YOUR NOTES ⬇

2.4 Enzymes

CONTENTS

2.4.1 THE ROLE OF ENZYMES

Enzymes as Catalysts

- Enzymes are **biological catalysts**
  - 'Biological' because they function in **living systems**
  - 'Catalysts' because they **speed up the rate of chemical reactions** without being **used up** or undergoing **permanent change**
- Enzymes are **globular proteins** with **complex tertiary structures**
  - Some are formed from a single polypeptide, whilst others are made up of two or more polypeptides and therefore have a **quaternary structure**
- **Metabolic pathways** are controlled by enzymes in a biochemical cascade of reactions
  - Virtually every metabolic reaction within living organisms is catalysed by an enzyme
  - Enzymes are therefore essential for life to exist

Site of action of enzymes

- All enzymes are **proteins** that are produced via the process of protein synthesis **inside cells**
- Some enzymes **remain inside** cells, whilst others are **secreted** to work **outside** of cells
- Enzymes can therefore be **intracellular** or **extracellular**, referring to whether they are active **inside** or **outside** the cell respectively
  - **Intracellular** enzymes are **produced** and **function inside the cell**
  - **Extracellular** enzymes are **secreted** by cells and catalyse reactions **outside** cells (eg. digestive enzymes in the gut)

2.4 Enzymes

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Intracellular and Extracellular Enzymes Table

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

- As well as amylase (which hydrolyses starch into maltose), another example of an extracellular digestive enzyme that is secreted by the pancreas and enters the small intestine is **trypsin**, which breaks **proteins** down into **peptides** and **amino acids**
- In fact, some organisms can only feed using a form of **extracellular digestion** in which the digestive enzymes are actually secreted **outside** of their **bodies**
    - For example, the **hyphae** of **fungi** secrete the necessary enzymes **directly onto the food** they are consuming (e.g. wood) so that the food is digested into smaller, simple molecules that the fungi can then **absorb** through the walls of the hyphae

2.4 Enzymes

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Exam Tip

Don’t forget that enzymes are proteins, meaning that anything that could denature a protein and make it non-operational (such as extremes of heat, temperature, pH etc.) would also denature an enzyme. This is one reason why homeostasis (the maintaining of internal body conditions) is so important: to ensure that enzymes within the body can function properly, as they are essential to the survival of organisms.

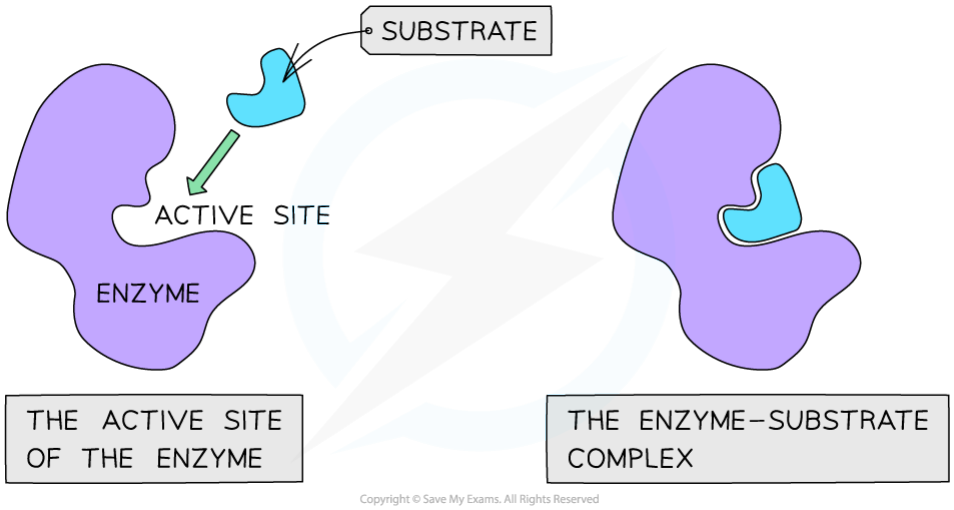
YOUR NOTES ⬇

2.4 Enzymes

2.4.2 ENZYME ACTION

Mechanism of Enzyme Action

- Enzymes have an **active site** where **specific substrates bind** forming an **enzyme-substrate complex**
- The **active site** of an enzyme has a **specific shape** to fit a **specific substrate**
- Extremes of **heat** or **pH** can **change the shape** of the active site, **preventing** substrate binding – this is called **denaturation** (the enzyme is said to be **denatured**)
- Substrates **collide** with the enzymes active site and this must happen at the **correct orientation and speed** in order for a reaction to occur



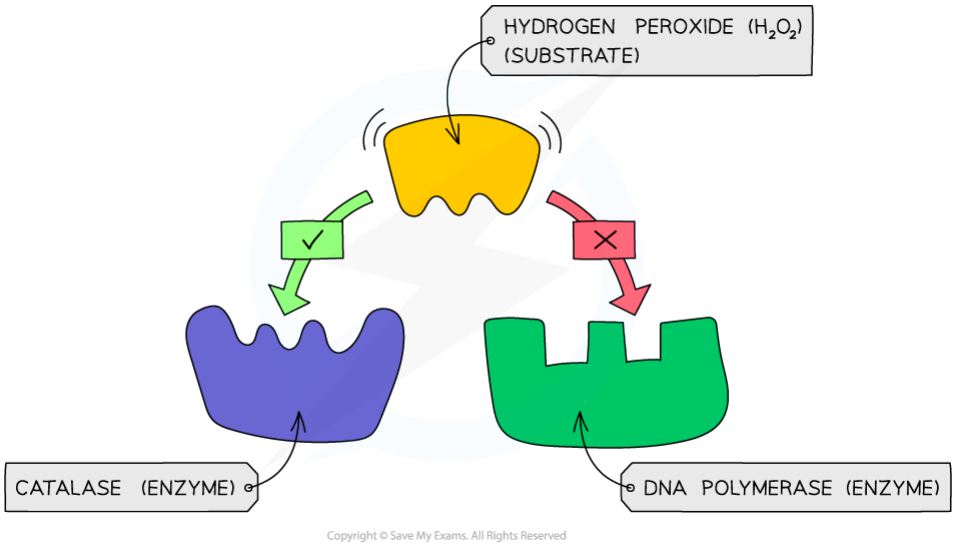
The active site of an enzyme has a specific shape to fit a specific substrate (when the substrate binds an enzyme-substrate complex is formed)

Enzyme specificity

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

2.4 Enzymes

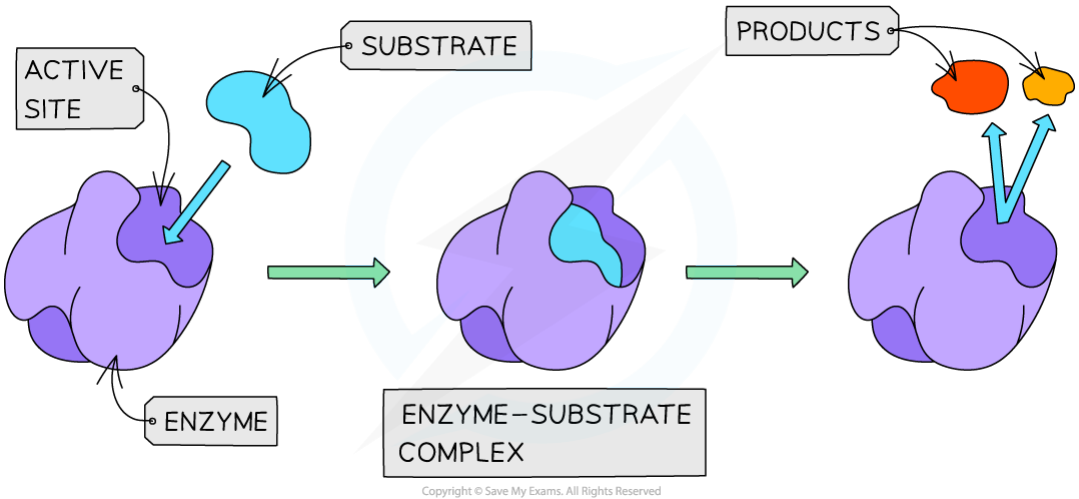
YOUR NOTES



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

The enzyme-substrate complex

- An **enzyme-substrate complex** forms when an enzyme and its substrate join together
- The enzyme-substrate complex is only formed **temporarily** before the enzyme catalyses the reaction and the **product(s)** are **released**



The temporary formation of an enzyme-substrate complex

2.4 Enzymes

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The lock-and-key hypothesis

- Enzymes are **globular proteins**
- This means their **shape** (as well as the shape of the **active site** of an enzyme) is determined by the **complex tertiary structure** of the protein that makes up the enzyme and is therefore **highly specific**
- In the 1890's the first model of enzyme activity was described by Emil Fischer:
  - He suggested that both enzymes and substrates were **rigid structures** that **locked** into each other very **precisely**, much like a key going into a lock
  - This is known as the '**lock-and-key hypothesis**'

