

# Monte Carlo simulations of lipid bilayers and lipid protein interactions in the light of recent experiments

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## Abstract

Statistical thermodynamics simulations in recent years have considerably altered the view of biological and model membranes. Fluctuations in concentration and state, as well as in area and volume, have led to the prediction of a domain structure and anomalies in the elastic constants. New experiments support the predictions made from these models. Domains have been found and elasticities have been measured, both in model and in biological systems. The concept of hydrophobic matching, underlying the simulations, has experimentally been shown to be relevant for the function of membrane proteins. The growing interaction of theoreticians with biologists will soon lead to a wide acceptance of the importance of these theoretical concepts. © 2000 Elsevier Science Ltd. All rights reserved.

**Keywords:** Chain melting; Fluctuations; Hydrophobic matching; Domain formation; Elastic constants; Shape transitions

## 1. Introduction

Monte Carlo (MC) simulations are a numerical thermodynamics technique designed to explore the thermodynamic equilibrium in complex systems when no analytical solution is available. In contrast to molecular dynamics (MD) simulations, they can be performed independent of time scales. This is especially important in the field of lipid membranes, because the relaxation times and correlation lengths of the lateral distribution of particles may be beyond the reach of MD simulations.

Important phenomena in membranes are of meso- and macroscopic nature [1•,2], meaning that they are on length scales which are large compared to single molecules. Furthermore, most biological lipids melt in a temperature range which is, in a broader sense, close to physiological temperatures (–20–60°C), usu-

ally affected by inclusions such as cholesterol [3••] and proteins [4•,5,6••,7•]. Close to transition events, fluctuations are high, influencing properties such as heat capacity, compressibility and elasticity [8••]. It has been theoretically predicted that, for membrane components of different nature and interface, non-ideal mixing behavior has to be expected, leading to phenomena such as domain formation, resulting in the clustering of lipids and proteins or lipid sorting close to protein interfaces [4•,9,10••]. It has also been proposed that domain formation and local protein clustering may affect diffusion pathways and reaction cascades in biological membranes [1•,11•].

Until recently, the theoretical analysis suffered from the lack of satisfying experiments that allowed us to relate the predictions (domain formation, area and curvature fluctuations) to experiments of biological and model membranes. Now that important experiments have been performed, the results from statistical thermodynamics simulations are likely to receive the attention that they deserve. It is highly probable

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that important properties of biological membranes are related to many-particle correlations, which are impossible to be understood on the single-molecule level.

## 2. Theoretical approaches

Most MC-simulations involving chain melting transitions focus on coarse-grained models simplifying lipids by either representative sub-ensembles such as the 10-state Pink model [1•,4•,12] or two-state Ising models [13•,14••]. Although mostly lattice models are employed, the recent utilization of off-lattice Ising models seems promising, since they are capable of describing distortions in crystalline order at low temperatures as they are introduced by cholesterol [3••]. These models are especially successful in analyzing critical phenomena and heat capacities in single-lipid membranes or lipid mixtures, as well as mixtures of lipids with proteins. The membrane Hamiltonian function is calculated by the summation of the energies of all lipids in their respective states (like gel and fluid states in Ising models) and their nearest neighbor interactions, which are the cause of co-operativity. The physical origin of the nearest neighbor interactions is partially due to the so-called hydrophobic mismatch [15••], which is related to the interaction of hydrophobic residues with the aqueous solvent when the neighboring molecules differ in length. A further contribution to nearest neighbor interactions may consist of head group interactions, for example, by the formation of hydrogen bonding patterns as in phosphatidyl ethanolamine membranes [16]. Some authors use models reducing lipids to chains of beads which may fluctuate in a defined force field [17–19]. These models predicted attraction between hard core proteins due to lipid chain ordering effects not directly related to hydrophobic matching.

