Effect of Line Tension on the Lateral Organization of Lipid Membranes

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The principles of organization and functioning of cellular membranes are currently not well understood. The raft hypothesis suggests the existence of domains or rafts in cell membranes, which behave as protein and lipid platforms. They have a functional role in important cellular processes, like protein sorting or cell signaling, among others. Theoretical work suggests that the interfacial energy at the domain edge, also known as line tension, is a key parameter determining the distribution of domain sizes, but there is little evidence of how line tension affects membrane organization. We have investigated the effects of line tension on the formation and stability of liquid ordered domains in model lipid bilayers with raft-like composition by means of time-lapse confocal microscopy coupled to atomic force microscopy. We varied the hydrophobic mismatch between the two phases, and consequently the line tension, by modifying the thickness of the disordered phase with phosphatidylcholines of different acyl chain length. The temperature of domain formation, the dynamics of domain growth, and the distribution of domain sizes depend strongly on the thickness difference between the domains and the surrounding membrane, which is related to line tension. When considering line tension calculated from a theoretical model, our results revealed a linear increase of the temperature of domain formation and domain growth rate with line tension. Domain budding was also shown to depend on height mismatch. Our experiments contribute significantly to our knowledge of the physical-chemical parameters that control membrane organization. Importantly, the general trends observed can be extended to cellular membranes.

Cellular membranes form closed volumes that define the cell and organelle identity, although ensuring the exchange of matter, energy, and information that is required for life. They do so by means of a complex protein and lipid composition that is actively regulated in time and varies not only among the different cellular membranes but also between the two leaflets that form the membrane bilayer (1). During the last years, it has also become clear that the existence of lateral heterogeneities or domains in membranes is essential for a number of cellular functions.

The raft theory predicts the existence of lipid assemblies that are enriched in sphingolipids and cholesterol. These membrane domains are thought to behave as protein and lipid platforms, important for protein trafficking and sorting, cell signaling, and other cellular processes (2–4). Recent findings suggest that rafts are dynamic structures of transient nature and sizes in the nanometer range (5). Though still debated, the widely accepted view implies a situation far from equilibrium, where signaling or sorting processes would induce the coalescence of these lipid assemblies into more stable larger platforms in the membrane (6).

However, the current knowledge of membrane organization is not sufficient to fully explain the behavior and functioning of cellular membranes. Precisely because of their complex composition and dynamics, it is difficult to understand the principles that govern the lateral organization of the cell membrane in relation to its function.

During the last years, some of the physical properties of the plasma membrane have been studied with model membranes that mimic the lipid composition of rafts. Model membranes are still far away from representing the intricacy found in cells, but they constitute simplistic systems that can help understanding the principles of the processes that happen in cellular membranes. In model lipid membranes with “raft-like” composition, large domains are observable by fluorescence microscopy or AFM (7–11). These domains are enriched in sphingolipids and cholesterol and appear as a liquid ordered (L_{o}) phase, coexisting with a liquid disordered (L_{d}) phase. In such membranes, domains exhibit a circular shape, which is rapidly recovered after a mechanical distortion (5, 9). This tendency to minimize the boundary length indicates the presence of line tension at the phase interface.

AFM and x-ray scattering measurements show that the L_{o} phase is thicker than the L_{d} one, giving rise to a “height mismatch” at the domain edge (13–15). The exposure of the hydrophobic tails of the lipids to the aqueous solvent would have a very unfavorable energetic effect, and as a consequence, the membrane distortions at the boundary to avoid it (16). This height mismatch has an energetic cost per unit length that is probably one of the main parameters contributing to the line tension at the phase boundary.

The abbreviations used are: L_{o}, liquid ordered; L_{d}, liquid ordered; PC, phosphatidylcholine; DPOPC, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine; DMoPC, 1,2-dimyristoyl-sn-glycero-3-phosphocholine; DOPC, 1,2-dioleoyl-sn-glycero-3-phosphocholine; DEiPC, 1,2-dieicosenoyl-sn-glycero-3-phosphocholine; DDIrPC, 1,2-dierucyl-sn-glycero-3-phosphocholine; DOPPC, 1,2-stearoyl-oleoyl-phosphatidylethanolamine; Chol, cholesterol; GUV, giant unilamellar vesicle; AFM, atomic force microscopy; A/P, area to perimeter ratio; DiD, 1,1’-dioctadecyl-3,3,3’,3’-tetramethylindodicarboxcyanine perchlorate; CtxB-488, cholera toxin-labeled with Alexa488.
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The distribution of domain sizes depends on the balance between line tension, which tends to increase size in order to reduce total boundary length and entropy and electrostatic repulsions, which oppose raft merger (16–18). As a consequence, line tension is probably a major factor in the regulation of raft size.

