Structural and energetic model of the mechanisms for reduced self-diffusion in a lipid bilayer with increasing ionic strength

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Ionic concentration of the buffer strongly affects properties of a lipid membrane, such as membrane durability (e.g., in electroporation experiments), lateral diffusion coefficient, and zeta potential. The effect of ionic strength is studied by Monte Carlo simulations based on the improved Pink model with explicitly included interactions between lipid heads. We examine the energetic profile of the membrane, conformation of lipid molecules, and molecular interactions. The study is provided for dipalmitoyl-phosphatidylcholine (DPPC) membrane in the gel (300 K) and fluid (330 K) temperatures for the ionic strength in the range 10–3000 mM at several values of dielectric constant. At high ionic strength, the simulations indicate an increase of the membrane stability due to the screening of the repulsive forces between lipid heads, more stable conformation of lipid chains, and denser packing of the molecules. These effects may account for reduced lateral diffusion in the membrane, as observed in experiments. The simulation also suggests that chains tend to assume a more straightened configuration and the number of standing polar heads increases, which may contribute to thickening of the membrane. An increase of the head tilt dependent on ionic strength may account for the greater value of zeta potential. The model shows stronger electropermebilization of the membrane in external electric field when ionic strength is low.

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I. INTRODUCTION

Experiments on lipid membranes show a strong effect of ionic concentration on the membrane properties. For example, a membrane located in electric field of high intensity is less susceptible to irreversible rupture, following electroporation in current-clamp conditions [1–5], when surrounded with buffers of high ionic strength [6–8]. This effect may be related to decreased self-diffusion in the membrane, which is experimentally observed. The mechanism leading to the reduction of the lateral diffusion and possible consequences of this phenomenon has not been revealed. One possibility is that some kind of aggregation of ions from the solution with the membrane may be involved. Since interactions between monovalent ions and uncharged or zwitterionic lipids are considered weak [9], a molecular mechanism of the reduced diffusion in high concentrations is not clear. Still little is known about interactions between ions from buffers with uncharged or zwitterionic lipids so the problem needs to be explored by different techniques.

The effect of ionic strength on lipid membrane was studied by molecular dynamics (MD) simulations by Böckman and co-workers [10]. The MD model showed that when the ionic concentration in the electrolyte is increased, cations from the buffer bind to carbonyl oxygen in polar part of the lipids, forming charged complexes of greater size and lower mobility. There are several consequences of this association. The anions remaining in the solution build a diffusive capacitor producing a considerable electric field alongside the lipid headgroups and the distance between membrane layers increases. Fatty acyl chains change their conformation increasing order parameter, consistent with experiments [11,12]. Lipid molecules tend to occupy smaller area so the membrane is more tightly packed. The lipid headgroups also change their configuration by altering their tilt into a more standing position. All these effects, following the charge separation and creation of the lipid-cation complexes, may account for the reduced self-diffusion of lipids and certain membrane thickening. Other MD studies [32–34] devoted to the same problem reported comparable effects, however, with some differences. For example, there is no agreement on sensitivity of the headgroups to ionic strength [33,34].

We study the effect of ionic strength from another perspective. The most energetically stable conformation of lipid membrane, with no cations bound to lipid molecules, is examined at the given ionic strength. The objective of this study is to test whether the experimentally observed reduction of self-diffusion and the increased durability of the membrane can be explained only by the appearance of the cation-lipid complexes and what is the contribution of the lipids alone. We hypothesize that the most favorable energetical state of the lipids, under conditions of increased ionic strength, may significantly contribute to the phenomenon by altering the interactions between lipid molecules and their conformation. The simulations use the Monte Carlo (MC) method based on the modified Pink model [13–24]. Our revised model, unlike the original Pink model, has explicitly included the contribution from interactions between lipid headgroups [14]. This modification provides insight into the influence of ions from the solution on the membrane. We also test the susceptibility of the membrane to electropermeabilization by considering the energy of interactions between lipid molecules and external electric field. The approach proposed here and MD simulations allow us to study the same

