Introduction to the Master thesis

We uploaded this document to the system, as we were not able to converge our LaTeX document containing the thesis into the system. Following paragraphs are, therefore, identical with the introduction presented in the theses. The original text of the theses is included as an attachment in the
*.zip file.

Abstract

Molecular dynamics simulations are an important tool for the study of biological systems, such as biomembranes. The missing electronic polarization in classical non-polarizable force fields, however, produces significant inaccuracies in the interactions of membranes with charged particles, such as ions. In this work, we implement the missing electronic polarization effects into CHARMM36 force field for phospatidylcholine lipids. This implementation is done in the mean field way by using electronic continuum correction (ECC) model. We will validate the strength of ion--membrane interactions using the electrometer concept. This concept connects the response of choline order parameters of lipid molecules with the amount of charge present in the surface of the membrane.

Abstrakt

Simulace molekuláru 11 dynamiky mohou poskytnout detailni informace o biologických systémech. Nicméně klasická silová pole, která nezohledňuj 11 elektronovou polarizaci atomů, nejsou schopná dostatečně přesně reprodukovat interakce mezi biologickými membránami a nabitými částicemi, jako jsou ionty. V této práci zahrneme efekt chybějici elektronové polarizace do silového pole CHARMM36 pro fosfatidylcholinové lipidy. Tato implementace bude provedena pomoc 11 aproximace středniho pole za využiti modelu elektronového kontinua (ECC). Správnou sulu interakci mezi ionty a membránou odvozujeme na základě tzv. konceptu elektrometru, který dává do souvislosti změnu v parametrech uspořádán 11 v cholinové části lipidů s množstv m 11 náboje, který je přitomen na povrchu membrány.

1. Introduction

Computer simulations represent an important tool for studying biological and other systems, as they provide atomic resolution insight into phenomena of interest. In biological membranes, which are involved in key cell homeostatic processes, one can gather dynamic and structural information with a resolution of their structure and molecular constituents that surpasses any known experimental technique. Computer simulations can provide critical information about interactions of membranes with

proteins and other important molecules which ultimately can help understand the phenomena of interest and hopefully develop better drugs. However, to take full advantage of this “computer microscope”, one needs to be sure that the interactions between different parts of a system are modelled accurately.The goal of this thesis is to improve the performance of one of the most widely used computer models for biosystems, the CHARMM36 force field, for phosphatidylcholine membranes in the presence of ions. Ions influence membrane properties and in some cases even facilitate membrane fusion; they may assist certain proteins’ binding to lipid membranes and among other things, some ions also function as signalling molecules themselves. It is therefore crucial to capture the ion-membrane interactions correctly if one wishes to model a biological system.

1.1 Biological membranes

Cells need to extract and transform nutrients from their surroundings and convert them into energy and work. To do so, thousands of reactions have to occur in the right order, with the right kinetics, and at the right place . Cell membranes play a critical role in all these processes. Biomembranes are fundamental for life, as they encapsulate every cell. In addition to defining the boundaries of cells, membranes also surround cell organelles, such as the mitochondrion, nucleus, endoplasmic reticulum or chloroplasts in plant cells. They are key for enabling different chemical reactions which happen on their surface. They also enable creation of a separete environment with unique chemical and electrical gradients, which can serve as driving forces. Membranes allow cells to intake nutrients selectively, to communicate with the outer environment, and send signals inside the cell.

Membranes mainly consist of lipids, proteins, and carbohydrates. Their exact molecular content varies from cell to cell and from organelle to organelle. Membrane proteins can be integral, spanning across the membrane, or peripheral, attached to the surface of the membrane, their main function being signalling, communication, and energy transfer. Both proteins and lipids can be further modified by covalently bound carbohydrates . Lipid composition varies not only between different species and cells, but also between the inner and outer leaflets. From all lipid classes, glycerophospholipids play the major structural role in membranes with phosphatidylcholine (PC) accounting for more than 50 % of lipids in most of the eukaryotic cells. In mammalian cells, PCs are mainly exoplasmic lipids, while phosphatidylserine (PS) and phosphatidylethanolamine (PE) can be mostly found in the cytosolic leaflet. Another important lipid class consists of sphingolipids with sphingomyelin (SM) being typically the most

abundant one. Sterols (cholesterol in mammals and ergosterol in yeast) are present in both leaflets in large quantities, over 30 % in mol ratio. They are largely responsible for maintaining of the fluidity of the membrane.. Altogether, there are over 1000 different lipid species in every eukaryotic cell. However, their specific function is not yet exactly known . Some of the membranes are mostly composed by lipids (80 % of lipids in case of myelin), while others mostly by proteins (80 % of proteins in case of mitochondria, nucleus or rough endoplasmatic reticulum).

