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Regulation of membrane proteins through local heterogeneity in lipid bilayer thickness

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Cell membranes show an intricate organization of lipids and membrane proteins into domains with distinct composition and hydrophobic thickness. Using mechanosensitive ion channels as a model system, we employ the membrane elasticity theory of lipid-protein interactions together with the Landau-Ginzburg theory of lipid domain formation to quantify protein-induced lipid bilayer thickness deformations in lipid bilayers with heterogeneous hydrophobic thickness. We show that protein-induced lipid bilayer thickness deformations yield, without any assumptions about preferential interactions between particular lipid and protein species, organization of lipids and membrane proteins according to their preferred hydrophobic thickness, and couple the conformational states of membrane proteins to the local membrane composition. Our calculations suggest that protein-induced lipid bilayer thickness deformations endow proteins in cell membranes with diverse and controlled mechanical environments that, in turn, allow targeted regulation of membrane proteins.

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Introduction. Cell membranes show an intricate supramolecular organization of membrane proteins and lipids into domains that vary in composition and hydrophobic thickness [1]. Heterogeneity in cell membrane structure and composition allows regulation of the interaction of membrane components and is crucial for a variety of cellular functions [2,3]. A wide range of experiments suggest [1–7] that membrane-actin interactions, preferential chemical interactions between particular lipid and protein species, and nonspecific, hydrophobic lipid-protein interactions provide key mechanisms for cell membrane organization. Membrane-actin interactions and preferential interactions between particular lipid and protein species have been explored in some detail through a combination of experiment and theory [8–15]. Furthermore, theoretical studies have shown [16–18] that lipid and protein composition can be linked, without requiring any specific interactions between particular lipid and protein species, through an elastic coupling between bilayer mean curvature and bilayer composition.

In recent experiments on lipid bilayers with heterogeneous hydrophobic thickness, it has been observed that lipid bilayer and protein composition couple through nonspecific lipid-protein interactions driven by local hydrophobic matching of bilayer and protein thickness, resulting in organization of lipid and protein species according to their energetically preferred hydrophobic thickness [1–3,19–21]. Motivated by these experimental observations, we combine here [17,22,23] the elasticity theory of lipid bilayer thickness deformations [24–30] with the Landau-Ginzburg (LG) theory of lipid domain formation [16,31–36] to quantify hydrophobic lipid-protein interactions in lipid bilayers with heterogeneous hydrophobic thickness [1–3,19–21]. We illustrate our theoretical approach for the mechanosensitive channel of large conductance (MscL), which provides a paradigm for the coupling of local lipid composition and membrane protein function [27–30,37–40]. Our calculations show that protein-induced lipid bilayer thickness deformations yield, without any assumptions about preferential interactions between particular lipid and protein species, key features of membrane organization observed in experiments [1–3,19–21,30,39], such as local organization of lipids and membrane proteins according to their preferred hydrophobic thickness, and a coupling between membrane protein conformational state and the local lipid environment. We find that protein-induced lipid bilayer thickness deformations allow MscL proteins to locally control their lipid environment in heterogeneous bilayers, with the local lipid environment strongly modifying the gating properties of MscL. Our results suggest that protein-induced lipid bilayer thickness deformations provide a general physical mechanism coupling lipid and protein organization in bilayers with heterogeneous hydrophobic thickness, endowing membrane proteins with diverse and controlled mechanical environments that, in turn, allow targeted regulation of membrane proteins.

