

## Calculation of the electrostatic potential of lipid bilayers from molecular dynamics simulations: Methodological issues

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The electrostatic properties of lipid membranes are of profound importance as they are directly associated with membrane potential and, consequently, with numerous membrane-mediated biological phenomena. Here we address a number of methodological issues related to the computation of the electrostatic potential from atomic-scale molecular dynamics simulations of lipid bilayers. We discuss two slightly different forms of Poisson equation that are normally used to calculate the membrane potential: (i) a classical form when the potential and the electric field are chosen to be zero on one of the sides of a simulation box and (ii) an alternative form, when the potential is set to be the same on the opposite sides of a simulation box. Both forms differ by a position-dependent correction term, which has been shown to be proportional to the overall dipole moment of a bilayer system (for neutral systems). For *symmetric* bilayers we demonstrate that both approaches give essentially the same potential profiles, provided that simulations are long enough (a production run of at least 100 ns is required) and that fluctuations of the center of mass of a bilayer are properly accounted for. In contrast, for *asymmetric* lipid bilayers, the second approach is no longer appropriate due to a nonzero net dipole moment across a simulation box with a single asymmetric bilayer. We demonstrate that in this case the electrostatic potential can adequately be described by the classical form of Poisson equation, provided that it is employed in conjunction with tin-foil boundary conditions, which exactly balance a nonzero surface charge of a periodically replicated multibilayer system. Furthermore, we show that vacuum boundary conditions give qualitatively similar potential profiles for asymmetric lipid bilayers as compared to the conventional periodic boundaries, but accurate determination of the transmembrane potential difference is then hindered due to detachment of some water dipoles from bulk aqueous solution to vacuum. © 2009 American Institute of Physics. [DOI: 10.1063/1.3148885]

### I. INTRODUCTION

Lipids constitute a broad class of molecules that give rise to a variety of fascinating structures. Of these, the lipid bilayer<sup>1</sup> is likely the most relevant one. In cells, lipid bilayers serve as the basic structure of cellular membranes, governing or mediating numerous cellular functions. Lipids themselves are also involved in many cellular processes, such as being part of membrane protein structures and functions or acting as second messengers in processes such as programmed cell death.

Of the many physical characteristics of membranes, the electrostatic properties are of special interest. The membrane potential, for instance, is crucial for a variety of membrane-mediated biological phenomena such as conductance of ionic channels, insertion and orientation of integral membrane proteins, transport across plasma membranes, and binding of therapeutic peptides to membranes.<sup>2</sup> The nature of the elec-

trostatic potential of model lipid bilayers in salt-free aqueous solutions is in most cases dipolar, originating from water dipoles and polar head groups of lipids. If charged (essentially always anionic) lipids are present in the system, then there is also an additional contribution due to counterions and the nonzero surface charge of a membrane.

Due to the important role of membranes in cellular phenomena, computational simulations and modeling have become an integral component of membrane research, complementing experiments especially in cases where insight of nanoscale phenomena is called for. In practice, atomic-scale molecular dynamics (MD) simulations of lipid membranes have reached a state where they are not only able to successfully complement experiments but also to provide added value by often being the only means to gain molecular-level insight into the system in question. Further, recent progress in both methodology and computer power made it possible to simulate bilayer systems comprised of more than 1000 lipids over a time scale of 100 ns at atomic resolution,<sup>3</sup> complemented by similar simulations of membrane proteins in lipid bilayers over microsecond time scales<sup>4</sup> (for a very recent

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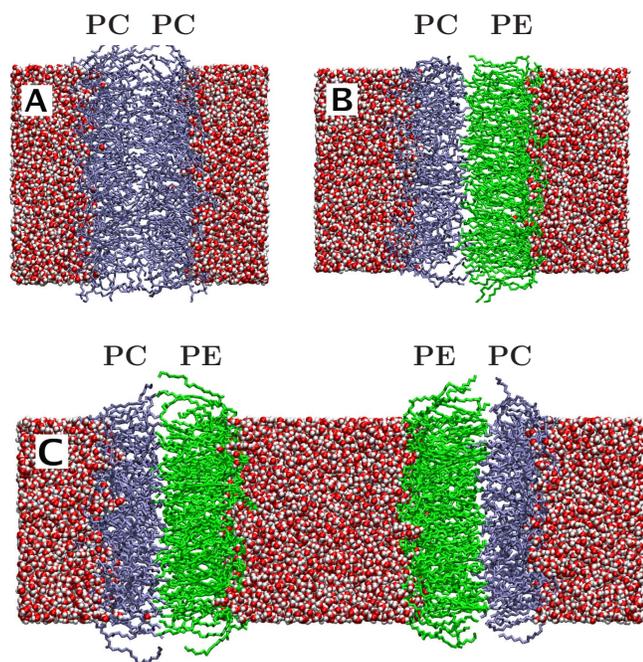


