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Abstract

Lipid molecules form thin biological membranes that envelop all living cells, and behave as two-dimensional liquid sheets immersed in bulk water. The interactions of such biomembranes with their environment lay the foundation of a plethora of biological processes rooted in the mesoscopic domain — length scales of 1–1000 nm and time scales of 1–1000 ns. Research in this intermediate regime has for a long time been out of reach for conventional experiments, but breakthroughs in computer simulation methods and scattering experimental techniques have made it possible to directly probe static and dynamic properties of biomembranes on these scales.

Biomembranes are soft, with a relatively low energy cost of bending, and are thereby influenced by random, thermal fluctuations of individual molecules. Molecular dynamics simulations show how in-plane (density fluctuations) and out-of-plane (undulations) motions are intertwined in the bilayer in the mesoscopic domain. By novel methods, the fluctuation spectra of lipid bilayers can be calculated with direct Fourier analysis. The interpretation of the fluctuation spectra reveals a picture where density fluctuations and undulations are most pronounced on different length scales, but coalesce in the mesoscopic regime. This analysis has significant consequences for comparison of simulation data to experiments. These new methods merge the molecular fluctuations on small wavelengths, with continuum fluctuations of the elastic membrane sheet on large wavelengths, allowing electron density profiles (EDP) and area per lipid to be extracted from simulations with high accuracy.

Molecular dynamics simulations also provide insight on the small-wavelength dynamics of lipid membranes. Rapidly decaying density fluctuations can be described as propagating sound waves in the framework of linearized hydrodynamics, but there is a slow, dispersive, contribution that needs to be described by a stretched exponential over a broad range of length- and time scales — recent experiments suggest that this behavior can prevail even on micrometer length scales. The origin of this behavior is discussed in the context of fluctuations of the bilayer interface and the molecular structure of the bilayer itself. Connections to recent neutron scattering experiments are highlighted.
Preface

This thesis is the condensed result of my work in the department of Theoretical Physics at KTH Royal Institute of Technology, during April 2007–November 2011. It is based on four papers that treat different aspects of fluctuations in fluid lipid membranes, with large-scale molecular dynamics simulations being an integral part of all projects. The first part of this thesis provides an introduction to, and expansion of, these papers. The purpose is to present how the papers fit into the field of membrane biophysics. The second part consists of the published material: The articles and their appurtenant Supplementary Material.

In the first part of the thesis, Chapter 1 introduces the reader to membrane biophysics with an emphasis on the lipid bilayer. The motivations underlying the research are formulated, and some applications are discussed. Chapter 2 reviews physical models for the lipid bilayer in rising order of complexity. These models serve as the foundation for interpreting simulation and experiment data, with the focus on the lipid bilayer fluctuation spectra. Chapter 3 is an overview of the molecular dynamics (MD) method that is used to simulate atomistic system. It is used in all papers in the second part. Chapter 4 elaborates on how MD simulations are performed on lipid bilayers, and how key properties are calculated. Simulations are emphasized but a brief overview of relevant experimental methods is given. Chapter 5 presents a new method to calculate the out-of-plane (undulations) fluctuation spectrum. It is shown that it is crucial to account for undulations to obtain accurate electron density profiles (EDP) and lipid areas. Chapter 6 turns to the dynamic aspects of bilayer fluctuations, extending the static models of Chapter 2 to account for time-dependent decay of fluctuations. The results are discussed in the light of simulation and experimental results. Chapter 7 summarizes the most important conclusions that have emerged from the current work with Chapter 8 pointing out possible directions for future work. Concise summaries of the publications included in the second part of the thesis are included in Chapter 9.

Readers digging into this thesis with an inclination for rigor will surely be disappointed. Throughout, the intention has been to follow the advice once attributed to John Wheeler: Never calculate without first knowing the answer. Haphazard mistakes possibly hiding in the text are solely due to the present author.
List of papers

The papers that form the basis of this thesis are referred to in the following order.


Papers not included in the thesis


The author’s contributions to the papers

*Paper I.* The project was a collaboration with A. Braun and J. N. Sachs from University of Minnesota, and J. F. Nagle from Carnegie Mellon University. The author performed the simulations and the data analysis, and worked out the correlation function formalism presented in the Supplementary Material of Paper I and in parts of Chapter 5. The author participated in the writing of the manuscript.

*Paper II.* The project was a collaboration with A. Braun and J. N. Sachs from University of Minnesota, and J. F. Nagle from Carnegie Mellon University. The author developed parts of the code for the analysis, was involved in the development of theory, and participated in the writing of the manuscript (and produced the Supplementary Material in collaboration with A. Braun).

*Paper III.* The project was suggested by Olle Edholm, to investigate how well the lipid bilayer is described in terms of linearized hydrodynamic theory (experimental data had recently been published by Weiss et al. (6), Chen et al. (7)). The author constructed the system, performed the simulations and did the data analysis. The author also suggested the double nature of the Rayleigh line. Writing the manuscript was a joint effort.
Paper IV. This project grew out of the findings of Paper III. The author performed all simulations and did the data analysis, and wrote the first draft of the manuscript.
Acknowledgments

Many people have supported me during my years at KTH, far more than can possibly be fitted onto a single page. First and foremost, my supervisor Olle Edholm, who awakened my interest for the fascinating area of membrane biophysics, and who has always made time to answer small and not-so-small questions. Thanks to all other staff at the Theoretical Physics department, for stimulating discussions and company during numerous lunches, coffee breaks and seminars. I want to acknowledge collaborators on present and past projects: John Nagle (and Stephanie Tristram-Nagle) for continuous support and for unrestricted hospitality during my visit. Jonathan Sachs and Anthony Braun from the University of Minnesota. Mikko Hellgren and Tomas Bergman from Karolinska Institutet. Tore Brinck from the Physical Chemistry department at KTH.

To past PhD students and the co-workers who welcomed me to the department, and now have moved on to other things: Jakob Wohlert, Martin Lindén, Marios Nikolaou, Anders Biltmo, Pedram Hekmati, and others.

To my roommates, for enduring my company on a daily basis: Qaiser, with whom I have shared office for four years. Richard, the most talented programmer I have ever met. My newest colleague, Mihail.

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To all my nearest friends, you know who you are. After all, most of us met here at KTH. And finally, to the members of my family. My sisters and their families. My father and my mother for their endless support, even during times when it has most certainly been unearned. You have truly deserved your own page in this thesis.

To Emma, my soon-to-be wife, for having enough faith to quit her job and follow me to the other side of the world. I won’t let you down.

Erik G. Brandt
November 2011

Insane in the membrane, insane in the brain.
— Cypress Hill
To my father and my mother.
For making this kid stay in school... cobbler, stick to thy last.

Come gather 'round people
Wherever you roam
And admit that the waters
Around you have grown
And accept it that soon
You'll be drenched to the bone
If your time to you is worth savin'
Then you better start swimmin' or you'll sink like a stone
For the times they are a-changin'

— Bob Dylan
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A demonstration convinces a reasonable man;
a proof, a stubborn one.
— Mark Kac

☐

(Even reasonable men might argue, of course, whether the following tentative considerations qualify for a demonstration rather than an expression of faith.)
— Dieter Forster
Part I

Synopsis
Chapter 1

Introduction

If one were to take a magnifying glass and direct it anywhere in nature, sooner or later a membrane would emerge. The simplicity of the membrane structure in combination with the ability to change shape have been taken up by evolution in numerous places. The most conspicuous appearance of the membrane in everyday biology is of course as an envelope for the cell — the smallest unit of life. Cells have evolved for billions of years and can be found in a variety of forms. The simplest cells are the prokaryotic ones found in bacteria, consisting only of a thin membrane with no, or few, internal structures. In contrast, the eukaryotic (animal) cell consists of a number of compartmentalized subunits, or organelles. Each subunit is responsible for a specific task: the cell nucleus handles the genetic material; mitochondria generate the chemical energy of the cell; the endoplasmic reticulum (ER) takes place in most of synthesis and metabolism; the Golgi apparatus is responsible for sorting and packing of macromolecules, etc. Although the organelles are structurally different and fulfill diverse functions, they are all enclosed with lipid bilayer membranes. Membranes are crucial in biology as barriers to protect and separate the hostile exterior environment from the ever ongoing life processes taking place in the interior of the cell (Fig. 1.1).

Despite the fact that the number of cells in complex organisms is far greater than the term “astronomical” can honor (a human body is comprised of more than $10^{14}$ cells), the number of different cell types are limited; a few hundreds out of that vast total number. Most cells follow the same basic structure as shown in Fig. 1.1 and described above: The cell and its internal compartments are enclosed by thin membranes with exterior filament networks to help the cell shape adapt as required. A particularly fascinating aspect of biomembranes is how thin they are. The lateral size of a typical cell is of the order of microns ($10^{-6}$ m) but the surrounding membrane is only a few nanometers ($10^{-9}$ m) thin. The membrane responds to applied stress by bending; biomembranes are soft. They are elastic materials but profoundly different from the thin sheets of solid material familiar from everyday life: aluminum foil, steel plates or even thin films of plastic materials.

The crucial differences lie in membrane fluidity and that typical energies required
Figure 1.1: (a) The prokaryotic cell common to bacteria has few internal structures. The genetic material is stored in an irregular DNA/protein complex. (b) The eukaryotic cell consists of a number of compartmentalized subunits that carry out different tasks, all surrounded by lipid bilayer membranes. In particular, the genetic material is stored within the cell nucleus. Lipid bilayers are extremely thin, only a thousandth of the extension of the cell itself. Images adapted from Wikipedia (http://en.wikipedia.org/wiki/Cell_(biology).

to bend a biological membrane are delicately balanced; distinctively larger than the energy associated with random molecular motion, but small enough that such molecular fluctuations are influential. Materials that respond to energies in this regime are called soft matter. A range of physical phenomena originate from this energy regime, because it means that biomembranes at room temperature are sensitive to the balance between energy (compression and bending) and entropy (fluctuations). This is a fact that will be revisited during the course of this thesis.

1.1 Cell membrane structure

Biomembranes are present in both animal and plant cells. There are many distinctions to be made in general between plant cells and other eukaryotic cells — not least with regard to the biochemical processes involved in photosynthesis — but perhaps most notable is the plant cell wall, which consists of the crystalline material cellulose. The animal eukaryotic cell shape is maintained by the cytoskeleton, which is a network of thin proteins, filaments, that resemble a bunch of entangled ropes. They act as a scaffold for the entire cell. In addition to its structural role, the cytoskeleton excludes volume in the interior of the cell, making the cytosol a very crowded environment where macromolecules can not diffuse freely.

The fundamental building block of the cell membrane is the lipid molecule (Fig. 1.2). Lipids are amphiphilic molecules, made up of a head group that favors water (is hydrophilic) and a tail of hydrocarbon groups that repels water (is hydrophobic). The
1.1. CELL MEMBRANE STRUCTURE

Figure 1.2: (Top) The cell membrane is comprised of different types of lipids, membrane proteins and small molecules. The lipids are organized in domains and are asymmetric, i.e., there are different lipid types in the opposing leaflets. Water molecules are present on both sides of the membrane and have been omitted in the pictures for clarity. (Bottom, left) The single-component lipid bilayer structure. (Bottom, right) A lipid molecule consists of a hydrophilic head group and a hydrophobic tail. Images adapted from Wikipedia (http://commons.wikimedia.org/wiki/File:Cell_membrane_detailed_diagram_en.svg).

Head group attracts polar solvents but the tail groups are uncharged and disfavor polar attractions. Lipid molecules therefore self-assemble in water. The nonpolar tail groups are shielded by the lipids folding into two opposing monolayers, with the head groups orientated towards the water. This hydrophobic effect is purely thermodynamic in nature, so a solution of randomly dispersed lipids in water will spontaneously form structured configurations. The equilibrium structures depend on the exact shape of the lipid molecules.

Cell membrane do not only consist of lipids, a fact that was recognized very early (8). To varying extents, eukaryotic membranes are interspersed with sterol molecules — small and compact lipid-like molecules with large impact on the membrane’s elastic properties. The most common sterol in animal cells is cholesterol. Proteins are also abundant and make up 20–80% of the membrane mass even though they are not as frequent in numbers as lipids (9). Proteins are attached to the lipid bilayer matrix in various ways. Some intersect the membrane and other anchor to the membrane surface. Singer and Nicholson proposed the classic picture of the cell membrane as a “fluid mosaic model” (Fig. 1.2) with proteins floating in a sea of lipids (10). Even though this concept may be valid on length scales ~10 nm, it has been established that there are domains in the cell membrane that is characterized by (i) protein-protein complexes (11), (ii) membranes attached to the cytoskeleton (12) and (iii) lipids or-
ganized in microdomains (rafts) (13).

In its role as a semi-permeable barrier, the cell membrane specifically permits molecules to pass easily, with difficulty, or not at all. The permeability depends primarily on the electric charge of the molecule and not on its mass. Hence, ions are virtually impermeable to the membrane, but small, non-charged compounds are able to pass by diffusion, as is easily understood from the energy cost associated with solvating a charged molecule (14) in the hydrophobic bilayer core. For charged compounds, specific mechanisms have evolved to facilitate transmembrane transport. These mechanisms are energy-driven (active), in contrast to the diffusion-driven (passive) ones. The cell’s osmotic pressure is kept in equilibrium by a constant water flow across the membrane, which takes place through membrane pores (water channels) formed by the protein aquaporin (15). Ions are transported similarly, by ion-specific channel proteins (16).

The preceding outline shows that the cell membrane is a complex system with countless interactions, between countless constituents: a description entrenched in physics seems but hopeless. At first glance that may be the case, but many of those countless interactions can be described by “effective” ones. The approach of this thesis is in this spirit: To study representative aspects that capture the physics of cell membranes, and attempt to draw general conclusions. The simplest realistic model of the cell membrane, the model that truly needs to be understood before proceeding to the cell membrane, is the pure lipid bilayer. Many physical processes of the cell membrane is directly due to the properties of the lipid bilayer. For example, it is well-known that membrane curvature is protein-regulated (by e.g., Bin-Amphiphysin-Rvs (BAR) domains) (17) but another highly influential factor is lipid shape. Another example is the reciprocal interactions between pore-forming proteins/peptides and lipid packing (18). In short, studying the pure lipid bilayer is the first step to understanding the behavior of real cell membranes. The rest of this thesis will therefore be devoted to the properties of lipid bilayers.

1.2 The lipid bilayer

The bilayer structure results spontaneously from lipid self-assembly, driven by the hydrophobic effect. There exists a plethora of lipid types in nature, and even more synthesized in laboratories (19). The key components in cell membranes are phospholipids, with a head group containing a negatively charged phosphate group, and two tails made of hydrocarbons. The phospholipid length is determined by the number of hydrocarbons in the tails. One or both of the tails may have no double bonds (saturated fat), one double bond (unsaturated fat) or many double bonds (polyunsaturated fat). The chains are connected to the head group via the glycerol backbone, a flexible construct that allows the head to rotate almost independently from the tails. Fig. 1.3 shows the atomistic structure of common biomembrane lipids. Of particular interest to this thesis are dymyristoylphosphatidylcholine (DMPC) and dipalmitoylphosphatidylcholine (DPPC). They are saturated lipids, identical except for the number of hydro-
1.2. THE LIPID BILAYER

Figure 1.3: The atomic structure of some biomembrane lipids. The chemical properties of the lipid is determined by its head group, the number of hydrocarbon groups in the tail, and the number of double bonds in the tail. From left to right: 1,2-dimyristoyl-sn-glycero-3-phosphatidylcholine (DMPC 14 hydrocarbon groups : 0 double bonds), 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine (DPPC 16:0) and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE 18:1). Cholesterol, shown to the right, is a small lipid-like molecule which is found embedded in lipid bilayers.

Carbon in the tails. DMPC has fourteen hydrocarbons while DPPC has sixteen. DPPC is the most common lipid in cell membranes, DMPC is less common. Their properties have been scrutinized and they are common model systems for cell membranes. Although the chemical properties of these lipids are very similar, DMPC is liquid around 30 °C but DPPC does not melt until 50 °C. Most experimentalists find it more convenient to work at the lower temperature.

The lipid bilayer has many remarkable properties. For one thing, it is one of few systems that can occupy a two-dimensional liquid phase, with no shear resistance or long-range internal order. Lipid membranes have a complex phase behavior over a series of intermediate stages between 10 °C and 80 °C (20). At the lowest temperatures the lipid bilayer is in the sub-gel (L_C phase, with fully ordered hydrocarbon chains. Increasing the hydration of the head groups leads to the gel (L_β′) phase. The gel phase is distinguished by ordered tail groups that are tilted with respect to the bilayer normal. Raising the temperature further, the bilayer surface starts to undulate with subdomains of ordered and disordered tails, the rippled (P_β′) phase. At higher temperatures the bilayer transcends to a phase with fully disordered chains—a fluid (L_α) phase. The P_β′ → L_α transition is known as the lipid bilayer main transition (21). For all biological purposes, the fluid phase is the relevant one; that said, the phase behavior is a fascinating problem of its own. For binary and/or tertiary (consisting of two and/or three different lipid types) membranes the phase diagrams become increasingly complex (22). The lipid bilayer structure can be measured in terms of head group/chain separation and orientation, chain tilt angle, area per lipid, etc., also in the
fluid phase, but the structure is highly dynamic as captured in the term “liquid crys-
tallography” (23), used in scattering experiments that seek to determine the structure of
fluid lipid bilayers. This implies that fluid lipid membranes as depicted in Fig. 1.2 are
only to be thought of as the average from a distribution of possible structures.

In contrast to true two-dimensional fluids, say, a liquid film spread on a solid sub-
strate, the membrane can bend in the normal (third) dimension, so that the lipid bi-
layer behavior is determined by the interplay between compression/stretching (area
change) and bending (crumpling). The origin of both phenomena are certainly mi-
croscopic, as the net effects of molecules moving individually as well as coherently,
to find favorable energetic minima. On the other hand, both stretching and bending
is perfectly defined in terms of macroscopic properties such as compressibilities and
bending moduli. This makes statistical mechanics, the physical theory relating macro-
scopic properties to averages of fluctuating microscopic interactions, a powerful tool
in the study of membranes (24). Although lipid membranes have been extensively
studied during the last 40 years, there are still properties that are not well understood.
This holds particularly true for dynamic properties of lipid diffusion, undulations and
area-thickness (peristaltic) fluctuations on small length scales (25).

1.3 Motivations and applications

The aim of the research presented in this thesis is twofold. First, it is to use mod-
ern theoretical and computational tools to extract properties of membranes that are
so accurate that one is able to test and improve different physical models of lipid bi-
layers. This is meant to serve as a complementary technique to the experiments that
provide structural and dynamical details of lipid bilayers. With this information as a
basis, the bilayer can provide a door to the mechanisms underlying the cell membrane.
More complex and biologically relevant systems can be studied once the lipid bilayer
is thoroughly understood. The long-term goal is hence to understand how the molec-
ular properties of the cell membrane shape the behavior of the cell as an entity. This
is the basis to understand a range of phenomena; from the uptake of pharmaceutical
compounds to the role of cell fusion and division in the early stages of reproduction.
Second, it is hoped that biotechnological applications are within reach from the sim-
ulation community. That is, to make simulations and theoretical arguments strong
and precise enough to manufacture biological membranes and related materials, such
as soaps, liquid films, foams and gels, with desired properties, directly by looking at
molecular compositions. This goal is realistic to reach within a not-too-distant future.

Hopefully, this introduction has illustrated why lipid membranes continue to in-
trigue the physicist, as rare model systems with high biological relevance, being one of
few genuine examples of a two-dimensional system. Motions in lipid membranes span
a vast range of length- and time scales, from the fastest hydrogen vibrations to the
slowest collective undulations of thousands of lipid molecules. Many of these phenom-
ena can be described by methods of modern theoretical physics, like non-equilibrium
statistical mechanics, differential geometry and phase transition theory.
Chapter 2

Simple models of biomembranes

Lipid bilayer membranes are closely related to two-dimensional fluids, but can in addition change shape by deforming into the normal dimension. To characterize the shape of the bilayer it is therefore necessary to include fluctuations in not only density, but also of the bilayer interface. The other characteristic feature of lipid membranes is how extremely thin they are in comparison to their lateral extensions. The simplest treatment of the bilayer is therefore one describing the physics of an infinitely thin elastic sheet (26). Based on this simplest model, refined descriptions capturing more of the details of the lipid bilayer are discussed. Only static properties are considered, as they lay the foundation of the dynamic properties which are discussed in Chapter 6. The focus is on the membrane fluctuation spectrum, which is the magnitude of the amplitudes of the Fourier representation of the bilayer surface. It is shown how fluctuations in density and molecular orientation couple to the bending.