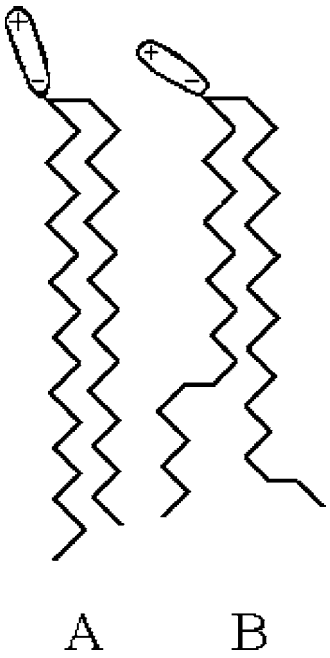

FIG. 1. Exemplary conformations. (A) Both chains are in all-trans conformation, the angle between C-C bond to the membrane normal is 35° or 145°. The head is in the standing position (78°). (B) Both chains have one gauche C-C bond which forms an angle 90° with the bilayer normal, the head is in the lying position (30°).

problems, however, from different perspectives. By the MC method tracking changes of all energy components is possible, showing how ionic strength affects each of them.

II. METHODS

The membrane was modeled as a triangular lattice where each node represented an acyl chain and two chains were attached to one lipid headgroup. The whole molecule could rotate 180° around the normal to the membrane surface. The chains were assumed as separate and each of them could take one of ten possible states representing ten levels of conformational energy. The number of actual conformations in each state α was represented by degeneracy D_α ranging from $D_1=1$ for all-trans conformation to $D_{10}=354\,294$ for the fluid state [21]. Chain conformation is defined by angles between C-C bonds. The angle was approximated as 35° or 145° to the bilayer normal for trans bonds and 90° for gauche bonds. The model takes into account that the chain closer to the polar head is effectively shorter of two C-C bonds (two bonds in the β chain are directed along the membrane surface) [25]. The distance between the positive charge at the N atom of the choline group and negative at the P atom was fixed, $d_{PN}=5\times10^{-10}$ m. Each chain from the lattice interacted with six nearest neighbors. Lipid heads were zwitterionic, represented as dipoles. They could assume one of two possible tilts toward the membrane surface 78° standing and 30° lying (Fig. 1).This assumption modeled two extreme positions of the heads [19] (and references therein). Polar heads could rotate towards their nearest six neighbors (nodes). Electrostatic interactions between dipoles included 14 neighboring dipoles [20]. Periodic conditions were imposed on the boundaries of the whole lattice.

The Hamiltonian of the studied system involved three terms—the energy of van der Waals interactions H_{vdW}, the conformational energy H_{conf}, and the energy of electrostatic interactions between polar heads H_{dip} [21],

$$H = H_{vdW} + H_{conf} + H_{dip}. \tag{1}$$

The van der Waals interactions were given by

$$H_{vdW} = -\frac{J_0^M}{2}\sum_{i,j=1}^{N}\sum_{n,m=1}^{10} f(r_{nm})S_nS_mL_{ni}L_{mj}. \tag{2}$$

J_0^M denotes the interaction energy between two parallel chains in all-trans conformation. Lattice co-ordinates are i (site index ranging from 1 to N) and j (index of six sites neighboring with site i). The indices of chain conformational state n and chain conformational state m range from 1 (all-trans) to 10 (fluid) [13]. The distance r_{ij} between two chains at sites i and j depends on their conformational states. State operator L_{ni} of the chain located in site i equals 1 if the ith chain assumes conformation n, and 0 otherwise.

The order parameter S_n for acyl chain in conformation n yields

$$S_n = \frac{\sum_p S_{np}}{\sum_p S_{1p}}, \tag{3}$$

where S_{np} order parameter of the pth C-C bond

$$S_{np} = \frac{1}{2}(3\cos^2\theta_{np} - 1), \tag{4}$$

and p is the index of C-C bond in the chain. The bond is characterized by the angle θ_{np} between the bilayer normal and the normal to the plane spanned by the pth CH_2 group of the chain.

Distance dependence of van der Waals interactions between chains in the mth and nth conformations r_{nm} is expressed by $f(r_{nm})$,

$$f(r_{nm}) = w_n\left(\frac{r_1^2}{r_n r_m}\right)^{5/2}, \tag{5}$$

where r_n denotes radius of the space occupied by an average chain in the nth conformation, w_n is a weakening factor, $w_{10}=0.4$ for chains in fluid state, and $w_n=1$ if $n\neq10$. The factor was introduced by Mouritsen and co-workers [21] in their modification of Pink's model to provide good agreement between the model and experimental data.

