# ENZYME KINETICS AND INHIBITION

# Learning Objectives

- To understand

  - The relationship between reaction velocity and substrate concentration of an enzyme catalyzed reaction.

  - The  irreversible and reversible enzyme inhibition.

  - The competitive inhibition.

  - The allosteric inhibition.

  - The enzyme denaturation.

  - The concept of assays of enzyme activity as a diagnostic tool.

# Introduction to enzyme kinetics

The word kinetics is derived from the Greek word *kinetos*, which means "moving".

- Using mathematical tool, we can describe an enzyme's catalytic power, its substrate affinity, and its response to inhibitors, etc.

- Study of this analytical part of the enzyme is known as enzyme kinetics.
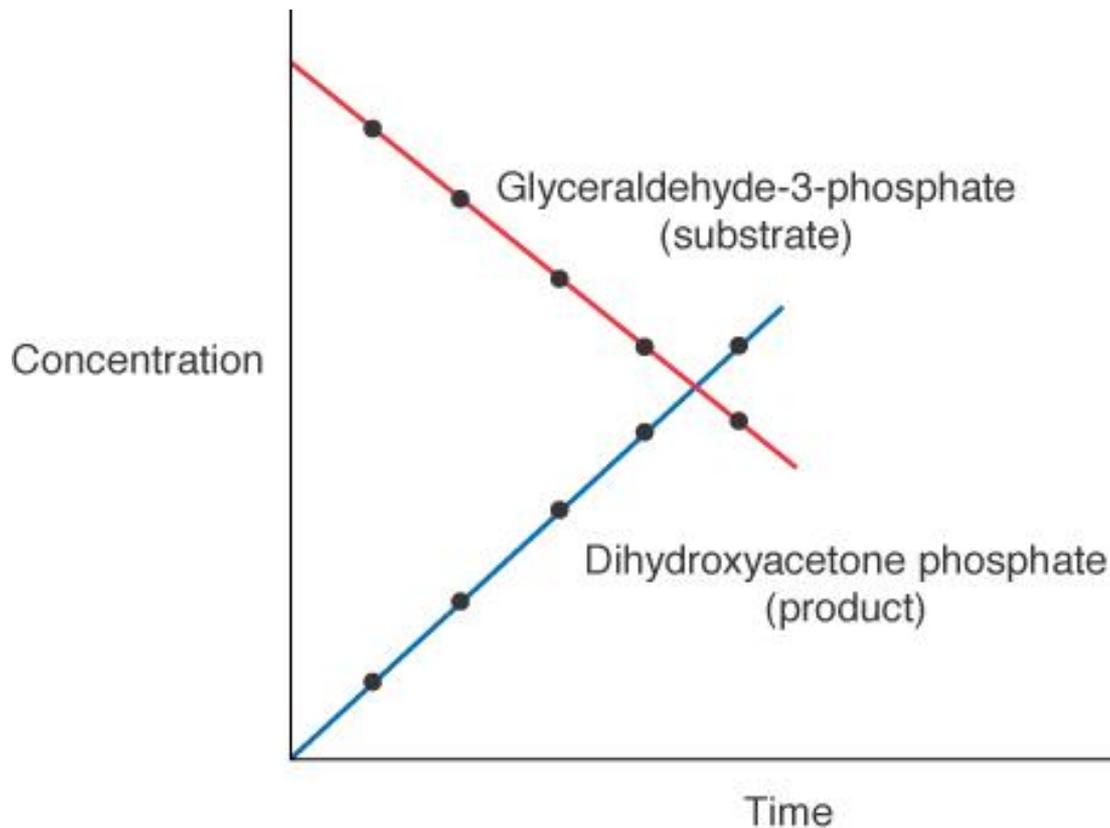
# Reaction velocity and substrate concentration…