Mean-field model. Our aim here is to explore generic features of the interplay between protein-induced lipid bilayer thickness deformations and local lipid-protein organization that are independent of most molecular details. To this end, we consider an idealized, cylindrical membrane protein of radius R at the center of a circular lipid bilayer patch representing the local lipid environment of the membrane protein (see Fig. 1). The energy of the bilayer patch, G, can be written as

$$G = G_h + G_c\,, \tag{1}$$

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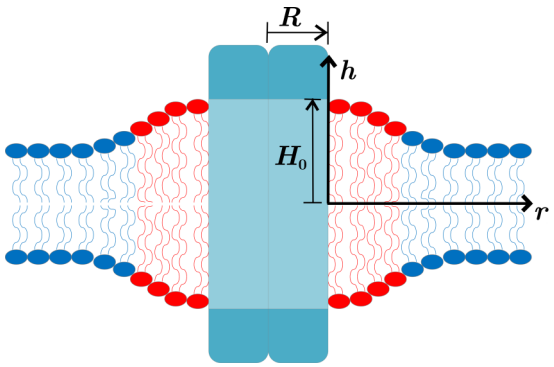

FIG. 1. Schematic of protein-induced lipid bilayer thickness deformations in heterogeneous lipid bilayers. We take the membrane protein at $r = R$ to induce the lipid bilayer leaflet thickness $h(R) = H_0$, with the outer bilayer patch boundary either being free or constrained by other membrane proteins. We denote the in-plane membrane protein radius by R and allow for two distinct lipid species, which are indicated in red and blue, in the lipid bilayer, with no chemical preference of the membrane proteins for a particular lipid species.

where G_h and G_c are the contributions to G due to protein-induced lipid bilayer thickness deformations and local lipid organization, respectively. We follow a mean-field approach and take the local lipid environment of the membrane protein to be approximately axisymmetric about the protein center. We do not account here for membrane protein shapes or lipid bilayer patch boundaries breaking rotational symmetry about the protein center [41]. Models assuming an axisymmetric lipid environment have been successfully used to describe bilayer-thickness-mediated protein interactions [17,25,26] and the observed dependence of the conformational state of MscL [27–29,37,38] and other membrane proteins [30,39] on lipid bilayer hydrophobic thickness. Similar mean-field models have also been successfully employed to describe protein interactions mediated by bilayer midplane deformations [42–44].

Membrane elasticity theory [24–30] implies that the leading order contributions to G_h in Eq. (1) can be obtained from

$$G_h = 2\pi \int_R^{R+L} \left\{ \frac{K_b}{2}\left[\frac{1}{r}D(rDh)\right]^2 + \frac{K_t}{2}\left(\frac{h-a}{a}\right)^2 + \frac{\tau}{2}\left[(Dh)^2 + 2\frac{h-a}{a}\right]\right\} rdr \qquad (2)$$

with $G_h = \bar{G}_h + 2\pi \int_R^{R+L} \bar{g}_h\, rdr$, where $r = R$ corresponds to the bilayer-protein boundary for the protein at the center of the bilayer patch, $r = R + L$ corresponds to the outer boundary of the bilayer patch, $D \equiv d/dr$ is the differential operator, $h(r)$ is one-half the lipid bilayer hydrophobic thickness (Fig. 1), K_b is the lipid bilayer bending rigidity, a is one-half the unperturbed bilayer thickness, K_t is the bilayer thickness deformation modulus, and τ is the (lateral) membrane tension. For a given lipid composition, the values of the parameters K_b, K_t, and a in Eq. (2) can be measured directly in experiments [45]. We set $\bar{g}_h = \tau^2/2K_t$ so that $G_h = 0$ for all extremal functions of G_h corresponding to a constant $h(r)$ [41].

We focus here on lipid bilayer patches that effectively consist of two lipid species [2–6], in a regime in which the two lipid species can form distinct domains in the membrane. We follow previous work on lipid organization [23,34,35] and take the lipid leaflet composition to be symmetric about the bilayer midplane. At the mean-field level, lipid domain formation in such binary systems is successfully described by the LG free energy [22,23,33–36] given by

$$G_c = 2\pi \int_R^{R+L} \left[\frac{\varepsilon}{2}(Dc)^2 + b_0 - \frac{b_1}{2}\eta^2 + \frac{b_2}{4}\eta^4\right] rdr\,, \qquad (3)$$

