Modeling lipid membranes

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Abstract

Modeling of membranes has become over the past fifteen years an emerging field that has benefited from developments on both the hardware and the software fronts. Large computer simulations, in particular molecular dynamics simulations are now able to provide novel insights into the structure and the dynamics of extremely complex, three–dimensional assemblies like lipid bilayers, thereby serving as an additional, complementary source of information to the current arsenal of experimental tools. An overview of the simulations performed in recent years to improve our understanding of the function of lipid membranes is presented. Successes, failures and limitations of the current methodology are discussed through a series of illustrative case examples. Last, a glimpse into new directions and future prospects is outlined, emphasizing on alternative approaches for modeling the complexity of the lipid environment.
1. Introduction

Membranes consist of an assembly of a wide variety of lipids, proteins and carbohydrates that self-organize to assume a host of biological functions in the cell machinery, like the passive and active transport of matter, the capture and storage of energy, the control of the ionic balance, or the intercellular recognition and signalling. In essence, membranes act as walls that delimit the interior of the cell from the outside environment, preventing the free translocation of small molecules from one side to the other. At an atomic level, knowledge of both the structure and the dynamics of membranes remains to a large extent fragmentary, on account of the remarkable fluidity of these systems under physiological conditions. As a result, the amount of experimental information that can be interpreted directly in terms of positions and motions is still rather limited.

A method that could provide the atomic detail of lipid bilayers, that is often inaccessible to conventional experimental techniques would, therefore, be extremely valuable for improving our understanding of how membranes function. It would further constitute a bridge between observations at the macroscopic and the microscopic levels, and possibly reconcile the two views. Atomic simulations, in general, and molecular dynamics (MD) simulations, in particular, have proven to be an effective approach for investigating lipid aggregates, providing new insights into both the structure and the dynamics of these systems.

Basic structural characteristics of the membrane are determined by the nature of the lipids, and how the latter self-organize into complex three-dimensional arrangements, exposing their polar head groups to the aqueous environment, while protecting the aliphatic domain to form the hydrophobic core. Atomic simulations have developed over the past two decades to such an extent that it possible to model with the desired accuracy these structural features.

Statistical simulations rely on models that have undeniably improved over the years, getting inexorably closer to the chemical, physical and biological reality of the systems investigated. Yet, they remain models, subject to a host of underlying approximations. It is, therefore, necessary to confront as systematically as possible the results of numerical simulations to the experimental data available. Only when the models have proven to have reached the appropriate robustness and reliability, can they serve as an explanatory, possibly predictive tool, capable of (i) rationalizing experimental findings, (ii) providing additional insights into experimentally observed phenomena, and (iii) suggesting new experiments. In the particular case of water–lipid assemblies, there is a considerable wealth of experimental information that can potentially be used to support or contradict in silico studies, albeit immediate confrontation often turns out to be rather cumbersome.

Modeling biological membranes raises a number of difficulties, that still have not found a satisfactory solution. A lipid bilayer is, in essence, a disordered liquid crystal of virtually infinite extent. Truncation of this system into a finite-size patch, to comply with the current limitations of molecular simulations, de facto rubs out significant ranges of the wavelength spectrum that corresponds,
for instance, to bending and splay motions. Current limitations in the available computational resources not only impose restrictions on the size of the system, but also on the time–scales explored. *In silico* experiments, like MD simulations, nonetheless, represent a powerful tool which is able to offer new insights into the structural and dynamical properties of lipid bilayers.

This chapter is aimed at introducing this field to non–specialists, yet providing the necessary guidance for setting up and understanding statistical simulations of lipid–water assemblies, together with key–references for further reading. Up–to–date comprehensive reviews on modeling membranes can be found elsewhere. After outlining the properties that govern self–organization, and the type of structural information accessible from experiment, the methodologies utilized to model these systems are described. Next, examples of atomic simulations of lipid bilayers are presented emphasizing how the results can be compared to experiment. Last, selected simulations of more complex membrane assemblies are described and discussed critically, with a glimpse into the future of this very promising research area.

2. lipid–water assemblies

2.1. What are the factors that determine the morphology ?

By and large, lipids and surfactants are amphipathic chemical species formed, roughly speaking, by a hydrophilic head group and a hydrophobic, alkyl tail. As a function of the chemical specie, this non–polar tail may be constituted by one or two aliphatic chains, either saturated or unsaturated. In the case of phospholipids, the head group usually consists of a phosphate group bonded to a variety of functional moieties, like a choline, an ethanolamine, a serine, or a glycerol fragment. Depending upon the type of fragment, the lipid is either charged — *e.g.* dimyristoylphosphatidylglycerol (DMPG), or neutral — *e.g.* dimyristoylphosphatidylcholine (DMPC). At the so–called *sn*–3 position, the phosphate group is attached to a glycerol hydroxyl moiety, the two remaining hydroxyl moieties being connected to aliphatic chains by means of ester linkages at position *sn*–1 and *sn*–2.

At low concentrations, lipids or surfactants in an aqueous medium usually remain in a monomeric state. Beyond the critical micelle concentration (CMC), they self–assemble into a wide variety of unique three–dimensional structures, that encompass micelles, inverse micelles, bilayers, hexagonal tubular phases and more complicated bicontinuous labyrinths (see Figure 1). The nature of the lipid determines the morphology of the three–dimensional arrangement. In water, lipids aggregate in such a fashion that the polar head group be hydrated adequately, while protecting the alkyl chains from exposure towards the aqueous environment. As a consequence, the cross–sections of both the head group and the chains dictate the morphology of the resulting lipid–water assembly. For instance, lipids featuring a large head group and a single alkyl chain usually form
direct micelles, whereas lipids characterized by a smaller head group and possibly two alkyl chains tend to self-organize into inverse micelles.\(^1\)

