Enzymes

Describe the mode of action of enzymes.	
Interaction and Binding	Enzymes have an **specific active site** which is **complementary in shape and charge** to the substrate. **Effective collisions** between the enzyme and substrate cause the substrate to bind to the enzyme to form a temporary **enzyme-substrate complex**. The enzyme-substrate complex is held together by **weak interactions** such as **hydrogen bonds**, **ionic bonds**, and **hydrophobic interactions**. Based on the lock and key hypothesis, **enzyme** is the **lock** and **substrate** is the **key**. Based on the **induced fit** hypothesis, the binding of the substrate **induces a conformational change** in the enzyme **active site** so that it becomes a **more precise fit** for the substrate for **more effective catalysis**.
Catalysis (Choose **2**)	Enzyme **lowers the activation energy** barrier by a. Aligning substrates **next to each other** in the active site, increasing the chances of reaction in the **proximity effect**. b. Causing **strain on the bonds** in the substrate to be broken as they bind to the active site, increasing the chances of bond breakage in the **strain effect**. c. Orientating the substrate such that its **bonds are exposed** to attack in the **orientation effect**. d. Providing a **favorable microenvironment** for reaction in the **microenvironment effect**. e. Participating **directly** in catalysis with **R groups** of amino acid residues in **acid-base catalysis**. This increases the **proportion** of **substrate molecules** with **energy greater than the activation energy**, allowing the reaction to proceed **faster** than an uncatalyzed reaction.
Release	Products **no longer fit active site** and are **released**. The **enzyme** is **unchanged** and can be used again.

Amino Acids Residues	Structural Residues	They interact with each other to maintain the overall 3D conformation of the protein.
	Contact Residues	They are found in the active site and hold the substrate in the correct orientation in the active site by weak interactions such as hydrogen bonds and ionic bonds.
	Catalytic Residues	They are found in the active site and act on the bonds in the substrate, catalyzing the conversion of substrate to product.
	Non-Essential Residues	Non-essential residues are found on the surface of the protein and have no specific function.
Active Site	Structure	<u>Describe how amino acid residues at different positions in the protein may be brought together in the active site.</u> The **primary structure** of the polypeptide is the sequence of amino acids which **determines** its **secondary** and **tertiary structure**. The polypeptide is folded into its **secondary structure** such as the **α-helix** and **β-pleated sheets** through **hydrogen bonding** between the **–CO and –NH groups** of the **polypeptide backbone**. The secondary structure is **further folded** into its **tertiary structure** via **hydrogen bonds**, **ionic bonds**, **hydrophobic interactions** and **disulfide linkages** which are formed between the **different R groups** of the amino acids. The **folding** of polypeptide thus results in a **globular structure** where the amino acids are brought closer together at the active site.
	Denaturation	<u>Explain the effect of denaturation on the enzyme active site.</u> Denaturation results when the **hydrogen bonds**, **ionic bonds** and **hydrophobic interactions** that **stabilize** the 3D conformation of the enzyme are **broken**. Hence, the conformation of the active site is **altered** such that it is **no longer complementary in shape and charge** to the substrate.
	K_m	The substrate concentration where the reaction proceeds at **half its maximum rate**. The **lower the K_m**, the **higher the affinity** of the enzyme for the substrate.

Enzyme Cofactors	Inorganic Ions	Mold the enzyme or substrate and allow the **enzyme-substrate complex** to **form more easily**. Example: **Mg^{2+}** in PCR.
	Prosthetic Group	**Permanently bound** to the enzyme and **transfers chemical groups** between the active site of the enzyme and another substance. Example: **Heme group** in **cytochrome oxidase** in the electron transport chain accepts electrons from cytochrome C and transfers them to water.
	Coenzymes	**Organic molecules** that are required by certain enzymes to carry out catalysis. They **bind to the active site** of the enzyme and participate in the catalysis but are not considered substrates. They function as **intermediate carriers** of electrons or atoms that are transferred in the reaction. Example: **NADH** in respiration.

Explain how temperature affects the rate of an enzyme catalyzed reaction.	
Before Optimum Temperature	Before the optimum temperature is reached, increasing temperature **increases the kinetic energy** of substrate and enzyme molecules, increasing the **frequency of effective collisions** between enzyme and substrate and hence the **rate of formation of enzyme-substrate complexes**. Increasing temperature also increases the **proportion of substrate molecules** with energy exceeding the **activation energy** necessary for the reaction to proceed. The rate of reaction typically **doubles** with every **10^{o}C increase** in temperature.
Optimum Temperature	**Maximum rate of reaction** is reached at the optimum temperature.
Beyond Optimum Temperature	Increases in temperature beyond the optimal results in a **drastic drop** in rate of reaction as it increases **intramolecular vibrations** within the enzyme, breaking **weak interactions** such as **hydrogen bonds**, **ionic bonds** and **hydrophobic interactions** that determine the enzyme conformation, resulting in **denaturation** of the enzyme. The active site is thus **no longer complementary in shape and charge** to the substrate. Hence, **less enzyme-substrate complexes** can be formed and the rate of reaction decreases.

