From 2D to 3D at the Air/Water Interface: The Self-Aggregation of the Acridine Dye in Mixed Monolayers

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ABSTRACT

The formation of well-defined supramolecular structures in the nanoscopic scale is a fundamental step in nanotechnology. The fine control of the layer-by-layer growth of the supramolecular assemblies at interfaces is most desirable. The collapse of a mixed monolayer composed of two surfactants in equimolar ratio: the organic dye N-10-dodecyl acridine (DAO) and stearic acid (SA) is analyzed herein. The collapse process of the DAO:SA mixed monolayer has been monitored using surface pressure-molecular area (π -A), surface potential isotherms, UV-visible reflection spectroscopy, Polarization-Modulated Infrared Reflection-Absorption Spectroscopy (PM-IRRAS), Brewster Angle Microscopy (BAM), and synchrotron-based in situ X-ray Reflectivity (XRR) measurements. The collapse of the DAO:SA mixed monolayer leads to an ordered trilayer. The growth of anisotropic 2D domains of micrometric size is observed during the formation of the trilayer, relating to the ordering of the acridine polar headgroups. The trilayer is organized with the first and third monolayers displaying the polar headgroups pointing to the aqueous subphase, whereas the intermediate layer display the polar headgroups pointing to the air. The trilayer is stabilized by the strong self-aggregation acridine dye group of the DAO molecule. The controlled transition from a monolayer to a trilayer described herein is proposed as a model for further interfacial supramolecular structures of tunable thickness comprising organic dyes.

INTRODUCTION

The “bottom-up” approach, i. e., the self-assembly of individual building blocks into larger functional structures is fundamental in nanotechnology.¹ In this regard, the controlled transformation of 2D entities into more complex 3D structures at the nanoscale has been highlighted as a suitable alternative for the construction of multifunctional material. The collapse of Langmuir monolayers is of high relevance from a nanotechnological point of view, given that during this process the molecules are self-assembled into 3D supramolecular structures.^{2,3} A computational study on the collapse of Langmuir monolayers of arachidic acid described the atomic interactions leading to the multilayer structure.⁴ On a subsequent study, the influence of divalent calcium cations on the collapse process was thoroughly studied by *in situ* X-ray reflectivity and neutron scattering. This study has been the first experimental proof of the formation of a well-defined trilayer structure formed by a monolayer of arachidic acid at the air/solution interface. This study is also providing interesting insights concerning the formation of 3D crystals at the air/liquid interface.⁵

Moreover, the collapse of a monolayer plays a fundamental role in highly relevant biophysical processes, e. g., pulmonary surfactants in the breathing cycle.^{6,7} Therefore, the rationale understanding of collapse process of a Langmuir monolayer including a quantitative model is highly desirable. The collapse of a Langmuir monolayer can be described as a phase change in which the Langmuir monolayer is transformed into bulk surfactant under conditions of film compression.⁸

We assume the absence of additional phenomena, e.g., the aggregation of surfactant molecules into soluble aggregates and chemical reactions. In this scenario, the spontaneous compression of a Langmuir monolayer under a constant value of surface pressure is interpreted as the collapse of the Langmuir monolayer.^{9,10}

Generally, the term “collapse” has been used to describe a disordering process. However, a number of examples have been reported in the literature describing the collapse of a Langmuir monolayer spontaneously leading to 3D ordered structures, either bilayers,¹¹⁻¹⁷ trilayers,^{3, 12, 16, 18-27} or multilayers.²⁸⁻³⁴ The study of the process of the collapse of an ordered monolayer is a subject of large

interest due to the possibility of controlling the growth of these 3D supramolecular structures by the understanding of the interplay of the involved short- and long- range intermolecular forces.

Two general mechanisms for the transformation of a Langmuir monolayer into a multilayer have been. In the first mechanism, the Langmuir monolayer folds over itself, leading to a trilayer. In the second mechanism, the trilayer is growing from the monolayer at the boundary of the micrometric domains.^{19, 21, 24, 26, 35, 36, 37}

The collapse of a mixed monolayer composed of two surfactants in equimolar ratio has been studied herein. The components of the mixed Langmuir monolayer are the organic dye N-10-dodecyl acridine (DAO) and the stearic acid (SA). See Figure 1A for the molecular structures of both components. The mixed Langmuir monolayer DAO:SA has been built by the cospreading method, i. e., mixing of both components in the same organic solvent prior to the spreading of the Langmuir monolayer on the air/water interface. A number of in situ experimental techniques for the study of Langmuir monolayers have been used. Surface pressure-molecular area (π -A) and surface potential isotherms provide information on the thermodynamical aspects of the collapse process. UV-visible reflection spectroscopy and Polarization-Modulated Infrared Reflection-Absorption Spectroscopy (PM-IRRAS) offer quantitative information on the polar headgroup and alkyl chain arrangement, respectively. Brewster Angle Microscopy (BAM) allows the direct visualization of the monolayer at the micrometric scale. Synchrotron-based in situ X-ray Reflectivity (XRR) provides the electron density profile of the monolayer.

Figure 1. A) Molecular structures of N-10-dodecyl acridine (DAO) and stearic acid (SA). B) Model of aggregation of DAO at the air-water interface. C) Rotation of the dihedral plane C(3-6) and tilt angle of the DAO headgroup.

A previous study in pre-collapse conditions on the mixed Langmuir monolayer DAO:SA showed a quite homogeneous appearance at the mesoscopic size, indicating the formation of an ionic pair DAO:SA.³⁸ In the absence of collapse, two main phenomena are present concerning the polar headgroup of the DAO molecule. First, the spontaneous formation of self-assembled H-aggregates was observed. Second, a change of tilting of the main dipole of the polar headgroup of the DAO molecule was monitored. The spontaneous formation of H-aggregates at the mixed monolayer DAO:SA is driven by the tendency of the DAO headgroups to self-assemble into large aggregates. The DAO molecule in all-trans conformation bears a large dipole moment. This large dipole moment results in the intermolecular repulsion between the adjacent DAO molecules in the mixed DAO:SA Langmuir monolayer.³⁹ Given the tendency of DAO to self-assemble into H-aggregates, a partial rotation of the DAO head groups was assumed to compensate this dipolar repulsion, see Figure 1B. H-aggregates of a dye occur for the case of a parallel arrangement of the dye units (face-to-face), leading to a blue shift of the absorption band.⁴⁰ The tilting angle formed between the main axis of the DAO head group and the normal to the air/water interface (θ) ranges from $\theta = 90^\circ$ (low applied surface pressure) to $\theta \approx 55^\circ$ (large applied surface pressure). From a structural point of view this tilt is caused by the rotation of dihedral angle formed by the atoms C(3)-C(4)-C(5)-C(6), see Figure 1C.³⁸ The different length of the alkyl chains of the DAO and SA molecules, and the bulkiness of the polar headgroup of the DAO molecule prevent the formation of a

highly condensed phase in the monolayer. In other words, the alkyl chains are not oriented fully perpendicular to the interface, adopting an ordered organization, in the mixed monolayer DAO:SA.

