GENERAL BIOCHEMISTRY - TOPICS

- **Biological macromolecules:**

✓ **Carbohydrates**

✓ **Lipids**

✓ **Aminoacids and Peptides**

✓ **Proteins**

- **Enzymes and enzymology**

The reversible binding of a ligand to a protein

- ✓ **The functions of many proteins involve the reversible binding of other molecules**. A molecule bound reversibly by a protein is called a **ligand** (any kind of molecule, including another protein). The transient nature of protein-ligand interactions is critical to life, allowing an organism to respond rapidly and reversibly to environmental changing and metabolic circumstances.

- ✓ A ligand binds at a site on the protein called the **binding site**, which is complementary to the ligand in size, shape, charge, and hydrophobic or hydrophilic character; the interaction is specific. A given protein may have separate binding sites for several different ligands.

- ✓ The binding of a protein and ligand is often coupled to a conformational change in the protein that makes the binding site more complementary to the ligand, permitting tighter binding. The structural adaptation that occurs between protein and ligand is called **induced fit**. In a multi-subunit protein, a conformational change in one subunit often affects the conformation of other subunits.

Enzymes

- With the exception of a few classes of catalytic RNA molecules (ribozymes, discovered in 1982), all enzymes are globular proteins.
- Enzymes have extraordinary catalytic power, often far greater than that of synthetic or inorganic catalysts.
- They have a high degree of specificity for their substrates, tremendously accelerating specific chemical reactions in aqueous solutions under very mild conditions of temperature and pH.
- Few nonbiological catalysts have all these properties.
- Their catalytic activity depends on the integrity of their native protein conformation. Thus, the primary, secondary, tertiary, and quaternary structures of enzymes are essential to their catalytic activity.

CATALYSTS

- A catalyst is a substance that increases the reaction rate without affecting the reaction equilibrium.

- If added to a reaction mixture, the catalyst undergoes no net change, nor does it alter the outcome of the reaction, but **it interacts with the reactants to create an alternative pathway for the formation of products**.

- **This alternative path has a lower activation energy**.

- This makes it easier for the reaction to take place and thus increases the rate.

Catalysts enhance reaction rates by lowering activation energies

Enzymes

TABLE 6-1 Some Inorganic Ions That Serve as Cofactors for Enzymes

Ions	Enzymes
Cu^{2+}	Cytochrome oxidase
Fe^{2+} or Fe^{3+}	Cytochrome oxidase, catalase, peroxidase
K^{+}	Pyruvate kinase
Mg^{2+}	Hexokinase, glucose 6-phosphatase, pyruvate kinase
Mn^{2+}	Arginase, ribonucleotide reductase
Mo	Dinitrogenase

- Catalysts for biological reactions
- Almost all are proteins
- Lower the activation energy
- Increase the rate of reaction
- Display specificity for a reaction (substrates)
- Activity is lost if enzymes are denatured
- May contain cofactors such as metal ions (Fe^{2+}, Mg^{2+}, Mn^{2+}, or Zn^{2+}) or organic molecules (vitamins) or work with coenzymes/cofactors (complex organic or metalloorganic molecules that act as transient carriers of specific functional groups).

Enzymes

Enzyme = *from the Greek en zymos, "leavened" (in* yeast)

- In 1850 Pasteur observed that the fermentation of sugar in alcohol was catalyzed by ferments: "theory of vitalism".
- Biochemists, by international agreement, have adopted a system for naming and classifying enzymes.
- This system divides enzymes into six classes, each with subclasses, based on the type of reaction catalyzed.

Class no.	Class name	Type of reaction catalyzed
1	Oxidoreductases	Transfer of electrons (hydride ions or H atoms)
2	Transferases	Group transfer reactions
3	Hydrolases	Hydrolysis reactions (transfer of functional groups to water)
4	Lyases	Cleavage of C—C, C—O, C—N, or other bonds by elimination, leaving double bonds or rings, or addition of groups to double bonds
5	Isomerases	Transfer of groups within molecules to yield isomeric forms
6	Ligases	Formation of C—C, C—S, C—O, and C—N bonds by condensation reactions coupled to cleavage of ATP or similar cofactor

Enzymes at work

- ✓ An enzyme-catalyzed reaction takes place within the confines of a pocket on the enzyme called **the active site**.
- ✓ The molecule that is bound in the active site and acted upon by the enzyme is called **the substrate**.
- ✓ The surface of the active site is lined with amino acid residues with substituent groups that bind the substrate and **catalyze its chemical transformation.**

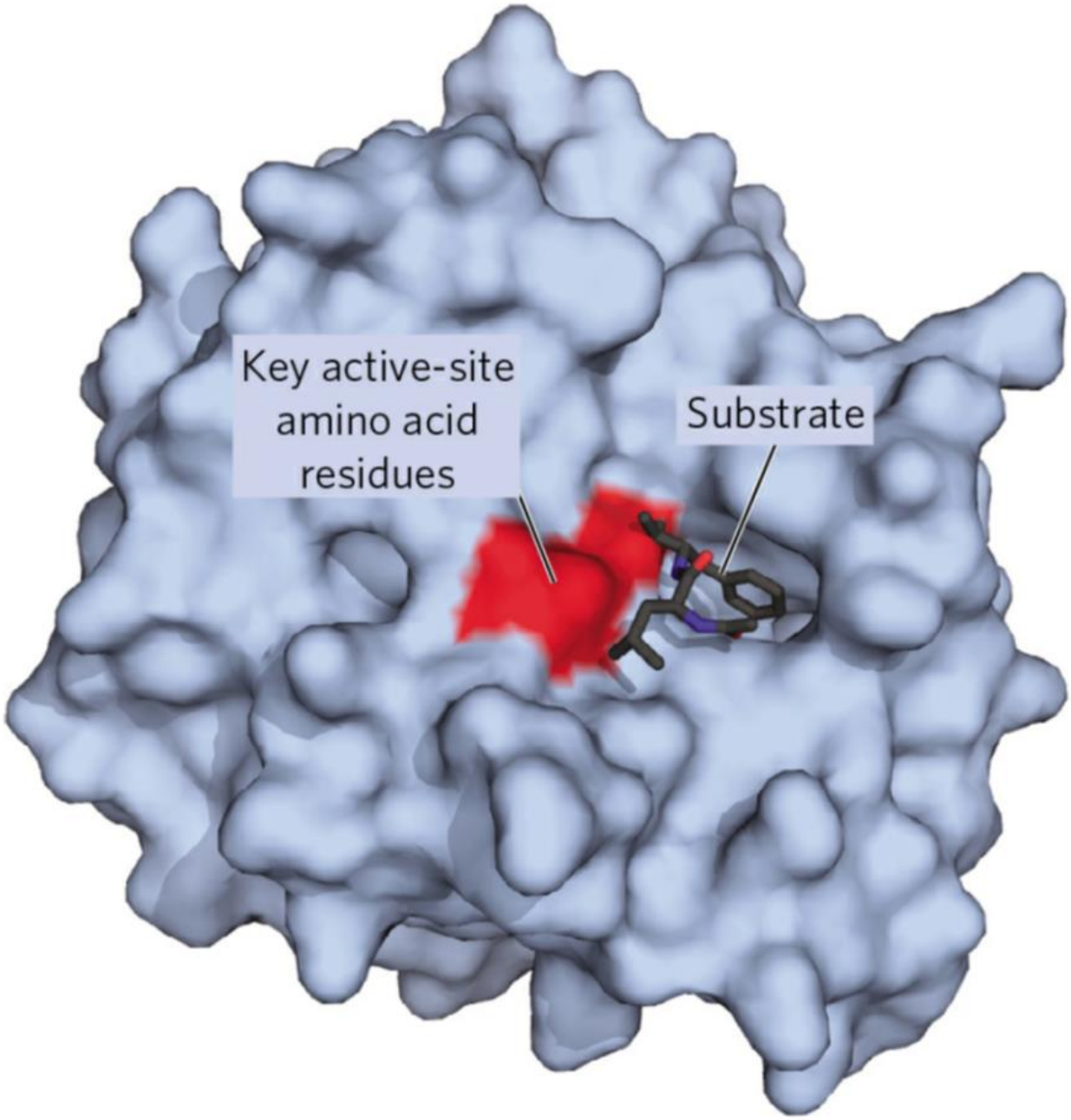
$$E + S \rightleftharpoons ES\ complex \rightleftharpoons E + P$$

E = enzyme

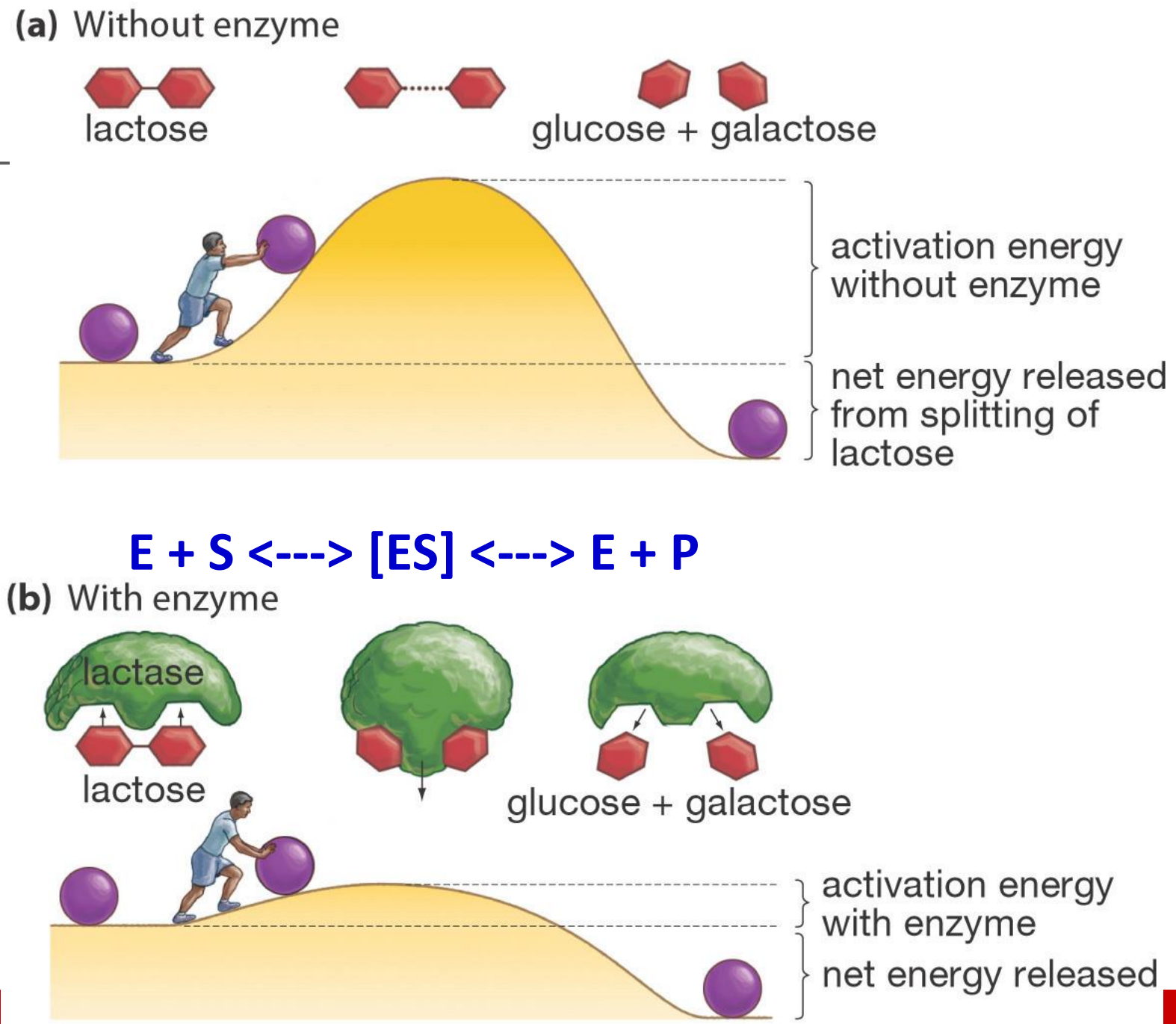
S = substrate

P = product

ES = enzyme-substrate complex


Key active-site amino acid residues
Substrate

Enzymes Lower a Reaction's Activation Energy



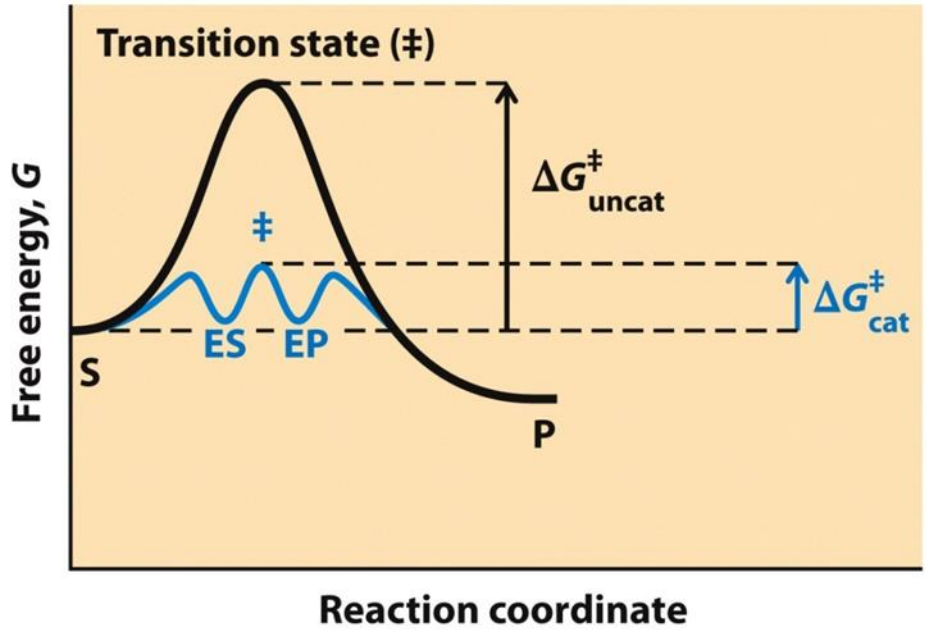


Figure 6-3
Lehninger Principles of Biochemistry, Fifth Edition

The transition state is a fleeting molecular moment in which bond breakage and bond formation proceed to the precise point at which decay to substrate or decay to product are equally likely (ES and EP can be considered intermediates).

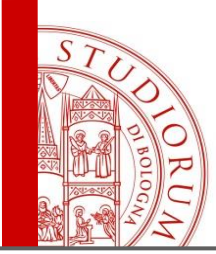
In the S → P reaction there is the formation and disappearance of chemical species called transient reaction intermediates (ES and EP).
Enzymes can increase the rate of a reaction by 5 to 17 orders of magnitude!!!!!!
For many enzymes, several steps may have similar activation energies, which means they are all partially rate-limiting.

What is the source of the energy for the dramatic lowering of the activation energies for specific reactions?

The interaction between substrate and enzyme in ES complex is mediated by the same forces that stabilize protein structure, including hydrogen bonds, ionic interactions, and the hydrophobic forces. **Formation of weak interaction in the ES complex is accompanied by release of a small amount of free energy that stabilizes the interaction, called binding energy** (ΔG_B). ***Binding energy is a major source of free energy used by enzymes to lower the activation energy of reactions.***

NONCOVALENT INTERACTIONS

Weak, noncovalent interactions help not only to stabilize protein structure and protein-protein interactions, but also for the formation of complexes between proteins and enzyme substrates**. Much of the energy required to lower activation energies is derived from weak, noncovalent interactions between the substrate and the enzyme.**



$$E + S \rightleftarrows ES\ complex \rightleftarrows E + P$$

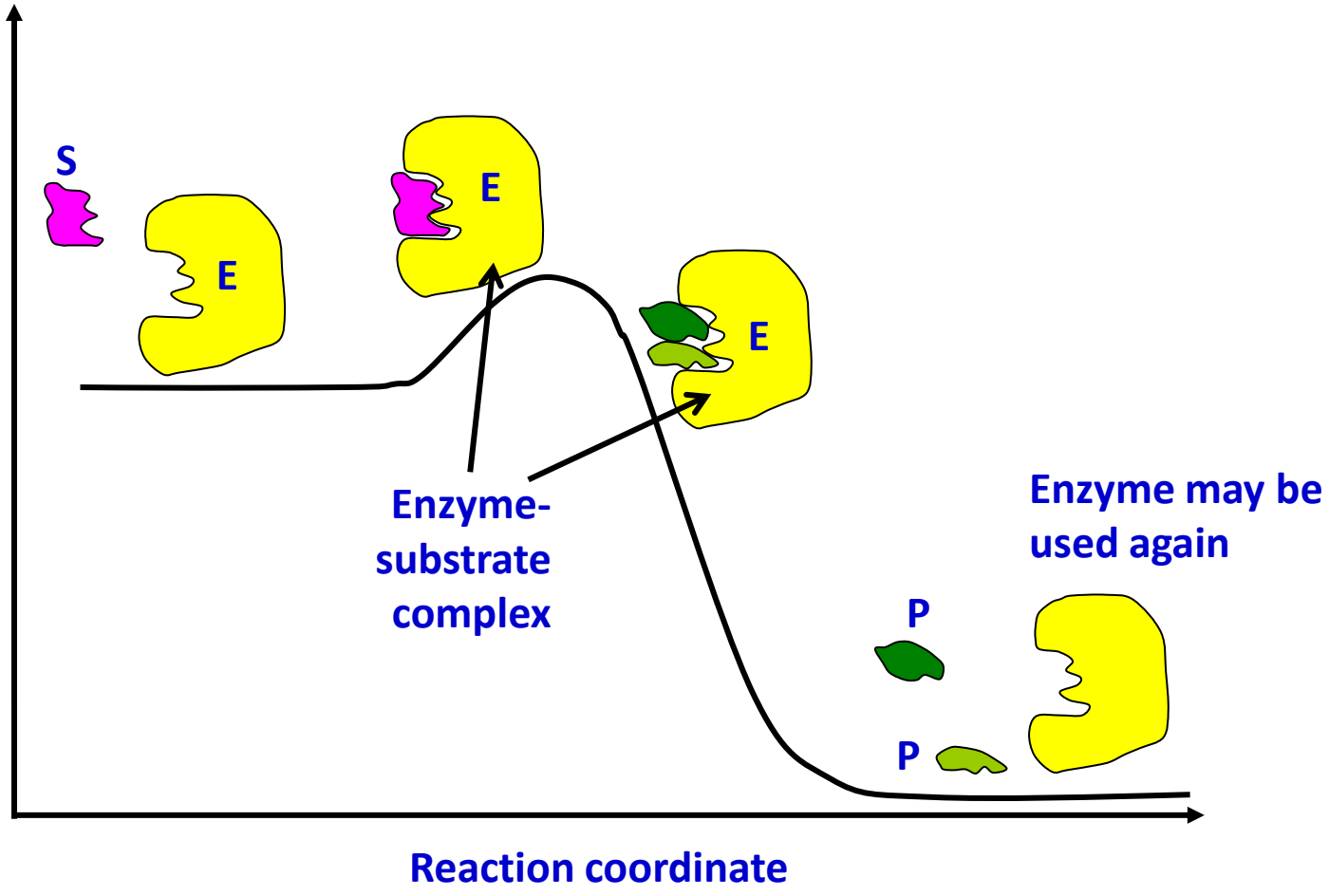
The catalytic power of enzymes comes from the energy released during the formation of many SPECIFIC bonds and weak interactions between the substrate and the enzyme.

The weak interactions remain optimal in the transition state of the reaction.

The active site is not complementary to the substrate, but to intermediates in the transition state.

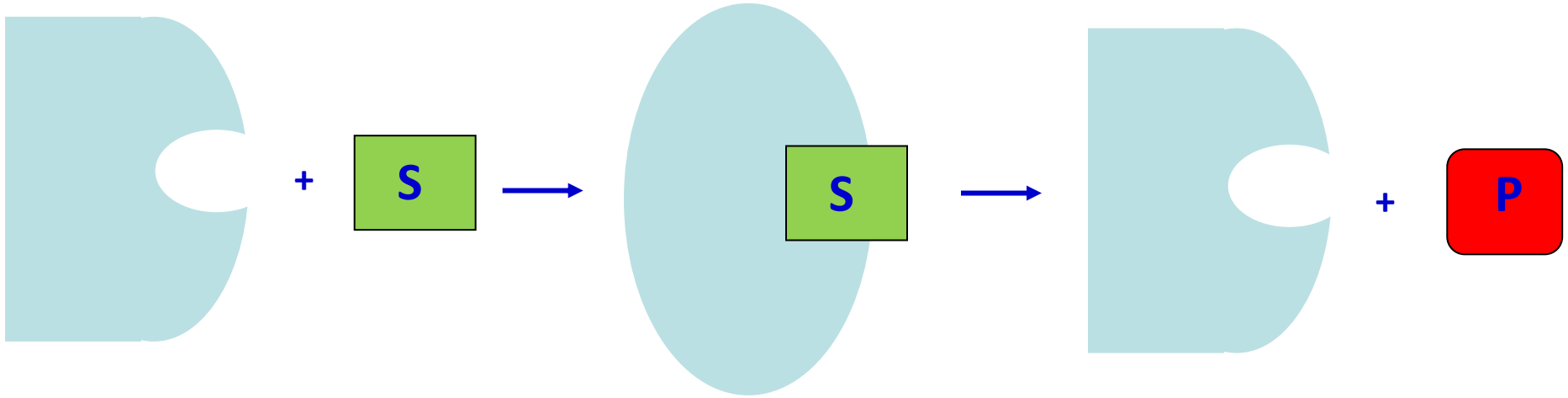
The Lock and Key Hypothesis
(Emil Fisher, 1894)

Enzymes were structurally complementary to their substrates, so that they fit together like a lock and key



Induced Fit Model

The "lock and key" hypothesis can be misleading when applied to enzymatic catalysis. An enzyme completely complementary to its substrate would be a very poor enzyme; in order to catalyze reactions, an enzyme must be complementary to intermediates in the transition state.

