# HL IB Biology

Your notes

## Enzymes & Metabolism

### Contents

# Metabolism: Enzymes & Reactions


Your notes

## Increasing Reaction Rates in Cells

### Enzymes as catalysts

- Most chemical reactions either **do not occur spontaneously** or **occur very slowly**
- In laboratory or industrial settings, some chemical reactions require some sort of catalyst in order to form a sufficient concentration of **product molecules**
  - Other conditions that may speed up the reaction rate include:
    - High temperatures or pressures
    - Extremes of pH
    - High concentrations of the reactants
- Cells are very sensitive to extreme temperatures, pressures and pH-levels, so the chemical reactions occurring in them cannot be sped up by these means
- **Enzymes** are proteins that **act as biological catalysts** in cells and allow chemical reactions to occur at a suitable rate in the conditions found in living organisms
  - They are **reusable**, so only a small number is needed to catalyse reactions
  - They **remain unchanged** by the reactions that they catalyse
- Without the presence of enzymes, the rate of chemical reactions in organisms would be **too low** to support life
- To form product molecules, the reactants would need to collide at the **correct angle and speed** in order for a reaction to occur
  - The chances of this occurring under normal conditions would be so low, that this would be an insignificant event
- Enzymes ensure that molecules (called substrate **molecules**) are orientated correctly and close enough for a reaction to occur
- The cell has control over the enzymes being produced, which in turn gives the cell **control** over the **chemical reactions** occurring in the cytoplasm

# Metabolism: Role of Enzymes


Your notes

## What is metabolism?

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

## Role of enzymes in metabolism

- Enzymes are **globular proteins**
- Critical to the enzyme's function is the **active site** where the substrate binds
- Enzymes are **specific** to the substrate
  - The shapes of the enzyme and substrate and their **chemical properties** are **complementary**, to allow the substrate to fit into the active site, like two jigsaw pieces fitting together
  - This is called **enzyme-substrate specificity**
- Due to this specificity, thousands of enzymes are needed throughout an organism, to carry out **individual chemical reactions**
- This means that **control over metabolism** can be exerted through these enzymes

### Examiner Tip

Avoid the common mistake in an exam to say that the shapes of the enzyme active site and substrate molecule are the same, they are not. Complementary means that they fit together because of the specific differences in their shapes.

# Anabolism & Catabolism

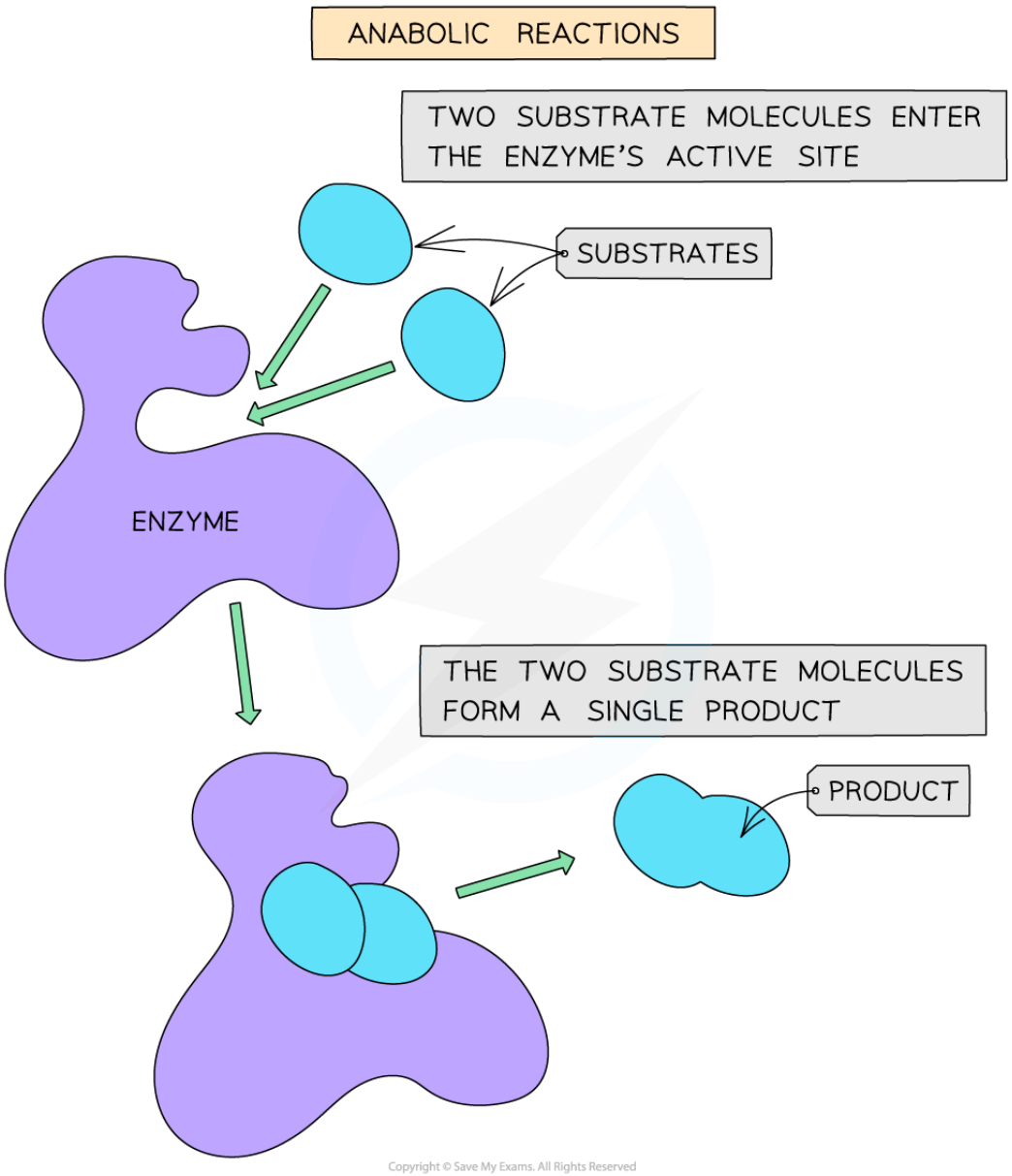
## Anabolic reactions

Your notes

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

**Enzyme-catalysed anabolic reactions diagram**

Your notes




***Anabolic reactions involve the linking of more than one substrate molecule to form a more complex product molecule***

## Catabolic reactions

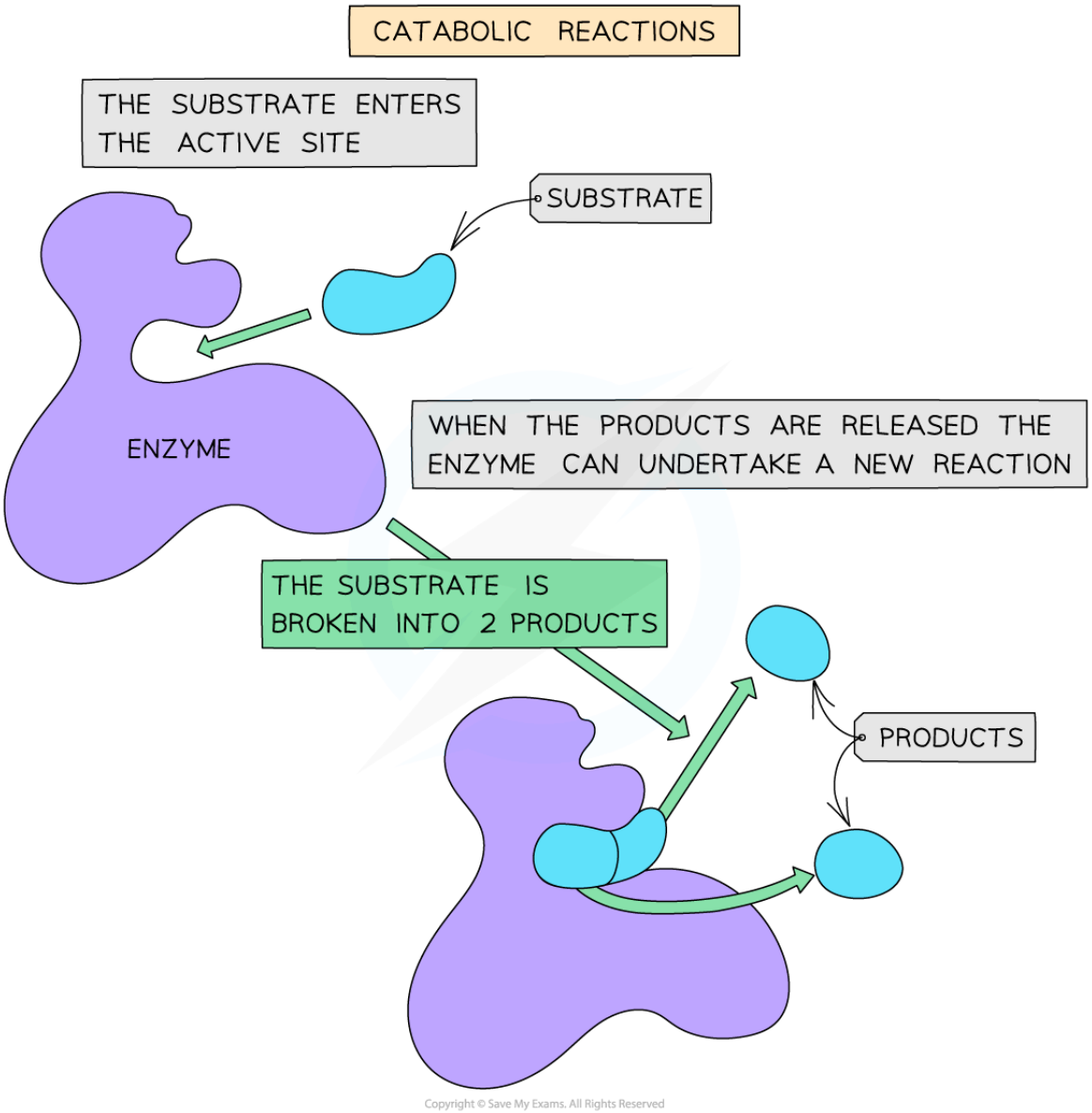
- Catabolic reactions are involved with **breaking down large molecules** into smaller, simpler ones
- These reactions are often carried out to **release energy** for cellular processes and for the **excretion** of waste
- Examples include:
  - **Respiration**, where $CO_2$ and water are produced from the oxidation of sugars

- **Deamination** of proteins to release urea
- **Breakdown of macromolecules** into monomers during digestion

- Catabolic reactions often include hydrolysis reactions
- Catabolic reactions are **exergonic** (free energy is released for cellular processes or as excess heat)

Your notes

**Enzyme-catalysed catabolic reactions diagram**





*Catabolic reactions happens when a single substrate molecule is drawn into the active site and broken apart into two or more product molecules*

