Membrane transport, Resting membrane potential

for pharmacy students

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SEMMELWEIS EGYETEM 1769

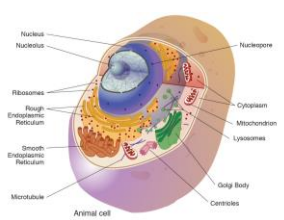
Lecture topics

Topics
- **Cell membrane** (function, structure, semipermeability)
- **Membrane transport**
  - Passive diffusion
    - uncharged particle and ion diffusion
    - permeability coefficient
  - Facilitated diffusion (channels, carriers, ionophores)
  - Active transport
- **Membrane potential**
  - Characteristics
  - Generation
    - Nerst equation
    - Donnan potential
    - Goldman-Hodgkin-Katz equation
- **Pharmaceutical importance**

Related practice topics
- Sensor
- ECG
- Diffusion

Textbook chapters
- III/4.1. Transport phenomena in resting cells
- III/4.2. Resting membrane potential

Cell membrane function

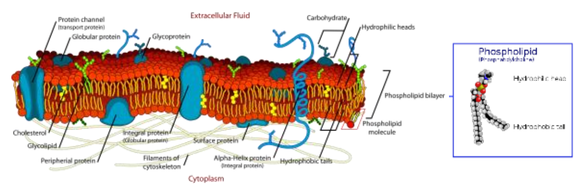


https://www.genome.gov/genetics-glossary/Cell

Cell
- The basic structural and functional unit of life.
- „cellula" (lt.) = small room
- Prokaryotic and eukaryotic cell types.
- Each cell has cytoplasm and cell membrane (plasma membrane)

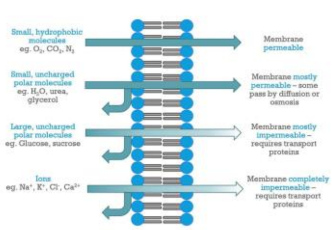
Function of cell membrane: barrier that precisely controls the level of solutes inside and outside the cell.

Cell membrane structure



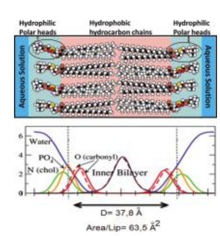
https://commons.wikimedia.org/wiki/File:Cell_membrane_detailed_diagram_en.svg

Semipermeability



https://www.wizeprep.com/online-courses/16916/chapter/3/core/3/1

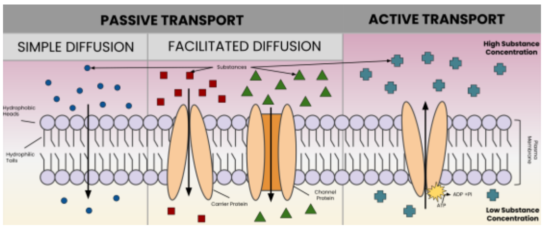
Structure and semipermeability



Disalvo, E; Frias, M (2019). The Role of Water in the Responsive Properties in Lipid Interphase of Biomimetic Systems.

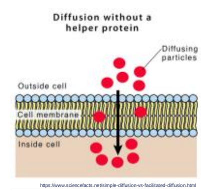
Aspects of semipermeability
- ~40 Å thick hydrophobic membrane core
- Permeability is composition dependent
- Affected by environmental factors
- Tighter packing of fatty acid chains lead to lower permeability
- Gel < liquid disordered < at T_m

Membrane transport types



https://commons.wikimedia.org/wiki/File:Passive_vs_Active_Membrane_Transport.svg

Passive diffusion of uncharged particles



https://www.sciencefacts.net/simple-diffusion-vs-facilitated-diffusion.html

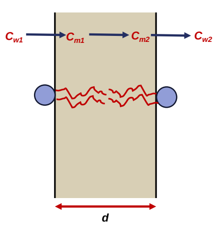
- Energy source: electrochemical gradient of the solute
- Passive diffusion requires no additional energy source.
- Steps:
  - solute must first lose its waters of hydration
  - diffuse across the membrane
  - and then regain its waters on the opposite side.
- The limiting step involves the energy required to lose the waters of hydration.

