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MINI REVIEW

Towards an Understanding of Complex Biological Membranes from Atomistic Molecular Dynamics Simulations

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Computer simulation has emerged as a powerful tool for studying the structural and functional properties of complex biological membranes. In the last few years, the use of recently developed simulation methodologies and current generation force fields has permitted novel applications of molecular dynamics simulations, which have enhanced our understanding of the different physical processes governing biomembrane structure and dynamics. This review focuses on frontier areas of research with important biomedical applications. We have paid special attention to polyunsaturated lipids, membrane proteins and ion channels, surfactant additives in membranes, and lipid–DNA gene transfer complexes.

KEY WORDS: Biomembranes; membrane proteins; lipid–DNA complexes; lipid–protein interactions; gene therapy; polyunsaturated lipids; surfactants; nicotinic acetylcholine receptor ion channel; peptide bundle; docosahexaenoic fatty acid; fluid lamellar phase.

INTRODUCTION

Biological membranes are sheetlike assemblies of amphipathic molecules that separate cells from their environment and form the boundaries of the different organelles inside the cells [1]. These physical barriers, however, allow a controlled interplay and exchange of material amongst the different parts of the cell and with the external world. Membrane structure and function is accomplished by a complex composition. Biomembranes are, thus, composed of a mixture of lipids, proteins, and carbohydrates. Lipids, in particular, constitute their main component and structural basis. The diversity existent in the lipid composition of cell membranes is a fundamental ingredient. For instance, since cells are usually constrained to an environment where temperature and pressure are fixed, membrane properties can be controlled by varying its composition (lipid type and cholesterol content, for example).

Lipid molecules are also interesting at the fundamental level. These amphiphilic molecules in water display a rich variety of mesophases, which are controlled mainly by temperature and lipid/water composition [1, 2]. Among these structures, the disordered fluid lamellar phase, L_α, is the most relevant in biology. Due to the complexity inherent in the biologically relevant systems, theoreticians, computer

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simulators, and experimentalists have adopted simplified models to aid them to understand the main properties of more complex membranes [1–3]. Pure lipid bilayers in the fluid lamellar phase represent the simplest of these biomembrane models.

Computer simulations provide a unique tool to analyze membrane properties from an atomic perspective and offer a direct connection between the microscopic details of the system and the macroscopic properties of experimental interest [4, 5]. Recent developments in the field of computer simulations of model biomembranes [5] have improved our understanding of the physical mechanisms governing their structure and dynamics. In particular, classical molecular dynamics (MD) simulation studies have been extensively used in the last decade to investigate pure lipid bilayer models [6–14]. Due to the excellent agreement with experiment, computer simulations are being used to probe more complex systems with potential biomedical applications (see, for instance, Refs. [15–19] for recent reviews and references therein). This complexity is achieved by introducing multiple components, such as small amphipathic molecules or other solutes, membrane peptides or proteins, or other biopolymers, into a simple model lipid bilayer. The level of detail available from atomic based computer simulations offers unique possibilities not only for the interpretation of experimental data but also to generate novel insights into crucial issues in membrane biophysics.

In this article, we focus on important complex (multicomponent) membranes from frontier areas of biomedical research. In particular, we consider subjects as diverse as highly unsaturated lipids, membrane peptides and ion channels, surfactant additives in membranes, and lipid–DNA gene transfer complexes. To illustrate each of these topics, we present four examples, which constitute the most recent developments in the field. Specifically, we will discuss the effect of highly polyunsaturated mixed-chain lipids on the membrane biophysical properties, the effect of surfactants on biomembranes, transmembrane proteins functioning as ion channels and their effect of the lipid environment, and the nature of lipid–DNA interactions in the lipid–DNA complexes that may be relevant in gene therapy.

METHODS: MD SIMULATIONS

Classical molecular dynamics simulations consist of the integration of the equations of motion for a many-body system of interacting particles [4, 5]. This method can provide direct information on the structure and dynamics of complex biological systems and a detailed picture of atomic and molecular motions subject to the limitations of classical mechanics and knowledge of the intermolecular interaction potentials (force fields). To handle the latter, effective pair potentials are commonly used, where the many-body interactions are reduced to the computation of interactions between pairs of molecules. Usually, they can be described as nonpolarizable pairwise additive interaction-site models. The parameters in the pairwise additive intermolecular models incoporate some of the nonpairwise additive effects of the surrounding medium and depend to some extent on the thermodynamic state of the system. AMBER [20], CHARMM [21], OPLS [22], and GROMACS [23] are examples of the most commonly used and better tested empirical force-fields. The

interaction sites in these models usually include all-atom descriptions, even though most of them have variants where groups of atoms are considered as single sites (united-atom models). In particular, the molecular and potential model used for the systems considered in this article was the recent version of the CHARMM all-atom force field (CHARMM27) for the different components of the biomembranes: lipids [24–26], nucleic acids [27], and proteins [28]. We used a rigid TIP3P model [29] for the water molecules, which is consistent with the force field chosen for the rest of the components. The intermolecular parts of the force fields are pairwise additive functions, which consist of simple Lennard-Jones plus Coulomb terms, while the intramolecular interactions consisted of bonded potentials (bond stretching, bond bending, torsional motions) and nonbonded potentials (Lennard-Jones and electrostatic interactions for atoms separated by more than two bonds).

In these complex biological systems, which usually are constituted by an increasingly large number of atoms (typically of the order of a few tens of thousands), the evaluation of the long-range electrostatic forces is the most computationally expensive part of the calculation. Recent developments of novel algorithms and the use of parallel machines, however, made these systems suitable for MD simulation studies. In systems with periodic boundary conditions, the long-range contributions are usually computed using the Ewald method. However, for very large systems, such as the one considered here, the Ewald summation is too slow and new algorithms with better scaling behavior have been developed and are currently being used. The particle mesh Ewald method [30], which has been used in the simulations reported in this article, and the fast multipole method (which scales as O(N)) are just a few examples [5]. Further improvement in the speed of the calculations can be achieved by using the so-called RESPA (reversible reference system propagator algorithm) algorithm, when multiple time scales are present in the studied systems [31]. For instance, the use of reversible multiple time step algorithm [31] with a three-stage force decomposition (into intramolecular, short-range, and long-range intermolecular interactions) permits the use of considerably larger time steps in lipid bilayer simulations. The short-range forces are usually computed using a cutoff (of about 10 Å) and methods like the minimum image convention [4, 5].

