Niels-Bohr Institute Copenhagen

Ionchannels in pure lipid membranes

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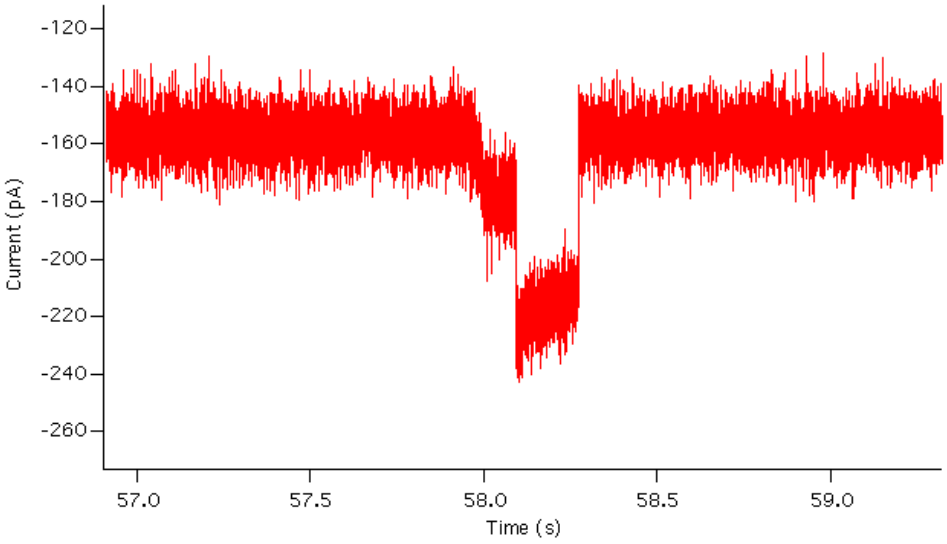

Supervisor: Thomas Heimburg

Abstract

A setup was built to investigate current and voltage over an artificial lipid bilayer membrane. The membrane was produced by folding two single monolayers, created on a water-air surface, over a $200\mu m$ hole in a teflon membrane. Voltage and current are controlled via an amplifier. With this setup one can show, that in pure lipid membranes ion channeling events can be observed when voltage is applied. The opening and closing of lipid pores is almost indistinguishable from the activity of ion channel proteins.

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1. Motivation

The first chapter, which shows why it is not only interesting but exciting, to do this experiment and to read this thesis, and why the project can be considered as successful.

To begin with something personal. The first chapter has the task to show why this project caught my interest so much, that I took the challenge to assemble a setup on a blank table, starting with making engineer drawings of several parts for the workshop, reading some exciting literature, making adjustments, failing tries and ending with measurements in a comperatively short time, not to forget that I learned a lot.

If one asks a student how he[1] imagines the structure and operation mode of a biological membrane (and choose a student who pays attention in his biology lessons) he will reproduce what he was taught and describe something close to the Fluid mosaic model of Singer and Nicolson[26]. According to this model from 1972, biological membranes consist of a phospholipid bilayer, in which a multiplicity of different proteins are embedded or attached, and various sugars are connected. In this model, the bilayer is a very passive, homogeneous constructed sheet, with the simple task to divide space into inside and outside, meanwhile every active business like ion exchange between the two sides, regulation of gradients and signal propagation (especially in excitable nerve cells) is considered as a function of proteins.
If one choose a student who has the luck to be a bit deeper into the matter, one will find out that this one has a more modern picture of the membrane, which includes that the bilayer does not consist of just one kind of lipids but of a mixture of up to several hundred different types of lipids, cholesterol and other molecules. The exact composition depends on the cell type and also on the part of the cell under investigation, furthermore the different lipids are not contributed randomly over the whole area, but somehow ordered, as described first in 1977 by Jain and White:

> "*... the biomembrane continuum is broken up into a number of relatively rigid plats or patches that are in relative motion with respect to each other*"[12]

This patches or domains are also not randomly distributed, over and above it seems that different kinds of proteins choose different lipid surroundings, and the properties of this rafts are highly dependent on the cell type and the part of the cell. An illustration of this membrane model is provided in figure 1.1 .

[1] Without any doubt the student could also be a female, but to keep language simple the "he" will be used substitutional for both genders.

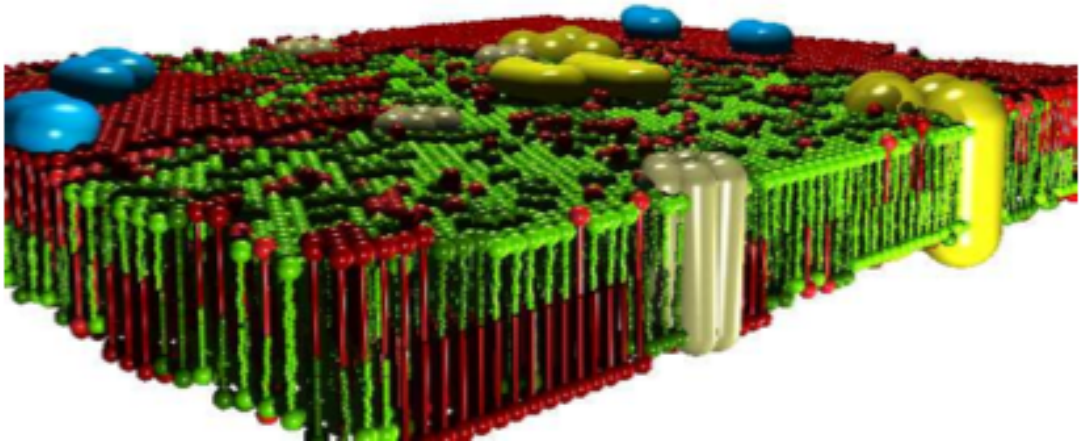


Figure 1.1.: structure of membrane according to fluid mosaik model. The membrane consists of a variety of different lipid types, forming clusters, proteins are attached to the surface or stick into the double layer. (figure taken from [25])

Walking home through the long danish night after learning this it should seem suspicious to our student, that nature creates a complex composed and cell-type specific bilayer only to fulfill the task of having a seal between inside and outside. Considering a bit about it could bring the idea, that a membrane as whole or also just taking the lipid bilayer is a system consisting of many particles, and therefore – even so it is two dimensional – can be described with thermodynamic methods in an appropriate manner. A look into literature shows that this actually is the case. Even so the classical biology and biochemistry books don´t mention words like entropy, energy, reversible heat, temperature or time scale, Membranes are physical systems and make phase transitions[22], thermodynamic properties like volume, area, enthalpy and especially heat capactity change their values dramatically at phase transition temperature[5]. The idea of using thermodynamics promises to describe the membrane quite successful.

One of the most remarkable and best investigated activities of biological membranes is the regulated exchange of ions between the two sides, dependent on cross membrane potential or triggered by the presence of special chemicals. In the mainly accepted model, this is done by channel proteins, which are either voltage gated, that means they open the channel when the membrane potential reaches a specific threshold, or ligand gated, so that the protein gets active in the case that a more or less specific molecule binds to the protein, causing a conformational change and opening a special pore for ions on this way. There is a good settled interpretation saying that a part of the molecule, called "pore" is responsible for conductance, a tunnel where the ions get through, and there are theories of moving parts, paddles, balls and chains and things like that.
The importance of ion channels became obvious when first measurements with the patch clamp method actually showed the current across the membrane and Hodgkin and Huxley 1952 presented their model to make a first explanation of signal transmission in nerve

pulses[11], which is basically completely driven by the correlated opening and closing of channels. Since that, people spent a lives time on investigating the structure and function of ion channel proteins, by isolating, cloning and sequencing it (Dr. Numa presented the sequence of the Na^+ channel of electric eel organs in 1982) finding hydrophobic and hydrophilic parts of the sequences to get a picture of which part is related to the membrane, and which part is on which side.
The idea, how this proteins are constructed in three dimensions became a bit more clear when McKinnon managed to crystalize the K^+ channel of a bacteria and made a X-ray spectroscopy on it (Nobel price 2003[4]) He also proposed an explanation of selectivity which assumes that there is a pore in the protein, and the structure of amino acids in this pore allows only K^+ ions to go through meanwhile other ions like Na^+ (which carries the same charge and is almost of the same size) can not pass this selectivity filter. [15]

All this models and explanations don´t include an active role of the membrane bilayer, they assume it as a matrix to hold the active proteins on their place, and increasing the probability of reactions by reducing the motion of membrane related substances to two dimensions. Furthermore, the Hodgkin-Huxley model is pure electrical, its theoretical background and the related calculations describe the membrane as Kirchhoff-circles with resistors and capacitors, thermodynamic terms like temperature, entropy, volume and transition are not included.
The patch clamp method shows the opening and closing of ion channels of some kind, but the acquired tracks (for example see also figure 2.4) do not show, if the ions go through the part of a protein considered to be the pore, it actually does not show clearly that the ions go through the proteins at all, the only thing one can conclude for sure is that charges cross the membrane.[2] The analysis of crystal structure of a ion channel protein gives the three dimensional structure of a protein in one conformation, but the task which is done by it makes at least two states (open and closed) necessary, and it can not be seen, if there are moving parts or special ways for ions or interactions because the x-ray picture does not show ions of movements at all. At physiological temperatures, a physics student would expect brownian motion, which should have a significant influence on contingently moving parts, but is not mentioned in any of the common explanations. Furthermore the structure provided by MacKinnon[4] is more or less the only one, meanwhile it seems clear that there is a enormous variety of different channel proteins.
Despite of that, one can find detailed drawings about the function of many different ion channel proteins, ion pathways, moving parts like “balls and chains”, paddles[16] and more in every biochemistry textbook. (Seemingly it would be a good advise to our curious student to see this drawings as a proposal.)Doubtless the proteins in biological membranes have some function and are important, also in a thermodynamic sense they influence the system significant. but what about the interaction with the surrounding membrane?

[2]This can be seen by patchclamping parts of the membrane, if it happens actually during the action potential is also not sure

If he takes a view over more recent published papers he can find out, that the influence of lipids surrounding the protein plays a extremely important role in the activity of the channels. In following some exemplarily papers are mentioned to show the development, these are not the only ones and perhaps even not the most remarkable, but the show the way.
Schmidt, Jiang and MacKinnon proved in 2006 that the function of the voltage gated K^+ channel is dependent of the charge of surrounding lipid headgroups [16]. They proposed that the electric interaction between the charge of the membrane and the charge of arginine groups in the protein[3] have to be balanced properly to guarantee channel function.

Investigations of the potassium channel from rabbit colon epithelium cells not only showed that the conductivity is different when channels are embedded in artificial membranes of different compositions, also artifacts like a decrease of open-state probability with time ("channel rundown"). Some of the results can be explained by the charge of the lipid head groups, but also other properties of bilayer constituents seem to play important roles [28]

As mentioned before, one should guess to find this "other properties" in the regime of thermodynamics. A paper from 2003 shows that the ryanodine receptor in the sarcoplasmatic reticulum, one of the well investigated, but more complex Ca^{2+} gated K^+-channels, has an activity maximum when the membrane surrounding it is close to a phase transition. [3] Also in this paper the similarity to domain borders appearing in phase transition is bespoken.

This and many other researches show that it is necessary to replace the accepted model, which is including only electrical aspects, with a model paying attention to thermodynamics. Most consequently this was done by Heimburg and Jackson.[9][17] for nerve cells and nerve pulse propagation. They investigated, that a special kind of waves, called solitons, can appear on biological membranes. The soliton model can have the potential to explain unanswered questions depending anesthetics[10], including the famous Meyer-Overton rule[19][20], which sets the critical dose of anesthetics in a direct dependence on its solubility in olive oil (which is similar to lipid chains) But before of all, it is almost free of parameters, includes all experimental results like patch clamp measurements and thermodynamics.
Because the interactions between proteins and membranes and the membrane activity are so basic, it seems consequent to have an eye on thermodynamic properties not only in nerves, but in all membranes in biology. For that, is is necessary, to investigate the physical behavior of membranes in more detail.

