Lateral dynamics of charged lipids and peripheral proteins in spatially heterogeneous membranes: Comparison of continuous and Monte Carlo approaches

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Biological membranes are complex environments whose physico-chemical properties are of utmost importance for the understanding of many crucial biological processes. Much attention has been given in the literature to the description of membranes along the z-axis perpendicular to the membrane. Here, we instead consider the lateral dynamics of lipids and peripheral proteins due to their electrostatic interaction. Previously, we constructed a Monte Carlo automaton capable of simulating mutual diffusive dynamics of charged lipids and associated positively charged peptides. Here, we derive and numerically analyze a system of Poisson-Boltzmann-Nernst-Planck (PBNP) equations that provide a mean-field approximation compatible with our Monte Carlo model. The thorough comparison between the mean-field PBNP equations and Monte Carlo simulations demonstrates that both the approaches are in a good qualitative agreement in all tested scenarios. We find that the two methods quantitatively deviate when the local charge density is high, presumably because the Poisson-Boltzmann formalism is applicable in the so-called weak coupling limit, whose validity is restricted to low charge densities. Nevertheless, we conclude that the mean-field PBNP approach provides a good approximation for the considerably more detailed Monte Carlo model at only a fraction of the associated computational cost and allows simulation of the membrane lateral dynamics on the space and time scales relevant for the realistic biological problems. © *2011 American Institute of Physics*. [doi:10.1063/1.3652958]

I. INTRODUCTION

Dynamics of biological membranes has been a matter of steadily increasing interest over the past several decades. Rapid advancement of experimental tools enables observation of subcellular structures and processes with ever increasing spatial and temporal resolution.[1] These studies demonstrated that biological membranes are fundamentally heterogeneous and consist of dynamically evolving domains with distinct protein-lipid composition. The characteristic physico-chemical properties of the membrane, such as fluidity, lipid packing density, and bilayer thickness, may differ significantly between the adjacent domains.[2] It has become increasingly clear that crucial biological processes, such as cell signaling, membrane trafficking, and cell motility, intimately depend on and themselves control the spatial heterogeneity of the membrane by altering its composition, curvature, and electric properties. Emergence and maintenance of membrane domains and other spatial heterogeneities depend on several types of lipid-lipid, protein-lipid, and protein-protein interactions that are commonly mediated by electrostatic and hydrophobic forces. Hydrophobic interactions that depend on the nature of non-polar lipid tails have received much experimental and theoretical attention in the context of lipid rafts[3] within which saturated lipids and cholesterol segregate away from the lipids with more disordered unsaturated tails. Importantly, many proteins show preference for localization either within or outside lipid rafts.[4] For instance, membrane proteins adorned by saturated lipid modifications, e.g., palmitoyl moieties, localize preferentially within lipid rafts.[5] Another manifestation of hydrophobic protein-lipid interaction known as the hydrophobic mismatch[6] has been predicted theoretically and has been shown to generate arrays of transmembrane proteins.[7] The role of hydrophobic interactions in the formation of membrane heterogeneities has been extensively studied elsewhere and will not be considered further in this paper.

Electrostatic forces have long been known to play a crucial role in the interaction of cytoplasmic proteins and lipid membranes.[8–10] Indeed, up to 40% of lipids in the cytoplasmic leaflet of the plasma membrane are represented by species whose headgroups carry negative charge,[11] while most of lipid-interacting protein domains are positively charged. A number of these lipid-interacting domains, such as PH, PX, PDZ, and FYVE, with variable degree of specificity for particular lipid species has been described in the literature.[12,13] Many biologically important proteins, however, interact

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with the negatively charged lipids non-specifically, by means of linear stretches of amino acid residues enriched in positively charged lysine (K) and arginine (R).[14,15] In the membrane-bound configuration, these polybasic domains directly interact with the negatively charged headgroups of lipids within the hydrated layer of the membrane. Since this interaction is essentially independent of the rest of the protein, short positively charged peptides, e.g., polylysine, have been extensively studied as convenient experimental and theoretical models of the membrane-interacting proteins.[15–19] These studies demonstrated that adsorption of charged peptides on the membrane results in lipid demixing, with a particularly strong effect observed for the multiply charged phosphoinositide species, such as PIP2 (−4) and PIP3 (−5).[12,17,20,21] Beyond the local lipid demixing that is restricted to the immediate footprint of the adsorbed peptide or protein,[19,20] little is known about the role of electrostatic interactions in the formation of spatially extended membrane heterogeneities. Spatial segregation of the adsorbed polybasic peptides and protein domains into a compact phase has been experimentally observed in several *in vitro* systems.[22] A study by Denisov *et al.*[9] proposed that this phenomenon is directly induced by the lipid demixing. Subsequent theoretical analyses,[23,24] however, demonstrated that such phase segregation would also require energetically favorable hydrophobic interaction between the recruited lipids and, thus, electrostatic interactions alone are not sufficient to generate a spatially extended membrane domain. Nevertheless, the role of electrostatic interactions in the formation of membrane heterogeneities *in vivo* deserves further careful consideration. Charged lipids can be rapidly produced at specific membrane loci due to the highly localized activity of lipid-modifying enzymes, such as lipid kinases and phospholipases, and destroyed elsewhere on the membrane by the opposing enzymes to prevent global accumulation of the charged lipid. Recent advent of live cell imaging with fluorescent lipid probes demonstrated that several charged lipid species may exhibit highly asymmetric localization with pronounced gradients of concentration along the membrane.[25] Inspired by these experimental observations, Kiselev *et al.*[19] have recently theoretically demonstrated that the electric field generated along the membrane surface by the gradient of charged lipid, even if short-living, could be sensed by the adsorbed peptides or proteins with polybasic domains. Resulting electrodiffusive drift of the peptides was predicted to occur along or against the lipid gradient depending on the cumulative charge of the peptide together with the adsorbed anionic lipids. This phenomenon with the potentially long-reaching biological implications is further considered in the present contribution.