In this work, the mesoscopic organization during the collapse of the mixed monolayer DAO: SA has been studied. The ordering within the micrometric domains, as well as the molecular organization of the film is described.

EXPERIMENTAL SECTION

Materials

DAO bromide salt and SA were purchased from Fluka and Sigma-Aldrich, respectively, and used as received. Their molecular structures are depicted in Figure 1. Chloroform was used as the spreading solvent for dissolving both components. The pure solvent was obtained without purification from Aldrich. Ultrapure water, produced by a Millipore Milli-Q unit, pretreated by a Millipore reverse osmosis system ($>18.2 \text{ M}\Omega \text{ cm}$), was used as a subphase. The subphase temperature was 21°C with pH 5.7.

Methods

Surface pressure-molecular area isotherms

Two different models of Nima troughs (NimaTechnology, Coventry, England) were used in this work, both provided with a Wilhelmy-type dynamometric system using a strip of filter paper: a NIMA 611D with one moving barrier for the measurement of the reflection spectra, and a NIMA 601, equipped with

two symmetrical barriers to record BAM images. The monolayers were compressed at a speed of ca. $0.1 \text{ nm}^2 \cdot \text{min}^{-1} \cdot \text{molecule}^{-1}$. The cospreading method has been used for preparing the mixed monolayer (1:1), mixing of the stock solutions of DAO and SA prior to spreading at the air/water interface.

Surface potencial curves

Surface potential at the air/water interface was measured with a surface-potential probe equipped with a vibrating plate (Kelvin Probe SP1, Accurion Technologies, Göttingen, Germany). Variation of the monolayer surface potential of the monolayer was obtained at the same time as the surface pressure/molecular area isotherm.

UV-vis reflection spectra

UV-vis reflection spectra at normal incidence as the difference in reflectivity (ΔR) of the dye film-covered water surface and the bare surface⁴⁰ were obtained with a Accurion Surface Analysis Spectrometer (RefSpec2 supplied by Accurion GmbH, Goettingen, Germany). All the surface pressure-molecular area isotherms and UV-vis reflections spectra presented herein have been performed at least three times, showing good reproducibility. No dependence of the number of molecules spread on the surface versus mean molecular area was observed for the different experiments.

BAM images

Images of the film morphology were obtained by BAM with a I-Elli2000 (Accurion GmbH), using a Nd:YAG diode laser with wavelength 532 nm and 50 mW, which can be recorded with a lateral resolution of 2 μm . The image processing procedure included a geometrical correction of the image, as well as a filtering operation to reduce interference fringes and noise. The microscope and the film balance were located on a table with vibration isolation (anti-vibration system MOD-2 S, Accurion, Goettingen, Germany) in a large class 100 clean room.

PM-IRRAS measurements

PM-IRRAS spectra were recorded using a KSV PMI 550 (KSV NIMA, Espoo, Finland) equipped with an MCT detector. A detailed description of the PM-IRRAS setup and the experimental procedure has already been given elsewhere.⁴¹ The setup consists of an IR source, a Michelson interferometer and

an external reflection unit. The infrared radiation intensity was modulated by the interferometer and polarized with a ZnSe polarizer. The beam was then passed through a ZnSe photoelastic modulator, which modulated it between polarization in the plane of incidence (p) and polarization perpendicular to this plane (s) with a fixed frequency of 100 kHz. From the detected intensity (using electronic filtering and demodulation) the two signals, $(R_p - R_s)$ and $(R_p + R_s)$, were obtained. Only anisotropic absorption contribute to the PM-IRRAS signal $S \approx (R_p - R_s)/(R_p + R_s)$. Normalized signals were obtained directly by using the following expression: $\Delta S = (S_d - S_o)/S_o$, where S_d and S_o are the signals in the presence and absence of a monolayer, respectively. The angle of incidence of the infrared beam with respect to the surface normal was 80° . Spectra were recorded with a spectral resolution of 8 cm^{-1} and collected using 3000-6000 scans during 5-10 min.

Specular X-Ray Reflectivity (XRR):

The specular X-Ray reflectivity (XRR) measurements were performed on the undulator beamline BW1 at HASYLAB, DESY (Hamburg, Germany). XRR provides an averaged electron density profile normal to the interface of all molecules. For reflectivity measurements, the angles of incidence α_i and reflected α_f beams are equal and varied in a range $0.5\alpha_c < \alpha_i (\alpha_f) < 30 \alpha_c$, where $\alpha_c = 0.138$. The reflected intensity was measured by a NaI scintillation detector in the plane of incidence as a function of the vertical scattering vector component Q_z . The electron density profile was obtained from the reflectivity curve using a linear combination of b-splines following the approach of Pedersen and Hamley. The electron density profiles were calculated using the StochFit software.⁴²

RESULTS AND DISCUSSION

Surface pressure – molecular area (π -A) isotherm

Figure 2 shows the π -A isotherm of the mixed monolayer DAO:SA, where A represents the average area per molecule, i. e., the DAO and SA molecules are considered equivalent. The onset of the surface pressure is observed at an average area per molecule of ca. 0.8 nm^2 . The starting point of the collapse is observed at a surface pressure of ca. 38 mN/m and an average area per molecule of ca. 0.24 nm^2 . During

the collapse process the surface pressure decreases slightly to a fixed value of ca. 37 mN/m, while simultaneously the area decreases. A slight increase of the surface pressure is observed at the final point of the collapse process at an average area per molecule of ca. 0.10 nm². A sharp increase in surface pressure is observed for a mean area per molecule of ca 0.08 nm². Therefore, the collapse of the mixed monolayer DAO:SA from a 2D to a 3D film takes place in the region of the π -A isotherm with a value of average area per molecule ranging from 0.24 to 0.08 nm². Note that the surface pressure is approximately constant along the complete process of collapse. Remarkably, the value of area per molecule at the final point of the collapse, i. e., 0.08 nm², is approximately 1/3 of the initial area, i. e., 0.24 nm², therefore suggesting the formation of a trilayer.

Figure 2. Surface pressure - area (π -A) (solid line) and apparent dipole moment - area (μ_A - A) (dashed line) isotherms of the monolayer DAO:SA.

