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Membrane Electrochemistry: Electrochemical Processes in Bilayer Lipid Membrane

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Additional information is available at the end of the chapter

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1. Introduction

Since the first report of reconstituted lipid bilayers or bilayer lipid membranes (BLMs) *in vitro*, more than 30 years have elapsed. It is informative, in retrospect, to mention in this introduction the crucial role played by the science of interfaces in the reconstitution experiments. In living cells, the tremendous interfacial areas that exist between the membrane and its surroundings not only provide ample loci for carrying out activities vital to the living system, but afford a clue for our understanding. Physically, an interface is characterized most uniquely by its interfacial free energy, which is a result of the orientation of the constituent molecules [1]. An ultra-thin film such as a lipid bilayer is a system whose interior is influenced by the proximity of its interfaces. In a sense, an interface can be thought of as a structure so that it has no homogenous interior. However, the kind of ultra-thin films (e.g., BLMs) under discussion here are heterogeneous from their contacting phases. This and other interfacial properties of membrane can be understood to a large extent in terms of the laws of interface chemistry and physics that govern them, in particular electrochemistry [2]. In this connection it is worth noting that within the last few years, several monographs on various aspects of electrochemistry have been published [2-4], attesting the importance as well as research activities on the state of the science. Indeed, electrochemistry embraces the field ranging from the theoretical to experimental, surface science, spectroscopy, and to ultra-thin film-based applications. For instance, cyclic voltammetry, one of the most elegant and powerful techniques of electrochemistry, was applied to the BLM research in 1984 [5]. A planar BLM is a 5 nm thick lipid bilayer structure separating two aqueous solutions [6], which along with the spherical liposome, has been extensively used as an experimental model of biomembranes [7-13]. In fact, the current understanding of the structure and function of biological membrane can be traced to the investigations of experimental model membranes which have been developed as a direct consequence of the applications of classical principles of interfaces advanced by Langmuir,

Adam, Harkins, McBain, Hartley, and others [1, 6, 7, 14]. In the last few years there have been a number of reports on self-assemblies of molecules as advanced materials or smart materials [15]. Without question, the inspiration for this exciting development comes from the biological world, where, for example, the lipid bilayer of cell membranes was among the foremost self-assembling systems. In this connection it should be stated that many other researchers have also reported self-assembling systems such as Langmuir-Blodgett multilayers and liposomes [6-11, 16, 17]. The cogent reason that self-assembled BLMs are of scientific and practical interest is owing to the fact that most physiological activities involve some kind of lipid bilayer-based ligand-receptor contact interactions. Outstanding examples among these are ion sensing, antigen-antibody binding, hormone-synapse response, light conversion and detection, and gated channels, to name a few.

Many physiologically important processes are accompanied by charge transfer across the membrane and adjacent layers, and the related electrostatic phenomena are commonly recognized as a fundamental aspect of membrane biophysics. Both, charge transfer and binding to the surface depend on the electric field distribution at the membrane boundary, which in most cases is determined by the presence of charged lipid species. These circumstances have stimulated extensive use of planar lipid bilayers (BLM) and liposomes as model systems for studies of electrostatic phenomena at the membrane boundaries induced by inorganic ions and substances of biological interest [18, 19]. The distribution of electric field across the membrane interface in a microscopic scale is extremely complex and generally may be only approximated by smooth changes of potential in both directions perpendicular and parallel to membrane. The goal of electrochemical methods is to evaluate the difference between the potential in a reference point, usually taken as zero in the bulk of solution, and the potential averaged over a certain plane parallel to the membrane interface. One important imaginary plane (membrane surface) separates the membrane from the aqueous solution. The total potential drop across the interface, referred to as boundary potential, η_b , can be defined as the potential difference between the points in two phases, one of which is in the bulk of electrolyte, another is inside the membrane, near its hydrophobic core. This potential η_b is the sum of two parts (η_s and η_i). The first is the potential drop in the diffuse part of the electrical double layer and defined here as the surface potential, η_s . It is determined by the processes of surface ionization and screening by ions of the electrolyte. The Gouy Chapman-Stern (GCS) model provides an adequate description of these phenomena on the surface of biological membranes [19]. The other component of the boundary potential corresponds to the voltage drop across the interface and can be defined as the potential difference between two imaginary planes placed in different phases: in the aqueous solution immediately adjacent to the membrane surface and in the hydrophobic core inside the membrane. In contrast to the surface potential formed by the double layer, this interfacial potential drop is not accessible by any experimental approach because the energy of charge transfer between different phases includes not only the electrostatic but also an unmeasurable chemical component. This important point had been discussed in many monographs and textbooks [20-22]. Only the change of this component and therefore the variation of the total boundary potential can be monitored by external devices. The exact potential distribution in this region of the membrane is unknown

and is presented in figure 1 as linear. As follows from many experiments [19, 23, 24] and numerous estimations [25], the interior of the membrane is more positive than the surrounding aqueous phase by about 200-300 mV. The physical nature of this potential difference reflects the molecular structure of the interface and may be ascribed to the orientation of dipole moments of lipid and water molecules or other moieties adsorbed or incorporated into lipid bilayer.

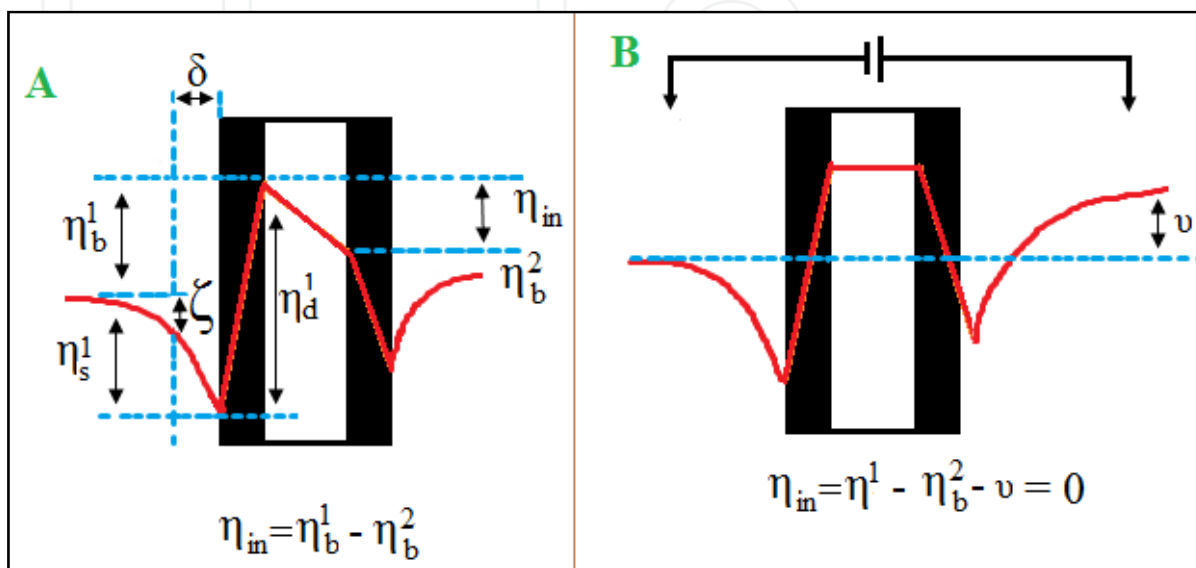


Figure 1. Electric potential distribution across the membrane under conditions of short-circuit (A) and intramembrane field compensation by an external voltage source (B). Hydrophobic core is indicated as light and polar regions as dark parts of the membrane. The vertical dashed line on the left shows the position of shear plane in the diffuse part of electrical double layer at distance δ from the surface of membrane.

2. Basic concepts and definitions

The discovery of galvanic by L. Galvani in 1786 occurred simultaneously with his study of a bioelectrochemical phenomenon which was the response of excitable tissue to an electric impulse. E. du Bois-Reymond found in 1849 that such electrical phenomena occur at the surface of the tissue, but it was not until almost half a century later that W. Ostwald demonstrated that the site of these processes are electrochemical semipermeable membranes. In the next decade, research on semipermeable membranes progressed in two directions—in the search for models of biological membranes and in the study of actual biological membranes. The search for models of biological membranes led to the formation of a separate branch of electrochemistry, i.e. membrane electrochemistry. The most important results obtained in this field include the theory and application of ion-exchanger membranes and the discovery of ion-selective electrodes (including glass electrodes) and bilayer lipid membranes. The study of biological membranes led to the conclusion that the great majority of the processes in biological systems occur at cell and organelle membranes. The electrochemical aspects of this subject form the basis of bioelectrochemistry, dealing with the processes of charge separation and transport in biological membranes and their

models, including electron and proton transfer in cell respiration and photosynthesis as well as ion transport in the channels of excitable cells. The electrokinetic phenomena (electrical double layer, interfacial tension of cells and organelles, cell membrane extension and contraction, etc.) also belong to this field.

