CHAPTER 5

Implicit Modeling of Membranes

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I. INTRODUCTION

Biological lipid membranes are central to many biological processes. They form selectively permeable barriers, allowing cells to control their contents and create concentration gradients. However, in contrast to the totally passive view espoused by standard undergraduate texts, biological membranes also actively modify cell behavior by altering the function of membrane proteins, modulating the stability of protein–protein associations, and altering the binding and distribution of small molecules including salts and osmolytes (Jensen and Mouritsen, 2004; Brown, 1994; White and Wimley, 1999; Epand, 1998, 2003; Mouritsen and Bloom, 1993; Nyholm *et al.*, 2007).

Membrane composition varies significantly in different tissues within a given organism, emphasizing that the distribution of specific lipid species is not a matter of simple abundance. For example, rod outer segment disk membrane, found in the mammalian visual system, contain roughly 50% polyunsaturated ω-3 fatty acids; this is an enormous enrichment considering their natural abundance is more like 5% (Boesze-Battaglia and Albert, 1989; Boesze-Battaglia *et al.*, 1989) Since humans are unable to synthesize ω-3s, this implies the body must be specifically trafficking them to the disk membranes. Polyunsaturated lipids have been shown

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to significantly enhance the function of rhodopsin (which constitutes more than 90% of the protein in the disk membranes) (Mitchell *et al.*, 2001; Niu *et al.*, 2001; Grossfield *et al.*, 2006), clearly demonstrating that the cell is manipulating membrane content in order to optimize function. The story gets even more interesting when the concentration of cholesterol, which inhibits rhodopsin function, is considered: the concentration is high (25–30 mol%) in immature disks found near the bottom of the rod outer segment stack, and is greatly reduced (5 mol%) in mature disks (Mitchell *et al.*, 2001; Niu *et al.*, 2001, 2002; Pitman *et al.*, 2005).

The last 20 years have seen a significant increase in attempts to model membranes, and in particular to model their interactions with proteins and other permeants. Conceptually, the simplest approach is to perform all-atom molecular dynamics simulations; to our knowledge, the first example of a protein modeled in an explicit lipid membrane was gramicidin in a DMPC bilayer, conducted by Woolf and Roux (1994). Recent improvements in computer speed have significantly changed the landscape of this field; where simulations were once run for 100s of picoseconds, present technology allows us to run explicit membrane systems for hundreds of nanoseconds (Grossfield *et al.*, 2006) or even microseconds (Martínez-Mayorga *et al.*, 2006; Grossfield *et al.*, 2007). However, in order to reach these timescales, the calculations require extremely powerful supercomputers (Allen *et al.*, 2001), and even then, running long enough to generate statistical convergence is difficult (Grossfield *et al.*, 2007; Faráldo-Gomez *et al.*, 2004).

As a result, many interesting calculations can be expected to remain out of reach of all-atom molecular dynamics for the foreseeable future. These include simulations of membrane protein folding and insertion, dimerization (or oligomerization) of integral membrane proteins, and membrane poration by antimicrobial peptides. In each case, the time- and length-scales involved would require prohibitively long simulations. In principle, one could trade temporal information for improved convergence by using enhanced sampling tools such as replica exchange dynamics (Okamoto, 2004). However, while some such calculations have been reported for membranes (Nymeyer *et al.*, 2005), this technique is difficult to apply to bilayers because of the higher temperatures tend to disrupt bilayer structure.

One approach which has garnered significant interest in recent years is the use of coarse-grained molecular models, where the number of atoms per molecular is strategically reduced and the interaction potential simplified, dramatically diminishing the computational cost of the calculations and concomitantly increasing the feasible simulation size and time. However, these methods will be discussed extensively elsewhere in this volume, and so we will not explore them here.

An alternative approach is to combine a continuum representation of the membrane with a atomic representations of the rest of the system. This scenario allows us to focus our computational effort on the portion of we are most interested in, for example the membrane protein whose folding we wish to explore. Rep-

resenting the membrane implicitly has a number of significant advantages, the most important of which is computational efficiency: as a rule, implicit models are dramatically less expensive per energy evaluation than the equivalent all-atom systems. Moreover, these models generally produce an approximation to the solvation free energy of the system, as opposed to simply the potential energy as computed in an all-atom system. As a result, no additional sampling of environmental degrees of freedom is required for a fixed solute structure. This savings is significant when one considers the nanosecond to microsecond relaxation and reorganization times of explicit lipid membranes. Finally, the absence of explicit "solvent" molecules simplifies conformational searching and enhances the power of sampling techniques like replica exchange.

The primary tradeoff in using implicit membrane models is their presumed lack of high-resolution accuracy; almost by definition, replacing all-atom models with analytic formulas sacrifices a certain level of detail. Thus, the key question becomes: what physical characteristics of the membrane must be reproduced in our membrane models to yield physically correct behavior of membrane permeants? The answer, of course, depends on the details of the system being considered, and the scientific questions asked. A model could simultaneously be well suited for some circumstances and wholly inadequate in others.

II. CLASSES OF MODELS

Lipid membranes self-assemble in an attempt to isolate hydrophobic acyl chains from the surrounding aqueous environment. Under appropriate conditions, this leads to the formation of stable bilayers, with a hydrophobic core, and interfacial region containing a mixture of polar headgroups and water, and the surrounding aqueous medium. Despite this apparent simplicity, biological membranes are capable of remarkable diversity of structure and dynamics. Although many models focus on a single property—the thickness of the hydrophobic core—real membranes have a broad range of physical characteristics which vary with lipid composition. These include structural quantities such as surface area per lipid, chain order, intrinsic curvature, and pressure profile, as well as dynamic properties such as the dielectric profile, diffusion coefficients, and chain and headgroup reorientation relaxation times.

Moreover, these properties are not independent of each other. For example, changing the hydration levels of model membranes modulates the surface area per lipid, and thus the chain order parameters. However, lipid surface area can also be controlled by varying the headgroup type, the length and degree of unsaturation of the chains, and the presence of other membrane permeants, such as cholesterol. The surface area per lipid in turn affects the magnitude of headgroup–headgroup interactions and, particularly in the case of charged headgroups, the distribution

of ions near the membrane surface. All of these properties can in principle affect the binding, conformation, stability, and oligomerization of peptides and proteins.

