Section 5
Biological Modeling

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# Modeling Signaling Processes across Cellular Membranes Using a Mesoscopic Approach

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## Abstract

Computational models are effective in providing quantitative predictions on processes across cellular membranes, thereby aiding experimental observations. Conventional computational tools, such as molecular dynamics or Monte Carlo simulation, offer significant insights when applicable. However, it remains extremely difficult to use these simulation methods to describe large

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macromolecular assemblies within timescales relevant to a vast majority of critical physiological processes. To overcome this outstanding challenge, alternative methods based on coarse-grained representations have more recently emerged. In this chapter, we review one such particular advanced methodology that is based on mean-field-type representations typically used for equilibrium thermodynamic calculations of lipids and proteins. The main advantages of this self-consistent scheme are in adding information concerning longer timescales and in gaining access to the steady state of the system without making a priori assumptions concerning protein–membrane interactions. We illustrate this methodology using several examples pertaining to interactions of peripheral signaling proteins with lipid membranes. These examples outline the current state of the computational strategy and allow us to discuss several future enhancements that should help the scheme become a powerful methodology complementary to other simulation techniques. With these extensions, the proposed methodology could enable quantitative description of large-scale membrane-associated interactions that are of major importance in physiological processes of the healthy and diseased cell.

Keywords: cell signaling; lipid rafts; BAR domains; membrane curvature; membrane elasticity; PIP2 diffusion; mean-field model; coarse-grained theory; Poisson–Boltzmann theory; Cahn–Hilliard equations

1. INTRODUCTION

1.1 Lipid rafts as platforms for cellular signaling

Overwhelming evidence indicates that function and organization of protein components of living cell membranes are orchestrated at specific spatial and temporal scales. In particular, structural, compositional, and mechanistic properties of lipid bilayers play a significant role in regulating the physiological function of membrane-associated proteins [1]. One of the best known examples is the existence of specialized plasma membrane domains, typically enriched in cholesterol and sphingolipids. These patches, termed “rafts”, have been implicated as platforms for various physiological processes, and specifically for cellular signal transduction [2].

As such, rafts have been shown to be important in regulating the function of both transmembranal (TM) and peripheral signaling proteins. For instance, evidence suggests that cholesterol-dependent separation of the TM signaling G-protein-coupled receptors (GPCRs) from their partners can be a determining factor for signaling efficacy [3]. Another example is the use of polyvalent phosphatidylinositol 4,5-bisphosphate (PIP2) lipids, also found to be enriched in rafts, for membrane targeting by various peripheral signaling motifs, such as C2 [4], PH [5], FERM [6], and BAR domains [7]. BAR domains present a particularly fascinating case because they have been found to act as mechanistic modules that are capable of locally reshaping plasma membranes as a part of cell signaling and other physiological functions such as endocytosis [7]. Importantly, BAR modules are involved synergistically with other protein domains, such as PDZ domains, in
interactions with GPCRs to direct subsequent steps in signaling through their effects on membrane remodeling. While this synergism has been proposed specifically for those proteins interacting with C-kinase 1 (PICK1) [8], the abundance of BAR domain containing proteins highlights the importance of this class of mechanisms and their putative physiological roles.

A fundamentally important question for the role of rafts in cellular signaling is whether such domains exist preformed in living cells so that they can be recognized by the cellular protein machinery, or alternatively, could rafts present structures that dynamically assemble, adopting specific lipid composition or membrane deformations around specific protein components in response to physiological function [2]. Biochemical and biophysical studies conducted in vitro on cell membranes, as well as on model lipid assemblies, established that rafts can exist as stable membrane domains in the liquid-ordered (L_α) phase surrounded by a relatively fluid (L_α) lipid environment (for example, see References [9–14] and references therein). These domains are physically different both structurally and mechanistically from the surrounding lipid matrix. In particular, rafts are generally thicker and more rigid compared to other membrane compartments [15–19]. These studies also identified additional putative raft components, such as glycosylphosphatidylinositol (GPI)-anchored proteins or TM domains [20,21]. However, despite the wealth of data collected in vitro, the challenge in the field still remains to link structural and mechanistic raft characteristics observed in artificial systems with those in living cell membranes under native conditions, where language borrowed from macroscopic phase transitions may become inadequate [2,16].

1.2 The need for large-scale quantitative models to describe complex signaling machinery

The difficulty with realistically describing rafts and associated interactions during signal transduction originates from the large number of concerted actions involved. When signaling proteins and other macromolecules adsorb, diffuse on cell membranes, penetrate into the membrane, and associate/dissociate in complexes within the membrane, they interact through intricate forces that ultimately determine biological function. This complexity of interaction makes it conceptually challenging and computationally very costly to quantify such encounters at the macromolecular level.

Computational models are powerful in aiding experimental observations by providing quantitative, testable predictions. However, while conventional computational tools, such as molecular dynamics (MD) or Monte Carlo (MC) simulations, offer significant insight when applicable, it remains very difficult to use them in order to describe large macromolecular assemblies within timescales relevant to a vast majority of critical biological processes. Even if the required force-fields are available, using current supercomputational resources, it is possible in exceptional cases to use MD simulations for ca. 1 μs for systems as large as 250,000 atoms. But even these relatively extended size and timescales do not permit the consideration of processes that include membrane reshaping, lipid
reorganization, and protein–protein interactions, which evolve concertedly at the lipid membrane interface.

Not surprisingly, methods have been devised in sustained efforts to address this perennial challenge. Recent computational strategies have attempted to coarse-grain the system, thus lowering the number of degrees of freedom addressed by the model, thereby also reducing the required computational effort (see, e.g., References [22–25]). However, most of these strategies rely on designing force-fields for specific mesoscopic models, a formidable task in itself.

