The Role of Minimal Models in the Context of a New Biomembrane Model[1]

Martin J. Zuckermann

Abstract

In recent years there have been performed a considerable number of excellent molecular dynamics (MD) simulations with realistic potentials which have made detailed predictions for the structure of lipid mono- and bilayers. However these simulations are restricted to a relatively small number of lipid molecules and it is therefore difficult to use such simulations to predict the detailed phase behaviour of lipid membranes. Here we discuss the role of minimal models in making such predictions. Furthermore we classify the models into two types and give one example for each of type of model. In the context of the type II model presented here, we describe some detailed results for the phase behaviour of lipid-sterol bilayers. This is followed by a discussion of the role of minimal models in the construction of a new biomembrane model.

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Introduction

The increase in speed and memory and the use of parallel computing in recent decades has given us the possibility of understanding the fundamental interactions between biomembrane components and at the same time allowed us to visualize their microscopic behavior in terms of real space snapshots. The improvement in computer power has gone hand-in-hand with the development of several software packages for molecular modeling based on molecular dynamics (MD) techniques and has often led to an analysis not just of structure but also of function of membrane proteins (Humphrey et al., 1995, Schulten, 1997). The inclusion of lipid and water molecules in the simulation of membrane proteins and peptides has been pioneered by Benoit Roux and his research group in particular with regard to gramicidin, melittin and Pf1 coat proteins (Roux and Woolf, 1995). Furthermore MD simulations were carried out by Benoit Roux, Jim Davis (1997) and colleagues and Shen et al.

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(1997) for the behavior of single α-helix intrinsic polypeptides which span a lipid bilayer, and the numerical results were used to understand related nuclear magnetic resonance (NMR) data by Davis. Clearly MD simulations based on detailed molecular interactions have been shown to be extremely successful for the understanding of structure and, in some cases, function of proteins at the molecular level.

A typical MD sample investigated by molecular dynamics is one gramicidin molecule surrounded by fifty lipid molecules plus water molecules in a hexagonal cell subject to periodic boundary conditions with a maximum time scale of 1 ns (Woolf and Roux, 1994). There now exist many results from MD simulations of lipid bilayers and monolayers at the molecular level (Zhou and Schulten, 1995; Pastor and Feller, 1996; Berger et al., 1997) for small samples for a maximum time of 1 ns. For example Zhou and Schulten (1995) performed a 200ps MD simulation for a dipalmitoylphosphatidylethanolamine (DPPE) bilayer composed of 202 lipid molecules and 8108 water molecules. They obtained data for the water polarization profile, the membrane dipole moment and the susceptibility profile. Recently Berger et al. (1997) used MD to simulate a fluid dipalmitoylphosphatidylcholine (DPPC) bilayer at full hydration and constant pressure and temperature. The bilayer comprised 64 DPPC molecules with 23 water molecules per lipid. As with previous simulations of this kind, they were able to examine the structure of the bilayer at the lipid-water interface and they obtained excellent agreement with experiment for the NMR order parameter along the acyl chains of the lipid molecules.

Even though the speed of numerical computation is increasing rapidly as we approach the second millennium, it will be some time before an analysis of collective behavior such as self-assembly, phase separation, clustering, etc. is possible at the molecular level since this requires a number of molecules of the order of a thousand at least. Such numerical studies at present require considerable simplification of the model. These simplifications could include for example the use of systems of 'monomers' in which each 'monomer' represents a complex molecular or sub-molecular component of the system whose details are not important to the processes under investigation. This is called a 'coarse grained' model where we simplify the interactions between the 'monomers'. The first step towards "minimal" modeling is to simulate such systems using MD without including full molecular details. A very successful example of such modeling is the work of Grest and Murat (1995) on many polymer systems using a 'ball and string' model for each polymer in conjunction with MD simulations. Consider a homopolymer brush (i.e. a flat plane containing a relatively dense system of end-grafted homopolymers) in a good solvent. If the solvent is changed from good to poor, the brush makes a transition from a homogeneous system to a lattice of pinned micelles (Soga et al., 1995) each of which is a collapsed cluster of many polymers. An MD simulation using the 'ball-and-string' model will at best result in the formation of a single cluster but there is no chance of simulating the collective nature of the system. This implies that collective behavior requires further 'simplifications' in the model, dare we say more 'minimality' in the model. The group at McGill is at present applying the minimal model for-end grafted polymer systems of reference (Soga et al, 1995) to a study of the properties of lipid vesicles containing lipopolymers i.e. homopolymers covalently bonded to the polar heads of some of the lipid molecules in the vesicle's bilayer (Rex at al., 1998).

