Advanced Higher Biology

Cell & Proteins Revision Notes

Laboratory Skills: Health and Safety

Lab Hazards

Harm caused to an individual when working in a laboratory

Types of Lab Hazards

1. Toxic or corrosive chemicals
2. Heat or flammable substances
3. Pathogenic organisms
4. Mechanical equipment.

Risk

The **likelihood** of harm arising from exposure to a hazard.

Risk assessment

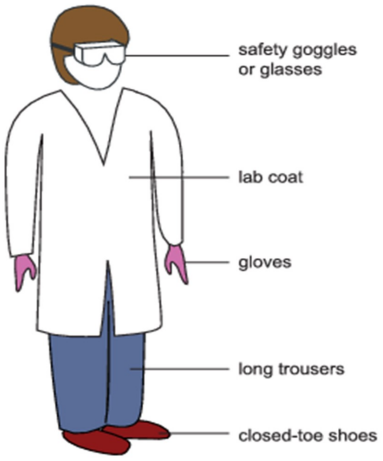
Involves identifying control measures to **minimise** the risk.

Control measures

Measures aim to reduce risk of harm caused by a hazard.

Types

1. Appropriate handling technique
2. Protective clothing and equipment
3. Aseptic technique.



Standard laboratory PPE

Dilutions

Logarithmic Dilution Series

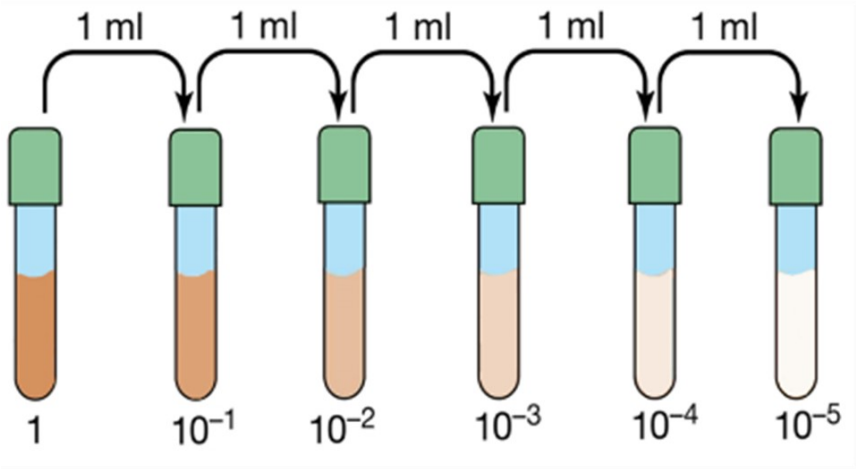
Differ by a constant proportion

E. g 10^{-1}, 10^{-2}, 10^{-3}

Serial Dilution

Taking 1 ml of a stock solution and adding this to 9ml of water. Then 1ml of the newly diluted solution is extracted and subsequently added to 9ml of water each time.

This dilutes the solution by a factor of 10 each time creating a logarithmic dilution series.



Linear Dilution Series

Differ by an equal interval

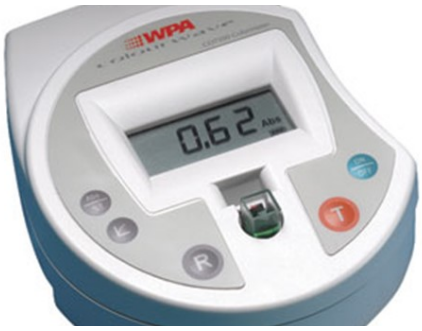
E.g 0·1M, 0·2M, 0·3M

Colorimeter/Spectrophotometer

Concentration of Unknown solution

Measured by a device called a spectrophotometer OR colorimeter by measuring the

absorbance/transmission of a solution at a certain wavelength of visible light.



Calibration

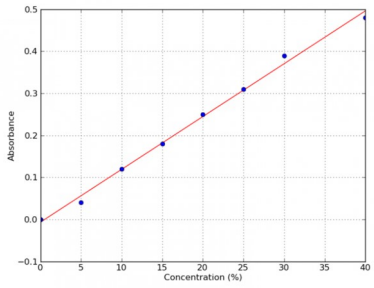
Calibration via a colourless solution should always be carried out to ensure the machine is working correctly.

Absorbance values 0.00 Transmission 100%

Colorimeter/Spectrophotometer

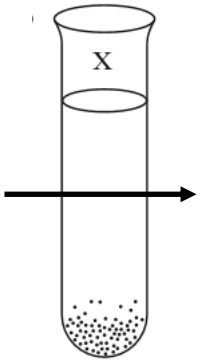
Absorbance

The more intense the colour (concentration) the higher the absorbance .



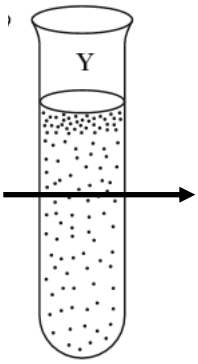
Transmission

As turbidity increases, less light passes through the sample and the transmission value is lower.



Low turbidity

High transmission value



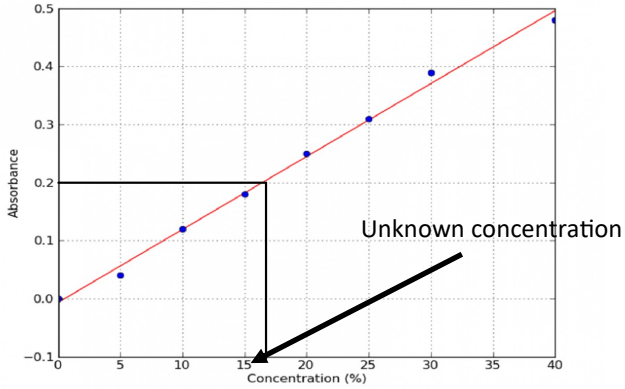
High turbidity

Low transmission value

Standard Curve

A standard curve can be created by measuring known concentrations of solution and taking readings to plot a line of best fit.

An unknown concentration can then be read off this to ascertain the unknown concentration.



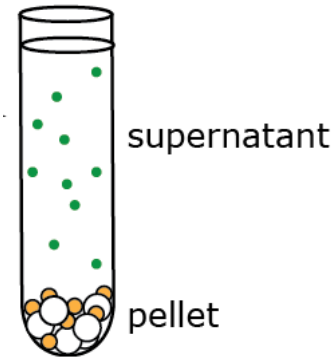
Laboratory Skills: Separating Techniques

Three Separating Techniques

1. Centrifuge
2. Chromatography
3. Gel Electrophoresis

Centrifuge

To separate substances of differing **density**



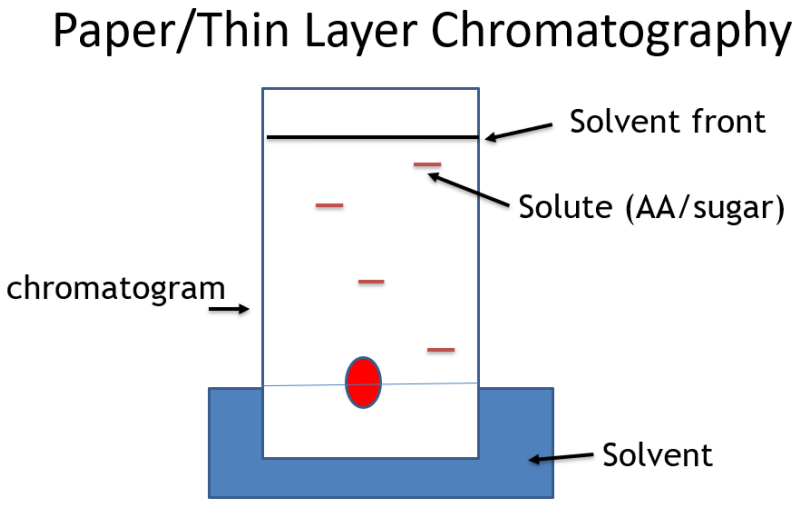
1. **More dense** components settle in the **pellet**
2. **Less dense** components remain in the **supernatant.**

Chromatography

1. Paper and thin layer chromatography

To separate substances such as amino acids or sugars.

The speed that each solute travels along the chromatogram depends on its differing solubility in the solvent used.

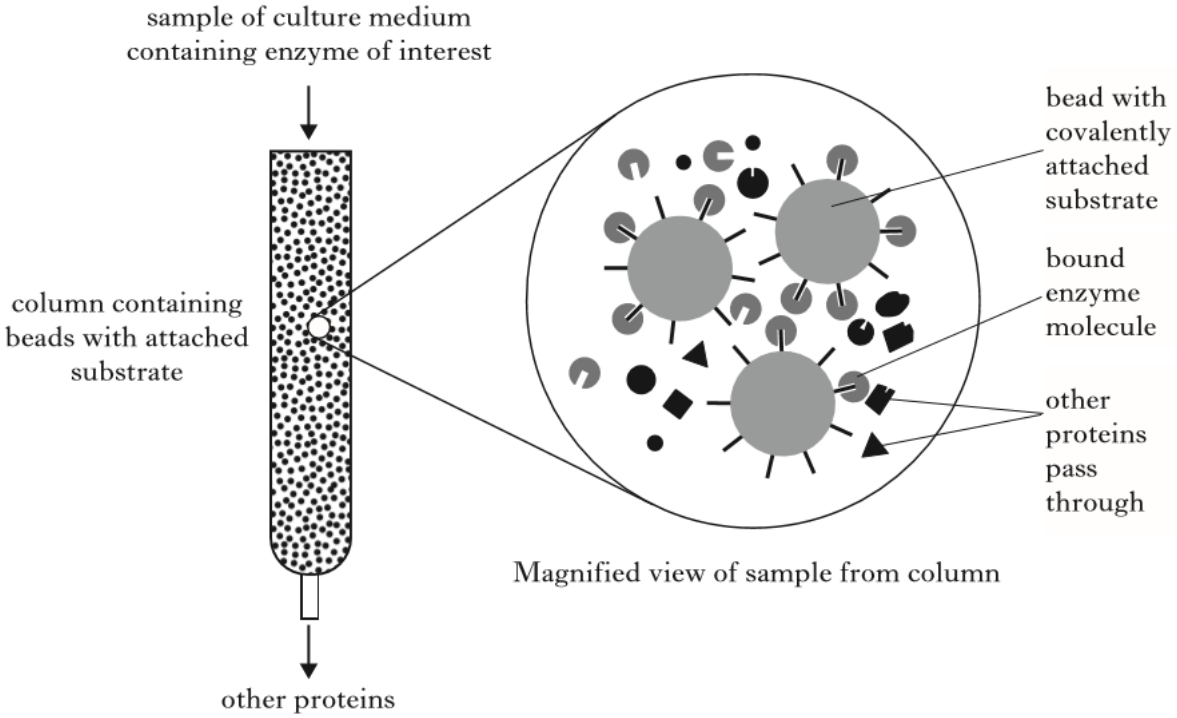


2. Affinity Chromatography

A solid matrix or gel column is created with specific molecules bound to the matrix/gel and mixture of proteins passed down column.

1. Soluble, **target proteins** with a **high affinity** molecules bound to gel/matrix become attached to them.

2. **Non-target** molecules with a **weaker affinity** are washed out



Laboratory Skills: Separating Techniques

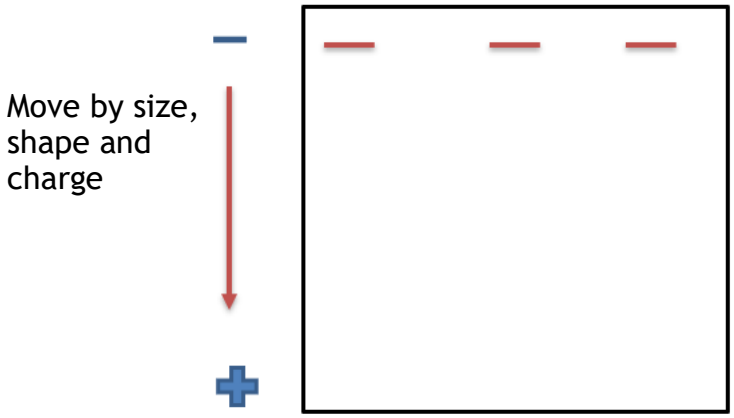
Gel Electrophoresis

Charged macromolecules move through an electric field applied to a gel matrix.

Native Gel Electrophoresis

Separates proteins by their **shape, size and charge**

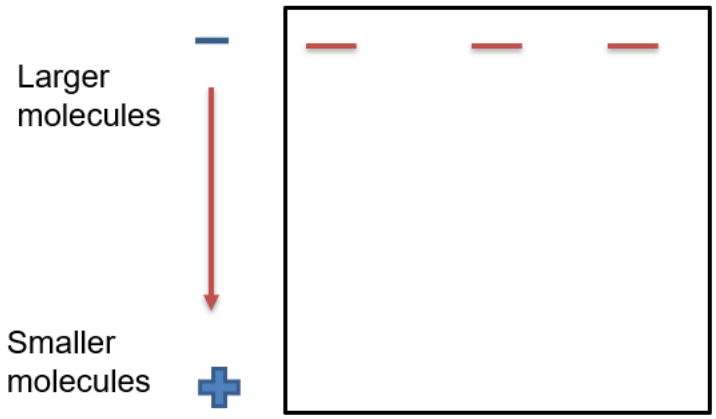
The proteins are not denatured in this technique.



SDS Page Electrophoresis

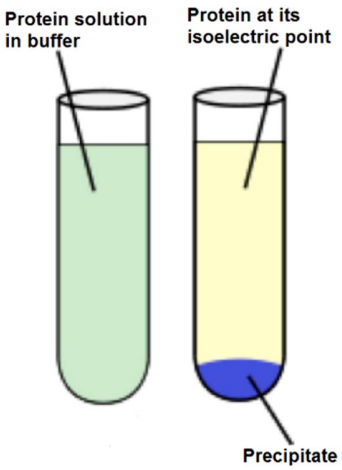
Separates proteins by **size alone** (smaller molecules travel further).

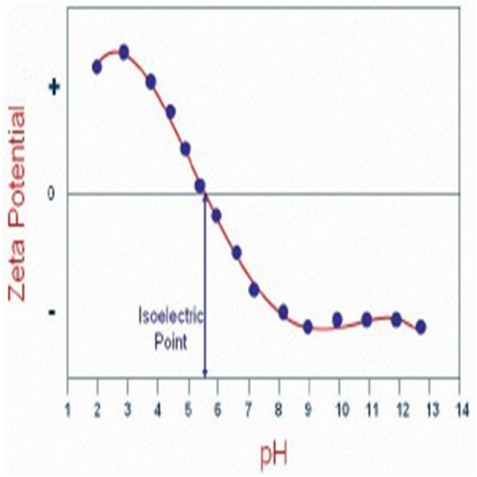
The proteins are denatured giving an equally negative charge

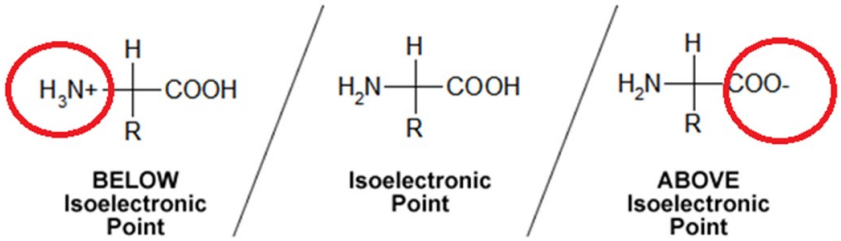


Iso electric Point

When there is **no net charge** on the protein and it will precipitate out of solution.







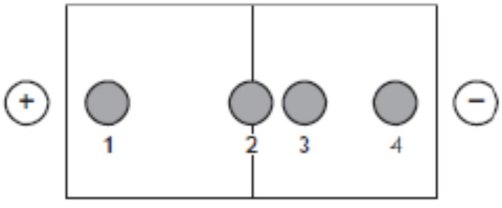
Isoelectric Focusing

Soluble proteins separated using their IEP in electrophoresis using an electric field and a pH gradient.

A protein stops migrating through the gel at its IEP in the **pH gradient because it has no net charge** and will **precipitate** out of solution.

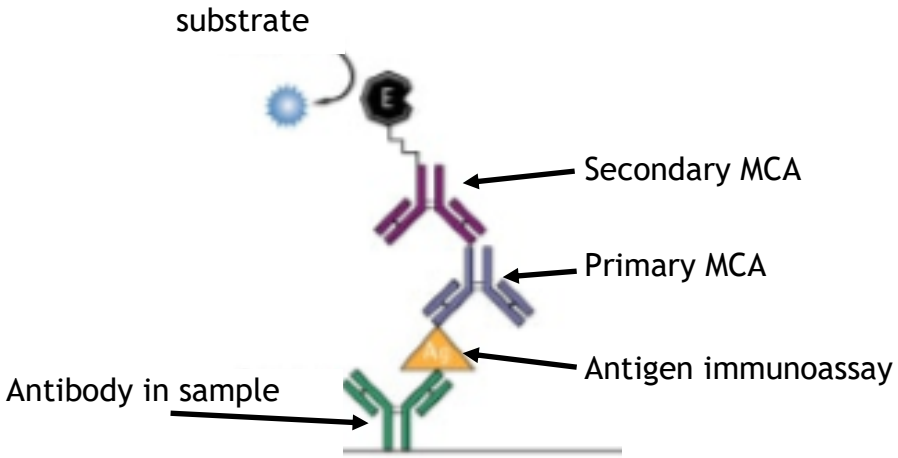
Buffers required:

Holds the pH constant despite adding small quantities of acid or alkali.



Laboratory Skills: Immuno assay

In some immunoassays, a **specific antigen** is used to detect the presence of antibodies rather than MCA's detecting specific antigens.



Western blotting

Allows a **single protein** to be identified from a **complex mix** using fluorescent labelled antibodies.

1. Separate a protein from a complex mix using **SDS-PAGE electrophoresis**

2. Probe for protein

The separated proteins from the gel are transferred (blotted) onto a solid medium

The proteins are identified using **specific antibodies** that have reporter enzymes attached.

Laboratory Skills: Immuno assay

Immunoassay techniques

Used to detect and identify specific proteins using monoclonal antibodies

Monoclonal antibodies

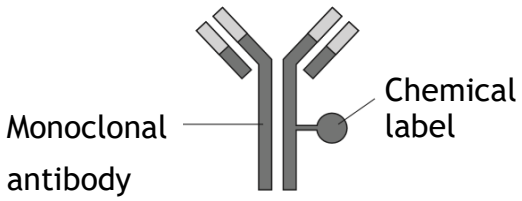
Stocks of antibodies with the same specificity to a particular antigen.

