Modeling of biomembranes: from computational toxicology to simulations of neurodegenerative diseases

Inna Ermilova

In this doctoral thesis computer simulation methods are discussed as good tools to investigate different biomedical problems on a molecular level. The problems were such as the nature of high content of cholesterol in different tissues, Alzheimer’s disease (or the aggregation of amyloid-ß peptides) and toxicity of flame retardants. Phospholipid bilayers of various compositions were used as models for cell-membranes and amyloid-ß peptides were serving as precursors of Alzheimer’s disease. The presence of unsaturated phospholipids in membranes affected the ability of different molecules to penetrate through lipid bilayers. Particularly, amyloid-ß peptides showed less aggregation on bilayer surfaces when higher amounts of omega-3 phospholipids were present in simulated systems. For membranes containing less omega-3 lipids higher aggregation of the peptides was observed. Higher aggregation of peptides is related to the development of Alzheimer’s diseases.
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Abstract
It was known from the middle of the last century that a cell-membrane is a lipid bilayer. Since that time a large number of experimental studies has been done in order to see how a certain molecule can penetrate through a membrane. Due to the complexity of laboratory experiments computational chemistry became a convenient tool for investigations involving this process. In a real life a compound has to pass through several membranes of different chemical composition before reaching the actual target. Such a diversity in constitution gives a various selectivity to cell-membranes: some molecules will penetrate through them and others will not. That is why the development and a choice of suitable models for lipid bilayers are important steps in such a research. In this thesis new all-atomistic models for polyunsaturated phospholipids in cis conformations have been derived and added to the SLipids force field. After a successful force field validation, the new lipid models were used in molecular dynamics and well-tempered metadynamics simulations of several problems, such as toxicity of hydroxylated polybrominated diphenyl ethers (OH-PBDE), behavior of cholesterol in various membranes, an aggregation of amyloid-β (Aβ) peptides. The significance of the presence of lipid unsaturation has been demonstrated by all computations. 2'-OH-BDE68 (ortho) showed the affinity to saturated lipid bilayer, but had more conformational variations in the center of the unsaturated membrane. Cholesterol did not exhibit the preference to polysaturated lipid bilayers from free energy calculations, but the diversity in orientations of this molecule, depending on its locations was observed. The behavior of Aβ peptides was dependent on membrane saturation as well. The insertion of Aβ peptides was detected in lipid bilayers containing higher amounts of polyunsaturated phospholipids, while in systems with more saturated membranes amyloids aggregated on membrane surfaces. Moreover, a comparison of simulations for quadro- and mono-component lipid bilayers showed that the membrane built of 18:0-22:6 PC can serve as a good model for the 'healthy' tissue of a human brain. Also the lipid bilayer built of 14:0-14:0 PC exhibited similar features as the quadro-lipid membrane representing the brain tissue affected by Alzheimer’s disease. Good agreement of some computational results with available experimental findings demonstrated the applicability of computer simulations to real life problems.

Keywords: biomembranes, lipid bilayers, Alzheimer's disease, Parkinson disease, computational toxicology, passive diffusion, hydroxylated polybrominated diphenyl ethers, omega-3, omega-6, amyloid beta peptide, molecular dynamics simulations, SLipids force field.

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MODELING OF BIOMEMBRANES: FROM COMPUTATIONAL TOXICOLOGY TO SIMULATIONS OF NEURODEGENERATIVE DISEASES

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To my parents and my supervisor, to Nikos and Diana and their hot summer of projects 2018, to my former bachelor students...
"... If you’ve done nothing wrong
You’ve got nothing to fear
If you’ve something to hide
You shouldn’t even be here
You’ve had your chance
Now we’ve got the mandate
If you’ve changed your mind
I’m afraid it’s too late

We’re concerned
You’re a threat
You’re not integral
To the project

Everyone has
Their own number
In the system that
We operate under
We’re moving to
A situation
Where your lives exist as
Information

One world
One life
One chance
One reason
All...

(Integral, Pet Shop Boys, 2007)
Abstract

It was known from the middle of the last century that a cell-membrane is a lipid bilayer. Since that time a large number of experimental studies has been done in order to see how a certain molecule can penetrate through a membrane. Due to the complexity of laboratory experiments computational chemistry became a convenient tool for investigations involving this process. In real life a compound has to pass through several membranes of different chemical composition before reaching the actual target. Such a diversity in constitution gives a various selectivity to cell-membranes: some molecules will penetrate through them and others will not. That is why the development and a choice of suitable models for lipid bilayers are important steps in such a research. In this thesis new all-atomistic models for polyunsaturated phospholipids in cis conformations have been derived and added to the SLipids force field. After a successful force field validation, the new lipid models were used in molecular dynamics and well-tempered metadynamics simulations of several problems, such as toxicity of hydroxylated polybrominated diphenyl ethers (OH-PBDE), behavior of cholesterol in various membranes, an aggregation of amyloid-β (Aβ) peptides. The significance of the presence of lipid unsaturation has been demonstrated by all computations. 2'-OH-BDE68 (ortho) showed the affinity to saturated lipid bilayer, but had more conformational variations in the center of the unsaturated membrane. Cholesterol did not exhibit the preference to polysaturated lipid bilayers from free energy calculations, but the diversity in orientations of this molecule, depending on its locations was observed. The behavior of Aβ peptides was dependent on membrane saturation as well. The insertion of Aβ peptides was detected in lipid bilayers containing higher amounts of polyunsaturated phospholipids, while in systems with more saturated membranes amyloids aggregated on membrane surfaces. Moreover, a comparison of simulations for quadro- and mono-component lipid bilayers showed that the membrane built of 18:0-22:6 PC can serve as a good model for the 'healthy' tissue of a human brain. Also the lipid bilayer built of 14:0-14:0 PC exhibited similar features as the quadrolipid membrane representing the brain tissue affected by Alzheimer’s disease. Good agreement of some computational results with available experimental findings demonstrated the applicability of computer simulations to real life problems.
List of Papers

The following papers, referred to in the text by their Roman numerals, are included in this thesis. Reprints were made with permissions from the publishers.

PAPER I: Extension of the SLipids force field to polyunsaturated lipids
I. Ermilova and A. P. Lyubartsev
*The Journal of Physical Chemistry B, 120 (50), 12826-12842 (2016).*
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PAPER II: Quantum chemical and molecular dynamics modelling of hydroxylated polybrominated diphenyl ethers
I. Ermilova, S. Stenberg A.P . Lyubartsev
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DOI: 10.1039/C7CP03471G

PAPER III: Cholesterol in phospholipid bilayers: positions and orientations inside membranes with different unsaturation degrees
I. Ermilova and A.P . Lyubartsev
*Soft Matter, 15, 78-93 (2019).*
DOI: 10.1039/C8SM019037A

PAPER IV: Effects of lipid saturation on amyloid-beta peptides partitioning and aggregation in neuronal membranes: molecular dynamics simulations
N. Ntarakas, I. Ermilova and A.P . Lyubartsev
*Submitted (2019)*

PAPER V: Modelling of interactions between Aβ(25 – 35) peptide and phospholipid bilayers: effects of cholesterol and lipid saturation
I. Ermilova and A.P . Lyubartsev
*Submitted (2019)*
The following is a list of papers by the author not included in this thesis.

PAPER VI: Molecular dynamics simulations of furfural and 5-hydroxymethylfurfural at ambient and hydrothermal conditions
F. Grote, I. Ermilova and A.P. Lyubartsev
DOI: 10.1021/acs.jpcb.8b03350
Abbreviations

MD - molecular dynamics
AD - Alzheimer’s disease
PD - Parkinson disease
FF - force field
EE - expanded ensemble
GAFF - General Amber force field
RDF - radial distribution function
Aβ - amyloid beta
SS-NMR - solid state nuclear magnetic resonance spectroscopy
PME - Particle Mesh Ewald
LJ - Lennard-Jones
DHA - docosahexaenoic acid
PUFA - polyunsaturated fatty acid
LOEC - lowest observed effect concentration
PMF - potential mean force
UV/VIS - ultraviolet-visible spectroscopy
fs - femtosecond ($10^{-15}$ s); ps - picosecond ($10^{-12}$ s)
ns - nanosecond ($10^{-9}$ s); µs - microsecond ($10^{-6}$ s)

Abbreviations and names for some lipids

DMPC - 1,2-dimyristoyl-sn-glycero-3-phosphocholine (14:0-14:0 PC)
POPC - 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (16:0-18:1 (cis) PC, ω-9)
DDPC - 1,2-didocosahexaenoyl-sn-glycero-3-phosphocholine (22:6-22:6 (cis) PC, ω-3)
DDPE - 1,2-didocosahexaenoyl-sn-glycero-3-phosphoethanolamine (22:6-22:6 (cis) PE, ω-3)
DSPE - 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (18:0-18:0 PE)
DPPC - 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (16:0-16:0 PC)
DOPC - 1,2-dioleoyl-sn-glycero-3-phosphocholine (18:1-18:1 (cis) PC)
DLPC - 1,2-dilauroyl-sn-glycero-3-phosphocholine (12:0-12:0 PC)
SOPC - 1-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine (18:0-18:1 (cis) PC, ω-9)
PiLPC - 1-hexadecanoyl-2-octadecadienoyl-sn-glycero-3-phosphocholine (16:0-18:2 (cis) PC, ω-9)
PDPC - 1-palmitoyl-2-docosahexaenoyl-sn-glycero-3-phosphocholine (16:0-22:6 (cis) PC, ω-3)
SAPC - 1-stearoyl-2-arachidonoyl-sn-glycero-3-phosphocholine (18:0-20:4 (cis) PC, ω-6)
DAPC - 1,2-diarachidonoyl-sn-glycero-3-phosphocholine (20:4-20:4 (cis) PC, ω-6)
PG - phosphatidylglycerol
PC/PCs - phosphatidylcholine/phosphatidylcholines (phosphocholine)
PE/PEs - phosphatidylethanolamine
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1. Introduction

Nowadays a life is not possible without computers. No matter what a human being wants to do, his desires will more likely be processed by machines. Moreover, it became quite popular to simulate real life events before performing them in reality. For instance, computer games like "Second Life" can let their users live different kinds of virtual lives, simulate various occasions before making crucial decisions [1, 2]. This kind of approach is useful in science as well. In fact, modern biophysics and biochemistry were strongly affected by computer simulations.

The first simulations were done in the middle of the twentieth century on small systems using primitive machines and even without taking into account real chemistry [3–5]. However, the scientific progress has gone quite fast, and within fifteen-twenty years since modeling the system containing of sixty-four particles [3], human beings became able to develop physically relevant models [6–8] and run more sophisticated simulations [7, 9], which could mimic real-life phenomena.

A dream of computational biochemists is to be able to reproduce a real functioning biological cell with all chemical reactions in it. Nevertheless, a cell is a quite big and complex specie, and all dreams can not come true: one will have to choose the right resolution for such a simulation and the fastest accurate model. In this thesis an all-atom level is going to be discussed with the simplified model of the cell-membrane which is a lipid bilayer. The latter one has been used successfully by experimentalists since 1950’s [10] and by the simulation community since 1970’s [8]. Regardless a pretty long history there still are unanswered questions about this simple model-membrane, because there are various kinds of lipids and every kind has subclasses. For instance, phosphatidylcholines, which have been used through this thesis, can be saturated and unsaturated. The different degree of saturation affects physical properties of lipids and membranes, where they are present. Most of models, which were developed earlier, have been made for saturated species or for ones containing an isolated double bond. The lack of good theoretical description has made them very attractive for the
SLipids force field extension, which will be discussed in Chapter 6.

Then the availability of accurate models for lipids can help to study processes of passive diffusion through a membrane, which are very essential in modern drug design as well as in computational toxicology. For instance, lipids have to be clinically approved for being used as drug carriers [11, 12]. Even if the drug carrier would be in a solid state the approval still would be needed. This makes medicines which can to pass through a lipid bilayer without any additional support more favorable, because in a real life such a substance must be able to penetrate through more than one membrane.

Furthermore, in computational toxicology the process of passive diffusion of a substance through a membrane is highly considered too. If a compound has an affinity to a membrane then there is a risk that it can be toxic for an organism.

However, the affinity of a certain chemical substance to a particular cell can depend on cell’s lipid composition. For example, considering Alzheimer’s disease one can notice that it affects certain regions of a human brain and the lipid composition of those regions differs for those who are suffering from it and people who are considered as healthy [13, 14]. Particularly, the change of amount of polyunsaturated phospholipids and cholesterol is related to the progress of this disease.