Thus, one critical question is, which of these membrane properties must be included to generate a successful implicit membrane model? Unfortunately, there is no simple answer to this question, as the answer depends largely on the model's intended use.

For example, if one plans to model a membrane protein using an all-atom representation, then effectively including the electrostatic effects of the membrane is probably paramount. If instead one wishes to use a simpler rigid-cylinder model for an embedded alpha helix then other issues become more important. As a direct result of this diversity, many different approaches to implicit modeling have emerged in the literature. For purposes of discussion, we have divided them into two broad classes: *Solute-focused* and *Membrane structure-focused*. Obviously, these labels represent something of a simplification, but we think this classification is on the whole helpful, in that it provides a context for understanding recent work.

A. Solute-Focused

This section will describe models best characterized as "solute-focused". By this, we mean models where the solute—typically a protein, peptide, or small molecule—is considered with atomic or near-atomic resolution, and the membrane is largely a backdrop intended to provide an appropriate venue. As a rule, these models neglect details of membrane structure other than the thickness of the hydrophobic low-dielectric core, and contain few provisions to account for the solute's disruption of membrane structure. Instead, these methods tend to focus on the membrane–solute interactions and the ways in which the membrane modifies solute–solute interactions, especially via electrostatics.

Although membrane electrostatics will be discussed in a separate chapter of this book, one cannot adequately introduce this class of implicit membrane models without first reviewing electrostatic and dielectric theory as applied to membranes. Indeed, one of the most pervasive concepts in membrane modeling is the notion of a low dielectric slab embedded between semi-inifinite regions of high dielectric. For this reason, we begin by considering the simplest circumstance, a spherical charge in an infinite uniform dielectric. The charging free energy for a charge q in a sphere of radius a embedded in a region of dielectric ϵ can be computed by integrating the electric field over all volume outside the sphere

$$\Delta G = \frac{1}{8\pi}\int \epsilon E^2\, dV = \frac{1}{8\pi}\int_a^\infty \epsilon\left(\frac{q}{\epsilon r^2}\right)^2 4\pi r^2\, dr = -\frac{q^2}{2\epsilon a} \qquad (1)$$

generating the familiar Born equation (Born, 1920). This derivation can be generalized for the case of a permanent (Bell, 1931) or polarizable (Bonner, 1951)

dipole (the so-called Onsager equation), or for an arbitrary charge distribution (Kirkwood, 1939).

However, the situation becomes somewhat more complex when the environment itself becomes heterogeneous, as is the case in a membrane. The electrostatic field due to a point charge approaching the barrier between two semi-infinite dielectric slabs is easily computed using the method of images (Jackson, 1962), and from this one can compute the charging energy. However, this solution contains an unphysical divergence as the charge approaches the dielectric interface due to the point charge approximation. This divergence, which appears repeatedly in the development of membrane models, was resolved by Ulstrup and coworkers by converting the volume integrals into surface integrals and directly accounting for the intersection between the ion surface and dielectric interface (Kharkats and Ulstrup, 1991). The result for an ion a distance $h > a$ from the interface is

$$\Delta G = -\frac{q^2}{8\epsilon_1 a}\left\{4 + \left(\frac{\epsilon_1 - \epsilon_2}{\epsilon_1 + \epsilon_2}\right)\left(\frac{2}{h/a}\right) + \left(\frac{\epsilon_1 - \epsilon_2}{\epsilon_1 + \epsilon_2}\right)^2\left[\frac{2}{1-(h/a)^2} + \frac{1}{2h/a}\ln\frac{(2h/a)+1}{(2h/a)-1}\right]\right\} \tag{2}$$

while the result for $0 \leqslant h \leqslant a$, where the ion overlaps the dielectric interface, is

$$\begin{aligned}\Delta G = & -\frac{q^2}{8\epsilon_1 a}\left\{\left(2 + \frac{2h}{a}\right) + \left(\frac{\epsilon_1 - \epsilon_2}{\epsilon_1 + \epsilon_2}\right)\left(4 - \frac{2h}{a}\right)\right. \\ & \left. + \left(\frac{\epsilon_1 - \epsilon_2}{\epsilon_1 + \epsilon_2}\right)^2\left[\frac{(1+h/a)(1-h/a)}{1+2h/a} + \frac{1}{2h/a}\ln(1+2h/a)\right]\right\} \\ & + \frac{q^2}{4\epsilon_2 a}\left(\frac{2\epsilon_2}{\epsilon_1 + \epsilon_2}\right)\left(1 - \frac{h}{a}\right).\end{aligned} \tag{3}$$

The same authors also derived analytic solutions for the charging free energy for a spherical charge in the presence of a slab of low dielectric surrounded by semi-infinite regions of higher dielectric, albeit without the finite ion size corrections (Iversen *et al.*, 1998). However, this solution involves several infinite sums, and is thus cumbersome to implement computationally, although Flewelling and Hubbell devised an efficient approximate solution (Flewelling and Hubbell, 1986). Krishtalik took this approach one step further, deriving an analytical solution for the case where there are 5 distinct dielectric regions (2 high-dielectric water regions, 2 moderate-dielectric interfacial regions, 1 low dielectric core) (Krishtalik, 1996). Previously, Parsegian published analytic solutions to several simple problems related to ion permeation through membranes (Parsegian, 1969).

Since analytic approaches are only readily applicable to simple geometries such as spheres, numerical methods are necessary in order to treat more biologically-relevant systems. The most obvious approach is to numerically solve the Poisson equation (or, if salt effects are to be included, the Poisson–Boltzmann equation)

(Schnitzer and Lambrakis, 1991; Sharp and Honig, 1990; Murray *et al.*, 1997; Lin *et al.*, 2002)

$$\nabla \cdot \epsilon(\vec{r}) \nabla \Phi(\vec{r}) = -4\pi\rho(\vec{r}), \tag{4}$$

where Φ is the electrostatic potential, $\epsilon(\vec{r})$ is the position-dependent dielectric constant, and ρ is the charge density (typically represented by a finite number of point charges q_i). Once this equation has been solved, the electrostatic free energy can be computed as

$$\Delta G_{\text{elec}} = \frac{1}{2} \int \rho(\vec{r}) \Phi(\vec{r})\, dV = \frac{1}{2} \sum_{i}^{\text{charges}} q_i \Phi_i, \tag{5}$$

where Φ_i is the electrostatic potential at the location of the ith charge. There is an extensive literature on the application of this formalism to biomolecular problems, so instead of reviewing it here we will simply suggest readers consult the recent review by Baker and the references cited there (Baker, 2005).

