Praktikumsversuch:
Black Lipid Membrane

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In diesem Versuch geht es um die Untersuchung von **Ionenkanälen** in **planaren Lipidmembranen**. Dabei werden Ionenkanäle durch das Peptidantibiotikum Gramicidin A in bimolekularen Lipidschichten generiert. Der Wirkmechanismus von Antibiotika gegen Bakterienmembrane kann dadurch modelhaft gezeigt werden. Im biologischen System wird durch das Einbauen von Kanälen in die Lipidmembran der Durchtritt von Kationen ermöglicht. Dadurch wird der elektrochemische Gradient in der Zelle zerstört und die Zelle stirbt. Betrachtet man Gramicidin A, so können nur Gramicidin A Dimere Kanäle in der Lipidmembran ausbilden.

Mit der **Black Lipid Membrane Methode** können künstliche Lipidmembrane hergestellt werden. Die zwei Kompartimente einer Küvette sind durch ein ca. 1 mm großes Loch miteinander verbunden. Über dieses kleine Loch wird ein Lipidfilm gestrichen, der eine wenige Nanometer dicke Lipid-Doppelschicht ausbildet. Beim Ausbilden der Lipid-Doppelschicht können zuerst im reflektierenden Licht Newtonsche Ringe beobachtet werden bis schließlich kein Licht mehr reflektiert wird und eine schwarze Membran - bei dunklem Hintergrund - sichtbar wird.

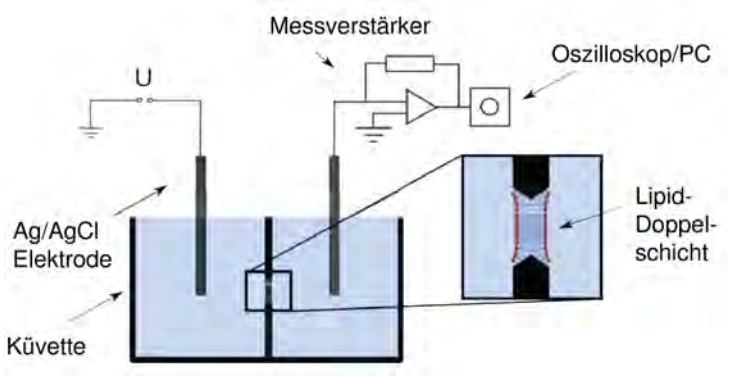

Fig. 1: Schematischer Aufbau der BLM-Apparatur.

Durch Anlegen von Rechteck- oder Gleichspannung kann mittels zweier Ag/AgCl-Elektroden eine Stromveränderung über der Membran gemessen werden. Die Rechteckspannung dient zur Bestimmung der Membrandicke. Der zeitliche Verlauf des Membranstromes wird bei fester Spannung untersucht. Einzelkanalleitfähigkeit und Kanallebensdauer werden bei

geringer Konzentration des Peptidantibiotikums Gramicidin A aus der Registrierung des quantisierten Stromes mittels Histogrammen und bei höherer Dotierung durch Auswertung der Autokorrelationsfunktion des fluktuierenden Stromes bestimmt.

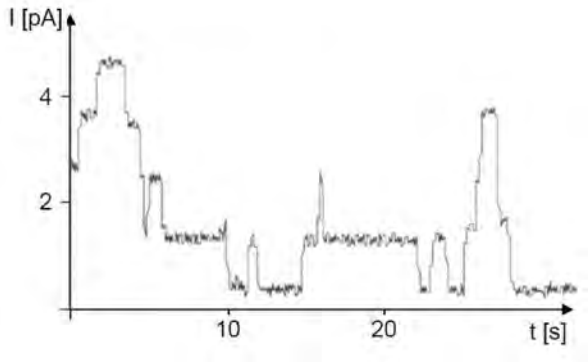

Fig.2: Beispielmessungen von Gramicidin A Ionenkanälen.

Practical course:

Black Lipid Membrane

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Contents

1 Overview

The goal of this practical course is to observe the formation of ion channels generated in lipid bilayers by a peptide antibiotic, gramicidin A. These pores and their formation are an essential part for the understanding of the reactions of antibiotics in our body. Pores produced by antibiotics lead to the destruction of the electrochemical gradient in bacterial cells and conduce to cell death. The measurements will be done by the so called 'Black Lipid Membrane' technique (Fig. 1).

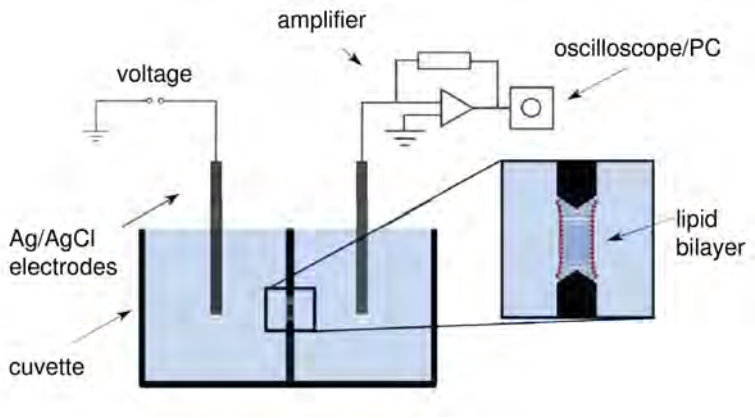

Figure 1: Experimental setup for studying BLMs.

For such measurements lipid bilayers are artifically created and show interference patterns on the few nanometer thick lipid bilayer. By recording the electrical current - decreasing and increasing over the membrane - two observations can be made in real time:

- The membrane thickness can be calculated, treating the membrane as a parallel-plate capacitor.
- Ion flux (=current) changes through the membrane show the formation of ion channels in real time (Fig. 2).

