

# Lateral Pressures in Cell Membranes: A Mechanism for Modulation of Protein Function

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Variations in the composition of cell membranes can strongly influence the function of proteins embedded therein. However, in most cases it is not known whether lipids and other membrane components act by binding directly to proteins or indirectly through changes in a structural or thermodynamic property of the fluid bilayer. In the present work, we develop a simple thermodynamic analysis based on the hypothesis that variations in membrane composition induce changes in the transverse pressure profile in lipid bilayers. If protein function involves a conformational transition accompanied by a depth-dependent change in its cross-sectional area, we predict that small changes in the lateral pressure can induce a large shift in the conformational distribution. The sensitivity of the conformational equilibrium to the lateral pressure profile arises in part from the localization of the large interfacial free energy within a domain of molecular thickness and also from the difference between the logarithmic dependence of the chemical potential of a protein conformational state on its own concentration and its linear dependence on small changes in the pressure profile.

## Introduction

Changes in the composition of cell membranes can significantly modulate the action of intrinsic membrane proteins.<sup>1–5</sup> Enzymes, ion and molecular pumps, and ion channel proteins are often sensitive to variation of lipid head groups and chain lengths or to the concentrations of cholesterol and smaller solutes such as general anesthetics. At present, the physical mechanisms by which membrane components influence embedded proteins are largely unknown. Putative mechanisms can be classified as either direct or indirect, depending on whether the membrane components are presumed to act by binding directly to a protein or by exerting their influence through variations in the properties of the fluid lipid bilayer in which the protein is embedded. A direct mechanism involving localized lipid–protein interactions (perhaps within a boundary layer of lipid around the protein) is probably uncommon, given the wide range of molecular characteristics among lipids, cholesterol, and other molecules solubilized in the membrane. Proposed indirect mechanisms have usually involved correlations of variations in bilayer composition with altered structural properties of the membrane such as thickness or orientational order parameters or with thermodynamic properties such as bending elasticity or proximity to phase transition boundaries. However, whereas small variations in concentration can alter protein function significantly, the accompanying changes in membrane structural properties are typically small and can often be achieved by small variations in temperature or other external variables that have relatively little effect on protein function.<sup>6</sup> It thus seems unlikely that such small structural variations *cause* any significant changes in protein function.

Is there another membrane property that is highly sensitive to (i.e., an amplifier of) small changes in membrane composition and has a clear *mechanistic* link to protein function? In the present work, we suggest that the lateral pressure profile within the membrane may serve as such a property for those intrinsic proteins whose function involves a conformational change accompanied by a depth-dependent variation in the cross-sectional area of the protein. We first apply simple thermodynamics to predict the shift in the protein conformational

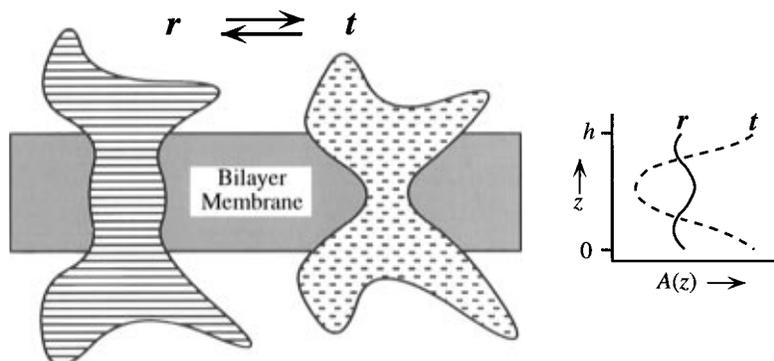
equilibrium resulting from changes in the pressure profile. Using an order-of-magnitude estimate for the variations in the protein cross-sectional area profile, we can then approximate the relative pressure changes necessary to alter the equilibrium significantly. The physical origins of the sensitivity of this equilibrium to a small relative change in the pressure profile are discussed.

