# Supported Monolayers Containing Preformed Binding Sites. Synthesis and Interfacial Binding Properties of a Thiolated $\beta$-Cyclodextrin Derivative

**María T. Rojas,[†] Rainer Königer,[‡] J. Fraser Stoddart,*[‡] and Angel E. Kaifer*[†]**

*Contribution from the Chemistry Department, University of Miami,
Coral Gables, Florida 33124, and School of Chemistry, University of Birmingham,
Edgbaston, Birmingham B15 2TT, United Kingdon*

*Received December 3, 1993*[⊗]

**Abstract:** Per-6-thio-$\beta$-cyclodextrin (**2**) was prepared in two steps from $\beta$-cyclodextrin. Receptor **2** is a $\beta$-cyclodextrin derivative in which all the primary hydroxyl groups have been replaced by thiol groups. As such, **2** chemisorbs onto gold surfaces forming at least six S–Au bonds per receptor molecule. Although this derivatization process leads to imperfect monolayers in which a substantial fraction of the gold surface remains uncovered, the monolayer defects can be covered by treatment with a solution of ferrocene and pentanethiol. Ferrocene, an excellent substrate for $\beta$-cyclodextrin, protects the monolayer binding sites, directing the pentanethiol molecules to seal defective sites instead of the cyclodextrin cavities. Electrodes derivatized by this procedure showed effective binding properties when immersed in aqueous solutions containing low concentrations ($<60\ \mu M$) of ferrocene. Their voltammetric response exhibited the waves anticipated for the reversible oxidation of the surface-confined (cyclodextrin-bound) ferrocene molecules. The process of interfacial ferrocene complexation was demonstrated to have the expected dynamic character by a series of competition experiments with *m*-toluic acid (mTA) in which the surface-confined voltammetric waves of ferrocene were gradually lost as increasing mTA concentrations were added to the solution.

## Introduction

Molecular recognition phenomena have been extensively studied in the last two decades, probably because of the realization of the widespread importance of noncovalent interactions in biochemistry.[1] In living systems, the molecular architecture evidently affects substrate recognition properties. Molecular organization and molecular recognition (binding phenomena) are intimately related and respond to the same kinds of intermolecular forces, such as ion–dipole, hydrogen bonding, van der Waals, aromatic $\pi$–$\pi$ stacking, and other similarly weak interactions. Therefore, detailed studies of the interplay between self-assembly forces and binding phenomena are of the utmost importance.

Self-assembled monolayers (SAM's) formed by the chemisorption of organosulfur compounds on gold exhibit highly organized molecular structures.[2] These systems may afford a convenient matrix for investigations on the interdependence between molecular organization and recognition processes. An additional advantage of such SAM's is that they are supported on a material suitable for applications in electrochemical and quartz crystal microbalance measurements. This point is important for the development of novel sensors. A few literature reports have described molecular recognition phenomena involving SAM's or components of SAM's. For instance, Rubinstein and co-workers reported[3] on SAM's prepared by the co-assembly of alkanethiols and thiolated ligands which impart to the monolayer their selectivity for Cu(II) ions. Creager et al. studied[4] ferrocene-containing SAM's which demonstrate ion pairing phenomena between the oxidized ferrocene residues and anions in the contacting solution. Crooks and co-workers reported[5] on hydrogen bonding between SAM monomers and appropriate substrates in the gas phase. Conspicuously absent from this body of work are the preparation and study of SAM's containing well-defined molecular cavities capable of inclusion complexation with solution species. Very recently, Reinhoudt and co-workers have described[6] the preparation of monolayers made by the self-assembly on gold of a receptor possessing four sulfide functionalities for surface attachment. However, the interfacial binding properties of these monolayer assemblies have not been reported yet. Our own approach relies on the synthetic modification of well-known receptors in order to drive their interfacial self-assembly. A prototypical example of well-studied hosts would be the cyclodextrins[7] (CD's), a class of naturally occurring receptors which are cyclic glucopyranose oligomers. Their toroidal shape and strong binding affinity for hydrophobic molecules in aqueous media are very well known. The inner surface of their molecular cavity is hydrophobic because it is lined by the glycosidic oxygen bridges. Conversely, the outer surface of CD's is hydrophilic due to the hydroxyl groups (see Scheme 1) that populate the two cavity openings. These openings are not identical; the wider opening is surrounded by the secondary hydroxyl groups on C-2 and C-3, while the narrower opening contains the primary hydroxyl groups on C-6.

Among the three unmodified CD's, $\beta$-CD is the most widely studied for reason of cost and also because its cavity has the

---

[†] University of Miami.

[‡] University of Birmingham.

[⊗] Abstract published in *Advance ACS Abstracts*, December 1, 1994.

(1) Creighton, T. E. *Proteins: Structures and Molecular Properties*; Freeman: New York, 1984; Chapter 4.

(2) For a recent review, see: Dubois, L. H.; Nuzzo, R. G. *Annu. Rev. Phys. Chem.* **1992**, *43*, 437.

(3) (a) Rubinstein, I.; Steinberg, S.; Tor, Y.; Shanzer, A.; Sagiv, J. *Nature* **1988**, *332*, 426. (b) Steinberg, S.; Tor, Y.; Sabatini, E.; Rubinstein, I. *J. Am. Chem. Soc.* **1991**, *113*, 5176.

(4) Rowe, G. K.; Creager, S. E. *Langmuir* **1991**, *7*, 2307.

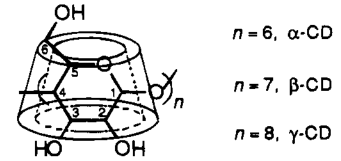
(5) (a) Sun, L.; Kepley, L. J.; Crooks, R. M. *Langmuir* **1992**, *8*, 2101. (b) Sun, L.; Crooks, R. M.; Ricco, A. J. *Langmuir* **1993**, *9*, 1775.

(6) Thioden Van Belzen, E. V.; Engbersen, J. F. J.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1994**, *116*, 3597.

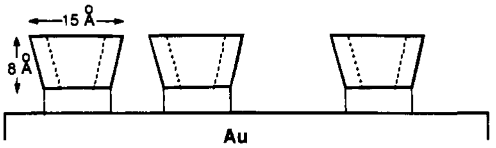
(7) (a) Saenger, W. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 344. (b) Szejtli, J. *Cyclodextrins and their Inclusion Complexes*; Akademiai Kiado: Budapest, 1982.

