

Enzymes

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Read first: [Biological molecules](#)

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What you need to know

By the end of this topic you should be able to:

How enzymes work

1. **State what enzymes are:** globular proteins that act as catalysts. Some work inside the cell that made them (**intracellular** enzymes); others are secreted and work outside it (**extracellular** enzymes).
2. **Explain how an enzyme speeds up a reaction** using the words active site, enzyme–substrate complex, activation energy and specificity, and describe both the **lock-and-key** and the **induced-fit** hypotheses.
3. **Follow the progress of an enzyme-catalysed reaction** in the lab in two ways: by measuring how fast a **product appears** (oxygen from hydrogen peroxide, using catalase) and how fast a **substrate disappears** (starch, using amylase).
4. **Outline how a colorimeter is used** to follow a reaction that involves a change in colour.

What changes the rate

5. **Investigate and explain the effect on the rate** of temperature, pH (kept steady with buffer solutions), enzyme concentration, substrate concentration and inhibitor concentration.
6. **Explain V_{max} and the Michaelis–Menten constant K_m :** how V_{max} is used to find K_m , and how K_m is used to compare how strongly different enzymes attract (their **affinity** for) their substrates.
7. **Explain how reversible inhibitors work**, both **competitive** and **non-competitive**, and what each does to enzyme activity.
8. **Compare an enzyme immobilised in alginate with the same enzyme free in solution**, and state the advantages of immobilised enzymes.

Understand it

What an enzyme is

An enzyme is a **biological catalyst**: it makes a reaction go faster, and it is **not used up or permanently changed**, so one enzyme molecule can be used again and again. Almost all enzymes are **globular proteins**: one or more polypeptide chains folded into a roughly spherical shape, with hydrophilic R groups on the outside

so they are soluble in water.

- **Intracellular** enzymes work inside the cell that made them, for example DNA polymerase in the nucleus, or catalase inside liver cells.
- **Extracellular** enzymes are secreted by the cell and work outside it, for example amylase in saliva, or trypsin and lipase in the lumen of the small intestine. Many bacteria and fungi also release extracellular enzymes to digest food around them.

The active site and specificity

Each enzyme has a small region called the **active site**, a pocket or cleft made of a few amino acids. The shape of the active site, and the charges and R groups lining it, come from the enzyme's **tertiary structure** (see Biological molecules): the hydrogen bonds, ionic bonds, disulfide bonds and hydrophobic interactions that hold the chain in its folded shape.

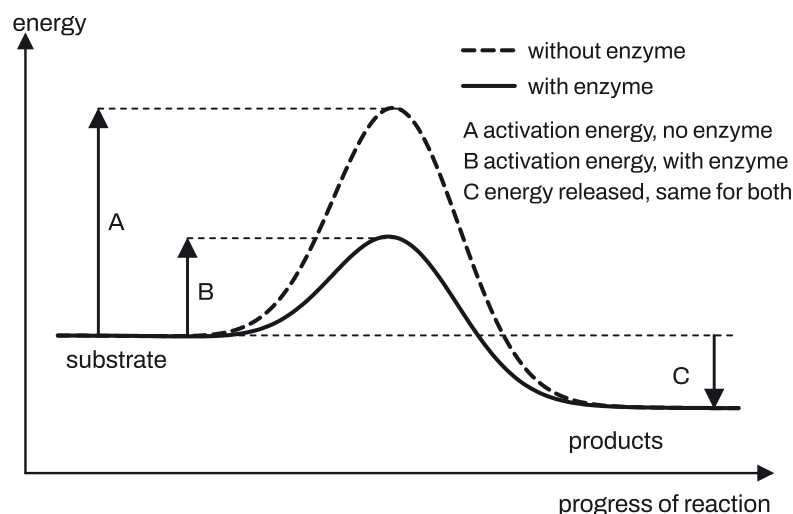
The substrate has a shape that is **complementary** to the active site: it fits into it the way a hand fits a glove, not "the same shape" as it. Only substrates with a complementary shape can bind, so each enzyme is **specific** to one reaction or a few similar ones.

When a substrate binds, it forms a short-lived **enzyme–substrate complex** (ES complex), held by temporary bonds between the substrate and R groups in the active site. The reaction happens, the **products** no longer fit, and they leave. The enzyme is free to bind another substrate.

Activation energy

Even a reaction that releases energy needs a push to get started: the reacting molecules must gain enough energy to reach an unstable in-between state before they can turn into products. This energy is the **activation energy**. An enzyme **lowers the activation energy**, so many more molecules can react at body temperature, and the reaction goes much faster.

The enzyme does this by holding the substrate in a position that puts **strain on its bonds**, or by holding two reacting molecules close together in the right orientation, so the reaction happens more easily.