Several groups have directly applied the Poisson–Boltzmann approach to membrane-protein association thermodynamics. For example, Ben-Tal *et al.* used it to examine the thermodynamics of α-helix insertion, representing the membrane as a simple low dielectric slab (Ben-Tal *et al.*, 1996). Murray and coworkers explicitly included the lipid headgroups in their calculations (Murray *et al.*, 1997); this was particularly important in later work examining the association of basic peptides with anionic lipid bilayers (Murray *et al.*, 1999).

Computing the electrostatic solvation free energy via the Poisson equation has a number of distinct advantages: it is directly seated in electrostatic theory, and within the limits of the dielectric assumption and numerical accuracy it is correct. However, there are a number of drawbacks. Historically, the finite-difference approaches typically used are relatively expensive, and computing forces sufficiently accurate for use in molecular dynamics was difficult. Recently, progress has been made in some of these areas (Lu *et al.*, 2005a, 2005b; Feig *et al.*, 2004), but for many applications rigorous Poisson electrostatics are still prohibitively expensive. Moreover, unless headgroups and some waters are explicitly included, this approach does not reproduce the correct sign of the electrostatic potential at the center of the lipid bilayer, which is thought to be crucial in the thermodynamics of many membrane permeants (Lin *et al.*, 2002).

As a result, significant effort has been invested in developing faster, if more approximate, methods for computing electrostatic energies in dielectric media. Many of the most commonly used methods are variants of the generalized Born approach originally developed by Still and coworkers (Still *et al.*, 1990). Although these developments have been the subject of several recent reviews (Bashford and Case, 2000; Feig *et al.*, 2004), the underlying techniques and assumptions become relevant when the formalism is expanded to cover membranes, so we will discuss it here as well.

Most generalized Born methods are built around the empirical solvation free energy expression suggested by Still et al. (Still *et al.*, 1990)

$$\Delta G = -\left(1 - \frac{1}{\epsilon}\right) \sum_{i,j}^{N_{\text{atoms}}} \frac{q_i q_j}{\sqrt{r_{ij}^2 + \alpha_i \alpha_j \exp(-r_{ij}^2/4\alpha_i\alpha_j)}}, \tag{6}$$

where q_i is the partial charge on the ith atom, the sum is over all atom pairs (including self-interaction) and α_i is the ith generalized Born radius. The radii are constructed by computing the electrostatic free energy to solvate each charge individually in the protein, and then plugging that free energy into the Born equation (Eq. (1)) to extract an effective radius. The free energy is computed assuming the protein is a region of low dielectric, usually 1, and only the atom under consideration is charged. As a rule, the Coulomb field approximation is invoked to simplify the calculation; that is, the electric field due to the charge is presumed to be undistorted by the surrounding dielectric boundary. The result is volume integral over all space excluding the atom itself. This can be converted to a difference between two volume integrals, one over all space outside the atom, and the other only over the volume outside the atom but inside the protein

$$\frac{1}{\alpha_i} = \frac{1}{R_i} - \frac{1}{4\pi} \int_{\text{solute}, r > R_i} \frac{1}{r^4}\, dV. \tag{7}$$

Although Eq. (6) appears to contain only pairwise interactions, many-body effects are included implicitly via the volume integral in Eq. (7).

The key to the effectiveness of the Generalized Born method is the calculation of the effective Born radii; recent work has shown that if "perfect" radii are used—the electrostatic free energy of each atom is computed numerically using a standard Poisson solver—then Eq. (6) does an excellent job reproducing the molecular solvation energies computed using the Poisson equation (Lee *et al.*, 2002), which in turn does a good job reproducing the electrostatic portion of the solute–solvent interaction from explicit solvent simulations (Wagoner and Baker, 2004). The original Still formulation used a numerical integration over the protein volume, which was expensive and was ill-suited to computing forces suitable for molecular dynamics calculations. Many groups developed better approaches to performing this integral, including pairwise approximations (Bashford and Case, 2000; Feig *et al.*, 2004), various numerical schemes (Srinivasan *et al.*, 1999; Lee *et al.*, 2002, 2003; Grycuk, 2003; Tjong and Zhou, 2007), and a reformulation as a surface integral (Ghosh *et al.*, 1998; Gallicchio *et al.*, 2002). Some groups also added corrections intended to improve on the Coulomb field assumption (Lee *et al.*, 2003; Grycuk, 2003; Tjong and Zhou, 2007)

The situation becomes significantly more complex when one considers a membrane environment, because in that instance energies and forces depend not only

on the relative position of the atoms but on their absolute location in the membrane. To our knowledge, the first membrane model explicitly based on the generalized Born formulation was due to Spassov et al. (Spassov *et al.*, 2002). Their approach, which they call GB/IM, includes the heterogeneous dielectric environment by considering the membrane interior to have the same dielectric as the protein, with the result that the volume integral over the embedded portion is replaced by an integral over the whole of the membrane interior, which is approximated by an analytic function fit to Poisson–Boltzmann results. The remaining protein volume is integrated using the efficient pairwise method of Dominy and Brooks (Dominy and Brooks, 1999). They applied their methodology rigid structures of bacteriorhodopsin and rhodopsin, and performed a short dynamics simulation of the influenza fusion peptide bound to the membrane.

Im *et al.* (2003a) took a related approach. They extended previous work from the Brooks group (Im *et al.*, 2003b), where numeric behavior of the volume integration was improve by use of a smoothing function, and, like Spassov *et al.* (2002), considered the membrane to be part of the protein interior. Their approach contained analytic corrections to the Coulomb field assumption originally designed for soluble proteins. They validated their results by examining the behavior of several membrane-binding peptides, including melittin, M2-TMP, and the glycophorin A dimer, comparing against Poisson–Boltzmann calculations and experimental structural information. The same model was later used to explore the folding and insertion of several designed helical transmembrane peptides (Im and Brooks, 2005).

