

Proteins and enzymes

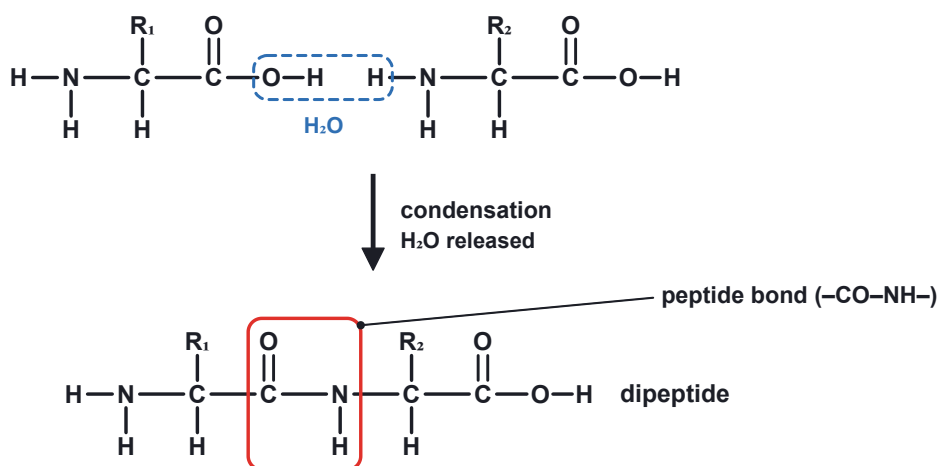
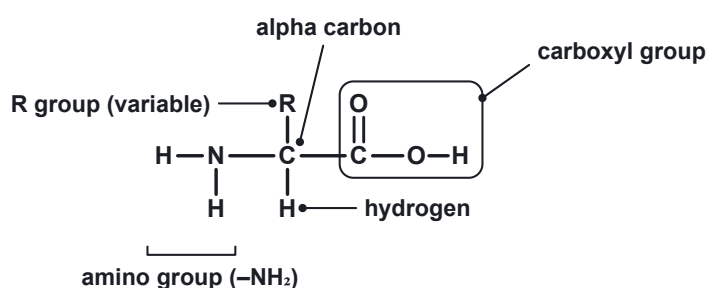
Revision notes · Amino acids, peptide bonds and protein structure; globular and fibrous proteins; how enzymes work and the factors that change their rate.

For Pearson Edexcel International AS Biology XBI11 and International A Level Biology YBI11. **A Level only** is IA2 content (Units 4 to 6), not needed for the International AS.

Proteins and enzymes Spec 2.6-2.8

Amino acids, peptide bonds and polypeptides Spec 2.6

Proteins are polymers made from **amino acid** monomers. All amino acids share the same basic structure around a central carbon atom: an **amino group** ($-\text{NH}_2$), a **carboxyl group** ($-\text{COOH}$), a hydrogen atom, and a variable **R group**. About 20 different amino acids are used to make proteins, and they differ only in their R group. (You do not need to learn the structures of individual amino acids.)



A **peptide bond** forms in a **condensation** reaction between the carboxyl group of one amino acid and the amino group of the next.

Condensation and hydrolysis

Two amino acids join in a **condensation reaction**. The carboxyl group of one amino acid reacts with the amino group of the next. A molecule of water is released, and a covalent **peptide bond** (C-N) forms between them, to give a **dipeptide**. Repeating this builds a chain of many amino acids, called a **polypeptide**. The reverse reaction, hydrolysis, uses water to break peptide bonds.

amino acid + amino acid → dipeptide + water (condensation, forms a peptide bond)

A **protein** is made of one or more polypeptide chains, folded into a precise three-dimensional shape. The sequence of amino acids in each chain is determined by the sequence of bases in a gene.

Practical skill: Recommended additional practical: use a semi-quantitative method to estimate protein concentration with biuret reagent and colour standards. Add sodium hydroxide, then copper(II) sulfate. A purple (lilac) colour shows peptide bonds; the more protein, the deeper the colour. Compare with standards of known concentration or use a colorimeter. Take care: sodium hydroxide is corrosive, so wear eye protection.

Common mistake: The peptide bond is between the carbon of one amino acid's carboxyl group and the nitrogen of the next amino group. A condensation reaction releases water, and does not use it up.