0002-7863/95/1517-0336$09.00/0

**Scheme 1.** Molecular Structure of the Unmodified Cyclodextrins



**Scheme 2.** Chemisorption of **2** on Gold



right size to bind a variety of aromatic and aliphatic residues. In this CD, the internal diameter of the cavity ranges from 6.0 to 6.5 Å, the average external diameter is ca. 15 Å, and the cavity depth is very close to 8 Å. Interestingly, its solubility in water (20 g/L at 25 °C) is the lowest among the three unmodified CD's. This fact has been recently attributed to the mismatch between the 7-fold symmetry of this receptor and the dynamic 6-fold symmetry of liquid water.[8]

A few preliminary reports have described attempts by other groups to modify CD's in such a way that they can be attached to electrode surfaces.[9] In this work, we report a synthetic procedure for the conversion of the seven primary hydroxyl groups of β-CD to thiol groups. The modified cyclodextrin product (receptor **2**) can be attached to a gold surface by a maximum of seven thiolate–gold covalent bonds. Upon surface attachment, the wider opening of the CD receptor will face the adjacent solution, which should facilitate binding of solution species inside the cavity. The resulting interfacial structure can be described as a monolayer containing preformed binding sites (see Scheme 2).

From the previously quoted value of 15 Å for the external diameter of β-CD, one can easily estimate a molecular surface of ~180 Å$^2$ for this receptor. This value is indeed quite large, especially compared to the surface area of straight *n*-alkane chains (~20 Å$^2$). Therefore, the self-assembly of these receptors into organized, defect-free monolayers on gold is anticipated to be much more difficult than the equivalent process for *n*-alkanethiols, owing to the more favorable lateral interactions among the latter monomers. Our results confirmed these expected difficulties and demonstrated that the design of surface-attachable molecular receptors is definitely challenging. In spite of these problems, we demonstrate here the chemisorption of receptor **2** on gold and the preparation of supported monolayers which exhibit effective binding properties toward hydrophobic substrates in the contacting solution.

## Experimental Section

**Materials.** Ferrocene (Aldrich) was sublimed twice before use. *m*-Toluic acid and *n*-pentanethiol were purchased from Aldrich and used without further purification. Hexaamineruthenium(III) chloride was obtained from Strem Chemicals. All reagents were of the best commercial quality available. All inorganic salts were Analytical Grade. Gold (99.999%) was purchased from Johnson Matthey. Distilled water was further purified with a Barnstead Nanopure four-cartridge system.

**Synthesis of Per-6-iodo-β-cyclodextrin (1).** This compound was prepared from β-CD as described by Defaye and Gadelle.[10] However, instead of precipitating the products with iced water, the reaction mixture was poured into 800 mL of methanol to form a precipitate, which was washed with methanol, dried superficially, and Soxhlet extracted with methanol for 20 h. After rigorous drying, compound **1** was recovered as a white powder in 90% yield: $^1$H NMR (300 MHz, $CD_3SOCD_3$) δ 3.24–3.47 (m, 21 H, H-2, H-4, H-6a), 3.54–3.68 (m, 14 H, H-3, H-5), 3.80 (bd, *J* = 9 Hz, 7 H, H-6b), 4.99 (d, *J* = 3 Hz, 7 H, H-1), 5.94 (d, *J* = 2 Hz, 7 H, OH-3), 6.05 (d, *J* = 6.5 Hz, 7 H, OH-2).

**Synthesis of Per-6-thio-β-cyclodextrin (2).** Compound **1** (0.965 g) was dissolved in DMF (10 mL); thiourea (0.301 g) was then added and the reaction mixture heated to 70 °C under a nitrogen atmosphere. After 19 h, the DMF was removed under reduced pressure to give a yellow oil, which was dissolved in water (50 mL). Sodium hydroxide (0.26 g) was added and the reaction mixture heated to a gentle reflux under a nitrogen atmosphere. After 1 h, the resulting suspension was acidified with aqueous $KHSO_4$ and the precipitate filtered off, washed thoroughly with distilled water, and dried. To remove the last traces of DMF, the product was suspended in water (50 mL) and the minimum amount of potassium hydroxide added to give a clear solution; the product was then reprecipitated by acidifying with aqueous $KHSO_4$. The resulting fine precipitate was carefully filtered off and dried under vacuum over $P_2O_5$ to yield **2** (0.536 g, 85%) as an off-white powder: mp 135 °C (dec): $^1$H NMR (300 MHz, $CD_3SOCD_3$) δ 2.16 (t, *J* = 6 Hz, 7 H, SH), 2.79 (m, 7 H, H-6a), 3.21 (br d, *J* = 14 Hz, 7 H, H-6b), 3.36–3.40 (m, 14 H, H-2, H-4), 3.60 (t, *J* = 9 Hz, 7 H, H-3), 3.68 (t, *J* = 8 Hz, 7 H, H-5), 4.95 (d, *J* = 2.5 Hz, 7 H, H-1), 5.83 (s, 7 H, 3-OH), 5.97 (d, *J* = 6 Hz, 7 H, 2-OH); $^{13}$C NMR (75 MHz, $CD_3SOCD_3$) δ 26.0 (C-6), 72.0 (C-5), 72.3 (C-3), 72.6 (C-2), 85.0 (C-4), 102.2 (C-1); MS *m/z* (positive ion FAB) 1269 for $[M + Na]^+$, calcd for ($C_{42}H_{70}O_{28}S_7$) 1247. Anal. Calcd for $C_{42}H_{70}O_{28}S_7$: C, 40.4; H, 5.66. Found: C, 37.70; H, 5.46.

**Equipment.** The electrochemical instrumentation has been described elsewhere.[11] NMR spectra were recorded in a Bruker AG300 NMR spectrometer. MS spectra were recorded in a Kratos MS80RF spectrometer.

**Procedures.** The gold bead working electrodes were made by annealing the tip of a gold wire (99.999%, 0.5 mm diameter) in a gas–oxygen flame. The hot gold bead was then cooled down in purified water. The voltammetric response of these electrodes was checked in 0.2 M $Na_2SO_4$. Flat background responses in the potential range +0.7 to −0.7 versus SSCE were commonly found. Otherwise, the electrode was discarded. Roughness factors for these electrons were measured to be in the 1.1–1.2 range and were used to obtain the actual surface area of the electrodes from their geometric or projected areas. Geometric areas were obtained from the slope of the linear plot of cathodic peak current versus (scan rate)$^{1/2}$ for the reversible reduction of $Ru(NH_3)_6^{3+}$. As previously reported,[12] we have utilized for this purpose a diffusion coefficient of 7.5 × $10^{-6}$ cm$^2$/s (at 25 °C in 0.1 M NaCl). Electrode capacitances were obtained from cyclic voltammetric data by dividing the difference between the anodic and cathodic current (measured at a given potential value) by twice the scan rate. The values thus obtained were normalized to unit area by using the measured area of the electrode.

Monolayer preparation was performed in several steps. First, the clean gold bead electrode was immersed in a deoxygenated 1.0 mM solution of **2** in DMSO/$H_2O$ (60:40 v/v) overnight. This step usually yielded a monolayer of chemisorbed cyclodextrins having a large density of defects (vide infra). In order to cover the defective areas,[3a] the electrode was then exposed to a 0.75 mM pentanethiol ($C_5SH$) + 0.75 mM ferrocene solution in EtOH/$H_2O$ (50:50 v/v). Before the cyclic voltammetric experiments, the gold bead electrode was rinsed with copious amounts of pure ethanol and purified water. Cyclic voltammetry (single-compartment cell, Pt flag counter electrode, home-made SSCE reference electrode) was performed with the monolayer-covered working electrode immersed in 0.2 M $Na_2SO_4$ (cell volume = 10 mL). A flat voltammetric background from 0 to 0.7 V versus SSCE indicated

---

(8) Szejtli, J. *Cyclodextrin Technology*; Kluwer: Dordrecht, 1988.