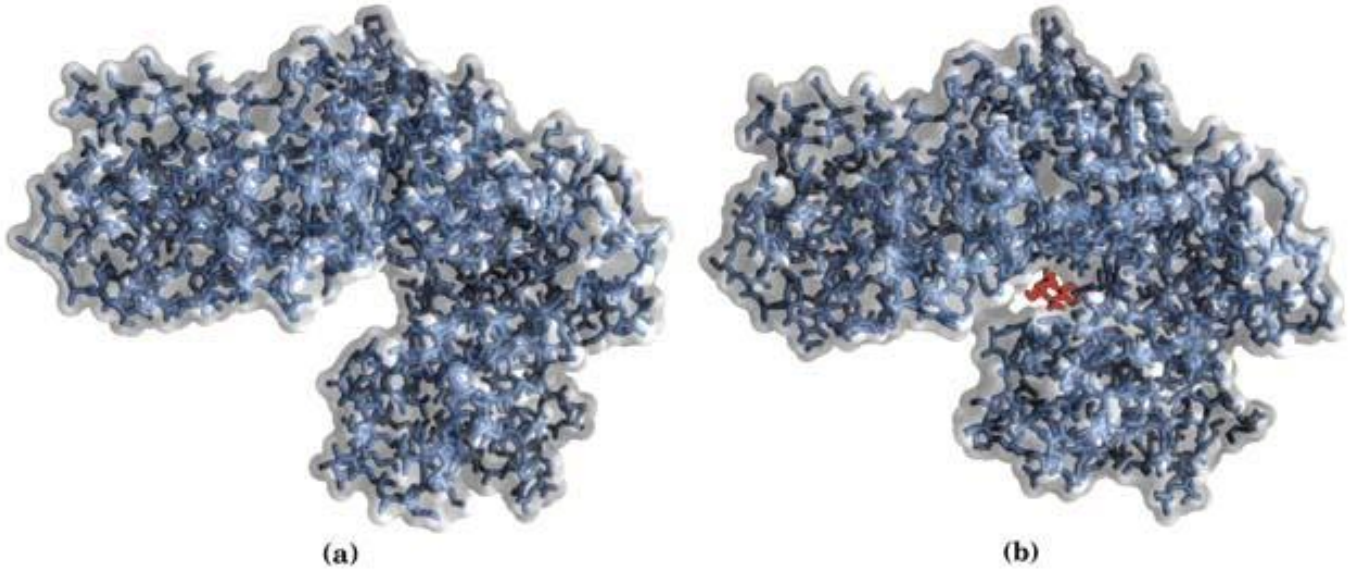


E + S ES complex E + P

Induced Fit Model
(Daniel Koshland, 1958)

- Enzyme structure is flexible, not rigid (specific active site)
- The active site of the enzyme has the “adjusted shape” to bind substrate and maximize interaction in the transition state
- Increases range of substrate specificity
- Shape changes also improve catalysis during reaction

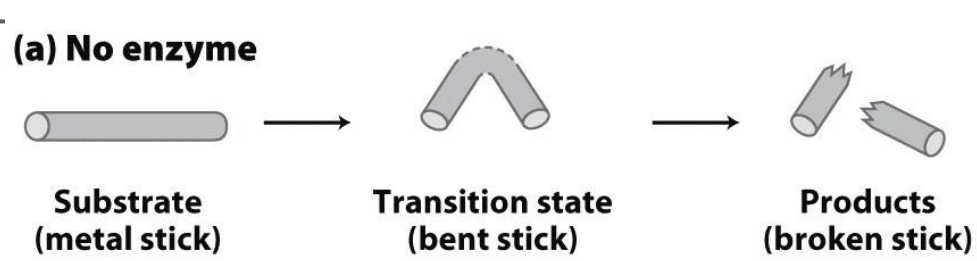
The Induced Fit Hypothesis

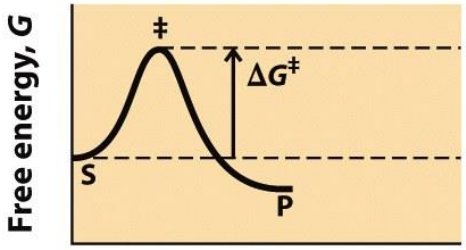


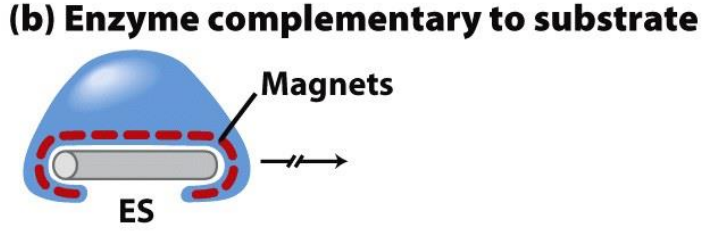
Hexokinase (a) without or (b) with glucose substrate

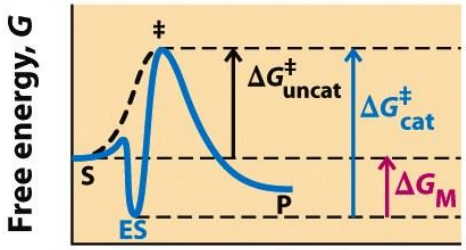
- Glucose + ATP ↔ Glucose-6-P + ADP
- Enzyme exists in open & closed forms
- Glucose induces a conversion to the closed form

TS (ES) Stabilization Cartoon

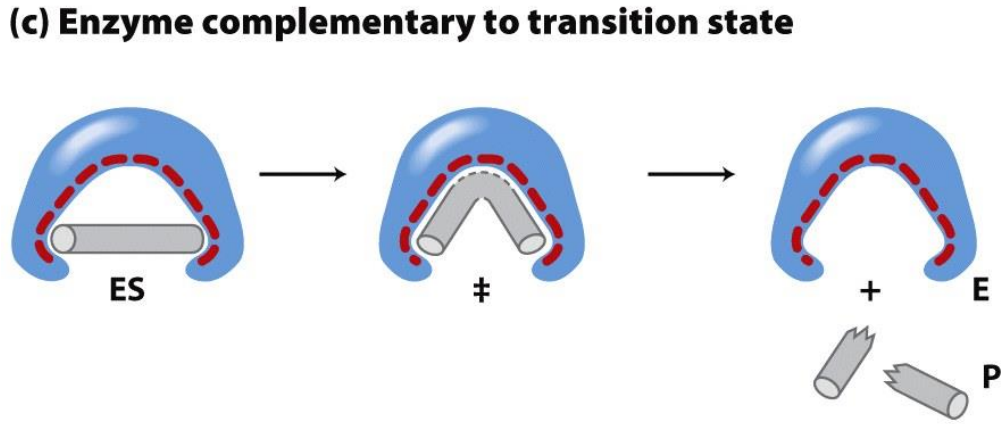


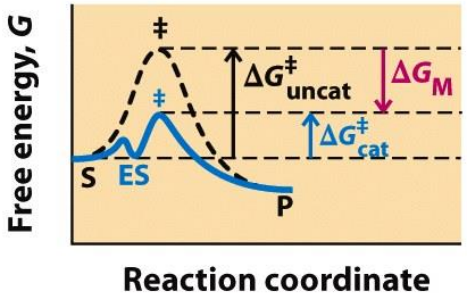






No catalysis is obtained by just binding substrate tightly!





BINDING SITE

- ✓ **Some weak interactions are formed in the ES complex, but the fully complementary interactions between substrate and enzyme is formed only when the substrate reaches the transition state.**

- ✓ **The enzyme active site** is structured so that some of the **weak interactions occur preferentially in the reaction transition state**.

- ✓ **The free energy (binding energy) released by the formation of these bonds partially offsets the energy required to reach the top of the energy hill (activation energy).**

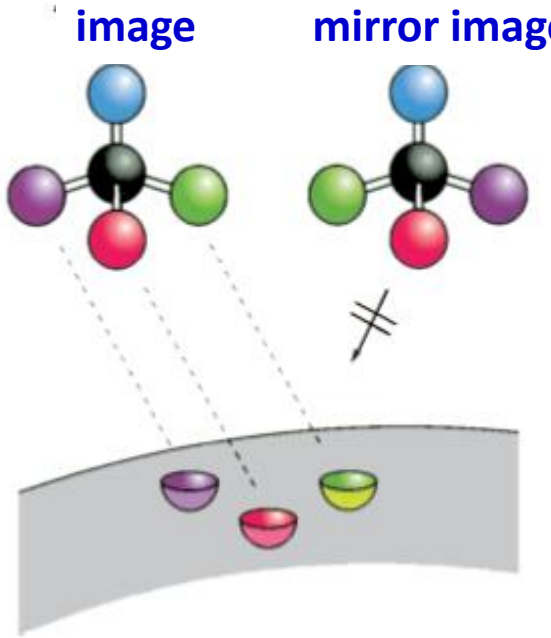
- ✓ **The sum of the unfavorable (positive) activation energy ΔG‡ and the favorable (negative) binding energy ΔGB results in a lower net activation energy.**

Properties of Enzymes

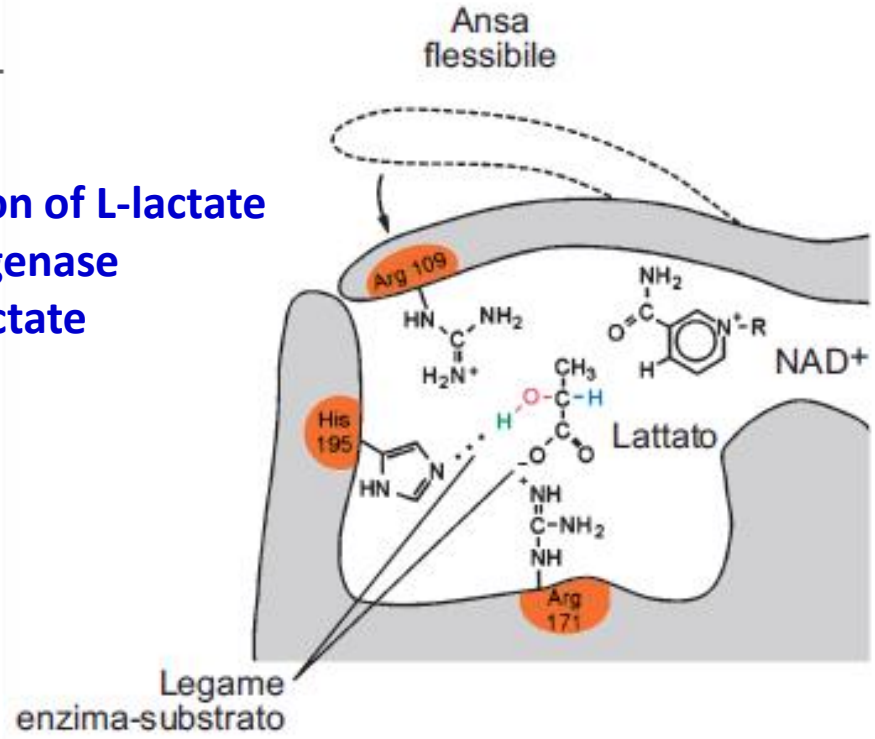
Specificity of enzymes

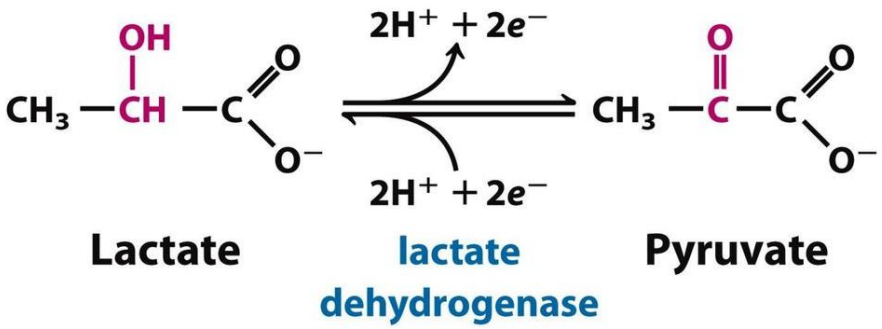
1. **Absolute –** one enzyme acts only on one substrate (example: urease decomposes only urea; arginase cleaves only arginine)
2. **Relative –** one enzyme acts on different substrates which have the same bond type (example: trypsin cleaves different proteins at the carboxyl side of amino acids lys or arg)
3. **Stereospecificity –** some enzymes can catalyze the transformation of only substrates which are in certain geometrical configuration, D or L, cis- or trans-

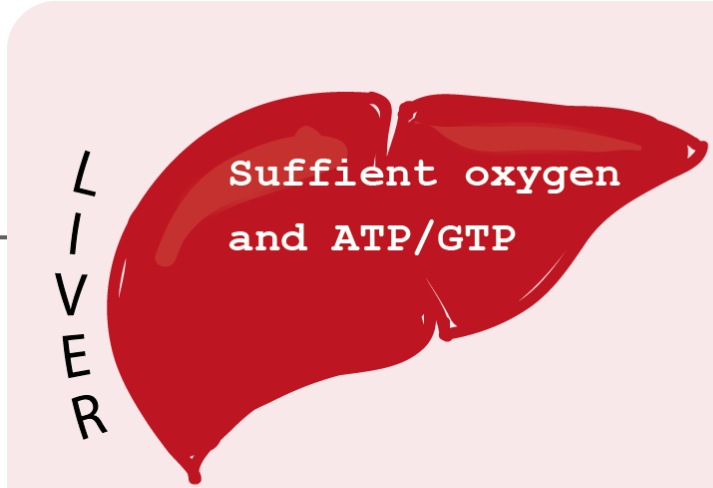
Many enzymes are stereospecific



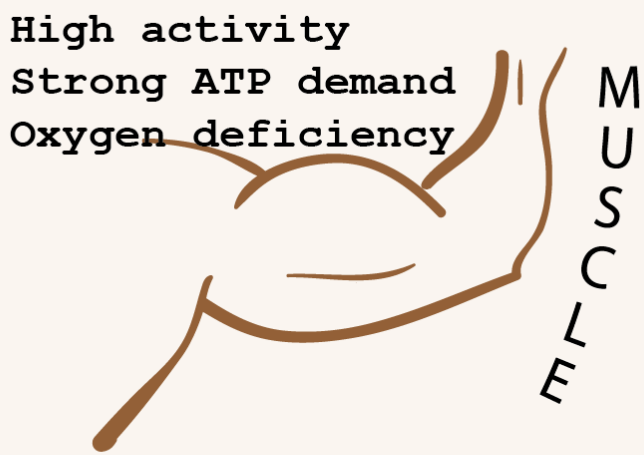
Interaction of L-lactate dehydrogenase with L-lactate



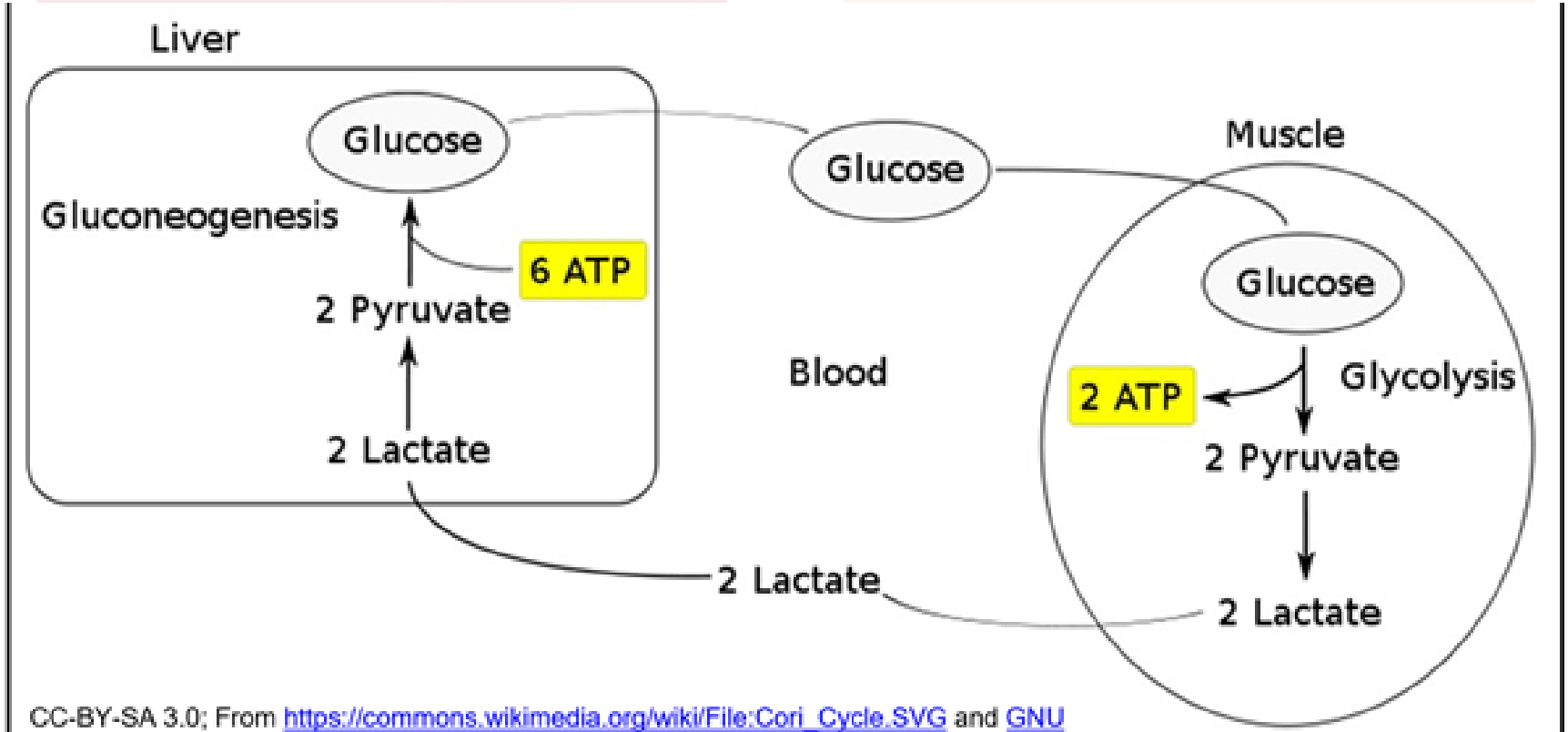




GLUCONEOGENESIS

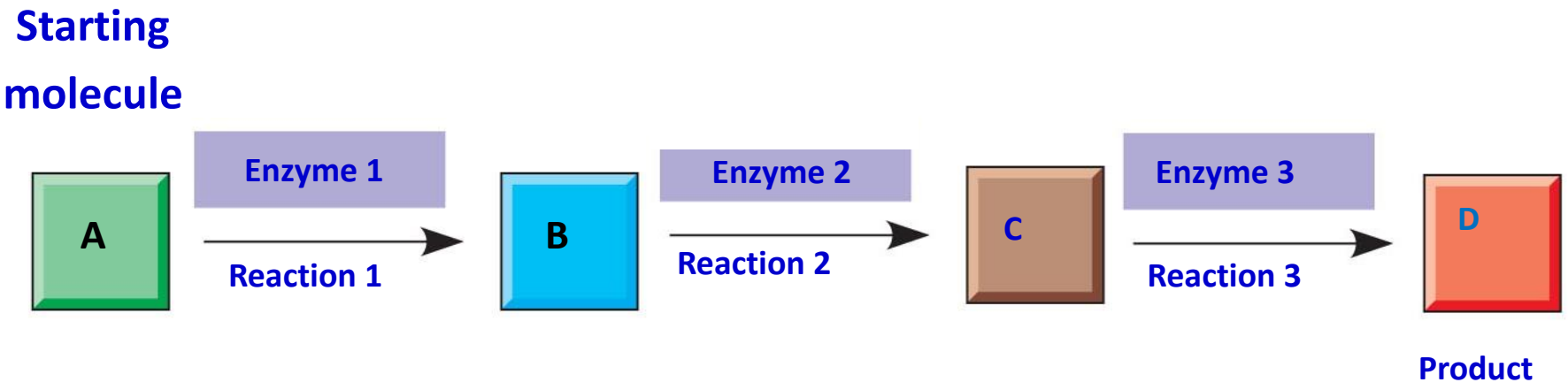


GLYCOGENOLYSIS, GLYCOLYSIS



ENZYMES CONTROL METABOLİSM BY REGULATİNG METABOLİC REACTİON RATES

- Act only upon a specific substrate (or substrate group)
- Do not change the equilibrium of the reaction but increase its rate
- Are considered catalysts that promote the formation of coherent reactions (each enzyme acts on its substrate), making possible the formation of metabolic pathways

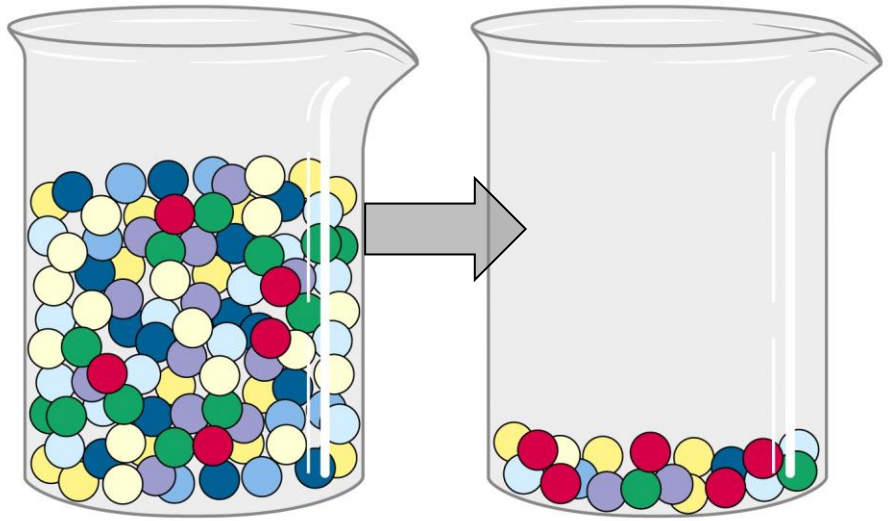


Enzyme kinetics

How to define the enzyme specific activity?

$$\text{Specific activity} = \frac{\text{Activity of enzyme (IU)}}{\text{total amount (mg) of proteins}}$$

Total activity of a specific enzyme in a sample (5 red balls)



specific activity increases (red balls/ total balls)

ACTIVITY= International unit (IU) amount of enzyme that converts 1 µmole of the substrate to the product in 1 minute (IU) unde defined conditions of T (25° C) and pH (7.4). e.g. 10 IU= 10 µmole/min

SPECIFIC ACTIVITY enzymatic activity (IU) per mg of proteins present in the preparation
e.g. 10 µmole/min/mg protein or 10 IU/mg protein

ENZYME KINETICS
Initial Velocity (v_o) and [S]

- The concentration of substrate [S] will greatly influence the rate of product formation, termed the velocity (v) of a reaction.

