IB Biology DP

YOUR NOTES ↓

2. Molecular Biology

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YOUR NOTES

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2.1 Metabolism & Water

2.1.1 Molecules

Molecular Biology

The substances of life

- There are **118 elements** in the Periodic Table
- Only the first **92 elements occur in Nature** ; the rest are artificially-synthesised in laboratories and are very unstable
- Only around **21 elements are required for life**
  - The rest have **no role** in sustaining life (some are poisonous eg. arsenic)
  - Some elements can be used in **medicine** eg. titanium for skeletal implants, thanks to its inertness, lightness and strength
- There are **4 ubiquitous elements** in biological systems (this means they are found everywhere)
- These 4 make up over 96% of living matter
  - Oxygen - 65% of body mass (humans)
  - Carbon - 18%
  - Hydrogen - 10%
  - Nitrogen - 3%
- Other **trace elements** found in organic compounds are: bromine, calcium, chlorine, chromium, copper, iodine, iron, magnesium, manganese, molybdenum, phosphorus, potassium, selenium, silicon and sodium
- There are **other trace elements found in certain phyla only** e.g. **strontium** in certain corals (*Cnidaria*)

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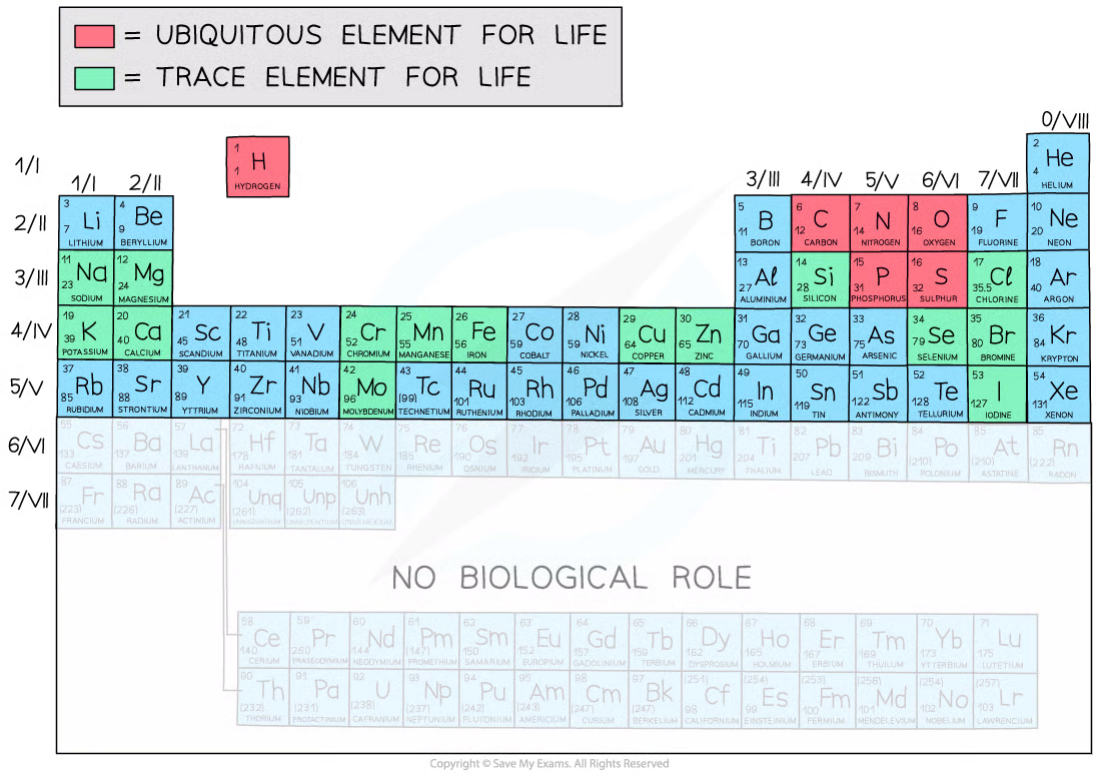


The elements of the Periodic Table that form parts of biological molecules

Elements in biology exist mainly in compounds

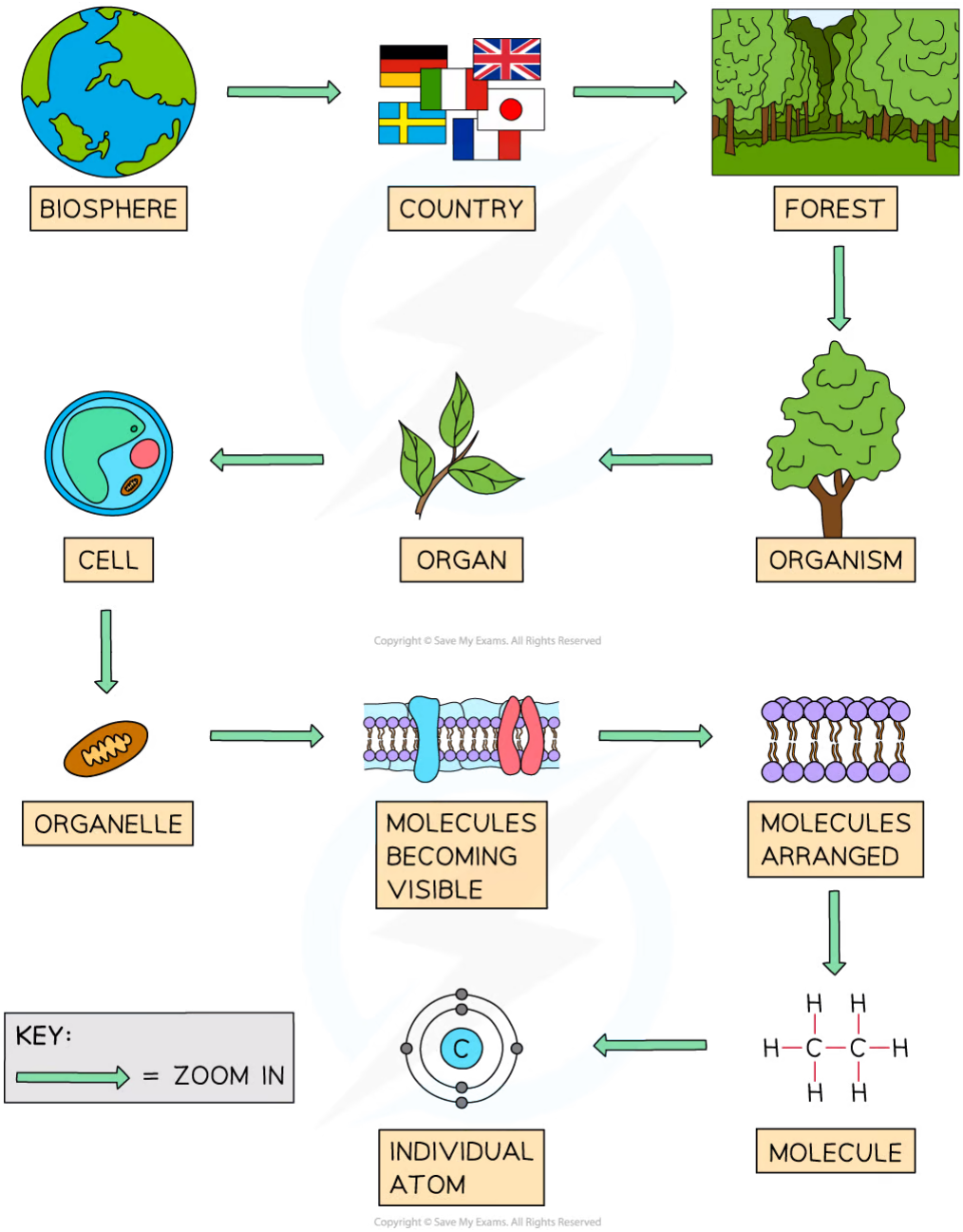
- Such compounds are **mainly covalent** compounds
  - **Electrons are shared** between atoms to generate **strong bonds** within compounds
  - For example, elemental carbon only exists as graphite and diamond, which are of no direct use to organisms
  - Carbon forms **millions of different covalently-bonded compounds**, mainly with hydrogen and oxygen
  - **Oxygen** is absorbed in elemental form but is quickly converted to its compounds during **transportation** and **respiration**
- Some are **ionic** eg. sodium chloride
- Some elements form **prosthetic groups** with larger organic molecules eg. magnesium in chlorophyll, iron in haemoglobin

All of Biology can be explained at a molecular level

- The molecules in cells, and the elements that go to form them, are **the basis of all events** that occur in Nature
- Everything that is observed has a **molecular explanation**
- Imagine an all-powerful '**zoom lens**' that could look into any level of detail of life
- Such a lens could start at its most zoomed-out, looking at our **biosphere**, the Earth
  - We assume that alien life does not exist because we haven't found evidence for it yet
- We see **habitats**, **populations**, **communities** and **individual organisms** coming into view in that order, as we zoom in

YOUR NOTES

- As we zoom in on one organism, we **enter its body** and see increasing levels of detail, right down to the molecular level
- The zoom model helps understand the **important interfaces** between chemistry, biology and physics
- We could zoom in further, to look at sub-atomic particles, although that begins to enter into the realms of physics!



We can zoom into any part of the biosphere to identify all of Biology at a molecular (and atomic) level

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Exam Tip

Please note that you do not need to know the specific details of the Periodic Table, it is provided here for context to support your understanding of important biological compounds

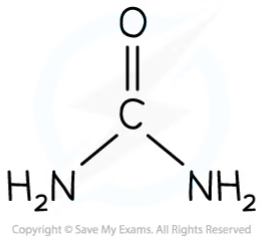
Synthesis of Organic Molecules

NOS: Falsification of theories; the artificial synthesis of urea helped to falsify vitalism

- When scientists do not have all the necessary information to understand or explain a biological process/phenomenon they use their knowledge and expertise to propose a **theory**
- Other scientists or researchers will often test theories through experiments and gathering data. Sometimes this can lead to an existing theory being disproved or replaced
- In the early 1800s, the theory of **vitalism** stated that a **living force**, a mysterious non-molecular entity, was necessary for the synthesis of all **organic molecules**
- This theory advocated that all biological molecules were **exclusive to living beings** and could not be found in other branches of science
- Frederick Wöhler, a German physician, was the first to synthesise a biological molecule, **urea**, from inorganic compounds
  - Urea was **thought to be synthesised only** in living organisms
- Wöhler heated ammonium cyanate and produced urea, a well-known organic constituent of blood and urine
  - Urea had been thought to be found only in living organisms
- The formation of urea from ammonium cyanate **helped to disprove the theory of vitalism**, which has been completely **falsified** by subsequent findings
- All of the observations of biology now have a **molecular explanation**, and that is now universally accepted

$$NH_4(OCN) \rightarrow NH_3 + HOCN \leftrightarrow (NH_2)_2CO$$

A balanced chemical equation showing the formation of urea



Chemical Structure of Urea

Carbon

YOUR NOTES

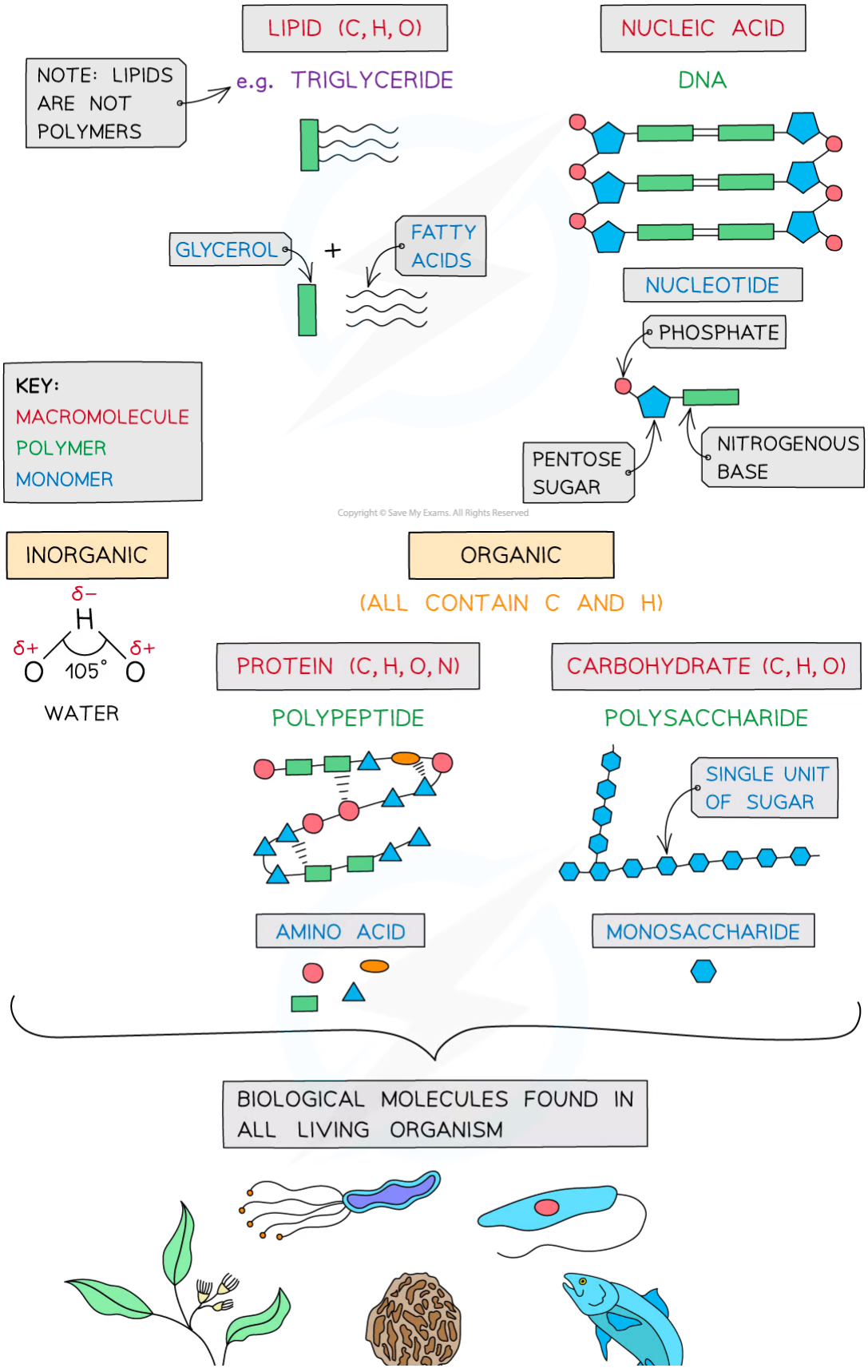
- Carbon's unique chemistry makes it the ideal basis of living systems
- The **structure and bonding** possibilities of **carbon** can be detailed as follows:
    - **Four electrons** in its outer (second) shell
    - Each atom can form four strong covalent bonds **using these 4 electrons and therefore forms very stable, large molecules**
- Bonds to **other carbon atoms**, or **other atoms** such as hydrogen, nitrogen, oxygen, sulfur and the halogens
- Forms **long-chain** and **cyclic** compounds that are stable, this allows a **very high number of possible organic compounds** to exist
- Produce a **tetrahedral structure**, due to the four bonds, which allows the formation of varied carbon compounds which have different 3-D shapes and hence, different biological properties
- **Double** and **triple bonds** can form with an adjacent carbon atom, allowing **unsaturated** compounds to form
- Can form part of (and join onto) many different **functional groups** that give organic compounds their individual properties
    - Alcohol groups
    - Hydroxyl groups
    - Ketone groups
    - Aldehyde groups
    - Carbonyl groups
    - Amino groups
    - Sulfhydryl groups
    - Phosphate groups

Carbon Compounds

YOUR NOTES

- The key molecules that are required to build structures that enable organisms to function are:
  - Carbohydrates
  - Proteins
  - Lipids
  - Nucleic Acids
  - Water
- All of these except water contain carbon

YOUR NOTES



YOUR NOTES

The key biological molecules for living organisms

- Carbohydrates, proteins, lipids and nucleic acids contain the elements **carbon** (C) and **hydrogen** (H) making them organic compounds
- Carbon atoms are key to organic compounds because:
  - Each carbon atom can form **four** covalent bonds – this makes the compounds very stable (as covalent bonds are so strong they require a large input of energy to break them)
  - Carbon atoms can form covalent bonds with oxygen, hydrogen, nitrogen and sulfur
  - Carbon atoms can bond to form **straight chains**, **branched chains** or **rings**
- Carbon compounds can form small single subunits **(monomers)** that bond with many repeating subunits to form large molecules **(polymers)** by a process called polymerisation
- **Macromolecules** are very large molecules that contain 1000 or more atoms therefore having a **high molecular mass**
  - Polymers can be macromolecules, however **not all** macromolecules are polymers as the subunits of polymers have to be the **same** repeating units

Exam Tip

When discussing monomers and polymers, you should be able to give the definition and also name specific examples eg. **nucleic acids** – the **monomer** is a **nucleotide.**

YOUR NOTES

Metabolism

- **Metabolism** is a catch-all term used to describe **all the chemical reactions** that take place within cells and organisms
- Metabolism can be thought of as **the chemical reactions of life**
  - The molecules involved are **metabolites**
- Many reactions of metabolism take place in **multiple stages**
  - Each stage is **catalysed** by a separate **enzyme**
- A series of interlinked metabolic reactions is called a **metabolic pathway**
- Metabolic reactions can be classified broadly as **anabolic** or **catabolic**

Anabolism

- Anabolic reactions are involved with the building of large molecules from smaller ones
- Examples include;
  - Photosynthesis, where CO_2 and water are built up into complex sugars
  - Protein synthesis, where amino acids are joined together in sequence
  - The buildup of fat stores ahead of animal hibernation
- Anabolic reactions often include **condensation** reactions
- Anabolic reactions are **endergonic** (they require an input of energy to take place)
  - Energy-storing products are the end result

YOUR NOTES

Catabolism

- Catabolic reactions are involved with **breaking down large molecules** into smaller ones
- These reactions are often carried out to **release energy** for cellular processes and for the **excretion** of waste
- Examples include:
  - **Respiration**, where CO_2 and water are produced from the breakdown of sugars
  - **Deamination** of proteins to release urea
  - The **depletion of fat stores** during animal hibernation
- Catabolic reactions often include **hydrolysis** reactions
- Catabolic reactions are **exergonic** (free energy is released for cellular processes or as excess heat)

Comparison of Anabolism and Catabolism Table

Anabolism	Catabolism
Requires an input of energy (endergonic)	Releases energy (exergonic)
Builds large molecules from small ones	Breaks down large molecules into smaller ones
Used to store energy in chemical form	Used to release chemical energy as heat and for movement, active transport etc
Involves condensation reactions	Involves hydrolysis reactions
Used for growth, repair, and energy storage	Performs several activities, eg. energy supply, digestion, excretion
Both are made up of enzyme–catalysed reactions	
Both are coupled to ATP, the principal energy carrier in cells	

Exam Tip

You may be familiar with the concept of anabolic steroid drugs used by bodybuilders. This is to build muscle mass and so is a good example to remember when trying to remember the difference between anabolic and catabolic reactions.

YOUR NOTES

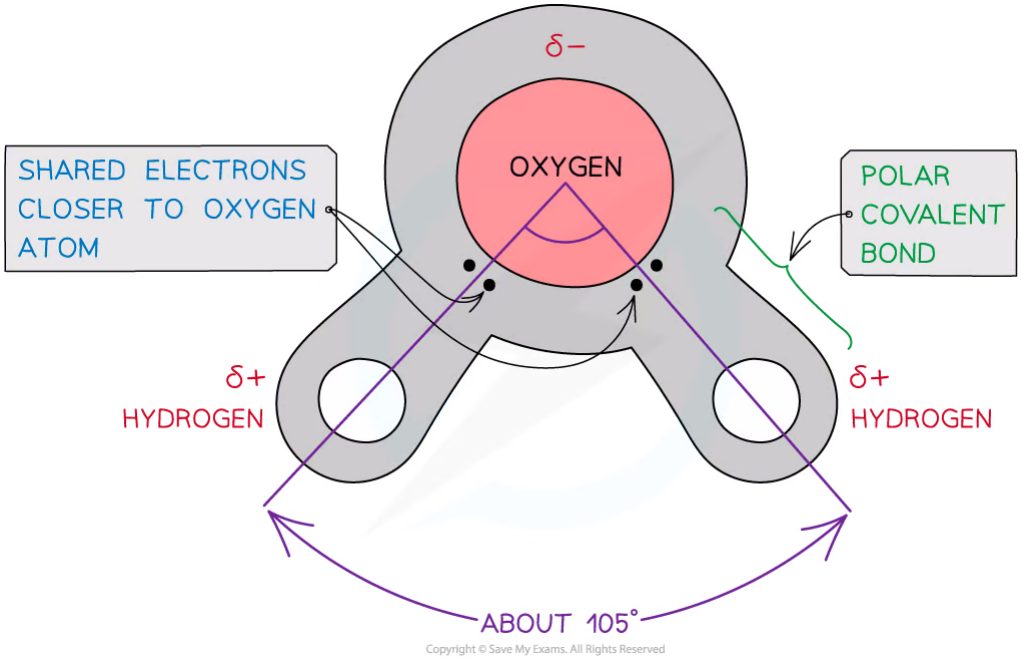
Hydrogen Bonds

- Hydrogen bonding plays an important role between many biological molecules
- Some key functions include:
  - **Dissolving of solutes** in water
  - The **cohesion** and **adhesion** of water molecules
    - These properties allow water to move up the trunks of really tall trees
  - **Base-pairing** between the two strands of DNA
  - Structure:
    - Hydrogen bonds help to form part of the **secondary** and **tertiary** levels of structure in proteins
    - The hydrogen bonds found between strands of **cellulose** and **collagen** give those molecules their **tensile strength**
  - Interactions between **mRNA** and **tRNA** during **protein synthesis**
  - **Surface effects** on membranes between **polar phosphate groups** and **water**

Hydrogen bonding in water

- Hydrogen bonding is a fundamental property of **water**
- Water is of the **utmost biological importance**
  - It is the **medium** in which all metabolic reactions take place in cells
  - Between 70% to 95% of the mass of a cell is water
  - Water is **so fundamental to life** that astronomers look for **signs of water** on other planets and moons, as indicators of possible extra-terrestrial life
  - As 71% of the Earth's surface is covered in water it is a **major habitat** for organisms
- Water is composed of atoms of hydrogen and oxygen
  - One atom of oxygen combines with two atoms of hydrogen by sharing electrons (**covalent bonding**)
- Although water as a whole is electrically neutral, the **sharing** of the **electrons** is **uneven** between the oxygen and hydrogen atoms
  - The oxygen atom attracts the electrons more strongly than the hydrogen atoms, resulting in a **weak negatively charged region** on the oxygen atom (δ^-) and a **weak positively charged region** on the hydrogen atoms(δ^+), this also results in the molecule's asymmetrical shape
- This separation of charge due to the electrons in the covalent bonds being unevenly shared is called a **dipole**
- When a molecule has **one** end that is negatively charged and **one** end that is positively charged it is also a **polar molecule**
- Water is therefore a **polar molecule**

YOUR NOTES



The covalent bonds of water make it a polar molecule

- **Hydrogen bonds** form between water molecules
  - As a result of the polarity of water, **hydrogen bonds form** between the positive and negatively charged regions of adjacent water molecules
- Hydrogen bonds are weak, when there are few, so they are **constantly breaking and reforming**
- However, when there are large numbers present they form a strong structure
- Hydrogen bonds **cause many of the properties of water molecules**, that make them so important to living organisms:
  - Excellent **solvent** – many polar substances can dissolve in water
  - A relatively **high specific heat capacity**
  - A relatively **high latent heat of vaporisation**
  - Water is less dense when a solid (ice floats, allowing aquatic life to flourish beneath)
  - Water has **high surface tension** and cohesion
  - It acts as a **reagent**

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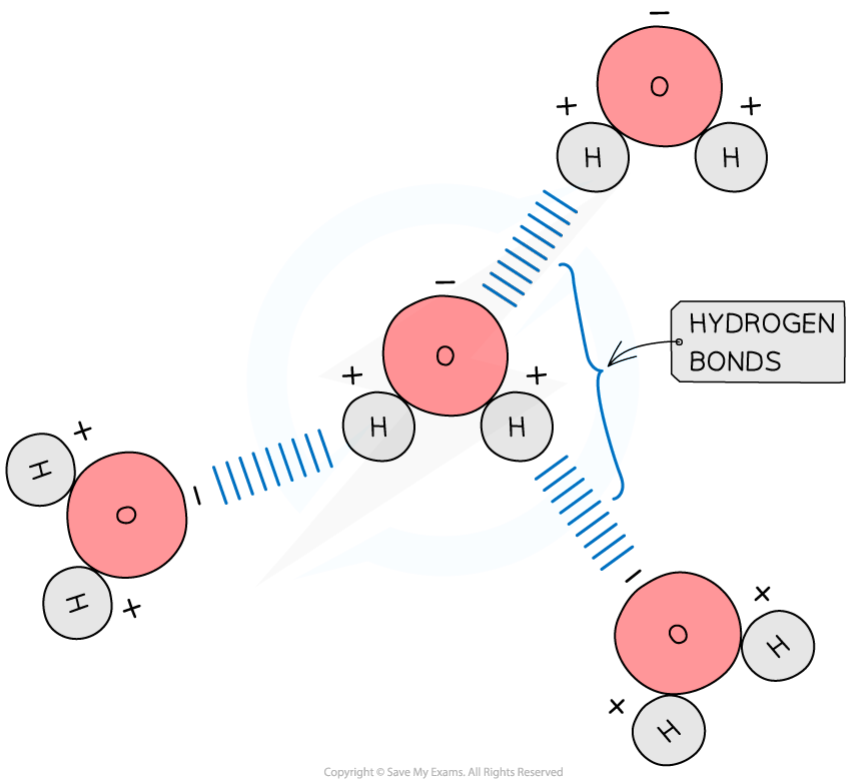
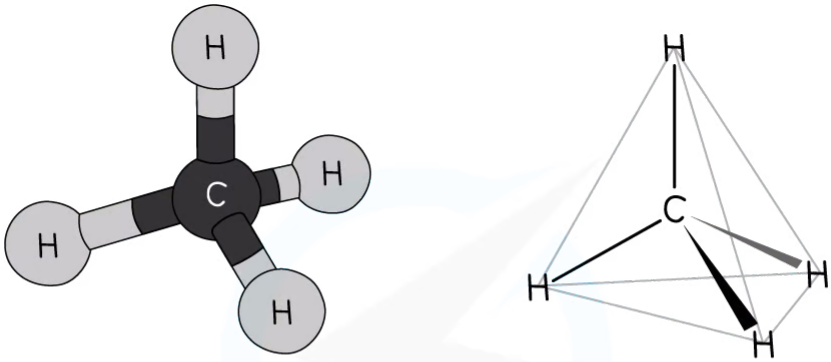


The polarity of water molecules allows hydrogen bonds to form between adjacent water molecules

YOUR NOTES

Comparison: Water & Methane

- Both **methane** (CH_4) and **water** (H_2O) are **small**, covalently-bonded molecules
- Methane is the **simplest organic compound**
- They have similar molecular weights, 16 and 18 respectively
- **But** water is polar and so forms hydrogen bonds, whereas methane is **not polar** and so does not form hydrogen bonds
- **Water is liquid** at room temperature, whereas **methane is a gas**
  - Other compounds, with a small molecular weight, such as ammonia (NH_3) and carbon dioxide (CO_2) are gases at room temperature
    - These compounds also **do not form hydrogen bonds**
  - This shows how **water molecules are held together by hydrogen bonds**, whereas the other gas molecules are free to move around in the gaseous state
  - Methane is a **fuel** with a **high energy content** in its bonds, whereas water is a **final product** of combustion and respiration
- Understanding the molecular properties of methane underlines **the significance of hydrogen bonding** in water



METHANE FORMS A TETRAHEDRAL STRUCTURE (A TRIANGULAR-BASED PYRAMID). IT HAS A H ATOM AT EACH CORNER AND THE C ATOM IN THE CENTRE.
THE BONDS ARE COMPLETELY NON-POLAR BECAUSE THE ELECTRONS ARE SPREAD EVENLY ALONG THE C-H COVALENT BONDS. THIS EXPLAINS WHY THERE IS NO COHESION BETWEEN METHANE MOLECULES AND WHY METHANE HAS A VERY LOW BOILING POINT (-162 °C).
THIS IS IN DIRECT CONTRAST TO WATER, WHICH HAS A HIGH BOILING POINT (100 °C).

Methane's structure determines its properties

Comparison of the Properties of Water and Methane Table

YOUR NOTES

	Water	Methane
Formula	H_2O	CH_4
Molecular Weight	18	16
Polarity	High	Low
Latent Heat of Vaporisation /kJ kg^{-1}	2 260	510
Specific Heat Capacity /kJ kg^{-1} $°C^{-1}$	4.2*	2.2
Melting Point / °C	0	−182
Boiling Point / °C	100	−162

* Water has the highest Specific Heat Capacity of any known substance

Exam Tip

It is important to know where the hydrogen bonds form between water molecules (oxygen of one water molecule to the hydrogen atom of another).

YOUR NOTES

Properties of Water

NOS: Use theories to explain natural phenomena; the theory that hydrogen bonds form between water molecules explains the properties of water

- When scientists observe natural phenomena they try to come up with **credible theories** to explain their occurrence
- A theory is presumed to be correct **if it explains the observation**, is supported by **experimental evidence** and has not been falsified
- As hydrogen bonds are not visible, scientists can never say for certain that they exist, however there is strong experimental evidence to suggest that they do
- The presence and number of **hydrogen bonds** between **polar** water molecules helps to explain water's unique properties

The properties of water

Solvent

- As water is a **polar molecule** many ions (e.g. sodium chloride) and covalently bonded polar substances (e.g. glucose) will dissolve in it
  - This allows **chemical reactions** to occur within the **cytoplasm** of cells (as the dissolved solutes are more chemically reactive when their individual molecules are free to move about)
  - **Metabolites** can be transported efficiently (except non-polar molecules which are hydrophobic)
- Water molecules 'surround' individual solute particles to ensure each solute particle is isolated from others
  - This explains why **solutions are clear** - we can't see individual molecules that are separated from their crystal structures
- This is also why concentrated solutions have a **lower water potential** or a higher **osmolarity**
  - Because many water particles are 'occupied' in keeping a solute molecule in solution, **fewer water molecules are free to diffuse** across partially permeable membranes

Water has a high specific heat capacity

- Specific heat capacity is a measure of the **energy required to raise the temperature of 1 kg of a substance by 1°C**
- Water has a **high** specific heat capacity of 4200 J/kg/°C meaning a relatively large amount of energy is required to raise its temperature
- The high specific heat capacity is due to the **many hydrogen bonds** present in water
  - It takes **a lot of thermal energy** to break these bonds and a lot of energy to build them, thus the temperature of water does not fluctuate greatly
- The advantage for living organisms is that it:
  - Provides suitable, **stable habitats**
  - Is able to maintain a **constant temperature** as water is able to absorb a lot of heat without wide temperature fluctuations

YOUR NOTES

- This is vital in maintaining temperatures that are optimal for **enzyme activity**

- Water in blood plasma is also essential in **transferring heat** around the body, helping to **maintain a fairly constant temperature**, especially at body extremities eg. fingertips
  - As blood passes through more metabolically active ('warmer') regions of the body, heat energy is absorbed but the temperature remains fairly constant
  - Water in **tissue fluid** also plays an important regulatory role in maintaining a constant body temperature

Water has a high latent heat of vaporisation

- In order to change state (from liquid to gas) a large amount of thermal energy must be absorbed by water to break the **hydrogen bonds** and allow individual gas particles to escape (evaporate)
- This explains water's **high boiling point** (100°C)
- Water is present on Earth in **all three physical states** (solid, liquid and gas) thanks to this characteristic
  - **Ice**, **liquid water** and **water vapour** all play a vital role in the biosphere
  - This is an advantage for living organisms as **only a little water is required** to evaporate for the organism **to dissipate a great amount of heat**
  - This provides a **cooling** effect for living organisms, for example, the transpiration from leaves or evaporation of water in sweat from the skin

Properties of Water & its Role in Living Organisms Table

Property	Role in living organisms	Reason
Solvent	◦ Allows chemical reactions to occur ◦ Transport medium	Polarity of water
High specific heat capacity	◦ Allows water to be a suitable habitat ◦ Optimal temperature maintained within cells and bodies	Presence of many hydrogen bonds
High latent heat of vaporisation	◦ Coolant	Presence of many hydrogen bonds

Cohesion and adhesion

- Hydrogen bonds between water molecules allows for strong **cohesion between water molecules**

YOUR NOTES

- Allowing **columns of water** to move (called **mass transport**) through the xylem of plants and through blood vessels in animals
- Enabling **surface tension** where a body of water meets the air, these hydrogen bonds occur between the top layer of water molecules to create a sort of film on the body of water
  - This layer is what allows insects such as **pond skaters** to move across the surface of water

- Water is also able to hydrogen bond to **other molecules**, such as cellulose, which is known as **adhesion**
  - This also enables water to move up the **xylem** during **transpiration**
  - Cohesion and adhesion both contribute to water forming a **meniscus** in glassware, where water molecules adhere to polar molecules in the glass
  - Water adheres to the xylem walls (made of lignin) by **capillary action**

Exam Tip

COhesion = water particles sticking to **each other**
ADhesion = water particles sticking to **other materials**

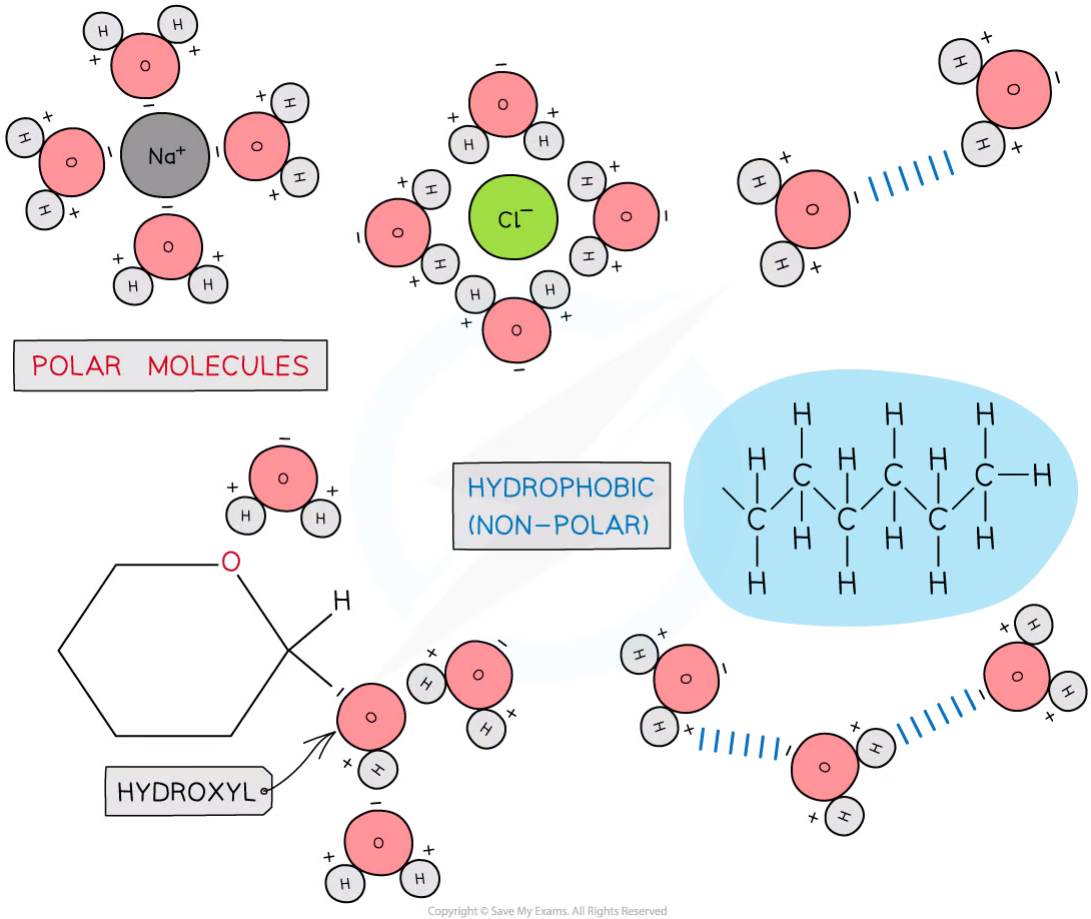
YOUR NOTES

Hydrophilic & Hydrophobic

- Biological molecules can be hydrophilic or hydrophobic (and sometimes both)
  - Hydrophilic = "water-loving"
  - Hydrophobic = "water-hating"

- **Polar molecules** and molecules with **positive or negative charges** can form hydrogen bonds with water (and dissolve) so are generally **hydrophilic**
- **Non-polar molecules** with no **positive or negative charge**, cannot form hydrogen bonds with water so are generally **hydrophobic**
  - These molecules tend to join together in groups due to **hydrophobic interactions** where hydrogen bonds form between water particles but not with the non-polar molecule

- Because most biological molecules are hydrophilic and can be dissolved, water is regarded as the **universal solvent**
- Some large molecules have **different groups** with **different characteristics**
  - **Phospholipids** have hydrophilic (phosphate group) heads and hydrophobic (hydrocarbon chain) tails. This dual character is a key feature in the structure and function of **cell membranes**

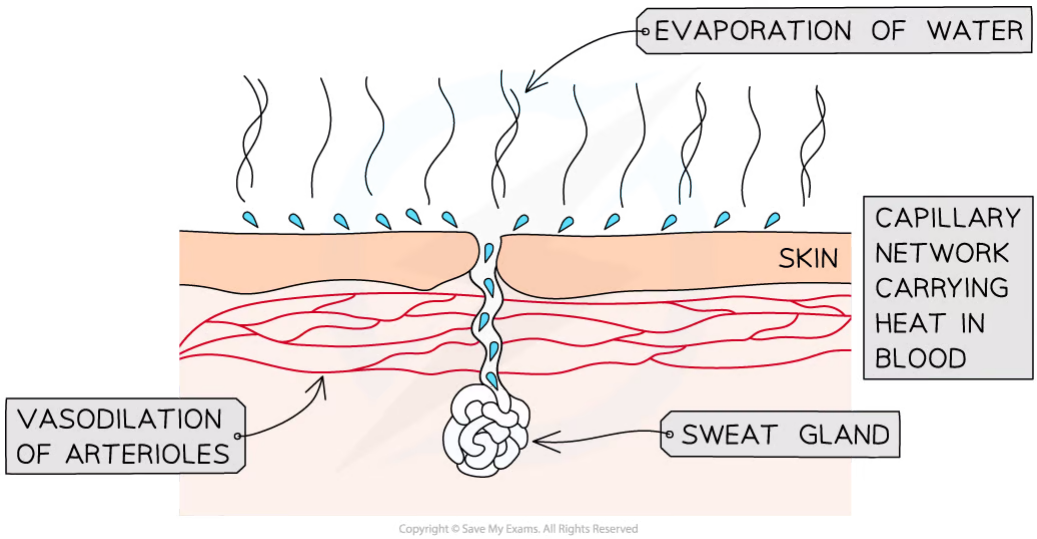


Due to its polarity water is considered a universal solvent

YOUR NOTES

Focus on Water as a Coolant

- Water's high latent heat of vaporisation makes it an excellent coolant
- **Animals** have evolved **sweating** (perspiration) as a way of disposing of excess heat generated through physical activity
- The **hypothalamus** detects changes to blood temperature and when temperatures rise, it stimulates the secretion of sweat
- Small droplets of water are secreted from **sweat glands** onto the skin's surface
- **Vasodilation** of arterioles just beneath the skin carries more blood close to the surface
- Sweat (mainly water, also contains salts and other solutes) **evaporates**, carrying the excess heat away into the surrounding air and reducing the temperature of the organism
- Water's high latent heat of vaporisation allows only **small volumes of water to be needed** to carry away a lot of heat



The excess heat carried in blood causes the evaporation of sweat from the skin surface

Water as a coolant in plants

- **Plants transpire**
- A large tree will stand in direct sunlight all day, so **will absorb a huge amount of heat** (as infra-red radiation from the Sun) on a hot day
  - A tree cannot seek shade, because it **requires light energy** for photosynthesis
  - A tree is also **immobile** and provide shade for other organisms
  - A **transpiration stream** of water flows up the tree, from roots to xylem to leaves, throughout the day
  - Water **evaporates inside the spongy mesophyll layer** of leaves, so water vapour can **diffuse out** via the stomata
  - For example, a large oak tree can absorb around 500 litres of water per day from the soil, around **90% of which is evaporated in transpiration** to dissipate heat
    - The remainder is used to keep cells turgid and as a raw material for photosynthesis

YOUR NOTES

Exam Tip

Sweat and **transpiration** have a lot of parallels in keeping animals and plants cool. This is why the French use the same word for both; the French word for "sweating" is "***transpiration***"!

Focus on Water as a Solvent

- Different solutes behave differently with water as a solvent
- Even though water is a universal solvent, different metabolites have **different solubilities in water**
- Different solutes have **different hydrophobic and hydrophilic properties** which affect their solubility in water

Highly soluble metabolites

- Some are **highly soluble** (eg. sodium chloride, urea), some are **insoluble** (eg. fats) and some have **intermediate solubility** (eg. oxygen and certain amino acids with a large R group)
- Highly soluble metabolites simply travel dissolved in the **blood plasma**
  - eg. salts, glucose, amino acids
    - Even the amino acids with hydrophobic R groups are soluble enough to be freely transported in water
- Different transport mechanisms have evolved to assist in the transportation of the **less soluble metabolites**

Less soluble metabolites

- A low solubility metabolite such as **oxygen requires assistance** through **combining with haemoglobin**, to allow more oxygen to be carried than directly in blood plasma
  - Oxygen is less soluble at body temperature (37°C) than at 20°C
  - Oxygen is **sparingly soluble** but **soluble enough to allow enough to dissolve** in oceans, rivers and lakes for aquatic animals to **breathe**
  - Haemoglobin can **bind oxygen** to allow sufficient oxygen to be transported to all body cells
- **Insoluble** metabolites like fats require **emulsification**, and transport in **lacteals**, or by being converted to **soluble phospholipids**
- **Cholesterol**, which is insoluble, is **converted to lipoproteins** by combining with proteins

YOUR NOTES

2.2 Carbohydrates & Lipids

2.2.1 Carbohydrates

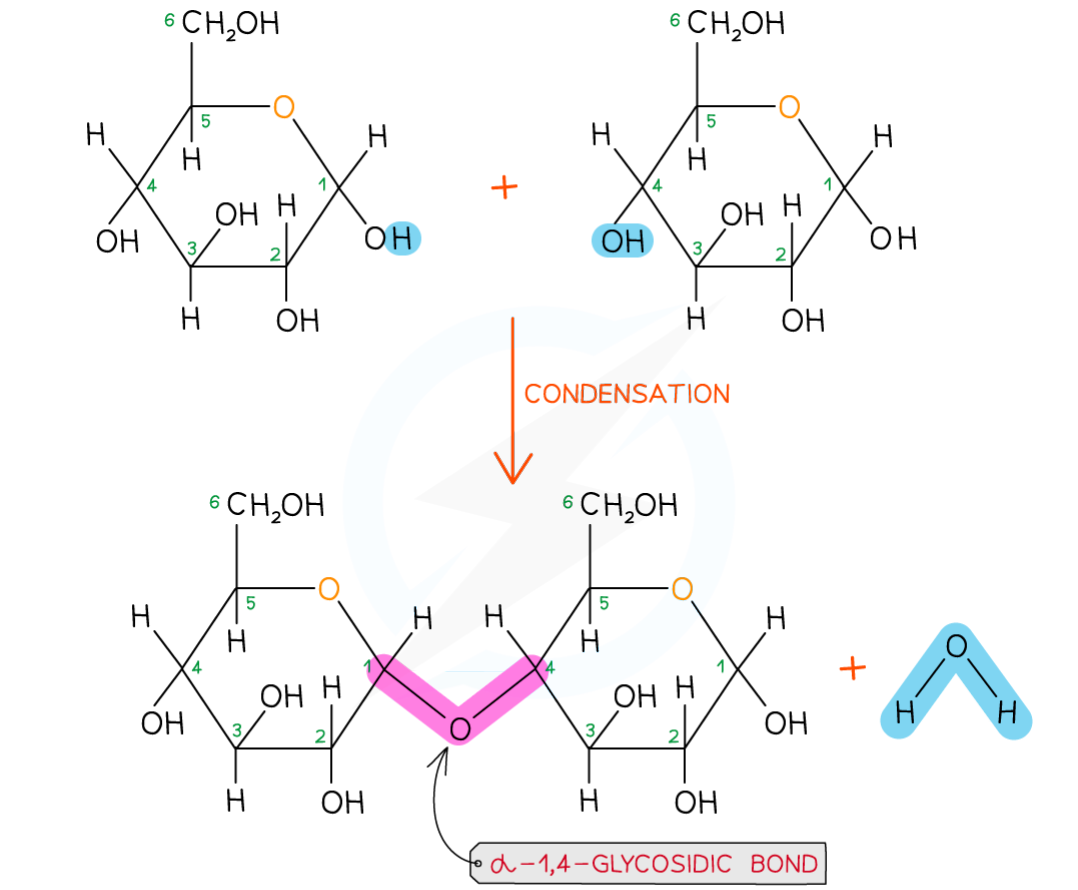
Formation of Sugars

- **Monosaccharides** can join together via **condensation reactions** to form **disaccharides**
  - A condensation reaction is one in which two molecules join together via the formation of a new chemical bond, with a **molecule of water** being **released** in the process
  - The new chemical bond that forms between two monosaccharides is known as a **glycosidic bond**
  - To calculate the chemical formula of a disaccharide, you add all the carbons, hydrogens and oxygens in both monomers then subtract 2 H and 1 O (for the water molecule lost)
- Common examples of disaccharides include:
  - **Maltose** (the sugar formed in the production and breakdown of starch)
  - **Sucrose** (the main sugar produced in plants)
  - **Lactose** (a sugar found only in milk)
- All three of the common examples above have the formula $C_{12}H_{22}O_{11}$

Common Disaccharides and their Monosaccharide Monomers Table

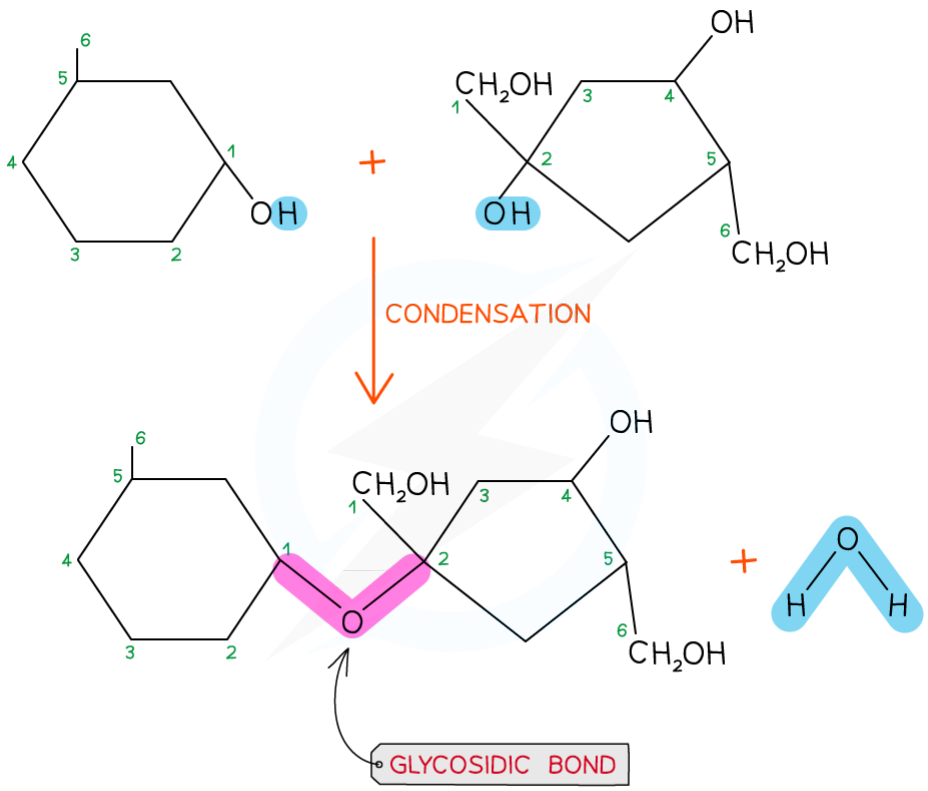
Name of disaccharide	Names of monosaccharide components	
Maltose	α-glucose	α-glucose
Sucrose	α-glucose	fructose
Lactose	α-glucose	galactose

YOUR NOTES



The disaccharide maltose is formed from two α-glucose monomers (sub-units)

YOUR NOTES



The disaccharide sucrose is formed from α-glucose and fructose monomers (sub-units)

Exam Tip

Galactose and fructose are monosaccharides and actually have the same molecular formula as glucose. However, the atoms that make up these three monosaccharides are arranged in different ways, meaning they each have slightly different molecular structures, giving them slightly different properties. For example, fructose is sweeter in taste than glucose. The three sugars are **isomers**.

