Polarization modulation infrared reflection absorption spectroscopy (PM-IRRAS) has been applied to determine the conformation, orientation, and hydration of a monolayer of 2,3-di-O-phytanyl-sn-glycerol-1-tetraethylene glycol-ti-α-lipoic acid ester (DPT) self-assembled at a gold electrode surface. This Archaea analogue thiolipid has been recently employed to build tethered lipid bilayers. By synthesizing DPT\textsubscript{4DEG}, a DPT molecule with a deuterium substituted tetraethylene glycol spacer, it was possible to differentiate the C–H stretch vibrations of the phytanyl chains from the tetraethylene glycol spacer and acquire the characteristic IR spectra for the chains, spacer, and lipoic acid headgroup separately. Our results show that the structure of the monolayer displays remarkable stability in a broad range of electrode potentials and that the phytanyl chains remain in a liquid crystalline state. The tetraethylene glycol chains are coiled, and the IR spectrum for this region shows that it is in the disordered state. The most significant result of this study is the information that in contrast to expectations the spacer region is poorly hydrated. Our results have implications for the design of a tethered lipid membrane based on this thiolipid.

1. Introduction

Tethered bilayer lipid membranes (tBLMs)\textsuperscript{[1–33]} are built by self-assembling a thiolipid monolayer consisting of a hydrophobic lipid tail and a hydrophilic spacer terminated with a mercapto functionality that anchors the thiolipid to a gold or mercury electrode surface. The second leaflet of the bilayer is formed by spreading unilamellar vesicles of phospholipids onto the thiolipid monolayer. This architecture is designed to separate the hydrophobic portion of the membrane from the metal surface by a hydrated hydrophilic spacer, mimicking the cytosol of a mammalian cell. Tethered lipid bilayers find applications in the basic research of transmembrane proteins\textsuperscript{[2–17,27]} and in the development of biosensors.\textsuperscript{[1]}

Recently, tBLMs assembled using the Archaea analogue thiolipid 2,3-di-O-phytanyl-sn-glycerol-1-tetraethylene glycol-ti-α-lipoic acid ester (DPT) shown as molecule I in Figure 1 became an attractive model of the biological membrane.\textsuperscript{[2–33]} The molecule

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In Situ PM-IRRAS Studies of an Archaea Analogue Thiolipid Assembled on a Au(111) Electrode Surface

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has a lipoic acid headgroup containing a dithiol which acts as a tether and an anchor to the Au surface. The middle part of the DPTL molecule consists of the tetraethylene glycol spacer, which was expected to have hydrophilic properties. The tail of the molecule is formed by two isoprenoid chains, which are highly hydrophobic. The bilayers formed by this thiolipid display very good barrier properties and the hydrophilic tetraethylene glycol sections of the molecules are believed to provide the hydrated layer that separates the hydrophobic phytanyl chains from the metal surface.

The objective of the present work is to determine the orientation, conformation, and extent of hydration of the polar headgroups of DPTL monolayers assembled at the Au(111) electrode surface using polarization modulation infrared reflection absorption spectroscopy (PM-IRRAS). To assist with the interpretation of the IR spectra of DPTL, additional transmission spectra for tetraethylene glycol (TEG) (hydrophilic spacer, molecule 2) and benzyl lipoate (BLA) (the tether, molecule 3) were measured. The molecular structures of these model compounds are given in Figure 1. In addition, DPTL with deuterium substituted hydrogens in the TEG spacer was employed to separate C−H vibrations in the tail and in the spacer. Various techniques have already been applied to study films of the DPTL monolayer and films of tethered lipid membranes. The thickness of these layers have been investigated by surface plasmon resonance (SPR) spectroscopy and quartz crystal microbalance measurements, the electronic properties have been studied by electrochemical impedance spectroscopy (EIS), and reflection absorption IR spectroscopy (RAIRS) characterizations have been performed on the monolayer in air. We will demonstrate that the PM-IRRAS technique employed in the present work allows for the performance of in situ experiments and determining the influence of the electric field applied to the electrode on the hydration of the polar region of the molecule and on the packing and conformation of its phytanyl chains.

