

## A Stripe-to-Droplet Transition Driven by Conformational Transitions in a Binary Lipid–Lipopolymer Mixture at the Air–Water Interface

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We report the observation of an unusual stripe–droplet transition in precompressed Langmuir monolayers consisting of mixtures of poly(ethylene) glycol (PEG) amphiphiles and phospholipids. This highly reproducible and fully reversible transition occurs at approximately zero surface pressure during expansion (or compression) of the monolayer following initial compression into a two-dimensional solid phase. It is characterized by spontaneous emergence of an extended, disordered stripe-like morphology from an optically homogeneous phase during gradual expansion. These stripe patterns appear as a transient feature and continuously progress, involving gradual coarsening and ultimate transformation into a droplet morphology upon further expansion. Furthermore, varying relative concentrations of the two amphiphiles and utilizing amphiphiles with considerably longer ethylene glycol headgroups reveal that this pattern evolution occurs in narrow concentration regimes, values of which depend on ethylene oxide headgroup size. These morphological transitions are reminiscent of those seen during a passage through a critical point by variations in thermodynamic parameters (e.g., temperature or pressure) as well as those involving spinodal decomposition. While the precise mechanism cannot be ascertained using present experiments alone, our observations can be reconciled in terms of modulations in competing interactions prompted by the pancake–mushroom–brush conformational transitions of the ethylene glycol headgroup. This in turn suggests that the conformational degree of freedom represents an independent order parameter, or a switch, which can induce large-scale structural reorganization in amphiphilic monolayers. Because molecular conformational changes are pervasive in biological membranes, we speculate that such conformational transition-induced pattern evolution might provide a physical mechanism by which membrane processes are amplified.

### Introduction

Liquid–liquid immiscibility in a rich variety of dipolar fluids produces strikingly similar morphologies in their equilibrated coexisting phases.<sup>1</sup> Fluid material systems as disparate as amphiphilic monolayers,<sup>2</sup> membrane bilayers,<sup>3</sup> thin films of magnetic fluids or ferrofluids,<sup>4</sup> self-assembled monolayers of immiscible ligands on nanoparticles,<sup>5</sup> and type I superconducting fluids<sup>6</sup> produce familiar spatial patterns primarily consisting of lamellar stripes and circular droplets. These patterns are understood, for some time now, to originate from a generic competition between long-range repulsive interactions and short-range attractive ones.<sup>1</sup> Specifically, interfacial tension, which relates to the cost of creating the pattern boundaries between the thermodynamically distinct coexisting fluids (e.g., expanded and condensed, compositionally different amphiphilic phases, oppositely magnetized domains, or normal and superconducting regions), favors the smallest interfacial length. This is opposed by a long-range repulsive dipolar force (e.g.,

molecular dipoles of amphiphilic headgroups, permanent or induced magnetization), which promotes an extended boundary between the phases. The balance of these two opposing free energy contributions then set the modulation period.

A key feature of coexisting fluid patterns is that the phase boundaries are not rigid. Rather they are highly dynamic—susceptible to a variety of shape, structural, and compositional distortions giving rise to interesting morphological instabilities.<sup>7,8</sup> Intrinsic thermal fluctuations, changes in thermodynamic parameters (e.g., pressure and temperature in monolayers), and external fields (e.g., hydrodynamic shear in amphiphilic monolayers) can all drive these patterns to evolve by altering the balance of competing interactions.<sup>8–10</sup> For instance, as the balance of competing interactions shifts toward a net increase in dipolar repulsion (or a reduction in line tension), initially circular domains (with line tension dictating the original shape) can undergo shape transitions, exhibiting elliptical, bending, and branching distortions. Conversely, an increase in line tension (or a reduction in dipolar repulsion) can induce rupture and coarsening of thin, continuous stripes producing disorder at the free ends. For larger or systematic shifts in the balance of competing interactions, these distortions can provide a critical mode for pattern evolution, driving interconversion between

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(1) Seul, M.; Andelman, D. *Science* **1995**, *267*, 476–483.

(2) McConnell, H. M. *Annu. Rev. Phys. Chem.* **1991**, *42*, 171–195.

(3) Veatch, S. L.; Cicuta, P.; Sengupta, P.; Honerkamp-Smith, A.; Holowka, D.; Baird, B. *ACS Chem. Biol.* **2008**, *3*, 287–293.

(4) Seul, M.; Monar, L. R.; Ogorman, L.; Wolfe, R. *Science* **1991**, *254*, 1616–1618.

(5) Singh, C.; Ghorai, P. K.; Horsch, M. A.; Jackson, A. M.; Larson, R. G.; Stellacci, F.; Glotzer, S. C. *Phys. Rev. Lett.* **2007**, *99*, 226106.

(6) Cebers, A.; Gourdon, C.; Jeudy, V.; Okada, T. *Phys. Rev. B* **2005**, *72*, 1–16.

(7) Benvegnu, D. J.; McConnell, H. M. *J. Phys. Chem.* **1992**, *96*, 6820–6824.

(8) Seul, M.; Sammon, M. *J. Phys. Rev. Lett.* **1990**, *64*, 1903–1906.

(9) Dickstein, A. J.; Erramilli, S.; Goldstein, R. E.; Jackson, D. P.; Langer, S. A. *Science* **1993**, *261*, 1012–1015.

