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Simulations of Biomembranes and Water: Important Technical Aspects

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Abstract

In this contribution, we discuss several important technical aspects which are relevant for the molecular modeling of biomembranes in aqueous environments. We study the effect of coarse-grained water models on free and supported systems and show that the choice of water model has dramatic repercussions on the phase behavior. We characterize the phase behavior of a widely used water model and discuss the technical implications of modeling solid supports for biomembrane simulations. Finally, we compare the effect of anisotropic pressure coupling with surface tension coupling in atomistic bilayer simulations including alcohols.

Introduction

Simulations of biomembranes that span a variety of length scales have become common over the last two decades [1–9]. This, however, does not imply that simulations can be performed without closely inspecting the simulation details as it has been shown that unfortunate choices in conditions or parameters can lead to unsuccessful simulations [10–12]. In this contribution, we discuss several technical aspects of simulations which are usually not the main focus of modeling studies. We initially assess the behavior of water molecules in conjunction with a membrane. The examination of water molecules is often neglected, even though it has a dramatic effect on the phase behavior and structure of biomembranes. We also discuss technical points that are relevant for modeling interactions with a support, where again the choice of water model is important. Finally, we conclude with a comparison between lipid membrane systems that are simulated either via constant pressure or constant surface tension.

Water is not only important in biomembrane simulations because it is the key ingredient in cellular environments, but it is also a unique and interesting compound that has chemical and physical characteristics which lead to complex phase behavior. This results in the well known anomalous density behavior in the liquid phase, the possible existence of a liquid-liquid phase transition and the existence of many solid phases [13]. Such behavior poses a severe challenge for computer modeling and many detailed water models have been used in molecular simulations [14–18]. This can be seen in the TIP5P model, where up to 5 interaction sites have been used to represent the triatomic molecule. In these classical models, water is treated as a non-polarizable molecule, where the positive charge location is the hydrogen atoms and the negative charge location varies. These models are adjusted to represent liquid water at ambient conditions and do not necessarily yield an accurate description of the solid phases. This can be problematic in studies involving low temperatures where freezing becomes important.

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All-atom simulations are very abundant, but they are restricted in simulation time and system size. This is especially true for water in biomembrane simulations where the main focus is the phospholipid system and not the water. However, to accurately represent a cellular bilayer, water must be included in the model and hence a significant amount of computational resources are spent on modeling the water. In order to minimize the resources spent on water, several approaches such as solvent free bilayer models using special interactions which mimic the water [7,19] and coarse grained models [20–22] have been used, which allow for larger time scales.

Most computer simulations of biomembranes have been performed on free standing bilayers in water, although a few freestanding monolayers have been examined [23–25]. Many experiments, however, are performed in supported bilayer systems [26–31]. There is clearly a disconnect between simulations and experiments in this respect. There are several technical aspects found in supported bilayer systems which do not exist in free mono- and bilayer systems. First, a model for the interaction between the support, the bilayer and the water has to be developed. The support can be modeled using either explicit atoms or through distance dependent interactions between the support and the bilayer and water atoms. In the first support scenario, constant pressure simulations in the bilayer plane are not possible because the interaction density of the support would change. The second part of this paper deals with such challenges.

Finally, we discuss how the treatment of pressure in different ensembles (constant pressure versus constant surface tension) leads to small but noticeable differences in simulations of atomistically detailed lipid bilayers with alcohol solutions. Comparisons between NPT (constant pressure) and $N\gamma T$ (constant surface tension) simulations have previously shown that when sampling a DPPC lipid bilayer, the two ensembles give similar results [32]. However, do the two ensembles also give similar results when a phase change occurs? To examine this question, we study a DPPC lipid bilayer that is solvated with an alcohol solution. It has been shown both experimentally and using dissipative particle dynamics (DPD) simulations that alcohol molecules have a biphasic effect on bilayers, which can result in the appearance of the interdigitated phase [33,34].