Despite the remarkable progress in the understanding of individual mechanisms that contribute to the membrane heterogeneity, a unified theoretical model of the membrane lateral dynamics has not yet been achieved.[26] This could be attributed in part to the largely irreducible complexity of the membrane dynamics that involves many types of molecular interactions and spans many orders of magnitude in space and time. Atomistic molecular dynamics simulations reproduce the membrane dynamics most faithfully since they have a potential to incorporate the entire spectrum of electrostatic, hydrophobic, and hydrodynamic interactions in the membrane. Due to the overwhelming amount of the molecular detail, these simulations, however, are limited to a few tens of nanometers in space and several microseconds in time. As the biological processes of interest may extend over several micrometers and last for seconds and even minutes, more coarse-grained approaches are required.

Most coarse-grained treatments typically fall within the two major categories: mesoscopic discrete particle methods, such as Monte Carlo and Brownian dynamics, and continuous mean-field equations. Both approaches have been extensively used to study the adsorption of polybasic peptides and the lipid demixing associated with it.[27] Much less attention, however, has been given in the literature to the description of the mutual diffusive dynamics of lipids and proteins. Adsorbed protein diffusion was considered in the Monte Carlo simulations by Hinderliter *et al.*[23] and by Khelashvili *et al.*[28] in a hybrid model with discrete dynamics of an adsorbed peptide and continuous evolution of the interacting lipid. Recently, Kiselev *et al.*[19] developed a dynamic Monte Carlo automaton that was designed specifically to simulate the lateral diffusion of pentalysine peptides (Lys-5, +5) on the membrane consisting of neutral (PC, 0), negatively charged monovalent (PS, −1) and polyvalent (PIP2, −4) lipids. The simulations were performed on two parallel overlying triangular lattices representing the planes of lipid headgroups and peptide residues, respectively. While the on-lattice Monte Carlo simulations offer a number of valuable advantages, such as the automatic satisfaction of the constraint of constant lipid density and a straightforward calculation of the electrostatic interaction energy, they also have obvious limitations. Thus, confining coordinates of all particles to the nodes of a spatially periodic lattice imposes restrictions on the geometry of the peptide-lipid interface, introduces lattice-specific artifacts, and makes simulation of the peptide rotational diffusion highly problematic. Besides, the associated heavy computational costs restrict the simulated dynamics to ∼100 nm in space and few milliseconds in time. Therefore, if the dynamics of multiple protein species on the membrane with complex lipid composition is sought after, further coarse-graining towards the mean-field approximation is highly desirable.

In this paper, we derive mean-field equations governing the dynamics of the membrane lipids and adsorbed peptides by minimization of the free energy functional. The resulting equations belong to the class of modified Poisson-Nernst-Planck (PNP) equations that have been extensively used in the literature to describe the dynamics of ionic and charged colloid systems. In application to biological membranes, PNP equations have been typically derived to describe the dynamics of ions in the dimension perpendicular to the plane of the membrane, such as in the models of ion channels[29] and the description of the gap between the membrane and the underlying substrate.[30] Here, we are concerned only with the dynamics in the plane of the membrane. All the involved ionic species can be naturally subdivided into two distinct classes: polar headgroups of lipids and peptide residues that constitute the membrane Stern layer[31] and the freely diffusing mobile ions. Since the diffusion coefficients of the mobile ions are approximately two orders of magnitude larger than those

of lipids, we assume their concentrations to be described by Poisson-Boltzmann distribution that rapidly evolves to fit the slowly changing concentrations of lipids and peptides. This assumption allows us to reduce the number of variables and exclude the explicit concentrations of the mobile ions from the equations in an approach similar to that recently developed by Zheng and Wei.[32] Since it involves solution of the coupled Poisson-Boltzmann and Nernst-Planck equations, following Zheng and Wei, we refer to it as the Poisson-Boltzmann-Nernst-Planck (PBNP) model. As has been done by others,[33] we utilize our earlier developed Monte Carlo automaton to inform the continuous model development. Both approaches are then thoroughly compared in a number of scenarios that involve electrostatic lipid-lipid as well as peptide-lipid interactions. Predictably, we find that the two methods quantitatively deviate when the local charge density is high since the Poisson-Boltzmann ansatz inherently employs the weak coupling approximation whose strict validity is restricted to low charge densities.[34,35] Nevertheless, the two approaches agree qualitatively well in all scenarios tested. We conclude that the mean-field PBNP approach provides a good approximation for the considerably more detailed Monte Carlo model at only a fraction of the associated computational cost and allows accounting for the lateral electrostatic interactions within the membrane on the space and time scales relevant for the realistic biological problems.

