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Review

Lipids on the move: Simulations of membrane pores, domains, stalks and curves

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ABSTRACT

In this review we describe the state-of-the-art of computer simulation studies of lipid membranes. We focus on collective lipid–lipid and lipid–protein interactions that trigger deformations of the natural lamellar membrane state, showing that many important biological processes including self-aggregation of membrane components into domains, the formation of non-lamellar phases, and membrane poration and curving, are now amenable to detailed simulation studies.

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Contents

Abbreviations: AFM, Atomic Force Microscope; AMP, antimicrobial peptide; BAR, Bin/amphiphysin/Rvs; CG, Coarse Grained; DAPC, diarachidonyl-PC; DMPC, dimyristoyl-PC; DMSO, dimethylsulfoxide; DOPC, Dioleoyl-PC; DPD, Dissipative Particle Dynamics; DPPC, Dipalmitoyl-PC; GMO, glycerol-monoolein; MC, Monte Carlo; MD, Molecular Dynamics; PA, phosphatic acid; PE, phosphatidylethanolamine; PMF, Potential of Mean Force; POPC, palmitoyl-oleoyl-PC; PS, phosphatidylserine; SSM, stearyl-sphingomyelin

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1. Introduction

Computer simulation is a powerful approach to studying the properties of lipid aggregates. Because lipid aggregates have a certain degree of intrinsic disorder in biologically relevant states, direct structural and spectroscopic experimental methods necessarily average over a large number of lipid conformations. In principle, simulation can be used to track the behavior of individual atoms, with the potential to give much more detail than can be measured experimentally. Computer simulations have reached a state where simulation times and model scale allow a direct comparison between simulation and experiment in well-characterized systems and have become sufficiently reliable to investigate states that are difficult to study experimentally.

This is especially true for non-lamellar bilayer states, the primary focus of this review. The importance of such non-lamellar bilayer states in biological systems has long been realized [1]. They occur in a large number of processes, including pore formation, membrane genesis, and in intermediates in fusion or fission as found in endocytosis, exocytosis and viral entry. In many of these processes the local membrane composition is far from homogeneous. Formation of non-lamellar phases is therefore intrinsically linked to the appearance of dynamical heterogeneities. In most cases, non-lamellar states are transient and only involve a fraction of the lipids in the aggregates. Both aspects complicate experimental studies, but computer simulations are well suited to study such problems.

Biomolecular simulation, initially focused on proteins, has seen a long development over the past 30 years [2]. Over the past 20 years, computer simulations of lipids have made great progress as well. Twenty years ago the first atomistic or near-atomistic simulations of surfactant bilayers in solution were published [3], followed by several papers on phospholid bilayers on the 100 ps time scale. Mixtures of lipids with cholesterol followed, as well as studies of the interactions between small molecules and lipids and the first steps towards membrane protein simulations. Detailed reviews of these early day bilayer simulations are available [4–9]. The scale of the systems that have been studied increased with increasing computer power as well as with more efficient simulation software. Groundbreaking simulations of bilayer undulations [10] and bilayer self-assembly [11] showed that collective events can be studied in atomic detail. At larger time and length scales atomistic detail may not be essential, however. Lipids and surfactants have therefore been an important area for development of coarse-grained models and simulation methods. Smit et al. [12] pioneered the use of simplified lipids based on a number of beads with Lennard-Jones type interactions, an approach that continues to be frequently used.

The current state of the art sees simulations of entire vesicles fusing, the transformation of monolayers into vesicles upon compression, simulation of domain formation, direct investigation of pore formation by antimicrobial peptides or electric fields, and large-scale remodelling of membranes by curvature-inducing proteins. The range of applications has become extremely broad, but generally speaking a reasonable limit on simulations at the moment is a million particles, corresponding to 5000 all-atom lipids or ca. 50,000 lipids in coarse-grained models. Simulation times of hundreds of nanoseconds have become routine in simple bilayer simulations, with microsecond simulations for all-atom and millisecond simulations at the coarse grained level at the edge of what is currently feasible. Both in terms of time and length scales simulations can now probe into the microscopic regime, allowing a direct bridge to new classes of experiments such as vesicle aspiration, fluorescence imaging and atomic force microscopy (AFM) measurements.

In this paper we review recent work in the past five years that used particle based (either all-atom or coarse-grained) molecular dynamics simulations as well as related methods such as dissipative particle dynamics and Brownian dynamics. We only consider studies in which lipids show relatively large changes in structure and orientation with respect to their equilibrium position in the bilayer. In other words, lipids on the move, leading to bilayer deformations in the broadest sense. We have tried to include as much of the literature as possible but with the current size of the field it has become very difficult to be comprehensive. Studies on micelles and monolayers, and the rapidly growing field of nanotube/bilayer interactions, are not covered. For the same reason we decided not to include references to experimental work, acknowledging the invaluable contribution of many of these papers to our field. We apologize to colleagues whose work we missed or were unable to include, and for a bias to the work from our own labs. For progress on related topics not covered in this work we refer to recent reviews on membrane protein simulations [13,14], the interaction of small compounds with bilayers [15], and peptide/lipid interactions [16].

Our review is organized as follows. We start with a brief overview of the main methods used to simulate lipid systems, including a brief description of the different levels of interaction models and approaches to solve the equations of motion. We proceed with describing the state of the field, subdivided into three sections: *i)* 'Phase transformations', covering lipid self-assembly, formation of inverted phases, and domain formation, *ii)* 'Poration', covering various means of inducing pores in lipid membranes and the associated process of ion permeation and lipid flip-flop, and *iii)* 'Bending, buckling, curving', including membrane undulations, vesicle formation and fusion, and large scale protein induced membrane remodeling. A short outlook concludes this review.

2. Methods

Several simulation methods to study membranes are in common use. In general, a simulation can be divided into the molecular model to describe interactions between atoms and molecules (the Hamiltonian, also named interaction model or force field) on the one hand and a computational technique to efficiently explore the consequences of that model.

2.1. Force fields

A force field is the set of parameters and the equations that describe the interactions between atoms or particles. Different force fields use different levels of detail, and can be categorized as 'all-atom', 'united-atom', or 'coarse-grained'. All-atom force fields treat every atom (including all hydrogen atoms) explicitly, united-atom force fields combine each aliphatic carbon and associated hydrogens into a single particle, and coarse-grained force fields describe larger molecular units (such as amino acid side chains and whole or multiple water molecules) as single particles. Fig. 1 gives an impression of the differences in detail between some of these models for a single lipid.

Traditionally, force fields for biomolecular simulation started as united-atom force fields (see Fig. 1A), although most commonly used force fields have moved toward all-atom models. In protein simulations the extra cost of simulating hydrogen atoms on carbons is negligible, because most of the atoms in the system are water. In lipid systems this is not true, and simulating all-atom lipids is much more expensive computationally than united-atom lipids. One reason is simply that there are more atoms for a given number of lipids, but a more important reason is that the particle density, and therefore the total number of

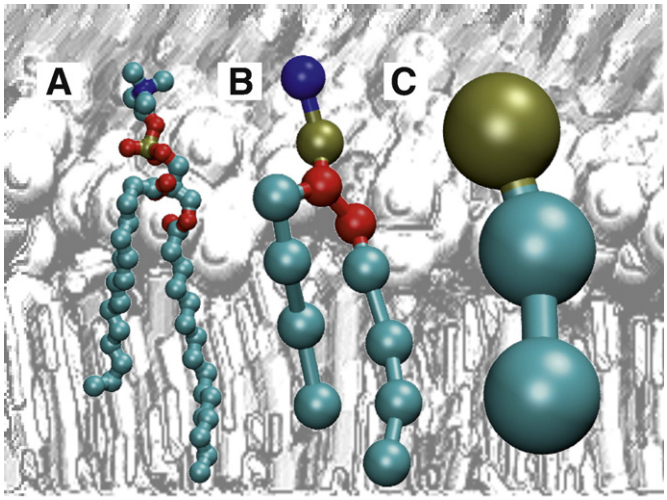

Fig. 1. Different levels of modeling. (A) A united-atom versus (B) a specific coarse grained and (C) a generic coarse grained lipid model.

interactions, is much higher. Tieleman et al. have argued for the desirability of combining all-atom proteins or other biomolecules with united-atom lipids [17]. A recent paper by Henin et al. tested united-atom CHARMM lipid parameters for combination with the all-atom CHARMM force field for other biomolecules [18]. United-atom models are in principle less accurate, but in practice there are other considerations that may be at least as important [17]. The most widely used atomistic lipid force fields are the all-atom CHARMM [19] force field and a united-atom force field originally based on a combination of OPLS and GROMOS [20], although there are other options under development, e.g. AMBER GAFF [21]. In [21] a number of these atomistic lipid force fields have recently been compared.

In the direction of more detail rather than less, there have been recent developments on polarizable force fields that have the potential to significantly improve biomolecular simulation in general. Polarizable force fields include some of the electronic detail that is averaged over by the fixed point charges used in classical force fields. To our knowledge no papers on the simulation of lipid bilayers with a polarizable force field have been published at the time of writing this review, but several efforts are underway. Allen and co-workers recently published a simulation of lipids with polarizable tails [22].

In a sense, the use of united-atom lipids is akin in spirit to coarse-graining details that are not likely to be relevant for the properties of interest, a sound principle of physical modelling [23]. Although coarse-grained (CG) lipid models go back a long time (e.g. tethered chains of beads mimicking a bilayer [24]), coarse-graining in the field of lipid membrane simulation has become a booming field only in the past few years. CG models can roughly be divided into two classes, namely generic and specific models (although the distinction between them is by no means a matter of black and white).

The first, the generic class, includes models that capture basic properties of lipid molecules. These can be as simple as a lipid consisting of two particles A and B tethered together that have different interaction strengths for the combinations AA, AB, and BB (see Fig. 1C). This will already lead to interesting phase behavior, given a judicious choice of parameters. Solvent may or may not be explicitly included, with the solvent-free approach leading to an obvious advantage in computational speed. Generic models vary significantly in complexity and scope of problems they are designed for, but all abstract from chemical details of specific lipids (for recent reviews see [25,26]).

The second, the specific class, concerns models that are usually more detailed (see Fig. 1B), and that are able to represent specific lipids. Such models are typically parameterized based on a comparison to atomistic simulations and/or detailed experimental data. Effective CG interaction potentials for lipids have been extracted from atomistic simulations using inverse Monte-Carlo (MC) schemes [27,28] or force matching [29,30] approaches. Alternatively, standard potential functions can be used which are calibrated based on thermodynamic data [31]. Advantage of the former approach is the higher level of accuracy and closer resemblance to atomistic simulations that can be obtained. However, this is only true for the state point at which the effective potentials were derived. Advantage of the thermodynamic approach is its broader range of applicability and the fact that it does not require additional atomistic simulations. In both cases, due to the reduction in number of particles and fast dynamics, the use of CG models makes simulations easily two to three orders of magnitude more efficient compared to their atomistic counterparts.

The CG dynamics is faster than the all-atom dynamics because the CG interactions are much smoother. The effective friction caused by the fine grained degrees of freedom is missing. In practice, time scales are estimated based on comparison of diffusion of water or lipids. However, the speed-up factor might be quite different in other systems or for other processes, and is likely not the same for different motions within a system. In general, the time scale of the CG simulations has to be interpreted with care. Widely used CG models include the generic model of Lipowsky et al. [32,33], the solvent-free model of Deserno et al. [34], and the specific models of the Klein group [35], the Voth group [29], and the Marrink group [36]. The latter model is now known as the MARTINI model.

2.2. Computational methods

Each of the methods below is essentially a method to sample conformational space for a specific model of atomic interactions and can be combined with different levels of detail to describe such interactions.

