# PRESSURE PERTURBATION CALORIMETRY

## MASTER THESIS



The Relation Between Enthalpy And Volume Change

Author: Frederik Becher Supervisor: Thomas Heimburg

Membrane Biophysics Group Niels Bohr Institute University of Copenhagen

April 2014 – version 1.0

Frederik Becher: Pressure Perturbation Calorimetry, The Relation Between Enthalpy And Volume Change , C April 2014

Several experiments have indicated a linear relation between the total enthalpy and volume change in lipid systems. In the current thesis we investigate if this behaviour is valid for the whole temperature range, and therefore for the heat capacity at constant pressure  $c_p$  and the thermal expansion coefficient  $\alpha_V$ . The phase transition of lipid membranes was measured using three different calorimetric methods. Subsequently the heat capacity at constant pressure  $c_p$  was compared with the thermal expansion coefficient  $\alpha_V$ . In addition, the effect of cholesterol and lidocaine was elucidated using different sample concentrations. The results, and their significance, will be discussed according to the theoretical predictions.

First of all I would like to thank my supervisor, Thomas Heimburg, for his good advice and for giving me the possibility to write my thesis about a topic I really love. Working on the topic broadened my horizon. Special thanks go as well to Alfredo Gonzalez-Perez and Henrike Sasse-Middelhoff. Alfredo, for his great help and answers to all my questions. Henrike always took the time to help as well but of course also a huge thanks for sharing her data with me. In general I want to say thank you to the whole biomembranes group. I experienced always a nice working atmosphere, and in between, time for really good conversations. I not have to mention that everybody was without any discussion helping if it was necessary.

During my writing I experienced once again how important my friends are. Therefore thanks for all the support and motivation during my writing and during the whole time of my master. I had always a place to sleep, even if for a while I had no apartment. And yes, also some thanks to all the life guards. I had it sometimes in my mind during my writing.

And last but not least I would like to thank my family. I never had any doubt about the support from home.

i	INT	RODUCTION 1			
1	MOTIVATION 3				
	1.1	Biological membranes 3			
	1.1.1 Life around melting points 7				
	1.2	Using thermodynamics in biology 7			
2	BACKGROUND AND THEORY 12				
	2.1	2.1 Lipids 13			
		2.1.1 Glycerophospholipids 13			
		2.1.2 Fatty acid 14			
		2.1.3 Aggregates, packing parameter and volume 15			
		2.1.4 Different kinds of vesicles 16			
		2.1.5 Phase transition 17			
		2.1.6 Cholesterol 18			
	2.2	Anaesthetics and the Meyer-Overton rule 19			
		2.2.1 Lidocaine 20			
	2.3	A short summary of thermodynamics 21			
	5	2.3.1 Some notes about internal energy, heat, and work 21			
		2.3.2 Entropy 23			
		2.3.3 Thermodynamic variables as statistical averages 26			
		2.3.4 Fluctuations and susceptibilities 27			
11	EXP	ERIMENT / RESEARCH 29			
3	MATERIALS AND METHODS 31				
	3.1	Buffer and sample preparation 31			
	3.2	Calorimetry 32			
		3.2.1 Differential Scanning Calorimetry 32			
		3.2.2 Pressure Perturbation Calorimetry 33			
		3.2.3 High Pressure Calorimetry 36			
4	RES	ULTS AND DISCUSSION 41			
	4.1	Heat change vs volume change 41			
		4.1.1 Hysteresis 41			
		4.1.2 Standard calorimetric experiment 41			
		4.1.3 DMPC - Lidocaine 46			
		4.1.4 DMPC - Cholesterol 52			
	4.2	High Pressure Calorimetry 54			
	4.3	Addendum - DMPC samples 56			
5	CON	ICLUSION 59			
		5.0.1 DMPC in non-equilibrium 59			
		5.0.2 Relation between $c_p$ and $\alpha_V$ 59			
	5.1 Outlook 60				

iii APPENDIX 61
A EVALUATION OF PPC DATA 63
B HIGH PRESSURE CALORIMETRY PICTURE 67
BIBLIOGRAPHY 69

Figure 1	Anatomy of an animal cell 3
Figure 2	Mosaic and mattress model 5
Figure 3	Modern interpretation of a bilayer membrane 6
Figure 4	Phase separation in DLPC-D $_{15}$ PC (2.5-7.5) vesi-
0	cle 7
Figure 5	Melting profile of the membranes of E.coli 8
Figure 6	The first recorded and published action poten-
-	tial 8
Figure 7	Electric circuit of a membrane suggested by
	H&H 9
Figure 8	Temperature change in a non-myelinated nerve10
Figure 9	Calculated solitary density pulse 12
Figure 10	Phospholipids structures in aqueous solution 13
Figure 11	Chemical structure of DMPC 14
Figure 12	Fatty acid examples 14
Figure 13	Aggregate structures in relation to the packing
	parameter P 15
Figure 14	Anatomy of an animal cell 16
Figure 15	Fatty acid examples 17
Figure 16	Solid-ordered, ripple phase, and liquid-disordered
	phase 18
Figure 17	Cholesterol 19
Figure 18	Meyer-Overton correlation 20
Figure 19	DSC Calorimeter scheme 32
Figure 20	Sketch PPC 33
Figure 21	PPC pressure jumps example 35
Figure 22	Sketch HPC 36
Figure 23	Pre-transition DMPC - Lidocaine 20 Mol% 41
Figure 24	Typical DSC scan 43
Figure 25	Typical PPC scan 43
Figure 26	Typical DSC scan, main-transition in detail 44
Figure 27	Typical PPC scan, main-transition in detail 44
Figure 28	Pre-transition DMPC-lidocaine 2 mM 45
Figure 29	DMPC-lidocaine, c <sub>p</sub> -curves 47
Figure 30	DMPC-lidocaine, $\alpha_V$ values 47
Figure 31	DMPC-lidocaine, $c_p$ -curves plus $\alpha_V$ values 48
Figure 32	Maximum values related to the lidocaine con-
	centrations 49
Figure 33	Correlation of the maximum values 50
Figure 34	Matching of data points 51
Figure 35	$\Delta c_p$ before and after the temperature shift 51

# x List of Figures

Figure 36	DMPC-cholesterol 13 Mol% 53
Figure 37	DMPC-cholesterol 13 Mol%, detail 53
Figure 38	DMPC-cholesterol 0, 13, and 25 Mol% 54
Figure 39	HPC DMPC 55
Figure 40	HPC DMPC with 5% Lidocaine 56
Figure 41	Aging of DMPC-lidocaine samples 57
Figure 42	Peak temperatures after storage 57
Figure 43	Maximum $c_p$ -values after storage 58
Figure 44	Shape comparison 60
Figure 45	HPC 67
Figure 46	HPC 67
Figure 47	HPC 68

# LIST OF TABLES

Table 1	State functions 23
Table 2	Peak temperatures of DMPC, pre- and main-
	transition 42
Table 3	Total heat change of DMPC, pre- and main-
	transition 45
Table 4	DMPC-lidocaine, heat capacity main-peak tem-
	peratures 46
Table 5	DMPC-lidocaine maximum values 48
Table 6	DMPC-lidocaine integrals 48
Table 7	DMPC-cholesterol maximum values 52
Table 8	DMPC-cholesterol integrals 52
Table 9	HPC DMPC: pressure, peak temperature, and
	time difference 55
Table 10	HPC DMPC-lidocaine: pressure, peak temper-
	ature, and time difference 55
Table 11	Storage influence on the DMPC-lidocaine peak
	temperatures 56
Table 12	DMPC-lidocaine maximum c <sub>p</sub> -values 58

## ACRONYMS

- DMPC 1,2-dimyristoyl-sn-glycero-3-phosphocholine [6]
- DPPC 1,2-dipalmitoyl-sn-glycero-3-phosphocholine [6]
- DSC Differential Scanning Calorimetry
- ER Endoplasmic Reticulum
- HPC High Pressure Calorimetry
- PPC Pressure Perturbation Calorimetry

# Part I

## INTRODUCTION

In the introduction the interested reader will find a presentation of the key points, and the respective motivation and context in which this thesis was written. Furthermore, the theoretical background of the thesis will be summarized.

### MOTIVATION

This section will start with an introduction about biological membranes. It will emphasise the impact of lipids in living cells and point out how elementary the understanding of lipids, as the bricks of life is. A second part of the motivation is about a possible interpretation of how the characteristics of lipids could lead to a new understanding of nerve propagation. Therefore a short summary about the Hodgkin & Huxley model is given as well as a short introduction to an alternative model describing nerve signals as solitons.

### 1.1 BIOLOGICAL MEMBRANES

Cells, as the smallest units of a plant or animal, are in several ways dependent on biological membranes (biomembranes) and lipids in general. Figure (1) shows a typical idealised drawing of an animal cell. Several elements of the cell are strongly dependent on the characteristics of lipids. There are several elements surrounded by or built out of lipids. Some examples are given in the following.



Figure 1: Diagram of an animal cell [1].

THE CELL MEMBRANE, also called plasma membrane, separates the interior of a cell from the outside environment. With its typical double bilayer, it has to control, actively or passively, what is going in or out of the cell. It is probably also involved in tasks like cell division [10] or the cell's ability to adhere to other surfaces [42].

4

- THE NUCLEUS, as usually the largest organelle is protected by two bilayer membranes, the so called nucleus envelope. Connected to the outer bilayer is the Endoplasmic Reticulum.
- THE ENDOPLASMIC RETICULUM (ER) is a membranous network, its structure reminds at the sponge phase aggregate of lipids. It is subdivided into the rough and smooth ER. They differ by the fact that the rough ER includes ribosomes, whereas the smooth does not.
- THE GOLGI APPARATUS is a single membrane compartment and is responsible for sorting, packaging, processing and modifying of proteins. The shape of the organelles changes from convex to concave depending on the distance to the rough ER, starting with a convex shape nearest to the rough ER.
- MITOCHONDRIA have similar chloroplasts to plants in their own DNA. In endosymbiotic theory the idea , that they originally derived from former prokaryotic organisms is supported. They are enclosed by a double bilayer membrane.

This is just a small selection of cell components, but it is already noticeable that lipids are essential for the structure and partitioning of a cell. To imagine that all these structures are created using their ability to self-organise competence is an interesting thought.

Now let's have a closer look at the research about cell membranes. The first statements were made based on observations of plant cells. In the 19th century it was experimentally shown that they separate their protoplasm from the environment by a osmotic barrier [64, 65, 45, 49]. One of the observations was that plant cell protoplasts were permeable to water, but impermeable to many other solutes, like sucrose [64]. The membrane structure, as well the material it was made of, was not known at that time. Even more impressive is the conclusion of Pfeffer in 1886 [50] who interpret the cell membrane as a protoplasmic organ which is able to regulate the exchange of solutes. His idea about the solubility as the main feature which determines the permeability of a solute in the membrane was adopted by Overton. After Quincke, who in 1888 as the first to suggest that a thin lipid layer is used as boundary for the protoplasm, it was Overton who suspected that the lipid material of this boundary had similar properties to cholesterol esters and phospholipids [51, 46]. Around that time Overton and Meyer developed, independently from each other, a theory about anaesthetics. Both suggested that the action of anaesthetics on nervous tissue is related to the solubility of anaesthetics in lipids [47, 46, 38]. More about the Meyer-Overton rule is presented in section (2.2).

The next important step was to figure out the structural nature of a cell membrane, because at the beginning of the twentieth century only the chemical composition was known. This was the case until 1925, when Fricke could measure the capacitance of erythrocyte solutions and determine the cell membrane thickness. His measurement of 3.3nm only determined the thickness of the hydrocarbon core, not the whole bilayer. But he was not aware of that and misinterpreted the results as single molecular layer [15]. Thus, also in 1925, Gorter and Grendel were the first who stated that a cell membrane is built as a lipid bilayer. They measured the relation between a monolayer made of cell lipids with the actual surface area of the cells, and found a relation of two to one [18].



(a) Mosaic model



Figure 2: a) The mosaic model introduced by Singer and Nicolson (1972) [57]. The phospholipids build a bilayer structure with their polar head regions exposed to the aqueous surrounding. Proteins can be partially or fully embedded into the lipid "mosaic" and are in general randomly distributed, or may form aggregates locally b) The mattress model of Mouritsen and Bloom (1984) [41]. Lipids are expected to accumulate around embedded proteins depending on the match of their hydrophobic regions.

About a decade later the bilayer model was extended, and Danielli and Davson suggested that the lipid bilayer is additionally coated either side by a layer of proteins [14]. The state of the membrane was expected to be homogeneous, and either liquid or solid.

With the help of electron microscopy, new interpretation came up in the late 1950s. For example Robertson, observed a characteristic trilaminar structure consisting of two darker as well a lighter inner region. He determined that the two darker electron dense bands were the headgroups and the proteins associated with it. With the concept of a "unit membrane", it was the first time that all cell membranes, as well as organelles, were assigned with bilayer membranes [52, 53]. A new interpretation of the structure, which still has a huge impact on our current picture of a membrane, was introduced by Singer and Nicolson in 1972. They came up with the model known as the fluid mosaic model. It was influenced by two important experiments. In

#### 6 MOTIVATION

1960 Mueller and Rudin could investigate for the first time characteristics of a simple artificial bilayer [43]. The so called BLM, standing for black lipid membrane or also for biomolecular lipid membrane [62, 61], revealed lateral fluidity, high electrical resistance and even self-healing in response to puncture. The second experiment was carried out by Frye and Edidin in 1970. They could show the fluidity of cell membranes with fluorescence by tagging the lipids with dyes. So Singer and Nicolson concluded that biological membranes are composed out of lipids, but are penetrated by proteins. They assumed that proteins can either penetrate half way or all the way through the membrane but, more importantly, that proteins are freely floating inside of a lipid's two dimensional surface [57]. An addition to the fluid mosaic model is the mattress model. It assumes that lipids may reorganize respective to the size of the embedded proteins. Around a protein with a long hydrophobic domain is it therefore more likely to find lipids with long carbon chains and vice versa in the case of shorter proteins. This accumulation of lipids with the right length around proteins was suggested by Mouritsen and Bloom in 1984 [41]. A sketch of the mosaic model and the mattress model is presented in figure (2a).