2. Experimental Section

Reagents, Solutions, and Electrode Materials. Gold single crystals, prepared as described in ref 34, were used as working electrodes for electrochemistry and PM-IRRAS measurements. Prior to each electrochemical experiment, the working electrode was flame-annealed, cooled first in air and then in water, and transferred to the electrochemical cell under protection of a drop of water covering its surface. A flame-annealed gold coil was used as the counter electrode. The reference electrode was an external saturated Ag/AgCl electrode (+197 mV vs SHE). For the PM-IRRAS experiments, the Au single crystal was flame-annealed, cooled, and thoroughly dried before it was mounted into the electrochemical IR cell equipped with a Pt foil as the counter electrode. A saturated Ag/AgCl reference electrode was used for the IR measurements as well (+197 mV vs SHE). In this paper, all potentials are referred to the Ag/AgCl scale.

Prior to all experiments, the glassware was cleaned in acid (1:3 mixture of HNO3 and H2SO4) and thoroughly rinsed with Milli-Q and Milli-Q UV plus (Millipore, Bedford, MA) water (≥18.2 MΩ cm). The electrochemical cell was soaked in Milli-Q water overnight and rinsed again. The IR cell was soaked in

Figure 1. Models of the molecules used for the IR studies. The following colors were used to mark various atoms: C, black; O, red; H, green; S, yellow.
Milli-Q water for several hours, rinsed again, and then dried overnight. A 0.1 M NaF (suprapur, EM Industries, Hawthorne, NY) solution was used as supporting electrolyte. The NaF was always cleaned for 30 min in an oxygen chamber (UVO cleaner, Geljet, Irvine, CA) before making the solutions. All aqueous solutions were prepared from ultrapure water purified by using a Milli-Q UV plus water system with resistivity > 18 MΩ cm. Some IR experiments were performed in deuterated water solutions (D₂O, Cambridge Isotope Laboratories, Cambridge, MA). All electrolyte solutions were deaerated by purging with argon (BOC Gases, Mississauga, Ontario, Canada) for at least 40 min before starting the experiments, and an argon blanket was maintained over the solution throughout the experiment.

The thiolipid DPTL was synthesized either at the Max Planck Institute for Polymer Research in Mainz, Germany or at the University of Guelph, Canada. The DPTL with deuterated tether and linker was synthesized using the procedure described in ref 35. The thiolipid was dissolved in methanol and deposited onto the Au(111) surface by self-assembly for a period of 12 h. After formation of the self-assembled monolayer (SAM), the electrode was rinsed with methanol and water (or deuterated water) before mounting it into the electrochemical or spectroelectrochemical cell. All measurements were carried out at room temperature (20 ± 2)°C.

**Electrochemical Measurements and Instrumentation.** All electrochemical measurements were carried out in an all glass three-electrode cell using the working electrode in the hanging meniscus configuration. Cyclic voltammetry and differential capacitance measurements were used to check if the electrode and the electrolyte solution were sufficiently clean. To measure the differential capacitance, an AC perturbation of 25 Hz frequency and 5 mV rms amplitude was used. All electrochemical experiments were performed using a computer controlled system consisting of a HEKA potentiostat/galvanostat PG590 (HEKA, Lambrecht/Pfalz, Germany) and a 7265 DSP lock-in amplifier (EG & G Instruments, Cypress, CA). Data acquisition was performed via a plug-in acquisition board (RC Electronics, Santa Barbara, CA) and in-house software. A series RC equivalent circuit was employed to calculate the differential capacity curves from the in-phase and the out-of-phase components of the AC current.

In addition, chronocoulometry was employed as a potentiostatic method to determine the charge density at the electrode surface. In this series of experiments, the Au(111) electrode was held at a base potential $E_b = +200$ mV for 120 s. Then the potential was stepped to a variable value of interest $E_s$ starting from the most positive potential (+400 mV), and held constant for another 120 s. The thiolipid was desorbed from the electrode surface by stepping the potential to $E_{des} = -1200$ mV. The current transient corresponding to desorption of DPTL was measured during 0.15 s. The potential was then stepped back to the base value of $E_b = +200$ mV. Integration of the current transients gives the difference between charge densities at potentials $E_s$ and $E_{des}$. The charge density curves measured with and without DPTL merged at the most negative potentials. The absolute charge densities for the electrode without DPTL were calculated assuming that at $E = -1.2$ V the charge density for the electrode initially covered by DPTL is equal to the charge density of the electrode without DPTL. The data processing is described in detail in refs 30 and 34. The SAM was formed on the Au(111) electrode immediately prior to each experiment.

