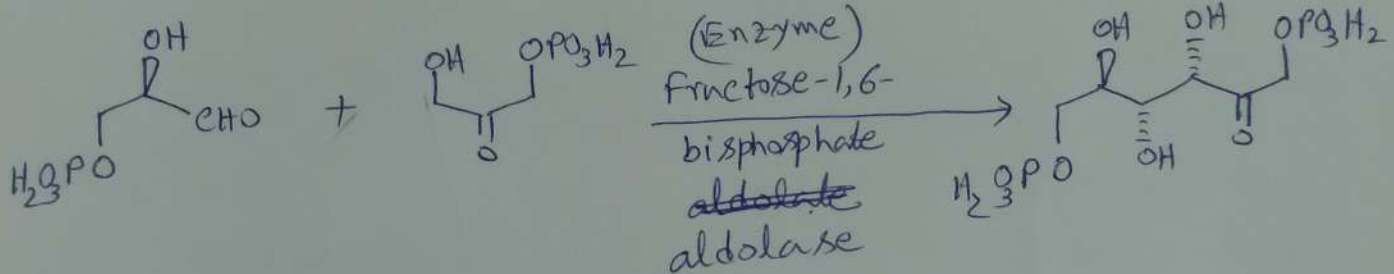
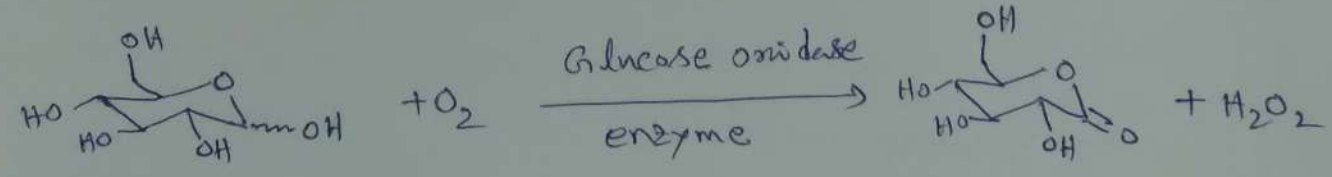
- Enzyme which is used for asymmetric aldol condensation.
- Enzyme which catalyzes asymmetric aldol reaction in metabolism is called 'aldolase'.

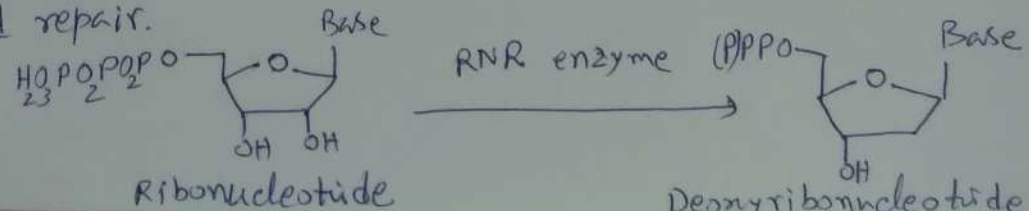
Example: In the sugar building biochemical pathway in the aldol reaction between glyceraldehyde-3-phosphate and dihydroxyacetone phosphate to form fructose-1,6-bisphosphate, we can see that fructose-1,6-bisphosphate aldolase acts an ~~an~~ enzyme.



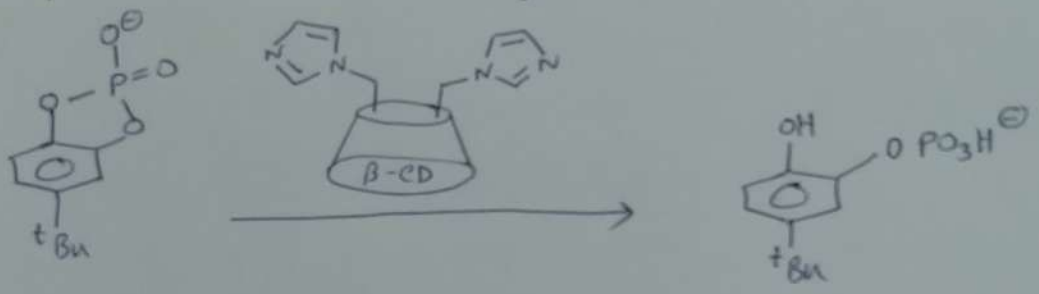
- Enzyme catalyzed oxidation reaction.
- D-Glucose is oxidized to D-glucono-1,5-lactone by glucose oxidase enzyme. This is an example of enzyme catalyzed oxidation reaction.



