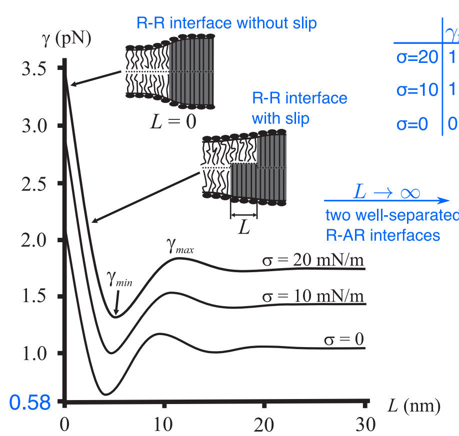
Comment on "Elastic Membrane Deformations Govern Interleaflet Coupling of Lipid-Ordered Domains"

Galimzyanov *et al.* [1] find that line tension between thick liquid-ordered (L_o) and thinner liquid-disordered (L_d) registered lipid bilayer phases is minimized by an asymmetric "slip region," length $L \sim 5$ nm (Fig. 1). They claim that line tensions alone explain domain registration, without "direct" (area-dependent) interleaflet interaction [2,3]. We show this is unfounded; without direct interaction their results would predict *antiregistration*, dependent on composition. To find equilibrium from line energies, line *tensions* must be combined with interfacial lengths for given states at given composition. This was not done in Ref. [1].

Figure 2(a) shows "registered" (R) and "antiregistered" (AR) bilayer phases in (ϕ^t, ϕ^b) (top and bottom-leaflet composition) space [2–4]. We assume $\phi^b = \phi^t \equiv \phi$ in each leaflet. "Domain registration" is R-R coexistence [Fig. 2(a)], with slip to minimize line tension (Fig. 1) [1].

Without direct interleaflet coupling, R and AR bulk free energies would be equal [3], so line energies alone would determine equilibrium. The authors claim perfect antiregistration (AR-AR state) is only possible for $\phi = 1/2$. They assume imperfect antiregistration involves *both* R-AR interfaces in Fig. 1. They find a minimum line tension $\gamma_{\min}$, and a limiting value $\gamma_\infty \equiv \gamma(L \to \infty) > \gamma_{\min}$ as the two R-AR interfaces in Fig. 1 (L_d/L_d to AR, AR to L_o/L_o) move apart. They claim that $\gamma_\infty > \gamma_{\min}$ favors an R-R state. However, $\phi \neq 1/2$ only forces one R phase [the AR-AR-R state, Fig. 2(a)], and the line tension of *one* isolated R-AR interface in Fig. 1 is $\gamma_{\text{R-AR}} \approx \gamma_\infty/2$.

In R-R, an L_o/L_o droplet, area ϕA, circumference $\ell_{\text{R-R}} = 2\sqrt{\pi\phi A}$ is surrounded by L_d/L_d [Fig. 2(c), A = total area]. The line energy is $W_{\text{R-R}} = \ell_{\text{R-R}}\gamma_{\min}$. For AR-AR-R, the area of L_o/L_d or L_d/L_o is $2\phi A$. (The equal-thickness phases are treated as a quasiuniform single phase.) Hence, for $\phi < 1/4$,



	$\gamma_{\min}$	$\gamma_{\text{R-AR}}$	$\sqrt{2}\gamma_{\text{R-AR}}$
σ=20	1.31	0.87	1.23
σ=10	1.00	0.71	1.01
σ=0	0.63	0.52	0.74

FIG. 1. Line tension versus slip length L [1], annotated (blue). $\gamma(L \to \infty) = 2\gamma_{\text{R-AR}}$ (see text). An axis tick is corrected.

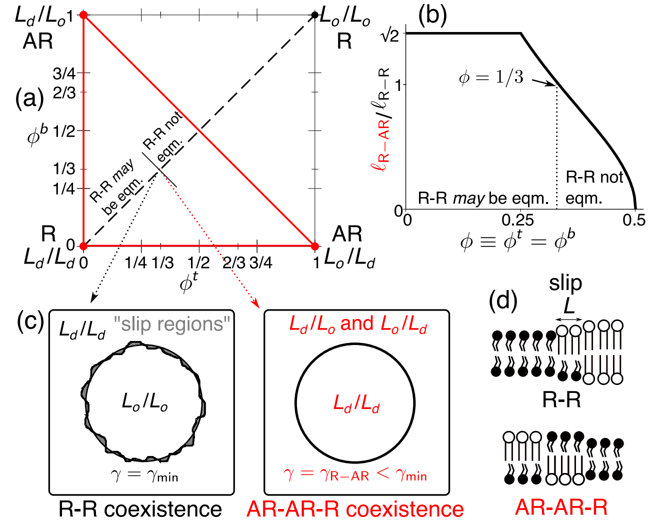


FIG. 2. (a) Partial diagram of two-phase R-R (black) and three-phase AR-AR-R (red) coexistences for $\phi^b = \phi^t \equiv \phi < 1/2$ [the full diagram is symmetric under inversion through (0.5,0.5) [4]]. (b) Minimal R-AR or R-R interface length, comparing AR-AR-R and R-R coexistence. (c) Morphology at $\phi = 1/3$, beyond which AR-AR-R *decreases* the thickness-mismatched interface length. (d) R-R coexistence; AR-AR-R.

L_d/L_d surrounds an AR domain ($\ell_{\text{R-AR}} = 2\sqrt{2\pi\phi A}$), with $W_{\text{R-AR}} = \ell_{\text{R-AR}}\gamma_{\text{R-AR}}$. $\ell_{\text{R-AR}} = \sqrt{2}\ell_{\text{R-R}}$ [Fig. 2(b)], so R-R would be equilibrium ($W_{\text{R-R}} < W_{\text{R-AR}}$) *if* $\gamma_{\min} < \sqrt{2}\gamma_{\text{R-AR}}$. This holds for $\sigma = 0$, but not $\sigma = 20$ mN/m (Fig. 1), and will depend on other parameters, e.g., the degree of hydrophobic mismatch.

For $1/4 < \phi < 1/2$, AR surrounds an L_d/L_d domain of $\ell_{\text{R-AR}} = 2\sqrt{\pi(1-2\phi)A}$. For $1/3 < \phi < 1/2$ (by symmetry $1/2 < \phi < 2/3$ [4]), $\ell_{\text{R-AR}} < \ell_{\text{R-R}}$; R-R would not be equilibrium for $\gamma_{\text{R-AR}} < \gamma_{\min}$. Indeed, as $\ell_{\text{R-AR}} \to 0$ *ever higher* $\gamma_{\text{R-AR}}$ would be needed to stabilize R-R. A full calculation of $\gamma_{\text{R-AR}}$ should use a single R-AR interface. Imposing flat boundary conditions could give an area-dependent cost, or make $\gamma_{\text{R-AR}}$ depend on where the boundary conditions are enforced.

Upon reinstating direct, area-dependent interleaflet coupling [undulations (see Reply) are one possible source], the *bulk* free energy of R is lower than AR, explaining equilibrium domain registration over all ϕ [2–4].

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