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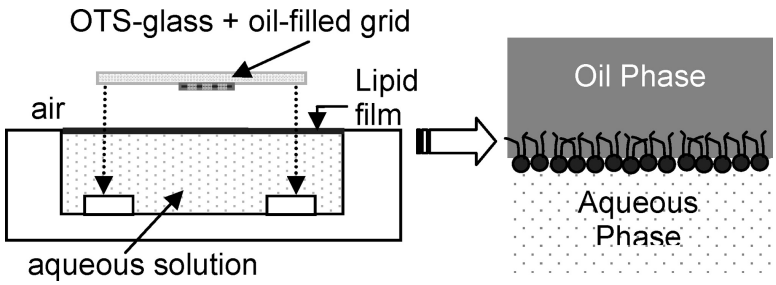


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# Preparation of Microscopic and Planar Oil−Water Interfaces That Are Decorated with Prescribed Densities of Insoluble Amphiphiles

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***Abstract:*** Langmuir monolayers (monolayers of insoluble molecules formed at the surface of water), and associated Langmuir−Blodgett/Schaefer monolayers prepared by transfer of Langmuir films to the surfaces of solids, are widely used in studies aimed at understanding the physicochemical properties of biological and synthetic molecules at interfaces. In this article, we report a general and facile procedure that permits transfer of Langmuir monolayers from the surface of water onto microscopic and planar interfaces between oil and aqueous phases. In these experiments, a metallic grid supported on a hydrophobic solid is used to form oil films with thicknesses of 20 $\mu$m and interfacial areas of 280 $\mu$m × 280 $\mu$m. Passage of the supported oil films through a Langmuir monolayer is shown to lead to quantitative transfer of insoluble amphiphiles onto the oil−water interfaces. The amphiphile-decorated oil−water interfaces hosted within the metallic grids (i) are approximately planar, (ii) are sufficiently robust mechanically so as to permit further characterization of the interfaces outside of the Langmuir trough, (iii) can be prepared with prescribed and well-defined densities of amphiphiles, and (iv) require only ∼200 nL of oil to prepare. The utility of this method is illustrated for the case of the liquid crystalline oil 4-pentyl-4′-cyanobiphenyl (5CB). Transfer of monolayers of either dilauroyl- or dipalmitoylphosphatidylcholine (DLPC and DPPC, respectively) to the nematic 5CB-aqueous interface is demonstrated by epifluorescence imaging of fluorescently labeled lipid and polarized light imaging of the orientational order within the thin film of nematic 5CB. Interfaces prepared in this manner are used to reveal key differences between the density-dependent phase properties of DLPC and DPPC monolayers formed at air−water as compared to that of nematic 5CB−aqueous interfaces. The methodology described in this article should be broadly useful in advancing studies of the interfacial behavior of synthetic and biological molecules at liquid−liquid interfaces.

## Introduction

The Langmuir film balance method, in which insoluble molecules are spread onto the surface of water and then compressed into a monolayer by lateral movement of a barrier that passes through the surface of the water, has led to a remarkable range of insights into the equations of state and morphologies of interfacial phases that can be formed by polymers,[1−4] amphiphilic molecules,[5,6] proteins,[7] and nanoparticles.[8−12] Langmuir monolayers of molecules have also been transferred from the surface of water to the surfaces of a variety of solids, via vertical (Langmuir−Blodgett)[13−15] or horizontal (Langmuir−Schaefer)[7,16] deposition. The resulting solid-supported films have been used to fabricate interfacial structures and devices[17−20] and have also been used to enable further characterization of the structure and properties of monolayer films by allowing transfer of the films to instruments for surface

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analysis (e.g., scanning probe instruments).[16,21,22] In this article, we report that it is possible to adapt the Langmuir−Schaefer technique to allow insoluble amphiphiles assembled at the surface of water in well-defined thermodynamic states within a Langmuir trough to be transferred to microscopic, planar, and mechanically robust oil−water interfaces. This procedure provides a general and facile method to prepare oil−water interfaces decorated with prescribed and well-defined densities of amphiphilic molecules, thus enabling the study of these buried interfaces. We illustrate the utility of this approach by demonstrating quantitative transfer of phospholipids from the surface of water onto the interface between a 20-$\mu$m-thick film of liquid crystalline oil and an aqueous phase, thereby providing insights into the phase behavior of the lipids at this interface as well as the coupling that occurs between the lipids and the orientational ordering of the liquid crystal.

Monolayer films of phospholipids have been widely used as biological membrane analogues to enable physicochemical studies of biomolecular interactions that occur at membrane surfaces.[5,6,23−25] In particular, measurements employing monolayers prepared using the Langmuir balance technique at the air−aqueous interface have provided important insights into a range of complex lipid behaviors, such as the formation of lipid domains and lipid−protein complexes.[23,26−33] Recently, interfaces formed between aqueous phases and water-immiscible, liquid crystalline oils have been identified as a novel and potentially useful subclass of interfaces for the study of biological membrane mimics.[34−39] The orientational ordering of the liquid crystal (LC) has been found to couple to the presence and organization of the lipids at this interface. Because the orientational ordering of molecules within liquid crystalline phases is highly cooperative and can extend over distances of tens of micrometers, the LC provides a means to amplify nanoscopic events into the optical domain. These prior studies also reveal that both protein binding events as well as enzymatic processing of the lipids can trigger ordering transitions in the liquid crystal that are readily observed by polarized light microscopy.[34,35,37,39] Many fundamental issues remain to be elucidated, however, such as the influence of the ordering of the LCs on interfacial organization of the lipids, including their phase behavior. The methodology reported in this article provides an avenue to such studies.