(10) Seul, M. *Adv. Mater.* **1992**, *4*, 521–523.

droplet and stripe patterns.<sup>11</sup> Understanding conditions that drive evolution of patterns of coexisting fluid phases is important for a variety of reasons. First, they allow assessing the role of kinetic (e.g., large activation barriers for nucleation, fission, or coalescence of domains) and thermodynamic (e.g., energy landscapes) factors in understanding disorder-mediated pattern transformations in broad classes of fluid materials systems with competing interactions.<sup>7,12</sup> Second, they afford a mechanism by which structural and compositional heterogeneities are maintained in fluid mixtures with little energetic expenditure, e.g., cellular membranes.<sup>13</sup>

Fundamental studies of such pattern emergence and evolution have been most extensively carried out using binary amphiphilic monolayers at the air–water interface, especially in the vicinity of their miscibility critical points.<sup>14</sup> Indeed, Langmuir monolayers of cholesterol–phospholipid mixtures at the air–water interface have proven particularly valuable.<sup>15,16</sup> Immiscibility in these binary interfacial mixtures is characterized by the formation of two coexisting phases: one rich in cholesterol and the other, phospholipid. In the vicinity of their critical points, epifluorescence microscopy reveals that these amphiphilic monolayers exhibit extensive striping due to low line tension flanked by an interspersed droplet phase at higher line tensions and a homogeneous phase at vanishing line tensions on either side of the critical point.<sup>2</sup> Previously, pattern evolution and dynamics in amphiphilic monolayers have been experimentally realized by traversing critical points via variations in thermodynamic conditions. For a given composition, temperature or surface pressure (or lateral density) can be varied to gain access to conditions for pattern evolution. Related types of pattern formation and evolution are also theoretically predicted and experimentally observed when line tension interactions compete with long-range repulsive interactions due to elastic deformations (e.g., curvature generation).<sup>17–20</sup> In this regard, it seems that perturbations in molecular properties that influence the competing line tension and electrostatic interactions (e.g., headgroup conformations, chain elongation, ligand binding, etc.) should also produce conditions for pattern evolution via shift in equilibration.<sup>21,22</sup> However, to our knowledge, examples of dynamic reorganization in fluid patterns induced by changes in molecular level properties, such as those likely to occur in membranes of living systems, are quite sparse.

In the work reported here, we study the phase behavior of interfacial Langmuir monolayers consisting of binary mixtures of poly(ethylene) glycol (PEG)-terminated surfactants and saturated phospholipids—a model composition for drug and therapeutic

delivery systems developed for practical biomedical applications.<sup>23–25</sup> We find that precompressed mixtures of PEG amphiphiles and phospholipids, upon expansion, reveal a remarkable stripe to droplet transition under conditions of fixed temperature ( $\sim 25^\circ\text{C}$ ) and a nominally constant surface pressure of 0 mN/m typically characterizing low-density liquid expanded/gas phase coexistence. Specifically, the homogeneous phase formed upon expansion of a precompressed monolayer in a narrow composition range about 75:25 of 1,2-dipalmitoyl-sn-glycerol-3-phosphocholine (DPPC) and polyoxyethylene stearate (PEG<sub>n</sub>S,  $n = 8$ ) is instantaneously replaced by an extended, disordered stripe or “labyrinthine”-like morphology when the surface pressure is at 0 mN/m. Upon further expansion, these stripes spontaneously coarsen in a manner reminiscent of reverse fingering instability<sup>1</sup> and ultimately are replaced by circular droplets. Additional experiments varying the size of the PEG unit and relative composition of the two amphiphiles in the mixture reveal that this pattern evolution occurs in narrow concentration regimes, whose values depend on the size of the PEG headgroup. All of our observations can be reconciled in terms of a unifying mechanistic picture wherein the coupling of molecular conformational transitions in the PEG surfactant with its phospholipid monolayer environment (rather than thermodynamic degrees of freedom) triggers the changes in the balance of competing interactions. Because intramolecular conformational dynamics are pervasive in biological membranes (such as during cell-surface signaling and ligand–receptor interactions), it seems tempting to speculate that such pattern evolution induced by conformational dynamics might serve as a physical mechanism by which membrane heterogeneity is reorganized and membrane processes amplified.

