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# QCM Study of the Aggregation of Starburst PAMAM Dendrimers on the Surface of Bare and Thiol-Modified Gold Electrodes

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The aggregation of starburst PAMAM (poly(amidoamine)) dendrimers (bearing either 64 carboxylic or amine peripheral groups) from micromolar aqueous neutral solutions on the surface of bare and thiol (HS(CH<sub>2</sub>)<sub>2</sub>COOH or HS(CH<sub>2</sub>)<sub>2</sub>NH<sub>3</sub>Cl) modified gold electrodes has been studied using the QCM technique. As expected, the electrostatic interaction between protonated amine groups and carboxylate functional groups has been shown to promote much larger surface coverage values than those obtained for naked clean gold substrates. Computer fitting of the obtained titration curves to Langmuir and Frumkin isotherm models suggests that the main effect of the surface attached thiol is not an increased overall adsorption energy but a favorable lateral interaction between the adsorbed dendrimer molecules.

## Introduction

The preparation and characterization of surface modified substrates has been the subject of a substantial research effort during the last two decades.<sup>1</sup> Among the many techniques reported for the synthesis of modified surfaces, the covalent attachment of thiolated species to the surface of clean gold substrates has proved to be one of the easiest and most reliable approaches developed so far.<sup>2</sup> The ability to form monolayer coatings of thiol molecules bearing specific terminal functional groups has, for instance, allowed researchers to prepare well-defined surfaces that have been used to design and construct electrochemical sensors,<sup>3</sup> catalysts,<sup>4</sup> and optoelectronic<sup>5</sup> and nanometer sized electronic devices<sup>6</sup> among many other applications. The careful positioning of specific terminal groups at the monolayer end of a given thiol has, on the other hand, allowed scientists to study and to exploit molecular recognition properties of potentially important systems at the electrode–solution interface. In this way, electroactive and non-electroactive molecules as well as supramolecular structures have been organized on an

interface on the basis of a modified surface that selectively interacts with a given species in solution.<sup>7</sup>

On the other hand, since their invention about 20 years ago, the dendrimers, also known as cascade or hyper-ramified polymers, have captured the interest and fascination of many research groups around the world.<sup>8</sup> The sequential incorporation of ramified monomers into a multifunctional core results in a radial gradient of properties that make these compounds an important new class of polymers with void regions and tailored functional internal and peripheric groups that allow a fine control of the interactions between these macromolecules and their environment. Although the number of recent publications in the scientific literature clearly reveal this fact, most of the work has been focused on the development of synthetic protocols and therefore has been carried out in solution. The use of dendrimers to modify the structure, properties, and behavior of surfaces and, in particular, of electrochemical interfaces is therefore a research field that has been barely explored.<sup>9</sup> In this work, we report the results of a study that is aimed to understand the factors that control the aggregation of commercial starburst PAMAM dendrimers on the surface of bare and thiol modified gold electrodes from aqueous neutral solutions. In particular, we have studied the aggregation of two similar starburst PAMAM dendrimers (each one bearing either 64 carboxylic acid or amine peripheral functional groups), on the surface of bare and modified substrates characterized by sub-monolayer coverages of either carboxylic acid (HS(CH<sub>2</sub>)<sub>2</sub>COOH) or amine terminated (HS(CH<sub>2</sub>)<sub>2</sub>NH<sub>3</sub>Cl) thiols.

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## Experimental Section

HS(CH<sub>2</sub>)<sub>2</sub>COOH (99%), **1**, HS(CH<sub>2</sub>)<sub>2</sub>NH<sub>3</sub>Cl (98%), 2<sup>+</sup>Cl<sup>-</sup>, as well as starburst (PAMAM) dendrimers Generation 4.0, **3** (64 terminal primary amine groups), and Generation 3.5, **4** (64 terminal carboxylic groups), were obtained from Aldrich and used without further purification. Ru(NH<sub>3</sub>)<sub>6</sub>Cl<sub>3</sub> was obtained from Strem Chemicals, and the electrolyte salts KH<sub>2</sub>PO<sub>4</sub>, Na<sub>2</sub>HPO<sub>4</sub>, and KOH as well as the organic solvents MeOH and EtOH, HPLC grade, all were obtained from J. T. Baker. Aqueous solutions were prepared with deionized water ( $\rho \geq 18 \text{ M}\Omega\cdot\text{cm}$ ) and further deoxygenated by bubbling N<sub>2</sub> (Praxair, grade 5.0) for at least 15 min before the electrochemical experiments were carried out.

Cyclic voltammetry (CV) experiments were performed using either a 263A-PAR (Princeton Applied Research) potentiostat/galvanostat or a PGZ301 VoltaLab potentiostat (Radiometer-Copenhagen). Whereas the PAR instrument was coupled to a M270 version 4.4. data acquisition program, the Volta Lab equipment was programmed and controlled by a computer loaded with the Volta Master 4.0 software. Resistance compensation for all the experiments was automatically computed and corrected for by the software. Electrochemical quartz crystal microbalance measurements were carried out by coupling to the PAR potentiostat a Maxtek PM-740 frequency monitor that is controlled with the PM-700 Data Log software.

Electrochemical quartz crystal microbalance (EQCM) experiments were performed in a glass cell (250 mL) fitted with a three electrode arrangement in which a SCE electrode, a gold mesh, and an AT-cut quartz Au covered crystal (5 MHz, MAXTEK, Torrance, CA) were used as reference, counter, and working electrodes, respectively. Before the experiments were made, the Au electrodes were polished with a Buehler's cloth that contained  $\alpha\text{-Al}_2\text{O}_3$  (0.1  $\mu\text{m}$ , aqueous suspension from Buehler), then washed with chromic mixture, rinsed with dilute nitric acid and plenty of deionized water, and dried under nitrogen. Deposited film masses from EQCM measurements were calculated using the Sauerbrey equation in which frequency changes  $\Delta f$  are linearly related (through the constant  $-C_f$ ) to mass changes ( $\Delta m_{\text{ads}}$ ) on the quartz crystal.<sup>10</sup>

$$\Delta m_{\text{ads}} = -C_f(\Delta f) \quad (1)$$

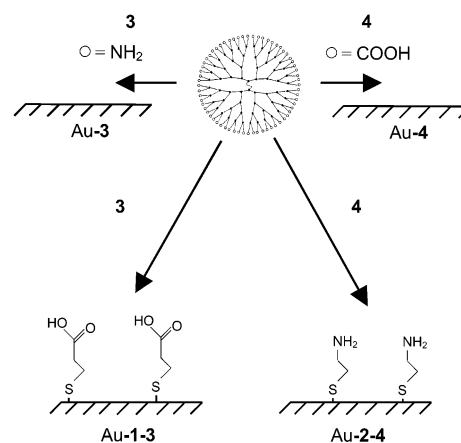
Gold beads for the CV experiments reported in this work were prepared from premium Au wire (99.999%, 0.5 mm diameter, Johnson Matthey) using the flame of a Bunsen burner according to reported procedures.<sup>11</sup> The geometric area for each one of these electrodes was calculated using the electrochemical response of a 2.0 mM Ru(NH<sub>3</sub>)<sub>6</sub><sup>3+</sup> ( $D = 5.5 \times 10^{-6} \text{ cm}^2\cdot\text{s}^{-1}$ ) solution in phosphate buffer pH = 7 ( $I = 0.1$ ) and the Randles-Sevcik equation.<sup>12</sup>

Self-assembled sub-monolayers of the thiol molecules on either the gold beads or the Au covered quartz crystals were prepared by overnight immersion of the metallic substrates in 1 mM deoxygenated ethanolic solutions of the appropriate thiol.