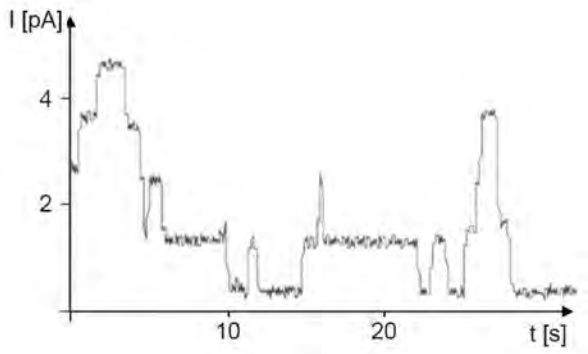

Figure 2: Quantized increased current flow by gramicidin A channel formation

For these experiments, gramicidin A shall be used as peptide antibiotic. In this introduction to the experiment, chapter 2 and 3 will cover the experimental setup and instructions how to conduct the experiment. The BLM method will be introduced in chapter 4. A general overview of the underlying techniques and terms of peptide antibiotics will be given in chapter 5. The mechanism of iontransport will be discussed in chapter 6.

2 Questions and to dos

2.1 Lipid membrane preparation

- Preparation of a lipid bilayer using glycerol-monooleate in tetradecane
- Electrical and optical monitoring of lipid bilayer generation
- Determination of membrane capacity, specific membrane capacity and membrane thickness
- Determination of the membrane resistance
- Determination of the avalanche voltage and the maximum E-field in the membrane

2.2 Measurement of single channels

- Modification of DC conductivity using a low concentration of gramicidin A
- Determination of the single channel current and the distribution of the mean channel life time (histogram) resulting from the current
- Test of Ohm's law for single channels

2.3 Measurement of multiple channels

- Monitoring channel formation using a high concentration of gramicidin A
- Determination of a single channel current by analysing a current histogram
- Determination of single channel current and mean channel opening times using autocorrelation function analysis of current noise.

2.4 Further questions

- Compare methods and results
- Determination of the dissociation of the rate coefficient of gramicidin A dimers
- Determination of the single channel conductivity of gramicidin A channels
- Calculation of a particle flow ($= \frac{\text{ions}}{\text{second}}$) through one gramicidin A channel

3 Experiment

The aim of this practical course is to prepare planar lipid bilayers and to monitor channel formation of gramicidin A. Gramicidin A dimers will generate channels through the lipid bilayer, which enables a penetration of cations.
The time dependent current through the membrane will be measured by applying constant voltage. Single channel conductance and life time of the formed channel can be determined by measurement of the quantized current. This will be done -using a low concentration of gramicidin A - by analyzing histograms. If a high concentration of gramicidin A is applied, an autocorrelation function of the fluctuating current will be analyzed to obtain the same information. 0.5 M potassium-chloride solution (KCl) as an electrolyte solution is used. The concentration of the solution is important. A higher concentration leads to more available ions which can diffuse through the membrane. To avoid destruction of the lipid bilayer and destruction of gramicidin A, we will use only 0.5 M KCl. For the lipid solution we will use a concentration of 3.5 $\frac{\text{mg}}{\text{ml}}$ in tetradecane. For the gramicidin A solution, we have to prepare a stock solution of 0.1 $\frac{\text{mg}}{\text{ml}}$ before we dilute with water to a 4 $\frac{\text{ng}}{\text{ml}}$ concentrated solution.

3.1 Black Lipid Membrane - BLM

3.1.1 Preparation of a planar lipid bilayer

- Series resistor: 100 kΩ (100 MΩ switched on equal to short-cicuited)
- Feedback resistor: 500 kΩ (500 kΩ switched on)
- Frequency: some 100 Hz
- Amplitude: 20 - 100 mV

3.1.2 Measurement of the membrane capacity

- Please insert the cuvette carefully.
- Add lipid to the cleaned hole and wait for a couple of minutes.
- Add electrolyte solution (KCl) to both compartments.
- Add some droplets of a 3.5 $\frac{\text{mg}}{\text{ml}}$ glycerine-monooleat in tetradecane solution to the teflon stick and spread it gently over the hole.

Using the objective and lamp, the dying out of the lipid bilayer can be monitored. The position of the lamp is important to enable an observation of the membrane (30 cm). If there are no Newton's ring, remove some of the lipid with another teflon stick. Avoid air bubbles! The generation of an intact membrane can be observed twice: First, the membrane can be observed with the objective and has to be black because of suppressed light reflection. Second, by monitoring the capacity with the oscilloscope, an increase will be measured. To calculate membrane capacity, the relaxation time of the membrane current will be analyzed. A rectangular, period voltage is applied for this measurement.
Please calculate the membrane capacity, specific membrane capacity (capacity/area) and membrane thickness.

3.2 Gramicidin A channels

- Series resistor: 100 MΩ (100 kΩ switched on equal to short-circuited)
- Feedback resistor: 500 kΩ (500 kΩ switched on)
- Voltage: DC, about 50 mV, applied to the membrane
- Observe the current profile after offset correction

3.2.1 Single channel measurements

1. Record the current through the membrane (constant voltage of 50 mV) using a small amount of gramicidin A.
2. Determine the current, flowing through a single gramicidin A channel.
3. Prepare a histogram of the channel life time by a selective analysis of the single channel events. Assuming an exponential distribution, determine a mean life time of a single channel from the histogram. What is the rate coefficient for dissociation of the gramicidin A dimer?