To get a better understanding about the role of thermodynamics in membranes it may be a good idea to build model systems out of pure lipids of one or just a few kinds, which allow control over lipid composition, temperature and electrolyte. One of the opportunities to create artificial membranes is to spread lipids in water in order to create vesicles, which can be treated like small model cells, one can clamp them, mark

[3]Parts of the protein with many positively charged arginines are thought to be responsible for the voltage-sensibility of the protein, these parts are called "voltage sensor" in literature.

it with fluorescence markers and observe them under the microscope. Another one is the so called "black lipid membrane cell" used in this setup. The results one can get out of these are amazing: As shown by K.Kaufmann[14], by Antonov[1] and others, it is possible to create ion channels in pure lipid membranes, in absence of any protein. From a thermodynamic point of view it is not surprising, that fluctuations in the system can cause holes in the membrane which open and close reversibly. The remarkable fact is, that the current measurements are almost indistinguishable from the patch-clamp measurements, which are the basics for the mentioned Hodgkin-Huxley model. The currents can be seen best, when the membrane close to a phase transition (which is not a necessary condition), but taking in consideration that biological membranes show a transition slightly under physiological temperatures [9] the relevance of this fact is perfectly given. At the latest here it should be clear to our student, that the widespread textbook picture leaves enough open questions, which are worth to spend some days and nights in a lab, this conclusion should be an adequate motivation to start a little project.

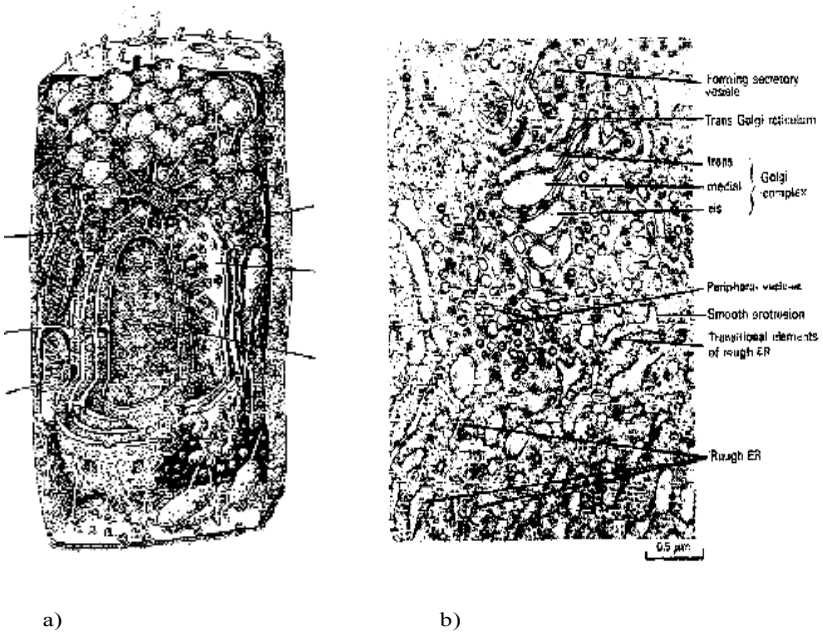
If the project was successful? To answer this question we should have a look on what it was supposed to do and what it did. The defined task was to build a working setup, which enables another investigator to acquire data systematically, and even so there is more fine tuning work to do, in general this task was fulfilled. My idea behind starting a bacheor-like project even so it is not required in my education was, to teach me the basics of lab work and to give me an idea of how experimental physics is done. After T. Heimburg defined the borders of the project, I started to collect adequate papers and to find out what the relevant questions are. I made some construction drawings for the workshop in order to get a flowcell and a fitting heat bath (here I was able to use some knowledge from my former educations), I experienced that time management is a necessary tool, got some experience with scientific software, (this includes standards like LaTeXand the widespread IGORpro as well as special software of the amplifier), I by heart learned how to handle lipids, how to deal with solvents, how to dose liquids in a μl range, how to acquire data with a DSC, and so much more by doing it and by watching and discussing with the members of the group. Furthermore, I was taught the very basic rules for beeing a physicist: Get resistance against frustration, if the experiment does not work, get a strong coffee and keep on searching, keep your smile when the table burns down, spend a splendid time in the lab, the more fun it is, the more useful it turns out to be. All in all, this should be a satisfactory answer to the question about success. Perhaps this view is very subjective, but at least one important thing was done by this project; it showed me the fascination of doing science.

2. Biological and physical background

The second chapter, which provides the reader with the knowledge needed to understand the experiment; the role of the membrane in cells, what the matter with ion channels is, and a short introduction into the thermodynamics of phase transitions and fluctuations.

2.1. Membranes in the Cell

A living cell is an extremely complex composition of different compartments, which accomplish different tasks and consists of solutions with different densities and concentrations of dissolved substances. In an electron-microscopic picture a cell looks like a somehow ordered system filled with big membrane bags and small vesicles. The assign-



a) b)

Figure 2.1.: Cells are dense packed with different comartments. a) Drawing of an eukaryotic epidelcell. b) Elelectronmicroscopic picture of a thin slide through a Pankreas cell (figure taken from[22])

ment of the cell against its environment as well as the definition of the single compart-

ments within the cytoplasma is performed by lipid bilayer membranes. This membrane has several tasks. Besides of the obvious fact, that it defines the shape and size of the enclosed volume, it is a border between watery solutions with different ingredients, concentrations and functions. It has to keep concentration gradients and being a seal for defined substances. Despite of that, interactions between inside and outside – defined by the membrane – are absolutely necessary for communication, metabolism and function of cells and cell compartments. Ions and other substances should be enabled to cross the membrane under defined circumstances, in adequate quantities and time scales. How can the membrane fulfill this seemingly contradictorily exigencies, to be a "permeable seal", so to say?

Structure of biological membranes

The most important constituents of the membrane are lipids. These are amphiphilic molecules, composed of a hydrophilic head group and an polar hydrophobic fatty acid tail. There are many different types of lipids, the properties of any type are defined by the length and the structure of the fatty acid tales, by number and position of contingently existent double bindings as well as by size, structure and charge of the head group. Biological membranes are a mixture of hundreds of different lipid types. In our experiment we also will use mixtures, in order to create membranes with confident transition properties. (See also section 2.4)

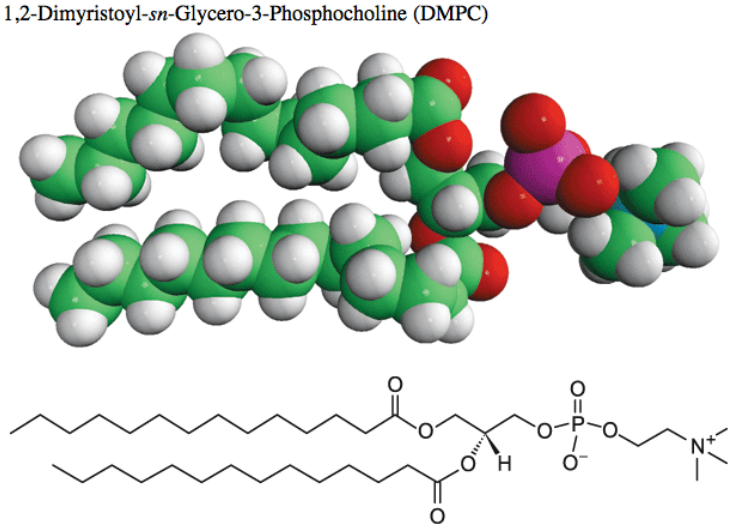


Figure 2.2.: molecular structure of lipids (figure taken from Avanti lipids[2]).

Set into watery environment, lipids form several different aggregates. Dependent on the lipid concentration complex systems of layers and pipes can develop in order to shield the hydrophobic tales from water. At low lipid concentrations the most important shape of self organisation is the bilayer. In this formation, two monomolecular layers develop, ordered in a way that the hydrophobic fatty acid chains are on one side of the plane,

meanwhile the head groups facing the other side. Two of this layers stick together, so that the hydrophobic sides lie on each other and the hydrophilic sides are in the watery surrounding (this can be seen in 2.3). Bilayers formed by selforganisation are about 5nm thick, dependent on the lipids they consist of and the phase they are in (See also chapter 2.3). Already at concentrations beneath the detection limit formation starts, due to the the fact that critical concentrations are exponential dependent on the work needed to bring a single lipid out of an aggregate into dilution in water. [22]

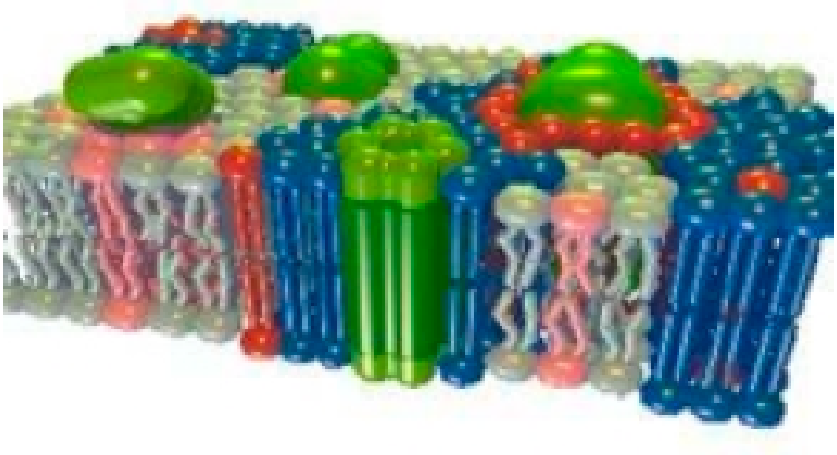


Figure 2.3.: Illustration of the modern picture of membranes. (*graphic friendly provided by Thomas Heimburg*)

Additional to lipids, there are several proteins dissolved into the membrane bilayer. Depending of their three-dimensional shape, these proteins are anchored on the inside or outside with hydrophobic parts (peripheral proteins) or cross the membrane, sticking out of it on both sides (integral proteins).

2.2. Ion channels

Obviously no cell is able to live without metabolism, the exchange with the environment is a basic element of life. Although many different substances are involved in this, the thesis in hand will be focussed on ions crossing the membrane. Many different mechanisms of the cell use the ion gradient over the membrane in order to transport other molecules, propagate signals, or do other tasks. But it is of no help, when the exchange is randomly or just follows the gradient as long as equilibrium is reached (which would mean that the cell is dead), the exchange of ions have to be selective and highly regulated.

For this exchange, in the currently accepted model so called ion channel proteins are thought to be responsible. There are different types, some are ruled by the cross-membrane voltage, opening a selective pore, when the potential difference between inside

and outside reaches a certain threshold. Other proteins open in presence of specific molecules or atoms, and open a channel for some other type of ion. The activity of those channels can be measured with a patch clamp. A small pipette, filled with an electrolyte and an electrode, is brought to the surface of a cell or a artificial membrane, in which the protein under investigation is embedded. Lowering the pressure in the pipette sucks the cell a bit into it and provides an extremely good seal at the edges of the pipette. Applying a suitable voltage offers the opportunity to measure currents over the membrane. A jump in the current represents a opening or closing event of an ion channel. The right side of Figure 2.4 shows a single channel event, a discrete step in conductance with equal height. Opening and closing of more than one channel could also be measured with this method.