*Triose phosphate isomerase*

Glyceraldehyde - 3 phosohate $\longrightarrow$ Dihydroxyacetone phosphate

Over the course of reaction, the concentration of the substrate falls as the concentration of the product rises.  The progress of this reaction or any other reaction can be expressed as **velocity** *(v); i.e, either the rate of disappearance of the substrate (S) or the rate of appearance of the product (P).*
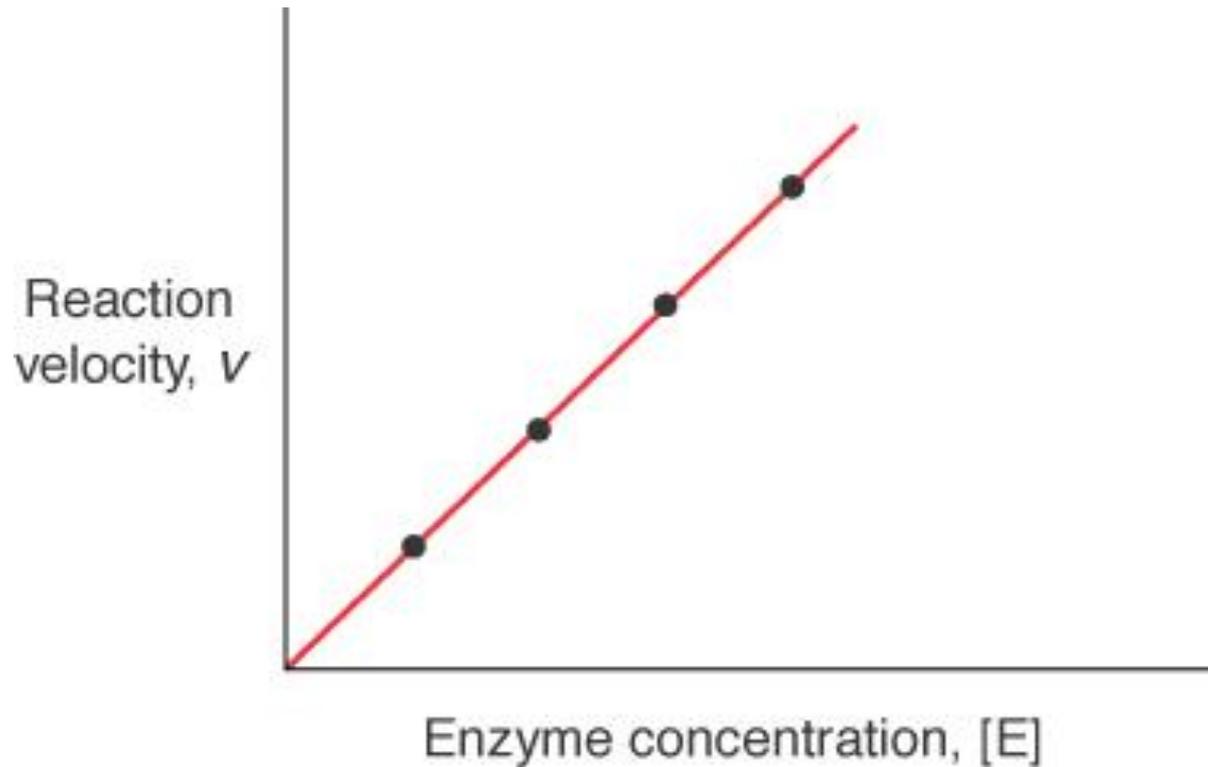
$$v = -\frac{d[S]}{dt} = \frac{d[P]}{dt}$$

# Progress of the triose phosphate isomerase reaction



Over time, the concentration of the substrate decreases and the concentration of the product increases.

# Relationship between enzyme concentration and reaction velocity



The more enzymes present, the faster the reaction.

# Reaction velocity and substrate concentration

When the enzyme concentration is held constant, the reaction velocity varies with the substrate concentration, but in a nonlinear fashion. As small amounts of substrate are added to the enzyme preparation, enzyme activity (measured as the reaction velocity) increases almost linearly.

However, enzyme activity increases less dramatically as more substrate is added.  Finally a point is reached beyond which enzyme activity appears to level off as it approaches a maximum value with increase in substrate. This plateau is known as maximum velocity ($V_{max}$). This behavior shows that at low substrate concentrations, the enzyme quickly converts all the substrate to product, but as more substrate is added, the enzyme becomes saturated with substrate resulting in formation of a hyperbola in the graph of a plot of reaction velocity verses substrate concentration.

The curve expressing this relationship has the same general shape for most enzymes (it approaches a rectangular hyperbola).