3.2.2 Multi channel measurements

1. Add an higher amount of gramicidin A to increase the concentration. More channels are open now and the quantization of the current is clear and visible.
2. Using the analysis software, create a current histogram and determine the current step heights and their means by measuring the distants of single peaks and calculation of the resulting current discrepance.

3.2.3 Noise analysis and autocorrelation function

1. Apply gramicidin A at a concentration that causes the generation of many channels. The baseline should be not visible; no current step should appear (20 - 50 pA). Take care that the mean of the current fluctuation is approximately constant. The equations for the autocorrelation are only valid for a constant mean value.
2. Compare the results achieved from the autocorrelation function and the life time histogram from the single channel measurements. Determine the mean value of the channel life time and the single channel current.
3. Describe some observations from the experiment and analysis which are not in accordance with the model of basic gramicidin A channels.

3.2.4 Further questions

1. What would you expect for the circular gramicidin S? Make a sketch of the current profile.
2. What are gram-positive and gram-negative bacterias and what is the connection to the gramicidine family? Please describe briefly.

4 Black Lipid Membrane Method

4.1 Introduction

An investigation of biological membranes and their interaction with molecules is often tricky and insolvable in vivo. Therefore, in vitro studies on artificial membranes are essential. One option is given by the so called 'Black Lipid Membrane' method [17, 18], which offers a versatile platform to study the interaction between membranes and proteins. Planar bilayers of different lipids can be prepared. Additional, the composition of the solutions on both sides of the bilayer can be controlled precisely [19]. The description 'Black Lipid Membrane' refers to the appearance of the prepared planar bilayer: Due to destructive interference of light reflected from both sides of this few nanometer thin bilayer, the membrane appears black. Physical properties such as membrane resistance or membrane capacity can be observed. Ion channel formation and changes in permeability for different molecules can be measured as well.

4.2 Settings

The experimental setup (Fig. 3) consists of the following items: cuvette, Ag/AgCl electrodes, current amplifier, oscilloscope and a PC to record the data.

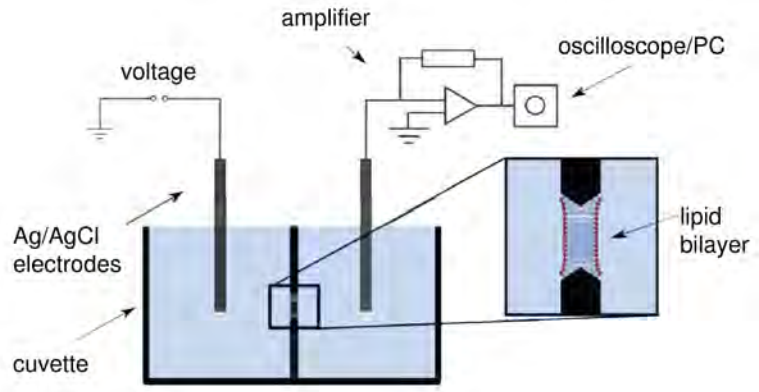

Figure 3: Experimental setup for studying BLMs.

The **cuvette** (Fig. 4) is made of teflon and consists of two compartments, which are separated by a wall, tilted by 10°. The volume for both compartments is 5 ml each. In the middle of the wall is a small circular hole of about 1 mm in diameter. To avoid any roughness around the hole, Stabilit epoxy glue (Pattex) was used to smoothen the teflon. Spreading lipids in the hole will lead to a planar bilayer.

To allow optical monitoring of the planar lipid bilayer, one side is made out of optical glass. A **telescope** with 10 x magnification enables optical monitoring of the membrane. To prepare planar lipid bilayers, Mueller and Rudin developed the following technique in 1963. Therefore, lipids are solved in an organic solvent. One droplet of the solution will be spread over the hole in the teflon cuvette. Depending on the thickness d of the lipid layer, the layer appears white for $d >> \lambda$, colourful (Newton's ring) for $d \approx \lambda$ or black for $d << \lambda$ (Fig. 5).

In this experiment **Ag/AgCl electrodes** are used. One electrode is connected to the power supply unit, the other one transfers the current through the membrane and is connected to

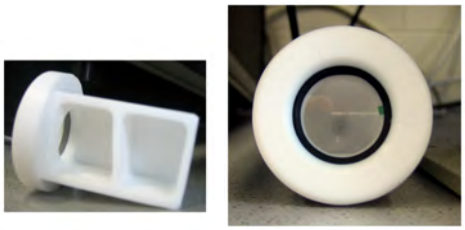

Figure 4: Cuvette

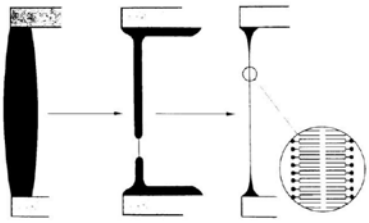

Figure 5: Depending on the membrane thickness, a white, colourful or black membrane appears.

a **signal amplifier**. Please see the circuit diagrams in the appendix. The signal is recorded by a computer and an oscilloscope. Voltage, which is proportional to the current flow I, can be measured as a function of time to reveal information about the conductivity G of the membrane:

$$G = \frac{\text{measured current}}{\text{applied voltage}} = \frac{I_{\text{measured}}}{U_{\text{ext}}} \tag{1}$$

The unit for the conductance is Siemens, where $1\text{S} = 1\frac{\text{A}}{\text{V}}$. Further, the **membrane** can be considered as a parallel-plate capacitor (Fig. 6). The lipid bilayer is an isulating layer between two conducting electrolyt solutions. All further characteristics of a parallel-plate capacitor can be applied:

$$C = \epsilon_0 \epsilon_m \frac{A}{d} \tag{2}$$

where ϵ is the dielectricity constant m for the membrane ($\epsilon_m \approx 2\frac{F}{m}$) and the constant $\epsilon_0 = 8.85 \cdot 10^{-12}\frac{F}{m}$. A refers to the area of the membrane and d is the thickness. The capacity of a membrane is between 0.3 and 0.8 $\frac{\mu\text{F}}{\text{cm}^2}$.

