Resting Membrane Potential

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- Transport model

Cell Membrane – Structure (1)

- Main constituent
  - Lipid bi-layer with build-in proteins
- Main constituents of the lipid bi-layer:
  - Phospholipids
    - Polar head groups – electrically charged
    - Apolar part – 2 hydrophobic chains of fatty acids
  - Cholesterol
    - „Cement“ among the molecules of phospholipids

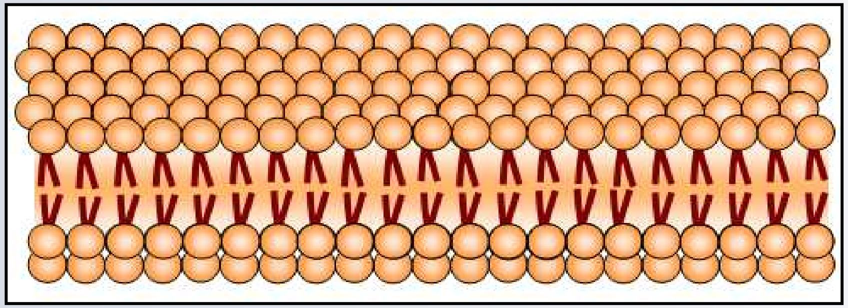

Fig. 1: Lipid bi-layer.
Phospholipids are turned towards each other with apolar parts.

Cell Membrane – Structure (2)

- Membrane proteins:
  - Peripheral
    - Are loosely bound to the membrane surface, can be easily loosen
    - Act as enzymes
  - Integral
    - Cross the membrane or are intercalated into the lipid bi-layer
    - Form ionic channels
    - Regulate the transport of the substances
    - Act as enzymes

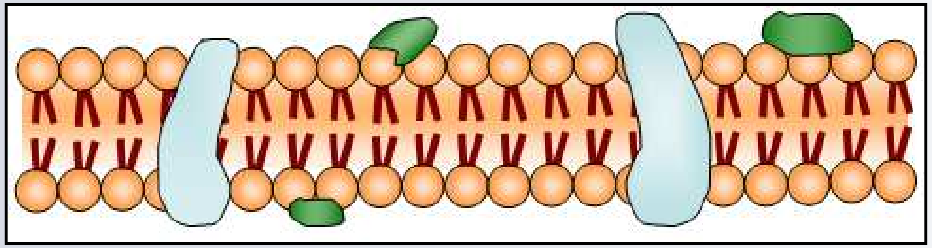

Fig. 2: Lipid bi-layer with membrane proteins.
Peripheral proteins – green, integral proteins – light blue.

Cell Membrane - Function

- Main structure of a living cell that is involved in realisation of primary functions of a living system, for example:
  - Cell transport
    - Exchange of substances between the intracellular and the extracellular environment
  - Excitability
  - Energetics of living systems
  - Immunity
  - Reproduction

Cell Membrane - Poperties

- Covers the cell and cell components
  - Separates the cytoplasm from the surroundings
- Functions as an obstacle for molecules and metabolic products, which do not diffuse in living processes within a continuous environment
- Is permeable to various extents to different substances
- Exerts a profound influence on transport processes

Resting Membrane Potential - Origin

- Originates as a consequence of different ionic concentration in the cell and outside the cell
  - A living cell is covered by a cytoplasmic membrane, that separates the extracellular and intracellular surroundings
- It is the potential difference (potential gradient) between both sides of the membrane, i.e. between the potential in the cell and potential outside the cell
- The membrane potential – an <u>electric phenomenon</u>

Resting Membrane Potential - Measurement

- The potential difference of the potential inside the cell (negative potential – a microelectrode) minus potential outside the cell (zero potential – a surface electrode) is always measured

