# Reversible Protection and Reactive Patterning of Amine- and Hydroxyl-Terminated Self-Assembled Monolayers on Gold Surfaces for the Fabrication of Biopolymer Arrays

Anthony G. Frutos, Jennifer M. Brockman, and Robert M. Corn*

*Department of Chemistry, University of Wisconsin−Madison, 1101 University Avenue, Madison, Wisconsin 53706-1396*

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The reversible protection of amine- and hydroxyl-terminated alkanethiol self-assembled monolayers (SAMs) on gold surfaces using the base-labile protecting group 9-fluorenylmethoxycarbonyl (Fmoc) and the acid-labile protecting group dimethoxytrityl (DMT) is described. When used in combination with UV photopatterning or $\mu$-contact printing techniques, this reversible SAM protection chemistry can be used to control the reactivity and wettability of different portions of the surface. Such spatial control is utilized in the fabrication of DNA arrays. Specifically, gold surfaces modified with the amine-terminated alkanethiol 11-mercaptoundecylamine (MUAM) are reacted with an *N*-hydroxysuccinimide (NHS) ester derivative of Fmoc, forming a covalent urethane (carbamate) linkage and converting the initially hydrophilic MUAM surface to a hydrophobic, Fmoc-terminated surface. Upon exposure of the Fmoc-modified surface to a solution of a secondary amine, the Fmoc is cleaved, regenerating the original hydrophilic MUAM surface. Fmoc is also utilized as a hydroxyl protecting group, as demonstrated by the reaction of Fmoc-Cl with a monolayer of 11-mercaptoundecanol (MUD). The hydrophobic protecting group DMT is reacted with the $\omega$-hydroxyl groups of both MUD and poly(ethylene glycol)-terminated alkanethiol (PEG-SH) SAMs to form an acid labile ether bond; removal of the DMT group is accomplished by soaking the surface in 3% trifluoracetic acid. A combination of polarization−modulation Fourier transform infrared reflection absorption spectroscopy (PM-FTIRRAS), surface plasmon resonance (SPR), and contact angle measurements is used to characterize the attachment and subsequent removal of the protecting groups. To demonstrate one application of this SAM protection chemistry and reactive patterning, a DNA array is fabricated and used in an SPR imaging measurement of the adsorption of the mismatch binding protein MutS.

## Introduction

Self-assembled monolayers (SAMs) of alkanethiols have been used extensively to modify and control the properties of gold surfaces.[1] For example, the wetting properties of a gold surface can be controlled by varying the end group of the alkanethiol or by the coadsorption of two or more thiols.[2−4] Patterned alkanethiol SAM surfaces have been created using $\mu$-contact printing[5] and UV photopatterning techniques;[6] however, the complexity of surfaces consisting of patterns of different alkanethiol SAMs is limited. To create more complex patterned surfaces, it is necessary to reversibly control both the spatial reactivity and wettability of different alkanethiols on the surface after self-assembly. In this reactive patterning technique, selected areas on a surface are rendered temporarily inert to chemical reactions (i.e. "protected"), and the remaining areas are "built up" through a series of chemical modification steps. At a later time, the reactive regions can be rendered inert, and the previously inert regions are rendered once again reactive and can then be chemically modified. Because these modification steps might involve the reaction of different solutions at different locations on the sample, it is often important that each solution be confined to the appropriate location to eliminate cross reactivity. This solution confinement can be accomplished by the spatial control of surface wettability. In order for this reactive patterning technique to succeed, it is necessary to develop a set of stable, easily reversible surface protection chemistries.

This paper demonstrates that several of the protecting groups commonly utilized in the solid-phase synthesis of peptides and DNA oligonucleotides can be conveniently reacted with SAMs of alkanethiols on gold surfaces. Specifically, 9-fluorenylmethoxycarbonyl (Fmoc) and di-*p*-methoxytrityl (DMT) groups are used for the reversible protection of amine- and hydroxyl-terminated alkanethiol SAMs. Introduced as an amine protecting group in 1972 by Carpino and Han,[7] Fmoc is routinely implemented in organic synthesis[8] and is crucial to the solid-phase synthesis of peptides.[9,10] It has also been effectively used as a 5′-OH protecting group in oligonucleotide synthesis.[11,12] Upon reaction of an Fmoc derivative (e.g. an *N*-hydroxysuccinimide ester) with an amine, a urethane (carbamate) linkage is formed which is stable under neutral to acidic conditions but is readily cleaved by treatment with simple bases such as ammonia, ethanolamine, or piperidine. These same conditions are used to cleave the carbonate linkage formed between Fmoc and hydroxyl groups. Like Fmoc, triphenylmethyl (trityl) is also used routinely in organic synthesis and is the

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protecting group of choice for 5′-OH protection during DNA oligonucleotide synthesis. In contrast to the urethane and carbonate linkages of Fmoc-protected amine and hydroxyl groups, the trityl ether linkage formed upon reaction of a hydroxyl with a trityl derivative is stable in base but is readily cleaved under acidic conditions. The acid lability of the trityl ether linkage depends on the number and type of substituents on the trityl group. For example, each *p*-methoxy substituent increases the rate of acid cleavage by about an order of magnitude;[13] trisubstituted derivatives have been found to be too unstable for oligonucleotide synthesis,[14] and consequently, the di-*p*-methoxytrityl (DMT) group is most commonly used.

