

# Boundary potential of lipid bilayers: methods and interpretations

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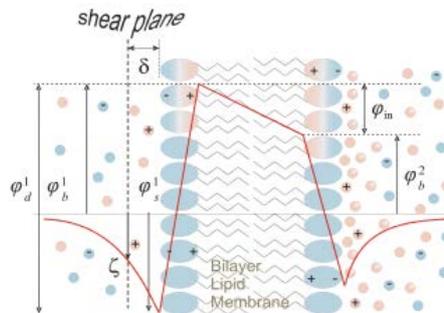
**Abstract.** The electric field distribution at the boundaries of cell membrane consists of diffuse part of the electrical double layer and the potential drop over polar area inside the membrane itself. The latter is generally attributed to the dipole effect, which depends on the lipid hydration and phase state. This report focuses on the experimental approaches developed to detect the relation between dipole effects and the bilayer structure, and to study their molecular nature. The total boundary potential (BP) of planar bilayer lipid membranes (BLM) can be controlled by Intramembranous Field Compensation (IFC) method developed in our laboratory. When combined with electrokinetic measurements in liposome suspension it allows detecting the changes of the dipole potential due to adsorption of inorganic cations and charged molecules. Multivalent inorganic cations increase the dipole potential up to 100-150 mV and make the membrane rigid. Most of these observations were simulated by Molecular Dynamics (MD) in order to visualize the relationship of electric field with the different structural factors (lipid structure, water orientation, ion adsorption etc.) responsible for its dipole component. Two principal contributors to BP – water and lipid molecules – create the opposite effects. The negative contribution with respect to the bulk is due to lipid itself and the inorganic cation penetration into the polar area of membrane. The positive contribution is caused by water orientation. Particularly, in the case of lysine adsorption, the contribution of water includes the rearrangement of H-bonds with the lipid phosphate group. This fact explains well the unusual kinetic phenomena registered by IFC in the case of polylysine adsorption at the BLM surface.

The electric field distribution at the boundaries of phospholipid membrane was intensively studied in many different aspects concerning its biological importance. Naturally, this field is sensitive to the ionic composition of the media and becomes essential for any membrane transport of ionized substances. In other hand, many water-soluble drugs primary interact with the outer surface of cells by electrostatic forces. Model lipid systems - liposomes, planar bilayer lipid membranes (BLM) and monolayers – are commonly used to study the electrostatic phenomena and their relation to structure of membrane interfaces. Our presentation focuses on the experimental approaches developed in our laboratory to differ two components of the boundary potential – the electric field in the water solution that corresponds to the diffuse part of the electrical double layer, and the electric potential drop over polar area at the lipid/water interface [1]. The first one, named here as the surface potential,  $\phi_s$ , is related to screening of surface charge by ionic media as it well defined by the classic Gouy-Chapman model. To describe the ion equilibrium at the membrane surface the classic model was supplemented with Langmuir-type isotherms. We do not present details of this classic theory, known as Gouy-Chapman-Stern (GCS), which is described in many original papers and reviews [2–4]. The second component of the boundary potential (BP),  $\phi_b$ , is generally attributed to dipoles oriented across the membrane interface and named as a dipole potential,  $\phi_d$ . This potential is determined by charged groups of lipids, adsorbed substances, and by inorganic ions placed under Helmholtz plane, which corresponds to the outer membrane surface. Naturally, the adsorption of cations shifts the surface potential to positive values but some of them may decrease the dipole potential by their effect on the lipid packing. In any of above-mentioned cases, the adjacent water molecules make the major



contribution to this potential. Several examples of this presentation are aimed to illustrate these situations with the aid of methods developed in our laboratory and applied to planar BLM.

Two principal components of the electric field at the membrane/water interfaces are qualitatively illustrated in figure 1.

