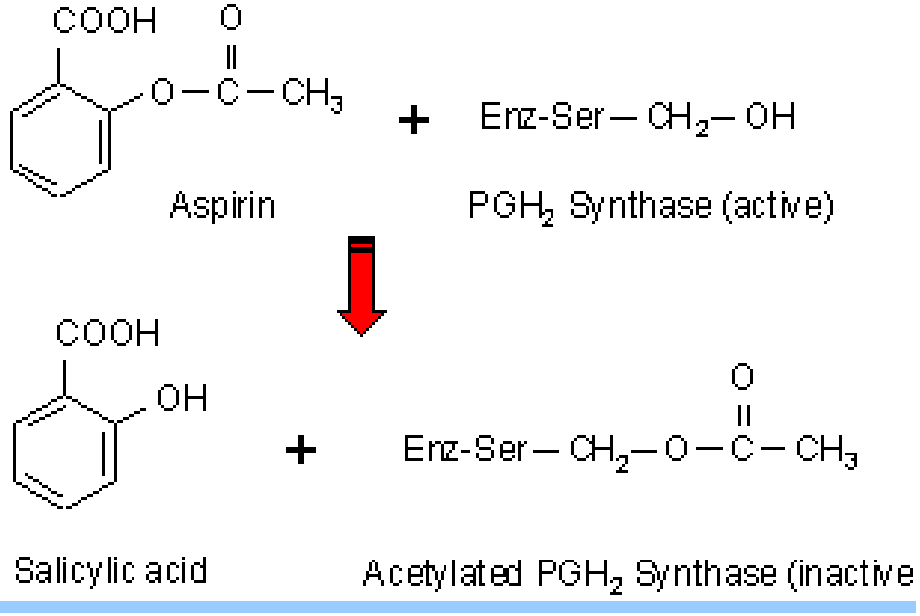
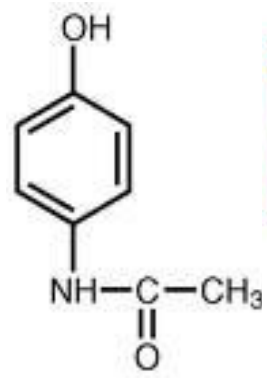
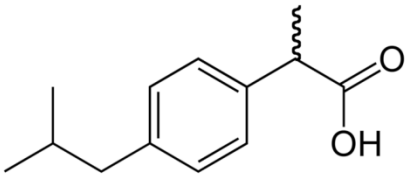
Non-steroidal anti-inflammatory drugs (**NSAID**s), such as aspirin and derivatives of ibuprofen, inhibit Cyclooxygenase activity of PGH_2 Synthase. They inhibit formation of prostaglandins involved in fever, pain and inflammation.





acetaminophen



Ibuprofen and related compounds act by blocking the hydrophobic channel by which arachidonate enters the active site.

Enzyme	Condition indicated by abnormal level
lactate dehydrogenase (LDH)	heart disease, liver disease
creatine phosphokinase (CPK)	heart disease
aspartate transaminase (AST)	heart disease, liver disease, muscle damage
alanine transaminase (ALT)	heart disease, liver disease, muscle damage
gamma-glutamyl transpeptidase (GGTP)	heart disease, liver disease
alkaline phosphatase (ALP)	bone disease, liver disease

Blood serum enzymes	*Pathological coditions*
Lipases, amylases	Pancreatitis
Glutamate-pyruvate transaminase (GPT)	Hepatitis
Glutamate-oxiloacetate Transaminase (GOP)	Heart attack Hepatitis
Acid phosphatase	Prostate cancer

Enzymes

I. General characteristics

II. Enzyme components

III. Names & classes

IV. Mechanism of enzyme action

V. Enzyme-Substrate Interaction

VI. Enzyme Kinetics

VII. Factors affecting enzyme activity

VIII. Inhibition of enzyme activity

IX. Regulation of enzyme activity

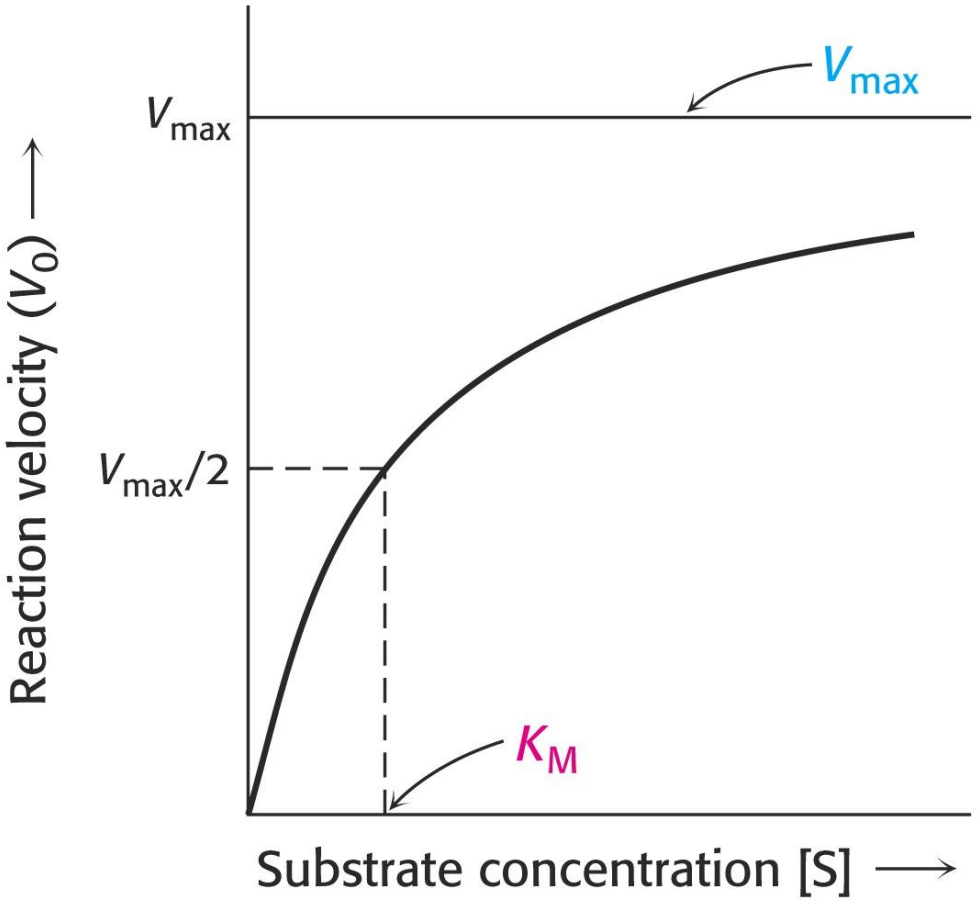
VI. Enzyme Kinetics (*reaction rate or Michaelis-Menten Kinetics*)

A plot of the reaction velocity (Vo) as a function of the substrate concentration [S]

V_o= the number of moles of product/per second

V_{max}= maximal velocity

K_m=Mechaelis constant = substrate concentration yielding the ½ V_{max}



Initial velocity (v_o)

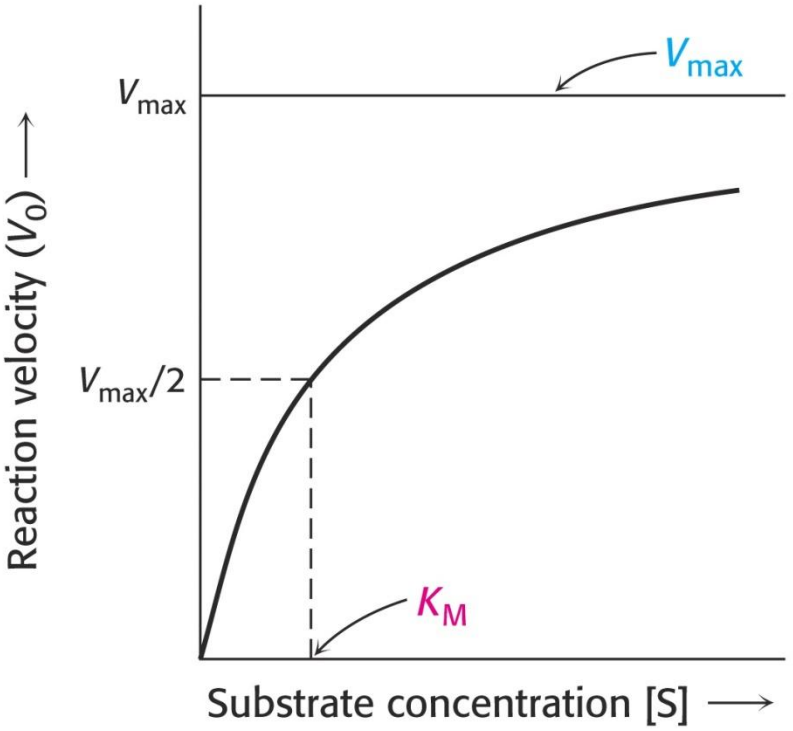
- Velocity at the beginning of an enzyme-catalyzed reaction is **v_o** (**initial velocity**)
- k_1 and k_{-1} represent rapid noncovalent association /dissociation of substrate from enzyme active site
- k_2 = rate constant for formation of product from ES

