Cell Biology

Unit 2 · Membranes and Membrane Transport

Every cell is wrapped in a membrane only about two molecules thick, and almost everything a cell does depends on what that membrane lets in and keeps out. This unit builds the membrane from its lipids and proteins, then works through each way molecules cross it: diffusion, carriers, pumps, channels and vesicles. After it you can draw the fluid mosaic model, explain why a red cell bursts in water, calculate the energy a pump needs, and name the transporter behind oral rehydration solution.

Taught at 7 of 10 | Usually Sem 3 | 7 chapters

About this unit

The unit starts with the structure of the plasma membrane (the fluid mosaic model) and the lipids and proteins that make it. With the structure in place, it turns to transport. Passive transport needs no energy and runs downhill. Active transport uses ATP or an ion gradient to push solutes uphill. The coupling of two solutes gives uniport, symport and antiport. Ion channels are the fastest route and the basis of electrical signals. Endocytosis and exocytosis move whole packages of material through the membrane in vesicles.

Chapters

1. Plasma membrane structure: fluid mosaic model
2. Membrane proteins and lipids ADVANCED
3. Passive transport: simple and facilitated diffusion, osmosis
4. Active transport: primary and secondary; ATP pumps
5. Uniport, symport and antiport systems ADVANCED
6. Ion channels: ligand-gated and voltage-gated
7. Endocytosis and exocytosis

How to use this book

- Read the lead paragraph and the figure of each chapter first; together they carry the one idea.
- Cover the solution of each worked example and do it yourself on paper, with units, before you check.
- Answer each "Check yourself" question in your head or on paper before you open it.

2.1 Plasma membrane structure: fluid mosaic model

The plasma membrane is a sheet of lipid two molecules thick, about 5 nm across, with proteins floating in it and sugar chains on its outer face. The fluid mosaic model, proposed by S. J. Singer and Garth Nicolson in 1972, says two things: the lipids form a fluid bilayer in which molecules move sideways freely ("fluid"), and proteins of many kinds sit in or on that bilayer like tiles in a moving floor ("mosaic"). Every transport process in this unit happens through this structure, so it is the picture to draw first in any membrane answer.

You need: the idea of polar and non-polar molecules and the cell compartments from Unit 1, Overview of cell organelles and compartments.

From sandwich to mosaic: how the model was built

In the 1890s Charles Overton noticed that substances which dissolve in oil enter cells faster than those which do not. He concluded that the cell surface is made of lipid.

In 1925 Gorter and Grendel extracted the lipids from red blood cells and spread them as a single layer on water. The layer covered about twice the surface area of the cells. Their conclusion: the membrane is a lipid **bilayer**.

In 1935 Davson and Danielli proposed a "sandwich": a lipid bilayer coated on both faces by flat sheets of protein. In the late 1950s J. D. Robertson's electron micrographs showed membranes as two dark lines with a light band between (the trilaminar **unit membrane**).

The sandwich failed on three points: many membrane proteins are globular and partly hydrophobic, freeze-fracture electron microscopy showed protein particles inside the bilayer, and in 1970 Frye and Edidin fused a mouse cell with a human cell, labelled the two sets of surface proteins with different fluorescent antibodies, and watched the colours mix over the whole hybrid cell within an hour at 37 °C. Proteins were moving sideways. Singer and Nicolson put these facts together into the fluid mosaic model.

The parts of the model

The lipid bilayer. A membrane phospholipid is **amphipathic**: it has a polar (hydrophilic) head and two non-polar (hydrophobic) fatty acid tails. In water, the tails hide from water and the heads face it, so the molecules assemble on their own into two layers (leaflets) with tails meeting in the middle. This hydrophobic core, about 3 nm thick, is the barrier to ions and large polar molecules. A bilayer also closes on itself to avoid exposed edges, so membranes always form sealed compartments.

Proteins. Integral proteins are embedded in the bilayer; most cross it completely (transmembrane proteins). **Peripheral proteins** sit on one face, held by ionic bonds and hydrogen bonds to the heads or to integral proteins. Proteins make up roughly half the mass of a typical plasma membrane (Alberts gives lipid as about 50% of the mass of most animal cell membranes). Their types are the subject of chapter 2.2.

Carbohydrates. Short sugar chains are attached to proteins (glycoproteins) and lipids (glycolipids) on the **outer face only**. Together they form the **glycocalyx**, a sugar coat used in cell recognition, for example the ABO blood group antigens on red cells.

Cholesterol. In animal plasma membranes cholesterol sits between the phospholipids, its small hydroxyl head at the surface and its rigid ring system among the tails. Alberts notes that eukaryotic plasma membranes can hold up to one cholesterol per phospholipid.

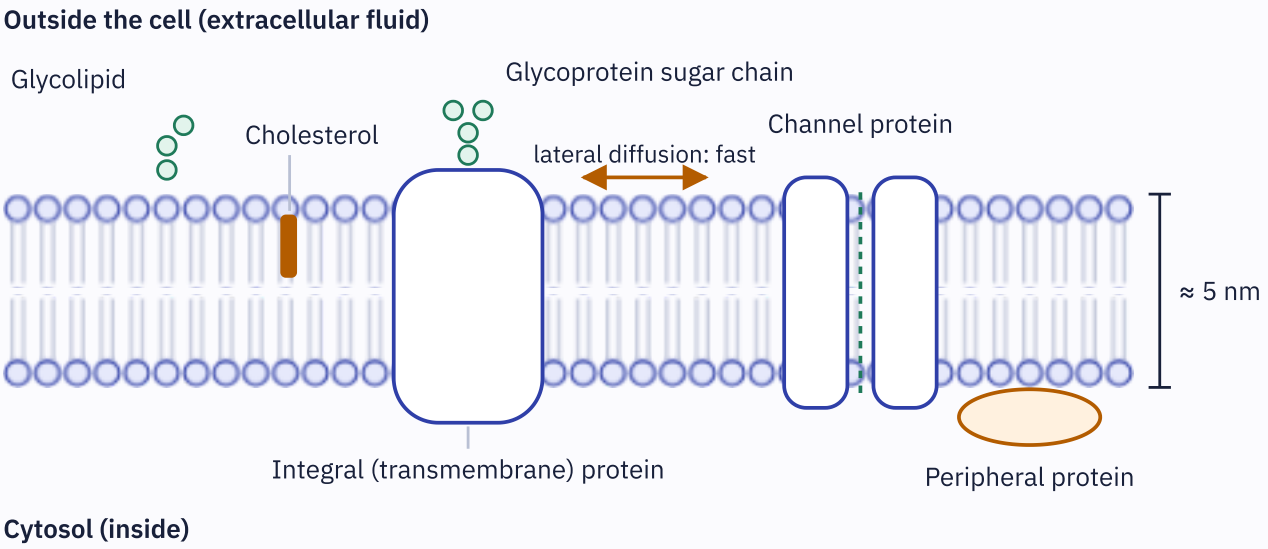


Figure 2.1.1 The fluid mosaic model. Blue circles are polar heads and grey lines are fatty acid tails; the tails form the hydrophobic core. Notice that sugar chains (green) are only on the outside, the peripheral protein sits only on the cytosolic face, and the integral and channel proteins cross the whole bilayer. Lipids and many proteins drift sideways within their own leaflet.

Fluidity and what controls it

The bilayer behaves like a two-dimensional liquid. A lipid swaps places with its neighbours about 10^7 times per second and has a lateral diffusion coefficient D of about 10^{-8} cm²/s (that is, 1 µm²/s). In contrast, **flip-flop**, the movement of a lipid from one leaflet to the other, is rare: for a given phospholipid it happens less than once a month unless an enzyme helps (Alberts). The polar head would have to pass through the oily core, which costs too much energy.

Four factors set fluidity:

1. **Temperature.** Cooling below a transition temperature makes the bilayer go from a fluid state to a rigid, crystal-like gel state.
2. **Unsaturation.** A cis double bond puts a kink in a tail, so tails pack loosely and the membrane stays fluid at lower temperature.
3. **Tail length.** Shorter tails have fewer contacts with each other, so they increase fluidity.
4. **Cholesterol.** It acts as a fluidity buffer. At body temperature its rigid rings restrain the tails and make the bilayer less fluid and less permeable to small water-soluble molecules. At low temperature it keeps the tails from packing into a gel.

Cells adjust these settings. Bacteria and fish grown in the cold make more unsaturated fatty acids, keeping their membranes fluid (homeoviscous adaptation). Many membrane proteins stop working when a membrane gels, so fluidity is not optional.

The modern view

The model is still correct in outline, with three refinements. The membrane is **asymmetric**: the two leaflets carry different lipids and proteins face one way only (chapter 2.2). Proteins are not all free: many are tethered to the cytoskeleton beneath or to the extracellular matrix, or fenced into regions such as the apical and basolateral faces of a gut epithelial cell. And cholesterol with sphingolipids can gather into small, short-lived ordered patches called **lipid rafts**.

WORKED EXAMPLE

Question. (a) The lipids extracted from 1.0×10^9 red blood cells are spread as a monolayer on water and cover 0.20 m^2. The surface area of one red cell is taken as 100 μm^2. What does this tell you about the membrane? (b) A lipid in a bilayer has $D = 1\ \mu m^2/s$. Using the two-dimensional diffusion relation $\langle r^2 \rangle = 4Dt$, how long does it take to travel 2 µm, the length of a bacterium? 6 marks

1. Total cell surface = $1.0 \times 10^9 \times 100\ \mu m^2 = 1.0 \times 10^{11}\ \mu m^2$.
2. Convert: $1\ m^2 = 10^{12}\ \mu m^2$, so $1.0 \times 10^{11}\ \mu m^2 = 0.10\ m^2$.
3. Ratio = monolayer area / cell surface = $0.20\ m^2 / 0.10\ m^2 = 2.0$.
4. Conclusion (a): there is enough lipid for exactly two layers over the cell surface, so the membrane is a lipid bilayer. This is the logic of Gorter and Grendel.
5. (b) Here r is the distance moved, t the time, D the diffusion coefficient. Rearranging, $t = r^2 / 4D = (2\ \mu m)^2 / (4 \times 1\ \mu m^2/s) = 4 / 4\ s = 1\ s$.
6. Conclusion (b): a lipid can cross a whole bacterium in about one second, which shows how fluid the bilayer is.

EXAM TRAPS

- "Fluid" refers to sideways movement within a leaflet. Flip-flop between leaflets is slow; do not write that lipids move freely between the two layers.
- Carbohydrate is only on the extracellular (non-cytosolic) face. A diagram with sugar chains facing the cytosol loses marks.
- Cholesterol does not simply "increase fluidity". It reduces fluidity at high temperature and prevents gelling at low temperature.
- Davson–Danielli is the protein–lipid–protein sandwich; Singer–Nicolson (1972) is the fluid mosaic. Examiners ask you to compare them.
- The favourite question: "Describe the fluid mosaic model with a neat labelled diagram, and give the evidence for it." Draw Figure 2.1.1 and quote the Frye–Edidin experiment.