Prior studies have revealed that a diverse range of interfacial phenomena involving surfactants,[38,40] lipids,[34−36,38] proteins,[34,35,37,39] and polymers[41] can occur at aqueous−LC interfaces. Advances in this area, and more generally advances in understanding the behaviors of molecules assembled at oil−water interfaces, however, have been limited by the absence of general methods that permit the preparation of these interfaces with defined densities of molecules in ways that enable their characterization. For example, although the Langmuir film balance has been used to prepare oil−water interfaces,[42−44] these interfaces are confined to the Langmuir trough (limiting the range of surface analytic methods that can be used to study these interfaces), they are large in area (cm × cm) and mechanically fragile, and they require the use of large volumes of oils (>mL). In addition, when working with liquid crystalline oils, it is very difficult (we did not succeed)[40] to prepare stable films of liquid crystalline oils that are sufficiently thin (<100 $\mu$m) to preserve orientational ordering of the LC across the entire thickness of the film. The procedure reported in this article is based on oil films that are hosted within microwells defined by a metallic grid supported on a hydrophobic solid. Passage of the supported oil film through a Langmuir monolayer is shown to lead to quantitative transfer of amphiphiles onto the oil−water interface. These interfaces are approximately planar, sufficiently robust mechanically so as to permit transfer of the interfaces out of the Langmuir trough, and can be prepared with prescribed and well-defined densities of amphiphiles. In addition, only small quantities of oil (∼200 nL) are required to prepare the interfaces. The utility of this method is illustrated for the case of a liquid crystalline oil 4-pentyl-4′-cyanobiphenyl (5CB). The amphiphiles used in the study are phospholipids, and the transfer of phospholipids at temperatures above and below their bilayer melting temperatures ($T_m$) is shown to enable studies of lipid phase behavior at this novel oil−aqueous interface. In this article, we also demonstrate the applicability of this methodology to oils other than LCs, thus establishing generality.

## Materials and Methods

**Materials.** L-DLPC and L-DPPC were purchased from Avanti Polar Lipids, Inc. Head group-labeled Texas Red-DPPE (Texas Red 1,2-dihexadecanoyl-*sn*-glycero-3-phosphatidylethanolamine, TR-DPPE) was obtained from Invitrogen. The LC 5CB (K15) was obtained from EMD Chemicals. Tris(hydroxymethyl)aminomethane (Tris), hydrochloric acid, sodium chloride, and octadecyltrichlorosilane (OTS) were obtained from Sigma-Aldrich. Chloroform (HPLC grade), hexadecane, and Fisher's Finest glass microscope slides were obtained from Fisher Scientific. Methanol and anhydrous ethanol were purchased from Aaper Alcohol and Chemical Co. Nondrying microscopy immersion oil (type B) was purchased from Cargille Labs. All chemicals were used as received. Deionization of a distilled water source was performed using a Milli-Q system (Millipore), yielding water with a resistivity of 18.2 MΩ. Gold

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specimen grids (20 $\mu$m thickness, 75 mesh, with square holes 283 $\mu$m $\times$ 283 $\mu$m) were obtained from Electron Microscopy Sciences.

**Preparation of LC-Filled Specimen Grids.** Glass slides were cleaned for 1 h at ~80 °C in piranha solution: 70% (v/v) sulfuric acid and 30% (v/v) hydrogen peroxide (*warning: piranha solution reacts strongly with organic compounds and should be handled with extreme caution. Do not store in closed containers).* The slides were rinsed with deionized water and methanol and dried for 12 h at 120 °C. The glass slides were then functionalized with OTS according to published procedures.[40]

Gold specimen grids were placed onto the OTS-treated glass slides. The grids were filled with approximately 200 nL of 5CB using a blunt-tipped syringe, such that the grid was evenly filled with LC. Care was taken to fill the grids to the appropriate level such that a flat film of LC was obtained with reproducible thickness (see Supporting Information for a more detailed description). The LCs (and other oils described below) were held within the grids by capillary forces and not gravity. The grids could be inverted without loss of LCs (oil) from the grids.

**Langmuir Film Preparation and Langmuir−Schaefer Transfer.** Materials were wrapped in aluminum foil and kept in the dark throughout the following procedure. All phospholipids were stored as powders at −8 °C, and solutions were prepared from powder and used for up to 3 days before being discarded. To make quantitative comparisons of the fluorescence intensity of lipid films prepared via Langmuir−Schaefer transfer and vesicle adsorption (as described below), we found it necessary to treat the lipid solutions in a similar fashion for both experiments, as handling of the TR-DPPE probe led to changes in the emission intensity of the dye (likely due to photobleaching). Therefore, before spreading a Langmuir film, a 1 mM TR-DPPE-doped DLPC solution was first dried under a gentle stream of nitrogen gas until it formed a thin film along the inner walls of the glass vial. The lipid film was then dried under vacuum for at least 2 h, before redissolving in chloroform for the formation of the Langmuir film. Langmuir films were prepared using a KSV Minimicro film balance equipped with a platinum Wilhelmy plate for surface pressure measurements. Ten microliters of a 1 mM chloroform solution of the phospholipids (DLPC, DLPC doped with 0.02% (mol) TR-DPPE, or DPPC doped with 0.1% TR-DPPE) was spread at the air−water interface in ca. 0.25-$\mu$L droplets at 40 °C. The solvent was allowed to evaporate for 20 min at 40 °C and then 40 min at 25 °C before film compression was initiated. Spreading of the 0.02% TR-DPPE/DLPC film from chloroform solution and subsequent solvent evaporation at 40 °C resulted in a homogeneously mixed monolayer, as determined by fluorescence microscopy. In contrast, spreading and drying at 25 °C resulted in inhomogeneous mixing with TR-rich and TR-poor regions. Here we also note that initial experiments were performed with BODIPY-labeled DPPE-doped films (0.5%) of DLPC at the air−water interface (Supporting Information). However, a nonlinear trend in fluorescence intensity as a function of area density was observed when using the BODIPY-labeled DPPE, likely a result of excimer formation and self-quenching at high area densities.[45] Symmetric film compression was performed at a rate of 5.0 mm/min (8.5 Å$^2$·mol·min$^{-1}$). Once a desired surface pressure was reached, the LC-filled grids (supported on OTS-treated glass) were lowered into horizontal contact with the air−water interface using tweezers and immediately submerged into the subphase for fluorescence and polarized light measurements. The lipid remaining at the air−water interface was removed by aspiration before imaging of the LC−water interface.

