

# Enzymes and Kinetics

By Thomas Dlugosz



Contents of Presentation:  
Introduction to Enzymes

Activation Energy

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)



# Introduction to Enzymes

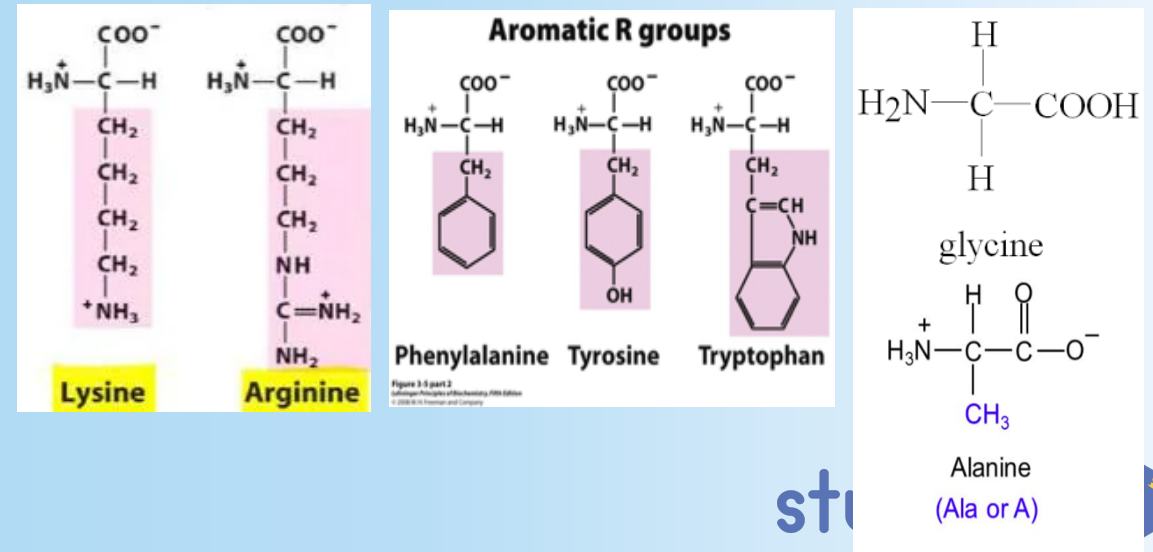
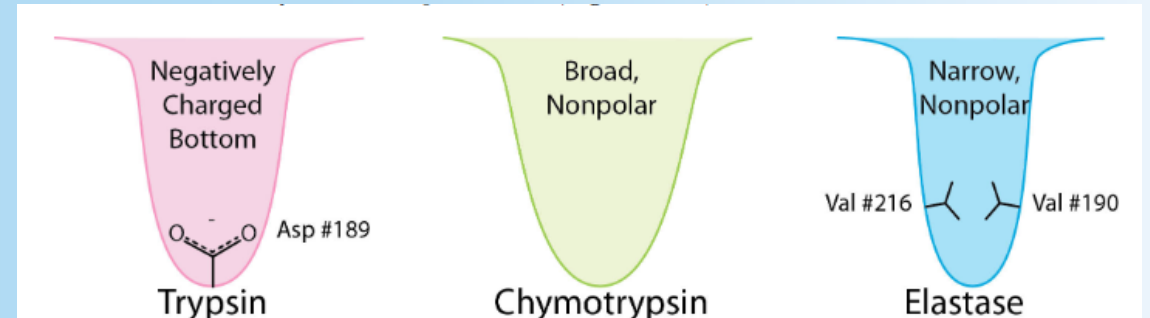
## Active Site

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 substrate they bind with

What amino acids would these proteases cleave?



# Introduction to Enzymes

## Allosteric Site

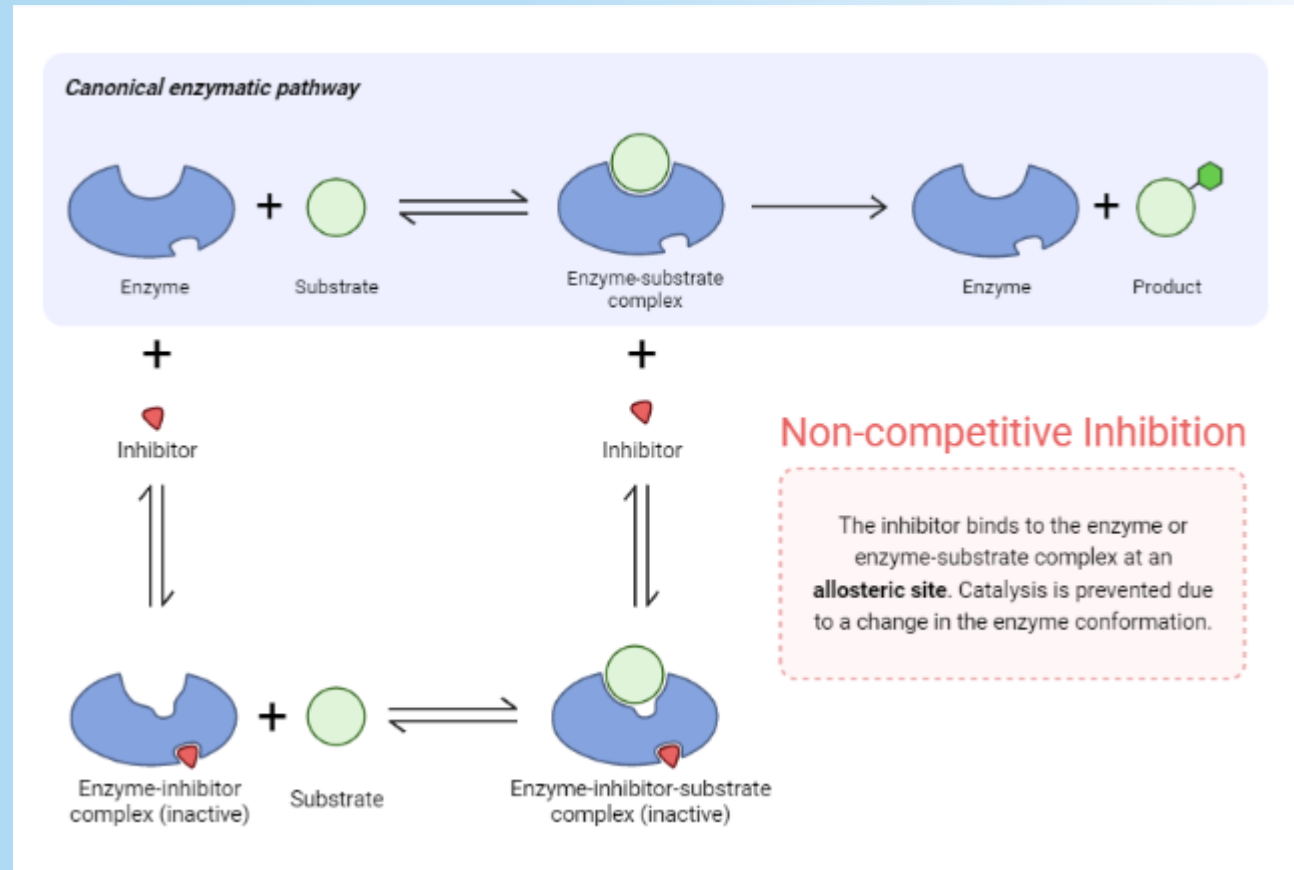
Non-catalytic regions of the enzyme that bind regulators.

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



# Activation Energy

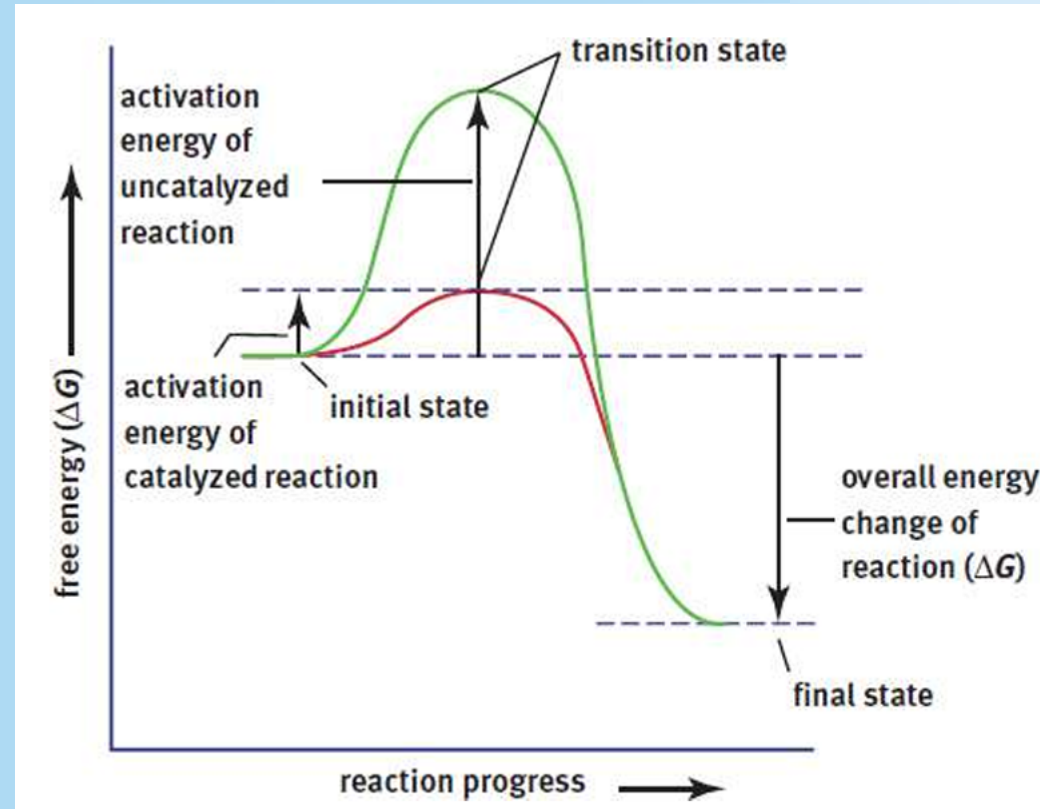


Enzymes speed up the rate of a reaction by **lowering the activation energy ( $E_a$ )**

$E_a$ : The energy required to overcome the instability of the transition state

**Enzymes lower activation energy by stabilizing the transition state**

Notice: Overall  $\Delta G$  and  $K_{eq}$  does not change!





