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A computer perspective of membranes: molecular dynamics studies of lipid bilayer systems

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1. Introduction

Knowledge of the structure and dynamics of membranes has traditionally been fragmentary at the atomic level. This is due partly to the fluid character of membranes under physiological conditions, and partly to the lack of experimental data that are directly interpretable in terms of positions and motions

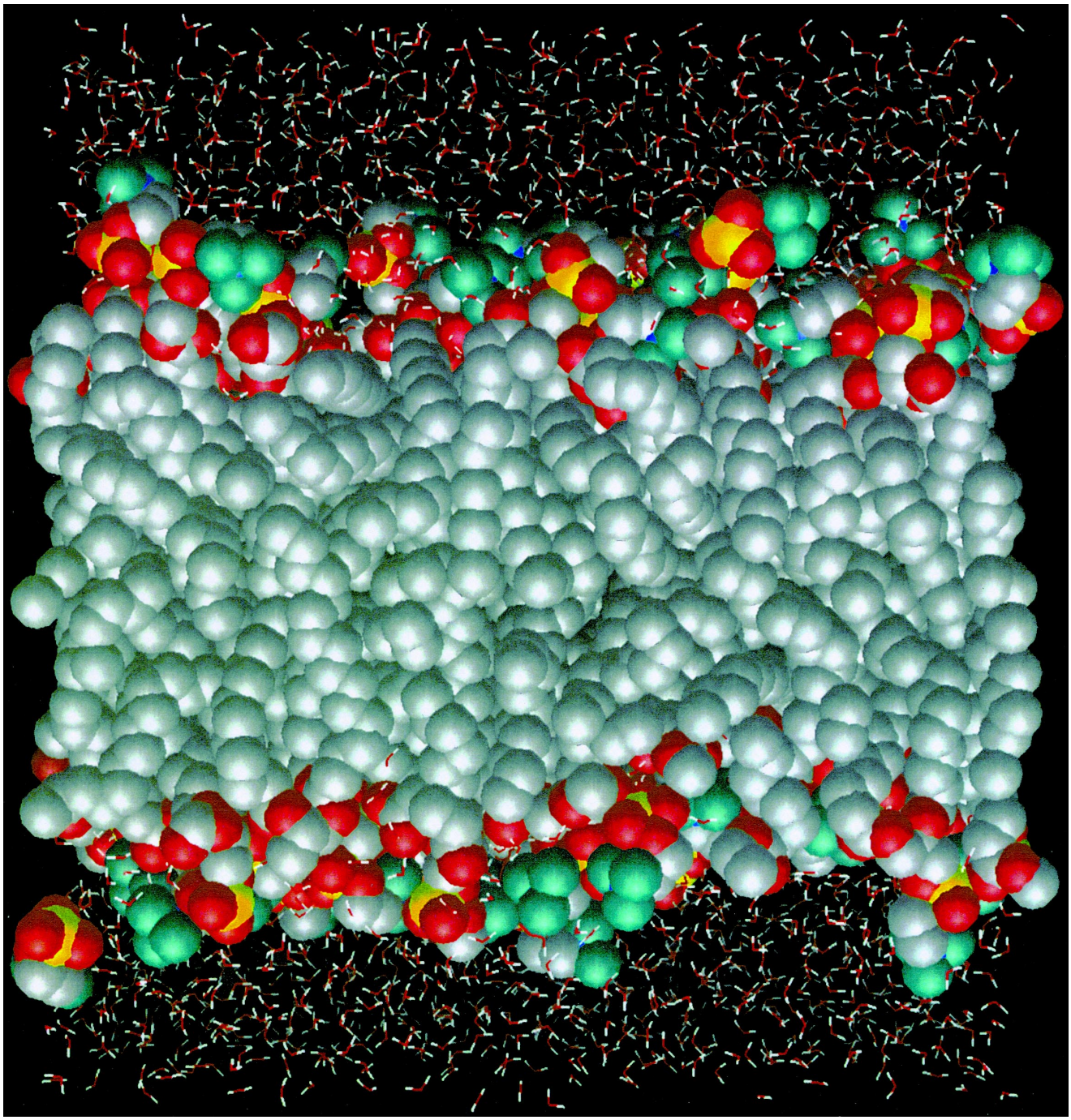


Fig. 1. A color picture of a typical system as studied by molecular dynamics. The system contains 128 DPPC lipids and 3910 water molecules [26]. Water molecules are drawn as sticks, hydrogens are white, oxygens red, phosphorous yellow, carbons grey, the three methyl groups on the choline blue and nitrogens dark blue.

of atoms. In the last decade the availability of powerful computers has opened new ways to study lipid bilayers in atomic detail. Computer simulations now offer a detailed picture of structure and dynamics of membranes. Fig. 1 shows a snapshot of a DPPC bilayer from a molecular dynamics (MD) simulation; a video film in fact leaves no questions unanswered about the behavior of a lipid membrane over a time span of 1 ns (10^{-9} s).

The important question to ask is: do simulations represent the truth? Is the apparent disorder, compared to most traditional textbook pictures, real? Do simulations have predictive power? What are the possibilities and limitations of these new techniques?

With these questions in mind, we will review simulation studies that use the molecular dynamics technique to study the structure and dynamics of lipid bilayers and molecules that interact with lipid bilayers in atomic detail. This excludes a number of other theoretical approaches, notably stochastic dynamics [1–3], continuum electrostatics methods [4], Monte Carlo approaches [1], simulations that treat peptides in atomic detail but the membrane as mean field [5] and more phenomenological approaches, which deal mainly with a larger time and length scale [6–8].

The molecular dynamics technique has developed over the last decades from a method to study the dynamics of liquids of solid spheres and Lennard–Jones particles to a versatile method to study many different types of systems at atomic resolution [9,10]. In the field of biophysics a large body of MD studies on proteins in vacuum or in solvents is available. The development of this particular use of molecular dynamics was greatly stimulated in the 1980s, when a number of general purpose force fields for water, proteins and DNA as well as some general purpose simulation computer programs became available, e.g. AMBER [11], CHARMM [12], GROMOS [13,14] and OPLS [15]. At about the same time the first studies of lipid systems appeared in the literature. Initially many of these studies were performed on simplified models for lipids and solvent was often not taken into account [16–18]. Within long however, the models were extended to represent all atoms of lipid molecules and water was included [19,20]. From these early studies it became clear that MD, subject to certain limitations, can give detailed insights into the motions of lipids and proteins.

In this review we will focus on the application of MD to biologically relevant lipid and lipid–protein systems. We start with a brief description of the MD technique, its potential for use in simulations of lipid bilayers and its main limitations. Then we proceed to a brief description of experimental data that can be used to validate the results of simulation studies. We review the structure of a pure DPPC liquid crystalline bilayer, the main model system thus far, as it emerges from simulations. Simulations of other phases, lipids, and mixtures of lipids with cholesterol are described. We review a number of current applications of MD, focusing on phenomena of biological importance: transport of small molecules across the bilayer, the connection between lipid structure and the so-called ‘hydration force’, and lipid–protein interactions. We conclude with a brief outlook on future developments.

2. Molecular dynamics

2.1. The molecular dynamics method

In a molecular dynamics simulation all atoms in the system under consideration are treated classically. Interactions between atoms are divided in non-bonded interactions, usually between any pair of atoms that are within a given cutoff radius, and bonded interactions between atoms connected by chemical bonds. For the non-bonded interactions (electrostatic and van der Waals), a partial charge and parameters for repulsion and attraction are assigned to each atom. The bonded interactions consist of bond, angle and dihedral terms. Bonds and angles are usually described as harmonic oscillators and dihedral angles are usually described by a suitable cosine expansion. A typical potential function is of the form

$$V = \sum_{i<j} \frac{q_i q_j}{4\pi\epsilon_0 r_{ij}} + \sum_{i<j} \frac{A_{ij}}{r_{ij}^{12}} - \frac{B_{ij}}{r_{ij}^{6}} + \sum_{\text{bonds}} \frac{1}{2} k_{ij}^{b}\left(r_{ij} - b_{ij}^{0}\right)^2 + \sum_{\text{angles}} \frac{1}{2} k_{ijk}^{\theta}\left(\theta_{ijk} - \theta_{ijk}^{0}\right)^2 + \sum_{\text{dihedrals}} k^{\phi}\left(1 + \cos\left(n\left(\phi - \phi^{0}\right)\right)\right). \quad (1)$$

Here r_{ij} is the distance between atoms (or pseudo-atoms when CH_n groups are treated as one atom) i and j, q_i is the partial charge on atom i, A_{ij} and B_{ij} are Lennard–Jones parameters, k^b, k_θ and k^ϕ are force constants for bonds, angles and dihedrals, n is the dihedral multiplicity and b^0, θ^0, ϕ^0 are equilibrium values for the bond lengths, angles and dihedral angles.

The precise form of this potential function is a choice for which there are many options. In particular, different forms for the van der Waals interactions and the dihedrals are in common use and the bonds are often constrained in simulations. However, the form given here is reasonably general and shows the most important assumptions that are made: only pair-additive interactions are taken into account (non-bonded interactions involving three or more atoms are neglected), atoms are represented as point charges (electronic polarizability is neglected) and simple quadratic forms are used for computational efficiency. The precise consequences of these assumptions are beyond the scope of the current paper and the reader is referred to standard treatments [9,10].

Using this potential function, we can solve the equations of motion for all atoms in the system by calculating the forces on all atoms and integrating in time. In principle it is possible to calculate the complete dynamics of any system that can be described in terms of a simple interaction potential. The main result of such a calculation is a trajectory of all atoms in time: the coordinates and velocities of all atoms at any of the integration steps. Potentially, this makes MD a powerful technique to study the motions of atoms in a detailed manner.

2.2. Limitations of molecular dynamics

MD has a number of important limitations. The potential function (Eq. (1)) requires a large number of parameters for partial charges, van der Waals interactions, equilibrium values for bonds, angles and dihedrals, and force constants. Many of these values can be obtained from either experiment (spectroscopy) or quantum mechanics, but because of the simplified form of the potential function compared to the 'real' function, there is no guarantee that these parameters will give good results. In particular, the omission of atomic polarizability in the commonly used force fields influences the force field parameters such that average effects of polarizibility are retained but detailed effects are not properly represented. Additionally, some parameters like the dispersion in the van der Waals interactions and the height of the barriers in the dihedral potentials are difficult to determine. This uncertainty in the parameters makes extensive testing of parameter sets on simple systems, which can be compared to experimental data, necessary. In fact, often parameters are treated as empirical values that can be obtained by fitting models to experimental data, e.g. a water model to experimental data on water.

The second limitation is the maximum timestep for which the integration of the equations of motion is still stable. A typical value in practice is 2 fs (10^{-15} s). This means that 500 000 computationally expensive integration steps are necessary (taking in the order of one to two weeks on a supercomputer for typical systems) to calculate the dynamics of a system during 1 ns. This limits the lengths of current simulations to the nanosecond time scale. The same practical limit on computer power dictates that the largest system that currently can be handled is of the order of tens of thousands of particles, corresponding to system sizes of roughly 5–10 nm.

The third major limitation of standard molecular dynamics is the classical treatment of the system. This makes it impossible to consider chemical reactions without describing at least part of the system quantum mechanically, but is currently of no consequence in simulations of lipid systems.

3. Molecular dynamics of lipid systems

What can we expect from molecular dynamics simulations of lipid systems, given the general possibilities and limitations of molecular dynamics described in the previous section? Obviously, it is important to know at which time and length scale the processes occur that we are interested in. A brief overview is given here, and a more elaborate account can be found in [21].

Apart from the fundamental considerations of time and length scale that have to be taken into account when planning a simulation, there are a number of

technical choices to be made. The most important technical choices are treated briefly below.

3.1. Time and length scales

The fastest motions are bond and angle vibrations and librational motions, small fluctuations of dihedral angles around a bond within the same molecular conformation. These types of motions occur on a time scale up to a few picoseconds. This is also the time scale for the diffusion and orientational correlation of water and other small molecules. Trans-gauche isomerizations of the dihedrals in the lipid tails are slower and occur on a time scale of tens of picoseconds. Trans-gauche isomerizations become slower closer towards the headgroup of a lipid, up to a few hundred picoseconds. The dynamics of some of the dihedrals in the headgroups is slower because of the strong interactions within and between headgroups.

If we turn to whole lipids the time scales become even longer. In a few nanoseconds, phospholipids might rotate around their long axis. For lateral diffusion, or two lipids switching place within one bilayer leaflet, tens of nanoseconds are needed. Even slower motions such as the cooperative motion in phase transitions, the insertion of large molecules like proteins, or the rare event of a lipid flipping over to the opposite membrane leaflet are well out of reach of MD simulations. The same would be true for the slow process of permeation of small molecules through bilayers, but sometimes there are ways to get around such limitations (see Section 8).

We can draw at least two conclusions. The first is that straightforward MD is an excellent method to study the dynamics of tails and individual lipids. This is an important application because MD can give detailed atomic pictures that can be used for the interpretation of, e.g., NMR studies on relaxations and diffraction studies on the rather disordered lipid membranes. It is also possible to study the behavior of solvent molecules in and near bilayers, as well as the differences in behavior of different types of lipids in terms of structure and solvent dynamics.

The second conclusion is that any simulation of a lipid bilayer at the current state of the art will stay relatively close to the initial configuration, since the rotational and translational motion of lipids is too slow to sample in a few nanoseconds. This is not necessarily a problem, but it cannot be expected for instance that phase separation is observed when two different types of lipids are mixed. This is an important consideration in the simulation of the interaction of phospholipids with cholesterol or the interaction between proteins and lipids, to name but two applications.