**Collection and Processing of Spectra.** The PM-IRRAS experiments were performed using a NEXUS 870 (Nicolet, Madison, WI) spectrometer, equipped with an external tabletop optical microscope, an ACT-A detector, with a photelastic modulator (PM-90 with II/ZSZ0 ZnSe 50 kHz optical head, Hinds Instruments, Hillsboro, OR) and a demodulator (Synchronous Sampling demodulator, GWC Instruments, Madison, WI). The spectra were acquired using in-house software, an Omnic macro, and a digital-to-analog converter (Omega, Stamford, CT) to control the potentiostat (HEKA PG285) and to collect the spectra. A BaF₂ 1° equiangular prism (Janos Technology, Townshend, VT) was used as the IR window. Prior to each experiment, the window was washed in methanol and in Milli-Q UV plus water and then cleaned for 10 min in an ozone chamber (UVO cleaner, Geljet, Irvine, CA). The Au(111) electrode was exposed to the methanolic thiolipid solution for ~12 h and then assembled in the IR cell which was sealed with the IR window and filled with the electrolyte solution. After deaeration of the electrolyte, the potential of the working electrode was set to a starting potential of $E = 400$ mV versus the Ag/AgCl reference electrode and spectra were measured for a series of potentials between +400 and −1100 mV by changing the potential using 100 mV increments and recording 8000 scans at every applied potential. The instrument resolution was 2 cm⁻¹. At the end of an experiment, the blocks of scans obtained at each value of the applied potential were individually checked for bad scans, which were removed before averaging.

Measurements of IR spectra were carried out with the photelastic modulator (PEM) set for half-wave retardation at either 2900 cm⁻¹ for the CH stretching region, 1600 cm⁻¹ for the C=O stretching, the CH bending, and the CH wagging, twisting, and rocking region, or 1200 cm⁻¹ for the C–O–C stretching region. In the CH stretching region, the angle of incidence of the infrared beam was set to 53° and the thickness of the electrolyte layer between the electrode and prism was ~2 μm. These parameters are chosen to obtain the maximal mean square electric field strength of p-polarized radiation at the electrode surface. In the C=O stretching and CH bending, wagging, and twisting region, the angle of incidence was set to 61° and the gap thickness was ~3.5 μm. In the C–O–C stretching region, the angle of incident light was 57° and the gap thickness was 3 μm. The thickness of the thin layer between the electrode surface and IR prism was determined by comparing the experimental reflectivity spectrum of the thin layer cell, which is attenuated due to the layer of solvent between the electrode and the IR window, to the reflectivity curve calculated from the optical constants of the cell constituents as described in ref 36.

The demodulation technique developed by Corn and co-workers was used in this work. After demodulation, two signals were measured: the intensity average $I_s(\omega)$ and the intensity difference $I_d(\omega).$ The two signals were convoluted with the PEM response functions. We used a modified version of the method described earlier by Buffeteau and co-workers to calculate the PEM response functions. The details of these calculations are described in ref 38.

After these corrections, the PM-IRRAS spectrum contains the absorption of the IR beam by both the adsorbed molecules and a background due to the absorption by the aqueous solution in the thin layer. To subtract the background from the spectrum, a baseline was constructed using a spline interpolation technique. The same wavenumber range was used to construct the spline for all of the spectra. After applying all of these corrections, the signal plot, ΔS, which is proportional to the absorbance A of the molecules adsorbed on the electrode surface (see eq 1), was used to calculate ΔS(ω) defined as

$$\Delta S(\omega) = \frac{2(I_s - I_d)}{I_s + I_d} = 2.34 \times 10^{-5} \epsilon$$  \[(1)\]

In eq 1, I, and I₀ are the intensities of s- and p-polarized light, ε is the molar absorption coefficient, and Γ is the surface concentration of the adsorbed species.