Levels of protein structure and the bonds involved Spec 2.6

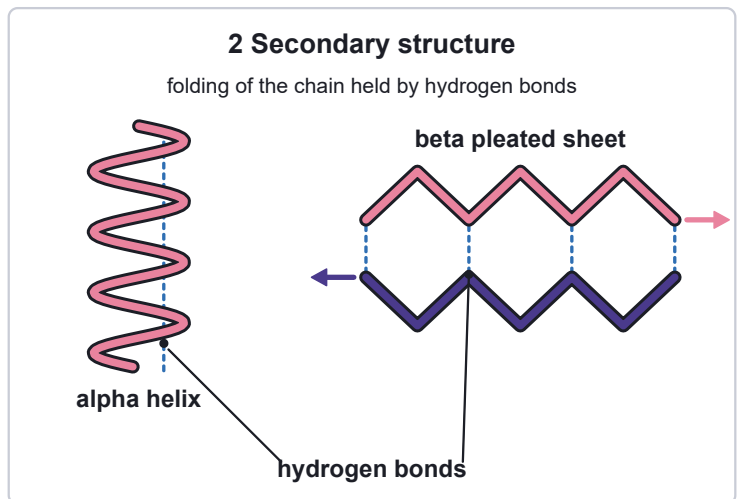
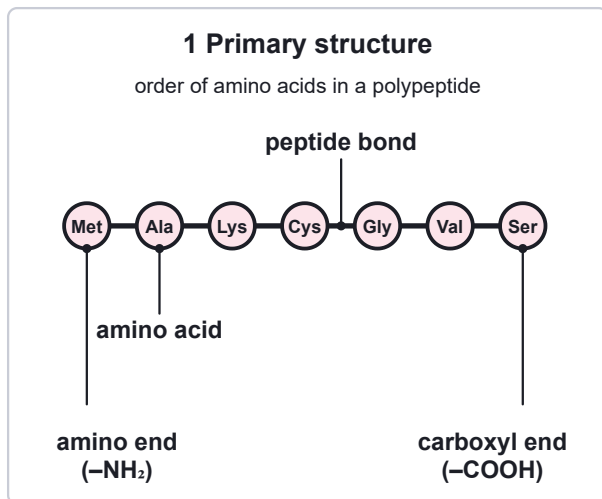
Levels of protein structure		
Level	Description	Bonds involved
Primary	The sequence of amino acids in the polypeptide chain.	Peptide bonds (covalent).
Secondary	Regular coiling or folding of the chain: the alpha helix and the beta pleated sheet.	Hydrogen bonds between the C=O and N-H groups of the polypeptide backbone, between amino acids near each other.
Tertiary	Further folding of the whole chain into a specific three-dimensional shape.	Hydrogen bonds, ionic bonds, disulfide bonds (covalent, between sulfur atoms of cysteine residues), and hydrophobic interactions between non-polar R groups.
Quaternary	Two or more polypeptide chains (and sometimes a non-protein group) together in one protein.	The same types of bond and interaction between the chains.

The **primary structure** decides everything else. The sequence of R groups determines where hydrogen bonds, ionic bonds and disulfide bonds can form, so it determines how the chain folds, and therefore the final shape and properties of the protein. Changing even one amino acid can change the shape, and so the function.