In practice, the size of a model bilayer in a simulation is currently limited to ca. 100–200 lipid molecules; 50–100 lipids is the most popular size. Usually, periodic boundary conditions are used to avoid strong artefacts from the presence of boundary planes, so that effectively a stack of bilayers with infinite dimensions is simulated. In the literature the length of simulations is limited to a few nanoseconds; most simulations are less than a nanosecond. Although many interesting phenomena occur on the nanosecond time scale, processes like phase transitions, phase separation in lipid mixtures, membrane fusion, protein folding or protein insertion into membranes are well out of reach of straightforward molecular dynamics.

3.2. Technical issues

3.2.1. Force fields

The force field is the description of interactions as in Eq. (1), and the parameter set that belongs to it. There are many choices in the literature; an extensive table is given in [22]. Parameters in different sets are internally consistent, but this is not necessarily true between different sets. An additional problem is that simulation-method details influence the parameters. Therefore, parameters may need to be adjusted when the simulation conditions or algorithms are changed.

An example of this is the behavior of a popular water model, TIP3P [23]. Feller et al. found rather drastic changes in the properties of TIP3P water when they used Ewald summation instead of a simple cutoff for electrostatic interactions (see below) and concluded that the model needed reparameterization [24].

We studied two other commonly used water models in water–lipid systems, namely SPC (simple point charge) and SPC/E (extended simple point charge). SPC/E has better bulk properties than SPC, which makes it a logical choice in molecular dynamics studies of bulk water. However, SPC has a better

chemical potential in mixed systems, which makes SPC the better choice in studies of interfaces [25,26]. For more details we refer to the original literature, e.g. [27–31].

3.2.2. Ensembles

There are different ways in a simulation to treat macroscopic boundary conditions. The temperature T, and number of particles N are almost always kept constant. However, there are several options for the volume or pressure, and we must distinguish dimensions and pressures in lateral and perpendicular directions. With l the perpendicular box size, A the lateral area, p the bulk pressure, and γ the surface tension, we can keep the dimensions (N,l,A,T), pressure and surface tension (N,p,γ,T) or the pressure and area per molecule (N,p,A,T) of the system constant [26,32–38].

For *NlAT* and *NpAT* simulations one needs an accurate value of the area per lipid from experiment, but only for liquid crystalline DPPC an accurate value is available. In addition, *NlAT* and *NpAT* simulations can give artefacts that may not be easily recognized as such [26,39]. Constant pressure algorithms allow the surface area per lipid to adjust. This makes it possible to verify the area per lipid obtained in simulations of liquid crystalline DPPC, and obtain the surface area per lipid as a result in simulations of other lipids or lipid–protein systems. For these reasons, it is generally accepted now that it has advantages to use constant pressure in simulations of lipid systems, although many simulations in the literature used constant volume conditions.

Historically, a pressure (both lateral and perpendicular component) in *NpT* simulations of 1 bar has been used (which is the same as zero bar within the numerical accuracy of pressure calculations) [20,28], implying there is no surface tension in the flat bilayers in simulations. Recently two groups proposed to use a surface tension. Chiu et al. argued that lipid bilayers have a surface tension based on a comparison with monolayers [37]. This seems unlikely since the main contribution to the surface tension in an air–monolayer–water systems comes from the air–monolayer interface. Jähnig has summarized the main physical arguments for a surface tension of zero in flat bilayers in [35]. Feller and Pastor argued that for technical reasons (artefacts introduced by the periodic boundary conditions in simulations) a small surface tension might be appropriate [34]. This would be a correction for a finite size effect, the magnitude of which is difficult to estimate. We found little difference between using a surface tension of 0 and $28\,\mathrm{mN\,m^{-1}}$ [26].

3.2.3. Pressure and temperature control

The temperature in a system is given by the kinetic energy of all atoms. Due to numerical inaccuracy and cutoff effects (see below) the temperature in a system will tend to drift away from the starting temperature. To prevent this, some type of temperature control is necessary. The pressure of a system depends on the forces and positions of all atoms and determines whether the system expands or contracts and, therefore, how the size of the simulation box fluctuates. Many algorithms for pressure and temperature control are discussed by Allen and Tildesly [10] and we will only describe two different approaches that are the most commonly used methods in lipid simulations.

A simple method to control both pressure and temperature is the weak coupling scheme [40], which means the system is coupled to a ‘bath’ of constant pressure or temperature via some suitable coupling parameters. The main advantages are that this method is simple and causes little perturbation of the system. The main drawback is that this method generates an unknown statistical mechanical ensemble; this makes it impossible to interpret fluctuations of thermodynamical averages and there is no conserved quantity. A second drawback is that a bad choice of coupling parameters can lead to unphysical temperature gradients in the system or fast fluctuations of the boxsize.

A second method is the so-called ‘extended system’ approach [38,41,42]. In this method additional degrees of freedom are included for a piston for pressure coupling and thermostats for temperature coupling. This system has the advantage of a well defined statistical mechanical ensemble, although this ensemble includes the unphysical piston and thermostat. Drawbacks include its greater complexity (the equations of motion become much more involved) and possibly oscillations depending on the mass of the piston, although there are ways around this [41].

3.2.4. Electrostatic interactions and cutoffs

Eq. (1) assumes we calculate the interaction of all atoms with all other atoms, but this is highly inefficient for large systems. Since the interactions between atoms become weaker at longer distances, it makes sense to cut them off at some point, i.e. no longer calculate interactions between atoms when the distance between them is more than a certain value. Such a cutoff means part of the interactions are neglected, but how serious this is depends on the type of interactions and the size of the cutoffs used.

van der Waals interactions rapidly decrease with increasing distance, but Coulomb interactions between dipoles, and especially between whole charges, are quite long-ranged. The simplest way to deal with these long-ranged interactions is by ignoring them, but in practice this does not work for systems with fully charged atoms.

In much of the work from our group a cylindrical cutoff was used. All interactions within a cylinder perpendicular to the lipid–water interface with a certain radius were calculated explicitly and for the remainder of the interactions the Poisson equation was solved, based on the average charge distribution in the direction perpendicular to the interface. This remaining part turned out to be so small that it was later neglected [20,28]. The problem with this method is that it becomes expensive for larger systems and does in principle not work at all when there is no cylindrical symmetry.

A popular approach is the use of a spherical double cutoff. This means that all interactions within a certain distance (typically 1 nm) are calculated every step, and every once in a while (typically 10 steps) the electrostatic contributions within a large sphere (typically 1.5–2.0 nm) are calculated and assumed to remain constant over the next ten steps. Varying results with this method have been reported but when applied with care we think it can give good results [43,30,26].

Schulten and co-workers used a multipole expansion method in combination with stochastic boundary conditions. Although this method gives a correct treatment of the coulomb forces, the stochastic boundary conditions are more awkward for technical reasons than standard periodic boundary conditions, and introduce inaccuracies at the surface [44,45].

The most accurate method to treat the electrostatic interactions in a periodic box is solving the Poisson equation for the complete system. Traditionally the Ewald method has been used to calculate the electrostatic interactions in crystals but when the charges in the system are distributed over a fine grid, this method can be applied to other systems too. The long-range electrostatic part then requires the solution of the Poisson equation on this grid, for which standard methods are available [46,47]. This method will no doubt in the near future be in universal use, although it may cause artificial correlations in some cases. In addition, it may involve reparameterizing (parts of) the currently used force fields.

In the remainder of this review we will generally ignore the technical conclusions from the reviewed articles and focus on the biophysically interesting conclusions. However, it should be realized that most of the current literature is dedicated at least in part to methodological questions. We refer to an excellent recent review by Tobias et al. [39] and to Berendsen and Tieleman [22] for more elaborate accounts.

4. Experimental data on lipid bilayers

Since molecular dynamics simulations are based on models, the results of such simulations have to be validated by experimental data. When the simulations yield good agreement with experimental data it is reasonable to trust the basic model and use the simulations to explain experimental results, enhance the models used for the interpretation of experimental data and study phenomena that cannot be studied by experiment.

What kind of data is available for comparison with simulations? Over the last decades a variety of experimental techniques has been applied to lipid systems. However, only a relatively small number of properties can be compared directly with simulation results. These include density profiles (electron and atom densities), cell parameters such as area per lipid, density and bilayer repeat distances in multi-lamellar bilayers, order parameters for the lipid chains, number of bound water molecules and electrostatic dipole potentials. Below we outline some of the experimental techniques.

4.1. Diffraction methods

Neutron and X-ray diffraction are probably the most powerful techniques to determine structures at atomic resolution. Unfortunately, the liquid crystalline phase is highly disordered and only a few diffraction peaks are observed. Nonetheless, considerable progress has been made in experimentally elucidating the structure of a bilayer.

Wiener and White published a series of papers in the early nineties on a method to combine neutron and X-ray diffraction data to arrive at one of the most detailed pictures of a fluid phase bilayer (DOPC) determined by experiments thus far [48]. One of the main new results of this study was the distribution of different atom types as function of the position perpendicular to the membrane interface. Unfortunately, the degree of hydration of this system was much lower than what is biologically interesting and what is used in most simulations. It is not immediately obvious that the structure of a bilayer at low hydration remains the same when more water is added. Another disadvantage of diffraction studies is that it is complicated to obtain data about the structure perpendicular to the interface, including the area per lipid. The area per lipid is an important parameter to check simulations against, and a crucial parameter to know when constant volume or constant surface area boundary conditions are used.

Recently, Nagle et al. determined the structure of a fully hydrated DPPC bilayer by a combination of high resolution X-ray diffraction and a theory to account for the substantial undulation fluctuations of the bilayer [49]. The main results of this study are bilayer form factors, which can be obtained by Fourier transforming electron density profiles, and a number of other structural parameters: the area (62.9 ± 1.3 Å^2) and volume per lipid, the peak-to-peak distance in the electron density profile, the number of water molecules per lipid at full hydration (29.1) and the bilayer repeat spacing (67.2 Å). These values will provide a stringent test for DPPC simulations. Nagle et al. also concluded that the change in electron density profiles as a function of the amount of water added is not caused by a change in the bilayer structure, at least up to a reasonably low hydration level, but rather is an artefact caused by neglecting the effect of fluctuations due to undulations in the interpretation of the experimental data. It turns out that the main results from older studies on less hydrated samples (such as the work on DOPC mentioned above) are relevant to the fully hydrated state when these fluctuations are taken into account.

The more ordered gel phase is easier to study by diffraction experiments and a detailed structure of a fully hydrated DPPC gel phase has been available for some time [50,51]. The available properties include the distribution of methylene groups in the bilayer, electron density profiles and form factors, and many derived structural parameters such as areas per lipid and chain tilt angles.

4.2. Nuclear magnetic resonance investigations

NMR spectroscopy yields ^{13}C–H relaxation times at many positions in lipids. The most elaborate study of such relaxations was reported by Brown and coworkers [52,53]. They studied DPPC vesicles at different field strengths. In principle, the fast motions obtained in these experiments can be compared with the fast motions in simulations, extracted from time correlation functions of C–H vectors. This provides a way to validate the dynamics of simulations, although simulators have rarely used these fast motions [54].

Much more attention has been paid to the order parameters which can be measured by NMR on specifically deuterated lipids. Order parameters are among the most accurately determined experimental properties (for reviews, see [55,56]) and are readily available from simulations. A more recent development is the determination of order parameter profiles from perdeuterated lipids (which are easier to prepare), giving the well known de-Paked spectra (for review, see [57]). Nagle has shown that estimates for the area per lipid can be derived from order parameter profiles [58]. The best estimate for the area per lipid in DPPC using this method was 62 ± 2 Å^2, in good agreement with the recently determined value from diffraction of 62.9 ± 1.3 Å^2 [49].

4.3. Other techniques

Rand and Parsegian have summarized a large number of structural parameters for many different types of lipids [59]. This list has been used to determine

box sizes in constant volume simulations but many of the values in the list have large error margins. In addition many other techniques have been used to determine specific properties: ESR spectroscopy, fluorescence measurements using fluorescent markers, black film measurements to determine permeabilities, IR/Raman spectroscopy to study tail dihedral gauche-defects and order parameters, measurements of membrane surface potentials, partitioning of small molecules, force measurements between membranes, differential scanning calorimetry to study phase transitions and doubtlessly countless others.

Although these results are important in specific studies, they are usually not general or accurate enough to provide critical tests for the validity of a simulation. With the continuous increase in computer power new experimental results come within reach of simulations. Examples of this are chemical shift calculations, which allow direct comparison with chemical shifts measured by NMR. Data from incoherent quasi-elastic neutron scattering experiments (see [60]) yield information on the same time scale (1 ps–10 ns) as MD simulations, which makes it interesting to compare the results from this technique to simulations. This has been done for the short-time mobility of lipids (rattling in a cage) but a more detailed comparison may be fruitful [39]. The simulations could also provide models to base the interpretation of scattering experiments on. We are not aware of such an effort but undoubtedly it will be made in the near future.

5. Liquid crystalline DPPC: a model system

DPPC is one of the best studied lipids, both by experiment and simulation and therefore has been termed the 'benchmark' of lipid simulations. Many simulations of DMPC [61–64,37] and DPPC [28,1,54,36,33,26,30,65–67] contributed to our overall view of this system. When general features discussed below are given without references, most of these studies describe them. We will first discuss the headgroup and tail regions separately and then describe the general structure in terms of the four region model (Fig. 5) that was proposed by Marrink and Berendsen [68].

5.1. The headgroup region

The most conspicuous features of the headgroup region are the considerable width of the distributions of headgroup atoms and water, the ordering of water around the headgroups (hydration shells) and the ordering induced by electrostatic effects.

The interfacial width, taken as the distance over which the headgroup density drops from 90% to 10% (Fig. 2) of its maximum value, is ca. 1.0–1.3 nm in most simulations of fully hydrated DPPC, although this value is sensitive to force field parameters [69,37,26]. This means there is a considerable degree of perpendicular motion of individual lipids and water penetration. The perpendicular lipid motion creates a rough membrane surface which in time is averaged to a smoothly decaying density profile.