- At the start of a reaction, [S] is in large excess, thus the **initial rate/velocity** (v_o) **of the reaction will be dependent on substrate concentration.**

$$E + S \rightleftharpoons ES\ complex \rightleftharpoons E + P$$

The rate (or velocity) of the reaction

The initial rate represents the amount of S that reacts per unit time. It is expressed by the equation:

$$V_0 = k\,[S]$$

$v_0 = t \rightarrow 0$ ⇒ **in kinetics experiments we measure the initial rate (or initial velocity), designated V_0**

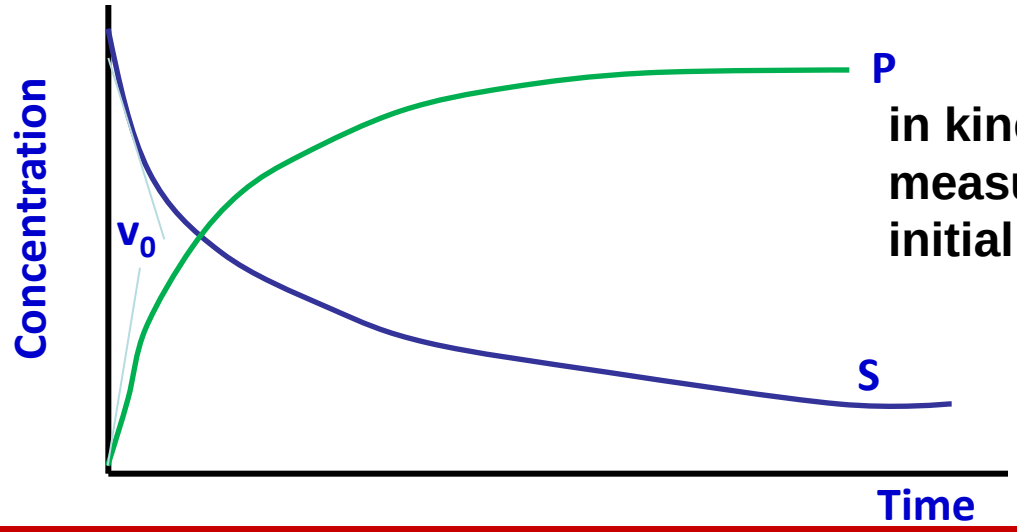
The factor *k* is a proportionality constant that reflects the probability of reaction under a given set of conditions (pH, temperature), so **the rate depends only on the concentration of S.**

REACTION RATE

$$S + E \rightleftharpoons ES \rightleftharpoons P + E$$

$$V = -\frac{d(S)}{dt} = \frac{d(P)}{dt}$$

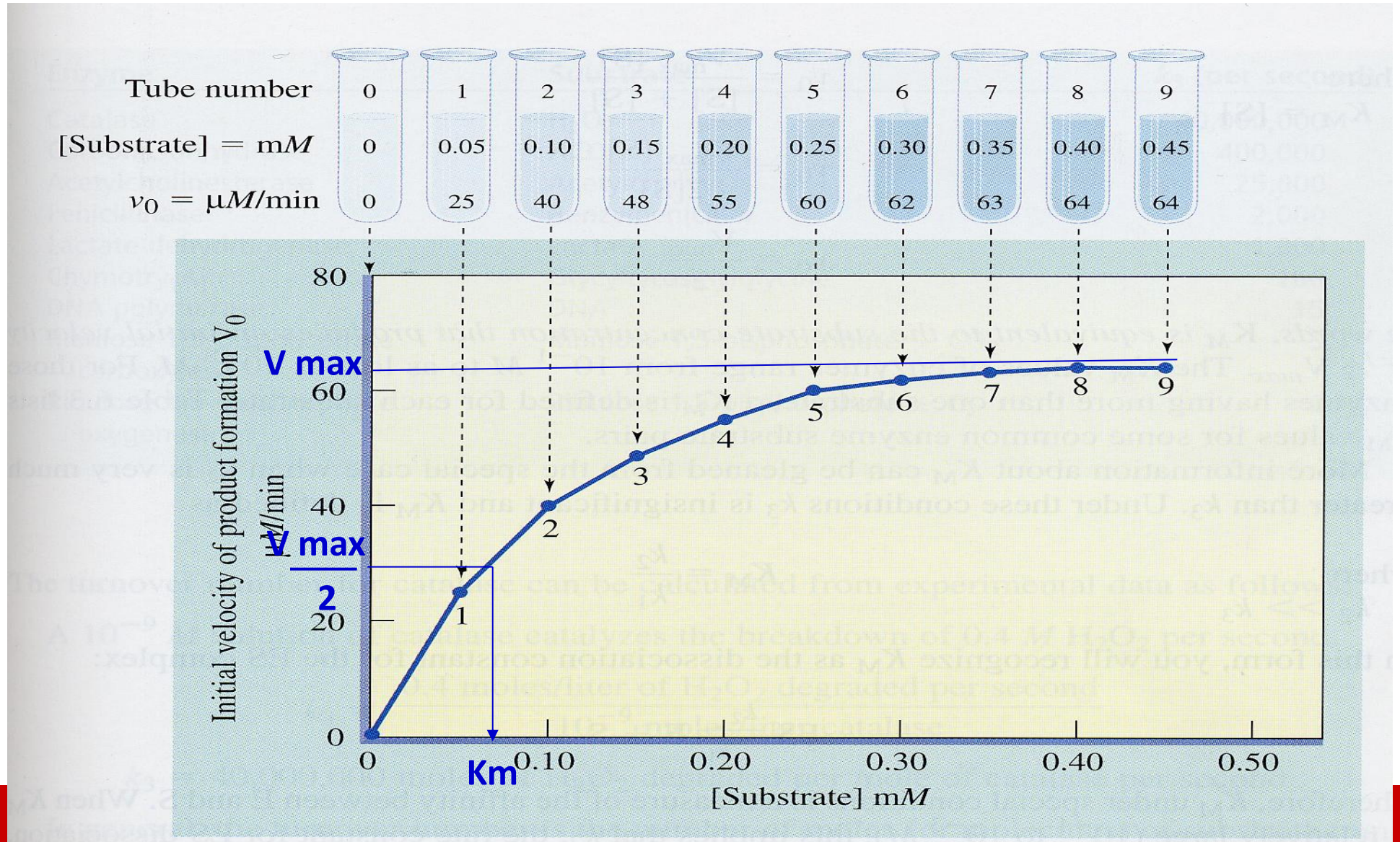
$v_0 = t \rightarrow 0$



in kinetics experiments we measure the initial rate (or initial velocity), designated V_0

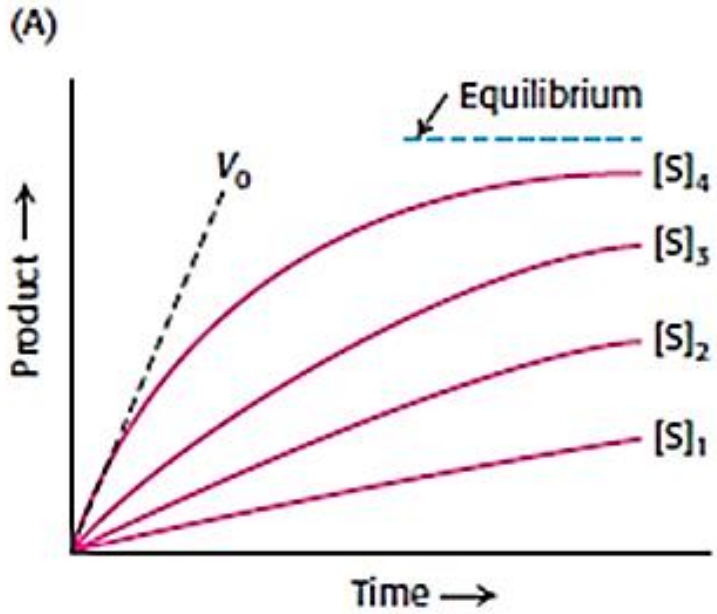
Study of the effect of substrate concentration on the rate of a reaction

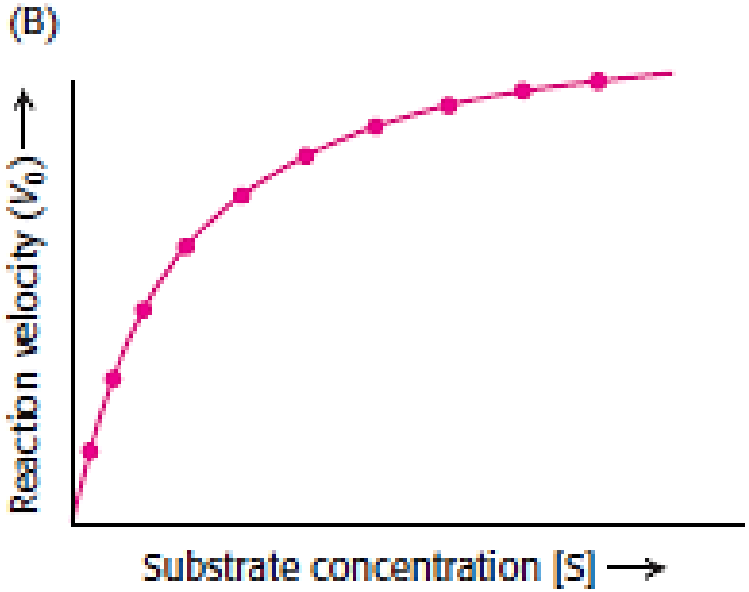
[E] = constant



- In a typical reaction, the enzyme may be present in nanomolar quantities, whereas [S] may be five or six orders of magnitude higher.
- The initial velocity of the reaction will be dependent on substrate concentration.

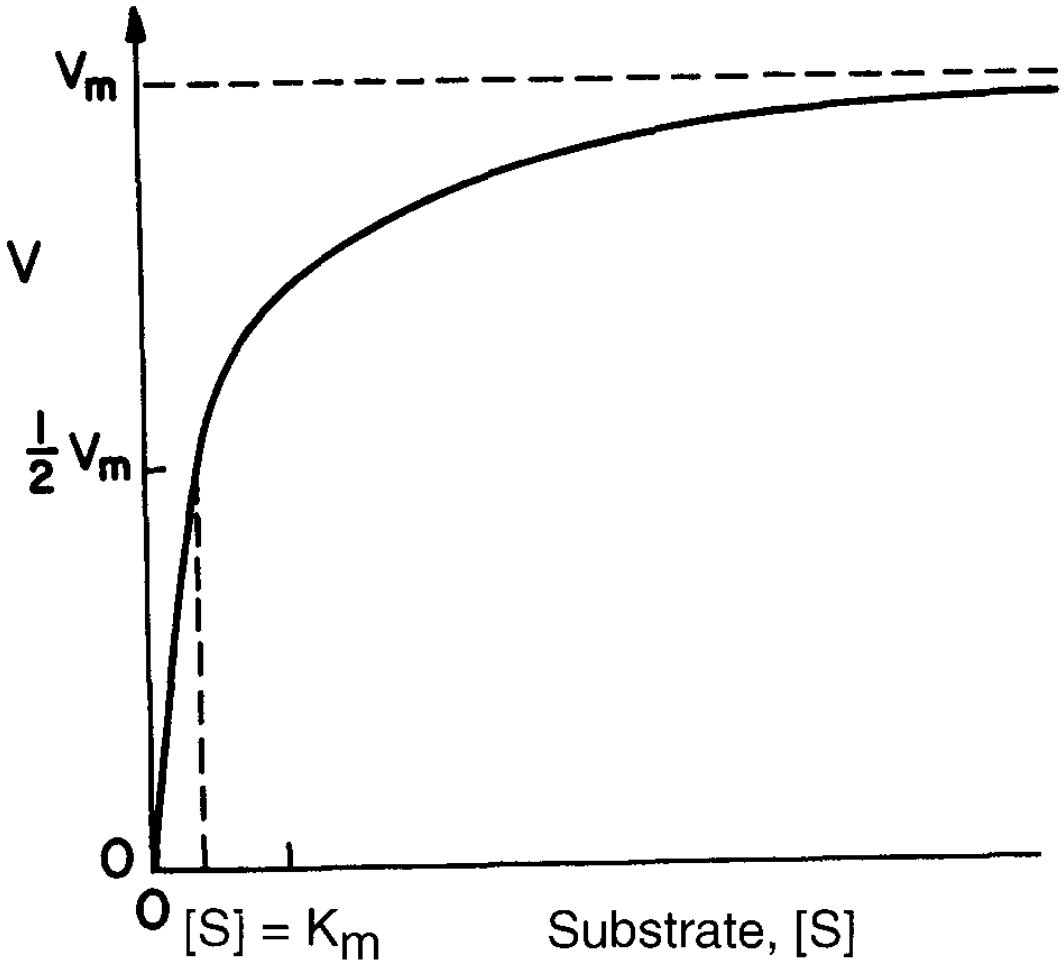
[E] = constant





$V_0 \longrightarrow t \longrightarrow 0$

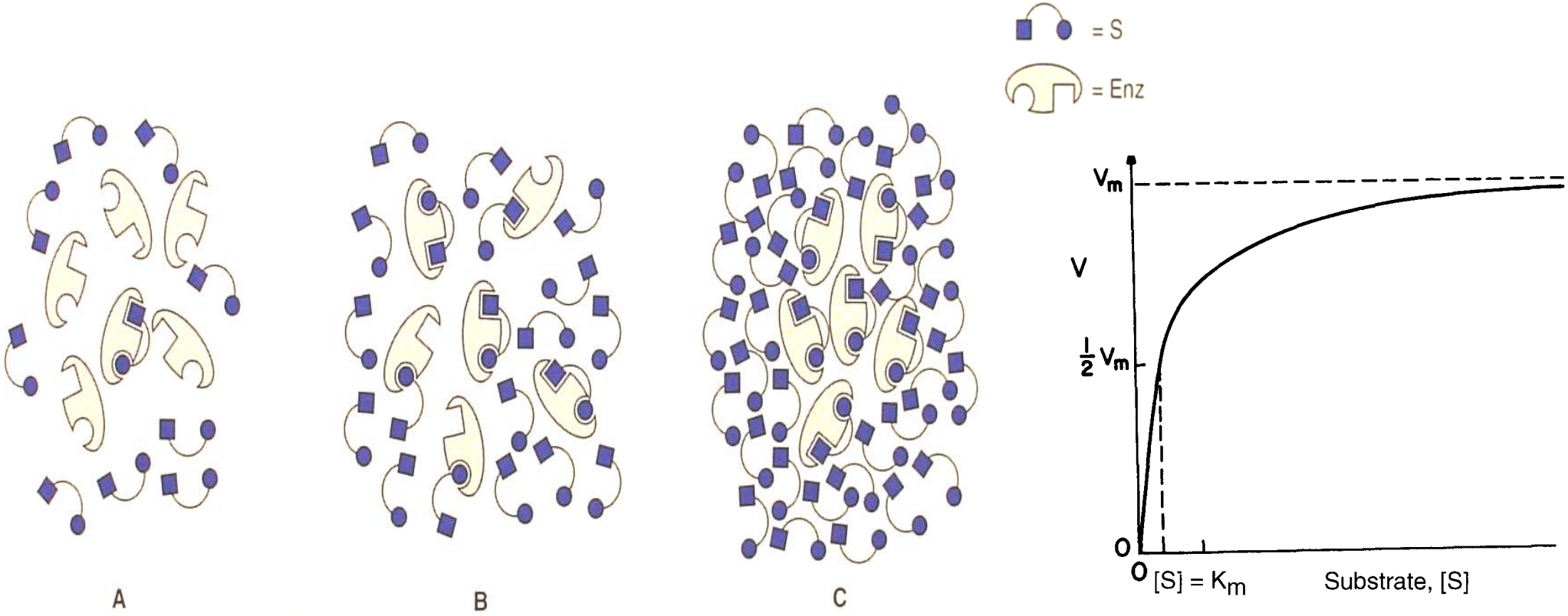
Saturation Enzyme Kinetics



When initial velocity (V_0) is plotted against [S], a hyperbolic curve results, where V_{max} represents the maximum reaction velocity.

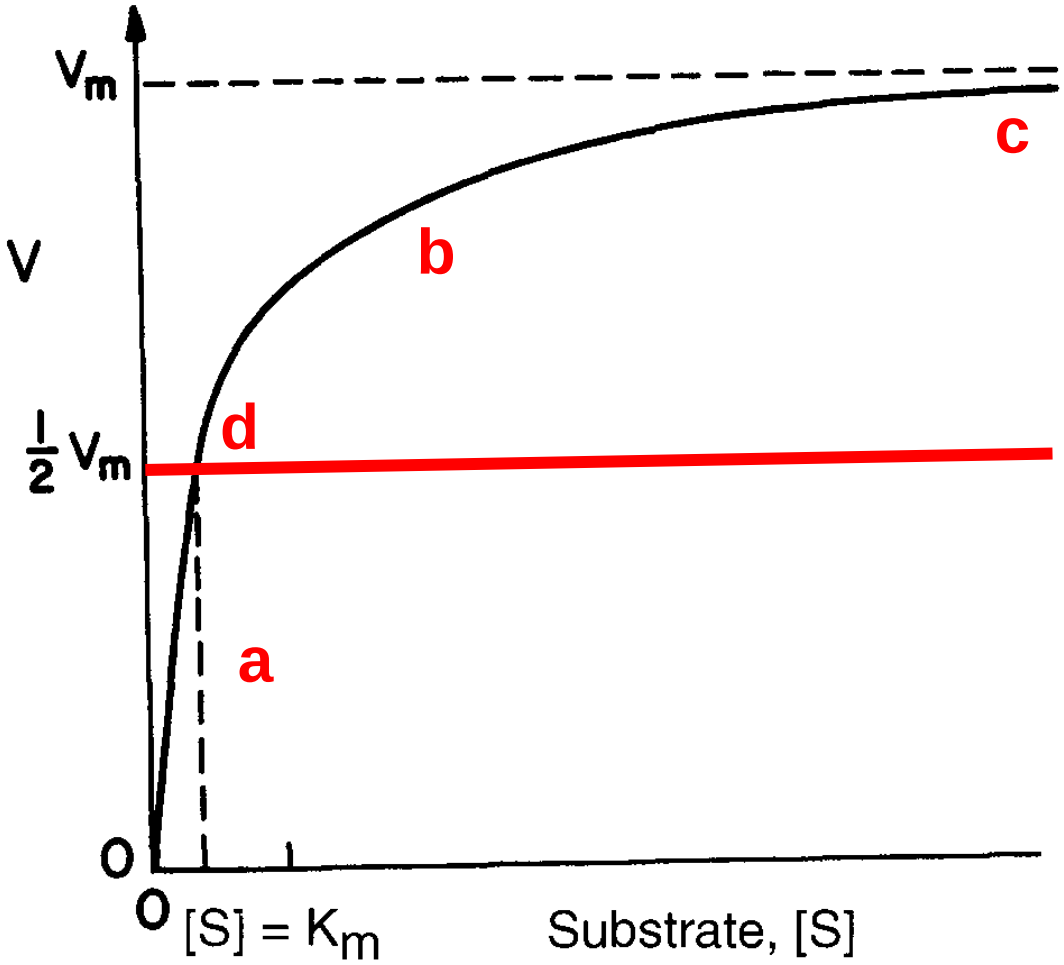
If [S] >> [E], all available enzyme is "saturated" with bound substrate. The rate of an enzyme-catalyzed reaction declines as substrate is converted to product.

Substrate Saturation of an Enzyme



A. Low [S] **B. [S]= K_m** **C. High, saturating [S]**

Saturation Enzyme Kinetics



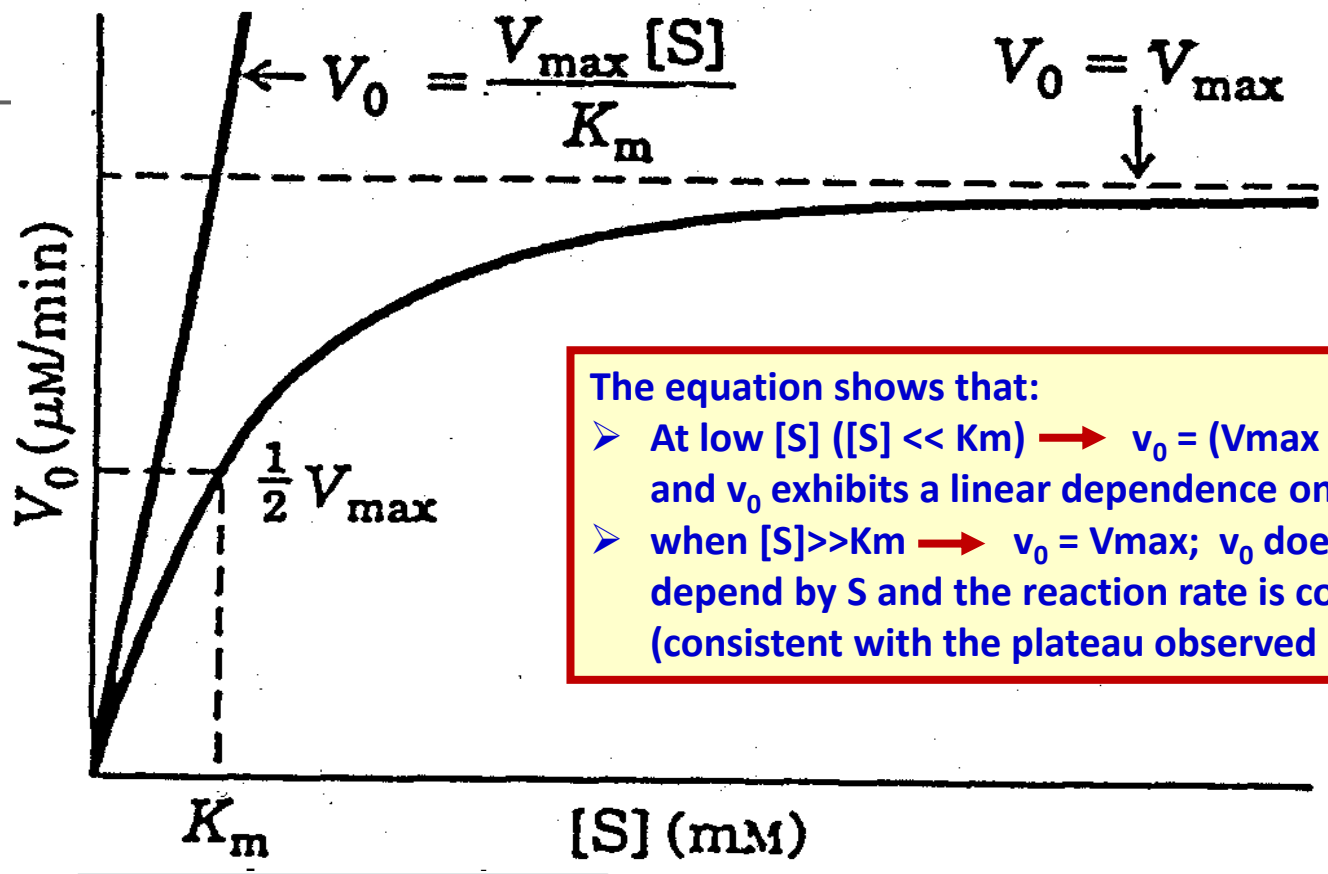
When initial velocity (V_0) is plotted against [S], a hyperbolic curve results, where V_{max} represents the maximum reaction velocity.

If [S] >> [E], all available enzyme is "saturated" with bound substrate. The rate of an enzyme-catalyzed reaction declines as substrate is converted to product.

[**a**] At relatively low concentrations of substrate, V_0 increases almost linearly with an increase in [S]. [**b**] At higher substrate concentrations, V_0 increases by smaller and smaller amounts in response to increases in [S]. [**c**] Finally, a plateau is reached (Vmax); the rate doesn't change increasing [S]. [**d**] The substrate concentration at which V_0 is half maximal velocity, the Michaelis constant (Km).

Michaelis-Menten Curve

MICHAELIS and MENTEN – first researchers who explained the shape of the rate curve (1913)



The equation shows that:

- At low [S] ([S] << Km) → v_0 = (Vmax [S])/ Km and v_0 exhibits a linear dependence on [S]
- when [S]>>Km → v_0 = Vmax; v_0 does not depend by S and the reaction rate is constant (consistent with the plateau observed at high [S]).