Polysaccharides: Structure & Function

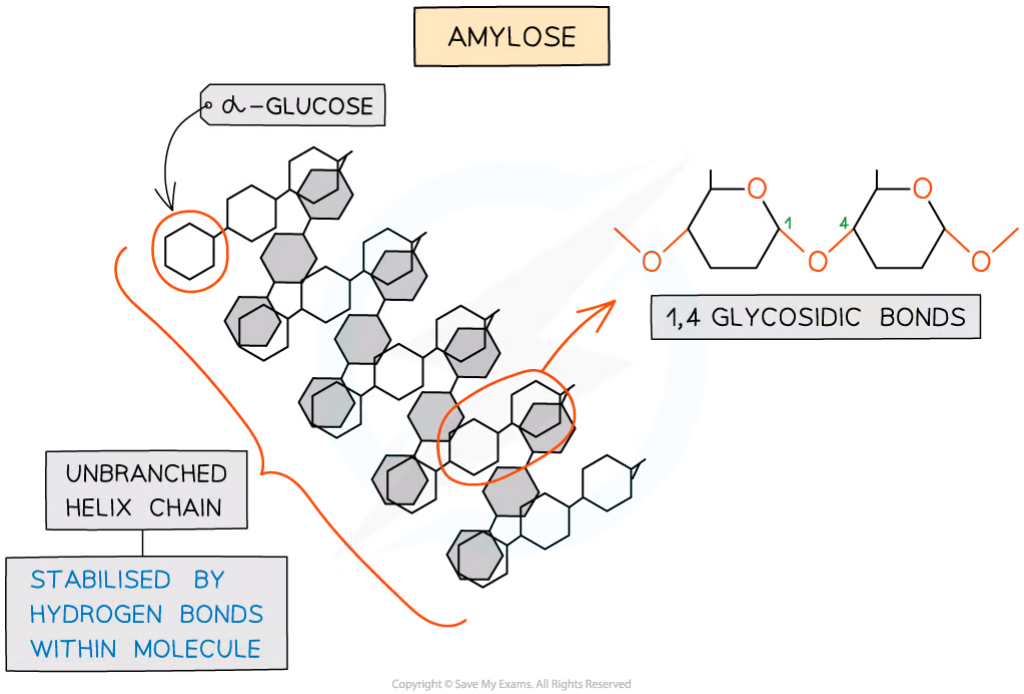
YOUR NOTES

- **Starch, cellulose** and **glycogen** are **polysaccharides**
- Polysaccharides are macromolecules that are **polymers** formed by **many** monosaccharides joined together by **glycosidic** bonds
- The bonds form from **condensation** reactions, resulting in polysaccharide chains
- These chains may be:
  - Branched or unbranched
  - Folded (making the molecule compact which is ideal for storage eg. starch and glycogen)
  - Straight (making the molecules suitable to construct cellular structures e.g. cellulose) or coiled
- **Starch** and **glycogen** are storage polysaccharides because they are:
  - **Compact** (so large quantities can be stored)
  - **Insoluble** (so will have **no osmotic effect)**
    - The monosaccharide glucose lowers the osmolarity of a cell causing water to move into cells
    - If too much water enters an animal cell it will burst
    - Plant cells have developed thicker cell walls to prevent this
- **Cellulose** is a structural polysaccharide because it is:
  - **Strong** and **durable**
  - **Insoluble** and slightly **elastic**
  - Chemically **inert** (hardly any organisms possess enzymes that can hydrolyse it)
  - Is an ideal material for plant cell walls
  - The main constituent of **dietary fibre** for animals that eat plants

Starch

- Starch is the **storage** polysaccharide of **plants**
- It is stored as granules in plastids (e.g. chloroplasts)
- Due to the many monomers in a starch molecule, it takes longer to digest than glucose
- Starch is constructed from **two different** polysaccharides:
  - Amylose (10 - 30% of starch)
    - **Unbranched** helix-shaped chain with 1,4 glycosidic bonds between **α-glucose molecules**
    - The helix shape enables it to be more **compact** and thus it is more resistant to digestion

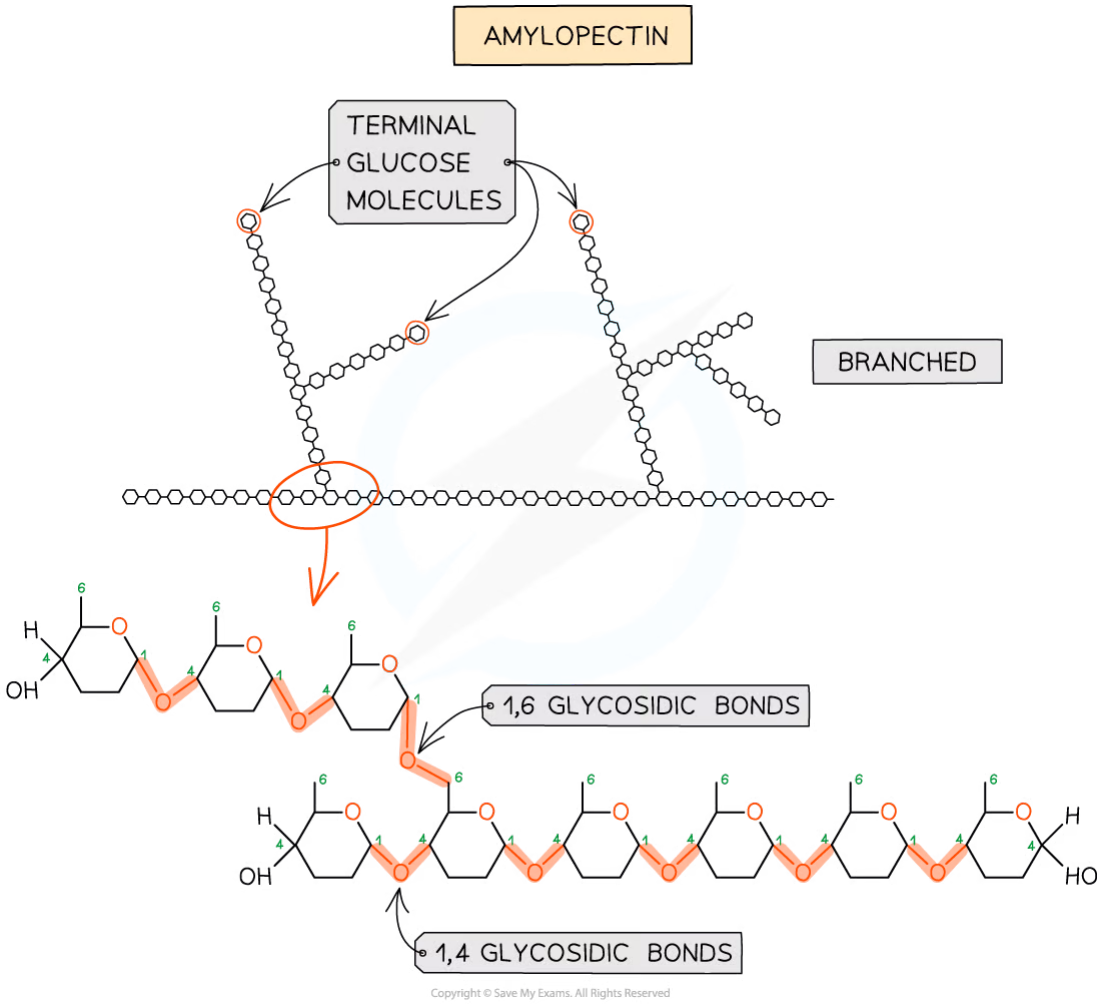
YOUR NOTES



Amylose – one of the two polysaccharides that is used to form starch (the storage polysaccharide in plants)

- Amylopectin (70 - 90% of starch)
  - 1,4 glycosidic bonds between α-glucose molecules **as well as 1,6 glycosidic bonds** creating a **branched** molecule
  - The branches result in **many terminal glucose molecules** that can be easily hydrolysed, for use during cellular respiration or added to for storage

YOUR NOTES

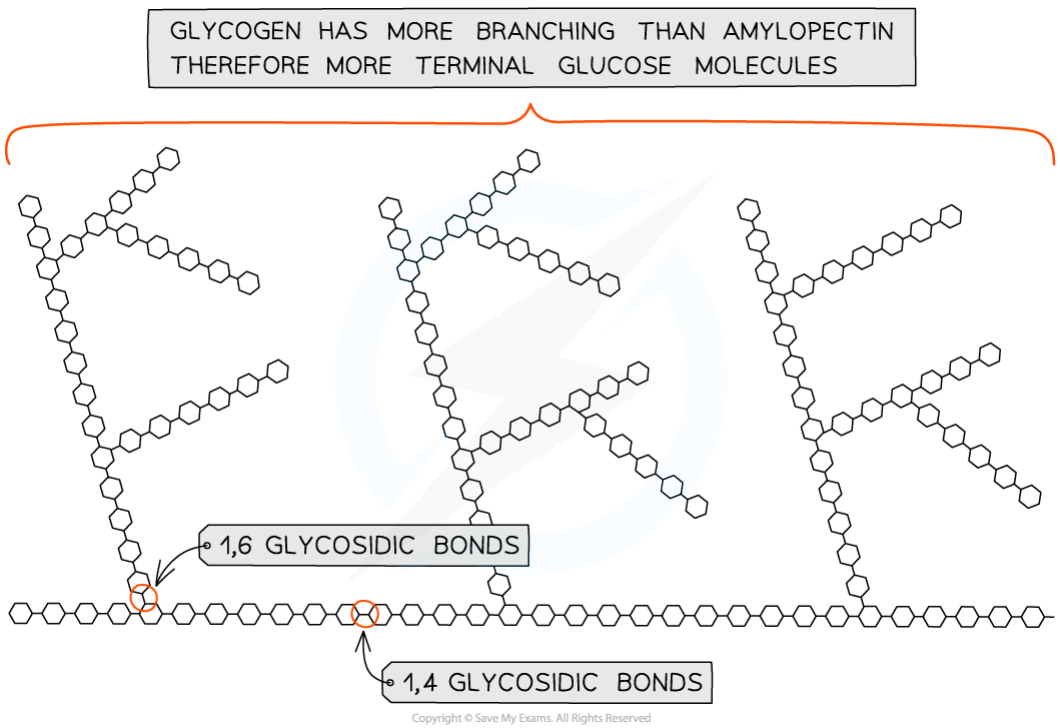


Amylopectin – one of the two polysaccharides that is used to form starch (the storage polysaccharide in plants)

Glycogen

- Glycogen is the **storage** polysaccharide of **animals** and **fungi,** it is highly branched and not coiled
- **Liver** and **muscles** cells have a high concentration of glycogen present as visible granules as the cellular respiration rate is high in these cells (due to animals being mobile)
- Glycogen is **more branched** than amylopectin making it **more compact** which helps animals **store the molecule more efficiently**
- The branching enables **more free ends** where glucose molecules can either be added or removed allowing for condensation and hydrolysis reactions to occur more rapidly – thus the storage or release of glucose can suit the demands of the cell

YOUR NOTES

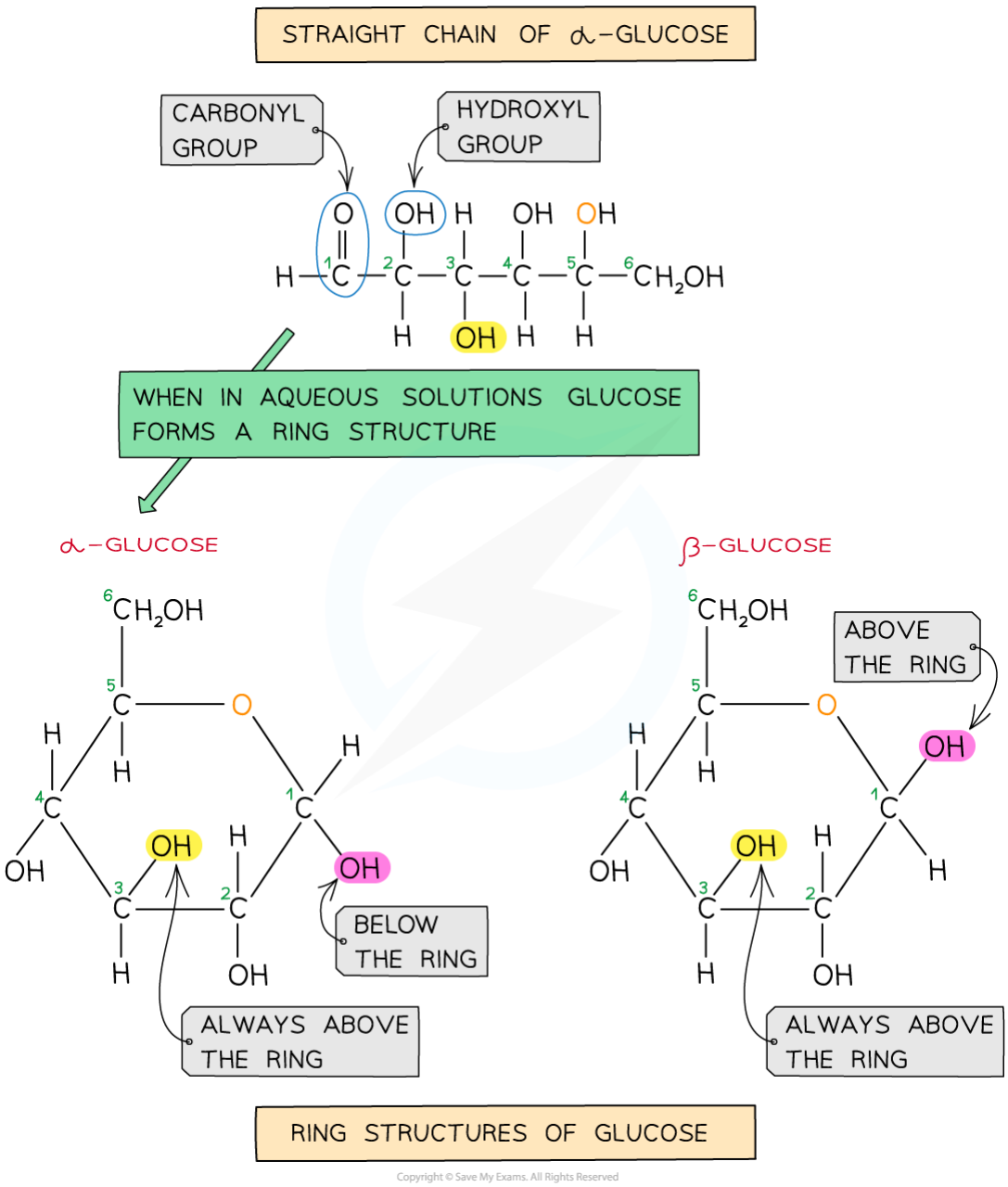


Glycogen, the highly branched molecule used as a storage polysaccharide in animals and fungi

Cellulose

- Cellulose is a polymer of **β-glucose** monomers
- β-glucose differs very slightly in structure to α-glucose
- The hydroxyl group on the C1 atom **sits above the carbon ring** in β-glucose, whereas it sits below the ring in α-glucose

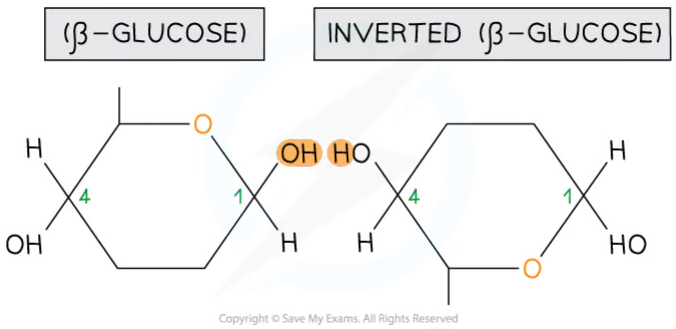
YOUR NOTES



A comparison of the structure of alpha-glucose and beta-glucose

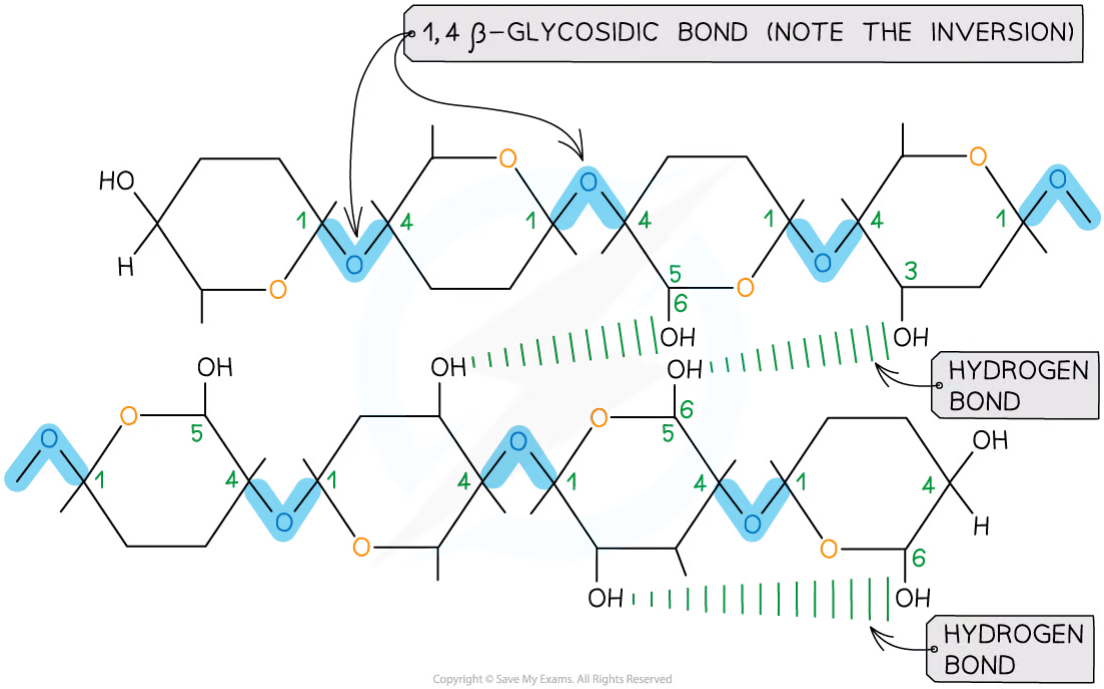
- Alpha-glucose and beta-glucose are isomers
- This **seemingly minor example of isomerism** has **far-reaching consequences** on the functions of the polymers
- It means that in order to form a glycosidic bond with a molecule of β-glucose, the next molecule of β-glucose in the chain must **invert itself**

YOUR NOTES



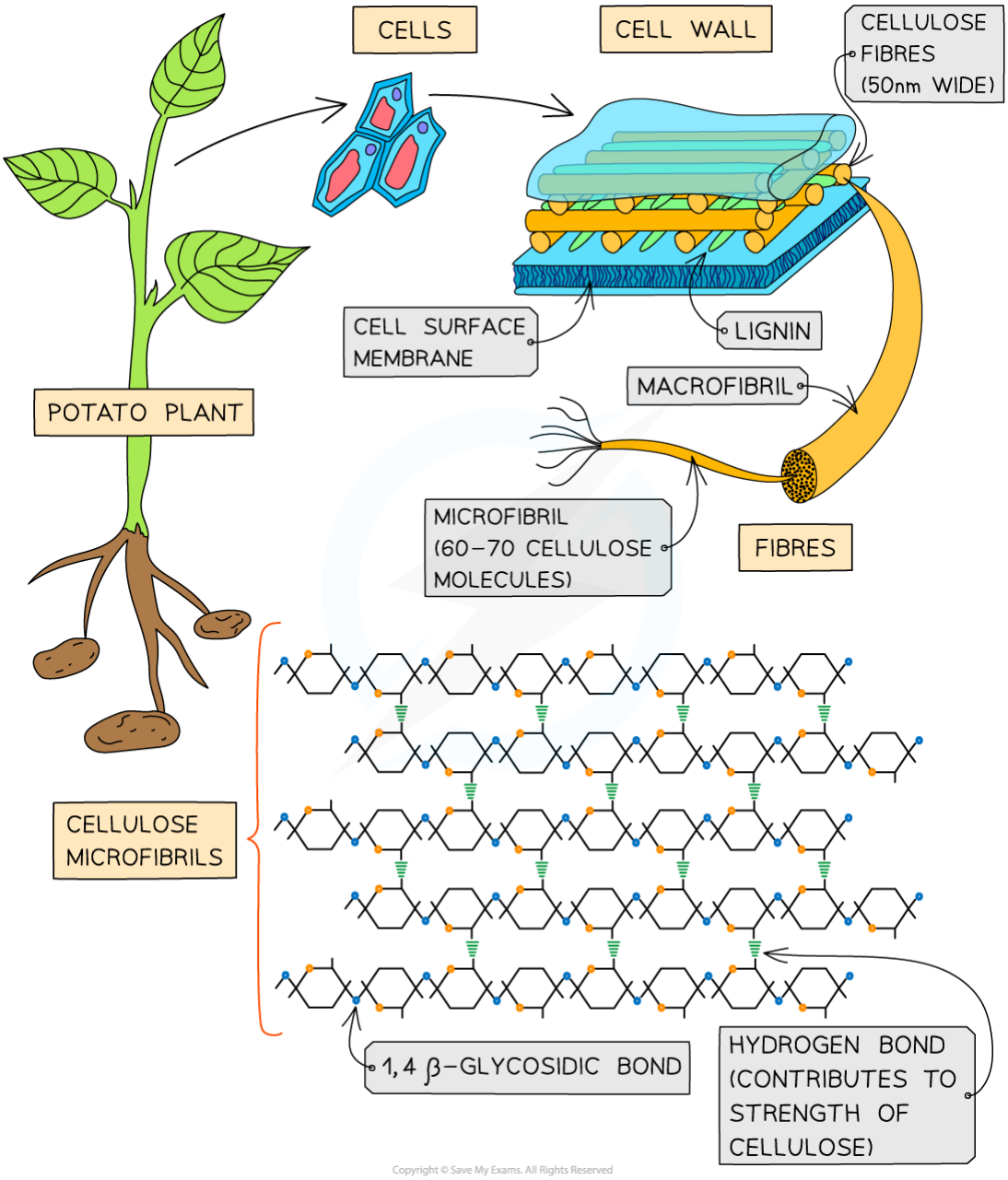
Two beta-glucose molecules orientation in a position where they are able to bond to each other

- This results in a chain of **repeatedly inverted** β-glucose monomers
- The alternating pattern of the monomers allows the chain to grow in **long, straight lengths** which gives **great fibrous strength**
- **Hydrogen bonding** occurs between strands of β-glucose monomers, adding strength to the polymer



The alternating pattern of glycosidic bonds in cellulose

YOUR NOTES



How cellulose fibres band together to provide plant strength

Summary of Polysaccharides Table

YOUR NOTES

Feature	Starch		Glycogen	Cellulose
	Amylose	Amylopectin		
Monomer	α-glucose	α-glucose	α-glucose	β-glucose
Branched	No	Yes (~every 20 monomers)	Yes (~every 10 monomers)	No
Helix (coiled) shape	Yes	No	No	No
Glycosidic bonds present	1,4	1,4 and 1,6	1,4 and 1,6	1,4
Source	plant	plant	animal	plant

Exam Tip

Be clear about the differences between starch (amylose and amylopectin), cellulose and glycogen.

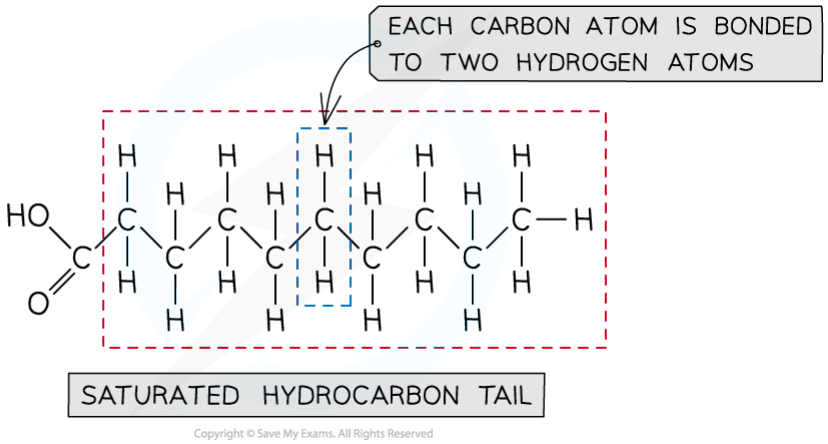
YOUR NOTES

Fatty Acids: Types

- Triglycerides are a form of lipid, made up of one molecule of **glycerol** with three **fatty acids** attached to it
- These fatty acids have **long hydrocarbon 'tails'**
- Fatty acids occur in **two** forms:
  - **Saturated** fatty acids
  - **Unsaturated** fatty acids
    - Unsaturated fatty acids can be **monounsaturated** or **polyunsaturated**
- The difference between these fatty acid types is found in their hydrocarbon tails

Saturated fatty acids

- In saturated fatty acids, the bonds between the carbon atoms in the hydrocarbon tail are **all single bonds**
- The fatty acid is said to be **'saturated' with hydrogen**
  - This means that each carbon atom in the hydrocarbon tail (except for the final carbon atom) is bonded to **two hydrogen atoms**
- Saturated fatty acids can be **synthesised industrially** by **hydrogenation** (reaction with hydrogen gas) of unsaturated fatty acids
- All the carbon-to-carbon bonds are **single bonds** in saturated fatty acids



An example of a saturated fatty acid

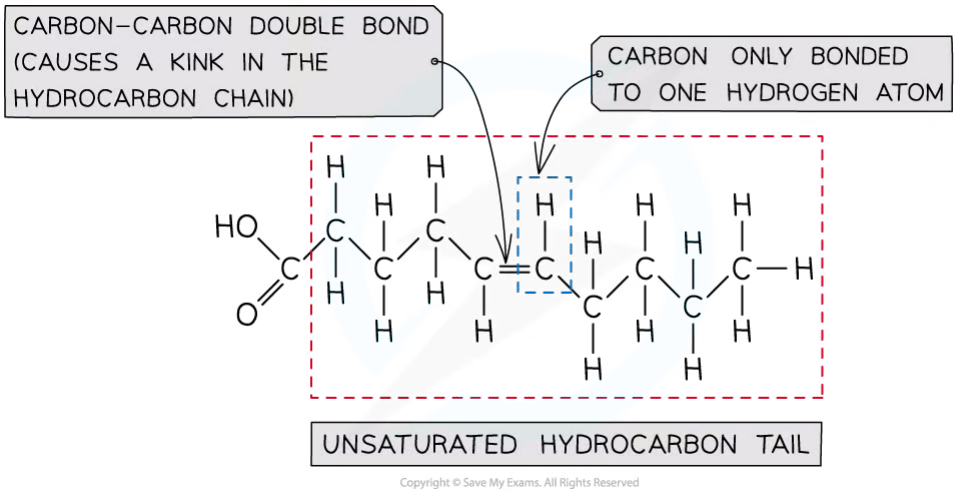
Unsaturated fatty acids

- In unsaturated fatty acids, the bonds between the carbon atoms in the hydrocarbon tail are **not all single bonds**
  - There is **at least one carbon-carbon double bond**; a fatty acid with one C=C double bond is known as **monounsaturated** fatty acid
  - In some unsaturated fatty acids, there are **many** carbon-carbon double bonds; these are known as **polyunsaturated** fatty acids

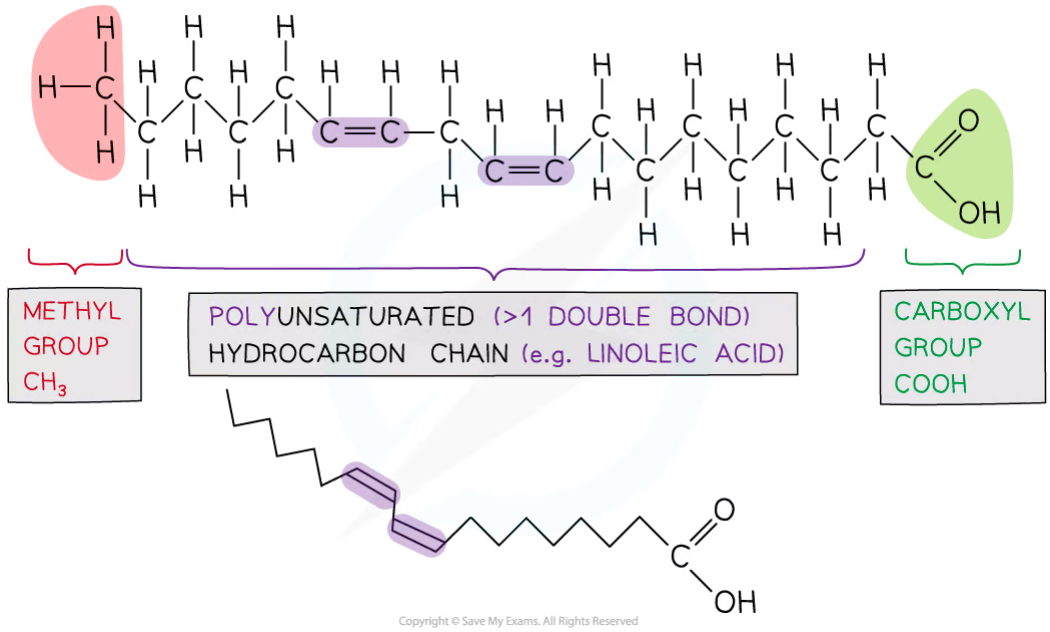
YOUR NOTES

- These double bonds can cause the hydrocarbon tail of unsaturated fatty acids to **kink** (**bend slightly**), meaning they are **not as straight as saturated fatty acids**

- The fatty acid is said to be **'unsaturated'** because the hydrocarbon tail **does not contain the maximum number of hydrogen atoms** possible
  - This is because each carbon atom in a carbon-carbon double bond **can only bond to one hydrogen atom** (instead of two)



An example of a monounsaturated fatty acid



An example of a polyunsaturated fatty acid

Exam Tip

You don't need to know the names of various fatty acids, but you should be able to recognise from a diagram whether a fatty acid is saturated, monounsaturated or polyunsaturated (look for any carbon-carbon double bonds)!

YOUR NOTES

Unsaturated Fatty Acids

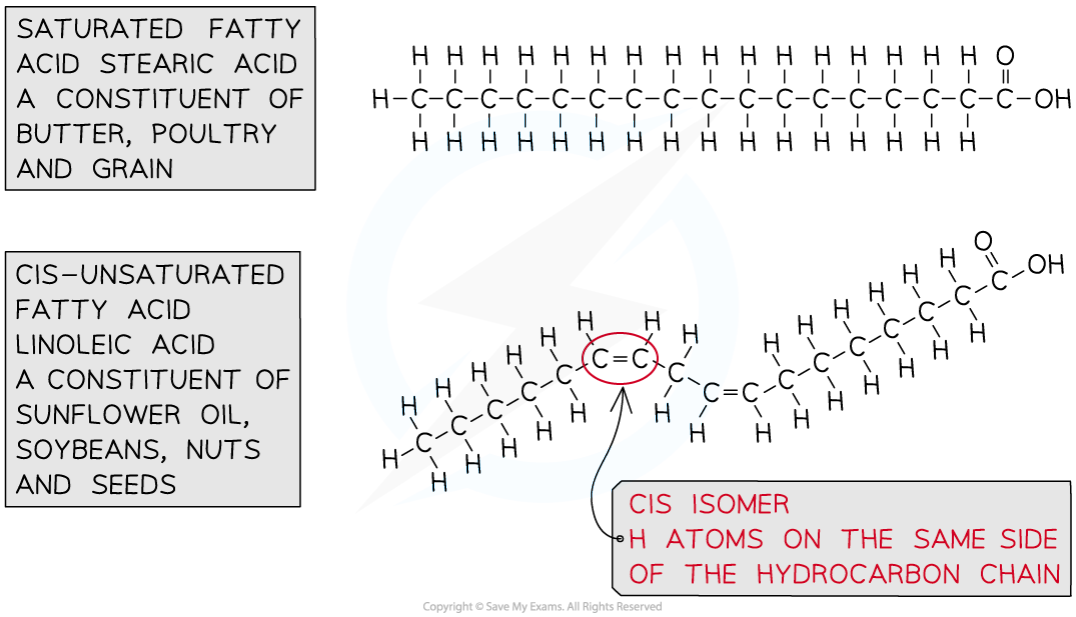
YOUR NOTES

The different isomers of unsaturated fatty acids

- A single C-C bond in a hydrocarbon chain is able to **rotate along its axis so** that rotation of one part of the molecule in relation to others is possible
- The rotation may cause conformational changes in molecules but they all remain **identical**
- However, when there is a **C=C double bond** in a hydrocarbon chain, **no rotation** is possible
- This causes **isomers** of fatty acids to be formed, each with different properties
- The isomers are labelled **cis** and **trans** isomers

Cis-isomers

- At a C=C double bond, the attached hydrogen atoms are on the **same side** of the hydrocarbon chain as each other
  - This causes a **kink in the fatty acid chain** which means the fatty acid chains are less tightly packed together, **lowering their melting point** (less kinetic energy is needed to break them apart)
  - Triglycerides that contain cis-unsaturated fatty acids are **liquid** (oils) at room temperature

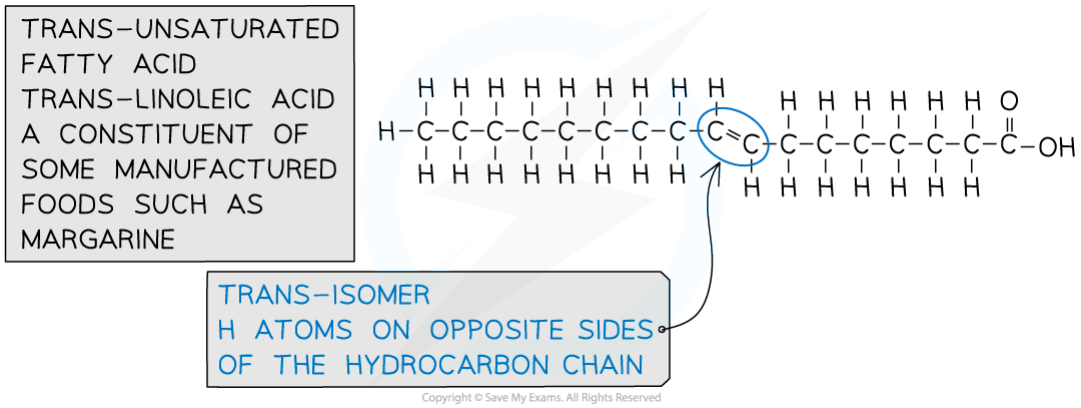


The Structural Differences Between a Saturated and a Cis-Unsaturated Fatty Acid

Trans-isomers

- At a C=C double bond, the attached hydrogen atoms are on the **opposite sides** of the hydrocarbon chain to each other
  - The lack of bend in the hydrocarbon chain allows them to pack more closely together meaning they have a **higher melting point** (this property makes them more attractive to food manufacturers)
  - Triglycerides that contain trans-saturated fatty acids are **solid** are room temperature

YOUR NOTES



The Structure of a Trans-Unsaturated Fatty Acid

Fatty Acids: Health Risks

YOUR NOTES

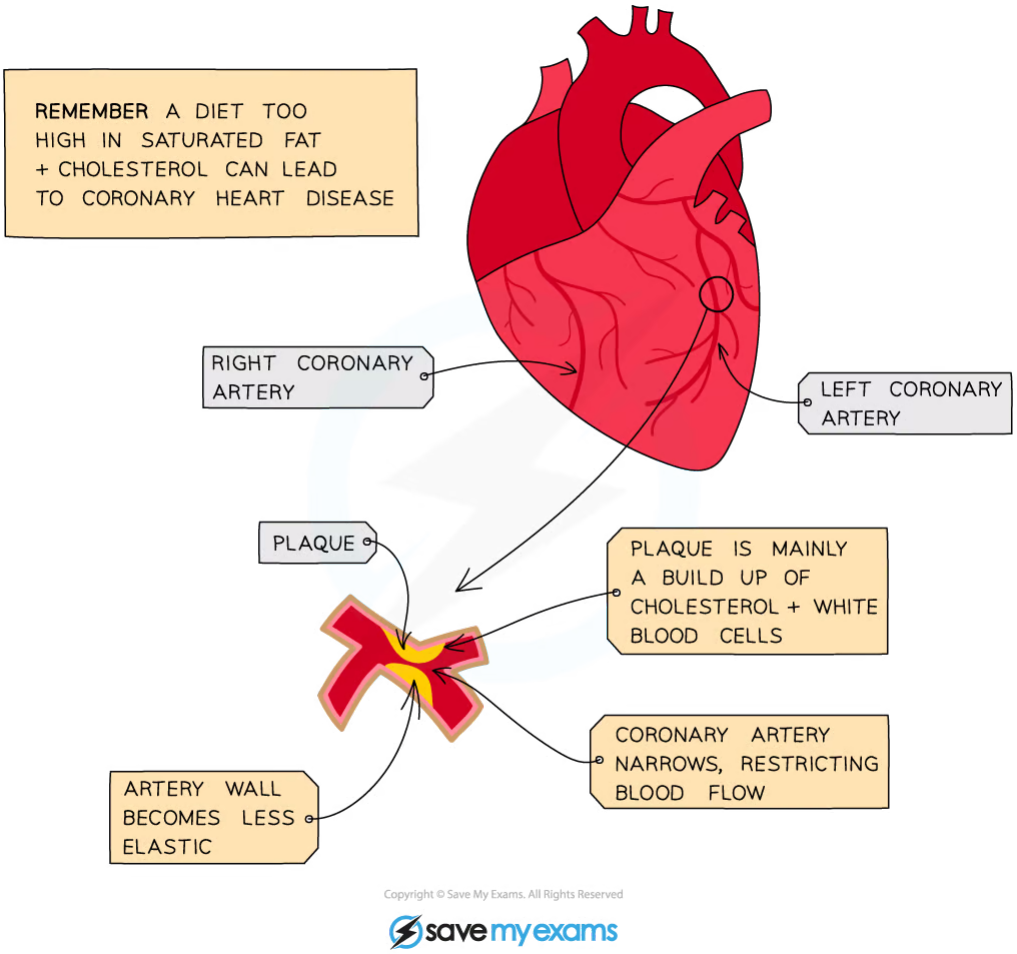
The use of trans-fatty acids in the food industry

- Trans-fatty acids occur in small quantities in natural products such as **dairy** and **red meat**
- Artificial trans-fats (which contain trans-fatty acids) are made industrially by the **hydrogenation** of liquid vegetable oils
- Trans-fats are **favoured by food manufacturers** for commercial reasons
  - They are more **solid at room temperature**
  - They create **more stable emulsions** in shortening agents
  - Food products with trans-fats appear (in their retail packaging) **drier** and **less 'greasy'** to consumers
  - Alleged **taste** benefits (though this is subjective)
  - They can be **reused** more times eg. in large-scale deep-fat fryers
  - Many countries have **legislated to restrict the use of trans-fats** in the foodservice industry
- Many foods that contains trans-fats (often labelled as '**partially hydrogenated vegetable oils**') are sold as processed products in supermarkets
  - Biscuits/cookies
  - Cakes
  - Doughnuts
  - Pie crusts
  - Crisps
  - Pizza bases
  - Certain kinds of margarine and spreads

Trans-fatty acids have associated health risks

- The two types of fat that lead to health problems are namely **saturated fats** and **trans-fats**
  - Doctors recommend limiting your intake of these types of fats
- Trans-fats alter the balance of various types of cholesterol
  - They **increase low-density lipoprotein (LDL)** levels in circulation (so-called 'bad' cholesterol)
  - They **decrease high-density lipoprotein (HDL)** levels in circulation (so-called 'good' cholesterol)
- LDLs are known **to increase the risk of coronary heart disease**, blood clotting and strokes
- Doctors recommend that the bulk of fats intake should come from **monounsaturated fats**, which **reduce** LDL levels
  - Omega-3 fats and oils are a well-publicised source of monounsaturated fats; these are found in fish, pulses and certain nuts

YOUR NOTES



Excess consumption of trans-fatty acids and saturated fats can lead to the buildup of cholesterol and the blockage of coronary arteries, causing Coronary Heart Disease (CHD)

Evaluating claims

- Evidence for the claims surrounding the health risks associated with trans-fats often comes from '**cohort studies**'
  - Eg. Greenland Eskimos, whose diet is rich in oily fish and meat, have a very low incidence of heart disease
- Other **epidemiological studies** can establish correlations between diet and incidence of disease
- Whilst **it is rare to find a direct causal link** between fat intake and heart disease, the many claims about fats **suggest strongly** that trans-fats have an overall **detrimental effect** on health when consumed in high quantities
- Other conditions linked to trans-fats include
  - Allergy
  - Breast cancer
  - Colonic cancer
  - Cardiovascular diseases
  - Premature birth

- Preeclampsia (a condition associated with pregnancy)
- Disorders of the nervous system
- Vision defects in infants
- Diabetes
- Obesity

YOUR NOTES

Exam Tip

- It is important to remember that correlation does not always mean causation
  - **Correlation** is an association or relationship between variables
  - **Causation** occurs when one variable has an influence or is influenced by, another
  - There is a clear distinction between correlation and causation: a correlation does not necessarily imply a causative relationship

YOUR NOTES

Long Term Storage

Lipids are excellent storage compounds

- Lipid macromolecules, like carbohydrates, contain **carbon, hydrogen** and **oxygen** atoms
- However, unlike carbohydrates, lipids contain **a low proportion of oxygen**
- More of the oxygen required for their respiration has to come from **the air**
- This allows lipids to be **energy-dense**, maximising the energy content per gram versus carbohydrates
  - They contain **2× more energy per gram** than most carbohydrates
  - **Less body mass** is required to store a given amount of energy
- Lipids are **insoluble** so do not affect osmosis, so do not risk upsetting the water balance of the organism
- When lipids are respired, **a lot of water is produced** compared to the respiration of carbohydrates
  - This is called **metabolic water** and can be used as a **dietary water source** when drinking water is unavailable
    - A **camel's hump** is not a water sac, it is a lipid-rich storage organ that yields metabolic water for the camel in its dry desert habitat
    - A **bird's egg** also makes use of lipid-rich yolk to provide energy and metabolic water to the growing chick
- All these features make lipids **ideal for long term energy storage**

Forms of lipid storage

- In **animals**, lipids are stored in various areas
  - **Subcutaneous** fats are stored below the skin
  - **Visceral** fats are stored around the major internal organs
- There are **genetic** and **gender differences** between how individuals store fat
- Fat is stored in **adipose cells**, which are specialised to contain large globules of fat
- **Adipose cells shrink** when the fat is respired to generate metabolic energy
- In many plants, **seeds** have evolved to store fats to provide energy for a growing seedling plant
- **Olives**, **sunflowers**, **nuts**, **coconuts** and **oilseed rape** are good examples of crops whose oils are harvested for edible oil production by humans

Other roles of lipids

- As well as energy storage molecules, lipids have a **number of other roles**
  - Physical **protection of soft organs** eg. visceral fat around the heart
  - **Thermal insulation** from subcutaneous fat eg. whale blubber
  - Subcutaneous fat as a **buoyancy aid** eg. in seals (fat is less dense than water so assists flotation)
  - **Waterproofing secretions** eg. birds' preening glands or waxy cuticles on leaf surfaces
  - **Electrical insulation** eg. the myelin sheath around certain nerve axons
  - Certain **photosynthetic pigments** eg. carotenoids

- **Glycolipids**, typically as cell-surface recognition molecules/receptors

YOUR NOTES

Exam Tip

Ensure that you are familiar with the structure of a triglyceride and that you can recognise whether the fatty acids are saturated or unsaturated.