The primary approach used to simulate the dynamical behavior of lipid systems is the Molecular Dynamics (MD) simulation technique, in which Newton's equations of motion are integrated numerically for a set of interacting particles to generate the time evolution of the system. Applying concepts from statistical mechanics, the resulting trajectory can be used to evaluate various time dependent structural, dynamic, and thermodynamic properties of the system. When the interaction model is of the all-atom or united-atom nature, we will use the term atomistic or fine-grained MD simulation, whereas the use of a CG interaction model is referred to as CG-MD. Another popular approach is Dissipative Particle Dynamics (DPD). DPD was originally introduced to simulate the hydrodynamic behavior of fluids in a computationally convenient particle-based approach. The elementary units of DPD simulations are beads with a mass and size, whose dynamics are governed by Newton's law supplemented by friction and random forces. Each bead represents the fluid volume of several molecules. A soft core potential and DPD thermostat permit larger time steps than in CG-MD simulations. An advantage of DPD is that it conserves the momentum of the system (both locally and globally), for which reason it is consistent with the hydrodynamic descriptions of Navier-Stokes. In simulations of lipids by DPD the resulting trajectories are often interpreted in terms of individual lipids, despite the origin of DPD in particles representing a fluid volume. However, DPD can also be applied to “hard” particles that interact via Lennard-Jones potentials, bringing it to a comparable level of CG-MD, both in terms of efficiency and accuracy. DPD is related to Langevin dynamics and its overdamped form Brownian Dynamics, two additional simulation techniques that are used in the field of membrane simulations, especially in combination with solvent-free models. A general overview of the simulation techniques and their relations is given by Berendsen [23].

In addition to these dynamic simulation methods, there are many other methods, including the flexible Monte Carlo method with its

many variations – particularly useful for studies of phase behavior and using arbitrary potentials that have no smooth derivatives for forces – and mean field methods such as density functional calculations on lipid aggregates. These are outside the scope of this review but are used in modeling aspects of membrane behavior. For reviews on mean field applications to lipid membranes see for instance [26,37].

3. Phase transformations

Cell membranes show an amazingly large compositional heterogeneity, including hundreds of different lipids and even more different proteins. Not surprisingly, such a mixture has a very rich polymorphism, both in model systems and in vivo. The normal, liquid-crystalline lamellar state, as known for single-component lipid systems, is in fact rather special, and many of the lipid components are probably required just to assure the membrane stays in its functional fluid state. However, non-lamellar states play important roles as intermediates in fusion and fission processes, and in the transport and storage of a broad range of biomolecules. Moreover, the ability of the lipid membrane to phase segregate may be important, as it is believed to be linked to protein sorting and signalling. To understand the details of these processes, simulation studies are becoming an increasingly powerful tool. Starting from self-assembly studies on simple systems more than ten years ago, complete phase diagrams can now be calculated for particle-based models, although they remain restricted to the generic kind of models. Detailed models, on the other hand, point to the roles of the individual membrane components in a growing number of phase transformation studies, most notably the formation of inverted phases and the formation of domains. An important side aspect of these studies is the opportunity to calibrate the force fields by their ability to reproduce phase boundaries. In contrast to a lot of structural data (e.g. the area/lipid), which often require a number of interpretation steps, phase boundaries are usually straightforward to interpret and experimentally well determined.

3.1. Bilayer self-assembly

The first reported studies on bilayer self-assembly date back to already almost ten years ago. Using generic models, the groups of Lipowsky [32] and Smit [38] showed that randomly dispersed lipids spontaneously form a bilayer. Subsequently, Marrink and coworkers simulated this process in atomic detail for DPPC [11] and mixed DPPC/DPPE [39] lipids. These pioneering studies showed that collective events involving hundreds of lipids can be simulated, even using detailed models. Although the randomly mixed starting configurations used in computational self-assembly studies are unusual compared to most commonly used experimental conditions, the process of self-assembly involves some realistic intermediate stages. Initially, the dispersed lipids quickly aggregate into small micelles, which subsequently fuse to form an interconnected network of worm-like micelles. Further condensation of this network eventually leads to the appearance of a bilayer state, with a metastable transmembrane pore (see Poration section) as the final intermediate before the bilayer actually seals. The micelle–interconnected micelle-bilayer pathway is likely to describe micellar to lamellar phase transformation taking place in real systems, including for instance the biologically relevant process of cholesterol nucleation in human bile. The onset of the latter process has been studied by CG simulations [40]. The ability of lipids to actually assemble into bilayers is, as expected, found to be strongly dependent on the relative strengths of the interactions in the head group and tail regions of the lipids. The actual range of stability of (fluid) bilayer structures is rather narrow, requiring substantial fine

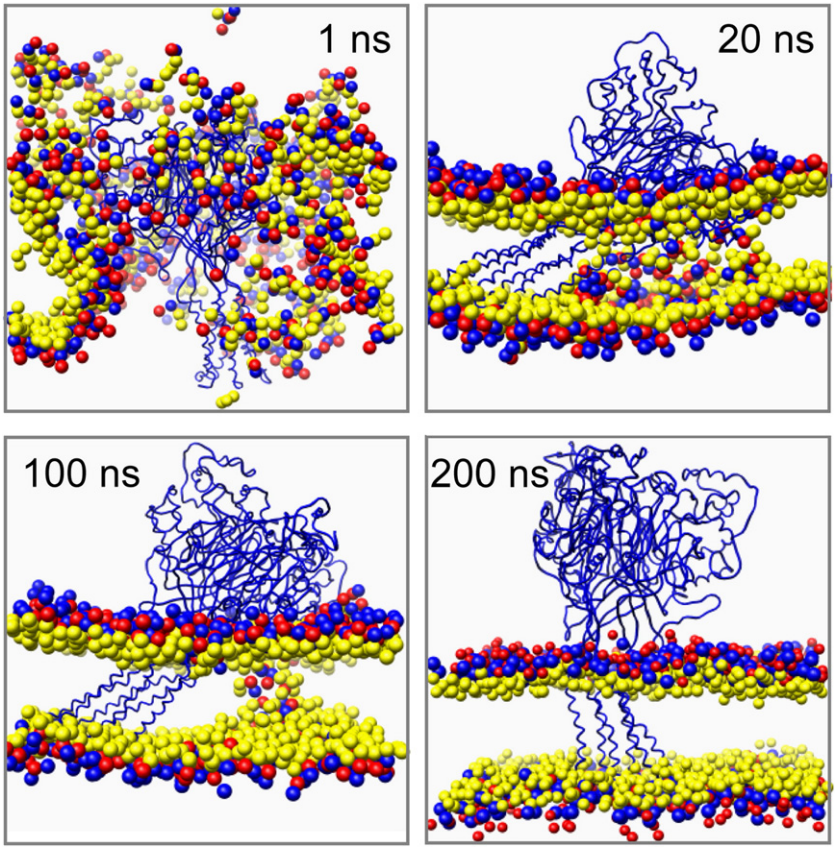

Fig. 2. Snapshots from a simulation (courtesy of M.S.P. Sansom) showing the spontaneous self-assembly of lipids together with the acid-sensing ion channel 2QTS into the native, membrane-embedded state. The protein is shown in blue (backbone only), the lipids (head group only) in blue (choline), red (phosphate), and yellow (glycerol). For similar processes, see other work of the Sansom's group [55–58].

tuning of the interactions [41]. Generic phase diagrams, largely based on self-assembly studies, have been reported by several groups[e.g. 42–46]. Notably, in the work of Kranenburg et al. [44], DPD simulations of lipid/alcohol mixtures showed an alcohol-induced interdigitated phase, in good agreement with experimental phase diagrams. The stability of the interdigitated phase was found to depend on the length of the alcohol. Other examples of the ability of current simulation studies to predict equilibrium lipid morphologies based on self-assembly protocols is given by the studies on cubic phase formation [47], nanodisc self-assembly [48] and formation of lipid–DNA complexes [49–52].

The ability to use self-assembly to generate bilayer structures has proven to be a powerful way to generate equilibrium structures in more complex systems, especially for systems in which the symmetry between the two monolayer leaflets is lost. Here, self-assembly allows the lipids to adopt a transmembrane equilibrium distribution, something which is not easily achieved using pre-assembled bilayers. This has proven a powerful method to obtain lipid vesicles, as will be discussed in the 'Bending, buckling, curving' section later on. The idea has also been applied to peptide/lipid complexes, as shown in recent studies by Esteban-Martin et al. [53] and by Leontiadou et al. [54]. The self-assembled equilibrium states of the peptide/membrane complexes, achieved in time ranges of 50–100 ns, show the peptides to behave as expected on the basis of experimental studies. A strongly hydrophobic poly-leucine model peptide is inserted in a transmembrane configuration and a marginally apolar, alanine-based model peptide is found either as transmembrane inserted or as adsorbed parallel to the membrane plane, both with similar stability [53]. The (amphiphilic) antimicrobial peptide magainin, however, is found to stabilize a transmembrane pore [54] (see Poration section). These studies demonstrate that the spontaneous assembly of peptides and lipids is an unbiased and reliable strategy to produce and study models of equilibrated peptide/lipid complexes of unknown membrane-binding mode and topology. This type of self-assembly simulations has been taken to new heights by a series of papers by Sansom and coworkers [55–58] in which they aim to predict the equilibrium structure of membrane proteins. Using coarse-grained models they show the self-assembly of membrane proteins together with randomly dispersed lipids into either a membrane bound state or a fully membrane embedded state, depending on the properties of the protein. An example of such a process for the membrane incorporation of an acid-sensing ion channel (2QTS) is given in Fig. 2. Although the process of insertion is not meant to model membrane protein insertion in vivo (for which the details are not known, but typically require a complex protein translocation/insertion machinery), the self-assembly route provides a powerful way to predict the protein position in the bilayer for membrane proteins with a range of different sizes and architectures [56]. Details of specific lipid–protein binding modes as well as global membrane deformations induced by the protein are thus revealed at near-atomic resolution.

3.2. Formation of inverted phases

The transition from the lamellar to inverted phase, such as the inverted hexagonal (H_{II}) phase or a variety of inverted cubic phases, is of biological relevance for two main reasons. First, the intermediates along the pathway are also relevant for membrane fusion and fission, and second, inverted phases play a role in compartmentalizing (sub)cellular space, offering a relatively large membrane surface for a given cellular volume. Specific roles of inverted phases furthermore include lipoplex formulations and templates for membrane protein crystallization.

An elaborate simulation study on the transformation between the lamellar and inverted hexagonal phase has been reported by Marrink and Mark [59]. Starting from multi-lamellar configurations of DOPE and DOPE/DOPC bilayers, the spontaneous formation of stalks between the bilayers is triggered either by increasing the temperature or by reducing the hydration level. The stalks subsequently elongate in a cooperative manner leading to the formation of an inverted hexagonal phase. Within a narrow hydration/temperature/composition range the stalks appear stable and rearrange into the rhombohedral phase (also called 'stalk' phase). Although the model employed in the above study is a coarse-grained one, the phase diagram is in good agreement with the experimental observations. This conclusion was also reached in another study based on the MARTINI model. Mixtures of di-oleoylphosphatidic acid (DOPA), lysophosphatidic acid (LPA), and DOPE lipids were simulated [60]. The increasing effect of LPA and the absence of an effect of DOPA on the lamellar to H_{II} transition temperature were reproduced. The same model was used by Dahlberg to study the polymorphism of cardiolipins [61], a negatively charged lipid with four acyl chains that is found in bacterial and mitochondrial membranes. It was found that a reduction of the charge, mimicking conditions of low pH or high ionic strength, leads to the formation of inverted phases. Similarly, the tendency to form an inverted phase is correlated with the number of acyl chains in cardiolipin derivatives. The inverted geometry allows the lipid tails to adopt a splayed conformation. The reverse transition, i.e. formation of a lamellar phase from an initial hexagonal arrangement, has been reported by Shinoda et al. [62]. Using a specific CG model for a $C_{12}E_6$ surfactant and a large overall system size of close to a million CG particles, they showed that the transformation takes place via a dislocated multi-lamellar intermediate phase.

The only two studies to date of inverted phase formation using an all-atom model are reported by Marrink and Tieleman [63] and by Knecht et al. [64]. In the former study [63], the transformation from an inverted cubic to an inverted hexagonal phase was reported for a glycerolmonoolein (GMO) system. Here, stalk formation is also the first step. The transformation, however, proceeded under non-equilibrium conditions because the inverted cubic phase proved difficult to stabilize in the first place [65]. In the latter study [64], a mixture of PC lipids and fatty acids has been simulated over a temperature range. At low temperatures the system favors a gel phase, at elevated temperatures the H_{II} phase is formed, in agreement with the experimental phase diagram. The study furthermore reveals a strong hysteresis in the kinetic pathway between the lamellar and inverted phase, which can be circumvented by starting from a pre-formed stalk.

