

Electrostatics of Membrane Systems — Complex, Heterogeneous Environments

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Abstract.

The cellular membrane is both a solvent for approximately 30% of known proteins, as well as a semi-permeable barrier which maintains differences between ionic concentrations inside and outside of the cell. Proper cell function depends heavily on this unique environment, and electrostatic properties are key to understanding the relationship between biomembrane structure and function. Any attempt to model the membrane bilayer requires a careful treatment of electrostatic calculations. We review general models of electrostatics which could be adapted to membrane systems, and examine existing approaches applied specifically to membrane systems. A new model for the bilayer using a lattice of dipoles with spatially varying properties is also presented.

I. INTRODUCTION

The treatment of long-range electrostatic interactions in biomolecular simulations remains a challenge. Many of the other chapters in this volume address the pertinent issues for homogeneous media. This article, on the other hand, will focus on cell membranes: lipid bilayers and their inclusions. These systems have particularly complex electrostatics, in part because the “solvent” (i.e., the lipid bilayer) is not isotropic. Instead, the electrostatic properties vary significantly depending on position in the bilayer. The membrane interior is frequently compared to a bulk hydrocarbon solution, while the headgroup region might be viewed as a solution of zwitterions. However, neither description is fully appropriate, because both images neglect the simultaneous heterogeneity and order found in a liquid crystalline membrane.

There is a growing body of experimental information about the motions and arrangements of the molecular components of membrane proteins and lipid bilayers [40,135]. Still, experimental knowledge about these systems lags far behind that for water and globular proteins [34,20]. Yet in parallel to the situation for globular proteins, a complete molecular understanding of membrane protein structure and function will require a detailed picture of how the bilayer solvent influences the protein properties, and how the protein in turn alters the bilayer. Computer modeling

will undoubtedly play a critical role in this pursuit, in part because many of the structural tools of molecular membrane biophysics, such as X-ray diffraction and nuclear magnetic resonance give only partial information about selected systems [40,135].

A wide variety of representations of the bilayer have been considered in the past. These range from models where the electrostatics is completely neglected (simpler polymer theories), to situations where the van der Waals interactions are completely neglected and only electrostatics are calculated [40,135,79]. We believe that an accurate model of the membrane bilayer must contain both. Because of the heterogeneity of the biomembrane, electrostatic interactions are difficult to calculate in a simultaneously realistic and tractable way — either analytically or numerically. The majority of electrostatic models have applied continuum electrostatics, treating the bilayer as a uniform slab with dielectric constant $\epsilon = 2$, embedded between two infinite water layers with $\epsilon = 80$. This “embedded slab” model is shown in Figure 1. Such an approach has the advantages of speed and simplicity. Indeed, for equally simple solutes, such as a single spherical charge, the equations are analytically tractable [94]. Moreover, small protein systems can be treated at reasonable cost [71,57].

The embedded slab model, however, neglects the crucial membrane-water in-

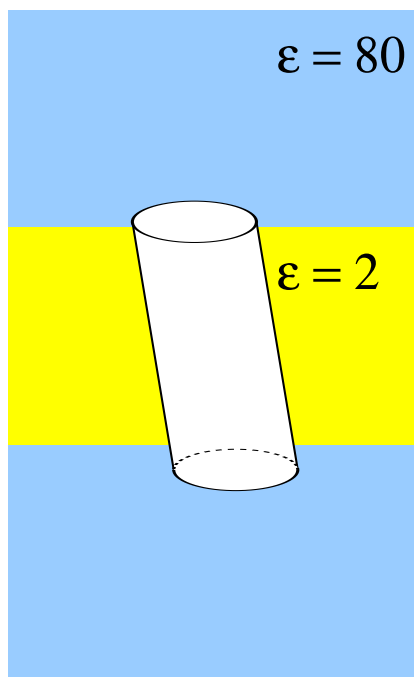


FIGURE 1. The membrane is often represented as a slab of low dielectric ($\epsilon = 2$) material embedded in regions of high dielectric ($\epsilon = 80$). In such a model, the membrane:water interface is infinitely thin. The cylinder schematically represents a membrane protein or alpha helix within the bilayer.

terface region. This 10-15 Å transition region is not well characterized by a step function, and actually accounts for as much as half of the total bilayer thickness [136]. The interface contains water molecules, zwitterionic or charged headgroups, lipid glycerol groups, and even hydrocarbon, and as such resembles neither bulk water nor a pure hydrocarbon solution. A realistic model of the membrane must include the properties of this interface zone.

There are many situations of practical and theoretical interest where a better ability to compute properties of the membrane bilayer is quite important. For example, drug compounds need to be able to “partition” across the bilayer in order to function. The ability to determine this “bio-availability” is often a key step in the process of rational drug design [6]. However, experimental screening of large libraries of compounds to directly determine their bio-availability is difficult and expensive. Present computational approaches, moreover, use a crude membrane model — usually based on data from bulk-solvent partitioning — and typically have limited predictive capability. An efficient and accurate computational membrane model, therefore, would be of great utility to the rational drug design process.

A better membrane model could also be used as part of a membrane protein structure prediction scheme. Many experimental techniques, such as cryo-EM, solid-state NMR, ESR, and FTIR yield partial structural information about membrane proteins [135]. Combined with sequence analysis, such data may be sufficient to generate families of candidate structures, which could be evaluated computationally. A fast but reasonably accurate membrane representation would be critical to the success of such an undertaking. Although there have been a small number of successful structure calculations where the membrane was neglected (e.g., the glycophorin A transmembrane dimer [124,70,93]), calculations on soluble proteins have clearly shown that discrimination of misfolded structures requires some representation of the solvent [89]. Moreover, recent experiments have shown that the membrane, and most particularly the membrane:water interface, can be critical to the folding of many peptides [136].

