Formulation, Spectroscopic Characterization, and Application of Sulfhydryl-Terminated Alkanethiol Monolayers for the Chemical Attachment of DNA onto Gold Surfaces

Emily A. Smith, Matt J. Wanat, Yufei Cheng, Sérgio V. P. Barreira, Anthony G. Frutos, and Robert M. Corn*

Department of Chemistry, University of Wisconsin, 1101 University Avenue, Madison, Wisconsin 53706

Received July 27, 2000. In Final Form: January 16, 2001

A novel surface modification procedure for the creation of sulfhydryl-terminated alkanethiol monolayers that can be used for the attachment of biomolecules onto gold surfaces is described. A self-assembled monolayer of the amine-terminated alkanethiol 11-mercaptoundecylamine (MUAM) is reacted with the heterobifunctional cross-linker N-succinimidyl 3-acetylthiopropionate (SATP) in order to create a protected sulfhydryl-terminated monolayer. This monolayer can then be deprotected in an alkaline solution to create an active sulfhydryl surface. Compounds that have been modified to contain a maleimide moiety can be easily attached onto the sulfhydryl-derivatized gold surface. In a second attachment strategy, the sulfhydryl-terminated monolayer is reacted with 2,2′-dipyridyl disulfide to form disulfide bonds on the surface. These disulfide bonds are then used in a thiol–disulfide exchange reaction with free sulfhydryls in order to attach biomolecules, such as thiol-modified DNA or cysteine-containing polypeptides, onto the surface. In contrast to the maleimide-attached monolayers, the disulfide-immobilized species can be cleaved in the presence of dithiothreitol (DTT) in order to regenerate the free sulfhydryl surface. Polarization modulation FTIR reflection–absorption spectroscopy (PM-FTIRRAS) has been used to characterize these surface reactions, and fluorescence “wash off” measurements provided an estimate of 1.5 × 10¹² molecules/cm² for the surface coverage of DNA immobilized using a thiol–disulfide exchange reaction. Surface plasmon resonance (SPR) imaging measurements were employed to monitor in situ hybridization onto DNA arrays fabricated using this surface immobilization reaction.

Introduction

Self-assembled monolayers of long chain alkanethiols that have ω-terminated functional groups such as NH₂, COOH, or OH have been used extensively for the robust attachment of biopolymers such as oligonucleotides and polypeptides onto gold surfaces.¹⁻⁴ Biopolymer films have been used previously in the areas of biomolecular adsorption sensors,²,⁴ enzyme-coated electrodes,²,⁴ biomolecular templating for the creation of novel nanostructured materials,⁵ and DNA computing.⁶,⁷ For example, amine-terminated alkanethiol monolayers on gold surfaces have been employed to attach thiol-modified oligonucleotides via a heterobifunctional cross-linker in an addressable array pattern to function as a biomolecular adsorption sensor.⁸,⁹ ω-Terminated alkanethiol monolayers have also been used to modify gold surfaces with molecules such as poly(ethylene glycol) (PEG) for the prevention of nonspecific adsorption of biomolecules onto the metal substrate.¹⁰⁻¹²

These previously demonstrated surface reaction schemes utilized amine-terminated surfaces that are formed either by the self-assembling alkanethiol monolayers of 11-mercaptoundecylamine (MUAM)¹³ or by a bilayer consisting of polylysine electrostatically adsorbed onto a self-assembled alkanethiol monolayer of 11-mercaptoundecanethiol (MUA).¹⁴ The amine-terminated monolayers are then reacted with the coupling molecule, sulfosuccinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), to create a maleimide-terminated surface, which can further react with thiol-containing molecules from solution. Such maleimide-modified surfaces can be used to immobilize a variety of tagged molecules, peptides, proteins, and antibodies, due to the facile reaction between the surface maleimide functionality and a free sulfhydryl group. For example, we attach commercially available thiol-modified oligonucleotides onto these surfaces to create DNA microarrays for surface plasmon resonance (SPR) imaging experiments.¹³,¹⁵

¹ Current address: Departamento de Química, Faculdade de Ciências da Universidade do Porto, Rua do Campo Alegre 687, 4150 Porto, Portugal.
Figure 1. Schematic presentation of the surface modification process with SATP: (A) MUAM surface; (B) thiolation with SATP; (C) sulfhydryl deprotection; (D) reaction of the sulfhydryl surface with maleimide derivatives (N-ethyl maleimide and maleimide-modified DNA); (E) activated sulfhydryl surface with pyridyl groups; (F) exchange reactions between the pyridyl disulfide surface and sulfhydryl-containing molecules (R-SH) in solution, producing a leaving group, pyridine-2-thione. When the surface (F) is exposed to DTT, the disulfide linkage is broken. The immobilized molecule is released, and the free sulfhydryl surface (C) is regenerated.