2.4 Enzymes

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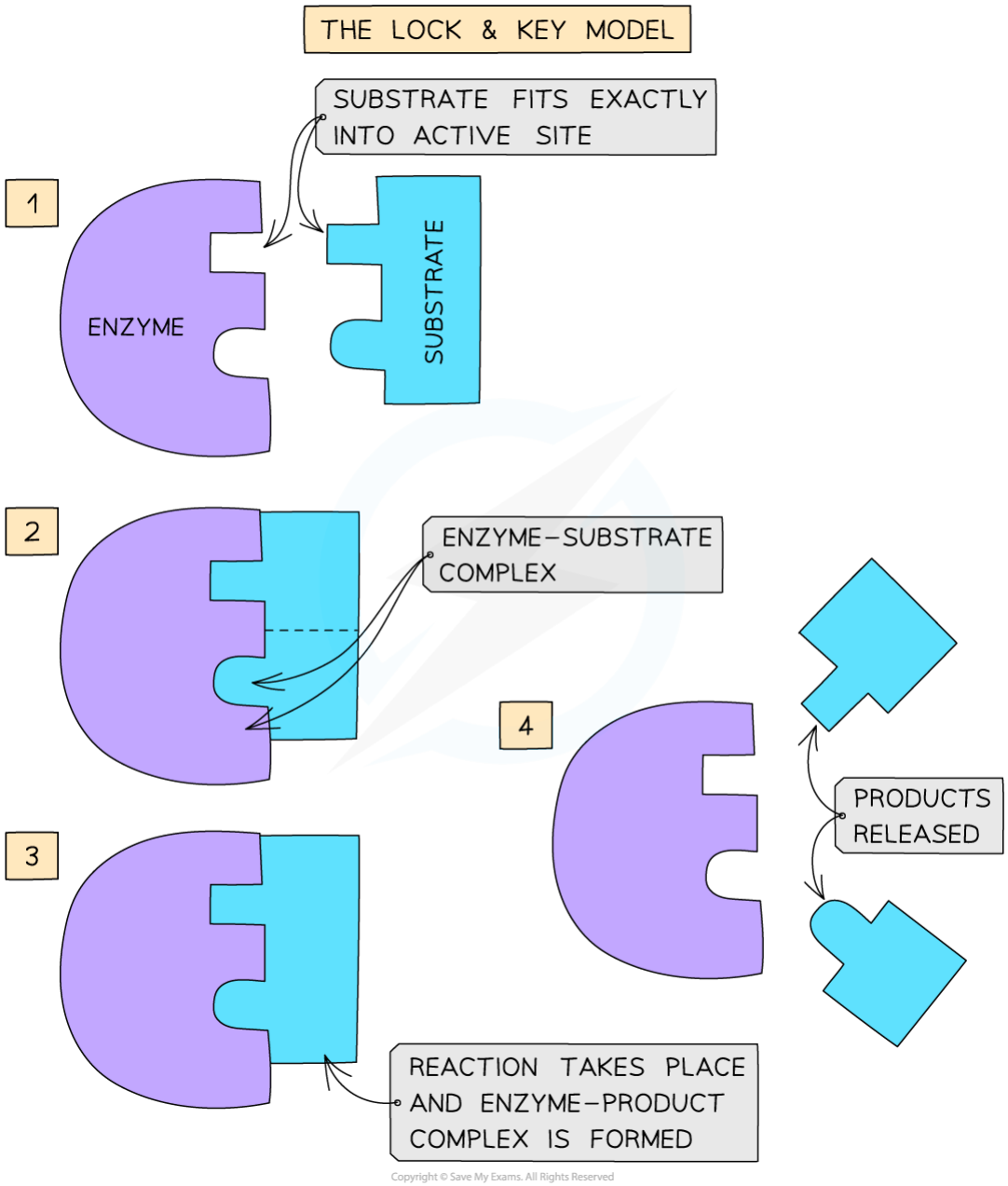

The lock-and-key hypothesis

2.4 Enzymes

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The induced-fit hypothesis

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

2.4 Enzymes

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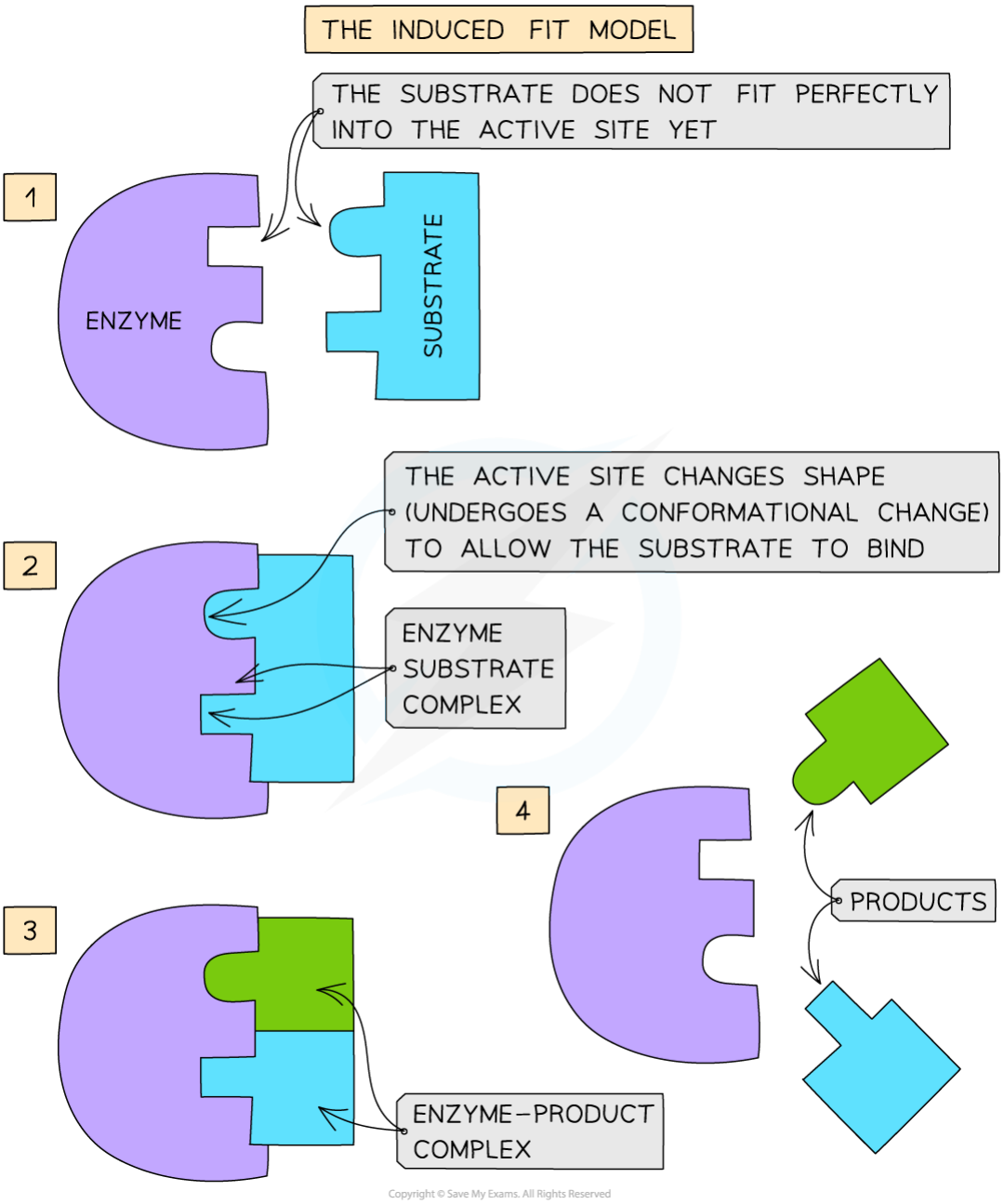


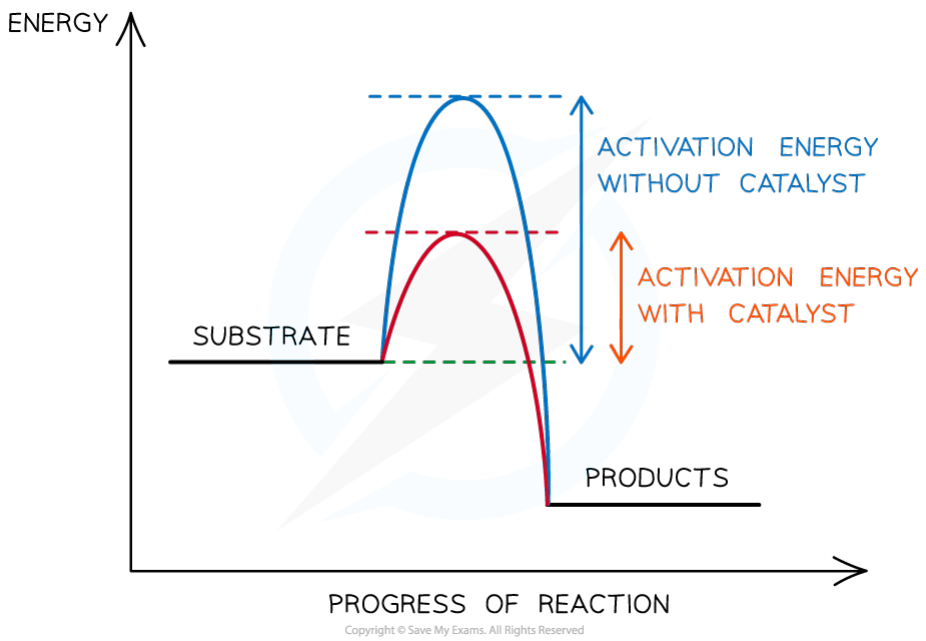
The induced-fit hypothesis

2.4 Enzymes

YOUR NOTES ⬇

Enzymes and the lowering of activation energy

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



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

2.4 Enzymes

YOUR NOTES ⬇

2.4.3 ENZYME ACTIVITY: PH

Enzyme Activity: pH

- All enzymes have an **optimum pH** or a pH at which they operate best
- Enzymes are **denatured** at extremes of pH
  - **Hydrogen and ionic bonds** hold the tertiary structure of the protein (ie. the enzyme) together
  - Below and above the optimum pH of an enzyme, solutions with an excess of H^+ ions (acidic solutions) and OH^- ions (alkaline solutions) can cause these **bonds** to **break**
  - The breaking of bonds **alters the shape of the active site**, which means enzyme-substrate complexes form less easily
  - Eventually, enzyme-substrate complexes can no longer form at all
  - At this point, **complete denaturation** of the enzyme has occurred
- Where an enzyme functions can be an indicator of its optimal environment:
  - Eg. **pepsin** is found in the stomach, an acidic environment at pH 2 (due to the presence of **hydrochloric acid** in the stomach's gastric juice)
  - Pepsin's optimum pH, not surprisingly, is pH 2