We have been pursuing a somewhat different approach, which takes advantage of the extensive knowledge and quantitative information accumulated on lipids, proteins, and their interactions. In particular, to model membrane-associated interactions during cellular signaling, we take advantage of available information on the elastic, entropic, and electrostatic properties of lipids and proteins [26,27]. Our starting point is mean-field-type theories that are typically used for equilibrium thermodynamic calculations of lipids and macromolecules such as proteins. Information resulting from these models is then fed as inputs to dynamic Cahn–Hilliard (CH) and stochastic Langevin formulations [28] that allow probing of the molecular interactions of membrane-associated proteins in time and space. With this algorithmic formulation, we concentrate on explicitly describing only a smaller number of important degrees of freedom, precluding the need to model individual lipid components.

In this review, we describe our modeling strategy and present several applications of the method. All the considered examples relate to interactions of peripheral signaling motifs, such as BAR domains or basic (hence positively charged) polypeptides with membranes of raft-like lipid compositions. We aim to illustrate the effectiveness of our approach in describing dynamic membrane processes that involve membrane remodeling upon protein adsorption, as well as lipid rearrangement and segregation following their interaction with adsorbing proteins.

The main advantages of this self-consistent scheme are in adding information concerning longer timescales and gaining access to the steady state of the system without making a priori assumptions on protein–membrane interactions. We end by discussing future perspectives and possible extensions of the model that will hopefully allow this to become a powerful methodology complementary to other simulation techniques, such as MD. Together, these strategies should enable the study of large-scale membrane-associated interactions that are of major importance in physiological processes of the cell in both healthy and diseased states.

2. MESOSCOPIC MODEL OF MEMBRANE-ASSOCIATED SIGNALING COMPLEXES

2.1 Overall strategy

We start by discussing our overall strategy to coarse-grain macromolecular representations by using available information from experiments and from results of atomistic simulations on the material properties of proteins, membranes, and lipid
components, as well as their interactions. This information can be used in order to
device models that treat explicitly only a smaller number of important degrees of
freedom. Thus, to quantify the combined kinetic effect of many lipid species
interacting with peripheral proteins, and to describe the concomitant membrane
shape perturbations, it is essential to be able to calculate the steady state of
adsorbing macromolecules in a way that will include all important degrees of
freedom in a self-consistent manner. These interactions include electrostatics
(Coulomb) forces, lipid mixing, and membrane elastic deformations. In our for­
mulation, self-consistency is achieved by minimizing the governing model free
energy density functional, which is based on the continuum Helfrich free energy
for membrane elasticity [29], and on the nonlinear Poisson–Boltzmann (PB) theory
of electrostatics [30–34]. By providing a realistic three-dimensional treatment of the
electrostatic problem, and requiring only a few phenomenological material con­
stants to describe the lipid bilayer, this simple formalism accounts for a number of
important membrane properties. Although this mesoscopic theory neglects most
atomic structural features of a lipid bilayer [34,35], similar membrane and mem­
brane–macromolecule models have been shown to yield reliable qualitative and
quantitative predictions [36–47].

2.2 System representation and governing free energy

Our method uses an atomic-level representation of the adsorbing protein in three
dimensions, and accounts for lateral reorganization and demixing of lipids, as
well as membrane deformations upon adsorption (see Figure 1). We consider the
limit of low surface density of adsorbing proteins, so that interactions between

![Figure 1](image_url)

**Figure 1** View of the BAR–membrane complex at steady state, as predicted from coarse-grained
mean-field-level calculations. BAR is shown in spacefill; the membrane interior is shaded gray. In
this hybrid model, BAR domain is represented in its all-atom detail, through partial charges and the
radii of each constituent atom. The membrane is represented as a two-dimensional
incompressible, tensionless, elastic medium comprised of 2D smooth charged surfaces (where the
lipid polar head-groups reside), and a low-dielectric hydrophobic core volume. Elastic properties of
the membrane are characterized by its bending modulus and locally defined spontaneous
curvatures. The system is driven toward equilibrium through the self-consistent minimization of
the free energy functional, the latter containing contributions from the system’s electrostatic
energy, mixing entropy of lipids and ions in the solution, and membrane deformation energy.
proteins are negligible. The adsorbing protein is represented in full-atomistic 3D details, whereas the membrane is considered as a two-dimensional fluid, allowing us to treat lipid head-group charges in the continuum representation, as usual in regular solution theory.

For simplicity of presentation, we assume here membranes containing binary mixtures of acidic and neutral (zwitterionic) lipids. The temporal evolution of the spatially varying charged-lipid compositions on the membrane upper (u) and lower (l) leaflets are linked to the Laplace–Beltrami (LB) operators acting on the corresponding electrochemical potentials through two CH equations (one for each leaflet) each of the form [28]:

\[
\frac{\partial \phi(\vec{r}, t)}{\partial t} = D_{\text{lip}} \nabla_{\text{LB}}^2 \mu(\vec{r}, t) = \frac{D_{\text{lip}}}{\sqrt{g}} \partial_i \left( \sqrt{g} g^{ij} \partial_j \mu(\vec{r}, t) \right)
\]  

(1)

Here, \( \phi \) and \( \mu \) denote respectively the local mole fraction and local electrochemical potential of the charged lipid species in that particular leaflet, \( g \) is the metric tensor defined on the leaflet surface, and \( D_{\text{lip}} \) represents the diffusion coefficient of charged lipids. Note that \( D_{\text{lip}} \) should not affect the equilibrium state. The local electrochemical potentials, in turn, are derived from the free energy functional that itself depends on local lipid component densities \( \phi \) and membrane curvature. This property results in a self-consistent formulation, which remains as the main computational task.