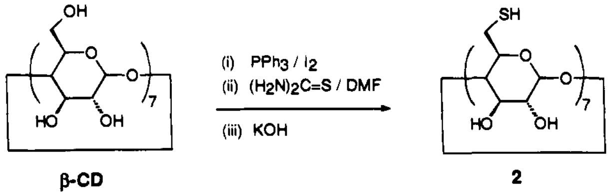
(9) (a) Porter and co-workers pioneered this research topic. See, for instance: *Chem. Eng. News* **1989** (May 1), 32–33. (b) Tamagaki, S.; Fukuda, K.; Sumita, H.; Tagaki, W. *Chem. Express* **1991**, *6*, 695.

(10) Defaye, J.; Gadelle, A. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 78.

(11) Bernardo, A. R.; Stoddart, J. F.; Kaifer, A. E. *J. Am. Chem. Soc.* **1992**, *114*, 10 624.

(12) Gomez, M. E.; Li, J.; Kaifer, A. E. *Langmuir* **1991**, *7*, 1797.

**Scheme 3.** Preparation of **2**



the effective ferrocene removal from the monolayer at this stage. Once this point was verified, the concentration of ferrocene in the 0.2 M $Na_2SO_4$ solution was adjusted (to the level required for the binding studies) by aliquot additions from an ethanolic stock solution. The concentration of ferrocene was always kept below 65 $\mu$M, which we determined as its solubility limit at 25 °C in this medium using absorbance measurements at 440 nm. The small (microliter) amounts of ethanol introduced in the aqueous solution did not have any effect on the voltammetric response of the monolayer-covered electrode, as verified by control experiments.

## Results

The thiolated $\beta$-CD derivative (**2**) was prepared from $\beta$-CD according to Scheme 3. In receptor **2**, the seven primary hydroxyl groups of $\beta$-CD have been replaced by thiol groups. Therefore, this receptor is anticipated to chemisorb strongly on gold surfaces through the formation of thiolate−gold bonds. Upon surface attachment, the wider opening of the CD cavity would be oriented away from the gold surface and face the adjacent solution. This adsorption configuration is expected to optimize the binding properties of the surface-attached receptor.

Exposure of a gold bead electrode to a 1.0 mM solution of thiolated receptor **2** in DMSO:$H_2O$ (60:40 v/v) results in the chemisorption of submonolayer amounts of **2**. The maximum cyclodextrin surface coverage ($7.5 \times 10^{-11}$ mol/cm$^2$) can be calculated by assuming a close-packed monolayer of cylindrical receptors with the same diameter as **2**. The fraction of electrode surface that is actually covered after the monolayer deposition step can be estimated by submitting the derivatized electrode to reductive desorption experiments. Porter and co-workers reported[13] that surface-attached thiolates undergo reductive desorption at accessible potentials in KOH solution. The corresponding process is represented by the following equation:

$$\text{R-S-Au} + e^- \rightleftharpoons \text{R-S}^- + \text{Au} \qquad (1)$$

Electrodes derivatized with **2** exhibit an irreversible cathodic wave ($E_{pc} = -0.95$ V versus SSCE) that is assigned to the reductive desorption of chemisorbed **2**. A typical voltammogram is shown in Figure 1. Integration of the cathodic wave, assuming that each CD receptor is covalently attached to the surface through *seven* Au−S bonds, indicates that approximately 64% of the surface is covered with surface-attached CD's. The surface coverage reveals a very imperfect monolayer, containing numerous defective sites or pinholes. If one assumes that each CD is attached to the surface through an average of *six* Au−S bonds, then the surface coverage goes up to ~75%. Still, this value clearly indicates a large density of defects in the monolayer. This outcome is probably the result of the large cross-section area of the thiolated cyclodextrins and the rather poor lateral interactions among them once they are chemisorbed. Thus, it is hardly surprising that the chemisorption of receptor **2** on gold surfaces yields submonolayer assemblies.

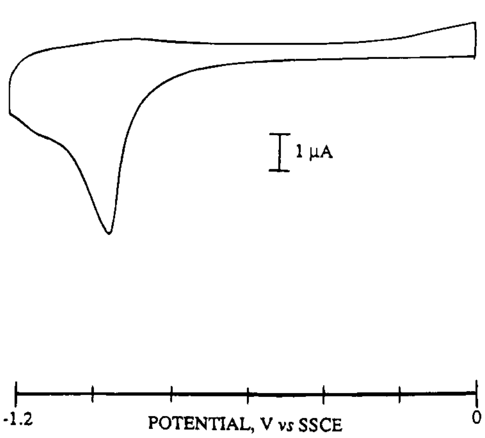


**Figure 1.** Voltammetric response in 0.5 M KOH of a gold electrode (0.037 cm$^2$) derivatized by overnight exposure to a 1.0 mM solution of **2** in DMSO/$H_2O$ (60:40 v/v); scan rate 0.1 V/s.

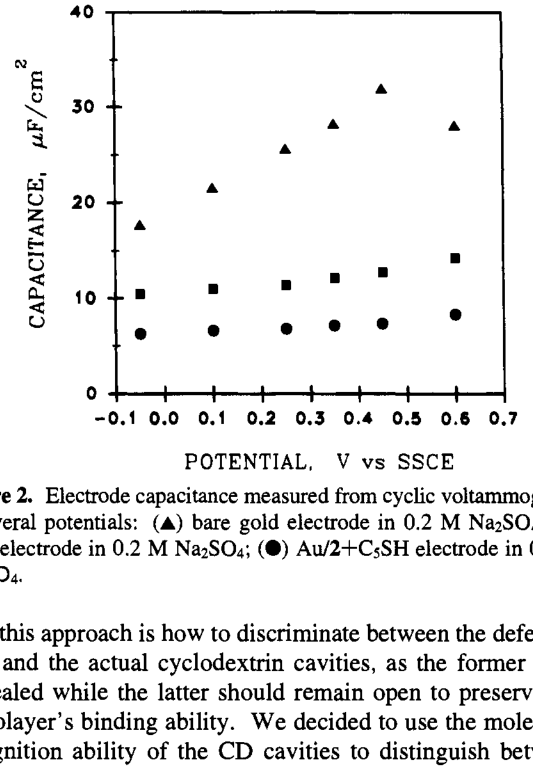


**Figure 2.** Electrode capacitance measured from cyclic voltammograms at several potentials: (▲) bare gold electrode in 0.2 M $Na_2SO_4$; (■) Au/**2** electrode in 0.2 M $Na_2SO_4$; (●) Au/**2**+$C_5SH$ electrode in 0.2 M $Na_2SO_4$.

