

Lipids Are Not Passive Bystanders in Membrane Protein Function

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Abstract

Recent structural and functional studies of membrane proteins show a direct link between the lipid environment and both the structure and function of these proteins. Although some of these interactions involve detailed chemical binding between lipids and protein residues, a whole class of these interactions are non-specific and can be described by coarse-grained models. The aim of our article is to show how quantitative analysis (both theory and experiments) can be used to explore the rich interplay between membrane proteins and the surrounding lipids. In particular, tension-gated ion channels are used as a case study illustrating how some membrane proteins can induce a membrane deformation footprint. We show how simple ideas from elasticity theory can be used to estimate the free energy cost of such deformations and the consequences of this free energy cost for membrane protein function.

Quantitative Analysis of Membrane Protein Function

Quantitative analysis is changing the face of biology. One area where quantitative thinking has provided useful insights is in the analysis of membrane protein function with specific reference to the interaction of such proteins with the surrounding lipid molecules. The plasma membrane is the first line of communication between the cell and the external world as well as a highly controlled barrier permitting the directed flux of molecules into and out of the cytoplasm itself. The membranes of cellular organelles, whose properties are key to intracellular trafficking, show a similar level of complexity.

These buzzing metropolises of membrane proteins and their lipid partners are a complicated environment whose understanding is constantly being refined by new generations of experiments [1]. Often, the data that emerges from such experiments reveal functional and quantitative relations between biologically interesting parameters (e.g. the open probability for ion channels as a function of driving forces such as voltage or membrane tension), and carry with them an imperative for models of the underlying phenomena. Each such generation of experiments brings concomitant refinements in the models used to describe membranes, a topic elegantly reviewed elsewhere [1].

In this article we provide a case study illustrating this kind of interplay between quantitative theory and experiment by showing how the conjunction of structural and functional data on certain mechanosensitive channels supports the idea that the physicochemical properties of the surrounding lipid bilayer yield a predictable and stereotyped consequence for the function of these channels. Though we highlight a particular case study, the effects considered here are of broad significance to the

understanding of channel function, as illustrated in Tables 1 and 2 of the recent review of Andersen and Koeppe [2] (see refs. [3, 4] for several other examples). The results presented below should have particular significance for any protein whose function requires remodeling of the protein-membrane interface and for proteins that function by oligomerization in the membrane.

The idea that sequence dictates structure, which in turn dictates consequence (i.e. function) is a second central dogma of biology [5]. One powerful example of this dictum is in the context of membrane-protein function. As illustrated by other articles in this issue, stunning structures of membrane machines of the cell ranging from the light-gathering apparatus of photosynthesis to the voltage-gated channels, that permit neurons to propagate electrical impulses, to the bacterial sensors that detect osmotic stress, paint a picture of the way proteins are threaded through the surrounding lipid bilayer and how they respond to stimuli such as light, voltage and membrane tension. In many cases, complementary structural and functional studies teach us that the lipid bilayer is not a passive bystander in membrane protein function as shown systematically elsewhere [2]. Though the word “structure” usually refers to the atomic positions associated with the macromolecules of the cell, a more coarse-grained picture of structure captured by ideas from continuum elasticity can also prove very useful. In this case, “structure” refers to quantities such as the local thickness and curvature of the bilayer membrane surrounding a membrane protein of interest and our aim in this article is to show how structure at this level leads to functional consequences for some membrane proteins.

To show this concretely, we use bacterial mechanosensitive channels as a case study. The structure, function and physiology

of mechanosensitive channels are reviewed in a number of papers some of which are cited here [6-9]. As shown in fig. 1, bacterial mechanosensitive channels are gated by membrane tension [10]. More precisely, *ex vivo* a patch of membrane containing these channels is grabbed using a pipette and the current passing across the protein-encumbered membrane is measured as a function of the pipette suction pressure. For our purposes, the most interesting feature of this data is the fact that the relation between the open probability of the channel and the pipette pressure depends upon the properties of the lipid membrane in which the proteins find themselves (such as the tail lengths of the lipids which result in a mismatch between the protein and the bilayer thickness) [10, 11]. Indeed, this observation serves as one of the key clues for the argument made throughout the remainder of the paper.

In addition to the hydrophobic thickness mismatch effect, a second clue pointing to the influence of the bilayer mechanical properties on membrane protein function is provided by the way channel activity can be modulated by the insertion of particular lipids, cholesterol or toxins (i.e. membrane doping) as indicated schematically in fig. 1b. In particular, recent studies show that instead of a specific pairing to a particular channel, some toxins exert their influence by altering the membrane properties. For example, some small molecules like capsaicin [12], and peptide toxins, like those found in spider venom [13], target membrane channels across many species by affecting the mechanical properties of the bilayer in which the proteins are embedded. It was also observed [10] that the opening of mechanosensitive channels can be triggered, even under small applied tensions, by the asymmetric membrane insertion of “conical” lysophosphatidylcholine (LPC) lipids. The role of asymmetric membrane properties such as a spontaneous curvature away from the LPC rich face, is generically difficult to explain within

the symmetrical hydrophobic mismatch model, but naturally follows from a “gating-by-tilt” hypothesis represented in fig.1b [14, 15]. The effectiveness of such schemes is intriguing when one considers the wide variety of lipid species across different cell types and species. While it is clear that certain lipids species and other membrane components such as cholesterol are required for proper protein function [16, 17], studies using toxins lend support to the notion that the membrane is also a generic mechanical medium with which proteins interact. The importance of the membrane composition for proper protein function might stem from their influence on the elastic properties of the membrane, in addition to more direct chemical interactions with the protein itself.