More specifically, we assume that the system’s free energy \( F \) consists of electrostatic energy, mobile salt ion translational entropy, lipid mixing entropy contributions, membrane bending energy, and a short-range repulsive interaction energy acting between protein and membrane interfaces [26,27,36,43,44]:

\[
F = F_{\text{el}} + F_{\text{IM}} + F_{\text{lip}} + F_b + F_{\text{rep}}
\]  

(2)

The system’s electrostatic (Coulomb) energy is given by

\[
F_{\text{el}} = \frac{1}{2} \varepsilon_0 \left( \frac{k_B T}{e^2} \right) \int_{V} \varepsilon_d (\nabla \Psi)^2 \, dv
\]  

(3)

Here, \( \Psi = e \Phi / k_B T \) is the dimensionless (reduced) electrostatic potential, with \( \Phi \) representing the electrostatic potential, \( k_B \) the Boltzmann’s constant, \( T \) the temperature, and \( e \) the elementary charge; \( \varepsilon_0 \) is the permeability of free space, while \( \varepsilon_d \) is the dielectric constant within the volume element \( dv \). We take \( \varepsilon_d \) as 2.0 inside the membrane and the protein and as 80.0 in the aqueous solution. The integration in Eq. (3) is performed over the volume \( V \) of the entire space.

The contribution from the translational entropy of mobile (salt) ions in solution is

\[
F_{\text{IM}} = k_B T \int_{V} \left[ n_+ \ln \frac{n_+}{n_0} + n_- \ln \frac{n_-}{n_0} - (n_+ + n_- - 2n_0) \right] \, dv
\]  

(4)
where \( n_+ \) and \( n_- \) are local concentrations of mobile cations (+) and anions (–), respectively, and \( n_0 \) is the electrolyte concentration in the bulk.

The contribution from the 2D mixing entropy due to the mobile lipid molecules within each leaflet is

\[
F_{\text{lip}} = \frac{k_B T}{a} \left[ \phi_u \ln \frac{\phi_u}{\phi_u^0} + (1 - \phi_u) \ln \frac{1 - \phi_u}{1 - \phi_u^0} \right] dA_u + \frac{k_B T}{a} \left[ \phi_l \ln \frac{\phi_l}{\phi_l^0} + (1 - \phi_l) \ln \frac{1 - \phi_l}{1 - \phi_l^0} \right] dA_l
\]

(5)

These integrals represent entropic penalties associated with lipid demixing due to possible lipid segregation, on the upper and the lower surfaces of the membrane, respectively. In Eq. (5), \( \phi_u^0 \) and \( \phi_l^0 \) denote the average compositions of charged lipids on the respective leaflets, and \( a \) represents the area per lipid head-group.

The membrane bending energy in Eq. (2) is the sum of local elastic energies associated with deformations of individual membrane leaflets away from their spontaneous curvatures, as described by the Helfrich free energy:

\[
F_u = \frac{1}{2} \kappa_m \left( c_u - c_u^0(\phi_u) \right)^2 dA_u + \frac{1}{2} \kappa_m \left( c_l - c_l^0(\phi_l) \right)^2 dA_l
\]

(6)

Here, \( c_u \) and \( c_l \) are the local mean curvatures of each of the two membrane monolayers, and \( \kappa_m \) denotes the bending rigidity of a single monolayer that is here assumed to be the same for each leaflet and for both lipid species. The spontaneous curvatures of the two leaflets, \( c_u^0 \) and \( c_l^0 \) are described as sums of the spontaneous curvatures of the pure lipid constituents weighted by their local compositions. This approximation has been previously validated [36,48].

Functionally minimizing \( F \) with respect to the compositional degree of freedom \( \delta F/\delta \phi = 0 \) results in an expression for the (local) electrochemical potential for charged lipid species on each leaflet [27,36]:

\[
\mu = \mu^0 + k_B T \left( \ln \frac{\phi(1 - \phi^0)}{\phi^0(1 - \phi)} + z\Psi \right) + a \kappa_m (c_n^0 - c_c^0)(c - c^0(\phi))
\]

(7)

where \( \phi^0 \) is the average mole fraction of charged lipids, \( z \) denotes the valency of charged lipid species, and \( c_n^0 \) and \( c_c^0 \) represent the spontaneous curvatures of the pure neutral and charged lipids, respectively. Adding lipid species to the formulation is straightforward, and simply involves modifying the free energies to include an additional compositional variable.

Finally, the short-ranged repulsive term \( F_{\text{rep}} \) accounts for the energy contribution related to excluded volume and hydration forces that appear when two surfaces (protein and membrane) come into close proximity of each other [49–52]. This term in the free energy functional is taken as a hard wall potential...
that restricts membrane–protein minimal approach to be \( \geq 2 \text{Å} \), and excludes any configuration that violates this limitation.

### 2.3 Free energy minimization

The free energy functional in Eq. (2) must be minimized with respect to all relevant degrees of freedom in a self-consistent manner. In particular, functionally minimizing \( F \) with respect to the mobile ion concentrations leads to the familiar nonlinear PB equation [26–36]:

\[
\nabla^2 \Psi = \lambda^{-2} \sinh \Psi \tag{8}
\]

where \( \lambda = (\varepsilon_0 \varepsilon_w k_B T / 2 e^2 n_0)^{1/2} \) is the Debye length. This equation is typically used to describe electrolyte solutions at the mean-field level. Solving Eq. (8) for the volume occupied by the aqueous solution yields the reduced electrostatic potential \( \Psi \) in space. Note that \( \Psi \), in turn, is linked to local lipid compositions in each leaflet through the boundary condition on the leaflet surface \( \partial \Psi / \partial r = -e \zeta \phi / (a \varepsilon_0 \varepsilon_d) \), where \( z \) and \( a \) are valency and lateral area per head-group of charged lipids, respectively.