where $c(r)$ is a continuous function capturing the (mean) lipid composition such that $c = 0$, 1 correspond to the two lipid species under consideration, the parameter ε specifies the energy penalty associated with lipid domain boundaries, and $\eta \equiv c(r) - 1/2$. From the LG theory of lipid domain formation [34], we have $b_1 = 4n_0k_BT/3$ and $b_2 = 16n_0k_BT/3$, where n_0 is the mean lipid number per unit area, so that the mean-field potential in Eq. (3) has minima at $c = 0$, 1. Furthermore, we set $b_0 = n_0k_BT/12$ so that the mean-field potential in Eq. (3) is zero at these minima. The two key physical parameters entering Eq. (3) are thus ε and n_0. Following Refs. [23,34], we use the values $\varepsilon = 1\ k_BT$ and $n_0 = 1\ \text{nm}^{-2}$, which successfully describe lipid domain formation in heterogeneous lipid bilayers [22,35,36]. We focus here on the local lipid environment of membrane proteins, with rapid lateral diffusion of lipids in the plane of the membrane. As a result, we allow for a free exchange of lipids between the bilayer patch surrounding the membrane protein and the remainder of the membrane, which means that the average lipid composition in the bilayer patch is not conserved. External constraints on the average lipid composition in the bilayer patch could be imposed by introducing a chemical potential in Eq. (3).

The effective elastic parameters K_b, K_t, and a in Eq. (2) depend on the lipid species under consideration [45], and thus couple h and c [22,23]. Recent experiments on lipid bilayers with heterogeneous hydrophobic thickness [1–3,19–21] indicate that lipid bilayer and protein composition couple through hydrophobic lipid-protein interactions driven by differences in the energetically preferred hydrophobic thickness of lipids and membrane proteins. This suggests that a in Eq. (2) provides the dominant coupling between h and c. Based on the experimental data on the dependence of bilayer elastic properties on lipid composition compiled in Ref. [45], we indeed find for all the scenarios considered here that the dependence of a on c dominates over the dependence of K_b and K_t on c. We therefore focus on the coupling of h and c through $a(c)$ [22,23]. In general, the bilayer mean curvature may also couple to the bilayer composition [16–18]. Experiments show that a depends crucially on the lipid chain length [45]. A major lipid component of cell membranes is provided by phospholipids [2], for which a roughly varies from $a \approx 1.7$ nm to $a \approx 2.2$ nm depending on the lipid chain length [45]. Following Refs. [27,28,45], we take $a(c)$ to be linear and, unless specified otherwise, identify the two lipid species in the bilayer with $a(c = 0) = 1.7$ nm (lipid species A) and $a(c = 1) = 2.2$ nm (lipid species B). We use the fixed values $K_b = 20\ k_BT$ and $K_t = 60\ k_BT/\text{nm}^2$ typical for lipid bilayer membranes [39,45].

We assume that the dominant $h(r)$ and $c(r)$ minimize G in Eq. (1) subject to suitable boundary conditions. For the bilayer-protein boundary at $r = R$, we follow previous work [24,27–30,46] and fix $h = H_0$ as well as $Dh = 0$ at $r = R$. The former boundary condition arises from bilayer-protein hydrophobic matching, with the protein offering a rigid interface to the lipid bilayer [24,27–30,46]. The results described here only change marginally if, at $r = R$, Dh is adjusted about $Dh \approx 0$ as part of the numerical minimization procedure [46]. We take the membrane proteins considered here to have no chemical preference for a particular lipid species, and therefore use the natural boundary condition $Dc = 0$ at $r = R$. For the outer bilayer patch boundary $r = R + L$ we consider, on the one hand, natural boundary conditions on $h(r)$,

$$rD\left\{\tau h - K_b\left[\frac{1}{r}D(rDh)\right]\right\}_{r=R+L} = 0\,,$$
$$[D(rDh)]_{r=R+L} = 0\,, \qquad (4)$$