To further improve the efficiency of the calculations, the motions involving hydrogen atoms of the different molecules are usually constrained to their equilibrium values. The SHAKE/ROLL and RATTLE/ROLL methods [32] have been employed here to use a longer time step in the integration of the classical equations of motion, when those fast degrees of freedom are not expected to be relevant for the properties analyzed.

Since cells are usually constrained to an environment where the ambient conditions are fixed, MD simulation are usually performed at constant temperature and/or pressure with flexible simulation so the system is able to evolve from an initial configuration to its equilibrium state. The precise control of temperature needed in biological systems can be obtained by the use of the Nosé–Hoover chain thermostat [33]. In this case, the MD simulations solve the extended equations of motions, sampling the canonical (in a system with constant volume, temperature, and number of particles) or the isothermal-isobaric (constant pressure, temperature, and number of particles) ensembles [32].

The simulations of the complex biological systems reported in this discussion were performed using the recently developed PINY–MD computational package [34], which includes all the aforementioned new methodologies.

PURE LIPID BILAYERS: LIPIDS WITH HIGHLY UNSTATURATED CHAINS

Fatty acids with multiple unsaturations or double bonds are quite abundant in brain gray matter, synaptic membranes, retinal tissue, and in the olfactory bulb [35–37]. The importance of polyunsaturated lipids, however, seems not to be limited to a mere structural role. In some situations polyunsaturated lipids are needed for the proper function of membrane embedded proteins, such as in the case of the G-protein coupled visual receptor rhodopsin [38]. By modifying the stability of Metarhodospin II (MII) vs. Metarhodospin I (MI), which are intermediate molecular forms of rhodopsin in the cascade that follows the absorption of a photon, the content of docosahexaenoic fatty acid (DHA) has been shown to affect rhodopsin's function [37–41]. Polyunsaturated fatty acids can also modify the activity of receptors by acting as ligands. For instance, the DHA has been recently identified as a ligand for the retinoid X receptor (RXR) in mouse brain, indicating that DHA may influence neural function through the activation of an RXR signaling pathway [42].

In simple model phospholipid bilayers, the presence of (*cis*) double bonds in one or both of the lipid fatty acid chains is known to affect a number of physical properties of the membranes. Examples of these effects are the low main order-disorder phase transition temperatures [43, 44], the enhanced permeability to water and small solutes [45, 46], the enhanced elasticity or decrease in area compressibility modulus [47–49] etc. The microscopic origin of these properties is, however, far from being well understood and although classical molecular dynamics simulation studies have been extensively used to investigate model membranes, earlier studies of water-lipid phosphatidycholine systems in the lamellar phase were restricted to disaturated lipids [3] or lipids or with a low degree of unsaturation [12, 24, 50]. Very recent MD simulation studies of a 1-stearoyl- 2-docosahexaenoyl- sn-glycero- 3-phosphocholine (SDPC, 18:0/22:6ω3 PC) lipid bilayer in the fluid lamellar phase [11, 51, 52] have improved our knowledge of the effect of highly polyunsaturated chains on the structure and dynamics of model membranes. This study was carried out at constant temperature ($T = 30°C$) and pressure ($p = 1$ atm) in a fully hydrated membrane consisting of 14,371 atoms (64 lipids and 1761 water molecules) [11].

In Fig. 1 (panel a), we show a snapshot of the SDPC lipid bilayer at the beginning of the equilibrium period, after a long (1.8 ns) equilibration period. The presence of the polyunsaturated chains (in green), which have the tendency to visit the lipid–water interface, increase the disorder of the membrane [11, 43, 51, 53, 54]. This trend was found also to enhance the interaction of water molecules with the end-carbon-atoms (and in general with the whole chain) of the polyunsaturated tail [11]. The different behavior of the polyunsaturated chains compared to that of the better studied saturated chains is due to a different intramolecular conformation and dynamics [11, 55, 56]. In particular, for the molecular bonds located between two (*cis*) double bonds, the dihedral angle distributions present two symmetric maxima

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at $\pm 120°$, which correspond to the so-called *skew*$^{\pm}$ conformations. In contrast, in saturated chains they can adopt three conformations: *gauche*$^{\pm}$ and *trans*; the latter being the one most energetically favorable. Calculations of the different conformations of the DHA chain [11] indicated that the helical and angle-iron conformations of the region of the polyunsaturated chains comprising three consecutive *cis* double bonds are quite stable for the studied thermodynamic state, representing 66% of the lipids. These conformations allow a relatively tight packing of the chains since consecutive *cis* double bonds are parallelly oriented. Nevertheless, a significant fraction of molecules (34%) with conformations (hairpin and other hairpin-like) where such a tight packing is not possible was observed. This leads to a high degree of inhomogeneity in the system. In fact, the results obtained for the conformations and intramolecular dynamics indicated a broad distribution of projected area per (polyunsaturated) chain and fairly large local fluctuations for transitions between the different molecular conformations.

Calculation of the experimentally measurable (by NMR) orientational order parameter profiles from the MD simulations resulted in values for the polyunsaturated chains significantly lower than those for the saturated chain [11]; in good agreement with experiment [57]. To illustrate these differences, we show in Fig. 2(a) orientational order parameter profiles for the two acyl chains of the SDPC lipid, which are given by, $S_{C\text{-}D}(n) = \frac{1}{2}\langle 3\cos^2\beta_n - 1\rangle$, where β_n is the angle between the orientation of the vector along a C–H bond of the n-th carbon atom of the saturated and/or the polyunsaturated chains and the bilayer normal. One of the advantages of computer simulation is that it allows one to calculate separately the different contributions to the order parameter profiles from the individual lipids with specific conformations, for instance, of the molecular segments comprising three consecutive *cis* double bonds of the polyunsaturated chains located at different positions along the chain [51]. By doing so, one can get insights into the molecular origin of the different theoretical and experimental findings on these complex organized systems. In particular, we plot in Fig. 2(b) the partial order parameter profiles for the saturated chain obtained as a function of the configuration of the region of the polyunsaturated chain close to the headgroup [51]. Figure 2 (b) shows that different polyunsaturated conformations have a distinct effect on the orientational order of their covalently bonded (saturated) chains. Individual conformations of the dihedral angles close to the lipid headgroup produce differences in the lower region of the saturated chain (Fig. 2(b)), in agreement with experimental observations [54]. In this region, the more linear structures (angle-iron and helical) increase the order, whereas the less straight conformations (hairpin and *other*) decrease the order of the saturated chains. Contrarily, the order parameters for the upper part of the saturated chain were only slightly changed as a function of the structure of the polyunsaturated chain.