For lipids forming planar bilayer assemblies, the net charge borne by the head group plays a noteworthy role in the self-organization process. Small, charged head groups show an interesting tendency to associate by means of intermolecular hydrogen bonds, resulting in compact structures with a small surface area per lipid — e.g. dilaureylphosphatidylethanolamine (DLPE).\(^{13}\) In larger, zwitterionic lipids, like phosphatidylcholine, lipid head groups are organized in inter and intra molecular charge pairs between the oppositely charged choline and phosphate groups.\(^{14}\)

![Figure 1: Polymorphism of lipid–water assemblies.](image)

Aside from the nature of the lipid, external conditions, like the concentration, the temperature, the pressure or the ionic strength of the solvent, strongly influence self-organization into a particular structure. Extensive variables, for instance, can be used to control the transition between phases. At low temperatures, lipid bilayers remain in the gel, L\(_{\beta'}\), phase, wherein the alkyl chains, mostly in an all-trans conformation, are well ordered and exhibit a reduced mobility. At higher temperatures, the gel phase transforms into a liquid crystal, L\(_{\alpha}\), phase characterized by an increase of the surface area per lipid and a decrease of the thickness of the bilayer, as a direct consequence of the “melting” of the participating alkyl chains. The transition temperature, depends on the chemical nature of the lipid. For instance, it increases with the length of the alkyl chains, but it decreases with the number of unsaturations.

Most cell membranes in vivo exist in the fluid, liquid crystal phase, barring a few cases, e.g. stratum corneum specialized membrane.\(^{15}\) It is, therefore, not too surprising that, at the exception
of a handful of simulations of lipid bilayers in the gel phase, most investigations have focused on the so-called, biologically relevant L_α phase.

2.2. Experimental available information

To this date, neutron and x-ray diffraction experiments probably remain the most powerful tools for determining structures of lipid bilayers at an atomic resolution.\(^{16-19}\) A particularly pertinent information supplied by diffraction experiments are density distributions,\(^{20}\) that can be deconvoluted in terms of atomic positions in the direction normal to the water–membrane interface, for different types of atoms.

High–resolution x–ray diffraction experiments may offer additional, valuable information, that can directly serve as a reference for computational studies. Such is the case of the surface area per lipid, that may be derived from gravimetric x–ray methods or from electron density profiles. It should be underlined, however, that the highly disordered nature of liquid crystal, L_α, phases, and their fluctuations makes the observation of such systems particularly difficult, and explains the large uncertainty in the values supplied by the literature.\(^{20}\).

Whereas x–ray and neutron diffraction on multilayered samples have historically been a source of high–resolution structural information of model membranes, neutron reflectivity has provided unique data on single lipid bilayers in contact with bulk water. Scattering length density (SLD) profiles along the normal to a layered system are deduced from the information collected as a function of the scattering wave–vector transfer (Q). Recently it has been shown that it is also possible to invert directly the reflectivity spectra to obtain SLD profiles.\(^{21}\) It is important to note, however, that only the total SLD profile is determined. For more complex systems, atomistic modeling can provide valuable insight into such structures, thereby complementing the experimental studies.\(^{22-24}\)

Nuclear magnetic resonance (NMR) techniques are also used extensively to probe the molecular organization in lipid membranes. Earlier on, \(^2\)H NMR experiments on oriented lipid matrices supplied lipid order parameters, against which the average orientational order along the acyl chains calculated from simulations could be confronted. Today, thanks to the introduction of magic angle spinning (MAS) techniques, a very large number of parameters from lipid bilayers are available, providing a wealth of information on the conformation of all lipid segments.\(^{25}\)

X–ray and neutron scattering measurements as well as NMR experiments may also be used as a possible source of comparison of dynamical properties against MD simulations. As will be seen in what follows, the significant computational effort involved in atomic simulations of large lipid–water assemblies limits, from a biological perspective, their length to relatively short times. Short time–scale dynamics is yet amenable to MD, and the data determined by this approach can
be confronted directly to scattering experiments\cite{26,4}, and, for instance, to nuclear Overhauser enhancement spectroscopy (NOESY) cross–relaxation rates\cite{27}, which probe motions occurring over comparable time–scales.

3. Modeling lipid bilayers

In order to eliminate edge effects and to mimic a macroscopic system, simulations of lipid bilayers consist of considering a small patch of lipid and water molecules confined in a central simulation cell, and replicating the latter using periodic boundary conditions (PBCs) in the three directions of Cartesian space, as is being done in the simulations of molecular liquids and crystals. In doing so, the simulated system corresponds to a small fragment of either a multi–lamellar liposome or of a multilamellar oriented lipid stack, similar to those deposited on a substrate (see Figure 2). The size of the simulated sample results in artefactual, symmetry–induced effects and the impossibility to witness collective phenomena like bending or splay motions that occur over length–scales above the size of the cell.\cite{28,29,20}

If needed, one may render a more biologically or physically meaningful picture, consistent with experimentally observed phenomena, by incorporating a large number of lipid and water molecules.\cite{30} Even then, the length of the simulation constitutes another critical aspect in the modeling of lipid–water assemblies, essentially because a number motions in lipid bilayers, occur over time–scales exceeding 10 ns (see Figure 2).

![Figure 2](image-url)

**Figure 2:** Left: Small patch of lipid bilayer replicated by PBCs. Right: Characteristic time–scales in lipid bilayers. Overall, motions occur on times that range between a few ps for the separation of \( sn \)–1 and \( sn \)–2 alkyl chains, to a few hours for the so–called flip–flop, where in a lipid unit migrates from one leaflet to the other.
3.1. Choice of the thermodynamic ensemble

From a technical perspective, the simplest thermodynamical ensemble for simulating lipid–water assemblies is undeniably the microcanonical, \((N, V, E)\), ensemble, or possibly the canonical, \((N, V, T)\), ensemble, wherein the temperature is controlled rigorously by means of a thermostat. In this event, the modeler may choose to fix the cross–sectional area per lipid unit to its experimental value and leave an appropriate head space of air in contact with the water lamellae, above and below the membrane. Whereas this protocol is \textit{ad hoc} in the case of a simple, homogeneous lipid bilayer, one may legitimately wonder how it will perform when additives — e.g. small solutes to large proteins, are introduced into the membrane or in its vicinity. A better adapted thermodynamic ensemble should then be employed to allow the participating lipid chains to relax in response to the modification of the surface tension imposed by the additive. A very tempting solution consists in turning to the isobaric–isothermal, \((N, P, T)\), ensemble, that makes use of rigorous barostats and thermostats to maintain, respectively, the pressure and the temperature at the desired values. This raises, however, difficulties of its own.

