How to escape from a tense situation
Folgering, Jozef Hendrik Arnold

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Channel electrophysiology: history, current applications and future prospects

Joost H.A. Folgering and Bert Poolman

Abbreviations: BLM: Black-lipid Membrane; GUV: Giant Unilamellar Vesicle; MS: Mechanosensitive; GOF: Gain of Function; LOF: Loss of Function; MTSET: [2-(trimethylammonium)ethyl]methanethiosulfonate; TM: Trans Membrane helix

Electrophysiology is the science concerned with the flow of ions in biological tissues and, in particular, the electrical recording techniques that enable the measurement of this flow (1). Ion channels are present in the membranes that surround all biological cells. By conducting and controlling the flow of ions, these pore-forming proteins influence the voltage (100-150mV) across the membrane of cells and they play an important role in the generation of action potentials in nerve cells. If one realizes that in the human genome around 350 genes code for channel proteins (2), it can be appreciated, that as a field of research, electrophysiology is very important. Thus far, around 40 hereditary channelopathies have been described and are currently the subject of intense research. These channelopathies can be divided into three main groups: muscular diseases (e.g., heart disease and muscular dystrophy), neuronal diseases (e.g., epilepsy and some forms of blindness) and internal diseases (e.g., cystic fibrosis and renal disorders); a more detailed description of hereditary channelopathies and the channels involved can be found in references 3 and 4. To fully appreciate current research in electrophysiology, two landmarks need to be discussed. The first is the development of the field of bioelectricity, the second the concept of the membrane bilayer. This introduction does not cover the complete field of electrophysiology, but indicates highlights that have directly or indirectly led to the work described in the following chapters.

History of bio-electricity

The first persons to study bio-electricity were Luigi Galvani and Alessandro Volta in the late 18th century. Galvani prepared frog legs, with their muscles and nerves exposed, and connected them to different sources of electricity (5, 6). The electrical stimuli resulted in twitching of the legs. These observations led to a discussion between Galvani and Volta on the nature of the stimulus that resulted in the activation of the leg muscles. Galvani thought the stimulation showed that a form of what he called "animal electricity" existed. We now understand the "animal electricity" as ion-fluxes across cell membranes. However, as the twitching could also be observed when a purely physical stimulus was applied, Volta was sure that it was caused by the electricity stemming from the touching of two different substances (metal touching tissue, 7). A simple experiment, where two nerves of separately prepared legs from the same frog were brought into contact and resulted in
muscle contraction showed that Volta's interpretation was incorrect (8 and Fig. 1). However, because of Volta's success with electricity generated from contact between dissimilar objects (in the so called "voltaic pile"), the experiments of Galvani, and their consequences, went unnoticed at that time. (9, 10)

During the early part of the 19th century, Galvani's experiments were often repeated but not interpreted correctly. After identifying the organ responsible for electrical discharges in electrical fish, Carlo Matteucci had turned his attention to the potentials that are responsible for muscle contraction. In 1838, he first reported his findings on muscle contractions (11). He showed that in rest there was a potential difference between the damaged and the intact surface of a severed muscle (i.e., the inside and the outside of a muscle cell). This potential was large enough to create a measurable current (12). He was the first to demonstrate that it is possible to induce muscle contraction by means of an action potential, and he showed that this action potential is associated with the depolarization of the muscle resting potential (13, 14). However, it was Emil Heinrich du Bois-Reymond, under the guidance of Alexander von Humboldt, who finally interpreted the membrane potential experiments correctly and refined the methods to record muscle currents from frogs and even humans (15-17). The last person that needs to be mentioned from the early days of bio-electricity is Hermann von Helmholz, who was able to determine the propagation speed of an electrical signal along the nerve cell. He found that nerve signals had a speed of "only" 10 - 100 meters per second. This is a lot slower than the speed of light, previously assumed to be the speed at which nerve impulses traveled (18, 19). The contribution of von Helmholz to the field of bio-electricity did not stop there; he also developed a number of instruments that made more accurate measurements of currents possible (9).
Membrane bilayers