Atom density profiles, which give the distribution of the position of a certain lipid atom along the axis perpendicular to the membrane, show that water molecules penetrate up to the carbonyl groups of the lipids (Fig. 2). The density in the headgroup region is the highest in the system and there is little free volume in this part of the bilayer [70,71].

From radial distribution functions of water molecules around the lipid headgroups hydration numbers can be calculated. Marrink and Berendsen

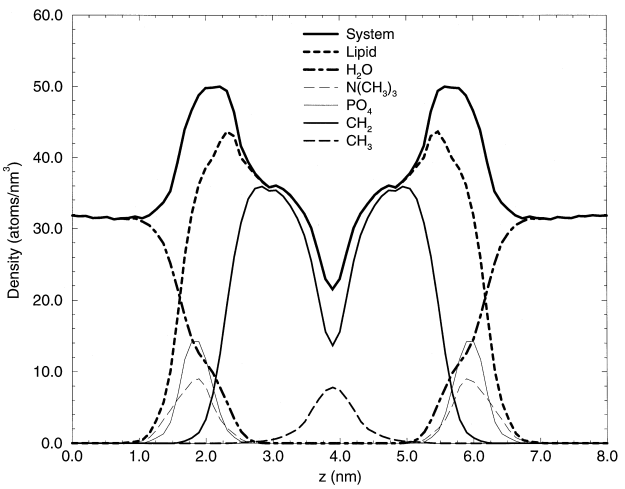


Fig. 2. Typical number density profiles for selected groups of atoms in a fully hydrated bilayer of DPPC at 325 K. The data has been averaged over both halves of the bilayer. For simulation conditions, see [26].

found that on an average 4.2 water molecules hydrate a choline methyl group, 10.2 water molecules hydrate the $N(CH_3)_3$ group, 4.0 water molecules the phosphate and 1.0 water molecules a carbonyl group [68]. This agrees with experimental data that suggest 11–16 water molecules per headgroup and results from Chiu et al. who found 14 water molecules for the entire headgroup [37].

From the mean square displacement of the headgroups and the lipids as a whole diffusion coefficients can be calculated. Due to their length, simulations probe the short time (ca. 20 ps) mobility of lipids, which is determined by restraints imposed by the neighbouring lipids. This motion has been termed 'rattling in a cage'. Short time diffusion coefficients can be compared to neutron scattering data which probes the same time scale. Marrink et al. found a diffusion coefficient for perpendicular motion $D_z = (6 \pm 1) \times 10^{-6}\,cm^2\,s^{-1}$, for lateral motion $D_{xy} = (4 \pm 1) \times 10^{-6}\,cm^2\,s^{-1}$, and for the motion of the headgroups only $D_{hg} = (10 \pm 1) \times 10^{-6}\,cm^2\,s^{-1}$ [69]. These values agree reasonably well with experimental data and show that the short time diffusion of lipids is faster perpendicular to the membrane than in the plane of the interface. The headgroups are considerably more mobile than the entire lipid.

Perhaps the most interesting property of phospholipids like DPPC is their large dipole moment. The tendency to orient their dipoles parallel to the surface, which is favorable in terms of the free energy of electrostatic interactions, and the larger degree of motional freedom in the water phase and steric requirements which oppose this parallel orientation, result in a wide distribution of tilt angles. For the average angle of tilt with respect to the membrane surface in DPPC different values have been found: 30° [28], 15–18° [72], 17° [44] (POPC) and 5° [62]. The large spread may be caused by the relatively short length of these simulations, but in all cases there is a net dipole. This average dipole causes a substantial electric field, which is largely compensated by the orientation of the water molecules.

Molecular dynamics simulations allow the unphysical splitting of the electrostatic potential into components caused by different types of molecules. Analysis of these components shows that the primary charge density of the phosphate and choline groups is completely compensated by the charge density due to water dipoles. The latter can be easily determined from the spatial distribution of partially charged atomic sites. Fig. 3 shows this charge compensation by comparing the cumulative charge distribution of the primary charges with that of the water and other polar groups [73]. In fact, we see that the orientational polarization of the water even overcompensates the primary charge density, thereby inverting the expected polarity of the membrane. This effect is rather subtle; it is sensitive to small variations in the force field used, but practically all simulation studies of uncharged lipids find this overcompensation. The reason is that water has an intrinsic tendency to orient slightly at interfaces, with its dipole tending to point outwards from the water phase.

5.2. The lipid chains

Generally speaking, properties of the lipid acyl chains can be categorized as static or dynamic. Dynamic properties include dihedral transition rates and

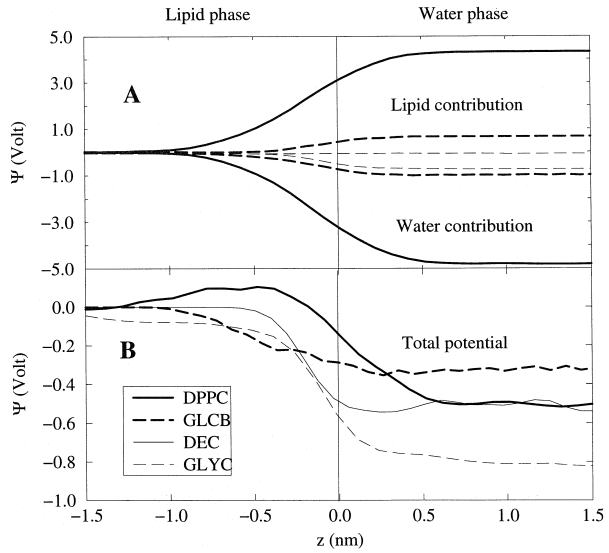


Fig. 3. The electrostatic potential across the interface for three types of lipid: DPPC, β-decylglucoside (GLCB), dilauroylglycerol (DLG) and decane. (A) Simulations allow the unphysical seperation into contributions to the total electrostatic potential from different molecules. The upper half shows the contribution of the lipids, the lower half the contribution of thc water molecules. Decane is not shown since the contribution from decane is zero. (B) The total electrostatic potential across the bilayer. For details on the simulations, see [86].

time correlation functions of the orientation of bonds. In principle such correlation functions contain a wealth of information on different motions in lipids but they are difficult to obtain and interpret accurately. The total motion of lipids in a bilayer is complex and includes slow motions with correlation times of up to nanoseconds [74]. Venable et al. showed that the fastest motions, with correlation times of up to a few tens of picoseconds, can be reproduced accurately when simulations are combined with experimental data on the slowest motions [54].

In addition, the fast motions (< 20 ps) are similar in hexadecane and DPPC bilayers. The microviscosity of the membrane interior is low, which means the measured high viscosity in bilayers is due to the headgroups. Analysis of dihedral transitions confirms the idea that isomerization rates in alkanes and liquid crystalline bilayers are similar. Such isomerizations become faster towards the middle of the bilayer [1,62,28,70].

The main static properties that can be obtained from simulations are order parameters and atom and electron density profiles.

Calculated density profiles can be compared with density profiles obtained by neutron diffraction [48] or electron density profiles obtained from X-ray diffraction [49]. An interesting feature of atom density profiles is the width of the distribution of the CH_3 segment: tails can fold back on themselves to give a noticeable CH_3 density even in the headgroup region (Fig. 2). The overall density of the tail region shows a decrease towards the middle of the bilayer. This decrease corresponds with an increase in free volume, which is important for the partitioning of solutes in the bilayer [75,70,71].

Order parameters are a measure of the spatial restriction of the motion of a CH vector. They can be characterized by a tensor S with elements

$$S_{ij} = \tfrac{1}{2}\left\langle 3\cos\theta_i \cos\theta_j - \delta_{ij}\right\rangle \quad (2)$$

in which θ_i is the angle between the ith molecular axis and the bilayer normal. The brackets denote an ensemble average. Usually, for the C_n methylene group the C_{n-1}–C_{n+1} direction is taken as z, and the C_{n-1}–C_n–C_{n+1} plane is the yz plane. S is a symmetric tensor with zero trace and with $S_{xy} = S_{xz} = 0$ due to molecular symmetry. However, S_{yz} is not necessarily zero and S_{xx} is not necessarily equal to S_{yy}. If the motion of the segments is symmetrical about the z-axis, as is often assumed, there is only one order parameter, $S_{zz} = S_{\text{chain}} = -2S_{xx} = -2S_{yy}$.

A typical order parameter profile for DPPC is given in Fig. 4. The deuterium order parameter S_{CD} can be calculated using

$$-S_{CD} = 2/3S_{xx} + 1/3S_{yy} \quad (3)$$

or can be evaluated directly if an all-atom force field has been used. It should be realized that order parameters in simulations converge only slowly, especially for the carbon segments close to the headgroup [2].

Dichroic ratios from attenuated total reflection infrared measurements depend on order parameters and can be used as a check on simulations [76].

The order parameters are related to the tilt angle of the chains and to the trans-gauche distribution of chain dihedrals, but the relation is indirect. In general it has been observed from simulations that the number of gauche defects in membranes above the phase transition is smaller than in liquid alkanes. With

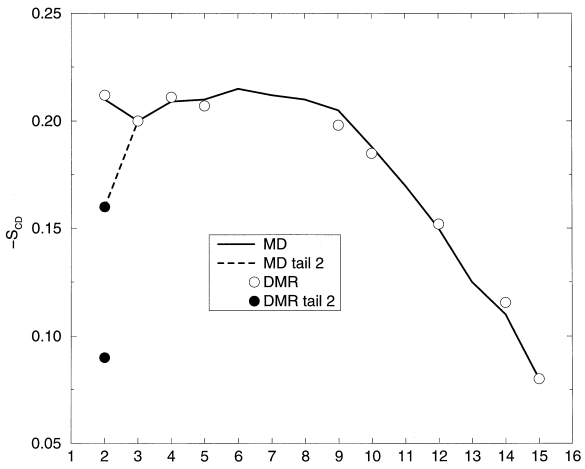


Fig. 4. A typical order parameter profile from a simulation of DPPC at 350 K and from NMR on selectively deuterated lipids. Atom #1 is the carbonyl, atom #16 the terminal methyl group. All values are averaged over both tails, except for carbon #2, where a significant difference between tail 1 and tail 2 is observed. Note that the experimental data indicate two long living conformations for carbon #2 at tail 2. An interesting feature of the experimental data is the slight dip in the order parameter profile right behind the headgroup. After [125].

decreasing temperature or increasing chain length the fraction of gauche dihedrals decreases. In order to explain the plateau region in the order parameter profile, the existence of 'kinks' (gauche ± -trans-gauche ∓) has been proposed [77]. Suchs kinks are indeed observed in simulations, but they are not the dominant factor of the chain defects [20].

5.3. Four region model of the lipid bilayer

Based on the data described above Marrink and Berendsen proposed a four region model of the lipid bilayer (Fig. 5). Although the model is based on simulations of DPPC it should be generally applicable to fluid phase bilayers.

5.3.1. Region 1: perturbed water

The first region, which can be quite broad, starts where the water molecules begin to feel the presence of the lipid headgroup and ends where the lipid and water densities are comparable. The lipids protrude into this region with their dipolar headgroups. The water molecules show a smoothly decreasing orientational profile, caused by the dipolar headgroups, from the interface towards the bulk water phase. This region is likely to be the most important part of the membrane for the interaction with other membranes or proteins.

5.3.2. Region 2: interphase

In the second region, which is roughly 0.8 nm broad in DPPC, the total water density decreases to almost nothing and the lipid density reaches its maximum. This region has the highest density in the system and all of the headgroup atoms and part of the tail methylenes spend most of their time here. All water molecules in this region are part of hydration

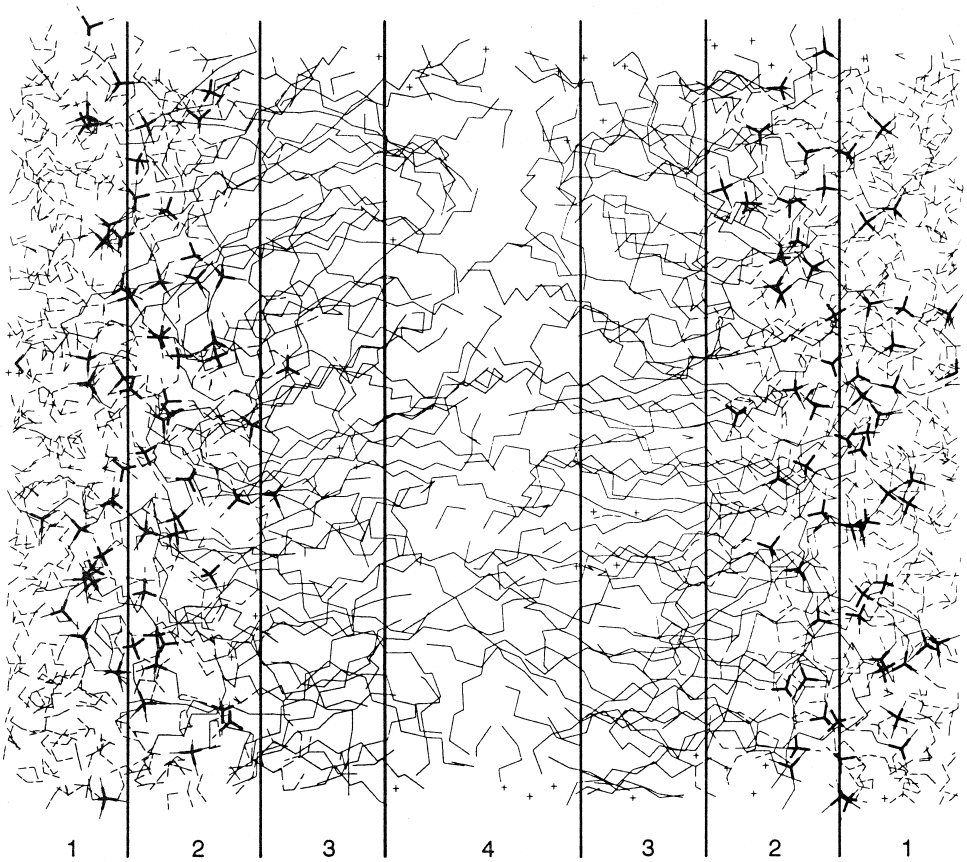


Fig. 5. A schematic overview of the structure of a DPPC bilayer in the context of the four region model. Dashed lines are used for water, bold lines for choline and phosphate groups. Vertical lines are the boundaries between the different regions. Crosses result from bonds cut by the boundary planes. Reproduced with permission from [68].

shells of the phospholipid headgroups. The diffusion coefficients of water and other small molecules and the free volume fraction are lowest in this part of the bilayer. The interactions in this region are complex due to the high density and the presence of many partial charges.