**Imaging of the Phospholipid Films.** The phospholipid films were imaged at the air−water and water−LC interface by epifluorescence microscopy using an Olympus IX71 inverted microscope equipped with a 100 W mercury lamp and filter cube with 560-nm excitation filter and 645-nm emission filter. Images were collected with a Hamamatsu 1394 ORCA-ER-CCD camera interfaced to a computer using SimplePCI software (Compix, Inc.). Quantitative fluorescence imaging was performed at 10× magnification with an exposure time of 0.3 s. Background fluorescence intensity was determined using a lipid monolayer that did not contain TR-DPPE. Fluorescence intensity was measured away from the edges of the grids. Polarized light microscopy was performed on the same microscope, using cross-polarizers in transmission mode and plane-polarized white light.

**Preparation of Vesicles.** Vesicles were prepared according to published procedures,[36] as briefly described below. Texas Red-DPPE from the stock solution used to prepare Langmuir−Schaefer films was used in the preparation of the vesicles (to address batch-to-batch variations in concentration and fluorescence activity of TR-DPPE). Phospholipids (and mixtures of phospholipids and 5CB) were dissolved in chloroform (1−50 mg/mL) and dispensed into brown glass vials in volumes that led to a final concentration of 0.1 mM phospholipid upon resuspension. Before resuspension, the lipid was dried under a gentle stream of $N_2$ (g) until it formed a thin film along the inner walls of the glass vial. The lipid was then dried under vacuum for at least 2 h. The dried lipid was hydrated by incubation in the aqueous phase (water or buffer) for at least 1 h and vortexed for 1 min. Subsequent sonication of the lipid suspension using a probe ultrasonicator (3 × 5 min at 8−9 W, with intermittent cooling to maintain the solution temperature below ~60 °C) resulted in a clear solution. The solutions were extruded three times through a 0.22-$\mu$m pore filter (Millipore) before use. The vesicles were used within 24 h of their preparation.

**Lipid Film Formation via Vesicle Fusion.** The LC-filled grids were submerged into ~2.5-mL wells containing 1 mL of the aqueous phase (water or TRIS buffer). An aliquot of the 100 $\mu$M phospholipid dispersion was added to the aqueous phase to yield a final concentration of 50 $\mu$M. The LC-filled grids were allowed to incubate in the lipid dispersion for 1−2 h. Epifluorescence measurements revealed no further change in fluorescence of the TR-DPPE-doped lipid-laden LC−aqueous interface after the first hour of lipid adsorption to the 5CB−aqueous interface. To stop lipid adsorption, the entire vessel was submerged into 1 L of lipid-free aqueous phase for 5 min. The LC-filled grids were then transferred into the aqueous subphase of the Langmuir trough and positioned at the same location used for imaging of samples prepared by Langmuir−Schaefer transfer.

**Fluorescence Spectroscopy of Vesicle Dispersions.** Fluorescence spectra were obtained using a Jobin Yvon Horiba Fluoromax-3 spectrometer. An excitation wavelength of 560 nm was used, and emission intensity was recorded in 0.5-nm increments.

## Results

**Transfer of Lipids and Characterization by Epifluorescence.** The experimental setup used to decorate oil−water interfaces with well-defined densities of amphiphiles is shown in Figure 1. In the first series of experiments described in this article, the oil is the nematic liquid crystal 5CB, and it is hosted within a gold specimen grid that is supported on a hydrophobic glass surface (OTS-treated glass, Figure 1A). We note here that direct application of the Langmuir balance technique to interfaces between liquid crystalline oils and water is not possible, as elastic stresses present in a liquid crystalline film will exert additional forces on a Wilhelmy plate and thus contribute to the measurement of an apparent surface pressure. Before attempting transfer of DLPC from the air−water interface to the aqueous−LC interface, we verified the surface pressure ($\pi$) versus area (A) isotherms at the air−water interface. Figure 2 shows representative $\pi$−A isotherms obtained using either DLPC (dashed line) or DLPC doped with 0.02% (mol) Texas Red-DPPE (solid line). These isotherms correspond to a lipid film in the liquid-expanded state throughout the compression

