LO#2: Passive transport mechanisms of the cell membrane

- Features of membrane: ingredients, molecular structure (lipid and protein components)

- Transport mechanisms: diffusion, ion channels, osmosis
LO#3: Active transport mechanisms of the cell membrane

- Primary pumps
- Transporters
- Transport mechanisms in polarized cells
- Vesicular transport

Functions of the membrane

- Diffusion barrier – controlled exchange of substances
- Electric insulation – resistor and capacitor
- Communication – signal transduction (receptors, ion channels, second messenger systems)
- Cell identity – cell-specific macromolecules (e.g.: MHC antigens, blood group antigens, etc.)
- Intercellular interactions – adhesion molecules (e.g.: immune response, gap junctions, tight junctions)
- Metabolism - lipid mediators stem from membrane lipids: phosphatidyl inositol (IP₃) – diacylglycerol, inositol trisphosphate; arachidonic acid: prostaglandins, leukotrienes, endogen cannabinoids
Structure of the plasma membrane

...historical background...

- 1665: Robert Hooke first uses „cellula”
- 1839: cell as a functional unit (Schleiden & Schwann)
- Early 20th century: chemistry of membrane, bilayer
- Davson & Danielli: globular protein envelop

Molecular structure of the membrane

- 1972: Singer & Nicolson, fluid mosaic model
- Lipid bilayer
A) Lipid bilayer

Phosphatidic acid

(a) Chemical structure of a phospholipid

(b) Simplified way to draw a phospholipid

Cell membrane
6-7 nm
Asymmetric distribution of lipid components of the plasma membrane in human red blood cells

<table>
<thead>
<tr>
<th>Membrane phospholipid</th>
<th>Percent of total membrane phospholipid</th>
<th>Distribution in membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphatidylethanolamine</td>
<td>30%</td>
<td>Inner monolayer 30</td>
</tr>
<tr>
<td>Phosphatidylcholine</td>
<td>27%</td>
<td>Outer monolayer 27</td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>23%</td>
<td>Outer monolayer 23</td>
</tr>
<tr>
<td>Phosphatidyserine</td>
<td>15%</td>
<td>Inner monolayer 15</td>
</tr>
<tr>
<td>Phosphatidylinositol</td>
<td>15%</td>
<td>Outer monolayer 15</td>
</tr>
<tr>
<td>Phosphatidylinositol 4-phosphate</td>
<td>5%</td>
<td>Outer monolayer 5</td>
</tr>
<tr>
<td>Phosphatidylinositol 4,5-bisphosphate</td>
<td>5%</td>
<td>Outer monolayer 5</td>
</tr>
<tr>
<td>Phosphatic acid</td>
<td>5%</td>
<td>Outer monolayer 5</td>
</tr>
</tbody>
</table>

Functions of phospholipids

- Interface - transport
- Precursors of secondary messengers (e.g. arachidonic acid, IP$_3$, DAG with the help of phospholipases
- Anchoring enzymes (e.g. acetylcholinesterase - glycosylphosphatidylinositol)
- Antioxidants (e.g. plasmalogen in peroxisome)
- Apoptosis: phosphatidylserine to the outer monolayer
- Membrane potential (e.g. phosphatidylserine in inner monolayer with its negative charge)
Glycolipids

• In the outer monolayer
• Lipid asymmetry
• Antigens (blood group), receptors (bacterial toxins), adhesion (neuron-myelin gangliosides)
• *Gangliosides*: ceramide-oligosaccharides-sialic acid
glycosphingolipid

Artificial lipid membrane

• *In vitro* experiments (transport mechanisms) – planar bilayer
• Diagnostics, therapy – liposome
Cell membrane is highly dynamic

Movements of membrane components: rotation, lateral diffusion, flip-flop, fatty acid chain (FAC) bends

- fluidity
  - Depends on:
    - Lipid arrangement; unsaturated FACs → “more fluid”)
      (poikilothermic animals in low temperature);
      rigidity: increased amount of saturated FAC
    - External temperature
    - Cholesterol: rigid tetracyclic ring, maintains membrane integrity, long-term temperature adaptation, lateral membrane fluidity, pathologic: arteriosclerosis

Lateral diffusion

Lateral movement of lipids and proteins found in the membrane, fairly quick ($10^7$/s), free to move laterally if they are not restricted by certain interactions