**Comparison of anabolism and catabolism table**

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


Your notes

# Enzyme Action

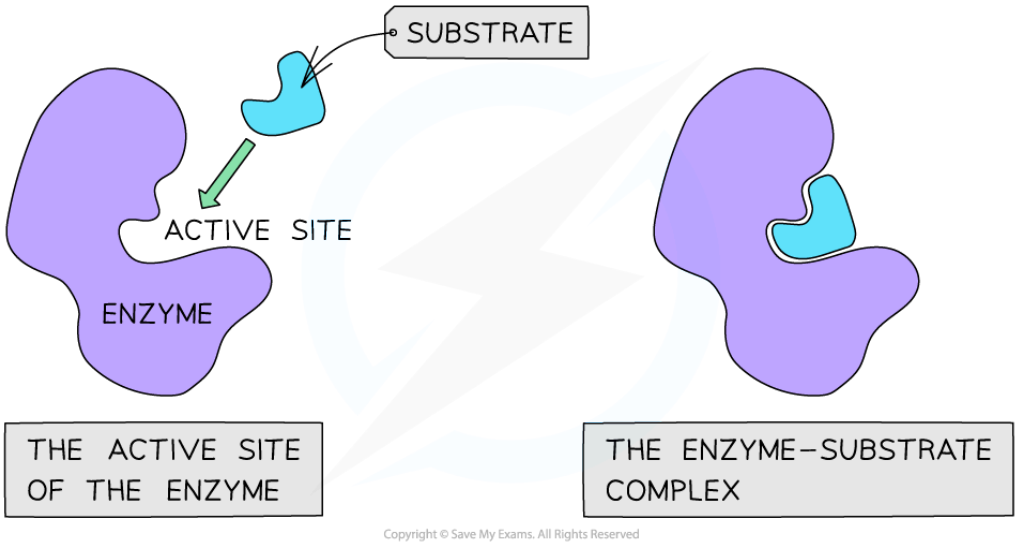
Your notes

## Structure of Enzymes

### The structure of enzymes

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

**Enzyme action diagram**



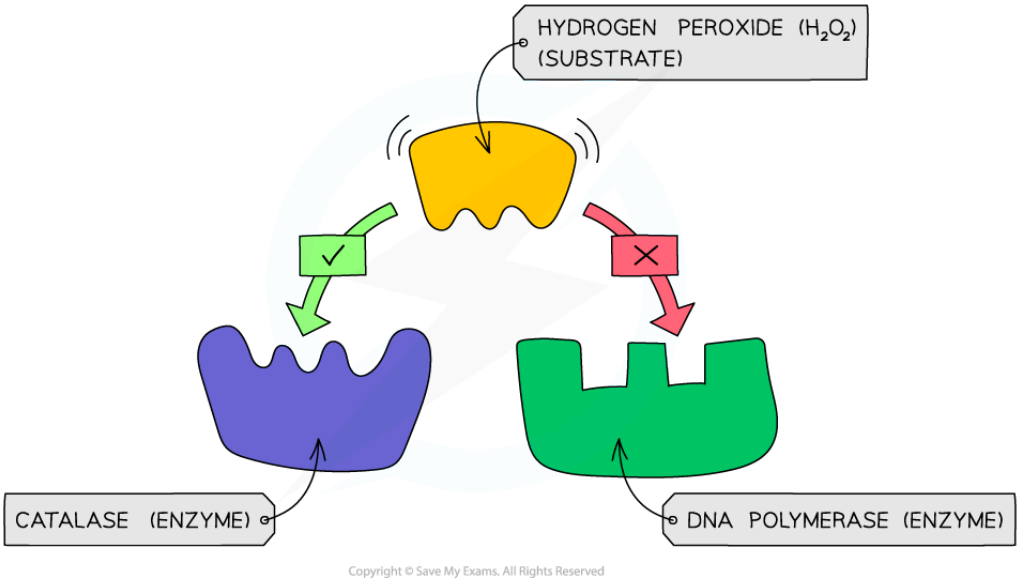


***The active site of an enzyme has a specific shape to fit a specific substrate (when the substrate binds an enzyme-substrate complex is formed)***

Your notes

- The **specificity** of an enzyme is a result of the **complementary nature** between the shape of the active site on the enzyme and its substrate(s)
- The **shape** of the active site (and therefore the specificity of the enzyme) is determined by the **complex 3D shape** of the protein that makes up the enzyme
  - The active site is made of only a few amino acids but the **interaction of these amino acids** within the 3D shape of the enzyme ensures that **catalysis** can occur
  - This is achieved by:
    - Binding to the **substrate molecule**
    - **Holding it in position** for a chemical reaction to occur
    - **Lowering the energy needed** for the reaction to occur

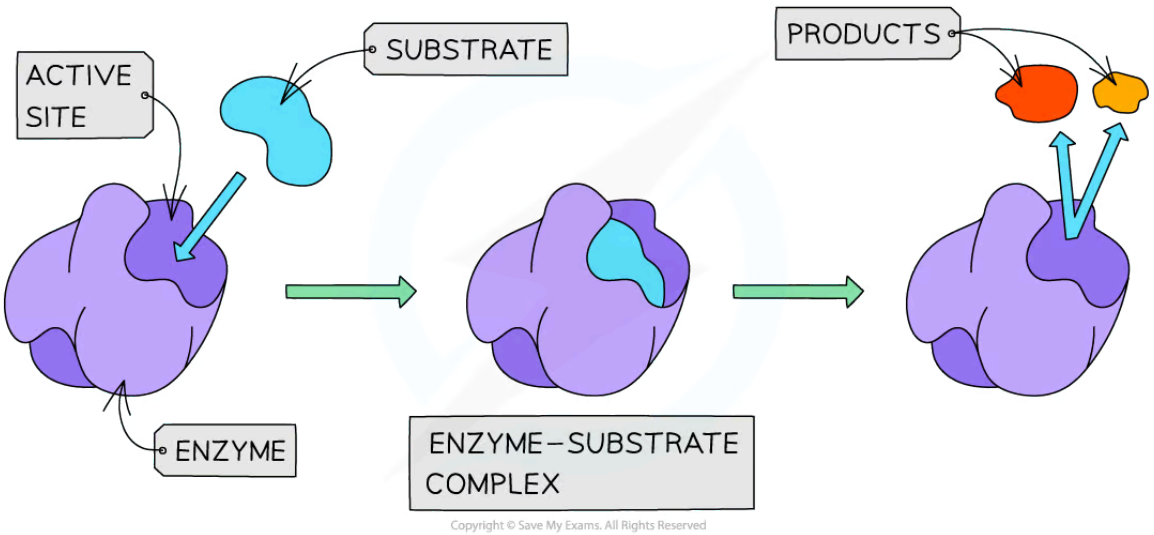
### Enzyme specificity diagram





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

### Formation of enzyme-substrate complex diagram




*The temporary formation of an enzyme-substrate complex*

## Examiner Tip

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

Your notes

# Induced-fit Binding

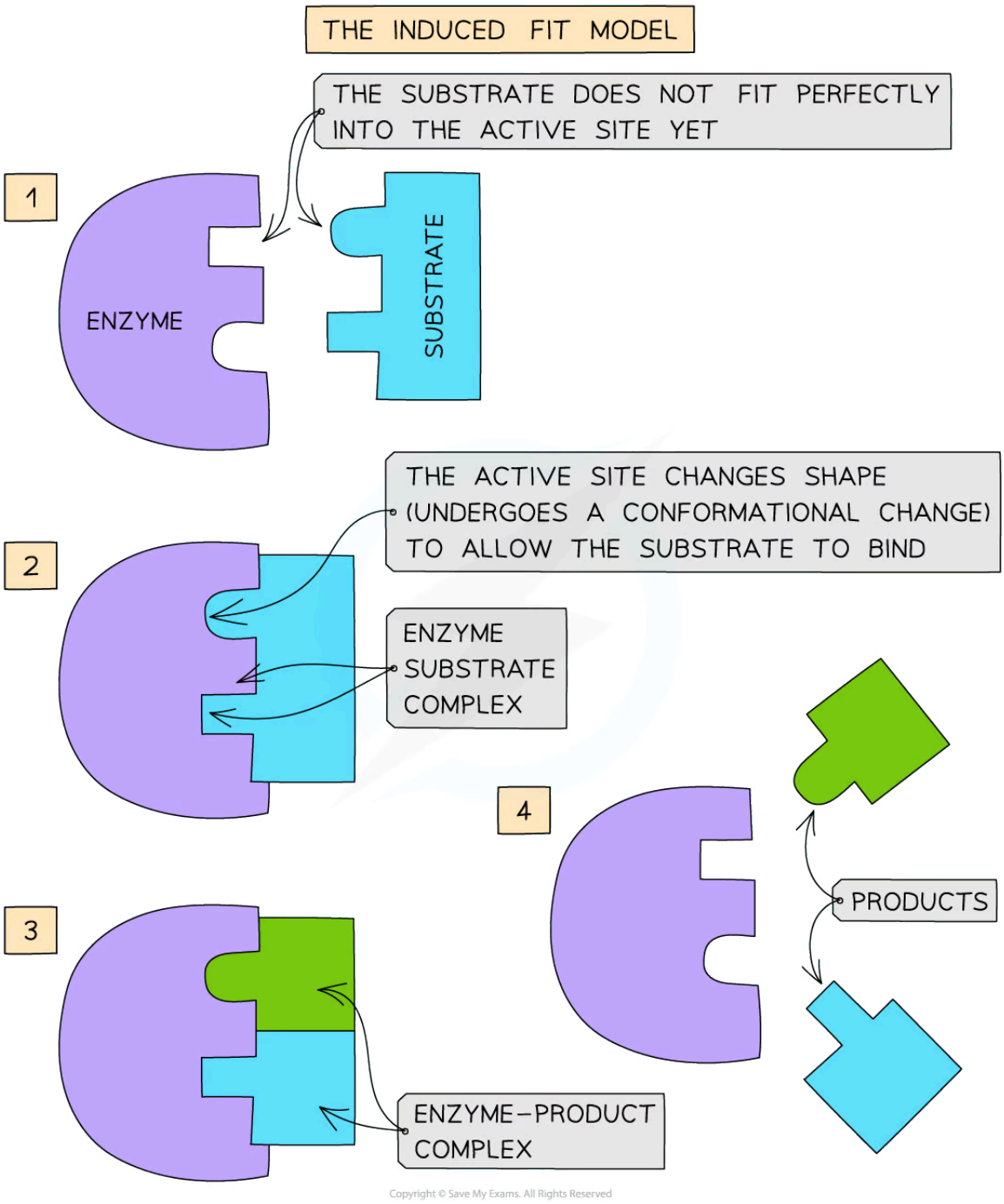
## The induced-fit hypothesis

Your notes

- The original model explaining interactions between enzymes and their substrate molecules was called the **lock-and-key model**
  - This model proposed that the enzyme active site is **precisely complementary** to the shape of the substrate molecule
  - The substrate molecule therefore fits into the active site like a key in a lock
- The **modified model** of enzyme activity 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**
  - This ensures an **ideal binding arrangement** between the enzyme and substrate is achieved
  - This **maximises the ability of the enzyme to catalyse the reaction**

### Induced-fit model diagram

Your notes




*The induced-fit hypothesis of enzyme action*

### Examiner Tip

Don't forget – our current understanding of enzyme-substrate interactions is based on the induced-fit hypothesis.

Your notes

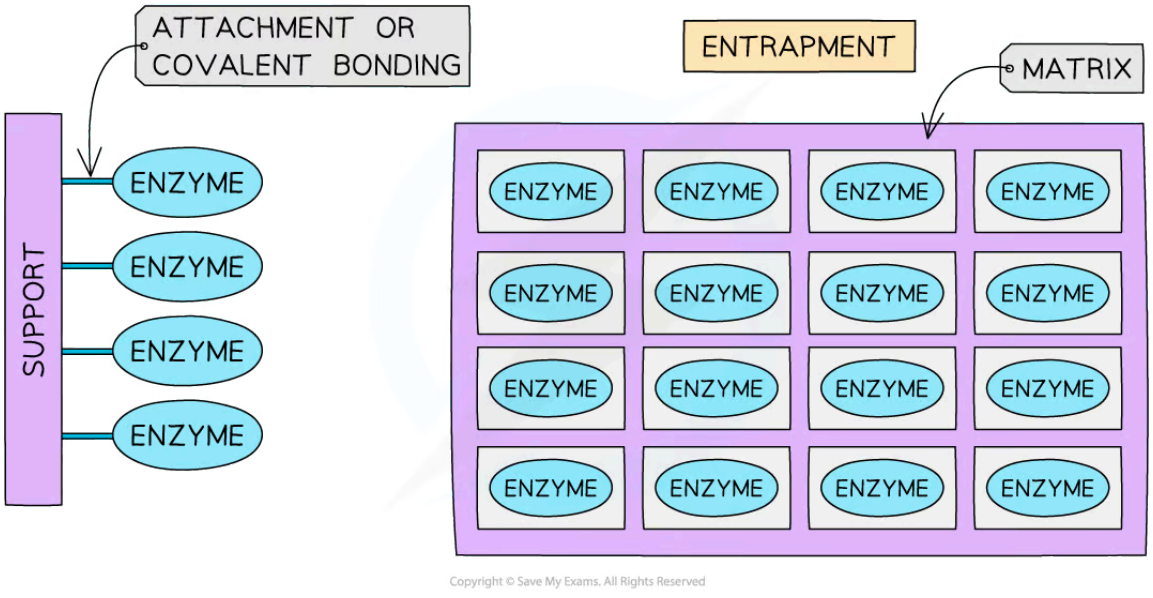
# Enzyme Catalysis

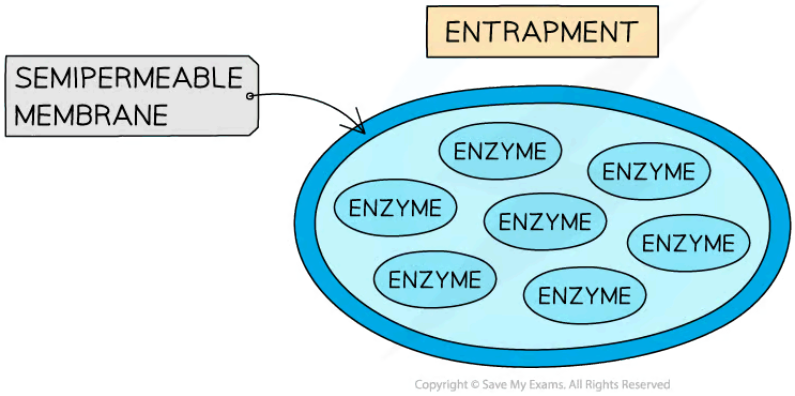
## The role of molecular motion and substrate-active site collisions