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

The Michaelis-Menten equation

1. What is t**he Michaelis-Menten equation?** What does it describe?

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

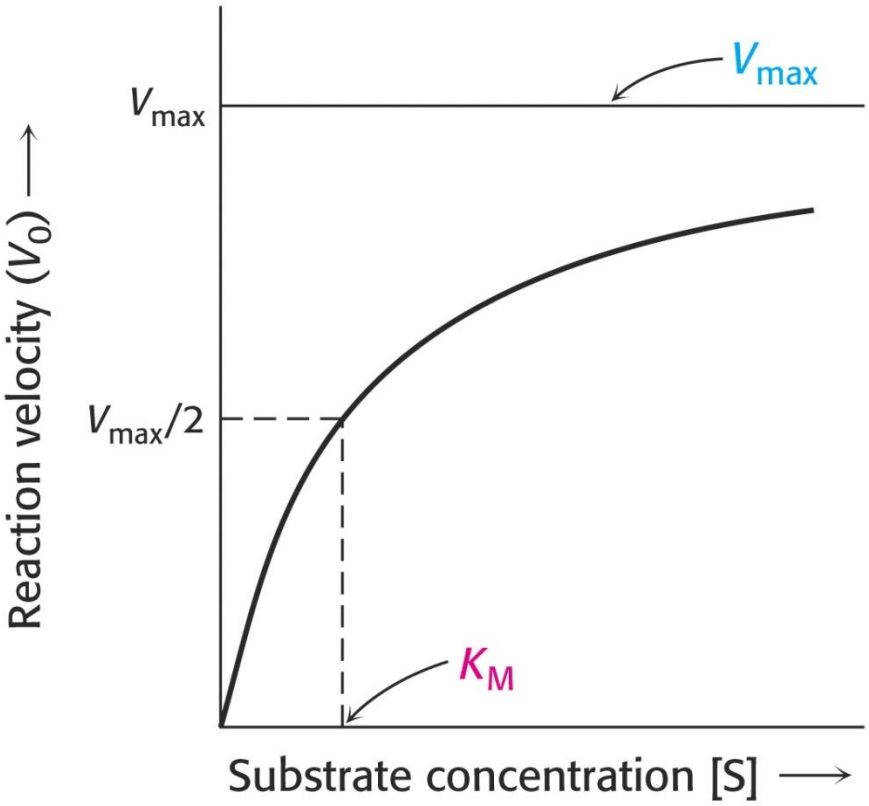


- Equation describes v_o versus [S] plots

the Michaelis constant

1. What is t**he Michaelis-Menten equation?** What does it describe?
2. What is the Michaelis constant? What does it mean?

- K_m = [S] when v_o = 1/2 Vmax
- $K_m \cong k_{-1} / k_1 = K_s$ (the enzyme-substrate dissociation constant) when $k_{cat} << $ either k_1 or k_{-1}
- The <u>lower</u> the value of K_m, the <u>tighter</u> the substrate binding
- K_m can be a measure of the <u>affinity</u> of E for S



Use t**he Michaelis-Menten equation to calculate reaction rate:**

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

What is the initial velocity for an enzyme reaction when V_{max} = 6.5 x 10^{-5} mol.sec^{-1}, [S] = 3.0 x 10^{-3} M, and Km = 4.5 x 10^{-3} M.

2.6 x 10^{-5} moles per sec.

Understand the meaning and use of K_m

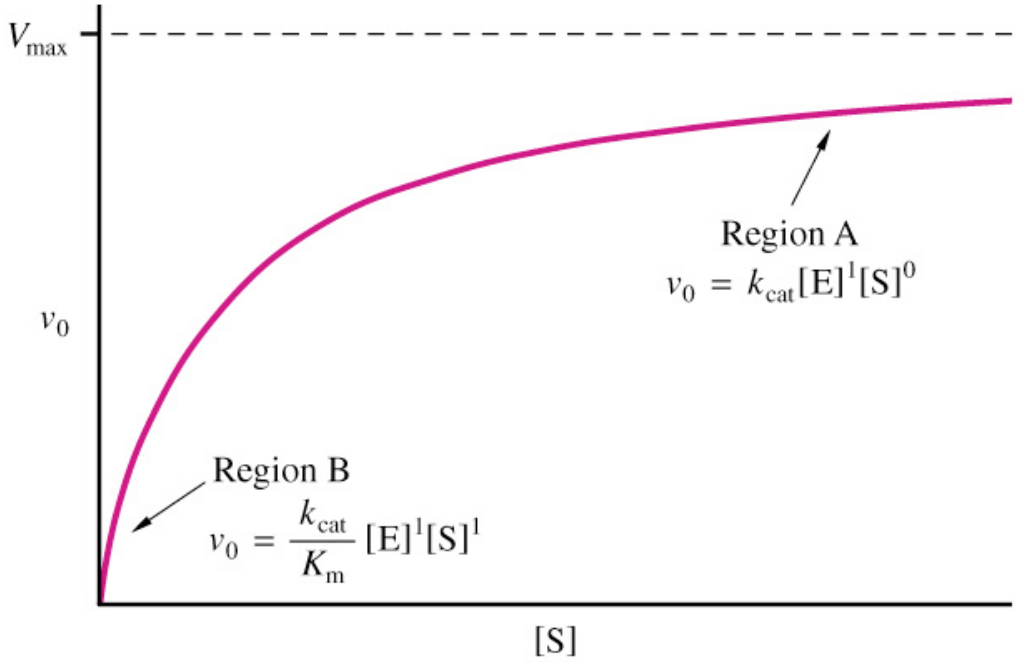
1. Given are five Km values for the binding of substrates to a particular enzyme. Which has the strongest affinity?
    a. 150 mM
    b. 0.15 mM
    c. 150 µM
    d. 1.5 nM
    e. 15000 pM

Catalytic Constants: k_{cat}

Turnover number (S^{-1})

- **Catalytic constant** (k_{cat}) - first order rate constant for conversion of ES complex to E + P
- k_{cat} most easily measured when the enzyme is saturated with S (region A)
- $K_{cat} = V_{max}/[E]_T$

$[E]_T$ = total enzyme concentration
[E] + [ES]