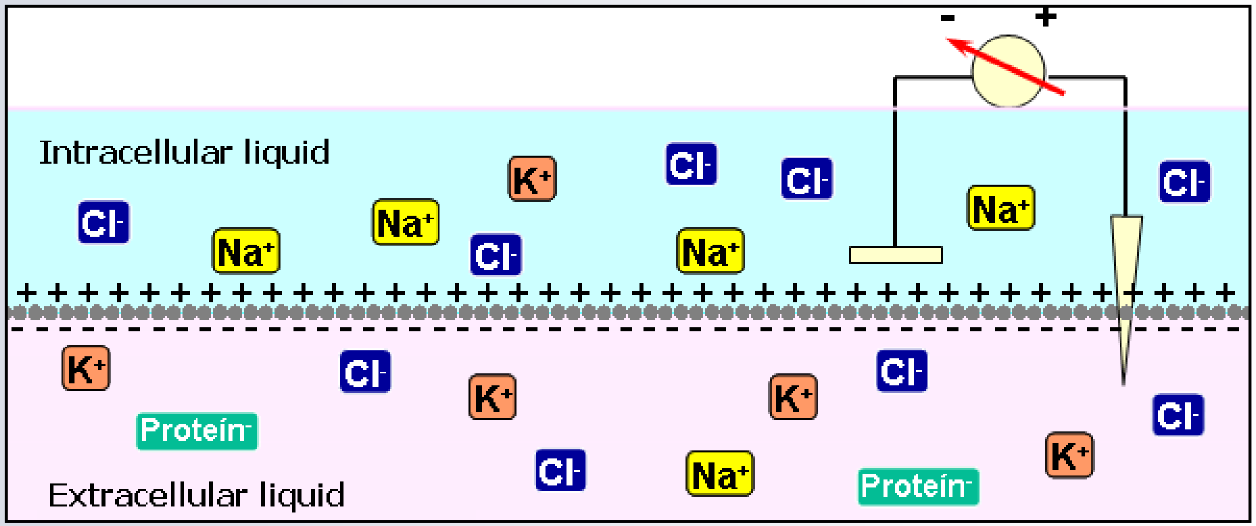

Fig. 3: Scheme of membrane potential measurement.

Resting Membrane Potential – Factors Influencing The Values

- Different values of resting membrane potential depend upon:
  - The cell type
  - The animal, from which the cell originates
  - The composition and concentration of the ionic constituents of the solution surrounding the cell (in identical cells)

Resting Membrane Potential – Typical Values

- For excitable cells, in normal ion composition of intracellular and extracellular space:
  - (-100 mV; -50 mV)

- *Comparison of the electric field intensity E of the Earth :*
  - *Across the membrane: E ~ 10^7 V/m*
    - *Thickness of the cell membrane ~ 10 nm*
  - *On the Earth surface: E ~ 10^2 V/m*
    - *The gaseous shell of the Earth behaves as a capacitor*
    - *„Electrodes" of the capacitor:*
      - *The Earth surface (negatively charged, earthed to zero potential)*
      - *Ionosphere (positively charged)*
      - *Distance: 50 000 km - 60 000 km*
      - *Total potential: 400 000 V*
      - *Total current: 2 000 A*
    - *Charging of the capacitor: by storms*

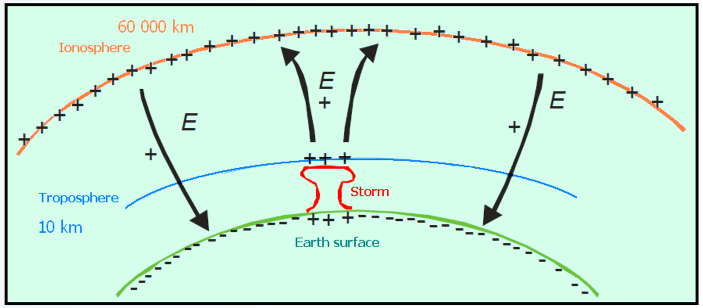

Fig. 4:
Electric capacitor of the Earth.

Resting Membrane Potential – Interpretation (1)

- Interpretation using models
- Models based on:
  - Electrodiffusion
  - Solid state (liquid crystals)
  - Equivalent electric circuits

Resting Membrane Potential – Interpretation (2)

- Electrodiffusion models
  - Describe the processes phenomenologically by thermodynamical reasoning
  - Connect the development of the potential with the diffusion of the ions across the membrane
  - Described models:
    - Donnan model
    - Model of ion transport

Resting Membrane Potential – Interpretation (3)

- Models based on the solid state (liquid crystals)
  - Describe the processes as the movement of the ions across the membrane and its blocking
  - Use characteristic properties of the structural elements of the membrane (lipids, proteins)
    - Molecular interpretation
    - Ionic channels

Resting Membrane Potential – Interpretation (4)

- Models based on equivalent electric circuits
  - Describe the behaviour of resting and exciting cells
  - Use electric properties of cells in accordance with electrodiffusion and solid-state models

Diffusion Potential In Nonliving Systems (1)

- Potential created by diffusion of charged particles across the membrane separating two environments
- Initial state:
  - Pure solutions of NaCl of different concentration separated by a membrane freely permeable to Na^+ and Cl^-
  - Electrically neutral compartments, but a concentration gradient exists because of different concentrations

Diffusion Potential In Nonliving Systems (2)

- Diffusion of ions from the environment with higher concentration NaCl to the environment with lower concentration of NaCl because of the concentration gradient (concentration fall)