The reactions of Fmoc and DMT protecting groups with amine- and hydroxyl-terminated alkanethiol SAMs are monitored and characterized in this paper with a combination of polarization−modulation Fourier transform infrared reflection absorption spectroscopy (PM-FTIR-RAS), surface plasmon resonance (SPR), and contact angle measurements. When used in combination with patterning techniques such as UV photopatterning or $\mu$-contact printing, this reversible SAM protection chemistry allows for the spatial control of surface reactivity and wettability that is employed in the fabrication of DNA arrays. We have utilized these arrays to monitor DNA−protein interactions,[15,16] and we demonstrate here SPR imaging measurements of the adsorption of the mismatch binding protein MutS onto a multicomponent DNA array.

## Experimental Considerations

**Materials.** The chemicals (3-mercaptopropyl)trimethoxysilane (MPS), triethanolamine (TEA), dimethyl sulfoxide (DMSO), *N*,*N*-dimethylformamide (DMF), dichloromethane (DCM), tris-(2-aminoethyl)amine (TAEA), piperidine, ethanolamine, 1,8-diazobicyclo[5.4.0]undec-7-ene (DBU), pyridine, and trifluoracetic acid (TFA) were all used as received from Aldrich; 9-fluorenylmethoxycarbonyl chloride (Fmoc-Cl) (Sigma), 4,4′-dimethoxytrityl chloride (DMT-Cl) (Sigma), 9-fluorenylmethoxycarbonyl-*N*-hydroxysuccinimide (Fmoc-NHS) (Nova Biochem), and *N*-hydroxysuccinimidyl ester of methoxypoly(ethylene glycol) propionic acid MW 2000 (PEG-NHS) (Shearwater Polymers, Inc.) were all used as received. The 11-mercaptoundecylamine (MUAM) and HS-$(CH_2)_{11}(OCH_2CH_2)_3OH$ (PEG-SH) were generous gifts from the George Whitesides group, and the 11-mercaptoundecanol (MUD) was synthesized as described elsewhere.[17] *Escherichia coli* MutS was purchased from Gene Check, and oligonucleotides were synthesized at the University of Wisconsin Biotechnology Center.

**Sample Preparation.** Gold substrates were prepared by vapor deposition of gold onto microscope slide covers (Fisher No. 2, 18 × 18 mm) that had been silanized with MPS in a manner similar to that described previously.[18] For some experiments commercial gold slides (Evaporated Metal Films, Ithaca, NY) were employed and were cleaned before use by soaking in a "piranha" etch solution (70:30 mixture of sulfuric acid: 30% hydrogen peroxide) for 10−15 s, followed by thorough rinsing with water. (**Caution**: *"piranha" etch solution reacts violently with many organic materials and should be used with extreme care.*) Gold surfaces were modified with alkanethiols by soaking in a 1 mM ethanolic solution of the alkanethiol for at least 1 h. After self-assembly, the surfaces were thoroughly rinsed with absolute ethanol and water and then dried in a stream of nitrogen.

**Surface Reactions.** *Fmoc-NHS*. The reaction of Fmoc-NHS with a MUAM-modified gold surface was accomplished by exposing the surface to an Fmoc-NHS solution (3 mM in 1:1 DMSO:100 mM TEA, pH 7) for 30 min followed by a 5 min rinse in DMSO, and finally rinsing with ethanol and water. Cleavage (deprotection) of the Fmoc was accomplished by soaking the slide for 20 min in a 1 M solution of either piperidine, ethanolamine, or TAEA in DMSO. This was followed by a 5 min rinse in DMSO and rinsing with ethanol and water.

*DMT-Cl*. The reaction of DMT-Cl with either PEG-SH or MUD-modified gold slides was achieved by exposing the slide to a DMF solution containing 25 mM DMT-Cl and ∼20 mM DBU (i.e. 1 drop from a Pasteur pipet in a total of 6−8 mL) for 1 h in a nitrogen glovebox. After the reaction was complete, the surface was soaked for 5 min in DMF and then rinsed with ethanol and water. Cleavage of the DMT was accomplished by soaking the slide for 2 min in a 3% solution of TFA in DCM, followed by thorough rinsing with ethanol and water.

