MOLECULAR SIMULATION OF MIXTURES IN LIPID MEMBRANES

Adrien Berthault

Molecular simulation of mixtures in lipid membranes

Doctoral thesis

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CERTIFICATION

I hereby state that the present study, entitled "Molecular simulation of mixtures in lipid bilayers", presented by Adrien Berthault for the award of the degree of Doctor, has been carried out under my supervision at the Department of Chemical Engineering of Universitat Rovira i Virgili and that it fulfils all the requirements to be eligible for the International Doctorate Award.

TARRAGONA, 8 JUNE 2018
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DEDICATION AND ACKNOWLEDGEMENTS

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LIST OF ABBREVIATIONS

Abbreviation Definition

AFM Atomic Force Microscopy

BFM Bond Fluctuation Model

DIC Digital Image Correlation

DMPC Dimyristoylphosphatidylcholine

DNA Deoxyribonucleic acid

EBSD Electron Backscatter Diffraction

ECCI Electron Channeling Contrast Imaging

MC Monte-Carlo

MD Molecular Dynamics

SCF Self-Consistent Field

SCMF Single Chain Mean Field

SEM Scanning Electron Microscopy

TEM Transmission Electron Microscopy

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SUMMARY

Lipid membranes have a major role in the defence of cells and their regulation processes with the external medium. Poorly understood because of their complexity, studies throughout years already brought hints which help to comprehend them and develop direct applications.

This work presents an extension of a fast and reliable method (Single Chain Mean Field (SCMF)) to study mixtures at equilibrium and in particular mixtures of lipids and small colloids inserted into lipid membranes, able to consider the presence of additional components and bridge molecular simulation models and elastic theories for amphiphilic membranes. During this work, we focused on the parametrisation of the parameters for the SCMF method to reproduce the features of DMPC lipid bilayers at equilibrium involving comparisons with previously published simulation results and experimental data. The thesis reports the work performed to achieve the specific objectives of this doctoral thesis: reliable fully parametrised molecular details able to reproduce the behaviour of lipid membranes made of a single type of component, the study of their equilibrium properties interacting with additional molecules and their effects on the line tension for the specific case of the pore creation and a dynamical approach to study the dynamics of membranes made of various amphiphilic chains, in particular in the presence of pores.

A general historical introduction showing scientific research interest in lipid membranes investigation is presented in Chapter I. Characterisation of amphiphilic membranes are obtained using the Single Chain Mean Field (SCMF) theory, designed to study systems at equilibrium. Combining it with the Helfrich model, we bridge the gap between analytical models and simulation methods in Chapter II. After establishing and verifying with experimental results the line tension of a pore formed with our DMPC model membrane system, a fully hydrophobic second molecule is studied. Various effects including line tension modification and membrane compressibility are observed and reported in Chapter III. To understand the behaviour of pores created in a bilayer made of two amphiphilic chains differing in their length, dynamical studies were performed with the Bond Fluctuation Model (BFM) and presented in Chapter IV. Finally, the last chapter shows general conclusions of the work carried in this thesis.

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CHAPTER 1

INTRODUCTION

1.1 History

One of the oldest questions asked by Mankind is the understanding of life. Mostly during the XVIII and XIX centuries, philosophers were promoting the vitalism theory, which considered life as an animated matter that living organisms possessed and was unrelated to physicochemical laws. While vitalist scientists were trying to corroborate their beliefs with experimental designs, others, like Hermann von Helmholtz, Carl Ludwig, Emil Du Bois-Reymond and Ernst von Briicke were arguing to refute such theory: investigations about cells and their compounds had begun[35].

Cells are the smallest known alive units able to autonomously replicate themselves. They are divided into two categories : procaryotes and eucaryotes. While bacteria and archaea belong to the first one and do not possess a nucleus, which contains DNA (*i.e.* free in the cytoplasm), the latter ones encompass multicellular and a few unicellular organisms. Each cell is composed of a cytoskeleton (used for cell shape preservation, cellular division, etc), DNA material, organelles (*e.g.* mitochondria, lysosomes, etc) and a membrane, which is the topic of our interest.

Already noticed in the second half of the XIX century for their regulation role (Nägeli and Cramer, de Vries, Pfeffer[23]), cellular membranes (Figure 1.1[34]) are complex biological setups, fundamental for the system[39], which aim to protect and regulate

exchanges between the cell and the extracellular medium through embedded structures (*e.g.* channels) and pore formation. Their composition is extremely diverse, combining proteins with a chemically broad range of lipids[21]. Each of them is amphiphilic and possesses an outwardly directed polar hydrophilic head and an inwardly directed apolar hydrophobic tail. They can self-organise into various structures: micelles[7], membranes[57] or liposomes[31]. This adaptability of lipid membranes involves possible modifications of their parameters set. For instance, the mechanism of endocytosis triggers a modification of the bilayer shape leading to a complete rearrangement of the lipids[38]. Likewise, the presence and the interactions with nano-objects create alterations of the structures[32, 43].