In a mixture of oil and water with a positive surface tension, the free energy increases monotonously with the surface area, as the system minimizes the contact area between the two liquids. In the case of lipids interacting with water — \textit{viz.} typically a hydrated lipid bilayer, the picture is somewhat more intricate. Just like for a mixture of oil and water, by virtue of the hydrophobic effect, the free energy increases with the surface area. This is evidently not the sole contribution governing the behavior of lipid bilayers, the surface area of which would be minimized regardless of the temperature, thereby forcing the system in the gel, \(L_{β′}\), phase. Small surface areas, indeed, restrain the alkyl chains in an ordered state, consequently decreasing the entropy of the lipid–water assembly. As a result, the free energy no longer increases with the surface area, but, on the contrary, exhibits a minimum that corresponds to an optimum surface area for a given temperature. This also implies that the surface tension, \(γ\), should be strictly zero, and, therefore, that the lateral pressure, \(P_∥\), be strictly equal to the pressure normal to the water–lipid interface, \(P_⊥\):

\[
γ = \int \left[ P_⊥ - P_∥(z) \right] \, dz = 0
\]  

(1)

This important result, which was expected for a self–organized system, prompted a host of authors to simulate lipid bilayers in the isotropic isobaric–isothermal ensemble, \((N, P, T)\).\textsuperscript{31} Whereas, strictly speaking, equation (1) is true for a lipid–water assembly of virtually infinite extent, it should be kept in mind that in atomic simulations one model patches of finite size. Feller and Pastor put forward that a finite surface tension should be introduced to compensate for such finite–size effects that eliminate the possibility to observe collective phenomena like undulations over significant length–scales,\textsuperscript{32,33} as in ripple, \(P_β\), phases, for instance. Tieleman and Berendsen argued that in the systems they investigated, the dependence of the surface tension with the surface area was marginal.\textsuperscript{34} Lindahl and Edholm later showed that an applied surface tension in the order of 10 mN/m would correct for large fluctuations in the surface area per lipid unit that are witnessed
in simulations of lipid–water assemblies of limited size.\textsuperscript{35} One thing is certain: In atomic simulations of lipid bilayers, $P_{\parallel}$ and $P_{\perp}$ are anticipated to vary differently on account of the anisotropy of the environment. It is, therefore, strongly recommended to adopt an algorithm that generates the $(N, P, T)$ distribution, so that the dimensions of the simulation cell are rescaled independently in the $x, y$ (in-plane) and in the $z$–directions.\textsuperscript{36,37,2}

### 3.2. The potential energy function

In atomic statistical simulations of membranes, all atoms pertaining to the system are treated classically as point masses, which, in the harmonic approximation, are connected to each other by means of springs. In some instances, for the sake of computational effort, certain groups of atoms, like methylene, $-\text{CH}_2-$, or methyl, $-\text{CH}_3,$ moieties, are represented as a single, “united” atom of appropriate van der Waals radius and well depth.\textsuperscript{38} Seminal simulations of lipid–water assemblies made use of the available multi–purpose force fields, often aimed at the modeling of solvated proteins and nucleic acids. It is, therefore, not too surprising that in early investigations, the agreement with experiment was either far from optimal, or clearly too good to not suspect a fortuitous cancellation of errors due to the conjunction of inadequate parameters and excessively short runs.

In the following years, it was realized that a specific potential energy function should be employed to mimic accurately the properties of lipids, like the subtle $\text{trans}$–$\text{gauche}$ equilibrium in the alkyl chains. A dearth of efforts was and is still invested to improve the representation of lipids and surfactants by means of an appropriate parameterization of the force–field contributions likely to affect the structural and dynamical features of these systems.\textsuperscript{39–43} In some of these force-fields, to obtain a better description of the ordering in the fatty aliphatic chains, that can be ascribed to $\text{trans}$–$\text{gauche}$ defects, the standard low–order Fourier series that is often used in conventional macromolecular force fields, was replaced by the more sophisticated Ryckaert–Bellemans torsional potential.\textsuperscript{44}

In addition, correct packing of the alkyl chains depends to a large extent on the quality of the van der Waals parameters utilized. One of the underlying assumptions made for the design of force fields is the transferability of these parameters between molecules — e.g. the van der Waals radius and well depth of an aliphatic $sp^3$ carbon should be the same regardless of the chemical environment. The interaction parameters of the united methylene and methyl groups were originally derived from statistical simulations of short hydrocarbons like $n$–butane, as is the case of the OPLS force field.\textsuperscript{45} The transferability hypothesis has proven to be inadequate when handling long alkyl chains, prompting a number of authors to reoptimize van der Waals interactions based on simulations of large hydrocarbons like pentadecane.\textsuperscript{31}

Determination of net atomic charges for lipids and surfactants from sophisticated quantum me-
mechanical calculations may turn out to be a difficult task, on account of the size of the molecules. Unquestionably, partial charges derived from the electrostatic potential constitute the most satisfactory solution among the arsenal of approaches available to the modeler. Yet, as has been demonstrated, point charges are inherently conformation–dependent, thus making the derivation of a unique set of charges representative of all possible conformations questionable. To circumvent the difficulties connected to the size of the molecules, it has been proposed to derive the net atomic charges as independent fragments, that are ultimately pieced together. This scheme, although tempting, should be considered with great care if local charges are delocalized over large spatial extents.