The history of lipid bilayers starts half-way through the 19th century, when three botanists, Karl von Nägeli, Karl Cramer and Wilhelm Pfeffer, realized that when plant cells were submerged in hypotonic solutions they would expand, whereas in hypertonic solutions they would shrink (20, 21). This observation led them to conclude that the cells were surrounded with some sort of membranous envelope. At the end of the 19th century, Ernest Overton had classified over 500 chemical compounds for their solubility in oil and in water and their ability to diffuse over the cell membrane (22-25). From this work, he concluded that the postulated membrane consisted of lipoid material and was semi-permeable. Inspired by the work of du Bois-Reymond, Overton subsequently studied the effect of extracellular sodium ion concentrations on nerve excitation and found that sodium ions were exchanged for internal potassium ions upon excitation of muscle cells (26, 27). Another scientist who contributed to the initial ideas on biological membranes, before actual models were proposed, was Alexander Langmuir (28). He was the first to quantitatively investigate the self-assembly of mono- and bi-layers on air-water interfaces (27-31).

The first real membrane model was proposed by Evert Gorter and F. Grendel in Utrecht in 1925 (32). They observed that lipids extracted from a single human red blood cell could cover an area twice the size of the cell surface area in a monolayer formation experiment. This indicated that the organization of the lipids in the membrane had to be that of a lipid bilayer (Fig. 2A). After studying membranes with an electron microscope, Hugh Davson, James Danielli and James David Robertson realized that this model was incomplete, as they found particles (proteins) associated with the membrane bilayer. They proposed a membrane model with a bilayer lipid core and a layer of proteins on either side of this so called unit-membrane (27, 29-31, 33, 34 and Fig. 2B).

The unit-membrane model was replaced in the early 1970s by a model from the biochemists Jonathan Singer and Garth Nicolson, known as the fluid mosaic model (35). This model (Fig. 2C) retains the basic lipid bilayer structure, first proposed by Gorter and Grendel and modified by Danielli and Davson and Robertson. The proteins, however, are thought to be globular and to float within the lipid bilayer rather than just on the membrane surface as in the sandwich-type model (27, 29-31). Owing to modern molecular dynamics and spectroscopic techniques, it is now possible to describe lipid bilayers at an even more detailed level. Molecular dynamics and spectroscopic studies have shown that the lipid water interface is not a solid barrier, but that water can penetrate the bilayer, and crystallographic studies have shown that proteins do not necessarily form globular structures within the membrane (Fig. 2D).
Chapter 1

Modern electrophysiology

In the 1930s, John Zachary Young discovered how to prepare squid giant neurons (36). The size of these axons made it very easy to apply external electrodes and to follow the depolarization of the membrane potential along the neurons. His work demonstrated that the membranes of the neurons became selectively permeable to sodium during depolarization. This was the first indication of the presence of ion-selective membrane channels.

Figure 2: Four different membrane models as proposed by A) Gorter and Grendel; B) Davson, Danielli and Robertson; C) Singer and Nicolson; and D) a molecular dynamics simulation of a lipid bilayer. Clearly the components (lipids, proteins and water) are not present in an ordered form as proposed in the Singer and Nicolson model. A membrane protein (MscL) is depicted by the light grey α-helices and linker regions, part of the lipid headgroups are shown in darker grey, and the hydrophobic lipid acyl chains are shown in the darkest grey. The image was kindly provided by Alex H. de Vries.
Around the same time, Alan Hodgkin and Andrew Huxley succeeded in inserting electrodes into these giant neurons and so in measuring the membrane potential in rest and during excitation (37). An even greater breakthrough came when they applied to these membranes a method devised by Kenneth Cole, which is now known as the voltage clamp (38). With this method, they could set a voltage across the neuronal membrane and then measure ion currents (9).