Michaelis-Menten equation or **rate equation**

$$v_o = \frac{[V_{max}][S]}{Km + [S]}$$

v_o = initial reaction velocity
V_{max} = maximal velocity
[S] = substrate concentration

Meaning of K_m

- **An important relationship that can be derived from the Michaelis-Menten equation is that when V_o is set equal to 1/2 V_{max}**

$$\frac{V_{max}}{2} = \frac{V_{max}[S]}{K_m+[S]} \longrightarrow \frac{1}{2} = \frac{[S]}{K_m+[S]} \longrightarrow K_m + [S] = 2[S].$$

$$K_m = [S], \quad \text{when} \quad V_0 = {}^1/_2 V_{max}$$

- **This means that at one half of the maximal velocity, the substrate concentration at this velocity will be equal to the K_m**

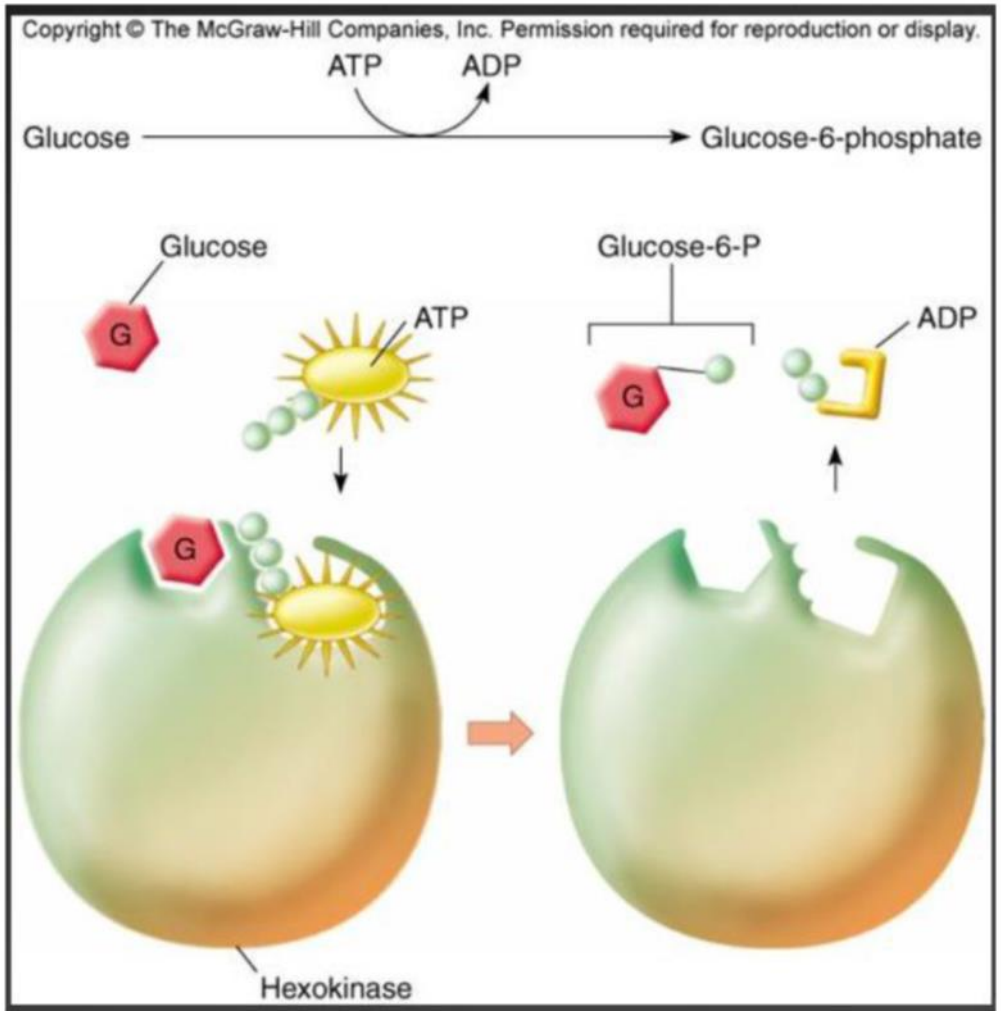
Nearly two-thirds of all enzymatic reactions have two substrates and two products

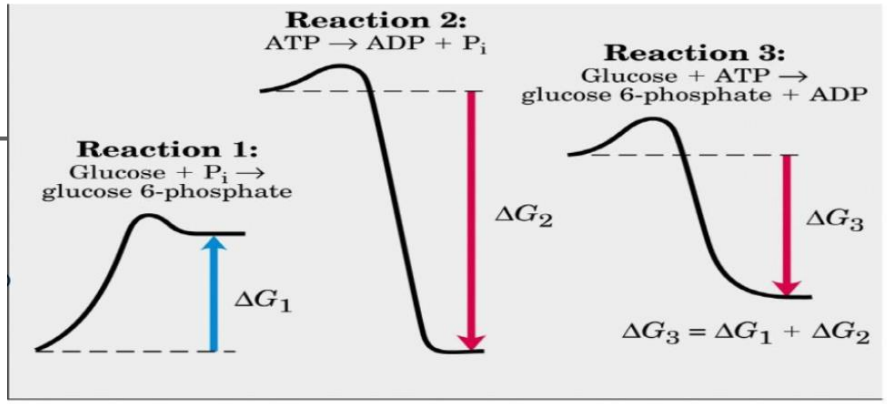
In the reaction catalyzed by hexokinase, ATP and glucose are the substrate molecules, and ADP and glucose 6-phosphate are the products:

$$\text{ATP} + \text{glucose} \rightarrow \text{ADP} + \text{glucose 6-phosphate}$$

Enzyme	Substrate	K_m (mM)
Hexokinase (brain)	ATP	0.4
	D-Glucose	0.05
	D-Fructose	1.5

D- Glucose has a greater affinity for hexokinase; hexokinase prefers glucose as substrate over fructose





1: glucose + Pi → glucose-6-P + H2O

ΔG°'= 13.8 kJ/mole

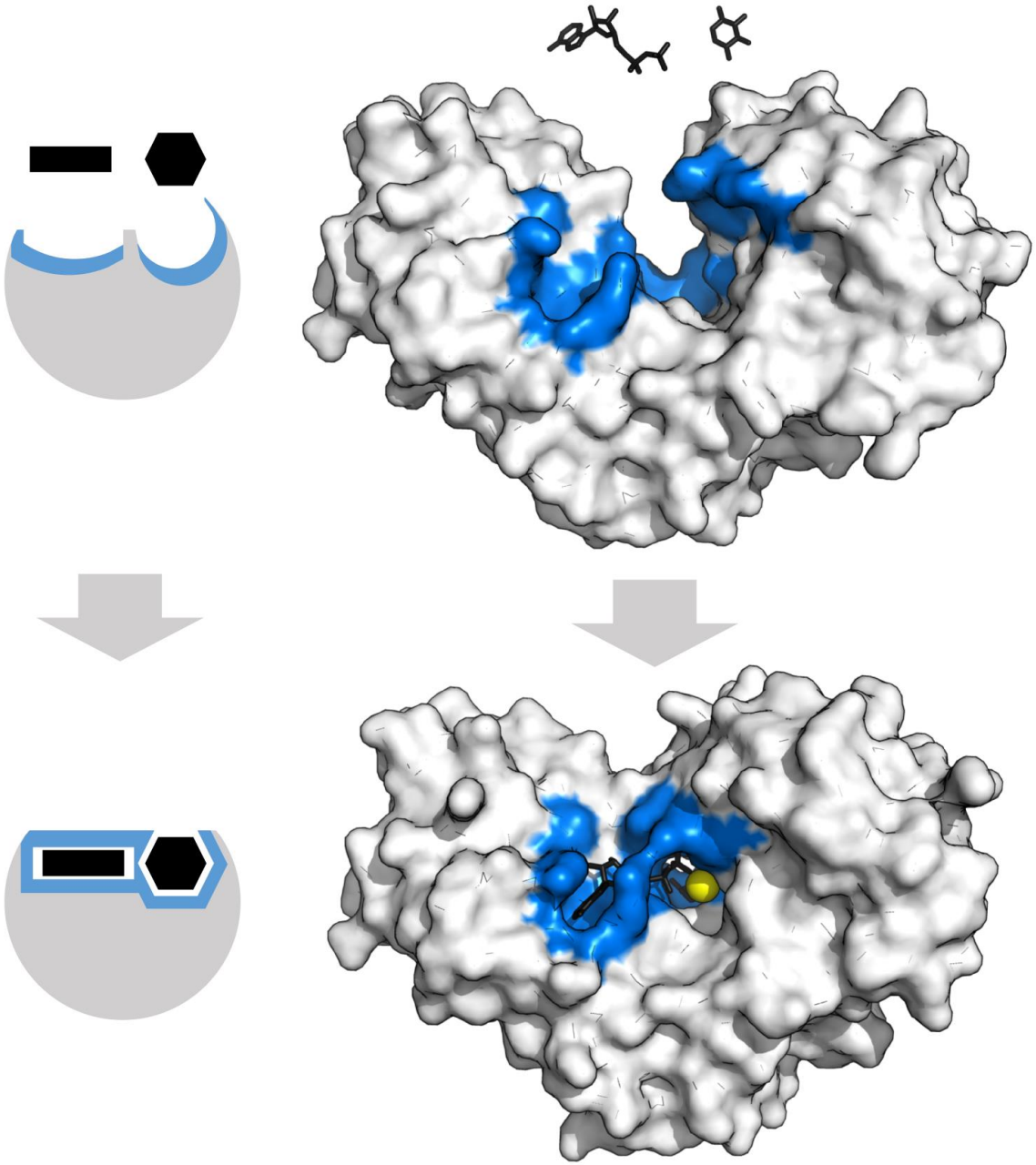
2: ATP + H2O → ADP + Pi

ΔG°'= -30.5 kJ/mole

- **Having two intermediates in common, the two reactions can be considered coupled.**
- **glucose + ATP → glucose-6-P + ADP**

$\Delta G^{\circ\prime}_{tot}$= -16.7 kJ/mole

- **$\Delta G^{\circ\prime}_{tot}$ has become negative, so the whole process is exoergonic and therefore spontaneous.**



Range of K_m values

Enzyme	Substrate	K_M(μM)
Chymotrypsin	Acetyl-L-tryptophanamide	5000
Lysozyme	Hexa-*N*-acetylglucosamine	6
β-Galactosidase	Lactose	4000
Threonine deaminase	Threonine	5000
Carbonic anhydrase	CO_2	8000
Penicillinase	Benzylpenicillin	50
Pyruvate carboxylase	Pyruvate	400
	HCO_3^-	1000
	ATP	60
Arginine-tRNA synthetase	Arginine	3
	tRNA	0.4
	ATP	300

Smaller values of Km indicate GREATER AFFINITY of the enzyme to the substrate

K_m provides an approximation of [S] for many enzymes *in vivo*

Uses of K_m

- All enzymes that exhibit a hyperbolic dependence of v_0 on [S] are said to follow the **Michaelis- Menten kinetics**.
- Experimentally, **K_m is a useful parameter to characterize the number and/or types of substrates for a given enzyme.**
- It is also useful for comparing **similar enzymes** from different tissues or different organisms.
- Also, the K_m of **the rate-limiting enzyme** in many of the biochemical metabolic pathways determines the amount of product and overall regulation of a given pathway.
- Clinically, K_m comparisons are useful for evaluating the effects of mutations on protein function for some inherited genetic diseases.

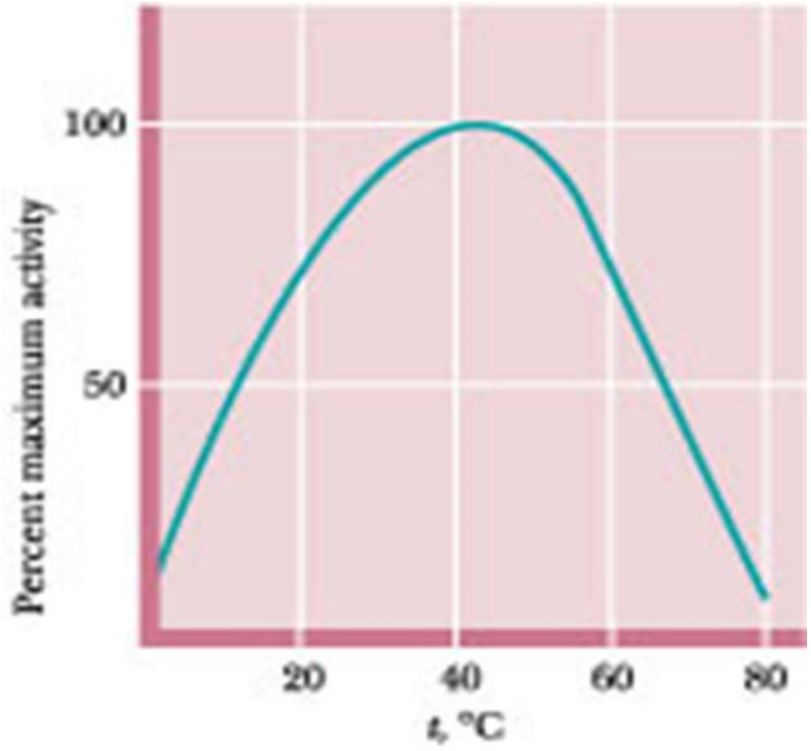
Factors Affecting Enzyme Kinetics

The rate of the reaction catalyzed by an enzyme depends on:

- **substrate concentration**
- **temperature**
- **pH**
- **ionic strength of the aqueous solution**

Effects of Temperature

- Reaction rate increases with temperature up to a limit
- Above a certain temperature, activity decreases with temperature due to denaturation

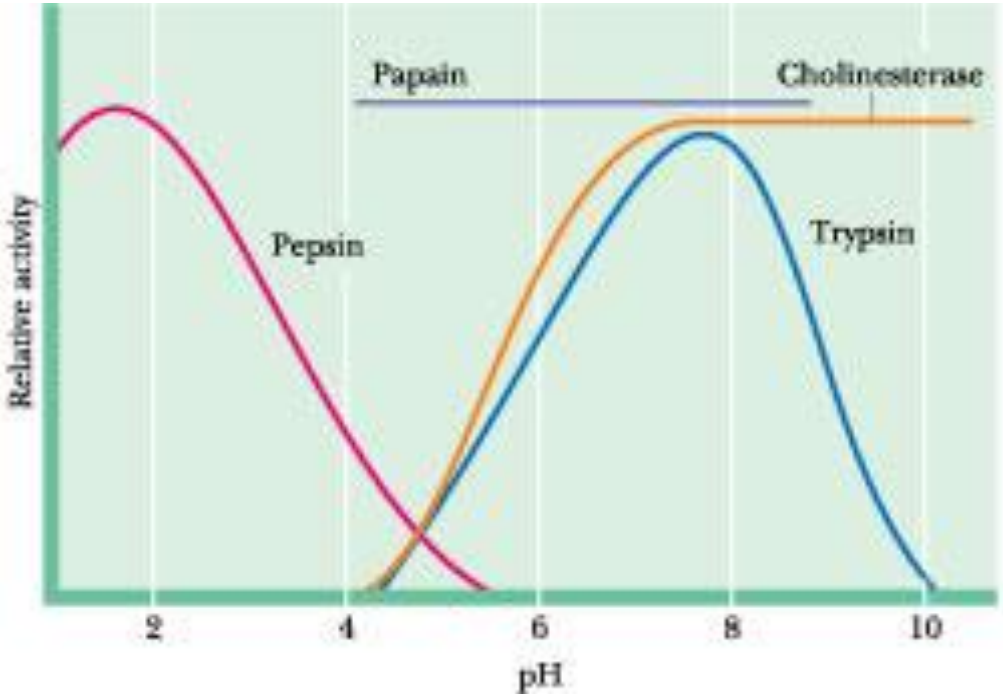


Effects of pH

- On the enzyme
    - enzymes have ionic groups on their active sites
    - variation of pH changes the ionic form of the active sites
    - pH changes the three-Dimensional structure of enzymes

- On the substrate
    - some substrates contain ionic groups
    - pH affects the ionic form of substrate affects the affinity of the substrate to the enzyme

Effects of pH



Optimum pH of Some Enzymes	
Enzyme	Optimum pH
Pepsin	1.5
Catalase	7.6
Trypsin	7.7
Fumarase	7.8
Ribonuclease	7.8
Arginase	9.7

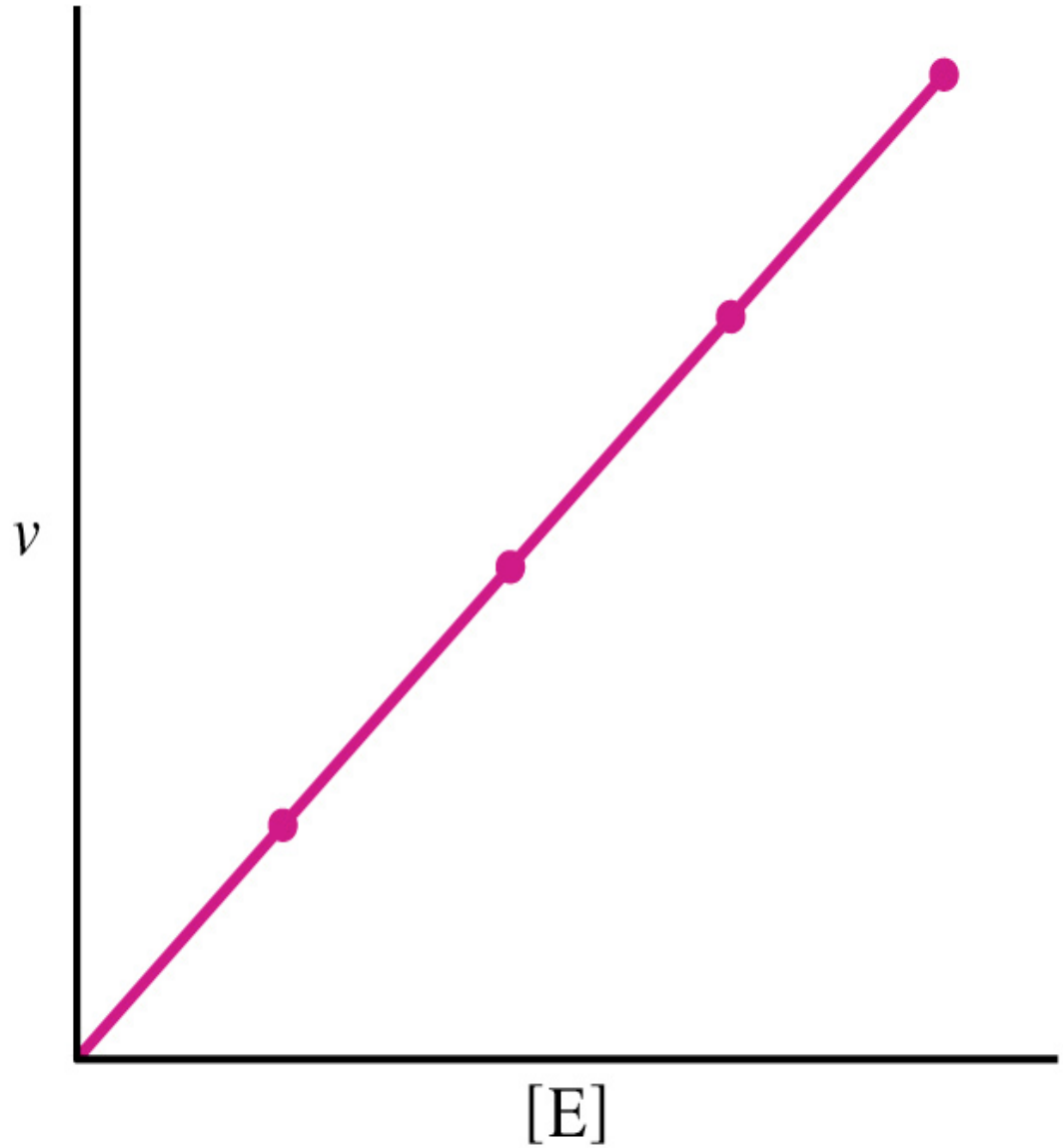
Enzyme-substrate recognition and the resulting catalytic responses largely depend on the pH.

Enzymes are generally only active within a limited pH range and most enzymes have optimal activity at an optimum pH value.

Effect of enzyme concentration [E] on velocity (v)

In fixed, saturating [S], the higher the concentration of enzyme, the greater the initial reaction rate

This relationship will hold as long as there is enough substrate present



Effect of the concentration of enzymes in the control of metabolism

The concentration of an enzyme can change the speed of the reaction catalyzed.

Therefore, conditions that increase (gene induction) or reduce (gene down-regulation) the concentration of an enzyme can change the speed of a metabolic pathway.

$$A \xrightarrow{[a]} B \xrightarrow{[b]} C \xrightarrow{[c]} D \ldots\ldots\ldots \xrightarrow{[z]} Z$$

Enzyme inhibition

Several agents (metabolites, substrate analogs, toxins, drugs, metal complexes, etc) can inhibit the enzyme activity. It is one of the fundamental mechanisms for the control of biological reactions.