The conformational energy H_{conf} is defined according to predefined [21] conformational energies of the chains E_n,

$$H_{conf} = \sum_{i=1}^{N}\sum_{n=1}^{10} E_nL_{ni}. \tag{6}$$

The interactions between polar heads H_{dip} that are due to electrostatic interactions are given by

$$H_{dip} = \frac{1}{2}\sum_{i=1}^{N}\sum_{j=1}^{14}\sum_{\alpha,\beta=1,-1}\frac{\alpha Q_{\alpha i}\beta Q_{\beta j}\exp(-\kappa r_{\alpha i\beta j})}{4\pi\varepsilon\varepsilon_0 r_{\alpha i\beta j}}, \quad (7)$$

where ε is the electrolyte dielectric constant, ε_0 is the permitivity constant, and $Q_{\alpha i}$ is the effective polar head charge with $Q_{\alpha i}=q/2$, where q is the actual dipole charge which equals an elementary charge [19] and α denotes the charge sign, $\alpha=1$ for a positive charge and $\alpha=-1$ for a negative. The distance between charges α and β of the dipoles at sites i and j is represented by $r_{\alpha i\beta j}$.

The inverse of Debye length κ defines the range of electrostatic interactions with screening,

$$\kappa = \sqrt{\frac{2z^2F^2c}{\varepsilon_0\varepsilon RT}}, \quad (8)$$

where $z=1$ is the valency of each polar head charge, F is Faraday's constant, T is the absolute temperature, c is the ionic strength of the solution, and R is the ideal gas constant.

To study an additional influence of electric field, the energy of interactions H_e between lipid molecules and electric field is also considered. Then, the Hamiltonian takes the form

$$H = H_{vdW} + H_{conf} + H_{dip} + H_e. \quad (9)$$

The energy H_e reflects interactions between the polar parts of the molecules and the electric field. H_e is calculated under the assumption that only the nitrogen end of the dipole is mobile and the other end is fixed. The direction of the electric field is assumed as perpendicular to the membrane surface,

$$H_e = -d\sum_{i=1}^{N} Q_i E[1-\cos(\theta)], \quad (10)$$

where d is a dipole length and θ is an angle between directions of the field E and the dipole. The field E represents an effective electric field through the membrane, which involves all possible effects (e.g., interfacial phenomena, divider effect, etc.) incorporated into one variable. Due to the full packing of the lattice, electroporation cannot be studied within this model. However, possible electropermeabilization of the membrane, resulting in the looser structure of the membrane, can be examined.

The simulations were carried out for a bilayer dipalmitoyl phosphatidylcholine (DPPC) membrane with 16 C atoms in each acyl chain, represented by a hexagonal lattice with 10×10 nodes and periodic conditions imposed on the boundaries of the lattice. A canonical ensamble was assumed. The system was equilibrated for 1000 Monte Carlo steps per site, then 10 000 steps per site were performed. A series of microconfigurations, which is a Markov process, was selected by means of Metropolis method [21]. The software for simulations was developed in Pascal programming language, software for analysis in MATLAB 6.5. Lines on the graphs were fitted to the simulation points.

