

Lecture 7: Cell Membrane: Structure & Transport

Aims:

- **Cell Membrane Structure**
 - Lipid bilayer
 - Membrane Proteins
- **Cell Membrane Transport**
 - Carrier Proteins & Their Functions
 - Ion Channels & The Membrane Potential

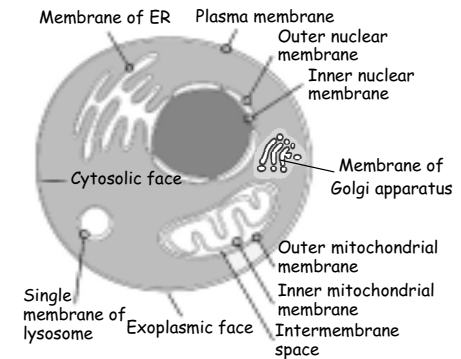
Alberts et al, Chap 11, 12

Cell Membrane Structure

Different Types of Membranes

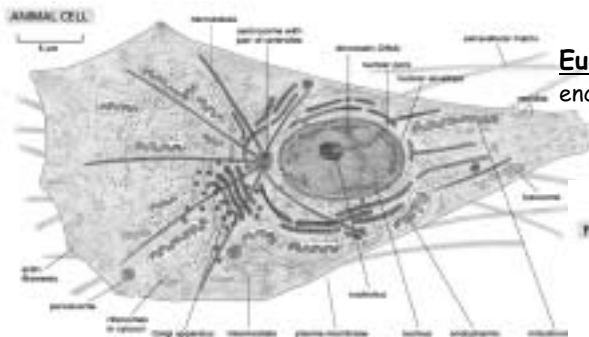
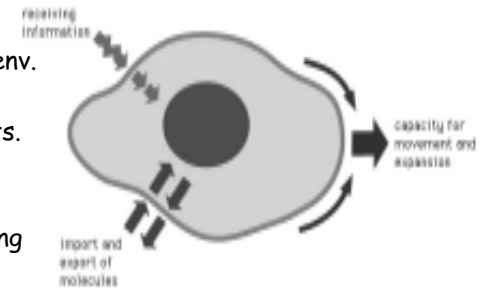
Plasma Membrane:

- membrane that surrounds a living cell
- thin, transparent fatty film
- not directly visible with a light microscope
- lipid sheet 5nm (~ 50 atoms) thick

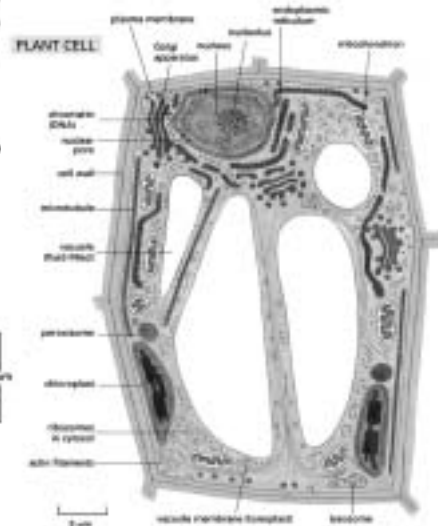


Function

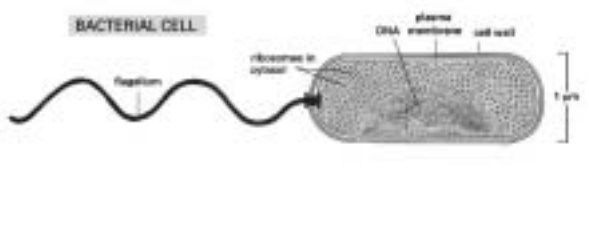
- encloses cell contents from surrounding env.
- nutrients in, waste out
 - > channels, pumps formed by prots.
- prots in memb as sensors to enable cell to respond to changes in env.
- capacity for movement, expansion, mending



Euc. cells: plasma memb + internal membs enclosing intracellular compartments (membrane bound organelles)



Proc. cells: single memb: plasma memb



Different Types of Membranes

Eucaryotic cells:

- > **selective barriers** between spaces containing distinct collections of molecules
- Differences in memb proteins for each organelle -> different characteristic

All cell membranes are composed of lipids and proteins

Lipids form a bilayer - basic structure & serves as a permeability barrier

Proteins - give membrane different characteristics

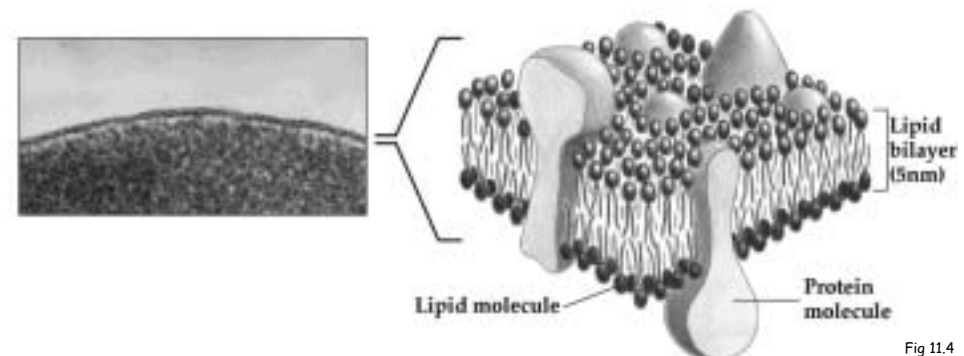
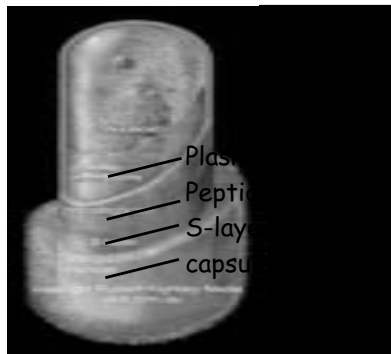


Fig 11.4

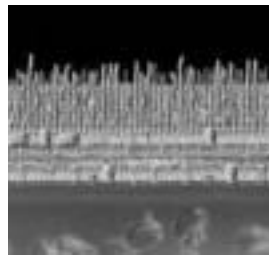


Bacillus anthracis

Bacterial cell wall



Gram positive
- much thicker cell wall



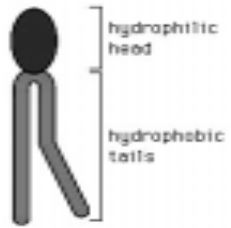
Gram negative
- has 2 layer cell wall

Gram = Klaus Gram

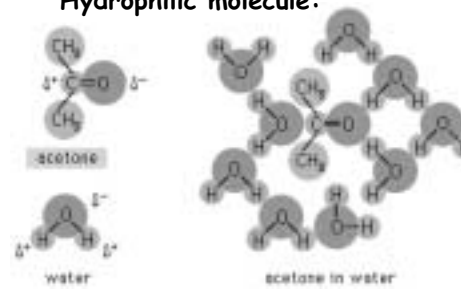
Peptidoglycan is a thick rigid layer that's found in both Gram+ and Gram- cells. It's composed of an overlapping lattice of 2 sugars (N-acetyl glucosamine (NAG) & N-acetyl muramic acid (NAM)) that are cross-linked by amino acid bridges. The exact molecular makeup of these layers is species specific.

Lipid

Amphipathic: hydrophilic & hydrophobic properties

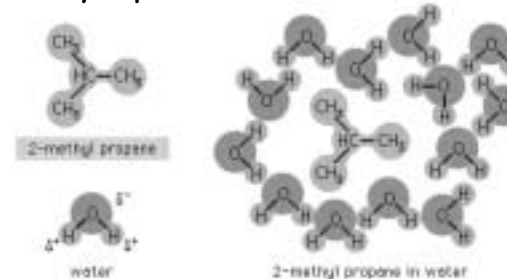


Hydrophilic molecule:



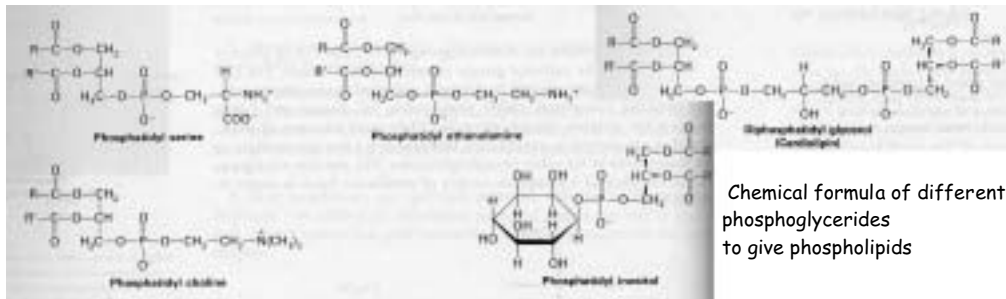
contain charged or polar groups;
can form electrostatic bonds or H bonds with water molecules (polar)

Hydrophobic molecule:



contain uncharged & nonpolar molecules;
insoluble in water
-> water forms a more ordered, cage-like, structure around molecule - needs energy
BUT if hydrophobic molecules cluster together -> less energy
-> droplet of fat/oil in water

Different types of phospholipids: head group varies



Chemical formula of different phosphoglycerides to give phospholipids

Membrane bilayers contain phospholipids.