Many studies show that bilayer thickness, bending stiffness and monolayer spontaneous curvature all affect the function of embedded proteins [2, 18]. For example, the dimerization kinetics of the channel-forming peptide gramicidin A can be controlled by an externally applied mechanical stress on the membrane, which results in membrane thinning and decreases the hydrophobic mismatch between the membrane and the gramicidin dimer [19]. Furthermore, using gramicidin A enantiomers as sensors for membrane mechanical properties, studies have shown that the small molecule capsaicin indirectly targets and triggers the pain receptor TRPV1 by decreasing the bending modulus of lipid bilayers, in a concentration-dependent manner (i.e. not with a certain stoichiometric relation between toxins and each channel, but rather progressively through alteration of the membrane mechanical response) [12]. Conversely, voltage-dependent sodium channels are inactivated by capsaicin with no significant change to the conductance properties of the channels, rather an alteration of the gating voltage itself suggesting that even channels that are ostensibly

indifferent to membrane mechanics can be altered by tuning membrane properties. In addition, it seems that some peptide toxins target multiple types of stretch-activated sodium channels not by changing membrane properties per se, but by changing the effective boundary conditions at or near the protein-lipid interface [20]—yet another generic method by which membrane mechanics can couple to protein function. In particular, it appears that either enantiomer of a peptide toxin is localized in the membrane close to the channel and shifts its dose-response curve.

Building on the experimental clues described above, in the remainder of the article we use quantitative models to explore the connection between membrane protein function and the mechanics of the surrounding membrane. A useful starting point to begin to flesh out a quantitative picture of such membranes is provided by simple order of magnitude estimates. For example, a proper lipid and membrane protein census gives a sense of how many lipids surround each membrane protein, how far apart those proteins are in the membrane and what this might imply about membrane-mediated interactions and corresponding cooperativity in protein function. Such a census gives us a framework for thinking about the molecular environment seen by membrane proteins, that in turn serves as a starting point for elastic analysis; for example, of membrane protein interaction and membrane-mediated cooperativity of channel gating.

Recent experiments on the occupancy of biological membranes, by lipids and their protein partners, provide a useful place to begin with our estimates [21]. As shown in fig. 2 (upper right), proteomic and lipidomic approaches have made it possible to survey the protein and lipid content of biological membranes. In the case shown in the figure, a survey of the contents of a

synaptic vesicle reveal a highly crowded and heterogeneous medium. Indeed, as noted amusingly in the presentation of the original experiments, “A picture is emerging in which the membrane resembles a cobblestone pavement, with the proteins organized in patches that are surrounded by lipidic rims, rather than icebergs floating in a sea of lipids.” [21]

To better understand the geometric constraints present in real biological membranes, we make a few simple estimates. The synaptic vesicle shown in fig. 2 tells a similar story to results found in other biological membranes such as in bacterial membranes or the protein census of the red blood cell membrane [22, 23]. The essence of the various membrane inventories is that biological membranes are as much protein as they are lipid, with typical protein to lipid mass ratios of order 60:40. Assuming that the areal mass density (i.e. the mass per unit area of membrane) is the same for proteins and lipids, we can estimate that the fraction of the membrane area occupied by proteins is thus of order $\phi_p = 60\%$. For an *E. coli* cell, this corresponds to roughly $4 \mu\text{m}^2$ of area occupied by proteins. If we assume a mean protein area of roughly 10 nm^2 , this tells us that there are roughly 400,000 proteins in the membrane. Detailed calculation should take into account the fact that a sizable fraction of the protein mass is actually not embedded in the membrane (see the sketch in Fig. 2). There are many different ways to perform these same estimates and it is possible to quibble over the details, but regardless of these details the message will be the same. Biological membranes are crowded! What these numbers tell us is that the mean center-to-center spacing between proteins (estimated by evaluating $1/\sqrt{c_A}$, where c_A is the areal density of membrane proteins) is less than 10 nm, a result which tells us in turn that these proteins thus have the possibility of exerting an influence over each other through the intervening membrane. Note that these spacings

between proteins are comparable to the distance between proteins in the cytoplasm[24, 25]. For the purposes of the rest of the article, the key point to emerge from the experiments described in the introduction is the idea that when thinking about the behavior of the proteins that riddle these membranes, interactions with the surrounding lipids and other membrane proteins both play a role in dictating the behavior of these proteins.

In the remainder of the paper, we focus on a more precise theoretical characterization of the way membrane proteins interact with the surrounding lipid bilayer and with each other. In the next section, we describe a single isolated channel in a lipid bilayer membrane, describing how the lipids surrounding that protein participate in determining the functional response of that channel. In the following section, we describe how channels might interact as a result of their membrane deformation footprint and how such interactions can influence the functional properties of these channels.

There are a variety of theoretical tools that can be used to explore the connection between structure and function that reach well beyond the confines of traditional biological analysis. Two of the most important classes of analysis of the structure-function linkage are atomistic models and continuum elasticity models. Although both of these classes hold an important place in the study of membrane channels, we will build our estimates using simple arguments from elasticity. Our conclusions are largely indifferent to the details of how one treats the energetics of the composite lipid and membrane protein system and an atomistic analysis would yield the same general picture of a deformed footprint of material around the protein of interest as already indicated schematically in fig. 2, though atomistic analyses can reveal features of membrane protein function that

are inaccessible to continuum analysis. Several representative examples can be found in refs. [26-29]. The generality of the concept follows because the key ideas have to do with the generic, *geometric* perturbations on the lipids present for any membrane protein and the *energetic* consequences of these perturbations, especially for those cases in which the membrane protein of interest undergoes some conformational change in the course of its functional activity. The key ideas are indicated schematically in fig. 2 where it is shown both in the “dilute” and “crowded” limits how membrane proteins perturb the surrounding lipids (and each other, if the membrane is sufficiently crowded) and what the geometrical and energetic implications are of such perturbations.