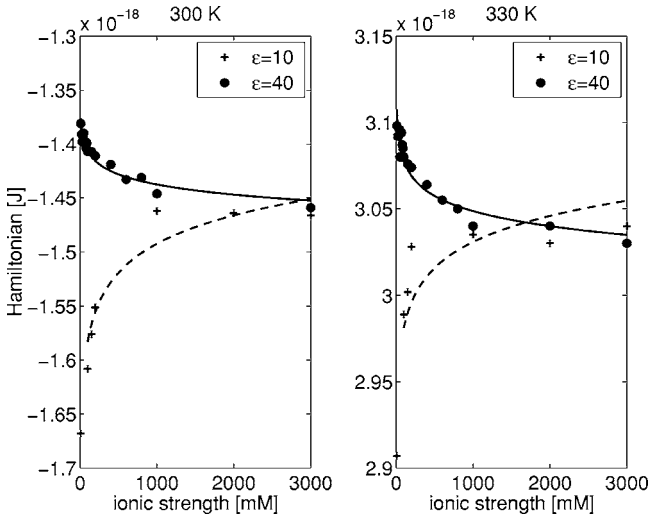

FIG. 2. Total energy H of the membrane assumes lower values as ionic strength is increased for dielectric constant $\varepsilon=40$. The membrane accepts energetically more stable configuration in higher ionic concentration. The result for $\varepsilon=10$ is in contradiction with experiments in aqueous buffers.

III. RESULTS AND DISCUSSION

The study of the influence of ionic strength on energetic state of the membrane and the interactions between molecules and the configuration of polar heads was performed. The membrane was examined at the gel and fluid temperatures, $T=300$ K and $T=330$ K. Since the effective dielectric constant in the plane of ion adsorbtion may depend on ionic strength [26], the influence of the dielectric constant ε of the electrolyte was tested as its value ranged from 10 to 80. A mechanism responsible for the increased durability of lipid membrane in higher ionic strength was investigated.

For both temperatures the Hamiltonian H of the membrane proved sensitive to ionic strength, decreasing as the ionic strength was increased ($\varepsilon=40$, Fig. 2, solid line). The dependence was remarkable only below 1000 mM. This result stands for better stability of the membrane, which is observed experimentally [4,5]. In the gel phase, as the ionic strength increases, the Hamiltonian assumes more negative values, which stands for stronger attraction between molecules. In the fluid state repulsion gets weaker for higher ionic concentration.

It is known that water organization changes in the vicinity of lipid bilayer, which has an effect on the effective dielectric constant of water, and $\varepsilon = 80$ may not represent the effective value appropriate for simulations. The value of ε depends on the location of water molecules relative to bilayer, changing from 4 to 78 [10,26]. It can be related to the number of water molecules hydrating certain part of a lipid (10.2 water molecules hydrate the $N(CH_3)_3$ group, 4.0 water molecules the phosphate, and 1.0 water molecules a carbonyl group [27]). While modeling the influence of ionic strength, we used the opportunity to test whether we could estimate the effective value of water dielectric constant applicable for such models. The stability decreased only at very low values of dielectric constant ($\varepsilon=10$, Fig. 2, dashed line), which for aqueous buff-

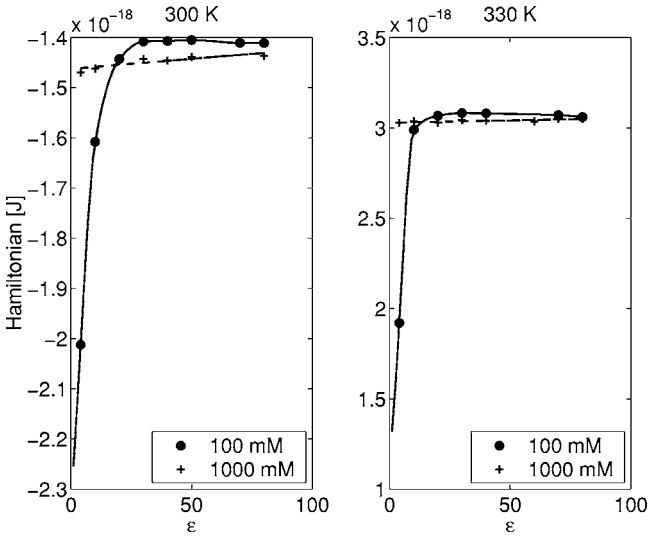

FIG. 3. Dependence of the total energy H value on the assumed dielectric constant.