The integrated intensity of the absorption bands of molecules adsorbed on a reflecting metal surface is proportional to the square of the dot product of the transition dipole moment µ and the electric field of the photon E:\(^2\): \[ \int A d\nu = |\mu E|^2 = |\mu|^2 |E|^2 \cos^2 \theta \] where \( \theta \) is the angle between the direction of the electric field of the photon corresponding to the surface normal if the light is p-polarized, and the transition dipole, \( \mu \), is the absolute value of the transition dipole moment and \( |E|^2 \) is the mean square electric field strength of the photon. As the electric field of p-polarized IR radiation is normal to the surface, the orientation of molecules at the metal surface can be found if the direction of the transition dipole can be related to the molecular geometry. For randomly oriented molecules, \( \cos^2 \theta = 1/3 \), and therefore, the orientation of adsorbed species at the electrode-solution interface can be determined by calculating the spectrum for randomly oriented molecules from the isotropic optical constants using the following relationship: \( \cos^2 \theta = \frac{A_{\text{exp}} dv}{3 \int A_{\text{cal}} dv} \) where \( A_{\text{exp}} \) is the measured, background-corrected absorbance of the DPTL film adsorbed on the Au(111) surface and \( A_{\text{cal}} \) is the absorbance calculated from the optical constants for reflection from the gold electrode surface using the optical matrix method.\(^{38,41} \) If the direction of the transition dipole moment is known, the orientation of the molecules forming the adsorbed layer can be determined by relating the direction of the transition dipole moment to the geometry of the molecule. Throughout the interpretation of the IR data, it is assumed that the static electric field at the surface does not affect the absolute value of the transition dipole, so that the changes in the integrated band intensities can be discussed in terms of the reorientation of the molecules. This approach is widely used in IR studies of electric-field-driven transformations in thin films at electrode surfaces.\(^{36} \)

For the calculation of \( A_{\text{cal}} \), a thickness of 4 nm is assumed for the DPTL monolayer, \( \tau_1 \), and similar plots published earlier for DPTL.\(^{26,30} \) The charge density data presented in Figure 2B provide additional information about the properties of the SAMs. The adsorption of the SAM leads to a significant increase of the charge density at the electrode surface. At \( E > -0.6 \) V for DPTL and \( E > -0.5 \) V for DPTL₆, the charge densities attain a value of \( \sim 20 \mu \text{C cm}^{-2} \). We have previously demonstrated that for DPTL this value of the charge density corresponds to the packing density of the SAM being equal to \( \Delta \sim 2.5 \times 10^{-10} \text{mol cm}^{-2} \). Since the same charge densities are observed for the electrode covered by monolayers of DPTL and DPTL₆, we can conclude that the films of these two thiolipids have the same packing densities. Overall, the monolayers formed by these two compounds display very similar behavior, although the SAM of DPTL₆ is desorbed at slightly less negative potentials than the SAM of DPTL.

PM-IRRAS Studies. PM-IRRAS measurements provide unique information regarding the changes in the conformation, orientation, and state of hydration of the thiolipid monolayer as a function of the applied potential. Below, the discussion of the IR spectra is divided into sections describing studies of the phytanyl chains, the tetraethylene glycol spacer unit, and the chiral acid headgroup.

The Phytanyl Chains. Figure 3A compares the spectra in the CH stretch region calculated using the optical constants of the three molecules whose structures are shown in Figure 1. Random orientation of the molecules was assumed in the calculations.
The deconvoluted bands are shown in Figure 3B. The CH stretching region of diphytanoylphospholipids contains more bands than the spectra of saturated or monounsaturated phospholipids.\textsuperscript{47,48} To assign these bands, the \textit{ab initio} normal coordinate quantum chemical calculations were performed. The calculations provided a display of animated vibrations at selected band frequencies which allowed the band assignment. The files showing animated methyl symmetric stretches at the terminal end and in the middle of the isoprenoid chains and the stretches of the methylene group are shown in the Supporting Information.

The calculations revealed that the band at \(\sim 2843\) cm\(^{-1}\), previously identified as a split symmetric CH\(_2\) stretch\textsuperscript{46} (\(\nu_s(CH_2)\)), actually corresponds to symmetric stretches (\(\nu_s(CH_2-t)\)) of terminal methyl groups of the phytanyln chains (two methyl groups at each chain). The band at \(\sim 2854\) cm\(^{-1}\) is chiefly due to symmetric stretches of the methylene groups. It overlaps with the band at \(\sim 2868\) cm\(^{-1}\) corresponding to the symmetric stretches of methyl groups in the middle of phytanoyln chains (\(\nu_s(CH_2-m)\)). The animations shown in the Supporting Information illustrate that these two last vibrations are significantly coupled.