Molecular dynamics (MD) simulations are presently restricted to the nanosecond time regime, whereas relaxation phenomena in lipid bilayers may be in the range from milliseconds to minutes close to transitions. Therefore, MD-simulations are not capable of describing correlated processes in membranes. For macroscopic phenomena, statistical thermodynamics means have to be employed. To shorten equilibration times, some authors have combined Monte-Carlo and MD simulations [20,21] or used plain Monte-Carlo approaches on the atomistic level [22,23]. Goetz and Lipowsky simulated the membrane using idealized lipids made from Lennard–Jones beads [24] with MC and MD simulations, and constructed stress profiles across the membrane. Such profiles were also studied with mean field approaches by Cantor [25,26], who proposed that pressure gradients may have the poten-

tial to affect protein conformations [27•]. Gompper and Kroll constructed membranes from tethered networks of beads [28•,29] to investigate long-range bilayer arrangements such as sponge phases, an approach which seems well suited to study structural changes of vesicles.

Close to melting transitions (to critical points), fluctuations may become so slow that even Monte-Carlo procedures may become difficult if the system under investigation gets trapped in local minima of the free energy landscape which are separated by barriers. One possibility to get around this problem is the flattening of the free energy barrier between coexisting states [30]. Another possibility which can be used for simple systems, such as Ising-models of single lipid melting or lipid-peptide mixtures, is the collection of the distribution of states into histograms which can be used to reconstruct probability distribution for many sets of parameters, including cases with first order transitions [Ivanova and Heimburg, A histogram method to obtain heat capacities in lipid monolayers, curved bilayers and membranes containing peptides (submitted 2000)].

## 3. Domain formation

As mentioned, one of the most important parameters in the commonly used statistical thermodynamics models of the Ising or Pink type are the nearest neighbor interactions between molecules of unlike state or chemical structure. One major contribution is the hydrophobic mismatch. A different hydrophobic length of two adjacent molecules was proposed to lead to an unfavorable free energy contribution stemming from solvent interactions at the interface [15••,31••]. The consequence of nearest neighbor interaction in Monte-Carlo simulations is the formation of domains. Although images of domains on monolayers have been known for many years, similar results for lipid bilayers have not been visualized until very recently. The first image of domain formation in vesicles is probably from confocal fluorescence microscopy [32••] (Fig. 1d). In this beautiful work, not only were domains in a two-component lipid mixture (DLPC/DPPC) investigated, but anomalous diffusion, which was probably caused by the lateral inhomogeneities, was also demonstrated with a fluorescence correlation spectroscopy (FCS) method. Other fluorescence results using two-photon fluorescence microscopy were reported in a recent series of papers, which equally demonstrated domains in simple lipid mixtures [33•,34•] and in biological lipid extracts [35•].

The demonstration of domains in a single lipid membrane was more demanding because diffusion processes do not limit the time scales of fluctuations