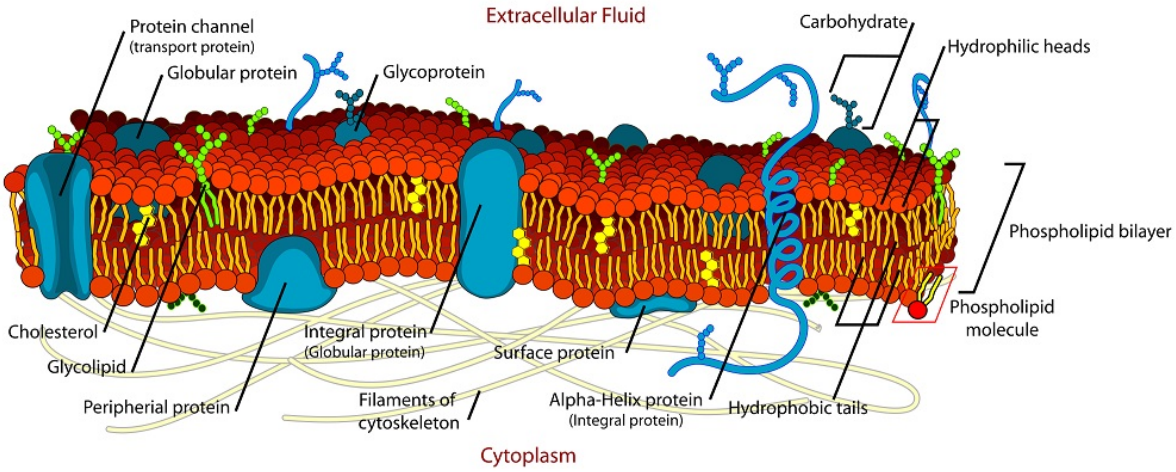


Figure 1.1: Scheme of a cellular membrane

Throughout the last decades, they have been deeply investigated due to their importance both from an academic standpoint and their industrial applications. Whilst lipid membranes are composed of multiple components in Nature, simplified models, restricted to a limited amount of phospholipids, have been studied and essential features, such as the thickness of the membranes, their stretching properties or their bending rigidity, have been determined.

1.2 Experimental methods

Developments of experimental and analysing tools ensued, so as theoretical models aiming to understand and predict membrane behaviours. Microscopy techniques (fluorescent[1,

33, 52], electron[10, 20, 25, 50] or Atomic Force Microscopy (AFM)[26, 29, 48, 54]) provide high resolution images which help to characterise lipid membranes. Additionally, methods such as neutron and X-ray scattering supply structural properties information, while others study the presence of pores, objects or the transportation of chemical compounds (electrical measurements)[36, 37]. However, each of them encounters limitations associated with required quantities, imaging artefacts or physical alterations related to experiments. These mainly descriptive results are combined with analytical theories which not only explain phenomena, but also generalise them.

1.3 Theoretical approaches

In addition with analytical theories which explicitly describe and predict the behaviour of lipid bilayers, simulation techniques are tools which aim to mimic experiments at low cost where we can control both time and focus. Apart from their application scale, one may divide them depending on their dynamics. On one side, dynamical methods like Molecular Dynamics (MD)[8, 15] or the BFM[58] inquire about the kinetics of a system or elements which constitute it. On the other side, methods like the Self-Consistent Field (SCF) theory[59] or the SCMF theory[3, 56] focus at a fixed time, a snapshot of a system configuration which will not undergo any alteration over time.

One the most famous among simulation methods, MD is a deterministic technique based on solving Newton equations of motion. Widely spread, teams of professionals maintain a properly implemented core of an open source code. Despite its reliability, due to countless simulations being able to reproduce experimental results, this one remains tricky to tune for unimplemented systems. Also, its range of applications is limited for computational reasons with system sizes and relaxation time. In fact, this method has shown the possibility to describe simple model membranes[4, 13], but the determination of their features remains complicated. The dynamics of the systems and their fluctuations, the level of coarse-graining and the constant incertitude not to be at equilibrium, are fundamental aspects that must be considered.

BFM is a Monte-Carlo based method (stochastic) used to study the dynamics of polymer systems on a lattice. Using the Carmesin and Kremer version[6, 12], polymer chains, represented by connected cubes with defined interactions, randomly move, interact and self-organise in a simulation box[45]. Further information regarding the model and

performed simulations are detailed in Chapter IV.

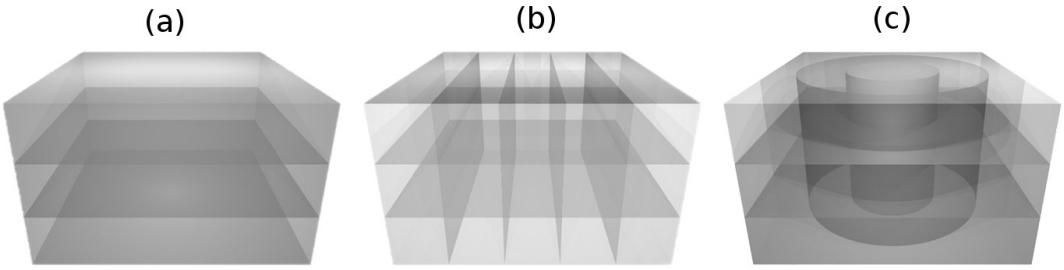


Figure 1.2: A few possibilities of simulation box division.
(a) 1D flat layers (b) 2D cells (c) 2D with axial symmetry

SCMF being similar to the SCF, is a fast numerical calculations method which considers a single molecule in a field. This technique follows two steps. First, a representative sampling of a molecule conformations (or set of molecules in the case of mixture) is randomly generated in a simulation box, which may be discretised according to various geometries (Figure 1.2). Then, numerical calculations are performed to calculate the most probable configuration of the system. Two assumptions which present both advantages and disadvantages are linked to this method: no fluctuation and no correlation. An exhaustive description of the method is given in Chapters II and III.
Other existing simulation methods such as the Single Chain in Mean Field[9] are mainly utilising a combination of these techniques or use complementary analysing tools.