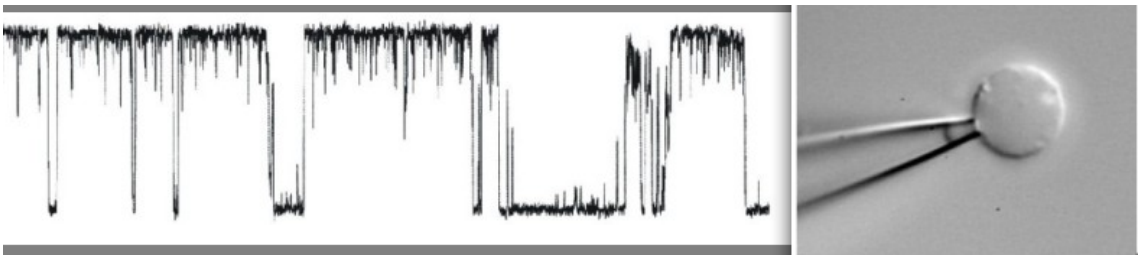


Figure 2.4.: typical measurement with a patch clamp and microscopic picture of the pipette dip with cell.[21]

In contrast to the interpretation that proteins are responsible for the existence of ion channels, recent investigations not only show, that the surrounding bilayer has a great influence in function of channels (see also chapter 1), it is also possible to measure channel events in pure lipid double layers, in completely absence of proteins.[1][14] To perform this experiments, the patch clamp can be used for sure, but also the setup described in chapter 3.1 offers a good alternative.
The appearance of channeling events in pure lipid membranes is highly dependent on the thermodynamic properties. Due to this it is worth to spend the next sections on giving an short introduction in the topic of phase transitions and also in fluctuations.

2.3. Phase transitions

This section intends to give the theoretical background about phase transitions in general and specified to the situation in lipid membranes.
In a physical sense, a phase is a set of states of a macroscopic system having more or less uniform chemical composition and physical properties. A daily life example would be the solid, liquid and gas aggregate state of water. In physical systems there are also other phases, but the theoretical treatment is more or less the same. Phase transition are classified via the *Ehrenfest classification*. Following this scheme, phase transitions are labelled by the *lowest derivative of the free energy (or an other relevant thermodynamic ptoential), which is not steady at the transition.*

First order phase transition

The thermodynamic potential for most experimental work (constant temperature T, pressure p, number of particles N) is the free enthalpy G.[1] Via the Gibbs-Duhem-Relation free enthalpy is connected to chemical potential:

$$G(T, p, N) = N \cdot \mu(T, p) \tag{2.1}$$

In equilibrium between two phases A and B chemical potentials μ are equal:

$$\mu_A(T, p) = \mu_B(T, p) \tag{2.2}$$

In case the chemical potential of one phase gets smaller then the other due to changes in temperature or pressure, the equilibrium goes in direction of the phase with smaller potential, which means also smaller enthalpy G. (as shown in figure 2.5)

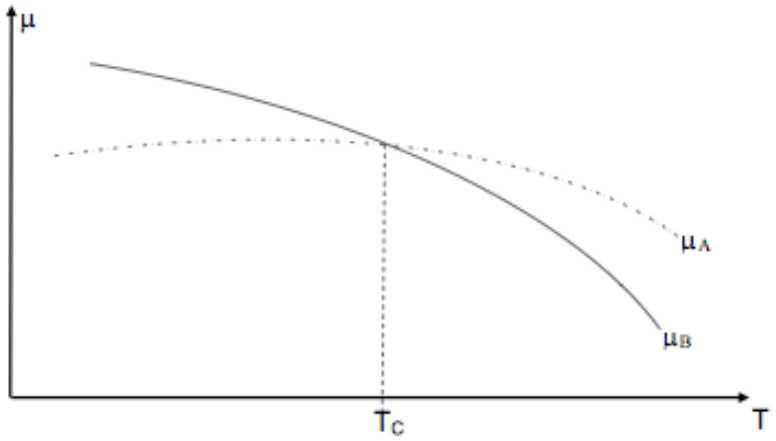


Figure 2.5.: Scheme of chemical potential depending on temperature. For $T < T_C$ it is $\mu_A < \mu_B$, the system is in phase A, for $T > T_C$ vice versa. T_C marks the phase transition temperature. Figure taken from [27]

To show the characteristic properties of *1st* order phase transitions – latent heat and volume jump – one should consider that from equation 2.2 an expression for p(T) can be derived, describing he line of coexistence of both phases. If one has a look at a small adjustment along this line, the changes in chemical potentials are still equal:

$$d\mu_A(T, p) = d\mu_B(T, p) \tag{2.3}$$

Using Gibbs-Duhem and equation 2.1, and assuming that the number of particles N does not change during the transition, this can be expressed as:

$$-S_A dT + V_A dp = -S_B dT + V_B dp \tag{2.4}$$

[1] For other given natural variables except of p and T the free enthalpy can be transformed into another confident thermodynamic potential via Legendre-transformation.

The gradient of the coexistence line therefore is given by:

$$\frac{dp}{dT} = \frac{(S_B - S_A)}{(V_B - V_A)} \tag{2.5}$$

The difference in entropy is connected to the latent heat via temperature:

$$\Delta Q = T \cdot (S_B - S_A) \tag{2.6}$$

With this, equation (2.5) becomes the *Clausius-Clapeyron-eqaution*:

$$\Delta Q = T \cdot \frac{dp}{dT}(V_B - V_A) \tag{2.7}$$

in which ΔQ is the *latent heat*, necessary to compensate cohesive forces between molecules and $(V_B - V_A)$ is the *change of volume* during the phase transition. This is only valid with the already used assumtion that

$$S_A \neq S_B \text{ and } V_A \neq V_B$$

This means that despite of similar chemical potentials and enthalpies, the derivatives with respect to natural variables are not equal at the phase transition point:

$$-\left(\frac{\delta G_A}{\delta T}\right)_p = S_A \neq S_B = -\left(\frac{\delta G_B}{\delta T}\right)_p \tag{2.8}$$

$$\left(\frac{\delta G_A}{\delta p}\right)_T = V_A \neq V_B = \left(\frac{\delta G_B}{\delta p}\right)_T \tag{2.9}$$

According to the Ehrenfest-classification this case is a phase transition of fist order, because the first derivatives of the thermodynamic potential with respect to according variables are not steady in the phase transition. Only such transitions need latent heat as calculated in equation (2.7), and a step in volume as well as in entropy appears at phase transition temperature. Examples of *1st* order phase transitions are not only water and ideal gases, but also membranes, as next section will impressively show.

Phase transitions in membranes

One of the amazing properties of lipid membranes is the ability to appear in different phases. A common method to investigate structural phase transitions and their properties is *differential scanning calorimetry (DSC)*. Two identical constructed but against each other insulated cells are heated simultanously by a constant heat flux. One cell includes a suspension of bilayer vesicles, the second one includes only buffer (or whatever one uses to dissolve the sample). The measured quantity is the rise of temperature. As shown in equation 2.7 temperature is not changing during an endotherm phase transition, because latent heat has to be provided. As long as one knows the heat capacity C_R^0 of the reference cell, the transition heat can be measured by comparing the temperature changes in both cells,

$$Q_t = C_S^0 \Delta T_R \tag{2.10}$$

while C_S^0 is the heat capacity of the sample and ΔT_R is the difference in temperature rising of the sample and reference chamber.

In modern microcaloriemeters the additional heat ΔC, which is necessary to heat the sample from T to $T + \Delta T$ can be measured directly. In figure 2.6 the additional heat ΔC is plotted against temperature for a sample of pure DPPC[2]. The figure shows two

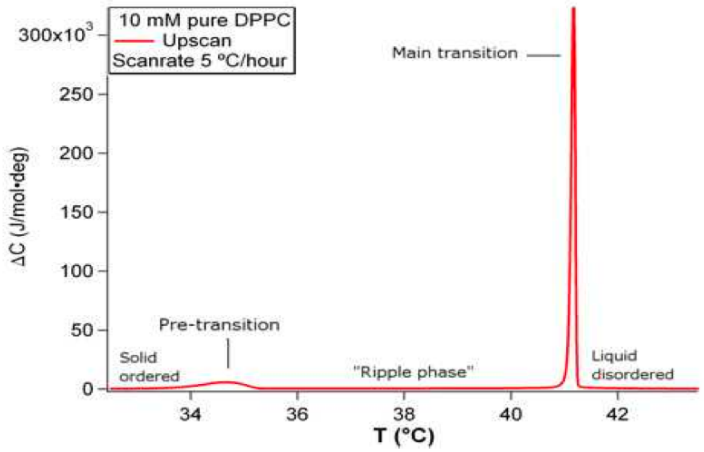


Figure 2.6.: heat capacity profile for DPPC, figure taken from [18]

peaks, marking a phase transition, which are called pre-transition and main-transition for historical reasons.

The three marked phases are called L_α , (this is a fluid phase or liquid disordered) P_β and L_β (these are the solid ordered and ripple phases of gel phases) [3] Beside this three, there is one more phase, called L_C appearing only at deeper temeratures, (it can be seen when lipids are stored in the fridge for several days) in which the index C stands for the really "crystalline" properties of this phase. With respect to biological relevance we only will discuss the behavior of the three high temperature phases in following.([22]) All transitions between this four phases are of *1st* order. Admittedly at high pressures, the $L_\beta \mapsto P_\beta$ intersection can be of *2nd* order (one can investigate this phenomena on lipid-films upon water/air surfaces) This transition can only be seen in lipids with comparatively big head groups and don´t appear in vesicles under high stress or in membranes attached on surfaces. The $L_\alpha \mapsto P_\beta$ transition (and in case the P_β does not appear also the $L_\alpha \mapsto L_\beta$ transition) is caused by the melting of the hydrophobic chains. In the gel phase, the CH_2 groups are in linear order and form a hexagonal lattice in the membrane plane.

During the melting process, the order of the fatty acid chains changes to more degrees of freedom due to rotation in the $C - C$ bond, as one can see in Figure 2.9 The

[2]The full names and further information about the used lipids are given in Appendix A.

[3]In the language of fluid crystal physics the lamellar lipid phase is a smectic phase, which means lipids can not leave the membrane-plane. Here L_α represents the fluid smectic-A phase, while P_β and L_β represent the crytsalline smectic-B phase.

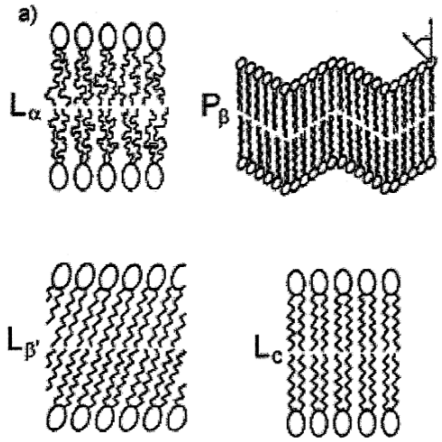


Figure 2.7.: Schematic structure of lipid phases according to [22]. The angle between the fatty acid chains and the membrane plane is about 30°. The L_α phase is also called "liquid disordered", the P_β is alternatively named "ripple phase", the L_β is called "solid ordered".

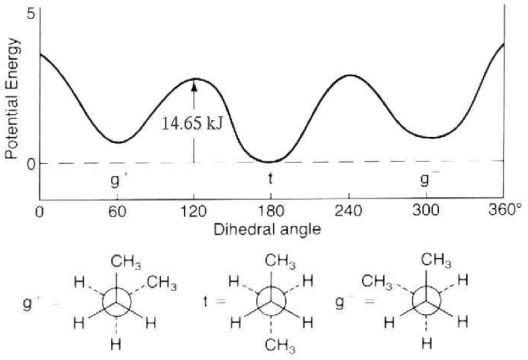


Figure 2.8.: Energy scheme for a carbon-carbon binding in a chain. The height of the barrier which has to be crossed to change conformation is $14.65kJ$.

graphic 2.8 shows the energy scheme of an ordinary carbon binding. In solid ordered state the energy in the chains is not enough to change between the configurations permanently, so the binding stays in the configuration it is with a high probability. (Most frequently this is the 180° configuration (see2.8) which means the chains are streched). Over the transition in liquid disordered state, the bindings are able to change conformation, this is an additional degree of freedom. The disorder in chains is the reason for the step in theromdynamic values. According to [8] the Enthalpy change ΔH of lipids is 25-40 kJ/mol, volume around 4%, surface 25% and Entropy around 80 to 130 $J/mol \cdot K$.