Lipids: Health Claims

YOUR NOTES

- Lipids have been **associated with poor health** for a long time, even though they perform vital functions in tissues and organs
- High-fat diets tend to supply **more chemical energy** than an individual needs
  - Consuming excess fat can cause an individual to become **overweight** or **obese** due to the **storage of fat** in adipose tissue
- **Body Mass Index** (BMI) is a rough and ready measure of a person's mass in relation to their height
- The calculation of BMI is as follows

$$\text{Body Mass Index} = \frac{\text{Body mass (kg)}}{\text{Height}^2 \text{ (metres)}}$$

  - A BMI below 18.5 is considered **underweight**
  - A BMI 18.5–24.9 is considered **normal**
  - A BMI of 25.0–29.9 is considered **overweight**
  - A BMI of 30.0–39.9 is considered **obese**
  - A BMI of 40.0 or more is considered **morbidly obese**
- BMI is a **crude measurement** as it works against individuals who are heavily muscular but who are also extremely lean
- Overweight and obese people have a higher risk of developing **type II diabetes** and **high blood pressure** and **coronary heart disease**
- Because many risk factors combine in the prevalence of these conditions, **lipids are by no means the only cause**

NOS: Evaluating claims; health claims made about lipids in diets need to be assessed

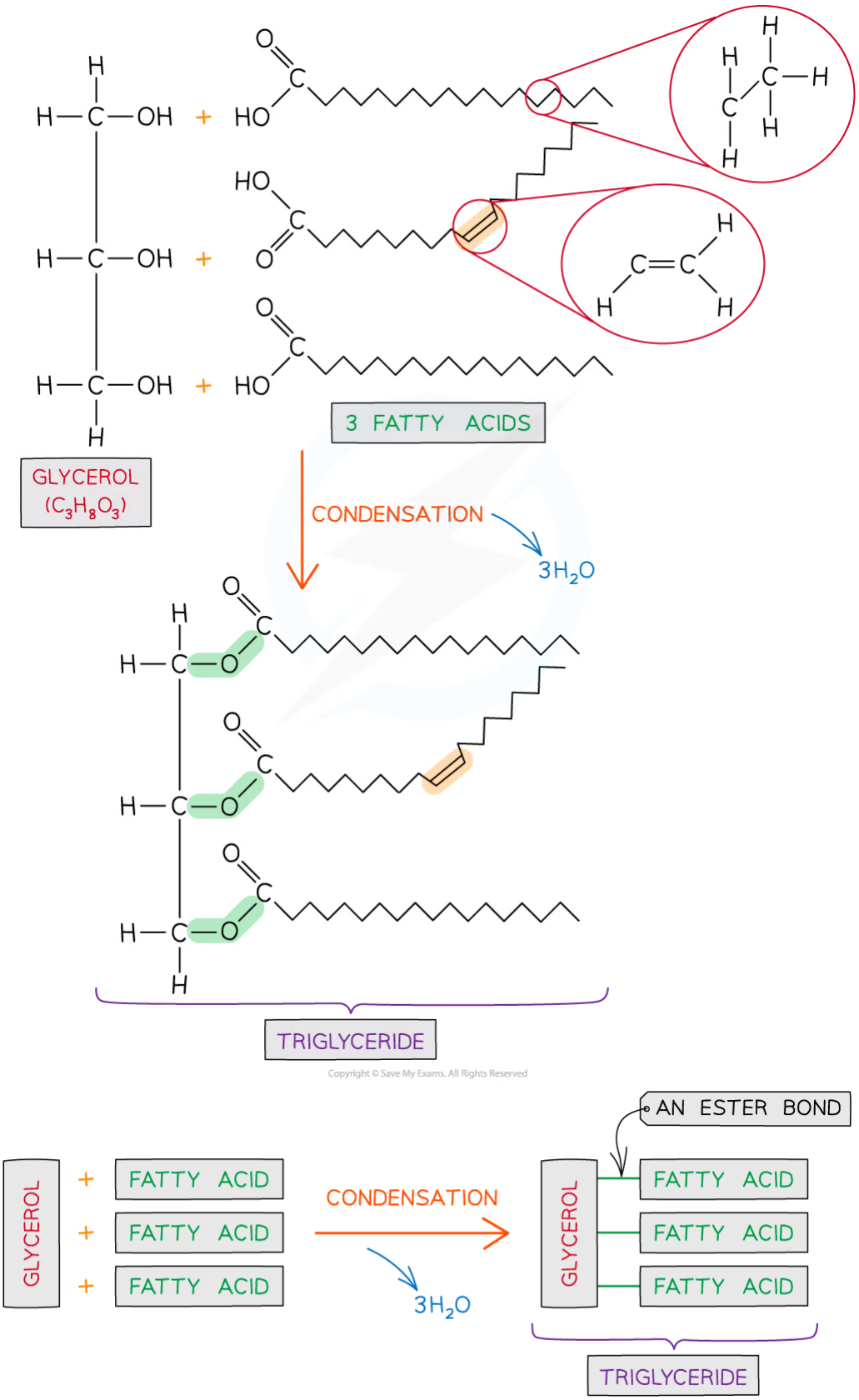
- Popular literature, TV and social media make **claims about various foods** and their health benefits
- A food product labelled, 'Low Sugar' may in fact contain a lot of trans-fats but **hides that information**, or doesn't label it at all!
- Many health claims are based on **pseudoscience**, or backed up with only very small trials or small samples sizes
- Only **scientifically controlled studies** are able to prove **causal links** between food choices and health risks
- Techniques such as **randomised clinical trials** provide data to **inform government policy** and consumers about their food choices
- There remain **complex challenges** for consumers, food producers and governments to ensure a food supply that puts people **at least risk of disease** whilst ensuring that enough food is produced

Formation of Lipids

YOUR NOTES

- Triglycerides are formed by esterification
- An **ester bond** forms when the hydroxyl (-OH) group of the glycerol bonds with the carboxyl group (-COOH) of the fatty acid
    - The formation of an ester bond is a **condensation reaction**
        - For each ester bond formed a water molecule is released
    - **Three fatty acids** join to **one glycerol** molecule to form a **tri**glyceride
    - Therefore for one triglyceride to form, **three water molecules** are released

YOUR NOTES



Formation of a triglyceride from a glycerol molecule and three fatty acid molecules by the process of esterification

YOUR NOTES

YOUR NOTES

Molecular Visualisation: Carbohydrates

- **Online learning tools** exist that can provide **visualisations** of large biomolecules
- Mouse functions can **zoom** and **rotate** molecules
- Many of these make use of **JMol** software, a database of large molecules that can be visualised in near-3D
- The differences between **cellulose, starch** and **glycogen** can be observed in close to 3-D quality
- An Internet search for '**molecular visualisation software**' will identify some good options
- Features include
  - Loading multiple molecules to show **independent movement**
  - **Surface topography**; as many biological reactions are on a theme of **3-D shapes fitting together**
  - **Cavity visualisation**; has applications when looking at structures eg. channel proteins in membranes
  - The **appearance of atoms can be adapted** to fill space or **show gaps in molecules** eg. the helical nature of amylose
- Having a visualisation of these molecules helps to understand **how they have evolved to fulfil their specific functions**

YOUR NOTES

Body Mass Index Calculations

- Body Mass Index (BMI) is a **rough and ready measure** of a person's mass in relation to their height
- The calculation of BMI is as follows

$$\text{Body Mass Index} = \frac{\text{Body mass (kg)}}{\text{Height}^2 \text{ (metres)}}$$

- A BMI below 18.5 is considered **underweight**
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- A BMI of 25.0–29.9 is considered **overweight**
- A BMI of 30.0–39.9 is considered **obese**
- A BMI of 40.0 or more is considered **morbidly obese**
- BMI is a crude measurement as it works against individuals who are heavily muscular but who are also extremely lean; their BMI might be an overestimate
- BMI may also be misleading in the case of elderly people who have lost a lot of muscle mass; their BMI might be an underestimate

Worked Example

Calculate the Body Mass Index (BMI) for an adult male whose mass is 77.3kg and who is 1.73m in height.

Comment on whether his BMI would be regarded as healthy or not.

Step 1: Ensure that mass and height are expressed in the correct units

Mass units are kg - this is correct

Height units are in metres - this is correct

Step 2: Use the formula

$$\text{Body Mass Index} = \frac{\text{Body mass (kg)}}{\text{Height}^2 \text{ (metres)}}$$

$$\text{BMI} = \frac{77.3}{1.73^2} = 25.8$$

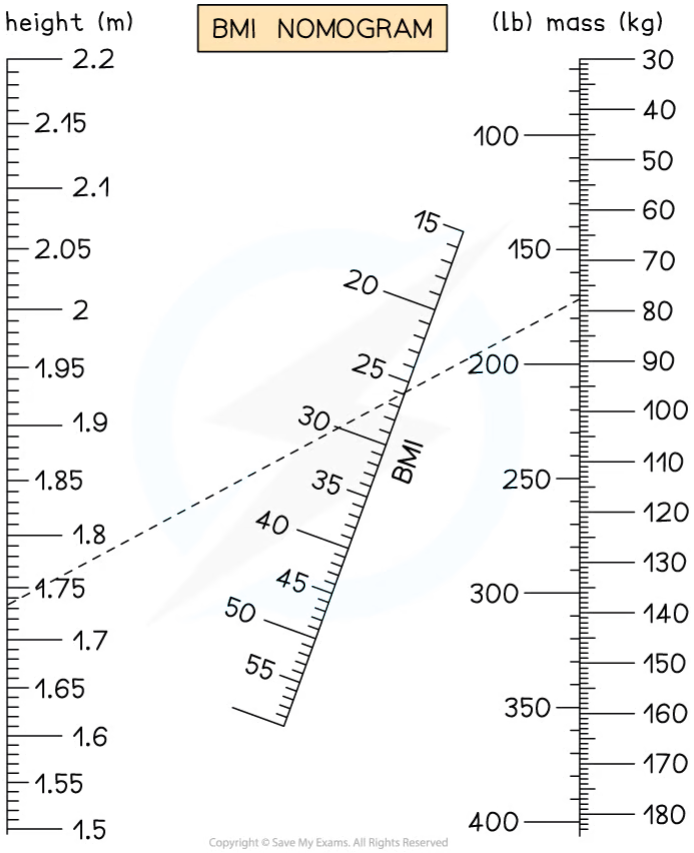
This man's BMI falls just into the 'overweight' category (25.0 - 29.9). It is not possible to judge his health by this measure alone, but if his BMI has been trending upwards over recent time, this might suggest to a doctor that there is an underlying cause of his weight gain.

A nomogram can help to calculate BMI

- A nomogram is a **two-dimensional chart** that allows rapid estimation of BMI by reading off two scales, one for mass and one for height
- A line is drawn, between the two scales, that **intersects a third axis** in the middle
  - This reveals the **BMI**

YOUR NOTES

- This **removes the need for a calculation** and requires **no mathematical expertise**
- Because the relationships between mass, height and BMI are **all fixed**, they can be represented on a chart like this
- Nomograms have been **largely superseded** by rapid **online calculators** or **smartphone apps**, but still have a use
    - For example, **doctors** and **health workers** will often have a BMI nomogram on the wall of their office for rapid reference when consulting a patient



A BMI nomogram. A line can be drawn from the height scale to the mass scale. Where the line intersects the BMI scale is the person's BMI (26 in this case)

Exam Tip

We commonly discuss a person's size as 'weight', though strictly speaking, we should refer to their 'mass'. In your written answers, use the scientifically correct term wherever possible.

YOUR NOTES

2.3 Proteins

2.3.1 Proteins

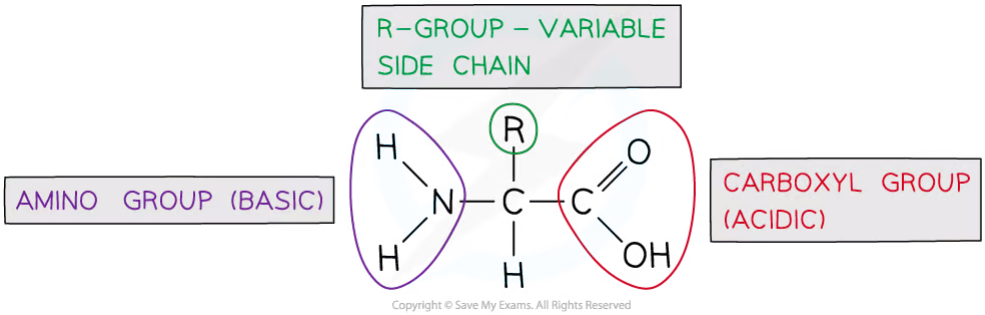
Amino Acids & Polypeptides

Proteins

- Proteins are polymers (and macromolecules) made of monomers called **amino acids**
- The **sequence, type** and **number** of the amino acids within a protein determines its shape and therefore its function
- Proteins **are extremely important in cells** because they form all of the following:
  - **Enzymes**
  - Cell membrane proteins (eg. carrier)
  - **Hormones**
  - Immunoproteins (eg. immunoglobulins)
  - **Transport** proteins (eg. haemoglobin)
  - **Structural** proteins (eg. keratin, collagen)
  - **Contractile** proteins (eg. myosin)
- Because all genes code for proteins, **all of the reactions necessary for life** are dependent on the function of proteins

Amino acids

- Amino acids are the **monomers** of polypeptides
- There are **20 amino acids** found in polypeptides common to all living organisms
- The general structure of all amino acids is a central carbon atom bonded to:
  - An **amine** group -NH_2
  - A **carboxylic acid** group -COOH
  - A **hydrogen** atom
  - An **R** group (which is how each amino acid differs and why amino acid properties differ e.g. whether they are acidic or basic or whether they are polar or non-polar)
  - The **R** group can be as simple as another hydrogen atom (glycine), right through to complex aromatic ring structures (eg. phenylalanine)

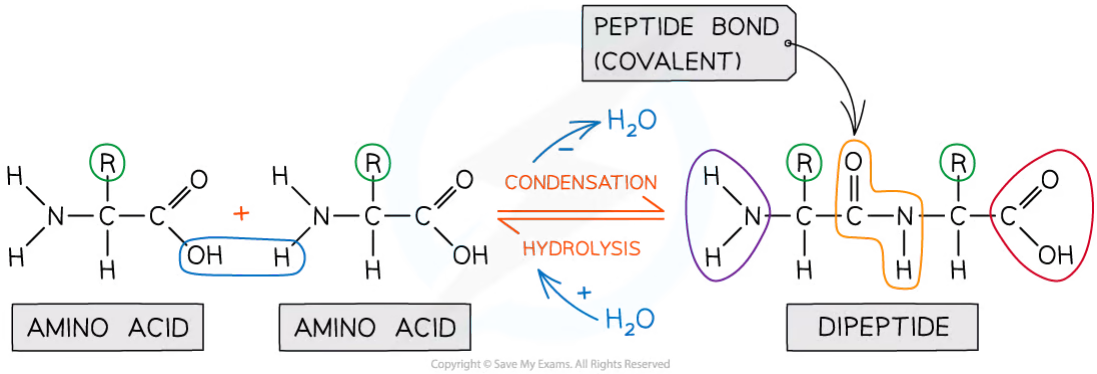


The generalised structure of an amino acid

Peptide bond

YOUR NOTES

- In order to form a **peptide bond** a hydroxyl group (-OH) is lost from the carboxylic group (-COOH) of one amino acid and a hydrogen atom is lost from the amine group (-NH2) of another amino acid
- The remaining carbon atom (with the double-bonded oxygen) from the first amino acid bonds to the nitrogen atom of the second amino acid
- This is a **condensation** reaction so water is released
- **Dipeptides** are formed by the condensation of **two** amino acids
- **Polypeptides** are formed by the condensation of **many** (3 or more) amino acids
- A protein may have only one polypeptide chain or it may have multiple chains interacting with each other
- During **hydrolysis** reactions, the addition of water **breaks the peptide bonds** resulting in polypeptides being broken down into amino acids



Amino acids are bonded together by covalent peptide bonds to form a dipeptide in a condensation reaction

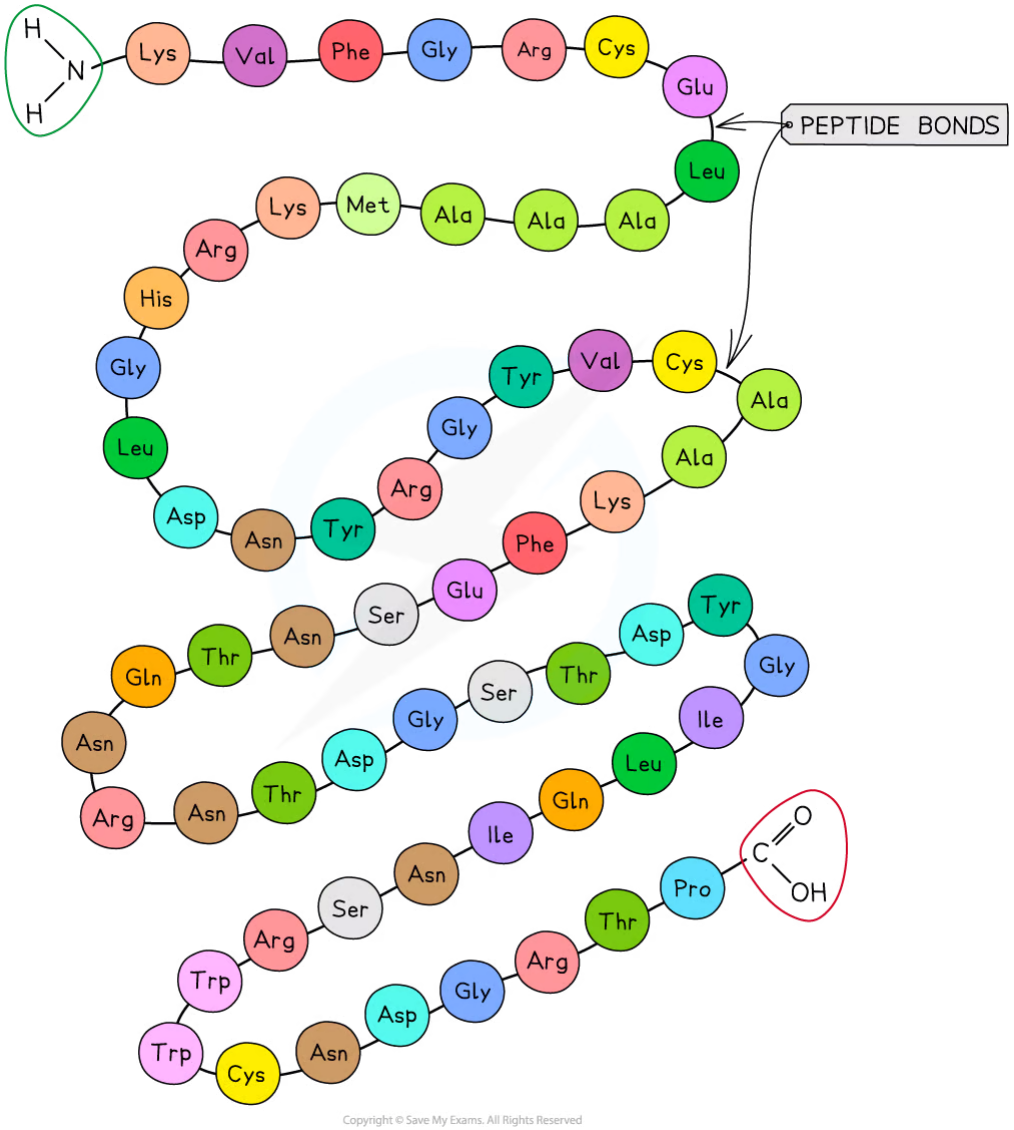
Exam Tip

You will be expected to recognise whether an unfamiliar molecule is an amino acid or polypeptide so look for the functional groups (amine and carboxyl). When asked to identify the location of the peptide bond, look for where nitrogen is bonded to a carbon that has a double bond with an oxygen atom, note the R group is not involved in the formation of a peptide bond.

Amino Acid Diversity

YOUR NOTES

- The same 20 amino acids make up most of the proteins found on Earth
- Around 500 amino acids have been found in nature, but only **20 are commonly found in proteins**
- **Eleven** of these can be naturally synthesised within cells by humans
- The other nine amino acids are **essential** (have to be in the human diet)
- **You don't need to remember the names of the amino acids**, but it's useful to see their names, which are usually **abbreviated to three letters**
  - Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His*, Ile*, Leu*, Lys*, Met*, Phe*, Pro, Ser, Thr*, Trp*, Tyr, Val*
  - * indicates the essential amino acids
- Because the R groups vary so much between the 20 amino acids, there is a **lot of chemical diversity** between the amino acids



YOUR NOTES

An amino acid sequence of a short polypeptide. The three-letter abbreviations indicate the specific amino acid (there are 20 commonly found in cells of living organisms).

NOS: Looking for patterns, trends and discrepancies; most (but not all) organisms build proteins from the same amino acids.

- **All life** except for a few species use these 20 amino acids
- The reason why only 20 amino acids are used has been the subject of a lot of differing hypotheses
  - That only these 20 were available at the origins of life, so have remained ever since, **OR**
  - That these 20 amino acids are diverse enough to give the wide range of functions that proteins possess, **OR**
  - Because of the theory that all organisms share a common ancestor, the link between the genetic code and amino acid sequence is fixed and is not easily altered, even by mutations
- The almost **infinite number of amino acid combinations** make polypeptides suitable to determine **all the characteristics of life**
- Only a few primitive, single-celled organisms use other amino acids
- One unusual amino acid includes the trace element **selenium** and is found in many polypeptides, though at **very low frequencies**
  - A discrepancy is that in some organisms, the stop codon UAG codes for this unusual amino acid containing selenium
- All life goes by the **Central Dogma** that **all genes code for proteins** and the actions of proteins determine all of an organism's characteristics

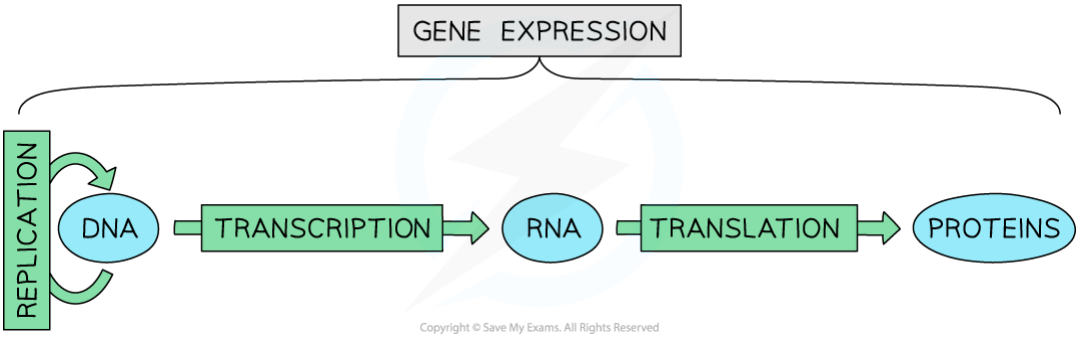
Polypeptide Diversity

- 20 amino acids can give an almost infinite number of polypeptides
- Polypeptides are assembled at a ribosome by condensing **individual amino acids** onto a growing chain, **one by one**
- This allows a **choice of 20 amino acids** each time one is added
- The **mRNA codon** determines which amino acid is added
- For a polypeptide chain of 50 amino acids in length (considered a very **short protein**), there would be $\mathbf{20^{50}}$ possible combinations of amino acids
  - This gives 1.13×10^{65} combinations
  - **Standard form** is preferable for showing such a large number, but writing it out in full shows its size, which is
  - 113,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000 combinations!
- Given that the average length of a protein is **300 amino acids**, the number of possible combinations is so large, we can consider it to be **infinite**

Genes & Polypeptides

YOUR NOTES

- The amino acid sequence of polypeptides is coded for by genes
- Despite the huge number of amino acid sequences that could be produced, only a **small fraction** of these are produced in nature
- Nevertheless, **many thousands of different polypeptide sequences** are synthesised
- The code for the sequence in which amino acids are joined together is the **genetic code**, held in a sequence of DNA bases in the **genome**
- The **expression** of a gene always results in the production of a polypeptide
- **Three consecutive DNA bases** are required to code for **each amino acid** in a polypeptide



The central dogma of gene expression. All genes code for proteins; proteins carry out the genes' instructions.

YOUR NOTES

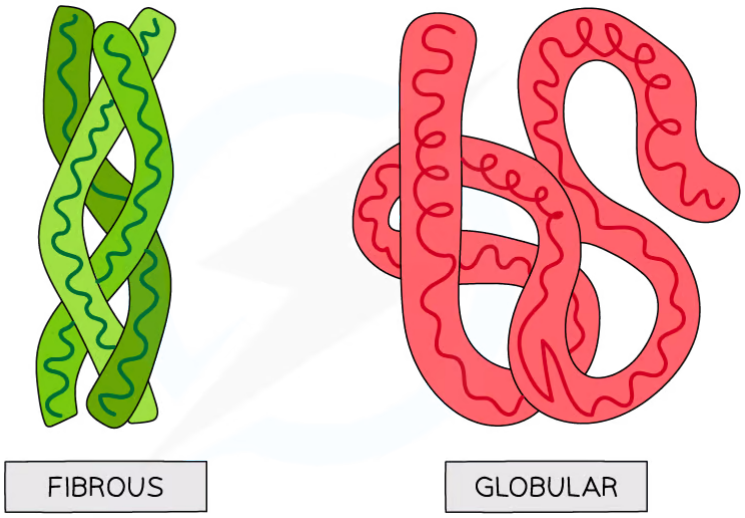
Protein structure

- A protein may consist of a single polypeptide or more than one polypeptide linked together
- Some proteins exist as a **single polypeptide chain** (of amino acids)
- Other proteins are made up of **two or more polypeptide chains** joined together
- **Single polypeptide chain** proteins include **lysozyme**, an enzyme present in mucus secretions and tears, that **kills bacteria** as part of our primary defences against pathogens
- Proteins with **two polypeptide chains** include
  - **insulin**, a hormone responsible for regulating blood glucose levels
  - **integrins**, a group of membrane proteins that span a **phospholipid bilayer** and act as a receptor
    - integrins' two polypeptide chains each have a **hydrophobic section** that sits in the membrane bilayer
- Proteins with **three polypeptide chains** include **collagen**, the main structural protein in skin, tendons, ligaments and the walls of blood vessels
- Proteins with **four polypeptide chains** include **haemoglobin**, which binds oxygen in red blood cells and delivers it from the lungs to respiring tissues
- Each polypeptide chain in a multi-polypeptide protein is referred to as a **subunit** of the protein

YOUR NOTES

3D Structure of Proteins

- The amino acid sequence determines the three-dimensional conformation of a protein
- Proteins perform their diverse roles because of their 3-D shape and structure
- This is known as the protein's **conformation**
- The **precise sequence of amino acids** determines how the protein **folds** and aligns itself **as the individual amino acids are being added** at the ribosome
  - Amino acids are always added in the same sequence so a **protein can start to form its shape even** before it is fully formed
  - **Bonds form** between parts of amino acids that can cause a **bridge** to form between one part of the chain and another
    - this creates **loops**, **sheets**, **helices** and **folds**
  - Many of the bonds that hold the protein's shape form between the various **R groups** of individual amino acids
    - If an amino acid is not present in its usual place in the chain due to **mutation**, this can drastically alter the protein's 3-D shape, and affect its function
- Haemoglobin is a **globular** protein (forms a globe-shaped protein)
  - Some of haemoglobin's outer parts are **hydrophilic** to be in contact with water whilst its inner parts are made up of amino acids with **hydrophobic** R groups
- Collagen is a **fibrous** protein (forms a rope-like protein for tensile strength)
  - It has a **repeating sequence of amino acids** to create a helical structure
  - The chain of amino acids **remains in an elongated conformation** to give fibrous strength

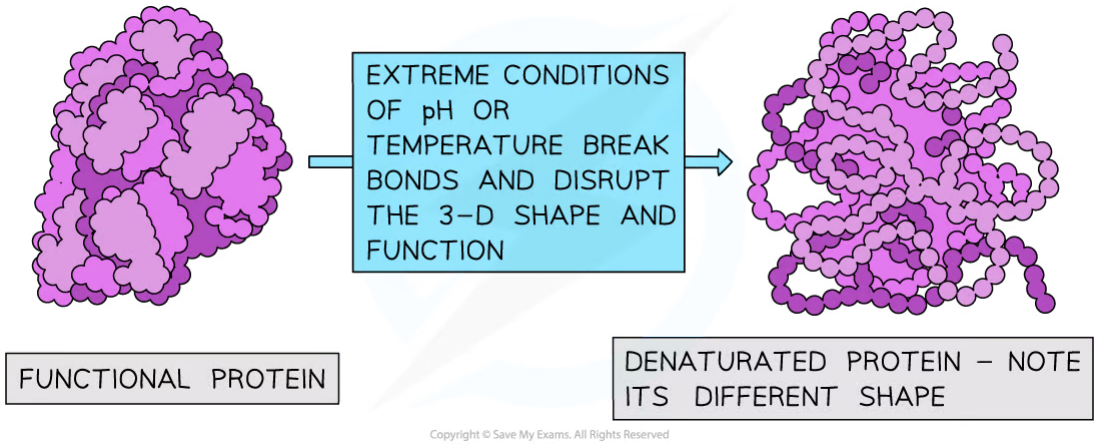


Globular and fibrous protein models illustrating the roughly spherical shape of globular proteins and the long, stranded shape of fibrous proteins

YOUR NOTES

Denaturation: Proteins

- Denaturation is the irreversible change of protein conformation caused by temperature and pH extremes
- The bonds that form **between different R groups** are **relatively weak** (compared to the peptide bonds that hold the amino acids in sequence)
- These bonds can be **broken easily,** which can cause the **conformation** of the protein to change
- The **altered protein shape** may affect its **function**, **physical state** and general usefulness in its original role
- This is called **denaturation**
- **Heat** and **extremes of pH** are the most common causes of denaturation
  - Both cause **breaking of the weak bonds** between R groups
- A certain pH is considered as an **optimum** for a particular protein, because at that pH, the protein's 3-D structure is not denatured
- Denaturation is almost always **irreversible**
  - The protein **cannot be re-formed** in its original conformation by reversing the change in conditions
  - However, **small denaturations** and **renaturations** are possible in certain proteins to respond to small fluctuations in pH eg. haemoglobin



The effect of heat and pH on the shape and function of a globular protein

Denaturation in action

- Denaturation can be seen most easily by looking at the **changes in an egg white** as the egg is fried or poached
- Egg white is mainly the protein **albumin**
- The **hydrophobic amino acids** in albumin are at the centre of the molecule in its normal state, so albumin is soluble
- Heating causes the hydrophobic amino acids to appear **at the edges**, where they cause the protein to become **insoluble**
- A harder, solid layer forms, which is the **cooked white**
- Similar events occur in the proteins of the **egg yolk** as it cooks

YOUR NOTES

- Denaturation also occurs in the **stomach**, where the low pH (pH2) causes **proteins in the diet to become denatured** on their way to being fully hydrolysed further down the digestive system
- The stomach enzyme **pepsin**, a protein-digesting enzyme has an optimum pH of 2 for this reason
- Certain **extremophiles** have evolved to have proteins that are stable even at extreme pH or temperature
  - eg. *Thermus aquaticus*, a **bacteria that lives in hot springs** at 80°C
  - This temperature would denature most other proteins
- **Denaturation of enzymes** can be used as part of experiments to measure enzyme activity
  - For example, **an experiment to establish the optimum pH or temperature** of an enzyme eg. pepsin or lipase
- Many drugs are proteins that **cannot be taken by mouth**, because the protein will be **denatured by stomach acid**
  - These drugs should be **delivered in another way** eg. by **direct injection** into the blood

Exam Tip

Remember to avoid confusing the bonds that hold a protein's shape together with the peptide bonds that attach each amino acid in sequence. Picture the peptide bonds holding the amino acids in a straight chain, then the other bonds holding the chain in its folded, 3-D structure.

YOUR NOTES

Functions of Proteins

- Living organisms synthesize many different proteins with a wide range of functions
- Proteins are so **versatile** that they have many different roles in cells, tissues and organs
- All of the following functions are performed by proteins:
  - Speeding up cellular reactions, or **catalysis**, is performed by **enzymes**
  - **Blood clotting**, where blood proteins interact with oxygen to form a gel-like scab across a wound
  - **Strengthening** fibres in skin, hair, tendons, blood vessels eg. **collagen, keratin**
  - **Transport** of vital metabolites eg. oxygen which is carried by **haemoglobin**
  - Formation of the **cytoskeleton**, a network of tubules within a cell that cause chromosomes to move during the cell cycle
  - **Cell adhesion**, where cells in the same tissue stick together
  - **Hormones**, chemical messengers that are secreted in one part of the body to have an effect elsewhere
  - **Compaction of DNA** in chromosomes for storage, caused by **histone** proteins
  - The immune response produces **antibodies**, the most diverse group of proteins
  - Membrane transport **channel and carrier proteins** that determine which substances can pass across a membrane
  - **Cell receptors**, which are binding sites for hormones, chemical stimuli such as tastes, and for other stimuli such as light and sound

Exam Tip

Many exam questions focus on enzymes but don't forget all the other types of protein when discussing protein functions.

Examples of Proteins

YOUR NOTES

Rubisco

- **R**ibu**l**ose **Bis**phosphate **C**arb**o**xylase
- An enzyme that catalyses the **fixing of CO_2 from the atmosphere** during photosynthesis
- Composed of **16 polypeptide chains** as a **globular** protein
- This is **the source of all organic carbon**, so Rubisco is arguably the most important enzyme in Nature!
- The **most abundant enzyme on Earth** as it's present in all leaves
- Rubisco is **a very slow catalyst**, but it's the most effective to have evolved so far to fulfil this vital function

Insulin

- A **hormone** produced and secreted by β-cells in the **pancreas**
- Binds to insulin receptors (on liver, fat and muscle cells) reversibly, causing **absorption of glucose from the blood**
- Composed of **2 polypeptide chains** as a **short, globular protein**

Immunoglobulins

- Also known as **antibodies**
- They have a **generic 'Y' shape**, with specific binding sites at the two tips of the 'Y'
- They bind to specific **antigens**
- The binding areas of immunoglobulins are **highly variable**, meaning that antibodies can be produced **against millions of different antigens**
- Immunoglobulins (as the name suggests) are **globular** and are the **most diverse range of proteins**

Rhodopsin

- A **pigment in the retina** of the eye
- A **membrane protein** that is expressed in rod cells
- Contains a light-sensitive part, **retinal**, which is derived from **Vitamin A**
- A photon of **light causes a conformational change** in rhodopsin, which sends a nerve impulse along the optic nerve to the **central nervous system**

Collagen

- A **fibrous protein** made of **three separate polypeptide chains**
- The **most abundant protein in the human body** - approx 25%
- Fibres **form a network** in skin, blood vessel walls and connective tissue that can **resist tearing forces**
- Plays a role in **teeth** and **bones**, helping to **reduce their brittleness**

Spider Silk

- The silk used by spiders to suspend themselves and create the spokes of their webs is as **strong as steel wire** though considerably lighter
- Contains **rope-like, fibrous parts** but also **coiled parts** that stretch when under tension, helping to **cause extension** and **resist breaking**
- Does not denature easily at extremes of temperature

YOUR NOTES

- Has many attractive aspects for **engineering** and **textile product design** thanks to its **strength** and **low weight**
- Can be **genetically engineered** to be **expressed in goats' milk** as spiders can't be farmed on a large enough scale
- Other kinds of spider silk protein are **tougher** though lack the tensile strength, eg. the silk they use to encase their prey after capture

Proteome

- The **proteome** is the full range of **proteins** that a cell or organism is able to produce
- By contrast, a **genome** is the complete set of **genes** present in a cell/organism
  - The full genome is present within every cell of an organism, but not every gene is **expressed** in every cell. Which genes are expressed, **depends on the cell type**
- The proteome is usually **larger** than the genome of an organism
- Every individual has a **different proteome**
  - Because of small differences in the amino acid sequence of proteins
- The **proteome varies during an organism's lifetime** as certain proteins are not needed throughout the organism's life
    - An example is **fetal haemoglobin**. The gene for that protein is not expressed after the baby is around 3 months old, as the baby expresses **adult forms of haemoglobin**, which are encoded by **separate genes**
  - This is also due to a large amount of **modification of proteins** that can take place after synthesis (often in the **Golgi** apparatus)
    - For example, adding a carbohydrate part to form **glycoproteins**, which are important in cell signalling
- **Splicing** of RNA during transcription can allow one gene to code for many proteins

Exam Tip

You don't need to know the details of splicing for Standard Level but it accounts for several proteins being produced from just one gene. Even though a lot of genes do not code for proteins, the proteome is larger than the genome because of the sheer range of proteins that can be produced from the DNA code.

YOUR NOTES

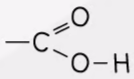
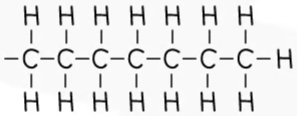
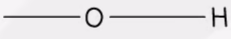
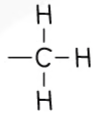
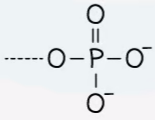
Molecular Diagrams: Drawing

Drawing biological molecules

- It is important to be able to **draw a few key molecules**
  - There is a huge variety of biological molecules, but **only the most important ones** are required

- **Element symbols** from the **Periodic Table** are used
- A **short, straight line** is used for a covalent bond, with **two lines** for a double bond
- Some chemical groups may be denoted by a **symbol** such as Ⓟ for a phosphate group
- The symbol **R** represents a **variable chemical group**, such as the variable side groups of amino acids
- An exam question may require you to **draw various molecules**
- It's advisable to break the task down **into stages**

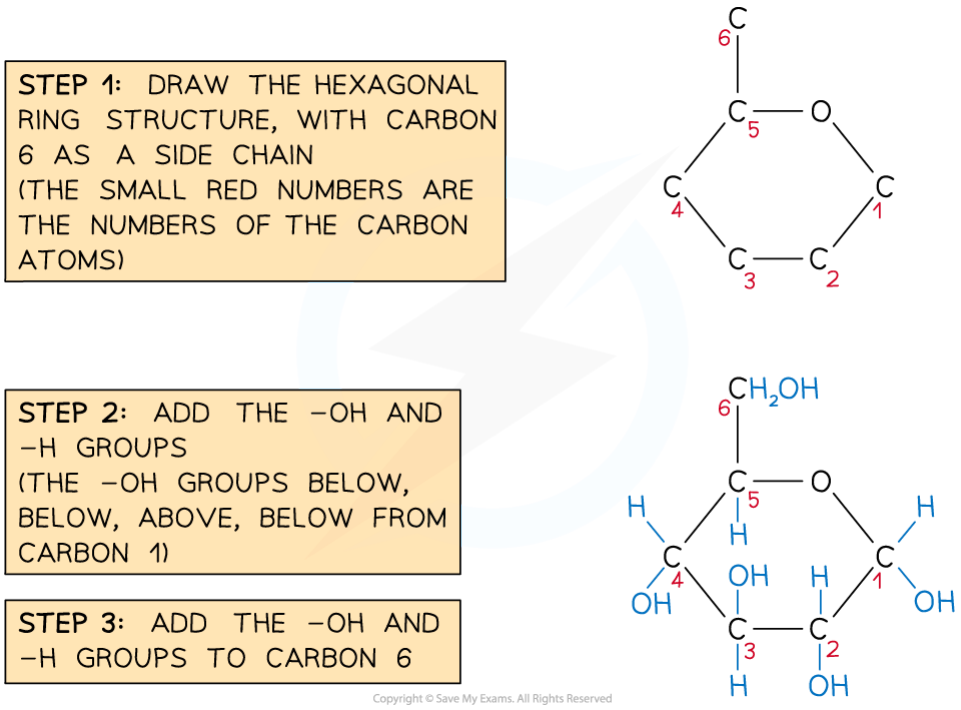
Symbols Used in Biological Molecule Drawings Table

Name of Group	Group Structure	Abbreviated Notation
Amine		$-NH_2$ or H_2N-
Carboxylic Acid		−COOH or HOOC−
Hydrocarbon Chain		
Hydroxyl		−OH or HO−
Methyl		$-CH_3$ or H_3C-
Phosphate		Ⓟ

Drawing α-D-glucose

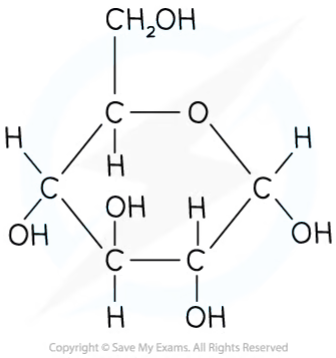
YOUR NOTES

- Aspects to remember
  - Glucose has the formula $C_6H_{12}O_6$
  - In solid form, glucose has a linear structure
  - It forms a **hexagonal** ring in an aqueous solution
    - As aqueous glucose is **the only state** that glucose exists in biology, it's the **ring structure** that should be learned
  - **One of the corners** of the ring (draw this in the top-right) is occupied by an **oxygen** atom
  - The 6th carbon occupies a **side chain** (top-left)
  - The **carbon atoms are numbered 1 to 6** starting on the right and working clockwise
  - The hydroxyl groups occupy positions above or below the ring as follows
    - Carbon atom 1 - below
    - Carbon atom 2 - below
    - Carbon atom 3 - above
    - Carbon atom 4 - below
- You can **ignore the 'D'** in the names alpha-D-glucose or beta-D-glucose
  - The only other version is L-glucose which plays **no significant role in biology**



Recommended steps to draw a molecule of α-D-glucose

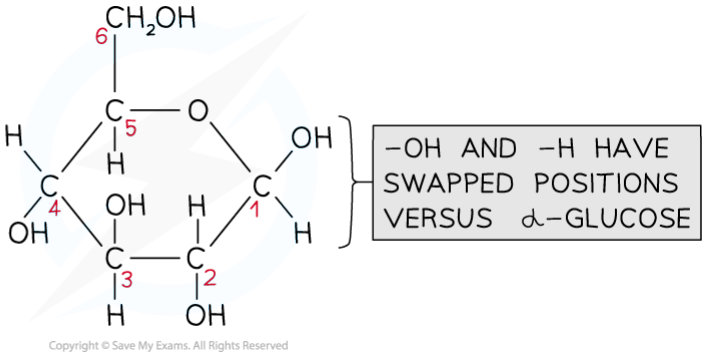
YOUR NOTES



Structure of α-D-glucose

β-D-glucose is very slightly different in structure

- Beta-glucose (β-glucose) has **a small, subtle difference** to α-glucose
- The hydroxyl group on carbon atom 1 **sits ABOVE the ring**, rather than below
- This sugar is the **monomer** of **cellulose**
- This example of two different **isomers** changes the properties of the polysaccharide formed from these monomers drastically
  - It accounts for all the many differences between starch and cellulose
- The hydroxyl groups occupy positions above or below the ring as follows
  - Carbon atom 1 - **above**
  - Carbon atom 2 - below
  - Carbon atom 3 - above
  - Carbon atom 4 - below



Structure of β-D-glucose

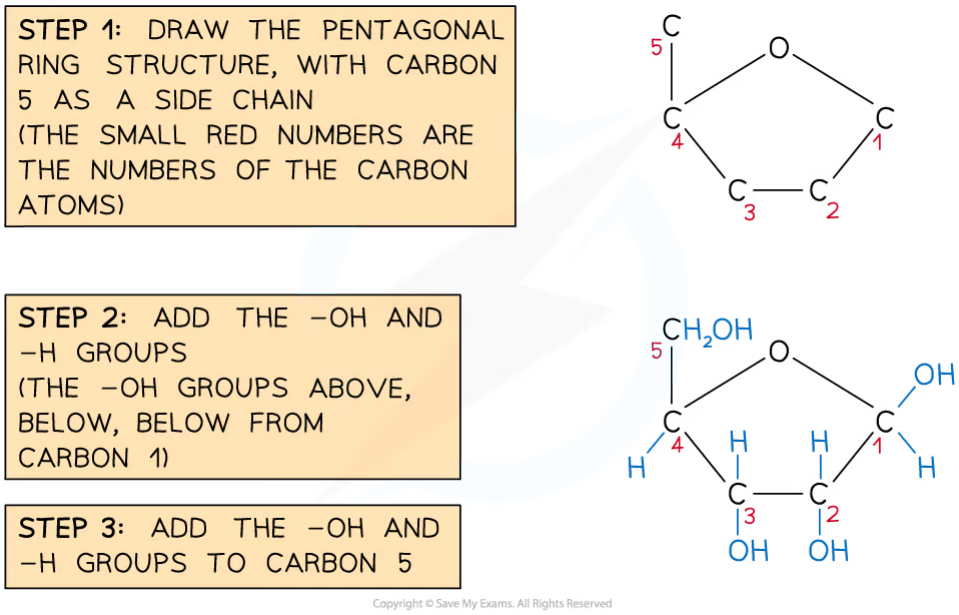
Drawing a ribose sugar

- This **family of sugars** play a role in DNA and RNA structure, as well as ATP
- Ribose is a form of **pentose** sugar (5 carbon atoms)
- Like glucose, ribose has a **ring** structure
- Aspects to remember
  - Ribose has the formula **$C_5H_{10}O_5$**
  - It forms a **pentagonal ring** in an aqueous solution
  - **One of the corners** of the ring (draw this in the top) is occupied by an **oxygen** atom
  - The 5th carbon occupies a **side chain** (top-left)

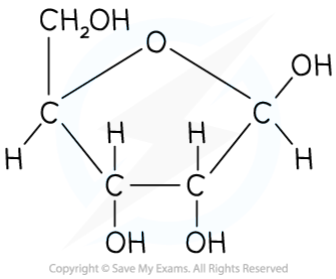
YOUR NOTES ⬇

- The **carbon atoms are numbered 1 to 5** starting on the right and working clockwise
- The hydroxyl groups occupy positions above or below the ring as follows
  - Carbon atom 1 - above
  - Carbon atom 2 - below
  - Carbon atom 3 - below

- Ribose sugars have an important close relative - **deoxyribose** sugars
  - Both are key components of **RNA** and **DNA** respectively
  - The 'R' and 'D' of **R**NA and **D**NA comes from the sugar in the structure, ribose or deoxyribose



Recommended steps to draw a molecule of ribose



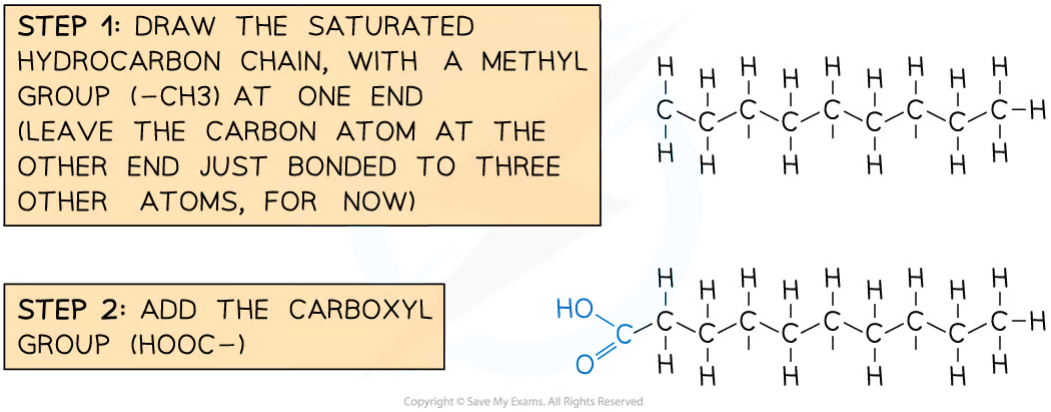
Structure of ribose

Drawing a saturated fatty acid

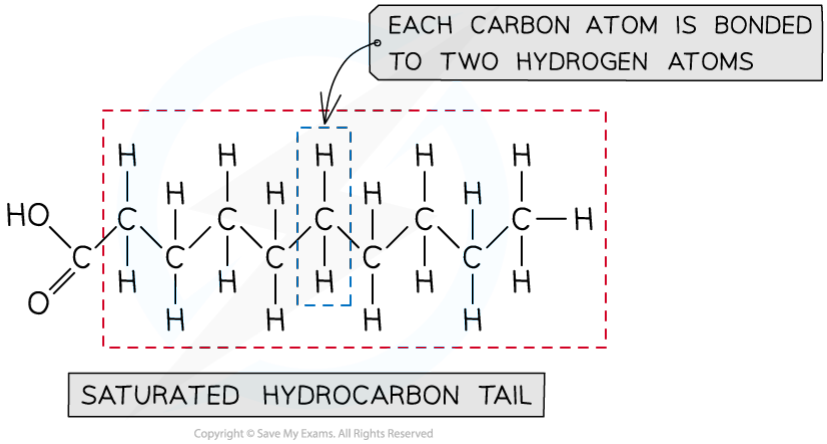
- There are two aspects to a saturated fatty acid
  - A **saturated** hydrocarbon chain
    - Contains **only C-C single bonds**
    - Each internal carbon atom is bonded to **2 hydrogen atoms**
  - A **carboxylic acid group** at one end

YOUR NOTES

- You don't need to memorise any names of saturated fatty acids
  - The number of carbon atoms in your chain is also not important, but greater than around 8 is advised.