Elasticity and the Isolated Channel

To get a feel for the interplay between ion channels and the surrounding lipids, we consider an idealization (like that shown in the top left corner of fig. 2) to an isolated channel in a single-component lipid bilayer. The elastician’s abstraction of several membrane proteins is shown more explicitly in fig. 3. Of course, such idealizations falls far short of the rich and varied landscape inhabited by channels in real cell membranes, but at the same time, these idealizations provides useful mechanistic insights into membrane protein function when the protein “footprint” changes during channel gating.

We can anticipate the results of the mathematical description of these channels using elasticity theory by once again appealing to fig. 2. For example, an ion channel might change its external radius (to the extent that the idealization of a “radius” is useful) during gating. Similarly, this same channel might change its hydrophobic thickness during the gating process [2]. The key point is that the red colored region of the membrane in the

figures corresponds to membrane that is disturbed, that is, it is not in the relaxed state it would adopt were there no protein present. This region of deformed material costs a certain amount of deformation free energy. Further, when the channel goes from the closed to the open state, the annulus of deformed material changes and with it so does the free energy penalty. This free energy of deformation favors the closed state and competes against the driving force (such as membrane tension) which favors the open state. To see how this works out explicitly, we turn to a description of the membrane as an elastic sheet.

Membrane as an elastic sheet. A convenient model for describing the interaction between membrane proteins and the surrounding membrane is to treat the membrane as a continuous elastic medium [14, 30-32]. The idea of such an elastic description is that there is a free energy cost that must be paid for perturbing the lipid bilayer away from some undeformed reference state as indicated schematically by the springs in figs. 3(c) and (d). In particular, we will emphasize several key modes of deformation (i.e. hydrophobic mismatch and bending) and their corresponding free energy cost. Though there is a well-defined mathematical theory of the free energy of membrane deformation, here we will emphasize a qualitative and intuitive description of these theoretical results and refer the interested reader to other sources for the mathematical details [33].

As highlighted in fig. 3, for different types of membrane deformation, there is a free energy cost that can be calculated in the form of an energy density (free energy per unit area of deformed membrane). For example, in the case of hydrophobic mismatch where there is a free energy penalty associated with “gluing” the hydrophobic lipid tails onto the hydrophobic region of the membrane protein, the free energy density grows as the

square of the hydrophobic mismatch [15, 34]. Similarly, some membrane proteins will bend the membrane in their vicinity incurring another class of free energy cost [15, 35]. As shown in the figure, this idea can be represented schematically by thinking of the membrane as a set of generalized springs. For every little patch of area on the membrane, we can ask by how much is the thickness different than the equilibrium thickness and by how much is the membrane bent away from the flat state (for the case in which there is no spontaneous curvature). Given the answer to these *geometric* questions, we can then use these generalizations of Hooke's law to assign an energy density (energy per unit area) to each such patch of membrane and can find the total free energy cost by summing over all such patches.

Energy and Length Scales. Essential to gauging the importance of the interplay between lipids and membrane proteins, as well as any subsequent membrane-mediated interactions, are estimates of the energetic costs of membrane deformation and the size of the region over which that deformation occurs. While membranes are composed of a plethora of different lipid species, on the length and time scales of interest several coarse-grained continuum material properties emerge [33]. For a homogenous lipid phase, those material parameters are a bending stiffness (with units of energy and measured here in units of $k_B T$, where k_B is Boltzmann's constant), a stretch stiffness (with units of energy/area and measured here in units of $k_B T/\text{nm}^2$), a bilayer thickness (nm), a membrane tension (with units of energy/area and measured here in units of $k_B T/\text{nm}^2$), and spontaneous curvature of the membrane (nm^{-1}). Though we will proceed as though these parameters are true material constants, the situation is more subtle since the lipid environment surrounding a protein of interest can change with its conformational state and hence, so too can parameters such as the bending stiffness.

Within the continuum elastic equations that describe the membrane deformation, certain ‘natural’ length and energy scales emerge that can serve as a guide to our thinking and in providing intuition about the relative importance of different effects [31, 36, 37]. We highlight these key scales in Table 1 below. We see that midplane bending and thickness deformation share a common energy scale proportional to the bending stiffness itself. Thus with all other membrane and protein properties fixed, intuitively, it is found that stiffer membranes will cost more energy to deform. Likewise, both modes of deformation share a common energy scaling with changes in the relevant boundary condition at the protein-lipid interface [38-40]. In midplane deformation, protein ‘shape’ dictates the angle at which the membrane contacts the protein (see fig. 3); the deformation energy increases quadratically in the contact angle, hence in some sense it acts like a classical Hookean spring. Likewise, in thickness deformation, as the degree of hydrophobic mismatch between the embedded protein and the bilayer increase, the deformation energy increases quadratically. In practice, a reasonable estimate of deformation energy around a protein is $\sim 10 k_B T$ in either case⁹.