Figure 3: Modern interpretation of a bilayer membrane [11].

Research in recent years also pointed out that lipids and proteins may distribute inhomogeneously, and that so called "rafts", made of spingholipids and cholesterol, may form in the membrane. This lateral heterogeneity seems to have a strong influence on diffusion, directed transport and signalling pathways [56].

Another important consideration regarding membranes is to take phase transition into account. In figure (4) one can see a DLPC- $D_{15}PC$ (2.5-7.5) vesicle at different temperatures. This lipid mixture has a region where the liquid and the gel phase coexist approximately between 19 and 30 °C. The temperature series clearly displays how the relation between lipids in the liquid and gel phase changes with decreasing temperature.

It seems evident that phase transition has a important influence on the membrane condition and the coefficients describing it. Two of these coefficients will be further analysed in this thesis.



Figure 4: Phase separation in DLPC-D<sub>15</sub>PC (2.5-7.5) vesicle visualized by fluorescence microscopy [34]. The images, taken in the temperature range where the liquid and gel coexist, show a clear trend in the relations between areas covered by lipids in the liquid (dark) phase compared to lipids in the gel phase (bright).

### 1.1.1 Life around melting points

It is obvious that, in general, life needs water in its liquid form. Life can only exist if water is above its freezing point. In a similar vein it seems that life can only exist if the required membranes, built out of lipids with their characteristic phase transition, are above their totally "frozen" state. Research indicate that bacteria grown at different temperatures adapt their composition of lipids to maintain the same difference between the growing temperature and their membrane melting point [?].

Such an alignment of the melting point also enables one to easily imagine how life could adapt to different temperature conditions. If this assumption is true, that life is strongly related to the melting temperature of the associated membranes, it could have a big influence on different interpretations of processes happening within a cell.

### 1.2 USING THERMODYNAMICS IN BIOLOGY

In the following sections one will find a short summary of the Hodkin-Huxley model together with some arguments about its interpretation. It will lead to an idea of how a thermodynamic view on living cells could change, for example, the understanding of nerve propagation.

### *Nerve Propagation and the Hodgkin-Huxley model*

In 1952 A. L. Hodkin and A. F. Huxley published a model to describe the nerve propagation in squid giant axons taking into account their



Figure 5: Melting profile of the memmbranes of E.coli grown at 37°C [25]. The peak on the left shows the melting behaviour of the membrane lipids in comparison to the peaks on the right, which are mainly associated with irreversible protein unfolding.

experimental research carried out in the years before. It is a mathematical model using fit parameters gained from a newly introduced experimental approach called voltage clamping. The aim of their model was to find a suitable description of an effect they recorded a couple of years before and was published in 1945: the so called action potential, which is shown in figure (6).



Figure 6: First recorded and published action potential of a squid axon [27]. The voltage difference (mv) is recorded between the inside and the outside. At the bottom the time marker has a width of 500 cyc./sec. The surrounding sea water was taken as zero potential.

9

In their model they treat each component of an excitable cell as an electrical element, as described in figure (7). The current flow is expected to consist of two different terms:

$$I = C_M \frac{dV}{dt} + I_i \tag{1}$$

In this case, I is the total membrane current density (inward current positive) and is composed of a capacity and an ionic current. The derivation of the capacity term is interesting. H&H assumed in their calculation that the capacity of the membrane stays constant. It means that in the H&H-model the lipid membrane plays no active role and is handled as an passive insulator. Meanwhile there is strong evidence that the capacity of a membrane is dependent on several factors, like pressure, temperature, membrane composition and applied potential. In 1975, for example, R.H. Adrian and W. Almers measured a capacity change around 50% in sartorius muscle fibres [8] caused by attended membrane potentials between -200 and +50 mV.



Figure 7: Suggested electric circuit by H&H. The membrane current is split into a capacity current and ionic currents (Labelled with sodium Na, potassium K, and leakage l)

The ionic current  $I_i$ , however, is expected to consist of mainly three currents, two caused by sodium ions (Na) and potassium ions(K), respectively and a third which combines the leakage currents,  $I_1$ . H&H determined the ion currents by an empirical fitting of voltage clamp experiments.

$$I_i = I_{Na} + I_K + I_l \tag{2}$$

The currents combined in equation (2) are all based on ion flows along gradients and are therefore by definition irreversible. The H&H model relies on such flux of charges through a membrane treated as a resistor. However, if charges flow through a resistor, one expects a temperature increase of the resistor. It is comparable to a light bulb,

#### 10 MOTIVATION

where flowing electrons produce heat, caused by friction. It means that one would expect a temperature increase around a nerve membrane while proceeding the action potential. The measured heat production, however, shows, within the range of experimental error, a nearly reversible heat production as it is seen in figure (8).



Figure 8: Recorded temperature change in the non-myelinated fibres of the garfish olfactory nerve at 0°C done by Howarth et al (1979)[28]. The response is caused by a single maximal stimulus (2 ms) applied at the stimulus artifact. The plotted curve is the result of an electronic averaging of 25 records.

Another observation which can not be explained by the H&H-model is mechanical displacements correlated with the Action potential. Iwasa, Tasaki, and Gibbons, for example, could measure a thickness change of the same magnitude as the length increase of a lipid while going from its gel into its fluid state [30]. Recent research also showed the ability of action potentials to penetrate each other. In these experiments pulses stimulated at both ends of isolated giant axons of an earthworm could pass through each other [17]. All of these observations are not, or only deficiently explained by the H&H model.

Despite the great technical and experimental achievements of H&H it seems that the whole process of nerve signal propagation could be interpreted differently. One potential explanation is presented in the following. To maintain a coherent picture is it important to remember the expected relation of life to the phase transition of lipids mentioned in section(1.1.1) as a frame condition.

### Soliton Model

The measured heat behaviour, mechanical changes, and the penetration of action potentials, sent in different directions, are not properly explained by the H&H model. Therefore an alternative, more complete model is desirable. The Soliton Model describes nerve propagation under the consideration of thermodynamic principles. First published in 2005 by Thomas Heimburg and Andrew D. Jackson [24], it assumes that signals within a nerve axon are transmitted by soliton waves. The interpretation of such a soliton wave is possible by comparing it to a wave propagated within the nerve cell membrane, with physical properties similar to a sound wave. Assuming that a system such as a membrane can be characterized in a thermodynamic manner<sup>1</sup>, and parameters describing the area density or melting transition can be determined with high precision, the following suggestions can be made:

To describe a soliton wave inside a medium like a lipid membrane, the well-known wave equation for area density change  $\Delta \rho^A$  is taken as a starting point. The equation originates from the Euler equations of compressible media (e.g., [31, 58]).

$$\frac{\partial^2}{\partial \tau^2} \Delta \rho^A = \frac{\partial}{\partial z} \left( c^2 \frac{\partial}{\partial z} \Delta \rho^A \right) \tag{3}$$

With  $\tau$  as time, *z* is the position along the nerve axon, and *c* is speed of sound. In the case of a constant velocity  $v = v_0$  the equation simplifies to the relation of sound propagation. However, it has been shown that in membranes sound velocity is a sensitive function of density [21, 55]. Therefore sound velocity is expanded around its value in the fluid phase up to the quadratic order

$$c^{2} = c_{0}^{2} + p\Delta\rho^{A} + q(\Delta\rho^{A})^{2} + \dots$$
(4)

The parameters q and p describe the dependence of sound velocity on density. They are fitted to experimental data [24].

To take the frequency dependence of the speed of sound into account a dispersion term is added to the equation (3). It has the form

$$-h\frac{\partial^4}{\partial z^4}\Delta\rho^A \tag{5}$$

with the constant h.

The result is a time and position dependent partial differential equation [24, 32]:

$$\frac{\partial^2}{\partial \tau^2} \Delta \rho^A = \frac{\partial}{\partial z} \left[ (c_0^2 + p \Delta \rho^A + q (\Delta \rho^A)^2 + ...) \frac{\partial}{\partial z} \Delta \rho^A \right] - h \frac{\partial^4}{\partial z^4} \Delta \rho^A$$
(6)

With the non-linearity and dispersive effects of the lipids taken into account, it describes the propagation of a longitudinal density pulse in a myelinated nerve. The following parameters of the membrane, except h, were experimentally determined:

<sup>1</sup> A further explanation of thermodynamic manner will be given in section (2.3)

• Area density change	$\Delta\rho^A=\rho^A-\rho^A_0$
• Area density	$\rho^{\mathcal{A}}$
• Equilibrium area density (fluid phase)	$ ho_0^{\mathcal{A}}$
• Velocity of small-amplitude sound	co
• Melting transition parameter	p, q
• Dispersion parameter	h

The parameter h parametrizes the dispersion, or in other words the frequency dependence of the speed of sound. It was not possible to determine h directly from a experiment, due to difficulties in measuring the velocity of sound in the kilohertz regime.



Figure 9: Calculation of a solitary density pulse. Plotted is the relative change in density  $\Delta \rho^A / \rho_0^A$  vs the lateral position z(m).

Figure (9) shows the expected shape of a soliton, propagating through a nerve cells. Under thermodynamic considerations it has the great advantage that the whole process is approximately reversible.

To verify or to falsify such a new theory it is important to understand the behaviour of lipids, and it is therefore a good motivation for this thesis.

## BACKGROUND AND THEORY

This section provides further background on lipids, anaesthetics and thermodynamics.

### 2.1 LIPIDS

Lipids are a complex group of natural occurring molecules, which typically have an amphipathic character. Accordingly they always have one end which is hydrophobic; one could say they are partly afraid of water. Some lipids, for example glycerophospholipids, have the ability to self assemble if they are surrounded by an aqueous solution. Possible aggregates are shown in figure (10). These complex structures are arranged by the simple aim to avoid contact between the hydrophobic tails of the lipids and water, and to find the most favourable energetic state. The lipid used in this thesis is the glycerophospholipid DMPC.



Figure 10: Diagram showing different self-assembled lipid structures in aqueous solution [7].

### 2.1.1 Glycerophospholipids

Glycerophopholipids, which are also known as phospholipids, are a main component of biological membranes. Recent research is indicating that phospholipids play an important role in cell division. It was shown that cells regulate their lipid composition and localization whilst dividing themselves [10]. The change in composition has a strong influence on the properties of the membrane, like its bending strength or flexibility. Knowledge about lipids and their different compositions in membranes also seems to be one of the main keys to understand questions of self-organisation in cells.



Figure 11: Chemical structure of DMPC

A typical structure for a Phospholipid is its hydrophobic tail consisting of two long fatty acid hydrocarbon chains plus a hydrophilic head group. The head group consist of an organic compound, such as choline, ethanolamine, serine and glycerol. The Phospholipid used in this thesis is DMPC, a Phosphatidylcholine (PC) using a choline at his head group. It is saturated, which means it does not contain carbon-carbon double bonds or triple bonds in its carbon chains.

### 2.1.2 Fatty acid

Fatty acids are a main component of lipids in plants, animals, and microorganisms. Naturally occurring fatty acids consist, as a rule, of an even number of carbon atoms and are accompanied by hydrogen atoms along its length. Chain lengths of 4 to 28 are common [4]. Fatty acids can occur as saturated or unsaturated. The possible scaling of the length and the possibility to have different saturations enables the fatty acid to change its properties that is reflected in changes in the phase transition.



(b) Linoleic acid - an unsaturated fatty acid

Figure 12: Example of fatty acids [5]. Stearic acid is a saturated fatty acid with 18 carbon atoms. Linoleic acid is also a 18-carbon chain but with two *cis* double bonds. Therefore it is a unsaturated fatty acid. Both sketches are simplifications and do not take the shape of the lipid into account. Double or triple carbon-carbon bonds within the chain lead to a lower melting point. Fatty acids with such bonds are called unsaturated. If there are no double or triple bonds at all it is called saturated. By contrast the chain length is positively correlated with the melting point, meaning that a longer chain length leads to phase transition at a higher temperature [3]. Experiments done with *escherichia coli* point out that, grown at different temperatures, they can use these parameters to adjust the melting point of their membrane lipid composition [63]. Fatty acids are usually not found on their own, instead they commonly exist in combination with a glycerol molecule.

### 2.1.3 Aggregates, packing parameter and volume

Due to its construction out of a head group, glycerol, and fatty acid, the shape of a phospholipid can, for instance, be described in simplified terms as a small tube or cone. If the volume v of the tube is given by the head area a times the length of the tube l one can define a packing parameter P [29]:

$$P = \frac{v}{a \cdot l}.$$
 (7)

This is then a measure for every aberration from the norm tube, and if the value is not equal to 1, the shape is conical. The shape of a component, which is repeated in the structure several times, has a huge influence on the shape of the finale aggregate.