To circumvent this difficulty, a second type of thiol can be used to "patch" the holes or defective sites.[3a] The key problem with this approach is how to discriminate between the defective sites and the actual cyclodextrin cavities, as the former must be sealed while the latter should remain open to preserve the monolayer's binding ability. We decided to use the molecular recognition ability of the CD cavities to distinguish between defects and binding sites. Thus, monolayer sealing or patching of defective sites was accomplished by exposing the gold electrode, after derivatization with **2**, to an EtOH/$H_2O$ solution (50:50 v/v) containing 0.75 mM $C_5SH$, as the "sealing" agent, and 0.75 mM ferrocene, which is expected to bind to the CD cavities[14] and keep the thiol from sealing them. This method is effective as reflected by the small differential capacitances recorded for the electrodes after exposure to the monolayer sealing step. Figure 2 shows the differential capacitances measured before electrode modification and after each one of the steps of the monolayer deposition process. The measured electrode capacitances decrease after derivatization with **2**. Subsequent treatment with $C_5SH$ and ferrocene yields electrode capacitance values that are essentially independent of the potential (in the range from −0.10 to +0.70 V versus SSCE) and exhibit an average value of 7.1 $\mu$F/cm$^2$, which is reasonably

---

(13) (a) Walczak, M. M.; Popenoe, D. D.; Deinhammer, R. S.; Lamp, B. D.; Chung, C.; Porter, M. D. *Langmuir* **1991**, *7*, 2687. (b) Widrig, C. A.; Chung, C.; Porter, M. D. *J. Electroanal. Chem.* **1991**, *310*, 335.

(14) (a) Siegel, B.; Breslow, R. *J. Am. Chem. Soc.* **1975**, *97*, 6869. (b) Harada, A.; Takahashi, S. *J. Chem. Soc., Chem. Commun.* **1984**, 645. (c) Matsue, T.; Evans, D. H.; Osa, T.; Kobayashi, N. *J. Am. Chem. Soc.* **1985**, *107*, 3411. (d) Isnin, R.; Salam, C.; Kaifer, A. E. *J. Org. Chem.* **1991**, *56*, 35.

**Table 1.** Thiolate Surface Coverages[a] and Cathodic Peak Potentials[b] Obtained from Reductive Desorption Experiments with Thiolated Monolayers in 0.5 M KOH

| monolayer | Γ (mol/cm²) | $E_{pc}$ (V vs SSCE)[a] |
|---|---|---|
| Au/**2** | $3.3 \times 10^{-10}$ | −0.95 |
| Au/**2**+$C_5SH$ | $6.9 \times 10^{-10}$ | −0.98 |
| Au/$C_5SH$ | $9.2 \times 10^{-10}$ | −1.18 |

[a] The error margin of these measurements is ±10%. [b] Measured at a scan rate of 0.1 V/s.

close to values reported for other alkanethiol monolayers of similar thickness.[15]

The data in the table qualitatively reveal that exposure of a monolayer of **2** to pentanethiol increases the surface coverage of thiolate while shifting the cathodic peak associated with the desorption process to more negative values. The cathodic potential shift, although small, suggests that the treatment with pentanethiol increases the degree of packing in the monolayer.[13] However, a comparison of the cathodic peak potentials for a mixed **2**+$C_5SH$ monolayer with those of a monolayer of pure pentanethiol suggests the existence of a greater surface density of pinholes (quite probably the CD cavities) in the former monolayer system. More quantitatively, the exposure of a Au/**2** monolayer to pentanethiol results in an increase of $3.6 \times 10^{-10}$ mol/cm² of thiolate surface coverage. Taking the surface coverage observed for a pure monolayer of $C_5SH$ as a reference, it can be concluded that the observed increase corresponds to 39% of a pentanethiol monolayer. This figure is in very good agreement with our previous estimate of uncovered surface after deposition of receptor **2** (36% assuming seven S−Au bonds per cyclodextrin).

The use of reductive desorption charge measurements to determine the surface coverage of thiolate monolayers has been recently questioned by Buttry and co-workers.[16] Their findings indicate that the large changes in electrode capacitance associated with alkanethiolate monolayer desorption may contribute to the charge measured in the cathodic process represented by eq 1. This capacitive contribution would cause large positive errors in the measurement of the faradaic charge associated with thiolate reduction and desorption. In contrast with these observations, our reductive desorption measurements yield values consistent with the molecular geometries of the monomeric thiols. For instance, the surface coverage that we determined for a pure monolayer of pentanethiol (see Table 1) is in excellent agreement with the value reported by Porter and co-workers[13] for *n*-alkanethiol monolayers and translates in a molecular surface area of ~18 Å² which is consistent with the cross-sectional area of extended aliphatic chains. We note that the thiol monomers used in our experiments cause substantial changes in electrode capacitance, but the capacitance changes are much lower than those observed with longer chain alkanethiols. However, as indicated by Schneider and Buttry,[16] extreme caution should be exercised in the interpretation of the reductive desorption data, as their agreement with geometrical expectations might be due to the "fortuitious summation of the double layer charge and the faradaic charge passed during reductive desorption."

Self-assembled alkanethiol monolayers create a lipophilic barrier for electron transfer between the electrode surface and hydrophilic electroactive species. The extent of kinetic hindrance to the electron transfer process imposed by the monolayer assembly increases with the thickness of the monolayer. For a monolayer of fixed thickness, the extent of hindrance (degree of electrode blocking) increases as the density of defects decreases.[17] It is then customary to assess the quality of self-assembled monolayers by investigating their effects on the voltammetric behavior of water-soluble electroactive species. Therefore, we investigated the barrier effects that monolayers of pure **2**, pure $C_5SH$, and mixed **2** and $C_5SH$ exert on the cyclic voltammetric response of $Fe(CN)_6^{4-}$ and $Ru(NH_3)_6^{3+}$. The results are given in Table 2.

**Table 2.** Potential Difference ($\Delta E_p$ in mV) between the Anodic and Cathodic Peaks in the Cyclic Voltammetric Responses of Water-Soluble Probes (1.0 mM Concentration)[a]

| | electroactive probe | |
|---|---|---|
| electrode | $Fe(CN)_6^{4-}$ | $Ru(NH_3)_6^{3+}$ |
| bare Au | 60 | 60 |
| Au/**2** | 100 | 65 |
| Au/**2**+$C_5SH$ | 180 | 80 |
| Au/$C_5SH$ | 250 | 110 |

[a] Scan rate = 0.2 V/s.

