** on modified Au–**4** substrates proved to be even more effective in the case of Au–**5** electrodes in which 5 μM of the receptor can block 95% of the Ru(III) electrochemical response. Just as in the case of bare gold electrodes, the effect on the aggregation of the number of sugar units in the amphiphilic CDs does not seem to be an important factor since receptors **1** and **3** yielded similar results to those presented in Figure 2 for compound **2**.

Surface-attached molecules of **4** on gold are partially deprotonated at neutral pH and, therefore, exert an important electrostatic interaction on the positively charged end of the CD amphiphiles in solution. Due to this interaction, the amphiphilic aggregation of the CD receptors must proceed with a favored orientation, approximately normal to the electrode surface, that is reflected in the experimentally observed enhancement of the voltammetric blocking effects. The same type of argument applies to gold substrates modified with the longer thioacid **5** in which packing of the carboxylic acid on the self-assembled monolayer (SAM) should be even better. It is worth noting that although the pK_a of **5** in a SAM has been reported to lie in the 8–9 range,^{21,22} electrostatic effects (ultimately responsible for templating effects on the aggregation of the amphiphilic CDs) arising

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Table 1. Contact Angles Measured with Drops of Pure Water and Hexadecane on Bare and Modified Gold Electrodes at 298 K^a

Surface	H ₂ O	CH ₃ (CH ₂) ₁₄ CH ₃
Au	<5	<5
Au-4	<5	<5
Au-5	<5	<5
Au + 1	72	9
Au + 2	75	9
Au + 3	72	12
Au-4 + 1	70	17
Au-4 + 2	68	24
Au-4 + 3	69	28
Au-5 + 1	69	22
Au-5 + 2	67	24
Au-5 + 3	66	22

^a Every value (in degrees) corresponds to an average of at least 3 readings after a 10 min equilibration period in the environmental chamber.

from deprotonated carboxylic groups have been clearly observed at neutral pH.²²

Characterization of the Amphiphilic Aggregates.

The marked electrode passivation effects that 1–3 exert on the voltammetric response of the Ru(III) electroactive probe clearly suggest that the structure of the aggregates on the carboxylate surfaces must be different than that on bare gold. In order to characterize the templating effect that the carboxylic acids 4 and 5 have on the orientation and packing of the amphiphilic CDs surveyed here, we carried out contact angle, FT-IR spectroscopy, and QCM measurements on these systems.

Contact angle measurements of drops of water and hexadecane on bare and modified gold substrates (Au-4 and Au-5) were carried out at 298 K as described in the Experimental Section. The results of these measurements are presented in Table 1. The low contact angle values of water on bare and carboxylic acid modified gold surfaces are in excellent agreement with previously reported data and reflect the strong hydrophilic character of the three surfaces.^{14,21,23} Upon exposure to the amphiphilic receptors 1–3 (40 μ M aqueous solutions for at least 2 h), the bare and modified gold substrates exhibit contact angle values which reveal an increased hydrophobic character. Since most of the external surface of compounds 1–3 is hydrophobic, it is reasonable to expect an increase of the contact angle of water when any of the gold substrates is covered with aggregates of these compounds. This is clearly the case as reflected by the 65° increase that, on the average, the bare and modified gold surfaces exhibit upon exposure to the amphiphilic receptors (see data in the first column of Table 1).

Further insight into the orientation that the amphiphilic molecules adopt on the gold surface was obtained by using hexadecane instead of water in the contact angle measurements. The extent of wetting measured with hexadecane drops is very sensitive to the type of hydrophobic surface contacted. For instance, whereas a "pure" CH₂ surface would be completely wet by a hexadecane drop (contact angle <5°), a contact angle of 46° would reveal the presence of a "pure" CH₃ hydrophobic surface.²⁴ As can be seen in the second column of Table 1, compounds 1–3 aggregated on bare gold surfaces (rows 4–6) yield contact angles with hexadecane that are very similar (approximately 10°). On the other hand, the values of the