# A plot of reaction velocity versus substrate concentration
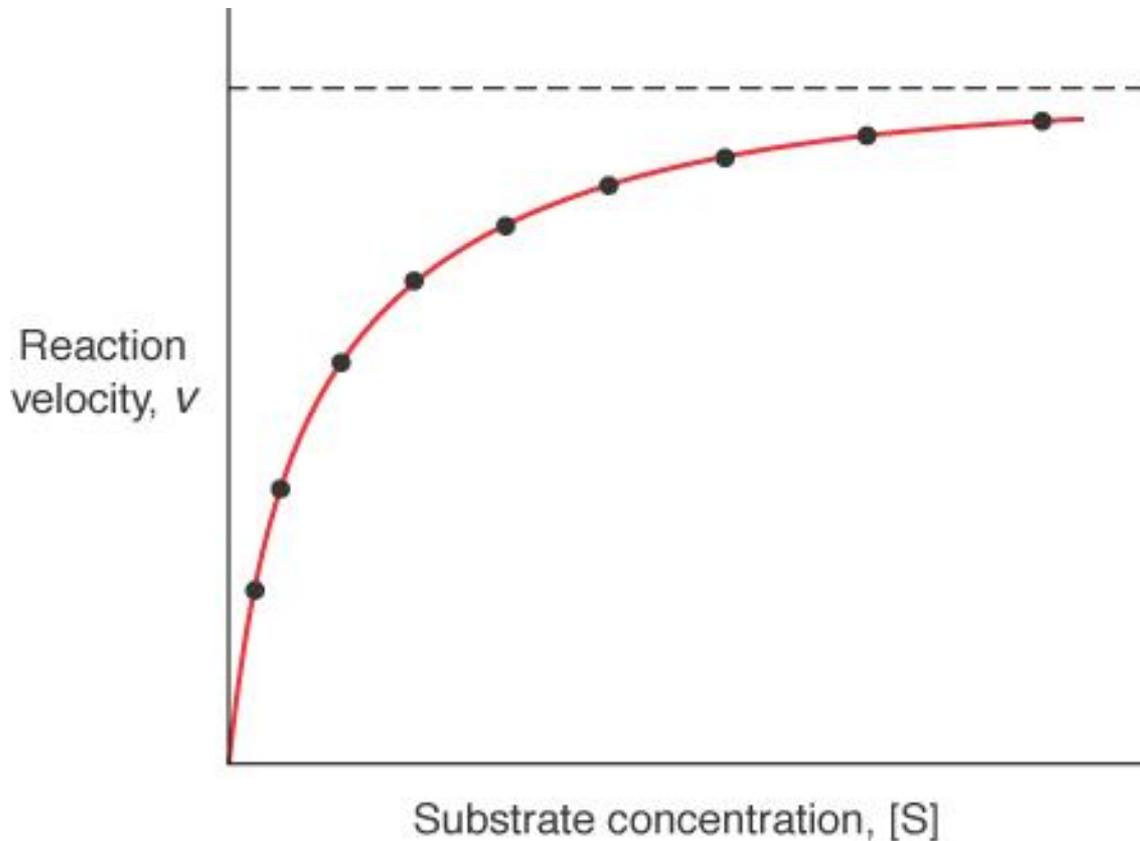


Here varying amounts of substrate are added to a fixed amount of enzyme. The reaction velocity is measured for each substrate concentration and plotted. The resulting curve takes the form of a hyperbola (a mathematical function in which the values initially increase steeply but eventually approach a maximum level).

# Michaelis-Menten equation...

- The curve described in the previous slide shows that:

    - ES complex is the key to understanding this kinetic behavior.

In 1903, this kinetic pattern led Victor Henry to propose that an enzyme combines with its substrate molecule to form ES complex as a necessary step in enzyme catalysis.

In 1913, Leonor Michaelis and Maud Menten expanded the idea of Victor Henry into a general theory of enzyme actions. Michaelis and Menten postulated that:

    - the enzyme first combines reversibly with its substrate to form a ES complex in a relatively fast reversible step.

    - the ES complex then breaks down in a slower second step(rate-limiting step) to yield the free enzyme and the product.

# Michaelis-Menten equation

- Michaelis-Menten equation is the rate equation for an enzyme – catalyzed reaction and is the mathematical description of the hyperbolic curve we have discussed earlier. The formula is