3.3. Intermolecular interactions

As has been mentioned previously, physically and biologically realistic simulations should involve a sufficiently large number of lipid and water molecules to minimize finite–size effects. Much of the computation effort involved in atomic simulations of lipid–water assemblies lies in the evaluation of pairwise interactions, the number of which increases dramatically with the number of particles in the system. Based upon the assumption that intermolecular interactions decay with the distance, earlier studies have employed a cut–off sphere, beyond which the interactions are truncated. This approximation is expected to be ad hoc for the short–range, van der Waals contribution. The use of a brute, finite spherical cut–off for truncating the short–range van der Waals interactions may, however, modulate the forces responsible for the cohesion of lipid–water assemblies. Accurate use of a cut–off requires to take into account the appropriate long–range corrections for both the energy and the pressure, based on the classical formulae utilized for Lennard–Jones fluids.

For Coulomb interactions, the range of which varies in $r^n$, where $n \leq 3$, truncation becomes particularly arguable. In this event, the long–range character of the participating charge–charge ($n = 1$) and charge–dipole ($n = 2$) interactions makes the use of a spherical truncation unsuitable. Probably the most accurate approach for handling the long–range nature of electrostatic interactions in spatially replicated simulation cells is solving the Poisson equation. The Ewald approach, that decomposes the conditionally convergent Coulombic sum over periodic boxes into two rapidly decaying contributions evaluated respectively in the direct and reciprocal spaces is the most used method. Formally, the computational effort involved in this method scales as $N^2$, thus making statistical simulations of large ensembles of atoms particularly costly. This effort can be reduced, scaling down the calculation to $N \ln N$, by solving the Poisson equation numerically on a grid of points, over which the position of the particles are interpolated. Such a scheme constitutes the central idea of algorithms like particle–mesh Ewald (PME) or particle–particle–particle–mesh (P³M). For completeness, while to our knowledge, it has not been yet applied in membrane simulations, it is worth mentioning the fast multipole approach, a method alternative to Ewald summation, that treats long–range interactions in a rigorous fashion, and scales
linearly with $N$ for very large systems — viz. on the order of 100,000 atoms.$^{52}$

The substantial computational investment required to attain a physically consistent description of the simulated molecular assembly may be further reduced by taking advantage of recent advances in the MD methodology. Considering that the different degrees of freedom involved in the system relax over distinct time–scales, it is not necessary that the corresponding force contributions be evaluated concurrently. This is, in essence, the basic principle of the so–called multiple time–step methods,$^{53,54}$ in which intramolecular, van der Waals and Coulomb forces can be updated with different frequencies.$^{55}$ In conjunction with constraint algorithms like SHAKE or RATTLE,$^{56}$ that virtually eliminate the vibrations due to hard degrees of freedom it is possible to explore large regions of the phase space for a lesser computational effort, thus making long simulations of large lipid–water assemblies somewhat more affordable — the reader is referred to the chapter of Tuckerman and Martyna dedicated to integrator and ensembles in statistical simulations.

4. Atomic simulations of lipid membranes

Traditionally, phospholipids have served as models for investigating in silico the structural and dynamical properties of membranes. From both a theoretical and an experimental perspective, zwitterionic phosphatidylcholine (PC) lipids constitute the best characterized systems. Hydrated DMPC,$^{13,57}$ and dipalmitoylphosphatidylcholine $^{58,34,31,59–61}$ (DPPC) bilayers have been so far probably the most extensively surveyed lipid membranes. Yet, on account of their intrinsic limitations — viz. the short alkyl chains in DMPC and the temperature of $L_{\beta'}$ to $L_{\alpha}$ phase transition in DPPC, above physiological conditions — several authors have turned to biologically more relevant lipids like palmitoyloleylphosphatidylcholine $^{62,63}$ (POPC), in particular for examining membrane proteins in a realistic environment, and lipids based on mixtures of saturated/polyunsaturated alkyl chains (SDPC, 18:0/22:6 PC).$^{64,43}$ A variety of alternative lipids, featuring different, possibly charged, head groups, have also been explored — e.g. DLPE$^{65,13}$ (DLPE), dipalmitoylphosphatidylinerine $^{66,67}$ (DMPS) or glycerolmonoolein $^{68,30}$ (GMO). In several cases, however, the modeller is faced with an absence of experimental data to which the results of atomic simulations can be confronted.

Bilayers built from PC lipids, nonetheless, represent remarkable test systems not only to probe the methodology, but also to gain additional insight into the physical properties of membranes. In this section, the derivation of these properties from MD trajectories and how a bridge with experiment can be established will be detailed.
4.1. Bilayer structure

4.1.1. Density distributions

As can be seen in Figure 3, the spatial extent encompassed by the head–group region of the DMPC units in a bilayer arrangement is remarkably broad. This is clearly seen in the number density profiles computed from the MD trajectory — an analysis along the direction normal to water–membrane interface of the in–plane densities of lipid and water atoms. A striking feature emerging from these distributions is the penetration of water far in the head–group region. The farthest extent of water molecules roughly coincides with the ester moieties of the lipids. The width of the interfacial region, on the order of 8 to 10 Å for a fully hydrated DMPC bilayer highlights the significant static and dynamic roughness of the membrane surface\textsuperscript{68,69}, therefore, refining the traditional textbook picture, like that of Figures 1 and 2.

Figure 3: Left: Snapshot taken from an MD simulation of a fully hydrated DMPC bilayer. Methylene and methyl carbon atoms of the alkyl chains are shown in grey and pink, respectively. Ester carbon and oxygen atoms are shown in blue and purple, respectively. Phosphate phosphorus and choline nitrogen atoms are shown in orange and green, respectively. Note the protrusion of head groups that results locally in a rough water–membrane interface. Right: Density distributions for selected groups of atoms in a fully hydrated DMPC bilayer examined at 303 K (from reference [70]).