Surface potential– area isotherm (ΔV -A)

The surface potential of a Langmuir monolayer is dependent on three main factors: a) the dipole moments of the components of the monolayer, b) the relative orientation of the water molecules, and c) the ionic environment and state of the headgroups and subphase. The contribution to the surface potential of the hydrophobic chains is similar for the DAO and SA molecules, given both are saturated

linear hydrocarbon chains. Thus, only the contribution of the dipole moments of the headgroups of DAO and SA is considered as relevant for the observed changes in the surface potential of the mixed monolayer DAO:SA. We adopt herein the general assumption to consider no significant reorientation of the water molecules with the compression of the monolayer. No ions have been added to the pure water subphase. Therefore, exclusively the ionic state of the DAO and SA polar headgroups is considered to have an impact on the ionic environment at the air/water interface. According to the Hemholtz model, the surface potential of a Langmuir monolayer is described by the equation:

$$\Delta V = \frac{\mu_{\perp}}{A \cdot \varepsilon_v \cdot \varepsilon_0} \quad (1)$$

where $\mu_{\perp}$ is the total dipole moment of the Langmuir monolayer with a perpendicular orientation to the air/water interface, and ε_v and ε_0 are the relative dielectric constant at the interface and the permittivity of free space, respectively. The apparent dipole moment, μ_A , is defined as:

$$\mu_A = \Delta V \cdot A \cdot \varepsilon_0 = \frac{\mu_{\perp}}{\varepsilon_v} \quad (2)$$

The plot $\mu_A - A$ is shown in Figure 2, dashed line. For $A > 0.5 \text{ nm}^2$, μ_A is ca. 0.58 D. By decreasing the area, μ_A decreases, reaching before the collapse a value of μ_A of ca. 0.25 D ($A = 0.24 \text{ nm}^2$). This decrease was related to the partial rotation of the acridine group, i. e., the polar headgroup of the DAO molecule. The alkyl chains of the DAO molecule are arranged in all-trans conformation, resulting in a large dipole moment of the whole molecule. The dipole moment of the hydrophobic chains in a compressed Langmuir monolayer has been appointed as the main contribution to the surface potential in several examples, e. g., a Langmuir monolayer of pure stearic acid. In that case, the contribution of the alkyl chains was assumed as ca. 230 mV in an overall value of 300 mV, with a minor negative contribution from the water layer underneath the monolayer. This last contribution might change as a function of the degree of ionization. In the case of the DAO molecule, the polar headgroup is also

bearing one charge per molecule. Despite the DAO headgroup is bulkier than the SA headgroup, we assumed a similar relative contribution to the overall surface potential of the mixed monolayer. Moreover, the charges of DAO and SA are of opposite sign. The cancellation of the contribution of the polar headgroups to the surface potential of the mixed monolayer is therefore expected.³⁶

This large dipole moment leads to a significant repulsion between adjacent DAO molecules. The DAO headgroups within the mixed DAO:SA monolayers have a certain degree of mobility. In other words, the DAO headgroups might change the relative orientation of the main axis of the DAO group, i. e., the axis connecting the amine groups, to reduce the intermolecular repulsion. Therefore, the DAO headgroups from neighbor DAO molecules within the mixed monolayer can rotate in opposite direction.³⁸ This rotation effectively reduces the intermolecular repulsion between the individual dipole moments of the DAO headgroups, see Figure 1B. The reduction of this intermolecular repulsion leads to a reduction in the overall value of μ_A value of the mixed monolayer DAO:SA.

For $A < 0.24 \text{ nm}^2$, μ_A decreases linearly with decreasing area with a slope approximately equal to unity. Thus, for A ca. 0.08 nm^2 , μ_A is approximately one third of the value at the beginning of the collapse, i. e., 0.09 D . This sharp decline of the dipole moment per molecule can exclusively be explained by the formation of a trilayer at the air/water interface. The top bilayer should have the two monolayers with opposite orientation, resulting in the cancelation of their contribution to the apparent dipole moment of the whole trilayer.

Brewster Angle Microscopy (BAM)

The Brewster Angle Microscopy (BAM) allows for the in situ imaging of the mixed monolayer DAO:SA at the air/water interface. The BAM can resolve the formation of domains with micrometer resolution. Figure 3 shows representative BAM images of the mixed monolayer DAO:SA. As previously mentioned, for $A \geq 0.24 \text{ nm}^2$, the mixed monolayer DAO:SA appear homogeneous in the BAM images,

with no apparent formation of domains. The homogeneity of the mixed monolayer DAO:SA has been related to the complete miscibility of the components. On the contrary, for $A \leq 0.24 \text{ nm}^2$, the formation of hexagonal domains is observed, see Fig. 3B,C. These domains increase in size with the decrease of the area, eventually deforming as shown in Fig. 3D.

Figure 3. BAM images of the mixed monolayer DAO:SA. A) Previous region to collapse. B-F) Collapse region. The width of each frame is $215 \mu\text{m}$.

Remarkably, the hexagonal domains display internal anisotropy, i.e., there are bright and dark regions inside the domain. The anisotropy in the orientation of dye headgroups led to inner textures within the domains in related cases, as previously studied by our group.^{43, 44} Note that the dark regions of the domains are always brighter than the surrounding. The dark areas of the domains are extended horizontally, i.e. perpendicular to the incidence direction of the laser used in the BAM experiments.

The existence of anisotropy inside the domains indicates the existence of distinct molecular ordering of the acridine dye moiety for the different regions within the domain, due to the enhancement of reflected radiation by certain absorption of the incoming radiation during the BAM experiment. Similar results have been reported during BAM experiments concerning the organization of the alkyl chains from Vollhardt et al. In such cases, either the different azimuthal angle of the alkyl chains of the lipids within the domains^{45, 46} or the effect of a chiral center of the surfactant, led to different inner textures always related to the organization of the alkyl chains.^{47, 48} However, in the case described herein, the anisotropy in the BAM images is caused by the different absorption of the dye headgroups.⁴⁴

UV-vis reflection spectra

The UV-vis reflection spectroscopy at the air/water interface offers in situ quantitative information on the presence and orientation of the chromophores at the air/water interface. The UV-vis reflection spectroscopy is exclusively sensitive to the molecules existing at the air/water interface, thus discarding any signal from the subphase. For a Langmuir monolayer containing UV-vis absorbing molecules, the reflection of an incident light at the interface is enhanced by⁴⁹

$$\Delta R = R_{D,S} - R_S = 2.303 \times 10^3 \cdot f_0 \cdot \varepsilon \cdot \sqrt{R_S} \cdot \Gamma \quad (3)$$

where $R_S = 0.02$ and $R_{D,S}$ are the reflectivity of the interface without and with the presence of the Langmuir monolayer, respectively, ε is the molar absorption coefficient in $\text{mol} \cdot \text{L}^{-1} \cdot \text{cm}^{-1}$ units, Γ is the surface concentration of the monolayer, in $\text{mol} \cdot \text{cm}^{-2}$ units, and f_0 is the orientation factor. In equation (3), ε coefficient is obtained from bulk measurements, i.e., DAO solution, therefore no preferred orientation is included. However, anisotropy in the orientation of the dipoles is expected at the air/water interface. Assuming a homogeneous orientation of all molecules at the interface, the orientation factor,

$$f_0 = \frac{3}{2} \langle \sin^2(\theta) \rangle$$

f_0 , accounts for the change in the absorption properties of the chromophore due to anisotropy. Given a nondegenerate absorption band, that is, with only one component of the transition dipole, the orientation factor is⁵⁰

$$(4)$$

where θ is the angle formed between the transition dipole and the Z axis, i.e., the normal axis to the air/water interface. The brackets indicate a mean value. UV-vis reflection spectra are often expressed in normalized units in order to discard any additional effect to the variation of the surface concentration of the chromophore units at the interface. The surface concentration of the dye, the DAO molecule, is expressed by

$$\Gamma = \frac{10^{14}}{N_A A_{DAO}} \quad (5)$$

where N_A is the Avogadro number, and A_{DAO} is the molecular area of DAO. A_{DAO} is obtained from the π -A isotherms by $A_{DAO} = A/X_{DAO}$, where $X_{DAO} = 0.5$ is the molar ratio of the DAO component. The normalized reflection signal ΔR_{norm} is the product of the absolute reflection signal and the molecular area of the chromophore:

$$\Delta R_{\text{norm}} = A_{DAO} \times \Delta R = \frac{2.303 \times 10^{17} f_0 \varepsilon}{N_A} \sqrt{R_s} = 5.41 \times 10^{-8} f_0 \varepsilon \quad (6)$$

ΔR_{norm} has $\text{nm}^2 \cdot \text{molec}^{-1}$ units. The normalized reflection spectra offer information on molecular aggregation, as well as molecular orientation.