Although both of these methods appear to perform well in practice, the assumption that the membrane and protein have the same dielectric is troubling. Because the protein charges are explicitly represented, one would expect the continuum dielectric inside the protein to be 1, as done for simulations where both solute and solvent are explicitly represented; the force field parameters are chosen with this application in mind. By contrast, the membrane interior has a dielectric of 2–4.

Feig and coworkers introduced a formalism to explicitly handle multiple dielectric environments (Feig *et al.*, 2004), and later applied it membrane modeling (Tanizaki and Feig, 2005, 2006). This model, called the heterogeneous dielectric generalized Born or HDGB, contains several notable technical advancements. First, the membrane representation is improved: the chemical heterogeneity of the membrane–water interface (Jacobs and White, 1989) is explicitly included in the calculation by modeling the membrane as a series of dielectric slabs, rather than just two regions. They computed the free energy profile for a test charge in this model using the Poisson–Boltzmann equation and used the results to spline-fit an effective dielectric constant profile to be used in the simulations. While the physical meaning of a bulk quantity like the dielectric varying smoothly on atomic lengthscales is unclear, the result is a formalism which accurately recapitulates a more realistic model for membrane electrostatics. Moreover, this method is built

on top of a rigorous volume integration scheme (Lee *et al.*, 2003), and has been very carefully parameterized and characterized.

However, this careful characterization revealed some unfortunate complications when applying this model to larger molecules, such as a bacteriorhodopsin monomer or trimer. Tanizaki and Feig found that the results were very sensitive to the long range electrostatics cutoff, oscillating over a range of hundreds of kcal/mol (Tanizaki and Feig, 2006); this can result in almost comic failures, where setting the electrostatic cutoff at a seemingly reasonable 16 Å causes the bacteriorhodopsin monomer to be most stable in a horizontal orientation, with the helices lying in the plane of the membrane and the loops embedded in the membrane core. These effects go away with a sufficiently long cutoff, in the range of 36–38 Å, but the result is a dramatic increase in the computational cost. Although this problem has not been reported with the other methods discussed here, it seems likely that the underlying mechanisms will be present in all of them.

To this point, we have focused entirely on the electrostatic components of these models. This is of course incomplete; all of these models, whether intended for bulk solvent or specific to membrane modeling, contain at least one additional term representing non-electrostatic effects. Most follow the traditional approach from bulk solvent modeling and assume that these interactions can be related to the solvent accessible surface area. This approximation makes intuitive sense, and has some theoretical basis in the scaled-particle theory of hard sphere solvation (Pierotti, 1976). However, several groups have argued that nonpolar solvation, which includes terms from cavitation, hydrophobic effects, and favorable van der Waal's interactions, requires a somewhat more subtle treatment (Gallicchio *et al.*, 2002; Levy *et al.*, 2003). Wagoner and Baker showed that a significant fraction of the error in continuum methods, when compared to explicit solvent calculations, was due to the treatment of the non-electrostatic components, and that including terms to explicitly account for volume effects and attractive solute–solvent interactions greatly improved the situation (Wagoner and Baker, 2006).

The situation is—at least in principle—far more complex in the context of a lipid bilayer. Lipid acyl chains are far larger than water molecules, and unlike bulk solvent, these chains have a net orientation. As a result, cavitation effects should arguably have some additional shape and location dependence. Furthermore, the largely anhydrous environment means that hydrophobic interactions should likely be neglected in the membrane core, but other nonpolar terms, such as favorable solute–solvent dispersion interactions and solvent entropy remain. Finally, molecules permeating the lipid bilayer feel a lateral pressure profile; this pressure varies significantly not only with location in the membrane but also with membrane composition (Carrillo-Tripp and Feller, 2005; Ollila *et al.*, 2007; Cantor, 1999a), and may have functional implications (Cantor, 1997a, 1997b, 1999b; Pitman *et al.*, 2005).

Still, most of the methods discussed above model nonpolar interactions strictly on the basis of solvent accessible surface area, usually parameterized to repro-

duce partitioning free energies from water to liquid alkane and combined with a position-dependent scaling factor which turns the interactions off in the membrane interior. However, even within this genre, there are some interesting variations. Tanizaki and Feig (2005) attempted to capture position dependent effects by fitting the effective surface tension in the membrane to the potential of mean force of O_2 permeating an explicit lipid bilayer (Marrink and Berendsen, 1996). As a result, this parameterization accounts for variations in cavitation and dispersion with membrane depth.

Ben-Tal *et al.* (1996) took a different approach in their calculations exploring rigid helix insertion into lipid bilayers; while they did use an area term, they also included a term to account for lipid disruption, based on chain statistics calculations from Fattal and Ben-Shaul (1995). However, the inclusion of this term relies on the rigid-body nature of the calculation, since it is derived by treating the helix as a featureless cylinder.

Lazaridis took an entirely different approach in developing his implicit membrane model. IMM1 (Lazaridis, 2003), which is a generalization of the EFF1 solvation model (Lazaridis and Karplus, 1999) to a membrane environment. EFF1 consists of a distance-dependent dielectric combined with a pairwise, distance-dependent solvation term. IMM1 adds an explicit position-dependent atomic potential, parameterized to reproduce liquid-hydrocarbon transfer free energies of model compounds, combined with an enhancement of the electrostatic interactions in the bilayer interior. Although there is little theoretical justification underlying the functional forms of the model, it is easy to compute, and dynamics trajectories on systems such as the glycophorin A dimer, a helix isolated from bacteriorhodopsin, and several membrane-binding peptides all produced qualitatively reasonable results. In a later paper, IMM1 was further generalized to represent the internal water in a simulation of a transmembrane β-barrel protein (Lazaridis, 2002).

Several groups have also developed purely empirical implicit membrane models. For example, IMPALA model of Ducarme *et al.* (1998) applies simple atom-restraints, parameterized to reproduce partitioning experiments, independent of molecular context. This method is essentially free computationally, but has a number of unphysical implications, most notably that atoms of a given type feel exactly the same membrane forces whether on the surface of the molecule or buried in the interior. As a result, such a method is completely incapable of reproducing basic phenomena such as the stabilization of helical structure by the membrane environment. The same complaint can be made about the model from Sanders and Schwonek, although that model succeeds admirably in its stated goal of reproducing binding thermodynamics of small rigid molecules (Sanders and Schwonek, 1993).