1.4 Problem statement and Objectives

Lipid membranes have a high level of complexity comprising various types of lipids interacting not only between each other, but also with other molecules (*e.g.* proteins). Previous work was done in order to understand the effects of a second compound[17, 49] or the influence of external nano-objects[19, 41, 44] onto a bilayer exclusively made of lipids. Experimentally, investigations regarding the effects and the characterisation of such newly formed systems are performed at significant cost considering the tremendous amount of molecules belonging to these high complexity systems. As a matter of fact, each experiment is done independently and therefore, brings a little piece of information to this big puzzle that is the understanding of cellular membranes. From a theoretical

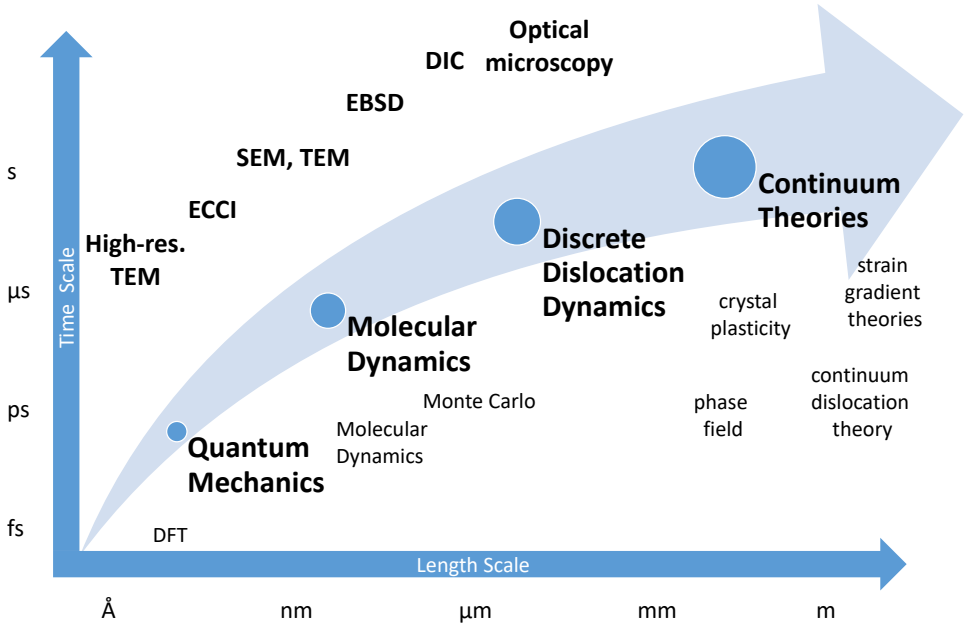


Figure 1.3: Possible methods that may be used depending on the time and length scales. Transmission electron microscopy (TEM), Electron channeling contrast imaging (ECCI), Scanning Electron Microscopy (SEM), Electron backscatter diffraction (EBSD), Digital Image Correlation (DIC)

standpoint, MD simulation technique was recently used to study the lipid organisation of the plasma membrane including 63 different species[27]. If such systems were hitherto never studied with any other method, we propose the SCMF theory to study mixed bilayers. Here, we consider a highly coarse-grained chain model to represent DMPC bilayers that we cross-check with experimental data.

This PhD thesis is focused on understanding how cellular membranes made of lipids respond to interactions with other compounds and their effects from an organisational, mechanical and thermodynamical standpoints. This work is done using simulation methods and analytical theories. In order to achieve this objective, we:

- Parametrised the molecular details for the SCMF method to reproduce the features of DMPC lipid bilayers at equilibrium involving comparison with previously published simulation results and experimental data. Thenceforward, this model was extended to obtain additional information such as the bending rigidity, proposing a method to link analytical theories and simulation models

- Studied the equilibrium properties of DMPC membranes interacting with hydrophobic molecules and their effects on the line tension of pores

- Observed the dynamics of membranes made of two amphiphilic chains of different length which can create pores

CHAPTER 2

BRIDGING MOLECULAR SIMULATION MODELS AND ELASTIC THEORIES FOR AMPHIPHILIC MEMBRANES

2.1 Abstract

The Single Chain Mean Field (SCMF) theory is used to link coarse-grained models of amphiphilic molecules with analytical models for membrane elasticity, where phenomenological parameters are deduced from explicit molecular models and force fields. We estimate the elastic constants based on the free energy of the amphiphilic bilayer in planar and cylindrical geometries on the example of four amphiphilic molecules that differ in length and stiffness. We study, how these variations affect the equilibrium bilayer structure, the equilibrium free energy and the elastic constants. Bending rigidities are obtained within the typical range of experimental values for phospholipid membranes in a liquid state.

2.2 Introduction

Microscopic molecular simulation models such as coarse-grained Molecular Dynamics (MD) or Monte Carlo (MC) methods operate in terms of force fields[16] in order to describe interactions between molecules, while analytical elasticity models such as Helfrich model of membranes deformations[5, 24] consider lipid membrane as a continuum elastic sheet described by phenomenological coefficients[28], which are not directly related to

molecular properties and describe the overall features of the membranes at a large scale. Connecting the two theoretical approaches for different length scales can be challenging since phenomenological models are mean field in nature and various contributions (or ingredients) to the total free energy are assumed to be independent. However, in molecular simulations getting the equilibrium free energy of the system is very time consuming[55], decoupling the resulting energy in contributions is not straightforward, especially at small length scales and the system fluctuations alter the resulting free energy[22]. Estimates of membrane deformation bending constants from MD simulations are obtained by comparing potentials of mean forces[22, 30, 55] or from fluctuation spectra of the membrane[13]. Both methods provide indirect estimates of mean field equilibrium constants.

In this work, we provide a theoretical framework allowing to relate consistently equilibrium mean field theories with coarse-grained molecular molecules represented by molecular interaction parameters. Our molecular model is the Single Chain Mean Field (SCMF) theory, where each molecule is described by a representative set of conformations and the molecule is represented by coarse-grained beads with the interactions between each type of bead given by potential wells. The SCMF method was proposed in 1985 by A. Ben-Shaul, I. Szleifer and W. M. Gelbart and was originally used to describe the micellisation processes of block copolymers[3, 56]. Further, it has been adapted to phospholipid membranes[42]. Various studies showed the efficiency of the method in the reproduction of the DMPC lipid bilayer at different levels of coarse-graining, but also for different general models of phospholipid bilayers[18] and, more recently, represented the translocation of nanoparticles through one of them[19]. This method is mean field by nature and gives directly the equilibrium free energy of the system without fluctuations. Thus, it has the same level of approximations as the widely used Helfrich model[24] for elastic properties of the membranes. The natural combination of the two methods provides the flexibility of analytical expressions of the Helfrich Hamiltonian and keeps the coarse-grained molecular details of the resulting structures.