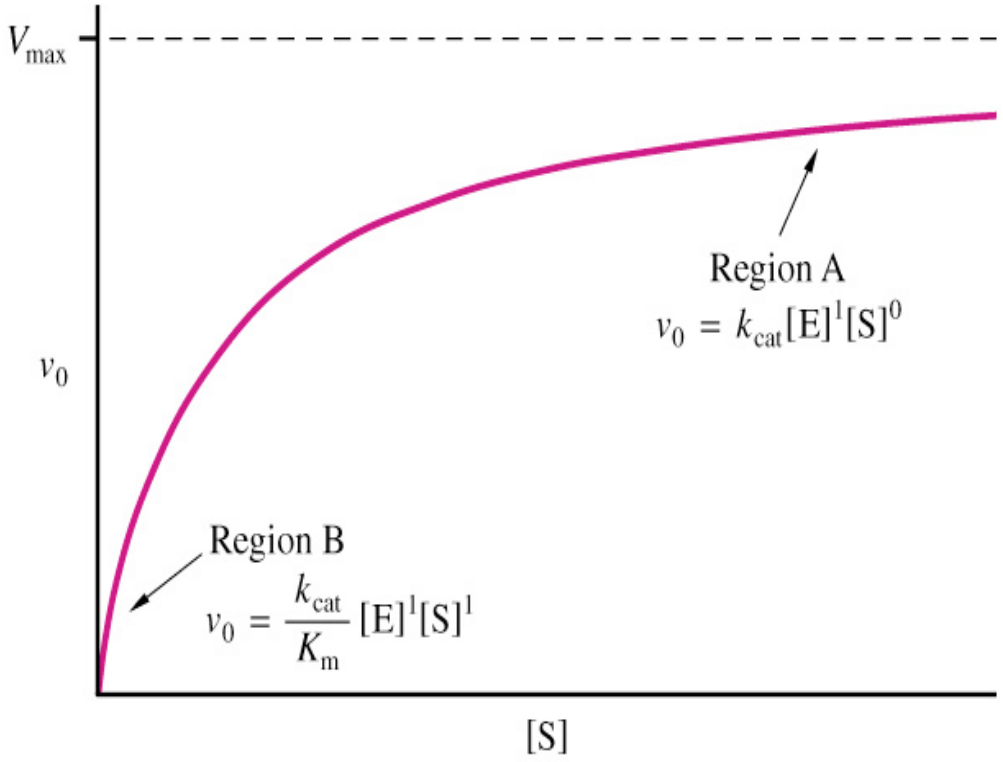


Region A: $ES \xrightarrow{k_{cat}} E + P$

Region B: $E + S \xrightarrow{\frac{k_{cat}}{K_m}} E + P$

$(E + S \longrightarrow ES \longrightarrow E + P)$

- Ratio k_{cat}/K_m is a rate constant for
  $E + S \rightarrow E + P$ at low [S] concentrations (region B)



Region A: $ES \xrightarrow{k_{cat}} E + P$

Region B: $E + S \xrightarrow{\frac{k_{cat}}{K_m}} E + P$

$(E + S \longrightarrow ES \longrightarrow E + P)$

When $[S] << K_m$

k_{cat}/K_m can be used as a measure of catalytic efficiency

- k_{cat}/K_m is also a measure of enzyme <u>specificity</u> for different substrates (**specificity constant**)

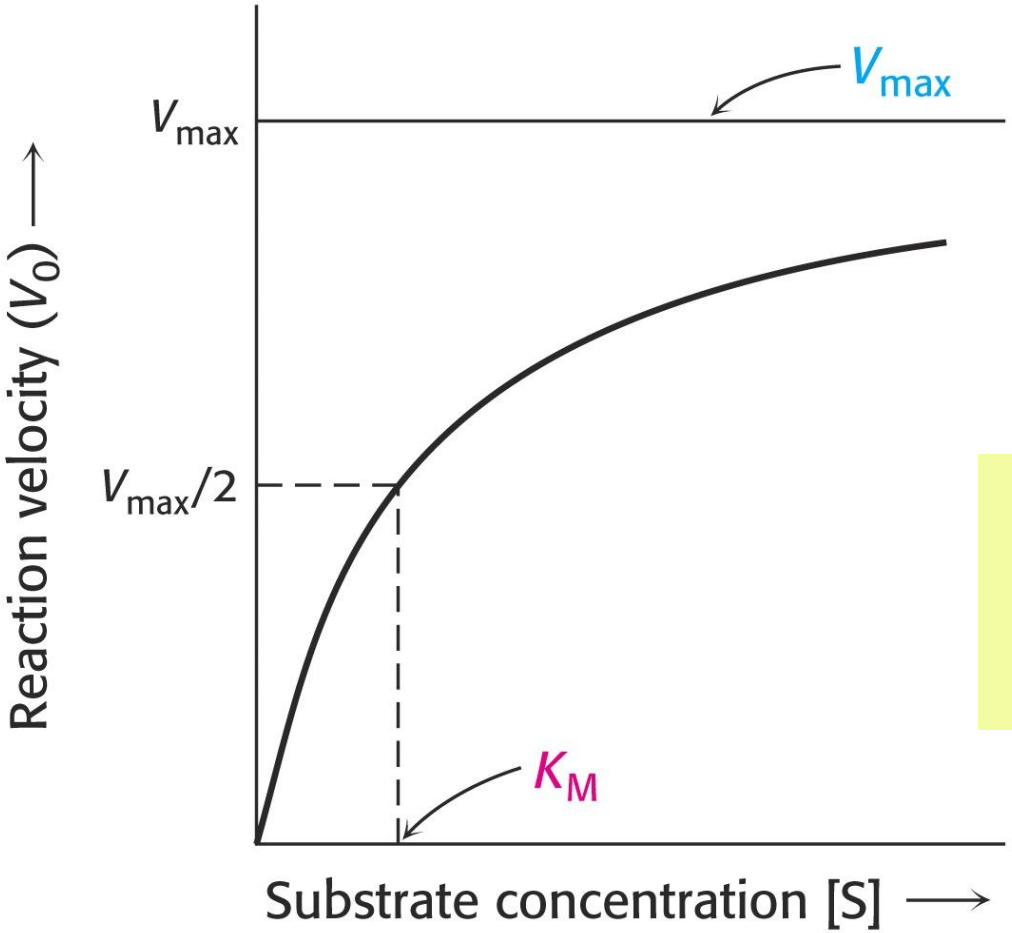
Table 12-1 The Values of K_M, k_{cat}, and k_{cat}/K_M for Some Enzymes and Substrates

Enzyme	Substrate	K_M (M)	k_{cat} (s^{-1})	k_{cat}/K_M ($M^{-1} \cdot s^{-1}$)
Acetylcholinesterase	Acetylcholine	9.5×10^{-5}	1.4×10^{4}	1.5×10^{8}
Carbonic anhydrase	CO_2	1.2×10^{-2}	1.0×10^{6}	8.3×10^{7}
	HCO_3^-	2.6×10^{-2}	4.0×10^{5}	1.5×10^{7}
Catalase	H_2O_2	2.5×10^{-2}	1.0×10^{7}	4.0×10^{8}
Chymotrypsin	*N*-Accetylglycine ethyl ester	4.4×10^{-1}	5.1×10^{-2}	1.2×10^{-1}
	N-Acetylvaline ethyl ester	8.8×10^{-2}	1.7×10^{-1}	1.9
	N-Acetyltyrosine ethyl ester	6.6×10^{-4}	1.9×10^{2}	2.9×10^{5}
Fumarase	Fumarate	5.0×10^{-6}	8.0×10^{2}	1.6×10^{8}
	Malate	2.5×10^{-5}	9.0×10^{2}	3.6×10^{7}
Urease	Urea	2.5×10^{-2}	1.0×10^{4}	4.0×10^{5}

Table 12-1 Fundamentals of Biochemistry, 2/e

- K_{cat} is a constant, whereas V_{max} depends on the concentration of the enzymes present in the experimental system. V_{max} increases as $[E]_T$ increases

