

# Bilayer Thickness and Membrane Protein Function: An Energetic Perspective

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## Key Words

bilayer deformation energy, bilayer material properties, elastic bilayer deformations, gramicidin A, intrinsic lipid curvature

## Abstract

The lipid bilayer component of biological membranes is important for the distribution, organization, and function of bilayer-spanning proteins. This regulation is due to both specific lipid-protein interactions and general bilayer-protein interactions, which modulate the energetics and kinetics of protein conformational transitions, as well as the protein distribution between different membrane compartments. The bilayer regulation of membrane protein function arises from the hydrophobic coupling between the protein's hydrophobic domains and the bilayer hydrophobic core, which causes protein conformational changes that involve the protein/bilayer boundary to perturb the adjacent bilayer. Such bilayer perturbations, or deformations, incur an energetic cost, which for a given conformational change varies as a function of the bilayer material properties (bilayer thickness, intrinsic lipid curvature, and the elastic compression and bending moduli). Protein function therefore is regulated by changes in bilayer material properties, which determine the free-energy changes caused by the protein-induced bilayer deformation. The lipid bilayer thus becomes an allosteric regulator of membrane function.

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## INTRODUCTION

The lipid bilayer component of biological membranes serves both as a barrier for the passage of polar solutes and as a solvent for bilayer-spanning membrane proteins that catalyze the transmembrane transfer of information and material across the permeability barrier provided by the bilayer. Studies on the uncatalyzed permeation of small solutes (33, 115) show that the bilayer barrier properties approximate those of a thin sheet of liquid hydrocarbon, which is ~30 Å thick. This approximation has proven useful for understanding the physical principles governing the uncatalyzed movement of small molecules across biological membranes. It does not, however, provide a realistic basis for understanding the regulation of membrane protein function by the lipid bilayer (11). For this pur-

pose, it is necessary to consider the lipid bilayer as a liquid-crystalline body (72, 105) with both short-range and long-range order and (locally) well-defined material properties (31, 73).

## REGULATION OF MEMBRANE PROTEIN FUNCTION BY THE LIPID BILAYER

The lipid bilayer component of biological membranes is important for the distribution, organization, and function of membrane proteins and thus for many cell functions (10, 25, 42, 59, 60, 72, 101, 106). Although the importance of the membrane lipids for different cell functions was appreciated long ago, mechanistic understanding of how the membrane lipid composition regulates membrane protein folding, trafficking, organization, and function is only beginning to emerge—notwithstanding seminal contributions by Israelachvili (46) and Sackmann (99). Not surprisingly, the membrane lipid regulation of membrane protein function has been formulated with different descriptors: bilayer fluidity (103); bilayer compression, or bilayer-protein hydrophobic mismatch (79); intrinsic lipid curvature (38) or curvature frustration (60); bilayer deformation energy (44); acyl chain packing (32); bilayer free volume (75); lateral pressure profile (14); lipid packing stress (9); or bilayer stiffness (68). These different descriptors constitute different attempts to parameterize the landscape of lateral interactions among the membrane lipids and the imbedded proteins.

One reason for this plethora of paradigms is that the lipid composition of biological membranes is diverse (12, 34) and differs among membrane compartments (94). Erythrocyte membranes are estimated to have more than 200 lipid species that differ in head group and acyl chain composition (80), and the number of distinct lipid species in the cellular lipidome is likely to be in the thousands (117). As pointed out elsewhere in this volume (32a), the membrane lipids

have a nonrandom distribution, which in its own right has consequences for membrane protein function. Given this diversity and complexity, one might expect membrane proteins to be regulated by both specific lipid-protein interactions and general bilayer-protein interactions. The distinction between specific (chemical) lipid-protein interactions and more general (physical) bilayer-protein interactions is often unclear, however, as genetic studies have revealed a remarkable plasticity in membrane lipid composition (26). In fact, when lipid molecules can be discerned and identified in membrane protein structures, they usually are resolved only in part (59, 60)—even at cryogenic temperatures—suggesting limited chemical specificity, significant disorder/mobility, or both.

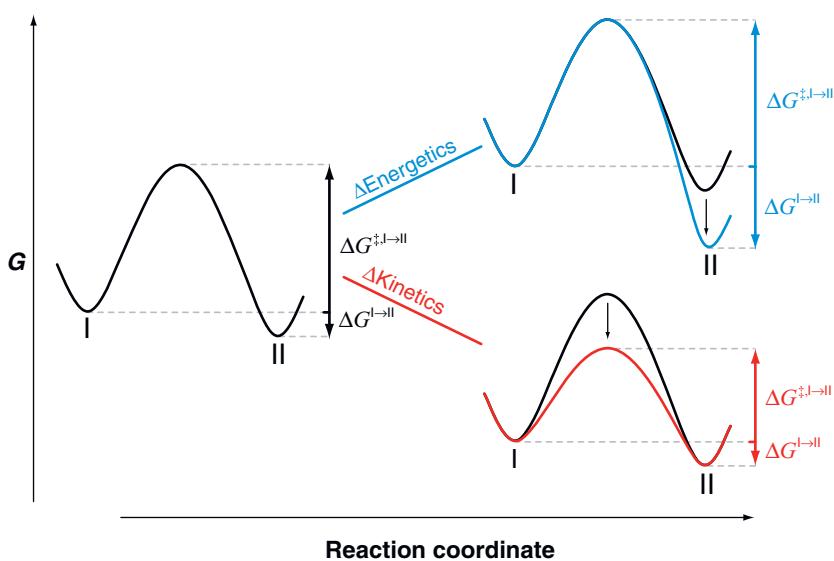
Whether the regulation is due to general or specific interactions, the membrane lipids regulate membrane protein function by altering the energetics and/or kinetics of the membrane protein conformational changes that underlie normal function (**Figure 1**). It is important that the lipid bilayer has a liquid-crystalline organization (72), which is necessary to allow for conformational changes that involve the protein-lipid bound-

ary. Yet, changes in lipid bilayer fluidity per se, which would alter only the kinetics of interconverting between different protein conformations, are unlikely to be important regulators of membrane protein function (58). Most importantly, changes in fluidity alone cannot alter the equilibrium distribution among different protein conformational states. The control of membrane protein function by the lipid bilayer becomes primarily a question of energetics—of changes in the equilibrium distribution among different conformations.

Moreover, the diversity of membrane lipids, and the limited chemical specificity of lipid-protein interactions, suggests that it often may be advantageous to dispense with the notion of specific lipid-protein interactions and adopt instead an energetic view of bilayer-protein interactions. In this view, the bilayer and the adjacent aqueous phases serve as a (highly structured) solvent for the bilayer-spanning proteins. In this approximation, it becomes useful to focus on the protein shape and the bilayer material properties. We consider the protein shape to be given by its radius ( $r_0$ ) or cross-sectional area (which may vary across the bilayer) and hydrophobic length ( $l$ );

$r_0$ : radius of bilayer-spanning protein

$l$ : hydrophobic length of a bilayer-spanning protein



**Figure 1**

Reaction coordinate diagrams for transitions between two protein conformation states (I and II). Changes in bilayer properties can alter the free energy between the states (top right), which alters both the equilibrium distribution as well as the kinetics of the transition, or the height of the transition state (lower right), which alters only the kinetics of the transition.

**$d_0$ :** average hydrophobic thickness of the unperturbed bilayer

**$c_0$ :** lipid intrinsic curvature

**$K_a, K_c$ :** bilayer compression and bending moduli

the bilayer material properties are given by the hydrophobic bilayer thickness ( $d_0$ ), the intrinsic lipid curvature ( $c_0$ ), and the elastic moduli for bilayer compression ( $K_a$ ) and bending ( $K_c$ ) (64, 73).

Support for such a physical view of the bilayer regulation of membrane protein function resides in the fact that it is often possible to reconstitute membrane proteins in lipid bilayers of defined composition, i.e., having only one or two components in addition to the protein. This suggests, in itself, that membrane protein function does not depend on specific lipid-protein interactions. One cannot exclude, however, that functionally important lipids bind so tightly that they should be considered structural cofactors. It also becomes possible to examine systematically whether a membrane protein's function varies as a function of the lipid bilayer thickness (Table 1) or the intrinsic lipid curvature (Table 2). Tables 1 and 2 show that membrane proteins are regulated by simple changes in bilayer properties, such as the bilayer (hydrophobic) thickness and intrinsic lipid curvature. Many proteins appear in Table 1 and Table 2,

which suggests a common underlying mechanism. Similar information would have been obtained if one had focused on proteins that are modulated by cholesterol or by reversibly adsorbing amphiphiles (64, 68, 122). We do not consider these membrane modifications here, because cholesterol and reversibly adsorbing amphiphiles tend to alter the bilayer elastic moduli (30, 70, 83, 124), in addition to their effects on bilayer thickness (84) and intrinsic curvature (19, 102). Also, we do not consider peripheral membrane proteins.

