Enzymes and Kinetics

By Thomas Dlugosz

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Introduction to Enzymes

Activation Energy

Mechanisms of Enzyme Catalysis

Coenzymes

Classification of Enzymes

Michaelis Menten Kinetics

Lineweaver- Burk Plot

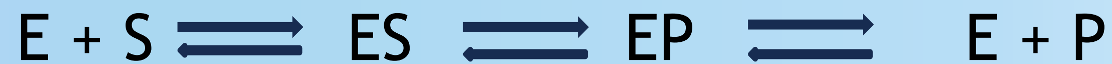
Enzyme Inhibitors

Regulation of Enzyme Activity

Introduction to Enzymes

- An enzyme is a **molecule** that is a catalyst to a chemical reaction

- A **catalyst** is a substance that speeds up the rate of reaction without being consumed or changed in the process
- Most enzymes are proteins, but not all (ribozymes: RNA based enzymes)



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Introduction to Enzymes

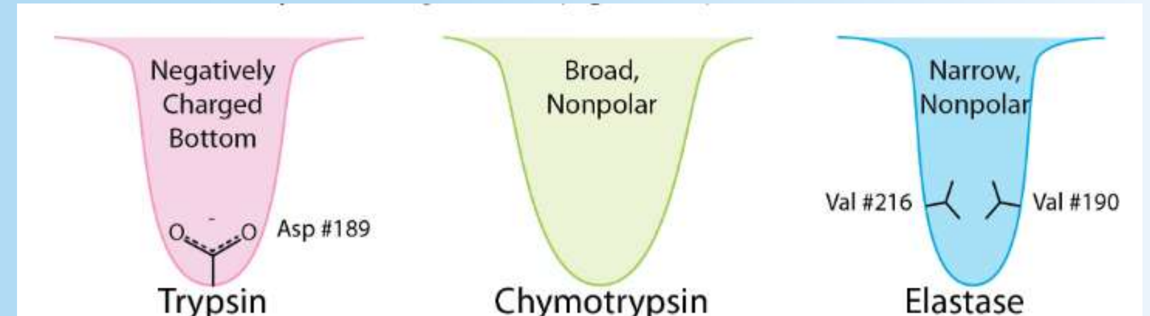
Active Site

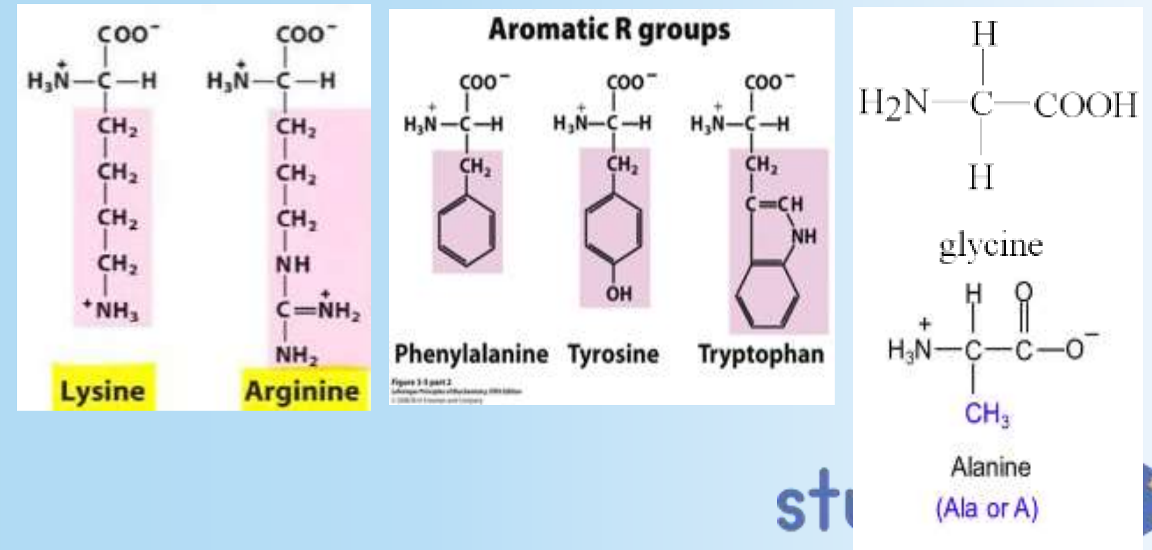
The active site is where the substrates bind and catalysis into product takes place

The active site is very specific to its substrates

Different amino acids form active sites. Their characteristics influence the **environment, 3D pocket dimensions, and substrate they bind with**

What amino acids would these proteases cleave?





Introduction to Enzymes

Allosteric Site

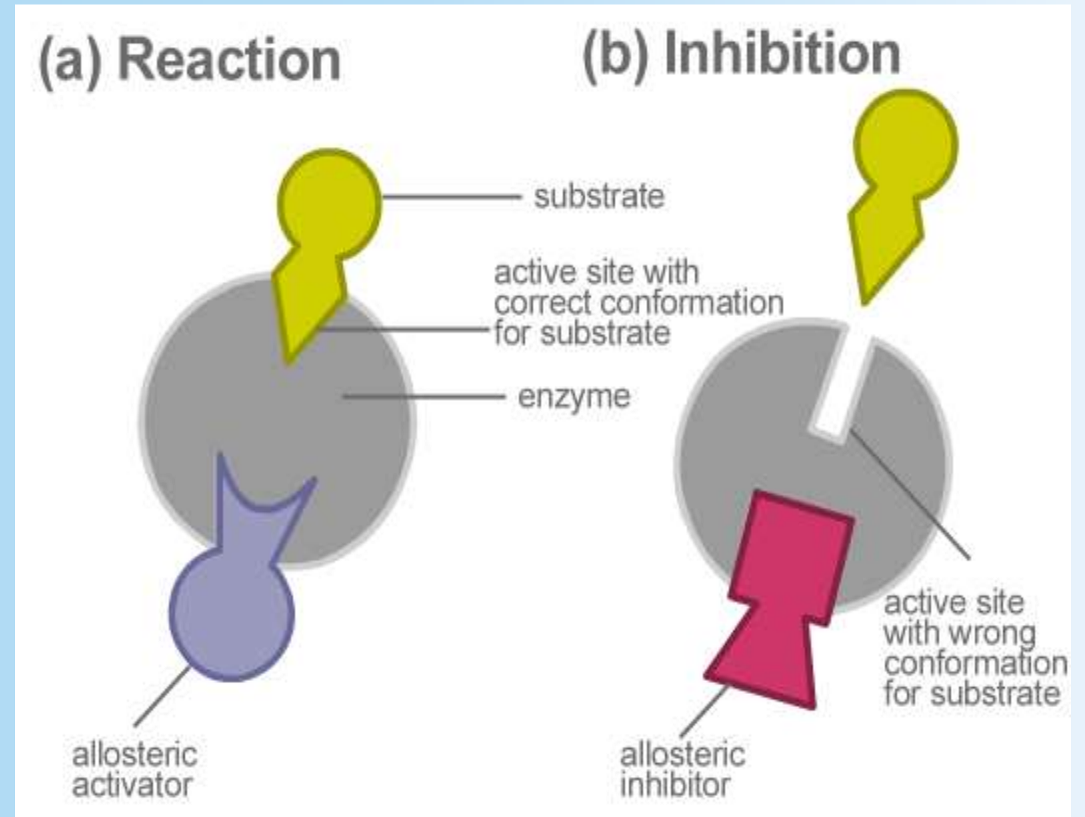
Non-catalytic regions of the enzyme that bind regulators.

The allosteric site is completely independent of the active site

Binding occurs via H-bonds, electrostatic interactions or hydrophobic interactions

If activity decreases → inhibitor

If activity increases → activator



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Activation Energy

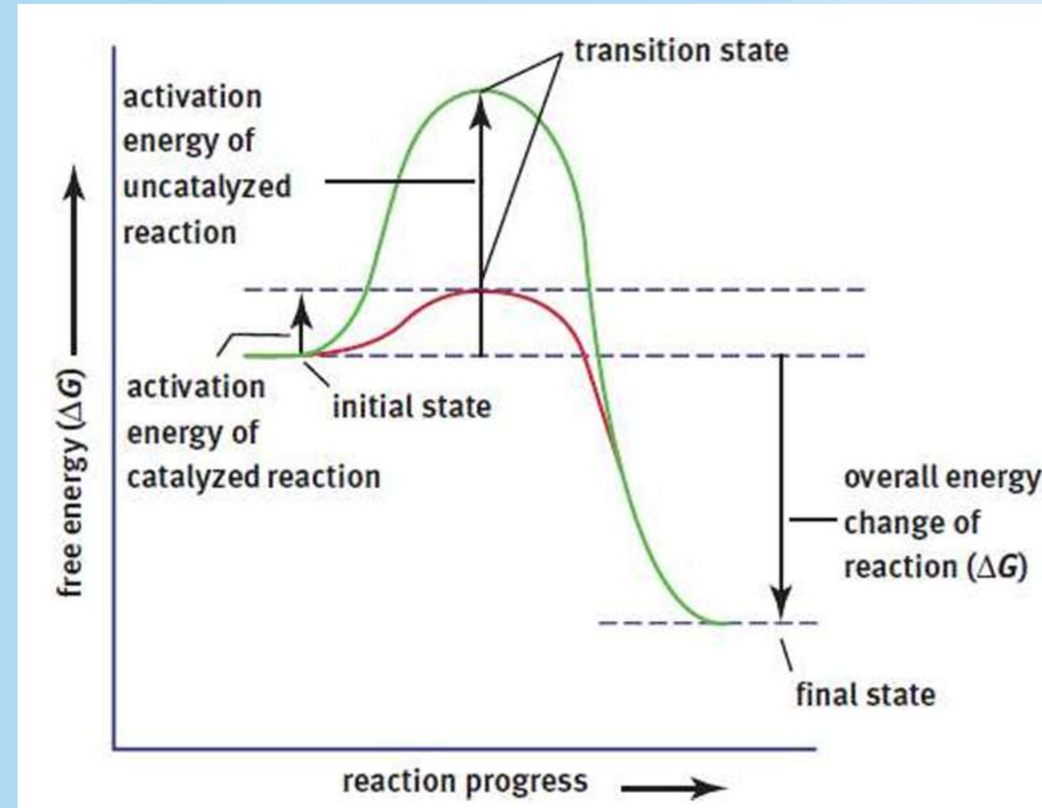
Catalysts speed up the rate of reaction by **lowering the activation energy (E_a)**

E_a : The energy required to overcome the barrier of forming product

- aligning reactants, electron repulsions, breaking bonds in reactants, **forming transition state**

Enzymes can ameliorate all these barriers to lower E_a

Notice: Overall ΔG and K_{eq} does not change!



