Membranes

Introduction

- **Cell membrane**
  - **Encloses** cell contents to make a functional unit
  - **Interfaces** with the cell's environment
    - **Senses** changes
    - **Signals** to the cell to respond
  - **Control board** for entry and exit of material
  - Central to its cell's nutrition and homeostasis
- **Endomembranes**
  - Enclose specialised organelles
  - Allow organelles to maintain their own internal environment
  - Site of communication with cytosol
- Can also be the site of specialised **chemical reactions** that can't take place in the cytosol
- Composition
  - **Lipids**
    - Defensive
    - Flexibility
    - Permeability barrier
    - Matrix for reactions
    - Active + passive transport
  - **Proteins**
    - Active + passive transport
    - Enzyme activity
    - Reception of regulatory molecules
  - **Polysaccharides**
    - No more than 10% by weight
    - Glycoproteins predominate in animals, glycolipids in plants
    - Cell recognition and adhesion
    - Recognition of regulatory molecules
  - Proportions vary depending on function
    - Nerve cells have lots of lipid
    - Mitochondria cells have lots of protein

Membrane lipids

- All **amphipathic** (to some extent)
  - One part is **non-polar and hydrophobic**
  - The other is **polar and hydrophilic**
- Make up the majority
- **Phospholipids** are the most common
  - Glycerol molecule
  - **Ester linkages** between -OH groups on C1 and C2 and **carboxyl groups** of long fatty acid chains
  - The -OH on carbon 3 forms an ester link to **phosphatic acid**
  - Phosphate group forms an **ester link** to the final component, **a charged group**
  - Hydrophobic + phosphatidic + charged group
- **Sphingophospholipids**
  - Formed around a long chain N-containing alcohol **sphingosine**
  - Use an **amino group** that can form an **amide link** to a long fatty acid to form a **ceramide**
  - The -OH group in C1 position can ester link to a phosphate which links to a polar headgroup
- The fatty acids
  - Comparatively simple, unbranched (14-20 carbons long)
  - May be **saturated** or **unsaturated**
  - In eukaryotes, one will usually be saturated, the other one not
- **Glycolipids**
  - Lipids that contain a carbohydrate group
  - May or may not contain a phosphate group
  - Most common type are **sphingolipids**, where the -OH group of a ceramide links to a carbohydrate moiety instead of phosphate
- **Sterols**
  - A class of steroids
  - Steroids comprise four interconnected rings of carbon atoms that have varying number of C=C bonds and side groups
  - -OH at C3 position and non-polar carbon chain at C17 position
  - Rigid ring structure, and only -OH is polar, so weakly amphipathic
  - Presence
    - Eukaryotes
      - Animals
        - Most abundant in **cholesterol**
      - Plants
        - Much greater diversity
        - Cholesterol is a minor part
    - Absent from eukaryotes
- **Bilayers**
  - Phospholipids in aqueous solution form a **lipid bilayer**
    - Hydrophobic moieties associate to form the core
    - Polar hydrophilic headgroups face solution
  - Pack closely together, via **van Der Waals** interactions
  - At physiological pH, they are **zwitterion**, so no charge repulsion
  - Ends can be left alone - spontaneously
  - Same together to form a **liposome**
    - Can incorporate purified membrane transport proteins and study
    - Can also deliver things to cells (e.g. corrected cystic fibrosis protein)
  - The degree of curvature is strongly influenced by glycolipid content
    - Glycolipid-rich - **support sharp bends**
    - E.g. thylakoid membranes are enriched in glycolipids
- Correct fluidity is essential
  - **Temperature** has a profound effect on cell function due to **fluidity** (studied through X-ray and differential calorimetry)
    - **Low temperature**