2.4 Enzymes

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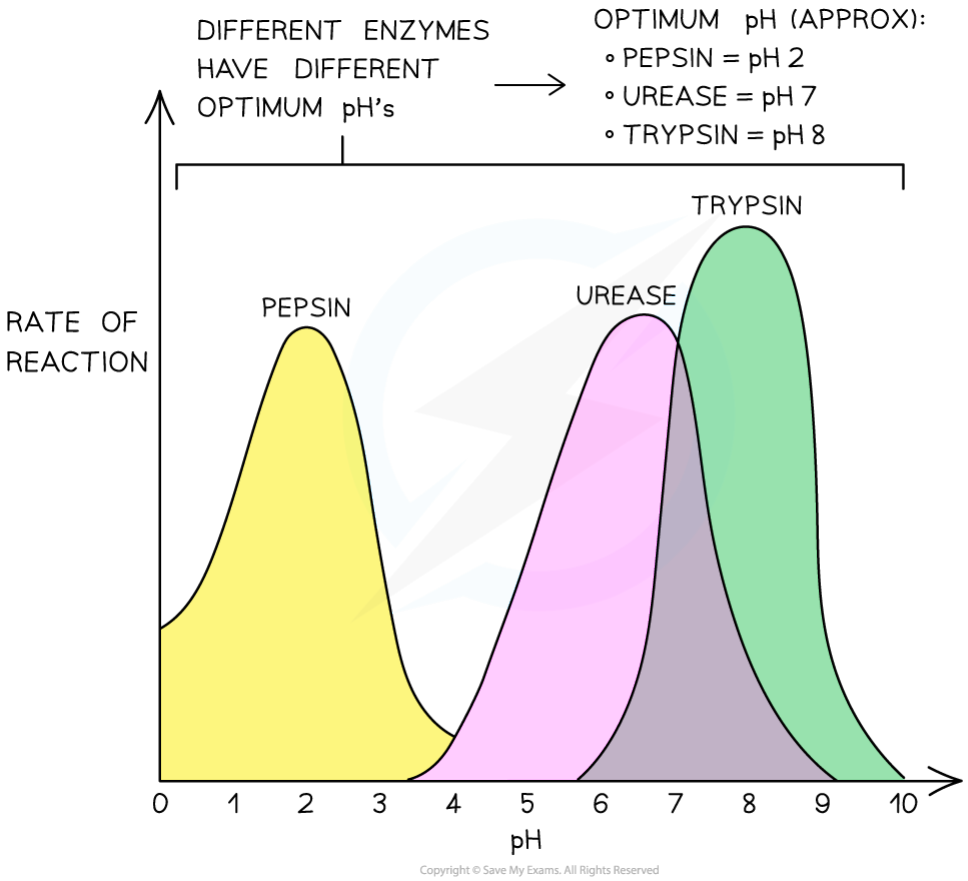

The effect of pH on the rate of an enzyme-catalysed reaction for three different enzymes (each with a different optimum pH)

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

2.4 Enzymes

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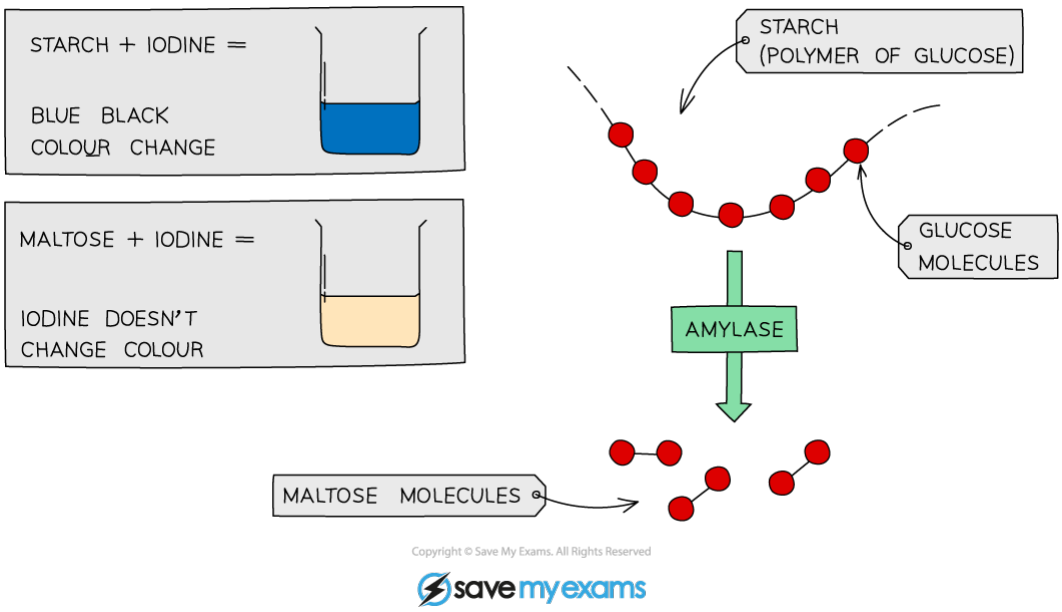
Investigating the effect of pH on enzyme reaction rates

- Use the enzyme amylase to breakdown starch at a range of pH values, using iodine solution as an indicator for the reaction occurring
  - Amylase is an enzyme that digests **starch** (a polysaccharide of glucose) **into maltose** (a disaccharide of glucose)
- A continuous sampling technique can monitor the progress of the reaction
  - Starch can be tested for using **iodine solution**



Iodine can be used qualitatively to indicate the presence or absence of starch from a sample

Apparatus

- Test tubes
- Buffer solutions at different pH levels
- Amylase solution
- Iodine solution
- Starch solution
- Pipettes
- Spotting tile
- Timer
- Gloves
- Goggles

2.4 Enzymes

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Method

- Wear goggles and gloves
  - Enzymes have the potential to cause allergic reactions if they come into direct contact with skin
- Place single drops of iodine solution in rows on the tile
  - Iodine solution is orange-brown
- Label a test tube with the pH to be tested
- Use the syringe to place $2cm^3$ of amylase in the test tube
  - Equal volume and concentration of enzyme should be used so these variables are controlled and the effect of changing pH can be measured
- Add $1cm^3$ of buffer solution to the test tube using a syringe
- Use another test tube to add $2cm^3$ of starch solution to the amylase and buffer solution, start the stopwatch whilst mixing using a pipette
  - Equal volume and concentration of the substrate (starch) should be used so these variables are controlled and the effect of changing pH can be measured
  - Mixing enables the enzymes and substrate to be equally mixed
- After 10 seconds, use a pipette to place one drop of the mixture on the first drop of iodine, which should turn blue-black
  - This test indicates whether starch is still present
- Wait another 10 seconds and place another drop of the mixture on the second drop of iodine
- Repeat every 10 seconds until iodine solution remains orange-brown
  - When the solution remains orange-brown it means amylase has broken down all of the starch so nothing is left to react with the iodine
- **Repeat experiment at different pH values**
  - The less time the iodine solution takes to remain orange-brown, the quicker all the starch has been digested and so the better the enzyme works at that pH