In the following, we present the coupling between the Helfrich model and the SCMF theory and show how the phenomenological elastic constants can be derived from coarse-grained models of molecules on the example of 4 slightly different amphiphilic molecules.

2.3 Helfrich model

Helfrich theory of membrane elasticity[24] is represented by the Helfrich Hamiltonian E which is written as a sum of bending, stretching and compression terms

$$E = \int \mathrm{d}S \left(\frac{\kappa}{2} (K_e - C_0)^2 + \overline{\kappa} K_G \right) + \int \sigma \mathrm{d}S \tag{2.1}$$

where σ is the membrane tension, K_e and K_G are the extrinsic and Gaussian curvatures, respectively, such that $K_e = c_1 + c_2$ is the sum and $K_G = c_1 c_2$ is the product of two principal curvatures c_1 and c_2 and C_0 is the spontaneous curvature. In this model three independent phenomenological parameters, membrane tension σ, the bending modulus κ and the saddle-splay modulus $\overline{\kappa}$ couple to the membrane deformations.

The Helfrich model is used to study membrane shape deformations at large length scales compared to the thickness of the membrane[2, 46], while molecular details and composition of the membrane are neglected. On the other hand, molecular simulations give precise information about the structure of the membrane[14] but are very time consuming[40] at scales $l > 10$ nm.

2.4 Single Chain Mean Field theory

In the SCMF theory[3, 56], molecules are described with molecular details at a coarse-grained level. Interactions between molecules and the resulting self-assembled structures are given by interactions of a single molecule with mean fields, which are self-consistently created by conformations of this molecule. Neglecting correlations between conformations and fluctuations of the resulting fields allows to get an equilibrium mean field structure with corresponding free energies as a direct result of the theory[42].

2.4.1 Method

The SCMF theory operates with a representative sampling, *i.e.* set of configurations Γ of a single molecule, which is generated in a simulation box with periodic boundary conditions following, for example, the Rosenbluth self-avoiding random walk[51]. This procedure allows to sample numerically the configurational space of a single molecule in contrast to Self Consistent Field (SCF) theories[53], where it is assumed that configurations are distributed with a Gaussian propagator; this assumption is valid only for long chains or in very concentrated solutions or melts. Thus, SCMF theory with numerical

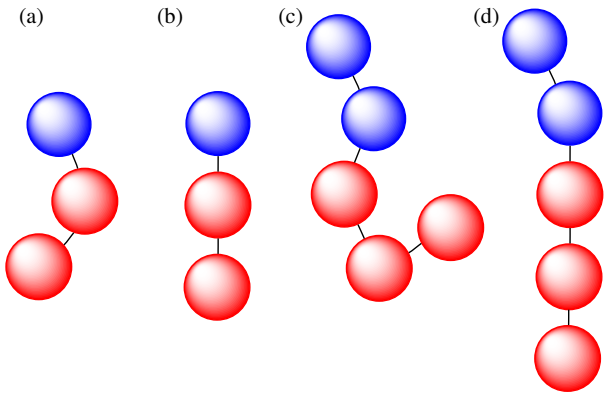


Figure 2.1: Representation of the four amphiphilic chains. From the left to the right: HT_2 fully flexible (DMPC), HT_2 fully rigid, H_2T_3 fully flexible, H_2T_3 semi-rigid.

sampling of conformational space is a more general method.

Free energy functional F of the SCMF theory is the variational function of probabilities of generated conformations, $P(\Gamma)$, embedded in the mean fields; it is the sum of entropy and energy contributions[42]. It is generally determined by the interactions of different types of segments or beads composing the molecules. We consider linear amphiphilic molecules comprised of two types of beads, type T, "hydrophobic", and type H, "hydrophilic" (see Figure 2.1), the free energy is given by

$$\begin{aligned} F = {} & N\langle H_{intra}\rangle + N\langle \ln(P(\Gamma)N)\rangle + \int c_w(\mathbf{r})\ln(c_w(\mathbf{r}))\,\mathrm{d}\mathbf{r} \\ & + \frac{N(N-1)}{2}\left(\int \langle u_T(\mathbf{r})\rangle\langle c_T(\mathbf{r})\rangle\,\mathrm{d}\mathbf{r} + \int \langle u_H(\mathbf{r})\rangle\langle c_H(\mathbf{r})\rangle\,\mathrm{d}\mathbf{r}\right) \\ & + N\int \langle u_w(\mathbf{r})\rangle c_w(\mathbf{r})\,\mathrm{d}\mathbf{r} + \int \lambda(\mathbf{r})\left(\phi_0 - N\langle\phi(\mathbf{r})\rangle\right)\mathrm{d}\mathbf{r} \end{aligned} \tag{2.2}$$

Here, N represents the number of molecules in the box, H_{intra} the intra-molecular interactions between the beads and u_T, u_H, u_w and ϕ the contributions to the interaction fields for two types of beads (H and T) and a solvent w at a point of the space $\mathbf{r}$, respectively. c_T, c_H and c_w are the corresponding concentrations and ϕ_0 is the bulk solvent volume fraction. The angular brackets denote the average weighted with the probability distribution $P(\Gamma)$ such as $\langle\ldots\rangle = \sum_{\Gamma}\ldots P(\Gamma)$. The Lagrange multiplier λ insures the incompressibility of the membrane and is given by

$$v_w\lambda(\mathbf{r}) = \ln(v_w c_w(\mathbf{r})) + N\int \langle u_w(\Gamma,\mathbf{r})\rangle\,\mathrm{d}\Gamma \tag{2.3}$$

where v_w is a volume of a solvent bead.