Line tension at the domain interface has been experimentally estimated in giant unilamellar vesicles with phase separation (8, 19) and very recently, in planar supported bilayers by means of nucleation rate measurements (20). Theoretical models have related line tension to physical properties of the membrane, like phase height mismatch, lateral tension, and spontaneous curvature (16, 21). According to them, line tension increases quadratically with phase height mismatch. However, there is little experimental evidence about how line tension affects the lateral membrane organization and the formation of domains in terms of kinetics of domain formation, domain size and shape, and domain dynamics and stability.

To address these questions, we have investigated the effects of the line tension on the formation of Ld domains in model lipid bilayers with raft–like composition. Given the link between line tension and phase height mismatch, we systematically varied the height mismatch between the two phases and consequently the line tension, by modifying the thickness of the Ld phase with PCs of different acyl chain length. Our studies involved measurements at non-equilibrium conditions. Using time-lapse confocal microscopy and AFM imaging, we analyzed the kinetics of domain formation, the domain shape and size, and the demixing temperature from Ld to Ld-Lo coexistence, as a function of the hydrophobic mismatch.

Our results indicate a great influence of the line tension on the physical-chemical properties of Ld domains. We observed that at higher hydrophobic mismatch, the increased line tension led to bigger domains that formed with significantly faster kinetics to minimize the interface length. Interestingly, both the demixing temperature and the domain growth rate increased linearly with line tension, calculated from phase height mismatch measurements according to the model in (16). Under conditions close to equilibrium, domains were bigger and more circular at higher line tension. In addition, experiments in giant unilamellar vesicles linked height mismatch to line tension and domain budding.

**EXPERIMENTAL PROCEDURES**

**Preparation of Supported Bilayers**—1,2-Dipalmitoleoyl-sn-glycero-3-phosphocholine (DPOPC), 1,2-dimyristoleoyl-sn-glycero-3-phosphocholine (DMoPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dieicosenoyl-sn-glycero-3-phosphocholine (DEiPC), 1,2-dieicosenoyl-sn-glycero-3-phosphocholine (DEruiPC), N-stearoyl-d-erythro-sphingosylphosphocholine (SM), and cholesterol (Chol) were purchased from Avanti Polar Lipids (Alabaster, AL).

Planar supported bilayers were prepared as described in (22). Briefly, lipids were dissolved in chloroform at the desired molar concentration, and 1,1’-diodotadecyl-3,3’,3’-tetramethyldindocarbocyanine perchlorate (DiD-C18) (Molecular Probes, Eugene, OR) was added to the lipid mixtures at a 0.01% (mol/mol) concentration. The solvent was evaporated under nitrogen flux followed by vacuum for 1 h. Lipid films were rehydrated to a final concentration of 10 mg/ml in 3 mM KCl, 1.5 mM KH2PO4, 8 mM Na2HPO4, 150 mM NaCl, pH 7.2 and vortexed for 5 min. A small aliquot (10 µl) of the suspension of multilamellar vesicles was diluted in 140 µl of 3 mM CaCl2, 150 mM NaCl, 10 mM Hepes, 3 mM NaN3, pH 7.4. The suspension was then bath-solicited at 60 °C until small unilamellar vesicles were obtained and then put in contact with freshly cleaved mica substrate, previously glued to a glass coverslip. The mixture was incubated at 40 °C for 2 min and then at 68 °C for 10 min. At this temperature, the samples were rinsed several times with 150 mM NaCl, 10 mM Hepes, 3 mM NaN3, pH 7.4, to remove the non-fused vesicles. Sample temperature was controlled with a BioCell (JPK Instruments, Berlin, Germany). The lipid content per sample was ~2 nmol, calculated assuming an average area per lipid molecule of 0.6 nm2 (4).