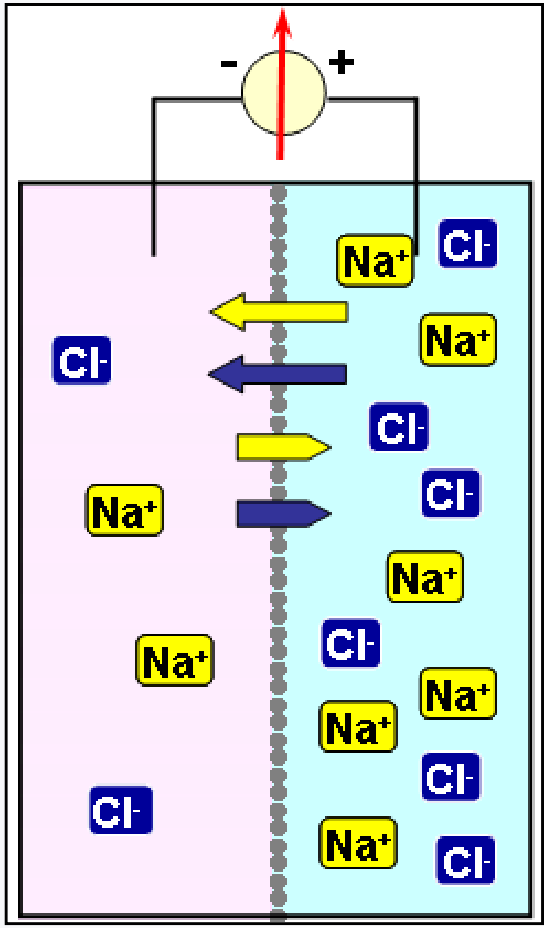

Fig. 5: Initial state for membrane potential generation in nonliving systems.

Used symbols:

Concentration gradient: 

Concentration fall: 

Diffusion Potential In Nonliving Systems (3)

- The mobility of ions is influenced by their hydration shells (water molecules attached to ions):
  - Na^+ (more molecules H_2O)
  - Cl^- (less molecules H_2O)
- Therefore, the diffusion of Cl^- down the concentration fall is more rapid than the diffusion of Na^+
- Consequence – surplus occurs for:
  - Positive charges in the environment of originally higher concentration
  - Negative charges in the environment of originally lower concentration

Diffusion Potential In Nonliving Systems (4)

- As a consequence of different mobility of ions, a temporary potential difference occurs between the two compartments – the <u>diffusion potential</u>

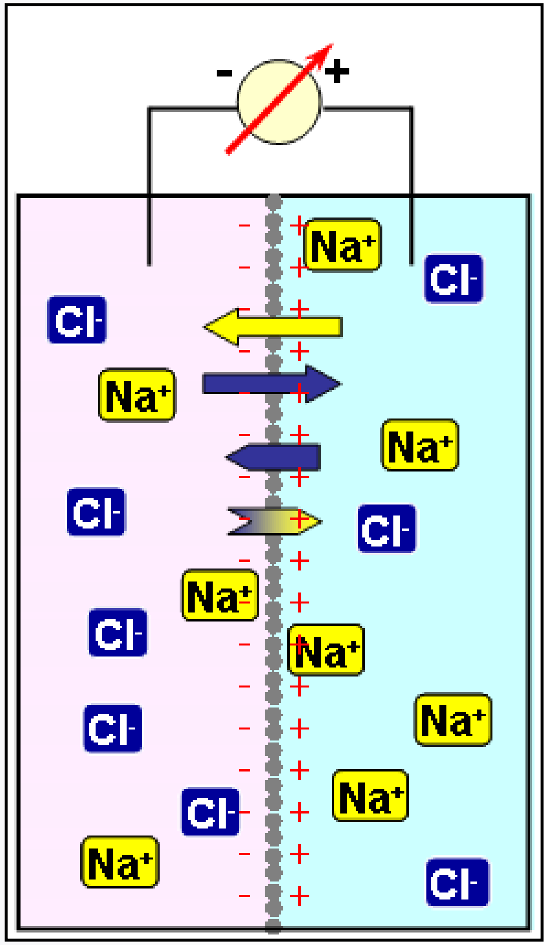

Fig. 6: Scheme of generation of a temporary membrane potential – diffusion potential.
Used symbols:
Electric gradient: 

Diffusion Potential In Nonliving Systems (5)

- Electric gradient
  - Repels Cl^- from the environment with the surplus of the negative charge
  - Acts against the concentration gradient until an equilibrium is reached
- An equilibrium is reached if there is no net flow of ions
  - The same amount of ions flow from the first environment into the second one as from the second environment into the first one
- As the membrane is permeable to both ions, the equilibrium is reached if ion concentrations on both sides of the membrane are equal, therefore, there is zero voltage across the membrane