However, these ion currents were the result of fluxes through an ensemble of membrane channels. It took until the early 1960s before ion fluxes through single pores or channels were measured. By using a fine brush, Paul Müller and Donald Rudin painted planar lipid bilayer membranes over a small aperture in a septum between two chambers filled with buffer (39, 40 and Fig. 3). After formation of these so-called black-lipid membranes (BLM, they have an optically black appearance under a microscope), channels could be inserted in two ways. Firstly, channels were solubilized from a membrane fraction and added to one of the chambers. Through diffusion the channels would reach the bilayer and insert, albeit with a very low efficiency. A second method involves the use of proteoliposomes, which can be prepared by, for instance, by detergent-mediated reconstitution (41). Through fusion of these proteoliposomes with the stabilized BLM, the channel can be inserted. All current applications of the BLM involve the same basic technique, but are often refined to counteract the disadvantages of the first generation of the BLM setup, that are: the small available bandwidth of sampling and filtering frequencies and the low signal to noise ratio (e.g. 42). Both these disadvantages are caused by the relatively large lipid area used in the system, which acts as a large antenna. Another disadvantage of the system is that the formed bilayer is usually stable for only 2-3h. The main advantages of BLMs are the ease with which buffer on either side of the membrane can be replaced and the fact that it can be used in combination with a large variety of lipids.

Finally, one remarkable recent application of BLMs needs to be mentioned. Generally ion pumps have a turnover-rate between 1 and 100s⁻¹, whereas the ion flow through a channel is in the order of $10^5 – 10^6$s⁻¹. This implies that one would need to reconstitute $10^4-10^5$ of these pumps in a single membrane to measure a current equivalent to that of a single channel. However, if one uses the BLM membrane as a sensitive capacitive electrode, it is possible to sense changes in charges inside an adsorbed proteoliposome containing ion pumps. As the charge needs to be equal on both sides of the "capacitive electrode", an opposing charge needs to be added for any ion transported into the adsorbed proteoliposome. The new charge distribution can be measured by charging and discharging the capacitor. The additional number of ions present in the proteoliposome determines the change in the time-constant for discharge and can be calculated from the time-constant. As the time between two charge and discharge events is known, the turnover number of the transporter can be determined. An added advantage is that any required substrate (e.g., ATP) can be added to the inside of the
proteoliposomes before the vesicles are added to the chamber in the BLM set-up. This method has been applied by Ernst Bamberg and colleagues to bacteriorhodopsin and a number of ATP dependent ion pumps (Na⁺/K⁺-ATPase and H⁺/K⁺-ATPase) as reviewed in (43).

Another approach to measure single channel ion currents was proposed by Erwin Neher and Bert Sakmann in 1976, that is, after many years of improving existing electrophysiological methods. By using blunt-tipped glass pipettes, gently pressed against a cell, and application of negative pressure inside the pipette (as suggested by Owen Hammill (44)), a part of the cell-membrane could be isolated (diameter ~ 1µm). This resulted in a very tight seal between pipette (containing one of the electrodes) and the membrane. The (giga-ohm) seal prevents leak currents to flow from the pipette electrode along the membrane to the bath electrode, and reduces the noise in the single channel measurements. This method, now called the patch-clamp, was first used to record activity of muscle acetylcholine receptors. In 1991 Neher and Sakmann were rewarded the Nobel Prize for physiology and medicine for the development of the patch-clamp technique.

Currently three forms of patch clamp are distinguished:
1) Whole cell recording: in which a single electrode is brought in contact with the cytoplasm of the cell to measure currents across the membrane of the whole cell. This technique is similar to voltage clamp, but can be applied to smaller and more fragile cells (Fig. 4B).
2) Cell attached recording: here the pipette is attached to the outside of a cell and channel activity in a patch of membrane is recorded. By formation of a giga-ohm seal, the patch is electrically isolated from the rest of the membrane; the behavior of channels trapped inside the pipette can be studied in relation to the native constituents of the living cell membrane and cytoplasm (Fig. 4C).