Using the expression for the chemical potential Eq. (7) together with the nonlinear PB Eq. (8) for electrostatic potential and the CH Eq. (1) that describes the temporal evolution of the system from any arbitrary (nonequilibrium) state, the total free energy can be minimized with respect to the local lipid compositions [53–55]. Practically, this is done by following the state of the system at long times, where steady state is reached. One tacit assumption behind our minimization strategy is that lipid diffusion is fast enough so that lipid compositions locally and continuously adapt to the electrostatic potential in space emanating from the macromolecular adsorbate.

In fact, \( F \) is also required to be at a minimum with respect to all possible membrane deformations, and this minimization with respect to membrane shape must be carried out self-consistently together with the electrostatic and lipid mixing contributions [27,36]. This presents a challenge, since in principle one has to consider all possible variations in membrane geometry, and these multiple shape deformations generally couple to other degrees of freedom.

To overcome this problem, we have designed a novel combined scheme that efficiently accounts for bilayer deformations together with the electrostatic PB solution self-consistently [27]. Our strategy is based on representing the membrane interface shape (contour) as a linear superposition of \( N \) Gaussian functions (used here as a basis set) centered at different locations on the surface of the membrane. In this manner, we can approximate the local membrane height \( h(x,y) \) at any point \((x,y)\) by the following sum [27]:

\[
h(x,y) = \sum_{i=1}^{N} A_i \exp \left[ - \left( \frac{(x-x_i^0)^2}{\sigma_{xi}^2} + \frac{(y-y_i^0)^2}{\sigma_{yi}^2} \right) \right], \tag{9}
\]
where $A_i$-s and $\sigma_i$-s denote the amplitude and variances of the $i$th Gaussian centered at $(x_i, y_i)$. With that, we sample membrane deformations by varying only the Gaussian amplitudes. Note that, $A_i$-s and $\sigma_i$-s are coupled to local membrane curvature through the relation $c = \nabla^2 \chi_{LB} h(x, y)$, while this local curvature itself is linked to the local lipid composition $\phi$ as described in Eq. (6).

The described minimization procedure significantly reduces the dimensionality of phase space that needs to be explored. In the minimization procedure, different Gaussian’s amplitudes are varied at random, and trial moves are accepted only if following the move the free energy is reduced. To ensure self-consistency, at each trial move we also solve the appropriate PB equation to obtain the electrostatic potential for the particular membrane shape. To couple shape changes to lipid mixing, we alternate between steps aimed at varying membrane deformations, with CH moves that spatially propagate local lipid compositions. This procedure allows the solution to converge to the (local) minimum of the total free energy.

2.4 Quantitative description of peripheral protein diffusion

To also follow the diffusion of an adsorbed protein on a membrane interface, our method implements a dynamic Monte Carlo (DMC) scheme [56–62]. This procedure is advantageous in that it directly relies on available free energies and does not require additional force calculations, which can be time-consuming. According to this scheme, the adsorbed protein diffuses on the membrane surface tracing a stochastic dynamic trajectory. This probabilistic path is generated in accordance with the fluctuation–dissipation theorem, as the adsorbent’s center of mass makes random displacements in the two directions of the membrane plane each of size $\alpha \sqrt{2 \Delta t'} D'$, where $\alpha$ is a Gaussian random number with zero mean and unit variance, $\Delta t'$ denotes the dimensionless time-step, and $D'$ represents the ratio of protein to lipid diffusion constants [26]. The random trial move is then accepted or rejected according to a Metropolis-like criterion employing the usual transition probability $W$ of value

$$W = \begin{cases} 1 & \text{if } F_{\text{new}} = F_{\text{old}}, \text{ and } F_{\text{new}} > F_{\text{old}} \\ e^{-\frac{(F_{\text{new}} - F_{\text{old}})}{k_B T}} & \text{if } F_{\text{new}} > F_{\text{old}} \end{cases}$$

Here, $F_{\text{old}}$ and $F_{\text{new}}$ are, respectively, the adsorption free energies of the “old” state (before the trial move) and “new” state of the protein–membrane system. If a trial move is accepted, the macromolecule is advanced to the “new” position, the CH equations for lipids are solved, and sampling of membrane deformations are performed for the newly accepted position of the adsorbate. If, on the other hand, the trial move is rejected, the protein remains at its previous position, and the same minimization step is conducted, only now with respect to the previous “old” location of the adsorbate.
2.5 Accounting for amphipathic helix insertions

An additional important force that should be considered corresponds to the effect of protein amphipathic helix membrane insertions that often play a critical role in attracting proteins to lipid membranes and in generating membrane curvature (see below). We have made use of an implicit representation of this effect by defining a membrane area (patch) of positive spontaneous curvature (defined as curving “toward” the adsorbing protein) that forms directly “under” an adsorbing protein at the interaction zone. We then use a phenomenological approach that assumes that the inclusions perturb the bilayer symmetry and its elastic properties primarily around the area of helical insertion [63,64]. We account for insertions to different membrane depths by varying the value for the spontaneous curvature assigned to this locally perturbed membrane region. For each insertion depth, the bilayer is allowed to adjust its geometry locally. The corresponding deformations at steady state for each penetration depth is found by minimizing the modified free energy functional, which now contains an additional (elastic) free energy term accounting for the nonzero spontaneous curvature region near the adsorbed protein.

3. MODEL APPLICATIONS

3.1 PIP$_2$ and cellular signaling—mechanisms of membrane targeting

In this section we present several model applications pertaining to the role of polyvalent PIP$_2$ lipids in membrane targeting of peripheral proteins. This targeting mechanism is of special interest for the link between cellular signaling and lipid rafts, because among their multiple functions, PIP$_2$ lipids are known to act as scaffolds for the recruitment of proteins with specific binding domains toward special cell membrane regions, namely rafts, during signal transduction [65]. Through this mechanism, PIP$_2$ lipids are thought to precisely regulate cell signaling both temporally and spatially.