This region could play the most important role in phase behavior of lipids; the (water mediated) interactions between the headgroups strongly influence the surface tension and surface curvature, which are main driving forces for phase transitions and phase separations.

5.3.3. Region 3: soft polymer

The third region (ca. 0.8 nm) is characterized by a high tail density and low free volume. It consists of partially ordered chains, resembling a soft polymer in density and properties. It starts at the carbonyl groups (which is also the limit for most penetrating water molecules) and ends where the chains have reached a density comparable to liquid hexadecane. The chain order parameters are at their plateau values and vary little over the first methylene groups.

This region is the main barrier to permeation of small molecules. Most of the anomalous effects of the permeation process originate in this region (see Section 8.2). The parallel alignment of the lipid tails may help to orient various trans-membrane molecules.

5.3.4. Region 4: decane

The fourth region, the middle of the bilayer, is characterized by a low density, comparable to decane, and a high fraction of free volume. Order parameters are close to zero and little perturbing effect from the interface is present.

The almost completely hydrophobic environment will favor the solution of hydrophobic molecules. The low density and corresponding large free volume allow for the incorporation of larger molecules in this region.

6. Other lipids

DPPC can be considered the benchmark lipid in the study of model bilayers, both experimentally and by simulation. However, a number of other lipids have been the subject of simulation studies as well. From the point of view of an MD simulation DMPC and DPPC are practically the same; the only significant differences are the slightly shorter acyl chains and a corresponding shift of the phase diagram to lower temperature by roughly 30 K. However, most biological membranes contain mixtures of lipids and proteins and the lipids are generally unsaturated. We can expect a shift of attention in simulation studies from the 'boring' DPPC to other lipids once DPPC can be accurately simulated (which is about at the present time). A few preliminary studies of unsaturated lipids are already available in the literature and they will be described below. Second to DPPC, DLPE has been a favorite lipid in the simulation community, partially because a crystal structure is available, and partially because of the interesting contrast of the PE headgroup with the PC headgroup.

6.1. Unsaturated lipids

Heller et al. studied POPC in a simulation of a large system of 200 lipids [44]. This represented an extraordinary computational effort by the standards of 1993, when the paper was published. The structure of POPC appeared much the same as DPPC and DMPC in many properties. The headgroups are the same and this turns out to be more important than the difference in the chains. The average angle between the double bond and the normal to the membrane was 38.5°. In an idealized geometry with an all-trans configuration this angle would be 26°. The order parameter profiles for both the saturated and unsaturated chains were in reasonable agreement with experimental values but the length of the trajectory that was used for analysis (ca. 100 ps) is too short for these order parameters to converge.

The only other unsaturated lipids that have been studied in the literature are DOPC and DOPE. In a comparative study of DOPC, DOPE and DLPE, Huang et al. simulated systems of 24 of these lipids, for 200 ps at 37° using constant pressure [78]. The main conclusion from these simulations was that the areas of the headgroups and the effective chain areas measured from the simulations were in agreement with the theoretical predictions for the phase these lipids will adopt: DOPC an inverted hexagonal phase, DOPE a bilayer. The area per lipid decreased from DOPC via DOPE to DLPE, and the same trend was

observed for the width of the interface. The calculated angles between the double bond and the bilayer normal in the DOPC simulation was 38.2°, close to the value obtained by Heller, and for DOPE 36.1°.

6.2. Phosphatidylethanolamine lipids

DLPE has been the topic of a large number of studies [78–81,64,45,67,82]. In a few of the earliest attempts to model a lipid bilayer Berkowitz and coworkers studied DPLE to try to explain the hydration force [79,80]. In the first paper the lipid headgroups were restrained and an oscillatory decay of the water density was found, but in later work with longer simulations on unconstrained lipids this decay became smooth again. Compared to PC lipids the width of the interface is typically somewhat smaller in DLPE.

The hydration of the headgroups is probably the most drastic difference between PC and PE. It is generally found that the simple substitution of three CH_3 groups by hydrogens has large consequences for the hydration of the headgroup and the structure of the water–lipid interface. Instead of clathrate like hydration shells now direct hydrogen bonds can be formed between the NH_3 group and water molecules. This results in a significant decrease in the area per lipid in PE bilayers. Inter- and intra-molecular hydrogen bonds are also possible now. A few groups noted that at low hydration levels, when two interfaces of two opposing bilayers approach each other, hydrogen bonds exist between lipids in opposing membranes. This obviously is not possible in PC lipids and explains the much lower swelling limit for PE. For a more detailed overview we refer to Section 9 on hydration forces, since much of the discussion on the differences between DLPE and PC deals with the origin of hydration forces.

6.3. Charged lipids

López Cascales et al. studied a bilayer of 64 DPPS lipids, 796 water molecules and 64 Na^+ ions [83,84]. The main conclusion, based on 360 ps equilibration and 184 ps production run, was that there are a few differences between DPPC and DPPS. In DPPC and many other dipolar interfaces [85,86] water overcompensates for the potential due to the lipid dipoles. In the charged DPPS the water did not fully compensate the potential caused by the lipids and ions and the potential is positive in the water layer with respect to the membrane interior. Salt bridges between lipid headgroups caused clustering of lipids. The atom density and charge density profiles for the headgroup atoms of DPPS are sharper than for DPPC. The order parameter profiles for DPPC and DPPS are very similar.

From a biological point of view it will be interesting to extend the work on charged lipids to a system of neutral lipids with a low concentration of charged lipids. Because of the slow equilibration times of ions, extending the simulations to a longer time scale will be interesting. The response of such a membrane to the presence of calcium ions is of biochemical interest, but may exceed the accessible time scale.

6.4. Cholesterol

Molecular dynamics studies of mixtures of lipids are rare. Edholm studied the influence of cholesterol on a model membrane, but this model used simplified headgroups and no solvent was present [87]. We are aware of only two recent works on mixtures, both dealing with the important interaction of cholesterol with PC lipids. Cholesterol is an essential component of eukaryotic plasma membranes that influences a variety of physical properties of the membrane. The physical effects of cholesterol depend on the phase of the membrane. Cholesterol breaks the structure of a gel phase, making it more disordered, but it increases the order in liquid crystalline phases, thus modulating the phase transition. The precise mode of interaction between cholesterol and other lipids is not known. Experimental structure determination of cholesterol/PC mixtures is complicated due to their disordered structure, which makes cholesterol an interesting case for simulations. For a recent review on the effects of cholesterol we refer to McMullen and McElhaney [88].

Robinson et al. simulated a system of 36 DMPC and four cholesterol molecules at 50° [89]. The 400 ps simulation was reasonably successful in reproducing some of the experimentally observed effects of cholesterol on PC lipids. The order parameters of the

lipid atoms adjacent to cholesterol were increased, together with an increased trans fraction of tail dihedrals and a decreased kink population, which could have an effect on the permeability of a cholesterol containing membrane. Cholesterol decreased the average tilt angle of the hydrocarbon chains with respect to the bilayer normal. Different populations of hydrogen bonds between the cholesterol hydroxyl group and other molecules were found: between cholesterol and the phosphate oxygens of DMPC, between cholesterol and water, and between cholesterol and carbonyl oxygens. This means that even on the short time scale of 400 ps cholesterol is quite mobile laterally. Although this is an interesting study, 400 ps is relatively short to study a lipid mixture and their data indicates that not all distributions of for instance trans-fractions and order parameters have converged yet. It would certainly be interesting to extend simulations of this or a similar system to a longer time scale, preferably under constant pressure conditions.

Gabdoulline et al. studied the influence of temperature and cholesterol on DMPC [90]. They studied two systems: a pure system of 32 DMPC and a mixed system of 16 DMPC and 16 cholesterol molecules. Both systems were simulated at 200 K for 100 ps, heated to 300 K in 300 ps, and finally heated to 325 K in 200 ps. The last 100 ps at 325 was used for analysis. Their main findings were an increase in the trans fraction in the tail dihedrals of the DMPC: cholesterol system, compared to the pure DMPC system. The distribution of the P–N vector in the mixed system was broader and the surface dipole potential decreased from -140 mV in the pure DMPC to -60 mV in the mixed system. This could indicate that the membrane permeability for ions is changed by the presence of cholesterol.

Unfortunately, 100 ps is too short to accurately sample the motions of cholesterol, especially in a small system. In addition, the area per lipid at 325 K for the pure DMPC system was only 50 $\AA^2$ and the gauche fraction of tail dihedrals was about 0.13, both values much too low for a liquid crystalline phase. Egberts et al. found the same behavior, which is an artefact caused by the carbon tail dihedral parameters of the GROMOS 87 force field [28]. Therefore, the results of this cholesterol/DMPC study should be regarded with caution.

7. Gel and crystal phase simulations

7.1. Gel phases

Two early attempts to model a liquid crystalline phase (of DPPC and POPC, respectively), led to highly ordered structures with a low area per lipid, close to gel phase structures [44,28]. In those studies a gel phase structure arose at temperatures above the main phase transition, due to force field artefacts. The main features that distinguish the gel phase from the liquid crystalline phase are the lower area per lipid, the higher fraction of dihedrals in the trans configuration, a corresponding increased hydrocarbon thickness and much higher chain order parameters.

Deliberate attempts to model the gel phase have been rare. This phase is biologically less interesting than the liquid crystalline phase, although there are indications that gel-like domains exist in cells. The gel phase is interesting from a methodological point of view; much more accurate structural data is available on gel phases [51], because of their higher degree of ordering compared to the liquid crystalline phase.

Essmann et al. studied four DPPC systems: two gel phase bilayers at 11°C with 11 and 20.5 waters per lipid, and two liquid crystalline bilayers at 60°C [67]. In a direct comparison of the gel and liquid crystalline bilayers they found that the distribution of carbonyl and phosphate groups is narrower in the gel phase. The distribution of the $N(CH_3)_3$ groups is broader and consists of two distinct populations. Essmann et al. observed that the tilt of the chains in both monolayers of the system is in different directions, which could be an artefact of the simulations.

A more detailed structural analysis of a DPPC gel phase is given by Tu et al. [91] They simulated a system of 64 DPPC lipids with 11.8 water molecules per lipid, at 19°C, close to the experimental conditions in [51]. After a run of over a nanosecond, the area per lipid, bilayer thickness, chain tilt angle and lamellar spacing were in good agreement with X-ray results. In addition, the atom distribution agreed closely with data from neutron diffraction. In contrast to liquid crystalline DPPC, water molecules did not penetrate as far as to the carbonyl groups. In an original analysis, the unit cell parameters of the distorted hexagonal lattice could be obtained from a

direct calculation of wide angle X-ray diffraction patterns. In agreement with the work of Essmann a bimodal headgroup distribution was found. A novel feature of the gel phase in this simulation is the asymmetric shape of the methylene density profiles (these are usually taken as Gaussians).

However, a pleated arrangement at the center of the bilayer was found, whereas the experimental X-ray structure shows a parallel arrangement. This may have been caused by the starting structure (the crystal structure of DMPC), in which case extending the simulation might improve the structure. An other reason could be a problem with the hydrocarbon parameters. Interestingly, a similar problem was encountered by Essmann et al., who used a different force field.

As part of their comparative study on DOPE, DOPC and DLPE, Huang and Loew also simulated a gel phase structure of 24 DLPE molecules and 11.8 water molecules per lipid [78]. They correctly reproduced the high order parameters and found a trans fraction for the tail dihedrals of almost unity along the chains, except for the first and last carbon segments. To test the size dependency of the results they did a test run of a larger system consisting of 60 DLPE molecules and a water layer of 6 Å. Essentially the results remained unchanged, indicating that, as long as a reasonable minimum size is used, the simulation results over at least shorter time scales (< 1 ns) are not much influenced by the size of the system.

7.2. Crystals

Crystal structures of lipids and lipid fragments have mainly been used to test force fields. Although it is nice if a force fields accurately reproduces the experimental structure, geometry and density of crystals, it is no guarantee that equally good results will be obtained in fluid phase simulations. Nonetheless, crystal simulations provide an interesting test.

Stouch et al. simulated crystals of glycerylphosphorylcholine (GPC), which is basically the headgroup of a PC lipid, and dilauroylglycerol (DLG), which is basically the tail part of any phospholipid. The deviations from the crystal structure were small, indicating a reasonable force field [27]. Later simulations of liquid crystalline DMPC bilayers using this force field also gave good results [61]. Tu et al. [31] tested this same force field on GPC, DLG and cyclopentylphosphorylcholine monohydrate (CPPC), using a much more critical fully flexible simulation cell. A fully flexible simulation cell makes it possible to test whether a force field gives the correct density and cell parameters. It turned out that the force field of Stouch and coworkers performed well in these simulations, giving deviations of a few percent from the experimental densities and geometries. The CHARMM22 force field [29] gave densities that were too high for GPC and CPPC, and reasonable for DLG. A newer version of CHARMM 22 improved the results to a level comparable to Stouch's force field [31].