Immunoassay e.g. ELISA

A MCA specific to the protein antigen is linked to a chemical 'label' to detect the presence/concentration of protein in the sample.

Types of chemical labels

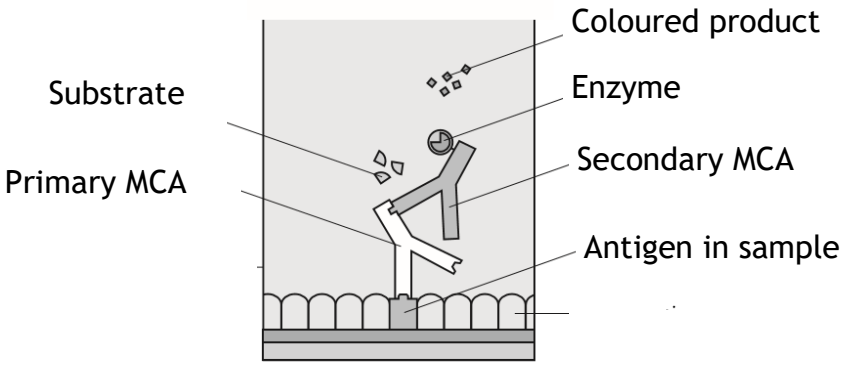
1. Reporter enzyme producing a colour change (ELISA technique)
2. Chemiluminescence Reporters
3. Fluorescence Reporters



Immunoassay

Used to detect the **presence/concentration** of antigen protein in a sample

Sometimes more than one MCA is used with the secondary MCA containing the chemical label.



Microscopy

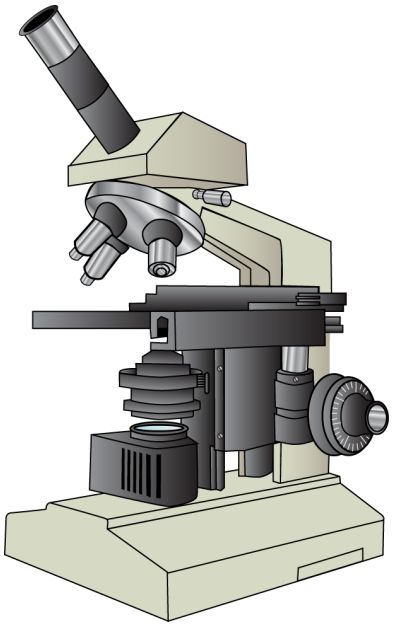
1. Bright Field Microscopy
   Allows the following to be viewed:

   (a) Whole organisms

   (b) Parts of organisms

   (c) Thin sections of dissected tissue

   (d) Individual cells



2. Fluorescence Microscopy

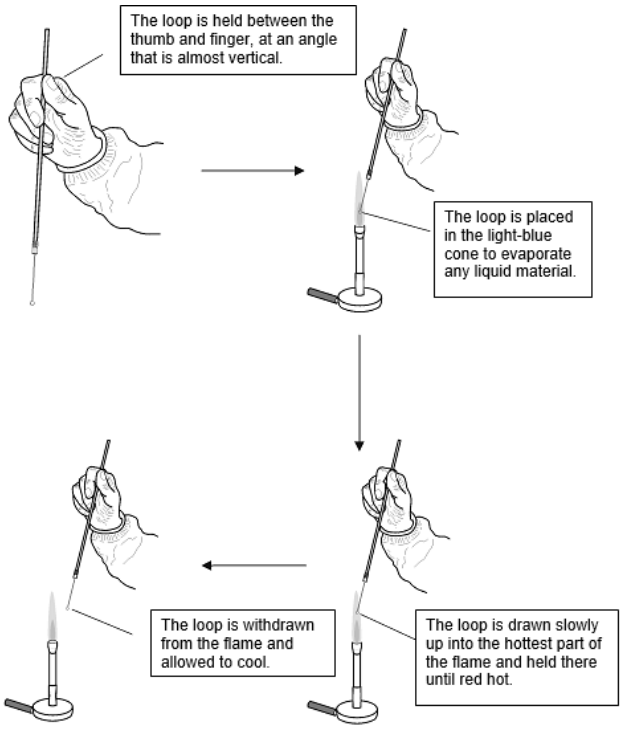
   Uses specific **fluorescent labels** to bind to and visualise certain molecules or structures **within cells/tissues.**

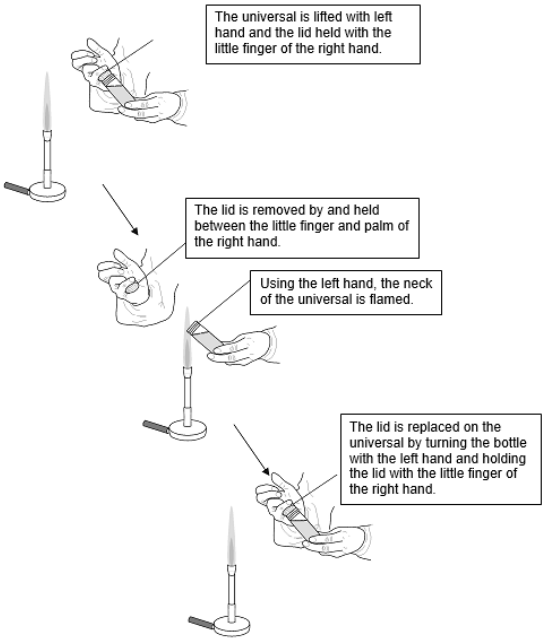
   Visualise structures that are **much smaller** in size (higher resolution/magnification).

Microbiology: Cell Culture & Aseptic Technique

Aseptic technique

Involves the **sterilisation** of equipment/culture media by **heat or chemical** means when carrying out cell culture experiments.





Why are aseptic techniques necessary?

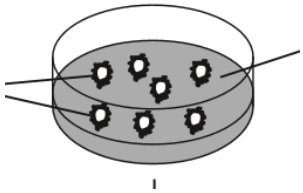
Eliminates unwanted **microbial contaminants** when culturing microorganisms /cells

Microobiology: Cell Culture & Aseptic Technique

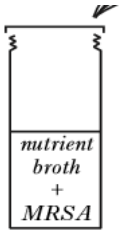
Types of Cell culture

A microbial culture can be started using an inoculum of microbial cells on:

1. Agar (solid media)



2. Broth (liquid media)



Nutrients in Cell Culture

Culture media contains suitable **nutrients** that promote the growth of **specific** cells/ microbes.

Example

Animal cells grown in media containing **growth factors** from **serum**.

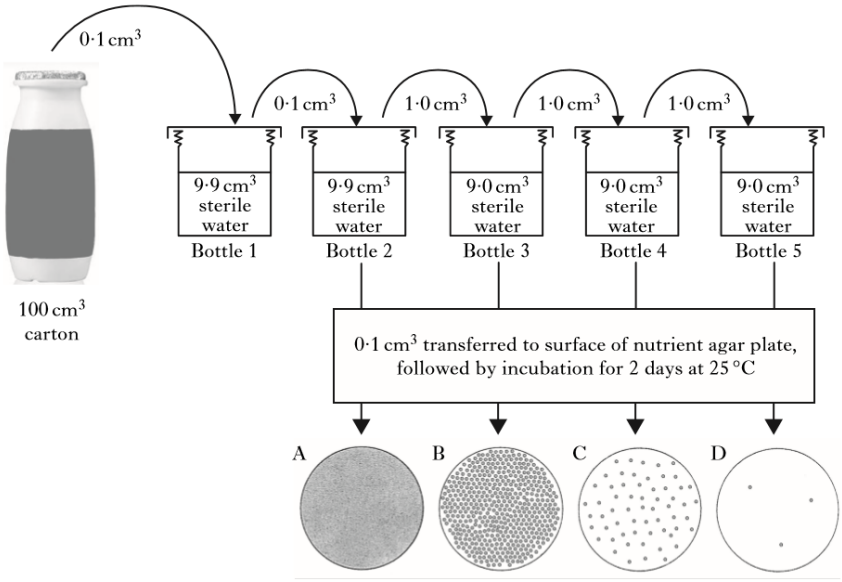
Growth factors are **proteins** that promote **cell growth** and **proliferation**.

Microbiology: Measuring Cell Growth

1. Colony forming units (CFU)

Plating out of a **liquid microbial culture** on solid media allows the number of CFU to be counted and the **density of cells** in the culture estimated.

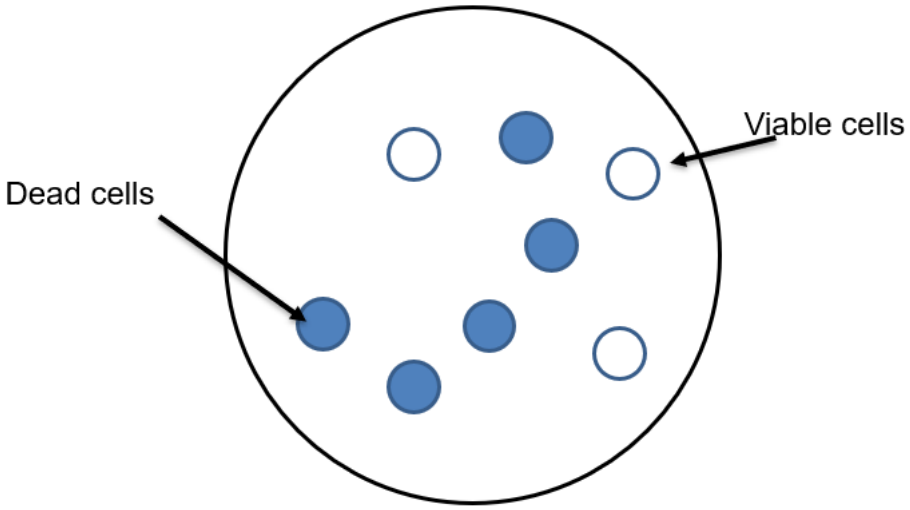
Serial dilution is often needed to achieve a suitable colony count.



Vital Staining of Cells

Measuring **viable** cell count.

Use of stains such as trypan/methylene blue that **only stain dead cells** as viable cells remove blue dye by active transport.



2. Haemocytometers

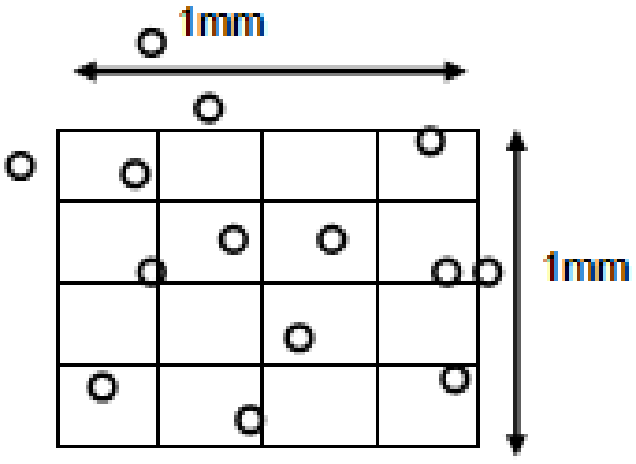
Use of haemocytometer to estimate cell numbers per cm^3 in a liquid broth culture.

Step 1 Work out volume of liquid placed in haemocytometer slide in cm^3.

Length x breadth x depth (remember if if mm divide by 10)

Step 2 Count number of cell colonies viewed in diagram (microscope).

Step 3 Proportion calculation to scale up per cm^3.



The Proteome

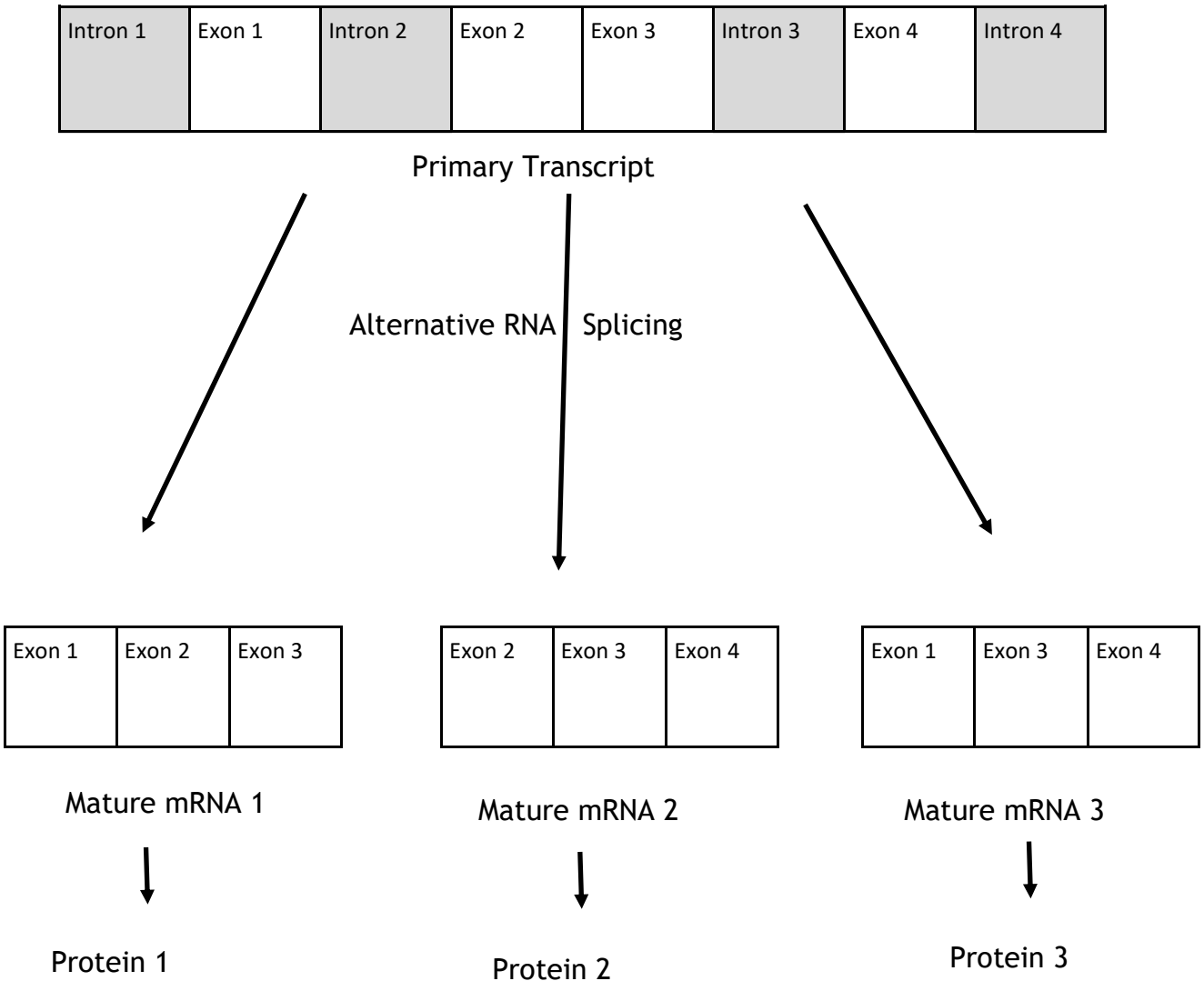
Proteome

Entire set of **proteins** expressed by a genome.

Proteome is larger than the genome particularly in eukaryotes as more than 1 protein produced per gene due to **alternative RNA splicing**.

Alternative RNA splicing

Introns removed and different combinations of exons make different mature mRNA's making different proteins.



The Proteome

The proteome (set of proteins) expressed by a cell can

1. Vary over time
2. Vary under different conditions such as :

    a) metabolic activity of the cell
    b) cellular stress
    c) diseased vs healthy cells
    d) response to signalling molecules

The Genome

Not all genes are expressed as proteins in a particular cell type.

Part of Genome	Function
Coding Genes	Code for specific proteins
Non Coding RNA genes	1. Transcribed to produce tRNA & rRNA 2. RNA molecules that control the ex-

Eukaryotic Internal Membranes

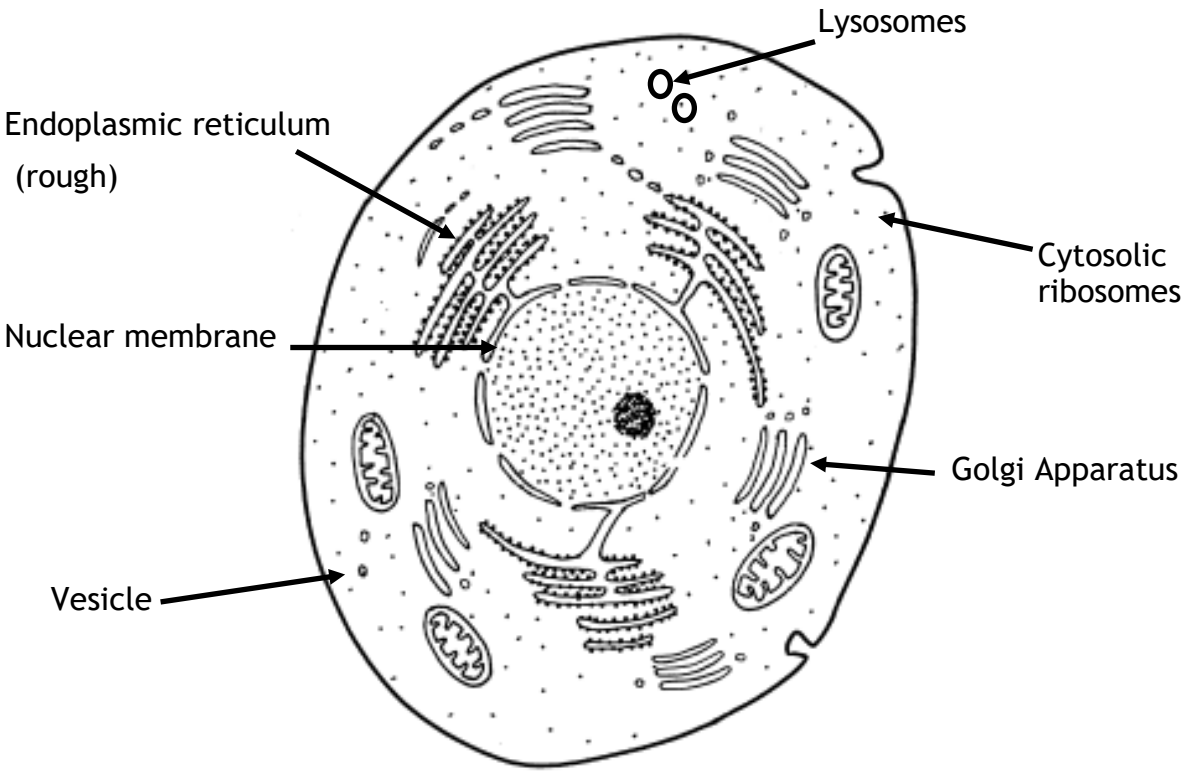
Eukaryotes have a relatively small surface area to volume ratio.