Your notes

- In order for the substrate molecule to collide with and ultimately bind to the enzyme active site, **movement is required**
  - This movement is the result of the **kinetic energy** that molecules have
  - The greater the kinetic energy of the molecules, the **faster the movement** and the higher the probability of enzyme and substrate colliding
  - This leads to **more enzyme-substrate complexes** forming and the production of more product molecules
- In some cases, **large substrate molecules** are immobilised, while in other cases it is possible to **immobilise enzymes** by embedding them in membranes
- These immobilised enzymes can be used in a **range of industries** such as food processing, environmental management, pharmaceuticals and manufacturing processes
- There are different methods by which enzymes can be immobilised including:
  - Attachment to an **inert substance** e.g. glass
  - Entrapment within a **matrix** e.g. alginate gel
  - Entrapment within a **partially permeable membrane**

**Examples of immobilised enzymes diagram**






*There are many different ways in which enzymes can be immobilised*

## Advantages of immobilised enzymes

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

Your notes

## Denaturation: Enzymes


Your notes

- Enzymes can be denatured when it is exposed to **high temperatures** or **extremes of pH**
- **Bonds** (e.g. hydrogen bonds) holding the enzyme molecule in its precise 3D shape start to **break**
  - Take note that the peptide bonds holding the amino acids together are not broken
- This causes the **3-dimensional shape** of the protein (i.e. the enzyme) to **change**
- This permanently **changes the shape** of the **active site**, preventing the **substrate** from **binding**
- **Denaturation** has occurred if the **substrate can no longer bind**
- The reaction that was previously catalysed **now no longer takes place**
- Denaturation often causes the enzyme to **become insoluble** and form a **precipitate**
- Very few human enzymes can function at temperatures **above 50°C**
  - This is because humans maintain a body temperature of about 37°C, therefore even temperatures exceeding 40°C will cause the denaturation of enzymes
  - High temperatures cause increased vibrations in the bonds **between the R-groups of amino acids** so they start to break, changing the conformation of the enzyme

### Examiner Tip

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

# Enzyme Activity: Skills

Your notes

## Effects of Temperature, pH & Substrate Concentration

### NOS: Describing patterns and trends in graphs

- You are required to **describe the relationship** between variables shown in graphs
- **Generalised sketches** of these relationships are **examples of models** in Biology
- Enzyme experiments can be conducted to **investigate the effects** of the following factors on the **rate of enzyme activity**:
  - Temperature
  - pH
  - Substrate concentration
- Sketch graphs can be drawn and evaluated using the results from these experiments

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

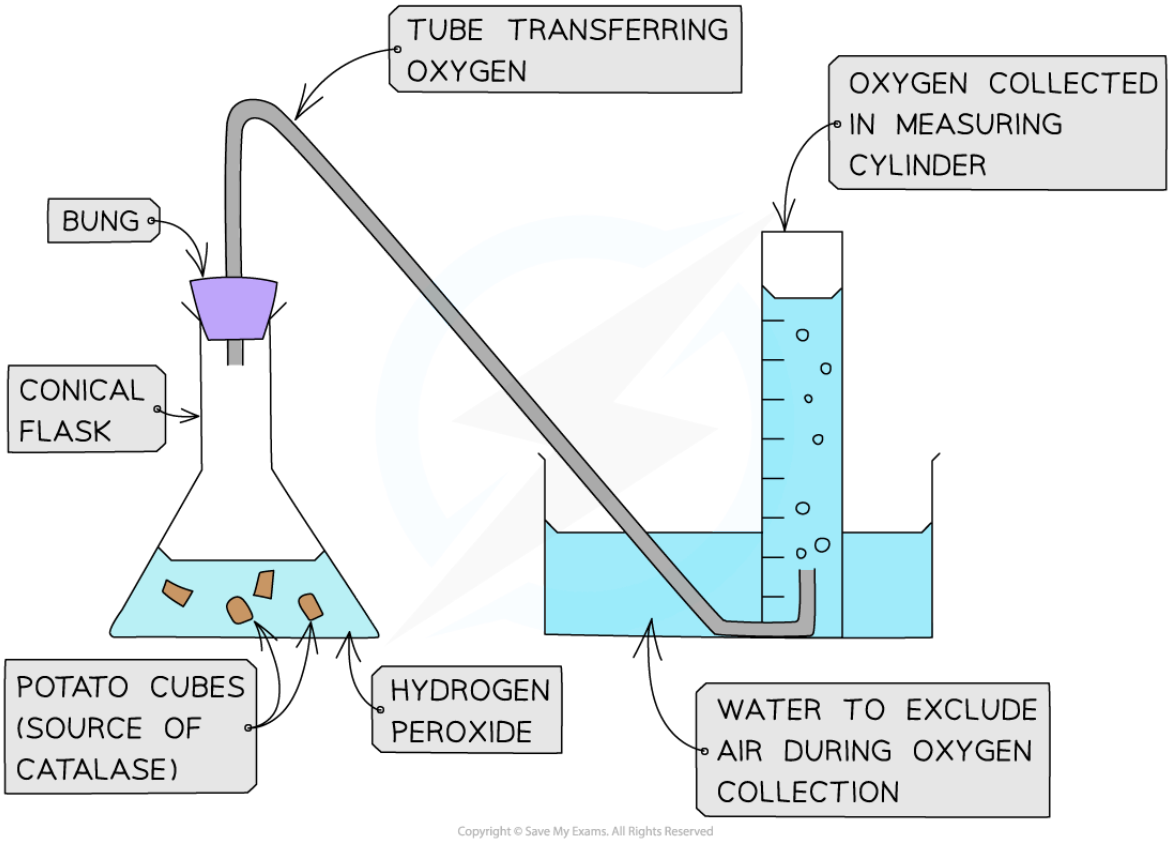
- Three different **independent variables** can be tested
  - Temperature
  - pH
  - Substrate concentration
- You should plan how the **dependent variable is going to be measured**
  - With appropriate units
- Also, what **intervals of the independent variable** are going to be chosen
- These factors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

### Investigating the effects of temperature or pH on catalase activity

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

**Investigating catalase diagram**

Your notes




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

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

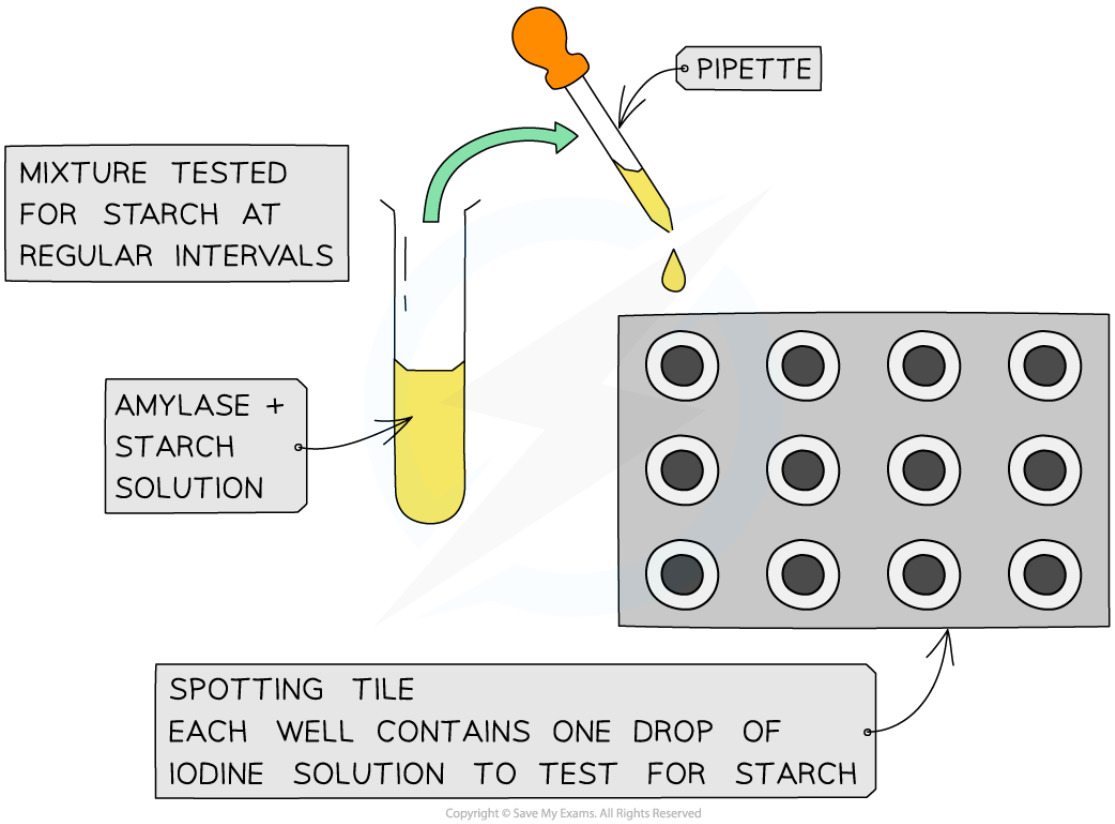
## Investigating the effect of substrate concentration on amylase activity

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

- In this way, the time taken for starch to be broken down can be measured
- The investigation can be **repeated under different starch concentrations** and the **reaction rates can then be compared**
  - This experiment also can be adapted to measure the effects of altering pH, temperature or enzyme concentration

Your notes

**Investigating amylase diagram**




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

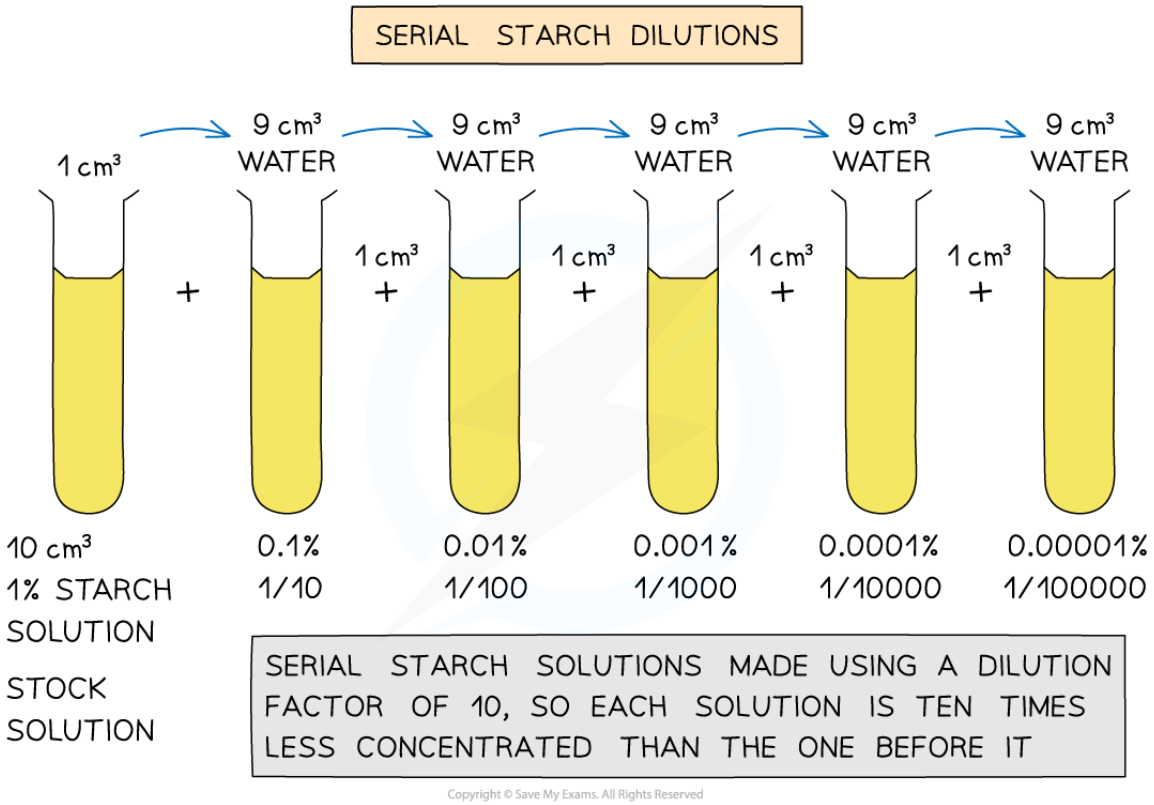
## Investigating the effect of starch concentration on amylase using colorimetry