Figure 13: Aggregat structures in relation to the packing parameter P. Drawings are taken from Mouritsen 2005 [42] but modified. The different structures are arranged with respect to their curvature and the value of their packing parameter.

#### BACKGROUND AND THEORY

16

Figure (13) shows several possible aggregates. They are arranged in an order associated with their packing parameter. If the shape changes, for example during the phase transition, the most likely aggregate state could also change.

In this thesis the total volume change of lipids will also be determined using pressure perturbation calorimetry. Within this context of lipid shape it is worth mentioning that the actual change in total volume and its influence on a structure is probably amplified or at least deviant from a simple increase of the structure size. Research has revealed that lipids in a bilayer membrane can increase their lateral area by about 24% and parallel decrease their thickness by about 16% [23, 44, 59] while crossing their phase transition. It would mean that besides their volume, their packing parameter is especially changing significantly.

### 2.1.4 Different kinds of vesicles

Another aggregate are vesicles, also called liposomes, if artificiallyprepared. They can in general be described as aqueos spheres surrounded by a lipid bilayer. One can distinguish further between the multilamellar vesicle (MLV), the large unilamellar vesicle (LUV), and the small unilamellar vesicle (SUV). They are not mentioned in figure (13) and should not be confused with micelles and reverse micelles. The latter are composed out of monolayers instead of bilayers.



Figure 14: Anatomy of an animal cell [60].

Measurements indicate that the shape of the different vesicles has an influence on the transition half-widths of their heat coefficient peaks. This can be explained by the existence of different cooperation factors.

#### 2.1.5 *Phase transition*

A fundamental property of lipids is that they have phase transitions. The heat capacity  $c_p$  of phospholipids has one characteristic main peak and in some cases a second small peak, interestingly around the temperature of living material. They are commonly associated with the pre- and main-transition. One expects that the pre-transition is caused by conformation changes happening in the head group of the lipid, whereas the main-transition is connected to the phase transition of the fatty-acid chains.



ordered and Eight dis (b) Einerent contormations of fatty acid

Figure 15: a) Illustration of a chain-melting process of a phospholipid bilayer [23]. An increase in temperature leads to a loss of lateral order. b)Different conformations of a fatty acid as part of a phospholipid [16]. The C-C bonds of a fatty acid show different conformation which can be excited. It is assumed that the chain can rotate around the C-C bonds. The figure describes the potential energy of different rotation angles. Three minima are indicated with a minimum potential at the trans position. If a bond changes to the gauche<sup>+</sup> or gauche<sup>-</sup> position the total potential energy increases. It is a transfer of thermal energy into potential energy.

The typical structure of a fatty acid, with its long chains of carbon and hydrogen atoms, enables it to adapt its melting temperature over a wide range. Figure (15 b) highlights the potential differences between different rotation positions of a fatty acid carbon connection. With increasing temperature there is a increase in enthalpy  $\Delta$ H and entropy  $\Delta$ S. A lipid aggregate present at a lower temperature than the pre-transition is in its solid-ordered (gel) phase. The state above the main-transition is called liquid-disordered (fluid) phase. Figure (15a) presents the gel and fluid phases schematically. If there is a temperature difference between the pre- and main transition a third phase is observed, which is called ripple phase. An example of a phase transition is shown in figure (16).

- THE SOLID-ORDERED (GEL) PHASE is built by lipids in their ground state. It is the phase with the highest packing density. The head groups are arranged in a solid triangular lattice and the hydrocarbon chains are in the all trans configuration. The system is well ordered and the entropy level is low.
- THE LIQUID-DISORDERED (FLUID) PHASE is characterized by randomly moving membrane lipids within a 2-dimensional lattice. Thermal energy can excite the lipid chains from the state with the lowest possible potential energy, all trans, to states with a higher potential energy. Entropy increases because more states are available. The exciting of the carbon chains is related to an increase in their volume.
- THE RIPPLE PHASE is named after its periodical wavelike (or as the name implies ripple) like surface structure. Observations indicate that they disappear, however, if secondary biomolecules are present. This suggests that this phase rarely emerges in biological membranes.



Figure 16: Heat capacity profile of DMCP. The dotted lines classify the different phases of lipid aggregates in solid-ordered, ripple phase, and liquid-disordered phase

### 2.1.6 Cholesterol

One of the chosen additives used in the experiment is the lipid Cholesterol. The name comes from the Ancient Greek words chole-(bile) and stereos (solid). It is one out of several members of the sterol family, like ergosterol in fungi or sitosterol in plants. They play an essential role in eukaryotes and they are therefore absolutely essentially for higher life forms. One assumes that without Cholesterol, or its relatives, no higher life forms could have evolved. In more basic organisms like prokaryotes, for example, which do not have a membrane bound nucleus, no cholesterol at all is found. This accounts for the high percentage of cholesterol in our plasma membrane, about 30-50%, and it is known for its strong influence on the physical properties of lipid bilayers.



Figure 17: Cholesterol [2], Polar hydroxyl group (-OH) together with a bulky, stiff and hydrophobic tail made out of a steroid skeleton and a small hydrocarbon.

Cholesterol differs from other lipids by the fact that it has no fatty acid. Instead it has a steroid ring structure and a small hydrocarbon chain as its hydrophobic tail. Its polar head is a simple hydroxyl group (OH). The structure of it is very similar to molecules like bile salt or vitamin D. Although Cholesterol is a polar lipid, it does not form aggregates in water on its own [42], but with its amphiphilic characteristic it easily incorporates itself into a lipid bilayer. If membranes contain cholesterol a fourth phase can be observed. In the liquid ordered phase the lipid chains are ordered, while the lateral order is random. With its strong influence on the lipid phase transition and its appearance in high concentration within plasma membranes in all animals, it is an optimal study subject and therefore used in one of the main test series here.

### 2.2 ANAESTHETICS AND THE MEYER-OVERTON RULE

For the most of us who are used getting dazed before an operation, at the dentist or in his/her free time by drinking a glass wine, it is probably quite surprising that modern science still has no confirmed and consistent theory about the mechanics and effects of anaesthetics. Noteworthy is the relation between the solubility of an anaesthetic in lipids relative to that in water, and its effect on an organism. The so called partition coefficient describes the relation between the concentration in the two solvents  $x_{lipid}$  and  $x_{water}$ .

$$K = \frac{\chi_{\text{lipid}}}{\chi_{\text{water}}} \tag{8}$$

This relation has been known since the 19th century and is still valid for the widest range of cases. The relation between solubility and effectiveness as an anaesthetic is strong evidence that membranes in general are an important factor for the anaesthetic process. Interestingly it has been found that the impact of anaesthetics is fully reversible under pressure [?]. It is interesting because anaesthetics and an increase in pressure show exactly the opposite effect, in respect to the direction of the temperature shift on the melting behaviour of lipids.



Figure 18: Meyer-Overton correlation [67]. The graph shows different gases and their linear relation between the oil/gas partition coefficient and the anaesthetic potency (MAC)

### 2.2.1 Lidocaine

Lidocaine was used in the second big test series. It is classified as a local anaesthetic and antiarrhythmic drug. Antiarrhytmic means that it is also used to suppress abnormal rhythms of the heart. But its main purpose is to relieve itching, burning and pain from skin inflammations, as well being injected as a dental or local anaesthetic for minor surgery.

#### 2.3 A SHORT SUMMARY OF THERMODYNAMICS

Characteristic of thermodynamics is that it uses mean values to describe procedures, and it is known as the branch of the natural science which deals with heat, other forms of energy and the relation between the two. Its history is strongly connected to the invention of the steam engine. It was used to describe heat's ability to generate motion.

### 2.3.1 Some notes about internal energy, heat, and work

In the middle of the 19th century James Prescott Joule showed through experiments that heat can be transformed into mechanical energy and vice versa [?]. He found that for each expended force performing work an equivalent amount of heat is obtained. This leads directly to the first law of thermodynamics:

THE FIRST LAW OF THERMODYNAMICS states that energy can not vanish, or emerge, out of nothing. It can only be transformed from one form to another. In a closed system with the internal energy dU it is valid that:

$$dU = dQ + dW \tag{9}$$

If dQ is heat absorbed or released, and if dW is the work performed on or by the system. Thus the total internal energy can be reduced to a heat term dQ and a term aligning all work terms dW. The latter consists of different kinds of work terms. The most common work term is mechanical work which is described by

•  $dW_{mech} = -pdV$ 

with pressure p as force per area multiplied by the volume change dV. Other work terms are:

- $dU_q = \varphi dq$
- $dU_{el} = -EdP$
- $dU_{mag} = -dM$

These include, for example, the transfer of charge, dq, across a potential difference  $\phi$ . The change of a dielectric dipole moment, dP, in an electric field E also counts towards it, as well as a change in magnetic dipole moment dM in a magnetic fied H. Altogether the internal energy in a closed system is given by

$$dU = dQ - pdV + \phi dq - EdP - HdM - \dots$$
(10)

In the case of a calorimeter without a magnetic field or similar, it is sufficient to write

$$dU = dQ - pdV.$$
<sup>(11)</sup>

There is one thing which all of these work terms have in common, they are all a product of a intensive property and a differential of a extensive property.

- INTENSIVE VARIABLES do not depend on the system size or amount of material of the system. Examples are temperature T, or pressure p.
- EXTENSIVE VARIABLES by contrast show a dependence on the system's size or the amount of material in the system. Typical are volume V, Area A, or charge q.

Interestingly the heat term dQ is also described by an intensive and an extensive variable. This will become clear as soon as entropy is introduced in subsection (2.3.2).

Internal energy, as the sum of the heat term and all the work terms has, however, something special. It is, unlike heat and work, which are dependent on the manner of transformation, a state function. This means that the property of internal energy is independent of the way in which it is acquired. It only depends on the initial and final state. In an equation it can be expressed as

$$\oint d\mathbf{U} = \mathbf{0} \tag{12}$$

for all cyclic processes. A nice visualization is to think of hiking and altitude. If one starts and arrives at the same point, the result of the integral corresponding to the meters in height is always o, independent of "the journey" and how many peaks one stood on during the round trip.

Several other state functions can be derived from internal energy. They can be calculated using Legendre transformations from an expression for U.

The differentials belonging to the state function are given as:

dU = dQ + dW	= TdS - pdV	(13)
dH = dU + d(pV)	= TdS + Vdp	(14)
dF = dU - d(TS)	=-SdT-pdV	(15)

dG = dH - d(TS) = -SdT + Vdp(16)

22

NAME	SYMBOL	FORMULA	VARIABLES
Internal energy	u	$\int ((TdS - pdV))$	S, V
Enthalpy	Н	U + pV	p, S
Helmholtz free energy	F	U – TS	Τ, V
Gibbs free energy	G	H - TS	р, Т

Table 1: State functions in a closed system

The next step cleary shows that, in systems with constant variables, differentials are powerful tools. For example one of the main properties in this thesis is the heat capacity c. The relation between heat absorption and temperature change is described by the heat capacity c. One can differentiate between two different cases either one with constant volume or one with constant pressure. The corresponding notation is  $c_V$  and  $c_p$  respectively.

If one is interested in heat capacity with either constant volume or pressure it leads to two different equations using equation (13) or (14).

$$c_{V} = \left(\frac{\partial Q}{\partial T}\right)_{V} = \left(\frac{\partial U}{\partial T}\right)_{V}$$
(17)

$$c_{p} = \left(\frac{\partial Q}{\partial T}\right)_{p} = \left(\frac{\partial H}{\partial T}\right)_{p}$$
(18)

This emphasises that the heat coefficient at constant pressure  $c_p$  is directly related to the enthalpy change dH. Thus it is possible to calculate the total change in enthalpy  $\Delta H$  by integration

$$\Delta H = \int_{T_1}^{T_1} c_p \, dT. \tag{19}$$

### 2.3.2 Entropy

To understand thermodynamics the concept of entropy is important. The first definition was made by Rudolf Clausius and alongside the concept of entropy, he introduced a fundamental and universal quantity as energy [?]. He referred to considerations produced by Sadi Carnot, who wrote about heat engines and the possibility of producing work if differences in temperature exist. Carnot also theorised that there is a maximum amount of work which can be done given

#### BACKGROUND AND THEORY

a certain temperature difference. This refers to the reversible cyclic process.

Carnot's theorem states

$$\eta = 1 - \frac{Q_2}{Q_1} = 1 - \frac{T_2}{T_1},\tag{20}$$

in which  $\eta$  is the efficiency of a reversible engine and  $T_1$  and  $T_2$  are the absolute temperatures of the cold and hot reservoir respectively. It is important to notice that efficiency is independent of the kind of transformation, undertaken chemical or physical.

From equation (20) follows that in a reversible heat engine process it is always valid that the ration between the heat and temperature of the cold reservoir is equal to the ratio of both quantities in the hot reservoir:

$$\frac{Q_1}{T_1} = \frac{Q_2}{T_2} \tag{21}$$

In a irreversible cycle, processes like friction will lead to a loss of heat,  $dQ_{irre}$  and the relations do not conform to the equation any longer. From this starting point Clausius introduced his idea of entropy, for which in a reversible case the following expression is valid

$$\oint \frac{\mathrm{d}Q}{\mathrm{T}} = 0. \tag{22}$$

Like internal energy, entropy is a function of state and only depends on its initial and final states in a reversible process

$$S_{\rm B} - S_{\rm A} = \int_{\rm A}^{\rm B} \frac{\mathrm{d}Q}{\mathrm{T}} \tag{23}$$

or

$$dS = \frac{dQ}{T}.$$
 (24)

The name entropy incidentally comes from a Greek word meaning transformation. Referring to Carnot's considerations, Clausius also laid the foundation for another important law.

