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Statistical Thermodynamics of Biomembranes

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Abstract

An overview of the major issues involved in the statistical thermodynamic treatment of phospholipid membranes at the atomistic level is summarized: thermodynamic ensembles, initial configuration (or the physical system being modeled), force field representation as well as the representation of long-range interactions. This is followed by a description of the various ways that the simulated ensembles can be analyzed: area of the lipid, mass density profiles, radial distribution functions (RDFs), water orientation profile, Deuterium order parameter, free energy profiles and void (pore) formation; with particular focus on the results obtained from our recent molecular dynamic (MD) simulations of phospholipids interacting with dimethylsulfoxide (Me₂SO), a commonly used cryoprotective agent (CPA).

Keywords

GROMACS; Dimethylsulfoxide; Lipid Bilayer Structure; Mass Density Profiles; Radial Distribution Function; Water Orientation Profile; Deuterium Order Parameter

Introduction

Understanding the basic principles of biomembranes (lipid bilayers), which govern and mediate various biologically relevant processes, on the microscopic level is one of the great challenges in biology. To investigate the characteristics of the membranes and to obtain the intriguing physicochemical aspects of membranes systems many experiments have been (and are still being) performed [21,52,70,108,117,150,153,186]. Recent development of new algorithms and revolutionary advances in the computational power has permitted large-scale molecular dynamic (MD) simulations of interaction(s) between biomembranes and small non-water polar molecules, as well as simulations of two component mixtures of phospholipid membranes and other natural amphiphiles [3,15,40,41,56,60,61,71,72,89,111,124,144,145,159]. Thus, allowing the development of a combination of computer simulations and experiments to analyze biomembrane properties with an even greater degree of detail [5,17,45,47,87,90,170].

Molecular dynamic (MD) simulations are well suited for detailed analysis of the interactions between lipid bilayers and various small molecules, including water, chemicals, co-enzymes, peptides, oligonucleotides and proteins, as evidenced by the extensive body of published

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literature [1,7,10,14,27,29,36,40,41,45,54,58,59,62,68,73,81–83,92,94,96,97,105,111,115,119,122,125–127,130,131,135,140,141,147,149,150,155,157,158,163,165,179,182,187,188,190]. Briefly, these studies describe the effect of cholesterol [29,40,68,86,126,127,140,141,158,172], of dimethylsulfoxide (Me_2SO) [27,122,157,179,182], of methanol [79,135,139], of ions [54,60,96,119,125,155], of proteins [37,58,76,94,107] and of disaccharides [130,131,163], on lipid bilayers; others describe the permeability coefficients of small organic molecules through lipid bilayers [7–10,103], the water/bilayer interface [14,41,73,104,106,111], the permeation of water across a lipid bilayer [13,30,81,102,188], lipid-DNA complexes [6,36,56], porating electric fields for various lipid bilayers [59,189], as well as the aquaporin-1 water channel in a lipid bilayer [30,58,62,63,73,187]. Clearly, a detailed description of all these studies is beyond the scope of this communication! The primary aim of this article is to describe the various steps involved in characterizing and analyzing a phospholipid membrane interacting with small molecules (like cryoprotective agents, CPAs) using statistical thermodynamics and large scale atomistic-level computer simulations.

Statistical Thermodynamics

Statistical treatment of a system, in general, assumes a large enough sample that the average behavior of the sample is representative of the full-size system. Thus, the statistical thermodynamic description of a system is based on the fact that the behavior of a system can be explained by sampling a collection of states, rather than a single state. A thermodynamic description of a system can use different sets of independent variables, like the number of particles (N), volume (V), energy (E), absolute temperature (T), chemical potential (μ) and pressure (p). Once the independent variables are chosen, other variables are determined by various thermodynamic relations (e.g., the equation of state, Maxwell relations) [16,18,19,48,66,134,151,160]. Each choice of independent variables, defines a different set of such relationships, and corresponding to each choice of independent variables, there is a statistical thermodynamic ensemble, with their respective formalism [11,23,48,88,98,109,110,151].

Choice of Thermodynamic Ensemble

The thermodynamic ensembles most frequently used, include the canonical (N,V,T), micro-canonical (N,V,E), isothermal-isobaric (N,p,T) and grand-canonical (μ,V,T) ensembles and in some cases, the surface tension (γ) can also be included as an additional variable, leading to simulations in the multiphase ensemble (N,p,γ,T) [3,28,44,61,89,149]. The selection of an ensemble is based on the thermodynamic property that is pre-set to the right value or guaranteed to agree with the experimental value. For example, setting V and N constant, the area/headgroup for lipid bilayers can be set to the known experimental value, although this cannot always be expected to result in the correct value (1 atm) for pressure [3,61,89,105]. Conversely, setting p and T constant requires periodic changes in the volume but allows the area of the lipid bilayer to fluctuate [135,149,172,175]. Using a constant μ , necessitates a change in the number of particles via the use of insertions or deletions but helps to rapidly equilibrate the system [37,94,97,113,177].