TABLE 19.2 Relationship Between the Waters of Hydration (Number of –OH Groups on a Homologous Series of Solutes) and the Activation Energy for Transmembrane Diffusion

Solute	Activation energy (kJ/mol)
Glycol (HO–CH_2–CH_2–OH)	60
Glycerol (HO–CH_2–CH(OH)–CH_2–OH)	77
Erythritol (HO–CH_2–CH(OH)–CH(OH)–CH_2–OH)	87

W. Stillwell: An Introduction to Biological Membranes (2016) http://dx.doi.org/10.1016/B978-0-444-63772-7.00019-1

Passive diffusion of uncharged particles



Fick's first law:

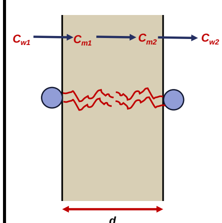
$$J_m = -D \cdot \frac{\Delta c}{\Delta x}$$

Adolf Fick 1829-1901

J_m: material flux density
D: diffusion coefficient
$\frac{\Delta c}{\Delta x}$: conc. gradient
D_m: membrane diff. coeff.

$$J_m = -D_m \cdot \frac{c_{m2} - c_{m1}}{d}$$

Permeability coefficient I.



$$J_m = -D_m \cdot \frac{c_{m2} - c_{m1}}{d}$$

$$p_m = \frac{D_m}{d}$$

membrane permeability constant $[\frac{m}{s}]$

$$J_m = -p_m \cdot (c_{m2} - c_{m1})$$

partition coefficient

$$\frac{c_{m1}}{c_{w1}} = \frac{c_{m2}}{c_{w2}} = constant = K$$

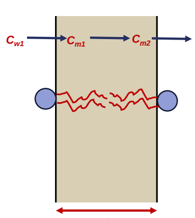
$$J_m = -p_m \cdot K(c_{w2} - c_{w1})$$

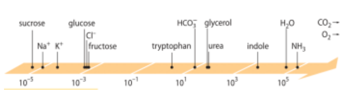
$$p = p_m \cdot K$$

permeability coefficient $[\frac{m}{s}]$

Material flux density (Fick I.): $J_m = -p(c_{w2} - c_{w1})$

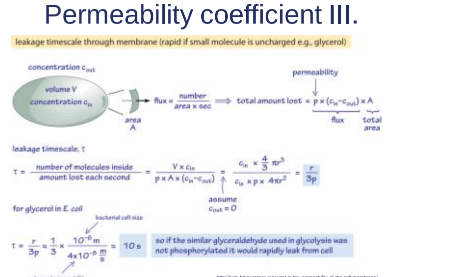
Permeability coefficient II.





http://book.bionumbers.org/what-is-the-permeability-of-the-cell-membrane/

Permeability coefficient III.



http://book.bionumbers.org/what-is-the-permeability-of-the-cell-membrane/

Passive diffusion of ions

Onsager equation:

$$J = L \cdot X$$
$$X = \frac{-\Delta\mu}{\Delta x}$$
$$\mu = \mu_0 + RT \cdot \ln c$$

Fick's first law

$$J = -\frac{LRT}{c} \cdot \frac{\Delta c}{\Delta x}$$

D

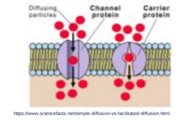
For a charged particle (k):

$$J_k = L_k \cdot X_k = -L_k \cdot \frac{-\Delta\mu_{ek}}{\Delta x} = -D_k\left(\frac{\Delta c_k}{\Delta x} + c_k \frac{z_k F}{RT}\frac{\Delta\varphi}{\Delta x}\right)$$

flux density of k^{th} particle: (J_k); *electrochemical potential gradient* ($\frac{-\Delta\mu_{ek}}{\Delta x}$); *concentration gradient* ($\frac{\Delta c_k}{\Delta x}$); *electric potential gradient* ($\frac{\Delta\varphi}{\Delta x}$)