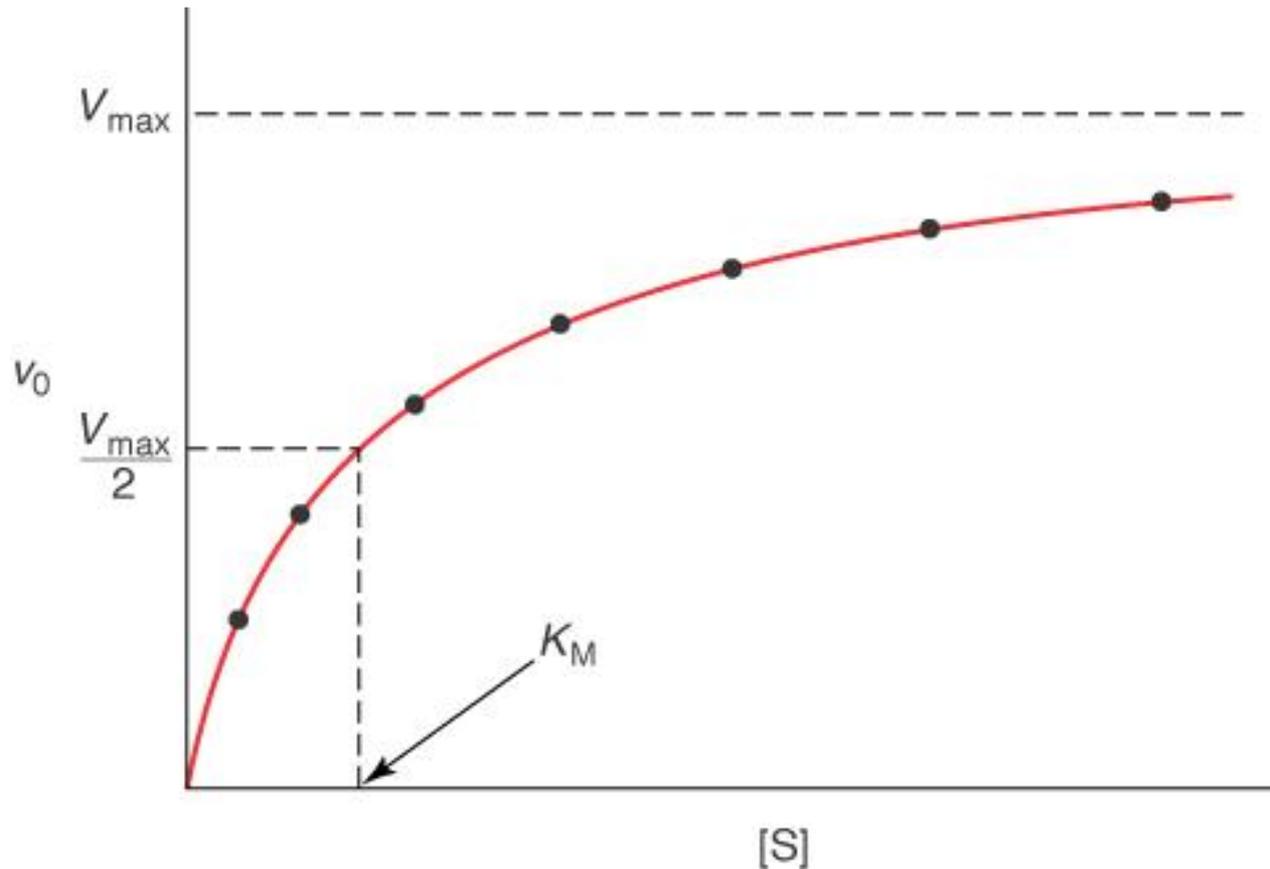$$V_0 = \frac{V_{\max}[S]}{K_m + [S]}$$

Where,

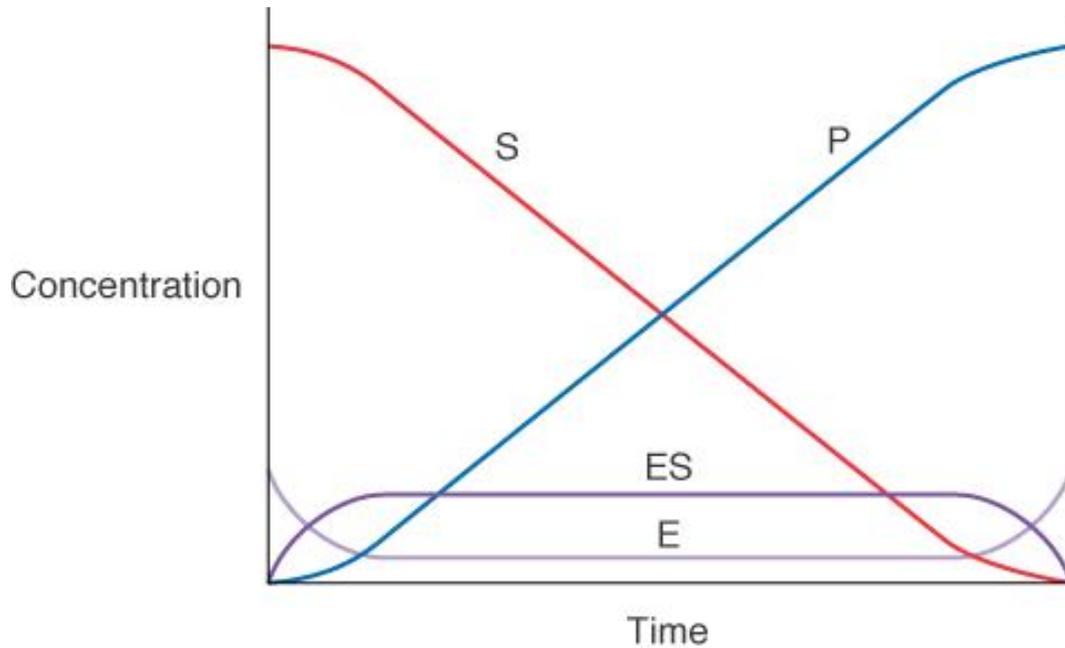    $V_0$ is the initial velocity

    $V_{max}$ is the maximum velocity

    [S] is the substrate concentration

    Km (Michaelis-Menten constant) is the substrate concentration     at which the reaction velocity is the half of the maximum velocity.

# Graphical determination of $K_M$

# Changes in concentration for a simple enzyme-catalyzed reaction



ES remains constant while S is converted to P.Here all the substrate is converted to P

# Control of enzyme activity

*Inhibitors and modulators help control enzyme activity in cells*

- Irreversible Inhibitior

- Competitive Inhibitior

- Mixed Inhibitior

- Allosteric inhibitior

# Irreversible Inhibition

- Certain compounds interact with enzyme so tightly that their effect is irreversible.

  - Any agent that covalently modifies the amino acid side chain in a protein can potentially act as an irreversible enzyme inhibitor.

# Irreversible inhibition



**Chymotrypsin (active)** + **DIPF** → **F** → **DIP-chymotrypsin (inactive)**
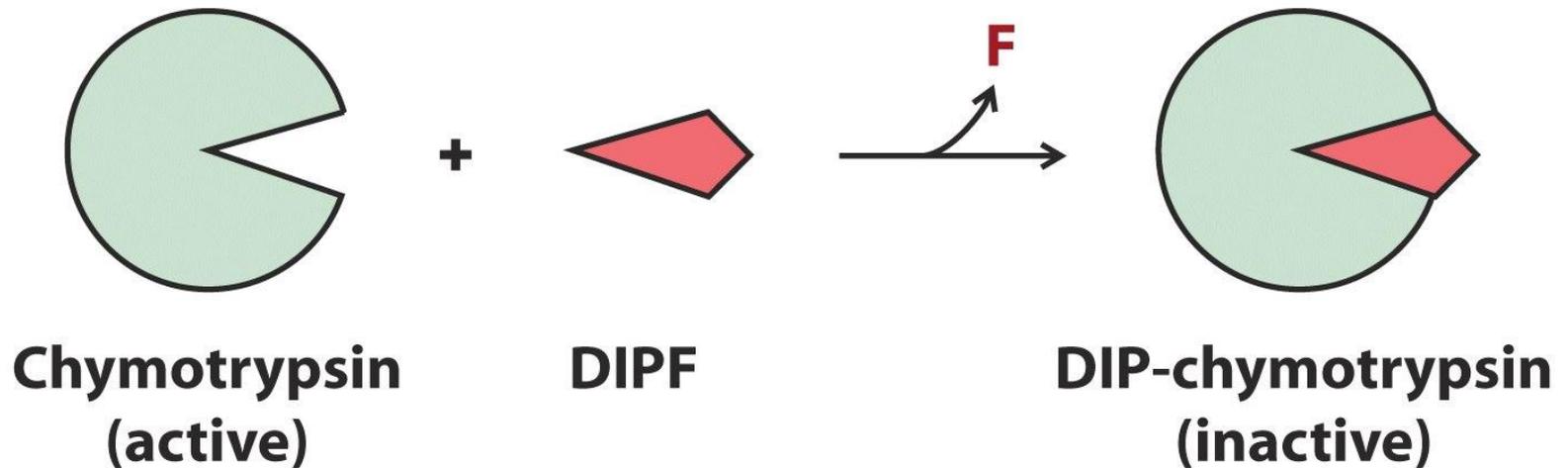
Diisopropylphosphofluoridate (DIPF) is an irreversible inhibitor of the enzyme chymotrypsin