Use of Computer Simulations

Analytical theories exist for the characterization of ensembles of simple systems; however, complex systems, like the phospholipid membranes, are not amenable to analytical treatment without extreme simplification. Such complex systems, however, are amenable to be modeled in full atomistic detail using computer simulations [3,61,82,83,89,111,149]. If the size of the computational domain is infinite, the results are independent of the thermodynamic ensemble. However, for a finite computational system, the results can differ by an amount that is proportional to $1/N$ [16,26,46,50,89]. Clearly, the larger the number of molecules in the model (N), the better the statistical representation, but the associated computational cost is also higher!

Fortunately, the accuracy of a computer model with limited number of molecules can be increased significantly by the use of periodic boundary conditions, i.e., a basic computational cell containing the system is surrounded by periodic replicas in all three dimensions [38,71,72,144,145,164,176,184].

Currently two major classes of computational methods are used for simulating soft condensed matter like the lipid bilayer membranes: molecular dynamics (MD) and the Monte Carlo (MC) approach. MD is based on the integration of Newton's law of motion [3,50,61,82,83,89,94,111] while MC uses a mathematical technique called the Markov chain [29,31,35,49,100,112,113,115,123,132,152,162,185]. Of these two techniques, MD has proven to be more popular, since it seems to work well and conversely, the few MC studies do not, as yet, seem to offer any significant advantage or ease of use. Additional techniques like Dissipative Particle Dynamics and Coarse-Grained models, still cannot be considered as predictive as all-atom molecular dynamic simulations [3,61,75,91,105,149,183]. Thus, MD simulations have emerged as one of the principal tools in the theoretical study of biological molecules [3,61,67,82,83,89,105,149].

Molecular dynamics is the science of simulating the motion of a large number of particles using Newton's laws of motion. Since, these simulations provide a description of the individual particle motion as a function of time, they can be analyzed in far more detail than actual experiments and generate a detailed atomistic description (and the physical properties) of the system being modeled. An essential requirement for performing these simulations is an *a priori* knowledge of the interaction potentials (force fields) for the particles [34,51,69,99,120,147]. The interaction potentials, although approximate, are under the control of the programmer and consequently, can be refined to represent the physical system being modeled as accurately as possible.

Initial Configuration – Biomembrane as a Lipid Bilayer

The cell membrane is a selectively permeable barrier that separates the intracellular components from the extracellular space and actively control the composition of the intracellular fluid. It contains a wide variety of biomolecules, primarily proteins and phospholipids. Generally, phospholipids (double chain amphiphiles) spontaneously arrange themselves in an aqueous solution into a bilayer, with the hydrophobic tail regions of the lipids orientated away from the water and the hydrophilic heads oriented towards the water, while other phospholipids (single chain amphiphiles) will form a closed sphere, i.e. a micelle [55,104,118,128,156,168]. These lipid bilayers (biomembranes) are routinely utilized in computer simulations as an idealized (and simplified) representation of the complex structure of cell membranes. With lipid simulations becoming more and more widespread, reasonably well-equilibrated initial configurations can be obtained from earlier simulations of the same or similar systems [7,10,14,27–29,31,34,37,38,40,45,47,58,71,72,92,111,129–131,145–147,149]. These can be directly imported using commercially and freely available MD computational software, including GROMACS, AMBER and CHARMM; Appendix 1 lists the major features of some of the commonly used MD software [20,24,25,80,93,114,133,174]. Alternatively, the initial structure of the phospholipid membrane can be constructed or created by placing the required number of lipids in a solution (water) with the appropriate force fields (described below) and to letting the system (lipids and water) equilibrate by running an initial simulation; this will result in the formation of the well known lipid bilayer, lipid heads oriented towards the water and tails away from it [41,101,104,118,128,136,137,156]. If the thermodynamic ensemble pre-selects (or inputs) p and T to a constant value, then the value of the area of the lipid will act as a test of the “equilibration” and to the validity of the initial lipid bilayer. Since the area per lipid of an equilibrated lipid bilayer can be measured from

experiments [5,17,21,52,70,153] and compared with the simulations [14,29,41,68–70,92,97,135,171].

Description of Force Fields

As stated earlier, the generation of an atomistic computer model for a phospholipid bilayer membrane involves the *a priori* representation of inter-molecular energies and/or forces. The most accurate representation obtained using quantum-mechanical techniques (*ab initio* calculations) is still prohibitively expensive [53,57,64,74,78,143,169]. Thus, the most commonly used approach to represent inter-molecular forces and/or energies is through the use of molecular mechanical force fields and treating non-bonded interactions in a pairwise additive manner [3,41,61,65,69,89,111,120,147]. Additionally, the intra-molecular interactions are described with bond stretching and bending as well as torsional terms [3,10,16,61,89,111,147,149].