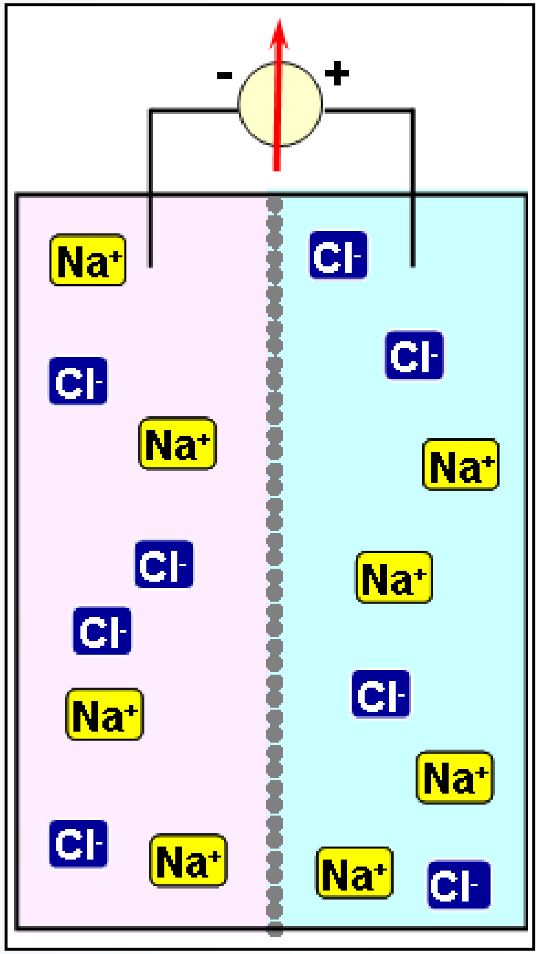

Fig. 7: Scheme of concentration equilibrium.

Diffusion Potential In Living Systems (1)

- Diffusion potential in living systems – solutions separated by a selective permeable membrane
- In such a system, equilibrium exists, if there is no net flow of a given ion
- Initial state:
  - Pure solutions of NaCl and KCl with equal concentration separated by a semipermeable membrane
  - Membrane:
    - Permeable to K^+
    - Impermeable to Na^+ and Cl^-
  - There exists a concentration gradient because of different compartments of both solutions although both solutions are electrically neutral

Diffusion Potential In Living Systems (2)

- Therefore, diffusion of K^+ only down its concentration fall, until an equal, but oppositely oriented electric gradient is created

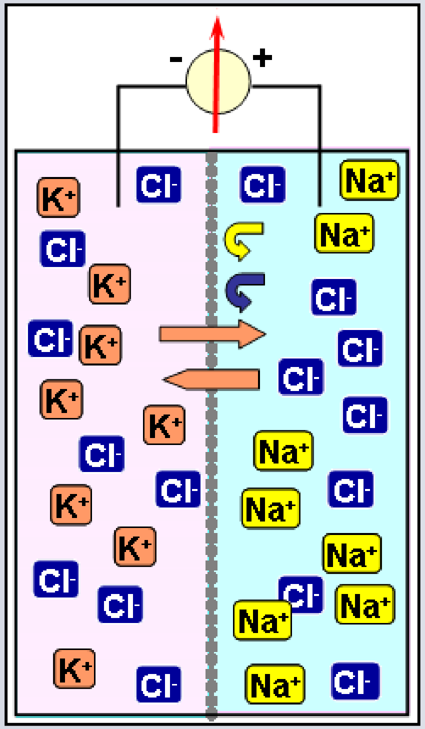

Fig. 8 (to the left):
Initial state for the generation of the membrane potential in living systems.

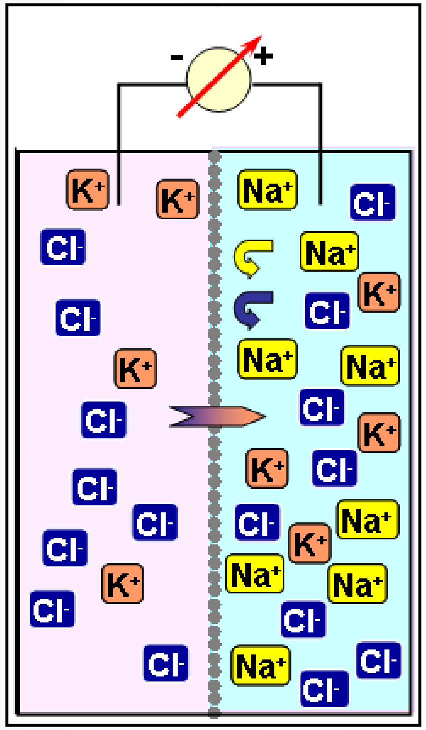

Fig. 9 (to the right):
Electrochemical gradient for the membrane potential in living systems.

Diffusion Potential In Living Systems (3)

- The electric and concentration gradients result into an equilibrium potential, when there is no net diffusion of ions