The tendency of the polyunsaturated chains to visit the lipid–water interface does not seem to strongly modify the interactions amongst lipid headgroups or between lipid headgroups and water molecules at the membrane surface compared with disaturated lipid bilayers [11, 52]. At the interface, which constitutes the most polar part of the membrane, lipid headgroups and water molecules are strongly organized [11, 52]. This organization is not only found along the bilayer normal,

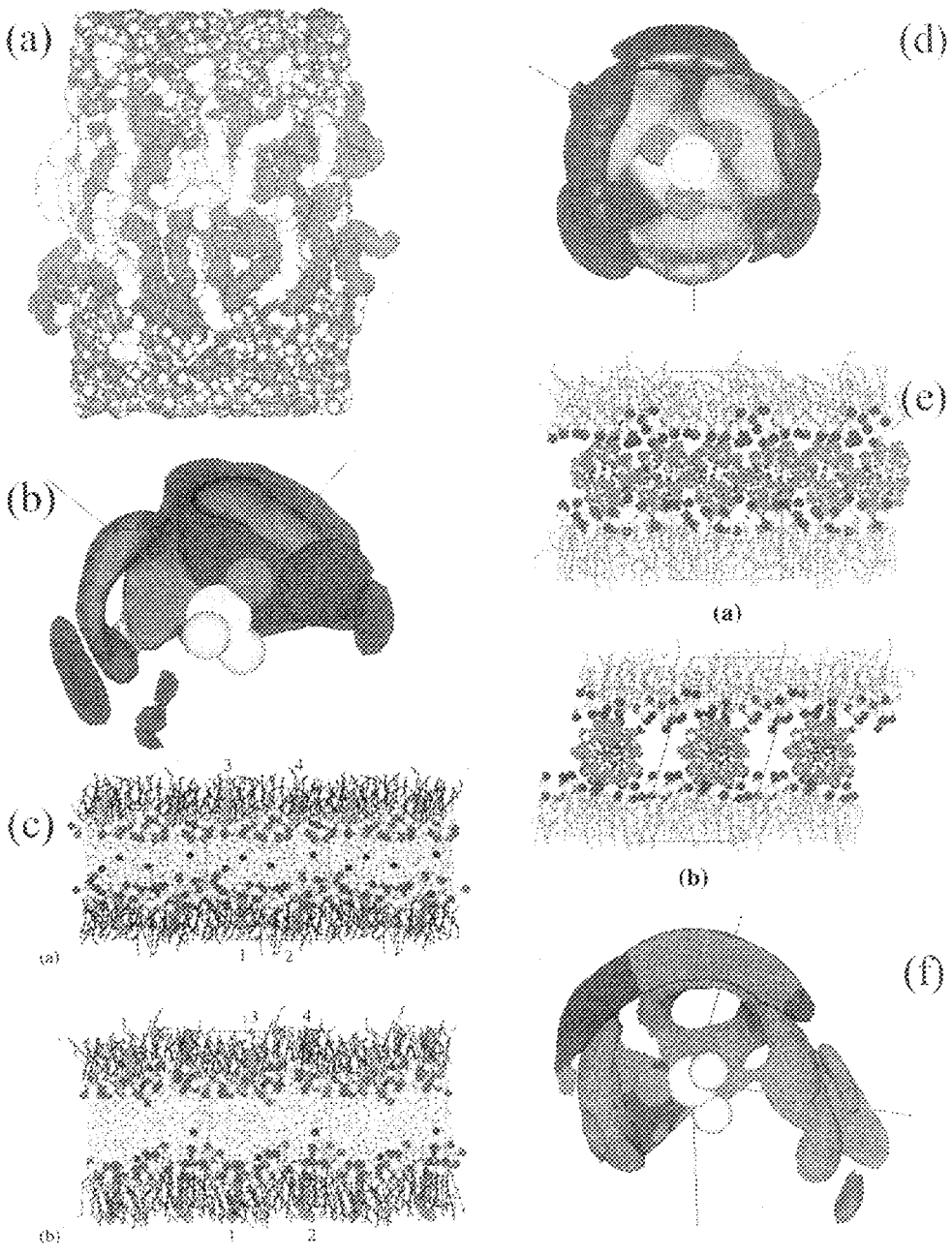
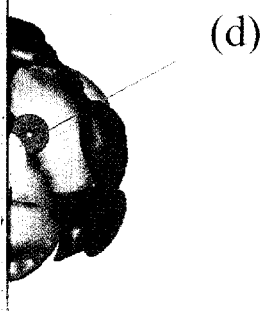


Fig. 1. (panels a–f). Panel a: Configuration of the highly unsaturated model SDPC lipid bilayer system after 2 ns at constant pressure and temperature (isothermal–isobaric ensemble). Only the molecules in the simulation cell are shown. Molecules are depicted by their atomic van der Waals radii, and, for the sake of clarity, the hydrogen atoms of the lipids are not displayed. The color code for the lipids is as follows:



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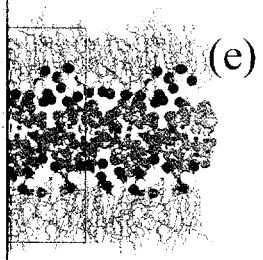

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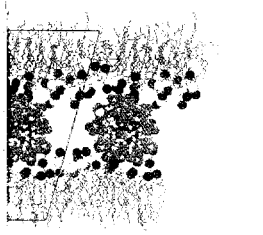

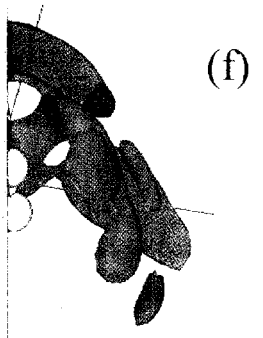

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where the water molecules are polarized to counteract the effect of the SDPC headgroup $P^- \rightarrow N^+$ dipoles, preferentially oriented forming 70° with the bilayer normal. At the interfacial plane, the lipid headgroups form a network of interacting moieties to minimize the electrostatic interactions via $P^- \ldots N^+$ charge pairs [11, 13, 52]. In this network, water molecules interact with the lipid headgroups and even form bridges among them. This behavior is illustrated in the three-dimensional intermolecular distribution (see Fig. 1, panel b) of the oxygen atoms of the water molecules (in red) and nitrogen atoms of the lipids (in blue) around the phosphate groups by the (red) water rings around the non-bonded oxygen atoms (in gray) of the phosphate group. Similar distances are preferentially adopted by those atoms within the same molecule [11], which agrees with the similarities found in the properties for free and nonfree ions in lipid bilayers. Examples of this phenomenon can be found, for instance, in the screening of charges in DNA complexes or planar surfaces of PCs, which leads to forces that does not depend critically on whether the phosphate and counterion are bonded, as in PCs, or not, as in DNA/tetramethylammonium systems [58], or in the equal screening of DNA charges by neutral and charged lipids in DNA/charged-lipid-bilayer complexes [59].