As expected both redox couples exhibit reversible behavior ($\Delta E_p = 57$ mV for $n = 1$ at 25 °C) on bare gold electrodes. The difference between this value and the $\Delta E_p$ value observed with a monolayer-covered electrode is a measure of the kinetic hindrance exerted by the monolayer on the electrode transfer process. Our results clearly show that the $Ru(NH_3)_6^{3+}$ couple is less affected than the $Fe(CN)_6^{4-}$ couple by all the monolayers surveyed here. This observation is in agreement with many others and reflects the inherently faster electron transfer kinetics associated with the Ru couple. In spite of these differences, both couples yield essentially the same qualitative results. A monolayer of receptor **2** moderately increases the $\Delta E_p$ values of both couples. The peak-to-peak potential splittings increase more after treatment with pentanethiol indicating that **2**+$C_5SH$ monolayers have less defective sites than monolayers of pure **2**. However, the kinetic hindrance introduced by the mixed monolayers is less pronounced than that observed with pure monolayers of pentanethiol. These observations are perfectly consistent with the expected composition of these monolayers and indicate that the two-step procedure employed for monolayer preparation works reasonably well.

In summary, the initial characterization of monolayers of **2** and **2**+$C_5SH$ has been performed using three different kinds of electrochemical experiments: electrode capacitance measurements, reductive desorption data, and electrode blocking effects on the voltammetric behavior of $Fe(CN)_6^{4-}$ and $Ru(NH_3)_6^{3+}$. All the electrochemical results clearly indicate that *receptor **2** can be attached to gold surfaces and that n-pentanethiol can be used to "repair" and cover the defective monolayer sites in the presence of ferrocene.* This compound is an excellent substrate for β-CD and, thus, protects the receptor cavities from clogging by pentanethiol chemisorption. We will refer to gold electrodes modified by this two-step procedure as Au/**2**+$C_5SH$ in the remainder of this work.

To investigate the binding ability of the mixed **2**+$C_5SH$ monolayer assemblies, the monolayer-covered electrodes were first treated as described in the Experimental Section to guarantee the removal of residual ferrocene from the derivatization procedure. Then, the Au/**2**+$C_5SH$ electrodes were submitted to voltammetric experiments in aqueous solutions containing 5 μM ferrocene (plus 0.2 M $Na_2SO_4$ as the supporting electrolyte). This ferrocene concentration is low enough so that

---

[15] (a) Porter, M. D.; Bright, T. B.; Allara, D. L.; Chidsey, C. D. *J. Am. Chem. Soc.* **1987**, *109*, 3559. (b) Sabatini, E.; Ribinstein, I.; Maoz, R.; Sagiv, J. *J. Electroanal. Chem.* **1987**, *219*, 365. (c) Sabatini, E.; Rubinstein, I. *J. Phys. Chem.* **1987**, *91*, 6663. (d) Miller, C.; Cuendet, P.; Grätzel, M. *J. Phys. Chem.* **1991**, *95*, 877.

[16] Schneider, T. W.; Buttry, D. A. *J. Am. Chem. Soc.* **1993**, *115*, 12 391.

[17] Bilewicz, R.; Majda, M. *J. Am. Chem. Soc.* **1991**, *113*, 5464.

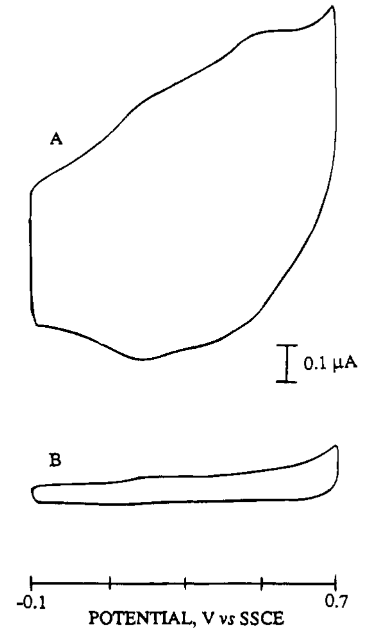


**Figure 3.** Voltammetric response in 5 μM ferrocene also containing 0.2 M $Na_2SO_4$: (A) bare gold electrode, scan rate 0.3 V/s; (B) Au/$C_5SH$ electrode, scan rate 0.5 V/s.

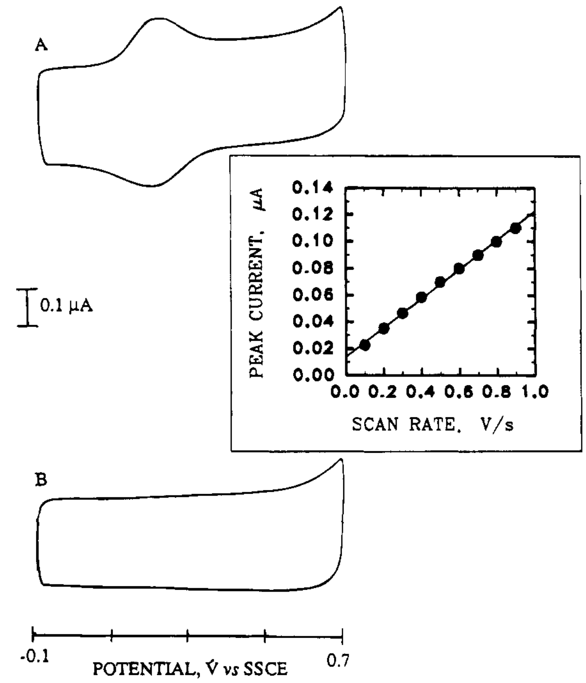


**Figure 4.** Voltammetric response of a Au/**2**+$C_5SH$ electrode (0.030 $cm^2$) at 0.5 V/s in (A) 5 μM ferrocene also containing 0.2 M $Na_2SO_4$ and (B) 0.2 M $Na_2SO_4$. Inset: Anodic peak current vs scan rate plot for the electrode of (A).

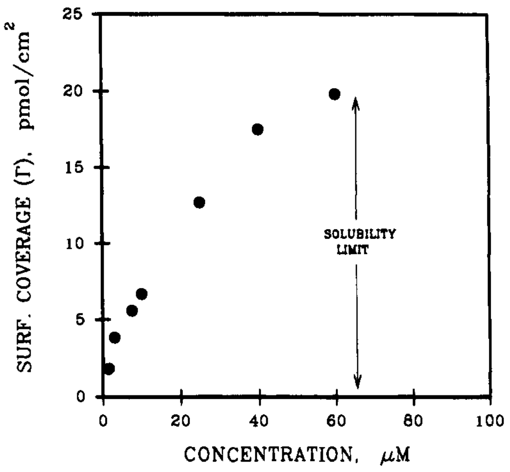


**Figure 5.** Measured ferrocene surface coverage on a Au/**2**+$C_5SH$ electrode as a function of the ferrocene concentration in the contacting solution (also containing 0.2 M $Na_2SO_4$).