THE SECOND LAW OF THERMODYNAMICS states, after Clausius, that heat cannot spontaneously flow from cold regions to hot regions without external work being performed on the system. In terms of entropy this means that in an isolated system entropy never decreases,

24
because the entropy in an isolated system tends to evolve towards a thermodynamic equilibrium, the state with the highest entropy.

Some considerations in this thesis will refer to some statistical rules. Therefore, and for a general understanding, a short explanation of the statistical interpretation of Entropy will follow.

THE STATISTICAL INTERPRETATION OF ENTROPY was introduced by Ludwig Boltzmann at the end of the 19th century. A statistical example based on a consideration of a canonical ensemble can be helpful to understand the process of entropy increase in the case of a lipid aggregates. A canonical ensemble is defined as a thermally isolated system with N independent subsystems inside, each with a equal number of molecules. It is assumed that there is no particle exchange between the subsystems, and that the total system is thermally equilibrated corresponding to the most likely state of the total system. Finally, a last constraint is required, as either the volume or the pressure of the subsystems is expected to be constant. In biology and biophysics, where the pressure can easily be kept constant, the enthalpy is normally the appropriate choice of state function. Accordingly, for each subsystem one defines a enthalpy H<sub>i</sub>. This could describe a experimental situation in which the system consists of equally sized unilamellar vesicles. If all n<sub>i</sub> vesicles have the same amount of molecules and a identical enthalpy  $H_{i}$ , an overall boundary condition of constant total enthalpy leads to

$$\sum n_i H_i = H_{tot}$$
(25)

and

$$\sum n_i = N.$$
(26)

The relation between  $P_1$  and  $P_0$ , where  $P_i$  is defined as the probability of finding subsystems with a enthalpy of  $H_i$ , is a Boltzmann distribution.

$$p_1 = \frac{P_1}{P_2} = \exp\left(-\frac{H_1 - H_0}{k_b T}\right).$$
 (27)

The distribution is defined relative to the ground state with the enthalpy  $H_0$ . One can generalize the probabilities  $P_i$  by

$$P_{i} = \frac{p_{i}}{\sum_{i} p_{i}} = \frac{exp(-\frac{H_{i}-H_{0}}{k_{b}T})}{\sum_{i} exp(-\frac{H_{i}-H_{0}}{k_{b}T})}.$$
(28)

#### BACKGROUND AND THEORY

26

The sum of all absolute probabilities has to fulfil the requirement  $\sum_{i} P_{i} = 1$ . We can simplify the equation using a definition  $\beta \equiv \frac{1}{k_{b}T}$ , which is sometimes called the inverse temperature. Additionally using the canonical partition function  $Z = \sum_{i} exp(-\beta(H_{i} - H_{0}))$  shortens equation (28) to

$$P_{i} = \frac{p_{i}}{Z} = \frac{exp(-\beta(H_{i} - H_{0}))}{Z}.$$
(29)

In a system with several different enthalpies and their associated probabilities possible to derive entropy as

$$S = -k \sum_{i} P_{i} ln P_{i}$$
(30)

and as it can be found specific statistical thermodynamics books [35, 26].

## 2.3.3 Thermodynamic variables as statistical averages

In biological systems it can be meaningful to determine observables as statistical averages. In the case of a system of a bulk of micro particles it is sometimes difficult or literally impossible to determine all the properties of a participating particle. But it is in many cases maintainable to work with variables defined as thermodynamic averages, such as, for example, the volume of lipids in a vesicle dispersion V, the surface of a vesicle out of lipids, or similar.

Accumulating the absolute probabilities of different enthalpy states  $P_i$  (29) multiplied by its corresponding enthalpy value  $H_i$  leads to the mean enthalpy of the system:

$$\langle H \rangle = \sum_{i} H_{i} P_{i} = \frac{\sum_{i} H_{i} exp(-\frac{H_{i}}{k_{b}T})}{Z}.$$
(31)

Likewise in the previously mentioned cases of the specific volume of the lipids in a vesicle dispersion, or the surface of a lipid vesicle, as it follows:

$$\langle V \rangle = \sum_{i} V_{i} P_{i} = \frac{\sum_{i} V_{i} exp(-\frac{H_{i}}{k_{b}T})}{Z}.$$
(32)

$$\langle A \rangle = \sum_{i} A_{i} P_{i} = \frac{\sum_{i} A_{i} exp(-\frac{H_{i}}{k_{b}T})}{Z}.$$
(33)

The average parameters are important for the calculation of fluctuations. This is explained in the next section as is, why all of the parameters are expected to be in relation to each other.

### 2.3.4 Fluctuations and susceptibilities

Thermodynamic functions in thermodynamics are often mean values describing a bulk of subsystems. Therefore they do not have sharp values, rather fluctuating around the actual mean. Susceptibilities quantify the change of an extensive property under variation of an intensive property. Examples are the heat capacity  $c_p$ , or the area compressibility  $\kappa_T^{area}$ . It is possible to calculate a relation to the fluctuation of the parameter they are describing.

Following equation (18) we know

$$c_{p} = \left(\frac{\partial \langle H \rangle}{\partial T}\right)_{p}.$$
(34)

If one now derives the equation using the mean enthalpy term from subsection (2.3.3) it follows:

$$\begin{split} \frac{d\langle H \rangle}{dT} &= \frac{d}{dT} \left( \frac{1}{Q} \sum_{i} H_{i} exp\left( -\frac{H_{i}}{k_{b}T} \right) \right) \\ &= -\frac{1}{Q^{2}} \frac{dQ}{dT} \sum_{i} H_{i} exp\left( -\frac{H_{i}}{k_{b}T} \right) \\ &+ \frac{1}{Q} \sum_{i} H_{i} exp\left( -\frac{H_{i}}{k_{b}T} \right) \frac{d}{dT} \left( exp\left( -\frac{H_{i}}{k_{b}T} \right) \right) \\ &= -\frac{1}{Q^{2}} \left( \sum_{i} \frac{H_{i}}{k_{b}T^{2}} exp\left( -\frac{H_{i}}{k_{b}T} \right) \right) \left( \sum_{i} H_{i} exp\left( -\frac{H_{i}}{k_{b}T} \right) \right) \\ &+ \frac{1}{Q} \sum_{i} H_{i} exp\left( -\frac{H_{i}}{k_{b}T} \right) \frac{H_{i}}{k_{b}T^{2}} \left( exp\left( -\frac{H_{i}}{k_{b}T} \right) \right) \end{split}$$

Thus, the heat capacity at constant pressure is equal to

$$c_{p} = \frac{\langle H^{2} \rangle - \langle H \rangle^{2}}{k_{b}T^{2}}.$$
(35)

Similar calculations can be done for other susceptibilities such as the area  $\kappa_T^A$  and the isothermal compressibility  $\kappa_T^V$  respectively. For the latter one we are using, as we already know, the enthalpy given as  $H_i = E_i + pV_i$  and it allows us to calculate the compressibility at constant temperature given by

$$\kappa_{\rm T} = \frac{1}{\langle V \rangle} \left( \frac{d \langle V \rangle}{dp} \right)_{\rm T} \tag{36}$$

## BACKGROUND AND THEORY

using the thermodynamic mean of the volume (at constant pressure)

$$\langle V \rangle = \frac{\sum_{i} V_{i} exp(-\frac{E_{i} + pV_{i}}{k_{b}T})}{Q}.$$
(37)

After performing a short calculation, one can show that this is equal to:

$$\left(\frac{\mathrm{d}\langle V\rangle}{\mathrm{d}p}\right)_{\mathrm{T}} = -\frac{\langle V^2 \rangle - \langle V \rangle^2}{k_{\mathrm{b}}\mathrm{T}}.$$
(38)

Altogether we get for the isothermal compressibility an equation showing the coupling between the parameter and the fluctuation of the volume:

$$\kappa_{\rm T} = -\frac{\langle V^2 \rangle - \langle V \rangle^2}{\langle V \rangle k_{\rm b} {\rm T}}.$$
(39)

## Relation between the heat capacity and the thermal volume expansion

In 1981 Anthony et al. [9] reported a linear relation between volume and enthalpy change in the chain melting regime, using densitometry and DSC measurements. Similar it was shown by Ebel et al. in 2001 [13]. The experimental results state:

$$\langle V(T) \rangle = \gamma \langle H(T) \rangle \tag{40}$$

In our experiments the aim is to find out if this is true for the whole temperature range. It should be shown that this is true for the whole temperature range

$$\left(\frac{\partial \langle V \rangle}{\partial T}\right)_{p} = \left(\frac{\partial \langle H \rangle}{\partial T}\right)_{p}.$$
(41)

Using the definition of the thermal expansion coefficient  $\alpha_V$ 

$$\alpha_{\rm V} = \frac{1}{\rm V} \left( \frac{\partial \langle \rm V \rangle}{\partial \rm T} \right)_{\rm p} \tag{42}$$

following relation has to be true as well

$$V \cdot \alpha_V = \gamma \cdot c_p. \tag{43}$$

Such a relation enables one to predict membrane characteristics using the measured heat capacity received from standard DSC experiments.

28

# Part II

# EXPERIMENT / RESEARCH

Everything about the experimental procedure, the settings, and a discussion of the results.

To determine the relation between enthalpy and volume change in lipid membranes, three different methods of calorimetry were used. Differential scanning calorimetry (DSC) was used to determine the heat capacity  $c_P$  of the samples. An add-on for the DSC calorimeter enables one to perform Pressure Perturbation Calorimetry (PPC) measurements. In this set-up the measured response of the samples to abrupt pressure changes is used to calculate the volume expansion coefficient  $\alpha_V$ . And a third approach, High Pressure Calorimetry (HPC) was performed in order to determine the change of melting point temperature caused by a pressure shift.

# 3.1 BUFFER AND SAMPLE PREPARATION

For the calorimetrical measurements, DMPC from Avanti Polar Lipids was used without further purification. The preparation itself has an influence on the shape of the phase transition peaks [23]. Therefore Multilamellar vesicles (MLV) are generally used. To weigh the lipids, a Sartorius balance with an accuracy of  $\pm 0.1$ mg was used. In the cases of lidocaine and cholesterol, a 20 mM Chloroform stock solution was prepared for each of the components.

The samples were prepared by dissolving the lipids in chloroform. Subsequent the desired volume of the stock solution was added to the prepared lipid cholesterol solution using a glass pipette. They were then dried under a gentle compressed air stream, followed by a night in an desiccator to assure that no chloroform residues remain in the sample. Finally, buffer or water was added and dissolved by heating it up to several times above the phase transition temperature of DMPC (approx. 24°C).

AN IMPORTANTINFORMATION about the DMPC-lidocaine samples, is that they got re-used. It means that the samples, after a first scan, were immediately frozen at -20 °C and stored for the length of three months before they got measured again.

A BUFFER was used to imitate the same pH (7.4) value as would be found within an organism. The components of the mixture used were:

• 150 mM KCl

- 1 mM EDTA
- 1 mM HEPES

#### 3.2 CALORIMETRY

# 3.2.1 Differential Scanning Calorimetry

Differential Scanning Calorimetry (DSC) is a thermoanalytical technique to measure the required power difference between a sample and reference cell while increasing the temperature. Generally a DSC, is designed to keep the increase in temperature linear. The idea of a DSC was introduced by Michael J. O'Neill and Emmett S. Watson in 1962[54].



Figure 19: Diagram of a DSC Calorimeter. Whilst linearly increasing the temperature of the two cells and keeping the temperature difference between the two cells constant at  $\Delta T = 0$ , it determines the power difference  $\Delta P$  between the two cells.

The main principle is to compare a reference with a well-defined heat capacity over the range of temperatures to be scanned. From the power difference it is possible to calculate the excess heat capacity of the sample substance. Integration for a short time interval  $\Delta t$  leads to the heat difference  $\Delta Q$ 

$$\Delta Q = \int_{t}^{t+\Delta t} \Delta P dt \approx \Delta P \Delta t.$$
(44)

Using the definition of heat capacity,

$$\Delta c_{\rm P} = \left(\frac{\partial Q}{\partial T}\right)_{\rm P} \approx \frac{\Delta Q}{\Delta T} \approx \frac{\Delta P}{\frac{\Delta T}{\Delta t}},\tag{45}$$

it is possible to determine the heat capacity profile.

All DSC experiments in this thesis were performed on a MicroCal (Northampton, MA) VP-DSC differential calorimeter. Scan rates are normally 5°/h unless otherwise noted. Two main test series were executed. In the first, DMPC was measured with different cholesterol concentrations. In the second test series DMPC was measured with different lidocaine concentrations. The pressure was held constant at approximately 35 psi (2.4 bar).

The obtained data was evaluated using an analysis program written in Igor Pro (DSC\_analysis\_v10.2). After converting the raw data received from the DSC from W to  $J \cdot mol^{-1} \cdot K^{-1}$  a baseline fit was executed. This was done by cutting out the peaks and applying a fit, ranging from a linear regression to a polynomial of fifth order.

## 3.2.2 *Pressure Perturbation Calorimetry*

Pressure Perturbation Calorimetry (PPC) is an official extension of the VP-DSC and allows one to include the volumetric properties of biopolymers in dilute solution.