CHECK YOURSELF

– REMEMBER **Who proposed the fluid mosaic model, and when?** 1 mark

S. J. Singer and G. L. Nicolson, in 1972.

– REMEMBER **Name the three kinds of molecule in a plasma membrane.** 2 marks

Lipids (phospholipids, cholesterol, glycolipids), proteins (integral and peripheral) and carbohydrates (as glycoproteins and glycolipids).

– UNDERSTAND **Why does a phospholipid bilayer form on its own in water?** 3 marks

Phospholipids are amphipathic. The hydrophobic tails are pushed together away from water (the hydrophobic effect) while the polar heads face water on both sides. Two leaflets tail to tail is the lowest-energy arrangement, and the sheet closes into a sealed compartment to avoid exposed edges.

– UNDERSTAND **How did the Frye–Edidin experiment support the fluid mosaic model?** 3 marks

Mouse and human cells were fused, and their surface proteins were tagged with two different fluorescent labels. Within about an hour at 37 °C the labels were mixed over the whole hybrid cell, showing that membrane proteins move sideways in a fluid bilayer. Mixing was blocked at low temperature.

– APPLY **How long does a lipid with D = 1 μm²/s take to move 6 μm by 2-D diffusion?** 2 marks

$t = r^2 / 4D = 36 / 4 =$ **9 s**. Note that tripling the distance multiplies the time by nine.

– APPLY **A bacterium is moved from 37 °C to 20 °C. Predict the change in its membrane lipids.** 2 marks

It makes more unsaturated (kinked) and shorter fatty acid tails so the membrane stays fluid at the lower temperature.

– ANALYZE **Give two observations the Davson–Danielli sandwich model cannot explain.** 4 marks

Freeze-fracture shows protein particles inside the bilayer, not only on its faces; many membrane proteins are hydrophobic and globular and could not lie on a water-facing surface; and proteins diffuse sideways (Frye–Edidin), which a fixed protein coat would not allow.

BEYOND THE SYLLABUS

This is not for the exam. Researchers now study membranes with cryo-electron microscopy, super-resolution fluorescence and molecular dynamics computer simulations that follow every lipid. Artificial bilayers are a technology in themselves: liposomes carry drugs, and the lipid nanoparticles that deliver mRNA vaccines are built on the same self-assembly you met here. Search next for "lipid nanoparticles mRNA", "FRAP fluorescence recovery after photobleaching" and "lipid rafts debate".

2.2 Membrane proteins and lipids

The bilayer of chapter 2.1 is built from three families of lipid, and its work is done by proteins attached to it in six distinct ways. Lipids set the barrier and its fluidity; proteins carry out transport, signalling, enzyme reactions and anchoring. Others cover it under "membrane structure".

The lipids and their asymmetry

- **Phosphoglycerides**: glycerol carrying two fatty acids and a phosphate-linked head. The main ones are phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS, negatively charged) and phosphatidylinositol (PI).
- **Sphingolipids**: built on sphingosine instead of glycerol. Sphingomyelin carries a phosphocholine head; glycolipids carry sugars (gangliosides carry sialic acid and are common in nerve cell membranes).
- **Sterols**: cholesterol in animals; plants use related phytosterols, and bacteria generally lack sterols.

The two leaflets differ. In the human red cell, the outer leaflet holds nearly all the PC, sphingomyelin and glycolipids, while the inner leaflet holds nearly all the PE and PS (Alberts). Enzymes keep it so: **flippases** use ATP to move PS and PE to the cytosolic leaflet, and **scramblases** mix the leaflets when activated. Asymmetry carries information: when a cell dies by apoptosis, PS appears on the outer face and acts as an "eat me" signal to macrophages (see Unit 4, Apoptosis), and inositol phospholipids on the inner face are raw material for second messengers (Unit 5).

Six ways a protein meets the bilayer

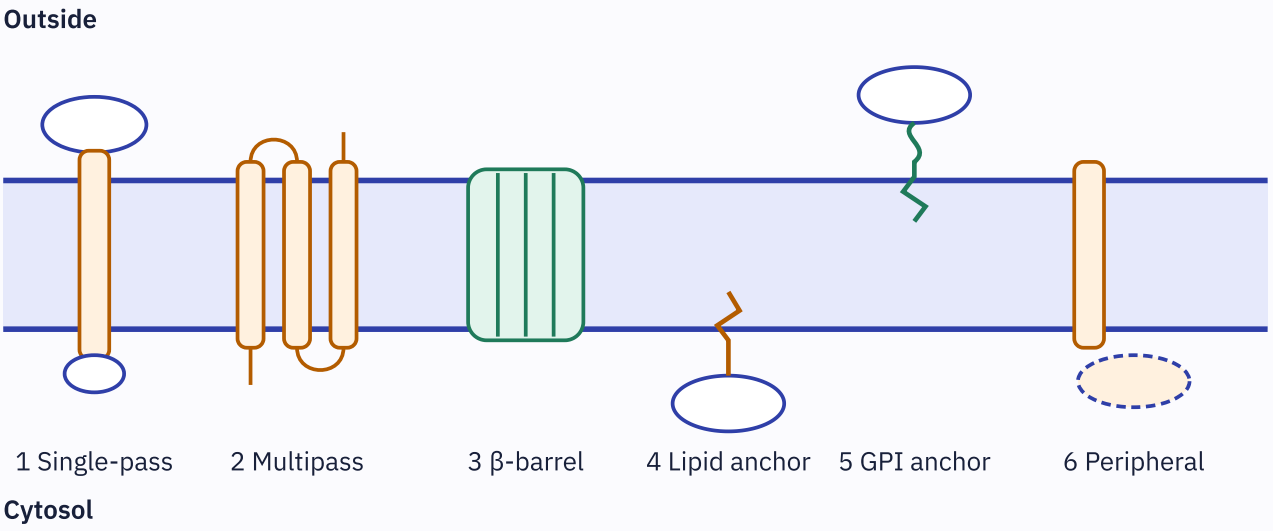


Figure 2.2.1 Orange bars are α-helices crossing the hydrophobic core; the green barrel is a rolled β-sheet. Types 1 to 3 are transmembrane (integral). Type 4 hangs on the cytosolic face by a fatty acid or prenyl chain, type 5 on the outer face by a GPI glycolipid. Type 6 (dashed) binds non-covalently to another protein and comes off with salt.

1. **Single-pass**: one α-helix of about 20 hydrophobic amino acids crosses the core. Example: glycophorin of the red cell.
2. **Multipass**: several helices, joined by loops. Most transporters and receptors are multipass; band 3 (the red cell Cl^-/HCO_3^- exchanger) and the seven-helix bacteriorhodopsin are examples.
3. **β-barrel**: a β-sheet rolled into a tube. Porins of the outer membranes of Gram-negative bacteria, mitochondria and chloroplasts are of this kind.
4. **Lipid-anchored on the cytosolic face**: a myristoyl, palmitoyl or prenyl chain is covalently attached. Ras (Unit 4) is held this way.
5. **GPI-anchored on the outer face**: attached to a glycosylphosphatidylinositol lipid; the enzyme phospholipase C can cut it free.
6. **Peripheral**: bound by ionic and hydrogen bonds to heads or to integral proteins. Spectrin, the network under the red cell membrane, is the classic case.

Integral proteins are removed only by **detergents** that dissolve the bilayer (SDS, which denatures, or the milder Triton X-100); peripheral proteins come off with high salt or a change of pH, leaving the membrane intact. This is the working definition of the two classes.

WORKED EXAMPLE

Question. An α-helix rises 0.15 nm per amino acid. The hydrophobic core of a bilayer is 3.0 nm thick. (a) How many residues are needed to span it? (b) A hydropathy plot of a new protein shows four stretches of hydrophobic residues of length 21, 8, 22 and 20. How many transmembrane helices does it probably have? 4 marks

1. (a) Residues = core thickness / rise per residue = 3.0 nm / 0.15 nm = **20 residues**.
2. (b) A stretch must be about 20 hydrophobic residues to cross the core. The stretches of 21, 22 and 20 qualify; the 8-residue stretch (8 × 0.15 = 1.2 nm) is too short.
3. Conclusion: probably **three** transmembrane helices, so a multipass protein.

EXAM TRAPS

- "Integral" means needs detergent to remove; it is not the same as "transmembrane". A lipid-anchored protein is integral by some books and peripheral by others; say which definition you use.
- GPI anchors are on the outer face; myristoyl and prenyl anchors are on the cytosolic face.
- PS and PE are inner-leaflet lipids; glycolipids are outer only.
- Examiners like: "Classify membrane proteins by their mode of association with a labelled diagram."

CHECK YOURSELF

– REMEMBER **Name the three main classes of membrane lipid.** 1 mark

Phosphoglycerides, sphingolipids and sterols.

– REMEMBER **Which lipids are mainly in the cytosolic leaflet of a red cell?** 1 mark

Phosphatidylethanolamine and phosphatidylserine.

– UNDERSTAND **Why does a transmembrane segment need to be hydrophobic?** 2 marks

It lies in the oily core of the bilayer. Hydrophobic side chains interact well with fatty acid tails; polar or charged groups there would cost a large amount of energy.

– APPLY **A protein is released from membranes by 1 M NaCl. Classify it.** 2 marks

Peripheral: salt breaks the ionic bonds that hold it, without dissolving the bilayer.

– ANALYZE **Why is PS appearing on the outer leaflet a useful death signal?** 3 marks

Living cells use flippases to keep PS inside, so outer PS is normally absent. In apoptosis the flippase stops and a scramblase acts, exposing PS; macrophage receptors recognise it, so dying cells are eaten before they burst and cause inflammation.

BEYOND THE SYLLABUS

This is not for the exam. Roughly a quarter to a third of human genes are commonly estimated to code for membrane proteins, and a large share of drug targets are membrane proteins. Their structures are now solved by cryo-EM in detergent micelles or lipid nanodiscs, and AlphaFold predicts transmembrane topology. Search next for "nanodisc membrane protein" and "TMHMM topology prediction".

2.3 Passive transport: simple and facilitated diffusion, osmosis

Passive transport is any movement across a membrane that runs downhill, from where a substance is more concentrated to where it is less, so it needs no ATP. Small non-polar molecules slip straight through the bilayer (simple diffusion). Glucose, ions and most nutrients need a protein door (facilitated diffusion). Water moves by osmosis, mostly through water channels. These three explain how oxygen reaches mitochondria, how glucose enters a red cell, and why a red cell bursts in pure water.

You need: the bilayer and its hydrophobic core from chapter 2.1.

Simple diffusion through the bilayer

Molecules move at random. Where there are more of them on one side, more cross from that side per second, so the net flow is down the concentration gradient. **Fick's first law** describes it:

$$J = -D \frac{dC}{dx}$$

Here J is the flux (amount crossing unit area per second), D the diffusion coefficient, C the concentration and x the distance. The minus sign says flow goes from high to low C. For a membrane of thickness x it becomes $J = P (C_1 - C_2)$, where C_1 and C_2 are the concentrations on the two sides and P is the **permeability coefficient** (units cm/s). P is larger when the molecule

dissolves well in lipid (a high oil–water partition coefficient K) and when the membrane is thin: P = K D / x.