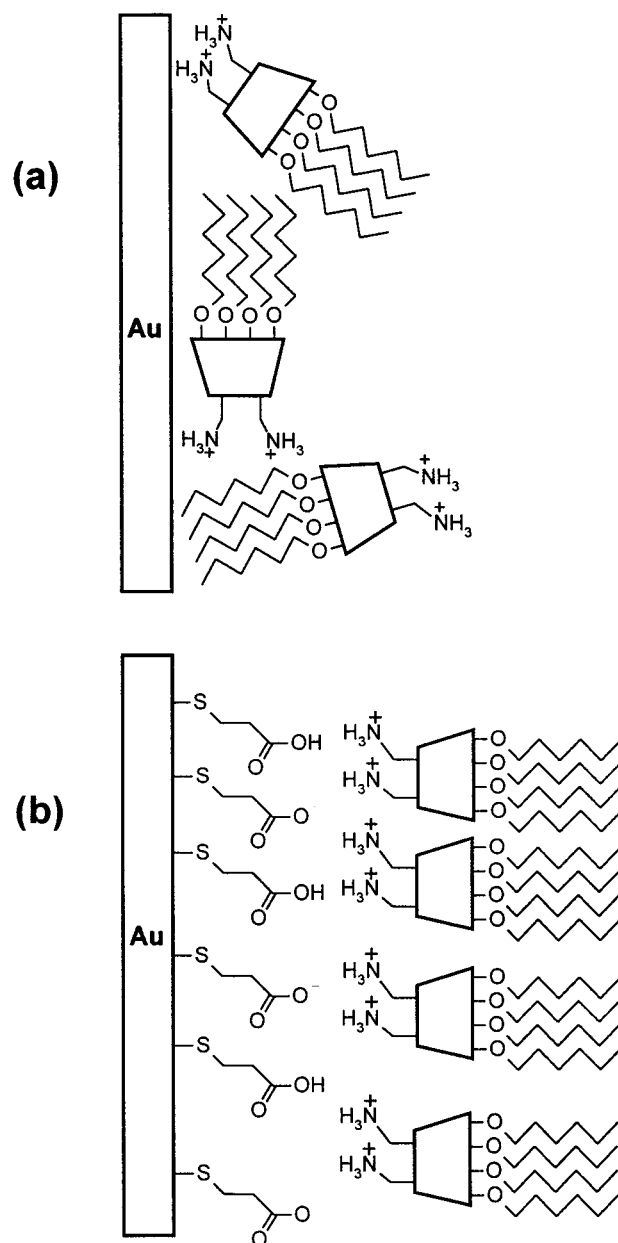


Figure 4. Schematic model for the interfacial aggregation of the modified CDs 1–3 on (a) bare gold and (b) Au-4 surfaces.

corresponding angles on the carboxylic acid modified surfaces are, on the average, larger by 12° and do not exhibit large differences between them. The larger angle observed in carboxylate modified gold substrates reflects the templating effect that the acids 4 and 5 have on the aggregation of the CD compounds 1–3. As proposed in Figure 4a, the aggregation of 1–3 on bare gold proceeds randomly (see below), a large fraction of the CH₂-like surface faces the hexadecane drop in the contact angle experiment and, therefore, a small contact angle results. In contrast, Figure 4b shows the templating effect that the carboxylic acid groups exert, orienting the positively charged amino groups toward the surface and increasing the contact angle reading by forcing a larger fraction of CH₃ groups to face the hexadecane drop.

FT-IR spectroscopic experiments further confirmed the general scheme presented in Figure 4a and 4b. As shown in Figure 5a, the C–H stretching region of the IR spectrum of 2 in a KBr pellet shows at least four resonance peaks at positions that define a randomly oriented, crystalline-like state of the alkyl groups. Such positions correspond