A remarkable feature of the bilayer regulation of many bilayer-spanning proteins is the biphasic changes in function with changes in a particular bilayer property, whether it be thickness, curvature (Tables 1 and 2), or cholesterol content (122). Figure 2 shows this pattern for ATP-driven pumps and other transporters. This biphasic behavior could arise for many reasons (60), but it represents a general feature of the host lipid bilayer regulation of membrane proteins that undergo conformational changes involving the protein/bilayer boundary. We return to this question at the end of this article.

**Table 1 Membrane proteins regulated by changes in lipid bilayer thickness**

Protein	Assay (pattern of regulation <sup>a</sup> )	Reference(s)
Sarcoplasmic reticulum $\text{Ca}^{2+}$ -ATPase	Enzyme activity (B), transport activity (B), $\text{Ca}^{2+}$ binding (B), enzyme kinetics (B)	13, 74, 107
$\text{Na}^+, \text{K}^+$ -ATPase	Enzyme activity (B)	49
Cytochrome <i>c</i> oxidase	Enzyme activity (B)	78
$\text{F}_1\text{-F}_0$ -ATP synthase	Oligomycin sensitivity (M)	78
<i>Pseudomonas aeruginosa</i> Leu- $\text{Na}^+$ cotransporter	Transport activity (B)	113
<i>Lactobacillus lactis</i> Leu- $\text{H}^+$ cotransporter	Transport activity (B)	45
<i>Escherichia coli</i> melibiose-cation cotransporter	Transport activity (B)	27
Erythrocyte glucose transporter	Transport activity (B)	15
Rhodopsin	MI/MII pseudoequilibrium (B)	11
<i>Escherichia coli</i> diacylglycerol kinase	Enzyme activity (B)	95
nAChR	Agonist binding (M)	18
MscL	Gating (M)	93
$\text{Ca}^{2+}$ -activated potassium channel	Single-channel conductance (B)	123

<sup>a</sup>M, monotonic; B, biphasic.

**Table 2** Bilayer-spanning proteins regulated by maneuvers that change lipid intrinsic curvature

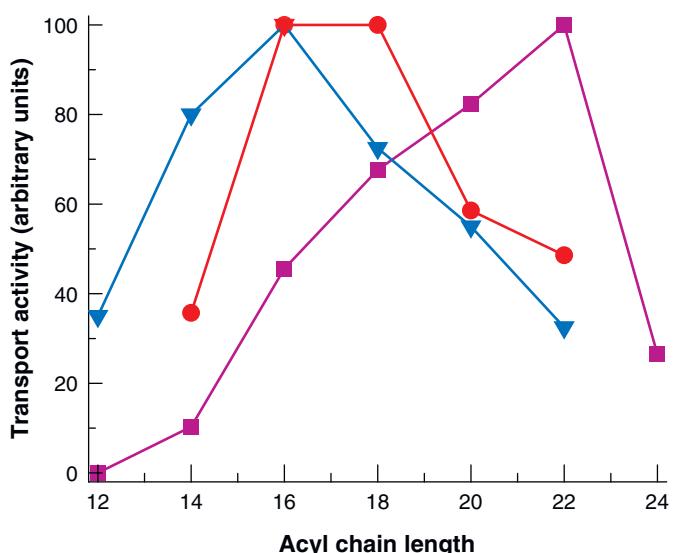
Protein	Assay (pattern of regulation <sup>a</sup> )	Reference(s)
Sarcoplasmic reticulum $\text{Ca}^{2+}$ -ATPase	Transport activity (M)	82
<i>Mycoplasma laidlawii</i> $\text{Mg}^{2+}$ -ATPase	Enzyme activity (M)	121
<i>Mycoplasma laidlawii</i> ubiquinol-cytochrome <i>c</i> reductase	Respiratory control (M)	121
<i>Escherichia coli</i> SecYEG	ATPase activity (M)	114
Cytochrome <i>c</i> oxidase	Enzyme activity (B)	71
<i>Escherichia coli</i> lactose- $\text{H}^+$ cotransporter	Transport activity (M), folding	17, 120
Adenine nucleotide transporter	Transport activity (B)	110
Rhodopsin	MI/MII pseudoequilibrium (M)	11
Bacteriorhodopsin	Folding (M)	1
OmpA	Folding (M)	43
Dolichyl-phosphomannose synthase	Enzyme activity (B)	47
MscL	Gating (M)	77
$\text{Ca}^{2+}$ -activated potassium channel	Gating (M), conductance	16, 90
VDAC	Gating (M)	98a

<sup>a</sup>M, monotonic; B, biphasic.

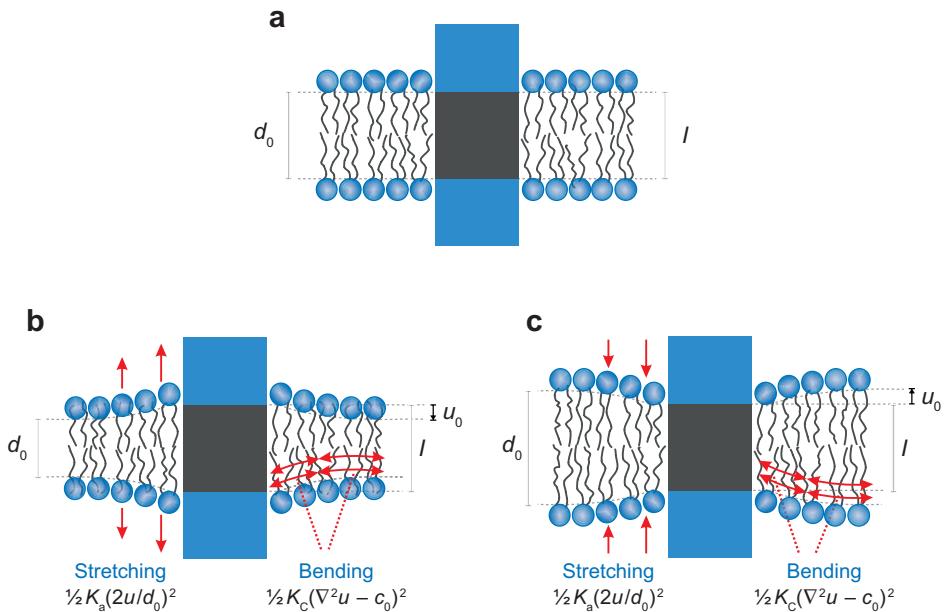
## HYDROPHOBIC MATCH AND MISMATCH

A central feature in models of lipid bilayer-integral membrane protein interactions, and bilayer regulation of membrane protein function, is the hydrophobic match between membrane proteins and their host lipid bilayer: The hydrophobic thickness ( $d$ ) of the host lipid bilayer in the immediate vicinity of a membrane-spanning protein should closely match the hydrophobic length ( $l$ ) of the protein's hydrophobic (bilayer-spanning) domain (**Figure 3**). This arrangement minimizes the energetic penalty associated with exposing a nonpolar/polar interface (50). For a given protein, if  $l$  differs from the average thickness of the unperturbed bilayer ( $d_0$ ), there is a bilayer-protein hydrophobic mismatch (32, 79). In response, the bilayer thickness in the vicinity of the protein may differ from the unperturbed bilayer thickness; this bilayer deformation may involve local compression or extension of the lipid acyl chains, bending of the bilayer/solution interface and splaying of the lipid acyl chains (**Figure 3**), and perhaps tilting of the acyl chain director relative to the bilayer normal. If  $l \neq d$  there is

not a hydrophobic match between protein and bilayer (7), but rather hydrophobic slippage (86), the energetically unfavorable exposure of hydrophobic amino acid residues to water

**Figure 2**

Transport activity as a function of lipid bilayer thickness: sarcoplasmic reticulum  $\text{Ca}^{2+}$ -ATPase (13) (purple); *Lactobacillus lactis*  $\text{Leu}-\text{H}^+$  cotransporter (45) (red); *Escherichia coli* melibiose-cation cotransporter (27) (blue).