The main focus of this thesis was to extend the existing force field with accurate numerical models for polyunsaturated phospholipids, then apply them to study effects of saturation on a behavior and a passive diffusion of different substances in membranes. The substances were related to various problems: the toxicity of hydroxylated polybrominated diphenyl ethers, the behavior of cholesterol in different lipid bilayers, the aggregation and partitioning of precursors of neurodegenerative diseases. Understanding the role of polyunsaturated lipids in development or inhibition of described problems could help in future drug design.
2. From lipid bilayers to cells

A human body and its functions are in the center of interests of all existing industries in the world. Manufacturing of any product has to be done by taking into account its toxicity, which is related to biochemical and biophysical processes happening in mammalian cells. The latter ones are known as structural units building tissues in living organisms.

Compositions of cells are different depending on tissues. Such a diversity makes it very difficult to investigate possible reaction mechanisms, which could take a place in case of environmental changes or a drug usage. Since it was discovered that a cell membrane (Figure 2.1) is a lipid bilayer [10, 16, 17] which can be stable and exist without proteins [18], simplified models of biological systems have become useful tools for medical research.

Because lipids are so important and "independent" in a sense of building steady structures, such as membranes, without the presence of other compounds a question might arise in a mind: what are these
molecules? There are many definitions can be found in literature. Foremost, lipids are molecules with biological origin which are known to be soluble in non-polar solvents [19]. Their nature is amphiphilic and one can divide them into following groups: glycerophospholipids, saccharolipids, sphingolipids, fatty acids, prenol lipids, sterol lipids, polyketides, glycerolipids (one lipid per group is presented on Figure 2.2). From this variety of species one shall select molecules which are able to build membranes, because the latter ones are of interest [20]. Such a choice shall be done depending on the problem, which is going to be studied, or, better to say, on cells where a particular phenomenon occurs. However, in this work the attention has gone to cholesterol (Figure 2.5) and glycerophospholipids (examples are on Figure 2.2(G) and Figure 2.3), which can be shortly called as "phospholipids" ("phosphatidylcholines" or "phosphatidylethanolamines").

Mono-lipid membranes were popular systems used by scientists which were built from the knowledge about the most represented species in the cell. Phosphatidylcholines are abundant in many tissues from human skin to a brain [14, 21–23]. Particularly, 1,2-dimyristoyl-sn-glycero-3-phosphatidylcholine (14:0-14:0 PC or DMPC) received a lot of attention from biophysicists. The presence of this lipid in many tissues of a human body and its fluidity at the room temperature make it more appropriate for working in the laboratory. Another reason for selecting 14:0-14:0 PC is because of its saturation which is resulting in chemical "stability" of bilayers formed only by this compound: lipid peroxidation (oxidative degradation), which happens in unsaturated lipids, does not occur in DMPC.

However, some other membrane-building lipids are used by researchers as well, but less frequently than 14:0-14:0 PC. Giving few more examples from PCs which are utilized in experiments, one can name 12:0-12:0 PC (DLPC), 16:0-16:0 PC (DPPC), 16:0-18:1 PC (POPC), 18:0-18:1 PC (SOPC).

2.1 The role of saturation in cells and lipid bilayers

The variety of lipids in a cell membrane is a known fact. Also within a class of lipids one can define sub-classes. For instance, phosphatidylcholines can be classified depending on lipid tails as following: saturated, unsaturated and with mixed acyl chains (Figure 2.3).

Saturation of lipid tails is important for a cell’s life. In case of breast
Figure 2.2: Examples of lipids (A) Prenol lipid (2E,6E-farnesol) (B) Sterol lipid (ergosterol) (C) Polyketide lipid (erythromycin, antibiotic) (D) Fatty acid (docosahexaenoic acid, DHA) (E) Glycerolipid (1-oleoyl-2-stearoyl-3-palmitoyl-sn-glycerol) (F) Sphingolipid (N-tetradecanoyl-sphing-4-enine) (G) Saccharolipid (lipid A from the bacteria E.coli) (H) Glycerophospholipid (1,2-dilauroyl-sn-glycero-3-phosphoethanolamine, DLPE)
Figure 2.3: Some phospholipids. (A) 14:0-14:0 PC, DMPC (saturated). (B) 18:0-22:6 PC, SDPC (with mixed acyl chains). (C) 22:6-22:6 PC, DDPC (unsaturated).

Figure 2.4: Fatty acids. (A) Stearic acid (saturated). (B) Elaidic acid (unsaturated, trans). (C) Oleic acid (unsaturated, cis).

cancer cells higher amount of eicosapentaenoic and docosahexaenoic fatty acids in lipid rafts can induce their apoptosis (cell’s death) [24] while in case of neuronal cells these particular species are vital [14, 22, 25–27].

Nevertheless, unsaturated phospholipids, depending on their conformations, can also be undesired compounds in a cell. These species can be divided into two sub-groups as well: cis and trans. The presence in a membrane of latter ones is seen as a possible cause for various diseases [28–30], while the higher content of cis configurations in some tissues is related to healthy conditions [14, 26].

Such a variety in fatty acids, which are present in lipids, raises
a question about their biophysical functions in cellular membranes. Firstly, one can mention structural similarities between saturated lipids and unsaturated ones in trans conformations (see Figure 2.4). This resemblance has a consequence on physical properties of membranes and lipid bilayers. Already in 1960’s it was discovered that unsaturated lipid bilayers have lower phase transition temperatures than saturated ones and, particularly, comparing unsaturated ones it was detected that trans conformations have higher melting point than cis [31–34]. Afterwards other groups of researchers have found that membranes built of unsaturated lipids in trans conformations have similar physical properties with those which contain saturated ones [35]. Lipid bilayers containing trans conformations had higher order and were affected more by cholesterol’s condensing effects than membranes consisting of more unsaturated lipids in cis conformations [35–37].

However, the health benefits and the abundance of cis polyunsaturated fatty acids in some human tissues made them very attractive for further studies by various methods: dilatometry, solid state nuclear magnetic resonance spectroscopy (SS-NMR), x-ray and neutron diffraction/scattering, electron spin resonance, computational methods etc [7, 38–42]. Higher content of these species in cell membranes is strongly related to disorder in lipid bilayers and fluidity, while the absence of polyunsaturated phospholipids can explain rigidity of a particular membrane [43–47]. For a biochemist or a pharmacologist it is important to know such properties when developing drugs or understanding possible interactions of various molecules with cells.

2.2 Cholesterol and lipid bilayers

Cholesterol became a subject for many debates. Fitness industry is all about getting rid of the fat from the body. Higher content of this species in some human tissues is related to diseases such as cancer, Alzheimer’s, Parkinson, HIV etc [13, 48–50]. At the same time low levels of cholesterol are associated with higher risks of getting ill as well [51]. So what is cholesterol?

In fact, cholesterol molecule (see Figure 2.5) is a lipid as well, but its structure is different from phospholipids, which are discussed in this thesis [52]. This molecule belongs to sterols, which were mentioned earlier in this chapter. It is quite rigid, what has an impact on cell-membranes, where it is present. However, studying the behavior
of cholesterol in real cells is difficult due to the complexity of the latter ones. Artificially created model membranes could help to answer questions regarding the behavior of cholesterol inside them and relate to the behavior of lipids depending on bilayers’ cholesterol content.

Already in the beginning of 1970’s it was known that cell membranes were heterogeneous where a coexistence of gel and liquid crystalline domains could be observed by various experimental techniques [34]. Studies with different lipid bilayers have confirmed that cholesterol can affect physical properties of a certain membrane [47, 53–55]. For example, the diffusion of lipids decreases with additional cholesterol [56, 57]. Moreover, it was observed that cholesterol can build domains in lipid bilayers at high concentrations which are similar to ones which were found in cells and were called as lipid rafts [58, 59]. These domains were ordered and they affected the order of membrane lipids as well [57, 60–62]. Comparing saturated with unsaturated phospholipid bilayers, various experiments [55, 63–66] have shown that cholesterol has an affinity to saturated acyl-chains of phospholipids and an aversion to unsaturated lipid tails.

2.3 Membranes and other molecules: the importance of a passive diffusion

Cell membrane permeability has to be considered while designing drugs, cosmetics or estimating a toxicity of a substance. There are many ways to deliver a medicine through a cell-membrane, but the passive diffusion (see Figure 2.6) through it is the most favorable, because this process can go spontaneously [67]. Such a process is of a high interest,
especially, if a drug can go to the target without any carrier [68, 69]. In case of toxicity studies, a better penetration of molecules through a cell by the passive diffusion can explain the aggregation of poisonous compounds inside cells, their ability to damage organisms and possible cell’s death [70–72].

Membrane permeability can be investigated experimentally by using living organisms as well as animals, what is called as in vivo experiments. One can feed animals with a drug, see an effect, then dissect them and control by using analytical experimental tools where the particular compound is concentrated, or which organs were affected [73, 74]. Regardless, that this method can give good results, it is not perfect: animals do not survive in such experiments.

Then one can perform in vitro experiments using cells taken from a living animal without sacrificing it. Here it is possible to learn about the cell’s death by "feeding" the substance to them [75, 76]. Further analysis using experimental techniques can give the desired information. Nevertheless, a detailed knowledge about mechanisms of reactions might not be obtained due to the complexity of cells.

However, there still is a way to simplify the experiment by conducting it on lipid bilayers, vesicles containing lipids or using certain
molecules from the cell in order to get more emphasized information about possible mechanisms [77–79]. Here the experiment will be also biased by a sample preparation, and maybe not so realistic environment comparing to a living organism, but a useful knowledge can be obtained.

Finally, in order to conduct a perfect research on such a biochemical topic the best solution could be to combine all experimental methods with computer simulations (in silico experiments). Then the computational world can offer various techniques for studying precisely a penetration of toxic and medical compounds through cell-membranes and their effect on properties of membranes [80–83].
3. Classical molecular dynamics simulations

Biological systems are built of molecules which consist of atoms. Every atom has a mass, coordinates and moves in the space with a certain velocity. Atomic motion has different time-scales comparing to a motion of large objects, like humans and cars, for example. Normally, it is considered that a position of an atom is changing within 1-2 fs. A knowledge of sizes and scales have been obtained experimentally. Lately, this information was used for building numerical models for computer simulation methods.

Molecular dynamics (MD) simulations is one of many methods which can be applied for studying biological systems on a molecular level. It is a computer based technique using Newtonian equations of motion which are solved numerically by some integrator, for example, leap-frog or velocity Verlet. During such a simulation a trajectory is produced in a form of coordinates of atoms or particles, which are written in so-called frames at every time-point. The input for a computation consists of starting coordinates and the force field.

Assume that there is a system, containing number of particles equal to $N$. Every particle has a mass $m_i$ and a spatial coordinate $r_i$ then one can write Newtonian equations as:

\[ \dot{r}_i = v_i, \]
\[ \dot{v}_i = \frac{F_i(r)}{m_i}, \] (3.1)

Here $v_i$ are velocities of particles at the time $t$, $F_i$ stands for forces which can be calculated from the total potential energy $E_{\text{pot}} = U(r)$ from the equation:

\[ F_i(r) = -\nabla_i U(r). \] (3.2)

The total energy of the system, which is the sum of potential and
kinetic energies ($E_{\text{total}} = E_{\text{pot}} + E_{\text{kin}}$), is conserved. This can be proved by a following equation [84]:

$$\frac{dE_{\text{total}}}{dt} = \frac{d}{dt} \left( \sum_i 0.5 \cdot m_i v_i^2 \right) + \frac{dU(r)}{dt} = \sum_i m_i v_i \cdot \dot{v}_i + \sum_i \frac{\partial U}{\partial r_i} \cdot v_i =$$

$$= \sum_i (v_i \cdot F_i + \frac{\partial U}{\partial r_i} \cdot v_i) = 0. \quad (3.3)$$

Considering that particles are constantly moving, one can assume that there is a defined finite time-step $\Delta t$ for their motion, described in equations (3.1). Then the leap-frog algorithm for solving equations (3.1) can be expressed in one dimension as following. Let’s say that the starting velocity and the position of a particle are written as $v(t - 0.5\Delta t)$ and $x(t)$ respectively, then after some $\Delta t$ the update for these two parameters will be [84]:

$$v(t + 0.5\Delta t) = v(t - 0.5\Delta t) + \frac{F_i(x(t))}{m_i} \Delta t,$$

$$x(t + \Delta t) = x(t) + v(t + 0.5\Delta t) \Delta t. \quad (3.4)$$

Another algorithm is the Verlet algorithm which employs a second derivative of the position without using any velocities:

$$\ddot{x}(t) \approx \frac{x(t - \Delta t) - 2x(t) + x(t + \Delta t)}{(\Delta t)^2}, \quad (3.5)$$

what follows by an approximated predicted position at a certain time-point:
\[ x(t + \Delta t) = 2x(t) - x(t - \Delta t) + f(t)(\Delta t)^2 + O((\Delta t)^4). \]  

(3.6)

Then the velocity can be calculated as:

\[ v(t) = \frac{x(t + \Delta t) - x(t - \Delta t)}{2\Delta t} + O((\Delta t)^2). \]

(3.7)

Here in equations (3.6) and (3.7) a letter O stands for a bound (an order) of the chosen approximation.