So the bilayer sorts molecules by size and polarity. Small non-polar gases (O_2, CO_2, N_2) and lipid-soluble drugs and steroid hormones cross fast. Small uncharged polar molecules (water, urea, ethanol, glycerol) cross slowly. Large polar molecules (glucose, amino acids) hardly cross. Ions (Na^+, K^+, Cl^-) are almost stopped, because a charge carries a shell of water that would have to be stripped off to enter the oily core.

Simple diffusion has three signatures: rate rises in a straight line with the concentration difference, it never saturates, and it has no specificity (no protein, so no binding site to compete for).

Facilitated diffusion: channels and permeases

Molecules the bilayer blocks cross passively through transport proteins. There are two kinds:

- **Carrier proteins** (also called **transporters** or **permeases**. The binding site is open to one side at a time, the **alternating access** mechanism. The glucose transporter GLUT1 of red cells is the textbook example. A carrier that moves one solute on its own is a uniporter (chapter 2.5).
- **Channel proteins** form a water-filled pore that is open to both sides at once when it is open. They select by size and charge and are much faster than carriers (chapter 2.6).

Facilitated diffusion is still passive: the direction is always down the gradient, and it stops when concentrations are equal (for an uncharged solute). The protein only lowers the barrier. Because a carrier has a limited number of binding sites, its rate follows the same curve as an enzyme:

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v = Vmax [S] / (Km + [S])
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Here v is the rate of transport, [S] the solute concentration, Vmax the maximum rate when every carrier is busy, and Km the concentration that gives half of Vmax (a low Km means high affinity). The carrier also shows **specificity** (GLUT1 carries D-glucose but not its mirror image L-glucose) and **competitive inhibition** by similar molecules.

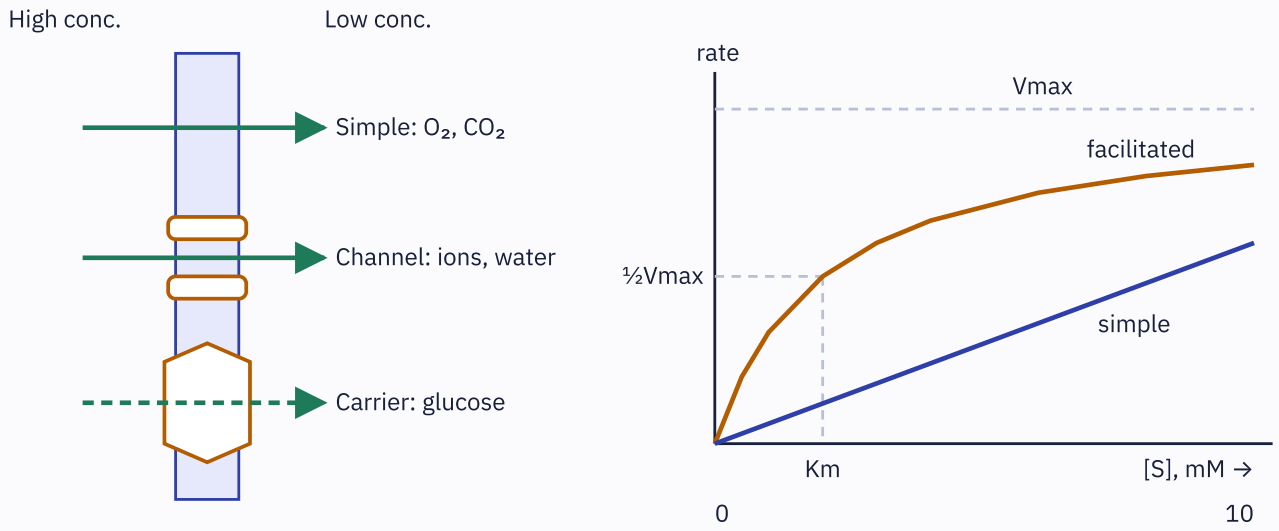


Figure 2.3.1 Left: the three passive routes, all running from high to low concentration. Right: rate against concentration, plotted from v = 100[S]/(2 + [S]) for a carrier (orange; Km = 2 mM) and v = 6[S] for simple diffusion (blue). Notice that the carrier is faster at low concentration but levels off at Vmax, while simple diffusion never saturates.

Osmosis

Osmosis is the net movement of water across a selectively permeable membrane from the side with lower solute concentration to the side with higher solute concentration. Water itself moves down its own concentration gradient. Some water diffuses through the bilayer, but most crosses through **aquaporins**, water channels discovered by Peter Agre (Nobel Prize in Chemistry, 2003). Kidney tubules and red cells are rich in them.

The pressure that would have to be applied to stop water entering is the **osmotic pressure**, π. For dilute solutions, the van 't Hoff equation gives:

$$\pi = i\,C\,R\,T$$

Here i is the number of particles each formula unit gives in solution (about 2 for NaCl, 1 for glucose), C the molar concentration, R the gas constant (0.0821 L·atm·mol^{-1}·K^{-1}) and T the absolute temperature. The product iC is the **osmolarity**.

Tonicity describes what a solution does to a cell, and it depends only on solutes that cannot cross the membrane:

- **Isotonic**: no net water flow; the cell keeps its volume (0.9% NaCl for human cells).
- **Hypotonic**: water enters. An animal cell swells and may burst (a red cell bursting is **haemolysis**). A plant cell swells against its wall and becomes **turgid**, which keeps leaves firm.

- **Hypertonic**: water leaves. A red cell shrinks and becomes spiky (**crenation**). In a plant cell the membrane pulls away from the wall (**plasmolysis**); this is why salted mango pickle draws water out of the fruit.

WORKED EXAMPLE 1

Question. Calculate the osmolarity and osmotic pressure of normal saline (0.9% w/v NaCl) at body temperature, 37 °C. Molar mass of NaCl = 58.44 g/mol; take i = 2. `4 marks`

1. 0.9% w/v means 0.9 g per 100 mL, that is 9 g/L.
2. Molarity C = 9 g/L ÷ 58.44 g/mol = 0.154 mol/L.
3. Osmolarity = iC = 2 × 0.154 = 0.308 osmol/L, about 308 mOsm/L, close to that of blood plasma, which is why this saline is isotonic.
4. T = 37 + 273 = 310 K.
5. π = iCRT = 0.308 × 0.0821 × 310 = **7.84 atm**.

WORKED EXAMPLE 2

Question. A glucose carrier has Km = 2 mM and Vmax = 100 µmol per minute per gram of cells. Find the rate at 1 mM and at 10 mM glucose. Why does a tenfold rise in glucose not give a tenfold rise in rate? `4 marks`

1. At 1 mM: v = 100 × 1 / (2 + 1) = 33.3 µmol/min/g.
2. At 10 mM: v = 100 × 10 / (2 + 10) = 83.3 µmol/min/g.
3. Ratio = 83.3 / 33.3 = 2.5, not 10.
4. Reason: at 10 mM most carriers are already occupied, so the rate approaches Vmax. Saturation is the mark of carrier-mediated transport.

EXAM TRAPS

- Facilitated diffusion uses a protein but no energy. Do not call it active because a protein is involved.
- In osmosis water moves towards the *higher* solute concentration. Writing "from high to low concentration" without saying of what is a common lost mark.
- Osmolarity counts particles: 0.1 M NaCl is about 0.2 osmol/L, 0.1 M glucose is 0.1 osmol/L.
- Tonicity is not the same as osmolarity. A solute that enters the cell (urea) does not hold water out, so an iso-osmotic urea solution is hypotonic.
- Remember to convert °C to kelvin in π = iCRT.

CHECK YOURSELF

– REMEMBER **State Fick's first law.** 1 mark

J = −D (dC/dx): flux is proportional to the concentration gradient, from high to low concentration.

– REMEMBER **What are aquaporins?** 1 mark

Channel proteins that let water cross the membrane rapidly; discovered by Peter Agre.

– UNDERSTAND **List three differences between simple and facilitated diffusion.** 3 marks

Facilitated diffusion uses a protein, simple does not; facilitated saturates (Vmax, Km), simple is linear; facilitated is specific and can be competitively inhibited, simple is not.

– UNDERSTAND **Why do ions not cross a pure lipid bilayer?** 2 marks

An ion is surrounded by a hydration shell; entering the non-polar core would mean stripping that water off, which costs a great deal of energy.

– APPLY **Find π for 0.30 M glucose at 27 °C.** 2 marks

i = 1, T = 300 K: π = 0.30 × 0.0821 × 300 = **7.39 atm**.

– APPLY **For a carrier with Km = 2 mM, at what concentration is the rate 80% of Vmax?** 2 marks

0.8 = S/(2 + S), so 0.8 × 2 = 0.2S, giving S = **8 mM**.

– ANALYZE **Red cells are put in 0.3 M sucrose and in 0.3 M urea. Predict and explain the result in each.** 4 marks

Both are close to the cell's osmolarity. Sucrose cannot cross, so it is isotonic and the cells keep their shape. Urea crosses the membrane; as it enters, water follows, so the cells swell and haemolyse: 0.3 M urea is iso-osmotic but hypotonic.

BEYOND THE SYLLABUS

This is not for the exam. Drug designers use permeability rules (small, not too polar, not too many hydrogen-bond donors) to predict which molecules can be taken as tablets, and screen compounds through artificial membranes in the PAMPA assay. Reverse osmosis, used in Indian household RO water purifiers and in desalination plants such as those near Chennai, pushes water against its osmotic pressure through a polymer membrane. Aquaporin-embedded membranes are being tested for water treatment. Search next for "Lipinski rule of five" and "aquaporin biomimetic membranes".

2.4 Active transport: primary and secondary; ATP pumps

Active transport moves a solute uphill, against its concentration or electrochemical gradient, so it needs an energy source. In **primary** active transport a pump spends ATP directly; the Na^+/K^+-ATPase is the model. In **secondary** active transport a carrier spends the energy stored in an ion gradient that a primary pump built, as when your intestine absorbs glucose together with Na^+. Every animal cell runs on this pair: pumps build gradients, and the gradients power the rest.

You need: passive transport and carrier kinetics from chapter 2.3.

Gradients and the energy to climb them

For a charged solute, two forces act: the concentration difference and the membrane voltage. Together they form the **electrochemical gradient**. The free-energy change for moving one mole of an ion into the cell is:

$$\Delta G_{in} = R\,T\,\ln\left(C_{in} / C_{out}\right) + z\,F\,V_m$$

Here R = 8.314 J·mol^{-1}·K^{-1} is the gas constant, T the absolute temperature, C_in and C_out the concentrations inside and outside, z the charge of the ion (+1 for Na^+, +2 for Ca^{2+}), F = 96 485 C/mol the Faraday constant, and Vm the membrane potential (inside minus outside, about −70 mV in many animal cells). If ΔG is negative the move is downhill and can be passive; if positive it must be driven.