Figure 20: Sketch of the PPC configuration[40]

Differently to the DSC setup, the PPC does not increase the temperature linearly. Instead, it is possible to program different temperatures and execute two pressure jumps at each of them with a pressure difference  $\Delta p$ . Whilst proceeding with the pressure jumps, the calorimeter keeps the temperature T constant and determines the required power. Integrating about the power curves leads to the heat change  $\Delta Q_r ev$  at each jump. The obtained data was evaluated using PPC Data Analysis in Origin software from MicroCal [39]. The main theoretical considerations are presented in the following subsection, and complementary informations are shown in appendix (A).

## 34 MATERIALS AND METHODS

## Basics of pressure pertubation calorimetry

The determined heat change  $\Delta Q_{rev}$  enables one to calculate the thermal expansion coefficient of volume  $\alpha_V$  using the relation between the heat change  $\Delta Q_{rev}$ , temperature T, volume of the cell V and the pressure change  $\Delta p$ :

$$\alpha_{\rm V} = -\frac{1}{\rm TV} \left(\frac{\Delta Q_{\rm rev}}{\Delta p}\right)_{\rm T} \tag{46}$$

This equation is obtained by following considerations: The heat of a reversible process,  $dQ_{rev}$  is related to the entropy change dS and the temperature T by

$$\mathrm{d}\mathrm{Q}_{\mathrm{rev}} = \mathrm{T}\mathrm{d}\mathrm{S}. \tag{47}$$

Differentiation with respect to pressure, p, yields

$$\left(\frac{\partial Q_{re\nu}}{\partial p}\right)_{\mathrm{T}} = \mathrm{T}\left(\frac{\partial S}{\partial p}\right)_{\mathrm{T}}.$$
(48)

Using the equation for Gibbs free energy dG = Vdp - SdT, together with the Maxwell relations it follows that

$$\frac{\partial^2 G}{\partial p \partial T} = \frac{\partial^2 G}{\partial T \partial p},\tag{49}$$

and therefore

$$\left(\frac{\partial S}{\partial p}\right)_{T} = -\left(\frac{\partial V}{\partial T}\right)_{p}.$$
(50)

Using equations (48) and (50), together with the definition of the thermal expansion coefficient of volume V

$$\alpha_{\rm V} = \frac{1}{\rm V} \left(\frac{\partial \rm V}{\partial \rm T}\right)_{\rm p},\tag{51}$$

we get equation (46).

In figure (21) a typical result of a PPC measurement at constant temperature is shown. The first peak is the response to a pressure decrease and the second to a pressure increase. At each temperature the value received from the decompression induced peaks has changed in sign to have the same sign as the compression peak. For the evaluation of  $\alpha_V$  of the lipid phase, the contribution of the buffer was taken into account. This was done on the basis of the volume fraction of the lipid in the cell, and the temperature-dependent  $\alpha_{Vbuffer}$  of the pure buffer.



Figure 21: PPC pressure jumps with fitted baseline, taken randomly from one PPC-scan. The time between the peak and the intersection of the power curve with the baseline is called the equilibration time. Integration about the Area enclosed by the peaks and the baseline leads to the heat difference  $\Delta Q$ . The primary unit used by the set-up is a calorie (1 cal = 4.184 J)

## Adjustment of the set-up

Before each PPC scan, a specific order of temperature steps was determined. Around the main transition the step density was increased up to a step size of 0.02 °C. A SLOPECHECKTIMEOUT of 600s maximum was chosen. This determines the maximum equilibration time after proceeding the pressure jump. After 600s a new pressure jump will be proceeded. Usually the upper limit of this time period was not fully exploited because of a slop criterion. The VPViewer <sup>1</sup> proceeds to the next phase of the experiment as soon as it determines that the slope of the measured signal is less than a certain threshold slope. The used SLOPEPASS value was 0.005. Whilst executing a PPC measurement an initial pressure of about 70 psi (4.82 bar) was applied at the PPC Accessory Box. This is slightly below the safe working pressure of 80 psi [40]. The filtering period, the time between data points saved to disk, was set to 5 sec. The instrument provides different feedback modes / gain. It determines the method and magnitude of the cell-cell compensation in response to temperature differences between the sample and the reference cell. It has an influence on the response time as well as on the short term baseline noise. Because of the relatively narrow peaks in our measurements, a high gain mode was chosen unless otherwise specified.

To avoid damaging of the cells a maximum pressure of 80 psi is recommended

<sup>1</sup> The corresponding application of the MicroCal Calorimeter

### 3.2.3 *High Pressure Calorimetry*

High Pressure Calorimetry (HPC) is a custom-made extension for the standard DSC set-up. First measurements were taken as in by Holger Ebel[12] and Peter Grabitz[19]. The HPC enables one to investigate the behaviour of a sample under high pressure. Because of the restriction to maximum pressure of the DSC-VP cells at approximately 5.5 bar (80 psi), the sample has to be in a custom-made capillary. The capillary itself was tested up to 150 bar. Theoretically higher pressures are possible, depending on the initial pressure of the nitrogen bottle. Because the capillaries are custom-made each new capillary has to be tested at the highest pressure outside of the cell before insertion into the DSC.



Figure 22: Sketch of a HPC Calorimeter[12]

A nitrogen bottle with a maximal output pressure of 200 bar is connected to a pressure reducer from Messer Griesheim (Frankfurt, Germany). The chosen output pressure was slightly above 100 bar. Following that there is a high pressure valve (Nova Swiss Effretikon, Switzerland), which is connected to a flexible tube. A union cross in the middle connects the tube with a flexible connection capillary leading to the sample capillary as well as to a pressure transducer (EBM 6045, Nova Swiss) to monitor the attached pressure. It uses 4 independent pressure dependent resistors to achieve a measurement accuracy of about 0.1 bar.

In the test series of DMPC-Lidocaine the sample was reduced from 1 ml to 0.3-0.4 ml by using a centrifuge and skimming off the excess solvent. The remaining sample solution was vortexed and inserted into the capillary. After filling the sample and reference cell with buffer, the pressure capillary and an empty capillary was inserted into the reference cell and the sample cell respectively. Subsequently, the opening of the calorimeter was closed using PARAFILM® M, avoiding gratuitous evaporation, and a standard DSC scan was executed. For comparison the scans were consecutively run with applied pressure as well as a without.

Because of the unknown sample volume inside the HPC capillary, there is a certain degree of inaccuracy regarding the amount of the sample. Therefore it is advisable to do HPC measurements in comparison to a DSC measurement. This allows a rescaling of the amount of substance measured after integration of the heat curve. The pressure itself is related to the temperature, but the observed influence is of the same order as the pressure change due to leaks in the setup. In total, the observed fluctuation in pressure whilst proceeding a calorimeter scan (approx. 2 h) is usually no bigger than 0.1 bar.

## Some notes regarding the set-up rebuilding

The most important detail of the set-up is to achieve a nearly leakage free installation. Therefore it is important to clean all components before use, especially the windings of the connections. Therefore small connection parts were cleaned using a sonication bath. The high pressure valves were rinsed with ethanol (96%). According to Nova Swiss one should not clean them with water, to avoid oxidation. After cleaning the windings were carefully greased to avoid getting grease inside the set-up. After pulling the connections it was helpful to let them settle for a while (1-2 days). After tightening the connections again, one got the best result.

## Pressure dependence of the enthalpy change

The following theoretical exposition of pressure dependence follows considerations from Heimburg 1998 [23] and Ebel et al. 2001 [13].

Starting with the experimentally confirmed relation between the enthalpy change  $\Delta H$ , and the volume change  $\Delta V$ 

$$\langle \Delta V(T) \rangle = \gamma_{\nu o l} \langle \Delta H(T) \rangle, \tag{52}$$

it is assumed that this relation is true for all temperatures close to the melting transition. Calculating the mean of the volume V, and using equation (52) leads to:

$$\langle \Delta V \rangle = \frac{1}{Q} \sum_{i} \Delta V_{i} \exp\left(-\frac{\Delta H_{i}}{k_{b}T}\right)$$
(53)

$$=\frac{\gamma_{\nu ol}}{Q}\sum_{i}\Delta H_{i}exp\left(-\frac{\Delta H_{i}}{k_{b}T}\right)$$
(54)

$$= \frac{1}{Q} \sum_{i} \gamma_{\nu o l} \Delta H_{i} exp\left(-\frac{\Delta H_{i}}{k_{b}T}\right), \ 0 \leqslant T \leqslant \infty.$$
(55)

Having presupposed that  $0 \le 1/RT \le \infty$ , this is the variable of a Laplacian space set by  $exp(-H_i/k_bT)$ . Consequently the pre-factors are well defined and unique, and the relation between enthalpy and volume change is not only valid for the mean values, but also for all available substates of the system.

$$\Delta V_{i} = \gamma_{vol} \Delta H_{i} \tag{56}$$

With equation (56) a dependency relation between pressure and phase transformation can be shown. To emphasize that the equation is pressure dependent, a new index is introduced and the equation is rewritten as:

$$\Delta V_i^0 = \gamma_{\nu o l}^0 \Delta H_i^0 \tag{57}$$

Further assumptions are made so that for the internal energy and volume change of state i, it hold that

$$\Delta U_i^p = \Delta U_i^0 \tag{58}$$

$$\Delta V_i^p = \Delta V_i^0. \tag{59}$$

Thus, for a pressure change  $\Delta p$  the enthalpy changes:

$$\Delta H_i^0 = \Delta U_i^0 + p_0 \Delta V_i^0 \tag{60}$$

$$\Delta H_{i}^{p} = \Delta U_{i}^{p} + (p_{0} + \Delta p) \Delta V_{i}^{p}$$
(61)

Using equations (57), (58), and (59) it follows that

$$\Delta H_{i}^{p} = \Delta U_{i}^{0} + p_{0} \Delta V_{i}^{0} + \Delta p \gamma_{\nu o l}^{0} \Delta H_{i}^{0}$$

$$= \Delta H_{i}^{0} (1 + \Delta p \gamma_{\nu o l}^{0})$$
(62)

Taking this into account, the mean excess enthalpy with temperature T and given pressure p can be written as

$$\langle \Delta H \rangle_{\rm T}^{\rm p} = (1 + \Delta p \gamma_{\rm vol}^{\rm 0}) \frac{\sum_{\rm i} \Delta H_{\rm i}^{\rm 0} \exp\left(-(1 + \Delta p \gamma_{\rm vol}^{\rm 0}) \frac{\Delta H_{\rm i}}{k_{\rm b} T}\right)}{\sum_{\rm i} \exp\left(-(1 + \Delta p \gamma_{\rm vol}^{\rm 0}) \frac{\Delta H_{\rm i}}{k_{\rm b} T}\right)}.$$
 (63)

When the partition function is inserted again to get the next step across, we define a new temperature

$$\mathsf{T}^* \equiv \frac{\mathsf{T}}{1 + \Delta p \gamma_{\nu o 1}^{\mathfrak{p}_0}},\tag{64}$$

and insert it into equation  $(6_3)$ . As a result we get

$$\langle \Delta H \rangle_{\rm T}^{\rm p} = (1 + \Delta p \gamma_{\rm vol}^{\rm 0}) \frac{\sum_{i} \Delta H_{i}^{\rm 0} \exp\left(-\frac{\Delta H_{i}}{k_{\rm b} T^{*}}\right)}{\sum_{i} \exp\left(-\frac{\Delta H_{i}}{k_{\rm b} T^{*}}\right)},\tag{65}$$

and

$$\langle \Delta \mathbf{H} \rangle_{\mathrm{T}}^{\mathrm{p}} = (1 + \Delta p \gamma_{\mathrm{vol}}^{0}) \langle \Delta \mathbf{H} \rangle_{\mathrm{T*}}^{0}$$
(66)

respectively.

The equations (65) and (66) show that enthalpy, after a pressure change with a final state  $p_0 + \Delta p$  and temperature  $T_0$ , is related to the enthalpy at  $p_0$  and the temperature T\* by a factor of  $(1 + \Delta p \gamma_{vol}^0)$ . This enables one to predict the melting temperature alteration of a lipid if a pressure change is occurring.

Three different methods of calorimetry where used in this thesis and gave us to following results:

# 4.1 HEAT CHANGE VS VOLUME CHANGE

## 4.1.1 Hysteresis

During the DSC scans, hysteresis was observed. The measured  $c_p$ -curves showed different peak-temperatures depending on whether it was an up-scan or a down-scan. In figure (23) the up- and down-scans of a 10 mM DMPC in buffer is plotted. The peak temperature changed from 23.94 °C on the up-scan to 23.82 °C on the down-scan. The hysteresis also had an effect on the total enthalpy change. Integration of the area under the curve led to 22.4 kJ/mol for the up-scan and 19.6 kJ/mol for the down-scan.



Figure 23: c<sub>p</sub> curves (main-peak) for an up- and down-scan of 10 mM DMPC in buffer

## 4.1.2 Standard calorimetric experiment

The main aim of this thesis was to provide experimental data to compare the relation between heat capacity  $c_p$  and the thermal volume

#### RESULTS AND DISCUSSION

expansion coefficient  $\alpha_V$  in the lipid phase transition. Therefore several measurements with the DSC and the PPC set-up were performed. The results of two typical experiments is presented in figures (24) and (25). The first figure shows a classical heat curve measured with a DSC calorimeter. The measured heat capacity  $c_p$  is plotted in respect to the temperature T. The measured peak temperature for the pre-transition  $T_{peak}^{pre}$  is 13.1 °C and for the main-transition  $T_{peak}^{main}$  it is 23.94 °C. In table (2) the values were compared with the literature, and a good agreement was found.

