# Elastic Energy-Driven Phase Separation of Phospholipid Monolayers at the Nematic Liquid-Crystal–Aqueous Interface

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Experimental measurements and a thermodynamic model reveal that nematic elasticity can induce lateral phase separation of amphiphilic molecules assembled at interfaces between thermotropic liquid crystals (LCs) and immiscible aqueous phases. The morphologies of the phase-separated domains of amphiphiles induced by nematic elasticity are shown to be strongly dependent on the nature of the deformation of the LC. This study provides important insight into the physics that controls the ordering of molecules at interfaces of soft anisotropic materials, and identifies a new mechanism of phase separation at these interfaces.

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The organization and dynamics of molecules assembled at interfaces between anisotropic and isotropic phases of matter underlie phenomena as diverse as nucleation and growth of crystalline solids, electronic transport properties of organic molecules deposited onto inorganic semiconductors, and the interactions between biological molecules at the surfaces of living cells, including formation of lipid rafts. Synthetic liquid crystals (LCs) define another important class of anisotropic material interfaces that exhibit particularly rich physics in micrometer-scale systems because the energetics associated with interfacial and bulk ordering are often comparable in their magnitudes. A number of past studies have reported on this interplay of surface and bulk effects in situations where monolayers of molecules are confined to interfaces between solids and LCs [1]. In contrast to the interfaces of solids, where the lateral mobility of molecules within surface-confined monolayers is typically low, interfaces between LCs and immiscible isotropic liquids are characterized by high mobility and thus provide an unusual opportunity to study coupling between the anisotropic ordering of a bulk material and the ordering of monolayers of molecules adsorbed at the surface of the material [2–4]. In the study described in this Letter, we report that the lateral organization of phospholipids at interfaces between isotropic aqueous phases and nematic LCs is coupled to the elastic energy stored within the LC. We demonstrate that the long-range nematic ordering of the LC phase can impose itself on the phospholipid monolayer to induce phase separation and lateral organizations of the resulting phospholipid domains that are not seen when the same lipids are assembled at interfaces between isotropic media (liquid-liquid and gas-liquid, as studied widely in the past [5,6]).

A number of previous studies have demonstrated that the nematic order of a LC can impose itself on the organization of small solute molecules [7], biological macromolecules [8], and mesoscopic particles that are hosted within the bulk of the LC [3,9]. In particular, topological defects and associated distortions of a LC induced by particles can mediate forces with dipolar and quadripolar symmetries that act between the particles to induce organizations not observed in isotropic liquids [10,11]. In this study, we report that distorted states of LCs can also generate effective forces between amphiphilic molecules that are assembled at interfaces of the LCs. We provide unambiguous evidence of a new mechanism that couples the nematic elasticity of LCs to the lateral organization of the amphiphiles.

We first performed an experiment to determine if the nematic ordering of a LC influences the assembly and organization of phospholipid molecules adsorbed to an interface formed between the LC and an aqueous phase. This experiment used the phospholipid dilaurylphosphatidylcholine (L-DLPC) and the LC 4′-pentyl-4-cyanobiphenyl (5CB). The ordering of L-DLPC has been widely studied at air-water and isotropic oil-water interfaces [5,6]: at 25 °C, L-DLPC forms liquid-like, homogeneous surface phases at those interfaces. In our experiments, we exploited the relatively low temperature at which nematic 5CB undergoes a bulk phase transition to an isotropic oil ($T_{NI}$ was measured to be $\simeq$33 °C in our experiments). We hosted 5CB in the pores of electron microscopy grids supported on octadecyltrichlorosilane (OTS)-treated glass slides: each pore defines an interface between the aqueous phase (Tris buffer saline) and 5CB that is 283 $\mu$m $\times$ 283 $\mu$m in area. The concentration of lipid at the aqueous-LC interface was controlled by varying the time of adsorption of lipid. The results shown in Fig. 1 were obtained by adsorbing lipid to the aqueous-5CB interface for 10 min from a 10 $\mu$M unilamellar vesicle solution of L-DLPC (doped with 0.1% Texas-Red-DPPE). Inspection of Fig. 1(a) (polarized light micrograph) and Fig. 1(b) (fluorescence micrograph) reveals the presence of birefringent and lipid-lean domains upon adsorption of