**Figure 3**

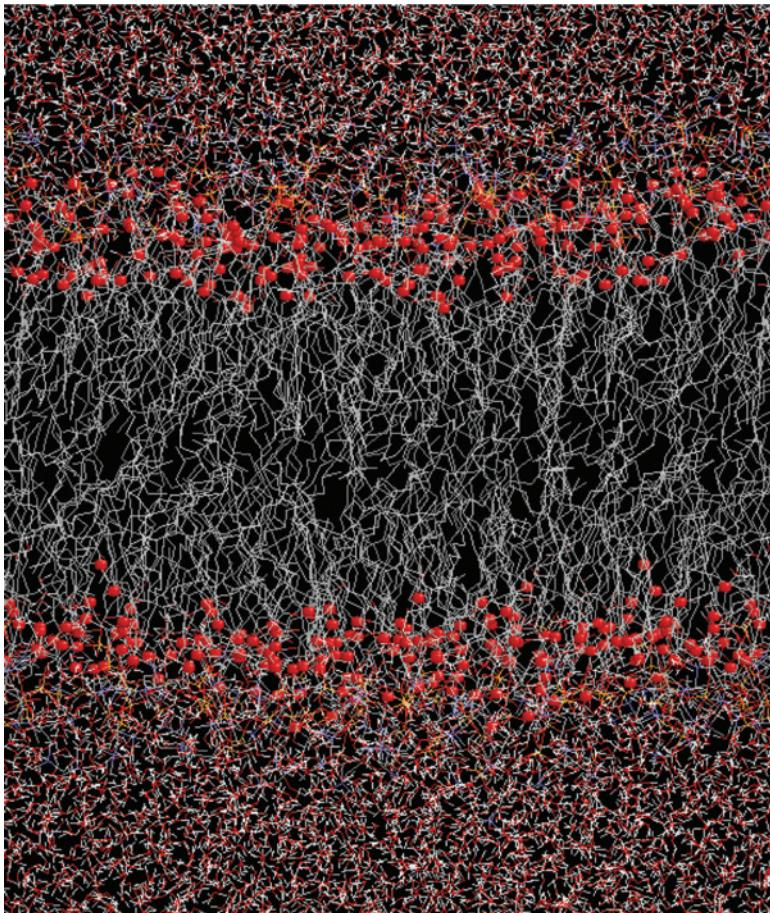
Bilayer-protein hydrophobic mismatch. Nonpolar residues are gray; polar residues are blue. (a) Perfect hydrophobic match: The protein hydrophobic length ( $l$ ) matches the thickness of the unperturbed bilayer ( $d_0$ ). (b, c) Hydrophobic mismatch: The protein's hydrophobic length is longer (b) or shorter (c) than the hydrophobic thickness of the unperturbed bilayer. The lipid bilayer hydrophobic core adjusts to the protein's hydrophobic exterior, which causes a local compression or stretching, with an energy density  $\frac{1}{2} \cdot K_a \cdot (2u/d_0)^2$  (cf. Reference 79), and bending of the bilayer/solution interface, with an energy density we approximate as  $\frac{1}{2} \cdot K_c \cdot (\nabla^2 u - c_0)^2$  (cf. Reference 38).

or of hydrophilic residues to the lipid bilayer hydrophobic core.

In principle, both the bilayer and the protein may deform in response to a hydrophobic mismatch,  $d_0 \neq l$ . In practice, membrane proteins are much less compressible than the bilayer. The volumetric compressibility moduli of globular proteins in water are  $10^{10}$ – $10^{11}$  N/m<sup>2</sup> (35)—one to two orders of magnitude larger than the volumetric compressibility moduli of liquid-crystalline phospholipid bilayers,  $\sim 10^9$  N/m<sup>2</sup> (62), and two to three orders of magnitude larger than the modulus for bilayer thickness compressibility,  $\sim 10^8$  N/m<sup>2</sup> (31). That is, lipid bilayers are 100- to 1000-fold softer than the imbedded proteins, meaning that hydrophobic matching implies that the bilayer adjusts to the protein. As noted below (see Bilayer Deforma-

tion Energy), though the bilayer is soft, the bilayer deformation nevertheless incurs a finite energetic cost, the bilayer deformation energy ( $\Delta G_{\text{def}}^0$ ), which in turn causes bilayer-spanning proteins to adjust their conformational preference in favor of conformations with smaller hydrophobic mismatches. (Individual  $\alpha$ -helices and protein domains are relatively rigid; the adjustment most likely involves small ratchet-like rotation and sliding movements of domains and helices relative to each other.) Thus, even when the hydrophobic mismatch between a bilayer-spanning protein and its host bilayer is small, the hydrophobic coupling between the protein and the bilayer may constrain the conformational landscape available to the protein—the bilayer serves as a splint to stabilize selected protein conformations.

$\Delta G_{\text{def}}^0$ : bilayer deformation energy



**Figure 4**

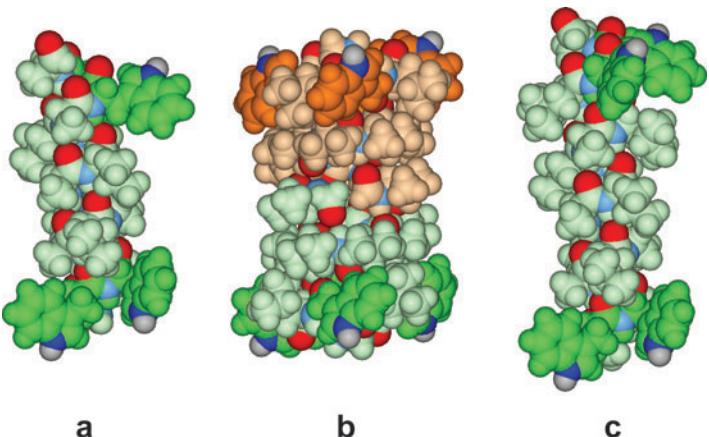
Lipid bilayers are dynamic structures. Snapshot from a molecular dynamics simulation of a DC<sub>16</sub>PC bilayer after a ~10-ns simulation at 330 K. The bilayer is composed of 96 DC<sub>16</sub>PC molecules and fully hydrated. The simulations were done with periodic boundary conditions using procedures described in Reference 2. Carbonyl oxygens, red spheres; acyl chains, gray lines. Blue, nitrogen; red, oxygen; white, hydrogen; yellow, phosphorus. Figure courtesy of Toby W. Allen.

Because lipid bilayers are soft, the bilayer/solution interface undergoes substantial thermal fluctuations (118), which involve the local movement of individual phospholipid molecules and more global bilayer undulations and peristaltic motions (61, 118) (**Figure 4**). The bilayer/solution interface thus is fuzzy. Nevertheless, the average bilayer thickness is a well-defined quantity, albeit with a somewhat gradual transition between the nonpolar bilayer core and the polar groups and water. Similarly, the thermal motion in bilayer-spanning proteins, in particular the side chain motions in the vicinity of the bilayer/solution interface, causes the transition between the protein's nonpolar and polar domains to be gradual. The hydrophobic mismatch between a bilayer-spanning protein and

the host bilayer thus is subject to uncertainty. Differences in hydrophobic mismatch, due to changes in phospholipid acyl chain length or the amino acid sequence of the bilayer-spanning protein domains, should be well-defined quantities.

## EVIDENCE FOR HYDROPHOBIC MATCH

What is the experimental evidence for bilayer (and protein) structural alterations in response to a hydrophobic mismatch? The best available evidence comes from defined models (**Figure 5**) such as  $\beta$ -helical gramicidin channels (6) and single-span  $\alpha$ -helical peptides like acetyl-GWW(LA)<sub>n</sub>LWVA-amide (WALP) (55, 56)



**Figure 5**

Model membrane proteins. Space-filling models of (a) WALP16, acetyl-GWW(LA)<sub>5</sub>WWA-amide, (b) gramicidin A, and (c) WALP19, acetyl-GWW(LA)<sub>6</sub>LWWA-amide. In the gramicidin dimer, the interfacial Trp residues are shown in dark green or dark orange (to highlight each subunit in the dimer), with the indole NH hydrogens colored gray, whereas the aliphatic side chains are shown in light green or light orange to identify the two subunits. In the WALPs, the indole nitrogens are dark blue within green rings, with the indole NH hydrogens colored gray. In all three models the peptide backbone is light blue, green, and red. Note how the peptide backbone is more exposed in the WALPs than in gramicidin A.

incorporated into lipid bilayers of different thicknesses.