Calculations that attempt to correlate molecular structure with functional properties also require a high quality model for the bilayer setting. For example, there is much interest in connecting the recent KcsA K^+ channel structure [25] to single channel electrophysiology measurements. This requires knowledge of the electrostatic responses of both the protein and the membrane. Some first steps in this direction were taken by the recent finite difference Poisson calculations of Roux and MacKinnon [109].

As another example, many biologically important processes, such as respiration and photosynthesis, involve charge transfer across biomembranes. One such system which has been characterized in some detail experimentally is the photosynthetic reaction center [22]. The structure for this protein has been solved to atomic resolution, and a great deal of mutagenesis work has also been performed [101,22]. Several groups have attempted to describe the charge transfer reaction in this protein using the solvent reorganization energy calculated from Marcus the-

ory [47,76,132]. These calculations could be further refined by the use of a better membrane model. Indeed, the photosynthetic reaction center system would be an excellent, if difficult, test for any new model since accurate calculations for this system will require correct representation of both the kinetics and thermodynamics of membrane dielectric response.

II. REQUIREMENTS FOR A SUCCESSFUL MEMBRANE MODEL

What properties should be contained in an ideal model for the electrostatics of the biomembrane environment? Given the variety of potential applications, this is a natural and important first question. Four elements are crucial:

1. The model should allow *accurate* calculation of electrostatic energies involving membranes and the molecules embedded in them. Of course, the degree of accuracy required will depend on the application. For instance, a different standard applies to calculating the free energy barrier to permeation for a drug candidate, as opposed to checking if a protein structure is likely to be stable in a membrane: the former would require quantitative accuracy, while a qualitative result would probably be sufficient for the latter.
2. The ideal model should capture the *essential features of membrane structure*. We argue that this cannot be done without some representation of the membrane interface. Otherwise, energetic calculations would necessarily misattribute the behavior of permeants to interactions with other parts of the bilayer. Lipids, after all, are large for solvent molecules (typically 100 or more atoms, as opposed to water with 3), and as such create an environment that is heterogeneous in all directions — but especially so along the bilayer normal. Furthermore, more detailed representations allow more accurate determination of the functional role of lipid components.
3. The model should, ideally, be capable of representing *kinetic responses* in addition to the equilibrium behavior. This is probably the most strenuous of our requirements, but follows naturally from a complete description of the bilayer setting. Although many processes can be described accurately while assuming the environment is in equilibrium, some, such as electron transfer, occur on a much faster timescale and require a model which can represent “solvent” relaxation directly.
4. Finally, the model must be *computationally tractable*. As with the first requirement, the standard for “reasonable” computational expense depends strongly on the application. A researcher trying to understand the molecular details of the membrane and protein response to light absorption by the chromophore in bacteriorhodopsin may be willing to invest months of computer time in a single calculation, while a drug researcher might want to screen dozens or even

hundreds of compounds per hour. The latter researcher would probably be willing to work with a less detailed and less accurate model, in exchange for computational efficiency.

The rest of this chapter will attempt to outline models that can and have been applied to membrane systems, and judge them in light of these criteria. However, the judgments must be regarded as preliminary, as many of the models are still in the development stage. Moreover, computer speed has been improving exponentially, so a calculation that appears prohibitively expensive today may be routine in a few years.

III. ELECTROSTATIC MODELS OF WATER AND THE MEMBRANE ENVIRONMENT

A wide variety of models have been used to represent the electrostatic interactions relevant to biomolecular simulation — both in aqueous and membrane environments. These models can be classified in five basic categories, as those using (i) “implicit” continuum representations, (ii) distribution-function/integral-equation methods, (iii) “explicit” all-atom representations, (iv) surface area approximations, or (v) dipole lattice models of the solvent. All of these approaches have been widely discussed in the literature, and have both proponents and detractors (e.g., [113,131,104,13]).

In surveying the models, we indicate the context where they have been applied. Most work has focused on bulk media, so we have attempted to review this area as well. However, it must be noted that the field of homogeneous electrostatic calculations is quite large and we can only comment (and then only briefly!) on those models and papers that seem most relevant to us. We apologize in advance for both the brevity of some descriptions and also to those authors whose work was not included in the following descriptions.

IIIa: The Simplest Models: Constant Dielectric, Born and related

The simplest implicit solvent model represents the entire solvent (typically water) *and* interactions among the atoms of the solute by use of a single dielectric constant. The intra-solute electrostatics are modeled simply as the Coulomb interaction divided by the dielectric constant [13]. Not surprisingly, there are significant problems with this model. It can describe neither temporal response nor spatial heterogeneity of the solvent. In particular, it does not capture the effect of solvent polarization due the solute, which can cause the calculation to deviate dramatically from the experimental results [133,131].