**Preparation of Giant Unilamellar Vesicles**—Giant unilamellar vesicles (GUVs) of the desired lipid composition were prepared according to the electroformation method as described in (10). Briefly, 5 µl of lipid mixture at 10 mg/ml was spread on indium tin oxide-coated coverslips at 65 °C. After solvent evaporation, the electrodes were assembled into custom-made perfusion chambers that were filled with 300 mM sucrose. Electroformation proceeded at 1.2 V and 10 Hz during ~1 h. Samples were equilibrated to room temperature and checked for phase separation with the confocal microscope. Then, 5 µg of B subunit of cholera toxin labeled with Alexa488 (CtxB-488) was added to the chamber, incubated for 30 min, and washed out with 300 mM sucrose solution. No apparent changes in the pattern of phase separation were observed upon CtxB-488 labeling (23).

**Confocal Microscopy**—We performed confocal fluorescence microscopy of supported lipid bilayers on a LSM Meta 510 instrument (Carl Zeiss, Jena, Germany). Confocal images were taken by using the excitation light of a He-Ne laser at 633 nm, which was reflected by a dichroic mirror (HTF488/633) and focused through a Zeiss C-Apochromat ×20, 0.75 numerical aperture objective onto the sample. The fluorescence signal was collected by the same objective, passed a 680/30 nm band pass filter and finally detected by a photomultiplier. Confocal geometry was ensured by a 100-µm pinhole in front of the photomultiplier.

GUVs were imaged in a commercial ConfoCor2 system (Carl Zeiss) using multi-track mode. Light from an Ar laser at 488 nm, and a He-Ne laser at 633 nm was reflected with a HFT UV/488/543/633 dichroic. A ×40 numerical aperture 1.2 C-Apochromat water immersion objective was used, and the pinhole size was set to 90 µm in the green channel, although adjusted in the red channel for the same z thickness. Emitted fluorescence was separated with a secondary dichroic beam splitter 570 dichroic and passed through 505 nm or 650 nm long pass filters to be finally detected with a photomultiplier. Image processing and analysis was carried out with ImageJ (rsb.info.nih.gov/ij/).

**Atomic Force Microscopy**—AFM measurements were performed using a NanoWizard system (JPK Instruments, Berlin, Germany) mounted on the same LSM Meta 510 setup used for microscopy. Contact mode topographic images were taken in the constant-deflection mode, using V-shaped silicon nitride cantilevers (Veeco, Santa Barbara, CA) with a typical spring constant of 0.08 newton/m. The force applied on the sample was maintained at the lowest possible value by continuously adjusting the set point during imaging. The scan rate was set to 1 Hz. Height and deflection were collected simultaneously in...
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TABLE 1
Lipid composition of the supported bilayers used in this study
The number of carbon atoms in the acyl chain of PC, the height difference between L\(_d\) and L\(_o\) phases, the demixing temperature, the average circularity of L\(_o\) domains and the line tension are shown.

| Lipid composition (2:2:1) | Carbon atoms in PC acyl chain | Phase height difference | Demixing temperature | Average domain circularity \(^{a,b}\) | Line tension |
|--------------------------|-------------------------------|------------------------|----------------------|----------------------------------------|-------------|
| DEruPC:SM:Chol           | 22                            | 170 ± 70               | 38 ± 1               | 0.6 ± 0.2                              | 0.06 ± 0.04 |
| DEiPC:SM:Chol            | 20                            | 670 ± 80               | 42 ± 1               | 0.78 ± 0.15                            | 0.8 ± 0.3   |
| DOPC:SM:Chol             | 18                            | 870 ± 100              | 46 ± 1               | 0.78 ± 0.14                            | 1.2 ± 0.5   |
| DPoPC:SM:Chol            | 16                            | 1330 ± 130             | 58 ± 3               | 0.90 ± 0.13                            | 4.1 ± 1.5   |
| DMoPC:SM:Chol            | 14                            | 1560 ± 130             | 66 ± 3               | 0.90 ± 0.11                            | 6 ± 2       |

\(^{a}\) The indicated errors correspond to the standard deviation of the mean in the case of phase height difference and average domain circularity, and the average \(\sigma\) of the Gaussian fittings in the height mismatch values.

\(^{b}\) Circularity was calculated as \(4\pi \text{area}/\text{perimeter}^2\) for every measured domain using the ImageJ software.

\(^{c}\) Calculated from the model in (16), assuming a soft domain, with \(B_0 = B_1 = 10 \text{kT}\), and \(k_1 = k_2 = 40 \text{mN/m}\), and no spontaneous curvature, \(f_s = 0\) (16). We considered an effective thickness of the L\(_d\) DOPC bilayer of 5.5 nm (26). Errors were estimated with Gaussian error propagation.

