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Indole Adsorption to a Lipid Monolayer Studied by Optical Second Harmonic Generation

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Adsorption of indole from an aqueous subphase to a lipid (DPPC) monolayer at the air/water interface is studied by nonresonant second harmonic generation (SHG) with $\lambda = 800$ nm and by observation of compression isotherms with a Langmuir trough. The nonlinear susceptibilities of the monolayers have been carefully measured, including corrections for the contributions of the lipid monolayer and subphase, calibration of absolute values by comparison with a quartz reference, and measurement of absolute phase by comparison with a clean air/water interface. Details of the calibrations involving z -cut quartz and the clean air/water interface are presented. The number density of adsorbed indole molecules has been estimated by comparison of nonlinear susceptibilities with a reference monolayer of hexadecyl 3-indoleacetate. The extent of adsorption of indole to the lipid monolayer is maximum at low surface pressure, decreasing from an indole/DPPC ratio near 2:1 as the monolayer is compressed. The free energy change for adsorption of indole to the monolayer is estimated as -8.1 kcal/mol, which is similar to a result reported previously for a lipid bilayer. Information on the orientation of adsorbed indole is derived by comparison of observed ratios of nonlinear susceptibilities with calculated ratios for model indole monolayers with assumed orientational distributions. Measurement and analysis procedures are described in detail for effective nonlinear susceptibilities in a s/p polarization basis. The calculations use as input the molecular hyperpolarizability tensor of indole, which is obtained by using several computational approaches including sum-over-states calculations and time-dependent Hartree–Fock and density functional methods. The analysis shows that the pyrrole ring of indole points toward the water subphase, and the tilt angle of the long axis of indole increases from near 50° to near 60° as the monolayer is compressed. The analysis also suggests that the plane of indole lies more nearly parallel than perpendicular to the plane of the interface.

I. Introduction

Indole is an important chromophore in biophysical studies of the amino acid tryptophan, which incorporates indole (as indolyl) within its side chain. The indole molecule is a planar aromatic system with fused benzene and pyrrole rings (Figure 1) and with a dipole moment near 2 D.¹ It has a rather low solubility in water and a favorable partition coefficient for transfer to cyclohexane.² A significant affinity of indole for water is nevertheless expected. This is due to the compact molecular structure of indole with associated dipole and quadrupole moments^{3–5} and the hydrogen bond donor property of the free imine $-\text{NH}-$ group.^{6,7} Indole may thus be considered to have an amphipathic nature. This is significant for biophysical studies that focus on the local environment of indole within biological structures. Examples of such studies include indolyl fluorescence as a probe of protein structure^{8,9} and studies of the specific role of the indolyl group in stabilizing tryptophan-rich peptides in lipid membranes.^{2–5,10–13}

Several reports have described the adsorption of indole and simple methylated derivatives of indole from bulk water to the interfacial region of lipid bilayers, in the vicinity of the polar head groups of the lipid molecules.^{2,10–13} It has been shown, by NMR spectroscopy, that indole is preferentially adsorbed in the region of the lipid head groups, as opposed to the hydrocarbon core of the lipid bilayer.¹⁰ Further NMR studies have addressed the orientation of indole in the adsorbed state near the surface of the bilayer.¹² The orientation of indole in model lipid membranes has also been studied by optical

Figure 1. Ball-and-stick rendering of the molecular structure of indole, $\text{C}_8\text{H}_7\text{N}$. The coordinate axes show the orientation used for hyperpolarizability calculations. In the standard orientation the pseudosymmetry axis is parallel to the z -axis.

spectroscopy, through observations of linear dichroism in deformed and aligned lipid vesicles.¹³ These optical and NMR studies have given contrasting results for the orientation of indole in the model membranes, differing on the alignment of the plane of indole with the plane of the bilayer. Furthermore, neither study was sensitive to the absolute orientation of indole, which refers to the benzene-to-pyrrole direction within indole compared with the hydrocarbon-to-water direction along the normal to the bilayer plane. The main features of the orientational distribution of indole adsorbed to a lipid bilayer are thus not resolved at present. This is a significant issue because of its relevance for understanding the stabilization of indole in the polar region of lipid membranes. It has been suggested that this

stabilization provides an important contribution to the overall stability of membrane-bound proteins that are rich in tryptophan residues.¹⁰

This work describes the application of second harmonic generation (SHG) for studies of adsorption of indole from bulk water to a 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) lipid monolayer at the air/water interface. As a second-order nonlinear optical technique, SHG is well suited for the study of monolayer films on a subphase in which the SHG chromophore is present. This is because SHG is specifically sensitive to surfaces and interfaces, where chromophores generally have a net polar alignment, and insensitive to chromophores in isotropic bulk phases.^{14–16} SHG is particularly useful for measurements of molecular orientation in monolayers, being intrinsically sensitive to polar alignment of chromophores.¹⁷ In the present study, measurements of the nonlinear susceptibility of indole adsorbed to a DPPC monolayer are combined with computations of the molecular hyperpolarizability tensor of indole to derive information on the orientation of adsorbed indole, including the absolute orientation. By comparison with measured susceptibilities for a reference indole film of known surface density, information on the extent of adsorption of indole to the DPPC monolayer has been obtained. This allows an estimate of the free energy change for indole adsorption, which is compared with previous measurements for adsorption of indole to lipid bilayers.²

The problem of characterizing the orientational distribution of indole adsorbed to a lipid monolayer or bilayer is challenging because the actual distribution may be complicated, requiring many parameters for its adequate description.¹⁷ In this work the technique of nonresonant SHG has been used, and an assessment has been made of its limitations. These limitations arise in part from the relative weakness of indole as an SHG chromophore, which means that background signals from the water subphase and lipid monolayer must be taken into consideration. A more fundamental difficulty arises from the unknown nature of the orientational distribution, and particularly the lack of an axis of symmetry of indole about which the rotational distribution may be assumed uniform. One approach to this problem is to rely on computational methods to provide reliable information on the molecular hyperpolarizability tensor. If certain properties of the molecular hyperpolarizability are known, then specific features of the orientational distribution may be deduced from measurements of the nonlinear susceptibilities.¹⁷ This approach has been used in the present study, with the monolayer modeled as a dilute gas of partially oriented chromophores. Considerable caution is needed in this approach because of uncertainties in the performance of the computational method, and also because interactions of indole with water and lipid are neglected in the computation of the hyperpolarizability tensor of indole. The present study is a continuation of previously published work from this laboratory on indole as chromophore for resonant and nonresonant SHG in monolayer films at the air/water interface.^{18,19}

II. Experimental Section

The experimental setup for SHG measurements on an air/water interface in a Langmuir trough is shown in Figure 2. Laser pulses with central wavelength $\lambda = 800$ nm, pulse duration ~ 80 fs, and repetition rate 1 kHz are from a regeneratively amplified (Positive Light, Spitfire) Ti:sapphire oscillator (Spectra-Physics, Tsunami). A laser beam with pulse energy $360 \mu\text{J}$ is split off from the output and reduced in size by using a 2:1 beam condensing telescope. A Berek's polarization compensator (BC)

Figure 2. Experimental setup for measurement of SHG at an air/water interface (adapted from ref 18). The polarization state of incident fundamental radiation is varied continuously by rotation of a quarter-wave retardation plate. Reflected second harmonic radiation is resolved in *s*, *p*, and *q* polarization components. See the text for details.

and Glan-laser prism (GP) are used to set the pulse energy ($\sim 200 \mu\text{J}$) and initial polarization state (*p* polarized) with respect to the air/water interface. A fraction of the beam is reflected by an uncoated BK7 beamsplitter (BS) and used to generate a reference SHG signal in a quartz waveplate. The reference signal is used to continuously monitor the stability of the laser pulses during data acquisition. The transmitted beam is directed toward the air/water interface by using a silver-coated mirror (M1). The polarization state of the beam incident on the interface is varied by rotation of a zero-order, quarter-wave retardation plate (QWP). A colored glass filter (F1) is mounted following QWP to ensure that no second harmonic radiation with $\lambda = 400$ nm is incident on the interface. The laser beam is collimated and forms a $1/e^2$ waist with radius 1.7 mm at the air/water interface. The angle of incidence is 60° . A second Glan-laser prism (GP2) selects the polarization state of second harmonic radiation reflected from the interface. A colored glass filter (F2) is inserted following GP2 to attenuate reflected fundamental radiation. This was necessary to avoid generation of a small SHG signal from the aluminum-coated mirror (M2) that redirects the reflected second harmonic beam toward the detector. A Pellin-Broca prism (PB) is used to separate fundamental and second harmonic beams, and colored glass filters and an interference filter (F3) are used to eliminate residual fundamental light. The second harmonic signal is detected on a 1P28 photomultiplier tube and amplified with a gain of 25 by a preamplifier coupled through 470 pF to a boxcar integrator (Stanford Research Systems). All SHG measurements were calibrated by using a signal that was recorded when a thick *z*-cut quartz wedge was substituted in the sample position.²⁰

The dichroic response of the detection optics and PMT for *s* and *p* polarized second harmonic radiation was calibrated by using a half-wave retardation plate. The detection efficiency was 1.44 for *s* polarized compared to *p* polarized light.

The air/water interface was formed in a Langmuir trough (Nima, Model 102M) fabricated from Teflon, and using Milli-Q water (resistivity $18 \text{ M}\Omega \text{ cm}$, total organic carbon <10 ppb). The trough was mounted on a translation stage that permitted small adjustments to the height of the air/water interface. Vibrations of the interface appeared to be minimal, as judged by observations of a reflected HeNe laser beam. The surface pressure was measured by using a calibrated sensor with a Wilhelmy plate made from chromatography paper. The absolute surface tension of the clean air/water interface was near 73 mN/m . Langmuir films of DPPC and hexadecyl 3-indoleacetate (HIA) were prepared by dropping $5 \mu\text{L}$ of $\sim 1 \text{ mg/mL}$ solutions in chloroform on the air/water interface. Indole solutions were prepared in Milli-Q water and stirred overnight. The pH of the unbuffered solutions was not measured but is estimated to be near 5.8 due to dissolution of CO_2 . The ionic strength of the solutions was low due to the absence of added electrolyte. All measurements were at room temperature, 22°C .