as well as $Dc = 0$ at $r = R + L$. From a physical perspective, Eq. (4) corresponds to scenarios in which the membrane protein at $r = R$ does not interact with any other membrane protein or constraint on the lipid bilayer, in which case the properties of the outer bilayer patch boundary can be adjusted freely so as to minimize G. On the other hand, we also consider scenarios in which the bilayer thickness is constrained at $r = R + L$ by an approximately uniform array of membrane proteins surrounding the membrane protein at the center of the bilayer patch [26,42–44]. In analogy to the bilayer-protein boundary condition at $r = R$ we then have, at the mean-field level, $h = H_L$, $Dh = 0$, and $Dc = 0$ at $r = R + L$. We employ MscL as a model system, for which $(H_{0,L}, R)$ take the values $(H^c, R^c) = (1.9, 2.5)$ nm and $(H^o, R^o) = (1.3, 3.5)$ nm in closed and open conformational states [29,37], respectively. We numerically minimize G in Eq. (1) using the L-BFGS-B solver [47,48].

Local lipid organization. We first consider situations in which the outer boundary of the bilayer patch surrounding the protein at $r = R$ is left unconstrained [see Eq. (4)], and choose a bilayer patch size large enough so that the bilayer energy density $g(r)$, defined as

$$G = 2\pi \int_R^{R+L} g(r) r dr\,, \qquad (5)$$

satisfies $g(R + L) \approx 0$ [see Fig. 2(a)]. We find that, even if the protein at $r = R$ does not show a chemical preference for a particular lipid species, the coupling of local lipid and protein composition through $a(c)$ [1–3,22,23,45] yields, depending on the value of H_0, accumulation of lipid species A or B around the membrane protein [Fig. 2(a)]. As the value of H_0 is changed through a critical value H_0^*, the dominant composition of the bilayer patch shows a sharp change from lipid species A to lipid species B. We find that the bilayer energy G in Eq. (1) has two minima as a function of protein hydrophobic thickness, at $H_0 = a(0)$ and $H_0 = a(1)$ [see Fig. 2(b)]. These two minima are associated with lipid species A and B, with a sharp change in G at $H_0 = H_0^*$ that mirrors the sharp change in local lipid composition at $H_0 = H_0^*$. As the membrane tension τ is increased, the preferred hydrophobic thickness of the lipid bilayer is reduced, thus shifting H_0^* to smaller values. For a membrane protein with $H_0 \approx H_0^*$, a change in membrane tension can therefore induce a change in the local lipid environment of the membrane protein and hence alter local membrane composition.