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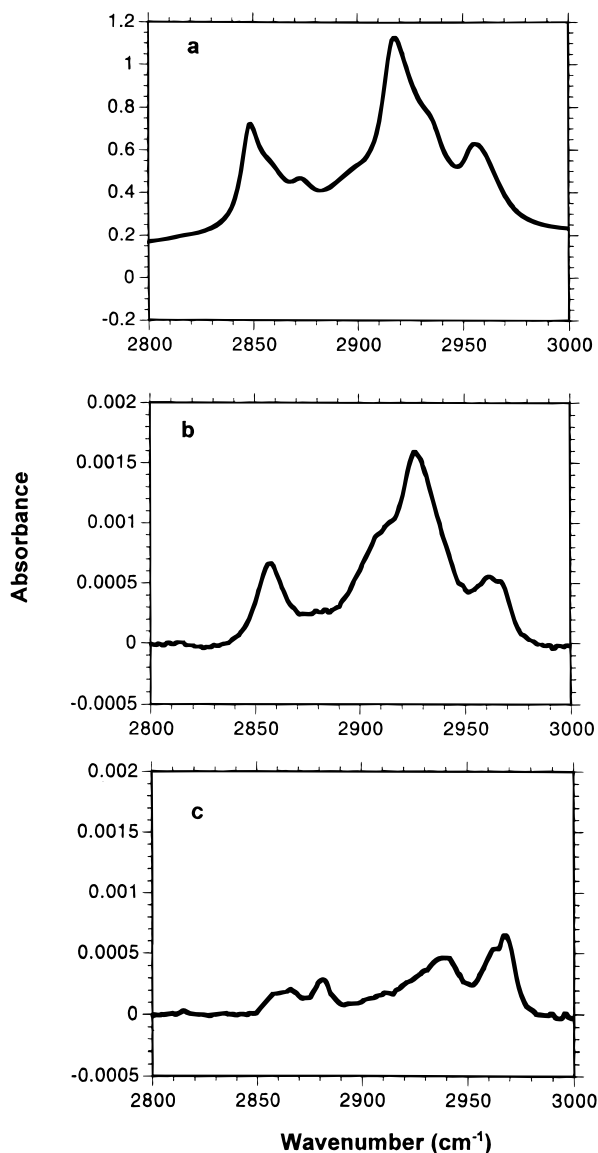


Figure 5. FT-IR spectra of the alkyl region of **2** (a) in a KBr pellet and adsorbed on (b) bare gold and (c) a Au-4 substrate.

to the symmetric ν_s (CH_2) mode at 2850 cm^{-1} , the asymmetric ν_a (CH_2) mode at 2918 cm^{-1} , the asymmetric ν_a (CH_3 , op) mode at 2956 cm^{-1} and the symmetric ν_s (CH_3 , FR) mode at 2879 cm^{-1} .²⁵ In a nonrigid environment, however, the liquid-like state of the methylene groups should be reflected by a shift of the peak positions to higher wavenumber values.²⁵ Figure 5b shows the IR spectrum of a gold substrate after exposure to a $40\text{ }\mu\text{M}$ aqueous solution of **2** for at least 2 h. The 6 cm^{-1} average positive shift in all the absorption peaks indicates that when compound **2** is adsorbed on the surface of a bare gold substrate, the methylene groups are in a nonrigid environment. Information on the orientation of the modified CD molecules on the surface of bare gold substrates was also obtained by comparison of parts a and b of Figure 5. The similarities in the relative intensities of the IR signals of **2** in a KBr pellet and adsorbed on bare gold suggest that the modified CD molecules do not adopt a preferred orientation in the

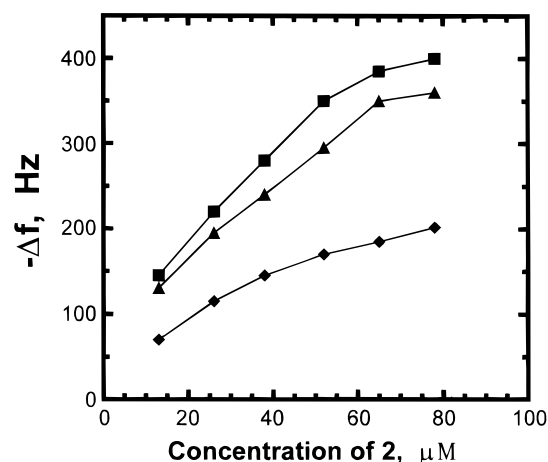


Figure 6. QCM frequency change ($f_0 - f$) vs concentration of **2** (pH = 7 Tris buffer solution, $I = 0.1$) on bare and modified gold surfaces.

aggregates and, therefore, supports the model sketched in Figure 4a.

Figure 5c presents the IR spectrum of **2** adsorbed on a Au-4 modified substrate after exposure to a $40\text{ }\mu\text{M}$ solution of **2**. As is evident by comparison with parts a and b of Figure 5, the intensities of the peaks corresponding to CH_2 groups ($2856, 2927\text{ cm}^{-1}$) are decreased relative to those related to the CH_3 groups ($2967, 2882\text{ cm}^{-1}$).²⁵ Such changes in the relative intensities of the C-H stretching modes indicate that there is a preferred orientation for the amphiphilic CD molecules in this type of aggregate. As in the previous case, this result complements the contact angle data and supports the oriented aggregation model depicted in Figure 4b. FT-IR spectra corresponding to the aggregation of **2** on Au-5 (not shown) are similar to that shown in Figure 5c. Similar intensity ratios of the spectroscopic signals also indicate a preferential orientation of the C-H stretching modes of **2** adsorbed on a mercaptotolanoic acid modified gold substrate.