The purpose of this introduction was to present the main concepts underlying minimal modeling in complex fluids. We now turn our attention to the minimal modeling of lipid bilayer systems.

Minimal Models for Lipid Phase Behaviour

There are at present several minimal models for the large scale behavior of lipid bilayers and they are based on a well tried philosophy. We could first simulate all possible configurations of a single lipid acyl chain and then model the interactions between chains in terms of a self-consistent field (SCF) which removes fluctuation effects. Let me call this a type I model. A second approach, which has been our philosophy, is to use multistate interacting lattice models typical of statistical physics to model lipid bilayers and their various phases in conjunction with Monte Carlo simulations. We were able to include the effects of cholesterol, hydration, drug molecules and proteins on such phases via specific interactions (Mouritsen, 1990). For these models, we used and still use a two-dimensional triangular lattice, the sites of the lattice represent single lipid acyl chains. The details of the lipid chain conformations (lateral area/chain, internal energy, degeneracy) are treated as state parameters. We will refer to these models as type II models. We have recently extended this philosophy to the off-lattice case (see below).

Type I Models

Some of the most recent models of type I were simulated by Szleifer et al. (1987; 1990). The lipid configurations of a single lipid acyl chain were evaluated by a Monte Carlo simulation using the rotational isomeric (RIS) model in which each C-C bond has three possible states, one *trans* and two *gauche*. The bilayer is divided into layers and the number of carbon atoms per layer is counted. The incompressibility condition is imposed via an osmotic pressure and the resulting equations are solved self-consistently in the sense of mean-field theory. The ends of the chains are pegged onto a flat or curved surface and properties of the liquid crystalline (fluid phase) of the bilayer are calculated including the elastic constant for the bending energy. This model has very recently been extended by Müller and Schick (1996) by first removing the constraint of a "pegging" surface and then adding a rigid polar head to the chain. Interactions between the acyl chain and the polar head and between the chain and water were taken as repulsive and were described by a single parameter. The volumes of the polar head and chain segments completed the parameterization of the model and again the system is constrained by an incompressibility condition. Müller and Schick used this model to calculate the phase diagram of monoacyl glycerol-water systems which exhibited lamellar, hexagonal, inverted hexagonal and Ia3d cubic phases. Agreement with experiment for one phase transition was found and detailed composition profiles were calculated. This example shows clearly the great advantage of minimal modeling. We have here three basic parameters plus the RIS model which represent the fundamental physics of the problem and these are then used to calculate and understand an extremely complex phase diagram which would have been virtually impossible to obtain via MD methods for a model containing the full molecular details. Müller and Schick plan to extend this method to the phase behavior of membrane lipids.

The mechanistic, thermodynamic explanation of general anesthesia proposed by Cantor (1997) represents a direct application of minimal modeling using a type I model. On the basis of lattice SCF calculations, Cantor predicts the incorporation of interfacially active solutes in bilayers to perturb the distribution of local lateral stresses, with a large pressure increase near the aqueous interface compensated by a decrease distributed through the bilayer interior. Although this perturbation is typically relatively small at clinical anesthetic concentrations, it is large in absolute magnitude since the lateral pressures are themselves enormous. Suppose, as is generally accepted, that general anesthesia involves a shift in the conformational equilibrium of an ion-channel receptor protein. If the opening of the channel is accompanied by a nonuniform change in protein cross-sectional area, then the mechanical work of channel opening will be altered by the anesthetic-induced re-