II. MODEL

A. Pure lipid membrane

The plasma membrane of biological cells has two 2–3 nm thick hydrated layers corresponding to its inner and outer leaflets that are separated by a hydrophobic core of ~2 nm. We consider the inner hydrated layer where the polar lipid headgroups reside accessible to the soluble cytoplasmic ions. Positively charged residues of the adsorbed polybasic peptides and biological proteins are also located within this layer.[18] We approximate this potentially complex environment by a continuous quasi 2D medium with spatially dependent concentrations of lipid headgroups c_i and monovalent soluble ions n_+ and n_- that satisfy the electroneutrality condition everywhere. To simplify the treatment, we first consider the case of a pure lipid membrane without adsorbed peptides.

To construct a meaningful mean-field theory, one has to explicitly take into account the fact that the lipids in the membrane are tightly packed without appreciable voids, so that the sum of their concentration is approximately constant—this constraint is implicit in the lattice-based Monte Carlo models. Note that this constraint also amounts to neglecting the differences in the lipid headgroup sizes and fluctuations in the packing density. We thus require that everywhere on the membrane

$$\sum_{i=1}^{N} c_i = C_m, \tag{1}$$

where N is the number of lipid species including electroneutral and C_m is the total headgroup concentration that corresponds to the average area per headgroup of 0.6 nm^2.[36] The membrane free energy functional

$$F = \int_V (u - Ts)\, dv$$

then can be explicitly defined following the standard procedure as follows:

$$\begin{aligned} F = k_B T \int_V \Bigg[& \sum_{i=1}^{N} (c_i \log(c_i V_0) - c_i) + n_+ \log(n_+ V_0) - n_+ \\ & + n_- \log(n_- V_0) - n_- + \left(\sum_{i=1}^{N} z_i c_i + n_+ - n_- \right) \psi \\ & - \frac{\varepsilon \varepsilon_0 k_B T}{2 e^2 N_A} |\vec{\nabla} \psi|^2 - \mu_+ n_+ - \mu_- n_- \\ & - \alpha \left(\sum_{i=1}^{N} c_i - C_m \right) \Bigg] dv, \end{aligned} \tag{2}$$

where z_i are the ionic valences of lipid headgroups, μ_+ and μ_- are chemical potentials of free ions and V_0 is a constant that corresponds to the cube of the thermal de Broglie wavelength multiplied by the Avogadro number N_A. Note that all concentrations are in molar units. A spatially dependent Lagrange multiplier α is introduced to enforce the dense packing condition (1). Minimizing F with respect to the nondimensional electrostatic potential $\psi = e\phi/k_B T$, we recover the Poisson equation

$$\vec{\nabla}^2 \psi = -\frac{e^2 N_A}{k_B T \varepsilon \varepsilon_0} \left(\sum_{i=1}^{N} z_i c_i + n_+ - n_- \right). \tag{3}$$

After a further minimization of the free energy functional over the cytoplasmic counterion densities n_+ and n_-, as routinely done in the Poisson-Boltzmann theory, we get that the freely moving counterions are distributed according to the Boltzmann distribution and obtain the following Poisson-Boltzmann equation:

$$\vec{\nabla}^2 \psi = \frac{2 e^2 n_0 N_A}{k_B T \varepsilon \varepsilon_0} \frac{e^{\psi} - e^{-\psi}}{2} - \frac{e^2 N_A}{k_B T \varepsilon \varepsilon_0} \sum_{i=1}^{N} z_i c_i$$

that can be further simplified with the introduction of Debye length λ

$$\vec{\nabla}^2 \psi = \frac{1}{\lambda^2} \sinh(\psi) - \frac{e^2 N_A}{k_B T \varepsilon \varepsilon_0} \sum_{i=1}^{N} z_i c_i. \tag{4}$$

Following a standard convention accepted in the literature,[19] we assume here that the free cytoplasmic ions are represented by 0.1 M solution of a monovalent salt and therefore the Debye length is ~1 nm.

Dynamics of lipids is defined by a system of mass-conservation equations

$$\frac{\partial c_i}{\partial t} = -\vec{\nabla} \mathbf{J}_i + R_i, \tag{5}$$

where R_i are chemical reaction terms and the expression for the fluxes $\mathbf{J}_i$ can be obtained from the free energy functional

F. Indeed, differentiating Eq. (2) with respect to c_i we get

$$\frac{\mu_i}{k_B T} = \frac{\mu_i^0}{k_B T} + \log(c_i V_0) + z_i \psi - \alpha,$$

where μ_i^0 are the standard chemical potentials of the lipid species. Therefore, the fluxes of all lipid species can be expressed through the gradients of their concentrations, electrostatic potential and the Lagrange multiplier as follows:

$$\vec{\mathbf{J}}_i = -D_i c_i \frac{\vec{\nabla}\mu_i}{k_B T} = -D_i(\vec{\nabla} c_i + z_i c_i \vec{\nabla}\psi - c_i \vec{\nabla}\alpha). \tag{6}$$

Now we can utilize the dense packing condition (1) to exclude the Lagrange multiplier from Eq. (6). Indeed, summing up Eq. (5) for all lipid species and taking into the consideration that all R_i are interconversions between the species, we get

$$\sum_{i=1}^{N} \vec{\mathbf{J}}_i = \text{const}, \tag{7}$$

where the constant can be taken as 0. Substituting Eq. (6) into Eq. (7) yields

$$\vec{\nabla}\alpha = \frac{\sum_{i=1}^{N} D_i \vec{\nabla} c_i + \vec{\nabla}\psi \sum_{i=1}^{N} D_i z_i c_i}{\sum_{i=1}^{N} D_i c_i}. \tag{8}$$