Molecular mechanical force fields express the energy of the system $E(X_N)$ as a sum of several terms, and calculate the force acting on each atom as the gradient of this energy: $E(X_N) = E_{\text{bond-stretch}} + E_{\text{bond-bend}} + E_{\text{rotate-along-bond}} + E_{\text{non-bonded}}$, where, $E_{\text{bond-stretch}}$, $E_{\text{bond-bend}}$, $E_{\text{rotate-along-bond}}$ represent the intra-molecular energies summed over all bonds, bond angles and torsions, respectively; $E_{\text{bond-stretch}}$ and $E_{\text{bond-bend}}$ are generally represented with harmonic

terms as: $E_{\text{bond-stretch}} = k_{ij}^b (r_{ij} - r_{ij}^0)^2$ and $E_{\text{bond-bend}} = k_{ijk}^a (\alpha_{ijk} - \alpha_{ijk}^0)^2$, where i and j are the atoms forming the bond of length r_{ij} , i , j , and k are the atoms forming the bond angle α_{ijk} , the superscript 0 refers to the equilibrium value and the parameters k^a and k^b are the respective harmonic strengths. In general, the $E_{\text{rotate-along-bond}}$ represents two types of terms: the first term, the contribution of the conformational state of a bond to the energy of the molecules is,

usually, expressed as, $E_{\text{rotate-along-bond, conformational}} = k_{ijkl} [1 + \cos(n_{ijkl}\delta_{ijkl} + \delta_{ijkl}^0)]$, where δ is the torsion angle, the parameter k_{ijkl} represents the strength of the interaction, and the parameters n_{ijkl} and δ_{ijkl}^0 depend on the type of the bond. The other type of $E_{\text{rotate-along-bond}}$ is described as improper torsion and is used to enforce either the chirality of an atom or to keep a bond in a plane (e.g., in the case of an aromatic ring). For an atom k with bonded neighbors i , j , and l , it is a harmonic function of the angle between the planes formed by atoms i , j , and k and by atoms j , k , and l [3,50,61,89].

The $E_{\text{non-bonded}}$ represents the non-bonded energy, summed over all pairs of atoms X_i and X_j , separated by the distance r_{ij} , that are on different molecules or in the same molecule but separated by more than three bonds and includes the contributions of Van der Waals forces and electrostatic interactions as: $E_{\text{non-bonded}}(X_i, X_j) = E_{\text{van-der-waals}} + E_{\text{electrostatic}}$. Typically,

$E_{\text{van-der-waals}} = 4\epsilon \left[\left(\frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}} \right)^6 \right]$ where ϵ_{ij} and σ_{ij} are the so-called Lennard-Jones parameters, representing the depth of the attraction due to dispersion forces and the extent of

exchange repulsion, respectively; and $E_{\text{electrostatic}} = \frac{q_i q_j}{4\pi\epsilon_0 r_{ij}}$ where q_i and q_j are the partial charges assigned to atoms i and j to represent the electrostatic interaction between them. Most force fields assign values for each atom type and obtain ϵ_{ij} and σ_{ij} as a combination of the two using the Lorentz-Berthelot rule: arithmetic mean for σ_{ij} and geometric mean for ϵ_{ij} . Partial charges are either obtained from empirical rules or from *ab initio* calculations, using a fitting procedure that finds partial charges by ensuring the best reproduction of the electric field around a molecule [39,65,77,99,165]. In general, the Lennard-Jones parameters are established independent of the molecule (i.e. specific to the atom), while partial charges are specifically assigned for each molecule [3,24,61,89,111,148,149,174]. Obviously, extreme care should be

taken to utilize force fields that have evolved as a whole. Since mixing terms from various sources will lead to an incorrect result.

Treatment of Long-Range Interactions

Accounting for the interactions between all atoms, even assuming pairwise additive potential, as described above, will require enormous computational resources (proportional to N^2). The computational time can be significantly reduced by treating the interactions between “distant” atoms separately from interactions between pairs of atoms that are “close” to each other. This observation lead to the introduction of a cutoff distance, R_{cutoff} , defined as the distance beyond which all interaction energies are assumed to be zero [3,4,24,85,111,174,178]. Obviously choosing a cutoff distance that is “too short” will lead to significant errors, as the contribution of small but numerous distant pairs can add up to a significant amount [32,129]. In addition, the force discontinuity at the cutoff distance, needs to be eliminated by the introduction of a switching function that continuously changes the function to be cut off to zero over a finite interval [32,149,174].