How to Measure K_m and V_{max}

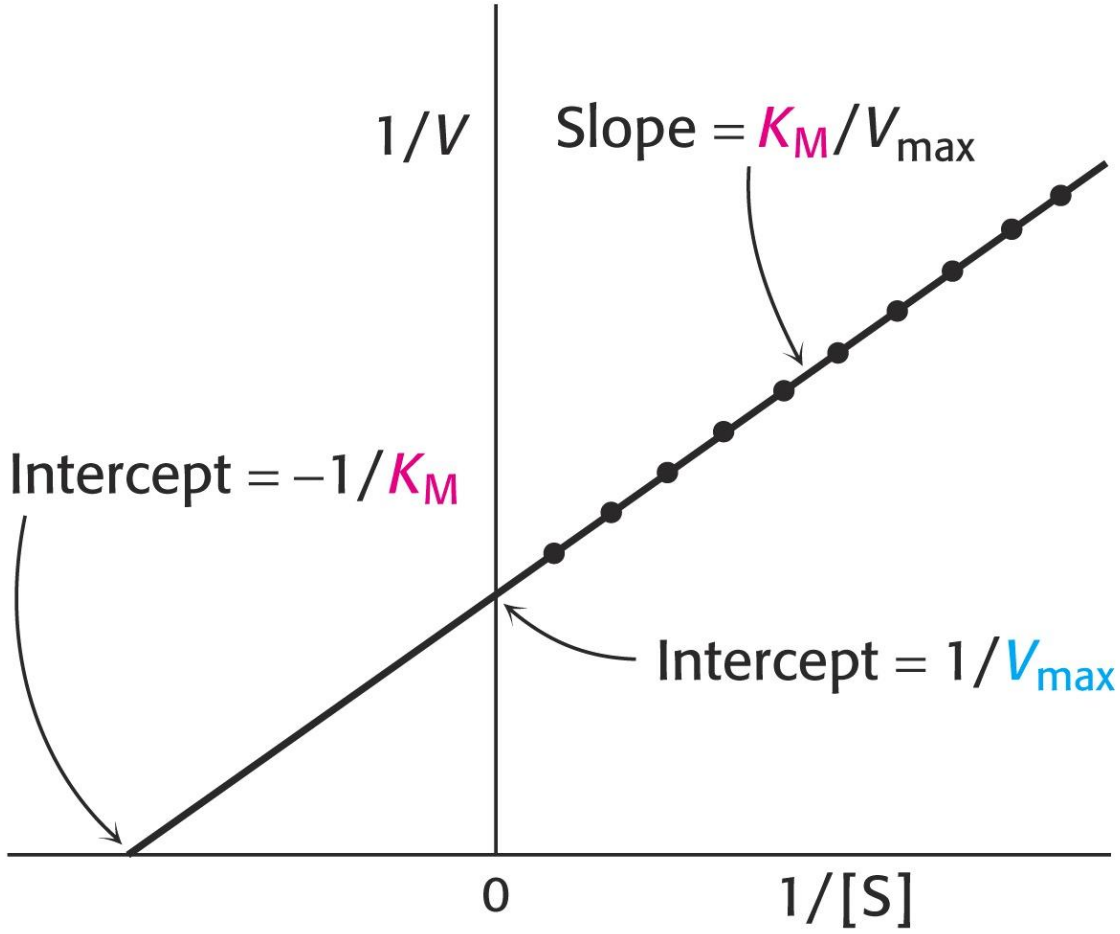


It is difficult to manually calculate enzyme kinetics data based a hyperbolic plot curve

How to Measure K_m and V_{max}

The double-reciprocal **Lineweaver-Burk plot** is a linear transformation of the Michaelis-Menten plot

($1/v_o$ versus 1/[S])



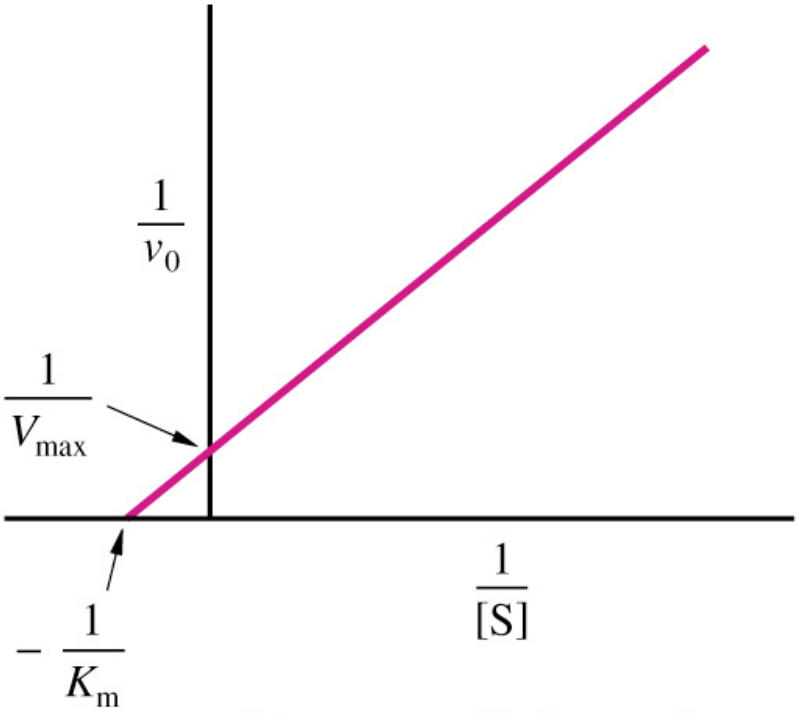
It is easier to extrapolate a straight line to locate the intercepts of the plot than to extend the curve on.

What is the double-reciprocal **Lineweaver-Burk plot?** **What is it used for?**

Lineweaver-Burk plot is a linear transformation of the Michaelis-Menten plot ($1/v_o$ versus $1/[S]$)

It is used to calculate K_m and V_{max}

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



Lineweaver-Burk equation:

$$\frac{1}{v_0} = \left(\frac{K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$

Determine K_m and Vmax using the Lineweaver-Burk plot:

[S] (uM)	1/[S]	Vo (mM/Sec)	1/Vo
0.1		0.34	
0.2		0.53	
0.4		0.74	
0.8		0.91	

1. Experimentally determined

2. The double-reciprocal conversion

3. **Lineweaver-Burk plot**

4. Find Y intercept = 1/Vmax

X intercept = 1/Km

In the double-reciprocal Lineweaver-Burk plot, the slope is

a. 1/[S]
b. 1/*V*max
c. 1/*K*m
d. *K*m/*V*max
e. 1/*V*0

Which of the parameter is used as specificity constant?

a. *k*cat
b. *k*cat/*K*m
c. *K*m
d. *V*o

When an enzyme is saturated with substrates, the rate of catalysis is equal to

a. K_d b. K_{cat} c. V_0 d. k_{cat}/K_m E. K_m

VI. Factors affecting enzyme activity

1) Enzyme concentration:

2) Substrate concentration:

3) Temperature

4) Effect of pH on enzyme's activity

*Discoloration of fruits (*Apples, pears, avocados, eggplants, potato, etc)

