Emerging roles for lipids in shaping membrane-protein function

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Studies of membrane proteins have revealed a direct link between the lipid environment and the structure and function of some of these proteins. Although some of these effects involve specific chemical interactions between lipids and protein residues, many can be understood in terms of protein-induced perturbations to the membrane shape. The free-energy cost of such perturbations can be estimated quantitatively, and measurements of channel gating in model systems of membrane proteins with their lipid partners are now confirming predictions of simple models.

Quantitative analysis is changing the face of biology. An area in which it has provided particularly useful insights is the analysis of the function of membrane proteins, specifically with respect to their interactions with the surrounding lipid molecules. Models and experiments show that rather than being a passive bystander in the function of membrane-bound proteins, the membrane can at times have an essential role in determining the function of these proteins.

Cell membranes are the barriers that separate the cytoplasm of the cell from the external world and internally compartmentalize eukaryotic cells into organelles. Far from being inert, biological membranes are key components in sensory and signalling pathways. They are highly controlled barriers that allow the directed flux of molecules into and out of the cytoplasm, and they have an analogous role in intracellular trafficking and energy production in cellular organelles.

At the microscopic scale, biological membranes are a crowded mix of membrane proteins and their lipid partners. Our understanding of this complicated environment is constantly being refined by new experiments[1]. The data that emerge often reveal functional and quantitative relations between biologically interesting parameters (for example, 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 generation of new experiments refines the models used to describe membranes, a topic elegantly reviewed elsewhere[1].

One case study that illustrates this interplay between quantitative models and experiments concerns the analysis of the structure and function of mechanosensitive channels[2], reconstituted in simple lipid bilayers[3]. The data demonstrate that the physicochemical properties of the surrounding lipid bilayer result in predictable and stereotyped consequences for channel function in artificial lipid membranes, although the interactions between lipids and membrane proteins have broader significance[4–6]. The concepts we present in this Review are predicted to have functional and structural significance for any protein whose function requires the remodelling of the protein–membrane interface. These models are used first to examine the properties of an isolated channel, followed by examples of an added layer of complexity resulting from membrane-mediated interactions between the channels.

Mechanosensitive channels and biological membranes

The idea that sequence dictates structure, which in turn dictates function, is a second central dogma of biology[7]. A powerful example of this dictum is in the context of membrane proteins. The stunning structures obtained of membrane machines, from the light-gathering apparatus of photosynthesis to the voltage-gated channels that allow neurons to propagate electrical impulses and the bacterial sensors that detect osmotic stress, provide key insights into the mechanisms by which these proteins respond to stimuli such as light, voltage and membrane tension. In many cases, complementary functional studies show us that the lipid bilayer is not a passive bystander in membrane protein function, as shown systematically elsewhere[4]. In this context, the word 'structure' usually refers to atomic positions, but a more coarse-grained picture of structure, captured by ideas from continuum elasticity, can reproduce many important membrane properties. In these models, 'structure' refers to quantities such as the local thickness and curvature of the lipid bilayer surrounding the membrane protein of interest. This is in contrast to molecular dynamics, which explicitly represents the position of every atom of both the protein and the lipid bilayer.

As a concrete example, we will consider bacterial mechanosensitive channels. The structure, function and physiology of mechanosensitive channels have been studied extensively. As shown in Fig. 1, bacterial mechanosensitive channels are gated by membrane tension[3]. More precisely, a pipette is used to grab a patch of membrane containing these channels and the current passing through the protein-encumbered membrane is measured as a function of the pipette suction pressure or membrane tension. These experiments demonstrate a relation between the open probability of the channel and the pipette pressure that is dependent on the properties of the lipid membrane in which the proteins find themselves (such as the tail lengths of the lipids, which can result in a mismatch between the protein and the bilayer thickness)[3,8]. Analysis of the gating free energy reveals that the quantitative dependence of the gating tension on the length of the lipid acyl tail matches the prediction from elastic bilayer models. Studies of mechanosensitive channels therefore reveal not only the importance of the lipid environment but show that, at least in the case of hydrophobic mismatch, where the hydrophobic core of the bilayer has a different thickness from the hydrophobic region of a transmembrane protein, the mechanism can be understood in terms of a coarse-grained elastic model of the bilayer.

