Edexcel International AS Biology

Your notes

Proteins

Contents

Your notes

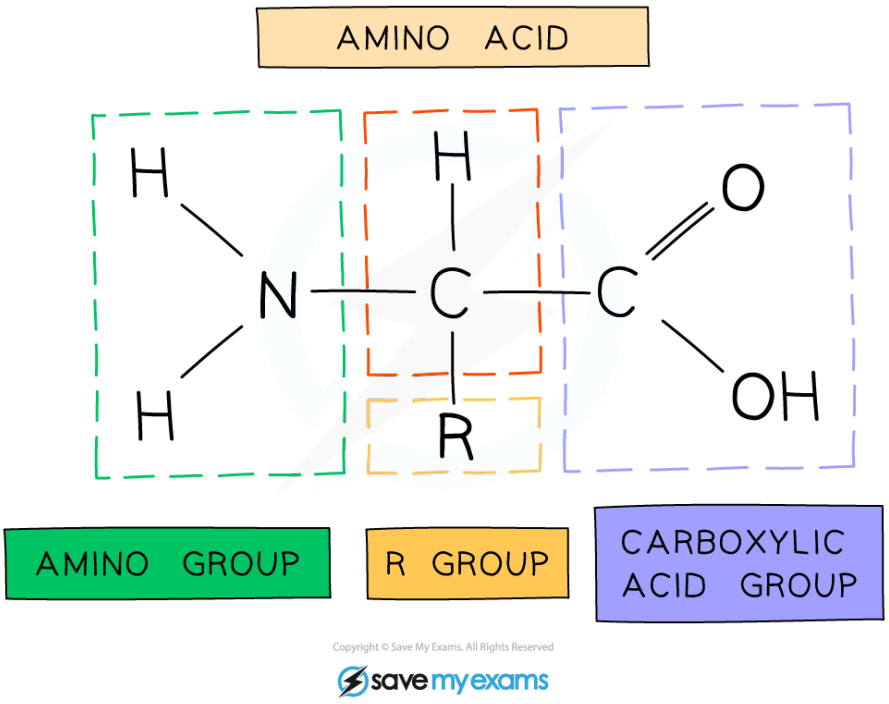
Amino Acids Structure

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)

Amino acids

- Amino acids are the **monomers** of polypeptides
- There are 20 amino acids found in proteins common to all living organisms
- The general structure of all amino acids is a central carbon atom bonded to:
  - An **amine** (also called amino) 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 general structure of an amino acid

Polypeptides & Protein Structure

- Peptide bonds form between amino acids
- Peptide bonds are **covalent bonds** and so involve the sharing of electrons
- In order to form a **peptide bond** :
  - A hydroxyl (-OH) is lost from the carboxylic group of one amino acid
  - A hydrogen atom is lost from the amine group 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 to amino acids

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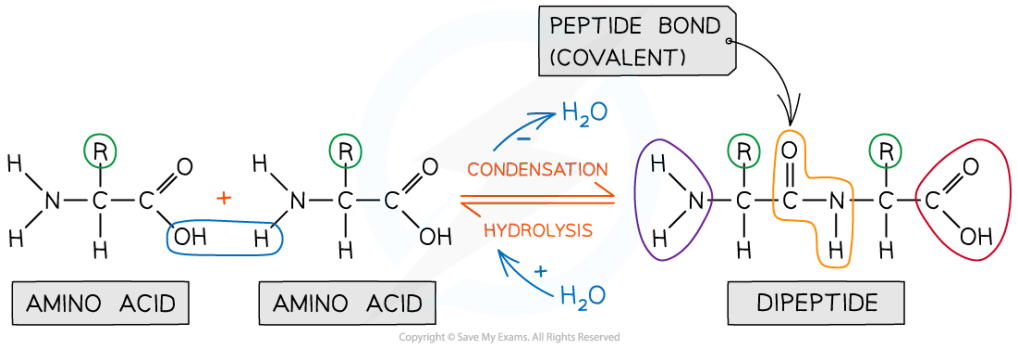

Peptide bonds are formed by condensation reactions (releasing a molecule of water) and broken by hydrolysis reactions (adding a molecule of water)

Examiner Tips and Tricks

When asked to identify the location of the peptide bond, look for where nitrogen is bonded to a carbon which has a double bond with an oxygen atom, note the R group is not involved in the formation of a peptide bond.

Structures of specific amino acids are not required.

Primary, Secondary & 3-D Structure of Proteins

- There are **four** levels of structure in proteins
  - Three are related to a single polypeptide chain
  - The fourth level relates to a protein that has two or more polypeptide chains
- Polypeptide or protein molecules can have anywhere from 3 amino acids (Glutathione) to more than 34,000 amino acids (Titin) bonded together in chains

Primary structure

- The sequence of amino acids bonded by covalent peptide bonds is the **primary structure** of a protein
- **The DNA** of a cell **determines** the primary structure of a protein by instructing the cell to add certain amino acids in specific quantities in a certain sequence. This affects the shape and therefore the function of the protein
- The primary structure is **specific** for each protein (one alteration in the sequence of amino acids can affect the function of the protein)

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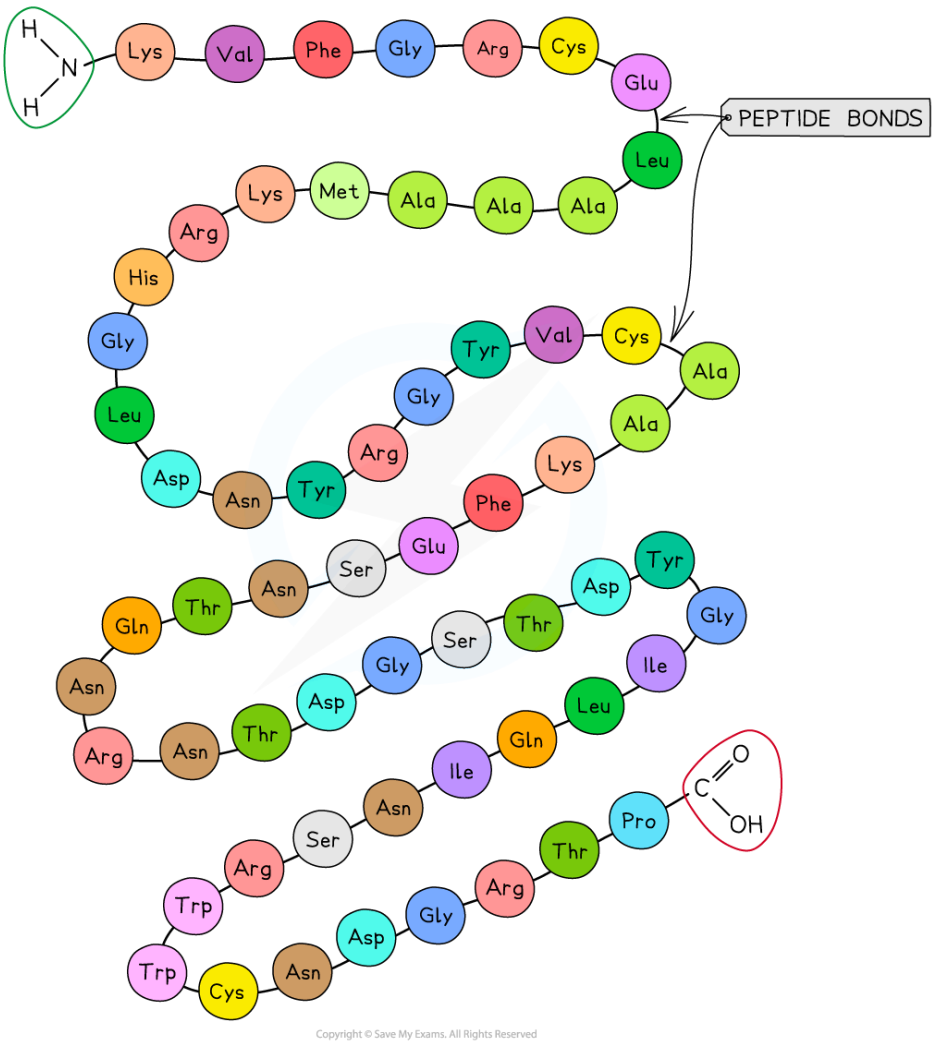


An example of the primary structure of proteins showing the three-letter abbreviation of specific amino acids

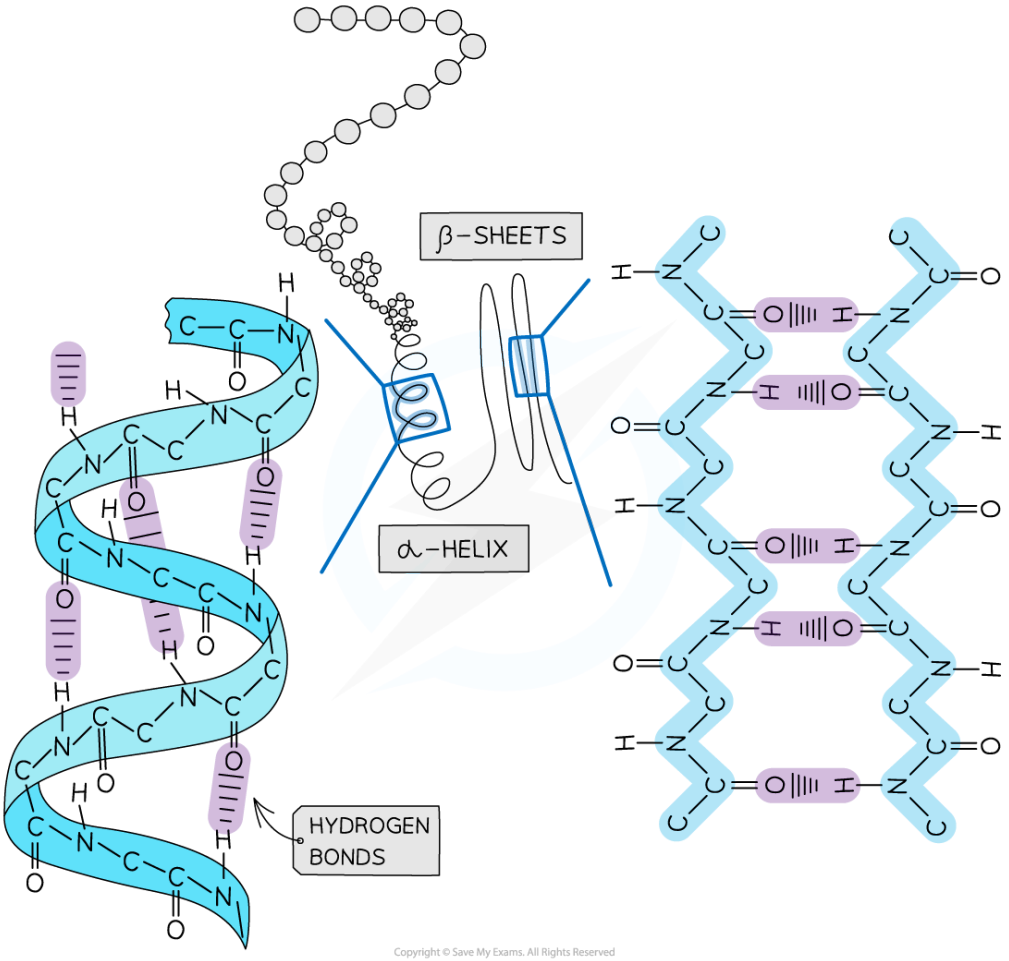
Secondary structure

- The **secondary structure** of a protein occurs when the weak negatively charged nitrogen and oxygen atoms interact with the weak positively charged hydrogen atoms to form **hydrogen bonds**
- There are two shapes that can form within proteins due to the hydrogen bonds:
  - **α-helix**
  - **β-pleated sheet**
- The **α-helix** shape occurs when the hydrogen bonds form between every **fourth** peptide bond (between the oxygen of the carboxyl group and the hydrogen of the amine group)
- The **β-pleated sheet** shape forms when the protein folds so that **two parts of the polypeptide chain** are **parallel** to each other enabling hydrogen bonds to form between parallel peptide bonds

Your notes

- Most **fibrous** proteins have secondary structures (e.g. collagen and keratin)
- The **secondary structure only** relates to **hydrogen bonds** forming between the **amino group** and the **carboxyl group** (the 'protein backbone')
- The hydrogen bonds can be broken by high temperatures and pH changes