- J_m: material flux density
- D: diffusion coefficient
- $\frac{\Delta c}{\Delta x}$: conc. gradient
- J: flux density
- L: conductivity coeff.
- X: thermodynamic force
- μ: chemical potential
- μ_0: molar free enthalpy
- R: univ. gas constant
- μ_e: electrochemical potential
- F: Faraday constant
- z: valency
- φ: electric potential

Facilitated diffusion



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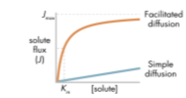


https://neurotext.library.stonybrook.edu/C1/C1_7/C1_7.html

- Energy source: inherent solute **electrochemical gradient**
- Gradient determines direction (**theoretically reversible**)
- No additional energy is required to transport the solute
- Final solute distribution reaches equilibrium across the membrane.
- Orders of magnitude **faster rate** than passive diffusion
- Protein-based **mediator molecules** embedded in the membrane
- **Strongly selective** for certain particles
- Exhibits Michaelis-Menten **saturation** kinetics
- Can be selectively **inhibited**
- Mediators: carriers, gated ion-channels, ionophores

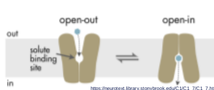
Facilitated diffusion

I. Channel proteins

- Transport mainly ions
- Supramolecular structures of several subunits → span the membrane → hydrophilic core is formed
- No conformational change during transport
- Gating: stimuli-responsive conformational change → opens or closes the channel
- Stimuli: voltage; ligand; second messenger; mechanics
- Rate cca. 10^6 s^{-1}

II. Carrier proteins

- Integral membrane proteins
- Bind specifically an ion or molecule
- Reversible conformational change enables the transport
- $E_{activation}$ is given by the bindig energy of substrate
- Min. 100x slower than channel proteins

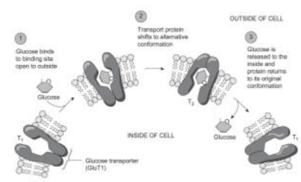


https://neurotext.library.stonybrook.edu/C1/C1_7/C1_7.html

Facilitated diffusion - examples

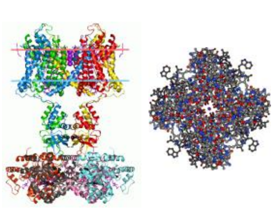
Glucose transporters (GLUT)

- Superfamily of carrier proteins
- Occur in nearly all cells
- Abundant in small intestines
- Integral membrane proteins
- 12 alpha helices in membrane spanning region.
- Activation energy of glucose should be > 100 kJ/mol (passive diffusion)
- BUT it is only 16 kJ/mol (with GLUT).

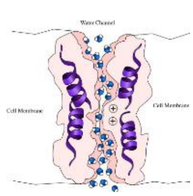


W. Stillwell: An introduction to Biological Membranes (2016) http://dx.doi.org/10.1016/B978-0-444-63772-7.00019-1

Facilitated diffusion - examples



Potassium channels

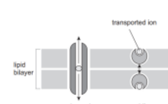


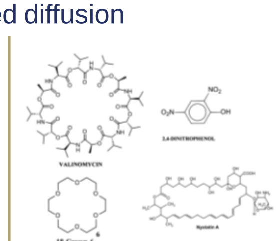
Aquaporin channels

Facilitated diffusion

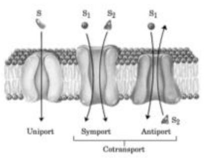
III. Ionophores („Ion bearers”)

- Small, lipid doluble molecules of usually microbial origin
- **Channel formers:** long lasting, stationary structures; many ions at a time; rapid flow across a membrane.
- **Mobile carriers:** ion binding on one side of a membrane; dissolving; membrane crossing; release. They can only carry one ion at a time.





