

The Dipole Potential of Lipid Membranes - An Overview

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Summer 2008

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1 Introduction

The motivation for writing this overview originated from the absence of an up-to-date summary of the findings regarding the dipole potential of lipids and lipid membranes. The last summary to my knowledge goes back to 2001 and was written by Clarke [1]. The attempt is to describe the development of the knowledge of the dipole potential from the very beginning over the nowadays forefront knowledge to suggestions for future experiments. The investigations on the dipole potential started with the discovery of a 3rd membrane potential in the middle of the 20th century and are now dominated by experiments like cyro-electro microscopy, emission ratiometric fluorescent methods and MD simulations.

The fact most striking is that the origin and composition of a membrane dipole moment is still not sufficiently known, despite all the technical nimbleness used to probe the dipole potential. The reason for this is quite technical as will be described later. The membrane dipole potential is however not the only potential of the lipid membrane. The total electrical potential across a lipid membrane is a superposition of three potentials, which are [1]:

- The transmembrane potential due to differences in the salt concentrations on both sides of the membrane. This is by far the best examined of the three potentials, since it is very easy to measure by electrodes on both sides of the membrane. The famous Nernst potential is an example of the transmembrane potential.
- The surface potential which is created by the charged head groups of the lipid molecules and the attracted ions that accumulate at the surface of the membrane. Using electrophoresis the potential can be determined quite easily.
- The dipole potential, Ψ_D , which in turn is a superposition of a few dipole potentials within and on the surface of the membrane. The details will be provided in the following parts of the article. We will also face the difficulties scientists had to overcome during their experiments (or sometimes skillfully avoided).

The dipole potential out values the two other potentials in electric field strength by one or two orders of magnitude. It is therefore likely that conformation and orientation, and hence the activity of membrane proteins is influenced by Ψ_D . Changes in Ψ_D , then, could induce changes in protein activity. In other words: They could have regulatory effects. Experiments on the effect of Ψ_D on the $\text{Na}^+\text{-K}^+\text{-ATPase}$ ion pump (Starke-Peterkovic et al., 2005 [59]), on amphiphilic peptides in membranes (Caldera and O'Shea, 1998 [60]) and on the phospholipase A_2 (Maggio, 1999 [61]) support this idea.

2 History

The aim of this section is to provide a path through important developments regarding the dipole potential of lipid membranes. The discoveries are numerous and resemble each other from time to time. Hence I decided to state only a few. My intention is not to favour these pieces of scientific work to others that are not mentioned here, but rather to pick some examples out of a huge stack of papers and to create a almost chronological journey through the history of the membrane dipole potential.

2.1 Discovery

The first hint for a existence of a third membrane potential (the transmembrane and surface potential were well known at this time) was discovered by Liberman and Topaly in 1969 [2]. They observed that fat-soluble, i.e. hydrophobic anions (tetraphenylborate, TPB^-) increase the electrical conductivity 10^5 times stronger than hydrophobic cations (tetraphenylphosphonium, TPP^+). Since the structure of these two molecules are very similar they assumed that the diffusion coefficients of the two ions are also approximately equal. They blamed the different effect on the conductivity on different values of the partition coefficients, i.e. the ratio of concentrations between the membrane and the aqueous phase. In other words: Under similar conditions 10^5 times more anions than cations dissolve in the membrane. To explain this high partition coefficient for anions they suggested a positive charged interior of the lipid membrane. Anions will decrease the positive charge and therefore increase the conductivity for cations. They were able to support this idea by experimental findings. Liberman and Topaly never actually used the word dipole potential.

This term was introduced for the first time 4 years later by Haydon. They compared monolayers of phosphatidylcholine and glycerylmonooleate with bilayers of these two lipids species. The observed differences in conductivity could not be explained by a difference in surface potential as initially expected, because the lipids did not carry a net charge. But what was the origin of the difference in conductivity? They introduced oriented molecular dipoles which were said to create a positive potential inside the lipid membrane.

2.2 A first estimation

The idea of Liberman and Topaly was picked up again in 1975 by Anderson and Fuchs [4] and three years later by Pickar and Benz [5]. They managed to calculate the absolute value of the membrane dipole potential by using the difference in conductivity between TPB^- and TPP^+ , as proposed by Liberman and Topaly. Assuming the membrane to be an energy barrier, they used the Arrhenius equation to relate the rate constant of ion diffusion to the free energy necessary to move an ion from one side to the other:

$$\ln k = -\frac{\Delta G^\#}{RT} \tag{1}$$

The free energy can be written in two terms. One of them accounts for the interaction of ions with water, van der Waals interactions and steric interactions. The second term accounts for interactions of the hydrophobic ions with the dipole potential:

$$\Delta G^\# = \Delta G_0^\# + \Delta G_D^\# \quad (2)$$

$$\Delta G_D^\# = zF\Psi_D \quad (3)$$

Following Liberman and Toplay there is no difference between the transfer of an anion and a kation beside the difference in the interactions with the dipole potential. $\Delta G_0^\#$ should therefore be the same for TPB^- and for TPP^+ . Subtracting the anion form of $\Delta G_D^\#$ from the kation form, one obtains an expression for the dipole potential:

$$\Psi_D = \frac{RT}{2F} \ln \frac{k_-}{k_+} \quad (4)$$

Instead of using the rate constants for anion and respectively kation transfer, it is more convenient to use the specific conductivities (units: $\text{S cm}^{-2}\text{M}^{-1}$):

$$\Psi_D = \frac{RT}{2F} \ln \frac{g_-}{g_+} \quad (5)$$

Anderson and Fuchs estimated the dipole potential to be +310mV. They investigated the transfer of TPB^- and TPP^+ through lipid membranes made of phosphatidylethanolamine (PE). In 1992 Gawrisch and Co.[6]estimated the dipole potential to be +227mV for dipalmitoylphosphatidylcholin (DPPC) membranes and +109mV for dihexadecylphosphocholine (DHPC). They used TPB^- and tetraphenylarsonium, TPA^+ instead of TPP^+ . Almost in the same breath both groups mentioned the uncertainty of their estimations. They suspected a slightly smaller covalent radius of the boron atom in comparison with the arsonium and respectively phosphonium atom and therefore a difference in hydration energies.