Interestingly enough, phosphate and choline groups lie approximately at the same depth in the bilayer, indicating that head groups are rather oriented in the plane of the bilayer. The average orientation of the head–group P—N bond dipoles with respect to the normal of the water–membrane interface arises around 70°, pointing towards the aqueous medium, albeit the orientational distribution is remarkably wide, and depends upon the temperature and, as expected, the potential energy function utilized.\textsuperscript{14} In addition, the level of lipid hydration has been shown to play a noteworthy role in the orientation of the head groups.\textsuperscript{9} Under any circumstances, it is crucial that the
slow reorientation of the lipid head groups be considered when interpreting results from short MD trajectories. Estimates from single–molecule anisotropy imaging for fluorophore–tagged POPC molecules\textsuperscript{71} indicate a rotational diffusion coefficient of ca. 0.7 rad\(^2\)/ns, slightly below estimates from MD simulations,\textsuperscript{72} suggesting that sampling of the whole rotational space for each molecule would necessitate over few tens of a nanosecond.

The information provided by the density distributions can be confronted directly to x–ray and neutron diffraction measurements\textsuperscript{17} (considering respectively the atomic scattering length densities, or the electron densities). The MD trajectories can further be used in conjunction with the scattering experiments in order to refine the data by, for instance including fraction volumes extracted from the simulation.\textsuperscript{73}

4.1.2. Lipid tail conformation

Deuterium quadrupole splitting measured by \(^2\)H NMR on non–oriented samples of membrane preparations, is mainly determined by the average conformation of the phospholipid molecules, and, as such, supplies valuable structural information about the system. Order parameters can be derived from MD trajectory, and can be expressed as a tensor, the elements of which write:\textsuperscript{74}

\[
S_{\alpha\beta} = \frac{1}{2} \left\langle 3 \cos \varphi_\alpha \cos \varphi_\beta - \delta_{\alpha\beta} \right\rangle
\]

Here, \(\varphi_\alpha\) is the angle formed by the \(\alpha\)–th molecular axis and the normal to the water–bilayer interface, and \(\langle \cdots \rangle\) is an ensemble average over all lipid chains. In most circumstances, based on symmetry relationships, it is assumed that the order parameters, \(S_{\text{CD}}\), for an alkyl chain bearing deuterium labels can be expressed as:

\[
S_{\text{CD}} = \frac{1}{2} \left\langle 3 \cos^2 \theta - 1 \right\rangle
\]

where \(\theta\) is simply the angle between the C—D chemical bond and the normal to the bilayer. When C—D is uniformly distributed, \(S_{\text{CD}} = 0\), and when the chain is all–trans, \(|S_{\text{CD}}| = 0.5\). In general for saturated lipids, \(|S_{\text{CD}}|\) exhibits a plateau value at ca. 0.2 for the upper chain segments. Force fields of the new generation reproduce quite well these order parameters, barring small discrepancies for the second carbon atom of the alkyl chains.

Further analysis of MD trajectories may be aimed at extracting additional information from the NMR experiments. For instance, one may refine those methods targeted at obtaining such quantities as the average chain length or the surface area per molecule.\textsuperscript{76} Another study exemplifies the successful combination of MD simulation with experiment to probe alkyl chain packing in lipid membranes. Such is the case of infrared (IR) data, that have been reinterpreted to estimate the concentrations of gauche–gauche, trans–gauche and trans–trans conformational sequences in a DPPC bilayer.\textsuperscript{77}. 

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\textsuperscript{71} Chipot, Tarek & Klein: Modeling lipid membranes
Figure 4: Order parameters of a fully hydrated DMPC bilayer at 323 K. red: derived from the simulation (conditions explicited in [75]) and black: experimental values.\textsuperscript{74}

4.1.3. Hydration of the head–group region

In atomic simulations, solvation properties are often measured by means of radial distribution or pair correlation functions (RDFs):

\[ g_{ij}(r) = \frac{\langle N_j(r; r + \delta r) \rangle}{4\pi \varrho_j \int r^2 dr} \]  

where \( N_j \) is the number of particles \( j \) at a distance from \( i \) comprised between \( r \) and \( r + \delta r \) and \( \varrho_j \) is the density of particles \( j \). In essence, this definition is targeted at isotropic fluids, and, in principle, should not be applied, as is, to anisotropic lipid–water assemblies,\textsuperscript{78} To estimate the coordination number of site \( i \) — e.g. PC head groups, it seems far more appropriate to merely evaluate \( \langle N_j(r; r + \delta r) \rangle \) as a function of the separation \( r \) and determine its value at the first minimum of a qualitative RDF computed using equation (4).

4.1.4. Transmembrane electrostatic potentials

Orientation of water molecules near the head–group region of the lipid bilayer is clearly anisotropic, compared to the bulk aqueous medium. This can be shown by measuring the average cosine of the angle formed by the dipole moment of the water molecules and the normal to the bilayer, as a function of the distance from its geometrical center. A marked peak emerges at a distance characteristic of the phosphate groups, emphasizing the orienting power exerted by this moiety on the surrounding aqueous environment. The preferential orientation of the dipole moment borne by the water molecules is at the origin of the vocabulary “dipole potential”, that has been employed
extensively to denote the electrostatic potential across the water–membrane interface.\textsuperscript{79,80} This conspicuous ordering of water molecules was recently directly evidenced using coherent anti–Stokes Raman scattering microscopy.\textsuperscript{81}

In a number of \textit{in silico} investigations, the electrostatic potential has been estimated from the knowledge of the charge density. In the spirit of atomic density distributions, charges are accumulated as a function of their position along the direction normal to water–bilayer interface. The negative of the first integral of the charge density yields the electric field. In turn, integral of the field provides the electrostatic potential. Not too surprisingly, the resulting “dipole potential” inherently depends upon the choice of the potential energy function and should, thus, be interpreted cautiously.\textsuperscript{4,31,57}

\textbf{4.2. Dynamics}

The increasing level of interaction between experimental studies and numerical simulations of lipid bilayers evidenced in the previous section also holds for the dynamics of lipid bilayers.