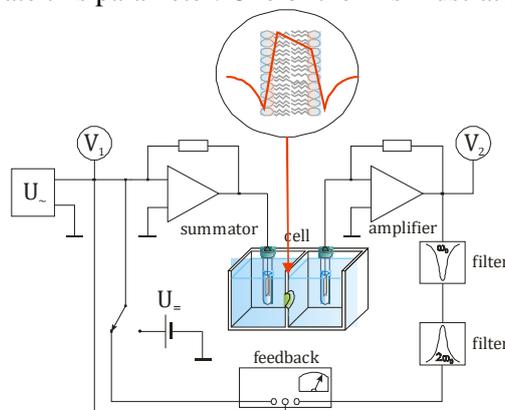


**Figure 1.** Electric field distribution (red line), the surface ( $\phi_s$ ) and dipole ( $\phi_d$ ) components of boundary potentials ( $\phi_b$ ) at the lipid membrane interfaces. Dotted line illustrate the position of shear plane in the electrokinetic measurements.

The electric field across the membrane is shown in figure 1 by red line. We assume that the membrane has negatively charged lipids and separates a different ionic media at both BLM sides. The electric potential has the same value in the bulk according to short circuit condition. The negative surface potentials ( $\phi_s^1$  and  $\phi_s^2$ ) drop at both sides up to zero in the bulk; the dipole components ( $\phi_d^1$  and  $\phi_d^2$ ) are directed to high but different positive value inside the membrane. The latter fact follows from many experimental works (i.e. [5–7]). Finally, trans-membrane potential is defined by difference between boundary potentials, which become equal to potential drop over the hydrophobic area of the membrane (intramembraneous field) in the short circuit condition:

$$\phi_{in} = \phi_b^1 - \phi_b^2 \tag{1}$$

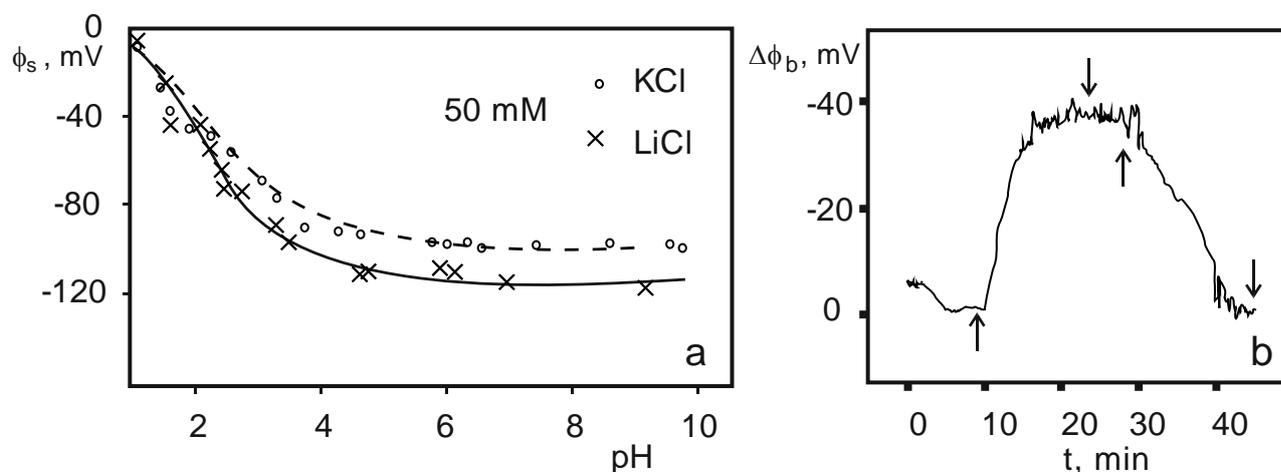
Some principal points are essential to note. The sign and absolute value of electric potential at the membrane surface is available in electrokinetic measurements at liposome suspension. Data of these measurements shown below were collected from dynamic light scattering experiments. The electrokinetic mobility of liposomes in the typical conditions is related to the electric potential (zeta-potential) at some distance,  $\delta$ , from the surface in so called “shear plane”. The alternative electrostatic methods are required to evaluate this parameter. One of them is illustrated below (figure 4).