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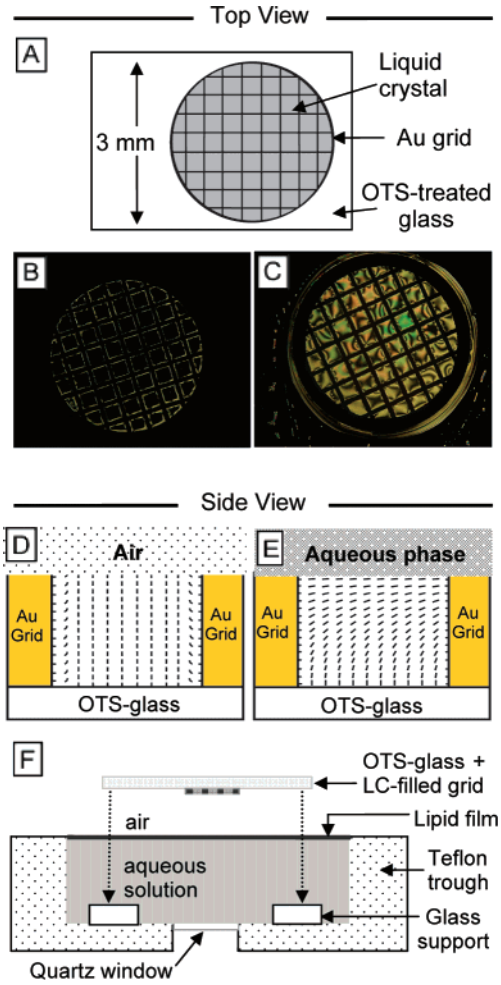


***Figure 1.*** Experimental setup used to create stable, flat, thin films of thermotropic liquid crystal 5CB for Langmuir−Schaefer transfer. Polarized light micrographs (B, C) and corresponding schematic side views of 5CB (D, E) confined to a gold grid in contact with air (B, D) or an aqueous phase (C, E). (A, F) Schematic representation of LC-filled grids (A) and configuration used for Langmuir−Schaefer transfer (F).

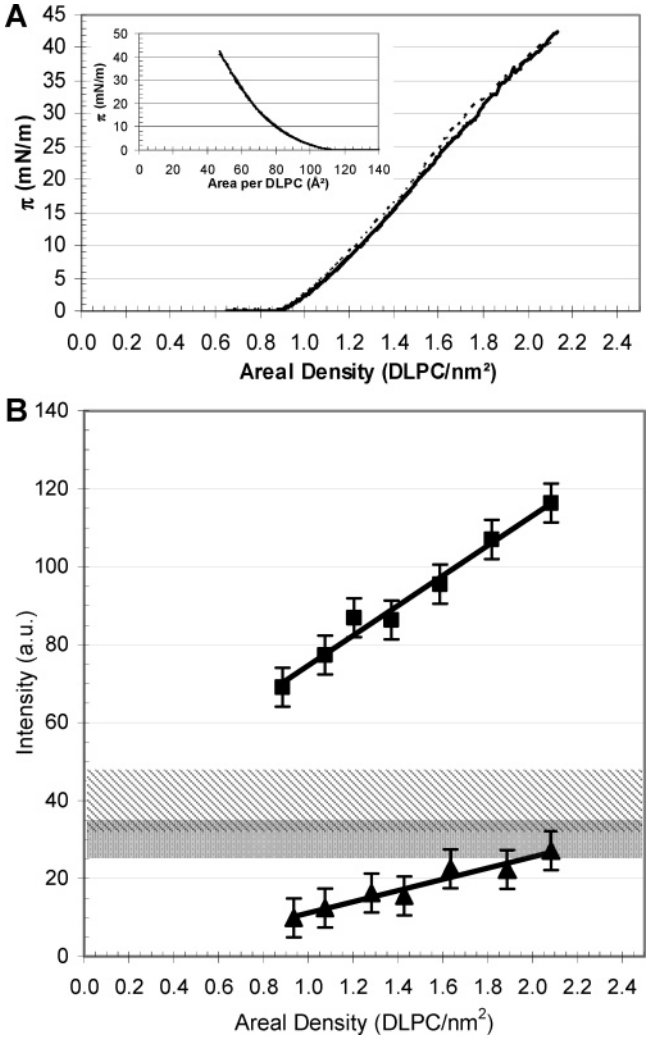


***Figure 2.*** (A) Surface pressure ($\pi$)−area density isotherms at 25 °C for pure DLPC monolayer (dashed line) and a DLPC monolayer doped with 0.02% TR-DPPE (solid line). (Inset) $\pi$−Area isotherms. (B) Fluorescence intensity of 0.02%TR-DPPE/DLPC films measured at the air−water interface (■) and at the LC−water interface (▲) with corresponding linear regressions of $y = 38x + 36$ and $r^2 = 0.9812$ and $y = 14x - 3.1$ and $r^2 = 0.9479$, respectively. The horizontal regions correspond to the range of fluorescence values observed on OTS glass (top, hatched bar) and on 5CB (bottom, solid bar) after exposure to a 50 $\mu$M vesicle solution for 2 h a.u. = arbitrary units.

range, as is typically observed for DLPC monolayers at 25 °C at the air−water interface.[44,46,47] Collapse of the film was observed at surface pressures in the range of 42−45 mN/m, corresponding to a mean molecular area of 47 ± 8 Å$^2$, while the onset of surface pressure was observed in the range of 105−115 ± 8 Å$^2$. The effect of the 0.02% (mol) TR-DPPE on the isothermal compression of DLPC was found to be minimal (inspection of Figure 2A reveals the isotherms of DLPC and TR-DPPE-doped DLPC to be almost co-incident).