 

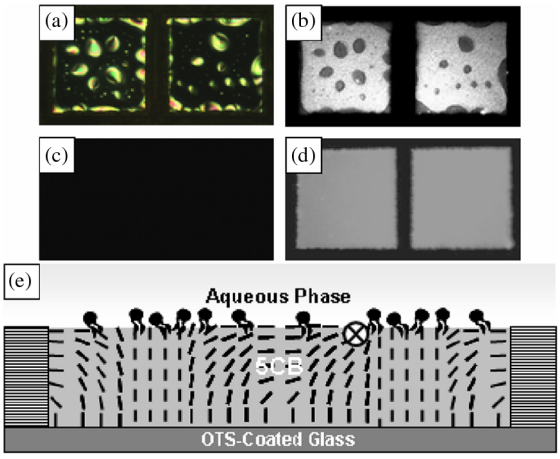


FIG. 1 (color online). (a) Polarized light micrograph (crossed polars) of nematic 5CB with L-DLPC-laden interface. (b) The corresponding fluorescent image. (c), (d) Images of the samples in (a) and (b) heated to 34 °C and equilibrated at that temperature for 2 h. Cartoon in (e) illustrates the lateral distribution of lipid and patterned orientation of 5CB corresponding to (a) and (b). The fluorescent micrographs were digitally enhanced to emphasize the contrast. The circled cross at the interface in (e) shows the location of a defect.

L-DLPC at 32 °C. This result demonstrates that the lipid-lean domains formed at 32 °C lead to patterned orientations of the nematic 5CB. As depicted in Fig. 1(e), the bright regions in Fig. 1(a) correspond to LC that is anchored perpendicular to the OTS-treated glass and parallel (planar) to the LC-aqueous interface, while the black regions correspond to LC that is anchored perpendicular to both the OTS-treated glass and LC-aqueous interface. Upon heating to 34 °C, however, the birefringent and lipid-lean domains were observed to disappear, as shown in Fig. 1(c) and 1(d). Control experiments indicate negligible uptake of lipid into 5CB above $T_{\mathrm{NI}}$. In addition, no lipid-lean domains were observed to form upon adsorption of L-DLPC to 5CB in its isotropic phase at 34 °C. Domains, however, were observed to form upon cooling of the lipid-decorated 5CB from 34 °C to 32 °C (and persiste for at least 38 h). Although lipid monolayers at interfaces of isotropic materials display temperature-dependent phase behavior [5], the close coincidence of the temperature of the bulk phase transition of the 5CB and surface phase transition of the lipid monolayer, as described above, suggests that the two transitions are closely coupled. This proposition is supported by the additional observations that (i) no lipid domains form above $T_{\mathrm{NI}}$ for any L-DLPC density at the aqueous-5CB interface, and (ii) that lipid-lean domains formed using the LC TL205 (a mixture of cyclohexane-fluorinated biphenyls and terphenyls, $T_{\mathrm{NI}} = 86\,^{\circ}\mathrm{C}$) persisted up to 60 °C. These observations, when combined, suggest that the nematic order in the film of LC induces phase separation of the adsorbed amphiphiles. Several past studies by us [12] and others [13] have hinted at a possible coupling between the organization of amphiphiles and the nematic order of LCs at these interfaces, but the mechanism of coupling was not clearly established.

We considered two mechanisms by which the nematic ordering of the 5CB may lead to the formation of lipid domains reported in Fig. 1. The first mechanism considers the nematic order to mediate an effective short-range attraction between the lipid tails (characterized by $\chi$ in the equation below). Past studies have demonstrated that nematic ordering leads to low solubility of alkanes [14] and flexible polymers due to constraints on conformational degrees of freedom [15]. The second mechanism considers the influence of the nematic elasticity of the film of LC, as described through a simple thermodynamic model [5] in which we write the free energy of a monolayer of lipid supported on a film of LC to include the elastic energy associated with distortion of the LC ($\Delta G_{\mathrm{elastic}}$) and surface anchoring energy ($\Delta G_{\mathrm{anchoring}}$) of the LC. For a film of LC supported on a substrate such as OTS-treated glass that causes strong perpendicular anchoring of the director, the free energy is written as