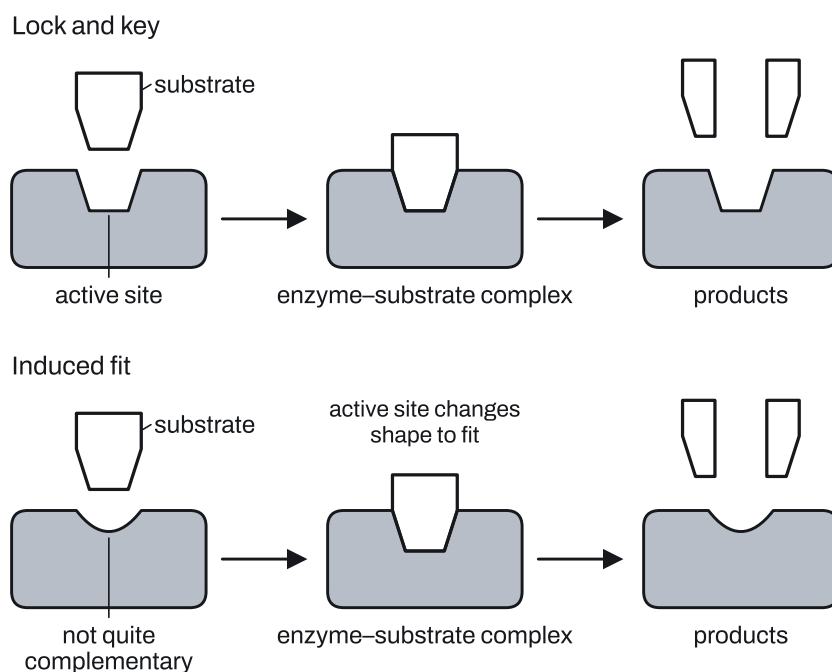


Notice what an enzyme does **not** change: the energy of the substrate, the energy of the products and so the **overall energy released**. It only lowers the hill in between.

Lock and key, and induced fit

There are two models of how the substrate binds.

- **Lock-and-key hypothesis.** The active site already has a shape exactly complementary to the substrate, like a lock and its key. The substrate slots in, the ES complex forms, the products leave.
- **Induced-fit hypothesis.** The active site is **not quite complementary** to begin with. As the substrate enters, the active site (and the enzyme around it) **changes shape**, moulding around the substrate so that the two become complementary and fit closely. This change of shape (a **conformational change**) can strain the substrate's bonds, lowering the activation energy. When the products leave, the active site goes back to its original shape.



The two models share a lot: in both the substrate binds to the active site, an ES complex forms, the activation energy is lowered, the enzyme is specific and it is unchanged at the end. They differ in **whether the active site changes shape** when the substrate binds. Note that in induced fit it is the **enzyme** that changes shape, not the substrate.

Measuring the progress of a reaction

You can follow a reaction by measuring a product appearing or a substrate disappearing.

- **Catalase** breaks down hydrogen peroxide: $2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$. Collect the oxygen in a **gas syringe** (or an upturned measuring cylinder full of water) and read the volume at regular times. Or time how long catalase-soaked filter-paper discs take to float up in hydrogen peroxide.

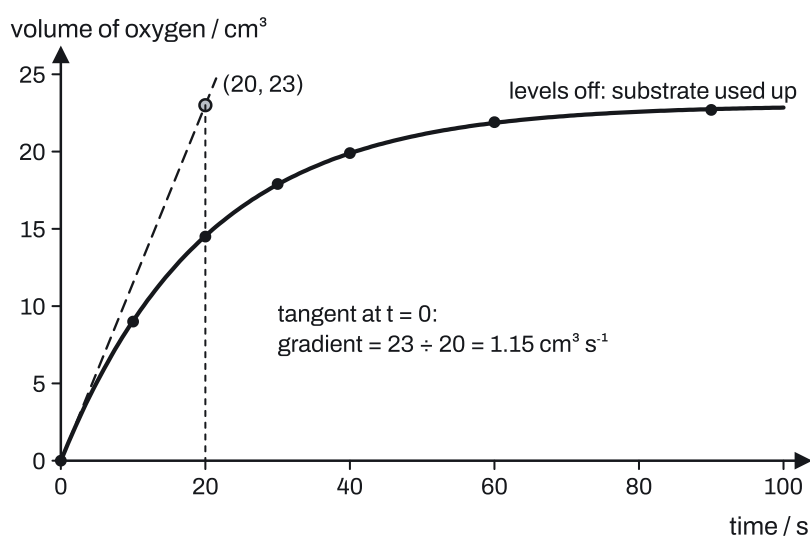
- **Amylase** hydrolyses starch to maltose. Every minute (or 30 s), put a drop of the reaction mixture onto a drop of **iodine solution** on a spotting tile. Iodine is **orange-brown**, and turns **blue-black** with starch. The time when the drop stays orange-brown is when all the starch has gone. A faster reaction gives a shorter time.

When you only have "time taken", turn it into a rate with

$$\text{rate} = \frac{1}{\text{time}}$$

so that a bigger number means a faster reaction. For example, starch gone in 40 s gives a rate of $\frac{1}{40} = 0.025 \text{ s}^{-1}$ (or $25 \times 10^{-3} \text{ s}^{-1}$).

Plot product against time and you get a **progress curve**. It is steepest at the start and then levels off.



The curve levels off because the **substrate is being used up**: fewer substrate molecules are left, so there are fewer collisions with active sites and fewer ES complexes form each second. The enzyme is **not** used up, and it has not denatured. More enzyme would reach the same final amount, only sooner.

Because the rate falls as the substrate runs out, compare experiments using the **initial rate**: the rate right at the start, when the substrate concentration is still the one you set. Find it from the **gradient of a tangent** drawn at time 0 (in the graph above, $23 \div 20 = 1.15 \text{ cm}^3 \text{ s}^{-1}$), or from the amount of product in a short, fixed time at the start.

