# THE INFLUENCE OF THE HYDRATION NUMBER IN A MIXED LIPID BILAYER WITH CHOLESTEROL

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### ABSTRACT

The main structural component of biological membranes are phospholipids. A large proportion of the constituent lipids have monounsaturated hydrophobic tails. The study of this type of system is adequate for the development of a number of pharmaceutical substances. Molecular dynamics (MD) is suitable for describing the structure of membranes at the molecular level. The degree of hydration of the molecules is of essential importance for the correct composition of the atomistic models of the lipid bilayers. For this reason, an atomistic MD model of a lipid bilayer composed of 128 SOPC molecules and 128 cholesterol molecules symmetrically placed in both monolayers at a temperature of 273K was constructed. The Slipids force field was used, showing good results in the simulation of lipid systems. Three degrees of hydration corresponding to a hydration number (Hn) of 25, 40 and 50 were studied with two different water models (TIP<sub>3</sub>P and TIP<sub>4</sub>P). The interaction between the lipids and the aqueous phase is described by radial distribution function (RDF) and the number of hydrogen bonds. The basic parameters of the lipid bilayer related to the mobility of the heads such as lateral diffusion coefficient were calculated. It was found that the 25 hydration number resulted in an unrealistic immersion of the lipid heads in the water molecules. The high degree of hydration (50 waters per lipid) gives a better description of water, but there is no significant difference in surface phenomena compared to hydration number 40. The model containing 40 waters per lipid in combination with water model TIP<sub>3</sub>P reproduces experimental data and is suitable for further consideration.

Keywords: membrane simulations, molecular dynamics, unsaturated lipid, cholesterol, water models.

# INTRODUCTION

Lipids build the cell membranes [1, 2], and the layers mainly contain glycerophospholipids [3]. The phosphatidylcholines PC are the most abundant [4] and SOPC (1-stearoyl-2-oleoyl-sn-glycero-3phosphocholine), a (mono)unsaturated lipid is a typical representative. Its properties have been examined in detail experimentally [5] and theoretically [6, 7]. Cholesterol is also an essential component in the structure of membranes. The main property is to magnify the arrangement of lipids in the bilayers [8, 9]. Its molecules are located between the lipid tails and increases the number of trans conformations of the chains [10].

Water models such as TIPS, SPC and TIP<sub>3</sub>P are suitable for studying the properties of liquid water [11 - 13]. With TIP<sub>3</sub>P, by calculating oxygen-oxygen correlation functions, the first hydration shell agrees well with experiment. This model is compatible with lipid bilayers and is widely used in biological simulations [14 - 16]. The TIP<sub>4</sub>P model describes the ice properties very well and shows a good mass density [17 - 19]. Both models give a tetrahedral arrangement of water molecules, but TIP<sub>3</sub>P is not validated with a triplet correlation function [20]. In the development of the Slipids force field (FF), the TIP<sub>3</sub>P water model is used with small modifications in the Lennard - Jones potential [21]. Some FFs also include a TIP<sub>4</sub>P water model [22 - 24], but for lipid bilayers the nonatomic sites are implemented only in the Drude FF [25, 26]. Fluctuating charges force fields (FQ FF) use TIP<sub>4</sub>P and the penetration of water molecules to the center of the membrane is achieved [21]. In this kind of simulations, the time step is 0.5 fs, which is smaller than the commonly used 2 fs for lipid membranes. When simulating lipid bilayers, it is not recommended to use water models other than TIP<sub>3</sub>P, even if they have better parameterization. Charge balance problems and a change in the free energy of solvation [21] are possible.

Of interest are the surface interactions between the aqueous phase and the lipid bilayer. The submergence depth of lipids from the double layer into water, the probability of hydrogen bond formation and the structuring of water molecules around the membrane surface are investigated [27 - 29]. The listed quantitative characteristics depend on the degree of hydration (hydration number - Hn). It is known that full hydration occurs at a value above 25 water molecules per lipid [30]. On the other hand, lower hydration provides a smaller expenditure of computational resources for the coverage of the water phase, but this is of less interest in lipid considerations.