The previous model does not account for the solvation (self) energies of solute atoms. The simplest way of doing so is to use the Born equation [11], which computes the work required to charge up a sphere embedded in a continuum dielectric. It can be used to estimate the solvation energy of a single spherical charge of magnitude q and “Born radius” a by calculating the difference in energies for charging in two uniform media:

$$\Delta G = \frac{332q^2}{2a} \left(\frac{1}{\epsilon_2} - \frac{1}{\epsilon_1} \right), \quad (1)$$

where ϵ_1 is the dielectric constant of the source medium (typically a vacuum, $\epsilon_1 \equiv 1$) and ϵ_2 characterizes the destination medium. The units of ΔG are kcal/mole when a is given in angstroms and q in electron units.

The Born result is characterized by a single adjustable parameter, the cavity radius. Over the years, there has been considerable effort to find a consistent and accurate method for defining it [77,122,103]. The issue is especially important when the solute is no longer a simple spherical charge, but has a more complex structure and charge distribution. Arguments have been made for determining the cavity radius from the arrangement of solvent around the solute in the context of integral equation theories [108,54]. If the radius is chosen “well,” the Born expression apparently provides reasonable estimates for the solvation energies of simple ions [103]. Moreover, the Born equation is commonly used to supplement more detailed models — for example, to correct for finite simulation size in explicit-atom calculations [3,60].

One of the first calculations exploring ionic charge transfer across a membrane pursued an approach similar in spirit to the Born model. Using the embedded slab model of the membrane, Parsegian computed the energy of a charged spherical solute at the center of the membrane model slab [94]. The analysis provided a rough estimate of the free energy barriers to ion permeation, underscoring the catalytic effect of ion channels in mediating the large ion fluxes measured under physiological conditions. Shortly thereafter, two groups extended the calculations to produce a profile for the electrostatic work required to cross the bilayer, both in the presence and absence of channel-like modifications, for all points during the transfer process [71,57]. When the gramicidin channel structure became available [5,59], it was used to compute similar electrostatic profiles using continuum dielectric theory [95].

Von Kitzing and Soumpasis recently advanced embedded slab calculations for the response of the bilayer to charge using a Green’s function approach [129]. Their method may provide a basis for efficient and accurate computations within the embedded slab model.

Another advance in embedded slab modeling and computation comes from the work of Krishtalik, which included an additional region of intermediate dielectric between water and membrane, to represent the interface [65]. The work determined analytic expressions for the self-energy of a charge embedded in such a membrane, calculated by summing the interactions between an infinite series of image charges.

Although the resulting expressions are extremely complicated, the model does represent the membrane-water interface and as such is a significant advance. On the other hand, the infinite sums would make it difficult to integrate this model into a molecular dynamics or Monte Carlo force field. We note, also, that the work was recently extended to examine charge transfer in the photosynthetic reaction center [66].

IIIb: Onsager and Related Models

A natural step beyond the solvation of point charges considers point dipoles. In the simplest case, Bell [7,12] derived the change in free energy upon transfer of point dipole of fixed magnitude μ in a spherical cavity of radius a and $\epsilon \equiv 1$ embedded in a region of uniform dielectric (ϵ), namely

$$\Delta G = -\frac{\mu^2}{a^3} \frac{\epsilon - 1}{2\epsilon + 1}. \quad (2)$$

Like the Born result (1), this solvation energy includes the interaction of the solute with the “reaction field” it induces in the dielectric material. Indeed, computation of the solvation energy hinges on deriving the reaction field [12].

Onsager [90] computed the reaction field for a polarizable dipole. The resulting solvation energy when the permanent dipole (μ) is additionally characterized by a polarizability, α , is found to be (e.g., [12])

$$\Delta G = -\frac{\mu^2}{a^3} \frac{\epsilon - 1}{2\epsilon + 1} \left(1 - \frac{2\alpha}{a^3} \frac{\epsilon - 1}{2\epsilon + 1}\right)^{-1}. \quad (3)$$

As with the Born model, and all dielectric continuum models, the definition of the solute-solvent interface is difficult. We note that ellipsoidal cavities have also been considered [12]. Furthermore, Bonner calculated the solvation energy for an arbitrary charge distribution in a spherical cavity [112,36].

Onsager’s result for the reaction field of a polarizable dipole also permitted the extension of the multi-component Clausius-Mossotti-Debye relation [17,84,21] for the dielectric constant in terms of microscopic properties of the “solvent.” The Clausius-Mossotti-Debye approach assumes the local reaction field to be the same as the bulk field, and estimates the dielectric constant as a function of the molecular dipole moments (μ_i), polarizabilities (α_i), and number densities (c_i) of the species present, according to

$$\frac{\epsilon - 1}{\epsilon + 2} = \frac{4\pi}{3} \sum_i c_i \left(\alpha_i + \frac{\mu_i^2}{3kT} \right) \quad (4)$$

Onsager realized that the cavity (reaction) field is not the same as the bulk field and thus modified the relationship:

$$\frac{\epsilon - 1}{\epsilon + 2} = 4\pi \sum_i \frac{c_i}{\epsilon + 2} \left[\frac{\epsilon(n_i^2 + 2)}{n_i^2 + 2\epsilon} \alpha_i + \frac{\epsilon(n_i^2 + 2)^2(2\epsilon + 1)}{(n_i^2 + 2\epsilon)^2} \frac{\mu_i^2}{9k_B T} \right] \quad (5)$$

In this expression, n_i is the internal index of refraction of the species i and is implicitly related to the induced polarizability of the solute [90].