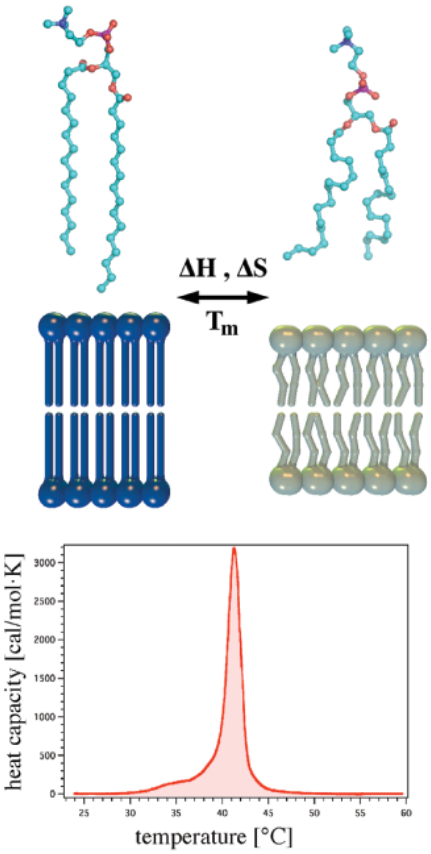


Figure 2.9.: Below the phase transition temperature, the lipid chains are ordered, above they are disordered. Therefore a jump in Enthalpy ΔH, Entropy ΔS and also in thickness and area of the membrane appears at melting temperature T_m. (Graphic friendly provided by Thomas Heimburg)

2.4. Lipid mixtures

In a model system containing only one kind of lipids, the phase transition has a very sharp temperature, the peak in the heat capacity profile is clear, but very narrow. An example is given in figure 2.6 where a measurement on a pure DPPC-samle is shown. In order to investigate the behavior of samples in the phase transition it is useful to use lipid mixtures instead of pure systems. In lipid mixtures the peak in the heat capacity peak is not sharp, but a broader interval.

In the phase transition, regardless if the sample consists of one or more components, the system is not clearly in one or another phase, the two phases above and below the transition point are in coexistence, that means parts of the system are in one phase, and other parts in the other. In the case of membranes one can observe the creation of domains, the domain size depends on the Energy of the system. These can be visualized with Fluorescence Microscopy due to the fact that one can find fluorescence markers with different solubility in different phases, giving contrast.[4] A picture made by this technique is shown in figure 2.11.

[4]The picture does NOT show different lipids in different colours! The lipids are mixed properly by dissolving it in chloroform, mixing it, drying it and dissolve again in water.

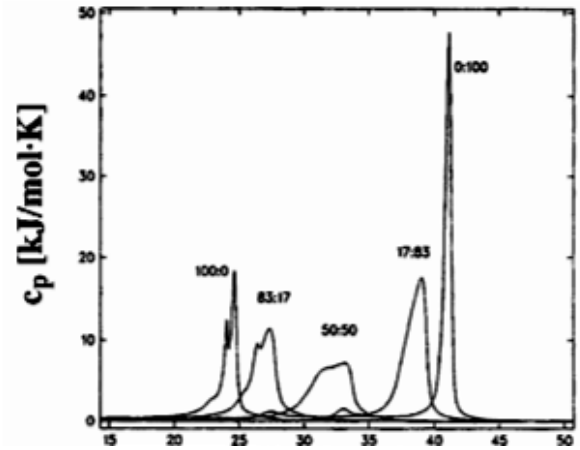


Figure 2.10.: Heat capacity profiles in dependance of mixture ratios of vesicles made of a mixture DMPC-DPPC (figure taken from [23])

In figure 2.10 it is shown, how the heat capacity profile is changed by using lipid mixtures on a system consisting of two components, DMPC and DPPC (Compare also to figure 4.2). Depending on ratio and properties of the included components the peak is not only switched in temperature, it is also broadened and gains some structure. The coexistence area, in which domains can be seen is not a sharp temperature point, but a temperature interval. In a more component system the size of the domains therefore is also dependent on the temperature. As shown in the next chapter, the fluctuations in a system are proportional to the heat capacity, this means that the lower peak of lipid mixtures compared to single component systems also create more stable systems, which is an additional reason, why lipid mixtures are used.

2.5. Fluctuations

The energy of a single particle in a many particle system with temperatures much higher than 0 Kelvin can not be considered as static, it fluctuates around a mean value.[5]
Let us consider a canonic ensemble, which is characterized by a temperature T, a volume V, a constant number of particles N and a density matrix ρ

$$\rho = \frac{e^{(-\beta H)}}{Z} \qquad with \qquad Z = Tr\left(e^{-\beta H}\right) \tag{2.11}$$

where Z is the partition sum. The mean value of energy is given by:

$$\overline{E} = \frac{1}{Z} \cdot Tr\left(e^{-\beta H}\right) \cdot H = \frac{1}{Z} \cdot \frac{\delta Z}{\delta(-\beta)} \tag{2.12}$$

[5]This derivation can be found in any good textbook about statistical mechanics as well as the definition of canonic ensemble, for instance in [24]

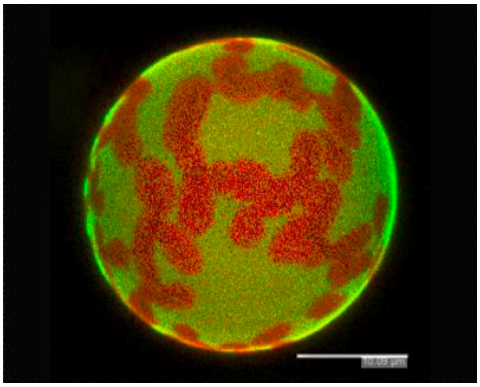


Figure 2.11.: Confocal flourescence picture of a giant unilamellar Vesicle. Two markers included into the lipid bilayer (DLPC:DPPC 67:33) emit different wave lengths after excitation. One of the markers prefer the L_α phase, the other is solvable in the L_β. The scale bar is $10\mu m$. Picture made by Mathias Fidorra[6]

Derivation with respect to temperature (which is included in β also) gives us :

$$\left(\frac{\delta\overline{E}}{\delta T}\right)_V = \frac{1}{kT^2}\frac{\delta\overline{E}}{\delta-\beta} = \frac{1}{kT^2}\langle H^2\rangle - \langle H\rangle^2 = \frac{1}{kT^2}(\Delta E)^2 \tag{2.13}$$

Using the maxwell relation

$$\left(\frac{\delta\overline{E}}{\delta T}\right)_V = C_V \tag{2.14}$$

provides a direct dependence between the heat capacity and the value of fluctuations around the energy:

$$C_V = \frac{1}{kT^2}(\Delta E)^2 \tag{2.15}$$

This means that the value of fluctuations is proportional to heat capacity, a maximum in heat capacity leads to big fluctuations.
In section 2.3 one can see that the heat capacity has a maximum in the phase transition. As shown in [7] the relaxation behavior close to chain-melting transition changes dramatically. The relaxation times are within experimental errors proportional to heat capacity and reach orders of seconds up to minutes.

Here one can try to find an explanation for the appearance of ion channels in pure lipid membranes. In the regime of phase transitions there are strong fluctuations, one could imagine that especially the edges of domains are involved in this. The membrane gets weak, and some holes open spontaneously. Some of them may be big enough to allow a Ion going through it. Due to the long relaxation times, this holes stay open for a while, so that currents, long and stable enough to be measured develop.This interpretation is not settled, and can be seen as a proposal, but what at least can be fixed is that the appearance of channel events show a strong dependence on thermodynamic properties.

3. Setups and Techniques

The third chapter, which is supposed to describe the setup so good, that it is easy to reconstruct a similar one, or at least to do a lot of exciting measurement with the existing one.

The build setup is a BLM cell, which is pronounced *bilayer membrane* or also *black lipid membrane* in literature. The second name comes from the fact, that people tried to observe the bilayer formation with an optical method, using interference of incoming and reflected beams. Using light of right wavelength, the interference picture turns black when a bilayer is created.
The second experiment used is the *Differential scanning calorimeter DSC*, a standard procedure to investigate thermodynamic properties of lipid samples. This chapter describes the build and the used setups.

3.1. Black lipid membrane setup

The properties of biological relevant lipid bilayers can mainly be studied using two different approaches. One is to probe pieces of Membrane by patch-clamping-techniques to observe currents and potentials across the membranes.This method enables the investigator to observe parts of living cells, but can also be used to testify artificial membranes of vesicles. The other one is to create an artificial lipid bilayer in a BLM cell as used here and observe all kinds of properties. The advantages of artificial lipid membranes are the simlpicity of the system compared to biological ones, which contain a huge diversity of proteins, ions, enzymes, acids and other molecules influencing the bilayer, and the precise control over properties like composition of lipidmix.

The setup should fulfil several requirements: It should offer an opportunity to create bilayers in macroscopic means and due to the fact that one expects very small signal amplitudes, it should be shielded against all outside interferences.
Figure 3.1 shows the complete setup used.

The heart of the setup is the chamber to produce the artificial lipid membrane,(1)[1] also shown in figure 3.2. It consists of a flowcell made of teflon, which is composed of two symmetric parts. Additional to the chambers taking up the electrolyte with lipids on the surface and the electrodes there are several pipette pathways in order to acess the electrolyte solution, and to rise the water level without touching the membrane or the monolayer on the water surface. The parts are separated by a teflon foil with a thickness of 25μm. In this sheet one drills a hole with a diameter of 200μm, this can

[1]The number in brackets in the text refer to the numeration in 3.1.

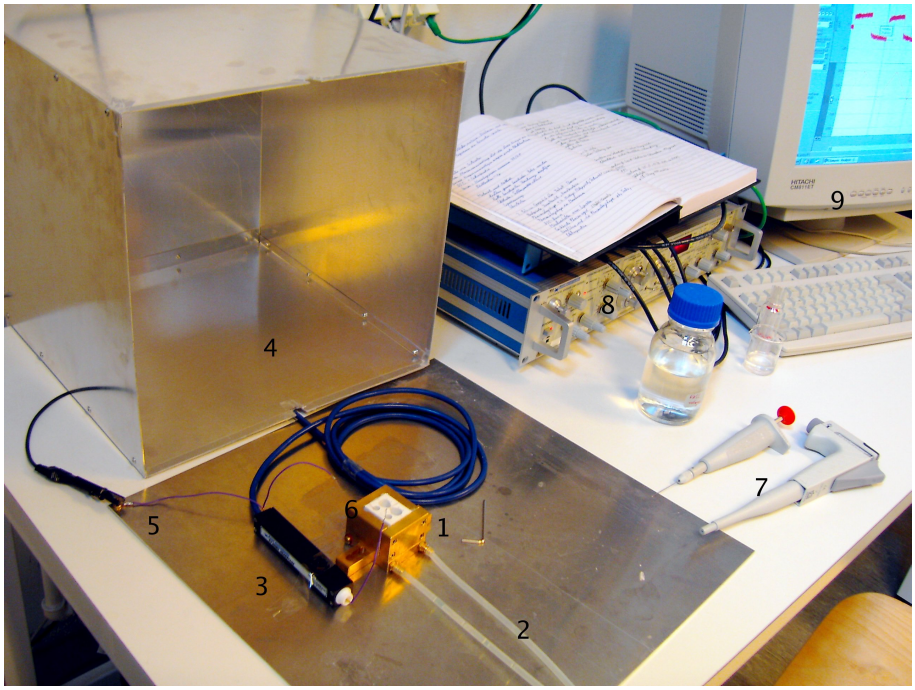


Figure 3.1.: Picture of whole setup: (1)flowcell in brass block; (2)connection to heatbath; (3)headstage; (4)faraday cage; (5) grounding; (6) electrodes; (7)pipets for lipid, hexadecane and electrolyte; (8)amplifier; (9)computer for analysis and data acquirement.

be done with an ordinary driller. In order to get an symmetric hole with sharp edges it is of advantage to clamp the foil between two thin sheets of metal or plastic while drilling. The hole should be checked under the microscope, experience also of other investigators show, that a symmetric hole with smooth and regular edges is extremely important for the creation of a bilayer. The foil is fixed between the teflon parts so, that the drilled hole lies on the chamber connecting one. Sealing material is not necessary between the two dices, but unfortunately the bottoms of the chambers are very thin, and the used material, a teflon-glass composition is porous[2], so it it necessary to seal them with vacuum grease. A thin plate of teflon ($1mm$) lies under the flowcells in the heat bath to prevent short circles caused by contingently leaking electrolyte.
The flowcell is enbedded in a brass-block(1) with four screws to press the two parts against each other consistently, and a cavity conected to a heat bath(2) (HAAKE F2) for temperature control. If one managed to find a working hole, it is not necessary to disperse the chamber again.

