

Hydrophobic Mismatch Triggering Texture Defects in Membrane Gel Domains

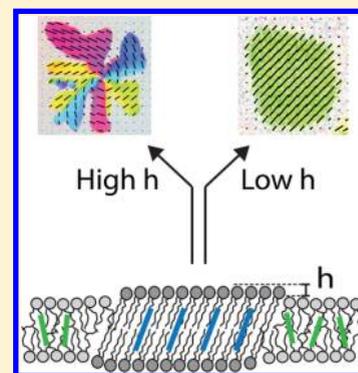
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S Supporting Information

ABSTRACT: The orientational texture of gel-phase lipid bilayers is a phenomenon that can structure membrane domains. Using two-photon polarized fluorescence microscopy and image analysis, we map the lateral variation of the lipid orientation (the texture) in single domains. With this method, we uncover a lipid-induced transition between vortex and uniform textures in binary phospholipid bilayers. By tuning the lipid composition, the hydrophobic mismatch at the domain boundary can be varied systematically as monitored by AFM. Low hydrophobic mismatch correlates with domains having uniform texture, while higher mismatch values correlate with a vortex-type texture. The defect pattern created during early growth persists in larger domains, and a minimal model incorporating the anisotropic line tension and the vortex energy can rationalize this finding. The results suggest that the lipid composition and the domain nucleation process are critical factors that determine the texture pattern of membrane domains.

SECTION: Biomaterials, Surfactants, and Membranes



Biological membranes have the basic architecture of a self-assembled lipid bilayer, but the physiological role associated with their compositional and structural complexity still remains incompletely understood. To help reveal the underlying physical principles governing biomembrane structure and function, model membranes with simple compositions have become indispensable experimental tools. Indeed, the physical underpinning of the raft concept in cell biology largely originates from studies of thermodynamic phase separation in bilayers containing a few lipid components.¹

The phases of lipid bilayers are characterized by different degrees of order in the acyl chain conformation and the in-plane position. The liquid disordered (ld) phase has a large acyl chain conformational freedom and high lateral mobility ($D \approx 1-2 \times 10^{-11} \text{ m}^2 \text{ s}^{-1}$).² Conversely, the gel phase is characterized by slow lateral diffusion ($D \approx 0.01-1 \times 10^{-13} \text{ m}^2 \text{ s}^{-1}$)^{3,4} and high acyl chain order with a conserved polar tilt angle normal to the bilayer.

The mechanisms for providing lateral structure to membranes extend beyond the information contained in the equilibrium phase diagram. The gel phase, in particular, can display a rich pattern of domain shapes and sizes regulated by the growth kinetics and the spatial arrangement of nucleation points.⁵ We have recently shown that gel domains in phospholipid membranes may contain long-range orientational texture patterns in the projection (director) of the acyl chains on the bilayer plane.^{6,7} The texture of gel domains can exhibit topological defects including a vortex, pairs of half-integer vortices, and line defects. As comparison, textures in domains of Langmuir monolayers are well-known⁸⁻¹⁰ and have been described by Landau-type models.¹¹ Bilayer and monolayer

textures share similarities with defects in Smectic-C liquid crystals,^{12,13} although these are effectively infinite systems as opposed to finite-sized domains with a boundary to an isotropic fluid phase. The fact that orientational textures are prevalent in gel-phase bilayers represents a previously hidden level of complexity. However, it is not yet known if this has significance as an organizing principle in cellular membranes.

Defects constitute sites of high local energy density with possible affinity for biomolecular binding and activity. In bilayers, the lipid composition is expected to be the major determinant of the defect structure. Texture with a single director orientation (uniform texture) will have the lowest bulk energy due to the absence of defects. In a simple picture, the domain edge contributes an additional energy term that, if sufficiently large, may trigger the generation of a vortex defect in order to satisfy boundary conditions at the domain edge. Because the lipid composition determines the line tension, the composition enables control over the transition between uniform and vortex textures. The magnitude of the line tension is closely linked to the hydrophobic mismatch at the domain border, which is directly accessible in AFM measurements. The hydrophobic mismatch has previously been coupled to line tension in modeling studies,¹⁴ and they have been correlated experimentally in nucleation studies.^{15,16}

In this Letter, we document a coupling between the hydrophobic mismatch and the resulting texture type in gel