FIGURE 1. Kinetics of L\(_o\) domain growth. Supported lipid bilayers containing 0.05% DiD were formed and warmed up to 67 °C, above the transition temperature. Then, the samples were cooled down to 21 °C while the formation of domains was imaged by confocal microscopy. Images were taken every second during 10 min. Only the indicated times and the corresponding temperatures are shown. The lipid composition of the membranes was DEruPC:SM:Chol (2:2:1) (A), DEiPC:SM:Chol (2:2:1) (B), DOPC:SM:Chol (2:2:1) (C), DPoPC:SM:Chol (2:2:1) (D), and DMoPC:SM:Chol (2:2:1) (E). All images are 46 × 46 \(\mu\)m.

both trace and retrace directions. Images were line-fitted as required with JPK processing software (JPK Instruments, Berlin, Germany). Occasionally, isolated scan lines were removed. We performed image analysis with ImageJ and OriginPro (OriginLab, Northampton, MA).

RESULTS

Line Tension Affects the Demixing Temperature into L\(_d\) and L\(_o\) Phases—To systematically investigate the role of line tension on the properties of raft-like domains, we modulated the height mismatch between the L\(_d\) and L\(_o\) phases by changing the thickness of the L\(_d\) phase, which is enriched in unsaturated PC (4). For this purpose, we used PC of varying acyl chain length in different samples (see Table 1). We prepared supported lipid bilayers composed by a doubly unsaturated phosphatidylcholine (PC), sphingomyelin (SM), and cholesterol (Chol) in a 2:2:1 ratio. We included 0.05% DiD, a fluorescent lipid analogue that partitions specifically to the L\(_d\) phase (7, 9, 10). Usually, model membranes with such lipid mixture exhibit coexistence of L\(_d\) and L\(_o\) phases that can be visualized by AFM or fluorescence microscopy (7, 9, 10).

To ensure sample homogeneity, all samples had the same thermal history. After bilayer formation, samples were warmed up to 67 °C, above the transition temperature, and then cooled down to 21 °C in ~5 min with a temperature controller. We performed this process only once for every membrane.

During the cooling process L\(_o\) domains appeared when the temperature of phase demixing was achieved. We measured the formation and growth of domains with confocal microscopy. Because the fluorescent dye DiD is excluded from the L\(_d\) phase, L\(_o\) domains can be identified as dark patches in the membrane. Fig. 1 shows the first 5 min of the process. The corresponding movies can be found in the Supplemental Data and include the first 10 min of the kinetics of domain formation and growth. Interestingly, no domains were discernible in the case of the sample containing the PC with the longest acyl chain (DEruPC). For the rest of the samples, domains grew faster when membranes contained PC of shorter acyl chains (see series B to E in Fig. 1). In addition, phase demixing took place at different moments and subsequently at different temperatures depending on the acyl chain length. We define “demixing temperature” as the measured temperature at which the appearance of L\(_o\) domains was observed in our system (see Table 1). In the case of the sample
After equilibration for 2.5 h at 21 °C, we scanned the same lipid membranes with AFM (see Fig. 2). We obtained topographical images of the sample surface at higher spatial resolution, from which we measured the height difference between the L_d and L_o phases for the different lipid compositions (see Table 1). In this case, domains with an average area of $0.056 \pm 0.003 \mu m^2$ and ~170 pm thicker than the surrounding L_d phase could be distinguished for the sample containing DErupC. These results, contradictory to the microscopy observations, can be explained by the fact that the domain size could be below our optical resolution, or that the DiD dye would not partition specifically to the L_d phase for this lipid composition, or a combination of both. The images in Fig. 2 show that the L_d domains tend to be bigger and the difference in thickness between the two phases higher as the acyl chain length of the PC contained in the lipid bilayers decreases, in agreement with a thinner L_d phase. The height mismatch values measured for the different lipid compositions are shown in Table 1.

If we combine the demixing temperatures measured for the different lipid compositions with the height mismatch that those lipid compositions exhibit between the L_o and L_d phases (see Table 1), we get an estimation of the dependence of the temperature of phase demixing with phase height mismatch. Fig. 3 depicts this relationship and shows that the demixing temperature strongly increases with height mismatch, and hence, line tension.