These doubts were confirmed by the work of Coetzee and Sharpe already in 1971 [7] and again in 1997 by Stranget and Kamienska-Piotriwicz [8]. They confirmed different interactions of solvent protons and even water molecules with the used hydrophobic anions TPB^- , TPP^+ and TPA^+ . The predicted differences in hydration energy span from 16mV to even 110mV. Eq.5 applied on the ions TPB^- and TPP^+ modifies to:

$$\Psi_D = \frac{RT}{2F} \ln \frac{g_{\text{TPB}}}{g_{\text{TPA}}} - \frac{\Delta G_{\text{hyd}}^{\text{TPB}} - \Delta G_{\text{hyd}}^{\text{TPA}}}{2F} \quad (6)$$

If one takes the best case of a hydration energy difference between the two ions of 16mV one finds differences in Ψ_D , that are of the same order as the absolute value of Ψ_D . This was of course not acceptable. Other ways to obtain the value of the dipole potential needed to be found.

2.3 An attempt to monolayers

A way to avoid the use of hydrophobic ions and the use of their vague defined hydration energies, is to measure the potential across the membrane directly with the help of two electrodes. It is however impossible to inject an electrode between the two layers of a lipid membrane. The thickness of the membrane is approximately 5 nm, whereas the tip of the electrode has a dimension of a few hundred nanometers. Measuring on both sides of the bilayer is not sufficient either. In this case one measures the sum of two monolayer dipole potentials which will - due to their opposite orientation - attenuate each other or even neutralize each other.

The way to go is therefore measuring the dipole potential of lipid monolayers. This was done by many groups in the early 90s, f. ex. by Smaby and Brockman [9, 10]. To measure the dipole potential in a monolayer they used two methods called 'The Vibrating Plate Method' and the 'The Ionized Electrode Method'. These methods are described in more detail later. The rough idea behind these methods was to measure the potential difference of an air/water interface first in the absence of lipids and then in the presence of a lipid monolayer between the phases. By combining these two results they obtained the difference in potentials in the presence and absence of the monolayer. Smaby and Brockmann found a value of approximately 400 mV for the dipole potential.

This was significantly higher than the value obtained for bilayers before. This arose doubts on the used method:

1. The first sore spot was the reorganization of water molecules while spreading the lipid on a water surface, which is a necessary process during the experiment. This reorganization gives rise to a potential jump of unknown magnitude.
2. Secondly one suspected a not constant potential of the hydrocarbon-chains/water interface. One assumed the potential to be due to the lipid head groups, i.e. the potential should rise in this area and then slowly decay towards the end of the hydrocarbon chains. It couldn't be proven that the decay is finished at this point.
3. Thirdly they came up with the idea, that differences in packing density between monolayers and bilayers could account for this difference. They abandoned this idea after calculating the packing density of lipids in bilayers that would be sufficient to account for the low measured dipole potential. They calculated value was not at all in agreement with former measurements on bilayer packing density (e.g. [22]).

Due to these uncertainties the measured value of the monolayer dipole potential was likely to be too high, since both the reorganization of water molecules and the non-constant, decaying potential increase the absolute value of the dipole potential. This was a promising insight and Smaby and Brockman took a closer look at the problem of the reorganization of water.

They suggested a lipid packing-independent component Ψ_{D0} of the monolayer dipole potential. The Helmholtz equation that was usually used to describe the dipole potential was extended by the area independent term Ψ_{0D} :

$$\Psi_D = \Psi_{0D} + 12\pi \frac{\mu_{\perp}}{A} \quad (7)$$

Where A is the lipid molecule area in $\text{\AA}^2/\text{molecule}$ and μ_{\perp} is the surface dipole moment in milliDebye. Eq.7 is however based on two assumptions. The dielectric constant of the medium is assumed to be 1 and the polarization of dipolar water molecules is neglected. Nevertheless Smaby and Brockman put a lot of effort in the analysis of Ψ_{0D} . They found this component to be dependent on the type of lipid used to build up the membrane. For phosphatidylcholines they determined Ψ_{0D} to be 100-125 mV. If this component of the monolayer dipole potential is taken into account, the difference to the bilayer dipole potential is very small and can be neglected. But what is the origin of this lipid area independent component? Here Smaby and Brockman were just able to guess. They supposed that the former mentioned reorganization of water molecules could be the reason for the lipid area independent component of the monolayer dipole potential. Since the experiments on bilayers are carried out on preexisting membranes no reorganization of water takes place and no component has to be added to the dipole potential. However technical obstacles and unfinished theoretical work (f. ex. the unknown decay of the dipole potential) prevented them from coming up with a complete theory that could prove their ideas.

2.4 Or better lipid vesicles?

2.4.1 Hydrophobic ions again

In 1986 the use of hydrophobic ions to probe the lipid bilayer was given another try. The object of interest was now a lipid vesicle. But why using a method that was sorted out years ago due to inaccuracy? Flewelling and Hubbell [11] improved the method by using a theoretical approach that described the translocation of the ions in two steps. First the ions bind to the membrane and secondly they have to overcome a energy barrier in the center of the membrane. This improved the accuracy of the measured value of the dipole potential which was then given by:

$$\Psi_D = \frac{\Delta G_{+}^{\#} + \Delta G_{-}^{\#} + \Delta G_{+}^0 + \Delta G_{-}^0}{2F} \quad (8)$$

Unfortunately the problem of unknown differences in hydration energy between the positively and negatively charged hydrophobic ions was still a unsolved issue. Flewelling and Hubbell were able to estimate the dipole potential of an egg phosphatidylcholine membrane to be 240 mV.

In 2002 Peterson et al. [44] dealt again with hydrophobic ions. Instead of using the voltage-clamp technique to measure the dipole potential, Peterson et al. used the charge pulse technique as described in section 4.2.2. The values of Ψ_D found for DPPC and DHPC were in good agreement with former results,

namely (243 ± 4) mV and (114 ± 7) mV respectively. Peterson et al. estimated also the Ψ_D of DPhPC to be (228 ± 5) mV.

2.4.2 Dyes instead of hydrophobic ions

In 1994 Gross et al. [12] and Zouni et al. [13] suggested independently of each other the use of voltage sensitive dyes to probe for the membrane dipole potential. They used styrylpyridinium dyes (f. ex. RH421 and di-8-ANEPPS), which bind to the lipids in the head group region. Depending on the electric field they are exposed to, their pK_a and their fluorescence excitation spectrum is shifted.