2.1 The Helfrich model

It was over 40 years ago that lipid bilayers were first treated by the principles of statistical mechanics. Helfrich (27), inspired by previous work on smectic liquid crystals (28) (that in turn built on elastic theory and statistical mechanics that had been developed up until then), proposed that the energetics of lipid bilayers could be described by a Hamiltonian of the form

\[ \mathcal{H} [S] = \int_S dS \left[ 2k_c (H - c_0)^2 + k_G K + \gamma_0 \right]. \tag{2.1} \]

Here, \( S \) is the particular configuration the bilayer has taken (with surface element \( dS \)). The curvature tensor, \( \mathcal{C} \), is formed from the derivatives of the bilayer surface. The mean curvature \( H \) and the Gaussian curvature \( K \) are invariants of \( \mathcal{C} \); namely (half) its trace, \( H = \frac{1}{2} \text{Tr } \mathcal{C} \), and determinant, \( K = \det \mathcal{C} \) (29). There are three material parameters in Eq. (2.1): the bending and Gaussian (saddle-splay) moduli, \( k_c \) and \( k_G \), and the spontaneous curvature \( c_0 \). The bending modulus, \( k_c \), describes the membrane's
resistance to bending, while the value of the Gaussian modulus, \( k_G \), describes the preference for convex/concave \( (k_G < -k_c) \) or saddle \( (k_G > -k_c) \) shapes. The spontaneous curvature, \( \epsilon_0 \), accounts for whether the lowest energy configuration of the membrane is bent (in units of inverse length). In addition, \( \gamma_0 \), is the surface tension of the membrane. Note that the lipid membrane is here an infinitely thin mathematical surface; no reference is made to its bilayer nature. Eq. (2.1) equally well describes monolayers as bilayers but with different moduli (30). For a planar bilayer to be stable to all kinds of undulations (to prevent the free energy, Eq. (2.1), from going to \(-\infty\)), it is required that \(-2k_c \leq k_G \leq 0\). This inequality may be relaxed by introducing higher order terms in the free energy expansion that stabilize the system against large curvatures. Since \( k_c \) is always positive, the Gaussian modulus is always negative.

Eq. (2.1) is too general for the present purposes, as it is written in terms of the shape, \( S \). It was not given in this form by Helfrich (27), who instead argued on physical grounds that various free energy contributions from the curvature tensor must vanish by symmetry. These arguments were very similar to the reasoning invoked in deriving the original Frank free energy for liquid crystals, a venture that took more than three decades to complete (some fascinating details about this tortuous derivation are told in the book by de Gennes and Prost (31)). Eq. (2.1) represents the first non-zero terms of an expansion in the derivatives of the surface, where linear terms vanish because of symmetry and first derivatives do not occur in the absence of a surface tension. (The small expansion parameter is the product of the curvatures and the membrane thickness.) Locally, the cell membrane geometry is planar and it proves convenient to parametrize its surface, \( S \), by the Monge gauge. In Cartesian coordinates the bilayer mid-surface is then described by a single-valued and slowly varying function \( u(x, y) \) (with \(|\nabla u| \ll 1\)) so that the position vector \( R = (x, y, u(x, y)) \) describes a point on the
2.1. THE HELFRICH MODEL

membrane (Fig. 2.1). The curvatures are to lowest order given by (32)

\[ H = \frac{1}{2} (u_{xx} + u_{yy}) = \frac{1}{2} \nabla^2 u(x, y) \quad (2.2) \]

\[ K = u_{xx} u_{yy} - u_{x y}^2, \quad (2.3) \]

and the surface element is \( dS = \sqrt{g} \, dx \, dy = \sqrt{1 + (\nabla u)^2} \, dx \, dy \) (\( g \) is the surface metric determinant). A symmetric bilayer has zero spontaneous curvature, although the individual \( c_0 \) for each leaflet may be non-zero. Further, a membrane in solvent is free to adapt its area to the equilibrium value and thus have zero surface tension (33). In simulations, this amounts to having equal pressures in the lateral \((x, y)\) as in the normal \(z\) direction. If the topology of the membrane remains fixed\(^1\) the integral of the Gaussian curvature over the membrane area is a constant:

\[ \int_S dS \, K = 2 \pi \chi(S) - \int_{\partial S} ds \, k_g. \quad (2.4) \]

This celebrated result from differential geometry is known as the Gauss-Bonnet theorem (32). The integral on the right hand side in Eq. (2.4) is over the geodesic curvature, \( k_g \), along the boundary of \( S \) with line element \( ds \). If the surface is closed, the line integral can be omitted, and the integral of \( K \) only depends on the Euler characteristic, \( \chi(S) \), of the surface. This is an invariant that describes the topological space. Regardless of its value, \( \chi(S) \) only adds a constant to the Hamiltonian which can be dropped.

With these considerations, Eq. (2.1) can be written

\[
\mathcal{H} = \int_{A_0} dx \, dy \, \sqrt{g(x, y)} \left[ 2 k_c \left[ H(x, y) \right]^2 + \gamma_0 \right],
\]

to be integrated over the flat reference plane (usually taken at \( z = 0 \)) and where \( \gamma_0 \) has been kept for completeness. Even in this simplified form, the physics described by Eq. (2.5) is nonlinear, owing to the metric determinant and the curvatures. \( \mathcal{H} \) is a functional of the derivatives of the bilayer interface, \( u(x, y) \). Averages in the statistical mechanics sense are to be taken over all possible conformations, Boltzmann-distributed according to Eq. (2.5). The equilibrium shape of the bilayer is the one that minimizes \( \mathcal{H} \) and can be found by standard calculus of variations (34). Although it can be performed on Eq. (2.5) it leads to a complicated fourth-order, non-linear, partial differential equation for \( u(x, y) \), that can only be solved for a few special cases (35, 36). In general, the partition function corresponding to \( \mathcal{H} \) can neither be calculated exactly (24).

For gentle undulations, a small gradient expansion yields to lowest order,

\[
\mathcal{H} = \frac{1}{2} \int_{A_0} dx \, dy \left[ k_c (\nabla^2 u)^2 + \gamma_0 (\nabla u)^2 \right],
\]

\(^1\)Constant topology excludes for example pore formation, membrane fusion, and processes where the membrane close up into a vesicle or vice versa.
up to an additive constant. This is the most common form of the Helfrich Hamiltonian, particularly appealing for its simplicity: The energy depends quadratically on the curvatures, implying that the statistical mechanics problem can be exactly solved. The most important observation regarding the harmonic model described by Eq. (2.6) is that the fluctuations of the lipid bilayer are completely determined by two types of deformations, out-of-plane bending (undulations) and in-plane compression. Furthermore, bending and compression are uncoupled. This amounts to choosing \( u(x, y) \) to follow a surface within the bilayer where cross-terms between bending and compression vanishes — the neutral surface. For a thorough discussion, see the book by Safran (30).

The fluctuation spectrum

The quadratic form of the Helfrich model, Eq. (2.6), means that various averages can be calculated exactly. In some cases the calculations will be involved, in others fairly straightforward. Fortunately, the fluctuation spectrum falls into the later category. It measures how undulations are correlated as a function of distance. In the literature, the fluctuation spectrum is confusedly often called static structure factor or form factor, but the later has an entirely different meaning within the scattering community (see Chapters 4 and 5). Here, the term fluctuation spectrum will be adhered to. The fluctuation spectrum was first used to explain the experimental observation of flickering contours in spherical lipid vesicles (37). Thermal fluctuations in lipid membranes have since then been confirmed in other experiments (38, 39), and can be used to determine the bending modulus of the membrane, by interpretation via the Helfrich model.

To proceed in the calculation of the fluctuation spectrum, it is most convenient to expand the bilayer interface in eigenfunctions of the Laplacian. For the planar membrane configuration considered here, with periodic boundary conditions (as in simulations), the eigenfunctions are the coefficients in a Fourier series. The Fourier pair to be used is

\[
\begin{align*}
  u(r) & = \sum_{q} u_q e^{i q \cdot r} \\
  u_q & = \frac{1}{A_0} \int_{A_0} dx \, dy \, u(r) e^{-i q \cdot r}
\end{align*}
\]  

(2.7)

(2.8)

where the integrals are over the base-plane area of the membrane, \( A_0 = L_x L_y \). The wave vectors are discrete in Fourier space, to be commensurate with the periodic boundary conditions, and are given by \( q = 2\pi(n/L_x, m/L_y) \) with \( n, m = 0, 1, \ldots, M \), up to a large-\( q \) cutoff wavenumber \( M \), corresponding to the smallest length scale in the continuum description. Here and henceforth, \( q \) is used as an index to emphasize discrete numbers. By Parseval’s theorem (40), the Hamiltonian decouples in Fourier
space as

$$\mathcal{H}_q = \frac{A_0}{2} \sum_q |u_q|^2 \left[ k_c q^4 + \gamma_0 q^2 \right],$$  

(2.9)

(the Fourier representation diagonalizes $\mathcal{H}$). Invoking the equipartition theorem (41), which states that a degree of freedom that is quadratic in the Hamiltonian (here $u_q$), contributes exactly $k_B T / 2$ (half the product of the Boltzmann constant and the absolute temperature) to the average energy, yields

$$S_u(q) = N \left\langle |u_q|^2 \right\rangle = \frac{k_B T}{a} \frac{1}{[k_c q^4 + \gamma_0 q^2]},$$  

(2.10)

where $N$ is the number of lipids per monolayer and $a$ is the area per lipid. This normalization is by convention and is done to give a size-independent spectrum. The above notation will be used in the rest of the thesis: $S$ for Fourier spectra and $F$ for corresponding real-space correlation functions. The index marks the quantity in question.

The Helfrich model predicts a fluctuation spectrum where bending modes ($q^{-4}$) are suppressed by surface tension ($q^{-2}$) for wave vectors smaller than $\sqrt{\gamma_0/k_c}$. With $\gamma_0 = 0.03$ N/m, typical to computer simulations (42), and $k_c = 7 \times 10^{-20}$ J (23), the crossover takes place around 0.65 nm$^{-1}$, corresponding to wavelengths of 10 nm. $S_u(q)$ calculated for a membrane under tension is dominated by the $q^{-2}$-term and eludes an accurate fit to $q^{-4}$ part of the spectrum. To determine $k_c$ from a fit to Eq. (2.10), the probed membrane should therefore be free from tension ($\gamma_0 = 0$).

### 2.2 Protrusions

$S_u(q)$ can be calculated from molecular dynamics simulations (43–47) (Chapter 4), and $q^{-4}$-behavior is found for vanishing surface tension on distances that are large compared to the membrane thickness (43). In practice this means below $\sim 1$ nm$^{-1}$. At progressively smaller wavelengths, or larger wave vectors, the spectrum levels out until the grid spacing limit is reached around 3–4 nm$^{-1}$. Deviations from Eq. (2.10) should come as no surprise given that the bilayer structure is ignored in the Helfrich model; neither is there any reference to the molecular structure. It has been established for a long time, that molecules protruding out the surface of which they take part, change the effective area of the membrane (48). Lipowsky and Grotehans (49, 50) proposed a model based on the concept of lipids as rigid rods, that included the energy penalty of protruding molecules, and showed that such a term is equivalent to a “microscopic” surface tension. Strictly, if added as an extra term to the Hamiltonian, this would lead to a fluctuation spectrum identical to Eq. (2.10) but with another coefficient $\gamma_p$ for the microscopic surface tension. However, this would lead to the unphysical notion of microscopic protrusions dominating the fluctuation spectrum for large wavelengths. (That result is also in conflict with simulations.) Phenomenological models have been used in the literature to confine protrusions to small wavelengths. Lindahl and Edholm
used
\( S_u(q) = k_B T a \left\{ \begin{array}{ll}
\left( k_c q^4 + \gamma_0 q^2 \right)^{-1} & q \leq q_0 \\
\left( \gamma_p q^2 \right)^{-1} & q > q_0
\end{array} \right. \) \tag{2.11}

to fit their simulation data. \( q_0 \) is a cutoff wave vector below which the protrusions do not contribute to the fluctuations and above which the bending does not contribute to the molecular fluctuations. \( \gamma_p \) is the microscopic surface tension. This is physically plausible, since bending is due to collective fluctuations of many lipid molecules while protrusions would be associated with a single (or a few) molecule(s). Goetz et al. (44) instead fitted their simulation data to a continuous crossover between the two regimes,
\[ S_u(q) = k_B T a \left[ \frac{1}{k_c q^4 + \gamma_0 q^2} + \frac{1}{\gamma_p q^2} \right]. \] \tag{2.12}
Both expressions are inconsistent with equipartition, but simulation data could be fit to either, yielding microscopic surface tensions \( \gamma_p \approx 0.05 \text{ N/m} \) or slightly less.

Protrusions have also been described as randomly fluctuating fields around the undulation surface (47). This gives a consistent picture of the microscopic protrusions as random noise, perturbing the smooth undulation surfaces. In particular, the protrusions are bounded at small wave vectors (with an additional constant in the denominator of the second term in Eq. (2.12)) relaxing the need of a rather arbitrary cutoff wave vector. Further discussions about the footprint of protrusions in the fluctuation spectrum can be found in Paper I and in Chapter 5. It remains an active area of research.

### 2.3 The Seifert-Langer model

The Helfrich model remains the foundation of membrane elasticity theory. Over the years a lot of effort has been put into extending the original Helfrich model. For an overview, see the review by Seifert (51). The first real attempt to take the bilayer aspect of lipid membranes into account was put forth by Seifert and Langer (52, 53) (and independently for spherical geometry of bilayer vesicles by Evans and Yeung (54, 55)). The idea was to combine the free energy costs of bending the membrane leaflets in a “coupled monolayer model”. Instead of a single mathematical surface, this picture starts from two neutral separated by a fixed distance (Fig. 2.2 (a)). The number densities along the surfaces are \( \phi_\pm \), which are projected onto the neutral surface of the bilayer (where bending and compression are uncoupled) and are to lowest order related to the monolayer surfaces by a well-known result of parallel surfaces (30), \( \phi_\pm = \phi^{\text{proj}}_\pm (1 \mp 2dH) \). \( d \) is the distance from the bilayer mid-surface to the neutral planes of the monolayers and \( H \) is the mean curvature of the bilayer. The reduced density deviations are defined as \( \rho_\pm = \phi^{\text{proj}}_\pm / \phi_0 - 1 \) in terms of the equilibrium density \( \phi_0 \). The expressions are more transparent with a change of variables to the reduced
2.3. THE SEIFERT-LANGER MODEL

Figure 2.2: (a) Schematic drawing of how the bilayer nature of the membrane is included in the Seifert-Langer (SL) model. The densities defined on the monolayer neutral surfaces $\phi_{\pm}$, located at distance $d$, are projected onto the bilayer neutral surface. On a more detailed level, it also costs energy to (b) have a lipid tilted with respect to the monolayer surface and (c) when the lipid tails of the two monolayers are entangled (interdigitation). Ideally, fluctuations in lipid shape should also be included. All these contributions are ignored in the SL model.

density difference,
$$\rho \equiv (\rho^+ - \rho^-)/2,$$

and the mean density deviation,
$$\bar{\rho} \equiv (\rho^+ + \rho^-)/2.$$

To lowest order in the energy, i.e., to quadratic order in the curvatures, the Hamiltonian can be expanded as

$$\mathcal{H} \left[ \nabla^2 u, \rho, \bar{\rho} \right] = \int_{A_0} \mathrm{d}x \mathrm{d}y \left\{ \frac{k_c}{2} (\nabla^2 u)^2 + k_m [\bar{\rho}^2 + (\rho - d \nabla^2 u)^2] \right\},$$

which is now a functional not only of the bilayer bending, $u(r)$, but also of the density difference, $\rho(r)$, and the mean density deviation, $\bar{\rho}(r)$. As before, the Gaussian curvature has been dropped and this time the tensionless state, $\gamma_0 = 0$, is considered. Here, $k_m$ is the monolayer compressibility modulus and $d$ is the distance from the bilayer mid-surface to the monolayer neutral surfaces. Again, the Hamiltonian is quadratic and is expanded in Fourier space as

$$\mathcal{H}_q \left[ u_q, \rho_q, \bar{\rho}_q \right] = \sum_q f_q, \text{ or explicitly,}$$

$$\mathcal{H}_q \left[ u_q, \rho_q, \bar{\rho}_q \right] = \frac{A_0}{2} \sum_q \left\{ \kappa q^4 |u_q|^2 + 2k_m \left( |\rho_q|^2 + |\bar{\rho}_q|^2 \right) - 4k_m d q^2 \rho_q u_q \right\},$$
with the renormalized bending modulus $\kappa \equiv k_c + 2k^m d^2$. Note that there can be a significant difference between $\kappa$ and $k_c$; a simple estimate from thin plate theory (26) gives $\kappa = 4k_c$. The modified fluctuation spectrum can be calculated by equipartition in diagonalized (normal) coordinates. Eq. (2.16) can be written on the form

$$\mathcal{K}_q[x] = \frac{A_0}{2} \sum_q x^T E(q) x,$$

(2.17)

where $x \equiv (u_q, \rho_q, \bar{\rho}_q)$ is a vector, $T$ denotes the transpose, and the energy matrix is symmetric and given by

$$E(q) = \begin{pmatrix} \kappa q^4 & -2k^m d q^2 & 0 \\ -2k^m d q^2 & 2k^m & 0 \\ 0 & 0 & 2k^m \end{pmatrix}.$$  

(2.18)

The normal coordinates $w = (w_1, w_2, w_3)$, are related to the original coordinates by the transform $w = P^{-1} x$, where $P$ is the matrix with columns formed from the eigenvectors of the energy matrix $E$. Expanding the energy matrix in an orthogonal transformation, $E = PD^p$, where $D$ is a diagonal matrix with the eigenvalues of $E$ as its elements, equipartition can be invoked as cross-terms are absent in normal coordinates, yielding $\langle w w^T \rangle = (k_B T/A_0)D^{-1}$.

The fluctuation spectra in the original coordinates are $S \equiv N \langle xx^T \rangle$. Transforming back and identifying $E^{-1} = PD^{-1}P^T$ gives the final result

$$S = \frac{k_B T}{A_0} E^{-1},$$

(2.19)

where the inverse of the energy matrix is

$$E^{-1}(q) = \begin{pmatrix} 1/(k_c q^4) & d/(k_c q^2) & 0 \\ d/(k_c q^2) & \kappa/(2k_c k^m) & 0 \\ 0 & 0 & 1/(2k^m) \end{pmatrix}.$$  

(2.20)

The bending modes are the same as in the original theory, growing with decreasing wave vector as $1/(k_c q^4)$. In addition there are compression modes for the density difference and the density deviations that are independent of wave vector. The constant is a combination of the bending and compression moduli. There is finally a coupling term between the bending and the density difference, scaling with wave vector as $d/(k_c q^2)$. Chapter 6 discusses the dynamic decay predicted by Seifert-Langer theory and how it shows up in simulations.
2.4 Fluctuations in molecular orientation

This chapter is finished by considering models that also account for molecular orientation. The aim is not rigor but to show how such terms can be included in the Hamiltonian, and thereby in the fluctuation spectrum. There are many possible ways in which individual lipid molecules can deform the local bilayer structure, but limitations are set due to geometric restrictions. (In what follows, protrusions are skipped as they have already been covered.) First and foremost, the collective elastic bending of the membrane sheet is treated with the classic Helfrich model. In addition, the orientation of individual molecules can deviate from the surface normal (Fig. 2.2 (b)), which is measured by the tilt vector

\[ t = \frac{n}{n \cdot N} - N, \]  

(2.21)

the deviation of the molecule director \( n \) from the surface normal \( N \). Further, neighboring lipid ‘rows’ tilted in different directions rise to a twist penalty, but this contribution is significantly smaller than the tilt and can be neglected to a good approximation. The monolayers also interact (Fig. 2.2 (c)), but the interdigitation between lipid tails is weak and can usually be left out as well.

Neglecting twist and interdigitation leads to the Hamm-Kozlov model (56), which extends the Helfrich Hamiltonian to include lipid tilt. The functional free energy depends on the derivatives of the surface, \( u(x,y) \), and of the molecular tilt vector \( t(x,y) = (t_x(x,y), t_y(x,y)) \),

\[ \mathcal{F}_{\text{HK}} \left[ \nabla^2 u, \nabla \cdot t, t \right] = \frac{1}{2} \int \mathcal{A}_0 \, \text{d}x \text{d}y \left\{ k_c (\nabla^2 u + \nabla \cdot t)^2 + k_\theta |t|^2 \right\}. \]  

(2.22)

The additional modulus, \( k_\theta \), measures the resistance to tilt. Employing the same formalism as in the previous section, i.e., switching to normal coordinates to invoke equipartition, Eq. (2.22) can be written on the same form as Eq. (2.17) but with the self-adjoint energy matrix,

\[ E_{\text{HK}}(q) = \begin{pmatrix} k_c q^4 & -i k_c q^2 q_x & -i k_c q^2 q_y \\ i k_c q^2 q_x & (k_c q_x^2 + k_\theta) & k_c q_x q_y \\ i k_c q^2 q_y & k_c q_y q_x & (k_c q_y^2 + k_\theta) \end{pmatrix}, \]  

(2.23)

being the generalization of a real symmetric matrix to the complex case. The fluctua-
tion spectra are as previously given by its inverse,

\[
E_{\text{inv}}^{-1}(q) = \begin{pmatrix}
\frac{1}{k_c q^4} + \frac{1}{k_\theta q^2} & \frac{i q_x}{k_\theta q^2} & \frac{i q_y}{k_\theta q^2} \\
-\frac{i q_x}{k_\theta q^2} & \frac{1}{k_\theta} & 0 \\
-\frac{i q_y}{k_\theta q^2} & 0 & \frac{1}{k_\theta}
\end{pmatrix}.
\] (2.24)

In particular,

\[
S_{x}(q) = \frac{k_B T}{a k_\theta} \
S_{y}(q) = \frac{k_B T}{a k_\theta} \
S_{u}(q) = \frac{k_B T}{a} \left[ \frac{1}{k_c q^4} + \frac{1}{k_\theta q^2} \right].
\] (2.25, 2.26, 2.27)

Interestingly, the undulation spectrum is given on the same form as the one including protrusions, Eq. (2.12), but with the tilt contribution taking the role of the protrusions. Explicitly adding a protrusion term to \( H_{\text{def}} \) amounts to a renormalization of the tilt modulus, \( \tilde{\kappa} = k_\theta + \gamma_\mu \). May et al. (57) found from simulations that the tilt contribution dominates over the protrusions and that the renormalization is weak. One of the strongest points of this formulation is that there are now two independent equations that can be used to verify the value of the tilt modulus, \( k_\theta \). This material constant should also, in principle, be possible to determine from experiments (58).

