Membrane Proteins

- Transmembrane proteins
  - cytosolic & extracellular part – hydrophilic
  - transmembrane part – hydrophobic (non polar amino acid chains)
    - for α-helices to maximise H-bonding in absence of H_2O – between polar peptide bonds

Single pass

- pass through memb. once
- form α-helices
- hydrophobicity calculated through hydropathy plots
- chain-bending not possible

Multipass

- multiple Traversions
- form β-barrels
- not possible because each Transmemb. Segment is too short
- contain regions that fold into memb. from either side.

Q: Do Transmembrane α-helices have functions other than anchoring?

Yes. Single pass proteins often form dimers. Interactions b/w their transmemb. α-helices determine protein-protein interaction.

Q: Why are transmemb. α-helices of multipass proteins hydrophobic, even if they are shielded by other helices?

Because, they are individually inserted into bilayer by protein translocator. Transient connection b/w with lipids in bilayer. This requires helix to be hydrophobic.

Q: Why are multipass membrane proteins easier to crystallise?

Memb. proteins are 2D-like, and the transmembrane parts are thin & delicate. Because they are hydrophob, they can get denatured by detergents. But multipass β-barrels are rigid — crystallise readily.

More about multipass memb. proteins

1. abundant in outer memb. of mitochondria, chloroplasts, many bacteria (esp. β-barrel proteins)
2. sometimes create waterfilled channel for hydrophilic molecules to pass through
3. porins, might be highly selective
4. might also function as receptors and enzymes & bac. plasma memb.
5. made of α-helices in eukaryotes (helices slide against each other & undergo conformational change) & β-barrels in prokaryotes (rigid with H-bonding)

Glycosylation

- most transmemb proteins are glycosylated on non-cytosolic side (because they are added in lumen of ER & golgi body)

Q: Why are S-S bonds not found on cytosolic side?

Because of reducing environment of cytosol.

- **Glycocalyx** – carbohydrate layer around cell membrane
  - composed of glyco-part of glycoprotein and glycolipids, lectins, secreted molecules adsorbed onto surface
  - mechanical and chemical protection, boundary b/w cells, preventing unwanted protein-protein interaction.

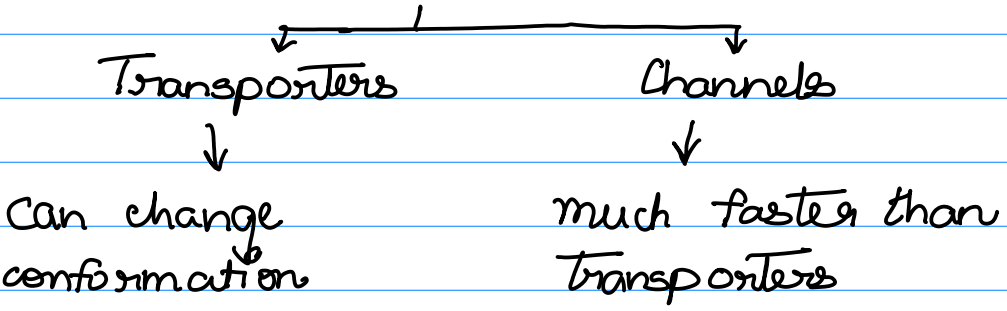
Detergents

- used to destroy the lipid bilayer & solubilise transmemb. proteins
- above CMC – form micelles (amphiphilic)
- when mixed with membranes, hydrophobic ends bind to hydrophobic regions of memb. proteins, displacing lipid molecules – detergent-protein complexes
- proteins analysed by SDS page
- strong detergents denature proteins, mild detergents

do not unfold proteins, just cover hydrophobic regions of memb spanning proteins

Membrane Transport

- exclusively multipass to create hydrophilic channels.



- Channels and many Transporters
  ↳ passive (facilitated diffusion)
  single uncharged molecule → conc. gradient
  charged → electrochemical gradient

Ion channels

- selective, fluctuate b/w open and closed states
- selectivity means pores are narrow enough to initiate contact with walls of channel (that can discriminate)

What are the distinguishing features of ion channels?