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Mechanisms of Enzyme Catalysis

A. Catalysis by proximity and Orientation

B. Acid-Base Catalysis

C. Covalent Catalysis

D. Catalysis by Distortion

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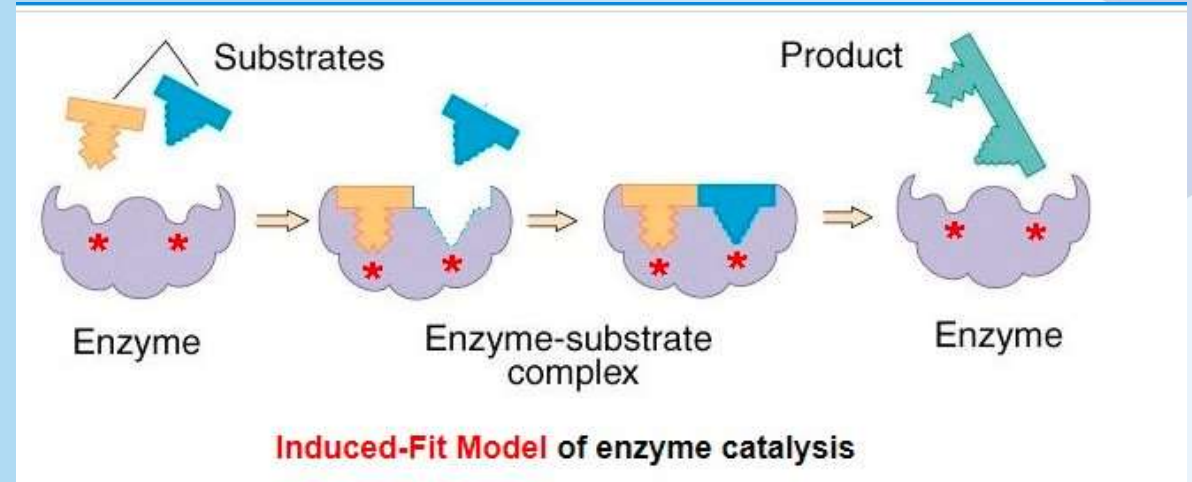
A. Catalysis by Proximity and Orientation

Enzyme holds molecules at the right distance and orientation to facilitate a reaction

- Substrate concentration is high in the active site
- Higher [substrate] → Higher collision frequency → Higher reaction rate



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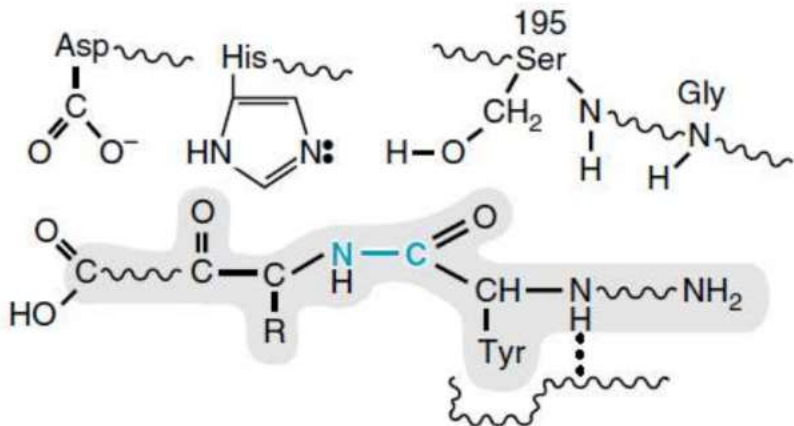
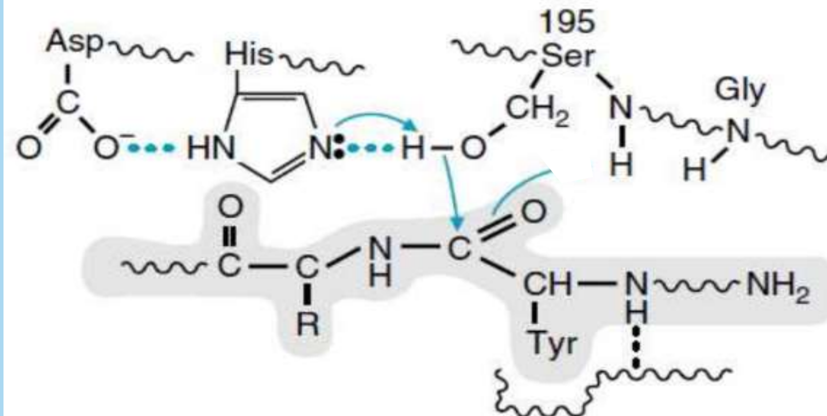
B. Acid Base Catalysis

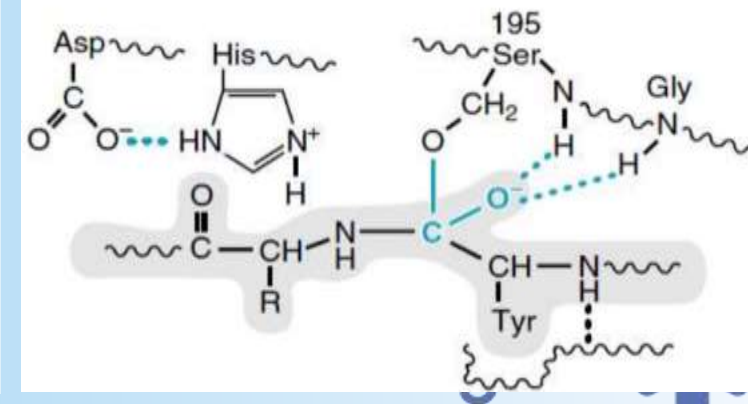
Enzyme side chains act as proton donors or acceptors to enhance reactivity or stabilize transition state intermediates

General Acid-Base Catalysis

Any acids (proton donors) or bases (proton acceptors) in solution participating in the reaction

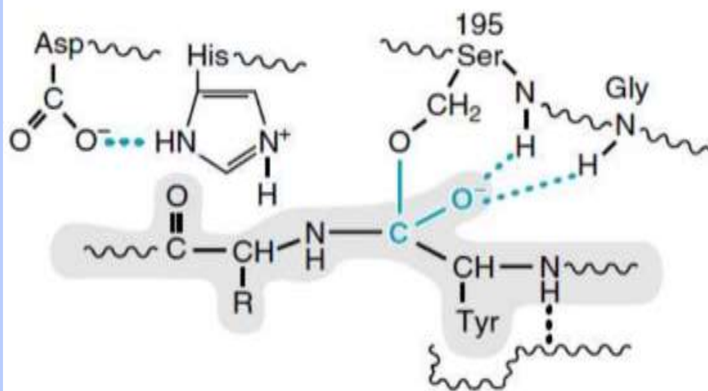


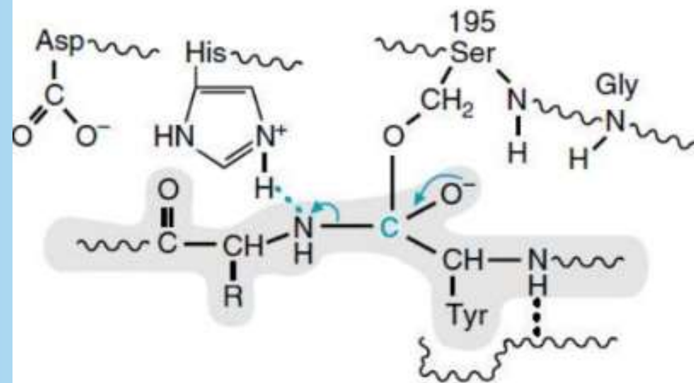


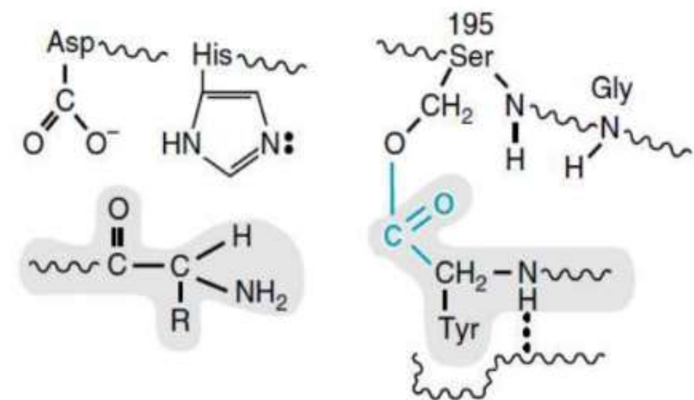


D. Covalent Catalysis

The enzyme forms a **temporary covalent bond** with the substrate via its active site residues. This creates an alternative reaction pathway with a lower activation energy.







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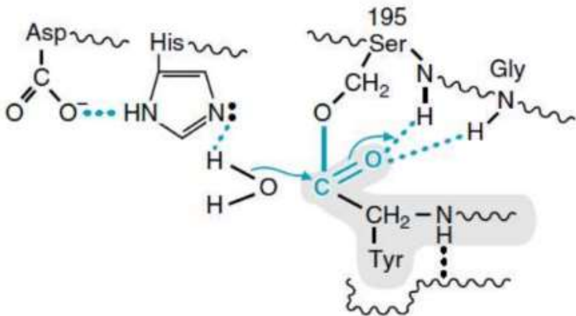

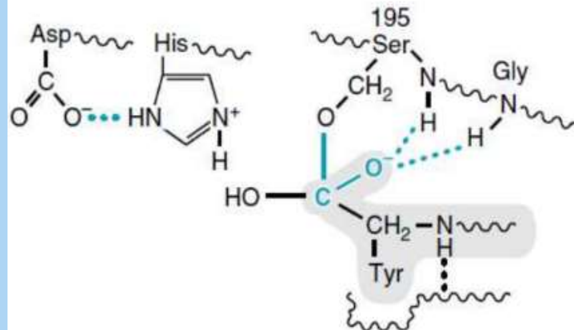

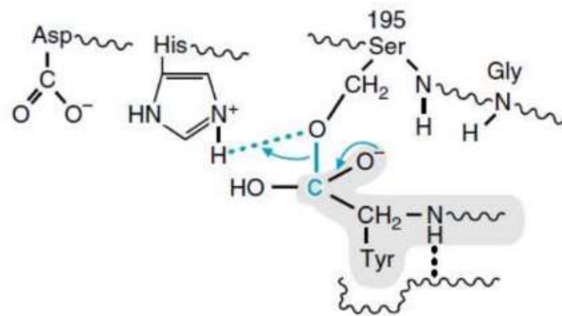

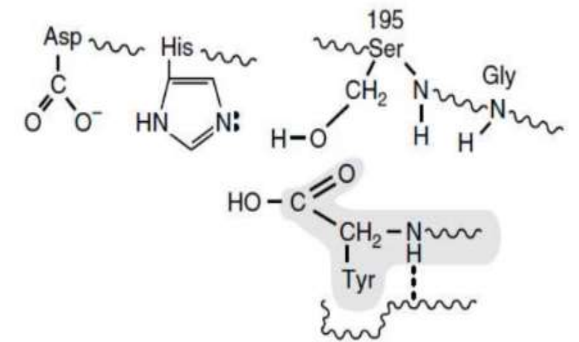

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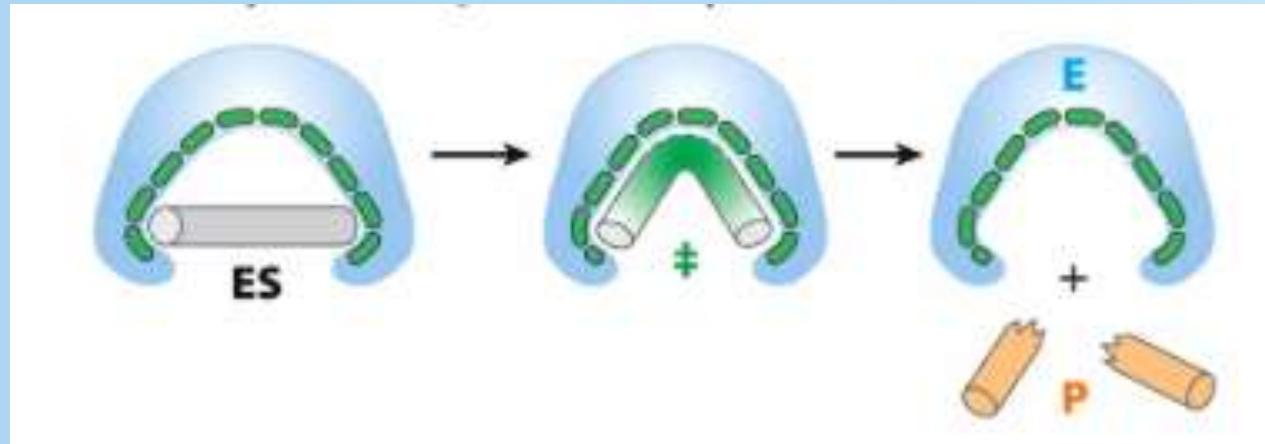
C. Catalysis by Distortion

Enzyme distorts a bond to weaken it and make it more susceptible to cleavage



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Coenzymes and Cofactors

Coenzymes and cofactors are small, non-proteinaceous molecules that bind enzymes and assist with reaction catalysis.