Indole (99+%) from Aldrich and DPPC from Avanti Polar Lipids were used as received. Hexadecyl 3-indoleacetate [$\text{CH}_3(\text{CH}_2)_{15}-\text{O}-(\text{CO})-\text{CH}_2-(3\text{-indolyl})$] was prepared from 1-hexadecanol (99%) and 3-indoleacetic acid (>98.0%), both from Aldrich, as follows: in a dry flask, 3-indoleacetic acid (0.500 g, 2.85 mmol) and 1-hexadecanol (1.038 g, 4.28 mmol) were dissolved in 50 mL of dry dichloromethane. 1,3-Diisopropylcarbodiimide (0.670 mL, 4.28 mmol) and 4-(dimethylamino)pyridine (0.035 g, 0.286 mmol) were added to the solution, and the reaction was allowed to stir overnight. The reaction mixture was then filtered and concentrated to an oil that was purified by flash chromatography (2% methanol in dichloromethane) to yield hexadecyl 3-indoleacetate (0.966 g, 85% yield) as a white solid.

III. Results and Discussion

A. Measurement of Effective Nonlinear Susceptibilities.

Our approach for measuring the effective nonlinear susceptibilities of an interface in an s/p polarization basis follows the work of Maki et al.^{21,22} and Geiger et al.²³ The technique involves continuous variation of the polarization state of input fundamental radiation by rotation of a quarter-wave retardation plate, with selection of s and p polarization states of reflected second harmonic radiation. Here and in Appendix 1 we provide essential equations that specify the analysis and notation used in this work. The efficiency of SHG is defined in eq 1 in terms of the irradiance I of the incident fundamental beam with frequency ω and the reflected second harmonic beam in polarization state j (where j is s or p).

$$R_j^{2\omega} \equiv \frac{I_j^{2\omega}}{[I_o^\omega]^2} = |f_j F + g_j G + h_j H|^2 \quad (1)$$

Here f_j , g_j , and h_j are the effective nonlinear susceptibilities, and the quantities F , G , and H are binary products of the fundamental field amplitudes incident on the monolayer in s and p polarization states. The field amplitudes are normalized to the total fundamental field E_o as shown in eq 2.

$$\begin{aligned} F &= E_p^2/E_o^2 \\ G &= E_s^2/E_o^2 \\ H &= E_s E_p/E_o^2 \end{aligned} \quad (2)$$

The s and p field amplitudes transmitted by the quarter-wave plate are given in eqs 3 and 4, where θ_{WP} is the angle between the fast axis of the wave-plate and the polarization

$$E_p/E_o = (1 - i \cos 2\theta_{\text{WP}})/\sqrt{2} \quad (3)$$

$$E_s/E_o = -i \sin 2\theta_{\text{WP}}/\sqrt{2} \quad (4)$$

direction of the p polarized fundamental field incident on the wave-plate. The efficiency of SHG in eq 1 can now be expressed in terms of θ_{WP} as shown in eqs 5 and 6.

$$R_j^{2\omega}(\theta_{\text{WP}}) = \text{QWP}_j(\theta_{\text{WP}}) \quad (5)$$

$$\begin{aligned} \text{QWP}_j(\theta_{\text{WP}}) &= \frac{1}{16} [g_{j1} - f_{j1} - 4f_{j2} \cos 2\theta_{\text{WP}} + \\ & (f_{j1} - g_{j1}) \cos 4\theta_{\text{WP}} + h_{j1} \sin 4\theta_{\text{WP}} - 2h_{j2} \sin 2\theta_{\text{WP}}]^2 + \\ & \frac{1}{16} [g_{j2} - f_{j2} + 4f_{j1} \cos 2\theta_{\text{WP}} + (f_{j2} - g_{j2}) \cos 4\theta_{\text{WP}} + \\ & 2h_{j1} \sin 2\theta_{\text{WP}} + h_{j2} \sin 4\theta_{\text{WP}}]^2 \end{aligned} \quad (6)$$

Here the real and imaginary components of the susceptibilities are given explicitly by using the notation $f_j = f_{j1} + if_{j2}$, with similar expressions for g_j and h_j .

In the case of a surface with macroscopic $C_{\infty v}$ symmetry, that is, an achiral surface with in-plane isotropy, and considering only electric dipole interactions, the unique nonzero susceptibilities are f_p , g_p , and h_s , with $f_s = h_p = g_s = 0$.²¹ In this case the expressions for $\text{QWP}_j(\theta_{\text{WP}})$ with $j = s$ and p are simplified as shown in eqs 7 and 8.

$$\text{QWP}_s(\theta_{\text{WP}}) = \frac{1}{16} h_{s1}^2 [\sin^2 4\theta_{\text{WP}} + 4 \sin^2 2\theta_{\text{WP}}] \quad (7)$$

$$\begin{aligned} \text{QWP}_p(\theta_{\text{WP}}) &= \frac{1}{16} [g_{p1} - f_{p1} - 4f_{p2} \cos 2\theta_{\text{WP}} + \\ & (f_{p1} - g_{p1}) \cos 4\theta_{\text{WP}}]^2 + \frac{1}{16} [g_{p2} - f_{p2} + 4f_{p1} \cos 2\theta_{\text{WP}} + \\ & (f_{p2} - g_{p2}) \cos 4\theta_{\text{WP}}]^2 \end{aligned} \quad (8)$$

An alternative expression for $\text{QWP}_p(\theta_{\text{WP}})$ is given in eq 9, where $|f_p|^2 = (f_{p1}^2 + f_{p2}^2)$ and $|f_p - g_p|^2 = (f_{p1} - g_{p1})^2 + (f_{p2} - g_{p2})^2$.

$$\begin{aligned} \text{QWP}_p(\theta_{\text{WP}}) &= \frac{1}{16} |f_p - g_p|^2 (1 - \cos 4\theta_{\text{WP}})^2 + \\ & \frac{1}{2} (f_{p1} g_{p2} - f_{p2} g_{p1}) \cos 2\theta_{\text{WP}} (1 - \cos 4\theta_{\text{WP}}) + \\ & |f_p|^2 \cos^2 2\theta_{\text{WP}} \end{aligned} \quad (9)$$

Equations 7–9 are used to fit experimental quarter-wave plate rotation traces by nonlinear regression. It can be seen from eq 7 that the quantity h_{s1} is uniquely determined by $\text{QWP}_s(\theta_{\text{WP}})$. Equation 9 is useful because it shows explicitly the quantities that are uniquely determined by $\text{QWP}_p(\theta_{\text{WP}})$ as the coefficients on the right-hand side. Note that g_p is not uniquely determined; rather, two values of g_p are in general consistent with eq 9. For a unique solution it is necessary to consider also the case where the second harmonic beam is selected in at least one plane-polarized state at $\pm 45^\circ$ to the plane of incidence. Such plane-polarized states are named $q_{\pm}$ states, and the corresponding nonlinear susceptibilities are $f_{q_{\pm}} = (f_s \pm f_p)/\sqrt{2}$, and similarly for $g_{q_{\pm}}$ and $h_{q_{\pm}}$. The detailed expressions for $\text{QWP}_{q_{\pm}}(\theta_{\text{WP}})$ in terms of f_p , g_p , and h_s follow directly from eq 6. The relative phases of the susceptibilities have been made explicit in eqs 7–9 by setting $h_{s2} = 0$, that is, h_s is real. There are thus five unique nonzero susceptibilities in general for a $C_{\infty v}$ surface (f_{p1} , f_{p2} , g_{p1} , g_{p2} , and h_{s1}), and only three (f_{p1} , g_{p1} , and h_{s1}) if all susceptibilities are real.

The effective nonlinear susceptibilities in the s/p basis are expressed in terms of corresponding susceptibilities in Cartesian coordinates as shown in Appendix 1.

1. z-Cut Quartz Crystal. Second harmonic reflection from the surface of a z -cut quartz crystal provides a useful reference standard for calibration of measured nonlinear susceptibilities

Figure 3. Quarter-wave plate rotation traces for the indicated polarization states of second harmonic radiation reflected from the surface of a *z*-cut quartz crystal, with $\phi = 0$. The second harmonic signal is in arbitrary units. The fit to the data is shown by the solid lines. See the text for details.

on an absolute scale. Such measurements are useful also for validating the experimental method for measurements of relative susceptibilities, as shown in the following. Detailed expressions for the effective nonlinear susceptibilities of eq 1 for the case of a *z*-cut quartz crystal with specified azimuthal orientation are given in Appendix 2. For the particular azimuthal angle $\phi = 0$, the susceptibilities $h_p^Q = f_s^Q = g_s^Q = 0$, and the ratios g_p^Q/f_p^Q and h_s^Q/f_p^Q are as given in eqs 10 and 11.

$$\frac{g_p^Q}{f_p^Q} = \frac{-t_{s12}^2}{t_{p12}^2 \cos^2 \theta_2} = -1.189 \quad (10)$$

$$\frac{h_s^Q}{f_p^Q} = \frac{-2A_s^Q t_{s12}}{\cos \theta_2 A_p^Q t_{p12}} = 2.353 \quad (11)$$

These ratios are fixed by the angle of incidence and the linear optical constants of the interface (see Appendix 2 for details). If the experimentally determined ratios do not agree with eqs 10 and 11, then systematic errors must be suspected in the measurements.

Figure 3 shows quarter-wave plate rotation traces for *s*, *p*, *q*₊, and *q*_− polarization states of second harmonic radiation reflected from the surface of a *z*-cut quartz crystal with $\phi = 0$, as in eqs 10 and 11, and with the fundamental wavelength $\lambda = 800$ nm. All traces were fit simultaneously by minimizing the sum of the squares of the deviations of the data from the appropriate form of eq 6 for each trace, with the effective susceptibilities f_p , g_p , and h_s taken as the only free parameters. All susceptibilities were assumed real, and thus $f_{p2} = g_{p2} = h_{s2} = 0$. The symmetric appearance of the traces indicates that $f_s = h_p = 0$ holds, which is consistent with $\phi = 0$. The fit to the data is excellent and yields $g_p^Q/f_p^Q = -1.18$ and $h_s^Q/f_p^Q = 2.32$, in satisfactory agreement with eqs 10 and 11. This confirms

TABLE 1: Absolute Values of Effective Nonlinear Susceptibilities for Second Harmonic Reflection from *z*-Cut Quartz Crystal and Clean Air/Water Interface, with $\lambda = 800$ nm^a

interface	f_p	g_p	h_s	χ_{zzz}	χ_{zxx}	χ_{xxz}
air/quartz	141	−168	332			
air/water	−4.37	−0.61	−8.75	−1.62	−0.082	−0.59

^a Measured susceptibilities for an air/water interface are given relative to calculated values for *z*-cut quartz. Susceptibilities f_p , g_p , and h_s in units 10^{-16} esu [esu = (cm² s/erg)^{1/2}]. Susceptibilities, χ , in units 10^{-16} esu [esu = (cm³/erg)^{1/2} cm; 1 esu = 4.189×10^{-6} m²/V]. Susceptibilities, χ , calculated from f_p , g_p , h_s (Appendix 1) with $n_3 = 1.163$ and $N_3 = 1.175$.

the orientation $\phi = 0$ and provides a validation of the experimental method.