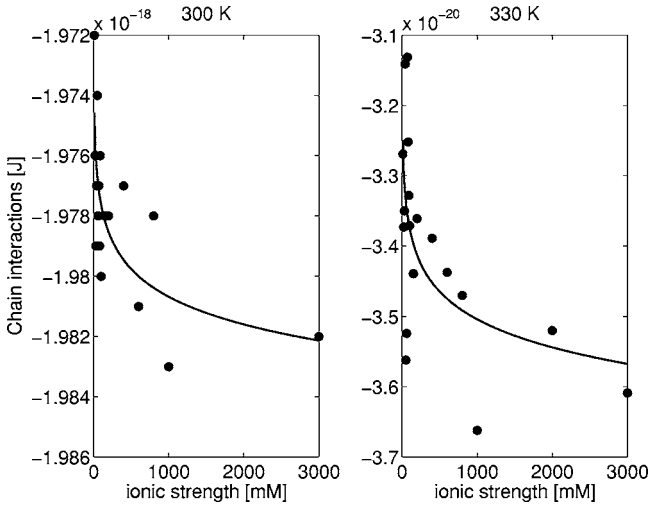

FIG. 5. Energy of van der Waals interactions H_{vdW} between acyl chains. Stronger attraction observed as ionic strength increases due to the tighter packing of the molecules.

ers is in contradiction to experimental evidence [4]. Since the value of dielectric constant had an effect on the result only for $\varepsilon < 30$ (Fig. 3), mostly at low ionic strength, for further experiments dielectric constant was fixed at $\varepsilon = 40$. This value was also validated as optimal for aqueous buffers by modeling membrane phase transition [20].

Three components contribute to the value of the Hamiltonian: (i) the energy of electrostatic interactions between polar heads H_{dip}, (ii) the energy of van der Waals interactions between acyl chains H_{vdW}, and (iii) the configurational energy of the chains H_{conf}, which is always a positive number. As expected, the change in the value H_{dip} revealed the screening effect from higher ionic concentration (Fig. 4, solid line). The repulsive interaction energy decreased approximately six-fold, while the ionic concentration was increased from 10 to 3000 mM. Although the repulsion between headgroups does not confirm MD results [28], it has been observed in different MC simulations from another group [29] and suggested as a source of ripple phase appearance.

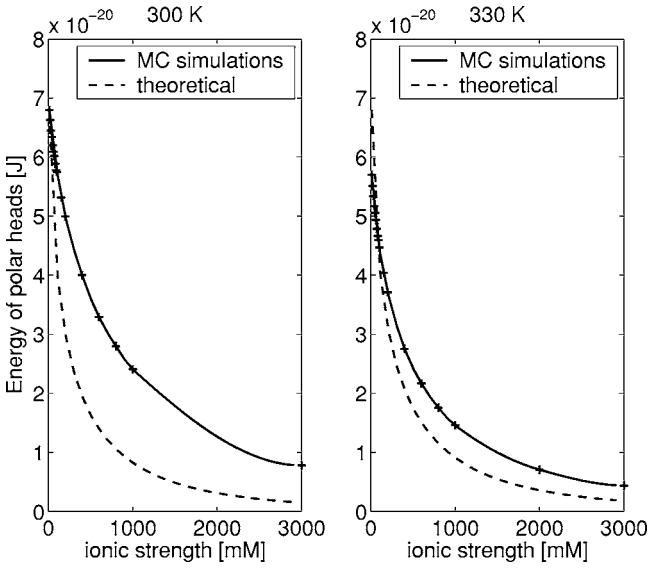

FIG. 4. The energy of interactions (repulsive) between polar heads H_{dip} decreases due to the screening effect from ions in the solution (solid line). Compared to the theoretical result (dashed line) when an average head-head distances were assumed constant.

The change of head-head interactions was slightly lower than expected. Theoretically, if the average distances between molecules were independent of the ionic strength, the decrease should be more significant, especially for the gel phase (Fig. 4, theoretical curve by dashed line and simulation by solid line). The weaker screening effect can be explained by closer packing of the molecules. The tighter packing of the molecules was confirmed by some increase in attracting van der Waals interactions (Fig. 5) at both $T = 300$ K and $T = 330$ K.

Another effect of ionic strength was a change of the chains conformation, which can be seen from H_{conf} value (Fig. 6). In the gel temperature most chains were in the all-trans conformation, therefore a considerable change towards lowering degeneracy factor of the chains (lower H_{conf}) is not expected, confirmed by the simulation at low ionic strength. However, at ionic strength of about 1000 mM the value of H_{conf} diminishes. The effect of ionic strength on chains conformations was more pronounced for the fluid phase below 1000 mM. A decrease of the conformational energy reflects straightening of the chains and lowering the degeneracy. It means that statistically more molecules are in the gel state, improving stability of the bilayer at the fluid temperature 330 K.