SURFACTANT IN A BIOMEMBRANE

The effect of foreign molecules on phospholipid membranes is a long standing issue in membrane science. Surfactants form an important class of such foreign

nitrogen (blue), phosphorus (yellow), oxygen (red), hydrogen (white), and carbon (gray) atoms. The carbon atoms of the polyunsaturated chains of the lipids are highlighted in green. The color code for the water molecules is as follows: oxygen (blue) and hydrogen (white) atoms.

Panel b: Three-dimensional average intermolecular density isosurfaces of the oxygen atoms of the water molecules (red surface) and the nitrogen atoms of the lipid molecules (blue surface) around the phosphate group of the SDPC molecule. The color code for the phosphate group of the lipid molecules is as follows: phosphorus (yellow), non-bonded oxygen (red), and bonded oxygen (gray) atoms.

Panel c: Snapshots of the configuration of the DMPC/SDS mixed system near the beginning (a), and after 3.2 ns of the simulation. The surfactant molecules and the lipid headgroup P and N atoms are drawn as spheres, while the lipid chain atoms and water molecules are drawn as sticks. The atom coloring scheme is N, blue; P, yellow; S, gray; O, red; C (surfactant), green; C (lipid), black; water molecules, blue; and Na ions, deep blue. The lipid and surfactant H atoms are not drawn. The surfactant DS chains are numbered for reference and the system is replicated once on both sides of the central simulation cell for visual clarity.

Panel d: Average density isosurfaces of the lipid headgroup nitrogen atoms (blue), and the water oxygen atoms (green) around a representative sulfate headgroup of the surfactant chain. The surfactant sulfate group is at the center of the figure, where the central sulfur atom is drawn in yellow, while the anionic and the ester oxygen atoms are drawn as red and gray spheres, respectively.

Panel e: Configuration of the DMPC/DMTAP-DNA complex after 5.5 ns of MD simulation. Two different views are shown: (a) perpendicular to, and (b) along the DNA axis. The DNA and the lipid head group P and N atoms are drawn as spheres, while the lipid chain atoms are drawn as sticks. The atom coloring scheme is N, blue; O, red; P, yellow; C (DNA), gray; C (lipid), green and H, dark gray. The water molecules and the lipid H atoms are not shown and the system is replicated once on both sides of the central simulation cell for visual clarity.

Panel f: Three-dimensional density isosurfaces of the lipid head group nitrogen atoms and the water oxygen atoms around a representative DNA phosphate group. The probability densities of the cationic lipid (DMTAP) and neutral lipid (DMPC) nitrogen atoms are drawn in red and blue, respectively, while the water density surface is drawn in green. The DNA phosphate group is at the center of the figure with the nonbonded and bonded oxygen atoms drawn as red and gray spheres, respectively.

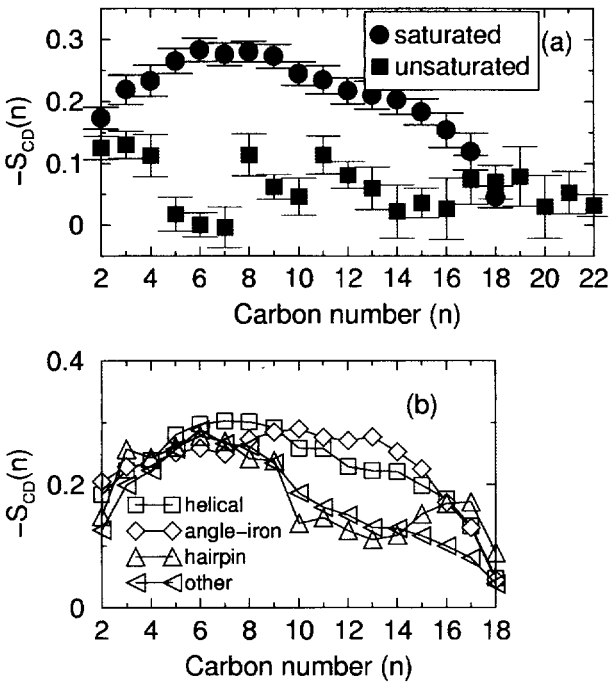

Fig. 2. (a) Orientational order parameter ($S_{CD}(n)$) as a function of the position of the carbon atom along the chains. The result of the saturated (black circles) and polyunsaturated (squares) chains are plotted separately. Error bars represent the standard deviations. (b) Orientational order parameter profiles of the SDPC saturated chains obtained by averaging over different conformations of the region of the polyunsaturated chains close to the headgroups. The lines are visual guides.

molecules. The study of phospholipid/surfactant mixed bilayer systems is important not only for many useful biochemical processes, such as, membrane solubilization [60–62], and protein extraction [63], but also as model systems for understanding crucial issues, such as, the structure and dynamic properties of such complex systems [64–66], and the partition of these foreign molecules in the bilayer matrix [67]. Besides, surfactants, being the major constituents of detergents, are released everyday into the environment, particularly into natural water, Therefore, the questions of how surfactants interact with biomembranes, and influence their properties are central issues for both toxicology and environmental science.

Due to technical limitations, it is difficult to carry out well-controlled experiments on lipid bilayer systems with additives. Klose and coworkers [60, 64–66, 68] have studied in detail the properties of mixed multilayers containing palmitoyloleoylphosphatidylcholine (POPC) lipids and non-ionic surfactants $C_{12}E_n$ (monodecyl ethers of poly(oxyethylene) glycols), using X-ray, neutron diffraction and NMR.

Because of the complex nature of the problem, very little theoretical modeling has been attempted in this area [67, 69]. Atomic based computer simulations can

play a powerful role in elucidating the properties of these systems at a microscopic level, and are therefore considered as natural complements to experiments. However, due to the inherent complexity, there are practically no atomistic simulations of lipid membranes containing long chain additives, such as surfactants. Only recently there were a few attempts at MD studies on phospholipid-surfactant mixtures [70, 71].