The detailed expressions in Appendix 2 give the absolute values of the effective nonlinear susceptibilities for second harmonic reflection from the surface of a *z*-cut quartz crystal, with the bulk nonlinear coefficient of quartz taken as $d_Q = 0.30$ pm/V.²⁴ The absolute values are given in Table 1, for the azimuthal angle $\phi = 0$ and the angle of incidence $\theta_1 = 60^\circ$. Note that only the relative signs of the susceptibilities are determined, and not the absolute signs.

2. Clean Air/Water Interface. Figure 4 shows quarter-wave plate rotation traces for *s*, *p*, *q*₊, and *q*_− polarization states of second harmonic radiation reflected from a clean air/water interface, with the fundamental wavelength $\lambda = 800$ nm. These data were fit in the same manner as described for *z*-cut quartz (Figure 3), with the effective nonlinear susceptibilities f_p , g_p , and h_s taken as the only free parameters and with all susceptibilities assumed real. The air/water and air/quartz interfaces were studied with the same sample position and orientation relative to the plane of incidence. In this way, the absolute values of the nonlinear susceptibilities of the clean air/water interface were measured relative to those of *z*-cut quartz. The absolute phases of the susceptibilities were fixed by taking $h_s = h_{s1}$ and

Figure 4. Quarter-wave plate rotation traces for the indicated polarization states of second harmonic radiation reflected from a clean air/water interface. The fit to the data is shown by the solid lines and the resulting effective nonlinear susceptibilities are given in Table 1.

$h_{s1} < 0.20$. The measured susceptibilities are given in Table 1, together with those for z-cut quartz.

By using the relationships in Appendix 1, the nonlinear susceptibilities for the clean air/water interface were converted to susceptibilities in Cartesian coordinates, as shown in Table 1. The linear optical constants of the interface for $\lambda = 800$ nm were taken as $n_1 = N_1 = 1.0$, $n_2 = 1.326$, $N_2 = 1.350$, $n_3 = (n_1 + n_2)/2$, and $N_3 = (N_1 + N_2)/2$. (Subscripts 1, 2, 3 refer to air, bulk water substrate, and interfacial water layer, respectively, and n (N) refers to the effective index of refraction at the fundamental (second harmonic) frequency; see Appendix 1.) From the results in Table 1 we find $\chi_{ZZX}/\chi_{XXZ} = 0.14$ (0.14) and $\chi_{ZZZ}/\chi_{XXZ} = 2.74$ (4.70). The values given in parentheses were obtained when n_3 and N_3 were taken as $n_3 = n_2$, and $N_3 = N_2$. Several previous reports have described measurements of the ratio χ_{ZZX}/χ_{XXZ} for the clean air/water interface consistently larger than the value reported here.^{25–27} In the same reports the reported ratio χ_{ZZZ}/χ_{XXZ} is close to our result. The origin of this discrepancy is not known. The absolute value of χ_{XXZ} in Table 1 (0.59×10^{-16} esu) is larger than the absolute values previously reported by Rasing et al.²⁸ (0.20×10^{-16} esu) and Antoine et al.²⁹ (0.06×10^{-16} esu), both with $\lambda = 532$ nm. However, both of these earlier studies made use of a calibration method involving SHG in a thin quartz plate measured in a transmission geometry, whereas a reflection geometry was used for the air/water interface. A difficulty with this approach is that the incident laser beam may not have the same irradiance in the sample and reference positions. The method used in the present study is preferred because the air/quartz and air/water interfaces are both measured in a precisely defined reflection geometry.

3. Monolayer of HIA at Air/Water Interface. Monolayer films of HIA (hexadecyl 3-indoleacetate) were studied to provide reference data for a monolayer with a known surface density of indole chromophores at the air/water interface. A typical compression isotherm for a HIA monolayer is shown in Figure 5. The isotherm shows a number of interesting features that are suggestive of monolayer phase transitions at molecular areas near 40 and 30 $\text{\AA}^2$ and film collapse for a molecular area near 20 $\text{\AA}^2$. An indication of the stability of the monolayer is given by the relatively high

Figure 5. Isotherm for Langmuir film of HIA, showing the variation of the surface pressure with the area per HIA molecule in the monolayer.

surface pressure ($\pi \approx 45 \text{ mN m}^{-1}$) attained before film collapse. SHG measurements on the monolayer showed that pronounced changes occurred in the effective nonlinear susceptibilities as the film was compressed. In this report we focus on the low surface pressure region ($\pi \approx 5 \text{ mN m}^{-1}$) of the isotherm with the molecular area $\sim 60 \text{ \AA}^2$. In this region the state of the film may be described as liquid-expanded, with a relatively low packing density of HIA molecules. The HIA monolayer described here is similar to the monolayer described by Abel et al.,³⁰ with an indolyl headgroup that confers stability to the monolayer.

Figure 6 shows quarter-wave plate rotation traces as in Figures 3 and 4 for a HIA monolayer at the air/water interface with the molecular area 60 $\text{\AA}^2$. Again, all four traces were fit simultaneously with the effective nonlinear susceptibilities f_p , g_p , and h_s taken as the only free parameters and with all susceptibilities assumed real. The fits to the data are shown in Figure 6, and the resulting effective nonlinear susceptibilities are given in Table 2. Also shown in Table 2 are the effective nonlinear susceptibilities of a clean air/water interface, in absolute units,

Figure 6. Quarter-wave plate rotation traces for the indicated polarization states of second harmonic radiation reflected from a monolayer of HIA at the air/water interface, with the area per HIA molecule $60 \text{ \AA}^2$. The fit to the data is shown by the solid lines and the resulting effective nonlinear susceptibilities are given in Table 2.

TABLE 2: Absolute Values of Effective Nonlinear Susceptibilities for Second Harmonic Reflection from a Monolayer of HIA at the Air/Water Interface, with $\lambda = 800 \text{ nm}$ ^a

interface	f_p	g_p	h_s
HIA monolayer ^b	-7.08	-5.11	-19.64
clean air/water	-4.37	-0.61	-8.75
HIA monolayer corrected ^c	-2.71	-4.50	-10.89

^a Susceptibilities in units 10^{-16} esu [$\text{esu} = (\text{cm}^2 \text{ s/erg})^{1/2}$]. ^b Area per HIA molecule, $A = 60 \text{ \AA}^2$, surface pressure $\pi = 6.5 \text{ mN/m}$. ^c Corrected for contribution of subphase.

from Table 1. By carefully comparing measurements for clean and monolayer covered air/water interfaces, all of the data were placed on the same absolute scale. It is seen in Table 2 that the magnitudes of the susceptibilities are similar for both clean and monolayer-covered interfaces, and thus the SHG response of the monolayer itself is rather weak. We shall assume that the response of the monolayer alone is adequately described by simply subtracting the response of the clean air/water interface, thereby neglecting possible monolayer-induced changes to the response of the substrate. The effective nonlinear susceptibilities of the monolayer alone obtained in this way are shown in Table 2. In subtracting the contribution of the air/water interface it has been assumed that the relative phases of the susceptibilities are such that both of the measured h_{s1} are negative. This has been confirmed experimentally as shown in Section 5 below.

4. Indole Adsorbed to a DPPC Monolayer at Air/Water Interface. The adsorption of indole to a DPPC monolayer at the air/water interface was inferred from compression isotherms shown in Figure 7. Isotherms are shown for varying concentrations of indole present in the aqueous subphase, ranging from no added indole (pure water) to $8.0 \times 10^{-3} \text{ M}$ indole, near the solubility limit at room temperature. With increasing concentration of indole in the subphase the isotherm is increasingly expanded, due to penetration of indole in the monolayer. In the absence of the DPPC monolayer the isotherms were flat, as expected, for the full range of indole concentrations.

The SHG response of the DPPC monolayer was studied for a range of indole concentrations in the subphase and at various levels of monolayer compression as controlled by the surface area (A) available per lipid molecule in the monolayer. For a pure water subphase the SHG response showed distinct breaks that were

Figure 7. Isotherms for DPPC monolayers on a subphase with varying concentration of indole, ranging from pure water to 8.1 mM indole, as indicated. The surface pressure at a constant area per DPPC molecule increases monotonically with the concentration of indole in the subphase.

Figure 8. Quarter-wave plate rotation traces for the indicated polarization states of second harmonic radiation reflected from a DPPC monolayer with $A = 127 \text{ \AA}^2$ and with a $4.0 \times 10^{-3} \text{ M}$ concentration of indole in the subphase. The surface pressure was $\pi = 8.6 \text{ mN/m}$. The fit to the data is shown by the solid lines, and the resulting effective nonlinear susceptibilities are given in Table 3.

correlated with known phase transitions of the DPPC monolayer. Transitions from the liquid-expanded/gas coexistence region (LE/G) to the liquid-expanded state (LE) near $A = 110 \text{ \AA}^2$, and from LE to liquid-expanded/liquid-condensed coexistence region (LE/LC) near $A = 80 \text{ \AA}^2$, were accompanied by small increases in the effective nonlinear susceptibilities. In the LE/G region ($A > 110 \text{ \AA}^2$) the susceptibilities differed insignificantly from those of a clean air/water interface. A marked increase in the nonlinear susceptibilities of the DPPC monolayer was observed when indole was present in the subphase. No such increase was observed in the absence of the DPPC monolayer. This confirms the adsorption of indole from the subphase to the DPPC monolayer.