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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<sup>+</sup>, thiamine, coenzyme A

## Cofactors

Metal ions

Ex: Mg<sup>2+</sup>, Cu<sup>+</sup>, Fe<sup>2+</sup>

## Cosubstrates

- Dissociate from enzyme in an altered form (NAD<sup>+</sup>, NADP<sup>+</sup>, CoA)

## Prosthetic Group

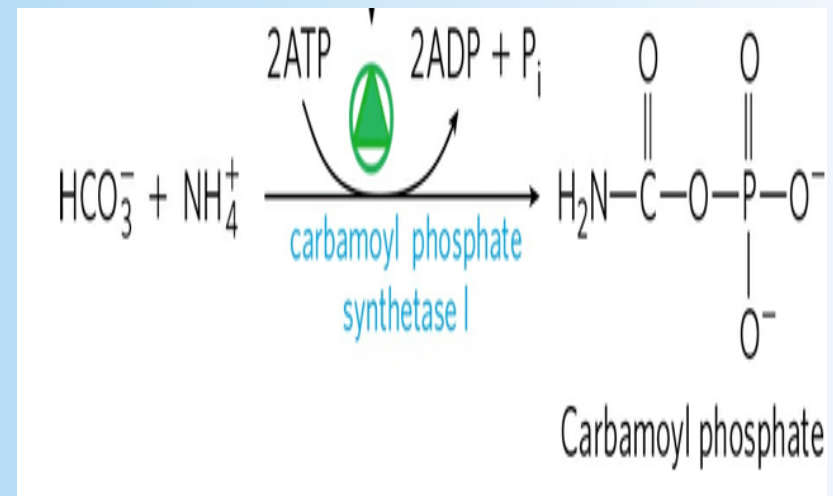
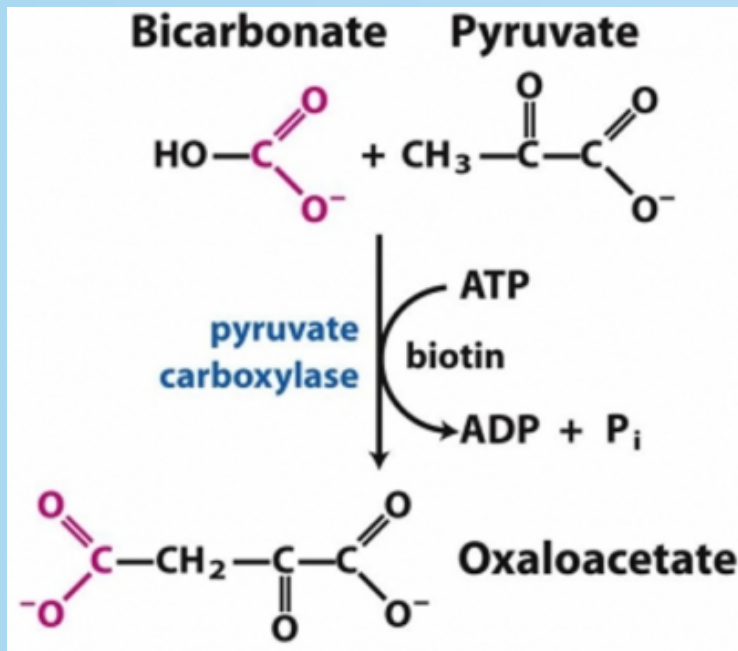
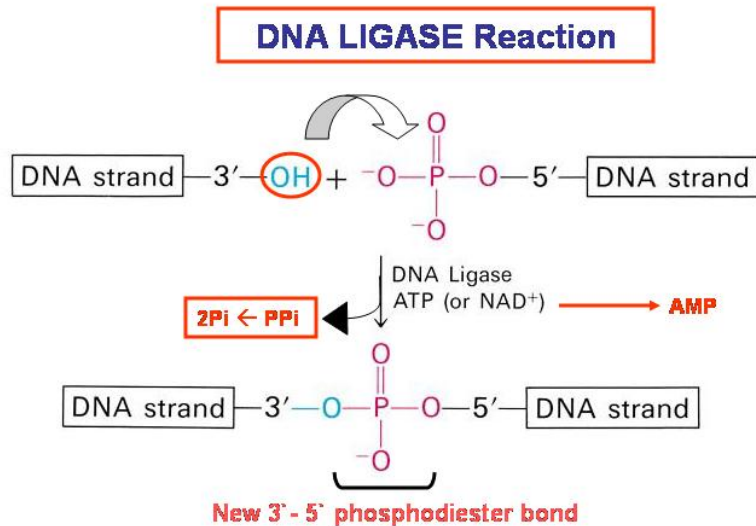
- Permanent part of enzyme (biotin, FAD, thiamine, Mg<sup>2+</sup>, Fe<sup>2+</sup>)

# Classification of Enzymes

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

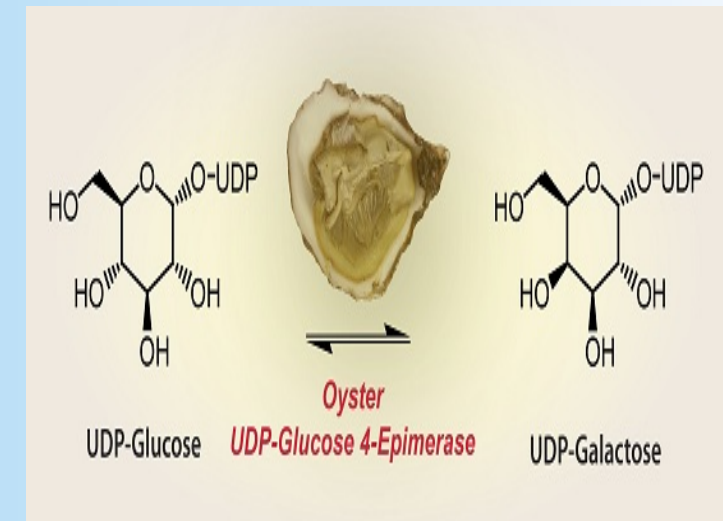
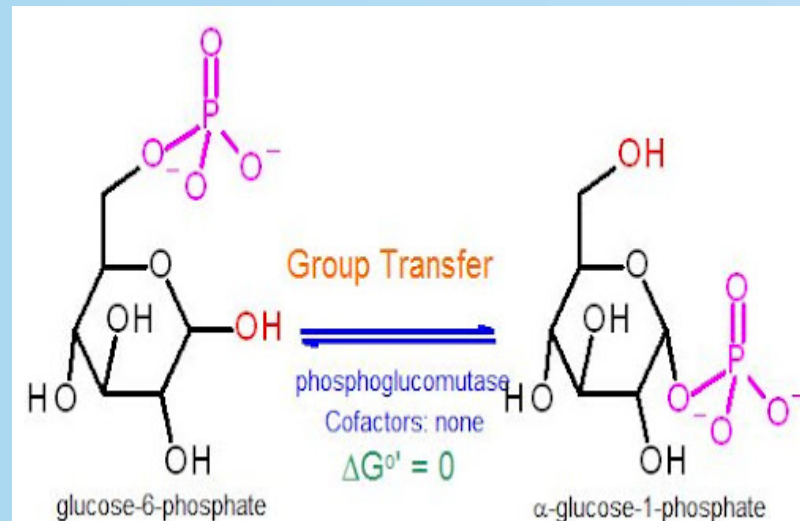
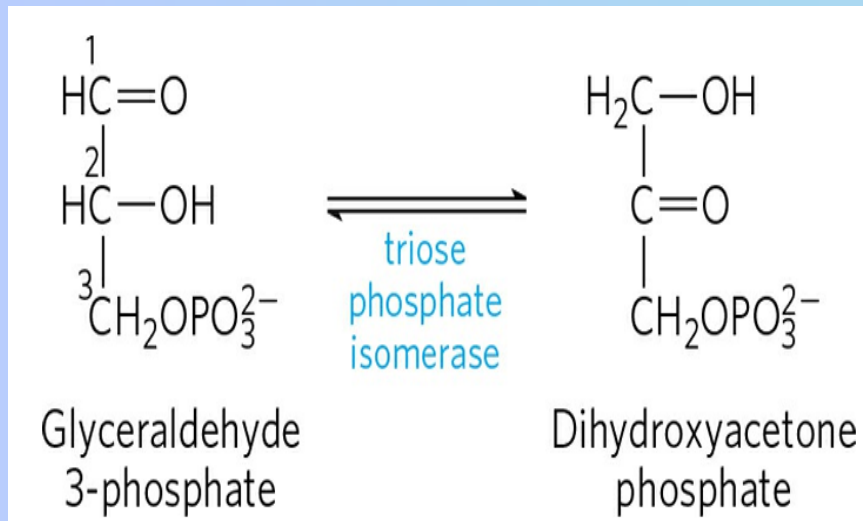
# Ligase

- Ligases** catalyze formation of a new covalent bond between two molecules, energized by hydrolysis of a nucleoside triphosphate



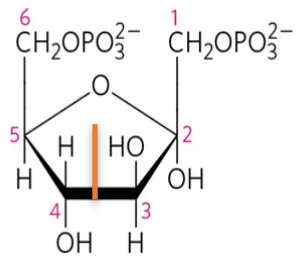
# Isomerase

- 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*.