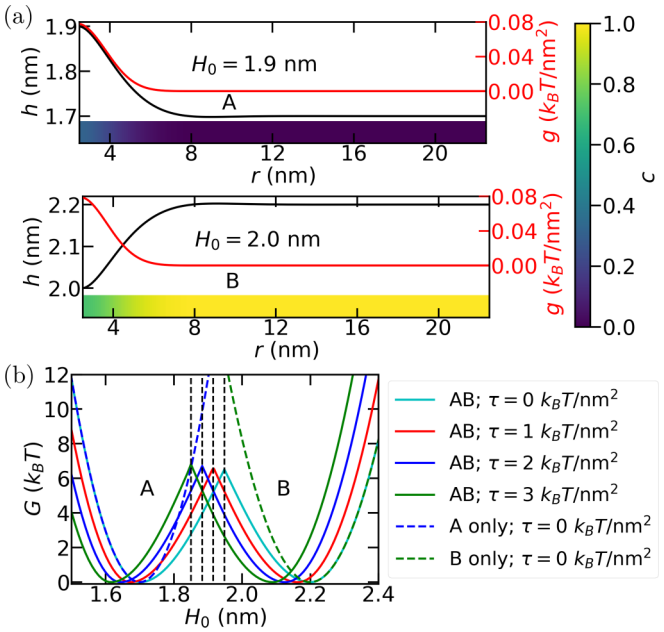

FIG. 2. Local organization of lipid species through protein-induced lipid bilayer thickness deformations. (a) Thickness deformation profile h, lipid composition c (color bars), and energy density g for $H_0 = 1.9$ nm (upper panel) and $H_0 = 2.0$ nm (lower panel) versus r at $\tau = 0$. (b) Bilayer free energy G in Eq. (1) as a function of H_0 for a heterogeneous bilayer containing lipid species A and B and homogeneous bilayers composed of lipid species A or B for $R = R^c$ and the indicated values of τ. The dashed vertical lines show $H_0 = H_0^*$ for $\tau = 0$, 1, 2, and 3 k_BT/nm^2 (right to left), for which the dominant lipid composition in the bilayer patch changes from lipid species A to lipid species B. At $r = R + L$ we use, for all panels, the boundary conditions in Eq. (4). We set $L = 20$ nm.

Protein conformational changes. Transitions in the conformational states of membrane proteins are often accompanied by changes in protein hydrophobic thickness [30,39]. In homogeneous lipid bilayers with constant a, $G(H_0)$ shows a minimum at $H_0 = a$ [Fig. 2(b)]. As a result, the protein is effectively biased toward conformational states with $H_0 \approx a$, which is thought to provide a general mechanism for membrane protein regulation [24,30,39,49,50] and, in particular, is crucial for MscL gating [27–29,38]. Heterogeneous lipid bilayers imply a strikingly different behavior: As shown in Fig. 2(b), the coupling of local lipid and protein composition through hydrophobic matching can drastically lower the energy cost of protein conformational changes. For instance, the contribution to the MscL gating energy due to bilayer thickness deformations can be written as [27–29]

$$\Delta G = G^o(H^o) - G^c(H^c)\,, \qquad (6)$$

where $G^{o,c}$ denote the values of G associated with the open and closed states of MscL, respectively. Figure 2(b) suggests that if, for example, the lipid composition is such that H_0 is offset from $a(1)$ in the closed state of MscL but $H_0 \approx a(0)$ in

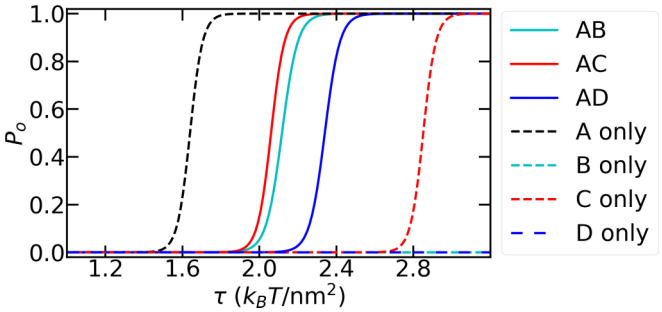

FIG. 3. MscL gating probability P_o in Eq. (7) as a function of τ for heterogeneous bilayers containing lipid species A and B, A and C, and A and D, and homogeneous bilayers containing only lipid species A, B, C, or D. All curves were calculated as in Fig. 2, with $a = 1.7$, 2.2, 1.8, and 2.0 nm for lipid species A, B, C, and D, respectively.

the open state of MscL, introduction of lipids corresponding to $c = 0$ into a bilayer containing originally only lipids corresponding to $c = 1$ can reverse the sign of ΔG in Eq. (6), and thus potentially gate MscL.