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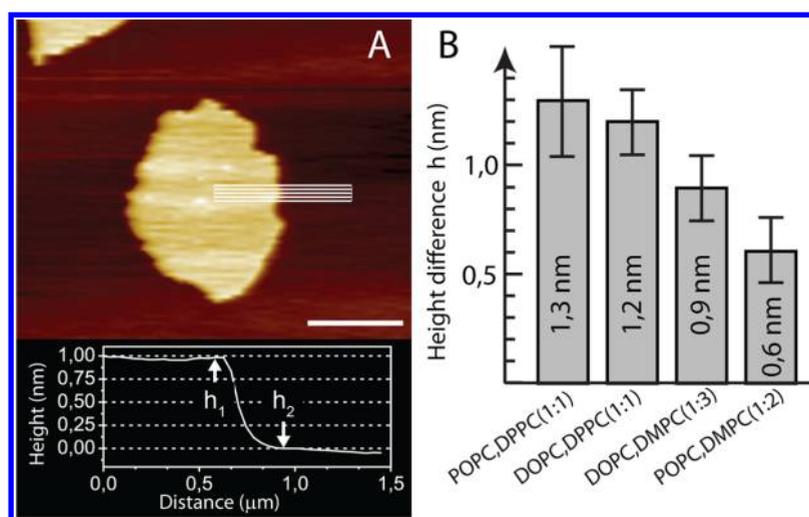


Figure 1. Measurement of hydrophobic mismatch by AFM. The image in (A) shows a typical gel domain in a bilayer with composition (DOPC/DMPC 1:3). The profile across the domain boundary (inset) is constructed as the average over parallel lines in the image, as indicated. The height difference across the domain edge represents the hydrophobic mismatch and is obtained as $h = h_1 - h_2$. Several ($N > 20$) domains were measured for each membrane composition with h values shown in (B). The error bars are \pm the standard deviation of the measured h values. The scale bar is 1 μm .

domains using binary supported lipid bilayers displaying ld/gel phase coexistence. We demonstrate that a transition from vortex to uniform texture takes place for low values of the thickness mismatch between the gel and ld phases. By varying the lipid acyl chain length and saturation, the mismatch is systematically modulated, while the lipid head groups are kept unchanged.

Atomic force microscopy (AFM) was used to measure the difference in height (h in Figure 2F) between the gel and ld phases as a quantitative measure of the hydrophobic mismatch. Samples were imaged in buffer with minimal contact force to minimize potential membrane deformations, as exemplified in Figure 1A. The height difference h was obtained using an average over parallel lines across the domain boundary. The measured height differences are summarized in Figure 1B. The relationship between the total membrane thickness and the AFM topography was recently examined using imaging ellipsometry.¹⁷ It was found that the total thickness difference for bilayer domains amounts to twice the height difference ($2h$) measured by AFM. We also note that the heights measured in this study compare well with previously reported values for binary membranes.^{18,19}

Examples of domain textures in membranes with varying hydrophobic mismatch are shown in Figure 2A–D. The lateral variation of the azimuth angle φ_c of the molecular director is color coded as shown in Figure 2A (inset), and the director is shown as black lines. For the two membrane compositions with the largest hydrophobic mismatch (Figure 2A,B), we find the same type of central defect. Both systems contain a vortex defect of $\text{index} = 1$, which is split into two closely spaced defects of $\text{index} = 1/2$, as previously described and analyzed in detail.⁶ There is a continuous deformation of the director field close to the center of the vortex while the peripheral regions of the domains are split into subdomains with distinct molecular orientations.

As the hydrophobic mismatch is lowered, the texture pattern converts to a different type. Examples for the two membrane compositions with the lower hydrophobic mismatch are shown in Figure 2C,D. For the (DOPC/DMPC, 1:3) membrane with

$h = 0.9$ nm, we find one population of domains displaying a uniform texture with no lateral variation in the azimuth angle. Another population of domains has a binary texture with two dominant molecular orientations. For membranes with composition (POPC/DMPC, 1:2), the hydrophobic mismatch is reduced to $h = 0.6$ nm. In this case, all domains have uniform texture, as is evident in Figure 2D. The orientation of the uniform director appears to vary randomly between domains, indicating that the mica substrate has no substantial influence on the domain orientations. It is also noted that domains in Figure 2C,D are more rounded and have a higher area-to-perimeter ratio than domains in Figure 2A,B. This could reflect that domain shapes are dominated by the growth kinetics, with the cooling rate and resulting growth speed for the systems in Figure 2C,D being lower.