      - Hydrocarbon chains are tightly packed
      - Restricts motion
      - **Gel state**
    - **Phase transition temperature**
      - **Peak of heat absorption**
      - Bilayer interior becomes more fluid
      - Hydrophilic headgroups are still closely associated
      - Overall integrity of layer remains, inside is a bit of a goo
      - **Liquid crystal state**
      - Normal membrane function occurs in this state
    - **High temperature**
      - Forces holding bilayer together disrupted
      - **Bilayer replaced by disordered liquid solution of individual liquid molecules**
    - Differential calorimetry shows that
      - One type of phospholipid (artificial) = discrete transition temp
      - More of them = larger range
    - So, transition temperature influenced by lipid composition
      - Those with shorter carbon chains undergo transition at lower temperature
      - Unsaturated chains have it at 45-53 (vs. 0) because majority of C=C bonds are in cis form which causes **kinks** and **restricts packing** of chains in the bilayer (weaker Van der Waals)
      - **Sterols** (**cholesterol**) phospholipids - polar hydrophilic group in in contact w/ aqueous solution w/ head groups, and hydrophobic core w/ rigid rings and hydrocarbon chains
        - Stabilises headgroups (restricts movement there)
        - Separates tails and causes inner region to become more fluid
        - **Below** transition temperature, helps maintain fluidity; **Above**, restricts movement of head groups and prevents bilayer from coming apart
        - **Act to restrict temperature-induced changes in bilayer structure**
    - **Homeoviscous adaptation**
      - In plant, bacterial and poikilothermic animal cells, membranes become adapted to lower temperature
      - Increase production of unsaturated fatty acid chains to maintain fluidity
      - And vice-versa
- Lipids move
  - In one half of bilayer (v. rapid)
    - Move laterally (changes places with neighbour)
      - Lipid rafts move together
    - Spin about longitudinal axis
  - Trans
    - **Flip-flop motion**
    - Rarely occurs in artificial membranes (thermodynamically unfavourable)
    - In biologically membranes, **flipases** catalyse process and make it(1) in more
    - Proteins **do not** flip
  - Across bilayer
  - Evidence for flip-flop
    - **Spin label** (absorb E at specific wavelength in magnetic field and produce spectrum)
      - ESR machine
    - Label all phospholipids in cell at phosphate
    - Add ascorbate - label at external surface reduced
    - Remove ascorbate and wait
    - Re-challenge with ascorbate
    - More reduced because of fresh inlt-labelled lipids that have flipped
  - Membranes make membranes
    - Cells can only make new membranes by expanding old ones
    - Enzymes for phospholipid synthesis membrane-bound with active site facing cytosol (receive substrates there)
      - New phospholipids go straight into membrane
    - Ways to make
      - **Bacteria** - synthesis at plasma membrane
      - **Plants, fungi + animals** - cytoplasmic side of smooth ER followed by movement of membrane
    - To achieve ordered structure, must "flip-flop" to other side (case incorporated in cytoplasmic half of bilayer
    - Special enzymes do this
      - **Flipases** - transport from side with highest conc. to other
      - **Phospholipid translocators** - translocate **specific phospholipids** from one half of bilayer to other, using energy. Can be against conc. gradient
- Lots of techniques have shown that membrane proteins and lipids are **asymmetrically distributed** in the two halves of the layer
- **Asymmetry**
  - Lipid asymmetry is no absolute (some of each in each side)
  - Protein and (probably) carbohydrate is absolute
    - Ends and exposures
    - Important in
      - Active transport
      - Cell recognition