The effect of ionic strength on the configuration of the lipid headgroups was also tested. Generally, there is no agreement between authors on the tilt sensitivity to ionic strength. Although there are experimental papers which claim that the headgroups reorient in high ionic strength [30,31], and this is confirmed by some MD results [10,32], other MD papers claim no sensitivity [33] or local sensitivity only, which does not affect the average [34]. In our simula-

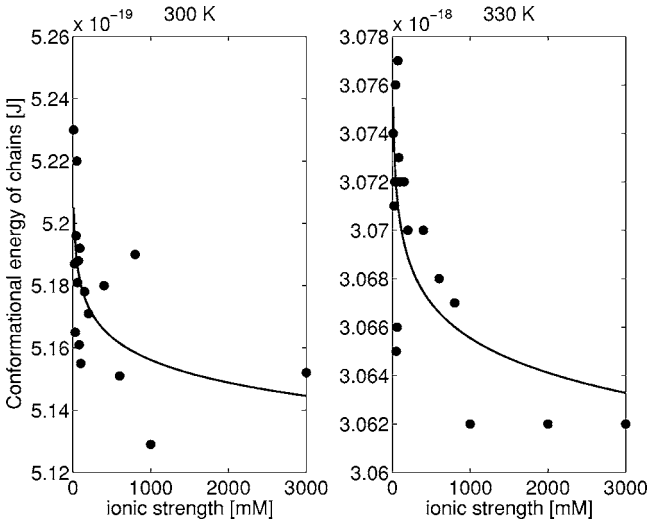

FIG. 6. Conformational energy of acyl chains H_{conf}. No significant dependence on ionic strength in the gel temperature observed. Acyl chains assume conformations with decreasing degeneracy for concentrations below 1000 mM in the fluid phase.

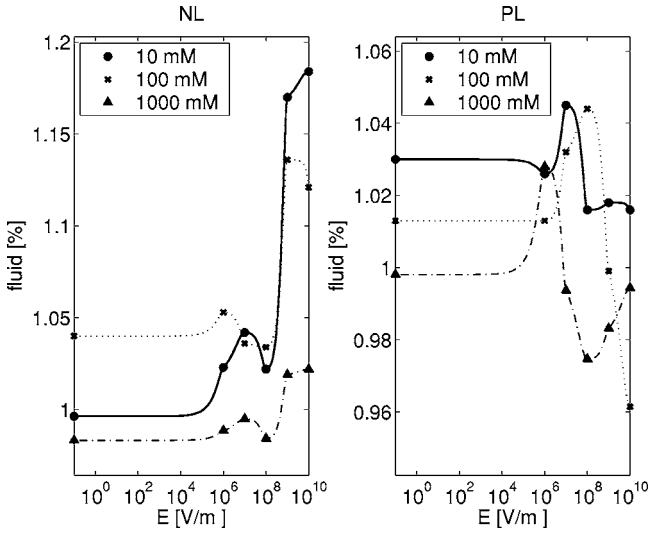

FIG. 8. Increase of the molecules in fluid conformation in the layer at the negative potential when high electric field is applied, stronger at low ionic strength.

tions the tilt of the dipoles showed sensitivity to the ionic strength (Fig. 7). At the gel temperature and low value of the ionic strength (10 mM) only 27% of polar heads assumed the standing configuration (78°). This number increased twofold at 3000 mM. The same dependence, although slightly weaker, could be observed at the fluid temperature. This result is in accordance with experimentally observed increase of zeta potential in buffers of higher ionic strength [30,35]. It has been also proposed that the increase of zeta potential results from the reorientation of the head groups [30].

Finally, an effect of ionic strength on the membrane in high external electric field was investigated. The experiments show that the electropores in higher ionic strength are smaller and the membrane is more stable. Therefore a chance for an irreversible rupture of the membrane is much lower if electroporation occurs at high ionic strength. Stronger interactions and smaller distances between lipid molecules could provide an explanation for an enhanced durability of the membrane at high ionic strength.