Single Bonds (Saturated)
Double Bonds (Unsaturated)

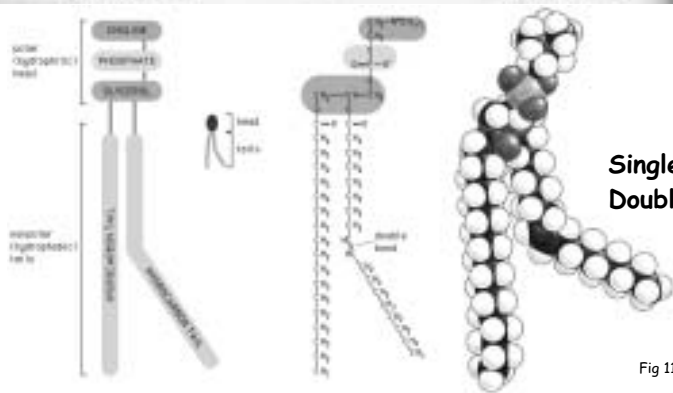


Fig 11.6

Other (non-phospholipid) membrane lipids:

- sterols (e.g cholesterol), glycolipids (sugar as hydrophilic head)

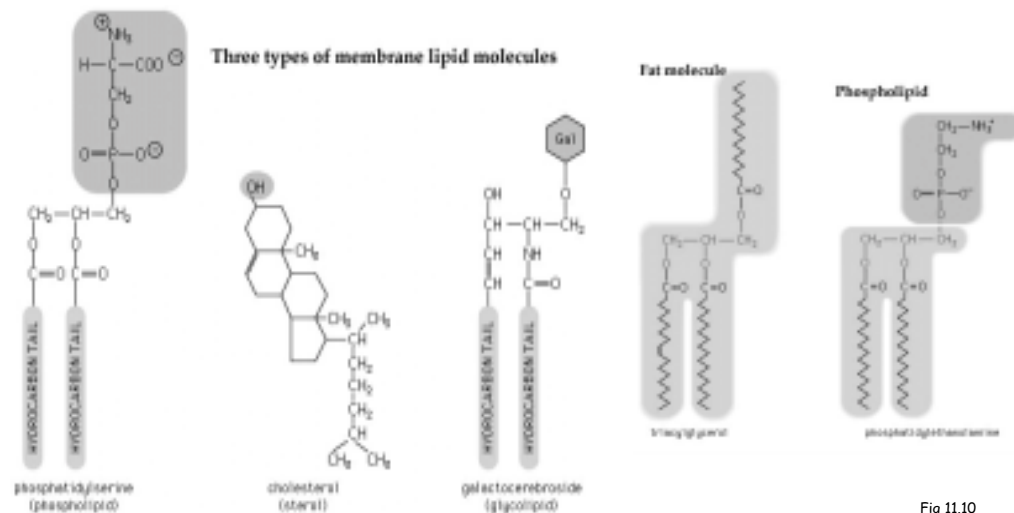


Fig 11.7

Fig 11.10

Phospholipid Bilayer:

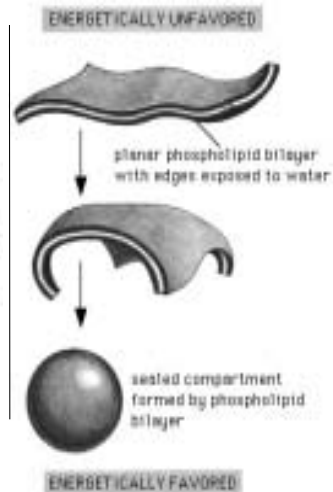
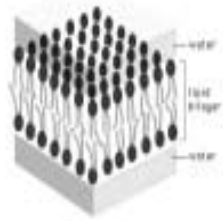
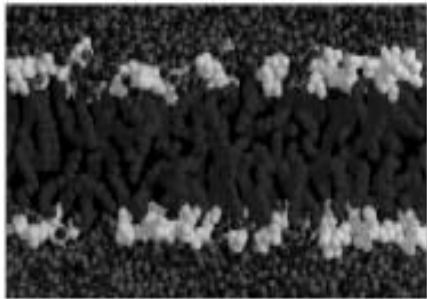


Fig 11.11

Phospholipids form bilayer as energetically favorable

Self-sealing property: tear in layer → free edge with water - not ideal

thus bilayer rearranges → repair of small tear

if large tear → sheet may break into separate vesicles

Sheet of lipid: free edges come together to form closed structure → cell

The Lipid Bilayer is a Two-Dimensional Fluid

Flexible: Lower limit of 25nm to the size of vesicle that cell memb can form

Liposomes: closed spherical bilayer vesicle of phospholipids in aqueous solution
(~ 25 nm to 1 mm diameter)

Flip-flop: phospholipid molecules can flip from one monolayer to the other

→ not spontaneous in cell: flip-flop occurs less than once a month per each lipid molecule

Thermal action → Lipid molecules move within their own monolayer

→ they rotate very rapidly about their long axis & also exchange places with their neighbors
→ fluidity

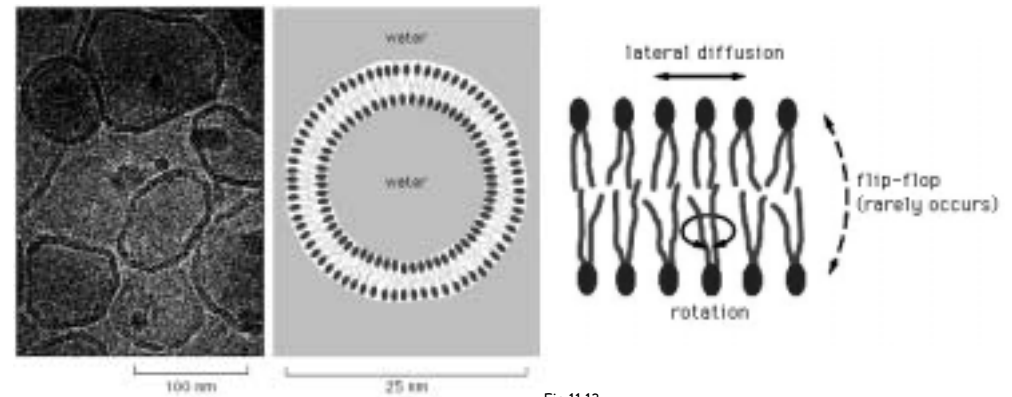


Fig 11.13

* Video memb fluidity *



Fluidity Depends on Bilayer Composition

Degree of Fluidity:

- depends on phospholipid composition & nature of hydrocarbon tails

Hydrocarbon tail:

- **length:** usually 18-20 C atoms
shorter → increases fluidity (less interaction with one another)
- **unsaturated:** number of double bonds (db) (no dbs = saturated)
1 db means 1 less H atom → unsaturated re H's
db → kink in tail → difficulty in packing tail → increase in fluidity

Importance in membrane function:

- diffusion of membrane proteins (cell signaling)
- distribution of lipids and proteins to other regions of cell
- fusion of membranes → mix molecules; even distribution of memb molecules between daughter cells during cell division

Margarine: unsaturated plant fat (usually liquid at room temp) is hydrogenated
vs Butter: saturated therefore solid at room temp

Cholesterol:

A sterol present in animal cell membranes, absent in plants, yeast, bacteria
Rigid molecule present in large amounts in plasma memb
Stiffens and less fluid bilayer → less permeable

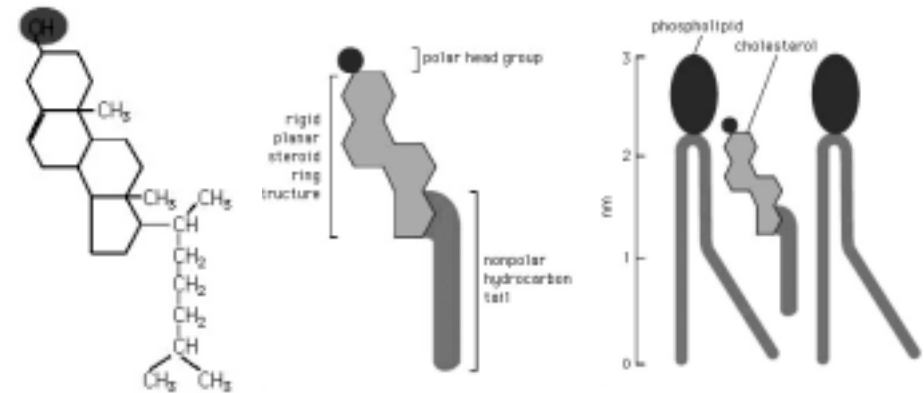


Fig 11.16

Major components of plasma membranes in various organisms:

	Components (% by weight)		
	Protein	Phospholipid	Sterol
Human myelin sheath	30	30	19
Mouse liver	45	27	25
Maize leaf	47	26	7
Yeast	52	7	4
<i>E. coli</i>	75	25	0

The Lipid Bilayer is Asymmetrical

- Face to the exterior of the cell is different than to the interior
- Two halves of bilayer have different PLs and glycolipids
- Specific orientation of proteins embedded in bilayer