Many of the architectural signaling proteins that use PIP$_2$ lipids for membrane targeting contain structured domains, through which specific binding to polyvalent lipids is achieved. Examples include the C2 [4], PH [5], FERM [6], and BAR domains [7]. However, an apparently different type of targeting is realized by numerous other proteins that contain natively unstructured clusters of basic residues, such as the well-studied examples of the GAP43, GTPase K-Ras, and MARCKS proteins or peptides [66–70]. Below, we describe our model application to both structured and unstructured protein domains interacting with PIP$_2$-containing membranes.

The use of positively charged residues for targeting may come as no surprise, as cellular plasma membranes typically contain ~20% anionic lipids. This affords a simple mechanism for protein–lipid binding that is essentially nonspecific, yet able to confine proteins to membrane interfaces. This simple molecular picture has been challenged by recent theoretical and experimental evidence suggesting
that the major anionic lipid component in many cells, phosphatidylserine (PS) (or phosphatidylglycerol), might not be the major participant in peripheral protein binding. Instead, polyvalent lipids such as PIP$_2$ are more likely implicated in segregation close to peripherally adsorbed proteins [45,71–73]. Despite the fact that phosphoinositides constitute typically only around 1% of membrane composition [65], these minority lipids can act at sites of regulation at least partly by electrostatic association with peripheral and embedded proteins. Concentrating PIP$_2$ at the site of protein adsorption is therefore a likely mechanism for local and specific recruitment. It has been suggested that segregated lipids can subsequently be released upon cellular changes, e.g., in Ca$^{2+}$ concentrations. This provides a way to control the amount of free PIP$_2$ in the membrane, and hence a mechanism for regulating PIP$_2$ known to participate in cellular signaling processes such as enzyme activation, endocytosis, and ion-channel activation [74].

To begin to understand why electrostatic targeting could primarily be achieved by polyvalent rather than the more abundant monovalent lipids, one must focus on the forces that underlie this protein–lipid interaction. Experiments have suggested that PIP$_2$ preferentially segregates at sites of charged protein adsorption. This is reasonable because multivalent lipids should incur a smaller lipid demixing penalty and larger counterion release entropy [75–78] per segregated lipid, simply because each of them carries a larger charge. Recent theoretical studies predict that multivalent lipids should indeed segregate more than monovalent ones, and that the binding free energy to rigid macromolecules as well as to polyelectrolytes is significantly stronger for such lipids [45,72,73].

But recognizing the dynamic nature of the adsorption problem raises the possibility that the kinetic energy of each adsorbing protein allows it to move so quickly on lipid membranes that some lipids rarely manage to segregate at all. Conversely, lipids may rearrange so quickly around an adsorbing protein that the protein appears stationary to them, creating a transient “binding site”. The result can be a dynamic assembly of a domain or “lipid raft” around a peripheral, adsorbed protein. Through association with the protein, this raft could then impede the protein’s motion in the membrane plane.

Our mean-field theory provides an opportunity to quantitatively approach this problem, and describe the combined kinetic effect of many lipid species interacting with peripheral proteins. As described in the next sections, our model allows us to conclude that it is the composition of the membrane on which the adsorbed proteins are diffusing that sensitively determines whether lipids will be effectively sequestered. The model predictions also suggest that protein domains that selectively target PIP$_2$-containing membrane regions can achieve such selectivity through electrostatic interactions alone, and without the need for any additional energy source. However, we also predict that, in order to deform spontaneously flat membrane patches, as required for physiological function, these proteins will utilize alternative mechanisms, such as amphipathic helix insertions. The role of electrostatics in this case appears to be the stabilization of locally deformed membrane structures, induced by amphipathic inclusions.
3.2 BAR–membrane interactions

As a first application example, we discuss the interactions of BAR domains with membranes. BAR domains have gained great interest in the study of cell physiological processes [79–81]. They are known to dimerize into a banana-like molecular structure [82] that adsorbs to and faces lipid membranes with its concave surface (see Figure 1). The interactions of BAR domain dimers with the cell membrane are associated with a curving of the interface regions that often contain a relatively higher concentration of negatively charged lipids [7,83–85]. The functional role of such membrane remodeling by BARs apparently is to cluster and localize proteins in specialized membrane regions, and is likely to be important for signaling [8]. When present at high concentrations, BAR is capable of tubulating and vesiculating lipid membranes both in vivo and in vitro [8,86]. Some BAR domains (termed N-BARs) have N-terminal regions that appear to fold into amphipathic helices upon BAR–membrane binding, and to insert into the polar head-group region of lipid membranes [86–95].

3.2.1 Elements of membrane remodeling by BAR domains

In transforming a membrane that is spontaneously flat at equilibrium into a highly curved structure, BAR appears to take advantage of a special set of structural features (Figure 1). First, by pulling the membrane toward, or away from the protein, the electrostatic interactions between positively charged residues on BAR’s concave surface and negative phospholipid head-groups may cause membrane deformations away from the flat bilayer plane. The same electrostatic interactions may also cause lateral sequestration of charged phospholipids near the protein [26,27,45–47,71–73]. This process of lipid demixing in the bilayer plane has been predicted to be particularly significant in membranes containing multivalent lipids, such as PIP2 lipids [26,71,72]. Segregation of such highly charged lipids (net head-group charge of −4.0 at neutral pH [65]) would not only enhance the overall electrostatic interactions between BAR and membrane, but also lead to significant entropic gains. The entropic gain is due to the release into the bulk solution of mobile counterions that were previously bound to each of the macromolecule (protein and membrane) [75,77]. Furthermore, these membrane deformations could lead to local asymmetry between the spontaneous curvatures of the two monolayers comprising a lipid membrane, simply because the head-group of PIP2 is larger than most monovalent lipids, such as PS, or zwitterionic lipids like phosphatidylcholine (PC). Such an asymmetry would be sufficient to produce a local positive curvature in the two bilayer leaflets toward the BAR [63,64,88].