Using a colorimeter

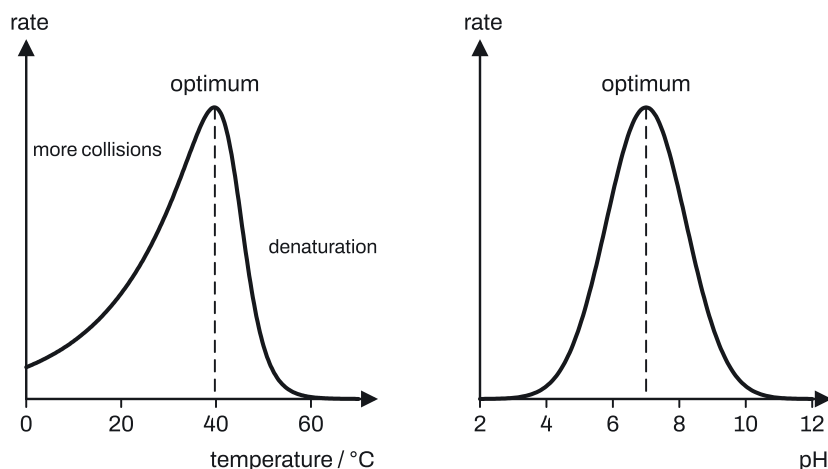
A **colorimeter** shines light of one colour through a sample in a small tube (a cuvette) and measures how much is absorbed (**absorbance**) or passes through (**transmission**). A darker or more intensely coloured solution has a **higher absorbance** and a lower transmission.

1. Choose a **filter** of the colour the solution absorbs most (the complementary colour, not the colour of the solution itself).
2. **Calibrate**: set the reading to zero absorbance with a blank, a tube of water or of the reaction mixture without the coloured substance.

3. Measure the absorbance of samples of the reaction at set times.
4. To turn absorbance into concentration, make a **calibration curve** from solutions of known concentration and read your values off it.

Use it for any reaction with a colour change, for example the blue-black starch–iodine colour fading as amylase works (absorbance falls), or a colourless substrate turning into a coloured product (absorbance rises). A colorimeter gives **numbers** instead of judging shades of colour by eye, so the results are **quantitative** and **objective**, and it can detect small changes in colour that the eye misses. It measures light **passing through** a solution, so it cannot be used on a solid object such as a leaf or skin.

Temperature



- **Below the optimum**, raising the temperature gives the enzyme and substrate molecules **more kinetic energy**. They move faster and collide more often, so **more ES complexes form per second** and the rate rises.
- At the **optimum temperature** the rate is highest.
- **Above the optimum**, the enzyme molecule vibrates so much that the bonds holding its tertiary structure (**hydrogen bonds and ionic bonds** in particular) break. The active site **changes shape**, the substrate no longer fits, and fewer ES complexes form. This is **denaturation**. It happens to more and more enzyme molecules as the temperature rises (partial, then complete), so the rate falls steeply. Denaturation is permanent: cooling does not restore the shape.
- At low temperatures the enzyme is only **inactive**, not denatured: warm it up and it works again.

Human enzymes usually have an optimum near 37–40 °C. Enzymes from organisms living in hot springs are **thermostable**: extra bonds (more hydrogen, ionic or disulfide bonds) hold their shape, so they have much higher optimum temperatures, such as 70–80 °C. Every enzyme, even a thermostable one, still has an optimum.

pH

Each enzyme works best at its **optimum pH**: about 2 for pepsin in the stomach, about 7 for salivary amylase, about 8 for trypsin in the small intestine. Away from the optimum, the extra or missing **hydrogen ions** (H^+) change the charges on the R groups. This breaks **ionic and hydrogen bonds** in the tertiary structure, and changes the charges in the active site, so the active site changes shape and the substrate binds less well: fewer ES complexes, lower rate. Extreme pH denatures the enzyme.

In an investigation, a **buffer solution** keeps the pH constant at the value you chose, even if the reaction itself makes acid (for example lipase releasing fatty acids).

Enzyme and substrate concentration

Enzyme concentration. With plenty of substrate, doubling the enzyme doubles the number of active sites, so the rate rises in a **straight line**. It only levels off if the substrate runs short and becomes the limiting factor.

Substrate concentration. With a fixed amount of enzyme:

- At **low substrate concentration**, many active sites are empty. More substrate means more collisions and more ES complexes, so the rate rises. Here **substrate concentration is the limiting factor**.
- At **high substrate concentration**, **all the active sites are occupied** (the enzyme is saturated) at any moment. Adding more substrate cannot make it faster, so the curve levels off at the **maximum rate**, V_{max} . Now the **enzyme concentration** is the limiting factor. Only a change in something else (more enzyme, a better temperature or pH) can raise the plateau.

V_{max} and the Michaelis–Menten constant K_m

The **Michaelis–Menten constant** K_m is the **substrate concentration** at which the rate is **half of** V_{max} . To find it from a graph of initial rate against substrate concentration:

1. Read V_{max} , the plateau, on the rate axis.
2. Halve it.
3. Go across from $\frac{1}{2}V_{max}$ to the curve, then down to the substrate-concentration axis. That value is K_m , in the **same units as the substrate concentration** (such as mmol dm^{-3}).

K_m is a **concentration**, not a rate. It tells you about the enzyme's **affinity** for its substrate: how readily the substrate binds and forms ES complexes.