We illustrate the effect of lipid heterogeneity on protein conformation for the tension-dependent gating of MscL. Assuming that contributions due to bilayer thickness deformations dominate the MscL gating energy [27–29,51], the probability of MscL being in its open state can be estimated from a two-state Boltzmann model,

$$P_o = \frac{1}{1 + e^{(\Delta G - \tau \Delta A)/k_B T}}, \tag{7}$$

where ΔG is given in Eq. (6) and $\Delta A = \pi[(R^o)^2 - (R^c)^2]$. A key functional characteristic of MscL is the gating tension $\bar{\tau}$, which we define here as the smallest τ with $P_o \geqslant 1/2$. As suggested by Fig. 2(b), introduction of lipid species A into a bilayer containing originally only lipid species B energetically biases MscL toward its open state, thus decreasing $\bar{\tau}$, and vice versa (see Fig. 3). The magnitudes and signs of the predicted shifts in $\bar{\tau}$ depend nonmonotonically on the unperturbed hydrophobic thickness of the lipids under consideration. For instance, introduction of lipid species C or D in Fig. 3, which both have smaller a than lipid species B, into a bilayer containing originally only lipid species A can result in a smaller as well as larger $\bar{\tau}$ than obtained with lipid species A and B. Figure 3 shows that, for lipids typically found in cell membranes [2,45], the coupling of local lipid and protein composition through $a(c)$ [1–3,22,23,45] can produce shifts in $\bar{\tau}$ that are a substantial fraction of the bilayer rupture tension $\tau_r \approx 3\ k_BT/\text{nm}^2$ [29], and can thus strongly affect MscL gating [38,40]. The gating tension of MscL is indeed known to depend on membrane elastic properties such as the bilayer hydrophobic thickness [27,28,38] with, for instance, a typical value $\bar{\tau} \approx 2.5\ k_BT/\text{nm}^2$ in *E. coli* giant spheroplasts [29,52].

Crowded membranes. Cell membranes are crowded with membrane proteins, with the mean protein separation and size being of the same order of magnitude [4,39]. To ascertain the effect of protein crowding on local lipid organization due to protein-induced lipid bilayer thickness deformations, we consider scenarios in which the membrane protein at the

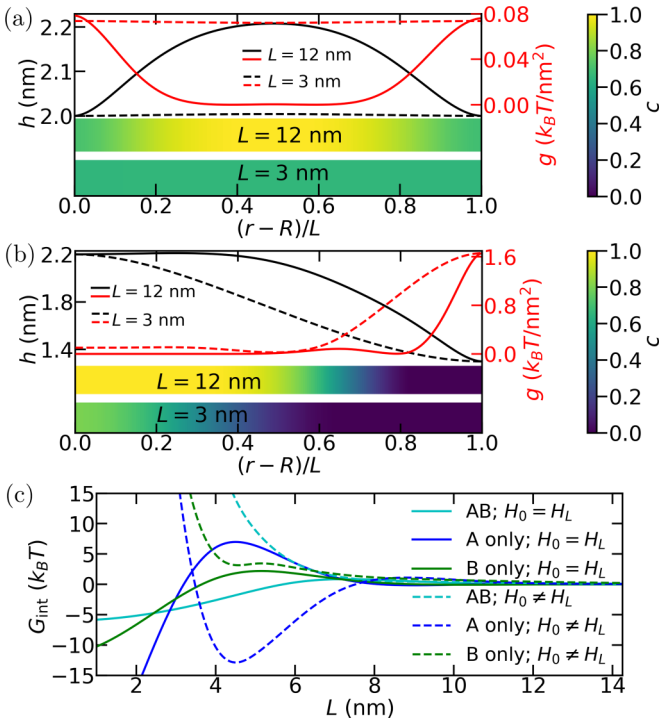

FIG. 4. Local lipid organization and bilayer-thickness-mediated protein interactions in crowded membranes. Thickness deformation profile h, lipid composition c (color bars), and energy density g at $L = 3$ nm and $L = 12$ nm vs $(r - R)/L$ with $R = R^c$ for (a) identical proteins with $H_0 = H_L = 2.0$ nm and (b) distinct proteins with $H_0 = 2.2$ nm and $H_L = 1.3$ nm in bilayers composed of lipid species A and B. (c) Bilayer-thickness-mediated protein interaction potentials G_{int} for identical ($H_0 = H_L$) and distinct ($H_0 \neq H_L$) proteins as in panels (a) and (b) with $R = R^c$ in a heterogeneous bilayer composed of lipid species A and B, and homogeneous bilayers containing only lipid species A or B. The interaction potentials G_{int} are obtained by subtracting from Eq. (1) the (noninteracting) large-L limit of Eq. (1), which is linear in L. We set $\tau = 0$ for all panels.