Irreversible

Covalent interactions

Reversible

non covalent (weak) interactions

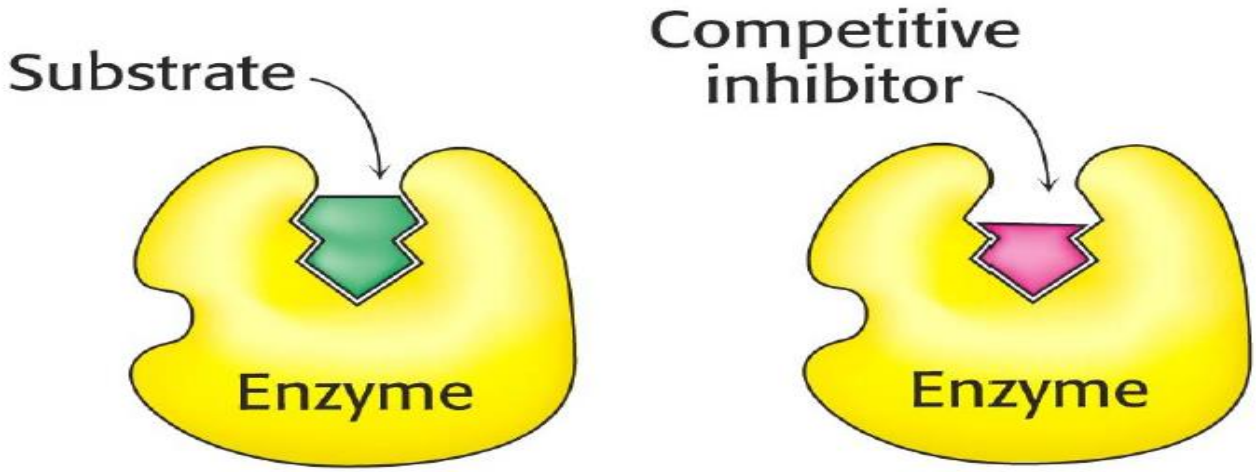
competitive **non-competitive**

Reversible inhibition

Competitive inhibition

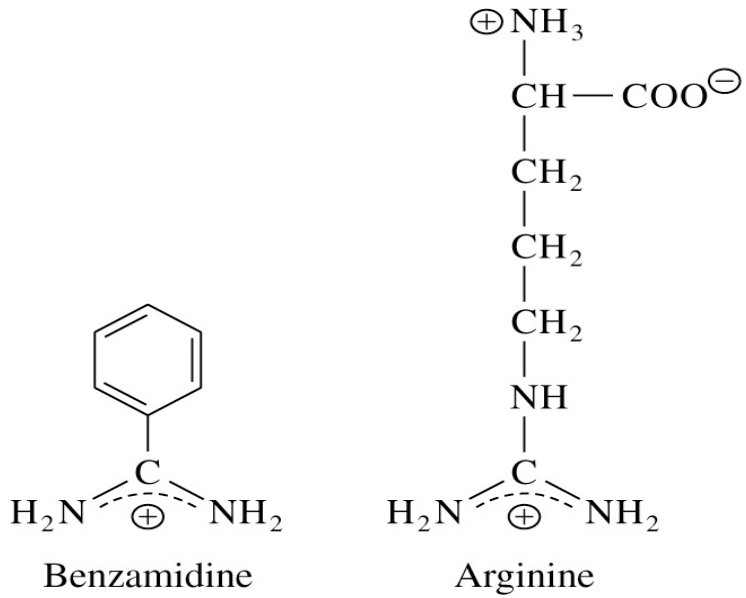
- Inhibitor has a structure similar to the substrate thus it can bind to the same active site
- The enzyme can hardly differentiate between the two compounds
- When inhibitor binds, binding of the substrate is prevented
- The inhibitor can be displaced by increasing substrate concentration

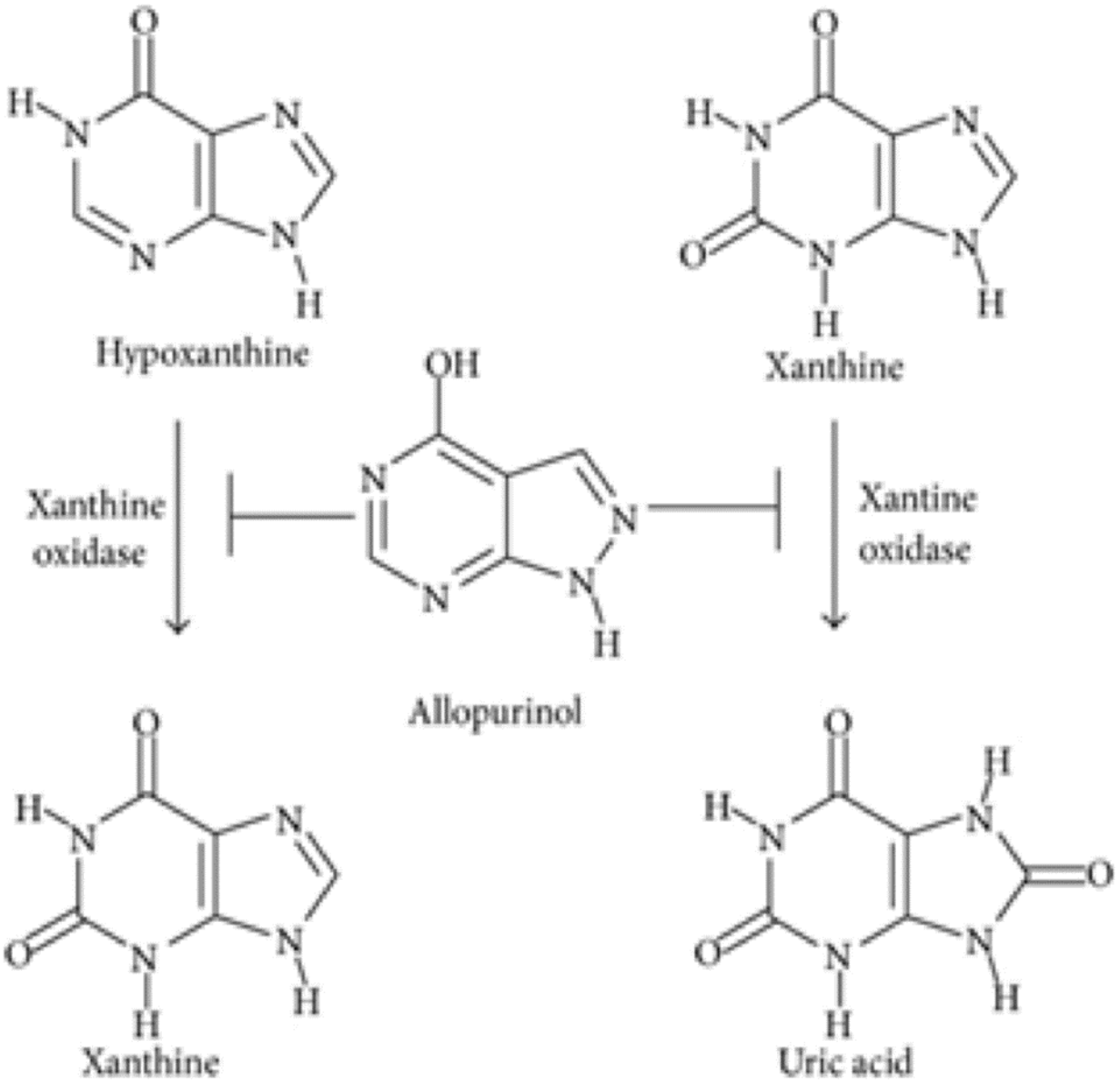
Competitive inhibition



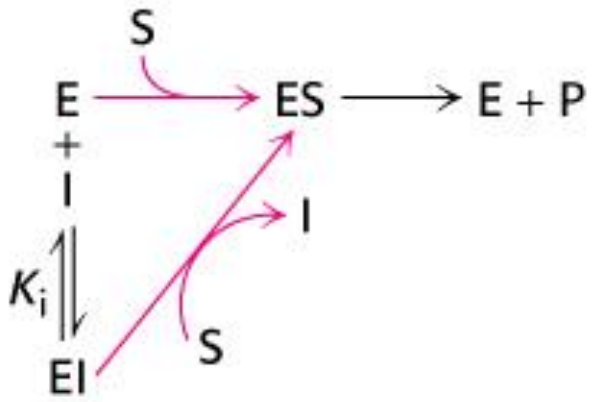
Example of competitive inhibition

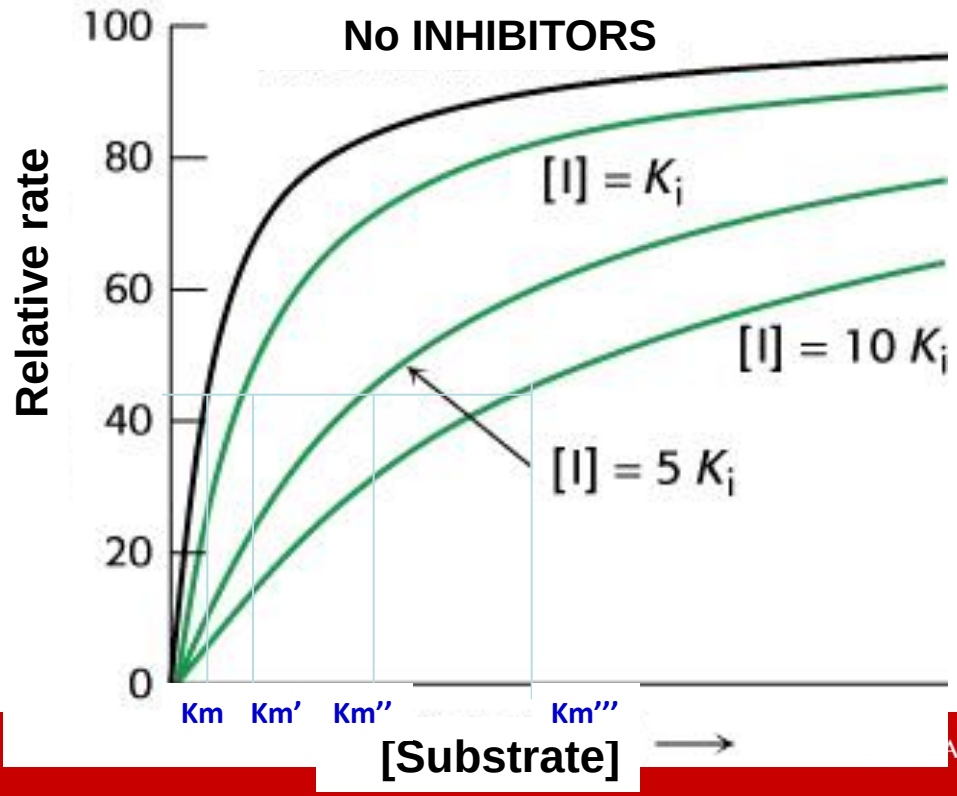
Benzamidine competes with arginine for binding to trypsin




Hypoxanthine
Xanthine
Xanthine oxidase
Allopurinol
Xantine oxidase
Xanthine
Uric acid

Competitive inhibition



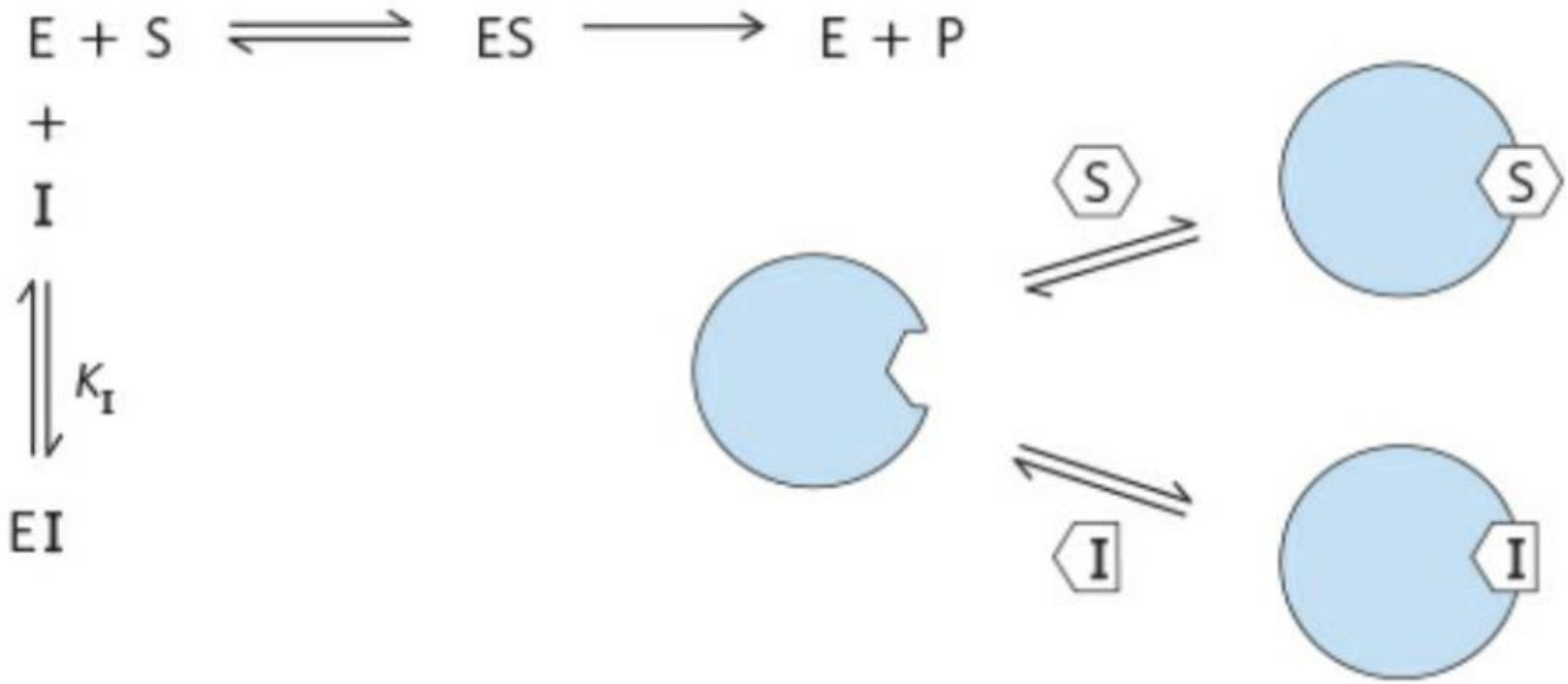


Since increased substrate concentration can remove inhibition, Vmax can be achieved in the presence of a competitive inhibitor by increasing [S].

However, the apparent Km value is altered as part of E will be occupied by I.

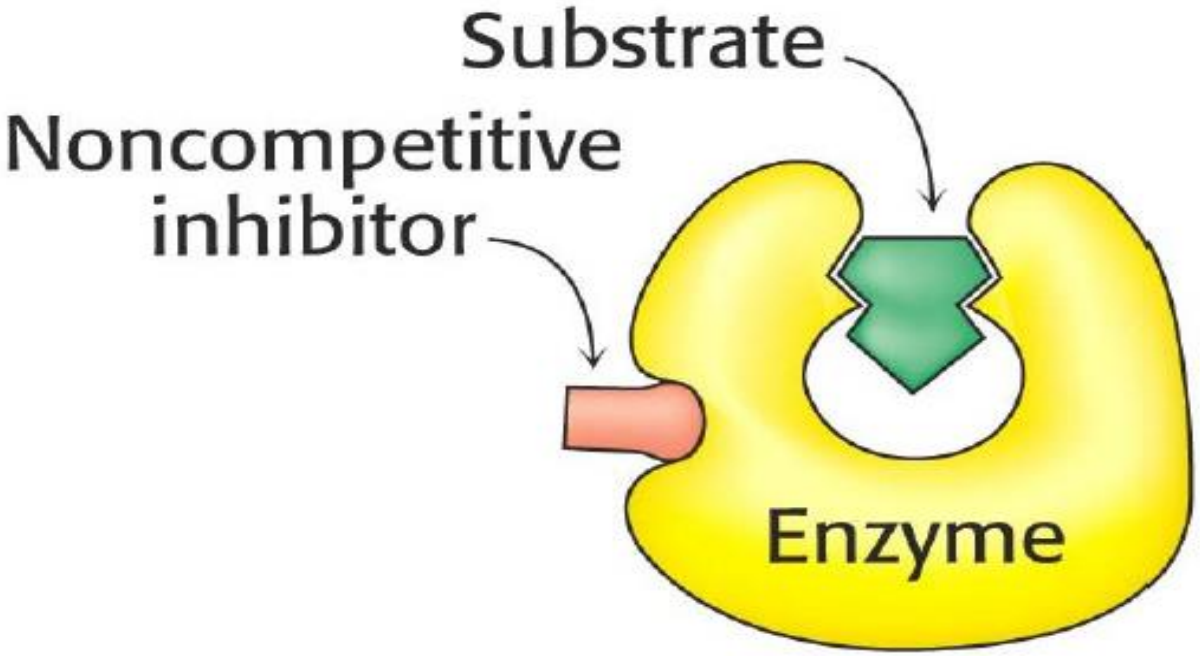
Increasing [I], Km^{app} increases

(a) Competitive inhibition

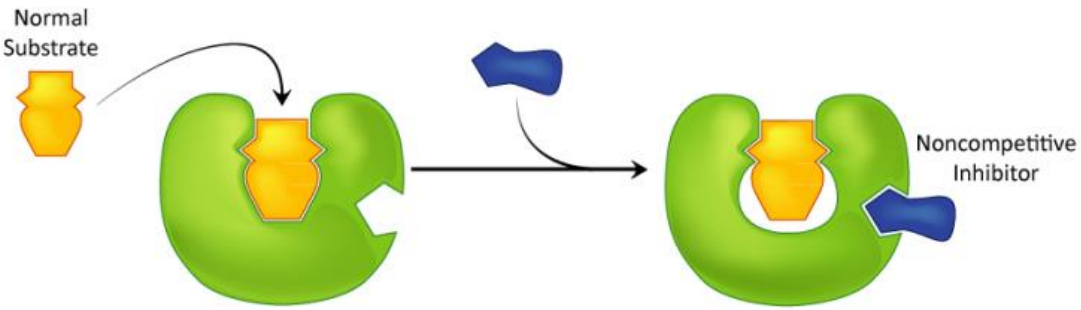


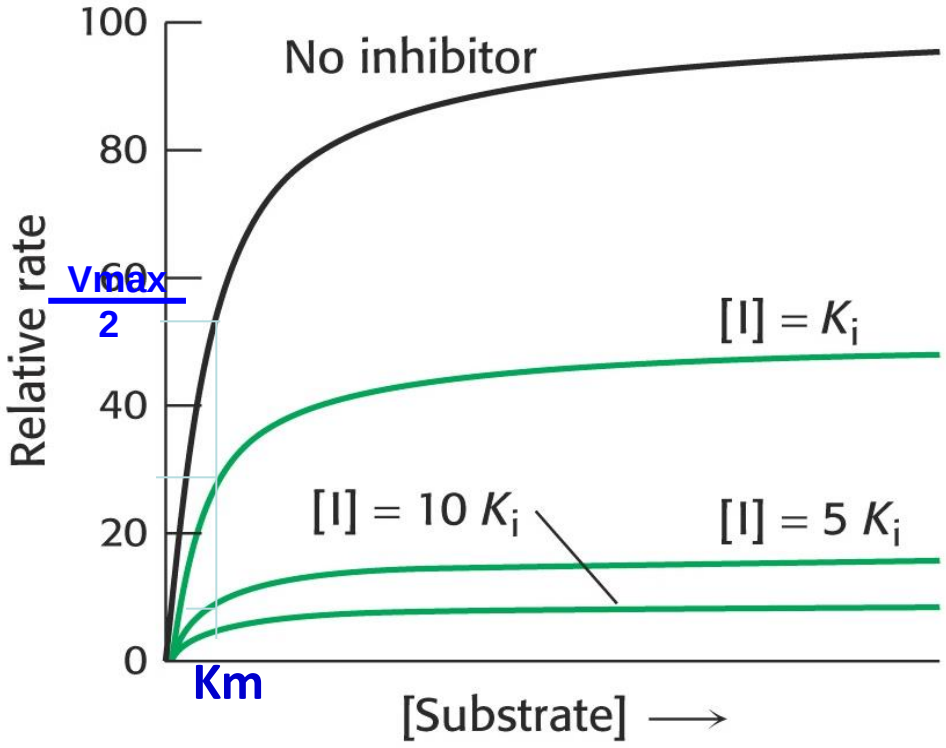
Non-competitive inhibition

- Inhibitor binds to an enzyme site different from the active site
- Inhibitor and substrate can bind enzyme at the same time
- Cannot be overcome by increasing the substrate concentration



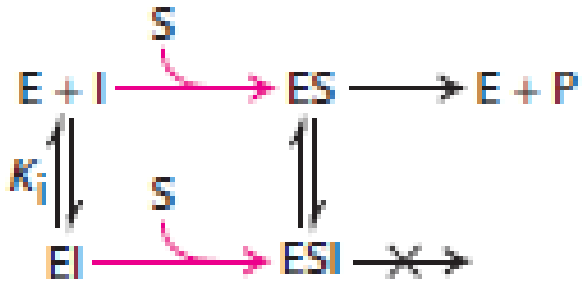
Kinetics of non-competitive inhibitors



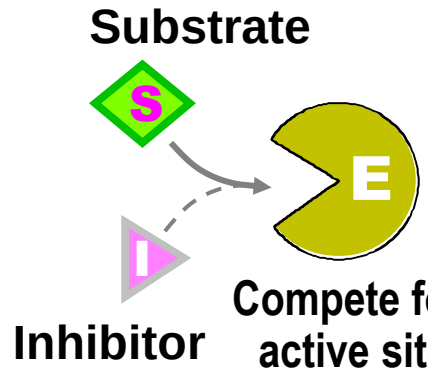
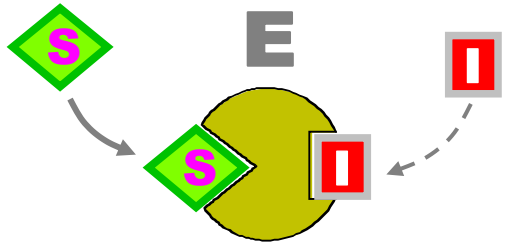


Increasing [S] cannot overcome inhibition, therefore V_{max} is lowered.

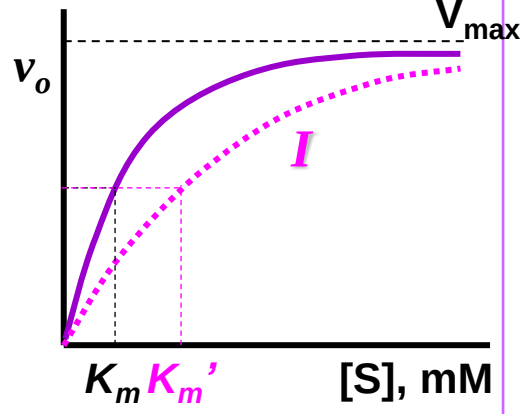
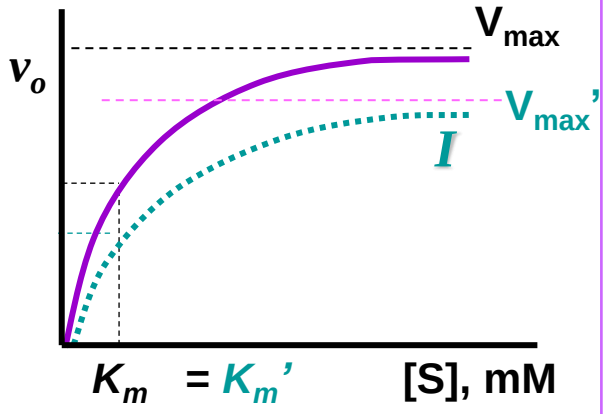
As the active site is free for the S to bind, K_m remains the same



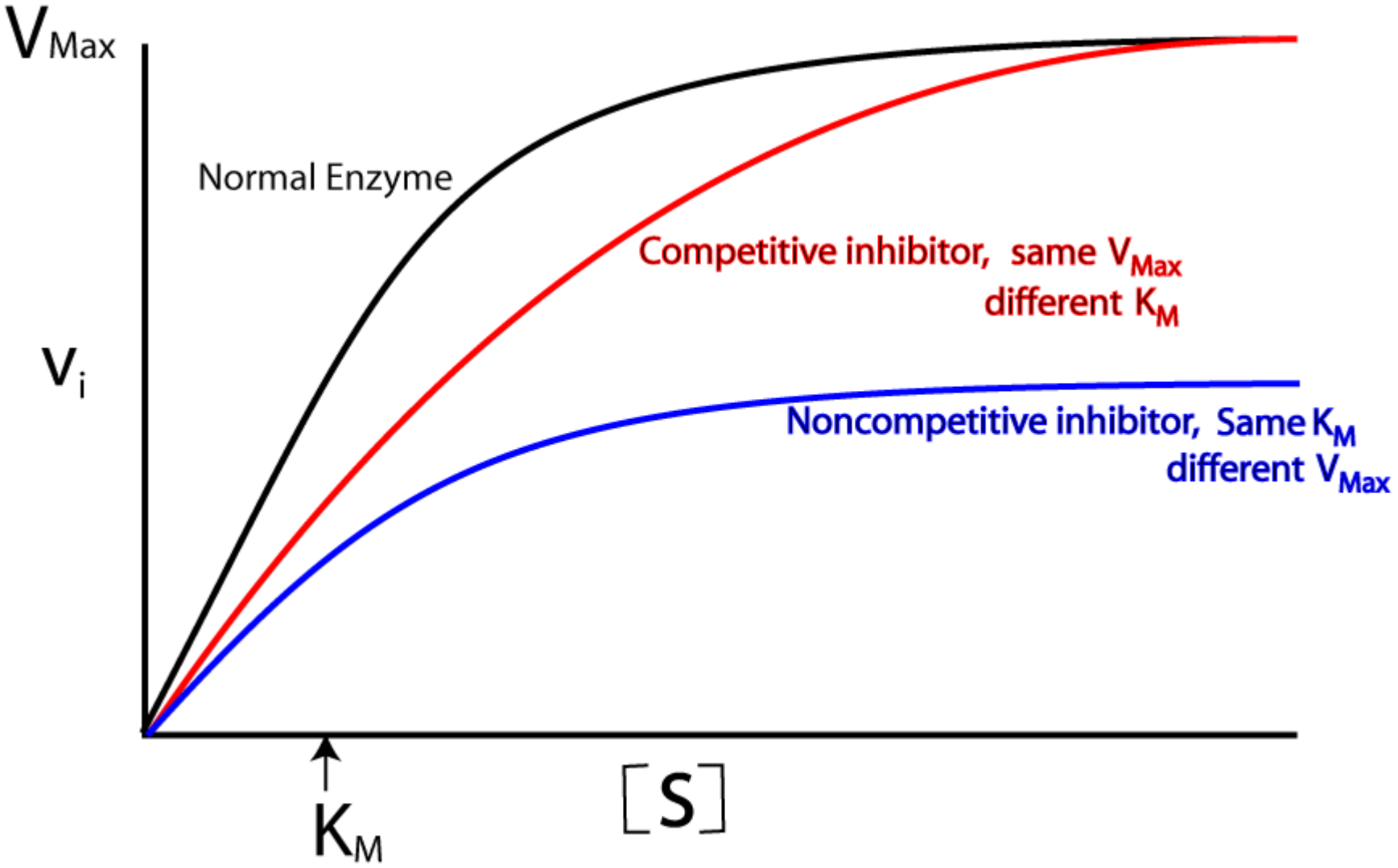
Enzyme Inhibition (Mechanism)

	Competitive	Non-competitive
Cartoon Guide		
Equation and Description	$E + S \rightleftarrows ES \rightarrow E + P$ $+$ I $\downarrow\uparrow$ EI	$E + S \rightleftarrows ES \rightarrow E + P$ $+$ $+$ I I $\downarrow\uparrow$ $\downarrow\uparrow$ $EI + S \rightarrow EIS$
	[*I*] binds to free [E] only, and competes with [S]; increasing [S] overcomes Inhibition by [*I*].	[*I*] binds to free [E] or [ES] complex; increasing [S] can not overcome [*I*] inhibition.

