Entropic Tension in Crowded Membranes

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Unlike their model membrane counterparts, biological membranes are richly decorated with a heterogeneous assembly of membrane proteins. These proteins are so tightly packed that their excluded area interactions can alter the free energy landscape associated with their conformational degrees of freedom. As a specific case study in these effects, we consider the impact of crowding on the gating tension for mechanosensitive channels. We show that crowding can alter the gating energies by more than $\approx 2\ k_BT$, a substantial fraction of the gating energies themselves in some cases.

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Cell membranes are packed full of proteins. The essence of various membrane inventories is that biological membranes are at least as much protein as they are lipid, with typical protein to lipid mass ratios of order $1-2.5$ [2–4]. Direct counting of the areal density of proteins in bacterial membranes tells a similar story with typical areal densities of $c_A = 10^5$ proteins/μm^2 or roughly $1/10$ nm^{-2} [2, 5]. There are other ways to think about the extent of membrane crowding, each with its own assumptions and merits, but regardless of these details the message will be the same. Biological membranes are crowded! (For an extreme example see Ref. [6].) For the purposes of this article, what these numbers tell us is that the mean spacing between proteins (estimated by evaluating $1/\sqrt{c_A}$) is only slightly larger than the proteins themselves, so that a significant fraction of the membrane area is occupied by proteins.

Experiments like those described above on the occupancy of biological membranes by lipids and their protein partners provide a useful basis for making estimates of the possible consequences of membrane crowding. The presence of such high areal fractions of protein means that there is the possibility that the "crowding" effect can alter the free energies of different membrane protein conformations and the dynamics of the changes between these conformations as well. Indeed, over the last several decades, the importance of crowding effects in general has become a theme of increasing concern in physical biology [7, 8].

The question of how the behavior of membrane proteins is altered by crowding effects has been explored much less thoroughly than their bulk counterparts [8, 9]. As a concrete example of the way crowding might play out in membranes, we consider transmembrane proteins that have several conformations with different areal footprint. One particularly fascinating class of proteins of this variety are the mechanosensitive ion channels. These proteins are thought to serve as safety valves for cells that are exposed to osmotic stress, opening up in response to increased membrane tension for the purpose of equilibrating the cells with their external environment [10].

To see how crowding might serve as an additional factor in the overall gating free energy balance for mechanosensitive channels, we consider the gating tension associated with the mechanosensitive channel of large conductance (MscL). Upon opening, at membrane tensions larger than $\sim 10^{-3}$ J/m^2, this channel undergoes a change in radius from roughly 2 nm to 3 nm [11, 12]. In the remainder of this paper, we explore the consequences of such gating for the free energy of the crowded proteins within the membrane, and the accompanying changes of the channel's gating tension.

The total free energy change upon gating, $\Delta G_{\rm tot} = G_{\rm open} - G_{\rm closed}$, can be thought of as arising from multiple contributions. In particular, we have

$$\Delta G_{\rm tot} = \Delta G_{\rm protein} + \Delta G_{\rm load} + \Delta G_{\rm mem} + \Delta G_{\rm crowd}, \quad (1)$$

where the first term reflects the free energy change associated with the protein degrees of freedom and their internal structural rearrangements, the second term refers to the potential energy of the loading device, and the third term characterizes the free energy of the deformed membrane surrounding the protein (and has been implicated as a key player in the gating of mechanosensitive channels [13, 14]). The last term is the crowding-induced term. A membrane protein with a large cytosolic domain, such as MscL, can potentially be crowded both by molecules in the cytoplasm, and by other membrane proteins. The latter effect forms the main substance of this paper.

The main conceptual point of the remainder of the paper can be stated simply as the idea that when the channel opens and changes its radius from "small" to "large", there will be a free energy cost for the surrounding membrane proteins, which we will refer to as crowders. In particular, these crowders will have their entropy reduced, which amounts to an effective pressure on the channel walls opposing its opening. To explore this claim, we will work in two distinct ensembles.