*Fmoc-Cl*. The reaction of Fmoc-Cl with a MUD-modified gold slide was accomplished by exposing the slide to a 25 mM solution of Fmoc-Cl in pyridine for 1 h in a nitrogen glovebox. The slide was then soaked for 5 min each in pyridine and DMSO and then rinsed with ethanol and water. Cleavage of the Fmoc was achieved by soaking the slide for 15 min in 1 M piperidine in DMSO.

*PEG-NHS*. The reaction of PEG-NHS with a MUAM SAM described in the last section of this paper was carried out by exposing the surface to a 4 mM solution of PEG-NHS in a pH 8, 100 mM TEA buffer for 30 min. This "pegylation" rendered the MUAM surface resistant to protein binding.

*DNA Array and MutS Adsorption*. The details of this experiment are described elsewhere.[16] Briefly, the following sequences of the two single-stranded DNA oligonucleotides were covalently attached on the array surface using the bifunctional linker sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SSMCC):

probe C:
5′ CGG TAG TTG GAG CAG ATG GTA TAT GGA GGC 3′

probe D:
5′ CGG TAG TTG GAG CAG GTG GTA TAT GGA GGC 3′

Both of these oligonucleotide probes were synthesized with a 5′ $HS(CH_2)_6(T)_{15}$ spacer. Hybridization to the array probes was accomplished by exposing the entire surface to a 2 $\mu$M solution of the complement to sequence C:

complement C*:
5′ GCC TCC ATA TAC CAT CTG CTC CAA CTA CCG 3′.

For the MutS adsorption, a solution of 200 nM *E. coli* MutS was allowed to react with the DNA array surface for 30 min. DNA hybridization and MutS adsorption were performed in situ using a pH 7.4 buffer consisting of 20 mM sodium phosphate, 100 mM NaCl, 1 mM EDTA, 1 mM DTT, and 5 mM $MgCl_2$.

**Surface Characterization.** *PM-FTIRRAS Measurements*. PM-FTIRRAS spectra were obtained from a Mattson RS-1 spectrometer with real-time interferogram sampling electronics and optical layout described previously.[19,20] Spectra in the mid-IR (2000−1000 $cm^{-1}$) were collected from 1000 scans at 2 $cm^{-1}$ resolution using a narrow-band HgCdTe detector; spectra in the CH stretch region (3300−2800 $cm^{-1}$) were collected from 1000 scans at 2 $cm^{-1}$ resolution using an InSb detector.

*SPR Measurements*. Scanning angle SPR measurements were performed on an instrument described in detail previously.[21,22] Briefly, the scanning angle SPR experiment measures the reflectivity of a HeNe laser (632.8 nm) as a function of incident angle from a prism/sample assembly. The sample consists of a 475 Å gold film which has been vapor deposited onto a microscope slide cover and is brought into optical contact with a BK-7 glass prism using ethylene glycol as an index matching fluid. SPR

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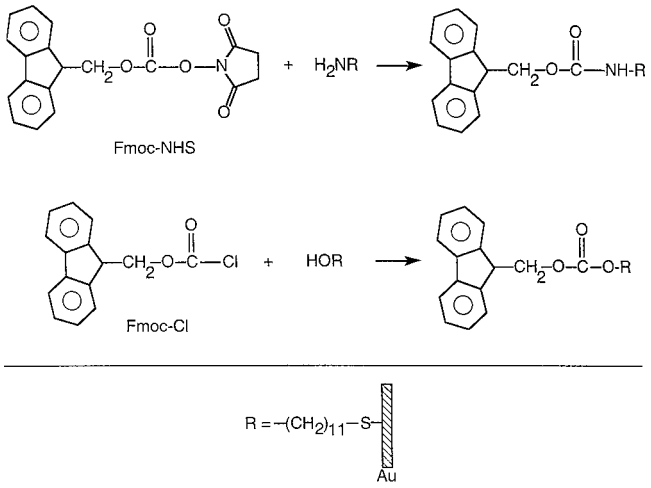


**Figure 1.** Fmoc protection reactions of amine- and hydroxyl-terminated alkanethiol SAMs. An NHS-ester derivative of Fmoc (Fmoc-NHS) is reacted with the terminal amines of an 11-mercaptoundecylamine (MUAM) monolayer on a gold surface, forming a covalent urethane (carbamate) linkage. The terminal hydroxyl groups of an 11-mercaptoundecanol (MUD) SAM are protected by reaction with Fmoc-Cl to form a carbonate bond on the surface. For both schemes the Fmoc is cleaved, regenerating the original surface, by exposure to a solution of a secondary amine.

