Membrane defect and water leakage caused by passive calcium permeation

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Abstract

Our previous simulations found that permeation of Na+ and K+, in contrast with Cl−, causes significant membrane deformation and formation of a water wire in the membrane hydrophobic domain. Clearly, positive charges have a propensity to interact more strongly with lipids and thus induce serious defects to membrane. Since calcium is one of the most common and important metals in biology, we take it as template of a +2 charge and made the unprecedented effort to bring it across the membrane in molecular simulations. It is observed that under the ion/lipid ratio of 1:126 permeation of a +2 charge would badly disturb the bilayer structure, even lipid “flip-flop” is quite common, and cause leakage of water molecules by a widely-reported “All or None” manner, suggesting unassisted permeation of small molecules with 2 or more positive charges to be severe threats to cell membranes. More importantly, we found “All or None” leakage may be closely related to the “game” of hydrophilic and hydrophobic forces caused by positive charges. Our findings may be enlightening for the exploration of underlying mechanisms of the antimicrobial peptides and similar positively charged molecules acting on membranes.

1. Introduction

Calcium is one of the most common metals in lots of animals. Besides being a major constituent of bone, teeth, and shells, calcium also plays a key role in the signaling of muscle and heart cells. In cell biology, calcium can be involved in functional activities of a variety of signaling proteins and channel proteins [1–7]. Being strongly positively charged, calcium can hardly permeate through lipid bilayers without the assistance of membrane channels.

We explored the unassisted monovalent ion permeation process, including Na+ and K+, and found significant membrane defect and also a hydrated pore-like conformation inside which a water wire/bridge/finger connecting both sides of lipid bilayers can significantly lower energy barriers of monovalent cation permeations [8–9]. However, no exchange or permeation of ions are found in this pore-like conformation, and even water molecules’ exchange is quite rare. Thus passive monovalent cation permeation cannot virtually create a complete pore or channel for cell substance exchange.

Probably there are some similarities in the unassisted permeations of 2 or more positive charges with the monovalent cations, as Bordi et al. found transient aqueous pores produced by thermal fluctuations facilitate Ca2+ and Mg2+ transport with the assistance of current-voltage characteristic measurements [10]. Also some experiments on permeation of some small molecules with 2 or more positive charges are demonstrated to improve the permeability of lipid bilayers by disrupting the physical integrity of the bilayer, via membrane thinning, transient poration and disruption of the barrier function, leading to cell death [11–15]. For instance, studies about positively charged antimicrobial peptides, Magainin, Cecropin and LL-37, have reported that enhancement of positive charges may help to effectively improve their potency disintegrating cell inner membrane [16–19]. Therefore, small molecules with 2 or more positive charges may preserve high potency to disrupt membrane barrier and permeate membrane.

According to current studies, there are two specific features of such positively charged small molecules to perturb membrane structure: a) likely to induce serious membrane surface defect and sometimes a pore/channel [16,20–25]. This may be caused by strong electrostatic interactions between the positive charges and lipid headgroups [20–21, 23,26–29], which make surrounding headgroups of lipids fold inward along with the positive charges [24,30–37] b) always hydrated [20–21,23–24]. Similar with the hydrated pore-like conformations and water wires previously observed in the passive potassium and sodium permeation [8], the permeation of such positively charged small molecules need the to be hydrated to cross the membrane inner core.

In this paper, we took calcium as template to study the unassisted permeation process of a +2 charge with a similar system of previous monovalent ion permeation studies. On the basis of two effective
sampling collective variables (described in Methods), a 2-dimensional potential of mean force (PMF) map was obtained, and based on the map we selected a typical pathway of passive calcium permeation to analyze related properties and situations as shown below. It’s noteworthy that our simulations are about one calcium permeates from membrane surface to the inner core, and how the calcium binds to the membrane surface is not the concern of this study. Also the permeation is under an ion/lipid ratio of 1:126; all our results and conclusions are under this premise.