Conductivity experiments were carried out on electrolyte-free aqueous solutions of the relevant dendrimer using a YSI conductimeter 3200 model at room temperature. Conductivity versus concentration measurements for each dendrimer allowed the determination of the conductivity at infinite dilution, which then was used to determine the  $pK_a$ .<sup>13</sup>

## Results and Discussion

As previously mentioned, the aim of this work was to understand the forces that a thiol modified gold surface exert on the aggregation of PAMAM starburst dendrimers in aqueous solution. As can be seen in Figure 1, we explored



**Figure 1.** Scheme showing the systems studied. Whereas **3** and **4** represent starburst (PAMAM) dendrimers generation 4.0 and generation 3.5, respectively, **1** and **2** correspond to the HS(CH<sub>2</sub>)<sub>2</sub>COOH and HS(CH<sub>2</sub>)<sub>2</sub>NH<sub>3</sub>Cl species that were used to modify gold substrates.

two dendrimers bearing either 64 amine or 64 carboxylic acid peripheral functional groups (**3** and **4**) and two thiol modified surfaces that are characterized by the same terminal organic functionalities (Au-1 and Au-2). Since all the species involved in the study are ionizable in aqueous media, the first stage of the project consisted in the preparation, characterization, and evaluation of the acid–base properties of the systems to be studied.

**i. Preparation and Characterization of the Systems To Be Studied.** The first step was therefore the preparation and characterization of the modified gold surfaces, Au-1 and Au-2, on which the aggregation of the dendrimers was going to be studied. Self-assembled monolayers of the relevant thiols on clean, freshly prepared gold bead electrodes were then obtained as described in the Experimental Section, and the surface coverage values of these thiol modified substrates were calculated from electrodesorption measurements in 0.5 M KOH, as reported in the literature.<sup>14</sup> The obtained values for the charge involved in the electrodesorption process, along with the roughness factor that for these gold electrodes has been reported to fall in the 1.1–1.2 range,<sup>15</sup> showed that sub-monolayer coverages of the relevant molecules were covalently formed in the conditions under study. In this way, surface coverage values ( $\Gamma_{\text{thiol}}$ ) of  $4.4 \times 10^{-11}$  and  $5.6 \times 10^{-11} \text{ mol/cm}^2$  were obtained for the Au-1 and Au-2 modified surfaces, respectively. As pointed out by Ulman,<sup>2a</sup> and consistent with previously reported surface coverages for the Au-2 system,<sup>14</sup> the sub-monolayer coverage values thus obtained were not surprising in view of the short alkyl chains that separate the thiol and the terminal functional groups for species **1** and **2**.

Au-covered quartz crystal substrates on which dendrimer aggregation was going to be studied were modified under the same conditions employed for gold bead electrodes, and electrodesorption measurements on these substrates were then carried out essentially in the same way as that previously described. The charge values obtained in these experiments, along with the geometric area of the Au-covered quartz crystals and the surface coverage values obtained with the gold bead electrodes, were then used to calculate the roughness factor (defined as real area/geometric area) of the Au quartz crystal

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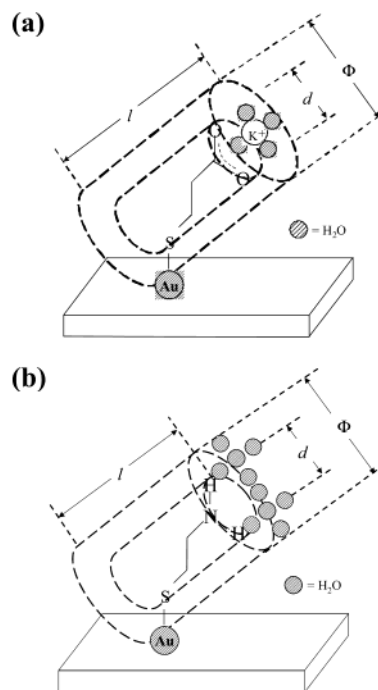
substrates. Several determinations of this factor gave an average value of  $4.9 \pm 0.7$ .

The proper analysis of the dendrimer aggregation data required another piece of information for these modified substrates. To find out whether the thiol molecules anchored on the gold electrode surface were "isolated", due to limited lateral van der Waals interaction between the short alkyl chains, or forming "islands" as a consequence of hydrogen bonding effects between the terminal functional groups of the thiols, we decided to perform a rough calculation based on the number of water molecules involved in the desorption process. This information was important because, depending on the actual state of the aggregated thiols, the  $pK_a$  of the terminal groups could be significantly different. Indeed, whereas the  $pK_a$  values of isolated carboxylic and amino terminal groups can be easily approximated to their corresponding values in solution, the  $pK_a$ 's are known to be seriously shifted when the terminal functional groups are positioned close to each other in a dense aggregate.<sup>11,16</sup> Therefore, while the electrochemical desorption experiments permitted the direct determination of the number of thiol molecules involved in the process, the coupled QCM measurement offered us the possibility of the simultaneous detection of the mass change associated with the desorption of the thiol along with the water molecules that were involved in its solvation. Equation 2 shows that the experimentally measured number of water molecules,  $Z_{\text{exp}}^{\text{H}_2\text{O}}$ , involved in the solvation per molecule of the thiol species is given by

$$Z_{\text{exp}}^{\text{H}_2\text{O}} = \frac{C_f(\Delta f) - \Gamma_{\text{thiol}} \text{MW}_{\text{thiol}}}{\text{MW}_{\text{H}_2\text{O}} \Gamma_{\text{thiol}}} \quad (2)$$

where  $C_f(\Delta f)$  corresponds to the negative mass change in the process (see eq 1),  $\Gamma_{\text{thiol}}$  is the thiol surface coverage, and  $\text{MW}_{\text{H}_2\text{O}}$  and  $\text{MW}_{\text{thiol}}$  correspond to the molecular weight of the water and the thiol molecules, respectively. The results of these calculations show that  $Z_{\text{exp}}^{\text{H}_2\text{O}}$  corresponds to 111 and 40 water molecules for **1** and **2**, respectively, and therefore, it was reasonable to expect that these numbers should somehow correlate with a theoretical solvation model. As can be schematically seen in Figure 2, the number of water molecules involved in the solvation of an isolated thiol molecule anchored on the gold surface can be approximated using the volume of the first solvation layer around the anchored thiol and the density of the water at room temperature. The number of water molecules using this model gave values of 119 and 41 for compounds **1** and **2**, which are very similar to the experimentally obtained values through the use of eq 2. This strongly suggests that, for the interfaces under study, the thiol molecules are essentially isolated and that, therefore, the use of the relevant  $pK_a$  value for each compound in solution (4.87 and 10.75 for **1** and **2**, respectively)<sup>17</sup> should constitute a valid approximation.