The plasma membrane of eukaryotic cells is therefore too small an area to carry out all the vital functions carried out by membranes.

Solution

System of internal membranes, which increases the total area of membrane

Type of Internal Membranes	Structure	Function
Endoplasmic Reticulum	Form a network of membrane tubules continuous with the nuclear membrane	Synthesis of lipids (SER) Synthesis of transmembrane proteins (RER)
Golgi Apparatus	Series of flattened membrane discs	Post translational modifications
Lysosomes	Membrane-bound organelles contain-ing a variety of hydrolases	Digestion of proteins, lipids, nucleic acids and carbohydrates
Vesicles	Membrane bound organelle that buds off ER/golgi	Transport materials between membrane compartments



Protein/Lipid Synthesis

Stage 1 : Protein synthesis

1. Begin synthesis in **cytosolic ribosomes.**
2. Carry a **signal sequence**, which halts translation and directs the ribosome synthesising the protein to dock with the ER, **forming RER.**

   A signal sequence is a short stretch of amino acids at one end of the polypeptide that determines the eventual location of a protein in a cell.

3. Translation continues after docking, and the protein is **inserted** into the membrane (lumen) of the RER

Stage 2: Movement to Golgi Apparatus

Once inside the ER, **vesicles** that bud off from the ER and fuse with the Golgi apparatus.

As proteins move through the Golgi apparatus, further **vesicles bud off** from one disc and fuse to the next one in the stack.

Enzymes in the golgi catalyse the addition of various sugars in multiple steps to **add a carbohydrate** to the protein as they undergo **post-translational modifications**

Step 3—Movement to plasma membrane/lysosomes

Vesicles that leave the Golgi apparatus move along **microtubules** to the **plasma membrane and lysosomes**.

Lipid Synthesis

Lipids are synthesised in the **smooth endoplasmic reticulum** (SER) and inserted in to its **membrane.**

Endoplasmic Reticulum

Secreted proteins

Translated in ribosomes on the RER and enter its lumen

↓

The proteins move through the Golgi apparatus and are then packaged into secretory vesicles

↓

These vesicles move to and fuse with the plasma membrane, releasing the proteins out of the cell

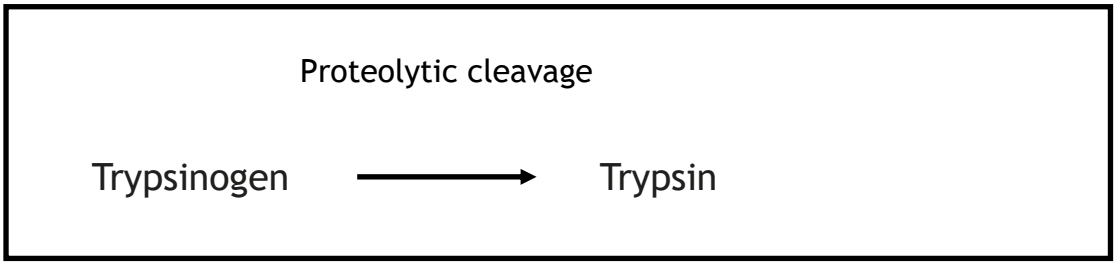
Examples of secreted proteins: peptide hormones and digestive enzymes

Proteolytic cleavage

Many secreted proteins are synthesised as **inactive precursors** and require proteolytic Cleavage to produce **active proteins**.

Proteolytic cleavage is another type of **post- translational modification**.

Example: Digestive enzymes



(inactive pre-cursor) (active enzyme)

Protein Structure

Proteins are polymers of amino acid monomers joined by peptide bonds to form polypeptide chains.

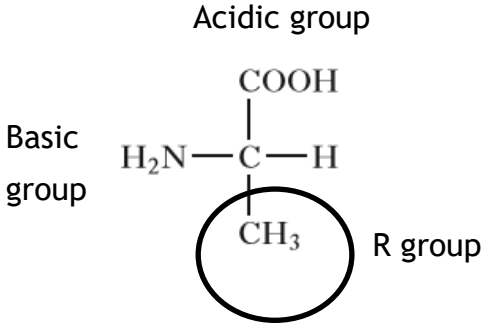
Amino Acid Structure

Amino acids have the same basic structure differing only in their R group.

The wide range of functions carried out by proteins results from the diversity of R groups

Differences in R groups

1. Size
2. Shape
3. Charge
4. Hydrogen Bonding capacity
5. Chemical reactivity



Type of Amino Acid	Hydrophilic/Hydrophobic	R group
Basic	Hydrophilic (+ve charge)	NH_3^+ group
Acidic	Hydrophilic (-ve charge)	COO^- group
Polar	Hydrophilic	OH group
Non Polar	Hydrophobic (no charge)	CH_3 group

Protein Structure

Primary Structure

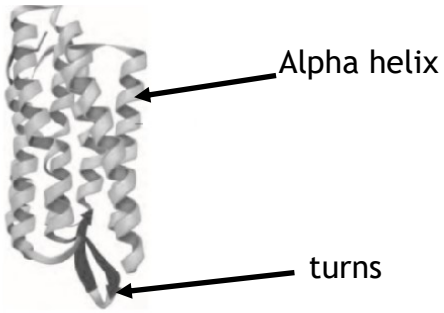
Sequence of amino acids in the polypeptide

Secondary Structure

Hydrogen bonding along the backbone of the protein strand results in secondary structure

Types of Secondary Structure

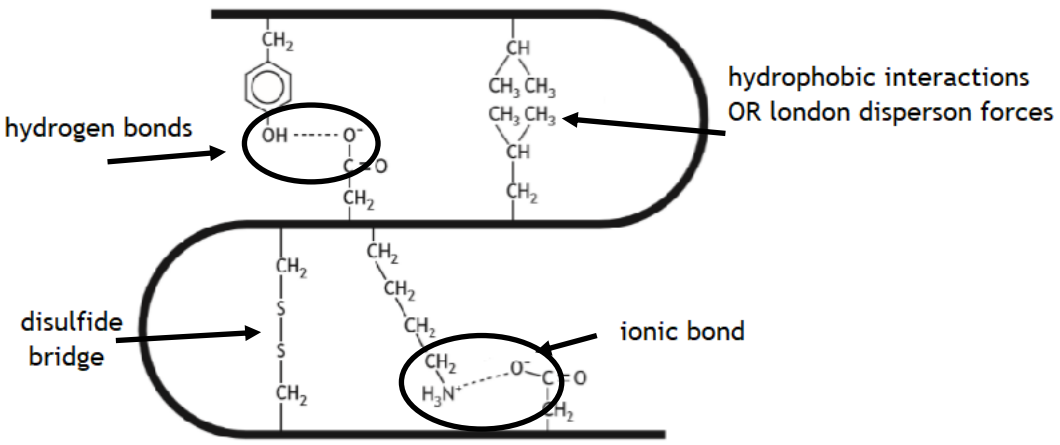
1. Alpha helices
2. Beta-pleated sheets (parallel/antiparallel)
3. Turns



Tertiary Stucture

Folding of polypeptide chain into 3D structure due to interactions between **R groups**

1. Hydrophobic interactions
2. Ionic bonds
3. London dispersion forces
4. Hydrogen bonds
5. Disulfide bridges (covalent bond between R groups containing Sulfur)



Ligand binding

A ligand is a substance that can **bind to R groups on proteins** not involved in protein folding.

Binding sites will have **complementary shape** and **chemistry** to the ligand

As a ligand binds to a protein-binding site the **conformation** of the protein changes

This change in conformation causes a **functional change** in the protein.

Quaternary structure

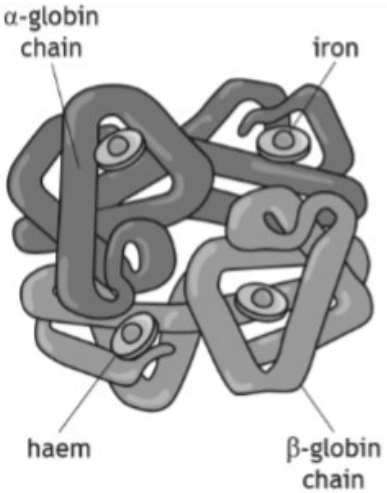
The **spatial arrangement** of the **subunits** in proteins with at least two connected polypeptide subunits

A prosthetic group

A **non-protein** unit tightly bound to a protein and necessary for its **function**

Example

Haemoglobin has 4 polypeptide sub-units with a prosthetic iron group required for oxygen binding to haemoglobin sub-units



haemoglobin

Denaturing Proteins: R Groups

Interactions of the R groups (tertiary level) can be influenced by temperature and pH.

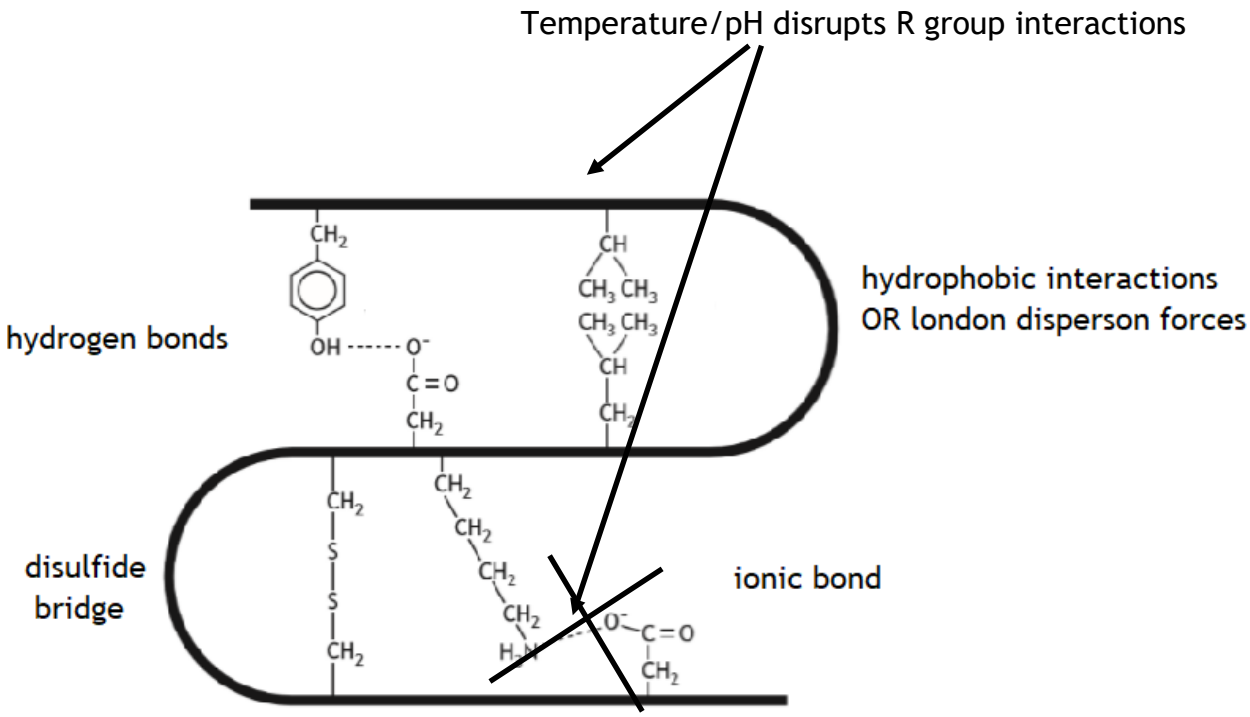
1. Increasing temperature

This disrupts the interactions that hold the protein in shape; the protein begins to unfold, eventually becoming denatured.

2. Altered pH from optimum

The **charges** on acidic and basic R groups are affected by pH.

As pH increases or decreases from the optimum, **ionic interactions** between charged groups are lost, which gradually changes the conformation of the protein until it becomes denatured



Allosteric Protein Reactions

Allosteric proteins

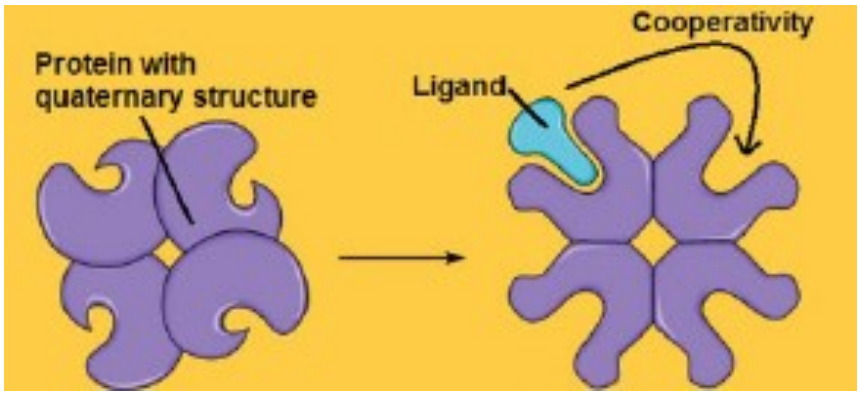
consist of multiple subunits (have quaternary structure) that have **allosteric interactions**

between **spatially distinct** sites

Allosteric proteins show co-operativity in binding.

Co-operativity

Changes in binding at one subunit alter the affinity of the remaining subunits



Allosteric Enzymes

The binding of a substrate molecule to one active site of an allosteric enzyme increases the affinity of other active sites for binding of substrate molecules.

The activity of allosteric enzymes can vary greatly with small changes in substrate concentration.

Allosteric Protein Reactions

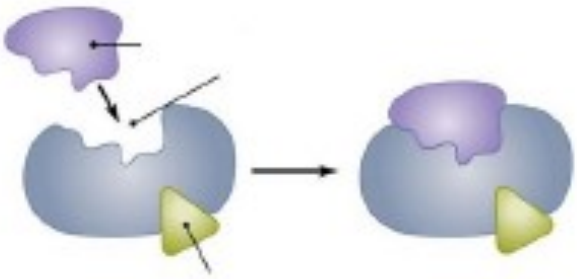
Allosteric Enzymes

Contain a **second binding site** called the allosteric site **spatially away** from the active site.

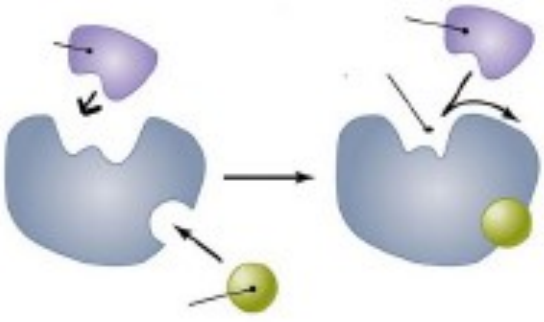
Modulators via the following process

1. Modulators bind to the allosteric site
2. The conformation of the enzyme changes
3. The affinity of the enzyme for the substrate increases (positive modulator).
   The affinity of the enzyme for the substrate decreases (negative modulator).

Positive Modulator



Negative Modulator

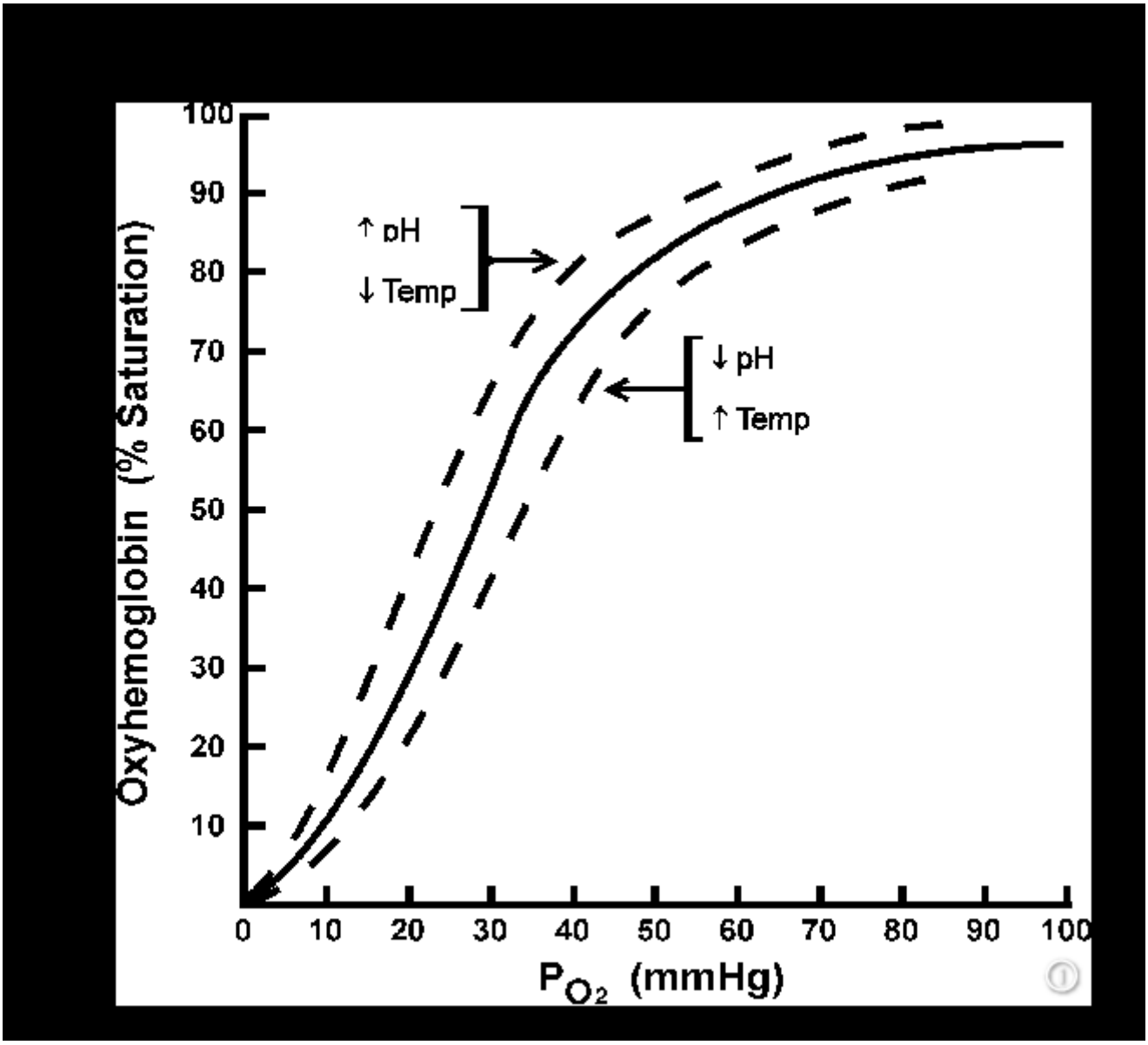


Haemoglobin dissocation Curve

Factors lowering affinity of haemoglobin for oxygen (rightward shift on graph)

1. Lower pH
2. Higher temperature

This promotes **increased oxygen delivery** to tissues.