the diffusion-controlled oxidation of dissolved ferrocene molecules does not give rise to detectable currents in conventional (linear sweep) voltammetric experiments. This situation is demonstrated by the voltammogram of Figure 3A which shows the response of a bare Au electrode in an aqueous solution containing 5 μM ferrocene plus 0.2 M $Na_2SO_4$. Similarly, a gold electrode covered with a monolayer of pure $C_5SH$ exhibits no detectable faradaic response in the same solution (see Figure 3B) even though a significant electrode capacitance drop is observed. In striking contrast, the voltammetric response of a Au/**2**+$C_5SH$ electrode in 5 μM ferrocene/0.2 M $Na_2SO_4$ clearly shows the reversible ferrocene/ferrocenium couple (see Figure 4A) centered at a half-wave potential of 0.19 V versus SSCE. This voltammetric response is sustained upon repetitive cycling. The inset of Figure 4 shows the linear plot of anodic peak current (for ferrocene oxidation) as a function of the scan rate. The linearity of this plot indicates that the ferrocene molecules responsible for the observed faradaic response are confined to the electrode surface; i.e., the response does not result from the oxidation of dissolved ferrocene molecules. The observed peak-to-peak potential splitting (30 mV at 200 mV/s) also indicates that the voltammetric response arises from surface-confined ferrocene. Integration of the anodic wave affords an electroactive ferrocene coverage of ca. $1 \times 10^{-11}$ mol/$cm^2$, based on the true surface area of the electrode. As expected, in the absence of dissolved ferrocene, the voltammetric response of Au/**2**+$C_5SH$ electrodes exhibits only an approximate constant level of charging current (see Figure 4B). Conversely, if these electrodes are exposed to higher concentrations of ferrocene, increasing faradaic currents are observed. However, the quantitation of the ferrocene surface coverage under these conditions ([ferrocene] > 10 μM) requires the use of faster scan rates as the recorded faradaic currents may contain appreciable contributions from the oxidation of dissolved ferrocene molecules. At 5 μM ferrocene, these contributions are negligible and, thus, clear surface-confined voltammetric behavior is observed. These voltammetric results firmly support the notion that the ferrocene molecules are bound into the cavities of the surface-attached cyclodextrin receptors. The monolayer assembly, which is primarily formed by a monomer possessing a well-defined cavity, maintains the solution binding properties of the monomeric receptor. Furthermore, these binding properties improve the detection limit of the electrode surface for the voltammetric determination of an appropriate substrate, such as ferrocene.

In order to further investigate the complexation of ferrocene by the surface-attached cyclodextrins, we performed experiments at faster scan rates (5 V/s) with a Au/**2**+$C_5SH$ electrode immersed in a solution containing increasing ferrocene concentrations. The electroactive surface coverages (determined from the anodic charge values associated with the ferrocene/ferrocenium conversion) are plotted in Figure 5 as a function of the solution ferrocene concentration. The graph exhibits a shape similar to that expected for a Langmuir adsorption isotherm. The low solubility of ferrocene in aqueous media prevented us from obtaining values at concentrations higher than 60 μM. This limitation made impossible the clear observation of the binding saturation region in which the ferrocene surface

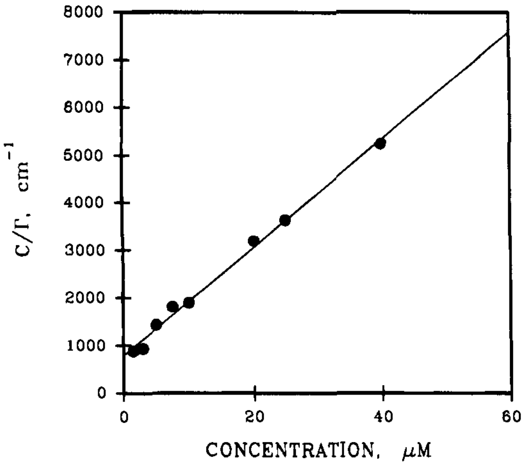


**Figure 6.** C/Γ vs C Langmuir plot for the data of Figure 5.

coverage would remain constant at increasing ferrocene concentrations in solution, reflecting the occupation of all the available surface binding sites. However, the data can be treated according to the following equation, which can be easily derived from the Langmuir isotherm,

$$\frac{C}{\Gamma} = \frac{1}{K\Gamma_{\max}} + \frac{C}{\Gamma_{\max}} \qquad (2)$$

where $C$ is the bulk concentration of ferrocene, $\Gamma$ is the surface coverage determined from the experimental data, $\Gamma_{\max}$ is the maximum surface coverage, and $K$ is the equilibrium constant for the adsorption process. The plot of $C/\Gamma$ versus $C$ was linear (see Figure 6) with a correlation coefficient of 0.977. Linear regression analysis of the data yields $\Gamma_{\max} = (2.5 \pm 1.0) \times 10^{-11}$ mol/cm$^2$ from the slope and $K = 3.9 \times 10^4$ M$^{-1}$ (or $\Delta G^\circ_{\text{ads}} = -6.4 \pm 0.5$ kcal/mol) from the intercept.

In the solution phase, inclusion complexation is a highly dynamic process in which the host and the guest undergo association and dissociation at relatively fast rates. We decided to investigate if the complexation of ferrocene by the surface-attached cyclodextrin receptors has the same dynamic character and performed a series of experiments in which Au/**2**+C$_5$SH electrodes were exposed to solutions containing mixtures of ferrocene and *m*-toluic acid (mTA). These two compounds are known to be excellent substrates for binding by β-CD receptors.[18] However, the association constant of β-CD with mTA is larger than the corresponding value for ferrocene derivatives. Figure 7 shows some typical results of these competition experiments. The response of a Au/**2**+C$_5$SH electrode contacting a 5 μM solution of ferrocene (Figure 7A) is identical with that already described. Addition of 5 μM mTA to the same solution causes a substantial decrease in the faradaic currents associated with the ferrocene/ferrocenium waves (Figure 7B). This result clearly indicates that mTA is competing for the same monolayer binding sites previously occupied by the ferrocene molecules. The monolayer-bound mTA molecules are not detectable by voltammetric means since mTA is not electroactive in the potential range surveyed. Integration of the anodic ferrocene wave indicates that 55% of the surface-bound ferrocene molecules are displaced from the monolayer by the presence of an equimolar concentration of mTA, which is qualitatively consistent with the larger binding constant of mTA (compared to ferrocene) with β-CD in aqueous solution. Furthermore, when the concentration of mTA in the solution reaches the level of 7.5 μM, the ferrocene/ferrocenium couple is no longer observed (see Figure 7C), indicating that the mTA molecules compete effectively with the ferrocene molecules, displacing them from the monolayer binding sites and preventing their voltammetric detection. These results clearly confirm the dynamic nature of the complexation process of these substrates by the immobilized cyclodextrin receptors in the monolayer.