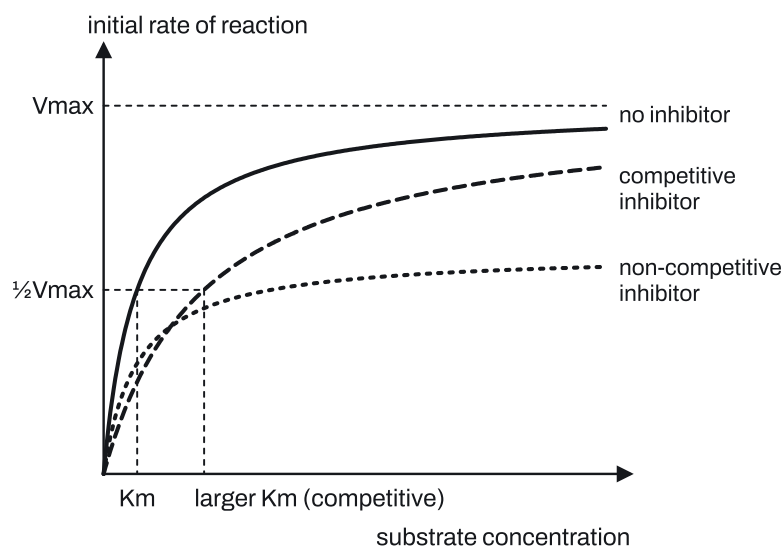
- A **low** K_m means the enzyme reaches half its maximum rate at a low substrate concentration: the substrate binds easily, so the enzyme has a **high affinity** for it. It works fast even when there is little substrate.
- A **high** K_m means a **low affinity**: a lot of substrate is needed before the enzyme gets going.

So K_m is used to **compare the affinity of different enzymes for the same substrate**, or of one enzyme for different substrates.

Inhibitors

An **inhibitor** is a molecule that reduces the rate of an enzyme-catalysed reaction. The syllabus deals with **reversible** inhibitors, which bind with weak bonds and can come off again.

- A **competitive inhibitor** has a shape **similar to the substrate**, so it can bind to the **active site**. While it is there, the substrate cannot get in. Substrate and inhibitor compete for the same site, so the effect depends on how many of each there are: **raising the substrate concentration reduces the inhibition**, because a substrate molecule is more likely to reach each free active site first. At very high substrate concentration the rate reaches the **same V_{max}** as without the inhibitor, but more substrate is needed to reach half of it, so **K_m increases** (the enzyme behaves as if its affinity were lower).
- A **non-competitive inhibitor** binds to the enzyme **at a site other than the active site** (an allosteric site). This changes the **tertiary structure**, so the **active site changes shape** and the substrate no longer fits (or the enzyme cannot catalyse the reaction). Adding more substrate **does not help**, because the inhibitor is not competing for the active site. It takes some enzyme molecules out of action, like having less enzyme, so **V_{max} falls**. The enzyme molecules that are still working have their normal affinity, so **K_m stays the same**.



Inhibitor concentration. With either kind, more inhibitor means more enzyme molecules are blocked at any moment, so the rate **falls** as inhibitor concentration rises, steeply at first and then more gradually.

Cells use inhibition to control their metabolism. In **end-product inhibition**, the final product of a chain of reactions inhibits the enzyme at the start of the chain, so when plenty of product has built up, the pathway slows down, and when the product is used up, the enzyme works again. Because the inhibitor is reversible, the control can switch on and off.

Immobilised enzymes

In industry, enzymes are often **immobilised**: trapped in, or attached to, an inert material so they don't mix freely with the product. A simple way in the lab:

1. Mix the enzyme with a solution of **sodium alginate**.

2. Drip the mixture from a syringe into **calcium chloride** solution. Each drop sets into a jelly-like **bead** of calcium alginate with enzyme trapped inside.
3. Rinse the beads and pack them into a column (a syringe barrel). Pour the substrate solution through and collect the product at the bottom.

To compare with the **free enzyme**, use the same mass of enzyme free in solution, the same substrate volume and concentration, the same temperature and pH, and measure the product formed (for example with Benedict's for the sugars from lactose, or iodine for starch left).

Advantages of immobilised enzymes:

- The enzyme can be **recovered and reused** many times, so it is cheaper.
- The **product is not contaminated** with enzyme, so it doesn't need purifying.
- The process can run **continuously**: substrate goes in at the top, product comes out at the bottom.
- The enzyme is **more stable**: the matrix holds the enzyme molecules in shape, so they work over a **wider range of temperature and pH** and denature less easily.

Typical results: the immobilised enzyme is less active at low temperatures, because the substrate must diffuse into the bead to reach the active sites; but it keeps working where the free enzyme denatures, so its optimum is often higher and its activity falls less steeply.

Key facts and formulas

None of these is in the formula list printed in the syllabus (that list is for A Level statistics and ecology), so you must learn them all.

Formula	Status
$\text{rate} = \frac{1}{\text{time}}$ (for "time taken" results; unit s^{-1})	Learn it
$\text{rate} = \frac{\text{change in amount of product (or substrate)}}{\text{time taken}}$	Learn it
Initial rate = gradient of the tangent to the progress curve at $t = 0$	Learn it
K_m = substrate concentration at which $\text{rate} = \frac{1}{2}V_{max}$ (units of concentration)	Learn it
Low K_m = high affinity; high K_m = low affinity	Learn it
Competitive inhibitor: V_{max} unchanged, K_m increases	Learn it
Non-competitive inhibitor: V_{max} decreases, K_m unchanged	Learn it
Percentage change = $\frac{\text{new} - \text{original}}{\text{original}} \times 100$	Learn it
$2\text{H}_2\text{O}_2 \xrightarrow{\text{catalase}} 2\text{H}_2\text{O} + \text{O}_2$	Learn it
Starch $\xrightarrow{\text{amylase}}$ maltose; iodine: orange-brown $\rightarrow$ blue-black with starch	Learn it

Digestive enzymes catalyse **hydrolysis**: amylase breaks **glycosidic** bonds, proteases (pepsin, trypsin) break **peptide** bonds, lipase breaks **ester** bonds between glycerol and fatty acids.