3. Model systems of the biological membrane

Artificial lipid membranes are useful models to gain insight into the processes occurring at the cell membrane, such as molecular recognition, signal transduction, ion transport across the membrane. These membranes are often used to characterize membrane proteins or to study membrane active substances [26-28]. These membranes with incorporated receptors have a great potential in biosensor applications [29-33]. Various methods have been used to create artificial lipid membranes including free suspended membranes as well as membranes supported on a solid surface. Bellow is the model used to create this artificial lipid membranes.

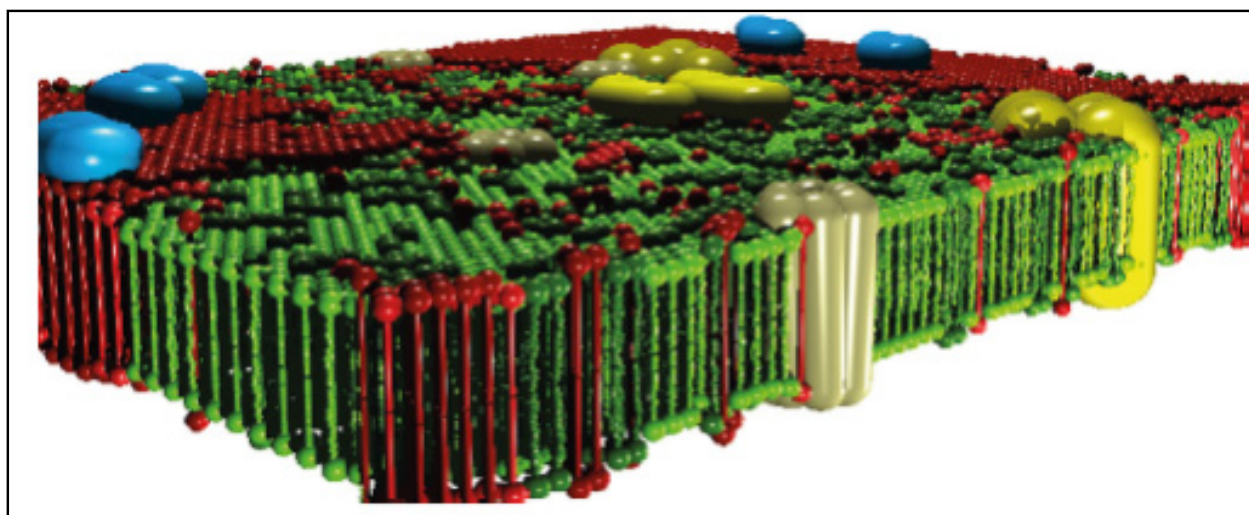


Figure 2. Modern view of biological membranes (Picture generated by H. Seeger from Monte Carlo simulations and kindly provided by T. heimburg, NBI Copenhagen).

3.1. Liposomes

Liposomes, or lipid vesicles, are spherical structures in which an aqueous volume is enclosed by one or several lipid bilayers. They are usually made from phospholipids, which form energy-favorable structures in an aqueous solution due to the hydrophilic and hydrophobic interactions. Depending on the size and the number of bilayers, liposomes are classified as large multilamellar vesicles (MLV's) or large and small unilamellar vesicles (LUV's and SUV's). The size of unilamellar liposomes vary between 20 nm and 500 nm and the thickness of one lipid bilayer is about 4 nm. The structure of liposomes makes it possible to either encapsulate water-soluble molecules in the water interior of the liposome or immobilise molecules within the lipid membrane. Liposomes can be modified in a desired manner through the choice of membrane components and this has made them attractive as

model system for cell membrane. In addition liposomes are frequently used as a delivery system for anticancer agents, increasing the effectiveness and circulation time of drugs. It is also possible to target specific cells by attaching an appropriate molecule at the liposome surface that binds specifically to the receptor site [34, 35]

3.2. Black lipid membranes (BLM)

In 1962 Müller et al pioneered the work of using black lipid membranes (BLM) as recognition elements. Unlike liposomes, BLMs are originally formed by spreading a lipid solution in a small hole ($\varnothing = 0.5\text{mm}$) of a wall separating two aqueous compartments. Evaporation or diffusion of the lipid solvent leads to thinning of the film to its final bilayer state. Black lipid membranes are very suitable for electro-chemical measurements, since there is easy electrochemical access to both sides of the membrane. They have the ability to control the constituents of each side of the membrane. In addition, there is no perturbing surface, and both the membrane and incorporated functionalities are likely to be close to their native state. The physical stability of these BLMs is very low and much effort has been put on improving the stability of this particular model membrane [36-38]. The residues left from solvent inside the bilayer have affected material properties of the bilayer, such as thickness [39], elasticity [40], and electrical properties [41]. The conduction of incorporated ion-channels is affected as well [42]. It is impossible to form large area BLMs because of the size limitation [43]. Nonetheless they have been extremely valuable in the history of membrane research.

3.3. Supported bilayer lipid membranes (sBLM)

An alternative to the BLM is to use lipid bilayers immobilized on a solid support. These membranes can be prepared on various surfaces, i.e., glass, silicon, mica, or on gold surfaces. The standard methods of preparing supported lipid membranes on planar solid surface are the Langmuir-Blodgett (LB) transfer and liposome spreading techniques. The major advantage of this membrane is its attachment to a solid support, resulting in a long-term and high mechanical stability. Solid supported membranes can be accessed by a variety of sensitive surface analysis tools such as surface Plasmon resonance spectroscopy, quartz crystal microbalance, scanning probe microscopy, as well as electrochemical measurements. But their close surface proximity between the artificial membrane and the bare solid surface onto which it is deposited restricts or even prevents the incorporation of large transmembrane-spanning proteins. The membrane-substrate distance is not sufficiently large to avoid direct contact between transmembrane proteins incorporated in the membrane and the solid surface. No ion transport can be detected with such membrane. Because embedded transmembrane proteins often have hydrophilic sections that protrude outside the lipid bilayer and may become immobile or denatured upon contact with the solid support. The lack of a well-defined ionic reservoir on the substrate side of the bilayer is a major drawback when studying membrane transport through ion carriers and channels. Hybrid bilayers have been developed to improve some of the above-mentioned shortcomings of the solid supported BLMs. In such sBLMs, the first monolayer is typically

an alkanethiol monolayer, covalently attached to a metal substrate, onto which a phospholipids monolayer is deposited either by LB transfer or vesicle fusion. Such thiol/lipid solid supported BLMs are stable in air and constitute an improved barrier towards charge transfer [44,45]. However, the rigidity of alkanethiol/phospholipids solid supported BLMs is much higher than that of fluid biological membranes. In addition, the structure of thiol-based solid supported BLMs prevents the formation of a water layer between the bilayer and the metal support. Because of these conformational restraints, they are unsuitable for the incorporation of integral proteins and studies of ion transport through ionophores [46].

3.4. Polymer-supported bilayer lipid membranes (psBLM)

Attempts have been made to separate the membrane from the solid substrate by polymer cushions that rest on the substrate and support the membrane. Strategies include attachment to water-rich gels [47, 48], linking with covalently bound spacers [49-53] and whole spanning membranes [54]. A water layer is formed between the support and the hydrophilic headgroups. Tamm [55] and co-worker reported on the successful formation of tethered polymer-supported planar lipid bilayers. In their work a linear polyethyleneglycol (PEG) polymer was attached at its two ends to the substrate and a lipid molecule, respectively. Polymer-supported BLMs were developed in order to combine the most benefits of unsupported BLMs and solid supported BLM, such as bilayer fluidity and stability, accessibility to various characterization methods, and the possibility of incorporation and investigation of membrane proteins. Polymer-supported BLMs are stable and the presence of a thin, lubricating water layer between the substrate and the inner monolayer allow the incorporation and characterization of proteins. Lower impedance of such membranes limits the application of polymer-supported BLMs for biosensor purposes. The sealing property of such membrane is not comparable to the BLMs. The defects of the membrane may also prevent the incorporation of channel peptides and proteins.