The same sample was measured with the PPC set-up to enable us to determine the thermal expansion coefficient  $\alpha_V$ . The result is plotted in the second figure (25). As in the  $c_p$ -curve, the main peak is easy to locate. However, the pre-transition seems difficult to distinguish from the background, as we will see in figure (28).

The peak temperature  $T_{peak}^{\alpha_V}$  at 23.94 °C perfectly matches the corresponding value from the DSC measurement.

PEAK TEMPERATURES	DSC	PPC	MABREY	PAIVA
Main transition [°C]	23.94	23.94	23.9	23.7
Pre transition [°C]	13.1	-	14.2	-

Table 2: Peak temperatures of pure 10 mM DMPC in comparison to Mabrey et al 1976[36] and Paiva 2012[48]

In figure (26) and (27) the main transition is shown in detail. The latter shows a small bulk of measured  $\alpha_V$ -values at around 23.72 °C, and subsequently a bigger gap between 23.75 and 23.86 °C. This is expected to be an anomaly caused by the calorimeter, because the data point look like they are disarranged from the original, more ordered scanning plan.



Figure 24: c<sub>p</sub>-curve of a 10 mM DMPC sample, main transition.



Figure 25:  $\alpha_V$  values obtained from a PPC scan of 10 mM DMPC sample, main transition.



Figure 26: c<sub>p</sub>-curve of a 10 mM DMPC sample.



Figure 27:  $\alpha_V$  values obtained from a PPC scan of 10 mM DMPC sample.

The integration of the area under the data curves gives us the total enthalpy change of the main transition in kJ/(mol) and the total volume change in % respectively. The calculated values are 22.4 kJ/mol, and a total volume change of 2.93 %. Again, we compared the data with values found in the literature, and especially the main transition is in a good accordance (3).

TOTAL HEAT CHANGE	DSC	MABREY	PAIVA
Main transition [kJ/mol]	22.40	22.59	21.34
Pre transition [kJ/mol]	2.6	4.18	-

Table 3: Total heat change of pure 10 mM DMPC in comparison to Mabrey et al 1976[?] and Paiva 2012[48]

# Pretransition

In both test series the scan range always included the whole transition range, including the pre-transition. Unfortunately, the received signal from the PPC-setup in the case of the pre-transition was not distinguishable from the baseline, as can be seen in figure (28) which shows the pre-transition of 10 mM DMPC. To analyse the pre-transition, a higher lipid concentration could be tested. Therefore, the emphasis of this analysis will be on the main transition.



Figure 28:  $c_p$  and  $\alpha_V$  data of 10 mM DMPC around the pre-transition.

## 4.1.3 DMPC - Lidocaine

In this test series, 10 mM DMPC in buffer was measured either pure, or with 0.5, 1, 2, 3, or 4 mM lidocaine. To investigate the relation between heat capacity,  $c_p$ , and the thermal expansion coefficient,  $\alpha_V$ , DSC scans and PPC measurements were taken for each sample.

The DSC-scans were taken with a scan rate of 5  $^{\circ}$ C/h and the obtained results are shown in figure (29). Both the up- and down-scans of the six different samples are plotted against temperature. The peak temperatures are given in table (4).

conc. in mM	0.0	0.5	1.0	2.0	3.0	4.0
T <sub>peak</sub> up [°C]	23.94	23.97	23.83	23.62	23.40	23.11
T <sub>peak</sub> down [°C]	23.82	23.86	23.72	23.46	23.22	22.82
ΔΤ	0.12	0.11	0.11	0.16	0.18	0.29

Table 4: Heat capacity peak temperatures of 10 mM DMPC with different lidocaine concentrations. The table shows the values for the up- and down-scans.

As was found by Hata et al 2000, Lee 1978, Paiva 2012, and Yi 2012 [22, 33, 48, 66] the peak temperatures  $T_{peak}$  shifted towards lower temperatures with an increase in lidocaine concentration. In our measurements we found one exception, the peak temperature of 10 mM DMPC with 0.5 mM lidocaine showing the opposite behaviour as it has a slightly higher peak temperature  $T_{peak}$  than the pure DMPC sample. The temperature difference  $\Delta T$  between the peaks of the upand down-scans caused by hysteresis seems constant at low concentrations. For the measurements of 2, 3 and 4 mM the  $\Delta T$  value seems to be slightly increasing.

The obtained  $\alpha_V$  for the different samples is presented in figure (30). The individual measurements at different temperatures were taken in increasing temperature order. Regarding hysteresis, as a first approximation we expect that it acts on the up-scan. Therefore we will first compare the DSC up-scans together with the  $\alpha_V$ -values. The combined data was plotted and is presented in figure (31). The  $\alpha_V$ -values and the  $c_p$ -curves seem to be in a linear relation and it is possible to align the data using a relation factor  $\gamma = 6.43 \cdot 10^{-4}$  ml/J, with the relation from equation (43) we know

$$\gamma = \frac{\alpha_{\rm V}}{c_{\rm p}} \cdot M \cdot v_{\rm s}, \tag{67}$$

using the molar mass M = 677.933 g/mol of DMPC and the solvent partial specific volume of approx.  $v_s = 1$  ml/g [20]. The scaling

factor is the average of the maximum calculated  $\gamma_m$  values from each peak, as is presented in table (5).



Figure 29: c<sub>p</sub>-curves of 10 mM DMPC with 0, 0.5, 1, 2, 3, and 4 mM lidocaine, received from DSC measurements. Up-scans are drawn as continous lines, down-scans are dashed.



Figure 30:  $\alpha_V$  data points of 10 mM DMPC with 0, 0.5, 1, 2, 3, and 4 mM lidocaine, received from PPC measurements.



Figure 31:  $c_p$  up-scans plus  $\alpha_V$  data of DMPC-Lidocaine with 0, 0.5, 1, 2, 3, and 4 mM, received from the PPC measurements.

conc. in mM	0.0	0.5	1.0	2.0	3.0	4.0
$c_p[kJ/(mol \cdot K)]$	226.1	117.3	110.3	91.5	63.1	35.1
$\alpha_V[1/K]$	0.193	0.116	0.113	0.091	0.057	0.032
$\gamma_m[10^{-4}ml/J]$	5.83	6.72	6.97	6.78	6.2	6.19
AVERAGE $\gamma_{m}$			6.43			

Table 5: Maximum values of the DMPC and DMPC-lidocaine curves.

From the integrals we get the total enthalpy change  $\Delta H$  and the total volume change respectively. Again, for each concentration a relation  $\gamma_i$  between the two values is calculated.

conc. in mM	0.0	0.5	1.0	2.0	3.0	4.0
$\Delta H[kJ/mol]$	22.4	20.6	16.4	24.0	23.6	22.6
$\Delta V[\%]$	2.93	2.56	2.30	2.92	2.74	2.40
$\gamma_i[10^{-4} ml/J]$	8.87	8.42	9.51	8.24	7.87	7.22
AVERAGE $\gamma_i$			8.36			

Table 6: Area integration of the DMPC and DMPC-lidocaine curves leads to the total enthalpy change  $\Delta H$  and the total volume change  $\Delta V$ 

The determined average value of  $\gamma_i = 8.36 \cdot 10^{-4} \cdot \text{ml/J}$  is about 30% higher than  $\gamma_m$  obtained from the maximum peak values.

## A closer look on the correlation between the maximum values

In figure (32) the maximum values are plotted against the lidocaine concentration. Therefore the  $\alpha_V$ -value is converted into its corresponding  $c_p$ -value, using equation (67) and the calculated average  $\gamma_m$ . The graph illustrates the decrease of the maximum values caused by increasing the lidocaine concentration. The first two data points belong to the pure DMPC sample and show a bigger displacement compared to the DMPC-lidocaine samples. It is assumed that in this case the calorimeter was overmodulated, caused by a too high signal from the pressure jumps at the peak of pure DMPC. A measurement with a lower DMPC concentration could quantify this assumption.

A second graph was created to present the correlation between the maximum values. Plotting the converted  $\alpha_V$ -values against the corresponding  $c_p$ -values, one generates the correlation graph shown in figure (33). In the case of a perfect relation, one could fit the data points with a straight line with a slope of 1. In the case of the maximum experimental values one finds a slightly smaller value of about 0.89. This could indicate that the  $\gamma_m$ -factor used was about 12 % too small, but the graph also indicates a big influence of the pure DMPC peak, which was the peak with the biggest displacement.



Figure 32: Maximum values related to the lidocaine concentration. One can observe a continuous decrease of the amplitude with an increasing concentration of anaesthetic.



Figure 33: Correlation of the maximum values of the thermal expansion coefficient  $\alpha_V$ - and the heat coefficient  $c_p$ -curves. The  $\alpha_V$ -values are converted. The data points stand for the different samples with different concentrations, starting with the highest lidocaine concentration in the left-hand corner.

# Shape comparison

We analysed the peaks of the 0.5 mM lidocaine sample further by manually matching each  $\alpha_V$ -value with its in respect to temperature closest  $c_p$ -value. The  $\alpha_v$  was converted using  $\gamma = 6.43 \cdot 10^{-4}$  ml/J The resulting plot is shown in figure (34) It allows to subtract the received values from each other and leads to a plot visualizing the interference of the data. The obtained values are plotted in figure (35) as the red curve. The two peaks are caused by a small temperature shift of the converted  $\alpha_V$ -values relative to the  $c_p$ -values. The blue curve is the result one gets after the curves got aligned.

The difference between the two curves is plotted in figure (35)



Figure 34:  $c_p$ -curve and converted  $\alpha_V$ -values after matching.



Figure 35:  $\Delta c_p$  before and after the temperature shift.

#### 4.1.4 DMPC - Cholesterol

In the second test series DMPC was measured pure, and with two different cholesterol concentrations. These measurements were motivated by a request from another research group. This explains the change of the concentration unit to Mol%. The corresponding mM values for the 13 Mol% sample are 10 mM DMPC and 1.5 mM cholesterol. In the case of 25 Mol% the amount of DMPC was increased to 30 mM, together with 10 mM cholesterol. The DSC scans were proceeded with a scan rate of  $5^{\circ}$ C/h.

In figure (36) the measured  $\alpha_V$ -data and the  $c_p$ -curves of the 13Mol% sample are plotted with respect to the temperature. Again the two different y-axis are in a linear relation to each other. The used scaling factor is  $\gamma = 8.72 \cdot 10-4$  ml/J, which was in this case chosen manually. Following Mabrey 1978, at 13.3 Mol% one can observe a slight shoulder on the high-temperature side of the peak [37], and indeed also in our sample with 13 Mol% a small shoulder is recognizable, as is shown in figure (37).

Selected data of the three measurements are presented in tables (7) and (8). The measured total enthalpy change seems to be different between the pure DMPC sample and the DMPC-cholesterol samples, and a decrease of nearly 50 % can be observed. The values of the total volume change stays constant at slightly above 2%.

CONCENTRATION	0  mol%	13 MOL%	25 MOL%
$c_p[kJ/(mol \cdot K)]$	169	6.72	0.654
$\alpha_V[1/K]$	0.202	0.00888	0.00192
$\gamma_m[10^{-4}ml/J]$	8.09	9.60	19.9
AVERAGE $\gamma_{m}$			12.53

Table 7: Maximum values of the DMPC and DMPC-cholesterol.

MOLE FRACTION	o mol%	13 MOL%	25 MOL%
$\Delta H[kJ/mol]$	23.6	12.8	12.8
$\Delta V[\%]$	2.2	2.1	2.1
$\gamma_i[10^{-4} \text{ml}/J]$	6.34	11.19	11.32
AVERAGE $\gamma_i$			9.62

Table 8: Area integration of the DMPC and DMPC-Lidocaine curves.



Figure 36:  $c_p$  and  $\alpha_V$  data of 10 mM DMPC with 13 Mol% cholesterol.



Figure 37:  $c_p$  and  $\alpha_V$  data of 10 mM DMPC with 13 Mol% cholesterol. The detail shows a shoulder on the high-temperature side of the narrow peak.

In summary, all of the results from the scans are visualized in figure (38).



Figure 38:  $c_p$  and  $\alpha_V$  data of DMPC-Cholesterol 0, 13, and 25 Mol%.

## 4.2 HIGH PRESSURE CALORIMETRY

For the HPC measurements the same DMPC-Lidocaine samples as in the DSC/PPC-measurements were used. Scans of the pre- and main-transition, as well as scans through the whole transition-range, were taken. The measurements can be used to calculate the linear relation factor  $\gamma$  in an alternative way.

In figure (39) five  $c_p$ -curves of pure DMPC are plotted. Because of the unknown amounts of lipid inside the pressure capillary, the height of the curves is arbitrary. For the sake of presentation, the curve was linearly rescaled using the total heat change determined by the previously performed DSC scan. In doing so, the exact position of each peak stays constant. The applied pressure-, and the peak temperatures are presented in table (9). In the third row one can see the different starting times of each scan. It seems as if the sample of DMPC and buffer is still in a non-equilibrium, and a slow equilibration processes inside the calorimeter cell can be observed. The calculated relation factor  $\gamma_{\Delta p}$  was calculated using equation (64) describing the temperature relation. The obtained value is  $7.68 \cdot 10^{-4}$  ml/J.

The DMPC sample with 0.5 mM lidocaine in the original solution shows similar behaviour. Its data is plotted in figure (40). As in the former case, under pressure a clear shift of the phase transition was observed.

HPC DMPC MEASUREMENT	Ι	II	III	IV	v
Pressure [bar]	101.7	101.7	0.3	0.3	101.2
Peak temperature [°C]	26.32	26.42	24.13	24.17	26.63
Time difference [h]	0	2.5	6	9	21
calculated $\gamma_{\Delta p}$			7.68		

Table 9: HPC data of DMPC 10 mMol.