**Figure 2.** Block-diagram of the setup to measure the difference between the boundary potentials of planar BLM by intramembraneous field compensation (IFC) with the help of second harmonic signal caused by membrane electrostriction [1].

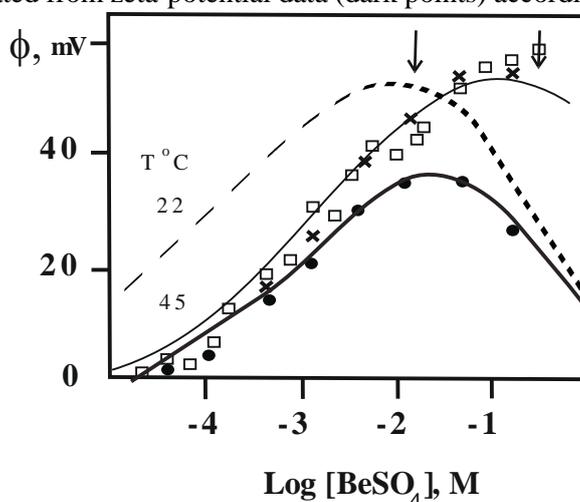
Block diagram in figure 2 illustrates the general experimental setup with planar BLM formed in the small hole (about 1 mm in diameter) and some electronics to detect higher harmonics signal of the circuit caused by electrostriction effect [1]. The real setup combines analog devices with their digital versions controlled by computer. It applies a combination of AC and DC voltages to the membrane and register high harmonic signals caused by BLM electrostriction property. In some experiments (figure 3), electric signals in the system registered during the variation of medium composition at one side of the BLM by permanent perfusion the proper cell compartment by peristaltic pump (not shown). Generally, the external DC voltage,  $V$ , is applied between the bulks at both membrane sides and compensates the potential drop  $\phi_{in}$  (1). As it follows from the simple analysis of the electric circuit, the capacitive current of “elastic” BLM have the second harmonic with amplitude proportional to difference  $(\phi_{in}-V)$  and the third harmonic – to BLM transversal elasticity. This method, named Intramembrane Field Compensation (IFC), was intensively used in combination with electrokinetic measurements in liposome suspension as a principal instrument to study the electrostatic effects attributed to dipole potential. A few examples listed below demonstrate some advantages of this approach.

Several experiments shown in figure 3 were performed with liposomes and BLM from phosphatidylserine (PS) in solutions of the same concentration (50 mM) but with different monovalent cations –  $Li^+$  and  $K^+$ . Left panel shows surface potential deduced from zeta potential due to electrokinetic measurements at different pH. Theoretical curves correspond to Gouy-Chapman model and Langmuir isotherm with parameters – dotted line and solid line. Experiment demonstrates the flat region for totally ionized charged PS with a difference explained by a different binding constants of these cations. Note, that adsorption of potassium cations seems more effective and surface potential is about 15 mV more positive than for lithium cations. Right panel of the figure demonstrates the direct comparison of these cations in perfusion experiment with planar BLM. Arrows indicate the moments of turn on (upper) and turn off (down) the pump, which replaces LiCl solution at one BLM side by KCl of the same concentration (50 mM) or replaces them back. If  $Li^+$  is replaced by  $K^+$  the changes of boundary potential is about 35 mV in negative direction in contrast to electrokinetic data. So, we may conclude that the difference between dipole potential at the membrane boundaries in these solutions was equal to 50 mV. The nature of this difference may be related to different position of these cations in respect to Helmholtz plane, to their effect on lipid packing and hydration of headgroups, to water orientation and H-bonds. All these phenomena defined here as dipole effect and well suitable for future analysis by methods of molecular dynamics.