Polyphenol oxidase

Phenolic compounds + O_2 ----------> browish oxidized products

Immerse slices in cold water — Low temp, O2 limit

Boil — High temp

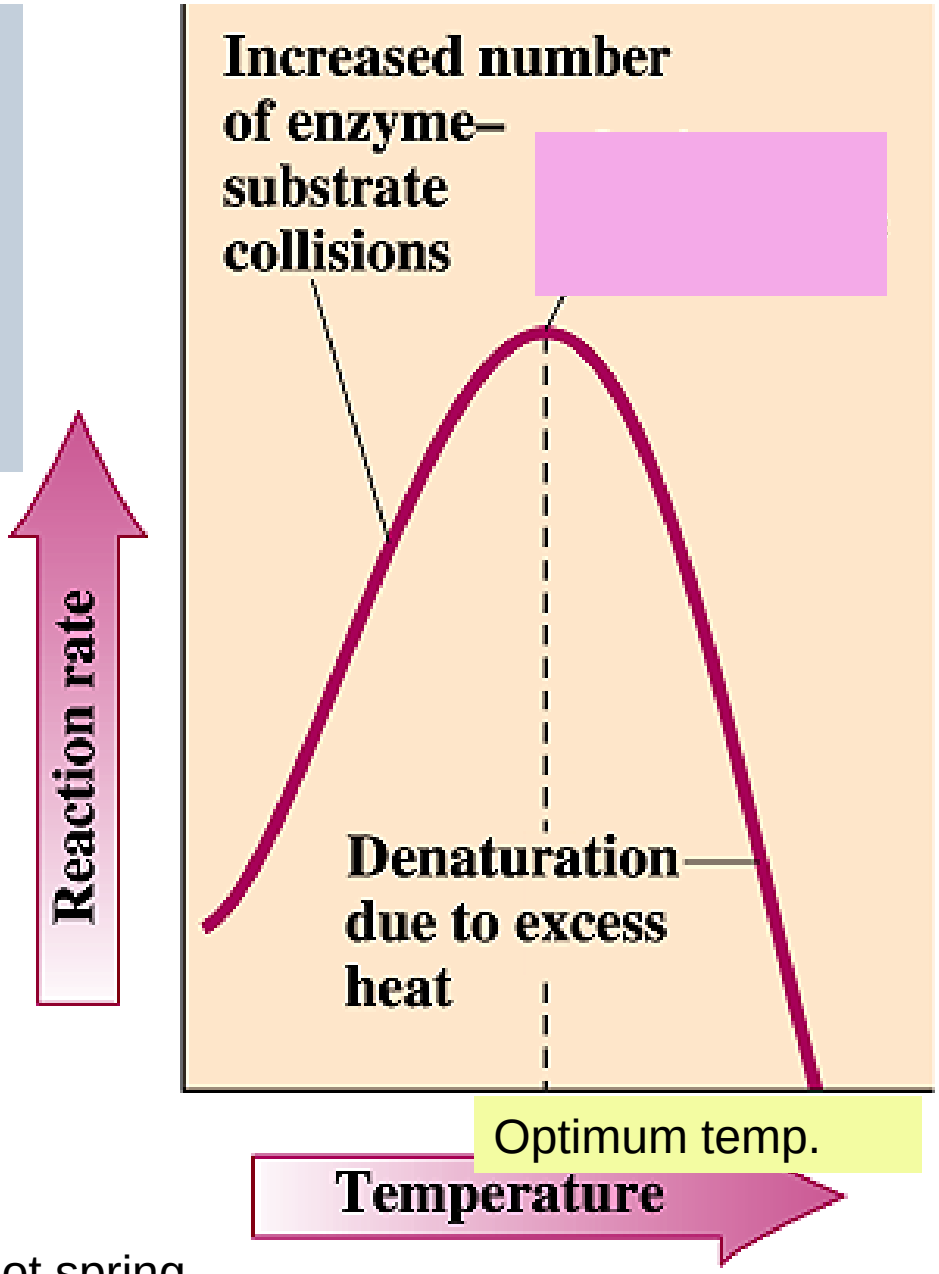
Spray lemon juice — Low pH, antioxidant

3) Temperature

Enzyme reaction rates increase with temperature.

Increase in temp. increases collisions between enzyme and substrates.

However, enzymes lose their structural stability and become denatured if the temperature is increased too much. So the reaction rate falls off sharply as protein unfolding occurs.



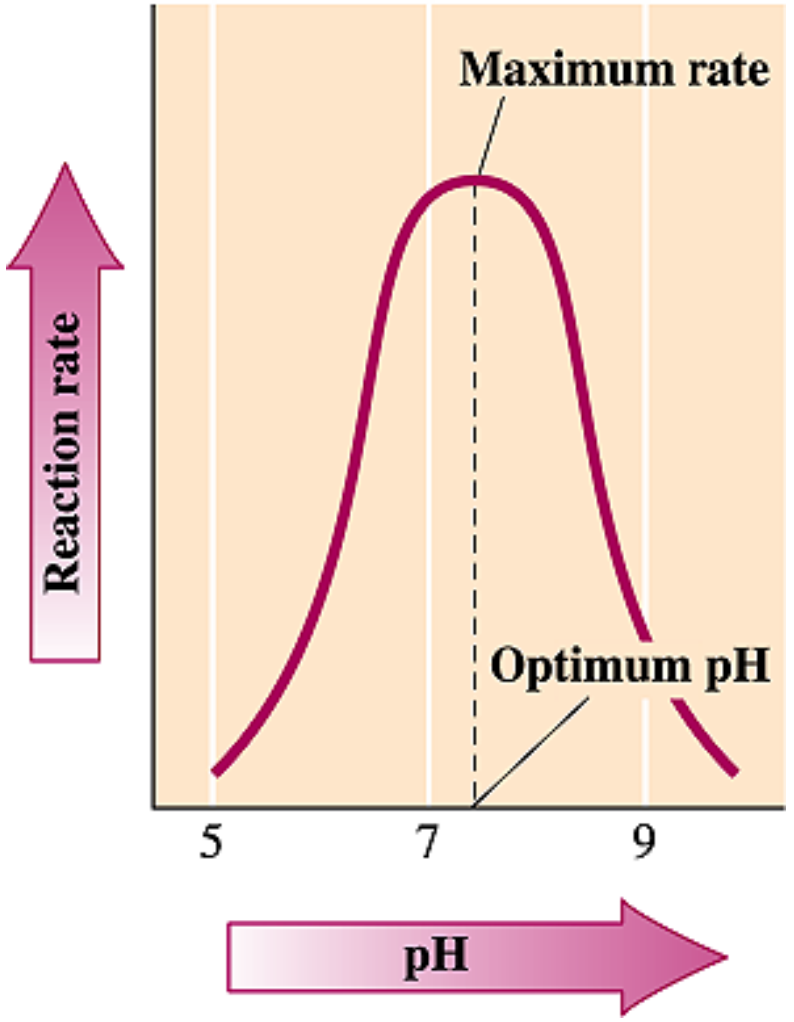
Optimum temp in human, organism in hot spring

Plants?

4) Effect of pH on an enzyme's activity

Enzymes often have very narrow pH optimums. This is because: **I.** pH affects substrate-enzyme interaction (*e.g. basic and acidic groups involved in catalysis on either the enzyme or the substrate need be in the right state (neutral or charged).*

II. The overall conformation of a protein (*e.g. an increase or decrease in pH may cause unfolding = denaturation of the protein.)*



Cellular cytoplasm pH=7, stomach pH=2, lysosome pH=5

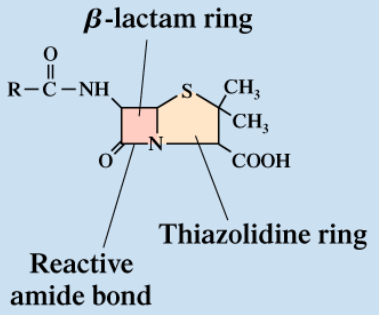
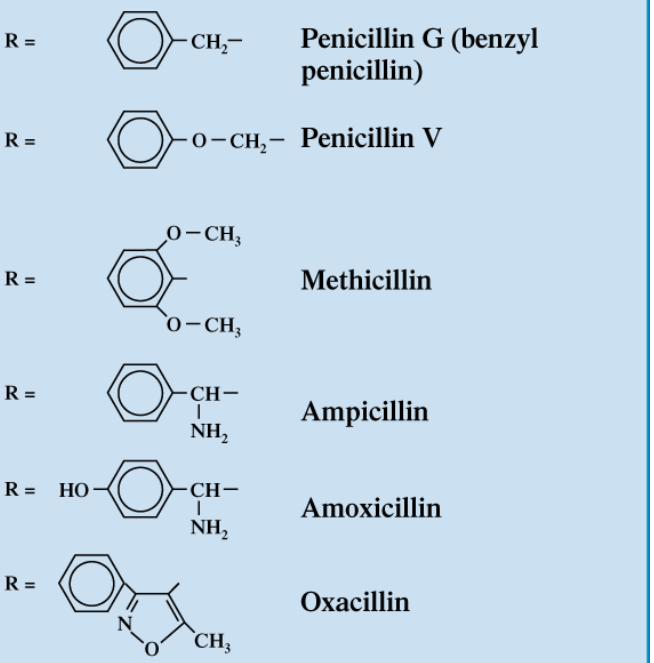
VII. Inhibition of enzyme activity

Enzyme inhibitor: A substance that can decrease the rate of an enzyme-catalyzed reaction.

- **Inhibitor** (**I**) binds to an enzyme & prevents formation of ES complex or breakdown to E + P

Many drugs & pharmaceutical compounds alter the activities of specific enzymes

Structures of selected penicillins

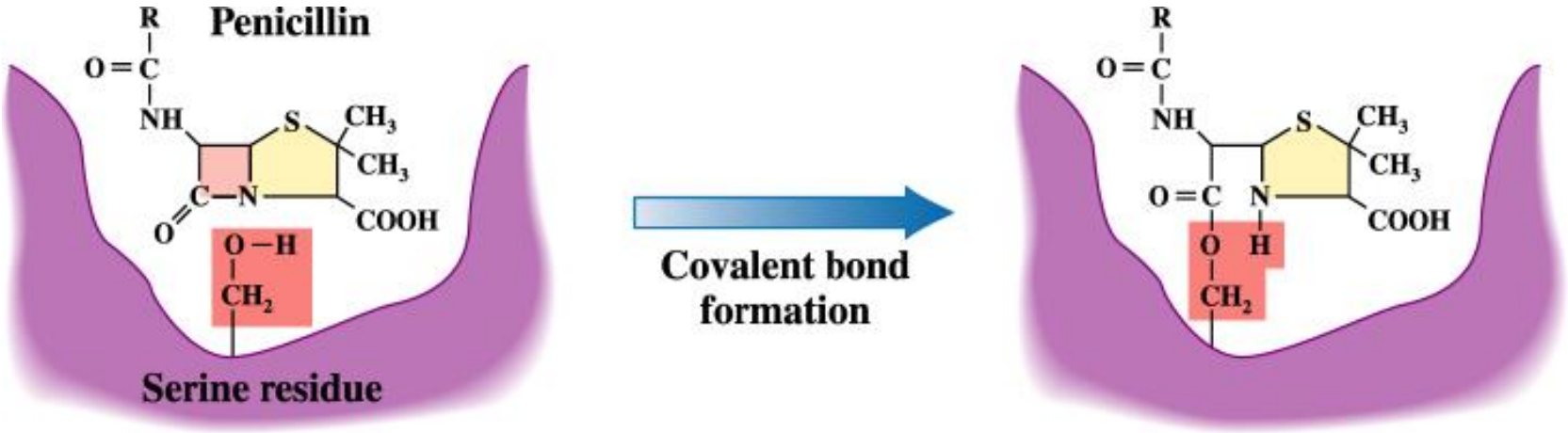
General Structure of Penicillin	R Group Variations in Penicillin Structures
	