center of the bilayer patch is surrounded by an approximately uniform array of membrane proteins [26,42–44] constraining the lipid bilayer thickness. We find that the local lipid organization due to protein-induced lipid bilayer thickness deformations not only depends crucially on the preferred lipid and protein hydrophobic thickness [1–3,19–21] but also on the (edge-to-edge) protein separation, L [see Figs. 4(a) and 4(b)]. In particular, proteins with identical hydrophobic thicknesses locally yield separate lipid domains for $L \gtrapprox 10$ nm, which merge into a single domain at small L [Fig. 4(a)]. In contrast, proteins with distinct hydrophobic thickness locally induce, at large L, lipid domains with distinct composition, but these domains tend to be dispersed at small L [Fig. 4(b)].

In bilayers with homogeneous lipid composition, membrane proteins with identical (distinct) hydrophobic thickness generally show favorable (unfavorable) bilayer-thickness-mediated interactions at small L [25,26,39,41,51]. We find that, depending on the lipid and protein hydrophobic thickness considered, heterogeneous lipid bilayers can yield similar bilayer-mediated protein interactions, or favorable (unfavorable) bilayer-mediated interactions between proteins with

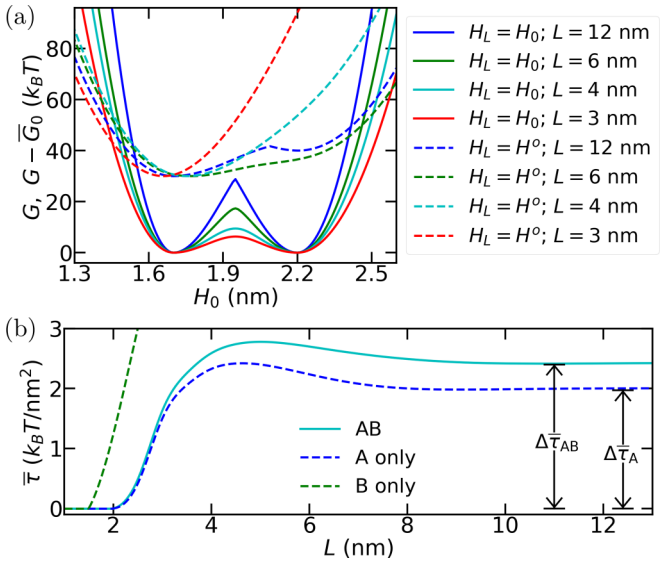

FIG. 5. Hydrophobic lipid-protein interactions in crowded membranes. (a) Bilayer free energy G in Eq. (1) as a function of H_0 for a heterogeneous bilayer composed of lipid species A and B with $H_L = H_0$ or $H_L = H^o$ and the indicated values of L. We set $\tau = 0$ and $R = R^c$ for both $H_L = H_0$ and $H_L = H^o$. For ease of visualization, we shifted the curves for $H_L = H^o$ by $\bar{G}_0 = G_{\min} - 30\ k_BT$, where $G_{\min}$ are the respective global minima of $G(H_0)$. (b) MscL gating tension $\bar{\tau}$ implied by Eq. (7) as a function of L for a heterogeneous bilayer containing lipid species A and B and homogeneous bilayers composed of lipid species A or B with open-state MscL proteins at $r = R + L$.

identical (distinct) hydrophobic thickness that are longer in range and smaller (greater) in magnitude than in homogeneous bilayers [see Fig. 4(c)]. Lipid heterogeneity thus expands the repertoire of bilayer-thickness-mediated protein interactions [25,26,39,41,51]. Experiments on a wide range of membrane proteins suggest that bilayer-thickness-mediated protein interactions provide a general mechanism for protein clustering [53–58]. Figure 4 predicts that such clustering of membrane proteins is accompanied by changes in local lipid organization, leading to colocalization of lipids and membrane proteins according to their preferred hydrophobic thickness [1–3,19–21,53–58].