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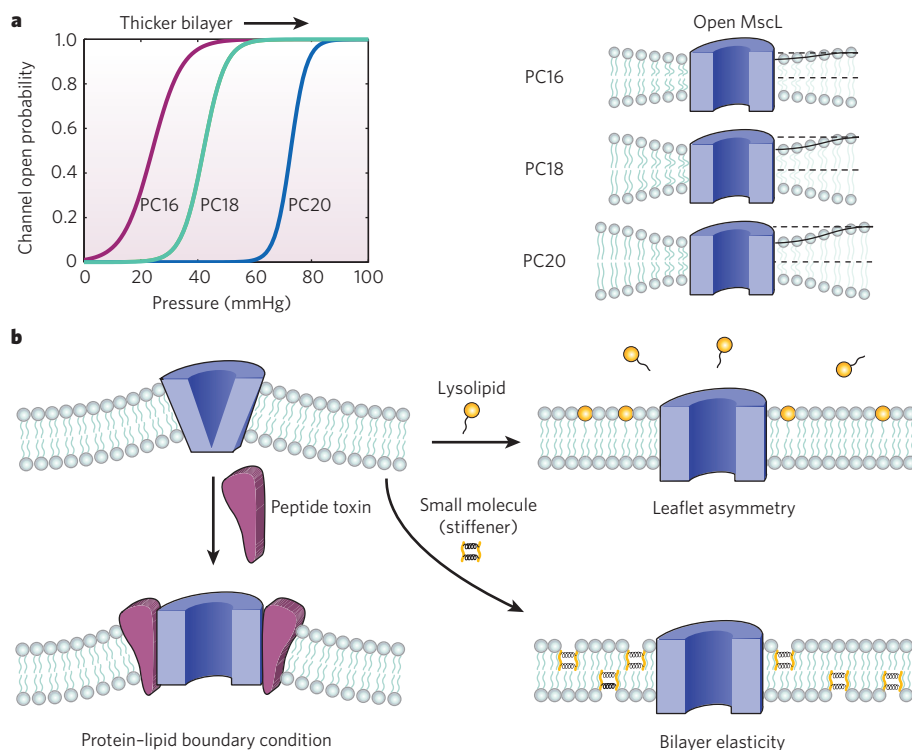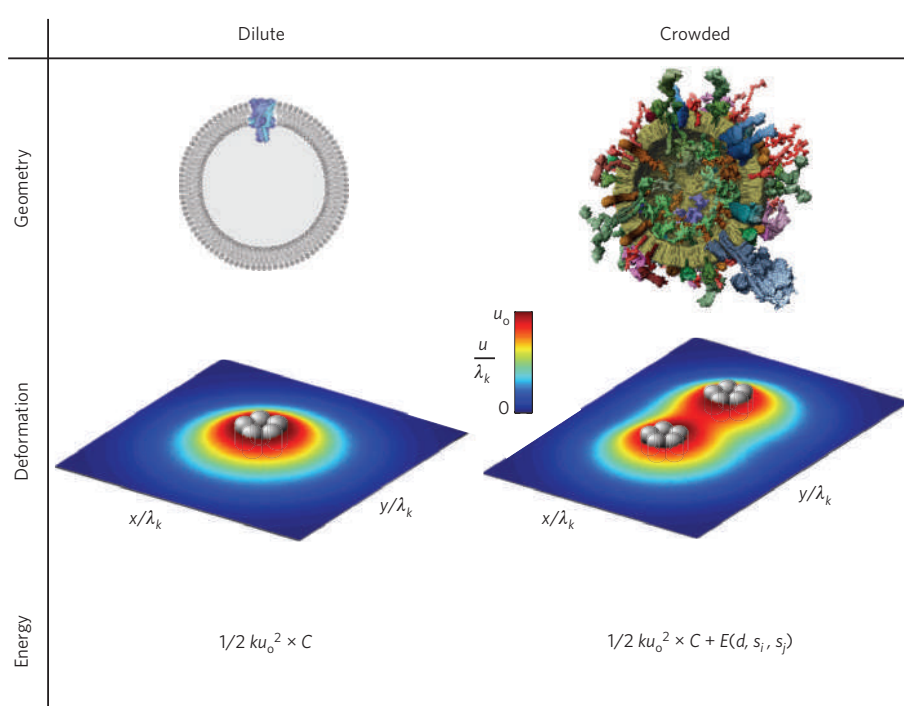
Figure 1 | Ion-channel function and membrane properties. a, Ion-channel open probability as a function of pipette pressure for mechanosensitive channels in lipids with different tail lengths. The data are adapted from ref. 3. The curves are an empirical fit to patch-clamp data using the functional form $p_{open} = 1/(1 + \exp(-\alpha(P-P_{1/2})))$, with the parameters α and $P_{1/2}$ as fitting parameters. The diagrams on the right show how different tail lengths imply a different hydrophobic mismatch as a result of the boundary conditions at the protein–lipid interface. MscL, mechanosensitive channel of large conductance. PC16, PC18 and PC20 are phospholipid bilayers with lipids with acyl chain lengths of 16, 18 and 20 carbons, respectively. **b,** Membrane doping and membrane protein function. The diagrams show hypothetical mechanisms whereby the insertion of various molecules can alter the protein–membrane interaction. For example, the asymmetrical insertion of lysolipids in the membrane produces a torque on the protein. The introduction of toxins can alter the boundary conditions between the protein and the surrounding lipids. Finally, small molecules can stiffen the membrane. In principle, all these effects could alter the gating characteristics of a channel.