**Figure 3.** Electrokinetic data (left) for PS liposomes in LiCl (crosses) and KCl (opened points) electrolytes and the boundary potential changes (right) when LiCl is replaced with KCl solution and back by permanent cell perfusion shown by up and down arrows.

The next example (figure 4) demonstrates another type of dipole effect concluded from electrokinetic and IFC experiments with liposomes and planar BLM from dipalmitoyl phosphatidylcholine (DPPC) [8]. Adsorption of divalent cations  $\text{Be}^{2+}$  at initially uncharged DPPC membranes creates the positive surface charge and potential both at the surface of liposomes and BLM both below and upper the temperature of phase transition. Theoretical curves correspond to Gouy-Chapman model and Langmuir isotherm of  $\text{Be}^{2+}$  adsorption at PC with a binding constant  $400 \text{ M}^{-1}$  ( $45^\circ \text{C}$ , black line)  $104$  ( $22^\circ \text{C}$ , dotted line) and shear plane position  $\delta=0.2 \text{ nm}$ . Crosses correspond to surface potential recalculated from zeta-potential data (dark points) according Gouy-Chapman theory.

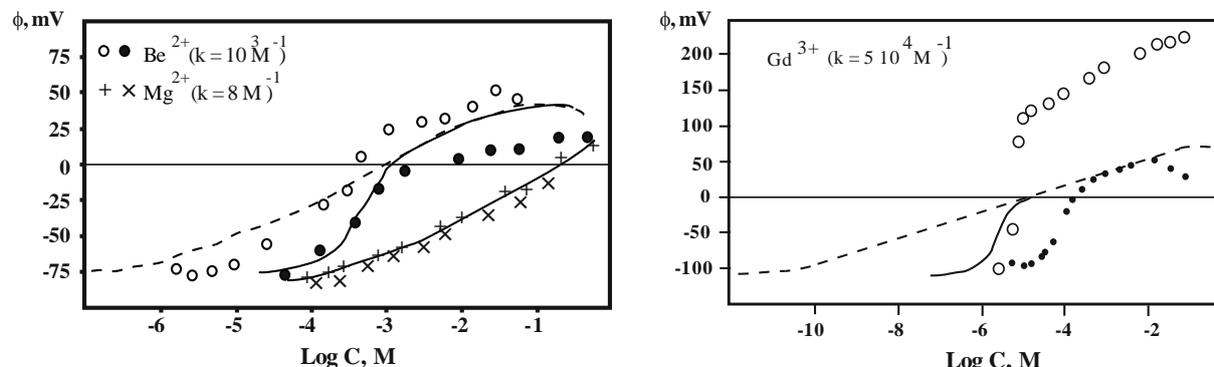


**Figure 4.** Electrokinetic data for DPPC liposomes (zeta potential) and boundary potential (BP) changes of planar BLM (black and opened points) measured at different  $\text{BeSO}_4$  concentration. Dotted and solid curves are calculated in the frame of GCS model for temperatures below and behind the point of phase transition. Arrow shows BP changes registered by cooling the experimental cell with BLM [8].

Two conclusions follow from data of figure 4: screening the surface charge by electrolyte of known ionic strength is well described by the model, and the surface potential recalculated from zeta potential with parameter  $\delta=0.2 \text{ nm}$  corresponds well to changes of the boundary potential at any salt concentration when the dipole effect is negligible. All measurements presented in figure 4 were done and compared at the temperature  $45^\circ \text{C}$  above the phase transition of DPPC because at low temperature planar “solid” BLM became unstable [9]. Parameter  $\delta$  determine the position of maximum of the curves, which in the case of surface potential is shifted to very high salt concentrations not available in the experiment. It looks natural because GCS model predicts the potential sensitivity to ionic strength of the media (screening effect) increased with a distance (in shear plane) from the surface in water solution. Moreover, the position of maximum of zeta potential depends on the ionic strength only but does not depend on the effectiveness of cation adsorption. Near the same position of maximum appeared for any salts with divalent cations of different affinity to lipid membranes [10].