Coarse-Grained Simulation Details

Verification of a Coarse-Grained Water Model for Biomembrane Simulations

A well characterized coarse-grained water model [20] is simulated using Molecular Dynamics at different temperatures. A group of 4 real water molecules is represented by one polar coarse-grained bead with a mass of 72 amu and an interaction distance of 0.47 nm. All beads interact through short-range Lennard-Jones potentials. The interaction of other particle types with water depends on their hydrophilic and hydrophobic nature. The GROMACS simulation suite [35] has been used to perform all simulations. Time scales have been renormalized by a factor of 4 to reproduce correct diffusion coefficients and the reported simulation times are real times. Unless stated, simulations lasted for 4 μ s with a time step of 160 fs. Temperature and pressure are kept constant using the weak-coupling formalism technique [36]. Pressure was maintained by coupling the three Cartesian directions to ambient pressure independently. Systems with 1500 water molecules (corresponding to 6000 real waters) in box sizes ranging from $4 \times 5 \times 8$ nm at 265 K to $4 \times 5.4 \times 8.5$ nm at 325 K and 1 bar were simulated.

We describe the importance of determining the freezing point of water for examinations of the lipid phase behavior and its dependency on thermal history. Knowledge of the ice/water interface for each thermal condition in the simulation is of high importance since a solid phase may distort the lipid phase behavior by constraining the lipid molecules. This may lead to incorrect interpretation of simulations when only the lipids are considered in the analysis.

We first evaluated the freezing point of the coarse-grained water by determining the inflection point in the density–temperature diagram at 1 bar for cooling and heating cycles (cf. Figure 1). A heating cycle is defined as the process of increasing the simulation temperature gradually to reach a desired temperature, i.e., the system changes from an ordered to a disordered state. Similarly in a cooling cycle, the simulation temperature is ramped down until the value of interest is reached. We observe a large hysteresis for systems of 1500 CG waters and find the freezing point using the cooling cycle to be between 260 K and 275 K and for the heating cycle to be between 325 K and 385 K. An earlier estimate of 290 K [20] was obtained for a system containing 400 waters. Supercooled water exists below 290 K, a spontaneous freezing is observed at lower temperatures. This confirms the importance of both thermal history and system size. The coarse-grained water model does not accurately describe the solid water phase due to the mesoscale nature of the water. Like some atomistic models [18], the experimental change in the slope of the density–temperature curve around 277 K is not observed. However, the model can be used with confidence for the liquid phase since it accurately predicts properties of liquid water [20]. For instance, water molecules have a self diffusion rate of $2 \times 10^{-5} \text{ cm}^2/\text{s}$ at 300 K, which is close to the experimental value of $2.3 \times 10^{-5} \text{ cm}^2/\text{s}$ at 300 K [20]. The coarse-grained approach is advantageous in the sense that testing the ice–water interface for specific simulation conditions can be achieved very quickly.

In terms of practicality, the determination of the freezing point of the coarse-grained water by identifying the inflection point in the density–temperature profile is a fast technique and the predicted values can be correlated with the evaluation of other properties, such as diffusion and structure factor. The changes in density with temperature (Figure 1) correlate with the changes in the static structure factor both for cooling and heating thermal cycles. The static structure factor

$$S(K) = 1 + 4\pi\rho \int_0^\infty dr r^2 \frac{\sin(kr)}{kr} (g(r) - 1). \quad (1)$$

can be obtained from the spatial Fourier transform of the radial distribution function [37]. In Figure 2, we show the structure factor for coarse grained water (heating cycle). The structure factor for the cooling cycle is shown elsewhere [38]. From the structure factor, we see that the water structure changes between 325 K and 385 K, which correlates well with the density (Figure 1) and the lateral diffusion (Figure 3 left). In fig. 3 left, the mean squared displacements were obtained at 265 K, 325 K and 385 K from 400 ns to 2000 ns, and the diffusion coefficients are approximately $8 \times 10^{-10} \text{ cm}^2/\text{s}$, $8 \times 10^{-9} \text{ cm}^2/\text{s}$, and $2 \times 10^{-5} \text{ cm}^2/\text{s}$ respectively. In Figure 4, the snapshots of the pure water simulations for cooling cycles are presented at 265 K, 275 K and 325 K. The snapshots depict frozen and fluid water lattices and they correlate with the density–temperature (Figure 1), structure factor [13] and diffusion (Figure 3, right) graphs. For instance, a frozen lattice structure corresponds to low diffusion values, as well as well defined peaks in the structure factor. This reinforces the importance in understanding the water phase behavior especially for hydrated systems. For instance simulations of fully hydrated bilayers of lipid mixtures of DLPC and DSPC were performed at different temperatures and compositions to address the phase behavior of this mixture. Biomembranes with high concentrations of DLPC were of particular interest because these simulations were executed at low temperatures since DLPC presents a low transition temperature [38]. For that, we addressed the study of the effect of temperature on water phase behavior in the absence and presence of lipid bilayers. In Figure 3 (right) the mean squared displacement shows increasing mobility for water at 275 K and 325 K, and it becomes clear that this coarse-grained water model freezes at ~265 K under cooling. It was observed that water molecules interacting with the bilayer at 265 K do not result in a frozen structure. This may be the result of bilayer/water interactions that disturb the ordering, a finite-size effect (the amount of water employed in water–bilayer simulations is more than four times the amount of water in pure water