① Selectivity filter - narrowest part of channel, ions shed all H_2O, limits rate of passage → saturates after a point

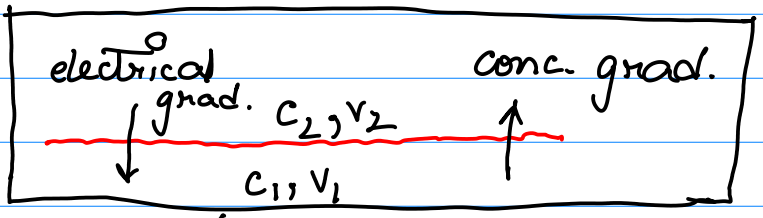
intra extra

② Gating – Voltage, mechanical, or ligand-gated, not continuously open — refractory period with prolonged activation

K^+ Ion Channel

- difference in memb. potential across two sides of memb → memb. potential
  - due to passive ion diffusion
  - pumping

For K^+



↳ when at equilibrium, no net flux of ions across memb.
⇒ resting potential / Nernst potential.

How do we find this?

$$\frac{c_1}{c_2} = \frac{e^{-ze v_1/k_B T}}{e^{-ze v_2/k_B T}}$$

Two players → Na^+-K^+ pump
Two players → K^+ leak channels

The story

No voltage gradient across cell

↓

$K^+ \downarrow$

$K^+ \uparrow$

↓

K^+ leaves through K^+ leak channels

↓

Unbalanced ⊖ charge inside (membrane potential)

↓

× Further efflux of K^+ when outward conc grad = inward electrical grad

↓

Resting membrane potential

Note: Resting pot. decays when $Na^+ - K^+$ pump stops as conc. grad decays

More permeable an ion is → more strongly memb. pot. decays as pump is stopped

How does this K^+ leak channel work?

↳ Selectivity

↳ not explained by pore size – why not the smaller Na^+ ion?

- Structure det. by X-ray crystallography

- Structure

1. Four identical transmembrane subunits.
2. cation-selective: negatively charged amino acids conc. at cytosolic entrance to repel anions
3. Each subunit

- 2 Transmembrane α-helices ⟹ tilted outward and form a cone.
- polypeptide chain that connects 2-alpha helices → forms selectivity filter (loop)

↳ 4 selectivity filters, form short narrow pore, lined by carbonyl Oxygen, to provide transient binding sites to dehydrated K^+.

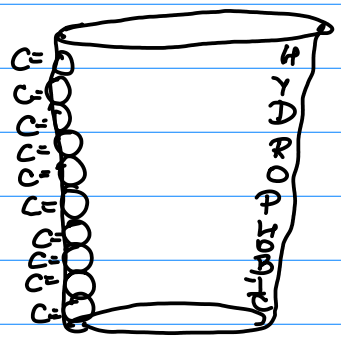
This explains selectivity against smaller Na^+

K^+ ion loses its water molecules to enter filter

Na^+ too small ⇒ carbonyl Os are too far away to compensate loss of energy to lose H_2O.

K^+ channels have α-helices → tilt & close to obstruct → hydrophobic, bulky amino acid chains close entry

Aquaporins



- how do they allow H_2O & not ions?
  - ↳ pore too narrow for hydrated ion to enter (even H_3O^+)
  - ↳ hydrophobic core cannot interact with dehydrated ion to compensate for loss of H_2O

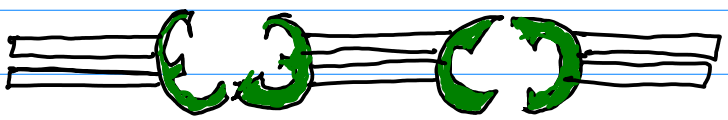
- rapid movement of H_2O, guided by C=O, without disrupting conc. grad.

- Strategically placed asparagines ⇒ binds to central O atom of H_2O → no valency available for H-bonding, preventing H^+ relay

PUMPS

Coupled Transporters / ATP-driven pumps / Light driven pumps

- transporter transfering solute — like an enzyme-substrate reaction
  - ↳ transfers by undergoing reversible conformation changes.