- Enzyme catalyzed reduction reaction.
- Ribonucleotide reductase (RNR) is a key enzyme that ribonucleotides to deoxyribonucleotides, the building blocks for DNA replication and repair.

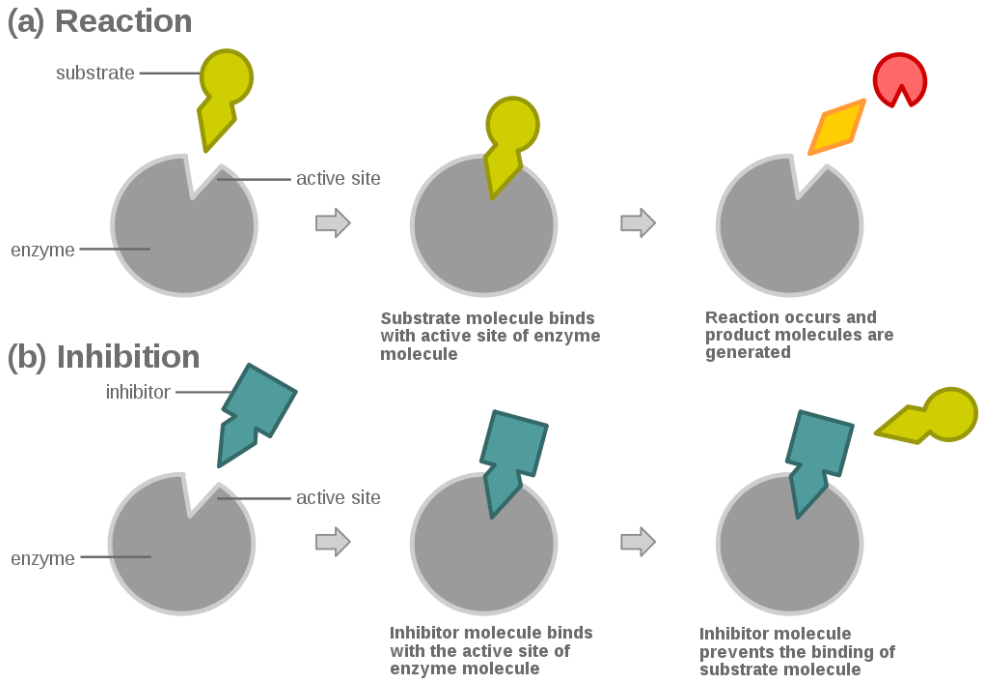


- Model enzyme based on cyclodextrin and its function.
- Cyclodextrin bis-imidazole is an example of model enzyme based on β-cyclodextrin which can catalyze the hydrolysis of cyclic phosphate ester, analogous to the process carried out by the enzyme ribonuclease-A.

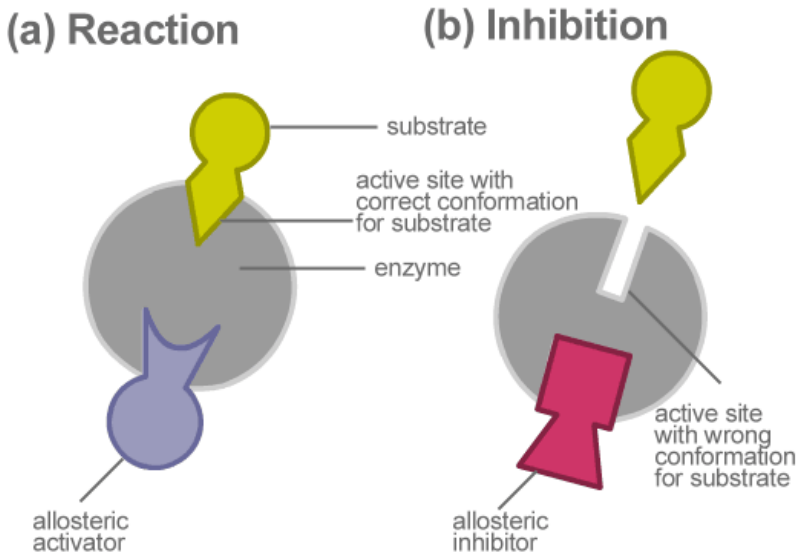


Q) What do you mean by competitive inhibition and allosteric inhibition?