**Domain Growth, Size, and Shape Depend on the Line Tension**—By image analysis, we quantified the distribution of domain sizes from the AFM images obtained for the different samples. Fig. 4 depicts the histograms of the logarithm of domain area and their corresponding fittings to Gaussian curves. There is a clear trend to domain enlargement with the increase in thickness difference between the L_d and L_o phases. Domain circularity, calculated as $4\pi (area/perimeter^2)$ (ImageJ), is shown in Table 1. In agreement with the observations above, there is a tendency to increase domain circularity with phase height mismatch. As expected, phase height mismatch is related to an increased line tension and to the formation of bigger and more circular domains to minimize the energetic cost associated to the domain interface length.

Though with a lower spatial resolution, we measured domain growth from the time series of domain formation obtained with the confocal microscope by image analysis. Fig. 5A shows that the average domain area increases approximately linearly with time for the different lipid mixtures. We calculated the rate of domain growth from the slope after 300 s, when the temperature of the sample could be considered constant. As observed in Fig. 5B, the rate of domain growth increased strongly with the height mismatch and thus, with the interfacial line tension, showing a similar dependence as the demixing temperature.

We compared the mechanism of domain growth during the 2.5 h of membrane equilibration for the different lipid compositions. We observed that domain fusion happens mostly dur-
very heterogeneous samples, we analyzed hundreds of vesicles and attempted to extract relevant information from the ensemble measurements.

Fig. 6 shows representative vesicles obtained for the different lipid compositions measured by confocal microscopy. In agreement with the observations made in planar supported bilayers, no phase separation could be distinguished in the GUVs containing DErUPC (Fig. 6, A and B), which had the longest acyl chain used in the experiments. If the AFM results are applicable, the presence of \( L_o \) domains could be again escaping us because of resolution and contrast issues.

Interestingly, bell-shaped \( L_o \) domains with negative curvature close to the domain edge could be observed in the cross-sections of some of the vesicles containing DEiPC, the longest acyl chain PC for which we observed phase separation (see Fig. 6, C and D). A similar shape has been reported previously for GUVs containing cholesterol sulfate, which is related to membrane budding-in processes (24). The presence of a small vesicle in the GUV in Fig. 6I, labeled with CtxB-488, which has no access to the interior of GUV, suggests that a budding-in process probably also occurred in this case. Almost no deviation of spherical shape was observed in samples containing DOPC (Fig. 6, E and F), whereas samples with shorter acyl chain PC exhibited budded-out structures (Fig. 6, G–J). In general, comparison of the vesicle shapes for the different lipid compositions shows a tendency to increase membrane curvature with phase height mismatch, in agreement with an increment in line tension (19, 25).

**DISCUSSION**

**Relationship between Height Mismatch and Line Tension—**

Cohen and coworkers have developed a theoretical model where line tension depends quadratically on phase height mismatch (16).

\[
\gamma = \sqrt{B_s K_c K_{fit}} \frac{\delta^2}{2} \left( J_{fit} - J_s \right)^2 \quad \text{(Eq. 1)}
\]

\( \gamma \) is the line tension, \( \delta \) is the phase mismatch, \( h_s = (h_r + h_l)/2 \), \( h \) is the monolayer thickness, \( B_s \) is the elastic splay modulus, \( K_c \) is the tilt modulus, and \( J \) is the spontaneous curvature of the monolayer. \( r \) and \( s \) refer to \( L_o \) and \( L_d \) phases, respectively. We used this model to calculate values of line tension for the samples with different height mismatch. We considered an effective thickness of the \( L_d \) DOPC bilayer of 5.5 nm (26), and calculated the thickness of the different phases from the height mismatch values measured. The values of the elastic moduli employed in the model are unknown, therefore we assumed the case of a “soft” domain, with \( B_s = B_L = 10 \, kT \), \( K_c = K_r = 40 \, mN/m \), and \( J_r = J_s = 0 \) (16, 27–30). We obtained values of line tension varying from 0.06 to 6 pN (see Table 1) in the same order of magnitude as the line tension values calculated in (8, 20).

Interestingly, when we plotted the measured demixing temperature against the calculated line tension, we obtained a linear relationship, as shown in Fig. 7A. Similarly, a linear dependence was found between domain growth rate and line tension, as depicted in Fig. 7B. Though the actual values of line tension may be shifted because of the estimation of the different moduli...
that we employed in our calculations, the dependencies obtained are still valid, at least in the context of the model used. This suggests the existence of a relationship between the thermodynamics of phase separation and growth as a function of the line tension.

