

Characterization of Poly-L-lysine Adsorption onto Alkanethiol-Modified Gold Surfaces with Polarization-Modulation Fourier Transform Infrared Spectroscopy and Surface Plasmon Resonance Measurements

Claire E. Jordan, Brian L. Frey, Steven Kornguth,^{*,†} and Robert M. Corn^{*}

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

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The electrostatic adsorption of poly-L-lysine molecules onto a vapor-deposited gold film modified with a carboxylic acid-terminated alkanethiol monolayer is monitored with the spectroscopic techniques of polarization-modulation Fourier transform infrared (PM-FTIR) spectroscopy and surface plasmon resonance (SPR). The PM-FTIR spectrum of a monolayer of poly-L-lysine (PL) adsorbed onto a self-assembled monolayer of 11-mercaptoundecanoic acid (MUA) indicates that the lysine residues and the MUA carboxylic acid moieties form ammonium-carboxylate ion pairs which electrostatically bind the polypeptide to the surface. The PL molecules can be desorbed from the surface by rinsing with a buffer solution at a pH that destroys the ion pairing (pH < 6.5 or pH > 12). Measurements of the shift in the SPR angle upon adsorption were used to determine the thicknesses of the adsorbed monolayers; the MUA and PL monolayers were found to be 17.0 and 10.5 Å thick, respectively. These thickness results suggest that the poly-L-lysine monolayer adsorbs onto the packed MUA surface in an extended conformation with the PL backbone lying parallel to the surface. Subsequent exposure of the PL monolayer to a solution of iron phthalocyanine tetracarboxylic acid (FePc) resulted in the adsorption of a third layer onto the surface. The ability of the adsorbed PL molecules to interact with FePc indicates the presence of free lysine residues available for interaction with molecules other than the MUA monolayer.

Introduction

The controlled adsorption of protein or polypeptide molecules onto a metal surface from aqueous solution is a crucial step in the fabrication of biochemical sensors and enzyme-modified electrodes.¹ In contrast, the undesirable adsorption of proteins can adversely affect the functioning of chemical sensors and devices in biological systems.² For these reasons, many researchers are looking for ways to control protein adsorption onto metal surfaces.³ One way a protein or polypeptide may adsorb onto a metal surface is by an electrostatic attraction between the surface and charged amino acid residues. This electrostatic adsorption mechanism has been both postulated⁴ and observed⁵⁻⁸ for a variety of systems. For multiply charged polypeptides, such as poly-L-lysine (PL), strong electrostatic adsorption is expected.⁹

Self-assembled monolayers have been used previously by a number of researchers to control the adsorption of proteins onto metal surfaces.^{10,11} A charged surface can

be created by the adsorption of a self-assembled monolayer of molecules that are terminated with an ionizable functional group. For instance, 11-mercaptoundecanoic acid (MUA, see Figure 1) forms a self-assembled monolayer on gold that can be negatively charged via the deprotonation of the carboxylic acid groups in the appropriate pH range; the properties of this surface have been extensively studied.¹²⁻¹⁴ This polyanionic MUA surface should be capable of binding polycationic molecules such as PL. In this paper, we demonstrate that a strong adsorption interaction between the MUA polyanionic surface and the polycationic PL does indeed occur. A schematic representation of this adsorption process is depicted in Figure 1.

Poly-L-lysine was selected as the adsorbed polycation for a variety of reasons. The chemical structure and conformation of PL are well characterized in bulk solution,¹⁵⁻¹⁸ and it exists as a random coil in its polycationic form in the same pH range that the carboxylic acid groups of the MUA monolayer are deprotonated. PL is known to bind to tumor cells with high affinity both in vivo^{19,20} and in vitro²⁰⁻²² and thereby may serve as a primary component in the construction of targeting

[†] Department of Neurology and Biomolecular Chemistry, 1500 Highland Ave., University of Wisconsin—Madison, Madison, WI 53706.

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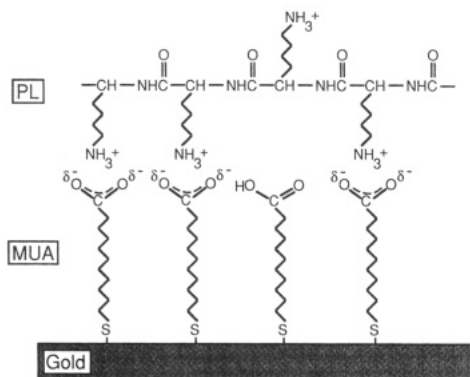


Figure 1. Schematic diagram of poly-L-lysine (PL) adsorbed onto a monolayer of 11-mercaptoundecanoic acid (MUA) formed on a gold substrate.

agents.²³ PL has also been found to interact with porphyrins and phthalocyanines to form stacked structures that exhibit unique conducting properties.^{24–26} PL can be modified either by reaction of the amine groups with various molecules or by the replacement of some of the lysine residues with other amino acids. In this way, modified poly-L-lysine may lead to a versatile route for the specific adsorption of protein molecules onto metal surfaces.

A combination of spectroscopic and chemical experiments was used to ascertain the structure of the adsorbed MUA and PL monolayers. The changes in the protonation of the carboxylic acid groups in the MUA monolayer before and after the adsorption of PL were followed by polarization-modulation Fourier transform infrared (PM-FTIR) spectroscopy. In addition, the presence of PL on the surface was verified via its characteristic amide bands. The spectroscopic measurements indicated that a MUA-PL bilayer held together by electrostatic ion pair interactions was formed on the gold surface. (Note that the use of a bilayer in this context refers to the PL adsorbed onto the MUA self-assembled monolayer and not to the classic lipid membrane bilayer structure.) In conjunction with the PM-FTIR experiments, we employed surface plasmon resonance (SPR) spectroscopy^{27–31} to measure the thickness of the MUA-PL bilayer. From these thickness measurements it is concluded that the MUA molecules are oriented nearly perpendicular to the surface and that the PL backbone is aligned parallel to the gold substrate. On the basis of these conclusions, a model of the MUA-PL bilayer is constructed as shown in Figure 1.