simulations), and the thermal history (since the hydrated bilayers were quenched to the desired temperature, and pure water simulations were ramped to the desired temperature). Figure 5 confirms this, where mean squared displacements for water in a pure DLPC bilayer (blue) and a two component DLPC-DSPC lipid bilayer (green) are shown at 265 K, and the diffusion coefficients for both cases are on the order of $10^{-6}\text{cm}^2/\text{s}$.

Modeling of Supported Bilayers

In order to examine supported lipid bilayers, we need to develop appropriate models for the interactions between the support and the water and the support and the lipids. We start from the above characterized coarse-grained water and develop an interaction model of this system with the support. Here, our model system contains 4129 CG water particles on a support. Four hundred and forty semi-hydrophilic particles form a relatively smooth supportive surface at a spacing of 0.3 nm. The surface particles interact with water at an interaction strength level III from ref. [20]. Note that the surface particles interact at the same level with lipid headgroups (see below). The surface was placed at the bottom ($z=0$ nm) of the simulation box. The system has periodic boundary conditions in all 3 directions leading to an effective slab of water and lipid bilayer between two surfaces. The insertion of the surface breaks the continuity of the bulk water and influences its properties. In order to fix the interaction density of the support, the lateral area of the simulation box is kept constant in the simulations. With that, only the box normal is coupled to the reference pressure (1 bar). At $T = 323$ K, clear layers of water particles were observed throughout the simulation box indicating nucleating ice at the support (Fig. 6 left). The black solid curve in Fig. 6 (left) representing the density of water exhibits multiple sharp peaks along the normal direction of the box. Those peaks correspond to the ordered water layers (i.e., ice-like structure) in the system, which is abnormal at 323K. As discussed in the previous paragraph and as reported in [20], the CG water particles in this model have a higher melting transition temperature, $T_{\text{melt}}=290\pm 5$ K, than real water (273 K), which also strongly depends on the thermal history. We now define the original water-water nonbonded interaction strength introduced by Marrink [20] as 100%. We have tested several lower interaction strengths (90%, 82%, 76%, and 68%) to examine the change of the structural and thermodynamic properties and possibly avoid the strong layering. For the two higher interaction strengths, water layers were observed from the vicinity of the surface towards the center of the simulation box, with the water molecules in the very middle being slightly less ordered than in the original model, but the ordering was still visible (Fig. 6 left). When the interaction strength between CG water particles was reduced to 76%, the center of the simulation box was mostly filled with fluid water (density of such system shown in green solid curve in Fig. 6 left). Within 2.5 nm of the surface, there were approximately 5 layers of water molecules. The confined water structure near the surface did not disappear as we decreased the water-water interaction strength. At 68%, approximately 5 layers of water molecules were found near the surface as well, but the bulk density in the center of the box was reduced to 800 kg/m^3 , which is too low for real water (blue solid curve in Fig. 6 left). At 76 % we find the best compromise between fluidity and density ($\sim 900\text{ kg/m}^3$). Kumar et al have studied thermodynamic and structural properties of water confined between two smooth hydrophobic plates [38]. They observed the emergence of a phase transition line separating liquid states of different densities, i.e., a liquid-liquid transition parallel to the surface. We have observed similar structural behavior. To verify that our water undergoes the same liquid-liquid transition induced by the surfaces, we defined three 2.5 nm slices of water molecules from the top, center and bottom of the simulation box. The top and bottom are equivalent for symmetry reasons. At 323 K, $D_{\text{top}} = (4.85\pm 0.39) \times 10^{-5}\text{ cm}^2/\text{s}$, $D_{\text{mid}} = (4.68\pm 0.49) \times 10^{-5}\text{ cm}^2/\text{s}$, $D_{\text{bot}} = (4.7\pm 0.03) \times 10^{-5}\text{ cm}^2/\text{s}$.