Explain how pH affects the rate of an enzyme catalyzed reaction.	
Optimum pH	Each enzyme has an **optimum pH** at which the rate of the enzyme-catalyzed reaction is **maximum** as the **shape and charge** of the **active site** is **most ideal for substrate binding**.
Deviation from Optimum pH	Any **deviation** from the optimum pH results in **excess H^+** or **OH^-** ions, affecting the **ionization** of R groups of **charged** amino acids, with **–COOH** groups become **$–COO^-$** groups in **excess OH^-** and **$–NH_2$** groups becoming **$–NH_3^+$** groups in **excess H^+**. When charges on R groups of **structural residues** are altered, **ionic bonds** and **hydrogen bonds** which maintain the overall **3D conformation** of the enzyme are disrupted, resulting in **denaturation** of the enzyme. The **interaction** between the **substrate** and the **enzyme active site** is also disrupted. The **specific charges** of the R groups of **catalytic residues** or **contact residues** in the enzyme active site may be changed, causing a **loss in catalytic activity** and affecting the **binding** of the enzyme and substrate respectively. Hence, the enzyme active site is no longer **complementary in shape and charge** to the substrate and **less enzyme-substrate complexes** are formed. Hence, there is a lower rate of reaction.

Explain how enzyme concentration affects the rate of an enzyme catalyzed reaction.	
Linear Portion	At low enzyme concentration, as enzyme concentration increases, the **frequency of effective collisions** between the enzyme and substrate molecules increases, increasing the rate of **enzyme-substrate complex formation** and hence increasing the rate of reaction **proportionally**.
Curved Portion	As enzyme concentration increases, it is **not the only limiting factor**, and some other factors are also limiting.
Plateau	Eventually, enzyme concentration is **no longer limiting** and some other factors are limiting. Increasing enzyme concentration will not increase the rate of reaction.

Explain how substrate concentration affects the rate of an enzyme catalyzed reaction.	
Linear Portion	At low substrate concentration, as substratecon centration increases, the **frequency of effective collisions** between the enzyme and substrate molecules increases, increasing the rate of **enzyme-substrate complex formation** and hence increasing the rate of reaction as the **active sites** of the enzymes are **readily available** to catalyze the reaction and **substrate concentration** is **limiting**
Curved Portion	As substrate concentration increases, the rate of reaction increases as **more active sites are occupied** by substrates.
Plateau	Eventually, all **active sites** of enzymes are **saturated** with substrate at **any one time** and hence **enzyme concentration** instead of substrate concentration becomes **limiting**. Further increase in substrate concentration will not increase the rate of reaction.

Compare competitive and non-competitive inhibitors.		
Point of Comparison	**Competitive Inhibitors**	**Non-Competitive Inhibitors**
Structure	**Similar** in **shape and charge** to the substrate.	**Not similar** in shape and charge to the substrate.
Binding Site	Competes with the substrate for the **active site**, binding **reversibly** to active site to **block** substrate binding.	Binds to **site other than active site**.
Effect	Decreases the rate of **enzyme-substrate complex formation** and hence the rate of reaction. K_m decreases.	Changes the conformation of the **active site**, such that it is **no longer complementary in shape and charge** to the substrate. Forms an **enzyme-inhibitor complex** hence decreases the availability of the enzymes for substrate binding and the rate of **enzyme-substrate complex formation** and hence the rate of reaction. K_m remains the same.
High Substrate Concentrations	At high substrate concentrations, the effects of the competitive inhibitor can be **overcome** as the **higher proportion** of substrate molecules can **outcompete** the inhibitor molecules for the **active site**. Hence, at high substrate concentrations, the rate of reaction is the **same** as that when no inhibitor is present. Hence, V_{max} is the same.	The effects of the non-competitive inhibitor **cannot be overcome** at high substrate concentrations. Hence, V_{max} decreases.

Describe the regulation of an allosteric enzyme.
An allosteric enzyme is typically **multimeric**, comprising of two or more subunits, each with their own **active sites** and **allosteric sites**. The enzyme oscillates between **two conformational states** – the **active conformation** and the **inactive conformation**. **Allosteric activators** and **inhibitors** are both **not similar** in shape and charge to the substrate and bind to the enzyme at the **allosteric sites**. The binding of an **allosteric activator stabilizes** the enzyme in its **active conformation** and increases the rate of reaction while binding of an **allosteric inhibitor stabilizes** the enzyme in its **inactive conformation** and decreases the rate of reaction.