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Membrane Potential and Dynamics in a Ternary Lipid Mixture: Insights from Molecular Dynamics Simulations

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Abstract

Transmembrane potential (V_m) plays critical roles in cell signaling and other functions. However, the impact of V_m on the structure and dynamics of membrane lipids and proteins, which are critical for the regulation of signaling, is still an open question. All-atom molecular dynamics (MD) simulation is emerging as a useful technique to address this issue. Previous atomistic MD simulations of pure or binary model membranes indicated that both ion imbalance and electric field can be used to generate V_m , but both approaches failed to yield structural changes of lipids with statistical significance. We hypothesized that a possible reason for this could be oversimplified membrane composition or limited sampling. In this work, we tested if and how V_m modulates the structure and dynamics of lipids in a physiologically relevant model membrane. Using a detailed side-by-side comparison, we first show that while both ion imbalance and electric field generate V_m in our complex membranes, only the latter could produce physiologically relevant V_m . We further show that double bonds in lipid acyl chains have a relatively large sensitivity to V_m . A single-bilayer model with electric field showed the highest sensitivity in simulations under the isothermal-isobaric (NPT) ensemble, reproducing expected responses of head-group dipoles to V_m , and suggesting that this approach may be more suitable for studying the structural effects of V_m . Our findings also shed light on the relationship between the macroscopic V_m and its atomic-level underpinnings.

Graphical Abstract

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The authors declare no competing financial interest.

Supporting Information

The convergence of V_m calculations, additional order parameters as well as system snapshots are available online.

Transmembrane potential modulates the structure and dynamics of lipid head-groups and acyl chains.

Introduction

The plasma membrane (PM) is a selectively permeable barrier between the intra- and extra-cellular environments of eukaryotic cells. Differential distribution of ions across the PM generates a transmembrane potential (V_m) of approximately -40 to -90 mV in non-proliferating cells¹. Proliferating cancer cells, on the other hand, tend to have a smaller V_m (i.e., are depolarized), which is believed to contribute to their increased migration and differentiation.^{1–3} Recently, Zhou *et al.* showed that PM depolarization enhances K-Ras clustering and increases mitogen-activated protein kinase (MAPK) signaling, promoting cell proliferation.⁴ It was proposed that V_m -induced change in K-Ras clustering is coupled with the reorganization of anionic lipids, especially phosphatidylserine (PS) and phosphatidylinositol 4,5-bisphosphate (PI(4,5)P₂ or PIP₂). Similarly, Chatterjee *et al.* found that PM depolarization triggers the activation of the PI3K/AKT signaling pathway and induces the generation of reactive oxygen species (ROS), which may contribute to carcinogenesis.⁵ The authors speculated that the voltage-sensitive domain of PI3K or phosphatase-mediated changes in substrate concentration might be responsible for the V_m -induced activation of PI3K. However, in both cases, the mechanisms by which V_m induces changes in the structure and/or dynamics of PM lipids remain unexplored.

There are various approaches to measure V_m experimentally, including patch clamping^{6–7} and *in situ* optical measurements with voltage-sensitive fluorescent probes^{8–9}. Computational approaches can complement these experiments by connecting macroscopic observations with the atomic origin of V_m . Atomistic molecular dynamics (MD) simulation is particularly suited for studying electrophysiology *in silico*^{10–14}. MD allows for studying the potential impact of V_m on the structure and dynamics of individual lipids and their reorganizations.¹⁵ When used in conjunction with a large pulsed electric field, MD can also reproduce membrane electroporation observed by low-volt, long-duration pulses in experiments.^{16–17} However, previous MD studies of V_m focused mostly on pure bilayers such as bilayers of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC)^{10, 14}, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC)^{12, 17} and 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC)¹⁸ lipids. It has been shown that application of ion imbalance or

external electric field to a model membrane can generate V_m during simulations. In both approaches, however, even V_m as large as $\sim 450\text{ mV}$ did not appear to result in significant structural changes in the bilayer.¹⁴ Thus, whether V_m can indeed induce measurable structural changes to membrane lipids during MD is still unclear. Experiments have shown that lipid head-groups and water dipoles align along electric fields¹⁹ and thereby induce membrane structural changes and even poration. Failure to observe structural effects of V_m in MD simulations may therefore be a consequence of limited sampling of configurational space or the use of small voltage amplitudes and oversimplified model membranes. In the current work, we included the anionic lipid 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine (POPS) and the highly charged PIP2 to previously simulated neutral POPC lipid bilayers. This mixture more faithfully models the PM of mammalian cells and was used in our all-atom MD simulations in the presence of a wide range of V_m generated by applied electric fields or externally imposed imbalance of ion distributions. Our goal was to examine subtle changes in the structure and dynamic of both lipid head-groups and acyl chains as a function of changes in V_m . We found that lipid head-groups and double bonds at the acyl chains are particularly sensitive to changes in V_m .

Methods

System construction

V_m can be generated during MD through inter-monolayer imbalance of ions (Q)^{10, 12–14} or via application of an external electric field (E)^{10, 14, 20–21}. The former can be modeled in a single-bilayer by adding a vacuum at each side of the bilayer¹⁰, or in a double-bilayer in which the inter-bilayer compartment has a different ion content than the outer chambers¹². Using a double bilayer lets us study two bilayers in a single simulation, resulting in better sampling. The goal in each case is to prevent spontaneous redistribution and equalization of the number of ions. The electric field, on the hand, can be applied directly to a single-bilayer system with or without vacuum^{20–21}. We tested all these approaches in the current work (Table 1 and Fig. 1a).