A second example of the influence of the lipid environment on the function of membrane proteins is provided by the effects of membrane doping (by toxins, lipids or cholesterol) on channel activity (Fig. 1b). Certain lipid species and other membrane components are clearly required for proper protein function[9,10], but studies using toxins support the idea that the membrane is also a generic mechanical medium with which proteins interact. Rather than having evolved to target a specific channel, some toxins impair the function of multiple membrane proteins, and some small molecules, such as capsaicin[11], and peptide toxins, like those found in spider venom[12], target membrane channels across many species. These broad-ranging effects favour a mechanism that targets a generic property of membrane proteins. It has therefore been proposed that these toxins affect the interactions with the membrane itself. But can these toxins be understood in terms of a coarse-grained membrane model?

Many studies have shown that bilayer thickness, bending stiffness and monolayer spontaneous curvature can affect the function of embedded proteins[4,13]. Indeed, although the role of certain proteins (such as mechanosensitive channels) is to respond to membrane mechanical stress, in principle this stress can alter the function of any membrane protein. For example, the dimerization kinetics of the channel-forming peptide gramicidin A can be controlled by an externally applied mechanical stress on the membrane, resulting in membrane thinning and decreasing the hydrophobic mismatch between the membrane and

Figure 2 | Geometry, deformations and energies of dilute and crowded membranes. The two columns correspond to the dilute (proteins do not interact) and crowded (proteins interact) membrane limits. Each column shows the class of geometries found, a diagram of the deformation field in the vicinity of the proteins, and a mathematical description of the energies. For the isolated channel in the dilute limit, the deformation height, u, surrounding a given membrane protein has an elastic decay length, λ_k, that is smaller than the protein size. The deformation energy around a protein depends on a generic 'spring constant', k, determined from membrane properties. The deformation energy scales quadratically with hydrophobic mismatch, u_o, and scales approximately linearly with protein circumference, C. For crowded membranes, proteins have a sufficiently small separation distance ($d \approx \lambda_k$) that the annulus of deformed material around the proteins overlaps, resulting in an interaction energy that depends on the conformational state, s_i, of the ith protein.
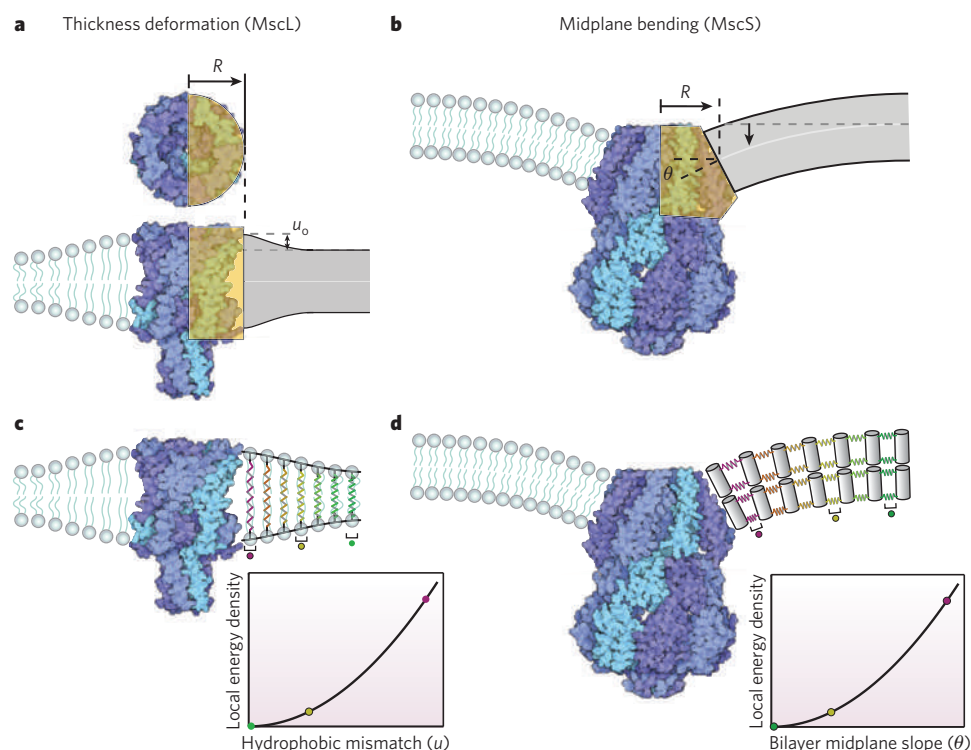
Figure 3 | Structure and energy at the protein–lipid interface. a, Atomic-level structure[63] and an elastic idealization of the mechanosensitive channel of large conductance (MscL) as a rigid cylinder with hydrophobic mismatch at the protein–lipid interface. R, effective radius of channel used in elastic model. u_o, hydrophobic mismatch between protein and equilibrium bilayer thickness. **b**, Atomic-level structure[64] and an elastic idealization of the mechanosensitive channel of small conductance (MscS) as a wedge with a slope that glues continuously onto the surrounding lipids. θ, midplane bending angle at protein–lipid interface. **c**, Membrane distortion and corresponding free energy of deformation per unit area of membrane surrounding MscL. **d**, Membrane distortion and corresponding free energy of deformation per unit area of membrane surrounding MscS. In **c** and **d** the elastic response of the lipids is captured with springs, and the colour coding indicates the local strain energy density at different distances from the proteins.