Recommended steps to draw a molecule of a saturated fatty acid

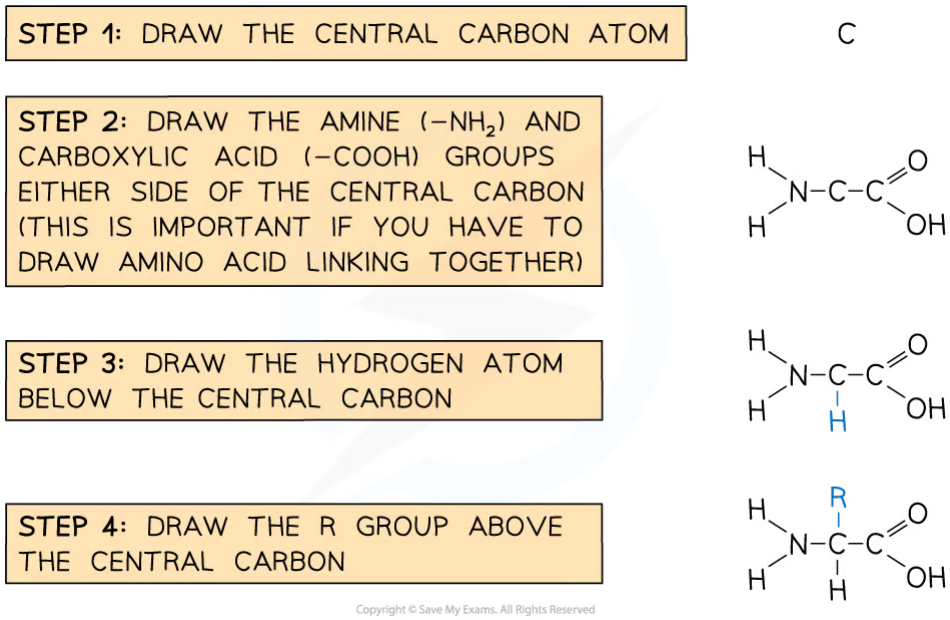


A saturated fatty acid

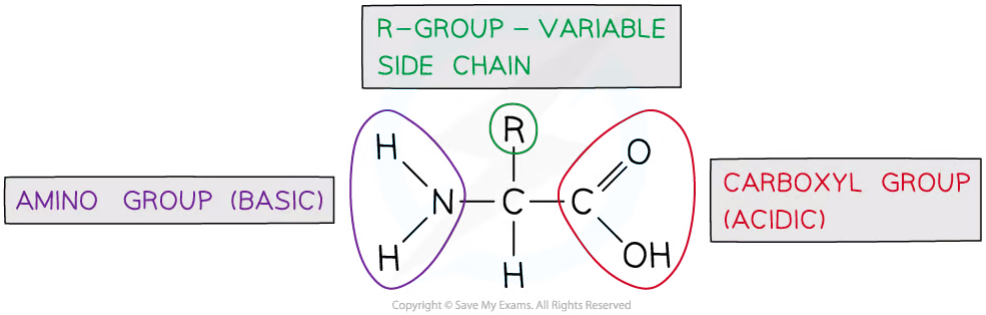
Drawing a generalised amino acid

- Each amino acid has **central carbon atom**
- Three of the bonds from the central carbon atom are occupied as follows
  - a **hydrogen atom**
  - a **carboxylic acid** group
  - an **amine** group
- The fourth bond attaches the central carbon to the **R group**
  - The R group is **variable** and determines the **identity of the amino acid**
  - You won't need to remember any of the R groups or amino acid names
- Drawing the 4 groups surrounding the central carbon in a **flat structure** is acceptable, although the real arrangement of bonds around a carbon atom is in a **tetrahedral shape**

YOUR NOTES



Recommended steps to draw a molecule of generalised amino acid



The generalised structure of an amino acid

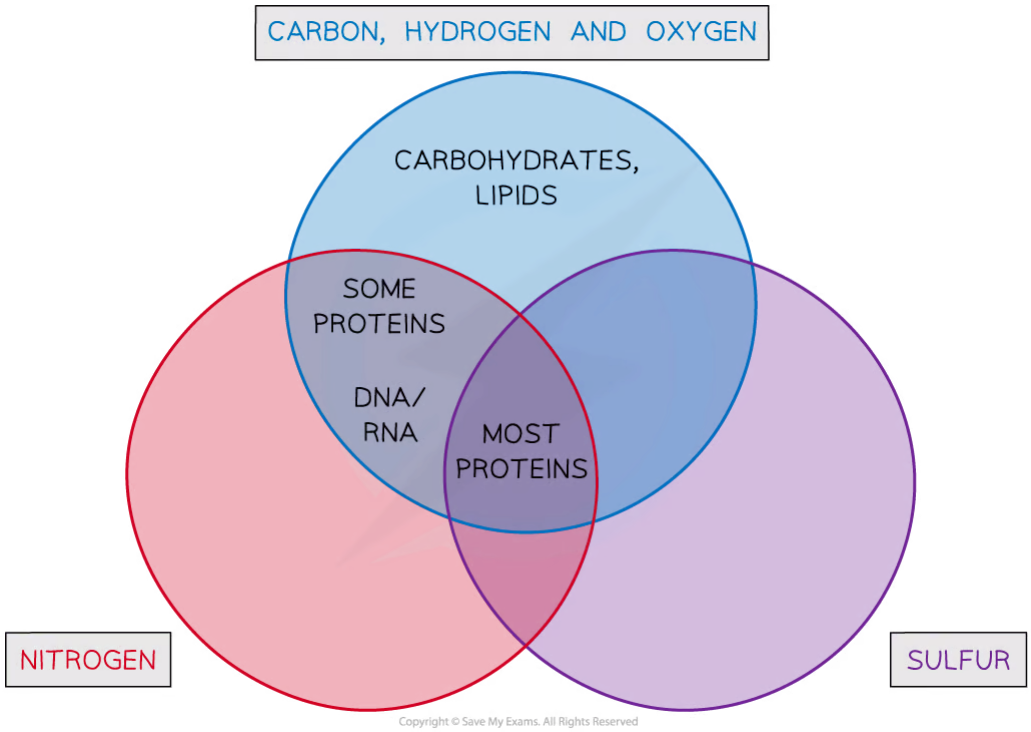
Exam Tip

The specification is very clear about what you should remember and equally importantly, what you **don't** have to memorise in this topic.With all these drawings, practise with a pencil and plenty of paper until you get it right. It WILL stick! Once you've memorised it, 'draw' questions should be easy marks!

Molecular Diagrams: Identification

YOUR NOTES

- As well as being able to draw certain molecules, an important skill is being able to **recognise certain biochemicals** from molecular diagrams
- There are **several features** that help to identify molecules
- The presence of **carbon, hydrogen, oxygen, nitrogen, sulfur** and **phosphorus** can help in the identification
- All biological macromolecules contain **carbon**, **hydrogen** and **oxygen**
- **Nitrogen** and **sulfur** are present in **proteins**
- **Nitrogen** is present in nucleic acids (DNA, RNA)
- **Phosphorus** is also present in certain molecules (DNA, RNA and phospholipids)
- The presence of **ring structures**, **hydrocarbon chains**, **carbon-to-carbon double bonds**, **double-stranded** areas and the **ratio of carbon to oxygen** in a molecule all give clues about the molecule's identity



Using the Presence of Various Atoms to Identify Biochemicals

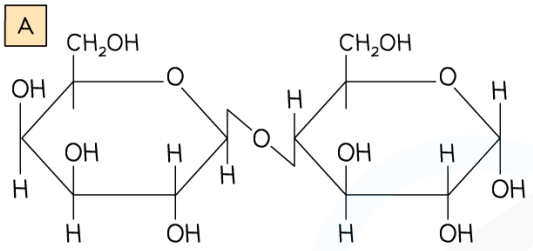
- **Glycosidic** bonds, **ester** bonds and **peptide** bonds all have a distinctive appearance in molecular drawings and will immediately **identify carbohydrates**, **lipids** and **proteins** respectively

YOUR NOTES

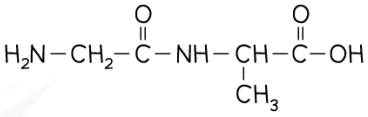
Worked Example

Identify the Diagram (A, B, C or D) which Shows a Triglyceride Structure

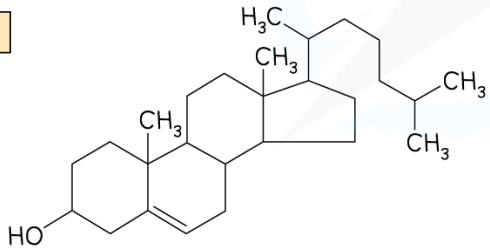
A



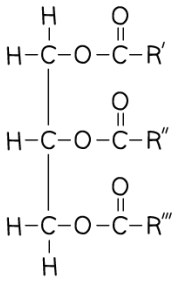
B



C



D



Step 1: Look at the elements present in all the diagrams

We're looking for a **triglyceride**, a type of lipid. Lipids contain C, H and O only. **B** also contains nitrogen (**B** is a dipeptide)

Eliminate Answer **B**

Step 2: Look for lipid structures

A contains ring structures, so is likely a carbohydrate (**A** is a disaccharide)

Eliminate Answer **A**

Step 3: Look for three hydrocarbon chains

D contains three hydrocarbon chains (attached by ester bonds to glycerol). **C** is cholesterol, which *is* a lipid, but not a triglyceride lipid.

Select Answer D

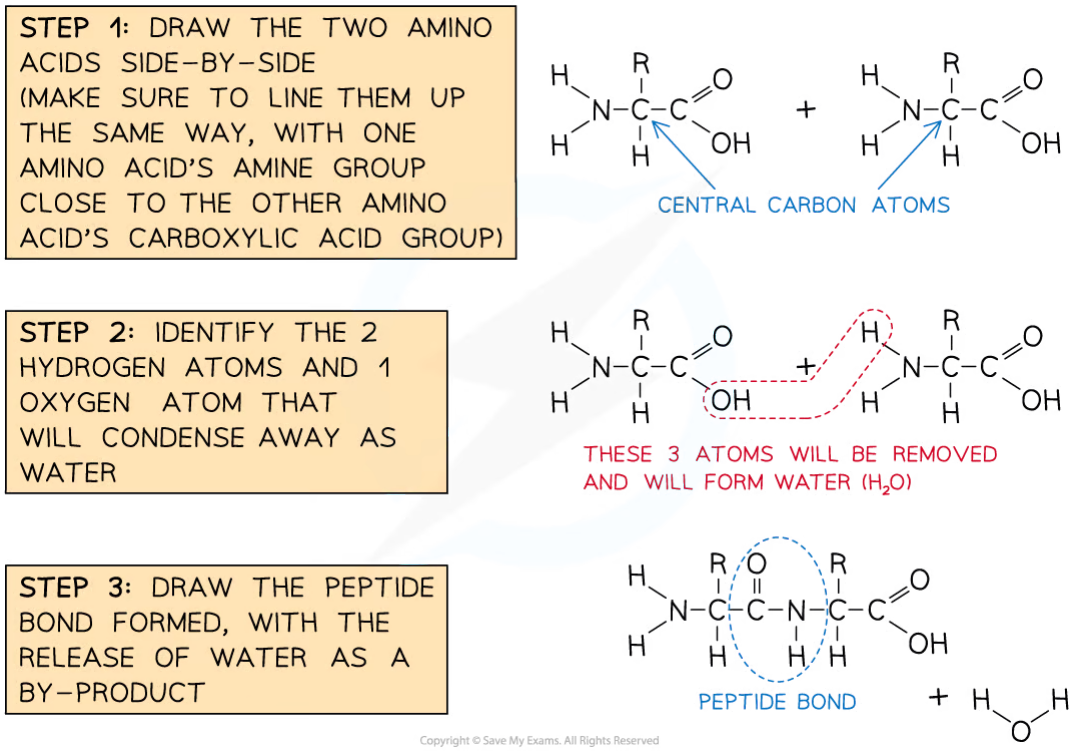
The ratio of hydrogen to oxygen

- The **numbers of hydrogen and oxygen atoms in a molecule** can help to identify it
- Carbohydrates contain **hydrogen and oxygen** in a **2:1 ratio**
  - Think of water, formula H_2O, and where the '-**hydrates**' part of the word 'carbo**hydrates**' comes from
- Lipids **contain a much lower proportion of oxygen** than carbohydrates eg. $C_{18}H_{34}O_2$, where the hydrogen to oxygen ratio is 17:1

Molecular Diagrams: Peptide Bond Formation

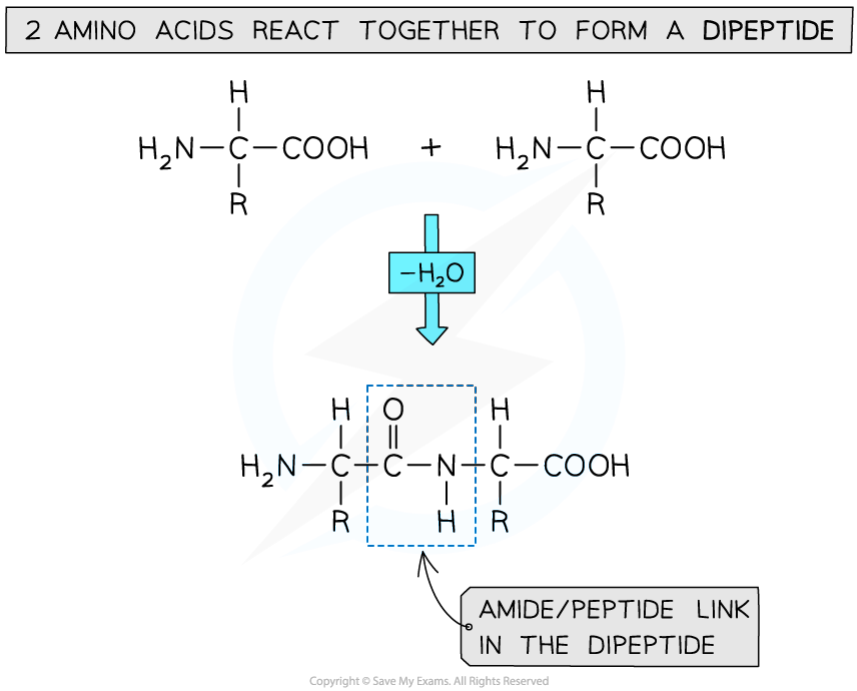
YOUR NOTES

- Having learned to draw the structure of a **generalised amino acid**, two or more of these can be joined together to **show how peptide bonds form** during protein synthesis
- Amino acid monomers link together via a **condensation** reaction
  - This releases a molecule of water (H_2O)
  - One **H** atom (of the released water) comes from **one amino acid's amine** group
  - The other **H** atom and an **O** atom come from the **other amino acid's carboxylic acid** group
- This knowledge can be **useful when drawing how two amino acids condense** to form a peptide bond



The recommended steps in drawing a peptide bond formation

YOUR NOTES



Formation of a dipeptide

YOUR NOTES

2.4 Enzymes

2.4.1 Enzymes

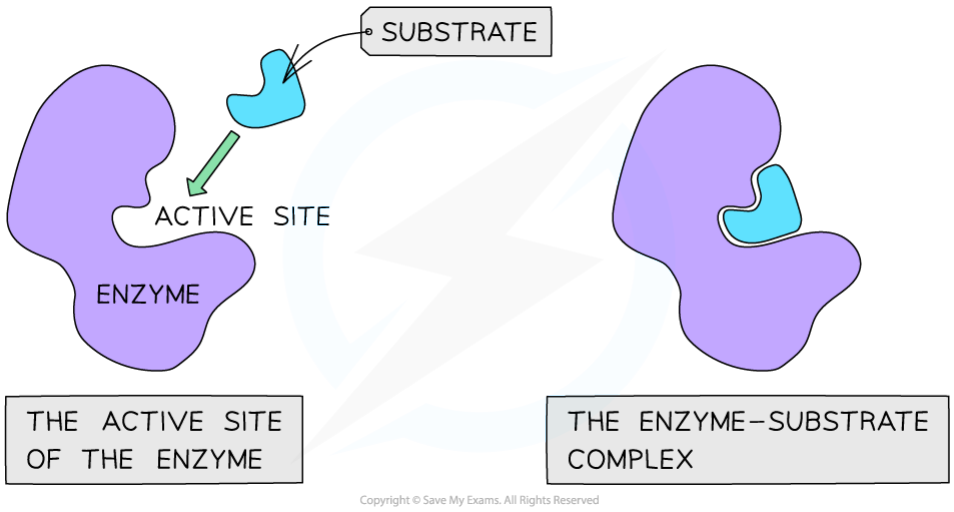
Structure of Enzymes

- Enzymes are **biological catalysts**
  - 'Biological' because they function in **living systems**
  - 'Catalysts' because they **speed up the rate of chemical reactions** without being used up or changed themselves
  - Enzymes have an active site to which specific substrates bind
- Enzymes are also **globular proteins**
- Critical to the enzyme's function is the **active site** where the **substrate** binds
- Enzymes are **specific** to the substrate
  - The shapes of the enzyme and substrate and their **chemical properties** are **complementary**, to allow the substrate to fit into the active site, like two jigsaw pieces fitting together
  - This is called **enzyme-substrate specificity**
- Due to this specificity, thousands of enzymes are needed throughout an organism, to carry out **individual chemical reactions**

Enzyme Activity

YOUR NOTES

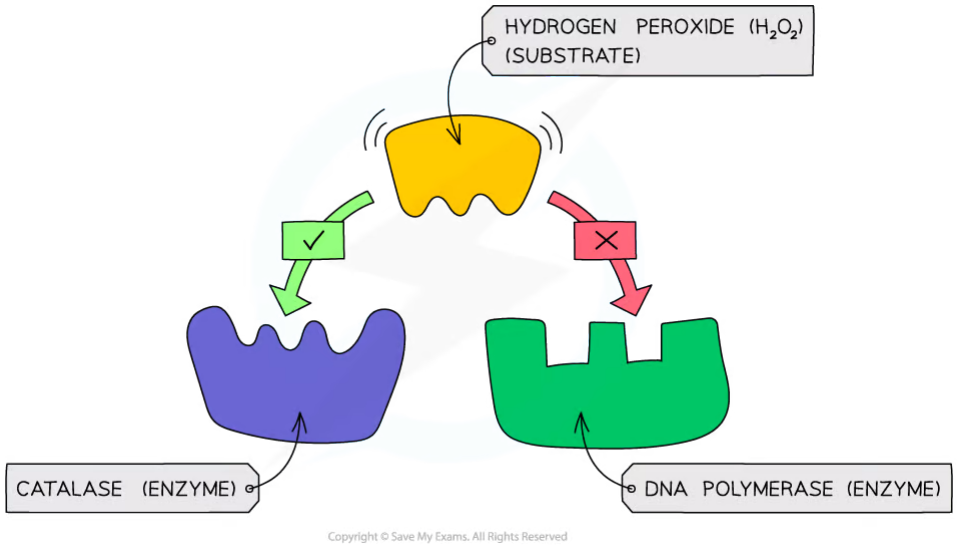
- Enzyme catalysis involves molecular motion and the collision of substrates with the active site
- For an enzyme-catalysed reaction to take place, substrates **collide at random** with the enzyme's active site
- This must happen at the correct **orientation** and **speed** in order for a reaction to occur
  - **Unsuccessful collisions** can occur when the molecules are not correctly aligned with each other at the moment of collision
  - The molecules 'bounce' off each other and **no reaction** takes place
- Some enzymes have **two substrates** that must each collide with a separate active site **at the same time**
- Substrates bind to enzymes, forming a temporary **enzyme-substrate complex**
- The **active site** of an enzyme has a **specific shape** and **chemical properties** to bind with a specific substrate
- The reaction occurs within the enzyme-substrate complex which leads to changes in the **chemical structure of the substrate**
- **Products** are formed, which **detach** and **move away** from the active site, which can be re-used



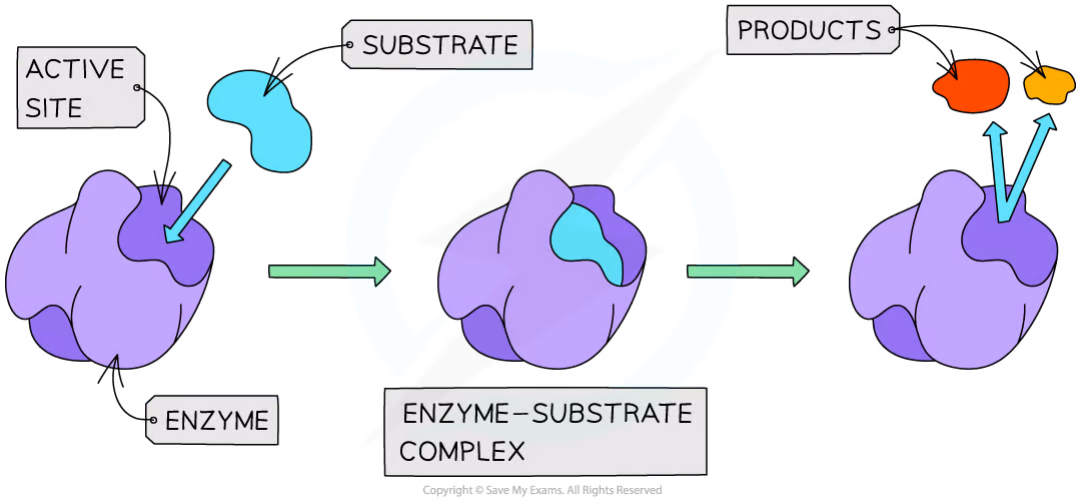
The active site of an enzyme has a specific shape to fit a specific substrate (when the substrate binds an enzyme-substrate complex is formed)

- The **specificity** of an enzyme is a result of the **complementary nature** between the shape of the active site on the enzyme and its substrate(s)
- The **shape** of the active site (and therefore the specificity of the enzyme) is determined by the **complex 3-D shape** of the protein that makes up the enzyme
  - Proteins are formed from **chains of amino acids** held together by peptide bonds
  - The order of amino acids in this chain determines the shape of an enzyme
  - If the order is altered, the resulting three-dimensional shape changes

YOUR NOTES



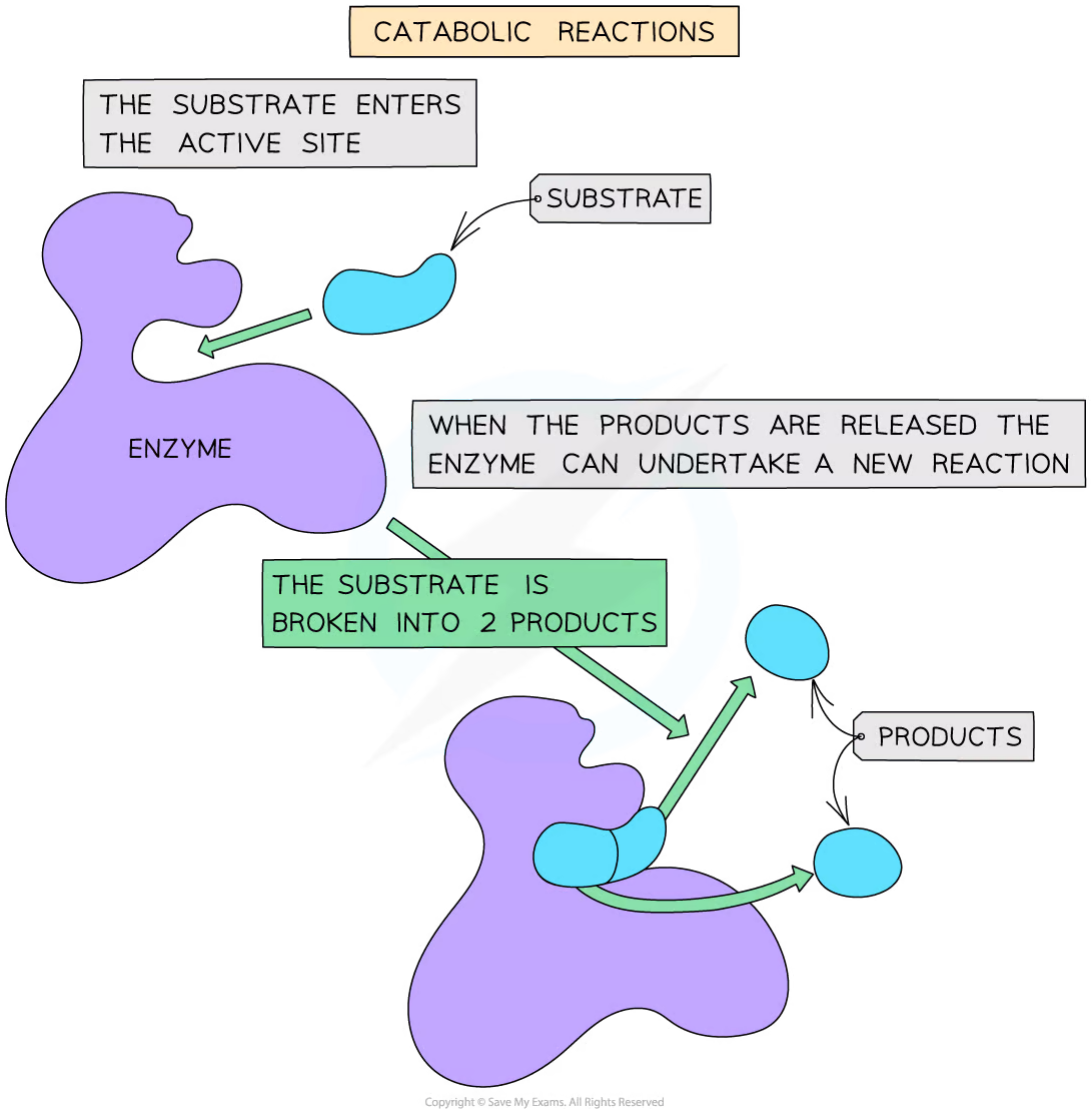
An example of enzyme specificity – the enzyme catalase can bind to its substrate hydrogen peroxide as they are complementary in shape, whereas DNA polymerase is not



The temporary formation of an enzyme-substrate complex

- Enzyme reactions can either be **catabolic** or **anabolic**
- **Catabolic** reactions involve the **breakdown** of complex molecules into simpler products, which happens when a single substrate is drawn into the active site and broken apart into two or more distinct molecules
- Examples of catabolic reactions include **cellular respiration** and **hydrolysis** reactions

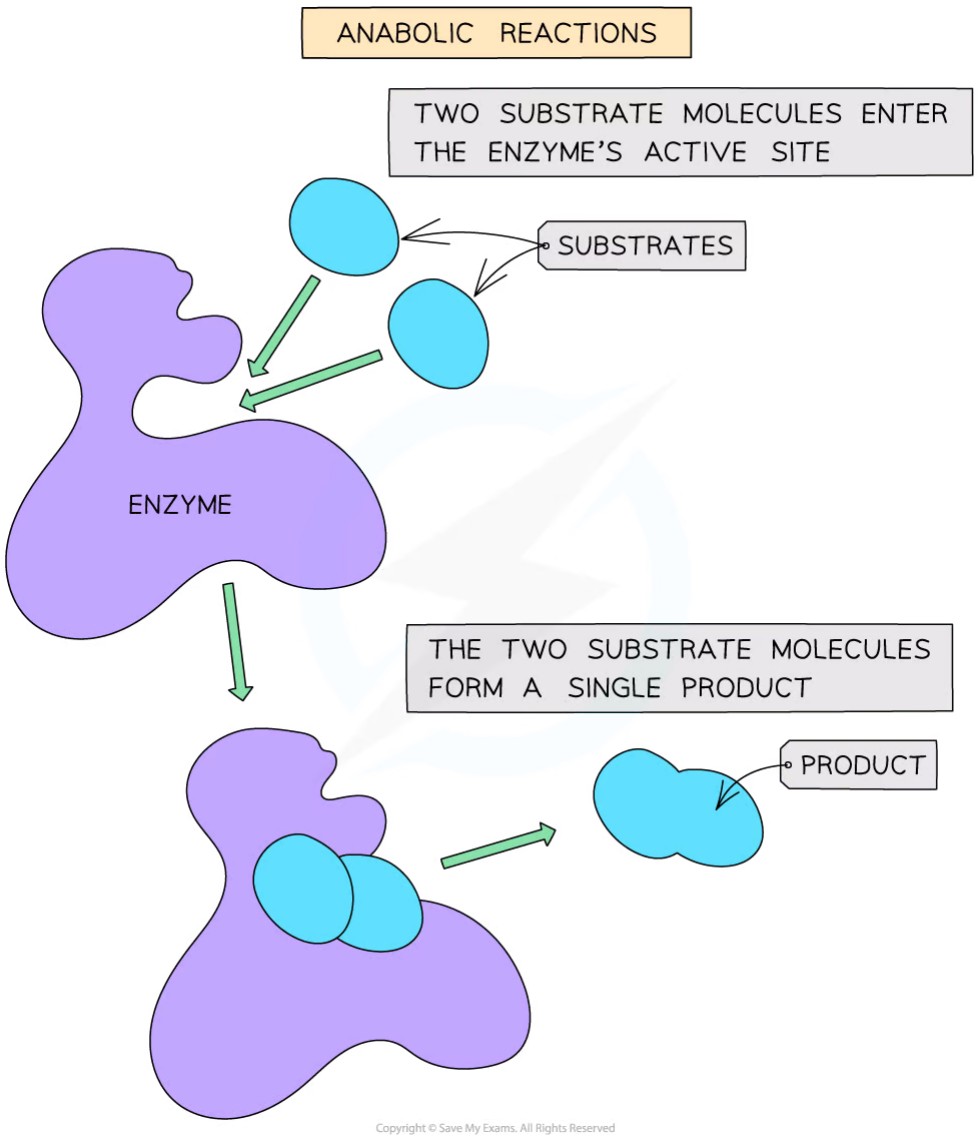
YOUR NOTES



A catabolic reaction

- **Anabolic** reactions involve the **building** of more complex molecules from simpler ones when two or more substrates are **held in the active site**, forming bonds between them and releasing a **single product**
- Examples of anabolic reactions include **protein synthesis** and **photosynthesis**

YOUR NOTES



An anabolic reaction

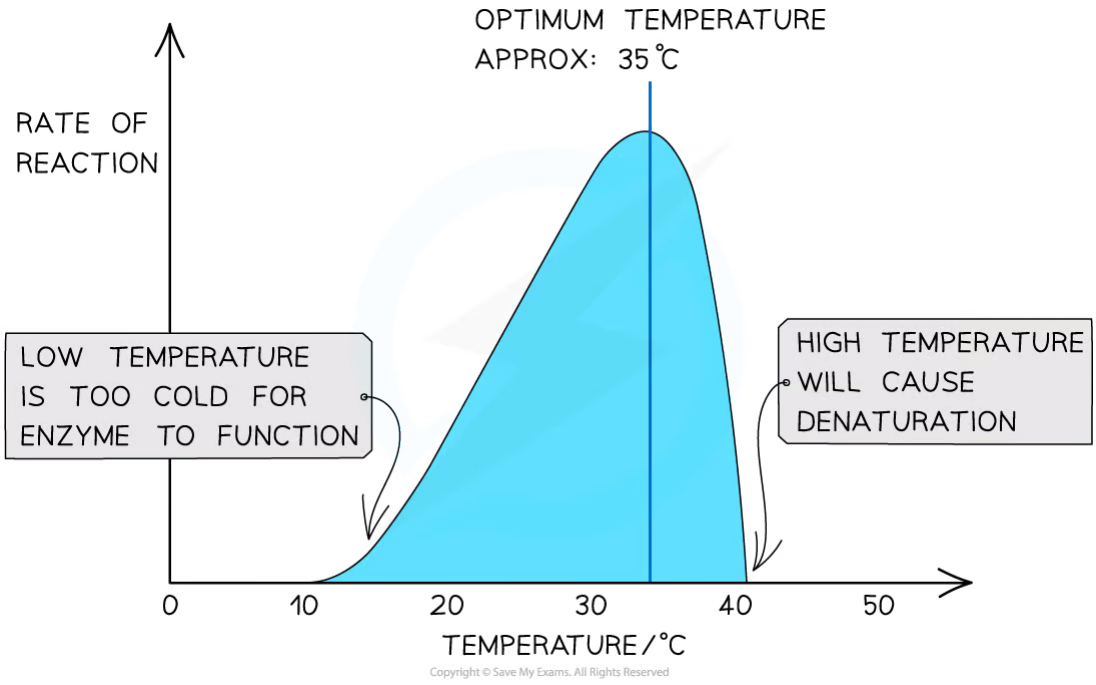
Exam Tip

Don't forget that both enzymes and their substrates are highly specific to each other – this is known as enzyme-substrate specificity.

Factors Affecting Enzyme Activity

YOUR NOTES

- Temperature, pH and substrate concentration affect the rate of activity of enzymes
- Enzymes have a **specific optimum temperature** – the temperature at which they catalyse a reaction at the **maximum rate**
- **Lower temperatures** either **prevent** reactions from proceeding or **slow them down**:
  - Molecules move relatively **slowly**
  - **Lower frequency of successful collisions** between a substrate molecule and the active site of enzyme
  - Less frequent enzyme-substrate complex formation
  - Substrate and enzyme collide with **less energy**, making it less likely for bonds to be formed or broken (stopping the reaction from occurring)
- **Higher temperatures speed up reactions**:
  - Molecules move more **quickly**
  - **Higher frequency successful collisions** between a substrate molecule and the active site of enzyme
  - More frequent enzyme-substrate complex formation
  - Substrate and enzyme collide with **more energy**, making it more likely for bonds to be formed or broken (allowing the reaction to occur)
- However, as temperatures continue to increase, the rate at which an enzyme catalyses a reaction **drops sharply**, as the enzyme begins to **denature**



The effect of temperature on the rate of an enzyme-catalysed reaction

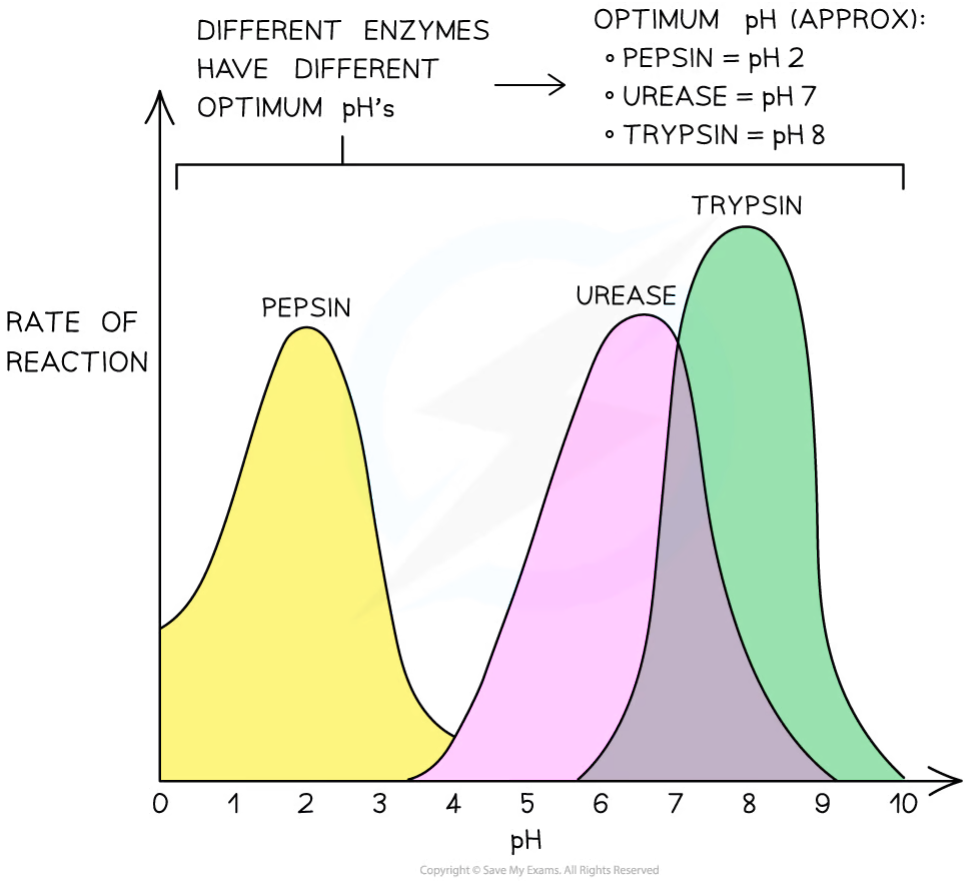
Changes in pH

- pH is a result of the **hydrogen ion concentration** in a solution
- A **low pH** is **acid** and has a **high** hydrogen ion concentration

YOUR NOTES

- A **high pH** is **alkaline** and has a **low** hydrogen ion concentration
- A **10× increase** in hydrogen ion concentration lowers the pH by **1 unit**
  - pH is therefore measured on a **logarithmic scale** of hydrogen ion concentration, **not a linear scale**

- Water has a pH of 7, regarded as **neutral**
- **Extremes of pH** can also alter hydrogen bonding within an enzyme's structure and cause irreversible **denaturation**
- Each enzyme has an **optimum pH**
- Not all enzymes have an optimum pH near to neutral. For example
  - The **stomach enzyme** pepsin is adapted to work best at **pH 2**
  - Certain bacterial enzymes work at **pH 9–10**, in line with the pH of the bacteria's main habitat



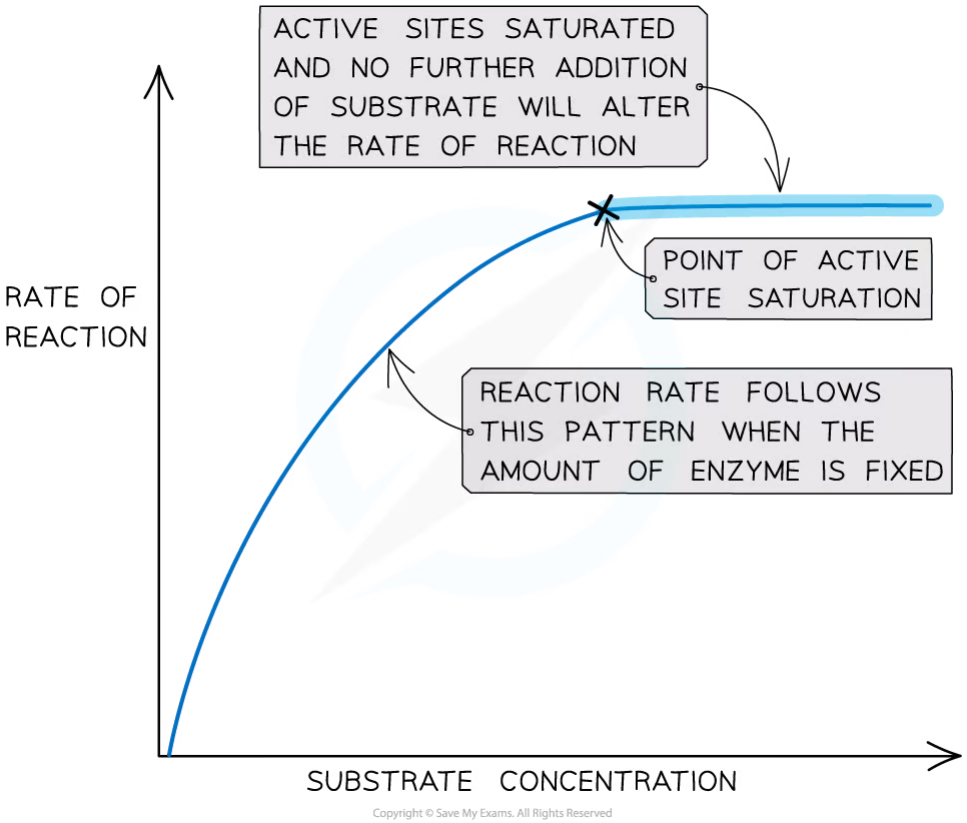
The effect of pH on three enzymes' rates of reaction

Changes in substrate concentration

- The more substrate molecules are present in a solution, this **increases the frequency of collisions** with the enzyme's active site
- Active sites are **occupied** or 'blocked' by substrates whilst the reaction is taking place
- The more active sites are occupied, **the fewer are available to catalyse other substrate** molecules

YOUR NOTES

- As substrate concentration rises, the slower the rise in the rate of the enzyme-catalysed reaction
- The active sites have become **saturated**
- At the **point of active site saturation**, increasing the substrate concentration will cause **no further increase** in the rate of reaction
- At the point of active site saturation, a method of increasing the rate of reaction would be to make more active sites available by **increasing the enzyme concentration**



The effect of substrate concentration on enzyme activity

Exam Tip

When answering questions about reaction rates for enzyme-catalysed reactions, make sure to explain how the temperature affects the speed at which the molecules (enzymes and substrates) are moving and how this, in turn, affects the number of **successful collisions**. You should memorise the sketch graphs of temperature, pH and substrate concentration and be able to sketch new curves for changed conditions.

Denaturation: Enzymes

YOUR NOTES

- Enzymes can be denatured
- **High temperatures** and **extremes of pH** cause denaturation
- **Bonds** (eg. hydrogen bonds) holding the enzyme molecule in its precise 3D shape start to **break**
- This causes the **3-dimensional shape** of the protein (ie. the enzyme) to **change**
- This permanently **damages** the **active site**, preventing the **substrate** from **binding**
- **Denaturation** has occurred if the **substrate can no longer bind**
- The reaction that was previously catalysed **now no longer takes place**
- Denaturation often causes the enzyme to **become insoluble** and form a **precipitate**
- Very few human enzymes can function at temperatures **above 50°C**
  - This is because humans maintain a body temperature of about 37°C, therefore even temperatures exceeding 40°C will cause the denaturation of enzymes
  - High temperatures cause increased vibrations in the bonds and the **hydrogen bonds between amino acids** start to break, changing the conformation of the enzyme

Exam Tip

Don't forget that enzymes are always proteins and so anything that could denature a protein, rendering it non-operational (extremes of heat, temperature, pH etc.) would also denature an enzyme.Avoid using the term 'destroyed' when describing the disruption to enzyme structure; the more accurate term is 'denatured'.

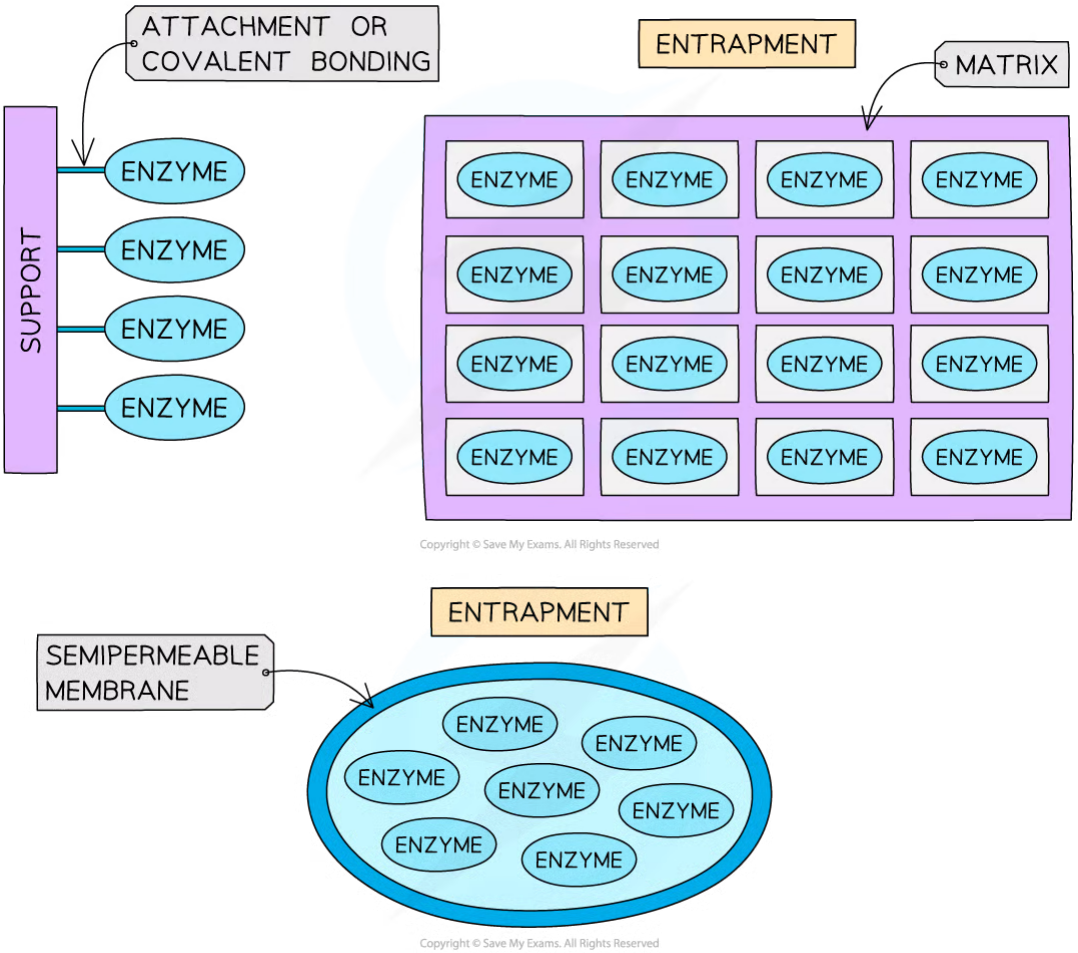
YOUR NOTES

Immobilised Enzymes

Immobilised enzymes are widely used in industry

- Just before 1900, it was discovered that enzymes could be used to catalyse production of alcohol in the absence of yeast cell
- Since then, hundreds of enzymes have been developed outside of living cells for **commercial purposes**
- Uses of enzymes in industrial processes can be **expensive,** so we need ways to **reuse** them in order to be cost-effective
- An **immobilised enzyme** is an enzyme that is attached to an **insoluble material** to prevent mixing with the product and through this method, the enzyme can be **reused** in subsequent reactions
- Immobilised enzymes are used in the following commercial processes
  - Agriculture
  - Biosensors (diagnosis, analysis eg. for impurities)
  - Manufacturing processes
  - Energy generation
  - Environmental management
  - Food/drinks industry
  - Medicines
- Methods by which enzymes can be immobilised include:
  - Attachment to an **inert substance** eg. glass
  - Entrapment within a **matrix** e.g. alginate gel
  - Entrapment within a **partially permeable membrane**

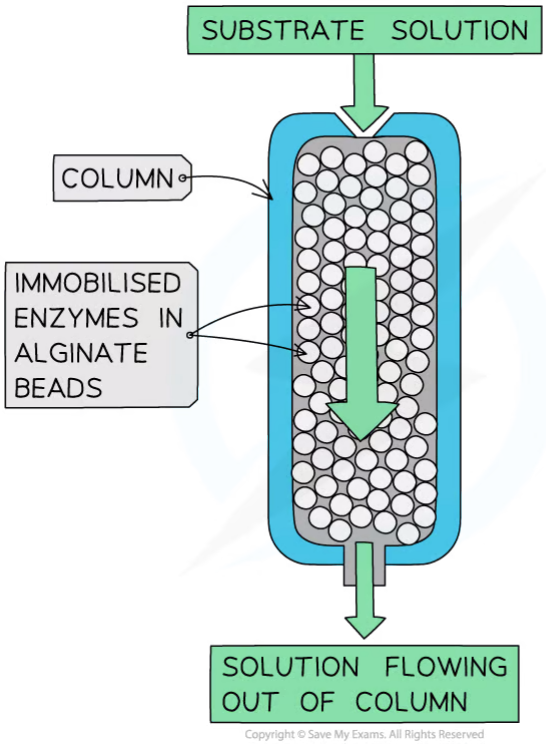
YOUR NOTES



Different ways in which enzymes can be immobilised

- The mechanism of immobilised enzyme use works as follows:
  - The immobilised enzymes are contained within a **column**
  - The substrate is **filtered through** this column in **solution**
  - As the substrate runs through the column, **enzyme-substrate complexes** are formed and products are produced
  - These products then **flow out** of the column, **leaving the enzymes behind** to catalyse the reaction again

YOUR NOTES



The immobilised enzymes are contained within a column

Advantages of immobilised enzymes

- There is **no enzyme in the product** (the product is **uncontaminated**) and therefore there is no need to further process or filter the end product
- The immobilised enzyme can be **reused multiple times** which is both efficient and **cost-effective** (many enzymes are **expensive**)
  - Reusing the enzyme also **avoids the need to separate** the enzyme from the product in downstream processing
- Immobilised enzymes have a **greater tolerance of temperature and pH changes** (immobilisation often makes enzymes more **stable**)
- Substrates can be exposed to **higher enzyme concentrations** than when using enzymes in solution, increasing the rate of throughput
- Conditions can be **controlled carefully**, allowing immobilised enzymes to function **close to their optimum conditions** and be **more stable**

Disadvantages of immobilised enzymes

- Specialist **expensive equipment** is required
- Immobilised enzymes are **more costly to buy**, so are unlikely to be financially worthwhile for **smaller industries**
- The **rate of reaction is sometimes lower** when using immobilised enzymes as the enzymes cannot mix freely with the substrate

Examples of immobilised enzymes in industry

YOUR NOTES

- There are many **industrial** and **medical** applications of immobilised enzymes, including production of the following:
  - **Lactose-free** dairy products such as milk
    - Enzyme: Lactase
    - Converts lactose to glucose and galactose
  - **Semi-synthetic penicillin** which overcomes issues of penicillin resistance
    - Enzyme: Penicillin acylase
    - Converts the original form of penicillin into one which is effective against penicillin-resistant organisms
  - **Glucose** products used to sweeten and thicken foods
    - Enzyme: Glucoamylase
    - Converts starch and other dextrins into glucose
  - **Fructose** for sweetening of foods where a lower quantity of sugar is necessary
    - Enzyme: Glucose isomerase
    - Converts glucose into the sweeter sugar, fructose
  - Purified samples of **L-amino acids** used in food production
    - Enzyme: Aminoacylase
    - Separates out L-amino acids from D-amino acids
  - **Acrylamide** required in disposable nappy/diaper production
    - Enzyme: Nitrilase
    - Converts acrylonitrile into acrylamide

Exam Tip

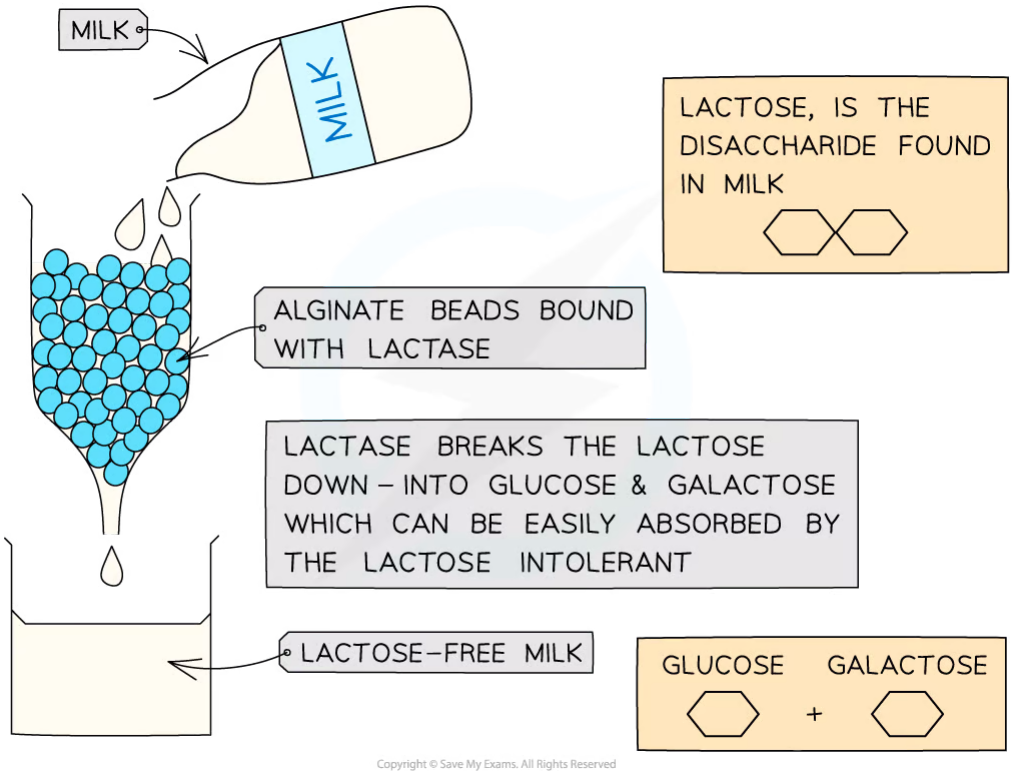
You will not necessarily be asked about these specific examples of industrial uses of immobilised enzymes (except for lactose modification), but it is useful to know of some uses in order to be able to apply your knowledge accurately in the exam.When discussing the advantages and disadvantages of immobilised enzymes, try to be specific about the cost implications as there are various considerations when it comes to the economical value of immobilising the enzymes.