Figure 4. A) UV-vis reflection spectra (ΔR) for the mixed monolayer DAO:SA = 1:1. B) Normalized reflection spectra ($\Delta R_{\text{norm}} = \Delta R \cdot A_{\text{DAO}}$). The values of total area per molecule, A , are noted in the Figure.

Figure 4A shows representative UV-vis reflection spectra corresponding to different values of molecular area, both before and during the collapse of the mixed monolayer DAO:SA. After the collapse of the mixed monolayer has taken place, the absolute value of the UV-vis reflection is increased approximately three-fold with respect to the value before the collapse. This increase of the UV-vis reflection signal is related to an increase in the surface concentration of the DAO molecule at the air/water interface, eq. 3 and 5.

The normalized UV-vis reflection spectra, ΔR_{norm} , are shown in Figure 4B. The spectra obtained immediately before the collapse (blue) and at the end of the collapse (red) are nearly coincident, therefore confirming that the previously described increase of the UV-vis reflection signal is due to an increase of the surface concentration of DAO molecules and not to a reorientation of the chromophore groups.

The value of the normalized UV-vis reflection spectra, ΔR_{norm} , displays almost no change with the collapse process. On the contrary, the apparent dipole moment per molecule, μ_A , diminishes to one third of the initial value with the collapse. The different trends of these two physical properties per molecule seem contradictory. The explanation for this difference is as follows: the apparent dipole moment per molecule diminishes due to 2/3 of the total molecules are in the top bilayer with opposite orientation, therefore their contribution to the surface potential is cancelled. On the other hand, the normalized UV-vis reflection spectra, ΔR_{norm} , are exclusively depending on the squared transition dipole. Therefore, the global value of reflection intensity is not depending on the orientation of the chromophore groups. In the case of the top bilayer, the chromophore groups have opposite orientations, not affecting the resultant value of the reflection signal.

Figure 5. Schematic model of the trilayer structure formed during the collapse process. DAO molecules are red colored (or green when are superimposed), and SA molecules are blue colored.

These data reinforce the hypothesis of the formation of a trilayer at the air/water interface, where a bilayer is positioned on the monolayer in direct contact with the aqueous phase, as shown schematically in Figure 5. This molecular arrangement agrees with the observed experimental data from the two commented techniques. First, the dipole moments of the top bilayer are cancelled against each monolayer leaflet. Second, the UV-vis reflection signal is increased by three-fold. The observed three-fold increase on the absolute value of the reflection signal corresponds to an increase of the number of absorbing molecules on the probed area by a factor of three. Given that the surface pressure is almost constant during this increase, and that the UV-vis reflection signal exclusively arises from the chromophores at the air/water interface, we assume that there is a multilayer formation, in this case a trilayer.

Synchrotron-based in situ X-ray Reflectivity (XRR).

XRR experiments allow the in situ determination of the vertical electron density profile of soft matter thin films.^{51, 52} Figure 6 shows the reflectivity data of the mixed monolayer DAO:SA monolayer before the collapse, Fig. 6A, $\pi = 34$ mN/m, $A = 0.28$ nm², and after the collapse, Fig. 6B, $\pi = 37$ mN/m, $A = 0.10$ nm².

Figure 6. A) Red circles: X-ray reflectivity for the DOA:SA mixed monolayer at $\pi = 34$ mN/m and $A = 0.28$ nm². Black line: numerical fit using a two box model. The fitting parameters are shown in Table 1. B) Red circles: X-ray reflectivity for DOA:SA mixed monolayer at $\pi = 37$ mN/m and $A = 0.10$ nm². Black line: numerical fit using five box model. The fitting parameters are shown in Table 2.

Before the collapse the reflectivity data have been fitted to a two-box model, being the fitting parameters shown in Table 1. The continuous line in Figure 6A shows the reflectivity using those mentioned parameters. The vertical profile of relative electron density (ρ/ρ_{water}) to the air/water interface plane was determined from the spatial distribution of different atoms using simulations with HyperChem.⁵³ An all-trans configuration of the alkyl chains and a tilting angle of the acridine group of 55°, according to the previously deduced value, have been assumed.³⁸

Figure 7 shows the detailed results of electron density. The relative contributions of the electron densities of the DAO and SA molecules to the total signal are shown as red and blue dashed lines, respectively. The electron density corresponding to the water subphase is modelled as a Gaussian distribution for the contact region with the polar group and plot as the dashed black line. The overall relative electron density is represented as a green solid line.

Table 1. Numerical fit of the reflectivity data obtained at $\pi = 30$ mN/m and $A = 0.28$ nm². Left: parameter obtained from the 2 box model. Right: Parameters obtained in the theoretical model develop in Figure 7. σ , interfacial roughness

	2 box model		Theoretical 3 box model		
	1 box	2 box	1 box	2 box	3 box
ρ/ρ_{water}	0.82 ± 0.07	1.12 ± 0.09	0.4	0.8	1.35
Length / nm	0.81 ± 0.01	1.72 ± 0.01	1.13	0.92	0.96
$\sigma / \text{\AA}$	3.19 ± 0.03		--		
Total Length	2.532 nm		3.01 nm		

A theoretical model using three boxes can be used for simulate the electron density profile based on the molecular arrangement obtained by HyperChem. This is a simple model, e. g., not including the hydrodynamic waves of the air/liquid interface. The polar headgroups of both DAO and SA occupy the closest box to the water, being noted as box 3. The alkyl chains are included in the box 2. Due to the shorter length of the alkyl chain of the DAO molecule when compared to the SA molecule, a segment of the alkyl chains of the SA molecules is included in box 1. Using this theoretical model the mixed monolayer DAO:SA is assumed to be composed of three boxes, see Figure 7. Note that the experimental data are fitted using a model including two boxes. The fitting parameters for both the theoretical model of three boxes and the model for fitting the experimental results of two boxes are shown in Figure 7 and Table 1.

Figure 7. Left: Theoretical vertical profile of the relative electron density for DAO (red dashed line), SA (blue dashed line), water (dashed black line) and overall (green solid line). The solid black line represent the three box theoretical model. Right: Vertical spatial distribution of DAO and SA.