Finally, combining the above expressions we arrive, after some term re-arrangements, at modified Nernst-Planck equations

$$\frac{\partial c_i}{\partial t} = \vec{\nabla}\left(D_i \left[\left(\vec{\nabla} c_i - c_i \frac{\sum_{i=1}^{N} D_j \vec{\nabla} c_j}{\sum_{i=1}^{N} D_j c_j} \right) + c_i \vec{\nabla}\psi \left(z_i - \frac{\sum_{i=1}^{N} D_j z_i c_j}{\sum_{i=1}^{N} D_j c_j} \right) \right] \right) + R_i. \tag{9}$$

Together with Eq. (4) these equations complete the Poisson-Boltzmann-Nernst-Planck description of the electrostatic dynamics in the pure lipid membrane. Note that in the derivation above, no assumptions have been made regarding the relative values of the lipid concentrations c_i. However, if the concentrations of all charged lipids are small everywhere, the dynamics of the membrane reduces to that of several dilute solutes (charged lipid) in the excess of the solvent (neutral lipid). In this case, condition (1) can be relaxed and Eq. (9) becomes the standard Nernst-Planck equation.

B. Membrane with adsorbed polypeptides

We now extend the PBNP description presented above for the pure lipid membrane to the case of a single adsorbed peptide species. While the following derivation can be readily generalized, for the sake of clarity, we consider a number of reasonable approximations that reduce the complexity of the formalism. Since we are interested in the lateral dynamics of the peptides, we do not explicitly consider adsorption-desorption of peptides from the bulk fluid phase above the membrane. Indeed, most of proteins with polybasic domains have additional means of attaching to the membrane, such as

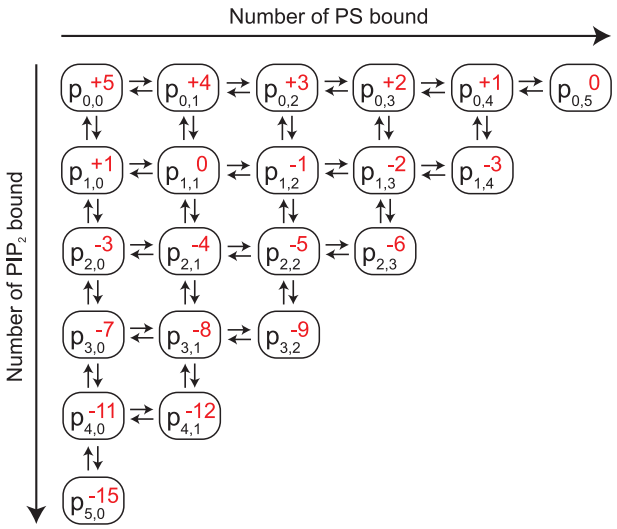

FIG. 1. Peptide-lipid complexes $p_{i,j}$ formed by pentalysine, and PS and PIP2 lipids. Total charge is shown in red.

lipid moieties and/or specific lipid-binding domains that extend their membrane residence time to ~1 s or longer.[37] At the same time, our Monte Carlo simulations[19] demonstrated that the peptides respond to the lipid gradients within few milliseconds; thus, the adsorption-desorption dynamics likely does not play a major role on this time scale.

Adsorbed polypeptides with M positively charged monovalent amino acid residues and negatively charged lipids form multiple stoichiometric peptide-lipid complexes with distinct total charge. On a membrane with biologically realistic composition, this may result in a combinatorial explosion of the number of all possible complexes, much alike the situation with multiply phosphorylated protein species or receptor complexes.[38] As an illustration let us consider pentalysine ($M = 5$) on a ternary membrane consisting of $N = 3$ electrostatically distinct lipid species: neutral (PC) with concentration c_1; monovalent negative (PS), c_2; and multivalent negative (PIP2, −4), c_3. All 21 possible peptide-lipid complexes together with their total charges are shown in Fig. 1. A system of Nernst-Planck equations with the reaction terms $R_{i,j}^p$ defined by the elementary transitions shown in Fig. 1 can be derived similar to Eq. (9) and solved in conjunction with the lipid Nernst-Planck equations and the Poisson-Boltzmann equation (4).

To proceed, we further reduce the complexity by considering pentalysine on a binary PC:PS membrane. In this scenario, adsorbed pentalysine is represented by 6 peptide-lipid species (see Fig. 1, top row) with concentrations p_i, $i = 0, M$, where the notation is simplified by dropping first index. The condition of dense packing (1) then takes the form

$$\sum_{i=1}^{N} c_i + \sum_{j=0}^{M} j p_j = C_m \tag{10}$$

resulting in the additional terms dependent on p_i in Eq. (8). Here, c_i are the molar concentrations of free lipids that are not bound to the peptides. In practice, however, the characteristic concentrations of proteins on biological membranes are typically 2–3 orders of magnitude lower than the C_m.

Leaving the case of dense protein lattices within which the protein local concentration is comparable to the C_m for the later consideration, in the following we assume that $\sum p_j \ll C_m$ and Eq. (9) thus remain unchanged.