FIG. 1. (Color online) Snapshots of the main three bilayer systems studied: (a) a symmetric POPC bilayer, (b) a single asymmetric POPC/POPE bilayer, and (c) a double POPC/POPE bilayer, two PC/PE bilayers being arranged in an antiparallel fashion.

overview of the area, see, e.g., Ref. 5). Larger bilayers over microsecond time scales have been successfully simulated using coarse-grained models.<sup>6,7</sup>

Significance of membrane potential for governing cellular functions underlines the importance of describing membrane electrostatics as accurately as possible. This in turn stresses the importance of methodological development to design novel and accurate tools for considering electrostatic properties of membranes. For example, while in principle the calculation of the electrostatic potential of a lipid bilayer is a seemingly straightforward task, in practice there are various issues one has to worry about. To highlight how sensitive this issue is, consider the membrane potential profile that can be computed using Poisson equation by twice integrating the charge density. In its classical form, the potential and the electric field are chosen to be nil in the middle of the water phase.<sup>8</sup> However, due to limiting sampling of atomic-scale MD simulations and fluctuations of the center of mass (CM) of a bilayer, the calculated electrostatic potential often has a nonsymmetric shape, sometimes also showing a nonzero potential drop across a symmetric lipid bilayer, which apparently is a pure artifact.

Recently, Sachs *et al.*<sup>9</sup> proposed a new form for Poisson equation and the related boundary conditions; the potential was chosen to be the same on the opposite sides of a simulation box along the direction of the bilayer normal. This automatically ensures that the potential difference across a bilayer is nil. A drawback of the above approach is that on definition it cannot be applied to asymmetric bilayer systems, which are characterized by a nonzero dipole moment across a simulation box. Such a situation is encountered, e.g., in computational studies of electroporation phenomena in lipid membranes under external electric field.<sup>10–15</sup> Another recent

example is related to the development of more realistic membrane models, which would account for transmembrane lipid asymmetry that is an inherent feature in plasma membranes of living cells.<sup>16,17</sup> Recently, it has been demonstrated that an asymmetric distribution of lipid molecules across a bilayer is able to give rise to a nonzero potential drop between the two membrane leaflets and, correspondingly, to a nonzero dipole moment across a simulation box.<sup>18,19</sup>

The aim of this study is twofold. First, we discuss application of the two different forms of Poisson equation for calculating the electrostatic potential of a *symmetric* lipid bilayer. We show that for sufficiently long simulations both approaches give essentially the same potential profiles. Criteria for matching these conditions are discussed. Second, we consider several means to compute the electrostatic potential for *asymmetric* bilayers. We demonstrate that the electrostatic potential can be computed reliably with the use of the classical form of Poisson equation provided that tin-foil boundary conditions are applied.

## II. METHODS

### A. Molecular dynamics simulations

We performed atomic-scale MD simulations for both symmetric and asymmetric lipid bilayers. As for symmetric bilayers, we considered a bilayer comprised of 128 palmitoyl-oleoyl-phosphatidylcholine (POPC) lipids solvated in a box with about 5100 water molecules. An asymmetric lipid bilayer was built from POPC and palmitoyl-oleoyl-phosphatidylethanolamine (POPE) single-component monolayers consisted of 51 POPC and 64 POPE lipids, respectively; the bilayer was solvated by  $\sim 5150$  water molecules. The number of lipids in the opposite leaflets of the PC/PE membrane was chosen such that the equilibrium monolayer areas were identical in the PC and PE leaflets; this was done by using data from previous work for the average area per lipid in single-component POPC and POPE bilayers.<sup>18</sup> There is reason to mention that such an asymmetric bilayer has been shown to generate a nonzero dipole moment across a simulation box.<sup>18</sup>

To explore how boundary conditions affect the electrostatic properties of asymmetric PC/PE membranes, we considered two options. First, we employed a double bilayer setup<sup>9,14,19–26</sup> and performed MD simulations of two PC/PE membranes arranged in an antiparallel (PC-PE to PE-PC) fashion in a box with  $\sim 10200$  water molecules. Such a setup nullifies an overall dipole moment of a simulation box and therefore is an excellent reference system to track down any possible artifacts related to the electrostatic properties of asymmetric membranes. Second, we studied a PC/PE membrane with vacuum boundaries (slab geometry).<sup>27</sup> For doing that the size of the simulation box in the direction normal to the membrane surface was increased from  $\sim 9$  to 30 nm.