3.5. Tethered bilayer lipid membranes (tBLM)

In an attempt to overcome the drawbacks of psBLM, sBLM and BLM, while preserving the stability provided by the covalently bound alkanethiol monolayer, so-called thiolipids have been synthesized [52, 56-59]. The thiolipids are composed of lipid derivatives, extended at their polar headgroups by hydrophilic spacers, which terminate in a thiol or disulfide group for covalent binding to the substrate. These molecules interact with gold surfaces, thus forming self-assembled monolayers hydrophobic to the outside. Once exposed to a suspension of liposomes, they tend to fusion and form lipid bilayers tethered to the gold surface by the hydrophilic spacer. Vogel and co-workers [56] were the first to introduce this concept. They synthesized so-called thiolipids with a head group composed of [60-62] ethyleneglycol units acting as a hydrogel and a thiol end group for covalent linkage to the gold surface. Bilayers were formed by depositing a second monolayer of different phosphocholines by the detergent dilution technique. Such bilayers exhibit a very high membrane resistance. Steinem et al [68] used different spacer and vesicle fusion technique to form a bilayer membrane with 0.8-1.0 μFcm^{-2} capacitance. Knoll and co-workers [58, 63]

were the first to introduce to use oligopeptide sequences as hydrophilic spacers attached to the head group of lipid, resulting in the formation of peptide-tethered bilayers. Cornell and coworkers [29, 52, 64-65] were the first using half-membrane spanning tether lipids with benzyl disulphide (DPL) and synthetic archaea analogue full membrane spanning lipids with phytanolychains to stabilize the structure and polyethyleneglycol units as a hydrophilic spacer. Bilayer formation was achieved by immersion of a gold electrode in an ethanolic solution of the lipid mixture for the outer leaflet.

4. Membrane potentials

One of the property of electrochemical membranes is the formation of an electric potential difference ($\Delta\eta_{mem}$) between the two sides of the membrane and this is termed the membrane potential, and this quantity is determined by subtracting the left phase electric potential from right phase electric potential,

$$\Delta\eta_{mem} = \eta(2) - \eta(1) \quad (1)$$

For cell membranes, the intracellular liquid is usually denoted as solution 2, while solution 1 is the extracellular liquid.

The formation of a membrane potential is connected with the presence of an electrical double layer at the surface of the membrane. For a thick, compact membrane, an electrical double layer is formed at both interfaces. The electrical double layer at a porous membrane is formed primarily in the membrane pores. The electrical double layer at thin membranes is formed on both membrane surfaces. It is formed by fixed ions on the surface of the membrane and the diffuse layer in the electrolyte, by considering the simple case where both sides of the membrane are in contact with a solution of symmetrical electrolyte of the form MX in a single solvent and the membrane is permeable for only one ionic species (cation or anion only). In equilibrium its electrochemical potential in both solutions adjacent to the membrane has the same value. Thus if the membrane is permeable for cation M^{z+} the equation is

$$\Delta\eta_{mem} = \frac{RT}{zF} \ln \frac{a_{M(1)}}{a_{M(2)}} \quad (2)$$

and if the membrane is permeable for anion X^{z-} the equation is:

$$\Delta\eta_{mem} = \frac{RT}{zF} \ln \frac{a_{X(2)}}{a_{X(1)}} \dots \quad (3)$$

The membrane potential expressed by Eqs (2 and 3) is termed the **Nernst membrane potential** as it originates from the analogous ideas as the Nernst equation of the electrode potential and the equation of the Nernst potential at ITIES (Interface between Two Immiscible Electrolyte Solutions) of the form

$$E = E^0 - \frac{RT}{nF} \ln Q \dots \quad (4)$$

Q is the reaction quotient.

Consider a system in which both solutions contain various ions for which the membrane is permeable and one type of ion that cannot pass through the membrane. The membrane is permeable for the solvent molecules to maintain the osmotic pressure across the two sides. The equilibrium conditions for the diffusible ions are

$$\mu'_i(1, P_1) = \mu'_i(2, P_2) \quad (5)$$

This condition expresses the fact that the two solutions are under different pressures, p_1 and p_2 , as a result of their different osmotic pressures. An analogous equation cannot be written for the non-diffusible ion as it cannot pass through the membrane and the 'equilibrium' concentrations cannot be established.

Consider dilute solutions, where it is possible to set $p_1 = p_2$ then the electrochemical potentials in Eq (5) are expanded in the usual manner, yielding for the diffusible cation

$$Ln \frac{a_{M(2)}}{a_{M(1)}} = -\frac{(Z_+)F}{RT} \Delta\eta_{mem} \dots \quad (6)$$

and for the diffusible anion,

$$Ln \frac{a_{X(2)}}{a_{X(1)}} = -\frac{(Z_-)F}{RT} \Delta\eta_{mem} \quad (7)$$

Rearranging these equations yielding,

$$\left[\frac{a_{M(2)}}{a_{M(1)}} \right]^{1/Z_+} = \exp \left[-\frac{(Z_+)F}{RT} \Delta\eta_{mem} \right]^{1/Z_+} = \lambda \quad (8)$$

i.e. for univalent, divalent, etc., cations and anions

$$\frac{a_{M(2)}}{a_{M(1)}} = \left[\frac{a_{M(2)}}{a_{M(1)}} \right]^{1/2} = \left[\frac{a_{M(2)}}{a_{M(1)}} \right]^{1/3} = \lambda \quad (9)$$

$$\frac{a_{X(2)}}{a_{X(1)}} = \left[\frac{a_{X(2)}}{a_{X(1)}} \right]^{1/2} = \left[\frac{a_{X(2)}}{a_{X(1)}} \right]^{1/3} = \lambda \quad (10)$$

The constant λ is termed the **Donnan distribution coefficient**.

In the simple case of a diffusible, univalent cation M^+ and anion X^- and non-diffusible anion N^- present in phase 2, the condition of electroneutrality in phase 2 gives,

$$[M^+]_{(2)} = [X^-]_{(2)} + [N^-]_{(2)} \dots \quad (11)$$

and in phase 1 gives,

$$[M^+]_{(1)} = [X^-]_{(1)} \dots \quad (12)$$

For dilute solutions, activities can be replaced by concentrations by taking activity coefficient equal to one, and then equations (9 and 10) give,

$$[M^+]_{(1)} [X^-]_{(1)} = [M^+]_{(2)} [X^-]_{(2)} \dots \quad (13)$$

The Donnan distribution constant λ from the combination of equations (11-13) is,

$$\lambda = \left(\frac{[X^-]_{(2)} [N^-]_{(2)}}{[X^-]_{(1)}} \right) \quad (14)$$

A more exact solution of the equilibrium conditions (Eq. 5) must consider that the standard term μ° depends on the pressure, which is different in the two solutions,

$$\mu^0(T, P) = \mu^*(T, P) = \int_0^P v dP \dots \quad (15)$$

where μ^* is the limiting value of μ° at $P \rightarrow 0$ and v is the molar volume of the component at pressure P . If the volume v is considered to be independent of the pressure (more accurate calculations employ a linear dependence) then,

$$\mu^0(T, P) = \mu^*(T) + vP. \quad (16)$$

The Donnan potential at dilute solution $\Delta\eta_D = \Delta\eta_{mem}$ can be found as,

$$\Delta\eta_D = -\frac{RT}{F} \ln \lambda \dots \quad (17)$$

The Donnan potentials contain the individual ionic activities and cannot be measured by using a purely thermodynamic procedure. In the concentration range where the Debye-Hückel limiting law is valid, the ionic activities can be replaced by the mean activities. The membrane potentials are measured by constructing a cell with a semipermeable membrane separating solutions 1 and 2:



5. Evidence for electronic processes in Membranes

The origin of the concept of electronic processes in membranes and related systems was first reviewed in 1971, in which the phenomenon known as "electrostenolysis" was stressed. In the language of membrane electrochemistry, electrostenolysis simply means that a reduction reaction takes place on one side of the membrane where the positive electrode is situated and the oxidation occurs on the other side of the membrane. Although electronic processes in BLM in the dark were mentioned in 1970, no conclusive evidence has been shown. This is because of the fact that an unmodified BLM is an excellent insulator (resistivity $>10^{15}$ ohms) incapable of either ionic or electronic conduction.

In order to demonstrate electronic conduction in BLM it seems that the membrane must, first of all, be modified to function as a redox or semiconductor electrode. Secondly, an appropriate method must be found for studies of electronic processes that can be applied to the BLM system. In the following paragraphs evidence of electronic processes in membranes, in particular in BLMs in the dark is described.

6. Electron-conducting BLMs

Consider an electron-conducting bilayer lipid membrane (BLM) is separating two aqueous solutions containing different redox couples, let the left side referred as the outside "out" (or Aqueous Solution 1) and the right side referred as the inside "in" (or Aqueous Solution 2). To facilitate the discussion, let's hold the outside constant and consider the interfacial electron transfer reaction on the inside,



where D and D⁺ denote an electron donor in its reduced and oxidized form, respectively, whose concentrations are [D⁺]₂ and [D]₂ in the bulk phase. The corresponding interfacial concentrations are [D⁺]₂^s and [D]₂^s. The forward and backward heterogeneous rate constants are k_f and k_b, respectively. The observed current is then given by

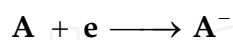
$$I_{net} = nFA(k_f[D]_2 - k_b[D^+]_2) \quad (20)$$

n is the number of moles of the electrons changed through the process, F is the faraday constant and A is the area of the electrode.