The region from 2875 to 3000 cm\(^{-1}\) of the PM-IRRAS spectra contains many peaks. Normal coordinate calculations demonstrated that the band at \(\sim 2958\) cm\(^{-1}\) corresponds to the asymmetric stretching of the methyl groups in the middle of the phytanyln chains (\(\nu_a(CH_3-m)\)). The strong band at \(\sim 2927\) cm\(^{-1}\) corresponds to the overlapping asymmetric methylene (\(\nu_a(CH_2)\)) and asymmetric methyl stretches of the terminal methyl groups (\(\nu_a(CH_3-t)\)). The intensity of this band changes little upon deuteration of eight CH\(_2\) groups in the tetraethylene glycol spacer, indicating that the contribution of the vibrations of the methyl groups in the phytanyln chains to the overall band intensity is significant. The low frequency shoulder of the \(\sim 2927\) cm\(^{-1}\) band consists of overlapping C–H stretches (\(\nu(CH)\)) of the C–H bonds adjacent to the methyl groups in phytanyln chains at \(\sim 2915\) cm\(^{-1}\) and Fermi resonances between the overtones of the symmetric bending mode and symmetric methyl and methylene stretches\textsuperscript{42} at \(\sim 2899\) cm\(^{-1}\). Due to the complex nature of this spectral region, its deconvolution is subject to significant uncertainty. Therefore, further discussion of the conformation and orientation of the phytanyln chains will be based on the analysis of the three bands in the 2825–2875 cm\(^{-1}\) region.

Figure 4 shows the PM-IRRAS spectra for a SAM of DPT\(_{d16}\)L determined for the selected electrode potentials indicated in the figure. For comparison, the top thick line corresponds to the spectrum calculated for a film of randomly oriented DPT\(_{d16}\)L molecules. For \(\nu(CH_2)\), \(\nu(CH_3-m)\), and \(\nu(CH_3-t)\) vibrations, the band centers and FWHM in the deconvoluted spectra are plotted in Figure S15 of the Supporting Information. There are two pieces of information one can glean from the inspection of these spectra. First, the SAM spectra do not change significantly with the electrode potential, even after desorption from the electrode surface. Second, the bands for CH\(_2\) stretches are apparently insensitive to the applied potential, independently of the applied potential.

The uncertainty of the band position reflects the uncertainty in the band position obtained by deconvolution the spectra, especially when the bands are close in wavenumber. As can be seen in Figures S14, S15, and S16 of the Supporting Information, the bands broaden with increasing deconvolution. Thereby, the uncertainty of the band position increases with increasing deconvolution, which results in a decrease in the band area. Therefore, the uncertainty of the band position increases with increasing deconvolution.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{(A) Differential capacity and (B) charge density curves for a film-free Au(111) electrode (black dotted line) and the electrode covered by self-assembled monolayers of DPTL (red line) and DPT\(_{d16}\)L (blue line) in a 0.1 M NaF supporting electrolyte solution.}
\end{figure}

\begin{table}[h]
\centering
\begin{tabular}{|c|c|}
\hline
Band & Wavenumber (cm\(^{-1}\)) \\
\hline
\hline
\end{tabular}
\caption{Summary of bands and wavenumbers for the PM-IRRAS spectra.}
\end{table}
associated with the deconvolution procedure, and within the experimental error it is equal to the frequency of the $v_s(CH_2)$ in a stack of multiple bilayers of diphytanoylphosphatidylcholine. For the $n$-alkanes, frequencies of the $v_s(CH_2)$ band smaller than 2850 cm$^{-1}$ are characteristic of the gel state of the bilayer in which the chains are fully stretched and assume an all-trans conformation. Higher frequencies indicate that the chains are partially melted and contain a fraction of gauche conformations. The phase transition temperature for DPTL was determined to be $-83^\circ C$. Therefore, the frequencies of the $v_s(CH_2)$ are consistent with the liquid state of the phytanyl chains.

For the symmetric methyl and methylene stretches, the integrated intensities of the deconvoluted bands were calculated and the angle between their transition dipoles and the surface normal were determined using eq 3. The tilt angles are plotted as a function of the electrode potential in Figure 5. The spread of the tilt angle values chiefly reflects the uncertainty associated with the deconvolution procedure for the bands. The tilt angles are essentially independent of the electrode potential. The average value of the angle for the symmetric stretch of the terminal CH$_3$ group is equal to 48$^\circ$ ± 5°. This number is close to the value of 54°, which is expected for random orientation of the terminal group and suggests that the terminal methyl groups can freely rotate in the monolayer.