3) Excised patch recording: the pipette is again brought into contact with the outside of a cell, but now the isolated patch is pulled free from the cell, allowing the experimenter to exchange the cell plasma for buffer of desired composition (Fig. 4D).

All three forms of patch-clamp can be applied to whole cells, provided their diameter is larger than the diameter of the pipette tip. Generally, bacteria with a diameter of 1-2µm are too small. However, a number of artificial systems have been developed. The first technique uses *Xenopus* (frog) oocytes, into which DNA is injected to enable overproduction and characterization of mutated or newly discovered channels in a eukaryotic system (45-47). For bacteria, it is possible to treat a growing cell culture with cephalexin, which prevents septation of the dividing cells. This causes the cells to grow into long snake-like structures. These snakes can subsequently be treated to form...
giant spheroplasts, with diameters ranging from 10-20µm. The procedure for making the giant spheroplasts is described in more detail in (48). A third artificial system for patch-clamp measurements concerns Giant Unilamellar Vesicles (GUVs). For this the channel of interest is purified and reconstituted into liposomes, and the so formed proteoliposomes are then used to form GUVs (chapter 3). Alternatively, one could isolate native membranes from cells expressing the channel of interest, fuse the membranes with lipid vesicles (48), and convert the hybrid membranes into GUVs.

Future

One of the important recent advances in the understanding of channel function has come from the crystallization of a number of ion channels. The first channel to be crystallized was the KcsA channel from Streptomyces lividans by the MacKinnon group (49). This was followed in the same year by Mscl from Mycobacterium tuberculosis by the Rees group (50). Since then the structures of a number of other channel proteins have been determined and without a doubt more will follow in the near future, enabling further insights in channel gating and selectivity. Combining the information retrieved from electrophysiology, crystallography, atomic force microscopy and spectroscopic techniques will increase our understanding of channel structure and function even more (4, 51). As for electrophysiology itself, part of the future developments may come from scaling-up of the existing techniques, so that multiple samples can be screened in parallel. For patch-clamp, the first requirement for a successful high-throughput system is automation of the formation of high resistance seals, which has been established in a number of cases. These systems are all based on a chip (or membrane) with very small (1-10µm) openings, made of glass, teflon or silicon polymers. The openings could be coated with lipid bilayers in which proteins were reconstituted (52-54). Alternatively, cells could be positioned on the apertures, and after high resistance seal formation, it was possible to apply suction and form the equivalent of the three patch configurations described in Fig. 4 (55-57). Recently, the Port-a-Patch© was introduced by Nanion Technologies GmbH, this is an automated electrophysiology workstation that enables patch clamp experiments on a single cell at a time. Patch clamp recordings in the whole cell configuration and single channel recordings can be performed with planar patch clamp chips, which are mounted in a workstation and to which samples can be applied in the form of a cell suspension. The process of seal formation is performed automatically, and recordings, under different conditions, are computer-controlled (58, 59). Also, the original patch-clamp set-up, using a glass pipette, has been adapted for high-throughput work, both in situ (multiple electrodes inserted into neuronal tissue for three-dimensional monitoring of signals; 60) and in model systems (drug screening in oocytes; 61, 62).

There are numerous examples, in which channels in supported bilayers may be used as biosensors. For instance, one could think of channels reacting to toxic compounds either as their natural ligand, or after they have
been rationally redesigned and modified to do so. Another potential application would be as light-sensitive or electrochemical devices that can store or generate energy (microelectronics). The biggest challenge at the moment for these applications is the stability of the supported bilayer, which is currently on the hour scale, but has to be longer for useful applications. (4, 27)

A medical application of channels may be found in the delivery of hydrophilic compounds (drugs) into human tissues. The compounds can be included in liposomes, which can be used as vehicles for the compound of interest to a target site in the human body. However, because of the hydrophilicity, the compound will not diffuse across the hydrophobic membrane. If a remotely switchable mechanosensitive (MS) channel (63 and chapter 4) would be incorporated into the liposome, the open channel may facilitate the outward diffusion of the compound. This concept is one of the current research topics within the BioMaDe Technology Foundation (64-66).