Let the observed membrane potential denoted by (Δη_{mem}), to explain this it is best to assume the membrane is behave like an ideal electron conductor just like platinum wire. From thermodynamics, the overall free energy change associated with an electrical cell reaction is given by

$$\Delta G = -nFDE \dots \quad (21)$$

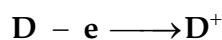
where E is the electromotive force of the cell. Consider the BLM system as an electrical cell, each solution/membrane interface is assumed to behave as a redox electrode. From eq. (21) the individual free energy terms are denoted by \check{G} . Thus, as indicated on the left side of the BLM,



Then

$$\check{G}_A + \check{G}_e = \check{G}_{A^-} \quad (22)$$

Similarly, on the right side,



Then

$$\check{G}_D - \check{G}_e = \check{G}_{D^+} \quad (23)$$

Here electrons involved are considered as one of the reactants. Since

$$\check{G}_i = \check{G}^0 + RT \ln a_i \dots \quad (24)$$

Taking the activity coefficient is equal to one, then

$$\check{G}_i = \check{G}^0 + RT \ln [i] \quad (25)$$

Where \check{G}^0 is a constant, R and T have the usual significance, [i] is the concentration of the redox species when the activity coefficient is taken to the unity. The free energy of the electron \check{G}_e for the outer solution/membrane interface, is given by

$$\check{G}_e = -FE_{out} = \check{G}_{A^-} - \check{G}_A = (\check{G}_{A^-}^0 - \check{G}_A^0) + RT \ln \frac{[A^-]}{[A]} \dots \quad (26)$$

where E_{out} is the electrode potential.

and the free energy of the electron \check{G}_e for the inner solution/membrane interface, we have

$$\check{G}_e = -FE_{in} = \check{G}_D - \check{G}_{D^+} = (\check{G}_D^0 - \check{G}_{D^+}^0) + RT \ln \frac{[D]}{[D^+]} \quad (27)$$

At equilibrium, the free energy difference of the electron across the BLM is equal to

$$\Delta\eta_{mem} = (E_{(A/A^-)}^0 - E_{(D^+/D)}^0) + \frac{RT}{F} \ln \frac{[A][D]}{[A^-][D^+]} \quad (28)$$

In case of $[A^-] = [A]$ and $[D^+] = [D]$ the logarithmic part will be equal to one, then $\Delta\eta_{mem}$ will be

$$\Delta\eta_{mem} = (E_{(A/A^-)}^0 - E_{(D^+/D)}^0) = E_{redox}^0 \dots \quad (29)$$

7. Examples of biological membrane processes, processes in the cells of excitable tissues

The transport of information from sensors to the central nervous system and of instructions from the central nervous system to the various organs occurs through electric impulses transported by nerve cells. These cells consist of a body with star-like projections and a long fibrous tail called an axon.

While in some mollusks the whole membrane is in contact with the intercellular liquid, in other animals it is covered with a multiple myeline layer which is interrupted in definite segments (nodes of Ranvier). The Na^+, K^+ -ATPase located in the membrane maintains marked ionic concentration differences in the nerve cell and in the intercellular liquid. The relationship between the electrical excitation of the axon and the membrane potential was clarified by A. L. Hodgkin and A. P. Huxley.

If the axon is not excited, the membrane potential

$$\Delta\eta_{mem} = \eta_{in} - \eta_{out} \quad (30)$$

has a rest value of about -90 mV. When the cell is excited by small square wave current impulses, a change occurs in the membrane potential roughly proportional to the magnitude of the excitation current impulses. If current flows from the interior of the cell to the exterior,

then the absolute value of the membrane potential increases and the membrane is hyperpolarized. Current flowing in the opposite direction has a depolarization effect and the absolute potential value decreases.

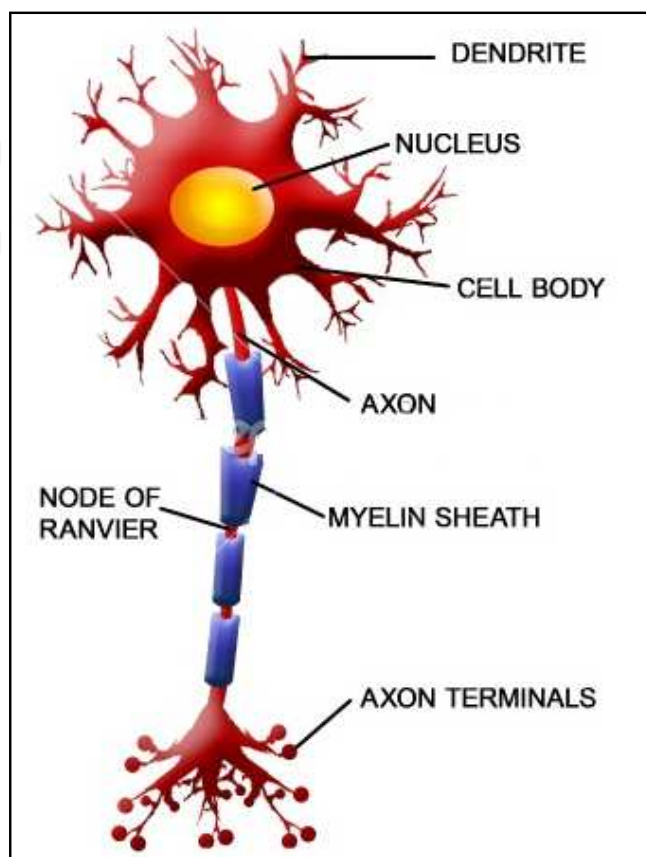


Figure 3. Structure of neuron cell.

When the depolarization impulse exceeds a certain 'threshold' value, the potential suddenly increases. The characteristic potential maximum is called a spike, figure 4, and its height no longer depends on a further increase in the excitation impulse. Sufficiently large excitation of the membrane results in a large increase in the membrane permeability for sodium ions so that, finally, the membrane potential almost acquires the value of the Nernst potential for sodium ions ($\Delta\eta_{\text{mem}} = +50 \text{ mV}$).

A potential drop to the rest value is accompanied by a temporary influx of sodium ions from the intercellular liquid into the axon. If the nerve is excited by a subthreshold current impulse, then a change in the membrane potential is produced that disappears at a small distance from the excitation site (at most 2 mm). A spike produced by a threshold or larger current impulse produces further excitation along the membrane, yielding further spikes that are propagated along the axon. As already pointed out, sodium ions are transferred from the intercellular liquid into the axon during the spike. This gradual formation and disappearance of positive charges corresponds to the flow of positive electric current along the axon. An adequate conductance of thick bare cephalopod axons allows the flow of sufficiently strong currents. In myelinated axons of vertebrates a much larger charge is

formed (due to the much higher density of sodium channels in the nodes of Ranvier) which moves at high speed through much thinner axons than those of cephalopods. The myelin sheath then insulates the nerve fibre, impeding in this way the induction of an opposite current in the intercellular liquid which would hinder current flow inside the axon.

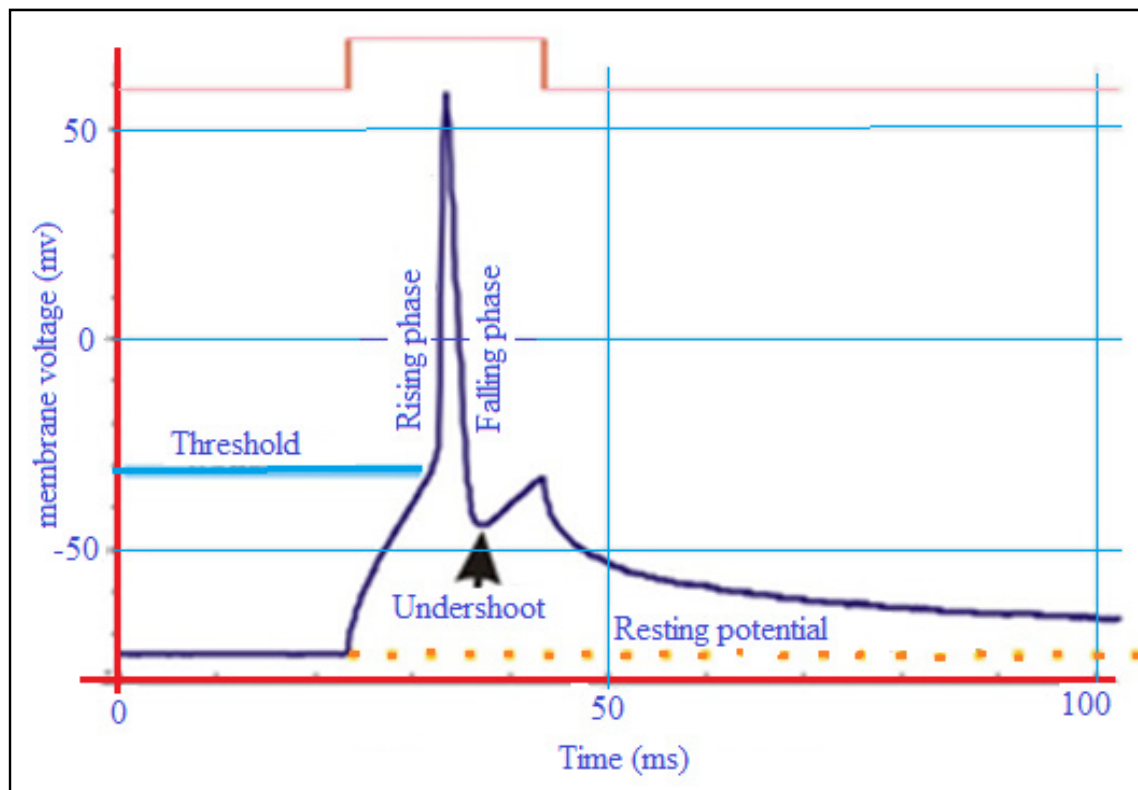


Figure 4. The characteristic potential maximum.