In the (mathematically) simpler case, we imagine a two-dimensional membrane "box" like that shown in

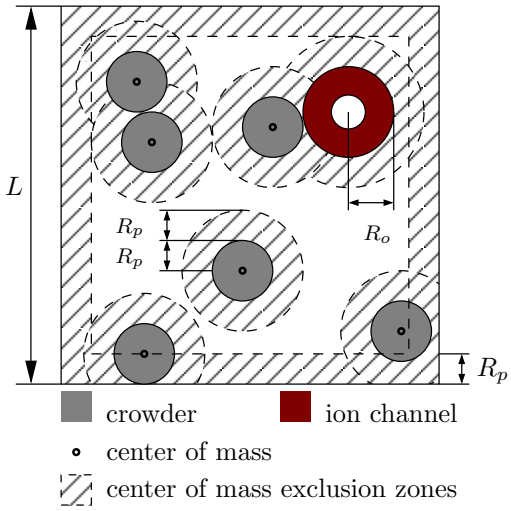

FIG. 1. (Color online) Excluded-area interactions and channel gating. For disk-shaped particles of finite size, the free area available for each center of mass is limited by the minimum distance between two centers of mass. This effect can be illustrated by exclusion zones of width R_p around each protein. The ion channel, in open configuration, is shown here as a red annulus.

Fig. 1, such that the overall area is fixed. When the channel goes from the closed to the open state, there is a net reduction in the available area for the remaining crowders, which results in an entropic tension that favors the closed state.

The second scenario imagines a loading device that subjects the membrane to some fixed tension on its perimeter, much like the springs that hold a trampoline under its state of tension. However, because of the change in the circumference of the protein, the exclusion annulus around the channel, indicated in Fig. 1, will be enlarged. Hence, there will still be an entropic tension, which favors the closed state. In both cases, we make the implicit assumption that the number of lipids in the membrane does not change on the time scale of protein conformational changes. For example, typical lifetimes of various conductance states of MscL near gating are of the order $10-100$ ms [11, 15], which is considerably faster than the time scale associated with lipid recycling *in vivo* (the formation of a recycling vesicles takes several seconds [16]).

To explore these two scenarios, we begin with the box of fixed area and use the simplest "ideal gas" physics to evaluate the change in entropy due to the loss of translational degrees of freedom when the channel goes from the closed to the open state. In particular, the translational entropy of one crowder can be computed as the logarithm of the area available to its center of mass,

$$g_{\rm crowd}(R) = -k_BT\ \ln\frac{\left(L^2-\pi(R+R_p)^2-A_{\rm edge}\right)}{A_{\rm lattice}},$$

where R_p is the radius of the crowder, and $A_{\rm edge}$ is the band of thickness R_p around the edge of the box in which crowders are forbidden (see Fig. 1). The denominator $A_{\rm lattice}$ refers to a discretization length scale used in a lattice model for the entropy [17]. Hence, the numerator is the effective area available to the crowder in the $L\times L$ membrane patch, recognizing that the minimal center-of-mass distance between the crowder and channel is R_p+R (see Fig. 1).

This expression can be simplified by expanding in the small parameter $(\pi(R+R_p)^2+A_{\rm edge})/L^2$. If we exploit this simplification and add the contributions from N non-interacting crowders, the difference in free energy between the open ($R=R_o$) and closed ($R=R_c$) states due to the crowding contributions can be written as $\Delta G_{\rm crowd}=N\Delta g_{\rm crowd}\approx c_Ak_BT\big(\pi(R_o^2-R_c^2)+2\pi R_p(R_o-R_c)\big)$, with $c_A=N/L^2$ being the crowder concentration. These two terms have simple and intuitive interpretations that are serviced by noting that we can rewrite the area and circumference change, respectively, as $\Delta A=\pi(R_o^2-R_c^2)$ and $\Delta C=2\pi(R_o-R_c)$. We can then divide the entropic crowding tension into a surface and a line tension, and write

$$\Delta G_{\rm crowd}\approx-\sigma_{\rm crowd}\Delta A+\tau_{\rm crowd}\Delta C. \qquad (2)$$

In our "ideal gas" approximation, the surface tension is $-\sigma_{\rm crowd}=c_Ak_BT$, the familiar ideal gas law. The line tension, $\tau_{\rm crowd}=c_AR_pk_BT$, originates in the fact that the annulus of exclusion shown in Fig. 1 changes size upon gating. This contribution vanishes in the limit that the size of the crowders goes to zero. For both terms, we will need to appeal to our earlier estimates of protein areal concentrations to set the scale of the effect.

We can now consider the second scenario in which there is a fixed applied tension σ. In this case, the lipid area in which the crowders wiggle around does not change, but the annulus of exclusion does, and hence the contribution of the entropy change to the free energy is given by the ΔC term only. At the same time, there is a relaxation in the energy of the loading device, which takes the form $\Delta G_{\rm load}=-\sigma\Delta A$.