In all of the above studies the formation of the initial stalk is reported to be the rate limiting step. This is not so surprising: after all it requires lipids to leave their surrounding lipid matrix. The potential of mean force (PMF) of a phospholipid in a bilayer is a key thermodynamic property that describes the energetic cost of localized lipid defects. This PMF has been calculated recently by umbrella sampling using molecular dynamics simulations [66]. The profile has a deep minimum at the equilibrium position in the bilayer and steeply rises for displacements both deeper into the bilayer and moving away from the bilayer (cf. Fig. 5). The energetic cost associated with the latter process is high, around 30–35 kT. Even more energy is required to pull a lipid out of a membrane under non-equilibrium conditions [67,68]. In that case the work required to remove a lipid is rate dependent, even at pull rates of the order of 0.2 nm/ns. The viscosity of the solvent becomes a dominant factor contributing to the adhesion force of lipids in membranes.

In addition to lipid composition, the polymorphism of lipid membranes may also be controlled by peptides. Especially fusion peptides (e.g. the N-terminal fragments of the HIV gp41 fusion protein and the influenza hemagglutinin) are known to induce formation of inverted phases, but peptides in general can modulate the phase behavior of lipid membranes. An example of this effect is shown by a coarse-grained MD study of Nielsen et al. [69]. It was found that a meniscus is formed around a model transmembrane peptide due to hydrophobic mismatch between the peptide and the lipids. This causes local dehydration of the lamellar sample in the region away from the peptide, triggering the formation of stalks and subsequent reorganization into an inverted hexagonal phase. Knecht and Grubmüller, using an atomistic

model, showed preliminary evidence for the membrane perturbing effect of the transmembrane peptide segment of syntaxin [70].

3.3. Lipid domain formation

Domains formed by ternary lipid mixtures including cholesterol have received a lot of attention as these so-called rafts are presumably linked to biological activity. In order to understand the complexity of multi-component lipid mixtures, much effort is also directed to study the phase behavior of simpler, two-component systems including mixtures of saturated/unsaturated lipids, short/long tail lipids, and lipids with different headgroups. In such systems, macroscopic domains (on a micrometer length scale) are visualized with confocal fluorescence microscopy on giant unilamellar vesicles whereas much smaller domains (down to tens of nanometers) are seen by AFM measurements. The geometry of the domains can change from striped patterns to quasi-circular; in the case of gel domains more complex shapes such as dendritic domains or percolated networks may appear. Whether or not these structures are true equilibrium structures is not clear. The kinetics of the phase separation process can become extremely slow, with correlation times reported on the order of hours or more. The presence of curvature (in vesicular studies), the sample type (supported versus unsupported bilayers), detection technique, and vicinity to the critical point may further contribute to the large variety of domain sizes and geometries observed experimentally.

Traditionally the domain of Monte-Carlo methods and grid based computational approaches, the recent development of coarse grained simulation models has made it computationally feasible to study the dynamics of a variety of domain formation processes in lipid membranes. In single-component membranes, Risselada et al. [71] showed the nucleation of gel domains in lipid membranes that were cooled down below the main phase transition temperature T_M. Once a nucleus of critical size had formed, the domain kept growing, converting the full sample to a gel phase. Closer to T_M, gel domains also formed but did not reach a critical size and vanished over time. No evidence for a hexatic phase was found, in contrast to work reported by Revalee et al. [72] based on a generic solvent free model. Using all-atom models, gel formation can also be observed upon cooling down samples [e.g. 73–75], however, the small system sizes prevent the observation of real nucleation events in these studies. It is interesting to note that also above T_M, in the fluid phase, transient domains can be formed. This has been clearly demonstrated in the work of Klauda et al. [76] and Murtola et al. [77,78], in which collective, correlated motions of lipids in the fluid phase were detected in all-atom MD simulations of DPPC membranes. These studies cast doubt on the traditional view of lipid diffusion as a simple two-stage process, described as rattling in a cage followed by a jump. Instead, the new picture sketches lipid diffusion predominantly arising from locally appearing collective flows of lipids. Correlated motions can also be induced in response to shear flow [79–83].

A special type of domain formation concerns the formation of the rippled gel phase ($P\beta'$), which has been observed experimentally for many years but lacked a detailed picture at the molecular level. A number of recent simulation studies have provided some surprising insights concerning the structure of this phase [75,84–87]. The first reported rippled phase by Kranenburg et al. [84,85] was observed with a generic CG model. Systematic modification of the head group-head group repulsion parameter revealed a regime with a symmetric peristaltic bilayer profile, which was interpreted as a coexistence between a tilted gel and a fluid phase. In contrast to ordinary coexistence, however, the interfacial area is not minimized in order for the head groups to be able to increase their effective hydration at the domain boundaries. Both symmetric and asymmetric ripple patterns were observed in another CG simulation study by Lenz and Schmid [86]. Here, the ripple develops through splaying of the lipid tails upon entering the gel phase. This splay can be either symmetric or asymmetric, leading to a symmetric or asymmetric ripple. In the asymmetric case, the splayed domains were found to be separated from each other by intervening domains of fully interdigitated lipids. The structure of the asymmetric ripple phase closely corresponds to the structure reported by De Vries et al. [75], who used an atomistic model. On a time scale of a few hundred ns, patches of 256 DPPC lipids were seen to transform from a fluid phase to an asymmetric gel phase. The initial stage of the process, involving the splaying of the gel domain, was relatively fast (10s of nanoseconds), followed by the slower interdigitation of the intervening domain. A small fraction of the lipids at the boundary of the two domains was observed to remain disordered, fluid like. Although the structural picture of the ripple phase emerging from these simulations is largely consistent with experimental information, an important restriction in all of the above studies is the fixed hydration level. In real systems, the gel phases are dehydrated compared to the fluid phase. Most simulation studies, however, are performed at a constant number of particles. An ensemble with constant chemical potential would in fact be more appropriate, but this is computationally much more demanding.

Although studies of lipid mixtures are getting more and more abundant, observation of the actual formation of (nano) domains in these systems is still very limited. Whereas experimental phase diagrams show a rich variety of beautiful and intriguing patterns forming in binary and ternary lipid mixtures, particle based simulations are seriously lagging behind. Part of the reason for this are the time and length scales involved, especially in the nucleation-and-growth regime. Faller and coworkers reported a number of studies [88,89] of lipid mixtures consisting of short and long tail lipids, indicating the existence of a small two-phase region consisting of a fluid domain enriched in the short tail lipid surrounded by a gel-phase matrix of the long tail lipid. The limited system sizes (mostly 256–512 lipids) combined with the small difference in phase transition temperature of the pure compounds hindered straightforward interpretation of the results. A much larger scale was probed by Stevens [90] in CG simulations of binary long tail/short tail lipid mixtures exceeding 12,000 lipids. Multiple gel domains are seen to form upon quenching of the system to low temperature. Interesting is the observation that the gel domain still contains a large fraction of the short tail lipid, and exhibits complementary matching between the short and long tail lipid (i.e. short tail lipids opposing long tail ones) to achieve a constant thickness.

More 'spectacular' results can be obtained by using even more simple lipid models. Laradji and Kumar reported a number of DPD simulation studies [91,92] considering a binary mixture of two otherwise identical classes of lipids except for a tunable repulsive interaction between them. The line tension associated with boundaries between these two lipid species triggered the formation of domains, both in planar membranes and in vesicles. For systems with a strong repulsion (and correspondingly large value of the line tension), spontaneous budding and vesiculation events were observed. Similar results were obtained by Hong et al. [93]. These authors introduced a variation of the standard DPD scheme, allowing for a change in the number of lipid particles during the simulations. This neat trick allowed them to study budding events at a fixed level of tension, more closely mimicking reality; it is believed that living cells contain lipid reservoirs keeping the surface tension of membranes at a constant level. In another set of DPD simulations [94] Illya et al. also studied domain formation, triggered by changing the preferred area per lipid of the two lipid molecules. The shape of the observed domains was found to depend strongly on the relative bending rigidities, with thin, elongated shapes adopted by the stiffer domain in vesicular systems. Another approach is taken by the Voth group, who bridge results obtained from particle based simulations to mesoscopic models. For instance, phase separation of mixed PC/PE lipid bilayers was simulated at a CG level for small membrane patches, and subsequently remodeled at a mesoscale level, reproducing the

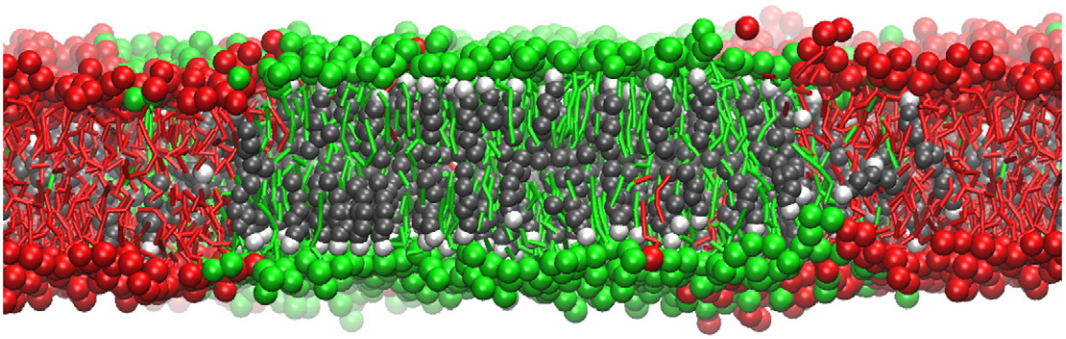

Fig. 3. Raft-like domain spontaneously formed in a ternary mixture of saturated PC (green), poly-unsaturated PC (red), and cholesterol (grey with white hydroxyl group), simulated at a CG level of resolution [100].

equilibrium properties of the CG system very well, including collective fluctuations in both phases, spatial correlation functions of the order parameter, and the line tension between the gel and fluid domains [95]. In the group of Vattulainen, atomistic simulations are also used to parameterize coarse-grained interactions between different lipids, using the inverse MC approach. Applications to cholesterol–lipid mixtures [96] show that the experimentally observed phase separation into cholesterol-rich and cholesterol-poor domains at intermediate cholesterol concentrations can be reproduced notwithstanding the very coarse-grained nature of their model.

Studies of three component systems are even rarer. Although there is a lot of interest in raft-like mixtures, particle-based simulation studies in which formation of raft-like domains is observed are virtually absent. The main obstacle seems to be the difficulty of parameterizing coarse-grained models that can mimic the detailed interaction of sphingomyelin, PC, and cholesterol molecules. All-atom models of course include such detail, but are necessarily limited to studies on pre-assembled rafts [e.g. 97,98]. An interesting atomistic study on the energetics of lipids in raft-like mixtures was reported by Zhang et al. [99]. These authors calculated a partial PMF for cholesterol in an unsaturated POPC bilayer and in a 18:0 sphingomyelin (SSM) bilayer to determine the free energy of transfer for cholesterol between these two bilayers. They found a favourable free energy of transfer to SSM by 2–3 kT, suggesting cholesterol prefers to associate with the saturated lipid, in agreement with calorimetric measurements. Interestingly, based on potential energies or local interaction energies the opposite conclusion would be reached, highlighting the danger of relying on indirect assessments including potential energies that ignore entropic contributions. There is clearly a need for a better understanding of the thermodynamics of the interactions between cholesterol and other membrane components.

Very recently Risselada and Marrink [100] observed the spontaneous domain formation in a raft-mimicking mixture composed of saturated PC, poly-unsaturated PC, and cholesterol, modeled with the MARTINI force field. The mixture phase separated into a liquid-ordered, raft-like phase and a liquid-disordered, fluid phase with structural and dynamic properties closely matching experimental data. The near-atomic resolution of the simulations reveals remarkable features of both domains, and of the boundary domain interface (see Fig. 3). Furthermore, the existence of a small surface tension between the monolayer leaflets was concluded, driving registration of the domains across the two monolayers.

3.4. Lipid mediated protein–protein self-assembly

The fluid mosaic model of biological membranes proposes free diffusion of single proteins in a sea of lipids. However, most membrane proteins are not unrestrictedly mobile, and spontaneous organisation into functional clusters is now well established, both in model liposomes and in vivo. The current challenge for simulation studies is to address the question of how the physicochemical properties of the membrane lipids might affect self-assembly of integral membrane proteins. Coarse-grained models appear ideally suited to target this question, yet this field of simulation is largely unexplored. Pioneering efforts, however, are now being made.