2. Methods

2.1. Redefinition of the collective variables for permeations

A reasonable way to define the process of ion permeations is by the relative position of ions and the membrane. Traditionally, the membrane was looked as a stationary plane in most simulations of transmembrane permeations, especially those through ion channels or across-membrane nanotubes. It was easy to transport the solute using a collective variable of the z-coordinate of its mass center, which could be also looked as the relative distance in z-axis between the solute and the horizontal plane with z coordinate as 0, generally the central plane of the membrane. However, the situations in the transmembrane passive ion permeations might be different. Earlier simulations have clearly shown possible local deformation of the membrane surface accompanying the movements of ions into the total membrane plane, so as to keep them out of the hydrophobic interior as possible as they can [9,30–31,33,38–39]. In these works, biased simulations using the traditional collective variable resulted in asymmetric concave of the monolayer on the side of ion entry, leading to inconsistency between the central horizontal plane of the total membrane and the mid-point of the upper and bottom hydrophobic-hydropathic interfaces at the permeation site, which is called local hydrophobic center here. This mid-point is the actual deepest position for the ion to permeate into the hydrophobic region, and possibly the transition state of the permeation. As in the earlier simulations, the hydrophobic center may have a dynamic position related to the local deformations of the bilayer, leading to a dynamic difference between the traditional reference point, the central plane and the hydrophobic-hydropathic interfaces at the permeation site, which is the cylindrical local region with 1 nm radius

\[
CV_1 = Z_{\text{ann}} - Z_{\text{hc}} = Z_{\text{ann}} - \frac{Z_a + Z_b}{2}
\]

where the position of the local hydrophobic core along z-axis, \(Z_{\text{hc}}\), is defined as the average of the weighted local heights of the upper and bottom hydrophobic-hydropathic interfaces, \(Z_a\) and \(Z_b\) respectively. The calculations on \(Z_a\) and \(Z_b\) used a successful equation in our earlier simulations [9]:

\[
Z_a = \frac{1}{\alpha} \sum_{i,j} \left[ \cos(\alpha \cdot r_{ij}) \right] Z_{i,j} 
\]

\[
Z_b = \frac{1}{\alpha} \sum_{i,j} \left[ \cos(\alpha \cdot r_{ij}) \right] Z_{i,j}
\]

where \(\alpha = \pi \text{ nm}^{-1}\) defines the cylindrical local region with 1 nm radius in which oxygen atoms of lipid molecules are used to calculate local heights, \(i\) and \(j\) are indices of the oxygen atoms, \(u\) and \(v\) denote the upper and bottom interfaces respectively, \(Z_{i,j}\) are the z-coordinates of oxygen atoms, and \(r_{ij}\) are the projected distance of oxygen atoms to the ion in the x-y plane.

Similarly, a second collective variable (CV2) in our earlier simulations is also used here to describe the continuity of water wire (or water bridge, which can be a pure water chain or a mixed polar chain of water molecules and lipid headgroups) in the hydrophobic part of bilayers by combining the distances of the oxygen atoms of adjacent water molecules along z-axis:

\[
CV_2 = \left[ \sum_i \left( Z_{i,0} - Z_{i+1,0} \right) \right]^{1/5}
\]

where \(\beta = 6\) and \(Z_{i,0}\) are the z-coordinates of the oxygen atoms with the index \(i\) in sorted order. The strong power of \(\beta = 6\) here makes the CV2 very sensitive to the breaking of the water wire. Large distances of the broken part of the water wire would contribute to CV2 mostly and increase CV2 importantly [8,9].

2.2. MD simulations and free energy changes

A solvated membrane of 126 Palmitoyloleoylphosphatidylcholine (POPC) lipids was simulated in a box of 6.41 × 6.41 × 7.63 nm³, with the membrane perpendicular to the z-axis. Also, one Ca²⁺, 19 Na⁺ and 21 Cl⁻ ions were placed in the system, together with 4905 water molecules. The lipids were modeled with the force field by Ulmschneider et al. [40], and OPLS-AA TIP3P model and ions’ model were used for water molecules and ions respectively. MD simulations were performed in the NPT,yT ensemble on Gromacs, at normal pressure (\(P_\theta = 1\) atm), with a Nose-Hoover thermostat (\(T = 310 K, \tau_T = 0.5\) ps). The time step is of 2 fento-second (fs), and this simulation lasts about 7000 nano-second (ns) in total. The non-bonded interactions were computed using particle mesh Ewald method [41–42].

Umbrella sampling was performed by harmonic potentials on the two collective variables, CV1 and CV2. The sampling on a square area (CV1 from 0 nm to 2.9 nm, CV2 from 0.29 nm to 2.34 nm) were completed by 132 windows, 9 columns of windows along CV1, 1000 kcal/mol constant force, 10 rows of windows along CV2, 2000 kcal/mol constant force, the locations of the windows are uniformly distributed. To avoid the lack of sampling in some space, we patched 42 extra windows between rows or columns. Each window was simulated for 50 ns including 10 ns equilibrium. All the conformational distributions in these simulations were integrated into overall free energy profiles with the biasing potentials adjusted by the weighted histogram analysis method (WHAM) [43], convergence tolerance for the WHAM calculations is set to 0.001 KJ/mol. Especially, a 2D WHAM software was used here [44].