A typical mammalian cell keeps (Alberts' standard values): Na^+ about 5–15 mM inside and 145 mM outside; K^+ about 140 mM inside and 5 mM outside; free Ca^{2+} about 10^{-4} mM (10^{-7} M) inside and 1–2 mM outside.

The ATP pumps

CLASS	HOW IT WORKS	EXAMPLES
P-type	Phosphorylates itself on an aspartate residue in every cycle; the phosphate switches its shape	Na^+/K^+-ATPase; Ca^{2+}-ATPases (SERCA of the sarcoplasmic reticulum, PMCA of the plasma membrane); gastric H^+/K^+-ATPase
V-type	Rotary pump; moves H^+ only, no phosphorylated intermediate	Acidifies lysosomes, endosomes and plant vacuoles
F-type	Rotary; related to V-type but normally runs backwards, using an H^+ gradient to make ATP	ATP synthase of mitochondria, chloroplasts, bacteria (Unit 3)
ABC transporters	Two ATP-binding cassettes; ATP binding brings them together, hydrolysis separates them	Multidrug resistance pump MDR1 (P-glycoprotein); CFTR, which acts as a Cl^- channel

The Na^+/K^+-ATPase

Discovered by Jens Skou (Nobel Prize in Chemistry, 1997), this P-type pump moves **3 Na^+ out and 2 K^+ in for each ATP** hydrolysed. Its cycle has two shapes, E1 (sites face the cytosol, high affinity for Na^+) and E2 (sites face outside, high affinity for K^+):

1. In the E1 form, 3 Na^+ bind from the cytosol.
2. ATP transfers its terminal phosphate to an aspartate on the pump; ADP leaves.
3. Phosphorylation flips the pump to E2; the Na^+ sites open outward and lose affinity, so 3 Na^+ are released outside.
4. 2 K^+ bind from outside.
5. The aspartyl phosphate is hydrolysed and Pi leaves.
6. The pump returns to E1 and releases 2 K^+ into the cytosol.

Because 3 positive charges go out and only 2 come in, the pump is **electrogenic**; it adds a little directly to the negative inside voltage, though most of the resting potential comes from K^+ leaking out through channels (chapter 2.6). The pump has three jobs: it keeps the Na^+ and K^+ gradients; it controls cell volume, because by keeping Na^+ out it balances the many impermeant molecules inside that would otherwise draw water in until the cell burst; and it supplies the Na^+ gradient for secondary transport. Alberts estimates that it uses almost a third of a typical animal cell's energy, and close to two-thirds in nerve cells. **Ouabain** and **digoxin** (cardiac glycosides, from plants) block it from the outside.

Ca^{2+} and H^+ pumps

Ca^{2+} is a signal (Unit 5), so its cytosolic level is kept about ten thousand times lower than outside. The plasma membrane Ca^{2+}-ATPase pumps it out, and SERCA pumps it into the sarcoplasmic reticulum of muscle, where it is stored for the next contraction; Alberts notes that this pump makes up about 90% of the membrane protein of that organelle. In the stomach, the H^+/K^+-ATPase of parietal cells pumps H^+ into the gastric lumen in exchange for K^+, making the acid of gastric juice. Proton pump inhibitors such as omeprazole, common acidity drugs in India, block it.

Secondary active transport

Na^+ outside "wants" to come in: both its concentration gradient and the negative inside pull it. A secondary carrier lets Na^+ in only if it brings another solute with it, or sends one out. The energy of Na^+ running downhill pays for the second solute running uphill. If the pump stops, secondary transport stops too.

- **SGLT1** (sodium–glucose linked transporter) on the gut-facing side of intestinal cells moves 2 Na^+ and 1 glucose in together. Glucose is concentrated inside, then leaves the other face of the cell passively through GLUT2.
- **Na^+/Ca^{2+} exchanger** (NCX) of heart muscle lets 3 Na^+ in and sends 1 Ca^{2+} out.
- In bacteria and plants the driving ion is usually H^+, not Na^+; the *E. coli* lactose permease brings in lactose with H^+.

The direction rules (uniport, symport, antiport) and the stoichiometry calculation are in chapter 2.5.

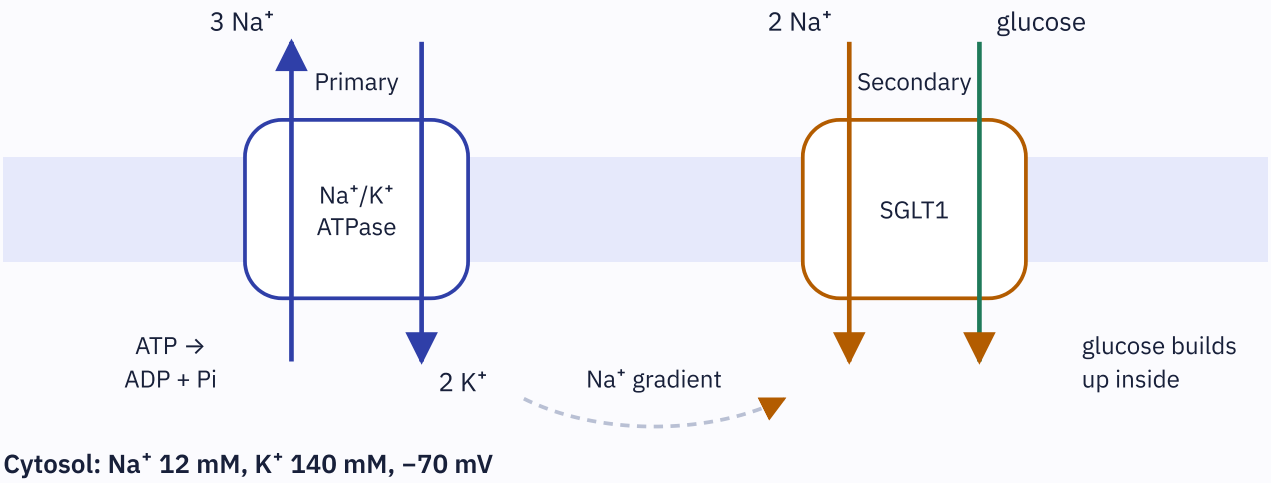


Figure 2.4.1 The pump (left) spends ATP to push Na^+ out and K^+ in, against both gradients. The symporter (right) uses no ATP: Na^+ flowing back in, down its gradient, drags glucose in against its gradient. The dashed arrow is the energy link: no pump, no gradient, no glucose uptake.

WORKED EXAMPLE

Question. Using Na^+ 12 mM inside and 145 mM outside, K^+ 140 mM inside and 5 mM outside, Vm = −70 mV and T = 310 K, find the free energy needed for one cycle of the Na^+/K^+-ATPase. Can the hydrolysis of one ATP, taken as −50 kJ/mol under cell conditions, pay for it? `8 marks`

1. RT = 8.314 × 310 = 2577 J/mol. zFVm for a +1 ion = 96 485 × (−0.070) = −6754 J/mol.
2. Na^+ moving *in*: ΔG = 2577 × ln(12/145) − 6754 = 2577 × (−2.492) − 6754 = −6422 − 6754 = −13 176 J/mol. Entry is downhill.
3. So moving Na^+ *out* costs +13.18 kJ/mol; for 3 Na^+: 3 × 13.18 = +39.5 kJ.
4. K^+ moving *in*: ΔG = 2577 × ln(140/5) − 6754 = 2577 × 3.332 − 6754 = 8588 − 6754 = +1834 J/mol. The voltage helps, but the concentration gradient wins, so it is slightly uphill. For 2 K^+: +3.7 kJ.
5. Total per cycle = 39.5 + 3.7 = **+43.2 kJ/mol**.
6. ATP supplies about 50 kJ/mol, more than 43.2, so one ATP per cycle is enough.

EXAM TRAPS

- The ratio is 3 Na^+ out : 2 K^+ in : 1 ATP. Swapping the 3 and 2, or the directions, is the commonest slip.
- Secondary active transport does use energy, from an ion gradient, not from ATP directly. Do not call it passive.
- F-type ATP synthase normally makes ATP; it is listed among pumps because it can run in reverse.
- Vm is inside minus outside, so it is negative. A wrong sign in zFVm changes the answer completely.
- The favourite question: "Explain the mechanism of the Na^+/K^+ pump with a neat diagram and state its significance."

CHECK YOURSELF

– REMEMBER **Name the four classes of ATP-powered pumps.** 2 marks

P-type, V-type, F-type and ABC transporters.

– REMEMBER **Which drug blocks the Na^+/K^+-ATPase from outside the cell?** 1 mark

Ouabain (and digoxin, a related cardiac glycoside).

– UNDERSTAND **Why is the Na^+/K^+ pump called electrogenic?** 2 marks

Each cycle moves 3 positive charges out and only 2 in, a net transfer of one charge out, which makes the inside slightly more negative.

– UNDERSTAND **Why does an animal cell swell and burst if its Na^+/K^+ pump is blocked for long?** 3 marks

Na^+ (and Cl^-) leak in and are no longer pumped out. Together with the impermeant molecules already inside, they raise the internal osmolarity, water follows by osmosis, and with no cell wall the cell swells until it lyses.

– APPLY **Find ΔG for moving one mole of Ca^{2+} into a cell: Ca^{2+} 10^{-4} mM inside, 1 mM outside, Vm = −70 mV, T = 310 K.** 3 marks

RT ln(10^{-4}) = 2577 × (−9.210) = −23 738 J; zFVm = 2 × 96 485 × (−0.070) = −13 508 J. ΔG = **−37.2 kJ/mol**: entry is strongly downhill, which is why opening a few Ca^{2+} channels floods the cytosol so quickly.

– APPLY **Why does oral rehydration solution (ORS) contain both glucose and salt?** 3 marks

SGLT1 in the gut wall absorbs Na^+ and glucose together (2 Na^+ per glucose), and this route keeps working in diarrhoea such as cholera. Water follows the absorbed solutes by osmosis, so the patient rehydrates.

– ANALYZE **Ouabain is added to intestinal cells. Explain why glucose uptake falls even though SGLT1 itself is not blocked.** 4 marks

Ouabain stops the Na^+/K^+ pump. Na^+ leaks in, the Na^+ gradient runs down, and SGLT1 loses its energy source. Uptake of glucose against its gradient fails, showing that secondary transport depends on the primary pump.

BEYOND THE SYLLABUS

This is not for the exam. SGLT2, the kidney's version of SGLT1, is the target of the gliflozin drugs (such as dapagliflozin and empagliflozin) used in type 2 diabetes: blocking it makes the kidney pass glucose in urine. ABC pumps such as P-glycoprotein throw anticancer drugs out of tumour cells and are a major cause of multidrug resistance. Cryo-EM structures of pumps caught in each step of their cycle are a current research theme. Search next for "SGLT2 inhibitors mechanism" and "P-glycoprotein multidrug resistance".