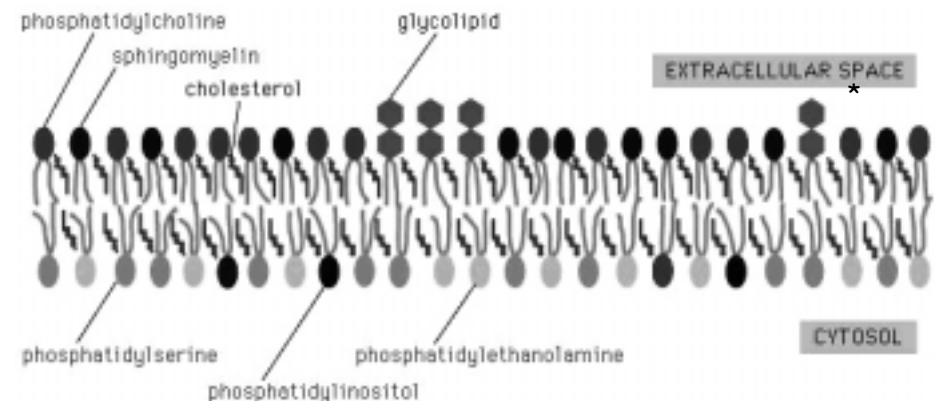


Fig 11.17

- Inositol phospholipids: face interior of cell; involved in cell signal

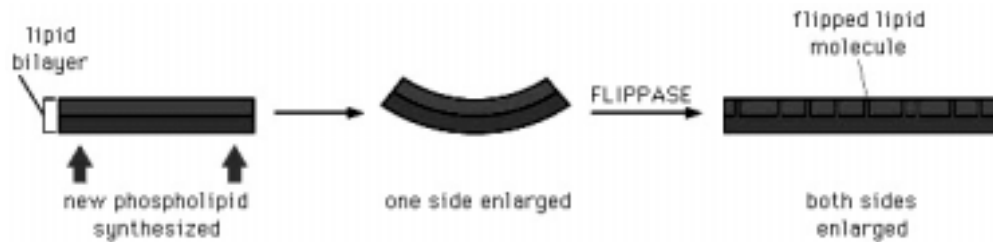
Flippases:

Enzymes transfer phospholipid molecules from one side of the bilayer to opposite monolayer (enables bilayer expansion in generation of memb)

New phospholipid molecules synthesized by membrane bound enzymes that use fatty acids on one half of bilayer (monolayer) as substrates.

These are released into the same monolayer.

BUT for membrane to expand as a whole, a proportion of lipid molecules are transferred to the opposite monolayer by flippases



In eucaryotic cells, most new membrane synthesis occurs in the ER:

- Bits of membrane pinch off ER -> form small vesicles
- Vesicles become incorporated into another membrane by fusing with it.

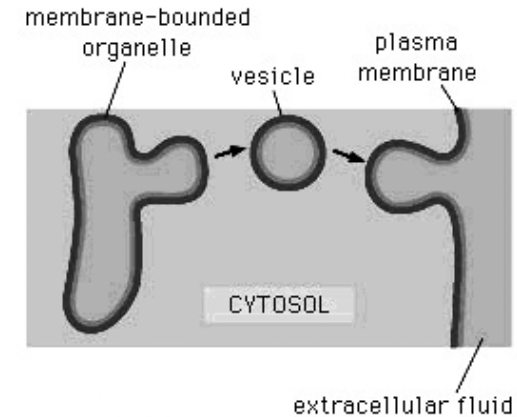
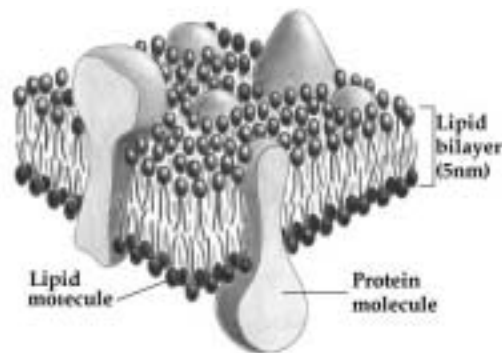


Fig 11.19

Membrane Proteins

In animals: proteins make up ~ 50% of the mass of most plasma membs, rest is lipid and carbohydrate

But 50x more lipid molecules than protein molecules because lipid molecules are smaller than protein molecules



Different memb prots: reflecting particular functions of the particular memb.

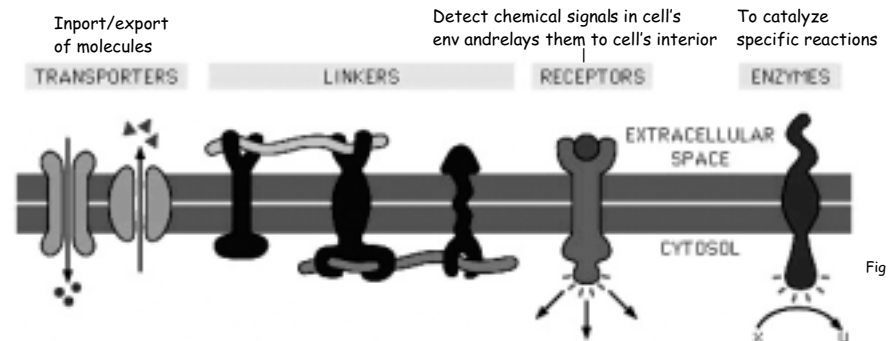


Fig 11.20

Examples of plasma membrane proteins and their functions:

Functional Class	Protein Example	Specific Function
Transporters	Na ⁺ pump	actively pumps Na ⁺ out of cells & K ⁺ in
Linkers	integrins	link intracellular actin filaments to extracellular matrix prots
Receptors	platelet-derived growth factor (PDGF) receptor	binds extracellular PDGF in doing so generates intracellular signal that cause the cell to grow & divide
Enzymes	adenylate cyclase	catalyzes the production of intracellular cyclic AMP in response to extracellular signals

Three main ways in which proteins associate with the cell membrane lipid bilayer

Transmembrane Proteins: extend through bilayer with part of it on either side

Have hydrophobic and hydrophilic regions

α helix, multiple α helices, closed β sheets (a β barrel) transverses lipid bilayer

Lipid-linked proteins: located entirely outside the bilayer, attached to it only by one or more covalently attached lipid group

Protein-attached: attached to membrane by weak, noncovalent interactions with other membrane proteins. Specific orientation for protein

Membrane-associated: Cytosolic proteins associated with the inner leaflet of the lipid bilayer

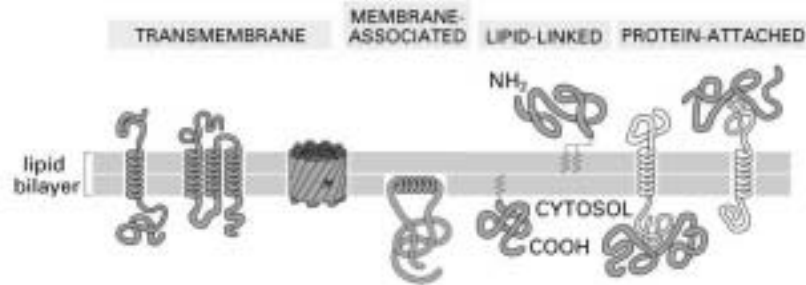
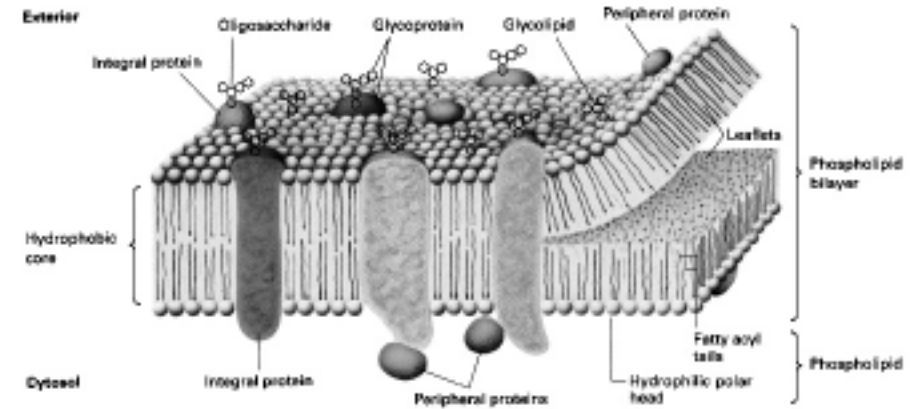


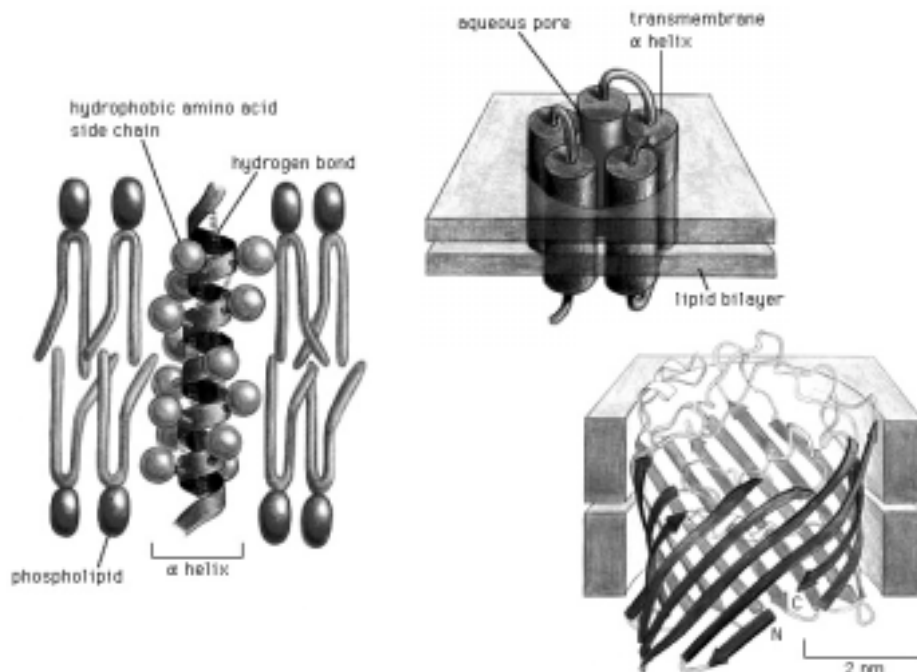
Fig 11.21

Integral membrane proteins: Proteins directly attached to membranes (transmembrane or lipid-linked) are dissociated from membranes by disrupting lipid bilayer with detergents