An alternative option to the use of cutoff distance, is to use a simplified representation of interactions between distant pairs, commonly denoted as Ewald sum [22,33,42,43,84,85,121,129,138,142,178]. For simulating lipid membranes this takes the form of using a formalism to obtain the interaction with simplified forms of all periodic cells, extending to infinity. However, the summation of the electrostatic interactions between a simulation cell and its periodic replicas, is nontrivial: the resulting infinite series is only conditionally convergent [3,61,89]. As a consequence, the final sum depends on the order of summation. Ewald introduced a technique that calculates the dipolar sum as two absolute convergent series, one of them in the reciprocal space, hence, the *nom de guerre* [22,42,43]. The use of Ewald sum has been further facilitated by using the particle mesh Ewald (PME) algorithm that has greatly reduced the associated computational cost and complexity [32,166,178,180].

Statistical Analysis of the Generated Ensemble

A description of the various ways that the simulated ensembles can be analyzed is provided below. To illustrate the analysis, a representative selection of results obtained from recent computer simulations, performed in collaboration with my colleague, Prof. D. Moldovan, and graduate students, D. Pinisetty and R. Alapati, of lipid bilayers interacting with 6 mol% Me₂SO are also shown. A detailed description of our MD simulations is provided elsewhere and, in the interest of brevity, will only be briefly described here [116,135]. We have simulated a Di-Myristoyl-Phosphatidyl-Choline (DMPC) lipid system consisting of 96 lipid molecules (48 lipids in each leaflet) in the presence of 5,422 water molecules (full hydration) and 326 Me₂SO molecules using the GROMACS 3.2.1. The force field parameters for both bonded and non-bonded interactions were taken from Berger et al. [14] while the partial charges were taken from Saiz and Klein [146]. Periodic boundary conditions were applied along the three space dimensions. The pressure was maintained at 1 atm using the semi-isotropic pressure coupling to a Berendsen barostat with a time constant of 1.0 ps [12,136,137]. The height of the simulation box (z direction) and the cross sectional area (xy-plane) was allowed to vary independently of each other, thereby allowing the area of the bilayers and the distance between the interfaces to fluctuate independently [41]. The nonbonded Lennard-Jones interactions were cut-off at a distance of 1.0 nm and the simulation time step was set to 2 fs. Long-range electrostatics were updated every 10 time steps and handled by particle-mesh Ewald (PME) algorithm [42,43,180]. An energy minimization procedure based on the steepest descent algorithm was initially applied to the initial structure prior to the actual MD run. The atomic coordinates were saved every 2ps for analysis and the total simulation time was 40 ns.

Area Per Lipid

The surface area per lipid is one of the most important quantities characterizing a bilayer membrane and is often monitored in simulations to assess whether or not the system has reached the equilibrium during the atomistic simulation [3,41,61,89,135,165]. Fig. 1 shows the time variation of the area per lipid in the DMPC bilayer systems in the presence and absence of Me₂SO over the total simulation time (40 ns). The simulation results show that after an initial transient regime of ~20 ns, the (data not shown). The illustrative figures shown for the mass density profiles, the radial distribution functions, the deuterium order parameters and the water orientation profile are generated by analyzing the simulations between 23 to 27 ns (and represent the equilibrated behavior of the DMPC lipid in the presence of Me₂SO at ~25 ns).

Mass Density Profiles

One of the most important tools in characterizing the average structure of phospholipid membranes at different regions along its normal axis is the mass density profile of various atoms or atomic groups. The calculation of density profiles is quite simple and requires only the counting of the average occurrence of the atoms of interest in different lateral slices of the membrane and then dividing it by the volume of the slice (Figs. 2 and 3). Generally, as shown in Figs. 2 and 3, the shape of the mass density profiles in phospholipid membranes shows that the highest density is near the region of the lipid headgroups while the lowest density part is in the middle region of the chain terminal methyl groups. Physically, the density profiles of an atom or atomic group shows how deeply these atoms or atomic groups can (and have) penetrate into the bilayer. Thus, the density at the center of the membranes is a measure of the ability of the component to penetrate into the center of the bilayer. Additionally, the membrane thickness can be estimated by the distance between the density peaks corresponding to the two headgroup regions. Thus, as shown in Fig. 2, the presence of Me₂SO in the system causes a decrease in the thickness of the phospholipids membrane (as seen by a comparison between the peaks of the two lines corresponding to the lipid density in the presence and absence of Me₂SO). And finally, the comparison of the density distribution of different atoms or atomic groups along the membrane normal axis can also give some indication on the average alignment of various parts of the lipid molecules [7,14,30,106].

Analysis of the Lipid Head Groups and Lipid Tails

The polar part of the lipid molecules along with the water molecules that might have penetrated into the bilayer constitute the dense head group region of the phospholipids membranes [41, 118,173]. Obviously, the intricate interactions between the lipid-lipid and lipid-water are of immense importance in determining the properties of the bilayer membrane [138,168,173]. A description of the headgroup region structure includes the analysis of the lipid headgroups (using radial distribution functions) as well as the structure of the interfacial water (water orientation profiles) while the lipid tails are analyzed via the profile of the methylene group order parameter along the lipid tails (deuterium order parameter).

