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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 (Me_2SO), a commonly used cryoprotective agent (CPA).

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 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

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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 A 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, $E_{\text{bond-stretch}}$, $E_{\text{bond-bend}}$,