Nevertheless, MD simulations are not only about solving the Newtonian equations of motion. Modeling real systems is an impossible task: they are too large for existing software and hardware and too complex to recreate. The only way to perform computational experiments with a sense is to put limits and to select a reasonable amount of molecules and atoms, which will not lose essential parts of a studied system. Normally, such simulations are restricted to a user-selected "box". Then this has implications on physics of a system. In order to take care of physical properties of systems during simulations, ensembles, force fields and bond optimization algorithms are used.

### 3.1 NVT and NPT ensembles

When designing an MD simulation, it is important to choose conditions for your system such as temperature, pressure, volume etc. Depending on what kind of biological system one likes to model, the choice of ensemble is one of the important parts particularly for so-called "production runs", which are used for the final analysis.

#### 3.1.1 Temperature coupling

For example, if a user knows exact parameters of the system such as volume (or density) at a defined temperature it might be convenient to perform calculation using canonical ensemble which is known as NVT ensemble. During such a calculation the volume, number of particles (atoms and molecules) and the temperature will be kept constant during the whole run. Originally J.W.Gibbs has defined it in 1902 as "ensembles of systems in which the index (or logarithm) of probability of phase is a linear function of the energy" [85]. In computer simulations this is implemented as temperature coupling algorithms (thermostats).
One of such algorithms is Berendsen temperature coupling scheme [86]. Assume that there is a given temperature $T_0$, then the weak-coupling thermostat can be written as:

$$\frac{dT}{dt} = \frac{T_0 - T}{\tau},$$  \hspace{1cm} (3.8)

where $\tau$ is a coupling constant in $\text{ps}$, $t$ is the time in a simulation. The kinetic energy for this thermostat can be computed as:

$$\Delta E_{\text{kin}} = (\lambda - 1)^2 E_{\text{kin}}$$  \hspace{1cm} (3.9)

here $\lambda$ is a time-dependent scaling factor for velocities (see the paper by Berendsen [86]) which can be calculated using the equation (3.10):

$$\lambda = \sqrt{1 + \frac{\Delta t}{\tau} \left( \frac{T_0}{T(t - 0.5\Delta t)} - 1 \right)}$$  \hspace{1cm} (3.10)

However, regardless the simplicity, this thermostat does not give an exact NVT ensemble.

The other thermostat is V-rescale which can be clarified as "velocity rescale" [87]. The average kinetic energy at a certain temperature can be determined as:

$$\bar{E}_{\text{kin}} = \frac{N_f}{2\beta},$$  \hspace{1cm} (3.11)

where $N_f$ is the number of degrees of freedom and $\beta = 1/(k_B T)$, $k_B$ is the Boltzmann's constant, $T$ is the temperature in [K]. Then the modified kinetic energy distribution for Velocity rescale can be written as:

$$dE_{\text{kin}} = (E_{\text{kin}} - \bar{E}_{\text{kin}}) \frac{dt}{\tau} + 2\sqrt{\frac{E_{\text{kin}} \bar{E}_{\text{kin}}}{N_f}} \frac{dW}{\sqrt{\tau}},$$  \hspace{1cm} (3.12)

where $E_{\text{kin}}$ is the current total kinetic energy, $dW$ is a Wiener process (a stochastic process continuous in time) [87]. Comparing to Berendsen thermostat, V-rescale gives exact NVT ensemble.

This thermostat and Berendsen thermostat have been used for many simulations which are discussed through the thesis, particularly, for equilibration of systems.

Then Nosé-Hover thermostat can be shortly described by a following equation:
\[ \frac{d^2 \ln T}{dt^2} = \omega^2 \left( 1 - \frac{T}{T_0} \right) \] 

(3.13)

This thermostat gives the exact NVT ensemble, but can be unstable in certain cases. For more details one can read papers by Nosé et al. [88] and Hoover et al. [89].

Andersen thermostat can be named as well [90]. It can not be used at the moment for systems with constraints.

3.1.2 Pressure coupling

Simulating a system in NPT ensemble means that following parameters will be kept constant: the number of particles (molecules and atoms), the pressure and the temperature. Comparing to earlier discussed NVT ensemble, in NPT ensemble the volume of the system is allowed to change during the simulation, and for starting such a calculation one does not need to know in advance such parameters as a density, what is very convenient when you start a computation from scratch. As for a temperature coupling, there are several algorithms for the pressure coupling can be defined and they are called as barostats. They are not going to be discussed in details, but briefly will be named here.

The first barostat is Berendsen barostat [91]. The equation describing it is very similar to Berendsen thermostat, except that there is the pressure in the equation, and the time constant \( \tau_p \) is referring to pressure as well:

\[ \frac{dP}{dt} = \frac{P_0 - P}{\tau_p}. \] 

(3.14)

In fact, this barostat can perform different scalings: isotropic scaling (the scaling goes in the same way for \( x, y, z \) directions), semi-isotropic (\( x/y \) directions in a simulation box are scaled separately from the \( z \)-direction) and fully anisotropic (scaling if performed separately for each direction). Berendsen barostat gives an approximate NPT ensemble. This algorithm has been used for all simulations in NPT ensemble performed for the thesis.

Another barostat is Parinello-Rahman [92, 93]. The problem with this barostat is that in case if the pressure of a simulation is quite far from equilibrium the oscillations of the box might get pretty high.
3.2 Bonds in MD simulations

MD simulations are desired to be as efficient as possible for studying an evolution of a particular system in time. In order to do it more efficient one could think of increasing the time step, but, unfortunately, this opportunity is limited by bond oscillations which have a quite high frequency and a low amplitude. Then for the sake of keeping the physical behavior of bonds holonomic constraints are used. These constraints are relations between position variables and can be expressed as a following equation:

\[ f(r_1, r_2, ..., t) = 0, \]  

(3.15)

where \( r_i \) is a position of a particle \( i \) and \( t \) is a time.

In MD programs such constraints are implemented in the following algorithms: SHAKE [94], RATTLE [95], LINCS [96], P-LINCS [97], SETTLE [98]. SHAKE and RATTLE are probably the oldest ones and have been developed back in 1970's-1980's. Both algorithms require the same amount of memory storage for integrating Cartesian equations.

Originally SHAKE was tested for such molecules as \( \text{N}_2, \text{H}_2\text{O} \) and \( \text{C}_4\text{H}_{10} \) [94]. The most suitable time step for this algorithm is the same as normally chosen for MD simulations, which is 1-2 fs.

RATTLE is pretty much similar to SHAKE. The distinction is that it computes positions and velocities at "the next time from the positions and velocities at the present time step" without demanding for any information from earlier steps [95].

SETTLE is a bit different comparing to the first two algorithms. For instance, it was originally made for rigid water models TIP3p and SPC which you will read about in the Section 3.4 of this chapter. This algorithm is known to be accurate. Surprisingly, it is up to 7 times faster than RATTLE on scalar machines and up to 98 times faster on vector machines. It can be parallelized if needed [98].

Regardless, all advantages of SETTLE it is too complicated for usage in calculations on large molecules. In the end of 1990's a new algorithm was developed in order to fulfill needs for a good and stable constraint solver for MD simulations of large molecules. This algorithm was LINCS which was also utilized for computations in the current thesis. That is why it is going to be described more detailed.

Let's say that Newtonian equations of motion are given in the following form:
\[ \frac{d^2 r}{dt^2} = M^{-1} F, \quad (3.16) \]

where \( F \) is the force vector of size \( 3N \), \( N \) is number of particles, \( r \) is a vector of positions of those particles, \( M \) is masses of particles stored in a diagonal matrix form of a size \( 3N \times 3N \). Then assume that the given system has \( K \) number of constraints \( g(r) \) given by the equation:

\[ g_i(r) = 0. \quad (3.17) \]

The system from the equation (3.16) can be rewritten as a constrained system:

\[ -M \frac{d^2 r}{dt^2} = \frac{\partial}{\partial r} (V - \lambda \cdot g), \quad (3.18) \]

where \( V(r) \) a potential which contains constraints as a zero term, \( \lambda \) is a vector of Lagrangian multipliers.

Then the equation (3.18) can be simplified into the following one:

\[ -M \frac{d^2 r}{dt^2} + B^T \lambda + F = 0, \quad (3.19) \]

where \( B \) is a matrix with elements \( B_{hi} = \frac{\partial g_h}{\partial r_i} \).

Then equations (3.16) - (3.19) can be implemented for the position update as:

\[ r_{n+1} = (I - T_n B_n) r_{n+1}^{unc} + T_n d =
\]

\[ = r_{n+1}^{unc} - M^{-1} B_n (B_n M^{-1} B_n^T)^{-1} (B_n r_{n+1}^{unc} - d), \quad (3.20) \]

where \( d \) is a vector of bond lengths between atoms \( i_1 \) and \( i_2 \); \( I - TB \) is a projection matrix which makes constraints equal to zero, \( r_{n+1}^{unc} \) stands for new positions after an unconstrained update; \( T \) is a transformation matrix of size \( 3N \times K \) which is turning motions from constrained coordinates in Cartesian ones without making any changes in equations of motion of unconstrained coordinates. For more detailed description of LINCS one can read a paper by Hess et al. [96].

LINCS is also known to be 4 times faster than SHAKE. Moreover, it can be used in parallel as P-LINCS since it was incorporated with domain decompositions for larger molecules [97] which is suitable for simulations of membranes. In this thesis LINCS has been used for all calculations.
3.3 Lipid models in MD simulations

For planning an MD simulation of a lipid bilayer it is important to decide about the resolution of the system and the time-scale. Particularly for lipids there are three kinds of representations can be defined: all-atom, united atom and a coarse-grained.

An all-atom representation contains all atoms (Figure 3.2 (A)) in the description. Using such a model one can study physical properties of systems more detailed, nevertheless, this detailed model puts limitations on the system size, which one wants to investigate. A united atom representation (Figure 3.2 (B)) uses less particles for the representation of a molecule, for instance, in lipids it incorporates hydrogens in heavier atoms to which they are bonded. Nevertheless, such a model will not give a user much better performance than all-atom ones, but still for some case studies it might be convenient.

At last but not least, coarse-grained models (Figure 3.2 (C,D)). They can incorporate larger groups of atoms depending on what one wants to simulate and what kind of physical properties are needed to be reproduced. These groups of atoms are called as beads.

On Figure 3.3 artificial structures can be observed. These structures can be built of lipids. For example, atomistic and united atom models are more suitable for simulations of smaller formations, such as micelles or lipid bilayers (Figure 3.3(A,B,E)). Coarse-grained models are more convenient for larger constructions, such as bicelles and lipo-
Figure 3.3: Different kinds of artificial structures which can be built by lipids (A) A micelle in an organic solvent (B) A micelle in a polar solvent (water, for example) (C) A liposome (D) A bicelle, has a form of an ellipsoid (green molecules are molecules of a stabilizing detergent, or a stabilizing lipid with shorter tails) (E) A lipid bilayer. Here lipids are cyan circles (lipid heads) with dark blue tails.

somes (Figure 3.3 (C, D)), but can also be used for micelles and lipid bilayers.