How to do it

In the 120 questions we have tagged for this topic (91 from Paper 1, 29 from Paper 2, 2021–2025), the types came up this often. 23 of the Paper 2 questions mix more than one type, so the counts add up to 154. Paper 1 questions are single multiple-choice marks; Paper 2 parts often sit inside a question on another topic. The methods below rely on the explanations in "Understand it"; they only add the steps and the traps.

Question type	Questions
Inhibitors: mechanisms, V_{max} and K_m , curves on rate–substrate graphs	32
Explain the mode of action: active site, ES complex, lock and key, induced fit	24
K_m : determine it from a graph and interpret it	22
Describe and explain the effects of temperature and pH	15
State what enzymes are: globular proteins, intracellular or extracellular	10
Immobilised and industrial enzymes: data and advantages	10
Explain rate–substrate and rate–enzyme concentration curves	10
Colorimeter use and absorbance results	8
Activation energy on an energy diagram	8
Progress curves and methods of measuring activity	7
Name the reaction or bond an enzyme catalyses	4
Calculate a rate or percentage change	4

1. Inhibitors: mechanisms, V_{max} and K_m , curves (32 questions)

- Decide the type** from the clues. Similar shape to the substrate, binds the active site, overcome by more substrate → **competitive**. Binds elsewhere, changes the active site's shape, not affected by substrate → **non-competitive**.
- Explain** in order: where it binds → what happens to the active site → fewer ES complexes → lower rate.
- State the effects**: competitive, V_{max} same and K_m higher; non-competitive, V_{max} lower and K_m same.
- Sketch** from the origin, below the original curve: competitive rises towards the **same** V_{max} ; non-competitive has the original's shape with a **lower plateau** (never S-shaped).

Variations you will meet:

- "Use the graph to support the conclusion that X is non-competitive"**: the marks are for data, not the mechanism: "the plateau with X is 40 au compared with 100 au without", " K_m is about 2 mmol dm⁻³ in both". A gap that shrinks at high substrate concentration means competitive.

- **Estimate K_m from the curve you drew:** halve your own curve's plateau, and mark K_m on the x-axis.
- **A named drug or poison:** apply the same steps. "Reversible" means it can leave, and the enzyme works again.
- **End-product inhibition:** the final product slows the first enzyme of the pathway. If it has a shape similar to the substrate, it is competitive.
- **Statements to judge:** inhibitors do not change the activation energy; more substrate does not reduce a non-competitive inhibitor's effect.
- Give **one** type and stick to it: listing features of both cancels the marks.

2. Explain the mode of action (24 questions)

1. The active site is **complementary** to the substrate (never "the same shape").
2. The substrate binds and an **enzyme–substrate complex** forms.
3. The enzyme **lowers the activation energy** (strain on bonds, or reactants held close together).
4. Products **leave the active site**; the enzyme is unchanged and reused.

For **induced fit**, add: the active site is **not fully complementary at first**; as the substrate enters, the **active site changes shape** and becomes complementary; after the products leave it **returns to its original shape**. Use "shape" or "conformation", and don't describe lock and key instead.

Variations you will meet:

- **One similarity and one difference** between the two models: see "Lock and key, and induced fit".
- **Why an enzyme won't act on a similar molecule:** its shape is not complementary to the active site, so no ES complex forms.
- **Where specificity comes from:** the amino acid sequence decides the folding (tertiary structure) and so the active site's shape.
- **Multiple-choice traps:** "same shape" is wrong; "binding causes a conformational change in the enzyme" is induced fit; enzymes never raise the activation energy.

3. K_m : determine it and interpret it (22 questions)

1. **Determine:** read V_{max} from the plateau, halve it, rule across to the curve and down to the x-axis. Give K_m with the **x-axis unit**, which often earns its own mark.
2. **Define** it fully: the **substrate concentration** at which the rate is **half V_{max}** . Both halves are needed.
3. **Compare:** lower K_m = higher affinity. Check your conclusion names the right enzyme.

Variations you will meet:

- **"Describe how you found K_m ":** give the values: " $V_{max} = 3.6$, half = 1.8, read across and down: $K_m = 150 \mu\text{mol dm}^{-3}$ ".
- **Explain a stated value**, such as 50 mmol dm^{-3} : at that substrate concentration the rate is half V_{max} . Not " K_m is half V_{max} ".

- **Tables of K_m values:** watch the powers of ten (1.5×10^{-2} is lower than 3.0×10^{-1}).
- **Why a low K_m helps:** the enzyme works fast even with little substrate.

4. Describe and explain the effects of temperature and pH (15 questions)

1. **Describe** with data and units: the rise, the optimum, the fall.
2. **Explain** with the chains in "Temperature" and "pH": kinetic energy → collisions → ES complexes; bonds break → active site changes shape → fewer ES complexes; denaturation.

Variations you will meet:

- **A very high optimum** (bacteria from hot springs): a **thermostable** enzyme with more bonds holding its shape; it still has an optimum.
- **Predict a human enzyme's curve:** optimum about 37–40 °C, narrower range, steeper fall to zero.
- **Two pH values compared on rate–substrate curves:** give the differences in rate and V_{max} with numbers, then explain that the pH nearer the optimum keeps the active site's shape best.
- **Time data:** time to digest against pH is U-shaped (lowest at the optimum); $\frac{1}{\text{time}}$ is a hump.
- **Valid conclusions** (multiple choice): a temperature is only "the optimum" if values either side were tested and were lower.