Enzyme Inhibition (Plots)

	Competitive	Non-competitive
Direct Plots		
	V_{max} unchanged K_m increased	V_{max} decreased K_m unchanged

Enzyme Inhibition (Plots)



Irreversible Inhibitors

- Irreversible inhibitors generally result in the MODIFICATION of an essential amino acid required for enzyme activity
- They covalently bind or destroy a functional group on an enzyme that is essential for the enzyme's activity
- These types of inhibitors range from fairly simple, broadly reacting chemical modifying reagents (like iodoacetamide that reacts with cysteines) to complex inhibitors that interact specifically and irreversibly with amino acids present in the binding site of the substrate (termed suicide inhibitors)
- Use of suicide inhibitors have proven to be very clinically effective (ex. Clavulanic acid)

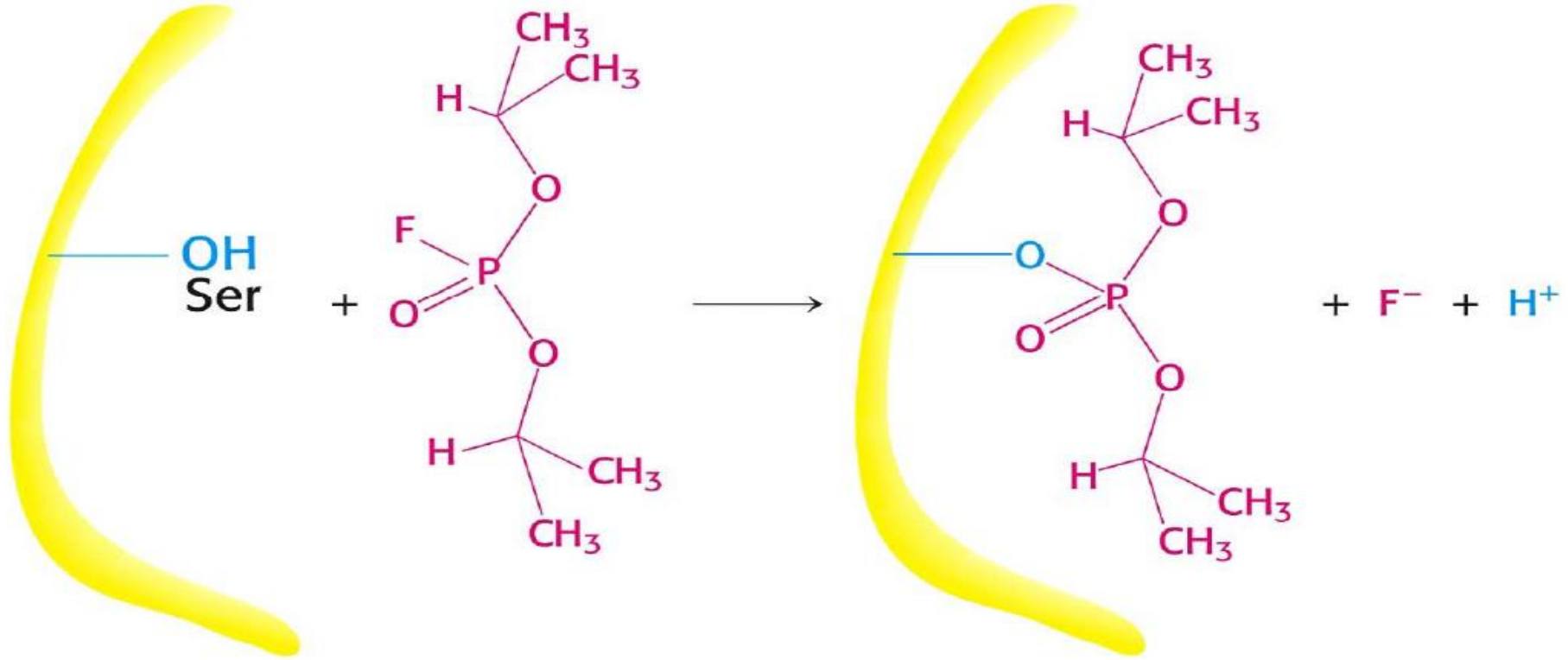
Irreversible Enzyme Inhibition

Irreversible inhibitors

- ***group-specific reagents***
- ***substrate analogs***
- ***suicide inhibitors (compounds relatively unreactive until they bind to the active site of a specific enzyme)***

1) Group-specific reagents

react with specific R groups of amino acids



Acetylcholin-esterase

DIPF
diisopropylphosphofluoridate
(Organophosphate pesticide)

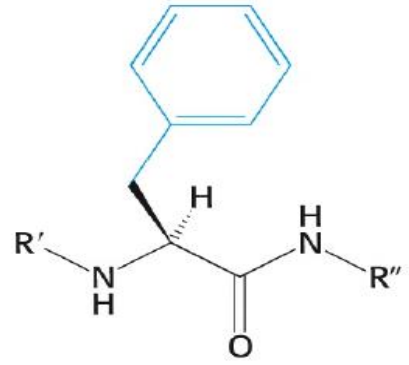
Inactivated enzyme

2) Substrate analogs

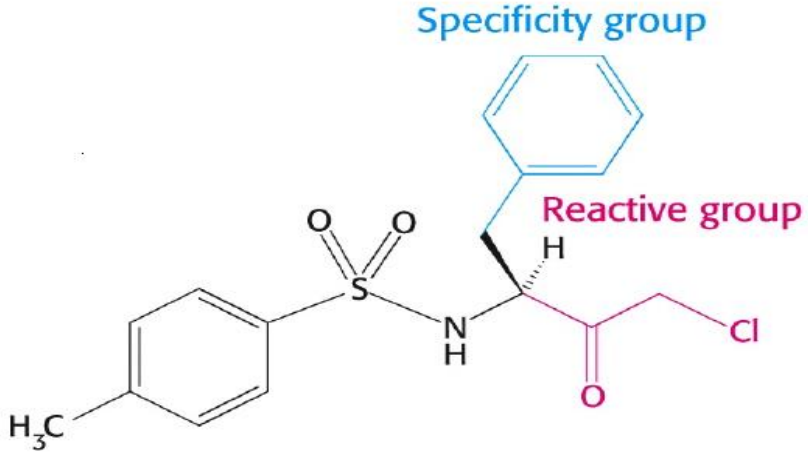
Structurally similar to the substrate for the enzyme

Covalently modify active site residues

(A)

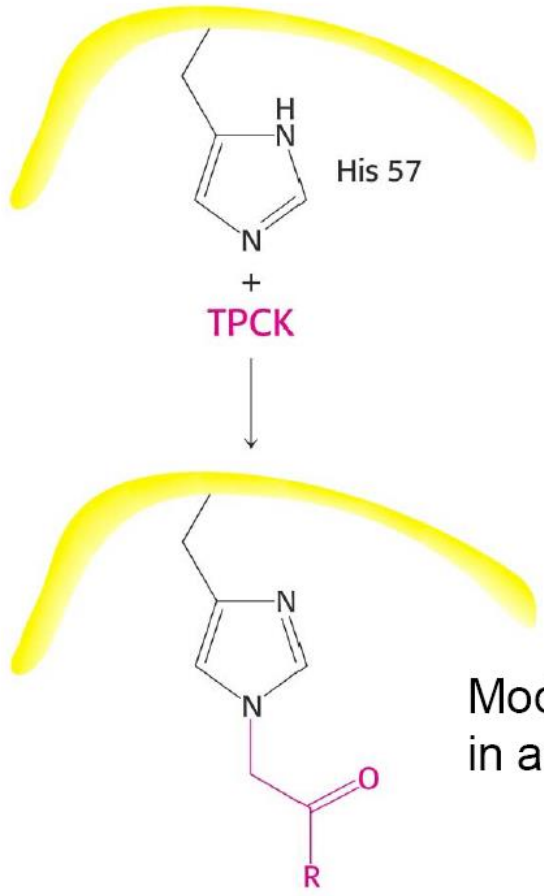


Natural substrate for chymotrypsin



Tosyl-L-phenylalanine chloromethyl ketone (TPCK)

(B) **Chymotrypsin**



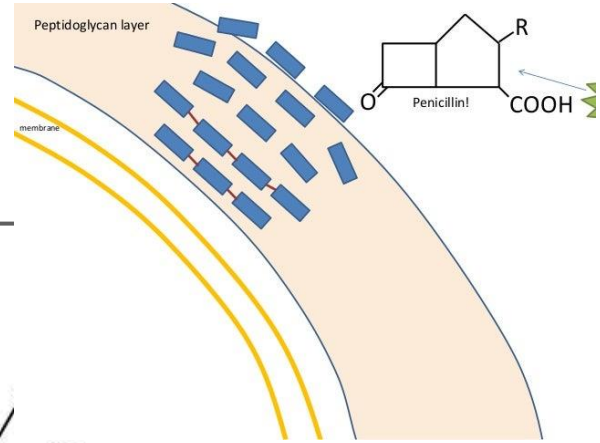
Modifies histidine in active site

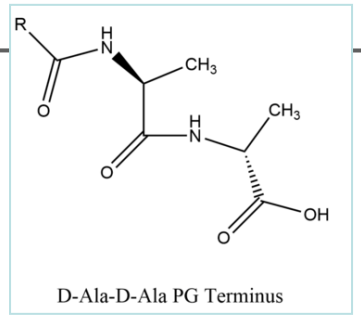
Suicide inhibitors/inactivators

- These compounds are relatively unreactive until they bind to the active site of a specific enzyme. A suicide inactivator undergoes the first few chemical steps of the normal enzymatic reaction, but instead of being transformed into the normal product, the inactivator is converted to a very reactive compound that combines irreversibly/covalently with the enzyme.

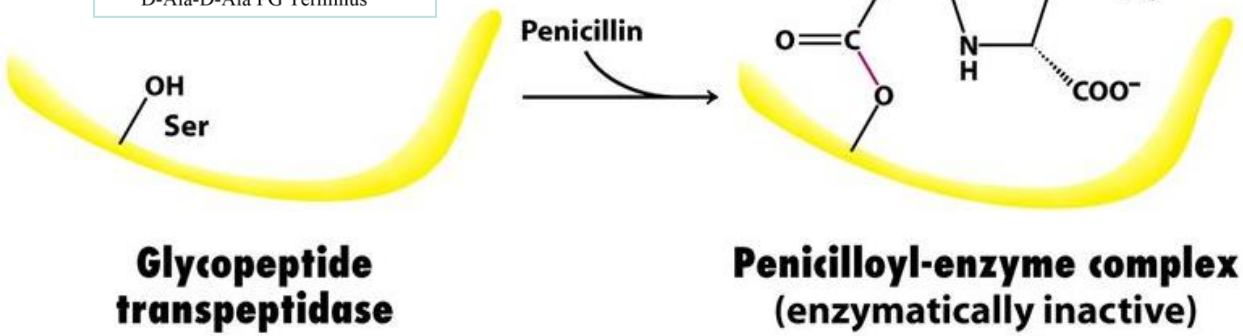
- Suicide because enzyme participates in its own irreversible inhibition.

Suicide inhibitors





D-Ala-D-Ala PG Terminus

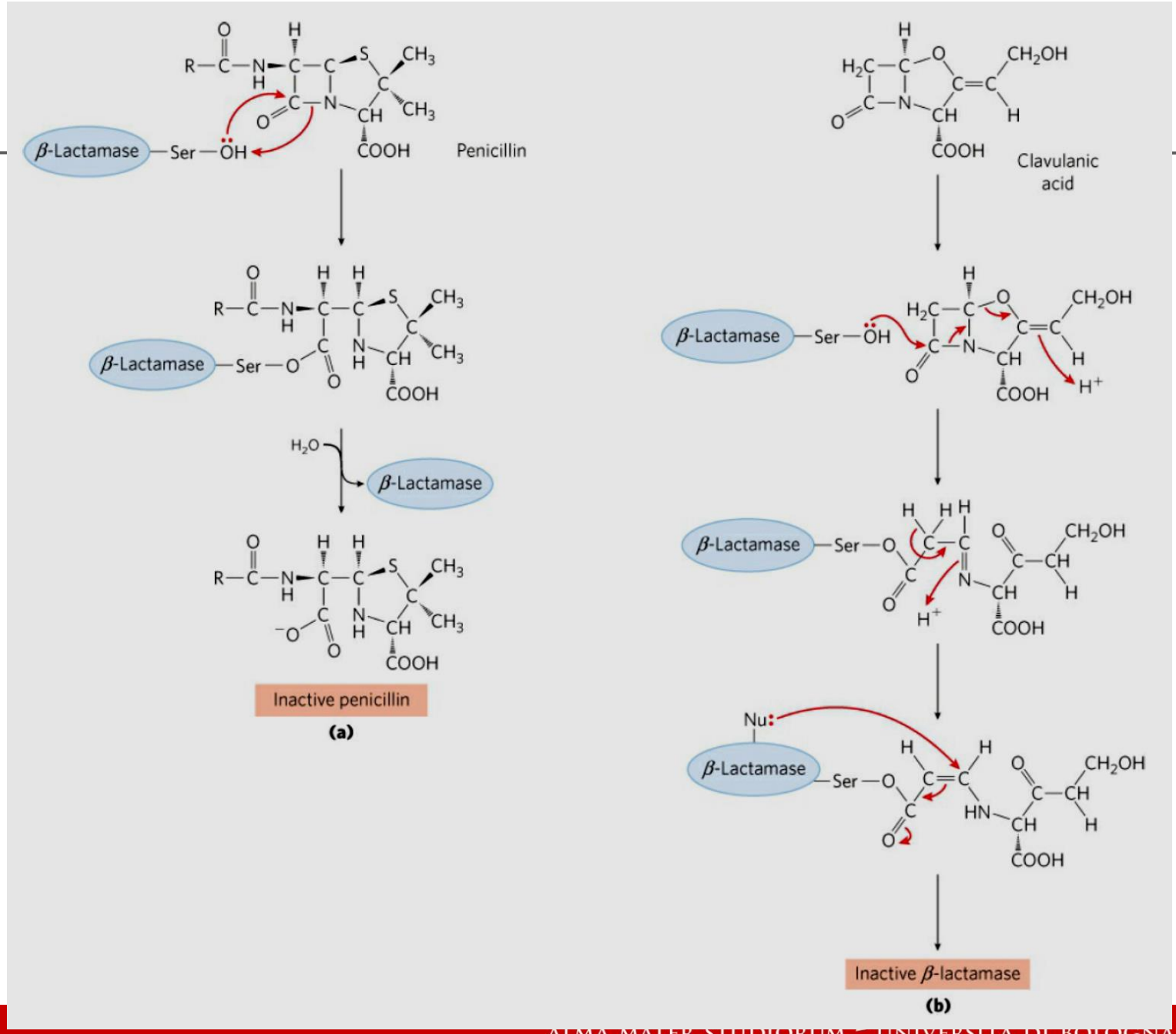


Glycopeptide transpeptidase

Penicilloyl-enzyme complex (enzymatically inactive)

Figure 8-35
Biochemistry, Sixth Edition

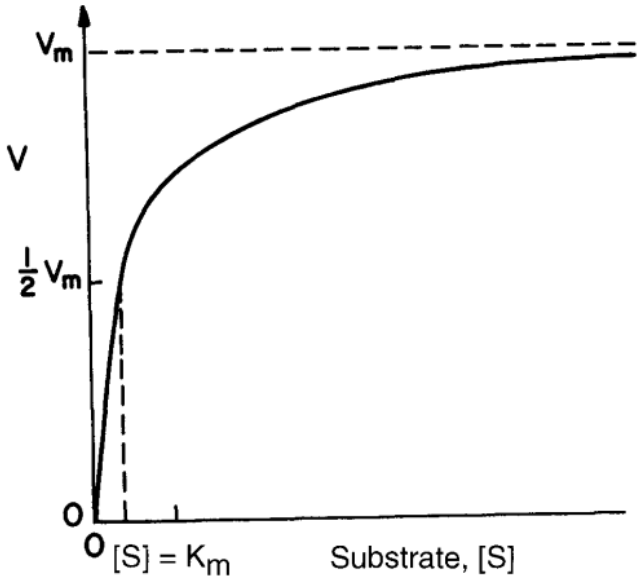
Glycopeptide transpeptidase catalyzes the formation of cross-links between D-amino acids in bacterial walls. Penicillin is used medicinally as an antibiotic in the treatment of many bacterial infections (especially, Gram+ bacteria). Penicillin binds irreversibly (covalently) to bacterial transpeptidase. Mechanistically, penicillin forms a penicilloyl-enzyme complex with a serine residue found in glycopeptide transpeptidase forming an ester, which is stably dysfunctional. The bacterial wall alteration leads to bacteria death.

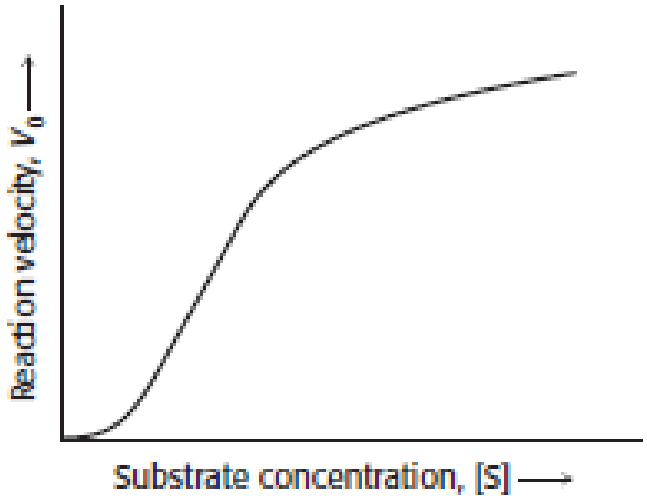

β-Lactamase
Ser
OH
Penicillin
H2O
Inactive penicillin
(a)
Clavulanic acid
H+
Nu:
Inactive β-lactamase
(b)

Regulation of enzyme activity

Mechanisms of Enzyme Regulation

1. Substrate concentration
2. Amount of enzyme present
3. Reversible inhibition
4. Allosteric inhibition or activation
5. Covalent Modifications





Regulation of Enzyme Concentrations

- **The overall synthesis and degradation of a particular enzyme, also termed its turnover number, is one way of regulating the quantity of an enzyme.**

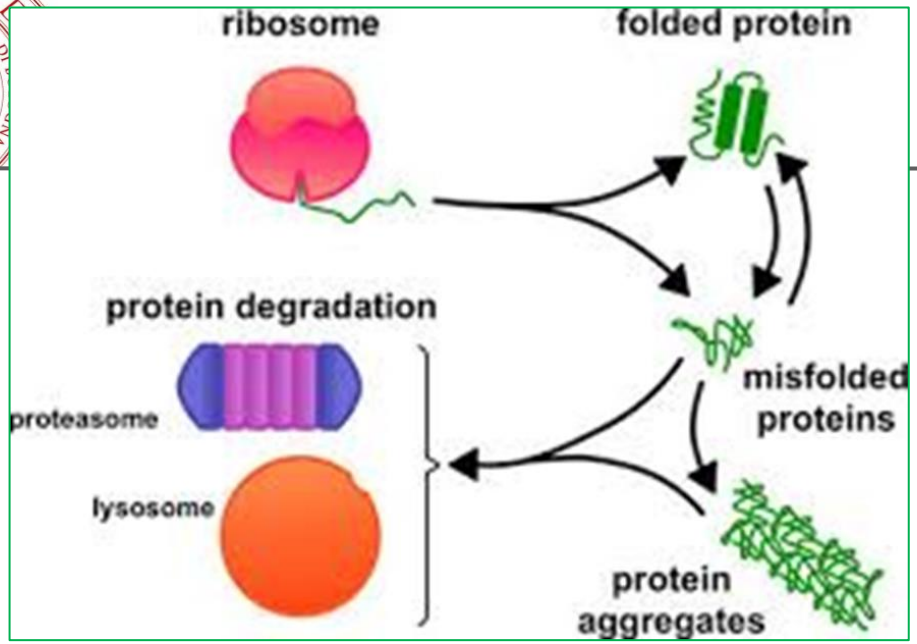
- **The amount of an enzyme in a cell can be increased by increasing its rate of synthesis, decreasing the rate of its degradation, or both.**