Using this approximation and repeating steps (2–8) we obtain the Poisson-Boltzmann equation

$$\vec{\nabla}^2\psi = \frac{1}{\lambda^2}\sinh(\psi) - \frac{e^2 N_A}{k_B T \varepsilon\varepsilon_0}\left(\sum_{i=1}^{2} z_i c_i - \sum_{j=0}^{5}(5-j)p_j\right) \tag{11}$$

and additional Nernst-Planck equations

$$\frac{\partial p_j}{\partial t} = \vec{\nabla}[D_j(\vec{\nabla}p_j + z_j^p p_j \vec{\nabla}\psi)] + R_j^p \tag{12}$$

that describe the dynamics of the peptide-lipid complexes and where the reaction terms R_j^p can be explicitly defined as

$$\begin{aligned} R_0^p &= h_1 p_1 - k_0 p_0 c_2, \\ R_j^p &= k_{j-1} p_{j-1} c_2 + h_{j+1} p_{j+1} - k_j p_j c_2 - h_j p_j, \quad j \in [1;4], \\ R_5^p &= k_4 p_4 c_2 - h_5 p_5. \end{aligned} \tag{13}$$

To enable the comparison of the two approaches, we determine quantitative values of the association k_j and dissociation h_j rates using our Monte Carlo model. In Ref. 19, we observed that the charged residues of polylysines interact with the monovalent PS lipids independently of each other. This non-cooperative character of their interaction suggests a simple association-dissociation model that allows us to express all k_j and h_j through only two empiric constants k^0 and h^0 that represent the on and off rates for a single peptide residue, respectively. Thus, in the assumption that the individual residues interact with lipids independently of each other, for a peptide-lipid complex with $m \le M$ residues bound to PS, we obtain

$$k_m \sim (M-m)k^0; \quad h_m \sim m h^0.$$

To fully define the reaction rates, dense lipid packing on the membrane should be taken into the consideration. Indeed, dissociation of a charged lipid from the peptide-lipid complex implies that it is displaced by a lipid molecule of another type, in our example, neutral PC. If the membrane is composed entirely of PS ($c_2 = C_m$), the peptide residue will remain in a complex with a negatively charged lipid regardless of the frequent association-dissociation reactions with the specific lipid molecules. Of several potentially applicable heuristic approaches to account for this, we chose to modify the dissociation rate, namely, we postulate

$$k_m = (M-m)k^0, \quad h_m = m h^0(1 - c_2/C_m). \tag{14}$$

Solving Eq. (11) with the reaction rates given in Eq. (14) in a spatially homogeneous regime with varying PC:PS ratios and comparing the results to the Monte Carlo simulations (see Fig. 2), we found that a single set of microscopic on and off rates k^0, h^0 provides one-to-one correspondence between the two approaches in a broad range of PS molar fractions.

Due to the dense packing of the membrane lipids, lateral diffusion of protein-lipid complexes, and surrounding lipids

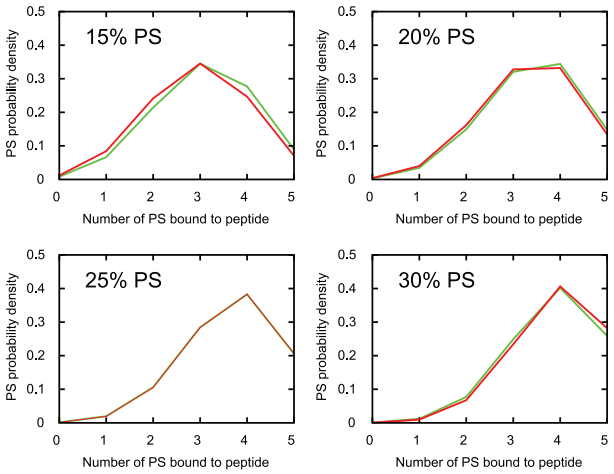

FIG. 2. Probability density functions of pentalysine peptide association with PS at various PS molar fractions. Results of Monte Carlo simulations are shown in green and the PBNP model in red.

are anticorrelated. This correlation, implicit in the on-lattice Monte Carlo simulations, needs to be explicitly incorporated into the membrane mean-field description constructed here. As shown in Ref. 19 this can be readily achieved by introducing effective protein-lipid complex charges z_j^p that together with the charges of lipids that are electrostatically associated with the peptide also account for the charges of lipids displaced by the lateral motion of the complex. Indeed, the effective charge of a single peptide residue adsorbed to the PC:PS bilayer was shown[19] to depend on the molar fraction of PS ρ as

$$Z^{eff} = 1 - p(\rho) + p(\rho)\cdot\rho,$$

where $p(\rho)$ is the probability of a peptide residue to be associated with a PS lipid. In the notations adopted in this paper, the effective charge of the peptide-lipid complex can be analogously defined as

$$z_m^p = (M-m)\cdot(+1) - m\frac{c_2}{C_m}\cdot(-1) = M - m\left(1 - \frac{c_2}{C_m}\right). \tag{15}$$

Figure 3 shows that the PBNP model with the z_j^p defined by Eq. (15) provides values of the average total and effective charges of the peptide on the PC:PS bilayer essentially identical to those computed in the Monte Carlo simulations.

C. Computational realization

The system of coupled Poisson-Boltzmann and Nernst-Planck equations was solved in one dimension using the FlexPDE software (PDE Solutions Inc.) that uses finite element method for solving partial differential equations. For simplicity, diffusion coefficients of all lipids and peptide-lipid complexes were chosen equal to $D_0 = 1 m\mu^2/s$. All Monte Carlo simulations were performed using the 2D double-lattice automaton as described in detail in Ref. 19. To compare the results of the two approaches, gradients of the lipid concentration were induced in the Monte Carlo automaton by the

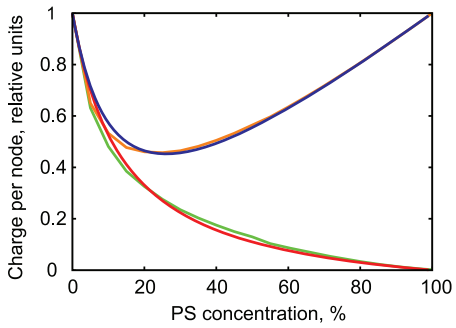

FIG. 3. Average total (red, green) and effective charge (orange, blue) of pentalysine on the PC:PS membrane at varying molar fractions of PS. Monte Carlo simulations (green, orange) show essentially the same profiles as the PBNP model (red, blue).

reaction source-sink terms along one dimension of the lattice and the output configurations were averaged along the other dimension. All results of Monte Carlo simulations were averaged over multiple stochastic realizations.