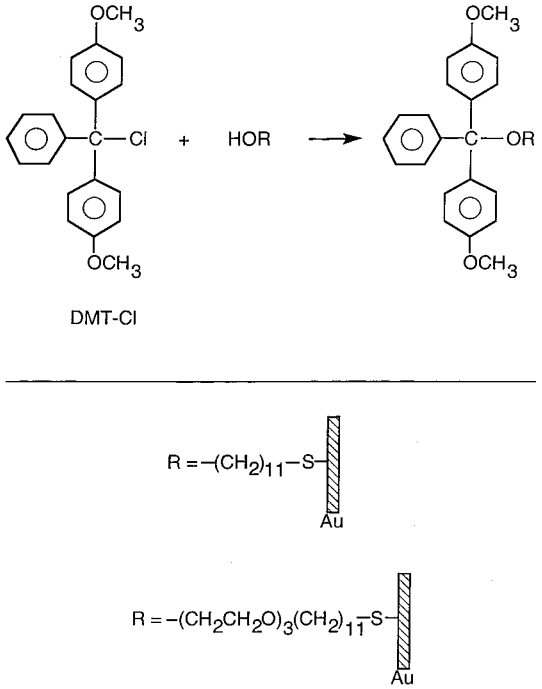


**Figure 2.** DMT protection reaction of hydroxyl-terminated alkanethiol SAMs. Dimethoxytrityl chloride (DMT-Cl) is reacted with the terminal hydroxyl groups of both 11-mercaptoundecanol (MUD) and PEG-SH monolayers on gold, forming an acid labile ether linkage. The original hydroxyl-terminated surface is regenerated by cleaving the trityl ether bond in a trifluoracetic acid solution.

reflectivity curves were fit using a four-phase (BK-7/Au/organic film/air) Fresnel calculation, assuming an index of refraction of 1.45 for the organic film. In situ SPR imaging measurements were performed using a collimated white light source and an 830 nm band-pass interference filter as described in detail elsewhere.[23]

*Contact Angle Measurements.* Water contact angle measurements were performed at ambient temperature using a Model 100-00 contact angle goniometer (Rame-Hart, Inc.). A 10 $\mu$L drop of Millipore water was delivered to the surface with an Eppendorf pipet and the contact angle was measured immediately. Contact angles were measured at three different locations on each sample.

## Results and Discussion

Figures 1 and 2 outline the three reactions schemes presented in this paper. In each case, an initially hydrophilic $NH_2$- or OH-terminated alkanethiol monolayer is reacted with a hydrophobic protecting group, rendering the surface hydrophobic. Depending on the protecting group employed, the original surface is regenerated by soaking the sample in either acid (to remove the DMT) or base (to remove the Fmoc). As shown in Figure 1, the reaction of an *N*-hydroxysuccinimide (NHS) ester of Fmoc with an amine-terminated alkanethiol SAM results in the formation of a urethane (carbamate) linkage to the surface. To attach Fmoc to a hydroxyl-terminated surface, a chloride derivative of Fmoc is employed, which reacts to form a carbonate bond. Figure 2 depicts the reaction of DMT chloride with two different hydroxyl-terminated alkanethiol SAMs to form an ether linkage to the surface. Numerous other amine and hydroxyl protecting groups are routinely used in organic synthesis.[8,24] For example, photolabile protecting groups[25] are successfully employed in the fabrication of DNA arrays on glass substrates.[26,27] These photolabile protecting groups are typically cleaved using UV−vis irradiation with a wavelength >200 nm, so their use with SAMs of alkanethiols on gold should not cause unwanted photooxidation of the gold−thiol bond.[6,28,29] For the studies reported in this paper, Fmoc and DMT were chosen because of their hydrophobicity, stability, and ease of cleavage using relatively mild reaction conditions. As illustrated in the final section of this paper, the hydrophobicity of these protecting groups is utilized to confine aqueous solutions of molecules to specific locations on a patterned surface during immobilization.

**Fmoc Protection of MUAM and MUD SAMs.** In a first demonstration of the use of Fmoc as a reversible SAM protecting group, the terminal amine groups of an 11-mercaptoundecylamine (MUAM) SAM were protected and deprotected. Figure 3A shows the PM-FTIRRAS spectrum of the initial MUAM monolayer. Peaks at 1610 and 1514 $cm^{-1}$ are assigned to the $NH_3^+$ asymmetric and symmetric deformation, respectively. The band at 1464 $cm^{-1}$ is assigned to the methylene scissors deformation and the band at 1258 $cm^{-1}$ is assigned to the methylene twist. The 17.5 ± 0.4 Å thickness of the MUAM layer, as determined by scanning angle SPR measurements, is consistent with a single monolayer of this molecule. (Though Tien et al.[30] reported that preliminary ellipsometry and contact measurements indicated that MUAM forms hydrophobic bilayers on gold, our SPR and contact angle measurements are consistent with the formation of a single, hydrophilic monolayer.) Upon reaction of the MUAM surface with Fmoc-NHS (see Figure 1) several new bands appear in the PM-FTIRRAS spectrum, indi-