In both scenarios, we neglect lipid elasticity. In the first case, the cost of squishing the lipids to access the open state is $\sim\kappa_A\Delta A^2/A_{\rm total}$, while in the second scenario, gating induces a small change in areal strain, which adds a correction of order σ/κ_A to the line tension. With an area compressibility $\kappa_A\sim60\ k_BT/{\rm nm}^2$ [18], $\Delta A\sim15$ nm^2 for MscL [11, 12], a patch area $A_{\rm total}\sim1\ \mu{\rm m}^2$, and a gating tension $\sigma\sim1\ k_BT/{\rm nm}^2$ [12, 15], these are indeed small corrections.

The treatment given above provides the simplest estimate of the crowding effect. However, as shown in Fig. 1, things become more complicated in the high concentration limit. In particular, the amount of available area is much less than is suggested by the simple estimate above,

	$-\sigma_{\rm crowd}/k_BT$	$\tau_{\rm crowd}/k_BT$
ideal gas	c_A	$c_A R_p$
SPT, uniform crowders	$\frac{c_A}{(1-\phi)^2}$	$\frac{c_A R_p}{1-\phi}$
SPT, non-uniform crowders	$\frac{c_A(1-\phi\eta^2)}{(1-\phi)^2}$	$\frac{c_A\langle R_p\rangle}{1-\phi}$

TABLE I. Entropic surface and line tension induced by crowders, estimated by an ideal gas calculation and scaled-particle theory (SPT). The results are derived for the case in which a single circular protein increases its radius from R_c to R_o in the presence of circular crowders with radius R_p. The non-uniform crowders case contains averages $\langle\cdot\rangle$ over the crowder radius distribution, and this size variation ($\eta^2 = {\rm Var}[R_p]/\langle R_p^2\rangle \geq 0$) leads to a smaller surface tension effect compared to uniform crowders with the same mean size.

	fixed area		fixed tension		
	IG	SPT	IG	SPT	units
$\Delta G_{\rm crowd}$	3.5	12	1	2	k_BT
$\Delta\sigma_{\rm crowd}$	0.22	0.76	0.06	0.13	$\frac{k_BT}{\rm nm^2}$

TABLE II. Different metrics for the effect of crowding on the gating behavior of a mechanosensitive ion channel. The first row shows the approximate gating energies. The second row shows the corresponding increase in gating tension, $\Delta\sigma_{\rm crowd} = \Delta G_{\rm crowd}/\Delta A$, which can be measured directly in patch-clamp experiments. For comparison, the typical gating tension for isolated MscL is 0.3-1.3 $k_BT/{\rm nm}^2$.

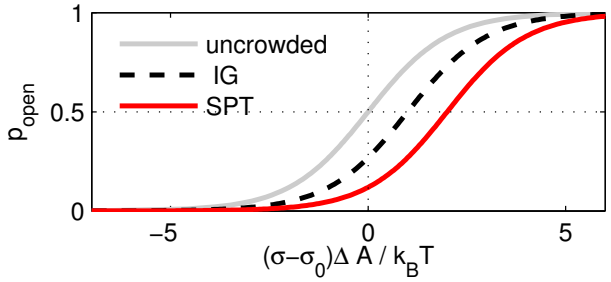

FIG. 2. (Color online) The effect of crowding on the open probability as a function of applied tension σ. The graphs illustrate the ideal gas (IG, $\Delta G_{\rm crowd} = 1k_BT$) and scaled-particle theory (SPT, $\Delta G_{\rm crowd} = 2k_BT$) results of table II, in the constant tension ensemble. The gating tension σ_0 includes all non-crowding contributions to the gating free energy.

where we made no reference to the way the crowders interact with each other. Neglecting these interactions underestimates the crowding effects. For 50% protein area coverage, the more accurate computations described below give increased surface and line tension terms by a factor of four and two, respectively. Note that the entropic effect increases in a highly non-linear fashion with the crowder area fraction, effectively diverging as one reaches the closed-packing limit. The effect we describe can thus be potentially much larger than the already substantial estimates of 1-10 k_BT given below.

One way to think about this, illustrated in Fig. 1, is in terms of exclusion zones around each crowder, analogous to the physics described by the van der Waals theory of gases. In the highly crowded regime, the theoretical difficulty is to compute the total size of the exclusion zones in a way that avoids double counting areas where multiple exclusion zones overlap. We use scaled-particle theory for mixtures of hard disks, an approximate equation of state that combines reasonable accuracy with analytical tractability [19], and has been widely applied to describe the effects of crowding [7–9].

