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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) LO and LD phases repelling each other [3] we propose that minimization of line tension \( \gamma \) 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 LD/LO–LD/LO and LD/LO–LD/LO interfaces (Cartoon 1), \( \gamma_{DD} \) and \( \gamma_{OO} \), respectively, exceed the line tension at the LD/LO–LD/LO interface, \( \gamma_{R} \), 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, \( \gamma_{R} < \gamma_{DD}, \gamma_{OO} \) because an isolated LD/LO 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 LD/LO boundary must be created in the upper monolayer to oppose the LD/LO boundary in the lower monolayer.

Cartoon 1. Calculated membrane shape at raft boundary for \( L = 100 \text{nm} \). The transitional LO/LD zone is tilted.

A flat membrane is assured in [1] by boundary conditions (Eq. 6), which set the LO/LD and LD/LD bilayers to a flat horizontal (in Cartoon 1 at \( x \rightarrow +\infty \) and \( x \rightarrow -\infty \), respectively). A tilt was only allowed for the transitional \( L \) zone to yield minimal \( W \). Accounting for the spontaneous curvatures of LO and LD, \( J_0 = -0.07 \text{ nm}^{-1} \) and \( J_D = -0.1 \text{ nm}^{-1} \), respectively, in a 1:1:1 mixture of dioleoylphosphatidylcholine:dipalmitoylphosphatidylcholine:cholesterol [4] and assuming \( h_D = 1.3 \text{ nm} \) (LD-phase) and \( h_O = 1.6 \text{ nm} \) (LO-phase) [5] yields the line tensions (in pN) of \( \gamma_{DD} = 1.06, \gamma_{OO} = 1.54 \), and \( \gamma_{R} = 0.52 \). This is in stark contrast to Williamson’s and Olmsted’s erroneous assumption [6] that \( \gamma_{R,AR} = \gamma_{DD} = \gamma_{OO} = \gamma_{\infty}/2 \). There \( \gamma_{\infty} \) was defined as \( \gamma_{\infty}(L \rightarrow \infty) \). For the specific lipid mixture \( \gamma_{\infty} \) is equal to 0.83 pN. Thus, for the physiological relevant case of small LO domains (signaling platforms = rafts) surrounded by a large area of LD lipids, the ratio \( W_R/W_{AR} \approx \gamma_R/(\sqrt{2}\gamma_{DD}) = 0.5/1.5 \approx 0.34 < 1 \), clearly favors registration. This is true for values of lateral tension \( \sigma \leq 6 \text{mN/m} \) per monolayer. Higher values of \( \sigma \) result in membrane rupture [7] and
may thus be disregarded. Experimental data are available also for a second 1:1:1 mixture of palmitoyloleoylphosphatidylcholine:sphingomyelin:cholesterol: For \( J_O = -0.2 \text{ nm}^{-1} \) and \( J_D = -0.1 \text{ nm}^{-1} \) [4] we find \( \gamma_{DD}=1.02, \gamma_{OO}=1.65, \gamma_R=0.6, \) and \( \gamma_\infty=0.74. \) 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 \( 1/4 < \phi < 1/2 \) we find:

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

where \( \times A \) is the area of the \( L_O \) domain. The ratio \( W_R/W_{AR} =1 \) for a critical value, \( \phi_{crit}: \)

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

to yield \( \phi_{crit} = 0.47 \) for both lipid mixtures. Thus, if only \( \gamma \) 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.

REFERENCES