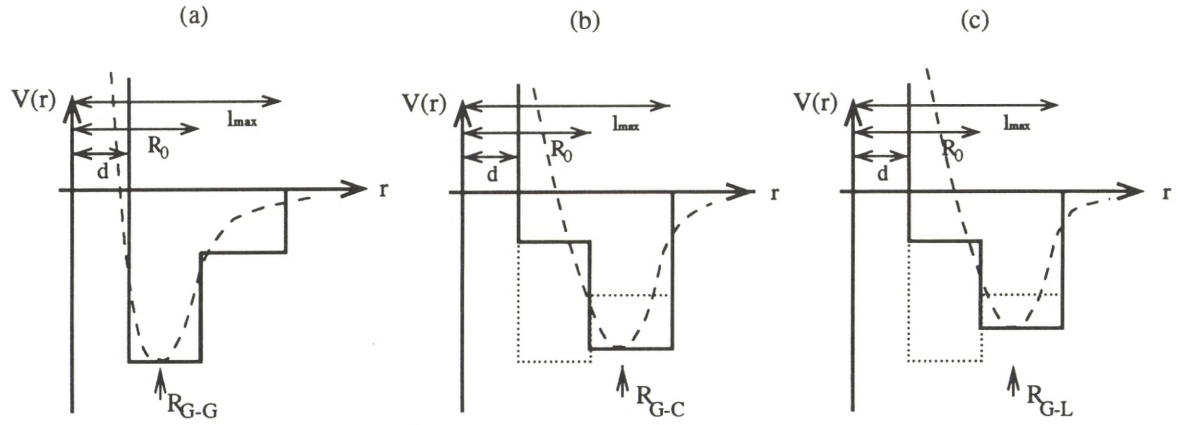

Figure 1. Schematic illustration of the interaction potential in the off-lattice model. The potential consists of a sum of a hard-disk potential and two square-well potentials. The hard-disk radius is d and the range and strength of the square-well potentials are $(l_{\max}, R_0)$ and (V_1, V_2), respectively. The dashed line illustrates a Lennard-Jones-like potential, to which the model potential is an approximation. (a) Interaction potential between two lipid chains in the gel state. (b) Interaction potential between a lipid chain in the gel state and a cholesterol molecule. (c) Interaction potential between a lipid chain in the gel state and a "lanosterol" molecule. R_{G-G}, R_{G-C} and R_{G-L} give the position of the minimum of the interaction potential for the gel-gel, gel-cholesterol and gel-lanosterol pairs. We chose $\frac{R_{G-G}}{R_{G-G}} \approx 1.3$ corresponding to the values of the lipid-cholesterol system. Interactions between lipid chains in the fluid state are weak and not shown in this figure.

distribution of lateral pressures, causing a shift in the equilibrium between the closed and open protein states. Calculations yield qualitative agreement with anesthetic potency at clinical anesthetic membrane concentrations, and predict the anomalously low potencies of long-chain alcohols and of strongly hydrophobic molecules with little or no attraction for the aqueous interface, such as perfluorocarbons. Clearly, there are many other processes involving conformational changes in membrane proteins whose sensitivity to altered membrane composition may also result from changes in the pressure profile. Cantor speculates that the homeostatic response of membranes may serve to restore the pressure profile, and thus reestablish the proper protein conformational distribution.

Lipid-Sterol Systems

We will now describe some recent work performed at the MEMPHYS group at the Technical University of Denmark (DTU) in collaboration with our group at McGill using a type II model (Zuckermann et al., 1993). We have worked for a considerable time using multi-state lattice models to describe the generic phase behavior of lipid-cholesterol bilayers. The physical principles defining the model were simply that cholesterol is an "ice breaker" which disrupts the gel phase of lipid bilayers but which is able at the same time to rigidify the acyl chains. This led to the classification of a phase with high cholesterol concentration which we called the **lo** (liquid ordered) phase. This is basically a 2d-fluid phase with relatively rigid chains and corresponds to the experimental β-phase of Vist and Davis (Davis, 1993). Using again very few additional parameters for the inclusion of cholesterol in our model for the main phase transition of lipid bilayers, we employed mean field theory to calculate the phase diagram of DPPC-cholesterol bilayers and it agreed with that of Vist and Davis. Since we had values for the fitted parameters from the phase diagram, it was possible to calculate a variety of physical properties, e.g. the specific heat and the hydrophobic thickness of the bilayer as function of temperature and concentration of cholesterol, which gave