Experimental Considerations

Substrate Preparation and Characterization. Silicon wafers were used as the substrate for the PM-FTIR experiments, and glass microscope slide covers were used in the SPR measurements. These substrates were rigorously cleaned and then silanized with (3-mercaptopropyl)trimethoxysilane, according to the method described by Majda.³² Gold (D. F.

Goldsmith, 99.99%) was vapor-deposited onto them by following a previously outlined procedure.³³ The silicon wafers were covered with a 120 nm gold film so that they would be highly reflective for the PM-FTIR experiments. The SPR measurements required a transparent substrate (glass slide covers) and a thinner gold coating (47 nm) to allow effective coupling of the incident laser light to the surface plasmons. The gold was deposited at room temperature, and some of the gold/glass samples were subsequently annealed at approximately 300 °C for 1 h. Scanning tunneling microscopy (STM) studies³⁴ of the unannealed gold/glass samples showed rolling hills with heights of 1–2 nm and diameters of about 40 nm, whereas the annealed samples exhibited atomically flat terraces of approximately 120 nm diameter. No differences in the SPR reflectivity curves were observed between the annealed and unannealed samples.

Chemicals and Adsorption Procedure. The 11-mercaptoundecanoic acid (Aldrich) monolayers were self-assembled onto the gold films from 1 mM ethanolic solutions for at least 18 h. The films were then rinsed with absolute ethanol (Quantum) and water (Millipore) and dried with nitrogen before use. Poly-L-lysine was adsorbed onto the MUA monolayer by dipping the substrate for 30 min in an aqueous solution of 0.2 mg/mL (1 mM per lysine residue) poly-L-lysine (Sigma, average molecular weight 14 000) buffered to a pH of 8.5. Buffers used throughout these experiments were made from aqueous 5 mM boric acid (Aldrich, 99.99%) and adjusted to the desired pH with 0.1 M NaOH (Fluka, 98.0%). Iron phthalocyaninetetracarboxylic acid (FePc) was synthesized using a modification²⁵ of the method of Shirai et al.³⁵ The iron tetracarboxyphthalocyanine was attached to the poly-L-lysine-coated film by dipping the substrate in a 0.3 mM solution of FePc (pH = 8.5) for 20 min. After both the PL and the FePc dips, the films were rinsed thoroughly with water and dried with nitrogen before the PM-FTIR and SPR experiments were performed.

PM-FTIR Measurements. A Mattson RS-1 spectrometer and previously developed real-time interferogram sampling methods were used to obtain PM-FTIR differential reflectance measurements ($\% \Delta R/R$) at a high angle of incidence ($77 \pm 5^\circ$) from the surface normal.^{36–38} The $\% \Delta R/R$ values were converted to absorbance units for comparison with conventional infrared reflection-absorption (IRRAS) data. The PM-FTIR method alleviates the need for a clean gold background. In the CH stretching region ($3400\text{--}2600\text{ cm}^{-1}$) an InSb detector was employed to obtain spectra of 1000 scans at 2 cm^{-1} resolution. A wide band HgCdTe detector was used to obtain spectra in the mid-IR region ($2000\text{--}1100\text{ cm}^{-1}$) of 1000–2000 scans at 2 cm^{-1} resolution.

SPR Experimental Apparatus. The SPR instrument is displayed schematically in Figure 2. For these experiments, the monolayers were adsorbed onto a thin (47 nm) gold film that was deposited onto a 0.2 mm thick glass microscope slide cover. This sample was brought into optical contact with a 25 mm diameter BK7 glass hemispherical prism using a thin layer of ethylene glycol. A 1 mW HeNe laser (Newport Corp.) at 632.8 nm was used to obtain reflectivity measurements from the prism-sample assembly as a function of incident angle θ . The laser beam was mechanically chopped at a frequency of 930 Hz (Stanford Research Systems SR540) and polarized parallel to the plane of incidence (p-polarization) with a calcite polarizer (Newport Corp., extinction ratio = 10^{-5}). The beam was polarized because only p-polarized light will excite surface plasmons at the gold-air interface.²⁸ The laser beam was collimated onto the gold surface by a lens and the hemispherical prism, creating a narrow range of incident angles on the gold film. The entire reflected beam was then collected and focused onto a photodiode (Hamamatsu).