To our knowledge, the Onsager framework has not yet been used for a model of membrane-bilayer electrostatics. Indeed, it would require a non-trivial determination of the effective cavity surface since in a membrane the dielectric is not homogeneous. But even with the cavity surface(s) defined, it is not clear that the resulting equations could be easily solved in the context of the embedded slab model for the membrane, let alone more realistic models. In principle, however, the Onsager approach should be more accurate than one stemming from the simple Born model, since it more correctly represents the reaction field due to a charge distribution more complex than a single sphere.

Kirkwood further extended the concept of Onsager by suggesting that the molecular distribution functions of the solvent around the cavity could be directly incorporated into the calculation [61]. In principle, this could be used to connect microscopic with the macroscopic measurements of the dielectric constant, since the distribution functions could be determined from molecular dynamics or other theoretical calculations. In fact, there has been considerable effort to derive the dielectric constant from models of statistical mechanics [35,12,120].

IIIc: Distribution Functions and Integral Equations

A formally exact way to calculate the polarization response of any medium to an arbitrary distribution of charges is to consider the distribution functions for solvent atoms about the solute in a suitable integral equation [14,48,104,63,98,64]. This approach has the advantage, at least formally, of generating a function that describes both the spatial and temporal properties of the solvent's response to a solute. Integral equations, moreover, bridge the gap between the continuum theories of Born and Onsager and molecular theories describing the behavior of atomic sites surrounding the solute. For example, a "Born-like" solvation energy can be calculated within the framework of integral equations with the following expression [105]:

$$\Delta G_{born} = \frac{q^2}{\pi} \int_0^\infty \left[\frac{1}{\epsilon_L(k)} - 1 \right] \left[\frac{\sin(k r_C)}{k r_C} \right]^2 dk \quad (6)$$

where $\epsilon_L(k)$ is the Fourier transform of the appropriate response function (which is computed from distribution functions [105]) and r_C is the effective cavity radius.

While the integral equation approach is formally exact, calculation of the distribution functions required as input can be difficult. Recently, though, an approach using molecular dynamics simulations and concepts from information theory has

suggested ways to make this method more practical [53,39]. In principle, a similar approach could be used for membrane systems. The necessary distribution functions for protein building blocks — such as alpha helices and beta sheets — could be estimated from molecular dynamics trajectories of these elements, considered individually in membrane environments.

Integral equation methods have already contributed concretely to the study of membrane electrostatics. For example, several groups have used this sort of approach to estimate the effects of surface charge distributions and different salt concentrations on the forces between membrane bilayers [45,62]. The calculations require as input the distribution functions describing the placement of charge near the bilayer surface. To our knowledge these methods have not yet been extended to describe a complete bilayer.

A recent calculation from the Roux group applies integral equation methods to the electrostatic response of a bilayer to the inclusion of membrane protein [67]. The calculation uses information from existing molecular dynamics trajectories in conjunction with the “hyper-netted chain” closure relation to derive a long-range solvation correction that can be applied to a new molecular dynamics simulation.

IIIId: Poisson-Boltzmann Calculations

The macroscopic equations of electrostatics can be used to solve for the electric fields for any distribution of charges in regions specified by uniform or step-wise discontinuous dielectric constants [55]. This approach has a long history in the physics community, but it does assume the existence of well-defined values for the dielectric constants [42]. That is, the solvent description is at the macroscopic level: it is implicitly assumed that the volume considered is large enough that specific molecular effects can be neglected and a relation such as (4) or (5) may be applied. This assumption is frequently violated when the equations of macroscopic electrostatics are applied to microscopic systems such as proteins, nucleic acids, and membranes. Moreover, the difficulties in defining the solute-solvent interface for continuum theories are present here as well, and are an immediate consequence of imposing a macroscopic theory. Despite these important theoretical flaws, macroscopic electrostatics calculations have been used with significant success in many areas of molecular biophysics [51].

The Poisson-Boltzmann equation is one of the most common approaches to computing solvation energies using continuum electrostatics. It approximates the electrostatic energy of a fixed system of solute charges, $\rho_{\text{int}}(\vec{r})$, embedded in a dielectric medium, including thermally averaged salt effects, by computing the total electrostatic potential, $\phi(\vec{r})$, according to

$$\nabla \cdot [\epsilon(\vec{r}) \nabla \phi(\vec{r})] - 4\pi \sum_s q_s c_s \exp[-\beta q_s \phi(\vec{r})] + 4\pi \rho_{\text{int}}(\vec{r}) = 0, \quad (7)$$

where the index s indicates salt species type, q_s is the charge of each species, c_s is the average number density of each species, and $\beta = 1/k_B T$. The sum of

exponentials reverts to the common sinh form for a 1:1 electrolyte. The Poisson-Boltzmann equation can be solved numerically for arbitrary solute charge distributions, using either self-consistent grid calculations or boundary element methods [42,52,72,100,130,134].

Applied to biomolecular systems, the Poisson-Boltzmann method typically divides space into a solute region and the surrounding solvent. The solvent, frequently water, is assigned a dielectric constant based on its bulk properties. An assignment must also be made for the solute region, but this is more difficult — as noted above — since the very concept of a dielectric concept assumes a bulk medium. In the case of proteins, low values (typically 2-4) were used initially, justified by measurements of the dielectric response of lyophilized protein powders. However, recent work has shown that a dielectric constant of 20 allows the model to better calculate pK values [41]. Still more recent work has shown that lower dielectric constants can be used, if conformational relaxation is factored into the calculations [128]. Finally, there has been considerable discussion of methods of directly calculating dielectric constants from all-atom molecular dynamics simulations [116,74].