To model the PM of mammalian cells as closely as possible^{22–23}, we built a ternary bilayer made up of 280 POPC (70%), 100 POPS (25%) and 20 PIP2 (5%) using the CHARMM-GUI membrane builder^{24–25} and added 28732 TIP3P water molecules plus 200 Na^+ counter ions to achieve overall charge neutrality. Based on this, we set up 18 simulation systems (plus 3 control simulations without PIP2) with either ion imbalance or electric field used to generate V_m (Table 1). To model ion imbalance (Q) while maintaining overall charge neutrality, we moved 1–5 Na^+ ions from one monolayer to the other, yielding $Q = 2, 4, 6, 8$ or 10 in the single-bilayer systems and $Q = 1$ or 2 in the double-bilayer systems. To model electric field, $\mathbf{f}_{i,q} = q_i \mathbf{E}_z$ was added to the classical (unmodified) force $\mathbf{f}_{i0}$ on each atom i with partial charge q_i , where $\mathbf{E}_z$ is the external electric field directed along the z axis (in this case Newton's equation of motion becomes $m\mathbf{\ddot{r}}_i = \mathbf{f}_{i0} + \mathbf{f}_{i,q}$). E_z is continuously adjustable. Here, we choose 8.8 mV/nm as the non-zero minimum value that may be able to generate physiologically relevant V_m . GROMACS 5.0.4 supports the application of both constant and time-varied electric fields;²⁶ we used constant electric field in the current work.

Molecular dynamics simulation

All simulations were conducted with GROMACS²⁶ using the CHARMM36 force field for lipids²⁷ and the standard CHARMM parameters for ions²⁸ and water²⁹. The Lennard-Jones potential was smoothly shifted to zero between 1.0 and 1.2 nm. Particle-Mesh Ewald (PME) electrostatics³⁰ was used with a real space cutoff of 1.2 nm. Lipids and solvent were coupled separately to a Nose-Hoover heat bath^{31–32} at $T = 310$ K (coupling constant $\tau = 1$ ps); a pressure of 1 bar was maintained by a semi-isotropic Parrinello-Rahman pressure coupling scheme³³ with a coupling constant $\tau = 5$ ps and compressibility of $4.5 \times 10^{-5} \text{ bar}^{-1}$. Simulations were conducted with a 2 fs time-step, restraining all bonds involving hydrogen atoms with the LINCS algorithm³⁴. Non-bonded neighbor lists were updated every 20 steps with a cutoff of 1.2 nm. Each simulation was run for 0.3 or 1 μs , saving coordinates every 2 ps for analysis. Snapshots were rendered by VMD³⁵.

Electrostatic potential across membrane

Electrostatic potential across membrane, $\psi(z)$, is calculated using the Poisson equation¹⁰ involving the double integral of the charge density $\rho(z)$ along the membrane normal (z):

$$\psi(z) = -\frac{1}{\epsilon_0} \int_{-z}^z dz' \int_{-z'}^{z'} \rho(z'') dz'' \quad (1)$$

To minimize the error from fluctuations of the simulation box in the *db* and *sb* systems, we set the center-of-mass of the bilayer to the origin and calculated the charge density $\rho(z)$ and $\psi(z)$ from $z = -4$ to $z = 4$ nm, which is wide enough to include bulk solvent while avoiding fluctuations at the edges of the box. As for the *sbv* systems, the box dimensions are fixed and therefore $\rho(z)$ and $\psi(z)$ were calculated along the entire z dimension from $z = -10$ to $z = 10$ nm after re-centering the membrane in the box for every frame of the trajectory.

Lipid acyl chain order parameter

Lipid chain order parameter, S_{CH} , which is a sensitive measure of structural flexibility of lipids in a bilayer, was calculated as:

$$S_{CH} = \frac{1}{2} \langle 3 \cos^2 \theta - 1 \rangle \quad (2)$$

where θ is the angle between the acyl chain C-H bond and the membrane normal. Calculated in this manner, S_{CH} can be directly compared to deuterium order parameter S_{CD} measured by experiments^{36–37}.

Lipid head-group orientation autocorrelation function

We define lipid head-group orientation, θ , as the membrane normal and the angle between a vector from atom P to atom N of a lipid, so that the head-group dipole rotation autocorrelation function, $C(\tau)$ can be calculated as³⁸:

$$C(\tau) = \frac{\langle \frac{\sum [\theta(t) - \overline{\theta(t)}] \cdot [\theta(t + \tau) - \overline{\theta(t)}]}{\sum [\theta(t) - \overline{\theta(t)}]^2} \rangle}{(3)} \quad (3)$$

where $\theta(t)$ and $\theta(t + \tau)$ represent instantaneous angles at time t and $t + \tau$, $\overline{\theta(t)}$ is the time-averaged angle and $\langle \rangle$ denotes averaging over all selected lipids.

Results

Application of charge imbalance (Q) and external electric field (E) to generate V_m in charged membranes

Charge imbalance across membranes generates an electric field and thus electrical potential difference. Therefore, constant E can be used to mimic effects of ion imbalance. In fact, both Q and E are frequently used to model transmembrane potential across bilayers. Our model membrane is a highly charged ternary mixture of POPC, POPS and PIP2 lipids that has not been studied by MD as extensively as simpler bilayer models made up of one or two lipid types^{10–12, 14, 39–40}. Previous studies have shown that Q and E generate similar V_m values in a POPC bilayer.^{10, 12} However, it is not clear whether this is still valid for highly charged membranes.