Lactose-free Milk Production

YOUR NOTES

A closer look at lactose-free milk production

- **Milk** is a valuable source of nutrients containing protein, fat and the carbohydrate **lactose**
- 5–10% of the UK population are **lactose intolerant**
  - They can't digest lactose and **suffer** from bloating, digestive problems, discomfort and pain
- Lactose is a **disaccharide** that is hydrolysed into **glucose** and **galactose**
- The yeast *Kluyveromyces lactis* grows naturally in milk and can be cultured as a source of the enzyme **lactase**
- Glucose and galactose are **sweeter than lactose**, so less sugar needs to be added to foods with modified lactose **to achieve the same sweetness**
- **Ice cream** and **yoghurt** production also benefit from having more glucose and galactose than lactose in the milk they are made from



Using the immobilised enzyme lactase to modify milk

YOUR NOTES

Practical 3: Enzyme Experiments

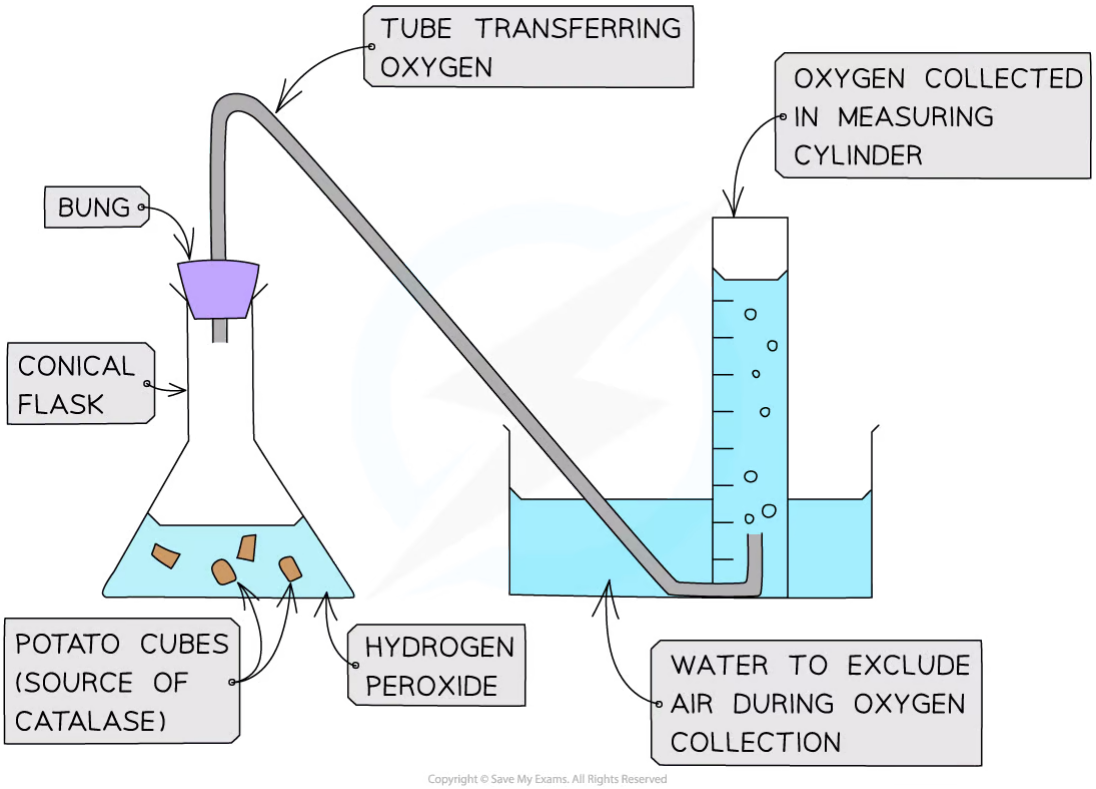
Design of experiments to test the effect of temperature, pH and substrate concentration on the activity of enzymes

- Three different **independent variables** can be tested
  - Temperature
  - pH
  - Substrate concentration
- You should plan how the **dependent variable is going to be measured**
  - With appropriate units
- Also, what **intervals of the independent variable** are going to be chosen
- These factors dictate the **choice of apparatus** and other equipment required for the experiment
- The **control variables** need to be identified and monitored eg. temperature when measuring the effect of pH

Investigating the effects of temperature or pH on catalase activity

- The **progress** of **enzyme-catalysed reactions** can be investigated by:
  - Measuring the **rate of formation of a product**
  - Measuring the **rate of disappearance of a substrate**
- In this investigation, the rate of **product formation** is used to measure the rate of an enzyme-controlled reaction:
  - **Hydrogen peroxide** is a common but **toxic** by-product of metabolism
  - This means it must be **broken down** quickly
  - **Catalase** is an enzyme found in the cells of most organisms that **breaks down hydrogen peroxide into water and oxygen**
  - Hydrogen peroxide and catalase are combined and the **volume of oxygen generated** is measured in a set time
  - The **rate of reaction** can then be calculated

YOUR NOTES



Experimental set-up for investigating the rate of formation of a product using catalase

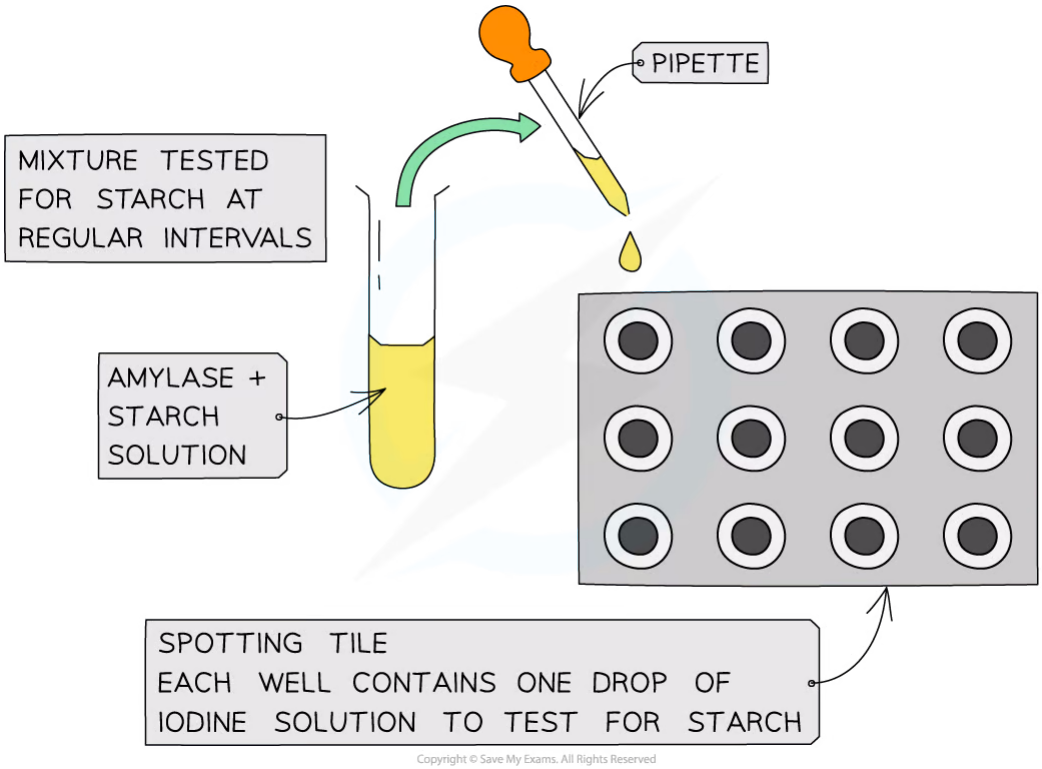
- If measuring the effect of temperature on enzyme activity, the conical flask containing potato pieces can be held in a water bath at the required temperature
  - The water level in the water bath must be higher than the level of H_2O_2 in the conical flask, to ensure even heating
  - The conical flask can also be swirled gently to mix the contents and maintain an even temperature

Investigating the effect of substrate concentration on amylase activity using iodine

- In this investigation, the rate of **substrate disappearance** is used to compare rates of reaction under different conditions
- **Amylase** is a digestive enzyme that **hydrolyses starch into maltose and glucose**
- Amylase functions best at pH 7 and 37°C (all enzymes operate best under specific conditions)
- **Amylase and starch are combined** and this reaction mixture is then **tested for starch** at regular time intervals
- This can be done by taking samples from the reaction mixture at each time interval and adding each sample to some **iodine in potassium iodide solution**
  - Starch forms a **blue-black** colour with this solution
  - If no starch is present, the iodine solution remains yellow-brown
- In this way, the time taken for starch to be broken down can be measured

YOUR NOTES

- The investigation can be **repeated under different starch concentrations** and the **reaction rates can then be compared**
  - This experiment also can be adapted to measure the effects of altering pH, temperature or enzyme concentration



Experimental set-up for investigating the rate of disappearance of a substrate using amylase

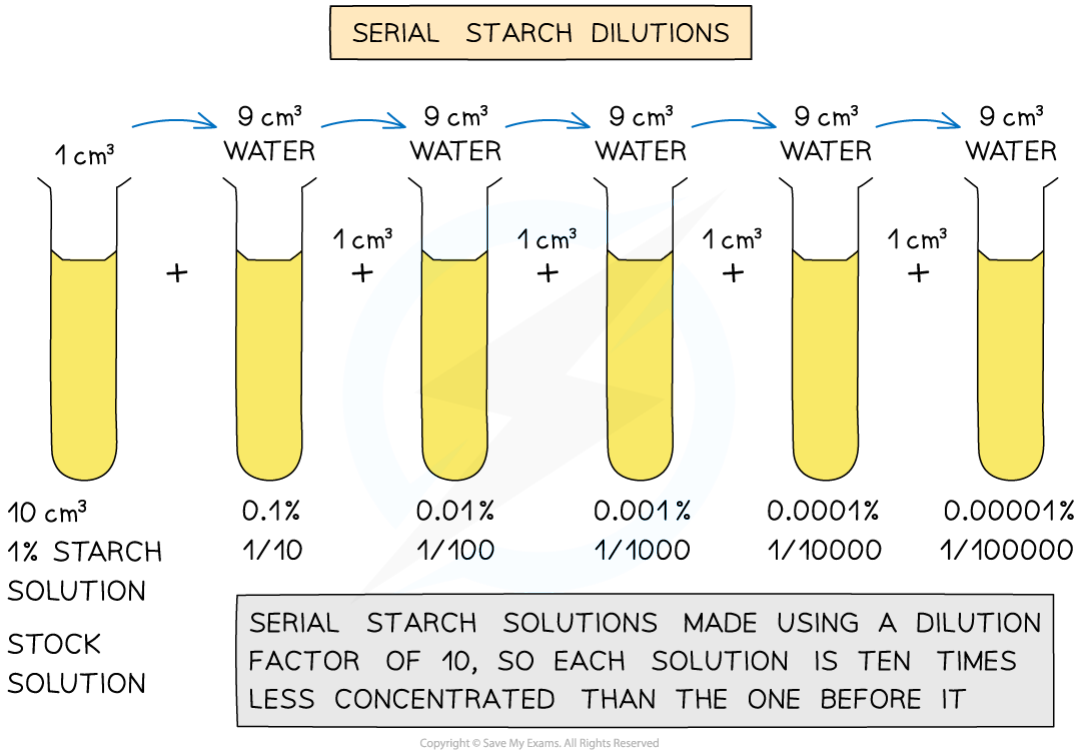
Investigating the effect of starch concentration on amylase activity using colorimetry

- A **colorimeter** is able to measure **light absorbance** (how much light is absorbed) or **light transmission** (how much light passes through) a substance
- Colorimetry can be used in any enzyme-catalysed reaction that involves a **colour change**
- As the colour breaks down the **transmission increases** or **light absorption decreases** and this can be used to **measure the rate of the reaction**
- For example, a colorimeter can be used to follow the progress of a **starch-amylase catalysed reaction** as the amylase breaks the starch down into maltose
- This can be carried out as follows:
  - **Colorimeter calibration**: this is an important step in a colorimetric investigation and in this case, a weak iodine solution can be used to calibrate the colorimeter as the endpoint (or 100% transmission)
  - Preparation of a starch solution of **known concentration** (stock solution), from which a range of concentrations are made using **serial dilutions** (method outlined in diagram below)
  - Following calibration and switching on the red filter (to maximise the percentage transmission or absorbance), the colorimeter is used to **measure the percentage**

YOUR NOTES

absorbance or percentage transmission values

- Sometimes a reagent or indicator is used to produce the colours detected by the colorimeter and sometimes the solutions themselves absorb light waves
- A **calibration graph** is then plotted of starch concentration (*x*-axis) vs percentage absorbance or percentage transmission (*y*-axis)



Serial dilution of starch to make a range of concentrations

NOS: Experimental design; accurate, quantitative measurements in enzyme experiments require replicates to ensure reliability

- **Accurate** measurements mean data that are close to the true value
- **Quantitative** measurements must be made
  - A **qualitative** measurement might state that, "the enzyme worked at a faster rate at the higher temperature", whereas
  - A **quantitative** measurement for the same experiment might state that, "the enzyme worked at a rate of 2.3 mmol product minute^{-1} at 40°C, versus 1.6 mmol product minute^{-1} at 25°C"
  - **Quantities**, using **numbers and appropriate units**, are quoted in the experimental results

- Reliable data are generated from repeated experiments
  - **Anomalies** can be identified and eliminated
  - A **reliable mean** can be calculated from the data that remain

YOUR NOTES

Exam Tip

RE-member: **RE**-peats bring **RE**-liability to experimental data.

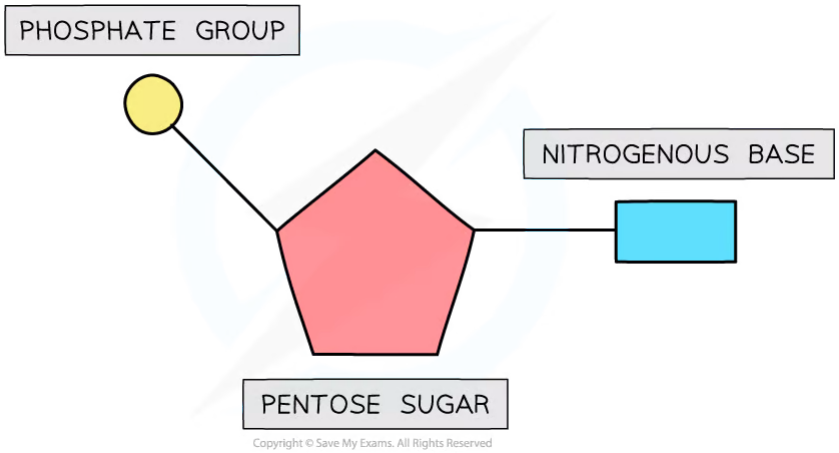
YOUR NOTES

2.5 Nucleic Acids: Structure & DNA Replication

2.5.1 DNA & RNA Structure

Nucleic Acids: Structure

- The nucleic acids DNA and RNA are polymers of nucleotides
- Both DNA and RNA are **polymers** that are made up of **many repeating units** called **nucleotides**
- Each nucleotide is formed from:
  - A **pentose sugar** (a sugar with 5 carbon atoms)
  - A nitrogen-containing **organic base** (with either 1 or 2 rings of atoms)
  - A **phosphate group** (this is acidic and negatively charged)
- The base and phosphate group are both **covalently bonded** to the sugar



The basic structure of a nucleotide

- Nucleotides join together in chains to form DNA or RNA strands
- The **phosphate group of one nucleotide** forms a covalent bond to the **pentose sugar of the next one**
  - This carries on to form a **large polymer**
- This forms a '**sugar-phosphate backbone**' with a base linked to each sugar
- The polymer of nucleotides is known as a **strand**
- DNA is double-stranded, RNA is usually single-stranded
- There are just **4 separate bases** that can be joined in **any combination/sequence**
  - Because the **sugar and phosphate are the same** in every nucleotide
- This sequence is the basis of the genetic code as a **store of genetic information**

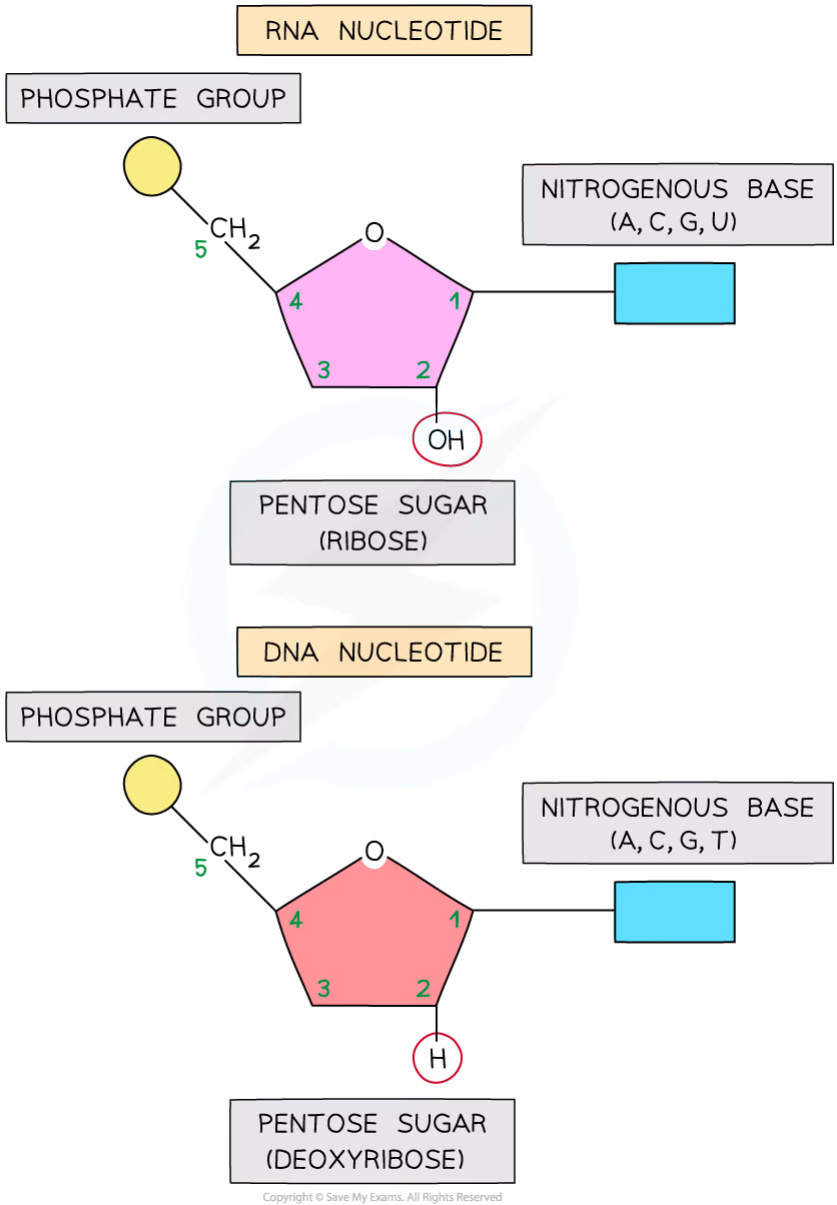
Exam Tip

A common error is to describe DNA or RNA as polymers of bases; more correctly, they are **polymers of nucleotides**

DNA & RNA: Comparison

YOUR NOTES

- **Like DNA**, the nucleic acid RNA (ribonucleic acid) is a **polynucleotide** – it is made up of **many nucleotides** linked together in a chain
- **Like DNA**, RNA nucleotides contain the nitrogenous bases adenine (A), guanine (G) and cytosine (C)
- **Unlike DNA**, RNA nucleotides **never contain** the nitrogenous base **thymine** (T) – in place of this they contain the nitrogenous base **uracil** (U)
- **Unlike DNA**, RNA nucleotides contain the pentose sugar **ribose** (instead of deoxyribose)

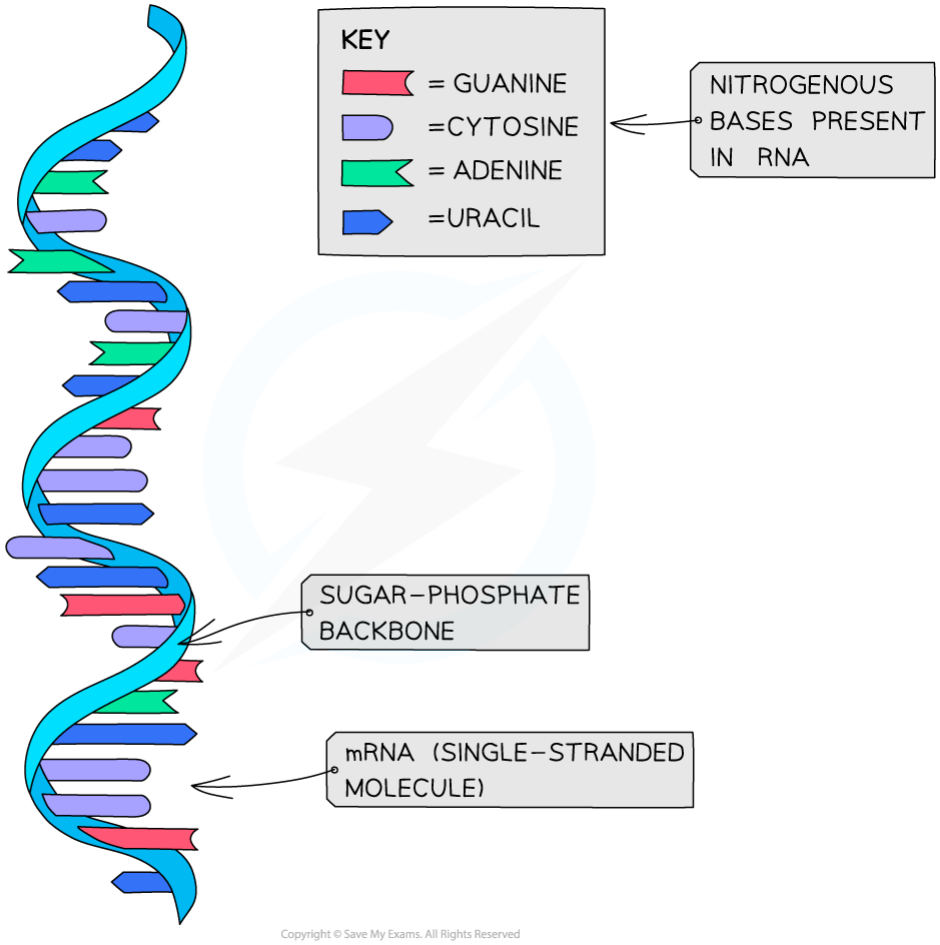


An RNA nucleotide compared with a DNA nucleotide

- **Unlike DNA**, RNA molecules are only made up of **one polynucleotide strand** (they are **single-stranded**)

YOUR NOTES

- **Unlike DNA,** RNA polynucleotide chains are relatively **short compared to DNA**
- **Like DNA,** the **sugar-phosphate bonds** (between different nucleotides in the same strand) are strong **covalent bonds**
- **Like DNA,** the nitrogenous bases **stick out sideways** from the sugar-phosphate backbone



The structure of RNA

Nucleotide Structure Summary Table

YOUR NOTES

Properties	DNA	RNA
Pentose sugar	Deoxyribose	Ribose
Bases	Adenine (A) **Thymine (T)** Cytosine (C) Guanine (G)	Adenine (A) **Uracil (U)** Cytosine (C) Guanine (G)
Number of strands	Double-stranded (double helix)	single-stranded

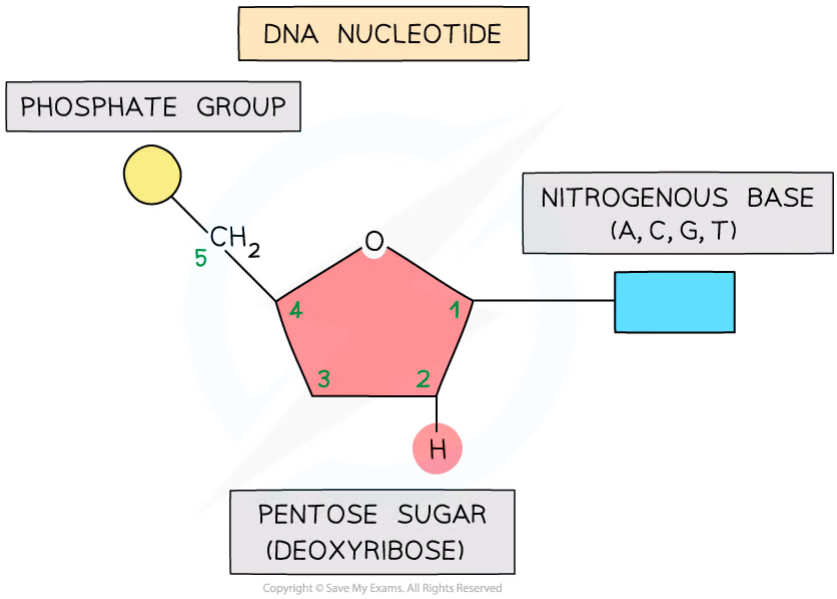
Exam Tip

You need to know the difference between DNA and RNA molecules (base composition, number of strands, pentose sugar present).

YOUR NOTES

Double Helix Structure

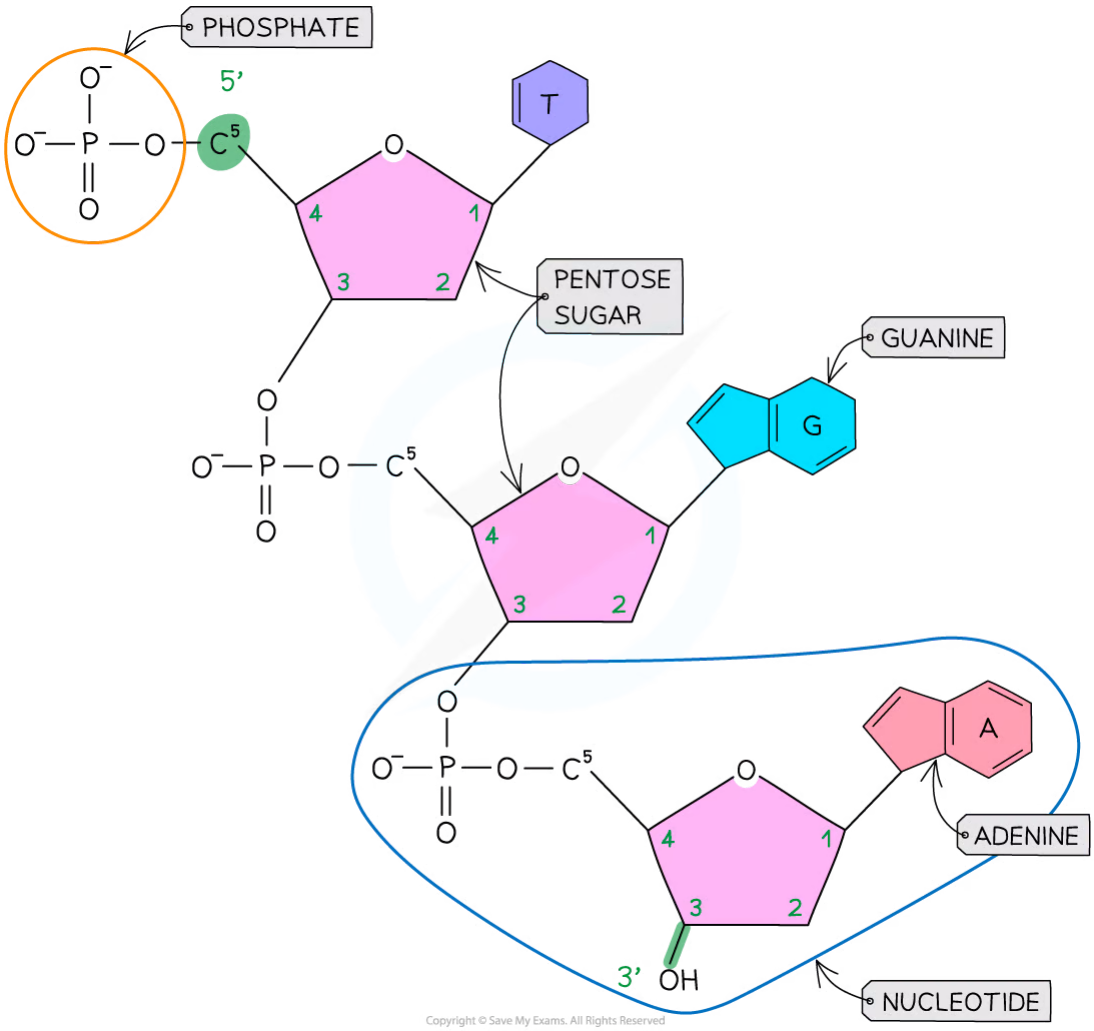
- DNA is a double helix made of two antiparallel strands of nucleotides linked by hydrogen bonding between complementary base pairs
- The nucleic acid DNA is a **polynucleotide** – it is made up of **many nucleotides** bonded together in a **long chain**



A DNA nucleotide

- DNA molecules are made up of **two polynucleotide strands** lying side by side, running in opposite directions – the strands are said to be **antiparallel**
- Each DNA polynucleotide strand is made up of **alternating deoxyribose sugars and phosphate groups bonded together** to form the **sugar-phosphate backbone**
- Each DNA polynucleotide strand is said to have a **3' end and a 5' end** (these numbers relate to which carbon atom on the pentose sugar could be bonded with another nucleotide)
- Because the strands run in opposite directions (they are **antiparallel**), one is known as the **5' to 3' strand** and the other is known as the **3' to 5' strand**
- The nitrogenous bases of each nucleotide project out from the backbone **towards the interior** of the double-stranded DNA molecule

YOUR NOTES

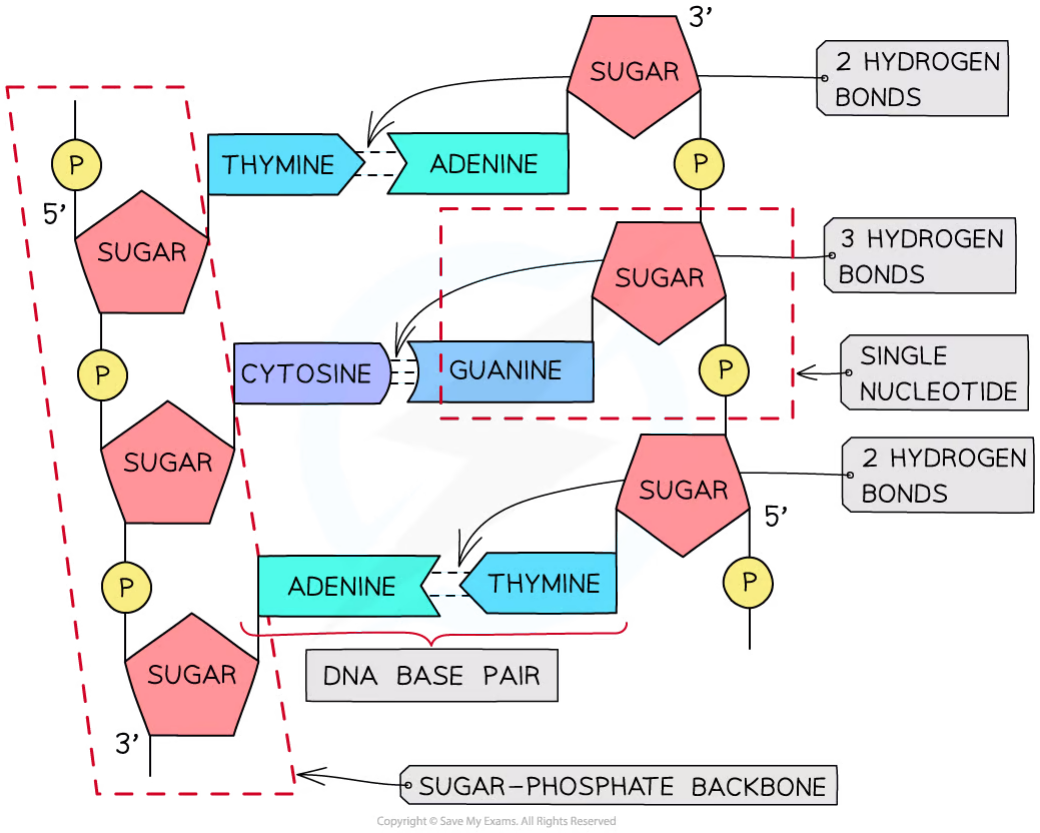


A single DNA polynucleotide strand showing 3 nucleotides in a sequence

Hydrogen bonding

- The two antiparallel DNA polynucleotide strands that make up the DNA molecule are **held together by hydrogen bonds** between the nitrogenous bases
- These hydrogen bonds always occur between the **same pairs of bases**:
  - The purine **adenine** (A) always pairs with the pyrimidine **thymine** (T) – two hydrogen bonds are formed between these bases
  - The purine **guanine** (G) always pairs with the pyrimidine **cytosine** (C) – three hydrogen bonds are formed between these bases
  - This is known as **complementary base pairing**
  - These pairs are known as **DNA base pairs**

YOUR NOTES

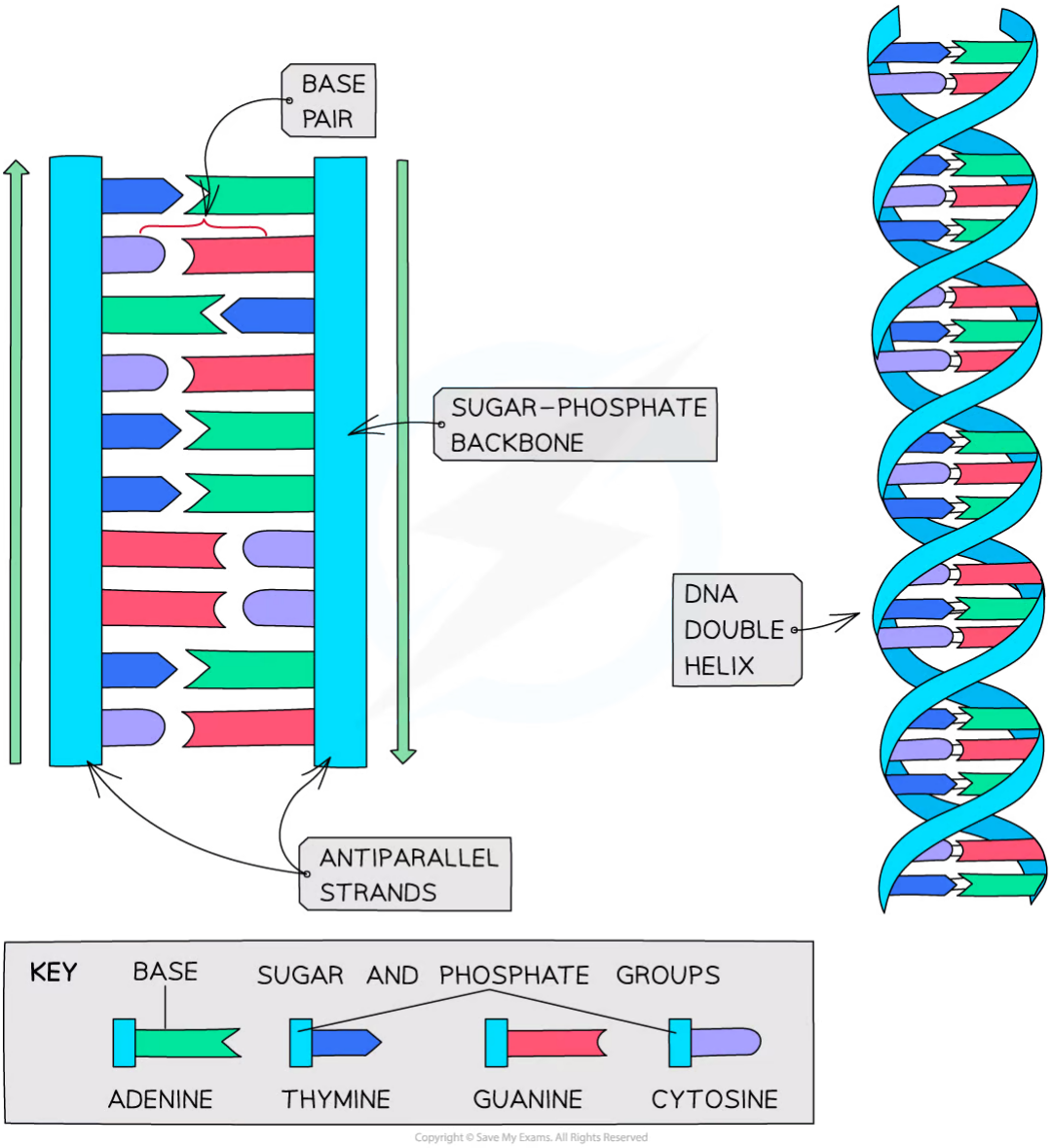


A section of DNA – two antiparallel DNA polynucleotide strands held together by hydrogen bonds

Double helix

- DNA is not two-dimensional as shown in the diagram above
- DNA is described as a **double helix**
- This refers to the **three-dimensional shape** that DNA molecules form

YOUR NOTES



DNA molecules form a three-dimensional structure known as a DNA double helix

Exam Tip

Make sure you can name the different components of a DNA molecule (sugar-phosphate backbone, nucleotide, complementary base pairs, hydrogen bonds) and make sure you are able to locate these on a diagramRemember that covalent bonds join the nucleotides in the sugar-phosphate backbone, and hydrogen bonds join the bases of the two complementary strands togetherRemember that the bases are complementary, so the number of A = T and C = G. You could be asked to determine how many bases are present in a DNA molecule if given the number of just one of the bases.

Crick & Watson

YOUR NOTES

- Francis Crick and James Watson were two Cambridge scientists who worked together to establish the **double helix** structure of DNA in 1953
- They used data from **their previous experiments** on the composition of DNA
- Published findings from **other research labs** played a role in developing their model
  - Rosalind Franklin, Edwin Chargaff and Linus Pauling, all of whom were leading research efforts in **other universities**, contributed important data to Crick & Watson's discovery
  - This suggests that there was a close-knit collaboration, but in fact, there was **a lot of competition between the groups** to make the breakthrough discovery
- Physical **model-making** played a large role in their success
- **Early versions of the model were rejected** for various reasons
  - It was **not compact enough**
  - It would have been too **unstable** (DNA is a highly stable molecule)
  - It did not allow **equivalent amounts** of A and T, and C and G bases to be present (Chargaff's findings)
  - It fitted together much better once the **second strand was flipped** to become antiparallel
- Their final model was constructed carefully with **clamps**, **metal rods** for bonds and with the **correct bond angles**
- Their model was **the basis of a lot of genetic research** globally in the years that followed
  - Notably, Crick and Watson's model sparked work to prove **the way in which DNA replicates** in cells

NOS: Using models as a representation of the real world; Crick and Watson used model making to discover the structure of DNA

- Models in science are built **to represent concepts** and ideas in a way that can be pictured by our brains
- Models can be **accepted or rejected** based on experimental data generated by further research
- Crick and Watson **built physical, scale** models of DNA to explain biological observations
  - Using **simple laboratory equipment** (clamps, stands, metal rods etc)
  - They **adapted their models** by making them more realistic eg. by building in the correct bond angles within molecules
- They built successive models, using **trial-and-error** to arrive at the finalised model
- Their **first model of DNA was rejected**, based on the findings of Rosalind Franklin
  - Crick and Watson **received the Nobel prize** for their work
  - Franklin died aged 37 so never received the recognition she deserved for **her significant role** in defining DNA structure
- Today, **sophisticated computer modelling** is performed, that
  - **Takes the place of physical model-making** and provides further explanation of the functions of various biomolecules
  - **Shortens the 'trial-and-error' cycles** of model-making as experienced by Crick and Watson

YOUR NOTES

Exam Tip

Crick and Watsons' model has been universally accepted because all further research findings have supported their model.

YOUR NOTES

DNA Replication

- The replication of DNA is semi-conservative and depends on complementary base pairing
- Semi-conservative means that **one strand of the 'parent' DNA is kept** in the 'daughter' molecule
- This is called the **template strand**
- The other half is determined by the code on the template strand and is built up from **free nucleotides** in the nuclear space around the chromosomes
- This takes place in the **nucleus**
- Nucleotides are added **one by one** to the new strand according to the rules of **complementary base-pairing**
  - If an **adenine** is the next exposed base on the original strand, a **thymine** nucleotide is added and *vice versa*
  - If a **cytosine** is the next exposed base on the original strand, a **guanine** nucleotide is added and *vice versa*
- **Hydrogen bonds** can only form between the template strand and the new strand if the **correct bases** are paired up
- Therefore, the new DNA molecule has **kept half** of the parent DNA and then used this to create a new, daughter strand

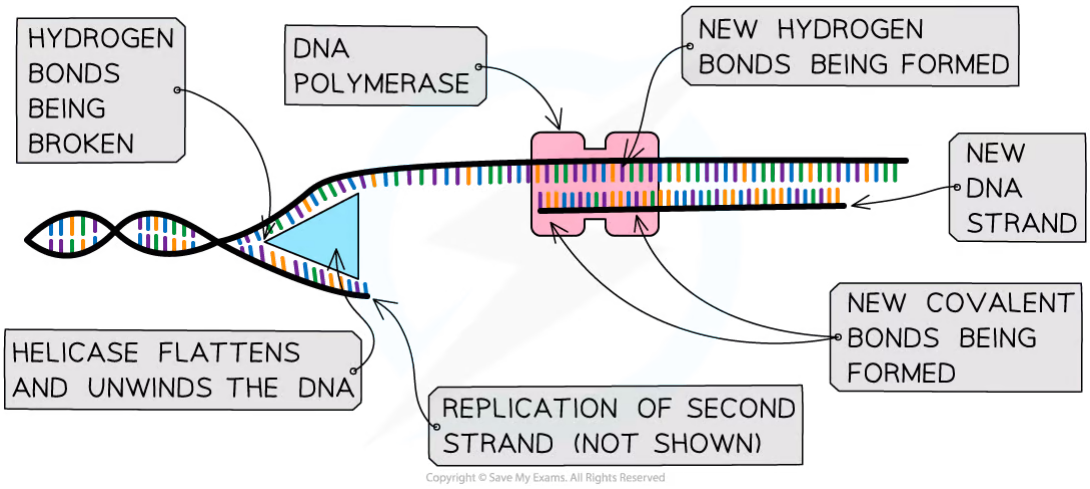
Exam Tip

Make sure you don't confuse 'parent cell' with 'parent organism'. A **parent cell** is any cell in the body that divides into two cells and the terminology is used to refer to the **'original' cell** that the DNA came from before it was split and replicated semi-conservatively.