The experimental diffusion constant of pure water $D_{\text{exp}} = 2.3 \times 10^{-5}\text{ cm}^2/\text{s}$ at 300 K. All water in the confined system is fluid, but it is more ordered close to a smooth surface. Using this

(weakened) water model, we have then studied a system of DPPC supported bilayers (with 64 lipids in each leaflet) where we placed 155 water molecules below and 2438 above the bilayer. Comparing to old results using the original Marrink model we have to keep this weakening in mind. The distribution of waters in the simulations was chosen because the thickness of the water slab between the support and the bilayer would correspond to about 2 layers of water molecules. We see very slow transport of water through the membrane. There is no direct experimental evidence on the exact amount of the water at the support, but two layers is a commonly hypothesized value. The DPPC model was taken without modification from ref. [20]. Figure 6 (right) shows the resulting density profile. We clearly see a strong effect of the support on the density profile in this system. Figure 7 shows a snapshot and Figure 8 the lateral and normal pressures, which in such a system are typically not the same. A surface tension comes from the difference between the lateral and the normal pressures of a system. Therefore, from Fig 8 where the pressure in normal and lateral direction is different and undergoes severe fluctuations, we can conclude that this supported system has a non-zero surface tension.

Comparison of Pressure Coupling Techniques

We will now discuss pressure effects in more detail. The test simulations in this case consist of fully hydrated bilayers with 128 DPPC molecules (64 per leaflet) and 3655 water molecules. The DPPC lipid structure is based on a united atom model where the lipid acyl chain hydrogen atoms are not explicitly represented. The starting configuration for the bilayer (*NPT*) was obtained from the Tieleman group website [40]. The GROMOS87 force field [41] was used for the lipid headgroups and the NERD force field [42] was used for the acyl chain tails. The alcohol structures are also based on a united atom model and therefore only the hydroxyl hydrogen is explicitly represented. The alcohol bonded and non-bonded parameters are from the GROMOS87 force field [41] and the water parameters come from the Simple Point Charge (SPC) model [14]. We compare lipid/ethanol behavior using the *NPT* and *N γ T* ensembles to determine if the ensemble has an impact on the membrane mechanical and structural properties. Two ethanol concentrations (0.85 M and 3.4 M ethanol) and a control system without ethanol are simulated in both ensembles. The *NPT* data have been discussed in detail elsewhere [43]. The ethanol concentration refers to the moles of ethanol initially located in the ethanol/water mixture. To distinguish between the two different ethanol concentrations, we refer to one as having a low concentration and the second as having a high concentration. The high concentration contains 280 ethanol molecules and the low contains 59. The ethanol molecules were added to the bilayer/water system by increasing the simulation box size in the *z* direction and inserting the ethanol molecules into the newly created volume. We concluded that the simulations were equilibrated when the area per lipid had stabilized which happened between 50 and 75 ns. The equilibration phase was followed by an additional 10 ns of simulations for data analysis. The starting configurations for the three *N γ T* simulations were taken from the configurations of the corresponding *NPT* simulations that existed at the end of the data collection period [43]. The center of mass motion of each leaflet was removed every time step during this phase. The time step was 2 fs. Bond lengths were constrained using the LINCS algorithm [44]. The Lennard-Jones interaction cutoff was 1.0 nm with a switch function starting at 0.8 nm. The electrostatics were calculated using the PME method [45] with a short range cutoff of 1.0 nm. The simulations were again performed with the MD package GROMACS 3.2 and some data was evaluated using standard GROMACS tools [35]. The simulations were coupled to a heat bath ($T = 325$ K) using a Berendsen thermostat [36] with a coupling time constant of 0.1 ps. For the *NPT* simulations, the system pressure was maintained at 1.0 bar anisotropically using a Berendsen barostat with a coupling constant of 0.2 ps. For the *N γ T* simulations, the height in the *z*-direction was fixed at the value at the end of the data collection for the *NPT* simulations. A Berendsen barostat was used with a coupling constant of 1.0 in the *xy* (lateral) direction. To determine the lateral pressure, we used equation (2) [46], where the surface tension of the water-lipid interface and the bilayer height are needed. We used a value