Dotted line in figure 4 corresponds to electrokinetic data for  $22^\circ \text{C}$  with liposomes in ‘solid’ state, which became more sensitive to  $\text{Be}^{2+}$  adsorption, and the surface potential is more positive. Unfortunately, at low temperature it is impossible to compare electrokinetic and IFC data directly. Nevertheless, if the experimental cell with “liquid” planar BLM is cooled we may fix the trend of boundary potential changes (shown by arrows at the top of the figure). In contrast to electrokinetic data, the boundary potential became more negative. This fact suggests a strong dipole effect induced

by adsorbed  $\text{Be}^{2+}$  in opposite direction to surface charge and surface component of boundary potential. Recently, this conclusion proved by our MD simulation of the same system [11].

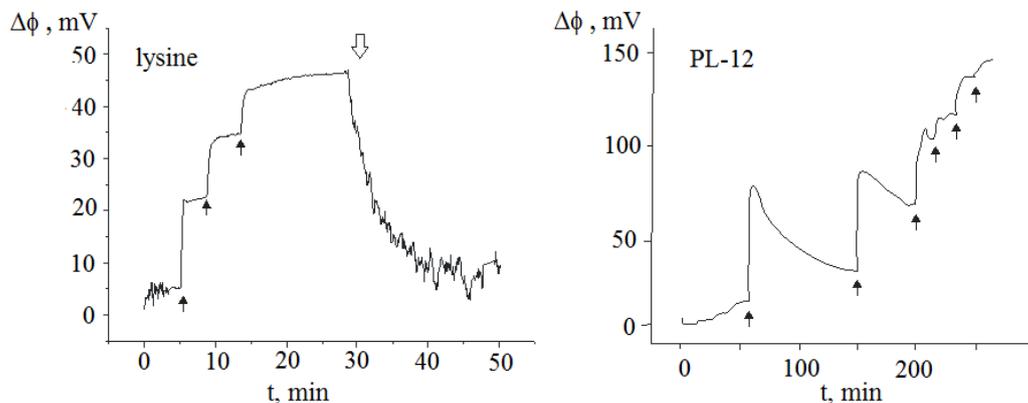


**Figure 5.** Effect of multivalent cations registered by electrokinetic measurements in liposome suspension (black points) and by boundary potential changes measured by IFC method applied to planar BLM (opened circles). Theoretical curves are calculated by classic GCS model for dotted curves at both panels and for  $\text{Mg}^{2+}$  adsorption or by modified model included the material balance condition – solid curves for  $\text{Be}^{2+}$  and  $\text{Gd}^{3+}$  adsorption [7,8].

Multivalent cations induce significant changes in both component of boundary potential at the membranes composed from negatively charged lipids, as phosphatidylserine in figure 5 [7]. If the cation affinity is not high, these changes are similar and well defined by classic GCS model ( $\text{Mg}^{2+}$  in figure 5). Cations of high affinity ( $\text{Be}^{2+}$  and  $\text{Gd}^{3+}$ ) demonstrate a high changes of boundary potential (opened points in both panels in figure 5) which exceeds much the changes of the surface potential recovered from electrokinetic measurements (closed points). Moreover, the slope of experimental curves (dotted lines in figures) does not correspond to GCS predictions. It was observed about 60 mV per decade in the experiment in contrast to the theory with 30 and 20 mV per decade for di- and tri-valent cations, correspondingly. To get the quantitative description of electrokinetic data one has to extend GCS model by the condition of mass balance, i.e. to sum the amount of ions in the bulk, in diffuse and compact parts of the electrical double layer. Note it means that the real bulk concentration of these cations remains unknown because of depletion effect and replaced in figures by their total amount in the experimental cell. According to the modified GCS model cations of high affinity to PS became “potential determining ions” at very low bulk concentration –  $10^{-6}$  or  $10^{-10}$  M for  $\text{Be}^{2+}$  and  $\text{Gd}^{3+}$  correspondingly [7,12]. This effect was related recently with the cooperative phenomenon of lipid phase transition of the membrane to “solid” gel state accompanied by lipid-cation cluster formation. This interpretation correlates well with the change of dipole potential induced by high-affinity cation adsorption at the membrane interface. In the examples of figure 5 the difference of boundary and surface potential is clearly observed. According to quantitative analysis of  $\text{Gd}^{3+}$  adsorption at the lipid membranes of varied composition and pH of the media, the dipole effect induced by these cations is directly related to the presence of PS molecule in their ionized form [7]. Moreover, their interaction initiates the compaction of lipids in the membrane and, as a result, the blocking effect of mechanosensitive channels incorporated into the membrane [13].