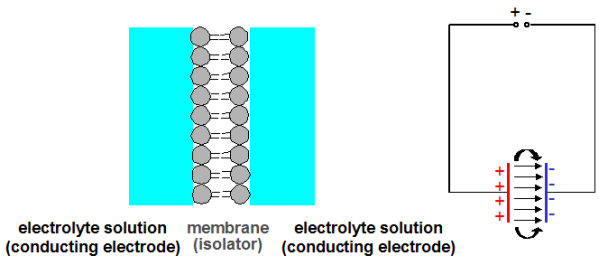

Figure 6: The membrane viewed as a parallel-plate capacitor.

5 Peptide antibiotics

5.1 Introduction

Peptide antibiotics are a group of peptides which interact with cell membranes [1, 2, 3]. A special group of peptide antibiotics are antimicrobial peptides. In contrast to peptides consisting of up to 100 amino acids, peptide antibiotics are built from 10 to 50 amino acids. As a part of the innate immune system, they are generated continuously in the human body to provide cover against infections [4]. One major advantage for drug design is that they can be produced artificially by solid-phase synthesis.
If peptide antibiotics are integrated in the bacterial cell membrane, the ion equilibrium will be destroyed and the cell will die. Different pharmaceutics are commercially available, for example, against ear pains or a runny nose.
In this practical course we will employ the peptide antibiotic gramicidin A. It is active against gram-positive bacteria and used for clinical application as an antibiotic in for example Angidin ®, Mycolog ®, Topsym ®, Neosporin ®, Sofradex ® (see Fig. 7).

Figure 7: Different pharmaceutics containing gramicidin A

Bulding blocks of antimicrobial peptides are the normal proteinogenic amino acids, but non-proteinogenic and D-amino acids are included as well. A high amount of hydrophobic amino acids is common. Concerning the molecular **structure**, α-helical, β-sheet, linear and even cyclic structures can be found [20].
Two main **interactions** lead to the high affinity between the peptide antibiotics and the phospholipids of a membrane:

- The hydrophobic interaction between the fatty acid chains of the membrane phospholipids and the hydrophobic peptide amino acids.
- The electrostatic interaction between negatively charged membrane phospholipids and positively charged peptide amino acid residuals.

5.2 Mechanism to destroy bacterial cells

How can peptide antibiotics destroy bacteria and defend our body? Different models have been proposed that describe in which way they integrate themselves into the membrane (Fig. 8) [5,6,7,8]:

- **A** 'Barrel stave' or 'helical bundle' model
  The 'barrel stave' model is widely used to explain pore formation by hydrophobic helical monomers. These associate in cells with the membrane. After channel formation,

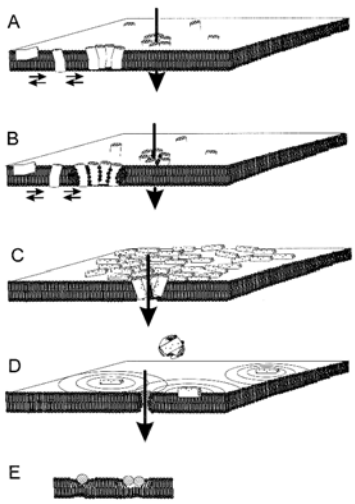

Figure 8: Different suggested models to explain the mechanisms by which peptide antibiotics destroy bacterial cells

ions and small molecules can travel through the membrane.
In a BLM measurement, this can be observed as a step-wise increase in conductivity. Conditioned by the number of monomers, the thickness of the formed pores are different.

- **B** 'Toroidal wormhole' model
  In contrast to the 'barrel stave' model, the 'toroidal wormhole' model describes pore formation in the presence of phosphatidylethanolamine/ phosphatidylserine (PE/PS) membranes. This leads to an association between lipids and peptides, and pore walls are formed by both, lipids and peptides. Thus, different and non-uniform pore sizes are generated.

- **C** 'Carpet' model
  The 'carpet' model describes, a concentration-dependent penetration of peptide antibiotics through the membrane. The molecules form carpet-like aggregates on the membrane surface. Upon increasing concentration of molecules on the surface, more and more molecules will penetrate into the membrane and the lipid bilayer will be destroyed.

- **D** 'Detergent similar' model
  In the 'detergent similar' model, micellular and bicellular patches are thought to be generated on the membrane. It is a similar mechanism as observed for detergents. Due to their amphiphilic properties, they associate with the phospholipids. The membrane becomes more permeable for different molecules and the cell will be damaged.

- **E** 'In-plane diffusion' model
  The 'in-plane diffusion' model proposes an approach in which the molecules can not span the whole membrane to form pores. Thus, they can strongly influence the phospholipid bilayer and lead to an effective thinner lipid bilayer. Consequently, the conductivity in the BLM measurements increases.

5.3 Gramicidin A

Gramicidin A belongs to the gramicidin family, which consists of different antibiotic compounds. For the practical course, we will investigate this molecule. Another molecule from the same family is the so called '**gramicidin D**'. It consists of a mixture of linear gramicidin A, B and C. Furthermore, the molecule '**gramicidin S**' shows a cyclic structure in contrast to the other gramicidin molecules. This leads to a completely different mechanism regarding the interaction with cell membranes.