2.4 Enzymes

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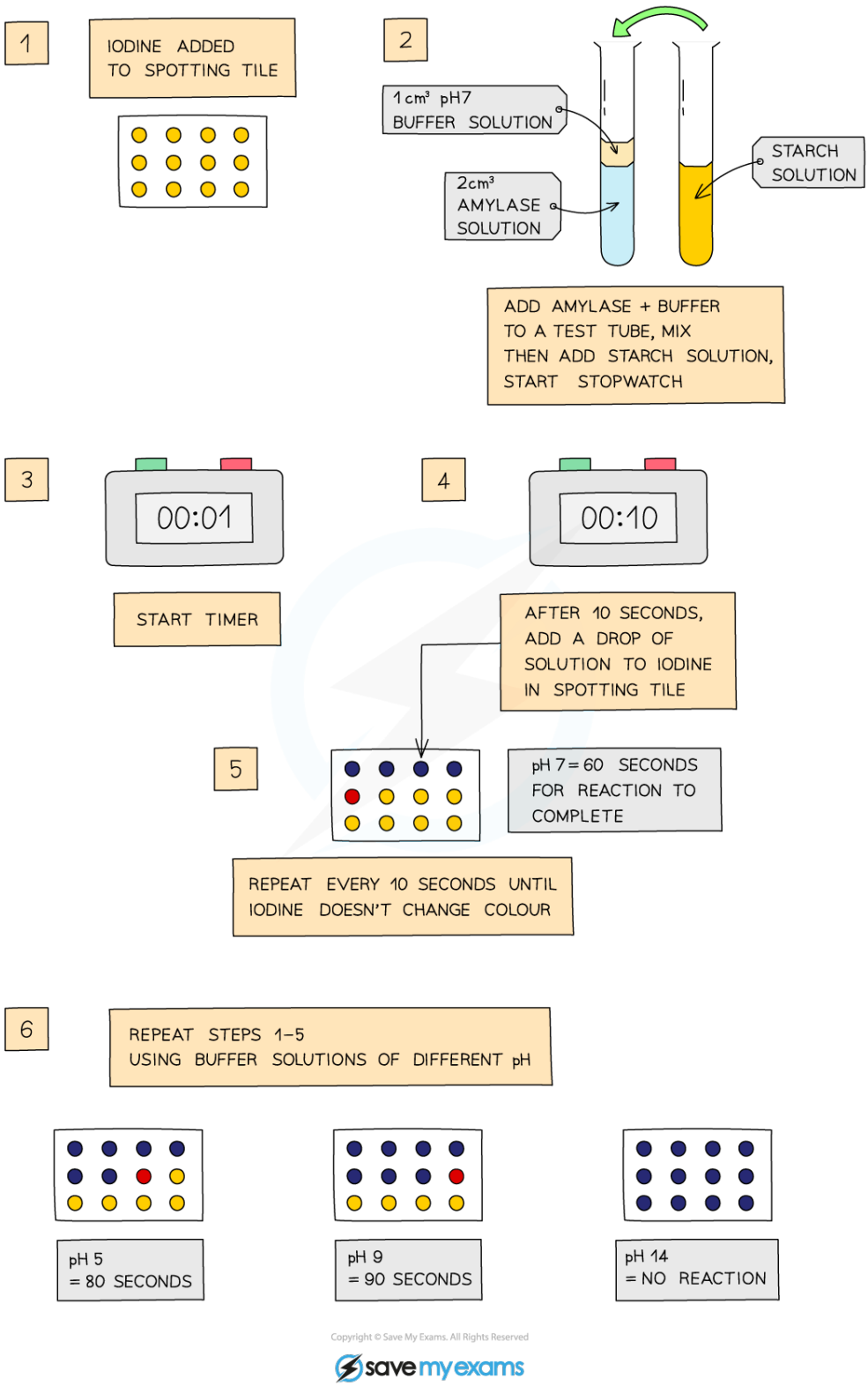

Investigating the effect of pH on enzyme activity

2.4 Enzymes

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Limitations

- The above method can be adapted to **control temperature** by using a **water bath** at 35℃
  - All solutions that need to be used (starch, amylase, pH buffers) should be placed in a water bath and allowed to reach the temperature (using a thermometer to check) before being used
- A **colorimeter** can be used to measure the progress of the reaction more accurately; with a solution containing starch being darker and glucose lighter (as a result of the colour-change of iodine) – this will affect the absorbance or transmission of light in a colorimeter

Exam Tip

Temperature can both affect the speed at which molecules are moving (and therefore the number of collisions between enzyme and substrate in a given time) and can denature enzymes (at high temperatures). pH, however, does not affect collision rate but only disrupts the ability of the substrate to bind with the enzyme, reducing the number of successful collisions until eventually, the active site changes shape so much that no more successful collisions can occur.

2.4 Enzymes

YOUR NOTES ⬇

2.4.4 ENZYME ACTIVITY: TEMPERATURE

Enzyme Activity: Temperature

- Enzymes have a specific **optimum temperature**
  - This is the temperature at which they catalyse a reaction at the **maximum rate**
- **Lower temperatures** either **prevent** reactions from proceeding or **slow them down** because:
  - Molecules move relatively **slowly** as they have **less kinetic energy**
  - Less kinetic energy results in a **lower frequency** of **successful collisions** between substrate molecules and the active sites of the enzymes which leads to less frequent **enzyme-substrate complex** formation
  - Substrates and enzymes also collide with **less energy**, making it less likely for bonds to be formed or broken (stopping the reaction from occurring)
- **Higher temperatures** cause **reactions** to **speed up** because:
  - Molecules move **more quickly** as they have **more kinetic energy**
  - Increased kinetic energy results in a **higher frequency** of **successful collisions** between substrate molecules and the active sites of the enzymes which leads to more frequent **enzyme-substrate complex** formation
  - Substrates and enzymes also collide with **more energy**, making it more likely for bonds to be formed or broken (allowing the reaction to occur)

Denaturation

- If temperatures continue to increase **past a certain point**, the rate at which an enzyme catalyses a reaction **drops sharply**, as the enzyme begins to **denature**:
  - The increased **kinetic energy** and **vibration** of the enzyme molecules puts a **strain** on them, eventually causing the **weaker hydrogen and ionic bonds** that hold the enzyme molecule in its **precise shape** to start to **break**
  - The breaking of bonds causes the **tertiary structure** of the protein (i.e. the enzyme) to **change**
  - The **active site** is **permanently damaged** and its shape is no longer **complementary to the substrate**, preventing the **substrate** from **binding**
  - **Denaturation** has occurred if the substrate can no longer bind

2.4 Enzymes

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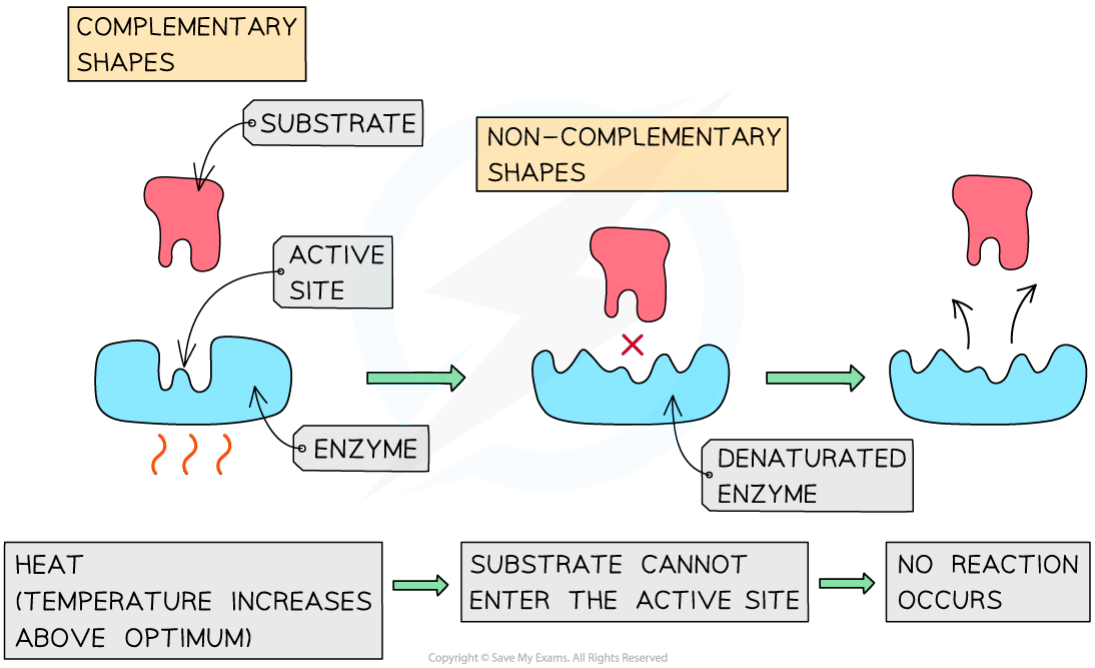
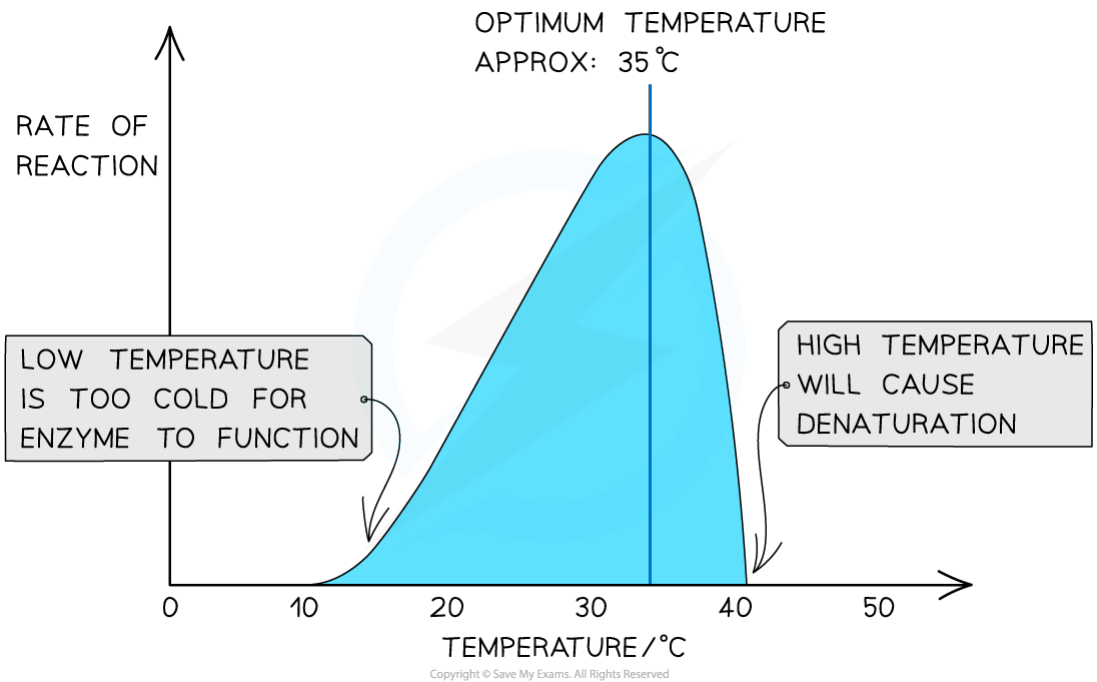


At high temperatures enzymes are denatured - the active site changes shape and is no longer complementary to the substrate. This change is irreversible.