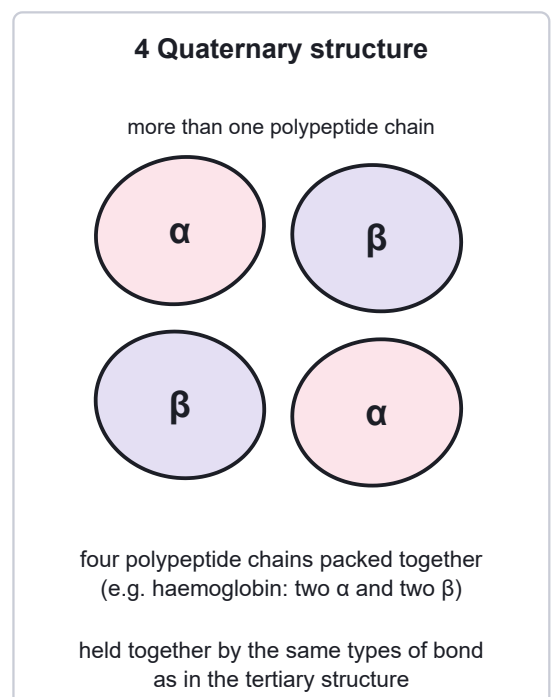
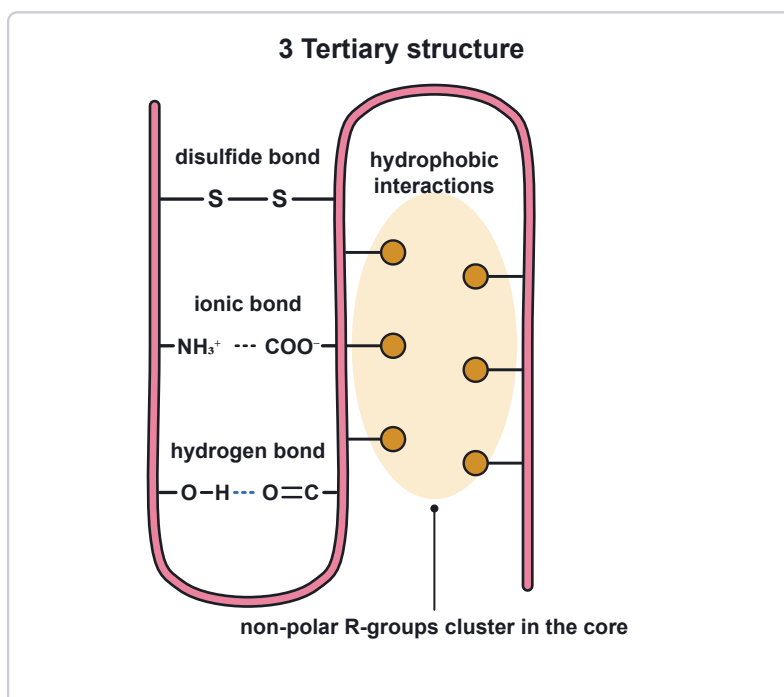
The bonds in the tertiary structure

- **Hydrogen bonds**: weak, but numerous. They form between polar R groups. They are broken by changes in temperature and pH.
- **Ionic bonds**: between positively and negatively charged R groups (for example -NH_3^+ and -COO^-). Stronger than hydrogen bonds but broken by changes in pH.
- **Disulfide bonds**: covalent bonds between the sulfur atoms of two cysteine R groups. Strong, and not easily broken.
- Hydrophobic interactions: non-polar R groups cluster in the centre of a globular protein, away from water.

Exam tip: Do not say the primary structure is 'held together by hydrogen bonds'. Peptide bonds hold the primary structure; hydrogen bonds hold the secondary structure and help hold the tertiary structure.



Primary structure is the amino acid sequence; **secondary structure** is the alpha helix or beta pleated sheet held by hydrogen bonds.



Tertiary structure is held by disulfide bonds, ionic bonds, hydrogen bonds and hydrophobic interactions; **quaternary structure** is more than one chain.

Globular and fibrous proteins: haemoglobin and collagen Spec 2.6

Globular and fibrous proteins compared		
Feature	Globular (for example haemoglobin)	Fibrous (for example collagen)
Shape	Compact, rounded, folded.	Long, rope-like fibres.
Solubility	Soluble in water: hydrophilic R groups face outwards, hydrophobic R groups face inwards.	Insoluble: many hydrophobic R groups on the outside.
Structure	Complex tertiary (and often quaternary) structure.	Repetitive amino acid sequence; little tertiary folding; chains wound together.
Role	Metabolic: transport, enzymes, hormones, antibodies.	Structural: strength and support.