Recently, an ambitious model that takes all these contributions into account have been proposed (59). It shows that all fluctuations can be separated into undulation (bending) and peristaltic (volume-conserving) fluctuations. Inevitably a general description makes for lengthy expressions for the fluctuation spectra, and therefore the limiting cases where the fluctuation spectra simplify are very useful. Summarizing the discussion, the bending contribution is most prominent due to that the bending modes are proportional \( q^{-4} \). The next order is \( q^{-2} \)-decay, which are dominated by molecular tilt fluctuations. The role of protrusions seem to have faded in recent years. In the model by Watson et al. (59) protrusions have been reduced to a constant in the spectrum, representing random noise. One should be a bit careful about decisive statements as it remains an active area of research.
Chapter 3

Molecular dynamics

Lipid bilayers are very thin; no more than a few nanometers. Therefore they elude not only the bare eye but also traditional light microscopy. Also conventional scattering experiments fail since the scattering signal from a single bilayer is too weak. However, what is a curse to the experimentalist, has become a blessing to computer-based studies. Such studies, designed to do microscopic calculations on fluid systems, were already called “computer experiments” by the pioneers performing them more than 40 years ago (60–64). This chapter is devoted to the molecular dynamics (MD) method, which has become a standard tool to assess the microscopic properties of lipid bilayers. There are of course many other computational methods relevant to soft matter physics, perhaps as many as there are practitioners. The focus is put on molecular dynamics because it is most relevant to the papers in Part II. The chapter presents the equations of motion along with the molecular interactions described by the force field. Ways to integrate the equations of motion are discussed, and also how simulations can be performed in other ensembles (characterized by certain fixed macroscopic parameters).

3.1 Computer experiments

Practicing theoretical physics, one quickly learns that there are very few realistic models around that can be solved exactly. Even though the equations used to formulate the problem are known, their solutions rapidly become too involved, a lesson that is particularly true for soft matter systems at room temperature, with few symmetries for the theoretical physicist to exploit. In general, the motion of a system is determined by the same number of (second-order) differential equations, as there are degrees of freedom. Even for a modest number, say $10^3$, solution by hand is not an option. In a computer simulation, which yields numerical solutions to a given physical model, it is a straightforward albeit time-consuming task. Computer simulations offer some appealing aspects. First, systems that are difficult to set up in experiments are easy to simulate on computers. Biological examples include single-molecule experiments and all kinds of nanoscale systems. Second, at least in principle, all variables that go into
the simulation are known in advance, which is not the case in experiments. Third, simulations provide all the atomic positions and velocities at all simulated times. Of course, computer simulations have their limitations. In particular, the length- and time scales that can be reached are directly limited by the available computer power. The methods are to varying extent dependent on a number of tunable parameters that go into the calculations in advance, so the results of computer simulations always have to be validated by experiments. Since simulations provide microscopic details that elude most experiments, they are especially powerful when used to validate and discriminate among theories, to determine model parameters, and to suggest new areas for experiments. Simulations are at present used to study virtually all kinds of systems in all scientific disciplines, but examples of systems related to biological physics are proteins, polymers, liquids, gels and colloids.

Roughly, simulations can be divided into particle-based and continuum methods. Hybrid methods exist and have been used successfully in a number of applications. Particle-based simulations follow the motion of discrete constituents, either atoms or interaction beads representing a number of particles. Continuum simulations focus on the dynamics of fields that usually represent a very large number of particles. Particle-based simulations can in turn (broadly) be classified by two categories; molecular dynamics (MD) or Monte Carlo (MC) simulations. These two are different in spirit and nature. MD simulations solve the equations of motion directly and follow the evolution of the positions and velocities of the particles. MC simulations generate random configurations from a specific distribution. Tentatively, one might say that MC simulations are most efficient to sample rare events and to pass high energy barriers, but MD simulations also provide dynamic information of the system.

3.2 The equations of motion

The method of molecular dynamics is started by formulating the differential equations that control how the particles move as functions of time. These are the equations of motion and goes back to Newton; although they can be written down in a number of different ways (34). In Cartesian coordinates, the equations of motion are summarized in Newton’s second law:

\[ m_i \frac{\partial^2 \mathbf{r}_i}{\partial t^2} = \mathbf{F}_i, \quad i = 1, \ldots, N. \]  

(3.1)

For an \(N\)-particle system there are hence three differential equations for each particle \(i\) of mass \(m_i\), located at point \(\mathbf{r}_i = (x_i, y_i, z_i)\) in space. The second law states that the rate of change of the particle velocity (its acceleration) is proportional to the force, \(\mathbf{F}_i\), acting on the particle. The forces describe how the particles interact, ergo how the system behaves. Given that the forces are known, the motion of the system as a function of time, \(\mathbf{r}_i(t)\), is obtained by solving the \(3N\) second-order differential equations constituted by Eq. (3.1), supplemented by \(6N\) initial conditions on the positions, \(\mathbf{r}_i(0)\), and on the velocities, \(\mathbf{v}_i(0) = \dot{\mathbf{r}}_i(0)\) (the dot denotes the time derivative). With a
straightforward generalization, Eq. (3.1) applies equally well to atoms as to the centers
of mass of molecules (65). Equivalently, these \(3N\) second-order differential equations
can be transformed into \(6N\) first-order differential equations for the positions \(r_i\)
and the momenta \(p_i = m_i v_i = m_i \dot{r}_i\). This is Hamilton's formulation. It reduces the order
of the differential equations from second to first for the cost of doubling the number of
degrees of freedom from \(3N\) to \(6N\). The mechanical solution to the \(N\)-body problem
as it follows the equations of motion may thus be thought of as a point tracing out a
path in a \(6N\)-dimensional phase space.

The equations of motion possess some general properties of interest. Based on
symmetry considerations a number of conservation laws can be proved for the equa-
tions of motion (66); that is, there exist a number of functions of the coordinates and
the velocities that stay constant during the motion. The most important of these laws
is the conservation of energy. If the forces are not explicitly time-dependent, the total
energy \(E\) of the system is conserved. The total energy can be monitored to determine
whether the integration of the equations of motion is accurate: if so, the total energy
is constant. Furthermore, the equations are time-reversible, implying that substituting
\(-t\) for \(t\) in Eq. (3.1) leads to unchanged motion of the system, unlike the irreversibil-
ity shown by macroscopic systems (for an in-depth discussion, see McQuarrie (67)).
Note that the actual phase space trajectories calculated from molecular dynamics sim-
ulations are not time reversible, because the limited precision of the computer causes
round-off errors in the calculations to spread with exponential rate (68).

3.3 The force field

Within the framework of classical mechanics (assuming that the forces depend only
on the coordinates and not explicitly on time), the forces can be derived as the gradient
of a potential function, \(F_i = \nabla_{r_i} U (r_1, \ldots, r_N)\), where the subscript emphasizes that the
gradient is to be taken with respect to the position of particle \(i\). Such forces are said
to be conservative, since the work done by moving a particle between two points is
independent of the path taken. Forces in classical physics can only be nonconservative
if degrees of freedoms are neglected; a well-known example is that of friction which
can be treated as conservative motion of the constituent atoms, although this requires
that every atom is tracked instead of treating the macroscopic system by statistical
methods.

Classically, the total potential of a system of \(N\) interacting point particles can be
written as,

\[
U(r_1, \ldots, r_N) = \sum_{i=1}^{N} u_i(r_i) + \sum_{i=1}^{N} \sum_{j>i}^{N} u_2(r_i, r_j) + \sum_{i=1}^{N} \sum_{j>i}^{N} \sum_{k>j>i}^{N} u_3(r_i, r_j, r_k) + \ldots, \tag{3.2}
\]

divided into interactions of single particles, pairs, triplets, and so on. The subscripts
on the sums mean that only distinct pairs, triplets, etc. are to be included. The first
term accounts for an external field that is applied on the system. Examples are the
interactions of the individual particles with the enclosing walls, or with electrical and
gravitational fields. This term is simple as it is not a many-body interaction. The second term represents interactions between particle pairs and contributes the major part to $U$. Assuming that the forces between two particles are equal and opposite (Newton’s third law) and lie along the line connecting the particles, a statement known as the strong law of action and reaction, the pair potential is reduced to a function of the pair separation vector, $r_{ij} = |\mathbf{r}_i - \mathbf{r}_j|$.

The higher order terms in Eq. (3.2) represent three-body, four-body, etc., interactions, resulting from the coarse-graining implicit in the classical approximation to the quantum mechanical interactions. In the density and temperature range of interest for classic liquids, the remaining many-body terms are smaller than the pair potential term, but can account for up to $\sim 10\%$ of the total potential energy, which is difficult to neglect. Evaluating triple sums as the one in Eq. (3.2) is extremely expensive in a computer simulation, and therefore the standard way is to avoid higher order terms by defining an effective pair potential that (partially) includes many-body interactions. This approach has turned out to be fruitful for most purposes, but means that the pair potential must be parametrized to reproduce experimental data, in a way that makes it a function of the state point (i.e., on the temperature, pressure, etc.). The ‘true’ pair potential, $u_2$, is independent of the state. Simulations including three-body interactions have been reported (69, 70) but are uncommon. In the light of these considerations, the total potential is approximately

$$U \approx N \sum_{i=1}^{N} u_1(\mathbf{r}_i) + \sum_{i=1}^{N} \sum_{j>i}^{N} u(r_{ij}),$$

where $u(r_{ij})$ is an effective pair-potential as discussed above.

The functional forms of the different contributions to the pair potential are shown in Fig. 3.1. At very short distances there is a strong repulsive force between two atoms due to that the electron clouds overlap (the Pauli principle). At long distances the atoms attract due to correlations between fluctuating multipoles (London dispersion). This attraction is quantum mechanical in nature and present even for particles without net charge. Perturbation theory shows that the asymptotic attraction between two atoms falls off as $\sim r_{ij}^{-6}$. There is no similar theoretical justification for the repulsive part, but an accurate representation is given by an exponential function with a suitable range parameter. Since the computation of the exponential function is fairly expensive, the usual strategy is to replace it with an inverse power law, $\sim r_{ij}^{-n}$; the most common choice being $n = 12$ as it is the square of $r_{ij}^{-6}$ and therefore cheap to evaluate. In practice this makes little difference. These features are incorporated into the famous Lennard-Jones (LJ) potential (71),

$$u_{\text{LJ}}(r_{ij}) = 4\epsilon_{ij} \left[ \left( \frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left( \frac{\sigma_{ij}}{r_{ij}} \right)^{6} \right],$$

where $\sigma_{ij}$ (the atomic diameter) and $\epsilon_{ij}$ (the interaction strength) are parameters to be fitted. If the particles have static electrostatic charges, as in the case of ions, the LJ
Interactions are insufficient and are to be supplemented with Coulomb charge-charge interactions,

\[ u_C(r_{ij}) = \frac{q_i q_j}{4\pi\varepsilon_0 r_{ij}}, \tag{3.5} \]

where the partial charges \( q_i \) and \( q_j \) are taken to represent the electron distributions of the atoms, and \( \varepsilon_0 \) is the permittivity of free space.

There is nothing in the presented treatment of atomic systems that do not apply to molecular systems as well. Chemical bonds can also be described as pairwise energy (or three-body, four-body, and so forth) terms from interatomic potentials. However, the classic approximations of forces for nearest neighbors break down completely because quantum mechanical effects are dominate. It is therefore assumed that in the molecule, the bond between atoms \( i \) and \( i+1 \), and the angle formed by atom \( i \), \( i+1 \) and \( i+2 \), can be modeled with harmonic potentials,

\[ u_{\text{bond}}(r_{i,i+1}) = \frac{1}{2} k_b^{i,i+1} \left( r_{i,i+1} - b_{i,i+1}^{0} \right)^2 \]
\[ u_{\text{angle}}(\theta_{i,i+1,i+2}) = \frac{1}{2} k_\theta^{i,i+1,i+2} \left( \theta_{i,i+1,i+2} - \theta_{i,i+1,i+2}^{0} \right)^2. \tag{3.6} \]

The force constants, \( k_b^{i,i+1} \) and \( k_\theta^{i,i+1,i+2} \), and the equilibrium distances, \( b_{i,i+1}^{0} \) and \( \theta_{i,i+1,i+2}^{0} \), are fit parameters. The vibration frequencies of these harmonic oscillators are so fast that a quantum-mechanical treatment would probably be most suitable, and therefore bonded atoms are commonly restrained to their equilibrium bond lengths and bond angles. For larger chain-like molecules, such as linear alkanes (or lipids),
torsional motions about the dihedral angle associated with the covalent bond \((i, i + 3)\) interactions give rise to energy changes of the same order as the thermal energies. This energy contribution can not be modeled with a harmonic function due to the steric repulsions that cause certain states to be favorable. A general dihedral potential have several minima that correspond to these states, which are (by usual convention) the \textit{trans} (dihedral angle \(\phi = 180^\circ\)), \textit{gauche} (\(\phi = 60^\circ\) and \(300^\circ\)) and the unusual \textit{cis} (\(\phi = 0^\circ\)) states. In biological molecules these states are not equally favorable, a feature that is captured by the empirical Ryckaert-Bellemans potential \((72)\)

\[
 u_{RB}(\psi_i) = \sum_{n=0}^{5} C_n \cos^n \psi_i, \tag{3.7}
\]

which is comprised of six cosine functions with the fitted coefficients \(C_n\) for the dihedral angle \(\psi = \phi - 180^\circ\) formed between atoms \(i\) to \(i + 3\), with respect to the bond between atoms \(i + 1\) and \(i + 2\).

These are the interactions necessary to describe most biomolecular systems. Note that since the energy contributions between bonded atoms \(i\) up until \(i + 3\) are included in the bond, angle and dihedral potentials, the nonbonded interactions between these atoms must be excluded in order to avoid double counting. Adding all energy terms together, yields the total potential energy

\[
 U = \sum_i u_1(r_i) + \sum_i u_{\text{bond}}(r_{i,i+1}) + \sum_i u_{\text{angle}}(\theta_{i,i+1,i+2}) + \sum_i u_{\text{RB}}(\psi_i) + \sum_{i<j} u_{\text{LJ}}(r_{ij}) + \sum_{i<j} u_C(r_{ij}). \tag{3.8}
\]

The fit parameters are hidden in this expression: the force constants, equilibrium values, interaction parameters \((\sigma_{ij} \text{ and } \epsilon_{ij})\) and partial charges \((q_i)\). These parameters are commonly collected under the label \textit{force field}. They are determined in different ways; directly from experimental data, from quantum mechanical calculations, or from semiempirical calculations on test systems where the parameters are varied to reproduce suitable experimental data \((73)\).

### 3.4 Boundary conditions and long-range forces

Molecular dynamics simulations are always performed on small numbers of particles (even \(N = 10^6\) is small compared to the Avogadro constant \(N_A = 6.02 \times 10^{23} \text{ mol}^{-1}\), the number of entities per mole of a substance). To avoid edge effects on bulk properties, periodic boundary conditions (PBC) are standard in molecular dynamics simulations. It has been found that a few hundred atoms or molecules are in general sufficient to avoid finite size effects related to PBC, so that the properties calculated from the simulation do not change when the number of particles is increased. When using PBC, only the interactions of each particle with its neighbors in the nearest box image are
3.5 Integrating the equations of motion

Up to now, a number of differential equations have been given that together with the force field dictates how the particles move. The simulation itself amounts to solving the equations numerically on a computer. The solution procedure is done iteratively to obtain the positions and velocities of as functions of time, or, in other words, the trajectory of the system in phase space. Many numerical algorithms have been designed to do this, with different advantages and drawbacks. A good molecular dynamics algorithm should fulfill several criteria. First, raw evaluation speed is not a deciding factor because most of the simulation time is spent evaluating forces. It is more preferable with an algorithm that allows a long time step so that the number of force evaluations is minimized. Of high importance is that the algorithm is time reversible and conserves total energy, since this is a general feature of the equations of motion. Most important is that there is no long-time drift in energy as the sampling is otherwise affected. Algorithms that conserve volume in phase space are called symplectic, and can be constructed systematically via the Liouville formulation of classical mechanics. For details of the Liouville approach, see the book by Tuckerman (79).

In practicality and for most purposes of molecular dynamics, the simplest integra-
tors based on Taylor expansions of the atomic positions are sufficient. Originally, Verlet constructed an iteratively updated scheme (61), corresponding to the discretized version of Eq. (3.1) but the algorithm was limited by cancellation errors from subtraction of two similarly sized numbers. This difficulty was circumvented with the use of half time steps, in the leap-frog algorithm proposed by Hockney (80). It is still a very popular algorithm due to its simplicity, and is implemented in some of the major simulation packages (81). Quantities that depend on both positions and velocities at the same time step, like the total energy, are a bit cumbersome to evaluate with the leap-frog algorithm. The discretized version of Eq. (3.1) can instead be recast as

\[ r_i(t + \Delta t) = r_i(t) + v_i(t)\Delta t + \frac{F_i(t)}{2m} \Delta t^2 + O(\Delta t^4) \]  
\[ v_i(t + \Delta t) = v_i(t) + \frac{F_i(t + \Delta t) + F_i(t)}{2m_i} \Delta t + O(\Delta t^2) \]  

yielding the velocity Verlet algorithm (82), with \( \Delta t \) being the time step used to propagate the equations of motion. Note that the order of the error given above is local, the long-time global error accumulated is \( O(\Delta t^2) \) for both positions and velocities (83). As is usually stated, “\( \Delta t \) is to be small in compared to the relevant time scales of the system”. In other words, the time step depends on the force field. For atomistic force fields, \( \Delta t = 1 \text{ fs} \) is common, with constrained bond lengths this might be doubled. For coarse-grained systems order-of-magnitude longer time steps are possible (see Chapter 4 for a further discussion on coarse-graining).

Many other, often highly sophisticated, integrators have been discussed in the literature (for an overview, see e.g., Allen and Tildesley (65), Tuckerman (79), Frenkel and Smit (84)). Worth mentioning are predictor-corrector algorithms and similar integrators that take higher order terms of the Taylor expansions into account, thus giving more accurate representations of Eq. (3.1). Although they can provide better short-time energy conservation, the clear advantage of the Verlet algorithms lie in their ability to minimize energy drift on long times.

### 3.6 Simulations in different ensembles

An ensemble is a conceptual collection of a large number of identical systems. Each member in the ensemble is specified by its microscopic parameters — classically the positions and velocities of the particles — but they all share the same macroscopic parameters. To be a bit more concrete: The standard equations of motion sample the microcanonical ensemble: Systems that are described by different positions and velocities but by the same (fixed) number of particles, \( N \), volume, \( V \), and total energy, \( E \). The microcanonical ensemble is useful to check integration accuracy and energy conservation but is less relevant in comparison to experimental conditions. There, the total energy is not constant, but the absolute temperature, \( T \), can be controlled with a thermostat. Similarly, the volume is difficult to fix in an experiment, but the pressure, \( p \), is easily controlled with a barostat. A number of different ensembles can be constructed
3.6. SIMULATIONS IN DIFFERENT ENSEMBLES

in this manner for various macroscopic parameters. Average values are ensemble independent in the thermodynamic limit \((N \to \infty, V \to \infty \text{ but the ratio } N/V \to \text{ constant})\) but the same is not true for fluctuations. A trivial example is energy fluctuations, which are zero in the microcanonical ensemble \((NVE \text{ constant})\) but proportional to the heat capacity in the canonical ensemble \((NVT \text{ constant})\). A few ensembles are of particular interest to membrane simulations. The first are the standard canonical \((NVT)\) and isothermal-isobaric \((NpT)\) ensembles. Of further interest are simulations in what has simply been called the “constant surface tension ensemble” \((42)\), at constant number of particles, \(N\), pressure in the normal direction, \(p_N\), surface tension, \(\gamma\), and absolute temperature, \(T\). Equal normal and lateral pressures amount to zero surface tension and the \(NpT\) ensemble is regained. For \(\gamma \neq 0\), the interface area per lipid head group is different from its equilibrium value. A membrane that is free to adjust its area will have no surface tension in equilibrium.

Constant temperature simulations can be achieved by different methods. Simplest is to scale the velocities to obtain the desired average kinetic energy \((85)\), but this leads to the so-called isokinetic ensemble rather than a true canonical ensemble. Average values are the same in the two ensembles but the fluctuations are different. Bussi et al. \((86)\) invented an algorithm to scale the velocities to the desired distribution, rather than instant value. This leads to sampling in the true \(NVT\) ensemble. Another approach is based on an extended Lagrangian formulation of the equations of motion, introduced by Nosé \((87)\) and later extended by Hoover \((88)\). There, an extra degree of freedom is supplemented to the equations of motion that mimics a heat reservoir, along with a friction term. The approach can be extended by coupling a chain of heat baths (a Nose-Hoover chain) \((89)\), which can be necessary to obtain ergodic behavior in some systems. A well-known example that requires this treatment is the harmonic oscillator.