$$\frac{\Delta G}{K_B T} = [\underbrace{n_1 \ln x + (N - n_1)\ln(1 - x)}_{\Delta S_{\mathrm{mixing}}} + \underbrace{\chi N x(1 - x)}_{\Delta H_{\mathrm{mixing}}}] + \left[\underbrace{\frac{1}{2}\frac{K(T)}{D}\theta_S^2}_{\Delta G_{\mathrm{elastic}}} + \underbrace{\frac{1}{2}W\sin^2(\theta_S - \phi(x))}_{\Delta G_{\mathrm{anchoring}}}\right]\frac{NA^*}{K_B T},$$

where $n_1$ is the number of molecules of lipid at the interface, $x$ is the fraction of saturation monolayer coverage, $N$ is the number of lipids on the interface at saturation, $\theta_S$ is the tilt of LC at the interface, $\phi$ is the easy angle of the LC, $A^*$ is the area per lipid molecule at saturation coverage, $\chi$ is a pairwise interaction parameter for lipid molecules at the interface, $K(T)$ is the temperature-dependent elastic constant of the LC (assuming splay and bend elastic constants to be equal [1]), $W$ is the anchoring strength coefficient, and $D$ is the thickness of the film of LC. Past experiments demonstrate that the easy axis of the LC changes from parallel to perpendicular to the interface with increasing density of lipid [4]: to approximate this observation, we used the sigmoidal function $\phi(x) = \pi/2[1 - 1/\{1 + \exp(-A(x - x_C))\}]$, where $A = 150$ and $x_c = 0.8$. We used the model to explore the consequences of strong ($W = \infty$) and weak ($W = 10^{-6}\ \mathrm{J/m^2}$) anchoring of the LC by the lipid. As shown in Fig. 2, the free energy calculated as a function of mole fraction of lipid adsorbed at the interface reveals that nematic elasticity in the presence of either strong or weak anchoring can lead to a miscibility gap. Phase separation is not observed in the absence of nematic elasticity, and the tendency to phase separate is lower for the case of weak anchoring as com-

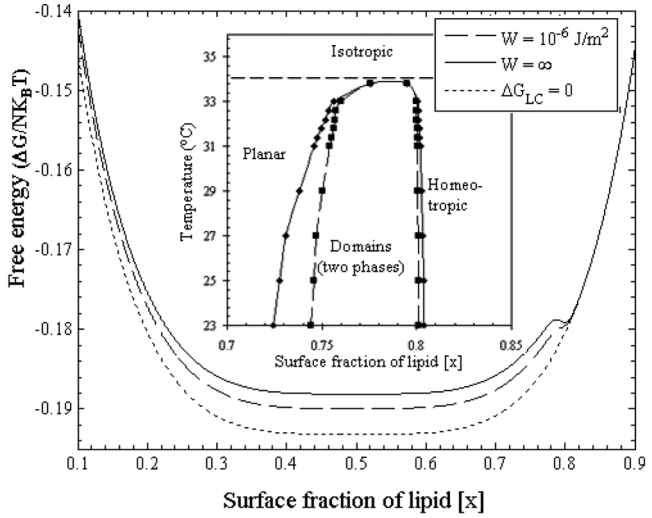


FIG. 2. Plot of dimensionless free energy versus mole fraction of lipid at the aqueous-5CB interface in the presence ($E = 0.005$; $W = \infty$ or $W = 10^{-6}$ J/m$^2$)/absence ($E = 0$; $\Delta G_{LC} = 0$) of elastic energy for $\chi = 2$. $E$ is the dimensionless elastic energy calculated as $K\pi^2 A^*/8DK_BT$. Inset: Phase diagram of lipid at aqueous-LC interface for the case of strong ($W = \infty$) and weak anchoring ($W = 10^{-6}$ J/m$^2$).