## Thermodynamic Analysis

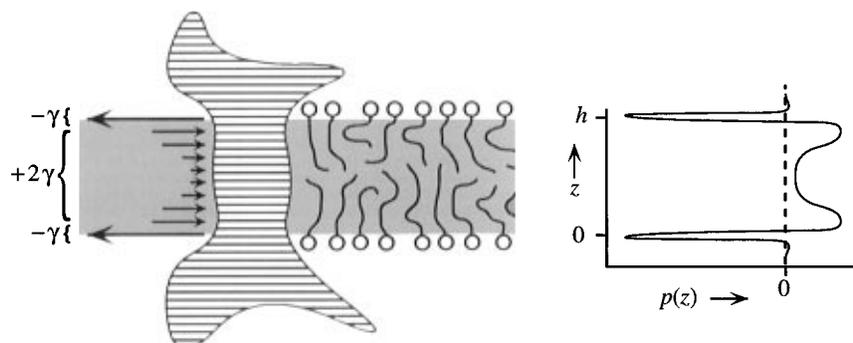
We consider those intrinsic membrane proteins whose function depends on a transition between conformational states. For simplicity, the protein is assumed to exist in only two such states, **r** and **t**, each of which is characterized by a cross-sectional area that varies, in general, with depth within the membrane. We let  $A_r(z)$  and  $A_t(z)$  represent these functions,  $z$  indicating the position along the perpendicular to the bilayer plane, as sketched in Figure 1. In general, the conformational shift **r** → **t** will be accompanied by a depth-dependent change in the cross-sectional area:  $\Delta A(z) = A_t(z) - A_r(z)$ .

The bilayer is a highly inhomogeneous region characterized by anisotropic stresses that vary with depth within the bilayer.<sup>7,8</sup> Since the membrane is of molecular thickness in the direction perpendicular to the bilayer plane, the large excess free energy characteristic of oil/water interfaces is concentrated within this narrow region, resulting in local lateral pressures (force per unit length transverse to the bilayer normal) of enormous magnitude. If we define  $\delta\pi(z)$  as the lateral pressure acting within a thin slice of the bilayer of thickness  $\delta z$ , then  $p(z) = \delta\pi/\delta z$  represents a lateral pressure density, with dimensions of bulk pressure. An example of a pressure profile that might be expected in a lipid bilayer is presented in Figure 2 (see also Figure 74 in ref 9). A large negative pressure is localized at the aqueous interfaces, similar to the tension found at the interface between bulk aqueous and hydrocarbon domains. The strong repulsions among the hydrocarbon chains (partially orientationally ordered, with accompanying loss of conformational entropy) result in large positive lateral pressures in the bilayer near the interface, decreasing toward the middle of the bilayer. Since the fluid membrane is self-assembled, there is no (or very little) *overall* stress. The large tensions at the interfaces are compensated by the predominantly positive pressures in the bilayer interior, with

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**Figure 1.** Slice through a bilayer membrane containing an intrinsic protein viewed in two different conformational states, **r** and **t**. At right, the cross-sectional area profile  $A(z)$  of each of the two states is plotted as a function of depth  $z$  within the membrane of thickness  $h$ .



**Figure 2.** Representative example of the pressure profile in a bilayer. The lipids on the right side of the embedded protein are sketched for simplicity as single-chain amphiphiles. On the left, the length and direction of the arrows represent the pressure profile of the fluid membrane acting on the protein: strong tensions at the interfaces balanced by positive pressures through the interior, largest near the interfaces. On the right the corresponding  $p(z)$  is plotted; the dashed line locates  $p = 0$ . Note that the area under the curve is zero.

additional contributions arising from head-group interactions. The total lateral pressure in the membrane is thus zero (or nearly so), i.e.,  $\pi = \int \delta\pi = \int p(z) \delta z \approx 0$ . [The integral implies a continuum approximation (i.e., in the limit of small  $\delta z$ ) that should be applied with caution when  $\delta z$  approaches the characteristic size of the methylene groups of which the lipid chains are comprised. While it provides a useful description of the spatial distribution of forces, it should be interpreted as a sum over terms corresponding to layers of finite thickness  $\delta z$ .]