Coenzymes

Organic molecules

Ex: NAD^+, thiamine, coenzyme A

Cofactors

Metal ions

Ex: Mg^{2+}, Cu^+, Fe^{2+}

Cosubstrates

- Dissociate from enzyme in an altered form (NAD^+, FAD^+ , CoA)

Prosthetic Group

- Permanent part of enzyme (biotin, FAD, thiamine, Mg^{2+} , Fe^{2+})

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Classification of Enzymes

- Ligase
- Isomerase
- Lyase
- Hydrolase
- Oxidoreductase
- Transferase

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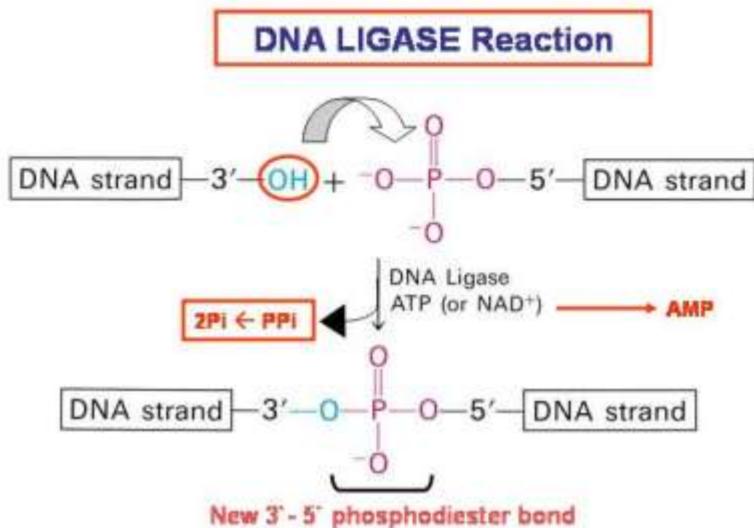
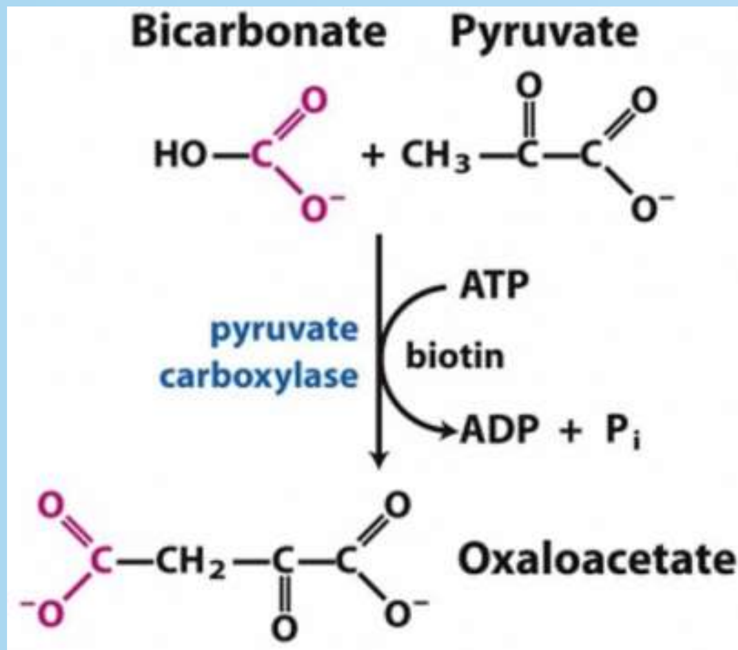
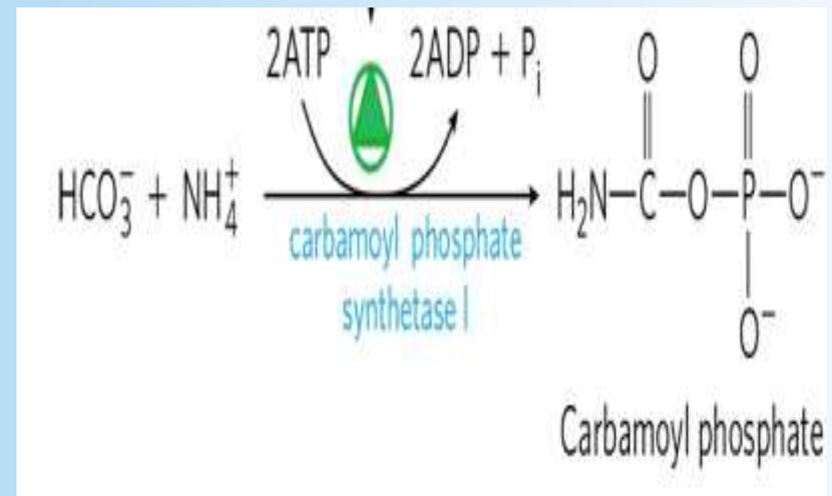
Ligase

- Join together two molecules by synthesis of a new bond **with simultaneous breakdown of nucleotide triphosphates**







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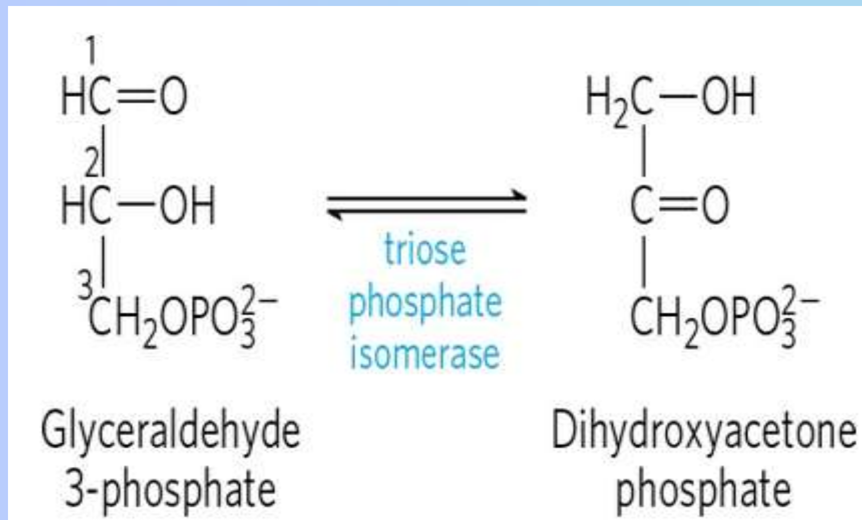
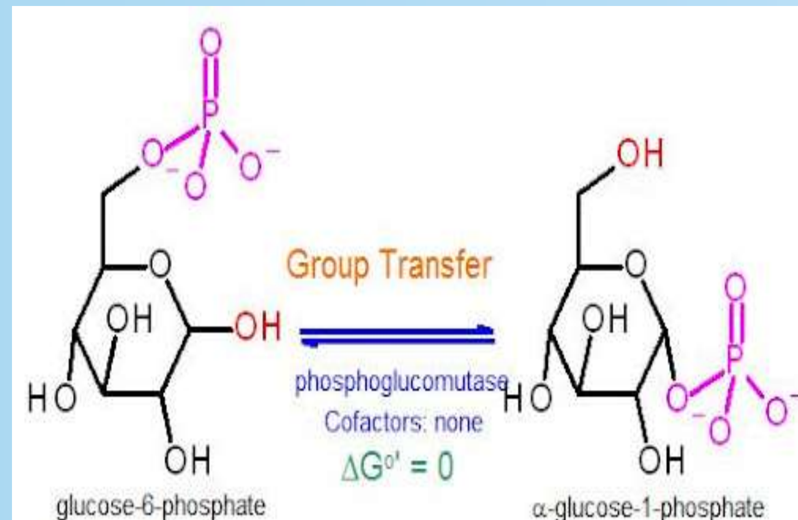
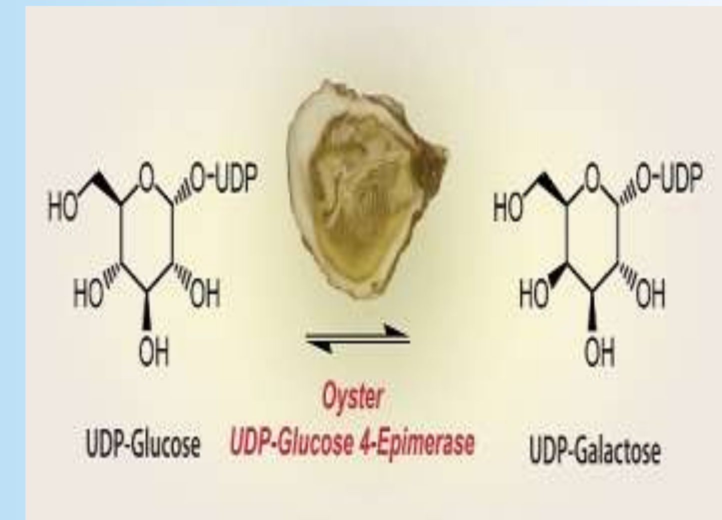
Isomerase

- Catalyze intramolecular rearrangement reactions
- If you see an entity move to a different spot or orientation, with no new atoms introduced or removed, it is an *isomerase*.