3.4 Force fields (FF) for lipids

Depending on selected resolution for an MD simulation a choice of a force field becomes a question number two. For every lipid representation mentioned earlier one can find more than one force field model. A force field (FF) is a set of parameters and equations which are describing inter- and intra-molecular interactions. Coarse-grained force fields for lipids such as MARTINI [99–101], SPICA [102] and Cooke’s [103, 104] do give a good computational advantage, but for simulations where hydrogens play a role they can not be used. A known united atom force fields [105–107] can not be used either for the same reasons, regardless, that polar hydrogens are considered explicitly. Then one has a plenty of choices for atomistic force fields what does not simplify the task of finding the right one.

One of such force fields is AMBER FF for lipids [108–110]. It was originally developed for simulations of peptides, proteins and nucleic acids. Later on the updates have been done even for lipids in order to make it compatible for simulations with proteins. It has been veri-
fied for lipid bilayers containing DMPC, DOPC and POPC lipids and cholesterol [110].

Another example from the AMBER "family" is an all-atom force field GAFFlipid [111, 112]. Originally, General AMBER force field (GAFF) was developed for simulations using small organic molecules by Wang et al. [113] but later on it was extended to phospholipids in order to be able to perform tensionless calculations with complex lipid bilayers, using NPT ensemble [112]. It was validated by MD simulations of membranes containing either DMPC or DOPC.

GLYCAM06 FF was designed with dependency on AMBER FF as well, according to Kirschner et al. [114], with phase-less dihedral terms which were fitted to quantum chemical data. It was also updated for phospholipids and glycolipids [115, 116].

Leaving AMBER FF "family", CHARMM FF can be discussed. Originally, CHARMM22 has been developed for saturated and monounsaturated phospholipids [117, 118]. Then the methodology for deriving torsional potentials was changed to more accurate methods what gave CHARMM27 [119] and then CHARMM36 [120]. The last version of CHARMM36 was improved for polyunsaturated phospholipids and cholesterol [121–126].

Figure 3.4: Representation of different energy terms for an all-atom force field using two molecules

Finally, to conclude the discussion about force fields, SLipids FF shall be named, which is the subject of this thesis. This FF originates from CHARMM FF. In the beginning of its development, parameters were derived and validated for saturated and monounsaturated phospholipids as well as some sphingolipids [127–129]. However, the need of a proper force field for polyunsaturated phospholipids has pushed to a development of a newer version of it [130] which will be discussed
in more details in Chapter 6 or Paper I.

All those different force fields have a lot in common. They all can be generalized into equations (3.21), visualized on Figure 3.4. The discussed FFs differ from each other by parameters in equations (3.21).

\[ U_{\text{bonds}} = \sum_{\text{bonds}} k_r (r - r_0)^2, \]
\[ U_{\text{Urey-Bradley}} = \sum_{\text{Urey-Bradley}} k_b (b - b_0)^2, \]
\[ U_{\text{angles}} = \sum_{\text{angles}} k_\theta (\theta - \theta_0)^2, \]
\[ U_{\text{torsions}} = \sum_{\text{torsions}} k_\phi (1 + \cos(n\phi - \delta)), \]
\[ U_{\text{Lennard-Jones}} = \sum_{i,j\neq i} 4\varepsilon_{ij} \left[ \left( \frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left( \frac{\sigma_{ij}}{r_{ij}} \right)^{6} \right], \]
\[ U_{\text{electrostatic}} = \sum_{i,j\neq i} \frac{q_i q_j}{4\pi\varepsilon_0 r_{ij}}, \]
\[ U_{\text{force-field}} = U_{\text{bonds}} + U_{\text{angles}} + U_{\text{torsions}} + U_{\text{Urey-Bradley}} + \]
\[ + U_{\text{Lennard-Jones}} + U_{\text{electrostatic}} \quad (3.21) \]

### 3.5 Atomistic water models

After making a choice about the model and the force field for lipids another question will be a selection of the right water model [131, 132]. Lipids are slightly simpler in this case, because all-atom representation is pretty much literal, while for water the number of sites is not limited to three atoms and might be higher or lower. Nevertheless, a random choice of a water-model is not recommended. Every FF has been developed and validated with a certain water-model. The selection shall always be based on a knowledge of what kind of water-model was used for a derivation of chosen FF.

There are many all-atom water models in MD simulations. Some of them are used more frequently for biomolecular modeling, others are not. For atomistic MD simulations of lipid bilayers water model consisting of two sites [133, 134] is not used at all.
Figure 3.5: Some water models (A) 3 sites. (B) 4 sites. (C) 5 sites. (D) 6 sites. Here atom M is a dummy atom which has a negative charges instead of having it on an oxygen atom. Pairs of atoms are lone pairs which represent a valence electron pair without bonding or sharing with other atoms. These L-pairs have negative charges.

Then one can identify three [135, 136], four [136], five [137, 138] and six [139] site water models (see Figure 3.5). Such a wide range of water models was made in order to reproduce different properties of water. For instance, the model with six sites was developed in order to reproduce properties of water on ice-water interface [139, 140]. Due to the big number of sites this model is not suitable for the all-atom lipid bilayer simulations. Models with five sites such as BNS, ST2 [141] and TIP5p [137, 138, 142] are not used for such calculations either, also due to the same reasons as for the previous model. However, TIP4p is employed more frequently for atomistic MD simulations on membranes [143, 144], but still is less popular comparing to three-site models.

Water models with three sites are more preferable in atomistic MD simulations have also more than one variation. One can differ between SPC (simple point charge) model [145], SPC/E [146] and TIP3p [136]. For the lipid bilayer simulations SPC and TIP3p have been mostly used. Moreover, since SLipids FF was dependent on CHARMM FF, and the latter one was designed using the modified water model TIP3p, this model became essential even for further developments of SLipids FF.

TIP3p is a rigid model with the fixed geometry. This water model can be described shortly by geometric and potential energy function parameters, where the latter ones are described by the equation:

\[
U_{\text{TIP3p}} = \sum_{i=1}^{n} \sum_{j=1}^{n} \left( k_{\text{e}} q_i q_j \frac{1}{r_{ij}} + 4 \varepsilon \left[ \left( \frac{\sigma}{r_{ij}} \right)^{12} - \left( \frac{\sigma}{r_{ij}} \right)^{6} \right] \right)
\]

(3.22)
where $k_e = \frac{1}{4\pi\varepsilon_0}$ is the electrostatic constant ($\varepsilon_0$ permittivity of vacuum), $r_{ij}$ is the distance apart of $i^{th}$ and $j^{th}$ atoms; $\sigma = 3.15061$ Å, $\varepsilon = 0.6364$ kJ/mol (Lennard-Jones parameters). Point-charges are $-0.834$ and $0.417$ on oxygen and hydrogen atoms respectively. The angle between atoms is $104.52^\circ$ and distances between oxygen and hydrogen are $0.9572$ Å. Perhaps, comparing this model to other models in a sense of reproducibility of experimental water properties, one can find out that it is not the most accurate one, but in a sense of reproducing experimental physical properties of lipid bilayers and regarding the performance, TIP3p might be the best option.
4. Free energy calculations

Free energy calculations can be used for various purposes. One can study a possibility to perform a chemical reaction, rotational barriers and, as in this thesis, the ability of different molecules to penetrate through cell membranes. The idea behind the usage of such methods is to determine if a process can go spontaneously (Gibbs free energy, $\Delta G < 0$) or not ($\Delta G > 0$).

There are many methods, incorporated with MD simulations, for computing the free energy of a particular process: umbrella sampling [147, 148], expanded ensemble [149, 150], replica exchange [151], metadynamics [152]. However, in this thesis two of them will be discussed more detailed.

4.1 Well-tempered metadynamics (MetaD)

Metadynamics simulation is a well-known method for calculating of a potential mean force (PMF) profile which can be integrated to a free energy of an investigated process [152–156]. The PMF might depend on many variables, but the user has to select only some of them which are in the area of an interest. Furthermore, metadynamics can be incorporated with various programs which perform different kinds of calculations: from quantum chemical to mesoscale simulations. In this thesis metadynamics is considered together with MD simulations.

There are more than one ways to perform this kind of simulation. For instance, the following methods can be identified: standard metadynamics [152], well-tempered metadynamics [153–156], $\mu$-tempered metadynamics [157] and parallel-tempered metadynamics [158]. Here only standard and well-tempered metadynamics simulations are going to be discussed further, since the latter one was employed for computations in the thesis.

So how the method actually works... Starting with the standard metadynamics, assume that there is a system which one wants to sim-
The system has many degrees of freedom, but suppose that only some of them are lying in the area of the interest. Then one selects those degrees of freedom $\mathbf{s}(\mathbf{q})$ (where $\mathbf{q}$ are microscopic coordinates) of the system which will be "included" in the history-dependent bias potential described by an equation:

$$V(\mathbf{s}, t) = \sum_{\tau<t} W \cdot \exp\left(-\sum_{i=1}^{d} \frac{(s_i - s_i(\mathbf{q}(\tau)))^2}{2\sigma_i^2}\right), \quad (4.1)$$

where $\tau$ represents the deposition stride for Gaussian functions, $\sigma_i$ stands for the width of the Gaussian function for the $i^{th}$ collective variable, $W$ is the height of the Gaussian function (it is a constant value in standard metadynamics). After some long-time simulation the approximate convergence of the bias potential can be observed, which is the negative free energy as a function of collective variables:

$$V(\mathbf{s}, t \rightarrow \infty) = -F(\mathbf{s}) + C. \quad (4.2)$$

Comparing well-tempered metadynamics with the standard one the
difference is in heights of Gaussian functions which are added to the potential. They are constant during the standard simulation and decreasing in a case of well-tempered one [156]. This has a consequence on the system’s behavior in oscillations of the free energy around a real value in case of the standard metadynamics. During the well-tempered simulation the motion slows down with the time and the potential is converging smoothly. The height of the Gaussian function in well-tempered metadynamics simulation can be written in the equation:

\[ W(\tau) = W_0 \cdot \exp\left(-\frac{V(\vec{s}(q(\tau)), \tau)}{k_B \Delta T}\right), \]

(4.3)

where \( W_0 \) is a user-determined initial height of the Gaussian function, \( \Delta T \) is an input parameter (has units of a temperature). Then the potential can be rewritten for the well-tempered metadynamics as:

\[ V(\vec{s}, t \to \infty) = -\frac{\Delta T}{T + \Delta T} F(\vec{s}) + C, \]

(4.4)

where \( T \) denotes the temperature of the system. Regardless the smooth convergence of the potential there is no full compensation of the underlying free energy. In case of \( \Delta T \) it is important to know that if \( \Delta T = 0 \) (or a too small value) then one has a standard MD simulation while \( \Delta T = \infty \) (too large value) means that the user will obtain results for a standard metadynamics simulation. Indeed, one has to think carefully when selecting parameters, that is why the "bias factor" is introduced as:

\[ \gamma = \frac{T + \Delta T}{T}. \]

(4.5)

Selecting the bias factor in settings for such a simulation is one of the most essential steps, because it will define both the efficiency and the accuracy.

After obtaining the PMF the difference in free energy along collective variables can be calculated. For example, for a one-dimensional case the standard binding free energy can be computed for a known PMF (\( w(z) \)):

\[ \Delta G_{\text{bind}}^\circ = -k_B T \cdot \ln\left(\frac{\int_B e^{-\beta w(z)} \, dz}{\int_U e^{-\beta w(z)} \, dz}\right), \]

(4.6)

where \( U \) denotes the unbounded state and \( B \) is the bounded state, \( z \) is the collective variable (see Figure 4.6), \( w(z) \) (PMF) is the same value.
as $F(\vec{s})$ in the equation (4.4), but for this particular 1-D case. For instance, if the penetration of a molecule through a membrane is studied the bounded state is determined when the molecule is inside the bilayer. The unbounded state is determined for the molecule in water.

No matter that the well-tempered approach is so attractive for simulations with many collective variables, modern users still prefer to choose no more than three variables for large systems. This is due to too low efficiency of the computation for several variables which makes the task of obtaining a stable bias potential unrealistic. In this thesis well-tempered metadynamics has been explored for one and two dimensional cases, but on a microsecond time-scale (papers II and III).

4.2 Computations of $\log(P)$ by the method of expanded ensembles

For an investigation of membrane permeability by other molecules, a membrane itself might not be needed for computational studies. One of the reasons to avoid using membranes for calculations can be a high computational cost for MD simulations due to the large number of particles in systems. Then it rises a question about what method is more suitable and how one can relate a possible result to experiments. Or if there are any experiments using other compounds than lipid bilayers which could successfully predict the permeability of a substance through a membrane.