HPC DMPC MEASUREMENT	I	II	III	IV
Pressure [bar]	101.7	0.3	0.3	101.1
Peak temperature [°C]	26.34	23.99	24.01	26.44
Time difference [h]	0	4.5	7	20
calculated $\gamma_{\Delta p}$			7.94	

Table 10: HPC data of DMPC 10 mMol.



Figure 39:  $c_p$  curves of pure DMPC 10 mMol. The left-hand peaks are taken at ambient pressure (1bar), whereas on the right-hand side the capillary is set under pressure (approx. 100 bar). The scans were taken in a certain time as order specified in the legend. If the curves are considered in the correct time order a slow equilibration process can be observed. The main peak shifts slowly at about 0.3 °C



Figure 40: c<sub>p</sub> curves of pure DMPC 10 mMol with 5% Lidocaine. The lefthand peaks were taken at ambient pressure (1bar), whereas on the right-hand side the capillary was set under pressure (approx. 100 bar). The scans were taken in a certain time order as specified in the legend.

## 4.3 ADDENDUM - DMPC SAMPLES

The samples used in the DMPC-lidocaine series, which were discussed in detail in section (4.1.3), were measured two times with a certain time delay. After a first DSC measurement by Henrike Sasse-Middelhoff, the samples were frozen for a time period of about 90 days. The influence of the storage is shown in figure (41).

conc. in mM	0.5	1.0	2.0	3.0	4.0
T <sub>peak</sub> Sasse-M. [°C]	23.87	23.78	23.51	23.31	22.79
T <sub>peak</sub> Thesis [°C]	23.97	23.83	23.62	23.40	23.11
$\Delta T [°C]$	0.10	0.05	0.11	0.11	0.32

Table 11: Heat capacity peak temperatures of 10 mM DMPC with different lidocaine concentrations. Two time separated measurements (approx. 90 days) of the same sample.

The  $c_p$ -curves of the second measurement are obviously all shifted towards a higher peak temperature. The values are presented in table (11) and plotted in figure (42). If one compares the maximum peak-values, one gets a different result. In this case, the values of the measurements, taken 90 days later, showed a diverse behaviour. The maximum  $c_p$ -values of the two measurements taken with 0.5 and 1.0



Figure 41: c<sub>p</sub>-curves of five different 10 mM DMPC in buffer samples with 0.5, 1.0, 2.0, 3.0, and 4.0 mM lidocaine. The measurements were taken with a time difference of approx. 90 days.

mM concentrations were lower after 90 days whereas, by contrast, at the three higher concentrations a greater maximum value than before was recorded.



Figure 42: Measured peak temperatures  $T_{peak}$  from the same DMPC sample, before and after 90 days.



Figure 43: Measured maximum  $c_p$ -values from the same DMPC sample, before and after 90 days.

conc. in mM	0.5	1.0	2.0	3.0	4.0
Sasse-M. $c_p[kJ/(mol \cdot K)]$	188.4	135.2	67.7	41.6	18.0
Thesis $c_p[kJ/(mol \cdot K)]$	117.3	110.3	91.5	63.1	35.1
$\Delta c_p c_p [kJ/(mol \cdot K)]$	-71.1	-24.9	23.8	21.5	17.1

Table 12: Maximum c - p-values of two time separated (approx. 90 days) DMPC-lidocaine measurements.

## 5.0.1 DMPC in non-equilibrium

The experiments carried out in this thesis demonstrate in several ways, that DMPC, and DMPC-mixtures with cholesterol or lidocaine, show an equilibration time longer than expected. Firstly, in the results from the two time separated DSC measurements of DMPC-lidocaine samples, the peak temperatures showed a uniform shift towards higher temperatures after a longer equilibration time. This is also a plausible explanation as to why the peak transition temperature of pure DMPC peak was located between the DMPC sample with the 1.0 mM and 0.5 mM lidocaine.

Furthermore, the DMPC-cholesterol measurement with 13 Mol% showed the expected shoulder on the high-temperature side of the main-peak. At a slightly higher concentration the shoulder should increase to an independent peak, following Mabrey et al. 1978. It seems obvious that in this case DMPC and cholesterol arrange themselves in at least two different states or aggregates.

In the HPC experiments a time dependence was also observed. It seems as if the high lipid concentration within the capillary used, and the small reaction volume, is perhaps reason enough to show a fast shift of the transition peak temperature. Finally one could state that the observed equilibration process of the DMPC-lidocaine samples seems to be reversible, again taking the in average lower peak temperatures of the DMPC-lidocaine sample compared to the pure sample in the HPC measurements as a basis for decision.

# 5.0.2 Relation between $c_p$ and $\alpha_V$

The main objective of this thesis determined the relation between heat capacity  $c_p$  and the thermal expansion coefficient  $\alpha_V$ . Despite some minor inconsistencies, such as the perceptible shift of the heat capacity curve and the thermal expansion coefficient curve of the DMPC samples with 3.0, 4.0 mM lidocaine, respectively, strong evidence for a linear relation was found.

In favour for a linear relation is also the good correlation between the maximum values of the  $c_p$  curves with the converted  $\alpha_V$ -values as shown in figure (33). The correlation even seems valid for small details as seen in the 13 Mol% DMPC-cholesterol measurement and the shoulder on the right-hand side of the main-peak. A striking argument in favour of a linear relation is the interference of the curve shapes as it is visualized in figure (44)



Figure 44: Shape comparison.  $c_p$ -curves and  $\alpha_V$ -values plotted relative to the peak temperature of the pure DMPC sample.

### 5.1 OUTLOOK

Measuring the relation between the heat capacity  $c_p$  and the thermal expansion coefficient  $\alpha_V$  was an essential task to underline the importance and use of thermodynamic models for biological systems. From the results showed in this thesis several new questions appeared.

It was for example not possible to determine the pre-transition for  $\alpha_V$ -values. It would be essential to measure samples with a higher concentration to examine if the linear relation is also true for the pre-transition.

Another interesting question could be to further investigate the equilibration behaviour of biomembranes. It would be possible by analysing the equilibration time of the measured power-curves of the PPC setup.

Our results may indicate that long equilibration times are needed in order to re-arrange the lipid-lidocaine system. Hence a time dependence investigation could be interesting to eludicate the equilibration dynamics.
Part III

APPENDIX

The following to sections describing the evaluation of the PPC data in detail. They are both from the MicroCal Tutorial Guide - PPC Data Analysis in Origin [39].

EXTENSION TO TWO COMPONENT SOLUTIONS

When a solution is formed by dissolving  $g_S$  grams of solute in  $g_0$  grams of a solvent, the total volume  $V_{total}$  of the resulting solution may be expressed as

$$V_{total} = g_0 V_0 + g_S \overline{V_S}$$
(68)

where  $V_0$  is the specific volume of pure solvent, and  $\overline{V_S}$  is the partial specific volume of the solute in the solution. The partial specific volume  $\overline{V_S}$  includes not just the intrinsic volume of the solute but also any volume changes induced in the solvent as the result of interactions with the solute.

Differentiating eq(68) with respect to temperature at constant pressure gives

$$\left(\frac{\partial V_{\text{total}}}{\partial T}\right)_{P} = g_{0} \left(\frac{\partial V_{0}}{\partial T}\right)_{P} + g_{S} \left(\frac{\partial \overline{V_{S}}}{\partial T}\right)_{P}$$
(69)

From equation (48) and (50) we know that

$$\left(\frac{\partial Q_{rev}}{\partial p}\right)_{T} = -T \left(\frac{\partial V}{\partial T}\right)_{p}.$$
(70)

After substituting the right hand side of eq(69) into eq(70), we obtain

$$\left(\frac{\partial Q_{rev}}{\partial P}\right)_{T} = -T \left[g_{0} \left(\frac{\partial V_{0}}{\partial T}\right)_{P} + g_{S} \left(\frac{\partial \overline{V_{S}}}{\partial T}\right)_{P}\right]$$
(71)

Multiplying and dividing the first term in brackets by  $V_0$  and multiplying and dividing the second term in brackets by  $V_S$  gives

$$\left(\frac{\partial Q_{re\nu}}{\partial P}\right)_{T} = -T \left[g_0 V_0 \alpha_0 + g_S \overline{V_S} \alpha_S\right]$$
(72)

According to eq(72), the heat arising from pressure perturbation of a solution can be viewed as the sum of that arising from the perturbation of the solvent and from perturbation of the solute in the solution.

Integration of eq(72) over a short pressure range leads to eq(73).

$$\delta Q_{rev} = -T \left[ g_0 V_0 \alpha_0 + g_S \overline{V_S} \alpha_S \right] \Delta P \tag{73}$$

In a differential experiment, sample solution in the reference cell and buffer in the sample cell, when both cells are subjected to the same  $\Delta P$  then the heat  $\Delta Q$  will be equal to the difference between eq(73) for the sample cell and for the reference cell. Assuming the two cells to be of identical volume, then the difference arises because the volume occupied by the solute in the sample cell,  $g_S \overline{V_S}$ , is occupied by solvent in the sample cell. i.e.

$$\Delta Q_{rev} = -T\Delta P \left[ g_S \overline{V_S} \alpha_S - g_S \overline{V_S} \alpha_0 \right]$$
(74)

which rearranges to eq(75).

$$\alpha_{\rm S} = \alpha_0 - \frac{\Delta Q_{\rm rev}}{T \Delta P g_{\rm S} \overline{V_{\rm S}}} \tag{75}$$

## CALCULATIONS IN ORIGIN

Four data sets ( $\Delta Q$  as a function of temperature t) must be read into the Origin project, each obtained at the same  $\Delta P$  value. These are the sample vs buffer (S/B), buffer vs buffer (B/B), buffer vs water (B/W), and water vs water (W/W). The three data sets B/B, B/W, and W/W will generally have small heats, and Origin fits each of these to a four order polynomial

$$\Delta Q_{W/W}(t) = a_{W/W} + b_{W/W} t + c_{W/W} t^2 + d_{W/W} t^3 + e_{W/W} t^4$$
(76)

The S/B data set is stored in a worksheet with the temperature (°C) in col(a) and the differential heats  $\Delta Q$  (µcal) in col(b). Before calculation of  $\alpha$  for the solute, the operator is given the chance to enter (or edit) four parameters, including solute concentration g (g/ml), pressure change  $\Delta P$  (psi), partial specific Volume  $\overline{V_S}$  (ml/g), and cell volume  $V_{tot}$ (ml).

The following worksheet calculations are then carried out by Origin:

- 1) col(c) = [S/B-B/B] = col(b) B/B
- 2) col(d) = [B/W-W/W]
- 3)  $col(e) = -T \alpha_{H_2O} = FIT$

 $FIT = 0,01821 - 0,00476 \cdot \text{col}(a) + 5,25126 \cdot 10^{-5} \cdot \text{col}(a)^2 - 6,66512 \cdot 10^{-7} \text{col}(a)^3 + 4,45806 \cdot 10^{-9} \cdot \text{col}(a)^4 - 1,265 \cdot 10^{-11} \cdot \text{col}(a)^5$ 

The equation on the right is a six-term power series in t (°C), previously obtained by fitting literature data for  $-T\alpha$  for pure water, where T is temperature in K. When  $-T\alpha_{H_2O}$  is multiplied by the volume (liters) of water times the pressure change  $\Delta P$  (atm) times the conversion factor 24, 2 · 10<sup>6</sup>, it gives the heat change in µcal

col(f) is the heat released by the extra buffer / water in the reference cell 4)  $\operatorname{col}(f) = g \cdot \overline{V_S} 10^{-3} \cdot (\operatorname{col}(d) + \operatorname{col}(e) \cdot V_{tot} 10^{-3} \cdot (\Delta P/14, 7) \cdot 24, 2 \cdot 10^6)$ 

Col(d) is the extra heat released by buffer in  $V_{tot}$  over and above that released by pure water in the same volume, while  $col(e) \cdot V_{tot} 10^{-3} \cdot (\Delta P/14, 7) \cdot 24, 2 \cdot 10^6$  is the heat which would be released by pure water in  $V_{tot}$ . The quantity in parenthesis is then the total heat released by buffer in  $V_{tot}$ . Multiplication by  $g \cdot \overline{V_S} 10^{-3}$  then gives the heat released by the excess volume of buffer in the reference cell.

5)  $\operatorname{col}(g) = \operatorname{col}(c) + \operatorname{col}(f)$ 

After adding back the heat released by the extra buffer in the reference cell, col(g) becomes the total heat released by the solute molecules in the sample cell

6)  $\operatorname{col}(h) = \alpha_{S} = \Delta Q / (-T\overline{V_{S}}10^{-3}\Delta P) = -\operatorname{col}(g) / ((273, 2 + \operatorname{col}(a)) \cdot g \cdot \overline{V_{S}}10^{-3} \cdot (\Delta P / 14, 7) \cdot 24, 2 \cdot 10^{6})$ 

The thermal coefficient of expansion of the solute partial volume is calculated in col(h), according to  $Q_{rev} = -TV\alpha_V \Delta P$  (compare to equation ??).

Finally, col(h) is plotted vs col(a) as a scatter graph with the y axis labeled Coefficient of Thermal Expansion (deg<sup>-1</sup>) and the x axis labeled Temperature (°C).