### Lipid Adaptations

In the case of a large hydrophobic mismatch ( $>4$  Å difference) between short bilayer-spanning peptides and thick host bilayers, the ensuing bilayer deformation tends to destabilize the lipid bilayer. At peptide/lipid ratios larger than  $\sim 1:100$ , the bilayer structure may be severely disrupted in favor of nonbilayer phases: an isotropic or an inverted hexagonal ( $H_{II}$ ) phase. In phosphatidylcholine bilayers, these lipid-phase transitions can be caused by both gramicidins (53) and WALP-like peptides including acetyl-GKK(LA)<sub>n</sub>LKKA-amide (KALP) (24, 56), as long as the peptides have interfacial anchoring residues, e.g., Trp indole rings or cationic Lys/Arg side chains, at either end of the peptides. Peptides lacking polar/charged anchors tend to have only little influence on the lipid phase behavior (54, 57).

A hydrophobic mismatch between long peptides and thin bilayers formed by phos-

phatidylcholines, which have little tendency to form nonbilayer phases, usually preserves the lipid bilayer phase (22). If the bilayer is formed by lipids that have a greater propensity to form nonbilayer phases, such as *N*-methyl-DC<sub>18:1</sub>PE, both short and long WALP peptides promote the formation of inverse cubic and  $H_{II}$  phases (104), indicating that a peptide-bilayer hydrophobic mismatch and an intrinsic lipid curvature act synergistically.

In cases in which only marginal hydrophobic mismatch occurs, the lipid bilayer phase is preserved even at peptide/lipid ratios of  $\sim 1:10$ . WALP peptides have modest influence on lipid acyl chain order or bilayer thickness: The effects tend to be smaller in magnitude than would be predicted *a priori* from the extent of hydrophobic mismatch (23, 116). On a per mole basis, the lipid adaptations to gramicidin channels are greater than those to single-span  $\alpha$ -helices (cf. References 39 and 116). The reason for this difference remains poorly understood, but examination of the structures in **Figure 5** shows that the peptide backbone residues are more exposed in the WALPs than

in the gramicidins. As a result, the energetic penalty for slippage between the peptide and the bilayer may be less for WALPs than for the gramicidins. Indeed, X-ray scattering measurements show that gramicidin channels, at a peptide-to-lipid molar ratio of 1:10, increase the thickness of DC<sub>12</sub>PC bilayers by 1.3 Å but decrease the thickness of DC<sub>14</sub>PC bilayers by 2.6 Å (39), whereas WALP peptides of comparable lengths and molar ratios have little effect on the thickness of either DC<sub>12</sub>PC or DC<sub>14</sub>PC bilayers (116). Consistent with these results, gramicidin channels increase the order of lipid acyl chains to a greater extent than do lipid-incorporated WALP peptides (23). Compared with single-span  $\alpha$ -helices, gramicidin channels are more robust (more rigid), have larger diameters, and therefore might be expected to better mimic larger bilayer-spanning proteins.

### Model Protein Adaptations

In cases in which the lipid bilayer phase is preserved, does a hydrophobic mismatch modulate the folding or orientation of embedded protein domains? The linear gramicidins can fold as single-stranded  $\beta$ -helical subunits (cf. Reference 6), which form the standard conducting channels by a transbilayer dimerization (88), as well as various double-stranded conformations (cf. Reference 6). The single-stranded, bilayer-spanning gramicidin channel structure is maintained in lipid bilayers having acyl chain lengths between 10 and 18 carbons. The single-stranded channel fold is maintained even in micelles formed by the 12-carbon, single-chain detergent sodium dodecyl sulfate (SDS) (8, 111). Molecular dynamics-based analysis of the structures deduced from solution NMR spectra of gramicidin in SDS micelles (8, 111) and solid-state NMR spectra of gramicidin incorporated into oriented lipid bilayers (52) shows that the structures are equivalent (2). In shorter (micelle-forming) six- to eight-carbon diacyl phosphatidylcholines, gramicidin refolds into double-stranded structures (37). At the

other extreme, in DC<sub>22:1</sub>PC bilayers, double-stranded gramicidin conformations again become prominent (76). In either case, the hydrophobic mismatch can become so large that the resulting bilayer deformation (and associated deformation energy) causes a switch in gramicidin's conformational preference. The interfacial tryptophans are important determinants of the gramicidin fold, presumably because the Trp residues serve to anchor the subunits to the interface (88), as analogues with Trp → Phe replacements tend to favor double-stranded conformations (28, 100).

WALP peptides tend to remain  $\alpha$ -helical in phospholipid bilayers with 12- to 18-carbon acyl chains, with a modest (4° to 8°) tilt of the helix axis with respect to the bilayer normal (108, 109, 114a). Very long, 23-amino-acid WALPs or KALPs remain largely  $\alpha$ -helical but adapt to short lipid bilayers, with a single distinct kink halfway through the membrane-spanning helix at the bilayer center (20).

### Integral Membrane Proteins

Integral membrane proteins show behavior similar to the gramicidins and WALPs/KALPs. When the  $\beta$ -barrel protein OmpF was reconstituted in lipid vesicles formed by DC<sub>n:1</sub>PC ( $12 \leq n \leq 24$ ), the protein appeared to be structurally invariant for  $14 \leq n \leq 20$ , with the hydrophobic match achieved by the lipid bilayer adapting to the protein's hydrophobic exterior (87); for  $n > 20$ , the protein appeared to deform in response to the hydrophobic mismatch (89). Similar experiments with the tetrameric  $\alpha$ -helical KcsA channel (119) and the pentameric  $\alpha$ -helical MscL (96) show that hydrophobic matching prevails, but that the protein organization varies as a function of the hydrophobic mismatch. It is not clear whether the changes in protein structure reflect a gradual change in the tilt of the  $\alpha$ -helices relative to each other, or a shift in the distribution between a few protein

conformations in which the subunits may slide and rotate relative to each other (63, 92).

Except for the extreme case of gramicidin channel refolding, the general picture is that hydrophobic matching is operative and that lipids, model proteins, and integral membrane proteins adapt to hydrophobic mismatch by rather small adjustments, which include a local bilayer deformation as well as shifts in the distribution between different protein conformations—with the major changes occurring in the lipid organization.  $\beta$ -barrel/ $\beta$ -helical proteins appear to be more rigid, imposing a greater restraint on the lipid packing;  $\alpha$ -helical proteins appear to be softer. Whether the changes in protein structure reflect gradual changes in the tilt of individual  $\alpha$ -helices relative to each other or shifts in the distribution among a few discrete conformations, the molecular responses to mismatch may have great functional significance while being modest in scope and difficult to detect.

## BILAYER DEFORMATION ENERGY

Although the bilayer is soft, the bilayer perturbation associated with a bilayer-protein hydrophobic mismatch incurs an energetic cost, the bilayer deformation energy ( $\Delta G_{\text{def}}^0$ ), which varies as a function of the hydrophobic mismatch ( $d_0 - l$ ) and the intrinsic lipid curvature ( $c_0$ ).