## Experimental Methods

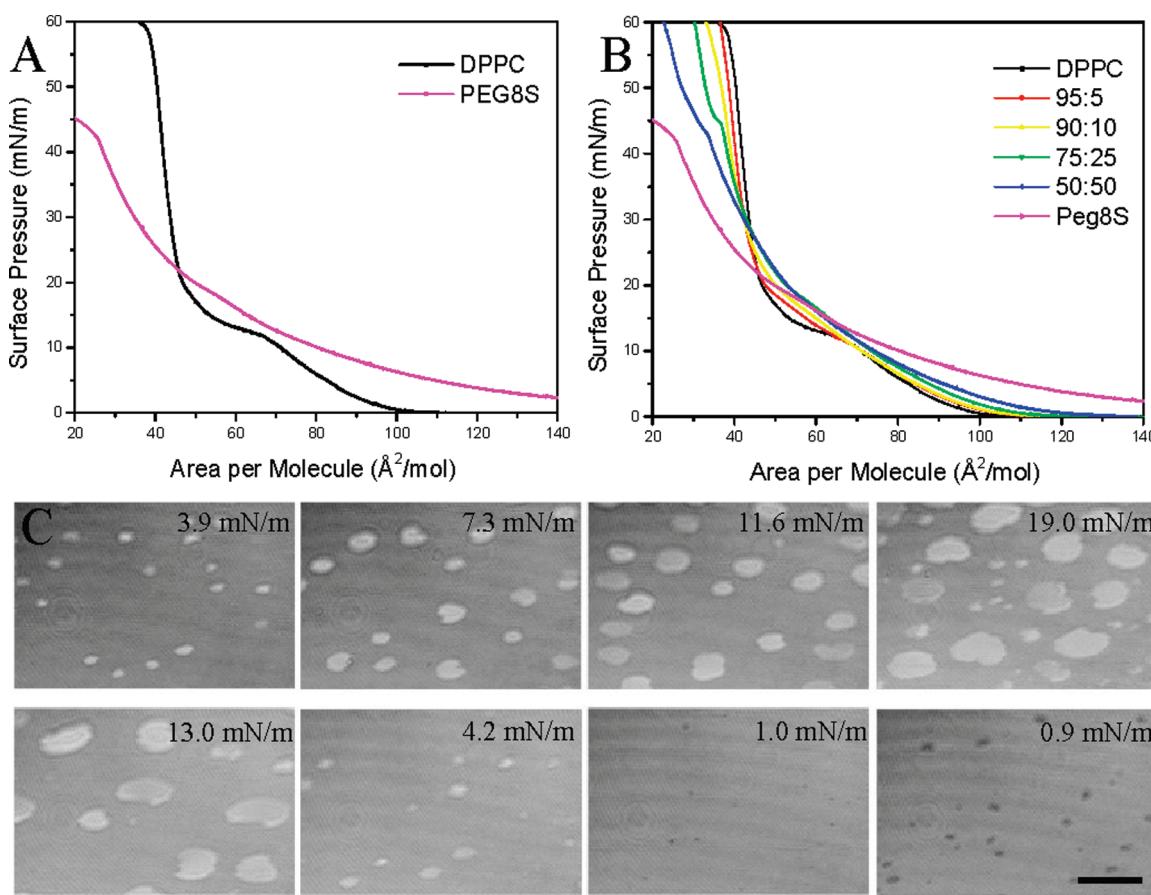
**Materials.** Chloroform solutions of high-purity 1,2-dipalmitoyl-sn-glycerol-3-phosphocholine (10 mg/mL, DPPC) and Texas Red 1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine, triethylammonium salt (TR-DHPE), were purchased from Avanti Polar Lipids (Alabaster, AL) and Invitrogen (Carlsbad, CA), respectively. Poly(oxyethylene) stearates (PEG8S, MW 623 g/mol and PEG40S, MW 2271 g/mol) were purchased from Aldrich (St. Louis, MO). All amphiphiles were used without further purification. Milli-Q purified deionized water (resistivity, 18.2 MΩ cm<sup>-1</sup>, pH  $\sim 6.4$ ) was used as the subphase for all experiments.

**Surface Pressure–Molecular Area ( $\pi$ –A) Isotherm Measurements.** All  $\pi$ –A isotherms obtained at Gettysburg College were recorded using a computer-controlled Teflon Langmuir trough (large inverted microscopy model, NIMA Technologies, Coventry, England). The moving barriers were Delrin-coated. A stationary Wilhelmy balance (NIMA Technologies, Coventry, England) was used to measure the surface pressure with a resolution of  $\pm 0.5$  mN/m. All isotherms were collected at  $25 \pm 0.5^\circ\text{C}$ , and the trough temperature was maintained at by a circulating water bath (Isotemp 3016D water circulator, Thermo Fisher Scientific).

The lipid monolayer was spread by dropwise addition of the spreading solution on the water surface, and the organic solvent was allowed to evaporate for 15 min. The barriers were then compressed with a linear speed of 6 cm<sup>2</sup>/min, and isotherm measurements in the form of surface pressure (mN/m) versus area per lipid molecule (Å<sup>2</sup>/molecule) were taken at 1 s intervals until the monolayer reached its collapse pressure. A resting period of 10 min was allowed between compression and expansion strokes of the isotherm cycle. The actual molar ratio mixtures prepared were the following: (DPPC:PEG8S) (mol:mol) 95:5, 90:10, 77.5:22.5,