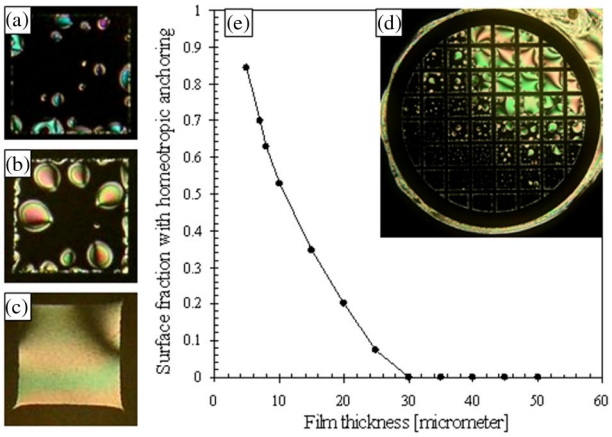


FIG. 3 (color online). Polarized light micrographs (crossed polars) of lipid-decorated films of 5CB hosted within grids with thicknesses of (a) 5 $\mu$m, (b) 20 $\mu$m, and (c) 40 $\mu$m. The concentric rings evident in the domains shown in (b) suggest continuous variation of the orientation of LC across the domains. The image in (d) shows a film of lipid-decorated LC having a continuous gradient in thickness (thicker region is top right). The plot in (e) shows the model-predicted decrease in the fraction of surface area with homeotropic anchoring as a function of LC film thickness (calculated for $x = 0.74$ and $W = \infty$).

pared to strong anchoring. The inset in Fig. 2 shows the phase diagram of the adsorbed lipid phase as a function of temperature. A key conclusion that emerges from the above model is that nematic elasticity of the LC can induce phase separation of the lipid monolayer under conditions that do not lead to phase separation in the absence of nematic elasticity (Fig. 2). Whereas in the absence of nematic order spinodal decomposition can occur only when $\chi > 2$, the model reveals that nematic elasticity leads to spinodal decomposition for values of $\chi$ that are less than 2. For example, if $E = 10^{-4}$ and $W = \infty$, spinodal decomposition occurs for $\chi > 1.64$.

The above model predicts that changes in the elastic energy stored within the LC, such as can be achieved by changing the LC film thickness or by changing the anchoring of the LC at the supporting solid surface, should lead to changes in the organization of the lipid. In a first experiment to test this proposition, L-DLPC was adsorbed at 35 °C from a 10 $\mu$M solution onto the interface of 5CB hosted in gold grids with thicknesses of 5, 20, or 40 $\mu$m. After adsorption of L-DLPC for 5 min, the lipid solution was displaced by buffer and the system was cooled to 25 °C. Figure 3(a)–3(c) shows polarized light micrographs of the three LC-filled grids after 2 h of equilibration. Inspection of Fig. 3(a)–3(c) reveals that domains are formed on the interfaces of the LCs with thicknesses of either 5 or 20 $\mu$m but that no domains of lipid are evident on the interface of the LC with a thickness of 40 $\mu$m. Furthermore, inspection of Fig. 3(d) reveals an evolution in the lateral distribution of lipid across a sample that varies continuously in LC film thickness. These observations are consistent with the prediction of our model [Fig. 3(e)], which shows a decrease in the fraction of surface area with homeotropic alignment upon increase in film thickness for a fixed amount of lipid at the interface ($x = 0.74$). The above experimental observations cannot be explained on the basis of the effect of nematic order on $\chi$, which is independent of film thicknesses. These experimental results lead us to conclude that elastic energy stored in LC films plays a role in the lateral phase separation of L-DLPC at these interfaces. Because quantitative interpretation of the fluorescent micrographs in Fig. 1 is complicated by the effects of chromophore orientation [16], we quantified the orientational response of the LC to known lipid densities using the Langmuir-Schaefer method to transfer L-DLPC monolayers prepared at known densities from the air-water interface onto the aqueous-5CB interface [17,18]. Using procedures similar to those described in past studies [18], a LC-filled grid was passed from air into water through a Langmuir monolayer of lipid (and was subsequently maintained under water) in order to transfer the lipid onto the aqueous-5CB interface. Using this technique, densities of L-DLPC ranging from 1.3 to 1.6 molecules/nm$^2$ were determined to give rise to lipid-lean domains and patterned orientations of the 5CB. Assuming an areal density of 2 molecules/nm$^2$ for saturation coverage of L-DLPC at the aqueous-LC interface, we observe good general agreement between the predictions of our model and our experimental observations (phase separation for $x = 0.65$ to 0.8). The above result also demonstrates that domains form when lipid is delivered to the interface via fusion of