Periole et al. [101] simulated the constitutive self-assembly process of the visual receptor rhodopsin, a 7 transmembrane-helix G protein-coupled receptor. Multi-copy rhodopsin simulations (16 proteins per unit cell) at a protein-to-lipid ratio of 1:100 in different bilayer environments were carried out. From an initial condition out-of-equilibrium, with the proteins fully dispersed in the bilayer cell, the formation of protein–protein contacts rapidly increased, with most monomers recruited within 1–2 μs. The final snapshots showed extended string-like clusters of eight monomers. Furthermore, from results obtained with different bilayers, a clear dependence on the lipid chain length could be appreciated, in line with fluorescence resonance energy transfer (FRET) experiments. A generic lipid/protein model was used by Reynwar and Deserno [102] to study the composition mediated interaction between proteins adsorbed onto a two-component lipid membrane. The short-range preferential solvation of the proteins by one of the two lipid components, which was put into the model, resulted in long-range protein–protein attraction. Simulations of membrane patches with 16 absorbed proteins showed formation of phase segregated protein clusters for lipid mixtures close to the critical point. In another study by the group of Deserno [103], capsid-like proteins also exhibit a membrane mediated attractive interaction. Here, the membrane was modeled as a single component membrane, however, and the attraction arises from curvature forces in the direction perpendicular to the protein–protein axis. At high enough capsid density, the curvature forces are strong enough to drive vesiculation of the membrane as will be discussed in more detail in the Bending, buckling, curving section. Systematic studies of lipid mediated protein–protein interactions are also performed in the group of Smit [104], showing that hydrophobic forces drive long-range protein–protein interactions and that the nature of these interactions depends on the length of the protein hydrophobic segment, on the three-dimensional structure of the protein and on the properties of the lipid bilayer. A specific study of the dimerization of glycophorin and some of its mutants has been reported by Sansom et al. [105].

4. Poration

Pores can be formed in membranes by several agents or environmental conditions. In the laboratory, electroporation is a common method to introduce foreign molecules, including genes, into cells. Ultra-high field electroporation permeabilizes internal membranes and can lead to apoptosis, with potentially therapeutical applications. Antimicrobial peptides in many cases cause pores in membranes, dissipating essential ionic gradients in the permeabilized cell. Pores also form spontaneously, at rates that can both be measured

and calculated, as normal but rare fluctuations that are essential for lipid flip-flop and unassisted transport of charged molecules, including ions and peptides. Pores can also be induced by mechanical stress such as osmotic swelling and are a common breakdown mode of membranes. Lastly, pores and pore-like structures occur as intermediates in large-scale remodeling of membranes. Because pores are normally transient, their structure is difficult to determine. Clear examples of this are the ongoing debates on the exact mode of action of antimicrobial peptides and the membrane states during electroporation. In a rapidly growing series of computational papers, the process of pore formation in lipid membranes has been revealed. The simulations greatly enhanced our understanding of the stability of membranes under various conditions of stress. Fig. 4 shows simulation snapshots of a number of different pores observed by different groups. Remarkably, independent of the type of model used (atomistic or coarse-grained) and the way in which the pore was created, the pores look very similar. They are toroidally shaped, with the lipid head groups lining the pore wall. The first description of such a pore was reported as an intermediate metastable state in the pathway of bilayer self-assembly [11] (see Fig. 4F and the Phase transformations section). Below we review different ways to create pores and the properties of those pores in more detail.

4.1. Pore formation by electric fields

Experimentally, pores are commonly formed using electroporation — the application of external potential differences to a membrane or cell. Traditionally this is done in relatively low-voltage simple lab setups, but recently high-energy ultra-short pulses have been applied to membranes in cells and organelles by powerful lasers. Voltage differences across a membrane due to application of nanosecond length pulses have been estimated at up to several volts. This setup can be mimicked in atomistic simulations of membranes by applying a constant electric field in the system [106]. The exact correspondence between the resulting field in the simulation cell and the field as found in experimental systems has been questioned [107], but has recently been clarified by Böckmann et al. [108] and by Roux [109].

Several recent papers have investigated electroporation in detail by simulation, initially by Tieleman et al. [107,110], followed by a significant number of additional simulations [108,111–116]. Animations of the pore formation process are available online, linked from ref. [107] at http://www.biomedcentral.com. In response to the electric field, water molecules are occasionally found in the interior of the membrane, which is normally, in the absence of applied electric fields, a very rare occasion. Such water molecules may form hydrogen-

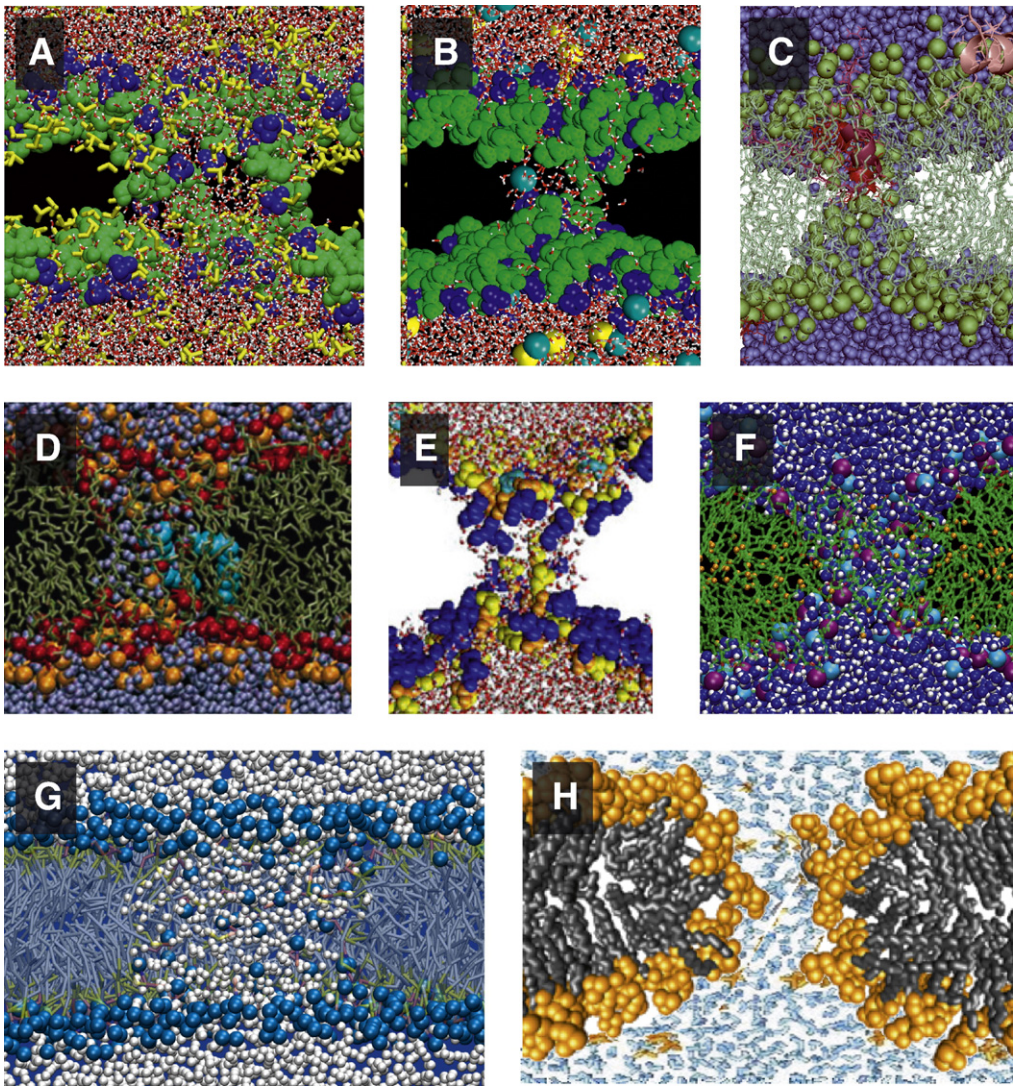

Fig. 4. Comparison of eight different transmembrane toroidal pore structures. Pores are induced by (A) the action of DMSO [130] (courtesy A. Gurtovenko), (B) an ion gradient [118] (courtesy A. Gurtovenko), (C) melittin peptides [136] (courtesy of D. Sengupta), (D) pulling of a lipid inside the bilayer [66], (E) electroporation [113] (courtesy of P.T. Vernier), (F) spontaneous aggregation [11,39], (G) application of lateral tension [127] (courtesy of A. Rzepiela), and (H) a local density based constraint [128] (courtesy W. den Otter).

bonded chains of several water molecules, which do, however, not necessarily lead to pores. Pores form from small water defects, which occur more commonly near head group defects, where the choline group of the lipid is located nearer the glycerol backbone region than normal. A stable pore forms when water defects begin to span the bilayer and head groups start moving towards this initial pore. This focuses the electric field across the defect and the process accelerates. The pore then expands and becomes lined with lipid head groups, although it takes some time before the head groups are evenly distributed over the pore 'surface'.

This atomistic description enables detailed analysis of the driving forces for pore formation. It appears that a key driving force is the motion of dipoles (water molecules and lipid head groups) in the strong field gradients at the interface. There always is a field gradient at the interface, even in the absence of an applied electric field, but this appears to be balanced by other forces to give a stable interface. In the presence of an electric field, this balance is broken and pore formation is the way to minimize the total free energy of the system in the presence of an applied electric field of sufficient strength. Thus, the simulations show how a pore forms in atomistic detail, starting from water defects involving one and then a few water molecules, with final pores involving significant rearrangement of lipids to form the characteristic toroidal, doughnut-shaped pore that is lined with head groups.

More recent work has expanded on the original picture, although this picture has not changed substantially. Vernier et al. have studied translocation of phosphatidylserine (PS) lipids through pores created by electric fields [113,114]. The pores look similar (see Fig. 4E), but the PS lipid is more likely to be moved across the membrane electrophoretically. Vernier et al. also investigated in detail the behavior of head groups in the presence of electric fields [112], the effect of lipid type on pore formation [116] and compared simulations with applied electric fields and ion gradients [114]. In an excellent recent paper, Böckmann et al. described a large set of simulations at different fields and provided a strong link between the experimental pore formation times as a function of applied potential difference and the simulated pore formation processes [108].

An alternative method to introducing a strong potential difference across a membrane in a simulation is by modifying the ionic concentration in two water compartments in the simulation cell. This requires two independent bilayers in a simulation cell because of the periodic boundary conditions normally used and is therefore computationally more expensive. Pioneered by the group of Vattulainen, it has been used by a number of groups to show that various forms of ion imbalance create a field that is strong enough to cause electroporation [114,117–119], with very similar qualitative result to simulations with a constant applied field (see Fig. 4B). In these simulations, pores form rapidly and expand in a similar manner as seen in simulations with applied external fields. Ions diffuse across the pore to approach (but generally not reach) equilibrium between both aqueous compartments. Pore closure is a slow process on a ca. 100 ns time scale, and full resealing of the bilayer has generally not been reached in these double bilayer simulations, although it is plausible that this will be the final result. Interestingly, these simulations have also given insight into the origins of experimentally measured potential differences. An example is a recent study by Baker and coworkers using the same concentration of NaCl and KCl on either side of the membrane, which resulted in a significant potential drop due to the binding of Na+ but not K+ to the membrane [120]. A clever way to simulate an ion imbalance, avoiding the double bilayer set-up, has been proposed by Tarek et al. [121]. Here two air/water interfaces are introduced on either side of the bilayer, preventing ions from crossing the periodic simulation cell.

Clearly, pore formation is linked to the likelihood of defects forming, which can be either aided or prevented by the presence of other molecules in the lipid bilayer. This can be tested by further simulations on bilayers with impurities, such as the simulation of Tarek on electroporation in the presence of proteins [111], as well as on bilayers with different water permeation properties, including mixed phospholipid/cholesterol bilayers and bilayers with oxidatively damaged lipids [122].

4.2. Pore formation by tension and energetics of pore formation

Membranes can also form pores in response to an applied tension. This is exploited in pipette aspiration experiments to measure mechanical properties of lipid mixtures. Simulations have explored the mechanical response to tension at a number of different levels. In older studies, highly simplified models were used, e.g. 2-dimensional membranes and a simple Hamiltonian [123], but more recently 3-dimensional particle-based simulations have been very successful. In simulation studies it is straightforward to vary the surface density of lipids, effectively changing the surface tension. At a certain threshold rate, pores form. An example of a tension stabilized pore simulated at a CG level of resolution [31] is show in Fig. 4G. Using a solvent-free generic coarse-grained model, Farago observed pores that could be explained by a simple model based on the area dependence of the free energy [124]. Briels and coworkers have reported extensive free energy calculations on pores [125,126]. They used constraint forces to calculate the free energy profile for pore formation as function of pore radius using a coarse-grained model. In this model, pore opening requires 15–20 kT, while no barrier to pore closure was found although such a barrier may exist. In the second study, they stretched bilayers to investigate the stability of pores and basically establish a pore phase diagram in terms of stable, metastable, or unstable. They also suggest a link between the number of lipids in the simulation and the observed stability, which may provide a closer link to experiment.