In the case of the acid–base properties of the dendrimer molecules under study, conductivity measurements in pure water, as described in the Experimental Section, allowed the computation of the average  $pK_a$  value of the relevant terminal groups of the polymer.  $pK_a$  values of 9.52 and 3.79 were thus obtained for dendrimers **3** and **4**, respectively, and it is worthy of note that the value corresponding to **3** is essentially the same as that previously reported by Crooks and co-workers.<sup>18</sup>



**Figure 2.** Cartoon showing the model employed to calculate the volume of the first solvation layer of gold anchored thiols (a) **1** and (b) **2**. The values of  $l$ ,  $d$ , and  $\Phi$  (14.94, 2.27, 10.83 and 9.43, 1.12, 9.40 Å for **1** and **2**, respectively) were obtained from the bond lengths found in the chemical literature<sup>29</sup> and assuming a 30° angle between the thiol molecule and the underlying gold substrate.<sup>2–b</sup>

**ii. Dendrimer Aggregation Studies.** Once the  $pK_a$  values for all the species involved in the study were calculated, phosphate buffered aqueous solutions ( $I = 0.1$ ) at pH values of 2, 7, and 12 were selected to study the aggregation of the corresponding dendrimers on bare and thiol modified Au surfaces. It was assumed that, at these pH values, either dendrimer or thiol molecules would be fully ionized or neutral and that, as a consequence, their electrostatically controlled aggregation could be maximized at neutral pH conditions in which the charges of the thiolated surfaces and dendrimers would be opposed and complementary.

The aggregation of **3** and **4** from micromolar aqueous solutions of the polymeric materials on the surface of bare and modified gold electrodes was therefore followed by QCM titration experiments. Frequency changes obtained after each dendrimer addition were then converted to mass values using eq 1 and further transformed to surface coverage values using the molecular weights of the corresponding dendrimer molecules.

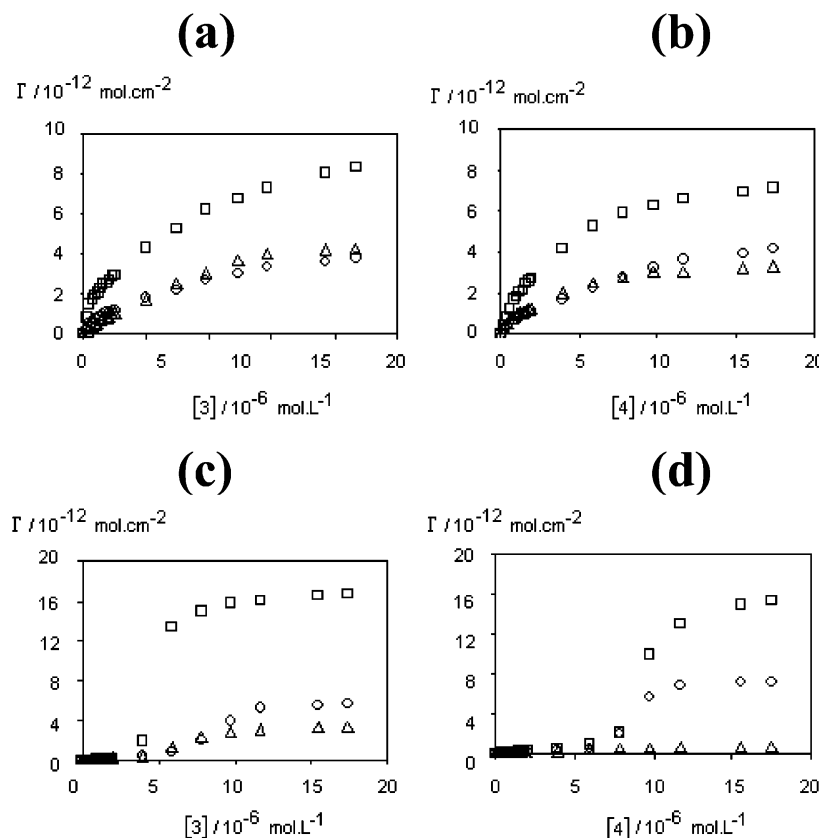
The resulting titration curves are presented in Figure 3, and as expected, the aggregation seems to be strongly dependent on the pH of the solution and on the presence of the pH sensitive thiols. Whereas aggregation of **3** and **4** on clean gold surfaces, at any pH condition, never reaches surface coverage values above  $9 \times 10^{-12}$  mol/cm<sup>2</sup>, the deposition under neutral conditions for complementary charged dendrimers and thiol modified substrates achieves surface coverage values close to  $1.6 \times 10^{-11}$  mol/cm<sup>2</sup> when the concentration of the polymer molecule is about 20  $\mu\text{M}$ .<sup>19</sup> In these two cases (Figure 3c and d), it was assumed that carboxylated and protonated amine groups are present in the dendrimer surfaces and on the functional

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**Figure 3.** Adsorption isotherms at pH values of 2 (O), 7 (□) and 12 (Δ) of dendrimers (a) **3** and (b) **4** on clean gold substrates and of (c) **3** on Au-1 and (d) **4** on Au-2 from micromolar phosphate aqueous buffer solutions ( $I = 0.1$ ) at 298 K.

groups of the adsorbed thiols and that the electrostatic interaction thus created should therefore be responsible for the larger dendrimer coverage values observed.

Another interesting difference that can be appreciated from comparison of the aggregation curves presented in Figure 3 is the shape of the titration isotherms. Whereas the high surface coverage curves for Au-1-**3** and Au-2-**4** at neutral pH conditions are essentially sigmoidal, the shapes of all the other curves suggest a regular adsorption isotherm that approaches saturation at surface coverage values smaller than  $9 \times 10^{-12}$  mol/cm<sup>2</sup>. The sigmoidal shape of the electrostatically induced adsorption isotherms suggests that, at low dendrimer concentrations, the polymeric material does not compete successfully for the ionic surface sites with the supporting electrolyte ions. Above a critical concentration, however, displacement from these surface sites takes place and the coverage of the dendritic material rapidly approaches a value almost twice as large as that observed for the adsorption at any of the other substrates surveyed.