Constant pressure simulations must allow for changes in the simulation box size. This can be done analogously to the weak-scaling algorithms used for the temperature, and has also been extended to stochastic rescaling methods by Bussi et al. \((90)\) to give the correct fluctuations. As for the temperature coupling, the box vector velocities can be introduced as additional degrees of freedom with their own equations of motion \((91)\). It is straightforward to generalize the constant pressure simulations to control the surface tension in membrane simulations, it requires only to treat the normal and lateral pressures independently. The Berendsen weak-coupling algorithms (and their stochastic counterparts) are usually most efficient in reaching desired reference values, compared to the second-order Nose-Hoover methods that can experience oscillations when started far from equilibrium. A good strategy in simulations is therefore to use a weak-coupling algorithm for equilibration and a second-order (or stochastic) algorithm for production simulations.
Chapter 4

Simulations and experiments on lipid bilayers

This chapter describes how bilayer properties are measured from molecular dynamics simulations and experiments. Emphasis is on simulations, but the chapter is concluded with a brief overview of the most relevant experimental techniques to membrane biophysics. Starting with lipid bilayer force fields and the concept of coarse-graining, it is shown how static and dynamic averages are calculated from simulations. A presentation is given on how the most important lipid bilayer properties are calculated. The chapter covers both static and dynamic properties and includes a description of the estimation of statistical errors from simulation. The survey of the experimental methods is given without details and is meant to convey to the reader how practical measurements are made on lipid bilayers in the laboratory. The chapter is summarized with an inspection of the length- and time scales involved with the simulations and the experiments, and to what extent they overlap. The major difficulties involved in comparison between simulations and experiments are discussed.

4.1 Coarse-graining

With the recipe of particle interactions described so far models can be built up in atomistic detail. Such models are very accurate in the sense that they include all microscopic interactions but for the price that their simulation is extremely time-consuming. All-atom simulations are restricted to small numbers of particles ($10^5$) and short times (100 ns). If the physics of interest takes place on length- and/or time scales large compared to the atomistic, it makes sense to remove the atomistic degrees of freedom from the calculations. Individual atomic interactions can hardly matter on large scales but only the result from the cooperative motions of a large number of atoms, which can then be described as "effective" interactions. Examples of biologically relevant problems that fit this description are vesicle fusion (17, 92), interactions with macromolecular structures like polymers (93) and lipid raft formation (94).
Figure 4.1: A coarse-grained (CG) lipid molecule in order of detail from left to right. The most detailed force field includes all atoms (AA), here CHARMM (95). The explicit hydrogens can be included into a hydrocarbon bead, in a united-atom (UA) description as given by e.g., the Berger force field (96). Simplifying further, each hydrocarbon group can be replaced with a single bead (the Martini force field (97, 98)). This represents a 4-to-1 mapping of the UA model. Proceeding further, there are a number of generic single-tail lipid models; that of Cooke et al. (99, 100) is shown.

Molecular dynamics simulations can be coarse-grained systematically by merging particles into progressively coarser interaction beads (Fig. 4.1). This must be done in a way that preserves the underlying physics. Coarse-grained interactions represent a smoother energy landscape with significantly faster dynamics than that of atomistic resolution. Therefore, the effective interactions must be properly scaled to account for the lost entropy. Even the (explicit) water molecules surrounding the bilayer may be removed and replaced with interactions between tail beads that are designed to mimic the hydrophobic effect (implicit water).

Coarse-graining reduce the number of particles in the simulation and allow a longer time step in the integration. Standard Lennard-Jones (LJ) potentials seem to be insufficient to describe self-assembling lipid bilayers using implicit water. The LJ potential is too short-ranged to account for the polar water interactions, and it is necessary to use a broader, attractive potential between the tail groups. The shape of the tail potential must be determined empirically. Brannigan et al. (101) reported a model with attracting tail beads according to a truncated $r^{-2}$ potential, while Cooke et al. (99, 100) used a broadened LJ- or tuned cosine-potential to achieve self-assembly. These simple models are able to accurately reproduce a remarkable number of lipid bilayer properties, from structure to phase behavior. The dynamic behavior of the generic models should be interpreted with care. Apparently, lipid bilayers can be described by a chain of different force fields, with ranging levels of detail. Coarse-grained simulations are capable of reaching as far as the micrometer length scale, and microsecond or longer times.
4.2 Averaging procedures

Measured properties are averages. How should this averaging be done? Classical mechanics gives the motion as a trajectory, \( \{ \mathbf{r}_i(t), \mathbf{p}_i(t) \}_{i=1}^{N} \), which can be thought of as a single point tracing out a path in a 6\( N \)-dimensional phase space with volume element \( \mathrm{d}\Gamma = \mathrm{d}^3\mathbf{r}_1 \mathrm{d}^3\mathbf{p}_1 \ldots \mathrm{d}^3\mathbf{r}_N \mathrm{d}^3\mathbf{p}_N \). A variable, \( a(t) = a(\mathbf{r}^N(t), \mathbf{p}^N(t)) = a(\{ \mathbf{r}_i(t), \mathbf{p}_i(t) \}_{i=1}^{N}) \), that is a function of the coordinates and momenta of all (or some of) the constituting particles, depends implicitly on time as the positions change during the motion. According to statistical mechanics the average of \( a \) can be calculated in two ways (102):

\[
\langle a \rangle_T = \frac{1}{T} \int_0^T \mathrm{d}t \, a(t) \quad \text{or} \quad \langle a \rangle_e = \int \mathrm{d}\Gamma \, f_0(\Gamma) a(\Gamma),
\]

where \( T \) is a long but finite time and \( f_0(\Gamma) \) is the distribution of states in phase space. The \( T \) and \( e \) subscripts denote time averaging and ensemble averaging, respectively. In the first case, the average is taken during the time interval \( T \) over a particular phase space trajectory, in the last case the averaging is done with respect to a large number of different systems, all tracing out different paths in the same phase space. The ensemble average is therefore calculated over phase space. The equilibrium distribution function, \( f_0(\Gamma) \), is proportional to the familiar Boltzmann factor. The point is that stated by the ergodic hypothesis: If the system visits the entire phase space during the time \( T \), these average are equal:

\[
\langle a \rangle \equiv \lim_{T \rightarrow \infty} \langle a \rangle_T = \langle a \rangle_e.
\]

For a statistical mechanics calculation, it will be easiest to perform ensemble averaging. From a molecular dynamics simulation, time averages are straightforward to calculate: the trajectory is the result of the MD simulation. An average calculated according to Eq. (4.1) is called a static property.

It is also possible to calculate the average value of a function at different times. Such an ensemble average takes the form (67)

\[
C_a(t = t' - t'') = \langle a(t') a(t'') \rangle_e = \int \mathrm{d}\Gamma f_0(\Gamma) a(t') a(t''),
\]

and is called the (auto)correlation function of \( a \). The correlation function only depends on the time difference \( t \), which is a consequence of Liouville’s theorem (103). The correlation function is thus said to be stationary and by convention, \( t'' = 0 \). (For two different functions \( a \) and \( b \), Eq. (4.3) is called the cross correlation, but the formulas go through unchanged.) The foregoing discussion can clearly be generalized, writing

\[
C_a(t) = \langle a(t) a(0) \rangle = \langle a(t + t_0) a(t_0) \rangle_{t_0} = \lim_{t_0 \rightarrow \infty} \frac{1}{T} \int_0^T \mathrm{d}t_0 a(t_0 + t) a(t_0),
\]

an average over different starting times. The autocorrelation function hence describes how \( a \) responds to being disturbed out of equilibrium, but in terms of the equilibrium
4.3 Lipid bilayer properties from simulations

Fig. 4.2 shows a snapshot from a MD simulation. The repeat distance, \( L_z \), needs to be large enough to prevent the bilayer from interacting with its periodic copies, preferably by simulating a cubic box. A bilayer patch containing \( \sim 1000 \) lipids corresponds to a lateral length of \( 17 \) nm, which is enough for large-wavelength undulations to develop.

**Area per lipid**

The ability to accurately reproduce the area per lipid is often taken as a force field hallmark. The area is highly sensitive to force field details and simulation conditions. Simulations performed at constant area (\( N_A T \) ensemble) — other than the equilibrium area \( A_0 \) — spawn surface tensions that suppress large-wavelength undulations. Contrariwise, constant pressure simulations relax the area to its equilibrium value, which is appropriate for comparison of \( A_0 \) to experimental values. The projected area per lipid is easily calculated from simulations of small membrane patches as \( A_0 = L_x L_y / N \) with \( N \) being the number of lipids per monolayer. Chapter 5 shows that undulations
make the true membrane area larger than the projected area. The true area diverges logarithmically with system size (10^4) which is a small correction even for large bilayer systems.

**Electron density profiles**

The electron density profile (EDP) is measured along the bilayer normal. Its Fourier transform is called the *form factor* and is proportional to the scattering intensity measured in experiments. EDP calculated for different atom types shows how they are distributed with respect to the bilayer center. EDP are calculated from simulations by collecting histograms of the atomic positions in the transverse (z) direction (Fig. 4.3). The histograms are normalized with the number of electrons present in each atom type. The histogram procedure is only straightforward for small systems because large-scale membrane undulations blur the EDP as the bilayer mid-plane becomes position-dependent. The transverse distances must then be defined from the undulating surface. Methods developed to overcome this problem are presented in Chapter 5 and in Paper II.

**Static correlation functions and structure factors**

Static correlation functions, $F_d(r)$, and structure factors (or fluctuation spectra), $S_d(q)$, form Fourier pairs:

$$F_d(r = |\mathbf{r}' - \mathbf{r}''|) = \langle a(\mathbf{r}') a(\mathbf{r}'') \rangle \quad \text{and} \quad S_d(q) = \langle |a(q)|^2 \rangle. \quad (4.6)$$
For isotropic and translation invariant systems like fluid lipid membranes, these functions only depend on the magnitude of the difference vectors, \( r \) and \( q \). \( F_\rho(r) \) and \( S_\rho(q) \) can be calculated from simulations only in a restricted range. In real space, the largest correlation distance is half the (smallest) box vector. Periodic boundary conditions (PBC) restrict the wave vectors to be discrete numbers. The wave vectors that are commensurate with the PBC are

\[
q = (q_x, q_y, q_z) = 2\pi \left( \frac{n}{L_x}, \frac{m}{L_y}, \frac{l}{L_z} \right),
\]

where \( n, m, l = 0, 1, 2, \ldots \) are integers and \( L_\alpha \) is the box size in the \( \alpha = x, y, z \)-direction. The small-\( q \) limit is thus \( q_{\text{min}} = \frac{2\pi}{L_{\text{max}}} \), where \( L_{\text{max}} \) is the largest box vector. (The zero mode, \( n = m = l = 0 \), corresponds to the system average and is of less interest.) This restriction is quite severe, for typical simulations \( L_{\text{max}} = 20 \text{ nm} \), which corresponds to \( q_{\text{min}} = \frac{2\pi}{20} = 0.31 \text{ nm}^{-1} \). Fluid bilayers are in-plane isotropic and cylindrical coordinates can be used in Fourier space, \( \mathbf{q} = (q_x, \sqrt{q_y^2 + q_z^2}) \). Correlation functions that are projected onto the bilayer mid-surface \( (q_z = 0) \) reduce to one-variable functions.

The most prominent correlation function is that of the (microscopic) density,

\[
\rho(r) = \sum_{i=1}^{N} \delta(r - r_i),
\]

which is defined by a sum of \( \delta \)-functions that mark out the atomic positions, \( r_i \). The density correlation function, or pair correlation function, \( g(r) \equiv F_\rho(r) \), states the probability that two particles are separated by a distance \( r = |r' - r''| \),

\[
g(r) = \{ \rho(r')\rho(r'') \}. \tag{4.9}
\]

\( \delta \)-functions are not easily implemented on a computer, but there is no trouble in evaluating Eq. (4.9) by keeping a histogram of pair distances. The calculation is done by keeping every particle in turn as a reference particle, counting the number of particles around it in concentric slices (shells in three dimensions) of thickness \( \Delta \). In the end, slice \( n \), corresponding to distance \( R_n = n\Delta \), is normalized with its area, \( 2\pi R_n \Delta \) (or volume \( 4\pi R_n^2 \Delta \), in three dimensions), accounting for the increasing number of pair separations for growing \( r \). The static structure factor for the density, the other half of the Fourier pair, is

\[
S_\rho(q) = \sum_{i=1}^{N} e^{-iq\cdot\mathbf{r}_i}, \tag{4.10}
\]

the \( \delta \)-functions translating to complex exponentials. Fig. 4.4 shows the in-plane pair correlation functions calculated for a DMPC bilayer, and the structure factors obtained by Fourier transformation of \( g(r) \). The structure depends on the atoms included in the sums in Eq. (4.8) and Eq. (4.10). In Chapter 5, expressions corresponding to Eq. (4.9) and Eq. (4.10), but for the undulating membrane surface will be discussed in detail.
4.3. LIPID BILAYER PROPERTIES FROM SIMULATIONS

Figure 4.4: (Left) In-plane pair correlation function, $g(r)$, of a united-atom DMPC bilayer, calculated for the head group phosphorus (P), the first oxygen of the glycerol backbone (OD), the terminal methyl groups (CH$_3$) and the center-of-mass of the entire DMPC lipid. (Right) The static structure factor, $S_q(q)$, calculated by Fourier transformation of $g(r)$ for the same atoms. The spurious oscillations at small $q$ are due to the truncation of the Fourier series.

**Time correlation functions**

Time correlation functions may be calculated as

$$C_a(t) = \langle a(t)a(0) \rangle = \frac{1}{T_{\text{max}}} \sum_{t_0=1}^{T_{\text{max}}} a(t_0)a(t_0 + t),$$

(4.11)

where $t$ is the time step and $T_{\text{max}}$ is the largest time separation to be considered, a number that is smaller than the total number of time steps since the statistics for long times is poor. Evaluating Eq. (4.11) directly requires $O(T_{\text{max}}^2)$ operations, but if the sum is instead evaluated using a Fast Fourier Transform (FFT) (105), only $O(T \log T)$ operations are involved. The Fourier approach calculates $C_a(t)$ with the following sequence of steps.

1. Calculate the Fourier coefficients, $a(t) \rightarrow a(\omega)$, using FFT.
2. Calculate the square modulus, $C_a(\omega) = |a(\omega)|^2$.
3. Invert the transform to get the unnormalized correlation function $\hat{C}_a(t)$.
4. Normalize $\hat{C}_a(t)$ with the factor $(T - t)^{-1}$ to obtain $C_a(t)$.

Time correlation functions of lipid bilayers are discussed in Paper III, Paper IV as well as in Chapter 6. Of particular interest are the Fourier representations of the density, $a(t) = \rho_a(t)$ (shown in Fig. 4.5), and of the undulations, $a(t) = u_a(t)$. These functions are not independent. On small length scales the density fluctuations will dominate but on progressively larger scales undulations force the density to relax along the fluctuating surface, which will be a slower process than for a flat interface.
error in the time correlation function itself can be expressed with a similar formula.

\[
\sigma_C^2 \approx \frac{1}{T} \sum_{t=1}^{T} (a(t) - \langle a \rangle)^2,
\]

where \( t \) denotes the time step of in total \( T \) steps and \( \langle a \rangle \) is the simulation average. If adjacent time steps are completely uncorrelated, the variance of the simulation data would be \( \sigma_{a,\text{MD}}^2 = \sigma_C^2 / T \). However, MD simulations generate a correlated time-series. A modified formula for the variance from a MD simulation is therefore \( \sigma_{a,\text{MD}}^2 = (1 + t_c) \sigma_C^2 / T \), accounting for how long time, \( t_c \), the variable \( a \) stays correlated. The correlation time is the integral of \( C_a(t) \) (65),

\[
t_c = 2 \int_0^\infty \text{d}t C_a(t),
\]

where the factor 2 comes from symmetry. Noting the relation \( \sigma_a^2 = C_a(0) \), the statistical error in the time correlation function itself can be expressed with a similar formula.

Figure 4.5: Time correlation function calculated from a molecular dynamics simulation of a lipid bilayer. The normalized density autocorrelation function, \( F(q,t)/F(q,0) \) is shown, at \( q = 0.8 \text{ nm}^{-1} \). The correlation functions starts at unity (full correlation) and decays to zero (no correlation). The shaded gray area shows the statistical error in the calculation of the correlation function.

**Estimating errors**

Molecular dynamics simulations produce a statistical description of the system. It is therefore important to estimate the errors related to the statistical fluctuations. In general, it is not known a priori how a given quantity fluctuates. However, properties that are calculated as sums of independently fluctuating parts (e.g., the density), follow Gaussian statistics to an excellent approximation, by the central limit theorem (40). A number of properties of Gaussian distributions can then be applied in the error estimation.

Statistical errors are estimated from the variance of the variable calculated in the simulation,

\[
\sigma_a^2 = \frac{1}{T} \sum_{i=1}^{T} (a(t) - \langle a \rangle)^2,
\]

where \( t \) denotes the time step of in total \( T \) steps and \( \langle a \rangle \) is the simulation average. If adjacent time steps are completely uncorrelated, the variance of the simulation data would be \( \sigma_{a,\text{MD}}^2 = \sigma_C^2 / T \). However, MD simulations generate a correlated time-series. A modified formula for the variance from a MD simulation is therefore \( \sigma_{a,\text{MD}}^2 = (1 + t_c) \sigma_C^2 / T \), accounting for how long time, \( t_c \), the variable \( a \) stays correlated. The correlation time is the integral of \( C_a(t) \) (65),

\[
t_c = 2 \int_0^\infty \text{d}t C_a(t),
\]

where the factor 2 comes from symmetry. Noting the relation \( \sigma_a^2 = C_a(0) \), the statistical error in the time correlation function itself can be expressed with a similar formula.
With the estimated time correlation function written on the form \( C_a(t) + \Delta C_a(t) \), a careful analysis shows that (106)

\[
\Delta C_a(t) = C_a(0) \sqrt{\frac{2\tau}{T}} \left( 1 - \frac{C_a(t)}{C_a(0)} \right),
\]

where the slightly different correlation time

\[
\tau = 2 \int_0^\infty dt \left[ \frac{C_a(t)}{C_a(0)} \right]^2,
\]

has been defined. The error starts out at zero for \( t = 0 \), and increases to its maximum value \( C_a(0) \sqrt{2\tau/T} \) as the time correlation function has decayed to zero. The factor \( \sqrt{\tau/T} \) makes it difficult to reduce statistical errors below 1%. In some cases it is possible to perform additional averaging to reduce the statistical error. The most prominent example of this situation is for the velocity autocorrelation function where it is possible to average not only over time differences but also over all particles in the system.

### 4.4 Experimental techniques

Experiments are preferably performed on systems in equilibrium (static properties), or near equilibrium (in the domain of linear response theory, such properties will here be referred to as dynamic), to facilitate comparison to theories. As simulations are performed on microscopic systems, they need to be validated by calculating macroscopic properties, in the fashion presented above, and compare them to the same values obtained from experiments. Often such comparison is nontrivial because the simulations and experiments reside in completely different length- and time scales. In addition, it is in many cases impossible to keep equivalent setups in experiments and simulations. The following sections list the most relevant experimental techniques in assessing lipid bilayer properties, and problems encountered when comparing simulation and experimental data.

**Nuclear Magnetic Resonance (NMR)**

NMR include a wide array of techniques. The common ingredient is spins of the atomic nuclei interacting with an applied magnetic field. Nuclei with zero spin are unaffected. Selective deuteration of atoms can be used to produce a signal as fit, in the case of lipid bilayers NMR experiments can be performed with deuterated tail groups. This spawns a splitting in the quadrupole contribution to the energy spectrum, which is proportional to the order parameter

\[
S_{CD}^i = \frac{1}{2} \left( 3 \cos^2 \theta_{CD}^i - 1 \right),
\]

measuring the ordering of lipid tail group \( i \). Here, \( \theta_{CD}^i \) is the angle between the C–D bond vector of the \( i \):th tail group and the bilayer normal (107). The angular brackets
denote an ensemble average. Dynamical properties of lipid bilayers can also be obtained with NMR by measuring the spin-relaxation rate (108–110). This information reflects the reorientation dynamics of the lipid chains.

**Elastic neutron and x-ray scattering**

Experimental methods collected under the label of ‘scattering experiments’ means no more than an experiment that bombards a sample with energetic particles and collects those that are scattered off the sample. If the scattered particles have lost/not lost energy in the process, the scattering is said to be inelastic/elastic. On lipid membranes (and other soft matter systems) neutrons or x rays are used as incoming particles since they penetrate into the bulk of the material (in contrast to electrons). Elastic scattering off a crystal is seen as a number of well-defined δ-spikes (Bragg peaks) which define the positions of the nuclei in the unit cell. Fluid lipid bilayers have far less structure than conventional crystals. This is an inherent property of lipid membranes being two-dimensional liquids and is not a limitation of the experimental techniques themselves. The molecules in the bilayer undergo strong fluctuations, which is seen as broadened Bragg peaks in the scattering spectrum. Depending on the geometry of the incoming beam and the sample, one can probe scattering along the bilayer normal (23) or in the plane of the bilayer (111). In the former case, the scattering intensity is the Fourier transform of the electron density along the bilayer normal, and in the latter case the in-plane structure factor is being probed. This information reveals the molecular structure of bilayers. In addition, the broadening of the Bragg peaks also provide the bilayer bending modulus and stack coupling constant (112).