The incident angle θ was scanned by rotating the prism sample on a fine rotation stage with a stepper motor such that one step

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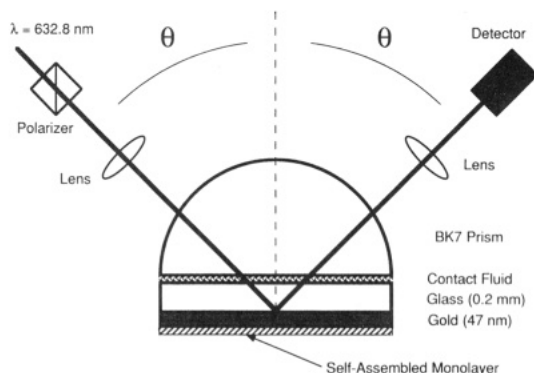


Figure 2. Optical layout for the surface plasmon resonance (SPR) instrument. A 47 nm gold film evaporated onto a glass microscope slide cover serves as the substrate for the formation of a self-assembled thin film. This sample is brought into contact with a hemispherical prism in order to couple the laser light with surface plasmons at the gold surface. Rotating the sample/prism assembly allows the reflectivity to be recorded as a function of the incident angle, θ .

corresponded to 0.004° . The collection lens and detector were rotated at an angle of 2θ in order to catch the reflected beam; and the output of the photodiode circuit was sent to a lock-in amplifier (Stanford Research Systems SR510) to demodulate the signal voltage. This reflectivity signal was then recorded as a function of incident angle by an IBM XT computer. In this manner, reflectivity versus incident angle curves were obtained first for the bare gold surface and then for the coated surfaces.

Results and Discussion

PM-FTIR Measurements of MUA and PL Adsorption. The formation of a self-assembled monolayer of MUA on a vapor-deposited gold film was accomplished by exposure of the gold surface to a 1 mM ethanolic MUA solution. The CH-stretching and mid-IR regions of the PM-FTIR spectrum of the resulting MUA monolayer are shown in Figures 3a and 4a, respectively. The vibrational band frequencies and assignments for the MUA monolayer are listed in Table 1 and agree with the results of previous researchers.^{14,39} The frequencies of the asymmetric and symmetric methylene stretching bands (2919 and 2849 cm^{-1}) and the intensity of the asymmetric methylene stretching band (5×10^{-4} abs. units) suggest a highly organized monolayer.⁴⁰ The mid-IR spectrum (Figure 4a) of the MUA monolayer contains bands at 1730, 1610, 1406, and 1200 cm^{-1} . The 1730 cm^{-1} band is assigned to the C=O stretch of the protonated form of the acid and has been shown to be a combination of two bands, one at 1739 and one at 1717 cm^{-1} due, respectively, to monomeric and laterally dimerized carboxylic acid groups.¹⁴ The position of this band is higher in frequency than that observed in the bulk compound (1699 cm^{-1}) due to the inability of the carboxylic acid groups to form head-to-head dimers in the closely packed film. The band at 1200 cm^{-1} is assigned to the C—OH stretching of the carboxylic acid groups.⁴¹ The band at 1610 cm^{-1} is assigned to the asymmetric COO⁻ stretching band of the carboxylate anion, and the band at 1406 cm^{-1} is assigned to a combination of the symmetric COO⁻ stretching band of the carboxylate anion and the α -CH₂ scissors deformation of the carboxylic acid.^{14,42} In this spectrum the two carboxylate bands at 1610 and 1406 cm^{-1} are much weaker than the C=O stretch at 1730 cm^{-1} ;

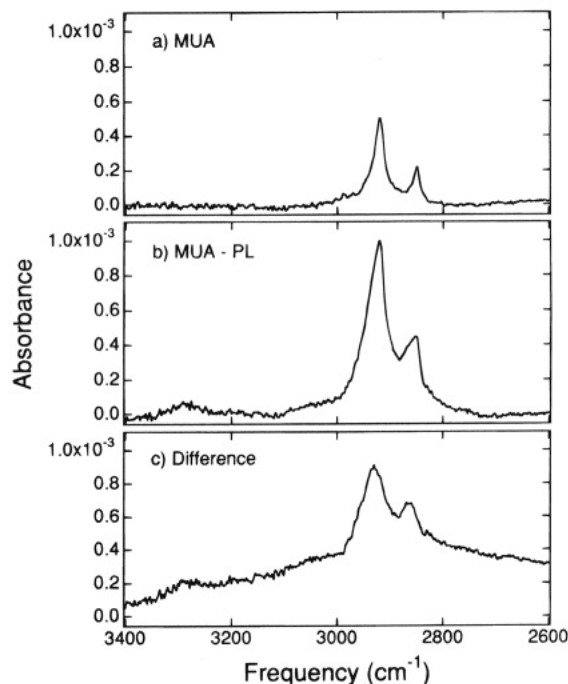


Figure 3. CH-stretching region of the polarization-modulation Fourier transform infrared (PM-FTIR) spectra for (a) a MUA monolayer on a vapor-deposited gold substrate and (b) PL adsorbed to the MUA monolayer. The difference between spectra a and b is shown in spectrum c, which is only the PL spectrum. The appearance of the NH stretch at 3290 cm^{-1} and the growth of the methylene stretching bands at 2930 and 2862 cm^{-1} indicate that PL is adsorbed to the surface.

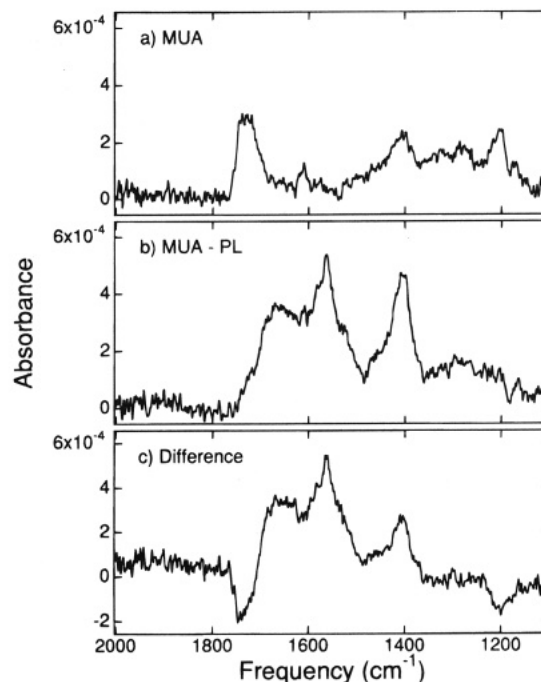


Figure 4. PM-FTIR spectra in the mid-IR region for (a) a MUA monolayer on a vapor-deposited gold substrate and (b) PL adsorbed to the MUA monolayer. The difference spectra between a and b is shown in spectrum c. Positions and assignment for the bands are shown in Table 1. The appearance of the Amide I and Amide II bands along with the loss of the COOH and the growth of the COO⁻ bands show the electrostatic adsorption of PL to the MUA monolayer.