Poisson-Boltzmann techniques have a few noteworthy drawbacks. First, as mentioned above, there is no theoretically rigorous way to define the surface of the solute. Second, solutions to the Poisson-Boltzmann equation are computationally expensive compared to evaluations of the potential functions typically used in molecular dynamics or Monte Carlo simulations. As a result, it is difficult to use these methods when conformational sampling of a large solute configuration space is required, although it must be noted that several groups are attempting to use them to perform long-range corrections for molecular dynamics simulations and ligand binding [118,115].

The Poisson-Boltzmann approach has had some notable success predicting the effects of varying salt concentrations on molecular binding. For example, one set of calculations was able to rationalize the experimentally measured salt dependence of the binding affinity of various drugs to protein and DNA [82,80,81,114].

A different series of calculations using Poisson-Boltzmann approaches were performed to elucidate the effect of salt on the binding of highly charged peptides to the membrane surface [97,8,88]. The calculations modeled the lipid surface as a regular array of lipid headgroups surrounding a slab of low dielectric. The results were successfully compared with Gouy-Chapman theory as well as experiment.

The Poisson-Boltzmann equation has also been used to compute the likelihood of ionization (pK) of protein sidechains. The approach has been applied to proteins in solution (for instance, the work of Gilson [41]), and to membrane proteins such as bacteriorhodopsin in an embedded slab environment [49,44].

As a final note, we address the tempting possibility to model the membrane “solvent” as a *non*-uniform continuum dielectric constant varying smoothly with position along the bilayer normal, $\epsilon = \epsilon(z)$. Superficially, this seems a reasonable way to consider a membrane with a “broad” interface (i.e., of finite thickness). Yet while the dielectric constant is a bulk property — implicitly averaged over a large volume — the membrane is a fundamentally molecular object: the properties of a

real interface change over a distance of roughly 10-15 Å [135]. Even if it is possible to define formally, the physical meaning of a dielectric constant which changes smoothly on atomic length scales is far from clear. One sees, then, a rationale — or at least an excuse — for the use of discontinuous interfaces: there is at least a possibility of intuitive, physical interpretation. From a microscopic viewpoint, nevertheless, the sharp interface in membrane models does not seem justifiable. Other paradigms are suggested: all-atom and lattice models, for instance, which are discussed below.

IIIe: The Generalized Born Equation

Several years ago, Still, *et al.* suggested a heuristic approach to the calculation of electrostatic solvation, typically referred to as the generalized Born approach [121]. More recently, several groups have published new versions of this technique, each implementing it slightly differently [111,119,26,24]. However, the basic underlying ideas are the same in each: treat the solute as region of low dielectric surrounded by a high dielectric solvent, calculate the “generalized Born radius” (σ_i) for each atom by estimating its electrostatic self-energy in the solute and calculating the atomic radius needed to produce that energy using the Born expression. These effective radii are then used to calculate the electrostatic solvation energy using the following semi-empirical expression:

$$\Delta G_{solv} = -166 \frac{\epsilon - 1}{\epsilon} \sum_{i,j}^{N_{atoms}} \frac{q_i q_j}{\sqrt{r_{ij}^2 + \sigma_i \sigma_j \exp\left(\frac{-r_{ij}^2}{4\sigma_i \sigma_j}\right)}} \quad (8)$$

where r_{ij} is the distance between solute atoms i and j , and the factor of 166 again yields the energy in kcal/mole.

This expression has no rigorous theoretical basis. However, it approximates a sum of the Born and Coulomb energies when two atoms are widely separated, and numerically resembles the Onsager term when two atoms are close together. More to the point, its results compare favorably to those calculated using the Poisson-Boltzmann equation at a fraction of the computational cost [26,52]. The generalized Born expression has moreover, recently been used to evaluate protein folding behavior and DNA dynamics [119,83,24].

Yet adapting this approach to represent membranes may be difficult, for the same reasons as with the Poisson-Boltzmann equation: a bulk dielectric constant is only physically meaningful when defined over a reasonably large region, but membrane properties change over small distances. Moreover, it is not clear how to consistently modify both the generalized Born expression and the calculation of the effective radii to represent a heterogeneous environment.

III f: Accessible Surface Area Models

There is a large family of solvent models which rest on the assumption that the solvation energy is proportional to the amount of surface area exposed to solvent [69]. Although not strictly electrostatics models, they are commonly used to estimate solvation energies, especially in certain fields such as protein structure prediction.

In simplest form, surface area methods work by calculating the accessible surface area for a given molecule and multiplying by an effective “surface tension” to produce a solvation free energy. This approximation works quite well for simple hydrocarbons in water, but does poorly when the solute has a more complex chemical structure [117]. This difficulty is typically overcome in one of two ways. First, the surface area model can be used to capture only the hydrophobic component of the solvation energy, while the electrostatic component is calculated using Poisson-Boltzmann, generalized Born, or other methods (e.g. Ref [121,42]). Alternatively, one can abandon the notion of a single “surface tension” in favor of so-called “atomic solvation parameters,” multipliers whose values depend on the chemical type of the atom or group [29].

The surface area approach has some significant advantages: it is inexpensive computationally (compared to Poisson-Boltzmann or explicit solvent calculations), relatively easy to implement, and seems to capture the hydrophobic effect, which is a dominant phenomenon in many biologically relevant situations. Recently, a pair of papers by Efremov *et al.* discussed an extension of this technique to membrane systems using a “group-parameter approach” [27,28]. However, in their modified version the “solvent” was chosen as isotropic and homogeneous, which would be more appropriately described as a bulk hydrocarbon model, rather than a bilayer model.