Phosphorylation/Dephosphorylation

The addition/removal of phosphate can cause **reversible conformational change** in proteins.

Adding a phosphate group adds **negative charges**. **Ionic interactions** in the unphosphorylated protein can be disrupted and new ones created.

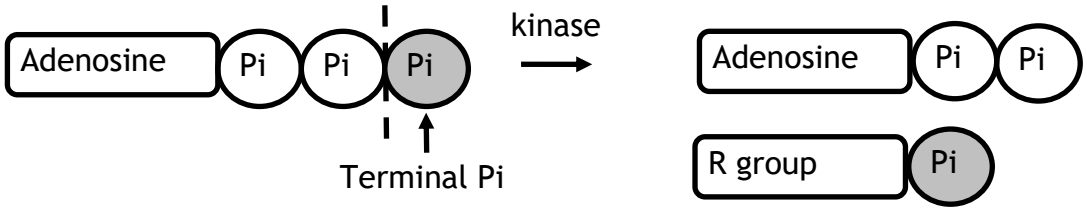
Phosphorylation can **activate/inhibit** proteins such as **enzymes/ receptors**.

This is a common form of **post-translational modification**

Two types of Enzymes

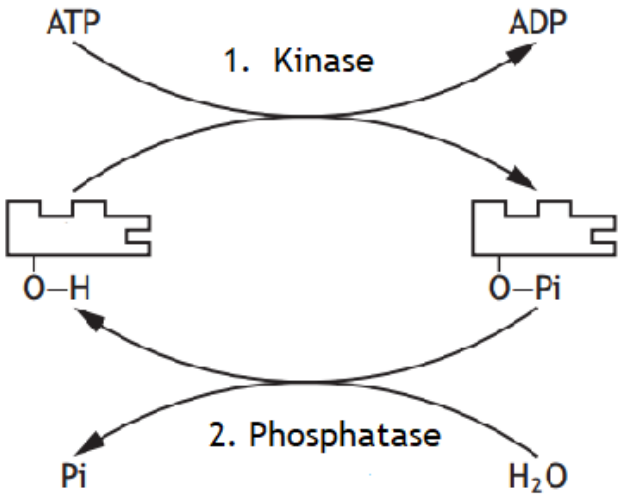
1. Protein kinases
   Catalyse the transfer of a phosphate group to other proteins

   The **terminal phosphate** of ATP is transferred to **specific R groups** on proteins



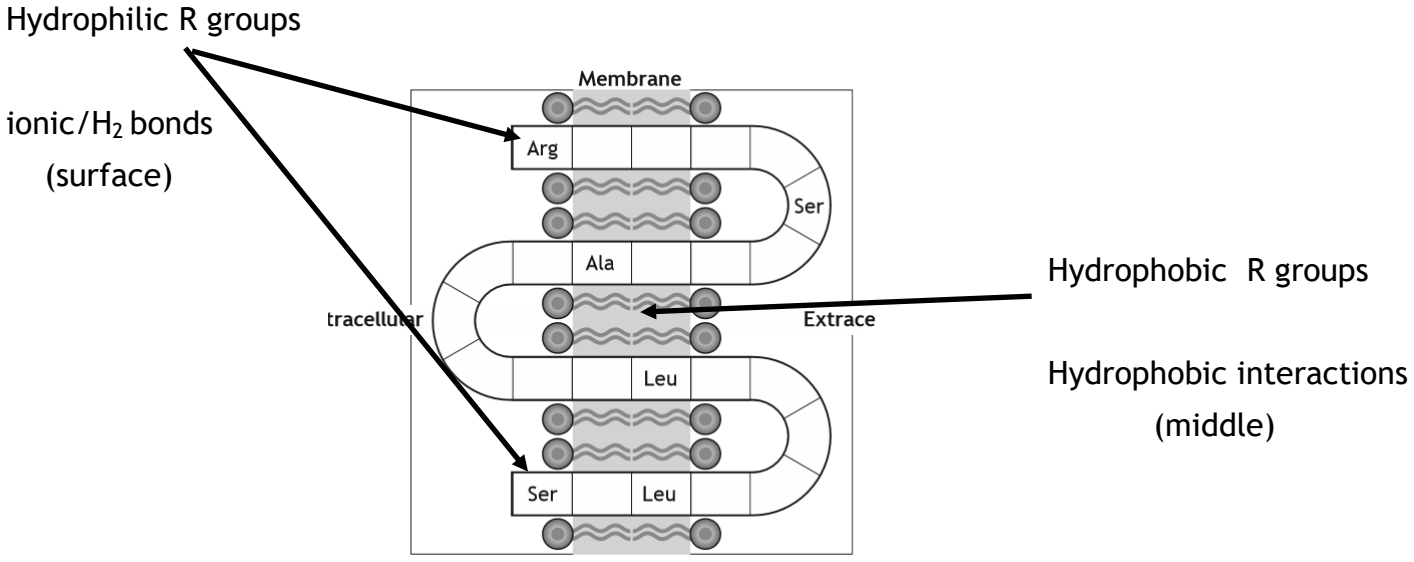
2. Protein phosphatases

   Catalyse the removal of a phosphate group from a protein.

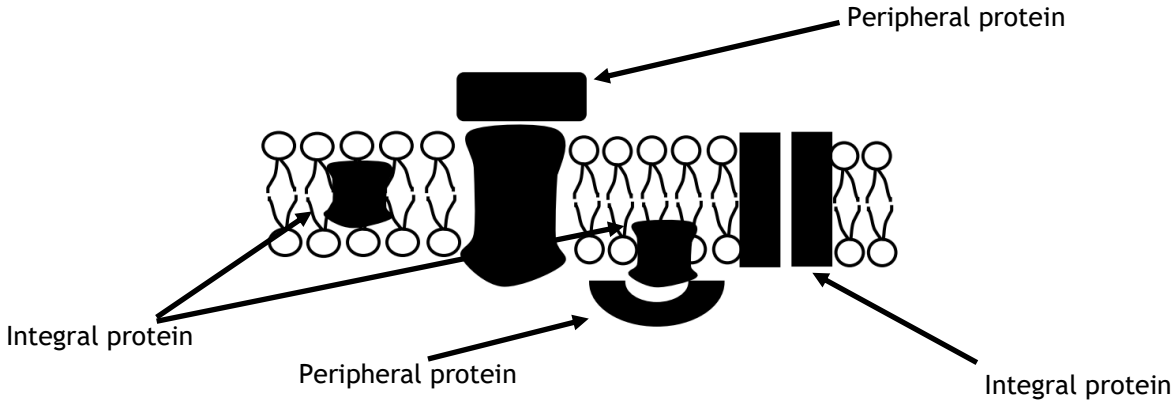


Integral/Peripheral Proteins

Type of Membrane protein	Description	Type of R groups on protein	Type of interactions
Integral	Held within the phospholipid bilayer Some are transmembrane	Hydrophobic R groups in middle of protein	Strong hydrophobic interactions
Peripheral	Bound to surface of membrane	Hydrophilic R groups at surface	Ionic or hydrogen bonds



Types of membrane protein

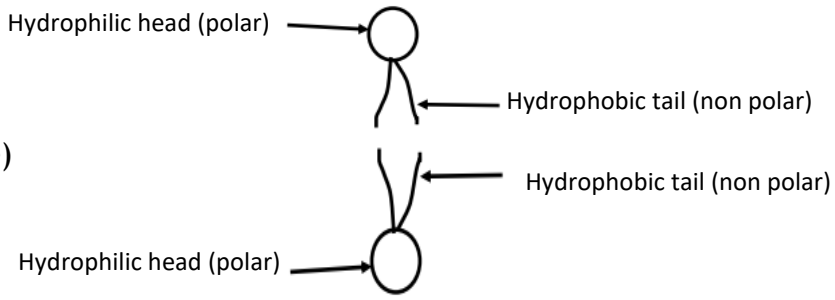


Many peripheral membrane proteins interact with the surfaces of integral membrane protein

Transport across the Membrane

Cell membrane

Made up of a phospholipid bilayer



1. Hydrophilic head (outer/inner edge)
2. Hydrophobic tail (middle)

Movement of Molecules across membrane

Phospholipid bilayer acts as a **BARRIER** across the membrane to **MOST** molecules.

STOPS

1. **Large uncharged (non polar) molecules**
2. **Ions/polar molecules**

GO

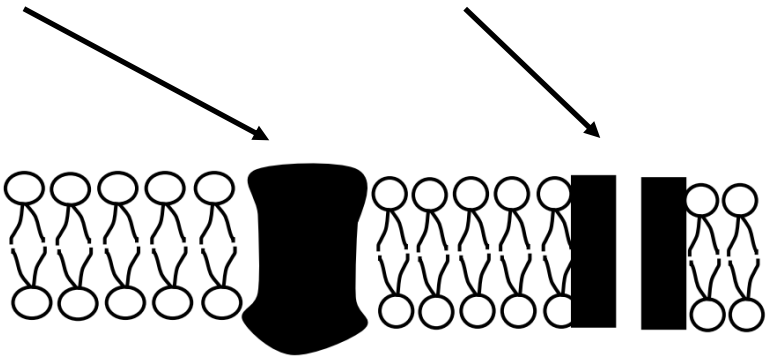
Some **small non polar** molecules (O_2/CO_2) can squeeze in between the phospholipid bilayer heads, diffusing across the membrane.

Facilitated Diffusion

Passive transport of substances across the membrane through specific **transmembrane** proteins

Transmembrane proteins

1. Transporters
2. Channel proteins



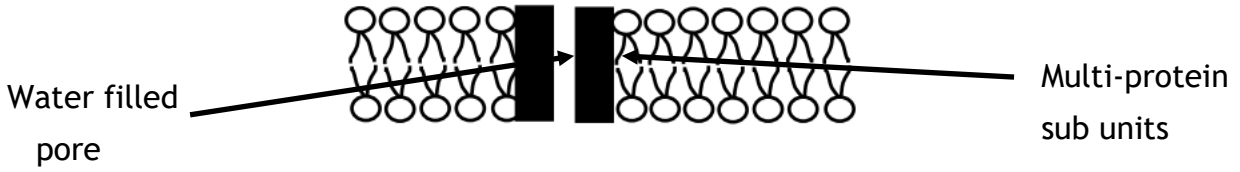
To perform **specialised functions**, different cell types have **different** channel & transporter proteins

Transport across the Membrane

Transmembrane protein	Process	Example of protein
Channel	Passive (diffusion)	Simple channels Ligand & Voltage gated channels
Transporter	Active transport Facilitated Diffusion	Sodium Potassium Pump Glucose Symport

Transmembrane Channel Proteins

Multi-subunit proteins with the subunits arranged to form **water-filled pores** that extend across the membrane



Function of channel proteins

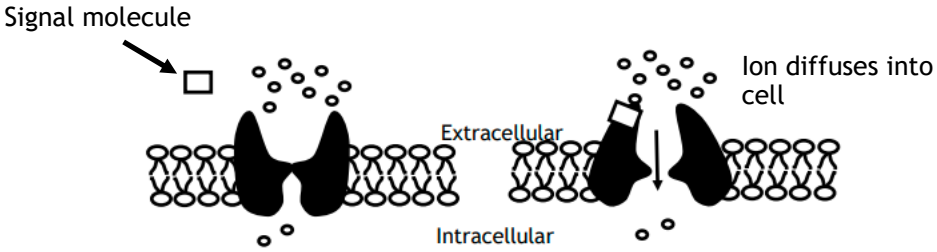
To move molecules across the membrane by **diffusion.**

Gated Channel Proteins

These channel proteins **change conformation** to allow/prevent **diffusion**,

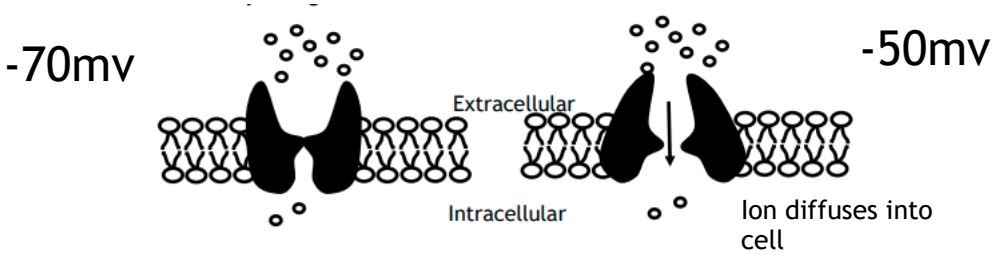
1. Ligand-gated channels

   Controlled by the binding of **signal molecules**. **Ion moves In/out cell by diffusion.**



2. Voltage gated channels

   Controlled by changes in **ion concentration** as this affects the **membrane potential**. **Ion moves In/out cell by diffusion.**



Transport across the Membrane

Transporter proteins

Operate between **two conformations** to move substances across the membrane.

1. A **specific** substance binds to the transporter protein
2 This results in a **conformational change** in the transporter protein
3. This releases the substance on the other side of the membrane

Pumps

One type of transporter proteins that is coupled to an **energy source** to enable **active transport.**

ATPases

Protein pumps that **directly hydrolyse ATP** to provide the energy for the conformational change required to move substances by active transport.

Example Na K ATPase

Na K ATPase/Pump

Function

Na/K pump establishes both **concentration gradients** and an **electrical gradient** (membrane potential) within the cell.

Energy Cost of Pump

The pump uses **energy directly** from ATP hydrolysis(ATPase) for active transport.

This accounts for a high proportion of the basal metabolic rate

Stages of Na K ATPase

1. In the **unphosphorylated stage**, the pump has **high affinity** for **Na^+ ions** and 3 Na^+ ions bind to the pump inside cell.

2. **Phosphorylation by ATP** causes a **conformation change** of the pump which lowers the affinity for Na^+ releasing **$3Na^+$** ions outside of the cell

3. **2 K^+ ions** bind outside the cell in the **phosphorylated state** as they have high affinity for the pump in the phosphorylated state.

4. **Dephosphorylation** causes a **further conformation change** which lowers the affinity of K^+ ions and 2 K^+ ions are released inside the cell

5. The pump returns to its original conformation.

Glucose Symport

Location—small intestinal epithelial cells

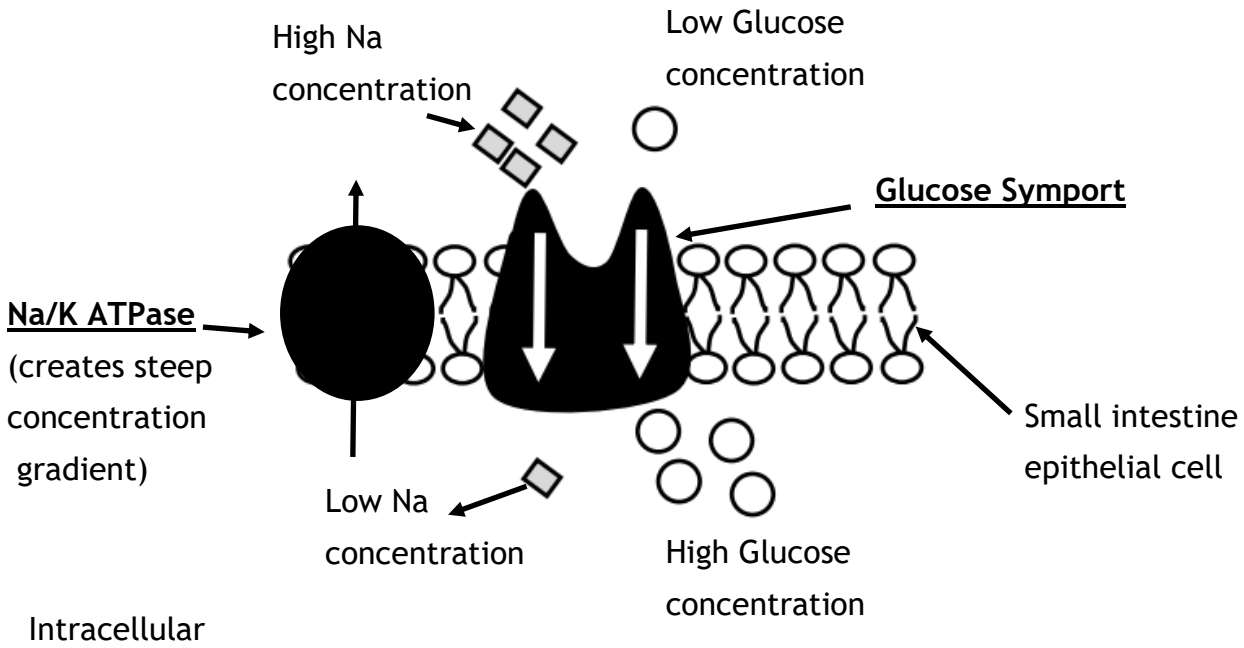
Glucose & Na^+ ions are transported **INTO** the cell at the same time via the glucose transporter.