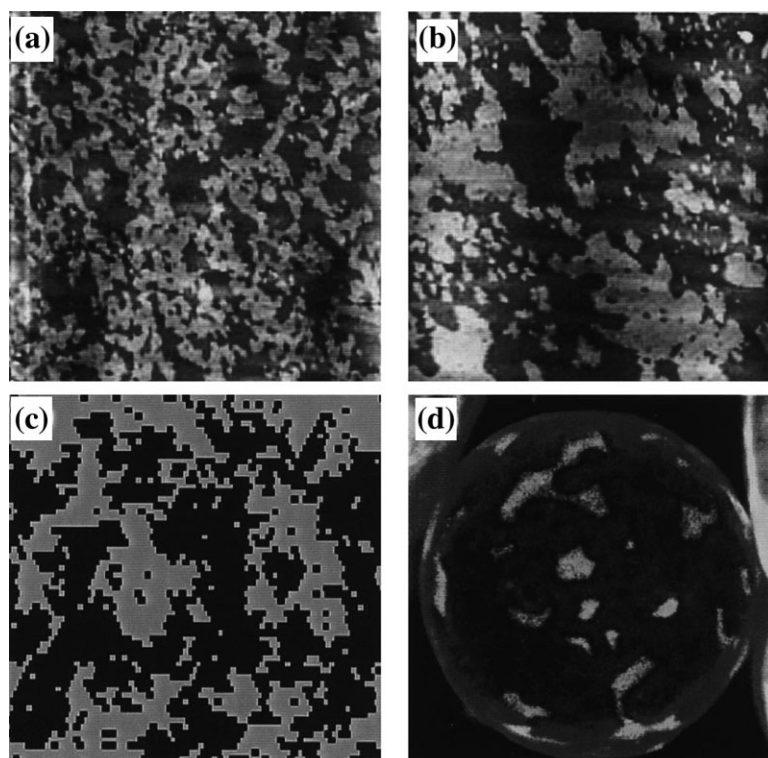


Fig. 1. Domain formation in experiments and in Monte-Carlo simulations. (a) AFM image of a supported monofilm of dimyristoyl phosphatidylcholine ( $25 \times 25 \mu\text{m}^2$  at the critical point (from [36••] with permission). (b) AFM image of a supported monofilm of dipalmitoyl phosphatidylcholine (DPPC) ( $20 \times 20 \mu\text{m}^2$  at the critical point [36••]). (c) Monte-Carlo simulation of a unilamellar dipalmitoyl phosphatidylcholine vesicle (approx.  $50 \times 50 \text{ nm}^2$ ) at the phase transition temperature employing an Ising model. (d) Fluorescence microscopy image of a dilauroyl phosphatidylcholine/dipalmitoyl phosphatidylcholine (20:80 mol/mol) giant vesicle ( $32 \times 32 \mu\text{m}^2$ ) in the phase separation regime (from [32••] with permission).

and the temperature range of large fluctuations is very small. First atomic force microscopy (AFM) images, obtained from immobilized monolayers of single lipids close to a critical point [36••,37•] (Fig. 1a and b) and from mixtures in the phase separation regime [37•] showed random, unstructured domains on the nanometer to micrometer scale. For comparison, Fig. 1c shows domain formation at the melting point of unilamellar DPPC vesicles calculated with an Ising model.

An important and unresolved question concerns the equilibrium shape of the domains. Domains in monolayers usually display a dendritic or spiral nature indicating slow growth processes and chirality. Simulations on membranes tend to yield domains with random interfaces and no dendritic growth is observed (Fig. 1c). This is probably caused by the equilibrium nature of these kinds of simulations and, possibly, the neglecting of molecular chirality. The recent fluorescence microscopy images were inconsistent in this respect. The domains observed by [32••] resemble those from calculations (Fig. 1c,d). The domains in the supported monolayer system studied by Nielsen et al. [36••,37•] also showed unstructured domains, whereas those seen by [33•,34•,35•] were of a rather

dendritic or snowflake nature. The shape of domains seemed to be further influenced by the magnitude of the hydrophobic mismatch [33•].

Domain formation influences diffusion properties and leads to anomalous transport properties as reviewed by Saxton and Jacobsen [38•] and Cherry et al. [39•]. Anomalous diffusion has been experimentally demonstrated for lipids in simple lipid mixtures [32••] with fluorescence correlation spectroscopy (FCS). Salome et al. [40•] presented a reevaluation of fluorescence recovery after photobleaching (frap) experiments using Monte-Carlo simulations. They concluded that, in biological membranes, connected domains of sub-micrometer size  $0.25\text{--}0.74 \mu\text{m}$  exist, both for proteins and lipids in human skin fibroblasts and in mouse hepatoma cells. Similar domain sizes in various cells were deduced from using single particle tracking (SPT) methods [38•]. The simulation of diffusion processes on the molecular level recently has been attempted with Monte-Carlo simulations using coarse-grained models [19].

An exciting new development is the detection of rafts in biological membranes. These are domains which are rich in cholesterol, sphingolipids, and proteins which are likely to be functionally relevant. They

have been identified in many biological systems [11•,41•,42–44].