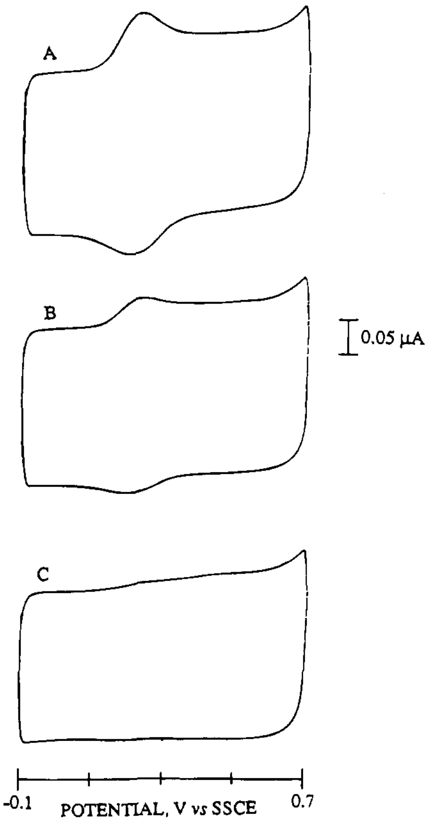


**Figure 7.** Voltammetric response of a Au/**2**+C$_5$SH electrode (0.035 cm$^2$) at 0.5 V/s in 0.2 M Na$_2$SO$_4$ also containing (A) 5 μM ferrocene, (B) 5 μM ferrocene + 5 μM mTA, and (C) 5 μM ferrocene + 7.5 μM mTA.

## Discussion

The electrochemical data presented here clearly indicate that the thiolated cyclodextrin receptor **2** chemisorbs readily on gold. However, our experiments did not provide any direct information on the number of S−Au bonds that can be formed between each CD and the gold surface. This point is of crucial importance to estimate the cyclodextrin surface coverage from reductive desorption experiments. In spite of the usual representation of cyclodextrins as rather rigid basket-shaped hosts, β-CD receptors are moderately flexible, and the group of thiol groups on the narrow opening of host **2** can depart from the symmetrical 7-fold conformation which would be expected for the undistorted cyclodextrin. Molecular mechanics calculations have suggested[19] that the cyclodextrins are continuously undergoing small distortions around their average symmetrical conformation. It is thus reasonable to assume that the rim of thiol groups in **2** can experience distortions that would lead to an increase in the number of covalent bonds between the cyclodextrin and the gold surface. Detailed investigations of thiolate chemisorption on gold surfaces suggest that the sulfur atoms preferentially attach to 3-fold hollow sites on the gold surface.[20] Inspection of CPK models of receptor **2** approaching

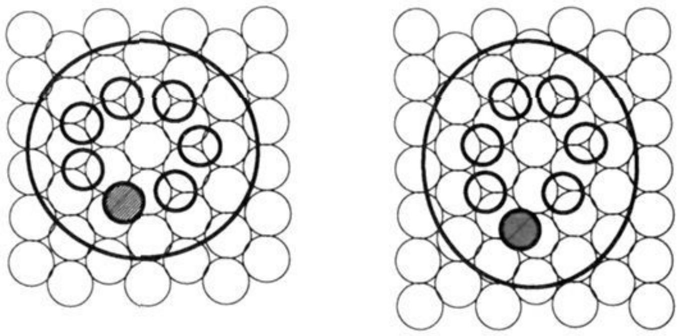
---

[18] Inoue, Y.; Hakushi, T.; Liu, Y.; Tong, L.-H.; Shen, B.-J.; Jin, D.-S. *J. Am. Chem. Soc.* **1993**, *115*, 475.

[19] Lipkowitz, K. B. *J. Org. Chem.* **1991**, *56*, 6357.

[20] Sellers, H.; Ulman, A.; Shnidman, Y.; Eilers, J. E. *J. Am. Chem. Soc.* **1993**, *115*, 9389.

**Scheme 4.** Examples of Possible Overlays of Sulfur Atoms (Represented by Thick-Line Circles) from Receptor **2** on the Gold Surface. The Patterned Sulfur Atom Does Not Fit Exactly on One of the 3-Fold Hollow Sites



a scaled model of the gold (111) surface indicates that six thiolate−gold bonds can be readily formed, requiring only small receptor distortions from the 7-fold symmetrical conformation. Two representative examples of possible overlays of sulfur atoms on the (111) gold surface are given in Scheme 4, showing that only relatively small deviations from 7-fold symmetry are required for the formation of at least six bonds per receptor. Therefore, the cathodic charge measured in the reductive desorption experiments probably represents a surface coverage in the range 64−75% (six to seven S−Au bonds per receptor). The uncovered surface area in submonolayers of **2** which can be sealed with pentanethiol is consistent with the formation of seven S−Au bonds per surface-attached cyclodextrin. The large fraction of uncovered surface is commensurate with the large individual surface area occupied by **2**, as the size of the individual defects is anticipated to correlate with the size of the self-assembling molecules. This is especially true if the adsorbate molecules cannot undergo lateral displacements on the surface after chemisorption. Furthermore, no favorable lateral (side-to-side) interactions among molecules of **2** are expected. These factors contribute to hamper the self-assembly of *compact* monolayers of **2**. On the other hand, each surface-attached receptor **2** should be strongly stabilized by the large number of S−Au bonds that can be formed per receptor molecule. Thus, self-assembly of **2** on gold surfaces leads to stable monolayers with numerous and rather large defective sites.

The differential capacitance of the electrode solution interface is extremely sensitive to the interfacial composition and, thus, it is commonly used to evaluate monolayer formation and quality. According to the parallel plate capacitance model, based on the Helmholtz theory, the electrode surface and the electrolyte solution are viewed as the capacitor conducting plates, while the monolayer represents the dielectric material. The electrode capacitance $C$ (per unit area) is then given by the simple equation

$$C = (\epsilon\epsilon_0)/d \qquad (3)$$

where $\epsilon$ is the dielectric constant of the insulating medium (the monolayer), $d$ is the distance between the conducting plates (the monolayer thickness), and $\epsilon_0$ is the vacuum permittivity. This simple model has been shown to describe reasonably well the capacitance of electrodes derivatized with alkanethiol monolayers.[15] Our capacitance measurements at several potentials within the range of interest demonstrate cyclodextrin chemisorption upon exposure of gold electrodes to solutions of **2**. However, the recorded capacitance values (in the range 10−14 $\mu$F/cm$^2$) are higher than those expected for compact monolayers, revealing the large density of defective areas in monolayers of pure **2**. After exposure of these electrodes to "sealing" solutions (containing pentanethiol and ferrocene), the measured capacitances decrease to values in the range 6.2−8.3 $\mu$F/cm$^2$. Literature values[15] for pentanethiol monolayers are slightly lower (in the range 2−7 $\mu$F/cm$^2$ depending on supporting electrolyte and electrode potential), but this is expected as our monolayers contain a reasonable density of well-defined pinholes (the cyclodextrin cavities). Therefore, the "sealing" step seems to be quite effective.