Peripheral memb prots: the remaining memb proteins which can be released from membrane by relatively gentle extraction procedures that interfere with protein-protein interactions leaving lipid bilayer intact

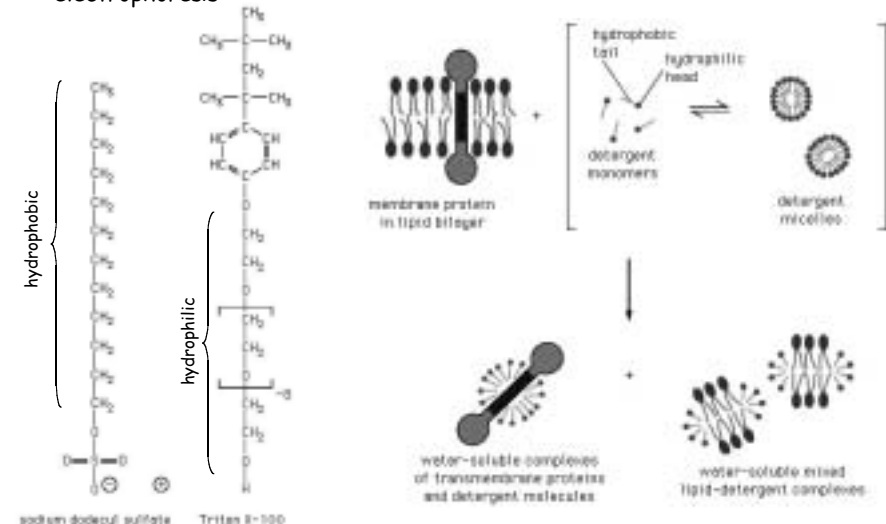


Transmembrane proteins: α helix, multiple α helices, closed β sheets (a β barrel)



Purification of Membrane Proteins:

- 1) **Solubilization** of membrane by disrupting hydrophobic associations using **detergents** - small amphipathic, lipid-like molecules **single HC tail** -> diff. behavior
- 2) **Separation** of protein-detergent complexes from lipid-detergent complexes via electrophoresis



SDS strong ionic detergent that displaces prot and unfolds prot

Fig 11.27



The plasma membrane is reinforced by the cell cortex

- Cell membranes are fragile on their own
- Most are supported by a cytosolic framework of fibrous proteins (cell cortex) attached to the memb by transmembrane proteins
 - e.g spectrin: long (~100nm), thin, flexible rod
- Cell cortex functions to:
 - provide mechanical strength (as RBCs are pumped through blood vessels)
 - allow cells to change shape actively and to move

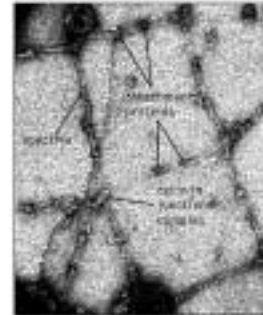
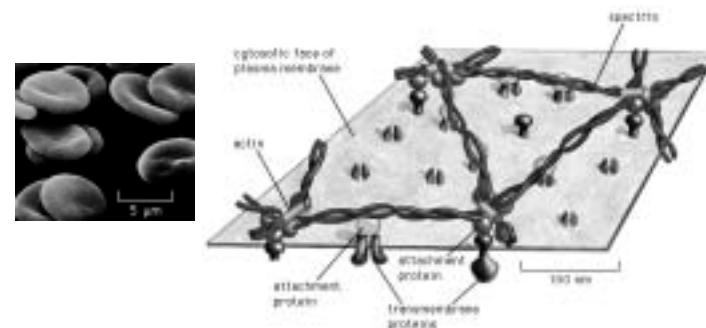
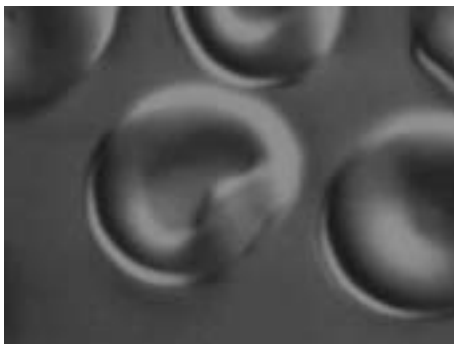


Fig 11.31

The spectrin-based cell cortex of human red blood cells
RBCs are flat shaped and lack nucleus & other organelles



The Cell Surface is Coated with Carbohydrate

Lipids on outer layer of plasma membrane have sugars covalently attached
Proteins in outer layer of plasma membrane also have sugars covalently attached

Oligosaccharides: short (diverse) chains of sugars (~15 sugar units)

Glycoprotein: protein with attached oligosaccharide.

Proteoglycans: protein with one or more long polysaccharide chain attached

Carbohydrates are located on one side of membrane: noncytosolic side

- forming a sugar coat called glycocalyx

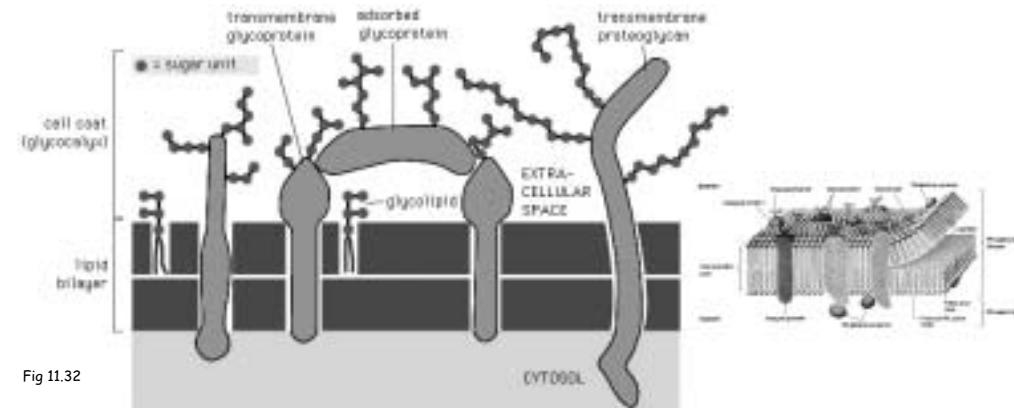


Fig 11.32

Glycocalyx: helps protect cell surface from mechanical & chemical damage

- the sugars absorb water giving cell slimy surface
- helps motile cells (e.g WBCs) squeeze through narrow spaces
- prevents blood cells from sticking to one another or to walls of blood vessels

So cell-surface CHs protect and lubricate the cell,
BUT also important in cell-cell recognition and adhesion

- lectins: proteins that bind oligosaccharide side chains

Thus glycocalyx can serve as a characteristic clothing that specializes cell for a particular function & cell is recognized by other cells with which they must interact

E.g Recognition of egg by sperm involves specific oligosaccharides in glycocalyx

Bacterial infections: lectins on cells lining blood vessels at site of infection recognize and adhere CHs on neutrophils (WBCs).

WBC then migrates from bloodstream into infected tissues to help remove bacteria

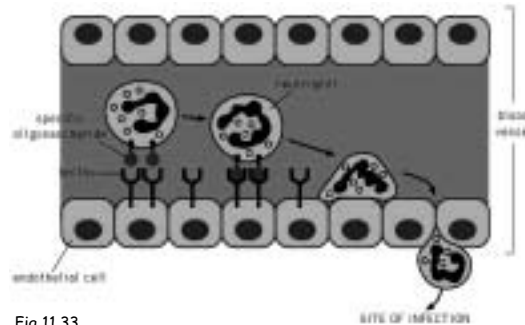


Fig 11.33

Cells can Restrict the Movement of Membrane Proteins

- Membrane is 2D fluid: many of its prots & lipids can move freely within plane of the lipid bilayer

Experiment: mouse cell fused to human cell

Proteins revealed using fluorescently tagged antibodies to human and mouse proteins. Visualized using fluorescent microscope.

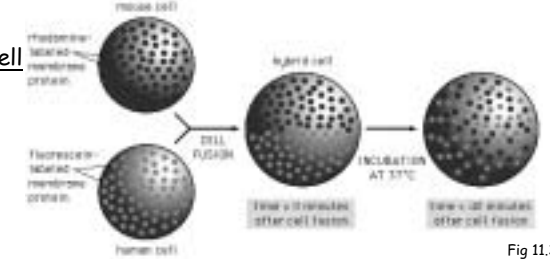


Fig 11.34

- Cells can restrict movement of certain plasma membrane proteins to certain areas (membrane domains) by four ways.