To better understand the adsorption process, we decided to model the aggregation events using the Langmuir and the Frumkin isotherms.<sup>20</sup> Following Abruña, Cuadrado, Morán, and co-workers,<sup>21</sup> the Langmuir isotherm,

$$\beta C = \frac{\Theta}{1 - \Theta} \quad (3)$$

(where  $\beta$  corresponds to the adsorption coefficient ( $\beta =$

$(1/55.5) \exp(-\Delta G_{\text{ads}}^{\theta=0}/RT)$ ) and  $\Delta G_{\text{ads}}^{\theta=0}$  is the free energy of adsorption in the limit where the surface coverage fraction  $\theta$  ( $\theta = \Gamma/\Gamma_m$ ) approaches zero) was used to model the aggregation of dendrimers on clean gold substrates (see Figure 4). From the fitting of the experimental data,<sup>22</sup> the monolayer surface coverage  $\Gamma_m$  and the adsorption coefficient  $\beta$  were obtained for each case and subsequently fed into the Frumkin isotherm

$$\beta C = \frac{\Theta}{1 - \Theta} \exp(a\Theta) \quad (4)$$

in order to obtain the parameter  $a$  that is related to the intermolecular interactions between adsorbate molecules.<sup>20</sup>

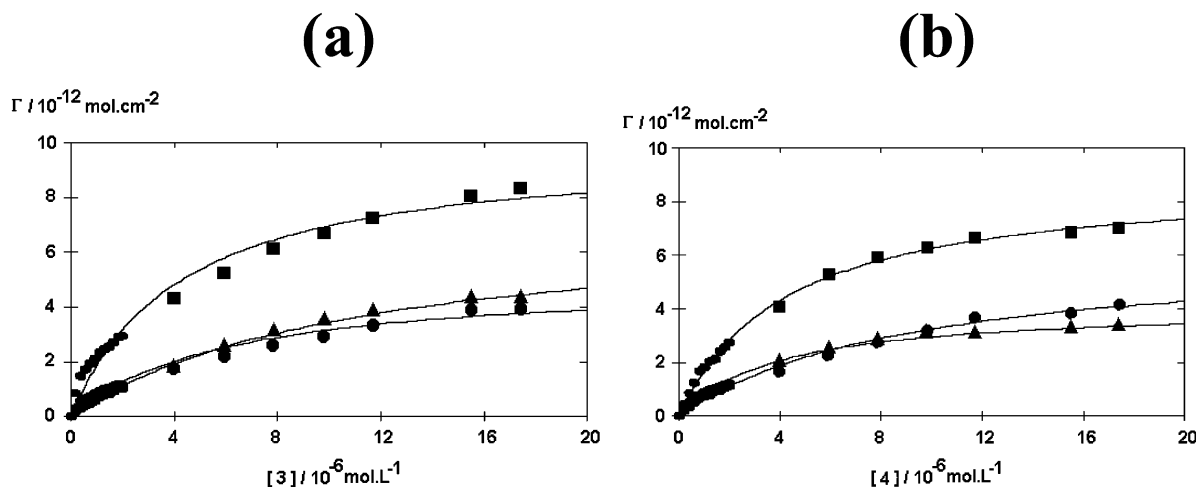
For clean gold substrates, the fitting of the relevant data is shown in Figure 5a and b, and as can be observed by comparison to Figure 3, the use of  $\Gamma_m$  to obtain  $\theta$  changes the relative effect of pH on the aggregation isotherms in such a way that the surface coverage fraction  $\theta$  is no longer very dependent on the pH of the solution. Inspection of the corresponding data presented in Table 1 also shows that, as expected, the monolayer surface coverage  $\Gamma_m$  is the smallest for the extreme pH values at which **3** and **4** are fully ionized, that is, at pH conditions of 2 and 12, respectively. In terms of the obtained free energies of adsorption, on the other hand, it can be observed that the most negative values for both cases are found at neutral pH conditions. The fact that at this pH value the driving force for adsorption is the most favorable is also consistent with the fact that the corresponding  $\Gamma_m$  values are also

(19) QCM control experiments with the Au-1 + **4** and the Au-2 + **3** systems did not show aggregation of the dendrimer molecules on the thiol modified surfaces.

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(22) Computer fitting of the data was carried out using the MINSQ software (MicroMath Scientific Software, Salt Lake City, UT).



**Figure 4.** Computer fitting of the aggregation data to the Langmuir isotherm model at pH values of 2 (●), 7 (■), and 12 (▲) for (a) **3** and (b) **4** on clean gold substrates at 298 K.

**Table 1. Thermodynamic Data Obtained from the Computer Fitting of Dendrimer Adsorption Using the (i) Langmuir and (ii) Frumkin Adsorption Isotherm Models at Room Temperature**

interface	pH	$i\Gamma_m / 10^{-12} \text{ mol}\cdot\text{cm}^{-2}$	$ii\Delta G_{\text{ads}}^{\theta=0} / \text{kJ}\cdot\text{mol}^{-1}$	$ii a$
Au- <b>3</b>	2	$5.07 \pm 0.27$	$-40.11 \pm 0.23$	$0.09 \pm 0.08$
	7	$9.87 \pm 0.41$	$-40.99 \pm 0.27$	$0.05 \pm 0.09$
	12	$7.27 \pm 0.21$	$-38.11 \pm 0.09$	$0.00 \pm 0.03$
Au- <b>4</b>	2	$6.25 \pm 0.25$	$-38.76 \pm 0.15$	$0.01 \pm 0.05$
	7	$8.87 \pm 0.12$	$-40.69 \pm 0.09$	$0.00 \pm 0.03$
	12	$4.11 \pm 0.10$	$-40.23 \pm 0.17$	$0.00 \pm 0.05$
Au- <b>1-3</b>	2	$5.07 \pm 0.27$	$-34.29 \pm 0.04$	$-3.83 \pm 0.04$
	7	$9.87 \pm 0.41$	$-34.70 \pm 0.60$	$-4.86 \pm 1.34$
	12	$7.27 \pm 0.21$	$-33.94 \pm 0.17$	$-4.14 \pm 0.23$
Au- <b>2-4</b>	2	$6.25 \pm 0.25$	$-33.65 \pm 0.08$	$-4.36 \pm 0.16$
	7	$8.87 \pm 0.12$	$-34.13 \pm 0.25$	$-4.01 \pm 0.38$
	12	$4.11 \pm 0.10$	$-34.01 \pm 0.20$	$-2.01 \pm 0.63$

the largest for both systems. The values obtained for the interaction parameter  $a$ , on the other hand, are in all cases positive and fairly small. This suggests that a small repulsive interaction takes place during the adsorption of both dendrimers at any pH value.

In the case of the adsorption of **3** and **4** on the surface of the thiolated gold substrates Au-**1** and Au-**2**, the data were directly fitted to the Frumkin isotherm using the values of  $\Gamma_m$  previously obtained. As can be seen by inspection of Figure 5c and d, only the region before saturation (below  $10 \mu\text{M}$  dendrimer concentration) was considered. The thermodynamic adsorption data thus obtained are also shown in Table 1, and it can be readily seen that, at any pH value, the intermolecular adsorbate interaction factor  $a$  is negative for both systems and quite large, as compared to the case of the adsorption of **3** and **4** on bare gold. This observation indicates that, according to the model employed, and opposed to the adsorption on bare gold substrates, there is a clear attractive interaction between adsorbate molecules when the supporting substrate is modified with thiols bearing ionizable and/or hydrogen bonding terminal groups. Comparison of the  $a$  values for dendrimer aggregation on the modified gold surfaces, on the other hand, also shows that the attractive interactions are basically controlled by the protonation state of the surface attached thiol species. In this way, whereas the intermolecular attracting effect between dendrimer molecules seems to be large when the thiol molecules are charged, this effect substantially decreases due to the combined effect of a neutral thiol species and

the maximized ionized dendrimer electrostatic repulsion. We can therefore speculate that when the surface anchored thiol is ionized, its terminal groups "screen" the repulsive lateral interactions between the ionized dendrimer adsorbate molecules rendering larger and more compact aggregates.