the gramicidin dimer[14]. Furthermore, using gramicidin A enantiomers as sensors for membrane mechanical properties, the small molecule capsaicin has been shown to target and trigger the pain receptor TRPV1 indirectly by decreasing the bending modulus of lipid bilayers in a concentration-dependent manner (not with a certain fixed stoichiometric relation between toxins and each channel, but progressively by altering the membrane's mechanical response)[11]. Conversely, voltage-dependent sodium channels are inactivated by capsaicin with no significant change to the conductance properties of the channels, but by an alteration of the gating voltage itself, suggesting that even channels that are not mechanically gated may still be subject to the effects of membrane mechanics through alterations of membrane properties[15–17]. In addition, it seems that some peptide toxins target multiple types of stretch-activated cation channels, not by changing membrane properties per se but by changing the effective boundary conditions at or near the protein–lipid interface[12]. This is yet another generic method by which membrane mechanics can couple to protein function (Fig. 1b). In particular, it seems that either enantiomer of a peptide toxin is localized in the membrane close to the channel and shifts its dose–response curve.

The experiments described above suggest ways of using quantitative models to explore the connection between membrane-protein function and the mechanics of the surrounding membrane. A useful starting point to flesh out a quantitative picture of such membranes is provided by simple order-of-magnitude estimates, and the derivation of scaling laws, for the free-energy costs associated with membrane deformations. For example, a simple 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.

Experiments on the occupancy of biological membranes by lipids and their protein partners provide a useful place to start[18]. As shown in Fig. 2, proteomic and lipidomic approaches have made it possible to survey the protein and lipid content of biological membranes. In the case shown in Fig. 2, a survey of the contents of a synaptic vesicle reveals a crowded and heterogeneous medium. Indeed, as noted 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"[18].

The synaptic vesicle in Fig. 2 tells a similar story to results from other biological membranes, such as bacterial membranes or the protein census of the red-blood-cell membrane[19,20]. The essence of the various membrane inventories is that biological membranes are as much protein as they are lipid, with typical protein:lipid mass ratios of around 60:40 (refs 19, 20). There are many ways to estimate the mean spacing between membrane proteins, and we can quibble over the details, but the message is always the same: biological membranes are crowded. The mean centre-to-centre spacing between proteins is estimated at about 10 nm (comparable to the distance between proteins in the cytoplasm[21,22]), which tells us that these proteins might be able to influence each other through the intervening membrane.