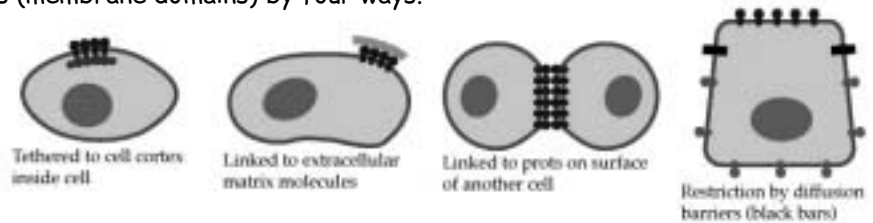


Fig 11.35

Example of membrane proteins restricted to particular domains of the plasma membrane of epithelial cells that line the gut:

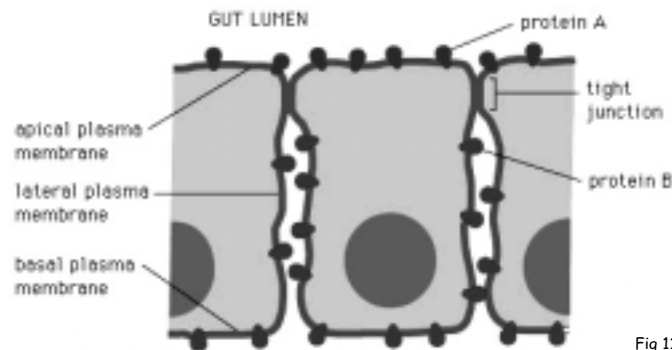


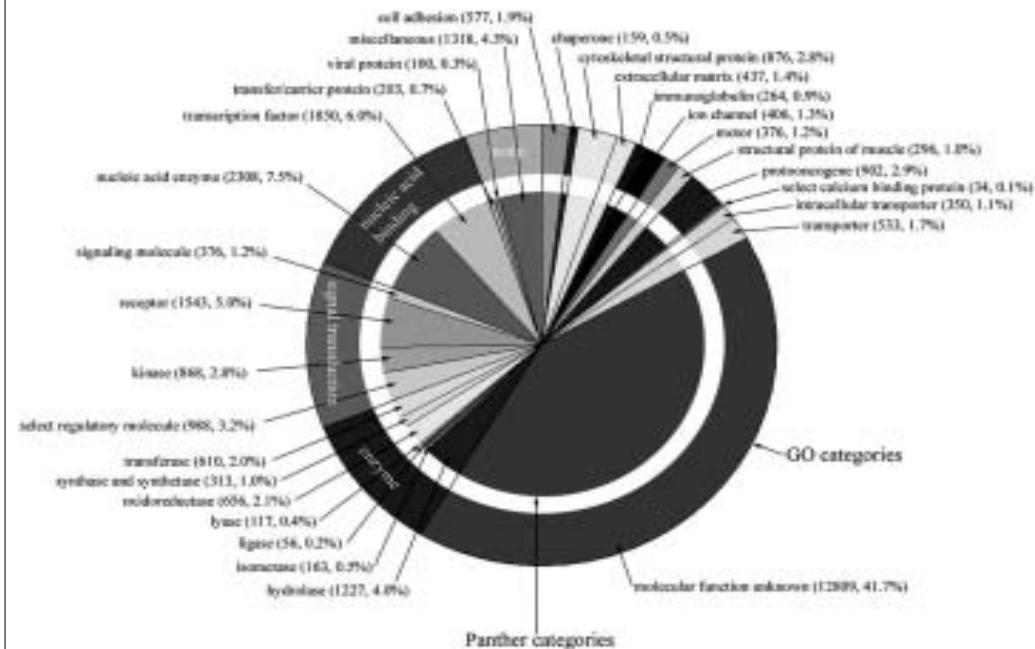
Fig 11.39

Apical surface: Transport proteins important in uptake of nutrients from the gut

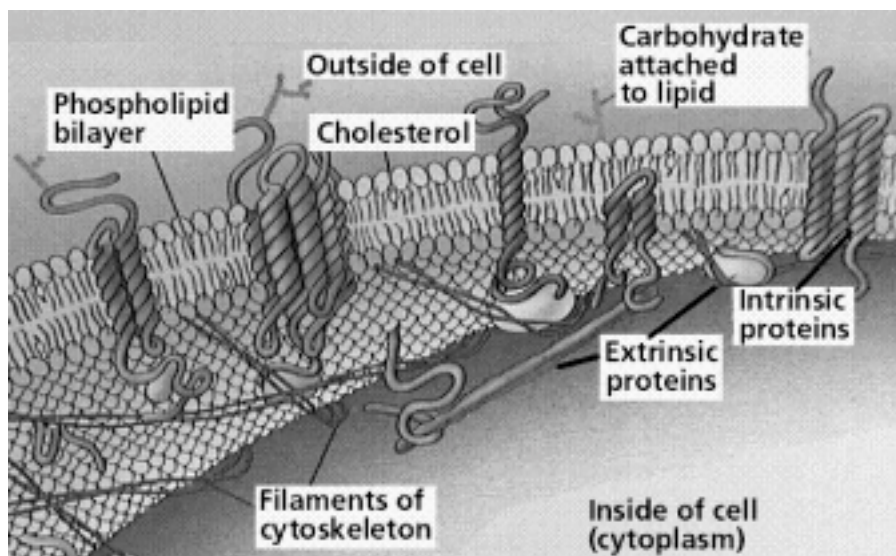
Basal & Lateral surface: proteins involved in transport of solutes out of epithelial cell into tissues and bloodstream

Tight Junction: cell sealed by adjacent cell; maintains asymmetric protein distribution

Complete Structure and Function is known for very few Membrane Proteins



Cell Membrane



Lipid Bilayers are Impermeable to Solutes and Ions

Function: controls passage of molecules across it Hydrophobic Interior

Rate of diffusion depends on molecule size and solubility properties

- smaller & more soluble in oil (degree of hydrophobicity/nonpolar)

-> more rapid diffusion

Small nonpolar molecules dissolve in lipid bilayers

-> rapid diffusion

e.g. O_2 & CO_2 , important in cellular respiration

Uncharged polar molecules

(uneven distribution of electric charge),

if small -> rapid diffusion e.g. ethanol, H_2O

c.t.: glycerol less rapid, glucose rarely diffuses

Ions & charged molecules:

impermeable regardless of molecule size (Na^+ , K^+)

molecule's charge & strong electrical

attraction to water inhibits entrance to HC bilayer

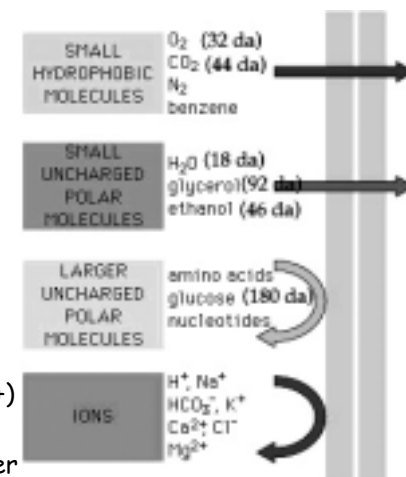


Fig 12.2

Plasma membrane is permeable to water

Osmosis

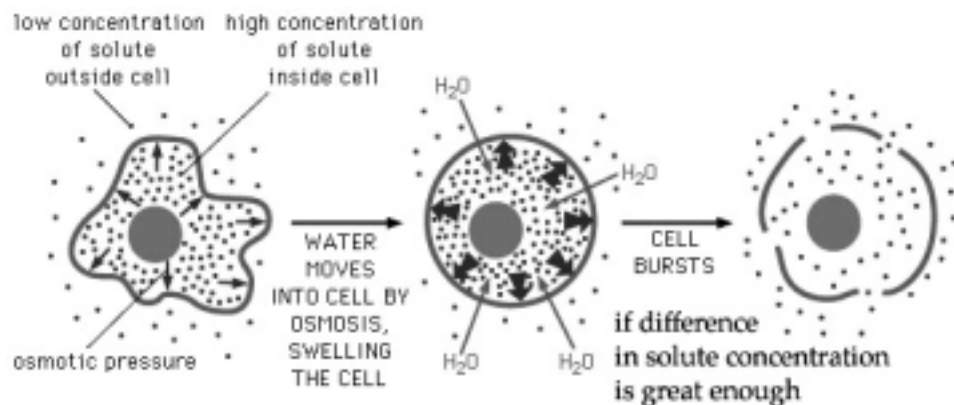


Fig 12.16

Cell membranes allow water & small nonpolar molecules to permeate by simple diffusion

BUT cell needs to uptake nutrients, release wastes

Passage: specialized transport proteins for transfer across cell membranes

General principles of how proteins associate with the lipid bilayer in cell membranes.

Membrane Transport

Membrane Transport Proteins

Proteins that span membrane providing private passages across it for **specific** substances

e.g ions, glucose

.... **Two Classes** -

Carrier Protein:

Prots that bind a solute on one side of memb & deliver it to the other side through a change in conformation of carrier prot

Solute: small inorganic molecule, or inorganic ions

Channel Protein:

- Prots form hydrophilic pores in memb through which solutes can pass by diffusion
- Ion Channels: only let inorganic ions through
- Exist in either open or closed conformation
- Opening and closing is usually controlled by an external stimulus or by conditions within the cell

Channel prots transport at a greater rate than carrier prots.