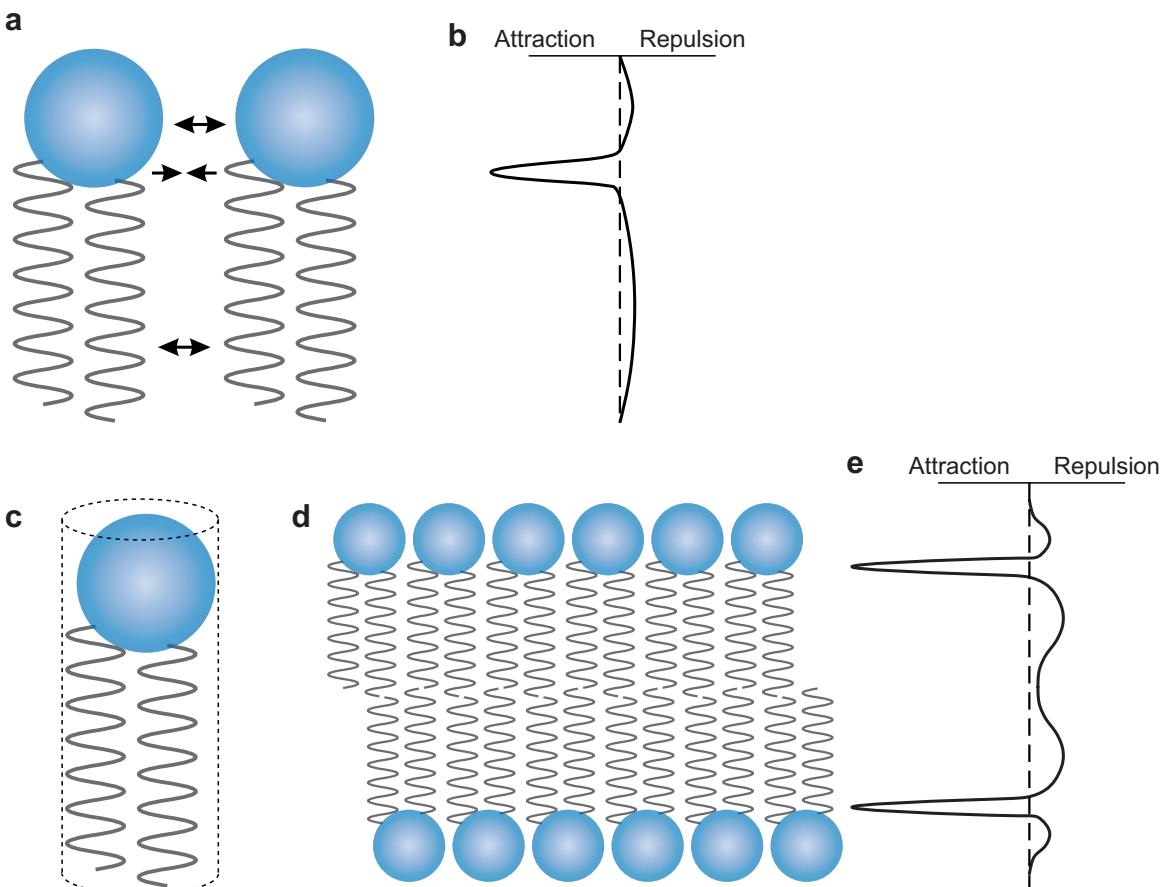
### Intrinsic Lipid Curvature

The intrinsic curvature of the membrane lipids denotes the tendency of lipids to form nonbilayer phases, i.e., the tendency for isolated lipid monolayers to form nonplanar, curved surfaces. The intrinsic curvature of a lipid monolayer is determined by the variation of intermolecular lateral interactions along the molecular axis (41, 102), which often is expressed in terms of the effective shape of the lipids in the monolayer (19, 46). There

are strong attractive interactions at the nonpolar/polar interface, to minimize the exposure of the hydrophobic acyl chains to water, and there are repulsive interactions between the polar head groups and between the acyl chains (Figure 6). The integral of the force profile along the lipids (across the monolayer) is zero. If there is no net torque across the monolayer, the lipids can be approximated as having a cylindrical shape (102). They form flat monolayers, and two monolayers come together to form a relaxed bilayer (Figure 6). If there is a torque across the monolayer, e.g., if the effective cross-sectional area of the polar head group region is larger than that of the acyl chains, the monolayer has a positive equilibrium curvature (Figure 7c), meaning that the monolayer is convex when viewed from the aqueous solution, and the intrinsic lipid curvature,  $c_0$ , is positive. If the effective cross-sectional area of the polar head groups is less than that of the acyl chains, the monolayer has a negative curvature (Figure 7d), meaning that the monolayer is concave when viewed from the aqueous solution, and the intrinsic lipid curvature,  $c_0$ , is negative.

In either case, whether  $c_0$  is positive, zero, or negative, two monolayer leaflets can form a bilayer if they have complementary curvatures (Figure 7e). The formation of a (planar) bilayer by lipids that by themselves would tend to form curved monolayers therefore changes the effective shape of the lipid molecules because of the requirement for a uniform cross-sectional area/molecule across a planar bilayer. The energy required to change the lipid shape causes a stress in the bilayer, with an energy density of  $(K_c/2) \cdot c_0^2$ , where  $K_c \approx 20-25 \text{ } k_B T$  for liquid-crystalline phospholipid bilayers that have saturated or monounsaturated acyl chains (98).  $c_0$  is likely to vary between  $-0.115 \text{ nm}^{-1}$  (for DC<sub>18:1</sub>PC) and  $-0.34 \text{ nm}^{-1}$  (for DC<sub>18:1</sub>PE) (97), meaning that the curvature frustration energy will vary between  $\sim 0.2$  and  $\sim 2 \text{ } k_B T \cdot \text{nm}^{-2}$ . For comparison, the area per lipid molecule is  $\sim 0.7 \text{ nm}^2$  (81).

The coupling between the two monolayers (or leaflets) in a bilayer, which forces them

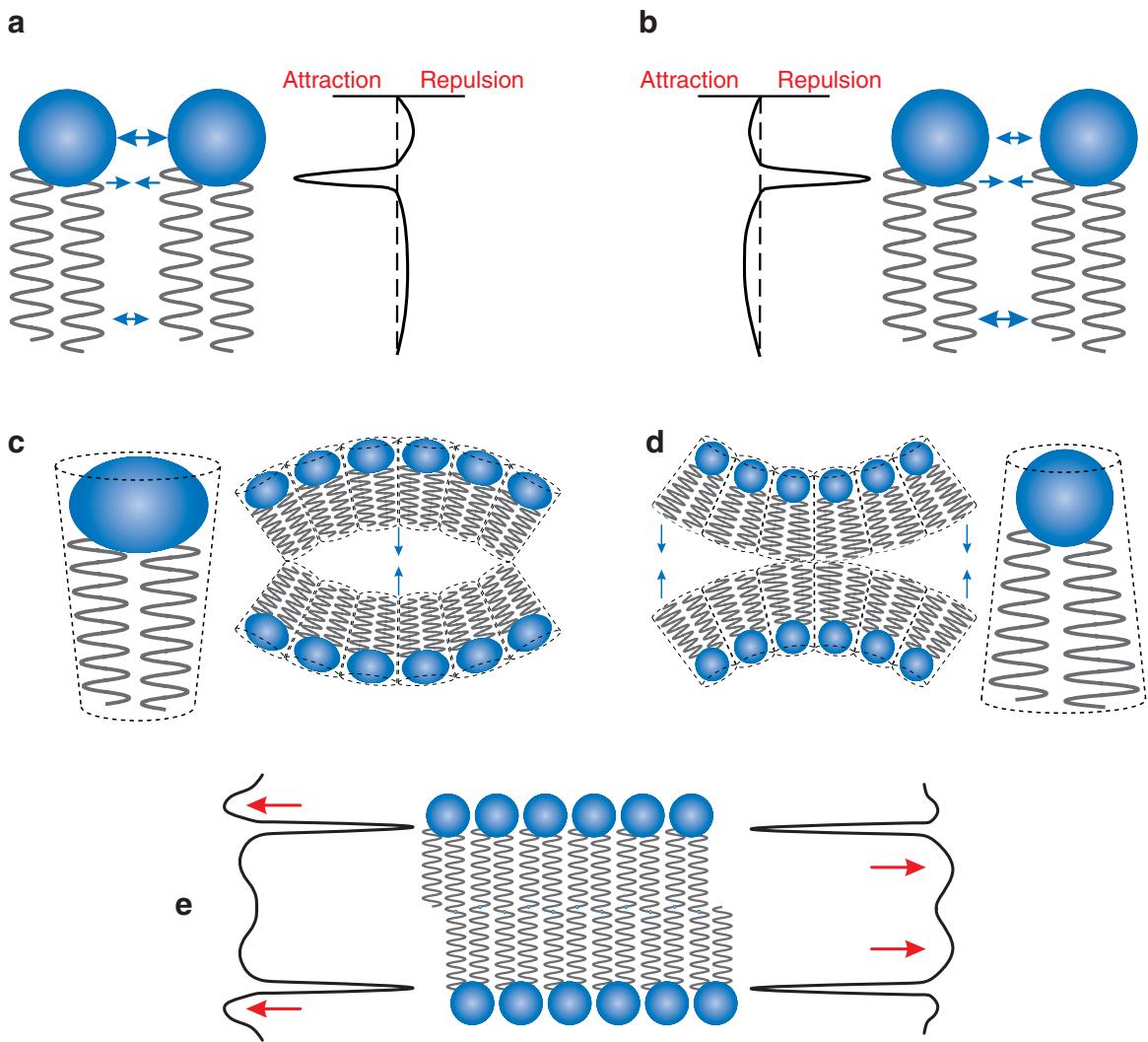


**Figure 6**

Lipid shape and bilayer curvature stress I. Formation of a relaxed bilayer, with no curvature stress, from lipid molecules that in isolation have a cylindrical shape. (a) The major contributions to the intermolecular force between adjacent molecules, with attractive interactions at the polar/nonpolar interface and repulsive interactions between the polar head groups and between the nonpolar acyl chains. (b) The profile of intermolecular interactions along the molecule length. (c) When the positive and negative forces balance, when there is no net torque across the monolayer, the lipid shape can be approximated as being cylindrical. (d) The isolated monolayer has zero curvature, and two monolayers form a relaxed bilayer. (e) The lateral-stress profile of the bilayer is indicated to the right of the bilayer.