Membrane Bilayer Properties		Channel Properties	Energy of Conformation change	
Bending stiffness $\kappa = 20k_B T$	Mismatch healing length $\lambda_k = \left(\frac{\kappa h_0^2}{4k_s} \right)^{1/4} = 1.1nm$	Radius (close-open) $R = 2.5 - 3.5nm$	Thickness Variation $\Delta E_h = \sqrt{2}\pi\kappa \frac{R}{\lambda_k} \left(\frac{\Delta h}{\lambda_k} \right)^2$	$\Delta E_h = 15k_B T$
Stretch stiffness $k_s = 60k_B T / nm^2$		Areal variation $\Delta A \approx 20nm^2$		
Thickness $h_o = 4nm$		Thickness variation $ \Delta h \approx 0.8nm$	Areal variation $\Delta E_\tau = \tau \Delta A$	$\Delta E_\tau = 2 \cdot 10^{-3} - 20k_B T$
Tension $\tau = 10^{-4} - 1k_B T / nm^2$		Tilt variation $ \theta \approx \pi / 4$	Tilt variation ($C_0 = 0$) $\Delta E_\theta = \pi(R\theta)^2 \tau \ln(\lambda/\lambda_a)$	$\Delta E_\theta = 5 \cdot 10^{-3} - 7k_B T$
Spontaneous curvature $C_0 \approx 0 - 1/25nm^{-1}$	Tilt healing length $\lambda_\tau = \left(\frac{\kappa}{\tau} \right)^{1/2} = 5 - 500nm$	Charge variation $\Delta Q = 12e$	Tilt variation ($C_0 \neq 0$) $\Delta E_\theta = 2\pi\kappa C_0 R \theta$	$\Delta E_\theta = 10k_B T$
Transmembrane potential $V_m = -40mV$			Charge variation $E_V = \Delta Q V_m$	$\Delta E_V = 20k_B T$

Table 1: Summary of various constants and scales associated with protein-lipid interactions. The first column gives characteristic values for key parameters describing the membrane environment surrounding a membrane protein. The mismatch and midplane bending healing lengths describe the scale over which the membrane returns to an unperturbed state. The second column gives characteristic values of key geometric and physical parameters associated with the elastician's abstraction of an ion channel. The values for the radius change approximate that for MscL. The third column gives the characteristic energy scales for the various membrane-

associated terms associated with the free energy of a membrane deformation during channel gating. The expressions for the energies of conformational change are given in the limit $\lambda_k \ll a \ll \lambda_\tau$. When applicable, the range of energy differences is for the range of tension given in the table.

Membrane elasticity and mechanosensitive channel gating. The ideas developed above can be used to understand the origin of effects like those shown in fig. 1. To see that, we exploit simple ideas from statistical mechanics to write the open probability of a channel as a function of the driving force of interest [14, 30]. In particular, we can write the channel open probability as

$$p_{open} = \frac{e^{-\beta \epsilon_{open}}}{e^{-\beta \epsilon_{closed}} + e^{-\beta \epsilon_{open}}} \quad (1)$$

where β is defined as $1/k_B T$ and ϵ_{open} and ϵ_{closed} refer to the free energies of the open and closed states, respectively. Of course, the free energies of the open and closed states are tuned by changing the contribution of the driving force to these two energies. This result can be specialized to the case of tension-driven ion channel gating by noting that the energies of the open and closed states are dictated by the coupling to the tension and by the free energy cost of the annulus of deformed material surrounding the channel. An example of the implementation of this logic is to imagine that the zero of energy is in the closed state and to assign a hydrophobic mismatch to the open state. This scenario results in an open probability of the form

$$p_{open} = \frac{1}{1 + e^{-\beta(\tau \Delta A + \Delta \epsilon_{membrane} + \Delta \epsilon_{protein})}}. \quad (2)$$

This kind of simple analysis has the explanatory reach to respond to experiments like those shown in fig. 1(a). The term $\tau \Delta A$ corresponds to the driving force that favors the open state. However, this driving force must compete with the free energy

penalty associated with the membrane deformation footprint introduced in fig. 2 and estimated in table 1. We also include the energy difference between open and closed state associated with the protein degrees of freedom. Direct comparison with the experimental results shown in fig. 1 are difficult since all that is reported experimentally is the pipette pressure whereas the membrane tension is the key driving force[11]. Assuming that the membrane radius of curvature in the pipette is of order $R_p = 1\mu m$, the gating tension is $\tau = \Delta P R_p = 5 \times 10^{-3} N/m$. For MscL, the areal change between open and close conformations is of order $\Delta A = 20 nm^2$, corresponding to a gating energy $E = \tau \Delta A = 25 k_B T$. As expected, this energy is much larger than the thermal energy, so that spontaneous channel opening under low tension almost never occurs.

Life in the Crowded Membrane: Interacting Membrane Proteins and Cooperativity

The previous section focused on the abstract (but useful) case of a single channel. Several key insights emerged from that discussion. First, general considerations tell us that membrane proteins will be surrounded by an annulus of deformed membrane with a corresponding free energy cost. Hence, within the confines of such models, any membrane protein that changes its radius, hydrophobic thickness, or contact angle during “gating”, for example, will incur a free energy cost associated with a change in the nature (i.e. radius, thickness, or tilt) of that annulus of deformed membrane. This free energy cost will reveal itself through an explicit dependence of the gating properties both on lipid character and on membrane tension.

A corollary to this description of the “isolated membrane protein” is that when different membrane proteins are within several elastic decay lengths of each other, they will interact in

much the same way that two Cheerios® in milk will attract each other as a result of the deformation on the liquid surface. In the case of membrane proteins, the membrane-mediated interactions we discuss below may however result in either attraction or repulsion [31, 36, 41-44]. Furthermore, at these small length scales where thermal fluctuations are important, membrane-mediated interactions between inclusions also stem from the fact that rigid proteins can perturb the fluctuation spectrum of membranes (Casimir effect) [45, 46]. These interactions are potentially long-ranged, but might be small and their physiological relevance to membrane protein has not been demonstrated.