Three main simulated systems (a symmetric PC bilayer and asymmetric single and double bilayer systems) are presented in Fig. 1. The trajectory for a single PC/PE bilayer was taken from Ref. 18.

Both POPC and POPE lipids were described by a united-atom force-field of Berger *et al.*<sup>28</sup> Water was modeled using

the simple point charge model.<sup>29</sup> The Lennard-Jones interactions were cut off at 1 nm. The electrostatic interactions were handled through the particle-mesh Ewald method (PME) method.<sup>30,31</sup> In the case of a PC/PE system with slab geometry a special correction term was applied to mimic the two-dimensional Ewald sum (EW3DC method).<sup>27</sup> The simulations were performed at the physiological temperature ( $T=310$  K). Pressure set to 1 bar was semi-isotropically coupled to a thermostat. The Berendsen scheme was employed for controlling both temperature and pressure.<sup>32</sup> Simulations of a PC/PE membrane with vacuum boundaries were performed in the *NVT* ensemble.<sup>27</sup> Periodic boundary conditions were applied in all three dimensions. The time step used was 2 fs. The simulation of a symmetric POPC bilayer, being based on the trajectory from our previous study,<sup>33</sup> was extended to 240 ns, where the first 40 ns were skipped from subsequent analysis. The simulations of all three PC/PE bilayer systems were performed over a period of 100 ns each, using the last 70 ns for analysis. The GROMACS suite was used for all the simulations.<sup>34</sup>

## B. Poisson equation

The electrostatic potential across a lipid bilayer  $\Psi$  is linked with the charge densities  $\rho$  via the Poisson equation:

$$\frac{d^2\Psi(z)}{dz^2} = -\frac{\rho(z)}{\epsilon_0}, \quad (1)$$

where  $\epsilon_0$  is the vacuum permittivity. We note that both  $\Psi$  and  $\rho$  are functions of position along the  $z$ -axis, which is perpendicular to the bilayer surface, i.e., Eq. (1) has already been averaged over  $x$  and  $y$  coordinates.

In its classical formulation, the electrostatic potential is computed by integrating Eq. (1) twice under the following boundary conditions:  $\Psi_{cl}(0)=0$  and  $E_{cl}(0)=0$ , i.e., the potential and the electric field are chosen to be zero at  $z=0$ , which is usually chosen to reside at the center of the bulk water phase or in the center of the membrane. These choices eliminate both integration constants and for  $\Psi_{cl}(z)$  one finds<sup>35</sup>

$$\Psi_{cl}(z) = -\frac{1}{\epsilon_0} \int_0^z dz' \int_0^{z'} \rho(z'') dz''. \quad (2)$$

Most lipid simulation studies employed Eq. (2) for computing the electrostatic potential.

Sachs *et al.*<sup>9</sup> recently derived another form of the Poisson equation. They assumed that the electrostatic potential is the same on the opposite sides of a simulation box, i.e.,  $\Psi_S(0)=\Psi_S(L)$ , where  $L$  is the length of a simulation box in the  $z$ -direction. In this case the condition  $\Psi_S(0)=0$  eliminates just one constant and the Poisson equation has the following form:<sup>9</sup>

$$\Psi_S(z) = \Psi_{cl}(z) + Cz. \quad (3)$$

Here  $C$  is some constant; it can easily be determined from Eq. (3) and the condition  $\Psi_S(0)=\Psi_S(L)$ , so one finally arrives at<sup>9</sup>

$$\Psi_S(z) = \Psi_{cl}(z) - \Delta(z), \quad (4)$$

where

$$\Delta(z) = \frac{z}{L} \Psi_{cl}(L). \quad (5)$$

Thus, both approaches give equations for the electrostatic potential which differ only by a position-dependent correction term,  $\Delta(z)$ .