We reported long all-atom MD simulations of the lamellar phase of a membrane/surfactant mixture containing dimyristoylphosphatidylcholine (DMPC) lipids and SDS surfactants [70]. The simulation was carried out at 30°C, with a mol fraction of 6.6% surfactant. The system contained 60 lipids, 4 SDS, and 1641 water molecules. The calculated properties of the mixed system were compared with the lamellar phase of pure DMPC lipids, and with available experimental data.

In Fig. 1 (panel c), we show snapshots of the configuration of the mixed system near the beginning and after 3.2 nanoseconds of the simulation. These snapshots showed how the nearly flat interface at the beginning had undergone perturbations due to the presence of the surfactants. Such perturbations indicated strong interactions between the surfactant and lipid headgroups. The location of the surfactant headgroups were obtained from the electron density profiles, as shown in Fig. 3. The overall distribution of the profiles for the lipid in the mixed system (Fig. 3(b)) is similar to that of the pure system (Fig. 3(a)). The headgroups of the surfactant chains were found to be located slightly deeper into the bilayer, near the carbonyl groups of the lipids. To obtain how the negatively charged surfactant headgroups interact with the zwitterionic phosphocholine (PC) groups, local density isosurfaces of the PC headgroup nitrogens and water molecules around the sulfate headgroups, were calculated (Fig. 1, panel d). These distributions reveal strong interactions between the negatively charged surfactant headgroups and the positively charged $N(CH_3)_3^+$ ends of PC groups. It was also shown that the lipid $N(CH_3)_3^+$ groups were bound to the surfactant headgroups either directly or bridged by water molecules. The strong interactions between the surfactant and lipid headgroups lead the $P^- \rightarrow N^+$ dipole vector of the lipid headgroups to reorient toward the interior of the bilayer (as shown in Fig. 4), with $\theta > 90°$), where θ is the angle between $P^- \rightarrow N^+$ vector and the bilayer normal.

MEMBRANE PROTEINS: ION CHANNELS

Ion channels are membrane proteins that regulate the flux of ions across cell membranes [72]. They are present in membranes of all cells and are responsible for the transmission of signals in many processes such as, excitation and electrical signaling in nerve and muscle synapses, detection of sounds and visual images, etc. Even though they play a fundamental role in biology, the structural motifs associated with function are just starting to emerge [72–79].

Since only a few of the structures of these ion channels have been solved with atomic resolution [73, 74, 80], experimentalists have approached this issue by studying minimalistic (simplified) synthetic peptide [81] channels or the sections of the protein that constitute the pore region of large native channels [77, 82–84) to get more insights into their function. These smaller systems, which still retain some or

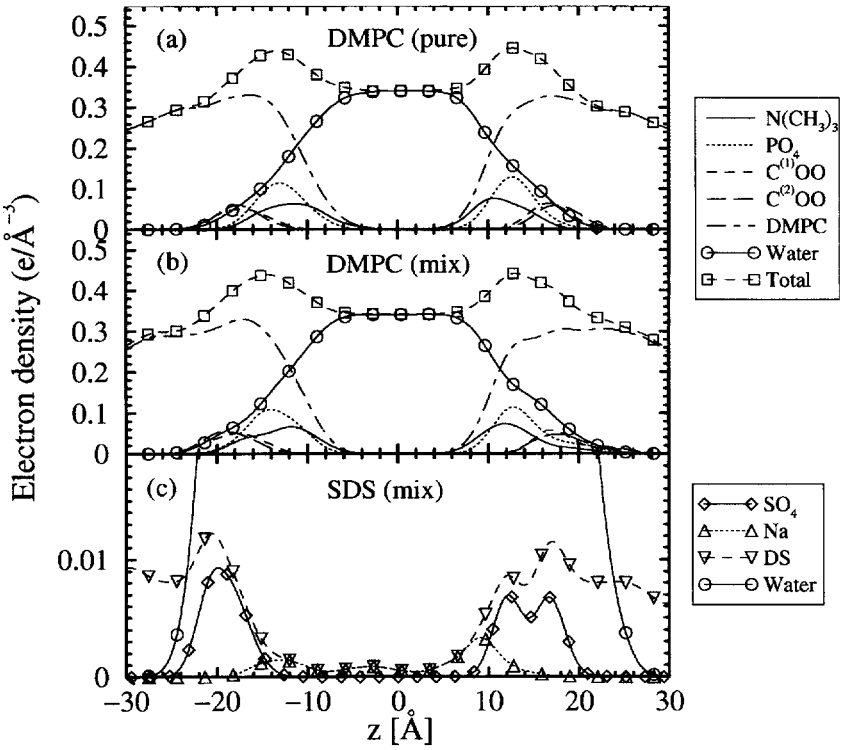

Fig. 3. Electron density profiles measured along the bilayer normal, z, for different components of the DMPC lipid in the pure (a), and in the mixed system (b). The total electron density distributions and that arising from water are also shown. The density profiles of the surfactant headgroups (SO_4), dodecylsulfate chains (DS), and the sodium counterions in the mixed system are shown in (c). The distribution for water is added in (c) for clarity.

most of the functionality of the channels, have been the focus of attention of atomistic molecular dynamics simulations [85–89].

The incorporation of relative large membrane proteins, such as ion channels, in membranes is expected to modify the physical properties of the lipid bilayer. However, due to the fundamental importance of these proteins most of the effort of the experimental and theoretical works has been devoted to the study of the effects of the lipid environment on the structure, dynamics, and function of membrane proteins. Two cases are of particular interest: the effects on the activity of the gramidicin A channel, which due to its simplicity has been extensively studied theoretically and by computer simulations [90–92], and the effect of specific phospholipids, in particular those with unsaturations [11], on the activity of the G-protein coupled visual receptor rhodopsin [39–41]. The key factor in this behavior seems to be the hydrophobic mismatch, i.e., the difference between the hydrophobic length measured perpendicular to the membrane surface of the lipid bilayer and the protein. The membrane curvature seems also to be an important factor, and lipids promoting inverted hexagonal phases have been shown to be fundamental in some systems.