VII. Inhibition of enzyme activity

1. Irreversible inhibition:

Inhibitors bind very tightly or covalently to enzymes and block enzyme-substrate complex formation.

-- Binding to the R groups of amino acids at the active site
-- An enzyme loses its activity permanently
e.g *antibiotics penicillin*; *pain killer aspirin*



organophosphorous inhibitors are used as insecticides or for enzyme research

VII. Inhibition of enzyme activity

1. **Irreversible inhibition:**

   Inhibitors bind very **tightly or covalently** to enzymes, which lead to **permanent loss** of enzyme's activity

2. **Reversible inhibition:**

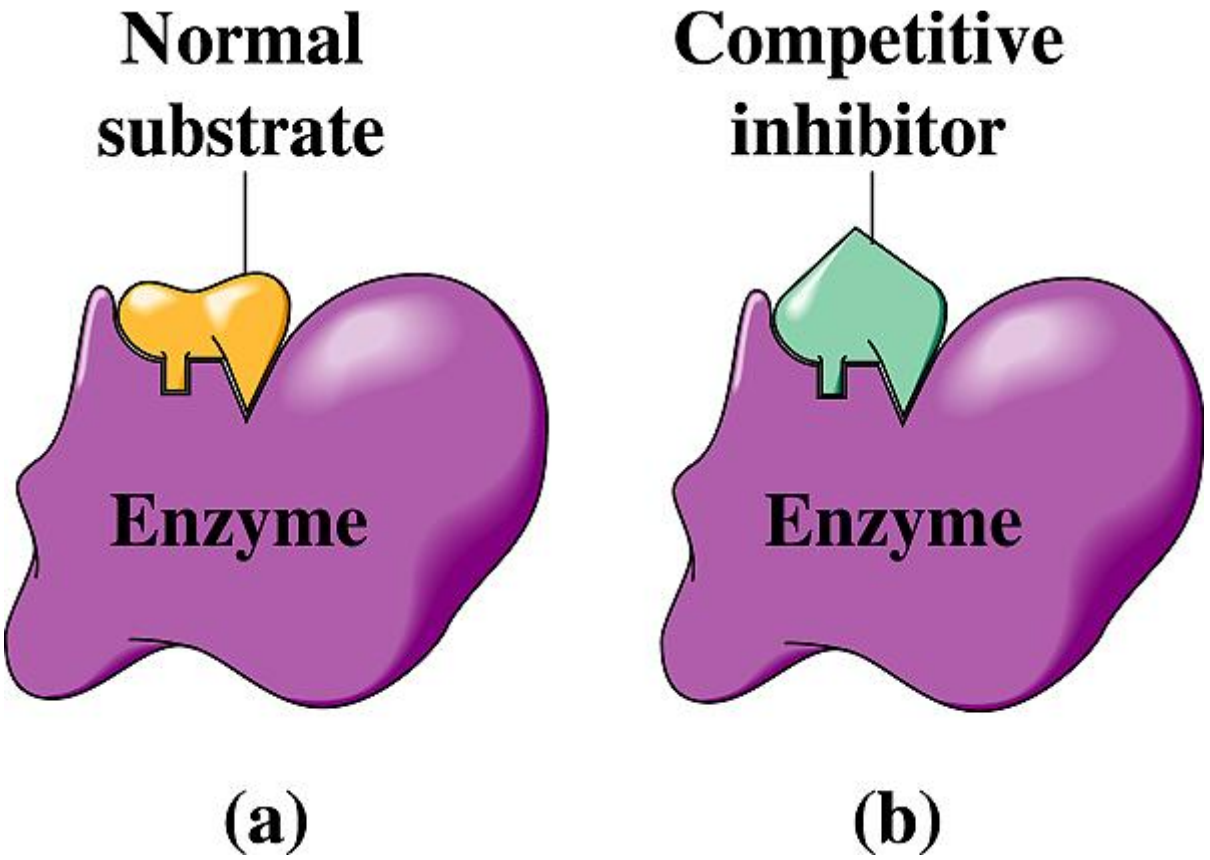
(Weak binding, such as hydrogen bonds)
*An enzyme activity can be **restored** when the inhibitor dissociates from the enzyme.*

1) competitive inhibition

2) non-competitive inhibition
or Mixed inhibition :

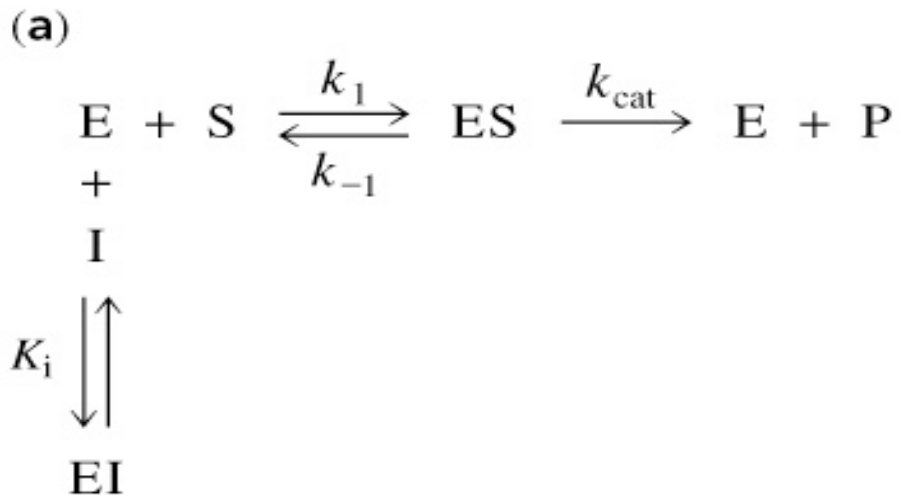
3) Uncompetitive inhibition

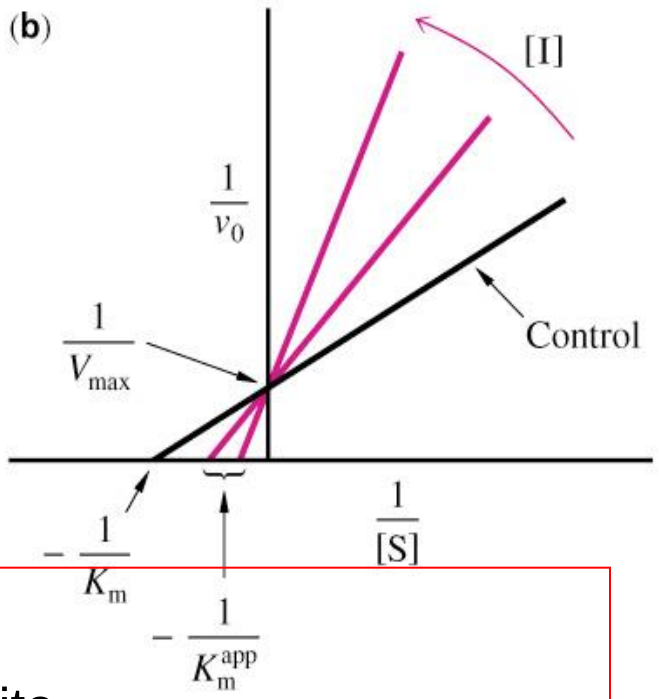
1) **competitive inhibitor:** Inhibitor is a structural analogue to substrate and binds to active site.