- A **colorimeter** is able to measure **light absorbance** (how much light is absorbed) or **light transmission** (how much light passes through) a substance
- Colorimetry can be used in any enzyme-catalysed reaction that involves a **colour change**
- As the colour breaks down the **transmission increases** or **light absorption decreases** and this can be used to **measure the rate of the reaction**
- For example, a colorimeter can be used to follow the progress of a **starch–amylase catalysed reaction** as the amylase breaks the starch down into maltose
- This can be carried out as follows:

Your notes

- **Colorimeter calibration**: this is an important step in a colorimetric investigation and in this case, a weak iodine solution can be used to calibrate the colorimeter as the endpoint (or 100% transmission)
- Preparation of a starch solution of **known concentration** (stock solution), from which a range of concentrations are made using **serial dilutions** (method outlined in diagram below)
- Following calibration and switching on the red filter (to maximise the percentage transmission or absorbance), the colorimeter is used to **measure the percentage absorbance or percentage transmission values**
- Sometimes a reagent or indicator is used to produce the colours detected by the colorimeter and sometimes the solutions themselves absorb light waves
- A **calibration graph** is then plotted of starch concentration (*x*-axis) vs percentage absorbance or percentage transmission (*y*-axis)

**Serial starch dilutions diagram**





***Serial dilution of starch to make a range of concentrations***

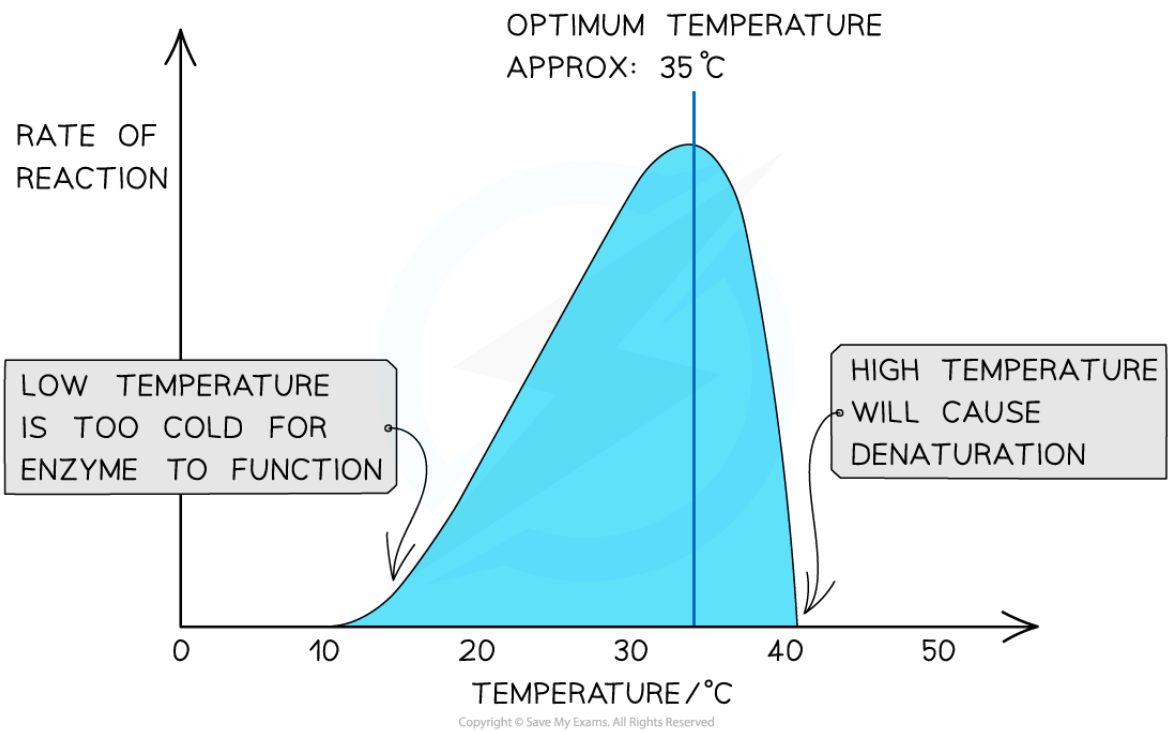
## Interpreting graphs on the effects of temperature, pH and substrate concentration on the rate of enzyme activity

- Temperature, pH and substrate concentration affect the rate of activity of enzymes

Your notes

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

**The effect of temperature on enzyme action diagram**



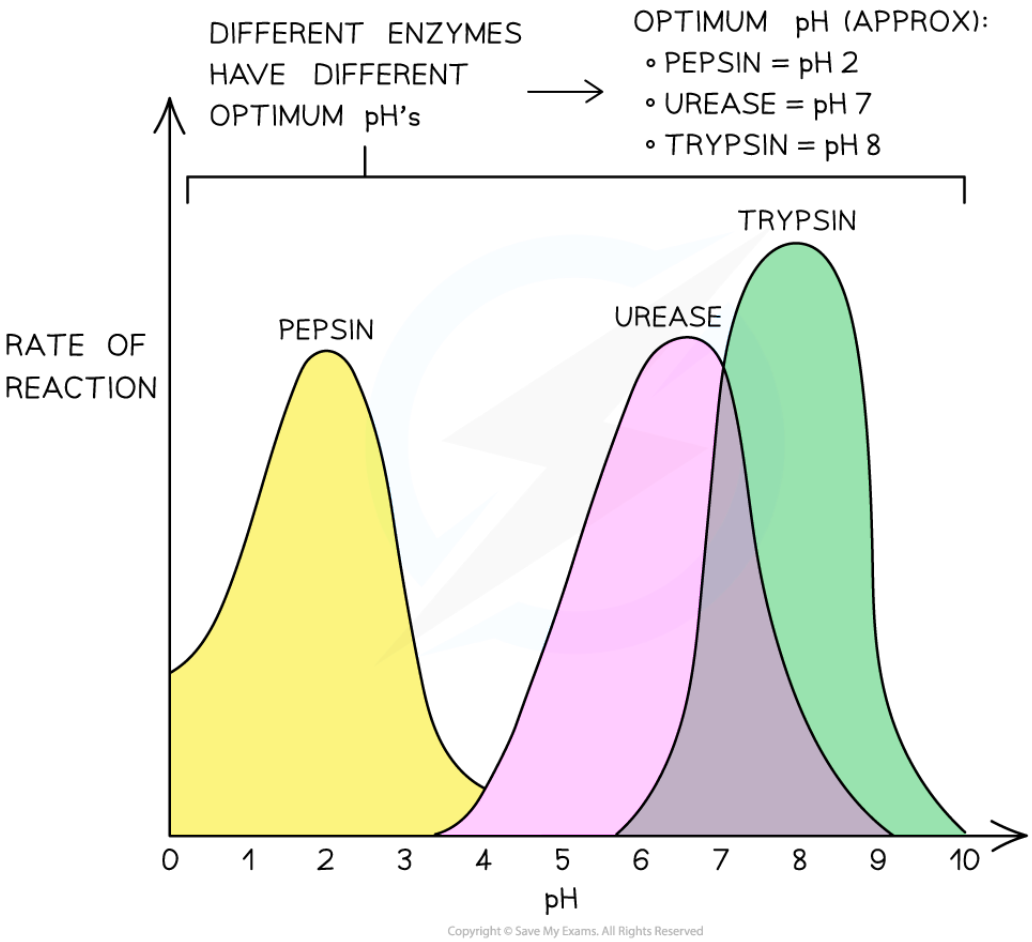

*The effect of temperature on the rate of an enzyme-catalysed reaction*

## Changes in pH

Your notes

- pH is a result of the **hydrogen ion concentration** in a solution
- A **low pH** is **acid** and has a **high** hydrogen ion concentration
- A **high pH** is **alkaline** and has a **low** hydrogen ion concentration
- A **10× increase** in hydrogen ion concentration lowers the pH by **1 unit**
  - pH is therefore measured on a **logarithmic scale** of hydrogen ion concentration, **not a linear scale**
- Water has a pH of 7, regarded as **neutral**
- **Extremes of pH** can also alter hydrogen bonding within an enzyme's structure and cause irreversible **denaturation**
- Each enzyme has an **optimum pH**
- Not all enzymes have an optimum pH near to neutral. For example
  - The **stomach enzyme** pepsin is adapted to work best at **pH 2**
  - Certain bacterial enzymes work at **pH 9–10**, in line with the pH of the bacteria's main habitat

**The effect of pH on enzyme action diagram**



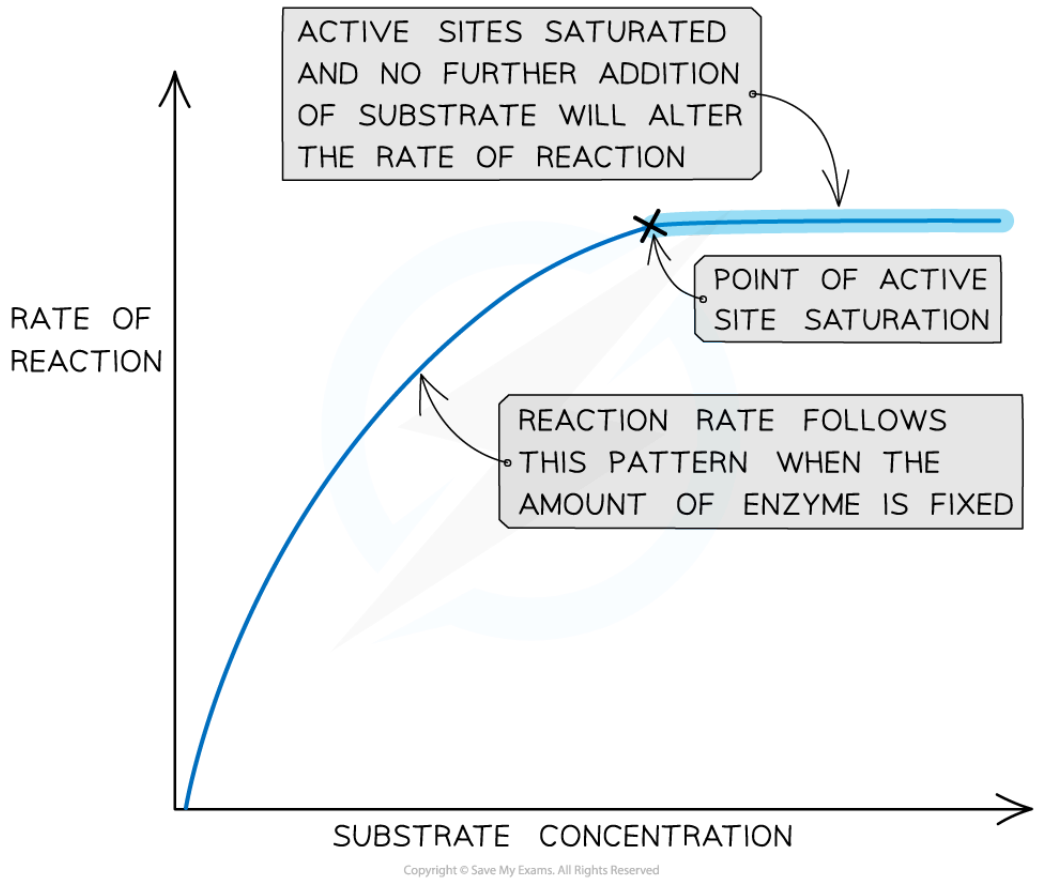
*The effect of pH on three enzymes' rates of reaction*