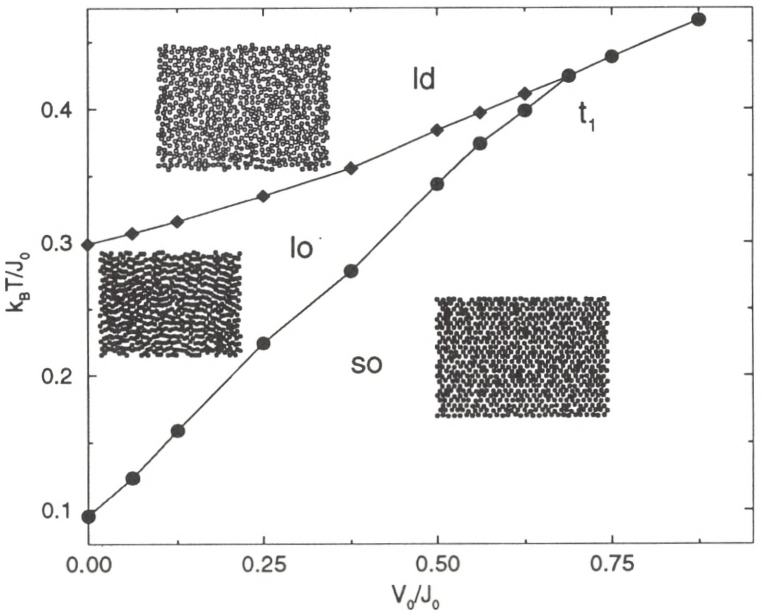

Figure 2. Phase diagram for the off-lattice model. All three phase boundaries are first-order phase boundaries. The insets show snapshots of typical micro-configurations for the three different phases labeled **so** (solid-ordered), **ld** (liquid-disordered), and **lo** (liquid-ordered). Chains in the disordered state are plotted as (○) and chains in the ordered chain state as (●). The three snapshots are not given to scale. t_1 is the triple point described in the text.

excellent quantitative agreement with experiment. Cruzeiro-Hansen and Mouritsen (Zuckermann et al., 1993) proposed and simulated (using Monte Carlo methods) a simplified version of this model which led to the understanding of the effect of cholesterol on the main phase transition and the ionic permeability at low cholesterol concentration (Zuckermann et al., 1993). One very basic problem with these models was that the **lo** phase was examined using a lattice model and there was therefore no qualitative difference between the **lo** phase and the **so** (solid ordered or gel) phase. Again in collaboration with the MEMPHYS group we have recently constructed the following minimal model to overcome this problem. This work is part of the Ph.D. thesis of Morten Nielsen, a graduate student of the McGill Physics Department.

The new model (Nielsen et al., 1996) is an off-lattice spin-$\frac{1}{2}$ Ising model in which the translational and internal (spin) degrees of freedom are coupled via microscopic interactions. The two internal (spin) degrees of freedom represent gel phase (rigid) and fluid phase acyl chain configurations respectively with the 'fluid phase' configuration being highly degenerate (Doniach, 1978). The translational degrees of freedom are unconventionally described in terms of a random lattice, which is structured by dynamically triangulating the spatial configurations. There is a 'hard core' interaction between all spin states and an attractive interaction between the 'gel' spin states only as shown in Fig. 1(a). This model leads to the phase diagram of Fig. 2 in which three phases are represented; an **so** phase ('gel' spin 2d-'solid'), an **ld** or liquid disordered phase ('fluid' spin, 2d-liquid), an **lo** ('gel' spin, 2d-liquid) and a triple point, t_1. We chose the interaction strength such that the main phase transition which lies on the **so-ld** phase line is located just beyond the triple point (see Fig. 2).

The next question is: how can we include lipid-cholesterol interactions into this minimal model with the least number of parameters, but in such a way that it retains its well-known properties