8. Transport of small molecules

The permeation process of small molecules across a lipid bilayer is an interesting topic for study with MD, as experimental techniques are unable to reveal the detailed motions of the small penetrants within the membrane interior. For most penetrants, the equilibrium concentration in the membrane is too low to be detected experimentally. Attaching special labels is not possible as this would change the permeation characteristics dramatically, especially for small penetrants. The best experimental data available are overall permeation rates, that can be obtained from osmotic, radio tracer, and NMR measurements.

Several studies compare a large number of permeation rates of different penetrants, from moderately polar to polar, e.g. [92–95]. The main conclusions drawn from these studies are the following: solubility of penetrants into the membrane interior is found to correlate best with slightly polar alkanes, the rate limiting step of permeation is likely to be located just behind the headgroups, and a steep size dependence is observed for the smallest penetrants, resembling the effect found in soft polymers. A major aim of the use of MD simulations is to understand these trends at a more detailed level.

Since early simulations mainly focused on how to realistically simulate membranes themselves, the study of transport properties has only just begun. As the permeation process of most molecules is slow, at least on the time scale available to MD, several

nanoseconds at most, we have to resort to special tricks in order to get statistically significant information. For instance, several seconds of simulation of a lipid membrane of ca. 4.0 nm × 4.0 nm lateral dimensions are needed to observe spontaneous transport of water across the membrane. Standard procedure therefore is to either drag the penetrants into the membrane or to put them within the membrane to start with. As is conclusively shown in all simulations of lipid membranes thus far, the membrane interior is far from homogeneous. Therefore, in order to get a full description of the permeation process, one has to make sure to sample the membrane-penetrant interactions across the whole membrane.

To do this we can either apply constraints to locally sample the membrane-penetrant interactions, or just perform normal MD and follow the trajectory of the penetrant in time. In order to describe the full permeation process, knowledge of both the local diffusion rates and the local solubility is required. Considering the large amount of computer time needed for such constraint simulations, most MD studies thus far have focused on diffusion only.

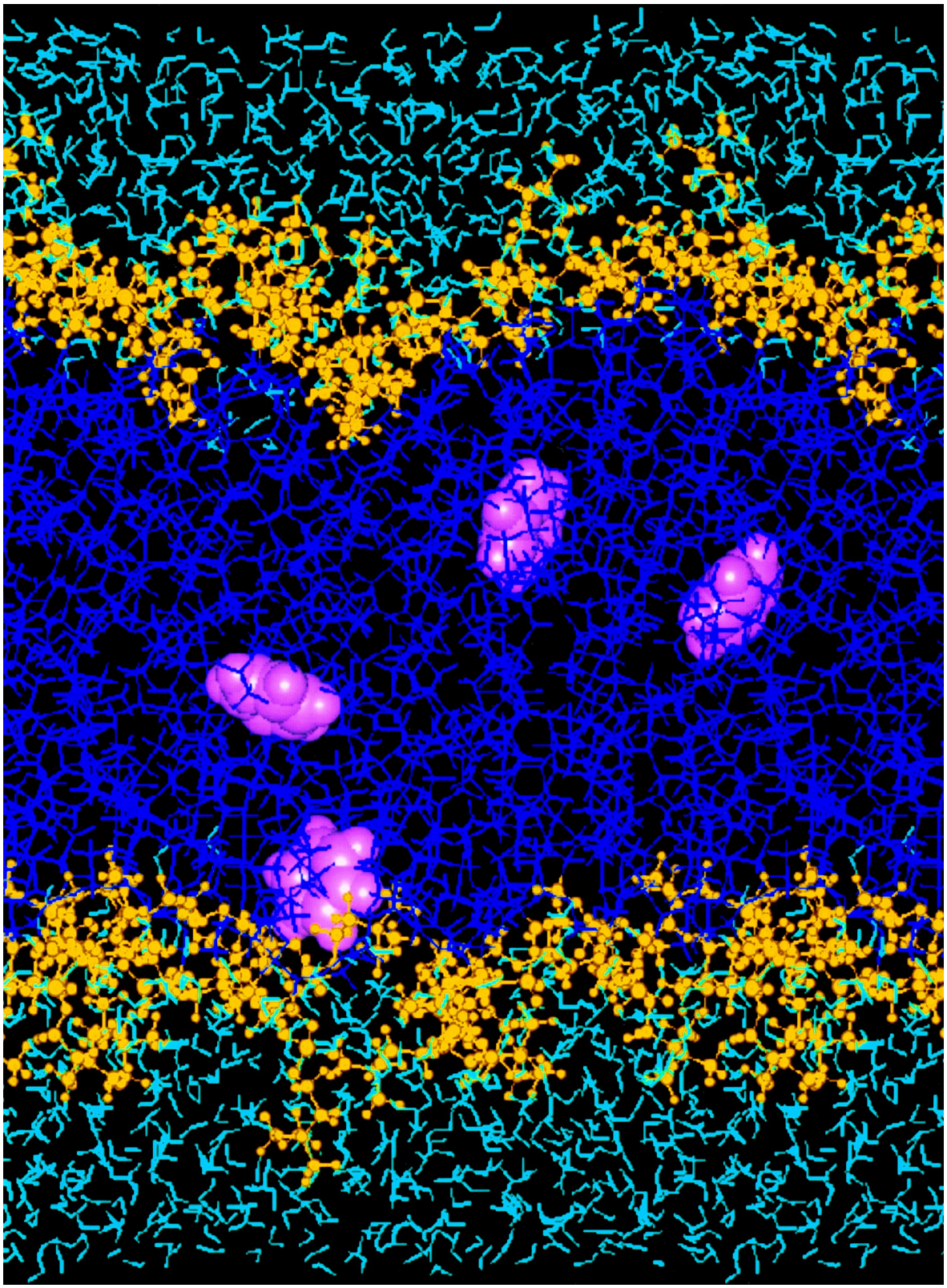
8.1. Diffusion

The first reported MD study in this field dates back to 1992 [96]. McKinnon et al. studied the diffusion of oxygen across lung membranes, and in particular the dependency of the diffusion rate on cholesterol concentration. However, the membrane model was crude, consisting of a monolayer of hexadecane molecules constrained at one end by a harmonic potential. Different concentrations of cholesterol were obtained by substitution of some of the hexadecane molecules. A harmonic restraining potential, which was shifted across the membrane during the simulation, was used to drive the oxygen through the monolayer. The diffusion rate can then be computed from the average friction force on the oxygen molecules. Although the membrane model was rather simple, the computed diffusion rate of oxygen across the hexadecane monolayer turned out to be in close agreement with an experimental estimate of oxygen diffusion in DPPC based on quench pyrene fluorescence. It was further shown that increasing cholesterol concentration resulted in a facilitated diffusion.

Bassolino et al. reported the first simulation of diffusion across a more realistic membrane in 1993 [75]. This paper was followed by two others from Stouch and coworkers, on the diffusion of small molecules in lipid membranes [70,97]. The membrane model explored in these studies is a fully hydrated DMPC bilayer, which was shown to compare favorably to most available experimental data [61]. The first paper deals with the diffusion process of benzene. Four simulations were conducted; three of them with a single benzene molecule placed at different positions in the membrane, and another simulation with four benzene molecules present at the same time (Fig. 6). Diffusion rates could be extracted by computing the mean square displacement (MSD) of the (unconstrained) molecules. For purely Brownian motion, the slope of the MSD curve at long times is proportional to the diffusion constant. The total simulation time, including equilibration, exceeded 4 ns. During the simulations it turned out that the benzene molecules did not have a strong preference for a particular part of the membrane. However, the rate of diffusion was found to depend on their location in the membrane, the benzene molecules diffusing 2–3 times slower in the headgroup region than in the center of the bilayer. The authors further concluded that the mechanism of diffusion is, at least partly, due to jumps of the benzene molecules between voids in the lipid matrix, similar to the diffusion process as observed in soft polymers. Since the number of free volume voids increases towards the bilayer interior, the higher diffusion rate originates from a higher probability of jumps.

In a subsequent paper [70] the effect of temperature on the diffusion rates and mechanism was studied. Comparison of four 1 ns simulations ranging in temperature from 310 to 340 K revealed that the

Fig. 6. Snapshot of system with benzenes. The benzene molecules are shown in pink as CPK models, the atoms in the headgroups in yellow, the hydrocarbon chains in blue, and the water molecules in cyan. Reproduced with permission from [75].



effect of temperature on the diffusion process is rather complicated. As is shown in the accompanying free volume analysis, the number and size of the voids changes significantly across this temperature range. As a result, different diffusional mechanisms dominate in different regions for different temperatures.

In some cases the simulation times turned out to be too short to obtain accurate local estimates. In addition, at lower temperatures the benzene shows a distinct preference to remain in the bilayer center, where the number of voids is relatively large. This implies that there is a thermodynamic gradient driving the benzene away from the interface, in which case the simple method of MSD is not applicable at all.

In the third paper [97], Alper et al. study the diffusion of a nifedipine analogue in the membrane using the same system and methods as in the benzene simulations. In contrast with benzene, the rate of diffusion of the nifedipine analogue did not vary with location in the bilayer. This clear difference is attributed to the observation that the nifedipine analogue is much larger and therefore unable to diffuse via the jump mechanism, which in the case of benzene facilitated diffusion toward the center of the bilayer.

Some smaller scale studies studying the transport process of other uncharged penetrants have also appeared in the literature recently. Paci et al. showed that the polar penetrant DMSO, upon entering the membrane, tries to drag a water molecule inside [98]. Unfortunately no reliable statistics could be obtained to compute the free energy profile from constrained simulation.

Jin and Hopfinger recently studied the diffusion process of two moderately polar molecules, methanol and propanol [99]. Using a rather small DMPC system, they show that the diffusion rates (however biased by the possible presence of thermodynamic gradients) increase towards the middle of the bilayer. The difference between diffusion rates of methanol and propanol is most pronounced in the dense region of the membrane, with methanol, being much smaller, diffusing faster. The authors speculate about the nature of the diffusion process in this region being through kink diffusion, but their data do not show any evidence for support.

8.2. Permeation

A series of detailed and systematic studies of permeation of small molecules across a DPPC bilayer has been performed by our own group [68,100]. These simulations are different in the sense that they study both the diffusional behavior of the penetrants and the solubility of the penetrant into the membrane. Both solubility and diffusion determine the overall permeation rate of a molecule across the membrane.

In the first paper [68] the permeation process of water was studied, a process that has a general role in establishing osmotic balance. The diffusion constant in the membrane was obtained by constraining a water molecule at a certain position within the membrane, allowing it to diffuse laterally only. The local friction coefficient, which is inversely proportional to the local diffusion coefficient, can be computed from the fluctuations in the forces that the constrained molecule experiences. The major advantage of this method is that it can be used in the presence of a thermodynamic gradient that would drive the molecule away in an unconstrained simulation.

In order to compute the solubility, one needs to know the excess free energy of a water molecule within the membrane. We employed three different methods to compute the excess free energy in order to increase the accuracy. In the interfacial region (regions #1 and #2 in the four region model), the local equilibrium density of water molecules directly provides an estimate of the excess free energy. In the dense part of the lipid tails (region #3), the average force exerted on constrained water molecules equals the slope of the local excess free energy curve. Finally, in the middle of the bilayer (region #4), the free energy was calculated from random particle insertions.

The computed over-all permeation rate fell within the range of values estimated from various experiments. More importantly, it was shown that both the diffusion constant and the solubility of water strongly depend on the location within the membrane. Therefore, application of a standard solubility-diffusion model to account for permeation rates of water greatly oversimplifies the nature of the lipid membrane. Furthermore, the rate limiting step in the total permeation process is the solvation of the water molecule into region #3 of the lipid membrane.

In the subsequent paper [73] the same membrane system was used to perform a more systematic study of the permeation process of different types of small penetrants. Using the same methods as in the water permeation study, some general conclusions could be drawn by comparing penetrants differing in hydrophobicity, size, and shape.

For apolar (oxygen) penetrants, the membrane behaves like a permeation 'accelerator' rather than a permeation barrier. Fig. 7 shows the striking difference between the membrane resistance to permeation for a strongly polar penetrant (water), a moderately polar one (ammonia), and an apolar one (oxygen). The origin of this effect is the large solubility of apolar penetrants in the lipid membrane. Within the context of the four region model it was concluded that the effect of size is dominant in region #3, as the lipid free volume is lowest in this region. Both the solubility step and the diffusion step in this region strongly depend on the size of the penetrant, and there is evidence for a hopping type of diffusion. The same region was also found to be the most important for discriminating between spherical and elongated molecules. Solubility data showed a relative stabilization of elongated molecules in this area. Together with an anticipated enhanced diffusion rate, we predict a relatively high permeability for nonspherically shaped penetrants.

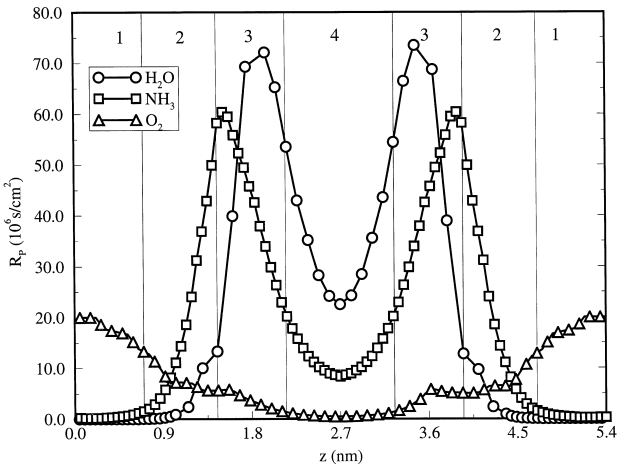


Fig. 7. Local resistance to permeation of water, ammonia, and oxygen in the membrane. The middle of the water layer is located in region 1. For reasons of clarity, the profiles of ammonia and oxygen are scaled by a factor of 10 and 10^4, respectively. Reproduced with permission from [100].