Ultimately, sequestering charged lipids could potentially lead to a new stable state, in which bilayer bending forces favor membranes with local nonzero curvature. Moreover, the mechanism for coupling local lipid composition with membrane curvature may be complemented by a “local spontaneous curvature” mechanism [88], whereby the asymmetry between the spontaneous shapes of two monolayers is achieved by insertion of amphipathic N-terminal helices of certain BAR domains into the lipid polar head-groups region on one side of the membrane [7,88–95]. According to this mechanism, the insertion of an amphipathic
peptide into one of the leaflets of a flat membrane produces an increase in the local spontaneous curvature of that leaflet because of the local bending of the monolayer where the helix is embedded [63,64,88]. Differences in the spontaneous curvatures of the two monolayers comprising a lipid membrane, one with and the other lacking helical insertions, establishes a new equilibrium state, in which bilayer elastic forces support a locally curved membrane shape.

Application of the mean-field theory outlined in the previous section to BAR–membrane systems is geared specifically to discern the role of electrostatic interactions and amphipathic helix insertions in the process of membrane remodeling by BAR domains, by accounting for the coupling between electrostatically driven lipid sequestration and local membrane curvature. By bringing an energetic perspective to the problem, the model quantitatively answers the following critical questions: Can BAR-induced segregation of polyvalent PIP2 lipids be the cause of substantial membrane deformation? And, how might N-helix insertions complement this coupling?

3.2.2 Lipid demixing upon Amphiphysin BAR dimer adsorption is insufficient on its own to induce significant membrane curvatures

Figure 2 shows a top view of the calculated lipid segregation and bilayer deformations for the equilibrium state of Amphiphysin BAR adsorbing on binary mixtures of 30:70 PS/PC and 4:96 PIP2/PC that are compositionally symmetric on both membrane leaflets. In panel 2C, the BAR domain is outlined for clarity. The results of the free energy minimization procedure reveal weak membrane deformations at equilibrium under the influence of the adsorbing BAR for both PS- and PIP2-containing membranes (Figure 2A–D). The largest membrane deformation, found in the center of the patches immediately under BAR’s “arch”, reaches only ~3–4 Å above the height of the planar membrane, a value comparable to the expected thermal undulations of the membrane at temperature $T = 300 \text{K}$ and bending rigidity $\kappa = 20k_B T$ [97].

These insignificant membrane curvatures are accompanied by only minor segregation of charged lipid around the adsorbing protein in both bilayer mixtures (Figure 2E–H). Thus, even in the regions of strongest aggregation (dark shades) on the BAR-facing leaflet of the PIP2-containing membrane, the PIP2 lipid levels are elevated by only ~1.3 times their 4% bulk value. These PIP2-enriched patches appear near the positively charged tips of the BAR domain, and their formation is the result of strong electrostatic interactions with negatively charged PIP2 lipid head-groups. At the same time, the concentration of PS lipid on the BAR-facing leaflet is minimally affected by the BAR domain. Interestingly, the lipid demixing on the lower monolayer of both membranes can be explained entirely by bending forces acting where the membrane is negatively curved. This curvature favors regions depleted of PS and PIP2 lipid (lighter shades) because these molecules have zero or even positive spontaneous curvature.

From the corresponding model binding free energy ($\Delta F$) we can conclude that lipid demixing and membrane deformations contribute to a lowering $\Delta F$ for BAR/PS/PC and BAR/PIP2/PC complexes by 1.9 $k_B T$ and 1.7 $k_B T$, respectively,
Figure 2  Adsorption of the Amphiphysin BAR domain on compositionally symmetric binary mixtures of PS/PC (lower panels) and PIP2/PC (upper panels) lipid membranes. The membrane patches are characterized by bending modulus of $\kappa = 20k_B T$, and contain 0.3 and 0.04 fraction of PS and PIP2 lipids, respectively. PS and PC lipids are described by spontaneous curvatures of $c_{ps}^0 = 1/144 \, \text{Å}^{-1}$, $c_{pc}^0 = -1/100 \, \text{Å}^{-1}$ [88]. The spontaneous curvature of PIP2 is not known from experimental measurements. We assume here $c_{pip2}^0 = 1/70 \, \text{Å}^{-1}$ in light of the substantial difference in head-group size between PIP2 and PS lipids. The BAR dimer orientation for both calculations is depicted in panel C as the projection of the BAR onto a membrane plane. Panels A–D show equilibrium membrane shapes of PIP2/PC and PS/PC membranes, respectively, with contours shown for the local heights of the upper and lower leaflets. Panels E–H depict steady-state lipid distributions on the upper and lower leaflets of both membranes ($a_{\alpha}, \alpha = \text{PS}, \text{PIP2}$). Shades for E–H panels represent ratios of local and average lipid fraction values. For all electrostatic calculations, we used a modified version of the APBS 0.4.0 software [96].