It is instructive to discuss the correction term  $\Delta(z)$  in more detail. With the use of Eq. (2) the term  $\Delta(z)$  reads

$$\Delta(z) = \frac{z}{L} \Psi_{cl}(L) = -\frac{z}{\epsilon_0 L} \int_0^L dz' \int_0^{z'} \rho(z'') dz''. \quad (6)$$

We now switch the order of integration in Eq. (6) and perform the integral over  $z'$ ,

$$\begin{aligned} \Delta(z) &= -\frac{z}{\epsilon_0 L} \int_0^L dz'' \int_{z''}^L \rho(z'') dz' \\ &= -\frac{z}{\epsilon_0 L} \int_0^L (L - z'') \rho(z'') dz''. \end{aligned} \quad (7)$$

The last expression can further be rewritten as follows:

$$\Delta(z) = -\frac{z}{\epsilon_0 L} \left[ L \int_0^L \rho(z'') dz'' - \int_0^L \rho(z'') z'' dz'' \right]. \quad (8)$$

The first integral in Eq. (8) is simply the overall charge of a lipid bilayer system; it is equal to zero for neutral systems. The second integral is related to the overall dipole moment of the system along the direction normal to the bilayer surface (the  $z$ -axis). To demonstrate this, let us divide the simulation box in small slices  $dz$  along the  $z$ -axis. The dipole moment  $dP(z)$  of a single slice located at position  $z$  is given by

$$dP(z) = [\rho(z) S_{xy} dz] z, \quad (9)$$

where  $S_{xy}$  is the bilayer area in the  $(x, y)$ -plane. Note that the expression in brackets is the charge of a slice. To get the total dipole moment of a system,  $P_{\text{total}}$ , one needs to integrate Eq. (9) over  $z$  from 0 to  $L$ . This way we arrive at

$$P_{\text{total}} = S_{xy} \int_0^L \rho(z) z dz. \quad (10)$$

Finally, combining Eqs. (10) and (8) gives us the following expression for the correction term  $\Delta(z)$ ,

$$\Delta(z) = \frac{z}{\epsilon_0 V_{\text{system}}} P_{\text{total}}, \quad (11)$$

where  $V_{\text{system}}$  is the volume of a simulation box. Thus, a difference between the electrostatic potentials,  $\Psi_{cl}(z)$  and  $\Psi_S(z)$ , computed with the use of the two forms of Poisson equation turns out to be proportional to the overall dipole moment of a system provided that the system is electroneutral.

### III. RESULTS AND DISCUSSION

#### A. Symmetric lipid bilayers

We start with a conventional symmetric lipid membrane, a POPC bilayer shown in Fig. 1(a) being a typical representative. To calculate the electrostatic potential, one needs to integrate the Poisson equation, see Eq. (1). In practice, a simulation box is divided in thin slices along the  $z$ -axis and the charge density of each slice is determined as the partial charges of all atoms within the slice over the slice volume. These charge densities are then numerically integrated twice over simulation box with appropriate boundary conditions. It has to be emphasized at this point that sufficient precision should be maintained during decomposing the partial charges over finite slices. Otherwise, one can expect significant integration errors in the potential and field, especially for large membrane systems. Another source of errors is related to the use of pressure coupling, so that the size of a simulation box in the direction perpendicular to the bilayer surface (the  $z$ -axis) fluctuates around its average value with time and can therefore affect potential profiles. This can be eliminated by scaling coordinates of all atoms in every simulation frame back to the initial size of the box. We use such a scaled coordinate system throughout the paper.

By far most simulation studies of lipid bilayers have used the classical form of the Poisson equation [Eq. (2)] with boundary conditions  $\Psi_{cl}(0)=0$  and  $E_{cl}(0)=0$ ,  $z$  being set to zero at the left-hand side of a simulation box along the  $z$ -axis.<sup>8</sup> In Fig. 2 (top) we plot the electrostatic potential calculated following this recipe for a symmetric POPC bilayer. Figure 2 (top) reveals a serious problem with the calculated potential profiles of a symmetric bilayer; the electrostatic potential turns out to be nonsymmetric. It is also very sensitive to the length of trajectory used for calculating the potential. Surprisingly, extending simulations to longer times does not make potential profiles more symmetric, see Fig. 2 (top).

The origin of this artifact is closely related to the fact that the position of the CM of a bilayer with respect to  $z=0$  can fluctuate over the course of simulations. To eliminate this effect, one needs to center the positions of all atoms in the system with respect to the CM of a bilayer in each frame of simulations; accordingly  $z=0$  should be chosen at the bilayer CM. Figure 2 (bottom) shows that such an approach indeed considerably improves the potential profile making it much more symmetric. Importantly, the electrostatic potential is now very slightly sensitive to the simulation time. In fact, one can observe such a sensitivity only in the water phase nearby the lipid/water interface, see inset in Fig. 2 (bottom); the potential profile turns out to almost fully converge within 100 ns of production run. Therefore, the classical form of the Poisson equation gives symmetrical potential profiles for symmetrical PC bilayers provided that all atoms are centered with respect to the bilayer CM and the simulations are long enough (a production run of at least 100 ns is required).