The pK_a -method had proven its value already. It was used to determine the value of the surface potential of lipid membranes. Depending on the strength of the surface potential the visible absorbance spectrum of the dyes changed. By applying this method on membranes consisting of zwitterionic lipids, i.e lipids with no surface potential, one made sure that changes in the absorbance spectrum are due to the dipole potential. Two conditions had to be ensured before a determination of the dipole potential with the pK_a -method was legitimate:

1. The absorbance spectra of the dyes must not be influenced by other membrane properties. Here the fluidity of the membrane was a controversial subject until 1997. Then Clarke et al. [14] found out that, if detected at an emission wavelength of 670 nm, the dyes absorbance spectra are independent of fluidity effects.
2. Voltage sensitive dyes need to be charged to function as probes for any kind of potential. It is therefore necessary to ask the question: Do the dyes themselves influence the membrane dipole potential? This question was first approached by Malkov and Sokolov in 1996 [15] and one year later solved by Clarke et al. [14]. They showed that, for molar ratios of lipid to dye ≥ 200 , the increase of dipole potential is negligible.

Disadvantages of this method were the strongly acidic pH values (pH 3 or lower), that were necessary to quantify the dipole potential. Under such acidic conditions the former zwitterionic lipids could get protonated and even the phase of the lipids could change. In the highly artificial membranes used in their experiments one went without the addition of membrane proteins. In a more realistic approach the protonation of these proteins needs to be taken into account.

They also investigated the effect of different substances on the dipole potential, by measuring the fluorescence ratio of the dye di-8-ANEPPS. They confirmed an increase of the dipole potential if 6-ketocholesterol or cholesterol was dissolved in the membrane. Phloretin, however was found to decrease the dipole potential. These effects were first observed by Bechinger and Seelig in 1991 [20] and Franklin and Cafiso in 1993 [21]. It is worth mentioning here, that cholesterol is a natural, widespread component of lipid membranes. The increase of dipole potential due to cholesterol results in a significantly higher conductance of hydrophobic anions compared to hydrophobic cations. It should

be kept in mind that a natural component of membranes can significantly change the electric properties of, and transport processes through the membrane.

As mentioned above Gross et al. measured shifts in the excitation spectrum of the dye di-8-ANEPPS. They related this spectral shift $\Delta\nu$ to a change in the dipole potential:

$$\Delta\nu = \left(-\frac{1}{h}\right)\Delta\mu E - \left(\frac{1}{2h}\right)\Delta\alpha E^2 \quad (9)$$

Where $\Delta\mu$ is the change in electric dipole moment of the dye upon electric excitation, $\Delta\alpha$ is the change in polarizability of the dye upon excitation, E is the electric field vector at the location of the chromophore and h is the Planck constant. The second term can be neglected and the first term describes frequency changes that depend linearly on the electric field. The electric field E is originates mainly from the dipole potential, because:

1. The transmembrane potential is smaller and decays over a larger area. They field due to this potential is therefore much weaker and can be neglected.
2. The electric field of the surface potential cannot effect the dye molecules, since they are dissolved in the membrane and the surface potential spans the surface and some layers of structured water attached to the surface. The potentials are sketched in fig.1.

Gross et al. determined the slope of the linear dependence between change in dipole potential and spectral shift. This data was then compared to data observed by Bedlack et al. describing the dependence between transmembrane potential and spectral shift. They found a 8 times stronger decay of dipole potential across the membrane compared to transmembrane potential. Since the transmembrane potential decays over the whole membrane, i.e. over a distance of approximately 40\AA , the dipole potential decays to zero already over the distance of 5\AA . This results in a very strong field of 10^7V/cm . This was a great result. For the first time one had an impression of the electrical field of the dipoles inside the bilayer membrane. The doubt mentioned in section 2.3, that the dipole potential may not be completely decayed over the thickness of the membrane, could now be neglected.

Unfortunately the voltage sensitive dye method could not be used to determine an absolute value of the dipole potential. Furthermore measurements in cells were not possible with the fluorescence microscopes. In 2007 Vitha and Clarke [58] took a closer look to the excitation ratiometric method using di-8-ANEPPS and found that this method is indeed suited for Ψ_D -measurement.

2.5 A new approach with monolayers

10 years ago, in 1998, Cseh and Benz [16] proposed a new method to determine the dipole potential. They measured the decrease of the surface potential of egg phosphatidylcholine monolayers, induced by titration of the membrane with phloretin. The negatively charged phloretin is supposed to decrease the surface

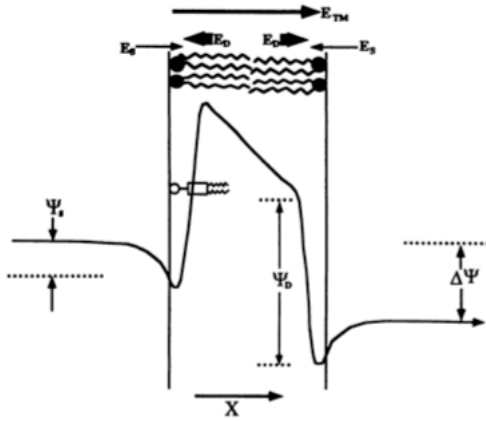


Figure 1: The electrostatic potential profile across a bilayer. The transmembrane potential, $\Delta\psi$, is the potential difference between the aqueous solutions on either side of the membrane. It arises from concentration differences of ions. The surface potential, ψ_S , is the potential difference between the membrane surface and the aqueous bulk. It arises from fixed charges at the membrane/water interface, f. ex. negatively charged head groups of lipid molecules. The dipole potential, ψ_D , is the potential difference between the center of the bilayer and the membrane/water interface. Electric fields of these potentials are represented by arrows in the upper part of the figure. Thickness reflects the field intensities, whereas the length reflects the regions over which the field is effective. To clarify the location of di-8-ANEPPS molecules one is sketched in the figure. (figure from [12])

potential, since it is a molecular dipole with the opposite direction compared to the membrane dipoles. If this is done until a saturation is reached, the reduce of the surface potential should equal the dipole potential. They however did not perform the experiment until saturation. The measured a dipole potential of 270 mV. The value under saturation is expected to be slightly higher.