In the present work, a lipid bilayer composed of SOPC lipids and cholesterol in a 1:1 ratio at a relatively low temperature of 273 K is considered. For this reason, it is appropriate to make a comparison between the water model TIP<sub>3</sub>P recommended for biological simulations and TIP<sub>4</sub>P giving good results for ice. Atomistic molecular dynamic simulations are performed and three degrees of hydration were considered: 25, 40 and 50 water molecules per lipid. The main goal of the study is to determine the most appropriate combination of water model/degree of hydration for this type of system.

### EXPERIMENTAL

### Models

Using the CHARMM-GUI tool [31, 32], the initial structure of a lipid bilayer composed of SOPC and cholesterol is generated. The membrane contains 256 molecules - 128 lipids in each monolayer, of which

56 are SOPC and 56 are cholesterol. The size of the box in the lateral direction (x and y) is consistent with the average area per lipid at temperature of 273 K and the given ratio of components [33, 34]. The built-in GROMACS tools are used to add water molecules to the system. In this way, the sharp interface between the lipid and water phases is avoided and good hydration of the lipid heads is achieved. Water molecules reach the first carbon atom of the glycerol ester. Systems with three different hydration numbers (25, 40 and 50 water molecules per lipid) are created, with the corresponding amounts of waters:  $\sim 6\,000 \sim 10\,000 \sim 12\,000$ . Hydration is achieved with two different water models: TIP, P and TIP<sub>4</sub>P. Fig. 1 shows the initial structure of the lipid-water bilayer system, with the solid line showing the thickness of the water phase for the corresponding hydration number. In all six systems, the size of the periodic box is large enough that there is no interaction with the periodic image.

#### Methods

With GROMACS [35, 36] version 2022.4 and the Slipids 2020 force field [37] applied at low temperatures [6, 7], atomistic molecular dynamics simulations are performed with the listed aqueous phase models (TIP<sub>3</sub>P and TIP<sub>4</sub>P). The integrator is a leap-frog [38] with a time step of 2 fs. For the long-range electrostatic interactions, PME [39] is used and for van der Waals a Lennard-Jones potential is applied, both with a cut-off of 1.2 nm. After



Fig. 1. Initial structure of a bilayer composed of SOPC lipid (in gray) and cholesterol (dark yellow) with different thicknesses of the aqueous phase, shown in dark red line for each hydration number.

the minimization of the systems, heating is for 10 ns in the NVT ensemble to temperature of 273 K. The V-rescale thermostat [40] and Berendsen barostat [41] combination is applied. The systems relaxation is 500 ns in the NPT ensemble at 273K and a pressure of 1 bar. A Nose-Hoover thermostat [42, 43] and a Parrinello-Rahman barostat [44, 45] with isotropic pressure scaling are used. Analyzes are performed on the last 50 ns of the trajectories with GROMACS built-in tools. VMD 1.9.4a is used to visualize the studied systems [46].

#### **RESULTS AND DISCUSSION**

Basic characteristics of the lipid bilayers, such as density profiles, are studied for the six bilayer models. Lipid mobility is determined by the lateral diffusion coefficient. The change in the distribution of lipids depending on hydration and the structuring of water near the surface of the membrane is examined. The average number of possible hydrogen bonds between lipids and water molecules is calculated.

# Mass density distribution

The mass density profiles is calculated perpendicular to the membrane surface, in the z direction. Data for all systems are shown in Fig. 2. The submergence depth of the lipid heads in water and the thickness of the bilayer are determined from the plots using the equimolecular dividing surface (EDS). The results are presented in Table 1.