3.2.3 N-helix insertions can potentially enhance membrane deformations
In order to explore whether insertions of the BAR dimer’s N-helices can enhance membrane curvature, various penetration depths of N-helices were examined, and the results are illustrated in Figure 3. We observe larger membrane deformations upon deeper insertion of N-helices (represented in the model by increasing the local spontaneous curvature). By performing quantitative analysis on binding compared to the binding free energies of BAR onto the flat PS/PC and PIP2/PC membranes of the same homogeneous compositions. Nevertheless, the combination of lipid segregation with the elastic forces within a membrane appears to be insufficient to produce significant compositional asymmetry between bilayer leaflets to drive significant bending deformations. Consequently, at steady state, the membrane remains near-flat, within fluctuations, upon BAR adsorption.
energies for the membrane patches shown in Figure 3, our model predicts that a single adsorbing Amphiphysin N-BAR dimer will stabilize membrane patches that have the inherent propensity for high curvature, reflected by the lipid tendency to create local distortions that closely match the curvature of the BAR dimer itself.

Additional calculations at different concentrations of charged lipids revealed that increasing PS lipid fraction from 0.3 to 0.5 resulted in stronger BAR binding with substantial (ca. $6k_B T$) strengthening of the adsorption free energy, but without noticeable changes in the equilibrium membrane deformation, shown in Figure 3. Taken together, the model results indicate that the N-helix insertions have a critical mechanistic role in the local perturbation and curving of the membrane, which is further stabilized by the electrostatic interaction with the BAR dimer.

Figure 3 is also a clear illustration that our method is able to accurately predict the experimentally observed and theoretically reproduced symmetry breaking
upon N-BAR dimer adsorption onto a membrane. Notably, our approach does so through the resulting self-consistent free energy minimization procedure, without a priori knowledge of any BAR-induced spontaneous curvature fields. This distinguishes our model from alternative mesoscopic approaches that assume BAR-generated nonisotropic curvature fields [25,98].

3.2.4 Membrane tubulation and vesiculation by arrays of BAR domains
A question that remains open is how the observed local deformations introduced by a single BAR translate into global changes in membrane shape observed upon binding of high concentrations of BARs [22,99–101]. Results of our calculations predict that, because of the interplay between electrostatic and elastic forces, a single BAR dimer deforms membranes so that the bilayer region under the BAR can be substantially curved. At the same time, the membrane remains flat within fluctuations beyond this interaction zone. Thus, it is clear that surrounding this high curvature area there must exist a narrow region, or “rim”, where the sign of the local membrane curvature changes from positive (under the BAR) to negative (outside the interaction zone) eventually decaying to zero. Although electrostatically advantageous, the formation of such a rim is opposed by bending forces within the membrane, because lipids in the rim zone pay an elastic penalty for bending away from the spontaneous curvature $c_0$. The larger the membrane deformations, the larger the expected free energy penalty exerted on the rim. To conclude, binding of an additional BAR will be most favorable energetically if, together with minimizing the electrostatic interactions, the BAR also alleviates the membrane stress introduced by the one already adsorbed. The optimal manner for achieving this effect with multiple BARs is clearly not a simple additive superposition of effects from a single BAR, but rather must include collective properties [22,99–101]. Bridging the gap between our calculations and experimental results showing membrane tubulation and vesiculation by arrays of BAR domains is one of the future challenges in the field of BAR/membrane modeling.

3.3 Adsorption of natively unstructured protein domains onto lipid membranes
As an illustration of membrane binding of natively unstructured protein domains, we describe the predictions from our calculations pertaining to the adsorption of basic lysine-13 (Lys13) peptides onto mixed lipid membranes. Basic polypeptides, such as Lys13, are well-studied simple yet realistic models to describe membrane anchoring of unstructured domains such as MARCKS [46,71]. We first present results for stationary adsorbed Lys13 peptides in the presence of diffusing lipids. Then, to consider the effect of protein mobility, we discuss how our predictions change if the adsorbate is also allowed to diffuse.

3.3.1 Sequestration of PIP$_2$ lipids by adsorbing basic polypeptides
Figure 4 shows the charged lipid organization for a ternary 74:25:1 PC/PS/PIP$_2$ mixture (Figure 4a and b) and binary 71:29 PC/PS mixture (Figure 4c) upon
Lys13 binding. Both lipid compositions are characterized by the same surface charge density, and the snapshots are taken after 500 ns (a point where steady state is achieved for lipid compositions) starting from a completely homogenous lipid distribution.

From Figure 4a we learn that the fraction of PIP2 lipid increases up to 4.5-fold near the adsorbed Lys13 side chains, where the positive charge is greatest. This area is surrounded by a region with lower PIP2 content, showing only 2.5–3-fold increase in multivalent lipid fraction. Because the peptide backbone is rich in both positive and negative charges, there are only minor changes in PIP2 content along the Lys13 backbone with respect to the bulk concentration. In contrast, Figure 4b reveals almost no sequestration of PS by the peptide. The highest increase in PS lipid is only 1.5-fold, observed, as expected, along the Lys13 side chains. For comparison, Figure 4c shows that even in PIP2-free membranes, the segregation of PS lipids around Lys13 is marginal.

Thus, in agreement with other theoretical predictions [45,46], our model indicates that an adsorbing stationary basic peptide will sequester primarily PIP2 lipids, and will only very weakly sequester PS lipids.

3.3.2 Diffusion of peripheral proteins on lipid membranes
The intriguing question that now arises is how the extent of PIP2 sequestration by Lys13 peptide described above will change when considering a more realistic
scenario, where the adsorbate is also allowed to diffuse on the membrane surface. In particular, we focus on how the macromolecule diffusion rates are affected by the acidic lipids in the membrane, and how different lipids can influence the apparent protein diffusion rates.

To address these questions, we follow a simplified spherical macroion that is allowed to move concomitantly with lipid diffusion. To do so, we extend our model to include protein diffusion and performed CH-DMC (see Section 2.4) calculations. We studied the same mixed membranes considered in Figure 4, focusing on two typical cases. In the first, the model protein has a diffusion constant much larger than that of lipids in the unperturbed (bare) membrane, with a ratio $D_0 = 10$ between the two, while in the second, the diffusion constant is comparable to that of the lipids, and $D_0 = 2$ (see Eq. 10). As we show, these two scenarios lead to different lipid and protein diffusion characteristics.