Xiang et al. used a combination of statistical mechanical theory (SMT) and MD in another indirect approach to the problem of penetrant transport [101]. An MD simulation of a simplified model lipid membrane is used to obtain a pressure profile across the membrane. From this profile the local excluded volume interaction is calculated which, using statistical mechanical theory, allows for the computation of molecular distributions across the membrane. The combined approach of MD and SMT results in a more realistic description of molecular distributions than ordinary mean field theory. In particular, the method is able to predict shape effects on solute partitioning. It is shown that, at a given molecular size, the partitioning of elongated molecules into the membrane is facilitated compared to more spherical molecules, especially in the densest part of the membrane (with the highest lateral pressure). However, this shape effect is shown to be small compared to size effects, which are also largest in the densest part of the membrane.

8.3. Ion transport

Unassisted ion transport has thus far only been studied in simplified systems, but since these systems will give an idea of the difficulties that we can expect in simulations of ion transport in phospholipid membranes we will briefly discuss them.

Benjamin and coworkers reported studies of ion transfer across a water/1,2-dichloroethane interface [102–104]. They compute the excess free energy (mainly used for qualitative predictions) of dragging a chloride ion across the interface by splitting the total free energy into a part arising from cavity work and into an electrostatic part. The first contribution is estimated using scaled particle theory, the second by a simplified perturbation computation on constrained ions at various positions across the interface.

They find that the computed free energy strongly depends on the hydration state of the ion as its position is moved from the apolar medium into the water layer. Due to the presence of spontaneous as well as ion-induced fluctuations of the interface, the hydration state of the ion will depend on how much time it can spend at a certain position trying to

encounter such a fluctuation ('water finger'). Once the ion becomes hydrated, it will keep its hydration shell in the apolar medium as this is energetically favorable.

One of the main conclusions is that the presence of interfacial water fingers is important for the transfer in both directions, from water to 1,2-dichloroethane and the other way around.

Wilson and Pohorille studied the unassisted ion transport across a glycerol 1-monooleate bilayer [105,106] and they also found that deformations of the membrane play an important role in this process. Permeation of ions is accompanied by the formation of deep, asymmetric thinning effects in the bilayer, with water and polar parts of the lipids penetrating into the bilayer. Na^+ and Cl^- ions remain well solvated at any position in the bilayer and are either surrounded by water molecules or polar atoms from the lipids. The authors calculate that the permeability of a membrane with the thinning defects is 14 orders of magnitude higher than that of a rigid intact membrane. In total 10 ns of simulations were needed for each type of ion.

Although neither the work of Benjamin and coworkers nor the work of Wilson and Pohorille deals directly with phospholipid membranes, their work shows that cooperative effects on a larger scale than for non-ionic penetrants play a crucial role. To extend their work to phospholipid membranes will present an interesting challenge.

The only other study to date that involves the transport of ions is the study of proton transport across a DPPC membrane [71]. In this simulation, the actual proton is not modelled at all, but instead the hypothesis that protons cross the membrane very efficiently via transient water pores is tested. Transport equations are derived for various mechanisms of the actual proton hopping across such a (hypothetical) pore. The likelihood of the mechanisms strongly depends on the formation rate and stability of the water pore. Therefore the free energy profile of the formation of a single file water pore is computed by pulling a loosely coupled strand of water molecules into the membrane (Fig. 8). It is concluded that the mechanism of fast proton transport across transient water pores can only account for the experimentally observed rates if the entrance of the proton at the pore is not rate-limiting.

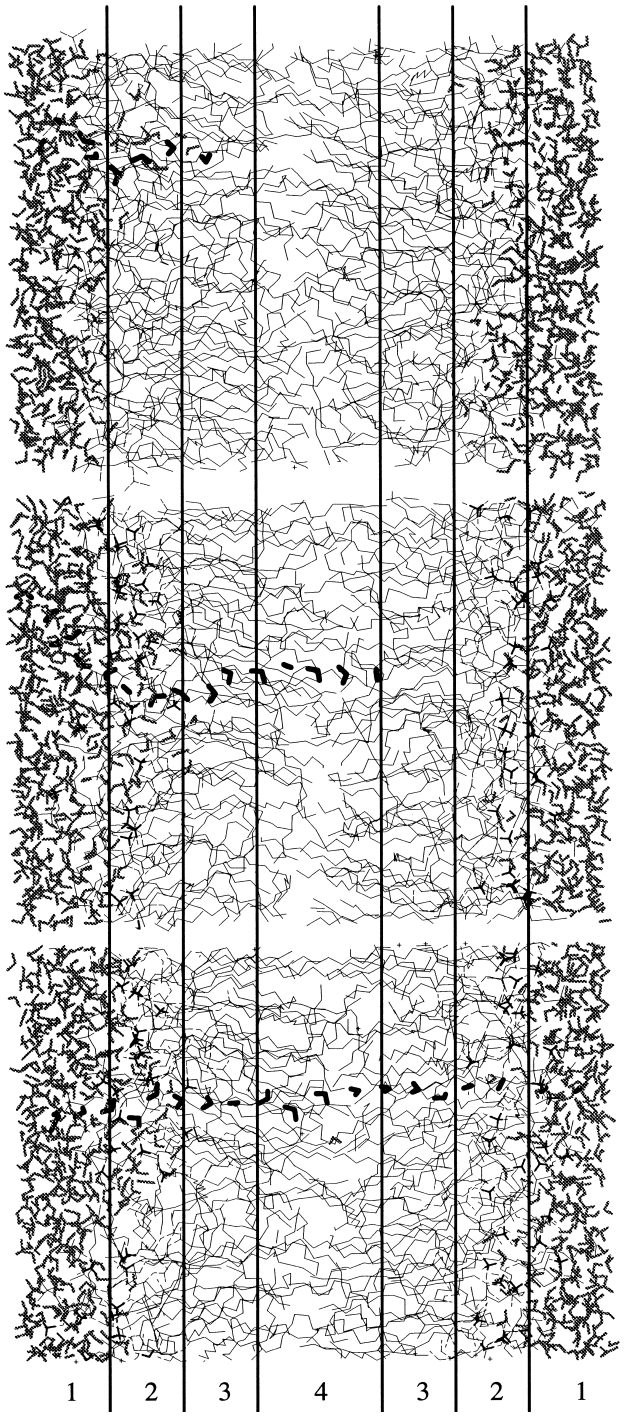


Fig. 8. Snapshots of a file of water molecules in a DPPC bilayer during the process of pore formation. The pore molecules are drawn with bold black lines; the other water molecules in lighter lines. The four regions correspond to those in Fig. 5. Reproduced with permission from [107].

8.4. Discussion

Although the field of penetrant permeation across lipid membranes, studied by MD, is young, already some general features have emerged. Qualitatively, all studies show that the inhomogeneous nature of the membrane is reflected in the interaction between the membrane and the penetrant. Both diffusion rates and solubilities [70,68,100,93,99] depend strongly on the position of the penetrant within the membrane. The studies of Marrink et al. and Xiang et al. [68,100,93] indicate that region #3, the dense tail region, is likely to be rate limiting in the permeation process of most penetrants. This confirms the patterns that arise from regression analysis of large series of experimental permeation rates [93]. Another hypothesis, based on experimental data, that the diffusion of small penetrants occurs via a hopping mechanism like in soft polymers [92], has found support in several simulations [75,68,100]. Furthermore, special size effects are seen in most studies.

It appears that the size of the penetrants is more discriminative in the denser part of the membrane [100,93,99], and that penetrants gradually change their diffusional mechanism from hopping to a Brownian type of diffusion upon increasing size [97,71]. Shape effects are pointed out by both [93] and [100], with both studies showing an enhanced solubility of non-spherical molecules especially in region #3. Experimentally this effect is probably hard to detect, as size effects are found to be more important.

For qualitative trends the simulations have thus far proven to be useful. Quantitative predictions are harder to make, as the techniques for studying penetrant transport still need improvement, and statistically significant results can be obtained only at the limit of currently available computer power. Especially when the complexity of the penetrants increases, either due to an increase in size (e.g. nifidrine [97]), or due to the presence of important electrostatic interactions (e.g. ions [103], DMSO [98]), current computer power is insufficient for quantitative predictions.

For the smallest molecules (e.g. water, oxygen, methanol), or larger apolar ones (benzene), quantitative comparison of MD results with available experimental data turns out quite favorable [96,75,68,99]. However, one has to keep in mind that experimental data are often measured at different conditions and/or in different systems than the simulated ones, and therefore an accurate quantitative comparison is often not possible.

Future work in this field should be aimed at more systematic studies to compare transport behavior of different penetrants using the same simulation parameters. Not only diffusional data, but also solubility data should be studied in more detail, as the solubility profile is the main determinant of the total permeation rate [68,107]. We believe that for more quantitative insights into diffusional behavior the method of MSD should be avoided. Diffusion rates computed with this method will include a bias if a thermodynamic gradient is present. Considering the inhomogeneous nature of the membrane this will almost always be the case. With increasing computer power, reliable free energy estimates may become possible for charged and/or bigger penetrants. However, it is possible that some of these processes, such as the hydration/dehydration of ions, require macroscopical simulation times. In that case, a non-equilibrium description of permeation is needed.

9. Hydration force

The existence of hydration forces between colloid systems in general, and between lipid membranes in particular, is still a controversial issue. Whereas some provide evidence that hydration forces arise from the ordering of water between two opposing surfaces [59], others emphasize that these forces are more likely to arise from suppressing surface protrusions [108]. More recently, even claims have been made that, at least in some cases, the so-called hydration forces are non-existent and can be accounted for by ordinary electrostatic interactions [108,109].

Several theoretical approaches exist to either explain the hydration forces in terms of water ordering only [109], surface protrusions only [108] or combinations of both [110,111]. According to the latter, more sophisticated, models the hydration force between lipid membranes depends on a delicate balance between lipid protrusions and water ordering, and one may dominate the other depending on specific properties of the surface. Since the hydration force (if it exists) is expected to arise from surface properties on

a molecular scale, the MD method seems suitable to obtain more insight into these forces.

Ideally one would like to compute the pressure between confined lipid membranes as a function of their distance directly from simulations. Unfortunately, accurate pressure calculations require long simulation times. Moreover, pressures are sensitive to the details of the force field, and current force fields are probably not sophisticated enough to realistically probe the pressure changes upon confining two membrane surfaces. Another problem with the direct computation of pressures is that, in order to parallel the experimental setups, we would have to simulate at constant chemical potential of water (either equal to the chemical potential of bulk water in case of SFA experiments, or equal to a distance dependent chemical potential, as in osmotic stress methods).

Nevertheless, valuable qualitative information can be obtained by analysing the lipid protrusions and the ordering of water between two opposing membrane surfaces. Reported MD studies thus far include comparison of lipid membranes consisting of different lipids, at varying water content, and in different phases.

9.1. Comparing different headgroups

Damodaran and Merz have published two papers on the comparison of PC and PE lipid membranes [81,64]. Experimentally it is found that the swelling limit for PC membranes is much larger than for PE membranes, possibly as a result of hydration interactions. Therefore, a direct comparison of these two lipid headgroups is interesting. The two simulated systems consisted of a (rather small) bilayer of 16 lipids per monolayer, together with a water layer close to the experimental swelling limits. Both DMPC and DLPE membranes were simulated in the liquid-crystalline phase during 200 ps, at NVT conditions. In the first paper [81], three important differences were reported between the surface/water properties of the two systems. The major difference is that around the PC headgroup waters orient themselves in a clathrate structure (optimizing inter water hydrogen bonding), characteristic of solvation shells of hydrophobic solutes. In contrast, a hydrophillic type of solvation (with waters hydrogen bonding with the PE headgroup) was observed for the PE membrane. This difference is a direct result from the larger hydrogen bonding possibilities of the PE headgroups (with easily available polar hydrogens) compared to the PC headgroup (with bulky methyl groups instead).

As a consequence of their weaker inter-headgroup hydrogen bonding, the PC headgroups were found to protrude further into the water layer than the PE headgroups did. Analysis of velocity autocorrelation functions of the headgroups showed that the PC headgroup moves in a much smoother way, whereas the PE headgroup has to break hydrogen bondings to undergo rotational or diffusional motions. From these observations the authors argue that PE membranes, upon bringing them together, are not likely to feel any hydration repulsion at all for two reasons.

First, the water molecules that remain between the bilayers can still make favorable hydrogen bonds with PE headgroups. Second, steric interactions between opposing PE headgroups are not likely to be important as they do not protrude much from the bilayer surface. In contrast, removing water molecules from in between two PC membrane surfaces would cause overlap between the clathrate shells of opposing headgroups, resulting in an extra repulsion compared to PE headgroups. Both unfavorable water hydration and entropic confinement may contribute to this repulsion. This repulsion is naturally expected to be longer ranged, because PC headgroups protrude more than PE headgroups. Thus, the larger swelling limit for PC can be accounted for at least in intuitive terms.

Another possible reason for the reduced swelling limit was noted in the second paper [64], which further analyzes the differences between the two lipids. Crosslinks were found between PE headgroups, likely stabilizing the close interaction between them. In order to get a more physical understanding of the enhanced repulsion between hydrated PC versus PE, the authors conducted some free energy calculations. Comparing the potentials of mean force between two ammonium ions, and between two tetramethylammonium ions, the authors did not find convincing evidence for any of the anticipated effects. This however might be largely due to the crudeness of the free energy calculations.