On the other hand, Longo and coworkers (17, 31) have proposed that the domain area to perimeter ratio (A/P) is proportional not only to line tension in conditions that assume equilibrium but also during nucleation and phase growth. Fig. 8 shows A/P as a function of phase height mismatch. Despite the considerable dispersion obtained in the A/P values, our results suggest a linear dependence of A/P with respect to phase height mismatch. This would imply a direct proportionality between line tension and height mismatch at the domain edge, in contradiction to Cohen and colleagues (16). Recently a method to measure line tension from nucleation rates was reported (20). It would be interesting to apply it to our system and find out the direct relation between phase separation and line tension.

Dynamics of Domain Formation and Growth—We have quantitatively analyzed the kinetics of domain growth in supported bilayers for different lipid compositions with varying height mismatch. Quantitative analyses are complicated in GUVs because of membrane curvature, and thus planar membranes constitute more convenient systems, with simpler experimental measurements and data analysis. Apart from some simulations (13, 14, 32–36), there are few experimental studies that investigate the dynamics of domain growth in lipid systems (5, 37, 38). In all cases, they report that it is a slow process, and hours are required to reach equilibrium, similar to our observations. In addition, our results suggest a linear increase of average domain area with time, \( A(t) \sim t \). This reveals a growth law \( r(t) \sim \sqrt{t} \), in agreement with interface-driven dynamics (39). Theoretical calculations consider an effect of the line tension on the growth of domains (40). However, the dependence of domain growth on line tension is difficult to estimate experimentally because of the superimposed mechanisms of domain growth. This dependence is confirmed by our results, which suggest a linear relationship between domain growth rate and line tension according to the model in (16), as shown in Fig. 8B.

Our results also show that line tension affects the demixing temperature of phase separation. Interestingly, the demixing temperature for small values of height mismatch is close to 37 °C. This prompts us to speculate that it may be advantageous for cells to stay close to the transition temperature to better control raft dynamics. In this situation, the action of proteins could have a great influence on the formation and stability of rafts. In fact, the demixing temperature in plasma membrane vesicles was found to vary between 10–25 °C (41). Though also a model system different from the situation in living cells, it suggests that a complex composition can cause a significant reduction of the line tension.

Theoretical models predict that in phase-separated membranes, domain bending reduces the edge length so that the interface energy decreases (42). Recently, Baumgart et al. (8) showed that line tension drives shape changes in vesicles that minimize domain boundary length. Our experiments with GUVs show a budding tendency that increases with phase

FIGURE 6. Membrane curvature at the domain boundary in GUVs is affected by the height mismatch between the \( \text{L}_{\alpha} \) and \( \text{L}_{\beta} \) phases. Vesicles were visualized with DiD (red), which partitions specifically to the \( \text{L}_{\alpha} \) phase, and with B-subunit of CtxB-488 (green), which binds preferentially to the \( \text{L}_{\beta} \) phase. The lipid composition of the vesicles was DEruPC:SM:Chol (2:2:1) (A and B), DEiPC:SM:Chol (2:2:1) (C and D), DOPC:SM:Chol (2:2:1) (E and F), DPPC:SM:Chol (2:2:1) (G and H), and DMPC:SM:Chol (2:2:1) (I and J). Image sections are shown in all cases, except for B, which is a three-dimensional projection to show the absence of phase separation in the whole vesicle. The bar corresponds to 10 μm.
of domains, their dynamics, and stability. As a consequence, line tension is likely to be one the key parameters controlling the size and dynamic properties of cellular rafts and other segregated domains.

The line tension of phase separated membranes can be modulated by altering the lipid composition or the protein content of the membrane. The lipid composition of cellular membranes is composed of hundreds of species and is temporally and spatially regulated (1, 46). Changes in the average length of the lipid molecules that modify height mismatch or the presence of lipids with intrinsic monolayer curvature will consequently affect the line tension at the phase boundary.

In the case of proteins, we have previously shown that an active peptide derived from the apoptotic protein Bax (47, 48) can decrease the line tension at the phase interface and alter domain morphology (49). A similar effect on the line tension has been also reported for other proteins, like PLA2 (50) and N-Ras (12). To further understand the behavior of cellular rafts, it will be interesting to investigate line tension effects in systems with high concentration of proteins and in the presence of lipids with surfactant properties.