however, these two bands became much more intense than the C=O stretch and dominated the mid-IR spectrum when the MUA monolayer was rinsed with a high pH aqueous solution. These observations indicate that the

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Table 1. Infrared Vibrational Assignments for 11-Mercaptoundecanoic Acid and Poly-L-lysine

frequency, cm ⁻¹	band	assignment
11-Mercaptoundecanoic Acid (MUA)		
2919 ± 2	$\nu_{as}(\text{CH}_2)$	methylene stretch ^a
2849	$\nu_s(\text{CH}_2)$	methylene stretch ^a
1730	$\nu(\text{C}=\text{O})$	carboxylic acid stretch ^a
1610	$\nu_{as}(\text{COO}^-)$	carboxylate stretch ^a
1406	$\nu_s(\text{COO}^-) + \delta(\alpha\text{-CH}_2)$	carboxylate stretch ^a + $\alpha\text{-CH}_2$ scissors def. ^d
1350–1150		CH ₂ rocking and wagging ^a
1200	$\nu(\text{C}-\text{OH})$	carboxylic acid stretch ^b
Poly-L-lysine (PL)		
3290 ± 2	$\nu(\text{NH})$	amide, amine NH stretch ^c
2930	$\nu_{as}(\text{CH}_2)$	methylene stretch ^a
2862	$\nu_s(\text{CH}_2)$	methylene stretch ^a
1655	$\nu(\text{C}=\text{O})$	Amide I ^a
1562	$\nu(\text{CN}) + \delta(\text{NH})$	Amide II ^a

^a From refs 14 and 39. ^b From ref 41. ^c From refs 57 and 42.

^d Note the $\alpha\text{-CH}_2$ scissors deformation is a relatively weak band, so at the higher pH values the carboxylate band dominates this region.

MUA monolayer is primarily in the protonated form after being rinsed with ethanol and Millipore water (pH \approx 6).

The adsorption of poly-L-lysine molecules onto the MUA monolayer occurred when the surface was exposed for 30 min to an aqueous PL solution buffered at a pH of 8.5, followed by a thorough rinsing with Millipore water. The CH-stretching and mid-IR regions of the PM-FTIR spectrum of the new MUA-PL bilayer structure are shown in Figures 3b and 4b, respectively. Increasing the exposure time or the PL concentration in solution did not alter the PM-FTIR spectrum of the MUA-PL bilayer. Assignments of the PL bands from these spectra are given in Table 1. To help the reader see the changes between the MUA and MUA-PL spectra, the differences between the MUA and MUA-PL spectra are also shown in Figures 3c and 4c. However, all band assignments and conclusions are taken directly from the MUA and MUA-PL PM-FTIR spectra. These spectra (Figures 3a, 4a and 3b, 4b) were highly reproducible from sample to sample and were very stable with time. In fact, the PM-FTIR spectrum did not change significantly after the bilayer was exposed to air for more than a month. It should also be noted that no background subtraction was used to obtain these PM-FTIR spectra. Actually, the use of the PM-FTIR methodology to obtain the differential reflectance spectrum of the adsorbed molecules removes the need for a background spectrum whatsoever.³⁷

The changes in the PM-FTIR spectra after exposure to PL show that PL is adsorbed onto the MUA surface. The main differences between the MUA and MUA-PL spectra are the following:

(i) Three new bands due to PL appear at 3290, 1655, and 1562 cm⁻¹. The band at 3290 cm⁻¹ is assigned to a combination of the amide and amine NH stretches. No bands due to NH₃⁺ stretch were identifiable in the spectra although they could be obscured by the methylene bands. The bands at 1655 and 1562 cm⁻¹ are assigned to the Amide I and Amide II bands of the polypeptide, respectively. The exact position of the Amide I band is difficult to obtain since it is strongly overlapped by MUA bands, but the Amide II band is well-separated and easily identifiable.

(ii) The intensities of the methylene stretching bands have increased due to the alkyl portions of the adsorbed PL. The asymmetric methylene stretch at 2930 cm⁻¹ in the difference spectrum has a reasonable position and intensity for approximately a monolayer of randomly ordered PL.

(iii) The intensities of the bands due to the protonated carboxylic acid at 1730 and 1200 cm⁻¹ in the MUA spectrum are substantially decreased in the MUA-PL spectrum. The loss of these bands indicates a substantial decrease in the number of protonated MUA carboxylic acid groups at the surface. Consistent with this decrease, the intensity of the symmetric stretching band of the carboxylate anion at 1406 cm⁻¹ increases significantly in the MUA-PL spectrum. (The asymmetric stretching band of the carboxylate anion at 1610 cm⁻¹ also increases but is overlapped by the amide bands of the PL.) These changes suggest that the adsorption of PL has led to the deprotonation of the MUA monolayer and the formation of COO⁻-NH₃⁺ ion pairs with the polypeptides.