Inspection of the free energies of adsorption in the limit of  $\theta = 0$  presented in Table 1, also reveal some insight into the aggregation processes under study. At a first glance, it can clearly be seen that the adsorption of **3** and **4** on the surface of the thiolated gold surfaces Au-**1** and Au-**2** is always favored but surprisingly smaller than the adsorption of any of the two dendrimers on naked gold electrodes. For dendrimer adsorption on any of the two thiolated gold electrodes, it can also be noted that although the most favorable driving force is always found at neutral conditions, the values obtained at extreme acidic or basic conditions are not very different and actually fall in some cases within the uncertainty computed from the fitted data.

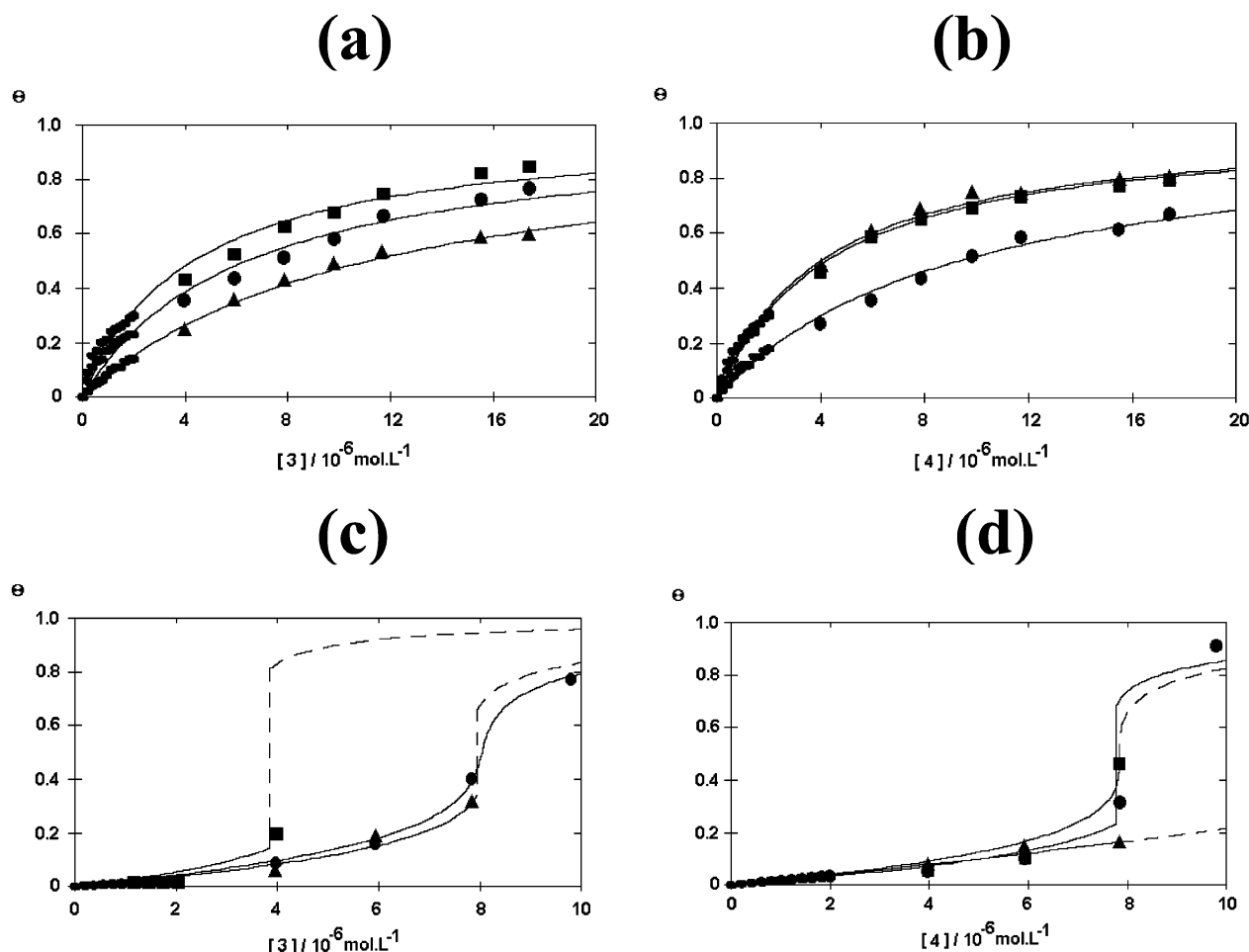
These observations were at first surprising because the larger and more compact aggregates observed on the surface of the two thiol modified gold surfaces at neutral pH conditions were expected to be controlled by pure electrostatic interactions, which, in turn, should have been reflected by large negative free energies of adsorption. Contrary to this, the results obtained clearly suggest that the main driving force for the observed efficient aggregation is not an improved free energy of adsorption in the limit of no coverage but instead a thiol induced attractive effect between the dendrimer adsorbate molecules.

In view of these observations, it was important to find out why the free energies for the adsorption of ionized dendrimers on the surface of oppositely charged, attached thiols were so low. We therefore decided to construct and analyze the thermodynamic cycles presented in Figures 6 and 7 and from them gain a deeper insight into the phenomena under study.

As can be seen in Figure 6, the cycle corresponding to the adsorption of dendrimers **3** and **4** on the surface of clean, bare gold electrodes shows three processes which, when coupled, are equivalent to that for which the experimental measurements presented in Table 1 were made. Equation 5 describes this relationship,

$$\Delta G_{\text{ads},\text{I}}^{\theta=0} = -n\Delta G_{\text{ads},\text{H}_2\text{O}}^{\theta=0} - j\Delta G_{\text{IP},\text{d}}^{\theta=0} + \Delta G_{\text{ads},\text{Au}}^{\theta=0} \quad (5)$$

where  $\Delta G_{\text{ads},\text{I}}^{\theta=0}$  represents the experimentally obtained



**Figure 5.** Computer fitting to the Frumkin isotherm model at pH values of 2 (●), 7 (■), and 12 (▲) for (a) **3** and (b) **4** on clean gold substrates and for (c) **3** on Au-1 and (d) **4** on Au-2 modified substrates at 298 K.

free energy of adsorption of the dendrimer at the surface of clean gold in the limit of zero surface coverage fraction,  $-n\Delta G_{\text{ads,H}_2\text{O}}^{\theta=0}$  is related to the free energy of desorption of the water molecules from the electrode surface,  $-j\Delta G_{\text{IP,d}}^{\theta=0}$  is the free energy associated with the removal of counterion molecules from the dendrimer surface ionized groups that adsorb on the electrode surface, and  $\Delta G_{\text{ads,Au}}^{\theta=0}$  corresponds to the free energy of association of the clean and unsolvated surfaces of the dendrimer molecule and the gold substrate.