to have complementary curvatures, means that it is necessary to distinguish among the intrinsic curvature of each monolayer, which is determined by the average lipid shape (cf. **Figure 7**); the curvature of the bilayer, which is determined by the coupling between the two leaflets; and the local monolayer curvature in the vicinity of a membrane protein, which is determined by protein–lipid bi-

layer interactions. The length scale for the decay of the local monolayer perturbations is  $\sim 1$  nm (48, 87). At first approximation, therefore, the bilayer can be considered to be a flat sheet. When the intrinsic lipid curvature differs from the bilayer curvature, however, the bilayer is under a curvature-induced stress, which modulates membrane protein function.



**Figure 7**

Lipid shape and bilayer curvature stress II. Formation of bilayers under curvature stress from lipid molecules that in isolation have a cone shape, as indicated by the profile of intermolecular interactions along the molecule length. Compared to Figure 6, panel *a* depicts increased repulsion between the lipid head groups, whereas panel *b* depicts increased repulsion between the acyl chains. Isolated monolayers formed by either type of molecule have nonplanar geometry, having a positive curvature (*c*) or a negative curvature (*d*). As a result, the two monolayers form a frustrated bilayer because the individual molecules are forced into an approximately cylindrical shape (*e*). The curvature-induced changes in the lateral-stress profile, relative to the relaxed bilayer (Figure 6), are denoted by red arrows.

### Estimating the Bilayer Deformation Energy

$\Delta G_{\text{def}}^0$  can be estimated using the theory of elastic bilayer deformations (44). Consider a

protein of hydrophobic length  $l$  imbedded in a bilayer of average thickness  $d_0$  (with  $l \neq d_0$ ), intrinsic monolayer curvature  $c_0$ , and bilayer compression and bending moduli  $K_a$  and  $K_c$ .

The local bilayer compression has an associated energy density that can be approximated as  $K_a \cdot (2u/d_0)^2$  (cf. Reference 79). The monolayer bending has an associated energy density that can be approximated as  $K_c \cdot (\nabla^2 u - c_0)^2$  (cf. Reference 38). [ $u = (d_0 - d)/2$  with  $d$  being the local bilayer thickness (cf. Figure 3).] Combining these contributions, one can estimate  $\Delta G_{\text{def}}^0$  for a cylindrical protein of radius  $r_0$  (21, 40, 44, 85, 87):

$$\begin{aligned} \Delta G_{\text{def}}^0 = & \int_{r_0}^{\infty} \left\{ K_a \cdot (2u/d_0)^2 \right. \\ & + K_c \cdot (\nabla^2 u - c_0)^2 \left. \right\} \cdot \pi \cdot r \cdot dr \\ & - \int_{r_0}^{\infty} K_c \cdot c_0^2 \cdot \pi \cdot r \cdot dr. \end{aligned} \quad 1.$$

Equation 1 can be expressed as a biquadratic form in  $(d - l)$  and  $c_0$  (67, 85, 87):

$$\begin{aligned} \Delta G_{\text{def}}^0 = & H_B \cdot (d_0 - l)^2 \\ & + H_X \cdot (d_0 - l) \cdot c_0 + H_C \cdot c_0^2, \end{aligned} \quad 2.$$

where the coefficients  $H_B$ ,  $H_X$ , and  $H_C$  are functions of  $K_a$ ,  $K_c$ ,  $d_0$ , and  $r_0$  (85, 87). Integral membrane proteins are not cylinders with smooth boundaries (60), which affects the local lipid packing and the tilt of the acyl chain director (the vector describing the time-averaged orientation of the acyl chains in a phospholipid) relative to the local bilayer normal (87) and thus the predicted value of  $\Delta G_{\text{def}}^0$  (85, 87). Moreover, the elastic moduli of the shell of lipids adjacent to the protein, the so-called annular lipids (60), may differ from the bulk values (91), which similarly affect the predicted  $\Delta G_{\text{def}}^0$ . Yet, the biquadratic organization of Equation 2 should remain correct to the first significant order (see below).

## Testing the Theory of Elastic Bilayer Deformations

The theory of elastic bilayer deformations, as expressed in Equations 1 and 2, provides quantitative insight into the bilayer-thickness dependence of gramicidin channel lifetimes (36, 44, 65). Gramicidin channels form by the transmembrane dimerization of two non-

conducting  $\beta^{6,3}$ -helical subunits (88); channel formation is visible as rectangular current transitions (Figure 8a).

The channels' hydrophobic length is less than the bilayer's hydrophobic thickness, meaning that channel formation causes a bilayer deformation with an associated  $\Delta G_{\text{def}}^0$ . In response, the bilayer pulls on the bilayer-spanning channel with the disjoining force,

$$\begin{aligned} F_{\text{dis}} = & - \left( \frac{\partial \Delta G_{\text{def}}^0}{\partial (d_0 - l)} \right) \\ = & 2H_B \cdot (d_0 - l) + H_X \cdot c_0. \end{aligned} \quad 3.$$

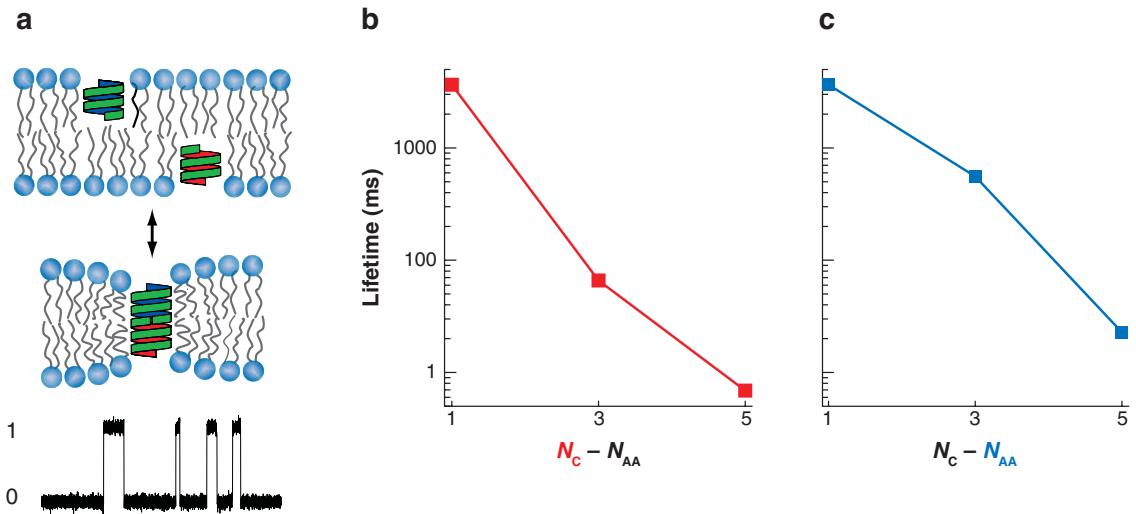
$F_{\text{dis}}$  varies as a function of the bilayer-channel hydrophobic mismatch, which means that the channel lifetime (the inverse of the dimer dissociation rate constant) varies as a function of the hydrophobic mismatch. The changes in channel lifetimes are comparable, whether the channel length (the number of residues in the sequence,  $N_{\text{AA}}$ ) or the bilayer thickness (the number of  $\text{CH}_2$  units in the acyl chains,  $N_C$ ) is varied. (Perfect agreement is not expected because the subunit-subunit interface, and therefore the intrinsic channel dynamics, varies with changes in channel length.) Thus, the gramicidin channels are suitable for use as molecular force transducers to monitor bilayer material properties (and protein-lipid bilayer interactions) (also see References 5 and 64).