Since scaled-particle theory supplies thermodynamically consistent approximations for the chemical potential and surface tension [8, 19], we divide the gating transition into several consecutive steps, corresponding to removal of the closed channel, area change (for constant tension), and insertion of an open channel, and add the contributions [20]. The central results, for circular crowders, are presented in table I, in terms of the concentration c_A, areal fraction ϕ, and, for non-uniform crowder sizes, relative size variance $\eta^2 = (\langle R_p^2\rangle - \langle R_p\rangle^2)/\langle R_p^2\rangle$. The crowding-induced changes in gating energy all take the form of Eq. (2), with only the line tension contributing in the constant tension ensemble. The more exact scaled particle theory gives larger crowding tensions.

With these analytical results in hand, we now turn to the question of the actual magnitude of the crowding effect. To be concrete, we consider the case in which we have a membrane where the area is half lipids and half proteins (i.e. $\phi = 1/2$), big enough to make the ideal gas estimates questionable. We consider a radius change of a single channel from 2 to 3 nm, and a crowder radius of 1 nm. For an estimate of the effect of protein size variability, we turn to distributions of the number of transmembrane helices, $n_{\rm TMH}$ [3, 21]. Approximating the protein radius R_p as proportional to $\sqrt{n_{\rm TMH}}$ leads to $\eta^2 \approx {\rm Var}[\sqrt{n_{\rm TMH}}]/\langle n_{\rm TMH}\rangle \approx 0.15 - 0.25$. This being a minor effect, we neglect it and use $\eta^2 = 0$. Using these numbers in the context of table I, we can quantify the influence of crowding on the gating behavior in different ways, shown in table II.

One way to present the significance of these results is by appealing directly to the curves that provide the probability of channel opening as a function of the driving force. For the case of a "two-state" ion channel, which transitions back and forth between distinct closed and open states, the open probability is $p_{\rm open} = (1 + \exp(\Delta G_{\rm tot}/k_BT))^{-1}$, where $\Delta G_{\rm tot}$ is the energy difference between the closed and open states, and depends upon the driving force (such as tension, voltage or ligand concentration). In our case, the driving force is the tension, and we can rewrite $\Delta G_{\rm tot} = \Delta G_0 - \sigma\Delta A + \Delta G_{\rm crowd}$. The first term corresponds to all contributions of Eq. (1)

that do not depend explicitly on crowding or applied tension. We can rewrite it in the simpler form $\Delta G_0 = \sigma_0 \Delta A$. Figure 2 shows the gating probability $p_{\rm open}$ as a function of σ both for a single isolated channel and for the case in which crowders are present.

An alternative way to decide if the effect is big or small, is to compare it to some reference energy (or tension). The first relevant energy scale for comparison is the thermal energy k_BT, the energy scale in the Boltzmann weight $\exp(-\Delta G/k_BT)$ in the open probability above. Our numerical examples in table II all change the gating free energy by $\geq 1\ k_BT$. A second relevant energy scale is that associated with the gating of various mechanosensitive ion channels. The gating properties of channels such as MscL have been measured for several different species of lipid molecules. The outcome of these elegant experiments is that the gating energies have typical values of 5-20 k_BT [12, 15] and corresponding gating tensions in the range of 0.3 - 1.3 k_BT/nm^2, though in the presence of spontaneous curvature inducing lipids these energies and tensions are even smaller (or even negative, meaning that the channel opens spontaneously without any applied tension) [12]. The change in gating tension due to crowding is $\Delta\sigma_{\rm crowd} = \Delta G_{\rm crowd}/\Delta A$, and we get numbers in the range 0.06-0.8 k_BT/nm^2.

The entropic cost of channel opening in a crowded solution of membrane proteins has so far been discussed only with reference to hard core repulsion between proteins. It is however well known that membrane-mediated interactions may emerge from the overlap of the membrane deformations surrounding neighboring proteins, such as those arising from a thickness mismatch between the hydrophobic protein core and the membrane average thickness[13], or a non-cylindrical shape of the transmembrane region [13, 22]. Beside the hydrophobic mismatch itself, the strength, and even the sign of such interactions depend on many factors, including membrane stiffness to bending and stretching, and the monolayer's spontaneous curvature, but the range of these interactions is comparable to the protein size itself and hence can increase the effective radii of both the channel and the crowder proteins, potentially increasing the surface fraction ϕ, and the gating energy.

The present analysis has as its key outcome the hypothesis that under sufficiently crowded conditions, membrane proteins can influence each others conformational changes through an entropic tension. Though we explored the consequences of that idea for one particular channel, given the great diversity of membrane proteins and the high degree of crowding in many different membrane types, we expect that such effects could be common.

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