As mentioned in *Methods*, both Q and E approaches can be applied to a single-bilayer model in the presence of vacuum (systems Q^{sbv} and E^{sbv} , Fig. 1a).¹⁰ In this approach, the only difference between systems Q^{sbv} and E^{sbv} is the way in which V_m is generated, which allows us to directly compare the Q and E approaches for modeling V_m of complex membranes. Therefore, we first evaluated the performance of Q and E in generating V_m in our ternary lipid bilayer using *sbv* modeling. To simulate a POPC/POPS/PIP2 bilayer with vacuum, we first built a symmetric bilayer of 280 POPC (70%), 100 POPS (25%) and 20 PIP2 (5%), as described in *Methods*. After a 100ns equilibrium run of this system under the isothermal-isobaric (NPT) ensemble, we placed it at the center of a new simulation box with a larger z dimension (20 nm), with the regions $z < -6$ nm and $z > 6$ nm representing the vacuum (Fig. 1b). To generate an ion imbalance while keeping the whole system neutral, 1–5 Na^+ ions were moved from the inner to the outer chamber ($z > 0$, Fig. 1b), and the number of water molecules was kept the same for both chambers to yield six systems:

$Q_0^{sbv}, Q_2^{sbv}, Q_4^{sbv}, Q_6^{sbv}, Q_8^{sbv}, Q_{10}^{sbv}$ where $Q = 0, 2, 4, 6, 8, 10 e$ (Table 1). Using the same

initial bilayer model with vacuum, we prepared systems of different E by applying a constant electric field E_z of different strength, resulting in systems

$E_0^{sbv}, E_{8.8}^{sbv}, E_{17.6}^{sbv}, E_{26.4}^{sbv}, E_{88}^{sbv}, E_{176}^{sbv}$ with $E_z = 0, 8.8, 17.6, 26.4, 88, 176$ mV/nm (Table 1). We simulated each of these systems under the isothermal-isochoric (NVT) ensemble for 300 ns and compared the V_m s derived from the electrostatic potential $\psi(z)$ based on charge density profiles (eq. 1).

To estimate convergence, we calculated $\psi(z)$ over different ranges of time. Fig. S1 and Fig. S2 show that 300 ns is adequate to obtain converged $\psi(z)$. A similar observation was made for a pure POPC bilayer¹⁴. We then compared the $\psi(z)$ obtained from systems Q^{sbv} and

E^{sbv} . Fig. 1c shows that the $\psi(z)$ profiles are qualitatively similar, supporting previous conclusions regarding the role of Q and E in modeling V_m . We defined the difference in the electrostatic potential at $z = -3.5$ nm and $z = +3.5$ nm as V_m , since $|z| < 3.5$ nm encompasses the whole membrane as well as the bilayer-water interface (Fig. S3a). We found that, irrespective of the way it was generated, V_m is linearly correlated to its source (i.e., Q or E ; Fig. 1d). In fact, in both cases, we observed an excellent fitting of the data to a linear model. This shows that either approach can be used to model V_m in charged membranes although, as we discuss in subsequent sections, the electric field approach appears to be more suitable for generating physiologically relevant V_m values.

The $\psi(z)$ profiles from simulations Q^{sbv} and E^{sbv} progressively diverge as V_m increases (Fig. 1c). For example, comparison of Q_8^{sbv} with E_{176}^{sbv} outside the membrane shows that $\psi(z)$ increases more rapidly in the latter. This is because electric field induces ordering of water molecules at the water-vacuum interface (data not shown). In the core of the bilayer, including the bilayer mid-plane ($z = 0$), the dependence of $\psi(z)$ on the source of the potential differs. While the shape of the $\psi(z)$ plot within the hydrophobic core remains unaffected by the strength of the electric field, it varies with Q . This is primarily because an increasing number of Na^+ gets clustered in the head-group and glycerol regions of the bilayer as Q increases (Fig. S7). This alters the local ion distribution and thereby the electrostatic potential.

Potential effects of V_m on the structure of membrane lipids in systems Q^{sbv} and E^{sbv}

As shown in Fig. S3a, V_m does not induce a major effect on the mass density profiles but, in the Q^{sbv} systems, there are small changes in the bilayer center and head-group regions that become noticeable when V_m is large (system Q_8^{sbv} and Q_{10}^{sbv} in Fig. S3a). This suggests that V_m may have strength-dependent structural effects on membrane lipids. Hence, we investigated the behavior of both lipid head-groups and acyl chains under different V_m values. Similar to POPC bilayers^{10, 14}, we did not observe V_m -induced effect on lipid head-group dynamics even with V_m of ~ 2.8 V (system Q_{10}^{sbv}). This may be an artifact of the NVT ensemble used for these systems, because the fixed volume limits collective changes in head-group orientation that would induce change in lateral area. This also explains the absence of membrane poration by the large V_m in system Q_{10}^{sbv} .

Interestingly, lipid acyl chain order parameter ($-S_{CH}$) analysis suggested that the double bond between carbon 9 and 10 in the sn-2 chain of POPC and POPS is somewhat sensitive to V_m (Fig. 2). Increasing V_m led to an increase of $-S_{CH}$ at carbon 9 and a decrease at carbon 10 in the outer leaflet; the opposite happened in the inner leaflet, reflecting the $\psi(z)$ gradient (Fig. 1c). This phenomenon has not been observed in previous V_m modeling of a POPC bilayer^{10, 14}. The difference may be due to the use of a united atom model in the previous study or the presence of PIP2 in the current work, which has a small effect on V_m (see below). No significant effect was observed on the saturated sn-1 chain (Fig. S4), and the structure of PIP2 could not be evaluated with confidence because of the poor statistics (the number of PIP2 in the membrane is small).