Two Ag–AgCl electrodes (6) are placed in the electrode cavities to define the relative potential difference between the two parts of the flowcell. Those electrodes consists of

[2] As it turned out, this is not the optimal material, if one produces new chambers, one should use pure teflon. See construction drawings in Appendix

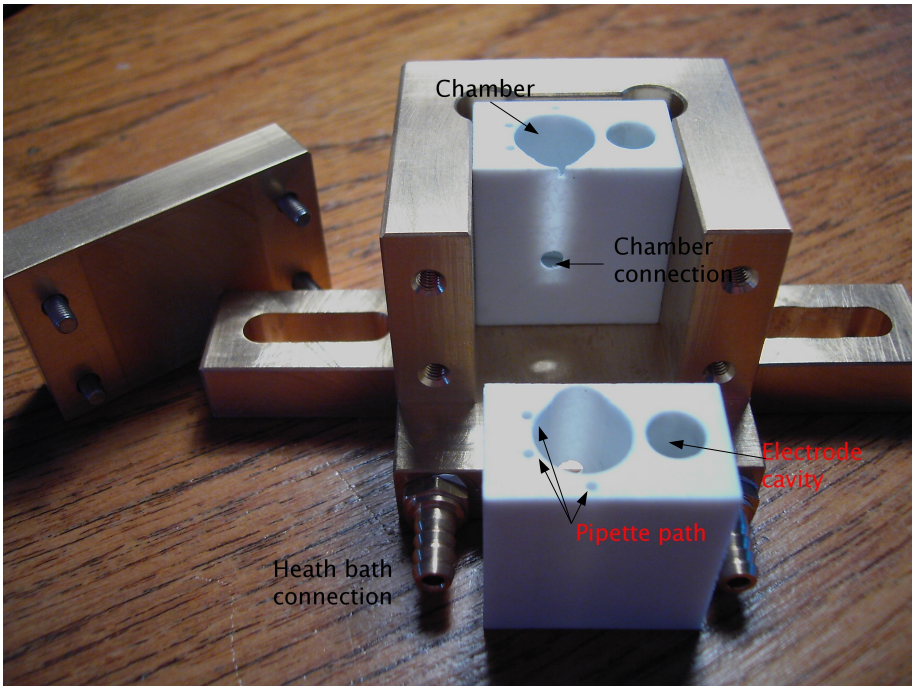


Figure 3.2.: Flowcell and heatbath, disjointed

a pure silver wire, which is chloridized in a solution of KCL[3]. To build the electrodes, one prepares a 3molar solution of KCl, connects the silver wire to the positive pole of a power supply and hold it into solution, until it turns black. The procedure has to be repeated after every experiment, before the covering the wire has to be cleaned with fine sandpaper. The other pole should be an electrode out of an inert material like gold or platin. For the grounded electrode (between (5) and (3)) one can use an ordinary cable. For the other one, a shielded cable is used, the silver shield is grounded by a connection to the bottom plate of the faraday cage. The connection can be realized with a drop of electrolyte.

Figure 3.1 shows the complete setup. Everything lying on the aluminum plate is supposed to be inside a Faraday cage (4), which is removed in the picture to give a sight to inside. The electrodes are connected to the Axon Headstage (3), which preamplifies the signal. It is supposed to be grounded as well as the aluminum plate and the cage. Best thing to do is to connect everything to the ground (5) of the amplifier. The headstage gives the signal to the Axon-amplifier (8) (Axon Instruments Tnc. Axopatch 200B) outside the faraday cage. The amplifier is connected to a computer (9) over a converter (Axon Digidata 1200 Series) where signals can be analized with appropriate software (patchex). If the settings on the amplifier are done once (see online manual) everything can be controlled via the software of the computer.

[3]It seems to be important to have a pure silver wire, otherwise one measures a static potential

Before it can be loaded the cell has to be cleaned properly. It is filled with acetone and rest for at least 10 Minutes and afterwards flushed with ethanol several times. In the end, it is dried properly under N_2 stream.

3.2. Differential scanning calorimeter

For the DSC measurements the calorimeter from *Microcal* in the lab is used. It is loaded and cleaned like described in the next section. It is connected to a computer which allows regulation of the setup as well as saving and analyzing data. The used software for interpreting data is IGORpro, and a small makro helping to substract the baseline, calculating Entropy and temperature of phase transition. (For background see also section 2.3

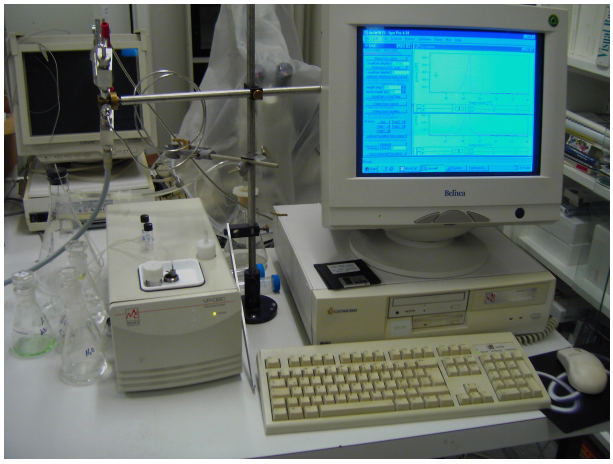


Figure 3.3.: differential scanning calorimeter while cleaning process.

3.3. Practical Items of Measurements

3.3.1. Lipid mixtures

Under investigation are mixtures of DMPC and DLPC and mixtures of DPPC and DOPC. We solved dry lipids in chloroform[4] in a concentration of $10mg/ml$ to create a stock of DMPC, DLPC, DPPC and DOPC. Then we prepared mixtures of DLPC in DMPC in ratios of $2,5:100; 5:100$ and $10:100$. DPPC and DOPC were mixed in a ratio of 100:10 and in 08:20.

3.3.2. Differential scanning calorimetry

In order gain information about the phase transition of our probes we took a scan in *DSC*. To prepare samples for the calorimeter we took 700μl out of the prepared lipid

[4]trivial name for tricholrmethan

mixture ($10mg/ml$) and dried it carefully under N_2 stream. To get all of the chloroform out of the sample, we dried it in vacuum for ca. two hours. After this procedure, we add 1 ml of the used electrolyte [5] to the sample (This is an ca. concentration of $10mmol/l$ which is in the optimal range for the calorimeter) and heat it to ca. 45°C in order to get vesicles. It helps to shake the sample once in a while. Before the sample is loaded to the DSC, it has do be degased in vacuum, same is valid for the reference. This can be done also with the small vacuum pump which is used for DSC cleaning.
The DSC has to be cleaned properly before every measurement. Therefore, each chamber is flushed with water Mucasol-solution and ethanol for ca.10 minutes per medium. The last medium is blown out with N_2 stream. If one wants to get sure, that all the ethanol is evaporated, one can heat the empty calorimeter before measurement.
The analysis of the data is done with IGORpro, which is installed on the DSC computer. A specialized experiment file (.pxp) helps to handle DSC measurements. It can also been found on this computer.

3.3.3. Creating and controlling a bilayer

In the next few chapters, some practical experiences are given, optimal work processes and suggestions, to show how experimental work was done, and of mayor interest for somebody who wants to repeat the experiment or do something related.

The general idea of creating an artificial bilayer in this setup is to fold two monolayers together. Figure 3.4 gives a sketch of the idea behind it. In step 1 a Teflon barrier dividing the two chambers with a hole in it is shown. The hydrophilic headgroups of the lipids are solved in electrolyte and the hydrophobic tales face the surrouding air. By rising the mirrow of electrolyte in one chamber higher than the level of the hole in the Teflon sheet, a single lipid layer is created (as shown in step 2) covering the wall and also the hole. In step 3 the level of electroyte is also increased in the second part of the chamber and the two monolayers form a bilayer when they are contacted into the hole. The energetic most favorable state is reached by joining the hydrophobic chains of both layers together.

To control the bilayer we evaluate its quality in term of its resistance and its thickness. A good seal should show resistances over 100 GΩ meanwhile resistances under 10GΩ are a beacon for leaks in the membrane. To estimate the thickness of the membrane we measure the capacitance and relate it just like a parallel plate capacitor to the distance between the plates. The capacitance of a plate capacitor is given by:

$$C = \frac{\epsilon_0 \epsilon_\tau \cdot A}{d} \tag{3.1}$$

in which ϵ_0 is the permeability of vaccum, ϵ_τ is the permeability of the material between the plates, A is the area of the plates and d is the distance between them. The thickness of the membrane ist therefore connected to its capacitance. The electric charge Q

[5] Theory says that the electrolyte shifts the phase transition slightly. Due to the small concentration on salt, in experiment the effect is very small

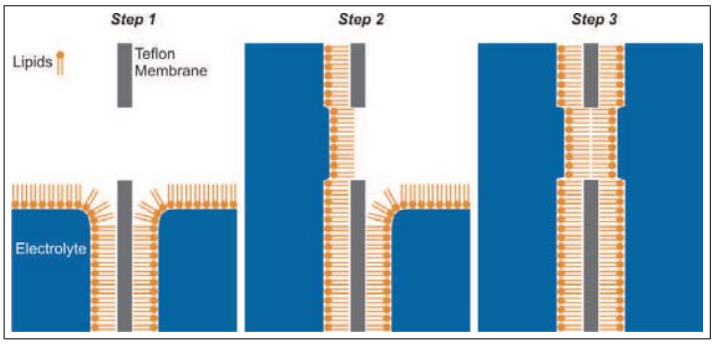


Figure 3.4.: Cartoon of bilayer production priciple: two monolayers are folded together by raising the liquid level. Figure taken from [29]

accumulated on the plates is given by the capacitance C and the applied voltage U by:

$$Q = C \cdot U \tag{3.2}$$

Differentiating (3.2) with respect to time provides us with an expression directly applicable to our current measurement:

$$\frac{dQ}{dt} = I = C\frac{dU}{dt} \tag{3.3}$$

If a triangular voltage is applied to the membrane, the measured current answer is constant due to $|dU/dt|$ is constant. The capacitance is now shows a direct dependance to the absolute value of current. An ideal picture is shown in Figure 3.5.

3.3.4. Filling the flowcell

It is necessary for the formation of membrane that the hole is wetted with a solution of 5% hexadecane in pentane. As soon as the pentane is evaporated, one can fill each side of the cell with approximately 750μl of electrolyte and add 15μl of dissolved lipids ($10mg/ml$) in chloroform. After 10 minutes, the chloroform also should be gone, and the water level is raised by adding of again 750μl of electrolyte to each chamber, very carefully and using the pipette path shown in figure 3.2 Success can be controlled by observing the screen in the gap free mode. If no membrane is created, a short cycle appears, the current is higher than can be measured with the amplifier. In the case lipids blocking the hole, the current stays at zero showing the normal noise. Now the faraday cage is closed, having a look at the capacitive reaction of the measurement like described above gives information if it is an actual bilayer which is created.