The inhibition **can be overcome** by increasing the substrate concentration.

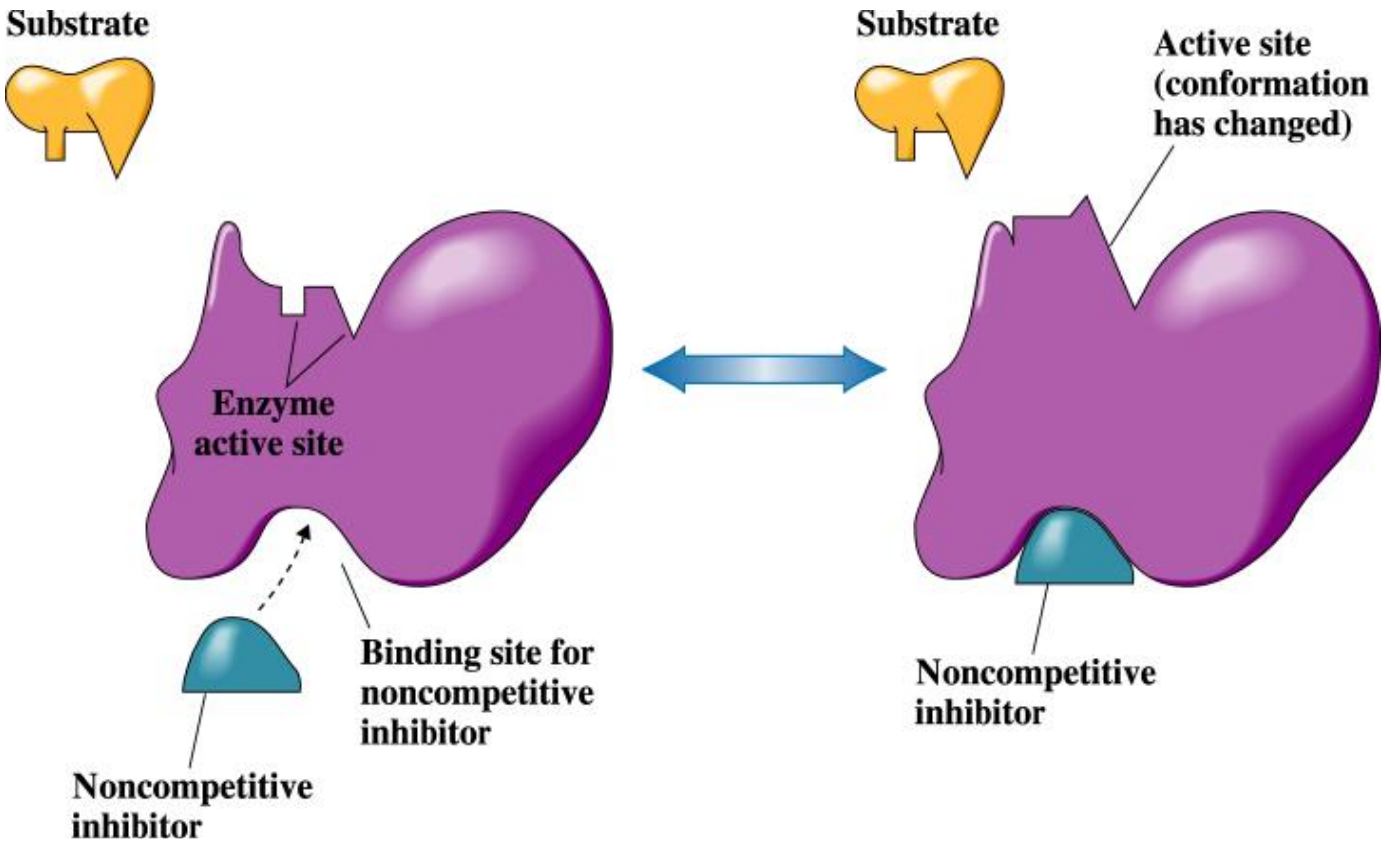
Competitive inhibition.
(a) Kinetic scheme. (b) Lineweaver-Burk plot





- Inhibitor binds only to free enzyme (E) not (ES)
- Substrate cannot bind when I is bound at active site (S and I "compete" for the enzyme active site)
- V_{max} remains the same with or without I (high S can still saturate the enzyme even in the presence of I)
- **K_m is increased**
- Competitive inhibitors usually resemble the substrate

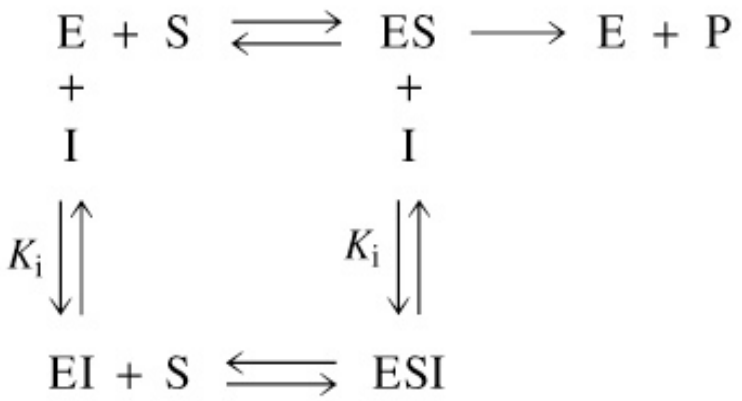
2) **non-competitive (mixed) inhibitor**:
inhibitor binds to a site on an enzyme that is not the active site, and this binding changes the shape of the enzyme and inhibits E+S interaction.



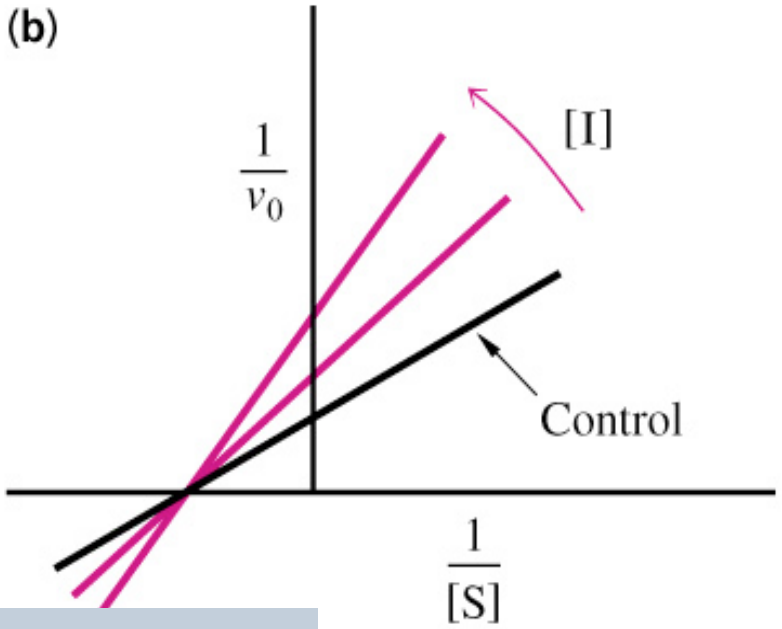
Unlike competitive inhibition, non-competitive inhibition **cannot be** overcome by increasing the substrate conc.