Minimisation of this functional with respect to $P(\Gamma)$, subjects to constraints of incompressibility conditions, gives the equilibrium distribution of probabilities $P(\Gamma)$ as follows[42]

$$P(\Gamma) = \frac{1}{ZW(\Gamma)} e^{-\frac{H_{eff}(\Gamma)}{k_B T}} \tag{2.4}$$

where Z is the normalisation constant and $W(\Gamma)$ is the Rosenbluth weight reflecting a bias of conformations generated with the Rosenbluth method. The effective Hamiltonian H_{eff} of a conformation Γ yields[42]

$$\begin{aligned} \frac{H_{eff}(\Gamma)}{k_B T} &= H_{intra}(\Gamma) \\ &+ (N-1)\left(\int u_T(\Gamma,\mathbf{r})\langle c_T(\mathbf{r})\rangle \mathrm{d}\mathbf{r} + \int u_H(\Gamma,\mathbf{r})\langle c_H(\mathbf{r})\rangle \mathrm{d}\mathbf{r}\right) \\ &+ \int u_w(\Gamma,\mathbf{r}) c_w(\mathbf{r}) \mathrm{d}\mathbf{r} - \int \lambda(\mathbf{r})\phi(\Gamma,\mathbf{r}) \mathrm{d}\mathbf{r} \end{aligned} \tag{2.5}$$

With Eq. (2.5), the equilibrium free energy per molecule f is given by:

$$fN = F - V\frac{\phi_0}{v_w}\ln\frac{\phi_0}{v_w} \tag{2.6}$$

where V is the volume of the simulation box.

2.4.2 Geometries

Eqs. (2.4)-(2.6) are valid for any geometry. The chosen symmetry of space discretisation determines the topology of the solution. If the object of the study is the stretching modulus K of a flat membrane, we therefore divide the space in 1D parallel layers, favouring flat structures and suppressing even occasional bending perpendicular to the layers (Figure 2.2a).

Choosing a division of space in concentric cylindrical layers forces cylindrical solutions, where the radius of the resulting equilibrium structures adjusts itself to the number of molecules placed in the box. Changing the number of molecules in the box, and thus, controlling the radius, one can obtain naturally and with no fluctuations the bending constant κ (Figure 2.2b).

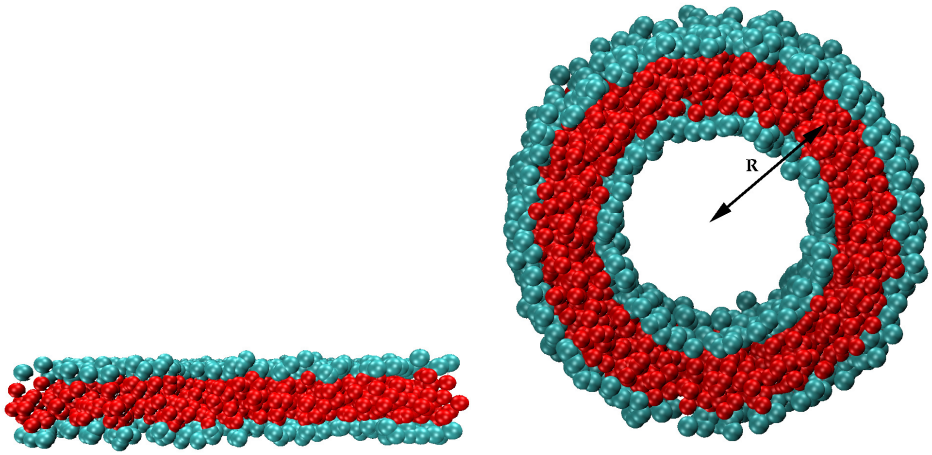


Figure 2.2: Typical representation of self-assembled membrane of fully flexible HT_2 (DMPC lipid) in two geometries: (a) planar (b) cylindrical. For visualisation of SCMF results we present most probable conformations of each geometry.

2.5 Elastic moduli

Changing the number of molecules in a flat geometry is equivalent to stretching and compression of the bilayer without bending. The second derivative of the free energy per molecule close to minimum f with respect to the area per molecule A gives the compressibility of the membrane[42]

$$K = 2Af''(A) \tag{2.7}$$

In turn, forcing cylindrical geometry allows to estimate the bending modulus κ in Helfrich expression (2.1). The radius of the resulting cylindrical bilayer is adjusted to accommodate a given number of molecules in the box, thus by changing the number of molecules in the box, one can control the degree of bending of the resulting tubular membrane.

We assume no spontaneous curvature, since there is no asymmetry between both leaflets, $C_0 = 0$. Then Eq. (2.8) for cylindrical bilayer of radius R yields in the form[11, 22]

$$E(R) = \frac{\pi\kappa L_z}{R} + \frac{K}{2}\frac{(A - A_0)^2}{A_0} \tag{2.8}$$

where L_z is the height of the simulation box, $A = 2\pi R L_z$ is the area of the mid-plane of the bilayer and $A_0 = NA_{eq}$ is the reference equilibrium area of the flat bilayer composed of the same number of molecules N. The excess of free energy E corresponds to the difference between free energies of the curved membrane, f_{curved} and that of flat membrane (f_{flat}) at equilibrium for the same number of molecules in the box, such that $E(R \to \infty) = 0$, *i.e.*

$$E(N) = N\left(f_{curved}(N) - f_{flat}\right) \tag{2.9}$$

The radius of the cylindrical membrane R (Figure 2.2b) is obtained from the calculation of the centre of mass of the bilayer given by

$$R(N) = \frac{\int \mathbf{r}\langle C_{all}(\mathbf{r})\rangle \mathrm{d}\mathbf{r}}{\int \langle C_{all}(\mathbf{r})\rangle \mathrm{d}\mathbf{r}} \tag{2.10}$$

where C_{all} is the total concentration of all beads in the bilayer.