III. RESULTS AND DISCUSSION

A. Gradient of a single charged lipid

We first sought to assess the mean-field and Monte Carlo approaches in the simplest scenario that involves spatial heterogeneity, a gradient of a single charged lipid, using PC:PS and PC:PIP_2 bilayers as models. As a preliminary step, we considered a neutral binary system (e.g., PC:PE) with lipids organized into the gradients of identical magnitude and opposite sign by the introduction of the interconversion reaction terms on the domain boundaries. As expected, in the absence of electrostatic interactions, both the PBNP equations and Monte Carlo automaton produced strictly linear steady state lipid distributions with constant gradient. The magnitude of the gradient, predictably, showed linear dependence on the source intensity providing a route to readily calibrate both techniques. As the concentration of one lipid was kept zero on the right boundary and the length of the spatial domain, $L = 80\,\text{nm}$, was kept constant in all simulations, it is convenient to refer to the magnitude of the gradients by the maximal molar fraction of a lipid that is achieved at the left boundary. Thus, we varied the source intensity to achieve the gradients of neutral lipid in the range 5–50%.

In the gradient of negatively charged PS or PIP_2 lipids, the electrostatic repulsion contributes to the lipid flux promoting the gradient dissipation. Since the electrostatic interaction is a function of lipid density, the shape of the gradient departs from linear (see Fig. 4). As the total lipid flux is fixed by the magnitude of the source terms, this implies that the steady state maximal value of the charged lipid concentration on the left boundary is lower than the value expected for the neutral lipid at the same source intensity. Figure 4(a) shows that for the monovalent PS, this effect becomes noticeable from a gradient of ~20%. However, for the multivalent PIP_2, this effect is already prominent at the smallest tested gradient of 5% resulting in the actual gradient with the maximal molar fraction of only ~3.4% (Fig. 4(b)).

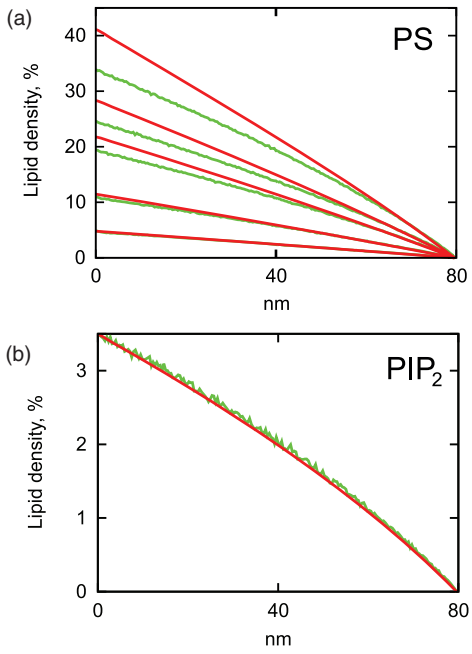

FIG. 4. Comparison of the Monte Carlo simulation (green) and the PBNP model (red) results in the scenario of a single charged lipid gradient. (a) PC:PS membrane. Gradients shown correspond to the neutral lipid gradients of 5%, 12.5%, 25%, and 33.5%. (b) PC:PIP2 membrane.

Comparison of the mean-field and Monte Carlo approaches shows that they quantitatively deviate at the high local charge densities. In all simulations, the Monte Carlo approach demonstrates larger contribution of the electrostatic interactions than the PBNP model. The Poisson-Boltzmann approach considers interaction of the ions through the common potential field and thus neglects the ion-ion correlations that become significant at high charge densities. Therefore the PBNP model derived in this paper belongs to the class of weak-coupling approximations that are known to fail at high charge densities where the strong coupling approximation becomes more appropriate.[35,39] We can qualitatively estimate the applicability of the weak coupling approximation by computing the Netz-Moreira electrostatic coupling parameter

$$\Xi = 2\pi q^3 l_B^2 \left(\frac{\sigma}{e_0}\right), \tag{16}$$

where $q = 1$ is the counterion valence and $l_B \simeq 0.7\,\text{nm}$ is the Bjerrum length.[35] Although Eq. (16) is typically associated with the adsorption of counterions on planar surfaces, it provides a useful dimensionless number also in our case; hence, we tentatively use it to assess the strength of the electrostatic coupling typical for the lateral dynamics of lipids and peptides on the membrane. Accordingly, we set the boundary of validity for the weak coupling approximation as $\Xi \simeq 1$. Since the typical lipid density on the membrane is ~1.67 lipid/nm^2, we find that $\Xi \simeq 1$ for the PS molar fraction of ~20%. This suggests that biological membranes may be positioned on the interface between the weak and strong coupling approximations and thus the predictions based on the Poisson-Boltzmann approximation should be considered