5. State what enzymes are (10 questions)

1. Globular proteins; catalysts; form temporary bonds with the substrate; unchanged at the end.
2. Acts outside the cell that made it → **extracellular** (the one-mark word); inside → intracellular.

Variations: digestion in the gut lumen is extracellular, DNA polymerase in the nucleus intracellular; not all enzymes break molecules down; the part of a membrane enzyme inside the bilayer has hydrophobic R groups; the enzyme is the substance whose amount stays the same during a reaction.

6. Immobilised and industrial enzymes (10 questions)

1. **Compare** immobilised with free at each condition, with numbers and units.
2. **Explain** using "Immobilised enzymes": the matrix holds the shape (less denaturation); substrate must diffuse into the bead (lower activity at low temperature).
3. **State advantages:** reusable, product not contaminated, continuous, more stable.

Variations you will meet:

- **Evaluate a study:** compare with the controls and between times, look for a trend with enzyme concentration, and note it is not 100 % effective, the time span is short, and there were no repeats.
- **Factors for a real-world trial:** enzyme concentration immobilised, the material it is fixed to, how long it stays active, the conditions it meets (temperature, pH, salt, pollutants that could inhibit it), other organisms.

- **Columns:** slower flow or a longer column → more contact time → more product; smaller beads → larger surface area.

7. Explain rate–substrate and rate–enzyme concentration curves (10 questions)

Use "Enzyme and substrate concentration": rising part, substrate limiting; plateau, **all active sites occupied**, enzyme limiting (not used up, not denatured); rate proportional to enzyme concentration while substrate is in excess.

Variations: only more enzyme, or a better temperature or pH, raises the plateau; rate goes on the y-axis; cells control rates by changing enzyme or substrate concentration, or by inhibition.

8. Colorimeter use and absorbance results (8 questions)

1. **Why a colorimeter:** quantitative, objective, detects slight colour changes, gives concentrations from a calibration curve. "More accurate" alone is too vague.
2. **Explain a curve as a chain:** more (or less) coloured substance → darker (or paler) → less (or more) light through → absorbance rises (or falls); it levels off when the substrate runs out.

Variations: an enzyme that **makes** starch gives rising absorbance with iodine; adding the product of a reversible reaction (such as phosphate) can drive it backwards; a colorimeter needs a solution light passes through, not skin or leaves.

9. Activation energy on an energy diagram (8 questions)

Activation energy runs from the **substrate level to the top of the peak**; the reduction is the difference between the peaks; the energy released (substrate level to product level) is the **same** with or without the enzyme. Pick graphs with a lower peak but the same start and end levels.

10. Progress curves and methods of measuring activity (7 questions)

Use "Measuring the progress of a reaction": product curves level off as the **substrate runs out**; more enzyme makes the start steeper but gives the **same final amount**; compare **initial rates**.

Variations: lipase in milk releases fatty acids, so the **pH falls**; to measure an enzyme's activity in a sample you need its substrate in excess, a set temperature and pH (buffer), and a way to measure product formed per unit time.

11. Name the reaction or bond an enzyme catalyses (4 questions)

Digestive enzymes catalyse **hydrolysis**. Carbohydrates → **glycosidic**, proteins → **peptide**, triglycerides → **ester** bonds. On a triglyceride, the arrow goes between the glycerol's O and the fatty acid's C=O carbon.

12. Calculate a rate or percentage change (4 questions)

rate = $\frac{1}{\text{time}}$ (convert first: 2 min 30 s = 150 s); rate from a graph = change $\div$ time, or the tangent's gradient, with units; percentage change = $\frac{\text{new} - \text{original}}{\text{original}} \times 100$, so a fall from 30 to 12 is a **60 %** decrease. Round only at the end.

Worked examples

The bold codes show where marks are earned, as in Cambridge mark schemes. The number is how many marks.

- **M1** method mark: for a correct method, even if the numbers slip.
- **A1** accuracy mark: for a correct answer; it needs the M mark before it.
- **B1** mark for a correct result on its own, with no method needed.

Example 1

Pepsin is made by cells in the lining of the stomach. It is released into the stomach, where it hydrolyses proteins.

- (a) State whether pepsin is an intracellular or an extracellular enzyme, and give a reason. **[1]**
- (b) Name the bond that pepsin breaks. **[1]**
- (c) Use the induced-fit hypothesis to describe how pepsin catalyses the hydrolysis of a protein. **[4]**
- (d) Explain why pepsin does not hydrolyse starch. **[2]**
- (a)** Extracellular, because it is secreted and acts outside the cells that made it. **B1**
- (b)** Peptide bond. **B1**
- (c)** Any four of:
- The active site of pepsin is **not fully complementary** to the protein at first. **B1**
 - As the protein (substrate) enters the active site, the **active site changes shape** (a conformational change) and moulds around it. **B1**
 - It becomes **complementary** and an **enzyme–substrate complex** forms. **B1**
 - The shape change puts strain on the peptide bond, **lowering the activation energy**. **B1**
 - The products leave and the active site **returns to its original shape**. **B1**
- (d)** The shape of starch is **not complementary** to the active site of pepsin, **B1** so starch cannot bind and no enzyme–substrate complex forms; enzymes are **specific**. **B1**

Example 2

A student measured the initial rate of a reaction catalysed by enzyme A at different substrate concentrations. At very high substrate concentrations the rate levels off at 60 au.