Membrane proteins

- Proteins can attach in various ways
  - **Fully traverse** the bilayer
    - Single ALPHA helix or BETA barrel
    - Or traverse it several times (several ALPHA-helices)
    - In these proteins, are also have fatty acid covalently attached to protein and inserted in bilayer
    - Such ALPHA helices are usually **amphipathic**
      - External hydrophobic face interacts with bilayer
      - Internal can be hydrophilic => can be transport proteins
  - **Attach** through a **covalently attached lipid**
    - Or attached via a **bridging oligosaccharide**, to one of the **phospholipids** in the **outer half** of the bilayer
    - Suitable for proteins that aren't hydrophobic
  - So far, these were **integral membrane proteins**
    - can only be detached by disrupting layer
  - Other proteins can attach through **covalent interactions** with other integral proteins
    - These are called **peripheral membrane proteins**
    - Can frequently be detached by gentle procedures which don't disrupt the membrane (e.g., changes in ionic strength, temperature shock)
- Determining whether we have a spanning protein
  - Add **lactoperoxidase**, **H2O2** and **radioactive iodide** to cells
  - This causes the H2O2-dependent **iodination** of the protein's **tyrosine residues**
  - Assumes, lactoperoxidase cross the membrane, so only the outer tyrosine residues are labelled
  - This is labelling, in-situ
  - Then, take another sample of cells and disrupt their membrane - then, label them
    - In situ
  - Extract proteins and digest with protease
  - Run the results on an **SDS polyacrylamide gel**
  - New bands will have appeared for membrane-spanning proteins
  - Linear membrane proteins must be removed before re-labelling in the second step, lest they spuriously create signals inside the cell
- SDS Polyacrylamide gel electrophoresis
  - Denature with a reducing agent (e.g., mercaptoethanol)
  - Unfold with detergent (e.g., SDS)
  - SDS is very charged and binds to the proteins, eliminating charge as a variable
  - Only mass then matters
- They moved
  - Association
    - Like lipids, proteins move **laterally** in the membrane
    - Cell fusion
      - Make antibodies to mouse and human membrane proteins, and conjugate them to different colour dyes
      - Conjugate these to mouse and human cells, and fuse the two cells (using, for e.g., an electrical field)
      - Originally, markers are segregated on opposite sides of the cell - eventually, they mix
    - Fluorescence recovery after photobleaching (FRAP)
      - Treat cell with **concanavalin A** (conA), conjugate w/ fluorescent dye
        - Plant protein
        - Binds to **carbohydrate moiety** of surface glycoproteins
      - Use a laser to **bleach** the dye in a certain part of the cell
        - The bleach molecules can never fluoresce again
      - Time the return of fluorescence
        - This corresponds to other proteins diffusing into that part
      - Results for mycoplasm
        - 50% of fluorescence regained
        - So 50% of glycoproteins are mobile
        - Others can be attached to the cytoskeleton
- The cytoskeleton
  - Some proteins need to be in a specific place in the cell to function correctly
  - The **cytoskeleton** can control protein position and reduce lateral mobility
    - Spectrins
    - Actin
    - Band 4.1
  - Can also control cell shape (e.g. if **erythrocytes**)
    - Plasma membrane contains **peripheral proteins** which form the **cytoskeleton**
    - Lie **below** the plasma membrane, and attached to it at regular intervals
    - Spectrin dimers join head-to head to form tetramers, bound at the tail to short actin filaments and Band 4.1 at junction complexes
    - Spectrin is anchored to the cytoplasmic surface by **ankyrin** which bind tightly to the **integral transmembrane protein Band 3** (an ion channel)
    - When erythrocytes are treated with **low ionic strength buffers**, the peripheral proteins dissociate
      - The cells then lose their shape
      - The proteins show lateral mobility
- Membrane protein traffic
  - Proteins not destined to stay in the cytosol contain **signal sequences** (usually N-terminus, 15-30 aa, usually 10 central hydrophobic AAs usually forming ALPHA-helix)
  - When 70-80 AAs have been polymerised the signal sequence has emerged
    - In eukaryotes, it different proteins bound to one molecule of tRNA
    - Into the cytosol, it is bound by an **SRP (Signal Recognition Particle)**
      - Slows down or even stops further synthesis until the next recognition event
  - **The SRP receptor**
    - Recognises SRP
    - Also contains **ribosome receptor**
    - Ribosome **docks** and SRP detaches and goes back into cytosol
    - Allows protein synthesis to continue
  - The signal sequence is then recognised by the ribosome receptor and acts as a **translocation tunnel** through the membrane
    - Synthesis resumes and the protein enters the lumen
    - Folding of new proteins in the lumen is **catalysed** by ATP-dependent **foldases**
    - Integral membrane proteins have a **hydrophobic internal "stop-transfer"** sequence which halts the translocation process through the tunnel
      - This leads to the fixing and orientation of the protein in the bilayer
      - Synthesis continues until the carboxy terminus outside the ER
    - If the protein is not going to be integral, the finished protein emerges into the ER lumen and the pore of the tunnel closes
  - **Signal peptidases** in the ER then cleave the N-terminal signal sequence from the new protein
  - **Redirection**
    - Those staying in the ER contain **recognition sequences**
    - Some contain **sorting sequence** to a particular cellular compartment
      - Leave the membrane inside **vesicles**
      - Or no sorting sequence => plasma membrane/secretory pathway
        - Bud from specialised ribosome-free regions of the ER near the golgi
        - Then bud to the cis cisternae followed by the other cisternae in the golgi, by repeated fusion and budding
        - Those with "stay-in-ER" signal sequence (KDEL) are re-directed to the ER thanks to an ER retrieval mechanism that recognises it
        - This is a highly conserved pathway
      - Others destined to go elsewhere
      - Pathways also exist to redirect cytoplasmically produced proteins to organelles - extra protein tags
- Glycosylation and covalent modification
  - Carbohydrates are attached almost exclusively to lipids and proteins of the plasma membrane and secreted proteins
  - These sugar residues are attached covalently to the amino acid chain
    - Usually contain 4-15 sugar residues
    - Can be branched
  - **Glycosylation** occurs in two stages
    - Sugars making up the basal structure added as a single oligosaccharide
      - Catalysed by **glycosyl transferases**
      - When tail is sticking out into the ER lumen
    - During passage through the golgi, additional side-branch residues are added
  - Covalent attachment to lipids also occurs in the ER