- (11) Tian, A. W.; Johnson, C.; Wang, W.; Baumgart, T. *Phys. Rev. Lett.* **2007**, 98, 208102.
- (12) Fisher, M. E.; Szpilka, A. M. *Phys. Rev. B* **1987**, 36, 644–666.
- (13) Lingwood, D.; Simons, K. *Science* **2010**, 327, 46–50.
- (14) Seul, M. *Europhys. Lett.* **1994**, 28(8), 557–563.
- (15) McConnell, H. M.; Vrljic, M. *Annu. Rev. Biophys. Biomol. Struct.* **2003**, 32, 469–492.
- (16) Veatch, S. L.; Keller, S. L. *BBA: Mol. Cel. Res.* **2005**, 1746, 172–185.
- (17) Baumgart, T.; Hess, S. T.; Webb, W. W. *Nature* **2003**, 425, 821–824.
- (18) Huang, K. C.; Mukhopadhyay, R.; Wingreen, N. S. *PLoS Comput. Biol.* **2006**, 2, 1357–1364.
- (19) Leibler, S.; Andelman, D. *J. Phys. (Paris)* **1987**, 48, 2013–2018.
- (20) Rozovsky, S.; Kaizuka, Y.; Groves, J. T. *J. Am. Chem. Soc.* **2005**, 127, 36–37.
- (21) Lipp, M. M.; Lee, K. Y. C.; Zasadzinski, J. A.; Waring, A. J. *Science* **1996**, 273, 1196–1199.
- (22) Christian, D. A.; Tian, A.; Ellenbroek, W. G.; Levental, I.; Rajagopal, K.; Janmey, P. A.; Liu, A. J.; Baumbart, T.; Discher, D. E. *Nature Mater.* **2009**, 8, 843–849.
- (23) Klibanov, A. L.; Maruyama, K.; Torchilin, V. P.; Huang, L. *FEBS Lett.* **1990**, 268, 235–237.
- (24) Papahadjopoulos, D.; Allen, T. M.; Gabizon, A.; Mayhew, E.; Matthay, K.; Huang, S. K.; Lee, K. D.; Woodle, M. C.; Lasic, D. D.; Redemann, C.; Martin, F. J. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, 88, 11460–11464.

- (25) Torchilin, V. P. *Nat. Rev. Drug Discov.* **2005**, 4, 145–160.



**Figure 1.** (A, B) Surface pressure versus molecular area isotherms of (A) pure DPPC and pure PEG8S and (B) DPPC:PEG8S mixtures. (C) Illustrative sequence of ellipsometric contrast images of DPPC:PEG8S (75:25 mol %) monolayer during compression (top panel) followed by expansion (bottom panel) at an arbitrarily selected set of surface pressures (scale bar, 50  $\mu\text{m}$ ).

75:25, 72.5:27.5, 50:50. Control experiments using mixtures with PEG40S and those containing 0.5 mol % TR-DHPE were performed as described. In all cases, reproducible isotherms were obtained in at least three consecutive isotherm cycles.

**Imaging and Characterization.** To visualize microstructure evolution in the Langmuir monolayers in *real time* as a function of surface pressure, two complementary imaging modalities were employed. Ellipsometric contrast imaging (ECI), which depends on the changes in the polarization of reflected light, provides refractive index-dependent image contrast between coexisting lipid phases. An additional benefit of the approach is that it enables a label-free and large-area characterization of Langmuir phases. ECI experiments were done on a Teflon Langmuir trough (Model 611, NIMA Technologies, Coventry, England). To eliminate air convection and dust settling, this trough (and ECI microscope described below) was enclosed in a Plexiglas box. Spatially resolved ECI measurements were carried out using a commercial system (Elli2000, Nanofilm Technologie, Göttingen, Germany) equipped with a frequency-doubled Nd:YAG laser (adjustable power up to 20 mW) at 532 nm and a motorized goniometer for an accurate selection of the incidence angle and an independently adjustable detector positions. The imaging is performed using a sensitive CCD camera. Parallel measurements were performed on a custom-made Teflon Langmuir trough equipped with an epifluorescence microscope (EFM),<sup>26</sup> which provides a contrast generated by differences in the partitioning preferences of the fluorophore used. Fluid-phase partitioning TR-DHPE at 0.5 mol % was used in all EFM measurements. Real-time EFM measurements were performed using an upright Nikon optical microscope (Fryer Co., Huntley, IL) positioned above the

trough and a 50 $\times$  extra-long working distance objective. A 100 W mercury lamp (Osram Sylvania, Danvers, MA) was used for fluorescence excitation, and a dichroic mirror/filter cube was used to direct light onto the monolayer and to filter the emitted fluorescence. Images were recorded on a digital recorder (Sony GVD 100 DV Recorder, B & H Photo-Video, New York, NY).

## Results and Discussion

The surface pressure–area ( $\pi$ – $A$ ) isotherm for pure DPPC on pure water subphase (25 °C, pH ~6.4) consists of well-isolated two-dimensional G (gas), LE (liquid expanded), and C (condensed) phases (Figure 1A).<sup>27–30</sup> An initial flat portion of the isotherm at 0 mN/m for molecular area > 95  $\text{\AA}^2/\text{molecule}$  corresponds to the two-dimensional G–LE coexistence. This is followed by the first limb of increasing surface pressure reflecting the compaction of the LE phase, which persists until about 78  $\text{\AA}^2/\text{molecule}$ . In these regimes, ellipsometric contrast imaging (ECI) and epifluorescence microscopy (EFM) data reveal a uniformly homogeneous surface (Supporting Information, S1). The LE region is then followed by an essentially flat plateau region between 78 and 55  $\text{\AA}^2/\text{molecule}$  at about 10–15 mN/m corresponding to coexisting LE–C phases. In this coexistence regime, ECI and EFM images reveal the appearance of small domains. Bright ellipsometric domains correspond to dark, fluorophore-deficient features in fluorescence data. Because the fluorescent probe

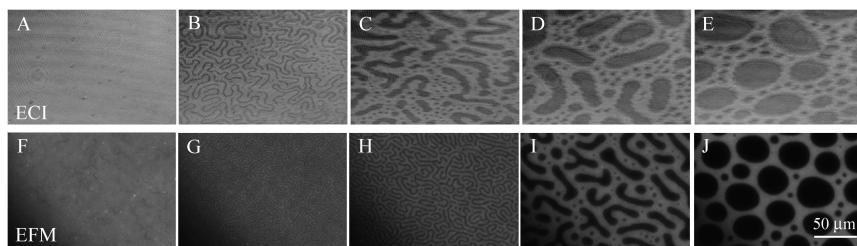
(26) Frey, S. L.; Lee, K. Y. C. *Langmuir* 2007, 23, 2631–2637.