A key question is whether the observed differences in the defect structure are governed by the early nucleation process for domains or if a global conversion and equilibration in the texture is possible for larger (micrometer-sized) domains. Because the domains are grown by cooling, temperature-dependent parameters such as Frank constants and line tension will change during growth, possibly altering the equilibrium texture of domains. In Figure 3, we trace the domain texture during growth for membranes displaying vortex textures (Figure 3A–C) and uniform textures (Figure 3D–F). The images in Figure 3A and D were acquired in the early stages of growth but with sufficiently large domains that internal orientation patterns could still be resolved optically. The round shape of the early domain (in Figure 3A) and the central vortex defect generates a specific orientation of the director relative to the boundary normal. This is in agreement with a boundary condition enforcing a specific orientation of the director. Close inspection of the vortex defect in Figure 3A–C reveals that the central part of the domain remains unchanged during subsequent growth (Figure 3B,C). Lipids condense epitaxially on the domain during growth and acquire the orientation of the local neighborhood. A topological defect present in the nucleation core or at early stages of growth remains in the domain as it grows. Conversely, if a uniform

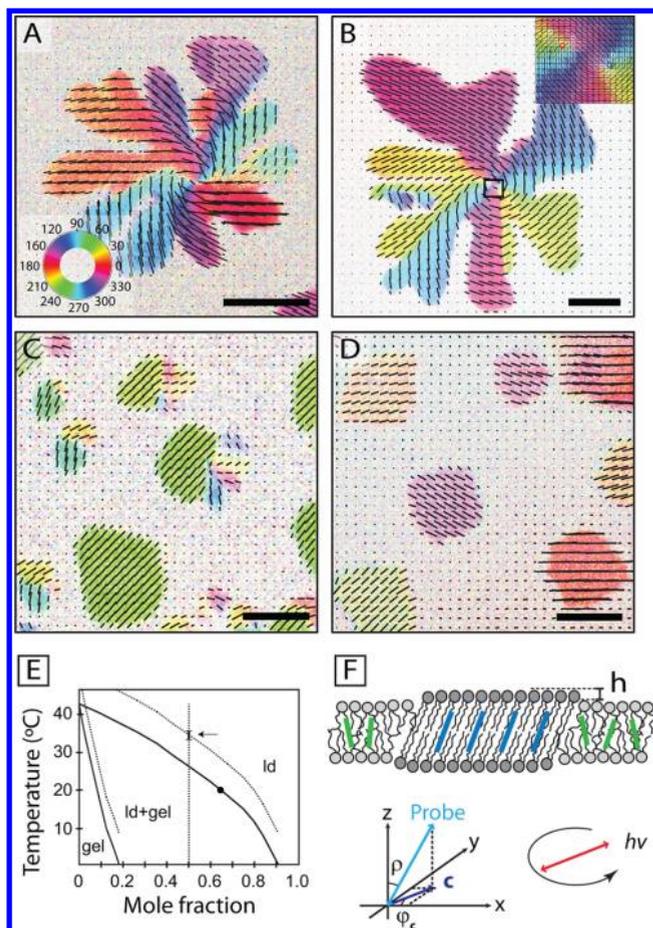


Figure 2. Typical textures observed in bilayer gel domains for four binary lipid compositions, (DOPC/DPPC, 1:1) (A), (POPC/DPPC 1:1) (B), (DOPC/DMPC, 1:3) (C), and (POPC/DMPC, 1:2)(D). The director \mathbf{c} is represented by black lines, and the in-plane orientation of the director is mapped by a color code according to the circular scheme (A, inset). A double vortex defect is present in (A,B) as indicated by inset in (B). In (D) and partly in (C), the domains display uniformly aligned textures. The composition–temperature phase diagram for (DOPC/DPPC) is shown in (E). Black line are from refs 25 and 26, while the dashed lines indicate phase boundaries corresponding to the nucleation temperature (arrow) found in fluorescence experiments.^{5,27} The bilayer configuration is illustrated in (F). The Laurdan probe in the gel phase (blue lines) aligns with the ordered lipid acyl chains, while Laurdan in the fluid phase (green lines) has a fluctuating orientation. The azimuth angle of the molecular director (φ_c) is experimentally measurable by rotating the polarization direction of the two-photon excitation light (red arrow). All scale bars are 5 μm .

domain texture is found in the early stages, the domains do not develop topological defects at later stages of the growth. Thus, such global rearrangements/conversions of the texture type for a large domain appear to be energetically constrained.