Passive transport

- This movement **against electrochemical gradients**
- **Primary active transport**
  - Usually **very specific**
    - Displays **saturation kinetics**
  - Can be used to accumulate solute against a gradient (important in nutritional uptake)
  - Can be used to transport a solute out of a cell against a gradient (important for detoxification)
  - The protein catalyses the hydrolysis AND the transport reaction
    - Energy is obtained by the hydrolysis of an energy-rich compound
  - The two reactions are COUPLED
  - The main types of energy donors are
    - ATP, GTP, PPi (inorganic pyrophosphate)
    - All have high energy phosphate bond
  - **Solute-translocating ATPases**
    - Called this because of conserved feature of formation of phosphorylated intermediate during the reaction
    - On example critical to sub function are **P-type ATPases**
  - Example is the Na+ – K+ translocating ATPase
    - Integral plasma membrane transporter
    - Widespread in animal cells
    - Animal cells are bathed in **high Na+ and low K+**
    - It is essential for K+ to be pumped in and for Na+ to be kept below toxic levels
    - This pump does just that - for each ATP hydrolysed, three Na+ ions are removed, and two K+ ions are added
    - All ATP in the cell can be used for this
  - **Mechanism**
    - Phosphorylation and binding of Na+ on exposed cytosolic side causes a **conformation change** that transfers the bound Na+ across the membrane to the outside
    - The pump is then returned to its original conformation by the **binding of K+** on the **extracellular surface** and **hydrolysis/dephosphorylation**
    - In the process, K+ is transferred into the cytosol and released
    - In fact more complex, because of 3:2 ratio
    - 1 bin out of the cell per ATP hydrolysed
    - Can take up to 1/2 of the cell's energy supply
    - Creates large inside negative membrane potential and pH gradient - crucial to cell function
  - **V-type ATPases**
    - In plants and fungi, H+ is the predominant ion (rather than Na+) for this kind of thing
    - Large ion-translocating pumps, ubiquitous in eukr
    - Found in endomembranes (vacuole, endosome...)
    - Existential to the acidification of compartments they border
  - **Secondary active transport**
    - In plants, another P-type ATPase predominates, and it translocates H+
    - Multi-subunit enzymes
    - The energy of existing gradients can be harnessed to the translocation of another solute against its gradient
    - e.g., Na+ in animals at H+ in cells and endosomes, vacuoles, etc.
    - If the transport of both solutes is in the **same direction**, then we call it a **symporter**
    - Otherwise, an **antiporter** (those that transport a single solute are **uniporters**)
  - **Na+ coupled glucose transport**
    - Transport of glucose from the gut lumen through the intestinal epithelium and into the blood
    - **Basolateral membrane Na+ – K+ ATPase** sets up an **electrochemical gradient** by pumping it out of the cell
    - This affects the other side where the entry of Na+ back into the cell is coupled to the uptake of glucose by an Na+ – glucose symporter
    - Glucose can then diffuse to the other side of the cell and be transported down its concentration gradient by a carrier
    - H+ coupled amino acid symport at fungal plasma membrane
    - H+ coupled Na+ antiport at the plant vacuole membrane
  - **Signaling**
    - Membrane proteins function in signaling events
    - Responses triggered without crossing membrane (receptor)
- **Lipid mediated**
  - The **partition coefficient** is (Amount dissolving in test lipid / amount dissolving in water)
    - We hope that the test lipid will be similar to the core of the bilayer
    - Use olive oil for experiments
  - Usually, the **higher the partition coefficient**, the higher the **permeability coefficient**