The same rules of thumb introduced in table 1 hold during membrane-mediated protein interactions; except that in this case we have to compare the spatial extent of the deformation field to the distance between proteins. Each type of deformation has a length scale over which the membrane returns to its unperturbed state [31, 36, 37]. Here the behavior of thickness and midplane deformations differ in two important ways. First, the length scale of thickness deformation is determined by a combination of bending stiffness, membrane thickness, stretch stiffness— all parameters that do not easily vary on the time scales of protein diffusion and conformational change. Additionally, this particular length scale is relatively indifferent to changes in the individual parameters [33], hence we might consider this length scale constant. On the other hand, the length scale of midplane deformation is a simple combination of bending stiffness and membrane tension that can be quite variable both within a cell through time and across different cell-types and environments [47]. Second, the real-world values of these two length scales are very different. While the length scale of thickness deformations is roughly a nanometer, the length scale of midplane deformations can be anywhere from $\sim 5\text{nm}$ to

~500 nm depending on the state of tension in the membrane, though when the elastic decay length is very large the interactions are very weak. With these characteristic scales in hand, we can now see how they play out in the competition between membrane deformation and driving force (i.e. tension) that determines channel opening.

This section now addresses some of the effects that can come into play in the case of the crowded membrane described in the introduction and captured in fig. 2. We explore several consequences of membrane-protein crowding. Our first example is a short account of the way in which the Brownian motion of proteins in a crowded membrane gives rise to an excluded area force that can affect the probability of a protein conformational change. We will then examine the concept of membrane-mediated interactions between channels (and lipids) and discuss their possible impact on the preferred conformational state of those proteins.

Biological membranes at physiological temperature are thought to be in the fluid state, which means that both lipids and proteins can diffuse laterally (provided they are not strongly interacting with the cytoskeleton). The diffusing proteins can be thought of as a gas, and there is an entropic tension (which we call τ_T), the equivalent of the pressure in a gas, acting on the external surface of each protein in the membrane due to the fact that the remaining proteins are jiggling around in the area available to them. The opening of membrane channels like MscL is typically associated with an increase ΔA of its area. One consequence of crowding is that this conformational change will cost a free energy $E_T = \tau_T \Delta A$ corresponding to the work done against the entropic tension during channel dilation. This energy decreases the probability of the channel open state because the surrounding proteins are effectively pushing on the channel

walls. A second way to think about this effect is that the other proteins are deprived of area to jiggle around in with an associated entropy cost that manifests itself as a tension acting to keep the channel closed. The effective mechanical tension acting on the channel in Eqn. 2 in the case of a crowded membrane, should thus be understood as the mechanical membrane tension (applied by the patch-clamp pipette) minus the entropic tension:

$$\tau_{eff} = \tau - \tau_T .$$

An estimate for the entropic tension can be obtained from the ideal gas law: $\tau_T = k_B T C_p$, where C_p is the concentration of "passive" proteins diffusing around the channel. Using the protein surface fraction $\phi_p = 60\%$ and an average protein area of 10 nm^2 , the entropic tension is of order $\tau_T = 2 \times 10^{-4} \text{ N/m}$ and the corresponding energy cost for the opening of MscL is of order $E_T \approx 1 - 2 k_B T$. This value is a lower bound, since we have assumed an ideal gas law for the entropic tension, which is a valid approximation for dilute systems only. In dense systems, the value of τ_T can easily be one order of magnitude larger, and can have a sizeable effect on the gating tension. Furthermore, the membrane deformation imposed by the channel conformation change can enhance this effect. As shown in Fig.1, MscL in the open conformation deforms the membrane, effectively creating an extended exclusion ring for proteins of larger thickness. The area difference ΔA subjected to the entropic tension might thus be larger than that estimated from the channel conformation alone, and will depend on the properties of the membrane.

In addition to the excluded area effect described above, other interesting effects arise through explicit interactions between adjacent channels as indicated schematically in fig. 4. The idea that the conformational states of two similar proteins can be coupled by the bilayer follows naturally from the previous

discussion of membrane deformation and has been explored in detail in a number of papers some of which are highlighted here. Two proteins in proximity (i.e. within a few elastic decay lengths) will have regions of bilayer deformation that overlap, thus one protein indirectly affects another via the lipids surrounding them both [31, 36, 41-44, 48]. When the proteins are coupled to the local membrane curvature, these interactions can lead to collective effects such as large-scale membrane deformations possibly responsible for membrane budding and the formation of vesicles [44, 49-51], as reviewed in [52]. Here, we concentrate on cooperative channel gating resulting from the fact that a conformational change in one protein will be 'felt' energetically via the surrounding lipids, influencing another protein's preference for a particular conformation [53]. Here too, certain rules of thumb emerge that depend on the nature of the interaction.

Proteins that cause thickness deformation tend to attract each other if they both increase or both decrease the bilayer thickness; conversely if one protein thickens the bilayer and another thins it, they will repel each other. Proteins that bend the midplane of the bilayer have the opposite behavior – those that bend the bilayer in the same direction tend to repel each other, while those that bend the bilayer in opposite directions tend to attract each other [48]. In either case, attraction arises because the amount of deformed material between the proteins decreases when proteins are in close proximity, hence lowering the deformation free energy. Proteins that attract each other have more deformation overlap and are more likely to be found within each other's circle of influence, and hence have more strongly coupled conformations.

Much work has been done on the nature of these interactions as an organizing principle for lipids and proteins. Our emphasis

here is on a second consequence of such interactions, namely, their ability to induce cooperativity in the gating of neighboring channels. Though we will not enter into the details here, the outcome of the interactions described here is a sort of slaving principle in which if one channel decides to gate, it increases the likelihood that its neighbors will gate as well[53]. The more severe the bilayer deformation, the stronger the interaction will be between similar proteins, thus more tightly coupling their conformations.