The minimisation of Eq. (2.8) with respect to the radius reveals the relationship between the radius R, the elastic area compressibility modulus K and the bending modulus κ as follows

$$R = \sqrt{[}3]{\frac{A_0^3}{216\pi^3 L_z^3} + \frac{A_0\kappa}{8\pi K L_z} + \frac{\sqrt{\Delta_1}}{2}} + \sqrt{[}3]{\frac{A_0^3}{216\pi^3 L_z^3} + \frac{A_0\kappa}{8\pi K L_z} - \frac{\sqrt{\Delta_1}}{2}} + \frac{A_0}{6\pi L_z} \tag{2.11}$$

where

$$\Delta_1 = \frac{A_0^4\kappa}{216\pi^4 L_z^4 K} + \frac{A_0^2\kappa^2}{16\pi^2 L_z^2 K^2} \tag{2.12}$$

2.6 Results

2.6.1 Molecular models

We focus on four types of amphiphilic chains with a similar but slightly different structure, see Figure 2.1. Each amphiphilic molecule is composed by two types of beads: a hydrophilic (H) and a hydrophobic (T). Both types of beads have the same radius 4.05Å and is connected with the bond length of 10Å. Interactions between the beads and with an implicit non-ionic solvent w is a potential well with interaction radius 12.15Å[42]. The

corresponding interaction energies per bead: between two tails $\varepsilon_{TT} = -0.58\,k_BT$, between head bead and solvent $\varepsilon_{Hw} = -0.15\,k_BT$, interactions between other combinations of beads are set to 0.

These interaction parameters and the dimensions of the beads were adjusted[42] in such a way that three beads model HT_2 (Figure 2.1a) with freely-joint beads (fully flexible) corresponds to DMPC phospholipid. 5-bead flexible amphiphilic chain Figure 2.1c is an extension of this model with additional H and T beads. Rigid versions of Figures 2.1a and 2.1c represent rigid rods in Figures 2.1b and 2.1d.

2.6.2 Comparison of the models

Figure 2.3 shows the distribution of the volume fractions reflecting mechanical characteristics of the lipid membranes (*e.g.* thickness, distance between head groups, etc). In both cases, HT_2 and H_2T_3, the membrane thickness, the distance between heads and the thickness of the hydrophobic core increases slightly for rigid chains. The interfacial area per molecule and the elastic area compressibility modulus, K, also vary. However, whereas the interfacial area per lipid decreases from flexible to rigid HT_2, this effect is not observed for the H_2T_3. This may be due to the flexibility of the additional hydrophilic bead, where an excess area is required as compared to a fully rigid chain.

Figure 2.4a represents the free energy per molecule as a function of the area per molecule A. In this plot we observe a shift of the minimum of the free energy per molecule between HT_2 flexible and HT_2 rigid. More molecules are needed for equilibrium in the rigid model, which reflects the higher molecule packing. The shape of the curves are different not only between HT_2 and H_2T_3, but also between the different rigidities with more pronounced minima (peaks are widened or sharpened). The fits attest on the good approximation of the chains parametrisation. The compressibility modulus tends to drop for both length from flexible to rigid. Thence, stiff chains require less free energy to be packed and compressed together than flexible chains, since their rod-like shape involves less entropy loss compelled by their stretching. Note that the mean field method does not include correlation effects by steric hindrance between the chains. A summary of the extracted information concerning the systems is listed in Table 2.1.

In Figure 2.4b we compare SCMF results for the excess of free energy (Eq. (2.9)) with the elastic energy given by Eq. (2.8), and $1/R$ given by Eq. (2.11) for equivalent ranges of

numbers of molecules, N. The values of κ have been fitted by a least square fit of $E(R(N))$ reusing the modulus K as obtained via Eq. (2.7), and N as an independent parameter only, while we did not involve SCMF results for the radius, R. Thereby, Figure 2.4b has to be seen as a test for the compatibility of SCMF results with the full theoretical picture. For all systems, we observe a good agreement in terms of the slope of the curves with respect to $1/R$. The radii obtained in SCMF, however, seem to be systematically larger than expected by the trivial expression $R = (A_0/2N)/(2\pi L_z)$ as well as the marginally larger expression (2.11). In particular for H_2T_3flex, a possible origin for the discrepancy is the geometric constraint for the inner head-group layer at the centre of the cylinder, and one will need to increase the number of lipids to reduce the effect. The results for κ are summarised in Table 2.1. All values of κ are in the typical range of several $10\,k_BT$ as known by experiments for phospholipid membranes. For the HT_2flex representing DMPC, our results are slighliy larger than recently reported atomistic simulation results, $\kappa = 34.7\,k_BT$ by Doktorova *et al.*[13, 47], and compatible for HT_2rig. For the latter model, however, compressibility moduli seem slightly underestimated as compared to typical experimental values for K between 200 and 300 mN/m[47].

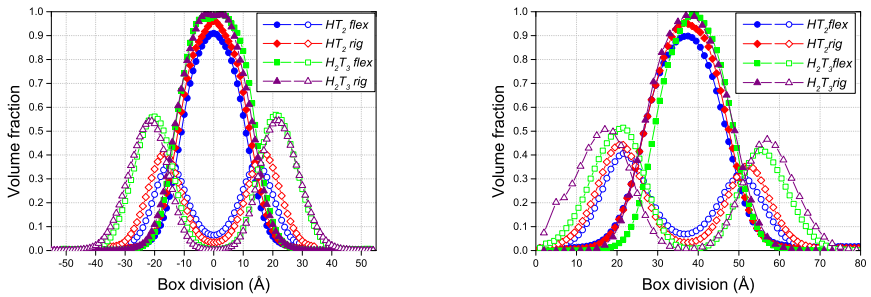
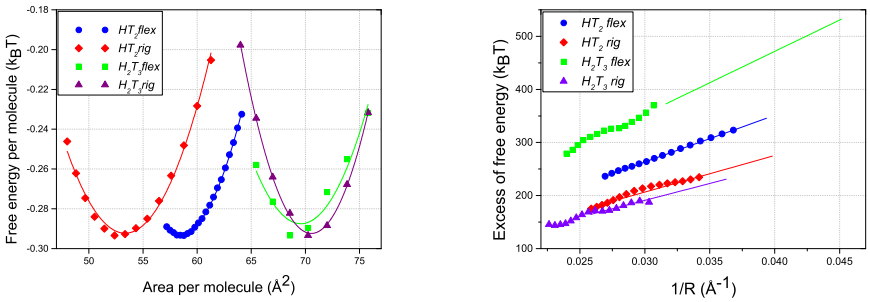


Figure 2.3: Volume fractions of the different amphiphilic bilayers implemented with a different geometry. (a) Volume fraction representations of the different types of chains associated by colours for flat membranes at their minimum of free energy per molecule. Symbols of the hydrophobic part are full and the hydrophilic one empty. (b) Volume fractions of molecule heads (empty) and tails (full) as a function of distance from the centre. These representations of the different types of chains are associated by colours for curved membranes at a close value of R.