We next sought to determine if it was possible to quantitatively transfer lipid from the air−water interface to the LC−aqueous interface. We used a method modified from that described previously for the transfer of monolayers to a silanized glass substrate.[16] We found that Langmuir−Schaefer transfer (from above the air−water interface) onto a fluid support, such as an oil or LC film, required the rapid and continuous passage of the support through the air−water interface. A slow passage, as well as attempts to hold the LC film in contact with the Langmuir film, resulted in the spreading of LC onto the air−water interface. The optimal transfer speed and angle at contact were estimated to be ∼10−20 cm/s and within 5° of horizontal contact, respectively. *In situ* epifluorescent microscopy of the lipid film before and after transfer from the air−water interface to the LC−aqueous interface was performed to assess the effect of Langmuir−Schaefer transfer on the area density of the film. At the air−water interface, DLPC doped with 0.02% TR-DPPE gave rise to a linear fluorescence intensity with increasing area density until monolayer collapse at 47 Å$^2$ (Figure 2B). The linear trend persisted for the films on the LC−water interface, consistent with transfer of lipid from the air−water interface to the LC−water interface. We note, however, that the intensity of fluorescence was found to be substantially lower at the LC−water interface, a point that we return to below.

**Optical Microscopy of the LC Films after Langmuir−Schaefer Transfer.** Past studies by Brake *et al.* have found that spontaneous adsorption of lipid monolayer films from

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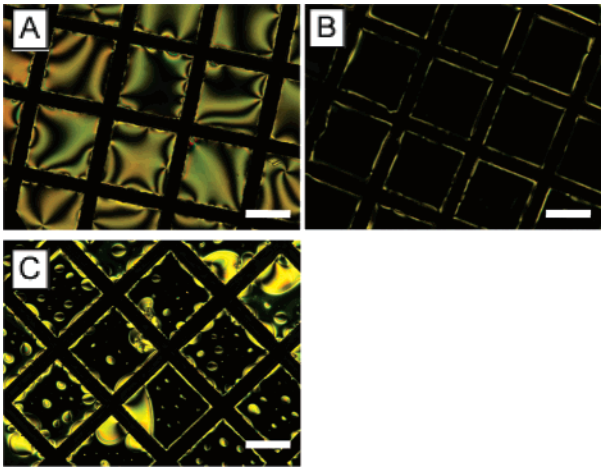


***Figure 3.*** Optical micrographs (cross-polars) of DLPC films at LC−water (A, B) and LC−aqueous (Tris buffer) interfaces (C). Lipid area densities corresponding to (A) ≤1.7 DLPC molecules/nm$^2$ (tilted/planar LC anchoring), (B) >1.8 DLPC molecules/nm$^2$ (homeotropic), and (C) 1.3−1.7 DLPC molecules/nm$^2$ (mixed planar/homeotropic). Scale bar corresponds to 200 $\mu$m.

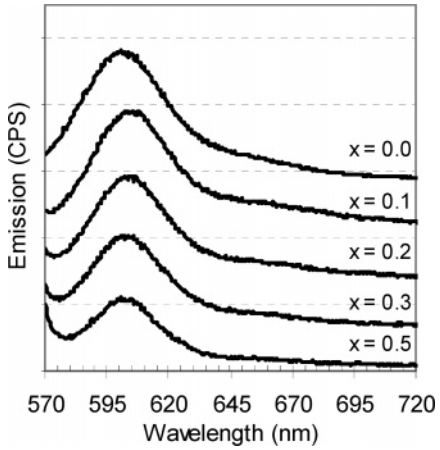


***Figure 4.*** Fluorescence emission spectra (560-nm excitation) corresponding to 0.02%TR-DPPE/DLPC vesicles containing mole fractions ($x$) of 5CB ranging from $x = 0$ to $x = 0.5$. Spectra have been offset for clarity.

aqueous suspensions of lipid onto the LC−aqueous interface leads to orientational ordering transitions in the LC film that can be readily observed by the transmission of polarized light through the LC.[34,36] The assembly of lipid at the LC−aqueous interface resulted in a reorientation of the LC at the interface from an initially near-planar orientation (parallel to the interface) to a homeotropic orientation (perpendicular to the interface). Here we report on the optical appearance of the LC by polarized light microscopy (transmission mode) to provide further evidence of lipid transfer from the air−water interface to the LC−aqueous interface. Transmission of polarized light (cross-polars) through the lipid-laden LC films revealed the optical appearance of the LC with area density of lipid less than 1.7 ± 0.2 DLPC/nm$^2$ (Figure 3A) to be similar to lipid-free films of LC in pure water (Figure 1C). Within these films of LC, the tilt angle of 5CB, relative to the surface normal, increases continuously from ∼0° (homeotropic orientation) at the OTS−5CB interface, to ∼90° (planar orientation) at the 5CB−aqueous interface through bend and splay distortions of the LC (Figure 1E). Variation in the azimuthal orientation of the 5CB results in dark brush patterns within the grid squares. When Langmuir films with densities close to monolayer collapse (2.1 ± 0.2 DLPC/nm$^2$) were transferred to the LC−aqueous interface, the LC was observed to orient homeotropically (Figure 3B; same LC director profile as shown in Figure 1D). In these samples, optical microscopy (cross-polars) revealed a uniformly dark region within the grid squares, with bright edges arising from 5CB interactions with the Au grid.[48] These results, when combined, provide additional evidence for Langmuir−Schaefer transfer of DLPC from the air−water to the LC−aqueous interface.