In nature, gramicidin A is provided by the soil bacterial species Bacillus brevis [9]. It can also be produced by chemical synthesis [10,11,12]. Since gramicidin A (Fig. 9) is integrated

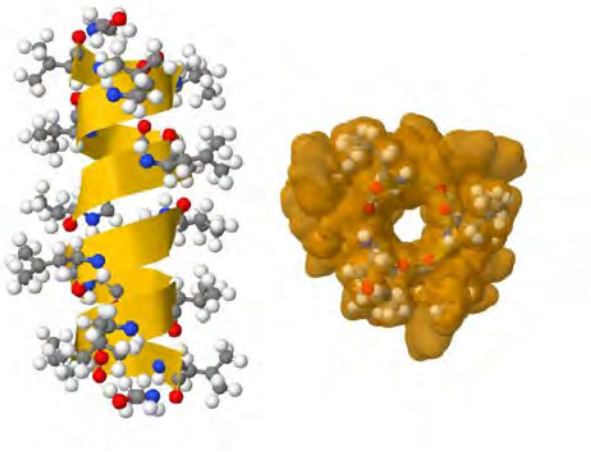

Figure 9: Structure of the gramicidin A ion channel, generated from two gramicidin A monomers in a DMPC bilayer (pdb: 1MAG)

into the bacterial cell membrane, it forms channels to transport ions. This molecule and its channel formation is well understood and has been often observed [7,13]. By opening a gate for ions into the cell, the ion gradient of the cell will be destroyed. Monovalent cations like K^+ or Na^+ ions can travel through the membrane. This leads to cell death due to lacking of electrochemical gradient required for cell vitality.
Gramicidin A is a linear petid built from 15 amino acids. The building blocks are the amino acids alanine, glycine, leucine, valine and tryptophan, with alternating L- and D-amino acid sequences, resulting in a β-sheet [13,14,15,16]. The sequence is

Formyl - L-Val - D-Gly - L-Ala - D-Leu - L-Ala - D-Val - L-Val - D-Val - L-Trp - D-Leu - L-Trp - D-Leu - L-Trp - D-Leu - L-Trp - ethanolamide

To anchor the molecule at the membrane-water interface, four tryptophan molecules bind to the charged phosphate group of the lipid bilayer (hydrogen bonding). This is a common strategy for membrane proteins to be integrated into membranes. It is important to realize, that only gramicidin A dimers form channels [14] (Fig. 10). The orientation of this dimer is parallel to the normal of the lipid bilayer [13]. Hydrogen bonds link two monomers to a dimer. A so called 'head-to-head-helix' is formed, connected by their N-formyl end groups [15]. The C termini instead point at the membrane surfaces. The size of one channel is around 30 Å in length and the outer diameter approximatly 15 Å with corresponds to an inner channel diameter of 3 - 4 Å [14]. The alternating L-D- conformation leads to an anti-parallel single strand $\beta^{6,3}$-helix, with 6.3 residues per turn [13]. Hydrophobic side

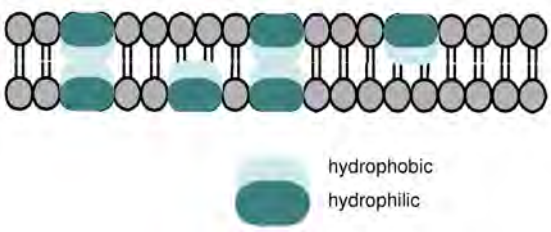

Figure 10: Model of gramicidin A dimer formation through a membrane. Only gramicidin A dimers but not monomers can span the whole membrane thickness.

chains form the outer cloak of the cylindrical cavity in contact with the hydrophobic interior of the membrane lipids. It is the energetically favoured conformation. The interior, i.e. the transport pathway for ions, is hydrophilic owing to the exposed carbonyl groups of the peptides [15].

6 Ion-Transport

The planar lipid bilayer separates two different aqueous solutions and is not permeable for ions due to the Born energy. By formation of pores using gramicidin A, the membrane is permeable for different cationic ions. Due to the negatively charged carbonyl groups inside the channels, many binding sites are offered inside the membrane. Figure 11 shows a model considering only two symmetric binding sites:

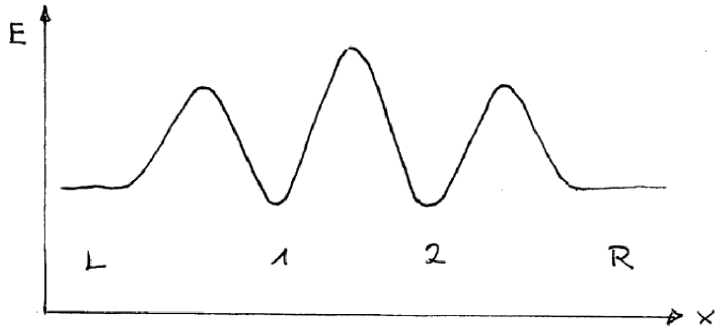

Figure 11: Symmetric energy scheme of a pore with two symmetric binding sites.