Rotation, flip-flop

- Rotation: an individual lipid molecule rotates very quickly around its axis
- Flip-flop: from one membrane surface to the other (very rarely) → phospholipid translocator (flippase)
B) Protein components of membrane

- Neighbouring (polar heads and FACs) → functioning properties
- Peripheral (extrinsic): binds to polar heads or integral proteins
  - Inner monolayer, maintains cell morphology, intracellular signal transduction
- Integral (intrinsic): penetrates into nonpolar parts
  - Transmembrane protein: embedded in the hydrophobic central core of the lipid bilayer
  - extracellular, transmembrane, intracellular domain
  - glycoproteins

- Stability: bindings between hydrophobic amino acids of proteins and fatty acid chains
- Recirculation of membrane proteins by transport vesicles
- **Intracellular** transport pathways: targeted movements of proteins, „**Trafficking**”, translocation
- „Lipid rafts”
Overview of intracellular protein transports

Influenced membrane function

Extreme importance in medicine
effects examples for drugs which influence the functions of the plasma 
membrane: Local and general anaesthetics, antiepileptic and 
antiarrhythmic drugs, diuretics, psychotropic drugs etc.:

Lidocaine, cocaine – Na-channel blocker
Antibiotics – polymyxin, Pseudomonas
Alcohol: short term → fluidity ↑, long-term → rigidity (cholesterol 
content ↑) + reactive oxygen species!
Membrane transport system

- Basic transport mechanisms
  - Diffusion, permeability
- Primary pumps
  - Uphill transport (against gradient), ion movement - ATP hydrolysis
- Passive carriers
  - Downhill transport (according to gradient), conformation alteration
- Transmembrane ion channels
  - Ion and water movement, osmotic gradient
  - Vesicular transport
  - Endocytosis, exocytosis, transcytosis

Diffusion

random motion of particles involved in the net movement of a substance from an area of high concentration to an area of low concentration

- Driving forces: concentration difference (ions: electrostatic force)
- Fick’s law:

\[ J = \frac{dQ_s}{dt} = -D \cdot A \cdot \frac{dc}{dx} \]

- Flux, or movement, of the molecules in a given time interval; \( D \): diff. Constant in fluid; \( A \): diff. area; \( dc \): concentration difference; \( x \): thickness of the membrane
- Permeability: rate of passive flow, \( dQ_s/dt = P(C_1 - C_2) \) \( P \): constant (cm/s)
  - Depends on temperature, thickness of the membrane, lipid solubility
  - Free diffusion: gases (O\(_2\), CO\(_2\), NO, N\(_2\)O), uncharged small molecules (ether, ethyl-alcohol)
  - Water soluble molecules and ions NOT!

In physiology: alveolar gas exchange, endothelial cells – microcirculation, absorption in the GI tract
Transport molecules

• General features:
  – Specificity (selective permeability or binding)
  – Saturation (transport velocity is proportional to the number of channels/carriers (see Michaelis-Menten kinetics)
  – Temperature dependency
  – Regulation/activation-inhibition (gating: channel subunit conformation change, covalent/non-covalent modification, gene expression change, translocation)
    • competitive inhibition, non competitive inhibition
• Ion channels and pores (porins, perforin, MAC-complement system)
• Passive carriers and active pumps
• Driving forces:
  – Passive: concentration or electrochemical gradient
  – Active: metabolic energy (ATP hydrolysis)

A) Passive carriers

• Downhill transport: according to the concentration difference(or electrochemical gradient)

• Types:
  – uniporters: e.g. glucose transporter
  – Symporter (cotransport): two or more ions/molecules transported in one direction, sodium-glucose cotransporter (SGLT)
  – Antiporter (cotransport, exchanger): two or more ions/molecules in opposite directions, Cl⁻-HCO₃⁻ exchanger

Ion transport:
  electrogenic (results in translocation of net charge)
  electroneutral (no translocation of net charge)
Uniporters

- **Facilitated diffusion**, GLUT

\[ V_{\text{max}} \] proportional to the number of carriers

No glucose specificity, competition with other monosacharides

<table>
<thead>
<tr>
<th>GLUT isoform</th>
<th>( K_m ) (substrate)</th>
<th>Tissue &amp; Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLUT-1</td>
<td>5 glucose</td>
<td>Resting glucose uptake in most cells, including muscle</td>
</tr>
<tr>
<td>GLUT-2</td>
<td>10-100 (glucose, [\text{galactose, fructose}])</td>
<td>Liver, monocytes, spleen, kidney, ovaries, erythrocytes</td>
</tr>
<tr>
<td>GLUT-3</td>
<td>1-2 (glucose)</td>
<td>Mainly brain (note low ( K_m )), also found at low levels in other tissues</td>
</tr>
<tr>
<td>GLUT-4</td>
<td>5-6 (glucose)</td>
<td>Insulin-sensitive tissues – Skeletal muscle, adipose tissue</td>
</tr>
<tr>
<td>GLUT-5</td>
<td>6 (fructose)</td>
<td>Adipose</td>
</tr>
</tbody>
</table>