Your notes

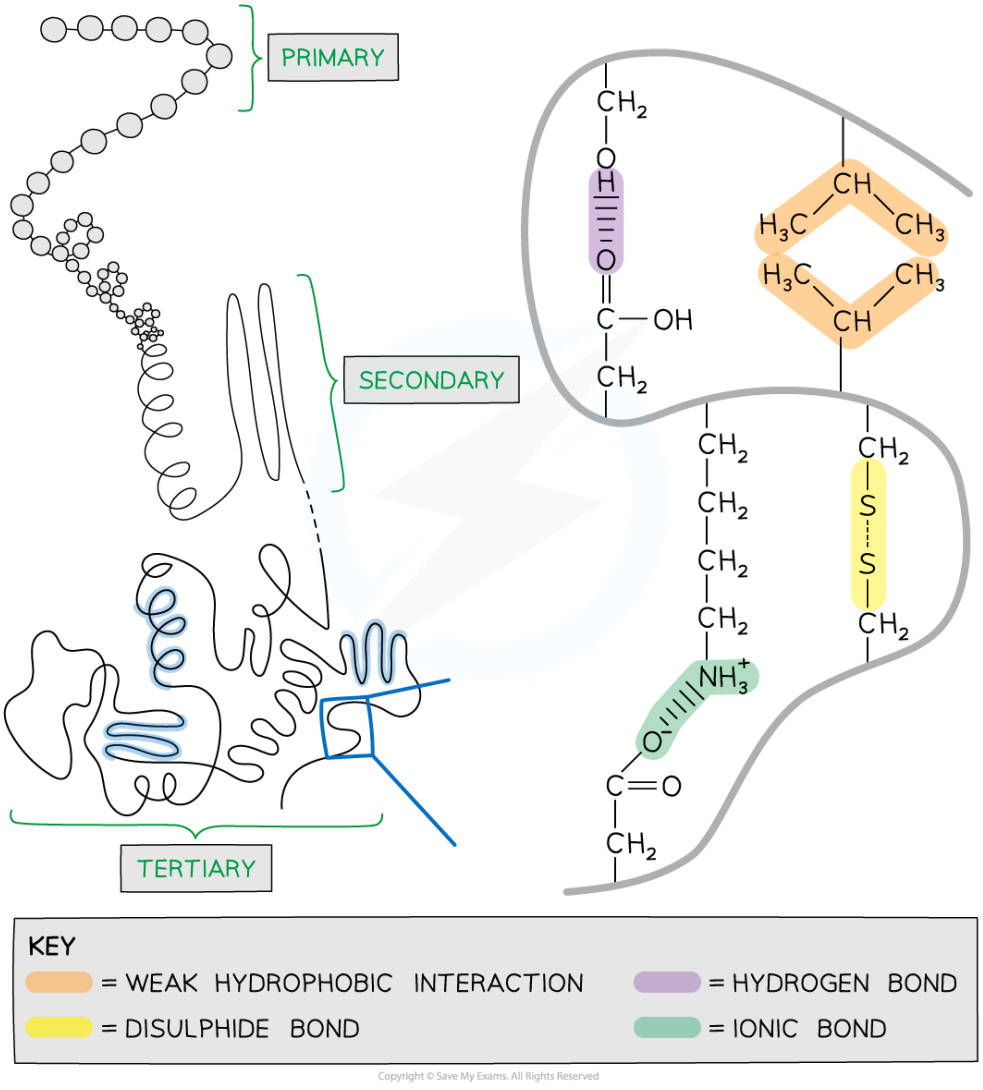


The secondary structure of a protein with the α-helix and β-pleated sheet shapes highlighted. The magnified regions illustrate how the hydrogen bonds form between the peptide bonds

Tertiary structure

- Further conformational change of the secondary structure leads to additional **bonds forming between the R groups** (side chains)
- The additional bonds are:
  - **Hydrogen** (these are between R groups)
  - **Disulphide** (only occurs between cysteine amino acids)
  - **Ionic** (occurs between charged R groups)
  - Weak **hydrophobic interactions** (between non-polar R groups)

- This structure is common in **3D globular** proteins

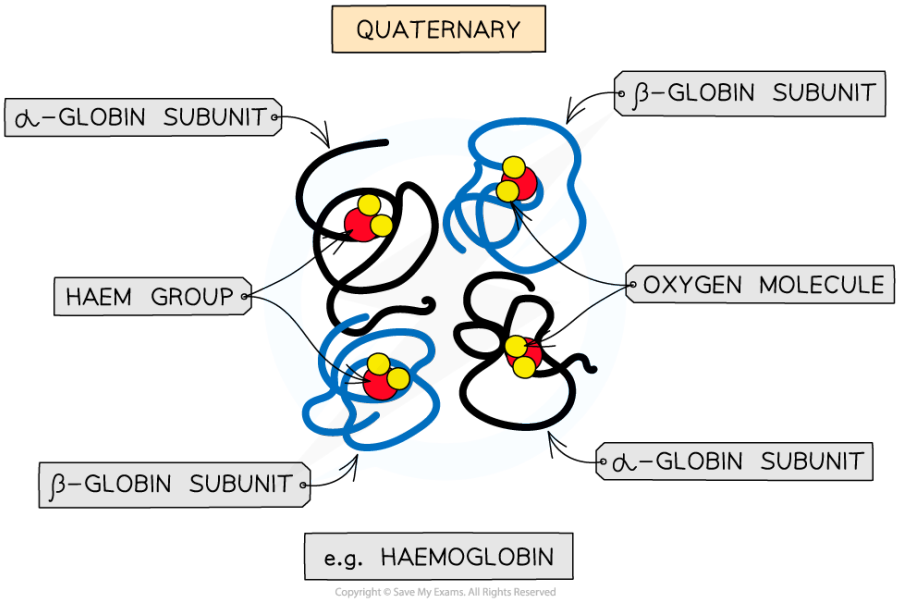


The 3D tertiary structure of proteins with hydrogen bonds, ionic bonds, disulphide bonds and hydrophobic interactions formed between the R groups of the amino acids

Quaternary structure

- Occurs in proteins that have **more than one** polypeptide chain working together as a functional macromolecule, for example, haemoglobin
- The same bonds responsible for maintaining the tertiary structure of a protein will also be involved in forming the quaternary structure
- Each polypeptide chain in the quaternary structure is referred to as a **subunit** of the protein

Your notes



The quaternary structure of a protein. This is an example of haemoglobin which contains four subunits (polypeptide chains) working together to carry oxygen

Summary of Bonds in Proteins Table

	Level		
Bonds	Primary	Secondary	Tertiary
Peptide	✓	✓	✓
Hydrogen		✓ (only between the amino and carboxyl groups)	✓ (R groups + amino and carboxyl groups)
Disulphide			✓
Ionic			✓
Hydrophobic interactions			✓

Your notes

Examiner Tips and Tricks

Familiarise yourself with the difference between the four structural levels found in proteins, noting which bonds are found at which level. Remember that the hydrogen bonds in tertiary structures are between the R groups whereas in secondary structures the hydrogen bonds form between the amino and carboxyl groups.

Your notes

Globular & Fibrous Proteins

Globular proteins: Structure

- **Globular proteins** are
  - **Compact**
  - Roughly **spherical** (circular) in shape
- Globular proteins form a spherical shape when folding into their tertiary structure because:
  - Their **non-polar hydrophobic R groups** are orientated towards the **centre** of the protein away from the aqueous surroundings
  - Their **polar hydrophilic R groups** orientate themselves on the **outside** of the protein
- The folding of the protein due to the interactions between the R groups results in globular proteins having **specific shapes**
- Some globular proteins are **conjugated protein** that contain a **prosthetic group**

Globular proteins: Function

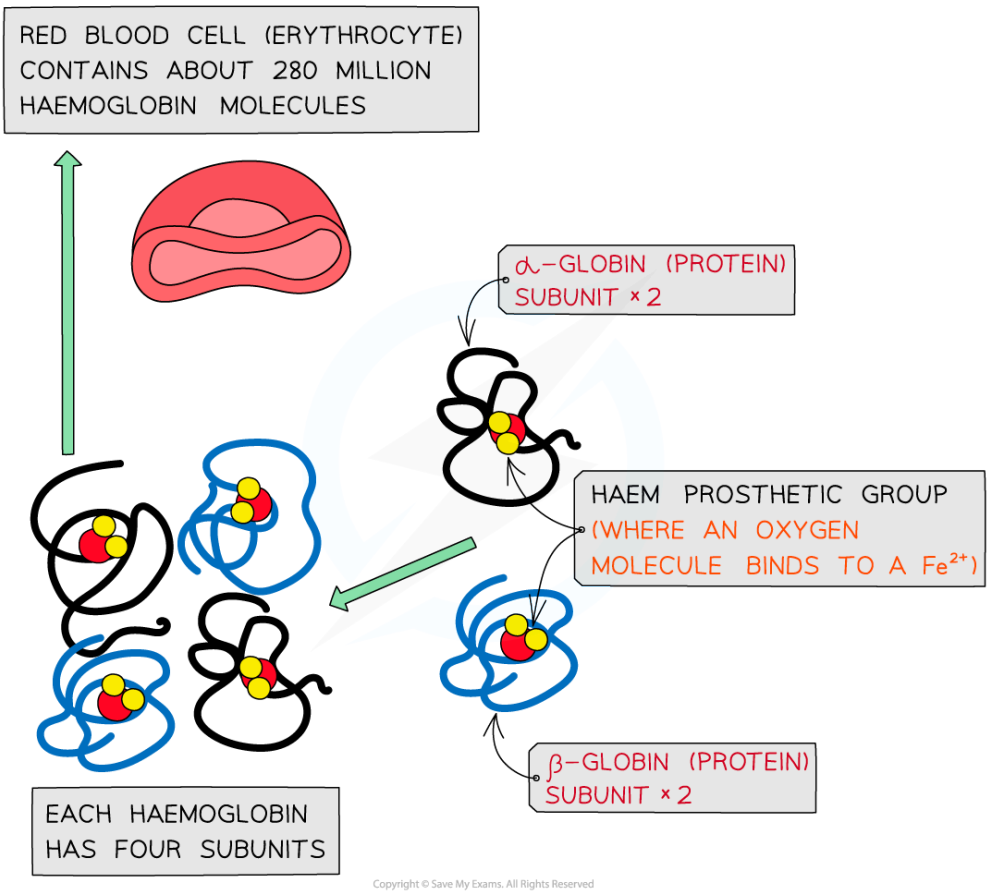
- The orientation of their R groups enables globular proteins to be (generally) **soluble** in water as the water molecules can surround the **polar hydrophilic R groups**
- The **solubility** of globular proteins in water means they play important **physiological** roles as they can be easily **transported** around organisms and be involved in **metabolic reactions**
  - For example, **enzymes** can catalyse specific reactions and **immunoglobulins** can respond to specific antigens

Haemoglobin

- Haemoglobin is a **globular** protein which is an oxygen-carrying pigment found in vast quantities in red blood cells
- It has a **quaternary** structure as there are **four polypeptide chains**
  - These chains or subunits are **globin** proteins (two **α-globins** and two **β-globins**) and each subunit has a prosthetic **haem** group
- The **four globin subunits** are held together by **disulphide bonds**
  - Their **hydrophobic R groups** are facing **inwards** (helping preserve the **three-dimensional spherical shape**)

- Their **hydrophilic R groups** are facing **outwards** (helping maintain its **solubility**)

- The arrangements of the R groups is important to the functioning of haemoglobin
- If changes occur to the sequence of amino acids in the subunits this can result in the properties of haemoglobin changing
  - This is what happens to cause **sickle cell anaemia** (where base substitution results in the amino acid valine (non-polar) replacing glutamic acid (polar) making haemoglobin less soluble)
- The prosthetic **haem** group contains an **iron** II ion (Fe^{2+}) which is able to **reversibly** combine with an **oxygen** molecule forming **oxyhaemoglobin** and results in the haemoglobin appearing bright red
- Each **haemoglobin** with the four haem groups can therefore **carry four oxygen molecules** (eight oxygen atoms)



The molecular structure of haemoglobin showing the α-globin and β-globin subunits, the prosthetic haem group with oxygen molecules bonded to form oxyhaemoglobin

- Haemoglobin is responsible for binding oxygen in the lungs and **transporting** the **oxygen** to tissue to be used in aerobic metabolic pathways
- As **oxygen is not very soluble** in water and haemoglobin is, oxygen can be carried more efficiently around the body when bound to the haemoglobin