However, there is yet another subtle detail which has not been brought about yet: the effects of undulations. Current state-of-the-art for lipid membrane simulations allows studies of bilayers that cover tens of nanometers<sup>3</sup> in the bilayer

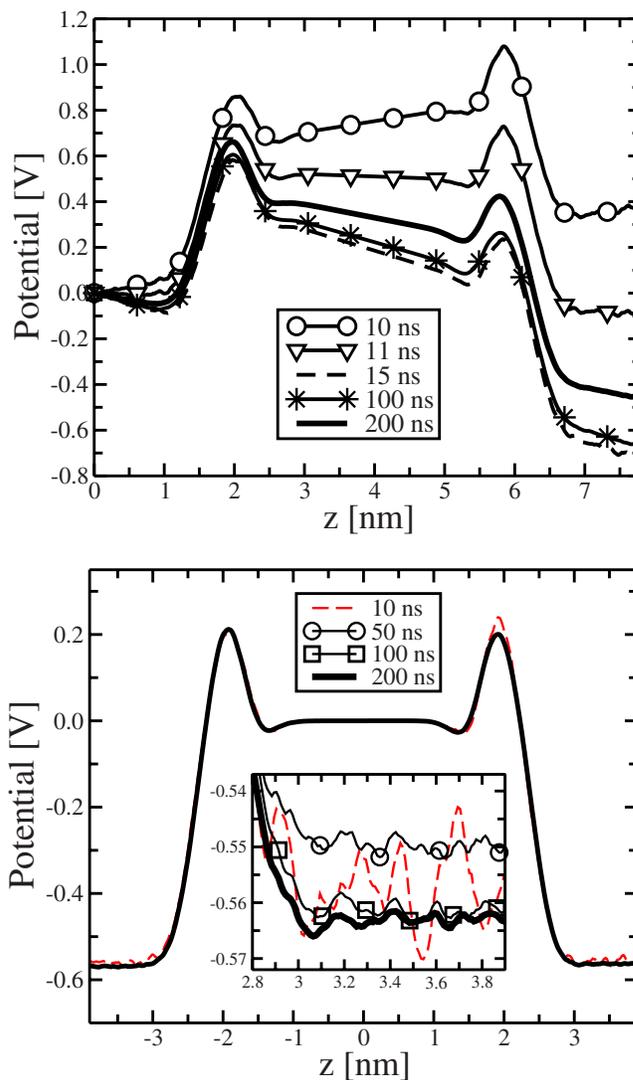


FIG. 2. (Color online) (Top) Electrostatic potential  $\Psi(z)$  of a symmetric POPC bilayer as a function of distance  $z$  from the left-hand side of a simulation box along the  $z$  axis. The potential is calculated from Eq. (2) with boundary conditions  $\Psi_{cl}(0)=0$  and  $E_{cl}(0)=0$ . Shown are the results for numerical integration of Eq. (2) when simulation trajectories of different lengths (from 10 to 200 ns) are employed. (Bottom) Electrostatic potential calculated from Eq. (2) as a function of distance  $z$  from the CM of a POPC bilayer, done individually for every frame of the simulation trajectory. The potential is chosen to be zero at the CM of the bilayer. Again, shown are results for simulation trajectories of different lengths (the inset shows a close-up view on the potential profiles in the water phase close to the interface).

plane. Even for membranes rich in cholesterol, undulations in systems of this size are rather pronounced, implying that a calculation of the charge density and the resulting potential profile is not a straightforward matter to do. Apparently, the most direct way to compute the electrostatic potential profile is to employ small systems that remain planar during the simulation time scale.

Note that due to the bilayer symmetry the essence of the membrane potential can be considered to be well characterized by the potential across a monolayer. For instance, for the POPC bilayer presented here we found an overall potential drop across a monolayer (from the bilayer center to bulk water) to be  $\sim 560$  mV, see Fig. 2 (bottom). In the past