PIP2 slightly affects the dependence of V_m on the magnitude of ion gradient

To check if 5% PIP2 content affects V_m generation, we compared POPC/POPS/PIP2 (Q^{db}) and POPC/POPS (Q^{db*}) double bilayer systems simulated under the NPT ensemble for 1 μ s (Fig. 3a). Comparison of the $\psi(z)$ profiles calculated separately for each bilayer of the double-bilayer and over nine 100-ns time blocks suggest a well-converged system (Fig. S5). Subsequent comparison of Q^{db} and Q^{db*} using the last 500 ns of the data revealed that incorporation of PIP2 slightly increases the membrane thickness (Fig. 3b) and decreases the sensitivity of $\psi(z)$ (Fig. 3c) and V_m (Fig. 3d) to charge gradient, Q . Similar to our Q^{sbv} and E^{sbv} systems (Fig. S3a), V_m has only a small effect on the thickness of the membrane even when the simulations are extended to 1 μ s. Again, as in Q^{sbv} and E^{sbv} , V_m exhibits a linear relationship with Q in systems Q^{db} and Q^{db*} . However, the slope in the latter is larger (Fig. 3d), suggesting that the presence of 5% PIP2 slightly decreased the dependence of V_m on Q . Though not the focus of this paper, the slope of the V_m vs. Q plot can be used to estimate the capacitance (C) of each bilayer model using $C = Q/V_m$. Such a calculation suggests that PIP2 slightly decreases membrane capacitance. However, this effect appears to be too small to result in measurable differences between db and db^* in terms of lipid dynamics (Fig. 4).

NPT simulations of single-bilayer models confirm that V_m modulates the structure of lipids

In order to more effectively capture potential V_m -induced changes in membrane structure or dynamics, we used single bilayer systems with relatively large E_z : E_{88}^{sb} and E_{176}^{sb} . These simulations resulted in larger V_m values (Fig 5) than our simulations with vacuum (system E^{sbv} , Fig. 1). In both cases, V_m is linearly correlated to E_z but the slopes are different: 8.6 in E^{sbv} and 10.1 in E^{sb} . The latter is very close to the average length of the simulation box along the z-axis ($L_z = 10.68$ nm), supporting the empirical relation $V_m = E_z \times L_z^{20-21}$ to estimate V_m in complex membranes.

To examine the overall structure of the bilayer in systems E_{88}^{sb} and E_{176}^{sb} relative to E_0^{sb} , we calculated the average area per lipid (A_{av}). As shown in Fig. 5d, A_{av} progressively increases with increasing V_m . The change in A_{av} is more notable in these simulations than in Q^{db} , suggesting that the double-bilayer system also limits lateral fluctuations, similar to the NVT runs in the presence of vacuum. This explains the differences in V_m generated by our single-bilayer and double bilayer models.

For a more detailed analysis of lipid dynamics, we focused on the fluctuations of the acyl chain and head-group of the POPC lipids that dominate the system. Fig. 6a and 6b show the time- and chain-averaged order parameter $\langle -S_{CH} \rangle$ for the sn-1 and sn-2 tails, indicating that V_m generally promotes disorder. To identify the region of the bilayer that is most sensitive to V_m , we show in Figs. 6c–d $-S_{CH}$ per carbon atom. For the sn-1 tail, there is a consistent V_m -dependent decrease in $-S_{CH}$ from the second carbon all the way to the 14th, especially for $V_m = 1839$ mV (system E_{176}^{sb}). There is a similar trend in the sn-2 tail, where we find that the cis double bond between the 9th and 10th carbon atoms is especially sensitive to changes in V_m (see inset in Fig. 6d). For the POPC lipids in the outer leaflet

where the electric field was applied, carbon 9 becomes progressively more ordered and carbon 10 disordered upon increasing V_m . The reverse is true for lipids in the lower leaflet.

We analyzed the POPC head-group orientation in terms of the angle θ between a P-to-N vector and the membrane normal (Fig. 7). The results show that V_m generally increases θ of the outer leaflet lipids and decreases θ of the inner leaflet lipids (Fig. 7b). This is an expected response of lipid head group dipoles to V_m , considering the direction of the applied electric field. However, V_m has a comparatively small and similar effect on the rate of the dipole fluctuations (slightly reducing the relaxation time) on the outer and inner leaflet lipids, as suggested by the rotational autocorrelation function $C(\tau)$ (Fig. 7c–d). These changes in the dynamics of dipoles at the head-group, though small, can have important consequences, such as on the dynamics of interfacial water molecules^{14, 41}. We note that previous MD simulation studies did not observe a major structural effect of V_m on lipid head-group dipoles^{10, 12, 14}, probably due to averaging out of opposite effects on the two monolayers. Our results show that V_m values larger than the physiologic range -- but not too large to cause pore formation -- have significant impact on the bilayer structure (Fig S5). The differential effect of V_m on the dipoles of the outer and inner monolayers could have a stronger effect on bilayer structure if the total lateral area of the monolayers were allowed to fluctuate independently; this is not the case in our setup but possible in real membranes. One approach to allow independent dynamics of monolayers would be to run NPT simulations with adaptively varied total number of lipids, along the line of N-varied dissipative particle dynamics simulation^{42–44}.