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

2.4 Enzymes

YOUR NOTES ⬇

- The optimum temperature of an enzyme and the temperature at which an enzyme is denatured **varies** according to the **habitat** to which an organism is **adapted**
  - Most enzymes present in living organisms denature at temperatures above 60 °C
- Very few human enzymes can function at temperatures above 50 °C
  - Humans maintain a body temperature of about 37 °C and even temperatures exceeding 40 °C can cause the denaturation of some enzymes
- Some bacteria that live in thermal springs have enzymes that can withstand temperatures in excess of 80 °C
  - These enzymes are **thermostable**

Temperature coefficient

- The **temperature coefficient** for a biological **reaction** is the **ratio** between the **rates** of that reaction at **two different temperatures**
  - For most enzyme-catalysed reactions the rate of the reaction **doubles** for every **10 °C increase** in temperature
  - The temperature coefficient (Q) for a reaction that follows this pattern is: **$Q_{10} = 2$**
- The temperature coefficient can be calculated using the following equation:

Temperature coefficient = (rate of reaction at (x + 10) °C) ÷ (rate of reaction at x °C)

2.4 Enzymes

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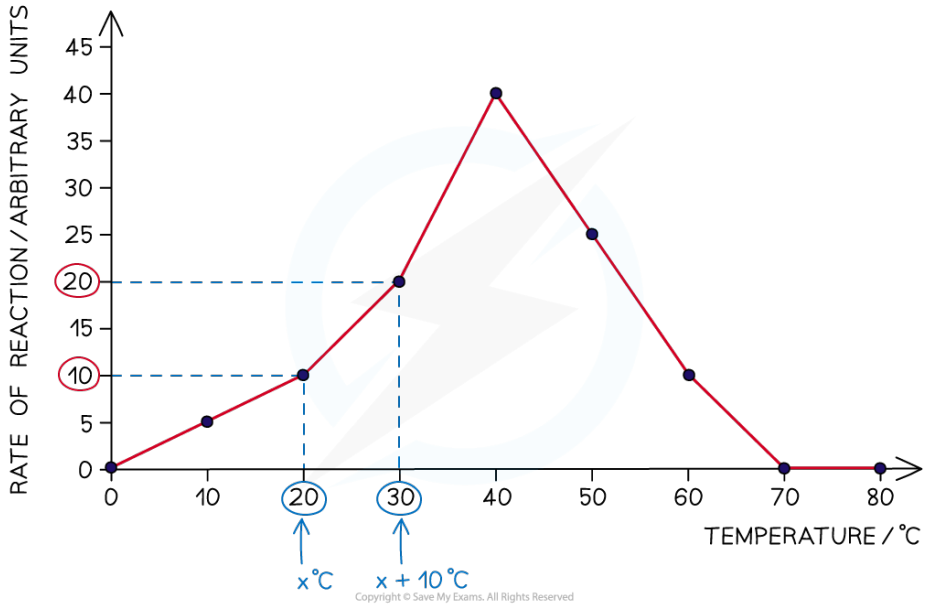
Worked Example

The graph below shows the effect of temperature on an enzyme-catalysed reaction. Using the information in the graph, calculate the temperature coefficient for the reaction between 20 °C and 30 °C.



Step One: Using the graph, note the intercepts on the vertical axis at 20 °C and 30 °C

At 20 °C the rate of reaction is 10 arbitrary units and at 30 °C the rate of reaction is 20 arbitrary units

Step Two: Write out the equation and substitute in the known values

Temperature coefficient = (rate of reaction at (x + 10) °C) ÷ (rate of reaction at x °C)

Q_{10} = rate of reaction at 30 °C ÷ rate of reaction at 20 °C

$Q_{10} = 20 \div 10$

Step Three: Calculate the temperature coefficient

$Q_{10} = 2$

(There is no unit for Q_{10} as it is a ratio)

2.4 Enzymes

YOUR NOTES

Exam Tip

When answering questions about reaction rates for enzyme-catalysed reactions, make sure to explain how the temperature affects the speed at which the molecules (enzymes and substrates) are moving (i.e. their kinetic energy) and how this, in turn, affects the number of successful collisions.

A common mistake in exams is to say that enzymes are 'killed' at high temperatures. This is not biologically accurate and you would be marked down for this, as enzymes are protein molecules, not living organisms. Enzymes are denatured, not killed.

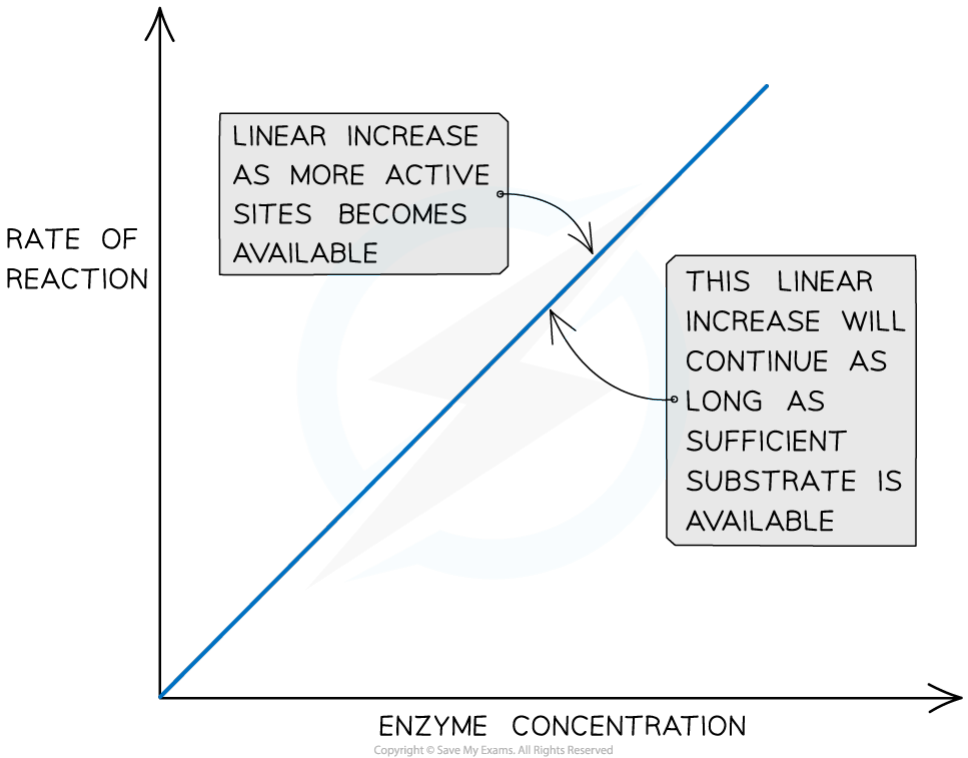
YOUR NOTES ⬇

2.4 Enzymes

2.4.5 ENZYME ACTIVITY: ENZYME CONCENTRATION

Enzyme Activity: Enzyme Concentration

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



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

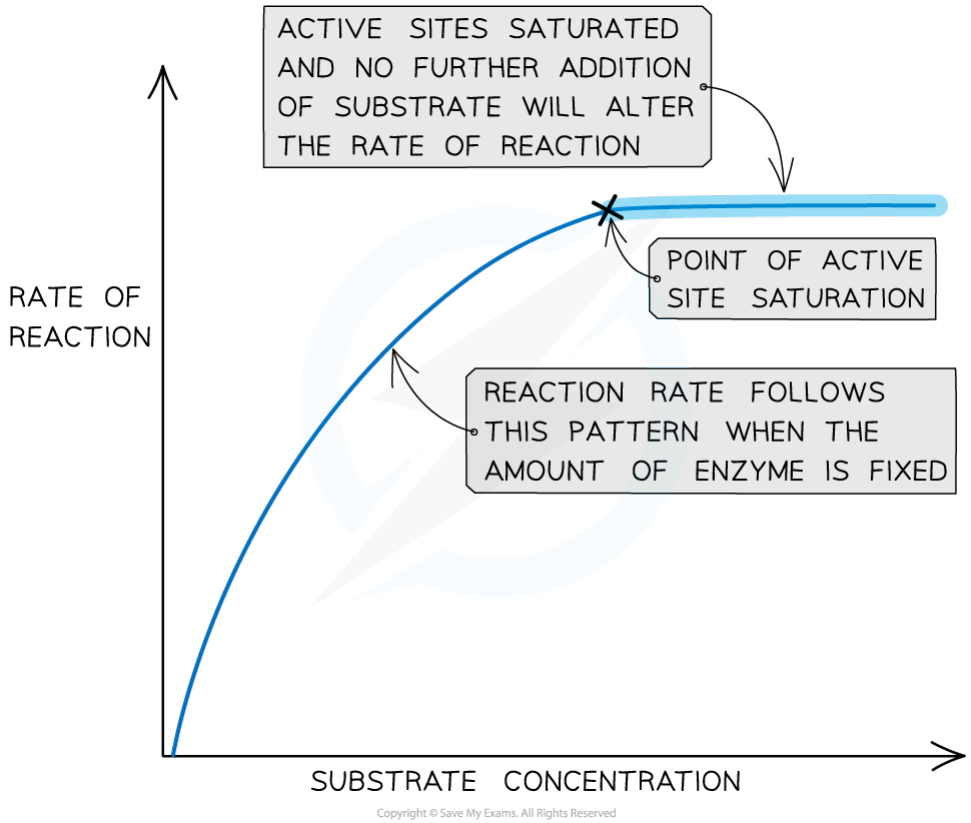
YOUR NOTES ⬇

2.4 Enzymes

2.4.6 ENZYME ACTIVITY: SUBSTRATE CONCENTRATION

Enzyme Activity: Substrate Concentration

- The **greater** the **substrate concentration**, the **higher** the **rate of reaction:**
  - As the number of substrate molecules increases, the likelihood of enzyme-substrate complex formation increases
  - If the enzyme concentration remains **fixed** but the amount of substrate is increased past a certain point, however, all available active sites eventually become **saturated** and any further increase in substrate concentration will **not increase** the reaction rate
  - When the active sites of the enzymes are all full, any substrate molecules that are added have **nowhere to bind** in order to form an **enzyme-substrate complex**
- For this reason, in the graph below there is a **linear increase** in reaction rate as substrate is added, which then **plateaus** when all active sites become occupied



The effect of substrate concentration on the rate of an enzyme-catalysed reaction

2.4 Enzymes

YOUR NOTES

Exam Tip

If substrate concentration is continually increased but enzyme concentration is kept constant, there eventually comes a point where every enzyme active site is working continuously. At this point, the substrate molecules are effectively 'queuing up' for an active site to become available.