In addition to control of line tension, regulation of raft size in cells probably depends also on the regulation of lipid exchange between cellular membranes. This implies a balance between line tension-driven processes and mechanisms like endocytosis, which would eliminate rafts from membranes once they reach a certain size.

To conclude, our experiments convincingly demonstrate a strong influence of line tension on the properties of raft-like domains. The temperature of phase separation, the dynamics of domain growth, and the distribution of domain sizes depend strongly on the phase height mismatch, a key parameter causing line tension at the phase interface. When considering line tension calculated from a theoretical model, our results revealed a linear increase of demixing temperature and domain growth rate with line tension. Domain budding was also shown to depend on the difference in thickness between the coexisting phases. Altogether, these observations point the importance of mechanisms of line tension control to ensure adequate membrane functionality and lateral organization in cells.

Acknowledgments—We thank E. Petrov and J. Suckale for useful discussions and careful reading of the manuscript.

REFERENCES
1. van Meer, G. (2005) EMBO J. 24, 3159–3165
2. Jacobson, K., Mouritsen, O. G., and Anderson, R. G. (2007) Nat. Cell Biol. 9, 7–14
3. Simons, K., and Ikonen, E. (1997) Nature 387, 569–572
4. Simons, K., and Vaz, W. L. (2004) Annu. Rev. Biophys. Biomol. Struct. 33, 269–295
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5. Samsonov, A. V., Mihalyov, I., and Cohen, F. S. (2001) *Biophys. J.* **81**, 1486–1500
6. Mayor, S., and Rao, M. (2004) *Traffic* **5**, 231–240
7. Bacia, K., Scherfeld, D., Kahya, N., and Schwille, P. (2004) *Biophys. J.* **87**, 1034–1043
8. Baumgart, T., Hess, S. T., and Webb, W. W. (2003) *Nature* **425**, 821–824
9. Chiantia, S., Kahya, N., Ries, J., and Schwille, P. (2006) *Biophys. J.* **90**, 4500–4508
10. Kahya, N., Scherfeld, D., Bacia, K., Poolman, B., and Schwille, P. (2003) *J. Biol. Chem.* **278**, 28109–28115
11. Veatch, S. L., and Keller, S. L. (2005) *Proc. Natl. Acad. Sci. U. S. A.* **102**, 1025–1030
12. Staneva, G., Angelova, M. I., and Koumanov, K. (2004) *Chem. Phys. Lipids* **129**, 53–62
13. Brown, D. A., and London, E. (2000) *J. Biol. Chem.* **275**, 17221–17224
14. Maulik, P. R., and Shipley, G. G. (1996) *Biophys. J.* **70**, 2256–2265
15. Saslowsky, D. E., Lawrence, J., Ren, X., Brown, D. A., Henderson, R. M., and Edwardson, J. M. (2002) *J. Biol. Chem.* **277**, 26966–26970
16. Kuzmin, P. I., Akimov, S. A., Chizmadzhev, Y. A., Zimmerberg, J., and Cohen, F. S. (2005) *Biophys. J.* **88**, 1120–1133
17. Blanchette, C. D., Lin, W. C., Ratto, T. V., and Longo, M. L. (2006) *Biophys. J.* **90**, 4466–4478
18. Veatch, S. L., and Keller, S. L. (2005) *Biochim. Biophys. Acta* **1746**, 172–185
19. Baumgart, T., Das, S., Webb, W. W., and Jenkins, J. T. (2005) *Biophys. J.* **89**, 1067–1080
20. Blanchette, C. D., Lin, W. C., Orme, C. A., Ratto, T. V., and Longo, M. L. (2007) *Langmuir* **23**, 5873–5877
21. Akimov, S. A., Kuzmin, P. I., Zimmerberg, J., and Cohen, F. S. (2007) *Phys. Rev. E Stat. Nonlin. Soft Matter Phys.* **75**, 011919