Your notes

## Changes in substrate concentration

- The more substrate molecules are present in a solution, this **increases the frequency of collisions** with the enzyme's active site
- Active sites are **occupied** or 'blocked' by substrates whilst the reaction is taking place
- The more active sites are occupied, **the fewer are available to catalyse other substrate** molecules
- As substrate concentration rises, the slower the rise in the rate of the enzyme-catalysed reaction
- The active sites have become **saturated**
- At the **point of active site saturation**, increasing the substrate concentration will cause **no further increase** in the rate of reaction
- At the point of active site saturation, a method of increasing the rate of reaction would be to make more active sites available by **increasing the enzyme concentration**

**The effect of substrate concentration on enzyme action diagram**





*The effect of substrate concentration on enzyme activity*

## Examiner Tip

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

Your notes

# Enzyme Reaction Rates: Skills

Your notes

## Determining Enzyme Reaction Rates

- Enzyme catalysed reactions can be affected by changes in **pH, temperature or substrate concentration**
- The **rate of reaction** can be determined by measuring the **rate of disappearance of a substrate** or the **rate of product accumulated** in a **given time period**
- This may be shown as a change in quantity (usually volume or mass) of substrate or product over a measured time period:

$$\text{RATE OF A REACTION} = \frac{\text{CHANGE IN AMOUNT OF REACTANTS OR PRODUCTS (mol dm}^{-3}\text{)}}{\text{TIME (s)}}$$



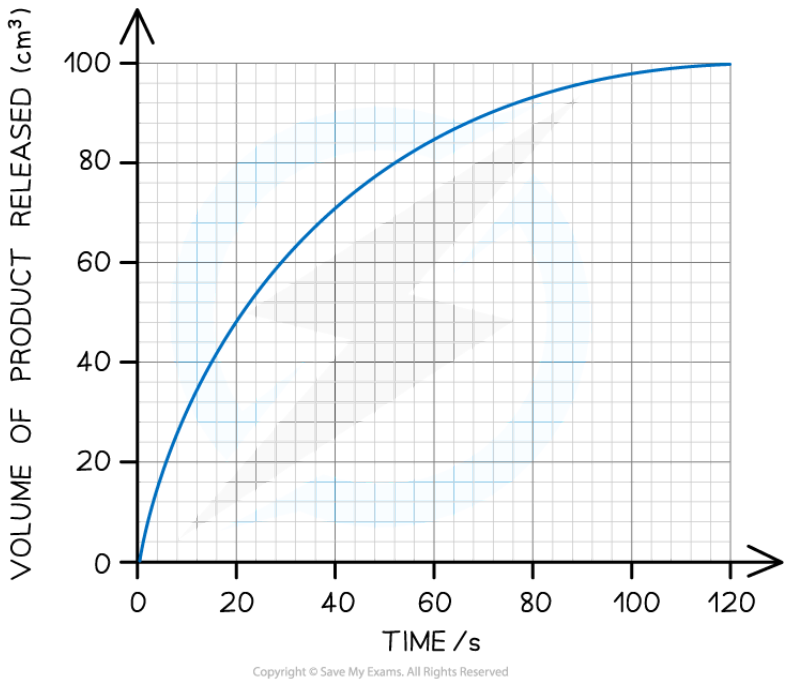
- **Or,** if we cannot collect quantitative data on the amount of substrate or product, we can calculate the rate of reaction **based on the time measured** using the following equation:

$$\text{RATE OF REACTION} = \frac{1}{\text{TIME TAKEN (s}^{-1}\text{)}}$$



  - **1 ÷ time taken (seconds)** and should include the units $s^{-1}$
- A **high** rate of reaction is when the reaction happens in **less time** i.e. it is faster
- A **low** rate of reaction is when the reaction happens in **more time** i.e. it is slower
- The rate of a reaction is likely to change throughout a reaction as the **substrate concentration will decrease** as the reaction proceeds
  - This leads to a graph that starts out as a **directly proportional** straight line (the value on the X increases at the same rate as the value on the Y) but then **plateaus as the reaction slows down**
- The **steeper the line the faster the rate of reaction**
- The rate of reaction can be calculated from a graph plotted where the reaction **time** is shown on the X-axis and the **quantity of product or substrate** is shown on the Y-axis

**Volume of a product produced against time graph**





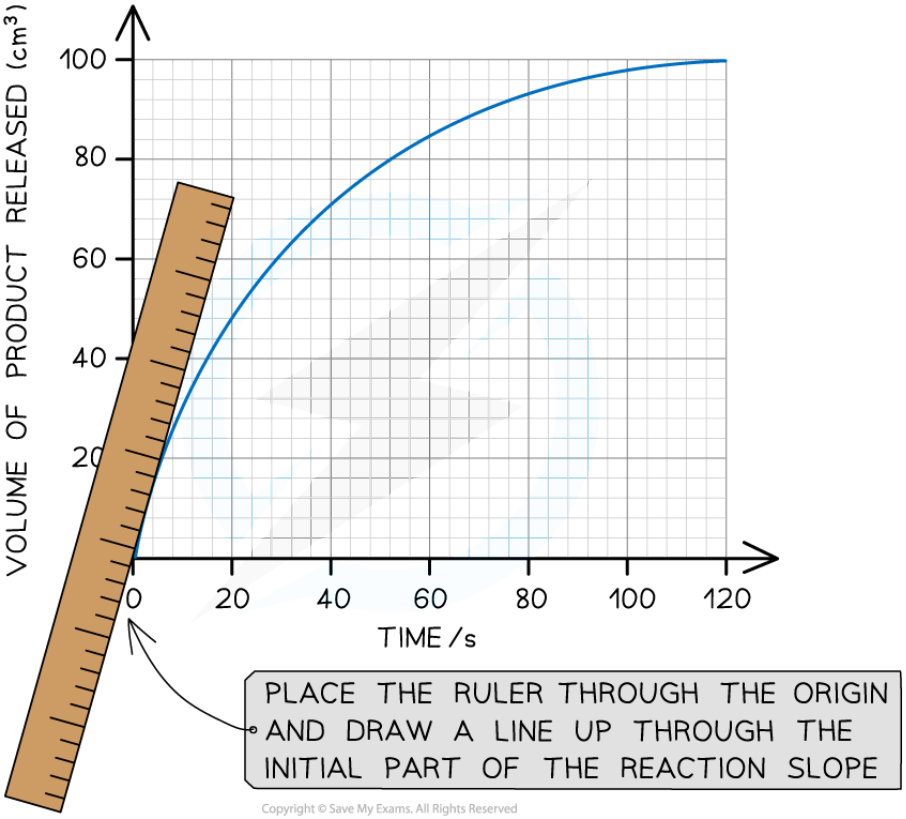
***Graph produced when plotting the volume of a product produced against time***

- The gradient is calculated from a point on the graph and used as a measure of the rate of reaction at that point in time
- A **tangent** must be drawn to calculate the change in x and y so the rate of reaction can be calculated
  - E.g. if calculating the initial rate of reaction
    - Place a ruler on the point of **origin** and draw a line that corresponds to the curve during the early part of the reaction
    - **Extend the line** as far as is convenient to perform the calculations e.g. to 60 seconds

**Drawing a tangent to calculate initial rate of reaction diagram**

Your notes

Your notes





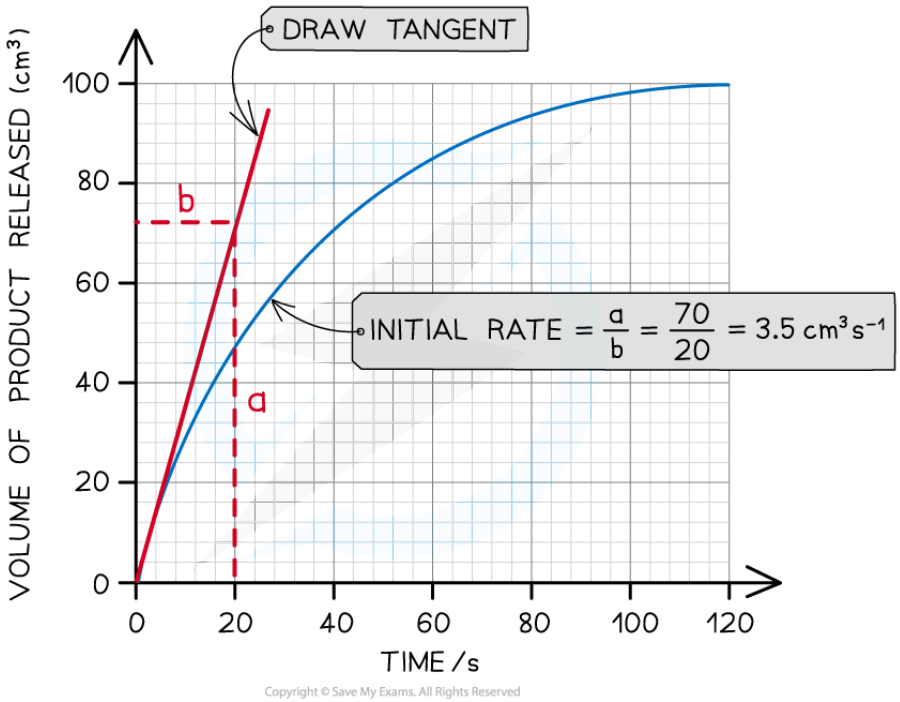
***Drawing a tangent against the line through the origin to calculate the initial rate of reaction***

## Calculating the rate of reaction

- Once the tangent is drawn you can calculate the **gradient** of the line which is equal to the rate of the reaction
  - Initial rate = a ÷ b
  - Where
    - a = change in volume and
    - b = change in time
  - The units will be **$cm^3$ $sec^{-1}$** (this means volume per sec)

Your notes

### Calculating the rate of reaction from the tangent diagram





***Rate of reaction is calculated by finding the gradient of the tangent from the origin***

# Activation Energy: Skills

Your notes

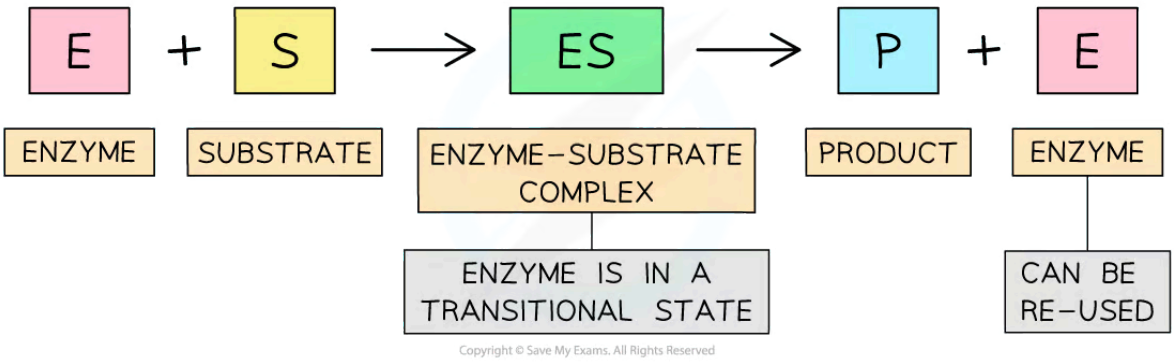
## Activation Energy

- **Metabolic pathways** are controlled by **enzymes** in a biochemical cascade of reactions
  - Virtually every metabolic reaction within living organisms is catalysed by an enzyme
  - Enzymes are therefore essential for life to exist
- 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