Competitive inhibition **occurs when molecules very similar to the substrate molecules bind to the active site and prevent binding of the actual substrate**. Penicillin, for example, is a competitive inhibitor that blocks the active site of an enzyme that many bacteria use to construct their cell

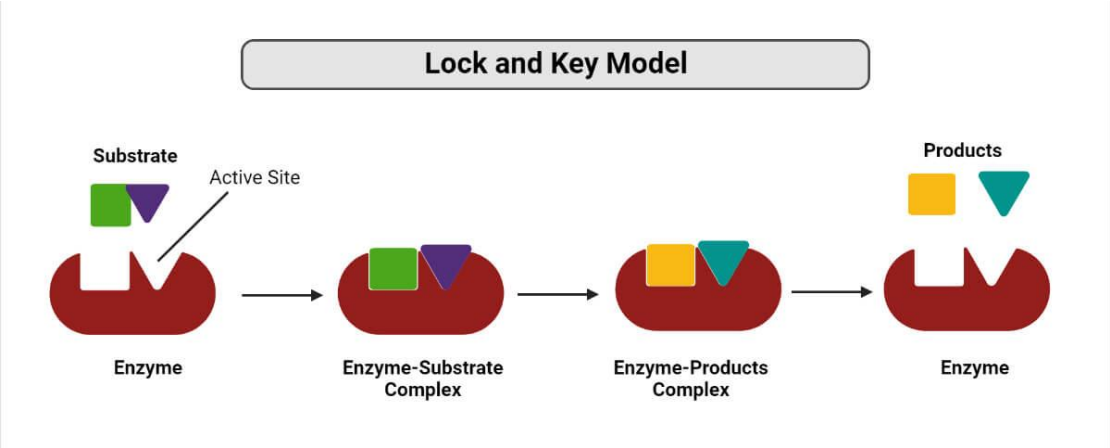


Allosteric Inhibition: **When an inhibitor binds to the enzyme, all the active sites of the protein complex of the enzyme undergo conformational changes so that the activity of the enzyme decreases**. In other words, an allosteric inhibitor is a type of molecule which binds to the enzyme specifically at an allosteric site.



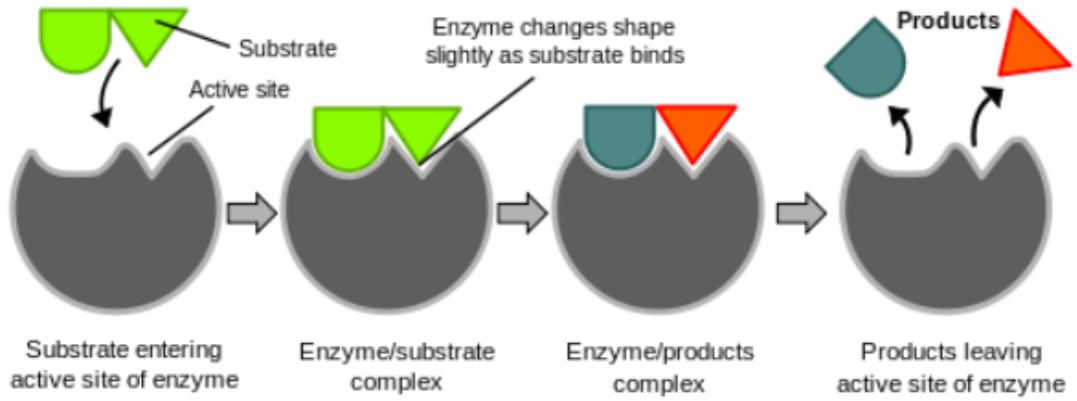
Q) Explain the Fischer lock and key model for enzyme substrate interaction.

To explain the observed specificity of enzymes, Emil Fischer proposed that both the enzyme and the substrate possess specific complementary geometric shapes that fit exactly into one another. This is often referred to as "the lock and key" model. This early model explains enzyme specificity, but fails to explain the stabilization of the transition state that enzymes achieve.[



Q) Describe the Koshland induced fit model for enzyme action on substrate.

Koshland suggested a modification to the lock and key model: since enzymes are rather flexible structures, the active site is continuously reshaped by interactions with the substrate as the substrate interacts with the enzyme. As a result, the substrate does not simply bind to a rigid active site; the amino acid side-chains that make up the active site are moulded into the precise positions that enable the enzyme to perform its catalytic function. In some cases, substrate molecule also changes shape slightly as it enters the active site. In this way the final product is formed.



Q) What are the factors that affect enzyme activity?

Enzyme activity is affected by a number of factors including the concentration of the enzyme, the concentration of the substrate, the temperature, the pH, and the salt concentration.

Michaelis-Menten (steady-state) Kinetics

The Michaelis-Menten model for enzyme kinetics presumes a simple 2-step reaction:

Step 1: Binding – the substrate binds to the enzyme

Step 2: Catalysis – the substrate is converted to product and released

(Note that enzymes not matching this reaction scheme may still show similar kinetics.)