**Epifluorescence and Optical Microscopy of Lipid Films Prepared by Vesicle Fusion.** A surprising result emerging from the above-described fluorescence microscopy (Figure 2B) was that the intensity of fluorescence at the aqueous−LC interface was substantially lower than that at the air−water interface. To provide insight into this observation, we prepared DLPC-laden (doped with 0.02% TR-DPPE) interfaces of 5CB by fusion of vesicles from bulk aqueous solutions, as reported previously.[36] We compared the fluorescence intensity of lipid-laden aqueous−LC interfaces prepared by vesicle fusion to that measured using Langmuir−Schaefer transfer (Figure 2B). Inspection of Figure 2B reveals close agreement in the overall fluorescence intensity obtained from the lipid films prepared by the two methods. This agreement, as well as the agreement in the resulting orientations of the LC films, suggests that the final lipid density prepared from the two methods is the same. The fluorescence intensity of the lipid films prepared via both Langmuir−Schaefer transfer and vesicle fusion was also measured on the OTS-glass adjacent to the LC-filled grids. In both cases, it was found that the net fluorescence measured on OTS-glass was slightly greater (∼5 a.u.) than that measured on the LC−aqueous interface (Figure 2B), and much less than that measured at the air−water interface (∼75 a.u.). We hypothesized that the differences in fluorescent intensity arose from the microenvironment of the Texas Red (TR), which has been reported to affect the net fluorescence of the TR (see below). To provide further insight into this proposition, in the next section we report an investigation of the influence of the microenvironment created by 5CB on fluorescence of TR-DPPE.

**Effect of 5CB on TR-DPPE Fluorescence.** To investigate the effect of 5CB on the local microenvironment of the TR-DPPE, we measured the fluorescence emission spectra of aqueous dispersions of DLPC vesicles with 0.02% (mol) TR-DPPE that were doped with 5CB (Figure 4).

Figure 4 shows that without incorporation of 5CB, TR-DPPE in DLPC vesicles exhibits emission peaks located at 602 and 660 nm (shoulder). Upon incorporation of 5CB into the vesicles, we observe that the two emission peaks are dramatically reduced in intensity, indicating quenching of the TR emission. The peak intensity is reduced by one-half upon incorporation of 5CB into the vesicles at a mole fraction of 0.5, indicating that quenching upon incorporation of 5CB into the vesicles is significant. This result is consistent with the loss in fluorescence intensity observed in Figure 2B upon transfer of TR-DPPE from the air−water interface to the 5CB−aqueous interface.

The interpretation of results obtained using fluorescent labels requires consideration of the inherent sensitivity of fluorophores toward changes in microenvironment (i.e., pH, ionic strength, solvent polarity) and their propensity to form aggregates that

(48) Occasionally, a few domains of LC with a planar orientation were observed within what was primarily (>90% area) a homeotropically aligned LC film.

may exhibit little to no fluorescence. The results shown in Figure 4 indicate that 5CB is likely promoting fluorescence quenching via the formation of nonfluorescent aggregates, a phenomenon that has been observed for Texas Red and other dyes incorporated into lipid monolayers or bilayers.[45,49,50] Texas Red, although considered to have relatively low sensitivity toward changes in solvent polarity and charge, does exhibit a tendency to form aggregated species even at low surface concentrations. Dutta *et al.* have reported a 23 nm shift in the TR-DPPE 0−0 emission band, and the emergence of a shoulder band, when it was mixed into DPPC monolayers at the air−water interface (as compared to its emission in chloroform solution).[50] The shift was determined to arise from the formation of (fluorescent) J-aggregates organized laterally within the monolayer. The intensity of the emission bands was found to decrease as the film was compressed to higher surface pressure (higher area density), indicating quenching via the effective energy transfer to a nonfluorescent aggregated state. In another study, Biadasz *et al.* found that mixing naphthalene-based fluorescent dyes in Langmuir−Blodgett (LB) films of arachidic acid and the nematic LC 4-octyl-4′-cyanobiphenyl (8CB) led to highly quenched fluorescence emission due to aggregate formation, along with a slightly red-shifted emission wavelength.[49]

**Phase Properties of Lipid Films at the LC−Aqueous Interface.** The above-described results obtained using fluorescence measurements (linear rise in fluorescence intensity to a value corresponding to saturation coverage, Figure 2B) indicate quantitative transfer of lipid from the aqueous−air interface onto the aqueous−LC interface. The quantitative transfer of lipid at known densities onto the interfaces of LCs provides a new experimental capability with which to investigate density-dependent phase properties of these monolayer systems. Previous reports of submonolayer DLPC films adsorbed to the LC−aqueous interface (Tris buffer, pH 8.9, 100 mM NaCl) describe formation of lipid-poor domains during film growth and corresponding patterning of the orientation of the LC.[36] Price and Schwartz have subsequently reported domains of similar optical appearance when using fatty acids at the LC−aqueous interface.[51] Although domains of lipid and associated patterning of the orientations of LCs have been observed, insights into their origins have been hindered by a lack of methods that permit formation of lipid films of known area densities at the LC−aqueous interface. In this context, we sought to illustrate the utility of the Langmuir−Schaefer method reported in this article by preparing DLPC films at various densities at the air−aqueous interface (TRIS buffer subphase) and transferring these films to the LC−aqueous interface via the Langmuir−Schaefer technique (Langmuir isotherm is provided in Supporting Information). After transfer, the films were allowed to equilibrate for 2 h and were found to be stable for up to at least 12 h. These investigations revealed that films of lipid with area densities ranging from 1.3 to 1.7 DLPC/nm$^2$ formed lipid-poor domains and led to associated patterning of the orientation of the LC (Figure 3C). Lipid films with area densities less than 1.3 DLPC/nm$^2$ caused uniformly planar LC orientations, while films with area densities of lipid greater than 1.7 DLPC/nm$^2$ caused uniformly homeotropic LC orientations. This result is an interesting one because DLPC has not been reported previously to phase separate within monolayers formed at air−water and isotropic oil interfaces.[5,6] The result suggests that the phase behavior of lipids at aqueous−LC interfaces can be substantially different from the interfaces of isotropic phases, a proposition that is supported by the experiment described below.