### The Enzyme-Substrate Complex

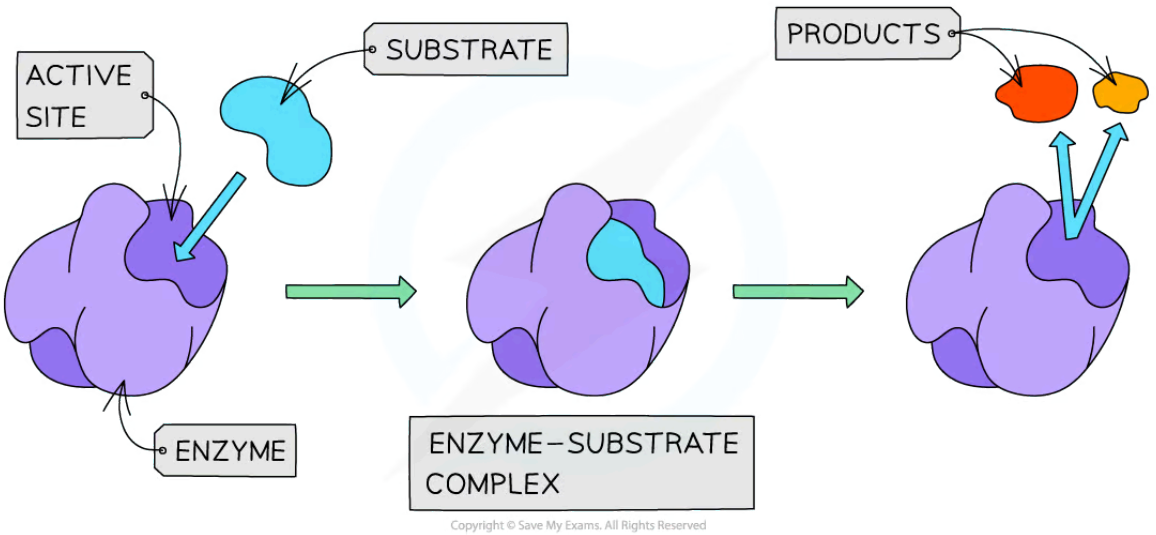
- The starting point of a metabolic pathway is a **substrate** which is converted to an end product
- The enzyme works by binding to the substrate at a special site on the enzyme called the **active site**
  - The active site of an enzyme has a specific shape to fit a specific substrate
- Substrates **collide** with the enzyme's active site and this must happen at the **correct orientation** and speed in order for a reaction to occur
- An **enzyme-substrate complex** is formed, temporarily, when the substrate binds to the active site
  - The substrate is said to be in a **transitional state** at this moment
- The product is formed and enzyme is released to take part in another reaction
- The reaction can be shortened to a simple equation

**Process of enzyme-catalysed reactions diagram**




***The simple equation can show how an enzyme reaction proceeds***

**Formation of enzyme-substrate complex diagram**




***The formation of the enzyme-substrate complex where the substrate is said to be in a transitional state, before forming the product(s)***

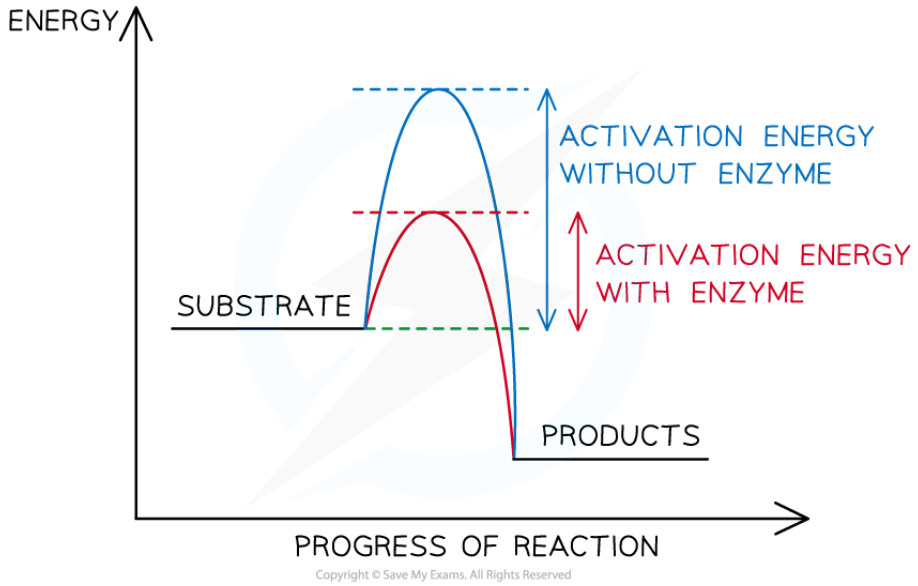
## Enzymes and the lowering of activation energy

- All chemical reactions, including metabolic pathways, are associated with **energy changes**
- Energy may either be released or absorbed during a reaction
  - If energy is released to the surroundings it is an **exergonic** reaction
  - If energy is absorbed from the surroundings it is an **endergonic** reaction
- For a reaction to proceed there must be enough **activation energy**
- Activation energy is the amount of **energy** needed by the substrate to become **unstable** enough for a reaction to occur and for **new products** to be formed
- Enzymes **speed up** chemical reactions because they reduce the **stability of bonds** in the substrate
- Enzymes **lower the activation energy** needed to catalyse a reaction
  - The energy released is unchanged but the activation energy required is lowered
  - The rate of reaction is therefore quicker

### The effect of enzyme action on activation energy diagram

Your notes

Your notes




***The graph shows how an enzyme lowers the activation energy required for a reaction***

## Examiner Tip

**Endergonic** and **exergonic** reactions are defined by the net the intake or output of energy (respectively) this differs from endo**thermic** and exo**thermic** reactions which are defined by the intake or output of **thermal energy** only.

# Metabolic Pathways (HL)


Your notes

## Enzyme Catalysed Reactions

### Intracellular and extracellular enzyme-catalysed reactions

- Enzymes can be **intracellular** or **extracellular** based on whether they are active inside or outside the cell respectively
- **Extracellular enzymes** are produced inside the cell and then packaged into vesicles before being **secreted** by the cell
  - These enzymes will **catalyse reactions outside** the cell
  - Examples of such enzymes are those involved in **chemical digestion** in the gut
- Most enzymes however are **intracellular**, meaning that they are **produced and function within** the cell
  - **Glycolysis** and the **Krebs cycle** are two important processes of respiration that are catalysed by intracellular enzymes

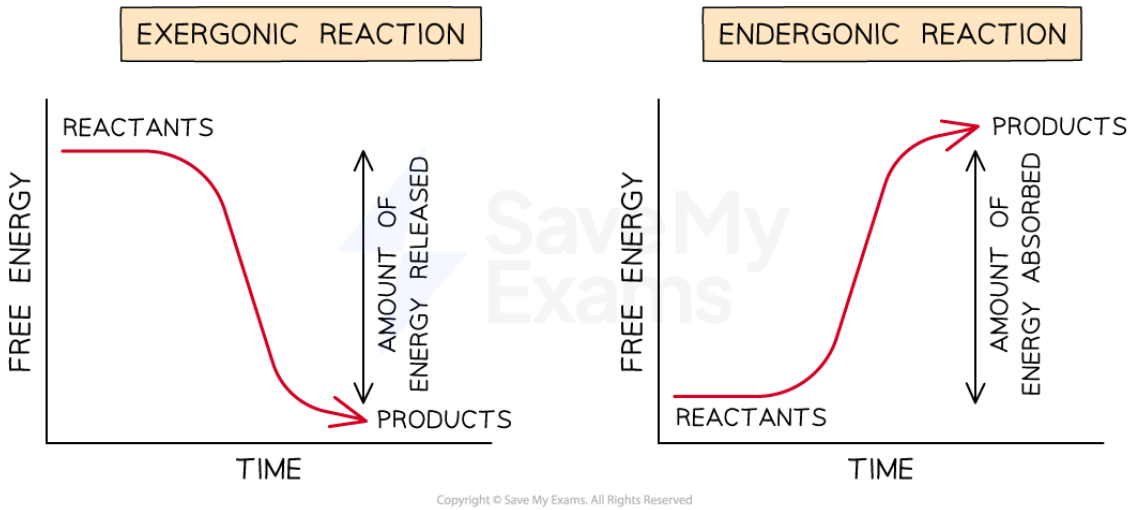
# Generation of Heat Energy

## Exergonic and endergonic reactions

Your notes

- During aerobic respiration, glucose is oxidised to carbon dioxide and water and in the process some of the **chemical potential energy** stored in the bonds of glucose is released
  - This called **free energy** and can be used to perform different functions
- Reactions such as these, that **release free energy**, are known as **exergonic reactions**
- Many metabolic reactions are exergonic, and some of the energy is released as **heat**
  - This is because the **energy transfer** in these reactions are **not 100% efficient**
- Organisms such as **birds and mammals** rely on the heat released by metabolic reactions to **regulate** their body temperature
  - They are called **endotherms** (or 'warm-blooded') and their body temperature remains **constant**
  - Those that are **unable to regulate** their body temperature this way are known as **ectotherms** (or 'cold-blooded' organisms)
- Reactions where energy is **absorbed** are called **endergonic reactions**
  - The products formed by these reactions will have more stored energy than the reactants
  - An example of this is the **synthesis of proteins** from amino acids
- Since endergonic reactions require an energy input, they are often **linked** to exergonic reactions in metabolism
- **Adenosine triphosphate** (ATP) acts as the intermediate that links the energy-yielding reactions to the energy-absorbing ones
- ATP therefore plays a very important role in metabolic processes in living organisms

### Exergonic and endergonic energy level diagrams





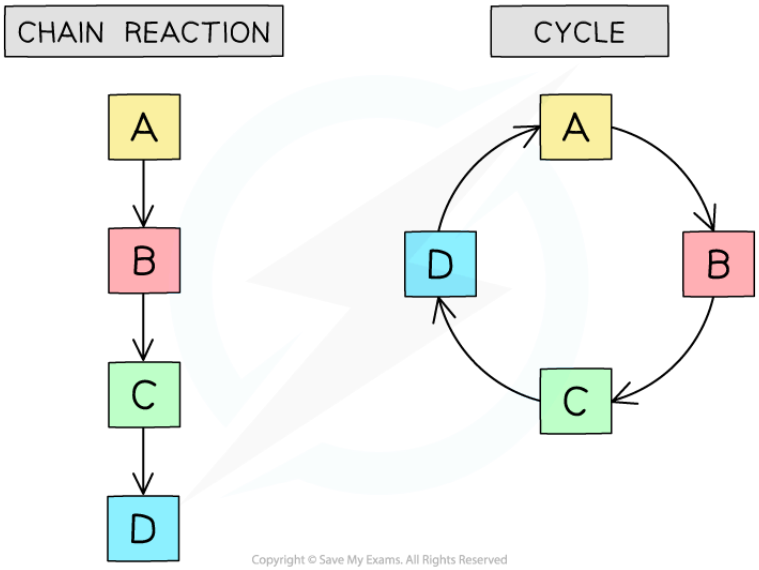

***Exergonic reactions release energy when products are formed, while endergonic reactions require the absorption of energy to form product molecules***

## Cyclic & Linear Metabolic Pathways

Your notes

- Metabolic pathways involve a **series of small steps**, each step involves a **chemical change**
- The **enzyme-catalysed** reactions that make up metabolic pathways usually consist of **linear** (chain) or **cyclical** reactions:
  - Linear (or chain) reactions are a linear sequence with a distinct beginning and end
    - **Glycolysis**, part of respiration, is an example of a linear metabolic pathway
  - Cycles involve the end product starting the next cycle, these are less common than chain reactions
    - **The Calvin cycle**, part of photosynthesis, is an example of a cyclical metabolic pathway
    - **The Krebs cycle**, part of aerobic respiration, is another example of a cyclical metabolic pathway

**Examples of types of metabolic pathways diagram**





***A chain metabolic pathway has a distinct start and finish, whereas in a cycle the end product feeds back into the starting reactant***

- Chemicals involved in metabolic pathways are called **metabolites** or **intermediates**
  - Some form new molecules within cells
  - Others breakdown molecules and involve an energy transfer

# Enzyme Inhibition (HL)

Your notes

## Competitive Inhibition

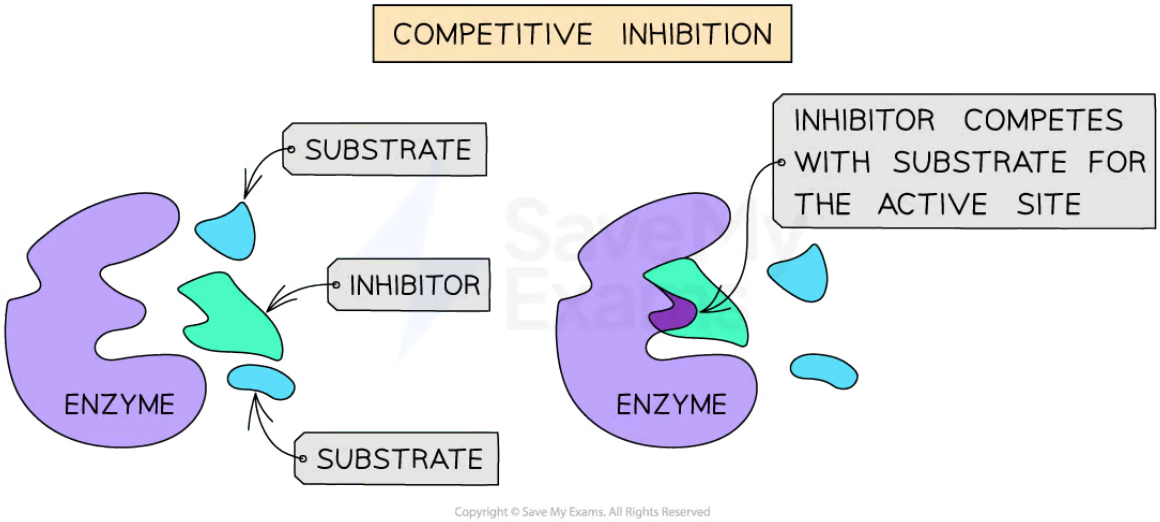
### Competitive inhibitors

- Competitive inhibitors have a **similar shape** to that of the substrate molecules
- They **bind to the active site** of the enzyme, interfering with it and **competing** with the substrate for the active site
- The substrate, therefore, cannot bind to the active site if a competitive inhibitor is already bound