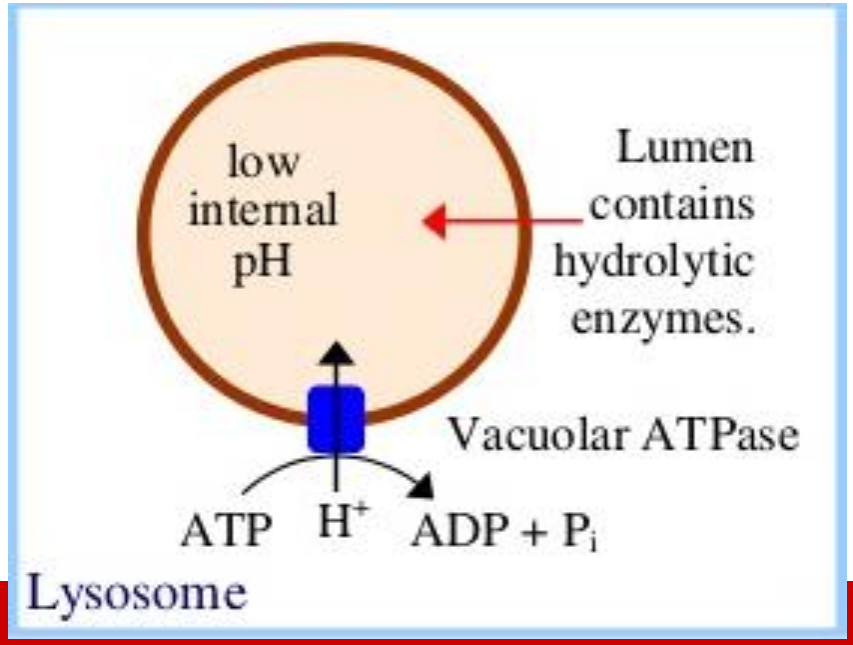
Regulation of Enzyme Concentrations: Induction

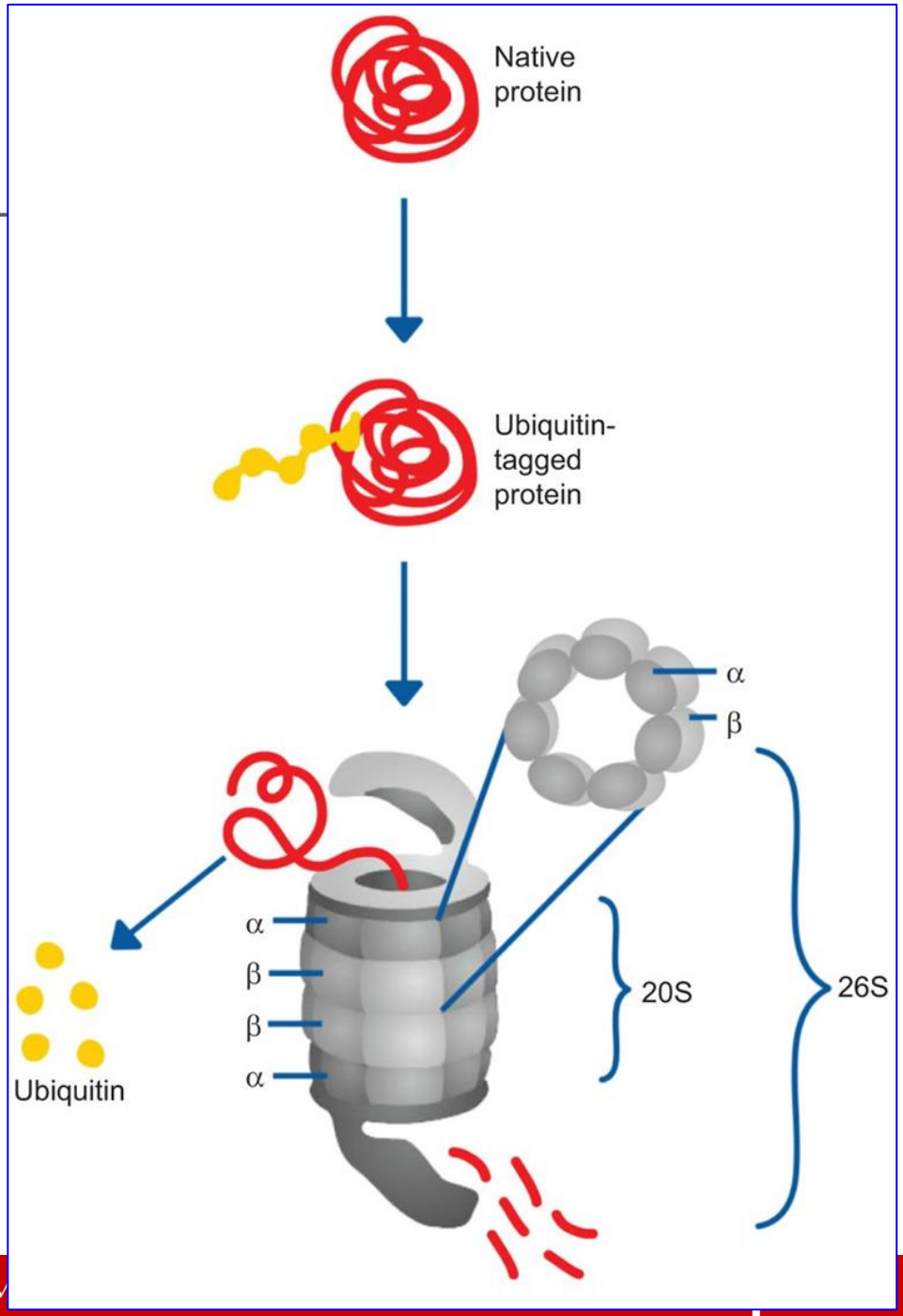
- Induction (an increase caused by an effector molecule) of enzyme synthesis is a common mechanism - this can manifest itself at the level of gene expression (transcription and translation)
- The actions of many hormones and/or growth factors on cells will ultimately lead to an increase in the expression and translation of "new" enzymes not present prior to the signal

Regulation of Enzyme Concentrations: Degradation

- Protein degradation by proteases is compartmentalized in the cell in the lysosome (which is generally non-specific)
- or in macromolecular complexes termed proteasomes. Degradation by proteasome is regulated by a complex pathway involving transfer of a 76 AA polypeptide, ubiquitin, to targeted proteins. Ubiquination of protein targets it for degradation by the proteasome.
- Proteolytic degradation is an irreversible mechanism.


ribosome
folded protein
protein degradation
misfolded proteins
proteasome
lysosome
protein aggregates


low internal pH
Lumen contains hydrolytic enzymes.
Vacuolar ATPase
ATP
H+
ADP + Pi
Lysosome


Native protein
Ubiquitin-tagged protein
α
β
β
α
20S
26S
Ubiquitin

ALLOSTERIC ENZYMES do NOT obey the MM kinetics

- **Allosteric proteins** - from the Greek *allos* for "other" and *stereos* for "shape" (or site) meaning "other site". An **allosteric protein** is one in which the binding of a ligand to one site affects the binding properties of another site on the same protein (e.g. the cooperative binding of oxygen to Hb is an allosteric binding).

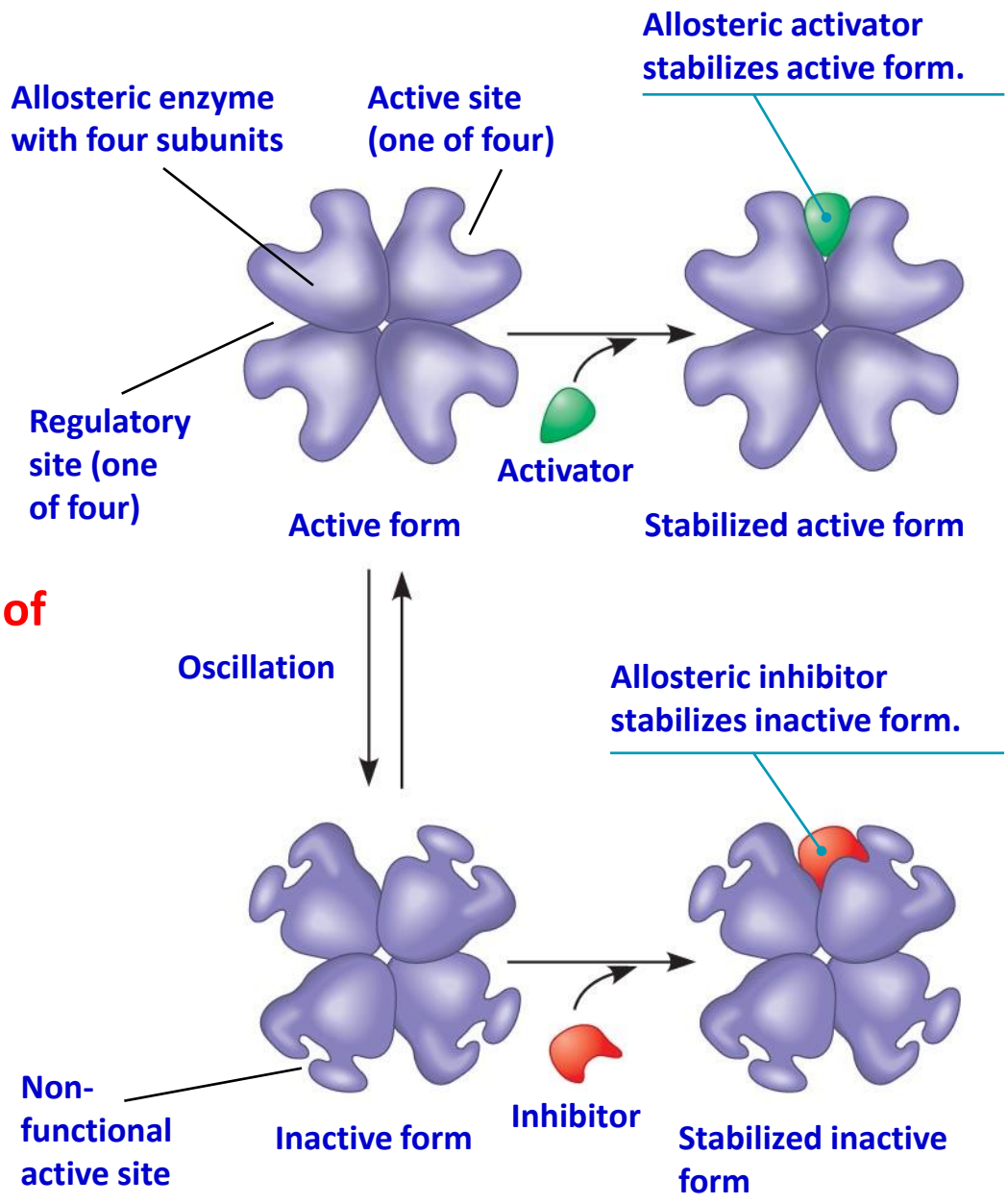
- **Allosteric enzymes** function through reversible, non-covalent binding of a ligand (**modulator**) at a site other than the catalytic, active site. Allosteric sites are binding sites distinct from an enzyme's active site or substrate-binding site.

- The **conformational changes** induced by the modulator(s) interconvert more active and less-active forms of the protein. The modulators for allosteric proteins may be either inhibitors or activators.

Allosteric Enzymes

- Allosteric enzymes are typically larger and more complex than nonallosteric enzymes, with two or more subunits. They have one or more allosteric sites
- Allosteric sites are binding sites distinct from an enzyme's active site or substrate-binding site
- Molecules that bind to allosteric sites are called effectors or modulators
- Binding to allosteric sites alters the activity of the enzyme. This is called cooperative binding. Allosteric enzymes display sigmoidal plot of Vo vs [S]
- Effectors may be positive or negative (inhibitors or activators)
- Regulatory enzymes of metabolic pathways are allosteric enzymes (eg: feedback inhibition)

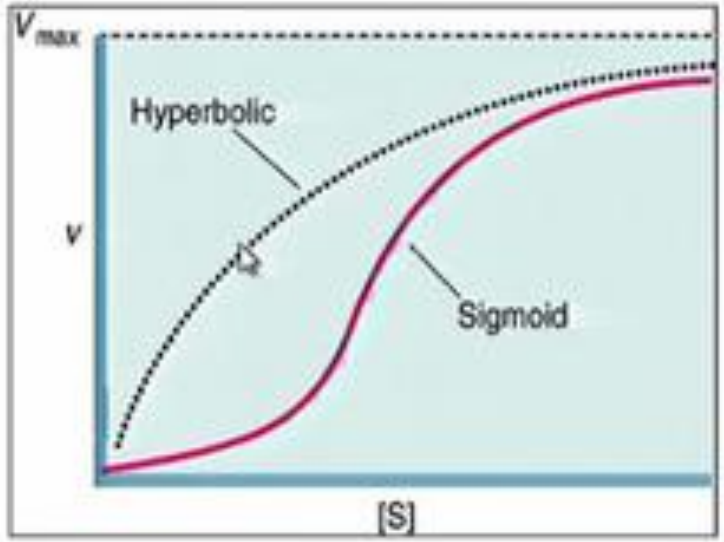
The concentrations of the modulators determine the final activity of the enzyme



Allosteric effects on enzyme kinetics

1. Cooperativity (sigmoid) indicates allosteric effects

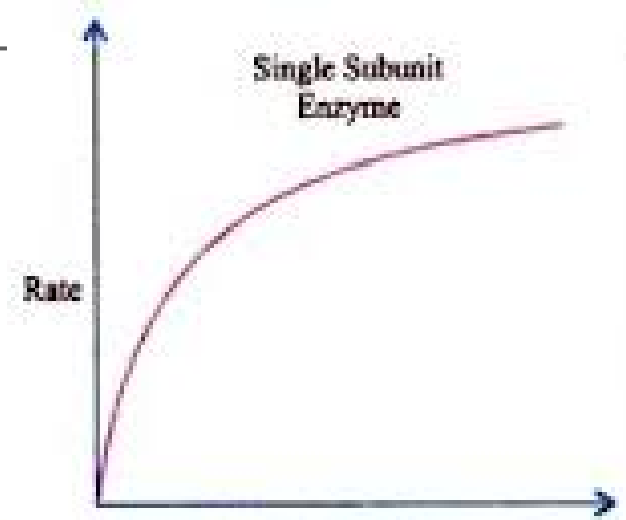
2. Allosteric enzymes do not follow Michaelis-Menten equation.

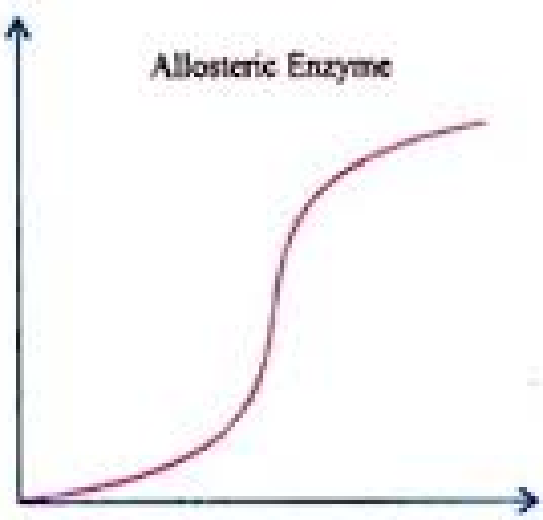


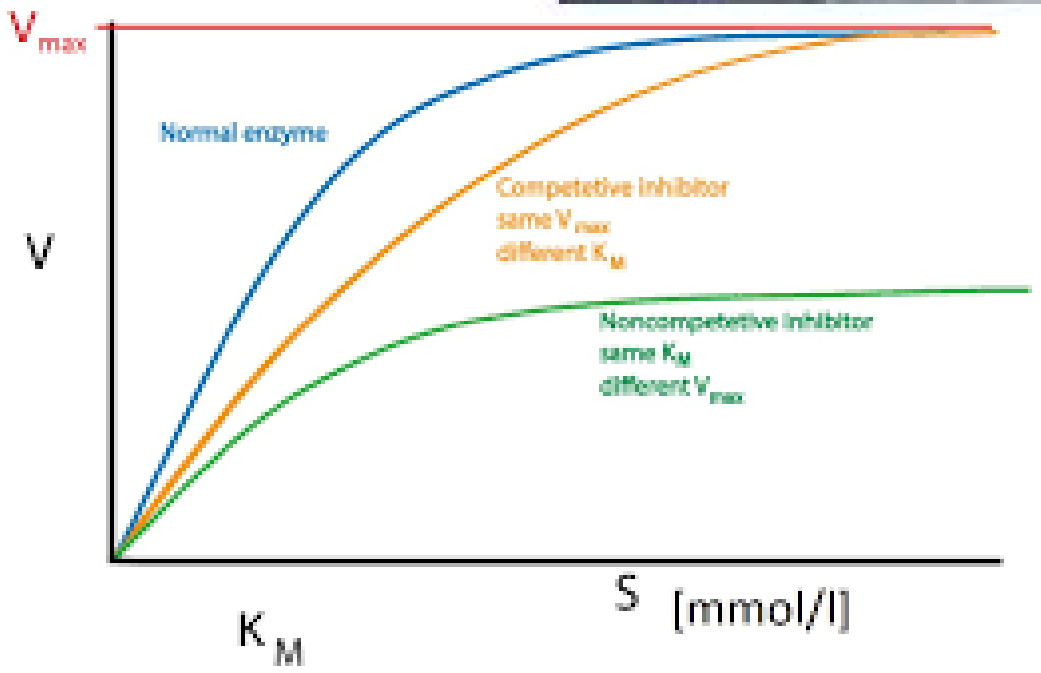
Allosteric Enzymes - Kinetics

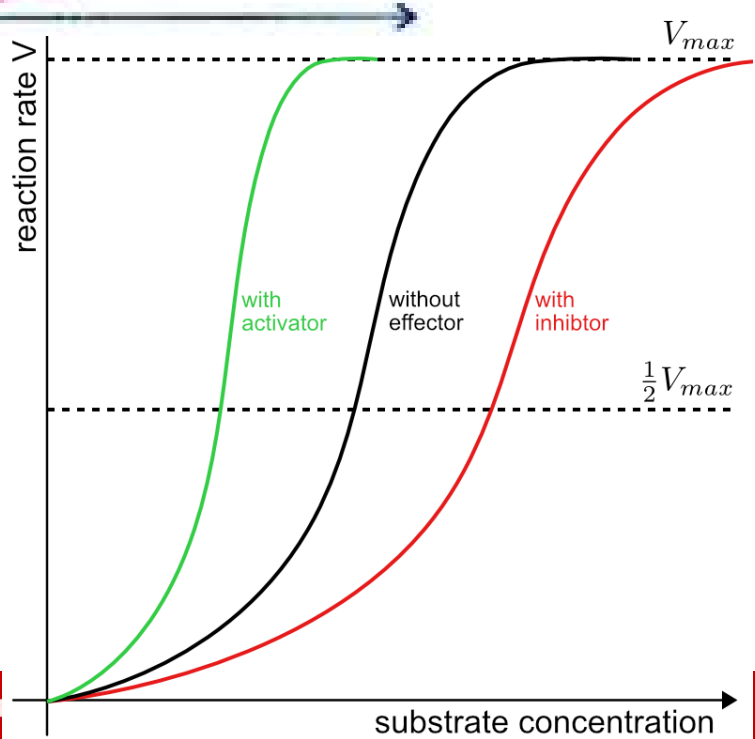
- Allosteric enzymes exhibit saturation kinetics at high [S], but they have a **characteristic sigmoidal saturation curve rather than hyperbolic curve when v_o is plotted versus [S]** (analogous to the oxygen saturation curves of myoglobin vs. hemoglobin)
- Addition of an allosteric activator (+) tends to shift the curve to a more hyperbolic profile (more like Michaelis-Menten curves), while an allosteric inhibitor (-) will result in more pronounced sigmoidal curves.
- The sigmoidicity is thought to result from **the cooperativity of structural changes between enzyme subunits** (similar to oxygen binding to hemoglobin)

V_o vs [S] for Allosteric Enzymes

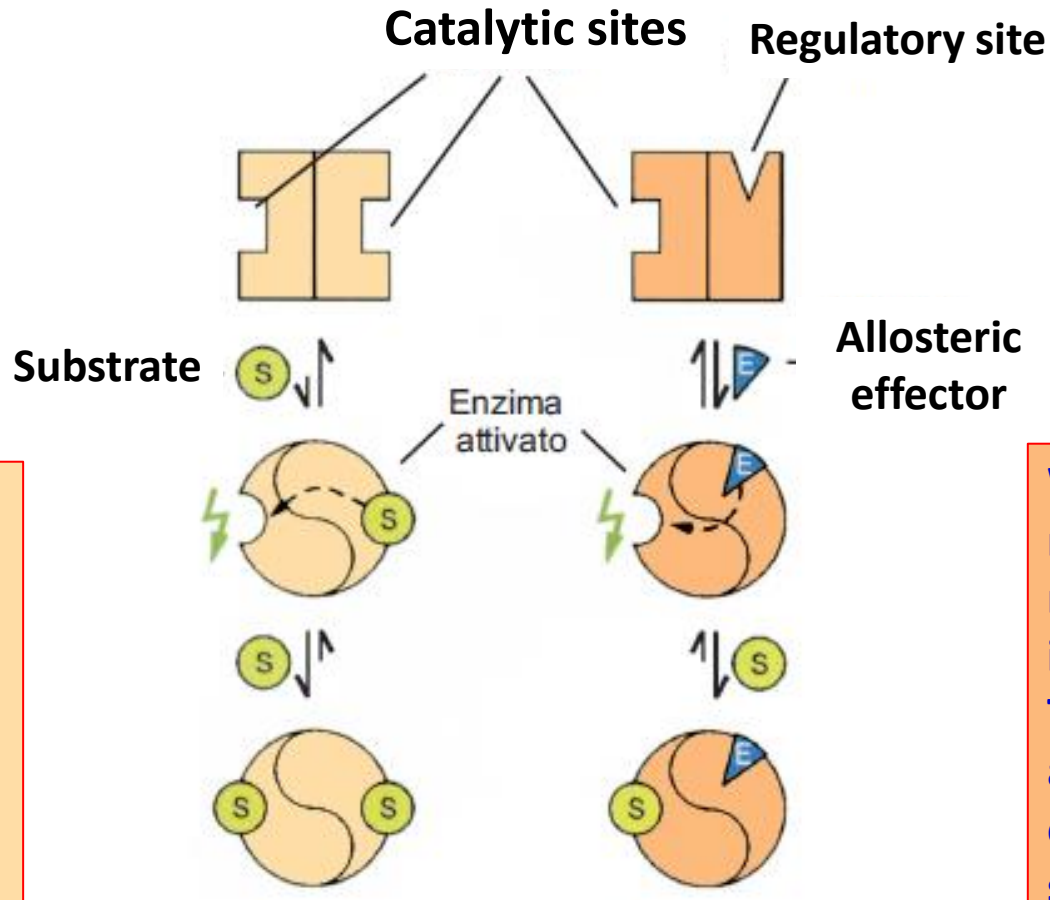








Types of allosteric regulators



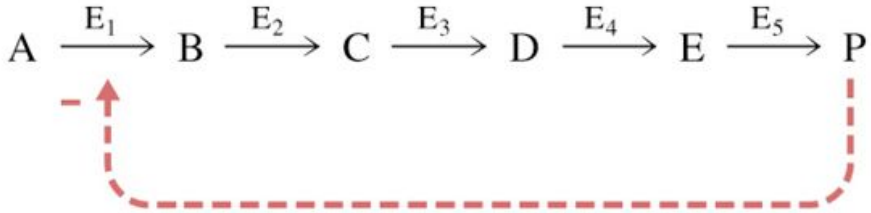
When the normal ligand/substrate and modulator are identical, the regulation is homo-allosteric. It provides the binding of the substrate to a catalytic center and requires multimeric enzymes.