**Preparation of Oil−Water Interfaces Decorated with Lipids with High Bilayer Melting Temperatures.** The development of the Langmuir−Schaefer method to prepare lipid-laden LC−aqueous interfaces, as reported in this article, was motivated by the observation that methods based on vesicle fusion do not provide a means to specify in advance the area density of lipid deposited onto the LC interface.[34−36] In addition, we comment here that the preparation of lipid films at the LC−aqueous interface via vesicle fusion is not applicable to all types of lipids and is complicated by the phase state of the lipid vesicles.[34−36] Unlike DLPC, which forms vesicles with bilayers in the liquid crystalline phase at room temperature, DPPC within the bilayer of a vesicle is in a gel phase (solid-like) at room temperature. DPPC films formed at the LC−aqueous interface via vesicle fusion at room temperature possess a heterogeneous appearance when viewed by fluorescence microscopy.[36] These results suggest that the fusion of lipid vesicles to the LC−aqueous interface is dependent upon the lipid-phase state. In light of this observation, we next sought to determine if the Langmuir−Schaefer transfer method described in this article could be used to prepare homogeneous and reproducible DPPC-laden interfaces of LC. The preparation of DPPC monolayers via Langmuir−Schaefer transfer from the air−water interface was performed at densities of 0.83, 1.1, and 2.5 DPPC/nm$^2$ (or 120, 90, and 40 Å$^2$/DPPC, respectively). As shown in Figure 5, these area densities correspond to the liquid expanded ($L_\alpha$), coexistence ($L_\beta$−$L_\alpha$), and condensed ($L_\beta$) phase of the DPPC isotherm at the air−water interface, respectively. A higher concentration of TR-DPPE (0.1%), which partitions to the $L_\alpha$ phase over the $L_\beta$ phase, was added to the DPPC to more readily observe the phase-separated domains associated with the $L_\alpha$−$L_\beta$ coexistence regime by epifluorescence microscopy. In each case, transfer of the monolayer of DPPC to the LC−aqueous interface resulted in a film with homogeneous appearance when imaged by epifluorescence and polarized light microscopy. The lower area density films of DPPC at the LC−aqueous interface gave rise to planar orientation of the LC (Figure 5B,C), while the highest area density lipid film transferred gave rise to a perpendicular (homeotropic) LC orientation (Figure 5H,I). Interestingly, the phase-separated DPPC films corresponding to coexisting $L_\alpha$−$L_\beta$ phases at the air−water interface (Figure 5D) also became *homogeneous* (of a single phase) upon transfer to the LC−water interface. In contrast, the same films, when transferred to OTS-treated glass, still exhibited the phase-separated domains (Figure 5E). These results, when combined with those obtained using DLPC films at the LC−aqueous interface described earlier, indicate that the phospholipid phase behavior at aqueous−LC interfaces differs in complex ways from the phase behavior at the air−water interface. These results also confirm that the LC−lipid interactions leading to the orientational ordering of the LC at its aqueous interface are highly dependent on lipid area density.

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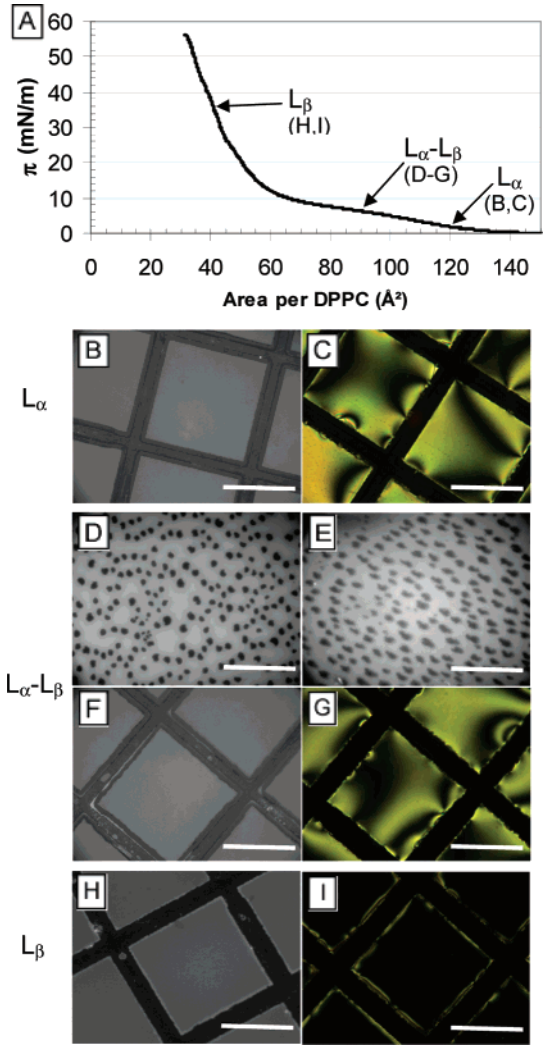