3.3.5. Noise minimation

In order to minimize the noise the hole setup is placed in a properly grounded Faraday cage, here an aluminum box with a bottom plate. The cage, the bottom plate, the

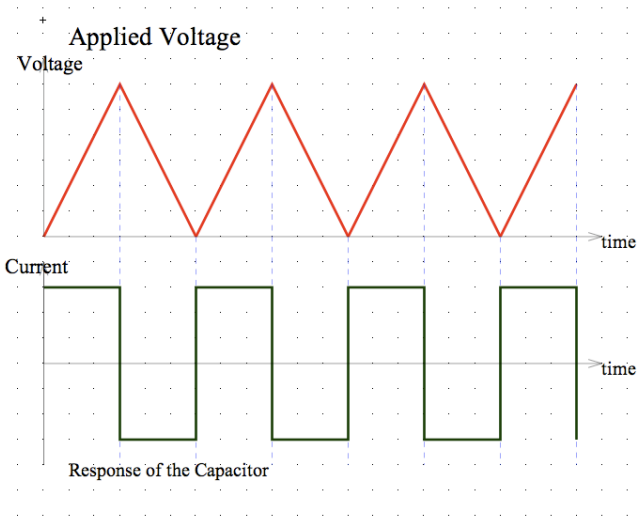


Figure 3.5.: Voltage signal and response of the membrane

heat bath of the cell and the headstage (one pole and housing) are connected with the grounding of the amplifier. For the not grounded electrode, a shielded cable is used, the shield is connected to the bottom plate of the faraday cage with a drop of electrolyte. One should also get sure, that the brass block has a connection to the faraday cage. The meter at the amplifier can be set to the V_{RMS}mode do observe the noise, the shown value is supposed to be smaller than 1.0 in order to guarantee good measurements. Experience shows, that the soldering connection on the silver wire electrodes can cause noise, to renew the soldering can help.

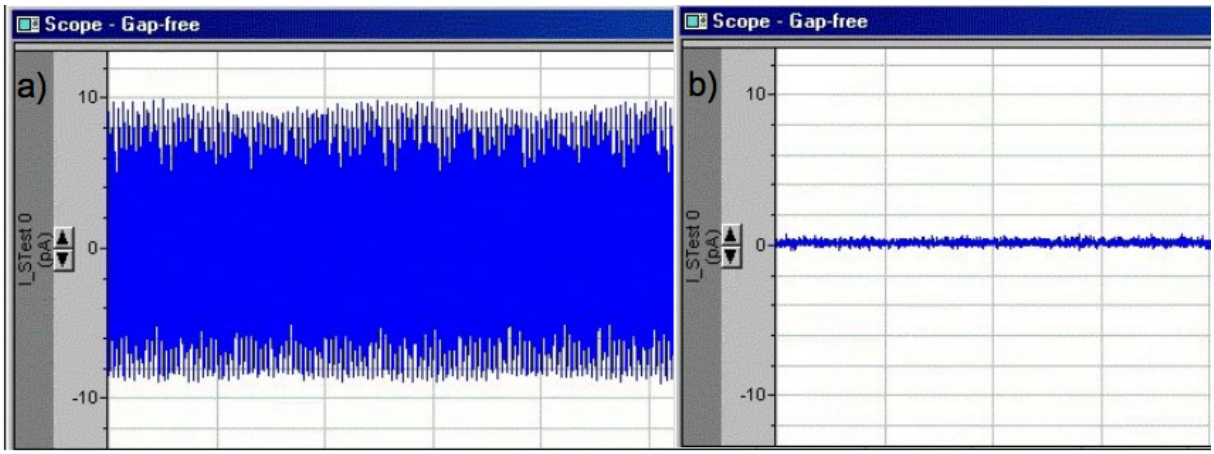


Figure 3.6.: a) unfiltered signal with empty flowcell and b) filtered with lowpass filter at 10kHz

The resolution of measurements is restricted from three parameters: frequency of sample, noise and filter settings. 3.6 shows the signal unfiltered and filtered with amplifiers intern lowpass Bessel filter at 10kHz. Because of this big interference the signal has to be filtered. The setting of the filter defines the shortest lenght of a detectable signal, here 100μs, which should be good enough to detect the opening and closing events.

3.3.6. Software

The amplifier software *patchex* is used to acquire the measurements. There are different modes, for measuring opening and closing events one uses the *gap-free mode*. Voltage can be applied with the help of the wheel on the amplifier panel. To measure the capacity, the *episodic mode*, which allows to apply signals of a defined shape. One can define the shape, slope and strength of the required triangular signal and measure the response. The specification of folder and name for saving acquired files is done in the *file* menu with the point *set data file names*. One should always keep in mind the settings of this menu in order to find the acquired data again. For further analysis the data can be exported in the *Axon text file format .atf* and be imported in any used analysis programme.
The program has a detailed online manual which is useful to read.

4. Results and discussion

The fourth chapter, in which the results of the measurements are assembled and interpreted.

4.1. Differential calorimetry

The differential calorimetry scan gives information about the heat capacity dependent on temperature, and so shows the temperature interval of phase transitions. Due to the fact that fluctuations are proportional to heat capacity one expects ion channeling in a regime of maximal heat capacity. In order to find a sample with a suitable phase transition behavior, different mixtures of lipid types were used. A scan was made of every used lipid mixture, the same sample was scanned upwards, downwards and again upwards in high resolution and with a scanrate of $5°C/hour$. Due to several equilibration processes, the first upscan sometimes shows artifacts and is therefor ignored.

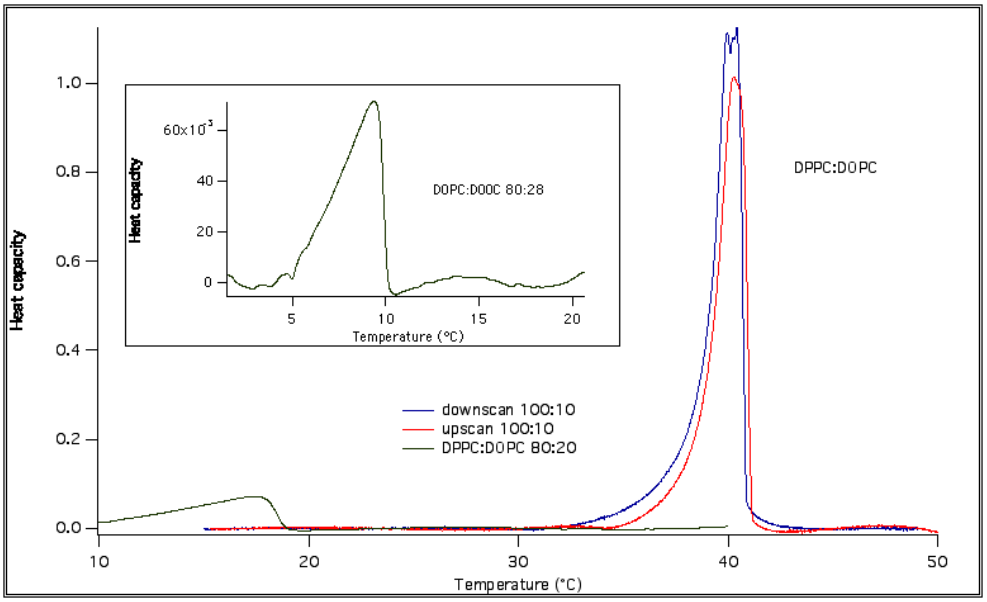


Figure 4.1.: Upscan and downscan of a sample of DPPC including DOPC in a ratio of 100:10. Both measurements are congruent in expected borders. The sample shows a transition at 40°C, see also melting temperatures in Appendix A.The small graph represents the mixture DPPC:DOPC 20:80. in a bigger scale

In figure 4.1 the upscan and the downscan of a sample consisting of DPPC with 10% DOPC is plotted to show that except of a small hysteresis and in the borders of experimental errors upscan and downscan are identical. Of course, in the original data the peak in downscan is negative, it was inverted in the graph to show the congruence. Furthermore, the scan of the mixture DPPC:DOPC 20:80 is included in this graphic. Comparison shows not only that the phase transition is shifted to much deeper temperatures, also the height of the peak in heat capacity is about 10 times smaller. Using this sample, the membrane is created not in the gel state but in the liquid state. As shown in section 2.5 the fluctuations are proportional to heat capacity, this means the membrane should be much more stable in this sample. DPPC shows a transition at 42°C as one can also see in figure 2.6, meanwhile DOPC is in liquid state above -20°C.
The DSC scans for the used DMPC-DLPC mixtures are shown in figure 4.2. The addi-

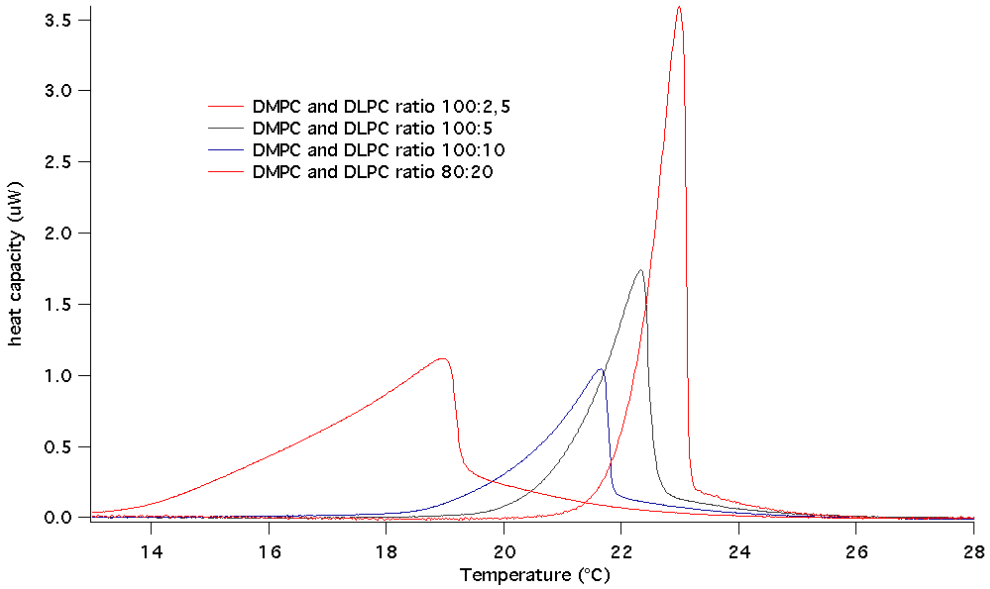


Figure 4.2.: Heat capacity profiles of lipid mixtures DMPC and DLPC in different ratios.

tion of DLPC (Phase transition temperature at -1°C) to the DMPC sample (transition temperature at 23°C, see also Appendix) shifts the peak in the heat capacity profile to lower temperatures and broadens the transition regime. Also the shoulders of the peak, especially on the lower temperature side gets more flat and widespread. The sample DMPC:DLPC 80:20 show comparable properties as the mixture DOPC DPPC in same ratio, one would expect, that it can also be used for creating bilayers in the BLM. The investigation of this mixture was not done in this project, but it could be interesting for the future.

4.2. Capacity

To control the development of an actual bilayer one meausures the capacity of the setup. Capacities are additive, so in order to gain information about what is in the hole, one has to subtract the contribution of the other setup components. According to figure 3.5 we applied a triangular voltage with a slope of $100mV/200ms$ and got the answer as shown in figure 4.3. The headstage has a capacity of $5pF$, the headstage and the connected

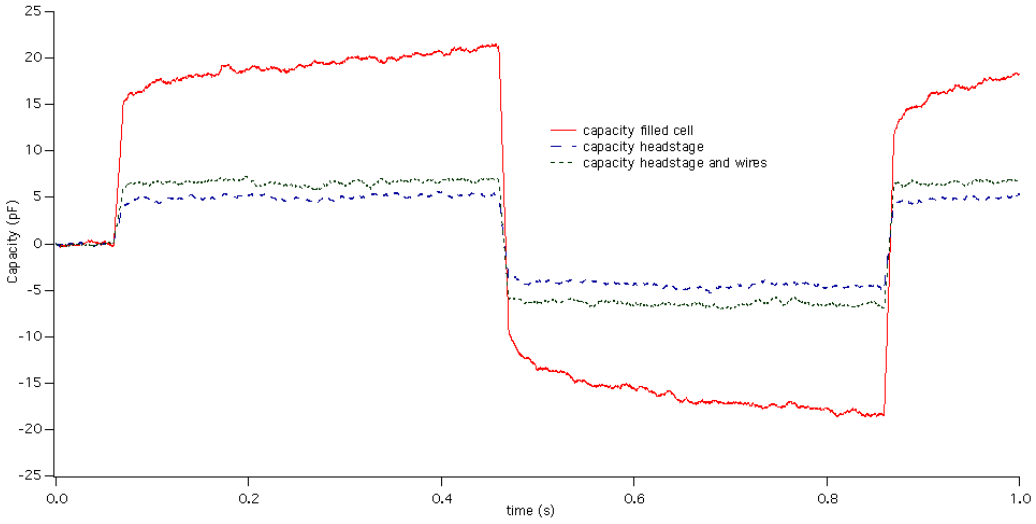


Figure 4.3.: Capacities of the headstage, headstage with wires and the filled flowcell (water level slightly under the connection hole). The curves are filtered by software to lower the noise.

wires approximately $7pF$ and the whole setup without the produced membrane shows a capacity of $18pF$. This is the value, which has to be subtracted to estimate the membrane thickness.