We consider the lipid bilayer as a fluid interfacial phase in which the protein acts as a solute that can exist in two different states  $s = \mathbf{r}$  or  $\mathbf{t}$ . At equilibrium, the chemical potentials of the two states must be equal:  $\mu_r = \mu_t$ . The dependence of the chemical potential of state  $s$  on its concentration  $[s]$  and on the pressure profile  $p(z)$  is obtained by analogy with three-dimensional isotropic systems. Consider a bulk solution, with ideal solute that can exist in two different states. Its chemical potential in state  $s$  at bulk pressure  $p$  would be

$$\mu_s = \mu_s^* + RT \ln [s] + V_s \Delta p \quad (1)$$

where  $\mu_s^*$  is the standard chemical potential, i.e., at unit concentration and standard pressure  $p_0$ , and  $V_s$  is the partial molar volume of the solute in state  $s$ , assumed invariant over the pressure range  $\Delta p = p - p_0$ . For each conformational state of the protein, the mechanical work accompanying a change  $dA(z)$  in the cross-sectional area profile of the protein is given by  $dw = -\int p(z) dA(z) \delta z$ , in analogy to the work in a bulk isotropic fluid  $dw = -p dV$ . In analogy with eq 1, the expression for the chemical potential of conformational state  $s$  is thus

$$\mu_s \approx \mu_s^* + RT \ln [s] + N_{Av} \int A_s(z) \Delta p(z) \delta z \quad (2)$$

where  $N_{Av}$  is Avogadro's number. By equating the chemical potentials of the two states at  $p(z)$  and also at  $p_0(z)$ , we eliminate the standard chemical potentials to obtain

$$[\mathbf{r}]/[\mathbf{t}] = [\mathbf{r}]_0/[\mathbf{t}]_0 e^{\alpha} \quad (3)$$

where  $[s]$  and  $[s]_0$  are the equilibrium concentrations of state  $s$  at pressures  $p(z)$  and  $p_0(z)$ , respectively, and  $\alpha = (k_B T)^{-1} \int \Delta A(z) \Delta p(z) \delta z$ . The fraction of protein in conformational state  $\mathbf{t}$  can then be written as  $F = [\mathbf{t}]/([\mathbf{r}] + [\mathbf{t}]) = (1 + y_0 e^{\alpha})^{-1}$ , where  $y_0 = [\mathbf{r}]_0/[\mathbf{t}]_0$ . At pressure profile  $p_0(z)$ ,  $F_0 = (1 + y_0)^{-1}$ , and thus

$$f = F/F_0 = (1 + y_0)/(1 + y_0 e^{\alpha}) \quad (4)$$

represents the fraction of protein in conformation  $\mathbf{t}$  at  $p(z)$  relative to that at  $p_0(z)$ .

For the special case of  $\Delta A$  independent of  $z$  (i.e., if the protein were to expand or contract uniformly), then the conformational equilibrium would be *unaffected* by a change in the pressure profile, since in that case  $\alpha = (k_B T)^{-1} \Delta A \int \Delta p(z) \delta z = 0$ . In general, however, the lateral expansion or contraction of the protein will vary significantly with  $z$ , as depicted in Figure 1. The sign of  $\alpha$  depends on the correlation of area and pressure changes. For the example sketched in Figure 1, if (as a result of some change in bilayer lipid composition) the pressure were to decrease near the bilayer center and increase near the interfaces,  $\alpha$  would be positive; if the pressure shifts were reversed,  $\alpha$  would be negative. Note that  $\alpha$  is determined only by the *variation* of  $\Delta A$  with  $z$ , while the *average* area change is irrelevant.

As an example, we consider inhibition of a protein that is active in conformation **t** but inactive in state **r**. We expect  $\Delta p$ , and thus  $\alpha$ , to vary approximately linearly with the change in the lipid composition if the changes are not too large. Because of the *exponential* dependence of  $f$  on  $\alpha$ , there is almost no shift in the protein conformational distribution until  $\alpha$  exceeds  $\ln(y_0^{-1})$ , beyond which the fraction of protein in the active state decreases rapidly. For example, suppose 95% of the protein is in the active conformation ( $y_0 = 1/19$ ) at  $p_0$ . Then  $f \approx 0.92$  (little inhibition) for  $\alpha = 1$ , decreasing to  $f \approx 0.0009$  at  $\alpha = 10$ . This strong exponential dependence on the perturbation contrasts with allosteric inhibition that would result from *direct binding* of a ligand **i** selectively to the **r** state. For  $n$  identical noninteracting binding sites with dissociation constant  $k_r$  (equivalent to the Monod-Wyman-Changeux model<sup>10</sup> in the limit of no ligand binding to the active state,<sup>11</sup> i.e.,  $k_t \rightarrow \infty$ ), it is easily shown that  $f_d = (1 + y_0)/[1 + y_0(1 + \alpha_d)^n]$ , where  $\alpha_d = [i]/k_r$ . Clearly,  $f$  decreases much more rapidly than does  $f_d$  with increasing perturbation  $\alpha$ .