Sukhorukov et al. (in collaboration with Benz) [17] pushed the experiments with the molecule phloretin further. In 2001 they achieved a more accurate determination of the dipole moment reduction through phloretin in biological cells and also great insights in the partition and translocation of lipophilic ions. They used a method called electro rotation. Here circular movement of the cell was induced by a rotating electric field. One obtained a dependence of the rotation speed of the cell on the frequency of the rotating electric field. Sukhorukov and Zimmermann [18] explained in their 1996 paper how one could use this dependence to obtain information about surface concentration, partition coefficient and translocation rates of an hydrophobic ion that was added to the system. However they did not use the hydrophobic ions TPB⁻ and TPP⁺, but the hydrophobic anion [W(CO)5CN]⁻. Already two years earlier Sukhorukov et al. found a relation between the dipole moment and the translocation of this anion.

$$\Delta\Psi_D = -\frac{RT}{F} \ln \frac{k_{i0}\beta_0}{k_i\beta} \quad (10)$$

Where k_i and k_{i0} are the translocation rate constants of the anion in the presence and absence of phloretin, respectively, with the corresponding partition coefficients $\beta_0 = N_{i0}/2c$ and $\beta = N_t/2c$; c is the anion concentration. N_{i0} and N_t are the membrane concentration of [W(CO)5CN]⁻ in presence and absence of phloretin, respectively. Sukhorukov et al. observed the translocation of the anion [W(CO)5CN]⁻ through mammal cells under the absence and presence of phloretin, respectively. They found a maximal decrease of the dipole moment, namely (10-15) mV, if the membrane was phloretin-saturated. This is an order of magnitude smaller than the decrease in artificial monolayers. The reason for that could not be fully cleared, but was supposed to be due to membrane proteins, which lower the lipid concentration and therefore also the dipole potential significantly. They mentioned furthermore, that the measured change of dipole moment was an average value measured over the whole cell. They considered the occurrence of strong local changes to be very likely. Finally they suggested a way to determine the absolute value of the dipole potential by using their electro rotation method: Given the future discovery of lipophilic ions detectable by the electro rotation method, a comparison of partition coefficients and translocation rates, as in the early experiments of Liberman and Toplay, could yield the absolute value.

Their second important achievement were some big steps in the understanding of the partition and translocation of lipophilic anions. First of all they showed that neither phloretin nor the anion [W(CO)5CN]⁻ disturb the integrity and viability of the cell. To show this they measured electrical properties of the membrane such as the membrane capacitance or conductivity. Hereby they confirmed the findings of Kürscher et al. [19] who already investigated these

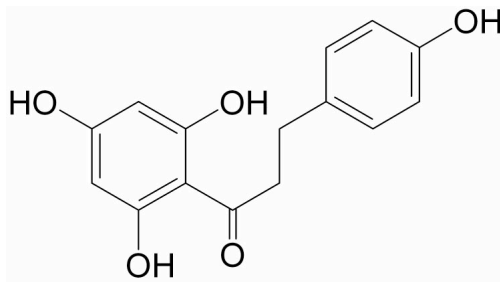


Figure 2: The structure of phloretin

effects on mammalian cells. Furthermore they found, that phloretin decreased the adsorption of $[W(CO)5CN]^-$ by lowering the intrinsic dipole potential of the cell membrane, whereas the translocation rate of the anion was found to be less sensitive to the treatment with phloretin.

The phloretin method was addressed again by Lairion and Disalvo in 2004 [50]. They correlated the change of Ψ_D , induced by phloretin, with the packing of lipids and with the formation of intermolecular hydrogen bonds. An alternation of lipid structure, more precisely a variation in the orientation of the ester carbonyls and/or the degree of water structuring, was proposed by Diaz et al. in 2001 [51]. Intermolecular hydrogen bonds between OH-group of phloretin and the P=O group of the phospholipid was proposed by Diaz et al. in 2001 and Disalvo et al. in 2002 [52].

Lairion and Disalvo confirmed the result of Cesh and Benz, that an increasing level of phloretin in the membrane decreases the dipole potential until a saturation occurs (some of their results in tab.1). They even extended their investigations and observed the effect of phloretin under different temperatures and surface pressures, i.e. in the gel and the fluid state of the membrane. They also found that the effect of phloretin is equal for ester and ether derivatives of PC. This yields that the effect of phloretin does not depend on the orientation of the carbonyl groups. The intermolecular hydrogen bonds however seem to have a large impact on the Ψ_D . By replacing water molecules around the P=O group and binding to it, phloretin decreases Ψ_D . Previous FTIR (Fourier transform spectroscopy) supported this theory: Diaz et al showed that phloretin promotes a pronounced downward shift of the frequency of the asymmetric vibrations of the phosphate group of PCs.

2.6 Maxwell-stress microscopy on Langmuir-Blodgett films

The scanning Maxwell-stress microscope (SSM) is a variant of the atomic force microscope (AFM) operated in the non contact mode, which can image the distribution of surface charge and potential over ultra thin films with a nanometer scale resolution. Improvements in the cantilever size made it possible to use the SSM also on biological samples such as Langmuir-Blodgett films (LB films).

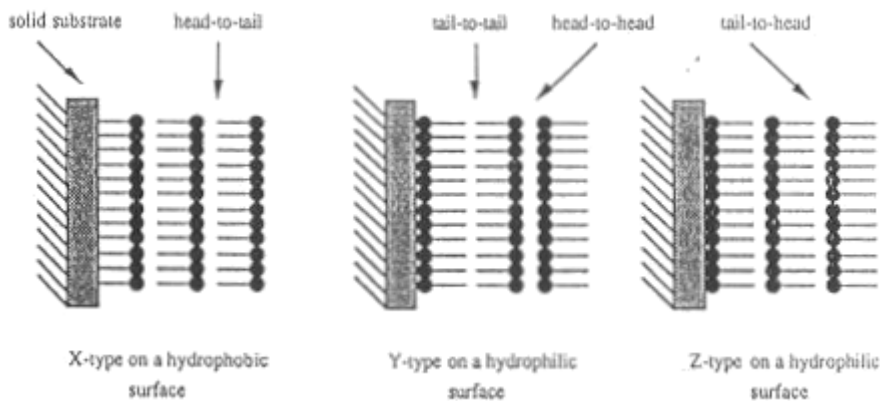


Figure 3: Three types of LB films. By varying the nature of the spread film, the sub phase composition and temperature, deposition speed and so on, one can obtain the different types of LB films shown here. The films are not necessary of lipid nature. Polymers, other water insoluble atoms or molecules can be used as well. Hundreds of monolayers can be piled up (from KSV Instruments Ltd.).