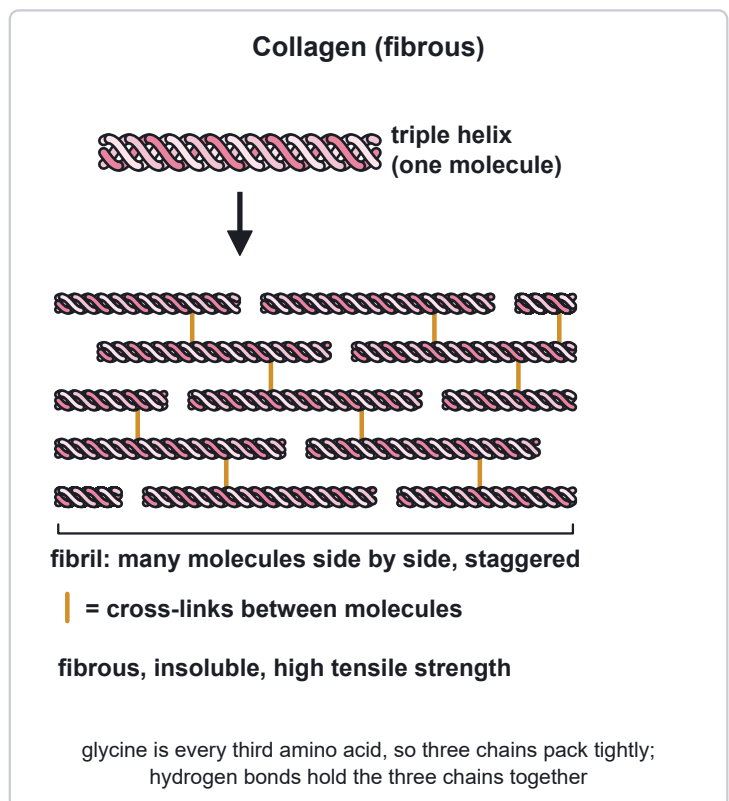
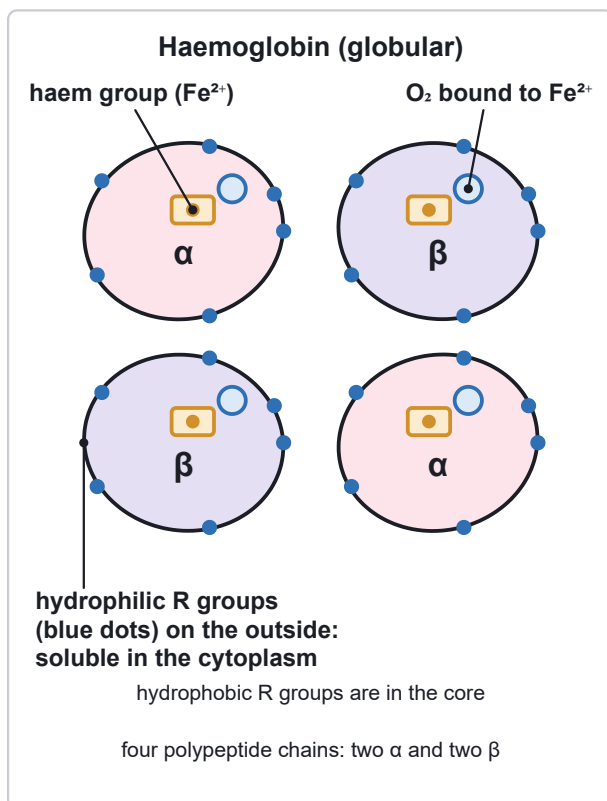
Haemoglobin

Haemoglobin is a globular protein with quaternary structure: four polypeptide chains (two alpha and two beta chains). Each chain is folded around a **haem group** that contains an iron ion (Fe^{2+}), which binds one oxygen molecule, so each haemoglobin molecule can carry four oxygen molecules. The hydrophilic R groups on the outside make haemoglobin soluble in the cytoplasm of red blood cells, and the precise shape allows it to bind and release oxygen.

Collagen

Collagen is a fibrous protein found in tendons, skin, bones, the walls of arteries and cartilage. Three polypeptide chains, each a left-handed helix, are wound together into a right-handed triple helix. Every third amino acid is glycine, the smallest amino acid, which allows the three chains to pack tightly together. The chains are held together by many hydrogen bonds, and neighbouring collagen molecules are linked by covalent cross-links, making fibrils and fibres. This gives collagen a very high tensile strength and makes it insoluble, which suits its structural function.

Exam tip: Always link structure to function: 'haemoglobin is soluble because hydrophilic R groups are on the outside, so it can be carried in the cytoplasm of red blood cells'; 'collagen has high tensile strength because of hydrogen bonds between three chains and cross-links between molecules'.



Haemoglobin is globular and soluble; **collagen** is fibrous with high tensile strength.

Enzymes: mode of action and specificity Spec 2.7

An **enzyme** is a globular protein that acts as a **biological catalyst**: it speeds up a chemical reaction without being used up. It does this by lowering the **activation energy**, the energy needed to start the reaction. The enzyme is not changed overall and can be used again.

The active site and specificity

An enzyme has an **active site**, a small region of the molecule that has a particular three-dimensional shape and charge pattern, set by the enzyme's tertiary structure. The substrate has a complementary

shape and binds to the active site, forming an **enzyme-substrate complex**. The products leave and the enzyme can bind another substrate.