In full atomistic detail it remains quite challenging to calculate line and surface tensions with sufficient accuracy to make a reliable link between the microscopic simulation and mesoscopic models, but this has been attempted in a number of cases. Leontiadou et al. showed that pores can be formed by applying a surface tension to unperturbed lipid bilayers [110,127]. These pores can also be simulated in a pre-formed state. Surface tension was applied in both cases to study the formation and stability of hydrophilic pores inside the bilayers. Below a critical threshold tension the pores are stabilized with a minimum radius of 0.7 nm. When the lateral pressure exceeds the threshold tension, the pores become unstable and start to expand causing the rupture of the membrane. In the simulations the mechanical threshold

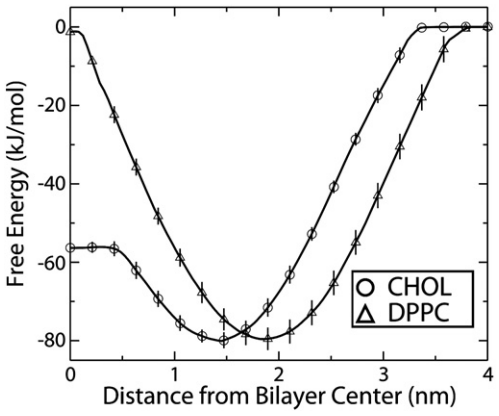

Fig. 5. Potential of mean force for a single cholesterol and a DPPC molecule in a liquid crystalline DPPC bilayer at 323 K. The relative free energy is set to 0 kJ/mol outside of the membrane in the fully solvated state. The barrier for moving the cholesterol to the center of the membrane, corresponding to the transition state for flip-flop, is 24 kJ/mol (Bennett, Tieleman, et al., in preparation), much smaller than the barrier of 78 kJ/mol required for the flip-flop of DPPC [66]. Extraction of the lipids from the membrane into the aqueous solvent requires about the same amount of energy (~80 kJ/mol) for both cholesterol and DPPC. Courtesy D. Bennet.

tension necessary to cause rupture of the membrane on a nanosecond timescale is much higher in the case of the equilibrated bilayers, as compared with membranes containing preexisting pores. In a very extensive calculation, Wohlert et al. determined the free energy for pore formation as a function of pore radius in a fully atomistic bilayer [128]. Depending on the pore radius, they found a quadratic shape below 0.3 nm and a linear shape for the free energy above that. Fig. 4H illustrates a pore right at the crossover point between these two regimes. For large pores, line tensions could be calculated and interpreted in terms of the energetic cost for deforming a part of the lipid bilayer into a hydrophilic pore. The region with small radii could be described and understood in terms of statistical mechanics of density fluctuations. An alternative approach to estimate the free energy of pore formation is by pushing a lipid reversibly into the bilayer interior [66]. During this process, a small water pore forms spontaneously. The energy required to form such a water pore is found to be almost 80 kJ/mol (Fig. 5). This maximum value corresponds to a water-filled pore, and will be similar to the work required to create such a pore with a lipid head group in the center of the membrane (see Fig. 4D).

4.3. Poration induced by surfactants and antimicrobial peptides

Membranes can also be porated by chemical means, of interest in e.g. biotechnology. Because surfactants can be changed arbitrarily by synthesis, they provide an interesting path to investigating membrane stability. Groot and Rabone [129] used DPD to study the interactions of surfactant with PE bilayers, varying both the hydrophobic/hydrophilic balance of the surfactant and its concentration in the membrane. More hydrophilic surfactants caused significantly larger perturbations for a given concentration. As the concentration increased, pore size and stability observed increased. Somewhat similar behavior was observed in bilayers containing the surfactant-like molecule dimethylsulfoxide (DMSO) in a series of simulations by Gurtovenko and Anwar [130–132]. At low concentrations, DMSO induces membrane thinning, while at higher concentrations, DMSO induces transient water pores into the membrane (see Fig. 4A). At still higher concentrations, individual lipid molecules are desorbed from the membrane and the bilayer breaks down [130]. Pore-mediated rupture of membranes consisting of short-tail lipids has also been observed [36]. Lee and Larson [133] studied pore formation by polyamidoamine (PAMAM) dendrimers in DMPC and DPPC bilayers using the MARTINI CG model. Third and 5th generation dendrimers interact with DPPC and may cause pore formation depending on the chemical details of the dendrimers and the salt concentration. In a follow-up study [134], 5th and 7th generation dendrimers were simulated and showed distinct behavior depending on their charges, in agreement with AFM experiments. Relatively subtle differences in structure caused significant differences in pore formation and water permeation through the bilayer, suggesting that the CG simulations are accurate enough to help design and understand this type of molecules.

Antimicrobial peptides (AMPs) have attracted significant interest as promising new candidates to fight the increasing bacterial resistance to existing antibiotics. Although the mode of action of antimicrobial peptides is not known in detail and is most likely manifold, most evidence suggests the formation of transmembrane pores. Depending on the type of pore they presumably form, AMPs have been classified into three main categories, namely barrel stave, toroidal pore and carpet-like. Simulations of antimicrobial peptides have become quite common, but it is difficult to obtain sufficient sampling to draw firm conclusions. A recent comprehensive review is available [16]. Simulations performed in the group of Marrink point to the danger of oversimplification in the hypothetical pore models [135]. Using atomic resolution, the mode of action of a specific AMP of the magainin family could be simulated. Similar pores were observed in extensive simulations of melittin interaction with DPPC bilayers [136]. The significance of these works is that pore formation was observed to occur spontaneously and was not induced by artificial constraints. Interestingly, the structure of the pore that formed in the simulations, while compatible with the available experimental data, differs significantly from current idealized models of a toroidal pore (see Fig. 4C). The term 'disordered toroidal pore' was coined to describe such pores. Pores only form above a critical peptide to lipid ratio and require local aggregation of peptides. Pores are toroidally shaped but only one or two peptides line the pore, in contrast to commonly depicted cartoons. In neither case does it appear to be required that the peptides remain helical (their solution structure), which may be of broad relevance given the large diversity in shapes of antimicrobial peptides. When positive charges on the side chains are removed pores no longer form, showing the importance of charges in the process. This conclusion was also reached from a combined approach [137] using all-atom simulation and electrophysiological measurements. Here, the mode of action of cateslytin, a natural beta-sheet forming AMP was investigated. The simulations underline the disordered nature of the transmembrane pores formed. Based on the simulation of a mutated peptide and the effects of small external electric fields, it was furthermore concluded that electrostatic forces play a crucial role in the process of pore formation. The hydrophobic peptide alamethicin formed pores at sufficiently high concentrations in combined atomistic/CG simulations [138]. The authors used CG simulations to equilibrate the distribution of alamethicin within the membrane and then converted the coarse-grained simulation to atomistic to investigate the details of water permeations. In the atomistic simulation based on the aggregated peptides significant water leakage through the membrane was observed. In the literature it has nearly always been assumed that alamethicin forms highly regular channels, unlike most antimicrobial peptides, but the simulations observed quite irregular structures. This is reminiscent of pores formed by magainin and mellitin (described above) and appears consistent with solid state NMR data in the same study, but needs to be reconciled with the experimental observation of reproducible discrete ion conduction levels for alamethicin in bilayers.

A nice illustration of the power of generic coarse-grained models is a recent paper by Ilya and Deserno [139]. They studied the interactions between lipid bilayers and amphiphilic peptides, using a coarse-grained lipid model and a rod-like peptide model that allowed tuning the hydrophilic/hydrophobic balance of the peptide as one might expect from changing the amino acid sequence. Not surprisingly, as the peptide–lipid attraction becomes stronger peptides are more likely to first adsorb and then insert. However, several peptides that individually only bind to the bilayer insert as a group. Pores involving multiple peptides formed when the peptides had hydrophilic strips, and the pore size and morphology could be tuned by the shape and extent of the hydrophilic strips as well as the strength of the peptide–peptide interactions.

4.4. Membrane defects due to charged residues

Extremely charged peptides interacting with membranes have also been simulated. A polyarginine or polylysine peptide bound preferentially to phosphatidic acid lipids in a mixed bilayer, but without forming pores using one peptide per bilayer leaflet [140]. Extension of 50 ns simulations to several 100 s of ns did not change this behavior (MacCallum, Bennett, Tieleman, unpublished observations). In contrast, a peptide derived from the HIV-1 Tat protein showed spontaneous translocation accompanied by large defects in the membranes [141]. The behavior of arginines in a membrane has been a recent topic of significant discussion in the context of voltage-gated potassium, sodium, and calcium channels. Several groups have shown that the voltage-sensing S1–S4 domains, or just the S4 helix, of potassium channels cause large water defects localized around arginines [142–144]. Simulations of small molecules mimicking each

amino acid side chain show that all charged side chains cause significant perturbation of the membrane as well as major shifts in pKa so that only arginine is likely to remain charged in the center of the membrane [145,146]. This was also found by independent calculations using different methods and a different force field [147,148]. Allen and coworkers calculated a potential of mean force for moving an arginine through the entire membrane as part of a polyleucine helix, and found similar energies and water/membrane defects [149]. Although the cost of burying a single arginine in the membrane is high, this cost is primarily associated with the creation of large-scale defects. Once formed, these defects may readily allow the translocation or partitioning of additional arginines or other charged molecules. This cooperative behavior has been observed in initial simulations (MacCallum, Bennett and Tieleman, unpublished) and may be biologically significant. The studies cited above all concern atomistic simulation studies. Whether CG simulations are also capable of predicting correct partitioning of charged residues remains to be seen. The potential pitfalls of CG models in this regard are nicely illustrated in a comparative paper on atomistic and coarse grained protein–lipid interactions [22].

4.5. Pore-mediated ion permeation and lipid flip-flop

Permeation of ions directly through the lipid bilayer, in the absence of fast transport through ion channels, has attracted significant attention. Different approaches have been taken. One approach is to calculate a potential of mean force for the ion of interest as function of its position in the membrane, or some other more complicated reaction coordinate. Tepper and Voth extended earlier work on the permeation of protons [150] to simulation of permeation of water, hydroxide ions, and sodium ions [151]. For protons, it was found that solvent structures form around the permeating proton that are distinctly different from the single-file water chains that were previously simulated [152] and also much longer lived. Furthermore, a transition state structure was proposed consisting of a small network of water molecules surrounding the proton, with the bilayer headgroup regions being relatively unperturbed. For sodium and hydroxide ions, the calculated PMF differed significantly from the proton case, although by umbrella sampling with windows of up to 1 ns no converged PMF could be calculated. This is consistent with other calculations on moving charges into the membrane, where equilibration times on the order of tens of nanoseconds were observed (see above). The authors argue that passive permeation of ions is a highly concerted process in which solvent, ion, and lipids are coupled [151].

A second approach studies pore formation coupled to ion transport. In nearly all simulations of pore formation, either by electric field or by ionic imbalance in double bilayers, ion permeation is observed at the same time. Generally, differences between cations and anions are observed, although obtaining accurate statistics is not possible in the highly non-equilibrium situation of a pore forming in a bilayer. Leontiadou et al. worked around this by creating pores first, and then studying transport of ions through these pores [153]. The presence of ions significantly reduced the stability of transient water pores, because the binding of in particular sodium ions to the lipid/water interface increases the line tension at the pore surface. Both sodium and chloride permeate slowly across small pores (radius <1.5 nm), but at larger radii anion flux is greatly enhanced relative to cation flux. Kandasamy and Larson also observed that chloride ions appear to have a lower residence time in pores than sodium ions [154]. Gurtovenko and Vattulainen observed similar permeation rates for sodium and chloride, while potassium appears to be faster than both [118].