These films consist of many lipid monolayers stacked on top of each other as shown in fig.3. Schwartz et al. [39] started the investigations of biological LB films in 1992. They used films made of cadmium arachidate (n-Eicosanoate), steric and arachidic acids.

More interesting in the context of this article is the investigation of LB films of lipid monolayers. In 1994 Inoue and Yokoyama [40] carried out SSM experiments on dipalmitoylphosphatidylcholine (DPPC) monolayers piled up in a LB film. Mainly the SSM experiments yielded results regarding the surface potential, the topography and the dielectric constant of the films. The surface potential Ψ_S however could be related to the perpendicular component of the molecular dipole moment P and the molecular density ρ :

$$\Psi_S = \frac{\rho P}{\epsilon_0} \quad (11)$$

Therefore P could be estimated from the surface potential images obtained by SSM. A typical surface potential image is shown in fig.4. Inoue and Yokoyama found significant differences in P between the liquid expanded and liquid condensed phase. During a phase transition however the maximal density change is 10%. The huge differences in surface potential between the two phases therefore could not be explained by a change in density only. They were rather due to changes in the effective dipole moment near the polar head group of lipid molecules during phase transition. This was in agreement with the former mentioned results from the Vibrating plate method. However the finding that changes in the dipole moment are connected to the phase transition was new and striking.

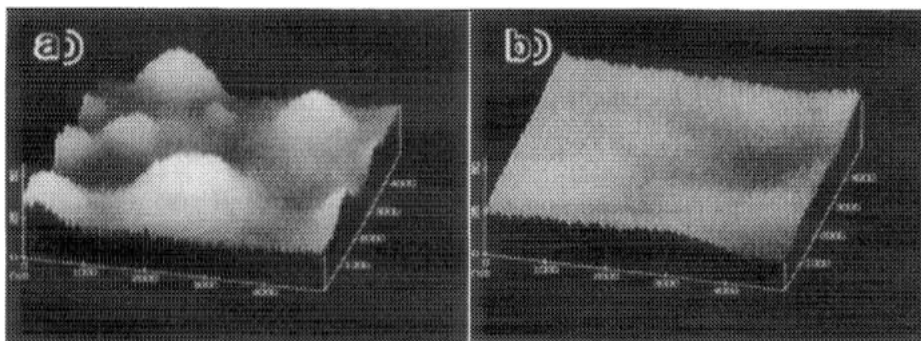


Figure 4: The surface potential images obtained from SSM of DPPC monolayers at a surface pressure of a) 10 nN/m and b) 40 nN/m. The monolayer in a) shows a mix of lipid areas in the so called liquid expanded phase (dark grey areas) and in the so-called liquid condensed state (white circular areas). The monolayer is in the phase transition regime. Differences in surface potential between these to phases up to 100 mV have been observed. Whereas the monolayer b) shows no such specific structure was observed. The measured surface potential was about 300 mV higher than the one measured for a) (from [40]).

2.7 Molecular dynamics simulations

In the last decade molecular dynamics (MD) simulations of lipid membranes have gotten more and more popular due to the rapid improvement in computational power. MD simulations had been quite successful in reproducing the membrane structure measured with X-ray and neutron scattering. However MD simulations yield more than static structural information. Elasticity, energy barriers and dynamical properties such as diffusion can be estimated by MD calculations.

Since the number of molecules that need to be modeled is fairly huge, the number of confirmations of these molecules is even a few orders of magnitude higher. Therefore MD simulations typically only range over a time span of pico- or nanoseconds. Furthermore - again caused by the limitation in computational power - MD predictions of electrostatic details are less reliable, because the models only use point charges and do not allow for the polarization of atoms and bonds in response to electric fields.

The results of some groups working with MD regarding the dipole potential are listed in tab.1.

2.8 The effect of cholesterol

Cholesterol is a major component of the lipid membrane and was known to increase Ψ_D (Szabo, 1974 [45]). In 2006 Starke-Peterkovic et al. [46] took a closer look to this phenomena. They used the voltage-sensitive fluorescent probe di-8-ANEPPS and the ratio metric method that was already used by Gross et

al. in 1994 [12]. Starke-Peterkovic et al. used however natural membrane lipids extracted from the kidney and brain of several vertebrate species, instead of artificial membranes.

The level of cholesterol in the membrane was regulated by cyclodextrin. A reduction of 80% of the initial cholesterol concentration resulted in a decrease of Ψ_D of 50 mV. The interesting observation was however that the intrinsic dipole moments of cholesterol molecules account only for 10-30% of the total change in dipole potential. This is due to the electric field they produce. The rest of the dipole potential change must arise from a further indirect effect of the cholesterol molecules.

One additional reason for a change in Ψ_D was suggested by Tu et al. [47] based on molecular dynamics simulations. They assume that the lipid head groups rotate toward the the bilayer to fill spaces left by the cholesterol molecules and therefore a reduced compensation of the oriented water to the head groups' dipole potential. Starke-Peterkovic et al. used different cholesterol derivatives with comparable sizes, but the results differed significantly. The contribution suggested by Tu et al. can therefore not be the only one beside the intrinsic dipole moment of cholesterol.

Starke-Peterkovic et al. called another mechanism to account for the change in dipole potential: Variations in lipid packing density. It had been known for a long time that cholesterol has a condensing effect on lipid membranes. Hence removing of cholesterol resulted in a decrease of packing density, $1/A$. According to Helmholtz equation 7 a decrease in packing density goes along with a increase in Ψ_D . Unfortunately not even the combination of intrinsic dipole moment of cholesterol and the change in packing density could account for the immense change in dipole potential.

Simon and McIntosh [48] showed that the removal of cholesterol from the bilayer membrane of bacterial phosphatidylethanolamine increased water penetration into the membrane. Due to this water penetration the dielectric constant, ϵ , in the region of the ester carbonyl groups decreased significantly. It was very likely that these effects also occur in phosphatidylcholine bilayers. A drop of the dielectric constant in this area increased the dipole potential.

The cooperation of these three effects was able to account for the Ψ_D -change.