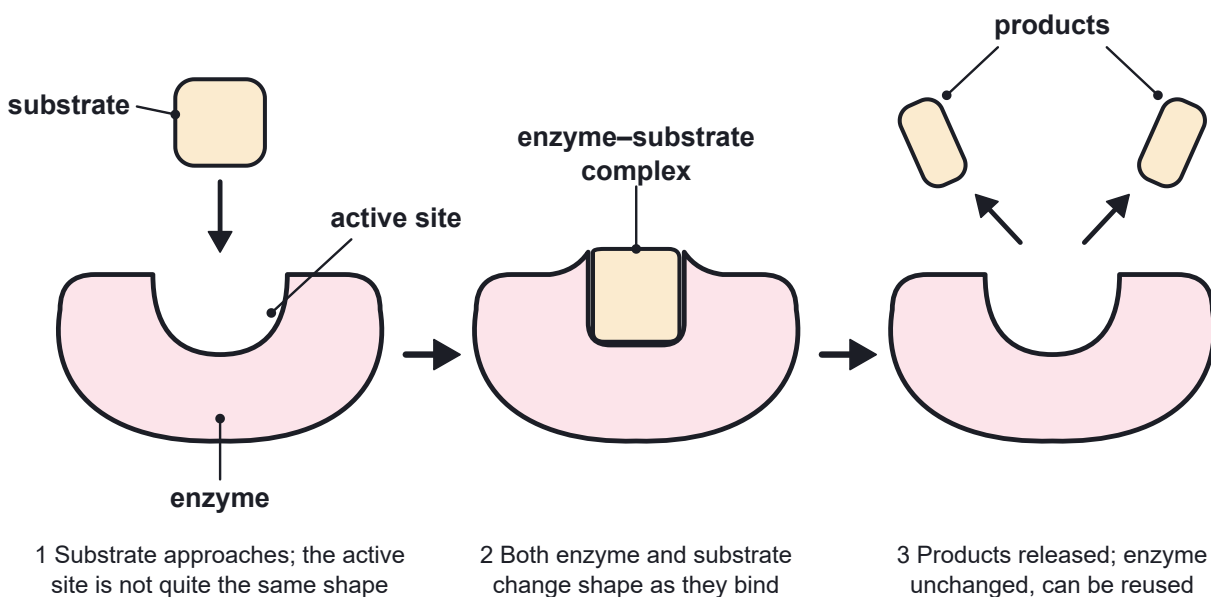
1. The substrate collides with the active site and binds, forming an enzyme-substrate complex.
2. The active site changes shape slightly to fit the substrate more closely (induced fit), which puts strain on bonds in the substrate.
3. This lowers the activation energy, so the substrate is converted to products.
4. The products leave the active site, which returns to its original shape.

Enzymes are **specific**: the shape of the active site only fits a particular substrate (or a small group of similar substrates). A different substrate cannot bind, so only one reaction is catalysed. The tertiary structure is determined by the primary structure, so a change in amino acid sequence can alter the active site and affect the enzyme's activity.