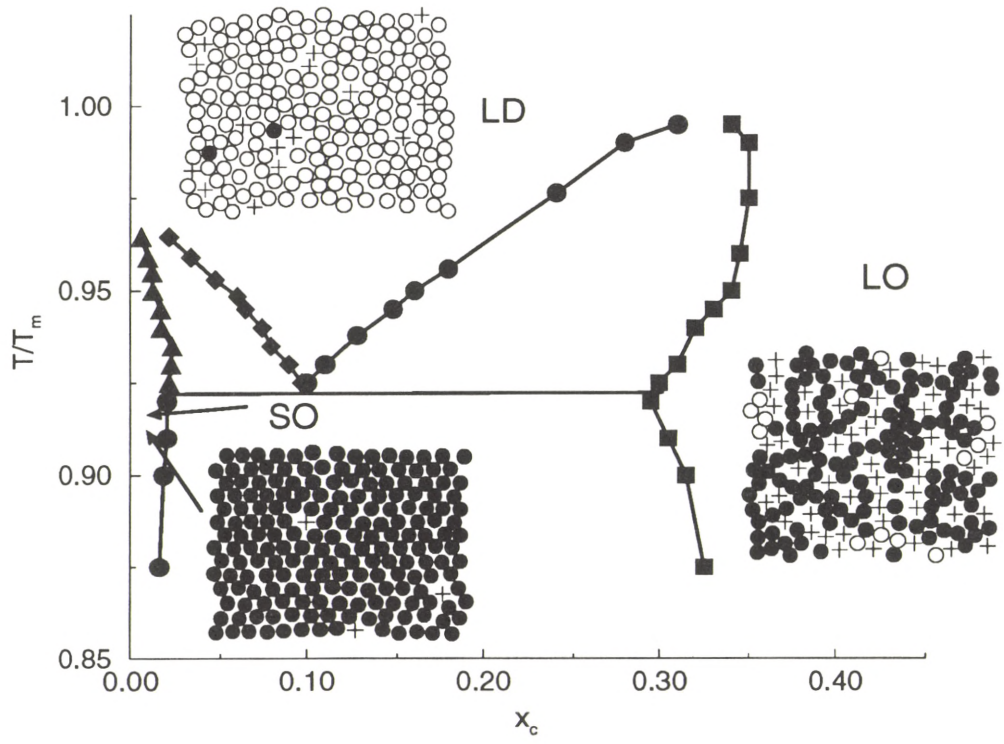

Figure 3. Lipid-cholesterol phase diagram. The insets show snapshots of micro configurations for the three different phases labeled **so** (solid-ordered), **ld** (liquid-disordered) and **lo** (liquid-ordered). The lipid chain conformational states are plotted as in the previous figure. Cholesterol molecules are plotted as +. The three snapshots are not given to scale. The concentration of cholesterol in each snapshot is: $c_{so} = 0.015$, $c_{ld} = 0.08$ and $c_{lo} = 0.31$. The x-axis gives the concentration of cholesterol, the y-axis the temperature measured in terms of T_m, the transition temperature for the off-lattice model.

of ice breaker and chain 'rigidifier'? The lipid-cholesterol interaction potential is shown in 1(b) where it can be seen that the basic difference is that the position of the minimum in the attractive interaction between 'gel' acyl chains and cholesterol is further out than that of the 'gel'-'gel' interaction in Fig. 1a. This potential brings one new variable parameter in the formalism, this being the depth of the potential well. Fig. 3 gives the resultant phase diagram for lipid-cholesterol systems and shows that, providing the position of the minimum of the 'gel'-cholesterol interaction has been correctly chosen, it exhibits the same generic behavior as the experimental phase diagram for DPPC-cholesterol bilayers. What do we gain from this?

This is where the philosophy of minimal models and their great advantage in terms of the description of the properties of lipid systems comes into play. Suppose we look at lipid bilayers containing sterols other than cholesterol. This is the subject of the work by Konrad Bloch and its interpretation by Bloom and Mouritsen (1991) These works deal with the evolution of eucaryotic membranes based on the hypothesis of Bloch that Nature optimizes membrane components and that cholesterol is optimized vis-a-vis precursor membrane sterols taking part in the evolutionary process with respect to its physical properties. Bloch pointed out that fluorescence experiments show that cholesterol gives the greatest decrease in fluidity in lipid bilayers. Bloom and Mouritsen interpreted this in terms of the increase in order of the bilayer, implying that cholesterol rigidifies neighboring chains more