#### 4. Lipid interactions with proteins

The hydrophobic matching condition provides the parameters for Monte-Carlo simulations on integral proteins (for earlier theoretical work on MC-analysis of protein arrangements, see Gil et al. [4•]). There is growing experimental evidence for the relevance of the hydrophobic matching condition and its consequences for protein arrangements and clustering [15•]. The hydrophobic matching of integral proteins may induce lipid-sorting around proteins, as has been shown by fluorescence methods for *Bacteriorhodopsin* [9]. Based on the lipid-sorting concept, Sabra et al. theoretically showed for the same protein, that one may expect proteins to aggregate into various quasi-crystalline arrangements depending on concentration and features of a binary lipid mixture [10•]. This work may provide a better understanding of how proteins crystallize within the membrane plane (see also [4]). A series of papers by Sintes and Baumgärtner explored non-specific interactions of proteins in lipid matrixes, simulated by a model for the lipid bilayer in which lipids were represented by chains of beads, which also predicted non-specific aggregation of membrane-imbedded proteins of various shapes by the effect of the proteins on the lipid fluctuations and by close contact (lipid depletion) [17–19]. The binding of peripheral proteins to lipid surfaces may also induce domain formation and rearrangement of lipids on a matrix. Based on binding studies and MC-calculations, the C2 binding motive of a class of proteins such as synaptotagmin has been proposed to induce a rearrangement of the lipid matrix [5], which is further influenced by calcium.

The hydrophobic matching condition may directly affect protein structure and function [15•]. An interesting paper by Dumas et al. demonstrates a pronounced influence of chain length on the function of melibiose permease [31•]. Fig. 2 shows that the activity of this protein is optimal for a membrane consisting of lipids with C-16 chains. This chain length is close to the hydrophobic length calculated for this protein. A further possible influence on protein function stems from the lateral pressure profile across the membrane [24,26•], which is influenced by small molecules such as anesthetics, alkanes and cholesterol [25,26•]. The stress profile has been discussed to be capable of influencing protein structure [27•].

In contrast to artificial membranes, the membrane of a living cell is probably not in equilibrium. The domain formation induced by proteins may be significantly altered when proteins change their shape

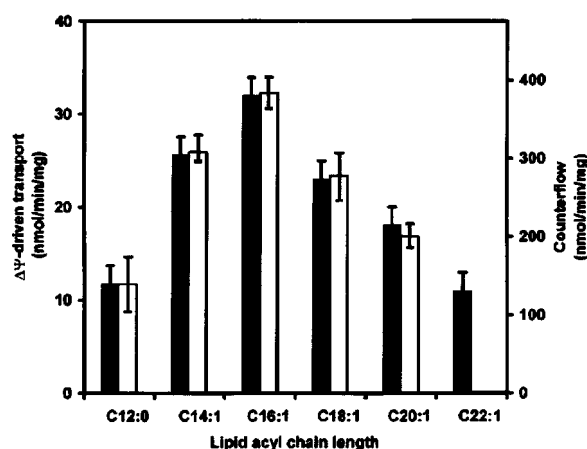


Fig. 2. Activity of melibiose permease in reconstituted lipid vesicles as a function of the lipid chain length (from [31•] with permission from *Biochemistry*). Plotted are the transport of melibiose driven by a transmembrane potential and the passive transport measured with a counterflow technique.

upon activation. In a very appealing based on M-C methods approach non-equilibrium it has been proposed, that when the proteins are activated with a constant drive, a steady state may form, in which the lateral organization of domains is significantly changed by the activity of inclusions [6•,7•] and organised domain patterns are induced.

#### 5. Elastic constants and shape transitions in membranes

Close to transitions, fluctuations, not only in enthalpy, but also in volume, are high. Volume and area fluctuations are related to the elastic constants. In the melting transition, the enthalpy and the volume have an exact proportional relationship [8•,45] (Ebel and Heimburg, in preparation). From this it can be concluded that the heat capacity is proportional to compressibility. Assuming the same for the area, it has been proposed that the increase of the curvature elasticity in the melting transition regime is also proportional to the heat capacity [8•]. This has been supported by Dimova et al. [46•] and Meleard et al. [47•], who found a temperature dependence of the bending rigidity of giant unilamellar vesicles very similar to that predicted by Heimburg [8•] for large unilamellar vesicles (Fig. 3). This means that the Ising model is sufficient to describe the coupling between elastic properties and the heat capacity.