Efremov et al. (1999a, 1999b) developed a model based entirely on solvent accessible surface area, generalizing the atomic solvation parameters of (Eisenberg and McLachlan, 1986). Initially, their models were parameterized to reproduce

low dielectric bulk solvent like hexane (Efremov *et al.*, 1999a) and octanol (Efremov *et al.*, 1999b), although later studies introduced spatial heterogeneity (Efremov *et al.*, 2002; Vereshaga *et al.*, 2007). These models produce qualitatively correct behavior, for example stabilizing α-helices, but as with the original atomic solvation parameters are not quantitatively accurate.

Of course, conceptually simple models are not to be disdained solely on that account. Rather, the goals and assumptions of the calculation must always be considered. For example, Pappu et al. showed they were able to find the correctly packed dimer structure of glycophorin A by representing the membrane as an infinite dielectric (no electrostatics at all) and a spring to prevent helix flipping (Pappu *et al.*, 1999).

B. Membrane-Focused

In contrast to the methods discussed in Section II.A, the methods presented in this chapter are largely focused on understanding the effects of membrane structure on the behavior of bound molecules. As a rule, the solute representations are not as detailed, but more care is taken to retain information about the membrane. In general, these models are intended in large part to describe the variation of membrane-solute interactions as a function of membrane composition, phenomena that are largely neglected by the models described previously.

The present class of models can be further divided into two subclasses: continuum models and chain models. The former class represents the membrane using some form of continuum mechanics based on some bulk property, *e.g.* hydrophobic thickness, while the latter attempts to build toward macroscopic predictions via a microscopic consideration of chain statistics.

The best known of the continuum models is the "mattress model" of Mouritsen and Bloom (Mouritsen and Bloom, 1984; Jensen and Mouritsen, 2004). In this approach, the lipids (and any additional membrane components, such as transmembrane proteins) are represented primarily as coupled springs with variable hydrophobic thickness. By assigning equilibrium thicknesses to the lipids and protein in specific phases, the elastic energy can be computed as

$$H^{\alpha}_{\text{Elastic}} = n^{\alpha}_{L} A^{\alpha}_{L} \left(d^{\alpha}_{L} - d^{0,\alpha}_{L}\right)^2 + n^{\alpha}_{P} A^{\alpha}_{P} \left(d^{\alpha}_{P} - d^{0,\alpha}_{P}\right)^2, \tag{8}$$

where n^{α}_{i} is the number of molecules of i in phase α, A is the effective force constant for thickness deformations, d_i is the hydrophobic thickness of molecules of type i, and $d^{0,\alpha}_{i}$ is the equilibrium thickness of molecules of type i in phase α. Specific protein–lipid interactions can also be included, such as hydrophobic mismatch

$$H^{\alpha}_{\text{hydro}} = \frac{n^{\alpha}_{L} n^{\alpha}_{P}}{n^{\alpha}_{L} + n^{\alpha}_{P}} B^{\alpha}_{LP} \left| d^{\alpha}_{L} - d^{\alpha}_{P} \right| \tag{9}$$

and favorable adhesion

$$H^{\alpha}_{\text{adhes}} = \frac{n^{\alpha}_{L} n^{\alpha}_{P}}{n^{\alpha}_{L} + n^{\alpha}_{P}} C^{\alpha}_{LP} \min\left(d^{\alpha}_{L}, d^{\alpha}_{P}\right), \tag{10}$$

where B and C are the positive and negative interaction coefficients for the respective terms. These energy terms constitute the excess enthalpy for the system, which can then be combined with the free energy for an ideal mixture to compute the free energies for different states. Thus, one can use the mattress model examine the effects of membrane permeants on lipid structure (and *vice versa*). For example, the original paper focuses on the effects of different "proteins" on the lipid phase diagram (Mouritsen and Bloom, 1984). In part because of its lack of atomic-level details, this model has been very successful in interpreting and suggesting experiments, particularly those involving designed single transmembrane helices such as the WALP and KALP families (Nyholm *et al.*, 2007).

However, the mattress model is neither the only nor the first continuum model for lipid–protein interactions. To pick one representative example, we consider the work of Owicki and McConnell, who used Landau–de Gennes theory to consider lipid–protein interactions in terms of order parameters related to the lipid gel–liquid phase transition (Owicki *et al.*, 1978; Owicki and McConnell, 1979). Their model describes mechanisms by which different lipid species could alter protein–lipid and even protein–protein interactions. However, the utility of the work is somewhat limited by its mandate that the protein be evenly distributed in the membrane, and by its focus on the notion of an annulus of boundary lipids surrounding the protein.

Brown and coworkers have proposed an alternative continuum formulation. Inspired by the unusual lipid composition of retinal rod outer segment disk membranes, with high concentrations of non-lamellar-forming lipids, they focused on spontaneous curvature of the membrane rather than simple hydrophobic matching (Gibson and Brown, 1993; Brown, 1994, 1997). When applied to the Meta-I/Meta-II equilibrium of photoactivated rhodopsin (Endress *et al.*, 2002), this model suggests that the lipid-composition dependent portion of the free energy change can be written as

$$\Delta G^{0} = \kappa\left[\left(H^{L}_{\text{MII}} - H^{L}_{0}\right)^{2} - \left(H^{L}_{\text{MI}} - H^{L}_{0}\right)^{2}\right] + \gamma_{\text{LP}}\left(A^{P}_{\text{MII}} - A^{P}_{\text{MI}}\right), \tag{11}$$

where H^{L}_{i} is the mean curvature of the membrane with the protein in state i, γ_{LP} is the lipid–protein surface tension, and A^{P} is the exposed area of the protein, believed to increase upon formation of Meta-II. More recently, this model was used to argue for the role of spontaneous curvature in modulating rhodopsin aggregation as well as function (Botelho *et al.*, 2006). This work proposes that increased protein concentration and decreasing bilayer thickness alter rhodopsin's properties via the same mechanism, a competition between curvature strain and hydrophobic matching.

By contrast, another subclass of models focused on the statistical physics of the lipid chains. In fairness, these two subclasses are not as distinct as they appear: chain states are invoked in the derivation and parameterization of the continuum models, and the results derived from the chain models, especially in the mean-field approximation, point back toward quantities used in the continuum methods.