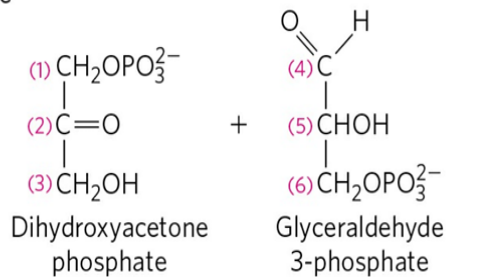


# Lyase

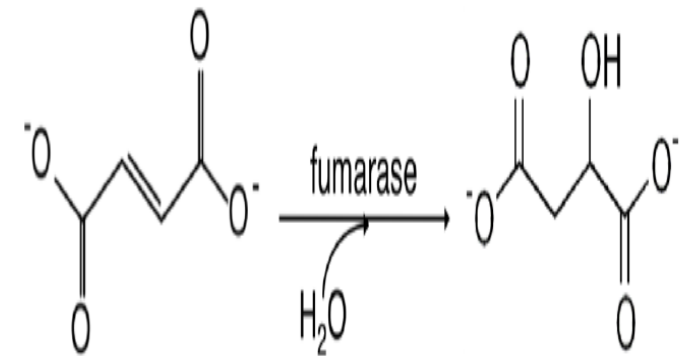
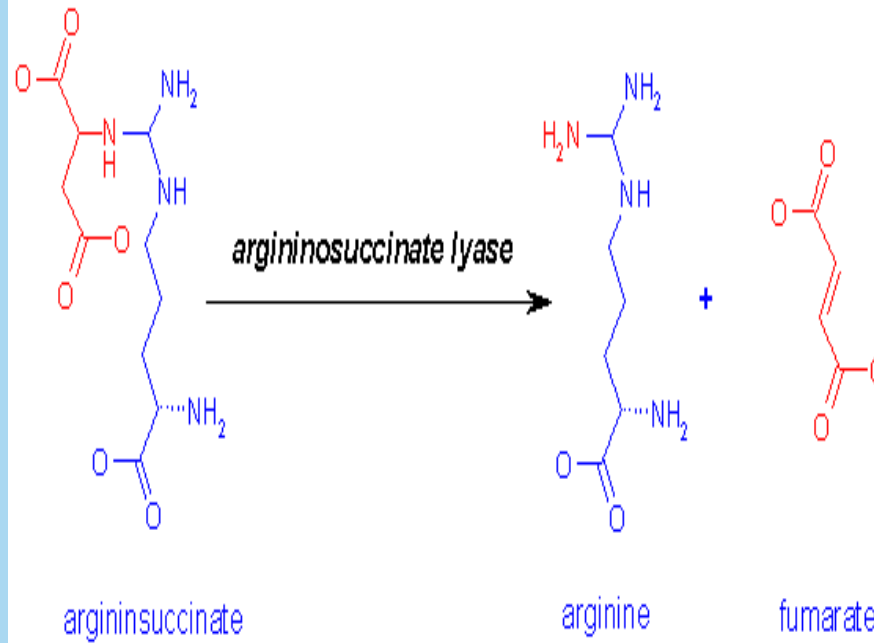
- **Catalyze Non-hydrolytic** addition or removal of groups from substrates.
- **Lyases** can break C-C, C-O, C-N or C-S bonds
- **Lyases are ATP independent!**