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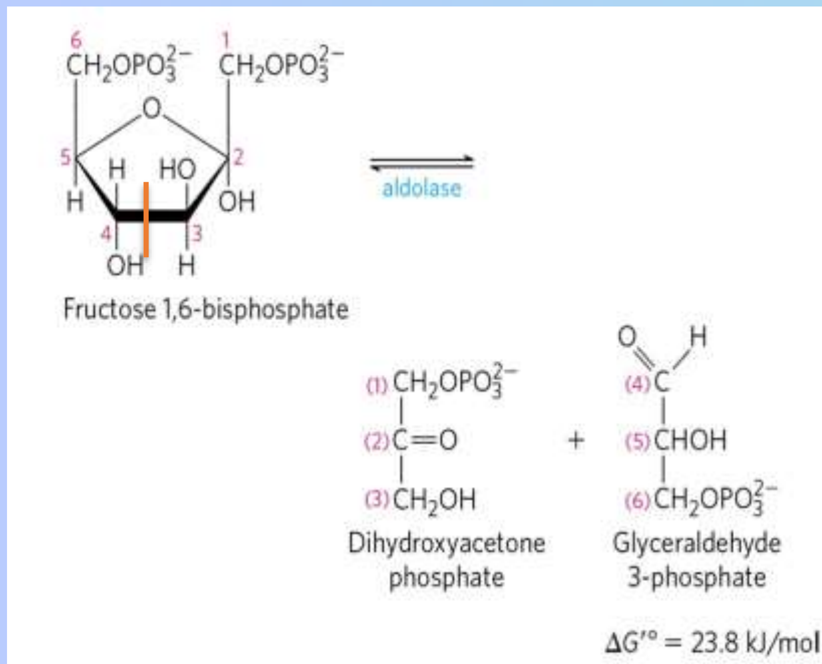
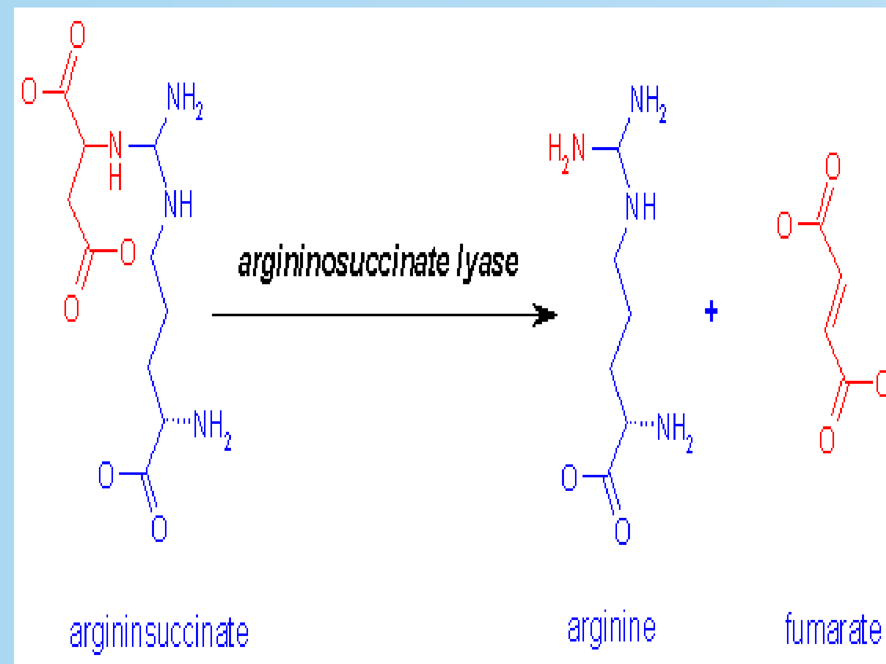
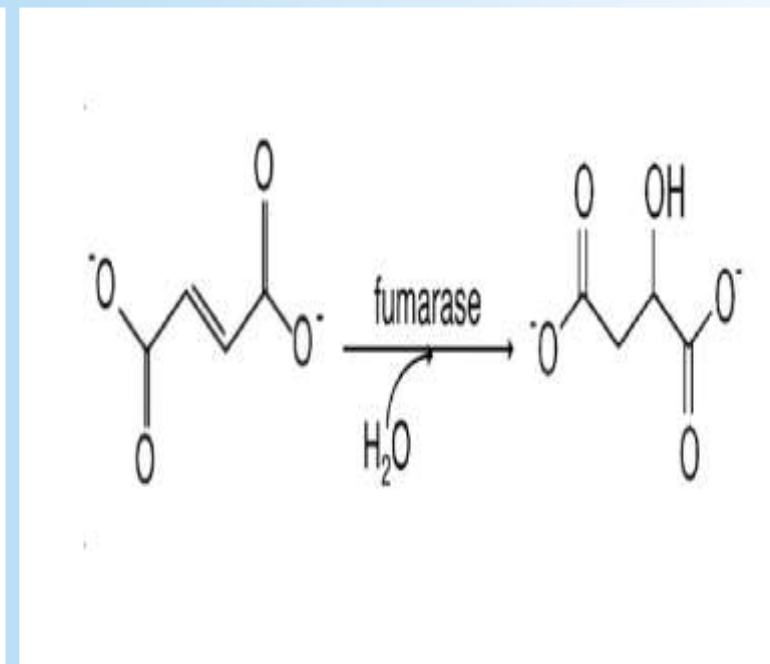
Lyase

- **Non-hydrolytic** addition or removal of groups from substrates.
- **Lyases** can break C-C, C-O, C-N or C-S bonds
- Lyases are ATP independent!

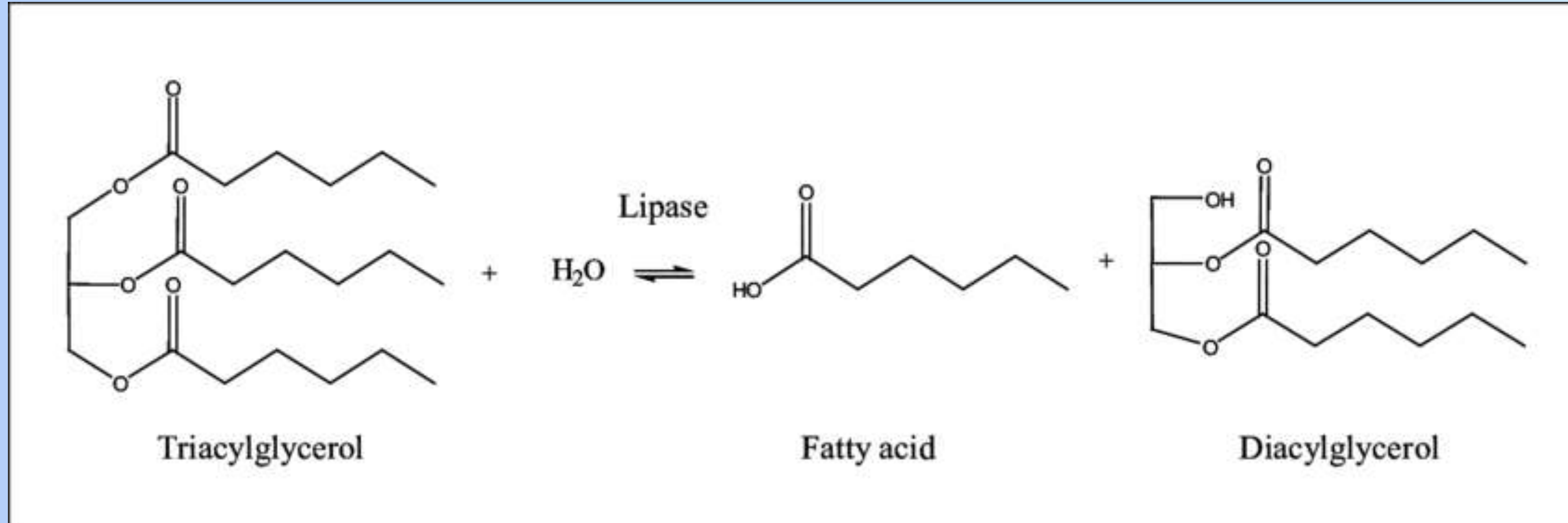


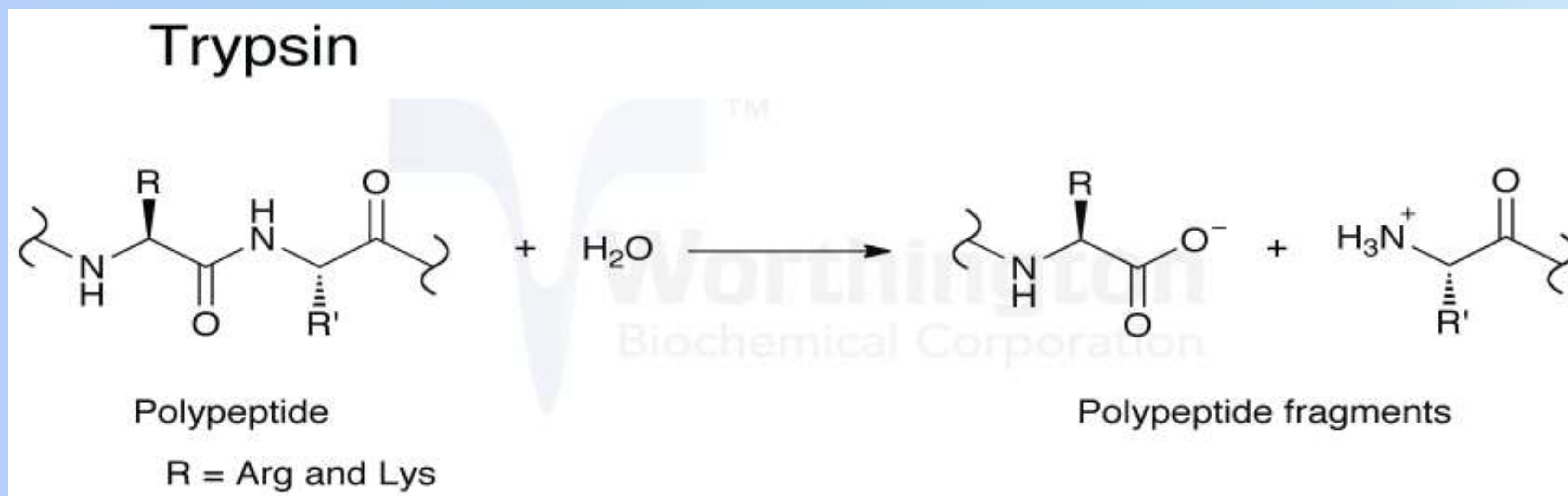




Hydrolases

- Water is used to break down a substrate into **2 products**





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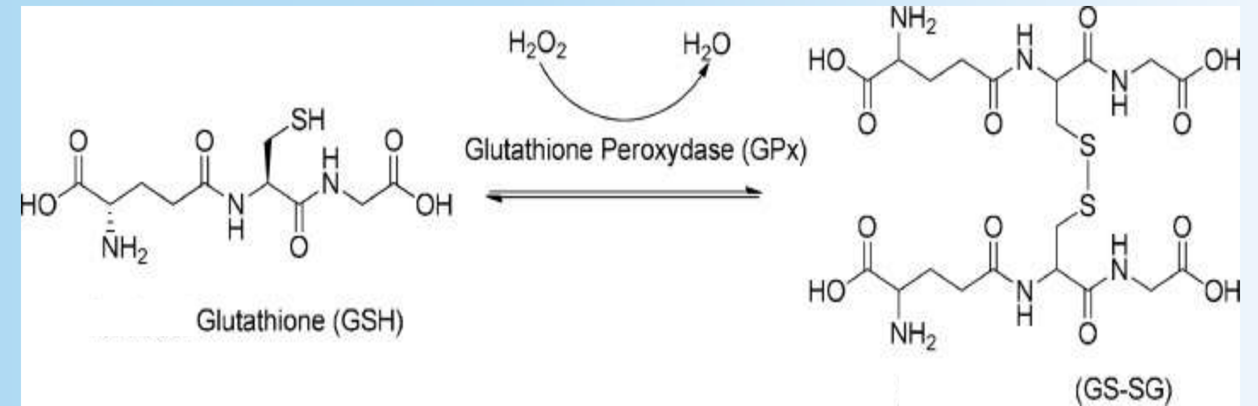
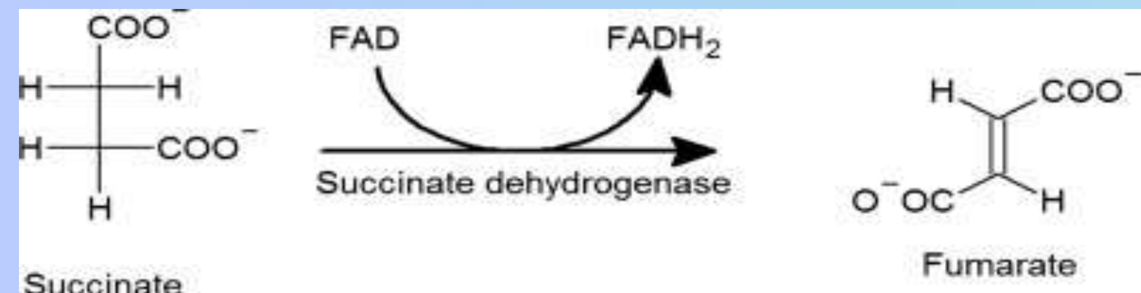
Oxidoreductases