Another way to study membrane electrochemistry is the so-called voltageclamp method which is based on polarizing the membrane by using a four-electrode potentiostatic arrangement. In this way, Cole [66] showed that individual currents linked to selective ion transfer across the membrane are responsible for impulse generation and propagation.

A typical current-time curve is shown in figure (5), obviously, the membrane ion transfer is activated at the start, but after some time it becomes gradually inhibited. The ion transfer rate typically depends on both the outer (bathing) and inner solution (the inside of a cephalopod membrane as much as 1 mm thick can be rinsed with an electrolyte solution without affecting its activity).

The assumption that the membrane currents are due to ion transfer through ion-specific channels was shown correct by means of experiments where the channels responsible for transfer of a certain ionic species were blocked by specific agents. Thus, the sodium transfer is inhibited by the toxin, tetrodotoxin, while the transport of potassium ions is blocked by the tetraalkylammonium ion with three ethyl groups and one longer alkyl group, such as a nonyl. The effect of toxins on the ion transport across the axon membrane, which occurs at very low concentrations, has led to the conclusion that the membrane contains ion-selective

channels responsible for ion transport. This assumption was confirmed by analysis of the noise level in ionic currents resulting from channel opening and closing.

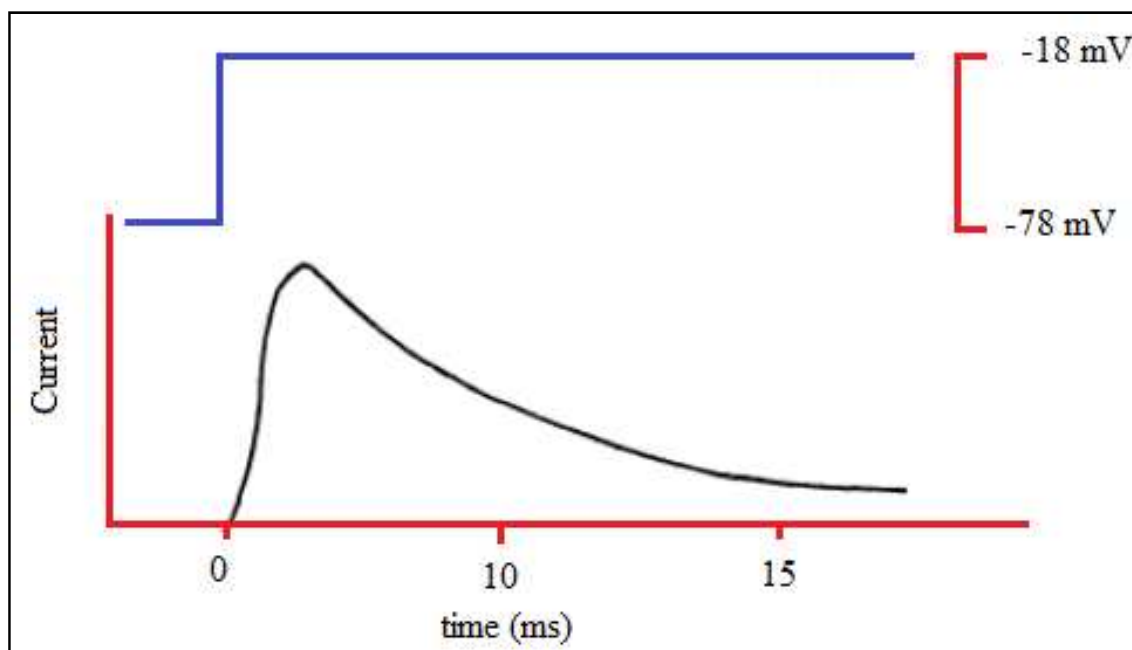


Figure 5. Time dependence of the membrane current. Since the potassium channel is blocked the current corresponds to sodium transport. The upper the represents the time course of the imposed potential difference. (According to W. Ulbricht)

In this regard we should mention here that the structure of the Na and K channels differ from type to type of animal classes, recently from our study on the conformational changes of Na⁺,K⁺-ATPase from some type of animal classes using stopped-flow techniques have revealed major differences in the kinetic mechanisms (and hence enzyme structure) of mammals and non-mammals enzymes [69,70], in the absence of ATP appear that the mammalian Na⁺,K⁺-ATPase exists in a diprotomeric state ($\alpha\beta$)₂, with protein-protein interactions between the α -subunits causing an inhibition of the transition, while binding of ATP to any of the enzyme conformational states induced the dissociation of the diprotomer into separate $\alpha\beta$ protomers and relieve the pre-existing inhibition, non-mammals exhibits no effect of ATP binding on the enzyme at all concentrations indicating a mono-protomeric structure ($\alpha\beta$ protomers).

Because of the very low extraneous noise level, the patch-clamp method permits the measurement of picoampere currents in the millisecond range figure (6).

Ion channels of excitable cells consist of a narrow pore, of a gate that opens and closes the access to the pore, and of a sensor that reacts to the stimuli from outside and issues instructions to the gate. The outer stimuli are either a potential change or binding of a specific compound to the sensor. The nerve axon sodium channel was studied in detail (in fact, as shown by the power spectrum analysis, there are two sorts of this channel: one with fast opening and slow inactivation and the other with opposite properties).

The rate of transport of sodium ions through the channel is considerable, when polarizing the membrane with a potential difference 4-60mV a current of approximately 1.5 pA flows through the channel which corresponds to 6×10^6 Na⁺ ions per second, practically the same value as with the gramicidin A channel.

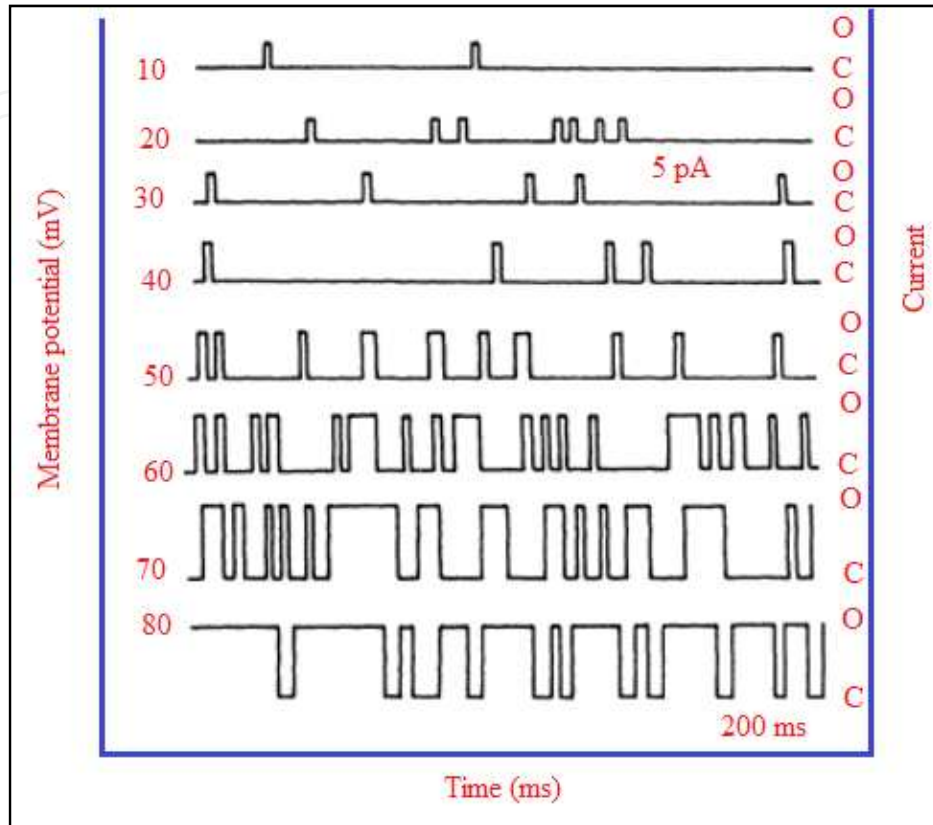


Figure 6. Joint application of patch-clamp and voltage-clamp methods to the study of single potassium channel present in the membrane of spinal-cord neuron cultivated in the tissue culture. The values indicated before each curve is potential differences imposed on the membrane. The ion channel is either closed (C) or opens (O). (According to B. Hille)

The sodium channel is only selective but not specific for sodium transport. It shows approximately the same permeability to lithium ions, whereas it is roughly ten times lower than for potassium. The density of sodium channels varies among different animals, being only $30 \mu\text{m}^{-2}$ in the case of some marine animals and $330 \mu\text{m}^{-2}$ in the squid axon, reaching $1.2 \times 10^4 \mu\text{m}^{-2}$ in the mammalian nodes of Ranvier. The potassium channel mentioned above, figure (6), is more specific for K⁺ than the sodium channel for Na⁺ being almost impermeable to Na⁺.