1. Na^+ ions move in by **diffusion**

2. Glucose moves in by **active transport.**

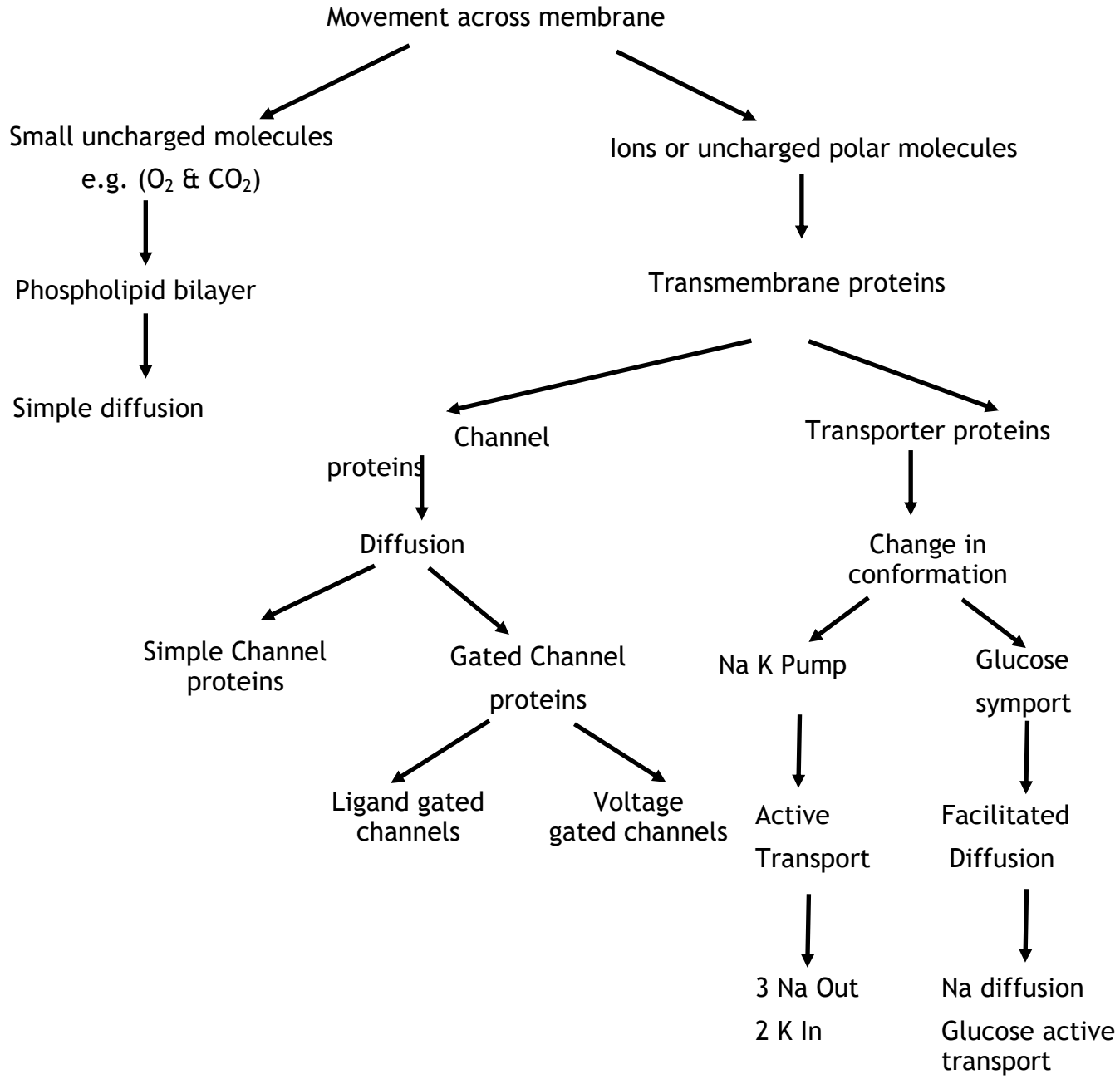
The **steep concentration gradient** of Na^+ due to the **Na K pump** causes the the simultaneous transport of glucose into the cell against its concentration gradient.

Extracellular



Intracellular

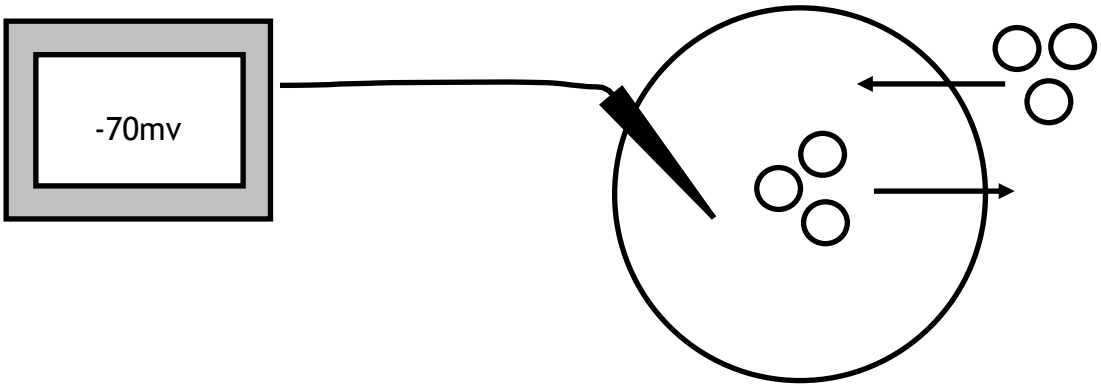
Transport across the Membrane



Nerve Impulse Transmission

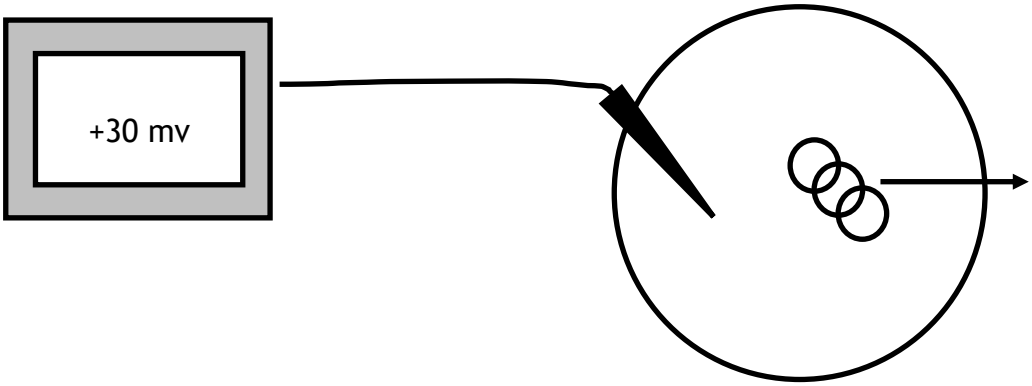
Resting membrane potential

A state where there is **no net flow** of ions across the neuron's plasma membrane.



Nerve Impulse Transmission

Change in the membrane potential of the neuron's plasma membrane.

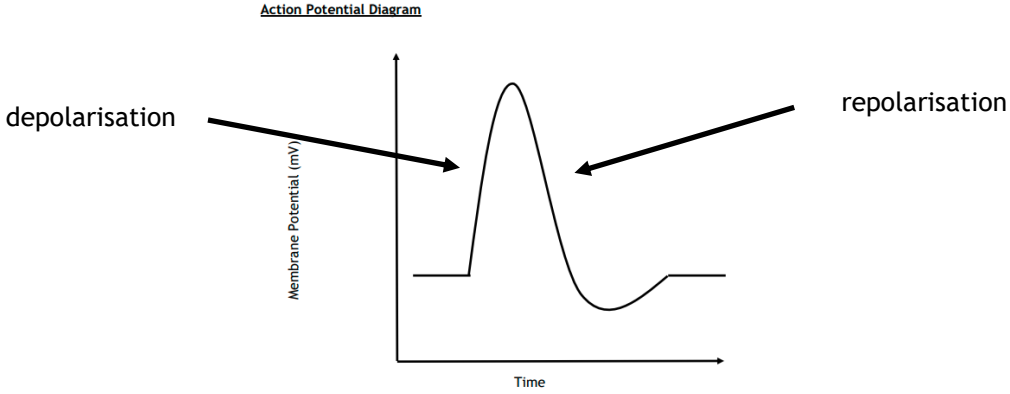


Depolarisation

Change in the membrane potential to a **more positive** value inside the neurone.

Repolarisaton

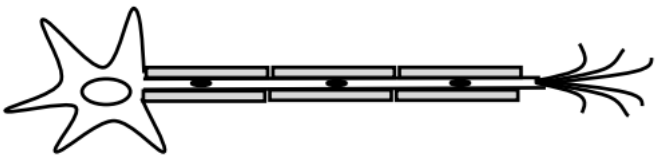
Change in the membrane potential to a **more negative** value inside the neurone .



Nerve Transmission

Action potential

Wave of electrical excitation along a neuron's plasma membrane.

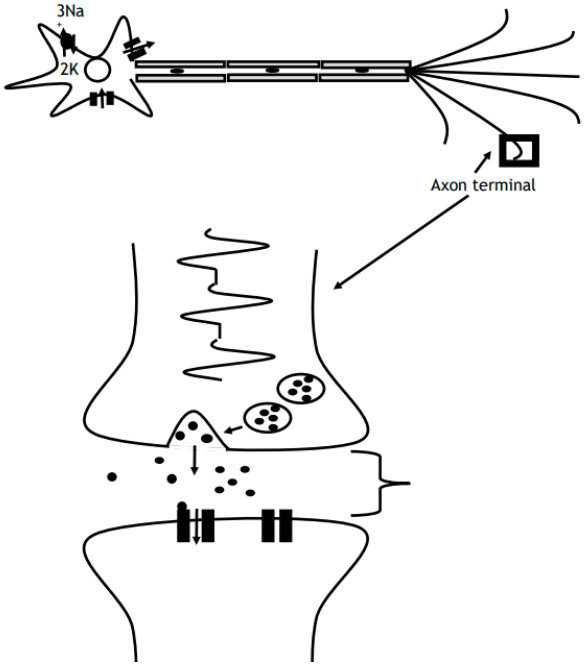


Pre/Post Synaptic Neurone

When the action potential reaches the end of the neurone, **vesicles** containing **neurotransmitters** fuse with the **pre-synaptic** membrane.

This releases neurotransmitter into the **synapse**.

The neurotransmitter will then bind to its receptor at the post synaptic neurone which is a **lig-** **and gated** channel.



Breakdown of neurotransmitter

To **prevent continual stimulation** of the neurone it is important to break down the neurotransmitter by

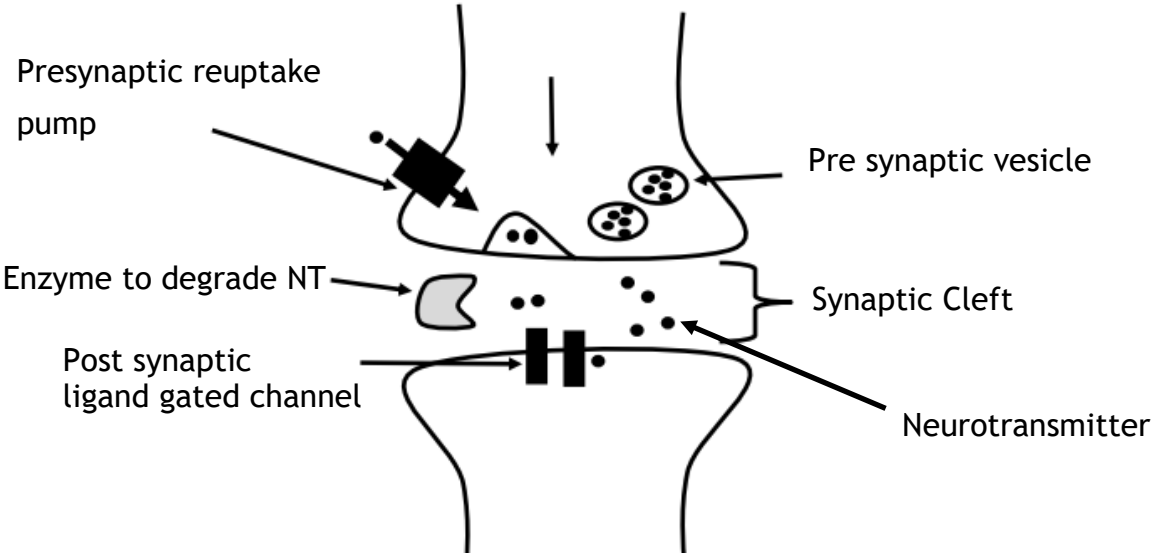
1. **Enzyme degradation** in synapse e.g. acetylcholinesterase

Nerve Transmission

Breakdown of neurotransmitter

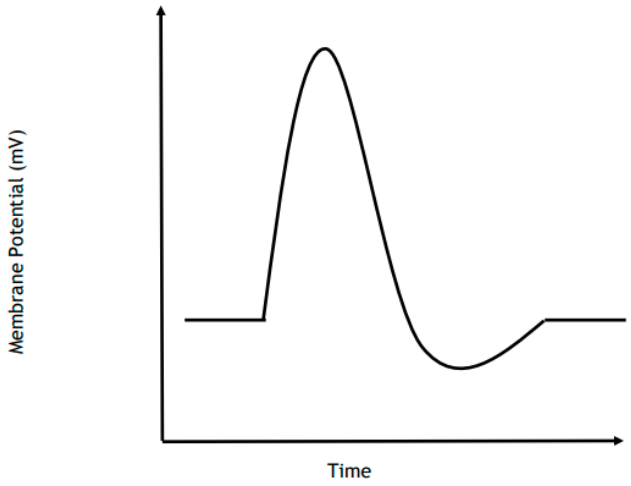
To **prevent continual stimulation** of the neurone it is important to break down the neurotransmitter by

1. **Enzyme degradation** in synapse e.g. acetylcholinesterase
2. **Reuptake** into **pre synaptic** neurone via a pump/vesicle



Nerve Transmission

Action Potential Diagram



1. Neurotransmitters bind to their receptors (**ligand gated** ion channels) at the post synaptic membrane resulting in **entry of Na^+** ions (depolarisation of the plasma membrane).

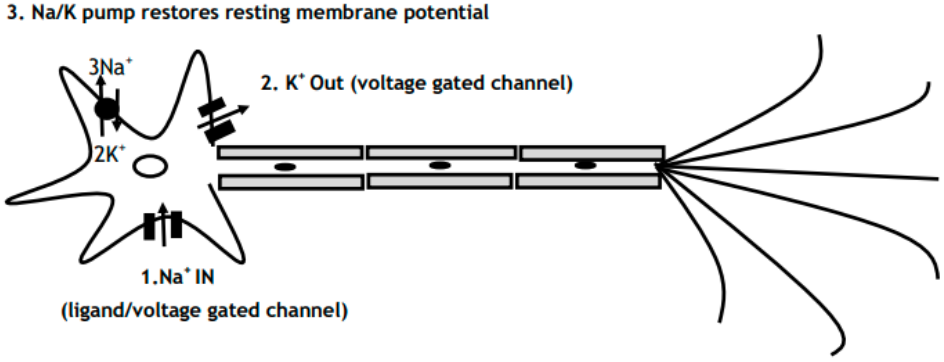
2. If **sufficient Na^+ ion movement** occurs, the membrane is depolarised beyond a threshold value (-55mv).

3. This triggers the opening of **voltage gated Na channels** causing much more Na^+ ions to enter the cell down their electrochemical gradient rsulting in a large rapid depolarisation of the membrane potential.

4. Na^+ channels close and **voltage gated K^+ ions open (**+30mv), causing **K^+ ions** to **leave** the cell , restoring the resting membrane potential (repolarisation).

5. This allows inactive voltage gated $Na^=$ channels to return to a conformation that allows them to open again in response to a further depolarisation.

6. Following repolarisation, K^+ and Na^+ ion concentration gradients are restored to the resting membrane potential due to the **Na-K pump**.



The vertebrate Eye: Nerve Transmission

Retina

Area of the eye that **detects light** via **rod** and **cone photoreceptor** cells:

1. Rod Photoreceptors

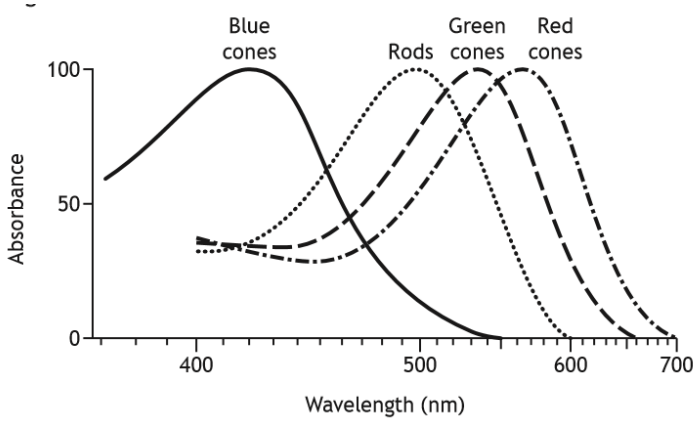
   Function in **dim light** but do not allow colour perception

   Adaptation— A **very high** degree of **amplification**

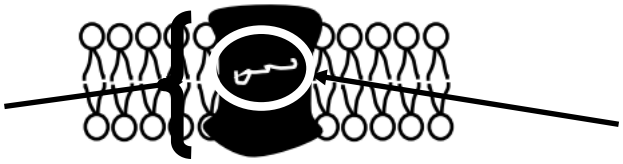
2. Cone Photoreceptors

   Enable **colour vision** but only function in bright light.

   Adaptation—**Different forms of opsin** enable different photoreceptor cells to have a maximal sensitivity to **red, green, blue or UV wavelengths (birds)**.



` Structure of Animal Photoreceptors



In rod cells the retinal-opsin complex is called **rhodopsin**

Function of Rhodopsin

When a photon of light is absorbed, a nerve impulse is generated.

The vertebrate Eye: Nerve Transmission

Nerve Transmission Mechanism in Rod Cones

Retinal absorbs a photon of light and rhodopsin changes conformation to form a single **photoexcited rhodopsin**

↓

A **cascade of proteins amplifies** the signal

↓

Photoexcited rhodopsin activates **hundreds** of **G protein transducing**

↓

This activates **hundreds of phosphodiesterase** (PDE) enzymes per second

.

↓

PDE catalyses the hydrolysis of **thousands of cyclic GMP** (cGMP) per second

↓

This **closes ion channels** in the membrane of rod cells.