Active transport



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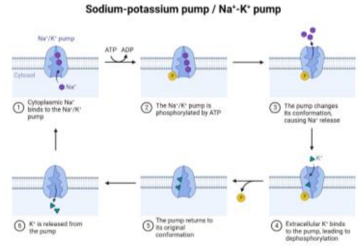
Characteristics

- Particles are transported against gradient → nonequilibrium distribution of solutes accross the membrane
- Requires energy! Possible sources:
  - ATP hydrolysis – ATPases
  - Light – photo transporters
  - Electrochemical gradient of another substrate – coupled (secondary) active transporters
- Uniporters / co-transporters
- Symporters / antiporters

Active transport - examples

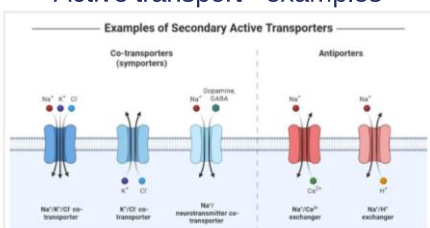
Sodium-potassium pump / Na^+-K^+ pump



https://thebiologynotes.com/active-transport/

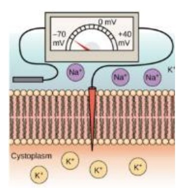
- ATPase
- antiporter
- accounts for one-third of human energy expenditure
- 3 Na^+ out / 2 K^+ in
- electrogenic
- Blocker: ouabain, digoxin

Active transport - examples



https://thebiologynotes.com/active-transport/

Membrane potential

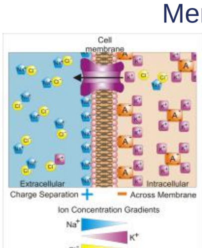


https://courses.lumenlearning.com/wm-biology2/chapter/resting-membrane-potential/

Transmembrane potential / Membrane voltage / „Resting membrane potential"

- Electric potential difference between inner and outer surface of the membrane
- Present in all living cell
- Varies among cell types (-30 mV to -90 mV)
- Negative sign: cell interior is negative compared to extracellular space
- Functions:
  - providing power to operate a variety of "molecular devices" embedded in the membrane (cell as battery)
  - in electrically excitable cells such as neurons and muscle cells, it is used for transmitting signals between different parts of a cell

Membrane potential



By Synaptidude, CC BY 3.0, https://commons.wikimedia.org/w/index.php?curid=21460910

- Two sides of the membrane has different ionic composition

	Intracellular concentration [mM]			Extracellular concentration [mM]		
Cell type	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-
Squid giant axon	72	345	61	455	10	540
Frog muscle	20	139	3,8	120	2,5	120
Rat muscle	12	180	3,8	150	4,5	110

- Large phosphate and protein anions inside – p ~ 0
- p is different for the different ions
- Electric and chemical potential difference occurs between the two sides.

Generation of membrane potential I.

Model 1

Presumptions:

- Closed thermodynamic system
- Membrane permable to ions
- Cytoplasm and extracellular space are in thermodynamic **equilibrium** – for **each** ion!
- No net transport of ions
- Thermodynamic force is 0
- Electrochemical potential is the same at the two sides for each type of ion:

$$\mu_{e,i}^{int} - \mu_{e,i}^{ext} = 0$$

↓

$$\mu_0 + RT ln c_i^{ext} + zF\varphi_i^{ext} = \mu_0 + RT ln c_i^{int} + zF\varphi_i^{int}$$

↓

$$\varphi_i^{int} - \varphi_i^{ext} = U_0 = \frac{RT}{z_i F} ln \frac{c_i^{ext}}{c_i^{int}}$$ Nerst equation

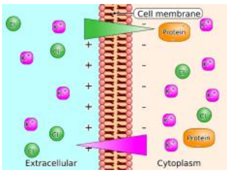
Electric potential of i^{th} ion in equilibrium = equiilibrium potential = Electromotive force of a concentration cell of the i^{th} ion

	Squid giant axon	Frog muscle
$U_{measured}$	-62 mV	-92 mV
U_{0Na+}	47 mV	46 mV
U_{0K+}	-91 mV	-103 mV
U_{0Cl-}	-56 mV	-88 mV

Results: model failed

- Nerst equation is inadequate to interpret resting potential
- It is not a closed system in equilibrium
- Transport of individual ions is not independent

Generation of membrane potential II.