**Inelastic scattering experiments**

In scattering processes where the incoming particle loses (or gains) energy, the formula $E = h\omega$ relates the energy difference, $E$, to the frequency shift, $\omega$, of the scattered particle. The proportionality constant, $h$, is the reduced Planck constant. Scanning a range in $\omega$ gives a frequency spectrum that describes the damping of density fluctuations within the sample. Depending on the properties of the scattered particle, density fluctuations on different scales are probed. Light has large wavelength and probe fluctuations of thousands of cooperatively moving particles (hydrodynamic fluctuations). Neutrons have small wavelengths and probe single (or a few) atoms fluctuation together. An inelastic scattering technique of particular importance to the study of lipid bilayers, is neutron spin-echo (NSE) (113) which is ideally suited to study fluctuations on distances 1–1000 nm and times 1–1000 ns. These are precisely the scales on which many biologically relevant fluctuations take place. In contrast to other inelastic scattering methods, which are performed in the frequency domain, NSE probe density fluctuations directly in the time domain. The energy resolution is extremely high, and the technique shares some features with dynamic NMR in that it monitors the relaxation of spins back to equilibrium. Relaxation in NMR is due the local fields of the
4.5 Comparing simulation data to experiments

It is often far from trivial to directly compare simulation and experimental data. The most common problem is related to differences in system size; most experiments are done on macroscopic systems but simulations are performed on microscopic systems. Fig. 4.6 shows the different length- and time scales probed by simulations and various experimental techniques. However, not only are lipid bilayers too thin to observe in an ordinary light microscope; the signal-to-noise ratio in scattering experiments is too low to be sufficient. Instead, oriented samples are stacked to strengthen the signal. The stacking introduces bilayer-bilayer interactions not present in simulations of bilayer patches with periodic boundary conditions. Only a sufficiently large repeat spacing in the experiments can minimize this difference. Another common experimental setup is vesicles with varying radii, either unilamellar (a shell consisting of a single bilayer) or multilamellar (shells consisting of many bilayers, like an onion). This also differs from the lamellar geometry of the simulations considered in the present work. The effects on bilayer structure and dynamics are not obvious. As long as the vesicle radius is large compared to the length scale of interest, the vesicle can be considered to be locally flat, and should be amenable for comparison to simulation results.

Simulation and experimental data have limited resolution in both length and time. Experiments are limited by the instrument used for the measurement, the simulations by the box size and the time step. Power spectra calculated from experiments are thus convoluted with a window function that is determined by the instrument resolution,
and thereby broadened compared to the ‘true’ spectrum. In some cases, e.g., NSE experiments, the window function is known and of simple form, and can simply be divided out. Under other circumstances deconvolution is not possible and the resolution function is an inherent limitation to the measurement.
Chapter 5

Membrane undulations

An ambitious reader that looks up the word “undulation” in the Merriam-Webster Dictionary finds: “a wave-like motion to and fro in a fluid or elastic medium propagated continuously among its particles but with little or no permanent translation of the particles in the direction of the propagation”. The particular property of the lipid bilayer that separates it from most other elastic materials is that such wave-like motion can be induced from random thermal collisions with the surrounding solvent molecules. The lipid bilayer is soft, with an energy cost of bending that is larger than, but comparable to, the thermal energy, $k_B T$. This chapter describes how to quantify undulations in lipid bilayer simulations, and their influence on local structure. First, methods to construct the membrane surface from the particle positions are presented. By measuring transverse distances with respect to the undulation surface, accurate EDP and areas can extracted from simulations of large membranes. The calculation of the fluctuation spectrum by direct Fourier analysis is presented in detail, in relation to the correlation function formalism introduced in Chapter 4. It is shown how molecular fluctuations coalesce with the wave-like fluctuations of the entire sheet in the mesoscopic regime. Limiting values of the spectra are presented, along with a tentative discussion on how the continuum and molecular descriptions can be reconciled.

5.1 Tracking the membrane surface

To assess the nature of undulations in simulations, a method is needed that continuously follows the membrane surface. The description starts from the lipid bilayer as two mathematical surfaces, $z_1(r)$ and $z_2(r)$, chosen so that $z_1(r) > z_2(r)$. They are uncoupled but prevented from overlapping due to steric repulsion. In the following, in-plane coordinates are denoted by $r = (x, y)$. The undulating surface is defined by

$$u(r) = \frac{1}{2} (z_1(r) + z_2(r)),$$  \hspace{1cm} (5.1)
and the thickness is defined by
\[ h(r) = \frac{1}{2} (z_1(r) - z_2(r)) \] (5.2)
The factor 1/2 is included to make the expressions symmetric. These functions are given for all values of \( r \). However, the bilayer consists of molecules, which are a discrete set of point particles. The task is therefore to reconstruct the optimal monolayer surfaces from the actual lipid positions. The optimal \( u(r) \) is called the Undulation Reference Surface (URS).

The real-space method

The most intuitive way to obtain a surface is to construct a uniform Cartesian grid and assign each lipid to its nearest grid cell. The advantages are simplicity and speed, the drawback is the “missing-data problem” (114): Too fine grids lead to empty cells. If there are few empty cells it is possible to average over neighbors. The minimum grid spacing becomes \( \sim 1.5 \) nm, or twice the lipid-lipid distance. To capture small-wavelength membrane fluctuations that reside in that regime, interpolating the surface between existing points has been used in early membrane simulations (43–45). It is still a fairly common procedure to reconstruct the undulation surface (115). There are an infinite number of surfaces that are compatible with the demand of passing through the available data points, so further prerequisites on the surface are needed. A good condition is to choose a surface that minimizes the curvature. A particularly appealing method was first developed in the earth sciences as a way to establish topographical contours from noisy satellite data (116).

The notion is to find a function with minimal curvature that passes through the data points. It can be shown (117) that a function minimizes the total squared curvature,
\[ C[u] = \int dS |\nabla^2 u|^2, \] (5.3)
of the surface \( S \) if and only if it satisfies the biharmonic equation,
\[ \nabla^4 u(r) = \sum_{j=1}^{N} \alpha_j \delta(r - r_j) \] (5.4)
\[ u(r_j) = u_j. \] (5.5)
The second line fixes the surface to pass through the data points, \( u_j \). The biharmonic equation, Eq. (5.4), is a linear partial differential equation and is solved (homogeneously) by the biharmonic Green function in two dimensions,
\[ \phi(r) = |r|^2 (\ln |r| - 1) . \] (5.6)
The general solution to Eq. (5.4) is a superposition of solutions to the homogeneous biharmonic equation,

\[ u(r) = \sum_{j=1}^{N} \alpha_j \phi(r - r_j), \quad (5.7) \]

where the coefficients, \( \alpha_j \), are determined by solving the linear equation system,

\[ u_i = \sum_{j=1}^{N} \alpha_j \phi(r_i - r_j), \quad (5.8) \]

fulfilling Eq. (5.5). The surface is obtained at arbitrary \( r \), but there is no new information, only interpolation between adjacent data points. Choosing \( u(r) \) to lie on a Cartesian grid facilitates the use of a FFT algorithm to calculate the Fourier spectrum.

The minimal curvature-method suffers from oscillation problems in the same way as cubic interpolation methods. This can be eliminated using a smaller number of fit coefficients than data points when solving Eq. (5.8). The linear system becomes overdetermined and is solved as a least-squares problem. The obtained surface is then a smooth ‘best fit’-function to the noisy data. The singularity present at the origin of Eq. (5.6) is problematic for strongly clustered data but not in membrane simulations. The largest drawback of this method is the computational load, which scales as \( N^3 \) to solve the linear equation system in Eq. (5.8). This becomes very slow when \( N \) is \( 10^3 \text{–} 10^4 \), especially since the procedure is to be repeated for many time frames.

**The direct Fourier method**

Because of the periodic boundary conditions (PBC), the surface can be expanded in a discrete Fourier series,

\[ u_q = u(n, m) = \frac{1}{N} \sum_{k=1}^{N} z_k e^{-i q_m \cdot r_k}, \quad (5.9) \]

with \( N \) being the number of points on the surface, positioned at \( R_k = (r_x, r_z) = (x_k, y_k, z_k) \) and \( q_m = 2\pi(n/L_x, m/L_y) \) is the usual two-dimensional wave vector. The surface is regained by an inverse Fourier transform. As for the real-space method the surface is obtained at arbitrary point by interpolation, which here amounts to approximating the Fourier integral with a sum (Eq. (5.9)). To obtain a smooth surface, the large-\( q \) modes must be filtered out of the sum. This is easily seen since the Fourier transform of Eq. (5.9) can be done analytically,

\[ u(r) = a_0 \sum_{k=1}^{N} z_k \delta(r - r_k), \quad (5.10) \]

as a sum of \( \delta \)-functions located at the data points. \( a_0 \) is the area per lipid, to get the correct surface dimension. Note the similarity to the microscopic density, Eq. (4.8).
Filtering the large-$q$ components makes the surface increasingly smooth, until a flat surface is obtained when all the modes are filtered. Applying the filter function $G(q/q_0)$ on the Fourier coefficients produce the filtered surface coefficients $\tilde{u}(q) = u(q)G(q/q_0)$. The parameter $q_0$ determines the transition wavenumber where the filtering starts. In Paper II it is shown that the simplest possible filter, the ideal (ID) filter $G_{ID} = \Theta(q - q_0)$, can be employed in membrane simulations ($\Theta(x)$ is the Heaviside step function). This works because the amplitudes of the large-$q$ modes are small. If not, spurious data oscillations (called ringing in signal processing) appears due to the truncation of the Fourier series. The Supplementary Material of Paper II compares several different filters and parameters.

Once the Fourier coefficients have been filtered a FFT transform can be used to regain the undulation surface. On a Cartesian grid of $N$-by-$M$ points distributed according to $x_k = \Delta k$ and $y_l = \Delta l$ ($k$ and $l$ are integers and $\Delta$ is the grid size), the transform is a discrete double sum,

$$ u(x_{k}, y_{l}) = \frac{1}{NM} \sum_{n=0}^{N-1} \sum_{m=0}^{M-1} \tilde{u}(q_{nm}) e^{-i2\pi(\frac{kn}{N} + \frac{lm}{M})}. $$

The tilde shows that the URS is obtained from the filtered coefficients. The raw speed of FFT will outperform real-space interpolation methods with orders of magnitudes. There is also a significant speed-up with the direct Fourier method because it is only the smallest (below $q_0$) Fourier modes that are needed to reconstruct the URS.

### 5.2 Electron density profiles and areas from undulating membranes

Fig. 5.1 shows the URS. The idea is to measure all transverse distances from the bilayer center with respect to the URS. This consideration is crucial in the calculation of
transmembrane profiles. Important examples are electron density profiles (EDP) and their concomitant form factors (described in Chapter 4), as well as the pressure profile (118). If the URS is ignored, the surface undulations submerge the structure in the profiles. The artifact is so severe that profiles calculated from mesoscopic membrane patches are useless. All structure is smeared out (See Fig. 1 in Paper II). The URS has been calculated and applied to calculations of EDP (and form factors) and areas for DMPC bilayers, and the results are presented in Paper II. It is shown that the artifact can be almost completely removed.

The URS can further be improved if local bilayer orientation is taken into account. This corresponds to calculating the distance from the atom to the red line rather than the black line in the magnified part (to the right) in Fig. 5.1. This geometric effect also needs to be taken into account in the interpretation of experimental data (23). The orientation correction is calculated from the angle between the bilayer normal and the z-axis,

\[ \theta_k = \arccos n_z = \arccos \left( \frac{1}{\sqrt{1 + (\nabla u)^2}} \right) \],

(5.12)

where the gradient can be calculated from the Fourier coefficients,

\[ \nabla u = i \sum_q (q_x, q_y) u(q) e^{-iq \cdot r}. \]

(5.13)

Analytically, the square root can be expanded for small gradients. In Paper II it is shown that it is sufficient to account for the orientation by scaling with the average angle,

\[ \left\langle \frac{1}{\cos \theta} \right\rangle \approx 1 + \frac{1}{2} \left\langle \theta^2 \right\rangle \approx 1 + \frac{k_B T}{4\pi k_c} \ln \left( \frac{L}{a} \right). \]

(5.14)

The calculation of the average in the last line follows directly from the Helfrich Hamiltonian, with \( a \) being a microscopic cutoff that corresponds to the largest Fourier mode in the system. \( a \) is roughly equal to the separation between molecules, and \( L \) is the linear system size. The continuum expression in Eq. (5.14) and direct calculation of the angle distribution from molecular dynamics agree to four significant digits. The corresponding root mean square angle is \( \sqrt{\left\langle \theta^2 \right\rangle} \approx 8.5^\circ \).

The undulation correction also enters when calculating areas. The projected area in the simulation is \( A_p = L_x L_y / N \) where \( N \) is the number of lipids per monolayer. The true area of an undulating membrane follows its surface and is larger than the projected area,

\[ A_{\text{true}} = \int_S dS = \int_{A_p} \sqrt{1 + (\nabla u)^2} \, dx \, dy. \]

(5.15)

Expanding the square root, the (average) true area becomes

\[ A_{\text{true}} = A_p + \frac{1}{2} \int \int (\nabla u)^2 \, dx \, dy = A_p \left( 1 + \frac{1}{2} \sum_q q^2 \left\langle |u(q)|^2 \right\rangle \right), \]

(5.16)
after the use of Parseval’s theorem. The second term in (5.16) can be calculated directly from the Fourier coefficients of the URS. The fluctuation spectrum of the Helfrich model predicts

$$A_{\text{true}} = A_0 \left( 1 + \frac{k_B T}{8\pi k_c} \ln N \right).$$

(5.17)

The results from simulations and the Helfrich model are in excellent agreement. The logarithmic growth of the correction term with system size is mildly divergent and small even for macroscopically sized systems. For a 1000-lipid system the second term in Eq. (5.17) is $\sim 1\%$.

### 5.3 Fluctuation spectra from the direct Fourier method

Eq. (5.9) makes it possible to calculate the undulation fluctuation spectrum,

$$S_u(q) = N \left( |u(q)|^2 \right),$$

(5.18)

without resorting to grid discretization. Since this calculation rests fully on Fourier analysis, it has been called the direct Fourier method. Some interesting results emerge from this method that are in contrast to the results that are obtained from grid methods. A more in-depth presentation of the theory involved is given in Paper I and its Supplementary Material. Briefly, each monolayer surface, $z(r)$, is described by the Fourier pair of Eq. (5.9) and Eq. (5.10), repeated here for convenience:

$$z(r) = a_0 \sum_{l=1}^{N} z_l \delta(r - r_l) \quad \text{and} \quad z(q) = \frac{1}{N} \sum_{l=1}^{N} z_l e^{-i q \cdot r_l}.$$

The two-dimensional fluctuation spectrum is constructed directly from simulations according to Eq. (5.9). Averaging over all time frames then yields $\langle |u(q_x, q_y)|^2 \rangle$, which can be reduced to a one-dimensional spectrum by additional averaging over circles of radii $q_r = \sqrt{q_x^2 + q_y^2}$ in Fourier space, with a typical bin width of 0.5 nm$^{-1}$.

Analytically, it can be shown (Supplementary Material of Paper I) that two surfaces, as in Eq. (5.1) and Eq. (5.2), each defined according to Eq. (5.9) lead to fluctuation spectra for the undulations and the thickness of the form

$$S_u(q) = \frac{1}{2N} \left( \langle u_0^2 \rangle + \langle h_0^2 \rangle \right) + \frac{\pi \langle h_0 \rangle^2}{a_0 N} \int_0^\infty dr J_0(qr) F_u(r),$$

(5.19)

$$S_h(q) = \frac{1}{2N} \left( \langle u_0^2 \rangle + \langle h_0^2 \rangle \right) + \frac{\pi \langle h_0 \rangle^2}{a_0 N} \int_0^\infty dr J_0(qr) F_h(r),$$

(5.20)

of the correlation functions

$$F_u(r) = F_{\text{mono}}(r) - F_{\text{bi}}(r)$$

(5.21)

$$F_h(r) = F_{\text{mono}}(r) + F_{\text{bi}}(r).$$

(5.22)
5.3. FLUCTUATION SPECTRA FROM THE DIRECT FOURIER METHOD

Figure 5.2: (Left) Fluctuation spectra calculated from simulations of UA and CG models. The small-q end can be fitted to the $q^{-4}$-line predicted by the Helfrich model. The large-q end can be fitted to the structure factor of the molecular density. (Right) The correlation functions corresponding to the Fourier spectra. In real space, the molecular structure is seen on small distances. At large distances the correlation functions diverge with different signs (top), so that the undulation spectrum diverges but the thickness spectrum is bounded (bottom).

In the above equations, $J_0(x)$ is the zeroth Bessel function of the first kind, $u_0 = (z_{01} + z_{02})/2$ is the mean bilayer mid-surface and $h_0 = (z_{01} - z_{02})/2$ is half the mean bilayer thickness. The Bessel functions constitute a general result for two-dimensional Fourier transforms with radial symmetry. The dimensionless correlation functions, $F_{\text{mono}}(r)$ and $F_{\text{bil}}(r)$, are defined in terms of the microscopic positions as

$$F_{\text{bil}}(r = |\mathbf{r} - \mathbf{r}'|) = \frac{\sigma^2_0}{\langle h_0 \rangle^2} \sum_{i=1}^{N} \sum_{m=1}^{N} \delta(\mathbf{r} - \mathbf{r}_i)\delta(\mathbf{r}' - \mathbf{r}_{2m}) \quad (5.23)$$

$$F_{\text{mono}}(r = |\mathbf{r} - \mathbf{r}'|) = \frac{\sigma^2_0}{\langle h_0 \rangle^2} \sum_{i \neq m}^{N} \delta(\mathbf{r} - \mathbf{r}_i)\delta(\mathbf{r}' - \mathbf{r}_m) \quad (5.24)$$

$F_{\text{mono}}(r)$ and $F_{\text{bil}}(r)$ measure the correlations between the height positions of two points in the same, and in the opposing leaflets, respectively. Therefore the bilayer aspect is completely contained in $F_{\text{bil}}(r)$ while $F_{\text{mono}}(r)$ only probes correlations within each leaflet. The bilayer has been chosen symmetrically centered around the $z = 0$ plane so that $\langle u_0 \rangle = 0$ and $\langle h_0 \rangle = \langle z_{01} \rangle = -\langle z_{02} \rangle$.

Both $F_{\text{mono}}(r)$ and $F_{\text{bil}}(r)$ diverge in the limit $r \to \infty$, because the undulations of the surfaces grow without bound with the system size. In Eq. (5.23) and Eq. (5.24), the terms in front of the sums have the same sign for $F_{\text{mono}}(r)$ but different signs for $F_{\text{bil}}(r)$. The result is that they tend to the same value in the large-$r$ limit but with opposite signs, implying that $F_{\text{b}}(r \to \infty) \to 0$ but $F_{\text{h}}(r \to \infty) \to 0$. The bilayer undulations are free to grow with system size, but the membrane thickness can not
fluctuate indefinitely without breaking.

The fluctuation spectra are shown in Fig. 5.2. The small-\(q\) divergence is adequately fitted to the \(q^{-4}\)-line predicted by Helfrich theory. A bending modulus of the order of \(10–20 \, k_B T\) can be extracted for the simulations of united-atom (UA) and coarse-grained (CG) models presented in Paper I. The undulation regime prevails up to \(\sim 0.7\) \(\text{nm}^{-1}\), corresponding to lateral distances of \(2\pi/0.7 = 9\) nm which is around ten lipid-lipid distances. For larger distances, the static structure of the density imprints on the spectrum. The reason for this is that the surfaces are defined directly from the molecular positions and are not interpolated on a regular grid. Interestingly, for the membrane models studied in Paper I, no other terms except the pure Helfrich contribution and the molecular density is needed to account for the spectrum, except for an oscillatory residual of a few percent. This residual most likely results from the density being projected in the plane and not defined along the undulation surface. No footprints of protrusions or molecular tilt are found. The small- and large-\(q\) limits of the spectra can be extracted analytically from Eq. (5.19) and Eq. (5.20). The calculations are outlined in the next section.

The different behaviors of the fluctuation spectra calculated with the real-space and direct Fourier methods for large \(q\) are expected. The methods define the spectra slightly different — in the direct Fourier method a microscopic definition based on the molecular positions is used but in the real-space method the positions are interpolated on a grid. The methods are in agreement in the undulation regime below \(1\) \(\text{nm}^{-1}\) and therefore gives approximately the same \(k_c\) when the small-\(q\) regime is fitted to a \(q^{-4}\) line. The real space analysis (by spline interpolation) in the other end of the spectrum goes smoothly to zero. This is a consequence of the numerical interpolation. Without interpolation the grid size restricts the wave vectors to \(q \lesssim 3\) \(\text{nm}^{-1}\). A continuous transition from \(q^{-4}\) to \(q^{-2}\) takes place up to large-\(q\) limit. In the absence of surface tension, this has been interpreted as an imprint from protrusions (44, 118) or lipid tilt (57). No \(q^{-2}\)-regime was found in the Fourier analysis presented in Paper I.

### 5.4 Limit values of the fluctuation spectra

The small- and large-\(q\) limits of the Fourier spectra can be calculated analytically. The following considers the undulation and thickness spectra introduced in this chapter, Eq. (5.19) and Eq. (5.20), and the density spectrum, \(S_\rho(q)\) which is given similarly to the other two. Detailed calculations are provided in the Supplementary Material of Paper I. These three fluctuation spectra are Bessel transforms of correlation functions. They all include an integral of the form (the second term in Eq. (5.19) and Eq. (5.20))

\[
\int_0^\infty dr \, r J_0(qr) F(r),
\]

which (since \(J_0(x \to \infty) \to 0\)) goes to zero as \(q \to \infty\). The self-terms in front of the integral that remain can be chosen by normalization.
In liquid state theory, it is conventional to let \( S(q \to \infty) = 1 \), but here the normalization is chosen to yield spectra with the same large-\( q \) limit. This facilitates comparison of different spectra in the same plot. The limits are \( \langle |u(q \to \infty)|^2 \rangle = \langle |h(q \to \infty)|^2 \rangle = (\langle u_0^2 \rangle + \langle h_0^2 \rangle)/2N \) and \( \langle |\rho(q \to \infty)|^2 \rangle = 1/(2Na^2) \) (see Paper I).

The small-\( q \) limit, \( q \to 0 \), is more subtle. Since \( J_0(x \to 0) \to 1 \), Eq. (5.25) reduces to a pure integral over the correlation function (in the language of statistical mechanics, a thermodynamic sum rule). The question is then (i) whether the integral is convergent and (ii) if so, what value it converges to. The undulation correlation, \( F_u(r) \), diverges with growing \( r \) (as shown in Fig. 5.2), implying that \( S_u(q) \) diverges with decreasing \( q \). The nature of this divergence cannot be inferred from the microscopic description, but the \( q^{-4} \)-divergence predicted by elastic theory (Chapter 2) leads to algebraic \( r^2 \)-increase.