    - Straight line of y = x on graph
    - **Water** and **urea are small**, and so diffuse well despite their low partition coefficient
    - **For polar molecules**, the smaller they are **higher their permeability coefficient**
    - Exceptions
      - for small uncharged solutes, the rate is determined by **Fick's First Law**
      - **Rate limiting step** is viscosity of the core
      - Water flow and gas exchange
      - Important in
  - Proportional to permeability coefficient and concentration gradient, inversely proportional to distance
    - **Rate limiting step** is viscosity of the core
  - Movement of **ethanol** for plant and fungal cells undergoing anaerobic respiration
  - Renders cells and organisms vulnerable to hydrophobic pollutants from industrial activity
  - Huge difference in rate of flow of ions in **artificial lipid bilayers** and **native layers**
  - **Evidence**
    - Especially rare
    - Catalyse the movement of solute from one side of the membrane to the other (e. conformational change, etc...)
    - **Highly specific**
    - **Can be inhibited**
    - ***Rate of reaction** is much higher* than would be in diffusion because of the **lowered activation energy**
    - Shows **saturation kinetics**
    - ***But*** substrate end up in the **same chemical state but in a different compartment**
    - Occurs **down an electrochemical gradient** - **not intrinsically vectorial**
    - So it's still diffusion, but we call it **facilitated diffusion**
    - Both powered by the electrochemical gradient (but lower activation energy of transport, and can be very specific
- **Protein mediated**
  - **Transport proteins** are regarded like enzymes
  - **Carriers**
    - Usually about 12 **ALPHA-helices**
    - Particularly useful for sugars and amino acids
    - Solute binds at the side of greater concentration
    - Mechanism
      - Conformational change which prevents further binding there and causes the release to the other membrane face
      - A return to the original conformation
    - It can then no longer fit the binding site
    - Fungi and RBCs prevent the release of glucose through this mechanism by **phosphorylating** it as soon as it enters the cell
  - **Channels**
    - **24 membrane spans for animal channels**
    - 10^6 - 10^8 per second
    - Probably because of tiny conformational changes involved
    - Fastest enzymes known
    - Fastest water soluble enzyme known is catalase (10^5 per second)
    - Range of specificity
      - Surface charge
      - Narrow filter
      - Residues lining the aqueous pore
    - Can be controlled
      - **Conformational "gate"** keeping channel in an **open** or **closed** state
      - This can be controlled by a **voltage sensor** (positively charged AA residues)
      - Channel activity can also be controlled by **phosphorylation** or binding of ligands such as cAMP
      - See diagram p 27
    - As charge flows, this generates **electrical current**
    - This can be measured using **patch clamp electrophysiology**
    - Glass microelectrode with a 1μm end is pushed against the membrane
    - This electrically isolates it from the rest of the cell, and currents can be measured
    - Signal transduction
    - Muscle contraction
    - Crucial for things like
    - Turgor relationships
- **Ionophores**
  - **Bacterial peptides**
    - Secreted by **left competing bacteria**
    - They **shuttle within a membrane**, and transport solutes
    - e.g., **Valinomycin** holds K+ in the **centre** of its structure, and **hydrophobic residues** on the outside permit **transit** through the layer
    - Valinomycin
    - Gramicidin
      - Example
- **Porins**
  - In bacterial cell membranes
  - Transmembrane BETA-barrel porins that allow passive transport

