



A MOLECULAR DYNAMICS SIMULATION STUDY ON THE EFFECT OF METHANOL ON THE STRUCTURAL CHARACTERISTICS OF DPPC AND POPC BILAYERS

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ABSTRACT

Understanding how cell membranes (or phospholipid bilayers) interact with small molecules and commonly used chemicals is of tremendous biological importance. Due to the inherent complexity of these systems their investigation by experimental techniques only is very difficult. However, recent development of new algorithms and revolutionary advances in the computational power available to scientists has permitted computer simulations of biological membranes to advance at a comparable pace with that of experiments [1]. The excellent agreement with the experiment obtained in various molecular dynamics (MD) studies [2] on simple model membranes has raised the confidence in applying the atomistic simulations to even more complex systems. To apply an atomistic simulation technique and obtain a molecular level understanding of the structural and dynamical aspects of lipid/water systems in the presence of methanol (a commonly used cryoprotective agent during cryopreservation) is not only important to the overall behavior and interaction of membranes, but also of great biological and medical interest.

Recently Patra et al. [3] investigated the structural changes in a fully hydrated dipalmitoylphosphatidylcholine (DPPC) and palmitoyloleoylphosphatidylcholine (POPC) lipid membranes in the presence of relatively small molar fractions (below 1.0 mol %) of ethanol and methanol in water using MD simulations. An another recent molecular dynamics study [4] has focused on the permeability of eight small organic molecules (representing the most common chemical functional groups) through a DPPC bilayer. Although, these earlier MD simulations [3, 4] have shed considerable light on the interactions between methanol and lipid bilayers, the assumed mol % (~1%) is considerably smaller than values used in typical cryopreservation protocols [5]. Thus, in this paper we have applied MD simulations to investigate and compare the structural changes in DPPC & POPC lipid membranes (in biologically relevant phase) in the presence of a

cryobiologically relevant molar ratio of methanol/water (~12.0 mol %).

SIMULATION MODEL AND METHODOLOGY

We have simulated DPPC & POPC bilayer systems separately consisting of 96 molecules (i.e. 48 lipids in each leaflet), together with 5422 water molecules (full hydration) and 612 methanol molecules. A snap shot of these two systems at time, $t = 20$ ns is shown in Fig. 1. We started the simulation with the DPPC and POPC bilayers immersed in a mixture of water and methanol. The DPPC and POPC lipid bilayers had an initial area per lipid of 0.625nm^2 and 0.67nm^2 , respectively. The simulation was run for 20ns at 323K for DPPC and 298K for POPC (at these temperatures both DPPC and POPC are in a biologically relevant liquid crystalline state). The simulations were performed with the GROMACS molecular dynamics package [6]. Periodic boundary conditions were applied along the three space dimensions. The pressure was controlled using Berendsen barostat with a time constant of 1.0 ps. We used the semi-isotropic pressure coupling. Accordingly the height of the simulation box (z direction) and the cross sectional area (xy-plane) were allowed to vary independently of each other. An energy minimization procedure based on the steepest descent algorithm was initially applied to the initial structure prior to the actual MD run.

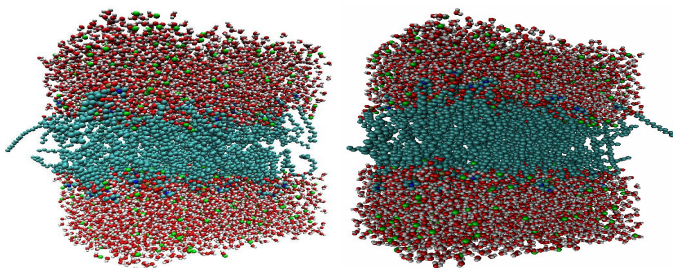


Fig. 1. A snapshot of the fully-hydrated DPPC bilayer (left) and POPC bilayer (right) in a mixture of water-methanol after 20 ns. The atom coloring scheme is N-blue, O-red, P-brown, C (lipid)- cyan, OH-light green, H(water)-white.