In this paper, we report the reverse of this maleimide-modified surface chemistry and the creation of active sulfhydryl-terminated monolayers that can be used to attach biopolymers onto gold surfaces. Other researchers have also created thiol-terminated monolayers on gold surfaces, but normally through the direct adsorption and self-assembly of an alkanedithiol. In contrast, the reaction scheme utilized in this paper (see Figure 1) employs a self-assembled MUAM monolayer that is then reacted with the molecule N-succinimidyl S-acetylthiopropionate (SATP). The N-hydroxysuccinimide (NHS) ester moiety of SATP forms an amide bond with the ω-amine groups of the monolayer and creates a protected sulfhydryl-terminated surface. The protecting group can be removed in alkaline solution in order to create an active surface.

Biopolymers can be attached onto these surfaces by two possible strategies: (i) the reaction of the sulfhydryl-terminated monolayer with maleimide-modified target biomolecules, or (ii) a thiol–disulfide exchange reaction with thiol-modified biomolecules to create a surface disulfide. The latter surface attachment scheme has the advantage of being reversible; the cleavage of the immobilized disulfide in the presence of dithiothreitol (DTT) releases the attached biomolecule and restores the sulfhydryl-terminated monolayer.

A variety of spectroscopic techniques were employed to characterize these two surface attachment chemistries. Polarization modulation FTIR reflection–absorption spectroscopy (PM-FTIRRAS) was used to monitor the chemical structure of the self-assembled monolayer at each step of the attachment process. The hybridization of fluorescently labeled DNA molecules onto oligonucleotides created by the thiol–disulfide exchange reaction was quantitated by fluorescence "wash-off" measurements. These solution fluorescence measurements provide an estimate of the surface coverage of immobilized DNA. In a final set of experiments, the thiol–disulfide exchange reaction was used to fabricate a two-component DNA microarray on a thin gold film, and SPR imaging measurements were used to monitor the hybridization of complementary DNA sequences onto this DNA array in situ.

Experimental Considerations

Materials. N-Succinimidyl S-acetylthiopropionate (SATP, Pierce), sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SSMCC, Pierce), N-ethyl maleimide (Aldrich), triethanolamine hydrochloride (TEA, Sigma), dithiothreitol (DTT, Aldrich), 2,2'-dipyridyl disulfide (DPDS, Aldrich), ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA, Aldrich), potassium hydroxide (EM Science), disodium hydrogen phosphate dihydrate (Fluka), sodium dihydrogen phosphate monohydrate (Fluka), N,N-dimethylformamide (DMF, Aldrich), 1-octadecanethiol (ODT, Aldrich), magnesium chloride (Sigma), and sodium dodecyl sulfate (SDS, Fluka) were used as received. The 5'-thiol-modified DNA sequences 1 = 5′-GTG TTA GCC TCA AGT G, 2 = 5′-GTG TAT GGC TGA ACT G, and 3 = 5′-GTG TAT CCG ACA TGT G were synthesized at the University of Wisconsin Biotechnology Center using a Glen Research 5'-Thiol Modifier C6 with a phosphoramidite linker, and were deprotected prior to use as outlined by Glen Research Corp. To verify the presence of thiol Elman's reagent was used. The oligonucleotide sequences 1 = 5′-CAC TTG AGG CTA ACA C, 2 = 5′-CAG TTC ACG CAT AGA C, and 3 = 5′-CAC ATG TCC GAT ACA C were also synthesized at the University of Wisconsin Biotechnology Center using a Glen Research 5′ ABI 6-FAM fluorescent tag. Amino-modified DNA (Integrated DNA Technologies) with a 5′ amino modifier C6 [5′-thiol-modified DNA sequences 5′-TGT CTC TGC CTC TGT] was used in the synthesis of maleimide-modified DNA. Before use, each oligonucleotide was purified using reversed phase binary elution HPLC (Shimadzu SCL10AVP) and the concentration was determined using an HP8452A UV–vis spectrophotometer to monitor the absorption at 260 nm. 11-Mercaptoundecylamine (MUAM) was a generous gift from Professor Whitesides at Harvard University; commercially available MUAM (Dojindo Laboratories) was also utilized for some experiments. Commercial gold slides (5 nm Cr and 100 nm Au) were purchased from Evaporated Metal Films (New York) and were used for all PM-FTIRRAS measurements.