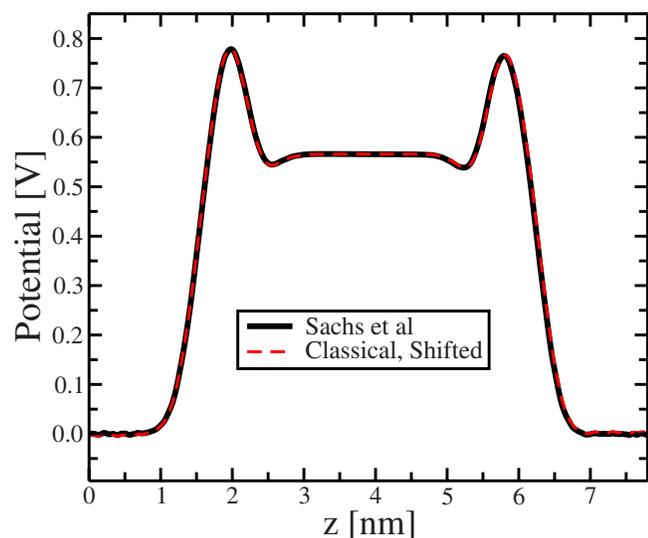


FIG. 3. (Color online) Electrostatic potential of a symmetric POPC bilayer as a function of distance  $z$  from the left-hand side of a simulation box. Shown are results for the potential calculated using the approach by Sachs *et al.* (solid line) and the classical form of the Poisson equation (dashed line), see Eqs. (4) and (2), respectively. The latter potential profile was centered with respect to the bilayer center and then shifted such that  $z=0$  was located at the left side of a box.

many computational studies simply symmetrized the electrostatic potential over two bilayer leaflets and discussed the potential across a monolayer only.<sup>33,35,36</sup> As we demonstrated, the potential profile of a symmetrical bilayer after centering with respect to the bilayer CM shows only a slight asymmetry, which disappears when simulation time goes up, see Fig. 2 (bottom). This justifies the use of symmetrization for getting reasonable potential profiles, especially when simulation time scales are limited.

We conclude this section with a calculation of the electrostatic potential following the approach proposed by Sachs *et al.*<sup>9</sup> The corresponding potential profile calculated over 200 ns trajectory is shown in Fig. 3. While the potential difference across a bilayer is by definition nil, i.e.,  $\Psi_S(0) = \Psi_S(L) = 0$ , the resulting potential turns out to be symmetric. Importantly, the approach by Sachs *et al.* and the classical method for calculating the electrostatic potential gives the same profiles, provided that the simulations are long enough and the potential calculated from Eq. (2) is centered with respect to the CM of a bilayer (note that in Fig. 3 the latter potential profile is also shifted for the sake of comparison).

We emphasize that this finding is not surprising and can readily be expected from Eqs. (4) and (11); the only difference between the two approaches lies in the correction term  $\Delta(z)$ , which is proportional to the total dipole moment of a system  $P_{\text{total}}$ . The dipole moment  $P_{\text{total}}$  of a symmetrical lipid bilayer can be nonzero only if the length of simulations is limited, so that the full phase space is not sampled and the effects due to different fluctuations in the two leaflets have not averaged out. Otherwise,  $P_{\text{total}}$  is essentially zero for symmetrical bilayers and there is no difference between the two approaches, see also Fig. 3.

## B. Asymmetric lipid bilayers

The situation becomes quite different when a bilayer system possesses a nonzero dipole moment across a simulation box. This can be encountered, e.g., in computational studies of electroporation phenomena in lipid bilayers; a constant external electric field is applied perpendicular to the bilayer surface to every atomic charge in the system, thereby inducing a nonzero potential difference at the opposite sides of a simulation box.<sup>10–15</sup> Another example is an asymmetric lipid bilayer; transmembrane asymmetry in distribution of (both zwitterionic and anionic) lipid molecules across the bilayer gives rise to a nonzero potential drop in water phases separated by the asymmetric bilayer.<sup>18,19,26</sup>

Because the condition  $\Psi_S(0) = \Psi_S(L)$  is no longer fulfilled, the approach by Sachs *et al.*<sup>9</sup> becomes inadequate for the bilayer systems with a nonzero net dipole moment and the classical formulation of the Poisson equation [Eq. (2)] should be used.

Note that if a simulation box has a nonzero dipole moment, it inevitably results in the appearance of surface charge on the box, or, for a system under periodic boundary conditions, on the periodically replicated multibilayer system. According to Tieleman,<sup>11</sup> such a surface charge does not require a correction, provided that the Ewald summation method is employed to handle electrostatic interactions: the infinite Ewald lattice is implicitly embedded in a conducting medium with a certain dielectric constant. Most computational studies set this dielectric constant to infinity, which corresponds to so-called tin-foil boundary conditions. In our case such a choice is also appropriate since the dielectric constant of the bulk medium (water) is high. Thus, the surface charge on the periodically replicated multibilayer system is exactly balanced by tin-foil boundary conditions used in the Ewald summation.<sup>11</sup> Below we demonstrate that the classical form of the Poisson equation, being combined with tin-foil boundary conditions, does provide an adequate description of the electrostatic properties of asymmetric lipid bilayers.