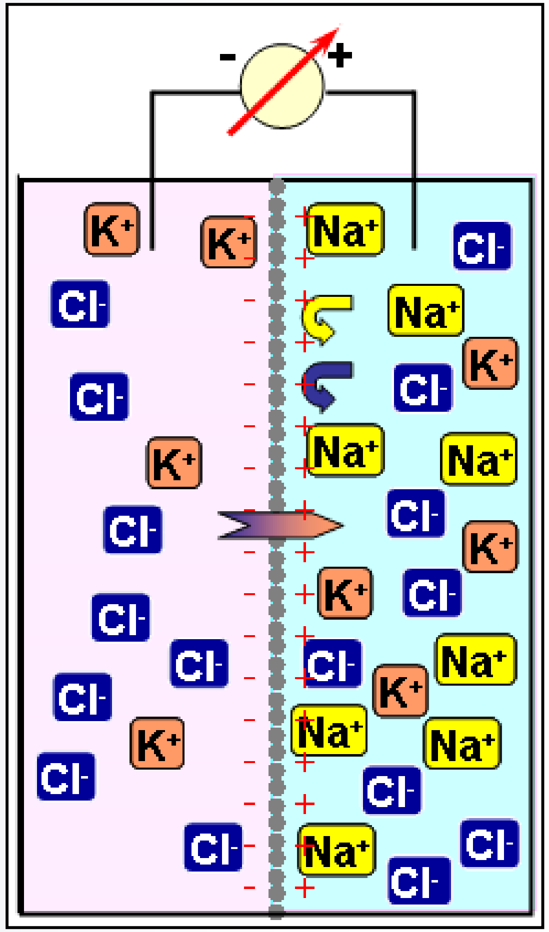

Fig. 10: Equilibrium state at the membrane potential in living systems.

Simple Case Of Membrane Equilibrium (1)

- Initial state:
  - The same electrolyte is on both sides of the membrane, but with different concentrations ($c_I > c_{II}$)
  - Membrane is permeable solely to cations

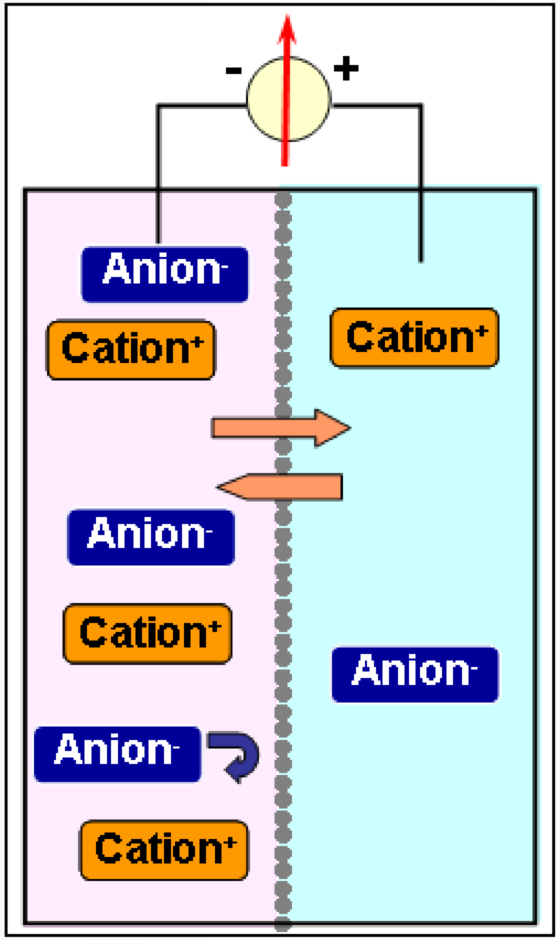

Fig. 11: Initial state for generation of membrane potential at a simple case membrane equilibrium.

Simple Case Of Membrane Equilibrium (2)

- Result:
  - An electric double layer is created across the membrane:
    - In electrolyte I:
      - The anions are stopped
    - In electrolyte II:
      - The cations are attracted to the anions
  - The concentration difference "drives" the cations, the electric field of the double layer "pulls them back"

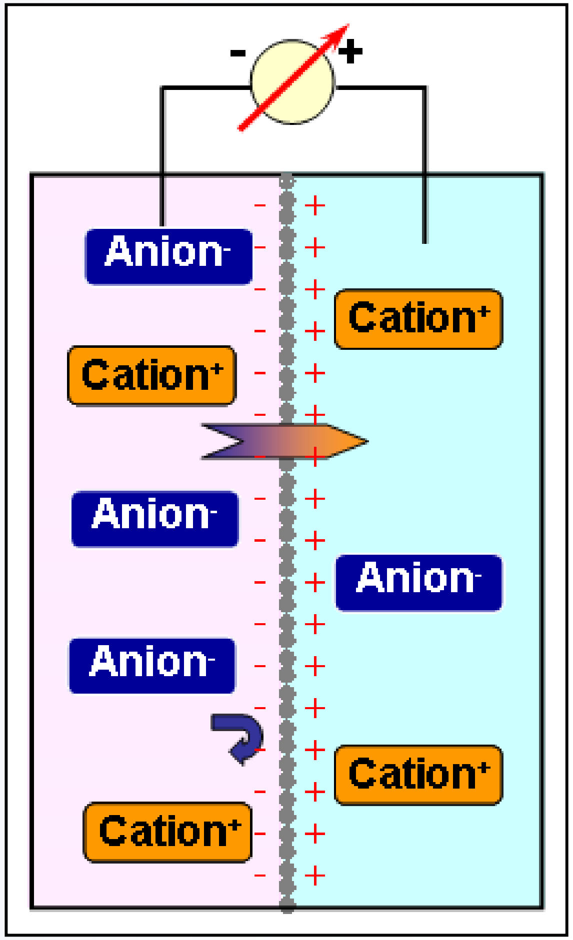

Fig. 12: Electric double layer forming the membrane potential in a simple case of membrane equilibrium.