- Catalyze oxidation-reduction reactions
- **In biochemistry: Loss of hydrogen is oxidation. Gain of hydrogens is reduction**





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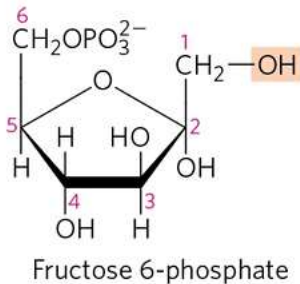
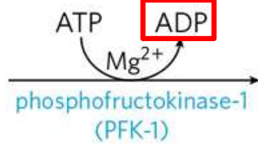
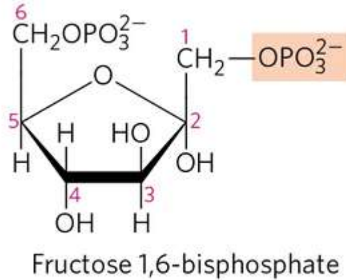
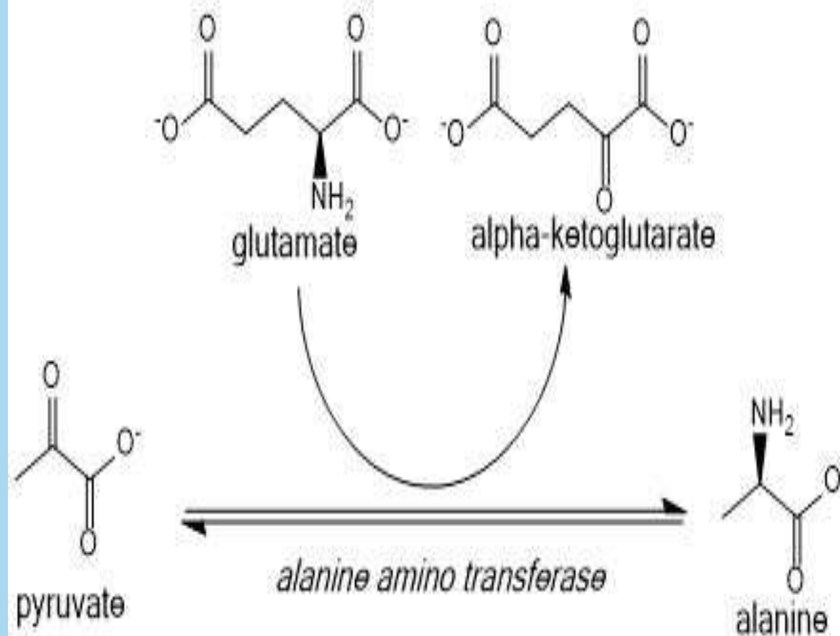
Transferase

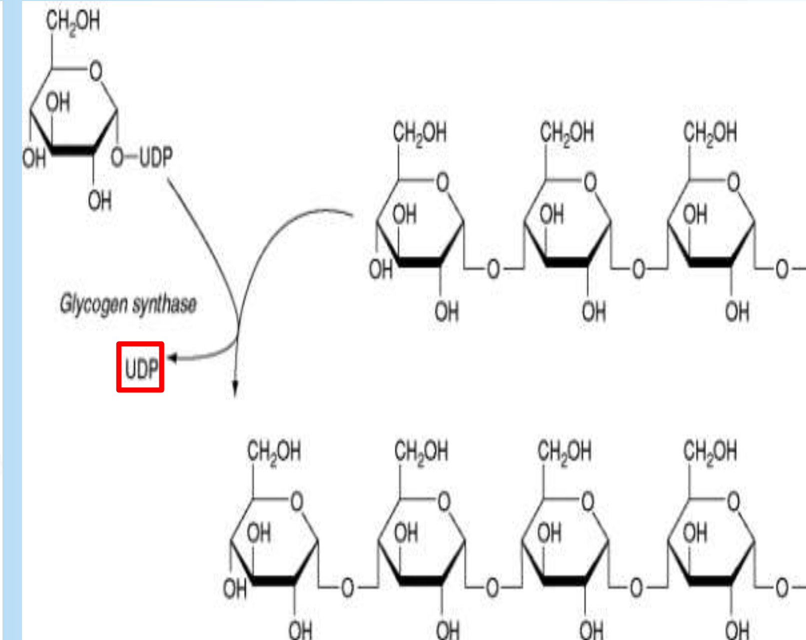
- Catalyze transfer of **functional groups** from one substance to another. Usually a phosphate, amino, methyl, or acyl group.
- To be a transferase, a product must remain after the transfer!











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Michaelis Menten Kinetics

Lineweaver- Burk Plot

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Regulation of Enzyme Activity

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5 minute break

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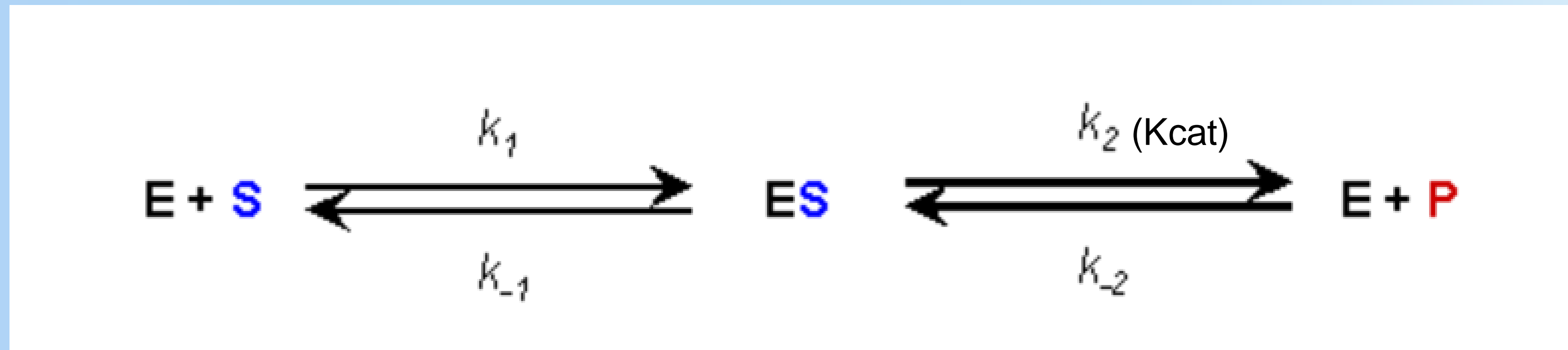
Michaelis Menten Kinetics

- Enzymes can catalyze reactions at different rates. Rates are dependent on [E] and[S]



Kcat- Number of substrate molecules converted to product per unit time when enzyme is fully saturated

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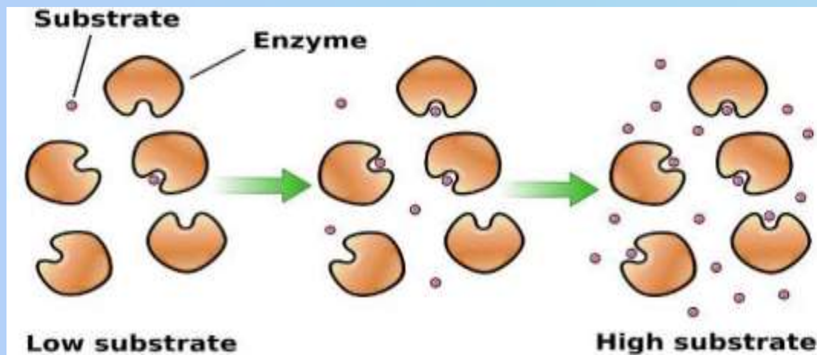
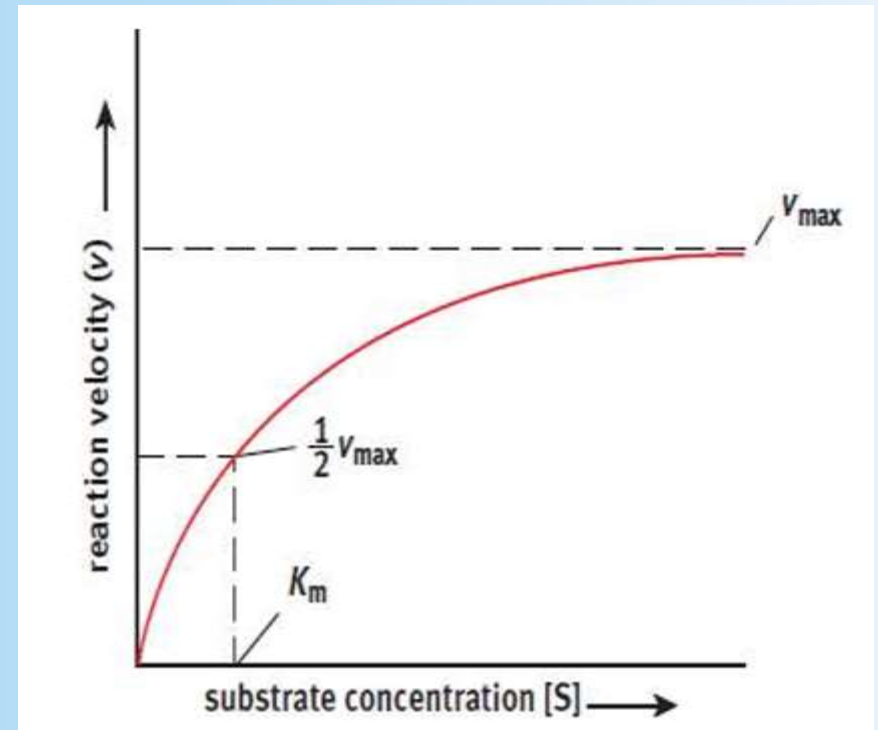
Michaelis Menten Kinetics

- If we have:
- 1. An enzyme that binds one substrate to make product
- 2. No cooperativity in the enzyme
- 3. [S] >> [E]
- 4. Constant [ES]

We get a Velocity- Concentration curve that looks like this!