In Figure 8, quarter-wave plate rotation traces are shown for s , p , q_+ and q_- polarization states of second harmonic radiation reflected from a DPPC monolayer with $A = 127 \text{ \AA}^2$ and with a $4.0 \times 10^{-3} \text{ M}$ concentration of indole in the subphase. The effective nonlinear susceptibilities obtained by simultaneously fitting all of the rotation traces are given in Table 3, together with data for the clean air/water interface from Table 1. Subtraction of the susceptibilities for the clean air/water interface as described previously for the HIA monolayer yields the

TABLE 3: Absolute Values of Effective Nonlinear Susceptibilities for Second Harmonic Reflection from a Monolayer of DPPC at the Air/Water Interface, with a 4.0×10^{-3} M Concentration of Indole in the Subphase and $\lambda = 800$ nm^a

interface	f_p	g_p	h_s
indole/DPPC monolayer ^b	-7.56	-5.82	-21.3
clean air/water	-4.37	-0.61	-8.75
indole/DPPC monolayer corrected ^c	-3.19	-5.21	-12.5

^a Susceptibilities in units 10^{-16} esu [esu = (cm² s/erg)^{1/2}]. ^b Area per DPPC molecule $A = 127$ Å², surface pressure $\pi = 8.6$ mN/m. ^c Corrected for contribution of subphase.

TABLE 4: Absolute Values of Effective Nonlinear Susceptibilities for Second Harmonic Reflection from a Monolayer of DPPC at the Air/Water Interface, with a 8.1×10^{-3} M Concentration of Indole in the Subphase and $\lambda = 800$ nm^a

interface	f_p	g_p	h_s
indole/DPPC monolayer ^b	-4.96	-7.09	-25.7
DPPC monolayer ^c	-6.14	-1.63	-10.8
indole/DPPC monolayer corrected ^d	1.18	-5.46	-14.9

^a Susceptibilities in units 10^{-16} esu [esu = (cm² s/erg)^{1/2}]. ^b Area per DPPC molecule $A = 60$ Å², surface pressure $\pi = 40$ mN/m. ^c DPPC monolayer on pure water subphase. Area per DPPC molecule $A = 60$ Å². ^d Corrected for contributions of subphase and lipid monolayer.

susceptibilities of the monolayer corrected for the contribution of the subphase. Because the contribution of the DPPC molecules is negligible for $A > 120$ Å², the corrected susceptibilities are specifically associated with indole molecules adsorbed to the monolayer. As shown in Table 3, the corrected susceptibilities are rather small, being comparable in magnitude with the susceptibilities of the clean air/water interface. It can also be seen that the corrected susceptibilities are remarkably similar to those of the HIA monolayer in Table 2, being in a nearly constant ratio of 1.16 ± 0.01 . From the quantitative similarity it can be concluded that both the orientational distribution and the interfacial density of indole chromophores must be similar for the HIA and indole/DPPC monolayers. This conclusion is based on the assumption that local field effects in the monolayers are not too dissimilar, which appears reasonable.

The effect of compressing the monolayer was observed in rotation traces for which $A = 60$ Å², with a 8.1×10^{-3} M concentration of indole in the subphase. The rotation traces are shown in Figure S1 of Supporting Information, and the corresponding effective nonlinear susceptibilities are given here in Table 4. In this case a correction for the subphase or background contribution was made by subtracting the susceptibilities measured for the same monolayer conditions but without added indole in the subphase. The corrected susceptibilities are thus specifically associated with indole molecules adsorbed to the monolayer. The effects of monolayer compression on the nonlinear susceptibilities are significant and are analyzed in terms of the orientation of indole in Section C below. In the following we describe qualitative effects on the susceptibilities that reveal significant aspects of the adsorption process. We use the sum of the susceptibilities f_p and h_s , corrected for the contribution of the subphase, as a crude measure of the number density of indole chromophores at the interface. By using the sum ($f_p + h_s$), variations in the individual susceptibilities f_p and h_s are approximately canceled. This is because the variations that occur individually in f_p and h_s over small changes in the average orientation of indole are, in a crude approximation, equal and opposite. This is shown in Section C below (see Figure 13b). The

Figure 9. Dependence of the ratio of the number densities of indole and DPPC molecules in the monolayer on the surface area A available per DPPC molecule, for three concentrations of indole in the subphase. **Figure 10.** Data from Figure 9 replotted to show the dependence of the ratio $\sigma_{\text{indole}}/\sigma_{\text{DPPC}}$ on the concentration of indole in the subphase, for three states of compression of the monolayer.

sum ($f_p + h_s$) may be converted to areal number density (cm⁻²) of adsorbed indole molecules by using the results in Table 2 to calibrate ($f_p + h_s$) against the known density of indole in the HIA monolayer. The ratio $\sigma_{\text{indole}}/\sigma_{\text{DPPC}}$ of the areal number densities of indole and DPPC molecules in the monolayer is obtained by using the known areal number density of DPPC molecules.

Figure 9 shows the dependence of the ratio $\sigma_{\text{indole}}/\sigma_{\text{DPPC}}$ on the surface area A available per DPPC molecule in the monolayer, for three concentrations of indole in the subphase. The maximum ratio $\sigma_{\text{indole}}/\sigma_{\text{DPPC}} \sim 2.5$ is observed for the highest indole concentration in the subphase and the highest value of A (most expanded DPPC monolayer). There is a pronounced decrease in the ratio $\sigma_{\text{indole}}/\sigma_{\text{DPPC}}$ with decreasing A , which means that more compressed monolayers accommodate less adsorbed indole relative to DPPC. Indole is thus squeezed out of the DPPC monolayer as it is compressed and the surface pressure increases. The data in Figure 9 for $A = 63, 85$, and 127 Å² are replotted in Figure 10 to show the dependence of the ratio $\sigma_{\text{indole}}/\sigma_{\text{DPPC}}$ on the concentration of indole in the subphase. These results show a saturation behavior in the adsorption of indole to the DPPC monolayer, with the saturated ratio $\sigma_{\text{indole}}/\sigma_{\text{DPPC}}$ decreasing from a value near 2:1 as the monolayer is compressed. It is interesting to note in this connection that well-defined adducts of indole derivatives with diacetyl phosphatidylcholine have been characterized in chloroform solu-

tion.³¹ These adducts have two indolyl groups bound to one phosphatidylcholine group (present in the headgroup of DPPC), that is, a 2:1 adduct.

The standard free energy change for transfer of indole from aqueous phase to the adsorbed state in the DPPC monolayer can be written as shown in eq 12.³² Here K is an equilibrium constant that describes partitioning of indole between bulk water and the DPPC monolayer,

$$\Delta G = -RT\ln(K) + RTV_{\text{indole}}\left(\frac{1}{V_{\text{DPPC}}} - \frac{1}{V_{\text{W}}}\right) \quad (12)$$

$K = [\text{indole}]_{\text{ads}}/[\text{indole}]$, and V_{indole} , V_{DPPC} , and V_{W} are molar volumes of indole, DPPC, and water, respectively. (The standard state for ΔG follows from the use of concentration units for the equilibrium constant K .³²) The concentration of indole in the adsorbed state can be expressed as $[\text{indole}]_{\text{ads}} = \sigma_{\text{indole}}/l$, where σ_{indole} is the areal number density of indole in the monolayer and l is the thickness of the monolayer in which indole is confined in the adsorbed state. From data in Figure 10, $\sigma_{\text{indole}}/\sigma_{\text{DPPC}} = 1.35$ for $[\text{indole}] = 1.20 \times 10^{18} \text{ cm}^{-3}$ (2.0 mM) and $\sigma_{\text{DPPC}} = 7.9 \times 10^{13} \text{ cm}^{-2}$ ($A = 127 \text{ \AA}^2$). Assuming that l is in the range $0.75 \pm 0.25 \text{ nm}$, and taking V_{indole} , V_{DPPC} , and V_{W} as suggested by Wimley and White,² this gives $\Delta G = -8.1 \pm 0.2 \text{ kcal/mol}$ ($-34 \pm 1 \text{ kJ/mol}$). To support this analysis it can be noted that the selected data point is close to the calibration point for the relation between $(f_p + h_s)$ and σ_{indole} , so the uncertainty in σ_{indole} is believed to be low. Also, the selected point corresponds with the lowest concentration of indole in the subphase shown in Figure 10, which is below the concentration that leads to saturation of $\sigma_{\text{indole}}/\sigma_{\text{DPPC}}$. The result for the free energy change, $\Delta G = -8.1 \pm 0.2 \text{ kcal/mol}$ is close to the result of Wimley and White for transfer of indole from aqueous phase to the surface of a lipid bilayer, $\Delta G = -7.6 \text{ kcal/mol}$.² This suggests that the adsorption site of indole is similar for the lipid monolayer and bilayer.

5. Absolute Phase of Nonlinear Susceptibility. In previous work from this laboratory the absolute phase of the effective nonlinear susceptibility f_p for the clean air/water interface was measured by comparison with f_p for a monolayer of 4-*n*-octyl-4'-cyanobiphenyl (8CB) at the air/water interface, for $\lambda = 800 \text{ nm}$.²⁰ The absolute phase of f_p for the 8CB monolayer was unambiguously determined from an analysis of the nonlinear susceptibilities of the monolayer in terms of the molecular hyperpolarizability and orientation of 8CB at the interface. It was shown that f_p is real and negative for both the clean air/water interface and the 8CB monolayer. Furthermore, g_p and h_s are also real and negative for the clean air/water interface. This is in agreement with previous work on the air/water interface that indicated that all of the effective nonlinear susceptibilities are negative.²⁵ In the present work the clean air/water interface was used as a reference for measurements of the absolute phase of f_p with $\lambda = 800 \text{ nm}$. For these measurements an interferometric technique was used in which a thin *z*-cut quartz plate is translated in the direction of propagation of fundamental (ω) and second harmonic (2ω) beams reflected from the air/water interface. For *p* polarized ω and 2ω , the SHG signal is associated with f_p ; its variation with the distance traveled by the quartz plate has the form of an interference trace that carries information on the phase of f_p .^{33,34}

Figure 11a shows interference traces obtained for a clean air/water interface and for a HIA monolayer at the air/water interface, with the area per HIA molecule similar to that for the results in Figure 6 and Table 2. These traces show that the relative phase of f_p is the same for the HIA monolayer and the

Figure 11. Interference traces showing that the effective nonlinear susceptibility f_p has the same relative phase for a clean air/water interface and (a) a HIA monolayer with the area per HIA molecule $60 \text{ \AA}^2$, and (b) a DPPC monolayer on a subphase with $[\text{indole}] = 4 \text{ mM}$, and with the area per DPPC molecule $165 \text{ \AA}^2$.