These three changes in the PM-FTIR spectra indicate that exposure of the MUA monolayer to a PL solution has created a MUA-PL bilayer held together with multiple electrostatic ion-pair interactions. Several chemical experiments were performed to verify this hypothesis:

(i) Although the MUA-PL bilayer was very stable in air or in aqueous solution buffered around pH = 8.5, the PL monolayer could be washed off the MUA monolayer by aqueous solutions buffered to a pH of less than 6.5 or greater than 12 due to the conversion of either the MUA carboxylate anion to COOH or the lysine ammonium moiety to the free amine.

(ii) The deprotonation of the MUA monolayer (as observed in the PM-FTIR spectrum) caused by the adsorption of PL was not seen when the MUA surface was dipped in pH 8.5 buffer without PL and then rinsed with Millipore water.

(iii) No biopolymer adsorption occurred when the PL solution was replaced with a polyglutamate solution buffered at pH = 8.5. At this pH the polyglutamic acid polypeptide contains one carboxylate anion per residue in contrast to one ammonium cation in PL.

(iv) No adsorption onto the MUA monolayer was observed after exposure of the surface to a solution of lysine under the same conditions as the PL adsorption. The inability of the lysine monomer to irreversibly adsorb onto the self-assembled film indicates the need for multiple ion-pair interactions to attach the PL from aqueous solution. The inability of the lysine monomer to adsorb differs from the results of previous work on anthraquinone-2,6-disulfonic acid, which has been observed to adsorb electrostatically onto self-assembled monolayers.⁶

Each of these chemical observations supports the model depicted in Figure 1 of a MUA-PL bilayer on the gold surface held together by multiple ion-pair interactions. These chemical observations are also in agreement with the interpretation of the PM-FTIR spectra in Figures 3 and 4. Electrostatic interactions with surface-confined molecules have also been investigated by Crooks et al. using gas and liquid phase adsorption.^{42,43} They recently demonstrated that monomeric alkyl amines will adsorb onto a MUA monolayer from the gas phase, forming a combination of hydrogen-bonded pairs and carboxylate-ammonium ion-pairs (no evidence for hydrogen-bonding pair formation was observed in our MUA-PL bilayers).

Our conclusion that PL binds to a self-assembled MUA monolayer via multiple ion-pair interactions implies that electrostatic binding can serve as a general route for the adsorption of polymers to a surface. Electrostatic binding mechanisms for the adsorption of biopolymers onto surfaces have been employed previously. Bowden et al. have adsorbed cytochrome *c* onto gold electrodes modified

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with self-assembled monolayers terminated with carboxylic acids and have suggested that the ion-pair interactions with the lysine residues of the protein are responsible for the adsorption.¹¹ Bottomley et al. have adsorbed DNA onto gold surfaces modified with positively charged self-assembled monolayers of *N,N'*-dimethyl-2-mercaptoethylamine via the negatively charged phosphate groups in the DNA backbone.⁴⁴ Decher et al. have used electrostatic adsorption to construct self-assembled thin films using oppositely charged layers;⁴⁵ one system of particular interest is the formation of multilayers of poly-(allylamine) and DNA on glass substrates.⁴⁶ In a related methodology, Rubner et al. have been able to form self-assembled films of conducting polymers in which electrostatic bonding within the bilayer is important.⁴⁷ Our PM-FTIR experiments on the MUA-PL bilayer confirm that the electrostatic adsorption of polyelectrolytes onto charged surfaces is a robust yet reversible method of adsorbing biopolymers.

SPR Measurements of Film Thickness. The PM-FTIR experiments unambiguously demonstrate that PL is adsorbed onto the MUA monolayer via ion-pair formation. One immediate question arises: how much PL is adsorbing onto the surface? The increase in intensity of the asymmetric methylene stretching band suggests approximately one monolayer, but quantitating FTIR peak intensity measurements from metal surfaces is difficult due to possible orientation effects. Ellipsometry has been used frequently as a method for measuring the thickness of self-assembled monolayer films on gold surfaces.^{13,14,40,48} In this paper, we employ a related method of measuring film thickness with surface plasmon resonance (SPR) spectroscopy. This method has been used previously to determine the thickness of adsorbed monolayers on metal surfaces,^{29,49} and we find that the SPR technique has a precision equal to or better than ellipsometric measurements.

Reflectivity curves were measured using the experimental arrangement shown in Figure 2. The reflectivity of p-polarized light at 632.8 nm was measured as a function of θ , the incident angle from the surface normal. Figure 5 displays the reflectivity curve obtained from a clean 47 nm gold film with the circles representing the experimental data points. At an angle of approximately 41.3°, a cusp in the reflectivity curve was observed due to the critical angle for the BK7 prism (refractive index $n_{\text{BK7}} = 1.515$). The position of this cusp did not change with the presence of monolayers on the gold film and was used to ensure a reproducible calibration of the incident angle. Beyond the critical angle, a sharp drop in the reflectivity was observed at about 43.7°. This drop is the well-documented coupling of the incident laser beam with the surface plasmons (sometimes called surface plasmon polaritons) on the thin gold film;^{27,28,30} the angle of minimum reflectivity is denoted the "SPR angle". Notice that the reflectivity plunged dramatically from >90% to <1%; the sharpness of the SPR minimum allows for a very accurate determination of the position of the SPR angle.