When the modulator is a molecule other than the normal ligand, the interaction is heterotropic. The adjustment hetero-allosteric provides a bond effector to a regulatory site distant from the catalytic site

Metabolic Pathway modulation: Feedback Inhibition/ Feedforward activation

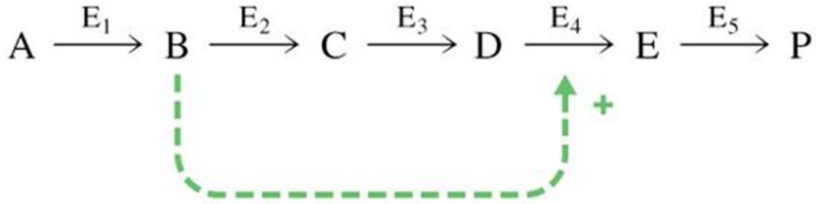
Feedback inhibition

- Product of a pathway controls the rate of its own synthesis by inhibiting an enzyme catalyzing an early step



Feed-forward activation

- Metabolite early in the pathway activates an enzyme further down the pathway



Regulation of Enzyme Activity by Covalent Modifications

- Another common regulatory mechanism is the reversible covalent modification of an enzyme
- Phosphorylation, whereby a phosphate is transferred from an activated donor (usually ATP) to an amino acid on the regulatory enzyme, is the most common example of this type of regulation
- Frequently this phosphorylation occurs in response to some stimulus (like a hormone or growth factor binding) that will either activate or inactivate target enzymes via changes in catalytic efficiency (e.g. K_m)

Phosphorylation of Enzymes Affects their Activity

- Protein phosphorylation is catalyzed by protein kinases
- Dephosphorylation is spontaneous, or catalyzed by protein phosphatases
- Typically, hydroxyl groups of Ser, Thr, or Tyr are phosphorylated

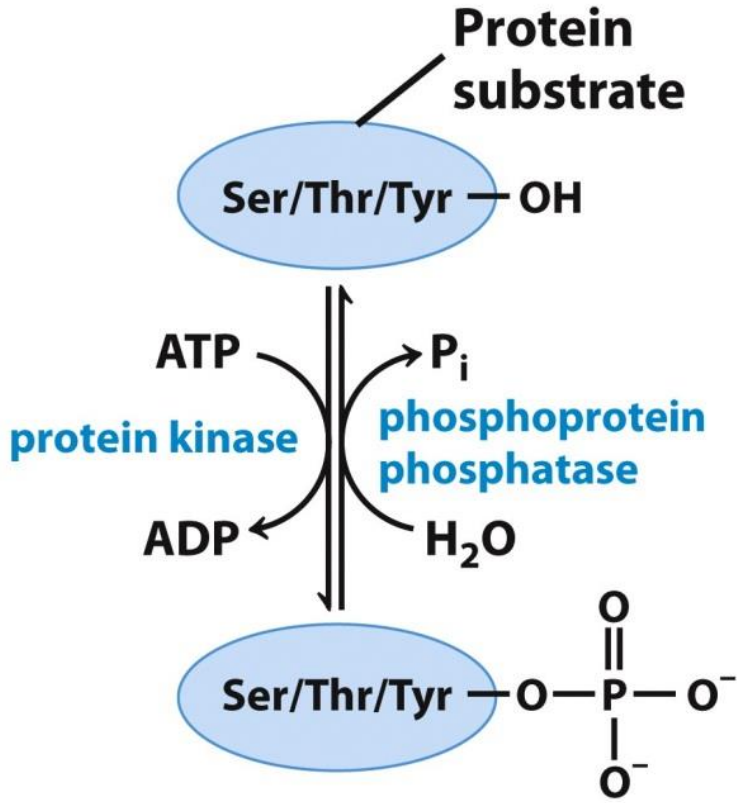
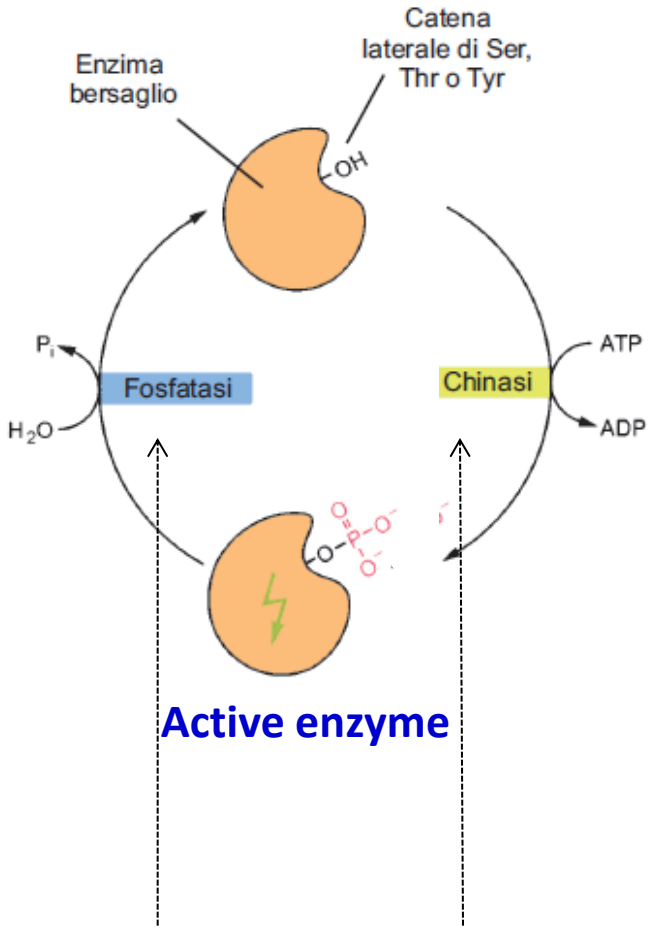


Figure 15-3
Lehninger Principles of Biochemistry, Fifth Edition

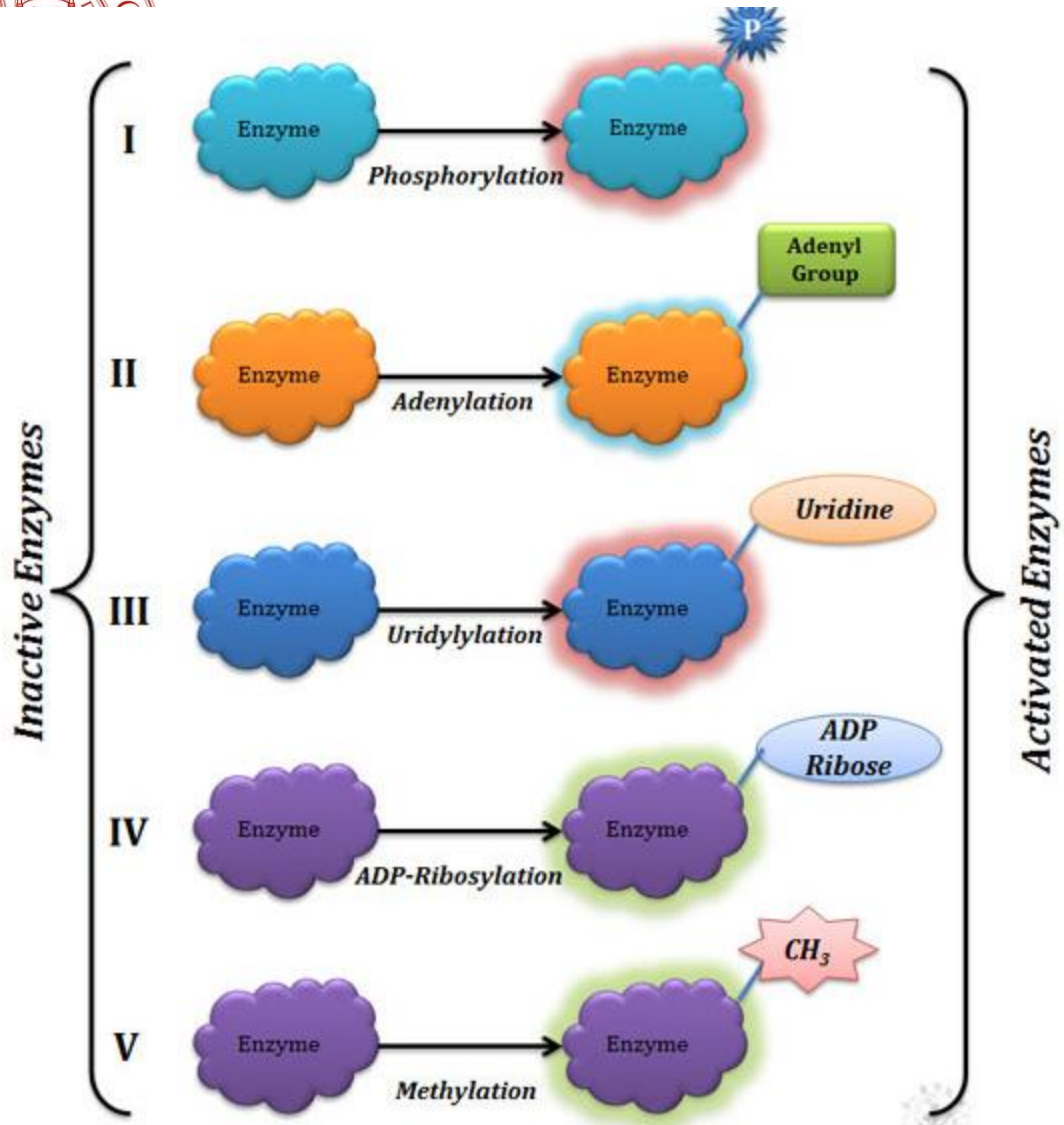
glycogen phosphorylase

Inactive enzyme

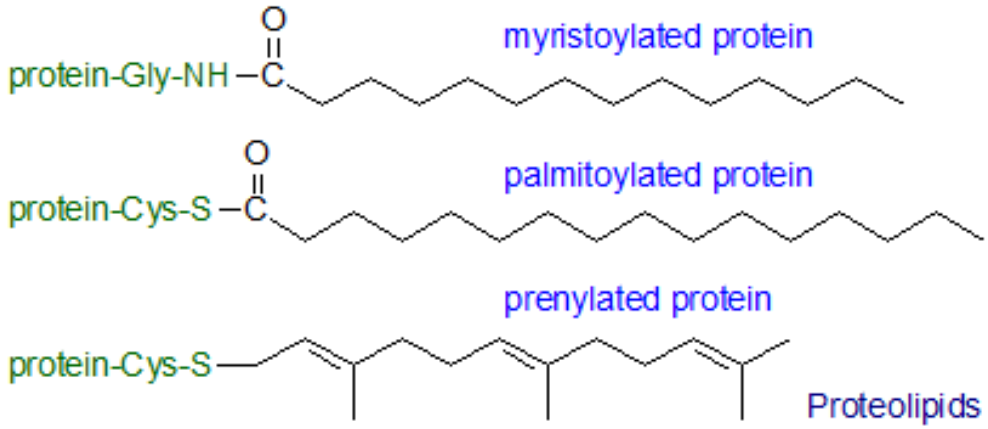


Active enzyme

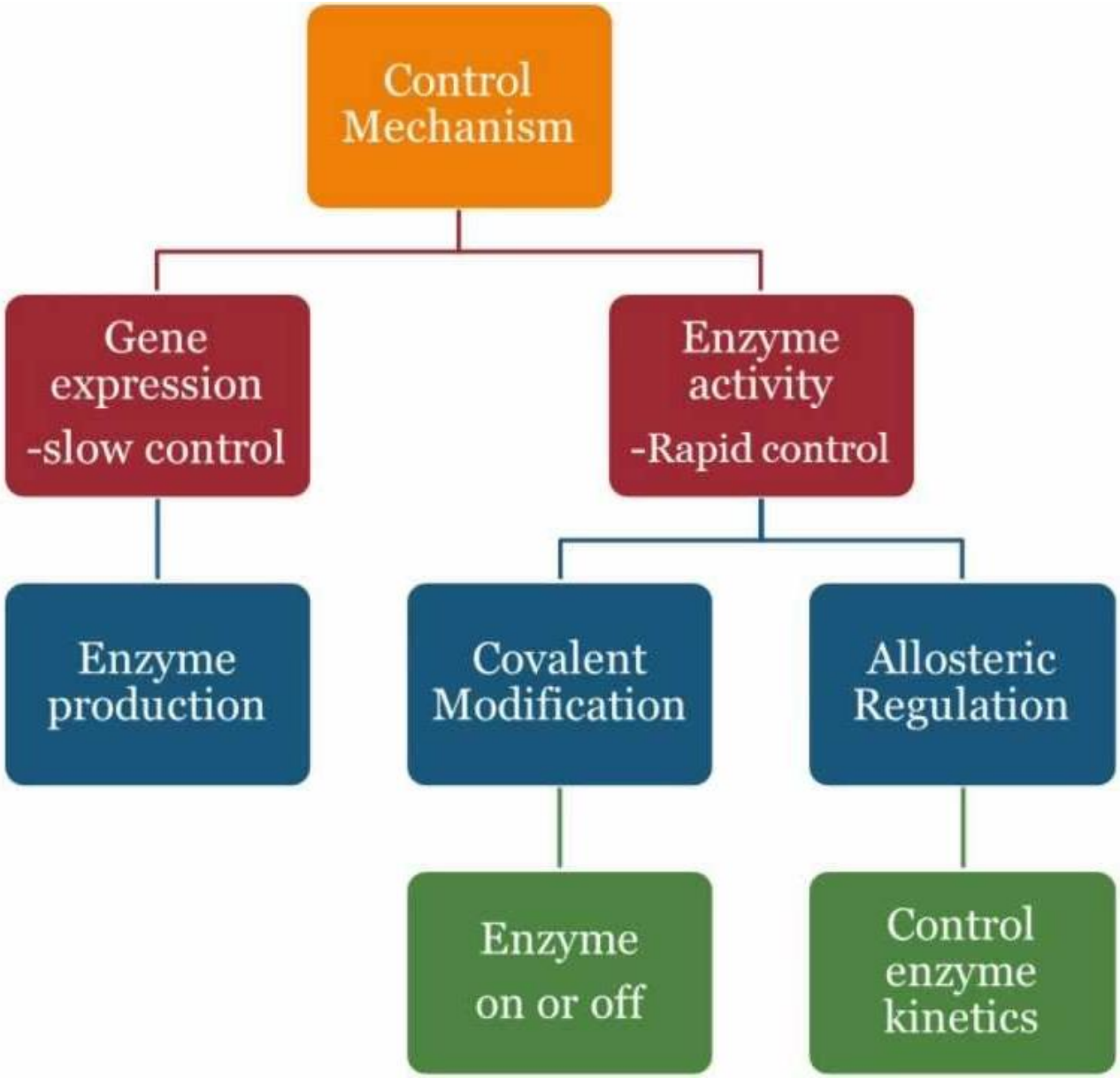
Other covalent modifications:



I: Phosphorylation, II: Adenylation, III: Uridylylation
IV: ADP-Ribosylation, V: Methylation



Proteolipids

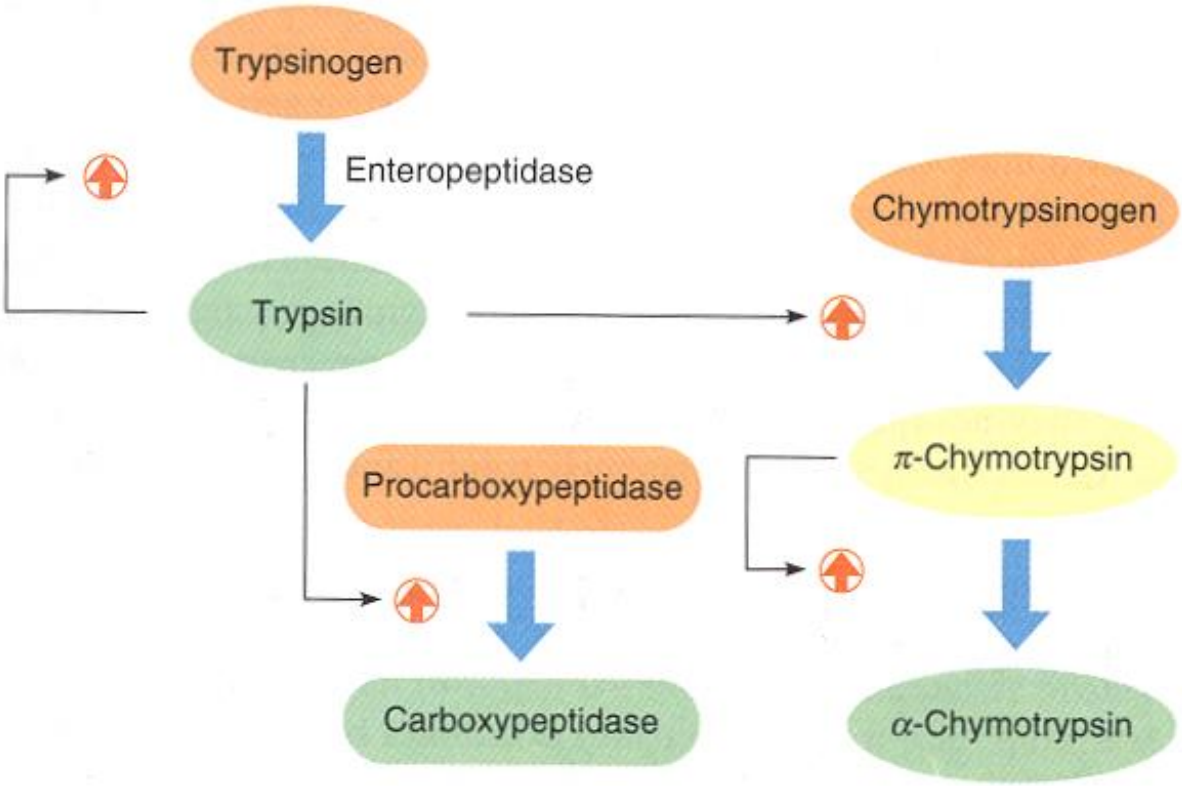

Control Mechanism
Gene expression -slow control
Enzyme activity -Rapid control
Enzyme production
Covalent Modification
Allosteric Regulation
Enzyme on or off
Control enzyme kinetics

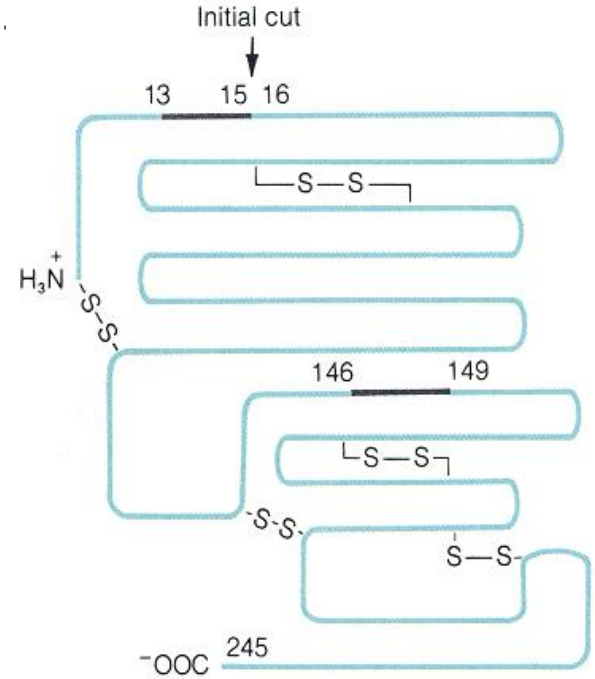
Zymogens: Inactive Precursor Proteins

- **A clinically important mechanism of controlling enzyme activity is the case of protease enzymes involved (predominantly) in food digestion and blood clotting**

Zymogen (from pancreas)		Active Enzyme (in small intestine)
Trypsinogen	*Enteropeptidase* →	trypsin + peptide
Chymotrypsinogen	*Trypsin* →	chymotrypsin + 2 dipeptides
Procarboxypeptidase	*Trypsin* →	carboxypeptidase + peptide

Zymogen Protease Examples

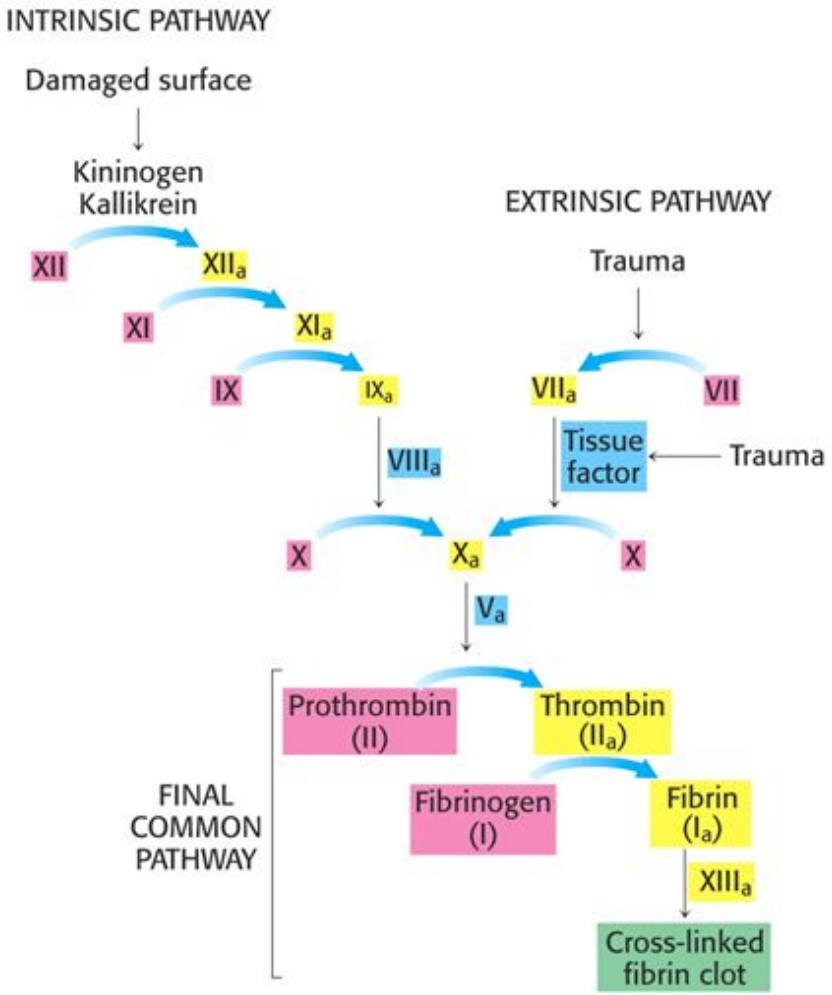




Chymotrypsinogen cleavage sites to yield active chymotrypsin

Blood Clotting: A Cascade of Zymogen Activations

The clotting of blood after injury must be rapid to avoid blood loss. The rapidity with which this is accomplished is due to a cascade of activation of blood clotting factors. Small amounts of the initial clotting factors amplify the response and result in the rapid formation of clots.



Clotting factors are referred to by Roman numerals.

These were named in the order that they were discovered, not for the order in which they act.

The inactive zymogen form is denoted by the Roman numeral, (e.g. Factor X) and the activated form is indicated by adding the suffix "a". (e.g. Factor Xa)


Kahoot!