(27) Ito, H.; Morton, T. H.; Vodyanoy, V. *Thin Solid Films* 1989, 180, 1–13.

(28) Phillips, M. C.; Chapman, D. *Biochim. Biophys. Acta* 1968, 163, 301–313.

(29) Möhwald, H. *Annu. Rev. Phys. Chem.* 1990, 41, 441–476.

(30) Knobler, C. M.; Desai, R. C. *Annu. Rev. Phys. Chem.* 1992, 43, 207–236.



**Figure 2.** Time-lapse (A–E) ellipsometric and (F–J) epifluorescence microscopy images obtained during the first expansion of a precompressed DPPC:PEG8S (75:25 mol %) monolayer in the vicinity of zero surface pressure. The images were obtained at arbitrary periods during the first 10 min when the film was at this surface pressure. The residual surface pressure was  $\sim$ 0 mN/m.

used in these measurements, Texas Red 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (TR-DHPE), is known to preferentially partition in the fluid phase of the monolayer,<sup>31</sup> its expulsion from the domains indicates that the domains represent condensed phase clusters. Upon further compression, the monolayer reaches a regime of steeply increasing surface pressure between 40 and 55 Å<sup>2</sup>/molecule (15–40 mN/m). Between 10 and 15 mN/m, the domains grow in size. The initial asymmetric domains adopt a characteristic bean-shaped character. Higher resolution EFM data reveal additional fingering and asymmetric branching producing a characteristic spiral shape, likely induced by the chirality of DPPC.<sup>29,32,33</sup> Beyond this pressure, the domains stop growing in size but are swept closer together at higher compression. These observations agree well with previous reports of the behavior of the DPPC at air–water interface under comparable temperature and subphase conditions.<sup>27,28</sup>

The isotherm for PEG8S monolayers, unlike DPPC, does not exhibit the classical break points, but rather a monotonic rise in surface pressure as the molecular area is reduced, suggesting the absence of typical phase transitions (Figure 1A). It is notable that even at large molecular areas ( $> 250 \text{ } \text{\AA}^2/\text{molecule}$ ) the PEG8S monolayer is characterized by a nonvanishing surface pressure and a homogeneous “bright” field as visualized by EFM consistent with LE phase, suggesting persistent intermolecular interactions even at large molecular areas. This is not surprising because the ethylene oxide (EO) monomers have a strong affinity for the air–water interface and are known to adopt extended interface-bound conformations.<sup>34,35</sup> On this basis, assuming the projected length of each EO monomer is  $\sim$ 3.7 Å, the average area per PEG8S in fully spread configuration becomes as large as  $\sim$ 690 Å<sup>2</sup>, thus allowing molecules to experience intermolecular forces at low molecular density, behavior consistent with nonzero surface pressure at these areas. Though no plateau in surface pressure is readily visible for PEG8S, domains appear at  $\sim$ 15 mN/m and form a distinctive dimer or trimer morphology that grows in size until  $\sim$ 45 mN/m, which marks PEG8S monolayer collapse (ECI and EFM images in Supporting Information, S2).

Upon addition of PEG8S to the DPPC monolayer in increasing relative amounts (1, 5, 10, 25, and 50 mol %), two major trends in the isotherms are evident (Figure 1B). First, the molecular areas at which initial liftoff occurs gradually shift to larger values when compared to the pure DPPC monolayer. This expansion of average area per molecules at liftoff can be attributed to the repulsive lateral interaction between EO headgroups. Upon

further compression, the isotherms of mixed DPPC:PEG8S monolayers shift to the left of pure DPPC. This can be attributed to the EO polymers of PEG8S partitioning to the subphase at high compression such that the surfactant tailgroups dictate the molecular cross-sectional area; all DPPC:PEG8S mixtures average  $< 2$  hydrocarbon tails per molecule. Second, the LE–C coexistence plateau region due to DPPC persists up to 25 mol % PEG8S. Together, these observations suggest that the lipopolymer is enriched in the disordered region, not readily mixing with condensed DPPC at the air–water interface. Additionally, the domain morphology for the mixtures observed in ECI and EFM images reveal the persistence of bean-shaped spiral domains (characteristic of a DPPC monolayer) above the liftoff pressure (Figure 1C), further suggesting that the PEG8S molecules preferentially partition out of the C phase domains resulting in domains that are DPPC enriched and LE phase enriched in PEG8S (for different composition ratio examples, refer to Supporting Information, S3–S5). The degree of partitioning should depend on the size of the EO headgroup with a longer polymer resulting in a smaller amount of lipopolymer in the C phase due to steric arguments. The expansion isotherms are generally similar to the shape of the  $\pi$ – $A$  compression isotherm for all the compositions but with a substantial hysteresis upon each expansion (Supporting Information, S6). ECI images for the sample system of 75:25 mol % DPPC:PEG8S (Figure 1C) reveal that as the mean molecular area is expanded and surface pressure decreased, the domains begin to shrink in size. Eventually around  $\sim$ 2 mN/m surface pressure, isolated C domains are no longer visible and the monolayer becomes optically homogeneous.