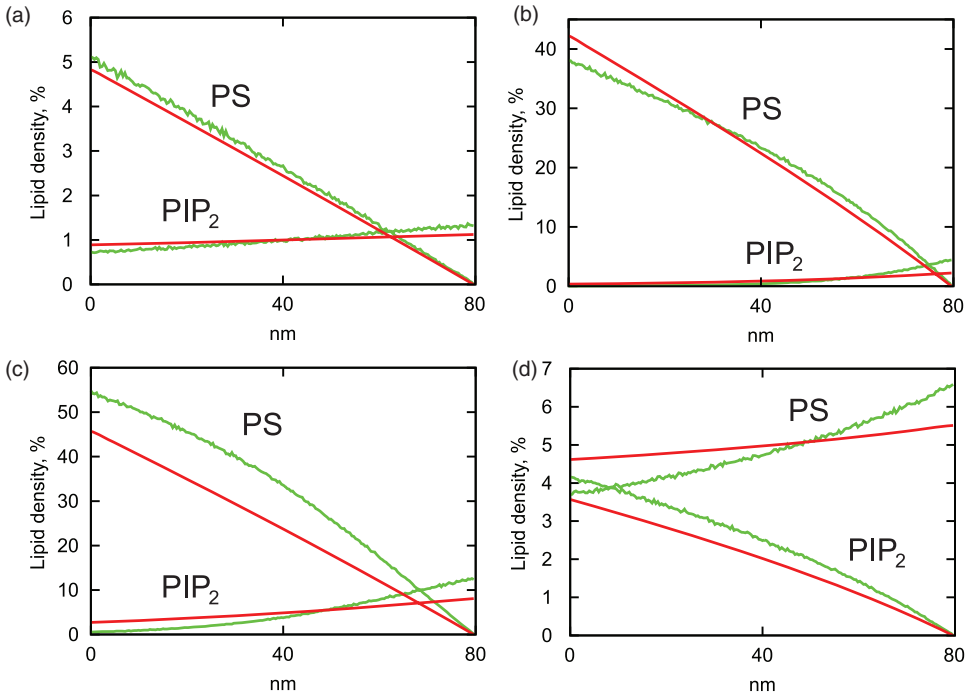

FIG. 5. Interaction of two charged lipid species on the ternary PC:PS:PIP2 membrane. Results of Monte Carlo simulations are shown in green and the PBNP model in red. (a) 5% gradient of PS interacts with homogeneously distributed 1% of PIP2; (b) 50% gradient of PS and 1% of PIP2; (c) 50% gradient of PS and 5% of PIP2; (d) 5% gradient of PIP2 interacts with homogeneously distributed 5% of PS.

with caution. Monte Carlo approach with its discrete representation of ions, presumably, is more quantitatively precise in the limit of high charge densities.

B. Gradients of interacting lipid species

When several types of charged lipid species are present on the membrane, as is the case for all biological membranes, formation of a gradient in the concentration of one species could induce response in the spatial distribution of other species. We next considered two electrostatically interacting lipids with distinct valences, PS and PIP2. The gradient of concentration was produced using the reaction source terms in the spatial distribution of the inducing lipid while no-flux boundary conditions were imposed on the responding lipid. The results of these simulations presented on the Fig. 5 show that a spatial heterogeneity induced in the distribution of one lipid can produce response in all components of the system. While a weak 5% gradient in the distribution of PS induces only very small perturbation to a homogeneous distribution of PIP2 (Fig. 5(a)), a 50% inducing gradient of PS significantly displaces PIP2 to the right boundary (Fig. 5(b)). Interestingly, the induced gradient of PIP2 generates electrokinetic force that pushes PS lipids back, thus, effectively increasing the PS gradient. This feedback is predictably more prominent at the higher PIP2 molar ratio in the bilayer (cf. Figs. 5(b) and 5(c)). As in the case of single lipid gradient, in all tested scenarios with two interacting lipids, Monte Carlo simulations systematically exhibited quantitatively larger effect due to the electrostatic interactions than the PBNP equations.

C. Dynamics of a peptide in the induced lipid gradient

Earlier study based on the dynamic Monte Carlo approach[19] offered an intriguing prediction that the polybasic peptides and proteins with polybasic domains can respond to the induced lipid gradients by drifting along or against the gradient depending on the total charge of the peptide together with the electrostatically bound lipids. Using the above derived PBNP model extended to describe the peptide-lipid complexes, we computed the spatial distribution of pentalysine on the membrane with varied PS and PIP2 gradients and compared the results with the Monte Carlo simulations. Figure 6(a) shows a good agreement between the two approaches in describing the redistribution of pentalysine in the presence of weak and steep gradients of PS.