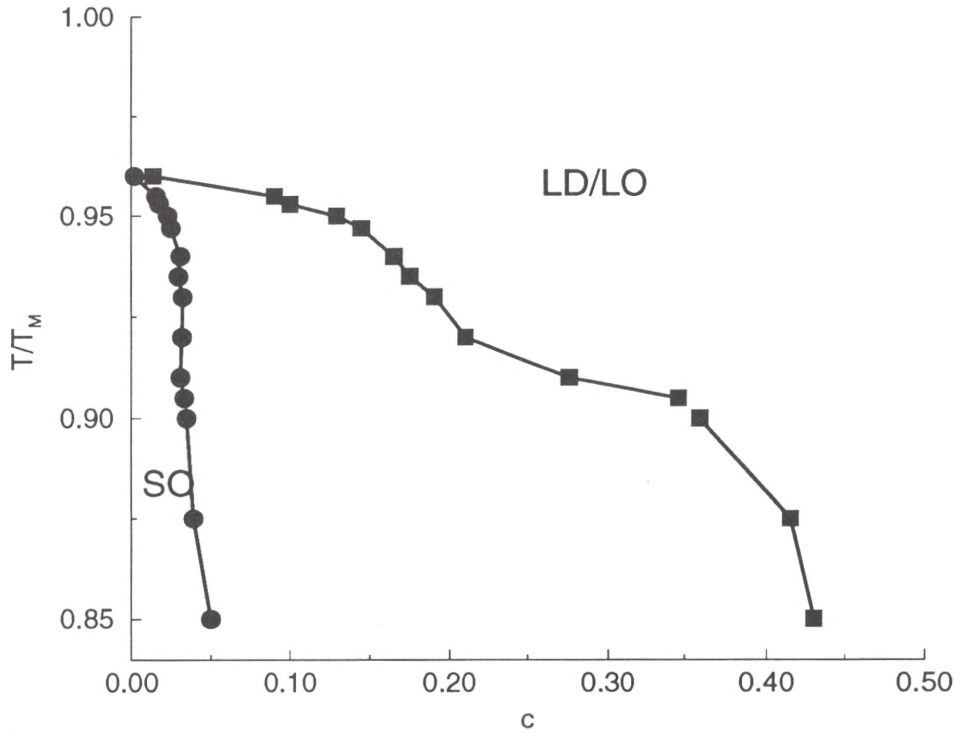

Figure 4. Lipid-lanosterol phase diagram. The x-axis gives the concentration of cholesterol, the y-axis gives the temperature measured in terms of T_m, theoretical transition temperature for the off-lattice model.

strongly than the other sterols in the evolutionary process. What does this mean in terms of our minimal model? Let us now consider lanosterol, which causes a smaller increase in chain order than cholesterol but larger than 'simpler' sterols. To explain this effect we appropriately adjusted the depth of the well of the lipid-cholesterol interaction shown in Fig. 1(b). By sufficiently reducing the depth of the well, we obtained the interaction used for lipid-lanosterol shown in Fig. 1(c) and the related phase diagram given in Fig. 4. It was quite pleasing to us that this phase diagram is qualitatively very similar to the experimental phase diagram for DPPC-lanosterol obtained from NMR experiments by Thewalt and Bloom (1997) even though the model is minimal. It should be mentioned that our theoretical phase diagram was found from Monte Carlo simulations which took time and effort on the part of Morten Nielsen. An article on our theoretical work on lipid-sterol phase diagrams is in preparation (Nielsen et al., in preparation).

Discussion and Conclusion

The following questions can now be posed:

- What information can we obtain from these simulations?
- What is the relation between numerical simulations and analytic methods for lipid bilayer properties?

- How does the information obtained from simulations lead to further improvements in the problem and in our knowledge of lipid systems in the context of a new biomembrane model?

We can calculate phase diagrams for precursor sterols between lanosterol and cholesterol. We can also (and are) calculating the specific heat and the hydrophobic thickness as a function of temperature and concentration for a qualitative comparison with experiment particularly for the case of DPPC-lanosterol bilayers. The calculations could be improved by increasing the number of conformations (states) per acyl chain and softening the potentials, which will allow quantitative comparison with experiment.

However the main aim of the simulations is considerably broader. We need to identify trends which will encourage new experiments. A specific example of this is the completely new picture of the main gel-fluid phase transition in terms of fluctuating gel (fluid) clusters in fluid (gel) phases above (below) the phase transition due to Ole Mouritsen (Mouritsen, 1990) This was only possible using a type II model since MD simulation would at most capture the interior of one cluster. Recent work in Paavo Kinnunen's group at Helsinki University has led to the experimental identification of this phenomenon. In our case of lipid-lanosterol systems, we hope that the phenomenological microscopic identification of the lipid-lanosterol interaction will encourage experimental workers to replace cholesterol by lanosterol in their samples. One example is the model system for stratum corneum (SC) of human skin which exhibits a low-temperature mixed phase with low hydration related to the fact that SC is impermeable to water. How would this phase and its characteristics change if cholesterol were replaced by lanosterol?