The selection of pentanethiol as the sealing agent deserves some comment. Why use pentanethiol as opposed to longer-chain *n*-alkanethiols? The answer is simply related to molecular dimensions. It seemed appropriate to patch the monolayer defects with an alkanethiol that would span to fit the monolayer thickness expected from the cyclodextrins (about 8 Å). The thickness of pure pentanethiol monolayers has been measured by ellipsometry as ~7 Å.[15a] Longer alkanethiols would generate monolayer patches thicker than the cyclodextrin receptors, creating an uneven monolayer interface with multiple steps. We decided to avoid this scenario as binding modes for hydrophobic molecules at the stepped surface could be envisaged. Pentanethiol was the ideal sealing agent to avoid this problem and guarantee that the cyclodextrin cavities were the only binding sites presented to the guests in the contacting solution.

Our data do not allow a quantitative determination of the effectiveness of ferrocene at protecting the CD cavities in the monolayer sealing process. In principle, the capacitance data indicate the perforated nature of the mixed **2**/C$_5$SH monolayers obtained after the sealing step. On the other hand, ferrocene cannot provide perfect protection because its binding to a CD cavity is a dynamic process and, when dissociation takes place, the cavity is exposed, at least for a short time, to the pentanethiol molecules. It seems reasonable to assume that a fraction of the surface-attached cyclodextrins will be clogged by through-cavity pentanethiol chemisorption and, thus, rendered useless for binding purposes. However, the observation of effective ferrocene binding (as demonstrated by the voltammogram of Figure 4A) reveals that a substantial fraction of surface-attached CD receptors are competently protected by ferrocene in the monolayer sealing step. The treatment of the ferrocene surface coverage data according to the Langmuir adsorption formalism yields a limiting surface coverage of $2.5 \times 10^{-11}$ mol/cm$^2$ which might reflect the surface density of open, ready-to-bind cyclodextrin cavities in the interfacial assembly. This value represents about 60% of the surface coverage of **2** (calculated from the reductive desorption data on the basis of seven S−Au bonds per molecule) before the pentanethiol sealing step. One may conclude that, during the treatment with the pentanethiol/ferrocene mixture, at least about 60% of the existing cyclodextrin cavities survive, i.e., remain unclogged because of the protection offered by ferrocene binding.

The serious problems associated with the quantitative interpretation of the reductive desorption data have been discussed already. However, the excellent qualitative agreement among the results obtained from three different kinds of electrochemical experiments strongly support the proposed models for the monolayers of **2** and **2**+C$_5$SH. Electrode capacitance measurements, reductive desorption charges, and monolayer blocking effects on the electron transfer reactions of hydrophilic electroactive probes indicate consistently that (i) the monolayers of **2** contain a large density of defects, (ii) the quality of the monolayers improves after the sealing step (exposure to the sealing agent, pentanethiol, while the cyclodextrin cavities are protected by ferrocene binding), and (iii) the mixed **2**+C$_5$SH monolayers contain more pinholes than the monolayers of pure pentanethiol.

In spite of the low solubility of ferrocene in aqueous media we could estimate thermodynamic parameters (based on the

Langmuir adsorption formalism) for the binding of ferrocene to the immobilized cyclodextrin cavities. We obtained a value for $\Delta G^\circ_{ads}$ of ca. −6.4 kcal/mol, which is slightly larger than literature values of $\Delta G^\circ$ for the complexation of ferrocene derivatives by β-CD in homogeneous aqueous solution. For instance, Evans and co-workers reported a $\Delta G^\circ$ value of −4.50 kcal/mol at 27 °C for the complexation of the ferrocenecarboxylate anion by unmodified β-CD in aqueous media.[14c] Our group obtained $\Delta G^\circ$ values of −4.47 and −4.29 kcal/mol at 25 °C for the β-CD complexation of methyl and heptyl(ferrocenylmethyl)ammonium cations in aqueous media.[14d] No thermodynamic values have been reported for the complexation of underivatized ferrocene, presumably because of its limited solubility in aqueous media. The origin of the additional free energy of complexation released in the interfacial situation is unclear at this point, but it might be related to the differences in guest structures.

In a very elegant study on the complexation of ferrocenecarboxylic acid by β-CD in aqueous solution, Evans and coworkers reported[14c] that the oxidation of the ferrocene derivative only takes place after complex dissociation; i.e., direct electron transfer from the cyclodextrin-bound species to the electrode was not observed. We have observed similar electrochemical oxidation mechanisms for several cationic ferrocene derivatives in the presence of β-CD.[14d] This body of work leads us to propose a similar CE mechanism (electrochemical step preceded by a chemical process) for our monolayer system. Electron transfer from the ferrocene molecules to the electrode surface takes place immediately after dissociation of the interfacial inclusion complex. The ferrocene molecules would then be oxidized as they leave the monolayer binding sites. This mechanism would imply that the electron transfer partners are separated by a distance equal to or larger than the monolayer thickness (~8 Å) when the transfer takes place. This is perfectly feasible as relatively fast electron transfer between monolayer-anchored ferrocene groups and the electrode surface has been observed across thicker monolayer assemblies.[21] The monolayer binding sites play a fundamental role as they are responsible for the accumulation of ferrocene molecules in the vicinity of the electrode surface. As the control voltammogram of Figure 3B demonstrates, ferrocene oxidation is not observed on an electrode derivatized with a monolayer lacking the built-in cyclodextrin cavities. The proposed mechanism is also consistent with the observed half-wave potential for the ferrocene/ferrocenium couple (0.19 V versus SSCE) which is more positive than the reported value[22] obtained with an unmodified electrode in sulfate-containing aqueous media (0.165 V versus SSCE).

## Conclusions

We have shown that an appropriately modified β-CD derivative chemisorbs on gold, generating stable, but imperfect, monolayers. We have developed a simple procedure to "patch" the defects of these monolayers, while the cyclodextrin cavities are protected. However, this procedure may also inactivate a fraction of the surface-attached cavities. The resulting monolayers exhibit excellent binding ability toward appropriate species in the contacting solution. Our voltammetric data demonstrate that ferrocene and mTA molecules are included in the cavities of the interfacial receptors. The relative binding strengths correlate well with those observed in the solution phase.

Our work reveals some of the difficulties involved in the preparation of self-assembled monolayers using large molecules as the monomeric components. Naturally, these monolayers are anticipated to contain large densities of defects, but procedures to repair the defective areas with other adsorbates can be devised. Future designs of interfacial hosts should attempt to optimize lateral interactions between the immobilized hosts in order to improve their self-assembly on metal substrates.

**Acknowledgment.** The generous support of this research by the NSF in the United States (CHE-9000532 and CHE-9304262) and SERC in the United Kingdom is gratefully acknowledged. A.E.K. acknowledges the award of a collaborative grant from the NATO Special Program on Supramolecular Chemistry. R.K. thanks Pfizer for a postgraduate studentship. A.E.K. also thanks the American Maize-Products Company for generous gifts of cyclodextrins.

JA9339662

---

(21) Chidsey, C. E. D. *Science* **1991**, *251*, 919.

(22) Oshawa, Y.; Aoyagui, S. *J. Electroanal. Chem.* **1982**, *136*, 353.