Several parameters could potentially influence domain texture and be responsible for triggering topological defects. The uniform and vortex textures observed in this study are experimentally associated with different cooling rates and changes in the lipid composition, which affects hydrophobic mismatch. We have previously demonstrated⁵ that the cooling rate is linearly related to the area density of nucleation sites N_s . In this study, we did not find a dependence of the texture defect pattern on the cooling rate. In other words, it is not possible to

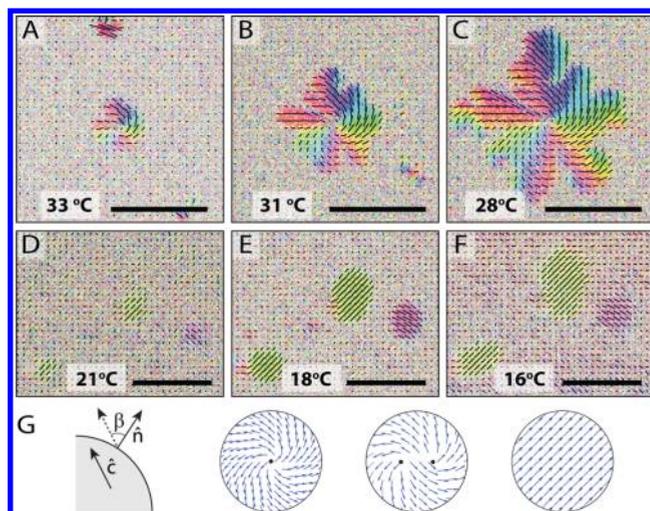


Figure 3. Textures recorded during growth (cooling) of gel domains for membranes with compositions (DOPC/DPPC, 1:1) (A–C) and (POPC/DMPC 1:2) (D–F). For each composition, the sequence shows the same sample region, and the sample temperature is given in each frame. The sample in (A–C) displays a vortex defect at all stages, while the sample in (D–F) has uniform alignment. Schematic illustration of the angle β between the director and the boundary normal and examples of possible textures for a circular nucleation core (G). All scale bars are 5 μm .

induce a transition between uniform and vortex textures only by changing the cooling rate. The polar tilt angle ρ (Figure 2F) of the pure lipids DMPC and DPPC in the gel phase has a value in the range of 31–33° as measured by X-ray techniques.^{20–22} Although the value for DPPC is slightly higher than that for DMPC,²³ the difference is too small to account for major changes in the texture pattern. This leads us to consider the influence of the hydrophobic mismatch on the texture type.

From the results in Figure 3, we establish that the texture type (uniform or vortex) in late micrometer-sized domains is determined by the structure of the nucleation core or of the very early domain. This implies that defects in late domains are governed by the nucleation and early growth process. In order to model this effect, we focus on possible mechanisms governing the texture of the nucleation core.

On experimental time scales, the texture in late domains will not reach equilibrium due to slow diffusion and dynamics of the gel phase. However, for the nucleation core, the time scale for equilibration is reduced significantly and becomes sufficiently short that equilibration of the texture during growth is possible. We therefore evaluate the energy criterion for generating vortex or uniform textures in the core.

The free energy of the nucleation core can, in a minimal model, be written as a bulk term plus an interface term, $E_{\text{nucleus}} = E_{\text{bulk}} + E_i$. For uniform textures, the bulk term vanishes, while for vortex textures, it contains the total energy associated with the formation of a vortex defect, $E_{\text{bulk}} = E_{\text{vortex}}$. The interface term is the integral of the anisotropic line tension $\gamma(\beta(\mathbf{r}))$ along the domain boundary

$$E_i = \oint ds \cdot \gamma(\beta) \quad (1)$$

Here, $\beta(\mathbf{r})$ is the angle between the boundary normal and the director \mathbf{c} . The line tension can be represented as a Fourier

series in β containing the constant isotropic line tension γ_0 and higher-order terms that depend on β

$$\gamma(\beta) = \gamma_0 + \sum_{n=1}^{\infty} (\Gamma_n \cos n\beta + \Lambda_n \sin n\beta) \quad (2)$$

The Fourier coefficients Γ_n and Λ_n determine the shape of $\gamma(\beta)$, and its global minimum corresponds to the preferred orientation of the director relative to the boundary producing the lowest line tension. Round nuclei with a vortex defect can fulfill the boundary condition everywhere on the border and obtain the lowest total boundary energy $E_{i,\text{aligned}}$, while nuclei with a uniform texture will violate boundary conditions, resulting in a higher boundary energy $E_{i,\text{uniform}}$. We assume that the isotropic component of the line tension γ_0 is primarily governed by the hydrophobic mismatch at the boundary, as previously suggested.^{15,16} Nucleation cores with vortex textures fulfill the inequality

$$E_{\text{vortex}} < E_{i,\text{uniform}} - E_{i,\text{aligned}} = \Delta_i \quad (3)$$