At this stage, the enzyme is working at its maximum possible rate, known as V_{max} (V stands for velocity).

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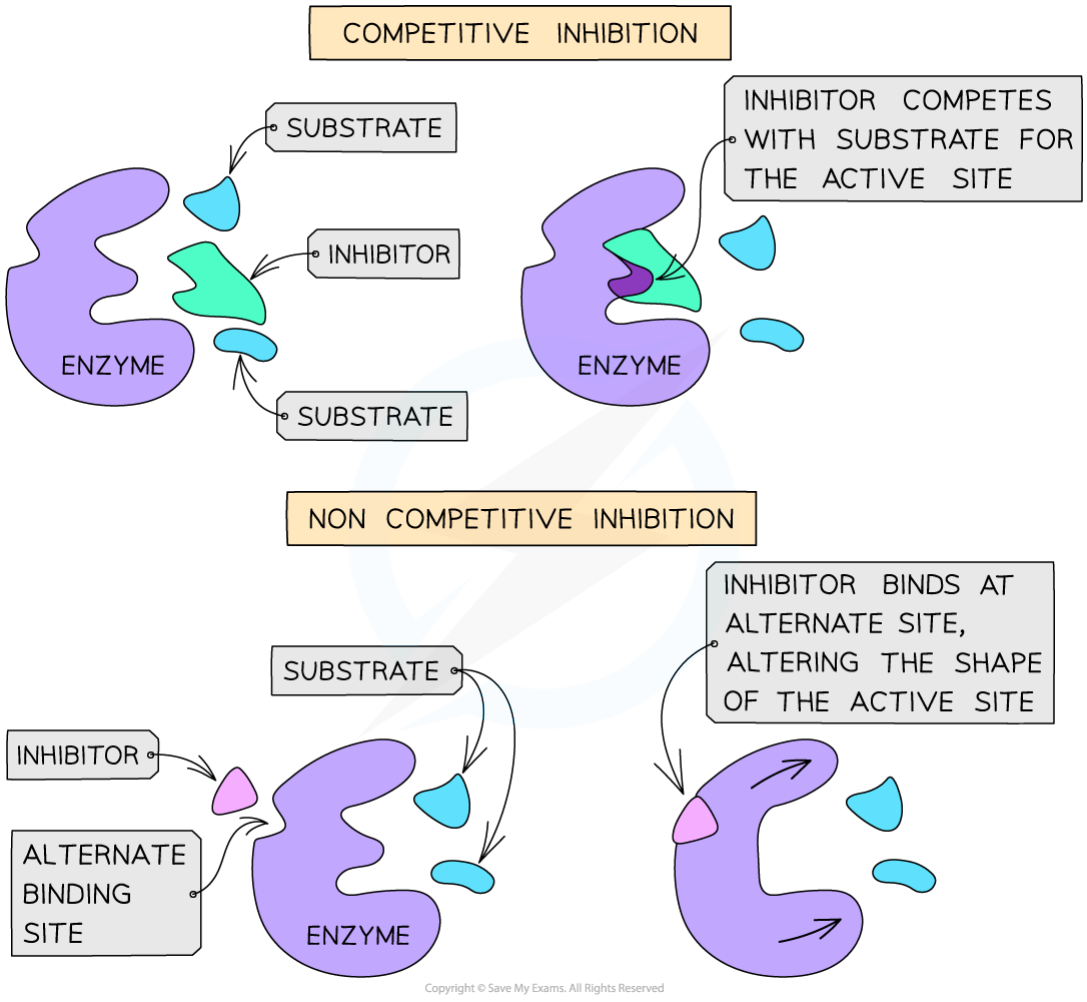
2.4 Enzymes

2.4.7 ENZYME ACTIVITY: ENZYME INHIBITORS

Enzyme Inhibitors

Reversible inhibitors

- An enzyme's activity can be reduced or stopped, **temporarily**, by a **reversible inhibitor**
- There are **two types** of reversible inhibitors:
  - **Competitive** inhibitors have a **similar shape** to that of the **substrate** molecules and therefore **compete** with the substrate for the active site
  - **Non-competitive** inhibitors **bind** to the enzyme at an **alternative site**, which **alters the shape of the active site** and therefore **prevents** the **substrate** from **binding** to it



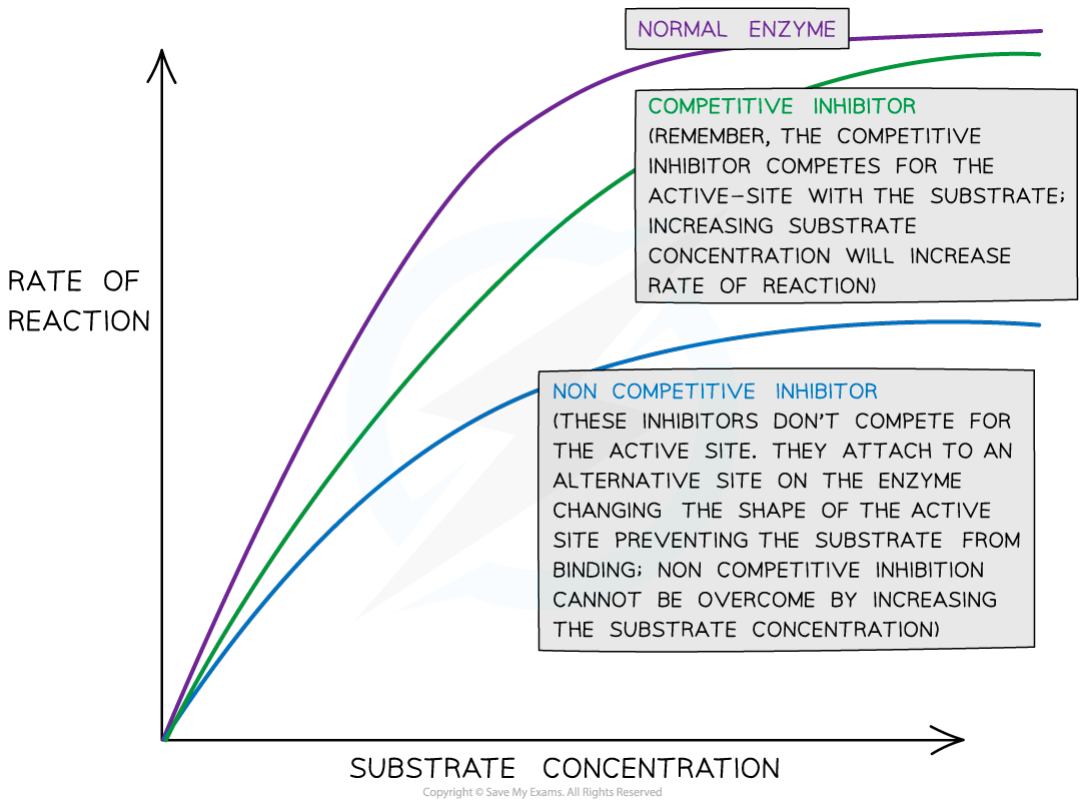
Competitive and non-competitive inhibition

2.4 Enzymes

YOUR NOTES

Reversible inhibitors and reaction rate

- Both types of reversible inhibitors **slow down** or **stop** enzyme activity, decreasing the rate of reaction
- **Increasing** the concentration of an inhibitor, therefore, **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 once more (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 once more, as the shape of the active site of the enzyme remains changed and enzyme-substrate complexes are still unable to form