2.9 Cryo-electron microscopy

Wang et al. in 2006 [49] proposed to record the phase shift of electrons, as they pass through regions with different electrostatic potentials in rapidly frozen phospholipid bilayers, to obtain a good estimate for the membrane dipole potential, Ψ_D . If the electrons pass through the sample they underwent a phase shift in the electron-wave function. Wang et al. used bright-field, phase contrast imaging to detect these phase shifts. Bases on structural information from MD simulations they were able to analyze the data, since the phase shift was proportional to the integrated electrostatic potential along the path of the electron. The electrons functioned as point charges, comparable to the hydrophobic ions or voltage-sensitive dyes used earlier by other groups to estimate Ψ_D . How-

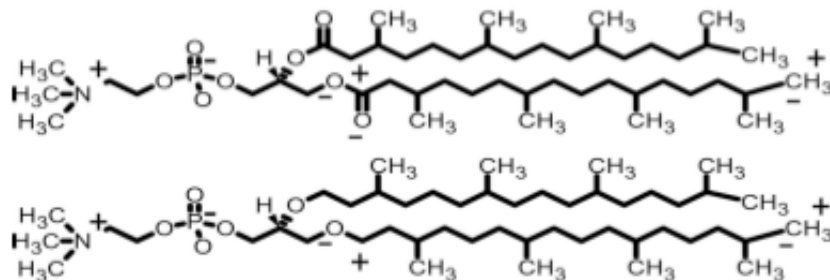


Figure 5: The two lipids used by Wang et al. to estimate the Ψ_D with the help of cryo electron microscopy. The upper is ester-diphytanyl PC and the lower is ether-diphytanyl PC. Note that the parts of the lipid molecules that contribute most to the dipole potential are labeled with '+' and '-' (from [49]).

ever the electrons had the big advantage of very small size and no differences in hydration energy over the two other probes. They investigated mainly to kinds of phosphatidylcholines (PCs) shown in fig.5 and obtained the following estimations for Ψ_D :

1. Diphytanyl PC an ester-PC, like the ones used in monolayer and bilayer studies described above. The peak dipole potential on the path of the electron through the membrane was 510 ± 70 mV.
2. Diphytanyl PC an ether-PC, like the ones used frequently in MD simulations of lipid membrane systems. The peak dipole potential was 260 ± 130 mV.

In this experiment Wang et al. determined the peak dipole potential, because the electron probes measure the electrostatic potential along their entire path. Therefore they also obtained a spatial potential profile as shown in fig.6. The dipole potential they measured was usually smaller than the one simulated by MD and was fluctuating. However it is larger than the Ψ_D 's obtained by monolayer and other bilayer techniques. Disadvantages are the low sensitivity that results in a error in the order of 100 mV and the fact that some assumptions have been used throughout the experiments. Wang et al. assumed that the electrostatic features of the specimen are preserved under the extreme temperature conditions of a cryo electron microscopy experiment and that the model they used for phase-contrast imaging is correct. This models relatively new and still under research. The big discrepancy of their Ψ_D values to the ones obtained by MD simulations are according to Wang et al. not at all surprising, since MD simulations do not take polarizability of atomic charges into account.

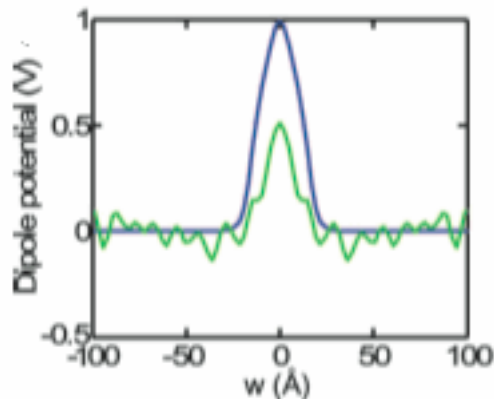


Figure 6: Comparison of Ψ_D profiles from the cryo-EM method (green curve) and MD simulation (blue curve) of ester-PC. The center of the bilayer membrane is represented by $w=0$ Å and the location of the head groups is around $w=\pm 20$ Å. The profile has its maximum at the center of the bilayer; a distinction between the single contributions to the dipole potential, i.e. head groups, ester linkage and terminal end, is not possible, due to the resolution of this technique (from [49]).

2.10 Overview

So far a lot of steps towards the determination of the dipole potential of lipid membranes, Ψ_D , have been discussed. It is time to provide you the reader with an overview over the estimated values for Ψ_D as shown in tab.1.

3 Origin of the dipole potential

3.1 The components of the dipole potential

The dipole potential of a lipid bilayer is nowadays supposed to consist of the following components [17]: The orientation of phospholipid carbonyls in the head group regions of the bilayer, the ester linkage between the head group and the fatty acid chains and the terminal end of the fatty acid chain. These three contributions are shown in fig.5. Plus layers of structured water attached to the surface of the bilayer, which cause a organization of the water dipoles.

The lipid head groups carry charges, even if they are zwitterionic and appear to be electrical neutral. Then they carry exactly one positive and one negative charge. An electric dipole is defined as a separation of positive and negative charge. This definition is fulfilled by all lipid head groups. In case of the zwitterionic lipids the charges are of equal magnitude. In 1979 Büldt et al. [23] used neutron diffraction to determine the orientation of the lipid dipoles. They found out that the P^-N^+ dipoles of a zwitterionic phosphatidylcholine

Method	PC (Ester) in mV	PC (Ether) in mV	Notes
Bilayer, hydrophobic ions	227±9 [6]	109±6 [6]	DPPC
Bilayer, hydrophobic ions	240±70 [11]		egg-PC
Bilayer, hydrophobic ions	310 [4]		PE
Bilayer, hydrophobic ions	227 [6], 243±4 [44]	228±5 [44]	DPPC
Bilayer, hydrophobic ions	109 [6], 114±7 [44]		DHPC
Bilayer, cryo-EM	510±70 [49]	260±130 [49]	DPhPC
Monolayer, Kelvin method	≈400 [9]		different PC's
Monolayer, phloretin method	270 [16]		egg-PC
Monolayer, phloretin method	449±24 [50]	360 [50]	DMPC
MD simulation of bilayer	1002 [54]	567 [54]	DPhPC
MD simulation of bilayer	600 [55], 557 [56]		DPPC
MD simulation of bilayer	500 [57]		DOPC

Table 1: An overview over the estimated values of Ψ_D

membrane are oriented almost parallel to the membrane surface. This result was confirmed by Seelig et. al [24] in 1987 by NMR studies on the same lipid.