Analysis of the interaction between pentalysine and the multivalent PIP2 lipids demonstrated that in the Monte Carlo automaton the individual peptide residues are not independent of each other in their interaction with PIP2 due to the non-negligible electrostatic repulsion between the PIP2 lipids. Therefore, simple model (14) is not applicable for the pentalysine-PIP2 interaction in a strict sense. To qualitatively compare the approaches in the presence of PIP2, we estimated the reaction rates k_0 and h_0 neglecting the cooperativity of binding. Also, to reduce the complexity we considered a bilayer consisting only of neutral PC and PIP2 itself. Figure 6(b) shows that pentalysine placed in the gradient of PIP2 exhibits strong repulsion by the gradient and accumulates on the left boundary. Predictably, due to the large negative charge of the peptide-PIP2 complexes, the repulsive effect of PIP2 is more prominent than the

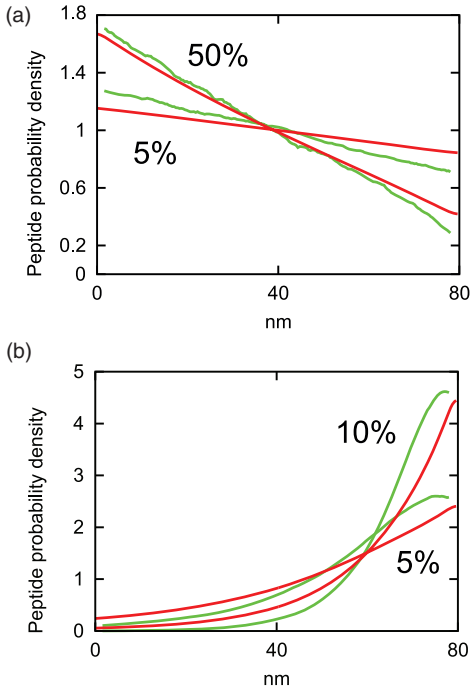

FIG. 6. Distribution of pentalysine in the gradient of a charged lipid. Results of Monte Carlo simulation are in green and the PBNP model in red. (a) On the 5% and 50% gradients of PS; (b) on the 5% and 10% gradients of PIP2.

attractive effect of the PS gradient. Consistently, Monte Carlo simulations exhibit stronger effect than the PBNB equations.

IV. CONCLUSIONS

Here, we derived and numerically investigated a system of Poisson-Boltzmann-Nernst-Planck equations that provide a mean-field approximation to the earlier proposed Monte Carlo model of electrostatic interactions between the charged lipids and proteins in the plane of the hydrated layer of a biological membrane. To reduce the complexity of this system, we, following other publications,[31,32] separated the ions into two classes: the explicitly considered charged lipid headgroups and peptide residues and mobile ions that are treated implicitly within the Poisson-Boltzmann framework. A particular property of lipid membranes that distinguishes them among other electrolyte-colloid systems is the dense packing of lipids. We explicitly considered this property by requiring that, the fluctuations being neglected, the sum of all lipid species concentrations on the membrane should be constant. Although we do not explicitly introduce the size of lipid head groups in our model, the condition of dense packing implies their finite size. The resulting modified Nernst-Planck equations contain additional terms that arise from this condition and effectively describe cross-diffusion fluxes associated with the excluded volume effect. In this regard, our approach is similar to other treatments where the finite size of ions was considered explicitly.[40] Numerical analysis demonstrated that the resulting correction terms become significant only for the lipid species with molar ratio above ~20%. This implies that if the system of interest consists of several minor (1%–5%) charged lipid species that are immersed in otherwise neutral membrane, the system of equations can be significantly simplified. With little error, charged lipids then can be considered as dilute solutes whose dynamics is described by regular Nernst-Planck equations and the concentration of neutral lipid ("solvent") computed simply from the condition of dense packing. The advantage of the full system of equations derived here, however, is that it treats all lipids species equally without any simplifying assumptions on their local concentrations. Although in the present contribution we restricted our consideration by assuming that the concentrations of protein-lipid complexes are negligible compared to the total lipid concentration, this restriction can be easily lifted by replacing the condition of dense packing given in Eq. (1) with Eq. (10). This will become important in the description of densely packed protein-lipid lattices, such as formed by annexin A5 in the presence of Ca,[41] or within other systems where negatively charged lipids and positively charged proteins segregate into distinct dense phases.[9,22]

The thorough comparison between the mean-field PBNP equations and Monte Carlo simulations demonstrated that both approaches are in a good qualitative agreement in all tested scenarios. Some quantitative discrepancy described above arises presumably from the mean-field nature of the PBNP model that ignores strong-coupling effects observed at high local charge densities. Transition to the coarse-grained mean-field description likely cannot be achieved without some loss of accuracy. Larger space and time scales come at the inevitable cost of introducing approximations that are necessary to achieve tractability of analytical models and computational feasibility of numerical approaches. At the same time, high charge densities at which the PBNP approach deviates from the Monte Carlo results are likely not observed in the biologically realistic conditions. This is because of the large abundance of proteins that use specific or non-specific electrostatic interactions to associate with negatively charged lipids. Under the realistic conditions, a large proportion of the charged lipids would be likely found within mostly electroneutral protein-lipid complexes that substantially reduce the effective charge density on the membrane. Thus, even though the total abundance of the negatively charged lipids on the inner leaflet of the plasma membrane may reach a staggering 40%–50%, the actual concentration of the free charged lipids is presumably only a minor fraction of this number. Taking this into consideration, we conclude that the PBNP model derived here provides a promising approach for the description of the lateral dynamics of biological membranes on the spatio-temporal scales commensurate with most important biological processes. Some examples of such processes in which charged lipids are dynamically generated in highly localized manner include, but are not limited to, the formation of actin-rich cell protrusions such as lamellipodia and dorsal ruffles.[25] Another relevant biological process of particular bio-medical relevance is the phagocytosis of pathogens. During the formation of a phagosome, charged lipid species are created and destroyed by lipid-modifying enzymes in a rapid succession;[10] therefore, during this process the formation of transient lipid gradients is highly likely. Understanding of these and many other biological phenomena will require development of a computationally

efficient continuous description of the membrane dynamics. The PBNB model offered here is a step towards the realization of this goal.

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