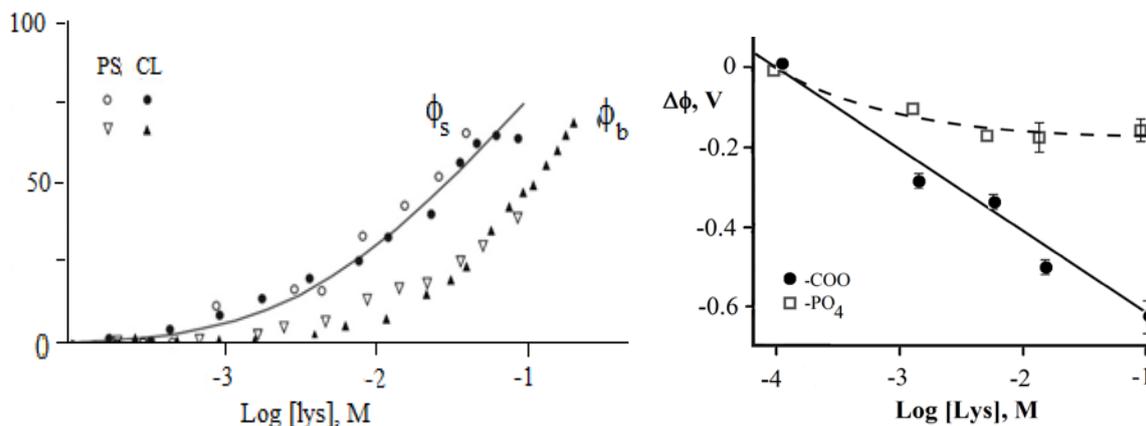
Molecular dynamic simulation reveals the opposite input of lipid and water molecules to boundary potential – lipids responsible for high negative and water - for high positive input to BP. The total boundary potential is always positive and is a small difference between two high contributions. MD simulation detects nanoclusters of lipids coordinated with the multivalent cations. Lipid head group hydration is altered significantly in these clusters [11].

The similar phenomena are responsible for electrostatic effects induced by adsorption of charged organic molecules – lysine and linear chains of polylysine - to the lipid surface [14]. Two principal facts are important to mention in this aspect (figure 6).