Substrate concentration / mmol dm ⁻³	0	2.5	5.0	10	20	40	160
Initial rate / au	0	20	30	40	48	53	58

- (a) State V_{max} and determine K_m for enzyme A. Give the unit of K_m . [3]
- (b) Another enzyme, B, acts on the same substrate and has a K_m of 12 mmol dm⁻³. Explain which enzyme has the higher affinity for the substrate. [2]
- (c) Explain why the rate increases less and less as the substrate concentration rises above 20 mmol dm⁻³. [2]
- (d) The experiment with enzyme A was repeated with a fixed concentration of an inhibitor. At 10 mmol dm⁻³ the rate fell to 24 au; at 160 mmol dm⁻³ it was 55 au. (i) Calculate the percentage decrease in rate at 10 mmol dm⁻³. [1] (ii) Identify the type of inhibitor and explain your answer, using the data. [3]

(a) $V_{max} = 60$ au. **B1** Half $V_{max} = 30$ au, which is reached at 5.0 mmol dm⁻³. **M1**

$K_m = 5.0$ mmol dm⁻³. **A1** (for the value with its unit)

(b) Enzyme A, because its K_m (5.0) is **lower** than B's (12 mmol dm⁻³). **B1** A reaches half its maximum rate at a lower substrate concentration, so its substrate binds more readily and ES complexes form more easily. **B1**

(c) As the substrate concentration rises, **more of the active sites are occupied** at any one time, so there are fewer free active sites for extra substrate to bind to; **B1** close to V_{max} almost all are occupied and the **enzyme concentration** becomes the limiting factor. **B1**

(d)(i) $\frac{24 - 40}{40} \times 100 = -40$, a **40 %** decrease. **B1**

(d)(ii) A **competitive** inhibitor. **B1** At a low substrate concentration the rate is much lower (24 au compared with 40 au, 40 % less), **B1** but at a very high substrate concentration it is only about 5 % lower (55 au compared with 58 au): **more substrate out-competes the inhibitor** for the active sites, so the rate approaches the same V_{max} . **B1**

Example 3

The activity of an enzyme was measured free in solution and immobilised in alginate beads, at different temperatures. Each value is a percentage of that form's maximum activity.

Temperature / °C	30	40	50	60	70
Free enzyme / %	64	100	58	15	0
Immobilised enzyme / %	52	81	100	74	35

- (a) Describe and explain the effect of temperature on the activity of the free enzyme. [5]

(b) Compare the activity of the immobilised enzyme with the free enzyme, and suggest explanations for the differences. [4]

(c) State **two** advantages, other than those shown by these data, of using an immobilised enzyme in industry. [2]

(a)

- Activity rises from 64 % at 30 °C to a maximum of 100 % at the **optimum, 40 °C**. **B1**
- It then falls to 15 % at 60 °C and 0 % at 70 °C (an 85 % fall between 40 °C and 60 °C). **B1**
- Below the optimum, molecules have **more kinetic energy**, so there are **more collisions** between enzyme and substrate and **more ES complexes** form per unit time. **B1**
- Above the optimum, **hydrogen and ionic bonds** in the tertiary structure break, so the **active site changes shape** and the substrate is no longer complementary: fewer ES complexes form. **B1**
- The enzyme is progressively **denatured**; at 70 °C it is completely denatured. **B1**

(b)

- The immobilised enzyme has a **higher optimum** (50 °C compared with 40 °C). **B1**
- It is more active at high temperatures, for example **74 % at 60 °C compared with 15 %**, about 4.9 times as much, and still active at 70 °C (35 % compared with 0 %). **B1**
- The alginate **holds the enzyme's shape**, so its bonds break less easily and it **denatures less** at high temperature. **B1**
- It is less active at 30–40 °C (52 % compared with 64 % at 30 °C), because the substrate has to **diffuse into the bead** and some active sites are hidden, so fewer ES complexes form. **B1**

(c) Any two: the enzyme can be **recovered and reused**; the **product is not contaminated** with enzyme; the process can run **continuously**. **B1 B1**

Example 4

Catalase was added to hydrogen peroxide at 25 °C and the oxygen was collected in a gas syringe.

Time / s	0	10	20	30	40	60	90
Volume of oxygen / cm ³	0	9.0	14.5	17.9	19.9	21.9	22.7

(a) Calculate the mean rate of oxygen production during the first 10 s and between 30 s and 60 s. [2]

(b) Explain why the rate decreases during the experiment. [2]

(c) The student plotted a graph and drew a tangent to the curve at 0 s. It passed through (20 s, 23 cm³). Calculate the initial rate and explain why the initial rate is used when comparing experiments. [3]

(d) A second student followed the hydrolysis of starch by amylase, taking a sample every minute, adding it to iodine solution and measuring its absorbance with a colorimeter. Describe and explain how the absorbance changes, and explain why a colorimeter was used. [4]

(a) First 10 s: $\frac{9.0}{10} = 0.90 \text{ cm}^3 \text{ s}^{-1}$. **B1** From 30 s to 60 s: $\frac{21.9 - 17.9}{30} = 0.13 \text{ cm}^3 \text{ s}^{-1}$. **B1**

(b) The **hydrogen peroxide (substrate) is used up**, so its concentration falls; **B1** there are fewer collisions between substrate and active sites, so fewer ES complexes form per second. **B1** (The enzyme is not used up or denatured.)