The solid line in Figure 5 is a theoretical calculation of the reflectivity curve using a three-phase (BK7, Au, air)

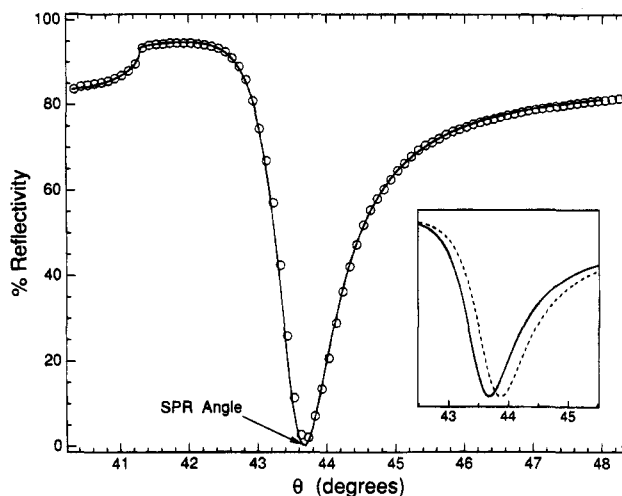


Figure 5. SPR reflectivity curve for a bare gold surface. The percent of reflected laser light is plotted versus the incident angle, θ . The circles give the experimentally measured values, and the solid line is the result of a three-phase (BK7, Au, air) phase complex Fresnel calculation. The tremendous decrease in reflectivity between about 43 and 45° is due to the laser light exciting surface plasmons on the gold surface, and the angle of minimum reflection is defined as the "SPR angle". The inset shows two theoretical curves: one for the bare gold film again (solid line), plus another of the calculated reflectivity curve for MUA monolayer adsorbed to a gold film (dashed line). The shift in the SPR angle can be used to determine the thickness of the adsorbed monolayer.

complex Fresnel calculation. This calculation was performed using the N-phase method as detailed by Hansen,⁵⁰ and the details of our specific implementation can be found elsewhere.⁵¹ The reflectance curve for this particular sample was best fit with a complex index of refraction of $\hat{n}_{\text{Au}} = 0.163 + 3.52i$. This index of refraction for the 47 nm gold film varied slightly from sample to sample and differed by approximately 15% from the bulk value, as noted previously.⁵²

At the reflectivity minimum, the light waves are coupled to the surface plasmons on the thin gold film. The intensity of the optical fields in these surface waves decays exponentially away from the surface on the order of 150 nm. This surface localization makes the position of the SPR angle extremely sensitive to the index of refraction of the adjacent medium, which was simply air in the case of the solid line in Figure 5. In contrast, the dashed curve in the inset of the figure represents the reflectivity curve expected after the adsorption of a monolayer onto the gold film. Notice the significant shift in the minimum for just one monolayer of material. The magnitude of this shift in the SPR angle, $\Delta\theta$, depends upon (i) the thickness of the monolayer and (ii) the optical index of refraction of the adsorbed molecules at 632.8 nm. Thus, the thickness of a monolayer can be obtained by measuring the SPR angle before and after absorption, provided the index of refraction is known.

Figure 6 depicts the experimental SPR curves near the reflectivity minimum for a vapor-deposited gold film and the reflectivity curves for the same film after the sequential deposition of a series of adsorbed monolayers. The experimentally measured shifts in the SPR angle and the calculated film thicknesses for the various monolayer films are listed in Table 2. A shift of $\Delta\theta = 0.179 \pm 0.009^\circ$ from the bare surface was observed after the adsorption of the self-assembled MUA monolayer. Through the use of a

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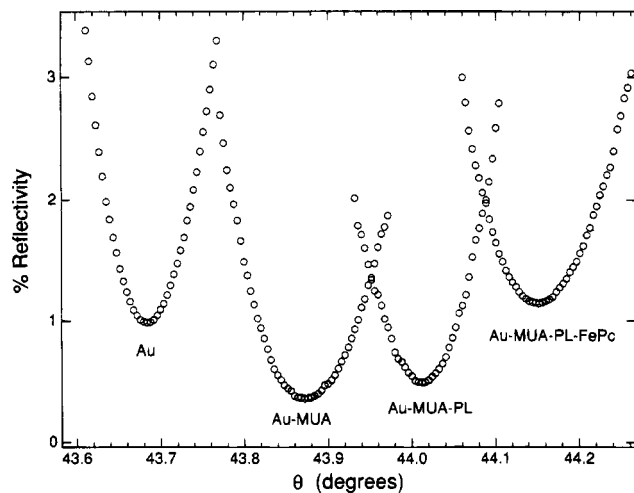


Figure 6. Expanded view of the experimental SPR reflectivity curves for a clean gold surface and the same surface after the adsorption of a MUA monolayer, then a PL monolayer, and finally a layer of FePc. The data points (circles) were taken every 0.004° to precisely determine the SPR angle. The observed shifts in the SPR angles correspond to a MUA thickness of 17.0 \AA and a PL thickness of 10.5 \AA (see text).

Table 2. SPR Angle Shifts and Calculated Film Thicknesses^a

self-assembled film	total $\Delta\theta$ (deg) ^b	additional $\Delta\theta$ (deg)	index of refraction	additional thickness (\AA)
MUA	0.179	0.179 ± 0.009	1.45 ^c	17.0 ± 0.9
MUA-PL	0.309	0.130 ± 0.021	1.52 ^d	10.5 ± 1.7
MUA-PL-FePc	0.430	0.121 ± 0.022	<i>e</i>	<i>e</i>

^a $\Delta\theta$ and thickness values are the average of six samples (MUA, PL) or four samples (FePc) with the errors being one standard deviation from the mean. ^b Total shift in SPR angle from that of the bare gold surface. ^c Estimated index of refraction.⁴⁸ ^d Bulk index of refraction.⁵³ ^e Since the complex index of refraction for the absorbing FePc is not known nor easily estimated, no attempt to convert $\Delta\theta$ to thickness was made.