Feller \textit{et al.}\textsuperscript{27} have used MD simulations to analyze NOESY cross relaxation rates in lipid bilayers. Magnetic dipole–dipole correlation in such systems occurs over a variety of time scales and depends upon the probability of close approach for proton–proton interactions. The relaxation rates have been calculated directly from a 10 ns MD simulation of DPPC. Fitting the autocorrelation functions yields characteristic correlation times and weight factors that determine the relative contributions of the individual type of motions. Combining simulations and experiments, relaxation rates may, therefore, be assigned to various motions — \textit{viz.} less than 1 ps for chemical bond vibrations, 50–100 ps for \textit{trans–gauche} isomerization, 1–2 ns for molecular rotation and wobble, and beyond 100 ns for lateral diffusion.

A model for the dynamics of individual lipid molecules has also been proposed based on a thorough comparison of simulation data and experimental measurements of the $^{13}$C NMR $T_1$ relaxation in DPPC alkyl chains.\textsuperscript{82} Employing Brownian dynamics and MD simulations associated to fits of experimental data, it was found that lipid molecules confine themselves into a cylinder within the 100 ps time scale, and wobble in a cone–like potential on the nanosecond time scale.

A similar model for lipid dynamics has emerged from an MD study aimed at interpreting inelastic neutron scattering (\textit{INS}) data. One particular aspect of such experiments, probing the motion of individual hydrogen nuclei — \textit{i.e.} self correlation of single particle, is that they are space– and time resolved. In the case of DPPC bilayers, a good agreement between simulations and experiments probing the 100 ps time scale is attained.\textsuperscript{83} The analysis corroborates the fact that the motion of the center of mass and the internal motions of lipid molecules are decoupled. Moreover, the former is well described as a diffusion in a confined space, \textit{i.e.} a cylinder. A refined picture of the internal
dynamics arising from the simulation shows that protons of the alkyl chains move according to a chain defect model, where kinks or chain defects form and disappear randomly — i.e. stochastic model — along the lipid tail, rather than diffuse along the chain.

Collective dynamics of lipid bilayers have also been examined carefully as simulations over increasingly significant time scales and length scales are feasible. Large systems involving 1,024 lipid molecules studied over 10 ns led to the direct observation of bilayer undulations and thickness fluctuations of mesoscopic nature.\textsuperscript{35} Continuum properties such as bending modulus, surface compressibility and mode relaxation times were calculated and agreed nicely with experiment. Several processes occurring at different length scales were identified. The undulatory motions could be separated in two regimes — one involving more than 50 lipids, that can be ascribed to mesoscopic undulations, and the other, involving less than 25 lipids, that is attributed to collective lipid protrusion. Peristaltic modes — i.e. anti–correlated modes between the two layers — could also be distinguished in two types: bending modes involving 50 to 400 lipids, and protrusion modes over shorter length scales.

Shorter wavelength collective dynamics may be probed using coherent inelastic, \textit{viz.} neutron or x–ray, scattering. Density fluctuations of length scales comparable to the interlipid distance are believed to play a pivotal role in the transport of small molecules across the bilayer. Recently, MD simulations have been used to complement inelastic x–ray data of lipid bilayers, both in the gel, L\textsubscript{\beta}', and the liquid crystal, L\textsubscript{\alpha}, phases.\textsuperscript{84} The results support the applicability of generalized hydrodynamics to describe the motion of carbon atoms in the hydrophobic core, thus allowing the modeler to extract key–parameters, such as sound mode propagation velocity, thermal diffusivity and kinematic longitudinal viscosity.

\section{4.3. Modeling transport phenomena}

Models of lipid bilayers have been employed widely to investigate diffusion properties across membranes through assisted and non–assisted mechanisms. Simple ions, \textit{e.g.} Na\textsuperscript{+}, K\textsuperscript{+}, Ca\textsuperscript{2+} or Cl\textsuperscript{−}, have been shown to play a significant role in the cell machinery, in particular at the level of intercellular communication. In order to enter the cell, the ion must preliminarily permeate the lipid bilayer that acts as a rampart towards the cytoplasm. Wilson and Pohorille have investigated the passive transport of Na\textsuperscript{+} and Cl\textsuperscript{−} ions across a lipid bilayer formed by glycerolmonoolein units, which undergoes severe deformations as the ions translocate across the water–membrane interface. This process is accompanied by thinning defects and the formation of water fingers that ensure an appropriate hydration of the ion as it penetrates in the non–polar environment.\textsuperscript{85}

Ideally, atomic simulations could also serve as a predictive tool for estimating water–membrane partition coefficients of small drugs, in strong connection with the so–called blood–brain barrier — the ultimate step in the \textit{de novo} design of pharmacologically active molecules. Diffusion of
small, organic solutes in lipid bilayers was examined for a variety of molecular species ranging from benzene\textsuperscript{86,87} to more complex anesthetics.\textsuperscript{88–90} Yet, access to partition coefficients by means of statistitical simulations implies the determination of the underlying free energy behavior along the direction normal to the interface.\textsuperscript{91} In the specific instance of inhaled anesthetics, an analysis of the variations of the free energy for translocating the solute from the aqueous medium into the interior of the bilayer suggests that potent anesthetics reside preferentially near the water–membrane interface. Contrary to the dogmatic Meyer–Overton hypothesis,\textsuperscript{92} potency is shown to correlate with the interfacial concentration of the anesthetic, rather than its sole lipophilicity.\textsuperscript{93}

The considerable free energy associated to the transfer of ions from the aqueous medium to the interior of the membrane rationalizes the use in cells of specific transmembrane channels, pumps or carriers that facilitate while controlling selectively the passage of ionic species across the lipid bilayer.\textsuperscript{94} Recent complete reviews of the theoretical developments and simulation capabilities in ion channels modelling can be found in references [95] and [96]. Here, we briefly describe some of the complex systems examined hitherto.