Concluding Perspective

Recent experiments reveal not only that biological membranes are exceedingly crowded, but also, that within these crowded environments, membrane protein function is in some cases strongly affected by the properties of the lipids that surround these proteins. Several of the most compelling case studies demonstrating the active role of lipids in membrane protein function are the cases of gramicidin and bacterial mechanosensitive channels, though there are a host of other intriguing examples highlighted elsewhere. On the other hand, both of these examples reveal *generic* effects related to the way that a membrane protein has a deformation footprint. These same arguments suggest that for any membrane protein that alters its deformation footprint during its conformational change, there will be a dependence of the protein function on the structure of the membrane (and possibly a tension dependence as well). Once the channel structure has been determined, the effect of the membrane properties on channel gating can be predicted quantitatively. As a result, we close with the speculation that the examination of lipid properties offers a promising direction and scope for biophysical investigation of the spectrum of interesting membrane proteins.

There is a broad variety of evidence for a number of different membrane proteins of the role played by the physical properties of the surrounding membrane. We advocate the idea that the regulatory effect of lipids on membrane protein function is a tool used not only by nature, but is also another way for scientists to dissect the structure-function relationship of membrane proteins. Concretely, the ideas described in this paper make concrete predictions for how channel gating will depend upon the properties of the surrounding lipids and proteins. Beyond this, as hinted at by the estimates in the final section of the paper, the role of “crowding” in the membrane setting may imply the same kinds of surprises and richness already seen for crowding effects in the bulk setting[24, 25, 54].

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NOTE: The reference list for this article was constrained by length limits and as a result the references presented here are representative rather than comprehensive. We apologize to those left off of this truncated list due either to space limitations or our ignorance.

Figure Captions:

Fig. 1: Ion channel function and membrane properties. (a) Ion channel opening probability as a function of pipette pressure for mechanosensitive channels in membranes with different tail lengths. The data is adapted from Perozo *et al.* [10]. The curves are an empirical fit to the patch clamp data using the functional form $p_{open} = 1/[1 + \exp(-\alpha(P - P_{1/2}))]$, with the parameters α and $P_{1/2}$ as fitting parameters. The schematics show how different tail lengths imply a different hydrophobic mismatch as a result of the boundary conditions at the protein-lipid interface. (b) Membrane doping and membrane protein function. The three schematics show hypothetical mechanisms whereby the insertion of amphiphilic molecules (small molecules or cholesterol, toxins/peptides, detergents or lysolipids) can alter the protein-membrane interaction. In the first case, asymmetric insertion of lipids in the membrane produces a torque on the protein, in the second case, hypothetical toxins alter the boundary conditions between the protein and the surrounding lipids, and in the third case, small molecules stiffen the membrane. In principle, all of these effects could alter the gating characteristics of a channel.

Fig. 2: Geometry, deformations and energies of dilute and crowded membranes. The two columns in this schematic correspond to the dilute and crowded membrane limits. Each column shows the class of geometries found, a schematic of the deformation field in the vicinity of the proteins, and a description of the energies. For the isolated channel case, it is seen that the

deformation height, h , surrounding a given membrane protein has an elastic decay length, λ , comparable to the protein size. The deformation energy around a protein depends on a generic ‘spring constant’, k , determined from membrane properties, scales quadratically with hydrophobic mismatch, u , and scales approximately linearly with the protein circumference C . For crowded membranes, proteins are sufficiently close ($d \approx \lambda$) that the annulus of deformed material around the proteins overlap resulting in an interaction energy that depends upon the conformational state, s_i , of the i^{th} protein.

Fig. 3: Structure and energy at the protein-lipid interface. (a) Atomic-level structure [55] and the elastician’s idealization of the mechanosensitive channel of large conductance (MscL) as a rigid cylinder with a hydrophobic mismatch with the surrounding lipids. (b) Atomic-level structure [56] and the elastician’s idealization of the mechanosensitive channel of small conductance (MscS) as a wedge with a slope that glues continuously onto the surrounding lipids. (c) Membrane distortion and corresponding free energy of deformation per unit area for membrane region surrounding MscL. (d) Membrane distortion and corresponding free energy of deformation per unit area for membrane region surrounding MscS. In (c) and (d) the elastic response of the lipids is captured with springs and the color coding indicates the local strain energy density at different distances from the proteins.

Fig. 4: Membrane protein interactions and conformational state. Overlap in the deformed membrane between proteins can cause proteins to attract or repel over distances comparable to the elastic decay length. Interestingly, the interaction energy between membrane proteins depends upon their conformational state and can induce cooperative conformational changes.