A case study: bacterial mechanosensitive channels

MS channels from bacteria have been the subject of research for over a decade now. The advances in patch clamp techniques enabled researchers to look at ion channel activity in bacteria. This resulted in the initial characterization of two types of so-called mechanosensitive channels in the cytoplasmic membrane of \textit{E. coli}. The channels were activated by a pressure decrease inside the patch pipette or the addition of amphipaths, indicating that it is the membrane tension (and not the pressure inside the patch pipette) that triggers channel activation (67, 68). In 1994, Sergei Sukharev, Paul Blount, Boris Martinac, Frederick Blattner and Ching Kung identified the protein and corresponding gene for one of the observed channel activities. This MS channel had a conductance of 2.5-4 nanoSiemens (at 200mM KCl bath solution), which was large compared to previously observed channels and resulted in the name MechanoSensitive Channel of Large conductance (MscL; 69). Based on its conductance the pore diameter of MscL was estimated to be around 40Å, which was confirmed by patch clamp measurements in the presence of poly-L-lysine molecules of different sizes that were screened for their ability to influence channel activity. MscL conductivity was reduced by poly-L-lysine molecules ranging in size from 27-42Å, but not by poly-L-lysine molecules smaller than 29Å (70). Soon after the initial discovery of MscL from \textit{E. coli}, homologous channels were identified in over 40 eubacteria and around 10 have been characterized (71-73 and chapter 2). More recently, MS channels have also been identified in archaea (74). The proteins and corresponding genes of the MS channel activity of ~1nanoSiemens have also been identified and are named MscK (coded by \textit{kefA}; 75, 76) and MscS (coded by \textit{yggB}; 77, 78).

As shown in Fig. 5, the crystal structures of both MscL (50) and MscS (79) have been determined. The structure of MscL most likely represents a largely closed state of the channel, whereas the structure of MscS probably represents an open state of the channel. The clear pentameric organization of MscL has been confirmed in a number of studies (81-83 and chapter 5).
availability of a crystal structure of the MscL channel made new directions of research possible, both experimentally and in silico. Using molecular dynamics, the response of MscL to membrane tension has been described (84-88). Unfortunately, there are no structures available of MscL in the open or intermediate conformations, so that the molecular dynamics models cannot be verified directly. However, an interesting mutant channel has recently been described that can be locked in the open state. This mutant, and the crystallization of MscL in the open conformation, may result in the elucidation of the channel gating mechanism (89).

The exact physiological function of the MS channels is not yet fully known. However, it is clear that they do play a role in cell survival upon osmotic downshift, i.e., when the cell environment suddenly changes from high to low osmolyte content (76, 90, 91 and chapter 2). Under these conditions, osmolytes may rapidly leave the cell via the MS channels and thereby prevent the cell from lysing. One of the still unanswered questions is:
why bacterial cells should have multiple "osmotically regulated osmolyte-release valves" that differ relatively little in threshold tension and selectivity. The gating mechanism of MscL is only mechanosensitive, whereas MscS activity is also partly membrane-potential dependent (79, 92) and MscK appears to be regulated by external ionic environment (i.e., MscK is activated only in the presence of high concentrations of K\(^+\), NH\(_4\)\(^+\), Rb\(^+\), or Cs\(^+\); 74). This leaves the question, whether these latter two channels represent true MS osmolyte release valves, or have developed mechanosensitivity as an additional functionality, in which case MscL would be the only genuine MS channel. The remaining part of this introduction will focus on MscL, as this is the most intensively studied bacterial MS channel and is also the topic of the rest of this thesis.