Answering both questions, there is a well-known method which has roots in the nineteenth century. This method is about measuring the partition coefficient of a certain substance between octanol and water environments [159]. Many experimental works have confirmed that the methodology is suitable for such a phenomena. These studies were about binding of solutes to serum albumin and enzymes [159], permeability of the rat brain capillary [160], membrane permeability [161, 162] etc. Octanol is an alcohol which can mimic the membrane environment, while water is acting as media on the outside surface of the lipid bilayer. Experimentally, this parameter can be determined using several different techniques: high performance liquid chromatography [161], UV/VIS spectroscopy which is co-called shake flask [163, 164], pHmetric [165] and electrochemical [166]. From the computational point of view the method of expanded ensemble (EE) serves as a perfect tool
Generally, the logarithm of partition coefficient (log(P)) can be calculated using the following formula:

$$\log(P) = \frac{\Delta G_{\text{water}} - \Delta G_{\text{octanol}}}{RT \cdot \ln(10)},$$

(4.7)

where $\Delta G_i$ denoting the solvation free energies of the compound in the selected environment (water of octanol), $R$ is the universal gas constant, $T$ is the temperature in K. $\Delta G$ can be computed by the EE method.

In EE method the partition function is presented as:

$$Z = \sum_{m=0}^{M} \int V \left( \prod_{i=1}^{N+1} dr_i \exp(-\beta[H_N(r_i) + H_{\text{int}}(r_{N+1}) + h(\alpha_m, r_i)] + \eta_m) \right),$$

(4.8)

where $\beta = 1/(k_B T)$, $T$ is the temperature in K, $k_B$ is the Boltzmann’s constant, $H_N$ denotes the potential energy of solvent molecules, $N$ is the number of solvent molecules, $H_{\text{int}}$ stands for the intramolecular potential energy of the solute, $h(\alpha_m, r_i)$ denotes the interaction of the
N+1-th molecule which is solute with the rest of particles which are the solvent, $\alpha_m$ represents the discrete set of points which are taken along the reaction coordinate, $\eta_m$ denotes the biased potential regulating the distribution (the gradual insertion of the solute) of all $\alpha$ points which is calculated using Wang-Landau algorithm [167], $r_i$ represents the set of atomic coordinates. During the simulations $\alpha$ parameter changes after a certain number of MD steps according to Monte Carlo Metropolis rule. Then one can calculate the excess free energy of solvation using the equation:

$$\beta G_{\text{solv}} = -\ln \frac{p_m}{p_0} + \eta_m - \eta_0, \quad (4.9)$$

here $p_m$ denotes probabilities of visiting sub-ensembles, which are determined during the simulation.
5. Connecting classical MD simulations with experiments

Figure 5.1: Different scales which can be accessed in various experiments and MD simulations (pink transparent area). Image adapted with the permission from ESS (Technical Design Report 2013 [168])

MD simulations can let their users calculate different properties of materials. When one wants to compare features obtained from analysis of MD trajectories with the same features, but determined experimentally, it is important to choose the right time and size scales. On Figure 5.1 overlaps in scales for various experimental methods and atomistic MD simulations are presented.

Since the comparison of experimental data with the outcome from
simulations was successful during decades, computational methods recommended themselves as good tools for predictions and understanding of various physical chemical phenomena. If experiments are difficult to perform, MD simulations can be used instead of them. For example, in this thesis attention goes to polyunsaturated phospholipids, which can go through a lipid peroxidation during experiments. In all-atom MD simulations, using reliable numerical models, this process can be avoided and features of the material can still be obtained with a good accuracy.

5.1 Area and volume per lipid

Area per lipid is an important property of a lipid bilayer. It indicates how tight is membrane’s packing. The formula for calculation is:

$$ A_L = \frac{\langle A_{xy} \rangle}{n_{L,leaflet}}, $$

(5.1)

here $A_{xy}$ is the average area of a simulation box in $xy$-plane, $n_{L,leaflet}$ is a number of lipids in a leaflet. In principle, one can monitor the area per lipid during the whole simulation time, but it is unreasonable to consider values for the non-equilibrated part of a simulation, as properties should be calculated only after the equilibration part is finished. Experimentally this value can be obtained [43, 169] by using one of following techniques: crystallographic (scattering techniques) [43, 170] or gravimetric methods [171].

Another property which is related to the area per lipid is the volume per lipid. It can be computed using the equation:
\[ V_L = \frac{\langle V_{\text{box}} \rangle - n_{\text{water}} V_{\text{water}}}{n_L}, \]  

(5.2)

where \( \langle V_{\text{box}} \rangle \) stands for the average volume of the simulation box, \( V_{\text{water}} \) and \( n_{\text{water}} \) are the average volume and the number of water molecules which can be calculated from separate MD simulations for the pure water. Experimentally \( V_L \) can also be determined using crystallographic methods such as neutron or X-ray scattering techniques [47, 172].

5.2 Electron and mass density profiles, form factors and membrane thickness

![Electron density profile illustration](image)

**Figure 5.3:** Illustration of an electron density profile

Electron and mass density profiles can give an idea about the structure of lipid bilayers. Knowledge of what atoms build a system helps to determine from electron and mass density distributions average positions of certain atoms and molecular parts. The same idea for determination of the structure is used in computer simulations in order to characterize sizes of simulated systems.
particularly in MD simulations computing electron density profile is a very simple task, since every frame of the trajectory is composed in the same way. Analyzing tools for simulations compute histograms of distributions of electrons and masses for every atom. Then the Fourier transform of the electron density profile will give a X-ray scattering form factor [172–174], which can be directly compared with experimentally determined form-factor:

\[ S(q) = \int_{-D/2}^{D/2} (\rho(z) - \rho_w) \cos(qz) \, dz, \] (5.3)

where \( \rho(z) \) stands for electron density along \( z \)-axis which is computed from the information about the atomic partial charges, \( \rho_w \) is the electron density of water, \( D \) denotes the size of the simulation box along \( z \)-axis which also corresponds to the \( D \)-spacing of membranes in scattering experiments.

Then knowledge of densities gives an opportunity to learn about such properties as Luzatti thickness, \( D_B \) [47, 106]:

\[ D_B = D - \int_{-D/2}^{D/2} \rho_w(z) \, dz, \] (5.4)

where \( \rho_w(z) \) denotes the probability distribution along \( z \)-direction of water (see Figure 5.3).

The thickness of the hydrophobic region, \( 2D_C \) of the membrane (see Figure 5.3) is an important property as well and can be computed as [47, 106]:

\[ 2D_C = \int_{-D/2}^{D/2} \rho_{CH}(z) \, dz, \] (5.5)

where \( \rho_{CH} \) (thickness of the hydrocarbon region) is computed as:

\[ \rho_{CH}(z) = \rho_{CH_2}(z) + \rho_{CH_3}(z). \] (5.6)

5.3 Parameters obtained from NMR experiments

A property of a lipid bilayer called as deuterium order parameters (\( S_{CD} \)) is used for validation of force fields, since experimental data from nuclear magnetic resonance spectroscopy is available for many lipid bilayers. This property can also be used to characterize the order in the
lipid bilayer: higher values are often determining a higher order [175–178]. However, values of order parameters include also orientational effects. Order parameters are computed using the formula:

$$|S_{\text{CD}}| = \frac{1}{2} \langle 3\cos^2\theta - 1 \rangle,$$  \hspace{1cm} (5.7)

where $\theta$ denotes the angle between the direction of the $C - H$ (or $C - D$ in experiments) bond and the bilayer normal (see Figure 5.4).

Another important parameter is the $^{13}$C spin-lattice relaxation time $T_1$ which can give an idea about the reorientational dynamics of $C - H$ bonds which normally has a time scale of pico- and nanoseconds. This scale makes this property accessible by MD simulations. Then the time $T_1$ has the following relation with the spectral density function $J(\omega)$ [179]:

$$\frac{1}{NT_1} = \frac{1}{10} \left( \frac{\gamma_C \gamma_H \hbar}{2\pi r_{\text{CH}}^2} \right)^2 \times (J(\omega_C - \omega_H) + 3J(\omega_C) + 6J(\omega_C + \omega_H))$$  \hspace{1cm} (5.8)

where $N$ determines the number of protons bound to the selected carbon atom, $\omega_C$ and $\omega_H$ are denoting the resonance frequencies of C and H nuclei respectively, $\gamma_C$ and $\gamma_H$ are gyromagnetic ratios for carbon and hydrogen respectively, $r_{\text{CH}}$ is the $C - H$ bond length.

The spectral density ($J(\omega)$) can be calculated in MD simulations from a reorientational time-correlation function $C(t) = \langle P_2(\mathbf{\mu}(0)\mathbf{\mu}(t)) \rangle$ of the $C - H$ unit-vector $\mathbf{\mu}(t)$ (here $P_2$ is the second Legendre polynomial):
\( J(\omega) = \int_0^\infty C(t) \cos(\omega t) dt, \) \hspace{1cm} (5.9)

where \( t \) denotes the time, \( \omega \) is the resonance frequency. For the limit of motional narrowing (where \( \tau \omega \ll 1 \) and \( \tau \) stands for the motional correlation time), \( T_1 \) becomes independent of the magnetic field and can be calculated directly from the decay constant of \( C(t) \) using the formula [109, 180]:

\[
\frac{1}{NT_1} = (1.855 \cdot 10^{10} \text{s}^{-2}) \int_0^\infty \left( \langle P_2(\vec{\mu}(0)\vec{\mu}(t)) \rangle - C(\infty) \right) dt \hspace{1cm} (5.10)
\]
6. Extending SLipids FF for polyunsaturated phospholipids (Paper I)

Figure 6.1: Examples of lipid peroxidation process (A) For linoleic fatty acid [181] (B) More general case for polyunsaturated lipids in cis–conformations [182]. Here $R_1$ and $R_2$ are radicals.

Simulations of biomembranes require reliable models for lipids. The presence and the quality of computational models have been strongly affected by the availability of the empirical data. For example, since saturated phospholipids are more convenient for laboratory experiments and they do not get affected by the lipid peroxidation, there is more available experimental data for these species. Polyunsaturated phospholipids can be affected by lipid peroxidation [181–183] (the schematic
process is in Figure 6.1) what follows by a smaller amount of experimental results, since that can cause difficulties in obtaining a reliable information. Another problem with experiments with polyunsaturated lipids is the difficulty in synthesis of $^2$H labeled unsaturated tails [184], which are needed for SS-NMR experiments. The lack of empirical data for polyunsaturated lipids had consequences on FF developments for these compounds. That is why originally all FF models have been made either for saturated lipids or for species containing one double bond in their tails.

However, modern pharmaceutical industry is struggling to find a cure for neurodegenerative diseases which are highly associated with a decrease in amounts of polyunsaturated phospholipids in neuronal cells of tissues in a human brain [14, 26]. Computer simulations of biomembranes containing polyunsaturated lipids could help to answer important biophysical questions regarding the connection of these molecules, for example, to the development of Alzheimer’s and Parkinson diseases. The need of an accurate FF for polyunsaturated lipids became a reason for the extension of SLipids FF for these compounds. Earlier SLipids FF was developed and successfully validated for saturated and mono-unsaturated phospholipids [127–129], but models parametrized for an isolated double bond were not accurate descriptors for lipids containing several double bonds in their tails. In this chapter and Paper I the methodology of extension of SLipids FF for polyunsaturated phospholipids is going to be discussed in more details.

### 6.1 Parametrization strategy

The parametrization strategy is based on dividing lipid molecules into smaller parts: lipid tails are parametrized separately from lipid head groups. Moreover, lipid tails are differentiated into saturated, mono-unsaturated and polyunsaturated. The principle of division of a big molecule into parts has roots in high costs of accurate quantum chemical computations for the whole lipid.

In order to mimic cis polyunsaturated lipid tails two dienes (cis – 3, cis – 6-nonadiene and cis – 3, cis – 6-dodecadiene) have been selected as representing components. These dienes were assembled into two separate simulation boxes where every box was containing 343 molecules of each component.

After equilibrating resulting systems and running MD simulations
for 5 ns 50 random conformations were taken from every box in order to calculate partial charges. These calculations were conducted using R.E.D. software [185] with B3LYP exchange-correlation functional [186, 187] with cc-pVTZ basis set [188]. The selected solvent model was IEF-PCM [189, 190] with the dielectric permittivity $\epsilon = 2.04$, which mimicked the hydrophobic environment. Resulting partial charges for every component were averaged over 50 conformations and transformed to SLipids FF for polyunsaturated lipid tails.