Your notes

- The **presence** of the **haem group** (and Fe^{2+}) enables small molecules like oxygen to be bound more easily because as each **oxygen molecule** binds, it **alters** the **quaternary structure** (due to alterations in the tertiary structure) of the protein which causes haemoglobin to have a higher affinity for the subsequent oxygen molecules and they bind more easily
- The **existence** of the iron II ion (**Fe^{2+}**) in the prosthetic haem group also allows **oxygen** to **reversibly bind** as none of the amino acids that make up the polypeptide chains in haemoglobin are well suited to binding with oxygen

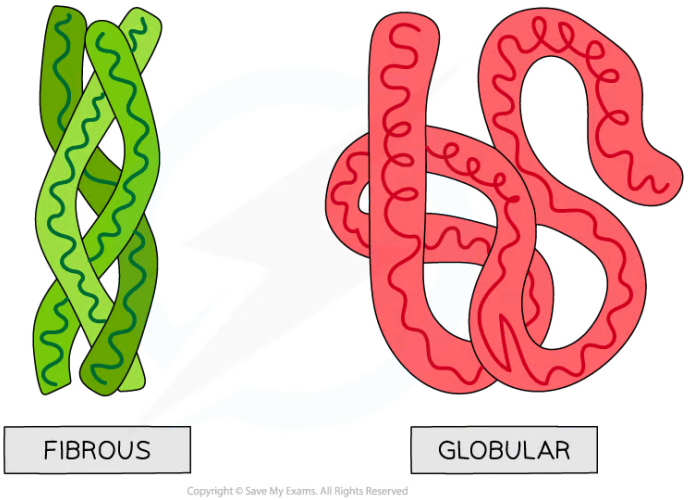
Your notes

Fibrous proteins: Structure

- **Fibrous proteins** are long strands of polypeptide chains that have cross-linkages due to hydrogen bonds
- These proteins have little or no tertiary structure
- Fibrous proteins have a limited number of amino acids with the sequence usually being highly repetitive
- The highly repetitive sequence creates **very organised structures**

Fibrous proteins: Function

- Due to a large number of **hydrophobic R groups**, fibrous proteins are **insoluble** in water
- Fibrous proteins are **strong** and this, along with their insolubility property, makes fibrous proteins very suitable for **structural roles**
- Examples of fibrous proteins:
  - **Keratin** makes up hair, nails, horns and feathers (it is a very tough fibrous protein)
  - **Elastin** is found in connective tissue, tendons, skin and bone (it can stretch and then return to its original shape)
  - **Collagen** is a connective tissue found in skin, tendons and ligaments



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

Your notes

Collagen

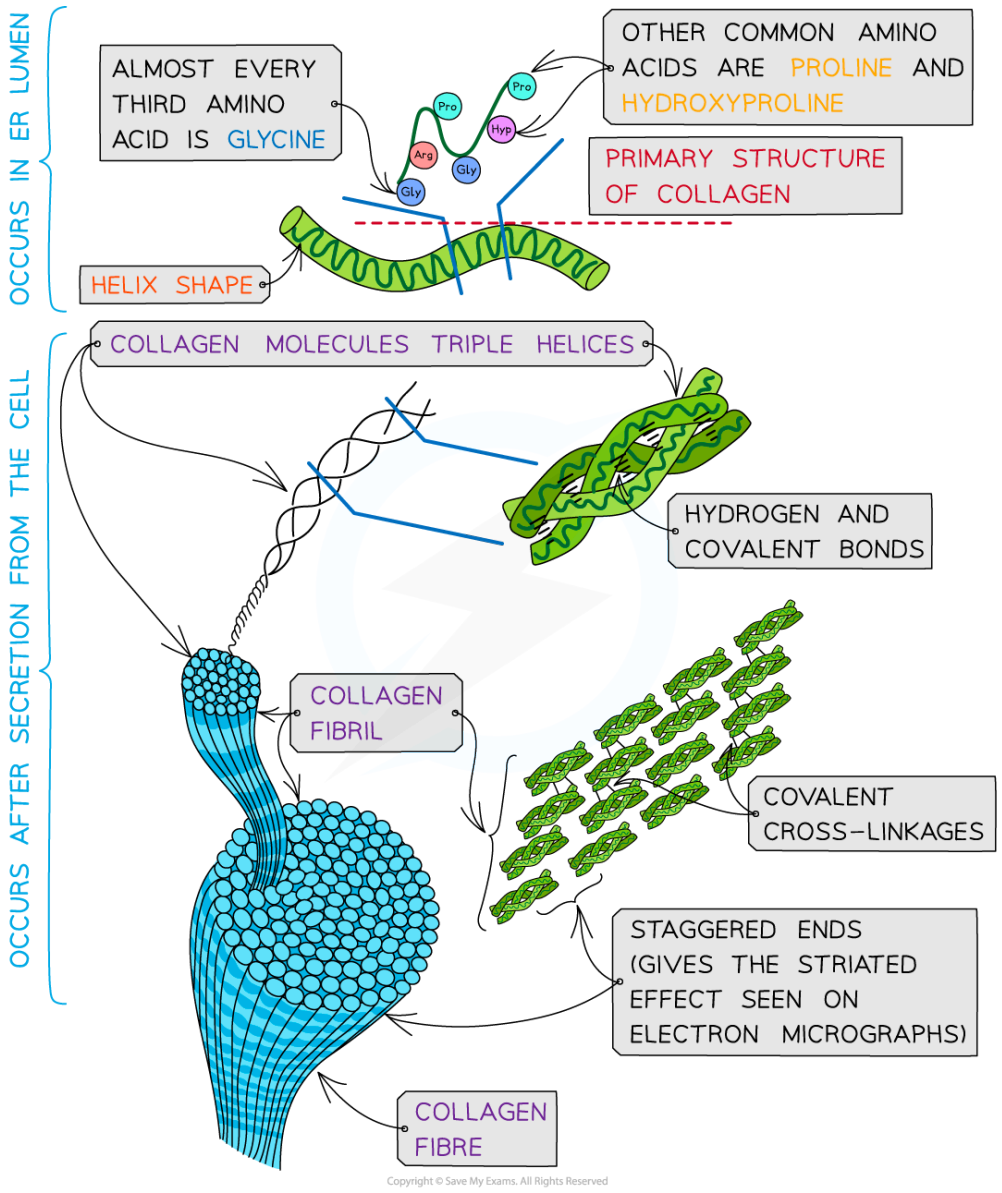
- **Collagen** is the most common **structural** protein found in **vertebrates**
- It provides structural **support**
- In vertebrates it is the component of **connective tissue** which forms:
  - Tendons
  - Cartilage
  - Ligaments
  - Bones
  - Teeth
  - Skin
  - Walls of blood vessels
  - Cornea of the eye
- Collagen is an **insoluble fibrous** protein

Structure of collagen

- Collagen is formed from **three polypeptide chains** closely held together by **hydrogen bonds** to form a **triple helix** (known as tropocollagen)
- Each polypeptide chain is a **helix shape** (but not α-helix as the chain is not as tightly wound) and contains about 1000 amino acids with glycine, proline and hydroxyproline being the most common
- In the primary structure of collagen almost **every third** amino acid is **glycine**
  - This is the smallest amino acid with a R group that contains a **single hydrogen atom**
  - Glycine tends to be found on the inside of the polypeptide chains allowing the three chains to be arranged closely together forming a **tight triple helix** structure
- Along with hydrogen bonds forming between the three chains there are also **covalent bonds** present
  - Covalent bonds also form **cross-links** between R groups of amino acids in interacting **triple helices** when they are arranged parallel to each other
  - The cross-links hold the collagen molecules together to form **fibrils**
- The collagen molecules are positioned in the **fibrils** so that there are **staggered ends** (this gives the striated effect seen in electron micrographs)
- When many fibrils are arranged together they form collagen **fibres**

- Collagen fibres are positioned so that they are lined up with the forces they are withstanding

Your notes



Collagen is a fibrous structural protein that is formed by triple helices. Collagen molecules arrange into collagen fibrils and finally into collagen fibres which have high tensile strength

Function of collagen

- Collagen is a flexible **structural** protein forming **connective tissues**
- The presence of the **many hydrogen bonds** within the **triple helix structure** of collagen results in **great tensile strength**. This enables collagen to be able to withstand large pulling forces without stretching or breaking
- The **staggered ends** of the collagen molecules within the **fibrils** provide **strength**

- Collagen is a **stable** protein due to the high proportion of proline and hydroxyproline amino acids present. These amino acids increase stability as their R groups repel each other
- The length of collagen molecules means they take too long to dissolve in water (making it insoluble in water)

Your notes

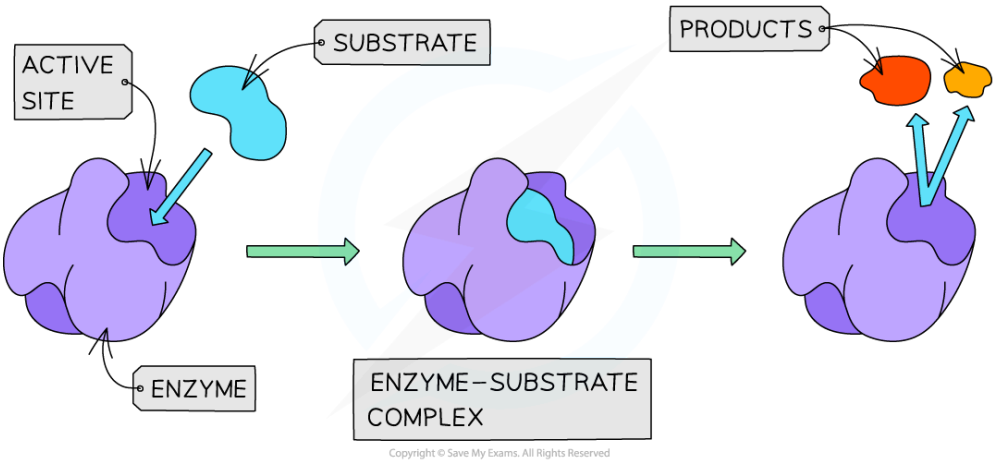
Comparison of Collagen & Haemoglobin Table

	Collagen	Haemoglobin
Number of polypeptide chains	3 (triple helix)	4 (2 x α−globin and 2 x β−globin)
Outline (Shape)	Long, thin	Spherical/Round
Type of protein	fibrous	globular
Main Function	Structural (connective tissue e.g. tendons, skin)	Functional (transport of oxygen)
Amino acid variation	Repetitive (every third amino acid is glycine)	Variable
Prosthetic group present	No	Yes (haem group)
Solubility	Insoluble in water	Soluble in water

Your notes

3-D Structure & Enzyme Function

- Enzymes are **globular proteins**
- This means their **3D shape** (as well as the shape of the **active site** of an enzyme) is determined by the complex tertiary structure of the protein that makes up the enzyme and is therefore **highly specific**
- Enzymes have a unique **active site** where **specific substrates bind** forming an **enzyme-substrate complex**
- The **active site** of an enzyme has a **specific shape** to fit a **specific substrate**
- Extremes of **heat** or **pH** can alter the protein structure and **change the shape** of the active site, **preventing** substrate binding – this is called **denaturation**
- Substrates **collide** with the enzymes active site and this must happen at the **correct orientation and speed** in order for a reaction to occur



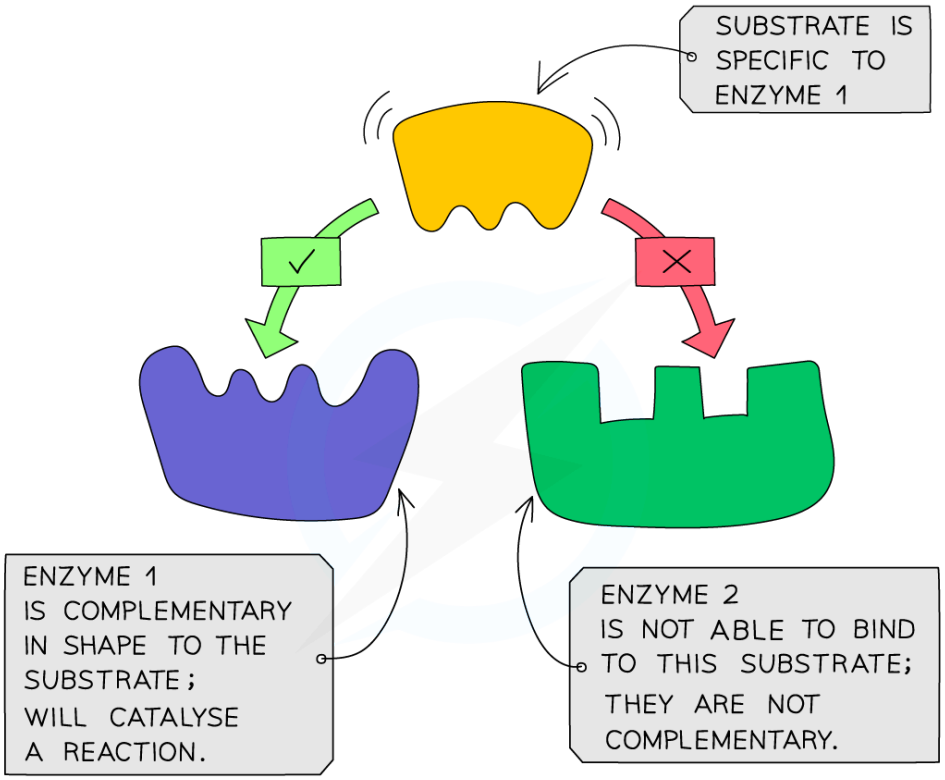
The active site of an enzyme has a specific shape to fit a specific substrate (when the substrate binds an enzyme-substrate complex is formed)