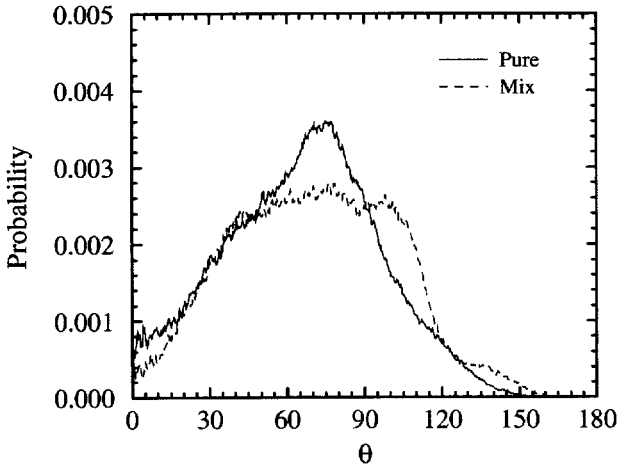

Fig. 4. The orientation distribution of the lipid $P^- \rightarrow N^+$ headgroup dipoles with respect to the bilayer normal, z, for pure DMPC lipids in L_α phase (solid line) and that in the mixture (dashed line).

Recent experiments have been directed to the study of the influence of simple membrane peptides on the membrane properties in an effort to get more insights into the structure and dynamics of model membranes. The effect of incorporating membrane proteins into lipid bilayers changes the phase behavior of the water-lipid system [93, 94] and can even promote nonbilayer phases [93], for instance, single transmembrane peptides decrease the temperature of the phase transition and increase the orientational order of the lipid chains [95]. Other NMR experiments performed on a mostly hydrophobic cationic peptide with two hydrophilic Lys^+ residues at each peptide end showed that the order parameters of the hydrophobic region (membrane interior) were not significantly modified by the peptide in DMPC lipid bilayers nor in DMPC/DMPS (5:1) but that the peptide affects the membrane interface by rotating the headgroup dipoles in a direction away from the membrane plane due to the (sign of the) "bound" surface charges [96]. In these experiments, even though a major influence is expected to occur in the lipids located closer to the peptide, the formation of lipid–protein complexes are usually ruled out at least in the time scale of the NMR experiments ($\approx 10^{-5}$ s) [96]. Systematic NMR and ESR spectroscopy studies using hydrophobic polypeptides of different lengths [93], however, indicate that peptides can induce a hydrophobic mismatch that perturbs the bilayer in a systematic manner (in an effort of the lipids to reduce this mismatch). Differences obtained for peptides with different shapes indicate also that the topology of the peptide surface modulates the effect of hydrophobic mismatch [93, 95].

To investigate the effect of the presence of ion-channel forming peptides on the physical properties of the lipid environment, atomistic MD simulations of the transmembrane homopentameric bundle of the α-helical M2 segments which are believed to form the pore region of the nicotinic acetylcholine receptor (nAChR) ion channel [83, 84] were recently performed [89]. The nAChR is the neurotransmitter-gated ion

channel responsible for the rapid propagation of electrical signals between cells at the nerve-muscle synapse [72]. Previous simulation studies on the nAChR were focused exclusively in protein–protein interactions of simplified models or the effects of α-helices environment on the secondary structure. The peptide bundle was embedded in a fully hydrated DMPC lipid bilayer in the fluid lamellar phase, L_α, at ambient conditions ($T = 303$ K and $p = 1$ atm) with a lipid/peptide molar ratio of 19:1 [89]. The M2 segments are characterized by the sequence GSEKMSTAISVLLAQAVFLLLTSQR and correspond to the δ subunit of the native nAChR ion channel of *Rattus norvegicus* [84].

In Fig. 5, we show a snapshot of the simulated system after 2 ns. Preliminary results [97] indicate that the main effect of the incorporation of the ion channel into the DMPC lipid bilayer consists of an increase of the bilayer thickness and of the orientational order of the DMPC lipid acyl chains. Other effects include: perturbation of the orientation of the lipid headgroup $P^- \rightarrow N^+$ dipoles at the membrane

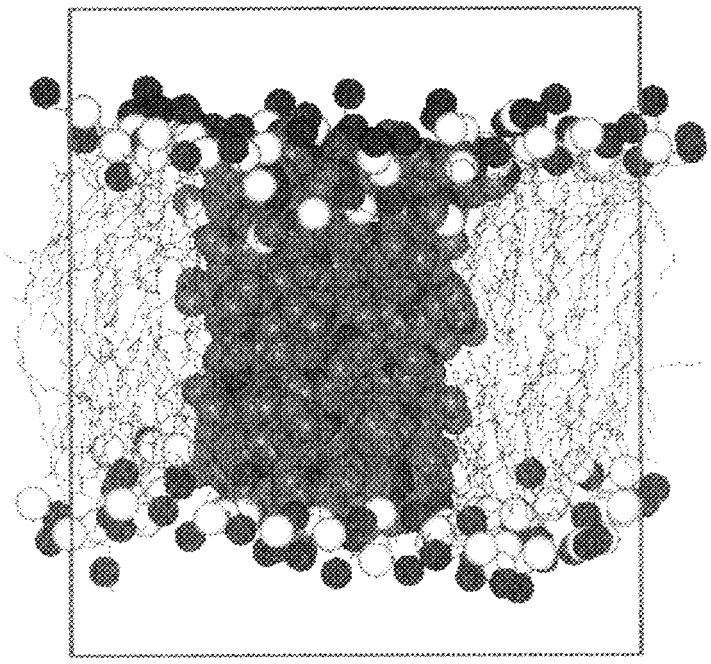

Fig. 5. Configuration of the pentameric bundle of the M2 segments, forming the pore of nicotinic acetylcholine receptor ion channel, in a DMPC lipid bilayer after 2 ns. The lipid molecules are shown as balls and sticks for clarity except the nitrogen and phosphorus atoms of the headgroups displayed as black and light gray spheres, respectively. The water molecules and hydrogen atoms are not shown for clarity. The M2 helices are shown in dark gray, except the sulfur (light gray) and nitrogen (black) atoms. The radii of the spheres correspond to the atomic van der Waals radii of the different species. The C-terminus [synaptic or extracellular, see Ref. 84] is located at the bottom of the bundle.

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interface; and a change in the analyzed properties as a function of the distance of the lipid from the bundle center of mass. The behavior of the lipids located at the two different leaflets differed. Some of these effects seem to be associated with the formation of lipid–protein complexes. Lipid–peptide interactions, mainly via Lys^+, which is located at the external surface of the peptides, with the lipid phosphate groups, have also been observed.