Using a three-box model to fit the experimental data requires a large number of numerical parameters into the model, allowing good fits for different collections of numerical parameters. However, there are significant similarities between the fitting parameters obtained by adjusting the reflectivity with a 2-box and the theoretical model. First, the thickness of the second box in the adjustment, 1.723 nm, is quite close to the sum of the thicknesses of the boxes 2 and 3 of the theoretical model, 1.88 nm. The relative electron density of this second box is $\rho/\rho_{\text{water}} = 1.12$, almost exactly the arithmetic mean value of those electron densities obtained in the theoretical model, $\rho/\rho_{\text{water}} = 0.8$ and 1.35. On the other hand, some discrepancies are found in the parameters of the first box. Note that the alkyl chains have been assumed to adopt an all-trans configuration, which is not correspondent with the experimental situation. A certain tilting of the alkyl chains with respect to the air/water interface plane is expected. The tilting of the alkyl chains leads to a reduction of the observed thickness by X-ray reflectivity, as well as a slight increase in the electronic density value. Both trends are observed in the parameters used for the fitting of the X-ray reflectivity data. Note that the total thickness of the films is in both cases within the same order of magnitude, 2.5 nm for the two-box model, compared to 3.0 nm for the theoretical model.

The reflectivity data for the mixed monolayer DAO:SA at the finalization of the collapse process, shown in Figure 6B as red circles, have been fitted using a 5-box model. In this case a theoretical model has been used based on the existence of a trilayer of DAO:SA at the air/water interface, as depicted in Figure 5 and Figure 8. The electron density of the mixed monolayer DAO:SA is shown as a green line in Figure 8. In the trilayer model, the alkyl chains of the DAO and SA molecules are interdigitated to a certain extent. The interdigitation is defined herein as the overlapping of the alkyl chains of two different monolayers in the Z-axis. The different length of the alkyl chains of the DAO and SA molecules favors the interdigitation, allowing the attractive interactions between the alkyl chains of both

monolayers. The interdigitation of the alkyl chains leads to a supramolecular network with no free space between the three monolayers, contributing to the stability of the trilayer. An all-trans configuration and a perpendicular orientation to the air/water interface of the alkyl chains are assumed. The total thickness of the trilayer is therefore ca. 8.7 nm. The thickness and the relative densities of each box are shown in Figure 8 and Table 2 (right).

Figure 8. Left: Theoretical vertical profile of the relative electron density for DAO (red dashed line), SA (blue dashed line), water (dashed black line) and overall (green solid line). The solid black line represent the five box theoretical model. Right: Vertical spatial distribution of DAO and SA. DAO molecules are red colored, and SA molecules are blue colored.

Figure 6B shows the experimental data and the numerical fitting of the reflectivity data for the DAO:SA trilayer, red circles and black line, respectively. The fitting parameters are shown in Table 2 (left). A certain discrepancy between the fitting parameters and the theoretical model is observed. The most relevant difference concerns the fitting parameters of the boxes 2 and 3. Comparing the fit parameters with the theoretical model (see Table 2), the values of thickness and electron density of box 2 increases by decreasing the parameters of the box 3, keeping approximate constant values of thickness and average electron density. This variation in the fitting parameters of the box 2 can be related to a separation between the boxes 2 and 3, rather rougher than that shown in Figure 8 (theoretical model). The total thickness of the trilayer obtained from the fitting of the reflectivity data is 7.57 nm. The thickness of the trilayer according to the previously described theoretical model is 8.72 nm. This discrepancy can be attributed to two reasons; the alkyl chains in the trilayer are not completely perpendicular to the air/water interface, and the inhomogeneity of the monolayer results in a certain coexistence of monolayer and trilayer at the air/water interface, even for $\pi = 37$ mN/m, $A = 0.10$ nm², see Figure 3.

Table 2. Numerical fit of the reflectivity data obtained at $\pi = 36.8$ mN/m and $A = 0.10$ nm². Left: parameter obtained from the 5 box model. Right: parameter obtained from the 5 box model by fixing the electron densities of the boxes 3 ($\rho/\rho_{\text{water}} = 1.3$), 4 ($\rho/\rho_{\text{water}} = 0.98$) and 5 ($\rho/\rho_{\text{water}} = 1.35$).

	5 box model					Theoretical model				
	Box 1	Box 2	Box 3	Box 4	Box 5	Box 1	Box 2	Box 3	Box 4	Box 5
ρ/ρ_{water}	0.36 ± 0.082	1.0 ± 0.13	1.3 ± 0.1	0.97 ± 0.08	1.2 ± 0.2	0.45	0.94	1.3	0.98	1.35
Length / nm	0.83 ± 0.01	1.73 ± 0.01	1.25 ± 0.01	2.71 ± 0.01	1.05 ± 0.01	1.35	0.95	2.15	3.2	1.07
$\sigma / \text{\AA}$	2.94 $\pm$ 0.05									
Total Length	7.584 nm					8.72 nm				

In spite of the found discrepancies between the experimental electronic density data and those calculated for an ideal trilayer of DAO:SA, the reflectivity data confirm the formation of the three-layer by obtaining thicknesses corresponding to approximately three times those obtained for the monolayer. It is noteworthy that the reflectivity data confirm the existence of a region (box 3) in which there is an increase of the electron density with respect to adjacent boxes (box 2 and 4), which can be exclusively explained if the formation of a trilayer takes place with structure similar to that proposed in Figures 5 and 8.

The dense packing of the alkyl chains suggests the ordering of those chains into a crystalline structure, which should lead to a significant signal in Grazing Incidence X-ray Diffraction (GIXD).⁵⁴ Indeed, we also expected a clear GIXD signal. However, there was no GIXD signal obtained during the synchrotron experiments in any case, either before or after the collapse. The experiments were repeated during a different beam time in order to check the reproducibility, obtaining similar results.

PM-IRRAS measurements

Figure 9 shows the PM-IRRAS spectrum in the 2700-3800 cm^{-1} region of the mixed monolayer DOA:SA. Prior to collapse ($\pi = 20$ mN/m and 30 mN/m), the asymmetric and symmetric stretching mode of methylene, $\nu_{\text{as}}(\text{CH}_2)$ and $\nu_{\text{s}}(\text{CH}_2)$, are barely distinguishable. This fact indicates that the alkyl chains are not oriented vertically to the interface and should not be in a crystalline state. Note also that for $\pi = 20$ mN/m, $A \approx 0.4$ nm^2 , whereas for $\pi = 30$ mN/m, $A \approx 0.3$ nm^2 per alkyl chain. Furthermore, as commented previously, the length of the alkyl chains of DAO and SA molecules differs.