Fructose 1,6-bisphosphate

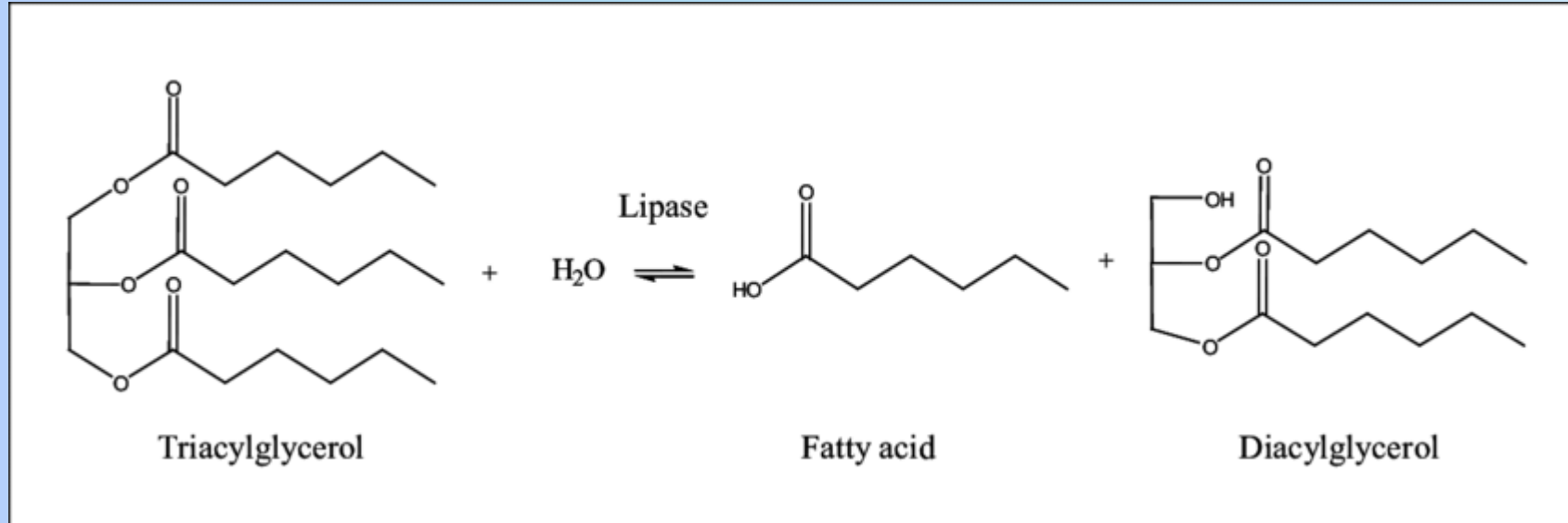


$\Delta G'^{\circ} = 23.8 \text{ kJ/mol}$

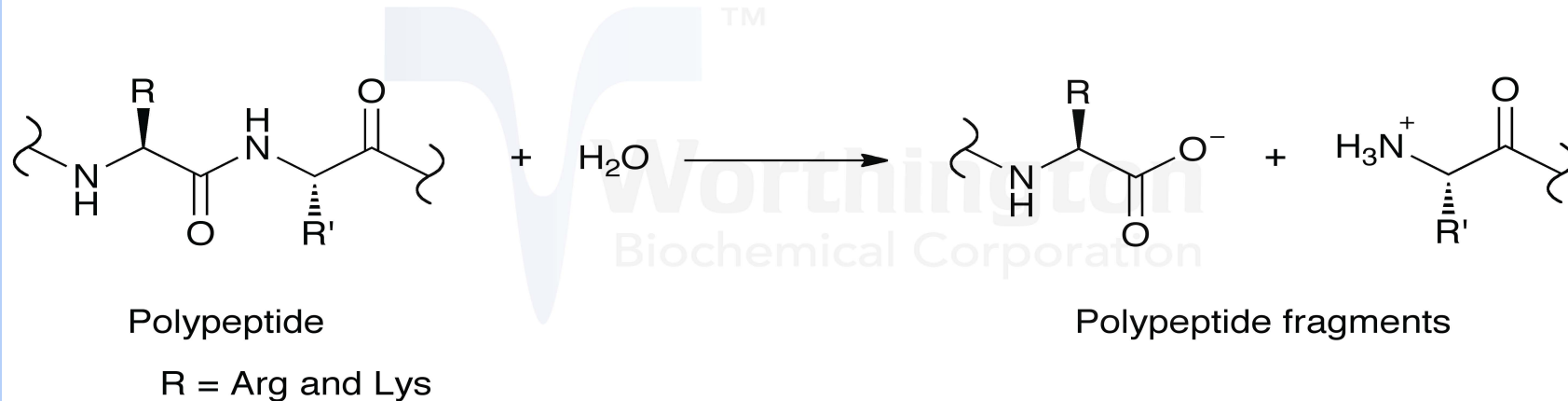


# Hydrolases

- Use water to break down a substrate into at least 2 products

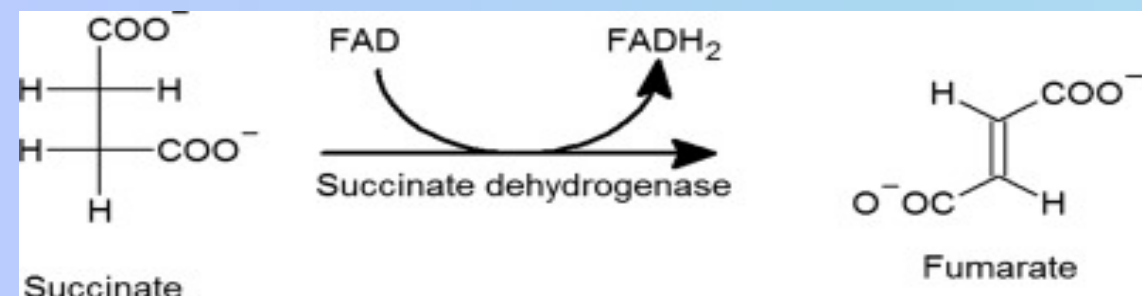
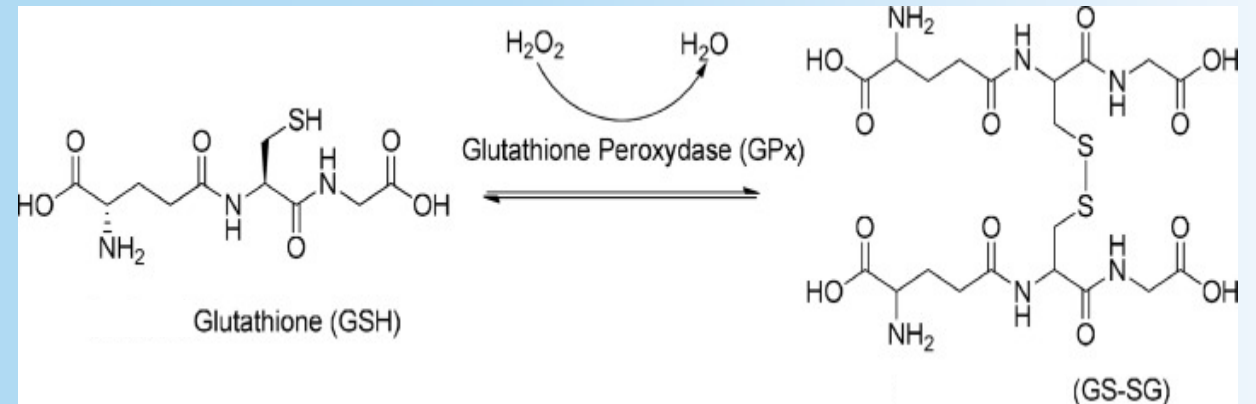


## Trypsin



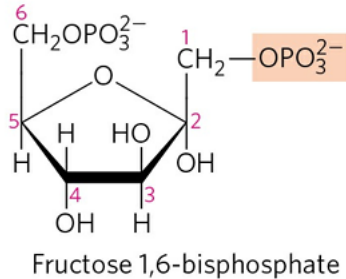
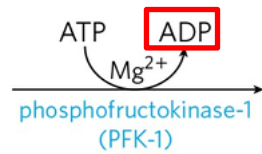
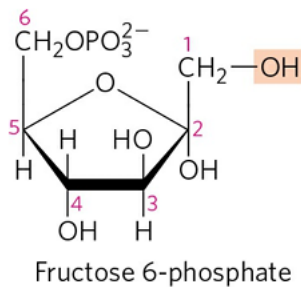
# Oxidoreductases

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

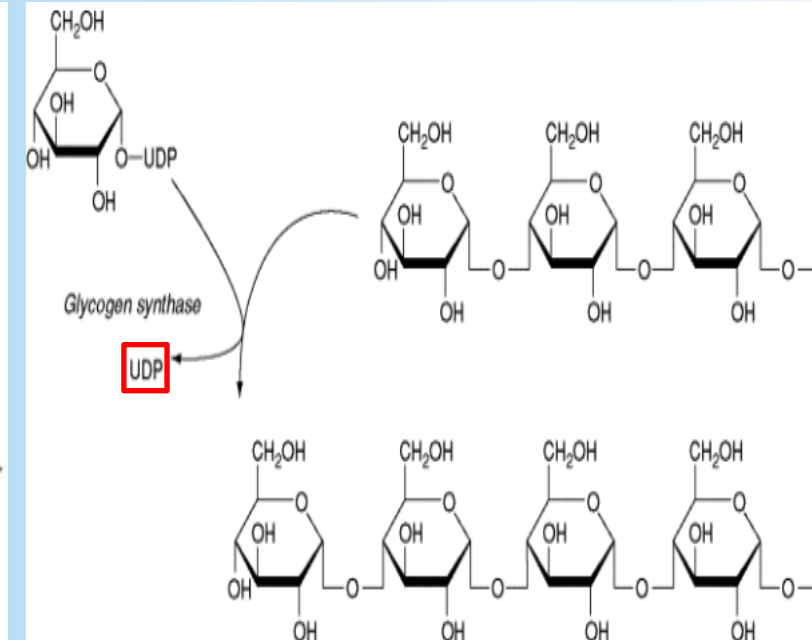
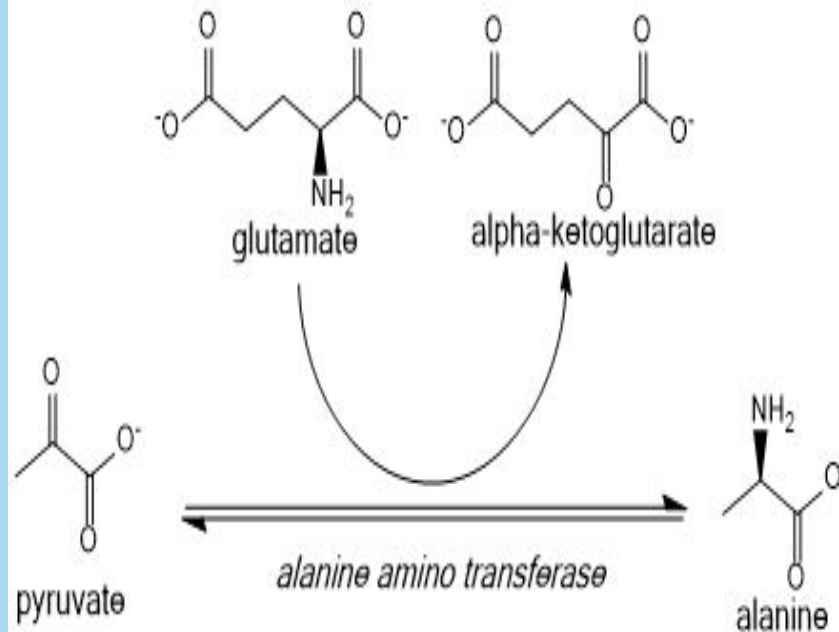


# Transferase

- Catalyze transfer of **functional groups** from one substance to another. Usually a phosphate, amino, methyl, or acyl group.
- May feature 1 swap or 2



$\Delta G'^{\circ} = -14.2 \text{ kJ/mol}$



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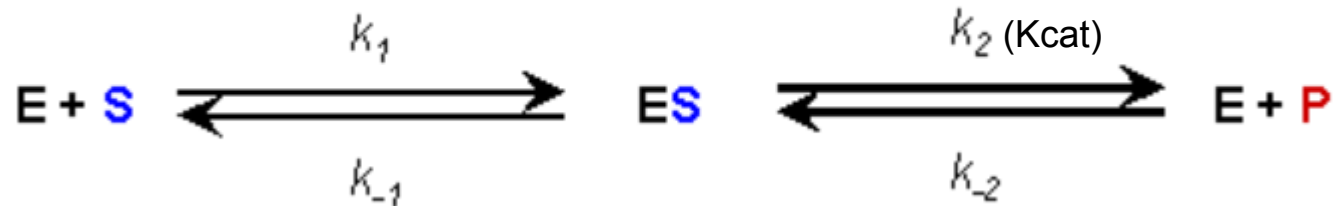
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# Michaelis Menten Kinetics