***Figure 5.*** (A) $\pi$−Area isotherm at 25 °C for a DPPC monolayer doped with 0.1% TR-DPPE. Arrows depict area densities corresponding to conditions under which Langmuir−Schaefer transfer was performed. (B, D−F, H) Fluorescence images of 0.1%TR-DPPE/DPPC monolayers (D) at the air−water interface, and after transfer to the LC−water interface (B, F, H) and to OTS glass (E). Corresponding polarized light micrographs (C, G, I) of films at the LC−water interface. The area densities of the DPPC films are 120 ($L_\alpha$ phase), 90 ($L_\alpha$−$L_\beta$ coexistence), and 40 Å$^2$/molecule ($L_\beta$ phase). Scale bar corresponds to 200 $\mu$m.

Prior studies by Kühnau and co-workers[52,53] as well as Hiltrop and Stegemeyer[54,55] investigated the orientational ordering of nematic 5CB on LB films of phospholipids supported on glass. These studies also identified the lipid area density as a key parameter influencing the orientational ordering of LCs at lipid-laden interfaces. In contrast to our studies, however, Hiltrop and Stegemeyer reported that homeotropic anchoring was only observed for LB films of phosphatidylcholine lipids transferred in the $L_\alpha$ phase, while those in the condensed phase $L_\beta$ result in either tilted or planar LC orientation.[54,55] Our results, when compared to these past studies of lipids supported on solid surfaces, suggest that the mobile and deformable nature of the LC−aqueous interface leads to lipid−LC interactions that differ from those at lipid monolayer-decorated surfaces of solids.

The results reported in this article also indicate that the lipid phase behavior at the LC−aqueous interface is markedly different from that observed at the air−water interface and air−glass interface. This conclusion is supported by the prior reports by Kühnau *et al.* using Langmuir monolayers of DPPC mixed with 5CB, where condensed phases of DPPC in the ($L_\alpha$−$L_\beta$) coexistence regime were lost upon incorporation of 5CB in the monolayer.[52] Möhwald and co-workers have also reported significant changes in the phase behavior of DPPC Langmuir monolayers in contact with films of isotropic oils.[56,57] Although a detailed description of the phase behavior of DLPC and DPPC monolayers at the LC−aqueous interface is beyond the scope of this study, our results suggest that systematic measurement of lipid phase behavior at the LC−aqueous interface is required to shed light on the nature of LC−lipid interactions leading to the orientational ordering of LCs at aqueous−LC interfaces. The generality of the Langmuir−Schaefer method for preparing lipid monolayers at the LC−aqueous interface should enable the pursuit of these and other studies.

**Transfer onto Isotropic Oil−Water Interfaces.** We end this article by demonstrating that the methodology we report for preparing lipid-laden interfaces is generally applicable to a range of oil−water interfaces, including isotropic oils. To this end, monolayers containing 0.02% TR-DPPE/DLPC at an area density 2.1 DLPC/nm$^2$ were transferred onto two isotropic oil films having different viscosities: hexadecane ($\eta$ = 3.0 mPa·s) and immersion oil ($\eta$ = 1.2 Pa·s). The oils were hosted in 20 $\mu$m-thick gold grids and supported on the same OTS glass slide to permit direct comparison with 5CB. Fluorescence imaging of the lipid films at the hexadecane−water and immersion oil−water interfaces yielded intensities of fluorescence that were greater (5−10 a.u., see Figure 2) than those measured at the 5CB−water interface but comparable within experimental error (±5 a.u.) to those measured on the OTS−glass interface. These results indicate that fluorescence quenching is occurring at these interfaces, as discussed above in the context of 5CB−water interfaces. Similar results were obtained for films prepared by vesicle fusion with these interfaces. From these results, we conclude that the Langmuir−Schaefer transfer method described in this article can also be used to transfer lipids onto aqueous interfaces of isotropic oils.

## Conclusions

The results reported in this article define a general and facile method to prepare planar, oil−aqueous interfaces that can be decorated with prescribed densities of insoluble amphiphiles. The method exploits the use of the Langmuir film balance to preorganize monolayers of molecules at the surface of water at well-defined densities and thermodynamic states, and the Langmuir−Shaefer method to transfer these monolayers to microscopic oil−water interfaces stabilized within microwells. We have demonstrated the general applicability of the methodology by using several oils and by preparing oil−water interfaces decorated with amphiphiles that do not readily transfer to the interface via spontaneous adsorption. The method is

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shown to be particularly well-suited to the study of interfaces of liquid crystalline oils because the sample volumes required are small (~200 nL) and the thickness of the film of oil can be controlled in the 20 $\mu$m range, thus defining the orientation of the LC across the entire film. By transferring DLPC and DPPC onto LC−aqueous interfaces at defined interfacial densities, we reveal that the area-dependent phase behaviors of these lipids are substantially altered in the presence of the LC. In summary, the methodology presented this article enables quantitative studies of interfacial molecular assemblies formed by synthetic and biological molecules at liquid−liquid interfaces.

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**Supporting Information Available:** Additional experimental details regarding the preparation of LC-filled grids, the fluorescence of BODIPY-doped DLPC monolayers, and the $\pi$−area isotherm for DLPC obtained using an aqueous solution of Tris buffer. This material is available free of charge via the Internet at http://pubs.acs.org.

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