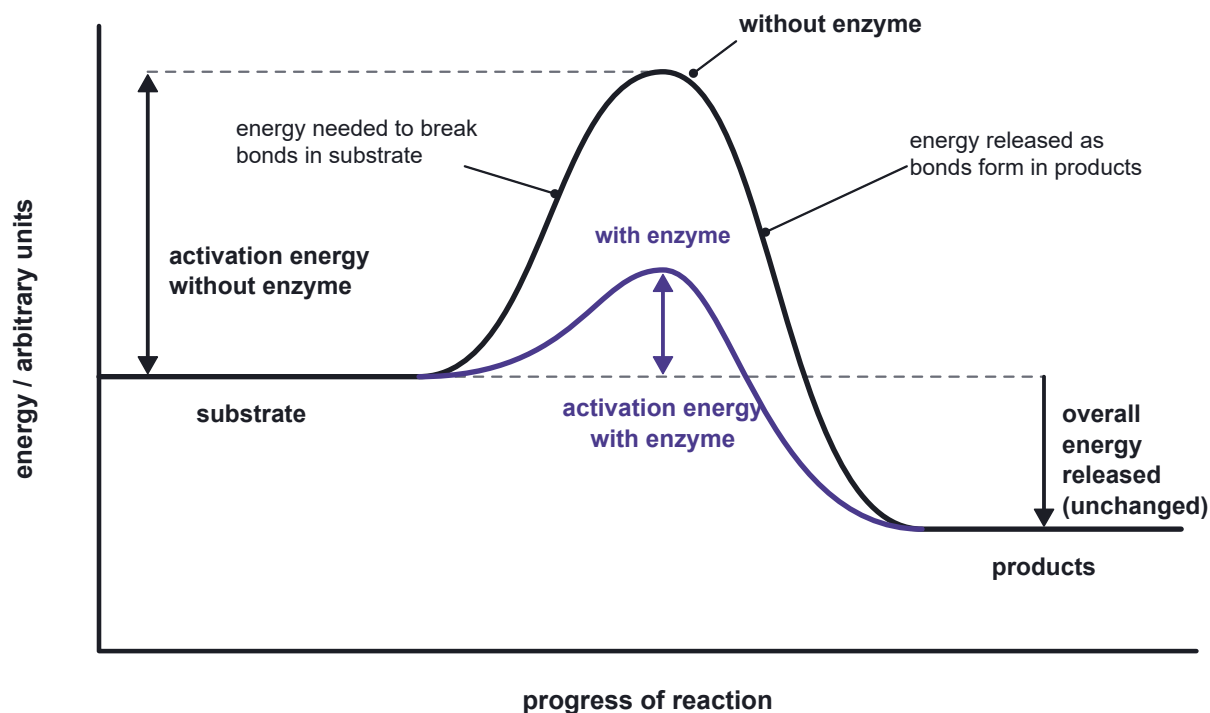
Intracellular and extracellular enzymes

- **Intracellular enzymes** catalyse reactions inside cells, for example catalase, which breaks down hydrogen peroxide inside cells, and the enzymes of respiration in the mitochondria.
- **Extracellular enzymes** are made in cells and secreted to catalyse reactions outside the cells, for example amylase and trypsin, which are secreted into the digestive system to digest starch and protein.

Exam tip: 'Lowers the activation energy' is the key mark point for how an enzyme works. Say that the substrate fits the active site because of its complementary shape, and that the shape is due to the tertiary structure.



The **enzyme-substrate complex**: the substrate fits the active site, and the enzyme is unchanged afterwards.



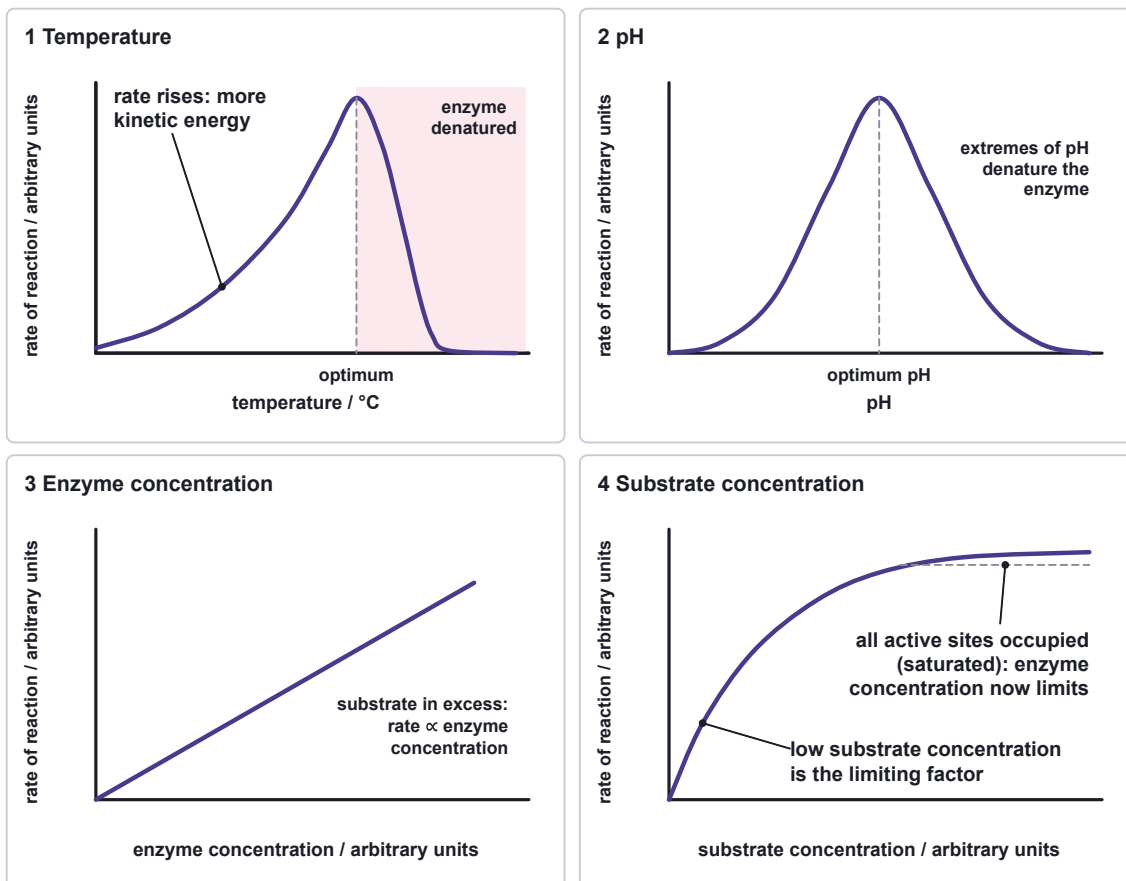
Enzymes **lower the activation energy** of a reaction.