Radial Distribution Functions (RDFs)

The radial distribution function $g(r)$ is often employed to identify close range ordering of neighboring atoms. In this regard, the radial distribution function, $g(r)$, is defined as: $g(r) = N(r)/4\pi r^2 \rho dr$, where, $N(r)$ is the number of atoms in a spherical shell at distance r and thickness dr from a reference atom, and ρ is the number density taken as the ratio of atoms to the volume of the computing box [3,61,82,83,89,94,111]. The calculations of $g(r)$ from a simulation are performed by sorting the neighbors around each atom or molecule into distance “bins” or histograms. The number of neighbors in each bin is then averaged over the entire simulation. Thus, $g(r)$ measures how atoms organize themselves around each other. Specifically, $g(r)$ gives the probability of finding an atom (or molecule, if simulating a molecular fluid) a distance, r ,

from another atom or molecule compared to the ideal gas distribution. $g(r)$ is thus dimensionless. Higher radial distribution functions (e.g. the triplet radial distribution function) can also be defined but are rarely calculated [3,19,50,89]. The radial distribution function, shown in Fig. 4, shows $g(r)$ is zero for short distances (less than the atomic diameter) and due to the strong repulsive forces. At long distances, $g(r)$, tends to one, indicating that there is no long-range order. In between the peak values represent the increased likelihood of the two molecules being within this distance (for example a $g(r)$ value of 4, means that it is 4 times more likely that two molecules would have this separation than average). Since, RDFs depend on density and temperature, $g(r)$ serves as an indicator of the nature of the phase of the simulated system [29,129,135,145,147,157,163,168]. For example, in regular crystal lattice structures, $g(r)$ will form a sequence of jagged, discrete, unequally spaced columns. If the atoms are vibrating rather than fixed the $g(r)$ will resolve into Gaussians. Note that a primary peak will always occur for any phase at σ_{ij} (the Lennard-Jones parameter representing the extent of exchange repulsion), since this is the most common radius recorded when two particles collide [3,61,89,94,111]. However, secondary peaks indicate that multiple layers of particles are packed together hinting at a crystalline structure [40]. RDFs can also be measured using X-ray diffraction, allowing us to verify/contradict the usefulness of the MD simulations [5,33,47, 51,108,118,168].

Structure of the Interfacial Water (Water Orientation)

The change of the orientation order of the interfacial water molecules along the membrane normal axis can be characterized by the profiles (i.e., the average values obtained in different lateral membrane slices) of appropriately chosen orientation parameters [3,24,27,58,83]. Thus, the water ordering in the vicinity of the bilayer-water interface (hydration layer) can be obtained from studying the mean cosine value, $\langle \cos\theta \rangle$, of the angle between the water dipolar moment

$\bar{\mu}$ and the bilayer normal unit vector $\bar{n}$. That is: $\langle \cos\theta(z) \rangle = \frac{1}{\bar{\mu}(z)} \langle \bar{\mu}(z) \cdot \bar{n} \rangle$, where z is the z -coordinate of the centre of mass of the water molecules. The mean cosine value is obtained by averaging, when the system is in the equilibrium regime, over dipolar orientations of all water molecules present in the system and over a large number of equilibrium states. Fig. 5 shows the results from our MD simulations for the ordering of water in the vicinity of DMPC bilayer systems, with and without Me₂SO. When generating the water orientation profile, the normal unit vector, $\bar{n}$ parallel to the z -axis was considered to have the same orientation for the solvent on both sides of the bilayer. Consequently, by symmetry, the cosine average has opposite sign in the two regions. As seen in Fig. 5, the presence of Me₂SO decreases both the ordering of water molecules in the hydration layer (as evidenced by the decrease in the peak heights) and the separation distance between the centers of the two hydration layers present on both sides of the membrane.

Lipid Tails Order Parameter

Generally, the conformation of the hydrocarbon tails of the lipids is highly disordered, since the membrane is in the lamellar or the liquid crystalline ($L\alpha$) phase. The conformational and orientation order/disorder can be quantified by various quantities, including the tilt angle of the C-C bonds along the hydrocarbon tails and the profile of the methylene group order parameter along the lipid tails [41,52,111,135,171,172]. The latter quantity (viz. the methylene group order parameter or the deuterium order parameter) can also be measured using nuclear magnetic resonance (NMR) spectroscopy, and therefore, can be used to corroborate or contradict the simulation results [21,51,82,90,170,173,186]. The deuterium order parameter, S_{CD} , is related to the components of the order parameter tensor, $S_{\alpha\beta}$, defined as:

$$S_{\alpha\beta} = \frac{1}{2} \langle 3 \cos\theta_\alpha \cos\theta_\beta - \delta_{\alpha\beta} \rangle, \text{ where } a, b = x, y, z \text{ and } \theta_\alpha \text{ is the angle between the molecular } \alpha\text{-}$$

axis and the bilayer normal (z-axis). The brackets “<>” denote a time average over an ensemble of configurations. The molecular axes must be defined separately for each segment of an acyl chain. Usually for the C_n methylene group the $C_{n-1} - C_{n+1}$ direction is taken as z, and the $C_{n-1} - C_n - C_{n+1}$ plane is the yz plane. Since the bilayer is symmetric with respect to the rotation around z-axis (perpendicular to the bilayer), we have $S_{xx} = S_{yy}$ and $S_{xx} + S_{yy} + S_{zz} = 0$. The relevant order parameter is the diagonal element, S_{zz} , which is related to the deuterium

order parameter, S_{CD} , as: $S_{CD} = -\frac{2}{3}S_{xx} - \frac{1}{3}S_{yy}$, or $S_{CD} = \left\langle \frac{3}{2}\cos^2\alpha - \frac{1}{2} \right\rangle$, where α is the angle formed by the C-H bond with the membrane normal. Given the system symmetry we can simply write $S_{CD} = 0.5*S_{zz}$. Noticeable deviation of the obtained S_{zz} values from $-2S_{xx}$ or $-2S_{yy}$ indicates rotational anisotropy along the molecular axis joining two C atoms that are separated by two C-C bonds. The deuterium order parameter, S_{CD} , typically ranges from 0 to 0.5. The value of 0.5 is associated with an all trans conformation state and a value of 0 is associated with the isotropically disordered state. From the simulations the order parameter is calculated separately for all carbon atoms along the acyl chain and is shown in Fig. 6 [40,136,137,173]. The simulation results clearly show that the presence of Me_2SO leads to a substantial decrease of ordering along both lipid tails, with the disordering effect being most pronounced for n being 2 to 7, close to the beginning and the middle of the lipid tails. Additionally, our simulations suggest that the disordering of the carbon atoms along the acyl chain keeps increasing as the concentration of Me_2SO is increased from 3 mol% to 11.3 mol% (data not shown).

Free Energy Profile of Small Molecules and Membrane Permeability

It is possible to analyze the free energy profile (and consequently, obtain the membrane permeability) of small, non-polar (neutral) physiologically important molecules like O_2 , CO_2 , NO , etc [7–10,103,119,155,177]. The profile of their solvation free energy across the membrane is determined by inserting the molecules into the system (particle insertion method) or by the method of constrained particles. The particle insertion method is well suited for the less dense parts of the lipid bilayer while the method of constrained particles is well suited for all parts of the lipid bilayer, but in comparison to the particle insertion method is computationally more expensive. The particle insertion method samples the relevant physical space through random insertions of the pre-selected molecule within the biomembrane and obtains the free energy profile of the molecule as the difference between its chemical potential in the membrane and in the bulk solution. The method of constrained particles constructs a potential of mean force by restricting the molecules to different regions within the biomembrane (and measures the average force, in the direction of the constraint, required to keep the molecule within the proscribed region). When the diffusion constant profile of the solute $D(Z)$ is also determined (e.g., by the force correlation method which relates the time fluctuations in the instantaneous force from the constrained particle method to the local time dependent friction coefficient and to the diffusion constant profile), the experimentally accessible permeability coefficient of the solute, P , can also be calculated using the inhomogeneous solubility-diffusion model of Marrink and Berendsen [103], as:

$$\frac{1}{P} = \int_{z_1}^{z_2} \frac{\exp(A(Z)/k_B T)}{D(Z)} dZ$$
, where $A(Z)$ is the free energy profile along the membrane normal axis, Z ; k_B is the Boltzmann constant [13,102,105,181]. The inhomogeneous solubility-diffusion model is valid as long as the thermodynamic gradients remain small. Given the extremely inhomogeneous and anisotropic nature of the bilayer, the permeation properties with the solubility-diffusion model of Marrink and Berendsen [103] are to be interpreted in a qualitative nature rather than a strictly quantitative result. However, the results of Marrink and Berendsen [103] do suggest that the resistance to the permeation of small molecules across biomembranes is characterized by the presence of four distinct regions: 1) bulk water: the resistance within this region is negligible (high permeability) for most molecules except for hydrophobic substances; 2) inter phase: a region with high permeability but unlike the bulk

phase is characterized by high viscosity and high dielectric constant. This region will only act as a rate limiting step for molecules that exhibit a hydrocarbon/water partition coefficient value of unity; 3) soft polymer/dense alkane: a highly anisotropic region of high density coupled with low solubility of hydrophilic molecule and a largely accessible free volume with an enhanced possibility for the dissolution of small molecules and is of extreme importance in determining the overall permeability of the molecule in the biomembrane; 4) fluid decane/low density alkane: a region characterized by low dielectric constant and low viscosity, with rapid diffusion.