A small statistical error is a good indicator for the convergence of the PMFs when the simulation time is comparable to or greater than the correlation time. In particular, if the simulation time is only comparable to the correlation time, the statistical error will be large. The statistical errors of the PMF profiles in all the sampling windows are shown in Figs. S7–S10. It can be seen that the most of the areas converges very well, except the area in red circles. The area in red circles has the highest energy barriers (probably larger than 100 kcal/mol), and is hard to get enough sampling technically. But also this area is far away from the transition pathway area, so it is of the least importance. All of our conclusions are not based on the results of this area.

2.3. Selection of a typical pathway

As we aimed to find and analyze the typical molecular process of unassisted calcium permeation in lipid bilayers, we determined to select a typical pathway from the hydrophobic core to the membrane surface of the calcium.

On a 2D energy map, if we assume there is only one energy barrier between two energy wells (A, B), the position (C) with the lowest free energy barrier is defined as the transition state from A to B. Because C has the lowest energy barrier, the transition path (ψ) through C is the most possible path to go from A to B. So ψ is the path A → C → B. Also along this transition path, C is the location of energy barrier, so it's the most difficult and important position on ψ.

To find the most possible path as the typical path, we must find the transition state at first. Because of the symmetry of lipid bilayers, transition state should be in the membrane center, where CV1 = 0. We fixed the calcium in the hydrophobic center for 120 ns (20 ns for equilibrium) to determine the transition-state configuration. As we know, transition state has the lowest energy barrier, so it appears the most times. 20 representative snapshots were randomly picked out of the ensemble of transition state as initial configurations to start unbiased release. We release the ion from its transition state (yellow star on the left side, Fig. S7), without any external force, the ion is supposed to go along the most energetically favorable path, which is its real transition path. So the unbiased release is aimed to let the ion goes freely to the membrane surface, and we record their path. The release simulation time is set as 20 ns, to allow almost all the calcium arrive their destination. But if calcium doesn’t, we extend the simulation time to 30–50 ns until it arrives. At last 20 unbiased release trajectories were obtained (black dots in Fig. S1).

As it’s clear that, the 20 unbiased release pathways are close to the “real” lowest free energy pathway. Based on the release results and also the PMF map, we selected a typical path as shown in Fig. S1. It is important to note that our typical pathway is theoretically close to, or at least comparable to the real lowest free energy pathway. Taking the typical pathway windows as the x-axis coordinate, we presented the interaction energies (Fig. 3), lipid positions (Fig. S3), and the distributions of related oxygens (Fig. S5).

3. Results and discussions

On the 2D PMF landscape in Fig. 1, the lowest free energy transition pathway (as shown in Figs. S1 and 1, chosen as the typical pathway as described in Method) of calcium is found to lower energy barrier by water wire (reflected by decrease in CV2, also shown as snapshots in Fig. 2) and membrane deformation (reflected by decrease in CV1, also shown as snapshots in Fig. 2), similar to the pathways of sodium and potassium. After calcium enters the hydrophobic domain of lipid bilayers, due to strong electrostatic interactions (as shown in Fig. 4, electrostatic energies contribute more than 90% in ion-POPC interaction energy), the surrounding lipid headgroups also move inward, which will increasingly cause concave deformation on the membrane surface. A cluster of water molecules was maintained inside the concave deformation, but there is little chance for water molecules to directly interact with lipid hydrocarbon tails; water molecules are still surrounded in the lipid headgroups. However, once calcium steps forward a little, lipid bilayers on the opposite side will respond with a symmetric deformation, shortening the distance of the bilayers. At this point, lipid tails have been disturbed, water molecules of both sides get chance to get rid of the surrounding lipid headgroups, and gathered to build a weak water wire, in most cases, just a single-file water wire at the beginning. As long as the water wire is formed, more and more water molecules scatter into the hydrophobic domain and gradually aggregate into the water wire, making the water wire much stronger until it grows into a remarkable water pore/channel. The radius of this pore/channel is not stable (Fig. S2): when lots of water molecules pass through, the radius becomes larger. During the formation and strengthening of the water wire, membrane concave deformations may also increase. Then with help of water wire the calcium slowly but continuously moves to the center of hydrophobic core, where lipids from both sides are connected by the calcium. Slowly the calcium mixed into the headgroups of the opposite side and move forward. Then the water wire becomes thin again, and finally breaks. Membrane surfaces will restore slowly after the ion gets out of the hydrophobic core.