22. Chiantia, S., Ries, J., Kahya, N., and Schwille, P. (2006) *Chem. Phys. Chem.* **7**, 2409–2418
23. Hammond, A. T., Heberle, F. A., Baumgart, T., Holowka, D., Baird, B., and Feigenson, G. W. (2005) *Proc. Natl. Acad. Sci. U. S. A.* **102**, 6320–6325
24. Bacia, K., Schwille, P., and Kurzchalia, T. (2005) *Proc. Natl. Acad. Sci. U. S. A.* **102**, 3272–3277
25. Kohyama, T., Kroll, D. M., and Gompper, G. (2003) *Phys. Rev. E Stat. Nonlin. Soft Matter Phys.* **68**, 061905
26. Leonenko, Z. V., Finot, E., Ma, H., Dahms, T. E., and Cramb, D. T. (2004) *Biophys. J.* **86**, 3783–3793
27. Cohen, F. S., and Melikyan, G. B. (2004) *J. of Membr. Biol.* **199**, 1–14
28. Hamm, M., and Kozlov, M. M. (1998) *European Physical Journal B* **6**, 519–528
29. Hamm, M., and Kozlov, M. M. (2000) *Eur. Phys. J. E, Soft Matter* **3**, 323–335
30. Rawicz, W., Olbrich, K. C., McIntosh, T., Needham, D., and Evans, E. (2000) *Biophys. J.* **79**, 328–339
31. Lin, W. C., Blanchette, C. D., and Longo, M. L. (2007) *Biophys. J.* **92**, 2831–2841
32. Laradji, M., and Kumar, P. B. (2006) *Phys. Rev. E Stat. Nonlin. Soft Matter Phys.* **73**, 040901
33. Wallace, E. J., Hooper, N. M., and Olmsted, P. D. (2006) *Biophys. J.* **90**, 4104–4118
34. Ayton, G. S., McWhirter, J. L., McMurtry, P., and Voth, G. A. (2005) *Biophys. J.* **88**, 3855–3869
35. Jorgensen, K., and Mouritsen, O. G. (1995) *Biophys. J.* **69**, 942–954
36. Laradji, M., and Sunil Kumar, P. B. (2004) *Phys. Rev. Lett.* **93**, 198105
37. Hu, Y., Meleson, K., and Israelachvili, J. (2006) *Biophys. J.* **91**, 444–453
38. Yanagisawa, M., Imai, M., Masui, T., Komura, S., and Ohta, T. (2007) *Biophys. J.* **92**, 115–125
39. Mouritsen, O. G. (1990) *Int. J. Modern Phys. B* **4**, 1925–1954
40. Frolov, V. A., Chizmadzhev, Y. A., Cohen, F. S., and Zimmerberg, J. (2006) *Biophys. J.* **91**, 189–205
41. Baumgart, T., Hammond, A. T., Sengupta, P., Hess, S. T., Holowka, D. A., Baird, B. A., and Webb, W. W. (2007) *Proc. Natl. Acad. Sci. U. S. A.* **104**, 3165–3170
42. Julicher, F., and Lipowsky, R. (1993) *Phys. Rev. Lett.* **70**, 2964–2967
43. Julicher, F., and Lipowsky, R. (1996) *Phys. Rev. E Stat. Phys. Plasmas Fluids Relat. Interdiscip. Topics* **53**, 2670–2683
44. Liang, Y., Fotiadis, D., Filipke, S., Saperstein, D. A., Palczewski, K., and Engel, A. (2003) *J. Biol. Chem.* **278**, 21655–21662
45. Quinn, P., Griffiths, G., and Warren, G. (1984) *J. Cell Biol.* **98**, 2142–2147
46. Garcia-Saez, A. J., and Schwille, P. (2007) *Appl. Microbiol. Biotechnol.* **75**, 663–671
47. Garcia-Saez, A. J., Coraiola, M., Dalla, S. M., Mingarro, I., Muller, P., and Salgado, J. (2005) *Biophys. J.* **88**, 3976–3990
48. Garcia-Saez, A. J., Coraiola, M., Serra, M. D., Mingarro, I., Menestrina, G., and Salgado, J. (2005) *Biophys. J.* **88**, 3976–3990
49. Garcia-Saez, A. J., Coraiola, M., Serra, M. D., Mingarro, I., Menestrina, G., and Salgado, J. (2005) *FEBS J.* **273**, 971–981
50. Garcia-Saez, A. J., Chiantia, S., Salgado, J., and Schwille, P. (2007) *Biophys. J.* **93**, 103–112
51. Nicolini, C., Baranski, J., Schlummer, S., Palomo, J., Lumbierres-Burgues, M., Kahms, M., Kuhlmann, J., Sanchez, S., Gratton, E., Waldmann, H., and Winter, R. (2006) *J. Am. Chem. Soc.* **128**, 192–201