A variety of theoretical tools can be used to explore the interactions of proteins and the surrounding membrane. Two of the most important classes of analysis of the link between structure and function are atomistic models, in which every atom is treated explicitly, and continuum elasticity models, in which the molecules of interest are represented by field variables that describe the height and thickness of the bilayer at each point. Although both are important, estimates can be built using simple arguments from elasticity. The conclusions are largely indifferent to the details of how the energetics of the composite lipid and membrane protein system are treated, and an atomistic analysis would yield the same general picture of a deformed footprint of material around the protein of interest, as indicated in Fig. 2. Even so, atomistic analyses can reveal features of membrane-protein function that are inaccessible to continuum analysis; several representative examples can be found in refs 23–26.

Additionally, certain theoretical constructs offer a correspondence between atomistic and continuum analysis. For instance, lipid pressure profiles are the statistical representation of fully atomistic bilayer forces, where the integral moments of the pressure profiles yield the continuum properties of lateral tension, bending rigidity, and monolayer and bilayer spontaneous curvature[27–29]. We refer to the generality of elasticity because the key ideas have to do with the kinds of generic, geometric perturbations on the lipids that can result from the presence of a membrane protein and the energetic consequences of the perturbations, especially where the membrane protein undergoes a conformational change in the course of its functional activity. The key ideas are indicated in Fig. 2, where both the 'dilute' and 'crowded' limits

This Box provides typical values of some key mechanical properties relating to the bilayer and illustrates how the protein–lipid boundary conditions are altered by conformational changes. It also gives approximate analytical expressions and numerical estimates for the energetic costs of different types of bilayer deformation[38,40,42].

Bilayer properties

Here are some typical values of the bending and stretch stiffness, as well as the membrane thickness, of phospholipid bilayers. These lead to a relatively constant value for the elastic decay length for thickness deformations. Lateral tension and spontaneous curvature depend heavily on the osmotic conditions and amphiphilic composition of the bilayer, respectively, and so are quoted over a range. Variability in lateral tension also leads to a range of values for the midplane decay length.

Bending stiffness, $\kappa = 20\,k_BT$,
  where k_B is the Boltzmann constant (1.38×10^{-23} J/K)
Stretch stiffness, $k_s = 60\,k_BT\,\text{nm}^{-2}$
Thickness, $h_0 = 4$ nm
Tension, $\tau = 10^{-4}$–$1\,k_BT\,\text{nm}^{-2}$
Spontaneous curvature, $C_0 = 0$–$0.04\,\text{nm}^{-1}$
Transmembrane potential, $V_m = -40$ mV
Mismatch decay length, $\lambda_k = (\kappa h_0^2/4k_s)^{1/4} = 1.1$ nm
Midplane decay length, $\lambda_\tau = (\kappa/\tau)^{1/2} = 5$–$500$ nm

Channel properties

These are estimates of the change in radius, area and hydrophobic mismatch for the two-state conformational change of the mechanosensitive channel of large conductance, MscL. A hypothetical value for the change in midplane tilt at the protein–lipid interface is given for a generic two-state protein. The gating charge of a typical voltage-gated potassium channel is given for use in an energetic comparison.

Radius (closed–open), $R = 2.5$–3.5 nm
Area variation, $\Delta A \approx 20\,\text{nm}^2$
Thickness variation, $|\Delta u_0| = 0.8$ nm
Midplane tilt variation, $|\Delta\theta| = \pi/4$
Charge variation, $\Delta Q = 12e$,
  where e is the elementary unit of charge (1.6×10^{-19} C)

Conformational energy

We have used approximate analytical expressions for bilayer mechanical properties and interfacial boundary conditions to estimate the energetic costs of bilayer thickness deformation, protein area change, midplane deformations, bilayer spontaneous curvature coupling to the midplane and, for comparison, the free energy of voltage gating. These expressions are given in the limit $\lambda_k < R < \lambda_\tau$.

Thickness variation, $\Delta E_u = 2^{1/2}\pi\kappa(R/\lambda_k)(\Delta u_0/\lambda_k)^2 = 15\,k_BT$
Area variation, $\Delta E_\tau = \tau\Delta A = 10^{-3}$–$20\,k_BT$
Tilt variation where $C_0 = 0$, $\Delta E_\theta = \pi\tau(R\theta)^2\ln(\lambda_\tau/R) = 10^{-3}$–$7\,k_BT$
Tilt variation where $C_0 \neq 0$, $\Delta E_\theta = 2\pi\kappa C_0 R\theta = 10\,k_BT$
Charge variation, $\Delta E_v = \Delta QV_m = 20\,k_BT$

show how membrane proteins perturb the surrounding lipids (and each other, if the membrane is sufficiently crowded).