To this end, we first calculate the electrostatic potential of a bilayer comprised of POPC and POPE single-component monolayers, see Fig. 1(b). The asymmetry in distribution of the zwitterionic phospholipids across the membrane gives rise to the asymmetric potential profile characterized by a nonzero potential difference of about 107 mV between the two water phases separated by the bilayer, see Fig. 4. This potential drop originates from the slight difference in dipole moments of the two leaflets of the asymmetric PC/PE bilayer.<sup>18</sup> The PME method in conjunction with tin-foil boundary conditions was used to handle the long-ranged electrostatic interactions and the potential presented in Fig. 4 was calculated with the use of Eq. (2) with  $z$  chosen to be zero at the CM of the bilayer.

To demonstrate that the method used for calculating the electrostatic potential does not suffer from artifacts related to the overall nonzero dipole moment across a simulation box, we performed additional MD simulations of *two* PC/PE membranes arranged in an antiparallel (PC-PE to PE-PC) fashion, see Fig. 1(c). Such a setup nullifies an overall dipole moment of a system,<sup>26</sup> so we can now use both Eqs. (2) and

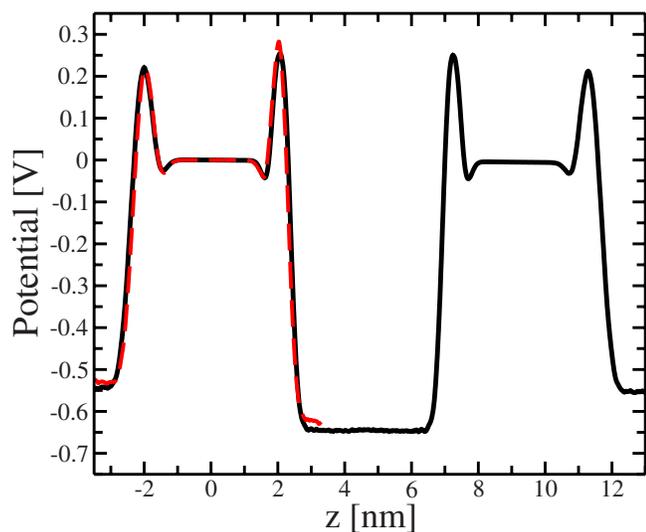


FIG. 4. (Color online) Electrostatic potential of two asymmetric POPC/POPE bilayers arranged in an antiparallel manner as a function of distance  $z$  from the CM of the left bilayer (solid line). The potential was centered and symmetrized with respect to the CM of the bilayer on the left-hand side. For comparison, the potential of a single POPC/POPE bilayer is shown (dashed line).

(4) for calculating the potential. In practice, we chose to employ Eq. (2) along with centering of the potential with respect to the CM of the bilayer on the left-hand side in a simulation box (the potential was symmetrized over two bilayers in a simulation box). The result of such calculations is shown in Fig. 4, while the overall potential drop across the simulation box is essentially zero, each of the PC/PE bilayers yield the asymmetric potential profile. Remarkably, the potential profile of a PC/PE bilayer, which is measured from the double bilayer simulations, turns out to be very similar to the potential profile calculated from single bilayer simulations (Fig. 4). What is more, the potential difference across a PC/PE bilayer was also found to be very close in both cases: 107 mV versus 100 mV for single and double bilayer simulations, respectively. This justifies the method used for calculating the electrostatic potential of a *single* asymmetric bilayer characterized by a nonzero net dipole moment.

To complete the picture, we also considered an alternative approach to nullify the overall dipole moment of a simulation box with an asymmetric lipid bilayer, namely, we performed simulations of a PC/PE bilayer with vacuum boundary conditions (slab geometry).<sup>27</sup> Two vacuum slabs of around 10 nm were adjoined to the water phases on both sides of a bilayer. Such large vacuum regions are meant to ensure that the potential difference, although being nonzero at the positions where water phases end, drops to zero in vacuum at the opposite sides of a simulation box. The electrostatic potential for the PC/PE bilayer with vacuum boundary conditions was calculated with the use of Eq. (2) and is presented in Fig. 5 (again, all atoms were centered with respect to the bilayer CM). First of all, the potential profile turns out to be qualitatively similar to what was observed for the system with conventional periodic boundary conditions. Second, it is seen that against expectations the potential does not return to zero at the opposite sides of a simulation box.