Remarkably, the optically homogeneous state obtained during the expansion of precompressed DPPC:PEG8S (75:25 mol %) mixtures spontaneously undergoes a rapid, unexpected morphological evolution as the surface pressure approaches 0 mN/m. This is in sharp contrast to single component DPPC (or PEG8S) monolayers which show stable circular G phase domains in coexistence with a bright LE phase upon expansion. This unusual structural transformation is documented both in ECI and EFM images shown in Figure 2. From a seemingly homogeneous mixed state at  $\sim$ 1 mN/m, dark spots in ECI and EFM images first appear. This surface morphology is not static upon further barrier expansion and exhibits a continuous evolution, first producing a striking pattern of stripe-like features covering the entire image. At this point, the surface pressure is 0 mN/m, and the only changing parameter is mean area per molecule. Here, optically bright background interspersed with thin serpentine features uniformly covers the surface. In addition to the long thin stripes, the image is also characterized by the presence of small circular domains. The presence of extensive interfaces in this monolayer morphology suggests low line tension between the coexisting phases. Upon further expansion, the dark stripes coarsen and shorten in length, forming branched and distorted, dog-bone-shaped domains.

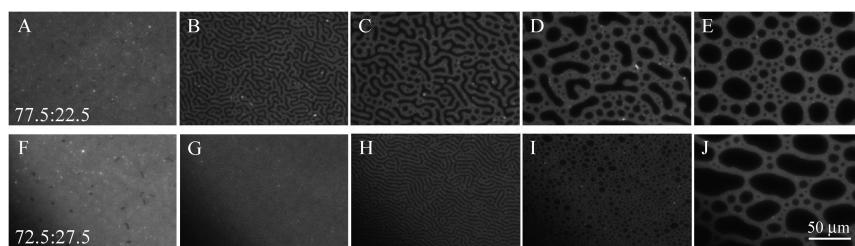
(31) Baumgart, T.; Hunt, G.; Farkas, E. R.; Webb, W. W.; Feigenson, G. W. *BBA: Biomembranes* **2007**, *1768*, 2182–2194.

(32) McConnell, H. M. *Annu. Rev. Phys. Chem.* **1991**, *42*, 171–195.

(33) Selinger, J. V.; Wang, Z. G.; Bruinsma, R. F.; Knobler, C. M. *Phys. Rev. Lett.* **1993**, *70*, 1139–1142.

(34) Baekmark, T. R.; Elender, G.; Lasic, D. D.; Sackmann, E. *Langmuir* **1995**, *11*, 3975–3987.

(35) Degennes, P. G. *Macromolecules* **1980**, *13*, 1069–1075.



**Figure 3.** Time-lapse epifluorescence microscopy images obtained during the expansion of DPPC:PEG8S Langmuir monolayers at (A–E) 77.5:22.5 and (F–J) 72.5:27.5 mol % composition ratios, respectively. The images were obtained at arbitrary periods during the first 10 min when the residual surface pressure was  $\sim 0$  mN/m.

At the same time, new dark specks appear and their number density increases. These features are ultimately replaced by a pattern consisting of essentially circular large dark droplets interspersed with smaller ones. It should be noted that if barrier expansion is stopped during this process, the distinct stripe morphology is stable (for up to several hours) and does not ripen, indicative of a thermodynamic equilibrium structure rather than a dynamic one. The progression of the stripe–droplet pattern fully reverses when the monolayer is recompressed after one full isotherm cycle, and this cyclical pattern of morphological transition can be reproduced in multiple cycles (subsequent compression images can be found in Supporting Information, S7). Qualitatively, similar behavior is observed within a  $\pm 2.5$  mol % range of PEG8S about the composition of 75:25 mol % DPPC:PEG8S (Figure 3). Because an identical transition is seen in both ECI and EFM images, we can safely conclude that this transition is intrinsic to the primary amphiphilic mixture (DPPC and PEG8S) and not influenced by the behavior of the probe. Taken together, these results reveal the existence of an unusual morphological transformation in precompressed Langmuir monolayers of mixtures of PEG8S and DPPC upon expansion at zero surface pressure and in a narrow composition window. The surface morphology evolves from an optically homogeneous state into a nested droplet phase characterized by nearly circular domains via a surface density mediated stripe-like pattern in a striking resemblance to the disorder-driven pattern evolution seen in the broader family of systems with competing interactions.

The observed spontaneous emergence of line-tension-dominated droplet morphology—via a stripe phase in interfacial monolayers of PEG8S and DPPC in 75:25 DPPC:PEG8S monolayers at room temperature and zero surface pressure—is an unusual morphological transition representing the first observation of its kind; we are unaware of other reports of similar phenomena at comparable surface pressures corresponding to LE–G regimes and at such low molecular densities. In these cases, the stripe phase is characterized by the presence of a large interfacial length between the coexisting domains, suggesting low line tension. As the stripe phase coarsens and transitions into the droplet phase, line tension gradually grows. This slow passage to the line-dominated state suggests the readjustment of the competing interactions and hence the modulation period. These morphological transitions closely mimic structural transitions such as occurs either during (1) a passage through a critical point by varying either the temperature or the pressure<sup>32</sup> or (2) those involving spinodal decomposition.<sup>36,37</sup> In the former case, when the thermodynamic parameters of temperature or pressure are changed spanning a critical point, a qualitative change in the morphologies is predicted based on their equilibrium phase diagrams. In our case, whether or not the morphological transitions