# Competitive inhibition

- In competitive inhibition, the inhibitor is a substance that directly competes with a substrate for binding to the enzyme's active site.

  - Competitive inhibitors are molecules which are similar in shape and chemical properties to substrates and capable of binding to enzyme active sites. It lacks the exact electronic charge that allows it to react

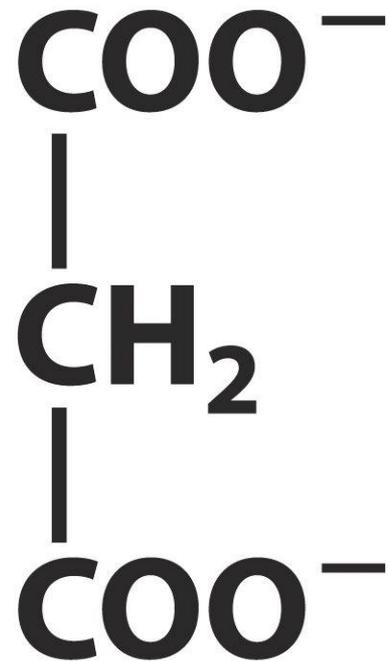# Competitive enzyme inhibition

# Succinate dehydrogenase
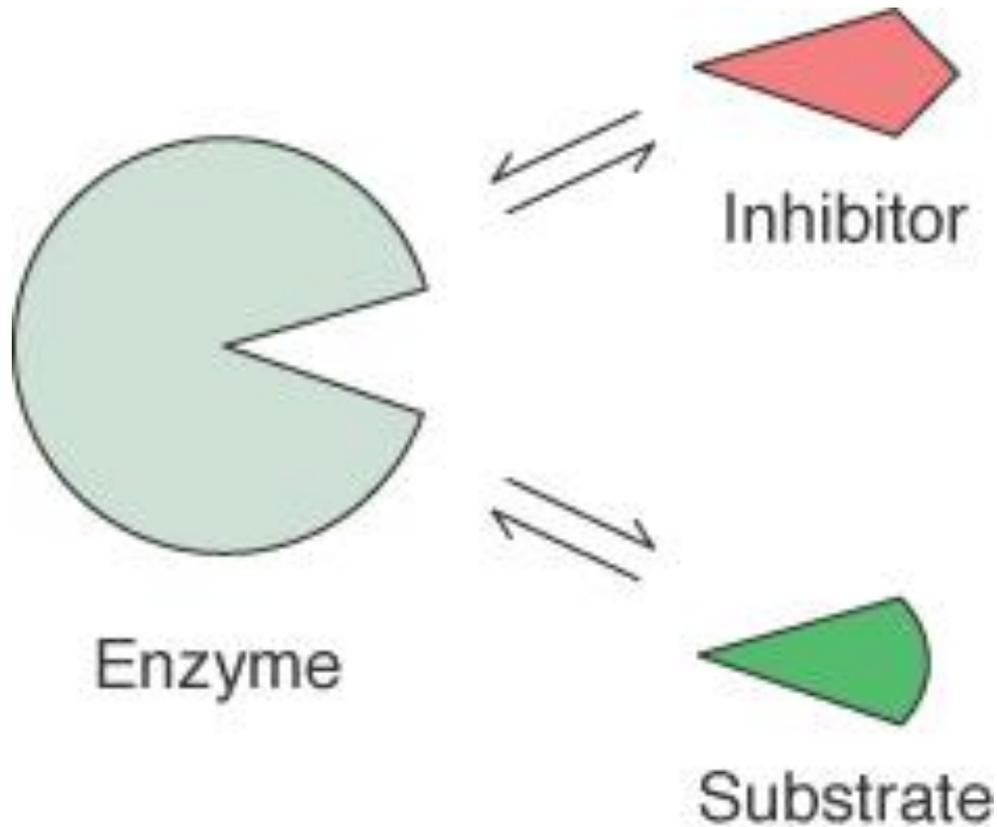


Succinate → (succinate dehydrogenase) → Fumarate

Malonate is a competitive inhibitor

# A competitive inhibitor

$$COO^-$$
$$|$$
$$CH_2$$
$$|$$
$$COO^-$$

**Malonate**

# Mixed inhibition

- In mixed inhibition, the inhibitor binds to a site of the enzyme other than the active site and elicits a conformational change. As a result, the apparent $V_{max}$ decreases and the apparent $K_m$ may increase or decrease.

  - When the $K_m$ is not affected by the inhibitor, it is said as non competitive.