Preparation of the Free Sulfhydryl Surface. The MUAM monolayer was prepared by soaking a Au slide in an ethanol solution of 1.0 mM MUAM from 0.1 M TEA (pH 7.0). The reaction of the amine surface with the NHS ester of SATP was carried out by spotting 2 mM SATP in 0.1 M TEA buffer (pH 7.0) to the surface overnight.

Sulfhydryl Surface Reactions. A 10 mM N-ethyl maleimide 0.1 M TEA buffer (pH 7.0) solution was reacted on the surface for 30 min for the N-ethyl maleimide addition to the sulfhydryl surface. Maleimide DNA was synthesized by reacting 50% 10 mM SSMCC and 50% amino-modified DNA, both in 0.1 M TEA buffer pH 7.0, for 3.5 h. This was followed by HPLC purification of the DNA. The maleimide-modified DNA addition to the sulfhydryl monolayer, a 30 μM solution of the modified DNA, was reacted on the surface overnight.

2,2'-Dipyridyl disulfide (1 mg/mL) was reacted with the thiol surface in a 1:1 mixture of 0.1 M TEA buffer solution (pH 8.0) and DTT for 2 h to create the intermediate disulfide linkage. The reaction of thiol-modified DNA on the disulfide surface was carried out by spotting 1 μL of 1 mM DNA in 0.1 M TEA (pH 8.0) buffer and then covering the surface with a glass slide in a humidity chamber for at least 6 h. The surface was then immersed for 1 h in a buffer solution containing 20 mM sodium phosphate, 100 mM NaCl, and 1 mM EDTA (pH 7.4) to remove any nonspecifically adsorbed DNA molecules from the surface. Reduction of the disulfide bonds between DNA and the sulfhydryl surface was accomplished by exposing the surface to 50 mM DTT in 0.1 M TEA (pH 7.0). Between each reaction step the samples were thoroughly rinsed with water and dried under a stream of nitrogen.


PM-FTIRRAS Surface Characterization. Between each reaction step, PM-FTIRRAS measurements were collected using a Mattson RS-1 spectrometer with real-time interferogram sampling electronics and optical layout as described previously. Spectra were collected from 1000 scans with a resolution of 4 cm⁻¹ using a narrow-band HgCdTe detector.

Fluorescence “Wash-Off” Measurements. The surface density of oligonucleotide monolayers created by the thio-disulfide exchange reaction was estimated by measuring the fluorescence of a solution used to “wash off” the fluorescently labeled complement to the surface-immobilized strand. To do this, a single-stranded DNA surface was coated with 2 μM fluorescently labeled complement and covered with a glass slide to evenly distribute the solution across the surface. After hybridizing for 20 min, the surface was washed in a solution of 0.2% SDS/2× SSPE for 10 min to remove the nonspecifically adsorbed fluorescent complement. Next, the slide was immersed for 20 min in 7 mL of a solution containing 50 mM KOH to denature the DNA. The fluorescence emission at 517 nm was measured with a Hitachi F-4500 fluorescence spectrophotometer for each denaturing solution.

Array Fabrication and Characterization. Gold films (45 nm) with a thin chromium underlayer (5 nm) on SiO₂ glass slides (Schott Glass Technologies) were used for SPR imaging measurements. A self-assembled monolayer of ODT was formed by soaking the sample overnight in a 1 mM ethanolic solution of the alkane thiol. The ODT monolayer was removed by irradiating the sample through a quartz mask with UV light from a mercury–xenon arc lamp for 1.5 h. The surface was then immersed in a 1 mM ethanolic solution of MUAM for 2 h. This resulted in a hydrophilic background of ODT and 500 nm thick MUAM multilayers. The acetyl protecting group is removed by soaking the sample overnight in a 1 mM ethanolic solution of MUAM for 2 h. The NHS ester functionality reacts with the primary amine surface of MUAM in a slightly alkaline solution (pH 7–9), forming amide bonds and a protected sulfhydryl group. These same reactions could also be used with amine-terminated alkanethiol and the NHS ester in SATP; (C) the active sulfhydryl surface obtained after deprotection of SATP; and (D) reaction of thiol groups with N-ethyl maleimide molecules to form stable thioether linkages. See Table 1 for band assignments.