Elasticity and the isolated channel

To understand the interplay between ion channels and the surrounding lipids, consider an idealized isolated channel, like that in the top left-hand corner of Fig. 2, in a single-component lipid bilayer. Such simplifications fall short of the rich and varied landscape inhabited by channels in real cell membranes, but they can still provide useful mechanistic insights into how membrane proteins function when the protein 'footprint' changes with the conformation.

Figure 2 can help us predict the results of a mathematical description of these channels using elasticity theory. For example, an ion channel might change its external radius during gating or the thickness of its

hydrophobic region[4]. The key point is that the region of the membrane coloured red in Fig. 2 corresponds to membrane that is deformed (not in the relaxed state it would adopt if no protein were present). This region of deformed material costs a certain amount of deformation free energy. Furthermore, when the protein changes conformation, the annulus of deformed material changes, and so does the free-energy penalty.

Membrane as an elastic sheet

A convenient model for describing the interaction between membrane proteins and the surrounding membrane is to consider the membrane as a continuous elastic medium[30–32]. The idea behind this description is that there is a cost in terms of free energy that must be paid for perturbing the lipid bilayer away from some undeformed reference state, as indicated by the coloured springs in Fig. 3c, d. We emphasize several key modes of deformation (notably hydrophobic mismatch and midplane bending) and their corresponding free-energy cost. There is a well-defined mathematical theory of the free energy of membrane deformation[33–36], but here we will emphasize instead a qualitative and intuitive description of these theoretical results.

As highlighted in Fig. 3, different types of membrane deformation have a free-energy cost that can be calculated in the form of energy density (the free energy per unit area of deformed membrane). For example, in the case of a hydrophobic mismatch, where there is a free-energy penalty associated with 'gluing' the hydrophobic lipid tails to the hydrophobic region of the membrane protein, the free-energy density increases as the square of the hydrophobic mismatch[36–38]. Similarly, some membrane proteins will bend the membrane bilayer in their vicinity, incurring another class of free-energy cost[37,38]. This idea is represented in Fig. 3 by thinking of the membrane as a set of generalized springs. For every patch of area on the membrane, we can ask how different the thickness is from the equilibrium thickness, and how much the membrane is bent away from the flat state (in which there is no spontaneous curvature). Given the answer to these geometric questions, we can use these generalizations of Hooke's law to assign an energy density (the energy per unit area) to each patch of membrane, so we 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, and of 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. Membranes are composed of a plethora of different lipid species, but on the length and time scales of interest, several coarse-grained continuum material properties emerge[33]. For a homogenous lipid phase, these material parameters are the bending stiffness (with units of energy, measured here in terms of k_BT, where k_B is the Boltzmann constant and $T = 300$ K), the stretch stiffness and the membrane tension (both with units of energy per unit area, measured here in units of $k_BT\,\text{nm}^{-2}$), the bilayer thickness (measured in nm) and the spontaneous curvature of the membrane (measured in nm^{-1}) (Box 1). We will proceed as though these parameters were true material constants, although the situation is more subtle because the lipid environment surrounding a protein can change with its conformational state, and so too can these parameters.

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 provide intuition about the relative importance of different effects[30,39,40] (Box 1). Midplane bending and thickness deformation share a common energy scale proportional to the bending stiffness. If all other membrane and protein properties are fixed, 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[41–43]. In midplane deformation, protein 'shape' dictates the angle at which
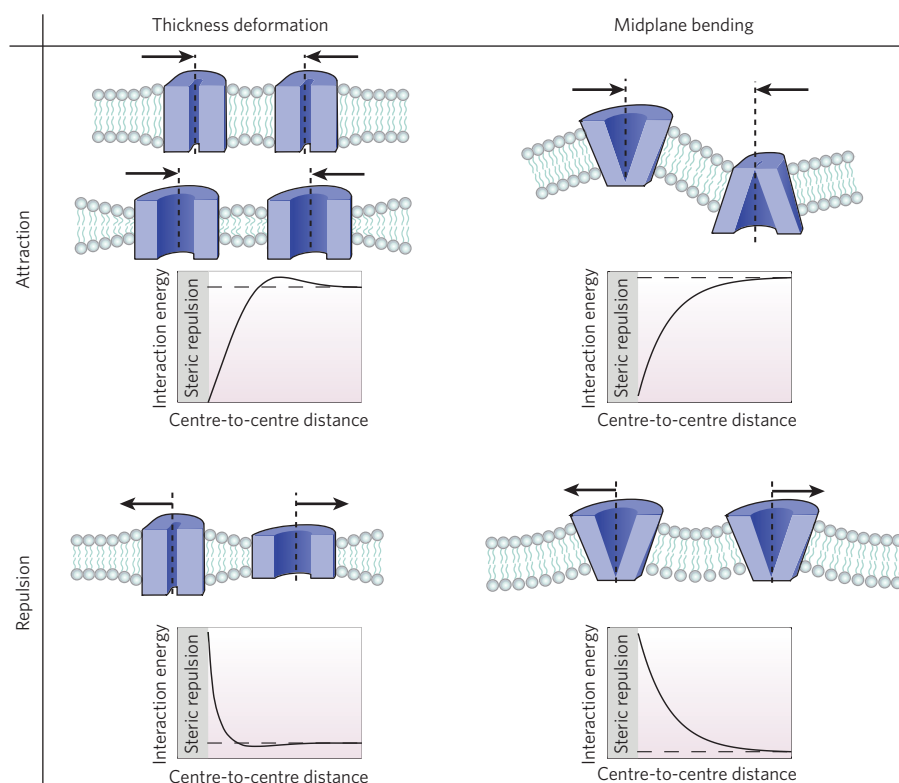
Figure 4 | Membrane–protein interactions and conformational state. Overlap in the deformed membrane between proteins can cause attraction or repulsion over distances comparable to the elastic decay length. The interaction energy between membrane proteins depends on their conformational state and can induce cooperative conformational changes.