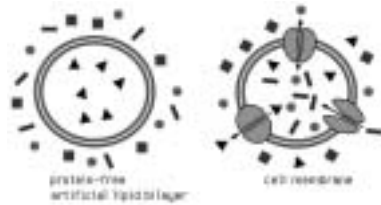
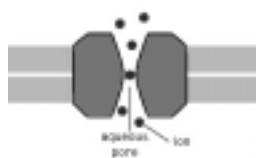
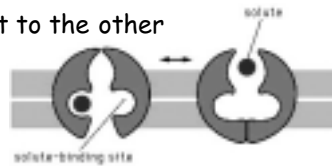


Fig 12.1



Carrier Proteins and their Functions

Carrier Protein:

Transport of **small inorganic molecules**, or **inorganic ions** across cell membranes (Remember fat-soluble molecules and small uncharged molecules [CO_2 , O_2 , ethanol] can pass directly through lipid bilayer by simple diffusion)

Transmembrane proteins: polypeptide chain **transverses** membrane

Transport is Selective: solute molecules fit into a binding site on prot

Transfers molecules one at a time across membrane by **changing its conformation** (similar binding as enzyme to its substrate -> selective) "Turnstile"

Compare to Channel protein:

Discriminates between solutes based on size & electric charge - "narrow trapdoor"

Examples of substances transported across cell membrane by carrier protein
Each cell membrane has its own characteristic set of carrier proteins

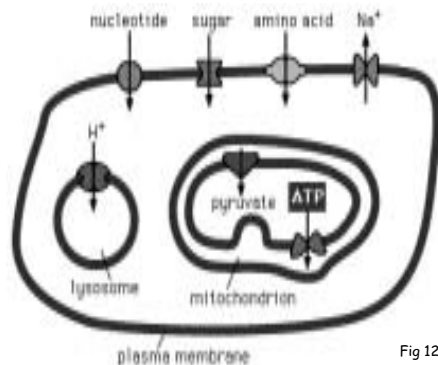


Fig 12.5

Ion Concentrations Inside Cell are Different from those Outside Cell

E.g Typical mammalian cell: Concentration of free ions inside & outside the cell

Component	Concentration (mM)	
	Intracellular	Extracellular
Cations		
Na^+	5-15	145
K^+	140	5
Mg^{2+}	0.5	1-2
Ca^{2+}	10-7	1-2
H^+	7×10^{-5} (pH 7.2)	4×10^{-5} (pH 7.4)
Anions		
Cl^-	5-15	110
Fixed anions	High	0

(negatively charged small and large organic molecules trapped inside cell, unable to cross plasma memb)

Note:

A solute (a substance e.g sugar crystal) dissolves in a solvent (e.g water) to make a solution (mixture). That is the sugar molecules separate from each other, each becoming surrounded by water molecules.

Carrier proteins: Solute cross membranes by Passive or Active Transport

Passive movement: no need for driving force

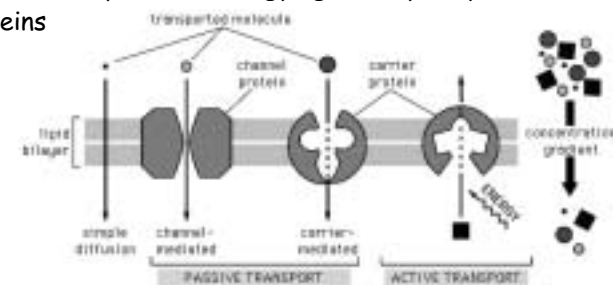
- same direction as concentration gradient
- **spontaneous** movement from high to low concentration
- movement of small molecule or ion across membrane is driven by a difference in concentration or electrical potential;
- no expenditure of energy by transport proteins
- sometimes called *facilitated diffusion*
- both carrier and channel prots can carry out passive transport

Active transport:

Transport of molecule against its concentration gradient requires work

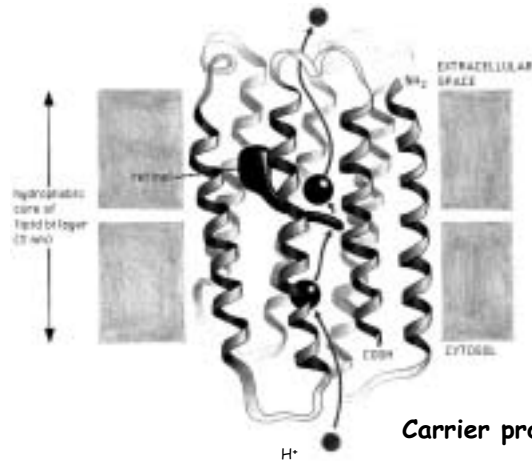
- coupled to some other process that provides energy eg ATP hydrolysis

Only carried out by carrier proteins
not channel



3D structure of a carrier prot bacteriorhodopsin

- Polypeptide chain crosses lipid bilayer as 7 helices
- pumps H^+ out of the bacterium *Halobacterium halobium* in response to light, which is captured by the covalently attached light absorbing group (a [purple] chromophore) retinal. Protons choose pathway avoiding contact with lipid bilayer.
- > Light activated H^+ pump



Carrier proteins are transmembrane proteins

Passive Transport:

Can be Driven by Electrical Forces as well as Concentration Gradients

Concentration gradient: concentration of molecule across the membrane
Carrier protein responds by change of conformation e.g glucose concentration
Uncharged molecules

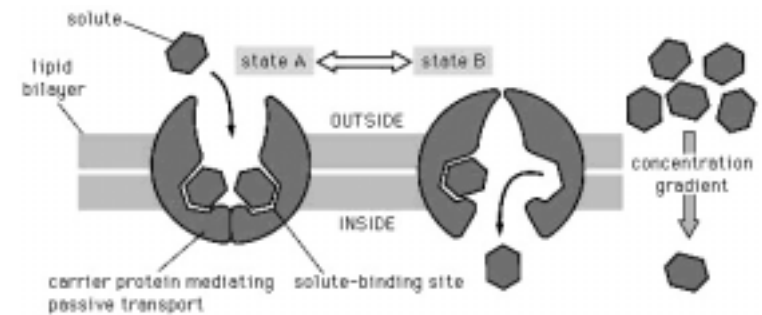


Fig 12.7

Need additional force for electrically charged molecules:

- small organic or inorganic ions

Membrane potential:

- Voltage difference across membrane due to slight excess of positive ions on one side & negative ions on the other.
 - > A difference in the electrical potential on each side of the membrane.
- Typical membrane potential for animal cell plasma membrane is -60mV (inside negative), measured relative to the surrounding fluid.
- Exerts a force on any molecule that carries an electric charge
- Cytosol usually negative potential relative to outside, thus pulls positively charged solutes into cell & drive negatively charged ones out
At the same time a charged solute will also tend to move down its conc. gradient.

Thus net force driving charge solute across memb is composite of two forces:
due to concentration gradient and due to voltage across membrane
Net force: **Electrochemical Gradient** for the given solute

Electrochemical Gradient = Concentration Gradient + Membrane Potential

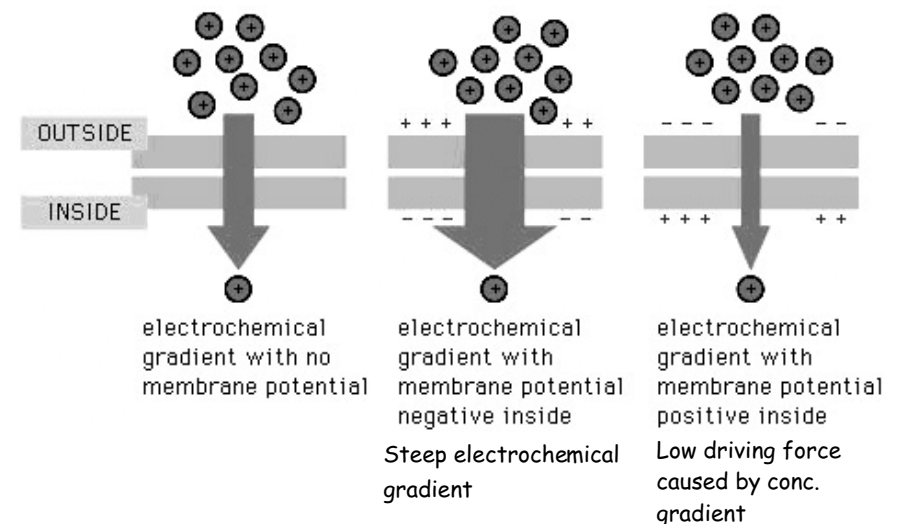


Fig 12.8

Active Transport:

Moves solutes Against Their Electrochemical Gradients

Three ways of Driving Active Transport:

Coupled Transporters: couple the uphill transport of one solute across memb to downhill transport of another

ATP-driven Pumps: couple uphill transport to the hydrolysis of ATP

Light-driven Pumps: couple uphill transport to an input of energy form light mainly in bacterial cells (e.g. bacteriorhodopsin)