Efremov *et al.* were not the first to attempt a group-parameter approach to membrane solvation energy calculations. Several years earlier, Sanders and Schwonek presented a membrane model using an empirical energy expression and a large body of experimental small-molecule partitioning data to fit solvation parameters for a wide variety of chemical groups [110]. Although this method was reasonably effective at calculating transfer energies, its applicability to larger molecules (e.g. membrane proteins) is doubtful, since group-wise additivity of solvation energies typically does not hold for large systems.

The main difficulty with surface area approaches is that, as mentioned above, they make no direct connection to underlying electrostatic theory. As a result, solvation energies calculated solely using these methods usually perform poorly when compared to Poisson-Boltzmann or even generalized Born calculations [26,52].

IIIg: Lattice Models

Between the microscopic detail of atomic solvent representations and the macroscopic continuum dielectric models lie mesoscopic models. These approaches maintain solvent discreteness without the cost of explicitly representing all the solvent atoms. Many of these models represent the solvent as a lattice of dipoles.

Dipole lattice models have several important advantages. As with continuum models, analytic results are readily available for several simple cases. Indeed, in the limit of an infinitely fine lattice, lattice dipole calculations will converge to the continuum results [91]. At the same time, because of the finite lattice spacing, these models share a real solvent's capacity for discrete local response, which in principle makes them more realistic.

Lattices of dipoles were first studied more than fifty years ago. One of the first steps was an analytic calculation by Lax and coworkers, extending earlier work by Berlin and Thomson, which allowed direct comparison between lattice calculations and continuum methods [9,68,106,123]. The key to their approach is the mean spherical approximation, where long-range dipole interactions are treated in an approximate way, while local interactions are included explicitly. This greatly simplifies calculations of otherwise intractable quantities such as the temperature dependence of the dielectric constant. The Lax papers showed that, up to a third order expansion in inverse temperature, lattice results for dipole solvation agree well with Onsager's continuum theory [90]. When the fourth order term was included, the lattice calculations were in better agreement with experiment than the continuum results.

Fulton further extended the lattice approach [37,38] using a method first applied by Van Vleck [126,127]. Basically, the lattice points are populated with harmonic oscillators, whose response to electric fields can be calculated using Green's functions. As a result, one can directly calculate the experimentally verifiable spectral properties of the system. Again, the lattice results were more accurate than the analogous continuum work.

Around the same time, a computational study by Adams and McDonald examined the dielectric properties and free energy of a range of simple cubic and face-centered cubic lattices of dipoles [1]. An interesting result was the sensitivity of the calculations to the treatment of long range effects. Calculations using "reaction fields" were compared to those using numerical evaluation of Ewald sums. Another point of comparison was with the results of Lax for the same lattice types. Intriguingly, the mean spherical approximation performed quite well against the numerical data.

In work suggestive of membrane geometry, Munn *et al.* have recently used lattice models to calculate surface properties of crystals and Langmuir-Blodgett films [86,85,87]. In particular, they apply a plane-wise approach to the sum of dipole lattice properties [23,99] that may have implications for any situation where the properties vary as a function of the plane location and/or type — perhaps for membranes. For example, the approach is capable of giving an estimate of the

local fields at lattice sites in the presence of an applied field and thus can be used to calculate optical properties from assumptions about the molecular arrangement.

Also taking a two-dimensional approach, lattice sums have been applied to calculations on electrorheological fluids (solutions of high dielectric particles in low dielectric media). For example, Clercx and Bossis [18,19] have developed methods to use lattice sums to calculate surface effects, again by summing contributions from different planes. These calculations were compared to experimental results and appear to provide insight into the molecular basis of the electrorheological behavior.

Another important application of lattice calculations has been to examine the temporal response of solvent to charge changes. This research direction appears to have been initiated in a combination of analytic and lattice computational work by Loring and Mukamel [73]. In particular, the study inspired two other groups to use Brownian lattice dipoles to examine the temporal response of a lattice to charge changes [139,92]. In the case of Bagchi's research [139], the computed properties of the lattice were directly compared against the temporal response derived by Zwanzig [140] from the high temperature expansion due to Lax. The group of Maroncelli has been particularly concerned with temporal response and compared simulations with the work of Fleming [33] and their own experimental work [92].

In an explicitly biological context, Warshel and co-workers have used dipole lattices to calculate solvation energies for protein systems, focusing on conformations and reaction mechanisms [133,131]. They used similar techniques to model ion permeation through channels [4]. Rather than explicitly model the reorientation of dipoles as was done by Maroncelli [92], most of these computations use the Langevin response function [50] to calculate the thermally averaged polarization of a dipole due to the solute electric field. Explicitly, the Langevin expression for the average dipole moment at lattice site i is

$$\vec{\mu}_i^L = \mu_o \left(\coth \chi_i - \frac{1}{\chi_i} \right) \vec{e}_i \quad (9)$$

where

$$\chi_i = \frac{\mu_o}{k_B T} |\vec{\xi}_i - \vec{\xi}_i^c| \quad (10)$$

In this context, $\vec{\mu}_i^L$ is the resulting dipole vector, μ_o is the magnitude of the dipole, $\vec{\xi}_i$ is the electric field at lattice point i , $\vec{\xi}_i^c$ is the electric field due to the nearest neighboring dipoles, and \vec{e}_i is a unit vector parallel to $\vec{\xi}_i$. Solvent polarization can be calculated by iterating the fields to self-consistency, or by the use of an empirical distant dependent field modulation [133].