To interpret the permeation process on the PMF map of two CVs, some clear identifications are added to the PMF map in Fig. 3. Since the permeation process is perfectly symmetric, we just make use of the right half of the PMF map to explain the process of calcium to permeate from the membrane surface to the membrane center. And the way it permeate out of the membrane is exactly the in the same way. CVz(CV1) reflects the distance between ion and the mid-point of the upper and bottom hydrophobic-hydrophilic interfaces at the permeation site (membrane center for short); that is, CVz reflects the ion movement relative to the membrane deformation. To put together the

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Fig. 1. 2-Dimensional free energy landscape of calcium permeation. CV1 is referred to the relative distance between the ion and the local hydrophobic core along z-axis, and CV2 is referred to the continuity of water wire (or water bridge, which can be a pure water chain or a mixed polar chain of water molecules and lipid headgroups) in the hydrophobic interior of bilayers. Also the typical pathway is presented by a black line, and the yellow arrows along it are to describe the permeation process. (The details should be referred to Methods part) When CV1 = 2.6 nm, calcium is on the surface of bilayers, beginning to get into membrane; when CV1 = 1.6 nm, calcium is beginning getting into hydrophobic core of membrane; when CV1 = 0 nm, calcium is in the membrane center. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

PMF and ion permeation trajectory, the black line is our typical pathway. The yellow star on the right represents the usual start position of ion, where CVz is large. It's the position where ion stays on the surface of membrane, far away from membrane center. And the yellow star on the left is the position where ion stays in the membrane center, CVz = 0, also the position of transition state. The region between red lines, A, represents the decreased CVz here is mostly caused by the membrane deformation. The region between red line and blue line, B, represents the decreased CVz here is mostly caused by the ion movement. This is concluded from our trajectories. (Fig. 2) And the dashed red line is to the decreased CVz here is mostly caused by the membrane deformation. The region between red line and blue line, B, represents the decreased CVz here is mostly caused by the ion movement. This is concluded from our trajectories. (Fig. 2) And the dashed red line is to allow some space for the variation of region A. CVwater(CV2) reflects how the water molecules are connected in z axis (perpendicular to membrane surface). If there is any gap between them, the larger the gap is, or the more the gaps are, the larger the value of CVwater is. And the minimum point of CVwater means that there is no big gap between water molecules. So when ion diffuse from membrane surface to the center, the water molecules of two sides get closer, CVwater decreases, and the water wire is beginning to be built. When the water molecules are all well-connected by each other, and the water wire is completed (CVz is about 1.15 nm), we saw the CVwater(CV2) reached its minimum (about 0.25 nm), as shown in Figs. 1 and 3. It's clear that once the water wire reaches its minimum, the well-connected water wire lasts for quite a long distance in CVz. This suggests that the permeation of calcium need to be assisted by the water wire, especially when it's close to the membrane center. Something to note, the decrease of CVWater as the green arrow shows in Fig. 3 during membrane deformation (in region A), is mainly caused by the approaching of two bilayers, the water molecules don't diffuse into hydrophobic domain here; and the decrease of CVWater in region B reflects the building of the water wire in membrane hydrophobic core.

In terms of free energy barrier (Fig. 1), it is very difficult for calcium to passively permeate through the lipid bilayers. The energy barrier of the lowest free energy transition pathway is as high as 57 kcal/mol, the highest energy barrier can be 120 kcal/mol (120 kcal/mol is estimated with large errors). Some experimental results on unassisted calcium permeation have been listed in Table 1, to provide a comparison with our results. Permeability coefficient of calcium in this study has been roughly estimated by a formula proposed by an earlier work [9]. Our result is significantly smaller than experiments, which indicates the energy barriers are higher. On one hand, experiments are under high ion concentration gradients and with different lipids, which may lead to the differences. Interestingly, the permeability coefficients of sodium in other experiments are also in similar orders of magnitude (-12 to -14) [45], which suggests large errors of permeability coefficients may exist extensively in the experiments. On the other hand, the force fields have been proved to create errors in the calculations of interaction energies by studies on polarizable force fields [46-48], the higher free energy barrier may also be related to the force field we used.