To determine the parts of the MscL channel that are involved in tension sensing and the parts that form the channel constriction site (and are therefore involved in channel gating and size-exclusion "selectivity"), a number of libraries with mutated mscL genes have been constructed and the mutant proteins have been studied (91, 93-95). These studies generally divide mutations into three groups: neutral mutations (i.e., mutants without altered tension sensitivity); Gain Of Function (GOF) mutations (i.e., channels that have a lower tension threshold for opening, or remain in the open conformation for long periods of time); and Loss Of Function (LOF) mutations (i.e., channels that have a higher tension threshold for opening, or remain in the open conformation for short periods of time).

One specific library was generated to elucidate the role of the α-amino acid positioned at the most constricted part of the channel: glycine 22 (95). If this residue was replaced by a hydrophilic (charged) residue the channel would generally become GOF, whereas more hydrophobic substitutions resulted in LOF phenotypes. A LOF channel is obtained when the glycine 22 is replaced by a cysteine, but this mutant becomes GOF upon labeling with the charged reagent [2-(trimethylammonium)ethyl]methanethiosulfonate (MTSET; 96). This was the first example of an engineered switchable channel. A very interesting modification to this switch is the replacement of MTSET with a label that changes charge when illuminated at a specific wavelength (63). A further cysteine library was scanned using MTSET, revealing that not only position 22 is very sensitive to charge modification, but also a number of other residues in the first transmembrane helix (TM 1). Interestingly, all these residues were found on the same face of the transmembrane α-helices that line the channel pore (97, 98). The corresponding residues were also modified to histidines. The five histidines of the subunits of the pentameric channel were found to form a metal binding site inside the pore, that is, when divalent cations (Ni\(^{2+}\), Cd\(^{2+}\), and Zn\(^{2+}\)) were added to the patch clamp bath-solution, it became increasingly harder to open the modified channel. The only exception was MscL L19H, which became harder to open on the addition of Cd\(^{2+}\), but easier to open in the presence of Ni\(^{2+}\). This suggested that L19 rather than G22 may reside at the most constricted part of the pore. (99)
Apart from defining the constriction site of the channel, a number of studies have been undertaken to identify the sensing part of the protein. Initially, this was done by removing α-amino acids from both N- and C-terminal ends of the protein. This showed that fully functional channels could still be formed when three N-terminal α-amino acids or twenty-seven C-terminal α-amino acids were removed (100, 101). Databases have been searched to identify channel regions that were highly conserved. However, it has not been possible to directly link any of the conserved regions directly to the sensing mechanism of the protein (102, 103). An interesting series of experiments involved the separate expression of polypeptides for the N-terminal TM 1 and the C-terminal part of the wild-type subunit, including TM 2. This study showed that TM 1 alone was able to form channels, albeit with reduced conductance, that gated completely tension-independent. The TM 2 part alone was not able to form channels, but when it was co-expressed with the TM 1 polypeptide, channel activity was observed. This activity had a normal conductance, but a gain of function phenotype (it needed less tension to gate) compared to the wild-type MscL channel. This indicates that the actual tension sensing region is most likely located in TM 2. It also emphasized the importance of the interaction between the two helices and the communication through the connecting loop (103). An earlier study, in which the loop connecting the two transmembrane helices was digested with a protease (trypsin), had shown that this loop played an important role in the transduction of the tension signal. When the loop was digested, the channel was still tension-sensitive, but gated at a lower tension. The loop has been proposed to work like a spring that sets the sensitivity threshold of the channel (104). Recently, a number of mutations at the rim of the channel (end of TM 1) have been made, which resulted in channels with a marked decrease in mechanosensation (LOF; 105). Together, these data suggest that the connecting loop plays an important role in the communication between the sensing (TM 2) and gating modules (TM 1) of the MscL subunits.