YOUR NOTES

Enzymes Involved in Replication

- Helicase unwinds the double helix and separates the two strands by breaking hydrogen bonds
- DNA replication occurs in preparation for **mitosis,** when DNA must be **doubled** before the parent cell can divide to produce two genetically identical daughter cells
- The enzyme **helicase** first **unwinds the DNA**, by flattening out its helical structure
  - Analogy - think about **untwisting a rope ladder**
- **Helicase** then causes the **hydrogen bonds to break** between pairs of bases, exposing bases on either strand
  - Analogy - **unzipping a zipper**
- Each of these single polynucleotide DNA strands acts as a **template** for the formation of a **new strand** made from free nucleotides that are attracted to the exposed DNA bases by **base pairing**



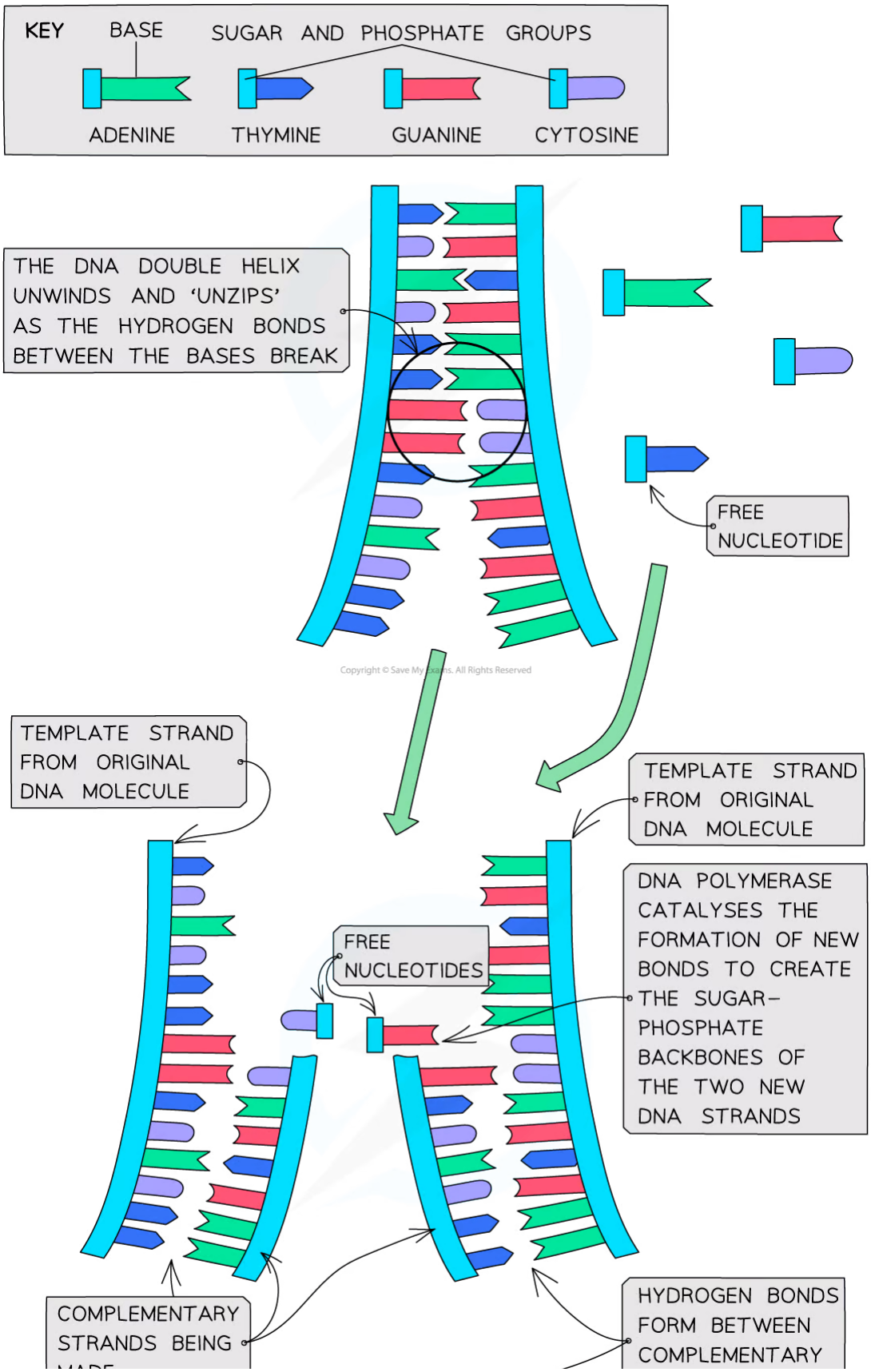
Helicase and DNA polymerase work together to replicate each strand of DNA

- DNA polymerase links nucleotides together to form a new strand, using the pre-existing strand as a template
- Following the action of helicase, the template strand is exposed and new nucleotides are **joined together by DNA polymerase,** which catalyses **condensation reactions,** to form a new strand
- The original strand and the new strand **join together through hydrogen bonding** between base pairs to form the new DNA molecule
- This method of replicating DNA is known as **semi-conservative replication** because **half of the original** DNA molecule is kept (**conserved**) in each of the two new DNA molecules
- The enzyme **DNA polymerase synthesises new DNA strands** from the two template strands
- It does this by **catalysing condensation reactions** between the **deoxyribose sugar and phosphate groups** of adjacent nucleotides within the new strands, creating the **sugar-phosphate backbone** of the new DNA strands
- DNA polymerase always works **in the same direction** along a strand of DNA, the 5' to 3' direction

- Adding the **5' terminal** of the new nucleotide to the **3' terminal** of the strand being built
- **Hydrogen bonds** then form between the **complementary base pairs** of the template and new DNA strands
- The **copying accuracy** of DNA polymerase is **very high**
  - Very few copying errors are made in DNA replication

YOUR NOTES

YOUR NOTES



The combined actions of helicase and DNA polymerase create new complementary DNA strands

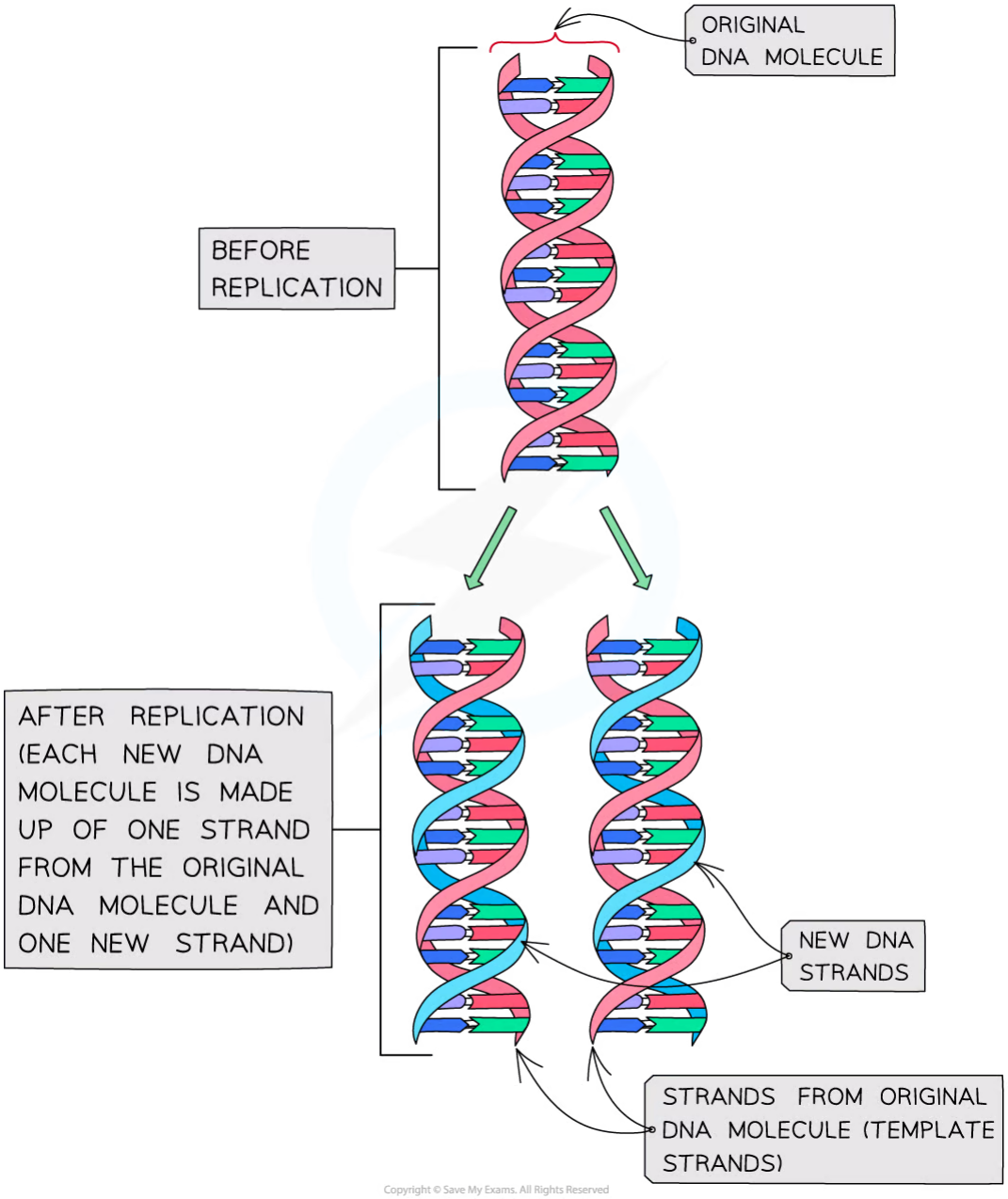
YOUR NOTES

Semi-Conservation Replication

YOUR NOTES

- DNA polymerase links nucleotides together to form a new strand, using the pre-existing strand as a template
- **Before** a (parent) cell **divides**, it needs to **copy the DNA** contained within it
  - This is so that the two new (daughter) cells produced will both receive the **full copies of the parental DNA**
- The DNA is copied via a process known as **semi-conservative replication** (half the DNA is kept)
  - The process is called so because **in each new DNA molecule** produced, one of the polynucleotide DNA strands (**half** of the new DNA molecule) is **from the original DNA molecule** being copied
  - The other polynucleotide DNA strand (the other half of the new DNA molecule) has to be **newly created** by the cell

YOUR NOTES



Semi-conservative replication of DNA

The importance of keeping one original DNA strand

- It **ensures** there is **genetic continuity** between generations of cells
  - In other words, it ensures that the new cells produced during cell division **inherit all their genes** from their parent cells
- This is important because cells in our body are **replaced regularly** and therefore we need the new cells to be able to do the same role as the old ones
  - Replication of DNA and cell division also occurs during **growth**

Crick and Watson proposed semi-conservative replication

- As part of their discovery of the double-helix structure of DNA, Crick and Watson **made a hypothesis about how DNA copies** during cell growth
- They proposed a **semi-conservative** model, but had **not provided the evidence**
- This was provided by two later scientists, **Meselson** and **Stahl**, in another award-winning piece of research
- Analysis of Meselson and Stahl's results **gave the necessary support for Crick & Watsons' hypothesis** of semi-conservative replication of DNA

YOUR NOTES

YOUR NOTES

Meselson & Stahl's Experiments

Analysis of Meselson and Stahl's results to obtain support for the theory of semi-conservative replication of DNA

- Crick and Watson, as they defined the shape of DNA, suggested a **credible explanation** of how the DNA molecule replicates itself
  - This was the theory of **semi-conservative replication**
- Like any scientific theory, this explanation **required evidence** to back up the claims
- Five years after their discovery, two other scientists, **Matthew Meselson** and **Franklin Stahl**, provided data to prove Crick and Watsons' theory

Meselson and Stahls' Experiment

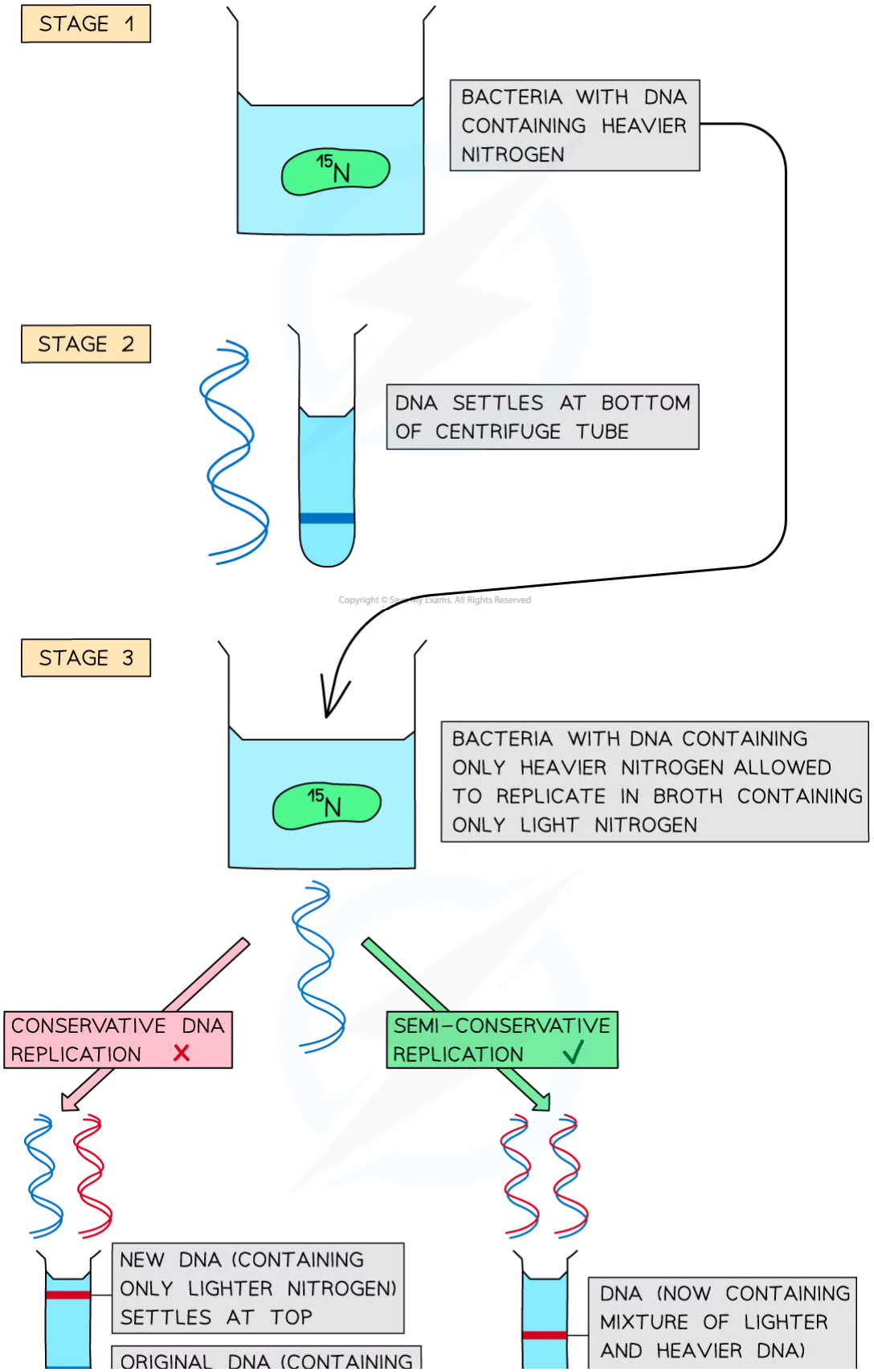
- Bacteria were grown in a broth containing the **heavy (^{15}N) nitrogen isotope**
  - DNA contains nitrogen in its bases
  - As the bacteria replicated, they used nitrogen from the broth to make **new DNA nucleotides**
  - After some time, the culture of bacteria had DNA containing **only heavy (^{15}N) nitrogen**
- A sample of **DNA** from the ^{15}N culture of bacteria was extracted and **spun in a centrifuge**
  - This showed that the DNA containing the heavy nitrogen settled near the bottom of the centrifuge tube
- The bacteria containing only ^{15}N DNA were then taken out of the ^{15}N broth and added to a broth containing **only the lighter ^{14}N nitrogen**. The bacteria were left for enough time for **one round of DNA replication** to occur before their DNA was extracted and **spun in a centrifuge**
  - If **conservative DNA replication** had occurred, the original template DNA molecules would only contain the heavier nitrogen and would settle at the bottom of the tube, whilst the new DNA molecules would only contain the lighter nitrogen and would settle at the top of the tube
  - If **semi-conservative replication** had occurred, **all** the DNA molecules would now contain **both** the **heavy ^{15}N** and **light ^{14}N** nitrogen and would therefore settle in the **middle of the tube** (one strand of each DNA molecule would be from the original DNA containing the heavier nitrogen and the other (new) strand would be made using only the lighter nitrogen)
- Meselson and Stahl confirmed that the bacterial DNA had undergone **semi-conservative replication**.
  - The DNA from this second round of centrifugation settled in the middle of the tube, showing that each DNA molecule contained a **mixture** of the **heavier and lighter nitrogen isotopes**
  - If more rounds of replication were allowed to take place, the **ratio of ^{15}N:^{14}N** would go from 1:1 after the first round of replication, to 3:1 after the second and 7:1 after the third
- This experiment **proved Crick and Watsons' theory correct**

NOS: Obtaining evidence for scientific theories; Meselson and Stahl obtained evidence for the semi-conservative replication of DNA

- Meselson and Stahl's experiment is a great example of how scientists can obtain evidence to back up a theory about a biological process

YOUR NOTES

YOUR NOTES



Meselson and Stahls' experiment provided unequivocal proof that DNA replicates via semi-conservative DNA replication

YOUR NOTES

YOUR NOTES

2.6 Transcription & Translation

2.6.1 Transcription

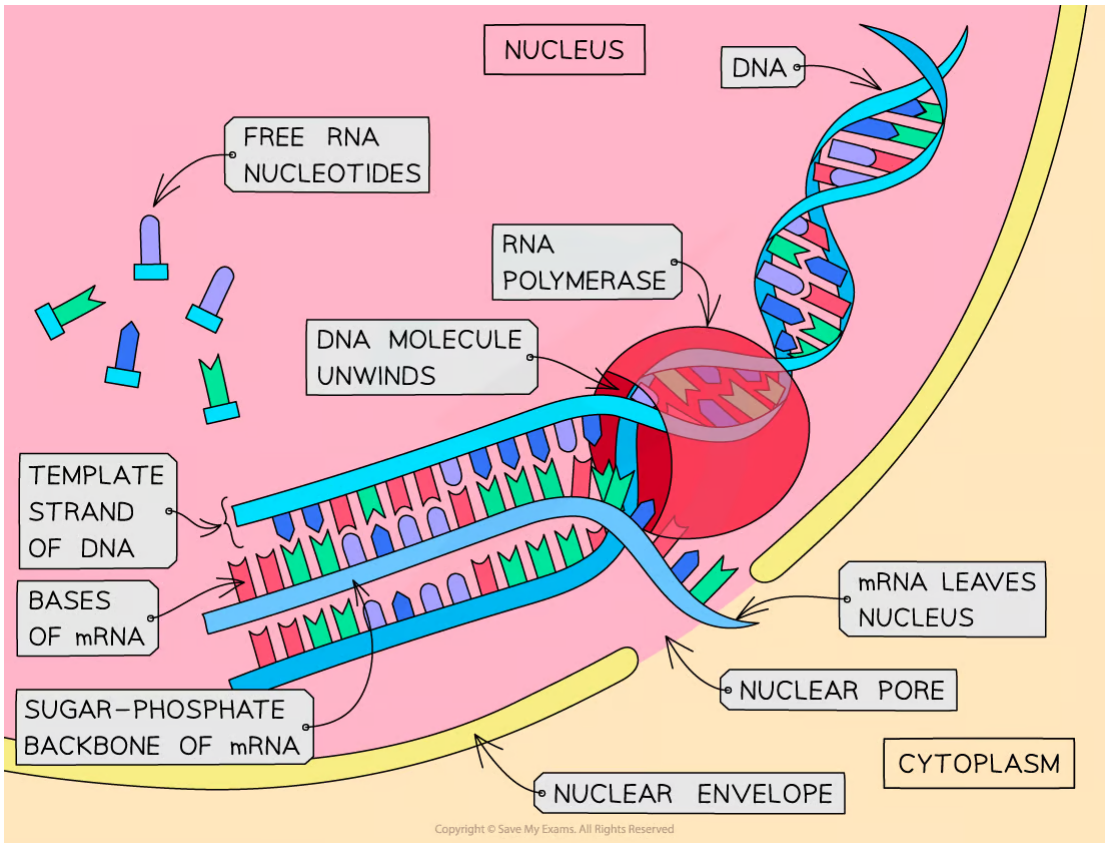
Transcription

- This process of protein synthesis occurs in **two stages**:
  - **Transcription** – **DNA** is transcribed and an **mRNA** molecule is produced
    - mRNA is a single stranded RNA molecule that transfers the information in DNA from the nucleus into the cytoplasm
    - mRNA production requires the enzyme RNA polymerase
  - **Translation** – **mRNA** (messenger RNA) is translated and an **amino acid sequence** is produced

The process of transcription

- This stage of protein synthesis occurs **in the nucleus** of the cell
- Part of a DNA molecule **unwinds** (the **hydrogen bonds** between the complementary base pairs **break**)
- This exposes the **gene** to be transcribed (the gene from which a particular polypeptide will be produced)
- A complementary copy of the code from the gene is made by building a **single-stranded nucleic acid molecule known as mRNA** (messenger RNA)
- **Free RNA nucleotides** pair up (via hydrogen bonds) with their complementary (now exposed) bases on one strand (the template strand) of the 'unzipped' DNA molecule
- The sugar-phosphate groups of these RNA nucleotides are then **bonded** together by the enzyme **RNA polymerase** to form the sugar-phosphate backbone of the mRNA molecule
- When the gene has been transcribed (when the mRNA molecule is complete), the hydrogen bonds between the mRNA and DNA strands break and the **double-stranded DNA molecule re-forms**
- The mRNA molecule then **leaves the nucleus** via a pore in the nuclear envelope
  - This is where the term *messenger* comes from - the mRNA is despatched, **carrying a message**, to another part of the cell
  - DNA can't make this journey; **it's too big to fit** through the pores in the nuclear envelope

YOUR NOTES

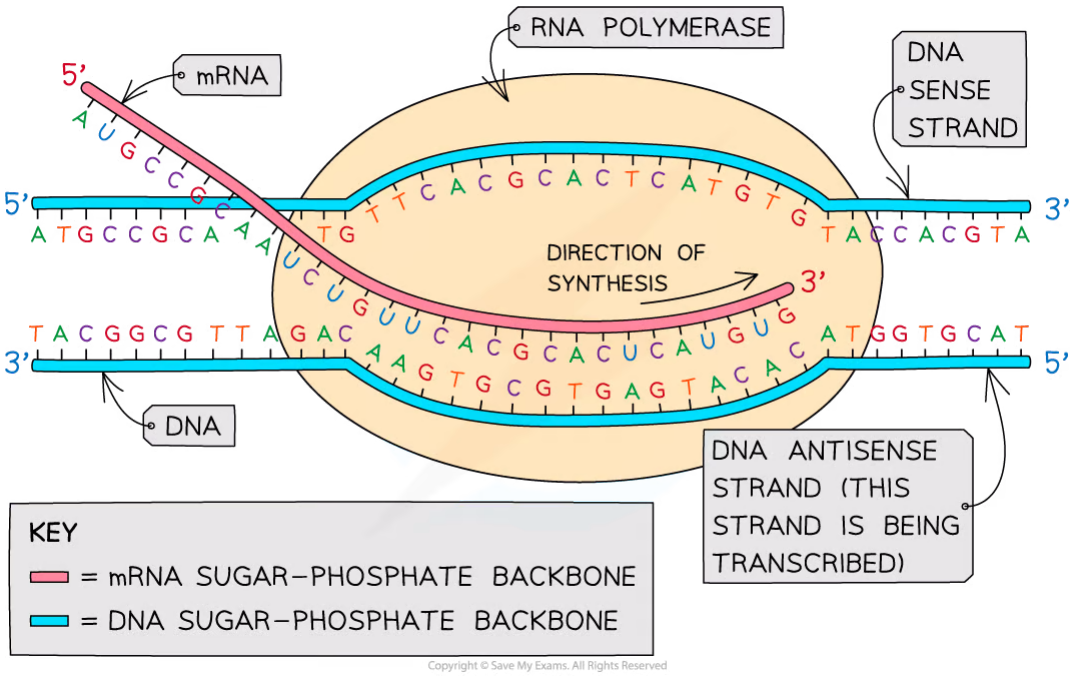


DNA is transcribed and an mRNA molecule is produced

Sense and anti-sense strands

- In the **transcription** stage of protein synthesis, free RNA nucleotides pair up with the exposed bases on the DNA molecule but **only with those bases on one strand of the DNA molecule**
- The RNA will have a complementary base sequence to the DNA strand (with the substitution of Thymine with Uracil)
- The strand of the DNA molecule that carries the genetic code is called the **sense strand**
- The opposite DNA strand is called the **antisense** strand
- To get **an RNA transcript of the sense strand**, the **antisense strand is the one that is transcribed** to form the mRNA molecule
    - This mRNA molecule will later be translated into an amino acid chain

YOUR NOTES



The antisense strand of the DNA molecule is the one that is transcribed

Analogy: Think of transcription and translation as being like converting between languages

- Each language has its **alphabet**, just as nucleic acids and proteins have their **monomers**
- **Transcription** is like converting text from **English** to **French**
  - The same characters are used, but there are slight differences
  - French uses the same alphabet as English but employs occasional accented characters like â, é, or ç
  - DNA and RNA employ largely the same monomers, but with the slight differences of the two pentose sugars and of U replacing T.
- **Translation** is like converting text from a western language to a language that uses a different alphabet, like **Japanese**
  - A completely **different set of characters** is used
  - The sequence of characters is **unrecognisable** from the original
  - If we could see them, a chain of amino acids would look nothing like a chain of nucleotides

Transcription and Translation Can be Likened to Conversion Between Languages Table

YOUR NOTES

Transcription	DNA → RNA	Similarities	English → French		Similarities
DNA → RNA	TTACAGCTC → AAUGUCGAG	Both use a similar set of monomers (with a slight difference: U replaces T)	"I received biology lessons at my school"	"J'ai reçu des cours de biologie à mon école"	Both use a similar alphabet (with slight differences: ç, à, é, Ô etc)

Translation	RNA → Protein	Differences	French → Japanese		Differences
RNA → protein	AAUGUCGAG → Asn–Val–Glu	Both use different monomers (nucleotides & amino acids)	"J'ai reçu des cours de biologie à mon école"	学校で生物学の授業を受けました	Both use different alphabets

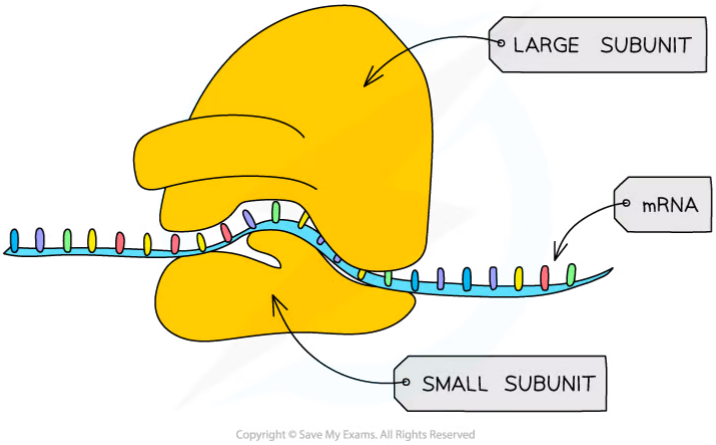
Exam Tip

Be careful – DNA polymerase is the enzyme involved in DNA replication; RNA polymerase is the enzyme involved in transcription – don't get these confused.

YOUR NOTES

Translation

- Translation is the synthesis of polypeptides on ribosomes
- This stage of protein synthesis occurs **in the cytoplasm** of the cell
- After leaving the nucleus, the **mRNA molecule attaches to a ribosome**
- A ribosome is a complex structure that is made of a large and small subunit
  - Ribosomes are themselves made of **proteins** and **RNA** (called ribosomal RNA or **rRNA**)
- There are **binding sites on the subunits** for the various other molecules involved in translation



A ribosome is built of large and small subunits, ribosomal RNA and an area on the surface that catalyses the formation of peptide bonds in a newly-synthesised protein

Exam Tip

Make sure you learn both stages of protein synthesis fully. Don't forget WHERE these reactions take place – transcription occurs in the nucleus but translation occurs in the cytoplasm!

Genetic Code & mRNA

YOUR NOTES

- The amino acid sequence of polypeptides is determined by mRNA according to the genetic code
- mRNA **varies in length**, depending on the length of the gene, but is around 2,000 nucleotides long, on average (in mammals)
- Only certain genes are transcribed in a particular cell, depending on the function of that cell
  - The gene for **rhodopsin** (a light-sensitive protein in the eye) is transcribed to mRNA in **retina cells**, but not transcribed in other body cells where rhodopsin is not required; that would be **a waste of cellular energy**

Exam Tip

Most RNA exists as mRNA but don't forget the other types; transfer RNA (tRNA) and ribosomal RNA (rRNA).

YOUR NOTES

Codons

- Codons of **three bases** on mRNA correspond to **one amino acid** in a polypeptide
- The four nucleotide bases in mRNA are not enough to code for **20 separate amino acids**
- Pairs of nucleotides would only give 16 combinations ($4^2 = 16$), which is **still not enough**
- Triplets of nucleotides would yield **64 combinations** ($4^3 = 64$), which is **more than enough**
- Different triplets code for **the same amino acid**, giving **some protection against mutation**
  - A **triplet** is a sequence of three DNA bases that codes for a specific amino acid
  - A **codon** is a sequence of three **mRNA** bases that codes for a specific amino acid
  - A codon is transcribed from the triplet and is complementary to it
- An **anticodon** is a sequence of three **transfer RNA** (tRNA) bases that are complementary to a codon
  - The transfer RNA **carries the appropriate amino acid** to the ribosome
  - The amino acid can then be condensed **onto the growing polypeptide chain**
- Certain codons carry the command to **stop translation** when the polypeptide chain is complete ('**Stop codons**')

mRNA Codons and Amino Acids Table

FIRST LETTER	SECOND LETTER: U	SECOND LETTER: C	SECOND LETTER: A	SECOND LETTER: G	THIRD LETTER
U	UUU, UUC: Phe UUA, UUG: Leu	UCU, UCC, UCA, UCG: Ser	UAU, UAC: Tyr UAA: Stop UAG: Stop	UGU, UGC: Cys UGA: Stop UGG: Trp	U C A G
C	CUU, CUC, CUA, CUG: Leu	CCU, CCC, CCA, CCG: Pro	CAU, CAC: His CAA, CAG: Gln	CGU, CGC, CGA, CGG: Arg	U C A G
A	AUU, AUC, AUA: Ile AUG: Met	ACU, ACC, ACA, ACG: Thr	AAU, AAC: Asn AAA, AAG: Lys	AGU, AGC: Ser AGA, AGG: Arg	U C A G
G	GUU, GUC, GUA, GUG: Val	GCU, GCC, GCA, GCG: Ala	GAU, GAC: Asp GAA, GAG: Glu	GGU, GGC, GGA, GGG: Gly	U C A G

YOUR NOTES

Worked Example

Use the **rules of base-pairing** and the **mRNA Codons and Amino Acids Table** (above) to deduce the amino acid sequence coded for by the following DNA **sense strand** sequenceTTC GAG CAT TAC GCC

Step 1: Work out the antisense sequence using A-T and C-G base pairing rules

AAG CTC GTA ATG CGG

Step 2: Work out the mRNA codons, complementary to the antisense strand

UUC GAG CAU UAC GCC

Step 3: Use the mRNA Codons and Amino Acids Table (above) to work out the first amino acid

First base in codon = U, second base = U, third base = C

So we're looking in the top-left box of the table; this amino acid is **Phe**

Step 4: Repeat for the remaining 4 codons

GAG = Glu

CAU = His

UAC = Tyr

GCC = Ala

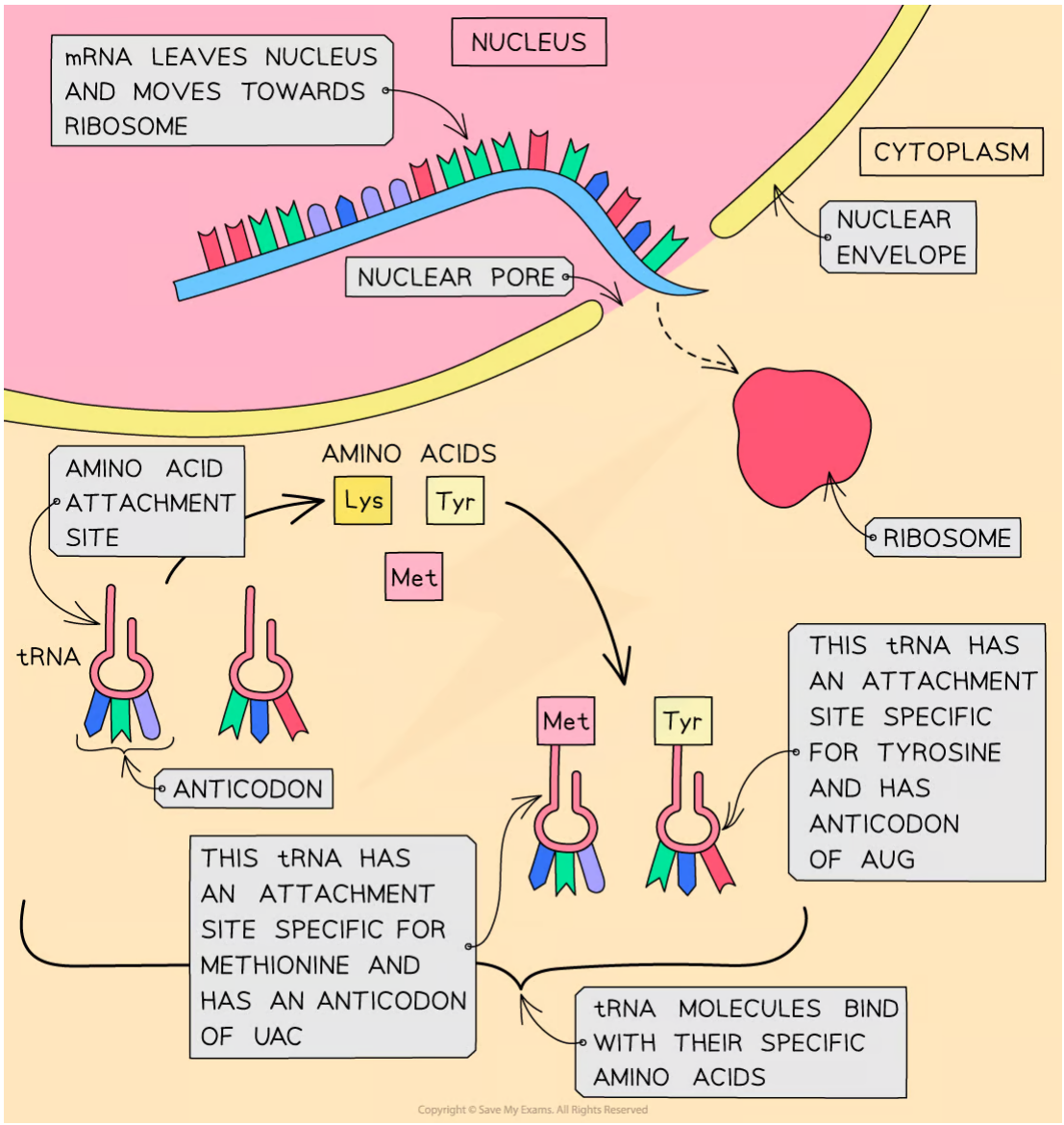
Answer: The final sequence of amino acids is Phe-Glu-His-Tyr-Ala

Codons & Anticodons

YOUR NOTES

- Translation depends on complementary base pairing between codons on mRNA and anticodons on tRNA
- In the cytoplasm, there are **free molecules of tRNA** (transfer RNA)
- The **tRNA molecules bind with their specific amino acids** (also in the cytoplasm) and bring them to the mRNA molecule on the **ribosome**
- The triplet of bases (anticodon) on each tRNA molecule pairs with a complementary triplet (codon) on the mRNA molecule
- **Two tRNA molecules fit onto the ribosome at any one time**, bringing the amino acid they are each carrying side by side
- A **peptide bond** is then formed (by condensation) between the two amino acids
    - The formation of a peptide bond between amino acids is an **anabolic** reaction
    - It **requires energy,** in the form of **ATP**
    - The ATP needed for translation is provided by the **mitochondria** within the cell
- This process continues until a **'stop' codon** on the mRNA molecule is reached – this acts as a signal for translation to stop and at this point the amino acid chain coded for by the mRNA molecule is complete
- This amino acid chain then **diffuses away** from the ribosome and forms the final polypeptide

YOUR NOTES



The translation stage of protein synthesis – tRNA molecules bind with their specific amino acids

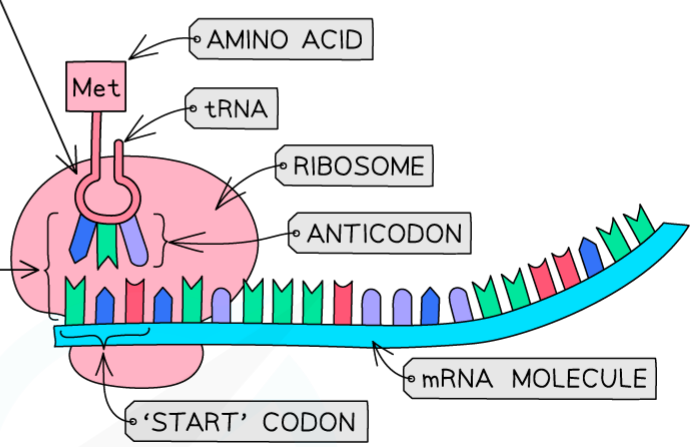
YOUR NOTES

YOUR NOTES

1 IN THE CYTOPLASM THE mRNA ATTACHES TO A RIBOSOME

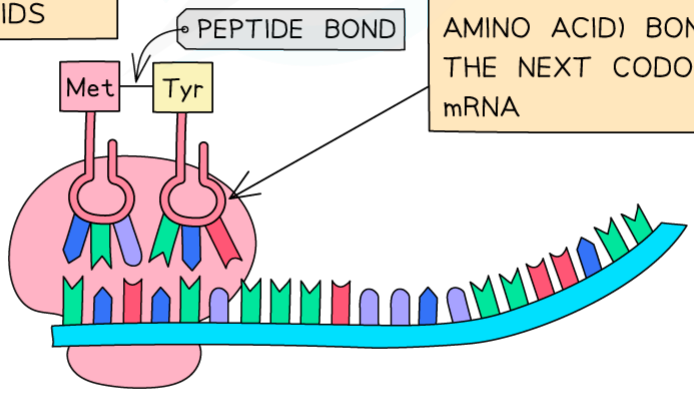
2 EACH tRNA HAS THE COMPLEMENTARY **ANTICODON** TO THE CODON ON THE mRNA

3 THE FIRST tRNA (WHICH ALWAYS CARRIES THE METHIONINE AMINO ACID) FORMS HYDROGEN BONDS WITH THE FIRST OR **'START' CODON** (AUG) ON THE mRNA.



5 A PEPTIDE BOND FORMS BETWEEN THE AMINO ACIDS

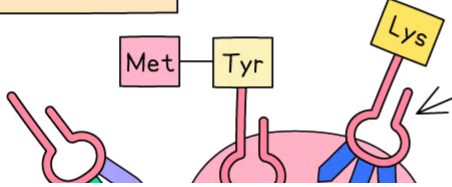
4 THE SECOND tRNA (BRINGING THE SECOND AMINO ACID) BONDS WITH THE NEXT CODON ON THE mRNA



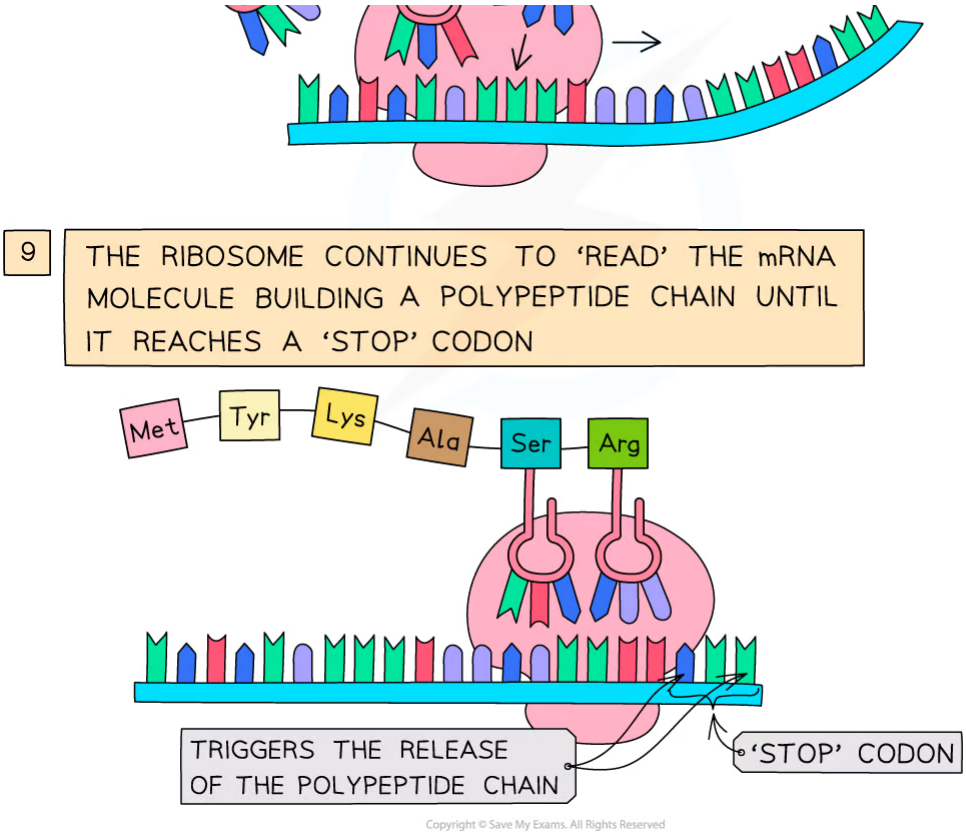
6 THE RIBOSOME MOVES ALONG THE mRNA (IN A 5' TO 3' DIRECTION) 'READING' THE NEXT CODON

8 THE FIRST tRNA (NOW WITHOUT AN AMINO ACID IS RELEASED)

7 THE THIRD tRNA (CARRYING THE THIRD AMINO ACID) BONDS WITH THE COMPLEMENTARY CODON

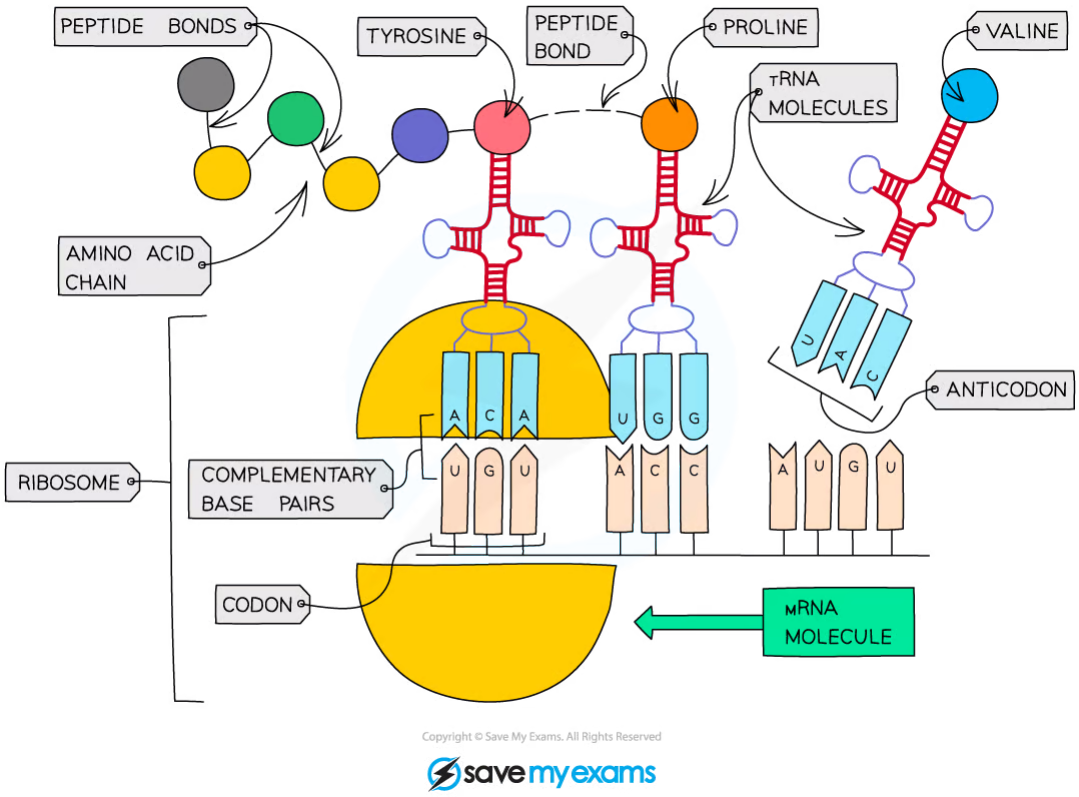


YOUR NOTES



The translation stage of protein synthesis – an amino acid chain is formed

YOUR NOTES



An polypeptide forms as peptide bonds are added in sequence

YOUR NOTES

Polymerase Chain Reaction

Use of Taq DNA polymerase to produce multiple copies of DNA rapidly by the polymerase chain reaction (PCR)

- **Polymerase chain reaction** (PCR) is a common molecular biology technique used in most applications of gene technology, for example, DNA profiling (eg. identification of criminals and determining paternity) or genetic engineering
  - PCR is also used in routine **COVID-19 testing** to detect and amplify small amounts of viral RNA
- It can be described as the ***in vitro* method of DNA amplification**
- It is used to produce **large quantities** of specific fragments of DNA or RNA from very small quantities (even just one molecule of DNA or RNA)
- By using PCR scientists can have **billions of identical copies** of the DNA or RNA sample within a few hours
- The PCR process involves **three key stages** per cycle
- In each cycle, DNA is doubled so, in a standard run of 20 cycles, 1 million DNA molecules are produced. The three stages are undertaken in a PCR instrument (or **thermal cycler**) which automatically provides the **optimal temperature** for each stage and controls the **length of time** spent at each stage

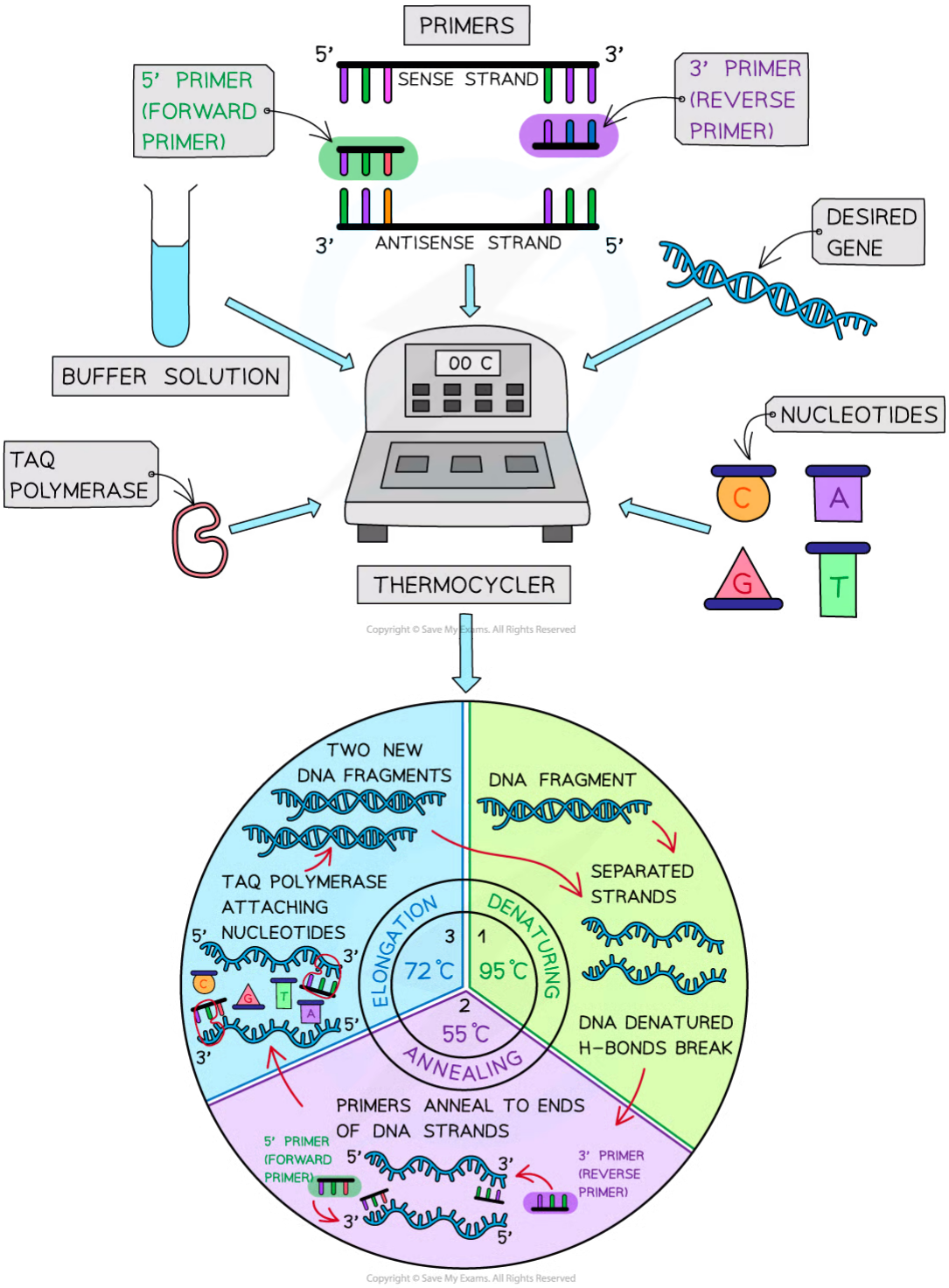
The process of PCR

- Each PCR reaction requires:
  - **Target DNA** or **RNA** that is being amplified
  - **Primers** (forward and reverse) – these are short sequences of single-stranded DNA that define the region that is to be amplified by showing the DNA polymerase **where to begin building** the new strands
  - **DNA polymerase** – is the enzyme used to build the new DNA or RNA strand.
    - The most commonly used polymerase is ***Taq* polymerase** as it comes from a thermophilic bacterium *Thermus aquaticus*
    - This bacterium lives in **hot springs** in geothermal areas
    - Taq polymerase **does not denature** at the high temperature involved during the first stage of the PCR reaction
    - The enzyme's optimum temperature is **high enough to prevent annealing** of the DNA strands that have not been copied yet
  - **Free nucleotides** – used in the construction of the DNA or RNA strands
  - **Buffer solution** – to provide the optimum pH for the reactions to occur in
- The three stages are:
  - **Denaturation** – the double-stranded DNA is heated to 95°C for 15 seconds, which breaks the hydrogen bonds that hold the two DNA strands together
  - **Annealing** – the temperature is decreased to between 50 - 60°C so that primers (forward and reverse ones) can attach to the ends of the single strands of DNA by hydrogen bonding
  - **Elongation / Extension –** the temperature is increased to 72°C for at least a minute

YOUR NOTES

- This is the optimum temperature for *Taq* polymerase to build the complementary strands of DNA
- To produce the new identical double-stranded DNA molecules

- The three stages of a cycle take 2–3 minutes, so many cycles can be completed in a short space of time



The substances required for the Polymerase Chain Reaction to occur and the three key stages of the reaction

YOUR NOTES

Exam Tip

It is important to know the three stages and the temperatures the reactions occur at during the different stages. You must also know why the *Taq* polymerase is used in PCR.