In Paper I and in previous papers for SLipids FF [127–129] the parametrization strategy included the control of a suitability of Lennard-Jones parameters. Unfortunately, for chosen dienes such a data was not available and the values were kept from previous versions of the FF, but densities were possible to verify comparing experimental values for some isomers of these molecules with values obtained from MD simulations for chosen dienes, using newly derived partial charges.

After the derivation of partial charges the same dienes have been used for the parametrization of torsional angles $-\text{HC} = \text{CH} - \text{CH}_2 - \text{CH} = \text{(in FF: CEL1 = CEL1 - CTL2 - CEL1)}$ and $-\text{HC} = \text{CH} - \text{CH}_2 - \text{CH}_2$ (in FF: CEL1 = CEL1 - CTL2 - CTL2) using quantum chemical calculations of the accuracy of coupled clusters CCSD(T) theory [191] (see Paper I for more details).

6.2 FF validation

The validation of an FF is the most important part of this work. The more properties can be reproduced for a bigger range of membranes the better the FF. SLipids FF was validated for several polyunsaturated lipid bilayers such as 16:0-18:2 ($\omega - 9$) PC, 16:0-22:6 ($\omega - 3$) PC, 18:0-18:3 ($\omega - 3$) PC, 18:0-22:6 ($\omega - 9$) PC etc. From analysis of MD simulations such properties as area per lipids, deuterium order parameters, scattering form factors were successfully reproduced.

On Figure 6.2 deuterium order parameters are shown for two selected lipid bilayers. Comparing $\text{sn} - 1$ with $\text{sn} - 2$ lipid tails a higher order for saturated chain than for polyunsaturated one can be observed. For 18:0-22:6 PC the availability of two different experiments illustrates the uncertainty of measurements. Moreover, for both membranes (18:0-22:5 PC and 18:0-22:6 PC) deuterium order parameters of $\text{sn} - 2$ chains are following the same trend around double bonds: $|S_{\text{CD}}|$ values are closer to zero.
Another property of lipid bilayers is the area per lipid. In all conducted MD simulations values obtained from trajectory analysis were in a good agreement with existing experimental data. For instance, for 18:0-22:6 PC the computed value was $68.6 \pm 0.7 \, \text{Å}^2$ [130] while the experimental value from NMR experiments was $68.8 \pm 0.4 \, \text{Å}^2$ [179]. In case of 18:0-20:4 PC the computed area per lipid was $69.6 \pm 0.6 \, \text{Å}^2$ [130] and the experimental value was $70.6 \, \text{Å}^2$ [192].

Remembering results for areas per lipids for saturated phosphatidyl-
cholines which have been done earlier by Jämbeck et al. [127] it is easy to see that $\omega - 3$, $\omega - 6$ and $\omega - 9$ phospholipids have a wider area, what can indicate together with deuterium order parameters a higher disorder in such membranes.

Further validation of the FF was done by MD simulations with DAPC and DDPC bilayers loaded with $33\%$ mol. of cholesterol [130]. It was demonstrated that in selected polyunsaturated membranes cholesterol could have three different orientations which were detected experimentally as well: "upright", lying flat in the center of the bilayer and with cholesterol’s head group oriented to the membrane’s center (see Figure 6.3). These findings were confirmed earlier by several experimental works [64, 65, 193].

6.3 Summary of Paper I

The derived FF for polyunsaturated phospholipids can accurately reproduce various properties of mono-component lipid bilayers as well as their behavior with additional cholesterol. Furthermore, since the
parametrization of lipid tails and head-groups was done separately, the parameters derived for lipid tails can be used for lipids with other head-groups. This can simplify the further extension for other similar lipids such as PE.

However, the extension of FF for phospholipids does not end here. In further development parametrization of torsional angles in the lipid head-groups is of importance, since current values were inherited from CHARMM FF.
7. Application of methods for studying the toxicity (Paper II)

7.1 Hydroxylated polybrominated diphenyl ethers

Figure 7.1: Polybrominated diphenyl ether (BDE or PBDE) (A) and hydroxylated polybrominated diphenyl ether (OH-BDE or OH-PBDE) (B)

Writing a FF is a meaningless task if it will never be applied for a real-life problem. As it was mentioned in Chapter 2, the passive diffusion of molecules through a lipid bilayer is playing a big role in biological systems. For instance, a toxicity of a chemical substance is strongly affected by thermodynamics of the cell-penetration process. In this project three different studies were done in order to relate physical chemical properties to toxicity of a certain type of a substances: quantum chemical calculations of pKₐ values, EE simulations for determining the partition coefficients, and well-tempered metadynamics simulations for obtaining binding free energies of compounds to membranes. Hydroxylated polybrominated diphenyl ethers (OH-BDEs) (see Figure 7.1), which are known as flame retardants, were selected for the study. These poisonous species are known from the industry and can be found in electronic devices as well as textiles. Also some OH-BDEs can be naturally produced by marine environment. Emissions from industrial products and the natural production made those compounds being able to enter a human body through a water and a food consum-
tion. However, the reasons why some of them are more and others are less toxic are not known, what made them attractive for toxicological and computational studies.

**Figure 7.2:** A simplified illustration of an uncoupling mechanism of OX- PHOS by OH-BDE molecules. Here OMM is the outer mitochondrial membrane, IMM is the inner mitochondrial membrane. I, II, III, IV and V are protein complexes. [194]

Experimentally the toxicity is determined using several different parameters or properties measured using various techniques. Particularly, for hydroxylated polybrominated diphenyl ethers and one polybrominated diphenyl ether there were four kinds of data sets used from in vivo experiments carried out by Legradi et al. [75]. These data sets were called as toxicity endpoints. Two of them were from a classic rat mitochondrial respiration assay (TPP assay) and two others were from the mitochondrial potential assay in zebrafish PAC2 cells (TMRM assay). TPP assays were made in order to detect a possible disruption of an oxidative phosphorylation via protonophoric uncoupling (see a simplified illustration of an uncoupling of OXPHOS on Figure 7.2) or inhibition (Figure 7.3) of the electron transport chain. For these assays the lowest observed effect concentrations (LOEC) were determined when a decrease of the membrane potential combined with an effect (positive or negative) on an oxygen intake was seen. TMRM assays were done in order to determine LOEC for the response to carbonyl cyanide...
Figure 7.3: A simplified illustration of an inhibition of adenosine triphosphate synthase mechanism by OH-BDE molecules. Here OMM is the outer mitochondrial membrane, IMM is the inner mitochondrial membrane. I, II, III, IV and V are protein complexes. [194]

4-(triluoromethoxy) phenylhydrazone (FCCP) and the half maximal effect concentration ($EC_{50}$) for the response to the same FCCP.

As a measure of correlation between experimental and computed values Pearson correlation coefficient ($r$) was used. Pearson correlation coefficient is a standard measure of a correlation between two data sets. It is successfully applied for studying various problems in biology and medicine. This coefficient has a value in the interval $[-1; 1]$. The value close to zero (the absolute value of $r$ belongs to the interval $[0; 0.1]$) will indicate no correlation between two variables (for example, sets of values for $\log(P)$ and $EC_{50}$ for OH-BDE molecules). The correlation between variables can be divided into three categories: strong if absolute values of $r$ belong to the interval $[0.5; 1.0]$, medium if $|r| \in [0.3; 0.5]$ and weak if $|r| \in [0.1; 0.3]$. Furthermore, the correlation can be either positive or negative, depending on the sign of Pearson correlation coefficient.
7.2 Calculations of $\log(P)$ and $pK_a$

In Chapter 4 EE method have been described as a good tool for calculations of $\log(P)$ values in order to determine if a molecule can penetrate through a cell membrane. Since in this project values from experiments on living organisms were used, values of $\log(P)$ can help to relate those toxicity endpoints with thermodynamics of the molecules uptake by membranes. This method can be combined with calculations of the logarithm of an acid dissociation constant ($pK_a$) for OH-BDE species (not for BDE though). $pK_a$ values are widely used in drug discovery in order to learn about the distribution of ionizable drugs in living organisms [195].

Experimental observations on living organisms by Legradi et al. [75] exhibited changes in membrane potentials, which could be related to dissociations of OH-BDE molecules. For example, one of the effects was connected to protonophoric uncoupling what made the computations of $pK_a$ values relevant.

For an ionizable molecule $pK_a$ is determined as:

$$pK_a = -\log(K_a) = -\log\left(\frac{[A^-][H^+]}{[AH]}\right),$$  \hspace{1cm} (7.1)

where the reaction can be written in an equation: $AH \leftrightarrow A^- + H^+$. According to classical physical chemistry equilibrium constant of a reaction is defined by the Gibbs free energy of a reaction ($G_m$ represents the molar Gibbs free energy):

$$pK_a = \frac{\Delta G_r}{RT \cdot \ln(10)},$$  \hspace{1cm} (7.2)

here $\Delta G_r = G_m(A^-) + G_m(H^+) - G_m(AH)$.

The equations (7.1)- (7.2) can not be used directly for a proton dissociation reaction happening in real systems, where a hydrogen ion exists as a mixture of various forms which can be $H_2O_2^+$ and $H_3O^+$. That is why the method described by Brown et al.[196] is better for such purposes. This convenient approach is using chemically similar species with experimentally determined $pK_a$ with the reaction of acid-base equilibrium written as:

$$AH + B^- \leftrightarrow A^- + BH$$  \hspace{1cm} (7.3)
Table 7.1: Pearson correlation coefficients describing the relation between calculated LogP and pKₐ values and toxicity endpoints. "all": computed for the whole set of molecules; "ortho": computed only over ortho-types of molecules without Cl-atoms. Table has been reproduced with a permission from Royal Society of Chemistry [197]

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>LogP</th>
<th>pKₐ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>all</td>
<td>ortho</td>
</tr>
<tr>
<td>Uncoupling, LOEC</td>
<td>0.03</td>
<td>-0.63</td>
</tr>
<tr>
<td>Inhibition, LOEC</td>
<td>-0.14</td>
<td>-0.77</td>
</tr>
<tr>
<td>Alt. mem. potential, LOEC</td>
<td>-0.45</td>
<td>-0.6</td>
</tr>
<tr>
<td>Alt. mem. potential, EC₅₀</td>
<td>-0.37</td>
<td>-0.56</td>
</tr>
</tbody>
</table>

where B represents a molecule with experimentally known pKₐ(B) and A is the compound of the interest. Then one can calculate pKₐ as:

$$pK_a(A) = pK_r + pK_a(B), \quad (7.4)$$

here pKₐ stands for the equilibrium constant of a reaction from the equation (7.3). It can be computed then as:

$$pK_r = \frac{G_m(A^-) + G_m(BH) - G_m(AH) - G_m(B^-)}{RT \cdot \ln(10)} . \quad (7.5)$$

The described methodology was seen as very promising one by Brown et al. [196], but for the nineteen species which were studied in this work no experimental values of pKₐ were available. That is why available pKₐ values for smaller molecules (which were containing one phenyl ring) were used for calibration of computational methods (as in the work by Brown et al. [196]) even in the case of OH-BDEs.

All calculations for the flame retardants were conducted using Gaussian09 [198]. Resulting computations for Pearson correlation coefficients for experimentally determined effects and computed pKₐ values showed no correlations (Table 7.1) [130].

Nevertheless, log(P) values exhibit generally better correlation with experimental toxicity data [75] comparing to correlation values for pKₐ (Table 7.1). After selecting only non-chlorinated molecules in ortho conformations, a strong and negative association between log(P) and experimental toxicity data was observed (|r| > 0.5). Larger values of log(P) meant that the compound would have a higher concentration in a membrane at the same "environmental" concentration.
**Figure 7.4:** Experimental toxicity endpoints from ref. [75] vs LogP of OH-PBDEs and least square linear fitting. (A): uncoupling, LOEC; (B): inhibition, LOEC, (C): Altered membrane potential, LOEC; (D): Altered membrane potential, EC$_{50}$. Points in blue corresponds to ortho structures without Cl atoms. Figure has been reproduced with a permission from Royal Society of Chemistry [197]
7.3 Well-tempered metadynamics for chosen species

As it was discussed in Chapter 4, well-tempered metadynamics simulations are convenient for investigations of the partitioning of solutes in lipid bilayers. Four compounds were selected for these computations due to limitations on available hardware resources. This choice was based on the differences in their toxic actions and similarities in their chemical compositions. For instance, 6-OH-BDE47 was seen as a disruptor of OXPHOS via inhibition of the electron transporting complex, while the similar compound to it, 6-OH-BDE85, acted via uncoupling of the protonophoric process [75]. Two other molecules, 3-OH-BDE155 and 2’-OH-BDE68, did not disrupt OXPHOS in zebrafish embryos, but 2’-OH-BDE68 was simulated in two different lipid bilayers in order to see the effect of saturation on its ability to penetrate through membranes. A collective variable for one-dimensional metadynamics simulations was the distance between the center of mass of a molecule and the center of mass of a membrane (see Figure 4.1).