The first term on the right-hand side of eq 5 actually consists of two terms,  $n$  and  $\Delta G_{\text{ads,H}_2\text{O}}^{\theta=0}$ , and the first term corresponds to the number of water molecules located on the surface of the electrode that occupy the area of one adsorbed dendrimer molecule. This term was calculated for each dendrimer at every pH condition using eq 6,

$$n = \left( \frac{A_{\text{dend}}}{A_{\text{H}_2\text{O}}} \right) \theta_{\text{H}_2\text{O}} = \left( \frac{r_{\text{dend}}}{r_{\text{H}_2\text{O}}} \right)^2 \theta_{\text{H}_2\text{O}} \quad (6)$$

where  $A_{\text{dend}}$  and  $A_{\text{H}_2\text{O}}$  correspond to the areas per molecule of dendrimer and water, respectively,  $r_{\text{dend}}$  and  $r_{\text{H}_2\text{O}}$  correspond to the radii of adsorbed dendrimer and water molecules, respectively, and  $\theta_{\text{H}_2\text{O}}$  is the fraction of the gold surface that at room temperature is covered with solvent molecules. The radius of water<sup>23</sup> and the fraction  $\theta_{\text{H}_2\text{O}}$ <sup>24</sup>

were directly obtained from the literature, and the value of  $r_{\text{dend}}$  was calculated through the use of eq 7 (where  $N_A$  corresponds to Avogadro's number).

$$r_{\text{dend}} = \sqrt{\frac{1}{\pi N_A \Gamma_{\text{m,av}}}} \quad (7)$$

In this equation, the monolayer surface coverage value ( $\Gamma_{\text{m,av}} = 9.37 \times 10^{-12}$  mol/cm<sup>2</sup>) corresponds to the average of those experimentally obtained from the QCM measurements at pH = 7. It is worthy of note that the adsorbed dendrimer area at this pH is also very similar to that obtained by Abruña and co-workers for dendrimer molecules that in solution exhibited the same radius as that of the compounds surveyed in this work.<sup>21</sup> The  $\Delta G_{\text{ads,H}_2\text{O}}^{\theta=0}$ , on the other hand, was obtained from the reported values of the free energy of adsorption of water molecules on gold from the gas phase<sup>25</sup> and from the free energy of condensation of this compound at room temperature.<sup>26</sup> The result of these calculations is shown in Table 2.

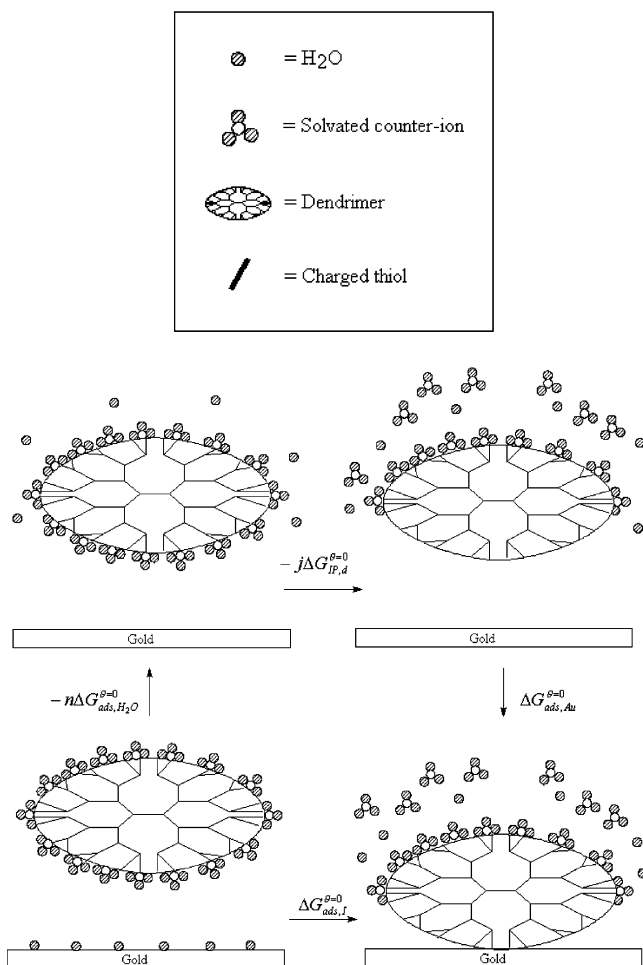
The second term on the right-hand side of eq 5 also contains two parameters:  $j$  and  $\Delta G_{\text{IP,d}}^{\theta=0}$ . Of these, the first

(24) (a) Bockris, J. O'M.; Reddy, A. K. N.; Gamboa-Aldego, M. *Modern Electrochemistry*, 2A, 2nd ed.; Kluwer Academic/Plenum Press: New York, 2000; p 895. (b) Berkowitz, M. L.; Yeh, I.; Spohr, E. In *Interfacial Electrochemistry: Theory, Experiment and Applications*; Wieckowski, Ed.; Marcel Dekker Inc.: New York, 1999; p 33.

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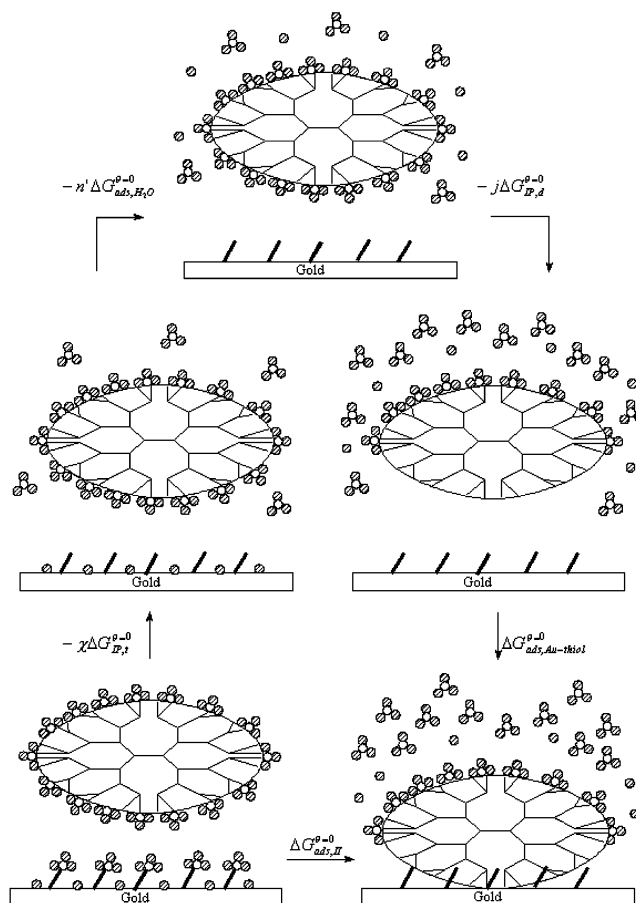
**Figure 6.** Thermodynamic cycle for the adsorption of dendrimer molecules **3** and **4** from dilute aqueous solutions on the surface of clean gold substrates.