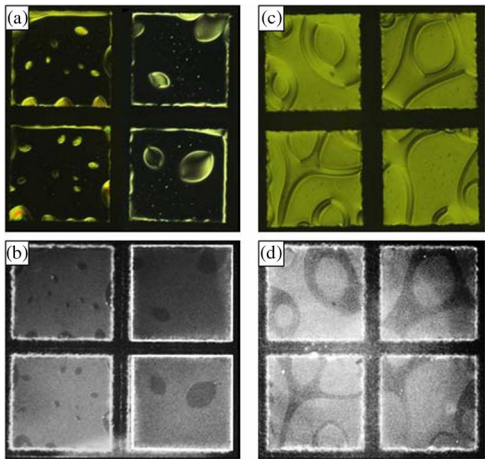


FIG. 4 (color online). Polarized light (a), (c) and fluorescence (b), (d) images of aqueous-TL205 interfaces decorated with L-DLPC (25 °C). The results shown in (a) and (b) correspond to a film of TL205 on a solid support that causes homeotropic anchoring, whereas (c) and (d) correspond to images obtained using a support that causes parallel anchoring. L-DLPC was adsorbed from a 5 $\mu$M solution at room temperature for 20 min for (a) and (b) and 35 min for (c) and (d).

vesicles as well as Langmuir-Schaefer transfer, a result that is consistent with equilibrium domain formation.

A final experiment that demonstrates that nematic elasticity can be used to manipulate the organization of phospholipids involved the use of nematic TL205. Similar to 5CB, when a monolayer of L-DLPC was formed at the aqueous interface of nematic TL205 supported on an OTS-treated glass substrate (homeotropic anchoring), discontinuous domains that were lean in L-DLPC were observed [Figs. 4(a) and 4(b)]. When nematic TL205 was anchored parallel to the supporting solid (by using a monolayer of pentadecanethiol on a gold film), we also observed domains of L-DLPC to form at the interface of the LC. In contrast to the case of OTS-treated glass, however, we observed the discontinuous surface phase to correspond to lipid-rich regions [Fig. 4(c) and 4(d)]. These results demonstrate that qualitative changes in the shapes of the coexisting surface phases accompany changes in the elastic energy stored in the supporting film of nematic LC. Whereas stored elastic energy is released from the nematic film by formation of lipid-rich regions in the experiment described in Fig. 4(a), the accumulation of lipid into localized regions of interface in Fig. 4(c) leads to storage of elastic energy in the LC. These results, when combined, lead to the observation that discontinuous surface phases tend to form on regions of the LC that are distorted. The distorted state of the LC beneath these surface phases may mediate forces that act to inhibit the coalescence of these phase domains, as has been observed with droplets of isotropic liquids at LC-air interfaces [19].

In conclusion, we report experimental observations and a simple thermodynamic model that provide clear evidence of a mechanism by which the nematic elasticity of a film of LC can trigger the phase separation and lateral organization of amphiphiles. Under conditions where phase separation will not occur in the absence of nematic order, we observe nematic elasticity to provide a mechanism leading to new surface phases. Furthermore, manipulation of the elastic energy stored or released within a film of LC is shown to cause changes in the morphology of the coexisting surfaces phases. The generality of the mechanism proposed defines an opportunity to control interfacial order via manipulation of bulk nematic order of a LC and also suggests that other surface phases likely exist in these systems. More generally, our study demonstrates that LCs can provide useful experimental systems with which to gain insights into the physics that control the ordering of molecules at interfaces of anisotropic materials. Whereas past studies [4,13] have demonstrated that molecular assemblies formed at aqueous-LC interfaces influence the ordering of the LC, the model and supporting experiments described in this Letter unmask the role that the LCs can play in directing the organization of the interfacial molecular assemblies.

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