The concave deformations of lipid bilayers make local membrane thickness decrease largely (as shown in Fig. S3), which agrees with an experimental study that Ca2+ binding results in a compression of the lipid bilayers [53]. In details, membrane thickness increases at hydrophilic interface, and then decreases to 1.25 from 1.64 nm up to 2.03 nm, while ion is in the hydrophobic core. Also, hydrocarbon tails of surrounding lipids become seriously disordered, shown as the decreases of |Scd| (Fig. S4), and some of the lipid tails are even parallel to the membrane surface, as shown in Fig. 5. According to the deuterium order parameters |Scd| we calculated (Fig. S4), there is an apparent decrease in |Scd| of both two lipid tails in the calcium permeation, especially the saturated one. Also, we found one or two lipid molecules close to the ion would follow the ion’s permeation all the way, from one side to the other, strongly assisting ion transport.

Table 1

<table>
<thead>
<tr>
<th>Vesicle lipids</th>
<th>P a (cm/s)</th>
<th>T °C</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horse bean lipids</td>
<td>2.5 × 10^{-11}</td>
<td>25</td>
<td>Rossignol et al. [49]</td>
</tr>
<tr>
<td>Egg PC</td>
<td>8 × 10^{-15}</td>
<td>30</td>
<td>Vanderkooi and Martonosi [50]</td>
</tr>
<tr>
<td>Dioley PC</td>
<td>5.3 × 10^{-14}</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>PC</td>
<td>4 × 10^{-11}</td>
<td>RT</td>
<td></td>
</tr>
<tr>
<td>Retinal lipid</td>
<td>5 × 10^{-11}</td>
<td>RT</td>
<td></td>
</tr>
<tr>
<td>PC/PS mixture</td>
<td>8 × 10^{-11}</td>
<td>RT</td>
<td></td>
</tr>
<tr>
<td>Lens lipids</td>
<td>4.5 × 10^{-13}</td>
<td>37</td>
<td>Zeng et al. [52]</td>
</tr>
<tr>
<td>Egg PC</td>
<td>9.8 × 10^{-14}</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>SM</td>
<td>9 × 10^{-13}</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>POPC</td>
<td>4 × 10^{-40}</td>
<td>37</td>
<td>Theoretical results of this study</td>
</tr>
</tbody>
</table>

a P for permeabilities.

b RT for Room Temperature.
Called “flip-flop”. Usually, the “flip-flop” lipid molecules turn their head to the other side in this process and finally mixed into the opposite side. Compared with sodium and potassium, permeation of calcium caused more significant membrane defect, and certainly more serious consequences. From the results of statistics (Fig. S5), a large quantity of polar oxygens swarmed into membrane hydrophobic domain. Especially after the water wire is formed, the number increases from 20.8 to 45 rapidly. The number of oxygens around the ion (within 0.5 nm) is even more surprising: in terms of number, calcium has about 20 oxygens around it in the membrane center, and sodium and potassium have 12; and in terms of trend, number of oxygens around ion would decrease significantly from 18 to 12 after sodium or potassium enters the hydrophobic core, but the number of oxygens around calcium is stabilized in a range of 20.1–22.5. Obviously, Entering the hydrophobic core doesn’t affect the polar shell of calcium. As we try to trace these changes to their sources, it is suggested that the electrostatic interactions of calcium with lipid and water oxygens are much stronger. As shown in Fig. 4, the interaction energies of calcium with both lipids and water are in the range of −289 to −353 kcal/mol, and those of sodium and potassium are in the range of −85 to −52 kcal/mol. The interaction energies of calcium are surprisingly strong. As we mentioned above, force field OPLS- AA is likely to overestimate the interaction energies of polar ions by as large as 100 kcal/mol [46–48], so we strongly recommend to pay attention to the comparative values, such as how the interaction energies change from the membrane surface to the inner core, how are the interactions of calcium compared with sodium of potassium (they are calculated in the same conditions), rather than their absolute values.