In deriving a relationship for the resting potential of the axon membrane it will be assumed that, in the vicinity of the resting potential, the frequency of opening of a definite kind of ion channel is not markedly dependent on the membrane potential. It will be assumed that the resting potential is determined by the transport of potassium, sodium and chloride ions alone. The constants k_i^θ are functions of the frequency of opening and closing of the gates of the ion-selective channels. The solution to this problem will be based on analogous

assumptions to those employed for the mixed potential. The material fluxes of the individual ions are given by the equations

$$J_{K^+} = k_{K^+}^{\theta} C_{K^+}(1) \exp\left(\frac{-F\Delta\eta_{mem}}{2RT}\right) - k_{K^+}^{\theta} C_{K^+}(2) \exp\left(\frac{F\Delta\eta_{mem}}{2RT}\right) \quad (31)$$

$$J_{Na^+} = k_{Na^+}^{\theta} C_{Na^+}(1) \exp\left(\frac{-F\Delta\eta_{mem}}{2RT}\right) - k_{Na^+}^{\theta} C_{Na^+}(2) \exp\left(\frac{F\Delta\eta_{mem}}{2RT}\right) \quad (32)$$

$$J_{Cl^-} = k_{Cl^-}^{\theta} C_{Cl^-}(1) \exp\left(\frac{F\Delta\eta_{mem}}{2RT}\right) - k_{Cl^-}^{\theta} C_{Cl^-}(2) \exp\left(\frac{-F\Delta\eta_{mem}}{2RT}\right) \quad (33)$$

At rest, no current passes through the membrane and thus the material flux of chloride ions compensates the material flux of sodium and potassium ions, so that,

$$J_{Na^+} + J_{K^+} = J_{Cl^-} \quad (34)$$

Equations (31, 32, 33 and 34) yield the membrane rest potential in the form

$$\Delta\eta_{mem} = \frac{RT}{F} \ln \frac{k_{K^+}^{\theta} C_{K^+}(1) + k_{Na^+}^{\theta} C_{Na^+}(1) + k_{Cl^-}^{\theta} C_{Cl^-}(2)}{k_{K^+}^{\theta} C_{K^+}(2) + k_{Na^+}^{\theta} C_{Na^+}(2) + k_{Cl^-}^{\theta} C_{Cl^-}(1)} \quad (35)$$

Ion transport is characterized by conditional rate constants $k_{K^+}^{\theta}$, $k_{Na^+}^{\theta}$ and $k_{Cl^-}^{\theta}$ which can be identified with the permeabilities of the membrane for these ions. These relationships can be improved by including the effect of the electrical double layer on the ion concentration at the membrane surface. Equation (35) is identical with the relationship derived by Katz [67].

It satisfactorily explains the experimental values of the membrane rest potential assuming that the permeability of the membrane for K^+ is greater than for Na^+ and Cl^- , so that the deviation of $\Delta\eta_{mem}$ from the Nernst potential for K^+ is not very large. However, the permeabilities for the other ions are not negligible. In this way the axon at rest would lose potassium ions and gain a corresponding concentration of sodium ions. This does not occur because of the action of Na^+, K^+ -ATPase, transferring potassium ions from the intercellular liquid into the axon and sodium ions in the opposite direction, through hydrolysis of ATP. When the nerve cells are excited by an electric impulse (either natural from another nerve cell or another site on the axon, or artificial from an electrode), the membrane potential changes, causing an increase in the frequency of opening of the gates of the sodium channels. Thus, the flux of sodium ions increases and the membrane potential is shifted towards the Nernst potential value determined by sodium ions, which considerably differs from that determined by potassium ions, as the concentration of sodium ions in the extracellular space is much greater than in the intracellular space, while the concentration ratio of potassium ions is the opposite. The potential shift in this direction leads to a further opening of the sodium gates and thus to 'autocatalysis' of the sodium flux, resulting in a spike which is stopped only by inactivation of the gates. In spite of the fact that the overall currents flowing across the cell membranes consist of tiny stochastic fluctuating components, the resulting dependences, as shown in figure (5), are smooth curves and can be used for further analysis (the situation is, in fact, analogous to most of phenomena occurring in nature). Thus, the formation of a spike can be shown to be a result of gradual opening and closing of many potassium and sodium channels figure (7).

According to figure (3) the nerve cell is linked to other excitable, both nerve and muscle, cells by structures called, in the case of other nerve cells, as partners, synapses, and in the case of striated muscle cells, motor endplates (*neuromuscular junctions*). The impulse, which is originally electric, is transformed into a chemical stimulus and again into an electrical impulse. The opening and closing of ion-selective channels present in these junctions depend on either electric or chemical actions. The substances that are active in the latter case are called *neurotransmitters*. A very important member of this family is acetylcholine which is transferred to the cell that receives the signal across the postsynaptic membrane or motor endplate through a specific channel, the nicotinic acetylcholine receptor. This channel has been investigated in detail, because, among other reasons, it can be isolated in considerable quantities as the membranes of the cells forming the electric organ of electric fish are filled with this species.

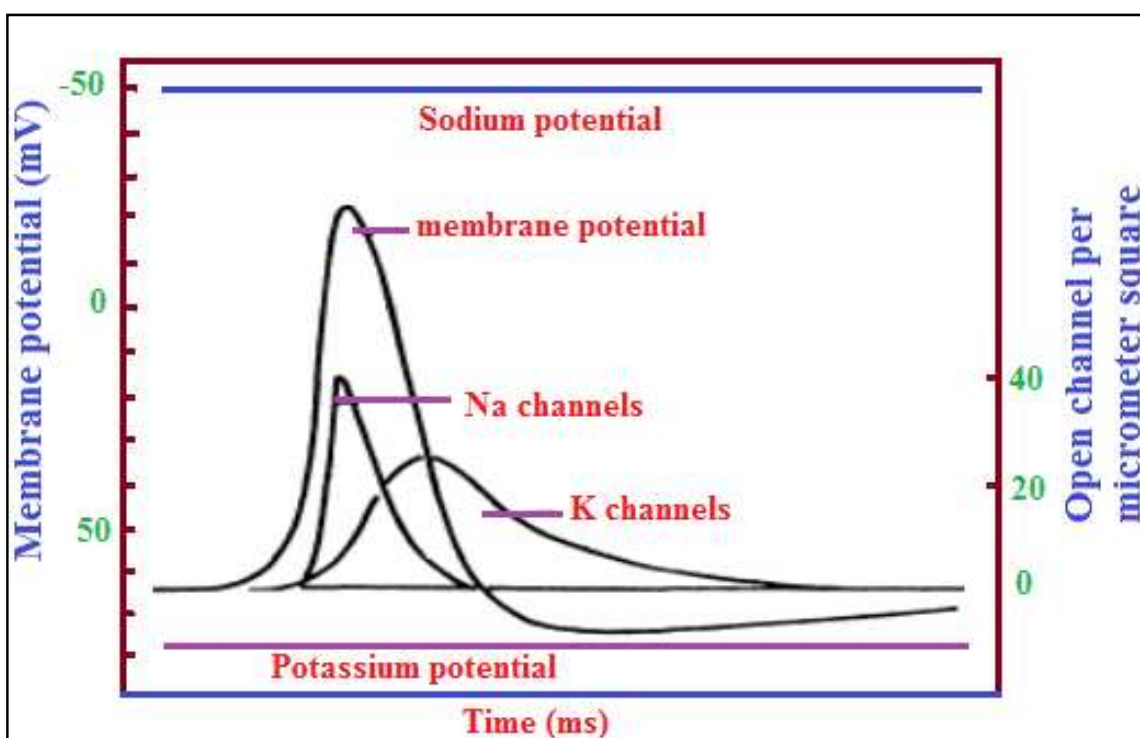


Figure 7. A hypothetical scheme of the time behavior of the spike linked to the opening and closing of sodium and potassium channels. After longer time intervals a temporary hyperpolarization of the membrane is induced by reversed transport of potassium ions inside the nerve cell. (According to A.L. Hodgkin and A.F. huxley)