Void (Pore) Formation

We have recently observed spontaneous formation of small hydrophobic pores across the phospholipids membranes at various times during the simulations (~15 ns for DMPC) in the presence of 11.3 mol% of Me₂SO (and also in the presence of 9 mol% of Me₂SO; but not at mol% concentrations lower than this) [116]. As there is no external stress applied to the membranes these hydrophobic pores are, presumably, nucleated by thermal fluctuations (Fig. 7). The initial hydrophobic pores transform into hydrophilic pores and continue to grow at an even higher rate, with the corresponding penetration of water and Me₂SO into the now hydrophilic pore [116]. The detailed mechanism and the energetics involved in the pore nucleation and growth are still subject to investigation. We postulated an extension to a well accepted Lister model of pore formation [95] by accounting for the entropic contribution to the overall driving force for pore nucleation and growth [154,167]. Utilizing this modified model, we were able to show that the membrane may become unstable to pore nucleation and growth even at zero surface tension provided the pore edge line tension, λ , is lower than the threshold value λ^* . The line tensions, λ , of a DMPC bilayer edge, in both water and in Me₂SO-water solutions, were calculated from separate “strip bilayer” simulations [167]. Briefly, ribbon-like (strip) bilayers, consisting of 96 lipids surrounded by water (or by Me₂SO-water) molecules in the normal (x) and one of the lateral (y) directions, were simulated using periodic boundary conditions in all three directions. The positive line tension, λ , was calculated from the diagonal elements P_{ij} of the pressure tensor, according to the relation,

$$\lambda = \frac{1}{2} \left\langle L_x L_y \left[\frac{1}{2} (P_{xx} + P_{yy}) - P_{zz} \right] \right\rangle$$

where, $\langle \dots \rangle$, stands for time averaging [116,167]. The threshold line tension value, λ^* , predicted by this simplified model is temperature and membrane specific, and for DMPC lipid bilayer at T = 300K is, $\lambda^* \approx 5.9$ pN. However, the line tension value for a DMPC bilayer edge in the presence of 11.3 mol% Me₂SO is $\sim 1.62 \pm 1.5$ pN; a value that is well below λ^* , and hence, pores were able to form and grow (as shown in Fig. 7). Our more recent simulations also suggest that the presence of Me₂SO greater than 7 mol% will reduce the line tension below λ^* (and the corresponding barrier for pore creation) and facilitate spontaneous and stable pore nucleation and growth in DMPC lipid bilayers (Fig. 8). From a broader perspective of the cryobiological community, these findings suggest that by using certain chemicals one can find ways of bringing biological membranes from the energy dominated stability regions into states in which hydrophilic pores are entropically favored (and consequently, further our understanding of the transport of molecules into and out of the intracellular spaces, a region of extreme interest to cryobiological applications).

Concluding Remarks

Starting with the initial Alder and Wainwright molecular dynamic studies on the interactions of hard spheres [2], to realistic simulations of water [161] and proteins [107], we now routinely find molecular dynamic simulations of solvated proteins, protein-DNA complexes, as well as lipid systems addressing a variety of issues, including, water channels, thermodynamics of ligand binding and permeation of molecules [10,30,37,58,62,73,76,83,94,104,105,120,147,165]. It is to be expected that as the available computational resources keep evolving and

improving, the ability of computer simulations to model the complex environment of living cells (and membranes) will also continue to improve. Conceivably, in the not too distant future, computer-based simulations might even enable us to understand and predict the behavior of complex living systems that are beyond the reach of our most powerful experimental probes or our most sophisticated theories.

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APPENDIX 1

AMBER (Assisted Model Building using Energy Refinement) is a suite of programs for molecular mechanics and molecular dynamics simulations and was designed primarily for proteins and nucleic acids [24]. Website: <http://ambermd.org/>

BOSS (Biochemical and Organic Simulation System) is a general purpose molecular modeling system that performs molecular mechanics (MM) calculations, Metropolis Monte Carlo (MC) statistical mechanics simulations, and semi-empirical quantum mechanics (QM) calculations [80]. Website: <http://zarbi.chem.yale.edu/software.html>

CHARMM (Chemistry at HARvard, Macromolecular mechanics) is a highly regarded and widely used simulation package and has been developed with a primary focus on the study of molecules of biological interest. CHARMM combines standard minimization and dynamics capabilities with features including free energy perturbation (FEP), correlation analysis and combined quantum, and molecular mechanics (QM/MM) methods [20,99,114]. Website: <http://www.charmm.org/>

GROMACS (GRONingen MOlecular Simulation package) is a versatile and popular package to perform molecular dynamics. It is primarily designed for biochemical molecules like proteins and lipids that have a lot of complicated bonded interactions, and is extremely fast at calculating the non-bonded interactions [174]. <http://www.gromacs.org/>