Gramicidin A, a prototypical channel for assisted ion transport, has been the object of thorough analyses from both experimental and theoretical perspectives. Dimerization of individual protein units results in membrane–spanning channels suitable for ion conduction. MD simulations of gramicidin A embedded in hydrated lipid bilayers, e.g. DMPC, were able to reproduce the structural features observed experimentally.\textsuperscript{97} Such studies have clearly shown that important questions related to ion selectivity, ion binding, gating and proton transfer mechanisms may be addressed with some confidence.

Internal arrangement of water molecules in single–file chain of water molecules, characteristic in complex transporters,\textsuperscript{98} was also witnessed in a somewhat more rudimentary, synthetic channel formed by stacked cyclic peptides of alternated D– and L–chiralities (see Figure 6).\textsuperscript{70} Such nanotubes have been recognized to modify in a selective fashion the permeability of cell membranes and are envisioned to act as potent therapeutic agents in response to bacterial resistance.\textsuperscript{99} Aquaporins, membrane channels ubiquitous to most living species controlling the water contents of the cells, have also focused much attention lately. They are formed of tetramers that organize to facilitate the transport of water, and possibly other small solutes, across the lipid bilayer. The resulting water pores remain, however, impervious to the passage of small ions to ensure a proper conservation of the electrochemical potential.\textsuperscript{100} As a final note, it is worth mentioning that, as expected, the determination of the high resolution structure of KscA, a bacterial K\textsuperscript{+} channel, has motivated a large number of realistic simulations taking into account the lipidic environment studies aimed at deciphering the underlying complex conduction mechanism.
**Figure 5**: Snapshot taken from an MD simulation of a synthetic channel formed of cyclic peptides of alternated D– and L–chiralities, embedded in a fully hydrated DMPC bilayer. Color coding of the atoms is identical to that in Figure 3. Note the antiparallel $\beta$–sheet like conformation of the nanotube spanning the membrane. Within a few hundreds of ps, a single–file chain of water molecules is established in the hollow tubular structure.

### 4.4. Interaction of small molecules, peptides and proteins with membranes

In most circumstances, the biological membrane is described at the theoretical level as a simple, homogeneous bilayer formed by a single type of lipid — usually the well–studied, zwitterionic PC lipids. Membranes, however, are infinitely more complex and consist of an heterogeneous assembly of different lipids, either charged or not, carbohydrates and proteins. Approaching the fine detail of the biological picture by incorporating in atomic simulations chemical species of different natures is evidently the direction towards which the modeler is evolving. From a modeling perspective, the influence of cholesterol,$^{101-105}$ and more generally sterols,$^{106}$ on the structure and dynamics of lipid bilayers has attracted a lot of attention in recent years. Although the limited sampling in some simulations calls into question the conclusions reached by the authors, cholesterol is shown to increase the order parameters of the alkyl chains while decreasing their tilt angle with respect to the normal to the water–membrane interface, in qualitative agreement with experiment.$^{107}$

Aside from transporters and channels that assist the transport of chemical species across lipid bilayers, a vast array of key–cellular functions are accomplished by proteins that interact with the membrane, either spanning the latter, or bound to its surface.$^{108}$ Yet, interfacial and transmembrane proteins generally play distinct roles in the cell machinery, albeit the frontier between these two classes of proteins remains somewhat fuzzy. A number of proteins, for instance, are only partially buried in the membrane — e.g. melittin or alamethicin, the insertion of which is conditioned by the transmembrane electric field.$^{109-112}$
The strength of in silico experiments is to provide glimpses into the atomic detail of biological membranes that conventional experimental techniques cannot capture. Of particular interest is the molecular interplay that govern membrane–protein association, accessible through large–scale atomic simulations. MD simulations illuminated, for instance, how the presence of a protein perturbs the structure of the lipid membrane. For example, the helices of the Influenza A M₂ channel tilt in a DMPC bilayer to maximize membrane–protein hydrophobic contacts. In the case of gramicidin A, key–residues located in the head–group region have been shown to stabilize the channel in the membrane.

The influence of the protein on the lipid bilayer can be viewed as the subtle balance between hydrophobic and hydrophilic contributions that, in principle, can be captured by MD simulations. Differences in the order parameters of lipid units adjacent to the protein and far from it have led to the concept of “boundary lipids”. In a vast number of instances, among which the Mycobacterium tuberculosis MscL channel, the Influenza A virus M₂ protein, and the Escherichia coli OmpF trimer, it was observed that the membrane protein induces an increasing disorder of the lipid alkyl chains in its neighborhood. In sharp contrast, alkyl chains close to the transporter gramicidin A tend to be more ordered, compared to those pertaining to the bulk lipid environment.

In the light of these computational investigations, it would, therefore, appear that trans–gauche equilibria in lipid chains are dictated by the very nature of the membrane protein. Yet, as was shown recently, drawing definitive conclusions based on limited simulation lengths may turn out to give a distorted vision of the actual behavior of the lipid bilayer. In principle, exceedingly short simulations do not permit the complete relaxation of lipid chains in the vicinity of the protein, and should, thus, be interpreted cautiously.