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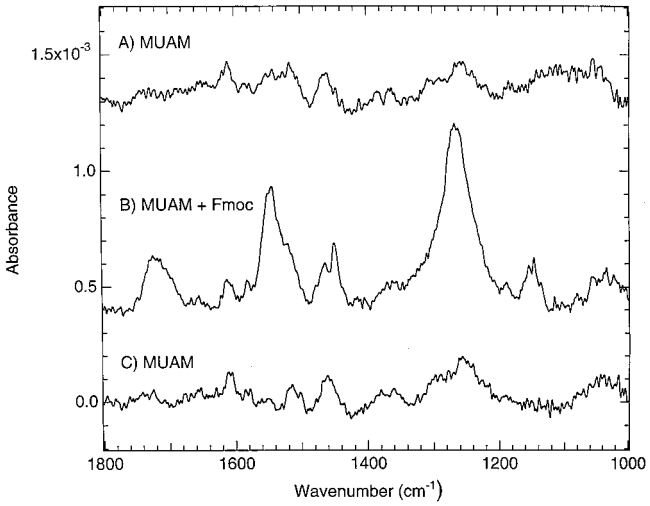


**Figure 3.** PM-FTIRRAS spectra showing the protection and deprotection of an 11-mercaptoundecylamine (MUAM) SAM. (A) The initial MUAM monolayer. (B) After the reaction of MUAM with Fmoc. The carbonyl band at 1720 $cm^{-1}$, the CHN band at 1544 $cm^{-1}$ and the coupled CN, CO band at 1267 $cm^{-1}$ indicate a covalent urethane linkage has been formed between the Fmoc and the MUAM monolayer. (C) After exposing the surface to a solution of a secondary amine to remove the Fmoc. The similarity between this spectrum and the original MUAM spectrum in part A indicates that the Fmoc has been completely removed.

**Table 1. Infrared Band Assignments for Fmoc Reactions**

| surface | wavenumber ($cm^{-1}$) | assignment |
|---|---|---|
| MUAM | 2925 | $CH_2$ asym stretch |
| | 2854 | $CH_2$ sym stretch |
| | 1610 | $NH_3^+$ asym deformation |
| | 1514 | $NH_3^+$ sym deformation |
| | 1464 | $CH_2$ scissors deformation |
| | 1258 | $CH_2$ twist |
| MUAM + Fmoc | 3069 | C−H ring stretch |
| | 2923 | $CH_2$ asym stretch |
| | 2851 | $CH_2$ sym stretch |
| | 1720 | amide I (C═O) |
| | 1544 | CHN vibration |
| | 1450 | C═C ring stretch |
| | 1267 | amide IV (CN, CO stretch) |
| | 1147 | C−O−C stretch |
| MUD + Fmoc | 1746 | C═O stretch |
| | 1452 | C═C ring strectch |
| | 1406 | |
| | 1282 | C−O−C asym stretch |

cating that a covalent attachment between the Fmoc and MUAM monolayer has occurred (see Figure 3B). Specifically, the bands at 1720 $cm^{-1}$ (assigned to the carbonyl stretch), 1544 $cm^{-1}$ (assigned to the CHN vibration), and 1267 $cm^{-1}$ (assigned to the coupled CN and CO stretch) are evidence that a urethane (carbamate) linkage has been formed between the Fmoc and the MUAM monolayer. Additional bands at 1450 and 1147 $cm^{-1}$ are due to the C═C ring stretch of the fluorene moiety and C−O−C stretch, respectively. In the CH stretch region (spectra not shown), the band intensities and positions of the methylene stretches changed after the Fmoc reaction (see Table 1). Specifically, the band intensities decreased ∼28% and the peak positions shifted to lower energies by 2−3 $cm^{-1}$, indicating that the Fmoc reaction induces a slight ordering of the underlying MUAM monolayer.[31] These changes were reversed upon removal of the Fmoc.

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**Table 2. Infrared Band Assignments for DMT Reactions**

| surface | wavenumber ($cm^{-1}$) | assignment |
|---|---|---|
| MUD | 2927 | $CH_2$ asym stretch |
| | 2880 | $CH_2$ stretch ($CH_2OH$ group) |
| | 2855 | $CH_2$ sym stretch |
| MUD + DMT | 3068 | C−H ring stretch |
| | 3005 | C−H ring stretch |
| | 2924 | $CH_2$ asym stretch |
| | 2850 | $CH_2$ sym stretch |
| | 1610 | C═C ring stretch |
| | 1511 | C═C ring stretch |
| | 1300 | |
| | 1253 | C−O−C asym. stretch |
| | 1177 | C−H ring deformation |
| | 1043 | C−O−C sym. stretch |
| PEG-SH | 1463 | EG+alkyl $CH_2$ scissor |
| | 1351 | EG $CH_2$ wag |
| | 1259 | EG $CH_2$ twist |
| | 1130 | EG C−O−C stretch |
| PEG-SH + DMT | 1607 | C═C ring stretch |
| | 1511 | C═C ring stretch |
| | 1300 | |
| | 1254 | C−O−C asym stretch |
| | 1178 | C−H ring deformation |
| | 1036 | C−O−C sym stretch |