Based on the estimated free energy of pore formation and closure [66] and the results of Leoniadou et al. [153] for ion diffusion through pre-existing pores, Tieleman and Marrink [66] predict membrane permeability rates of sodium ions in close agreement with experiment, on a time scale of a day, ca. 14 orders of magnitude longer than the actual simulations. These results show that basal permeability of ions is pore mediated. A number of simulation studies [54,155,156] have also shown that lipids can use pores to flip-flop from one leaflet to the other. This process was first observed for a metastable pore formed during the self-assembly of lipids into a vesicle [155]. Flip-flops through this pore occur on a nanosecond time scale. The flip-flop process was studied more systematically by Gurtovenko and Vattulainen, in a setup that created pores and then looked at flip-flop through pre-existing pores [156]. Taking into account the rate at which pores form, the predicted [66] lipid flip-flop rate is of the same order of magnitude as the permeation rate of sodium, in agreement with experiment. Current MD simulations thus convincingly show that the most likely pathway of lipid flip-flop as well as ion permeation (at least cations) is through transient pores that stochastically form in lipid membranes. Although the formation rate of such pores is low, they are frequent enough to account for the observed macroscopic ion permeabilities and lipid flip-flop. In biological membranes, the formation rate of the pores will be affected by lipid composition and the presence of other molecules in general, offering the cell a natural way to control osmotic pressure and membrane asymmetry. Simulation studies show that pores formed in the presence of DMSO, for instance, facilitate both ion permeation through the membrane [131] and lipid flip-flop [132].

In contrast to lipid flip-flop, cholesterol flip-flop most likely does not proceed via pores. Potential of mean force calculations for cholesterol movement across the bilayer can be used to quantify flip-flop for cholesterol based on the height of the free energy barrier. To get an estimate of the barrier for cholesterol flip-flop, a full PMF including the center of the membrane has been calculated (Bennett et al., in preparation). An example is shown for cholesterol in a DPPC membrane in Fig. 5, with a barrier of ca. 24 kJ/mol. This is much lower than for DPPC flip-flop (78 kJ/mol, see discussion of pore energetics above). The steroid hormone cortisone has a chemical structure similar to cholesterol although it contains more hydrophilic groups. Vijayan and Biggin calculated the distribution of this steroid hormone in a POPC bilayer and found a barrier of ca. 29 kJ/mol for translocation, similar but somewhat higher than for cholesterol as would be expected based on the structure of cortisone [157]. These barriers in DPPC are sufficiently high that spontaneous translocation is unlikely to be observed in nanosecond scale simulations, but this barrier can easily be lowered considering different lipid types or cholesterol analogues. Simulations of ketosterone, a less hydrophilic variant of cholesterol with a ketone instead of a hydroxyl group in the headgroup, in DPPC showed that ketosterone has a less well defined position in the bilayer and actually showed spontaneous translocation during equilibrium simulations, suggesting the barrier for translocation is greatly reduced compared to cholesterol [158]. Simulations and experiments on a number of different types of bilayers have shown that a certain fraction of cholesterol may even be found in the center of the bilayer, parallel to the plane of the membrane. In a comparison of C22:1 and C14:1 bilayers, cholesterol was found in the center of the membrane experimentally in the C14:1 case but not in the C22:1 case. Water permeation was also increased in the C14:1 case, suggesting the existence of hydrogen-bonded defects linked to the cholesterol [159]. Simulations of bilayers formed by the poly-unsaturated diarachidonyl (DAPC) lipids [160] showed fast flip-flop rates for cholesterol and a measurable population of cholesterol in the center of the membrane, in agreement with previous neutron-scattering experiments that suggested cholesterol has a strong aversion from poly-unsaturated fatty acids. See also Fig. 3 in which the different packing of cholesterol in poly-unsaturated versus saturated lipids clearly shows up.

Combined, the studies to date suggest that cholesterol flip-flop depends strongly on the details of a bilayer, but is fast on a physiological time scale (milliseconds), and for poly-unsaturated even fast enough to observe on the nanosecond time scale accessible to atomistic simulations.

5. Bending, buckling, curving

Cell membranes are not simply flat. In fact, a completely planar state would be difficult to achieve. For entropic reasons alone, any bilayer undulates. Besides, a finite patch has a tendency to close upon itself (i.e. form a vesicle) in order to minimize the line tension arising at the bilayer edge. Furthermore, membrane asymmetry induces a spontaneous curvature in the bilayer, favoring the formation of bent geometries. Such asymmetry can be of lipid origin only, but can also be induced by the adsorption or aggregation of peptides and proteins. Many structures in the cell involve strongly curved membrane surfaces, such as the nuclear pore, mitochondrial cists, the Golgi apparatus, the endoplasmatic reticulum, and the endosomal membrane. In addition, important cell membrane related processes require the transient formation of bent lamellar structures, especially during exo- and endocytosis.

5.1. Membrane undulations

Bilayers, like any elastic material, exhibit temperature induced fluctuations. Fluctuations in the average position of the bilayer surface, called undulations, are governed by the bending rigidity of the bilayer. Lindahl and Edholm [10] were the first to show the spontaneous appearance of such undulations in their benchmark study of a DPPC membrane, modeled at all-atom resolution. From the amplitude of the undulations the bending rigidity of the DPPC membrane patch was estimated to be 4×10^{-20} J, within the range of experimental values. Their study has inspired a range of similar studies using atomistic models [161,162], and subsequently coarse-grained models [163–165] to be able to access larger bilayer patches and longer simulation times. The results of a number of these studies were cast into an elastic model by Brown [166], showing that the inclusion of a finite monolayer spontaneous curvature is essential to obtain fully consistent agreement between theoretical and available simulation/experimental data. An important conclusion drawn from the work of Shkulipa et al. [163] is the observation that much longer time scales are required for undulatory modes to relax than hitherto assumed. An alternative approach to measure the bending rigidity of lipid bilayers has been reported by Harmandaris and Deserno [167]. These authors simulated membrane tethers (i.e. cylindrical bilayer tubes). The tensile force along the tether, which can be easily measured during a simulation, is proportional to the bending modulus. Even for tethers with a curvature radius comparable to the bilayer thickness this relation was shown to still hold. A clear advantage of this method to determine the bending rigidity is that it does not require large system sizes nor long simulation times; a disadvantage is the difficulty to equilibrate the inner and outer monolayers with respect to the relative amount of lipids, which can be alleviated by using a less realistic model in which lipids can easily flip-flop between the monolayers.

An ongoing debate since the late nineties has been whether or not the periodic boundary conditions used in simulation studies of membrane patches introduces an artificial tension. Feller and Pastor, followed by several others, argued that such a tension would arise from the suppression of long wavelength undulatory modes [168]. This has been questioned in [161], presenting simulation results which exhibit only a weak correlation between surface tension and projected area. To complicate the issue, finite size effects did appear in other simulation studies [10]. The cause of the finite size effect in the latter study, however, has been blamed on artifacts associated with the heat bath [169]. CG models in principle offer a way to provide clear insights into the matter. In a recent re-examination of the problem, the group of Pastor simulated CG DPPC patches in size varying between 72 and more than 30,000 lipids in excess solvent (see Fig. 6). The preliminary results show that the surface tension is not very sensitive to the size of the membrane patch (Lerner, Lee, Marrink, Pastor, in preparation),

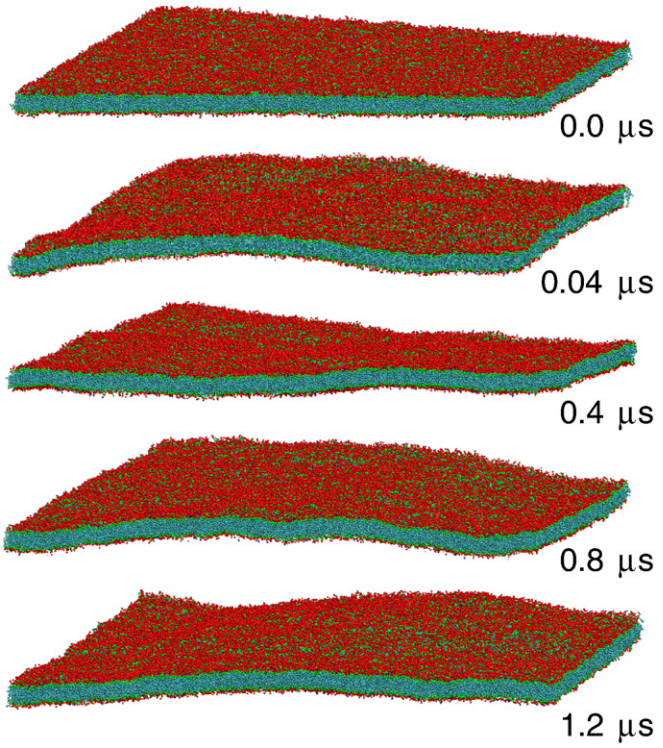

Fig. 6. Large scale undulations of a coarse grained DPPC bilayer (courtesy H. Lee and R.W. Pastor). From top to bottom, a time series is shown starting from a completely flat bilayer state. Lipid head groups are colored red, the glycerol group green, and lipid tails cyan. Solvent is omitted for clarity. The patch contains more than 30,000 lipids with an area of approximately 100×100 nm^2.

indicating that the suppression of long wavelength undulations in small systems has little effect on the projected membrane area. In accord with these new insights, the recent re-parameterization of the CHARMM lipid force field (Pastor, personal communication) has led to a reduced surface tension for small membrane patches. The issue of the coupling between surface tension and membrane area is still not fully resolved, however. For instance, recent simulations show that two types of tension need to be distinguished, one associated with stretching the membrane, and the other for bending [170]. Related work shows that the two types of tension may also appear as a consequence of the way the tension is calculated, either along the projected area, or along the curved membrane midsurface [171].

5.2. Formation and structure of vesicles

Vesicles, or liposomes, are widely used in in vitro studies as model systems for either complete cells or cell organelles such as endosomes or transport vesicles. In the realm of synthetic biology they furthermore play an important role as drug carriers, sensors and many more potential applications, limited only by imagination. Whereas particle based simulation studies traditionally have been looking at small planar membrane patches artificially extended to quasi-infinite size by the use of periodic boundary conditions, simulations of vesicles have only appeared on stage during the last five years. The main reason for this apparent gap in the field of membrane simulations is the fact that even for the smallest vesicles one already needs close to 1,000 lipids and substantially more water molecules per lipid than in bilayer systems, resulting in an until recently challenging number of particles. Even today's simulation studies on vesicles concern vesicle sizes not exceeding 40 nm in diameter, at or even below the limit of vesicle sizes that can be made in vitro. Simulation studies of vesicles have some clear advantages compared to planar membranes: the absence of a periodicity effect (at

least through the bilayer), the use of curvature as an additional parameter, and of course the closer correspondence to many experimental studies performed with vesicular systems.

In contrast to the experimental formation of vesicles, in simulations vesicles can in principle be pre-assembled. However, it is difficult to determine in advance the total number of lipids, the ratio between lipids in inner and outer monolayers, and the water content required to obtain a realistic vesicle model. Not surprisingly, the ability of the lipids to self-assemble has been exploited to form vesicles right from the beginning. Pioneering efforts in this direction were made by Drouffe et al. [172], who used single 'lipid' particles with anisotropic pair interactions and a multibody hydrophobic driving force. MD simulations showed spontaneous aggregation of such particles into vesicles. A more realistic lipid model consisting of three connected particles (one modeling the headgroup, two modeling the tail) was used to study vesicle formation by Noguchi et al. [173,174] using Brownian dynamics. Explicit solvent was included for the first time in a DPD study of vesicle formation by Yamamoto [175], followed by studies using more sophisticated CG models [176,177] and even an atomistic model [155]. Although the models and simulation techniques used by the different groups cited above differ considerably, vesicle formation is observed in each case to proceed similarly, via a bicellar like intermediate. Once formed, the bicelle encapsulates water and eventually forms a closed vesicle, driven by the line tension arising at the bilayer edge. Markvoort et al. [177] provided evidence that not an enthalpic, but rather an entropic effect drives closure of the vesicle. One might also start from a bicellar intermediate, for instance by removing the periodic boundaries of a planar membrane, and observe the closure into a vesicle [176,177]. For large membrane patches, however, this process can become tediously slow. A quick way to obtain vesicles was recently demonstrated by Risselada et al. [178]. In this study a mean field boundary potential was used to replace the bulk solvent both inside and outside the vesicle, leaving a small layer of solvent around the membrane. Aided by the molding effect of this set-up, and the reduced number of (solvent) particles, vesicles up to 60 nm diameter were formed on a nanosecond time scale. The same study also pointed to a caveat of the self-assembly method to generate vesicles. Due to the fast nature of lipid self-assembly, the vesicles, once sealed, may still not be equilibrated. In fact, even if this process is slowed down, the vesicular membrane remains under expansive stress as a consequence of the line tension that surrounds it, right up to the moment of closure. The total membrane area is therefore too large, and the vesicle formed too big in comparison to the expected equilibrium state. The non-equilibrium state of self-assembled vesicles was demonstrated in [178] by the introduction of artificial pores in the vesicle wall, allowing flip-flopping of lipids after the vesicle has formed. Depending on system composition, hundreds of nanoseconds may be required before the 'flip' and 'flop' rates are the same, and hence the vesicle equilibrated (Risselada and Marrink, manuscript in preparation). In fact most studies on vesicular systems reported up to date are likely based on insufficiently equilibrated systems, except for studies using generic CG models in which lipids unrealistically readily flip-flop between the monolayers.