LIPID–DNA COMPLEXES

Human gene transfer is an important clinical strategy in which a segment of extracellular DNA is transferred to the nucleus of cells to replace or add genes [98–100]. Gene transfer involves the delivery of a cassette made up of one or more genes and the sequences controlling their expression to target cells. At present, the most common method of gene delivery uses viral-based carriers of DNA [98, 101, 102]. It has been known for some time that binary mixtures of suitable cationic and neutral lipids can form stable complexes with DNA and hence can potentially be used as synthetic carriers of DNA [103]. Recently, the study of lipid–DNA complexes has received considerable attention [99–102, 104–116]. X-ray studies by Safinya *et al.* [104, 105] discovered that mixtures containing the unsaturated neutral DOPC (dioleoylphosphatidylcholine) and the cationic DOTAP (dioleoyltrimethylammonium propane) lipids form a novel multilayer structure with alternating lipid bilayers and DNA monolayers, in which the DNA chains form a two-dimensional smectic phase, intercalculated between lipid bilayers. Rädler and coworkers [106, 107] used binary mixtures of the saturated neutral and cationic lipid DMPC and DMTAP to complex with DNA. These intercalated complexes exist in two lamellar phases, the gel phase (L_β^c) at low temperatures and the fluid-like lamellar phase (L_α^c) at higher temperatures.

Due to the lack of understanding of the lipid–DNA complexes, and the nature of interactions between the DNA and the lipids, the development of synthetic non-viral carriers remains preliminary. Few theoretical calculations have been carried out that help rationalize the observed structures of the lipid–DNA complexes [111–115]. Atomistic based MD simulations can play an important role in elucidating the structural and other properties of such systems. However, due to the complex nature of the problem, no atomistic computer simulation study has been attempted so far to study such systems. Only recently, we performed an all-atom MD simulations of lipid–DNA complexes containing DMPC and DMTAP lipids [59]. This simulation was carried out in the liquid crystalline L_α^c phase of the complex ($T = 50°C$). The simulation was carried out at an isoelectric point containing 24 DMPC, 20 DMTAP, 1003 water molecules, and a DNA duplex $d(CCAACGTTG)_2$. The details of the system setup and simulation details can be found elsewhere [59].

Figure 1 (panel e) shows the configuration of the system after a long 5.5 nanoseconds of simulation. The most interesting and important feature to note from Fig. 1 is the structure of the lipid–DNA interface. It is clear that significant undulations developed near the lipid head group region of the interface as compared to the nearly flat interface of the lipid bilayer.

Calculation of the three-dimensional local density isosurfaces of the lipid head group nitrogen atoms as well as the water molecules around the DNA phosphate groups (Fig. 1, panel f) reveal that a large fraction of the cationic lipids, are bound to the DNA phosphate groups either directly or bridged by water molecules. However, the most interesting feature was the significant population of the zwitterionic PC head group nitrogens of DMPC in proximity to a DNA phosphate group. Our results predicted the existence of TAP and PC groups with about equal probability around the DNA phosphates. From a similar estimation, we found that there are roughly 2.5 water molecules per non-bonded DNA phosphate oxygen atom within a typical nearest neighbor distance of ~3.3 Å, which confirms the existence of water molecules bridged between the lipid head groups and the DNA phosphates.

The reason behind such an apparently surprising distribution as shown in Fig. 1 (panel f) is the attractive electrostatic interaction between the cationic TAP head group and the anionic phosphate of the zwitterionic PC head group of DMPC. In Fig. 6 we show a snapshot from the simulation illustrating the lipid–lipid and lipid–DNA contacts (salt bridges). For clarity, we have shown only the N^+ and P^- atoms of the PC and N^+ atoms of the TAP groups corresponding to one monolayer and the phosphate groups of the DNA, which are close to that layer. Figure 6 clearly shows the existence of three distinct types of contact bridges as marked by circular regions. *Region 1*, shows a P^-–N^+–P^-N^+ type of bridge between the PC groups, typical of a pure lipid bilayer structure [13]. Similar bridged configurations are identified in *Region 2*, were the N^+ of the PC group is replaced by the cationic N^+ of the TAP group. The existence of such configurations unambiguously demonstrates the presence of strong electrostatic interactions between the PC and TAP head groups. This leads to the formation of contact pairs of DMPC-DMTAP lipids with the P^- end of the P^-–N^+ dipole approximately at the same distance (~4.5 Å) from the PC head group N^+ as from the N^+ of the TAP group. Such interactions also induce a change in orientation of the P^-–N^+ head group dipoles of DMPC with the N^+ part pointing away from the bilayer plane and hence coming into more effective contract with the anionic DNA phosphate, as illustrated in *Region 3* of the snapshot. The explicit arrangements of the atoms in these three regions are shown in the insets of Fig. 6.

This study showed that the current generation of simulation methodologies and force fields have the ability to offer valuable insights into such complex systems with potential biomedical applications.

PERSPECTIVES

The examples considered in the previous sections illustrate the current ability of computer simulations to describe model membranes of a considerable degree of complexity. The membranes of cells are, however, composed of an heterogeneous mixture of various components and one would like also to learn about their lateral organization. Recently, there have been a few attempts to study model systems with a mixture of lipids [117, 59] and special attention has been devoted to cholesterol [118–124] and its role in the formation of microdomains [125]. This is particularly

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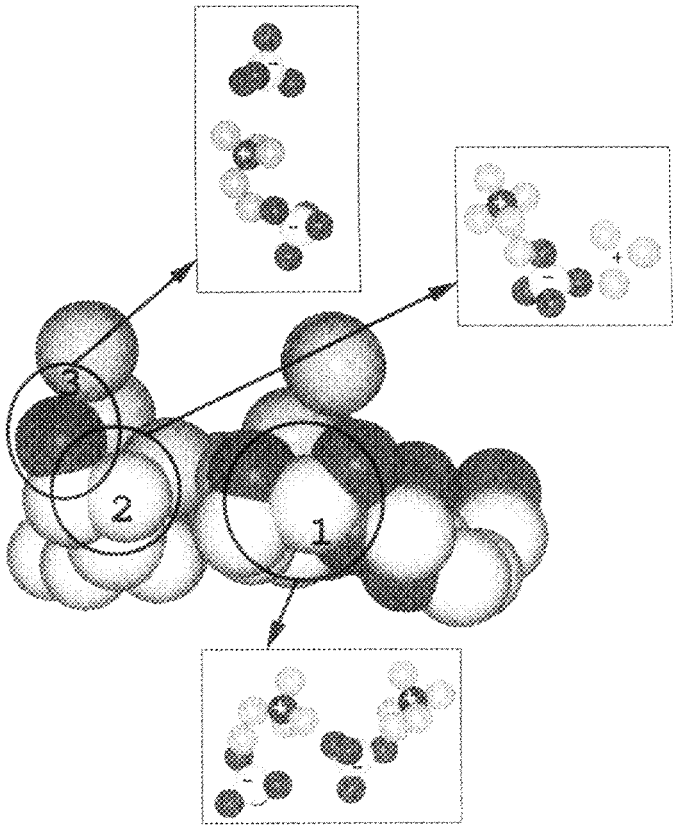