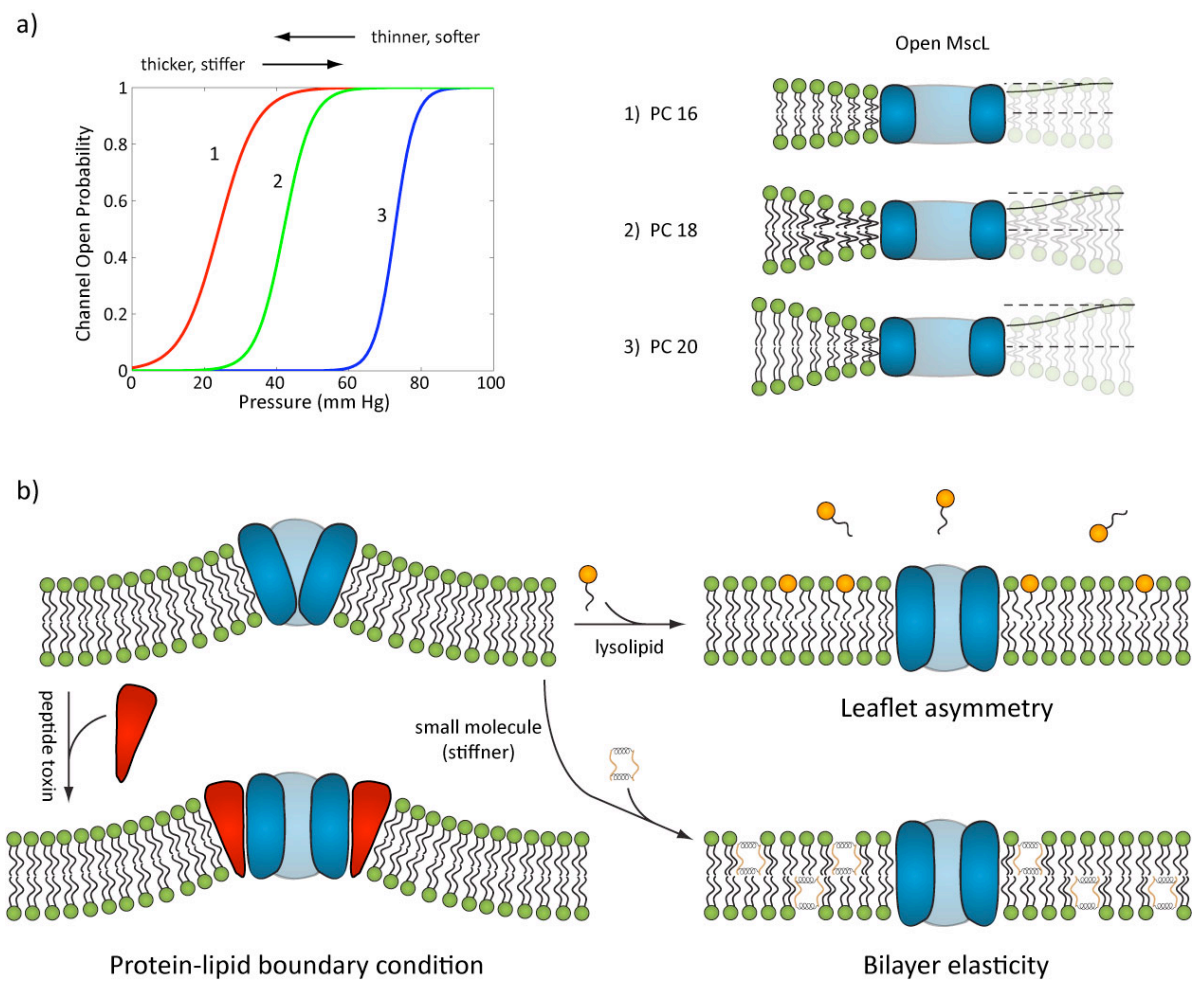


Fig. 1

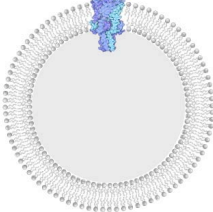
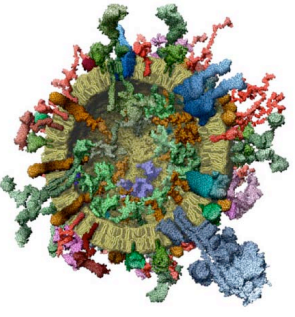
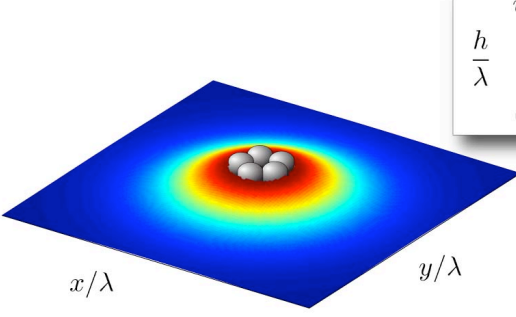
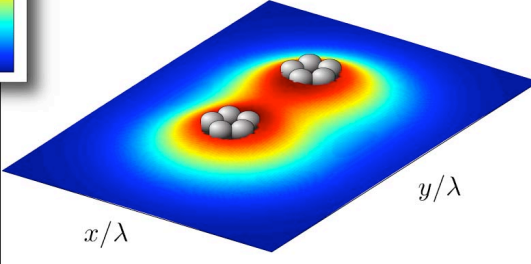
	Dilute	Crowded
Geometry		
Deformation		
Energy	$\frac{1}{2}ku^2 \cdot C$	$\frac{1}{2}ku^2 \cdot C + E(d, s_i, s_j)$