The shape transitions of vesicles are dominated by the elastic constants of the bilayers [28•,29]. Since bending rigidity is largely reduced close to the melting transition, the likelihood of structural transitions is increased. Several experimentally observed phenomena have been attributed to this effect. For anionic lipid dispersions, it has been reported that structural

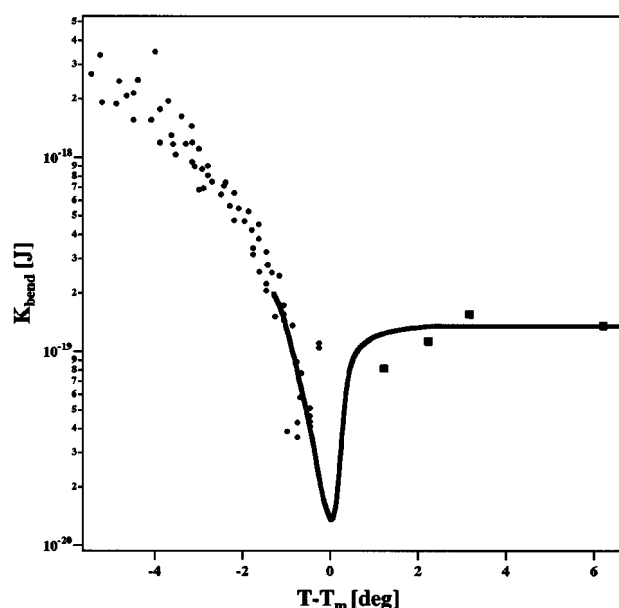


Fig. 3. The bending modulus of dimyristoyl giant vesicles adapted from [46] (circles), and [47] (squares). The solid line indicates the predictions for dipalmitoyl phosphatidylcholine large unilamellar vesicles from the heat capacity profile [8]. Data are plotted vs. the reduced temperature.

transitions occur from a vesicular state to a continuous bilayer network of different mean curvature [48] and back to vesicles (Fig. 4a). This has been explained by the coupling of the heat capacity with membrane

elasticity, further promoted by favorable solvent interactions of the long range structure (Fig. 4b), based on Monte-Carlo simulations of the melting profiles of membranes with different mean curvature [48] (Ivanova and Heimburg, submitted). Since this transition involves the spontaneous and reversible fusion of vesicles, it has been suggested that this kind of phenomena may be significant for the fusion of synaptic vesicles in exocytosis (for a review see [49]).

In membranes constrained by hard or soft walls, surface modes or undulations are hindered [50,51]. A related phenomenon, which had been unresolved for decades, is the lipid pre-transition. By employing one-dimensional Monte-Carlo simulations it has been linked to the melting process, explaining the ripple phase as a periodic pattern of gel and fluid domains [14] (Fig. 4c), caused by undulations of membranes in geometrically constrained membranes. Due to the coupling of structure with the heat capacity, structural changes become evident in a splitting of the melting profile into several  $c_p$ -maxima, resulting in a three-peak profile for the anionic lipid network formation (Fig. 4a,b) and a two-peak profile for the ripple phase formation (pre- and main transition) (Fig. 4c). This seems also to be the case for the sub-main transition: a very low enthalpy transition close to the main transition of long-chain phosphatidylcholines which has been attributed to local curvature differences [52]. Strong fluctuations in shape close to transitions have