# Mixed enzyme inhibition



Enzyme        Enzyme + Substrate

Enzyme + Inhibitor        Enzyme + Substrate + Inhibitor
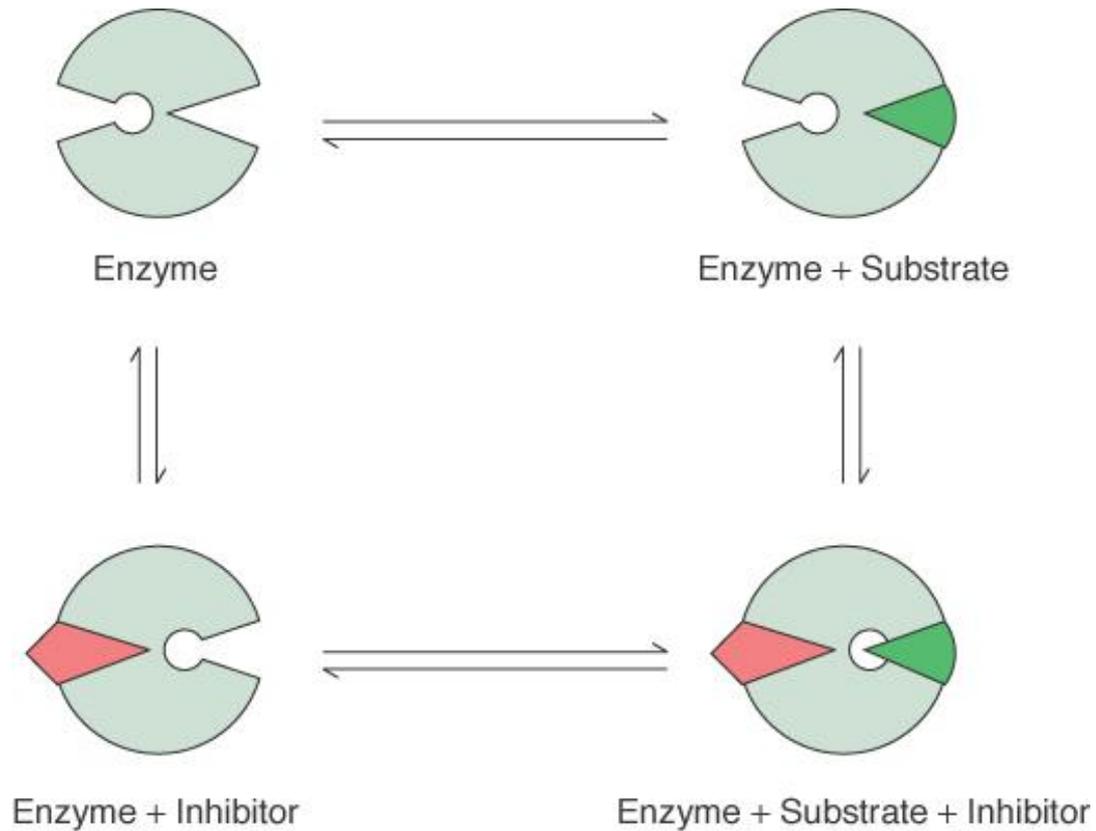
# Allosteric Inhibition

- Oligomeric enzymes –those with multiple active sites in one multi subunit protein- are commonly subjected to allosteric inhibition. Catalytic proteins containing both an active site and a modulator site are called allosteric enzymes.

  - In allosteric inhibition, the inhibitor binding to one subunit of an enzyme may decrease the catalytic activity of the of all the subunits.
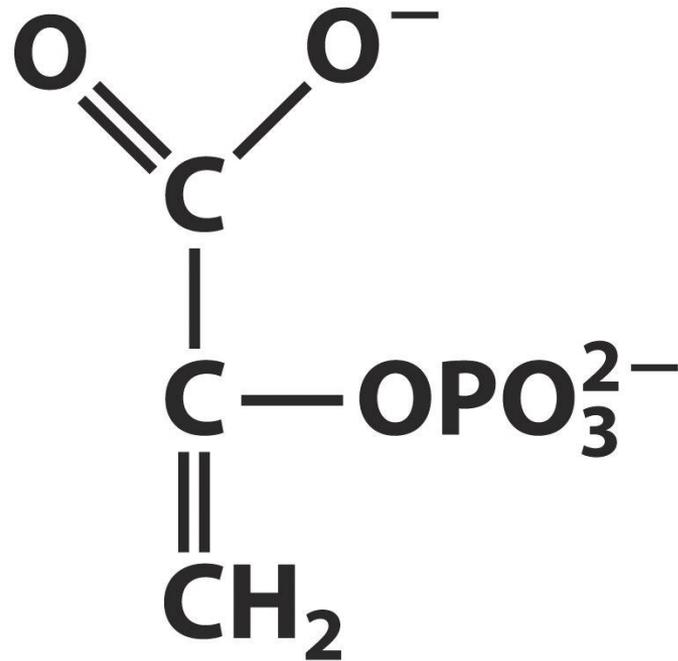
# Feedback inhibition…