3.3.3 Modeling a fast protein diffusing over PIP$_2$-containing versus PIP$_2$-depleted membranes

Following the time evolution of the system, depicted in Figure 5c, reveals significant local PIP$_2$ lipid segregation around the fast-diffusing protein as it moves over a ternary PC/PS/PIP$_2$ membrane. Quantitative analysis of protein diffusion rates predicts a prominent concomitant retardation in the macroion’s movement. Due to lipid rearrangement, the adsorbate diffusion becomes confined, for a limited time, to an area rich in PIP$_2$. However, due to the model protein’s high mobility compared to that of lipids, the adsorbate occasionally and temporarily escapes, leaving behind the multivalent lipid cloud that had segregated around it.

The free diffusion of the macroion does not last very long, because PIP$_2$ lipids quickly segregate again around the new protein position. This segregation is due to the large forces acting on the PIP$_2$ lipids by the electrostatic field emanating from the adsorbate. Essentially, the macroion diffuses and drags PIP$_2$ lipids along its way, while the PIP$_2$ units that are segregated retard the free diffusion of the protein. In contrast to the strong PIP$_2$ segregation, we found that PS segregation in the ternary mixtures is very weak, in accordance with our previous findings for the stationary peptide (Figure 4b).

We compare this diffusion process with the same rapid model protein diffusing on a binary PIP$_2$-depleted membrane containing only monovalent (PS) lipids (Figure 5a). Clearly, acidic (PS) lipids segregate around the macroion to a much lesser extent compared to the ternary mixture, resulting in low energetic barriers to adsorbate motion. Hence, the diffusion of the macroion here is less restricted compared to that seen for the ternary mixture.

3.3.4 Slow protein diffusing over PIP$_2$-containing versus PIP$_2$-depleted membranes

Diffusion of a slower model protein, $D' = 2$, on the same binary and ternary membranes (Figure 5b and d, respectively) shows qualitatively similar behavior to that observed for $D' = 10$. However, due to the lower mobility of the macroion, the acidic lipids have more time to effectively segregate near the adsorbate, and
therefore segregate more strongly. The result is that a majority of the macroion moves are restricted to the acidic lipid-rich patch that forms close to the protein. This is particularly noticeable for the ternary system, where the macroion practically never escapes to go beyond the circular patch formed by PIP$_2$ lipids, but rather diffuses together and within it. Whereas for the fast protein on ternary mixtures we observed the creation and destruction of macroion/PIP$_2$ “binding sites”, for the slower protein this lipid–protein “complex” stays intact for the entire trajectory. In a sense, we find that there are always PIP$_2$ lipids associated with the macroion as it diffuses on the membrane.
3.3.5 Implications for the role of PIP$_2$ in anchoring proteins to specialized membrane domains

Our results suggest that PIP$_2$ lipids can diffuse in concert with adsorbed molecules even when the diffusion of the adsorbate is much faster than lipid diffusion. In contrast, monovalent PS lipids segregate only weakly, so that macromolecule and lipid diffusion will remain largely uncorrelated. The difference in behavior between different lipid species arises because PIP$_2$ lipids, in the presence of the protein electric field, are much more mobile than PS, due to their higher charge and hence larger chemical potential.

Predictions from our model bear interesting implications for the role of PIP$_2$ lipids in anchoring natively unstructured domains (and other peripherally bound proteins) to lipid membranes. Clearly, to carry out their function, peripheral proteins must often remain localized in certain regions on the membrane (say, in rafts) for some duration of time. This requires a mechanism that would slow down diffusion across the membrane in the region in which these proteins must act. In agreement with recent experimental observations [70,71], our model predicts segregation of PIP$_2$ lipids around the diffusing charged protein, keeping these lipids effectively “bound” to the protein vicinity, and retarding the protein’s diffusive motion.

4. FUTURE PROSPECTS

We have presented a new computational modeling approach to describe membrane-associated interactions that evolve at mesoscales and over long times. Through illustrative examples, we showed how this self-consistent strategy is successful in elucidating some of the fundamental mechanistic aspects of cellular signaling. Specifically, we have shown how this method can not only help to discern the role of polyvalent lipids in recruitment and confinement of signaling proteins to specialized membrane regions to carry out their physiological function, but also can illuminate mechanisms responsible for membrane remodeling by signaling motifs such as BAR domains.

Toward developing a powerful and complete methodology, we are now pursuing several key enhancements and extensions to the model, to enable quantitative description of large-scale membrane-associated processes set in action by complex signaling machinery. Two major improvements are especially noteworthy in the context of lipid rafts and cell signaling. The first involves adding to the model quantitative details on TM protein–membrane interactions and connecting these degrees of freedom with the current knowledge on interactions between peripheral proteins and membranes. The second is to enable a description of phase separating elastic lipid membranes to capture the formation and dynamics of membrane rafts.

Obviously, these two enhancements are closely related through the well-known impact of cholesterol on both raft formation and functioning of raft-containing TM domains, such as GPCR proteins. Thus, with the intended modifications, the model should be able to elucidate the role of cholesterol and
other synergetic raft modulators in the function and organization of signaling proteins. With that, the extended methodology should become complementary to other simulation techniques, covering temporal and spatial regimes that are currently not readily accessible by existing techniques. The main gain for this type of approach (and a potential reason for the wider applicability) is that the method adds information concerning longer timescales and can reach the steady state of the system. Together with the opportunity to discuss protein–membrane interactions in terms of model free energies, the new methodology can, for the first time, begin to access a quantitative view of large-scale interactions during cellular signaling that act across the plasma membrane interface.

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REFERENCES