Calculations using the Langevin-dipole lattice have been used to predict the changes in solvent reorganization energy for different stages of the photosynthetic reaction center electron transfer cycle [131,2]. This reorganization energy is important for the Marcus theory of electron transfer [76], and a good estimate of

the solvent response to the solute charge is central to understanding the rates of electron transfer measured experimentally. The calculations assumed an embedded slab membrane model and started from the X-ray structures of the reaction center [22]. The results of these calculations disagree with a similar set of calculations using the Poisson-Boltzmann formalism [47], leaving the relative merits of the two models, in this context, a matter of debate.

In work with relatively transparent implications for membrane electrostatics, Macdonald and coworkers used lattices to model an electrical double layer, with solvent ions and dipoles in competition for lattice sites [75,58]. The model assumes a series of coupled lattice planes parallel to a planar electrode: planar averages are made self-consistent by requiring electrostatic continuity. In the limit of low applied fields, the resulting series of equations can be solved for the effective charges, potential and electric field within each plane. For higher fields a numeric approach is adopted. The results appear to be an improvement over the earlier continuum models [43,15], but are still lacking in comparison to electro-chemistry experiment. Nevertheless, the potential for membrane applications appears quite strong.

IIIh: Explicit-Atom Solvent Models

All-atom simulations are the most computationally expensive models of the solvent setting, because they represent the solvent at the same level of detail as the solute. Explicit solvent simulations of proteins and peptides date back to the early 1980s [13]. However, explicit membrane representations have only been performed for the last 10 years [96]. In fact, the first simulations of a protein in an explicit bilayer was only performed 5 years ago, a simulation of gramicidin in a DMPC bilayer [138].

These calculations yield an unprecedented quantity of information about the molecular-level behavior of membranes. However, there are still some methodological issues that need to be resolved. First, it is not clear what thermodynamic ensemble should be used for these simulations. The earliest membrane simulations used the microcanonical ensemble (NVE – constant atom number, volume, and energy). This is the simplest to implement, but as a result there is no direct mechanism to control the temperature and pressure. As a result, the normal pressure and surface tension are determined entirely by the choice of unit cell dimensions made when the system is constructed. More recently, simulations of the canonical ensemble (NVT – constant atom number, volume, and temperature), as well as constant pressure and temperature ensembles, have become possible. Although the choice of pressure tensor is obvious when simulating a protein in water (1 atm in all directions), it is considerably less clear in a membrane, which may have a non-zero surface tension in the plane of the bilayer. Indeed, this point is still being actively debated in the lipid simulation literature [31,16,32,125,56].

The other major drawback to this sort of simulation is the computational expense. Even running on a fast workstation or cluster of workstations, calculating

a one nanosecond trajectory for 10^4 atoms would take weeks to months, consume roughly a hundred megabytes of memory for the life of the simulation, and produce several gigabytes of data. Unfortunately, 10^4 atoms translates only to a small solvated bilayer patch, perhaps 20 lipids per leaflet. Much larger systems will be needed to accurately calculate many bilayer properties, such as undulation. Moreover, many important phenomena like lipid translocation, ion permeation, and even simple rearrangement take place on microsecond to millisecond timescales, which are not sampled in the current simulations.

IIIi: Evaluation of Membrane Electrostatic Models

Table 1 summarizes the characteristics of the various membrane models considered above. Several points should be noted while examining the table. First, we scored the models based on their best possible performance in each category. For example, the temporal response of dipole lattices was scored as “Good”, because while Langevin dipoles represent only equilibrium behavior, Brownian dipoles can accurately model solvent relaxation. Similarly, there are a wide variety of explicit atom models available, running from “united atom” pictures to mixed quantum mechanical and all-atom simulations. All of these models would capture some aspects of relaxation dynamics, but only the mixed quantum simulations could directly model the fastest relaxation processes involving the electronic degrees of freedom.

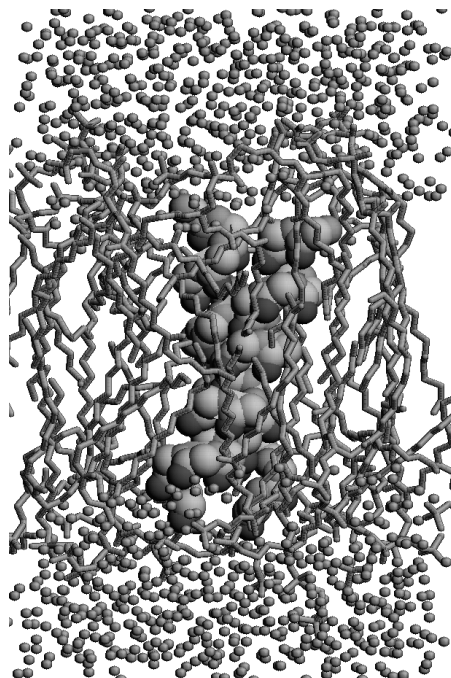


FIGURE 2. Explicit all-atom simulation of a glycoprotein A transmembrane domain dimer embedded in a DMPC lipid bilayer.

TABLE 1. Comparison of models for the electrostatics of the membrane bilayer.