- Cells have other ways of controlling the activity of enzymes. A fine slowdown control of enzyme activity built into some enzymes relies on **feed back inhibition**; *i.e., the concentration of a product at the end of a long series of steps builds up and then "feed back" to inhibit the enzyme in a preceding step.*
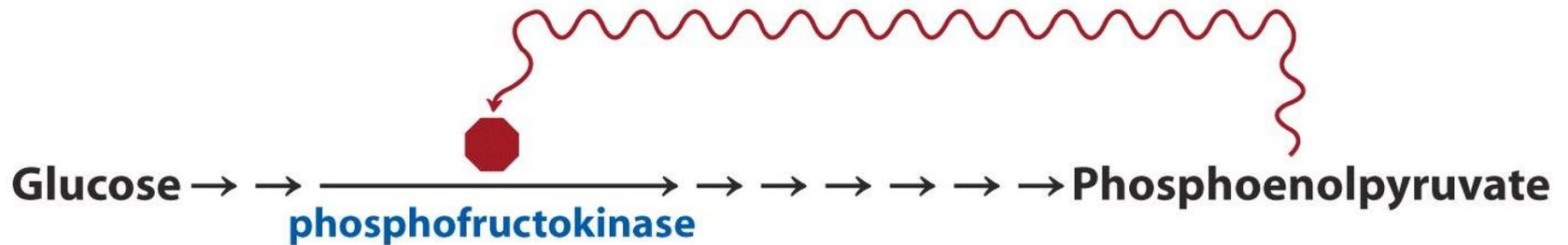
# Feedback inhibition…



Fructose-6-phosphate + ATP → (phosphofructokinase) → Fructose-1,6-bisphosphate + ADP
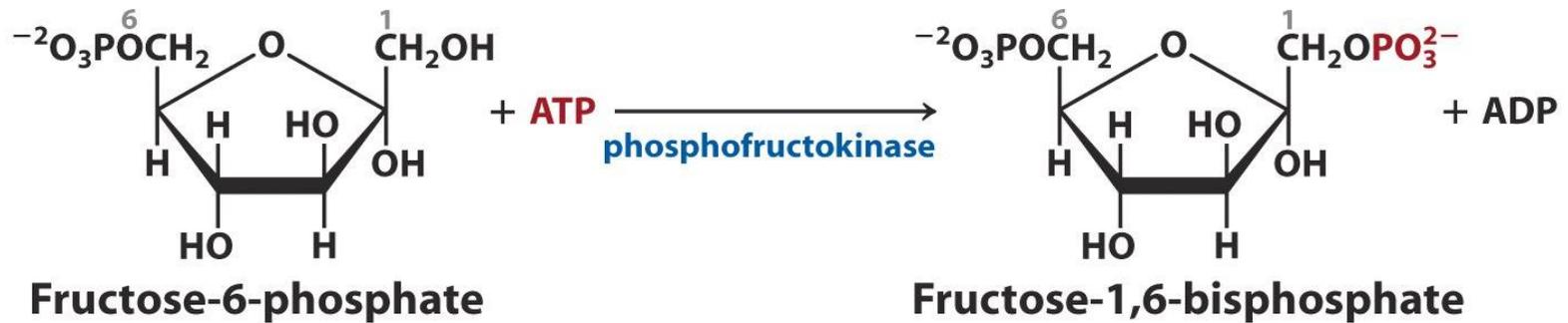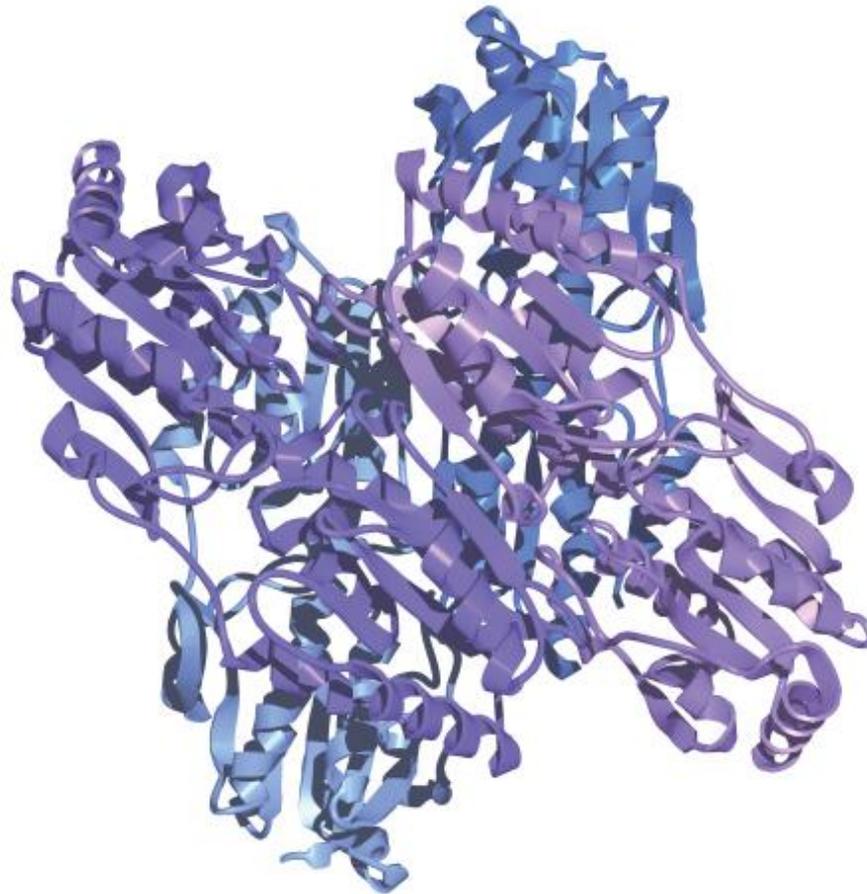
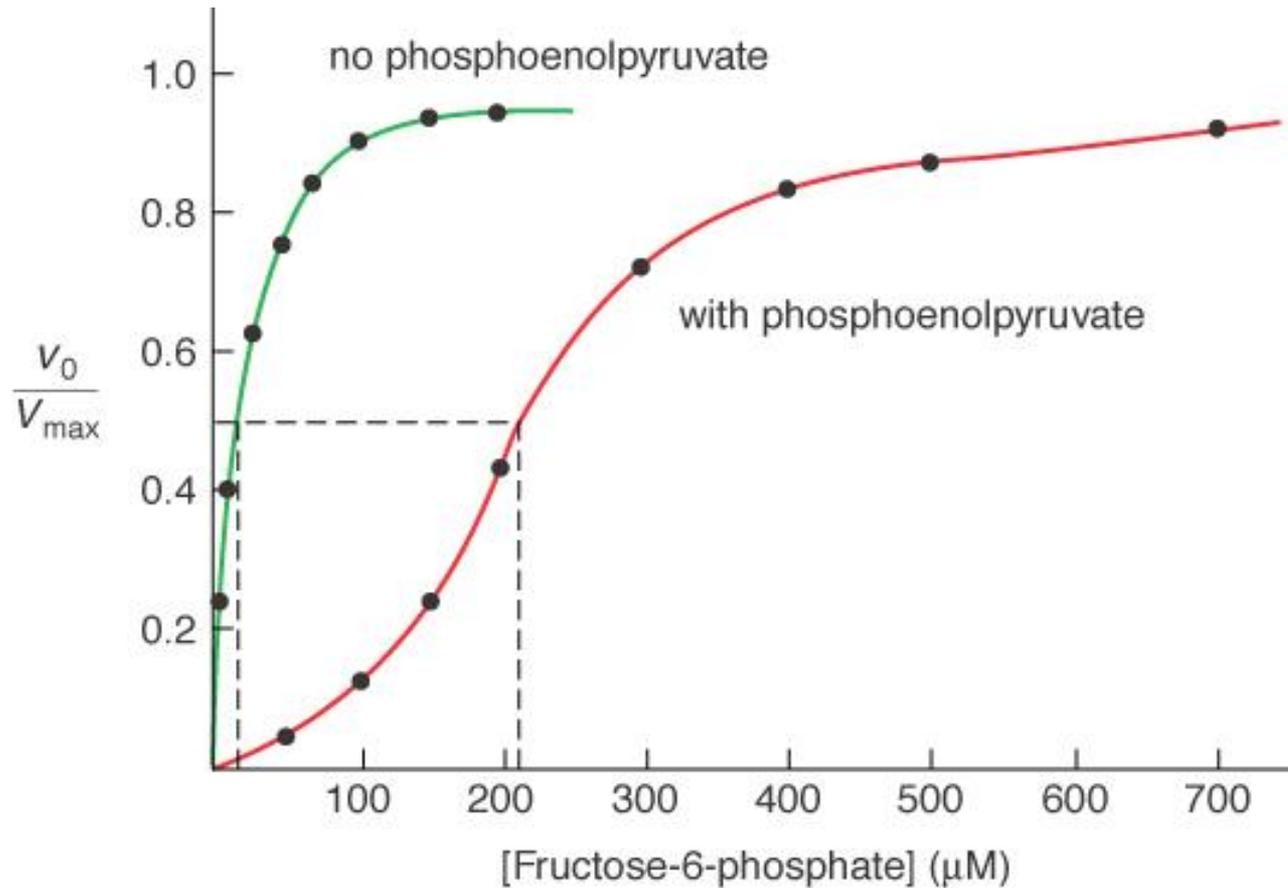Feed back inhibitor