Competitive inhibition: \( V_{\text{max}} \) doesn’t change, \( K_m \) increases

Non-competitive inhibition: \( V_{\text{max}} \) decreases, \( K_m \) doesn’t change

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Antiporters

1. **Cloride-hydrocarbonate exchanger in red blood cells:**

2. **Sodium-calcium exchanger ensures low calcium level:**
Symporters

• Requires all the substrate particles for functioning
• E.g.: Sodium-glucose, Na-phosphate-, Na-K-2Cl-cotransporters in kidney

Transmembrane channels – water channels, AQUAPORINS

• Transmembrane proteins through which water molecules flow according to the concentration gradient (passive!)
• Ensures basic and increased (regulated) water permeability of cell membranes
• Constantly open (gating in plants only)
Special diffusion: osmosis

• 1748 Jean-Antoine Nollet abbé, vine cooling in urinary bladder
• 1865 van’t Hoff: theory
• Driving force: chemical potential difference
• In balance net movement of solvent molecules is 0.
• Osmotic pressure: \( \text{"that would have to be applied to prevent the net flow of solvent molecules across the membrane (mm Hg or Pa)\} \ P = RT/n/V} \) (van’t Hoff)
• The force of the column of water (hydrostatic pressure) on the hypertonic side of the semipermeable membrane will equal the force of diffusion (osmotic pressure) on the hypotonic side, creating equilibrium

1 osmol: number of molecules in 1 mol \( (6*10^{23}) \)
Blood plasma osmotic concentration: 290 mosm/l
In case of ions – number change due to the dissociation!
OzmolaLity: molar concentration \( \text{(osm/kg H}_2\text{O}) \) vs.
OzmolaRity: molar concentration \( \text{(osm/l)}, \) temperature!
Tonicity: described due to the response of cells immersed in an external solution \( \rightarrow \) RBCs (AQP1), practice!

Solutions: iso-, hypo-, hyperosmotic
Colloidosmotic (oncotic) pressure: elicited by the macromolecules; filtration!

Filtration

• Semipermeable barrier layer
• Driving forces: pressure gradients
  – (colloid osmotic (oncotic) pressure: macromolecules)
• 2 sides of cells
• E.g. filtrate (kidney), tissue capillaries
Transmembrane channels – ion channels

- Different ion channels are presented in cells: determines functional properties, excitable or non excitable cells
- Conductivity \(10^6-10^9\) ion/s
- Transmembrane proteins, ions flow through according to their gradients
- Functional regions: pore, selectivity filter, gate subunits
- Rectification: conductivity depends on the ion flux direction
- Activation (probability) – gating mechanisms:
  - Voltage gated (sodium, calcium, heart, nervous system)
  - Ligand gated (nicotinic-acetylcholine receptor, skeletal muscle)
  - Mechanosensitive (touch)
  - Chemo sensitive (ASICs, low extracellular pH, nervous system)
  - Thermoreceptors
  - Intracellular signal sensitive (G-protein activation)
- Leaky: constantly open

Medical importance of ion channels

The electric (and other functional) properties of excitable and non-excitable cells are determined by the expression pattern of the ion channels

- Ion channels are targets of many different drugs:
  - Voltage dependent sodium channels: local anaesthetics, anti-arrhythmic and antiepileptic drugs
  - Ionotropic acetylcholine receptors: muscle relaxants
  - ATP-sensitive K⁺ channels: oral antidiabetic drugs
  - GABA₄ receptors (ionotropic Cl⁻ channels): hypnotic, sedative drugs
- Congenital malfunctions of ion channels (or carriers):
  - „Channelopathies“ Congenital arrhythmias of the heart (e.g.: long QT syndrome) - K⁺ channels
  - Myotony (delayed relaxation of the muscles): - Cl⁻ channels
- Not strictly ion channels:
  - Renal type diabetes insipidus (failure of water conservation, polyuria): failure of the aquaporin-2 function
  - Cystic fibrosis: CFTR Protein (Cystic Fibrosis Transepithelial conductance Regulator – dysfunctional Cl⁻ transport)
**Examples**