Discussion

In this work, all-atom MD simulations were used in three different approaches to model V_m : (1) single-bilayer with vacuum using either $Q(Q^{sbv})$ or $E(E^{sbv})$ under NVT; (2) double-bilayer with $Q(Q^{db})$ under NPT; (3) single-bilayer with $E(E^{sb})$ under NPT. In all cases, the generated V_m was linearly correlated to the source Q or E_z . Both Q and E yielded similar electrostatic potential profiles in simulations with NVT or NPT, confirming the usefulness of Q or E to generate V_m as reported for pure POPC bilayers^{10, 14}. Q is discrete in the single-point charge model used in this work, and has a non-zero minimum value of 2 in the NVT simulations (system Q_2^{sbv}) and 1 in the NPT simulations (system Q_1^{db}). Both yielded a relatively large V_m (554 mV and 240 mV). In contrast, the continuous E_z yielded V_m close to physiological values (Figs. 1 & 3). Hence, we propose that E rather than Q is more suitable for generating physiologically relevant V_m (10 – 100 mV) by MD simulations.

The shape of the electrostatic potential $\psi(z)$ profiles vary among different model systems (Fig. 1, Fig. 3 and Fig. 5) and among force fields (e.g. coarse-grained models⁴⁵; united-atom models¹⁰; modified *Slipids* all-atom model with virtual interaction sites¹⁴; and CHARMM Drude polarizable model⁴⁶). An intriguing finding in the current work using the CHARMM36 all-atom model^{27–29} is the significant effect of V_m on the dynamics of unsaturated lipids (especially cis double bonds), which may be ascribed to the charge distribution (Fig. S8). This finding provides clues about how V_m preferentially induces

electroporation in the liquid disordered phase⁴⁷, and why it promotes membrane phase separation⁴⁸. Our findings may thus inspire experimental measurements of deuterium order parameter S_{CD} with NMR or ESR^{36–37} in the presence of V_m . If systematically validated and proven to be universally true, the dynamics of cis double bonds may be a useful proxy to measure V_m in complex systems. In the MD front, whether polarization of cis double bonds may increase the effects of V_m is an interesting topic that requires a polarizable force field to test.

One issue raised by the current as well as previous simulations¹⁰ is the nonzero V_m (typically several to tens of millivolts) in systems with no net Q or E_z (Table 1), where V_m should be zero since the bilayer is symmetric. This could be explained by differential lipid clustering in the outer and inner leaflets, which requires longer simulations to average out^{49–51}. However, extending simulation Q_0^{db} to 1 μ s did not improve the results significantly (Fig. S5c,d), and the deviation from zero is always positive. These observations, along with the fact that the initial lipid distribution was similar among the various systems, suggest that the nonzero V_m is a systematic error likely caused by local asymmetry in ion distribution whose concentration was large in our systems. This, however, will have little effect on the trends observed in our comparative analyses of V_m , or on its impact on lipid dynamics.

Conclusion

We have investigated the potential effects of transmembrane potential (V_m) on the structure and dynamics of a complex bilayer made up of POPC, POPS and PIP2 lipids. We used three different MD simulation approaches: single-bilayer models with vacuum under the NVT ensemble (Q^{sbv} and E^{sbv}), a double-bilayer model with ion imbalance (Q^{db}) and a single-bilayer with constant electric field (E^{sb}) under NPT ensemble. Our results indicated that both the Q and E approaches generate qualitatively similar V_m s for the model membranes tested. We further found that V_m decreases lipid acyl chain order, particularly at the cis double bond of the sn-2 tail. Moreover, using NPT simulations with system E^{sb} , we observed V_m -induced fluctuations of lipid head-groups with opposite effects on the orientation of dipoles in the two monolayers. Generally, the adjustable electric field approach enables modeling of more physiologically relevant V_m , suggesting that it is a better means of studying V_m by MD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Yang M, Brackenbury WJ. Membrane Potential and Cancer Progression. *Front Physiol.* 2013;4. [PubMed: 23404365]