Fig. 6. A snapshot from the simulation illustrating how $N(CH_3)_3^+$ (black) and PO_4^- (light gray) of the zwitterionic PC head groups and $N(CH_3)_3^+$ of the TAP groups interact with the phosphate groups (dark gray) of the DNA. *Region 1* shows P^-–N^+–P^-–N^+ contacts between the PC groups. *Region 2* shows substitution of N^+ of a PC group by the N^+ of a TAP group, while *Region 3* demonstrates how such substitution leads to change in orientation of the PC group, bringing it into close proximity with the DNA phosphate. For clarity, the spheres are drawn with artificially enhanced radii of 3.6 Å (N) and 3.9 Å (P). The insets show the explicit arrangement of the atoms in the PC and TAP groups as well as the DNA phosphate group within the three regions. Hydrogen atoms and water molecules are omitted for visual clarity.

relevant since cholesterol seems to be crucial in the sorting of membrane heterogeneities. One example is the formation of the so-called lipid rafts [126] in plasma membranes, which act as platforms of adhesion and signaling. Even though this kind of model is still too complex for the present capabilities of atomistic scale computer simulations, the properties of mixed lipid bilayers can help understand the lateral organization of these membrane heterogeneities. An instance of the current efforts towards the study of such multicomponent systems with atomistic detail is

the modeling of human bile, which consists of a mixture of phospholipids, bile salts, and cholesterol, and forms micelles under supersaturated conditions [127].

Lipid-protein and protein–protein interactions in membrane environments play a fundamental role not only in the folding of natural proteins or association of synthetic peptides but also in the mechanisms of action of toxins and antimicrobial peptides or other peptides with lytic activity, which are lethal to the cells. Peptide aggregation in membranes and the formation of pores is, thus, becoming a very challenging problem, for both computer and experimental studies. Recent experiments using novel X-ray methods to study peptide aggregation (at low concentrations) and its role in pore formation provide structural evidence that self-association of amphipathic helices at the membrane surface may be the crucial initial step toward bilayer destabilization and, consequently, the formation of pores [128]. The two state model proposed recently for the action of antimicrobial peptides [129], in contrast, reviews the latest experimental literature in the field and found no indication of helix association of peptides at the membrane interface for the intermediate state between a regime (at low lipid/peptide concentration) where the peptide adsorption at the interface occurs and a regime (at high lipid/peptide concentration) where the pores are formed. Similarly, in membrane protein folding, a reasonable scenario is the two stage model [130]. In a first stage, the helical regions of the protein or model (synthetic) peptides insert in the bilayer, and then oligomerization takes place. However, it is worth emphasizing that this thermodynamic model is just one possibility (reviewed in Ref. [131]) and that the process of insertion and assembly are far from being well understood.

Recent computer simulations demonstrate that processes such as spontaneous aggregation of phospholipids into bilayers or collective phenomena, such as the appearance of spontaneous undulations, can be followed and studied with atomistic detail. In particular, Marrink *et al.* [132] followed the formation of a DPPC lipid bilayer from a random configuration of lipids during 10–100 ns and identified the reduction and subsequent disappearance of transmembrane water pores as the rate-limiting process of the aggregation. The extension of the size of the system to lengths larger than the thickness of the bilayer permits the development of spontaneous undulations and, thus, the study of collective phenomena. Bending modes have been extensively studied and a spectral decomposition of the mesoscopic undulations and thickness fluctuation modes (into peristaltic, undulatory, and protrusions) was performed [133] and mesoscopic properties, such as the bilayer bending modulus, have been accurately calculated. Simulations show that the membrane properties in the long wavelength limit can be reproduced by continuum models. These two examples are illustrative of the latest achievement on time and length scales available for molecular dynamics simulations. These mesoscopic regimes reached nowadays by atomistic simulations were only available to simplified models. Among these models, those of the coarse-grain type [134, 135], for instance, which are adjusted to mimic a specific system instead of a general phenomenon, are still useful since they are significantly more efficient than atomistic models to study collective phenomena.

There are many issues from a more theoretical/methodological point of view that should be addressed, even though it is clear that most of the effort is being devoted to the modeling of more complex (multicomponent) systems. The main

concern is the ability of computer simulations to reproduce experiments and experimental conditions. Due to the finite size of the computer simulations, the study of system size on the physical properties of the model membranes has always been an important topic [6, 17, 133, 136, 137]. The extension of temporal and spatial scales up to mesoscopic regimes has permitted the further evaluation of finite-size effects. In the case of the surface tension, for instance, earlier simulations [6] indicated that the surface tension decreases with increasing size of the systems. Surface tension, thus, depends strongly on surface area and a decrease in the surface compressibility has been observed due to undulatory modes [133, 136]. This effect, however, appears to depend on the stress conditions [136]. Even in very simple model systems large deviations are present between literature values for structural quantities, such as the surface area per lipid, due to the presence of fluctuations, inherent in disordered lamellar phases. Recently, these structural parameters and material properties are being updated, reviewed, and adjusted, and are becoming available [48, 138].

CONCLUSION

The past decade has yielded revolutionary advances in computer hardware technology along with concomitant development of state-of-the-art simulation methodologies. These two factors have enabled the successful application of computer simulation methods in general, and molecular dynamics (MD) in particular, in frontier areas of biomedical research. In this article, we reviewed recent progress that has been made in computer simulation studies of model biomembranes and other important membrane related structures. We discussed several examples where, in the recent past, large scale atomistic MD techniques have been successfully employed. These studies include highly polyunsaturated lipid bilayers, membrane proteins forming ion-channels, effects of surfactant impurities on biomembranes, and lipid–DNA gene transfer complexes. The results obtained from all these studies were in excellent agreement with experiments. This clearly indicates that MD simulations can provide valuable insights in elucidating the structural, dynamical, and functional properties of such complex systems and can act as powerful complements to real experiments and much less detailed theoretical models. The successes achieved in the past decade indicate that the use of atomistic computer simulations as a means of probing even more complex biological systems will inevitably continue.

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