Osmosis

- Three types of transport
  - **Diffusion** within a compartment
  - **Bulk flow** - mass movement of water molecules, usually in response to a pressure gradient
    - Also passive
    - Best example is water movement in **xylem vessels**
  - **Osmosis** - movement of water through a **differentially permeable membrane** from an area of **high water potential** to one of **low water potential**
    - This leads to a **solute potential**
    - For dilute solutions and a non-dissociating **solute**, this is given by the **van't Hoff equation**
      - $\psi_s = -RTc_s$
      - Concentration gradient of water
      - For dissociating solute, this is multiplied by the number of dissociating particles
      - Diluting solute in water increase disorder = decreases free energy = decreases potential
    - Leads to a **pressure/hydrostatic/turgor potential**
    - Negative pressure (suction) **decreases** the water potential
    - Important for walled cells, where the protoplast pushes against an inflexible wall which pushes back with an equal force
    - Pressure gradient
    - Only has appreciable impact above 5m
    - Gravity
      - Usually ignored
    - Do it **relative** to that of **pure water at sea level at standard temperature and pressure**
    - Sum all these potentials
    - We usually divide the potential by the **molar volume** of water to give the **water potential** (free energy per unit volume)
    - Chemical potential depends on three things
    - No pressure difference across membrane
    - Water potential will be due to solute potential only
    - Wall-less cells
      - Placing the cell in equally concentrated **(isotonic)** more concentrated **(hypertonic)** or more dilute **(hypotonic)** solution will cause size changes to dilute or concentrate solute inside the cell and reduce the potential difference to 0
      - To keep a constant volume and prevent cells from bursting, cells use their membrane proteins to **adjust ionic concentration in the cytosol**
    - Walled cells
      - In a hypotonic solution, the cell water resists expansion which leads to an increase in the turgor potential. This pressure increases until the potential difference is 0 again
      - In a hypertonic solution, water leaves the cell - this usually means that the membrane peels away from the wall, leading to pre- **plasmolysis**
      - Solute concentration is also regulated in plants, like in animals
      - Activity of many antibiotics is to weaken the cell wall -> lyse
    - Weird that with relatively low lipid permeability, water can go so quickly through membranes
    - Water-permeable channel proteins (**aquaporins** - involves ALPHA-helices) have been identified in a variety of cell types (plants are stuffed full of them)
    - Regulated by phosphorylation and intracellular Ca^{2+} to control rate of water flow
    - Current thinking - osmosis is a combination of diffusion of single water molecules through the membrane and bulk flow through the pores
    - **In action**
    - **Mechanism**