of 56 dynes/cm for the interface surface tension as was done by Chiu et al. [46]. The bilayer height depends on the ethanol concentration. The resulting lateral pressures were −82 bar for the control simulation, −87 bar for the 0.85 M ethanol concentration, and −99 bar for the 3.4 M ethanol concentration.

$$\int_{Z1}^{Z2} \frac{P_T(Z)dZ}{Z2 - Z1} = 1 \text{ atm} - \frac{\gamma}{Z2 - Z1} \quad (2)$$

The main phase transition temperature for DPPC is at 314 K [47] and since the simulations were maintained at 325 K, the bilayer was in the fluid phase. In our simulations, the area per lipid is determined by dividing the x and y dimensions of a simulation cell by the number of lipids per leaflet. The average area per lipid for the control system (no alcohol molecules) using *NPT* during the 10 ns data collection period was 0.639 nm². This value is similar to previous values for DPPC, where an experimental measurement found an area per lipid of (0.629 ± 0.013) nm² [48] and a simulation with a different force-field reported 0.630 nm² [49]. The fluctuations in the area per lipid for the simulations are depicted in Figure 9 and show that the area per lipid fluctuates less in *N_γT* simulations than in *NPT* simulations, thus the *N_γT* appears stiffer. However, the difference between the average areas per lipid for the two ensembles is small, with the largest difference between the *N_γT* and *NPT* systems being 0.014 nm² for the high concentration ethanol system. Tieleman and Berendsen also found that bilayers composed of DPPC lipids in the *N_γT* and *NPT* ensembles show similar values for the area per lipid [49]. To determine if the ethanol molecules in the two different ensembles have the same placement relative to the bilayer interface, we examine the density profiles. Figure 10 (Left) shows that there is no significant difference between the *NPT* and *N_γT* DPPC density profiles in the 3.4 M ethanol concentration systems. Similarly, Figure 10 (Right) shows little difference between the locations of the ethanol density peaks in the *N_γT* and *NPT* simulations. To examine the hydrophobic interactions between the ethanol molecules and the lipid acyl chains, we calculate the chain order parameters. In simulations, the order parameter is a useful measurement because it can be compared with the experimental deuterium order parameter, which can be determined through nuclear magnetic resonance spectroscopy measurements. Since the hydrocarbon chain structures are based on the united atom model, hydrogen atoms are not explicitly represented and the C-H bonds are reconstructed assuming tetrahedral geometry of the CH₂ groups. The order parameter is defined as $S_{CD} = 0.5 \langle 3\cos^2 \Theta_{CD} - 1 \rangle$ where Θ_{CD} is the angle between the CD-bond and the bilayer normal in experiments and in simulations the CD-bond is replaced by the CH-bond. The order parameters are defined for carbon atoms C_{*n*−1} through C_{*n*+1} and thus for DPPC, order parameters are calculated for atoms C₂ through C₁₅. Figure 11 shows that the high concentration ethanol simulation for the *NPT* ensemble has a significantly smaller s_{n2} chain order than the control and the low concentration ethanol (*NPT*) simulations. The same trend was seen for the *N_γT* low and high concentration ethanol simulations (not shown). This however is not particularly surprising since the area per lipid for the high concentration ethanol simulation is significantly higher than that of the low ethanol concentration simulations. Figure 11 shows that the *N_γT* control simulation has a higher chain order than that of the *NPT* control simulation, and that the *N_γT* control order parameter more closely matches experimental values than that of the *NPT* [50]. One focus of this work is to examine how the *NPT* and *N_γT* ensembles respond to changes in phase behavior. In the phosphatidylcholine/ alcohol phase diagram, at high alcohol concentrations, a bilayer in the gel state will pass through the interdigitated state before reaching the fluid phase with an increase in temperature [33]. Interdigitation will arise when lipid chains from one bilayer leaflet are found in a region of space normally occupied only by lipid chains from the opposite leaflet. One effect of interdigitation is a decrease in bilayer thickness. Using atomistic simulations, we examine how interdigitation between bilayer leaflets differs in the *NPT* and *N_γT* ensembles. The bilayers were solvated with a 3.4 M concentration ethanol solution and Figure 12 shows that the *NPT*