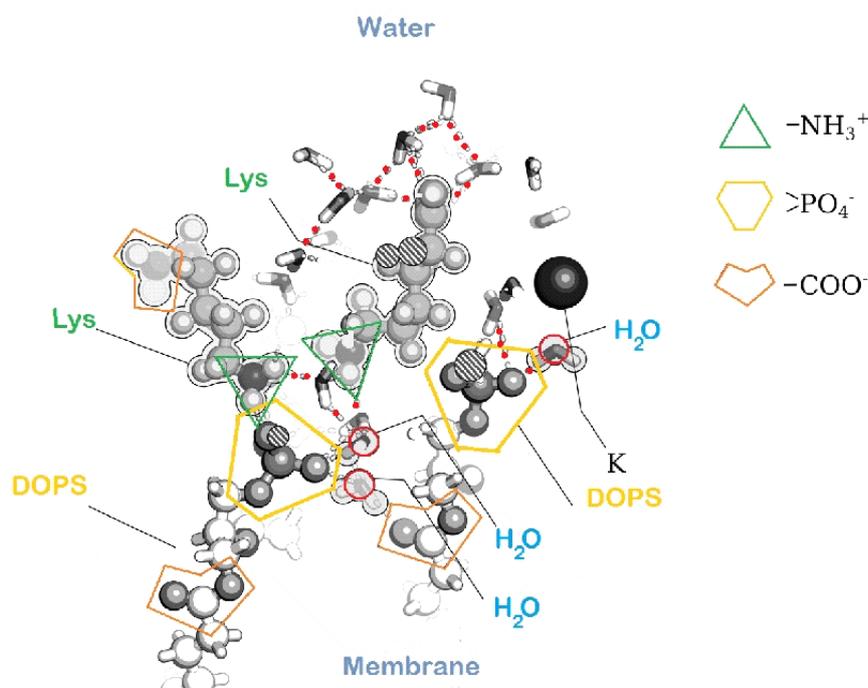
**Figure 6.** Boundary potential of planar BLM registered by IFC method when lysine added to cell or removed by permanent perfusion at the moment shown by thin and thick arrows in left panel. Right panel illustrate kinetics of boundary potential changes when polylysine stepwise added to one BLM side [14].

Boundary potential at one side of BLM (made from PS) were measured and depicted at figure 6 when lysine or polylysine PL-12 concentration was increased stepwise at moments pointed by black arrows. The solution in the cell was intensively stirred during the experiment and became uniform in a scale of minute or less. The opened arrow in left paned shows the start of cell perfusion by background electrolyte (10 mM KCl). At this step, water solution at both BLM sides returns to their initial symmetry and the magnitude of potential drops to its zero value because the lysine molecules desorbed and were removed from the cell. The same perfusion procedure with polylysines adsorbed at BLM have no effect on boundary potential. This is a general property of many polycations studied in our laboratory, which clearly show their irreversible adsorption at the negatively charged surface of membranes. If a drop of stock solution of polylysines is added to one BLM side (right panel), potential demonstrate two phases of kinetics – fast changes to positive direction and slow return back. The total amplitude of fast steps corresponds to maximal changes of surface potential up to overcharge liposome surface at saturation level detected in electrokinetic measurements. Boundary potential shifted to negative direction (figure 6) indicates the dipole effect. This idea is qualitatively supported by comparison data of two electrostatic methods applied to mono-lysine solutions (figure 7) [14].



**Figure 7.** Surface and boundary potentials measured at different lysine concentration in liposome suspension and by IFC method applied to planar BLM composed from PS or CL (left panel). MD simulation data for dipole effect in DOPS bilayers due to H-bonds to carboxyl and phosphate groups modified by lysine adsorption at the membrane surface (right pane) [15].

Experimental data (left panel) presents surface and boundary potential values measured at different lysine concentration in liposome suspension (circles) and in the cell with planar BLM made from two types of anionic lipids (triangles). It is important, that for both lipid membranes data of boundary potential measurements shifted to high concentration for about order in comparison to electrokinetic data. It means that boundary potential ‘insensitive’ to lysine adsorption up to its concentration in the cell corresponded to about 50 mV of zeta potential of liposomes. It suggests that dipole component of boundary potential compensate in some extent adsorption of positively charged lysine molecules. Note, no difference observed between these lipids, which have a different structure of polar heads: CL have no carboxyl groups presented in PS head groups.



**Figure 8.** MD simulation data for structural elements in polar area of DOPS membrane in the presence of adsorbed lysine molecules. Dashed lines illustrate bonds directed to water phase, dark sphere is inorganic cation located close to the membrane surface [15].