Simple Case Of Membrane Equilibrium (3)

- At equilibrium a potential difference – a voltage *U* occurs, which:
  - Represents the difference between the potential φ_I of the electrolyte I and the potential φ_{II} of the electrolyte II

$$U = \varphi_I - \varphi_{II}$$

  - Expresses the mutual relation of cation concentrations at given temperature *T*

$$U = -\frac{R \cdot T}{z \cdot F} \cdot \ln \frac{c_I^+}{c_{II}^+}$$

Simple Case Of Membrane Equilibrium (4)

- Used symbols:
  - U : potential difference [V; mV]
  - φ_i : potential of the i^th^ electrolyte [V; mV]
  - R : mole gas constant: $R = 8.314\ \mathrm{J \cdot K^{-1} \cdot mol^{-1}}$
  - T : thermodynamic temperature [K]
  - z : valence of cations
  - F : Faraday constant: $F = 9.65 \cdot 10^4\ \mathrm{C \cdot mol^{-1}}$
  - c_i^+: concentration of cations in the i^{th} electrolyte [$\mathrm{mol \cdot l^{-1}}$]
  - c_i^-: concentration of anions in the i^{th} electrolyte [$\mathrm{mol \cdot l^{-1}}$]

Donnan Equilibrium (1)

- Initial state:
  - The same electrolyte on both sides of the membrane
  - Different concentrations ($c_i > c_{ii}$)
  - Membrane
    - Permeable to monovalent ions
      - They can freely diffuse
    - Impermeable to large anions
      - They are stopped at the membrane

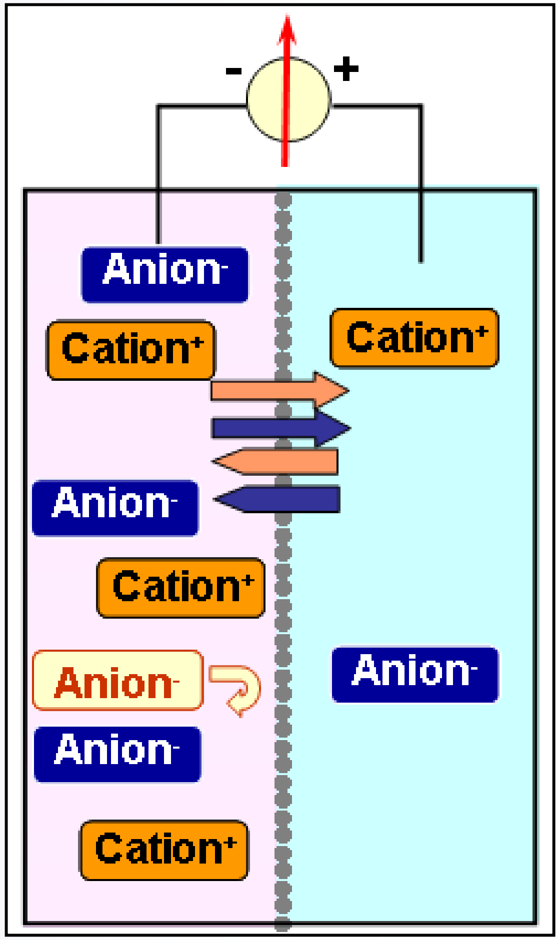

Fig. 13: Initial state for generation of the Donnan equilibrium.

Donnan Equilibrium (2)

- The consequence of immobile ions presence :
  - An equal distribution neither of mobile cations nor of mobile anions will be established, but a special equilibrium occurs – the Donnan equilibrium
- Equilibrium concentration at the Donnan equilibrium:
  - The product of concentrations of cations and anions on one side of the membrane equals the product of concentrations of cations and anions on the other side of the membrane

$$c_I^+ \cdot c_I^- = c_{II}^+ \cdot c_{II}^-$$

Donnan Equilibrium (3)

- Equilibrium concentrations can be expressed by their mutual ratio called the Donnan ratio
- Donnan ratio:
  - For monovalent ions:

$$\frac{c_I^+}{c_{II}^+} = \frac{c_{II}^-}{c_I^-} (= r)$$

  - In general, for any ion with valence *z*:

$$r = \sqrt[z]{\frac{c_I}{c_{II}}}$$

Donnan Equilibrium (4)