Phosphoenolpyruvate

# Feedback inhibition



Here phosphoenolpyruvate is a negative modulator

# Structure of phosphofructokinase from *B. stearothermophilus*

# Effect of phosphoenolpyruvate on phosphofructokinase activity

# Denaturation of enzymes

### *Enzymes are sensitive to pH and temperature*

- Enzyme's activity  vary with changes in pH. Most enzymes exhibit maximum activity at a pH near 7, the pH of the most body fluids. The pH where maximum activity occurs is called the optimum pH of the enzyme.

- Enzyme activity usually varies with temperature. At temperature near $20^oC$, most enzymes are active and relatively stable. An enzyme's activity decreases beyond the optimum temperature. The decrease in activity is usually due to  heat denaturation of the enzyme. In case of nonbiologic catalyst an increase in temperature increases the reaction rate.

# Enzyme assay

***Assays of enzyme activity are useful in the diagnosis of disease***

**Enzyme activity** *is expressed as the rate at which the enzyme catalyzes the conversion of a substrate to products.* Activity depends on the concentration of the enzyme and substrate, and reaction conditions (pH, temperature).

*Experiments in which the enzyme activity is measured are called* **enzyme assays.** Many diagnostic procedures consist of assays for blood serum enzymes. The concept of this diagnostic procedure is simple: slight leakage of of enzymes from tissues and organs to the circulatory system is normal; additional leakage is abnormal and indicates damage or disease. For instance, damage to the heart muscle in heart attacks results in the release of the enzyme *creatine phosphokinase* (CPK) into the blood stream. Over 40 other blood serum enzymes can be subjected to clinical testing. Please refer to the hand-out regarding information about a few selected groups of serum enzymes.