Digital simulation of PS membrane by methods of molecular dynamics visualizes the structure of polar area in the presence of adsorbed lysine molecules (figure 8) [15]. Dipole effect calculated for this structure reveals a different input of H-bonds between lysine and two negatively charged groups. Comparison with real experiments leads to conclusion that H-bonds with phosphate (but not ones with to carboxyl) shows the saturation effect as it follows from real experiments (figure 7). This conclusion was proved by the similar data observed in the electrostatic experiments with membranes formed from anion lipids PS and CL with the different charged groups.

### Conclusions and speculations.

Multivalent cation adsorption induces a strong effect to both BP components and leads to surface overcharging when the cation is bounded to the negatively charged membranes. The effect of

multivalent cations on the lipid lateral interaction is responsible for the dipole potential alterations and for the phase transition of lipids to the solid state. Near the same phenomena are revealed by boundary potential registration during the perfusion procedure. Lysine adsorption was observed at the negatively charged membranes and show the same effect for membranes composed from PS and CL. In both cases, lysine effect to boundary potential became significant at the concentration for about 1.5 order higher than in the electrokinetic measurements. We may propose the compensation between lysine effect on surface and dipole components of BP in the range of several tens of mV. Near the same changes of BP (but to the negative direction) were detected by IFC method in second slow phase of polylysine adsorption at the surface of planar BLM. Probably, these negative changes of PB (figure 6 right panel) are related to the same changes in membrane polar region as is was induced by mono-lysine adsorption. MD simulation of BLM reproduces this lysine effect and demonstrates is relation to H-bonds between water molecules and lipid head groups. Moreover, MD suggests the phosphate groups to be responsible for dipole effects of negative sign in both cases.

### Acknowledgements

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### References:

- [1] Ermakov Yu A, and Sokolov V S 2003 *Planar lipid bilayers (BLMs) and their applications* vol 7 ed H T Tien and A Ottova (Amsterdam: Elsevier) chapter 3 p 109
- [2] Cevc G, and Marsh D 1987 *Phospholipid Bilayers. Physical Principles and Models* (New York: Willey-Interscience)
- [3] Cevc G 1990 *Biochim. Biophys. Acta* **1031** 311
- [4] McLaughlin S 1989 *Annu. Rev. Biophys. Biophys. Chem.* **18** 113
- [5] Wang L 2012 *Annu. Rev. Biochem.* **81** 615
- [6] Brockman H 1994 *Chemistry & Physics of Lipids* **73** 57
- [7] Ermakov Y A, Averbakh A Z, Yusipovich A I and Sukharev S 2001 *Biophys J.* **80** 851
- [8] Ermakov Y A, Makhmudova S S and A Z Averbakh 1998 *Colloids and Surfaces A- Physicochemical and Engineering Aspects* **140** 13
- [9] Ermakov Y A, Mahmudova S S, Shevchenko E V, and Lobyshev V I 1993 *Biologicheskie membrany* **10** 212
- [10] Tatulian S A 1993 *Phospholipid Handbook* ed G Cevc (New York: Marcel Dekker) p 511
- [11] Nesterenko A M and Ermakov Y A 2012 *Biochemistry (Moscow) Supplement Series A: Membrane and Cell Biology* **6** 320
- [12] Ermakov Yu A 2000 *Colloid Journal* **62** 389
- [13] Ermakov Y A, Kamaraju K, Sengupta K, and Sukharev S 2010 *Biophys J.* **98** 1018
- [14] Marukovich N, McMurray M, Finogenova O, Nesterenko A, Batishchev O and Ermakov Yu 2013 *Advances in Planar Lipid Bilayers and Liposomes. A Tribute to Marin D. Mitov* vol 17 ed A Iglic and J Genova (Amsterdam: Elsevier) chapter 6 p 139
- [15] Marukovich N, Nesterenko A and Ermakov Yu A 2015 *Biochemistry (Moscow) Supplement Series A: Membrane and Cell Biology* **9** 40