### Statins as an example of competitive inhibition

- **Statins** are drugs that are prescribed to lower the cholesterol levels of patients with high cholesterol levels in their blood
  - They bind to the **active site** of the enzyme needed to **synthesise cholesterol**
- Binding to the active site is possible because statins have a shape that is **similar to the substrate** of this enzyme
  - It therefore **blocks access** to the active site and the substrate is **unable to bind**
- The enzyme **cannot catalyse** the reaction that synthesises cholesterol, leading to cholesterol levels **decreasing** in the blood
  - Note that the cholesterol that is being referred to here is called **low-density lipoproteins** (LDLs), more commonly known as "bad cholesterol"
  - High LDL levels have been linked to **atherosclerosis** and may increase the risk of developing **coronary heart disease**

**Competitive inhibition diagram**





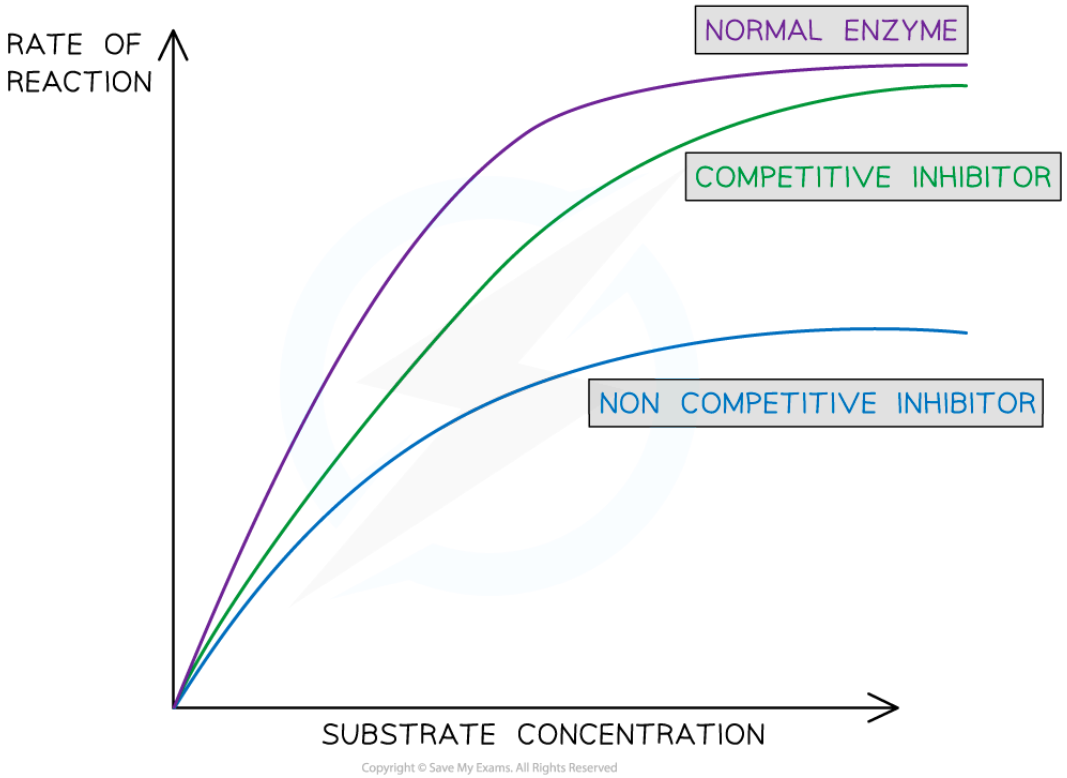
*Competitive inhibition is a consequence of the reversible binding of an inhibitor to the active site*

## Identifying types of inhibition

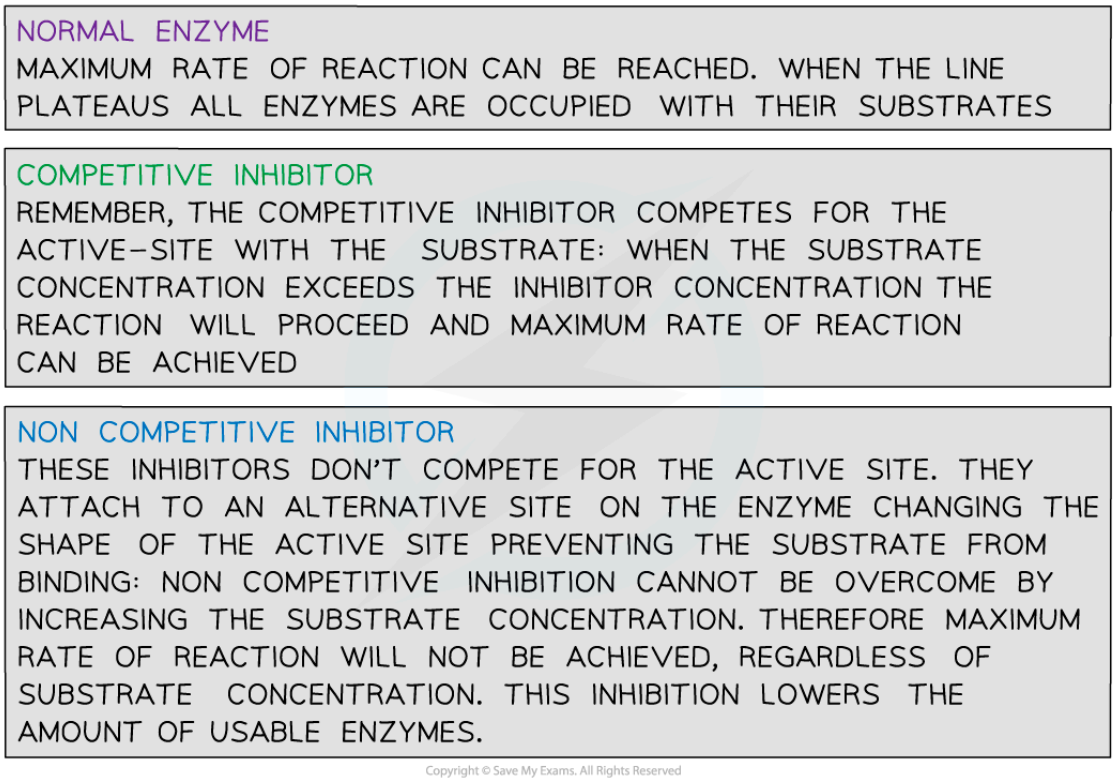
Your notes

- The effect of competitive and non-competitive inhibitors on enzyme controlled reactions can be represented graphically
- Both types of inhibitors **slow down** or **stop** enzyme activity, decreasing the rate of reaction
- **Increasing** the **concentration** of an inhibitor **reduces** the rate of reaction and eventually, if inhibitor concentration continues to be increased, the reaction will **stop completely**
  - For **competitive inhibitors** countering the increase in inhibitor concentration, by increasing the substrate concentration, **can increase** the rate of reaction but the substrate needs to reach a high enough concentration in order to displace the inhibitor (more substrate molecules mean they are more likely to collide with enzymes and form enzyme-substrate complexes)
  - For **non-competitive inhibitors** increasing the substrate concentration **cannot increase** the rate of reaction, as the shape of the active site of the enzyme remains changed and enzyme-substrate complexes are still unable to form
- A graph can be used to distinguish between the two different types of inhibitors and their effect on the rate of reaction
- The patterns shown are **notably different for each type of inhibitor** and also for an **uninhibited enzyme**

**Rate of reaction of enzyme-catalysed reaction with inhibitors present diagram**



Your notes





***Graph showing different types of inhibitors and their effect on rate of reaction***

- A competitive inhibitor will lower the initial rate of reaction (by occupying some of the available active sites), whilst the maximal rate is not affected
  - **Eventually,** the same amount of product will be produced as would have been produced without the competitive inhibitor
- Non-competitive inhibitors lower the initial rate of reaction **and the maximal** rate of reaction
  - A lower amount of product is produced than would normally be produced

**Comparing Competitive and Non-competitive Inhibitors Table**

| Competitive Inhibitors | Non-competitive Inhibitors |
|---|---|
| Bind to the active site | Bind to an allosteric site on the enzyme |
| Chemically resemble the substrate | Chemically unlike the substrate |
| Block the active site | Change the shape of the active site |

| | |
|---|---|
| Low concentration allows high substrate concentration to overcome inhibitors | Low concentration doesn't allow high substrate concentration to overcome inhibitors |


Your notes

# Non-competitive Inhibition

Your notes

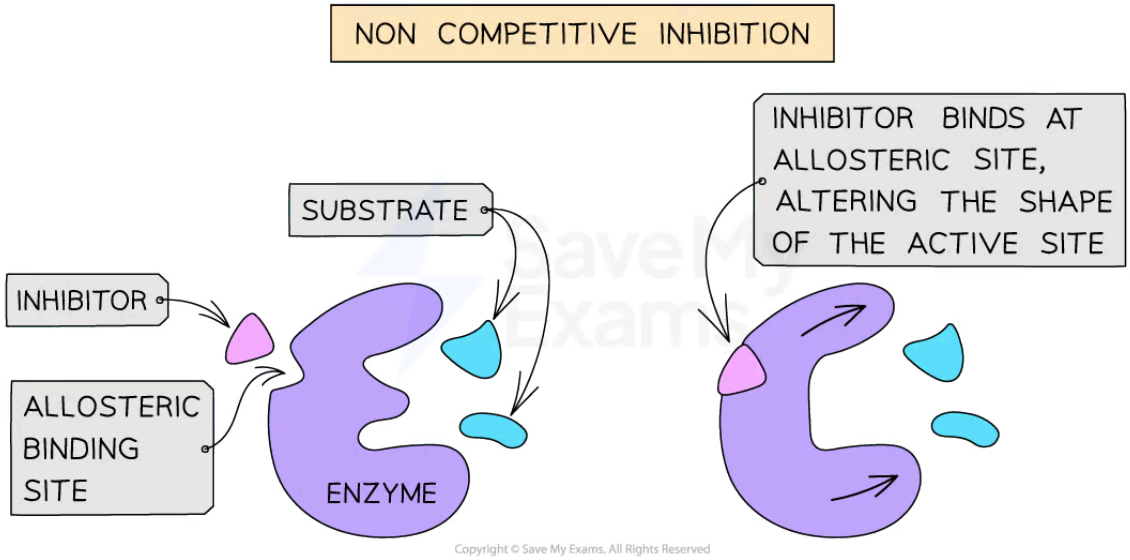
## Enzyme inhibitors

- **Inhibitors** are chemical substances that can bind to an enzyme and **reduce its activity**
- Inhibitors can be formed from **within the cell** or can be introduced from the **external environment**
- An enzyme's activity can be **reduced** or **stopped,** temporarily, by an inhibitor
- There are two types of inhibitors: **competitive** and **non-competitive**

## Allosteric sites and non-competitive inhibitors

- Non-competitive inhibitors bind to the enzyme at an **alternative site** which is not the active site
  - These sites are called **allosteric sites** and they are usually located quite far from the active site
  - Only **specific substances** (called effectors) can bind to an allosteric site
  - Binding to the allosteric site is **reversible**
- Binding to the allosteric site causes interactions within an enzyme which leads to **conformational changes**
  - These conformational changes will **alter** the **shape of the active site**
- This therefore **prevents** the substrate from binding to the active site
  - This will apply for as long as the effector is bound to the allosteric site