four-phase (BK7, Au, MUA, air) complex Fresnel calculation, $\Delta\theta$ was converted to a thickness of $17.0 \pm 0.9 \text{ \AA}$ by assuming a MUA index of refraction of $n_{\text{MUA}} = 1.45$.⁴⁸ This film thickness is in excellent agreement with previous ellipsometric film thickness measurements of $16\text{--}19 \text{ \AA}$ for the MUA monolayer^{13,14,48} and confirms the accuracy of the SPR technique. A theoretical thickness of about $18\text{--}19 \text{ \AA}$ is expected for a fully extended MUA monolayer oriented normal to the surface,^{13,48} and so our measured value of 17.0 \AA is in agreement with a picture of the MUA molecules forming a close-packed self-assembled monolayer where the methylene chains are oriented nearly perpendicular (i.e., with an average tilt angle of $\leq 30^\circ$) to the surface.

In principle, the index of refraction of the monolayer (n) can be extracted from the shape of the entire SPR reflectivity curve. However, for the experiments in this paper we will focus only on the shift in the SPR angle and simply estimate n with bulk values. Similar uncertainties are present in the determination of film thickness from ellipsometric measurements and have been discussed in the literature.⁴⁰ To examine the uncertainty caused by estimating the index of refraction, a four-phase (BK7, Au, film, air) complex Fresnel calculation was performed for a range of values of n . Figure 7 plots the results of these calculations and shows the shift in the SPR angle as a function of film thickness for thin films with $n = 1.40, 1.45, 1.50, \text{ and } 1.55$. A variation of 0.05 in the monolayer index of refraction leads to an error of approximately $\pm 7\%$ (i.e. 1 \AA for every 15 \AA of film thickness). Although n may

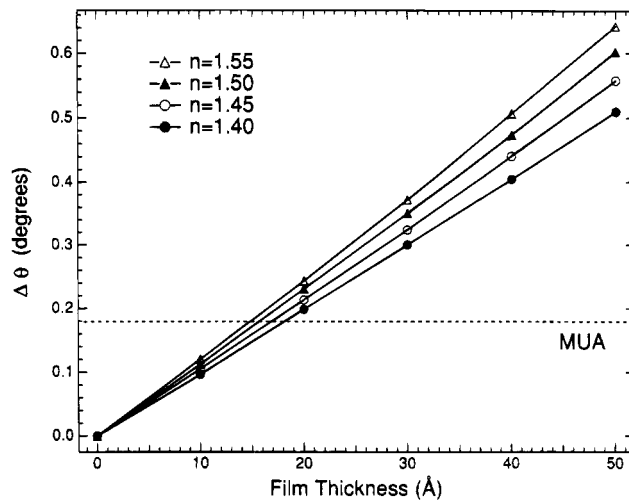


Figure 7. Results of a four-phase (BK7, Au, film, air) complex Fresnel calculation giving the expected change in SPR angle, $\Delta\theta$, for thin films with thicknesses up to 50 \AA and different refractive indices: $n = 1.40, 1.45, 1.50, \text{ and } 1.55$. The sensitivity of the SPR technique is given by the slope of approximately $0.012^\circ/\text{\AA}$. However, the slope depends somewhat on the thin film thickness and refractive index, therefore requiring Fresnel calculations for a more accurate conversion of experimental $\Delta\theta$ values to thicknesses. The dashed line indicates the experimentally observed shift for a MUA monolayer, and the intersections of this line with the solid lines establish the error in thickness associated with an uncertainty in the refractive index. For example, an uncertainty of ± 0.05 in the thin film index of refraction for MUA gives an error of roughly 1 \AA in the calculated MUA thickness.

vary somewhat from the bulk index of refraction, it is unlikely to change by more than ± 0.05 . If the bulk value of n is not known for a given compound, it can usually be estimated within these same limits from the indices of refraction for related compounds.

In addition to the error in estimating the monolayer index of refraction, two other sources of error are present in the SPR thickness measurements. The shift in SPR angle for our instrument can be determined to an accuracy of $\pm 0.005^\circ$. This error in $\Delta\theta$ corresponds to an error of approximately $\pm 0.4 \text{ \AA}$. Note that this instrumental error is constant regardless of the film thickness; in contrast, the error in n is proportional to the film thickness. A third source of error arises from sample variations such as surface roughness, contamination, and relative humidity. This error is reported for our SPR measurements as the standard deviation in the mean of multiple samples (see Table 2).

After adsorption of poly-L-lysine onto the MUA-coated gold surface, an additional shift in the SPR angle of $0.130 \pm 0.021^\circ$ was observed (see Figure 6). From the bulk PL index of refraction of $n_{\text{PL}} = 1.52$,⁵³ a film thickness of 10.5 \AA was determined from a five-phase (BK7, Au, MUA, PL, air) complex Fresnel calculation. A thickness of 10.5 \AA for the adsorbed PL suggests that the polypeptide monolayer is adsorbed parallel to the surface. This conclusion is in agreement with surface force measurements on mica surfaces,^{8,54} which indicate a thickness on the order of 10 \AA for a PL monolayer in an extended configuration. The SPR thickness measurements, in conjunction with the structural information obtained from the PM-FTIR experiments, confirm the model of the electrostatically adsorbed MUA-PL bilayer depicted in Figure 1.