2.5 Uniport, symport and antiport systems

Carriers are named by how many solutes they move and in which direction. A **uniporter** moves one solute. A **symporter** moves two solutes in the same direction, and an **antiporter** moves two in opposite directions; together these two are called **cotransporters**, because the movement of one solute is tied to the other. At other universities it appears inside passive and active transport, so this chapter is kept short.

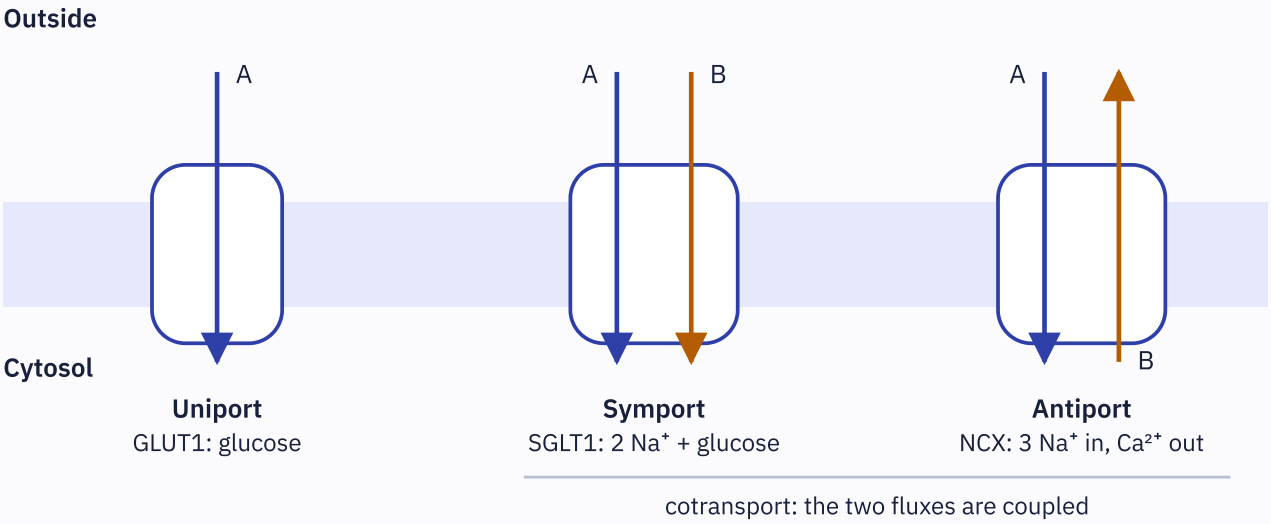


Figure 2.5.1 The three carrier systems. In symport and antiport, A is usually the driving ion (Na^+ in animals, H^+ in bacteria and plants) moving down its gradient, and B is the solute pushed up its gradient. Notice that the arrows show direction only; whether a move is uphill depends on the gradients.

The three systems

- **Uniport**: one solute, facilitated diffusion, always downhill. GLUT1 (glucose into red cells) and GLUT4 (glucose into muscle and fat when insulin moves it to the surface).
- **Symport**: both solutes the same way. SGLT1 in the intestine (2 Na^+ with 1 glucose); Na^+–amino acid symporters; the *E. coli* lactose permease (H^+ with lactose).
- **Antiport**: solutes in opposite directions. NCX in heart muscle (3 Na^+ in, 1 Ca^{2+} out); the Na^+/H^+ exchanger that pushes acid out and controls cytosolic pH; band 3 of the red cell (Cl^- in, HCO_3^- out), which helps blood carry CO_2; the mitochondrial ADP/ATP carrier.

A carrier cannot "half work": a symporter will not move B unless A binds too. That strict coupling is what lets the downhill flow of A pay for the uphill flow of B. Uniporters and cotransporters share the alternating access mechanism of chapter 2.3; only the number of binding sites differs.

The words describe direction, not energy. Some books also call the Na^+/K^+-ATPase an antiport pump, because Na^+ and K^+ move in opposite directions; its energy still comes from ATP, so it is primary active transport (chapter 2.4).

How far can a symporter push?

At the limit, the energy released by the driving ions just equals the energy needed by the solute. For a symporter bringing n Na^+ in with one uncharged solute S:

$$R\,T\,\ln([S]_{in} / [S]_{out}) = n \times |\Delta G(Na^+ \text{ in})|$$

So each extra Na^+ in the stoichiometry multiplies the possible concentration ratio, it does not just add to it.

WORKED EXAMPLE

Question. In an intestinal cell, ΔG for Na^+ entry is −13.18 kJ/mol (from the worked example of chapter 2.4) and RT = 2.577 kJ/mol at 310 K. Find the largest glucose ratio [glucose]in / [glucose]out that a symporter can build with (a) 1 Na^+ per glucose, (b) 2 Na^+ per glucose. `5 marks`

1. (a) ln(ratio) = 1 × 13.18 / 2.577 = 5.11. Ratio = $e^{5.11} \approx$ **166**.
2. (b) ln(ratio) = 2 × 13.18 / 2.577 = 10.22. Ratio = $e^{10.22} \approx$ **2.8×10^4**.
3. Glucose is uncharged, so no voltage term is needed for it.
4. Conclusion: doubling the Na^+ per glucose squares the possible ratio ($166^2 \approx 2.8 \times 10^4$). That is why the 2 : 1 SGLT1 can absorb almost all the glucose from the gut, even when gut glucose is low.

EXAM TRAPS

- "Symport" and "antiport" say nothing about ATP. Name the energy source separately.
- Cotransport covers both symport and antiport; uniport is not cotransport.
- Band 3 is an antiporter (Cl^-/HCO_3^- exchange), not a channel.
- Examiners ask: "Distinguish uniport, symport and antiport with one example each and a diagram."

CHECK YOURSELF

– `REMEMBER` **Define antiport and give one example.** `2 marks`

A carrier that moves two solutes in opposite directions, such as the Na^+/Ca^{2+} exchanger (3 Na^+ in, 1 Ca^{2+} out).

– `REMEMBER` **Which ion drives most symporters in bacteria?** `1 mark`

H^+ (protons), as in the lactose permease.

– `UNDERSTAND` **Why must a cotransporter be strictly coupled?** `2 marks`

If the driving ion could cross alone, its gradient would run down without moving the second solute; coupling makes every downhill ion pay for an uphill solute.

– APPLY **With $|\Delta G(Na^+ \text{ in})|$ = 13.18 kJ/mol and RT = 2.577 kJ/mol, what amino acid ratio can a 1 : 1 Na^+ symporter reach?** 2 marks

ln(ratio) = 5.11, ratio ≈ **166** (for an uncharged amino acid).

– ANALYZE **Digoxin partly blocks the Na^+/K^+ pump in heart cells, and the heart beats more strongly. Explain using NCX.** 4 marks

Less pumping lets cytosolic Na^+ rise, so the Na^+ gradient that drives NCX becomes smaller. NCX removes Ca^{2+} more slowly, Ca^{2+} builds up in the cell and its stores, and more Ca^{2+} is available for each contraction.

BEYOND THE SYLLABUS

This is not for the exam. Transporters of this kind belong to large families named in the SLC (solute carrier) classification, which counts several hundred human genes; many are drug targets or decide how drugs enter cells. Crystal and cryo-EM structures of LacY and LeuT showed the "rocking bundle" form of alternating access. Search next for "SLC transporter family" and "LeuT alternating access".

2.6 Ion channels: ligand-gated and voltage-gated

An ion channel is a membrane protein with a water-filled pore that lets a particular ion flow down its electrochemical gradient. Channels are not open all the time: they are **gated**, opening when a chemical binds (ligand-gated) or when the membrane voltage changes (voltage-gated). Because millions of ions can rush through a single open channel each second, channels are the basis of every fast electrical event in the body, from a nerve impulse to a heartbeat.

You need: the electrochemical gradient and the ion concentrations from chapter 2.4.

Channels versus carriers

A carrier binds its solute and changes shape for every molecule moved; a channel, once open, is a continuous pore. So a channel is much faster: Lodish gives about 10^7–10^8 ions per second for a channel against about 10^2–10^4 molecules per second for carriers (from memory; check). Channel transport is always passive; there is no channel that pumps.

A channel has two key parts:

- The **selectivity filter**, the narrowest part of the pore, which lets one kind of ion through. In K^+ channels, a ring of backbone carbonyl oxygens sits exactly where the water molecules around a K^+ ion would be. A K^+ ion can shed its water and sit comfortably in the filter; the smaller Na^+ ion does not fit those oxygens well and stays out. Roderick MacKinnon solved this structure and shared the 2003 Nobel Prize in Chemistry.
- The **gate**, which opens and closes the pore in response to a stimulus.

Erwin Neher and Bert Sakmann's **patch clamp** (Nobel Prize 1991) records the current through a single channel. It shows that each channel snaps between fully closed and fully open; a stimulus changes how much of the time it spends open.

Membrane potential and the Nernst equation

When only one ion can cross, it flows until the voltage it builds balances its concentration gradient. That voltage is its **equilibrium potential**, given by the Nernst equation:

$$E = \frac{R\,T}{z\,F}\ln\frac{C_{out}}{C_{in}} \approx \frac{61.5\ \text{mV}}{z}\log_{10}\frac{C_{out}}{C_{in}} \quad \text{at } 37\ ^\circ\text{C}$$

Here E is the equilibrium potential (inside relative to outside), z the ion's charge, and C_out, C_in the concentrations outside and inside. Resting cells have many open K^+ **leak channels**, so the resting potential (about −70 mV) lies close to E_K. Opening Na^+ channels pushes the voltage towards E_Na, which is positive.

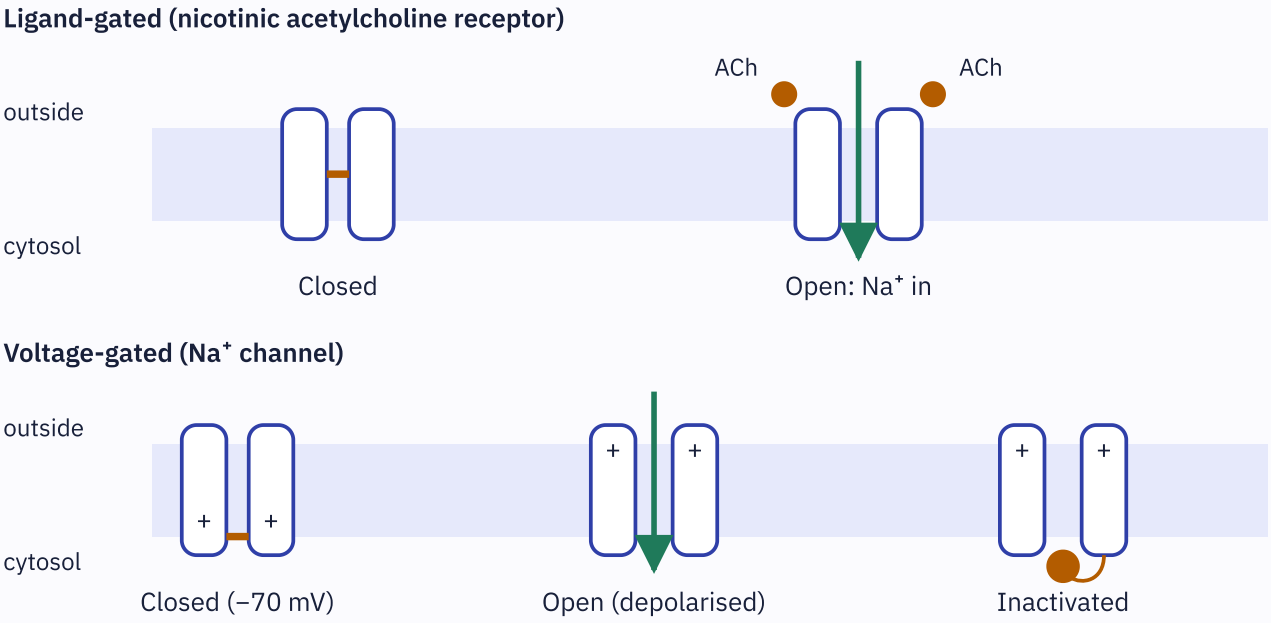


Figure 2.6.1 Top: the nicotinic receptor stays shut until two acetylcholine (ACh) molecules bind, then lets Na^+ flow in. Bottom: in a voltage-gated Na^+ channel the "+" marks the charged S4 voltage sensors; depolarisation moves them outward and opens the gate. Within about a millisecond a cytosolic loop (orange ball) plugs the pore: the channel is inactivated and cannot reopen until the membrane returns to rest.