To investigate further differences between lipid models (Figure 2.1), we calculate the orientational order parameter S for the planar geometry defined as

$$S = \langle P_2(cos\theta)\rangle = \left\langle \frac{3cos^2\theta - 1}{2} \right\rangle \tag{2.13}$$



a b

Figure 2.4: Free energy. (a) Free energy per molecule representation for the different chain types in the case of flat membranes. (b) Excess of free energy (Eq. (2.9)) for the different types of chains obtained from bent membranes.

Table 2.1: Comparison between the two different types of chain.
Membrane thickness (MT), Thickness of the hydrophobic core (THC), Distance between heads (D_{heads}), Interfacial area per molecule at equilibrium (A_{eq}), Compressibility constant (K), Order Parameter (S), Bending Rigidity (κ)

	HT_2		H_2T_3	
	Flexible (DMPC)	Rigid	Flexible	Semi-Rigid
MT (Å)	46	54	69	72
THC (Å)	28	30	33	32
D_{heads} (Å)	38	44	49	50
A_{eq} (Å^2)	59 ± 1	52 ± 1	69 ± 3	70 ± 1
K (dyn/cm)	256 ± 11	144 ± 11	303 ± 61	262 ± 30
S	0.145	0.333	0.165	0.339
κ (k_BT)	46.5 ± 6	36.5 ± 3	25 ± 4	13.5 ± 4
$\frac{\kappa}{K}$ (Å^2)	75.2	104.9	34.2	21.3

where θ is the angle between the chain and the normal of the membrane. In both cases, the order parameter value is higher for rigid chains in comparison with the flexible ones, which is coherent with the decrease of their area per molecule. Unlike rigid HT_2 chains, the semi-rigid H_2T_3 ones have their additional H-group flexible. While the order parameter confirms a stronger alignment of the rigid chains (parallel to the normal of the bilayer) than the flexible ones, the minimum of interfacial area per molecule (A_{eq}) remains unchanged, suggesting that this additional flexible hydrophilic group requires excess area. The different shape of the molecules may have led to a modification of the bilayer structure into a new geometry (*e.g.* toroidal structures, micelles, flexible bilayers...) as predicted by phenomenological models[28].

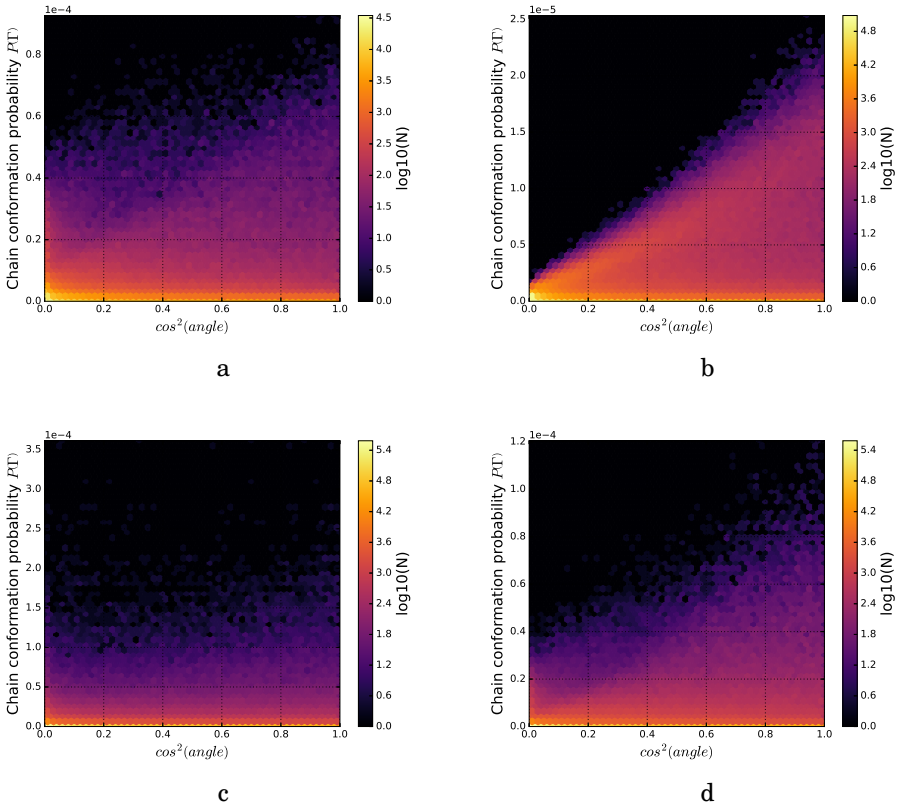


Figure 2.5: Probabilities of conformations $P(\Gamma)$ as a function of $\cos^2\theta$ of angles θ between the normal to the membrane and the block of each generated chain for models in Figure 2.1: (a) HT_2flex (b) HT_2rig (c) H_2T_3flex (d) H_2T_3rig

We report $\cos^2(\theta)$ of each model in Figure 2.1 associated to the probabilities for chains to be in particular configurations (Figure 2.5). The distributions between flexible and rigid chains are modified with narrower spectra.