Note. There are three temperature-dependent parameters in the above equations, which are treated as temperature-independent in the calculations.  $V_{tot}$  is only marginally dependent on temperature, since the coefficient of cubic expansion of tantalum is only  $2 \cdot 10^{-5} \text{deg}^{-1}$ . The solute concentration g (g/ml) decreases with increasing temperature as the solvent expands, while the solute partial volume  $\overline{V_S}$  (ml/g) increases with increasing temperature according to the value of its thermal coefficient of expansion. These latter two factors work in opposite directions and tend to cancel. For proteins in aqueous solvents, the effect of neglecting these temperature dependencies introduces errors in  $\alpha$  of ca. 1 % over the temperature range o to 100°C which is well within experimental errors.

## B

## HIGH PRESSURE CALORIMETRY PICTURE



Figure 45



Figure 46



Figure 47: Capillary

- [1] Diagram of an animal cell. URL http://www. b2b.invitrogen.com/site/us/en/home/References/ Molecular-Probes-The-Handbook/Probes-for-Organelles/ A-Diverse-Selection-of-Organelle-Probes.html.
- [2] Cholesterol. URL http://en.wikipedia.org/wiki/ Cholesterol.
- [3] Virtual chembook, elmhurst college, o2. 2014. URL http://www. elmhurst.edu/~chm/vchembook/551fattyacids.html.
- [4] Iupac. compendium of chemical terminology, 2nd ed. (the "gold book"). compiled by a. d. mcnaught and a. wilkinson. blackwell scientific publications, oxford (1997). xml on-line corrected version: http://goldbook.iupac.org (2006-) created by m. nic, j. jirat, b. kosata; updates compiled by a. jenkins. isbn 0-9678550-9-8. doi:10.1351/goldbook., 02. 2014. URL http://goldbook.iupac.org/F02330.html.
- [5] Hyperphysics, 02. 2014. URL http://hyperphysics.phy-astr. gsu.edu/hbase/organic/fataci.html.
- [6] Lipidbook, 02. 2014. URL http://lipidbook.bioch.ox.ac.uk.
- [7] Phospholipids aqueous solution structure, o2. 2014. URL http://en.wikipedia.org/wiki/File:Phospholipids\_ aqueous\_solution\_structures.svg.
- [8] R. H. Adrian and W. Almers. The voltage dependence of membrane capacity. J. Physiol., 254:317–338, 1976.
- [9] F.H. Anthony, R. L. Biltonen, and E. Freire. Modification of a vibrating-tube density meter for precise temperature scanning. *Anal. Biochemistry*, 116:161–167, 1981.
- [10] G. Ekin Atilla-Gokcumen, Eleonora Muro, Josep Relat-Goberna, Sofia Sasse, Anne Bedigian, Margaret L. Coughlin, Sergi Garcia-Manyes, and Ulrike S. Eggert. Dividing cells regulate their lipid composition and localization. *Cell*, 156:428–439, 2014.
- [11] Andreas Blicher. *Electrical aspects of lipid membranes*. PhD thesis, University of Copenhagen, 2011.
- [12] Holger Ebel. Kalorische und strukturelle umwandlungen von lipidmembranen: Kopplung von wärmekapazität und volumenexpansionskoeffizienten. Master's thesis, Physics Dept., Univ. Göttingen, 1999.

- [13] Holger Ebel, Peter Grabitz, and Thomas Heimburg. Enthalpy and volume changes in lipid membranes. i. the proportionality of heat and volume changes in the lipid melting transition and its implication for the elastic constants. *J. Phys. Chem.*, 105:7353– 7360, 2001.
- [14] Danielli J. F. and Davson H. A contribution to the theory of permeability of thin films. *Journal of Cellular and Comparative Physi*ology, 5 (4):495, 1935.
- [15] H. Fricke. The electrical capacity of suspensions with special reference to blood. *Journal of General Physiology*, 9:137–152, 1925.
- [16] R. B. Gennis. *Biomembranes Molecular Structure and Function*. Springer, 1989.
- [17] Alfredo Gonzalez-Perez, Rima Budvytyte, Lars D. Mosgaard, Marius T. Stauning, Søren Nissen, and Thomas Heimburg. Penetration of action potentials during collision in the medial giant axon of the earthworm. *arXiv.org*, 2014.
- [18] E. Gorter and F. Grendel. On bimolecular layers of lipids on the chromocytes of the blood. *Journal of Experimental Medicine*, 41: 439–443, 1925.
- [19] Peter Grabitz. Dynamische vorgänge in phospholipidmembranen. relaxationsprozesse und viskoelastizität in der nähe der schmelzumwandlung. Master's thesis, Physics Dept., Univ. Göttingen, 2001.
- [20] Alexander I. Greenwood, Stephanie Tristram-Nagle, and John F. Nagle. Partial molecular volumes of lipids and cholesterol. *Chem Phys Lipids*, 143(1-2):1–10, 2006.
- [21] S. Halstenberg, T. Heimburg, T. Hianik, U. Kaatze, and R. Krivanek. Cholesterol-induced variations in the volume and enthalpy fluctuations of lipid bilayers. *Biophysical Journal*, 75:264– 271, 1998.
- [22] Takashi Hata, Hitoshi Matsuki, and Shoji Kaneshina. Effect of local anesthetics on the bilayer membrane of dipalmitoylphosphatidylcholine: interdigitation of lipid bilayer and vesicle]micelle transition. *Biophysical Chemistry*, 87:25–36, 2000.
- [23] Thomas Heimburg. Mechanical aspects of membrane thermodynamics. estimation of the mechanical properties of lipid membranes close to the chain melting transition from calorimetry. *Biochimica et Biophysica Acta*, 1415:147–162, 1998.
- [24] Thomas Heimburg and Andrew D. Jackson. On soliton propagation in biomembranes and nerves. *PNAS*, 102:9790–9795, 2005.

- [25] Thomas Heimburg and Andrew D. Jackson. On the action potential as a propagating density pulse and the role of anesthetics. *Biophys. Rev. Lett.*, 2:57–78, 2007.
- [26] Terrell L. Hill. *An Introduction to Statistical Thermodynamics*. Dover Publications, 1960.
- [27] A. L. Hodgkin and A. F. Huxley. Resting and action ppotential in single nerve fibres. J. Physiol., 104:176–195, 1945.
- [28] J. V. Howarth, J.M. Ritchie, F.R.S., and D. Stagg. The initial heat production in garfish olfactory nerve fibres. *Proc. R. Soc. Lond.*, B 205:347–367, 1979.
- [29] Jacob Israelachvili. The science and application of emulsions – an overwiew. *Physicochemical and Engineering Aspects*, 91:1–8, 1994.
- [30] Kunihiko Iwasa, Ichiji Tasaki, and Robert C. Gibbons. Swelling of nerve fibers associated with action potentials. *Science*, 210: 338–339, 1980.
- [31] L.D. Landau and E. M. Lifshitz. *Fluid Mechanics*. Pergamon Books Ltd., 1987.
- [32] B. Lautrup, R. Appali, A.D. Jackson, and T. Heimburg. The stability of solitions in biomembranes and nerves. *The European Physical Journal E*, 34:57, 2011.
- [33] A.G. Lee. Effects of charged drugs on the phase transition ttemperature of phospholipid bilayers. *Biochimica et Biophysica Acta*, 514:95–104, 1978.
- [34] Christian T. Leirer. *Dynamik und Struktur in der Phasenkoexistenz* von Lipidmembranen. PhD thesis, Universität Augsburg, 2008.
- [35] E.M. Lifschitz and L.P. Pitajewski. Lehrbuch der Theoretische Physik
   Statistische Physik Teil 1, volume V of 8., berichtigte Auflage. Akademie - Verlag Berlin, 1979/1987.
- [36] Susan Mabrey and Julian M. Sturtevant. Investigation of phase transition of lipids and lipid mixtures by high sensitivity differential scanning calorimetry. *Proc. Natl. Acad. Sci. USA*, 73:3862– 3866, 1976.
- [37] Susan Mabrey, Pedro I. Mateo, and Julian M. Sturtevant. Highsensitivity scanning calorimetric study of mixtures of cholesterol with dirnyristoyl- and dipalmitoylphosphatidylcholines. *Biochemistry*, 78:2464–2468, 1978.
- [38] H. Meyer. Arch. Exp. Pathol. Pharmacol., 42:109–118, 1899.

- [39] PPC Data Analysis in Origin Tutorial Guide. Micro Cal, .
- [40] Pressure Perturbation Calorimetry (PPC) User's Manual. MicroCal, LLC, .
- [41] O. G. Mouritsen and M. Bloom. Mattress model of lipid-protein interactions in membranes. *Biophysical Journal*, 46 (2):141–153, 1984.
- [42] Prof. Ole G. Mouritsen. Life As a matter of fat. Springer, 2005.
- [43] P. Mueller, D. O. Rudin, H. I. Tien, and W.C: Wescott. Reconstruction of cell membrane structure in vitro and its transformation into an excitable system. *Nature*, 194:979–980, 1962.
- [44] J.F. Nagle, R. Zhang, S. Tristram-Nagle, W. Sun, H.I. Petrache, and R.M. Suter. X-ray structure determination of fully hydrated l alpha phase dipalmitoylphosphatidylcholine bilayers. *Biophysical Journal*, 70(3):1419–1431, 1996.
- [45] C. Nägeli and C.Cramer. Pflanzenphysiologische untersuchungen. Shulthess Zürich, 1855.
- [46] E. Overton. Vieteljahrschr. Naturforsch. Ges. Zürich, 44:88–114.
- [47] E. Overton. Studien über die narkoze, zugleich ein beitrag zur allgemainen pharmakologie. *Gustav Fisher Jena*, 1855.
- [48] José Gabriel Paiva, Patrizia Paradisoa, Ana Paula Serroa, Anabela Fernandesa, and Benilde Saramagoa. Interaction of local and gerneral anaesthetics with liposomal membrane mmodel: A qcm-d and dsc study. *Biointerfaces*, 95:65–74, 2012.
- [49] W. Pfeffer. Osmotische untersuchungen. W. Engelmann Zuerich, 1877.
- [50] W. Pfeffer. Unters. a. d. Bot. Inst. Tübingen, 2:179–331, 1886.
- [51] G. Quincke. Ann. Phys. (Leipzig), 35:580-642, 1888.
- [52] J. D. Robertson. The ultrastructure of cell membranes and their derivatives. *Biochemical Society Symposia*, 16:3–43, 1959.
- [53] J. D. Robertson. The molecular structure and contact relationships of cell membranes. *Progress Biophysics and Biophysical Chemistry*, 10:343–418, 1960.
- [54] O'neill Michael J & Watson Emmett S. Differential microcalorimeter, 1966.
- [55] W. Schrader, H. Ebel, P. Grabitz, E. Hanke, T. Heimburg, M. Hoeckel, M. Kahle, F. Wente, and U. Kaatze. Compressibility of lipid mixtures studied by calorimetry and ultrasonic velocity measurements. *J. Phys. Chem.*, 106:6581–6586, 2002.

- [56] K. Simons and E. Ikonen. Fufunction rafts in cell membranes. *Nature*, 387(6633):569–572, 1997.
- [57] S. J. Singer and G. L. Nicolson. The fluid mosaic model of the structure of cell membranes. *Science*, 175:720–731, 1972.
- [58] A. Sommerfeld. Vorlesungen über Theoretische Physik, Bd.2, Mechanik der derformierbaren Medien. Deutsch Harri, 1969 (Nachdruck 1992).
- [59] W.J. Sun, S. Tristram-Nagle, R.M. Suter, and J.F. Nagle. Structure of gel phase saturated lecithin bilayers: temperature and chain length dependence. *Biophysical Journal*, 71(2):885–891, 1996.
- [60] et al. Sávia Caldeira de Araújo Lopes. Cancer Treatment Conventional and Innovative Approaches. Number DOI: 10.5772/55290.
   2013.
- [61] H. T. Tien and A. L. Diana. Some physical properties of biomolecular lipid membranes produced from new lipid solutions. *Nature*, 215:1199–1200, 1967.
- [62] H. T. Tien, S. Carbone, and E. A. Dawidowicz. Formation of "black" lipid membranes by oxidation products of cholesterol. *Nature*, 212:718–719, 1966.
- [63] Kaku Uehara, Hideo Akutsu, Yoshimasa Kyogoku, and Yuzuru Akamatsu. Phase transition of phospholipid bilayers from an unsaturated fatty acid auxotroph of escherichia coli. *Biochimica et Biophysica Acta*, 466:393–401, 1977.
- [64] H. Vries de. Arch.Neerl.Sci.Exactes Nat., 6:117–126, 1871.
- [65] H. Vries de. Jahrb.Wiss.Bot., 14:429-601, 1884.
- [66] Zheng Yi, Michihiro Nagao, and Dobrin P. Bossev. Effect of charged lidocaine on static and dynamic properties of model bio-membranes. *Biophysical Chemistry*, 160:20–27, 2012.
- [67] Tanfiuji Z, Eger EI, and Terrell RC. Some characteristics of an exceptionally potent inhaled anesthetic: thiomethoxyflurane. *Anesth Analg.*, 56:387, 1977.

## COLOPHON

This document was typeset using the typographical look-and-feel classicthesis developed by André Miede. The style was inspired by Robert Bringhurst's seminal book on typography "*The Elements of Typographic Style*". classicthesis is available for both LATEX and LYX:

http://code.google.com/p/classicthesis/

Happy users of classicthesis usually send a real postcard to the author, a collection of postcards received so far is featured here:

http://postcards.miede.de/

*Final Version* as of April 22, 2014 (classicthesis version 1.0).