The angle between the transition dipole of the methyl groups in the middle of the chain and surface normal is much larger, 66$^\circ$ ± 5°. It has a magnitude that is comparable to the angle between the transition dipole of the symmetric methylene stretch which is equal to 61$^\circ$ ± 5°. The transition dipole of the $v_s(CH_2)$ band is oriented along C$_2$ of the methylene group. The direction of the transition dipole of the $v_s(CH_3-t)$ coincides with the direction of the C$_3$ axis of the methyl group. Consequently, the transition dipoles of these two vibrations are oriented at an angle of 90° with respect to the all-trans isoprenoid chain. Assuming the all-trans conformation for the chains, their tilt with respect to the surface normal should be between 29$^\circ$ ± 5° and 25$^\circ$ ± 5°. However, the chains are in the liquid state, and hence, these tilt angles should be used only as a very rough approximation.

The Tetraethylene Glycol Spacer. The IR spectra of the ethylene glycol spacer consist of $\nu(CC)$ and $\nu(CO)$ stretches in combination with CH$_2$ stretching, wagging, rocking, and twisting. The CH$_2$ stretching bands of the spacer overlap with the stretches of CH$_3$ groups in phytanyl chains. However, in DPT$_{d16}$L, the CD$_2$ stretches are not present in the spectrum. The IR bands of the tetraethylene glycol spacer are listed in Table S1.
vibrations of the spacer are shifted to lower wavenumbers and may be used to determine the orientation of this segment of the molecule. Figure SI 6 of the Supporting Information shows the spectra of the SAM in the CD₂ stretch region acquired at selected electrode potentials. The signal-to-noise is poor, since these bands are clearly weak; however, they do not change with the electrode potential. Therefore, the bands recorded at individual potentials were added to give the average spectrum shown in Figure 6A. For comparison, Figure 6B plots the spectrum calculated from the optical constants for a monolayer of randomly oriented molecules at the Au(111) electrode surface calculated using the optical constants reported in the Supporting Information. Lorentzian functions were assumed for the band deconvolution.

Figure 6. For the C–D stretching region in the IR spectrum of DPT₉₅L: (A) PM-IRRAS spectrum for the SAM of DPT₉₅L at the Au(111) electrode surface measured in 0.1 M NaF in H₂O solution. This is the average spectrum averaged for all potentials measured. (B) Spectrum for a monolayer of randomly oriented DPT₉₅L molecules at the Au(111) electrode surface calculated using the optical constants reported in the Supporting Information. Lorentzian functions were assumed for the band deconvolution.

Figure 7. For the 1500–900 cm⁻¹ spectral region: IR spectra of monolayers of randomly oriented molecules at the Au(111) electrode surface calculated using optical constants: DPTL (black line); TEG (red line); BLA (green line). The C–O–C stretch bands located in the 1400–900 cm⁻¹ region provide further information concerning the orientation of the ester group and about the conformation of the polyethylene glycol spacer. This is a dense spectral region which also contains bands of the lipoprotein anchor and wagging bands of the phytanyl chains. This point is illustrated by Figure 7 which compares spectra for randomly oriented monolayers of DPTL, TEG, and BLA calculated using the optical constants determined for solutions of these species. The following bands from the BLA anchor may overlap with the bands of the PEG spacer: 1384, 1355, 1316, 1235, 1214, 1170, 1092, 1083, 1035 (shoulder), 1018 cm⁻¹.

Figure 8A shows the deconvolution of the spectrum of randomly oriented DPTL molecules, and Figure 8B shows the PM-IRRAS spectrum of the SAM of DPTL. The PM-IRRAS spectra recorded at different potentials are shown in Figure SI 7 of the Supporting Information. These spectra do not change with potential. Therefore, the spectra were added to improve S/N and averaged. Figure 8A plots the average spectrum. When compared with the RAIRS spectrum of the SAM of DPTL measured in air previously,26a the PM-IRRAS spectrum of the SAM in solution shows bands at ~1040 and 945 cm⁻¹ that were not observed in the RAIRS spectrum. Consequently, Figure 8B gives a more complete fingerprint of the tetraethylene glycol spacer region in the IR spectrum of DPTL. The frequencies of the bands assigned to the TEG spacer in the solution of DPTL in CCl₄ and in the SAM are listed in Table 1 where they are compared to the frequencies of the bands in the 7/2 crystalline and melted (amorphous) polyethylene glycol (PEG).