On the contrary, after the collapse, especially when the collapse is completed ($\pi = 40$ mN/m, $A = 0.09$ nm^2), the $\nu_{\text{as}}(\text{CH}_2)$ mode at 2919 cm^{-1} and $\nu_{\text{s}}(\text{CH}_2)$ mode at 2849 cm^{-1} are clearly distinguishable. Previous IR studies have shown that the location of $\nu_{\text{as}}(\text{CH}_2)$ and $\nu_{\text{s}}(\text{CH}_2)$ modes are sensitive indicators for the lateral interactions between alkyl chains.⁵⁵⁻⁵⁹ Thus, the band position for the $\nu_{\text{as}}(\text{CH}_2)$ mode is 8 cm^{-1} lower in the crystalline state (2920 cm^{-1}) than that for the liquid state (2928 cm^{-1}). whereas for the $\nu_{\text{s}}(\text{CH}_2)$ mode the band position is 6 cm^{-1} lower in the crystalline sample (2850 cm^{-1}) than in the liquid (2856 cm^{-1}).^{59, 60} The low wave numbers observed for the $\nu_{\text{as}}(\text{CH}_2)$ and $\nu_{\text{s}}(\text{CH}_2)$ modes are indicative of a tightly packing of the hydrocarbon chains of both DAO and SA at the end of collapse, see Figure 9.

Figure 9. PM-IRRAS spectrum (2700-3800 cm^{-1} region) of the DOA:SA mixed monolayer at different values of surface pressures.

Likewise, the band width, i.e, fwhm full width at half-maximum, of $\nu_{\text{as}}(\text{CH}_2)$ mode has been used to provide a measure of adlayer order.⁵⁹⁻⁶¹ Whereas those bands for the crystalline state are narrower ($\leq 20\text{cm}^{-1}$), bandwidths for the disordered liquid state are very broad ($\geq 28\text{cm}^{-1}$). In this case, the fwhm of $\nu_{\text{as}}(\text{CH}_2)$ band reaches a value of 32 cm^{-1} when the collapse is completed ($\pi = 40\text{ mN/m}$, $A = 0.09\text{ nm}^2$). This value of fwhm indicates that the alkyl chains are rather disordered in liquid state. This experimental observation is in apparent contradiction with the solid state of the alkyl chains as described above by the values of maximum wavenumber of the PM-IRRAS bands, as well as the BAM and X-ray reflectivity. This apparent contradiction is related to the two different environments of the alkyl chains in the trilayer. In the first environment, the alkyls chains of the two monolayers closest to the aqueous subphase are interdigitated, see Figure 5. Therefore a highly packed, quasi-crystalline state is expected. In the second environment, the alkyl chains of the outermost monolayer are in a disordered state, quasi-liquid state. The latter scenario represents approximately 1/3 of the total number of alkyl chains. The position of the $\nu_{\text{as}}(\text{CH}_2)$ mode at 2919 cm^{-1} indicates the quasi-crystalline state of the interdigitated alkyl chains, while the bandwidths indicates the state of disordered chains outer monolayer.

At 3572 cm^{-1} there is a negative band that arises from the OH stretch of water, see Figure 9. The water O-H stretching vibration present in the reference signal is reduced in the reflectivity signal from the monolayer-covered surface because the surfactant layer replaces a water layer and masks partially the OH stretching vibration.³⁷ The result is a strong negative band that is related to the effective thickness of the monolayer. The increase in the intensity of this band to pass from the previous to the posterior collapse region, should be related to increased thickness of the material in the air/water interface, and therefore with the trilayer formation.

CONCLUSION

The collapse process of the mixed Langmuir monolayer DAO:SA has been analyzed by a number of in situ experimental techniques at the air/water interface. The experimental results have demonstrated the formation of an ordered trilayer as a result of the collapse. X-ray reflectivity measurements showed a value of thickness of the trilayer, being ca. 7.6 nm. This value of thickness of the trilayer is three times the value of thickness for the monolayer, ca. 2.5 nm.

In the proposed trilayer model, the first and third monolayers orient its polar group toward the aqueous subphase, while the intermediate layer has an inverted orientation in order to promote interactions between polar groups and between alkyl chains. This molecular arrangement is particularly favored by the strong tendency to self-aggregation of the DAO polar headgroup and by the different length of the alkyl chains of DAO and SA molecules. The interdigitation of the alkyl chains from DAO and SA molecule takes place, as observed by PM-IRRAS, detecting a strong increase of the methylene (CH_2) IR signals during the trilayer formation, being related to a vertical orientation and a quasi-crystalline order of these imbricate alkyl chains.

A striking growth of hexagonal domains with internal anisotropy is observed by BAM during the trilayer formation. The anisotropy has been related to the existence of order due to the acridine polar groups, i.e. both in the monolayer in contact with the aqueous subphase, as in the two additional monolayers.

The in situ visualization of the molecular aggregates formed by the acridine group is feasible thanks to the absorption of radiation from the aggregates at the wavelength used in the laser on BAM experiments. Similar hexagonal domains with internal anisotropy have been observed in the mixed monolayers of dimyristoyl-phosphatidic acid (DMPA) and methylene blue (MB).⁴³ However, in that case the domains are formed during the compression of the monolayer, whereas herein the domains are formed and grow during the collapse process. The same physical phenomenon is responsible for the inner textures inside

the domains in both cases. The dark and bright regions of domains can be related with different orientations of the transition dipoles projections of the acridine group on the air/water interface.

The strong tendency to self-aggregation of the acridine group in the DAO polar headgroup is the main driving force leading to the formation of a trilayer. The interdigitation of the alkyl chains of DAO and SA molecules contributes significantly to the stability of 3D structure of the trilayer, observed as hexagonal domains.

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REFERENCES

1. Lehn, J.-M., Perspectives in Supramolecular Chemistry-From Molecular Recognition towards Molecular Information Processing and Self-Organization. *Angew. Chem. Int. Ed.* **1990**, 29, 1304-1319.
2. Ungar, G.; Tschierske, C.; Abetz, V.; Holyst, R.; Bates, M. A.; Liu, F.; Prehm, M.; Kieffer, R.; Zeng, X.; Walker, M.; Glettner, B.; Zywockinski, A., Self-Assembly at Different Length Scales: Polyphilic Star-Branched Liquid Crystals and Miktoarm Star Copolymers. *Adv. Funct. Mater.* **2011**, 21, (Adv. Funct. Mater.), 1296-1323.
3. Nitoń, P.; Żywociński, A.; Paczesny, J.; Fiałkowski, M.; Hołyst, R.; Glettner, B.; Kieffer, R.; Tschierske, C.; Pocięcha, D.; Górecka, E., Aggregation and Layering Transitions in Thin Films of X-, T-, and Anchor-Shaped Bolaamphiphiles at the Air-Water Interface. *Chem. Eur. J.* **2011**, 17, 5861-5873.
4. Lorenz, C. D.; Travasset, A., Atomistic Simulations of Langmuir Monolayer Collapse. *Langmuir* **2006**, 22, 10016-10024.