Ligand-gated channels

These channels open when a specific molecule, the **ligand**, binds. Most are receptors for neurotransmitters at synapses, so they turn a chemical signal into an electrical one in under a millisecond.

- **Nicotinic acetylcholine receptor** (nAChR) at the nerve–muscle junction: five subunits around a central pore (two α plus β, δ, and γ or ε). Acetylcholine binds at two sites on the α subunits; both must be filled for the channel to open. The open pore passes cations, mainly Na^+ inward, which depolarises the muscle cell.
- **GABA-A and glycine receptors**: Cl^- channels that inhibit nerve cells.
- **Glutamate receptors** (AMPA, NMDA): excitatory cation channels in the brain; NMDA receptors also let in Ca^{2+}.

An **agonist** binds the receptor and opens it like the natural ligand: nicotine opens nAChR. An **antagonist** binds without opening it and blocks the natural ligand: d-tubocurarine (the arrow poison curare) and α-bungarotoxin, from the venom of the many-banded krait (*Bungarus multicinctus*, a relative of the Indian common krait), block nAChR and paralyse muscle. Benzodiazepines are different again: they do not open GABA-A receptors themselves but make

GABA open them more easily. Receptor pharmacology in general is in Unit 5, Agonists and antagonists.

Voltage-gated channels

Voltage-gated Na^+, K^+ and Ca^{2+} channels share one plan. A voltage-gated K^+ channel is four separate subunits; the Na^+ and Ca^{2+} channels are a single long protein with four similar domains (I to IV). Each subunit or domain has six transmembrane helices, S1 to S6:

1. **Sensing.** Helix S4 carries a positive amino acid (arginine or lysine) at about every third position. At rest the negative inside holds S4 in.
2. **Opening.** Depolarisation (the inside becoming less negative) pushes the S4 helices outward, and the movement pulls open the gate formed by S5 and S6. The loop between S5 and S6 lines the selectivity filter.
3. **Inactivation.** In Na^+ channels a cytosolic loop between domains III and IV swings in and blocks the pore within about a millisecond, even though the membrane is still depolarised. Only after repolarisation does the channel return to closed and become ready again.

In a nerve impulse, Na^+ channels open first and Na^+ rushes in; then they inactivate, and slower K^+ channels open and let K^+ out, bringing the voltage back. Inactivation is why an impulse travels one way and why there is a refractory period (details in Unit 6, Nerve cells). At nerve terminals, voltage-gated Ca^{2+} channels let in the Ca^{2+} that triggers exocytosis of neurotransmitter (chapter 2.7). Tetrodotoxin from pufferfish blocks Na^+ channels; local anaesthetics such as lidocaine block them from the inside, which is how a dentist numbs a tooth.

WORKED EXAMPLE

Question. A nerve cell has K^+ 140 mM inside and 5 mM outside, Na^+ 12 mM inside and 145 mM outside. Calculate E_K and E_Na at 37 °C, and explain why the resting potential is about −70 mV. 6 marks

1. At 37 °C, RT/F × ln 10 = 61.5 mV. For K^+ and Na^+, z = +1.
2. E_K = 61.5 × $\log_{10}(5/140)$ = 61.5 × (−1.447) = **−89 mV**.
3. E_Na = 61.5 × $\log_{10}(145/12)$ = 61.5 × (1.082) = **+67 mV**.
4. At rest, many K^+ leak channels are open and very few Na^+ channels, so the membrane is much more permeable to K^+. The resting potential lies near E_K (−89 mV), pulled a little towards E_Na by the small Na^+ leak, giving about −70 mV.
5. When voltage-gated Na^+ channels open, Na^+ permeability dominates and the potential swings towards +67 mV: the rising phase of the action potential.

EXAM TRAPS

- Channels are passive. "Ion channel pumps ions" is wrong; pumps are carriers that use ATP (chapter 2.4).
- Inactivated is not the same as closed. An inactivated Na^+ channel cannot be opened by depolarisation; a closed one can.
- Nernst uses C_out/C_in for the inside potential. Flipping the ratio gives the wrong sign.
- For Cl^-, z = −1, which flips the sign of the equation.
- The favourite question: "Explain the structure and working of ligand-gated and voltage-gated channels with examples." Give nAChR for one and the Na^+ channel with its S4 sensor for the other.

CHECK YOURSELF

– REMEMBER **Which helix is the voltage sensor in voltage-gated channels?** 1 mark

S4, which carries positively charged arginine or lysine residues.

– REMEMBER **Name one agonist and one antagonist of the nicotinic acetylcholine receptor.** 2 marks

Agonist: nicotine (or acetylcholine itself). Antagonist: d-tubocurarine (curare) or α-bungarotoxin.

– UNDERSTAND **How does a K^+ channel let K^+ through but keep out the smaller Na^+?** 3 marks

The filter's carbonyl oxygens are spaced to replace exactly the water shell of a K^+ ion, so K^+ loses its water at little cost. Na^+ is too small to touch all those oxygens at once, so shedding its water is costly and it stays out.

– UNDERSTAND **Why does the resting potential lie close to E_K?** 2 marks

At rest the membrane is far more permeable to K^+ (open leak channels) than to other ions, so the K^+ equilibrium dominates.

– APPLY **Cl^- is 10 mM inside and 110 mM outside. Find E_Cl at 37 °C.** 2 marks

$z = -1$: $E = (61.5 / -1) \times \log_{10}(110/10) = -61.5 \times 1.041 =$ **−64 mV**.

– APPLY **Outside K^+ rises from 5 mM to 10 mM (inside 140 mM). What happens to E_K?** 2 marks

$E_K = 61.5 \times \log_{10}(10/140) =$ **−70 mV**, about 19 mV less negative: the cell depolarises. This is why high blood K^+ is dangerous for the heart.

– ANALYZE **α-Bungarotoxin from krait venom causes paralysis. Which step of nerve–muscle signalling does it block, and why are breathing muscles at risk?** 4 marks

α-Bungarotoxin binds the nicotinic ACh receptors of the muscle end-plate and stops ACh opening them. No Na^+ enters, the muscle does not depolarise and cannot contract. The diaphragm is a skeletal muscle with the same receptors, so breathing fails.

BEYOND THE SYLLABUS

This is not for the exam. Mechanically gated channels, the Piezo proteins, sense touch and pressure; their discovery was part of the 2021 Nobel Prize in Physiology or Medicine. Light-gated channels such as channelrhodopsin let neuroscientists switch chosen neurons on with a flash (optogenetics). Many diseases are channelopathies, from cystic fibrosis (the CFTR Cl^- channel) to some epilepsies and heart rhythm disorders, and automated patch clamp is now a standard drug-safety screen. Search next for "optogenetics channelrhodopsin" and "hERG channel drug safety".

2.7 Endocytosis and exocytosis

Proteins, particles and large volumes of fluid are too big for any carrier or channel, so cells move them inside membrane bubbles called vesicles. In **endocytosis** a patch of plasma membrane folds inward and pinches off, carrying material into the cell. In **exocytosis** a vesicle from inside fuses with the plasma membrane and releases its contents outside. A macrophage swallowing bacteria, a liver cell taking up cholesterol, a pancreatic β cell releasing insulin and a nerve releasing a transmitter all use these two processes.

You need: membrane fluidity from chapter 2.1. The organelles that sort vesicles (Golgi, lysosomes) are in Unit 3.

Kinds of endocytosis

- **Phagocytosis** ("cell eating"): specialised cells such as macrophages and neutrophils extend arm-like pseudopods, driven by actin, around a large particle (a bacterium, a dead cell) and enclose it in a **phagosome**, which then fuses with a lysosome for digestion. It is triggered by receptors that recognise the particle. In protozoa such as *Amoeba* it is a way of feeding.
- **Pinocytosis** ("cell drinking"): nearly all cells continually take in small vesicles of extracellular fluid and whatever is dissolved in it, with no selection. Macropinocytosis takes larger gulps.
- **Receptor-mediated endocytosis**: specific molecules bind receptors that gather in **clathrin-coated pits**, so the cell takes up chosen cargo in concentrated form, even when it is scarce outside.
- **Caveolae**: small flask-shaped pits coated with the protein caveolin, rich in cholesterol and sphingolipids, a clathrin-independent route.

Receptor-mediated endocytosis step by step

1. **Binding.** The ligand, for example a low-density lipoprotein (LDL) particle carrying cholesterol, binds its receptor on the cell surface.
2. **Coat assembly.** Adaptor proteins (AP2) bind the receptor's cytosolic tail and recruit **clathrin**. Clathrin is a three-legged unit (a triskelion of three heavy and three light chains) that assembles into a basket of pentagons and hexagons, bending the membrane into a coated pit.
3. **Pinching off.** The GTPase **dynamin** forms a ring around the neck of the pit and, by hydrolysing GTP, pinches the vesicle free.
4. **Uncoating.** Within seconds the clathrin coat is removed (an ATP-using Hsc70 chaperone helps) and recycled.

5. **Sorting in the early endosome.** The vesicle fuses with an early endosome, kept acidic (about pH 6) by V-type H^+ pumps (chapter 2.4). The acid makes LDL fall off its receptor.
6. **Recycling and digestion.** Empty receptors bud off in vesicles and return to the plasma membrane for reuse. The LDL goes on through late endosomes to lysosomes (about pH 5), where it is broken down and cholesterol is released to the cytosol.

Michael Brown and Joseph Goldstein worked out this pathway from patients with **familial hypercholesterolaemia**, whose LDL receptors are missing or cannot gather in coated pits; their blood cholesterol is very high and they develop heart disease early (Nobel Prize in Physiology or Medicine, 1985). Iron enters cells the same way, bound to transferrin. Many viruses, including influenza, exploit the route: they are taken in by endocytosis and use the acid of the endosome to trigger fusion with its membrane.