A useful starting place to review chain-based models is the work of Marčelja (1974, 1976). His model considers the membrane as a set hexagonal of lattice sites, each of which contains a single lipid molecule. Each lipid is characterized by a molecular order parameter

$$\eta_j = \left\langle \frac{1}{n} \sum_m \left(\frac{3}{2} \cos^2 \nu_m - \frac{1}{2} \right) \right\rangle_j , \tag{12}$$

where n is the number of carbon segments in the chain ν_m is the orientation of the mth segment. Lipid–lipid interaction is represented in the molecular field approximation, summing over nearest neighbors

$$\Phi_i = \frac{1}{6} \sum_{j=1}^{6} V_0 \phi_j , \tag{13}$$

where

$$\phi_j = \left\langle \frac{n_{\mathrm{tr}}}{n^2} \sum_m \left(\frac{3}{2} \cos^2 \nu_m - \frac{1}{2} \right) \right\rangle_j \tag{14}$$

and n_{tr}/n is the fraction of trans states, V_0 is the coupling constant, and Φ_i describes the strength of the molecular field acting to orient the molecule at site i. If there is a protein molecule at a neighboring site, a term in Eq. (13) is replaced by the lipid–protein interaction V_{lp} (Marčelja, 1976). Thus, the total energy for the ith position in the lattice is

$$E_i(\Phi_i, P) = E_{\mathrm{int}} - \Phi_i \left(n_{\mathrm{tr}}/n^2 \right) \sum_m \left(\frac{3}{2} \cos^2 \nu_m - \frac{1}{2} \right) + PA \tag{15}$$

is dependent on the chain's internal energy E_{int}, the cross-sectional area A and lateral pressure P. The partition function for the ith chain is

$$Z_i = \sum_{\mathrm{confs}} \exp\left[-E_i(\Phi_i, P)/k_{\mathrm{B}}T \right]. \tag{16}$$

Thus, the average orientations for all chains can be calculated by solving the following set of coupled non-linear differential equations

$$\phi_i = \frac{1}{Z_i} \sum_{\mathrm{confs}} \frac{n_{\mathrm{tr}}}{n} \sum_m \left(\frac{3}{2} \cos^2 \nu_m - \frac{1}{2} \right) \exp\left[-E_i(\Phi_i, P)/k_{\mathrm{B}}T \right]. \tag{17}$$

The total internal energy thus becomes

$$U = \sum_i \left[E_{\text{int}} - n\Phi_i\phi_i/2\right] + V_{\text{lp}} \sum_{lp \text{ pairs}} n\phi_i/12, \tag{18}$$

where the latter term is necessary to correctly account for protein–lipid interactions in the hexagonal lattice.

Writing the total system partition function as the product of the individual partition function in Eq. (16), and combining with Eq. (18), it is straightforward to write the system's entropy and Gibbs free energy.

This formalism can be used to investigate the effects of a protein on lipid structure as a function of temperature, protein size, and concentration. Most interestingly, one can compute the effective protein–protein potential of mean force; in this manner, a Marčelja-type model can be used to explore the effects of lipid composition on protein oligomerization and aggregation. Several other groups have explored similar models, differing primarily in the details of lipid chain representation and the manner in which the resulting equations are solved (Meraldi and Schlitter, 1981; Pink and Chapman, 1979).

More recently, Ben-Shaul and coworkers have developed a comprehensive membrane model which explicitly account for chain statistics (Fattal and Ben-Shaul, 1993, 1994, 1995). They begin by expressing the system's free energy as a sum of three terms

$$F = 2N(f_{\text{t}} + f_{\text{s}} + f_{\text{h}}), \tag{19}$$

where N is the number of lipids per leaflet (the present equation assumes a symmetric planar bilayer), f_{t} is the free energy per lipid chain, f_{s} the surface free energy due water–chain interaction, and f_{h} is the free energy due to headgroup–headgroup and water–headgroup interactions. Describing individual chain conformations using the rotational isomeric approximation combined with an overall tilt vector, the probability distribution P over all conformations α can be constructed, and the chain free energy computed as

$$f_{\text{t}} = \sum_\alpha P(\alpha)\epsilon(\alpha) + k_{\text{B}}T \sum_\alpha P(\alpha) \ln P(\alpha), \tag{20}$$

where $\epsilon(\alpha)$ is the internal energy of conformation α. $P(\alpha)$ is constrained to obey

$$\sum_\alpha P(\alpha) = 1, \tag{21}$$

$$\sum_\alpha P(\alpha)\left[\phi(z_i;\alpha) + \phi(-z_i;\alpha)\right] = a\rho, \quad \text{for all } z_i. \tag{22}$$

The first equation represents a simple normalization of probability. In the latter, $\phi(z_i;\alpha)$ is the atomic number density of conformation α in the membrane slice z_i, a is the area per chain, and ρ is density of the bilayer interior. Equation (22)

explicitly assumes constant density in the hydrophobic interior of a symmetric planar bilayer, but can be easily generalized to account for heterogeneous density drawn from experiment, or to deal with more complex geometries such as curved bilayers or micelles.

Minimizing Eq. (20) with respect to Eqs. (21) and (22) gives

$$P(\alpha) = \frac{\exp[\beta\epsilon(\alpha) - \beta \sum_{z_i} \pi(z_i)\phi(z_i;\alpha)\Delta z]}{\sum_{\alpha} \exp[\beta\epsilon(\alpha) - \beta \sum_{z_i} \pi(z_i)\phi(z_i;\alpha)\Delta z]}, \tag{23}$$

where $\beta \equiv 1/k_{\mathrm{B}}T$, the denominator is the chain partition function Z, and $\pi(z_i)$ are a series of Lagrange multipliers physically corresponding to the lateral pressure profile along the membrane normal. Substituting back into Eq. (20) generates

$$f_{\mathrm{t}} = k_{\mathrm{B}}T \ln Z - a\rho \sum_{z_i} \pi(z_i)\Delta z. \tag{24}$$

Thus, calculating any chain property amounts to computing the appropriate $\pi(z_i)$ values for the system. This model has a number of desirable properties, most notably the direct dependence of the chain thermodynamics on the headgroup type, via the surface area per chain a. This area can in turn be considered as a variable, which is where the latter two terms in Eq. (19) come into play.