Another important finding in the permeation of calcium is the formation of a complete water pore/channel. Water wires in the permeation of sodium and potassium have no other functions or effects, except providing a hydrophilic environment to help the ion move through the hydrophobic membrane, but it is significantly different in the permeation of calcium. We found lots of water molecules pass through this water wire (or water pore/channel in this case) by a “All or None” manner. “All or None” is widely-reported in AMP experiments, [16,54] that there are only two cases for the membrane leakages of substances, they leak a large amount in very short time, or they don’t leak at all. In our simulation, about 127 water molecules leakages were found in randomly picked-up 500 frames, about 1 ns, as shown in Fig. S6. As we observed from the trajectories, water molecules passed through the channel in an instant, and the channel then becomes too small to be permeated. By the short-time permeations of water molecules, the radius of the channel increases or decreases accordingly. Apparently, the membrane deformation discussed above has a close relationship with the formation of this water channel. The calcium and submerged lipid headgroups help water molecules reside in the hydrophobic domain, and create a relatively comfortable hydrophilic environment for the water channel. It is important to note that the lipid headgroups, however, do not constitute the water channel mentioned here; the water channel we meant always consists of water molecules in the hydrophobic domain. In another perspective, despite the fact that the number and distribution of lipid oxygens in the hydrophobic domain are relatively stable (ranging from 21.2 to 29, shown in Fig. S5), the radius of water channel changes erratically (Fig. S2). And the reason may be simple: on one hand, the water channel/pore are “attracted” by the polar calcium and lipid oxygens (indicated by the strong electrostatic energies); on the other side, it is also surrounded by unstable hydrocarbon tails outside (Fig. 5), suffering serious and turbulent hydrophobic forces. The totally different forces on the water channel make it hard to reach a balance, therefore cause the leakages in an extremely unstable “All or None” manner.

Lots of experiments report leakages caused by membrane-permeating antimicrobial peptides (usually +3 +9) have doubted about their “All or None” manner [16,54], which is completely consistent with our findings in the calcium permeation. Probably it can be considered that high electropositivity (at least more than +2) is the root cause of “All or None” leakage manner.

4. Conclusion

Compared with +1 charged sodium and potassium, apart from the differences in the Born radius or their chemical properties, calcium has one more positive charge, but its permeation process has greatly changed under the same ion/lipid ratio. The unassisted permeations of sodium and potassium in our previous study are similar, which indicates the differences caused by different electrostatic or chemical properties are still not enough to compare those caused by different charges. In the passive permeation of calcium, more significant membrane defect and a complete water channel/pore are found. In view of free energy barriers, it is very difficult for calcium to passively permeate through lipid bilayers for its relatively strong electropositivity. Calcium in the membrane can attract oxygens steadily and largely, including lipid and water oxygens, to create a relatively hydrophilic local environment, where a water channel/pore is formed. On one hand, the water channel is built on the basis of electrostatic forces; on the other hand, it has to suffer serious and turbulent hydrophobic forces from hydrocarbon tails outside. The “game” of hydrophilic and hydrophobic forces on the water channel finally introduces extremely unstable leakages, “All or None”. This leakage manner is quite similar with what was found in AMP experiments, suggesting “All or None” leakage may be closely related to high positive charges. Also, unassisted permeations of small

![Fig. 4](https://doi.org/10.1016/j.molliq.2017.12.127)
always brought me to the beach on Saturday. I had many opportunities to discuss science with Lesser. We published 5 good papers in two year time, and also I completed my Ph.D. in record time in the university history. I once told Lesser that most of the difficult problems were solved in the beach.

The last time I saw Lesser and Elisa was in 2008. I was happy to have them with me at the 2008 TACC conference (Theory and Application of Computational Chemistry), where I served as the chairman. They visited my home, and city of Shanghai. Lesser was in good health and high spirit. I enjoyed so much because it was a way for me to express my appreciation for what he had done for me.

Lesser wrote me a few times after going back to the states. He told he had new ways to do GMSA, it will include properly the correlation MAS misses. I feel sorry I did not do much in this direction.

Now Lesser, the great man in science is gone and I have to carry on. This whole day I could not do much, and I tried to call a few times but could not reach anyone. We shall remember Lesser for his great works in statistical mechanics, a dear friend with kind heart.

Dong-Qing Wei, Shanghai Jiao Tong University

Appendix A. Supplementary data

Selected typical pathway of calcium permeation from the hydrophobic core to the equilibrated state out of the membrane; radii (Å) of transient water pores caused by calcium permeation in four different trajectories; Positions of ion (Zion), upper bilayer (Zup) and bottom bilayer (Zdown) by CV1; deuterium order parameters |Scd| of unsaturated tails sn1 and saturated tails sn2 of POPC lipids; number of water and lipid oxygens; number of water molecules permeating through the pores in 500 frames; the differences in PMF between 10–20 ns sampling and 20–30 ns sampling; the differences in PMF between 30–40 ns sampling and 30–40 ns sampling; the differences in PMF between 30–40 ns sampling and 40–50 ns sampling; the differences in PMF between 10–20 ns sampling and 40–50 ns sampling. (PDF). Supplementary data associated with this article can be found in the online version, at https://doi.org/10.1016/j.molliq.2017.12.127.

References