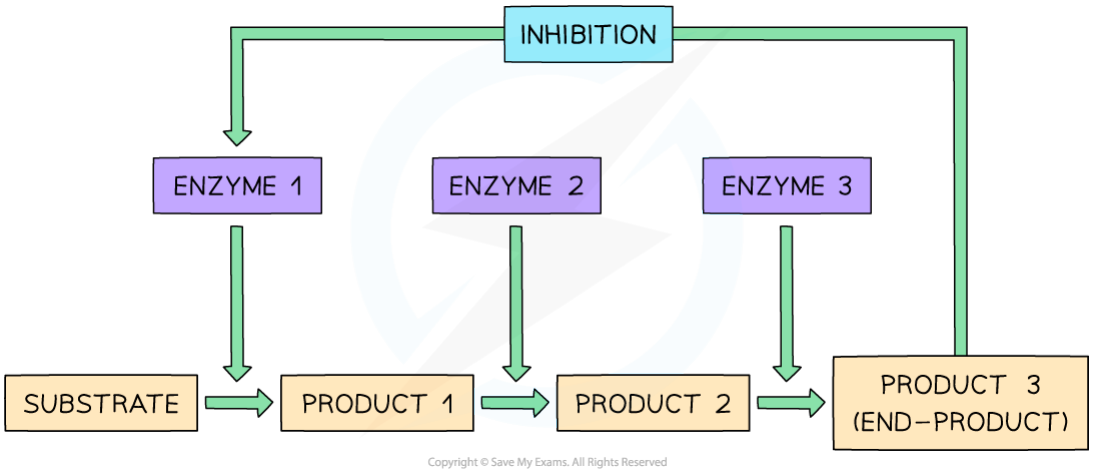
The effect of inhibitor concentration on the rate of an enzyme-catalysed reaction

2.4 Enzymes

YOUR NOTES

End-product inhibition and the control of metabolic pathways

- Reversible inhibitors can act as **regulators** in **metabolic pathways**
- Metabolic reactions must be very **tightly controlled** and **balanced**, so that no single enzyme can 'run wild' and continuously and uncontrollably generate more and more of a particular product
- Metabolic reactions can be **controlled** by using the **end-product** of a particular sequence of metabolic reactions as a **non-competitive, reversible inhibitor**:
  - As the enzyme converts the substrate into product, the process is itself **slowed down** as the **end-product** of the reaction chain binds to an alternative site on the original enzyme, changing the shape of the active site and preventing the formation of further enzyme-substrate complexes
  - The end-product can then **detach** from the enzyme and be used elsewhere, allowing the active site to **reform** and the enzyme to return to an **active state**
  - This means that as product levels fall, the enzyme begins catalysing the reaction once again, in a continuous **feedback loop**
  - This process is known as **end-product inhibition**



End-product inhibition

2.4 Enzymes

YOUR NOTES ⬇

Non-reversible inhibitors

- Some inhibitors can form **covalent bonds** with enzymes, inhibiting them **permanently**
  - These are known as **non-reversible** or **irreversible inhibitors**
- If this type of inhibition occurs in a living cell or organism it will result in the **complete inactivation** of the enzyme
  - This can be **dangerous** as can cause the biological reaction the enzyme is catalysing to be **completely stopped**
  - The only way to avoid this is for the cell or organism to **produce more of the enzyme** being inhibited, which can only be achieved by transcribing and translating the gene(s) for that enzyme, which is a **relatively slow** process
- This is why some non-reversible inhibitors are considered to be **metabolic poisons**
- For example, **cyanide** acts as a non-reversible inhibitor of **cytochrome oxidase**, a **mitochondrial enzyme** that catalyses one of the key reactions in **aerobic respiration**
  - This can be **fatal** as it takes **too long** to produce new enzymes and the organism will **die** before this can occur
  - As it stops a **metabolic reaction**, cyanide is known as a **metabolic poison**
- Other non-reversible inhibitors, such as **lead** and **mercury**, are also serious poisons
  - For example, lead acts as a non-reversible inhibitor of **ferrochelatase**, an enzyme involved in the production of **haem** for **haemoglobin**
- Some non-reversible inhibitors can be **beneficial** if they can be used, in a **medical context**, to inhibit enzymes that cause **harm** to some individuals

Examples of Non-reversible Inhibitors as Medicinal drugs

Medicinal drug	Inhibitory action	Medical benefit
Penicillin	Non-reversible inhibition of transpeptidase (the enzyme that helps form the cross-links in bacterial cell walls)	Results in the destruction of bacteria as their cell walls break down
Aspirin (acetylsalicylic acid)	Non-reversible inhibition of COX (the enzyme that helps produce prostaglandins for stimulating inflammation and pain)	Results in the reduction of inflammation and provides pain relief
Eflornithine	Non-reversible inhibition of ornithine decarboxylase (an enzyme essential to cell growth)	Used in the treatment of sleeping sickness (African trypanosomiasis)

2.4 Enzymes

YOUR NOTES

Exam Tip

While a competitive inhibitor will lower the initial rate of reaction (by occupying some of the available active sites), eventually the same amount of product will be produced as would have been produced without the competitive inhibitor (the maximal rate is not affected). 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).

2.4 Enzymes

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2.4.8 COENZYMES, COFACTORS & PROSTHETIC GROUPS

Coenzymes, Cofactors & Prosthetic Groups

- There are substances **other than** substrates and inhibitors that **interact** with **enzymes**
  - Some enzymes can only **function properly** if another **non-protein substance** is present
  - For example, some enzymes are **inactive** until they combine with a non-protein substance that **changes** their **tertiary structure** (allowing the **active site** to **bind** correctly with the **substrate**)
- These substances are broadly known as **cofactors**

Cofactors

- Some enzymes require **inorganic ions** to function properly
- Particular inorganic ions may help to **stabilise** the **structure** of the enzyme or may actually **take part in the reaction** at the **active site**
  - For example, **chloride ions** act as a cofactor for **amylase**
  - This means that in order for amylase to be able to digest starch into maltose, chloride ions must be present
- The inorganic ions that an enzyme requires in order to function are known as **inorganic cofactors**

2.4 Enzymes

YOUR NOTES ⬇

Coenzymes

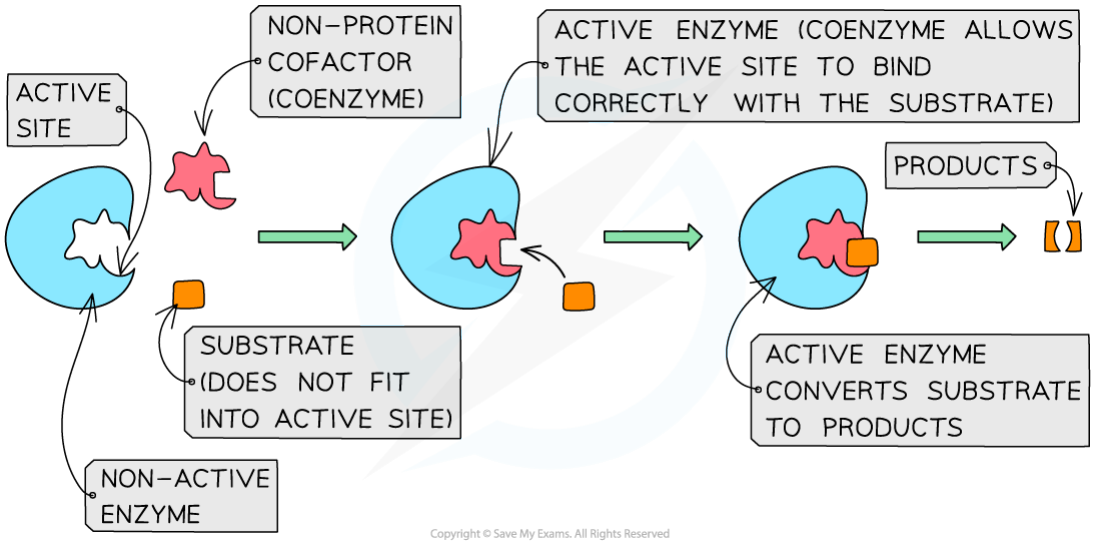
- Larger **organic** (carbon-containing) **cofactors** are known as **coenzymes**
  - Some coenzymes are **permanently bound** to the enzyme they assist, often **in** or **near** the **active site**
  - Some coenzymes only **bind temporarily** during the reaction
- Coenzymes link different enzyme-catalysed reactions into a sequence during **metabolic processes**, such as **photosynthesis** and **respiration**
- **Vitamins** are an important **source** of coenzymes. For example, many vitamins in the B vitamin group are used in the production of important coenzymes, including:
  - **Pantothenic acid**, a key component of **coenzyme A** (a coenzyme required for the oxidation of pyruvate during the link reaction that occurs between the glycolysis and Krebs cycle stages of respiration)
  - **Nicotinic acid**, used to produce the coenzymes **NAD** and **NADP** (coenzymes required in many different metabolic reactions, including many of the reactions that take place during photosynthesis and respiration)
  - **Vitamin B_1** (riboflavin), used to produce the coenzyme **FAD** (a coenzyme required in the Krebs cycle during respiration)

Examples of coenzyme functions

- During many of the reactions in **respiration**, the coenzymes **NAD** and **FAD** are alternately **reduced** and **oxidised**, **transferring energy** in the form of **hydrogen ions**
- The coenzyme **NADP** fulfils this same role in chloroplasts during **photosynthesis**
- The coenzymes **ATP** and **coenzyme A** act in a different way, by **transferring chemical groups**. For example:
  - ATP is responsible for the transfer of **phosphate groups** between respiration and energy-consuming processes in cells
  - Coenzyme A is responsible for the transfer of an **acetyl group** (-CH_3CO) from fatty acids and glucose during respiration

2.4 Enzymes

YOUR NOTES



Coenzymes are a type of cofactor that interact with enzymes and allow them to function properly

Prosthetic groups

- Some cofactors are actually a **permanent part** of the structure of the enzyme they assist
  - These cofactors are known as **prosthetic groups**
- Prosthetic groups are essential to the enzyme **functioning properly**, as they help to form the final **3D shape** of the enzyme
  - For example, by forming part of the **active site** of the enzyme, a **zinc ion** acts as the prosthetic group for **carbonic anhydrase** (an enzyme found in red blood cells that converts CO_2 and H_2O into carbonic acid, H_2CO_3)

2.4 Enzymes

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Summary

- **Cofactors** are **non-protein** substances (i.e. not made from amino acids) that enzymes require in order to function properly. Cofactors can be a **temporary** part of the enzyme or a **permanent** part (known as a **prosthetic group**)
- **Coenzymes** are **organic** non-protein cofactors. Coenzymes contribute to enzyme-catalysed reactions by **accepting** or **donating hydrogen ions** or **chemical groups** (e.g. phosphate groups)

Exam Tip

For exam questions on this topic, you do not need to learn the names of the various coenzymes described above (although these will need to be learnt for later A level topics on respiration and photosynthesis)! In addition, you do not need to learn the names of the specific vitamins here, just that vitamins of the B group are used in the production of several important coenzymes.