S readily binds E V α [S] → 1st order kinetics.	Half saturation. Mixed reaction kinetics	Saturated with substrate. V is independent of [S] → 0-order enzyme kinetics



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Michaelis Menten Kinetics

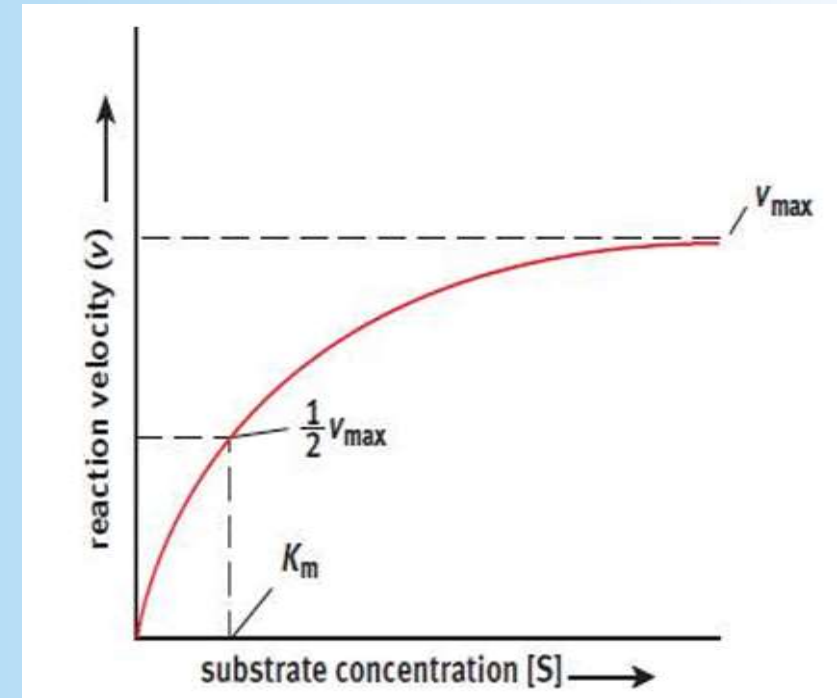
- Vmax= Maximal enzyme velocity (M/S)

The maximal rate of catalysis. At Vmax:
1. The enzyme is saturated with S
2. All active sites are filled with S
3. V cannot increase unless we add E

- Km= Michaelis Menten Constant

Km is a measure of affinity of the enzyme for its substrate.

- Km is equal to the [S] when V= 1/2Vmax
- The higher the Km, the lower the affinity
- The lower the Km, the higher the affinity

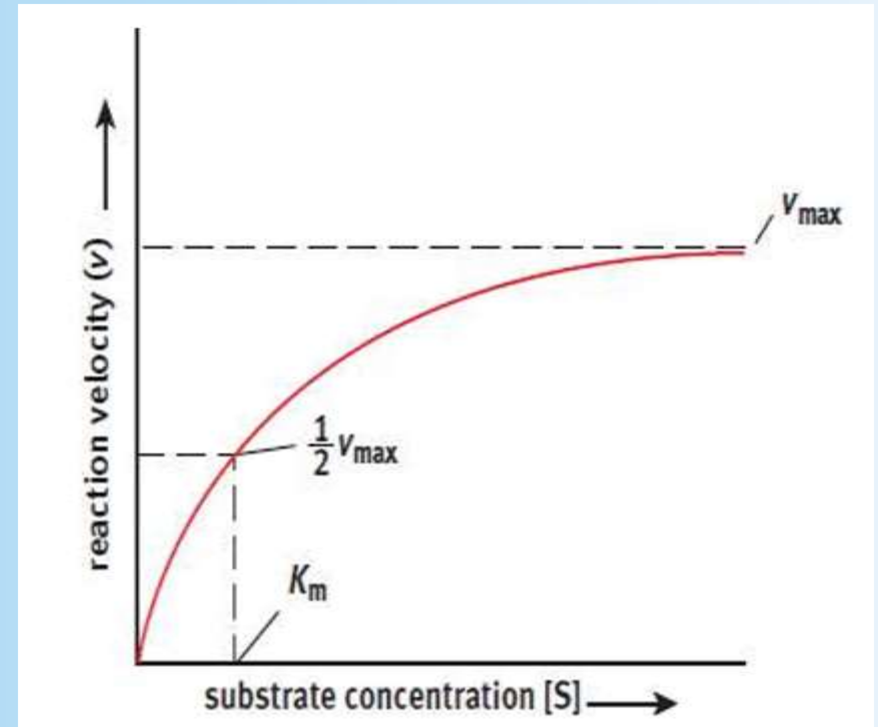


Michaelis-Menten Kinetics

- We have an equation to model this curve, called the **Michaelis-Menten Equation**

$$v = \frac{v_{max}[S]}{[S] + K_m}$$

- Vmax= Maximal enzyme velocity
- Km= Michaelis Menten Constant
- [S] = Substrate Concentration
- V= Initial Enzyme velocity



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Michaelis Menten Kinetics

Determine the initial enzyme velocity if Vmax=15M/s , Km= 5M , and [S]= 5mol

$$v = \frac{v_{max}[S]}{[S] + K_m}$$

$$V = \frac{(15M/s)(5M)}{5M + 5M}$$

V=75/10

V= 7.5 s^{-1}

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Michaelis-Menten Kinetics

- Another equation we can derive:

$$V_{\max} = k_{\text{cat}} \times [E]$$

- Kcat- Number of substrate molecules converted to product per unit time when enzyme is fully saturated

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Lineweaver Burk Plot

- Let's take a reciprocal of the Michaelis Menten Equation and simplify

$$v = \frac{V_{max}[S]}{K_m + [S]}$$

$$\frac{1}{v} = \frac{K_m + [S]}{V_{max}[S]}$$

$$\frac{1}{v} = \frac{K_m}{V_{max}[S]} + \frac{[S]}{V_{max}[S]}$$

$$\frac{1}{v} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$$

$$\frac{1}{v} = \frac{K_m}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}}$$

y = mx + b

We have made a **hyperbolic** equation into a **linear** equation!

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Lineweaver Burk Plot

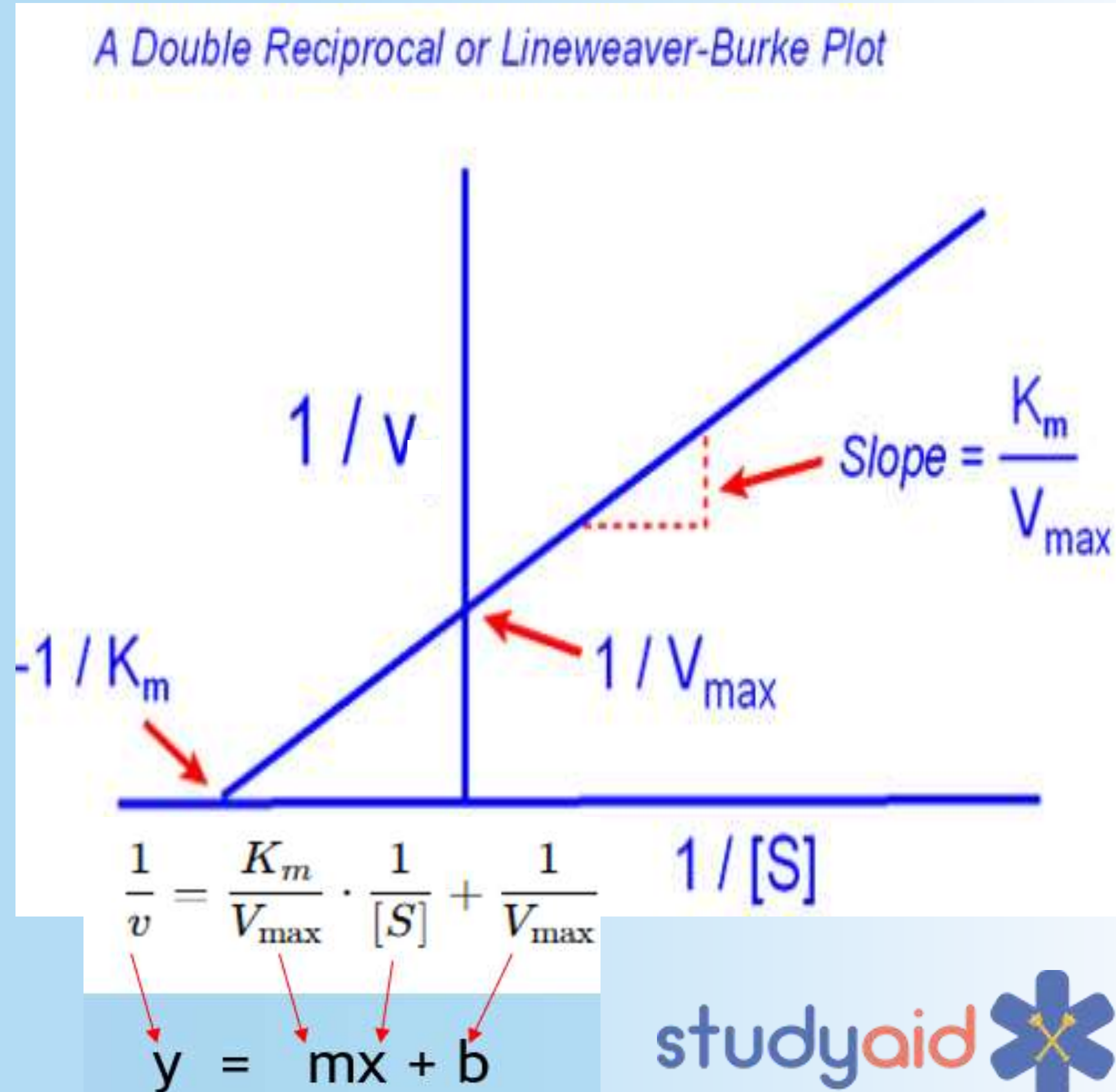
- **The crossing of the x-axis is equal to -1/Km**

$$0 = \frac{K_m}{V_{\max}} \cdot \frac{1}{[S]} + \frac{1}{V_{\max}}$$

$$\frac{Vmax}{Km} * \quad -\frac{1}{V_{\max}} = \frac{K_m}{V_{\max}} \cdot \frac{1}{[S]} * \frac{Vmax}{Km}$$

$$\frac{1}{[S]} = -\frac{1}{K_m}$$

- **The crossing of the y-axis is equal to 1/Vmax**
  - Why? [S] approaches infinity which is where the enzyme would be saturated!