**Table 3. Contact Angle and SPR Thickness Measurements**

| surface | contact angle (deg) | thickness (Å) |
|---|---|---|
| MUAM | 36.2 ± 2.5 | 17.5 ± 0.4 |
| MUAM + Fmoc | 74.4 ± 2.5 | 22.8 ± 0.5 |
| MUAM + PEG | 37.3 ± 2.6 | 23.8 ± 0.8 |
| MUD | 34.2 ± 2.9 | 16.0 ± 1.0 |
| MUD + DMT | 61.0 ± 1.8 | 21.8 ± 0.5 |
| MUD + Fmoc | 75.4 ± 1.5 | 23.3 ± 0.5 |
| PEG-SH | 31.3 ± 0.7 | 21.5 ± 0.9 |
| PEG-SH + DMT | 69.2 ± 0.9 | 28.0 ± 0.9 |

Attendant to these spectral changes were changes in the wettability of the surface. After reaction with Fmoc-NHS, the initially hydrophilic amine-terminated surface (contact angle of 36.2° ± 2.5°) was converted to a hydrophobic, Fmoc-terminated surface with a measured contact angle of 74° ± 2.5°. In addition, the thickness of the Fmoc layer of ∼5 Å (see Table 3), as determined by scanning angle SPR measurements, corresponds to roughly 70% of a monolayer based on the bond lengths for a fully extended Fmoc molecule.

Cleavage of the Fmoc group from the surface (i.e. deprotection) was easily accomplished by soaking the sample in a 1 M solution of a secondary amine in DMSO. Specifically, piperidine, ethanolamine, and TAEA were all successfully used as deprotection reagents. The spectrum of a MUAM/Fmoc surface after deprotection in 1M piperidine is shown in Figure 3C. Note the similarity between this spectrum and the initial MUAM spectrum (Figure 3A), which indicates that the Fmoc has been completely removed. Additionally, SPR measurements performed on these deprotected surfaces showed that the measured layer thickness returned to within ∼1 Å of the original thickness. The amine groups of a MUAM surface protected and deprotected in the manner described here retain their reactivity and have been successfully reacted a second time with Fmoc-NHS (data not shown) as well as with other amine reactive molecules.[15]

In another demonstration of the use of Fmoc as a SAM protecting group, the $\omega$-hydroxyl groups of an 11-mercaptoundecanol (MUD) SAM were reacted with a chloride derivative of Fmoc. Figure 4A shows the spectrum of the

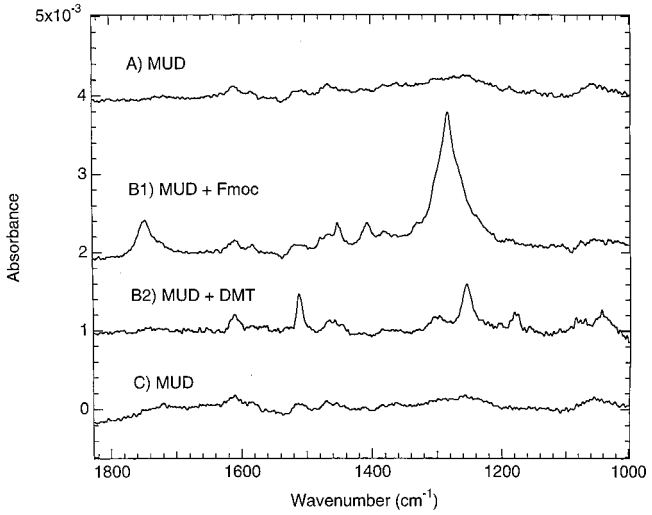


**Figure 4.** PM-FTIRRAS spectra showing the protection of an 11-mercaptoundecanol (MUD) SAM using either Fmoc or DMT. (A) Initial MUD spectrum. The MUD monolayer is reacted with either (B1) Fmoc-Cl or (B2) DMT-Cl, which protects the hydroxyl groups and renders the entire surface hydrophobic. (C) The spectrum of a MUD/Fmoc surface which has been deprotected in a solution of a secondary amine. The similarity between this spectrum and the initial MUD spectrum in part A indicates that the Fmoc has been completely removed. To cleave the DMT group, the surface is exposed to a 3% trifluoracetic acid solution.