Figure 12. Illustration of Euler angles ϕ , θ , and ψ that specify the transformation between laboratory (X,Y,Z) and molecular (x,y,z) coordinate systems (adapted from ref 19).

clean air/water interface. Similar measurements were done for a DPPC monolayer with adsorbed indole, under conditions close to those for the results in Figure 8 and Table 3. The interference traces for this case are shown in Figure 11b. Here again the results show no difference in the relative phase of f_p for the monolayer relative to the clean air/water interface. Note that the shift in the traces between panels a and b in Figure 11 reflects slight changes in the optical setup. Care was taken to maintain identical conditions for each measurement of the relative phase of f_p . The conclusion from these measurements is that f_p is real and negative for the HIA and DPPC/indole monolayers under the conditions investigated. Because f_p is negative also for the clean air/water interface, the correction of the effective nonlinear susceptibilities of the monolayers for the contribution of the subphase is appropriate as shown Tables 2 and 3, that is, the

TABLE 5: Unique Nonzero Elements of Molecular Hyperpolarizability Tensor of Indole for SHG with $\lambda = 800$ nm, Calculated by Using Three Computational Approaches^a

β_{ijk}	ZINDO/S ^b	TDHF	TDDFT/B3LYP
xxx	-74.0	-10.9	-13.4
xxz	31.8	-0.1	-4.1
xyy	2.0	-18.5	-20.6
xzz	-51.0	-8.2	-12.4
yxy	2.4	-19.9	-22.5
yyz	5.2	6.9	14.5
zxx	7.4	-6.8	-11.4
zxz	-12.8	0.2	5.2
zyy	4.8	6.8	13.9
zzz	-431.0	-55.2	-79.0

^a Hyperpolarizabilities are given in atomic units in convention B of Willetts et al.⁴⁵ (1 atomic unit = 8.639×10^{-33} esu). The applicable coordinate axes are shown in Figure 1. ^b Real values of hyperpolarizabilities calculated by using ZINDO/S method.

assumption that h_s is negative in all cases is justified. It is shown in Section C below that the negative sign of the corrected f_p for both monolayers shows that the absolute orientation of indole is such that the pyrrole ring points toward the water subphase.

B. Microscopic Description of Effective Nonlinear Susceptibilities. The effective nonlinear susceptibilities of an interface can often be described in terms of the molecular hyperpolarizability and orientational distribution of a particular chromophore. Equation 13 shows how the elements of the macroscopic nonlinear susceptibility tensor χ are related to the elements of the molecular hyperpolarizability tensor β , where the χ_{IJK} are expressed in Cartesian coordinates X, Y, Z fixed in the laboratory and the β_{ijk} are expressed in coordinates x, y, z attached to the chromophore.

$$\chi_{IJK} = N_S \sum_{i,j,k=x,y,z} \langle R_{Ii} R_{Jj} R_{Kk} \rangle \beta_{ijk} \quad (I, J, K = X, Y, Z) \quad (13)$$

In eq 13 N_S is the surface density of molecules, R_{Ii} is the direction cosine between laboratory axis I and molecular axis i , and the angular brackets denote averaging over the molecular orientational distribution.¹⁷ Detailed expressions for the χ_{IJK} may be obtained by writing the direction cosines R_{Ii} in terms of Euler angles ϕ , θ , and ψ that specify the transformation between laboratory and molecular coordinate systems,³⁵ as illustrated in Figure 12.

1. Hyperpolarizability Tensor of Indole. The elements β_{ijk} of the molecular hyperpolarizability tensor of indole were calculated by using three distinct computational approaches: (1) sum-over-states method based on time-dependent perturbation theory, with molecular parameters from semiempirical ZINDO/S calculations, as described previously;¹⁹ (2) time-dependent Hartree-Fock method (TDHF);³⁶ and (3) time-dependent density functional theory (TDDFT)³⁷ using the B3LYP hybrid three-parameter functional.³⁸ The calculations in the second and third approaches were performed with the ab initio program PC GAMESS,^{39,40} and using the augmented polarized double- ζ (aug-cc-pVDZ) basis set of Dunning.⁴¹ This basis set has been shown to be suitable for calculations of molecular hyperpolarizabilities.⁴² Preliminary calculations on the molecule *para*-nitroaniline using the TDHF and TDDFT approaches gave results for the hyperpolarizability tensor for second harmonic generation with the fundamental frequency $\hbar\omega = 1.17$ eV ($\lambda = 1060$ nm) in good agreement with calculations reported by Karna et al.⁴³ and Salek et al.⁴⁴ using similar methods and basis sets. The results of the present work for β_{ijk} of indole with the fundamental frequency $\hbar\omega = 1.55$ eV ($\lambda = 800$ nm) are

summarized in Table 5, for the three computational approaches. The structure of indole was the optimized planar structure found by using the respective computational method, except that a structure from an ab initio approach was used for the ZINDO/S calculations.¹⁹ The hyperpolarizabilities in Table 5 are given in the convention B described by Willetts et al.⁴⁵ The applicable coordinate axes are shown in Figure 1 as (x, z) .

The results in Table 5 show that the ZINDO/S method produced much larger values of the hyperpolarizabilities than TDHF and TDDFT, in particular for the dominant element β_{zzz} . The same trend was observed in calculations on *para*-nitroaniline. However, similar calculations on the molecule 4-methyl-4'-cyanobiphenyl (1CB) studied in earlier work from this laboratory²⁰ produced much smaller differences among the β_{zzz} calculated by these three approaches. Further calculations on indole using the more elaborate ab initio method MP2 and with the aug-cc-pVDZ basis set gave the result $\beta_{zzz} = -78$ atomic units for second harmonic generation in the static limit ($\hbar\omega = 0$). It has been shown that this level of theory gives reasonable results for *para*-nitroaniline.⁴⁶⁻⁴⁸ Guthmuller and Simon have reported $\beta_{zzz} = -52$ atomic units for indole in the static limit, from DFT calculations.⁴⁹ The MP2 result for indole from the present study suggests that the TDHF and TDDFT results for β_{zzz} with $\hbar\omega = 1.55$ eV in Table 5 may be too low, because dispersion should lead to an increase in the hyperpolarizability for $\hbar\omega > 0$.

2. Orientational Distribution. Consider a monolayer composed of indole chromophores and having macroscopic $C_{\infty v}$ symmetry. With the orientational distribution of indole described in terms of Euler angles illustrated in Figure 12, eq 13 gives the following expressions for the unique nonzero elements of the effective nonlinear susceptibility tensor.

$$\chi_{zzz} = N_S \left\{ \begin{aligned} &\langle \cos^3 \theta \rangle \beta_{zzz} + \langle \sin^2 \theta \cos \theta \cos^2 \psi \rangle (2\beta_{xxz} + \beta_{zzx}) \\ &+ \langle \sin^2 \theta \cos \theta \sin^2 \psi \rangle (2\beta_{yyz} + \beta_{zzx}) - \\ &\quad \langle \sin \theta \cos \psi \rangle (2\beta_{zxx} + \beta_{zzz}) \\ &- \langle \sin^3 \theta \cos \psi \rangle (2\beta_{yxy} + \beta_{xyy} - 2\beta_{zxx} - \beta_{xzz}) \\ &- \langle \sin^3 \theta \cos^3 \psi \rangle (\beta_{xxx} - \beta_{xyy} - 2\beta_{yxy}) \end{aligned} \right\} \quad (14)$$

$$\chi_{zxx} = \frac{N_S}{2} \left\{ \begin{aligned} &\langle \sin^2 \theta \cos \theta \rangle \beta_{zzz} + \langle \cos^3 \theta \rangle (\beta_{zxx} + \beta_{zyy}) \\ &+ \langle \sin^2 \theta \cos \theta \sin^2 \psi \rangle (\beta_{zxx} - 2\beta_{yyz}) \\ &+ \langle \sin^2 \theta \cos \theta \cos^2 \psi \rangle (\beta_{zyy} - 2\beta_{xxz}) \\ &+ \langle \sin \theta \cos \psi \rangle (2\beta_{zxx} - \beta_{xyy} - \beta_{xxx}) \\ &+ \langle \sin^3 \theta \cos \psi \rangle (2\beta_{yxy} + \beta_{xyy} - 2\beta_{zxx} - \beta_{xzz}) \\ &+ \langle \sin^3 \theta \cos^3 \psi \rangle (\beta_{xxx} - \beta_{xyy} - 2\beta_{yxy}) \end{aligned} \right\} \quad (15)$$

$$\chi_{xxz} = \frac{N_S}{2} \left\{ \begin{aligned} &\langle \sin^2 \theta \cos \theta \rangle \beta_{zzz} + \langle \cos^3 \theta \rangle (\beta_{xxz} + \beta_{yyz}) \\ &+ \langle \sin^2 \theta \cos \theta \sin^2 \psi \rangle (\beta_{xxz} - \beta_{yyz} - \beta_{zyy}) \\ &- \langle \sin^2 \theta \cos \theta \cos^2 \psi \rangle (\beta_{xxz} + \beta_{zxx} - \beta_{yyz}) \\ &- \langle \sin \theta \cos \psi \rangle (\beta_{xxx} - \beta_{xzz} + \beta_{yxy} - \beta_{zxx}) \\ &- \langle \sin^3 \theta \cos \psi \rangle (\beta_{xzz} - \beta_{xyy} + 2\beta_{zxx} - 2\beta_{yxy}) \\ &- \langle \sin^3 \theta \cos^3 \psi \rangle (\beta_{xyy} + 2\beta_{yxy} - \beta_{xxx}) \end{aligned} \right\} \quad (16)$$

All of the nonzero elements of the molecular hyperpolarizability tensor of indole are present in these equations. A considerable simplification results if the distribution of the twist angle ψ is uniform in the range $0-2\pi$, and thus $\langle \cos \psi \rangle = \langle \cos^3 \psi \rangle = 0$.