Only few studies to date address the structure of lipid vesicles [43,176]. The curvature, especially for small vesicles, is shown to affect the packing of the lipids and to induce a transmembrane asymmetry. In simulations of mixed vesicles composed of PC, PE and lysoPC lipids [176], three key features that solve the geometrical constraints imposed by the strong curvature were observed: enrichment of the outer monolayer by the lyso lipid, dehydration of the inner monolayer interface, and either extension or backfolding of the lipid tails in the outer monolayer. The asymmetry between the inner and outer monolayer of vesicles is reflected in the stress distribution across the bilayer, as is illustrated in Fig. 7. Both the stress profile across a tensionless vesicle and across a hypo-osmotically stressed vesicle are shown. The latter was obtained simply by adding additional solvent to the vesicle interior [179]. In a study reported by Markvoort et al. [180], asymmetry between the inner and outer monolayer was enforced by changing the nature of the headgroups of the CG lipids. The resulting spontaneous curvature dramatically alters the preferred shapes of the vesicles, from ellipsoids, pear-shaped to cup-shaped geometries. Even fission of these vesicles into two separate vesicles has been observed [181].

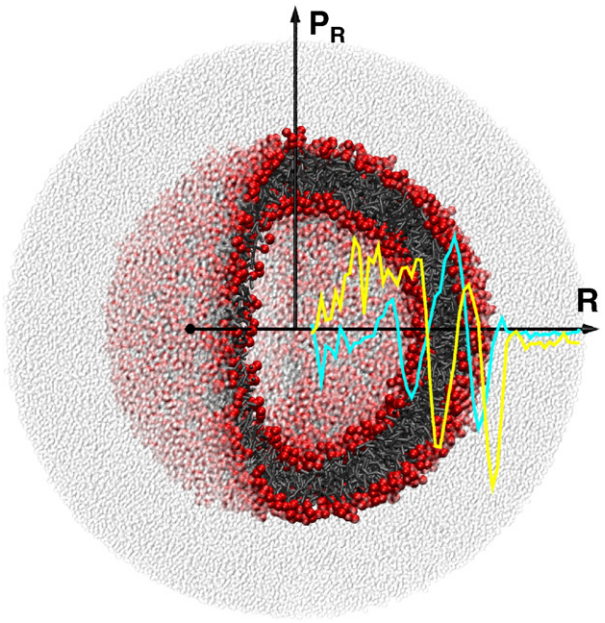

Fig. 7. Pressure profile of an equilibrated vesicle (cyan) and an osmotically swollen vesicle (yellow). The level of stress (vertical axis) versus the distance from the center of the vesicle (horizontal axis) is plotted, showing the characteristic negative peaks at the membrane/water interfaces and the positive peak in the membrane interior. For the equilibrated vesicle, the pressure inside and outside of the vesicle are equal, whereas in the hypo-osmotically stressed vesicle a large pressure difference is observed. Also noticeable is a shift in the location of the pressure peaks in the stressed vesicle due to the swelling, and a clear asymmetry in the profile for both vesicles as a result of the membrane curvature. See [179] for more details (courtesy of M. Louhivuori).

5.3. Curvature induced fusion

The fusion and fission of membranes is an essential process in cell biophysics, occurring during exo- and endocytosis, intracellular trafficking and enveloped virus infection. Membrane fusion is also important in a range of biotechnological applications such as in gene or drug delivery. A wide range of regulatory protein complexes exist in vivo. Due to the wide variety and complexity of fusion protein arrays, the molecular picture of protein-mediated fusion and fission is largely unclear. The basic mechanism, however, is believed to be primarily determined by the physics of lipid–lipid interactions.

The protein-free fusion process has been simulated in detail over the past five years by a number of groups [182–190]. In each of these studies, small vesicles (~20 nm diameter) have been used. Although artificially small, such vesicles have the advantage that they store a high curvature energy leading to a large fusogenic propensity. On the other hand, it remains to be seen whether the small vesicle size does not bias the fusion pathways too much, and is therefore representative of fusion in real systems. Yet, even though the studies cited above vary in the coarseness of the representation of the lipids, use either explicit or implicit solvent, and use different methods to bring the vesicles into close juxtaposition, the general picture emerging from these simulations is rather independent of system details. The fusion intermediates found are in general agreement with the stalk–pore mechanism; the fusion process starts with the formation of a stalk between the

adjacent monolayers, and continues toward a hemifused state, in which the inner monolayers are in contact, by means of expansion of the stalk. Opening of a fusion pore completes the fusion process. The first step, formation of the stalk, involves a kinetic barrier in which a splayed lipid bridges the two contacting monolayers [182,188,191]. This splayed lipid transition state quickly relaxes to a stalk, similar to the stalks observed in the transition pathway from lamellar to inverted phases (see section Phase transformations). Expansion of the stalk can happen either in a two-dimensional fashion, by a radial expansion, or in a one-dimensional linear expansion. The first constitutes the more direct route, but requires stretching of the tails of the lipids of the inner leaflet to fill the otherwise empty void appearing in the center of the expanding stalk. The linear expansion, first observed in Brownian dynamics simulations [182], avoids the energetic cost associated with the required stretching of the lipid tails. Here, the expansion proceeds in a bent ("banana-shaped") manner until both ends of the linear expanded stalk meet, at which point the two ends fuse and form a closed circular stalk. This gives rise to an intermediate structure in which an inverted micelle is trapped in between the two vesicles (see Fig. 8C). The formation of a pore, through rupture of one of the membranes separating the vesicle interiors from the water bubble, then leads to the hemifused state. This type of pore formation is observed to happen either before or after the closure of the circular stalk, but always in the near vicinity of the stalk. As a consequence of the transient pore formation, both leakage of vesicle contents and mixing of lipids from the outer and inner monolayers are observed, offering a molecular explanation for those effects sometimes seen in experimental fusion assays. Surprisingly, simulations show that both the non-porous and porous pathways may be followed for a single composition [184,188]. However, a recent set of simulations using vesicles of increased size show different behavior [192]. A clear bias towards the bent expansion route is observed, pointing to an interesting size effect.

The rate of stalk formation and the opening of the fusion pore can be modulated by altering the lipid composition in qualitative agreement with experimental observations. Based on the fusion assay of Marrink et al. [184], Kasson and Pande [186] used distributed computing to generate large ensembles of fusion trajectories in which the ratio of PC:PE lipids was systematically varied (see Fig. 8B). From their detailed kinetic analysis it was concluded that a 1:1 PC:PE composition offers the fastest rate to full fusion. Lowering the PE content drastically reduces the rate of stalk formation, and consequently many vesicles remain unfused. Increasing the PE content, on the other hand, while increasing the speed of stalk formation leads to

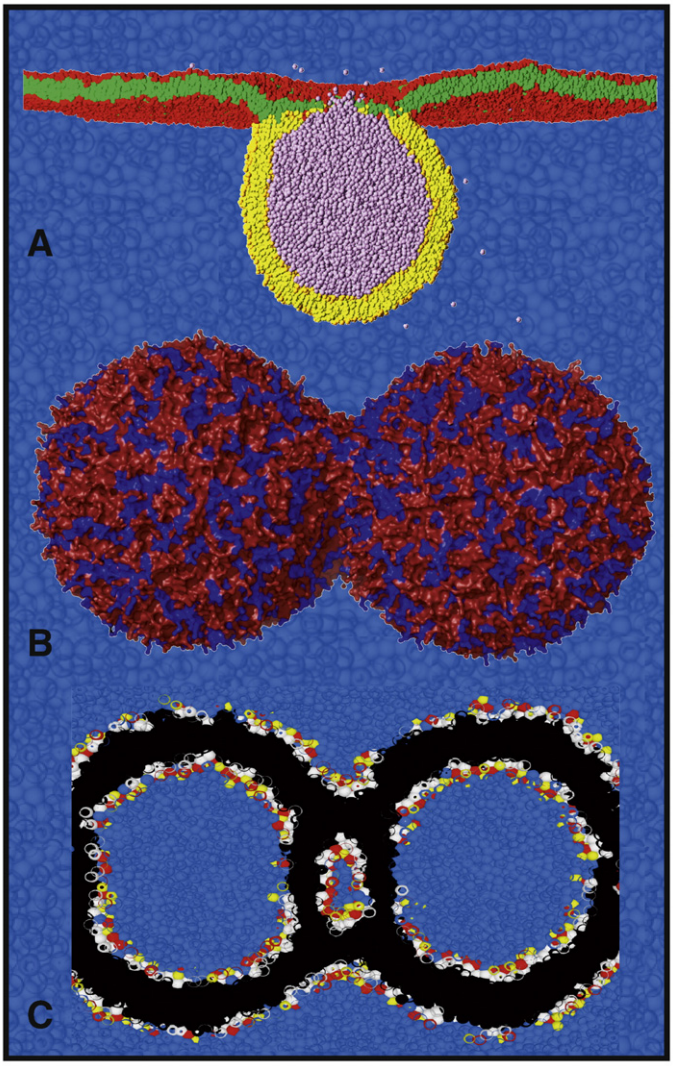

Fig. 8. Comparison of different fusion assays used to study the molecular details of fusion intermediates. (A) DPD simulation of fusion of a vesicle with a planar membrane ($100 \times 100\ nm^2$) triggered by the pulling of SNARE mimicking, membrane embedded proteins (not visible in the picture) [195] (courtesy of J.C. Shillcock), (B) CG-MD simulation of two mixed PC/PE (blue/red) vesicles of 15 nm diameter, showing the hemifused state [186], (courtesy of P.M. Kasson), and (C) another CG-MD simulation of mixed PC/PE vesicles measuring 20 nm in diameter, showing a cut through an intermediate stage in which an inverted micelle is trapped in between the fusing vesicles [192] (courtesy of M. Fuhrmans).

a metastable hemifused state rather than a fully fused state. Furthermore, DPD simulations [193] of two juxtaposed planar membranes indicate that lipids with more negative curvature (such as PE) favor the bent expansion of the stalks with concomitant pore formation. For vesicles with asymmetric lipid composition the rate of fusion depends not only on the overall composition but also on the concentration of the lipid species in the respective monolayers. For instance lysoPC in the outer monolayer prevents the formation of stalks, whereas in the inner monolayer it accelerates fusion by inducing rupture of the hemifusion diaphragm [184]. In addition to lipid composition, the tension inside the vesicle is a key determinant of the apparent fusion rate. Shillcock, Lipowsky and coworkers [185,190,194] systematically studied the fusion of small vesicles with either other small vesicles or planar membranes, using DPD simulations at an impressive system size reaching more than 30,000 lipids in total. They found that tension in both the vesicle and the planar membrane are required to observe fusion on a sub-microsecond time scale. Sometimes, however, tension was released by rupture of either the vesicular or the planar membrane, prior to complete fusion. In an extension of this work [195] the tension in the planar membrane was generated by pulling 6 artificial barrel proteins, mimicking the action of SNARE proteins (see Fig. 8A). Smeijers et al. also explored the effect of small coarse grained protein mimetics on the process of fusion [196]. Especially interesting is their finding that a so-called scramblase, i.e. a transmembrane protein with a hydrophilic strip which allows rapid lipid flip-flopping, strongly promotes vesicle fusion. The authors conclude that it does so by providing a way to relieve membrane tension that results from crowding of the outer monolayer due to growth of the stalk. After the fusion process has completed, the presence of the scramblase proteins also helps the vesicles to relax to a spherical shape. Furthermore, Noguchi and Takasu [197] showed that small nanoparticles that adhere to the membrane surface can promote fusion by bending of the stalk. For more elaborate reviews on computational modeling of membrane fusion see [26,195].