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Lineweaver Burk Plot Practice

- Determine the Vmax and the Km value from the following Lineweaver Burk Plot:

Vmax:

$$\frac{1}{Vmax} = 5$$

$$\frac{1}{5} = Vmax$$

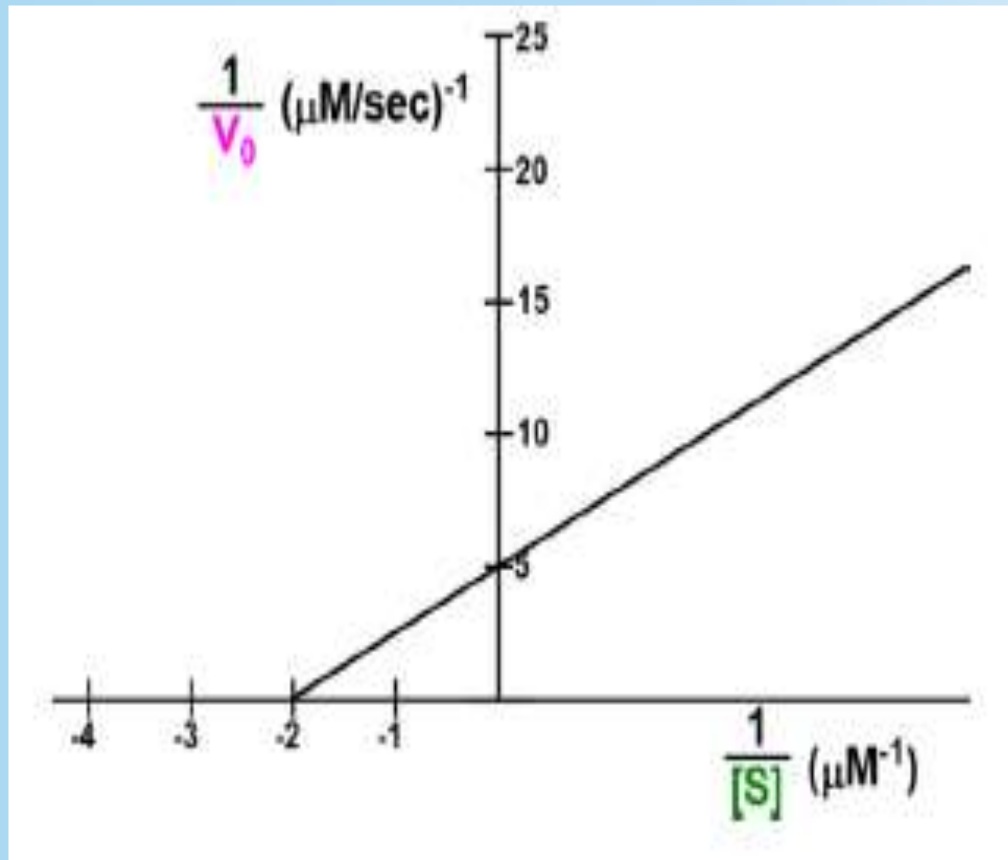
$$0.2\mu M/s = Vmax$$

Km:

$$\frac{-1}{Km} = -2$$

$$\frac{-1}{-2} = Km$$

$$0.5\mu M = Km$$



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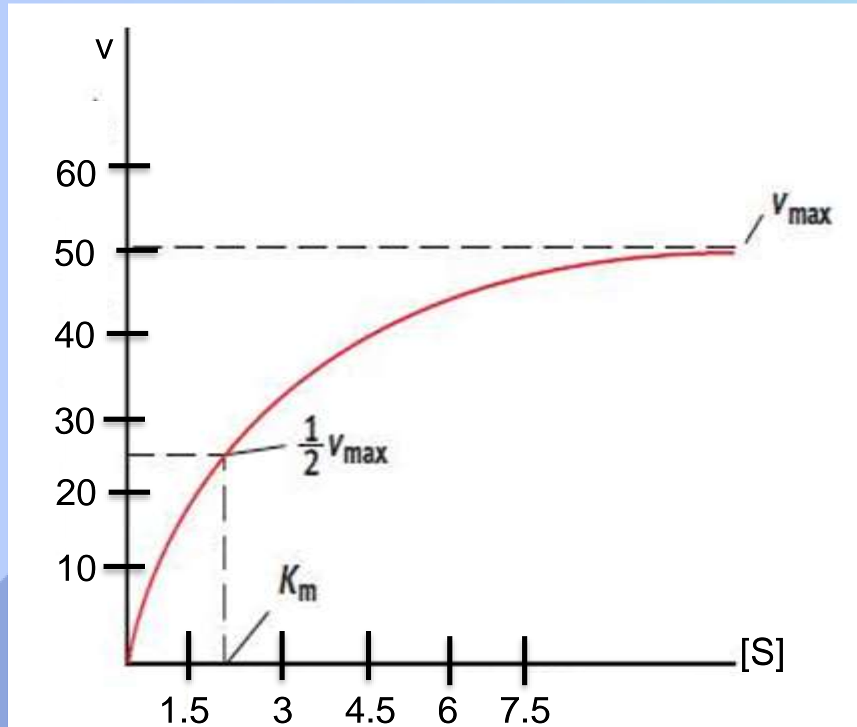
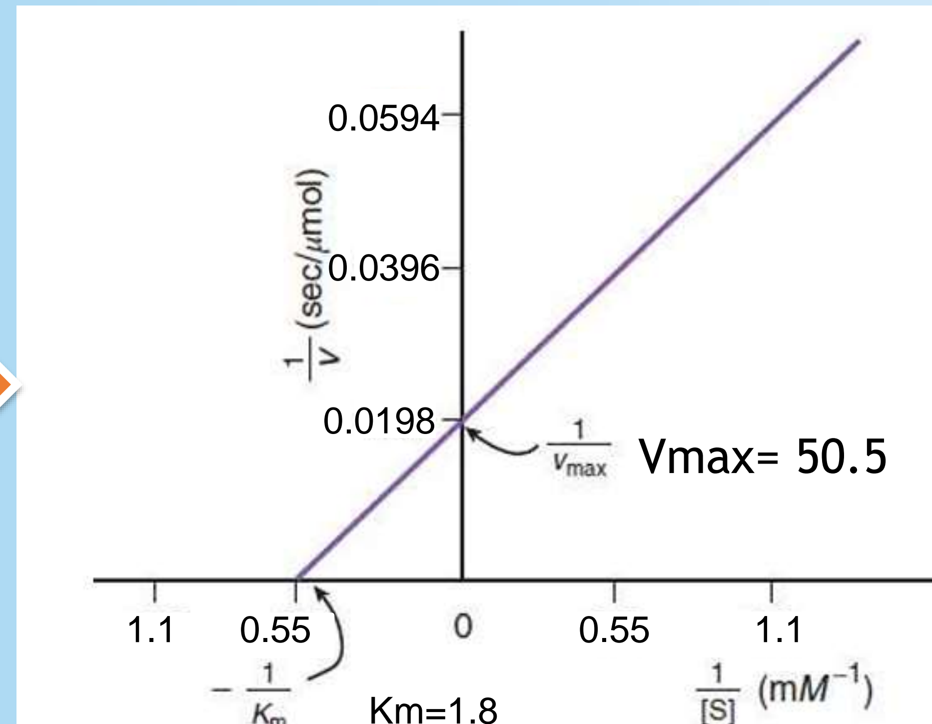
Lineweaver Burk Plot

- Why do we even bother with Lineweaver Burk Plots?
- It can be hard to see the exact value of Vmax and Km sometimes from the Michaelis Menten Plot. This is not the case for the Lineweaver Burk Plot!





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Enzyme Inhibition

- 2 kinds of inhibition is important to know

Competitive Inhibition

Noncompetitive Inhibition

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Competitive Inhibition

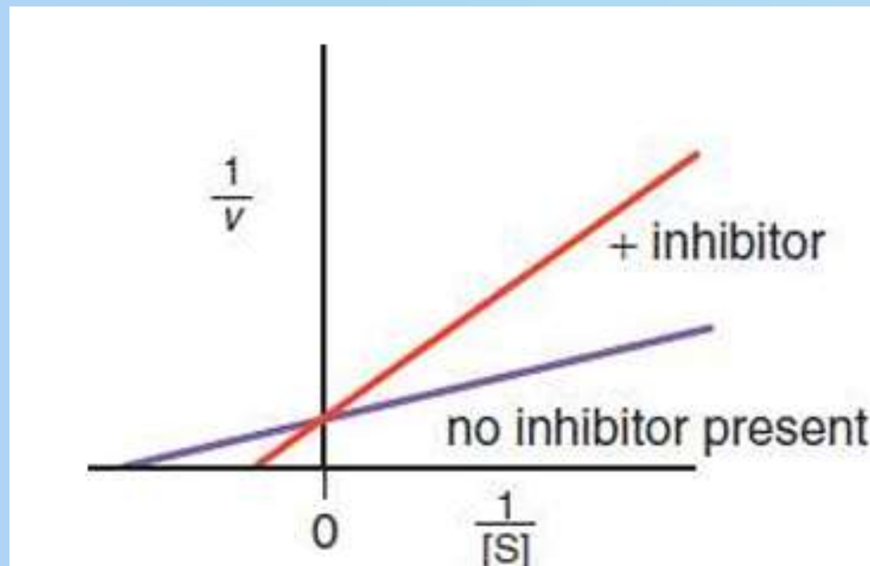
- **Competitive Inhibition** simply is competition between the inhibitor and substrate for occupancy of the active site.
- Competitive inhibition can be overcome by adding more substrate so that the substrate: inhibitor ratio is higher

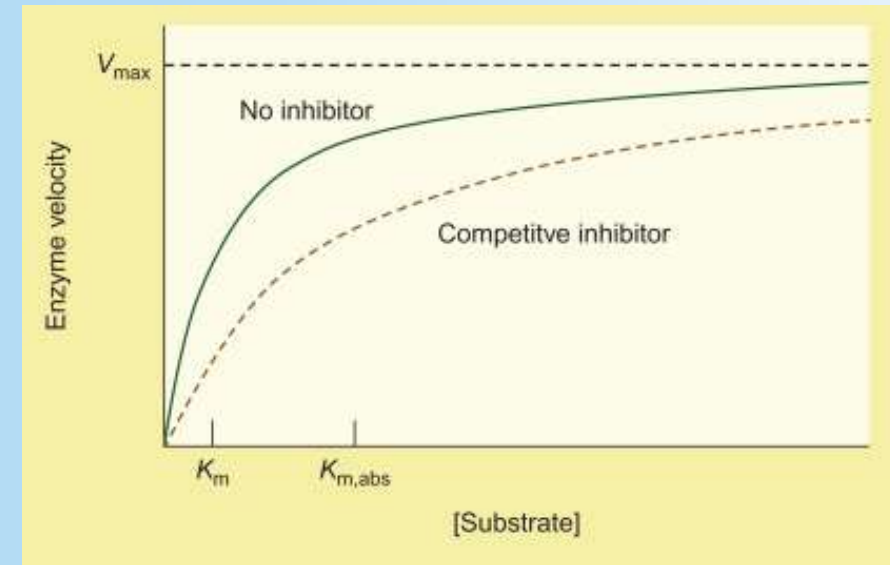
Features

- Vmax is the same
- Km is increased
- Inhibitor line has increased slope

$$\frac{1}{v} = \frac{K_m}{V_{\max}} \cdot \frac{1}{[S]} + \frac{1}{V_{\max}}$$

$$y = mx + b$$





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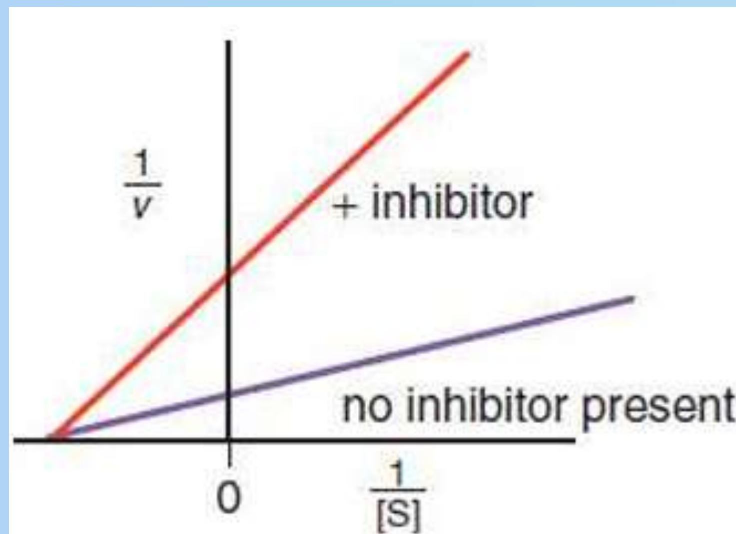
Noncompetitive Inhibition