↓

Nerve impulse triggered in retinal neurones

Nerve Transmission Summary

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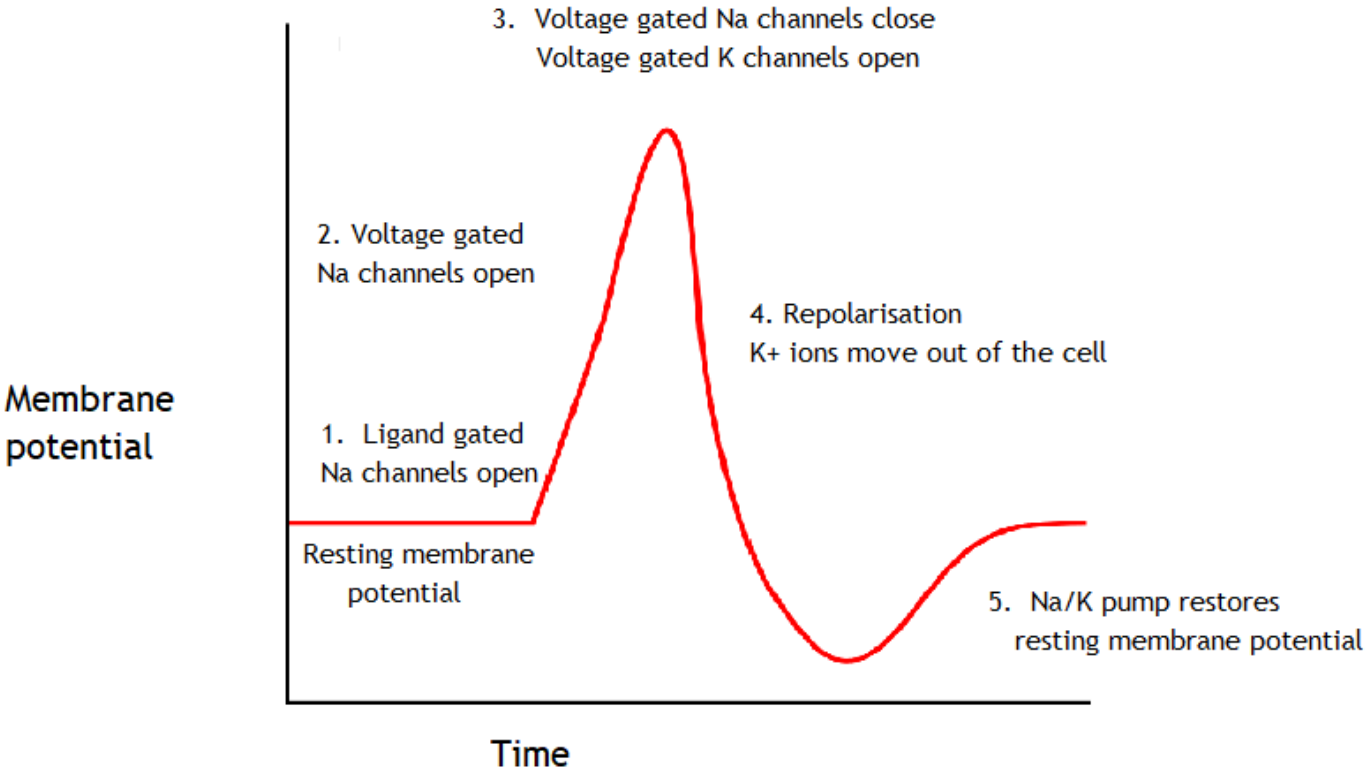


Communication & Signalling

Multicellular organisms

Cells in multicellular organism communicate using **extracellular signalling** molecules

Signal Molecule Examples

1. Steroid Hormones (testosterone & oestrogen)
2. Peptide Hormones (insulin)
3. Neurotransmitters

General Mechanism

1. Receptor molecules are **proteins** found on the surface of the plasma membrane or within the cytosol/nucleus of the target cell.

2. These receptors have a binding site for a **specific** extracellular signal molecule

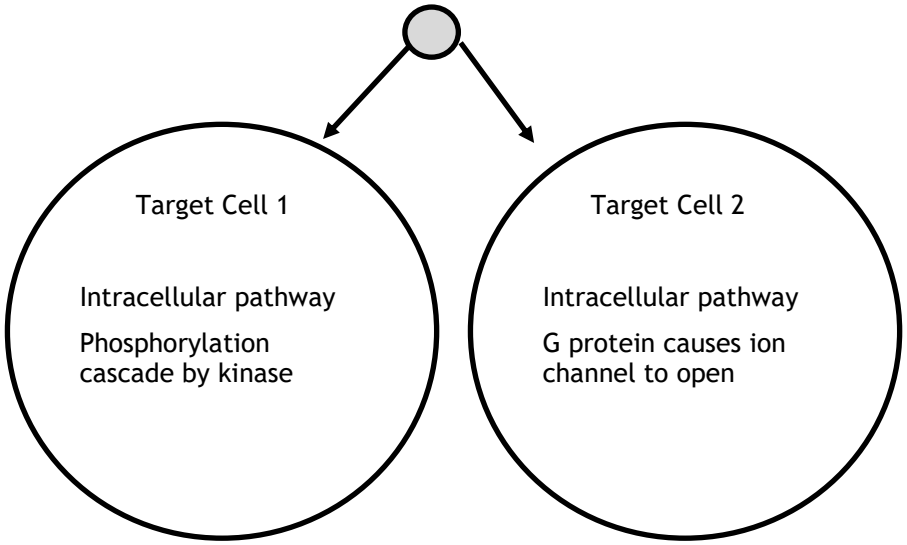
3. The binding of the signal molecule (ligand) changes the **conformation** of the receptor

4. This initiates an **intracellular response** within the cell.

Type of Signalling Molecule	Molecule can pass through membrane	Location of receptor	Examples of extracellular signal
Hydrophilic	no	Plasma membrane	Peptide hormones (insulin) Neurotransmitters
Hydrophobic	yes	Cytosol or nucleus	Steroid Hormones (testosterone and oestrogen)

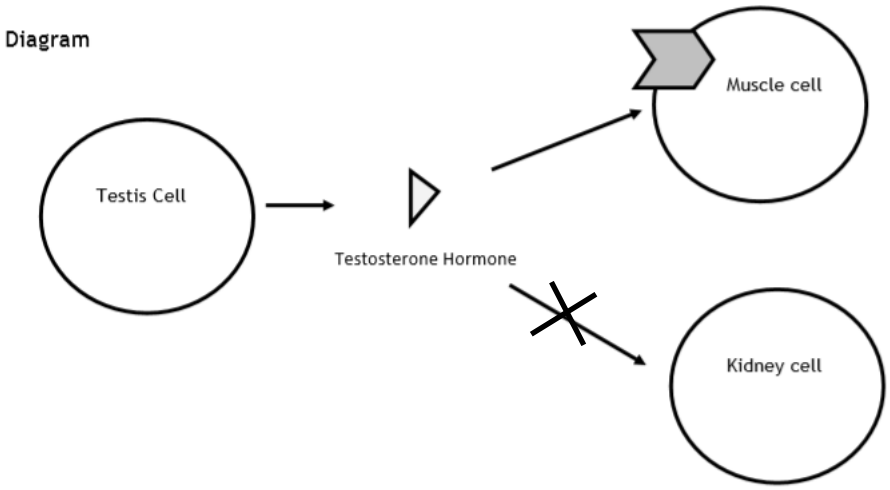
Tissue Specific Response

The **same signal** may have **different effects** on different target cell types due to differences in the **intracellular signalling molecules/pathways** .



Target vs Non Target Cells

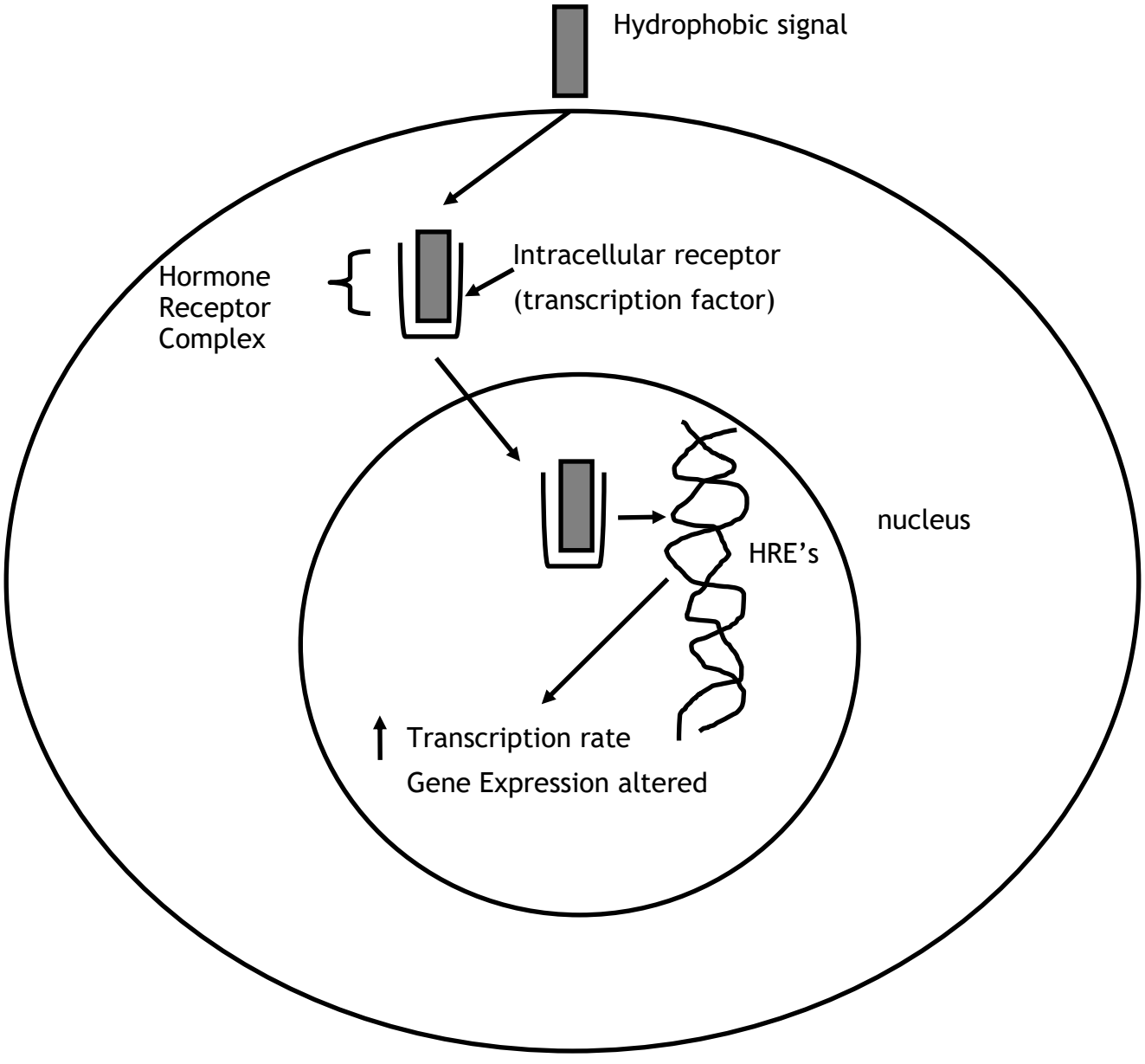
Different cells produce specific signals that can only be detected and responded to by cells with the **specific receptor**



Hydrophobic Signalling

Extracellular hydrophobic signals (steroid hormones oestrogen/ testosterone)

1. Hydrophobic signalling molecules can diffuse directly through the phospholipid bilayers of membrane
2. They bind to specific intracellular receptors (transcription factors) found in the cytosol/nucleus forming the hormone receptor complex.
3. The hormone-receptor complex moves to the nucleus where it binds to specific sites on DNA called hormone response elements (HREs).
4. This influences the rate of transcription affecting the gene expression of many different genes.



Hydrophilic Signalling

Extracellular hydrophilic signals (peptide hormones/neurotransmitters)

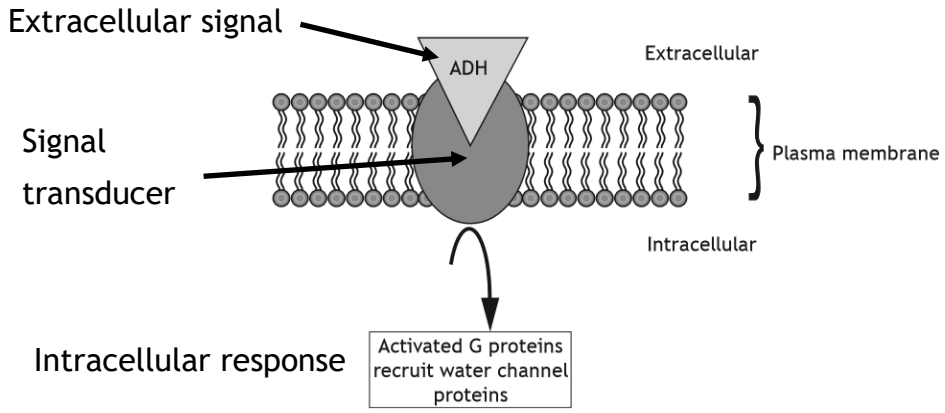
1. These signal molecules (ligands) **cannot enter the cytosol** but instead bind to the extracellular face of transmembrane surface receptors, changing their conformation.

2. The activated transmembrane receptors then act as a **signal transducers** by converting the extracellular ligand-binding event into intracellular signals i.e. transducing the signa;/

3. These intracellular signals involve **G proteins** or **cascades of phosphorylation** by kinases

   (a) G protein intracellular Mechanism

   G-proteins relay signals from activated receptors to target proteins (enzymes/ ion channels)



   (b) Phosphorylation cascade Intracellular Mechanism

   One kinase activates the next in the sequence through phosphorylation. These cascade result in

   (i) Phosphorylation of many proteins as a result of the original signalling event.

   (ii) Activation of more than one intracellular signalling pathway

Hydrophilic Signalling: Insulin and Diabetes Mellitus

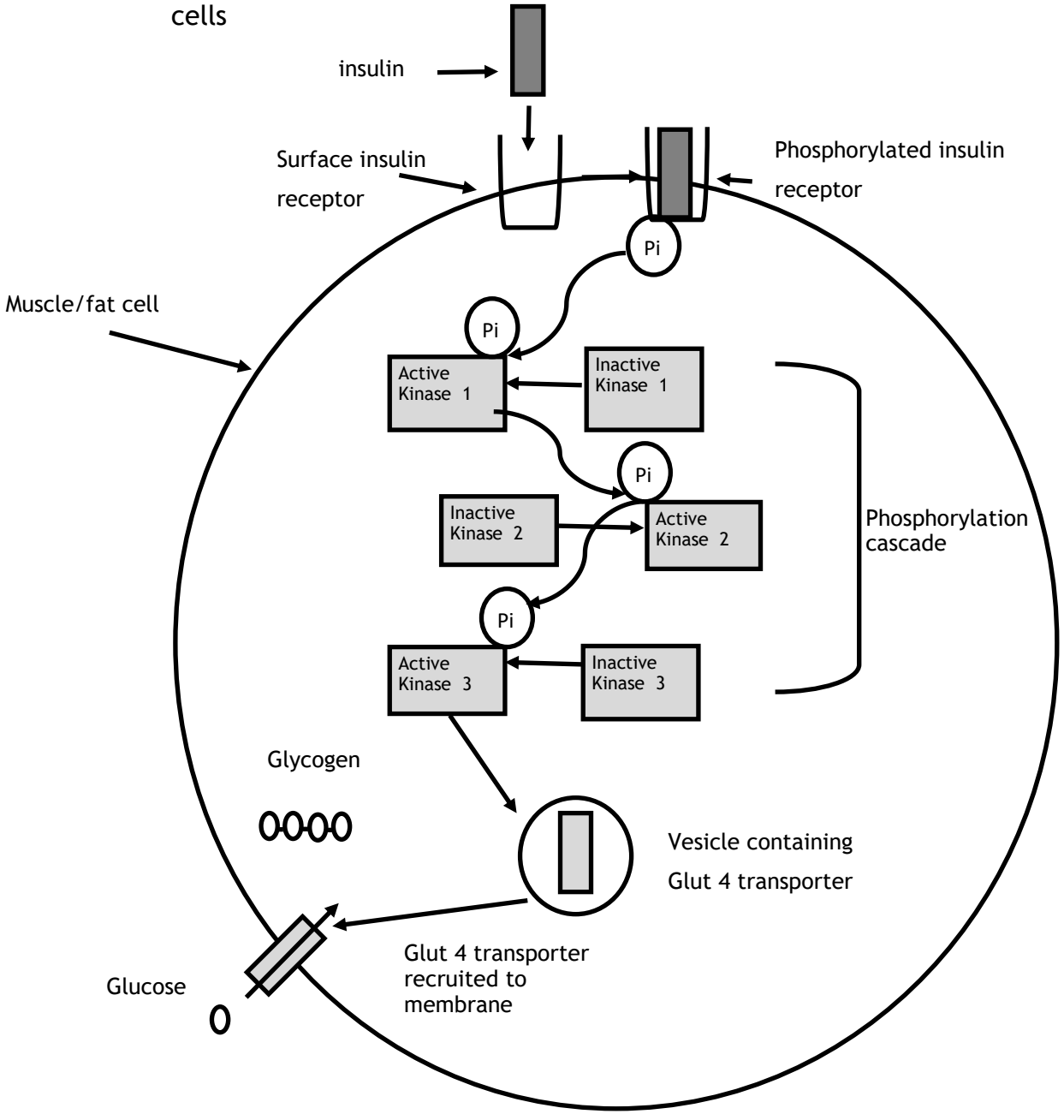
Insulin Signalling Mechanism

1. Insulin cannot enter the cytosol but instead binds to the extracellular face of its **transmembrane surface insulin** receptor on muscle/fat cells.

2. This causes a **conformational change** in the insulin receptor that triggers a **phosphorylation cascade** as the intracellular response.

3. The insulin receptor is phosphorylated and acts as a kinase by phosphorylating the next kinase in the series of reactions.

4. This phosphorylation cascade eventually causes **GLUT4 glucose transporter proteins** contained within vesicles being transported to the cell membrane of fat and muscle cells



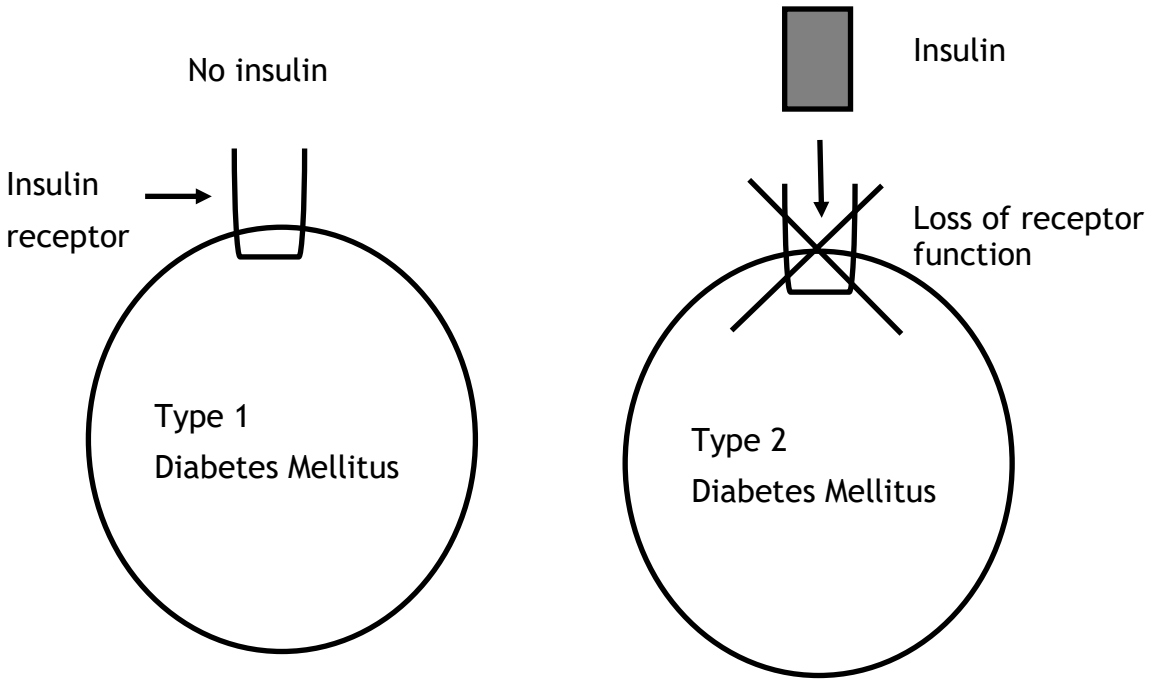
Hydrophilic Signalling: Insulin and Diabetes Mellitus

Diabetes mellitus

Type 1
Failure to produce insulin

Type 2
Loss of receptor function
Generally associated with obesity

Solution
Exercise triggers recruitment of GLUT4 improving glucose uptake in fat/ muscle cells

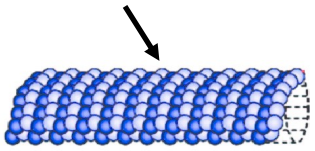


The Cytoskeleton

The cytoskeleton provides **mechanical support** and **shape** to cells.