Although contact angle and FT-IR spectroscopic measurements can give information on the templating effect that the carboxylic acids **4** and **5** have on the aggregation of the CD derivatives **1–3**, these ex-situ techniques do not provide information on the size of the CD aggregates when the gold substrates are in contact with dilute CD solutions and, therefore, cannot completely explain the blocking effects that these amphiphilic CDs exert on the Ru(III) electrochemical response.

QCM Measurements. In order to investigate the surface coverage afforded by the interfacial CD aggregates, we performed QCM measurements on the three gold surfaces under study. As described in the experimental part, exposure of the bare and modified gold substrates to increasing concentrations of **2** (in the $0\text{--}75\text{ }\mu\text{M}$ range) gave rise to frequency changes that can be correlated to CD surface coverages.^{17,26}

The QCM data showed that the apparent mass of the interfacial aggregate formed by the amphiphilic receptor was dependent on the nature of the substrate's surface (see Figure 6). For instance, the frequency change measured for the adsorption of **2** at a concentration of $75\text{ }\mu\text{M}$ on a Au-4 substrate is roughly 1.8 times larger than that measured on bare gold and about 10% smaller than on Au-5 electrodes. The direct application of the Sauerbrey equation to the observed frequency changes measured (after correction for the amounts of DMSO introduced)

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yields a surface coverage of 1.4×10^{-9} mol/cm² at the largest concentration of **2** surveyed (75 μ M). According to Coleman and co-workers, a compact monolayer of **2** (250 Å²/molecule oriented perpendicularly to the surface) would result in a surface coverage of approximately 6.6×10^{-11} mol/cm².^{9a} Therefore, the measured frequency change would represent about 21 layers of a compact and perpendicularly oriented amphiphile CD aggregate with an approximate thickness of 400 Å. For randomly oriented CD molecules, however, as suggested by the contact angle and FT-IR experimental data, the area taken up by each molecule should be larger. This, in turn, suggests that the interfacial aggregate could be even thicker and less compact. Indeed, the presence of an electrochemical signal in the Ru(III) blocking experiments at concentrations of **2** as large as 75 μ M strongly suggests that a dense aggregate is unlikely since it would completely block the electron transfer process. Instead, a more disorganized, and therefore thick, aggregate with lower density should feature holes and channels that make it partially permeable to the Ru electroactive probe. In this context, a comparison between the shape of the electrochemical and QCM curves presented in Figures 2 and 6, respectively, is consistent with this picture since a monotonic increase in the concentration of **2** results in both a larger CD aggregate on the surface of the electrode and a reduction of the Ru electrochemical response. On the basis of these arguments, the experimental QCM measurements for the aggregation of **2** on bare gold surfaces seem to describe, in conjunction with contact angle and FT-IR spectroscopy data, a randomly oriented and highly disorganized multilayer of the amphiphilic CD.

As previously mentioned, QCM measurements of aggregates of **2** in carboxylic acid modified gold surfaces showed increased surface coverages as compared to bare gold substrates. For instance, the apparent mass of the aggregates of **2** (from a 75 μ M solution) on Au-4 surfaces turned out to be about 1.8 times larger than that on clean gold ($\Gamma = 2.5 \times 10^{-9}$ mol/cm²). On the basis of the estimate of Coleman and co-workers for a compact monolayer of **2** oriented perpendicularly to the surface,^{9a} this result represents about 38 layers of the modified CD with an approximate thickness of about 700 Å. For the aggregation of **2** on Au-5 substrates, the templating effect of the long chain acid on the surface coverage seems to be the largest one at all the concentrations surveyed. A surface coverage value of $\Gamma = 2.8 \times 10^{-9}$ mol/cm² (at 75 μ M **2**) represents under the same criteria a deposit of 42 layers held as a compact aggregate close to the modified electrode surface with a thickness of roughly 780 Å.