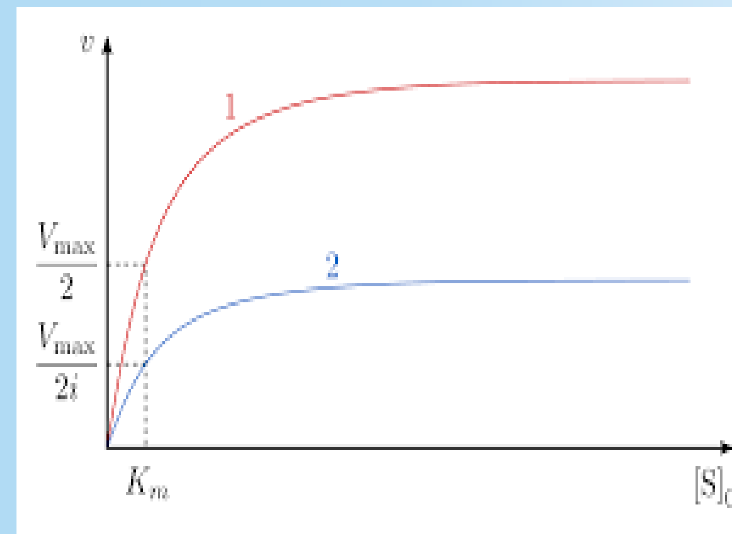
- Noncompetitive inhibition features an inhibitor binding at an **allosteric site**, which changes enzyme conformation at the active site.
- **While Substrate binding is not interfered with, it cannot be made into product**
- Any enzyme molecules that have not bound the inhibitor are still active

Features

-Vmax is decreased

-Km is the same





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Enzyme Regulation

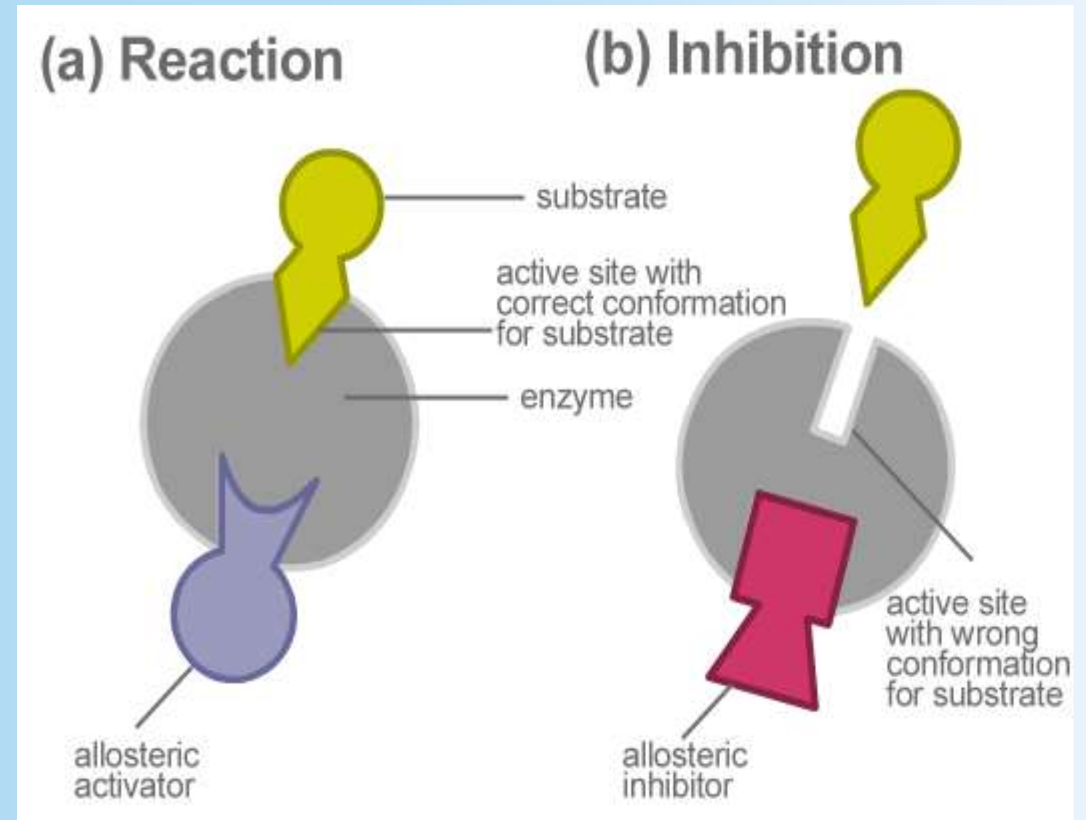
Enzymes are regulated in multiple ways:

Allosteric Regulation – Regulators bind allosteric site which changes conformation of active site.

If activity decreases → inhibitor

If activity increases → activator

Feedback Inhibition usually works by allosteric regulation to prevent overproduction of product.



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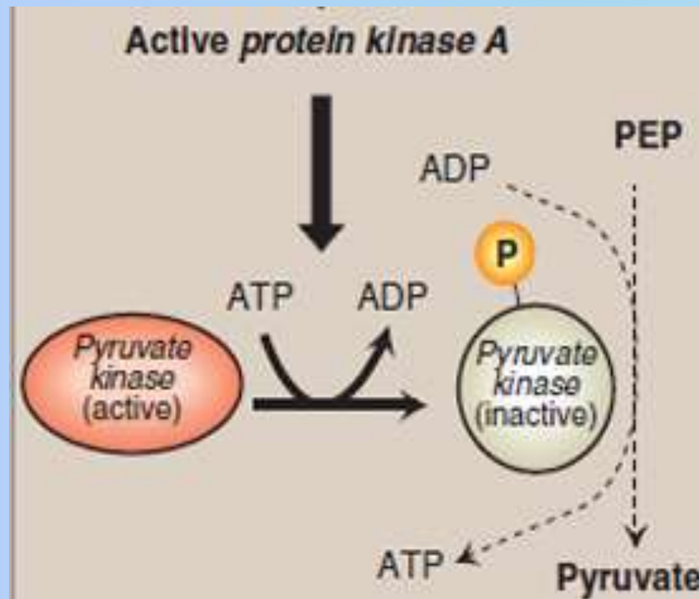
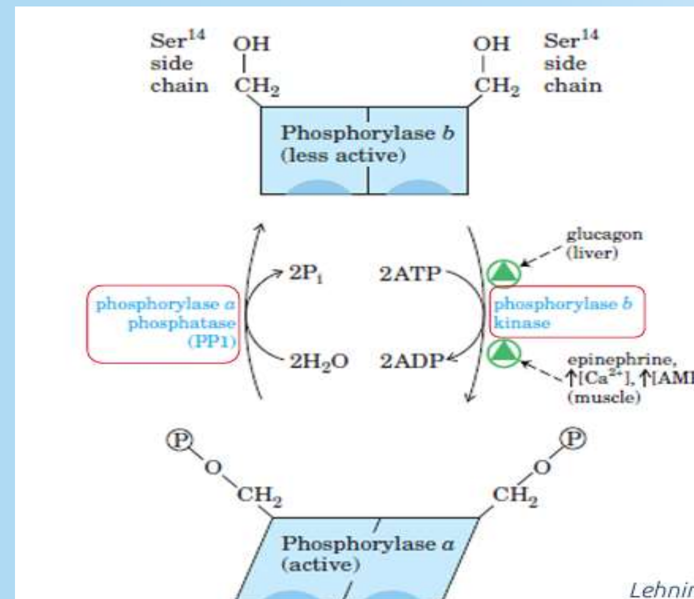
Enzyme Regulation

Covalent Modification

- New covalent bonds are formed on the enzyme
- Main Modification is Phosphorylation



Inactivating Phosphorylation



Activating Phosphorylation

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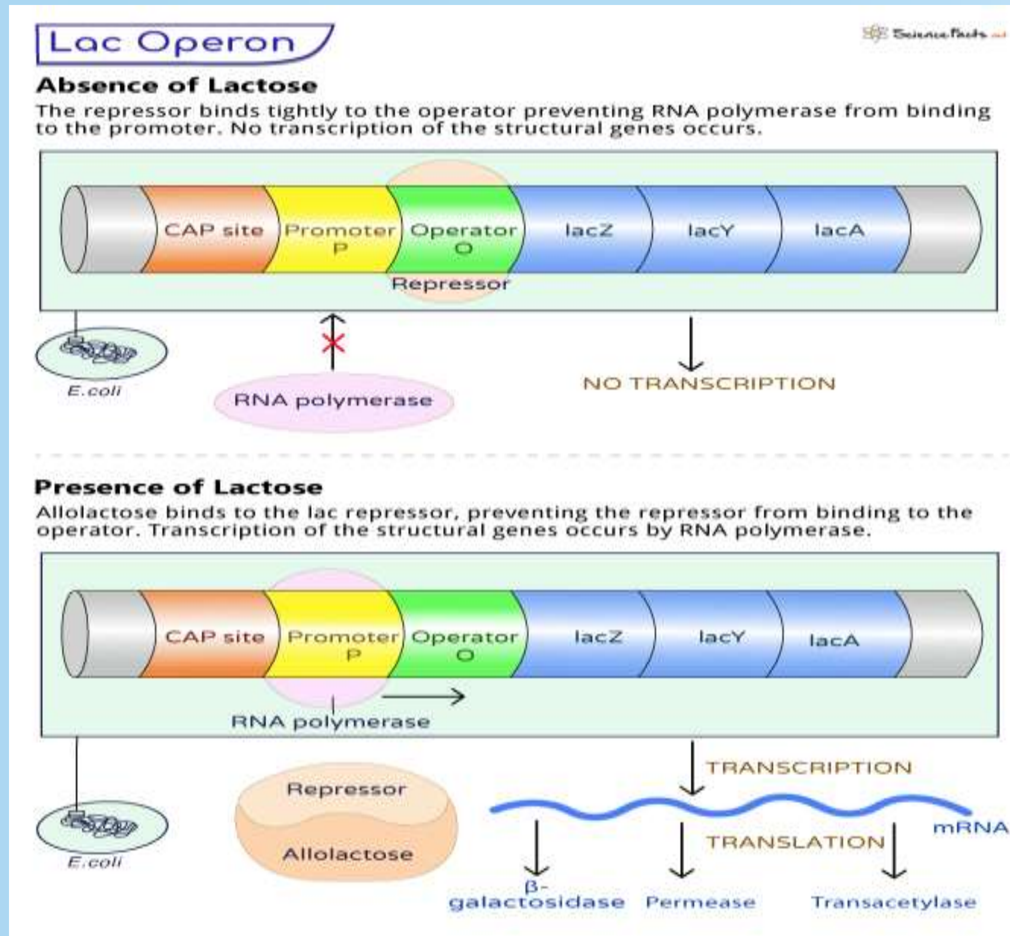
Enzyme Regulation

Gene Expression Changes

- Transcription factors control expression of various enzymes
- Example : lac operon



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Contents of Presentation:

~~Introduction to Enzymes~~

~~Activation Energy~~

~~Mechanisms of Enzyme Catalysis~~

~~Coenzymes~~

~~Classification of Enzymes~~

~~Michaelis Menten Kinetics~~

~~Lineweaver- Burk Plot~~

~~Enzyme Inhibitors~~

~~Regulation of Enzyme Activity~~

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