Table 7.2: Binding free energies of OH-PBDEs to a lipid bilayer ($\Delta G_{\text{bind}}$), and the difference of solvation free energies in octanol and water phase. All values are given in kJ/mol. Table was reprinted with a permission of Royal Society of Chemistry [197]

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Molecule</th>
<th>$\Delta G_{\text{bind}}$</th>
<th>$G_{\text{oct}} - G_{\text{water}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>POPC</td>
<td>2’-OH-BDE68</td>
<td>-40.6</td>
<td>-</td>
</tr>
<tr>
<td>DMPC</td>
<td>2’-OH-BDE68</td>
<td>-47.1</td>
<td>-47.</td>
</tr>
<tr>
<td>DMPC</td>
<td>6-OH-BDE47</td>
<td>-50.5</td>
<td>-46.6</td>
</tr>
<tr>
<td>DMPC</td>
<td>6-OH-BDE85</td>
<td>-60.2</td>
<td>-53.8</td>
</tr>
<tr>
<td>DMPC</td>
<td>3-OH-BDE155</td>
<td>-46.7</td>
<td>-53.6</td>
</tr>
</tbody>
</table>

In Table 7.2 binding free energies ($\Delta G_{\text{bind}}$) as well as differences in free energies in octanol and water are presented [197]. The solvation free energies are less negative than membrane binding free energies. From the Table 7.2 it is not easy to understand why the toxicity of a molecule can be so different from others of the same kind. However, a clear effect of a presence of one double bond in a lipid tail of 16:0-18:1 PC on the value of the binding free energy for 2’-OH-BDE68 can be observed. The molecule shows the affinity to DMPC rather than to POPC. Conformational analysis of this specie in two simulations has revealed differences in orientations of hydroxyl group towards oxygen...
situated in the ether group, depending on a position of this substance in the simulation box. The distribution of O−H distance on Figure 7.5 shows that in 14:0-14:0 PC the location of the molecule does not play a role, while in 16:0-18:1 PC hydroxyl group had more motional freedom in the center of a membrane. Such a motional freedom in the center of the unsaturated lipid bilayer can be related to a disorder of the membrane, but in this work order parameters were not computed.

7.4 Summary of Paper II

For the considered set of molecules, computed values for pK\textsubscript{a} have not shown strong correlations with experimental toxicity data. Anyway, these calculations were done without any calibration set for studied species, using available data for smaller molecules.

Well-tempered metadynamics has shown promising results which were similar with ones obtained for octanol systems. The effect of one double bond in a lipid tail was well-pronounced in values for binding free energies. Conformational analysis has shown that the orientation of hydroxyl group in 2’-OH-BDE68 (ortho) differs depending on a saturation of a lipid bilayer where the molecule is situated.

Computations of log(P) revealed that molecules with higher value would have a higher concentration in a membrane at the same "envi-
In addition a suggestion can be made for future experiments. One shall choose and sort substances according to their chemical composition and structure. Gathering different species, more likely, will not show any significant correlations. For example, when the set of non-chlorinated molecules in ortho conformations was isolated for computations of Pearson correlation coefficients, a stronger correlation between experimental toxicity data and calculated values was observed. Selecting only species in meta conformations with the same chemical composition could improve the toxicity analysis for this particular group. However, there have not been enough molecules in meta conformations in the set of studied species.
8. Cholesterol in membranes with different grade of saturation (Paper III)

It was already discussed in Chapter 1 and Paper I that polyunsaturated phospholipids have differences from the saturated ones and play an important role in biological systems. Nevertheless, there were not so many computational studies on polyunsaturated membranes loaded with different amount of cholesterol. The curiosity regarding the question has roots in a composition of lipid rafts in different tissues of a human body, for example, in a brain, where one can find both: polyunsaturated phospholipids and a high content of cholesterol.

Paper III addresses several questions on behavior of cholesterol in lipid membranes and its effect on membrane’s properties. First, the idea was to see how the degree of saturation can affect the orientation and the position of a single cholesterol molecule in a lipid bilayer. For this purpose free energy calculations were done. Then the interest was to study the ability of cholesterol molecules to build clusters and it’s orientations at higher concentrations, depending on lipid chain length and the number of double bonds in a single lipid tail.

8.1 Lessons from classical MD simulations

In previous experiments and simulations of saturated phospholipid bilayers it was found that the presence of cholesterol in a membrane is leading to so-called condensing effect: the area per lipid was decreasing with increasing concentration of cholesterol. In this project, such an effect has been observed for all simulated membranes. On Figure 8.1 two membranes are compared with each other in terms of changing areas per lipids depending on cholesterol content (the data is taken from Table 3 in Paper III [199]). Almost a linear dependency of the area with an increasing concentration of cholesterol is seen for the saturated bi-
layer while in case of polyunsaturated one a strongly non-linear behavior is observed. Earlier Mitchell et al.[200] have seen a different effect of cholesterol at the concentration 30% mol. for 22:6-22:6 PC comparing it to 20:4-20:4 PC and even 16:0-22:6 PC. Two polyunsaturated acyl chains with six double bonds according to them interact weaker with cholesterol than other kinds of lipid tails what condenses the membrane less at the cholesterol content equal to 30 % mol. A similar trend can be seen for the cholesterol content equal to 33 % mol. on Figure 8.1: the membrane containing 22:6-22:6 PC is less condensed than the one with 14:0-14:0 PC.

![Figure 8.1: Simulated areas per lipid for 14:0-14:0 PC and 22:6-22:6 PC](image)

Due to such different trends in values of areas per lipids a question can arise regarding locations and orientations of cholesterol in various lipid bilayers. This can be partially answered by contributions to mass density profiles from whole molecules of cholesterol as well as their parts: OH- (the "head group" of cholesterol) and terminating CH3-groups (the "tail" of cholesterol). Such profiles are computed using the same principle as for electron density profiles, described in Chapter 5, but masses of certain molecules and their parts are considered for computations.

Figure 8.2 shows that in both saturated membranes cholesterol molecules are situated in their "traditional" orientations: with OH-group towards lipid head groups, but in 18:0-18:0 PC the separation into leaflets is more obvious, than in 14:0-14:0 PC. When the number
Figure 8.2: Contribution from cholesterol molecules to mass density profiles for different lipid bilayers (50 % mol. cholesterol in each system). (A) 14:0-14:0 PC  (B) 18:0-18:0 PC  (C) 18:0-18:2 PC  (D) 18:2-18:2 PC  (E) 20:4-20:4 PC  (F) 22:6-22:6 PC Here: "whole" - a contribution from the whole cholesterol molecule, "CH\textsubscript{3}-groups" - contributions from atoms from methyl-groups on cholesterol molecules, "OH-group" - atoms from hydroxyl group on cholesterol molecules. The image is reproduced by permission of Royal Society of Chemistry [199]
of double bonds in acyl chains was increased for lipids containing 18 carbons in each tail cholesterol CH$_3$-groups are more likely to be observed in the bilayers’ centers, than in a case of 18:0-18:0 PC. A similar distribution is seen for 20:4-20:4 PC, but in 22:6-22:6 PC cholesterol molecules seem to reside in inverse positions too, because both OH and CH$_3$ groups can be seen in the bilayer’s center and closer to lipid head groups (Figure 8.3). Such an usual orientation and location at high con-
centration can be explained by a bad mobility of cholesterol in 22:6-22:6 (cis) PC, since areas per lipids have shown a stronger condensing effect at 50 % mol. of cholesterol for this lipid bilayer. A graphic explanation of contributions to mass density profiles can be seen in Figure 8.3 for 14:0-14:0 PC and 22:6-22:6 PC.

A number of radial distribution functions was computed for studied systems in order to investigate possible associations including those which could indicate possible hydrogen bonding between pairs of atoms. Hydrogen bonding between lipids and cholesterol seems to play a big role in possible orientations and locations of cholesterol inside a lipid bilayer. Strong associations were detected from RDFs between oxygens in ester groups on lipid tails and hydrogens in hydroxyl groups of cholesterol molecules. Furthermore, cholesterol-cholesterol associations were different depending on studied system, and in 22:6-22:6 PC coordinations between methyl groups and OH-groups were observed (see Paper III).

8.2 Two-dimensional well-tempered metadynamics

Figure 8.4: Illustration for selection of collective variables and how to read the PMF profile in 2-D well-tempered metadynamics. CV1 is the first collective variable (Z-distance between centers of mass of cholesterol molecule and the lipid bilayer). CV2 is the second collective variable. [199]
In Chapter 4 and Paper II well-tempered metadynamics simulations were already discussed. Here two-dimensional calculations were carried out in order to compare free energy profiles of a single cholesterol molecule in bilayers composed of different phosphatidylcholines. The aim was to investigate the ability of cholesterol to go through membranes and reorient itself depending on saturation of lipid tails. For this purpose two collective variables were chosen. The first collective variable was the $Z$-distance between the center of mass of cholesterol molecule and the center of mass of the lipid bilayer. The second collective variable was the $Z$-projection the $C-O$ vector in cholesterol (see the illustration of collective variables on Figure 8.4), which determined the orientation of the molecule.

On Figure 8.5 two-dimensional PMF maps are shown for six selected simulations. Better mobility of a single cholesterol molecule was indicated for 22:6-22:6 PC, then 18:2-18:2 PC and 20:4-20:4 PC (images F, D, E), what was reflected by different conformations depicted by points of minima on the PMF maps (lower free energy barriers). For these lipid bilayers one can see cholesterol lying flat in the center of the bilayer. In systems built of lipids with mixed acyl chains, the variation of locations with orientations was smaller. However, the same PMF maps show lower affinity of cholesterol to a membrane containing 22:6-22:6 PC, than to other polyunsaturated lipid bilayers.

8.3 Summary of Paper III

Regardless, that set-ups were the same for all simulations, several important differences were observed in behavior of cholesterol in bilayers composed of various lipids. The most unsaturated lipid, 22:6-22:6 PC, gives unusual properties to bilayers which it forms. Areas per lipids follow a non-linear trend for 22:6-22:6 PC with an addition of cholesterol, while in case of 14:0-14:0 PC this property has a linear behavior with increasing cholesterol content. A single molecule of cholesterol has a better mobility inside polyunsaturated lipid bilayers comparing to saturated ones.

Saturated membranes have a lot in common in a sense of cholesterol’s behavior: the molecule prefers the same positions and orientations inside those bilayers and shows the affinity to them rather than to polyunsaturated ones (see Paper III).
Figure 8.5: Free energy profiles for selected lipid bilayers after 6 µs (the minimum is set to zero). (A) 14:0-14:0 PC (B) 18:0-18:0 PC (C) 18:0-18:2 PC (D) 18:2-18:2 PC (E) 20:4-20:4 PC (F) 22:6-22:6 PC \( \cos(\alpha) = \frac{CV2}{CV2_{\text{max}}} \) and \( \alpha \) is the angle between the selected vector and the membrane normal. The image is reproduced by permission of Royal Society of Chemistry [199].
Alzheimer’s disease is known to be a cause for the most of dementia cases [201]. There are several existing hypotheses of occurrence of this disease: genetic [202, 203], cholinergic [204, 205], homeostasis of biometals [206, 207], tau hypothesis [208–210], gum disease [211, 212], amyloid [213, 214] etc. From all existing hypotheses amyloid hypothesis seems to be one of the most trustworthy than others due to the large number of studies proving the adequacy of a theory. In this hypothesis the extracellular amyloid-β deposits (or Aβ peptides) are seen...
as the major cause of the illness. These deposits are known to come from amyloid precursor protein (APP).