Comparing the data with the two water models, a sharpening of the peak corresponding to cholesterol (blue line -2) in the  $\text{TIP}_4P$  systems is noticed. The lipid profiles also show the formation of more peaks, while

Table 1. Thickness of the lipid bilayer and the submergence depth of the lipids head determined from the mass density profiles of the studied model systems (see Fig. 2).

	Thickness [nm]			Submergence depth [nm]		
Model	25 Hn	40 Hn	50 Hn	25 Hn	40 Hn	50 Hn
TIP <sub>3</sub> P	$4.19\pm0.08$	$4.16\pm0.09$	$4.09\pm0.07$	$1.46\pm0.05$	$1.59\pm0.03$	$1.60\pm0.03$
TIP <sub>4</sub> P	$4.26\pm0.09$	$4.20\pm0.07$	$4.11\pm0.08$	$1.51\pm0.02$	$1.53\pm0.02$	$1.59\pm0.02$



Fig. 2. The mass density profiles in the normal direction of the bilayer for the two water models at the studied hydrations. Dotted line shows equimolecular dividing surface (EDS).

in the TIP<sub>3</sub>P systems the membrane is relatively smooth. The profile of the tails (in red -3) is similar in all six systems with the exception of the one with 40 hydration number in TIP<sub>3</sub>P. The minimum is smooth and wide, i.e. there is no penetration of water molecules in this part of the membrane. The hydration number does not have a significant effect on the general profile of the studied parts of the lipid molecule. There is an increase in water density at 50 hydration number in both water models. With a thicker water layer, the effects of the periodic box are also reduced. The most significant difference is in the interaction of the heads with the water phase.

The determination of the thickness of the lipid layers is by the equimolecular dividing surface defined at 500 kg m<sup>-3</sup> for the aqueous phase [47]. The distance between the two surfaces corresponds to the thickness of the bilayer. The difference between the beginning of the profile of the heads (green line -4) and the EDS gives the submergence depth of the lipid head in water molecules. Calculated data are presented in Table 1 with corresponding standard errors.

The thickness of the lipid bilayer with TIP<sub>2</sub>P is smaller than that of  $TIP_{A}P$ , but both values are close and fall within the experimental range 4 - 6 nm [33]. In the two investigated cases, as the volume of the water layer increases, a slight shrinkage is observed in the membranes. At lower hydration in TIP, P water model, smaller submergence depth is realized, while the values at high hydrations are similar. In TIP<sub>4</sub>P there is a similar weak trend, but the values for head immersion are close. There is no clear dependence in the degree of submergence depth on the water model. According to this indicator, the hydration number has a greater influence. At a temperature of 283K, a thickness of SOPC of 4.08 nm was achieved [48]. A layer thickness of 4.1 nm was obtained from CHARM36 FF simulations [49]. These values are close to those reported in this study and indicate that the models are valid. With the high values of hydration, a realistic immersion of the heads in the water environment is achieved.

#### **Radial distribution functions**

The most probable distances between the phosphorus atoms in the lipid heads are calculated using the radial distribution function (RDF). This gives information about the arrangement of the lipids inside the bilayer. The distances between the phosphorus atom of the hydrophilic head and the oxygen atom of water are also calculated. In this way, it can determine the arrangement of water molecules close to the surface of the membrane. The calculated profiles are shown in Fig. 3.

Three peaks present in the RDF profiles  $(g_{p,p})$  covering the subrange in the lipids for both water models. Systems in TIP<sub>3</sub>P have higher peaks compared to TIP<sub>4</sub>P. An increase in hydration also leads to an increase in peak height. It can be said that in the long range the arrangement of the lipids is weaker and the interaction takes place between close neighbors.

In the RDF profiles  $(g_{p,O})$  of the water with the membrane, there is an increase in the intensity of the first peak with an reduce in the hydration number in both water models. The reverse relation is reported in the previous RDF profiles  $(g_{p,P})$ . At TIP<sub>4</sub>P the height of the peaks is greater. The next two peaks are of comparable intensity and clearly show that the structuring of water near the membrane affects the arrangement of water molecules in the bulk of the water phase. Here again, the degree of hydration of the systems has a greater influence on the organization of molecules around the phase surface than the type of water model used.