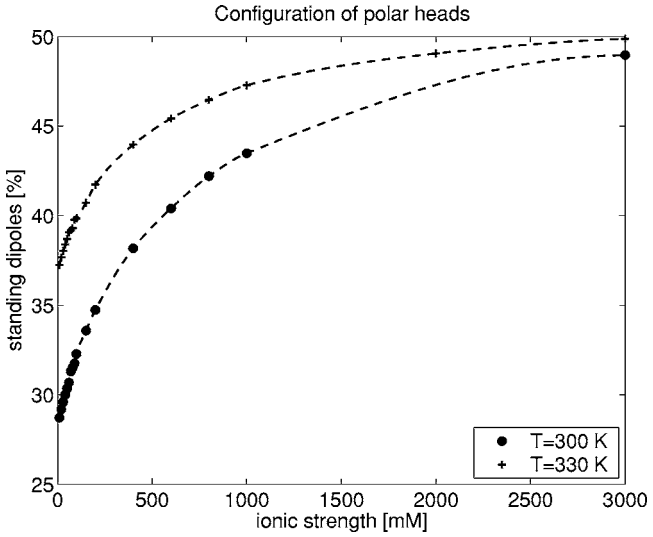

FIG. 7. The number of standing dipoles significantly increases with the ionic strength.

Our simulations showed that, due to the interactions between the field and the membrane, some molecules from the layer at the negative electric potential (NL) make transition into the fluid phase at $T=300$ K (Fig. 8). The lower the ionic strength, the stronger was this effect. The layer at the positive electric potential (PL) did not show any significant change in the number of fluid molecules, a certain decrease of fluid molecules appears at high ionic strength only. Note that the results for PL may be less reliable since the model lattice is fully occupied and a dramatic rearrangement of lipids, such as the flip-flop process that could be expected here, is not possible in the model. The net effect of the high electric field shows appearance of the spots with fluid conformation. The two effects observed in the membrane at low ionic strength, loosening of the membrane structure due to weaker interactions between lipids, and local appearance of the fluid conformation in electric field, may contribute to higher electropermealization of the membrane.

IV. CONCLUSIONS

The Monte Carlo simulations based on the modified Pink model proved the influence of ionic strength on the conformation of lipid molecules and their interactions, which has an effect on the membrane integrity and stability. We searched for the mechanism responsible for reduced self-diffusion in the membrane at higher ionic strength, which may contribute to lower susceptibility of the membrane to electroporation. The question was if creation of lipid-cation complexes is the only source of this phenomenon and what is the contribution of the energetical effects originating in the membrane alone. Our study provided another perspective, complementing MD modeling.

The simulations suggest that the membrane becomes more stable in higher ionic strength. The most significant change in the membrane stability comes from lower repulsive electrostatic interactions between polar heads as ionic strength increases. However, the change of these interactions is less significant than theoretically expected from the screening, mostly due to the tighter packing of the lipid molecules. Therefore the van der Waals interactions between hydrophobic chains increase, binding the internal part of the membrane more strongly. Both effects may contribute to the observed reduction of lipid self-diffusion.

In the case with fluid temperature and the buffer concentration below 1 M, acyl chains increase their order as they tend to assume more energetically stable conformation with decreased degeneracy coefficient, consistent with experimental data.

The effect of ionic strength on polar heads configuration was predicted, in agreement with MD simulations and experimental results on increasing zeta potential. The number of standing dipoles with the tilt 78° increased twofold while comparing 10- and 3000-mM solutions. This may indeed elevate accessibility of ions from the solution in higher ionic strength, sodium or chloride ions, as well as other molecules or compounds present in the solution.

The model suggests that the reduced self-diffusion in high ionic strength is related to the conformations of lipid molecules and their interactions when the most favorable energetic state is assumed by the membrane. The membrane structure gets tighter in high ionic strength. This result was achieved without assuming the appearance of lipid-cation complexes.

Finally, the simulations showed that in high electric field, the membrane fluidity locally increases and this effect is stronger at low ionic strength. It may additionally contribute to the increase of membrane electropermeability, already facilitated by looser structure of the membrane at low ionic concentration.

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