The simplified expressions include only the elements β_{zzz} , β_{iiz} , and β_{zii} ($i = x, y$), as shown in eqs 17–19, where the quantities a and b are defined in eqs 20 and 21.

$$\chi_{zzz} = \frac{N_s}{2} \beta_{zzz} \{2\langle \cos^3 \theta \rangle + \langle \sin^2 \theta \cos \theta \rangle (2a + b)\} \quad (17)$$

$$\chi_{zxx} = \frac{N_s}{2} \beta_{zzz} \left\{ \langle \cos^3 \theta \rangle b + \langle \sin^2 \theta \cos \theta \rangle \left(1 + \frac{b}{2} - a\right) \right\} \quad (18)$$

$$\chi_{xxz} = \frac{N_s}{2} \beta_{zzz} \left\{ \langle \cos^3 \theta \rangle a + \langle \sin^2 \theta \cos \theta \rangle \left(1 - \frac{b}{2}\right) \right\} \quad (19)$$

$$a = (\beta_{xxz} + \beta_{yyz})/\beta_{zzz} \quad (20)$$

$$b = (\beta_{zxx} + \beta_{zyy})/\beta_{zzz} \quad (21)$$

Note that all three nonzero elements of χ include only terms in $\langle \cos^3 \theta \rangle$ and $\langle \sin^2 \theta \cos \theta \rangle$. The same is true of the effective nonlinear susceptibilities f_p , g_p , and h_s , since each one of these is a linear combination of the elements of χ in eqs 17–19 (see Appendix 1). Considering now certain ratios of the effective nonlinear susceptibilities, it is clear that all such ratios have the same general form of the dependence on tilt angle θ , with specific details determined by the quantities a and b in eqs 20 and 21 and also by Fresnel factors given in Appendix 1. Of particular interest in the following are the ratios f_p/h_s , f_p/g_p , and g_p/h_s , with the latter closely related to χ_{zxx}/χ_{xxz} . The general form of the θ -dependence is shown for χ_{zxx}/χ_{xxz} in Figure 13a, for two choices of the parameters a and b . There is typically a dispersion in the ratio χ_{zxx}/χ_{xxz} about a singularity at $\theta = \theta_{ab} = \tan^{-1}(\sqrt{2a/(b-2)})$, the form of the dispersion governed by the sign and magnitude of the parameter $\delta_{ab} = 2(a-b)/(a+b)$, as shown in Figure 13a. The quantities θ_{ab} and δ_{ab} are introduced as descriptive parameters of the variation of χ_{zxx}/χ_{xxz} with the tilt angle θ . Analogous parameters can be defined for the ratios f_p/h_s , f_p/g_p , and g_p/h_s , for which the θ -dependence has the general form of Figure 13a.

C. Orientation of Indole in Monolayers at Air/Water Interface. In this section we present an analysis of the experimentally determined effective nonlinear susceptibilities of the indole/DPPC monolayers in terms of the orientational distribution of indole chromophores. The general scheme of the analysis is to compare measured ratios of the effective nonlinear susceptibilities with calculated ratios, using as input to the calculations the molecular hyperpolarizability tensor of indole from Table 5 and an assumed orientational distribution. Equations 14–16 are used to calculate the nonzero elements of χ , with the distribution functions of the tilt angle θ and twist angle ψ (Figure 12) modeled as square distributions with full widths σ_θ and σ_ψ , respectively. The effective nonlinear susceptibilities in the s/p polarization basis are obtained from the calculated elements of χ from eqs 14–16 as shown in Appendix 1. This requires as additional input the linear optical constants of the interface at the fundamental and second harmonic frequencies.

Figure 14 shows calculated ratios f_p/h_s , f_p/g_p , and g_p/h_s obtained for assumed tilt angles θ in the range 0–90°, in comparison with observed ratios for the indole/DPPC monolayer. Observed ratios from data in Tables 3 and 4 are shown

Figure 13. (a) Curves illustrating the dependence of the ratio χ_{zxx}/χ_{xxz} on the tilt angle θ for two choices of the parameters a and b . For the solid curve $a = -0.135$ and $b = -0.034$, and the associated descriptive parameters are $\theta_{ab} = 20^\circ$ and $\delta_{ab} = 1.2$. For the broken curve $a = -0.144$ and $b = -0.176$, and the associated descriptive parameters are $\theta_{ab} = 20^\circ$ and $\delta_{ab} = -0.2$. The curves were calculated by using eqs 18–21 in the text, assuming delta-function distributions of the tilt angle. The horizontal line shows $\chi_{zxx}/\chi_{xxz} = 1$. (b) Typical form of the dependence of the effective nonlinear susceptibilities (as indicated) on the tilt angle θ . The curves were calculated by using analysis I described in the text (see also Table 6) and using the TDDFT computational method.

for two states of compression of the DPPC monolayer, corresponding with the area per DPPC molecule in the monolayer $A = 127$ and $60 \text{ \AA}^2$. The calculated ratios are curves with the general form shown in Figure 13a, whereas the observed ratios are solid (broken) horizontal lines corresponding to the data for the higher (lower) value of A . The intersections of the calculated curves with the horizontal lines are circled in Figure 14. They indicate the tilt angles θ_o for which agreement is found between the calculated and observed ratios of effective nonlinear susceptibilities. The calculated curves in Figure 14 were obtained for assumed widths of the square distributions of θ and ψ , $\sigma_\theta = 30^\circ$ and $\sigma_\psi = 360^\circ$, respectively. Note that two sets of calculated ratios are shown in Figure 14 as solid and broken curves. These correspond with different assignments of the tilt axis of indole in the molecular plane. The solid curves in Figure 14 were calculated for the tilt axis parallel to the pseudosymmetry axis of indole, indicated as the z axis in Figure 1. This is the coordinate system that was used for all calculations of the hyperpolarizability tensor of indole summarized in Table 5. The alternate choice of the tilt axis of indole represented by the broken curves in Figure 14 is discussed later.

The typical form of the θ -dependence of the effective nonlinear susceptibilities f_p , g_p , and h_s is illustrated in Figure 13b, for the case of the calculation corresponding with the solid curves in Figure 14. Note that the curves for f_p and h_s in Figure

Figure 14. Calculated ratios of effective nonlinear susceptibilities (as indicated) for assumed tilt angles in the range 0–90°, in comparison with observed ratios for indole/DPPC monolayers described in Tables 3 and 4. Observed ratios are shown as solid and broken horizontal lines, for monolayers with $A = 127$ and $60 \text{ \AA}^2$, respectively. Calculated ratios were obtained by using analysis I described in Table 6 (solid curves) and analysis II described in Table 8 (broken curves), and using the TDDFT computational method. The intersections of the calculated curves with the observed ratios are circled.

13b are in a rough approximation balanced such that their sum ($f_p + h_s$) is nearly constant with θ . This behavior is typical and justifies the approach used in Section III.A.4 for calibrating the sum ($f_p + h_s$) with the areal number density of indole.

Tables 6 and 7 present summaries of the tilt angles θ_o obtained by comparison of calculated and observed ratios of effective nonlinear susceptibilities as shown in Figure 14. Separate tables are given for the monolayers with $A = 127$ and $60 \text{ \AA}^2$. In each table, results for θ_o are presented for three methods used for calculating the hyperpolarizability tensor of indole: TDDFT, TDHF, and ZINDO/S, as discussed with reference to Table 5. For each method, optimized tilt angles $\theta_o(f_p/h_s)$ and $\theta_o(f_p/g_p)$ are given for the comparisons with f_p/h_s and f_p/g_p , respectively. Also shown in Tables 6 and 7 are the ratios g_p/h_s calculated for $\theta = \theta_o(f_p/h_s)$, which should be compared with the observed values of g_p/h_s given in the headings of the tables. Tables 6 and 7 show that closely similar results

are obtained for the optimized tilt angles $\theta_o(f_p/h_s)$ and $\theta_o(f_p/g_p)$ for the three computational methods. Furthermore, these pairs of optimized tilt angles are nearly in agreement within each method. The indication from these results, independent of the computational method, is that the tilt angle of indole is near 48° in the monolayer with $A = 127 \text{ \AA}^2$ and near 60° in the monolayer with $A = 60 \text{ \AA}^2$. However, all three methods show similarly large disagreements between calculated and observed g_p/h_s . In fact, Figure 14 (solid curve) shows that the calculated θ -dependence of g_p/h_s for the TDDFT method never approaches the observed values. The same applies for the TDHF and ZINDO/S methods. The similarity in the results obtained by using the three computational methods is shown also in the parameters a and b associated with the respective hyperpolarizability tensors. As discussed in connection with eqs 20–21, these parameters govern the θ -dependence of both χ_{ZZX}/χ_{XXZ} and g_p/h_s . Table 6 gives the parameters a and b and also their descriptive counterparts θ_{ab} and δ_{ab} for the three computational methods. It is remarkable that the variations in these parameters among the methods are small, even though striking differences are present in the full hyperpolarizability tensors given in Table 5.

The analysis summarized in Tables 6 and 7 (described hereafter as analysis I) assumed that the orientational distribution of indole in the monolayers is such that the twist angle ψ is uniformly distributed in the range 0– 2π about a tilt axis that coincides with the pseudosymmetry axis (z) of indole, as illustrated in Figure 12. The particular choice of the pseudosymmetry axis of indole as the tilt axis (and hence also the twist axis) is arbitrary and difficult to justify on physical grounds. Alternate choices for the tilt axis within the molecular plane of indole were investigated by repeating the analysis with suitably transformed elements of the molecular hyperpolarizability tensor. The transformation is a rotation in the molecular plane such that the new tilt axis (z') makes an angle φ with the original tilt axis z , as illustrated in Figure 1. A range of the angle φ was investigated for each of the three computational methods used to calculate the molecular hyperpolarizabilities. In each case the angle φ was selected to produce agreement between calculated and observed g_p/h_s , specifically for the tilt angle $\theta = \theta'_o(f_p/h_s)$ of axis z' that produced agreement between calculated and observed f_p/h_s . This ensured that the optimum tilt angles $\theta'_o(f_p/h_s)$ and $\theta'_o(f_p/g_p)$ were identical, in contrast with analysis I of Tables 6 and 7. Typical results of the analysis with variable angle φ , described hereafter as analysis II, are shown in Figure 14 (broken curves), for the case of the TDDFT computational method. In this case $\varphi = 36^\circ$ was selected for the indole/DPPC monolayer with $A = 127 \text{ \AA}^2$. The results of analysis II are summarized in Table 8, for the monolayer with $A = 127 \text{ \AA}^2$. The corresponding table for the monolayer with $A = 60 \text{ \AA}^2$ is given in Table S2 of Supporting Information. Table 8 has the same form as Table 6, and includes in addition the selected angle φ between axes z and z' . Note that the optimized tilt angles $\theta'_o(f_p/h_s) = \theta'_o(f_p/g_p) = \theta'_o$ refer to the tilt axis z' , about which the twist angle ψ is uniformly distributed in the range 0– 2π . The average tilt angle of the pseudosymmetry axis z is also given in Table 8, under the heading θ_o , and is directly comparable with $\theta_o(f_p/h_s)$ and $\theta_o(f_p/g_p)$ in Table 6. As Table 8 and Table S2 of Supporting Information show, analysis II is successful in finding a unique tilt angle (θ'_o) of a z' axis such that all of the calculated ratios of effective nonlinear susceptibilities are in agreement with the measured ratios.