2. Ly JD, Grubb D, Lawen A. The Mitochondrial Membrane Potential (ψ M) in Apoptosis; an Update. *Apoptosis*. 2003; 8:115–128. [PubMed: 12766472]
3. Rosen LB, Ginty DD, Weber MJ, Greenberg ME. Membrane Depolarization and Calcium Influx Stimulate Mek and Map Kinase Via Activation of Ras. *Neuron*. 1994; 12:1207–1221. [PubMed: 8011335]
4. Zhou Y, et al. Membrane Potential Modulates Plasma Membrane Phospholipid Dynamics and K-Ras Signaling. *Science*. 2015; 349:873–876. [PubMed: 26293964]
5. Chatterjee S, Browning EA, Hong N, DeBolt K, Sorokina EM, Liu W, Birnbaum MJ, Fisher AB. Membrane Depolarization Is the Trigger for Pi3k/Akt Activation and Leads to the Generation of Ros. *Am J Physiol Heart Circ Physiol*. 2012; 302:H105–H114. [PubMed: 22003059]
6. Sakmann B, Neher E. Patch Clamp Techniques for Studying Ionic Channels in Excitable Membranes. *Annu Rev Physiol*. 1984; 46:455–472. [PubMed: 6143532]
7. Cadwell CR, Palasantza A, Jiang X, Berens P, Deng Q, Yilmaz M, Reimer J, Shen S, Bethge M, Tolias KF. Electrophysiological, Transcriptomic and Morphologic Profiling of Single Neurons Using Patch-Seq. *Nat Biotechnol*. 2016; 34:199. [PubMed: 26689543]
8. Steinberg BE, Touret N, Vargas-Caballero M, Grinstein S. In Situ Measurement of the Electrical Potential across the Phagosomal Membrane Using FRET and Its Contribution to the Proton-Motive Force. *Proc Natl Acad Sci US A*. 2007; 104:9523–9528.
9. Koivusalo M, Steinberg BE, Mason D, Grinstein S. In Situ Measurement of the Electrical Potential across the Lysosomal Membrane Using FRET. *Traffic*. 2011; 12:972–982. [PubMed: 21554506]
10. Delemotte L, Dehez F, Treptow W, Tarek M. Modeling Membranes under a Transmembrane Potential. *J Phys Chem B*. 2008; 112:5547–5550. [PubMed: 18412411]
11. Böckmann RA, Hac A, Heimburg T, Grubmüller H. Effect of Sodium Chloride on a Lipid Bilayer. *Biophys J*. 2003; 85:1647–1655. [PubMed: 12944279]
12. Lee SJ, Song Y, Baker NA. Molecular Dynamics Simulations of Asymmetric NaCl and KCl Solutions Separated by Phosphatidylcholine Bilayers: Potential Drops and Structural Changes Induced by Strong Na⁺-Lipid Interactions and Finite Size Effects. *Biophys J*. 2008; 94:3565–3576. [PubMed: 18222999]
13. Sachs JN, Crozier PS, Woolf TB. Atomistic Simulations of Biologically Realistic Transmembrane Potential Gradients. *J Chem Phys*. 2004; 121:10847–10851. [PubMed: 15634036]
14. Melcr J, Bonhenry D, Timr Stpn, Jungwirth P. Transmembrane Potential Modeling: Comparison between Methods of Constant Electric Field and Ion Imbalance. *J Chem Theory Comput*. 2016; 12:2418–2425. [PubMed: 27014925]
15. Lin X, Lorent JH, Skinkle AD, Levental KR, Waxham MN, Gorfe AA, Levental I. Domain Stability in Biomimetic Membranes Driven by Lipid Polyunsaturation. *J Phys Chem B*. 2016; 120:11930–11941. [PubMed: 27797198]
16. Delemotte L, Tarek M. Molecular Dynamics Simulations of Lipid Membrane Electroporation. *J Membr Biol*. 2012; 245:531–543. [PubMed: 22644388]
17. Polak A, Bonhenry D, Dehez F, Kramar P, Miklav i D, Tarek M. On the Electroporation Thresholds of Lipid Bilayers: Molecular Dynamics Simulation Investigations. *J Membr Biol*. 2013; 246:843–850. [PubMed: 23780415]
18. Tarek M. Membrane Electroporation: A Molecular Dynamics Simulation. *Biophys J*. 2005; 88:4045–4053. [PubMed: 15764667]
19. Vernier PT, Ziegler MJ. Nanosecond Field Alignment of Head Group and Water Dipoles in Electroporating Phospholipid Bilayers. *J Phys Chem B*. 2007; 111:12993–12996. [PubMed: 17949035]
20. Roux B. The Membrane Potential and Its Representation by a Constant Electric Field in Computer Simulations. *Biophys J*. 2008; 95:4205–4216. [PubMed: 18641071]
21. Gumbart J, Khalili-Araghi F, Sotomayor M, Roux B. Constant Electric Field Simulations of the Membrane Potential Illustrated with Simple Systems. *Biochim Biophys Acta-Biomembr*. 2012; 1818:294–302.
22. Van Meer G, Voelker DR, Feigenson GW. Membrane Lipids: Where They Are and How They Behave. *Nat Rev Mol Cell Biol*. 2008; 9:112. [PubMed: 18216768]