What is the connection between numerical simulations and analytic results for lipid bilayers? Using recent terminology, sterols can be viewed as small inclusions in lipid bilayers and they have a considerable perturbative effect. The most important inclusions biologically are proteins and the application of computational methods to the examination of lipid-protein interactions has been described in detail by Mouritsen et al. (1993) The proteins in this case are amphiphilic transmembrane proteins, i.e. their hydrophobic amino-acid residues have an affinity for the hydrophobic core of the lipid bilayer while their hydrophilic residues are screened from the hydrophobic core by being inside the protein or they lie in the aqueous medium surrounding the bilayer or else they are positioned in the polar head region of the bilayer. The interactions between lipids and proteins are given in terms of (i) direct hydrophobic interactions between neighbouring lipids and proteins and also between neighbouring proteins themselves as well as (ii) mismatch interactions between a protein and an adjacent lipid acyl chain. The mismatch interactions describe the effect of mismatch between the hydrophobic thickness of the lipid bilayer and the hydrophobic length of the transmembrane protein. The reader is referred to Mouritsen et al. (1993) for details. These interactions are basically phenomenological. The numerical simulations using these models have been very successful in predicting nanoscale organisation of lipid-protein bilayers, and protein selectivity.

The analytic theories are not able to give a full picture of lateral bilayer organisation and protein selectivity. Their advantage, however, is that they can give a detailed description of the interactions between proteins mediated by the lipid molecules. For example, the excellent paper by Aranda-Espinosa et al. (1996) is the latest in a series of papers by this group which builds on the seminal work of Huang (1986) on the deformation energy of lipid bilayers. These authors use analytic expressions for the bending stiffness and spontaneous curvature of fluid lipid bilayers to write an expression for the effective interaction between protein inclusions in the lipid bilayer. The local thickness itself is determined from an Euler-Lagrange equation which is solved using matching boundary conditions at the surface of the inclusion. The resultant interaction between inclusions is then used together with the Ornstein-Zernicke equation for liquid structure under Percus-Yevick closure to calculate the radial distribution function of the inclusions. They find that, when the spontaneous curvature is zero, the interaction be-

tween inclusions is similar to a hard core interaction. However for finite spontaneous curvature, a positive spontaneous curvature leads to conditions favorable for the adsorption of inclusions in the bilayer.

Clearly such an effective interaction between proteins induced by the bilayer can be used directly in simulations to determine the microscopic lateral organisation in the bilayer. Thus analytic methods and numerical simulation can often be regarded as giving complementary information. A recent example of this complementarity is the work of Gil et al. (1997) on wetting and capillary condensation as a means of protein organisation in membranes. In this case the analytic theories of Gil and Mikheev (1995) and Gil and Ipsen (1996) for wetting of proteins by one lipid component of a binary lipid mixture and the resultant effective interaction between proteins due to this wetting has led to the construction of a minimal microscopic model for wetting and protein induced phase equilibrium. Using this model in conjunction with Monte Carlo simulations, Gil et al. (1997) were able to examine the conditions for the formation of protein aggregates in lipid bilayers containing proteins.

How does all this relate to investigations for a new biomembrane model? Clearly minimal models for lipids alone are insufficient to result in such a new model directly. However such minimal models are essential in order to understand and describe collective behavior of molecules in lipid systems, both pure and mixed. It is our hypothesis that such behavior is biologically important in the context of (i) lipid protein interactions and (ii) systems where lipids alone play an important role such as the impermeability of the stratum corneum of mammalian skin to water. Thus minimal models for lipid systems must in general be used as 'sub-models' in the context of more complex models which describe biological phenomena. Minimal models of this type are then useful for such more complex models in the sense that they provide an understanding of the basic nature of the interactions between lipids (regarded as membrane components) important for collective behavior.

It should be pointed out that up to now we have in no sense exhausted the range of minimal models for lipid systems. For example, one vital area is the effect of the internal degrees of freedom of the lipid molecules on the phenomena arising from the curvature elasticity of lipid bilayers (Lemmich et al., 1994, Hansen, 1997) Consider for example the case of the phase separation of two lipids species in a curvature model of this type. Now include in the bilayer the presence of intrinsic proteins which strongly prefer one lipid species in its neighborhood over the other species. This will cause clustering of the proteins in the bilayer and such clustering may result in the collective (biological) activation of the proteins given the right external conditions. Clearly in order to understand the protein case, we must first understand the underlying lipid behavior. This is the reason for minimal modeling and its role in the development of new biomembrane models. A second area deals with minimal modeling of rotational isomerism and electrostatics in the polar head region (Belaya et al., 1994) of lipid bilayers and there are many more areas. The opportunities for development and application of such models are extremely wide, and the challenge is to find out using these models where the physical properties of lipid systems have biological significance.

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