we witness involve a critical point cannot be ascertained using the results presented here alone. The stripe-like phase, which emerges during the early breakup of the homogeneous phase, also resembles the bicontinuous patterns occurring at the initial state of spinodal decomposition in two-component systems. However, in our case, since the composition-dependent variations in headgroup conformations (see below) are involved, much more complicated intermediate metastable phases might be stabilized. Extending the analogy with passage through a critical point requires unambiguous experiments demonstrating scale invariance in compositional fluctuations.<sup>38</sup> Nevertheless, because the area expansion in our experiment occurs in a regime with no significant qualitative changes in the phase diagram, we propose the existence of an independent order parameter, possibly arising from the headgroup conformational landscape, such as discussed below.

We postulate that the compression-induced phase separation in the DPPC:PEG8S monolayers, and attendant headgroup conformational transition which relaxes at slow time scales during expansion, are responsible for the observed morphological transitions. It is now well-established that the extended headgroups of PEG-conjugated surfactants and lipids exhibit multiple headgroup conformations dependent on the packing density.<sup>34</sup> At low densities, or high area per molecule, the polymer headgroups spread on the two-dimensional air–water interface in a “pancake”-like conformation with an area that scales with the number of adhesive EO monomers.<sup>35</sup> Upon compression, repulsive interactions between the EO units drive the headgroups into the third dimension, namely, the subphase, forming a “mushroom” like globular headgroup conformation with a characteristic size akin to the Flory radius ( $R_F \propto N^{3/5}$  where  $N$  is the number of EO monomers). For lateral distances smaller than  $R_F$ , the chains further stretch normal to the air–water interface forming a “brush” conformation with the polymer forming a linear chain of compact regions with the size, determined by both the number of monomers and the packing density ( $D_{\text{brush}} \propto N A_p^{-1/3}$  where  $A_p$  is molecular area).<sup>34</sup> The nonideal mixing behavior evident in isotherm data and domain morphology in Figure 1 suggest that PEG8S preferentially partitions out of the condensed phase domains which form during the plateau in the  $\pi$ – $A$  isotherm. This compression-induced phase segregation enhances the polymer concentration in a PEG-rich phase creating conditions for conformational transitions even at molecular densities as low as 25 mol %. The  $R_F$  of 8 ethylene oxide units for Peg8S is  $\sim 12$  Å, corresponding to a maximal mushroom cross-sectional area of  $\sim 450$  Å<sup>2</sup>/molecule. The polymer component of a DPPC monolayer containing  $\sim 25$  mol % PEG8S in the phase-separated state would be expected to undergo a mushroom-to-brush like transition as the monolayer is compressed. This is supported by the behavior of the isotherm compared to pure DPPC (Figure 1).

(36) Bates, C. S. *Science* **1991**, *251*, 898–905.

(37) Cahn, J. W. *J. Chem. Phys.* **1965**, *42*, 93–99.

(38) Nielsen, L. K.; Bjørnholm, T.; Mouritsen, O. G. *Nature* **2000**, *404*, 352.



**Figure 4.** (A–E) Time-lapse epifluorescence microscopy images obtained during the expansion of DPPC:PEG40S Langmuir monolayers at 95:5 mol % compositional ratios. The images were obtained at arbitrary periods during the first 10 min when the residual surface pressure was  $\sim 0$  mN/m.

Furthermore, because PEG brush formation is stabilized by considerable molecular entanglement and significant interdigitation, presumably to ease packing stress,<sup>39</sup> its relaxation, upon reduction in packing density, to mushroom and eventually the pancake conformation upon lowering the surface pressure is invariably met with large hysteresis which is echoed in the isotherm (Supporting Information, S6). This slow relaxation of PEG8S conformation can plausibly explain the long time scales associated with the structural evolution we observe at nominally zero surface pressure during monolayer expansion.

A similar striped phase pattern was reported by Ahrens et al. where monolayers of the pegylated phospholipid, DSPE-EO<sub>44</sub>, were deposited from the air–water interface onto a solid substrate and imaged with atomic force microscopy.<sup>40</sup> In this related single-component system, lateral superstructure defined by 100 nm wide stripes of ordered gel phase alkyl chains islands embedded in solubilized polymer regions was not evident until compression crossed a phase transition at  $\sim 20$  mN/m. This is in contrast to our binary system, where passage through the micrometer-sized striped morphology instead occurs at a low molecular density. At a surface pressure of 0 mN/m, a monolayer composed of a typical amphiphile adopts a G/LE coexistence at the interface with regions of space defined by a low molecular density (referred to as the G phase) becoming larger upon further expansion. In our binary phospholipid:lipopolymer system, this introduces an independent order parameter where available space at the interface (normally the G phase) allows for polymer structure flexibility, opening the avenue for a conformational change. In the case of our lipopolymer, the mushroom to pancake transition occurs in this low-pressure regime. Because of the polymer entanglement at high compression, we postulate the dark spots forming at  $\sim 0$  mN/m are polymer-rich regions which grow in size as EO groups relocate from the subphase to the interface. Despite low molecular densities, pancakes of interfacially bound PEG headgroups produce a polymer-rich phase, interspersed with a polymer-depleted LE phase enriched in DPPC. This is manifest in our isotherm and EFM data where, upon expansion, at molecular areas as large as 250 Å<sup>2</sup>/molecule, the monolayer exists in two distinct phases: a more traditional bright LE phase coexisting with a dark phase where lipopolymer has phase separated out due to an extended pancake conformational change similar to the proposed polymer and alkyl chain phase separated structure in Ahrens' DSPE-EO<sub>44</sub> system.<sup>40</sup> The passage through a low line tension limit or stripe-like phase is mediated by the remaining polymer equilibrating from a mushroom to a pancake conformation, a conformational transition which also alters the domain line tension, until the stable droplet phase, enriched with polymer in the pancake conformation at the air–water interface, has formed. This is better understood by recognizing that for pure DPPC the structural transition in the monolayer that occurs during expansion to zero surface pressure is due to a coexistence between the