one, related to the number of counterion molecules that must be removed from the surface ionized groups on a dendrimer molecule upon adsorption, was calculated considering that each adsorbed dendrimer molecule has a maximum of 32 ionizable groups on its adsorbing half, which, depending on the  $pK_a$  and pH value of the solution, could be either in a neutral or in an ionized state. Taking the  $pK_a$  values obtained from conductometric measurements as described in the Experimental Section, the values of  $j$  for dendrimers **3** and **4** at each pH condition were calculated, and as shown in Table 2, it is clear that the dendrimer surface is fully ionized at pH values of 2 and 12 for compounds **3** and **4**, respectively. The  $\Delta G_{IP,d}^{\theta=0}$  values, on the other hand, were obtained using the Fuoss relationship (eq 8),<sup>27,28</sup>

$$K_{IP} = K_A C^\circ = 4\pi N_A a^3 \exp\left(\frac{2e_0^2 |z_+ z_-|}{a 8\pi \epsilon_0 \epsilon k T}\right) C^\circ$$

$$\Delta G_{IP,d}^{\theta=0} = -RT \ln K_{IP} \quad (8)$$

where  $a$  is the sum of the ionic radii,  $e_0$  is the fundamental charge,  $z_i$  corresponds to the charge of the corresponding



**Figure 7.** Thermodynamic cycle for the adsorption of dendrimer molecules **3** and **4** from dilute aqueous solutions on the surface of thiol modified gold substrates. The legend in this figure is the same as that in Figure 6.

ion,  $k$  and  $T$  are the Boltzmann constant and the temperature, and  $\epsilon$  and  $\epsilon_0$  are related to the dielectric constant of water in the interfacial region (40)<sup>28</sup> and to the permittivity constant in a vacuum, respectively. It is important to note, however, that the values of  $a$  and  $z_i$  were functions of the composition of the buffer solution and the nature of the dendrimer surface ionic group at each pH condition studied and that, therefore, the obtained values of  $\Delta G_{IP,d}^{\theta=0}$  are, as shown in Table 2, slightly different for each system.

With this information in hand, it was then possible to use eq 5 to compute the values of  $\Delta G_{ads,Au}^{\theta=0}$  for the adsorption of desolvated dendrimers on clean bare gold substrates, as shown in the last column of Table 2.

Inspection of the values presented in this table shows that although the experimentally obtained free energies of adsorption  $\Delta G_{ads,I}^{\theta=0}$  are similar for both dendrimers at any of the pH conditions surveyed, the relative contributions  $j\Delta G_{IP,d}^{\theta=0}$  are very small when the dendritic molecules are essentially not ionized, that is, at the extreme pH conditions of 12 and 2 for dendrimers **3** and **4**, respectively. In this way, it is interesting to note that the small free energies of counterion desolvation  $j\Delta G_{IP,d}^{\theta=0}$  for the non-ionized dendrimers are compensated by small free energies for the adsorption  $\Delta G_{ads,Au}^{\theta=0}$  for these systems.

In the case of dendrimer adsorption on the surface of thiol modified gold substrates, the thermodynamic cycle is presented in Figure 7. As can be seen by comparison to the cycle corresponding to adsorption on bare gold electrodes (Figure 6), this thermodynamic construction

(27) Barthel, J. M. G.; Krinke, H.; Kunz, W. In *Topics in Physical Chemistry*, Vol. 5; Baumgartel, H., Franck, E. O., Grunbein, W., Eds.; Springer-Verlag: Germany, 1998.

(28) Bockris, J. O'M.; Reddy, A. K. N. *Modern Electrochemistry*, Vol. 1; Plenum Press: New York, 1970.



**Table 2. Free Energy Contributions for the Thermodynamic Cycle Presented in Figure 6**

substrate	pH	$\Delta G_{\text{ads,I}}^{\theta=0}/\text{kJ}\cdot\text{mol}^{-1}$	$n$	$\Delta G_{\text{ads,H}_2\text{O}}^{\theta=0}/\text{kJ}\cdot\text{mol}^{-1}$	$j$	$\Delta G_{\text{IP,d}}^{\theta=0}/\text{kJ}\cdot\text{mol}^{-1}$	$\Delta G_{\text{ads,Au}}^{\theta=0}/\text{kJ}\cdot\text{mol}^{-1}$
Au-3	2	$-40.11 \pm 0.23$	245.83	-4.96 -1219.32	32	-17.44 -558.08	-1817.51
	7	$-40.99 \pm 0.27$	245.83	-4.96 -1219.32	32	-20.50 -656.00	-1916.31
	12	$-38.11 \pm 0.09$	245.83	-4.96 -1219.32	0.09	-27.01 -2.43	-1259.86
Au-4	2	$-38.76 \pm 0.15$	245.83	-4.96 -1219.32	0.51	-17.71 -9.03	-1267.11
	7	$-40.69 \pm 0.09$	245.83	-4.96 -1219.32	32	-17.84 -570.88	-1830.89
	12	$-40.23 \pm 0.17$	245.83	-4.96 -1219.32	32	-18.40 -588.80	-1848.35

**Table 3. Free Energy Contributions for the Thermodynamic Cycle Presented in Figure 7**

substrate	pH	$\Delta G_{\text{ads,II}}^{\theta=0}/\text{kJ}\cdot\text{mol}^{-1}$	$\chi$	$\chi\Delta G_{\text{IP,t}}^{\theta=0}/\text{kJ}\cdot\text{mol}^{-1}$	$n'$	$n'\Delta G_{\text{ads,H}_2\text{O}}^{\theta=0}/\text{kJ}\cdot\text{mol}^{-1}$	$j$	$j\Delta G_{\text{IP,d}}^{\theta=0}/\text{kJ}\cdot\text{mol}^{-1}$	$\Delta G_{\text{ads,Au-thiol}}^{\theta=0}/\text{kJ}\cdot\text{mol}^{-1}$
Au-1-3	2	$-34.29 \pm 0.04$	0.01	-17.71 -0.18	243.73	-4.96 -1208.90	32	-17.44 -558.08	-1801.45
	7	$-34.70 \pm 0.60$	4.69	-17.84 -83.67	243.73	-4.96 -1208.90	32	-20.50 -656.00	-1983.27
	12	$-33.94 \pm 0.17$	4.69	-18.40 -86.30	243.73	-4.96 -1208.90	0.09	-27.01 -2.43	-1331.57
Au-2-4	2	$-33.65 \pm 0.08$	5.98	-17.44 -104.29	243.15	-4.96 -1206.02	0.51	-17.71 -9.03	-1352.99
	7	$-34.13 \pm 0.25$	5.98	-20.50 -122.60	243.15	-4.96 -1206.02	32	-17.84 -570.88	-1933.63
	12	$-34.01 \pm 0.20$	0.33	-27.01 -8.91	243.15	-4.96 -1206.02	32	-18.40 -588.80	-1837.74

involves one more step. Equation 9 describes the relationship between these processes,

$$\Delta G_{\text{ads,II}}^{\theta=0} = -\chi\Delta G_{\text{IP,t}}^{\theta=0} - n'\Delta G_{\text{ads,H}_2\text{O}}^{\theta=0} - j\Delta G_{\text{IP,d}}^{\theta=0} + \Delta G_{\text{ads,Au-thiol}}^{\theta=0} \quad (9)$$

where  $\Delta G_{\text{ads,II}}^{\theta=0}$  corresponds to the experimentally measured free energy of adsorption in the limit of zero dendrimer surface coverage,  $-\chi\Delta G_{\text{IP,t}}^{\theta=0}$  and  $-j\Delta G_{\text{IP,d}}^{\theta=0}$  are the energies associated with the removal of counterions from the ionized terminal groups of the thiol molecules and from the adsorbing dendrimer species, respectively,  $-n'\Delta G_{\text{ads,H}_2\text{O}}^{\theta=0}$  represents the free energy associated with the removal of water molecules from the naked gold surface, and  $\Delta G_{\text{ads,Au-thiol}}^{\theta=0}$  corresponds to the free energy of adsorption of unsolvated dendrimer molecules on the thiol modified surface.