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

Binding Catalysis

The Michaelis-Menten equation shows how the initial rate of this reaction, V_o, depends on the substrate concentration, [S]: $V_o = \frac{V_{max}[S]}{K_m + [S]}$

Several simplifying assumptions allow for the derivation of the Michaelis-Menten equation:

(1) The binding step ($E + S \rightleftharpoons ES$) is fast, allowing the reaction to quickly reach equilibrium ratios of [E], [S], and [ES]. The catalytic step ($ES \rightleftharpoons E + P$) is slower, and thus rate-limiting.

(2) At early time points, where initial velocity (V_o) is measured, [P] ≈ 0.

(3) ES immediately comes to steady state, so [ES] is constant (throughout the measured portion of the reaction).

(4) [S] >> [E_T], so the fraction of S that binds to E (to form ES) is negligible, and [S] is constant at early time points.

(5) The enzyme exists in only two forms: free (E), and substrate-bound (ES). Thus, the total enzyme concentration (E_T) is the sum of the free and substrate-bound concentrations: [E_T] = [E] + [ES]

A derivation of the Michaelis-Menten equation shows how to use the above assumptions to describe the rate of the enzyme-catalyzed reaction in terms of measurable quantities:

From (1), we know the overall rate of the reaction is determined by the rate of the catalytic step:	$V_o = k_2[\mathrm{ES}] - k_{-2}[\mathrm{E}][\mathrm{P}]$
From (2), the second term equals zero, so we are left with:	$V_o = k_2[\mathrm{ES}]$
We want to describe V_o in measurable quantities, but [ES] is not easy to measure. However [S] is known, from (4). To express [ES] in terms of [S], we can start from (3):	Rate of formation of ES = Rate of breakdown of ES $k_1[\mathrm{E}][\mathrm{S}] + k_{-2}[\mathrm{E}][\mathrm{P}] = k_{-1}[\mathrm{ES}] + k_2[\mathrm{ES}]$
From (2), this simplifies to:	$k_1[\mathrm{E}][\mathrm{S}] = k_{-1}[\mathrm{ES}] + k_2[\mathrm{ES}]$
We can factor out [ES] and group the rate constants:	$k_1[\mathrm{E}][\mathrm{S}] = [\mathrm{ES}]\{k_{-1} + k_2\}$ $[\mathrm{E}][\mathrm{S}] = [\mathrm{ES}]\left\{\frac{k_{-1} + k_2}{k_1}\right\}$
This ratio of rate constants is defined as **the Michaelis Constant, K_m**:	$\boldsymbol{K_m = \frac{k_{-1} + k_2}{k_1}}$
Substituting in K_m for the rate-constant ratio gives:	$[\mathrm{E}][\mathrm{S}] = [\mathrm{ES}]K_m$
Just as [ES] is not easy to measure, [E] is also not easy to measure. However, [E_T] is known. Rearranging (5) for [E] and substituting, we get:	$\{[\mathrm{E_T}] - [\mathrm{ES}]\}[\mathrm{S}] = [\mathrm{ES}]K_m$
We are still trying to get an expression for [ES] in terms of measurable quantities. Here we can multiply, rearrange, factor, and divide, to get [ES] in terms of [E_T], [S], and K_m:	$[\mathrm{E_T}][\mathrm{S}] - [\mathrm{ES}][\mathrm{S}] = [\mathrm{ES}]K_m$ $[\mathrm{E_T}][\mathrm{S}] = [\mathrm{ES}]K_m + [\mathrm{ES}][\mathrm{S}]$ $[\mathrm{E_T}][\mathrm{S}] = [\mathrm{ES}]\{K_m + [\mathrm{S}]\}$ $\frac{[\mathrm{E_T}][\mathrm{S}]}{K_m + [\mathrm{S}]} = [\mathrm{ES}]$
Now we can substitute our expression for [ES] into the rate equation:	$V_o = k_2[\mathrm{ES}] = \frac{k_2[\mathrm{E_T}][\mathrm{S}]}{K_m + [\mathrm{S}]}$
At high [S] (when [S] >>> K_m), nearly all enzyme will have substrate bound, and [ES] approaches [E_T]. This is when V_o approaches V_{max}. Since $V_o = k_2$[ES], (Or, mathematically, when [S] >>> K_m, K_m is negligible, and the equation simplifies to:)	$V_{max} = k_2[E_T]$ $\left(V_{max} = \frac{k_2[\mathrm{E_T}][\mathrm{S}]}{[\mathrm{S}]} = k_2[\mathrm{E_T}]\right)$
Substituting V_{max} in to the rate equation gives **the Michaelis-Menten equation**:	$\boldsymbol{V_o = \frac{V_{max}[\mathrm{S}]}{K_m + [\mathrm{S}]}}$