To examine whether small variations in the pressure profile are capable of inducing a significant shift in the protein conformational distribution, an order-of-magnitude estimate for  $\alpha$  is useful. Unfortunately, almost no information is available on  $\Delta A(z)$ , even for the relatively few intrinsic membrane proteins for which  $A_r(z)$  is known for the resting state of the protein, such as the nicotinic acetylcholine receptor (a well-studied ion channel protein), which has an average radius of about 35 Å. We can speculate that the magnitude of the average shift in the radius might be about 5 Å; the corresponding average area change would be of magnitude  $\langle \Delta A \rangle \approx 1000 \text{ \AA}^2$ . What about  $\Delta p$ ? The (negative) lateral pressure localized at each aqueous interface is of order  $-\gamma$ , where  $\gamma \approx 50 \text{ dyn/cm}$  (a typical oil/water interfacial tension), presumed to be unaffected by changes in membrane composition. As shown in Figure 2, these interfacial tensions are compensated by a positive lateral pressure of order  $+2\gamma$  spread predominantly (and unevenly) over the membrane interior of width  $h \approx 30 \text{ \AA}$ , corresponding to an average pressure density  $\langle p \rangle \sim 3 \text{ dyn cm}^{-1} \text{ \AA}^{-1} \approx 300 \text{ atm}$ , but likely to be much larger near the aqueous interface. We let  $\langle \Delta p/p \rangle$  represent the magnitude of typical relative pressure changes. Of course, we expect  $|f \Delta A(z) \Delta p(z) \delta z| < \langle \Delta A \rangle \langle p \rangle h \langle \Delta p/p \rangle$ , the reduction in magnitude minimized if the largest variations in the  $z$ -dependence of  $\Delta A(z)$  coincide with those of  $\Delta p(z)$ . Arbitrarily assuming a reduction by a factor of 4, we estimate  $\alpha \approx 0.25 \langle \Delta A \rangle \langle p \rangle (h/k_B T) \langle \Delta p/p \rangle \approx 60 \langle \Delta p/p \rangle$ . Using the example above ( $y_0 \approx .05$ ), a change of order 10% in the pressure profile gives  $f < 0.05$ , i.e., an overwhelming shift in the conformational population distribution. While this is (at best) an order-of-magnitude estimate, it illustrates the sensitivity of the equilibrium to relatively small changes in  $p(z)$ .

## Discussion

There are two reasons for the sensitivity of the protein conformational transition to small changes in the pressure profile. The concentration of large interfacial free energies within the microscopically narrow region, as in a monolayer or bilayer film, leads to enormous local lateral pressure densities

corresponding to bulk pressures of hundreds of atmospheres or more. Although the perturbation of the local pressures by small changes in membrane composition might be *relatively* small, it is large in absolute magnitude since the pressure densities are enormous. Also, the chemical potential of the protein depends approximately *logarithmically* on its own concentration, but *linearly* (through  $\Delta p$ ) on small compositional changes in the bilayer. Thus, a relatively small variation in the pressure profile requires a much larger compensating shift in the concentrations of the protein conformational states to maintain equality of their chemical potentials.

Transverse pressure profiles of monolayer and bilayer films have been studied theoretically,<sup>7</sup> in part because the moments of the profile and its curvature derivatives determine the bending elastic properties of the film<sup>12</sup> and thus strongly influence the phase diagrams of surfactant solutions. In the present work, we suggest a simple thermodynamic approach to investigate a different consequence of variations in the lateral pressure profile: the sensitivity of conformational equilibria of intrinsic membrane proteins to bilayer composition. For simplicity, we have ignored many other effects that would contribute to these variations. For example, for mixed films, it has been shown<sup>13,14</sup> that elastic contributions to the free energy can result in phase separation, which would likely alter the pressure profile significantly.

In recent work<sup>15</sup> we have used lattice statistical thermodynamic methods<sup>16</sup> to determine how clinical concentrations of general anesthetics might perturb the pressure profile sufficiently to inhibit the opening of postsynaptic ion channel proteins. In principle, changes in the pressure profile upon incorporation of a wide range of other bilayer constituents can be calculated and used to correlate experimentally observed modulation (inhibition or potentiation) of a membrane protein with changes in the cross-sectional area profile that accompany the conformational transition required for its function.

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