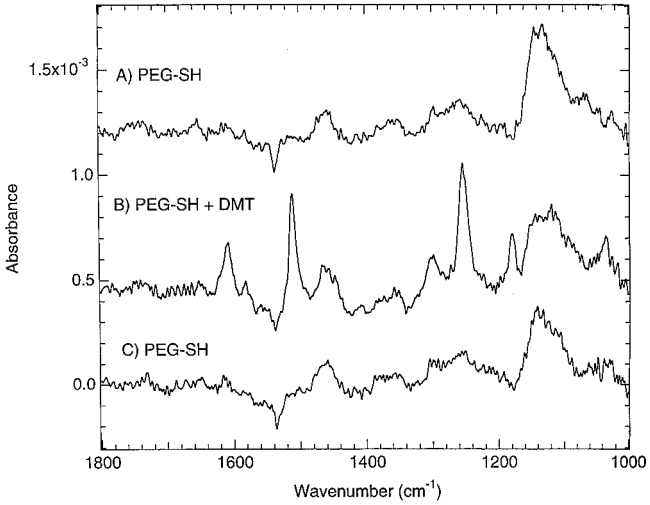


**Figure 5.** PM-FTIRRAS spectra showing the protection of the terminal hydroxyl groups of PEG-SH with DMT. (A) The initial PEG-SH spectrum. (B) Spectrum after the reaction with DMT-Cl. The bands at 1254 and 1036 $cm^{-1}$ (assigned to the C−O−C asymmetric and symmetric stretch, respectively) are evidence that an ether bond has been formed between the terminal hydroxyl groups and the DMT. (C) After removal of the DMT group by exposing the surface to a 3% trifluoracetic acid solution.

initial MUD monolayer and Figure 4B1 shows the spectrum after the reaction with Fmoc-Cl. The bands at 1746 and 1282 $cm^{-1}$ are consistent with the formation of a carbonate linkage (see Figure 1) and are assigned to the carbonyl stretch and C−O−C stretch, respectively. If the Fmoc-Cl were merely nonspecifically adsorbed onto the surface, the carbonyl stretch would occur at higher wavenumbers (∼1770 $cm^{-1}$ in the bulk spectrum). Contact angle and SPR thickness measurements of Fmoc-modified MUD SAMs are listed in Table 3 and provide further evidence that the surface was modified in the expected manner. The Fmoc group was readily cleaved by soaking the sample in 1 M piperidine in DMF for 15 min. Notice that the spectrum of the surface after this deprotection shown in Figure 4C is almost identical to the original MUD spectrum (Figure 4A), which indicates that the Fmoc has been completely removed.

**DMT Protection of MUD and PEG-SH SAMs.** The use of DMT as an acid-labile, hydroxyl-protecting group was demonstrated by reaction of DMT-Cl with a SAM of MUD as depicted in Figure 2. Similar to the Fmoc protecting group, DMT is a hydrophobic molecule, and reaction with a hydrophilic, hydroxyl-terminated surface causes the surface to become hydrophobic. Specifically, the contact angle of the MUD surface increased from 34.2° to 61.0° upon reaction with DMT. A corresponding ∼6 Å change in the thickness of the monolayer was measured by SPR. The spectrum of a DMT-modified MUD surface is shown in Figure 4B2. Bands at 1253 and 1043 $cm^{-1}$, assigned to the asymmetric and symmetric C−O−C stretches, are strong evidence of the formation of an ether bond. Additional bands at 1610 and 1511 $cm^{-1}$ are assigned to the C═C ring stretch, and the band at 1177 $cm^{-1}$ is due to the C−H ring deformation. In the CH stretch region (see Table 2), a band at 2880 $cm^{-1}$ (assigned to the C−H stretch of the hydroxymethyl group[32]) observed in the original MUD spectrum disappeared after reaction with DMT, indicating conversion of the terminal alcohol group to an ether.[17] Ordering of the underlying MUD monolayer after reaction with DMT was observed, similar to the case of the MUAM/Fmoc reaction, as a decrease in both the peak intensity (∼22%) and position (∼3 $cm^{-1}$) of the methylene stretches (see Table 2). Two small bands associated with the C−H ring stretch were also observed at 3067 and 3006 $cm^{-1}$. Complete removal of the DMT group was accomplished by cleaving the trityl ether bond in a DCM solution containing 3% trifluoracetic acid. The spectrum of the surface after this deprotection step looked essentially identical to the original MUD surface.

The use of DMT as a SAM protecting group was extended to other hydroxyl-terminated SAMs. For example, Figure 5 shows the PM-FTIRRAS spectra of a poly(ethylene glycol)-modified alkanethiol [$HS(CH_2)_{11}(OCH_2CH_2)_3OH$, (PEG-SH)] monolayer before and after reaction with DMT-Cl. SAMs of alkanethiols modified with poly(ethylene glycol) have been shown to render the surface resistant to protein adsorption.[33] The spectrum in Figure 5A is consistent with similar PEG-SH monolayers reported in the literature[34] (see Table 2 for band assignments). Bands due to the covalent attachment of the DMT group seen in Figure 5B are similar in intensity and position to those shown in Figure 4B2 for the reaction of DMT with MUD (see Table 2). A contact angle of 31.7° was measured for the unmodified PEG-SH monolayer, which increased to 69.2° after DMT protection. From SPR measurements, the thickness of the DMT layer was 6.5 Å. A loss in intensity of the 1130 $cm^{-1}$ band [assigned to the ethylene glycol (EG) C−O−C stretch] was observed after reaction with, and subsequent removal of, the DMT. SPR thickness measurements of five different samples showed that after deprotection, the PEG-SH monolayer thickness returned to within 1 Å of the original thickness, suggesting that the observed decrease in 1130 $cm^{-1}$ band intensity is primarily due to a reorientation of the EG groups on the surface. Furthermore, preliminary experiments indicate that PEG-SH monolayers protected and deprotected as

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described here maintain their ability to resist the adsorption of proteins.