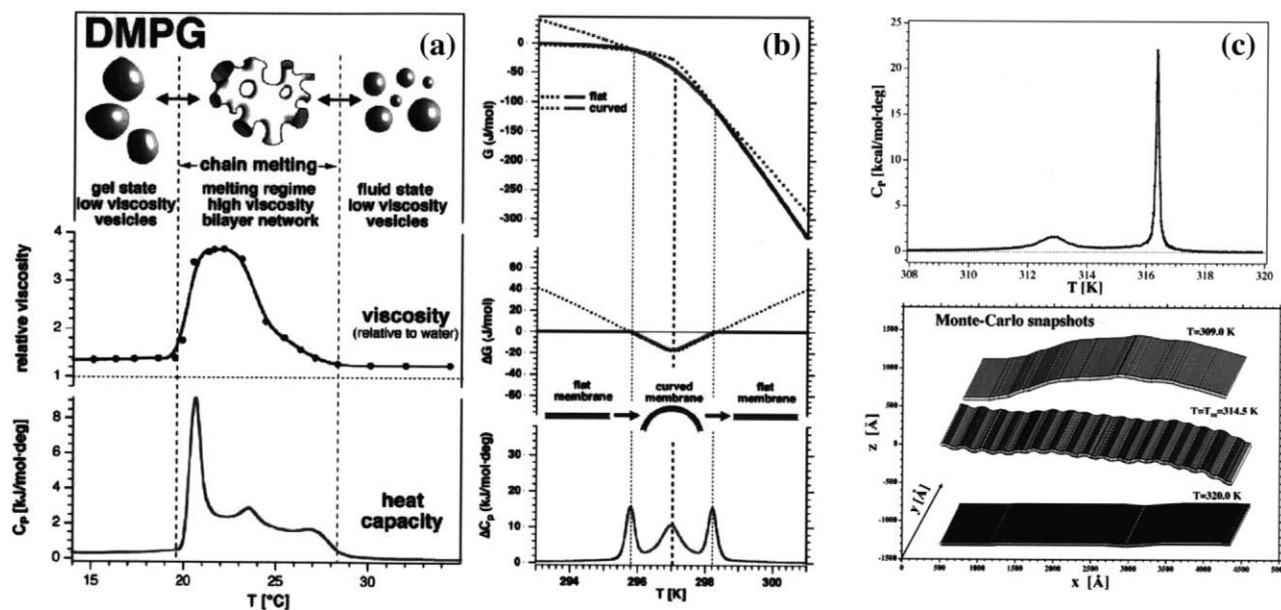


Fig. 4. Structural transitions in lipid vesicles close to the melting temperature. (a) Reversible fusion of vesicles to extended membrane networks of dimyristoyl phosphatidylglycerol at low ionic strength (from [48] with permission). Plotted are viscosities (center) and the heat capacity profiles (bottom). (b) Theoretical rationalization of the splitting of the heat capacity profiles from Monte-Carlo simulations. Assumed are two different vesicular states with a different dependence of the free energy on temperature. The outer heat capacity maxima correspond to the intersection of the free energy profiles (from [48]). (c) Monte-Carlo simulations of the ripple phase formation, showing calculated heat capacity profiles (top) and Monte-Carlo snapshots below the pre-transition, in the ripple phase regime and above the main transition (bottom) (from [14] with permission).

also been observed with two-photon fluorescence microscopy [53•]. The heat capacity profile of extruded unilamellar vesicles of DMPC splits into two maxima [8••], accompanied by an increase in viscosity (unpublished results from our lab). This is further evidence for an increased likelihood of structural changes close to the melting transition. As a summary, local curvature influences heat capacity profiles. In this respect, it should be mentioned that lipids with a large head-group, for example when coated with polyethyleneglycol [54,55] and surface adsorbed proteins [56], exert a lateral pressure on surfaces and may also influence local curvature and heat capacity profiles.

## 6. Conclusions

The field of membrane simulation has highly profited from recent progress in experiments, supporting the major predictions of the models. Domains have been found in model systems and in biological membranes. The coupling of melting processes with the elastic constants provides these studies with a new dimension. Different hydrophobic lengths have been shown to affect protein function. This will most definitely lead to a new understanding of the role of biological membranes. Recently several studies have started to focus on measuring diffusion processes with the aim of understanding signaling cascades. It seems likely that Monte-Carlo simulations, taking into account the thermodynamic parameters of the systems, will accompany these experiments. The application of these concepts on non-equilibrium systems seems promising. Further work will be necessary to prove the relevance of these concepts for biological function. This includes studies on the effects of drugs, protein binding and cations (calcium) on the pattern formation in membranes.

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