#### Hydrogen bonds

The arrangement of water molecules with the membrane can also be described by the hydrogen bonds between the two phases. The average number of bonds formed between the hydroxyl group of the water molecule and the oxygen atom of the hydrophilic head of SOPC are determined. Data are presented in Table 2 with corresponding standard errors.

Naturally, as the number of water molecules increases, so does the number of hydrogen bonds. In TIP<sub>3</sub>P the values for the two higher hydration numbers are close and at 50 waters per lipid there is no practical difference between the two water models. Values in the TIP<sub>4</sub>P model are similar for all three degrees of hydration. In general, more bonds are formed in TIP<sub>4</sub>P, but there is also a water density gradient in these systems. The smallest number of hydrogen bonds are formed at 25 hydration number in TIP<sub>3</sub>P water model. The lowest degree of immersion of the hydrophilic lipid heads in the aqueous phase is also reported in this system (Table 1). The probability of forming hydrogen bonds (the average number) is consistent with the submergence depth values.



Fig. 3. Radial distribution functions for the distances between: the phosphorus atoms (P-P) in SOPC lipids (right); phosphorus atom from SOPC and oxygen atom (P-O) of the water (left) in the three hydrations studied.

Table 2. Average number of hydrogen bonds between the oxygen atom of SOPC lipid and water molecules in all six systems.

Model	25 Hn	40 Hn	50 Hn
TIP <sub>3</sub> P	$42.05\pm0.13$	$58.32\pm0.12$	$59.15\pm0.15$
TIP <sub>4</sub> P	$61.15 \pm 0.12$	$60.26 \pm 0.14$	$59.16 \pm 0.12$

#### Lateral diffusion coefficient

Lipid mobility can be determined by the lateral diffusion coefficient,  $D_L$ . Note that only the movements in the xy direction are taken into account and the movements normal to the surface are excluded. The classical Einstein relation [50] with the root-mean-square (rms) shifted is calculated. Data are plotted in Fig. 4 with standard errors.

There is no clear trend in the dependence of  $D_L$  on the water model in the time intervals.

At a hydration number of 40 waters per lipid, the values for the two models are the closest. At low hydration, the largest difference in  $D_L$  value is reported for the water models. In TIP<sub>3</sub>P as the degree of hydration increases, there is a slight decrease in the mobility of the lipid heads. It can be said that in TIP<sub>4</sub>P model the inverse dependence is present, although the calculated coefficients are close and within the standard error. This trend was also observed in the other investigated parameters of the systems with different amounts of water molecules. For the systems shown, the  $D_L$  data correspond well with experimentally obtained, for similar lipids at low temperatures, values ranging is from 10<sup>-8</sup> cm<sup>2</sup> s<sup>-1</sup> to 10<sup>-11</sup> cm<sup>2</sup> s<sup>-1</sup> [51].



Fig. 4. Lateral diffusion coefficient for SOPC lipid heads in the two water models at the studied hydration numbers.

#### CONCLUSIONS

In the present study, two water models TIP, P and TIP<sub>4</sub>P and different degrees of hydration are analyzed. The results of the lipid bilayers agree well with the experimental data. The influence of the hydration number is greater in the described analyses. There is no significant difference in the results between the two water models in the analyzes performed except for the 25 Hn system in TIP, P. At the high value (40 waters - 50 waters) there is an overlap of the obtained results. 25 waters per lipid hydration number resulted in an unrealistic immersion of the lipid heads, which affects the number of hydrogen bonds. The high degree of hydration (50 waters per lipid) requires more computational resources. The system containing 40 waters per lipid is fully hydrated and there is a good reproduction of the results. This bilayer (40 Hn) in combination with water model TIP, P is suitable for further consideration.

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