Using Eqs. (2.14) and (2.15), we calculated the free energy depending on the chain angles (Figure 2.6). The free energy reaches a maximum when the chain is parallel to the membrane and decreases with its perpendicularity to the normal vector.

$$Q_0 = \sum_{i,\theta_1 \leq \theta < \theta_2} P(\Gamma_i) \tag{2.14}$$

$$F(\theta) = -\ln Q_0 \tag{2.15}$$

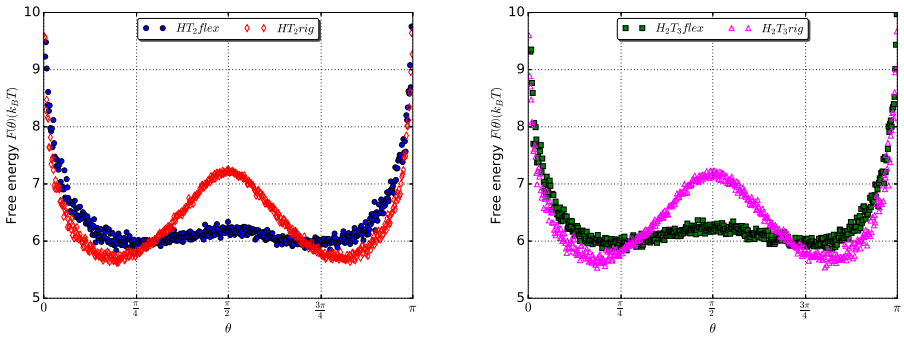


Figure 2.6: Free energy per molecule f depending on the chain angle. (a) HT_2 (b) H_2T_3

We investigate the evolution of the order parameter depending on the excess area per molecule (Figure 2.7). We both stretch and compress the flat membranes in order to calculate the corresponding order parameter for each type of molecule. We observe in Figure 2.7 the possibility for the amphiphilic bilayers to reorganise and demonstrate the variation of molecular packing under stress. A study of the free energy variations in case of stretching or compression shows the difference of free energy between chains oriented in parallel and perpendicular to the membrane. This difference decreases when the bilayer is stretched and increases in a compressed state. We also note that these differences are more significant for rigid chains than for flexible ones.

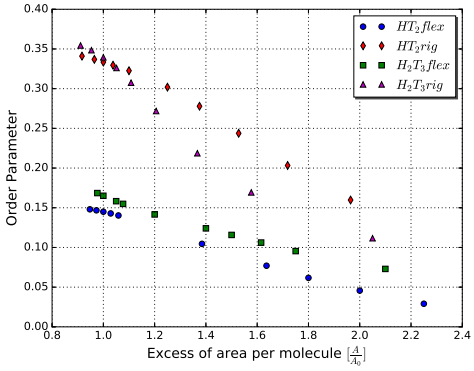


Figure 2.7: Order parameter depending on the excess area per molecule.

2.7 Conclusion

Many force fields for Molecular Dynamics and Monte Carlo methods have been used to describe lipid membranes. However, these dynamical methods are limited to the description of lipid membranes at small length scales only. In addition, it is not straightforward to access the free energies of a resulting self-assembled systems and, consequently, the equilibrium properties, including stretching and the bending moduli. The Single Chain Mean Field (SCMF) method is a complementary strategy to provide the equilibrium free energy of a membrane directly from self-consistent equations as well as equilibrium properties at the minimum of the free energy. Stretching and bending moduli can be calculated without the necessity to measure the forces under constraints, and are direct estimates for the thermodynamic limit. Thus, SCMF allows to bridge between analytical theories and simulation methods by extending the applicability of the force fields obtained from molecular simulations to much larger length scales, where mathematical surface descriptions such as the Helfrich hamiltonian are applicable.

We estimated elastic constants for two coarse-grained linear chains with both flexible and rigid backbones on the basis of DMPC lipids (fully flexible HT_2). The obtained values for bending rigidity are compatible with the typical order of several $10 k_B T$ as observed in experimental studies.

Our theoretical approach can be extended for other systems using force fields to fix equilibrium constants and link experimental data from different length scales.

UNIVERSITAT ROVIRA I VIRGILI
MOLECULAR SIMULATION OF MIXTURES IN LIPID MEMBRANES
Adrien Berthault

CHAPTER 3

GENERAL CONCLUSIONS

First, we bridged the gap between molecular simulation models and elastic theories for amphiphilic membranes with the SCMF theory and characterised, at equilibrium, lipid membranes features. This leads to the possibility to study any type of lipid at different levels of coarse-graining and extract fundamental information such as the compressibility of membranes, their area per lipid or their thickness. To ensure the description quality, we chose DMPC lipids as a proof of concept and compared with experimental data. Three other chain types were also considered varying by their length and rigidity.

Further, we extended the method and studied the formation of pores in lipid bilayers and calculated their associated line tension, initially for a single type of chains and then, in the case of mixture via the presence of fully hydrophobic monomers. A range of the DMPC line tension was found, compared with experimental techniques and validated. We observed the modification of both the pore line tension and membrane compressibility in the presence of hydrophobic molecules. Moreover, volume fraction distributions of each element type can be followed before and after the membrane was stretched, underlining their possible roles.

The dynamics of systems was studied with the Monte-Carlo based BFM method during my secondment at the Leibniz-Institut für Polymerforschung Dresden. We considered two types of amphiphilic chains which differ with their length and aggregate

to form membranes. Similarly to the procedure which was used with the SCMF theory, we decreased the amount of molecules in the simulation box to form a pore, mimicking mechanical stretchings performed experimentally. The study of the distribution of molecules over time brought us first insights to understand the role of each type of chain.

The general objective of this doctoral thesis was to use molecular simulation methods to study mixture in lipid membranes. Using two complementary techniques that we adapted to our issues, we are now able to provide multiple information to describe systems both at equilibrium and considering their dynamics. We are now studying the insertion of long fully flexible hydrophobic polymer chains in membranes and their mutual effects on each other, including the chains folding.

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