The theory of elastic bilayer deformations has been tested by examining how the single-channel lifetimes vary as a function of lipid bilayer thickness (29, 44, 65) or bilayer tension (36). It is possible to account quantitatively for lifetime changes using independently measured elastic moduli—but only by assuming that the energetic penalty for tilting the acyl chain director relative to the bilayer normal is high (44, 65), which constrains the lipid packing and thereby increases the value of  $\Delta G_{\text{def}}^0$  (and thus the magnitude of  $F_{\text{dis}}$ ). A similar conclusion was reached by examining how the channel lifetimes (and appearance rates) vary as a function of the bilayer tension (36). It is not clear, however, if the conclusion about lipid tilt reflects the uncertainties

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**$H_B$ ,  $H_X$ , and  $H_C$ :** phenomenological spring coefficients describing the energetics of a protein-induced bilayer deformation

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**Figure 8**

Effect of a bilayer-channel hydrophobic mismatch on gramicidin channel lifetimes. (a) Gramicidin channels form by means of the transmembrane dimerization of nonconducting subunits, which is observable as discrete current transition between two levels: 0, in which there is no conducting channel, and 1, in which there is one conducting channel. Because channel formation causes a local bilayer thinning, the bilayer responds by imposing a disjoining force on the channel. The magnitude of this force varies as a function of the channel-bilayer hydrophobic mismatch, which means that the average channel lifetimes vary as a function of the hydrophobic mismatch. (b, c) Gramicidin channel lifetimes vary as a function of the hydrophobic mismatch, characterized by  $N_C - N_{AA}$ , where  $N_C$  denotes the number of carbon atoms in the acyl chains of the bilayer-forming monounsaturated phosphatidylcholines and  $N_{AA}$  denotes the number of amino acids in the sequence. In panels b and c, the parameter that is changed is in red and blue, respectively. Modified after Reference 5.

associated with using a continuum description at the molecular level (also see Reference 91). [An alternative description of elastic bilayer deformations, based on lipid acyl chain splay and tilt, can be found in Reference 57a. In its present implementation the predicted bilayer deformation energies are too low to be compatible with experimental results (cf. References 57a, 65, and 87).]

In other tests, which examine more general features of the model, the relative distribution of the different conductance levels in alamethicin channels varies as an approximately linear function of  $c_0$  (and, contrary to what might have been expected, not  $c_0^2$ , see also Equation 4) (51), and the gramicidin channel lifetimes vary as a function of  $c_0$  (69). The theory of elastic bilayer deformations provides a basis for understanding the energetics of bilayer-protein interactions.

### Energetics of a Hydrophobic Mismatch

To calculate the energetic cost of a change in hydrophobic mismatch, consider the transfer of a bilayer-spanning protein of hydrophobic length  $l$  from a bilayer with a hydrophobic thickness  $d_1$  to a bilayer with a hydrophobic thickness  $d_2$  ( $\neq d_1$ ) (cf. Reference 66). Using Equation 2, and assuming that  $H_B$ ,  $H_X$ , and  $H_C$  vary little between the two bilayers, the free energy of transfer ( $\Delta\Delta G_{\text{def}}^{d_1 \rightarrow d_2}$ ) becomes

$$\begin{aligned}
 \Delta\Delta G_{\text{def}}^{d_1 \rightarrow d_2} &= H_B \cdot (d_2 - l)^2 + H_X \cdot (d_2 - l) \\
 &\quad \cdot c_0 + H_C \cdot c_0^2 - (H_B \cdot (d_1 - l)^2 \\
 &\quad + H_X \cdot (d_1 - l) \cdot c_0 + H_C \cdot c_0^2) \\
 &= H_B \cdot (d_2 - d_1) \cdot (d_2 + d_1 - 2 \cdot l) \\
 &\quad + H_X \cdot (d_2 - d_1) \cdot c_0. \quad 4.
 \end{aligned}$$

The term reflecting the bilayer curvature frustration energy,  $H_C \cdot c_0^2$ , does not enter into the expression for  $\Delta\Delta G_{\text{def}}^{d_1 \rightarrow d_2}$ , qualitatively consistent with experimental results (51). Rather, it is the product of  $(d_0 - l)$  and  $c_0$  (with  $H_X$ ) that confers the  $c_0$  sensitivity. The same reasoning applies to membrane protein conformational changes (see below).

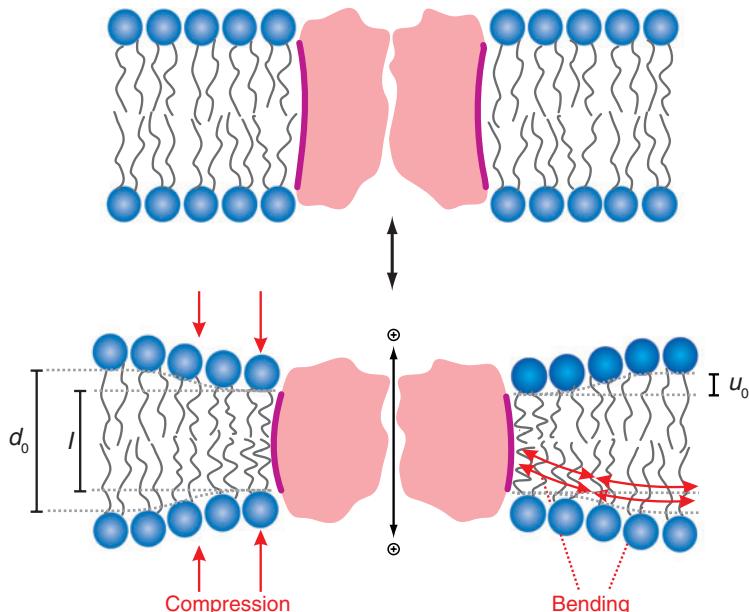
## INTEGRAL MEMBRANE PROTEINS

Integral membrane proteins are not smooth cylinders (**Figure 9**). Nevertheless, the bilayer deformation associated with a bilayer-protein hydrophobic mismatch involves a local bilayer compression/extension and monolayer bending, and the deformation energy should vary as a function of the hydrophobic mismatch and intrinsic lipid curvature.

It therefore should be possible to express  $\Delta G_{\text{def}}^0$  as a function of  $(d_0 - l)$  and  $c_0$ . Using a Taylor expansion in  $(d_0 - l)$  and  $c_0$ ,  $\Delta G_{\text{def}}^0$  becomes

$$\begin{aligned} \Delta G_{\text{def}}^0(d_0 - l, c_0) &= \Delta G_{\text{def}}^0(0, 0) + \frac{\partial (\Delta G_{\text{def}}^0)}{\partial (d_0 - l)} \\ &\quad \cdot (d_0 - l) + \frac{\partial (\Delta G_{\text{def}}^0)}{\partial c_0} \cdot c_0 + \frac{1}{2} \frac{\partial^2 (\Delta G_{\text{def}}^0)}{\partial (d_0 - l)^2} \\ &\quad \cdot (d_0 - l)^2 + \frac{\partial^2 (\Delta G_{\text{def}}^0)}{\partial (d_0 - l) \partial c_0} \cdot (d_0 - l) \cdot c_0 \\ &\quad + \frac{1}{2} \cdot \frac{\partial^2 (\Delta G_{\text{def}}^0)}{\partial c_0^2} \cdot c_0^2 + \dots, \end{aligned} \quad 5.$$

where the first-order terms are zero [because the  $\Delta G_{\text{def}}^0$  for small decreases in  $(d_0 - l)$  should be equal to that for small increases, with a similar argument holding for  $c_0$ ]. The biquadratic form for  $\Delta G_{\text{def}}^0$ , Equation 2, and thus Equations 3 and 4 should be valid



**Figure 9**

Hydrophobic coupling between membrane protein conformational changes and lipid bilayer deformations/perturbations. Protein conformational changes that involve the hydrophobic protein/bilayer boundary (heavy purple lines) cause a local bilayer deformation, which can be described in terms of the compression and bending of the two bilayer leaflets. The bilayer itself is flat; the two bilayer leaflets bend.

generally with the following assignment:

$$H_B = \frac{1}{2} \cdot \frac{\partial^2 (\Delta G_{\text{def}}^0)}{\partial (d_0 - l)^2}$$