Transport - introduction

- Homeostasis - **protein structure and function sensitive to surrounding medium. Must maintain internal environment against a different and changing external environment**
- Purpose
  - "Trade" and communication - **nutrients, exporting waste products, signal particles (messaging)**
  - Volume regulation - **by regulating the amount of water**
  - Compartmentation - **organelles provide the eukaryotic cell with areas of specialised functions, each of which need their own internal environment**
- Vesicle-mediated transport
  - **Exocytosis** is the process by which small proteins of the membrane blebs and pinch off to form vesicles
  - **Endocytosis** is the process by which vesicles fuse with an existing membrane
  - These allow the control of membrane composition, but also the import and export of cargo
  - Endocytosis can be **receptor-stimulated**
    - When this is **solid**, it's **phagocytosis**
    - When it's **liquid**, it's **pinocytosis**
    - Endocytosis involves "capturing" the external medium in a vesicle
    - Membranes undergo "turnover" and modification.
    - **Solute** to be transported binds to **extracellular domain** of a **specific transmembrane protein**
    - This in turn causes a conformational change in the **intercellular domain** of the protein
    - Consists of three heavy and three light chains, which form a **trisskelion**
    - Adaptin binds to the free end of the heavy chain
    - A **protein adaptor** (**adaptin**) can then bind, which interacts with another cytoplasmic protein - **clathrin**
    - The clathrin assemblies just below the membrane and spontaneously **polymerises** to form **coated pits** of **invaginated membrane**
    - This continues until it **excises** to form a **clathrin-coated vesicle**
    - New receptors can diffuse into the area of the bit and be "captured"
    - The vesicle is then **uncoated** by a specific **chaperone protein** and the clathrin and adaptin are recycled
    - The vesicle is now the **"early endosome"**
    - Can be up to 2000 of them per minute
    - The cargo is then **unloaded**
    - **Early endosome** fuses with the **late endosome** (or **CURL**)
    - Core of cholesterol ester-linked to fatty acids
    - Insoluble so transported as water-soluble
    - Surrounded by layer of phospholipids
    - **LOW DENSITY LIPOPROTEIN (LDL)**
    - Contains protein allowing recognition by LDL receptor
    - E.g. **cholesterol**
    - **CURL** lumen is at lower pH (pH 5.5) - LDL dissociates from receptor
    - Receptors return to membrane via vesicles
    - LDL transported to **lysosome** by other vesicles - contain hydrolytic enzyme to cleave fatty acids from cholesterol
  - Exocytosis
    - Can release cargo to the outside of the cell
    - **Capsaicin** in chili peppers
    - Example
    - ALPHA-amylase secreted by specialised alleurone cells during germination, to break down starch supplies
    - **Solute remains at the same membrane face during transport**
- Transmembrane transport
  - Movement of solute **through** a membrane
  - **Through the bilayer**
    - Generally, the more lipid soluble the better it will permeate the layer
    - Non-polar and hydrophobic molecules (oxygen, fatty acids) easily permeate
    - **Small** polar molecules also do (important example **water**)
    - Will only occur if there is a **concentration gradient**
    - This is **passive transport**
    - Net movement will occur **down the gradient (diffusion)**
    - There is a **chemical potential gradient** (free energy difference)
  - **Protein mediated**
    - Hydrophilic, charged and polar solutes require the assistance of proteins to permeate the membrane
    - Can be passive or active
    - $$\Delta G = RT\ln\left[\frac{[X]_{outside}}{[X]_{inside}}\right] + zFE_m$$
    - This is for **INWARDS TRANSPORT** of material
    - z is the charge on the ion
  - **Energetics**
    - Notes
      - Most membrane potentials are negative
      - Eo is the potential of the **INSIDE RELATIVE TO THE OUTSIDE**
      - i.e. the inside the negative relative to the outside
      - -90 mV in animal cells, -150 mV in plant cells and -200 mV to -250 mV for fungal cells
    - $$E_m = \frac{-RT}{zF}\ln\left[\frac{[X]_{inside}}{[X]_{outside}}\right]$$
    - $$E_m = \frac{RT}{zF}\ln\left[\frac{[X]_{outside}}{[X]_{inside}}\right]$$
    - If we re-arrange, change logs and include constants, we get the NERNST EQUATION
    - This is for a temperature of 20 degrees = 293 K
    - If our gradient is **different** to that predicted by this equation, then active transport's got to be playing a role
    - DELTA G for ATP hydrolysis is **-50 kJ/mol**

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