Inspection of Figure 7 also shows that  $\Delta G_{\text{ads,H}_2\text{O}}^{\theta=0}$  and  $j\Delta G_{\text{IP,d}}^{\theta=0}$  have the same values as those previously calculated for dendrimer adsorption on clean gold substrates. In a fashion similar to that employed for the calculation of the counterion removal free energy for the dendrimer adsorbate  $-\Delta G_{\text{IP,d}}^{\theta=0}$ , the Fuoss model described in eq 8 allowed the computation of the  $\Delta G_{\text{IP,t}}^{\theta=0}$  term corresponding to the analogous process on the surface attached ionized thiols. The values of  $\chi$ , on the other hand (see Table 3), which correspond to the number of ionized thiol molecules per molecule of adsorbed dendrimer, were calculated using the following equation,

$$\chi = \frac{\Gamma_{\text{thiol}}}{\Gamma_{\text{m,av}}} \alpha \quad (10)$$

where  $\Gamma_{\text{thiol}}$  corresponds to the relevant thiol surface coverage and  $\alpha$  is the thiol dissociation fraction, which is a function of the  $\text{p}K_{\text{a}}$  of the surface modifier and the pH

of the aqueous solution. The value of  $n'$ , on the other hand, was related to the number of water molecules that must be displaced from the electrode surface upon adsorption of one mole of dendrimer molecules. Its calculation involved the use of eq 11

$$n' = \left[ \left( \frac{r_{\text{dend}}}{r_{\text{H}_2\text{O}}} \right)^2 - \frac{\Gamma_{\text{thiol}}}{\Gamma_{\text{m,av}}} \left( \frac{r_{\text{thiol}}}{r_{\text{H}_2\text{O}}} \right)^2 \right] \theta_{\text{H}_2\text{O}} \quad (11)$$

where  $r_{\text{thiol}}$  corresponds to the radius of one sulfur atom attached on the surface of the gold substrate.<sup>29</sup> Equation 11 is similar to eq 6 and involves an additional term that accounts for the number of water molecules that the surface attached thiol displaces per molecule of adsorbed dendrimer.

As previously shown for the case of dendrimer adsorption on naked gold substrates, knowledge of  $\Delta G_{\text{ads,II}}^{\theta=0}$ ,  $\chi\Delta G_{\text{IP,t}}^{\theta=0}$ ,  $n'\Delta G_{\text{ads,H}_2\text{O}}^{\theta=0}$ , and  $j\Delta G_{\text{IP,d}}^{\theta=0}$  allowed the calculation of the free energies for the adsorption of unsolvated dendrimer molecules on the surface of the thiol modified substrates,  $\Delta G_{\text{ads,Au-thiol}}^{\theta=0}$ , at each pH condition through the use of eq 9.

The relevant data are presented in Table 3, and since the values for  $\Delta G_{\text{ads,II}}^{\theta=0}$  are similar for all the systems studied (within an average of approximately 1 kJ/mol), it can be clearly seen that the sum of  $\chi\Delta G_{\text{IP,t}}^{\theta=0}$ ,  $n'\Delta G_{\text{ads,H}_2\text{O}}^{\theta=0}$ , and  $j\Delta G_{\text{IP,d}}^{\theta=0}$  (see eq 9) gives rise to the value of  $\Delta G_{\text{ads,Au-thiol}}^{\theta=0}$ . Inspection of the relevant values for each case shows that, as expected, at the extreme pH conditions of 2 or 12, either the dendrimer ( $j\Delta G_{\text{IP,d}}^{\theta=0}$ ) or the thiol ( $\chi\Delta G_{\text{IP,t}}^{\theta=0}$ ) counterion free energy contribution is seriously decreased by a very small ionization fraction. At neutral pH conditions, on the other hand, the free energies

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$\chi\Delta G_{\text{IP,t}}^{\theta=0}$ ,  $n'\Delta G_{\text{ads,H}_2\text{O}}^{\theta=0}$ , and  $j\Delta G_{\text{IP,d}}^{\theta=0}$  all have relatively large negative values that result in the most negative  $\Delta G_{\text{ads,Au-thiol}}^{\theta=0}$  observed. This, of course, reflects that the free energy of adsorption  $\Delta G_{\text{ads,Au-thiol}}^{\theta=0}$  is the most thermodynamically favored process and that this is a consequence of the fact that, at this pH value, the dendrimer and the surface attached thiol molecules are ionized with complementary and opposed charges.

### Conclusions

We have shown that the aggregation of the two PAMAM dendrimer molecules under study on the surface of clean and thiol modified gold electrodes takes place at micromolar level concentrations from aqueous solutions and that, as expected, this process is strongly influenced by the pH of the solution. Whereas the aggregation of both dendrimers on the surface of clean gold substrates never achieves a surface coverage larger than  $9 \times 10^{-12}$  mol/cm<sup>2</sup>, the surface coverage on thiol modified gold electrodes at pH = 7 showed values almost twice as large that at a dendrimer concentration of 20  $\mu$ M. On the other hand, the increase in dendrimer aggregation at neutral pH is not the only difference exerted by surface modification. Covalent attachment of ionized thiol molecules on the gold substrate results in curves whose sigmoidal shape suggests that at very low dendrimer concentration the adsorbate does not compete successfully with supporting

electrolyte counterions and that, above a critical concentration, dendrimer adsorption takes place in a much larger fashion.

Computer fitting of the adsorption isotherms obtained revealed that the free energy of adsorption in the limit of zero surface coverage is relatively similar (within 7.5 kJ/mol) for all the systems at any of the pH conditions surveyed and that the presence of the short thiol on the surface of the gold electrode results in negative  $a$  terms, which suggest favorable interactions between the dendrimer molecules. Analysis of the free energy data obtained from the computer fitting also showed that although opposite charged dendrimer and thiol interactions account for a large favorable adsorption energy, this is always compensated by large unfavorable counterion removal free energy terms.

It is important to point out that these results are based on a series of assumptions that, although reasonable, are not necessarily exact, and therefore, the limitations of the reported data and the relevant conclusions should be considered.

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