In addition to considering alternative membrane geometries, this model can also be generalized to include the effects of other molecules included in the bilayer. Ben-Shaul and coworkers considered simple protein models such as impermeable walls and cylinders (Fattal and Ben-Shaul, 1995), while other groups have used analogous approaches to consider protein–protein interaction (May and Ben-Shaul, 2000; Bohinc *et al.*, 2003). The lateral pressure profile computed in this model can be directly connected to the intrinsic curvature described in the models from Brown and coworkers, in that heterogeneity in the pressure leads directly to a preference for intrinsic curvature. As we will see below, this is a repeating theme in chain-based models of membranes.

Frink and Freschknecht (2005a, 2005b) have used a simple coarse-grained chain representation combined with density functional theory to compute lipid bilayer properties. Although the details of their formulation are too complex to describe here, it is interesting to note that once again the lateral pressure profile plays a central role. They have applied their model to explore the effects of alcohols on lipid structure (Frischknecht and Frink, 2006), and to explore pore-formation due to the binding of rigid helices (Frink and Frischknecht, 2006). By contrast to the models described in Section II.A, their model allowed them to compare different modes of pore-formation.

Over the last 15 years, Cantor has presented a series of papers describing another chain-based model focused on the lateral pressure profile (Cantor, 1993, 1996, 1997a, 1997b, 1999a, 1999b, 2002). His approach uses a lattice model to account for chain conformations, with a constant density assumption similar to that of Ben-Shaul. He has used his model to explore the effects of chain length

and unsaturation on the equilibrium width, area fluctuations, and lateral pressure profile of planar lipid bilayers (Cantor, 1999a). He has also proposed that lateral pressure is a common mechanism by which bilayer composition can be used to regulate protein function (Cantor, 1999b, 2002). Specifically, if a protein has two states with different shapes in the membrane, then the free energy difference between the two states is

$$\Delta G_{12} = \Delta G_{12}^0 - \int \pi(z)\big(A_2(z) - A_1(z)\big)\, dz, \tag{25}$$

where ΔG_{12}^0 is the intrinsic free energy difference between the states, excluding bilayer effects, $A_i(z)$ is the area profile for the protein in state i, and $\pi(z)$ is the lateral pressure profile. The last term in Eq. (25) provides a direct mechanistic coupling between the lateral pressure profile (and thus lipid composition) and the protein's conformation equilibrium even in the total absence of specific lipid–protein interactions. Cantor has also proposed that this model provides a simple framework for understanding the mechanism of most general anesthetics (Cantor, 1997b, 2001).

III. INTERESTING PROBLEMS IN IMPLICIT MEMBRANE MODELING

There has been a great deal of work to develop models for implicitly modeling membrane–protein interactions. This work spans a broad range of different approaches, each with different strengths and weaknesses. In particular, the methods described in Section II.A have the advantage of using atomic descriptions of the solute of interest; this means that subtle differences, such as the effects of mutations or chemical modifications, can be directly examined. However, the membrane is usually represented in a very simple manner, and non-electrostatic effects in particular are not included in detail. As a result, these models do not as a rule capture the effects of lipid composition, except crudely via the hydrophobic thickness of the membrane. Moreover, if solute binding is correlated with disruption of membrane structure, these models will not capture it, since these models explicitly assume membrane structure is invariant. By contrast, the models in II.B represent membrane-bound solutes in far less detail, but do far more to include the effects of lipid chain structure. However, they typically lack atomic detail and tend to represent lipid–protein interactions phenomenologically. As a result, they cannot easily be used to resolve questions which depend on the details of solute structure. This means that there are many interesting problems which will require new approaches combining the strengths of the existing solute-centric models with better representations of membrane structure.

A. Antimicrobial Peptides

Antimicrobial peptides (AMPs) are an ancient immune mechanism, ubiquitous in multicellular organisms and even found in some bacteria (Zhang *et al.*, 2000; Risso, 2000; Zasloff, 2002a, 2002c, 2002b). In humans, they are found mostly on exposed organs, such as the eyes, skin, and mouth. Unlike the rest of the immune system, these peptides generally act in a non-inflammatory way. This is critical, as organs such as the eyes are constantly exposed to pathogens, and permanent inflammation would seriously degrade their performance.

AMPs exhibit a broad diversity of structures, ranging from single helices to β-strands to small globules (Zasloff, 2002c). However, the vast majority of them share two characteristics: amphipathic structure and positive charge. The former quality encourages binding to the membrane–water interface, while the latter enhances selectivity toward bacterial membranes, which tend to be enriched in anionic lipids compared to the zwitterionic lipids most common in mammalian cells. Interestingly, transformed cancer cells also have a higher concentration of anionic lipids, and some AMPs have been shown to have antitumor activity (Jacob and Zasloff, 1994; Mader and Hoskins, 2006). Lipopeptides have also found use in the development of vaccines (BenMohamed *et al.*, 2002). The biophysics of AMPs binding to lipid membranes have been extensively reviewed (Epand and Vogel, 1999; Shai, 1999; Huang, 2000, 2006; Doherty *et al.*, 2006; Chan *et al.*, 2006).

Unlike most other classes of drugs, AMPs do not inhibit enzymatic pathways, or indeed specifically bind any proteins in their targets. Rather, they operate by binding to and disrupting the membrane (Boman *et al.*, 1994). As a direct result, pathogens such as bacteria, fungi and viruses are far less likely to evolve immunity to them, because doing so would require changing the lipid composition of their membranes and likely disrupting many of their own native proteins.

Despite this, AMPs have not for the most part found much use as antibiotic drugs, because they are relatively hard to synthesize and tend to break down rapidly in the body. However, in recent years, scientists have borrowed from the basic properties of AMPs to design new potential drugs, for example using peptide mimetics (see for example Ref. (Ishitsuka *et al.*, 2006)). Shai and coworkers have had remarkable successes by combining two strategies: including D-amino acids to foil peptidases (Avrahami and Shai, 2003), and conjugating shorter peptides to fatty acids (Avrahami *et al.*, 2001; Avrahami and Shai, 2002, 2004; Makovitzki *et al.*, 2006; Makovitzki and Shai, 2005; Malina and Shai, 2005). The essential insight into the value of lipidization is that hydrophobicity is relatively non-specific. That is, most of the sequence in AMPs is devoted to making them hydrophobic enough to bind to membranes, as opposed to lysing them after binding. Thus, one can dispense with most of the sequence if the peptide is attached to an acyl chain; Shai and coworkers found that sequences

as short as 4 amino acids had strong antimicrobial activity against fungi and bacteria, without significantly damaging human cells (Makovitzki *et al.*, 2006).