The density spectrum limit can be calculated in the absence of monolayer-monolayer coupling. The spectrum is (the derivation of this expression is given by Hansen and McDonald (103)),

\[
\langle |\rho(q \to 0)|^2 \rangle = \frac{1}{2a_0^4 N} \left[ 1 + \frac{2\pi}{a_0} \int_0^\infty dr F_\rho(r) \right],
\]

(5.26)

where \( F_\rho(r) \) is the pair correlation function introduced in Chapter 4. Making no distinction between the canonical and grand canonical distribution functions, it is possible to identify the (two-dimensional) compressibility equation of statistical mechanics (119),

\[
\frac{2k_BT}{a_0 K_A} = 1 + \frac{2\pi}{a_0} \int_0^\infty dr F_\rho(r),
\]

(5.27)

where \( k_B \) is the Boltzmann constant, \( T \) is the absolute temperature and \( K_A \) is the bilayer area compressibility. The small-wave vector limit of the number density spectrum is thus given by

\[
\langle |\rho(q \to 0)|^2 \rangle = \frac{1}{a_0^2 N} \frac{k_B T}{a_0 K_A}.
\]

(5.28)

or, rearranging,

\[
K_A = \frac{k_B T}{a_0^2 N} \langle |\rho(q \to 0)|^2 \rangle.
\]

(5.29)

For a full derivation of the compressibility equation and a more complete discussion about the distinction between the canonical and grand canonical ensembles in this context, see Hansen and McDonald (103, Chapter 2) and Forster (120).

The same analysis as for the density spectrum can be done for the thickness spectrum. The discussion goes through unchanged but with material constants related to the thickness. However, since it is not the number density correlation function (pair correlation) in the integral, but the thickness correlation function, the compressibility modulus is not related to the area but to the thickness. This “thickness modulus” is not measured in experiments and thus of less importance. Numerical data, presented
in the Supplementary Material of Paper I, suggests that the thickness modulus is of the
same order of magnitude as the area compressibility, but about a factor of two larger.

5.5 Bridging the gap

The simple models described in Chapter 2 are to various degree continuum models
based on an elastic description of the membrane, while the direct Fourier method gives
a molecular description of the membrane. The direct Fourier method corresponds to
the elastic models in the large-wavelength limit, which is confirmed by Fig. 5.2 and
Fig. 3–5 in Paper I. This chapter is concluded with an attempt to reconcile some
aspects of the elastic and molecular descriptions.

For simplicity, leaflet coupling is neglected by considering a single (monolayer)
surface. Its correlation function, corresponding to Eq. (5.24), can be written

\[ F_u(r) = \frac{a_0^2}{\langle h_0 \rangle^2} \langle z(r') z(r'') \rho(r') \rho(r') \rangle , \]  

(5.30)

by recognizing the molecular density as given by Eq. (4.8). This is a mixed average
of the continuum theory and the microscopic density, and can in general not be cal-
culated analytically. First, take some limiting cases. Neglecting density fluctuations
with respect to the undulations by inserting \( \rho(r) = \rho_0 = a_0^{-1} \) in Eq. (5.30), regains the
result of elastic theory,

\[ F_u(r) = \frac{\langle z(r') z(r') \rangle}{\langle h_0 \rangle^2} . \]  

(5.31)

The other limit, a flat membrane with \( z(r) = z_0 = h_0 \), regains the two-dimensional pair
correlation function familiar from liquid theory,

\[ F_u(r) = \frac{\langle \rho(r') \rho(r') \rangle}{\langle \rho_0 \rangle^2} . \]  

(5.32)

A lipid membrane with coupled undulations and density fluctuations gives a result
in between. For the case of Gaussian fluctuations (a quadratic Hamiltonian like Hel-
frich’s), Wick’s theorem (121) can be used to calculate the average in Eq. (5.30),

\[ F_u(r) = \frac{a_0^2}{\langle h_0 \rangle^2} \left[ \langle z(r') z(r') \rangle \langle \rho(r') \rho(r') \rangle + \langle z(r') \rho(r') \rangle \langle z(r') \rho(r') \rangle 
+ \langle z(r') \rho(r') \rangle \langle z(r') \rho(r') \rangle \right] . \]  

(5.33)

as a sum of variable pair averages. The real-space correlation functions,

\[ Z(r = |r' - r'|) = \langle z(r') z(r') \rangle \]  

(5.34)
\[ \Phi(r = |r' - r'|) = \langle \rho(r') \rho(r') \rangle \]  

(5.35)
\[ \Psi(r = |r' - r'|) = \langle z(r') \rho(r') \rangle \]  

(5.36)
for pure undulations, pure density fluctuations and for correlations in undulations and density, respectively, can be used to give a completely microscopic description. Both averages in the second term in Eq. (5.33) are constant, $\Psi_0 \equiv \Psi(0)$, because they are evaluated for the same point. The third term is $\Psi(r)\Psi(-r)$. The constant, $\Psi_0$, only contributes to the fluctuation spectrum with a $\delta$-function at $q = 0$ and can be ignored. The result from the remaining two terms is

$$F_u(r) = F_u^0 + \frac{a_0^2}{\langle h_0 \rangle^2} \left[ Z(r)\Phi(r) + \Psi(r)\Psi(-r) \right].$$

Both terms are convolutions in Fourier space; albeit the last one is a special case. If the undulations-density correlations are symmetric, i.e., $\Psi(r) = \Psi(-r)$ the fluctuation spectrum is given by the autocorrelation theorem as

$$S_u(q) = \frac{a_0^2}{\langle h_0 \rangle^2} \left[ \int_{-\infty}^{\infty} dq' Z(q - q')\Phi(q') + \int_{-\infty}^{\infty} dq' \Psi(q + q')\Psi(q') \right].$$

Here, $Z(q)$ is the fluctuation spectrum obtained from an elastic theory while $\Phi(q)$ is the “static structure factor” (pair correlation function). If density-undulations correlation, $\Psi(q)$, are short-ranged the second term is small for small $q$. Similarly, since the static structure factor is bounded in the small-$q$ limit by the compressibility relation, the small-$q$ regime is dominated by $Z(q)$. There, the results of elasticity theory are regained. In the intermediate-$q$ regime there is a convolution between undulations and density. Working out this formalism in the general case of monolayer-monolayer coupling remains to be done in future work.
Chapter 6
Dynamic properties of lipid bilayers

The decay of spontaneous fluctuations in equilibrium also describe how a system responds to a weak, applied perturbation (122). Even though the dynamics of many-body systems is immensely involved, the overwhelming majority of the system’s degrees of freedom follow “molecular chaos”. Such fluctuations can decay locally and are short-lived. Only a few fluctuation modes result from cooperative motion of many molecules and lead to slow relaxation. These hydrodynamic fluctuations can only relax by slowly spreading throughout the system. If a hydrodynamic fluctuation needs to be transported a long distance, the relaxation time will also be large (being infinite for an infinite system). Hydrodynamic modes are very rare, in fact, for a normal fluid consisting of $\sim 10^{23}$ point-like particles there are only five such collective modes: The density, the velocity (a vector of three components) and the energy (123).

The lipid bilayer is more involved, for two reasons. First, it is a molecular fluid with internal modes that couple to the relaxation. Second, the bilayer interfaces to another bulk fluid, water. The hydrodynamics of the lipid bilayer thus depends on the length- and the time scale. The aim of this chapter is to provide a background of lipid bilayers and hydrodynamic theory, to facilitate the work presented in Paper III and Paper IV. Starting from the microscopic definition of the two-dimensional liquid and the linearized hydrodynamic equations, fluctuations of the interface are included as natural results of the models introduced in Chapter 2. Nonlinear behavior is discussed in two regimes, hydrodynamics on a curved surface in the Zilman-Granek model, and the decay on molecular wavelengths. It is shown that the relaxation is well-described by stretched exponentials in both domains, but on different physical grounds.

6.1 Microscopic description of a two-dimensional fluid

The physics of the fluid state has proven far from trivial to describe theoretically. The treatment starts from a collection of point-like particles that interact via a pair potential. In two dimensions, a liquid in equilibrium (consisting of $N$ particles that are contained within an area $A$) is characterized by time-dependent thermal fluctuations
in the local number density $\rho$. The most fundamental description is the van Hove correlation function (124),

$$G(r, t) = \frac{A}{N} \langle \rho(r_0 + r, t_0 + t) | \rho(r_0, t_0) \rangle_{r_0, t_0},$$  

(6.1)

which is a direct generalization of the pair correlation function introduced in Chapter 4. Here, $G(r, t)$ gives the probability to find a particle at location $r$ and time $t$ given that there was a particle at the starting conditions. The bracket is an ensemble average over all starting times and starting positions. In a translational invariant liquid, $G(r, t)$ is origin-independent and only conditioned by the elapsed time and the relative position. The isotropic symmetry of the fluid lipid bilayer make, as usual, real space correlations functions only of $r \equiv |r|$. The spatial Fourier transform of the van Hove correlation function is

$$F(q, t) = \int_{\mathbb{R}^2} d^2 r e^{-iqr} G(r, t),$$  

(6.2)

usually referred to as the intermediate scattering function in the literature (103, 125) (although dynamic structure factor is also used), and is measured in spin-echo neutron scattering experiments (126, 127). The intermediate scattering function provides a straightforward way to monitor the time propagation of density fluctuations for different wave vectors $q$. The space and time Fourier transform of the van Hove correlation function is the dynamic structure factor,

$$S(q, \omega) = \int_{-\infty}^{\infty} dt \int_{\mathbb{R}^2} d^2 r e^{i(\omega t - qr)} G(r, t) = \int_{-\infty}^{\infty} dt e^{i\omega t} F(q, t).$$  

(6.3)

probed in inelastic scattering experiments using neutrons (128) or x rays (6, 7). These correlation functions may also be calculated directly from computer simulations with the techniques described in Chapter 4. Generalizing that discussion, the time-dependent microscopic density operator is

$$\rho(r, t) = \sum_{l=1}^{N} \delta(r - r_l(t)).$$  

(6.4)

The $l$ index refers to the individual particle and the sum is over all particles in the sample. The time and space Fourier representations of the number density are

$$\rho_q(t) = \int_{\mathbb{R}^2} d^2 r e^{-iqr} \rho(r, t) = \sum_{l=1}^{N} e^{-iqr_l(t)}$$  

(6.5a)

$$\rho_q(\omega) = \int_{-\infty}^{\infty} dt e^{i\omega t} \rho_q(t) = \sum_{l=1}^{N} \int_{-\infty}^{\infty} dt e^{i(\omega t - qr_l(t))},$$  

(6.5b)

respectively. The $q$-subscript for the Fourier functions emphasizes that wave vectors are discrete. From molecular dynamics simulations, the scattering functions are calculated
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by invoking the Wiener-Khinchin theorem (41) to write the time average in Eq. (6.1) as an integral over a finite time interval $T$:

$$ F(q, t) = \frac{1}{NT} \int_0^T \rightdel{t_0 \rho_q(t_0 + t)} \rho_q^*(t_0). \tag{6.6} $$

The convolution integral translates to a Fourier product,

$$ S(q, \omega) = \frac{1}{N T} \rho_q(\omega) \rho_q^*(-\omega) = \frac{1}{NT} |\rho_q(\omega)|^2. \tag{6.7} $$

6.2 The dynamic structure factor from linearized hydrodynamics

The time and space correlation functions can be derived from hydrodynamic theory. A condensed review of this derivation is stated in Paper III, here some more details are outlined. The lipid bilayer is considered to be a continuum material in a regime of time and space where the variations of local properties are slow compared to microscopic scales (for a more thorough discussion of the hydrodynamic regime, see Hansen and McDonald (103, chapter 8)). This implies length- and time scales that contain a large number of atomic collisions, a statement that makes the hydrodynamic approximation valid down to surprisingly short lengths and times. The hydrodynamic limit is related to the mean free path in the liquid, which is very small, comparable to the interatomic spacing (125). Transport processes in the hydrodynamic regime are governed by the hydrodynamic equations, which maintain conservation of certain state variables. Many choices of state variables are possible, but it is simple to consider conservation of particle number density $\rho(r, t)$, particle momentum/velocity $p(r, t) = m v(r, t)$ and absolute temperature $T(r, t)$. For a fluid in thermal equilibrium, $\rho$ and $T$ can be split into $\rho(r, t) = \rho_0 + \rho_1(r, t)$ and $T(r, t) = T_0 + T_1(r, t)$. The fluctuating parts $\rho_1(r, t)$ and $T_1(r, t)$ describe the (small) deviations from the equilibrium values $\rho_0$ and $T_0$.

The variables obey the linearized hydrodynamic equations (129):

$$ \frac{\partial \rho_1}{\partial t} + \rho_0 \nabla \cdot \mathbf{v} = 0 \tag{6.8a} $$

$$ \rho_0 \frac{\partial \mathbf{v}}{\partial t} + \frac{c^2}{\gamma} \left[ \nabla \rho_1 + \alpha \rho_0 \nabla T_1 \right] - \left( \frac{4}{3} \eta_i + \eta_B \right) \nabla (\nabla \cdot \mathbf{v}) = 0 \tag{6.8b} $$

$$ \rho_0 c_v \frac{\partial T_1}{\partial t} - \frac{c_v (\gamma - 1)}{\alpha} \frac{\partial \rho_1}{\partial t} - \lambda \nabla^2 T_1 = 0. \tag{6.8c} $$

These are the continuity equation, the (linearized) Navier-Stokes equation and the energy transport equation. In these equations, $c$ is the sound velocity and $\gamma = c_p/c_v$ is the ratio between the specific heats at constant pressure, $c_p$, and constant volume, $c_v$. The shear and bulk viscosities (per molecular mass), $\eta_i$ and $\eta_B$, are also present as well as the thermal conductivity $\lambda$ and the thermal expansion coefficient $\alpha$. It is important to emphasize that linearizing the equations decouples the number density from
the transversal velocity and that replacement of the entropy and energy deviations in the last two equations by the corresponding number density $\rho_1$ and temperature $T_1$ gradients, requires local equilibrium for the necessary thermodynamic relations to be valid.

The aim is to construct the (macroscopic) density autocorrelation functions, the route is by Fourier-Laplace transformation of the hydrodynamic equations. The velocity is eliminated using $\nabla \cdot \mathbf{v}$ from Eq. (6.8a). The remaining two equations for $\rho_1$ and $T_1$ are:

\[
\frac{\partial \rho_1}{\partial t} + \frac{c_s^2}{\gamma} \left[ \nabla^2 \rho_1 + a \rho_0 \nabla^2 T_1 \right] + \left( \frac{4 \eta_s + \eta_B}{\rho_0} \right) \nabla^2 \left( \frac{\partial \rho_1}{\partial t} \right) = 0 \quad (6.9a)
\]

\[
\rho_0 c_v \frac{\partial T_1}{\partial t} - \frac{c_v (\gamma - 1)}{a} \frac{\partial \rho_1}{\partial t} - \lambda \nabla^2 T_1 = 0. \quad (6.9b)
\]

The Laplace-Fourier transformations of the number density and the temperature,

\[
\rho_q(s) = \int_0^\infty dt \ e^{-st} \int_{-\infty}^\infty d^2r \ e^{-iqr} \rho_1(r, t) \quad (6.10a)
\]

\[
T_q(s) = \int_0^\infty dt \ e^{-st} \int_{-\infty}^\infty d^2r \ e^{-iqr} T_1(r, t), \quad (6.10b)
\]

(dropping the subscripts for brevity) where $s = \epsilon + i\omega$ is the complex Laplace variable, result in two linear equations involving the initial values $\rho_q \equiv \rho_q(t = 0)$ and $T_q \equiv T_q(t = 0)$,

\[
\begin{pmatrix} \rho_q(s) \\ T_q(s) \end{pmatrix} \begin{pmatrix} s^2 + \rho_0^{-1} \left( \frac{4 \eta_s + \eta_B}{\alpha} \right) q^2 s + \gamma^{-1} c_s^2 q^2 \\ a \gamma^{-1} \rho_0 c_s^2 q^2 \end{pmatrix} \begin{pmatrix} \rho_0 c_v s + \lambda q^2 \\ \frac{\rho_0^4 c_v (1 - \gamma) s - \rho_0^4 c_v (1 - \gamma) + T_q \rho_0 c_v} \end{pmatrix} = \begin{pmatrix} \rho_q(s) + \rho_0^{-1} \left( \frac{4 \eta_s + \eta_B}{\alpha} \right)q^2 \rho_q^\star \\ \rho_q^\star \gamma^{-1} c_s^2 (1 - \gamma) + T_q \rho_0 c_v \end{pmatrix}. \quad (6.11)
\]

Terms involving $T_q$ are dropped since the number density and the temperature are independent variables. This leaves the thermal expansion coefficient $\alpha$ out of the solution for the number density. Solving for the number density yields,

\[
\rho_q(s) = \rho_q^\star \frac{\rho_0^3 s^3 + (a + b) q^2 s^2 + c_s^2 (1 - \gamma) q^2 + (c_s^2 q^2 + abq^4) s + \gamma^{-1} c_s^2 aq^4)}{\rho_0^3 + (a + b) q^2 s^2 + (c_s^2 q^2 + abq^4) s + \gamma^{-1} c_s^2 aq^4}, \quad (6.12)
\]

with a third-order dispersion equation for the complex Laplace variable $s$ in the denominator. To condense the expressions, the shorthands $a = \lambda / \rho_0 c_v$ and $b = \left( \frac{4 \eta_s + \eta_B}{\rho_0} \right)$ have been introduced. From Eq. (6.12) the Laplace-Fourier transformed density autocorrelation function, $(\rho_q(s) \rho_q^\star)$, can be constructed. Classic autocorrelation functions are real and even in time (and power spectra are real and even in frequency), so the dynamic structure factor is obtained from the relation

\[
S(q, \omega) = 2 \text{Re} \lim_{\epsilon \to 0} \left( \rho_q(s = \epsilon + i\omega) \rho_q^\star \right). \quad (6.13)
\]
The expression is evaluated by identifying the static structure factor,

$$S(q) = \frac{1}{2\pi} \int_{-\infty}^{\infty} d\omega S(q, \omega) = \langle \rho_q \rho^*_q \rangle,$$

and performing a partial fraction expansion of Eq. (6.12), requiring the roots of the dispersion equation

$$s^3 + (a + b)q^2s^2 + (c^2q^2 + abq^4)s + \gamma^{-1}c^2aq^4 = 0. \quad (6.15)$$

The exact solutions to the cubic equation are roots in Cardano’s formula (130), but their algebraic complexity does not make them very useful. If \( q \ll c/a \) and \( q \ll c/b \), the roots simplify and can be determined with a perturbation calculation. As shown in Appendix A, this gives the three roots

$$s_0 = -D_T q^2 \quad \text{and} \quad s_\pm = \pm icq - \beta q^2,$$

with \( D_T = \gamma^{-1}a \) being the thermal diffusivity and \( \beta = \frac{1}{2} \left[ a(1 - \gamma^{-1}) + b \right] \) being the sound attenuation coefficient. Given the roots, the dynamic structure factor is to order \( q^2 \),

$$\frac{S(q, \omega)}{S(q)} = 2\text{Re} \lim_{\epsilon \to 0} \left[ \frac{s^2 + (a + b)q^2s + (1 - \gamma^{-1})c^2q^4}{(s - s_0)(s - s_+)(s - s_-)} \right]_{s = \epsilon + i\omega}. \quad (6.17)$$

All that remains is to perform the partial fraction decomposition, and then setting \( \epsilon = 0 \), keeping only the lowest order terms. The calculations are straightforward but lengthy; the interested reader may find them in Appendix A. Cleaning up and rearranging, the dynamic structure factor can be written compactly,

$$\frac{S(q, \omega)}{S(q)} = A_0 \frac{\Gamma_h}{\omega^2 + \Gamma_h^2} + A_s \left[ \frac{\Gamma_s - \xi(\omega - \omega_s)}{(\omega - \omega_s)^2 + \Gamma_s^2} + \frac{\Gamma_s + \xi(\omega + \omega_s)}{(\omega + \omega_s)^2 + \Gamma_s^2} \right]. \quad (6.18)$$

The amplitudes are denoted by \( A_0 = 2(\gamma - 1)/\gamma \) and \( A_s = 1/\gamma \), whereas the linewidths are \( \Gamma_h(q) = D_T q^2 \) and \( \Gamma_s(q) = \beta q^2 \). In addition \( \omega_s(q) = cq \) and the asymmetry parameter is \( \xi(q) = [\beta + D_T (\gamma - 1)]^{-1} q \). This form of the power spectrum is the well-known Rayleigh-Brillouin triplet (103, 125), consisting of three Lorentzian functions with halfwidths \( \Gamma_h(q) \) and \( \Gamma_s(q) \), centered at \( \omega = 0 \) and \( \omega = \pm \omega_s \), where the asymmetry parameter \( \xi(q) \) causes the side peaks to slightly shift towards the central line. Fig. 6.1 is taken from paper (3) and plots Eq. (6.18) as a function of \( \omega \).