Perera et al. also studied the differences between PC and PE headgroups [82]. They compared two simulations (from [67]) of a liquid-crystalline DPPC

bilayer with 11 and 20.5 water molecules per lipids, respectively, to two simulations of DLPE under the same conditions and level of hydration. Again it was found that the PE headgroups protrude less far into the water layer, and that, just as PC, PE headgroup distributions are touching at the hydration level corresponding to the experimental swelling limit. The water layer in the PE system at the higher hydration level has bulk properties.

Thus, in accordance with the conclusions drawn by Damodaran and Merz, the authors state that any repulsion force arising from bringing the membrane surfaces to within their equilibrium distance (i.e. the distance at the swelling limit) is intimately connected with the presence of lipid headgroups. Furthermore, they underline the conclusions of the same authors that, next to a steric repulsion term arising from confining the headgroups, the difference in solvation structure also might contribute to the larger repulsion observed experimentally for PC. Fig. 9 shows a graphical impression of the difference in solvation structure between PC versus PE headgroups.

A recent paper from our group [86] compares the interface properties of lipid membranes to those of more hydrophobic surfaces. Previous simulations of DPPC, GLCB, glycerol, and decane – increasingly hydrophobic molecules – are compared to each other to obtain a better understanding of general principles that govern hydration forces. The main conclusion is that hydrophilic surfaces not only have broader interfaces (which can be expected) but that the decay of the protrusions shifts from Gaussian towards stretched exponential when the lipids become more hydrophilic.

In all cases the decay of water order closely follows the decay of the interfacial density, even in the case of low charge density. No evidence is found in any system for an intrinsic type of water ordering that could propagate a repulsive force much further than the length scale of the protrusions.

9.2. Comparing different hydration levels

Marrink et al. studied water/surface properties as a function of hydration level through a comparison of three DPPC simulations with different levels of hydration: one at low hydration level (11.5 waters/lipid), one at full hydration (20.5 water/lipid) and one well above the swelling limit (29.1 waters/lipid) [69].

In all three simulations large protrusions of the lipid headgroups into the water layer are observed. This results in interdigitation of opposite headgroups in the system with the lowest level of hydration. Interestingly, at the swelling limit, the two headgroup distributions just touch each other, whereas at the largest separation a layer of bulk water exists. This might point at a repulsion due to lipid protrusions as the cause for the observed swelling limit of DPPC. However, analysis of the mobility of the lipid headgroups did not reveal any constraining effect at lower hydration levels.

To test whether water molecules cause the hydration repulsion, the ordering of water was analyzed in terms of its polarization orientation, hydrogen bonding characteristics and diffusional properties. In all three simulations, the water is clearly ordered and the ordering decays roughly exponentially away from the surface. Important is the observation that this ordering of water is not intrinsic, but directly related to the decay of the lipid density itself. More specifically, the interfacial charge distribution is directly responsible for the observed water ordering. Therefore, the authors conclude that, if hydration forces arise from water ordering at all, indirectly the properties of the interface play a major role.

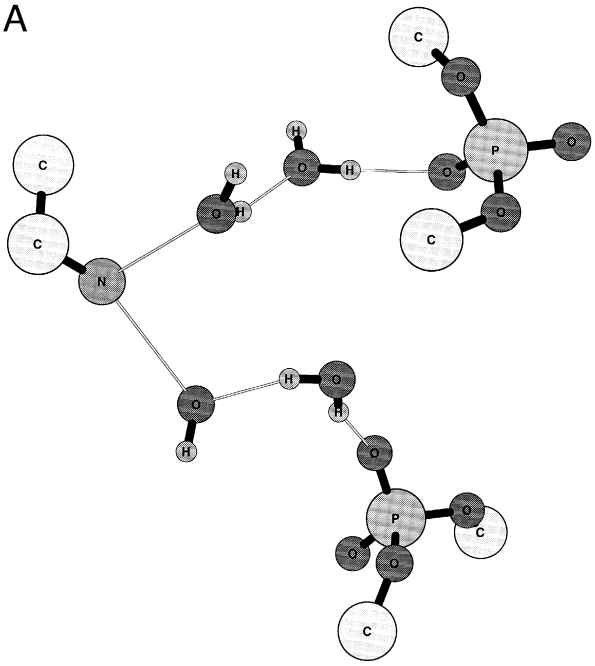
Analysis of the type of decay of the interface properties revealed that any type of repulsion resulting from closely approaching interfaces is more likely to be of a stretched exponential, or multi exponential, rather than of a single exponential nature.

9.3. Effect of lipid phase

Berkowitz and coworkers compared the gel and liquid-crystalline phases in a recent paper [67]. They did simulations (NVT, 300 ps) of DPPC in the gel and in the liquid-crystalline phase at both 11 and 20.5 waters/lipid, close to the swelling limit of, respectively, the gel and the liquid-crystalline phase of DPPC.

Generally, the effects observed for DPPC in the liquid-crystalline phase are the same as earlier findings [81,69]. The gel phase of DPPC is particularly interesting because experimentally a relatively large hydration repulsion is observed in this system. The

A



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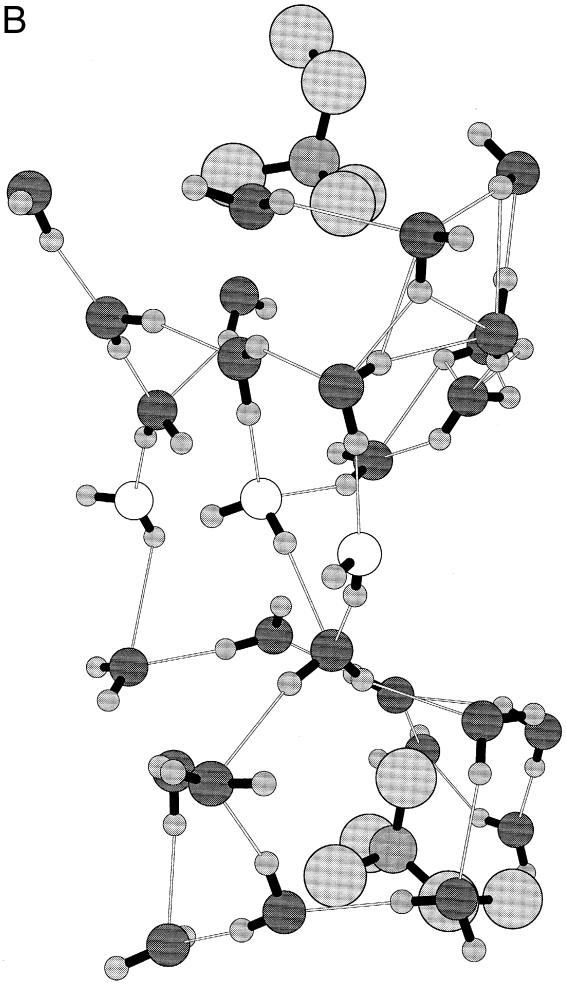

simulations provide a clear clue why: even in the gel phase there is a substantial protrusion of the lipids, which leaves no bulk water at the swelling limit. In contrast, the gel phase at the higher hydration level shows a layer of water with bulk properties.

Therefore, it seems that just as in the liquid-crystalline phase, the swelling limit of gel phase DPPC coincides with the point at which the headgroups start to overlap.

9.4. Discussion

When we compare all of the reviewed simulations, some general properties emerge that give us a better understanding of the type of forces that are important between membranes below their swelling limit. All simulations observe a broad lipid/water interface, the degree of lipid protrusion varies, depending on lipid headgroup and membrane phase, and a direct correlation seems to exist between the length scale of the protrusions and the experimental swelling limit [69,67,82]. These observations support the proposed steric confinement model [108] as the cause of the hydration repulsion. However, an attempt to actually show this confinement has not provided any direct evidence [69].

Is there a role for water too? The simulations all show that water is ordered, the ordering smoothly decaying away from the membrane surface. Oscillatory decaying profiles are not observed, due to the dynamical nature of the lipid/water interface. An important general observation is that the ordering of water is not caused by some intrinsic property of water, but is strongly correlated with the length scale of the lipid protrusions. This implies that if water ordering contributes to the repulsive interaction between lipid membranes, the range of the interaction will depend on the properties of the surface rather than on the properties of the solvent.

Both Cevc's [110] and Lipowsky's [111] theoretical models show that, when the intrinsic ordering of water is small (or negligible compared to the ordering imposed by the surface), the range of the hydration repulsion will be entirely determined by the length scale of the surface protrusions. Depending on the details of the lipid membrane surface, however, a more specific role of water, which is not incorporated into theoretical models, is likely to modulate the range and magnitude of the forces between two membranes. Examples are the observation of clathrate types of solvation shell opposed to hydrophilic hydration shells, or the existence of water mediated bridging interactions between opposite surfaces [81,64,67,82].

It is difficult to estimate the various contributions arising from steric headgroup–headgroup repulsion, entropic headgroup confinement, and surface induced water ordering, although it seems clear from the MD simulations that the actual surface properties are likely to be important for the interactions between opposing membrane surfaces. Apart from these general, repulsive, contributions, attractive forces might arise from specific water ordering or from headgroup–headgroup electrostatic correlations across the water layer.

Given the broadness of most lipid/water interfaces, all of the contributions mentioned above are likely to contribute in the distance range over which hydration forces are measured. As a further complication, MD simulations show that surface protrusions of lipids do not decay in a nice Gaussian or single exponential way, but more likely as a stretched exponential [69,86].

With these complications in mind, it seems unlikely that any theoretical model not incorporating the molecular details of the lipid membrane is able to fully account for the experimental data on hydration forces. As long as quantitatively reliable pressure-distance profiles cannot be obtained from simulations, future work in this field should aim at more systematic studies of changing surface properties. Careful analysis of surface protrusions as a function of membrane separation would further enhance our qualitative understanding of hydration forces. Continuation of potential of mean force computations between single lipids, as initialized by [64], might provide us with some quantitative clues as well.

Fig. 9. (A) Snapshot showing water bridges between opposing headgroups from a DLPE system. (B) Snapshot from a DPPC system depicting the clathrate cages around $N(CH_3)_3$ groups from two lipid headgroups from opposing bilayers. Water molecules which bridge clathrate cages are shown with white oxygen centers. Reproduced with permission from [82].

10. Lipid–protein interactions

Recently, researchers have begun to include proteins in lipid systems. Obviously, this is a major advance towards studying more realistic biological systems. Although the number of studies is still small, widely varying proteins such as a membrane spanning channel (gramicidin A), an integral membrane protein (bacteriorhodopsin), a membrane-binding helix (CRF) and a phospholipase that is active on lipid surfaces (PLA_2) have been studied. Below we will discuss the most important findings.

10.1. Surface bound peptides

Damodaran et al. studied the interactions of the tripeptide Ala–Phe–Ala–*O-tert*-butyl with a DMPC bilayer [112]. Two starting structures were used, based on different experimental data. In the first, the peptide backbone was placed parallel to the bilayer surface, which exposes the *tert*-butyl group to solvent somewhat. In the second, the *tert*-butyl group was placed so that it interacts with the acyl chains of the lipids. Neither the dynamics nor the average structure of the bilayer appeared to be perturbed much by the presence of the peptide. Compared to the same peptide in solution, the dynamics of the peptide are much slower. In 450 ps both starting structures remained stable, indicating the presence of multiple stable conformations of these peptides. This can help to explain the different results from diffraction and NMR studies, since these two methods average over different time scales. It also means the choice of starting structure in a lipid–protein simulation is important; no path connecting the two structures was found in a simulation of 450 ps.

In the second paper on the interaction between a small peptide and a bilayer Damodaran and Merz Jr. studied the fusion inhibiting peptide carbobenzoxy–

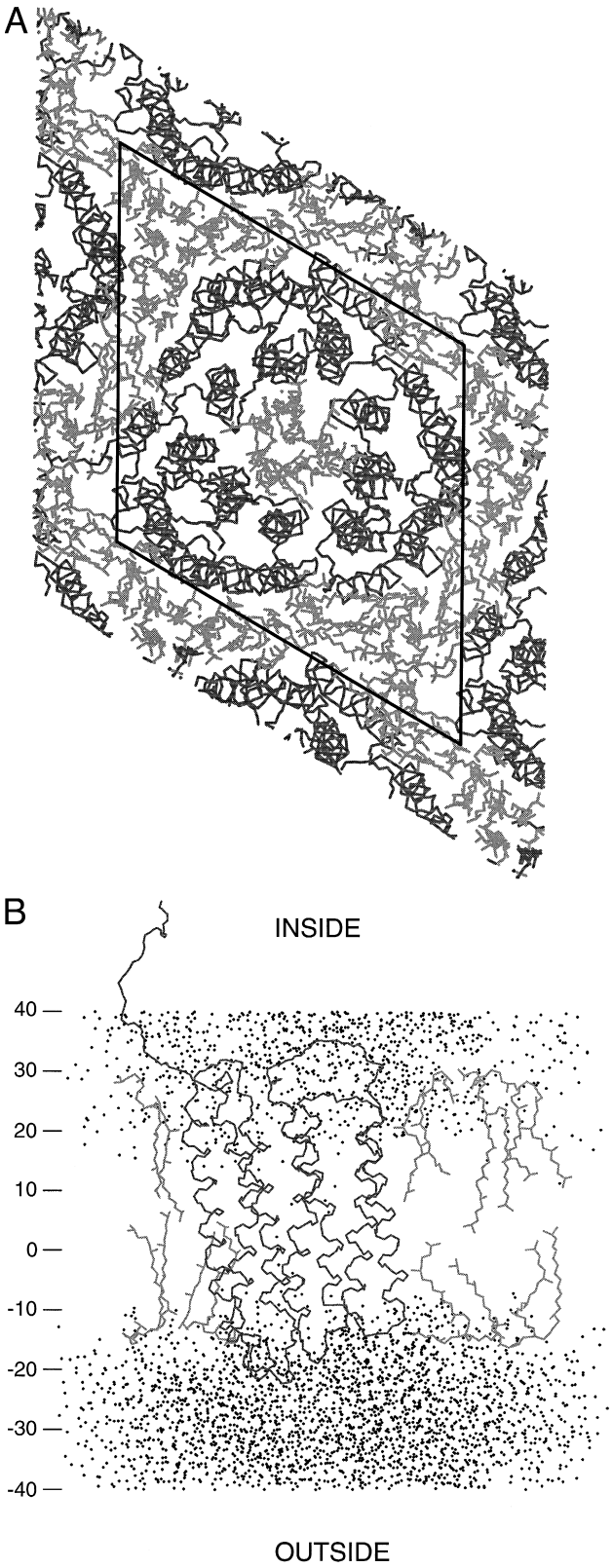

Fig. 10. (A) Time-averaged structure of a bacteriorhodopsin–lipid system, projected into the membrane plane, and (B) viewed from the front. Backbones of protein molecules are shown in black, lipid molecules in grey, water molecules as dots, and the boundaries of the unit cell in black. In (A) water has been omitted and in (B) only one monomer of the protein trimer and a few selected lipids are included. The inside is the cytoplasmic side, the outside the periplasmic site. Reproduced with permission from [122].