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

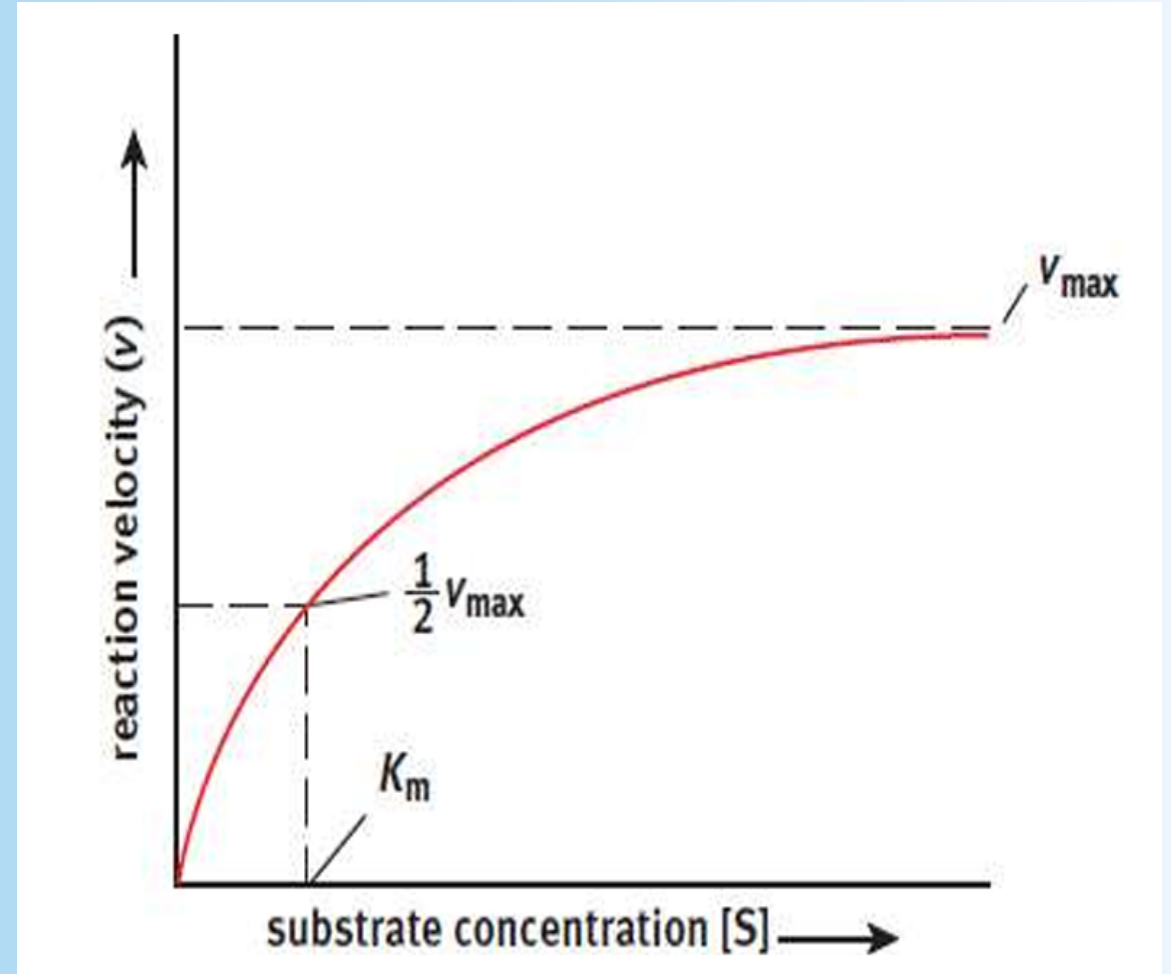


**Kcat**- Number of substrate molecules converted to product per unit time when enzyme is fully saturated

# Michaelis Menten Kinetics

- If we have:
- 1. An enzyme that only forms one ES complex
- 2. No cooperativity in the enzyme
- 3.  $[S] \gg [E]$
- 4. Constant  $[ES]$

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



# Michaelis Menten Kinetics

- **$V_{max}$** = Maximal enzyme velocity

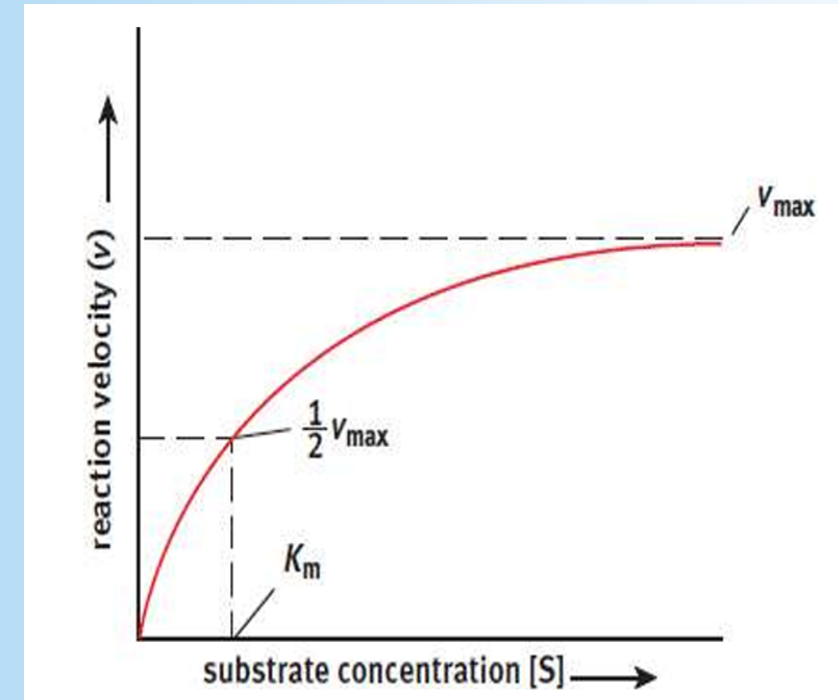
The maximal rate of catalysis. At  $V_{max}$ :

1. The enzyme is saturated with S
2. All active sites are filled with S
3. V cannot increase unless we add E

- **$K_m$** = Michaelis Menten Constant

$K_m$  is a measure of affinity of the enzyme for its substrate.

- **$K_m$  is equal to the  $[S]$  when  $V = 1/2V_{max}$**
- **The higher the  $K_m$ , the lower the affinity**
- **The lower the  $K_m$ , the higher the affinity**

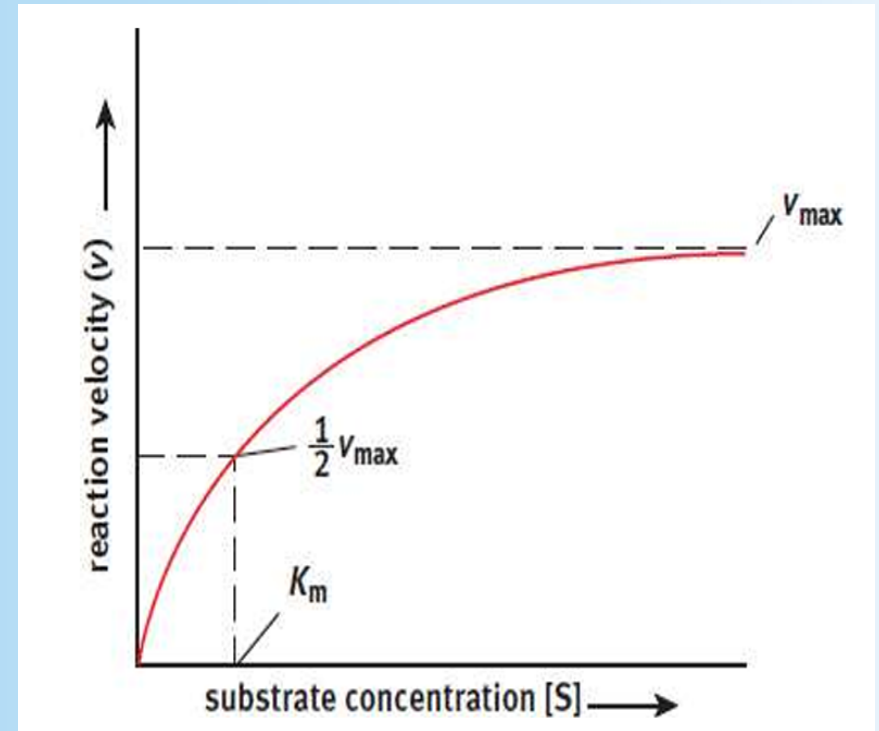


# Michaelis-Menten Kinetics

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

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

- **V<sub>max</sub>**= Maximal enzyme velocity
- **K<sub>m</sub>**= Michaelis Menten Constant
- **[S]** = Substrate Concentration
- **V**= Initial Enzyme velocity



# Michaelis Menten Kinetics

Determine the initial enzyme velocity if  $V_{max}=15M/s$  ,  $K_m= 5M$  , and  $[S]=5mol$

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

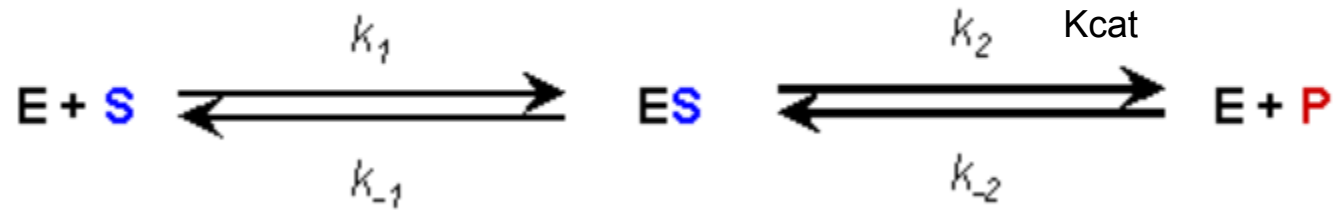
$$V = \frac{(15M/s)(5M)}{5mol + 5M}$$

$$V = 75/10$$

$$V = 7.5 \text{ s}^{-1}$$

# Michaelis-Menten Kinetics

- Another equation we can derive:



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

- **K<sub>cat</sub>**- Number of substrate molecules converted to product per unit time when enzyme is fully saturated

# 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!

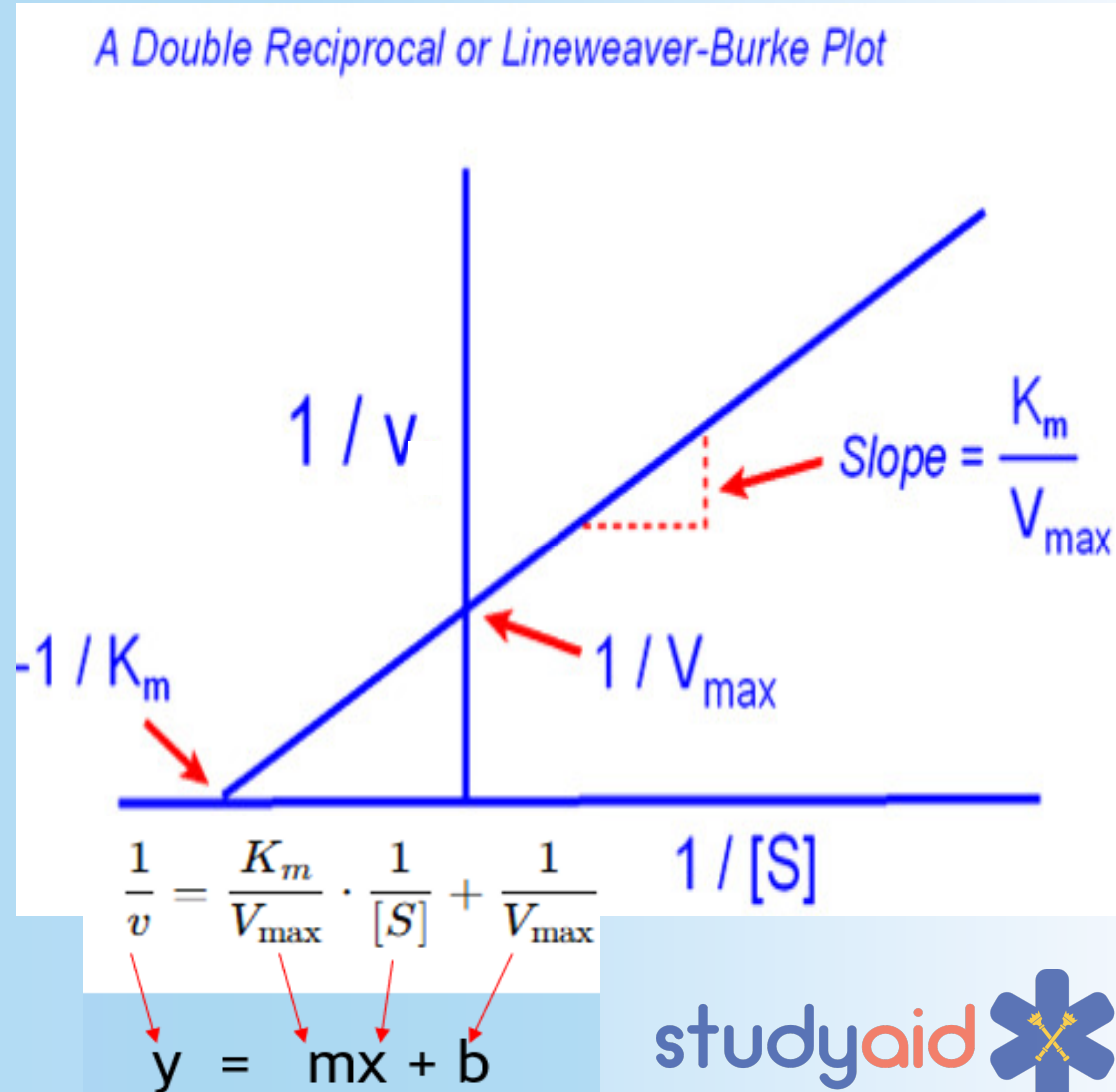
# Lineweaver Burk Plot

- The crossing of the x-axis is equal to  $-1/K_m$

$$0 = \frac{K_m}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}} \quad \xrightarrow{\text{multiply by } \frac{V_{max}}{K_m}} \quad -\frac{1}{V_{max}} = \frac{K_m}{V_{max}} \cdot \frac{1}{[S]} \quad * \frac{V_{max}}{K_m}$$

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

- The crossing of the y-axis is equal to  $1/V_{max}$ 
  - Why?  $[S]$  approaches infinity which is where the enzyme would be saturated!



# Lineweaver Burk Plot Practice

- Determine the  $V_{max}$  and the  $K_m$  value from the following Lineweaver Burk Plot:

$V_{max}$ :

$$\frac{1}{V_{max}} = 5$$

$$\frac{1}{5} = V_{max}$$

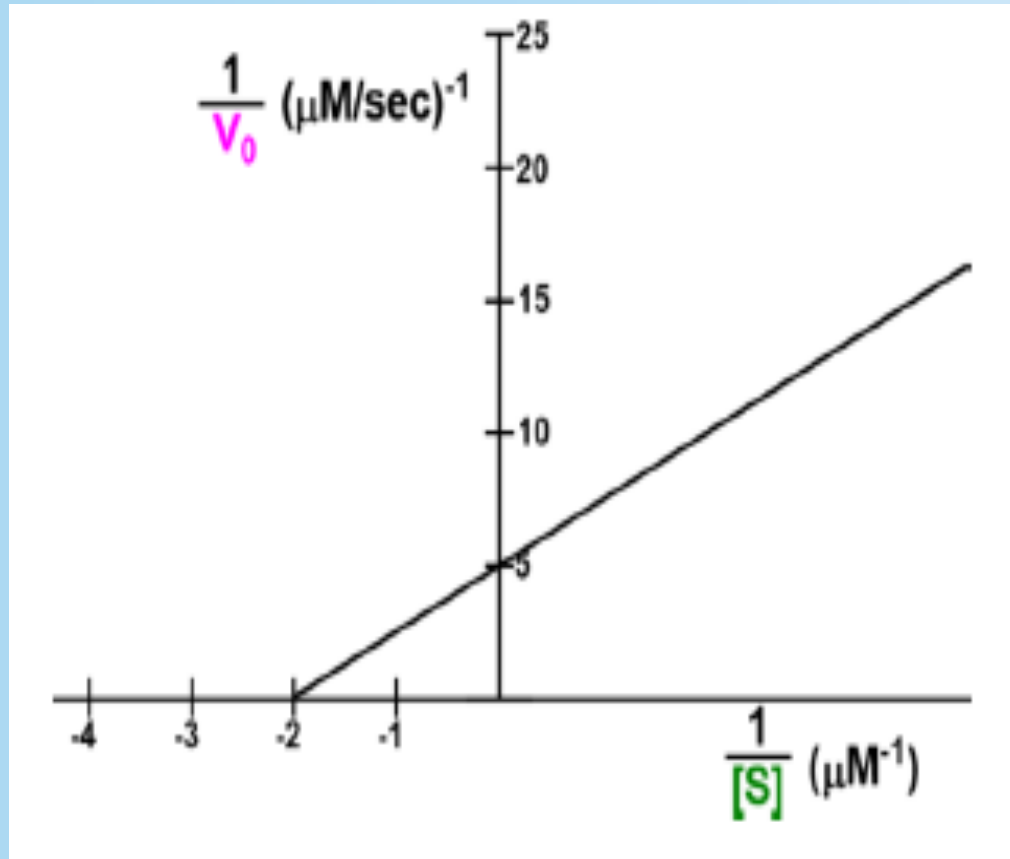
$$0.2 \mu M/s = V_{max}$$

$K_m$ :

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

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

$$0.5 \mu M = K_m$$





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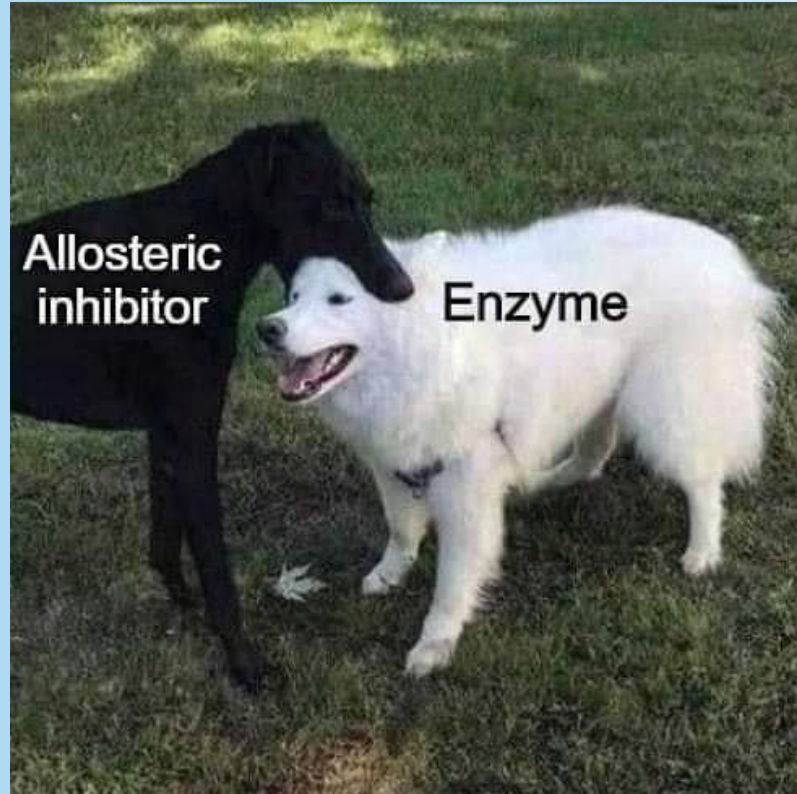
Regulation of Enzyme Activity