The central Rayleigh line is due to nonpropagating diffusive relaxation of the particles in the liquid. The lifetimes of such fluctuations are \( \Gamma_h^{-1} \). In contrast, the shifted Brillouin doublet comes from propagating sound waves and is connected to the fluctuating longitudinal velocity field in the liquid. These density fluctuations move with the sound velocity \( c \) and are damped with rate \( \Gamma_s^{-1} \). The sound modes and the thermal diffusivity mode are uncoupled in the hydrodynamic theory. In real space, the corresponding intermediate scattering function, \( F(q, t) \), is the inverse time Fourier transform of Eq. (6.18),

$$\frac{F(q, t)}{F(q)} = A_0 e^{-\Gamma_0(q)t} + A_s e^{-\Gamma_s(q)t} \left[ \cos(\omega_s(q)t) + \xi(q) \sin(\omega_s(q)t) \right], \quad (6.19)$$
manifested in the Brillouin doublet occur on ps times. In the intermediate- and large-
ongular. The Rayleigh line describes relaxation on the ns timescale but the sound waves
om domains the lines overlap and can not be separated.

a combination of exponential decay and damped oscillations.

**Lipid bilayer density fluctuations probed by simulations**

The hydrodynamic equations are valid on large distances and for long times, when
system is settled near equilibrium. Dynamic light scattering (DLS) experiments
can probe the hydrodynamic limit but in the mesoscopic regime of nanometers and
nanoseconds, neutron spin-echo (NSE) experiments (127) are required. There are two
reasons why hydrodynamic fluctuations are difficult to study in simulations. First and
most obvious, the hydrodynamic description requires large systems to be valid. Second
and perhaps more subtle, hydrodynamic fluctuations decay slowly and therefore also
require long simulations. The dyadic need of large-scale and long-time simulations
is seldom overcome by computer power, and neither can the statistics be improved
by averaging because the hydrodynamic modes are collective properties of the system
as a whole. The first lipid bilayer simulations that probed collective dynamics (131)
reached wavelengths of $\sim 1.5$ nm, corresponding to wave vectors $\sim 4.5$ nm$^{-1}$; that
proved enough to resolve some hydrodynamic properties. In Paper III, to the author’s
best knowledge, the most extensive calculations of dynamic structure factors for lipid
bilayers were presented.

The fit procedure of calculated $S(q, \omega)$ to the Rayleigh-Brillouin triplet is nontriv-
ial. The Rayleigh line describes relaxation on the ns time scale but the sound waves
manifested in the Brillouin doublet occur on ps times. In the intermediate- and large-$q$
domains the lines overlap and can not be separated. $S(q, \omega)$ can roughly be divided

![Figure 6.1: Schematic view of the dynamic structure factor obtained from the linearized hydrodynamic equations. Rayleigh and Brillouin lines are drawn with solid lines and also Brillouin lines for larger values of $q$ where the asymmetry appears (dashed and dotted lines). The inset shows its time Fourier transform, $F(q,t)$. The Brillouin doublet are oscillations in the time domain, on so small scales that they are invisible on a linear scale.](image)
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Figure 6.2: The domains of the dynamic structure factor by representative wave vectors $q = 1.0$ nm$^{-1}$ (small), $q = 7.1$ nm$^{-1}$ (intermediate) and $q = 20.0$ nm$^{-1}$ (large). The Rayleigh-Brillouin triplet shape is clear in the small domain but is progressively smeared out in the intermediate domain as the Brillouin lines become shoulders on the Rayleigh line. The large-$q$ part of the spectrum is dominated by the thermal mode.

into three domains according to wave vector (Fig. 6.2). The transitions are continuous and the domains overlap to varying extent. The classification loosely characterize the coherent (collective) and incoherent (single-particle) parts of the density fluctuations.

In the small domain, $q < 5$ nm$^{-1}$ (wavelengths $\lambda > 1.2$ nm), density fluctuations origin from collective motion of many lipids. The fluid membrane is expected to adhere to a hydrodynamic description. For wave vectors in the intermediate domain, $5 < q < 20$ nm$^{-1}$ ($1.2 > \lambda > 0.3$ nm), single or few lipids fluctuate together, and deviations from conventional hydrodynamic theory are to be expected. Hydrodynamic relations need to be revised into generalized hydrodynamic theories, i.e., lineshapes with positions and widths that are free to vary with wave vector. The Brillouin lines take the shapes of shoulders on the central Rayleigh line whereas the spectrum tends to three Lorentzians at $\omega = 0$, leaving the Brillouin doublet inseparable from the Rayleigh mode. Density fluctuations in the large domain, $q > 20$ nm$^{-1}$ ($\lambda < 0.3$ nm), are exclusively due to single-particle motions, which cause a thermal mode to dominate the spectrum.

The data that was available in Paper III could not adequately fit the Rayleigh line to a single Lorentzian function (Fig. 6.2), or equivalently, a single exponential in the time domain. Although the lineshape resembled a Lorentzian, the base was too broad to fit the narrow top. A double exponential form was suggested, as earlier had been proposed from neutron scattering experiments on the same lipid (DMPC) (132). The improved data reported in Paper IV showed convincingly that the functional form was a stretched exponential. The discussion of these data is postponed to the end of this Chapter.

The hydrodynamic equations also describe sound waves in fluid membranes, prop-
agating with velocity $c$, independently of the dispersive fluctuations. The sound waves are pressure fluctuations at constant entropy, analog to phonon modes in solids. The sound waves are characterized by angular frequency, $\omega_s$, and damping rate, $\Gamma_s$. The sound wave propagation is influenced by the lipid bilayer structure and slows down at distances corresponding to the chain-chain and head group-head group separations (in liquid state theory, this is known as de Gennes narrowing (133) of the power spectrum). Mapping the dispersion relation, $\omega_s(q)$, is of fundamental interest to understand the propagation of the sound waves. Hydrodynamic theory predicts a linear relation between the angular frequency and the wave vector, $\omega_s(q) = cq$. The validity of the approximations leading to the hydrodynamic roots to the dispersion equation, Eq. (6.16), can be extended into the intermediate domain by working out correction terms in a systematic fashion (134). The roots were derived assuming $q \ll q_1 = c/a = c \rho_0 c_V / \lambda$ and $q \ll q_2 = c/b = c \rho_0 M_L / \eta$. With the experimental numbers, $c = 1.5 \times 10^3$ m/s (7), $M_L = 677$ u (135), $\rho_0 = 7.5 \times 10^{26}$ m$^{-3}$ (23), $c_V = 2.5 \times 10^{-21}$ J/K (136), $\lambda = 0.14$ W/(m K) (n-hexadecane (137)) and $\eta = 0.1$ Pa s (138), one finds $q_1 \approx 20$ nm$^{-1}$ and $q_2 \approx 0.02$ nm$^{-1}$. This shows that wave vectors are always small compared to $q_1$ but not $q_2$, a result of that lipid membranes are very viscous fluids. The viscosity of the lipid bilayer is about two orders-of-magnitude larger than that of water. Hence, although the approximate solutions to the dispersion equation (Eq. (6.15)) accurately describe the diffusive behavior of the lipid bilayer, it is not appropriate for the sound damping at the length scales probed by simulations. This is most likely the reason for the deviations from the expected hydrodynamic results that are observed in the simulations of Paper III.

### 6.3 Including fluctuations of the bilayer interface

The hydrodynamic equations outlined in the previous section are accurate in describing how spontaneous fluctuations decay in the bulk of a simple liquid made up of point-particles. This is a plausible statement on sufficiently small length scales within the membrane. However, there are a range of hydrodynamic phenomenon that raise due to membrane undulations (139). The most general hydrodynamic description of the bilayer is a thin (but finite) layer of viscous fluid, sandwiched between two infinitely thick slabs of a fluid with another viscosity (water). Thermal fluctuations must of course be included. Modeling this geometry has been attempted, but the dynamic equations are highly nonlinear. The linearized equations treat the membrane as an infinitely thin surface that responds instantaneously to changes in the surrounding water. This leads to exponential relaxation for the bilayer surface (the internal structure of the membrane is ignored), and can be done similarly to the hydrodynamic derivation presented above, by including the equations of motion of the water in combination with boundary conditions for the interface and the bulk. The calculations are involved (140). A more transparent presentation of the hydrodynamics of the membrane sheet can be done via the Oseen tensor.
6.3. INCLUDING FLUCTUATIONS OF THE BILAYER INTERFACE

Stokes’ approximation for the membrane surface

On cellular length scales, flows are dominated by the viscous, rather than the inertial, terms in the Navier-Stokes equations. This flow at low Reynolds number (141, 142) is the ground for Stokes’ approximation (121) to the Navier-Stokes equation. For a three-dimensional fluid of infinite extent, Stokes’ equations are, in combination with the incompressibility condition,

\[ \eta \nabla^2 \mathbf{v}(\mathbf{R}) = \nabla p(\mathbf{R}) - \mathbf{F}_{\text{ext}}(\mathbf{R}) \]  
\[ \nabla \cdot \mathbf{v}(\mathbf{R}) = 0. \]  

The flow is the same regardless of time! \( \eta \) is the fluid viscosity. \( \mathbf{R} = (x, y, z) \) is a three-dimensional position in the fluid, and \( \mathbf{v}(\mathbf{R}) \) and \( p(\mathbf{R}) \) are the velocity and pressure, respectively, in presence of the external forces, \( \mathbf{F}_{\text{ext}} \). The Stokes’ equations are conveniently solved by Fourier transformation in the absence of boundary conditions. The incompressibility condition, Eq. (6.21), can be used to eliminate the pressure in favor of the forces. The Fourier transformed velocity becomes then

\[ \mathbf{v}(\mathbf{Q}) = \frac{1}{\eta Q^2} \left( I - \hat{\mathbf{Q}} \hat{\mathbf{Q}} \right) \cdot \mathbf{F}_{\text{ext}}(\mathbf{Q}) \equiv \mathbf{H}(\mathbf{Q}) \cdot \mathbf{F}_{\text{ext}}(\mathbf{Q}), \]  

defining the Oseen tensor, \( \mathbf{H}(\mathbf{Q}) \), in Fourier space. \( I \) is the unit tensor, and \( \hat{\mathbf{Q}} = \mathbf{Q}/|\mathbf{Q}| \) is a unit vector in Fourier space. The velocity in real space is regained by inverse transformation,

\[ \mathbf{v}(\mathbf{R}) = \int_{-\infty}^{\infty} \, d^3\mathbf{R}' \, \mathbf{H}(\mathbf{R} - \mathbf{R}') \cdot \mathbf{F}_{\text{ext}}(\mathbf{R}') \]  

a convolution of the real-space Oseen tensor and the external forces. Apparently, \( \mathbf{H}(\mathbf{R}) \) mediates the response to the fluid velocity from applied external forces. The Oseen tensor is hence the Green function for the velocity in Stokes’ approximation. Eq. (6.23) is general but the functional form of \( \mathbf{H}(\mathbf{R}) \) depends on the hydrodynamic geometry. Often the Oseen tensor is simplest in Fourier space and it will be convenient to work throughout there. Inverting the three-dimensional Oseen tensor gives (121)

\[ \mathbf{H}(\mathbf{R}) = \frac{1}{(2\pi)^3} \int_{-\infty}^{\infty} d^3\mathbf{Q} \, e^{i\mathbf{Q} \cdot \mathbf{R}} \, \frac{1}{\eta Q^2} \left( I - \hat{\mathbf{Q}} \hat{\mathbf{Q}} \right) = \frac{1}{8\pi \eta R} \left( I + \hat{\mathbf{R}} \hat{\mathbf{R}} \right). \]  

This equation makes the physical interpretation of \( \mathbf{H}(\mathbf{R}) \) clear. The Oseen tensor mediates a long-range hydrodynamic interaction, a fluid “back-flow” created by the forces, that slowly decays with distance as \( 1/R \).

The Oseen tensor can be used to derive an approximate equation of motion for out-of-plane membrane fluctuations (undulations). The membrane is assumed to be impermeable so that the interface moves with the fluid. Further, the membrane velocity is taken to coincide with the surrounding fluid, but is always evaluated at \( z = 0 \).
This simplifies the equations and is a justified approximation for small membrane fluctuations. Using Eq. (6.23) for the velocity,

\[ \mathbf{v}(r, z = 0) = \frac{\partial u(r)}{\partial t} = \int_{-\infty}^{\infty} d^3r' H_{zz}(r - r', z = 0) \left[ F(r', t) + \zeta(r', t') \right], \tag{6.25} \]

with \( r = (x, y) \) and \( H_{zz}(r) \) being the \( zz \)-component of the Oseen tensor. Note that the problem is now two-dimensional. It has been assumed that the forces act along the membrane normal and can be divided into two parts. A random force, \( \zeta(r, t) \), spawned by molecular collisions, is described as white noise obeying the fluctuation-dissipation theorem,

\[ \langle \zeta(r, t) \rangle = 0 \quad \text{and} \quad \langle \zeta(r, t) \zeta(r', t') \rangle = 2k_B T H_{zz}^{-1}(r - r') \delta(t - t'), \tag{6.26} \]

with the inverse of the Oseen tensor defined by the relation

\[ \int d^3r H_{zz}(r - r') H_{zz}^{-1}(r') = \delta(r). \tag{6.27} \]

The bending force is derived from the Hamiltonian, \( F(r, t) = \delta \mathcal{H} / \delta u(r, t) \), which is given by an elastic theory as presented in Chapter 2. Eq. (6.25) is a linear Langevin equation and can easily be generalized to include time-dependent hydrodynamic kernels. Here the Oseen tensor derived from Stokes’ approximation shall be sufficient. Fourier transforming Eq. (6.25), \( (F_q(t) = -\delta \mathcal{H} / \delta u_q(t)) \),

\[ \frac{\partial u_q}{\partial t} = H_{zz}(q) \left( F_q \{ u_q(t) \} + \zeta_q(t) \right), \tag{6.28} \]

where the Fourier transformed forces in general depend on the entire set of amplitudes \( \{ u_q(t) \} \). If \( \mathcal{H} \) is quadratic in \( u_q(t) \) different modes are uncoupled and \( F_q(t) = E(q) u_q(t) \), with the form of \( E(q) \) depending on the Hamiltonian being used. Multiplying Eq. (6.28) with \( u_q(0) \) and taking the ensemble average (assuming the velocity and the noise are uncorrelated) results in

\[ \frac{\partial}{\partial t} \langle u_q(t) u_q(0) \rangle = H_{zz}(q) E(q) \langle u_q(t) u_q(0) \rangle. \tag{6.29} \]

This simple differential equation is immediately solved to give

\[ \langle u_q(t) u_q(0) \rangle = \langle |u_q|^2 \rangle e^{-\omega(q)t}, \tag{6.30} \]

with the dispersion relation given by \( \omega(q) = H_{zz}(q) E(q) \). The work that remains to be done is to calculate \( H_{zz}(q) \) and \( E(q) \). The first is done by Fourier transforming the Oseen tensor, given in Eq. (6.24), in the plane,

\[ H_{zz}(q) = \int_{-\infty}^{\infty} d^2r e^{-iqr} \frac{1}{8\pi\eta r} = \frac{1}{4\eta q}. \tag{6.31} \]
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The energy relation, $E(q)$, depends on the Hamiltonian. Using the Helfrich model, Eq. (2.9), gives $E(q) = k_c q^4$. The dispersion relation becomes

$$\omega(q) = \frac{k_c}{4\eta} q^3,$$

(6.32)

the product of the bending energy and the hydrodynamic back-flow from the Oseen tensor. This result is identical to the large wavelength-limit of the hydrodynamic equations of an interface surrounded by infinite fluids, as first shown by Kramer (140). Stokes' approximation thus leads to exponential decay of membrane undulations, with damping rate $\omega(q)$. This behavior has been confirmed by experiments on large wavelengths (143). As wave vectors comparable to the membrane thickness are reached, the bilayer nature of the membrane, and its internal structure, will be pronounced.

**Dynamics of the refined models**

The dynamic description can be improved in a similar manner as the static properties in Chapter 2. Additional terms in the Hamiltonian (as for protrusions) that are functions of the undulating surface, change $E(q)$, and consequently, $\omega(q)$. For example, an applied surface tension leads to the dispersion relation

$$\omega(q) = H_{zz}(q)E(q) = \frac{k_c q^3 + \gamma_0 q}{4\eta}.$$  

(6.33)

The introduction of additional fields into the Hamiltonian, as in the Kozlov-Hamm and Seifert-Langer models, can change the functional form of the decay. Solving the coupled hydrodynamic equations of the Seifert-Langer model, for example, leads to bi-exponential decay for the undulations,

$$\langle u_q(t) u_q(0) \rangle = A_0(q) e^{-\tau_1(q) t} + (1 - A_0(q)) e^{-\tau_2(q) t}.$$  

(6.34)

The rates and the amplitude are complicated functions of $q$, but can be inferred in three regions, $q \ll q_1$, $q_1 \ll q \ll q_2$, and $q \gg q_2$. The regions are separated by the crossover wave vectors $q_1 = 2\eta k_m / b \kappa$ and $q_2 = \sqrt{2b/\mu}$. The material constants are the same as introduced in Chapter 2 with two additional contributions, $b$, the friction between the monolayers, and $\mu$, the monolayer viscosity (which is inversely related to the lateral diffusion coefficient).

The amplitude of one exponential dominates in the small-$q$ region and the previous hydrodynamic rate, $(k_c/4\eta)q^3$, is regained. The question is what values to assign to $q_1$ and $q_2$. The membrane material parameters are obtained in agreement from simulations and experiments, with one notable exception. The monolayer-monolayer friction, $b$, differs with almost two orders of magnitude between experiments and simulations (144, 145). This means that experiment data gives $q_1 < q_2$ but simulation data the other way around without an intermediate regime. Simulation values ($\eta = 10^{-3}$ Pa s, $k_m = 0.07$ J m$^{-2}$, $b = 0.7 \times 10^6$ N s m$^{-3}$, $\kappa = 10^{-19}$ J and $\mu = 6 \times 10^{-10}$
\[ q_1 \approx 2 \text{ nm}^{-1} \text{ and } q_2 \approx 0.05 \text{ nm}^{-1}. \] There, Seifert-Langer theory predicts a true bi-exponential decay with similar amplitudes. The first rate, \( \gamma_1(q) \), is expected to interpolate between \( q^3 \) and a constant, while the second rate, \( \gamma_2(q) \), should follow a power law in the wave vector, with negative slope interpolating between 2 and 3. Considering the difference in value of the crucial parameter \( b \) from simulations and experiments, this interpretation should be taken with care. It also seems that simulation data, presented in Paper IV, favors stretched exponential relaxation over bi-exponential decay. It should be noted that regardless of shape of the decay function, the overall small-\( q \) rates (or correlation times) obtained from simulations are orders-of-magnitude faster than those obtained from experiments.

### 6.4 Stretched exponential relaxation

Experiments show that relaxation dynamics in biological membranes is not described by single exponential form on mesoscopic regimes (146–150) but is instead well-fitted to a stretched exponential function,

\[ F(q, t) = F(q, 0) e^{-\left( \Gamma(q) t \right)^\beta(q)}. \] (6.35)

Stretched exponential (SE) behavior in complex systems is well-known and has a long history, first used more than 150 years ago by Kohlrausch to describe charge decay on a glass Leyden jar (151). A universal origin to SE relaxation has never been given, but it is often (phenomenologically) attributed to a broad distribution of relaxation processes with different decay times (152). It is clear that nothing is present in the linearized hydrodynamic description to account for SE behavior, but some more physics is required. Such physics could be provided by time-dependent transport coefficient (viscoelasticity), but the question on whether bilayers are viscoelastic is controversial (153). Nevertheless, complex forms of the lipid bilayer intermediate scattering function have been shown for dehydrated lipids (154).

The molecular dynamics simulation data reported in Paper III and Paper IV, and also independently by Flenner et al. (155), was not adequately described by single-exponential decay for the intermediate scattering function, \( F(q, t) \). Although experimental data on the smallest wavelengths (132) is not always good enough to distinguish between, say, stretched exponential or bi-exponential decay, it is interesting to note that nonexponential relaxation is found in lipid bilayers both in the mesoscopic and the truly microscopic domains, and the physical origins on the different length scales are clearly not the same. Stretched exponential behavior in the mesoscopic domain is often attributed to membrane undulations and interpreted by the theory of Zilman and Granek (156, 157). On microscopic scales, not only the intermediate scattering function, but also fluctuations in the orientation of the fatty acid chains in the bilayer hydrophobic core, relax slowly; algebraically \( (t^{-1/2}) \) (158), or as stretched exponentials (145). These fluctuations decay locally and are similar in nature, but a consistent theory to explain the stretched behavior on microscopic scales in lipid membranes has not yet been given.
The final sections of this chapter describe these different types of stretched exponential decay, starting with Zilman-Granek theory, and finishing with some observations from the microscopic domain.