Our calculations predict that protein crowding affects the energy landscape of hydrophobic lipid-protein interactions in heterogeneous lipid bilayers [see Fig. 5(a)]. For membrane proteins with identical hydrophobic thickness, we find that the interaction of lipid domains smoothes the transition in lipid patch composition at the critical $H_0 = H_L = H^*$ [solid curves in Fig. 5(a)]. Calculating G as a function of H_0 for fixed H_L, we find at large L a global minimum of G corresponding to $H_0 = H_L$ and a local minimum with $H_0 \neq H_L$ for which, as illustrated in Fig. 4(b), the two proteins have distinct local lipid environments [dashed curves in Fig. 5(a)]. As L is decreased, the lipid domains induced by proteins with $H_0 \neq H_L$ tend to be dispersed, and the corresponding local minimum in $G(H_L)$ is suppressed.

The interplay of local lipid organization and protein crowding can have intricate effects on membrane protein cooperativity, which we illustrate in Fig. 5(b) for MscL. As in Fig. 3, we thereby follow Refs. [27–29,51] and assume that contributions due to bilayer thickness deformations dominate the MscL gating energy. Figure 5(b) implies that, compared to dilute membrane protein environments, the presence of open-state MscL proteins with $L \lessapprox 2.0$ nm leads to a decrease in the MscL gating tension $\bar{\tau}$ by $\Delta\bar{\tau}_{AB} \approx 2.4\ k_BT/\mathrm{nm}^2$ for heterogeneous bilayers composed of lipid species A and B, but by $\Delta\bar{\tau}_A \approx 2.0\ k_BT/\mathrm{nm}^2$ for homogeneous bilayers composed of lipid species A. In contrast, Fig. 5(b) suggests that bilayers composed of lipid species B only yield an MscL gating tension smaller than the membrane rupture tension, $\bar{\tau} < \tau_r$, for $L \lessapprox 2.5$ nm or after introduction of some other lipid species, such as lipid species A, into the membrane. Thus, we find that the coupling of local lipid and protein composition through $a(c)$ [1–3,22,23,45] can strongly affect bilayer-mediated protein cooperativity in crowded membranes.

Conclusion. Employing [17,22,23] the elasticity theory of bilayer thickness deformations [24–30] together with the LG theory of lipid domain formation [16,31–36], we have quantified the coupling of lipid and protein composition through nonspecific, hydrophobic matching in bilayers with heterogeneous hydrophobic thickness. In agreement with a variety of experiments [1–3,19–21,30,39], our calculations suggest that membrane hydrophobic thickness is a generic regulator of lipid and protein organization independent of most molecular details, and couples the conformational states of membrane proteins to the local lipid and protein organization. The mechanics of lipid bilayers implies that protein-induced bilayer thickness deformations are localized over a scale of approximately 4 nm about each membrane protein, which corresponds to roughly one-half the typical protein-protein separation in cell membranes [4,39]. Bilayer thickness deformations are therefore well placed to influence local membrane organization and function. Our calculations suggest that protein-induced lipid bilayer thickness deformations endow proteins in cell membranes with diverse and controlled mechanical environments that, in turn, allow targeted regulation of membrane proteins. A wide range of experiments have demonstrated that cell membranes contain lipid domains distinguished by their hydrophobic thickness [1–3,19–21] and that membrane protein conformation elastically couples to lipid bilayer thickness deformations [24,27–30,38–40,46]. The physical mechanisms and principles for membrane organization and function explored here may thus be broadly applicable to cell membranes.

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