**Application of Fmoc Protection Chemistry in DNA Array Fabrication.** As an example of one application of the protection chemistries described in this paper, we have created multicomponent DNA arrays to monitor protein−DNA interactions using SPR imaging.[15,16] To spatially confine each array element (DNA sequence) during immobilization, an initially hydrophobic surface is required. However, once the oligonucleotides have been immobilized, the array background must be rendered resistant to protein binding. A DNA array consisting of a pattern of two single-stranded oligonucleotides which differed from each other in one base location was fabricated using the following steps (a more detailed description can be found elsewhere[15]):

(1) A MUAM-modified gold surface was reacted with Fmoc-NHS.

(2) Squares of MUAM/Fmoc were removed by UV photopatterning, creating a surface containing squares of bare gold surrounded by a hydrophobic background of MUAM/Fmoc.

(3) The surface was immersed in a solution of MUAM, producing a surface with squares of MUAM surrounded by a hydrophobic background of MUAM/Fmoc.

(4) Two different single-stranded, thiol-modified DNA oligonucleotides were covalently attached in a checkerboard pattern onto these MUAM squares using a bifunctional linker[35−37] and were confined to their respective array locations by the hydrophobic background.

(5) The Fmoc was cleaved from the background, regenerating the original MUAM surface.

(6) The MUAM background was reacted with PEG-NHS, rendering it resistant to protein binding.

Figure 6B shows the results of an SPR imaging experiment using this DNA array which monitored the adsorption of the mismatch binding protein MutS. MutS is a DNA-binding protein known to bind to DNA duplexes containing single-base mismatches.[38] The array consisted of two different 30-basepair DNA duplexes arranged in the pattern shown in Figure 6A. The locations labeled P contain perfectly matched DNA duplexes and the locations labeled M contain duplexes with a single-base (G/T) mismatch. These duplexes were created by exposing the entire two-component, single-stranded DNA array prepared as described above to a solution containing the perfectly complementary sequence to one of the oligonucleotides. The image in Figure 6B was produced by taking the difference between the SPR images of the surface obtained before and after exposure to MutS. Notice that binding occurs predominantly in those array locations that contain single-base mismatches and that there is minimal binding to the background, thus demonstrating that the reversible protection chemistry and reactive patterning were successful.

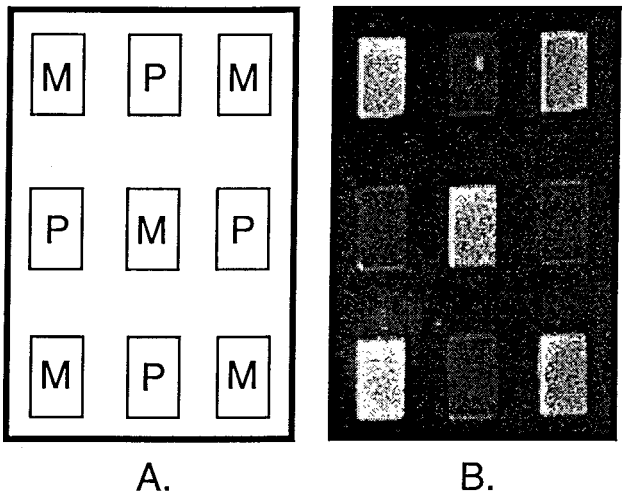


**Figure 6.** SPR image showing the binding of MutS to a two-component DNA array. (A) A DNA array composed of two different DNA duplexes arranged in the pattern shown here was created. The locations labeled P contain a perfectly matched DNA duplex and the locations labeled M contain a DNA duplex with a single-base (G/T) mismatch. (B) SPR difference image obtained by subtracting the images of the surface obtained before and after MutS binding. MutS is a protein known to bind to DNA duplexes containing single-base mismatches.

## Summary and Conclusions

The reversible protection of amine- and hydroxyl-terminated alkanethiol SAMs on gold using the protecting groups Fmoc and DMT has been demonstrated. Utilization of these SAM protection chemistries affords an easy, reversible means to control surface wettability and reactivity and can be used for the creation of complex, patterned surfaces. Future experiments will investigate the protection of carboxylic acid-terminated alkanethiol SAMs.

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