# Enzyme Inhibition

- 2 kinds of inhibition is important to know

**Competitive Inhibition**

**Noncompetitive Inhibition**



# Competitive Inhibition

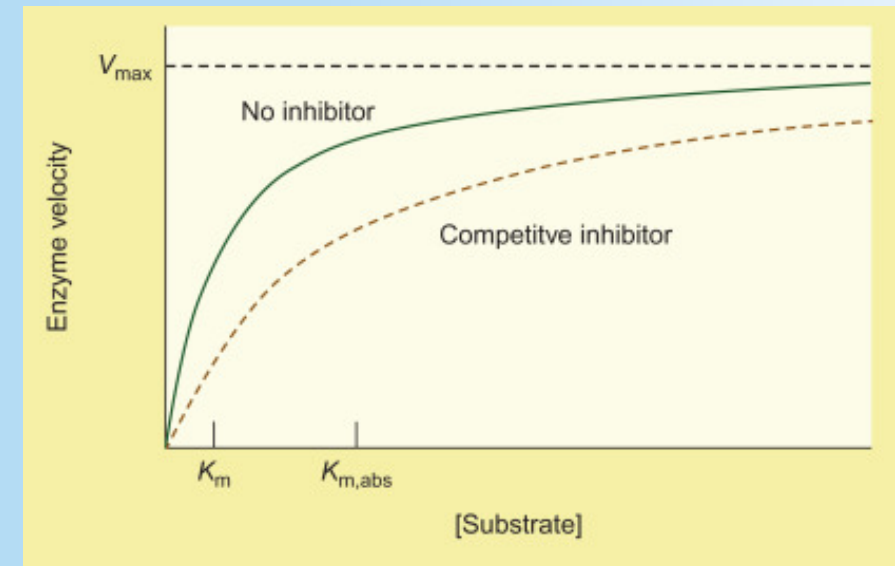
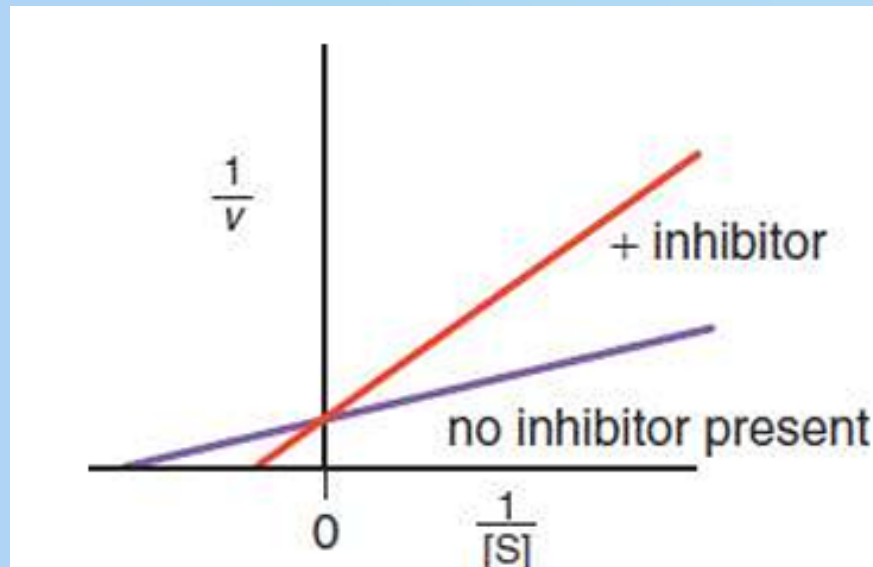
- **Competitive Inhibition** simply is competition between the inhibitor and substrate for occupancy of the active site.
- Competitive inhibition can be overcome by adding more substrate so that the substrate: inhibitor ratio is higher

## Features

- $V_{max}$  is the same
- $K_m$  is increased
- Inhibitor line has increased slope

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

$$y = mx + b$$

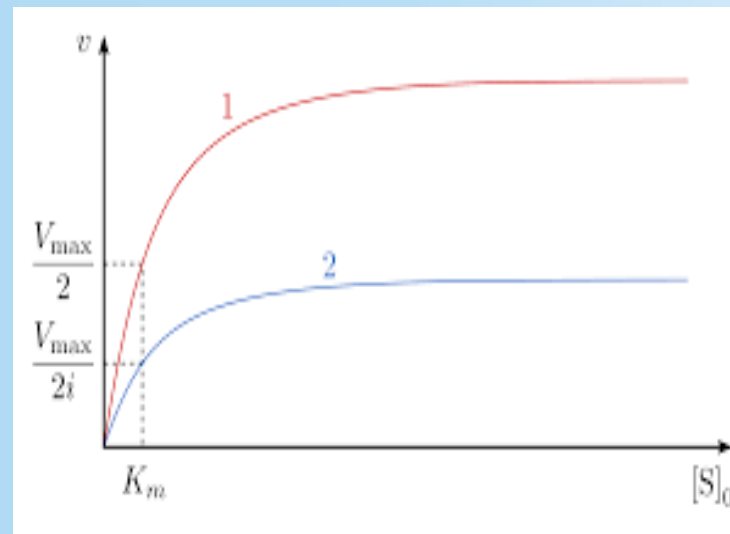
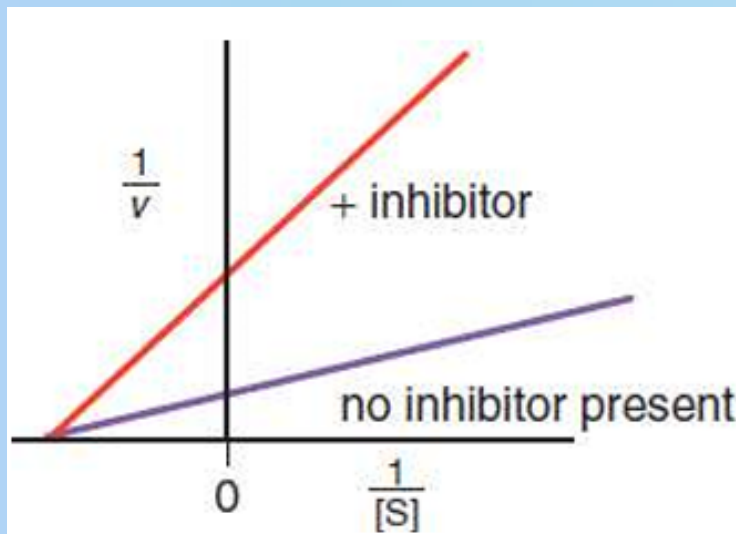