The SATP attachment onto a MUAM self-assembled monolayer and the SATP deacetylation reaction were characterized by PM-FTIRRAS. The PM-FTIRRAS spectrum of a MUAM monolayer (Figure 2A) shows several weak bands in the region from 2000 to 1000 cm⁻¹. Bands at 1615 and 1517 cm⁻¹ are assigned to the NH₂⁺ asymmetric and symmetric deformations, respectively; while the methylene scissor deformation and twisting modes are seen at 1467 and 1264 cm⁻¹ (see Table 1 for band assignments). This spectrum is similar to that previously reported. The covalent attachment of SATP to the MUAM monolayer gives characteristic amide bands (amides I and II) that result from the formation of an amide linkage between the NHS ester of the SATP molecule and free amine groups on the surface. Also, a prominent carbonyl stretching feature arises from the acetyl protecting group on SATP at 1696 cm⁻¹. Two additional bands at 1360 and 1140 cm⁻¹ are assigned to the CH₂ wagging and C–O stretching modes of the acetyl group, respectively. After deprotection, the carbonyl band (1696 cm⁻¹) (Figure 2C) disappears, as expected, due to the removal of the acetyl protecting groups from the surface. A difference spectrum between the deprotected and protected SATP monolayers shows the elimination of the bands at 1796, 1554, 1360, and 1140 cm⁻¹, suggesting that a successful deprotection reaction has occurred (Figure 3, bottom). There was no change in the amide I and II bands during the deprotection process, indicating that SATP did not desorb from the MUAM surface. These measurements suggest SATP has robustly added to the primary amine groups on the surface and that the sulfhydryl group can be deprotected without affecting the amide bond. These same reactions could also be used with amine-terminated bilayers created from 11-mercaptopoundecanoic acid and poly-L-lysine as a second method for creating sulfhydryl-terminated gold surfaces.

B. Maleimide Attachment Chemistry. Once the sulfhydryl monolayer has been formed, the terminal thiol is free to undergo alkylation with molecules that contain maleimide groups, as shown in Figure 1 (step D). The double bond of the maleimide group undergoes an alkylation reaction by forming a stable thioether bond with sulfhydryls at a neutral pH with reaction rates 1000 times greater than that for a maleimide’s reaction with amino or hydroxyl groups. Two maleimide-containing molecules were immobilized onto the surface using this attachment strategy: N-ethyl maleimide and maleimide-modified DNA. The N-ethyl maleimide addition to the surface was complete in 30 min at a concentration of 10 mM, while the maleimide-modified DNA was reacted with the surface overnight.

Figure 2D shows the PM-FTIRRAS spectrum for the attachment of N-ethyl maleimide to the sulfhydryl surface, and Figure 3 (top) shows the difference PM-FTIRRAS spectrum for this reaction. The attachment of maleimide groups onto the free sulfhydryl surface is shown by the appearance of the asymmetrical carbonyl stretching band at 1710 cm\(^{-1}\). This band is due to the coupled in-phase stretching of the two maleimide carbonyls. It has a strong intensity and is very sharp for the reaction with N-ethyl maleimide, as well as other maleimide probe molecules that were studied. In addition, there is a less intense maleimide carbonyl symmetrical stretching mode located at \(\sim 1780\) cm\(^{-1}\). The difference spectrum also shows extra bands featuring methylene scissoring and twisting deformations (1405 cm\(^{-1}\)) and a wagging mode (1360 cm\(^{-1}\)).

The band at 1228 cm\(^{-1}\) is assigned to the asymmetric CNC stretching mode.

C. Disulfide Attachment Chemistry. In addition to the maleimide surface attachment strategy, thiol-containing biomolecules can be immobilized by a disulfide exchange reaction with the pyridyl disulfide surface and thiol-modified DNA; (top) maleimide-modified DNA addition to the deprotected sulfhydryl surface. Letters refer to the reaction steps shown in Figure 1.