The probability k to jump over this energy barrier with a height E^* - so called activation energy - per unit time (rate coefficient) is given for 1-dimensional transport by the Arrhenius relation:

$$k = k_0 \cdot e^{\frac{-E^*}{kT}} \tag{3}$$

Regarding the entire transport procedure, the following kinetic scheme applies

$$\begin{array}{ccccc} & k_A & & k & & k_D & \\ \text{ion left} & \rightleftarrows & \text{ion position 1} & \rightleftarrows & \text{ion position 2} & \rightleftarrows & \text{ion right} \\ & k_D & & k & & k_A & \end{array}$$

It is described by the rate coefficient k_A for binding, k_D for dissociation and k for the ion transport. k_A refers to the standard concentration in the solution.
The populations of ions in the four kinetic states L, 1, 2, and R are governed by rate equations. For positions 1 and 2, they are given by

$$\frac{dN_1}{dt} = k_A \cdot c_L - (k_D + k) \cdot N_1 + k \cdot N_2 = 0 \tag{4}$$

$$\frac{dN_2}{dt} = k_A \cdot c_R - (k_D + k) \cdot N_2 + k \cdot N_1 = 0 \tag{5}$$

Here, c_L and c_R refer to the ion concentrations right hand side of the channel, N_1 and N_2 are the populations in states 1 and 2. In steady state, N_1 and N_2 are constant, so $\frac{dN_1}{dt}$ and $\frac{dN_2}{dt}$ are set to 0.

The ion flux j from the left hand position to the right, is given by

$$j = k \cdot (N_1 - N_2) \tag{6}$$

$$j = \frac{k_A}{k_D} \cdot (c_L - c_R) \tag{7}$$

For this approach, saturation of the ion pore is neglected. Entrance into the channel, transport through the channel and leaving out of the channel is taken into account. An electrical potential V_M applied to the membrane will lead to a tilted potential profile if the pore is symmetrical whith an approximately constant slope. The resulting reaction scheme is asymmetric (Fig. 12).

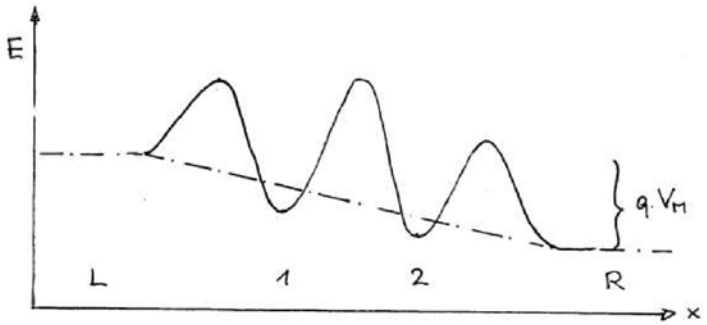

Figure 12: Asymmetric pore potential.

$$\begin{array}{ccccc} & k_A^+ & & k^+ & & k_D^+ & \\ \text{ion left} & \leftrightarrows & \text{ion position 1} & \leftrightarrows & \text{ion position 2} & \leftrightarrows & \text{ion right} \\ & k_D^- & & k^- & & k_A^- & \end{array}$$

Let $\frac{q \cdot V_M}{kT} = u$ with ion charge q. Regarding two positions, there will be a decrease of each slope of about a sixth of $q \cdot V_M$ resulting in the following rate equation:

$$k_A^+ = k_A \cdot e^{\frac{u}{6}} \qquad k^+ = k \cdot e^{\frac{u}{6}} \qquad k_D^+ = k_D \cdot e^{\frac{u}{6}} \tag{8}$$

$$k_A^- = k_A \cdot e^{\frac{-u}{6}} \qquad k^- = k \cdot e^{\frac{-u}{6}} \qquad k_D^- = k_D \cdot e^{\frac{-u}{6}} \tag{9}$$

As an example, consider the marginal case of small potentials with a decay of $\frac{u}{6} << 1$. In this case, Ohm's law is obtained for the stationary electrical current. Let Λ be the single channel conductivity; consider only equal concentrations on both sides, with $c_L = c_R = c$, and neglect saturation.

$$q \cdot j = \Lambda \cdot V_M \tag{10}$$

$$\Lambda = \frac{k_A}{k_D} \cdot \frac{c}{k_D^{-1} + k^{-1} + k_D^{-1}} \cdot \frac{q^2}{kT}. \tag{11}$$

The conductivity of the channel depends on the structure of the channel profile. It is linear in the ion concentration. The measured total current I_M of a membrane is given by

$$I_M = \lambda_M \cdot V_M, \tag{12}$$

$$\lambda_M = \Lambda \cdot N_P, \tag{13}$$

with the total conductivity λ_M.

6.1 Gating of a single channel

As discussed before, only dimerization of gramicidin A leads to ion channel formation. The forces between two dimers are weak (hydrogen bonds). Dimers form and break down stochastically in chemical equilibrium. Channels are opened and closed spontaneously (spontaneous gating). The number of pores N_P changes over time. The total conductivity of the membrane fluctuates about Λ due to closing or opening a channel. If there are only few gramicidin A molecules in the membrane, there will be only one or no channel opened in the membrane at any point in time. The total conductivity - which means the current I_M applying constant voltage V_M - fluctuates in a step-wise fashion by one quantum current. A single channel can be seen macroscopically. The usuall disturbing fluctuation is a essential support for this observation using an autocorrelation function for further analysis. To describe this phenomenon quantitative, the dimerisation reaction can be written as

$$G_1 + G_1 \underset{k_d}{\overset{k_r}{\leftrightarrows}} G_2.$$

The rate coefficient k_d describes the probability of a dimer dissociation per time unit. The coefficient k_r describes the probability of a reaction of two gramicidin molecules to form a dimer. Beginning at the moment of the monomer-monomer interaction, the dimer decays. The probability of decaying follows an exponential decay law. Channel generation and decay can be followed by observing the current profile (Fig. 13). Analysing the life time of the pores, the exponential decay law can be found and, therefore, the rate coefficient, k_d, can be measured.