- Created potential is called the Donnan potential
- It is calculated as the difference of potential inside the cell φ_{in} minus the potential outside the cell φ_{ex}
  - In agreement with the measurement arrangement of the potential difference across the membrane
    - In Fig. 14 is the cell inside situated on the left side

$$U = \varphi_{in} - \varphi_{ex}$$

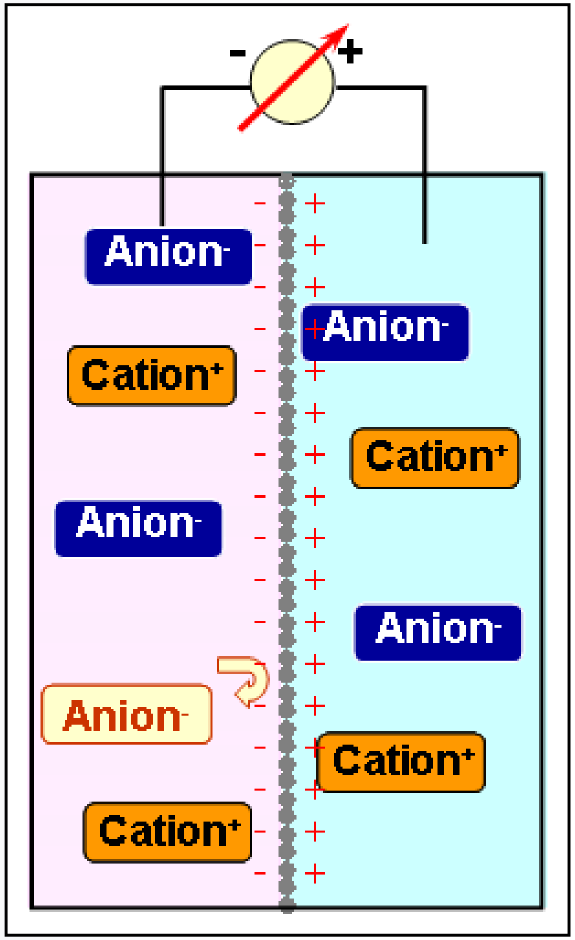

Fig. 14: Donnan potential.

Donnan Equilibrium (5)

- Different possibilities how to calculate the Donnan potential:
  - By means of cation concentrations: $$U = -\frac{R \cdot T}{F} \cdot \ln \frac{c_I^+}{c_{II}^+}$$
  - By means of anion concentrations: $$U = -\frac{R \cdot T}{F} \cdot \ln \frac{c_{II}^-}{c_I^-}$$
  - By means of arbitrary ion concentrations using the general expression of the Donnan ratio: $$U = -\frac{R \cdot T}{F} \cdot \ln r$$

Donnan Model In A Living Cell (1)

- Situation in a living cell:
  - Mobile ions: K^+, Cl^-
  - Immobile ions: Na^+, anions (phosphate, protein)
  - Concentration of ions inside the cell (in) and outside the cell (ex):
    - $[K^+]_{in} > [K^+]_{ex}$
    - $[Cl^-]_{in} < [Cl^-]_{ex}$
  - Donnan ratio:

$$\frac{[K^+]_{in}}{[K^+]_{ex}} = \frac{[Cl^-]_{ex}}{[Cl^-]_{in}}$$

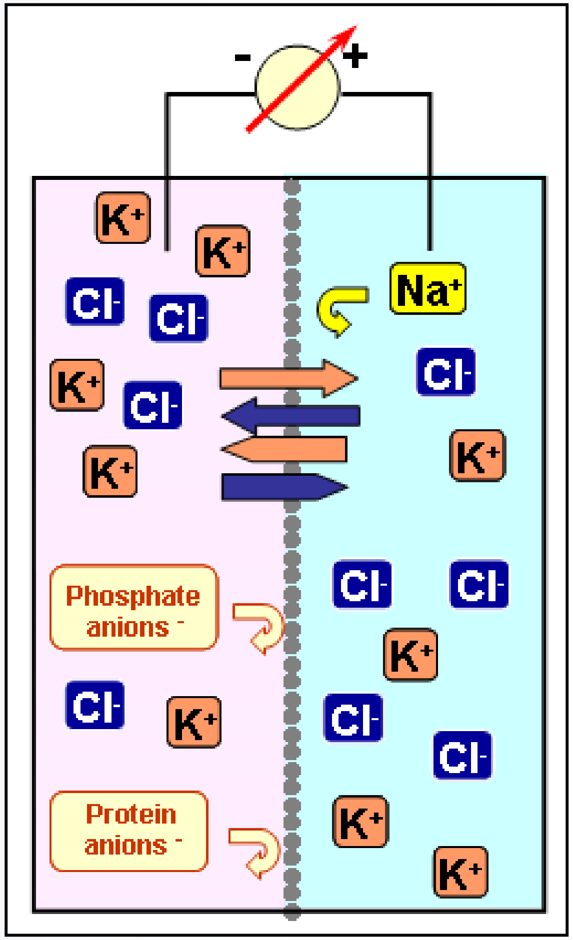

Fig. 15: Donnan equilibrium in a living cell.