Enzyme specificity

- 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)
- Only **one specific substrate will fit into one specific active site**
- The shape of the active site (and therefore the specificity of the enzyme) is determined by the **complex tertiary structure** 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 determines the shape of an enzyme

- If the order is altered, the resulting three-dimensional shape changes

- If the **tertiary structure of the protein is altered** in any way, the shape of the **active site will change** and the substrate will no longer fit the active site
- This means that an enzyme-substrate complex will not be able to form and the product(s) will not be produced: **the enzyme will not be able to carry out its function**

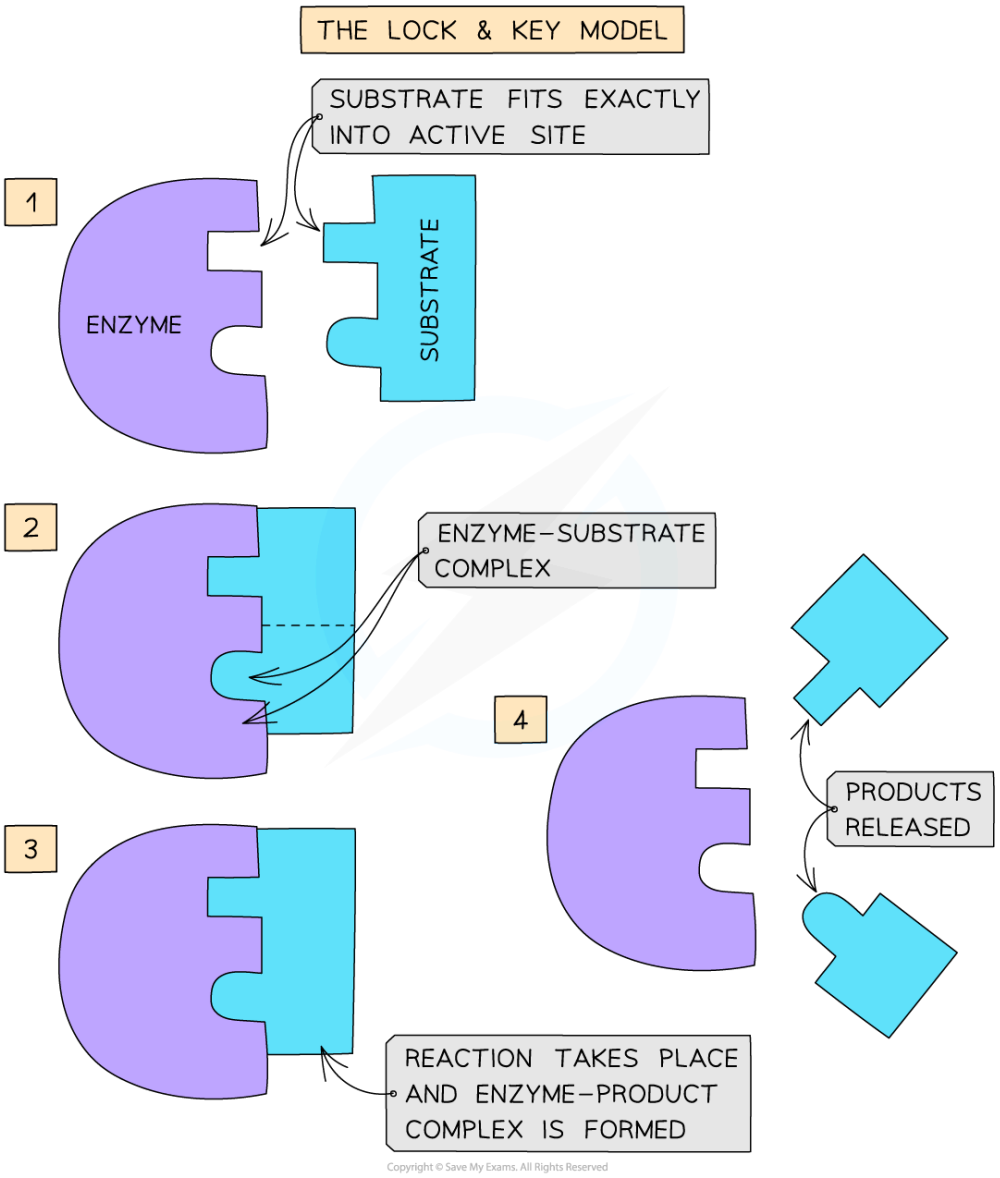


An example of enzyme specificity

The lock-and-key hypothesis

- In the 1890's the first model of enzyme activity was described by Emil Fischer:
  - He suggested that both enzymes and substrates were **rigid structures** that **locked** into each other very **precisely**, much like a key going into a lock
  - This is known as the '**lock-and-key hypothesis**'

Your notes



The Lock and Key hypothesis

The induced-fit hypothesis

- The lock-and-key model was later **modified** and adapted to our current understanding of enzyme activity, permitted by advances in techniques in the molecular sciences
- The **modified model** of enzyme activity (first proposed in 1959) is known as the '**induced-fit hypothesis**'
- Although it is very similar to the lock and key hypothesis, in this model the enzyme and substrate **interact** with each other:
  - The enzyme and its active site (and sometimes the substrate) can **change shape** slightly as the substrate molecule enters the enzyme
  - These changes in shape are known as **conformational changes**

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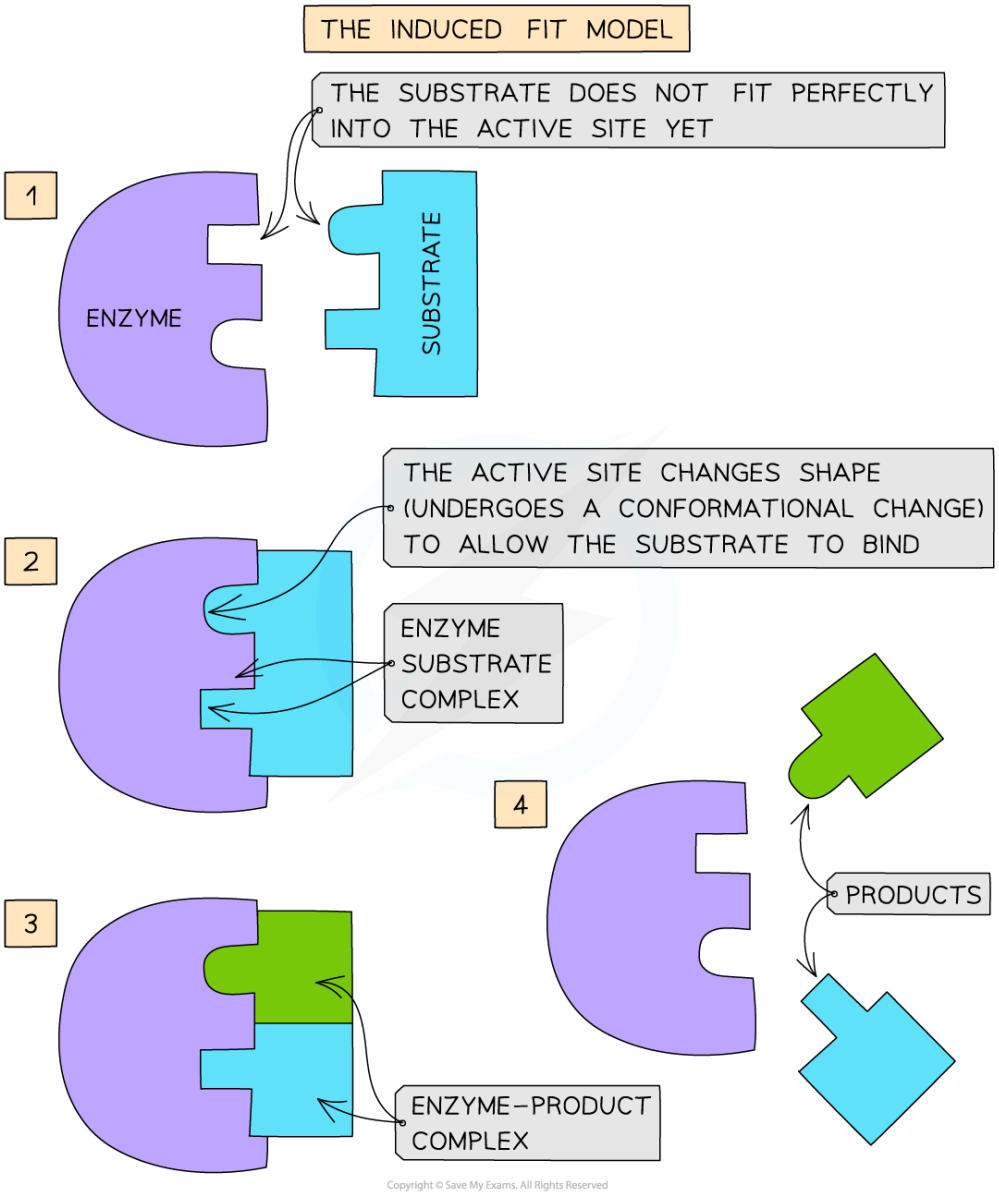
- The conformational changes ensure an **ideal binding arrangement** between the enzyme and substrate is achieved
- This **maximises the ability of the enzyme to catalyse the reaction**

Your notes



The Induced Fit model of enzyme action

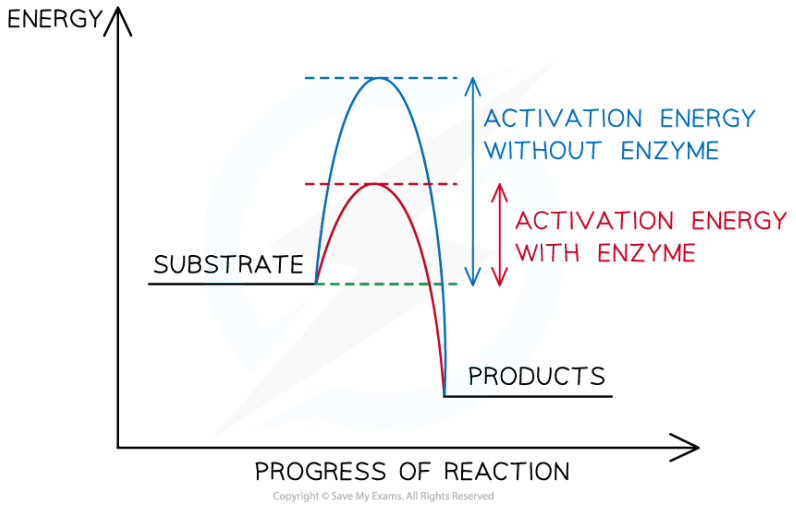
Enzymes are Catalysts

- 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 undergoing **permanent change**
  - They speed up reactions by **reducing the activation energy** of reactions

Enzymes and the lowering of activation energy

Your notes

- All chemical reactions are associated with **energy changes**
- For a reaction to proceed there must be enough **activation energy**
- Activation energy is the **amount of energy needed** by the substrate to become just **unstable** enough for a **reaction to occur** and for **products to be formed**
  - Enzymes speed up chemical reactions because they reduce the **stability** of **bonds** in the reactants
  - The **destabilisation of bonds** in the substrate makes it **more reactive**
- Rather than lowering the overall energy change of the reaction, enzymes work by providing an **alternative energy pathway** with a **lower activation energy**
- Without enzymes, **extremely high temperatures** or **pressures** would be needed to reach the activation energy for many biological reactions
  - Enzymes avoid the need for these **extreme conditions** (that would otherwise **kill cells**)



The activation energy of a chemical reaction is lowered by the presence of a catalyst (i.e. an enzyme)

Examiner Tips and Tricks

Don't forget that enzymes are proteins and so anything that could denature a protein, rendering it non-operational (extremes of heat, temperature, pH etc.) would also denature an enzyme.