D-Phe–L-Phe–Gly with *N*-methyl–dioleoyl-phosphatidylethanolamine lipids [113]. They conclude that the insertion of the phenylalanyl side chains into the lipid hydrocarbon region cause a significant increase in the order parameters near the carbonyl groups and a decrease in the water penetration in the headgroup region. This gives the affected region gel-like properties, which may be the molecular mechanism for the fusion inhibition that is observed experimentally.

One of the first published studies on a larger peptide interacting with a membrane concerned an amphipathic helix from Cortico-tropin-releasing factor [114]. Huang and Loew simulated residues 13–41, modeled as ideal helix, bound to a DOPC bilayer. The peptide remained mostly helical during the 510 ps of simulation although the ends started to unravel. In vacuum the helix rapidly unfolded, but this is not a stringent test since single helices are not usually stable in vacuum. The effect of the peptide on the membrane was small: the first methylene segment of the tails was disordered compared to the pure bilayer and the lipid headgroup region was broadened towards the water region. The lack of experimental data for this system makes it difficult to assess the validity of the results and the conclusions are modest, but it is a start.

10.2. Membrane spanning peptides

Woolf and Roux studied the gramicidin A channel in DMPC [115,116]. This is probably the best characterized system experimentally, with a wealth of NMR data from several sources available. The dimers form membrane spanning channels that conduct ions. The system under consideration contained 16 DMPC, two gramicidin A proteins and about 650 water molecules. By averaging over a series of six simulations many of the available experimental data on order parameters and backbone conformations were reproduced. The presence of gramicidin A causes an increase in ordering of neighbouring lipids, in agreement with experimental findings and the concept of ‘boundary lipids’. However, the protein concentration in this system is very high and no ‘bulk’ lipids are present. Tryptophan residues appeared to form the boundary between the glycerol groups and the acyl chains: they are hydrogen bonded to the glycerol backbone or water and the bulky hydrophobic part is in contact with the acyl chains. This seems to be a general feature of membrane proteins, judging from the available crystal structures.

Another project from the same authors concerns the bacteriophage Pf1 coat protein [117]. This 46 residue protein consists of a membrane spanning hydrophobic helix, a short amphipathic helix and a disordered connecting loop and termini. The amphipathic helix oriented itself as expected, with the hydrophobic side facing the membrane interior. Once a high resolution structure of this peptide in a bilayer becomes available we will know how well the structure has been predicted from the simulations.

10.3. Surface active proteins

The important enzyme phospholipase A_2 is a water soluble protein, that is active at the water–lipid interface. It hydrolyses the *sn-2* ester bond of phospholipids, plays an important role in many processes and occurs in a variety of forms in a variety of organisms. We refer to [118] for a list of review articles on PLA_2. There exist several molecular dynamics studies on the interaction of types of PLA_2 with monolayers (for computational efficiency).

Berendsen et al. explored the role of the lipids in binding to PLA_2. Preliminary simulations of PLA_2 in a system with lipids and solvent revealed problems with the modelling of the calcium ion in the enzyme [119]. Jones et al. modelled the structure of a porcine PLA_2–lipid complex and simulated this system for 48 ps. The predicted structure closely resembled a structure of the enzyme/inhibitor complex found experimentally, but the simulation is too short to give much insight into the dynamics of the system [120]. A short account of a simulation of PLA_2 on a monolayer is also given by Ahlström et al. [121]. A lipid molecule was placed halfway into the enzyme and over the course of the simulation an increasing van der Waals interaction between lipid and protein was observed. The protein kept most of its structure but some force field problems related to the calcium prohibited more extensive work.

More recently, Zhou and Schulten studied the structure of PLA_2 on a DLPE monolayer and mechanisms that enhance the catalytic activity of the enzyme [118]. Zhou and Schulten modeled two lipid–enzyme complexes. In the first complex PLA_2 was

placed loosely on top of the bilayer, in the second complex PLA_2 was placed much deeper into the bilayer, which they refer to as the 'tight complex'. For purpose of comparison a third simulation was done of the enzyme in water, without interface. Using a free energy perturbation method [9] and knowledge of the dielectric susceptibility calculated by a method developed in an earlier study [45] they show that the lipids at the interface between the protein and the bilayer become desolvted in the tight, but not in the loose complex. The desolvated lipids can interact with a number of hydrophobic residues lining the active site of the enzyme. The combination of molecular dynamics, free energy perturbation methods and continuum electrostatics used in this work is innovating but could use further tests on convergence and accuracy.

10.4. Integral membrane proteins

Edholm et al. studied bacteriorhodopsin in a lipid bilayer [122] (Fig. 10). This work includes the only published simulation of an integral membrane protein in a bilayer in one of the biggest systems in the literature to date. The study aimed at two principal goals: (1) Testing the feasibility of MD simulations of integral membrane proteins surrounded by water and lipids. (2) Studying the thermal fluctuations of bacteriorhodopsin to gain insight into the dynamics of the protein.

For the first purpose they performed a series of simulations of increasingly complex systems, starting with a monomer of BR in vacuum, then the trimer plus six crucial lipids in vacuum, the trimer in a lipid bilayer and finally a unit cell of the hexagonal lattice, containing the trimer, lipids and water. In all of the simulations the structure of the protein moved away 2–3 Å from the starting structure, but the r.m.s. averaged over all three monomers in the full system was much smaller. These deviations suggest that the structure of the protein is stable in the simulations. It also shows the advantage of a trimer, which provides three approximately independent structures. This suggests that doing multiple simulations starting from the same structure but with different initial velocities can result in better sampling than doing one longer run. A similar conclusion was reached by Woolf and Roux in their study of gramicidin A [116].

A careful analysis of the r.m.s. deviations from the simulations with respect to their average structures and electron cryo-microscopy structure revealed good agreement with data from NMR on flexible parts of the protein and with the crystal structure for the more rigid helices.

Although these results have no immediate biological significance, they are important methodologically: they demonstrate that a state-of-the-art treatment of a membrane protein, which means including lipids and solvent, is comparable in reliability with simulations of water soluble proteins. This is an important result, since there is a considerable amount of experience with simulations of water soluble proteins. With increasing computer power we can expect interesting simulations of membrane proteins in the future as more structures of membrane proteins become available experimentally. This can be a particularly fruitful use of MD since experimentally the fast dynamics of membrane proteins is less accessible: NMR techniques are not able yet to deal efficiently with membrane imbedded proteins.

The second goal was to study the fluctuations of the protein structure to get insight into the dynamics of the system. As expected, the membrane-spanning helices of the protein fluctuate less than the peripheral loops. Surprisingly, the fluctuations of both the lipids and the protein were much stronger in the inner side of the membrane. This appears to be caused by the structure of the protein: water penetrated deeper and often into the bilayer and protein structure at the inner side, which may cause the lipid and proteins atoms in that area to become more mobile. In the absence of water molecules, in a system with just lipids and protein, this asymmetry is not found. Interestingly, a similar asymmetry appears to be present in the high resolution crystal structures of bacteriorhodopsin from *Rhodobacter capsulatus* [123] and *Rhodobacter blastica* [124]. Whether this asymmetry has any biological relevance remains open.

11. Outlook and conclusions

Molecular dynamics simulations of lipid systems have come a long way since the first studies of highly simplified systems. It is now possible to study a variety of phospholipid systems, including systems

with solutes, cholesterol and membrane proteins. At the same time, the limitations of the current simulations are becoming more obvious. We will mention a few areas of innovation and future projects.

11.1. Technological advances

Eventually the currently used potential functions combined with the force field parameters, which are at the heart of any molecular dynamics simulation, will prove to be too limited in several aspects. Many of the currently used lipid parameters are not consistent with current protein force fields, which limits their applicability in mixed lipid–protein systems. The improvement of the treatment of long-range forces and other methodological issues will make it necessary to construct and test new force fields. Ultimately polarizability will have to be included in the force fields, lifting one of the most important limitations of MD.

The algorithms that are used in simulations form a second area of innovation. An increasing number of studies suggests it is necessary to use more accurate methods than cutoff schemes to calculate long-ranged forces. These include Ewald or other lattice-sum methods to calculate Coulomb forces and mean field approximations for the long-range part of van der Waals or Coulomb interactions. The precise effects of different algorithms for pressure and temperature control are also a matter of concern. Multiple timestep algorithms, designed to have different timesteps for integrating fast and slow motions, can increase the total time scale for simulations. Klein and co-workers developed and studied many relevant algorithms [38,42], but these are not in general use yet and more work is needed.

Most biological questions concern much larger time and length scales than can be investigated by straightforward molecular dynamics. In principle the combination of molecular dynamics to study local motion and biased Monte Carlo methods that allow large motions, including lipids exchanging position, could provide access to a longer time scale. This concept will have to be worked out in more detail.

Such processes require further development of sophisticated simulation techniques, including non-equilibrium methods and dynamics with forces derived from potentials of mean force and stochastic forces. In addition, Monte-Carlo type sampling procedures could be used to generate adequate ensembles of starting structures.

The time scale that is accessible for simulations will increase with the increase of computer power, but to adequately sample mixtures of lipids, lateral lipid motion and protein–lipid interaction, an increase in speed of several orders of magnitude is needed. Together with the increasing complexity of algorithms and increasing system size, demand for computational power will remain tremendous.

11.2. Biological issues

The previous section deals with rather technical issues, but what can we expect from a biological or biophysical point of view from simulations?

Generally speaking, the more recent simulations of liquid crystalline and gel phase DPPC and DLPE come close to reproducing all available experimental data. They have provided a detailed picture of the structure and dynamics of fluid phase model bilayers. In particular, practically all experimentally available data has been reproduced in some simulation; the problem is to reproduce all data at once. A notorious feature of the order parameter profiles for DPPC, a dip in the profile directly behind the headgroups, has never been reliably reproduced. However, our knowledge of the general physics of DPPC and DLPE is unlikely to increase much further from straightforward simulations of these systems. They do remain excellent systems to test new methods on, of course.

The detailed study of other lipids than DPPC (or DMPC) and DLPE has only yet begun, with just a few studies available that generally describe short simulations. From a number of longer simulations it is clear that structural parameters and properties such as order parameters converge on the nanosecond time scale; in addition, details of the structure and dynamics of lipid systems, as they are found from simulations, are sensitive to the simulation methods used. It will therefore be interesting to directly compare longer simulations of different lipids, simulated under the same conditions. Although we did not go into the technical details behind the simulations described here, it is somewhat surprising, and reassuring, that

in spite of the considerable differences in simulation methodology and force fields the results from most studies on similar systems are very comparable.

Three particularly interesting types of lipid systems, due to their biological relevance, are mixtures with cholesterol, mixtures with charged lipids, and unsaturated lipids. When much of the work to date is viewed as an attempt to develop the methods and the parameters, using two well-studied lipids, the study of biologically relevant model membranes has just started. Plenty of relevant systems are waiting.

The partitioning of small molecules, such as anesthetics or ions, between medium and membrane, and the influence of such molecules on membrane structure and fluidity can in principle be investigated by present-day techniques. The same applies to the effect of lipids such as cholesterol on bilayers. We can expect more and longer simulations on such mixed systems in the future. Eventually, the gap between atomic detailed simulations using molecular dynamics and the phenomenological microscale simulations should be bridged by a combination of techniques.

Currently many groups are working on simulations of membrane proteins or peptides imbedded in bilayers. The study of this complicated type of system is still in its infancy and more experience is needed. The development of algorithms and force fields that can increase the time scale that is accessible by simulations will be particularly important here. The low number of membrane proteins for which accurate structures are available also limits the applicability of simulations, but more structures are expected to be solved by X-ray, NMR or electron-microscopy.

What kind of new experimental data would be interesting to researchers in the field of MD? In general, any experimental data that is accurate enough to provide a critical test on the angstrom and picosecond length and time scales is interesting. The same applies to data that can be derived, using suitable statistical mechanical relations, from simulations at this scale. Experimental data on the structure of other lipids than DOPC and DPPC, on the structure of lipid mixtures and on the detailed structure of peptides and proteins bound to or incorporated in model bilayers will be most welcome. In return, molecular dynamics can aid in interpreting the averages over many lipids and long time scales that are typical in most experiments in terms of models at the molecular level and provide insight into experimentally less accessible phenomena.

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