The close adequation between the thickness of the lipid bilayer and the length of the hydrophobic segment of the protein spanning the latter constitutes yet another important facet of the protein–membrane interplay. By providing the microscopic detail of the interactions of integral proteins with the lipid environment, atomic statistical simulations may contribute to advance our understanding of the underlying physical principles that govern the function and structure of membranes. In the light of a series of experimental investigations on model peptides embedded in PC membranes with alkyl chains of increasing length, it was found that if the hydrophobic thickness of the peptide is greater than that of the bilayer, the latter becomes thinner, and vice versa. A similar phenomenon was observed recently in the MD simulation of a single peptide nanotube inserted in a hydrated DMPC bilayer. The hydrophobic thickness of the membrane adjusts itself as the synthetic channel tilts concurrently to adapt to its host lipid environment. Whereas the so–called hydrophobic mismatch does not appear to induce perturbations in peptide nanotubes, it can, however, modulate strongly the function of more complex proteins. As was observed recently for gramicidin A, minute changes in the length of the lipid alkyl chains — viz. from the 18–carbon oleyl– to the 20–carbon eicosenoylphosphatidylcholine, switch the protein from a stretch–activated to a stretch–inactivated channel. Symmetrically, the hydrophobic mismatch
may alter the phase behavior of the membrane, as demonstrated in the case of WALP peptides that promote the formation of non-lamellar phases.\textsuperscript{122} These remarkable results should, therefore, incline the modeler to be cautious when solvating membrane proteins in lipid surroundings. The choice of the lipid unit for a given protein may turn out as a genuine leap of faith if attention has not been paid to the possible imbalance in the hydrophobic thicknesses of the membrane and the protein, likely to render a physically unrealistic picture of the assembly. When devised appropriately, atomic simulations can, nonetheless, shed new light on the nature of the protein–membrane interplay, by allowing the modeler to not only visualize, but also possibly quantify the strength of the participating interactions. Of particular interest, the non–covalent chemical bonds formed by L–Trp residues and acceptor moieties of the head–group region have been recognized to act as anchoring points of the protein into the lipid bilayer.\textsuperscript{123,124} As has been shown in the case of gramicidin A, the presence of several L–Trp amino acids at the level of the lipid head groups is expected to mediate the overall stabilization of the channel in the membrane.\textsuperscript{97}

5. Discussion, outlook and future prospects

Retrospectively, with about fifteen years of hindsight, it has become clear that atomic simulations, in particular MD simulations of lipid–water assemblies have contributed in a large measure to improve our knowledge of these very complex systems from both a structural and a dynamical point of view. It is also obvious that the successes of pioneering, tantalizing investigations, which not only ignited the field of lipid simulations, but were also rapidly fueled by many studies on larger assemblies, often reflected as much good fortune as they did science. Yet, major advances on both the hardware and the software, algorithmic fronts progressively allowed the modeler to tackle systems of increasing complexity over time–scales compatible with the physical, chemical and biological reality. Among these advances, the development of specific methods for performing the simulation in apt thermodynamic ensembles, the improvement of potential energy functions targeted at the specific modeling of lipid–water assemblies, and the continuous decrease of the price/performance ratio of modern computers have helped pushing back the intrinsic limitations of MD simulations. More recent studies have demonstrated that simulations at least an order of magnitude longer than those reported when the field was only in its infancy, were required to obtain reliable and reproducible results.\textsuperscript{125}

Simulation of lipid–water systems still constitutes a research area seething with excitement. The development of all the ingredients to investigate \textit{in silico} lipid bilayers with full confidence opens new perspectives, in particular on the biological front, and should rapidly allow the modeler to use lipids in a routine fashion, just like any other solvent. In this spirit, theoretical studies of membrane proteins in a realistic environment should continue to flourish in the near future. Unfortunately, as the level of sophistication of atomic simulations increases, together with the available computational power, so does the ambition of the modeler, attempting to deal with molecular systems yet
even more complex, both in terms of size– and time–scales. This explains the current teeming activity in the development of approximate schemes that could serve as alternatives to a full–atomic description for the modeling of large lipid–water assemblies over long times.

Among these alternatives, a dearth of effort has been invested in recent years in the field of implicit solvation.8 Since the seminal work of Onsager on continuum electrostatics,126 the temptation to represent explicit surroundings by a simple dielectric medium for a myriad of chemical systems has been the object of tremendous interest. Modeling the complexity of lipid bilayers by means of a continuum description has been used, for example, to investigate the insertion of α–helical peptides in a membrane,127 or the interaction of a small toxin with the latter.111 Results of continuum electrostatics simulations, which are based on solving the Poisson–Boltzmann equation numerically, are, in general, in qualitative agreement with atomic simulations. Yet, not too unexpectedly, this approximate description cannot capture subtle, specific interactions that govern the stability of the solute — e.g. a short peptide, at the water–membrane interface. As was underlined recently by Lin et al., the reproduction of membrane dipole potentials based on a sole continuum electrostatics representation is usually erroneous, but can be significantly improved by inclusion of explicit layers of water molecules near the head–group region.128

Aside from implicit solvation approaches, the use of coarse–grained representations, wherein each lipid unit is described by a limited number of interacting sites, is probably the most promising. The underlying assumption that the formation of a lipid vesicle is a sufficiently robust process to be simulated by simplified models of lipids was ascertained recently by Marrink and Mark through a study of the aggregation of DPPC units into small unilamellar vesicles.129 By and large, the strength of coarse–grained models resides in their ability to make simulations self–assembly processes substantially more affordable than conventional all– or even united–atom models.130 The level of representation offered by this alternative is, in sharp contrast, incompatible with the fine description of specific interactions of the participating lipid units with small solutes, like anesthetics. It is obvious that much attention should still be paid in the optimization of the potentials of these rudimentary models to warrant compatibility with a full–atomic descriptions.
References


[75] Constant surface area molecular dynamics simulation of a fully hydrated dimyristoyl phosphatidylcholine (DMPC) bilayer. The system consisted of 64 lipid units in contact with 1,825 water molecules in each lamella, above and below the bilayer. The temperature was maintained at 323 K by means of a Nosé–Hoover thermostat. The surface area was fixed at 62.9 Å². Equilibrium properties were averaged over a period of 2 ns.