Model	Accuracy	Molec. Reality	Temporal Effects	Comput. Time
Constant Dielectric	Poor	Poor	Not possible	Very Rapid
Born	Poor	Poor	Poor	Very Rapid
Onsager	Poor	Poor	Poor	Rapid
Integral Equation	Good	Good	Good	Slow
Poisson Boltzmann	Fair	Fair	Fair	Slow
Generalized Born	Fair	Fair	Fair	Rapid
Atomic Solv. Param.	Poor	Poor	Poor	Rapid
Dipole Lattices	Good	Fair	Good	Slow
Explicit Atoms	Excellent	Excellent	Excellent	Extremely Slow

Furthermore, as has been stressed several times, the acceptability of a given model depends strongly on what problem is being considered, the degree of accuracy required, and how much computer power is available. For some problems, such as estimation of drug partitioning or evaluation of a models for membrane protein structures, a quick and relatively rough calculation may suffice.

IV: LATTICE DIPOLE MODEL FOR THE MEMBRANE BILAYER

We (Grossfield and Woolf) are currently pursuing a lattice dipole model for the membrane setting. Essentially, we construct a lattice of Langevin dipoles, varying the magnitude of the dipoles (μ_0 in equation (9)) as a function of position along the z -axis, chosen to be the bilayer normal. Varying the magnitude of the dipoles allows us to accurately represent atomic-scale variations of the bilayer environment: the relatively nonpolar center of the membrane, the polar water region outside the membrane, and most importantly, the region of intermediate polarizability in between. The dipole lattice model could be used on its own, or in conjunction with a small number of explicit lipids and waters, depending on the desired level of realism.

The model could be parameterized in two separate ways. The first way is to fit the model parameters to reproduce experimental small molecule partitioning data. This would guarantee that the model reproduced at least some subset of the available data. However, for most of the compounds measured, the equilibrium location (or, more generally, distribution) in the membrane is not known, complicating parameterization. Moreover, there is significantly less data available for membrane partitioning than for bulk hydrophobic solvents such as octanol or cyclohexane [30,102,137]. Bulk solvent partitioning data could be used to tune the parameters for the water region and the hydrophobic core, but this approach yields few constraints on the interfacial parameters, and does not guarantee a self-consistent

bilayer parameterization.

The model could also be parameterized to reproduce the polarization behavior of a real bilayer, as represented in a molecular dynamics simulation. This would in principle allow us to capture more of the atomic properties of a bilayer. However, it would tie the model to a specific lipid type. More importantly, the limited timescale of current molecular dynamics simulations makes it difficult to be sure all of the physically relevant polarization is sampled.

For this last reason, we are currently pursuing the first method and parameterizing our lattice dipole model against the experimental literature values, while taking care to produce self-consistent parameters. However, we may use long dynamics simulations to help us refine the parameters at a later date.

V: CONCLUSION

One of the most critical aspects of solvent modeling is the representation of electrostatic response — and it is an especially acute problem for the simulation of membrane proteins. These proteins “live” in a highly heterogeneous environment very different from the isotropic media usually considered by solvation theories. This review has attempted to assess an array of existing and potential models in terms of the context-sensitive criteria set out in Sec. II: accuracy, molecular reality, temporal response, and computational efficiency. A summary of the results is given

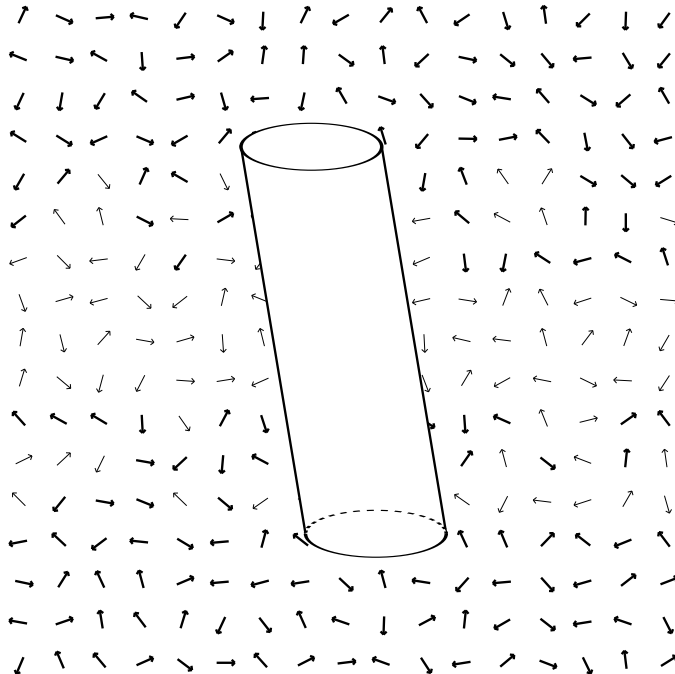


FIGURE 3. A lattice of dipoles with properties changing as a function of position with respect to the bilayer normal

in Table 1. Although a significant amount of useful and interesting information is available for membrane systems, many more calculations are needed to determine what kinds of representations will ultimately be most effective in rationalizing the available experimental information.

Perhaps the most important and difficult aspect of the bilayer environment, from a modeler's point of view, is the membrane-water interface. This broad region can cover as much as half the total width of the bilayer, but is frequently modeled as vanishingly thin. While such approximations make computations more tractable, they miss a crucial element in the bilayer's structure and function. This review stresses interface modeling in membrane electrostatics.

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