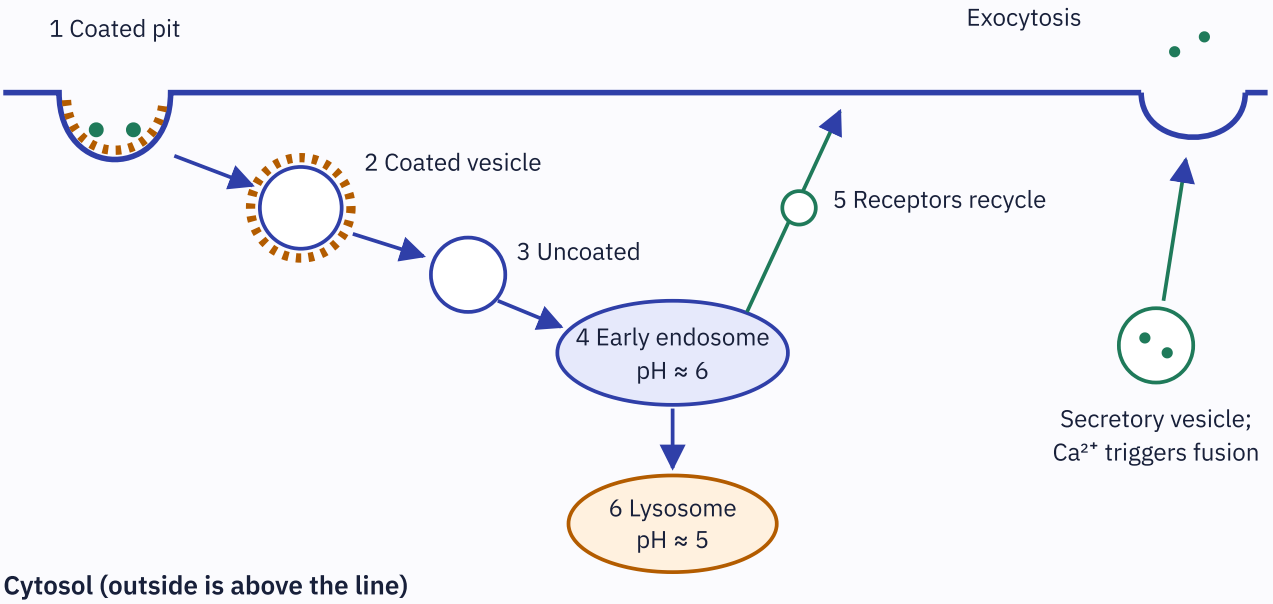


Figure 2.7.1 Left to centre: receptor-mediated endocytosis. Receptors with bound cargo (green dots) gather in a clathrin-coated pit (orange dashes), which pinches off, loses its coat and fuses with the early endosome. The acid releases the cargo; receptors return to the surface (5) while cargo goes on to the lysosome (6). Right: regulated exocytosis, where a secretory vesicle fuses with the plasma membrane and releases its contents outside.

Exocytosis

Proteins made for export are packed into vesicles in the Golgi apparatus (Unit 3). Two routes lead to the surface:

- **Constitutive exocytosis** runs all the time in every cell. It delivers new lipids and membrane proteins to the plasma membrane and secretes material such as extracellular matrix proteins and, in plasma cells, antibodies.

- **Regulated exocytosis** happens only in secretory cells and only on a signal. Vesicles full of product wait near the membrane until a signal, usually a rise in cytosolic Ca^{2+}, triggers fusion. Examples: insulin from pancreatic β cells when blood glucose rises, digestive enzymes from pancreatic acinar cells, and neurotransmitters at a synapse after voltage-gated Ca^{2+} channels open (chapter 2.6).

Fusion is done by **SNARE** proteins. A v-SNARE on the vesicle (synaptobrevin, also called VAMP) twists together with t-SNAREs on the target membrane (syntaxin and SNAP-25) into a tight four-helix bundle, pulling the two bilayers so close that they merge. Rab GTPases help the vesicle find the right target first. At synapses, the Ca^{2+} sensor **synaptotagmin** releases the final step. After fusion the ATPase NSF pulls the SNARE bundle apart for reuse. Botulinum and tetanus toxins are enzymes that cut SNAREs, which is why they paralyse. James Rothman, Randy Schekman and Thomas Südhof shared the 2013 Nobel Prize in Physiology or Medicine for this vesicle traffic machinery.

Endocytosis removes membrane from the surface, and exocytosis adds it back. The two must balance, or the cell would shrink or grow. In **transcytosis** a vesicle taken in on one face of an epithelial cell is carried across and released on the other face; this is how antibodies from a mother's milk cross the gut wall of a newborn rodent.

WORKED EXAMPLE

Question. A cultured fibroblast has a plasma membrane area of 1500 μm^2. It forms 2500 coated vesicles per minute, each a sphere 100 nm in diameter. (a) What area of membrane is taken in per minute? (b) What percentage of the surface is this, and how long would it take to internalise an area equal to the whole surface? (c) What does this imply? `6 marks`

1. Surface area of one vesicle = $4\pi r^2 = \pi d^2$, with d = 100 nm = 0.1 μm. Area = $3.142 \times (0.1)^2 = 0.0314\ \mu m^2$.
2. (a) Per minute: $2500 \times 0.0314 =$ **$78.5\ \mu m^2$**.
3. (b) Fraction = 78.5 / 1500 = 0.052, about **5.2% per minute**. Time for the whole surface = 1500 / 78.5 ≈ **19 minutes**.
4. (c) The cell does not shrink, so membrane must return to the surface at the same rate, by receptor recycling and exocytosis. Membrane flows in a continuous loop.

EXAM TRAPS

- Pinocytosis is non-selective; receptor-mediated endocytosis is selective. Do not call clathrin-coated uptake "pinocytosis" without the qualifier.
- Dynamin uses GTP, not ATP, to pinch off the vesicle.
- The receptor is not digested; it recycles. Only the ligand goes to the lysosome (for LDL).
- Constitutive exocytosis needs no signal; regulated exocytosis needs one, usually Ca^{2+}.
- The favourite question: "Explain receptor-mediated endocytosis with the LDL example and a neat diagram."

CHECK YOURSELF

– REMEMBER **Name the coat protein of coated pits and the protein that pinches the vesicle off.** 2 marks

Clathrin; dynamin (a GTPase).

– REMEMBER **Name the v-SNARE and the two t-SNAREs of a synapse.** 2 marks

v-SNARE: synaptobrevin (VAMP). t-SNAREs: syntaxin and SNAP-25.

– UNDERSTAND **Distinguish phagocytosis from pinocytosis.** 3 marks

Phagocytosis takes up large particles, is done by specialised cells, is triggered by receptors and uses actin-driven pseudopods to form a phagosome. Pinocytosis takes up fluid and solutes in small vesicles, continuously and in almost all cells, without selection.

– UNDERSTAND **Why does the early endosome need to be acidic?** 2 marks

The lower pH weakens ligand–receptor binding, so cargo such as LDL falls off and the empty receptor can recycle to the surface.

– APPLY **A cell forms 1000 vesicles per minute, each 80 nm across. How much membrane area is that per minute?** 2 marks

Each: $\pi \times (0.08\ \mu m)^2 = 0.0201\ \mu m^2$. Total = **20.1 µm² per minute**.

– APPLY **Predict what happens to insulin release if Ca^{2+} is removed from the medium around β cells.** 2 marks

Release falls sharply, because regulated exocytosis needs a rise in cytosolic Ca^{2+} from outside to trigger vesicle fusion.

– ANALYZE **A patient's LDL receptors bind LDL normally but cannot gather in coated pits. Explain why the blood cholesterol is high.** 4 marks

Binding alone does not bring LDL in; receptors must enter coated pits to be endocytosed. Without that step, LDL stays on the surface and in the blood, cells sense low cholesterol and make more of their own, and plasma LDL cholesterol rises: a form of familial hypercholesterolaemia.

BEYOND THE SYLLABUS

This is not for the exam. Drug delivery depends on endocytosis: antibody–drug conjugates and lipid nanoparticles enter cells this way, and a major challenge is helping their cargo escape the endosome before it is digested. Cells also release tiny vesicles called exosomes, now studied as disease markers in "liquid biopsy". Biotech production of therapeutic proteins in CHO cells relies on the constitutive secretory pathway. Search next for "endosomal escape lipid nanoparticles" and "exosomes liquid biopsy".

Unit summary

TERM	MEANING OR FORMULA	CHAPTER
Fluid mosaic model	Fluid lipid bilayer with proteins embedded in or attached to it; Singer and Nicolson, 1972	2.1
2-D diffusion	$\langle r^2 \rangle = 4Dt$; lipid $D \approx 1\ \mu m^2/s$	2.1
Integral vs peripheral	Removed only by detergent vs removed by salt or pH change	2.2
Fick's law	$J = -D\, dC/dx$; across a membrane $J = P(C_1 - C_2)$	2.3
Carrier kinetics	$v = Vmax[S]/(Km + [S])$	2.3
Osmotic pressure	$\pi = iCRT$ ($R = 0.0821$ L·atm·mol^{-1}·K^{-1})	2.3
Ion transport energy	$\Delta G(in) = RT \ln(C_in/C_out) + zFVm$	2.4
Na^+/K^+-ATPase	3 Na^+ out, 2 K^+ in per ATP; P-type; electrogenic; blocked by ouabain	2.4
Symport limit	$RT \ln([S]in/[S]out) = n\lvert\Delta G(Na^+ in)\rvert$	2.5
Nernst equation	$E = (61.5\ mV/z) \log_{10}(C_out/C_in)$ at 37 °C	2.6
Receptor-mediated endocytosis	Receptor → clathrin-coated pit → dynamin → endosome (pH ≈ 6) → lysosome; receptor recycles	2.7
SNAREs	v-SNARE (synaptobrevin) + t-SNAREs (syntaxin, SNAP-25) drive fusion	2.7

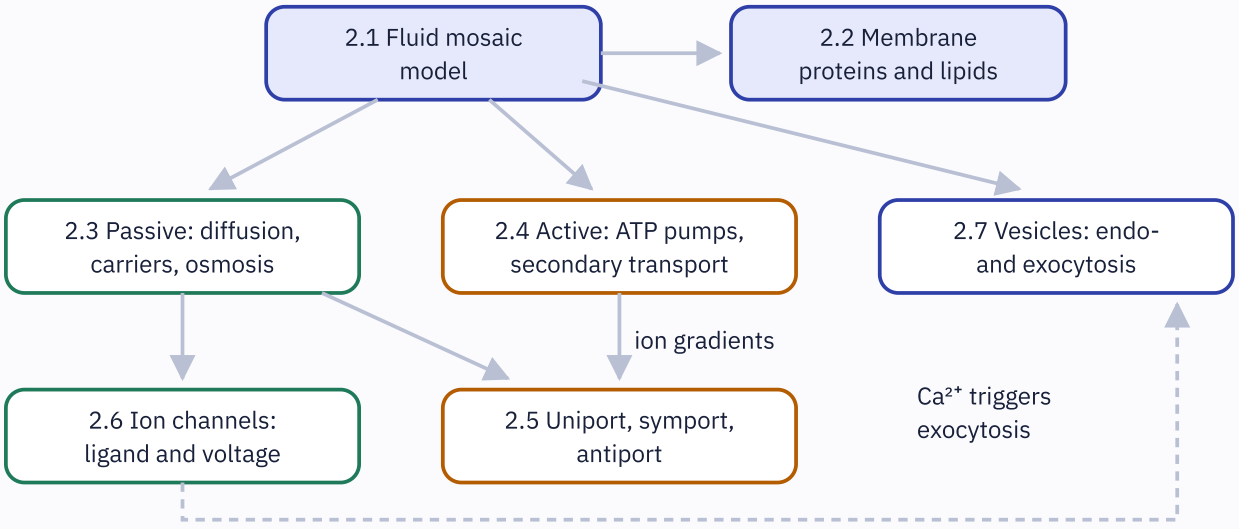


Figure 2.S.1 How the chapters connect. Structure (top) sets what can cross. Passive routes (green) run downhill; active routes (orange) climb uphill using ATP or ion gradients; vesicles (blue) carry bulk cargo. The diagonal from 2.3 to 2.5 is uniport; the dashed line links Ca^{2+} channels to regulated exocytosis.