liquid phase and the gas phase. This results in high interfacial line energy or line tension, and the gas phase domains are circular. When the same transition occurs in the DPPC/PEG8S system, the dark phase is likely PEG8S enriched—it is more gas-like because the effective area for each polymer headgroup is substantially larger. As one expands to higher molecular areas, a greater proportion of the mushroom phase transitions into pancake configuration. On the basis of the above, we suggest that the interfacial line energy is thus not between the DPPC domains and bare surface (as is the case for pure DPPC), but that between DPPC-rich domains and PEG pancake-rich domains at the interface. This persists until the last mushroom to pancake transition has occurred. Further increase in area will lead to bare interface (gas phase) and that further increases the interfacial line energy, giving rise to the transition from stripe-like morphology to the one characterized by circular droplets. It should also be noted, at polymer concentrations higher (7:3) and lower (8:2) than the 75:25 DPPC:PEG8S ratio, the monolayer also forms a similar droplet morphology at high area per molecule upon expansion, but this occurs simply via growth of dark spots at nominal surface pressure. The narrow concentration range (77.5–72.5% DPPC) of the effect can be attributed to a specific density of polymer in the subphase which dictates the equilibration of the polymer at the interface.

To further validate the proposed role of PEG headgroup conformational transitions in driving the observed morphological transitions, we carried out additional experiments replacing PEG8S with PEG40S—a molecule with an identical aliphatic tail but a considerably larger headgroup consisting of 40 repeating EO units. On the basis of the rationale presented above, we reason that a much smaller concentration of PEG40S with a larger resultant cross-sectional area should be sufficient to induce the morphological transitions observed. A structural instability and passage through stripe phase (with somewhat altered morphology compared to 75:25 DPPC:PEG8S) is also seen when the relative mole fractions fall in the range of 5–10 mol % PEG40S (Figure 4). A longer EO chain would have a larger Flory radius and a higher molecular density of polymer in the subphase at a similar lipid:lipopolymer ratio. Thus, in comparison with PEG8S, a much lower mole fraction of PEG40S can induce the comparable spatial equilibration from the mushroom to the pancake regime. No such transition is observed in the 75:25 mixture using PEG40S. This simple experiment lends additional credence to the foregoing inference that the observed structural evolution is dependent on the packing of as well as the conformational changes in the EO headgroup.

In summary, results presented here provide a reproducible observation of an unusual morphological evolution in mixtures of a PEGylated lipid and a saturated phospholipid. The transition includes a gradual and continuous breakup of an initial homogeneous phase into line-tension-dominated coexisting droplet morphologies. Curiously, this breakup reveals a passage through a stripe phase akin to that found in equilibrated Langmuir monolayers via modulation of competing line tension and electrostatic interactions. We postulate that this transition is enabled

(39) Majewski, J.; Kuhl, T. L.; Kjaer; Gerstenberg, M. C.; Als-Nielsen, J.; Israelchivili, J. N.; Smith, G. S. *J. Am. Chem. Soc.* **1998**, *120*, 1469–1473.

(40) Ahrens, H.; Graf, K.; Helm, C. A. *Langmuir* **2001**, *17*, 3113–3115.

by the conformational change of amphiphilic PEG headgroups. Because cellular membranes are believed to be poised close to a critical point, it seems tempting to speculate that a conformation-induced morphological change such as observed here may provide a mechanism by which cell-surface signals result in large-scale reorganization and signal amplification. For instance, it has been broadly suggested that cholesterol-dependent compositional heterogeneities (or so-called lipid rafts) in lipid and cellular membranes may undergo large-scale reorganization (e.g., coalescence and capping) following activation during many cellular processes (e.g., signaling) which induce conformational change in raft-partitioning molecules.<sup>12</sup>

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**Supporting Information Available:** Isotherms for pure DPPC and pure PEG8S and their corresponding ECI and EFM micrographs for a set of arbitrarily selected surface pressures; images of mixed DPPC:PEG8S monolayers for three different compositions, 90:10, 75:25, and 77.5:22.5 mol %, for a set of selected pressures during monolayer compression; compression and expansion cyclic isotherms for 75:25 and 50:50 mol % DPPC:PEG8S demonstrating how the magnitude of hysteresis scales with the mol % of PEG8S; corresponding micrographs of the expansion and compression of the 75:25 DPPC:PEG8S system, confirming reversibility of the observed stripe-to-droplet transition. This material is available free of charge via the Internet at <http://pubs.acs.org>.