Donnan Model In A Living Cell (2)

- How to calculate the Donnan potential:
  - By means of Potassium cations concentrations:

$$U = -\frac{R \cdot T}{F} \cdot ln \frac{[K^+]_{in}}{[K^+]_{ex}}$$

  - By means of Chlorine anions concentrations :

$$U = -\frac{R \cdot T}{F} \cdot ln \frac{[Cl^-]_{ex}}{[Cl^-]_{in}}$$

- Donnan potential has the same form as has the Nernst equation

Concentrations of ions in the intracellular and extracellular liquids

Table 1: Concentration of ions in the intracellular and extracellular liquids – examples. Concentration values are taken from Rontó and Tarján, 1997

Intracellular liquid		Extracellular liquid	
Ion	Concentration [mmol/l]	Ion	Concentration [mmol/l]
Squid giant axon			
Na^+	72	Na^+	455
K^+	345	K^+	10
Cl^-	61	Cl^-	540
Frog muscle			
Na^+	20	Na^+	120
K^+	130	K^+	2.5
Cl^-	3.8	Cl^-	120
Rat muscle			
Na^+	12	Na^+	150
K^+	180	K^+	4.5
Cl^-	3.8	Cl^-	110

Donnan Model In A Living Cell (3)

- Comparison of the Donnan potential obtained in calculations and by measuring the resting membrane potential

Table 2: Donnan potential – examples.

Cell	Potential value [mV]		
	Calculated with K^+	Calculated with Cl^-	Measured
Squid giant axon	- 91	- 103	- 62
Frog muscle	- 56	- 59	- 92
Rat muscle	- 95	- 86	- 92

Comment: Concentration values from Table 1 were used.

Donnan Model In A Living Cell – Summary Of Assumptions

- Donnan model differs from real situation, because:
  - The cell and its environment is regarded as a thermodynamically closed system
  - Immobile ions are assumed to be perfectly immobile, the membrane is no obstacle for mobile ions
  - It omits the effect of the ionic pump in the concentrations of ions
  - Interactions between the membrane and ions are not taken into account (although they may vary)

Transport Model (1)

- Electrodiffusion model with fewer simplifications
- Main characteristics:
  - Constant concentration differences between the outer and the inner side of the membrane ensures constant material transport across the membrane
  - Migration of ions across the membrane creates the electric double layer on both sides of the membrane

Transport Model (2)

- Main characteristics - continued:
  - The resting potential is equal to the potential difference characterising the double layer
  - All of the ion species on both sides can be considered simultaneously
  - An empirical fact – the membrane is neither perfectly permeable, nor perfectly impermeable for any of the ions
  - The membrane permeability differs for different ions

Transport Model (3)

- Calculation of the membrane potential in the transport model
- Potential difference $U = \varphi_{in} - \varphi_{ex}$
- Form as the Goldman - Hodgkin - Huxley – Katz equation (general form, p_k (p_l) s the membrane permeability of the k[th] (l[th]) ion)

$$U = -\frac{R \cdot T}{F} \cdot \ln \frac{\sum_{k=1}^{m} p_k^+ \cdot c_{k,in}^+ + \sum_{l=1}^{n} p_l^- \cdot c_{l,ex}^-}{\sum_{k=1}^{m} p_k^+ \cdot c_{k,ex}^+ + \sum_{l=1}^{n} p_l^- \cdot c_{l,in}^-}$$

Transport Model (4)

- In a real situation, it is enough to consider solely the ions of Potassium, Natrium and Chlorine
  - This equation is usually called the Goldman (- Hodgkin - Katz) equation

$$U = -\frac{R \cdot T}{F} \cdot ln \frac{p_K^+ \cdot c_{K,in}^+ + p_{Na}^+ \cdot c_{Na,in}^+ + p_{Cl}^- \cdot c_{Cl,ex}^-}{p_K^+ \cdot c_{K,ex}^+ + p_{Na}^+ \cdot c_{Na,ex}^+ + p_{Cl}^- \cdot c_{Cl,in}^-}$$

An animated model of membrane potential calculation is, for example, on page:
Goldman-Hodgkin-Katz Equation. [Cit.: 14. 2. 2012]
Available at: http://thevirtualheart.org/GHKindex.html

Transport Model (5)

- Giant squid axon ($t = 25°C$):
  - Relative permeability: $p_K : p_{Na} : p_{Cl} = 1 : 0.04 : 0.45$
    - Calculation: $U = -61$ mV
    - Measurement: $U = -62$ mV
- Frog Muscle ($t = 25°C$):
  - Relative permeability : $p_K : p_{Na} : p_{Cl} = 1 : 0.01 : 2$
    - Calculation : $U = -90$ mV
    - Measurement : $U = -92$ mV
- The agreement between the calculated and the measured values is in both cases at the level of measurement errors; the model can be regarded as a sufficiently accurate one.

Comment: Concentrations of ions from Table 1 were used for calculation.

Literature

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Comment:

If not stated else, the author of the text is the author of figures as well.