The templating effect of the chemisorbed carboxylic acids on the aggregation of **2** is reflected not only by a larger mass of the aggregate—as compared to deposition on bare gold—but also by an improved structural order of the amphiphilic film. This effect, which is consistent with the contact angle and FT-IR spectroscopic results, is evident if one compares the QCM and Ru(III) voltammetric blocking curves on bare gold at high concentrations of **2** with those obtained on the derivatized gold surfaces at low concentrations of the amphiphilic CD. Whereas on bare gold the voltammetric response to Ru(III) shows a 60% decrease at the highest concentrations of **2** (about 75 μ M), the corresponding signal almost disappears at concentrations as low as 10 μ M when carboxylic acid modified gold surfaces are used. In contrast, QCM measurements indicate that the apparent total mass of the interfacial aggregate formed by **2** (at 75 μ M) on bare gold is larger than that formed by the amphiphilic CD at 10 μ M on the carboxylic acid modified gold surfaces. This

finding clearly indicates that the improved Ru(III) blocking exhibited by **2** at low concentrations on the carboxylic acid modified gold surfaces is due to a more structured aggregate since more massive deposits on bare gold at higher concentrations show less effective electrochemical blocking properties.

We must note however, that the structural information from QCM measurements described in this section involves some drastic approximations that must be taken into account. For instance, the thickness values of the interfacial aggregates were computed with the assumption that no solvent and/or supporting electrolyte molecules or ions were incorporated in the aggregates. This approximation is quite extreme and the number of monolayers as well as the corresponding thickness of the aggregates should be smaller than the calculated values. Furthermore, the quantitative interpretation of QCM measurements on very thick films presents serious difficulties which have been described in detail.²⁷ Frequency changes can be associated to changes in roughness, thickness, and other film properties and, thus, may reflect other phenomena in addition to simple mass loading on the quartz oscillator. Even with these limitations, the QCM experiments reported in this section lead to some important findings. First, the aggregation of **2** on the three types of surfaces surveyed clearly yields thick multilayer aggregates. A comparison between the QCM data obtained with bare and modified gold surfaces clearly reveals the templating effect of the mercaptocarboxylic acids on the aggregation of the amphiphilic CD **2**. These results, obtained using *in situ* measurements, are in excellent agreement with the *ex situ* data from contact angle and FT-IR spectroscopic measurements.

Mechanical Stability of the Amphiphilic Aggregates. The stability of the aggregates was also studied by repeating some of the FT-IR and contact angle measurements of aggregates of **2** on gold and Au-4 substrates after thoroughly rinsing with water and drying with a nitrogen gas flow. To our surprise, the modified CD aggregates proved to be fairly robust on the surfaces studied. For instance, the high contact angle readings with water drops did not decrease by more than 7° on the average upon rinsing and drying, and the IR response showed that, for a particular sample, every spectral signal before and after rinsing and drying was exactly the same. On the other hand, the voltammetric blocking properties of the amphiphilic CD aggregates were not maintained after transfer of the gold substrates to solutions containing no CD. These results indicate that the adsorption of the first layer(s) of the amphiphilic receptors is rather irreversible. However, the thick multilayer aggregates that are formed when the substrates contact solutions with micromolar concentration levels of compounds **1–3** are more labile and redissolve quickly in the absence of the amphiphilic receptor.

Conclusions

In this work, we have shown that the amphiphilic receptors **1–3** readily form multilayer aggregates on the surface of gold electrodes from neutral aqueous media at relatively low concentrations (micromoles per liter range). On the basis of FT-IR, QCM, and contact angle measurements, the CD derivatives cover the bare gold surface, forming a highly disorganized multilayer characterized by the liquid-like random orientation of the CD amphiphilic molecules. Chemical modification of the gold surface by the adsorption of the mercaptocarboxylic acids

gives rise to the Au-4 and Au-5 surfaces that exert a template effect on the aggregation of compounds 1-3 by modifying not only the degree of packing and thickness of the resulting multilayer assembly but also the orientation of the adsorbed amphiphiles.

Since the design and construction of well-defined molecular architectures on electrode surfaces have typically relied on techniques and procedures that are usually complicated and time-consuming, the templating effect exerted by the carboxylic groups on the hydrophobic aggregation of amphiphilic receptors such as the CD compounds studied here is certainly important in the

context of the preparation and design of modified electrodes with molecular recognition properties.

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