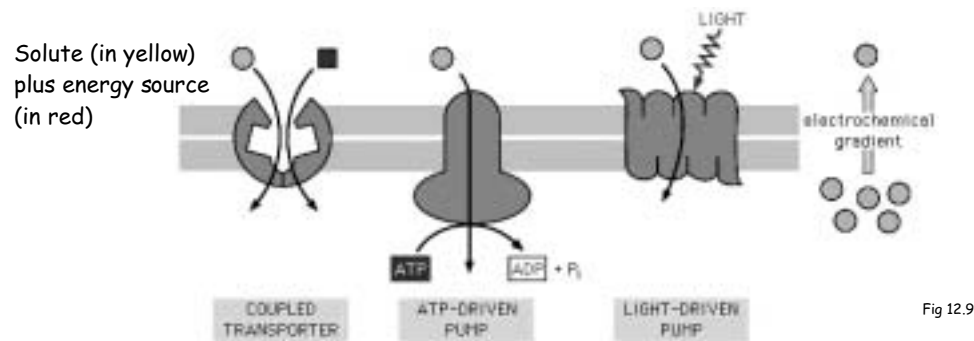


Fig 12.9

Linking of active transport

- substance needs to be carried up hill before can flow downhill

Example: Na⁺-K⁺ pump

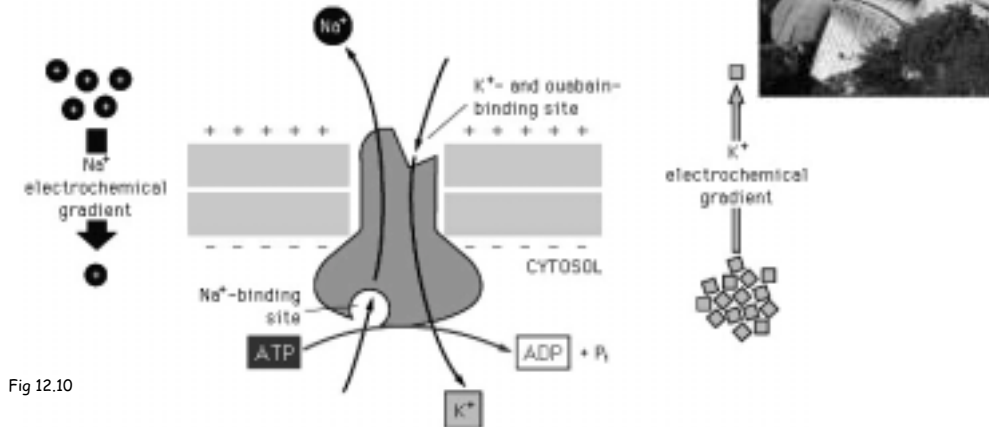


Fig 12.10

Carrier protein uses energy of ATP hydrolysis to pump Na⁺ out of cell and K⁺ in, both against their electrochemical gradients. Thus alternative name: **Na⁺-K⁺ ATPase**
 Drug: Ouabain, binds to pump and inhibits pump activity by preventing K⁺ binding

Na⁺-K⁺ pump is driven by the transient addition of a phosphate group

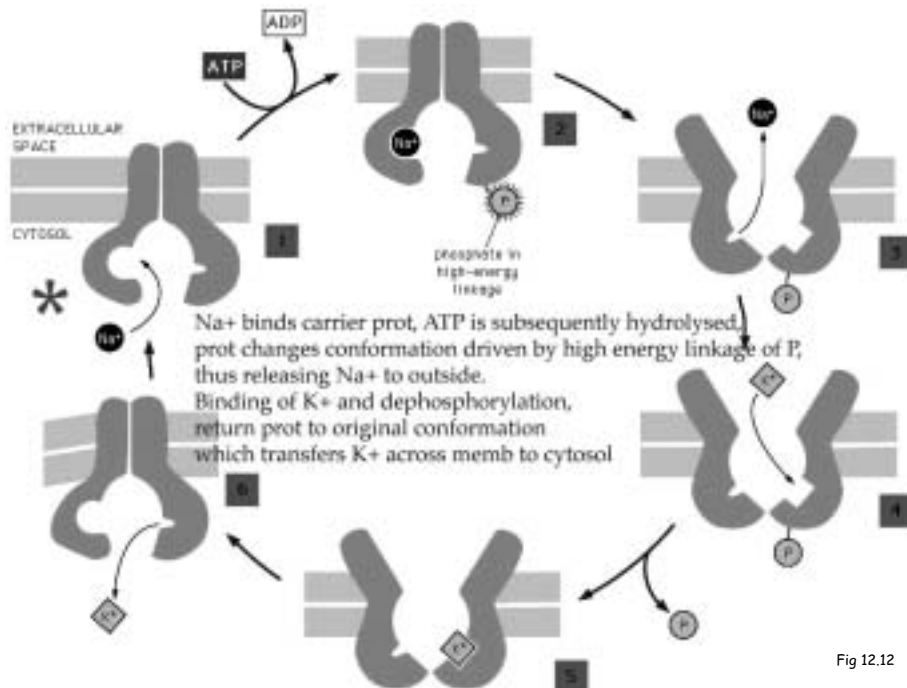


Fig 12.12

Na⁺-K⁺ pump generates Na⁺ Gradient which can be used by animal cells to take up nutrients actively by coupled transporter proteins

Symport: a transporter that moves solutes across membrane in the **same direction**

Antiport: a transporter that moves solutes in **opposite direction**

Uniport: carrier protein that moves only one type of solute across membrane so is not a coupled transporter e.g. passive glucose transporter

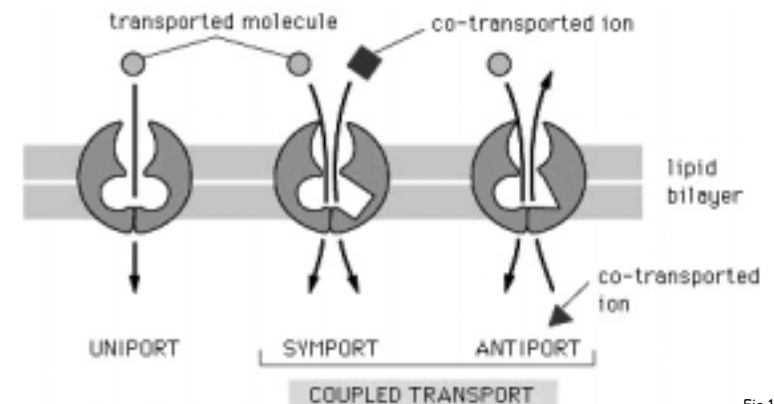
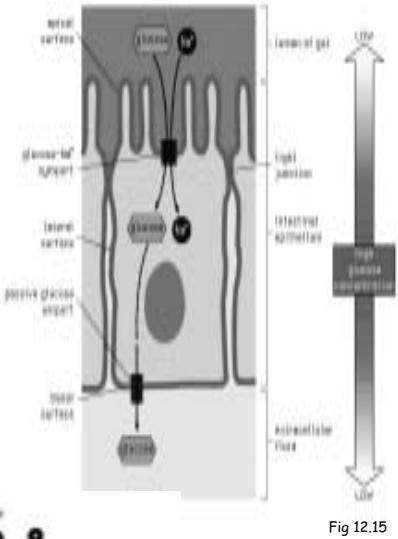
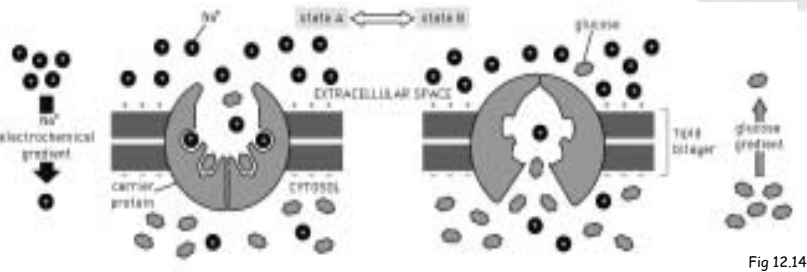


Fig 12.13

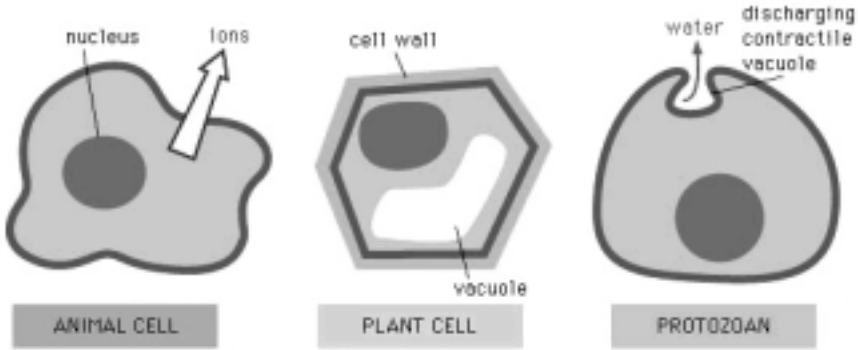
Example:
Model of glucose pump driven by Na⁺ gradient

Two types of glucose carriers enable gut epithelial cells to transfer glucose across the gut lining



Na⁺-K⁺ Pump Helps Maintain the Osmotic Balance of Animal Cells

Cells avoid osmotic swelling by three ways:



Keeps intracellular [solute] low by pumping out ions

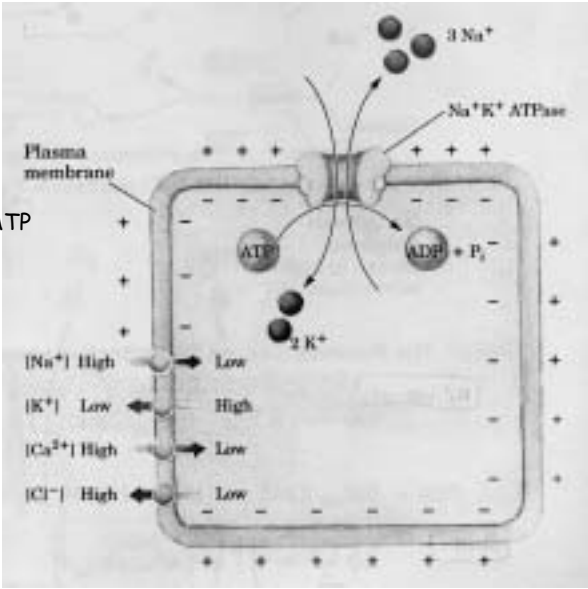
Tough cell wall

Periodically ejects the water that moves into the cell

Other pumps similar to Na⁺-K⁺ Pump
Eg Ca²⁺

Na⁺-K⁺ATPase polarizes memb using ATP
 Ion Channels move Na⁺, K⁺, Ca²⁺ & Cl⁻
 Na⁺ Influx or Cl⁻ Efflux
 → depolarization
 K⁺ Efflux → hyperpolarization

Charge balance



Intracellular H⁺ gradient is used to drive memb transport in plants, fungi & bacteria:
 Electrochemical proton gradient: H⁺ higher outside than inside of cell
 → acid pH in medium surrounding cell