Still, when studying biological membranes, their native complexity calls for more simplified models. The basic model of a biomembrane is a lipid bilayer, which is composed only by lipids. It results in a hydrophilic surface, where the polar headgroups of the lipids reside in contact with the water phase and the hydrophobic core, where the chains of the lipids and cholesterol reside.

In the biological environment, cell membranes are usually in a fluid phase. This means that their components experience rotational and translational diffusion in the membrane plane. This results in a broad distribution function of the positions of the lipids.

1.2 Ions in Cell Biology

Ions play a crucial role in cells. They propagate electrical signals (e.g., in neuron signalling), serve as cofactors for proteins, are involved in muscle contraction, and maintain the transmembrane potential that is further used to drive metabolism. Notably, ionic concentrations differ in the extracellular and intracellular regions of the cells and also vary in specialized cells. These concentrations of ions is carefully maintained and controlled in cells by complex mechanisms involving pumps, transporters and ion channels.

Furthermore, ions can directly interact with proteins, modulate their function, or promote a conformational change. Some ions also affect the properties of lipid bilayers and promote their aggregation and fusion.

2 Experimental methods for studying biomembranes

In 1925, Gorter and Grendel first proposed lipid bilayer model for a membrane . It took another 47 years until Singer and Nicolson proposed the model of a fluid mosaic. In this model the membrane is a soup where all components randomly diffuse and distribute. We know now that the membrane organization is not as random as it was initially postulated , however, the proposed model is still conceptually important for understanding the molecular organization of membranes. All these advances in understanding of the membrane structure and its interactions with ions required the development of experimental techniques and experimental setups .

Determination of the structure and behavior of lipids in cell membranes using simple model membranes is justified by similar lipid order parameters for cell membranes, lipid extracts, and model systems measured by NMR. These model membranes are usually composed by few lipid moieties, including single lipid bilayers, and their topology depends on the target experiment. Two experimental methods to study membranes that we use in this work are solid state nuclear magnetic resonance and neutron and X-ray scattering. Deuterium NMR can accurately measure order parameters of different methylene groups in which characterize their average conformation. Scattering methods provide information about the average properties of the whole lipid system, such as the area per lipid or bilayer thickness .

2.1 Nuclear magnetic resonance

Nuclear magnetic resonance (NMR) is a powerful technique to study the molecular structure of various materials. The method is based on the measurement of the interaction of nuclei immersed in a strong magnetic field with an oscillating radio-frequency electromagnetic field. Different NMR techniques were used to describe the structure of cell membranes and lipid bilayers. In this section, we briefly introduce the NMR experiment and its applications for lipid bilayers.

Atoms are characterized by quantum numbers, one of them being a spin quantum number. To detect an NMR signal, it is necessary for the atom to have an unpaired spin from neutrons and protons. This means that only atoms with an odd number of neutrons and/or an odd number of protons can be measured. Some atoms can have higher spin than 1/2 leading to more than 2 spin orientations and an electric quadrupole. This last property is the basic for deuterium NMR applied to membranes. The energy, E, of different spin states, m, is the same in the absence of external magnetic field.

Small variations of the local magnetic field are present as a response to the external magnetic field. This local field is dependent on the molecular structure and can be different in different parts of the system. The external and local magnetic fields add to each other and result in the actual field that nuclei feel. As different nuclei are in different local fields, this results in a different split in the energy levels of the spins and, therefore, in a different frequency of photons that can be absorbed. This is known as the chemical shift . When dealing with biomembranes, chemical shift measurements can be used to identify differences between leaflets in vesicles, impermeability of lipid bilayers to paramagnetic ions, or the effect of certain ions on the structure of the bilayers .

Every spin under the effect of an external magnetic field wobbles with Larmor frequency. When immersed into the magnetic field, this precession is not immediate. Its delay is characterized by a relaxation time, T1. ^{13}C relaxation times T1 can be used to characterize lipid-lipid and lipid-protein interactions. Another property of interest in NMR spectra is the spin-spin coupling arising when non-equivalent NMR-active nuclei are present in the vicinity of the studied nucleus.

Classical proton and ^{13}C NMR spectra are usually complicated because of the splitting of the peaks caused by spin-spin coupling. Fortunately, in deuterium NMR this dipolar coupling is significantly reduced and spectra are much easier to interpret. Moreover, molecules can be deuterated specifically yielding straightforward information about particular parts of molecules. Also, ^{2}H NMR spectra provide information about the anisotropy and the orientation of the sample. Unoriented samples result in a single peak whereas spectra of oriented samples are dominated by quadrupole interactions resulting in one doublet called quadrupolar splitting.