5. Vaknin, D.; Bu, W.; Satija, S. K.; Travesset, A., Ordering by Collapse: Formation of Bilayer and Trilayer Crystals by Folding Langmuir Monolayers. *Langmuir* **2007**, 23, 1888-1897.
6. Lee, K. Y. C., Collapse Mechanisms of Langmuir Monolayers. *Annu. Rev. Phys. Chem.* **2008**, 59, 771-791.
7. Rugonyi, S.; Biswas, S. C.; Hall, S. B., The biophysical function of pulmonary surfactant. *Respir. Physiol. Neurobiol.* **2008**, 163, 244-255.
8. Gaines, G. L., *Insoluble Monolayers at Liquid-Gas Interface*. Interscience: New York, 1966.
9. Smith, R. D.; Berg, J. C., The collapse of surfactant monolayers at the air-water interface. *J. Colloid Interface Sci.* **1980**, 74, 273-286.
10. Vollhardt, D.; Retter, U., Nucleation in insoluble monolayers. 1. Nucleation and growth model for relaxation of metastable monolayers. *J. Phys. Chem.* **1991**, 95, 3723-3727.
11. Wagner, J.; Michel, T.; Nitsch, W., Relaxation Behavior and Bilayer Formation of Poly(4-vinylpyridinium) Type Polyelectrolytes at the Air/Water Interface. *Langmuir* **1996**, 12, 2807-2812.
12. Adams, J.; Buske, A.; Duran, R. S., Viscoelastic properties and collapse behavior of a smectic liquid-crystalline polymer at the air/water interface. *Macromolecules* **1993**, 26, 2871-2877.
13. Friedenbergh, M. C.; Fuller, G. G.; Frank, C. W.; Robertson, C. R., Formation of Bilayer Disks and Two-Dimensional Foams on a Collapsing/Expanding Liquid-Crystal Monolayer. *Langmuir* **1994**, 10, 1251-1256.
14. Chen, X.; Wiehle, S.; Weygand, M.; Brezesinski, G.; Klenz, U.; Galla, H.-J.; Fuchs, H.; Haufe, G.; Chi, L., Unconventional Air-Stable Interdigitated Bilayer Formed by 2,3-Disubstituted Fatty Acid Methyl Esters. *J. Phys. Chem. B* **2005**, 109, 19866-19875.
15. Seitz, M.; Struth, B.; Preece, J. A.; Plesnivý, T.; Brezesinski, G.; Ringsdorf, H., Self-organization of amphiphilic N-acylated linear polyethyleneimines: investigation of a reversible monolayer collapse. *Thin Solid Films* **1996**, 284-285, 304-307.
16. Bu, W.; Vaknin, D., Bilayer and Trilayer Crystalline Formation by Collapsing Behenic Acid Monolayers at Gas/Aqueous Interfaces. *Langmuir* **2008**, 24, 441-447.
17. Krafft, M. P., Large Organized Surface Domains Self-Assembled from Nonpolar Amphiphiles. *Acc. Chem. Res.* **2012**, 45, 514-524.
18. Deng, J.; Viers, B. D.; Esker, A. R.; Anseth, J. W.; Fuller, G. G., Phase Behavior and Viscoelastic Properties of Trisilanolcyclohexyl-POSS at the Air/Water Interface. *Langmuir* **2005**, 21, 2375-2385.
19. Gourier, C.; Knobler, C. M.; Daillant, J.; Chatenay, D., Collapse of Monolayers of 10,12-Pentacosadiyonic Acid: Kinetics and Structure. *Langmuir* **2002**, 18, 9434-9440.
20. Haycraft, J. J.; DeVries, C. A.; Garcia-Flores, H.; Lech, A.; Hagen, J. P.; Eckhardt, C. J., Experimental investigation of the reversible collapse of a capped amphiphile Langmuir monolayer. *Thin Solid Films* **2007**, 515, 2990-2997.
21. Nikomarov, E. S., A slow collapse of a monolayer spread on an aqueous surface. *Langmuir* **1990**, 6, 410-414.
22. Pérez-Morales, M.; Pedrosa, J. M.; Martín-Romero, M. T.; Möbius, D.; Camacho, L., Reversible Trilayer Formation at the Air–Water Interface from a Mixed Monolayer Containing a Cationic Lipid and an Anionic Porphyrin. *J. Phys. Chem. B* **2004**, 108, 4457-4465.
23. Plehnert, R.; Schröter, J. A.; Tschierske, C., Influence of the Position of the Hydrophilic Group on the Monolayer Properties of Rigid Amphiphiles with Laterally Attached Headgroups. *Langmuir* **1999**, 15, 3773-3781.
24. Rugonyi, S.; Smith, E. C.; Hall, S. B., Kinetics for the Collapse of Trilayer Liquid-Crystalline Disks from a Monolayer at an Air–Water Interface. *Langmuir* **2005**, 21, 7303-7307.
25. Salfer, R.; Michel, T.; Nitsch, W., About the formation and characterisation of a new phase of polycationic monolayers at the air/water interface. *Colloids Surf., A* **2002**, 210, 253-264.
26. Schief, W. R.; Antia, M.; Discher, B. M.; Hall, S. B.; Vogel, V., Liquid-Crystalline Collapse of Pulmonary Surfactant Monolayers. *Biophys. J.* **2003**, 84, 3792-3806.