23. Di Paolo G, De Camilli P. Phosphoinositides in Cell Regulation and Membrane Dynamics. *Nature*. 2006; 443:651–657. [PubMed: 17035995]
24. Jo S, Kim T, Iyer VG, Im W. Charmm-Gui: A Web-Based Graphical User Interface for Charmm. *J Comput Chem*. 2008; 29:1859–1865. [PubMed: 18351591]
25. Wu EL, Cheng X, Jo S, Rui H, Song KC, Dávila-Contreras EM, Qi Y, Lee J, Monje-Galvan V, Venable RM. Charmm-Gui Membrane Builder toward Realistic Biological Membrane Simulations. *J Comput Chem*. 2014; 35:1997–2004. [PubMed: 25130509]
26. Abraham MJ, Murtola T, Schulz R, Páll S, Smith JC, Hess B, Lindahl E. Gromacs: High Performance Molecular Simulations through Multi-Level Parallelism from Laptops to Supercomputers. *SoftwareX*. 2015; 1:19–25.
27. Klauda JB, Venable RM, Freites JA, O'Connor JW, Tobias DJ, Mondragon-Ramirez C, Vorobyov I, MacKerell AD Jr, Pastor RW. Update of the Charmm All-Atom Additive Force Field for Lipids: Validation on Six Lipid Types. *J Phys Chem B*. 2010; 114:7830–7843. [PubMed: 20496934]
28. Venable RM, Luo Y, Gawrisch K, Roux B, Pastor RW. Simulations of Anionic Lipid Membranes: Development of Interaction-Specific Ion Parameters and Validation Using Nmr Data. *J Phys Chem B*. 2013; 117:10183–10192. [PubMed: 23924441]
29. Jorgensen WL, Chandrasekhar J, Madura JD, Impey RW, Klein ML. Comparison of Simple Potential Functions for Simulating Liquid Water. *J Chem Phys*. 1983; 79:926–935.
30. Essmann U, Perera L, Berkowitz ML, Darden T, Lee H, Pedersen LG. A Smooth Particle Mesh Ewald Method. *J Chem Phys*. 1995; 103:8577–8593.
31. Nosé S. A Molecular Dynamics Method for Simulations in the Canonical Ensemble. *Mol Phys*. 1984; 52:255–268.
32. Hoover WG. Canonical Dynamics: Equilibrium Phase-Space Distributions. *Phys Rev A*. 1985; 31:1695.
33. Parrinello M, Rahman A. Polymorphic Transitions in Single Crystals: A New Molecular Dynamics Method. *J Appl Phys*. 1981; 52:7182–7190.
34. Hess B, Bekker H, Berendsen HJ, Fraaije JG. Lincs: A Linear Constraint Solver for Molecular Simulations. *J Comput Chem*. 1997; 18:1463–1472.
35. Humphrey W, Dalke A, Schulten K. Vmd: Visual Molecular Dynamics. *J Mol Graph*. 1996; 14:33–38. [PubMed: 8744570]
36. Davis JH. The Description of Membrane Lipid Conformation, Order and Dynamics by 2h-Nmr. *Biochim Biophys Acta*. 1983; 737:117–171. [PubMed: 6337629]
37. de Planque MR, Greathouse DV, Koeppe RE, Schäfer H, Marsh D, Killian JA. Influence of Lipid/Peptide Hydrophobic Mismatch on the Thickness of Diacylphosphatidylcholine Bilayers. A 2h Nmr and Esr Study Using Designed Transmembrane A-Helical Peptides and Gramicidin A. *Biochemistry*. 1998; 37:9333–9345. [PubMed: 9649314]
38. Cote Y, Senet P, Delarue P, Maisuradze GG, Scheraga HA. Nonexponential Decay of Internal Rotational Correlation Functions of Native Proteins and Self-Similar Structural Fluctuations. *Proc Natl Acad Sci US A*. 2010; 107:19844–19849.
39. Prakash P, Zhou Y, Liang H, Hancock JF, Gorge AA. Oncogenic K-Ras Binds to an Anionic Membrane in Two Distinct Orientations: A Molecular Dynamics Analysis. *Biophys J*. 2016; 110:1125–1138. [PubMed: 26958889]
40. Li ZL, Buck M. Computational Modeling Reveals That Signaling Lipids Modulate the Orientation of K-Ras4a at the Membrane Reflecting Protein Topology. *Structure*. 2017; 25:679–689.e2. [PubMed: 28286004]
41. Janosi L, Gorge AA. Simulating Popc and Popc/Popg Bilayers: Conserved Packing and Altered Surface Reactivity. *J Chem Theory Comput*. 2010; 6:3267–3273. [PubMed: 26616788]
42. Hong B, Qiu F, Zhang H, Yang Y. Budding Dynamics of Individual Domains in Multicomponent Membranes Simulated by N-Varied Dissipative Particle Dynamics. *J Phys Chem B*. 2007; 111:5837–5849. [PubMed: 17487994]
43. Yue T, Zhang X. Molecular Understanding of Receptor-Mediated Membrane Responses to Ligand-Coated Nanoparticles. *Soft Matter*. 2011; 7:9104–9112.
44. Yue T, Li S, Zhang X, Wang W. The Relationship between Membrane Curvature Generation and Clustering of Anchored Proteins: A Computer Simulation Study. *Soft Matter*. 2010; 6:6109–6118.

45. Bruhn DS, Lomholt MA, Khandelia H. Quantifying the Relationship between Curvature and Electric Potential in Lipid Bilayers. *J Phys Chem B*. 2016; 120:4812–4817. [PubMed: 27163659]
46. Li H, Chowdhary J, Huang L, He X, MacKerell AD, Roux B. Drude Polarizable Force Field for Molecular Dynamics Simulations of Saturated and Unsaturated Zwitterionic Lipids. *J Chem Theory Comput*. 2017
47. Sengel JT, Wallace MI. Imaging the Dynamics of Individual Electropores. *Proc Natl Acad Sci US A*. 2016; 113:5281–5286.
48. Malinsky J, Tanner W, Opekarova M. Transmembrane Voltage: Potential to Induce Lateral Microdomains. *Biochim Biophys Acta-Mol Cell Biol Lipids*. 2016; 1861:806–811.
49. Janosi L, Li Z, Hancock JF, Gorfe AA. Organization, Dynamics, and Segregation of Ras Nanoclusters in Membrane Domains. *Proc Natl Acad Sci U S A*. 2012; 109:8097–8102. [PubMed: 22562795]
50. Li Z, Janosi L, Gorfe AA. Formation and Domain Partitioning of H-Ras Peptide Nanoclusters: Effects of Peptide Concentration and Lipid Composition. *J Am Chem Soc*. 2012; 134:17278–17285. [PubMed: 22994893]
51. Lin X, Li Z, Gorfe AA. Reversible Effects of Peptide Concentration and Lipid Composition on H-Ras Lipid Anchor Clustering. *Biophys J*. 2015; 109:2467–2470. [PubMed: 26682805]

Figure 1. V_m modeling using Q^{sbv} and E^{sbv} approaches

(a) The approaches used in the current work for modeling V_m (see Table 1 for abbreviations). (b) A system snapshot at $t=0$ ns with POPC in green, POPS in pink, PIP2 in red, Na^+ in yellow and water in grey. (c) Electrostatic potential profile $\psi(z)$ along the z -axis for 12 simulation systems using charge imbalance (top) and electric field (bottom). (d) A linear relationship between V_m measured as the potential difference at $z = -3.5$ nm and $z = +3.5$ nm (highlighted in green dashed lines in panel c) and the source of the V_m : Q (top) or E_z (bottom). $|z| \leq 3.5$ nm encompasses the entire membrane as well as the bilayer-water interface as shown by the mass density profiles in Fig. S3a. In panels (c–d), the analysis involved data in the last 250 ns of each 300 ns trajectory.