It consists of different **protein** structures including **microtubules**, which are found in all eukaryotic cells.

Tubulin protein



Microtubules Structure

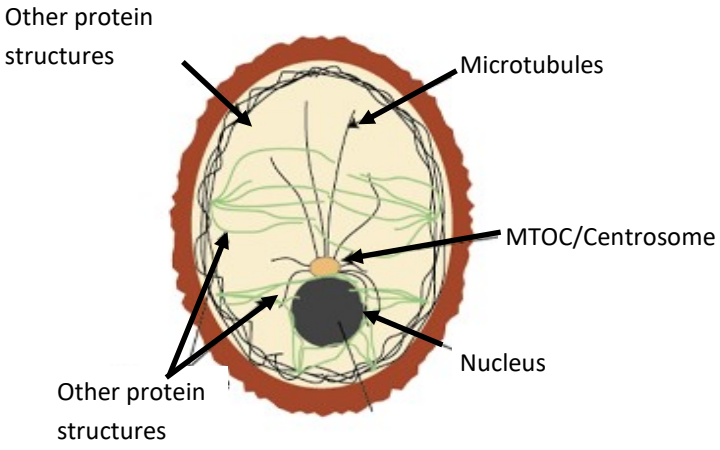
Hollow cylinders composed of the protein **tubulin**.

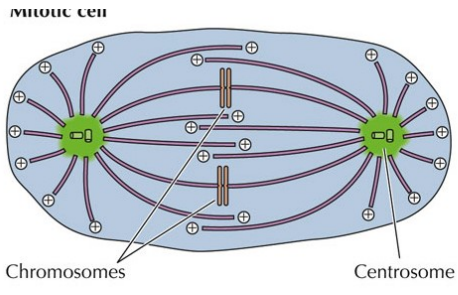
Microtubules Function

1. Control the movement of **membrane-bound organelles.**

2. Control the movement of **chromosomes** though formation of **spindle fibres** that are active during cell division.

Microtubules radiate from the **microtubule organising centre** (MTOC)/ **centrosome** near the nucleus.



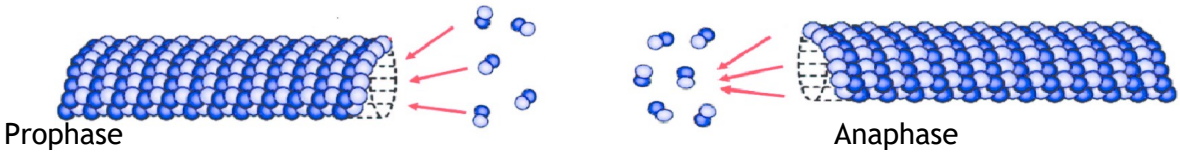


Cell Division & cytoskeleton

Cell Division requires **remodelling** of the cytoskeleton through **polymerisation.** and **depolymerisation** of **tubulin** in the formation/ break down of **spindle fibres**.

Formation of Spindle fibres (polymerisation)

Breakdown of Spindle fibres (depolymerisation)



Prophase

Anaphase

Likely applied knowledge Question

Chemotherapy cancer drugs such as colchicine and paclitaxel are designed to destroy microtubule formation in tumour cells that show an increase rate of cell division.

Disadvantage

Cell division in normal cells also inhibited OR cannot move organelles within cell.

Other drugs interfere with another stage of the cell cycle such as cisplatin which prevents successful DNA replication.

The Cell Cycle

The Cell Cycle

Consists of **interphase** and **mitotic phase**.

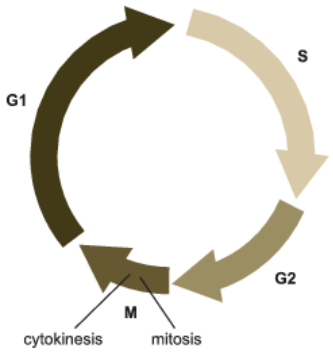
a) Interphase

This Phase involves growth of the cell and DNA synthesis.

- G1 - Growth phase

- S - DNA replication

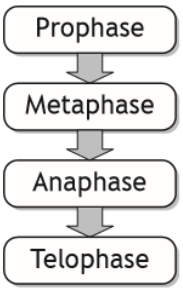
- G2 - Second growth phase



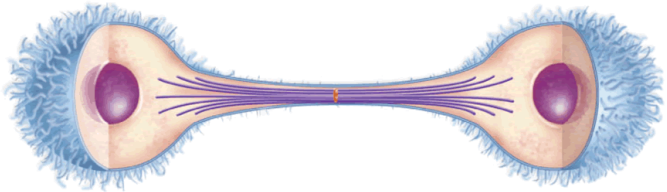
Cell cycle

b) Mitotic (M) Phase

1. Mitosis — The chromosomal material is separated by the spindle fibres in a **4 stage** process.



2. Cytokinesis — The **cytoplasm** is **separated** into two daughter cells.



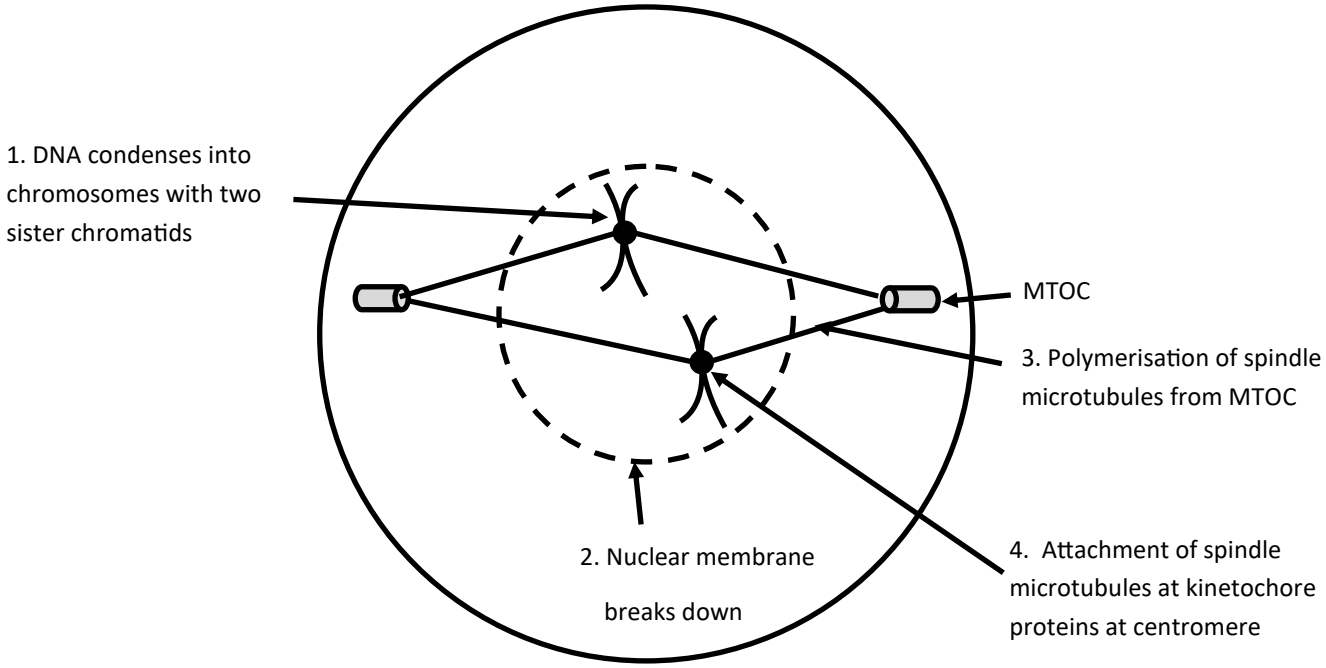
Stages of Mitosis

1.Prophase

a. DNA **condenses** into chromosomes each consisting of two **sister chromatids**.

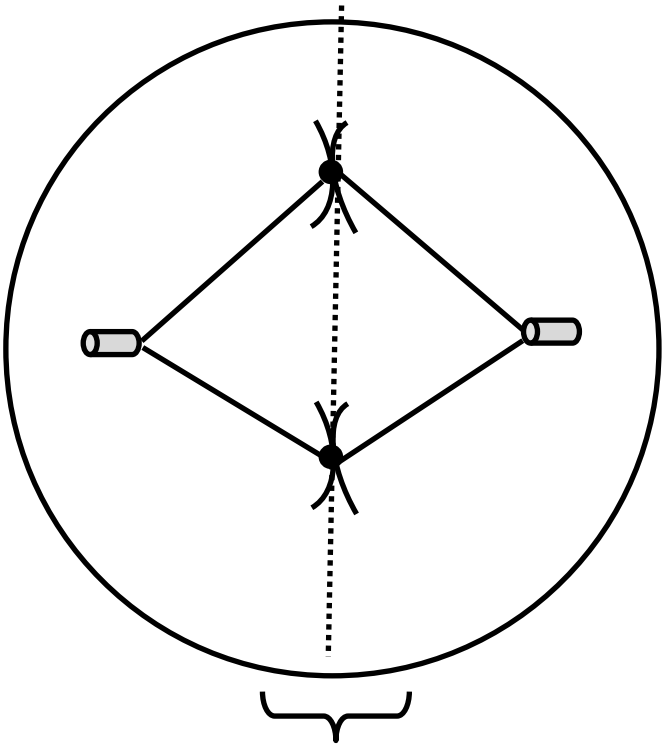
b. The **nuclear membrane** breaks down.

c. **Spindle microtubules** extend from the MTOC by **polymerisation** attach to chromosomes via their **kinetochores** in the centromere region.



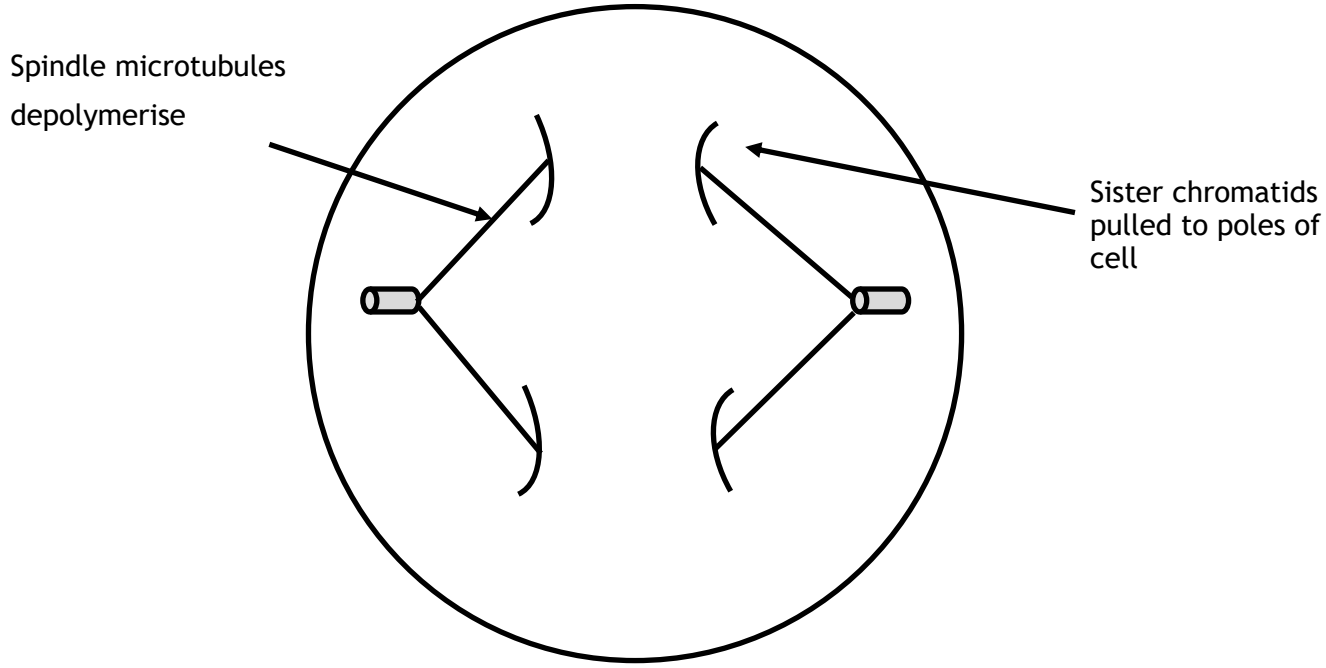
2. Metaphase

Chromosomes are aligned at the **metaphase plate** (equator of the spindle).



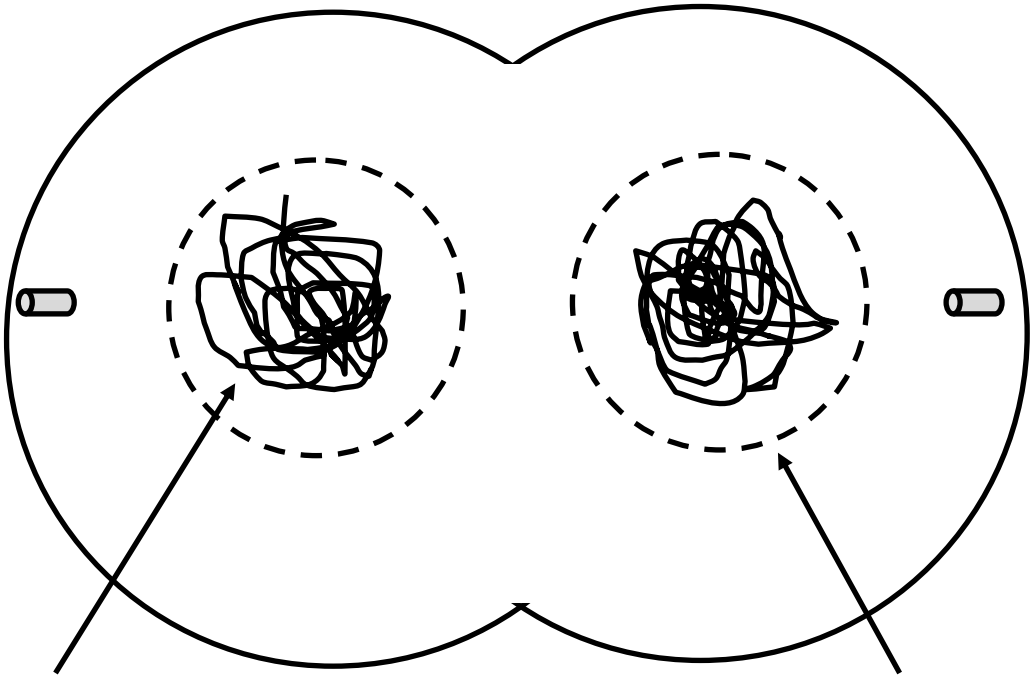
3. Anaphase

Spindle microtubules shorten by **depolymerisation**, separating **sister chromatids** which are pulled to **opposite poles**.



4. Telophase

The chromosomes **decondense** and **nuclear membranes** are formed around them.



Cell Cycle Checkpoints

Three Cell Cycle Checkpoints

Mechanisms within the cell that assess the condition of the cell during the cell cycle and **halt progression** to the **next phase** until certain requirements are met.