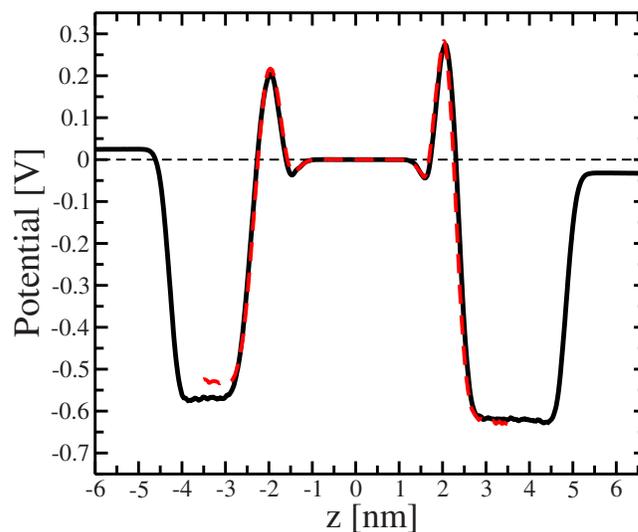


FIG. 5. (Color online) Electrostatic potential of an asymmetric POPC/POPE bilayer with vacuum boundary conditions as a function of distance  $z$  from the bilayer CM (solid line). For comparison, the potential of a POPC/POPE bilayer system with conventional periodic boundary conditions is shown by dashed line.

This is due to the fact that some of water molecules get detached from bulk solution during the course of simulations and can travel across the vacuum regions. This makes the charge density of the vacuum slabs nonzero. The implication is that the overall dipole moment of a simulation box again deviates from nil, this time due to the dipoles of detached water molecules. Such a “water noise” prohibits accurate determination of the intrinsic potential drop of an asymmetric PC/PE bilayer.

#### IV. CONCLUSIONS

In this article, we addressed methodological issues of calculating the electrostatic potential from atomic-scale MD simulations of lipid bilayers. Two forms of the Poisson equation were explored. (i) A classical form, where the potential and the electric field are chosen to be zero at a certain position in a simulation box (normally, on one of the sides of the box along the direction of the bilayer normal). (ii) An alternative form, where the potential is set to be the same (equal to zero) on the opposite sides of a simulation box, as derived by Sachs *et al.*<sup>9</sup> Both forms differ by a position-dependent correction term, which was shown to be proportional to the overall dipole moment of a bilayer system (for neutral systems).

For conventional *symmetric* bilayers, we have shown that both approaches give essentially the same potential profiles, provided that MD simulations are long enough and the fluctuation of the CM of a bilayer is properly taken into account in the classical method. In other words, if a symmetrical bilayer is properly equilibrated and the analyzed trajectory covers a time scale of at least 100 ns, the total dipole moment of the system is essentially zero, implying that the above mentioned correction term vanishes (and so does the difference between the two approaches).

In contrast with the symmetric bilayers, a simulation box with an *asymmetric* lipid bilayer is characterized by a non-

zero dipole moment across the box. Therefore, the approach (ii) is no longer appropriate as it is essentially based on the assumption that  $\Psi_S(0) - \Psi_S(L) = 0$ , which is not the case in asymmetric membranes, and the classical form of the Poisson equation should be employed. With the help of a double bilayer setup, we demonstrated that the classical approach indeed provides an adequate description of the electrostatic potential of a single asymmetric lipid membrane, provided that it is used in conjunction with tin-foil boundary conditions, which are implemented in the PME method and exactly balance the nonzero surface charge of a periodically replicated multibilayer system. Furthermore, we have shown that the potential of an asymmetric bilayer with vacuum boundary conditions is qualitatively similar to what was observed with the use of the conventional periodic boundary conditions. However, accurate measurement of the intrinsic potential drop across an asymmetric bilayer is hindered in this case by water molecules, which occasionally can get detached from bulk aqueous solution and travel across the vacuum slabs.

The present results highlight the importance of accounting for a number of methodological details in simulations of lipid membranes and in derivation of electrostatic membrane potential profiles. First, the simulation times even for simple systems should be at least of the order of 100 ns. Otherwise, insufficient sampling may give rise to artifacts whose magnitude is difficult to assess. This condition becomes more severe for complex membranes that model circumstances in biomembranes under physiological conditions, since binding and unbinding events of ions are slow processes, and sampling of their phase space likely requires time scales that are considerably larger than the limit of 100 ns found in this work. Second, the position of a membrane in the simulation box fluctuates in time, leading to prominent artifacts in the electrostatic profile unless the profile is computed with respect to instantaneous CM positions of the bilayer. Third, for large membranes, undulations come into play and may complicate the analysis of the potential profile. This issue becomes topical in the near future when simulations of large membranes with, e.g., voltage-gated ion channels together with asymmetric transmembrane ion distributions become more common. This last issue remains to be explored in future studies.

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