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Reply to Comment to "Elastic membrane deformations govern interleaflet coupling of lipid-ordered domains"

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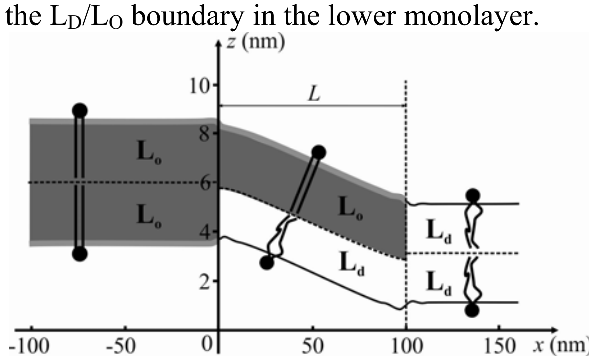
Our recent publication in this journal [1] challenges the concept that domains in opposing membrane leaflets are in register because of interactions at a membrane midplane. Compelled by the lack of direct experimental proof for (i) midplane interaction via an overhang [2] or (ii) L_O and L_D phases repelling each other [3] we propose that minimization of line tension γ drives registration (R) [1]. We dismiss antiregistration (AR) as an unlikely event because its twofold larger domain area translates into a $\sqrt{2}$-fold larger boundary length. Moreover, the line tensions at the L_D/L_D–L_D/L_O and L_O/L_O–L_D/L_O interfaces (Cartoon 1), γ_{DD} and γ_{OO}, respectively, exceed the line tension at the L_D/L_D–L_O/L_O interface, γ_R rendering the elastic energy W_R of the registered state smaller than the elastic energy W_{AR} of the antiregistered state. Consequently, registration is energetically favorable. Also, $\gamma_R < \gamma_{DD}$, γ_{OO} because an isolated L_D/L_O boundary in only one leaflet leads to membrane bending. As readily observed in the Cartoon, for the membrane to remain flat, a substantial torque must be applied or an L_D/L_O boundary must be created in the upper monolayer to oppose the L_D/L_O boundary in the lower monolayer.



Cartoon 1. Calculated membrane shape at raft boundary for L = 100nm. The transitional L_O/L_D zone is tilted.

A flat membrane is assured in [1] by boundary conditions (Eq. 6), which set the L_O/L_O and L_D/L_D bilayers to a flat horizontal (in Cartoon 1 at $x \to +\infty$ and $x \to -\infty$, respectively). A tilt was only allowed for the transitional L zone to yield minimal W. Accounting for the spontaneous curvatures of L_O and L_D, $J_O = -0.07$ nm^{-1} and $J_D = -0.1$ nm^{-1}, respectively, in a 1:1:1 mixture of dioleoylphosphatdiylcholine:dipalmitoylphatdiylcholine:cholesterol [4] and assuming h_D = 1.3 nm (L_D-phase) and h_O = 1.6 nm (L_O-phase) [5] yields the line tensions (in pN) of γ_{DD}=1.06, γ_{OO}=1.54, and γ_R=0.52. This is in stark contrast to Williamson's and Olmsted's erroneous assumption [6] that $\gamma_{R\text{-}AR} = \gamma_{DD} = \gamma_{OO} = \gamma_\infty/2$. There γ_∞ was defined as $\gamma_R(L \to \infty)$. For the specific lipid mixture γ_∞ is equal to 0.83 pN. Thus, for the physiological relevant case of small L_O domains (signaling platforms = rafts) surrounded by a large area of L_D lipids, the ratio $W_R/W_{AR} = \gamma_R/(\sqrt{2}\gamma_{DD}) = 0.5/1.5 \approx 0.34 < 1$, clearly favors registration. This is true for values of lateral tension $\sigma \leq 6$mN/m per monolayer. Higher values of σ result in membrane rupture [7] and may thus be disregarded. Experimental data are available also for a second 1:1:1 mixture of palmitoyloleoylphosphatdiylcholine:sphingomyelin:cholesterol: For J_O = –0.2 nm^{-1} and J_D = –0.1 nm^{-1} [4] we find γ_{DD}=1.02, γ_{OO}=1.65, γ_R=0.6, and γ_∞= 0.74. For small L_O domains within a sea of L_D lipids, $W_R/W_{AR} \approx 0.41 < 1$, indicating that antiregistration does not occur. We conclude that our theory works well for all physiologically relevant cases.

Williamson and Olmsted [6] raised the issue of large L_O domains occupying an area fraction □ that is comparable to that of L_D phases. Although such a configuration precludes the L_O phase from functioning as a signaling platform (raft), their analysis may be helpful for a generalization of the theory. For 1/4<□<1/2 we find:

$$W_R = \gamma_R 2\sqrt{\phi \pi A}, \quad W_{AR} = \gamma_{OO} 2\sqrt{(1-2\phi)\pi A}$$

where □×A is the area of the L_O domain. The ratio W_R/W_{AR} =1 for a critical □ value, □$_{crit}$:

$$\phi_{crit} = \frac{\gamma_{OO}^2}{2\gamma_{OO}^2 + \gamma_R^2}$$

to yield □ = 0.47 for both lipid mixtures. Thus, if only γ causes domain registration, registration might not occur in the interval $0.47<\phi<0.53$. Therefore, our theory should be extended to account for these rare cases. In [1] we ignored the doubling of the area that is stiff if antiregistration occurs. Because stiff L_O areas show reduced undulations, antiregistration violates the tendency of the system toward maximum entropy. In contrast, the mutual attraction of stiff membrane regions from both monolayers maximizes the membrane area in which the membrane is free to undulate, thereby providing a gain in free energy [8]. Since energy is required to prevent the membrane from undulating [9], we envision that accounting for it will rule out antiregistration for all □ values. A paper in preparation will provide a full quantitative analysis.

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