Factors affecting the rate of enzyme-catalysed reactions Spec 2.8

The **initial rate** of reaction is the rate at the very start, when the substrate concentration is highest and little product has built up. Measuring the initial rate makes comparisons fair, because the rate falls later as substrate is used up.

Effect of each factor		
Factor	Effect on rate	Explanation
Temperature	Rate rises to an optimum, then falls rapidly.	Below the optimum, molecules have less kinetic energy, so there are fewer collisions and fewer enzyme-substrate complexes form. Above the optimum, hydrogen and ionic bonds in the tertiary structure break, the active site changes shape and the enzyme is denatured, and substrate no longer fits.
pH	Rate is highest at the optimum pH and falls on either side.	Changes in H^+ concentration change the charges on R groups and disrupt ionic and hydrogen bonds, so the active site changes shape and fewer enzyme-substrate complexes form. Far from the optimum pH the enzyme is denatured.
Enzyme concentration	Rate is proportional to enzyme concentration, if substrate is in excess.	More active sites are available, so more enzyme-substrate complexes form per second. At high enzyme concentration, substrate becomes limiting and the rate levels off.
Substrate concentration	Rate rises, then levels off at a maximum.	More substrate gives more collisions with active sites. When all the active sites are occupied (saturated), enzyme concentration is the limiting factor and rate stops rising.

Common mistake: Enzymes at low temperature are inactive, not dead: the active site is not changed and activity returns on warming. Above the optimum, denaturation is permanent. Do not say enzymes are 'killed' or 'destroyed', because enzymes are not alive.



Factors affecting the rate of an enzyme-controlled reaction. **Illustrative curves.**

Core practical 4: the initial rate of enzyme-catalysed reactions Spec 2.8

Core practical: Core practical 4: investigate the effect of temperature, pH, enzyme concentration and substrate concentration on the initial rate of enzyme-catalysed reactions.

A suitable system is catalase breaking down hydrogen peroxide into water and oxygen. The oxygen is collected, and the volume is measured over time. (Other systems also work, for example amylase and starch, where the time for the iodine colour to disappear is measured, or the breakdown of milk protein by a protease.)

1. Place a fixed volume of hydrogen peroxide solution, adjusted to the chosen pH with a buffer, in a boiling tube and bring it to the chosen temperature in a water bath.
2. Add a fixed volume of catalase solution (for example from yeast or liver) and immediately connect a delivery tube to a gas syringe or an upturned measuring cylinder over water.
3. Start a stopwatch. Record the volume of oxygen at regular intervals (for example every 10 seconds) for several minutes.
4. Repeat at least three times for each value of the independent variable, and calculate a mean.

Variables for each version of the investigation

Independent variable	How to change it	Keep constant
Temperature	Water baths at, for example, 10, 20, 30, 40, 50 and 60 °C.	pH (buffer), enzyme concentration and volume, substrate concentration and volume.
pH	Buffer solutions of different pH, for example pH 3 to 10.	Temperature, enzyme and substrate concentrations and volumes.
Enzyme concentration	Dilute the enzyme stock to give a range of concentrations (serial dilution).	Temperature, pH, substrate concentration and volume.
Substrate concentration	Dilute hydrogen peroxide stock to a range of concentrations.	Temperature, pH, enzyme concentration and volume.

Risk management

- Hydrogen peroxide is an irritant (and stronger solutions are corrosive): wear eye protection and gloves, and wash splashes off skin.
- Do not use a very high concentration of enzyme with high hydrogen peroxide, because the gas is evolved quickly: use a gas syringe or a measuring cylinder that is big enough.
- Hot water baths can scald.