YOUR NOTES ⬇

2.4 Enzymes

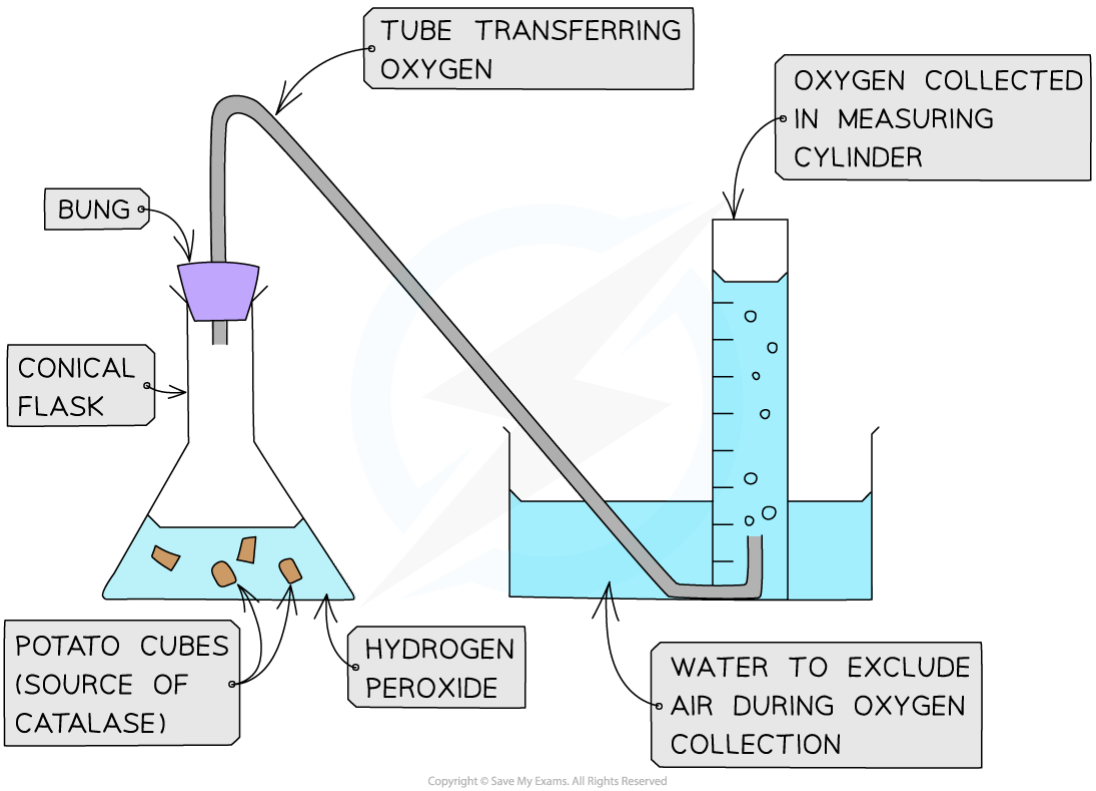
2.4.9 PRACTICAL: MEASURING ENZYME ACTIVITY

Practical: Measuring Enzyme 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**

Investigating catalase activity

- 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



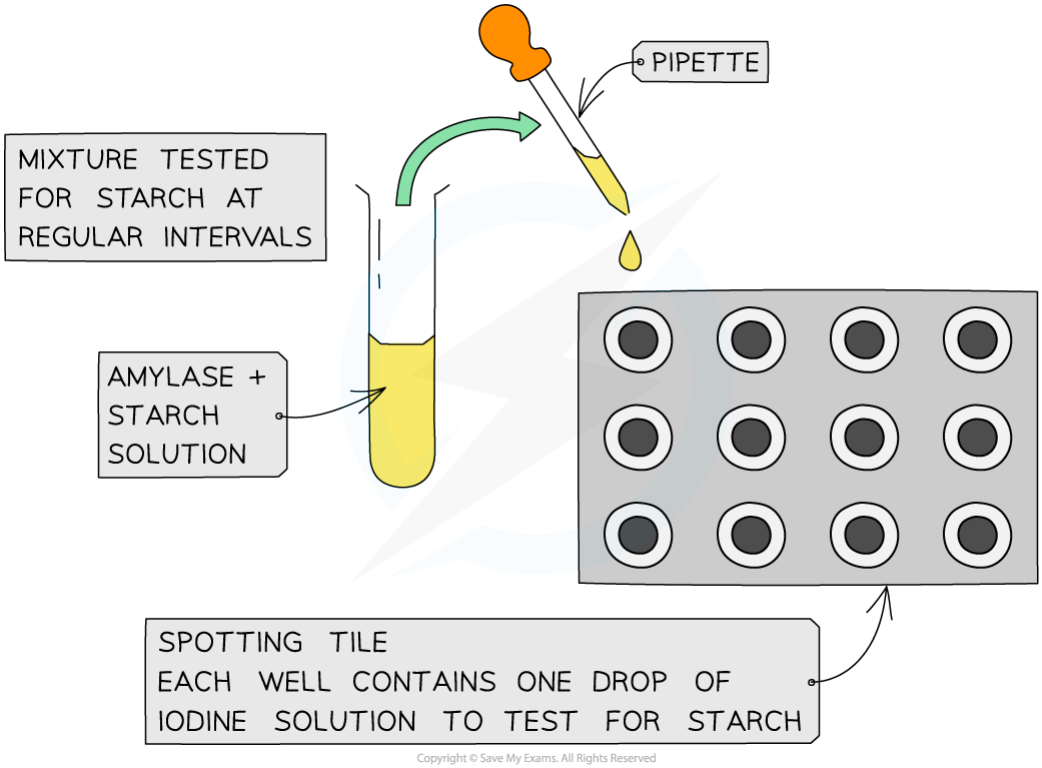
Experimental set-up for investigating the rate of formation of a product using catalase

2.4 Enzymes

YOUR NOTES ⬇

Investigating amylase activity using iodine

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



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

2.4 Enzymes

YOUR NOTES ⬇

Investigating the effect of starch concentration on amylase activity using colorimetry

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

2.4 Enzymes

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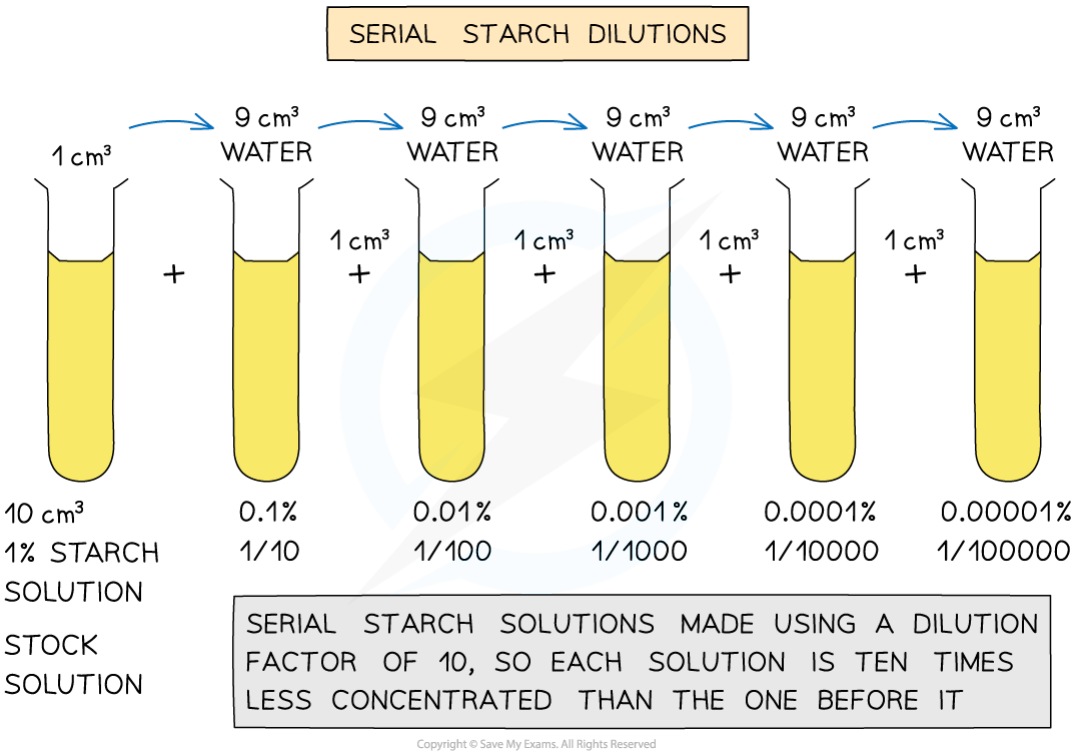

Serial dilution of starch to make a range of concentrations