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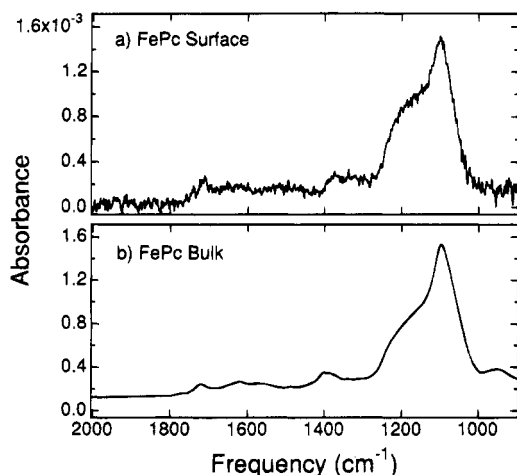
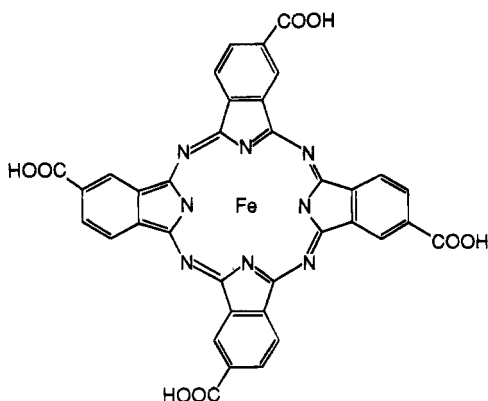


Figure 8. (a) Mid-IR region of the PM-FTIR difference spectrum between the MUA-PL bilayer and the MUA-PL-FePc trilayer on a vapor-deposited gold surface. (b) Bulk FTIR spectrum of the same region for FePc in a KBr pellet. The similarity of these spectra indicates the presence of FePc adsorbed onto the MUA-PL bilayer.

Adsorption Experiments onto the MUA-PL Bilayer. The ability of the PL to be modified while on the surface via free ammonium moieties is critically important for the implementation of the MUA-PL bilayer in chemical sensor applications. If the poly-L-lysine is adsorbed onto the MUA monolayer in an extended configuration, then some of the lysine residues may not be able to interact with the surface but may remain available for interaction or reaction with molecules from solution. As a test of the availability of lysine residues, the MUA-PL bilayer was exposed to a solution of iron phthalocyaninetetracarboxylic acid (FePc):



FePc was chosen because it is known to associate with PL in solution. Optical absorption studies by Kornguth et al. have demonstrated that tetracarboxyphthalocyanines will interact electrostatically with PL in its polycationic form.²⁵ X-ray photoelectron spectroscopy (XPS) measurements⁵⁵ on the MUA-PL bilayer after exposure to a FePc solution verified that iron was present on the surface. The difference between the PM-FTIR spectra for the surface before and after exposure to FePc is plotted in Figure 8a; the bulk spectrum of FePc in a KBr pellet is shown in Figure 8b. In both spectra a large broad band is observed from 1250 to 1000 cm⁻¹; this band is assigned to a combination of C=N and C=C stretching, and CH in-plane bending modes.⁵⁶ The similarity of the two spectra

in Figure 8a and 8b confirms the presence of the FePc on the surface, most likely in a random orientation. It was observed that the FePc does not adsorb to the MUA monolayer in the absence of a PL overlayer.

The adsorption of FePc molecules onto the surface also resulted in a change in the SPR angle. In Figure 6, the SPR reflectivity curve is plotted for the MUA-PL-FePc trilayer, showing an additional shift of $0.121 \pm 0.022^\circ$. Unfortunately, the thickness of the FePc layer is more difficult to determine from $\Delta\theta$ since the FePc absorbs 632.8 nm light and therefore requires the use of a complex index of refraction in the Fresnel calculation. However, the shift of 0.12° is similar to that observed for the PL monolayer, implying that the FePc layer has a thickness comparable to that of PL.

Both the PM-FTIR and SPR measurements conclusively demonstrate that some fraction of the lysine residues in the adsorbed PL is available for interaction with species in solution. These results suggest that the functionalization of the MUA-PL bilayer for chemical sensor applications via the free lysine residues is feasible.

Summary and Conclusions

Through a combination of vibrational spectroscopy and surface plasmon experiments, we have demonstrated that multiply charged polypeptides such as poly-L-lysine can be strongly adsorbed onto gold surfaces that have been modified with a self-assembled monolayer terminated by a charged functional group such as a carboxylate anion. Specifically, a robust MUA-PL bilayer can be created on the gold surface when a self-assembled MUA monolayer is exposed to a poly-L-lysine solution at an appropriate pH. The PM-FTIR spectroscopic measurements indicate that the PL adsorbs onto the MUA monolayer via the formation of multiple carboxylate-ammonium ion pairs and that the PL can be released from this surface by rinsing with a buffer solution adjusted to a pH which destroys the ion pairing interactions. The SPR measurements suggest that the MUA-PL bilayer consists of a 17.0 Å monolayer of MUA oriented nearly perpendicular to the surface which is coated with a 10.5 Å monolayer of PL adsorbed in an extended chain conformation lying essentially parallel to the surface. The formation of a robust MUA-PL bilayer via electrostatic adsorption is in agreement with the work of previous authors on related self-assembled monolayer systems.⁴⁵

Subsequent adsorption measurements of an iron phthalocyanine probe molecule indicate that not all of the lysine residues of PL are interacting with the MUA monolayer. This result implies that some ammonium groups remain available for the possible attachment of enzymes or other molecules to the surface in order to create chemical sensors. The combination of the PM-FTIR and the SPR measurements employed in these studies has proven to be a very sensitive and complementary pair of experiments for the analysis of the molecular structure of ultrathin molecular films at metal surfaces.

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