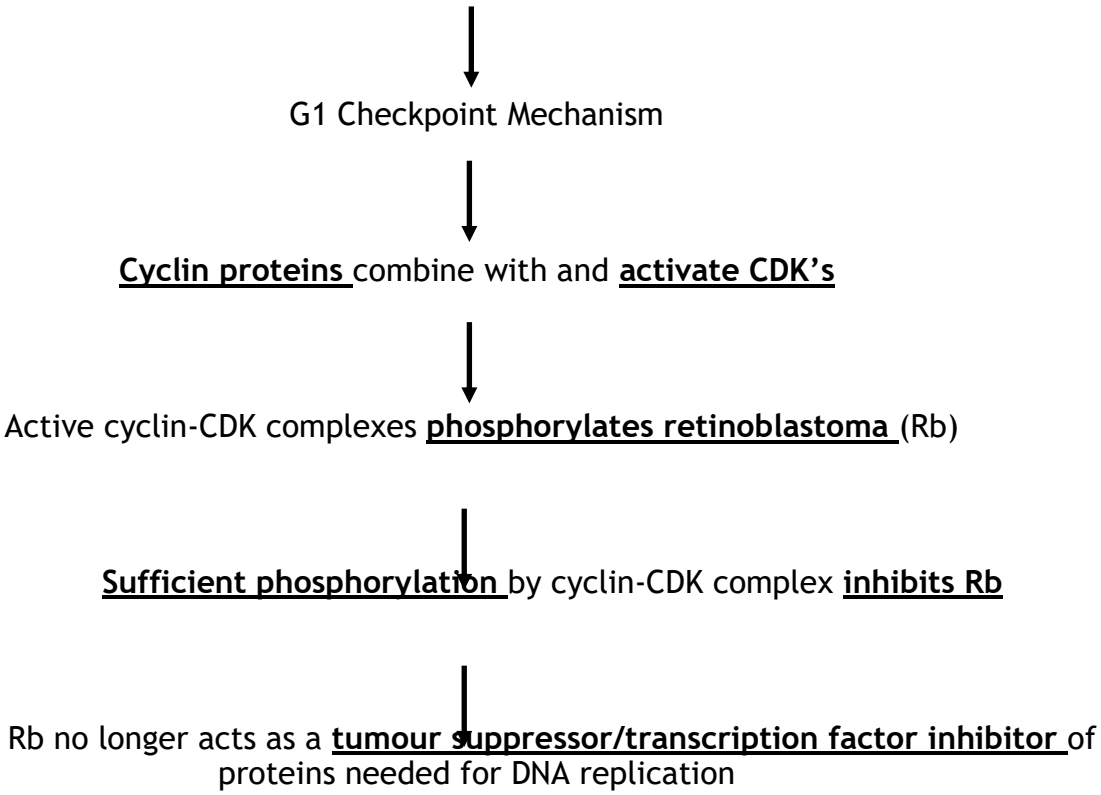
Name of Checkpoint	Cell Checkpoint	Halts entry before
G1 checkpoint	Assesses cell size through sufficient cyclin proteins that accumulate during **cell growth**	S phase
G2 Checkpoint	Success of **DNA replication** & any **DNA damage** is assessed.	M phase
M Checkpoint	Checks whether chromosomes have aligned correctly on the **metaphase plate** and attached to the spindle microtubules	Anaphase

Cyclin proteins

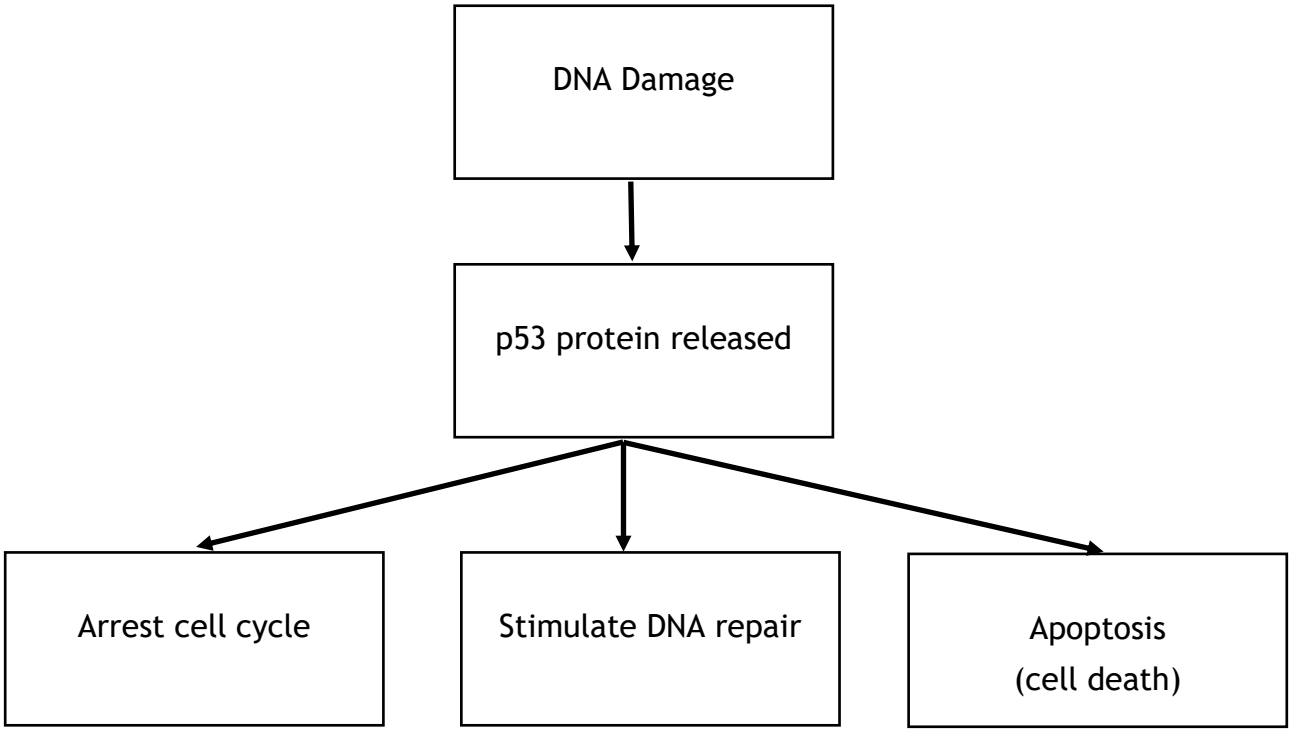
Accumulate during **cell growth** and are involved in **regulating** the cell cycle.

Cyclins combine with and activate **CDK's**.

Active cyclin-CDK complexes **phosphorylate specific proteins** that regulate progression through the cycle. If **sufficient phosphorylation** is reached, progression occurs.



DNA Damage & p53



Cell Cycle Rate

1. **Uncontrolled reduction** in the rate of the cell cycle
   - may result in **degenerative disease.**

2. **Uncontrolled increase** in the rate of the cell cycle
   - may result in **tumour formation.**

Proto-oncogene → Onco gene

Normal gene
Involved in **cell growth/division**

Mutated gene
Promotes **tumour formation**

Apoptosis

Apoptosis

1. External Cell death signal - **Lymphocytes**

2. Internal Cell death signal — **DNA damage/absence of growth factors (GF)**

.

External death signal molecules

1. **Lymphocyte** releases cell death signal
2. This signal molecule bind to a **surface receptor** protein
3. A **protein cascade** is triggered within the cytoplasm
4. Resulting in activation of **caspases (proteases)** that cause destruction of the cell

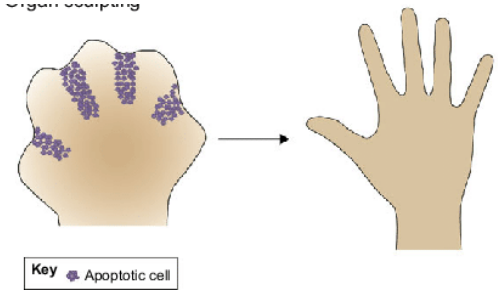
Internal death signal

1. DNA damage/absence of GF activates **p53 tumour suppressor protein.**
2. Resulting in activation of **caspases** that cause destruction of the cell

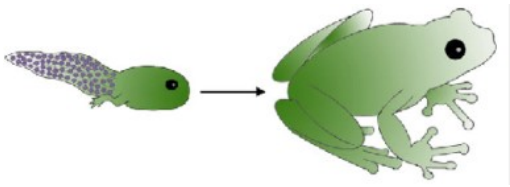
Importance of Apoptosis

Remove cells no longer required during the process of **development/metamorphosis**

Organism development



Metamorphosis



Health & Safety, Solutions, Microscopy & Immunoassay

Health and Safety

1. **Define the terms Hazard/risk/control measures in terms of risk assessment .**

   Hazard

   Harm caused to an individual when working in a laboratory

   Risk

   Likelihood of harm arising from exposure to a hazard

   Control measures

   Measures to reduce risk

2. **State two examples of hazards.**

   Toxic/*corrosive chemicals/flammable substances OR heat/pathogenic organisms /mechanical equipment*

3. **List the three types of control measures.**

   Appropriate handling technique/Protective clothing/equipment/Aseptic technique

Solutions

1. **State the form of dilution that differs by an equal interval.**

   Linear dilution

2. **State the form of dilution that differs by a constant proportion**

   for example 10^{-1}, 10^{-2}, 10^{-3}

   Logarithmic dilution

3. **State which type of separating technique separates substances based on density.**

   Centrifugation

4. **Explain the difference in densities between the pellet and supernatant.**

   Pellet solid at bottom higher density

   Supernatant liquid at top lower density

5. **Explain why In colorimeter a colourless solution called a blank is used before taking an absorbency measurement.**

   To ensure calibration of measurement equipment for accurate results.

6. **State what an absorbance measurement of a coloured solution is an prediction of in colorimeter.**

   Concentration

7. **State the effect on transmission if solution B has a higher turbidity than solution A**

   Transmission reading decreases with increasing turbidity

8. **State the name for the technique of plotting measured values for known concentrations to predict the concentration of an unknown to be determined.**

   Standard curve/line

Separating Substances & Cell Culture

Separating Substances

1. **State the three types of chromatography.**

   Paper, thin layer and affinity.

2. **State which types of chromatography separate substances based on their solubility.**

   Paper & thin layer

3. **Explain how soluble target proteins are separated from non target proteins in affinity chromatography.**

4. **State what charged macromolecules moving through an electric field applied to a gel matrix is a definition of.**

   Gel electrophoresis

5. **State one type of molecule that can be separated by gel electrophoresis.**

   DNA or proteins

6. **State the two types of gel electrophoresis and by which property/properties they separate substances by.**

   Native Gel Electrophoresis—size, charge and shape

   SDS Page—Size only

7. **Describe the difference in the way the two types of electrophoresis separate substances.**

   Native Gel electrophoresis does not denature substances.

   SDS page gel electrophoresis denatures substances giving them a uniform negative charge.

   Move towards positive electrode based on size (bigger proteins move less).

8. **Define the term isoelectric point.**

   When a protein has no net charge and precipitates out of solution.

9. **State the type of chemical that enables a solution to be kept at a specific pH to enable a protein to precipitate out of solution.**

   Buffers

Cell Culture Microbiology

1. **State one example of aseptic technique.**

   Sterilisation by heat or chemical means.

2. **Explain why aseptic technique is necessary.**

   Eliminates unwanted microbial contaminants.

3. **State the two types of cell culture that can be inoculated with microbes.**

   Agar OR broth

4. **State one substance mammalian cells need in cell culture and explain why this substance is necessary.**

   Growth factors from serum for cell growth/proliferation

5. **State the technique used to count viable cells.**

   Vital Staining

6. **State the name of the technique used to count cells in a liquid culture via its volume.**

   Haemocytometer

7. **State what can be counted by plating out a liquid microbial culture on solid agar media via serial dilution to allows the density of cells in the original culture to be estimated.**

   Colony Forming Units

Microscopy & Immunoassay

Microscopy

1. **State the two types of microscopy and give an example of what can be viewed under each.**

   Bright field microscope

   - whole/parts of organism/dissected tissue/parts of cells

   Fluorescent Microscope *(higher magnification)*

   Can detect specific molecules (proteins) OR individual cell structures

   Immunoassay

1. **State the term for stocks of antibodies with the same specificity.**

   Monoclonal antibodies

2. **Give two examples of chemical labels found on antibody Immunoassays**

   Reporter enzymes/chemiluminescence reporters/fluorescence reporters

3. **In some assay state the molecule uses to detect the presence of a specific antibody in a sample.**

   Antigen

4. **Describe the ELISA test.**

   Specific antibodies in sample bind to antigens bound to assay plate.

   Other non specific antibodies do not bind & washed away with buffer

   Second MCA added linked to reporter enzyme added

   The plate is washed again with a buffer & substrate added causing a colour change.

5. **State the name for the immunoassay used to detect a specific protein from a mixture following SDS page.**

   Western blotting

6. **Describe what happens after SDS page to allow detection by antibodies possible of separated proteins**

   Transferred/blotted onto a solid medium (nylon membrane).

Movement of molecules across membrane

Movement of molecules across membrane

1. **State how small uncharged molecules pass through the membrane.**

   Phospholipid bilayer

2. **State how charged ions or uncharged polar molecules pass through the membrane.**

   Transmembrane proteins

3. **State the two types of transmembrane proteins.**

   Channel proteins and transporters

4. **Describe the process of facilitated diffusion**

5. Passive transport through specific transmembrane proteins (channel proteins & transporters

6. **State what process all channel proteins move molecules through the membrane by.**

   Diffusion

5. **State the three types of channel proteins.**

   Simple channel, ligand and voltage gated channels.

6. **Describe what is meant by a gated channel.**

   Changes conformation to move molecules across membrane by diffusion

7. **State what changes the conformation of ligand and voltage gated channels.**

   Ligand gated— binding of signal molecules

   Voltage gated—changes in ion concentrations

8. **Describe the structure of a channel protein.**

   Multi subunits with a water filled pore

9. **Describe what feature all transporter proteins have in common**.

   They must change conformation/operate between two conformations across the membrane.

10. **State the two types of transporters**.

    Na K Pump and Glucose symports

11. **Describe what is meant by a pump.**

    A transporter protein that is coupled to an energy souyrce to enable active transport

12. **Define what is meant by an ATPase.**

    A protein pump that directly hydrolyses ATP to provide energy

13. **Explain the importance of the Na K pump.**

    Restores/maintains resting membrane potential.

14. **State the affinity the Na K pump has for K and Na in the unphosphorylated and phosphorylated state**.

    Unphosphorylated—Na phosphorylated—K

15. **Describe the number of Na and K ions and the direction of movement by the Na K pump.**

    3 Na out 2 K in

16. **State the location of the glucose symport**.

    Small intestine epithelial cells.

17. **State the process by which glucose and Na move into the cell via the glucose symport**.

    Na moves in by diffusion Glucose moves in by active transport.

18. **Describe what enables the movement of glucose into the cell against its concentration in the glucose symport.**

    Na K pump creates the steep concentration gradient of Na

Nerve Impulse

Nerve Impulse

1. **Describe what is meant by the resting membrane potential.**

   No net flow of ions across the membrane.

2. **Define an action potential**.

   Wave of electrical excitation along a neuron's plasma membrane.

3. **State the effect of depolarisation and repolarisation on the membrane potential.**

   Depolarisation—more positive value inside neurone

   Repolarisation—more negative value inside neurone

4. **Define a nerve impulse**

   Change in the membrane potential of the neurone's plasma membrane (depolarisation)

5. **State one way of degrading neurotransmitters in the synapse and explain why this is necessary.**

   Enzymes OR reuptake into pre-synaptic neurone

   Prevents continual stimulation of nerve impulse by neurotransmitter degradation.

6. **State the first two steps in nerve transmission.**

   Neurotransmitter initiate response by binding to ligand gated channel on post synaptic membrane

   This causes influx of Na ions (positive ions) to enter cell.

7. **State what is required for voltage gated Na channels to open.**

   Sufficient Na ion movement into neurone/membrane depolarised beyond threshold value

8. **State what type of Na channel is responsible for the large rapid depolarisation of the membrane**

   Voltage gated Na channel

9. **State what type of channel causes repolarisation of the membrane potential.**

   Voltage gated K channels.

10. **State how the ion concentration gradients are restored following repolarisation**.

    Na/K pump

Photoreceptors in the Retina

Photoreceptors in the retina

1. **State the two components of rhodopsin.**

   Retinal and opsin

2. **Describe the function of retina**l?

   Light sensitive molecule in membrane.

3. **State the name for the membrane proteins in rhodopsin**.

   Opsin proteins

4. **Describe the function of rod photoreceptors.**

   Absorb light at low light intensities.

5. **Describe the function of cone photoreceptors**.

   Detects colour

6. **State the 4 different types of opsin?**

   red, blue, green and UV (birds)

7. **Explain how rods are well adapted to their function.**

   Rods have a very high degree of amplification resulting in them being sensitivities at low light intensities.

8. **Explain how cones are well adapted to their function.**

   Cones have more than one type of opsin whereas rods only have one type of opsin.

9. **Explain how rhodopsin absorbs light in the human eye.**

   One photon of light is absorbed by rhodopsin changing its conformation to photoexcited rhodopsin. A cascade of proteins amplify the signal. This activates 100's of G protein molecules called transducin which activate 100's of the enzyme PDE. This hydrolyses 1000's of molecules of cyclic GMP which closes ion channels, generating a nerve impulse.

10. **Do rods or cones contain rhodopsin?**

    Rods only

Cytoskeleton & Cell Cycle

1. **State which type of organisms have cytoskeletons.**
   Eukaryotes
2. **Describe the two roles of the cytoskeleton.**
   Provides mechanical support and shape to cell
3. **Name the protein structures found in the cytoskeleton.**
   Microtubules
4. **Which protein are microtubules made from?**
   Tubulin
5. ***Describe the structure of microtubules***
   Hollow cylinders made of tubulin protein
6. ***Describe the two functions of microtubules.***
   1.Movement of membrane bound organelles
   2.Movement of chromosomes through formation of spindle fibres
7. **State where microtubules radiate from**
   MTOC/centrosome
8. ***The cell cycle requires remodelling of the cytoskeleton through which two processes.***
   Polymerisation and depolymerisation
9. **State the 2 key stages of the cell cycle.**
   Interphase and mitosis
10. **State the three stages to interphase.**
    G1, S, G2 phase
11. **Explain what happens at each stage of interphase.**
    G1 – Initial growth phase, S -cell copies its chromosomes via DNA replication
    G2 – further growth phase
12. **Name and describe the 4 phases of mitosis in the correct order.**
    Prophase
    Nuclear membrane breaks down. Chromosomes condense into sister chromatids
    Spindle microtubules undergo polymerisation to extend from MTOC to kinetochore
    Proteins at centromere of chromosomes
    Metaphase
    Chromosomes align by spindle fibres on metaphase plate
    Anaphase
    Sister chromatids pulled to opposite poles when spindle fibres depolymerise.
    Telophase
    Chromosomes decondense and nuclear membranes form.
13. **Describe what is meant by cytokinesis**
    Splitting of the cytoplasm

Cell Cycle Checkpoints & p53/apoptosis

14. **Name the 3 cell cycle checkpoints and describe what happens at each stage.**

    G1 – checks cell size (allows entry into S phase)

    G2 – Checks DNA replication and DNA damage (allows entry into mitosis)

    M – Ensure chromosomes are aligned on the metaphase plate (allows entry into ana phase)

15. **Active cyclin CKD complexes regulate cell cycle checkpoints by**

    Phosphorylating specific proteins

16. **Explain what happens during G1 checkpoint**

    Cyclin proteins accumulate throughout G1, they combine and activate CDK's forming cyclin CDK complex. They phosphorylate retinoblastoma. If sufficient phosphorylation occurs, transcription of genes necessary for *DNA replication occurs and the cell moves on to S phase. If insufficient phosphorylation occurs, the cell cycle is paused*

17. **What stage of the cell cycle does Rb control entry into.**

    S phase

18. ***State another name for Rb***

    Transcription factor inhibitor or tumour suppressor protein

19. **What does an uncontrolled increase in the rate of the cell cycle cause?**

    Tumour formation

20. ***Name the mutated gene that causes tumour formation.***

    Onco

21 *State the function of proto onco genes*

    Cell growth/division

22. **What does an uncontrolled decrease in the rate of the cell cycle cause?**

    Degenerative disease

23. **State the name for the cell death signal.**

    p53

24. **State the 3 roles of p53 proteins following DNA damage**

    DNA repair, arrest of the cell cycle, programmed cell death (apoptosis)

25. **Describe the process of intrinsic apoptosis.**

    Cell death signals are produced which causes a cascade of caspases (proteases) which causes protein breakdown causing cell destruction.

26. **State a substrate for caspases?**

    Protein

27. **Give an example of an intrinsic cell death signal**

    DNA damage.

28. **Give an example of extrinsic cell death signal**

    Molecules from lymphocytes OR absence of growth factors

29. ***Explain the importance of apoptosis in development.***

    Removes unnecessary cells during embryo organ development

    Remove unnecessary cells during metamorphosis.

    Remove damaged/diseased cells