Acetylcholine, which is set free from vesicles present in the neighborhood of the presynaptic membrane, is transferred into the recipient cell through this channel figure (8). Once transferred it stimulates generation of a spike at the membrane of the recipient cell. The action of acetylcholine is inhibited by the enzyme, acetylcholinesterase, which splits acetylcholine to choline and acetic acid. Locomotion in higher organisms and other mechanical actions are made possible by the striated skeletal muscles. The basic structural unit of muscles is the muscle cell-muscle fibre which is enclosed by a sarcoplasmatic membrane. This membrane invaginates into the interior of the fibre through transversal

tubules which are filled with the intercellular liquid. The inside of the fibre consists of the actual sarcoplasm with inserted mitochondria, sarcoplasmic reticulum and minute fibres called myofibrils, which are the organs of muscle contraction and relaxation. The membrane of the sarcoplasmic reticulum contains Ca^{2+} -ATPase, maintaining a concentration of calcium ions in the sarcoplasm of the relaxed muscle below $10^{-7} \text{ mol.dm}^{-3}$. Under these conditions, the proteins, actin and myosin, forming the myofibrils lie in relative positions such that the muscle is relaxed. When a spike is transferred from the nerve fibre to the sarcoplasmic membrane, another spike is also formed there which continues through the transverse tubules to the membrane of the sarcoplasmic reticulum, increasing the permeability of the membrane for calcium ions by five orders within 1 millisecond, so that the concentration of Ca^{2+} ions in the sarcoplasm increases above $10^{-3} \text{ mol.dm}^{-3}$. This produces a relative shift of actin and myosin molecules and contraction of the muscle fibre, and after disappearance of the spike, Ca^{2+} -ATPase renews the original situation and the muscle is relaxed.

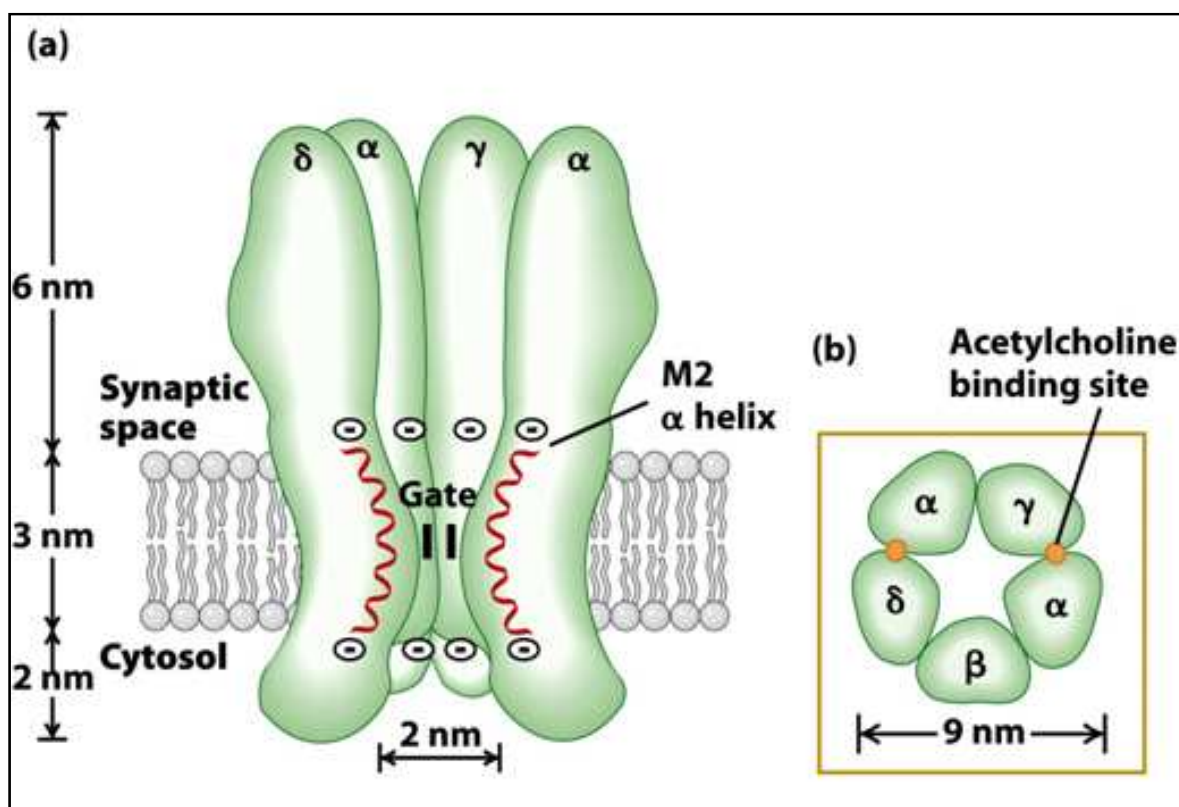


Figure 8. The nicotinic acetylcholine receptor in a membrane. The deciphering of the structure is based on X-ray diffraction and electron microscopy. (Molecular cell biology, sixth edition, 2008, W. H. freeman and company).

Abbreviations

BLMs	bilayer lipid membranes
GCS	Gouy Chapman-Stern model
MLV's	multilamellar vesicles

LUV's	large unilamellar vesicles
SUV's	small unilamellar vesicles
BLM	Black lipid membranes
LB	Langmuir-Blodgett transfer
psBLM	Polymer-supported bilayer lipid membranes
tBLM	Tethered bilayer lipid membranes
ITIES	Interface between Two Immiscible Electrolyte Solutions
$\Delta\eta_{\text{mem}}$	membrane potential
T	Absolute temperature
R	Universal gas constant
F	Faraday constant
Z	Ion charge
a	Activity
η_b	boundary potential
η_s	surface potential
E	Electrode potential
E_0	Electrode standard potential
P	Pressure
λ	Donnan distribution coefficient
Q	Reaction quotient
N	non-diffusible anion
v	is the molar volume of the component at pressure P
$\Delta\eta_p$	Donnan potential at dilute solution
I_{net}	The observed current

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8. References

- [1] K.L. Mittal and D.O. Shah (Eds.), *Surfactants in Solution*, Plenum Press. NY. 8 (1989) 133; II (1991) 61.
- [2] M. Blank (Ed.) *Biomembrane Electrochemistry*, Adv. Chem, Series No. 235, American Chemical Society, Washington. DC. 1994.
- [3] J.O'M. Bockris and S.U.M. Khan. *Surface Electrochemistry*, Plenum Press, NY. 1993.
- [4] J. Wang, *Analytical Electrochemistry*, VCH Publishers, NY.1994
- [5] H.T. Tien, *J. Phys. Chem.* 88 (1994) 3172; *J. Electroanal. Chem.* 174 (1984) 299
- [6] A. ottova-Leitmannuva and H.T. Tien, *Prog. Surf. Sci.*, 41 (1992) 337.