The results of analysis II indicate that the average tilt angle θ_o of the pseudosymmetry axis z is in the range 55 – 62° for the

TABLE 6: Tilt Angle θ_o of Indole Adsorbed to a DPPC Monolayer with $A = 127 \text{ \AA}^2$, Obtained by Comparison of Calculated and Observed Ratios of Effective Nonlinear Susceptibilities and Using Three Computational Methods to Calculate the Hyperpolarizability Tensor of Indole^a

computational method	$\theta_o(f_p/h_s)$	$\theta_o(f_p/g_p)$	g_p/h_s (obs. = 0.415) ^b	a^c	b	θ_{ab}	δ_{ab}
TDDFT/B3LYP	48	46	0.593	-0.131	-0.032	19.8	1.22
TDHF	49	46	0.613	-0.123	0.00	19.3	1.99
ZINDO/S	48	46	0.549	-0.086	-0.025	16.2	1.01

^a Tilt angle (degrees) of pseudosymmetry axis z relative to interface normal, according to analysis I described in the text. Parameters used in the calculations: $\sigma_\theta = 30^\circ$, $\sigma_\psi = 360^\circ$, $n_3 = 1.20$, and $N_3 = 1.222$. ^b Calculated ratio g_p/h_s for $\theta = \theta_o(f_p/h_s)$. The observed value is 0.415 (from results in Table 3). ^c Parameters a and b of the hyperpolarizability tensor (eqs 20, 21) are given together with their descriptive counterparts θ_{ab} and δ_{ab} (see the text).

TABLE 7: Tilt Angle θ_o of Indole Adsorbed to a DPPC Monolayer with $A = 60 \text{ \AA}^2$, Obtained by Comparison of Calculated and Observed Ratios of Effective Nonlinear Susceptibilities and Using Three Computational Methods to Calculate the Hyperpolarizability Tensor of Indole^a

computational method	$\theta_o(f_p/h_s)$	$\theta_o(f_p/g_p)$	g_p/h_s (obs. = 0.359) ^b
TDDFT/B3LYP	59	61	0.567
TDHF	60	63	0.582
ZINDO/S	59	61	0.535

^a Tilt angle (degrees) of pseudosymmetry axis z relative to interface normal, according to analysis I described in the text. Parameters used in the calculations: $\sigma_\theta = 30^\circ$, $\sigma_\psi = 360^\circ$, $n_3 = 1.20$, and $N_3 = 1.222$. ^b Calculated ratio g_p/h_s for $\theta = \theta_o(f_p/h_s)$. The observed value is 0.359 (from results in Table 4).

monolayer with $A = 127 \text{ \AA}^2$ and is in the range $63\text{--}66^\circ$ for the monolayer with $A = 60 \text{ \AA}^2$. The ranges given span the variation in θ_o among the three computational methods considered. These ranges are relatively small, so the results can be said to be nearly independent of the computational method. A further similarity among the methods is seen in the selected rotation angles φ . These angles are in narrow ranges spanning 15° in Table 8 and 17° for the monolayer with $A = 60 \text{ \AA}^2$. However, there are significant differences in the parameters a and b (or θ_{ab} and δ_{ab}) among the three methods, particularly for the ZINDO/S method. The effect of rotating the coordinate axes is particularly significant for the parameter δ_{ab} . The sign of this parameter is associated with the sign of the dispersion in the ratio g_p/h_s about the singularity at $\theta = \theta_{ab}$. This is illustrated in Figure 14 for the case of the TDDFT computational method, where it is seen that the change in sign of δ_{ab} upon rotation of the axes is an essential feature of the agreement between calculated and observed g_p/h_s .

The success of analysis II comes at a cost. It is clear that a nonzero angle φ between the tilt/twist axis z' and the pseudosymmetry axis z means that the orientation of the z -axis depends on the twist angle ψ , the distribution of which is taken to be uniform in the range $0\text{--}2\pi$ (see Figure 1). In the example of analysis II illustrated in Figure 14 (broken curves), the tilt angle of the z -axis varies between 6 and 94° (with $\theta'_o = 50^\circ$), and the average tilt angle is 63° as reported in Table S2 of Supporting Information. Such a large variation in the tilt angle of the pseudosymmetry axis may not be realistic. Steric interactions in the crowded headgroup region of the monolayer could favor a narrower distribution of the tilt angle, particularly at higher compression of the monolayer. A broad distribution of the tilt angle also implies broad distributions of both the dipole moment direction and the direction of the free imine N-H bond. It seems likely that anisotropic forces at the interface would favor one extreme of the z -axis orientation over the other, considering that such forces are responsible for a net polar orientation of indole at the interface. Instead of assuming that the potential is flat for the variation of the twist angle ψ in the range $0\text{--}2\pi$, we may

also consider a restricted range for ψ . This approach was used in a third analysis of the effective nonlinear susceptibilities of the indole/DPPC monolayers. By restricting the range of the twist angle such that the molecular plane of indole remained more nearly parallel to the plane of the interface, it was found that the success of analysis II could be replicated with the range of the tilt angle of the z -axis of indole much reduced. In the example of Figure 14, a restricted range of the twist angle $\psi = 90 \pm 30^\circ$ had an associated range for the tilt angle of the z -axis $37\text{--}60^\circ$, which seems more reasonable than the range $6\text{--}94^\circ$ noted above. The results of the calculations for this third analysis, analysis III, are summarized in Table 9 for the monolayer with $A = 127 \text{ \AA}^2$. Corresponding results for the monolayer with $A = 60 \text{ \AA}^2$ are given in Table S3 of Supporting Information. Note that the orientational distribution of ψ is expected to be achiral, so the range of the twist angle in the calculations was taken to be symmetric with components $\psi = 90 \pm 30^\circ$ and $\psi = 270 \pm 30^\circ$.

The three types of analysis described here produced rather similar results for the average tilt angle θ_o of the pseudosymmetry axis of indole in the indole/DPPC monolayers, as shown in Tables 6–9. Furthermore, the three methods used for calculating the molecular hyperpolarizability tensor of indole produced broadly similar results within each analysis. The only exception is the ZINDO/S method in analysis III, for which the tilt angles θ_o appear anomalous. Several parameters used throughout the analysis affect the finer details of the results but not the general trend. They include the effective index of refraction of the monolayer at the fundamental and second harmonic frequency and the widths of the square distributions of the tilt and twist angles, as specified in Tables 6–9. The overall analysis indicates that the average tilt angle θ_o is near 50° in the monolayer with $A = 127 \text{ \AA}^2$ and increases by $5\text{--}10^\circ$ in the monolayer with $A = 60 \text{ \AA}^2$. By including in the analysis a variable orientation of the tilt/twist axis in the molecular plane of indole, good agreement has been found between calculated and observed values of all ratios of the effective nonlinear susceptibilities. It is argued that a nonzero angle between the tilt/twist axis and the pseudosymmetry axis of indole is more readily acceptable if the distribution of the twist angle ψ about the tilt/twist axis is restricted in a range with the plane of indole more nearly parallel than perpendicular to the plane of the interface. Such an orientational distribution is consistent with conclusions based on linear dichroism measurements on indole in model lipid membranes,¹³ but contrasts with conclusions based on NMR studies.¹²

Similar considerations apply for the HIA monolayer with nonlinear susceptibilities closely similar to those of the indole/DPPC monolayer with $A = 127 \text{ \AA}^2$. In the case of the HIA monolayer the orientational distribution of indolyl is constrained by conformational degrees of freedom of the HIA molecule. By considering molecular models for HIA, and through

TABLE 8: Tilt angle θ'_0 of Indole Adsorbed to a DPPC Monolayer with $A = 127 \text{ \AA}^2$, Obtained by Comparison of Calculated and Observed Ratios of Effective Nonlinear Susceptibilities and Using Three Computational Methods to Calculate the Hyperpolarizability Tensor of Indole^a

method	φ^b	$\theta'_0 (f_p/h_s)$	θ_0^c	g_p/h_s (obs. = 0.415) ^d	a^e	b	θ_{ab}	δ_{ab}
TDDFT/B3LYP	36	43	55	0.419	-0.145	-0.212	19.9	-0.38
TDHF	48	43	62	0.420	-0.192	-0.256	22.4	-0.29
ZINDO/S	51	32	59	0.419	7.0	6.4	60.7	0.10

^a Tilt angle (degrees) of tilt/twist axis z' relative to interface normal, according to analysis II described in the text. $\theta'_0(f_p/h_s) = \theta'_0(f_p/g_p)$ obtained in all cases. Parameters used in the calculations: $\sigma_\theta = 30^\circ$, $\sigma_\psi = 360^\circ$, $n_3 = 1.20$, and $N_3 = 1.222$. ^b Angle (degrees) of tilt/twist axis z' relative to pseudosymmetry axis z (see Figure 1). ^c Average tilt angle of pseudosymmetry axis z . ^d Calculated ratio g_p/h_s for $\theta = \theta'_0(f_p/h_s)$. The observed value is 0.415 (from results in Table 3). ^e Parameters a and b of the hyperpolarizability tensor (eqs 20, 21) are given together with their descriptive counterparts θ_{ab} and δ_{ab} (see the text).

TABLE 9: Tilt Angle θ'_0 of Indole Adsorbed to a DPPC Monolayer with $A = 127 \text{ \AA}^2$, Obtained by Comparison of Calculated and Observed Ratios of Effective Nonlinear Susceptibilities and Using Three Computational Methods to Calculate the Hyperpolarizability Tensor of Indole^a

method	φ^b	$\theta'_0(f_p/h_s)$	θ_0^c	g_p/h_s (obs. = 0.415) ^d
TDDFT/B3LYP	36	27	46	0.420
TDHF	48	15	51	0.420
ZINDO/S	51	72	79	0.417

^a Tilt angle (degrees) of tilt/twist axis z' relative to interface normal, according to analysis III described in the text. $\theta'_0(f_p/h_s) = \theta'_0(f_p/g_p)$ obtained in all cases. Parameters used in the calculations: $\sigma_\theta = 30^\circ$, $\psi = 90^\circ/270^\circ$, $\sigma_\psi = 60^\circ$, except $\sigma_\psi = 100^\circ$ was used with ZINDO/S, $n_3 = 1.20$, and $N_3 = 1.222$. ^b Angle (degrees) of tilt/twist axis z' relative to pseudosymmetry axis z (see Figure 1). ^c Average tilt angle of pseudosymmetry axis z . ^d Calculated ratio g_p/h_s for $\theta = \theta'_0(f_p/h_s)$. The observed value is 0.415 (from results in Table 3).

computations using the semiempirical AM1 method, it was seen that constraints on the distribution of ψ of the type described in analysis III are plausible consequences of the conformational constraints.