27. Schröter, J. A.; Plehnert, R.; Tschierske, C.; Andreas, J.; Janietz, D.; Penacorada, F.; Brehmer, L., Monolayer Properties of a New Family of Amphiphiles with an Unusual Head-Group Topology. *Langmuir* **1997**, 13, 796-800.
28. Buzin, A. I.; Godovsky, Y. K.; Makarova, N. N.; Fang, J.; Wang, X.; Knobler, C. M., Stepwise Collapse of Monolayers of Cycloliner Poly(organosiloxane)s at the Air/Water Interface: A Brewster-Angle Microscopy and Scanning Force Microscopy Study. *J. Phys. Chem. B* **1999**, 103, 11372-11381.
29. de Mul, M. N. G.; Mann Jr., A. M., Multilayer Formation in Thin Films of Thermotropic Liquid Crystals at the Air-Water Interface. *Langmuir* **1994**, 10, 2311-2316.
30. Fang, J.; Dennin, M.; Knobler, C. M.; Godovsky, Y. K.; Makarova, N. N.; Yokoyama, H., Structures of Collapsed Polysiloxane Monolayers Investigated by Scanning Force Microscopy. *J. Phys. Chem. B* **1997**, 101, 3147-3154.
31. Kundu, S., Collapse of preformed cobalt stearate film on water surface. *Colloids Surf., A* **2009**, 348, 196-204.
32. Kundu, S.; Datta, A.; Hazra, S., Effect of Metal Ions on Monolayer Collapses. *Langmuir* **2005**, 21, 5894-5900.
33. Paczesny, J.; Nitoń, P.; Żywociński, A.; Sozański, K.; Holyst, R.; Fiałkowski, M.; Kieffer, R.; Glettner, B.; Tschierske, C.; Pocięcha, D.; Górecka, E., Stable, ordered multilayers of partially fluorinated bolaamphiphiles at the air-water interface. *Soft Matter* **2012**, 8, 5262-5272.
34. Zou, L.; Wang, J.; Beleva, V. J.; Kooijman, E. E.; Primak, S. V.; Risse, J.; Weissflog, W.; Jákl, A.; Mann, E. K., Langmuir Monolayers of Bent-Core Molecules. *Langmuir* **2004**, 20, 2772-2780.
35. Baoukina, S.; Monticelli, L.; Risselada, H. J.; Marrink, S. J.; Tieleman, D. P., The molecular mechanism of lipid monolayer collapse. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, 105, 10803-10808.
36. Dynarowicz-Latka, P.; Dhanabalan, A.; Oliveira Jr., O. N., Modern physicochemical research on Langmuir monolayers. *Adv. Colloid Interface Sci.* **2001**, 91, 221-293.
37. González-Delgado, A. M.; Pérez-Morales, M.; Giner-Casares, J. J.; Martín-Romero, M. T.; Camacho, L., Reversible Collapse of Insoluble Monolayers: New Insights on the Influence of the Anisotropic Line Tension of the Domain. *J. Phys. Chem. B* **2009**, 113, 13249-13256.
38. Jiménez-Millán, E.; Giner-Casares, J. J.; Muñoz, E.; Martín-Romero, M. T.; Camacho, L., Self-Assembly of Acridine Orange into H-Aggregates at the Air/Water Interface: Tuning of Orientation of Headgroup. *Langmuir* **2011**, 27, 14888-14899.
39. Thirumorthy, K.; Nandi, N.; Vollhardt, D., Role of Dipolar Interaction in the Mesoscopic Domains of Phospholipid Monolayers: Dipalmitoylphosphatidylcholine and Dipalmitoylphosphatidylethanolamine. *Langmuir* **2007**, 23, 6991-6996.
40. Valdes-Aguilera, O.; Neckers, D. C., Aggregation phenomena in xanthene dyes. *Acc. Chem. Res.* **1989**, 22, (), 171-177.
41. Buffeteau, T.; Desbat, B.; Turler, J. M., Polarization Modulation FT-IR Spectroscopy of Surfaces and Ultra-thin Films: Experimental Procedure and Quantitative Analysis. **1991**, 45, 380-389.
42. Danauskas, S. M.; Li, D.; Meron, M.; Lin, B.; Lee, K. Y. C., Stochastic fitting of specular X-ray reflectivity data using StochFit *J. Appl. Cryst.* **2008**, 41, 1187-1193.
43. Giner-Casares, J. J.; de Miguel, G.; Pérez-Morales, M.; Martín-Romero, M. T.; Camacho, L.; Muñoz, E., Effect of the Molecular Methylene Blue Aggregation on the Mesoscopic Domain Morphology in Mixed Monolayers with DMPA. *J. Phys. Chem. C* **2009**, 113, 5711-5720.
44. Roldán-Carmona, C.; Giner-Casares, J. J.; Pérez-Morales, M.; Martín-Romero, M. T.; Camacho, L., Revisiting the Brewster Angle Microscopy: The relevance of the polar headgroup. *Adv. Colloid Interface Sci.* **2012**, 173, 12-22.
45. Weidemann, G.; Gehlert, U.; Vollhardt, D., Inner Structure of Condensed Phase Domains in Monolayers at the Air-Water Interface. *Langmuir* **1996**, 11, 864-871.
46. Weidemann, G.; Vollhardt, D., Long-range tilt orientational order in phospholipid monolayers: the inner structure of dimyristoyl-phosphatidyl-ethanolamine domains. *Thin Solid Films* **1995**, 264, 94-103.

47. Gehlert, U.; Vollhardt, D.; Brezesinski, G.; Möhwald, H., Chiral Discrimination in Monolayers of Monoglycerides. *Langmuir* **1996**, 12, 4892-4896.
48. Nandi, N.; Vollhardt, D.; Brezesinski, G., Chiral Discrimination Effects in Langmuir Monolayers of 1-O-Hexadecyl Glycerol. *J. Phys. Chem. B* **2004**, 108, 327-335.
49. Grüniger, H.; Möbius, D.; Meyer, H., Enhanced light reflection by dye monolayers at the air-water interface. *J. Chem. Phys.* **1983**, 79, 3701-3711.
50. Pedrosa, J. M.; Martín-Romero, M. T.; Camacho, L.; Möbius, D., Organization of an Amphiphilic Azobenzene Derivative in Monolayers at the Air–Water Interface. *J. Phys. Chem. B* **2002**, 106, 2583-2591.
51. Ege, C.; Majewski, J.; Wu, G.; Kjaer, K.; Lee, K. Y. C., Templating Effect of Lipid Membranes on Alzheimer's Amyloid Beta Peptide. *ChemPhysChem* **2005**, 6, 226-229.
52. Evers, F.; Shokuie, K.; Paulus, M.; Sternemann, C.; Czeslik, C.; Tolan, M., Exploring the Interfacial Structure of Protein Adsorbates and the Kinetics of Protein Adsorption: An In Situ High-Energy X-ray Reflectivity Study. *Langmuir* **2008**, 24, 10216-10221.
53. HyperChem 7.51 ed.; 1115 NW 4th Street, Gainesville, 32601, Florida, USA, 1999.
54. Kaganer, V. M.; Möhwald, H.; Dutta, P., Structure and phase transitions in Langmuir monolayers. *Rev. Mod. Phys.* **1999**, 71, 779-819.
55. Fernández, A. J.; Ruiz, J. J.; Camacho, L.; Martín, M. T.; Muñoz, E., Orientation and Organization of Langmuir–Blodgett Mixed Films Consisting of a Phospholipid and a Viologen Studied by Infrared Spectroscopy. *J. Phys. Chem. B* **2000**, 104, 5573-5578.
56. Gao, W.; Dickinson, L.; Grozinger, C.; Morin, F. G.; Reven, L., Self-Assembled Monolayers of Alkylphosphonic Acids on Metal Oxides. *Langmuir* **1996**, 12, 6429-6435.
57. Lozano, P.; Fernández, A. J.; Ruiz, J. J.; Camacho, L.; Martín, M. T.; Muñoz, E., Molecular Organization of LB Multilayers of Phospholipid and Mixed Phospholipid/Viologen By FTIR Spectroscopy. *J. Phys. Chem. B* **2002**, 106, 6507-6514.
58. Sinniah, K.; Cheng, J.; Terrettaz, S.; Reutt-Robey, J. E.; Miller, C. J., Self-Assembled .omega.-Hydroxyalkanethiol Monolayers with Internal Functionalities: Electrochemical and Infrared Structural Characterizations of Ether-Containing Monolayers. *J. Phys. Chem.* **1995**, 99, 14500-14505.
59. Snyder, R. G.; Maroncelli, M.; Strauss, H. L.; Hallmark, V. M., Temperature and phase behavior of infrared intensities: the poly(methylene) chain. *J. Phys. Chem.* **1986**, 90, 5623-5630.
60. Snyder, R. G.; Strauss, H. L.; Elliger, C. A., Carbon-hydrogen stretching modes and the structure of n-alkyl chains. 1. Long, disordered chains. *J. Phys. Chem.* **1982**, 86, 5145-5150.
61. Katayama, N.; Enomoto, S.; Sato, T.; Ozaki, Y.; Kuramoto, N., Order-disorder transition in Langmuir-Blodgett films of 2-(4'-(dioctadecylamino)phenylazo)-N-methylbenzothiazolium perchlorate studied by infrared, visible absorption, and resonance Raman spectroscopies. *J. Phys. Chem.* **1993**, 97, 6880-6884.

SYNOPSIS TOC