(c) Initial rate = gradient of the tangent = $\frac{23}{20} = 1.15 \text{ cm}^3 \text{ s}^{-1}$. **M1 A1** This is the rate when the substrate concentration is still the one that was set, before it has been used up, so it is a **fair comparison** between experiments. **B1** (See the progress-curve diagram in "Understand it".)

(d)

- At first the samples are **blue-black** with iodine, because starch is present, so the absorbance is high. **B1**
- As amylase hydrolyses the starch, less starch is left, the colour becomes **paler**, more light passes through, so the **absorbance falls**. **B1**
- It levels off at a low value (the orange-brown of iodine) when all the starch has gone. **B1**
- The colorimeter gives **quantitative, objective** readings and detects small changes in colour that the eye cannot judge. **B1**

Common mistakes

- **Writing "the same shape" instead of "complementary"**. The substrate and active site fit together; they are not identical. Examiners say this every year, and multiple-choice options use it as a trap.
- **Describing lock and key when induced fit is asked for**, or saying the **substrate** changes shape. In induced fit the **active site (enzyme)** changes shape, and the answer must mention shape or conformation.
- **"Km is half Vmax"**. K_m is a **substrate concentration**: the concentration at which the rate is half V_{max} . Mark it on the x-axis, and give the x-axis unit.
- **Stating the rule and then contradicting it**. "A lower K_m means higher affinity, so the enzyme with the higher K_m has more affinity" loses the mark. Check which enzyme has the lower value.
- **Saying the plateau is because the enzyme is used up or denatured**. On a rate–substrate graph the plateau means all the active sites are occupied; on a progress curve it means the substrate has run out.
- **Mixing up the two inhibitors, or giving features of both**. Competitive: same V_{max} , bigger K_m , overcome by more substrate. Non-competitive: lower V_{max} , same K_m . Hedging between them scores nothing.
- **Vague temperature answers**. Say "more kinetic energy, more collisions, more ES complexes"; for the fall, say which bonds break and that the active site changes shape. Include data with units when you describe.
- **Forgetting that a thermostable enzyme still has an optimum**. It is higher, not absent.

How to get the marks

- **"Describe"** a graph means the trend **with data and units**: the optimum, values at the start and end, where it rises and falls. Quote pairs of readings when you compare (such as "74 % compared with 15 % at 60 °C").
- **"Explain"** needs the reason, linked step by step: kinetic energy → collisions → ES complexes; bonds break → active site shape changes → not complementary → fewer ES complexes.
- **"Suggest"** means apply what you know to an unfamiliar enzyme or situation; any sensible, biological answer is credited.
- **"Determine" or "calculate"**: show the working (the V_{max} you read, its half), then the answer with its **unit**; the unit is often a separate mark.
- **"Discuss" or evaluate data**: give points for and against: compare with the controls, note trends (or no trend), and comment on repeats, sample size and time span.
- **Key words the mark scheme looks for**: complementary, active site, enzyme–substrate complex, activation energy, tertiary structure, denatured, V_{max} , K_m , affinity, "all active sites occupied", "limiting factor".
- **Stick to the question**. Mode-of-action detail in a question about something else earns nothing, and a list of contradictory answers loses marks you would otherwise get.

Quick check

1. Classify each enzyme as intracellular or extracellular: (a) DNA polymerase in the nucleus; (b) trypsin in the lumen of the small intestine; (c) catalase inside liver cells.
2. Enzymes P and Q act on the same substrate. P has $K_m = 0.4 \text{ mmol dm}^{-3}$ and Q has $K_m = 2.0 \text{ mmol dm}^{-3}$. Which has the higher affinity? At a substrate concentration of 0.4 mmol dm^{-3} , what fraction of its V_{max} does P reach?
3. Starch disappeared in 50 s with amylase at pH 7 and in 125 s at pH 5. Calculate the rate at each pH, and the percentage decrease in rate from pH 7 to pH 5.
4. A reversible non-competitive inhibitor is added to an enzyme. State its effect on V_{max} and on K_m , and explain why adding more substrate does not bring the rate back up.
5. On an energy diagram the substrate is at 40 units, the peak without enzyme at 120, the peak with enzyme at 75 and the products at 10. Find the activation energy with and without the enzyme, and the energy released in each case.

Answers

1. (a) Intracellular; (b) extracellular (secreted into the gut and acts outside cells); (c) intracellular.
2. P, because its K_m is lower. At a substrate concentration equal to its K_m , P works at **half** its V_{max} ($\frac{1}{2}$).
3. pH 7: $\frac{1}{50} = 0.020 \text{ s}^{-1}$; pH 5: $\frac{1}{125} = 0.008 \text{ s}^{-1}$. Decrease = $\frac{0.020-0.008}{0.020} \times 100 = 60 \%$.
4. V_{max} decreases; K_m stays the same. The inhibitor binds at a site away from the active site and changes the active site's shape, so it is not competing with the substrate: extra substrate cannot displace it, and the affected enzyme molecules stay inactive until the inhibitor leaves.
5. Without enzyme: $120 - 40 = 80$ units. With enzyme: $75 - 40 = 35$ units. Energy released: $40 - 10 = 30$ units in both cases; the enzyme does not change it.

Past questions to try

- 9700/23/M/J/25 Q4: determine K_m with its unit, then compare results at two pH values.
- 9700/22/M/J/25 Q6: sketch a non-competitive inhibitor's curve and estimate K_m from it.
- 9700/21/M/J/21 Q4: explain the induced-fit hypothesis, then the rate–substrate curve and K_m .
- 9700/22/O/N/21 Q6: immobilised amylase in a column.