the membrane contacts the protein (Fig. 3); the deformation energy here increases quadratically in the contact angle, so it acts rather like a classical Hookean spring. Similarly, in thickness deformation, as the degree of hydrophobic mismatch between the embedded protein and the bilayer increases, the deformation energy increases quadratically. In practice, a reasonable estimate of deformation energy around a protein is $10\,k_{B}T$ in either case[40,43] (Box 1).

Membrane elasticity and mechanosensitive channel gating
The ideas developed above can be used to understand the origin of the effects shown in Fig. 1. To see how, we use ideas from statistical mechanics to write the open probability of a channel as a function of the driving force of interest[31,43,44], resulting in:

$$p_{\text{open}} = \frac{e^{-\beta\varepsilon_{\text{open}}}}{e^{-\beta\varepsilon_{\text{closed}}} + e^{-\beta\varepsilon_{\text{open}}}} \qquad (1)$$

where β is $1/k_{B}T$ and $\varepsilon_{\text{open}}$ and $\varepsilon_{\text{closed}}$ refer to the free energies of the open and closed states, respectively. 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, such that

$$p_{\text{open}} = \frac{1}{1 + e^{-\beta(\tau\Delta A + \Delta\varepsilon_{\text{membrane}} + \Delta\varepsilon_{\text{protein}})}} \qquad (2)$$

This kind of analysis can respond to experiments like those in Fig. 1a. The term $\tau\Delta A$ corresponds to the driving force that favours the open state (τ is the membrane tension and ΔA is the change in protein area). However, this driving force must compete with the free-energy penalty associated with the membrane deformation footprint ($\Delta\varepsilon_{\text{membrane}}$) introduced in Fig. 2. We also include the energy difference ($\Delta\varepsilon_{\text{protein}}$) between the open and closed states associated with the protein's internal

degrees of freedom. Direct comparison with the experimental results in Fig. 1a is difficult because only the pipette pressure is reported experimentally, whereas the membrane tension is the key driving force[8]. Experiments that measure bilayer tension show that gating the mechanosensitive channel of large conductance (MscL) requires $\sim 2\,k_{B}T\,\text{nm}^{-2}$, depending on the lipid, and is accompanied by a change in area between open and closed conformations, ΔA, of around $20\,\text{nm}^{2}$, corresponding to a gating energy, $\tau\Delta A = 40\,k_{B}T$. As expected, this energy is much larger than the thermal energy, so spontaneous channel opening under low tension rarely occurs. Box 1 provides estimates for the membrane-associated energy penalty, and quantitatively shows the main point of this Review, namely that this membrane-deformation free energy can be reasonably expected to compete with the driving force, and can thereby influence the protein conformation.