Donnan equilibrium (Gibbs-Donnan effect)

- Closed thermodynamic system
- Presence of nonpermeable charged particles in one side of a membrane leads to noneven distribution of ions.
- Membrane is permeable only to K^+ and Cl^-
- Electroneutrality at the two sides,
- Electrochemical potential is the same at the two sides for each type of ion.

Donnan potential:

- Equilibrium potential difference due to ionic concentration differences (determinded by the presence of nondiffusible anions)
- Typical value: U_{Donnan} ~ -14 mV → relatively small contribution to membrane potential.

Generation of membrane potential III.

Goldman-Hodgkin-Katz equation

- Passive ion diffusion maintains an electric potential difference
- Permeability is different for different ions
- Flux of individual ions ≠ 0
- At rest the transmembrane potential difference is constant →, total electric charge and particle flux must be 0.
- Thus, flux of ions may depend on each other

Steady state elecrodiffusion:

- Constant electrochemical potential gradient → constant flow of ions → electrically compensate each other → no net charge accumulation
- Na^+/K^+ pumps maintains constant ion gradients

Result: good agrrement with experimental data

flux density of k^{th} particle:

$$J_k = -u_k kt\left(\frac{\Delta c_k}{\Delta x} + c_k \frac{z_k F}{RT}\frac{\Delta\varphi}{\Delta x}\right)$$

D ($u_k kt$); *electric potential difference* ($\Delta\varphi$); *electrochemical potential gradient* (bracketed term)

$$\Sigma J_k = 0$$

$$U = \Delta\varphi = \frac{RT}{F} ln \frac{p_{K^+}[K^+]_{ext} + p_{Na^+}[Na^+]_{ext} + p_{Cl^-}[Cl^+]_{int}}{p_{K^+}[K^+]_{int} + p_{Na^+}[Na^+]_{int} + p_{Cl^-}[Cl^+]_{ext}}$$

	Squid giant axon	Frog muscle
$U_{measured}$	-62 mV	-92 mV
U_{GHK}	-61.3 mV	-89.2 mV

Pharmaceutical importance - examples

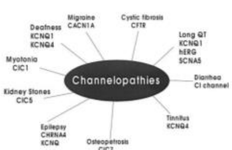


FIG. 2. Channelopathies. Examples of diseases associated with dysfunction of ion channels and the specific ion channel species involved.

TABLE 3
Ion channel modulator drugs on the market

Ion channel mechanism	Drug	Indication
Cl^- channel activators	Benzodiazepines:	Anti-anxiety
	Nitrazepam	Insomnia
	Lorazepam	Epilepsy
	Clonazepam	Anti-spastics
	Diazepam	
K_{ATP} channel blockers	Sulphonylureas:	Type II diabetes
	Tolbutamide	
	Glibenclamide	
	Glipizide	
K^+ channel blocker	Amiodarone	Cardiac arrhythmia
	Dofetilide	
	Sotalol	
Ca^{2+} channel blockers	Verapamil	Cardiac arrhythmia
	Nifedipine	Arterial hypertension
	Diltiazem	
	Amlodipine	
	Felodipine	
	Lacidipine	
Na^+ channel blockers	Lidocaine	Local anesthesia
	Lamotrigine	Epilepsy
	Phenytoin	
	Carbamazepine	
	Chinidine	Cardiac arrhythmia
	Disopyramide	
	Mexiletine	

Willumsen, Niels & Bech, Morten & Olesen, Søren-Peter & Jensen, Bo & Korsgaard, Mads & Christophersen, Palle. (2003). High Throughput Electrophysiology: New Perspectives for Ion Channel Drug Discovery. Receptors & channels. 9. 3-12. 10.1080/10606820308259.

Thanks for your attention!

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