- the rate of maximum conformational change – V_{max}
  (flipping between two conformations)

Minor modification

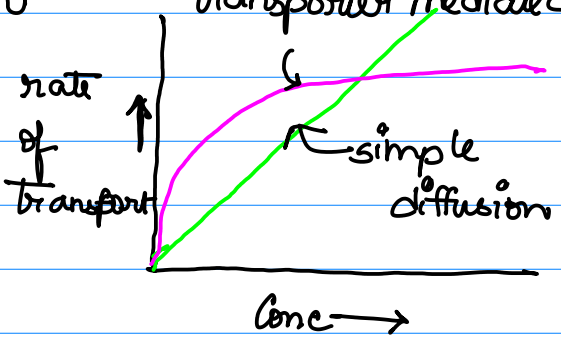
↳ linking transporter to source of energy
↳ allows pumping of solute against electrochemical gradient

⇓

Active Transport

Coupled Transporters	ATP-driven pumps	Light-driven pumps
uphill transport of one coupled with downhill transport of another	uphill transport of one coupled with ATP hydrolysis	uphill transport of one coupled with input of energy from light

- interestingly, passive transporters and active transporters have same evolutionary origin



Transporters driven by ion-gradients (Coupled Transport)

1. Uniporters – simply mediate movement of solute down grad
2. Symporters – coupled, simultaneous transporter in same direction
3. Antiporters – coupled, simultaneous Transporter in opp direction

- Tight coupling b/w the Transfer of two solutes
  – harvest the energy stored in electrochemical gradient of one solute to transport another

- Movement of solutes down electrochemical gradient
  ↓ Free energy release
  Movement of second solute uphill

- Na^+ – usually the one transported down electrochemical gradient (large) – large driving force

Indirect Transport of ions by Na^+ ⇒ SECONDARY ACTIVE TRANSPORT

↳ many active transporters use H^+ gradient instead of Na^+ gradient.

Regulation of cytosolic pH

- cytosolic pH ~ 7.2, lysozyme pH ~ 5

- Na^+ driven antiporters in plasma membrane – used to maintain cytosolic pH

↓

Na^+ gradient used to pump out excess H^+

↓

Na^+–H^+ exchanger

↓

influx of Na^+ & efflux of H^+

Na^+ driven Cl^-–HCO_3^- exchanger

↓

influx of Na^+ & HCO_3^- & efflux of Cl^- & H^+

2x more effective

Transcellular transport of absorbed solutes

↳ asymmetric distribution of transporters across membrane.

ATP-Driven Pumps

1. P-type pumps – • multipass • phosphorylate during pump cycle • ion pumps maintaining grad
2. F-type pumps – • multiple subunits • use H^+ gradient to drive ATP synthesis
3. ABC Transporters – Pump small molecules across cell membranes

Ca^{2+} Pump – P-type ATPase

- need To pump out Ca^{2+} out of cell in order to maintain Ca^{2+} gradient
- present in sarcoplasmic reticulum – Ca^{2+} released into cytosol through Ca^{2+} release channels (stimulus for contraction)
  ↳ pumps back into sarcoplasmic reticulum

Structure (determined by X-ray crystallography)

- 10 Transmembrane α-helices – 3 line central channel that spans lipid bilayer
- in unphosphorylated state, helices bind 2 Ca^{2+} ions, accessible from cytosolic side of membrane
- binding of ATP to a binding site on same side of memb

↓

Transfer of phosphate group to aspartic acid of an adjacent domain

↓

drastic rearrangement of Transmembrane helices

↓

Ca^{2+} ions released on other side of membrane

$Na^+ - K^+$ pump

out: $Na^+ \uparrow$

in: $K^+ \uparrow$

- ATP-driven antiporter
- pumps Na^+ out and K^+ in to maintain conc. difference
- can be driven in reverse, To produce ATP

↓

if Na^+ & K^+ gradients are increased such that energy stored in electrochemical grad > energy stored in chemical hydrolysis of ATP

↓

ions move down gradients, free energy used to synthesise ATP

- electrogenic – creates membrane potential

ABC Transporters

- each specific for a single class of molecules
- harness energy of ATP

CFTR

- Cl^- transport protein
- regulates ion conc. of ECF.
- gated channel.