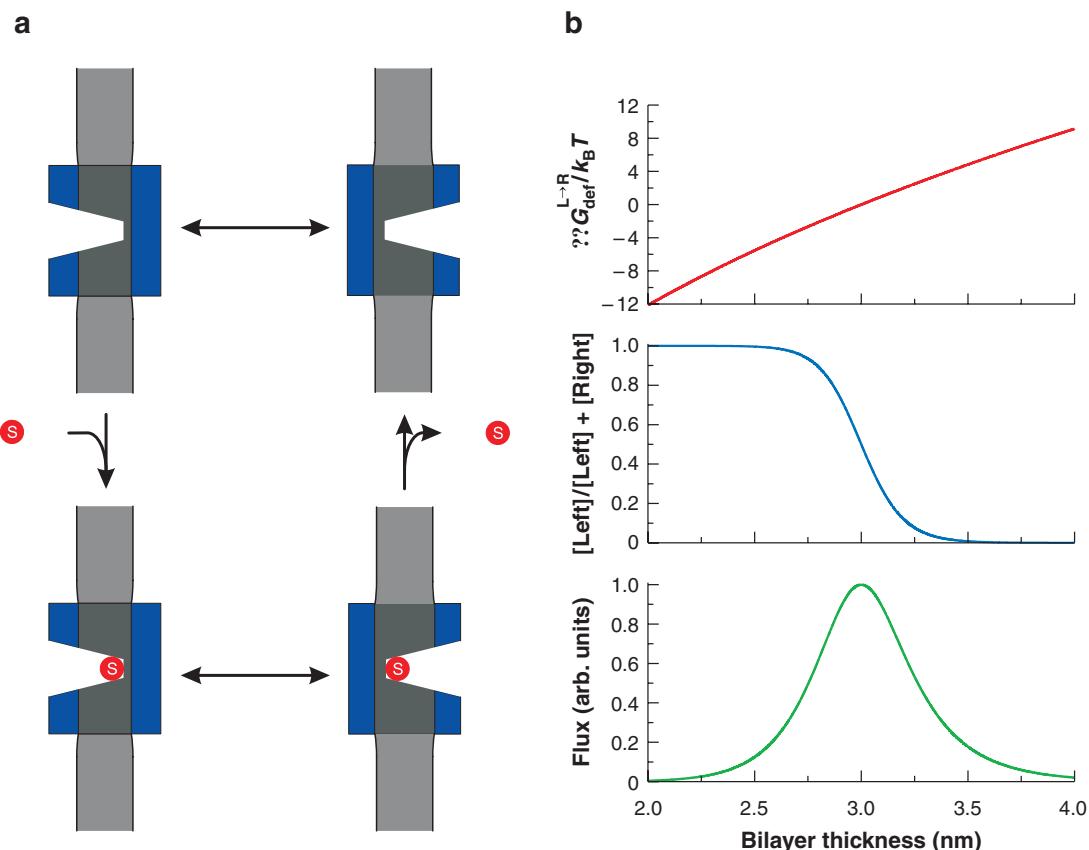
$$H_X = \frac{\partial^2 (\Delta G_{\text{def}}^0)}{\partial (d_0 - l) \partial c_0}$$

$$H_C = \frac{1}{2} \cdot \frac{\partial^2 (\Delta G_{\text{def}}^0)}{\partial c_0^2}$$

Equation 1 is needed if one wishes to understand (semi)quantitatively how membrane function is regulated by the host bilayer,

e.g., using the scaling relation developed by Nielsen and colleagues (85, 87).

To illustrate this approach, we consider the biphasic changes in solute transport by ATP-driven pumps and other conformational transporters as a function of bilayer thickness (**Figure 10**). In conformational transporters (or carriers), a solute binds to a recognition site on the membrane-bound carrier molecule. The solute-transporter complex then undergoes a conformational change,



**Figure 10**

Bilayer regulation of solute transporter function. Conformational transporter function involves protein conformational changes that may couple transporter function to the bilayer material properties. (a) Kinetic scheme for transporter-mediated solute movement; the shift in accessibility of the solute binding is associated with a slight change in bilayer-protein hydrophobic mismatch. (b) Graphs illustrating the predicted changes in the  $\Delta G_{\text{def}}^0$  contribution to the free-energy difference between the two protein conformations (top), the equilibrium distribution between the two conformations (middle), and the solute flux (bottom). The calculations were done (4) using the standard parameters used by Nielsen & Andersen (85), in which a protein ( $r_0 = 3 \text{ nm}$ ,  $l_1 = 2.85 \text{ nm}$ ,  $l_2 = 2.85 \text{ nm}$ ) is imbedded in a C<sub>18</sub>C<sub>18:1</sub>PC bilayer ( $d_0 = 3 \text{ nm}$ ).

such that the solute (and the solute binding site) has access to the other aqueous solution. The solute is released to that solution, and the empty transporter undergoes another conformational change, such that the binding site again becomes accessible from the first solution, ready for a new cycle. These conformational changes involve the protein/bilayer interface (112), meaning that  $\Delta G_{\text{def}}^0$  may be different for transporters having their binding site exposed to the left and to the right solution.

Approximating the conformational change associated with the shift in binding site accessibility as a change in the protein's hydrophobic length, the bilayer contribution to the free-energy difference between two protein conformations of lengths  $l_1$  and  $l_2$  is given by (cf. Equation 4)

$$\Delta \Delta G_{\text{def}}^{l_1 \rightarrow l_2} = H_B \cdot (l_2 - l_1) \cdot (l_2 + l_1 - 2 \cdot d_0) - H_X \cdot (l_2 - l_1) \cdot c_0. \quad 7.$$

$H_B$  and  $H_X$  can be evaluated in a manner similar to that used in Reference 85, and Equation 7 then provides estimates for  $\Delta \Delta G_{\text{def}}^{l_1 \rightarrow l_2}$  and the equilibrium distribution between the two conformations as a function of  $d_0$  (**Figure 10b**, top and middle). Using a standard conformational transporter model (3), the flux- $d_0$  relation can be calculated (**Figure 10b**, bottom). Though the assumed change in the protein's hydrophobic length is modest (0.03 nm), the flux is a biphasic function of bilayer thickness (cf. **Figure 2**). In the calculations used to generate **Figure 10**, the flux is maximal when the  $d_0$  is equal to the protein's average hydrophobic length  $(l_1 + l_2)/2$ , when the kinetic consequences of the protein-bilayer hydrophobic mismatch are minimal. The biphasic relation between transporter function and bilayer thickness arises as a gen-

eral consequence of the hydrophobic coupling between membrane protein function and bilayer material properties.

## CONCLUSIONS

The theory of elastic bilayer deformations provides a robust framework for understanding the bilayer regulation of membrane protein function. The key element is that not only is there hydrophobic matching between a bilayer-spanning protein and the host bilayer, but there is hydrophobic coupling of protein function to the bilayer material properties (64). The lipid bilayer thus becomes an allosteric regulator of membrane function. A key element in the continuum model of elastic bilayer deformations, as represented in Equations 2–4, is that it is possible to develop quantitative estimates for the protein-induced bilayer deformation energy, which in turn allows for predictions regarding how a membrane's lipid bilayer component will regulate the function of bilayer-spanning proteins. Even when applied to proteins with complex geometries, Equations 2–4, although approximate, should apply generally and allow for the prediction of complex changes in membrane protein function.

Future challenges will be to develop a continuum model of elastic bilayer deformation that includes bilayer compression, monolayer bending, and acyl chain tilt; to go beyond the continuum models and develop semimicroscopic descriptions that still allow for fairly straightforward estimates of the bilayer deformation energy; to understand better the energetic consequences of the local lipid packing around integral membrane proteins; and to incorporate more specific lipid-protein interactions.

### SUMMARY POINTS

- When membrane proteins undergo conformational changes, they tend to perturb the surrounding bilayer. The associated bilayer deformation energy contributes to the overall free-energy difference between different protein conformations.

2. The bilayer deformation energy varies as a function of bilayer thickness and intrinsic lipid curvature; optimal protein function therefore occurs when the lipid bilayer and the protein are well adapted to each other.
3. The lipid bilayer, with its associated material properties (thickness, intrinsic lipid curvature, and the elastic compression and bending moduli), thus serves as an allosteric regulator of membrane protein function.

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## Errata

An online log of corrections to *Annual Review of Biophysics and Biomolecular Structure* chapters (if any, 1997 to the present) may be found at  
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