Locations of Enzyme Reactions

- Enzymes are **globular proteins** with **complex tertiary structures**
  - Some are formed from a single polypeptide, whilst others are made up of two or more polypeptides and therefore have a **quaternary structure**
- **Metabolic pathways** are controlled by enzymes in a biochemical cascade of reactions
  - Metabolism is a combination of **anabolic** and **catabolic** reactions
    - **New molecules** are **built up** during anabolic reactions
    - Large, complex molecules are **broken down** into smaller, simpler ones during catabolic reactions
  - Virtually every metabolic reaction within living organisms is catalysed by an enzyme
  - Enzymes are therefore essential for life to exist
- All enzymes are **proteins** that are produced via the process of protein synthesis **inside cells**
- Some enzymes **remain inside** cells, whilst others are **secreted** to work **outside** of cells
- Enzymes can be **intracellular** or **extracellular** referring to whether they are active inside or outside the cell respectively
  - **Intracellular** enzymes are **produced** and **function inside the cell**
  - **Extracellular** enzymes are **secreted** by cells and catalyse reactions **outside** cells (eg. digestive enzymes in the gut)

Intracellular & Extracellular Enzymes Table

Your notes

Type of enzyme	Intracellular	Extracellular
Example	Catalase	Amylase
Function of enzyme	○ Hydrogen peroxide is produced as a by-product of many metabolic reactions. ○ It is harmful to cells. ○ Catalase converts hydrogen peroxide into water and oxygen, preventing any damage to cells or tissues.	○ Digestion is usually carried out by extracellular enzymes. ○ This is because the macromolecules being digested are too large to enter the cell. ○ Amylase is involved in carbohydrate digestion it hydrolyses starch into simple sugars. ○ It is secreted by the salivary glands and the pancreas, for digestion of starch in the mouth and small intestine respectively.

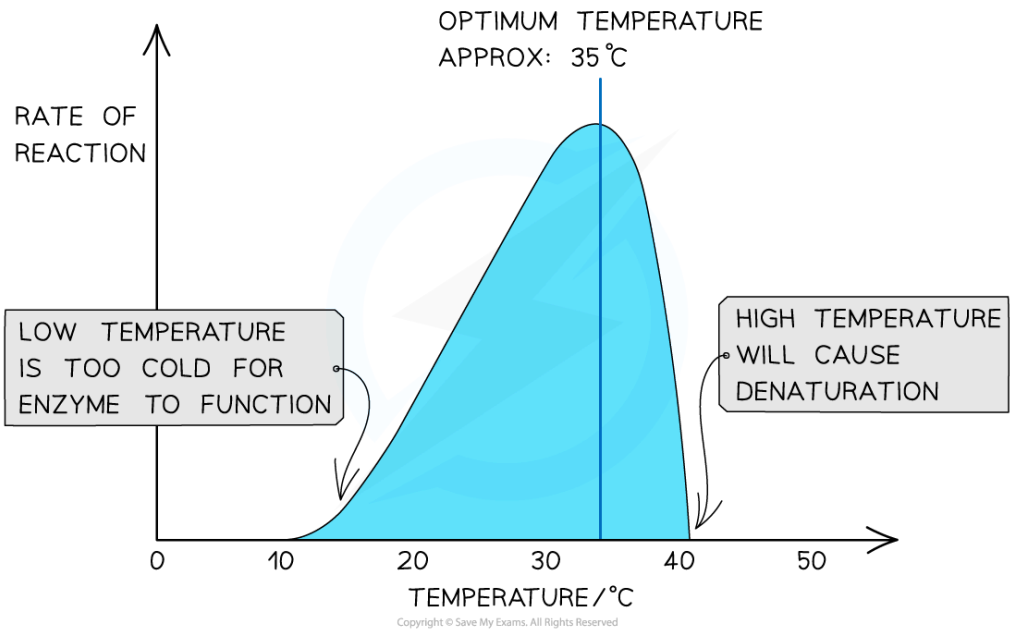
Your notes

Your notes

Investigating the Rate of Enzyme Reactions

Temperature

- 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 **slow**
  - **Lower frequency of successful collisions** between substrate molecules and 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 substrate molecules and 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**:
  - **Bonds** (eg. hydrogen bonds) holding the enzyme molecule in its precise shape start to **break**
  - This causes the **tertiary structure** 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
  - 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 causes the hydrogen bonds between amino acids to break, changing the conformation of the enzyme



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

Examiner Tips and Tricks

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.

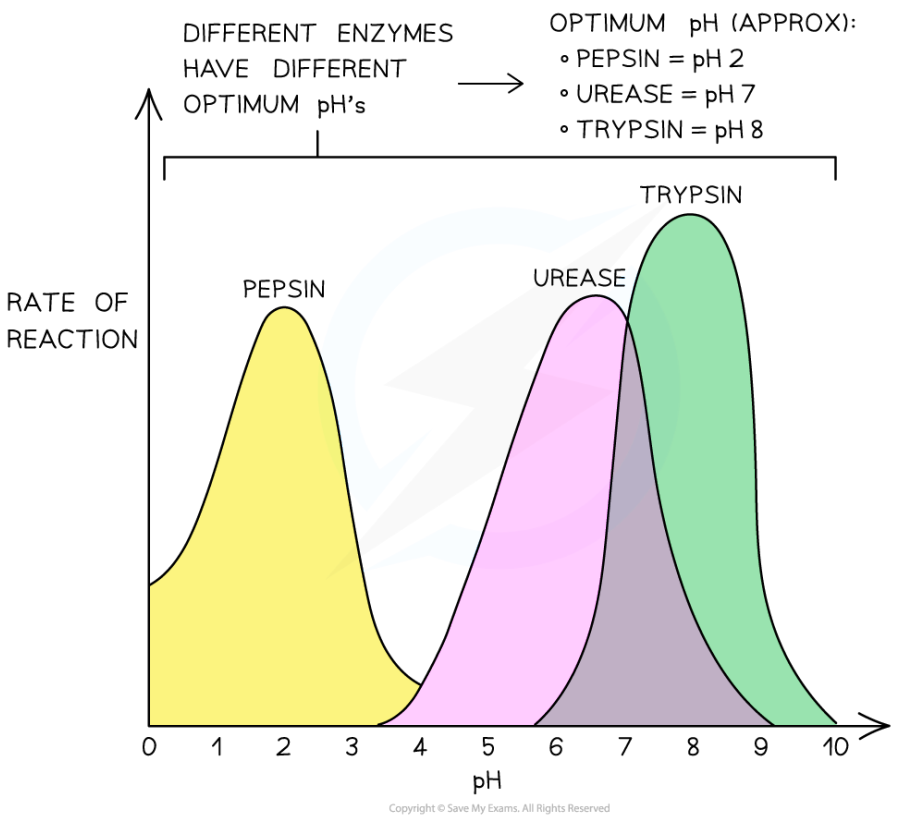
pH

- All enzymes have an **optimum pH** or a pH at which they operate best
- Enzymes are **denatured** at extremes of pH
  - **Hydrogen and ionic bonds** hold the tertiary structure of the protein (ie. the enzyme) together
  - Below and above the optimum pH of an enzyme, solutions with an excess of H^+ ions (acidic solutions) and OH^- ions (alkaline solutions) can cause these **bonds** to **break**
  - This **alters the shape of the active site**, which means enzyme-substrate complexes form less easily
  - Eventually, enzyme-substrate complexes can no longer form at all
  - At this point, **complete denaturation** of the enzyme has occurred
- Where an enzyme functions can be an indicator of its optimal environment:

Your notes

- Eg. **pepsin** is found in the stomach, an acidic environment at pH 2 (due to the presence of **hydrochloric acid** in the stomach's gastric juice)
- Pepsin's optimum pH, not surprisingly, is pH 2

Your notes



The effect of pH on the rate of an enzyme-catalysed reaction for three different enzymes (each with a different optimum pH)

- When investigating the effect of pH on the rate of an enzyme-catalysed reaction, you can use **buffer solutions** to measure the rate of reaction at **different pH values**:
  - Buffer solutions each have a **specific pH**
  - Buffer solutions **maintain** this specific pH, even if the reaction taking place would otherwise cause the pH of the reaction mixture to **change**
  - A **measured volume** of the buffer solution is added to the reaction mixture
  - This **same volume** (of each buffer solution being used) should be added for each pH value that is being investigated

Examiner Tips and Tricks

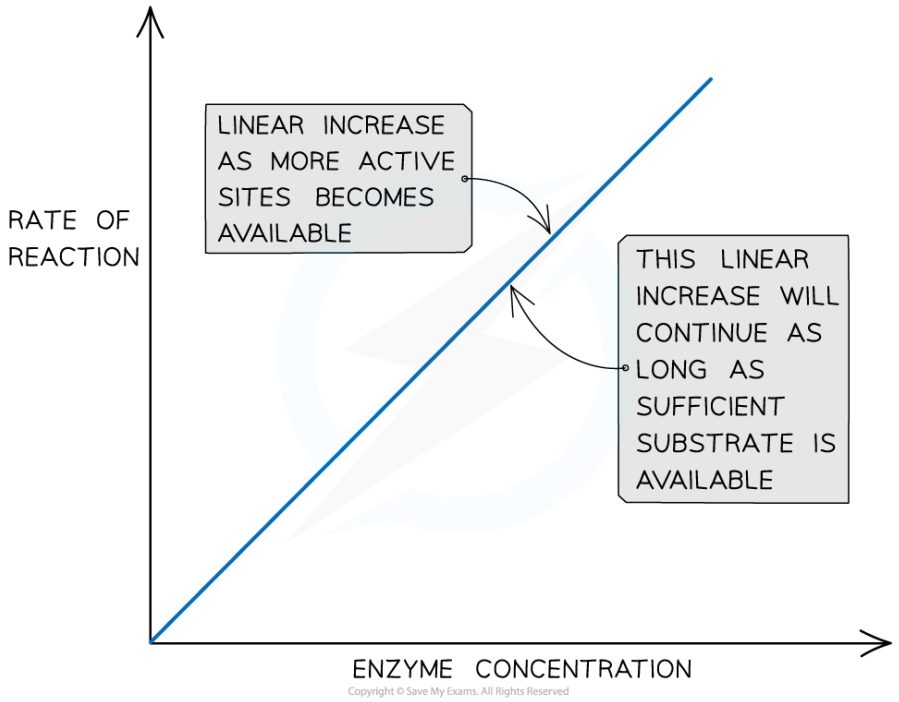
Temperature can both affect the speed at which molecules are moving (and therefore the number of collisions between enzyme and substrate in a given time) and can denature enzymes (at high temperatures). pH, however, does not affect collision rate

but only disrupts the ability of the substrate to bind with the enzyme, reducing the number of successful collisions until eventually, the active site changes shape so much that no more successful collisions can occur.