Some examples of Carrier Proteins:

Table 12-2 Some Examples of Carrier Proteins			
Carrier Protein	Location	Energy Source	Function
Glucose carrier	plasma membrane of most animal cells	none	passive import of glucose
Na ⁺ -driven glucose pump	apical plasma membrane of kidney and intestinal cells	Na ⁺ gradient	active import of glucose
Na ⁺ -H ⁺ exchanger	plasma membrane of animal cells	Na ⁺ gradient	active export of H ⁺ ions, pH regulation
Na ⁺ -K ⁺ pump (Na ⁺ -K ⁺ ATPase)	plasma membrane of most animal cells	ATP hydrolysis	active export of Na ⁺ and import of K ⁺
Ca ²⁺ pump (Ca ²⁺ ATPase)	plasma membrane of eucaryotic cells	ATP hydrolysis	active export of Ca ²⁺
H ⁺ pump (H ⁺ ATPase)	plasma membrane of plant cells, fungi, and some bacteria	ATP hydrolysis	active export of H ⁺ from cell
H ⁺ pump (H ⁺ ATPase)	membranes of lysosomes in animal cells and of vacuoles in plant and fungal cells	ATP hydrolysis	active export of H ⁺ from cytosol into vacuole
Bacteriorhodopsin	plasma membrane of some bacteria	light	active export of H ⁺ out of the cell

Ion Channels and the Membrane Potential

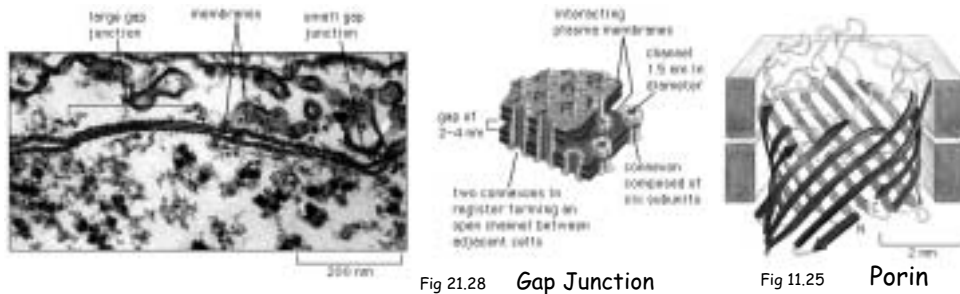
Channel Protein: Protein that forms a narrow hydrophilic pore across memb, allowing ions or small molecules to move passively from one side to the other.

→ passive movement of small water-soluble molecules in/out of cell/organelle

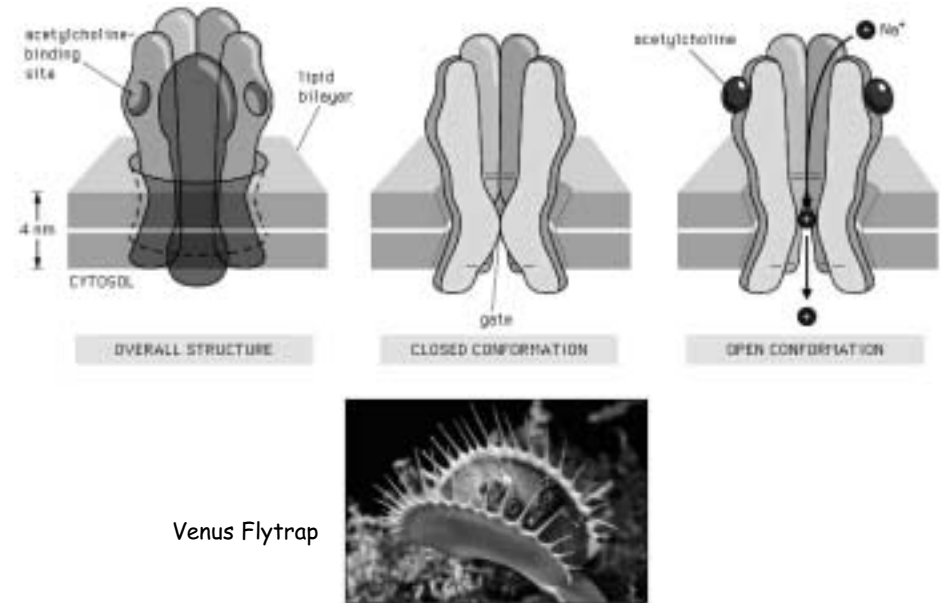
Gap Junctions: communicating cell-cell junction that allow ions & small molecules pass from cytoplasm of one cell to cytoplasm of the next

Large pores: Porins → cell leakage!

SO.... most channel proteins in plasma memb of animal & plant cells are narrow, highly selective pores e.g ion channels



Ion Channels: transport of inorganic ions, mainly Na^+ , K^+ , Cl^- and Ca^{2+}
- Ion selective and Gated (switch between open & closed state)



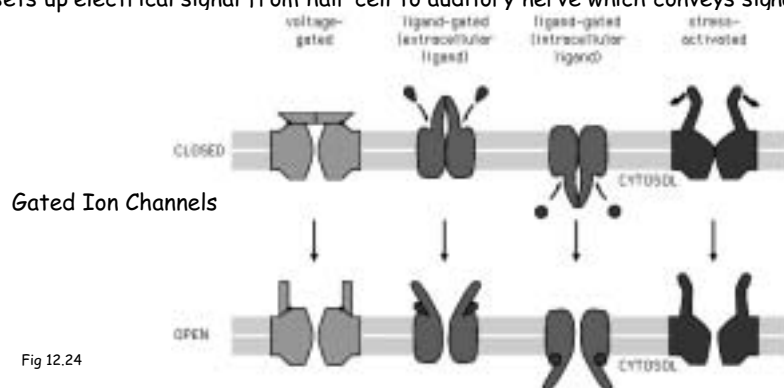
Ion channels differ in their ion selectivity and gating

Gating: the conditions that influence their opening and closing

Voltage-gated channels: probability of being open is controlled by memb potential (e.g in nerve muscle cells, plant cells)

Ligand-gated channel: controlled by binding of ligand to channel prot (e.g receptor)

Stress-activated channel: opening controlled by mechanical force applied to channel (e.g auditory hair cells: vibrations open st.act.channel, ions flow in to hair cell, sets up electrical signal from hair cell to auditory nerve which conveys signal to brain)



Voltage-gated ion channels:

- enable signals to travel from one part of cell, or organism, to another
- have voltage sensors: specialized charged protein domains sensitive to changes in membrane potential

**Ion Channels -> Membrane Potential -> Ion Channel Loop
results in electrical signaling in cells**

Membrane potential is governed by membrane permeability to specific ions

Resting membrane potential is the membrane potential in steady-state conditions, in which the flow of positive and negative ions across the plasma membrane is precisely balanced, so that no further difference in charge accumulates across the membrane.

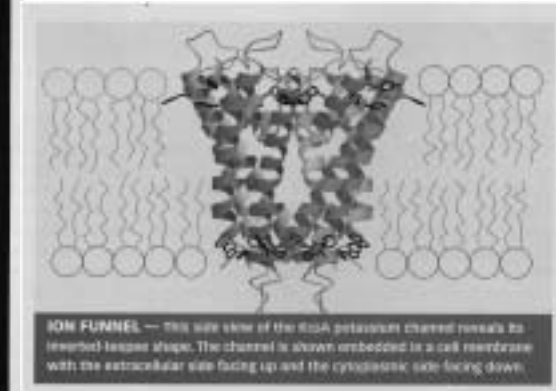
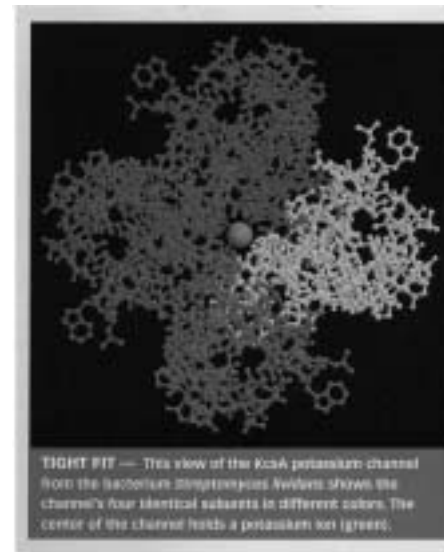
Commercially: Why are pumps important?

Proton Pump Inhibitors (PPI)

- Largest selling drug in the world

(Heartburn -Nexium, Prilosec)

\$13 Billion in US sales alone (2002)



Summary: Cell Membrane Structure & Transport

- Bilayer phospholipid cell membrane
- Lipid bilayer is a fluid
- Fluidity is dependent on its composition
- Asymmetrical lipid bilayer - phospholipids and proteins
- Lipid bilayers allow water and small nonpolar molecules to permeate by diffusion
 - impermeable to solutes and ions
- Various membrane proteins
- The plasma membrane is reinforced by cell cortex
- The cell surface is coated with carbohydrate
- Ion concentration inside cell is different to that outside
- Carrier proteins: solutes cross membranes by passive or active transport
- Passive transport - driven by electrical and concentration gradients
- Active transport - moves solutes against their electrochemical gradients
- Various "pump" systems for active transport
- Ion Channels are ion selective and gated
- Voltage-gated ion channels respond to the membrane potential
- The membrane potential is governed by membrane permeability to specific ions

How To Read A Scientific Journal Article

Abstract

Aim

Materials and Methods

Results

Discussion

Acknowledgements

References