Figure 9.2: $A\beta(1-42)$ peptide with labeled peptides which were used for MD simulations

APP is a specie present in human tissues and, particularly, in the 21st chromosome. This specie has received a lot of attention nowadays not only because it was highly related to AD, but to many different diseases such as amyloidosis [215, 216], Parkinson disease [26], cancer [217], diabetes [218] etc. Proteolysis of APP (Figure 9.1) is known to be a process which is catalyzed by proteases [219]. During this process the proteins get broken into smaller polypeptides or even aminoacids. Proteolysis of APP is blamed nowadays for creating amyloid-$\beta$ ($A\beta$) peptides which are seen as causes for the diseases mentioned above [217, 220, 221].

However, regardless that the problem is known for decades, the cytotoxic mechanisms of actions of $A\beta$ peptides with cell-membranes, which cause cell deaths, are not known. Many experiments were done with different peptides in cells and phospholipid bilayers, but lipids which were seen as "signaling" species (containing 22:6 (cis) fatty acids in their tails) in AD have not been used in such studies, probably, due to possible implications of lipid peroxidation. Computationally $A\beta$ pep-
tides were not studied on all-atomistic level in a membrane environment. Some MD simulations were done using united atom FFs for peptides in lipid bilayers.

Anyway, the majority of computational studies were performed on these species in different solvents in order to verify solved by NMR structures or to investigate the ability of different peptides to aggregate, depending on an environment surrounding them. The fact that Aβ peptides are known for their cytotoxicity makes them very alluring species for computational studies on various lipid bilayers. Since it is not clear which parts of the Aβ(1–42) are causing AD or playing a key role in its development, it is important to investigate different parts of the sequence.

That is why projects for papers IV and V were done for three different peptides (Aβ(1–28), Aβ(26–40) and Aβ(25–35)) taken from the sequence Aβ(1–42) (Figure 9.2).

9.1 Simple lipid bilayers and three different peptides

First, four MD simulations, where each was 1 µs long, were done for 14:0-14:0 PC and 18:0-22:6 (cis) PC with Aβ(1–28) and Aβ(26–40) as model-peptides. The idea behind selection of phospholipids was inspired by an observation from the work by Söderberg et al. [14] that AD brain has much lower amount of polyunsaturated lipids (particularly, the ones containing DHA) than a healthy one. 14:0-14:0 PC has the lowest gel-liquid phase transition from all saturated phosphatidylcholines with a longer acyl chain, what could serve as a model for a cell-membrane from the gray matter of an AD brain, while a lipid bilayer containing of 18:0-22:6 PC has played a role of a "healthy" gray matter tissue due to abundance of 22:6(cis) fatty acid in there. Generally, the interest was to see where peptides were situated in the simulation box, and how different grade of membrane’s saturation would affect the ability of these species to aggregate.

The results for contributions to mass density profiles from three selected aminoacids in different parts of sequences revealed that Aβ(1–28) could partially enter only polyunsaturated lipid bilayer, while Aβ(26–40) was entering both membranes, but partially residing outside of saturated membrane (Figure 9.3).

For Aβ(25–35) two MD simulations were done with 14:0-14:0 PC and 22:6-22:6 PC bilayers. Simulations were slightly longer than for...
two other peptides, but in both cases no insertion in the center of any bilayer was detected on mass density profiles for this specie, regardless, that system sizes of calculations have been smaller than for the first two peptides (see Paper V).

9.2 Complex membranes and peptides

Two more membranes were created containing four different lipids in each. The number of lipids of each type was set according to the paper of Söderberg et al. [14] in order to mimic the compositions of most abundant lipids in the gray matter of a human brain. Four simulations were done with one kind of peptides (Aβ(1−28) or Aβ(26−40)), while
Figure 9.4: Snapshots and mass density profiles of selected amino acids of Aβ(1−28) peptide in two complex membranes (A) "Healthy" model membrane, (B) "Sick" model membrane. For the peptide three amino acids were taken from the beginning, middle and end of the sequence: ASP₁, HIS₁₃ and LYS₂₆. The image was adapted from Paper IV.

Results from simulations for one particular type of the peptides showed similarities to what was observed from computations of Aβ(1−28) and Aβ(26−40) in monocomponent lipid bilayers (Figure 9.4). The membrane which was supposed to be a model for the sick tissue (Figure 9.4(B)) of a gray matter in a human brain acted similarly on peptides location as the lipid bilayer containing 14:0-14:0 PC, while the one which was used as a model for a healthy tissue was acting as the membrane containing 18:0-22:6 PC (Figure 9.4(A)). In case of more saturated 'sick' membrane Aβ(1−28) did not enter the lipid bilayer and for the more unsaturated 'healthy' system the insertion was detected. This can be observed on contributions to mass density profiles and snapshots from simulations (Figure 9.4).

However, when both kinds of peptides are present, the situation is changing. Due to interactions between two different peptides Aβ(1−
Figure 9.5: Snapshots and mass density profiles of selected amino acids of Aβ(1−28) peptide in two complex membranes when Aβ(26−40) is present as well (A) "Healthy" model-membrane, (B) "Sick" model-membrane. For the peptide three amino-acids were taken from the beginning, middle and end of the sequence: ASP₁, HIS₁₃ and LYS₂₆. The image was adapted from Paper IV.

28) can partially enter the membrane by locating itself closer to lipid tails in a sick model membrane (Figure 9.5 (B)), while in case of a healthy one, smaller amounts of it are placed outside of the lipid bilayer (Figure 9.5(A)).

9.3 Cholesterol loaded bilayers

Addition of 50% mol. of cholesterol in mono-component membranes is changing their properties. The ratio Cholesterol/PC equal to 1 is the typical one for lipid rafts in neuronal membranes [222]. Two MD simulations were done for Aβ(25−35) in 14:0-14:0 PC and 22:6-22:6 PC loaded with extra cholesterol. Both lipid bilayers became more ordered and more rigid. This was reflected in deuterium order parameters of lipid tails (see Paper V).
Figure 9.6: Snapshots and mass density profiles of selected amino acids of Aβ(25 – 35) is present as well (A) 14:0-14:0 PC, (B) 22:6-22:6 PC. For the peptide three amino-acids were taken from the beginning, middle and end of the sequence: GLY\textsubscript{25}, ALA\textsubscript{30}, MET\textsubscript{35}. The image was adapted from Paper V.

Regarding the behavior of peptides, mass density profiles and snapshots of systems on Figure 9.6 show that three selected amino-acids and the whole peptide do not enter the membrane deeper than ester groups of lipid tails. The aggregation of the species was higher in cholesterol loaded lipid bilayers, perhaps, because of the rigidity of cholesterol loaded membranes.

9.4 Summary of Paper IV and Paper V

Starting from relating the membrane properties to peptides’ aggregation, it is clear that in systems with saturated lipid bilayers peptides aggregate more on membrane surfaces, while in more unsaturated membranes the insertion can be observed and even the accumulation inside the bilayer for some species. This can be explained by the order of
membranes: more saturated phospholipid bilayers have a higher order, while polyunsaturated ones are known to be more disordered. Order can cause a rigidity of a membrane, which can inhibit the insertion of peptides and promote their aggregation outside of lipid bilayers.

Another interesting thing is what kind of peptides can enter bilayers and which ones will never do that. The most toxic part of Aβ(1–42) which is Aβ(25–35) did not penetrate through any of bilayers. It was partially inserted in membranes containing no cholesterol but it has never been reaching the center of a membrane. One could think of the reason which would be too short simulation time, but comparing that with two longer peptides in larger systems, it was easy to notice that if peptides are supposed to enter the membrane, they would do that. This can be also explained by the amount of polar versus the amount of hydrophobic groups in these peptides. Furthermore, in case of Aβ(1–28) and Aβ(26–40), when they were mixed in more complex systems, both could enter, while when computations were done using single types of peptides, one of them would never enter any of saturated bilayers.

Computational results were coherent with many experimental findings. Aβ(25–35) were seen partially inserted in the lipid bilayer but not really penetrating it [223–225]. Lau et al. [223] related the possible membrane penetration by this peptide to the sample preparation technique, i.e. when the specie was present in the mixture during the bilayer formation, otherwise, it was not seen in the membrane’s center. Ionov et al. [226] showed that Aβ(1–28) was residing outside of the bilayer containing 14:0-14:0 PC and 16:0-16:0 PG (98/2 w/w), while Aβ(25–40) was inserted in the same membrane. This result was in agreement with the outcome from MD simulations for Aβ(1–28) and Aβ(26–40) in 14:0-14:0 PC.
10. Conclusions

Behavior of various molecules in different cell membranes and the ability of species to penetrate through lipid bilayers are very important to investigate. However, as this work shows, one shall select model-membranes very carefully. Often mono-component lipid bilayers with species containing one double bond per fatty acid in their tails are used as models for polyunsaturated phospholipids which is not correct to do. This statement can be proved by taking into consideration several different properties of various membranes. Considering deuterium order parameters (calculated for bilayers in Paper I) for lipid tails with different saturation, it is clear that with the decrease of the degree of saturation, deuterium order parameters are decreasing as well. This was observed in both experimental and computed profiles for membranes containing 18:0-18:2 PC, 18:0-22:6 PC, 18:0-22:5 PC, where the most "disordered" lipid bilayer was the one built of 18:0-22:6 PC (containing DHA in the unsaturated tail). The disorder can affect the motion of lipid tails and on a larger scale properties of a membrane.

The disorder and the fluidity are very essential for diffusion and aggregation of various molecules in membranes [200, 227, 228]. For example, only one double bond in a lipid tail could affect the binding free energy of 2'-OH-BDE68 (ortho) as well as its conformational variety [197]. In a case of cholesterol loaded membranes it was observed that the presence of DHA gave more freedom in orientations and locations for cholesterol in those lipid bilayers, what was even coherent with results of free energy calculations for single cholesterol molecules in mono-component membranes (Paper III).

Finally, studies with Aβ peptides in neuronal membranes showed that the amount of polyunsaturated phospholipids affected the behavior of precursors of Alzheimer’s disease (papers IV and V). Earlier such peptides were investigated experimentally in various lipid bilayers, but even though nobody has made a research on how amyloids behave in a presence of DHA, which is an abundant specie in a gray matter of a human brain tissue [14].
Results presented in this thesis show that the saturation of a lipid bilayer as well as high cholesterol content prevent the $\text{A}^\beta(25-35)$ from entering a membrane, while in case of mono-component bilayer containing 22:6-22:6 PC the partial insertion of the peptide has been observed, but no penetration was detected.

Longer peptides were used in other studies with mono-component and neuronal membranes where it was shown that DHA could be a preventing compound in the development of AD. This statement was proved even by results from MD simulations for more complex membranes. Higher saturation has promoted the aggregation of $\text{A}^\beta(1-28)$ outside of lipid bilayers while in more unsaturated membranes species $\text{A}^\beta(1-28)$ and $\text{A}^\beta(26-40)$ could partially enter or be completely inserted in the center of bilayers.

The MD simulations of mono-component bilayers and complex membranes (healthy and sick) demonstrate that 14:0-14:0 PC can serve as a good model for the sick tissue of a gray matter in a human brain while 18:0-22:6 PC shall be considered as the lipid bilayers representing the healthy one.

This thesis shows how the composition and, particularly, saturation of a lipid bilayer can affect the distribution of different molecules through model-membranes what shall be highly considered in drug development process.

Computer simulation methods showed again that they can be very promising techniques for studying various problems from toxicological to development of neurodegenerative diseases on a molecular level.
Sammanfattning

och Aβ(26 − 40) har studerats även i mer komplexa lipid bilager som hade 4 typer av lipider. Syftet var att imitera den gråa hjärnsubstansen från människans hjärnan som påverkas påverkat av Alzheimers sjukdom och en annan som var helt frisk från sjukdomen. Det visade sig att enkla modeller som bestod av 14:0-14:0 PC och 18:0-22:6 PC kan korrekt representera den ’sjuka’ och, repettive, den ’friska’ delar av hjärnsubstansen. Samtidigt kunde man se att lipiders mättningsgrad i alla system har stark påverkan på fömågan av peptider att gå igenom membraner. Lipid bilager som innehöll hög mättningsindex hade minst förmåga att absorbera peptider, medan omättade lipid bilager uppvisade högre upptag av peptider. Metodik som involverar användning av komplexa bilager, samt enkla bor utvecklas vidare för andra substanser i människans kropp med användning av SLipid's kraftfält.
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