Your notes

Enzyme concentration

- Enzyme concentration affects the rate of reaction
- The **higher** the **enzyme concentration** in a reaction mixture, the **greater the number of active sites** available and the **greater the likelihood of enzyme-substrate complex formation**
- As long as there is **sufficient substrate available**, the initial rate of reaction **increases linearly** with enzyme concentration
- If the **amount of substrate is limited**, at a certain point any further increase in enzyme concentration will **not increase** the reaction rate as the amount of substrate becomes a **limiting factor**



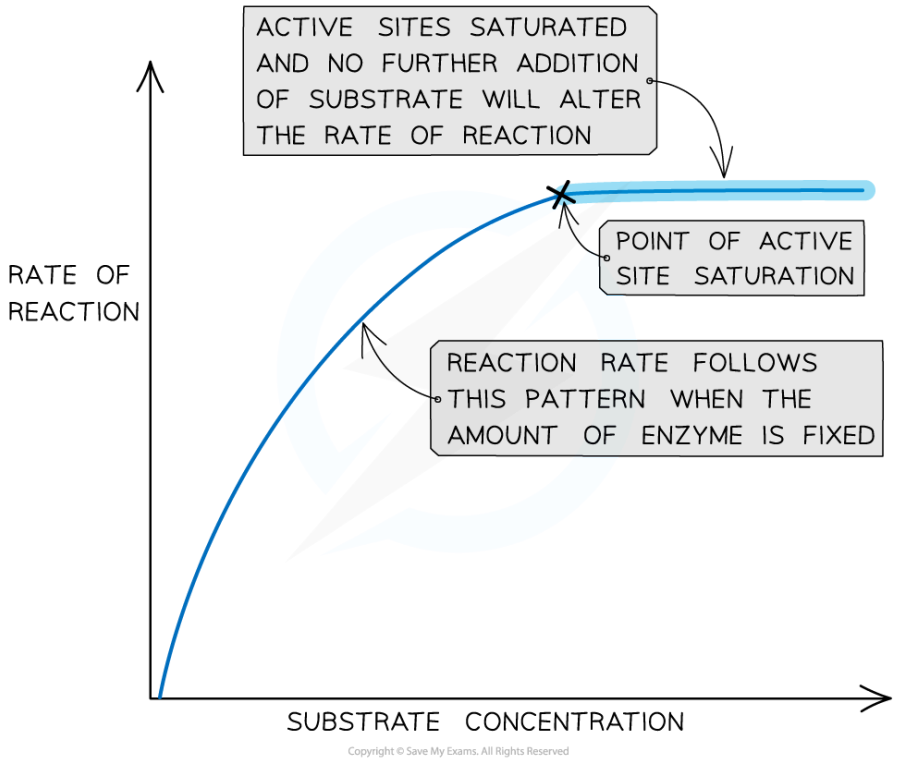
The effect of enzyme concentration on the rate of an enzyme controlled reaction

Substrate concentration

- Substrate concentration affects the rate of reaction
- The **higher the substrate concentration the faster the rate of reaction**
- More substrate molecules means **more collision between enzyme and substrate** so the more likely an active site will be used by a substrate
- The is only the case up until a certain concentration of substrate, at which point a **saturation point** is said to have been reached

- At this point **all active sites are occupied** and increasing the substrate concentration will not affect the rate of the reaction

- **Substrate concentration will decrease over time** (if no new substrate is added)
- The **rate of reaction** will therefore **decrease** over time
- This means the **initial rate of reaction will be fastest** throughout the reaction



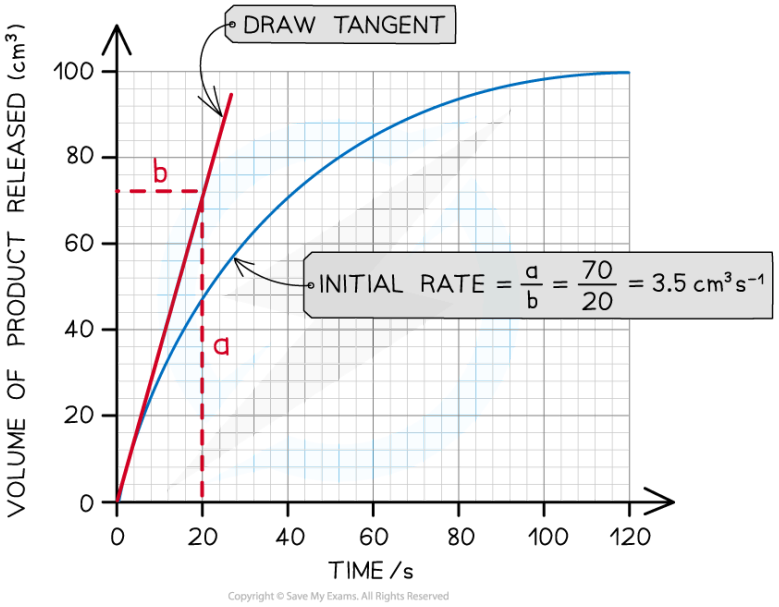
The effect of substrate concentration on the rate of an enzyme controlled reaction

Practical: Investigating the rate of enzyme reactions

- 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**
- There are many enzymes that can be used in this practical; some common examples are catalase, amylase and protease
- The initial rate of reaction can be calculated to determine the effect of changing enzyme or substrate concentrations
  - The initial rate of reaction is at the start of the reaction
  - You can **calculate the initial rate of reaction using a graph** of results showing volume of product/substrate against time
  - **Draw a tangent** to the graph through the origin

Your notes

- **Calculate the gradient of the tangent** - this is the **initial rate of reaction**



How to calculate the initial rate of reaction from a graph

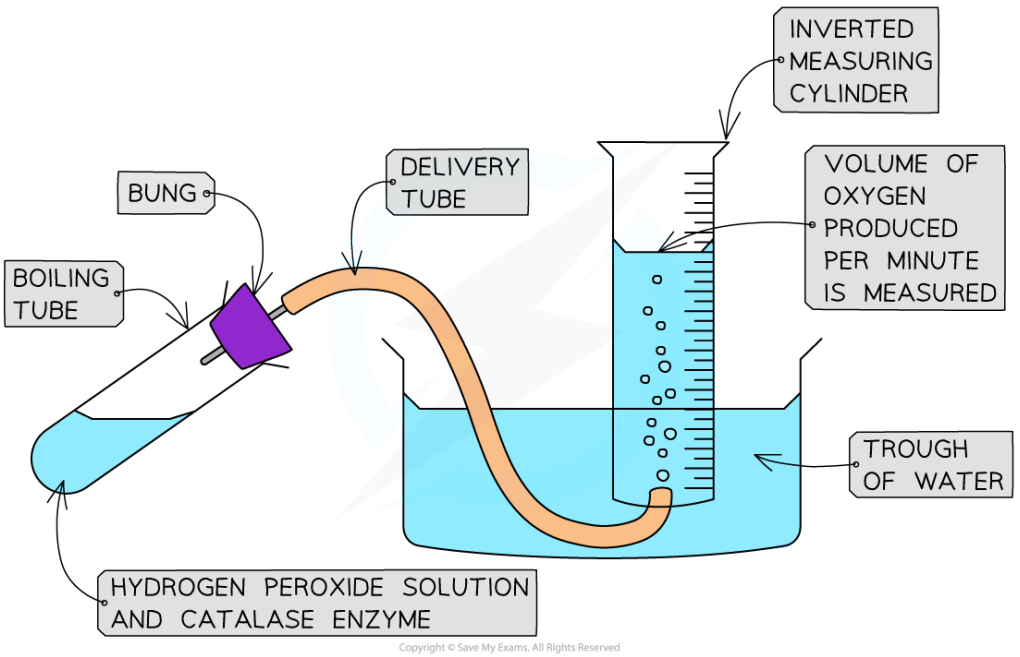
Effect of enzyme concentration on the rate of reaction

- You can measure how fast a product is made in a reaction

Apparatus

- Catalase solution at five different concentrations (enzyme)
- Hydrogen peroxide solution (substrate)
- Buffer solution (to keep the pH constant)
- Boiling tube
- Bung and delivery tube
- Measuring cylinder
- Water trough
- Stopwatch

Your notes



The apparatus set up to investigate how changing the concentration of catalase affects the volume of oxygen produced

Method

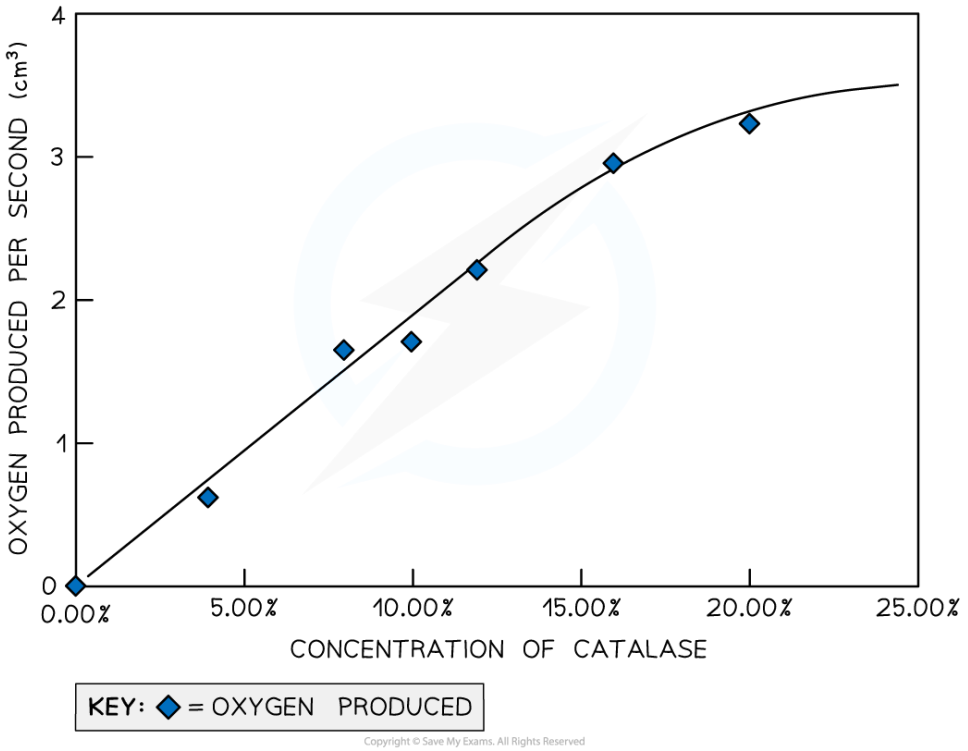
1. Add a set volume of hydrogen peroxide solution to a boiling tube
2. Add a set volume of buffer solution to the same boiling tube
3. Invert a full measuring cylinder into a trough of water
4. Place the end of the delivery tube into the open end of the measuring cylinder and attach the other end to a bung
5. Add a set volume of one concentration of catalase to the boiling tube and quickly place the bung into the boiling tube
6. Record the volume of oxygen collected in the measuring cylinder by the water displaced every 10 seconds for 60 seconds
7. Repeat the experiment twice more and calculate the average volume of oxygen produced at each 10 second interval
8. Repeat the whole experiment for the different concentrations of catalase
9. Plot the average volume of gas produced against time for each concentration
10. Compare the initial rate of reaction of each of the concentrations

Results

- As the concentration of catalase increases the volume of oxygen produced would increase
- This is because there would be more available active sites for hydrogen peroxide to use

Your notes

- The volume of oxygen would plateau out after the initial rate of reaction due to the substrate decreasing, having been converted into the product (oxygen)



An example of a set of results for one concentration of catalase showing the volume of oxygen produced per second

Effect of substrate concentration, temperature and pH on the rate of reaction

- Another investigation is to measure how fast a substrate is removed from a reaction
- This can be done using a range of substrate concentrations to investigate how changing concentration effects the rate of the reaction
- The breakdown of starch by amylase is a good example of how to investigate the effect of substrate concentration on the rate of the reaction
- Iodine solution can be added to a starch solution to create a solution with a blue-black appearance
    - This will provide a measurable way of determining the rate at which starch is broken down to maltose using a colorimeter
- The colorimeter will measure how the absorbance of the starch solution change over a period of time once amylase is added to it
- This can be repeated for a range of different starch concentrations and a graph of absorbance against time can be plotted

Your notes

- Results should show a fast initial rate of reaction and then plateau out as the substrate is converted into product(s) and all available active sites become occupied by the increasing concentration of substrate
- The investigation can be repeated by altering the pH (use buffer solutions) or temperature

Your notes

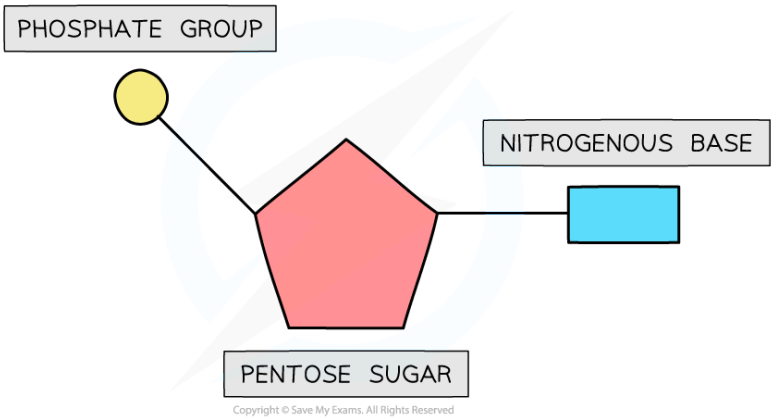
Examiner Tips and Tricks

When investigating the effect of enzyme concentration, pH or temperature on the rate of an enzyme catalysed reaction, it is important to provide an abundance of substrate to prevent it from becoming a limiting factor.