LAMMPS (Large-scale Atomic/Molecular Massively Parallel Simulator) is a classical molecular dynamics code that is somewhat limited in its ability to interface with visualization software [56]. <http://lammps.sandia.gov/>

NAMD (NAnoscae Molecular Dynamics) is a molecular dynamics program designed to provide high performance simulations for large biological molecular systems. NAMD uses the popular molecular graphics program Visual Molecular Dynamics (VMD) for simulation setup and trajectory analysis, but is also file-compatible with AMBER and CHARMM [133]. <http://www.ks.uiuc.edu/Research/namd/>

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Fig. 1. Time dependence of the area per lipid for DMPC bilayers in the presence of 6.0 mol% water-Me₂SO mixtures. For reference and comparison the temporal evolution in pure water (or 0 mol % Me₂SO) is also included. The system equilibration time is shown as a vertical dotted-dashed line.

Fig. 2. Mass density profiles of DMPC lipid (blue) and water (red) across the whole bilayer in the presence (solid line) and absence (dotted line) of 6.0 mol% Me₂SO. The size of the lipid bilayer in the z-direction is shown on x-axis and mass density is shown on y-axis. The lipid component is divided into the contribution from two separate leaflets with the center of the lipid bilayer system lying at '0' on the x-axis.

Fig. 3.

Mass density profiles across the bilayer. The densities given are the nitrogen, N (brown) and phosphorous, P (green) of the DMPC lipid in the presence (solid line) and absence (dotted line) of 6.0 mol% Me₂SO. The mass density profile of Me₂SO is also shown. The size of the lipid bilayer in the z-direction is shown on x-axis and mass density is shown on y-axis. The center of the lipid bilayer system lies at '0' on the x-axis.

Fig. 4. Radial distribution functions (RDF) between nitrogen, N-N (blue) and phosphorous, P-P (red color) atoms present in the present in the lipid head group in the presence (solid line) and absence (dotted line) of 6.0 mol% Me₂SO. The spherical radius r is shown on x-axis and radius of gyration ($g(r)$) is shown on y-axis.

Fig. 5. Water orientation profiles across the vicinity of the DMPC lipid/water interface in the presence (solid line) and absence (dotted line) of 6.0 mol% Me₂SO. The size of the lipid bilayer in the z-direction is shown on x-axis and mean cosine angle between water dipolar movement and bilayer normal vector is shown on y-axis. The center of the lipid bilayer system lies at '0' on the x-axis.

Fig. 6. Ordering of DMPC lipid's acyl sn-1 chain in the presence (solid line) and absence (dotted line) of 6.0 mol% Me₂SO. The atom number of carbon atoms is shown on x-axis and deuterium order parameter (S_{cd}) is shown on y-axis.

Fig. 7.

Three snapshots of a DMPC bilayer membrane in the presence of 11.3 mol% Me₂SO depicting pore formation due to thermal fluctuations. Both top and selected side view areas of the DMPC bilayer are shown. The left figures show the top view of the DMPC bilayers in which only the lipid molecules are shown as space filling, with red for the head groups and blue for the tails. Water and Me₂SO molecules are not shown in the top views. For clarity in addition to the simulation cell three additional periodic images of the cell are also shown. One can clearly see that after about 10 ns a hydrophobic pore is nucleated in the DMPC bilayer. The pore continues to grow and after about 15 ns becomes a hydrophilic pore filled with water. The right figures depict cross-sectional side views of selected bilayer areas crossing the pore (as indicated in the left figures by the white dashed lines). Both the water and lipid molecules are shown in the side view pictures. The waters are shown as space filling with red (oxygen) and white (hydrogen) and the lipids are shown as licorice. The side view figures clearly indicate that the pore nucleated after 10 ns, is indeed of hydrophobic nature. This is substantiated by the fact that no water molecules seem to penetrate inside this pore. However as the nucleated pore grows and the lipid hydrophilic heads rearrange around its rim this evolves into a hydrophilic pore (see the substantial water penetration inside the pore at $t = 15$ ns).

Fig. 8.

Variation of DMPC lipid bilayer line tension, λ , with the mol% of Me₂SO in the water solution. The temperature ($T = 300$ K) and membrane (DMPC bilayer) specific value of the threshold line tension value, $\lambda^* \approx 5.9$ pN is shown as a dashed-dotted horizontal line. Pores nucleate and growth even at zero surface tension provided the pore edge line tension, λ , is lower than the threshold value λ^* ; this corresponds to a Me₂SO mol% concentration greater than ~7% (shown as the dashed vertical line). DMPC lipid bilayer line tension, λ is shown on the y-axis while the mol% of Me₂SO is shown on the x-axis. Note that the error bars represent the standard deviation(s) in the simulated value(s) of the line tension.