Table 1. PM-FTIRRAS Band Assignments for Surface Modification with Maleimide Reaction Probes

<table>
<thead>
<tr>
<th>surface</th>
<th>wavenumber (cm(^{-1}))</th>
<th>assignment</th>
<th>figure</th>
</tr>
</thead>
<tbody>
<tr>
<td>MUAM</td>
<td>1615</td>
<td>NH(^{3+}) asymmetric deformation</td>
<td>2A</td>
</tr>
<tr>
<td></td>
<td>1517</td>
<td>NH(^{3+}) symmetric deformation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1467</td>
<td>CH(_2) scissor deformation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1264</td>
<td>CH(_2) twist</td>
<td></td>
</tr>
<tr>
<td>SATP</td>
<td>1750</td>
<td>C=O symmetric stretch</td>
<td>2B</td>
</tr>
<tr>
<td></td>
<td>1696</td>
<td>C=O asymmetric stretch</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1660</td>
<td>amide I</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1550</td>
<td>amide II</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1444</td>
<td>CH(_2) scissor deformation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1360</td>
<td>CH(_2) wagging</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1140</td>
<td>C=O stretch of tertiary amine</td>
<td></td>
</tr>
<tr>
<td>N-ethyl maleimide</td>
<td>1787</td>
<td>C=O symmetric stretch</td>
<td>3 (top)</td>
</tr>
<tr>
<td></td>
<td>1710</td>
<td>C=O asymmetric stretch</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1405</td>
<td>CH(_2) scissor deformation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1360</td>
<td>CH(_2) wagging</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1228</td>
<td>C=O stretch</td>
<td></td>
</tr>
<tr>
<td>maleimide-modified DNA</td>
<td>1780</td>
<td>C=O symmetric stretch</td>
<td>4 (top)</td>
</tr>
<tr>
<td></td>
<td>1709</td>
<td>C=O asymmetric stretch and double-bond stretching vibrations of the DNA bases</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1273</td>
<td>NH bending of thymine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1221</td>
<td>asymmetric stretch of phosphate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1074</td>
<td>symmetric stretch of phosphate</td>
<td></td>
</tr>
</tbody>
</table>

Figure 3. Difference PM-FTIRRAS spectra of (bottom) the deprotected SATP surface after removal of the acetyl protecting group, and (top) N-ethyl maleimide addition to the deprotected sulfhydryl surface. Letters refer to the reaction steps shown in Figure 1.

Figure 4. Difference PM-FTIRRAS spectra of DNA immobilization on the surface: (bottom) thiol–disulfide exchange reaction with the pyridyl disulfide surface and thiol-modified DNA; (top) maleimide-modified DNA addition to the deprotected sulfhydryl surface. Letters refer to the reaction steps shown in Figure 1.
linkage onto the surface. This surface disulfide attachment of a biomolecule is created in a two-step process shown in Figure 1 (steps E and F). The first step (step E) is a thiol–disulfide exchange reaction of the sulfhydryl surface with the disulfide DPDS to create a pyridyl disulfide surface. The leaving group for this surface reaction, pyridine-2-thione, is not capable of reacting with the resulting disulfide on the surface.27 In the second reaction step (step F), the pyridyl disulfide on the surface is exchanged with a thiol-containing biomolecule in solution. Once again, the pyridine-2-thione leaving group of this second reaction does not react with the surface.

The absorptions at 1704 and 1278 cm\(^{-1}\) indicates that DNA has been immobilized on the surface. The appearance of four IR absorption bands in the spectrum suggests that DNA has been immobilized on the surface. The absorptions at 1704 and 1278 cm\(^{-1}\) are assigned to the double-bond stretching vibration of the DNA bases and the NH bending vibration on the base thymine, respectively. The bands at 1220 and 1070 cm\(^{-1}\) are assigned to the asymmetric and symmetric stretching vibrations of phosphate, respectively.