Production of Human Insulin

YOUR NOTES

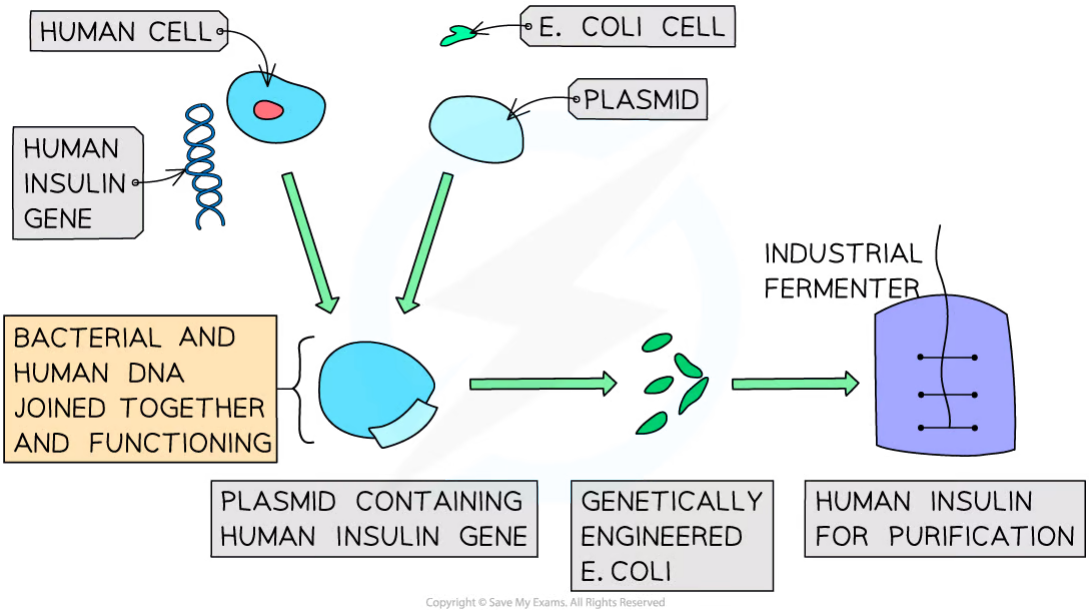
Production of human insulin in bacteria as an example of the universality of the genetic code allowing gene transfer between species

- Prior to the mid-1980s, some insulin-dependent diabetics would need to inject **pig or cattle insulin** as a substitute for human insulin to control their blood sugars
  - Some diabetics developed an **allergy** so could not use it
- Human insulin can be produced by other organisms **by transferring the human insulin gene to them** for **large-scale expression** of that gene
- DNA can be transferred from a human to a prokaryote (eg. *E. coli*), a eukaryote single-celled organism (eg. yeast) or a plant (eg. safflower species)
  - All these organisms express the human insulin gene
  - The insulin produced can be **harvested for medical use**
- The fact that DNA can be transferred from one organism to another **across kingdoms** (and can still do the same job) demonstrates the **universality of the genetic code**
  - The presence of nucleotides is a marker between living and non-living entities
- This was an early successful example of **genetic modification**

Human and Bacterial DNA working together

- In 1982, **insulin** was the first genetically engineered human protein to be approved for use in **diabetes** treatment
- Bacterial **plasmids** are modified to incorporate the human insulin gene
- These genetically modified plasmids are then **inserted into *Escherichia coli***
- The newly-adjusted bacteria are isolated, purified and placed into large scale **fermenters** that provide **optimal conditions**
- The genetically engineered bacteria multiply by binary fission, and express the human protein - insulin, which is eventually extracted and purified
- The advantages for scientists to use genetically engineered insulin are:
  - It is **identical to human insulin**, unless modified to have different properties (eg. act faster, which is useful for taking immediately after eating or to act more slowly)
  - There is a **reliable supply available** to meet demand (no need to depend on the availability of meat stock)
  - **Fewer ethical**, **moral** or **religious** concerns (proteins are not extracted from cows or pigs)
  - **Fewer rejection problems** or **side effects** or **allergic reactions**
  - **Cheaper** to produce in large volumes
  - That it is useful for people who have **animal insulin intolerance**

YOUR NOTES



The production of human insulin; the combination of DNA from two widely different organisms demonstrates the universality of the genetic code

Exam Tip

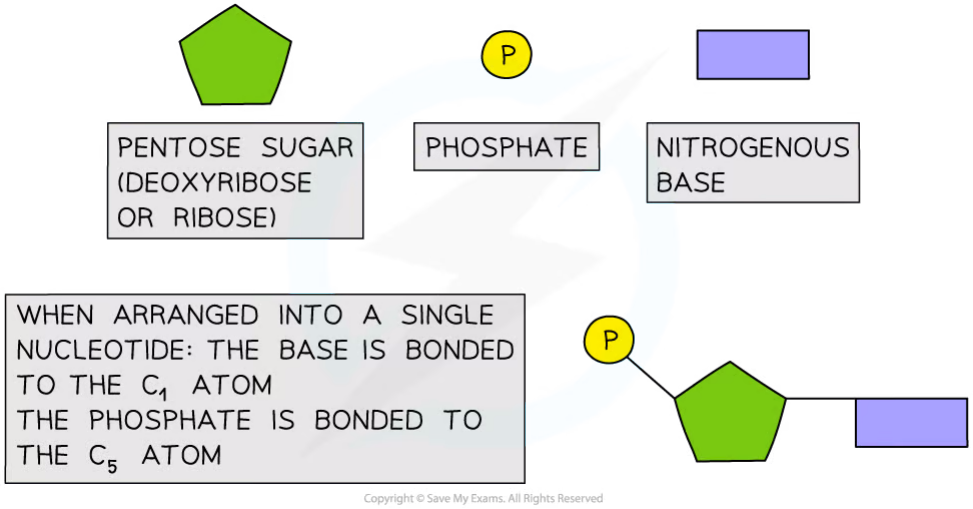
The details of the steps of production of human insulin are not required here. The main learning is that DNA and RNA are a universal code that applies to **all life forms**, as demonstrated by our ability to transfer genes successfully across species and kingdoms.

YOUR NOTES

Drawing DNA & RNA Nucleotides & DNA Double Helix

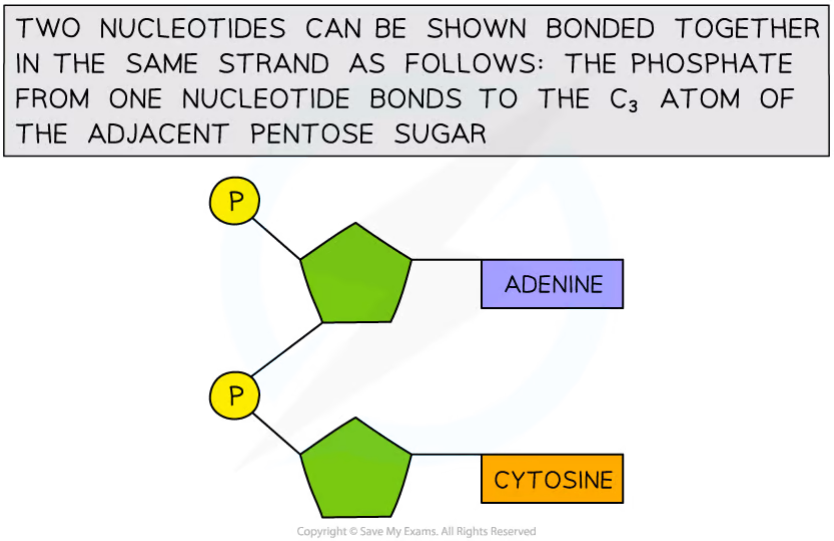
Drawing simple diagrams of the structure of single nucleotides of DNA and RNA

- **Simple shapes** can be used to draw the **main building blocks of nucleotides** and the DNA double helix
  - Advanced drawing skills are not required!
- **Pentagons** can represent **pentose sugars**
- **Circles** can represent **phosphates**
  - Often shown as a circle with the letter P inside: Ⓟ
- **Rectangles** can represent **bases**
- **Covalent bonds** can be shown with **solid lines**
- **Hydrogen bonds** can be shown with **dashed lines**
  - Or with complementary shapes that fit together (see diagrams)

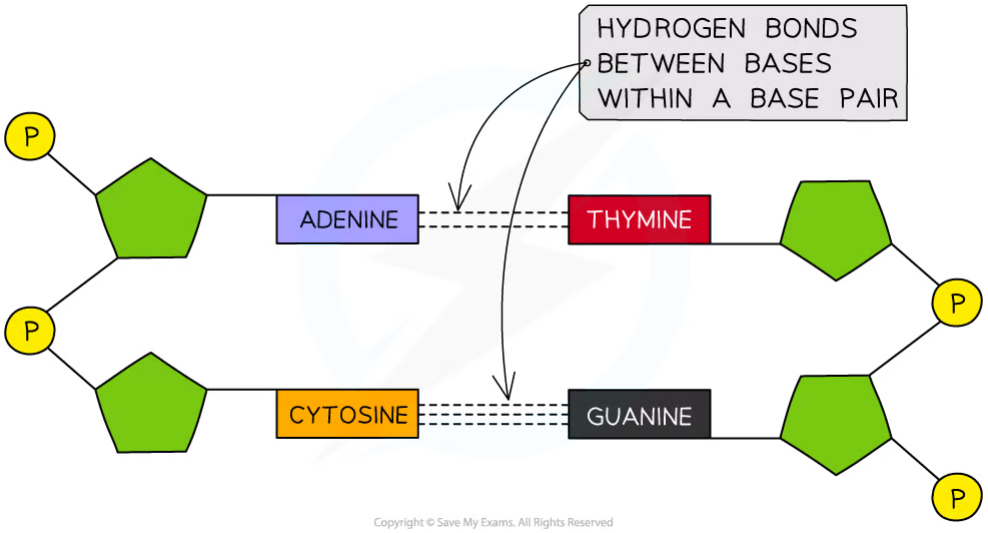


Simple shapes can be used to represent parts of nucleotide molecules

YOUR NOTES

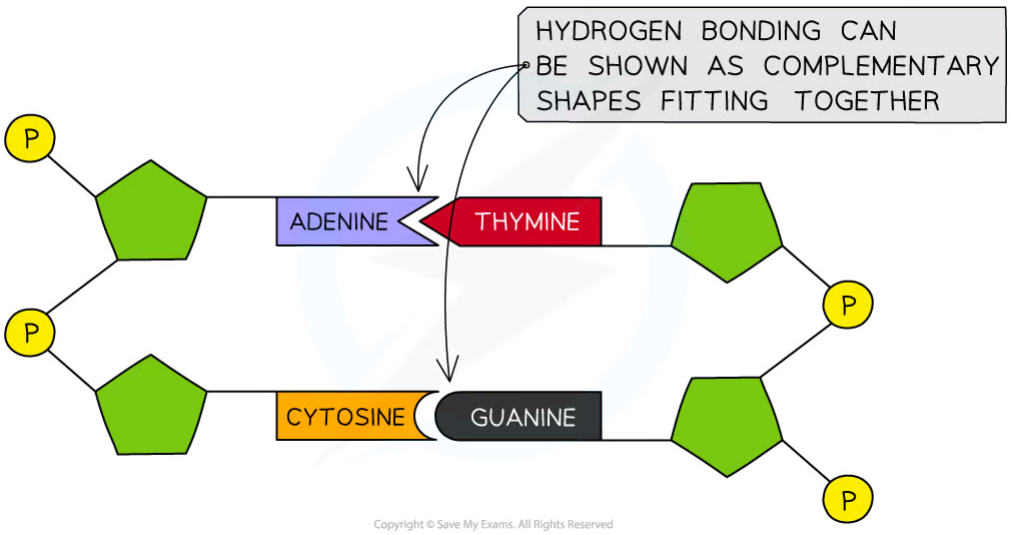


Two nucleotides shown bonded together covalently within a strand



When drawing the base pairing, the opposite strand should be antiparallel to the first. The presence of hydrogen bonding is shown, but the numbers/lengths of bonds is not required

YOUR NOTES



An alternative way to draw a DNA strand is to use complementary shapes for the bases

Exam Tip

Simple, hand-drawn shapes will suffice in an exam. Expert tip - a **large** drawing is always easier for an examiner to read (and award marks for) than a small one!Read the question carefully; examiners often want a **whole nucleotide** to be identified in your diagram and to ensure your diagram includes **all 4 complementary bases.** You don't have to remember the number of hydrogen bonds between the bases.Also, remember to draw DNA strands as **antiparallel** (one upside-down versus the other) but you don't have to be able to draw a helix shape!

YOUR NOTES

Determination of mRNA Base Sequence

- The **rules of base pairing** and the **table of mRNA codons** are needed to be able to convert between DNA sequences, mRNA base sequences and amino acid sequences
- A **triplet** is a sequence of three DNA bases that codes for a specific amino acid
- A **codon** is a sequence of three **mRNA** bases that codes for a specific amino acid
- A codon is transcribed from the triplet and is complementary to it
- When comparing the genetic code to amino acid sequences, **mRNA codons are often used**
- The four bases found in RNA molecules (adenine, uracil, cytosine and guanine) have the ability to form **64** different codons
- Multiple mRNA codons can encode the same amino acid
  - This means that a **change in the genetic code doesn't necessarily result in a change in the amino acid sequence**
    - For example, UGU and UGC both code for the amino acid, cysteine
- Some send important signals to the transcription machinery
  - The **START codon** initiates the process of transcription and ensures it starts in the right location (this is always the amino acid **methionine** in eukaryotic cells, coded for by the codon AUG)
  - **STOP codons** cause transcription to terminate and do not code for an amino acid e.g. UAA
- The genetic code is **non-overlapping**
  - Each base is only read once in the codon it is part of

The rules of base pairing

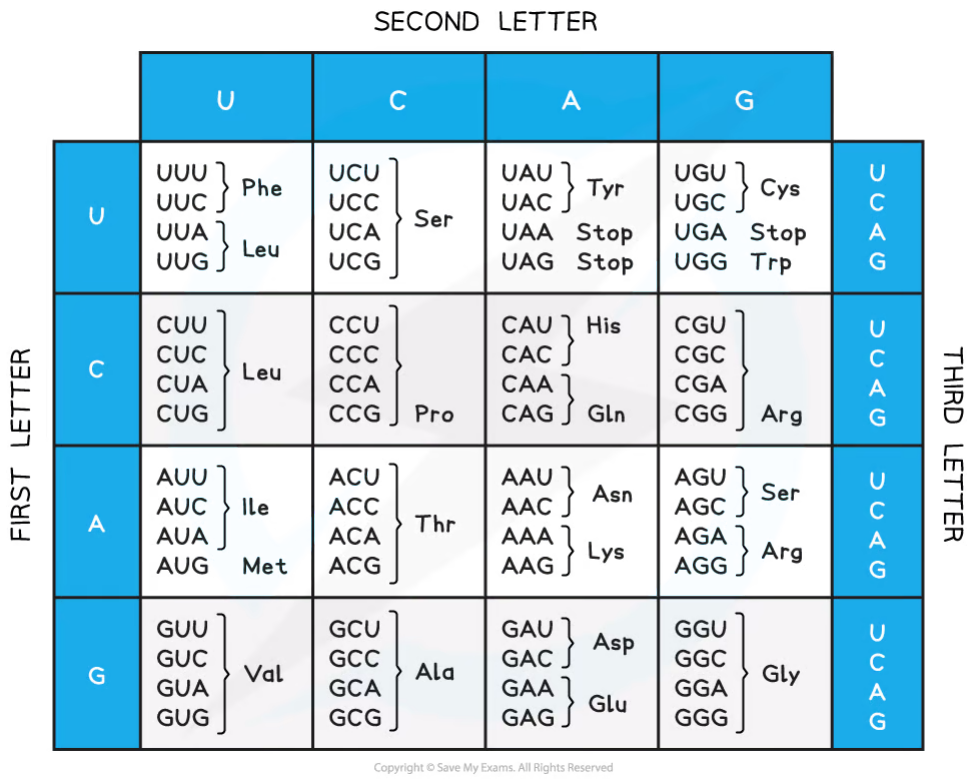
- In **DNA**,
  - **Adenine** (A) always pairs with **Thymine** (T)
  - **Cytosine** (C) always pairs with **Guanine** (G)
- In **RNA**, Thymine (T) is replaced by **Uracil** (U)
  - This means that the base Adenine (A) in DNA is transcribed to Uracil (U) in the mRNA strand

The mRNA Codons and Amino Acids table

- The first three bases of an mRNA strand form the first codon
- The **first base** of the codon is read from the **first column** of the table
- The **second base** of the codon is read from the **top row** of the table
- The **third base** of the codon is read from the **final column** of the table

mRNA Codons and Amino Acids Table

YOUR NOTES



Intrepreting the Genetic Code

YOUR NOTES

- Use a table of the genetic code to deduce which codon(s) corresponds to which amino acid
- The **20 amino acids** are all coded for in the Table of mRNA Codons
- Some amino acids are **only coded for by one codon** eg. methionine (Met), coded by AUG
- Other amino acids **have several codons that code for them** eg. arginine (Arg), coded by CGU, CGC, CGA, CGG, AGA and AGG

Worked Example

Use the table of mRNA Codons and Amino Acids to identify the mRNA codons that code for the following amino acids:

1. Histidine (His)
2. Tryptophan (Trp)
3. Glycine (Gly)
4. Leucine (Leu)

Step 1: Look up His in the table

Codon CAU (no others)

Step 2: Look up Trp in the table

Codon UGG (no others)

Step 3: Look up Gly in the table

Codons GGU, GGC, GGA and GGG

Step 4: Look up Leu in the table

Codons CUU, CUC, CUA, CUG, UUA, UUG

Deducing the Sequences

YOUR NOTES

- Use a table of mRNA codons and their corresponding amino acids to deduce the sequence of amino acids coded by a short mRNA strand of a known base sequence

Worked Example

Deduce the amino acid sequence coded for by the mRNA sequenceAUGACUGGGCCUCCCCAAUAUUAG

Step 1: split the mRNA sequence into triplets

AUG ACU GGG CCU CCC CAA UAU UAG

Step 2: look up the first triplet in the mRNA Codons and Amino Acids Table:

AUG = Met

Step 3: repeat for the remaining triplets

ACU = Thr

GGG = Gly

CCU = Pro

CCC = Pro

CAA = Gln

UAU = Tyr

UAG = Stop

Step 4 : link the amino acids together

Met-Thr-Gly-Pro-Pro-Gln-Tyr

Exam Tip

In an exam, you may be asked to predict the effect of specific mutations in the genetic code. Remember that the genetic code allows more than one amino acid to be coded for by triplets and is non-overlapping!You will not be required to memorise specific codons and the amino acids that they code for.

YOUR NOTES

2.7 Cellular Respiration

2.7.1 Cellular Respiration

Cellular Respiration Defined

- Cell respiration is the controlled release of energy from organic compounds to produce ATP
- Respiration is a **series of chemical reactions** that happens in **every cell**
- Its purpose is to **release energy** in usable forms from the chemical energy stored in food e.g. glucose
- Respiration is a **catabolic** process
- **Glucose** is the main respiratory fuel used in cells
  - **Lipids** and **proteins** can also be used
- **Organic** food substances contain **a lot of chemical energy**
- This **energy cannot be released in one, uncontrolled step** in cells, which would cause cell damage and tissue death
- Enzymes **control the release of energy** through a series of chemical reactions called a **pathway**
- This ends in the production of **ATP** (adenosine triphosphate)
  - To make ATP, a **phosphate group** is linked to adenosine diphosphate (**ADP**)
  - This process **requires energy** which comes from the breakdown of organic molecules
- The energy that is released is used for
  - Fuelling **anabolic** processes
  - Muscle contraction
  - Fuelling **active transport**
  - Moving molecules around the cell
  - **Generating heat** to maintain body temperature in warm-blooded animals

Exam Tip

Respiration is **often confused with breathing,** but remember, respiration is a chemical process, breathing is a method of moving air in and out of the body

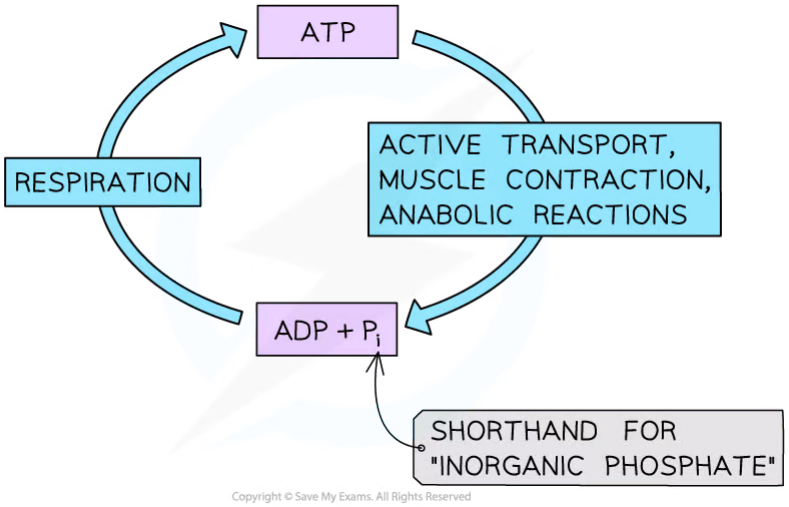
YOUR NOTES

ATP

- ATP is a **source of energy** for cellular processes
- The energy can be released **immediately**, exactly when it is required
- All organisms require a **constant supply of energy** to maintain their cells and stay alive
- This energy is required:
  - In **anabolic** reactions – synthesizing larger molecules from smaller molecules
  - To **move molecules** across the cell membrane (active transport)
  - To move substances and organelles within the cell
  - In animals, energy is required:
    - For **muscle contraction** – to coordinate movement at the whole-organism level
    - In the **conduction of nerve impulses**, as well as many other cellular processes

- In all known forms of life, ATP from respiration is used to transfer energy in **all energy-requiring processes** in cells
- ATP is converted to **ADP** and **phosphate** when releasing its energy
  - ADP and phosphate can then be **re-converted to ATP** during respiration

- Organisms require a **constant supply of ATP** because much of the energy is dissipated (lost to the surroundings) as **heat**



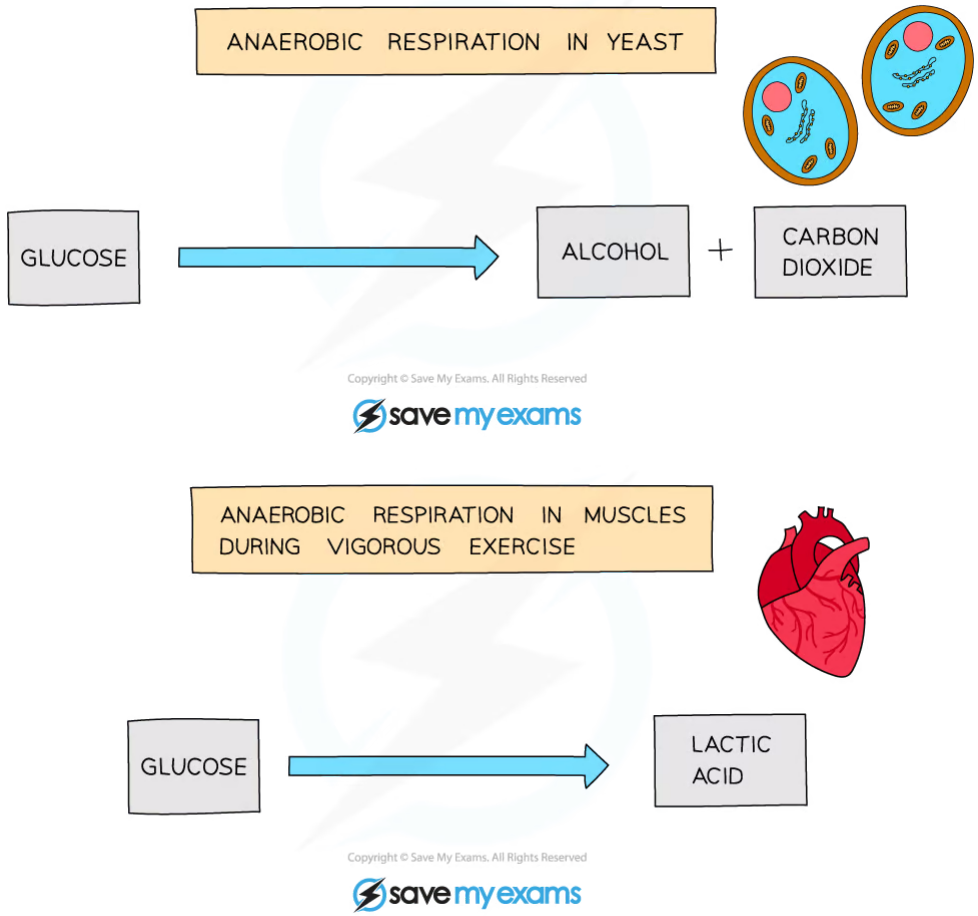
The constant cycling of ATP and ADP+Pi within a cell

YOUR NOTES

Anaerobic Respiration: ATP Yield

- In cells, there is a much **lower energy yield** from respiration in **anaerobic conditions** than in aerobic conditions
- There can be different ways in which oxygen becomes unavailable
  - When **oxygen supply can't keep up with demand** in heavily respiring cells
    - But a short supply of ATP is still required eg. **vigorous exercise** requiring a lot of muscle contraction
  - In conditions where oxygen **cannot reach the organisms** eg. in waterlogged soil
- In anaerobic respiration, **glucose is only partially oxidised** meaning only **a small part of its chemical energy is released** and transferred to ATP
  - The only ATP-producing reaction that continues is the first stage of respiration (around 2 ATP molecules per molecule of glucose)
- As there is no oxygen, **none of the remaining reactions** (of aerobic respiration) can take place
  - This means that around **36 ATP molecules are not produced anaerobically** that would otherwise have been produced in the presence of oxygen
  - 2 ATP molecules is better than zero ATP molecules, so anaerobic respiration can give **a short discharge of energy** when oxygen runs out
- Different types of organisms produce **different products** when respiring anaerobically
  - **Plants and yeasts** produce **ethanol** and **CO_2**
  - **Animals** produce **lactate**

YOUR NOTES

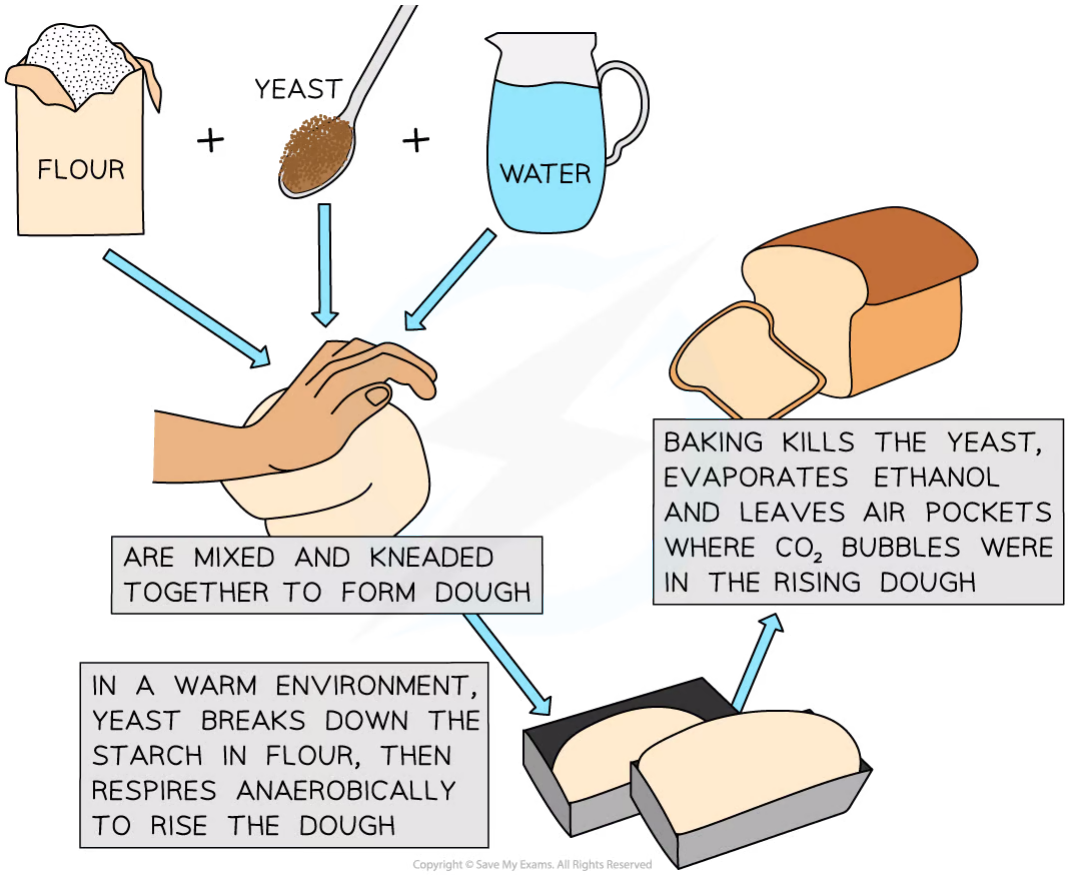


Anaerobic respiration in yeast produces different products to anaerobic respiration in animals

Anaerobic Respiration: Yeast

YOUR NOTES

- Bakers can make use of anaerobic cell respiration in yeasts to produce ethanol and carbon dioxide in baking
- Yeasts are **single-celled fungi** that live in areas where sugars are present eg. on fruit or on leaves
- They can respire **aerobically** or **anaerobically**
- **Flour** contains starch, and when mixed with **water** and **yeast** can form a bread dough
- The dough is **kneaded** to mix everything together
- The dough is then **left in a warm place** to encourage the yeast to respire
- Yeast cells **grow rapidly in number** while oxygen is still present in the dough
    - The yeast **hydrolyses the starch** into maltose and glucose and respire the sugars, **aerobically at first**
- The dough soon **becomes anaerobic** (all the oxygen within it is used up aerobically by the yeast)
- **Anaerobic respiration takes over** and **CO_2 bubbles** begin to form in the dough
- These bubbles allow the dough to **rise** (swell up)
- Baking the dough **kills the yeast** and the bubbles form the fluffy texture of the finished bread
- **Ethanol**, the other product of anaerobic respiration of yeast, is produced but **evaporates** during the final baking stage



The role of anaerobic respiration of yeast in breadmaking to cause bread dough to rise

Anaerobic Respiration: Lactate Production

YOUR NOTES

- Animals have evolved a system to provide **a short burst of energy** in exceptional circumstances
  - Anaerobic respiration can help to **generate powerful muscle contractions** in the short term eg. to escape from a predator or to catch prey
- For humans, anaerobic respiration plays a role in **sport and exercise** more than in survival
- Glucose is metabolised to **lactate** when oxygen can't be supplied quickly enough to muscle cells
- Lactate **accumulates** in those cells and tissues
- Lactate is **toxic above a certain level** and can cause discomfort and even pain (**cramp**)
- This **limits how long an athlete can perform anaerobically for** eg. sprinters, who typically only race over 400 metres or less
- After lactate is produced it has to be **broken down aerobically**
- The breakdown of lactate needs extra oxygen
  - This extra oxygen is referred to as an **oxygen debt**
  - It explains why animals **breathe deeper and faster** for a period of time **after exercise**

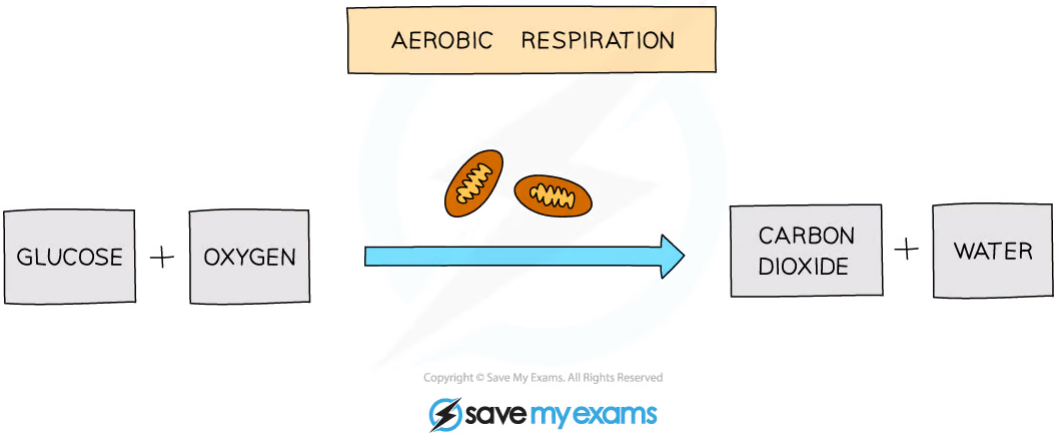
Exam Tip

You won't be expected to know the total yield of ATP from each type of respiration in detail but be prepared to explain why anaerobic respiration produces substantially less ATP than aerobic respiration.

YOUR NOTES

Aerobic Respiration

- Aerobic cell respiration requires oxygen and gives a large yield of ATP from glucose
- The presence of oxygen allows glucose to be **broken down fully** into carbon dioxide and water
- This yields far more energy (approx. 36 ATP molecules) than anaerobic respiration (2 ATP molecules) per molecule of glucose
- CO_2 is a **waste product** and has to be excreted
  - Except in plants where it is used for photosynthesis
- Water is a **by-product** and contributes to the organism's water needs
  - Some animals that live in deserts **drink very little** but survive on this water
- Most of the reactions of aerobic respiration, in eukaryotes, take place in the **mitochondria**



Aerobic respiration releases energy during the reaction between glucose and oxygen

Comparing combustion and respiration

- There are **important similarities** between the **burning** (combustion) of fuels and the **two forms of respiration**
- Both **require oxygen** and produce **CO_2** and **water**
- Both **release heat** from the breakdown of chemical bonds in the fuel

Comparing Respiration and Combustion Table

		Fuel	Reaction	Oxygen supply	Products	Energy Output
Bunsen Burner		Methane	Combustion	High (collar open)	CO_2 and water	High (blue flame)
A cell		Glucose	Respiration	High (aerobic)	CO_2 and water	High (36 ATPs)
Bunsen Burner		Methane	Combustion	Low (collar closed)	Carbon, carbon monoxide	Low (yellow flame)
A cell		Glucose	Respiration	Low (anaerobic)	Lactate	Low (2 ATPs)

YOUR NOTES

YOUR NOTES

Respirometer

Analysis of results from experiments involving measurement of respiration rates in germinating seeds or invertebrates using a respirometer

- Respirometers are used to measure and investigate the **rate of oxygen consumption** during respiration in organisms
- The experiments usually **require live organisms** such as seeds or invertebrates
  - **Use of animals should be minimised** when seeds can provide excellent data
- There are **many different designs of respirometers**, though they all have certain features in common
  - A **sealed container** containing **live organisms** and **air**
  - An alkaline solution (eg. potassium hydroxide) to absorb CO_2
  - A **capillary tube** connected to the container and set **against a graduated scale** (a **manometer**)
- The organisms **respire aerobically** and **absorb oxygen** from the air
- The **CO_2** they release is **absorbed by the alkali**
- This **reduces the air pressure** inside the sealed chamber
- The manometer fluid (shown in red below) **moves towards the organisms** because of the pressure drop inside the chamber
- The respirometer must be kept in **very temperature-controlled conditions** because slight fluctuations in temperature can affect the air pressure
  - A **thermostatically controlled water bath** is the best way to maintain a constant temperature
- **Repeat readings** should be carried out for each set of experimental conditions, in order to **identify** and **eliminate anomalies**
  - **Repeat** readings give a **reliable** mean

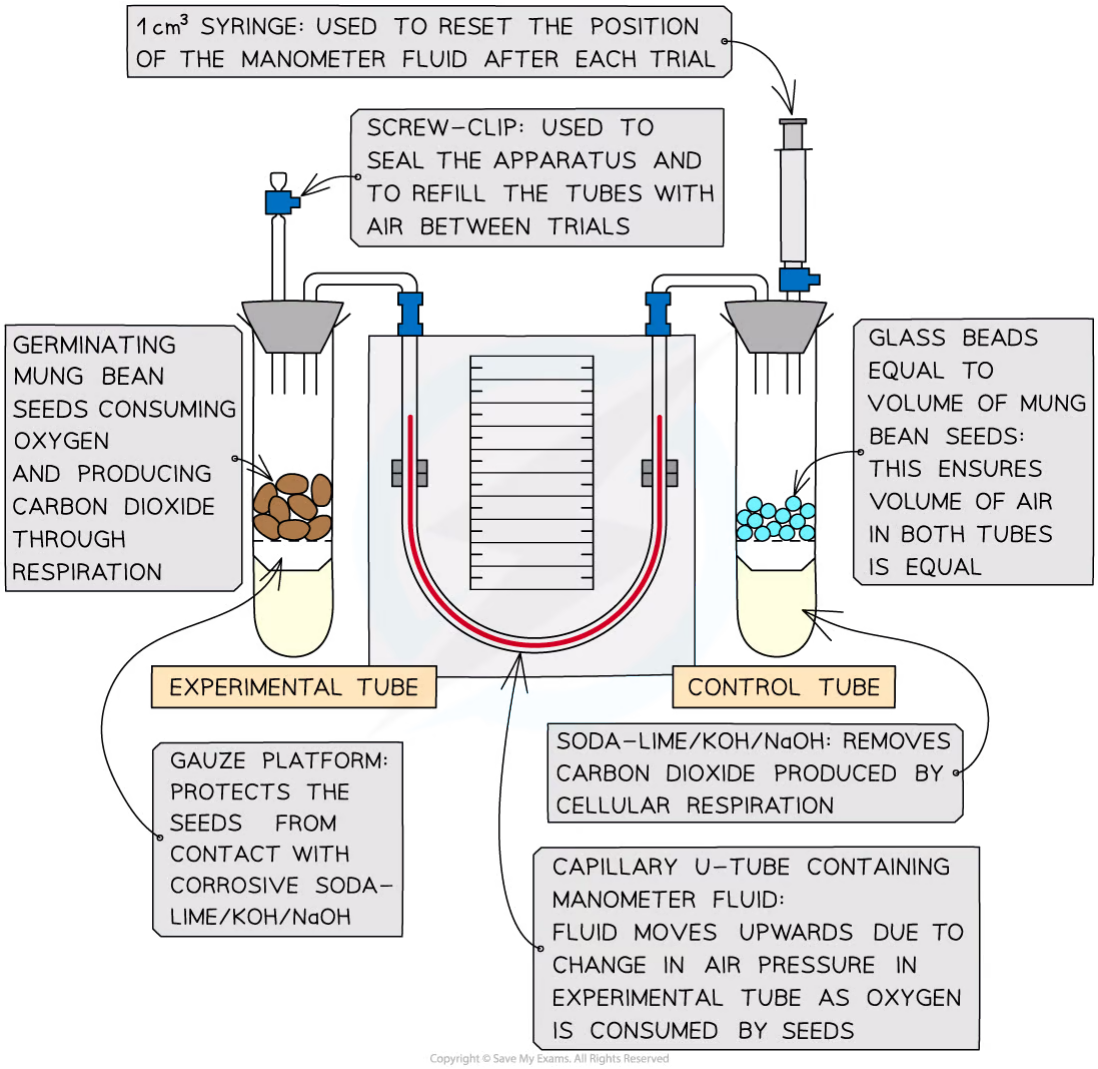
Analysis

  - Respirometers can be used in experiments **to investigate how different factors affect the rate of respiration** of organisms over time
    - Eg. temperature – using a series of water baths

Use of technology to measure rate of respiration

- **Technological devices** can automate and make the measurement of respiration rate easier
  - Not to be confused with **breathing rate**
- **Oxygen sensors** and **CO_2 monitors** can measure oxygen and CO_2 concentration in real-time
  - Without the need to expose the subject to hazards such as strong alkalis
- **Dataloggers** can record data over a period of time for analysis later

YOUR NOTES



The typical set-up of a respirometer

The equation for calculating a change in gas volume

- The volume of oxygen consumed ($mm^3\ min^{-1}$) can be worked out using the radius of the lumen of the capillary tube *r* (mm) and the distance moved by the manometer fluid *h* (mm) in a minute using the formula:

$$\pi r^2 h$$

Worked Example

A respirometer was set up with germinating mung beans in the experimental tube. After a period of equilibration, the liquid in the capillary was measured to move by 2.3 cm in 25 minutes 30 seconds. The capillary tube had an internal diameter of 0.30 mm.Calculate the rate of respiration of the mung beans, measured as the rate oxygen uptake, in $mm^3\ hr^{-1}$Use the value of pi (π) = 3.141 and state your final answer to 2 significant figures

YOUR NOTES

Step 1: Calculate the cross-sectional area of the capillary tube

Diameter = 0.30mm, so radius = 0.30 ÷ 2 = 0.15 mm

Cross sectional area = $\pi r^2 = 3.141 \times 0.15^2 = 0.0707\ mm^2$

Step 2: Calculate the volume of oxygen that had been taken up

The liquid moved 2.3 cm, which is 23mm

Volume of liquid moved in 25 minutes 30 seconds =

$\pi r^2 h$, where h = 23 mm
$= 0.0707 \times 23 = 1.625\ mm^3$

Step 3: Calculate the rate of oxygen consumption per hour

25 minutes 30 seconds = 25.5 minutes

Rate per hour = $1.625 \times (60 \div 25.5)$
$3.824\ mm^3\ hr^{-1}$

To 2 sf = **$3.8\ mm^3\ hr^{-1}$**

NOS: Assessing the ethics of scientific research: the use of invertebrates in respirometer experiments has ethical implications

- The use of live animals in experiments has raised **ethical concerns**
- Should we be removing animals from their natural habitat?
  - Does **human learning** outweigh the suffering that may be caused?
- Will the animals suffer or feel **pain**?
- How can **exposure to hazards be minimised** for the animals eg. avoiding contact with the alkali
- Animals must be **returned** to their natural habitat **directly after** the readings have been taken
- Can an **alternative method** that uses other non-animal species be found that still provides learning eg. the use of **germinating seeds**?
- There must be **no laboratory work** that **causes pain or suffering** to animals or humans

Exam Tip

There are several ways you can manage variables and increase the reliability of results in respirometer experiments:

- Use a controlled water bath to keep the **temperature** constant
- Have a control tube with an equal volume of inert material to the volume of the organisms to compensate for changes in atmospheric **pressure**
- Repeat the experiment multiple times for reliability and calculate a **mean**

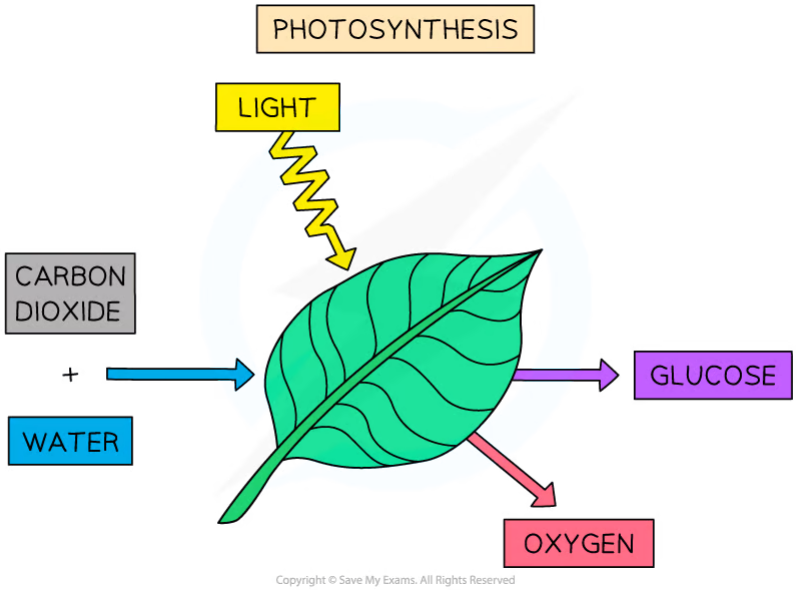
YOUR NOTES

2.8 Photosynthesis

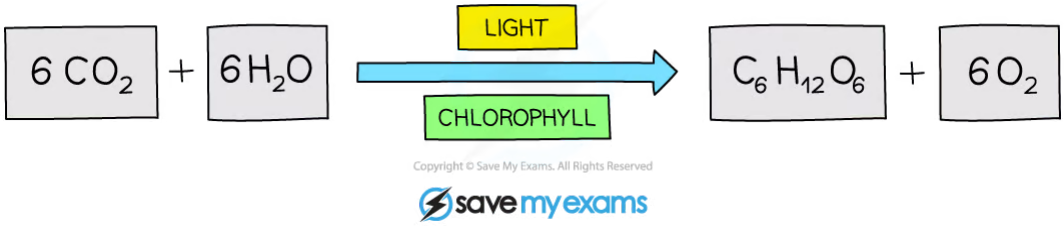
2.8.1 Photosynthesis

Photosynthesis Defined

- **Simple, inorganic compounds** are converted into complex organic ones by photosynthesis
  - The energy required is provided by **light**
- Photosynthesis occurs in autotrophic organisms such as **plants**, **algae** and **cyanobacteria**
  - H_2O and CO_2 are the **raw materials**
- Photosynthesis is a form of **energy conversion**, from **light energy** to **chemical energy,** stored in biomass
- **Energy is stored** within the bonds of these organic compounds
- Photosynthesis can be thought of as the **exact reverse of respiration**
  - Respiration is the process by which **energy is *released*** from organic molecules in living cells
- The overall **chemical equation** for photosynthesis is as follows:



The basic equation of photosynthesis as it takes place in a leaf



The chemical equation for photosynthesis

YOUR NOTES

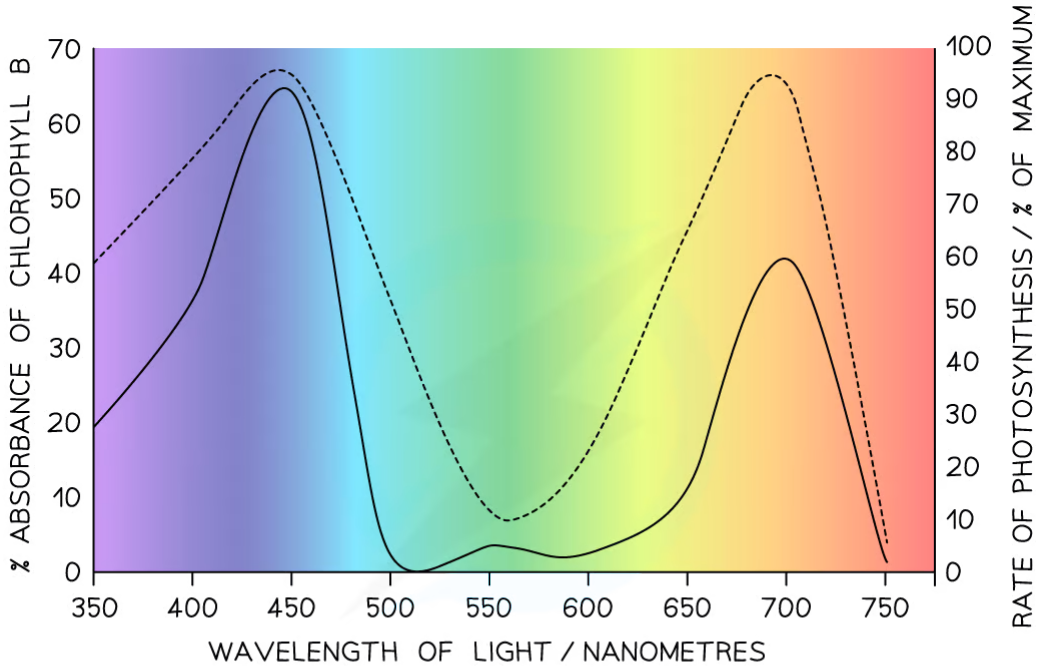
Exam Tip

Remember, energy is never created or destroyed; it is only ever converted from one form to another!