and NyT upper and lower leaflets show identical density profiles, indicating that the level of interdigitation between the two ensembles is very similar. These density profiles can be compared with that of a control simulation that does not contain alcohol molecules and in Figure 12, we see that the lipids in this simulation show significantly less overlap.

Conclusions

We characterize the phase behavior of a coarse-grained water model [20] through the construction of density versus temperature curves for cooling and heating cycles. We also address the effect of thermal history of water in hydrated bilayer simulations and derive a water model from this which can be used in studies of supported bilayers. The support induces an ordering of the water close to it but does not severely decrease its mobility. We find a coexistence of an ordered and a disordered fluid. Supports also have a strong influence on the density profile of the bilayer. Finally, we compare atomistic simulations using different pressure coupling schemes where we can conclude that the differences between NPT and NyT simulations is only minor. The main difference is that constant surface tension leads to a higher area compressibility modulus.

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Figure 1.
Coarse grained water density at 1 bar for cooling (red) and heating (black) cycles.

Figure 2.
Water structure factor for heating cycles at 265 K, 325 K and 385 K.

Figure 3.

Left: Water diffusion for heating cycle. Long range diffusion at 385 K corresponds to fluid water.

Right: Water MSD for cooling cycles. Low diffusion at 265 K contrasts the higher diffusion at 275 K and 325 K.

Figure 4.

Water snapshots for cooling cycles. Ordered water at 265 K, and fluid water structure at 275 K and 325 K.

Figure 5.

Diffusion of water in contact with biomembranes after quenching. It is evident that water in contact with the bilayer preserves its fluidity at 265 K.

Figure 6.

Left: Density profile of water between two confining plates depending on interaction strength (100% corresponds to the original strength [20])

Right: Density profile of a supported DPPC bilayer separated into leaflets.

Figure 7.
Snapshot of a supported DPPC bilayer using a coarse-grained model.

Figure 8.
Lateral and normal pressure in a supported coarse-grained DPPC system showing the appearance of a surface tension

Fig 9.

The DPPC area per lipid for the two control (both ensembles) and four ethanol simulations. The six simulation systems are DPPC (no ethanol molecules) – *NPT* (black), DPPC (no ethanol molecules) – *NγT* (red), DPPC w/ 0.85 M ethanol concentration – *NPT* (green), DPPC w/ 0.85 M ethanol concentration – *NγT* (blue), DPPC w/ 3.4 M ethanol concentration – *NPT* (orange), DPPC w/ 3.4 M ethanol concentration – *NγT* (brown)

Figure 10.

Left: DPPC density profiles for $N\gamma T$ (red) and NPT (blue) M ethanol concentration systems. Density profiles have been centered at 2.8 nm.

Right: Ethanol molecule density profiles for the $N\gamma T$ 0.85 M (blue) and 3.4 M (green) and NPT 0.85 M (red) and 3.4 M (black) ethanol concentration simulations. Density profiles have been centered at 2.8 nm.

Fig 11.

Order parameter profiles for the DPPC sn_2 chains in NPT and $N\gamma T$ simulations. The five simulation systems in the figure are DPPC (no ethanol molecules) – NPT (red), DPPC (no ethanol molecules) – $N\gamma T$ (black), DPPC w/ 0.85 M ethanol concentration – NPT (green), DPPC w/ 3.4 M ethanol concentration – NPT (blue), DPPC experimental values at 323 K [50] (orange).

Fig 12.

Density profiles for the upper and lower leaflets in the control NPT simulation that included no alcohol molecules (black, red solid), the $N\gamma T$ (green, blue circles) and NPT (blue, pink dashed) 3.4 M concentration ethanol simulations. The figure shows that the 3.4 M concentration systems have higher lipid density in the chain region of the bilayer than the control system. Density profiles have been centered at 2.8 nm.