The surface coverage of DNA immobilized using the thiol–disulfide exchange reaction was estimated by quantifying the “washed-off” fluorescently labeled complement with solution fluorescence measurements. The fluorescently labeled complement was hybridized to the surface and then removed in a denaturing solution and quantitated versus standards. Using this method, the surface coverage of the immobilized DNA is estimated to be \(1.5 \times 10^{12}\) molecules/cm\(^2\), assuming a 100% hybridization efficiency. The hybridization efficiency of surface-immobilized DNA has typically been found to be 60% 
\(\pm 20\%\)^9 therefore, the above number should be considered the lower limit for the surface coverage of DNA immobilized in this manner. Typical DNA surface coverages using other attachment strategies have been measured in the range 1.0 \(\times 10^{12}\) to 1.5 \(\times 10^{13}\) molecules/cm\(^2\).9

The thiol-modified DNA attached to the surface with a disulfide linkage can be removed when the surface is exposed to DTT. The PM-FTIR RAS difference spectra taken before and after exposing the disulfide-attached DNA surface to DTT showed the elimination of the characteristic DNA bands (spectra not shown). This suggests that the DNA was cleaved from the surface and that the sulfhydryl surface was regenerated. These results imply that the disulfide-modified surfaces prepared at present can be used to reversibly immobilize thiol-containing biomolecules.

**D. DNA Array Fabrication and SPR Imaging Measurements.** An important application of the DNA surface attachment chemistry described in this paper is the fabrication of DNA microarrays on thin gold films for SPR imaging.15,28 In this final section we demonstrate that the DNA immobilization chemistry can be used in a multistep fabrication process to create DNA microarrays and that these arrays can be used in a reversible fashion to monitor the hybridization adsorption of complementary DNA molecules onto the gold surface with SPR imaging.

A DNA microarray was fabricated on a 45 nm thin gold film using a combination of self-assembly, photopatterning, and the disulfide attachment chemistry described in the previous section. This fabrication process was similar to one previously published.13 On a thin gold film, a hydrophobic background was created by the self-assembly of a monolayer of octadecanethiol (ODT). This monolayer was photopatterned to create 500 μm \(\times 500\) μm squares that were filled in with MUAM. Each MUAM array element could then be separately reacted by spotting approximately 40 nL of solution to each array spot. The ODT background prevented the spread of the reaction solutions outside the array elements; other hydrophobic monolayers have also been used with the same effect.13

The DNA array was created by reacting each MUAM square with SATP, deprotecting the SATP to generate the sulfhydryl surface, followed by the formation of the surface disulfide using DPDS, and finally, the thiol–disulfide exchange with thiol-modified oligonucleotides. Two sequences (1 and 2) of 16-base thiol-modified DNA were reacted on the disulfide surface following the pattern depicted in Figure 5.

The results of SPR imaging measurements obtained from this DNA microarray are shown in Figure 5. The pictures are SPR reflectance difference images obtained by subtracting the SPR image taken before exposing the array to one of the complementary DNA sequences from that taken after (top, sequence 1) and from that taken after denaturing the first complement and introducing the second complement (bottom, sequence 2). The images show that an array fabricated in this manner has good hybridization specificity, that is, there is very little binding
of the complement to the mismatched sequence, and that the hybridization is reversible. It is possible to perform several hybridization cycles using the same array without degradation of the signal. With the addition of DTT to the surface, there was a decrease in the SPR signal for all array spots, confirming the release of DNA from the surface and the cleavage of the disulfide bond (data not shown).

Conclusions

In this paper, a simple approach to the attachment of biomolecules onto gold surfaces utilizing sulfhydryl-terminated monolayers was described. It was demonstrated that the sulfhydryl surfaces could react with compounds containing a maleimide functionality to form a thioether bond. In a second surface attachment strategy, the sulfhydryl surfaces were used to attach thiol-containing compounds via a disulfide linkage. This attachment strategy is useful for the preparation of DNA arrays, as well as arrays of other biomolecules such as cysteine-containing polypeptides. In addition, the ability to cleave the surface disulfide bond and the subsequent release of the biomolecule will be useful for both the characterization of these films and their implementation in various biosensor applications.

Acknowledgment. This research is funded by the NSF (Grant CHE-9820742) and the NIH (Grant GM59622-02). Sérgio Barreira acknowledges the Portuguese Foundation for Science and Technology for a Ph.D. grant. The authors also wish to thank Professor G. Whitesides for the gift of the 11-mercaptoundecylamine (MUAM).

LA001064Q