Fig. 2

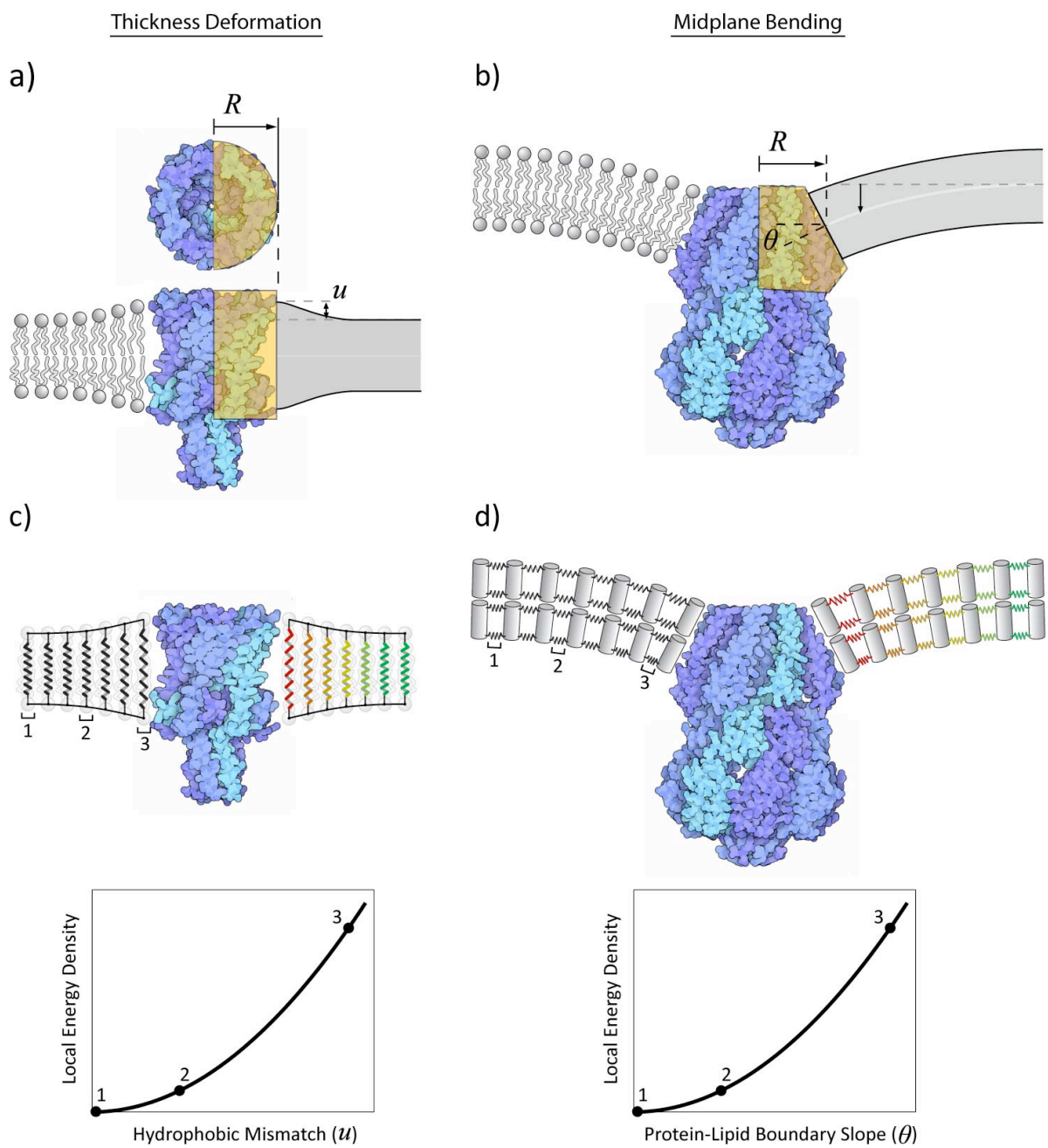


Fig. 3

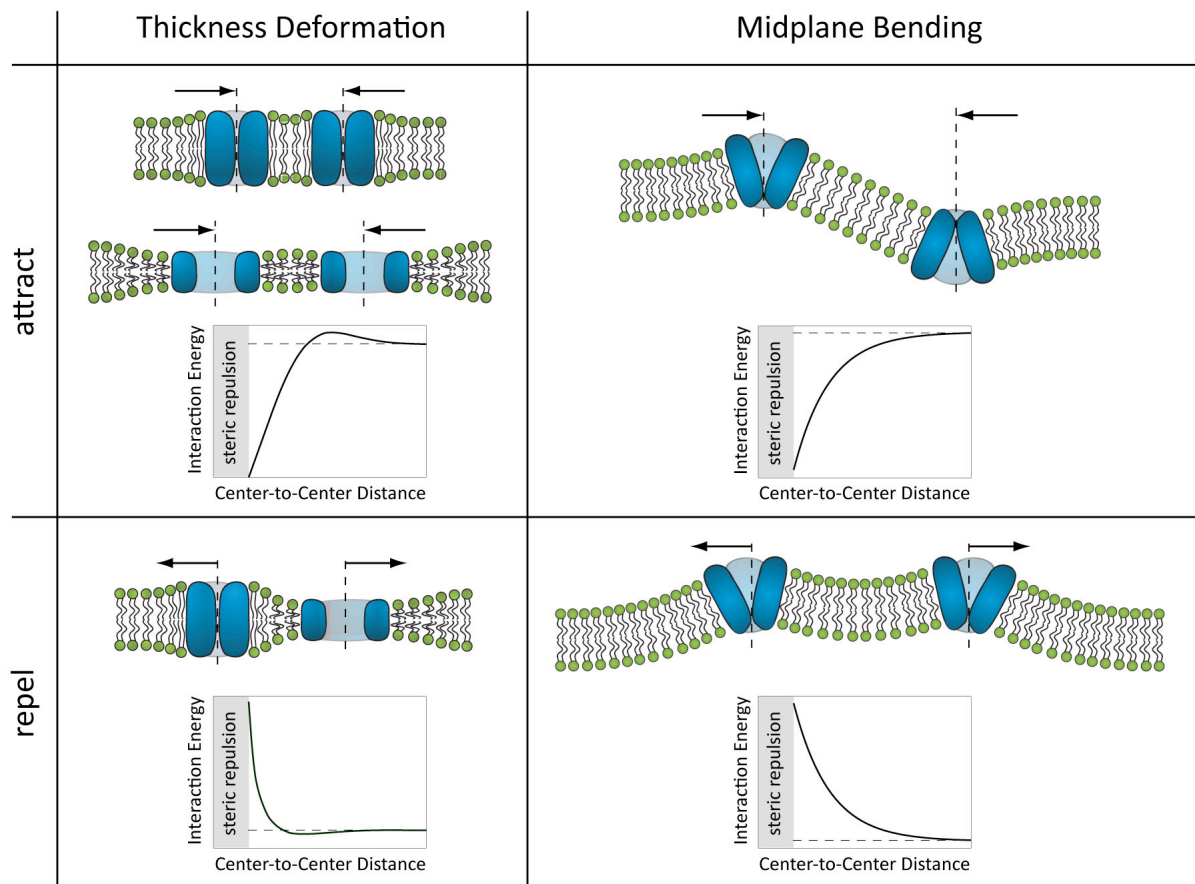


Fig. 4