# Noncompetitive Inhibition

- 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

- $V_{max}$  is decreased
- $K_m$  is the same



# Enzyme Regulation

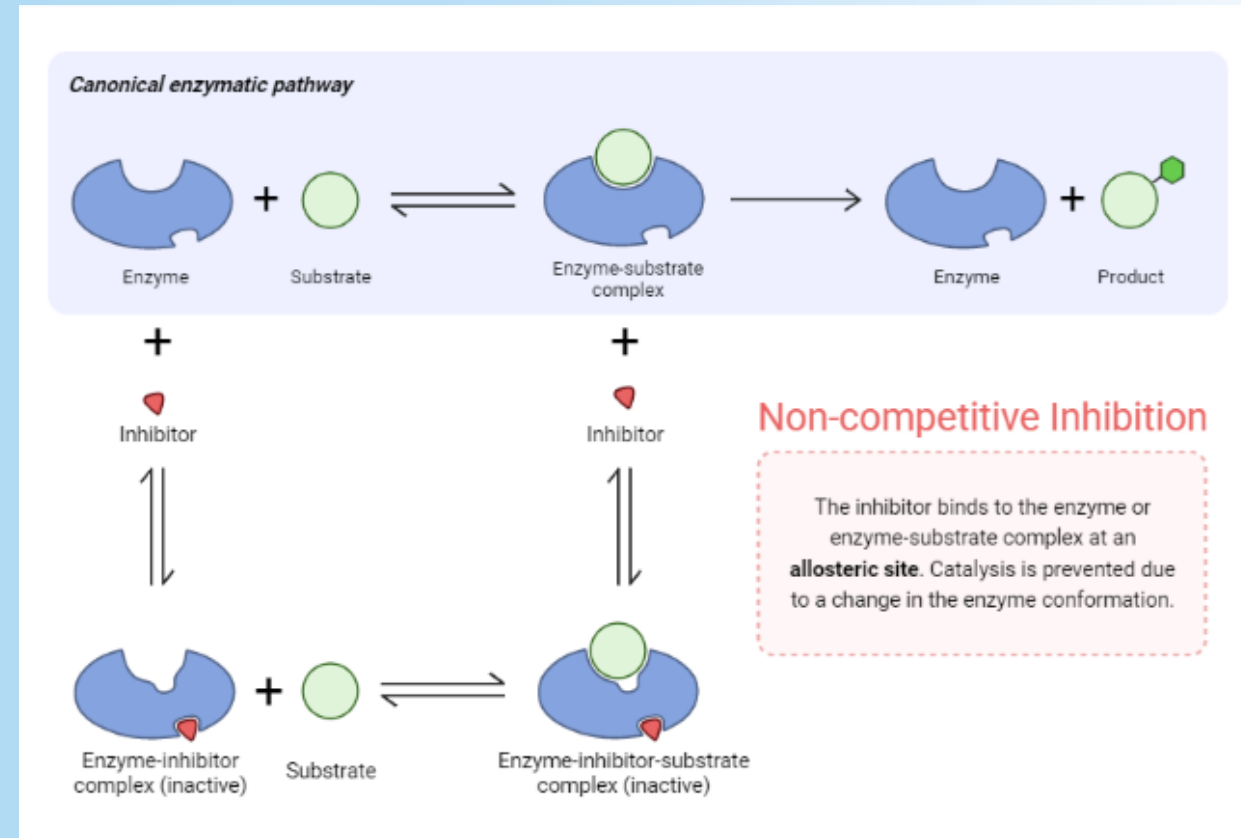
Enzymes are regulated in multiple ways:

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

If activity decreases → inhibitor

If activity increases → activator

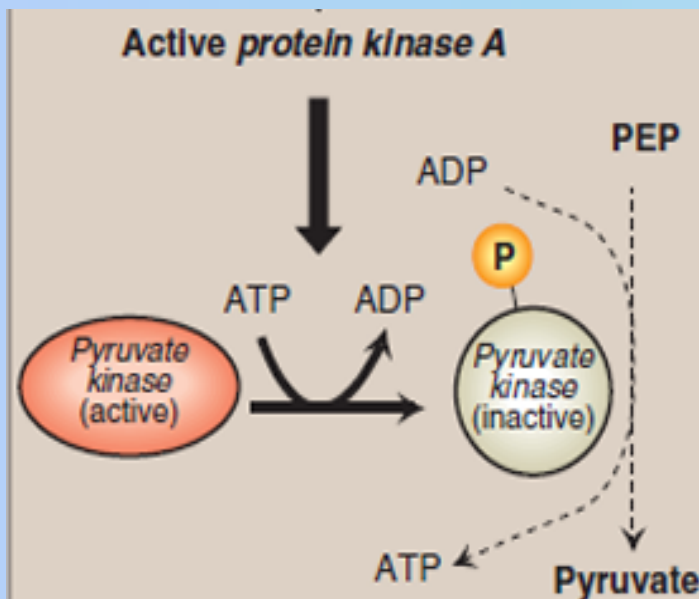
**Feedback Inhibition** usually works by allosteric regulation to prevent overproduction of product.



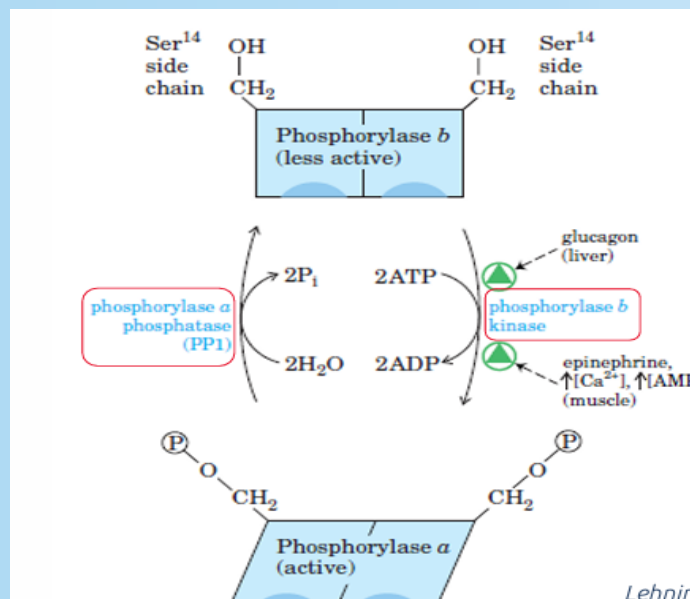
# Enzyme Regulation

## Covalent Modification

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



Inactivating Phosphorylation

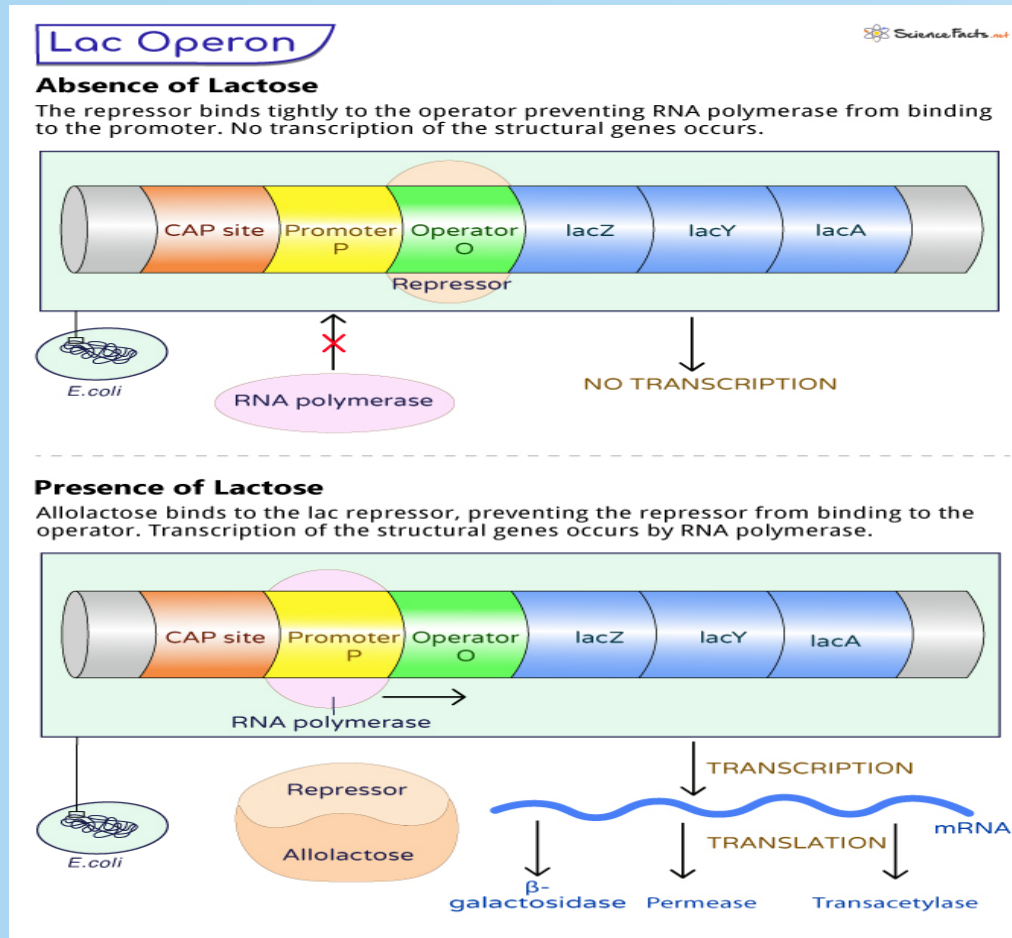


Activating Phosphorylation

# Enzyme Regulation

## Gene Expression Changes

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



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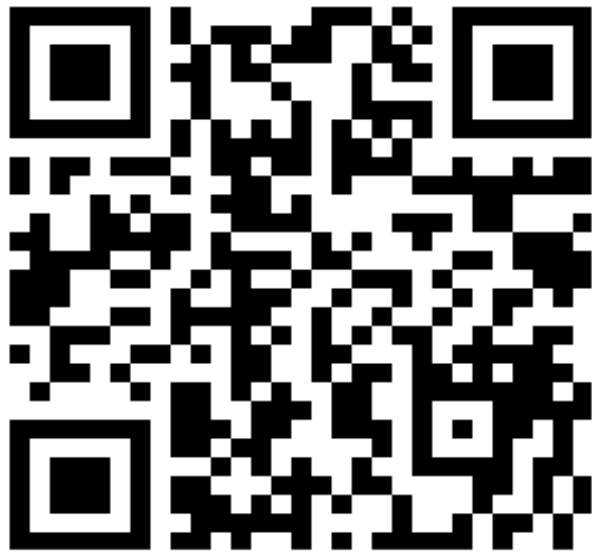
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Event code

**RIRUGX**