It is known that the area per lipid, $\langle A_{\text{DPPC}} \rangle$, is one of the most important quantities characterizing a bilayer membrane and it is often monitored in simulations to assess whether or not the system has reached equilibrium during the subsequent MD run. Fig. 2 shows the time variation of $\langle A_{\text{DPPC}} \rangle$ in the presence of methanol over the 20 ns simulation time of DPPC and POPC bilayer systems. The area per lipid $\langle A_{\text{DPPC}} \rangle$ reaches a plateau and fluctuates around the average value $\langle A_{\text{DPPC}} \rangle = 0.750 \text{ nm}^2$ for DPPC bilayer and $\langle A_{\text{DPPC}} \rangle = 0.718 \text{ nm}^2$ for POPC bilayer. Our study reports 9.5% and 3.5% increase in the area per lipid values for DPPC and POPC respectively. This increase in the area per lipid due to increase in concentrations of methanol would result in higher membrane permeabilities. This might be the primary reason for the ability to successfully cryopreserve biological systems at a higher cooling rate in the presence of chemicals (like methanol) than in their absence. The profile of the methanol mass density across the bilayer system of DPPC and POPC are shown in Figure 3. Accordingly the methanol molecules are distributed fairly symmetric with respect to the horizontal

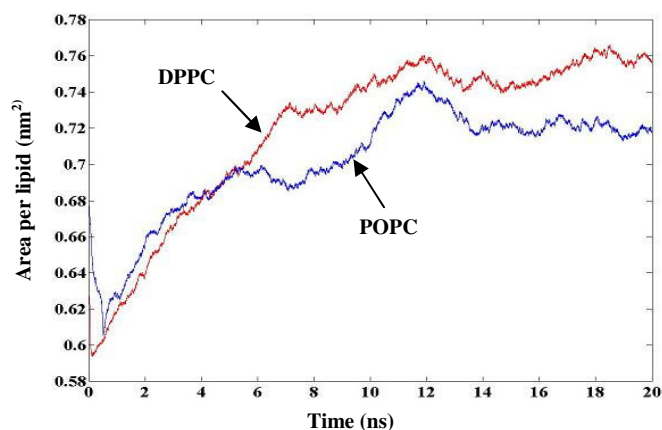


Fig. 2. Time dependence of the area per lipid for the DPPC bilayer (red) and POPC bilayer (blue) in the presence of the 12 mol % water-methanol mixture.

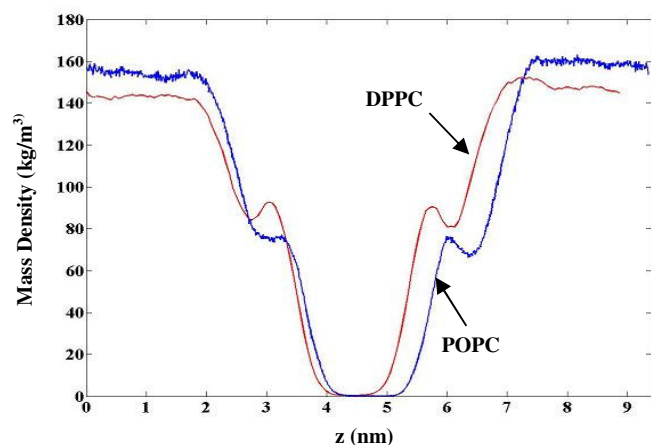


Fig. 3. Methanol mass density profile across the DPPC bilayer (red) and POPC bilayer (blue).

plane passing through $z = 4.5 \text{ nm}$ which is likely to coincide with the median plane of the lipid bilayer. Moreover from Fig. 3 one may also infer that there are very few methanol molecules that penetrate even deeper in the hydrophobic tail region. Patra et al. [3] showed that while ethanol molecules are able to penetrate through the bilayer membrane over very short time scales typical for MD studies (50 ns) no methanol molecule penetrates the membranes on this time scale.

CONCLUSION

In this paper, we used molecular dynamics simulations to investigate the effect of methanol on the structural properties of a DPPC and POPC lipid bilayer membrane. Our study shows that in the presence of the methanol the DPPC and POPC bilayer thickness decreases (and thus results in a higher membrane permeability). We postulate that this increase in the membrane permeability in the presence of methanol might be the primary reason for the higher values of optimal freezing rates in the presence of methanol for most cells than its absence. Future studies will investigate the effect of other commonly used chemicals (like dimethylsulfoxide, glycerol) and sugars (like trehalose and sucrose) at cryobiologically relevant concentrations on DPPC and POPC membranes.

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