Your notes

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**
  - A **phosphate group**



The basic structure of a mononucleotide

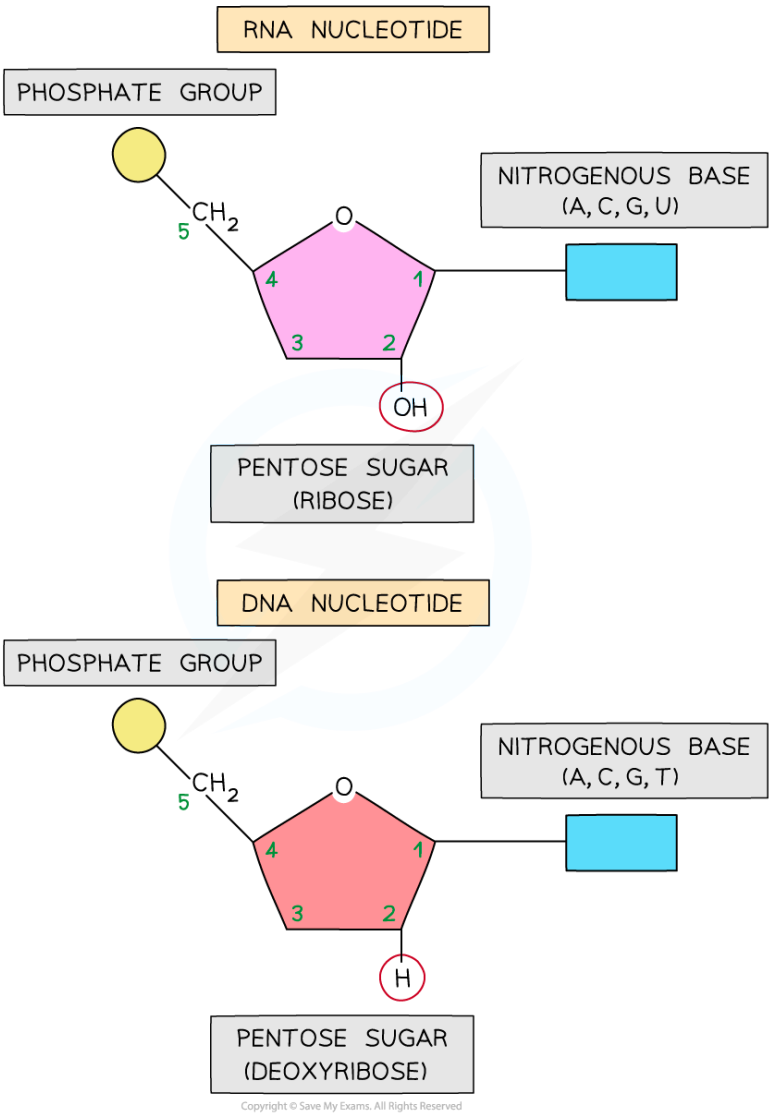
DNA nucleotides

- The components of a DNA nucleotide are:
  - A **deoxyribose** sugar with **hydrogen** at the 2' position
  - A phosphate group
  - One of four nitrogenous bases - adenine (A), cytosine(C), guanine(G) or thymine(T)

RNA nucleotides

- The components of an RNA nucleotide are:
  - A **ribose** sugar with a hydroxyl (OH) group at the 2' position
  - A phosphate group
  - One of four nitrogenous bases - adenine (A), cytosine(C), guanine(G) or **uracil (U)**
- The presence of the **2' hydroxyl group** makes **RNA more susceptible to hydrolysis**

- This is why DNA is the storage molecule and RNA is the transport molecule with a shorter molecular lifespan

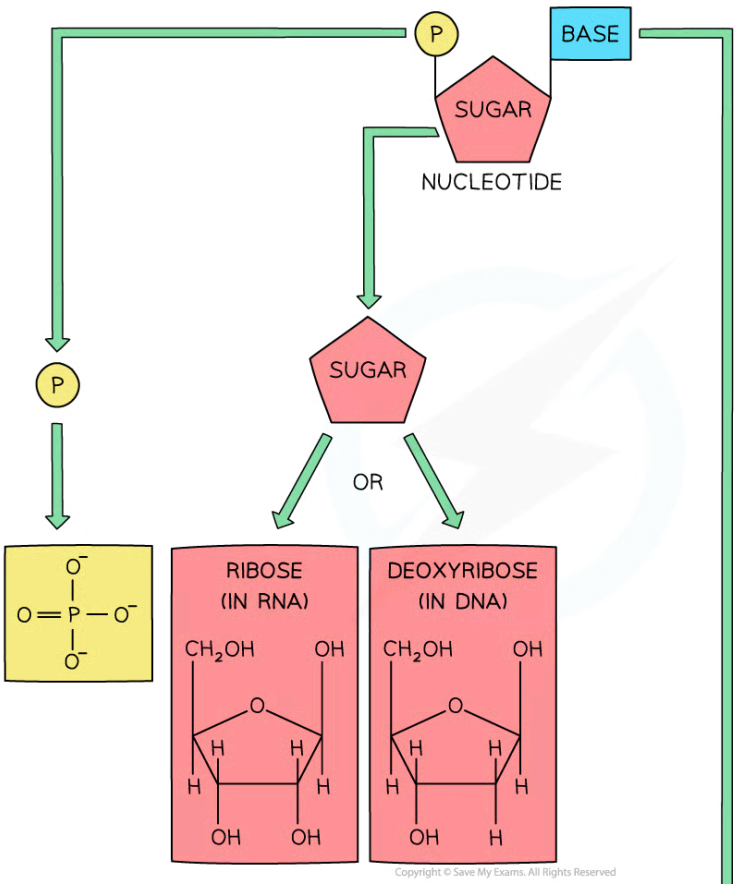


RNA nucleotide compared with a DNA nucleotide

Purines & Pyrimidines

- The **nitrogenous base** molecules that are found in the nucleotides of DNA (A, T, C, G) and RNA (A, U, C, G) occur in **two structural forms**: **purines** and **pyrimidines**
  - The bases **adenine** and **guanine** are **purines** – they have a **double ring structure**
  - The bases **cytosine**, **thymine** and **uracil** are **pyrimidines** – they have a **single ring structure**

Your notes


P
BASE
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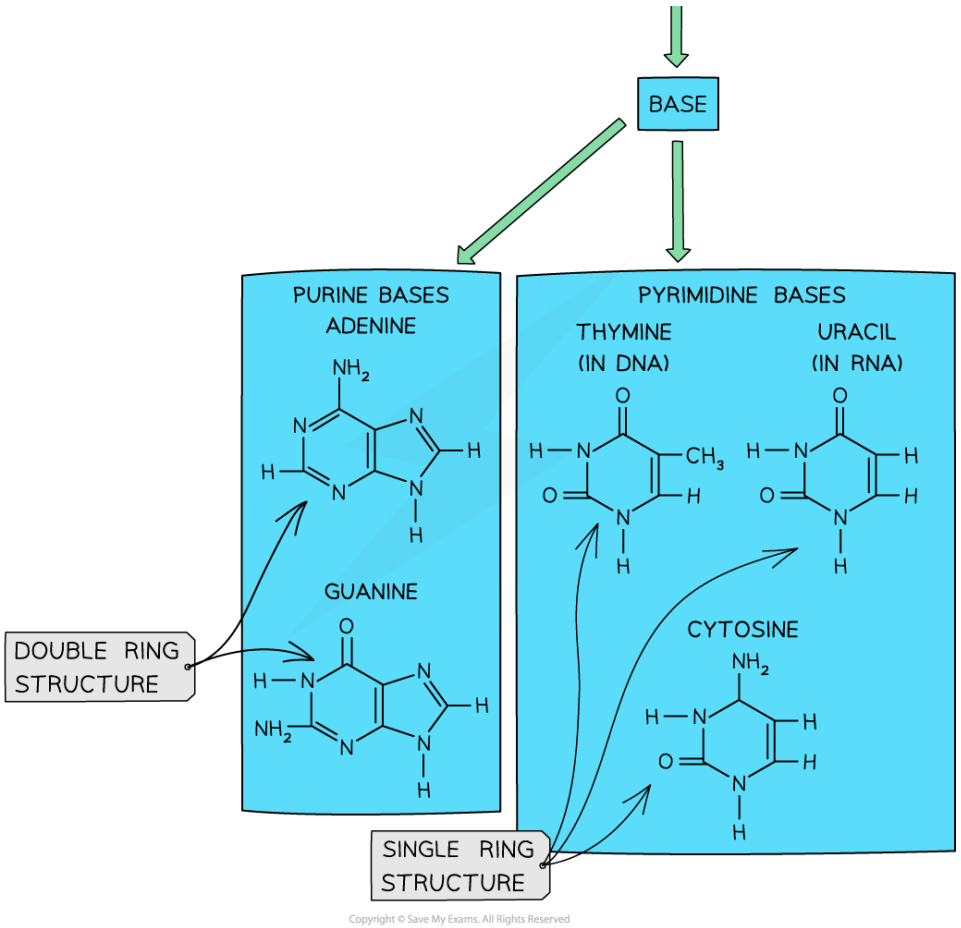
Your notes

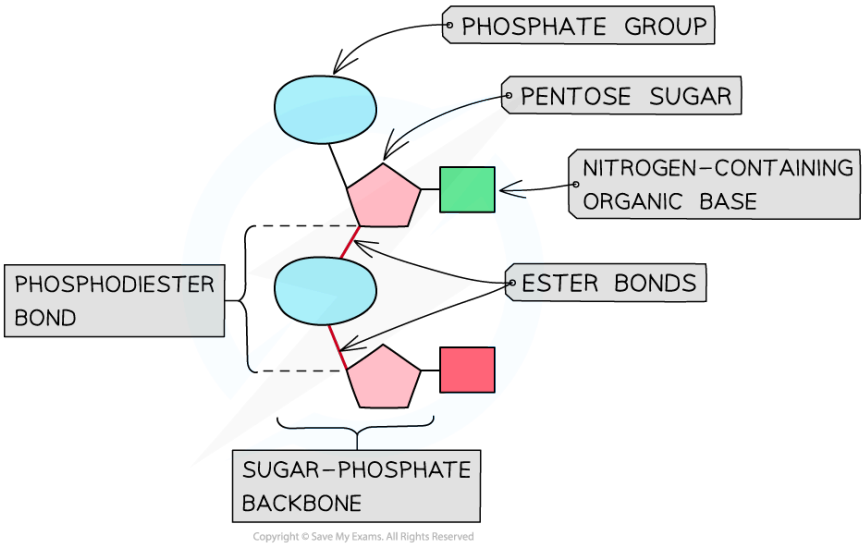


The molecular structure of each base is different, depending on whether they are a purine or pyrimidine

Polynucleotides: DNA & RNA

- DNA and RNA are polymers (**polynucleotides**), meaning that they are made up of many nucleotides joined together in long chains
- Separate **nucleotides are joined via condensation reactions**
  - These condensation reactions occur between the **phosphate group** of one nucleotide and the **pentose sugar** of the next nucleotide
- A condensation reaction between two nucleotides forms a **phosphodiester bond**
  - It is called a phospho**di**ester bond because it consists of a phosphate group and **two** ester bonds (phosphate with double bond oxygen attached - oxygen - carbon)
- The chain of alternating phosphate groups and pentose sugars produced as a result of many phosphodiester bonds is known as the **sugar-phosphate backbone** (of the DNA or RNA molecule)