Finding the initial rate

Plot the volume of product (y axis) against time (x axis). The curve is steepest at the start, then levels off as substrate is used up. Draw a **tangent** to the curve at time zero, and calculate its gradient. The gradient is the initial rate.

initial rate = change in volume of product ÷ change in time (gradient of the tangent at t = 0)

Initial rate from a graph

A tangent drawn to the curve at time zero passes through the points (0 s, 0 cm³) and (20 s, 14 cm³). Calculate the initial rate of the reaction.

1. Initial rate = change in volume ÷ change in time.
2. = (14 – 0) cm³ ÷ (20 – 0) s.
3. = 0.70 cm³ s⁻¹.

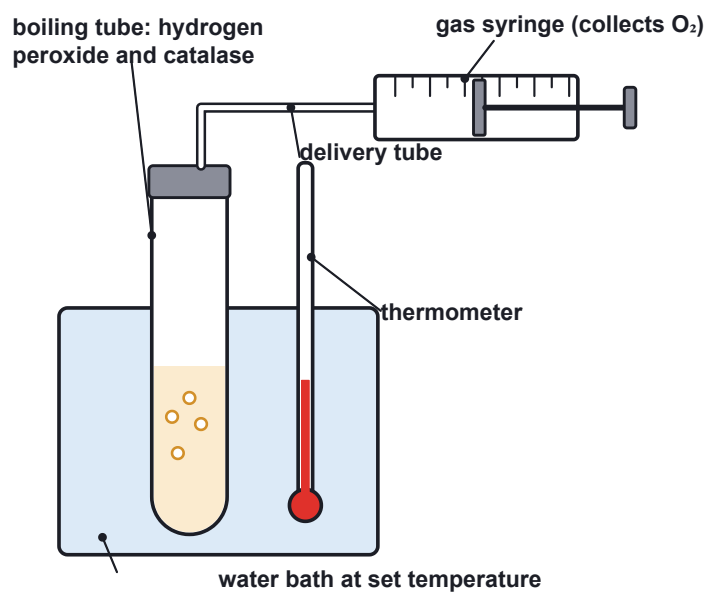
Answer: 0.70 cm³ s⁻¹ (the unit is cm³ per second).

Maths skill: Use a large triangle on the tangent to find the gradient. If the rate is taken from a data table instead, use the first, steepest interval. Give the unit (for example cm³ s⁻¹ or mm³ min⁻¹).

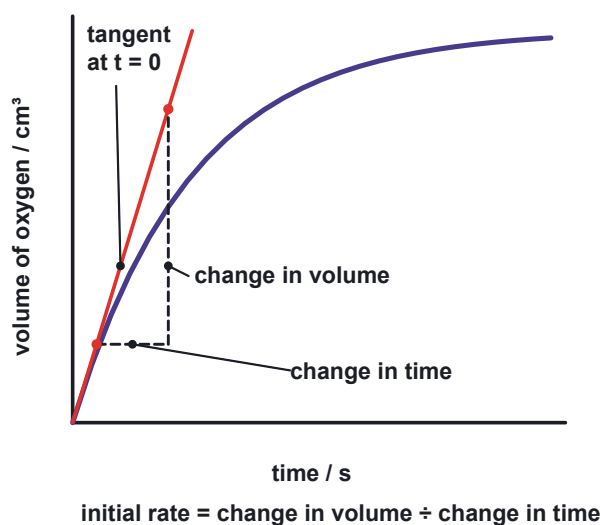
Percentage change is also used to compare rates between treatments: (new – original) ÷ original × 100.

Exam tip: In an evaluation, enzymes at different temperatures must be brought to temperature before they are mixed. Otherwise the reaction starts at the wrong temperature and the initial rate is not valid.

Core practical 4 apparatus



Initial rate from the graph



Core practical 4: find the **initial rate** from the **gradient of a tangent** at $t = 0$. Illustrative graph.

Quick check

1. Name the bond that links two amino acids.
2. Which bonds hold the tertiary structure of a protein?
3. Why is haemoglobin soluble?
4. What does an enzyme do to the activation energy of a reaction?
5. How is the initial rate of reaction found from a graph?

Quick check answers

Proteins and enzymes

1. A peptide bond (covalent), formed in a condensation reaction that releases water.
2. Hydrogen bonds, ionic bonds and disulfide bonds (plus hydrophobic interactions).
3. Hydrophilic R groups face outwards, so it dissolves in the cytoplasm of red blood cells.
4. It lowers it.
5. From the gradient of the tangent drawn at time zero on a graph of product against time.