6.2 Gating of multiple channels

With increasing gramicidin A concentration, single channel generation can not be observed any more. Instead, a noise signal is found in the current measurement, which arises from stochastical opening and closing of multiple channels. The total activity (dynamic) of a pore entity is described by a rate equation considering the mean value of the number of molecules

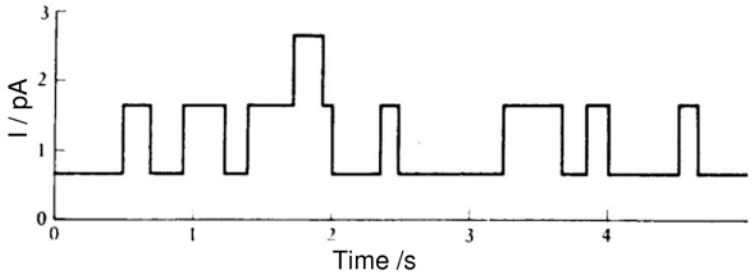

Figure 13: Representativ depiction of opening and closing of ion channels by a dimerisation reaction of gramicidin A.

(eq. 14). Let G_1 be the monomer, G_2 be the dimer, G be the total molecule number. In equilibrium, eq. 15 is obtained (law of mass action).

$$\frac{dG_2}{dt} = -k_d \cdot G_2 + k_r \cdot G_1^2 \tag{14}$$

and $G_1 + 2 \cdot G_2 = G$.

$$\frac{G_2}{G_1^2} = \frac{k_r}{k_d} \tag{15}$$

The dynamic process of dimerisation can be observed by changing the equilibrium. Two different methods can be chosen which are equivalent regarding the fluctuation-dissipation theorem:

- Rapid changes of external parameters such as temperature, electrical field and monitoring of the relaxation to a new equilibrium.
- Monitoring of the time-dependent development of spontaneous changes due to small particle number in terms of an autocorrelation function of the noise.

The exponential relaxation can be calculated using equation 14-15 for small deviations from equilibrium. Both rate coefficients influence the relaxation time:

$$\frac{1}{\tau} = k_d + 4 \cdot k_r \cdot G_1 \tag{16}$$

Substitution of G_1 and G_2 due to the law of mass action and replacement of $G_2 = N_P$ by the measured conductivity of the membrane (eq. 12-13) leads to the equation,

$$\frac{1}{\tau} = k_d + 4 \cdot \sqrt{k_d \cdot \frac{k_r}{\Lambda}} \cdot \sqrt{\lambda_M}. \tag{17}$$

There is an increase in relaxation velocity in correspondence with the square root. For small conductivities, the relaxation is defined by a decay constant of a single channel.

6.3 Autocorrelation function

An autocorrelation function describes a cross-corelation between a signal and itself. Therefore, regarding a basic stochastic process, such as an opening or closing of channels, the relaxation to the equilibrium is identical with the time profil of the autocorrelation function of the stationary noise-induced process by itself. Repeating patterns can be observed which

could not be observed due to noise. This means, that by the autocorrelation function, the time until the fluctuation is elapsed can be gained. As it is equal to eq. 17, this equation can be taken in account.
The self-similarity of the noise can be monitored for particular separations in observation time. Let's observe the noise signal of the variable *x(t)* about 0 (Fig. 14). A comparison of

Figure 14: Noise signal of the variable *x(t)* about 0

the signals *x(t)* and *x(t+T)* leads to an equal average probability for odd or even signs. The expected value of the product $\langle x(t) \cdot x(t+T) \rangle$ is small. If small time intervals are compared, the expected value is large. The autocorrelation function decreases

- from the value of the variance (at T = 0),

$$\langle x(t) \cdot x(t+T) \rangle = \langle x^2 \rangle$$

- to zero at T = ∞

$$\langle x(t) \cdot x(t+T) \rangle = 0.$$

According to eq. 17, the relaxation time is defined by the channel life time $\frac{1}{k_D}$. By analysing the autocorrelation function, the life time of the channels can be calculated without observing individual channels.

6.4 Potential characteristics of a membrane

To understand the potential barrier of the membrane in more detail, a short overview will be given in this section. The characteristics of the electrical potential across our artificial membrane depends on its building blocks. Different electrostatic and physicochemical models are applied to describe the electric potential. Each of them describes single aspects and details which are necessary to understand the potential characteristics of a membrane [21].

1. The strongest effect is given by the **Born energy**,

$$W_B(r) = \frac{z^2 e^2}{8\pi\epsilon_0 r}(\frac{1}{\epsilon_r^M} - \frac{1}{\epsilon_r^{H_2O}}, \tag{18}$$

   where r is the ion radius, z is the valence, e the elementary charge, ϵ_0 is the dielectric constant of the vacuum and ϵ_r the dielectric constant in water $\epsilon_r^{H_2O}$ or in the membrane ϵ_r^M.

2. The surface charge density and the ion concentration in the solution is another important factor. The **Gouy Chapman theory** describes the correlation between the surface charge density σ, the ion concentration c in solution and the potential at the interface,

$$\Psi = 2\frac{kT}{e} arcsinh(\frac{\sigma}{A\sqrt{c}}). \tag{19}$$

Here, A = $\sqrt{8NkT\epsilon_0\epsilon_r^{H_2O}}$, and k is the Boltzmann constant, c is the concentration in the solution, T is the absolute temperature and N is the Avogadro number.

3. Furthermore, also have an effect on the potential the **dipole moment** of the phospholipids. Phospholipids are amphiphilic, leading to a positively charged inner membrane potential.