As illustrated above, there are generic reasons to expect that for any membrane protein that alters its deformation footprint during a conformational change, protein function will be dependent on the structure of the membrane (and possibly on the tension as well). Our discussion has focused on general principles, rather than specific examples, and we have not dwelled on the contribution to the free energy from protein conformation ($\Delta\varepsilon_{\text{protein}}$ in eqn (2)). It should be emphasized that although lipid membranes generally exert a (composition-dependent) mechanical stress on embedded membrane proteins, the way that a given protein responds to this stress is highly specific. The mechanical stress from membrane deformation is likely to have little or no effect on proteins that offer a rigid (non-deformable) interface to the lipid membrane, or on proteins that show high affinity for particular lipids and that will not be influenced by the overall membrane composition if those lipids are present in the membrane.

Interacting membrane proteins and cooperativity
Several key insights from the discussion of the isolated channel can help us examine what happens when there are multiple membrane proteins. When different membrane proteins are within several elastic decay lengths of each other, they will interact. However, depending on protein shape, these membrane-mediated interactions may result in either attraction or repulsion[30,39,45–48]. Furthermore, at these small length

scales, where thermal fluctuations are important, membrane-mediated interactions between proteins can arise from rigid proteins perturbing the allowed jiggling motions of the membrane (the Casimir effect)[49,50]. These interactions are potentially long-ranged, but might be small, and their physiological relevance to membrane proteins has not been demonstrated.

The rules introduced in Box 1 hold during membrane-mediated protein interactions, except that we must compare the spatial extent of the deformation field with the distance between proteins. Each type of deformation has a length scale over which the membrane returns to its unperturbed state[30,39,40], although the interactions from thickness and midplane deformations behave qualitatively differently. The length scale of thickness deformations is mainly constant[33] and short (about a nanometre), but the length scale of midplane bending interactions is variable and longer (5–500 nm)[50], and tends to be weaker. These characteristic length scales are important in determining the balance between membrane deformation and the generic driving force that determines the conformation of the interacting proteins.

Biological membranes at physiological temperatures are generally in a fluid state, so both lipids and proteins can diffuse laterally (provided that they do not strongly interact with the cytoskeleton[51]). The diffusing proteins can be thought of as a two-dimensional gas with an entropic tension, equivalent to the pressure in a gas, that acts on the external surface of each protein in the membrane because the remaining proteins are jiggling around in the area available to them. The opening of membrane channels is typically associated with a change in channel area. One potential consequence of membrane crowding is that this conformational change could cause a change in free energy that is associated with the area available to the rest of the proteins, resulting in depletion forces.

Other effects can also arise through explicit interactions between adjacent channels, as shown in Fig. 4. The idea that the conformational states of two similar proteins can be coupled by the bilayer follows naturally from the discussion of membrane deformation and has been explored in detail elsewhere. Two proteins in proximity (within a few elastic decay lengths) will have regions of bilayer deformation that overlap, so one protein indirectly affects another through the lipids that surround them both[30,39,45–48,52,53]. One interesting outcome of these interactions is cooperative channel gating, as a conformational change in one protein will be 'felt' energetically through the surrounding lipids, influencing another protein's preference for a particular conformation[54]. Alternatively, the binding of membrane-associated proteins may impose boundary conditions that deform the membrane midplane, similar to the embedded proteins mentioned previously[55]. Several studies have shown that large-scale membrane deformations, such as budding[56] and tubulation[57], result from the collective mechanical interactions of such proteins[48,58–60], as reviewed in ref. 61. Here, too, certain rules emerge that depend on the nature of the interaction.

Within these elastic models, 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 behaviour: those that bend the bilayer in the same direction tend to repel each other, whereas those that bend the bilayer in opposite directions tend to attract each other[52]. In either case, attraction arises because the amount of deformed material between the proteins decreases when proteins are in close proximity, 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, so they 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: their ability to induce cooperativity in the conformational changes of neighbouring membrane proteins. We will not discuss the details here, but the outcome of the interactions is the principle that if one channel decides to gate,

this increases the likelihood that its neighbours will gate as well[54]. The more severe the bilayer deformation, the stronger the interaction will be between similar proteins, and the more tightly their conformations will couple.

Concluding perspective

A range of evidence for several different membrane proteins reveals the role played by the character of the surrounding membrane. We suggest that the natural regulatory effect of lipids on membrane-protein function can be used to dissect the structure–function relationship of membrane proteins. To make further progress in understanding the richness of the interface between membrane proteins and lipids, one useful avenue might be to exploit the generic predictions resulting from the kinds of theoretical analysis described here for the way in which conformational changes depend on the properties of the surrounding lipids and proteins. Beyond this, the role of membrane crowding should be explored more systematically because the proximity of membrane proteins may result in the same kinds of surprises already seen for crowding effects in the bulk setting[21,22,62]. ∎

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