Interaction between the sensing module of the protein (TM 2) and the surrounding lipids is also thought to be important for proper channel functioning. It is therefore not surprising that protein-lipid interactions have been the subject of a number of investigations, both in silico (106-109) and in vitro, using MscL reconstituted in proteoliposomes (110-112) and chapter 3). These studies showed the importance of lateral tension in the membrane (68), asymmetric bending, and membrane thinning (which was nicely demonstrated using a combination of electron spin resonance and lipids with different acyl chain lengths (112)) on the activation of the MscL channel. An overview of the role of membrane lipids in the regulation of osmotic downshift-activated MS channels (i.e., MscL and MscS) and osmotic upshift-activated transporters (i.e., BetP, OpuA and ProP) is available (113).

For a bacterial cell, it is important to have the appropriate number of channels present in the cell membrane. Too many channels may cause unwanted leakage of vital cytoplasmic components. Too few channels may lead to cell lyses under conditions of osmotic downshift. Regulation of the
expression of genes coding for MS channels is therefore important to the cell. A few papers have so far been published on this subject. The most rigorous study showed that expression of MS channels is up-regulated under the influence of rpoS during stationary growth phase. It also showed that in strains that lacked the MscL channel, the MscS channel was expressed to higher levels and vice versa (114). Interestingly, high external salt concentration did not have an effect on the level of mRNA for MscL or MscS in L. lactis (chapter 2).

A very promising recent development, in the light of the application of the channel in drug delivery, is the possibility to produce the channel in a cell free system. Using an enhanced E. coli cell lysate and mRNA coding for the MscL from E. coli, it was possible to produce channels in a detergent environment and reconstitute them into liposomes. These biologically produced channels showed normal channel activity in patch clamp (115). Chemically, the channel has been produced by linking synthetically-prepared polypeptides, using modified cysteines, and thereby forming functional channel subunits (116, 117). One final study worth mentioning, concerns the preparation of a series of TM 2 cysteine mutants that allowed the conversion of a lipid-soluble protein into a water soluble one by chemically linking amphipaths to the cysteines. This water soluble form has been investigated by circular dichroism, size exclusion chromatography and electron microscopy, which showed that the water soluble form resembled the lipid soluble form of the channel both in structure and in size. The electron micrographs obtained with MscL from E. coli showed a five-fold symmetry, indicating that it is structurally very similar to MscL from M. tuberculosis (83).

In conclusion: MscL is a versatile and flexible channel that, because of its relatively simple structural and functional properties, is easy to study. The channel is the paradigm of our understanding of the gating mechanism of MS channels, and it may be a good candidate for future applications in bio(nano)technology.

Outline of this thesis
Chapter 2: deals with the identification and characterization of the MS channels from Lactococcus lactis. It also shows that Lactococcus lactis uses MscL as the main mechanosensitive solute-release system to protect the cells under conditions of osmotic downshift.

Chapter 3: describes a new method for the formation of membrane protein-containing Giant Unilamellar Vesicles. The formed proteo-GUVs were applied in patch-clamp measurements to monitor channel activity and in fluorescence correlation spectroscopy experiments to monitor mobility of a number of model membrane proteins.

Chapter 4: describes the reversible activation of MscL via a light-sensitive lipid mimic. For these experiments MscL containing GUVs were prepared from bilayers composed of 80mol% 1,2-dioleoyl-sn-glycero-3-phosphocholine and 20mol% of di-(5-[[4-(4-butylphenyl)azo]-phenoxy]-penty1)phosphate (4-Azo-5P). Light-
induced isomerization of the azobenzene moiety of 4-Azo-5P from trans to cis was used to activate MscL.

Chapter 5: shows the construction of covalent oligomers by gene fusion; up to six copies of the mscL gene were fused in tandem. Only the covalent pentamer opened at the same relative pressure (compared to the pressure required to open MscS) as the wild-type MscL channel. In a size exclusion chromatography, the wild-type channels and the covalent pentameric channel migrated similarly. Overall, the data strongly suggest that the pentameric MscL represents the functional state of the channel.

Chapter 6: presents a summary of the work carried out during the Ph.D. project, including some unpublished experiments, combined with future prospects. The chapter also contains information on the techniques that were used and developed for the work presented in this thesis.
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