1. The membrane is a fluid lipid bilayer about 5 nm thick with proteins in it and sugars only on its outer face; lipids move sideways fast and flip-flop rarely.
2. Fluidity rises with temperature, unsaturation and shorter tails; cholesterol buffers it in both directions.
3. The two leaflets differ: PS and PE inside, PC, sphingomyelin and glycolipids outside. Proteins meet the bilayer in six ways, from single-pass to peripheral.
4. Passive transport runs down the gradient: simple diffusion is linear and unselective; carriers saturate (Km, Vmax) and are specific; water moves by osmosis, mostly through aquaporins.
5. Active transport needs energy: P-, V-, F-type and ABC pumps use ATP; secondary carriers use the Na^+ or H^+ gradient that pumps build.
6. The Na^+/K^+-ATPase moves 3 Na^+ out and 2 K^+ in per ATP; it sets the gradients, controls cell volume and powers secondary transport.
7. Uniport, symport and antiport name directions, not energy sources; each extra coupled ion multiplies the concentration ratio a symporter can build.
8. Ion channels are fast, selective, passive pores; ligand-gated ones respond to transmitters, voltage-gated ones to depolarisation through the S4 sensor, and Na^+ channels then inactivate.
9. Endocytosis brings cargo in through clathrin-coated pits, endosomes and lysosomes; exocytosis, driven by SNAREs and often triggered by Ca^{2+}, returns membrane and secretes products.

Exam question bank

Remember and understand

- UNDERSTAND **Describe the fluid mosaic model of the plasma membrane with a labelled diagram, and give two pieces of experimental evidence for it.** 12 marks

  Points: amphipathic phospholipid bilayer, hydrophobic core; integral, peripheral and lipid-anchored proteins; carbohydrates on outer face (glycocalyx); cholesterol; lateral diffusion fast, flip-flop rare; asymmetry. Evidence: Frye–Edidin cell fusion; freeze-fracture particles inside the bilayer; Gorter–Grendel 2 : 1 lipid area. Diagram as Figure 2.1.1.

- UNDERSTAND **Explain the factors that control membrane fluidity, with the special role of cholesterol.** 8 marks

  Temperature and phase transition; unsaturation and cis kinks; tail length; cholesterol as buffer (rigid rings restrain tails at high temperature, prevent packing at low); homeoviscous adaptation in bacteria; why fluidity matters for protein function.

- REMEMBER **Classify membrane proteins by their association with the bilayer, with examples and a diagram.** 8 marks

  Single-pass (glycophorin), multipass (band 3, bacteriorhodopsin), β-barrel (porins), lipid-anchored inside (Ras) and outside (GPI), peripheral (spectrin). Detergent vs salt extraction. Diagram as Figure 2.2.1.

- UNDERSTAND **Compare simple diffusion, facilitated diffusion and active transport in a table, and sketch rate against concentration.** 10 marks

  Direction, energy source, protein needed, saturation, specificity, inhibition, examples (O_2; GLUT1; Na^+/K^+ pump). Sketch: straight line vs hyperbola with Vmax and Km marked (Figure 2.3.1). Spans chapters 2.3 and 2.4.

Apply

– APPLY **Explain the mechanism of the Na^+/K^+-ATPase, and calculate the free energy for one cycle given Na^+ 12/145 mM, K^+ 140/5 mM (in/out), Vm = −70 mV, T = 310 K.** 14 marks

E1/E2 cycle in six steps with aspartyl phosphate; 3 : 2 : 1 stoichiometry; electrogenic; volume control; ouabain. Calculation: Na^+ out +13.18 kJ/mol each (×3 = 39.5), K^+ in +1.83 each (×2 = 3.7); total +43.2 kJ/mol, less than ~50 kJ/mol from ATP.

– APPLY **Calculate the osmolarity and osmotic pressure of 0.9% NaCl at 37 °C, and explain what happens to red cells in water, in 0.9% NaCl and in 3% NaCl.** 10 marks

0.154 M, 0.308 osmol/L, π = 7.84 atm. Water: hypotonic, swelling, haemolysis. 0.9%: isotonic, no change. 3%: hypertonic, water leaves, crenation.

– APPLY **Using the Nernst equation, calculate E_K and E_Na for a neuron and use them to explain the resting potential and the rising phase of the action potential.** 10 marks

E_K = −89 mV, E_Na = +67 mV for the standard concentrations; resting potential near E_K because of K^+ leak channels; opening voltage-gated Na^+ channels drives the potential towards E_Na; inactivation and K^+ channels end the spike.

– APPLY **Explain with a diagram how glucose is absorbed from the intestinal lumen into blood, naming every transporter and its energy source. Why does ORS work?** 12 marks

Apical SGLT1 symport (2 Na^+ : 1 glucose, secondary active); basolateral GLUT2 uniport (passive); basolateral Na^+/K^+ pump (primary, keeps Na^+ low inside). Max ratio calculation (≈ 2.8×10^4 for 2 : 1). ORS: Na^+ and glucose absorbed together, water follows by osmosis. Spans chapters 2.3, 2.4 and 2.5.

Analyze and evaluate

– ANALYZE **Compare ligand-gated and voltage-gated ion channels in structure, gating and function, using the nicotinic acetylcholine receptor and the voltage-gated Na^+ channel. Explain the action of one agonist and two antagonists.** 14 marks

nAChR pentamer, two ACh sites on α subunits, cation pore; Na^+ channel four domains × six helices, S4 sensor, P loop filter, III–IV inactivation loop; closed/open/inactivated states. Agonist: nicotine. Antagonists: d-tubocurarine, α-bungarotoxin; also tetrodotoxin and lidocaine as Na^+ channel blockers.

– ANALYZE **Describe receptor-mediated endocytosis of LDL, and explain the defect in familial hypercholesterolaemia.** 12 marks

LDL receptor, AP2, clathrin triskelion, dynamin (GTP), uncoating, early endosome at pH ≈ 6, receptor recycling, lysosome digestion and cholesterol release. FH: receptors missing or unable to cluster in coated pits, so LDL stays in blood; Brown and Goldstein.

– ANALYZE **A cell is treated with ouabain. Trace the effects on membrane potential, cell volume, glucose uptake by SGLT1 and Ca^{2+} removal by NCX.** 12 marks

Pump stops: Na^+ rises and K^+ falls inside; gradients run down and membrane depolarises slowly; Na^+ and water enter, cell swells; SGLT1 loses its driving force; NCX slows, Ca^{2+} rises (the basis of digoxin's effect on the heart). Spans chapters 2.3 to 2.6.

– ANALYZE **Compare constitutive and regulated exocytosis, and explain the role of SNAREs, synaptotagmin and Ca^{2+} at a synapse. Why do botulinum toxins paralyse?** 12 marks

Constitutive: all cells, continuous, no signal, delivers membrane and ECM. Regulated: secretory cells, stored vesicles, Ca^{2+} signal (insulin, enzymes, neurotransmitters). v- and t-SNARE four-helix bundle, Rab targeting, synaptotagmin as Ca^{2+} sensor, NSF disassembly; voltage-gated Ca^{2+} channels at the terminal. Toxins cut SNAREs, so ACh is not released.

Glossary

Amphipathic Having both a water-loving (polar) part and a water-avoiding (non-polar) part, like a phospholipid.

Antiport Carrier that moves two solutes in opposite directions.

Aquaporin	Channel protein for fast water movement across membranes.
Clathrin	Three-legged protein that forms the basket-like coat of coated pits and vesicles.
Cotransport	Coupled transport of two solutes: symport or antiport.
Crenation	Shrinking and wrinkling of a red cell in a hypertonic solution.
Dynamin	GTPase that pinches a coated vesicle off the membrane.
Electrochemical gradient	Combined driving force on an ion from its concentration difference and the membrane voltage.
Electrogenic	Moving net charge across the membrane, as the Na^+/K^+ pump does.
Endosome	Acidic compartment that receives endocytosed vesicles and sorts their contents.
Equilibrium potential	Voltage at which an ion's electrical and concentration forces balance; given by the Nernst equation.
Flip-flop	Movement of a lipid from one leaflet of the bilayer to the other; rare without enzymes.
Flippase	ATP-using enzyme that moves specific lipids (PS, PE) to the cytosolic leaflet.
Glycocalyx	Sugar coat on the outer cell surface formed by glycoproteins and glycolipids.
Haemolysis	Bursting of red cells, for example in a hypotonic solution.
Inactivation	Channel state in which the pore is blocked despite an open-favouring stimulus.
Integral protein	Membrane protein that can be removed only by dissolving the bilayer with detergent.
Km	Solute concentration at which a carrier works at half its maximum rate.
Ligand	Molecule that binds specifically to a receptor or channel.
Osmosis	Net movement of water across a selectively permeable membrane towards higher solute concentration.
Peripheral protein	Protein bound to a membrane surface by non-covalent bonds; removable by salt or pH.
Permease	Older name for a carrier protein (transporter).
Phagocytosis	Uptake of large particles by specialised cells into a phagosome.
Pinocytosis	Non-selective uptake of extracellular fluid in small vesicles.
Selectivity filter	Narrowest part of a channel pore, which picks the ion that passes.

SNARE	Proteins on vesicle (v-SNARE) and target (t-SNARE) membranes that zip together to drive fusion.
Symport	Carrier that moves two solutes in the same direction.
Tonicity	Effect of a solution on cell volume, set by solutes that cannot cross the membrane.
Uniport	Carrier that moves a single solute down its gradient.

Sources and further reading

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