The 7/2 crystalline PEG has a helical structure made of seven CH₂CH₂O units and two helical turns per fibre period.53 In the helix, the internal rotations around the O–CH₂, CH₂–CH₂, and CH₂–O bonds are trans, gauche, and trans, respectively. Therefore, in a crystalline PEG, the repeating COCCOC adopts the TGT conformation. The transition dipoles of IR bands for the helical conformation are oriented either parallel (A₂ symmetry) or
perpendicular (E₁ symmetry) to the helix axis.⁵³,⁵⁴ In solution⁵⁵ or upon melting,⁵⁶,⁵⁷ the helical structure is lost and the repeating unit adopts a distribution of TGT, TGG, GGG, TTG, TGG, and GTG conformations. The frequency of the IR bands in the 1400–900 cm⁻¹ region of the molten PEG or PEG in solution can be correlated with either trans or gauche conformation of the O–CH₂–CH₂–O unit. This assignment of the IR bands for the spacer layer from the analysis of the C=O stretching band at 1650 cm⁻¹ corresponding to the trans conformation of the O=C–C=O moiety is a further indication that this unit adopts predominantly the gauche conformation in the SAM. In contrast to the SAM, these bands are present in the spectrum of DPTL in CCl₄ solution where the spacer unit has apparently a mixture of gauche and trans conformations. The differences between the spectra for DPTL in the SAM and in the solution indicate that the distribution of the O=C–C=O unit conformations in these two systems is different. In addition, bands with larger components of the transition dipole in the direction normal to the surface appear stronger in the spectrum of the SAM due to the surface selection rule.

The spectrum of the SAM of DPTL is significantly different than the spectrum of a linear thiol having the structure HS(CH₂CH₂O)₅R with R being the n-alkyl group self-assembled on gold, when the number of the repeat units in the oligo-EG chain is larger than six.⁵⁶,⁶⁰ In these films, the oligo-EG segment adopts the helical 7/2 conformation and the spectra are characteristic of the ordered crystalline structure. However, the conformation of the TEG spacer in DPTL is in reasonable agreement with the conformation of the oligo-ethylene glycol chain in SAMs of oligo-EG terminated thiols with short chains consisting of three to four repeat units. The IR spectra of these SAMs are characteristic of a disordered state of the EG chains.⁵⁶–⁵⁹ The conformation of oligo-EG chains in SAMs is also influenced by their packing density. When thiols with terminal oligo-EG chains are self-assembled at a silver surface, which allows for a more close packing of thiol molecules, the oligo-EG chains adopt the all-trans conformation.⁵⁶ The TEG spacer is coiled, although in the spacer layer the chains are packed irregularly to form a disordered state. Recently, Skoda et al.⁶⁰ demonstrated that the C–O–C stretching band in EG terminated thiols is red-shifted upon hydration. In the case of DPTL, it is more convenient to extract information concerning hydration of the spacer layer from the analysis of the C=O stretching bands described below.

The Lipoic Acid Headgroup. The C=O stretching of the lipoic acid ester group can provide useful information concerning hydration of the headgroup.⁶¹–⁶⁵ The top line in Figure 9 plots the C=O stretching band calculated for a monolayer of randomly oriented DMPC molecules from optical constants determined for a solution of DPTL in CD₃OD. The bottom trace in Figure 9 plots the band calculated from optical constants determined for a solution of DPTL in CCl₄. These two traces show a dramatic effect of the solvent on the C=O stretching band. In the aprotic solvent, CCl₄, a single narrow and symmetric band centered at 1742 cm⁻¹ is observed. In contrast, in the CD₃OD solution, this mode splits into three overlapping bands at 1740, 1720, and 1700 cm⁻¹. The shift of the C=O stretching band to lower frequencies is caused by hydrogen bond formation between the C=O group and the solvent.⁶¹–⁶⁵ (To avoid the overlap of the C=O stretching band with a very strong scissoring band of water

**Figure 8.** Deconvolution of the IR spectra of DPTL in the 1425–900 cm⁻¹ spectral region: (A) monolayer of randomly oriented molecules calculated using the optical constants; (B) average PM-IRRAS spectrum for the SAM averaged for all potentials investigated. The deconvoluted bands plotted in color are discussed in the text.

of nonhydrated C–O groups is ~50%. The fraction decreases as the potential is decreased, attaining a value of ~30% at $E = -600$ mV, which indicates increasing hydration of the spacer layer at these negative potentials. This change in hydration with potential correlates well with a slow increase of the differential capacitance of the interface shown earlier in Figure 2. At potentials that are more negative than ~600 mV, the monolayer is desorbed from the gold surface and the spacer region is apparently somewhat less hydrated in the desorbed state than at $E = -600$ mV.