Zilman-Granek theory

Zilman and Granek (ZG) calculated the intermediate scattering function of an undulating membrane patch in the continuum limit (156, 157). The calculations are involved and here, only an outline is provided. ZG theory starts from the definition of the density time correlation function, the intermediate scattering function,

$$ F_\rho(Q, t) = \frac{1}{N} \left< \sum_{i,j=1}^{N} e^{-iQ(R_i(t) - R_j(0))} \right> , \quad (6.36) $$

An undulating membrane patch with projected area $A_0 = L^2$, is comprised of $N$ molecules with positions $R_i(t) = (x_i(t), y_i(t), z_i(t))$. The three-dimensional wave vector is $Q = (q_x, q_y, q_z) = (q, q_z)$, where $q$ is the in-plane scattering vector and $q_z$ is the scattering vector along the membrane normal. ZG theory amounts to taking the continuum limit of Eq. (6.36). The molecular position $i$ is then replaced with the position on the undulating membrane surface. This approximation is only justified for distances and times that are large in comparison to molecular scales. For gentle undulations, the normal position is described by the usual height function $z_i(t) = u(x_i(t), y_i(t))$. The sums are replaced with integrals over the time-averaged lateral positions $r_0 = (r(t))$,

$$ F_{ZG}(Q, t) = \frac{1}{N a^2} \int_{A_0} d^2 r_0 d^2 r_0' \left< \sqrt{\frac{\sigma}{\tau}} \sqrt{\frac{\sigma}{\tau}} e^{-iQ(r(r_0, t) - r(r_0', t))} e^{-iq_u(u(r_0, t) - u(r_0, t))} \right> . \quad (6.37) $$

Only the lowest order terms have been retained in these calculations, which means that local bilayer orientation has been ignored. Corrections to this approximation, which were calculated in Chapter 5, are of order $\left< (\nabla u)^2 \right>$. How the calculations proceed from here depend on the scattering geometry. The simplest case is that of nearly perpendicular scattering, $q_z \gg q$ because then the approximation $\sqrt{\frac{\sigma}{\tau}} \approx 1$ is adequate. For nearly parallel scattering, $q \gg q_z$ the lowest order terms from expanding the metrics must be kept (157). A general result for Gaussian distributions (121) is $\left< iQ \cdot R \right> = \prod_{a=x,y,z} \exp[-\frac{1}{2} Q_a^2 \langle |R|^2 \rangle]$. Then, for $q_z \gg q$, the intermediate scattering function becomes (156),

$$ F_{ZG}(Q, t) = \frac{1}{N a^2} \int_{A_0} d^2 r_0 d^2 r_0' e^{iQ(r(r_0, t) - r(r_0', t))} \Phi_f(|r(r_0, t) - r(r_0', t)|), \quad (6.38) $$

with the definition of the functions

$$ \Phi_f(|r_0 - r_0'|, t) = \left< (u(r_0, t) - u(r_0', 0))^2 \right> \quad (6.39) $$

$$ \Phi_i(|r_0 - r_0'|, t) = \left< (r(r_0, t) - r(r_0', 0) - (r_0 - r_0'))^2 \right> . \quad (6.40) $
These are the transversal, $\Phi_t$, and lateral, $\Phi_l$, correlators of two different points $r_0$ and $r'_0$, on the membrane at two different times, $t$ and $t'$. A self-consistent calculation relates them as $\Phi_t(|r_0 - r'_0|, t) = \frac{k_B T}{4k_c} \Phi_l(|r_0 - r'_0|, 2t)$ (159). Note the argument 2$t$, which is due to that the variance of $r \propto u^2$. Asymptotically they depend on the distance between two points as $|r_0 - r'_0|^2$, with the typical $r^2$ behavior that is characteristic to bending-dominated membranes (30). Expanding the undulating surface in a Fourier series and using the dispersion relation from Eq. (6.32), $\Phi_t$ can be written as a dimensionless integral,

$$\Phi_t(|r_0 - r'_0|, t) = \frac{1}{\pi} \left[ \frac{k_B T}{k_c} \right]^{1/2} \frac{k_B T t}{4\eta}^{2/3} \times \int_{(2\pi^2 k_c t / \eta)^{1/3}} dz \frac{1}{z^2} J_0 \left( \frac{z|r_0 - r'_0|}{\lambda(t)} \right) \left[ 1 - e^{-z^2} \right],$$

(6.41)

where $\lambda(t) = (k_c t / 4\eta)^{1/3}$ is a time-dependent length scale, $l$ is the smallest wavelength in the continuum description, and $L$ is the lateral system size. For times $\eta L^3 / k_c \ll t \ll \eta L^3 / k_c$, the integral limits may be replaced with zero and infinity, respectively, and it is possible to approximate the integral with an asymptotic form (156). Interestingly, this shows that a tagged monomer (with $r_0 = r'_0$) diffuses anomalously as $t^{2/3}$ on the membrane surface. This is caused by the undulations causing diffusion to occur over a larger length scale compared to a flat interface. In general cases a closed expression is not available for the integral in Eq. (6.41) and numerical evaluation is necessary (160).

Since $\Phi_t$ and $\Phi_l$ only depend on the difference variable $|r_0 - r'_0|$ one of the integrals in Eq. (6.38) is trivial. The other integral can be done without too much trouble with the mentioned asymptotic expansion. The result for the perpendicular scattering is

$$F_{ZG}(q, q_z, t) \propto e^{-\Gamma(q_0) t^{2/3} - \Gamma(q) q_z^{2/3}}, \quad q \ll q_z,$$

(6.42)
a stretched exponential decay in $t$. Including the metric correlations for the parallel geometry yields a slightly different but more complicated result,

$$F_{ZG}(q, q_z, t) \propto e^{-\Gamma(q_0) t^{1/3} - \Gamma(q) q_z^{1/3}} Y \left[ \frac{k_c q^3}{4\eta} t \right], \quad q \gg q_z,$$

(6.43)

where $Y[x]$ is a scaling function with the asymptotic forms (157)

$$Y[x] = \begin{cases} \pi \ln \left( \frac{2q}{2\pi} \right) - \frac{6\pi(4/3)^{2/3}}{2^{1/3}} x^{2/3} & x \ll 1 \\ \pi e^{-\gamma \left[ \ln \left( \frac{2q}{2\pi} \right) - \frac{x}{2} \right]} & 1 \ll x \ll \left( \frac{2q}{2\pi} \right)^3 \end{cases} \quad (\gamma \approx 0.577 \text{ is the Euler constant})$$

For short and intermediate times this is still a stretched exponential but in combination with an ordinary exponential, that will dom-
institute the long-time decay. There is no analytic solution for a general scattering geometry. The decay rates are

\[ \Gamma_t(q_z) = 0.025 \left( \frac{k_B T}{k_c} \right)^{1/2} \frac{k_B T}{\eta} q_z^3 \]  
(6.45)

\[ \Gamma_l(q) = 0.0022 \left( \frac{k_B T}{k_c} \right)^{4/3} \frac{k_B T}{\eta} q^3. \]  
(6.46)

The \( q^3 \)-relaxation is a result from combining the \( q^2 \)-terms in Eq. (6.38) with the anomalous diffusion result, \( t^{2/3} \).

Neutron spin-echo experiments (146–148, 150) have been interpreted with the formula derived from the nearly-perpendicular scattering geometry, Eq. (6.42). Although the scattering functions are well-fitted to stretched exponentials with stretching exponents close to 2/3, the obtained rates yield bending moduli that are roughly a factor of three too small compared to other experimental methods. Experiments have for a long time been interpreted with a higher viscosity than that of bulk water, typically \( \eta = 3\eta_{\text{H}_2\text{O}} \) (161). Monkenbusch et al. (160) pointed out that the integrals involved in the original Zilman-Granek calculation were sensitive to the cutoff values and showed that a careful numerical evaluation of the integrals leads to bending rigidities in better agreement with other measurements. Recently, Watson and Brown (162), proposed an improvement to ZG theory. They pointed out that the dynamics is determined by the relaxation of undulations, and that the original Kramer expression had been used. If the bi-exponential decay predicted by Seifert-Langer theory is used in Eq. (6.39), nothing changes on the NSE time scale, except that the bending modulus in all formulas are replaced with the renormalized Seifert-Langer rigidity \( \kappa \) (below Eq. (2.16)).

When it comes to testing ZG in simulations, few efforts have been carried out. The problem is that the length- and time scales involved are substantial to simulations. To ensure correct hydrodynamics, only wave vectors in the regime \( 2\pi/d \ll q \ll 2\pi/l \) should be probed. Here, \( d \) is the box repeat spacing and \( l \) is the ubiquitous small-wavelength cutoff. The upper limit is \( q_{\text{max}} = 6 \text{ nm}^{-1} \) using \( l = 1 \text{ nm} \), while the lower limit is set by the box size. A state-of-the-art computer simulation with a box of side 20 nm yields a small-\( q \) cutoff at 0.3 nm\(^{-1} \), which mostly erases the eligible wave vector interval. Further, the question is to what extent the correct hydrodynamics in the simulations is captured. There are clear differences between the correlation times presented in Paper IV and the available NSE data (146–148) in the small-\( q \) limit. For large \( q \) the simulation data is consistent with inelastic scattering experiments (132) and other molecular dynamics simulations (155). Testing ZG theory would require bilayer systems spanning roughly 100 nm, simulated for microseconds. This does not seem realistic for all-atom or united-atom simulations, but could be reached with coarse-grained simulations. It should be kept in mind, however, that the present coarse-grained water models are rather crude.
Stretched decay on molecular wavelengths

Smaller length scales are more amenable to simulations. Motions in membranes on these distances are probed in experiments by inelastic scattering of neutrons (128) or x rays (6) (as described in Chapter 4). On wavelengths comparable to the bilayer thickness and smaller, undulations can be neglected in the relaxation dynamics. The dynamics is determined by the molecular structure of the bilayer, as shown in Paper IV. However, fluctuations still decay as stretched exponentials, even at wavelengths that are clearly below ZG theory!

Small-wavelength stretched exponential dynamics is well-known to occur in glass-forming liquids (163), as a result of a degenerate energy landscape, making specific liquid configurations metastable and violating ergodicity. Polymeric liquids are glass-forming at low temperatures but lipid bilayers are fluid at substantially higher temperatures. Nevertheless, stretched exponential decay of the intermediate scattering function has been observed in lipid bilayers in neutron scattering experiments (164) and also in simulations (155) but no microscopic theory has been given.

In Paper IV, the data on the Rayleigh line is extended to investigate the intermediate scattering function over a broad range of wave vectors. The deviations from the single-exponential (Lorentzian) shape is found to be accurately accounted for by a stretched exponential function. Fig. 6.3 shows the dispersion relations and stretching exponents that have been calculated by fitting MD data to Eq. (6.35). The stretching exponents are found to only weakly depend on wave vector, taking on the constant values 0.45 for the united-atom model and 0.60 for the coarse-grained Martini model for most wave vectors. The dispersion relations are well-described by power laws in the wave vector. A crossover between two different regimes, \( q^{-2} \) and \( q^{-1} \), is found \( \sim 5 \text{ nm}^{-1} \) (Fig. 6.3). The undulations and thickness are practically indistinguishable from the density fluctuations for all but the smallest wave vectors. Only for the lowest modes can the undulations be separated. The thickness and density fluctuations are highly correlated for all wave vectors, which implies that fluctuations take place at constant volume. An extensive discussion about the dynamic correlation functions for undulations, thickness and density fluctuations are found in Paper IV.

The dispersion relations calculated from simulations differ from the experimental ones in the small-\( q \) limit. Given the fair agreement in the large-\( q \) regime, the hydrodynamic behavior is a likely source for this discrepancy, as discussed in the previous section. Other factors that could affect the small-\( q \) dynamics are differences in the scattering geometry between simulations and experiments and/or the multilamellarity employed in the experiments. Future experimental and theoretical work is surely to be aimed at giving a microscopic theory that accounts for the stretched behavior in lipid bilayers at large \( q \).
Figure 6.3: Dispersion relations (functions of the in-plane wave vector $q$) and stretching exponents obtained by fitting the intermediate scattering function to a stretched exponential function. $F(q, t)$ was calculated for undulations (black), thickness (red) and in-plane number density (green). Simulations were done on Berger lipids (united-atom, UA) and Martini lipids (coarse-grained, CG).
Chapter 7

Conclusions

Lipid membranes are simple but realistic models of cell membranes. Shape formation and motions in lipid bilayers take place on a wide range of different length- and time scales. Spatial fluctuations in biomembranes range from collective motions of thousands of lipids spanning micrometers, to individual molecular motions on fractions of nanometers. Temporal fluctuations stretch from pore formation taking seconds, to the rapid vibrations in the hydrogen bonds, occurring in femtoseconds. Lipid membranes are peculiar in that they are relatively easy to bend, soft, and therefore largely influenced by the random thermal motions of individual molecules. Computer simulations have emerged, in combination with advances in scattering techniques, as an invaluable tool to study systems that reside in the mesoscopic regime — where the relevant distances are nm and the relevant times are ns. Recent improvements in computer hardware and development of novel simulation algorithms make it possible to simulate systems with millions of particles for millions of time steps. Whether the trend of increasing computer power will continue is for the future to tell.

Molecular dynamics simulations on large patches of lipid bilayers have been performed that allow the membranes to develop realistic large-scale fluctuations as present in real cell membranes. Newly developed methods, presented in this thesis, makes it possible to efficiently analyze these fluctuations in Fourier space. It is shown, that by taking the molecular structure of lipid membranes into account, a formalism emerges that unifies the innate molecular bilayer structure with elastic continuum theories. Further, it is shown that taking membrane undulations into account is crucial to obtain accurate values when calculating local bilayer properties, like electron density profiles (EDP) and lipid areas. This will be of great value to facilitate the future comparison of simulation data to experiments.

The liquid properties of lipid membranes are also unique due to the fluctuating bilayer interface, and the molecular structure. A careful investigation and comparison of membrane dynamics to the predictions of generalized hydrodynamics show that although the small-wavelength behavior of lipid bilayers can be described largely by the linearized hydrodynamic equations, important differences occur on longer scales.
In contrast to simple liquids, fluctuations in fluid membranes decay as stretched exponentials. Dispersion relations and stretching exponents of lipid bilayers have been calculated from molecular dynamics simulations, and the decay rates are found to be power laws in the wave vector, but with approximately constant stretching exponents. Future work is needed to give a microscopic theory for this stretched decay in the framework of generalized hydrodynamics.
Chapter 8

Outlook

Molecular dynamics can at the time of writing be performed on detailed membrane models to provide accurate data on the atomistic structure of lipid membranes. However, due to that the simulations are so time-consuming, they are restricted to small systems and short times. Future work will be directed into extending simulations to make accurate predictions for larger scales and longer times where neutron scattering data is available. This is out of reach for all-atom simulations, and new, efficient models and algorithms need to be developed. In particular, including hydrodynamic effects is crucial to obtain the correct membrane dynamics (139). This is especially true for phenomena relevant to the cell as a whole. As more experimental data is made available, accurately modeling membrane hydrodynamics will be a major challenge to computer simulations. Simulations will be an integral part in underpinning new theories, as well as to guide interpretations of future scattering experiments. An important stepping stone is to find a common way to analyze membrane fluctuations over all length scales. In addition, new scattering theories are needed to account for more complex systems, most prominently to include membrane-protein interactions.

The local static and dynamic properties of the lipid bilayer, where its continuum description breaks down, still lack a microscopic theory. Such a theory should be able to predict and account for the stretched exponential decay of the intermediate scattering function, that has been reported from experiments (132, 164) and simulations (4, 155). Once the details of such a theory has been worked out, it should be generalized to other geometries; in particular the spherical geometry of vesicles is of interest. One problem to overcome lies in that the eigenfunctions of the surface in spherical geometry are not Fourier series but spherical harmonics, and require an analog to the Fast Fourier transform for efficient calculations.
Chapter 9

Summary of the papers

9.1 Paper I

The paper introduces a novel method to calculate the fluctuation spectrum from lipid bilayer simulation, by direct analysis in Fourier space. The approach underlines how the molecular fluctuations present on the smallest wavelengths coalesce with the collective fluctuations that are predicted from elastic theory. The implications for interpreting the fluctuation spectrum in light of continuum theories are discussed, along with limitations imposed by the membrane material constants. Fluctuation spectra calculated for two different membrane models show no footprint from molecular protrusions or tilt. Except for pure bending, other contributions are submerged by fluctuations in the molecular density.

9.2 Paper II

The paper builds on the theoretical results derived in Paper I to develop novel methods that can extract accurate electron density profiles (EDP) and lipid areas from simulations of large, undulating membranes. Undulations are shown to blur out all transverse membrane profiles. Correcting for this artifact is crucial to facilitate comparison between simulations and experiments. An array of different methodologies are compared to find the optimal way to create an undulation reference surface. Finite size effects in the calculations of EDP and areas from simulations are also investigated in detail.

9.3 Paper III

The small-wavelength density fluctuations of the lipid bilayer are calculated from molecular dynamics simulations. The results are compared to inelastic neutron and x-ray scattering data, and to the predictions of linearized hydrodynamic theory. The dynamic structure factor is found to have the same shape as predicted by linearized hydrodynamics, but the long-time decay of the central Rayleigh line is found to deviate
from single-exponential behavior. Membrane material constants are determined and discussed.

9.4 Paper IV

The slow relaxation dynamics in lipid bilayers, which was discovered in Paper III to deviate from a single exponential, is investigated in detail. It is found to be well-described by a stretched exponential over a broad range of length- and time scales. The dispersion relations and stretching exponents are calculated for different membrane models, and are shown to obey power laws in the wave vector, while the exponents are approximately constant. The exponents are determined to 0.45 for the united-atom model, and 0.60 for the coarse-grained model.
Appendix A

Derivation of the Rayleigh-Brillouin triplet

A.1 Perturbation solution of the dispersion equation

The hydrodynamic dispersion equation, Eq. (6.15), can be written to order $q^2$,

$$z^3 + (u + v)z^2 + z + \gamma^{-1}u = 0,$$  \hspace{2em} (A.1)

using the reduced variables $z = s/cq$, $u = aq^2/cq$ and $v = bq^2/cq$. Treating $u$ and $v$ as small quantities allow for a perturbation approach to find approximate solutions to Eq. (A.1), valid as long as $aq^2$ and $bq^2$ are small compared to $cq$. With the small perturbation terms $\epsilon_1 = u + v$ and $\epsilon_2 = \gamma^{-1}u$, the approximate solution is $z \approx z_a + \epsilon_1 z_b + \epsilon_2 z_c$ to lowest order in $\epsilon_1$ and $\epsilon_2$ (linear in $u$ and $v$ or $q^2$). Keeping only linear terms and inserting into Eq. (A.1) gives:

$$z^3_a + z_a + \epsilon_1 \left[ 3z_a^2 z_b + z_a^2 + z_b \right] + \epsilon_2 \left[ 3z_c^2 z_c + z_c + 1 \right] = 0.$$  \hspace{2em} (A.2)

Equating the coefficients of $\epsilon_1$ and $\epsilon_2$ to zero so that Eq. (A.2) is satisfied for all values of $\epsilon_1$ and $\epsilon_2$, yields three equations:

$$z_a^3 + z_a = 0 \hspace{2em} (A.3a)$$
$$3z_a^2 z_b + z_a^2 + z_b = 0 \hspace{2em} (A.3b)$$
$$3z_c^2 z_c + z_c + 1 = 0, \hspace{2em} (A.3c)$$

from which the unknown numbers $z_a$, $z_b$ and $z_c$ can be determined. The first equation gives $z_{a,0} = 0$ and $z_{a,\pm} = \pm i$, the second $z_{b,0} = 0$ and $z_{b,\pm} = -\frac{1}{2}$ and the third $z_{c,0} = -1$ and $z_{c,\pm} = \frac{1}{2}$. Putting everything together the approximate solutions to Eq. (A.1) are:

$$z_0 = -\gamma^{-1}u \quad \text{and} \quad z_{\pm} = \pm i - \frac{1}{2} \left[ u(1 - \gamma^{-1}) + v \right],$$  \hspace{2em} (A.4)
or, in terms of the original variables,

\[ s_0 = -D_T q^2 \quad \text{and} \quad s_{\pm} = \pm icq - \beta q^2. \]  

(A.5)

In these expressions, \( D_T = \gamma^{-1} a \) is the thermal diffusivity and \( \beta = \frac{1}{2} \left[ a(1 - \gamma^{-1}) + b \right] \) is the sound attenuation coefficient.

### A.2 Partial fraction of Eq. (6.17)

The partial fraction of Eq. (6.17) is

\[
S(q, \omega) = 2\text{Re} \left[ \frac{A_1(q)}{\omega - s_0} + \frac{A_2(q)}{i\omega - s_+} + \frac{A_3(q)}{i\omega - s_-} \right],
\]

(A.6)

and the \( q \)-dependent coefficients are to be identified from solving the linear equation system,

\[
\begin{pmatrix}
1 & -2\beta q^2 & \frac{1}{c^2 q^2 + \beta^2 q^4} \\
-icq - (D_T + \beta)q^2 & D_T q^2 (\beta q^2 + icq) & \frac{1}{D_T q^2 (\beta q^2 + icq)} \\
c^2 q^2 + \beta^2 q^4 & \frac{1}{(D_T q^2 + \beta q^2 + icq)} & \frac{1}{(D_T q^2 + \beta q^2 + icq)}
\end{pmatrix}
\begin{pmatrix} A_1 \\ A_2 \\ A_3 \end{pmatrix}
= \frac{1}{(1 - \gamma^{-1}) c^2 q^2}.
\]

(A.7)

The real and imaginary parts of the coefficients are to lowest order in \( q \):

\[
\text{Re}A_1 = \frac{\gamma - 1}{\gamma},
\]

(A.8a)

\[
\text{Im}A_1 = 0
\]

(A.8b)

\[
\text{Re}A_2(q) = \text{Re}A_3(q) = \frac{1}{2\gamma}
\]

(A.8c)

\[
\text{Im}A_2(q) = -\text{Im}A_3(q) = -\frac{1}{2\gamma} \left[ \beta + D_T (\gamma - 1) \right] c^{-1} q.
\]

(A.8d)

Inserting these coefficients in Eq. (A.6) and keeping only the real valued parts, one obtains:

\[
\frac{S(q, \omega)}{S(q)} = \frac{2(\gamma - 1)}{\gamma} \frac{D_T q^2}{\omega^2 + (D_T q^2)^2} + \frac{1}{\gamma} \left[ \frac{\beta q^2 - (\beta + D_T (\gamma - 1) c^{-1} q)(\omega - cq)}{(\omega - cq)^2 + (\beta q^2)^2} + \frac{\beta q^2 + (\beta + D_T (\gamma - 1) c^{-1} q)(\omega + cq)}{(\omega + cq)^2 + (\beta q^2)^2} \right].
\]

(A.9)

Collecting terms and rewriting yields Eq. (6.18).
References