Your notes

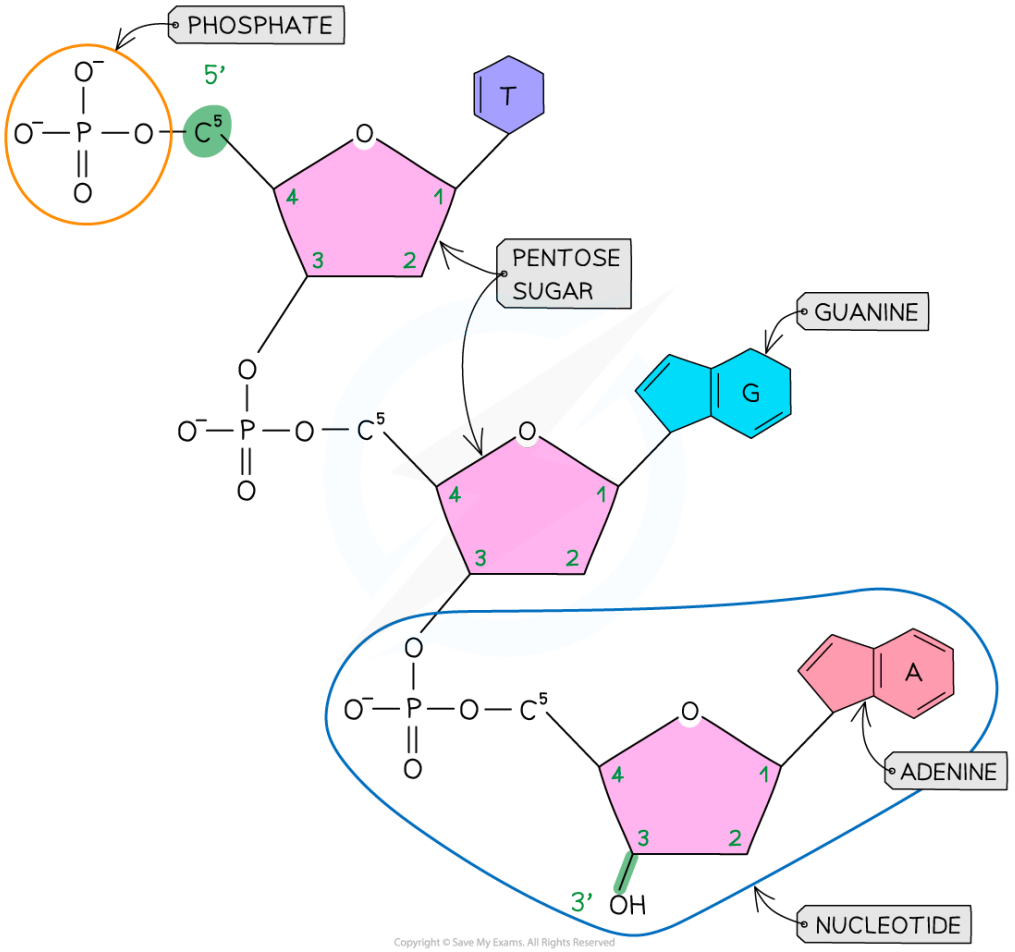


A section of a polynucleotide showing a single phosphodiester bond (and the positioning of the two ester bonds and the phosphate group that make up the phosphodiester bond)

DNA structure

- The nucleic acid DNA is a **polynucleotide** – it is made up of **many nucleotides** bonded together in a **long chain**
- 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.** These bonds are **covalent bonds** known as **phosphodiester bonds**
  - The phosphodiester bonds link the **5-carbon of one deoxyribose sugar** molecule to the phosphate group from the same nucleotide, which is itself linked by another phosphodiester bond to the **3-carbon of the deoxyribose sugar molecule of the next nucleotide** in the strand
  - Each DNA polynucleotide strand is said to have a **3' end and a 5' end** (these numbers relate to which carbon on the pentose sugar could be bonded with another nucleotide)
  - As 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



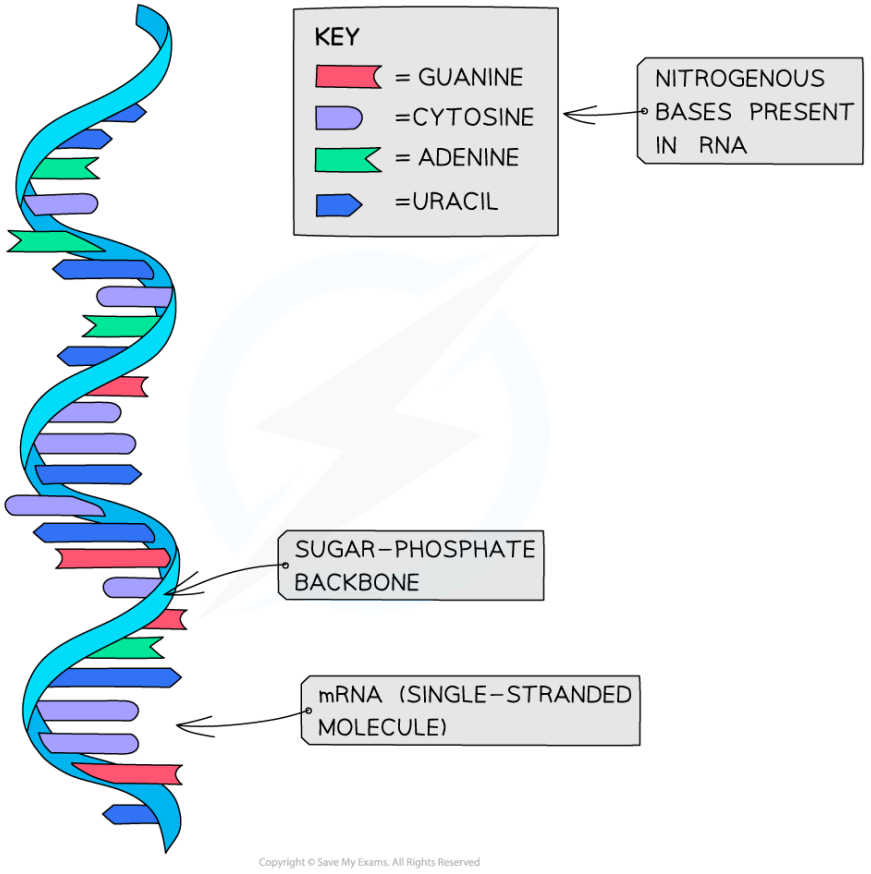
A single DNA polynucleotide strand

RNA structure

- **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)
- Unlike DNA, RNA molecules are only made up of **one polynucleotide strand** (they are single-stranded)
- RNA polynucleotide chains are relatively **short compared to DNA**
- Each RNA polynucleotide strand is made up of **alternating ribose sugars and phosphate groups linked together**, with the nitrogenous bases of each nucleotide projecting out sideways from the single-stranded RNA molecule

Your notes

- The **sugar-phosphate bonds** (between different nucleotides in the same strand) are **covalent bonds** known as **phosphodiester bonds**
  - These bonds form what is known as the **sugar-phosphate backbone** of the RNA polynucleotide strand
  - The phosphodiester bonds link the **5-carbon of one ribose sugar** molecule to the phosphate group from the same nucleotide, which is itself linked by another phosphodiester bond to the **3-carbon of the ribose sugar molecule of the next nucleotide** in the strand
- An example of an RNA molecule is **messenger RNA** (**mRNA**), which is the transcript copy of a gene that encodes a specific polypeptide. Two other examples are **transfer RNA (tRNA)** and **ribosomal RNA (rRNA)**



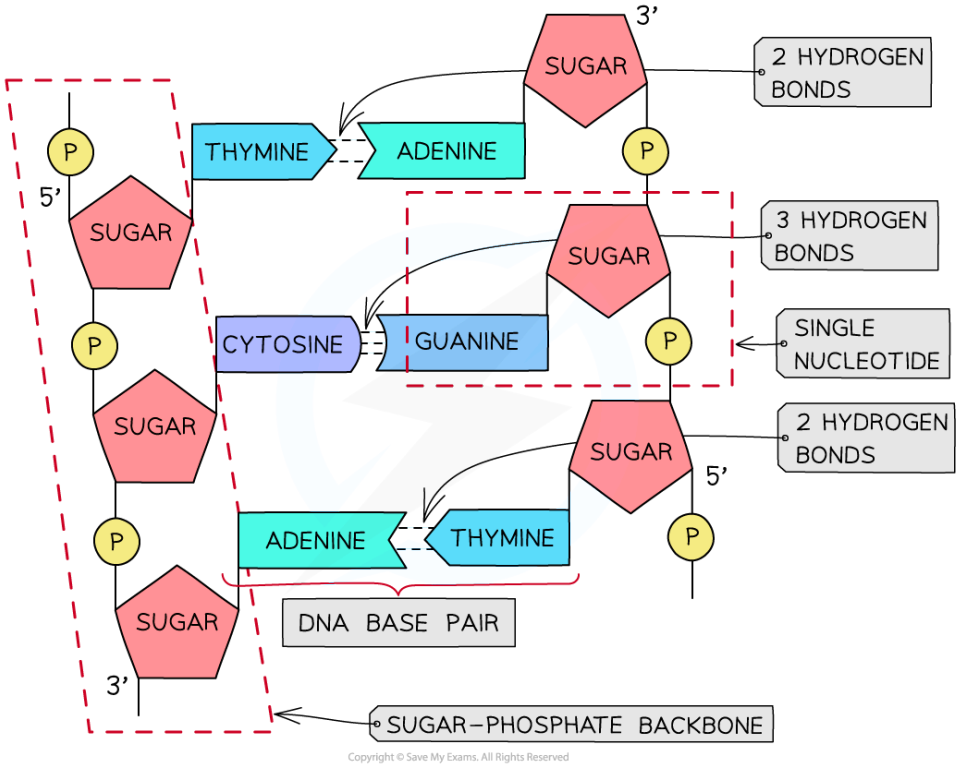
Messenger RNA (mRNA) is an example of the structure of RNA

Base Pairing in the DNA Double Helix

- 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

Your notes

- 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**

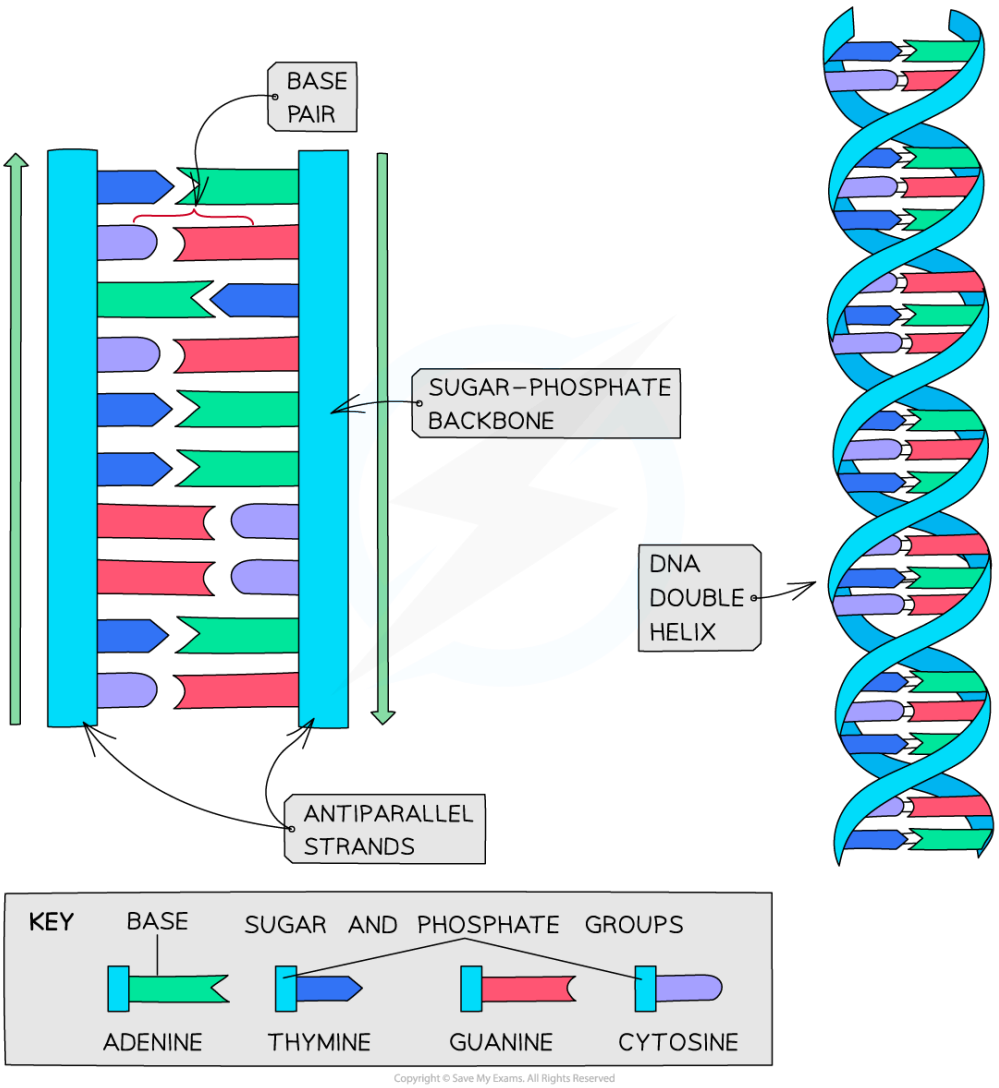


A section of DNA showing hydrogen bonding between base pairs

Double helix

- DNA is not two-dimensional as seen 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 3D double helix structure

Your notes

Examiner Tips and Tricks

Make sure you can name the different components of a DNA molecule (sugar-phosphate backbone, nucleotide, complementary base pairs, phosphodiester bonds, hydrogen bonds) and make sure you are able to locate these on a diagram.

Remember that **phosphodiester bonds** join the nucleotides in the sugar-phosphate backbone, and **hydrogen bonds** join the bases of the two complementary strands together.

Remember 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.