Ligand-gated: nicotinic-acetylcholine receptor:

- Voltage gated sodium channel:
  - Intracellular activation (G-protein)
  - m-Ach-R & K⁺-channel, heart, Frv.↓

Transmembrane channels – antibiotics, antimicrobics and toxins

- Gramicidin
- Nystatin (perforated patch clamp method)
- Streptococcus pneumoniae, pneumolysin
B) Active transporters - Primary pumps

- ATPase activity (ATP is required)
- P-type ATPase: become phosphorylated, V-type: vacuolar (no phosphorylation, e.g., H⁺-pump cell organelle)
- Against concentration gradient, uphill transport
- Sodium/potassium pump

Active transporters – ABC transporters

- „ATP Binding cassette” sequence, transmembrane and nucleotide-binding domain (cytosol)
- CFTR: only one ion channel
- MDR (multidrug transporter): responsible for chemoresistance, protecting tissues from toxic xenobiotics and endogenous metabolites, low specificity, therapy-resistance
Primary pumps

<table>
<thead>
<tr>
<th>Group</th>
<th>Member</th>
<th>Location</th>
<th>Substrate(s)</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-ATPase (coupling factor)</td>
<td>H^+ - Pase</td>
<td>Mitochondrial inner membrane</td>
<td>H^+</td>
<td>ATP synthesis driven by electrochemical gradient of H^+</td>
</tr>
<tr>
<td>V-ATPase (vacuolar)</td>
<td>H^+ - Pase</td>
<td>Cytoplasmic vesicles (lysosomes, secretory granules), plasma membranes (ruffled border of respiratory, kidney epithelial cell)</td>
<td>H^+</td>
<td>Activation of lysosomal enzymes, accumulation of neurotransmitters, turnover of bone, acidification of urine</td>
</tr>
<tr>
<td></td>
<td>H^+ / K^+ - Pase</td>
<td>Stomach (parietal cell in gastric gland)</td>
<td>H^+ and K^+</td>
<td>Acidification of stomach lumen</td>
</tr>
<tr>
<td></td>
<td>Ca^2+ - Pase</td>
<td>Sarcoplasmic reticulum and endoplasmic reticulum</td>
<td>Ca^2+</td>
<td>Ca^2+ sequestration into sarcoplasmic (endoplasmic) reticulum</td>
</tr>
<tr>
<td></td>
<td>Ca^2+ - Pase</td>
<td>Plasma membrane</td>
<td>Ca^2+</td>
<td>Ca^2+ excretion to outside of the cell</td>
</tr>
<tr>
<td></td>
<td>Ca^2+ - Pase</td>
<td>Plasma membrane and cytoplasmic vesicles</td>
<td>Ca^2+</td>
<td>Ca^2+ absorption from intestine and excretion from liver</td>
</tr>
<tr>
<td>ABC ATP-binding cassette (transporter)</td>
<td>Polyprotein</td>
<td>Plasma membrane</td>
<td>Various drugs</td>
<td>Excretion of harmful substances, multidrug resistance for anticancer drugs</td>
</tr>
<tr>
<td></td>
<td>MRP</td>
<td>Plasma membrane</td>
<td>Glutathione conjugate</td>
<td>Detoxification, multidrug resistance</td>
</tr>
<tr>
<td></td>
<td>CFTR</td>
<td>Plasma membrane</td>
<td>Cl^-</td>
<td>cAMP-dependent chloride-Dannel, regulation of other channels</td>
</tr>
<tr>
<td></td>
<td>TAP</td>
<td>Endoplasmic reticulum</td>
<td>Peptide</td>
<td>Pintratination of peptides for immune response</td>
</tr>
</tbody>
</table>

Active transports – polarized cells

Secondary and tertiary active transport

Distal tubule epithelial cell

2 membranes 3 compartments
Vesicular transports: endocytosis (pinocytosis, fluid-phase endocytosis with less specialised and larger amount, pl. thyreocyte, phagocytosis), exocytosis, transcytosis

James Rothman, Randy Schekman, and Thomas Südhof were awarded the 2013 Nobel Prize in Physiology or Medicine ‘for their discoveries of machinery regulating vesicle traffic, a major transport system in our cells’.