**Non-competitive inhibition diagram**



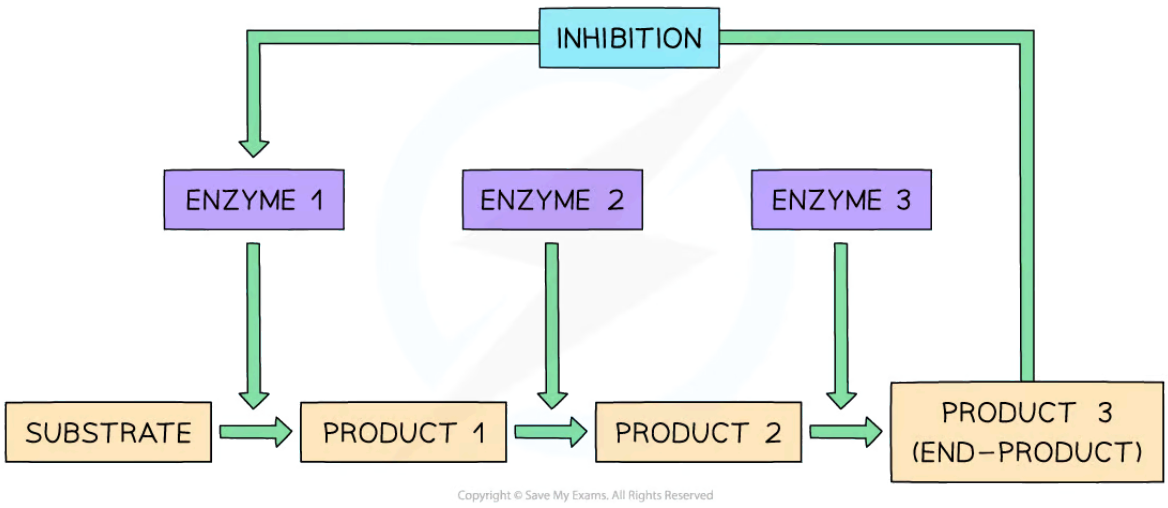

***Non-competitive inhibitors bind to the allosteric site of an enzyme to alter the active site***

## Feedback Inhibition

Your notes

- **End-product inhibition** occurs when the end product from a reaction is present in excess and itself acts as a **non-competitive inhibitor** of the enzyme
- The end product binds to an **allosteric site** on the enzyme and causes inhibition of the pathway, so they are referred to as **allosteric inhibitors**
- Allosteric inhibitors are important to prevent the build-up of **intermediate products** in a metabolic pathway, as each small step of the pathway may produce a new product
- The product therefore does not accumulate and the pathway can continue
  - An outline of the process is as follows:
    - As the enzyme converts substrate to an end product, the process is itself slowed down as the **end-product** of the reaction chain binds to an allosteric site on the original enzyme, changing the shape of the active site and preventing the formation of further **enzyme-substrate complexes**
    - The inhibition of the enzyme means that product levels fall, at which point the enzyme begins catalysing the reaction once again; this is a continuous **feedback loop**
      - The end-product inhibitor eventually detaches from the enzyme to be used elsewhere; this is what allows the active site to **reform** and the enzyme to return to an **active state**

**End-product inhibition diagram**





***End-product inhibition where the end-product of an enzyme controlled pathway inhibits the starting enzyme and limits the reactions***

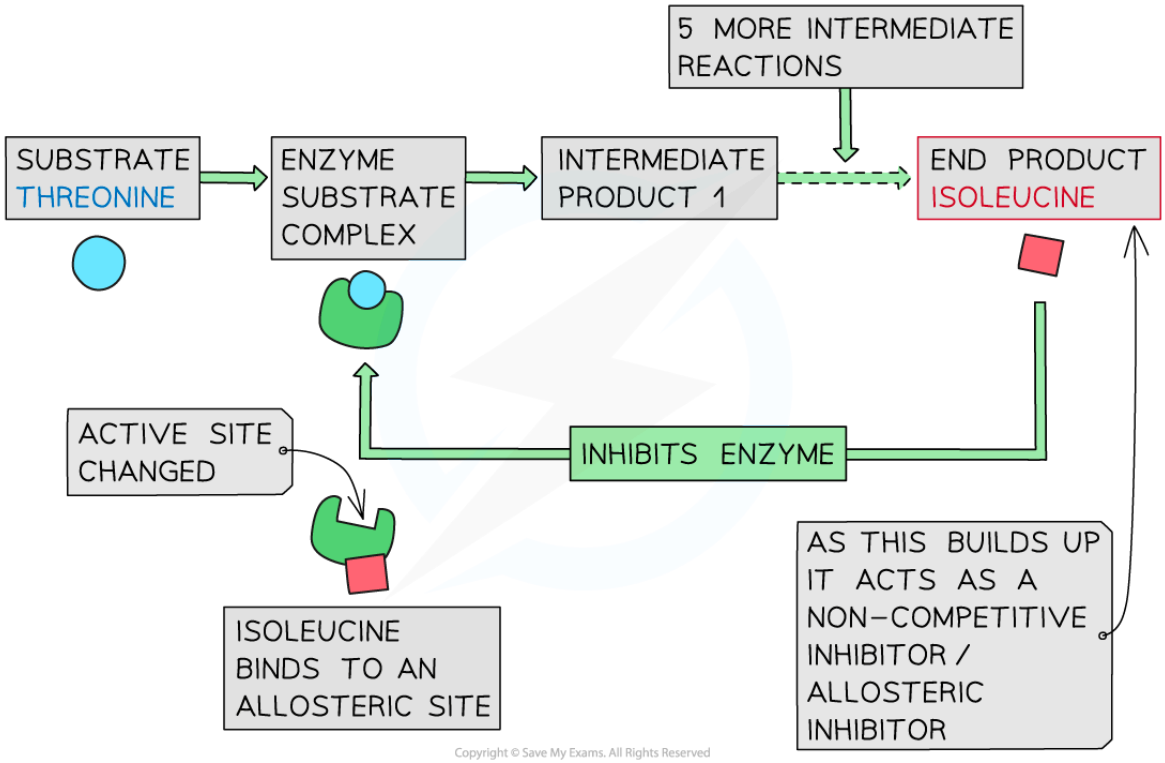
## An example of end-product inhibition

- The amino acid **isoleucine** can be synthesised from **threonine** in bacteria
- Isoleucine can bind to the **allosteric site** of the enzyme **threonine deaminase**
  - Threonine deaminase catalyses the first stage of the metabolic pathway that produces isoleucine
  - If the enzyme is inhibited, then the production of isoleucine stops

- At the start of the process, isoleucine levels are **low** so the metabolic pathway can proceed without being inhibited too much
- However, as the concentration of isoleucine **increases**, it begins to regulate the metabolic pathway by **acting as a non-competitive inhibitor**
- Isoleucine is an essential amino acid, so as it is **used by cells** for protein synthesis, its **concentration decreases** which decreases the number of allosteric sites occupied
- **More** enzymes are free to bind to threonine, and the production of isoleucine can continue

Your notes

**Example of end-product inhibition diagram**




***Example of end-product inhibition between threonine and isoleucine***

## Examiner Tip

You need to know the specific example of end-product inhibition of **threonine** and **isoleucine.**

## Mechanism-based Inhibition

Your notes

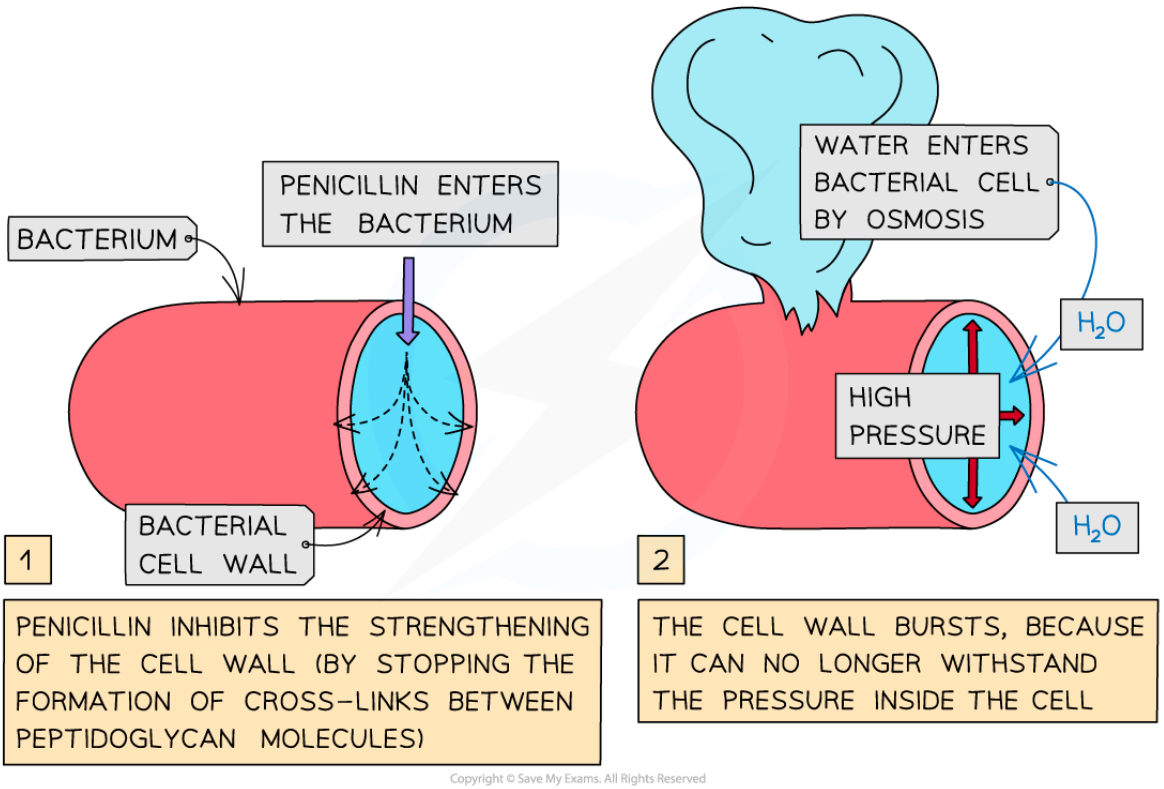
- Molecules that are able to form **covalent bonds** with the **active site** of an enzyme are known as a **substrate analogue**
- The substrate analogue can now be changed by the enzyme to produce a **reactive group**
  - This reactive group leads to the formation of a **stable inhibitor-enzyme complex**
- This form of inhibition is called **mechanism-based inhibition** and it is **irreversible**

### Penicillin as an example of mechanism-based inhibition

- Penicillin is an antibiotic that is very effective at killing bacteria
- Bacterial cell walls are composed of **peptidoglycans** (long molecules of peptides and sugars)
- These peptidoglycan molecules are **held together** by **cross-links** that form between them
- When a new bacterial cell is growing, it secretes enzymes known as **autolysins** that create **small holes** in the bacterial cell wall
- These holes allow the bacterial cell wall to **stretch**, with new peptidoglycan molecules then joining up via the **cross-links** described above
- Penicillin stops these cross-links forming by **inhibiting** the enzymes (**DD-transpeptidase**) that catalyse their formation
  - This happens because penicillin has a **similar structure** to parts of the growing peptide chain of the cell wall
  - DD-transpeptidase will **bind to penicillin** and modify it to form a stable enzyme-penicillin complex
  - This will **permanently block** the enzyme from creating more cross-links
- However, the autolysins keep creating holes in the bacterial cell wall, making the walls **weaker and weaker**
- As bacteria live in watery environments and **take up water by osmosis**, their weakened cell walls eventually **burst** as they can no longer withstand the **pressure** exerted on them from within the cell
  - This is known as death by **lysis**
- This means **penicillin is only effective against bacteria that are still growing** (autolysins no longer create holes and no more cross-links between peptidoglycan molecules are formed once the growth of a bacterium is complete, as the bacterial cell wall no longer needs to expand)

**The affect of penicillin on bacteria diagram**

Your notes





***Penicillin works by irreversibly inhibiting transpeptidase and thereby prevents the formation of cross-links between peptidoglycan molecules***

- Bacteria however, may develop **resistance** to penicillin
- **DNA mutations** in the bacterial genome may lead to changes in the **shape of the active site** of DD-transpeptidase, which will make it difficult for penicillin to bind and inhibit the enzyme
- Bacteria are able to share these mutations that promote antibiotic resistance quickly if it occurred in **plasmid DNA**
  - This is because bacteria are able to transfer plasmids to other bacteria by the process of **conjugation**
  - These antibiotic resistant mutations could even be shared between different species of bacteria

## Examiner Tip

Make sure that you are able to describe the process of mechanism-based inhibition by using penicillin as an example.