Estimation of membrane thickness

With some easy assumptions one can estimate what the membrane itself contributes to the capacity of the setup. Using formulas 3.1 and 4.1

$$A = \pi r^2 \qquad (4.1)$$

ϵ_0	$8,812 * 10^{-12} F/m$
ϵ_τ	3
A	$\pi \cdot 10^{-8} m^2$
d	$5 * 10^{-9} m$

and plug in the values in table 4.1 one ends up with an estimated membrane capacity of $160pF$. When the conductance does not show a short cycle after raising the water level and the capacity measurement shows around $180pF$ one can be assured, that a membrane was created. As figure 4.4 shows, an estimation of capacitance is difficult,

because the capacitive part of the current is overlayed by a constant resistor-like current. Following equation 4.2 the resistance of the membrane is $0,3G\Omega$ which is in the expected range.
When the high resistance is archieved, one can start to apply voltage in order to create electroporation.

$$R = \frac{U}{I} \qquad \frac{200mV}{700pA} = 0,3G\Omega \tag{4.2}$$

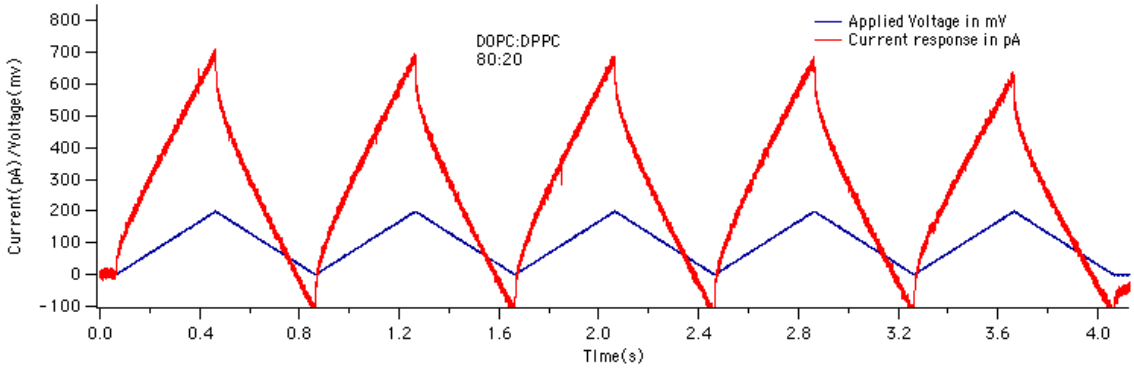


Figure 4.4.: Applied trianguar voltage (see also figure3.5) and response. The sample does not show a resistance in the $G\Omega$-range, there is a permanent current additional to the channeling events.

4.3. Electroporation

To prove that the setup works, the sample DOPC:DPPC in a ratio of 80:20 was used. In two experiments it was possible to observe opening-closing events. In both cases, the

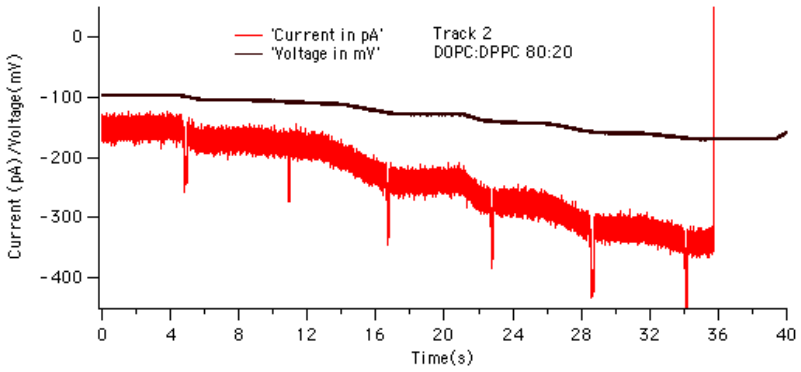


Figure 4.5.: Opening and closing of ionchannels and applied voltage.

capacity test showed a picture like figure 4.4, one can assume that the resistance is high

enough and the lipids formed a layer.

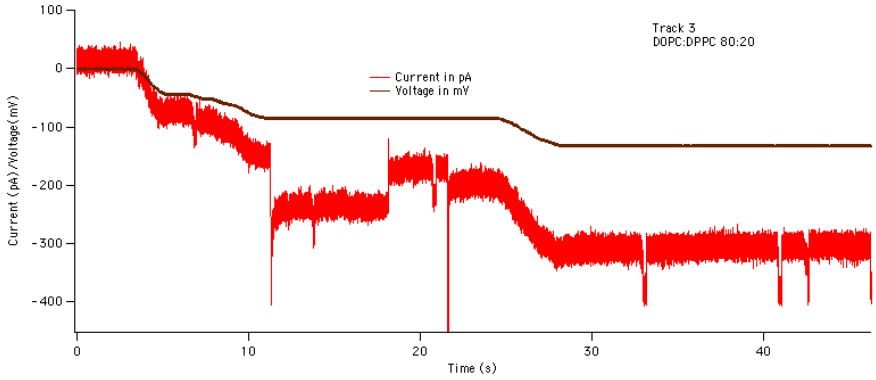


Figure 4.6.: Second try on the same sample the opening-closing events are still there, they are no longer periodic.

Opening and closing events has been observed clearly. At a room temperature of 18°C, the events started already at $100mV$ voltage. Figure 4.5 shows one of the observed tracks, showing the applied voltage in brown and the current response in red. The value of the opening currents scale with the applied voltage.
In this track, the noise is relatively loud, nevertheless, one can clearly see the events. The events in this measurement seem to be very periodic, which is by coincidence and can not be confirmed in other tracks (compare with figure 4.6). At the end of the track, the membrane was destroyed, a short cycle appears via the electrolyte. Figure 4.6 shows the experiment on the same sample which was made to guarantee the reproducibility. The periodicity disappeared, the jumps in the track are caused by touching the table slightly and show, how extremely sensitive the setup is against disturbtion. Taking a closer look

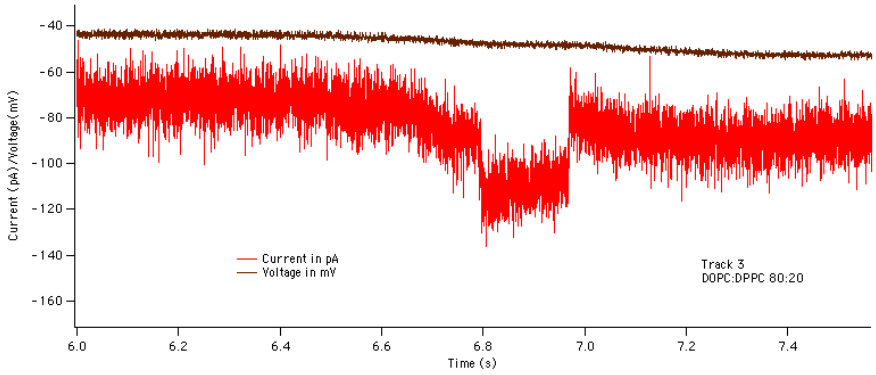


Figure 4.7.: Opening and closing of a single ionchannel.

at the single events and zooming into the acquired tracks shows, that the opening events have a lifetime of approximately $100ms$, the strength scales with the applied voltage

and is also a function of the salt concentration in the electrolyte. Figure 4.7 shows the appearance of a single ionchannel, which opend and closes again after $150\mu s$, the slight slope in the current is due to the fact that the applied voltage was increased permanently in this measurement. These events are more rare, the majority of the measured events are more than one channel opening at the same time, looking like the example in figure 4.8. The appearing current is additive, so this example it reaches a strength of ca. $80pA$ (dependent also on the applied voltage). Interestingly, the channels close synchronous, this fact is also known from path clamp measurements on ion channel proteins.

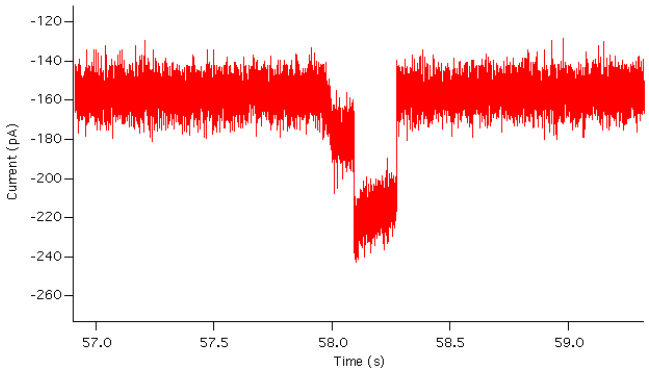


Figure 4.8.: Opening and closing of ionchannel.

5. Conclusion

The fifth chapter, in which everything is summed up and a future prospect is given

A setup was assembled to create small pieces of artificial lipid bilayer membranes, where voltage can be applied and the current response can be measured. Starting with ordering single parts from companies and in the workshop, the setup was assembled part by part after descriptions in literature and advise of the supervisor.
Differential scanning calorimetry was used to gain information about samples of usable lipid mixtures, different mixtures of DMPC and DLPC were used as well as compositions of DOPC and DPPC. The expected opening and closing events could be seen in a sample of DOPC and DPPC in a ratio 80:20. Even so some fine tuning work has to be done, the setup enables an investigator to observe ion channel events in pure lipid membranes. Besides the investigation of lipid double layers close to phase transition, one can also create stable layers with embedded proteins and investigate the properties of these, as shown in [29].

Although the phenomena of ion pores in pure lipid membranes is already known, it can be interesting to change the conditions like temperature, applied voltage, lipid composition and composition and concentration of the electrolyte systematically and try to come closer to biological relevant conditions step by step. The existence of this events clearly puts many question marks in the field of biochemistry, where ion channels play an extremely important role not only in nerve pulse propagation and excitable cells, but also in many other mechanisms in nature. Ion channels are involved in pain, in the olfactory system, in the visual and audio system, proteins with channel functions are thought to be involved in diseases like schizophrenia and depression, and lots of other biological principles.
Nevertheless, by far most attempts in research are exclusively focussed on the investigation of channel proteins. In recent time it has got more and more accepted that the lipid bilayer shows some interaction with the proteins and has some influence (remember chapter 1) on its function, but the fact that the membrane could cover the important task of ion channeling by itself could still hit the field unexpected.
By far is it not the aim to claim that Ion channel proteins don´t have a function, all the BLM setup can do is to show that the exclusive point of view apparently falls to short.

The project is closed with the prove that the setup is working in general, but the actual work begins now: To understand what is happening and to make a waterproof theory out of it, one now can start to ask the right questions to the experiment, acquire data by changing the properties systematically and also improve the efficiency of the setup. Here, one can spend some exciting time in future.

6. Acknowledgements

The sixth, last (but not least) chapter, in which people are named who deserve it.

The whole Biophysics membrane group really deserves my gratefulness for welcome me very warm in Denmark, for helping me with the project and with general orientation, for contentful discussions in the lab and in the cantine. My special gratitude goes to Christian Leirer for his guiding and to Thomas Heimburg for good advise as well as to Eric and Niels in the workshop for preparing the measuring cell and the heat bath after my drawings.
All people who made my stay in Denmark a exciting and good experience I want to thank very much. The greatest thanks go to Maj-Britt, for giving me a cosy accommodation, to Claudia and Marc for being and staying friends, to Alex for the tea and Peter for the jazz and to Lena for being a fabulous mentor.