- [7] S.G. Davison (Ed.) *Progress in Surface Science*, Pergamon Press, NY, 4 (1973): 19 (1985) 169: 23 (1986) 317:30 (1989) 1.
- [8] A.D. Bangham. *BioEssays*, 17 (1995) 1081.
- [9] P.-A. Ohlsson, T. Tjamhage, E. Iterbai, S. Lofas and G. Puu. *Bioelectrochem. Bioenerg.*, 38 (1995) 137
- [10] T. Vo-Dinh, *Sensors Actuators B*, 29 (1995) 183.
- [11] F.M. Menger and K.D. Gabriels, *Angew. Chem.*, 34 (1995) 2091.
- [12] B.W. Koenig, S. Krueger, W.J. Orts, C.F. Majkrzak, N.F. Berk, J.V. Silverton and K. Gawrisch, *Langmuir*, 12 (1996) 1343.
- [13] N. Marakami, S.S. Singh, V.P.S. Chauhan and M. Elziaga, *Biochem.*, 34 (1995) 6046.
- [14] Y.Q. Liang, Z.Q. Zhang, L.X. Wu, Y.C. Tian and H.D. Chen, *J. Coll. Int. Sci.*, 178 (1996) 714.
- [15] H.T. Tien, *Mat. Sci. Eng. C*, 3 (1995) 7; in *Proc. 5th Int. Symp. On Bioelectronics and Molecular Electronic Devices*, 28-30 November 1995, Okinawa, Japan. p. 81.
- [16] P. Tancrede, P. Paquin, A. Houle and R.M. LeBlanc, *J. Biochem. Biophys. Meth.*, 7 (1983) 299.
- [17] T.D. Osborn, and P Yager, *Langmuir*, 11 (1995) 8.
- [18] G. Cevc and D.Marsh. *Phospholipid Bilayers, Physical Principles and Models*, Vol.5 (1987), New York, Willey-Interscience Publication, *Cell Biology: A Series of Monographs*. Bittar, E. E. (ed.)
- [19] S. McLaughlin, *Electrostatic Potentials at Membrane-Solution Interfaces*, Vol.9, 71-144 (1977) New York. *Current Topics Membranes and Transport*. Bronnen, F. and Kleinzeller, A. (eds.)
- [20] E.A. Guggenheim, *Thermodynamics*, Wiley, New York, (1950).
- [21] A.W. Adamson, *Physical Chemistry of Surfaces*, Wiley&Sons, (1967).
- [22] J.T. Davies and E.K. Rideal, *Interfacial Phenomena*, Academic Press, 2nd ed., New York, (1963).
- [23] A.D. Pickar and R. Benz, *J. Membrane Biol.*, 44 (1978) 353.
- [24] K. Gawrisch, D. Ruston, J. Zimmerberg, A. Parsegian, R.P. Rand, and N. Fuller. *Biophys. J.*, 61 (1992)1213.
- [25] R.F. Flewelling and W.L. Hubbell., *Biophys. J.*, 49 (1986) 541.
- [26] A. Charbit, C. Andersen, J. Wang, B. Schiffler, V. Michel, R. Benz and M. Hofnung, *Mol. Microbiol.*, 35 (2000), 777.
- [27] T. K. Rostvtseva, T. T. Liu, M. Colombini, V. A. Parsegian and S. M. Bezrukov, *Proc. Nat. Acad. Sci. USA*, 97 (2000), 7819.
- [28] M. Akeson, D. Branton, J. J. Kasianowics, E. Brandin and D. W Deamer, *J. Biophys.*, 77 (1999), 3227.
- [29] B. A. Cornell, V. L. Braach-Maksvytis, L. G. King, P. D. Osman, B. Raguse, L. Wiczorek and R. J. Pace, *Nature*, 387 (1997), 580.
- [30] S. Heyse, T. Stora, E. Schmid, J. H. Lakey and H. Vogel, *Biochim. Biophys. Acta*, 1376 (1998), 319.
- [31] K. Seifert, K. Fendler and E. Bamberg, *Biophys. J.*, 64 (1993), 384
- [32] T. Stora, J. H. Lakey and H. Vogel, *Angew. Chem. Int. Ed. Engl.*, 38 (1999), 389

- [33] M. Winterhalter, *Colloids Surf. A*, 149 (1999), 547.
- [34] R. J. Banerjee, *Biomater. Appl.* 16 (2001), 3-21.
- [35] D.D. Lasic, *Trends Biotechnol.*, 1998, 16, 307-321
- [36] H.T. Tien, and A. Ottova-Leitmannova, Elsevier, Amsterdam, (2003)
- [37] J.D. Castillo, A. Rodriguez, C.A. Romero, and V. Sanchez, *Science*, 153 (1966) 185-188
- [38] R.G. Ashcroft, H.G.L. Coster, D.R. Laver, and J.R. Smith, *Biochimica et Biophysica Acta*, 730 (1983) 231-238.
- [39] R. Fettiplace, D.M. Andrews, and D.A. Haydon, *J. Membrane Biol.*, 5 (1971) 277-
- [40] T. Hianik, J. Miklovicova, A. Bajci, D. Chorvat, and V. Sajter, *Gen. Physiol. Biophys.*, 3 (1984) 79-84.
- [41] H.G.L. Coster, and D.R. Laver, *Biochim. Biophys. Acta*, 857 (1986) 95-104.
- [42] B.M. Hendry, B.W. Urban, and D.A. Haydon, *Biochim. Biophys. Acta*, 513 (1978) 106-116.
- [43] O. Purucker, H. Hillebrandt, K. Adlkofer, and M. Tanaka, *Electrochim. Acta*, 47 (2001) 791-798
- [44] A.L. Plant, *Langmuir*, 9 (1993) 2764-2767.
- [45] S. Lingler, I. Rubinsten, W. Knoll, A. Offenhäusser, *Langmuir*, 13 (1997) 8085-7091.
- [46] R. Guidelli, G. Aloisi, L. Becucci, A. Dolfi, M.R. Moncelli; F. T. Buoninsegni, *J. Electroanal. Chem.*, 504 (2001), 1-28.
- [47] H.T. Tien, and A.L. Ottova, *Colloids and Surfaces A. Physicochemical and Engineering Aspects*, 149 (1999) 217-233.
- [48] T. Ide, and T. Yanagida, *Biochemical and Biophysical Research Communications*, 265 (1999) 595-599.
- [49] Y. Cheng, N. Boden, R.J. Bushby, S. Clarkson, S.D. Evans, P.F. Knowles, A. Marsh, and R.E. Miles, *Langmuir*, 14 (1998) 839-844.
- [50] N. Bunjes, E.K. Schmidt, A. Jonczyk, F. Rippmann, D. Beyer, H. Ringsdorf, P. Gräber, W. Knoll, R. Naumann, *Langmuir*, 13 (1997) 6188-6194.
- [51] B.A. Cornell, V.L.B. Braach-Maksvytis, L.G. King, P.D.J. Osman, B. Raguse, L. Wiczorek, R.J. Pace, *Science*, 387 (1997) 580-583.
- [52] B. Raguse, V. Braach-Maksvytis, B.A. Cornell, L.G. King, P.D.J. Osman, R.J. Pace, L. Wiczorek, *Langmuir*, 14 (1998) 648-659.
- [53] A.T.A. Jenkins, R.J. Bushby, N. Boden, S.D. Evans, P.F. Knowles, Q. Liu, R.E. Miles, S.D. Ogier, *Langmuir*, 14 (1998) 4675-4678
- [54] C. Schmidt, M. Mayer, and H. Vogel, *Angew. Chem. Int. Ed.*, 39 (2000) 3137-3140
- [55] V. Kiessling, L.K. Tamm, *Biophys J.*, 47 (2003) 105-113.
- [56] H. Lang, C. Duschl, H. Vogel, *Langmuir*, 10 (1994) 197.
- [57] C. Steinem, A. Janshoff, J. Goossens, H-J. Galla, *Bioelectrochem. Bioenergetics*, 45 (1998) 17-26.
- [58] R. Naumann, A. Jonczyk, R. Kopp, v. Esch, J. Ringsdorf, H. Knoll, W. Gräber, P. *Angew., Chem.*, 34 (1995) 2056.
- [59] L.M. Williams, S.D. Evans, T.M. Flynn, A. Marsh, P.F. Knowles, R. J. Bushby, N. Boden, *Langmuir* 13 (1997) 751.
- [60] S.J. Singer, G.L. Nicolson, *Science*, 175 (1972) 720-731

- [61] O.G. Mouristsen, In *Life-as a matter of fate: The Emerging science of lipidomics*; D. Dragomann, M. Dragomann, A.C. Elitzur, M.P. Silverman, J. Tuszynski, and H.D.Zeh, Eds., The Frontiers Collection, Springer, Germany, Vol.1 (2005) pp 27.
- [62] A. Charbit, C. Andersen, J. Wang, B. Schiffler, V. Michel, R. Benz and M. Hofnung, *Mol. Microbiol.*, 35 (2000) 777
- [63] R. Naumann, E.K. Schmidt, A. Jonczyk, K. Fendler, B. Kadenbach, T. Lieberman, A. Offenhäusser, W. Knoll, *Biosens Bioelectronics*, 14 (1999) 651-662
- [64] G.E. Woodhouse, L.G. King, L. Wieczorek, B.A. Cornell, *Faraday Disc.*, 111 (1998) 247.
- [65] B.A. Cornell, G. Krishna, P.D. Osman, R.J. Pace, L. Wieczorek, *Biochemical Soc. Transactions*, 29 (2001) 613.
- [66] K.S.Cole, *Membranes, Ions and Impulses*, University of California Press, Berkeley, (1968).
- [67] B. Katz, *Nerve, Muscle and Synapse*, McGraw-Hill, New York, (1966).
- [68] C. Steinem, A. Janshoff, J. Goossens, H-J. Galla, *Bioelectrochem Bioenergetics*, 45, (1998)17- 26.
- [69] M. Khalid, G. Fouassier, H-J. Apell, F. Cornelius, and R.J. Clarke, *Interaction of ATP with the Phosphoenzyme of the Na,K-ATPase*, *Biochemistry*, 49, (2010) 1248-1258.
- [70] M. Khalid, F. Cornelius, and R.J. Clarke, *Dual mechanisms of allosteric acceleration of the Na⁺,K⁺-ATPase by ATP*, *Biophysial journal*, 98, (2010), 2290-2298.