5.4. Stress induced membrane curving

In addition to membrane curvature induced by temperature (undulations) and curvature as a response to minimization of line tension (vesicle formation), curvature may be induced by local stress. The stress may arise from a variety of sources, including lipid asymmetry, the adsorption of peptides or proteins, and the presence of a compressive lateral tension. Simulation studies are now beginning to explore these effects in detail.

In Fig. 9 two examples are shown from one of our groups in which the asymmetric addition of a second component to a homogeneous one-component membrane results in strong bending deformations. Whether the added component is a surfactant (resorcinol [198]), or a cell penetrating peptide (penetratin, [199]), in each case the response of the membrane is similar: strong buckling to accommodate the stress induced by the asymmetric adsorption. Note, however, that other kinds of responses are also possible, such as the formation of pores (see Poration section). Asymmetry-induced membrane curvature has also been observed by Markvoort [180] in mixed bilayers consisting of two types of CG lipids with different headgroups. An asymmetric positioning of lipid domains leads to buckling, in line with Helfrich's spontaneous curvature model. The reverse situation was probed in simulations by Wang et al. [200], in which tension was applied to force a periodic bilayer patch to buckle. Using a mixed MC/MD approach the equilibrium distribution of the two components (long and short tailed lipids at atomic resolution) of the bilayer could be achieved. The anticipated correlation between local curvature and the mean local lipid composition could not be resolved, however. Tension induced buckling and collapsing of monolayers was studied by Baoukina et al. [201]. Lipid monolayer collapse plays an important role in the regulation of surface tension at the air–liquid interface in the lungs. The large scale CG simulations of 50×50 nm^2 patches reveal the molecular mechanism of monolayer collapse. On lateral compression, the collapse begins with the buckling of the monolayer, followed by folding of the buckle into a bilayer in the water phase. The folds undergo further transformation and form either flat circular bilayers or vesicles, depending on the monolayer composition.

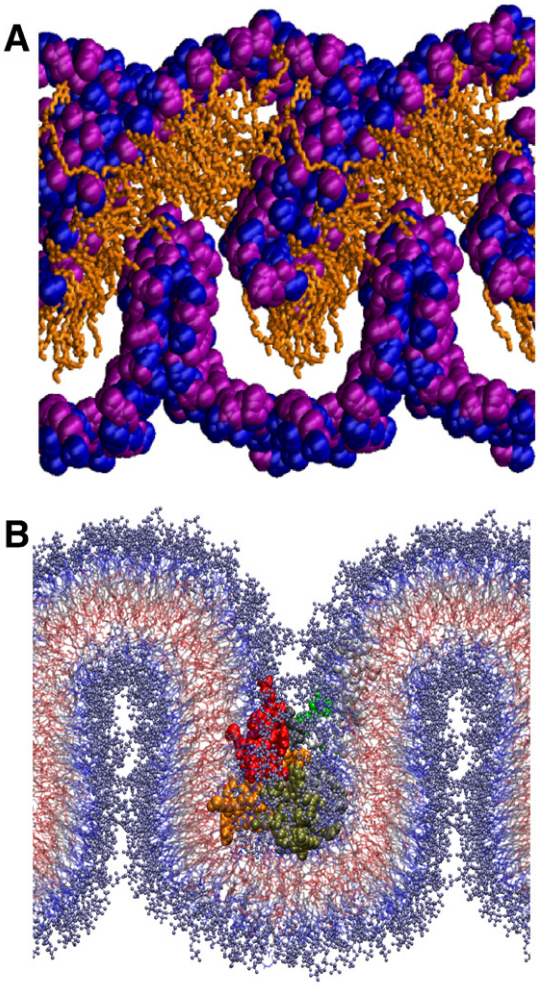

Fig. 9. Membranes spontaneously buckled by (A) resorcinols [198] (courtesy of M. Siwko), and (B) penetratin peptides [199] (courtesy of S. Yesylevskyy). The additional component was adsorbed spontaneously from the aqueous solution to one of the monolayer leaflets only (the top monolayer in the images). In each case, the large deformations shown developed over a period of 100 s of nanoseconds.

In order to regulate membrane curvature in vivo, nature has selected some dedicated peptides and proteins. A well-known example is given by the so-called BAR (Bin/amphiphysin/Rvs) domains, banana shaped proteins that are hypothesized to either sense or impose membrane curvature, thereby initiating and regulating the fission process in cooperation with other proteins such as clathrin and dynamin. The recently resolved crystal structure for the *Drosophila* BAR domain revealed a crescent-shaped dimer with a high density of positively charged residues on its concave surface. Atomistic MD simulations showed that the protein induces a strong curvature upon binding to a negatively charged membrane [202]. This study is ground breaking, being the first detailed simulation of a protein-induced large scale response of a lipid membrane. In fact, two binding modes were observed, each inducing a slightly different curvature, depending on the initial simulation conditions. Although the simulations nicely show that BAR domains can indeed induce membrane curvature, the curvature observed in the simulations (~6.7 nm) shows a large discrepancy with the global curvature

observed in experiments (~25 nm). In an extension of this study [203], another puzzling effect was observed, namely the ability of the N-terminal helices of the BAR domains to induce curvature by themselves. These observations raise the question to which extent cooperative effects play an important role. Experiments indicate association of the BAR domains on tubulated liposomes. Combining models of different resolution, both the groups of Voth [204] and Schulten [205] recently addressed the cooperative nature of BAR domain induced membrane bending (Fig. 10). Due to the cooperative action of these BAR domains, curvatures are induced with radii closer to the experimental values. Only an anisotropic curvature field, which requires a collective orientational ordering of the BAR domains, is predicted to lead to tubulation of liposomes [204]. The modeling studies [205] furthermore reveal that two different arrangements of the BAR domains lead to distinct membrane curvatures. Protein induced membrane curvature was also seen in simulations modeling chromatophores [206]. Most notably, a membrane-embedded array of light harvesting complexes LH2s was found to relax to a curved state. Membrane curvature, in turn, might also affect the behavior of proteins. In a pioneering atomistic simulation by Meyer et al. [207], small but noticeable structural changes of a mechanosensitive channel upon embedding in a curved bilayer were observed.

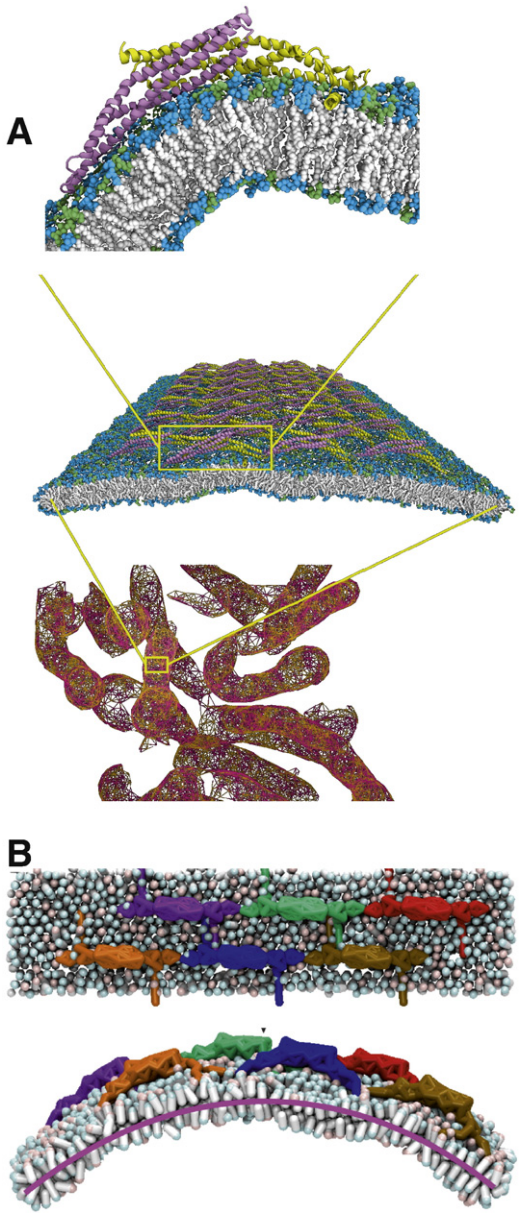

Fig. 10. Impression of BAR domain induced curvature simulations. Multi-scale simulations show that individual BAR domains already induce strong curvature in lipid membranes, however, their collective interaction is required to trigger the formation of tubular or vesicular systems. (A) Models of the atomic (upper) resolution are coupled to an intermediate resolution coarse-grained model (middle) or to a mesoscopic mean field model (bottom) [202,204], courtesy of G.A. Voth. (B) Starting configuration (upper, top view) and final configuration (bottom, side view with curvature radius of 33 nm indicated by pink line) after 5 μs simulation using a coarse grained model [205], courtesy A. Arkhipov and K. Schulten.

One of the largest-scale membrane simulations up to date has been reported by Reynwar et al. [103]. The use of a generic solvent-free model allowed simulations of membrane patches exceeding 46,000 lipids, about 160 nm length in both lateral directions. The effect of curvature-inducing proteins is included through addition of small 'cap' or 'capsid' molecules, which have an intrinsic curvature radius of about 5 nm. Attractive interactions between the caps or capsids and the lipid head groups drives their adsorption to the membrane, and subsequent aggregation (see also the Phase transformations section). The collective effect arising from the coupling between the protein-like inclusions and membrane lipids shows up at an impressive level. Depending on the details of the caps/capsids, large scale membrane deformations occur, leading to spontaneous vesiculation and budding events.

6. Outlook

The power of computer simulations lies in the detailed understanding they offer at the molecular level. Experimentally observed behavior can be explained, for example, in the case of membrane electroporation [107], the slow rates of transmembrane lipid flip-flop and permeation of cations [66], or the aggregation tendency of specific membrane proteins [101]. At the same time, simulation studies offer predictions, new food for experimentalists to digest. Examples are the predicted disordered nature of toroidal pores formed by antimicrobial peptides [135,136], unanticipated new mechanisms for membrane fusion involving bent stalks [182,184,188], and the remarkable lipid organization in rippled gel phases [75,85,86].

The challenge for the next ten years is to use wisely the expected increase in computational resources due to the ongoing development of both hard- and software. For the next decade, we anticipate the feasibility of simulating millisecond events with all atom models, and truly macroscopic time scales for simplified models. Spatially, however, the microscopic limitations will not be overcome as system sizes of 10s or even 100s of millions of particles are still restricted to femto-liter volumes. With the increasing number of particles that can be simulated, we expect studies on vesicles to become gradually more dominant. The ability to mimic hypo- or hyper-osmotic conditions [179] opens the way to study more realistic processes such as the gating of membrane embedded protein channels in response to osmotic shock. Actual simulations of small transport vesicles [208] may become possible, and we expect to see an increasing collaboration between computational groups and synthetic biologists in their aim toward the design of artificial cells. Bigger is not always better, however. We foresee a large benefit from smaller scale studies performed in massive parallel fashion, with systematic exploration of parameter space. Parameters to be explored can either be literally understood as force field parameters or other simulation details, or as state conditions such as composition and temperature. New areas that will certainly benefit from this systematic approach are the interface between nanomaterials and lipid membranes, bio-engineered novel lipid types, the behavior of multi-component lipid mixtures, the polymorphism of lipid/peptide systems, and self-aggregation of membrane-embedded or anchored protein complexes.

Of equal importance is the continuation of force field development, on all levels of resolution. The parameterization for polarizable lipid force fields is needed, but is still in its infancy. At the atomistic level,

progress has been made in the development of compatible lipid/peptide force fields derived along the same principles, but more work and especially testing is still required. The growing ability to construct phase diagrams for lipid systems offers a tempting alternative route to calibrate lipid force fields. Transition temperatures from the fluid to gel phase, or toward inverted phases, are readily available, and often more straightforward to interpret compared to structural parameters. One of the challenges for coarse grained models is to get away from the use of a uniform dielectric permittivity throughout the system. Solutions might be found in assigning explicit dipolar charges to the CG water models [209] or by introducing polarization at the CG level (Yesylevskyy and Marrink, in preparation). Multi-level optimisation strategies, in which models of lower resolution are systematically derived from more fine grained models, are indispensible, and offer the best route toward real multiscale applications [210]. The first applications we have already seen with the multiscale descriptions of membrane remodelling by BAR domains [204,205].

Together these methods will form the start of a toolbox for model-based design in structural membrane biology. With macroscopic time scales in reach, and compatible multi-level simulation models at hand, the future of membrane simulation studies looks brighter than ever.

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