4. Finally, the **image point charge effect** decreases with the increasing distant of the distance to the interface.

Considering all these influences, the potential barrier for an ion is given by Fig. 15.

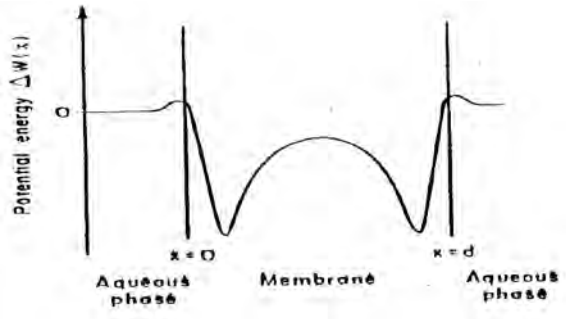

Figure 15: Membrane potential for an ion [21].

7 Literature

1 R. Balser. Antimikrobielle Peptide und Peptidantibiotika. Medizinische Klinik - Intensivmedizin und Notfallmedizin, Springer Verlag 2000

2 M. Zasloff (2000). Antimicrobial Peptides of Multicellular Organisms. Nature 415.389-395

3 D.S. Chapple, R.E.W. Hancock (1999). Peptide Antibiotics. Am Soc Microbiol 43.1317-1323

4 R.E. Hancock, N. Mookherjee (2007). Cationic Host Defence Peptides: Innate Immune Regulatory Peptides as a novel Approach for Treating Infections. Cell Mol Life Sci. 64. 922-933

5 A. Giuliani, A. Rinaldi. Antimicrobial Peptides - Methods and Protocols. Springer-Verlag 2010

6 B. Bechinger (1999). The structure, dynamics and orientation of antimicrobial Peptides in Membranes by multidimensional Solid-State NMR Spectroscopy. Biochim Biophys Acta 1462. 157-183

7 M. Berditsch. Membranaktives Peptidantibiotikum Gramicidin S - Untersuchungen zur Struktur und Funktion. Theorie für das Biochemie Praktikum am Lehrstuhl für Biochemie, Universität Karlsruhe

8 K. Matsuzaki (2008). Control of Cell Selectivity of Antimicrobial Peptides. Biochim Biophys Acta 8.1687-92

9 A.S. Ulrich, M. Berditsch, S. Afonin (2007). The Ability of Aneurinibacillus Migulanus (Bacillus Brevis) to produce the Antibiotic Gramicidin S is correlated with Phenotyp Variation. Appl Environm Microbiol 73.6620-6628

10 F. Lipmann (1971). Polypeptide Synthesis on Protein Templates. Enzymic Synthesis of Gramicidin S and Tyrocidine. Adv Enzymol RAMB 35.1-34

11 G.F. Gause and M.G.Brazhnikowa (1944). Institut für Tropische Medizin. Lancet, 247.715-716

12 M. Ieronimo, J. Buerck, A.S. Ulrich, P. Wadhwani, S. Afonin (2006). Optimized Protocol for Synthesis of Cyclic Gramicidin S: Starting Amino Acid is Key to High Yield. J Org Chem 71.55-61

13 V. Krishnamurthy, C. Shin-Ho, O.S. Andersen (2007). Biological Membrane Ion Channels Dynamics, Structure, and Applications. Springer Science + Business media

14 B.A. Wallace (1986). Structure of Gramicidin A. Biophys J 49.295-306

15 B.A. Wallace (1998). Recent Advances in the high Resolution Structures of Bacterial Channels: Gramicidin A. J Struct Biol 121.123-141

16 T.A. Cross, A.S. Ulrich, S.L. Grage, J. Wang (2002). Structure Analysis of Fluorine-Labelled Tryptophan Side-Chains in Gramicidin A by Solid-State 19F-NMR. Biophys J 83.3336-3350

15 G. Stark, G. Adam, P. Läuger (2003). Biophysikalische Chemie und Biophysik. Springer-Verlag

17 A.L. Ottova, H. Ti Tien (2001). The Lipid Bilayer Concept and its Experimental Realization: From Soap Bubbles, Kitchen Sink, to Bilayer Lipid Membranes. J Membrane Sci 189.83-117

18 M. Winterhalter (2000). Black Lipid Membranes. Cur Opin Colloid Int 5.250-255

19 P. Novak, M. Gaburjakova, I. Zahradnik (2007). BLM Analyzer: a software tool for experiments on planar lipid bilayers. BioTechniques 42 (3)

20 R.N. McElhaney, Md. Ashrafuzzaman, O.S. Andersen (2008). The Antimicrobial Peptide Gramicidin S permeabilizes Phospholipid Bilayer Membranes without forming discrete Ion Channels. Biochim Biophys Acta 1778.2814-2822

21 http://www2.uni-jena.de/biologie/biochemphys/biophys/Lehre/blmbiolo.pdf (17/12/2011)

Further Literature:

- S.B. Hladky, D.A. Haydon, Nature 225 (1970) 451
- H.P. Zingsheim, E. Neher, Biophys Chem 2 (1974) 197
- E. Bamberg, R. Benz, P. Läuger, G.Stark,Chemie in unseres Zeit 8 (1974) 33
- E. Neher, C.P.Stevens, Ann.Rev.Biophys.Bioeng. 6 (1977) 345
- B.A. Wallace, Ann. Rev. Biophys. Biophys. Chem. 19 (1990) 127
- D.A. Kelkar, A. Chattopadhyay, Biochim. Biophys. Acta 1768 (2007) 2011
- Image titel: http://www.heise.de/imagine/igS43DwvAa2yrz9bjMNexEMYJWA/gallery/Seifenblasen.jpg

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