while cores with uniform texture have the inequality sign reversed. Assuming that E_{vortex} is nearly constant for our lipid compositions, it is the magnitude of the energy difference Δ_i relative to E_{vortex} that determines the formation of uniform or vortex textures. Note that Δ_i is controlled exclusively by the Fourier coefficients Γ_n , Λ_n because the γ_0 term cancels. The coefficients are subject to the constraint that the line tension $\gamma(\beta)$ must always be positive, and consequently, larger numerical values $|\Gamma_n|$ and $|\Lambda_n|$ are allowed for domains with a larger isotropic line tension γ_0 and a larger hydrophobic mismatch. This coupling sets an upper bound on Δ_i , which will be smaller for domains with smaller hydrophobic mismatch and isotropic line tension γ_0 . According to our experimental results, the membranes displaying uniform texture indeed have smaller hydrophobic mismatch correlating with smaller values of the isotropic line tension γ_0 .

The argument given above lends support to the hypothesis that the texture of the nucleation core or of the early domain is governed by energy minimization while larger domains are dominated by growth kinetics without sufficient time to reach the global energy minimum. For the lipid compositions studied here, the central vortex is split into a pair of half-integer disclinations, as previously analyzed.⁶ As schematically illustrated in Figure 3G, splitting of the central vortex is possible while, at the same time, the texture orientation β relative to the boundary remains relatively unaffected. The energy argument given above is therefore valid for both a single integer and a pair of closely spaced half-integer disclinations.

In conclusion we have demonstrated that the hydrophobic mismatch at the boundary of bilayer gel domains correlates with the onset of a central vortex defect. For domains with sufficiently low hydrophobic mismatch, domains adopt a uniform texture with a single orientation. We trace the effect to the structure of the nucleation core that governs the defect pattern in subsequent large domains. A simple energy argument based on the anisotropic line tension and the vortex energy can rationalize this effect. Our results point to the importance of the nucleation process and the lipid composition for creating topological texture defects in membranes.

EXPERIMENTAL SECTION

Binary supported membranes on mica were fabricated by spin coating, using a previously established procedure.^{5,19} The

membranes were composed of a lipid with high melting temperature (T_m) and a low T_m lipid characterized by phase diagrams with a gel–ld coexistence region covering a wide temperature range. The high T_m lipids were 1,2-dimyristoyl-*sn*-glycero-3-phosphocoline (DMPC, C14:0/C14:0, $T_m = 23$ °C) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocoline (DPPC, C16:0/C16:0, $T_m = 41$ °C), and the low T_m lipids were 1,2-dioleoyl-*sn*-glycero-3-phosphocoline (DOPC, C18:1/C18:1, $T_m = -20$ °C) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocoline (POPC, C16:0/C18:1, $T_m = -2$ °C). All lipids were obtained from Avanti Polar Lipids Inc. (Alabaster, AL). The samples were hydrated in a HEPES buffer at pH = 7 and heated to above the phase transition temperature, followed by a cooling at a controlled rate into the two-phase coexisting region. Gel domains nucleate when the temperature crosses the phase boundary. As the sample is further cooled, the gel domains grow in size according to the lever rule, while the number of domains remains constant.^{5,24} The temperature ramp determines the density of nucleation sites and was 0.8 °C/min for the DOPC/DPPC and POPC/DPPC mixtures and 0.1 °C/min for DOPC/DMPC and POPC/DMPC mixtures. The lower-temperature ramp employed for the systems containing DMPC was used to ensure that sufficiently few and large domains would form in these systems. Atomic force microscopy (AFM) was performed in soft contact mode using a JPK Nanowizard (JPK Instruments AG, Berlin, Germany) at ambient temperature (20–23 °C). Cantilevers were of the type MSCT, lever C, with a spring constant of 0.01 N/m (Bruker Corporation). The domain textures were investigated in a custom-built polarization two-photon fluorescence microscope using 6-dodecanoyl-2-dimethylaminonaphthalene (Laurdan, 0.5 mol %) as the fluorescence probe. Each texture map is based on 36 fluorescence images acquired with 10° increments in the polarization angle of the 780 nm excitation light. A Fourier decomposition of the pixel intensities enables construction of texture maps that spatially resolve the azimuth angle φ_c of the molecular director. Further details are provided as Supporting Information and in ref 6.

ASSOCIATED CONTENT

Supporting Information

Experimental details of the polarized fluorescence microscopy setup. Description of the image analysis procedure and the calculation of texture maps from fluorescence image stacks. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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