As a next step many groups investigated the effect of binding of positively charged molecules to the membrane on the dipole orientation. They used metal ions (Brown and Seelig, 1977 [25]), local anesthetics (Boulanger et al., 1981 [26]; Seelig et al., 1988 [27]) and peptides (Dempsey et al., 1989 [28], Roux et al., 1989 [29]). In all cases the N⁺ end of the head group moved towards the water phase, i.e. away from the membrane surface. Scherer and Seelig [30] used dialkyl phosphates to generate a negative surface charge. As expected the N⁺ end moved towards the hydrocarbon phase. The mechanism that changes the dipole potential is therefore based on electrostatic attraction and respectively repulsion. By changing the angle of the dipole with respect to the membrane surface the dipole moment is varied and therefore also the measured value of the dipole potential.

$$\vec{P} = q\vec{r} \quad (12)$$

Where \vec{r} is the displacement vector pointing from the negative charge to the positive charge. This implies that the electric dipole moment vector \vec{P} points from the negative charge to the positive charge too; q is the charge of the dipole. The electrostatic potential at position \vec{R} due to an electric dipole at the origin is given by:

$$\Psi_D = \frac{1}{4\pi\epsilon_0} \frac{\vec{P}\hat{R}}{R^2} \quad (13)$$

The definition of a dipole is also fulfilled for the ester linkage and the terminal end of the fatty acid chain, although there are no charged atoms as in the head group. The atoms that form these bonds have different electronegativities and therefore carry partial charges of opposite sign. The contribution of these two bonds is even greater than the contribution of the head groups, since the water dipoles partly compensate the dipole potential of the head groups.

3.2 What about the fatty acid chains?

Already in 1955 Davies and Rideal [41] suggested that another component is required to account for the measured dipole potential, Ψ_D , beside the contribution of the head groups, terminal CH₃-group and the ester linkage. This component was supposed to be a contribution from the CH₂-bonds in the fatty acid chain. Numerous experiments to evaluate the contribution of the fatty acid chains have given controversial results. The results reached from a contribution of 9 MV per CH₂-bond (Mingins et al., 1992 [42]) to a contribution only from terminal methyl group (Vogel and Möbius, 1988 [43]).

In 2002 Peterson et al. [44] addressed the problem again. The investigated alterations in Ψ_D induced by substitution of a sulfur atom for a methylene group in one of the hydrocarbon chains. The also took into account that the incorporation of sulfur atoms slightly varies the packing density of the lipids in the membrane. They used the charge pulse method (described below) to determine Ψ_D of unlabeled DPPC membranes and sulfur-labeled DPPC membranes. A

comparison of the results led Peterson et al. to the conclusion that the only contribution of the fatty acid chains to Ψ_D is a steric one, i.e. a change of lipid packing density results in a change of dipole density and also dipole moment (if the steric effect causes a rotation of the lipid molecules). A contribution beside the steric one, i.e. an intrinsic dipole moment of the fatty acid chains could not be confirmed for the CH₂-bonds.

4 Some techniques

4.1 Monolayer techniques

4.1.1 The vibrating plate (Kelvin) method

In this method, that was introduced for the first time by Lord Kelvin and improved by Zisman [33], a metal plate functioning as an electrode was brought close to the surface of the aqueous phase. The non-conducting air gap between the plate and the aqueous phase acted as a capacitor. Vibrations of the metal plate around 200 MHz (Piezo) gave rise to an AC current in the circuit due to the potential difference between the metal plate and the aqueous phase $\Psi_{MP} - \Psi_W$. Application of a DC voltage, V , opposite to that between the electrodes, compensated the inter facial potential. This was done until no measurable current flew through the circuit, i.e. the applied voltage matched the inter facial potential.

$$V = \Psi_{MP} - \Psi_W \quad (14)$$

The voltage V was measured in the absence and in the presence of a lipid monolayer in the aqueous phase. The difference between this two measurements was an estimation of the dipole potential of this specific monolayer. This method was used f. ex. by Smaby and Brockman [9] as described in section 2.3. A more contemporary variation of the Kelvin method was an electro chemically edged tonsten wire as in the scanning tunnel microscopy instead of the plate electrode to observe a very small area of membrane only (lateral resolution of 5 μm). This was done by Nonnenmacher et al. in 1991 [31] and Mäckel et al. in 1993 [32]. A big advantage of the Kelvin method over other monolayer techniques was the fact that the Kelvin method could also be used for an oil/water interface.

4.1.2 The ionizing electrode method

This method was also used by Smaby and Brockman and was closely linked to the Kelvin method described above. A potential difference between metal plate and aqueous phase $\Psi_{MP} - \Psi_W$, as in the Kelvin method, gave rise to a current. In this method however the current was not induced by a vibrating plate, but by a ionizing material that coated the plate electrode. The ions made the air gap conductive. Traditionally a compensating voltage was used to determine the potential difference. More modern amplifiers with a very high resistance (up to $10^{14}\Omega$) made it possible to measure this current very precisely. Therefore the compensating DC voltage circuit used in the Kelvin method became

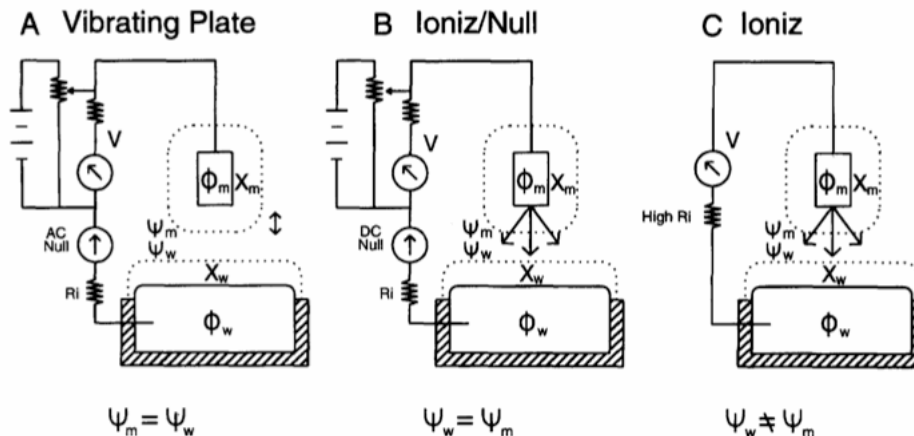


Figure 7: Simplified circuits for the measurement of air-water or oil-water dipole potentials. X denotes the difference between the potential, Φ , of the bulk phase metal electrode (m) or water (w) and the potential of the bulk air or oil phase, Ψ , adjacent to it. Part A shows the Kelvin method. Part B the traditional ionizing electrode method with the compensating DC voltage, whereas C shows the modern version without the DC circuit (from Brockman [10]).

unnecessary. Again the potential difference was measured in the presence and absence of the lipid monolayer to determine the dipole potential. However the sample had to be cleaned during longer experiments or the experiments had to be kept short, since the ions contaminated the sample.