Unsurprisingly, there has been a great deal of interest in modeling the binding of AMPs to model lipid membranes (La Rocca *et al.*, 1999). Indeed, membrane-disrupting peptides such as gramicidin, alamethicin, and melittin have become standard test cases in the development of implicit membrane models. In fact, much of the interest in modeling isolated helices and helical aggregates in membranes derives from the classic "barrel-stave" model, where amphipathic helical peptides initially bind to the membrane interface, then cooperatively associate and fully insert to form pores; this process has been explored via theoretical means (Frink and Frischknecht, 2005a; Bohinc *et al.*, 2003) and by explicit molecular dynamics simulations (Tieleman *et al.*, 1999b, 1999a).

However, there is significant evidence that many if not most AMPs do not operate via the "barrel-stave" mechanism. Rather, the "carpet model" appears more prevalent (Chan *et al.*, 2006); in this view, peptides bind interfacially and stabilize highly curved lipid structures, leading to the formation of toroidal pores ("wormholes") and even micellization of the membrane.

This case is a difficult one to treat computationally. Poration due to lipid binding occurs on too long a time scale for all-atom molecular dynamics; the calculations which have been done have typically begun by preforming a particular pore-forming oligomeric structure (Tieleman *et al.*, 1999b; La Rocca *et al.*, 1999). In principle, solute-focused implicit membrane models can reach the necessary time- and length-scales for spontaneous oligomerization, and as described above have had some successes in describing barrel-stave-type pore formation. However, these models cannot represent the kinds of membrane disruption expected according to the carpet model, and so cannot be used to elucidate which if either model applies to a given solute. The only example we are aware of where an implicit membrane model was used to examine the mechanism of poration is the work of Frink and Frischknecht, described above (Frink and Frischknecht, 2005a, 2005b), which applied density functional theory. In this model, however, protein helices are represented as impermeable hard cylinders, neglecting other phenomena such as electrostatics and amphipathicity. As such, these calculations, while highly instructive, cannot be used to reveal the binding mode of. for example, a particular amino acid sequence.

An implicit membrane model which could successfully attack this problem would most likely need the following properties:

- Atomic- or near-atomic-level solute description.
- Accurate electrostatics, including both dielectric effects and effects due to headgroup charge and dipoles.
- Membrane which responds to solute structure.

Several models from Section II.A have the first property, and could readily be extended to have the second. However, none of the existing models can readily

meet the third requirement, which should make this an interesting future research problem.

B. Protein–Protein Association

Although biophysical experiments frequently focus on the behavior of isolated proteins, biologically many if not most membrane proteins function as part of complexes (Alberts *et al.*, 1994). Of these complexes, homodimers are most easily studied by biophysical techniques, since only a single protein needs to be overexpressed. In particular, there is great interest in the dimerization of G protein-coupled receptors (GPCRs) (Park *et al.*, 2004), the largest superfamily of proteins in the human genome. These proteins are responsible for a broad array of physiological processes involving signaling (Bockaert and Pin, 1999), and as a result are commonly targetted in drug development (Ma and Zemmel, 2002). Among GPCRs, only one protein, rhodopsin, has been structurally resolved at atomic resolution (Edwards *et al.*, 2004; Li *et al.*, 2004; Okada *et al.*, 2002, 2004; Palczewski *et al.*, 2000; Schertler *et al.*, 1993). In recent years, significant controversy has erupted over the oligomeric state of rhodopsin in its native membrane environment. Several groups have demonstrated that rhodopsin dimerizes in non-native membranes (Kota *et al.*, 2006; Mansoor *et al.*, 2006), and Palczewski and coworkers showed striking images from atomic force microscopy showing ordered rows of rhodopsin dimers (Fotiadis *et al.*, 2003a). However, others have argued that these dimers are artifacts of sample preparation conditions (Chabre *et al.*, 2003; Fotiadis *et al.*, 2003b), and have argued that the functional form of the protein is most likely monomeric (Chabre and le Maire, 2005).

In principle, one way resolve this controversy would be an unambiguous determination of the dimeric structure of rhodopsin. However, this seems unlikely, because any crystal structure could be countered by the argument that the dimer was stabilized only by crystal packing. Instead, various groups have attempted to map a generic GPCR dimer interface using mutagenesis studies (Javitch, 2004; Guo *et al.*, 2005; Fanelli and De Benedetti, 2005; Filizola *et al.*, 2002; Filizola and Weinstein, 2005b).

Ideally, this sort of strategy would be complemented by molecular-level simulation, to flesh out the details and validate the interactions. Indeed, there have been several efforts along these lines (see for example references (Filizola *et al.*, 2006; Filizola and Weinstein, 2005a)). However, such efforts are complicated by the very long time scales necessary to sample large-scale rearrangement of protein–protein interfaces; it is to be expected that even a totally incorrect protein–protein docking would be stable on the 10–100 ns timescale readily accessible by all-atom molecular dynamics. Using existing implicit membrane models is more

appealing, since conventional molecular dynamics simulations could be abandoned in favor more efficient searching and sampling techniques. However, the best of the existing solute-focused are expensive for larger systems; for example, Feig's work on bacteriorhodopsin trimers showed that the need for very long electrostatics cutoffs greatly increased the computational cost (Tanizaki and Feig, 2006). Moreover, these models are not capable of capturing the effects of specific lipid species. This could be critical to assessing the stability of dimers, since we know that rhodopsin is both highly sensitive to and capable of perturbing its lipid environment (Brown, 1994; Botelho *et al.*, 2006; Polozova and Litman, 2000). As such, we once again reach a point where new models will be needed in order to resolve the problem.

IV. CONCLUSION

Lipid membranes are critically important biologically, both as passive barriers and as active participants in membrane protein function. Molecular modeling has already made significant contributions to our understanding of their roles, and can be expected to be even more valuable as structures of more membrane proteins become available. However, for many applications, explicit all-atom calculations are prohibitively expensive, and will remain so for the foreseeable future. In this context, the development of new models for representing protein–lipid interactions implicitly becomes extremely important. A great deal of impressive work has been done, but still more remains.

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