Figure 2. V_m slightly affects lipid chain order

In both systems Q^{sbv} and E^{sbv} , V_m has a small but significant effect on the order of the sn-2 chain, especially at the double bond (green dashed box). Though small, this effect is dependent upon V_m and the inner and outer leaflets appear to respond oppositely. The data is shown as mean $\pm$ s.e.m. The statistics is poor for the minor lipid PIP2, whose error (s.e.m.) is not shown here.

Figure 3. PIP2 slightly affects the dependence of V_m on the magnitude of ion gradient
 (a) A snapshot of a POPC/POPS/PIP2 double-bilayer system at $t=0$ ns with POPC in green, POPS in pink, PIP2 in red, ions in yellow and water in grey. (b, c) Mass density (b) and $\psi(z)$ (c) profiles for systems POPC/POPS/PIP2 (Q^{db} , solid line) and POPC/POPS (Q^{db*} , dashed line), at different ion gradients (Q). (d) Relationship of V_m to Q .

Figure 4. No significant difference in lipid chain order between system Q^{db} (POPC/POPS/PIP2) and Q^{db*} (POPC/POPS)

Lipid chain order parameters are shown as mean $\pm$ s.e.m. V_m has no effect on lipid dynamics in systems Q^{db} and Q^{db*} likely due to its small magnitude.

Figure 5. V_m modeling with system E^{sb}

(a) A snapshot at $t = 0$ ns with POPC in green, POPS in pink, PIP2 in red, ions in yellow and water in grey. (b) Electrostatic potential profiles $\psi(z)$ generated by different electric field strengths E_z . (c) Linear correlation between V_m and E_z for the sb (red line) and sbv (blue line) systems. (d) Average area per lipid (A_{av}) versus V_m for sb (red line) and sbv (blue line) systems.

Figure 6. V_m promotes the dynamics of acyl chains

(a, b) Ensemble- and chain-averaged order parameters for the sn-1 (a) and sn-2 (b) tails of each lipid type at three different V_m values from systems E_0^{sb} (black and red for the outer and inner leaflets), E_{88}^{sb} (blue and magenta) and E_{176}^{sb} (green and dark blue). (c, d) Ensemble-averaged order parameter per carbon atom for the sn-1 (c) and sn-2 (d) tails of each lipid type at the same three different V_m values, where the data is shown as mean $\pm$ s.e.m. Note the identical (i.e., converged) average order parameter for the POPC lipids in the outer and inner leaflets in the absence of V_m (cyan dashed circle in a and b).

Figure 7. Effect of V_m on the dynamics of lipid head-groups

(a) Illustration of head group tilt angle (θ) defined as the angle between a P-N vector (blue arrow) and the membrane normal (light blue arrow), with the direction of the applied electric field shown in red arrow. (b) P-N vector tilt angle averaged over POPC lipids in each leaflet from three simulations. (c) First rank rotational autocorrelation function ($C(\tau)$) of the tilt angle for POPC lipids in the outer (left) and inner (right) leaflet. The insets show a zoom-in of the faster timescale fluctuations.

Table 1

Summary of the simulations performed in this work.

System*	Q_0^{sbv}	Q_2^{sbv}	Q_4^{sbv}	Q_6^{sbv}	Q_8^{sbv}	Q_{10}^{sbv}	E_0^{sbv}	$E_{8,8}^{sbv}$	$E_{17,6}^{sbv}$	$E_{26,4}^{sbv}$	E_{88}^{sbv}	E_{176}^{sbv}	Q_0^{db}	Q_1^{db}	Q_2^{db}	E_0^{sb}	E_{88}^{sb}	E_{176}^{sb}
Q (e)	0	2	4	6	8	10	-	-	-	-	-	-	0	1	2	-	-	-
E_z (mV/nm)	-	-	-	-	-	-	0	8.8	17.6	26.4	88	176	-	-	-	0	88	176
Length (μ s)	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	1	1	1	0.3	0.3	0.3
V_m (V)	0.00	0.55	1.14	1.68	2.26	2.79	0.02	0.08	0.14	0.21	0.74	1.51	0.07	0.24	0.41	0.07	0.98	1.84

* Each system is named as Q_x^{model} or E_x^{model} where Q represents ion imbalance and E constant electric field; $model$ equals sbv (single-bilayer with vacuum), db (double-bilayer), or sb (single-bilayer); x is the value of Q per bilayer in e or the strength of E (E_d) in mV/nm . All the single-bilayer systems consist of 280 POPC, 100 POPS, 20 PIP2, 28732 TIP3P water plus 200 Na^+ , and were simulated for 300ns each. The double-bilayer systems are twice as large and therefore the simulations were extended to 1 μ s for better sampling. In order to examine the role of PIP2, three 1 μ s-long control simulations of the double-bilayer systems were run without PIP2, referred to as Q_0^{db*} , Q_1^{db*} and Q_2^{db*} (not listed in the table).