The direction of the transition dipole of the C=O stretching band coincides with the direction of the C=O bond. The integrated intensity of the band in a randomly oriented monolayer calculated from optical constants determined for a solution of DPTL in CD$_3$OD (top trace, solid line) and in CCl$_4$ (bottom trace, dotted line).

The five traces in the middle of Figure 9, collected at different values of the electrode potential, correspond to PM-IRRAS spectra of the SAM of DPTL molecules. The spectra display a maximum at ~1737 cm$^{-1}$ and a broad shoulder at lower frequencies, which is analogous to the spectrum of DPTL in CD$_3$OD calculated from optical constants determined for a solution of DPTL in CD$_3$OD (top trace, solid line) and in CCl$_4$ (bottom trace, dotted line).

The tilt angle corresponding to a random orientation is equal to 54°. A decrease of the tilt angle therefore may indicate a more random orientation of the ester group at the negative potentials.

### 4. Summary and Conclusions

We have applied PM-IRRAS to obtain molecular level information concerning the orientation, conformation, and hydration of the self-assembled monolayer of DPTL at a Au(111) gold electrode surface. The PM-IRRAS technique allowed us to collect the IR spectra for the SAM at the electrode in solution under potential control. In this way, the effect of the electrode potential on the structure of the SAM could be assessed. The PM-IRRAS spectra reported in this work are more complete than the RAIRS spectrum of the DPTL SAM previously measured in air.$^{26a}$ Using both DPTL and DPT$_{14}$L, a molecule with deuterium substituted hydrogen atoms in the tetraethylene spacer, we were able to separately analyze the properties of the phytanyl chains, the tetraethylene spacer, and the lipoyc acid headgroup of this complex molecule.

Our results show that the SAM of DPTL displays a remarkable stability in a broad range of potentials applied to the gold surface.
The orientation of the phytanyl chains does not change even upon desorption of the monolayer from the electrode surface. In the desorbed state, the monolayer most likely remains in close proximity of the electrode, retaining the laminar structure. The O—C—C—O unit of the spacer exists predominantly in the gauche conformation. The TEG is coiled but this section of the monolayer is disordered. The large value of the angle between transition dipoles of the CD$_2$ bands in the TEG unit indicates that the coils are extended and that their axis forms a small angle with respect to surface normal. This conformation and the structure of the spacer region are weakly dependent on the electrode potential.

The C—O stretching bands of the lipoic acid ester group provided significant information concerning the hydration of the headgroup region. The IR data demonstrated that a significant fraction of the ester group is not hydrated, which indicates that the amount of water molecules in the spacer region is small. DPTL monolayers have been initially employed to build biomimetic systems assuming that the polyethylene glycol spacer behaves as a water-rich hydrogel. Our results indicate that pure DPTL monolayers do not provide a water-rich spacer separating a biomimetic membrane from the metal surface. This conclusion is supported by independent neutron reflectivity measurements by Vockenroth et al.

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**Supporting Information Available:** Isotropic optical constants for benzyl lipoate (BLA) (Figure SI 1); isotropic optical constants for tetraethylene glycol (TEG) (Figure SI 2); isotropic optical constants for DPTL (Figure SI 3); isotropic optical constants for DPTL$_{d16}$L (Figure SI 4); peak centers and fwhm values of the deconvoluted $v_6$(CH3-t), $v_6$(CH3-m), and $v_4$(CH2) bands plotted as a function of the electrode potential (Figure SI 5); PM-Irras spectra for the C—D stretching region of the DPTL$_{d16}$L molecules self-assembled at the Au(111) electrode surface at selected electrode potentials (Figure SI 6); PM-Irras spectra for the SAM of DPTL at the Au(111) surface in the 1400—900 cm$^{-1}$ spectral region, recorded at selected potentials (Figure SI 7); description of the ab initio computational method (Figure SI 8); animations of molecular vibrations. This material is available free of charge via the Internet at http://pubs.acs.org.