Visible Light Wavelengths

YOUR NOTES

- Chloroplasts contain **pigments** in order to **absorb light**
- Pigments are **coloured**, which means they absorb **some wavelengths** (or colours) of the white light that the Sun radiates
  - The remaining light is **reflected**, giving the pigment its colour
- Chloroplasts contain several different **photosynthetic pigments**, so that they can **absorb multiple different wavelengths of light**
  - The main photosynthetic pigment is **chlorophyll**
- **Violet light** has the shortest wavelength of light in the visible spectrum (around 400nm)
- **Red light** has the longest wavelength of light in the visible spectrum (around 700nm)
- **Green light** has a wavelength in the middle of this range (around 550nm)
- The **absorption of light varies with wavelength**, as does the **rate of photosynthesis** that a plant can carry out
- When plants are exposed to light of a specific wavelength, the **rate of photosynthesis** can be measured as well as the **absorbance** (the % of the light that is absorbed by the plants)
- There are peaks in both plots at the **blue** and **red** ends of the spectrum, where photosynthesis can occur
- There are troughs in both plots for **green** light, which is **not absorbed** and so **cannot provide energy for photosynthesis**



The effect of visible light wavelength on the % absorbance of chlorophyll b and the rate of photosynthesis

YOUR NOTES

Exam Tip

You don't have to memorise the wavelengths of different colours of light, but you need to know that visible light has a wavelength of between 400 and 700 nanometres (nm).

Chlorophyll

- Plant cells contain **chloroplasts** which are the site of photosynthesis
- The main photosynthetic pigment is **chlorophyll**
- Chlorophyll absorbs red and blue light most effectively and reflects green light more than other colours
  - Chlorophyll appears green because it **absorbs red and blue light**
  - The **green light is reflected away** and so leaves appear green to the eye
  - This explains **why the majority of plants are green** (with variations in the shades of green that we can see)
- Red and blue light provides the energy needed for photosynthesis
- Chlorophyll exists in **two main forms**, ***a*** and ***b***
- There are **two groups** of pigments: primary pigments known as **chlorophylls** and accessory pigments known as **carotenoids**
- **Chlorophylls** absorb wavelengths in the **blue-violet and red regions** of the light spectrum
- **Carotenoids** absorb wavelengths of light mainly in the **blue-violet region** of the spectrum
- The combination of pigments **maximises the amount of white light energy** that can be captured

Exam Tip

Remember – chlorophyll is not the only photosynthetic pigment, others exist to maximise light energy absorption.

Photolysis of Water

- Oxygen is produced in photosynthesis from the photolysis of water
  - **Photo** - means 'with light'
  - **Lysis** - means 'breaking apart'
- Water is **broken apart using light** energy; this is called **photolysis**
- This releases **electrons** (e^-), **protons** (H^+) and the waste product, **oxygen gas**

$$2H_2O \rightarrow 4e^- + 4H^+ + O_2$$

- Whilst oxygen is a waste product, the **electrons and protons** play a crucial role in the **further reactions** of photosynthesis
  - Though oxygen is a waste product, in practice, a plant will use some of the oxygen it produces in photosynthesis for its own respiration (during the day)

YOUR NOTES

Effects of Photosynthesis

- Changes to the Earth's atmosphere, oceans and rock deposition occur due to photosynthesis
- The **first life forms** emerged around 4 billion years ago
  - At the time, there was **no oxygen in the atmosphere**
- About 3.5 billion years ago photosynthetic prokaryotes became the first organisms to carry out photosynthesis
  - **This began the release of oxygen** into the atmosphere
- Millions of years later algae and plants evolved and also carried out photosynthesis
- Around 2.2 billion years ago, the oxygen concentration in the atmosphere reached 2%
  - This is known as the Great Oxidation Event
- Other changes to the Earth occurred due to photosynthesis
  - **Minerals** in the oceans were **oxidised**
    - Photosynthetic bacteria released oxygen into the ocean
    - When dissolved iron was oxidised it formed iron oxide which is a red precipitate that lies on the sea bed
    - Over time a distinctive rock formation was produced - the banded iron formation. Layers of red iron oxide alternate with other mineral oxides
    - Banded iron formations are the most important source of iron **ores** (and consequently our supply of steel)
  - Methane and CO_2 levels in the air fell, which resulted in an **Ice Age**
    - Because methane and CO_2 are important **greenhouse gases**
- By 600 million years ago, life had evolved into **large multicellular organisms**, many of which were photosynthetic (plants)
- This pushed the oxygen concentration of the air up to 20%, **peaking at 35%** 300 million years ago
  - This contributed to the **large size of the animals** that roamed the Earth at that time
- The current atmospheric oxygen level is around 21%, due to **increased human activity** eg. burning of fossil fuels, deforestation which remove oxygen from the atmosphere

Energy Requirements

- Chemical reactions can be **exothermic** or **endothermic**
- Photosynthesis is an example of an endothermic reaction and an anabolic reaction, where the required energy input is in the form of light energy
- Energy is needed to produce carbohydrates and other carbon compounds from carbon dioxide
- The energy is not lost - it is **stored in chemical form** in the carbohydrates that are produced

Limiting Factors

YOUR NOTES

Temperature, light intensity and carbon dioxide concentration are possible limiting factors on the rate of photosynthesis

- Each of these factors can **limit the rate of photosynthesis** when they are below the optimal level
  - Temperature
  - Light intensity
  - Carbon dioxide concentration
- These are known as the **limiting factors** of photosynthesis
  - A limiting factor is a variable that holds back the rate of a chemical reaction
  - If that variable is increased, the reaction rate also increases
- Under any set of conditions, only one of these factors will be limiting the rate of photosynthesis
  - At night, **light intensity** will be very low, so that is the limiting factor
  - On a cold, sunny day, **temperature** will be the limiting factor
    - An increase in the light intensity will not increase the rate of photosynthesis because the temperature is, at that point, the limiting factor

Analogy - Limiting Factors

Imagine you're on a group hike and you have to stick together with your two teammates. You are not allowed to finish the hike separately. If your legs are feeling weak, you will be walking slowly. The other two hikers will have to slow down to walk at your speed; the team progresses at the speed of the slowest member. However, after lunch, you may be feeling strengthened by the food and your legs feel good; this time, it's your teammate who is the slowest, so has taken over from you as the limiting factor. Your team only finishes when all three of you finish, and that determines your overall rate of progress.

Exam Tip

When writing about limiting factors, it's important to mention '**light intensity**', not just 'light'. Other aspects of light such as wavelength can play a role so it's important to specific about intensity.

YOUR NOTES

Absorption & Action Spectrums

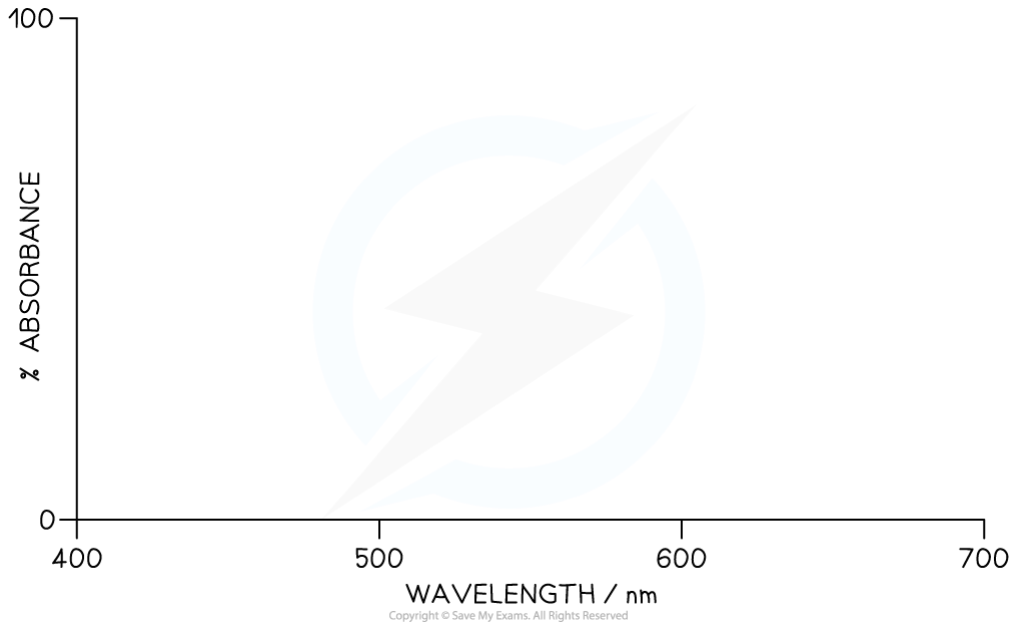
- An **absorption spectrum** (for a particular pigment) shows how much light of different wavelengths is absorbed by the pigment
- An **action spectrum** shows how each wavelength of light affects the rate of photosynthesis that it can power
- These are two graphs that use the wavelength of light on the x-axis
  - **Violet** light is the **lowest wavelength** of the visible spectrum, at 400 nanometres (nm)
  - **Red** light is the **highest wavelength** of the visible spectrum, at 700 nanometres (nm)

Drawing an absorption spectrum for chlorophyll

Step 1: Draw and label the axes

- Draw an x-axis
- Label the axis **wavelength**
- Add the units / **nm**
- Make 400 the smallest value and 700 the largest value
  - Label 500 and 600 nm on the x-axis

- Draw a y-axis
- Label it **% absorption**
- Make 0 the lowest value and 100 the highest value
  - No units are required because the y-axis is showing a percentage scale



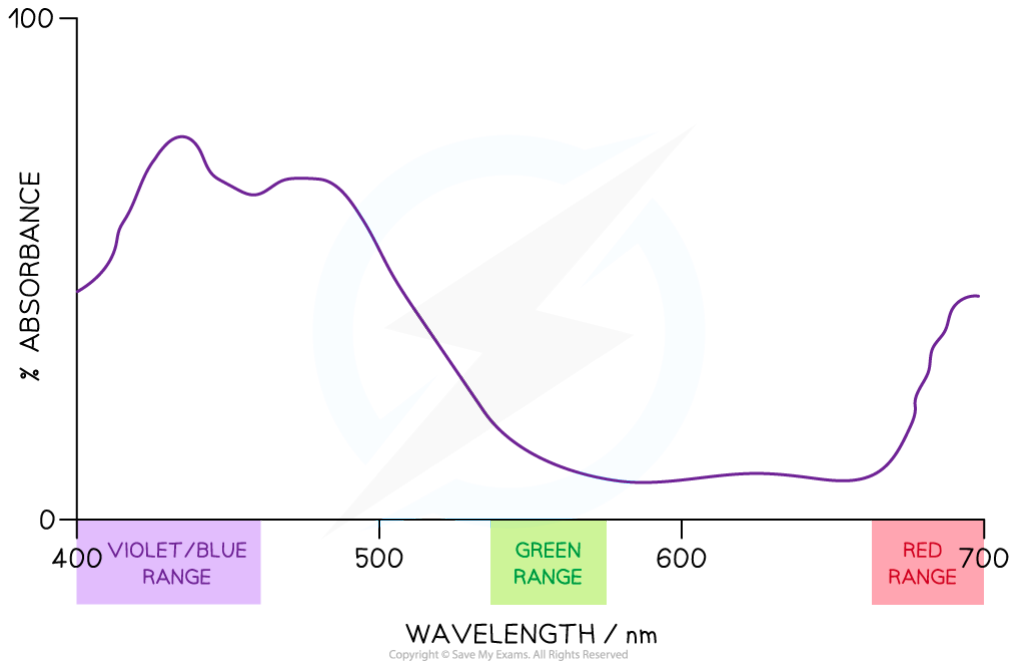
Step 1 : Draw and label the axes

Step 2: Draw the Plot

- There should be **two absorbance peaks**
  - One peak at either end, in the blue and red areas of the spectrum

YOUR NOTES

- And a **trough** in the middle, which represents green light
- As below, with a smooth curve



Step 2: Sketch the Curve

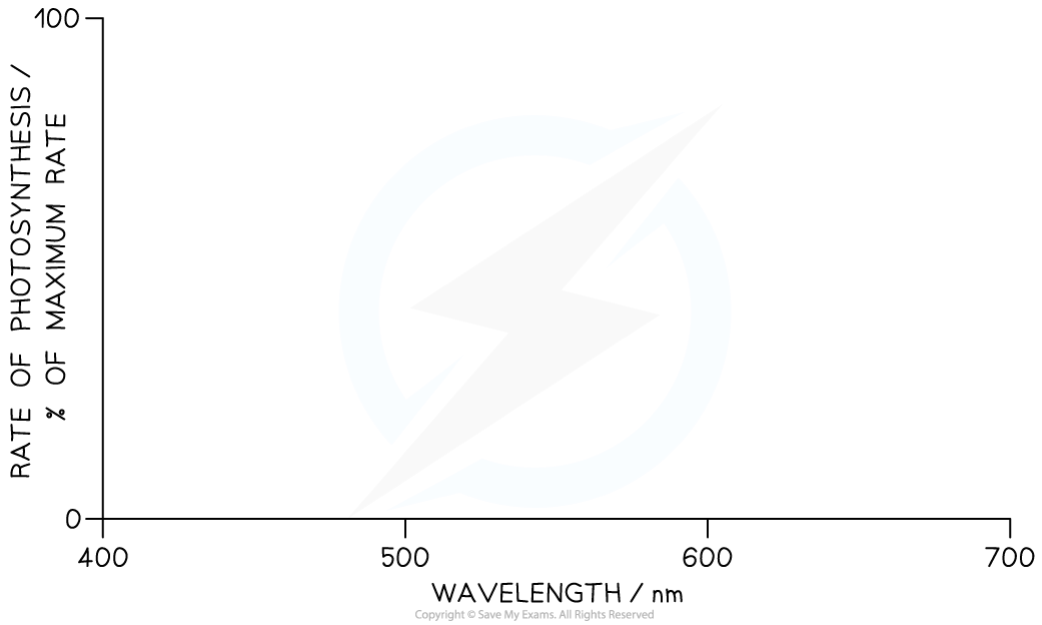
An absorbance spectrum for photosynthesis (colour range labels are not required)

Drawing an action spectrum for photosynthesis

Step 1: Draw and label the axes

- Draw an x-axis
- Label the axis **wavelength**
- Add the units / **nm**
- Make 400 the smallest value and 700 the largest value
  - Label 500 and 600 nm on the x-axis
- Draw a y-axis
- Label it **Rate of photosynthesis / % of maximum rate**
- Make 0 the lowest value and 100 the highest value
  - No units are required because the y-axis is showing a percentage scale

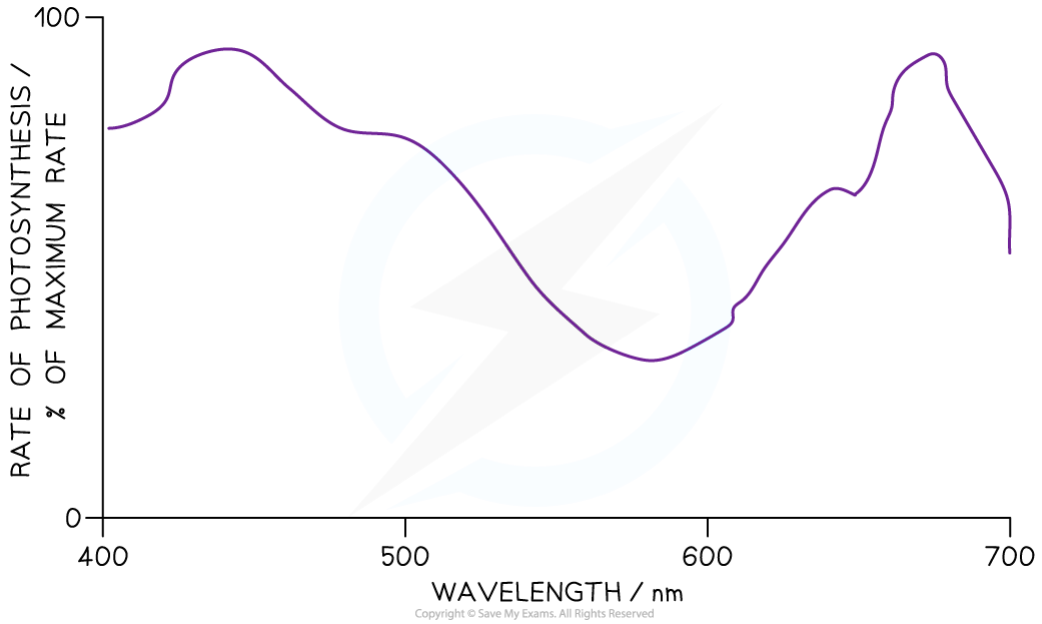
YOUR NOTES



Step 1 : Draw and label the axes

Step 2: Draw the plot

- There should be **two peaks of rate of photosynthesis**
  - One peak at either end, in the blue and red areas of the spectrum
  - And a **trough** in the middle, which represents green light
  - As below, with a smooth curve



Step 2: Sketch the Curve. An absorbance spectrum for photosynthesis (colour range labels are not required)

YOUR NOTES

Exam Tip

Remember – the pigments themselves have a distinctive colour. This is different from the colours of light that they *absorb*.Key points to remember:

1. Label 400 - 700nm on the x-axis, in 100nm increments
2. Use a % scale on the y-axis
3. Smooth curve
4. Peaks at either end
5. Trough in the middle for green light

Investigating Photosynthesis

YOUR NOTES

- An **aquatic plant** such as *Elodea* or *Camboba* is a good choice for investigating photosynthesis in plants, because the rate of photosynthesis can be measured by **counting oxygen bubbles** that come off a cutting of this plant
  - Oxygen output from terrestrial plants (that grow on land) would not be observable

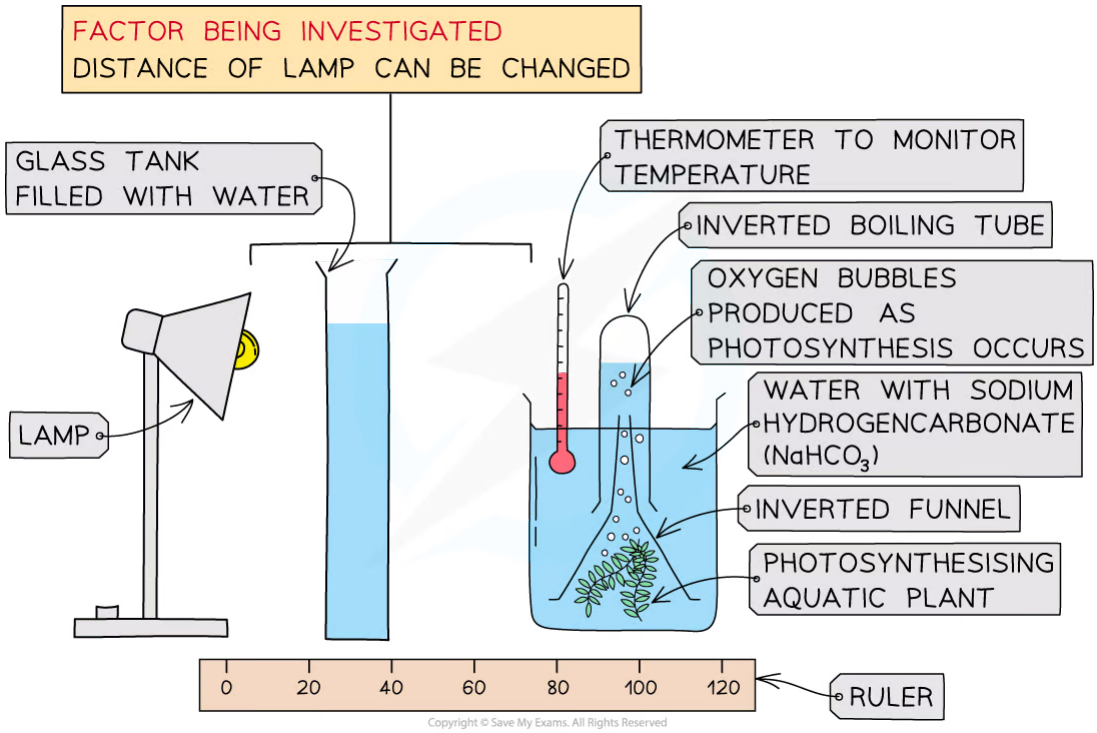
NOS: Experimental design - controlling relevant variables in photosynthesis experiments is essential

- When designing an experiment it is crucial that all variables (apart from the independent and dependent variables being investigated) are controlled
  - The independent variable is the factor that is **deliberately manipulated** between a specific range throughout the experiment
  - The dependent variable is the factor that is **measured** during the experiment (to see if it is affected by the changes to the independent variable)
- Other variables must be controlled so that it can be said the independent variable is the only factor affecting the dependent variable during the experiment
- **Changes** in **light intensity**, **carbon dioxide concentration** and **temperature** are all limiting factors that affect the rate of photosynthesis and **can be altered experimentally** to measure the effect on the rate of photosynthesis

Effect of light intensity - experimental design

- Basic Experimental Setup
  - Aquatic plant cutting in water
  - Powdered sodium hydrogencarbonate ($NaHCO_3$)
  - Glass funnel
  - Boiling tube
  - Lamp for illumination
  - Glass tank filled with water

YOUR NOTES



Measuring the Effect of Light Intensity on the Rate of Photosynthesis in Pondweed

Research Question

Does the rate of photosynthesis (number of bubbles released per min) of *Elodea* increase as the light intensity increases?

Method

- Place a piece of aquatic plant (*Elodea* or *Cabomba* are often used), into a beaker of water
- Use a light a set distance from the plant
- Record the number of bubbles observed in three minutes
- Repeat steps for different distances

Improvements

- Use a **gas syringe** to collect and measure the volume of gas produced
- For **reliability** of data, **repeat** the experiment at least twice for each distance and calculate the mean number of bubbles
- Use of a **data logger** to measure results continuously

Variables to Be Controlled

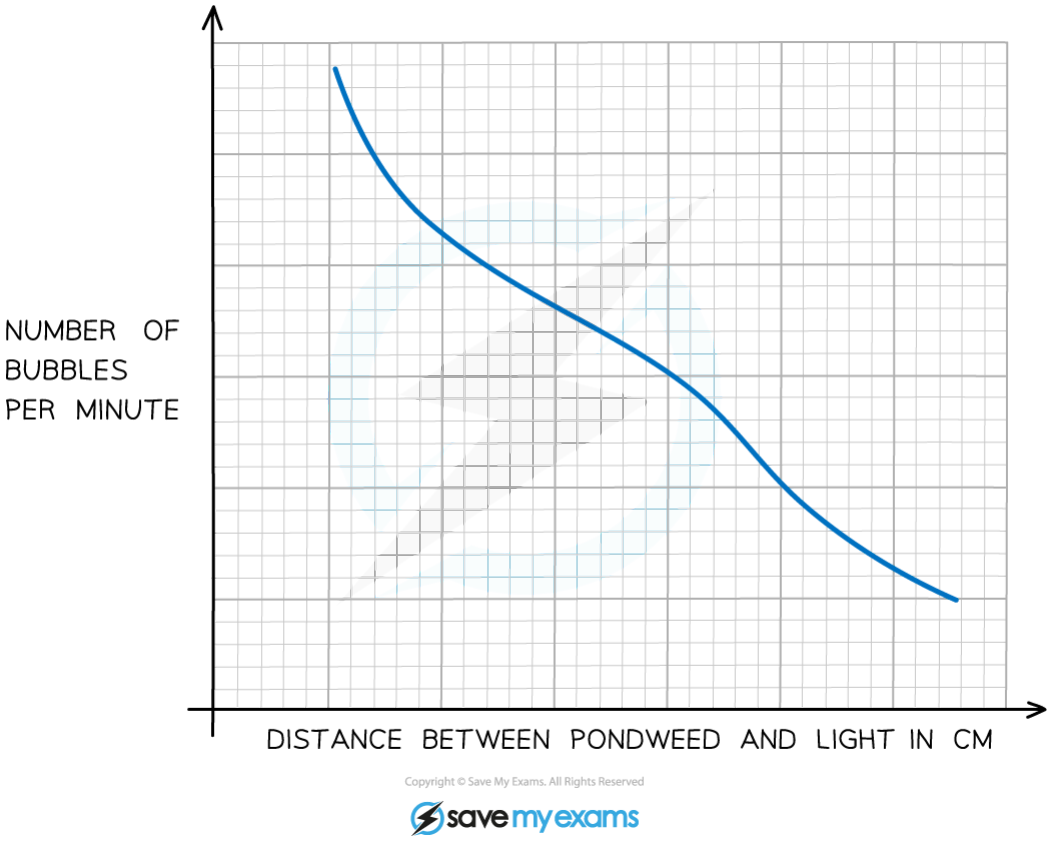
- **Temperature**
  - The glass tank filled with water absorbs any heat that is emitted from the lamp
  - Modern LED bulbs can be used as they give off less heat than filament bulbs
- **CO_2 concentration**
  - The water used around the plant is first **boiled and re-cooled** to remove any dissolved carbon dioxide

YOUR NOTES

- A **set mass of sodium hydrogencarbonate** is added to the water that surrounds the plant
- To make the concentration approx. 0.1 mol dm^{-3}, which is not a limiting concentration

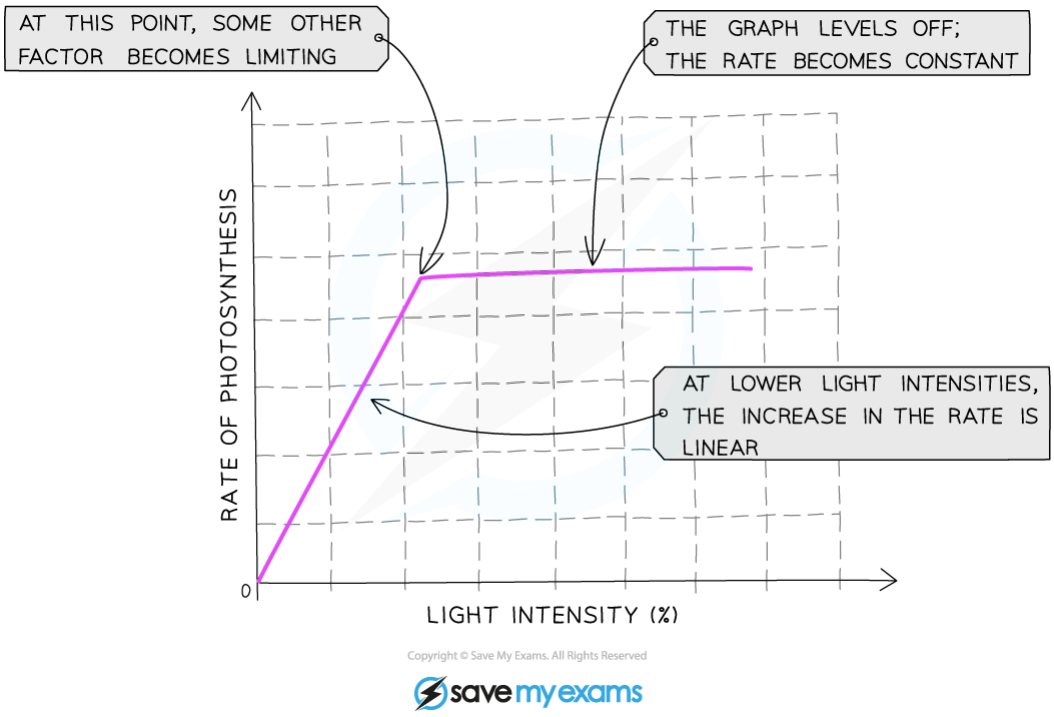
Results

- A graph of the **number of bubbles produced per minute** against the **distance between the lamp and the plant** used can be drawn to see the pattern or trend
  - Distance between the lamp and the plant is linked to the light intensity



A graph of distance from the lamp against number of bubbles per minute

YOUR NOTES

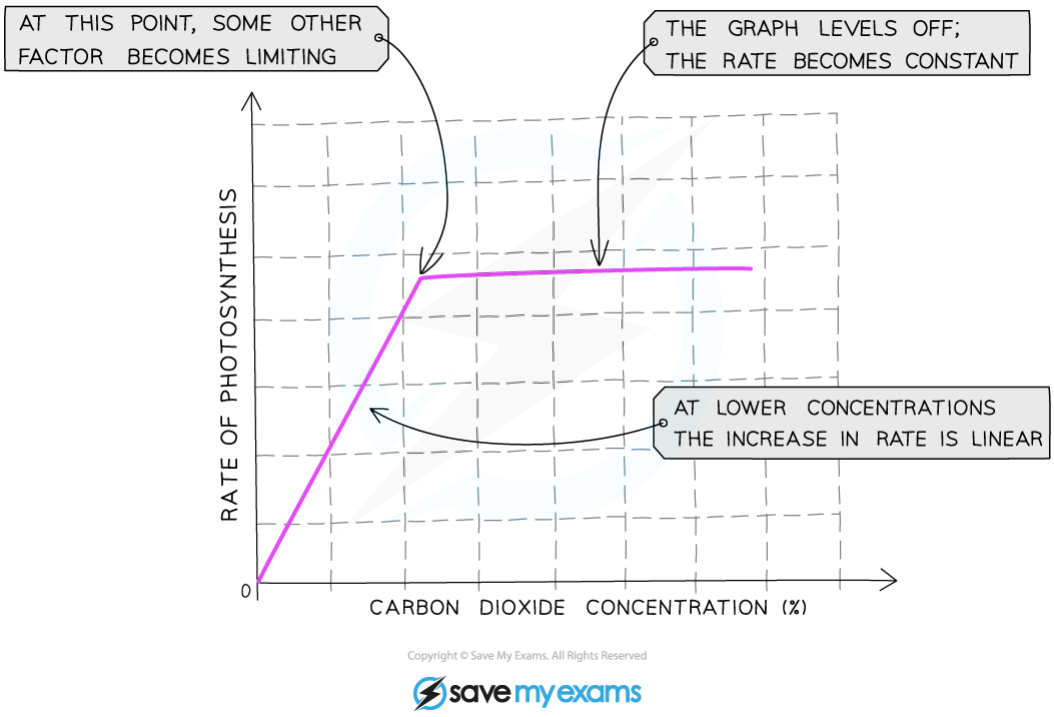


The effect of light intensity on the rate of photosynthesis

Carbon dioxide concentration

- The **same basic experimental setup** can be used, but with varying use of the following variables
- Start with boiled and re-cooled water as before
- Add **successive masses of sodium hydrogencarbonate** to increase the concentration in increments of 0.01 mol dm^{-3}, and record the rate of photosynthesis in bubbles $minute^{-1}$
- **Keep the temperature constant** at 25°C using a water bath, monitoring with a thermometer in the water surrounding the aquatic plant
- **Keep the light intensity constant** by keeping the lamp a fixed distance from the plant

YOUR NOTES

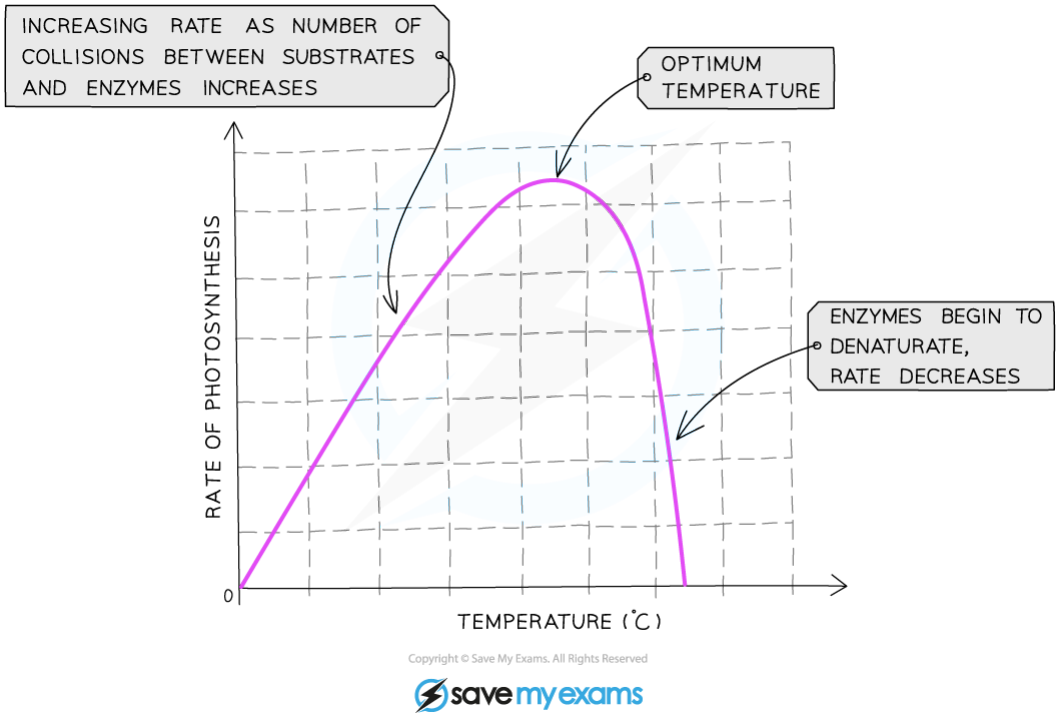


The effect of carbon dioxide concentration on the rate of photosynthesis

Temperature

- The **same basic experimental setup** can be used, but with varying use of the following variables
- Start with boiled and re-cooled water as before, with **sodium hydrogencarbonate at a fixed concentration** of 0.1 mol dm^{-3}, and record the rate of photosynthesis in bubbles $minute^{-1}$
- **Vary the temperature from** 5°C to 50°C using water baths, monitoring with a thermometer in the water surrounding the plant
- **Keep the light intensity constant** by keeping the lamp a fixed distance from the plant
- The rate will tail off after around 40°C due to the denaturation of important proteins involved in photosynthesis

YOUR NOTES



The effect of temperature on the rate of photosynthesis

Exam Tip

The key to this part of the spec is to appreciate how an experimental investigation can be controlled so that any effects we observe **are directly due to the one variable** that we are deliberately changing.

YOUR NOTES

Practical 4: Separation of Photosynthetic Pigments

Separation of photosynthetic pigments by chromatography

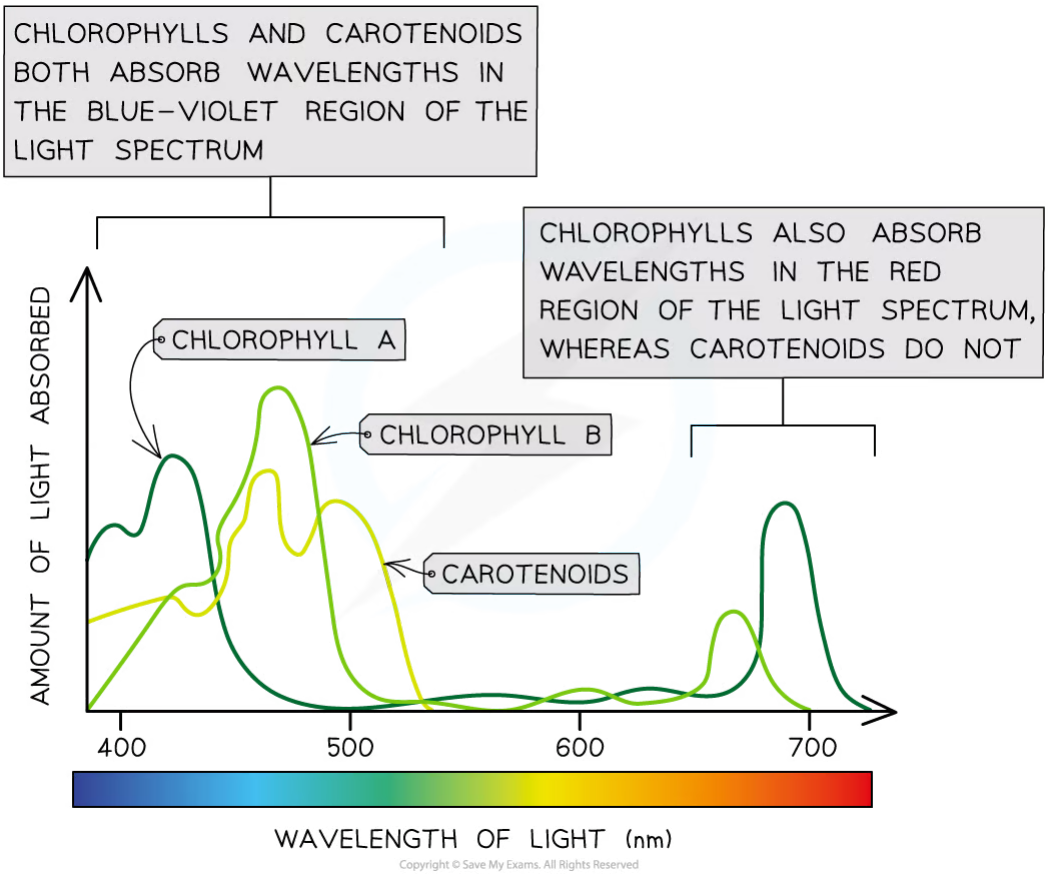
- Plants contain several different **photosynthetic pigments**, which **absorb different wavelengths of light**
- There are **two groups** of pigments: **chlorophylls** and **carotenoids**
- Carotenoids surround the chlorophyll and absorb both similar and different wavelengths of light to chlorophyll
  - This **expands the range of wavelengths** that can be absorbed from light for use in photosynthesis

Chloroplast Pigments Table

Pigment group	Name of pigment	Colour of pigment
Chlorophylls	Chlorophyll a Chlorophyll b	Yellow–green Blue–green
Carotenoids	β carotene Xanthophyll	Orange Yellow

- **Chlorophylls** absorb wavelengths in the **blue-violet and red regions** of the light spectrum
  - They reflect green light, causing plants to appear green
- **Carotenoids** absorb wavelengths of light mainly in the **blue-violet region** of the spectrum

YOUR NOTES



Chlorophyll and carotenoids absorb light across the visible light spectrum to use in the light-dependent reaction of photosynthesis

Chromatography

- **Chromatography** is an experimental technique that is used to **separate mixtures**
  - **Different components** within the mixture travel through the material at **different speeds**
  - This causes the different components to **separate**
  - A retardation factor (**R_f value**) can be calculated for each component of the mixture
- Two of the most common techniques for separating these photosynthetic pigments are:
  - **Paper chromatography** – the mixture of pigments is passed through paper (cellulose)
  - **Thin-layer chromatography (TLC)**– the mixture of pigments is passed through a thin layer of adsorbent (eg. silica gel), through which the mixture travels faster and separates more distinctly
- Paper chromatography can be used to separate photosynthetic pigments although **TLC gives better results**

Apparatus

- Leaf sample
- Distilled water
- Pestle and mortar

YOUR NOTES ⬇

- Filter paper
- Capillary tube
- Chromatography solvent
- Propanone
- Pencil
- Ruler

Method

- Draw a straight line in pencil approximately 1cm above the bottom of the filter paper being used
  - **Do not use a pen** as the ink will separate into pigments within the experiment and obscure the results
- Cut a section of leaf and place it in a mortar
  - It is important to **choose a healthy leaf** that has been in direct sunlight so you can be sure it contains many active photosynthetic cells
- Add 20 drops of propanone and use the pestle to grind up the leaf sample and release the pigments
  - Propanone is an organic solvent and therefore fats, such as the lipid membrane, dissolve in it
  - The combination of propanone and mechanical pressure breaks down the cell and chloroplasts to **release the pigments**
- Extract some of the pigment using a capillary tube and spot it onto the centre of the pencil line you have drawn
- Suspend the paper in the chromatography solvent so that **the level of the solvent is below the pencil line** and leave the paper until the solvent has reached the top of the paper
  - The mixture is **dissolved** in the **solvent** (called the mobile phase) and the dissolved mixture then passes through a static material (called the stationary phase)
- Remove the paper from the solvent and draw a pencil line marking where the solvent moved up to
  - The pigment should have separated out and there should be different spots on the paper at different heights above the pencil line, these are the separate pigments
- Calculate the Rf value for each spot

$$R_f \text{ value} = \frac{\text{distance travelled by component (pigment)}}{\text{distance travelled by the solvent}}$$

  - Always measure to the centre of each spot

Results

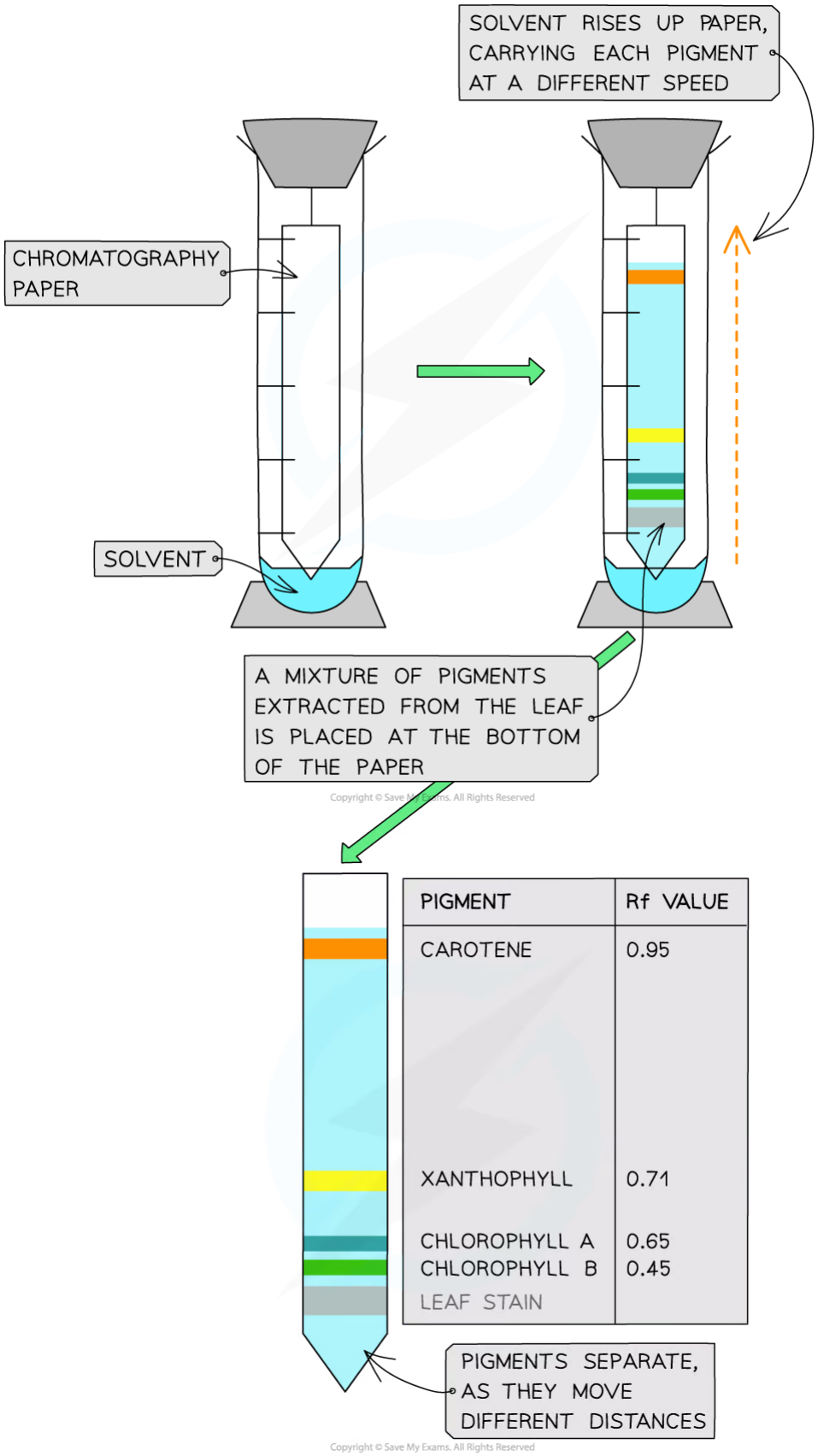
- Chromatography can be used to **separate and identify chloroplast pigments** that have been extracted from a leaf as each pigment will have a unique R_f value
- The R_f value demonstrates how far a dissolved pigment travels through the stationary phase
  - Molecules with a higher affinity to the stationary phase, such as large molecules, will travel slower and therefore have a **smaller R_f value**

YOUR NOTES

- Molecules that are more soluble in the mobile phase will travel faster and therefore have a **larger R_f value**

- Although specific R_f values depend on the solvent that is being used, in general:
  - **Carotenoids** have the **highest R_f values** (usually close to 1)
  - **Chlorophyll *b*** has a **much lower R_f value**
  - **Chlorophyll *a*** has an R_f value somewhere **between** those of carotenoids and chlorophyll *b*
  - **Small R_f values** indicate the pigment is **less soluble** and/or **larger** in size

YOUR NOTES



PIGMENT	Rf VALUE
CAROTENE	0.95
XANTHOPHYLL	0.71
CHLOROPHYLL A	0.65
CHLOROPHYLL B	0.45
LEAF STAIN	

YOUR NOTES

Paper chromatography is used to separate photosynthetic pigments. These pigments can be identified by their R_f values. In this example, a line of the mixture (rather than a spot) is added to the paper.

Limitations

- Paper chromatography is not as specific as other chromatography techniques
  - It is sufficient to separate and distinguish different pigments and to calculate their R_f value
- Chromatography does not give data on the amount of each pigment present or the wavelengths that they absorb
  - Colorimetry can be used to calculate these values

Exam Tip

Remember – the pigments themselves have colour (as described in the table). This is different from the colours of light that they *absorb*. You don't have to remember specific R_f values, just know that they differ between each type of pigment.