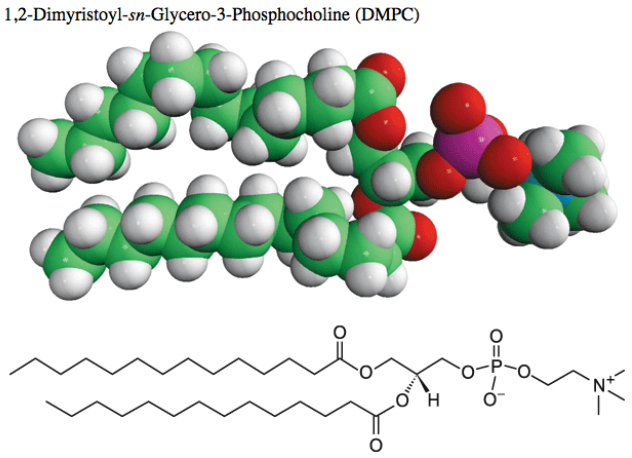
A special thank goes to Maria Hogger for endless patience, which I do not deserve, I only can take it as a gift.

A. Technical information about lipids

In this appendix the abbreviations used in the thesis are fully expressed and the structure and technical Data of used lipids are given. All information is taken by www.avantilipids.com, and all used Lipids are ordered from Avanti Polar lipids, Inc.[2]

DMPC

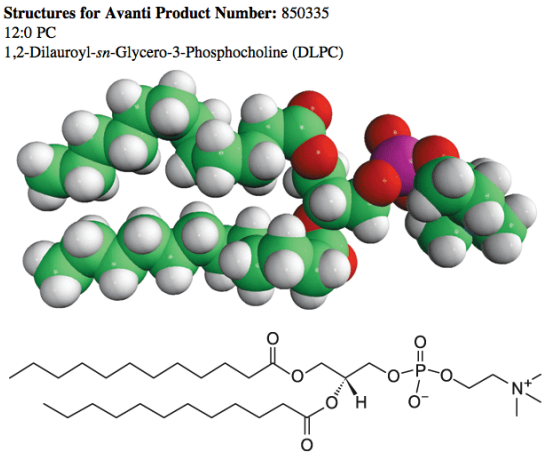
Name: 1,2-Dimyristoyl-*sn*-Glycero-3-Phosphocholine



AVANTI Product number	850345
Phase transition temperature	$23°C$
Molar weight	677.940
Molecular formula	$C_{36}H_{872}NO_8P$
Charge of headgroup	+

DLPC

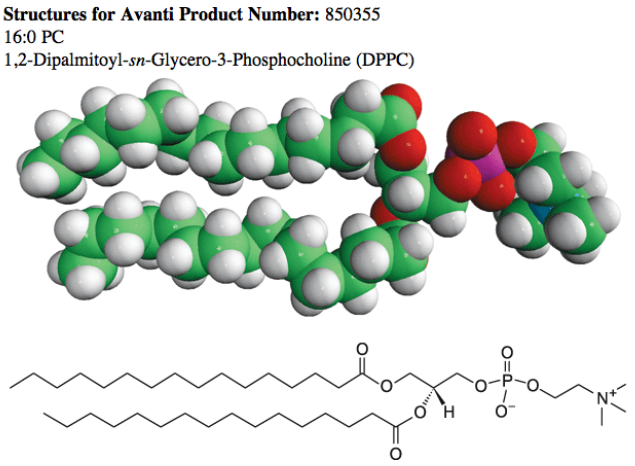
Name:1,2-Dilauroyl-*sn*-Glycero-3-Phosphocholine



AVANTI Product number	850335
Phase transition temperature	$-1°C$
Molecular weight	621.84
Molecular mass	$C_{32}H_{64}NO_8P$
Charge of headgroup	*uncharged*

DPPC

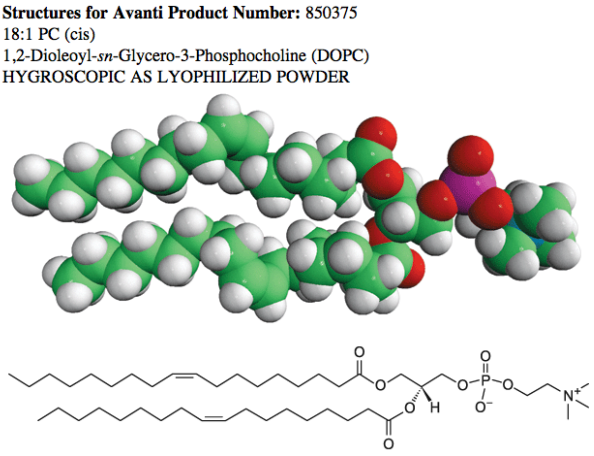
Name: 1,2-Dipalmitoyl-*sn*-Glycero-3-Phosphocholine



AVANTI Product number	850355
Phase transition temperature	$41°C$
Molecular weight	734.05
Molecular Formula	$C_{40}H_{80}NO_8P$
Charge of headgroup	*uncharged*

DOPC

Name: 1.2-Dioleoyl-*sn*-Glycero-3-Phosphocholine



AVANTI Product number	850375
Phase transition temperature	$-20°C$
Molecular weight	786.150
Molecular Formula	$C_{44}H_{84}NO_8P$
Charge of headgroup	*uncharged*

B. Engineer drawings

This are pictures of the construction drawings, given to the workshop. The pictures are not in scale.

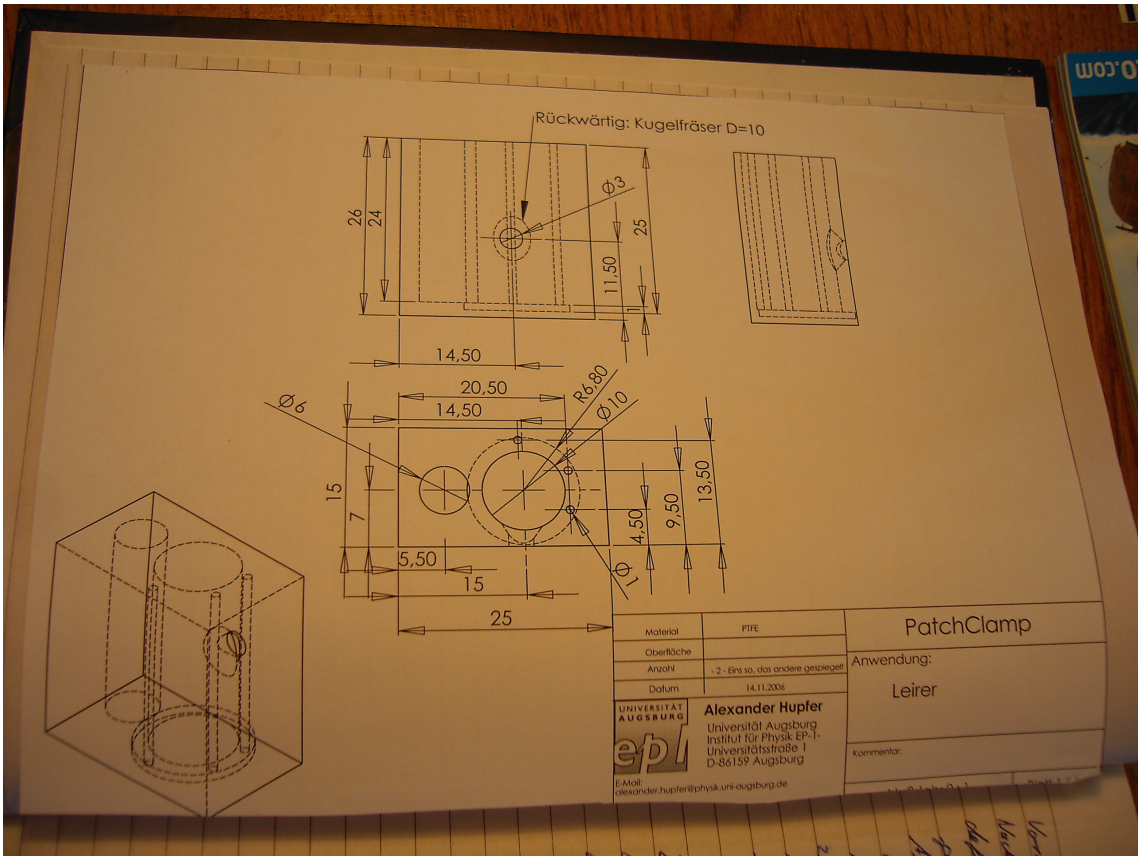


Figure B.1.: drawing of the flowcells, provided by Christian Leirer, university of Augsburg

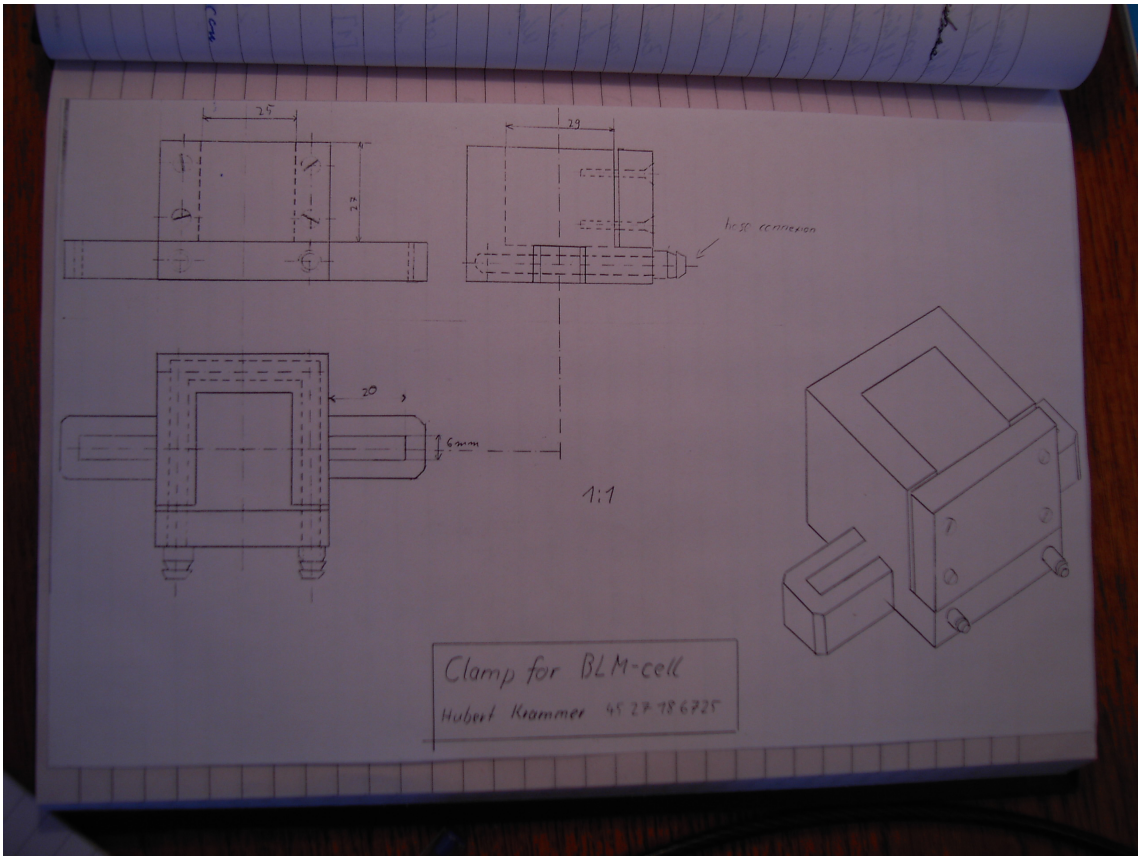


Figure B.2.: drawing of the heat bath, Hubert Krammer

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