D. Absolute Values of Nonlinear Susceptibilities. The absolute values of calculated and observed effective nonlinear susceptibilities for the indole/DPPC monolayer with the area per DPPC molecule $A = 127 \text{ \AA}^2$ are given in Table S4 of Supporting Information. Results are presented for three computational methods used to calculate the hyperpolarizability tensor of indole. As noted, all model monolayers with nonzero φ are successful in providing agreement between all calculated and observed ratios of the effective nonlinear susceptibilities. The absolute values of the calculated nonlinear susceptibilities for the model monolayers are generally smaller than the observed values, and there is considerable variation among the results for the model monolayers. The absolute orientation of indole in the monolayer with the pyrrole ring facing toward the water subphase is confirmed in this analysis by the negative signs of the calculated nonlinear susceptibilities for all of the model monolayers considered.

IV. Summary and Conclusions

Adsorption of indole from an aqueous subphase to a DPPC monolayer at the air/water interface has been studied by SHG. Indole is a relatively weak SHG chromophore, and thus the contributions of the water subphase and lipid monolayer must be accounted for in the total SHG response. Precise measurements of the relative values of the effective nonlinear susceptibilities of indole in the DPPC monolayer have been measured for two states of compression of the monolayer. Relative nonlinear susceptibilities were placed on an absolute scale by comparison with SHG in reflection from a z -cut quartz crystal,

for which the absolute magnitude of the nonlinear susceptibility is known, and by comparison with a clean air/water interface, for which the absolute phase of the nonlinear susceptibility is known. The surface density of indole molecules in the monolayer was measured by comparison with a reference monolayer of HIA with a known surface density of indole chromophores. The extent of indole adsorption to the DPPC monolayer decreases as the monolayer is compressed, falling from a maximum indole/DPPC ratio in the monolayer near 2:1. The free energy change for adsorption of indole has been estimated as $\Delta G = -8.1 \text{ kcal/mol}$, similar to the value measured previously for a lipid bilayer.² An analysis of the nonlinear susceptibilities is presented in which the susceptibilities are calculated for model monolayers with assumed orientational distributions of indole and with molecular hyperpolarizabilities calculated by using one of three distinct computational approaches. The analysis provides information on the tilt and twist angles of indole in the monolayer. The average tilt angle of the pseudosymmetry axis of indole relative to the interface normal is near 50° , increasing by $5\text{--}10^\circ$ as the monolayer is compressed. The absolute orientation of indole is with the pyrrole ring facing toward the water subphase. An orientational distribution is proposed in which there is restricted rotation about an axis in the plane of indole and noncollinear with the pseudosymmetry axis. It is suggested that the distribution of the twist angle about this axis is such that the plane of indole remains nearly parallel to the plane of the interface. Similar conclusions regarding the orientational distribution of indole were drawn for each of the three methods for computation of the molecular hyperpolarizability tensor of indole. Measured nonlinear susceptibilities are significantly larger than calculated values obtained by using the TDDFT approach. Further work is needed to interpret this result in terms of the accuracy of the computational method for the molecular hyperpolarizability and the possible role of local fields and/or specific molecular interactions in the monolayer.

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Appendix 1

The effective nonlinear susceptibilities in the s/p polarization basis are related to the elements χ_{ijk} of the effective nonlinear susceptibility tensor in Cartesian coordinates as shown in eqs A1.1–A1.3.²¹

$$f_p = A_o A_p \left(\frac{t_{p13}}{1 - r_{p31} r_{p32}} \right)^2 \{ -2\chi_{XXZ}(1 - R_{p32}) \cos \Theta_3 \times \\ (1 + r_{p32})(1 - r_{p32}) \sin \theta_3 \cos \theta_3 + \chi_{ZXX}(1 + R_{p32}) \times \\ \sin \Theta_3 (1 - r_{p32})^2 \cos^2 \theta_3 + \chi_{ZZZ}(1 + R_{p32}) \sin \Theta_3 \times \\ (1 + r_{p32})^2 \sin^2 \theta_3 \} \quad (\text{A1.1})$$

$$g_p = A_o A_p \left(\frac{t_{s13}}{1 - r_{s31} r_{s32}} \right)^2 \chi_{ZXX}(1 + R_{p32}) \sin \Theta_3 (1 + r_{s32})^2 \quad (\text{A1.2})$$

$$h_s = A_o A_s \frac{t_{s13}}{1 - r_{s31} r_{s32}} \frac{t_{p13}}{1 - r_{p31} r_{p32}} 2\chi_{XXZ} \times \\ (1 + R_{s32})(1 + r_{s32})(1 + r_{p32}) \sin \theta_3 \quad (\text{A1.3})$$

In these equations A_o , A_p and A_s are constants that incorporate Fresnel factors as shown in eqs A1.4, with c the speed of light in vacuum and λ the wavelength of the fundamental.

$$A_o = \sqrt{\frac{2\pi}{c}} \frac{i8\pi^2}{\lambda N_3 \cos \Theta_3} \\ A_s = \frac{T_{s31}}{1 - R_{s31} R_{s32}} \\ A_p = \frac{T_{p31}}{1 - R_{p31} R_{p32}} \quad (\text{A1.4})$$

The Fresnel factors are given in eqs A1.5–A1.8,

$$t_{sij} = \frac{2n_i \cos \theta_i}{n_i \cos \theta_i + n_j \cos \theta_j} \quad (\text{A1.5})$$

$$t_{pij} = \frac{2n_i \cos \theta_i}{n_i \cos \theta_j + n_j \cos \theta_i} \quad (\text{A1.6})$$

$$r_{sij} = t_{sij} - 1 \quad (\text{A1.7})$$

$$r_{pij} = \frac{n_j}{n_i} t_{pij} - 1 \quad (\text{A1.8})$$

where the indices ij here specify the propagation direction from medium i to medium j , with i and j equal to 1, 2, or 3, corresponding to ambient (air), substrate (water), and nonlinear layers, respectively. The angle θ_i of propagation in medium i relative to the interface normal is given by eq A1.9,

$$n_i \sin \theta_i = \sin \theta_1 \quad (\text{A1.9})$$

where n_i is the index of refraction of medium i . Equations A1.5–A1.9 are given explicitly for the fundamental frequency. The same relationships apply for the second harmonic frequency, with t , r , n , and θ (with appropriate subscripts) replaced by the corresponding upper case quantities T , R , N , and Θ . In this work $\theta_1 = 60^\circ$ was used, and the optical constants n_2 and N_2 were taken from ref 50.

It should be noted that the effective nonlinear susceptibilities used in this work differ from those of Maki et al.,²¹ in that the prefactors $A_o A_j$ (j is s or p) in eqs A1.1–A1.3 are not included in the definitions of Maki et al. They differ also from those of previous work from this laboratory,^{19,20} in which the prefactor A_o in eqs A1.4 was defined with the factor $i8\pi^2/\lambda$ omitted.

Appendix 2

From the analysis described in a previous report from this laboratory,²⁰ the effective nonlinear susceptibilities in eq 1 can be expressed as shown in eqs A2.1–A2.6

$$f_p^Q = \cos^2 \theta_2 \cos \phi (\cos^2 \phi - 3 \sin^2 \phi) A_o^Q A_p^Q t_{p12}^2 \quad (\text{A2.1})$$

$$g_p^Q = -\cos \phi (\cos^2 \phi - 3 \sin^2 \phi) A_o^Q A_p^Q t_{s12}^2 \quad (\text{A2.2})$$

$$h_p^Q = 2 \cos \theta_2 \sin \phi (\sin^2 \phi - 3 \cos^2 \phi) A_o^Q A_p^Q t_{s12} t_{p12} \quad (\text{A2.3})$$

$$f_s^Q = \cos^2 \theta_2 \sin \phi (\sin^2 \phi - 3 \cos^2 \phi) A_o^Q A_s^Q t_{p12}^2 \quad (\text{A2.4})$$

$$g_s^Q = -\sin \phi (\sin^2 \phi - 3 \cos^2 \phi) A_o^Q A_s^Q t_{s12}^2 \quad (\text{A2.5})$$

$$h_s^Q = -2 \cos \theta_2 \cos \phi (\cos^2 \phi - 3 \sin^2 \phi) A_o^Q A_s^Q t_{s12} t_{p12} \quad (\text{A2.6})$$

In these equations ϕ represents the azimuthal angle of the z -cut quartz crystal, A_o^Q , A_s^Q , and A_p^Q are constants given in eqs A2.7–A2.9, and all other quantities are defined in Appendix 1, with the indices i and j equal to 1 or 2, corresponding to ambient (air) and substrate (quartz), respectively.

$$A_o^Q = d_Q \sqrt{\frac{8\pi}{c}} \quad (\text{A2.7})$$

$$A_s^Q = \frac{-4\pi \sin \theta_2 \sin^2 \Theta_2}{\sin \theta_1 \sin(\Theta_2 + \theta_2) \sin(\Theta_2 + \theta_1)} \quad (\text{A2.8})$$

$$A_p^Q = \frac{4\pi \sin \theta_2 \sin^2 \Theta_2 \cos \Theta_2}{\sin \theta_1 \sin(\Theta_2 + \theta_2) \sin(\Theta_2 + \theta_1) \cos(\Theta_2 - \theta_1)} \quad (\text{A2.9})$$

Note that the factor 8 in eq A2.7 is due to the convention used for the electric field.⁵¹ This factor was set to 2 in ref 20, and the nonlinear coefficient d_Q was adjusted accordingly to give the same result for A_o^Q in eq A2.7. The angles θ_i and Θ_i are given by eq A1.9 with $n_2 = 1.53837$ and $N_2 = 1.55772$ (for $\lambda = 800$ nm).⁵²

Supporting Information Available: Figure showing rotation traces for compressed DPPC monolayer with $A = 60 \text{ \AA}^2$ and with a $8.1 \times 10^{-3} \text{ M}$ concentration of indole in the subphase, Table of results of analysis II for the monolayer with $A = 60 \text{ \AA}^2$, and Table of results of analysis III for the monolayer with $A = 60 \text{ \AA}^2$. Table of absolute values of calculated and observed

effective nonlinear susceptibilities for the indole/DPPC monolayer with the area per DPPC molecule $A = 127 \text{ \AA}^2$. A brief discussion of the absolute values of the nonlinear susceptibilities is included. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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