4.1.3 A current measuring method

In 1989 M. Iwamoto and Y. Majima [34] developed a new current-measuring technique to replace the conventional surface potential method, i.e. the direct measurement of the capacitance that developed across an air/water interface. In this newer technique, two electrodes parallel to the water surface were used. One electrode was placed in the water while the other was suspended in the air. Both electrodes were grounded. They investigated physical properties of lipid monolayers and determined the vertical component of the dipole moment of the acid molecules.

4.1.4 Field gradient electrophoresis

In 1993 Klinger and McConnell [36] suggested an method based on electrophoresis to examine the dipole potential of lipid monolayers. They used the fact that lipid monolayers at the air-water interface frequently showed coexisting lipid phases, which could be visualized by epifluorescence microscopy. The interaction of such lipid phases was thought to be governed by electrostatic forces

related to differences in the dipole moment density between the phases. They exposed single lipid domains in a drift-free monolayer to an inhomogeneous electric field of known geometry and strength. By observing the dynamics of the motion of a single domain, both sign and magnitude of the differences in dipole moment density between the phases could be measured.

4.1.5 Ratio metric fluorescence measurement

This method was used f. ex. by Gross et al. in 1994 [12]. They used the potential-sensitive fluorescent dye di-8-ANEPPS. As a result of altering the dipole potential, the ratio of the dye fluorescence excited at 440 nm to that excited at 530 nm changed too. The excitation ratio was found to be independent of fluidity effects. This was essential since the alteration of dipole potential was usually achieved by incorporating cholesterol or phloretin into the membrane, i.e. substances that were known to change the membranes fluidity.

Vitha and Clarke in 2006 [58] confirmed these findings, but also found that emission ratio measurements were not suitable for the measurement of the dipole potential. In contrast to the excitation process the time scale of the emission process was long enough, so that neighboring solvent molecules could reorient to stabilize the excited species (a process called relaxation). This relaxation led to a decrease of the energy gap between excited and ground state. The emitted light's energy was therefore shifted, by the so-called Stokes' shift.

4.1.6 Scanning Maxwell stress microscope

This technique is related to the former described advanced Kelvin method, i.e. the one that used the tonsten wire. However there is one great difference: Instead of detecting a null current, the Maxwell stress microscope uses harmonic analysis of the electric field-induced oscillations of a cantilever of the type used in atomic force microscopy. From this analysis surface potential, surface dielectric constant and surface topography differences can be detected with sub micron resolution [38]. First it was only demonstrated to operate in air and with non-organic samples, but improvements in cantilever size allowed for the usage of it with biological samples under water. Modern Maxwell-stress microscopes offer a resolution on nanometer scale. These microscopes were used by H.K. Shin et al. in 1999 [37] Langmuir-Blodgett films. With this technique H.K. Shin et al. were able to determine the topography and the surface potential distribution of the monolayer piles. They also investigated the domain formation of metal ions within the membrane. Due to the high resolution of the microscope, the ions were easy to localize (see fig.4).

4.2 Bilayer Techniques

4.2.1 Translocation of hydrophobic ions

Since bilayer membranes did not lean themselves to direct potentiometric measurements, other techniques had to be developed. The use of hydrophobic an-

ions and cations with similar physical and chemical properties was one of these techniques and was also the choice for the first attempt to estimate the dipole potential of lipid membranes [2, 4]. The basis for these measurements was the energy barrier to ion transport caused by the potential in the center of the membrane relative to the bulk phase. This produced differences in transport rate constants for anions and cations which could differ by five orders of magnitude. The sum of dipole potential and surface potential (as during all monolayer experiments) could be measured if all other factors were assumed to be constant. Plus the partitioning of the ions between the aqueous and membrane phases had to be measured independently, so that binding and transport data could be modeled. The value of the surface potential could be measured with other methods and was then subtracted to obtain the dipole potential.

4.2.2 The charge pulse method

To measure the potential across a bilayer the voltage clamp technique was normally used. Pickar and Benz in 1978 [5] however suggested an alternative technique. In this so-called charge pulse technique the membrane capacitance was charged to a voltage of approximately 10 mV by a short-lived current pulse through silver/silver-chloride electrodes. After charging the membrane got isolated from the external circuit so that the following decay resulted from charge transport processes across the membrane only. Time constants and relaxation times could be determined. The advantages of this technique to the voltage clamp were:

1. Only very small voltages had to be applied on the very voltage-sensitive membrane.
2. Relaxations fast as 1 μ s could be measured.

The transport processes across the membrane were usually represented by the translocations of hydrophobic ions such as TPB⁻ and TPP⁺.

4.2.3 Cryo-electron microscopy

See in paragraph 2.9.

5 To be continued..

Despite numerous attempts to estimate the dipole potential - from which only a few have been mentioned here - the differences in estimated Ψ_D -values are significant. They are of the same order of magnitude as the dipole potential itself. The discrepancy becomes even worse if MD simulations are taken into account. Nevertheless MD simulations seem to be necessary, since the measurement of the dipole potential is still difficult and based on a lot of assumptions. Improvement is expected with the increase of computational power, that will allow for more detailed measurements.

Another promising approach seems to be the cryo-EM technique, that is able to provide not only a peak dipole potential, but even more important a dipole potential profile. With more sensitive measurements one could distinguish between the three contributions to the total dipole potential. Although it seems that there is a general agreement regarding the origin of the dipole potential nowadays, more sensitive EM would make new discoveries possible, or confirm the present idea. The effect of rapid freezing and low temperatures on the electrostatics of the membrane need more investigation. However cryo-EM cannot provide any dynamical information. To obtain dynamical information MD simulations are a better choice.

The importance of the exact determination of the dipole potential profile across the membrane (some reasons for that are stated in the introduction) makes further experiments, simulations and theoretical work mandatory. I am therefore convinced that this summary needs to be updated in the near future.

6 Acknowledgments

I hereby thank Thomas Heimburg for the inspiration he gave me to start this work. Furthermore I thank H.L. Brockman and R.J. Clarke for their great prior overviews over the membrane dipole potential, that helped me a lot during my work on this paper.

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