Noncompetitive inhibition (*or mixed inhibition*)

(a)

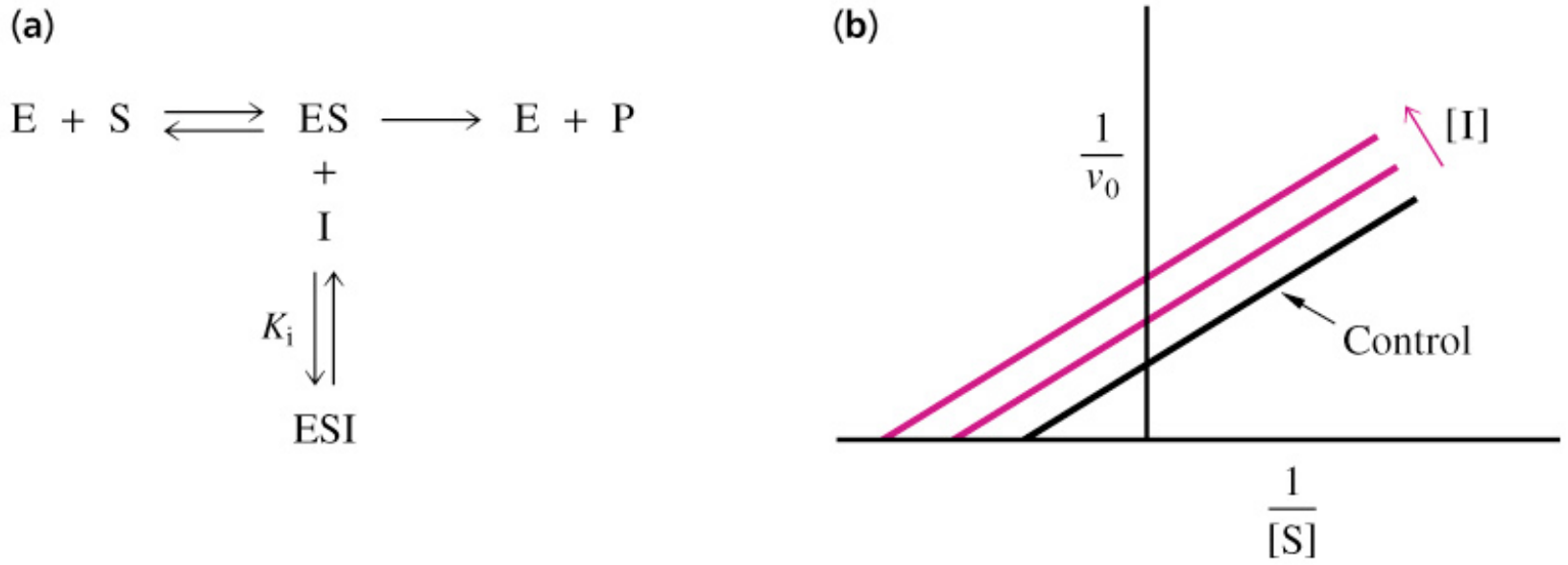


(b)



- Noncompetitive inhibitors bind to <u>both</u> E and ES
- Inhibitors do not bind at the same site as S
- **V_{max} is decreased**
- K_m does not change
- Inhibition cannot be overcome by addition of S
- Lines on double-reciprocal plot intersect on x axis

3) Uncompetitive inhibition



- Uncompetitive inhibitors bind to ES <u>not</u> to free E
- V_{max} is decreased by conversion of some E to ESI
- K_m is also decreased
- Lines on double-reciprocal plots are parallel
- This type of inhibition usually only occurs in multisubstrate reactions

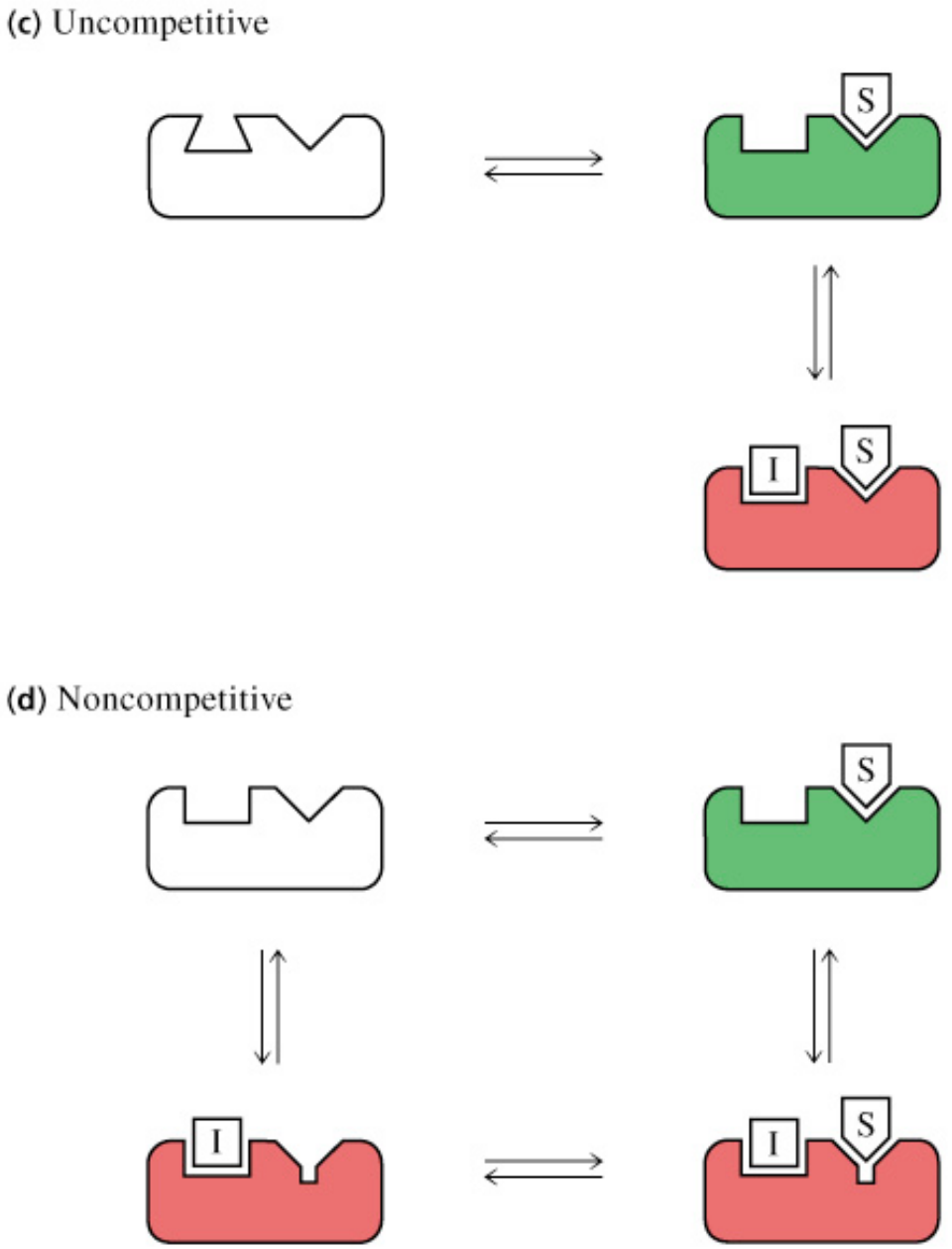
Uncompetitive.

I binds only to ES (inactivates E)

vs

Noncompetitive.

I binds to either E or ES to inactivate the enzyme



Many drugs & pharmaceutical compounds are inhibitors that decrease the activities of specific enzymes

Q? Suppose you had discovered a compound that could block the activity of an enzyme of your interest, and you wanted to determine how a compound inhibits the enzyme activity. Outline an analytical strategy by which you could determine what type inhibitor of this compound is.

Assay enzyme activity in the presence and absence of the inhibitor at different concentrations of the inhibitor. Then plot the data using the Double-Reciprocal plots to determine whether Km, Vmax, or both of them have been changed.

If it decreases Vmax	Non-competitive
If it increases Km	Competitive
If it decreases both Vmax and Km	Uncompetitive

VII. Regulation of Enzyme Activity

1. **Noncovalent allosteric regulation**

2. **Regulation by Covalent modification**

3. **Activation of Zymogen (proenzyme):**

VII. Regulation of Enzyme Activity

1. Noncovalent allosteric regulation

Allosteric regulation: an interaction in which the binding of a regulator (effector) at one site on a protein affects the protein's ability to bind another molecule at a different site

Allosteric enzyme: an enzyme whose activity is changed by the binding of an effector.

Positive allosterism: Binding of an effector to an enzyme increases the enzyme activity.

Negative allosterism: Binding of an effector to an enzyme decreases the enzyme activity.

Allosteric enzymes have a second **regulatory site** (allosteric site) distinct from the active site
Allosteric inhibitors or activators bind to this site and regulate enzyme activity via conformational changes

1) Feedback inhibition

- **Regulatory enzymes** - activity can be reversibly modulated by effectors
- Such enzymes are usually found at the first unique step in a metabolic pathway (the first "committed" step)
- Regulation at this step conserves material and energy and prevents accumulation of intermediates

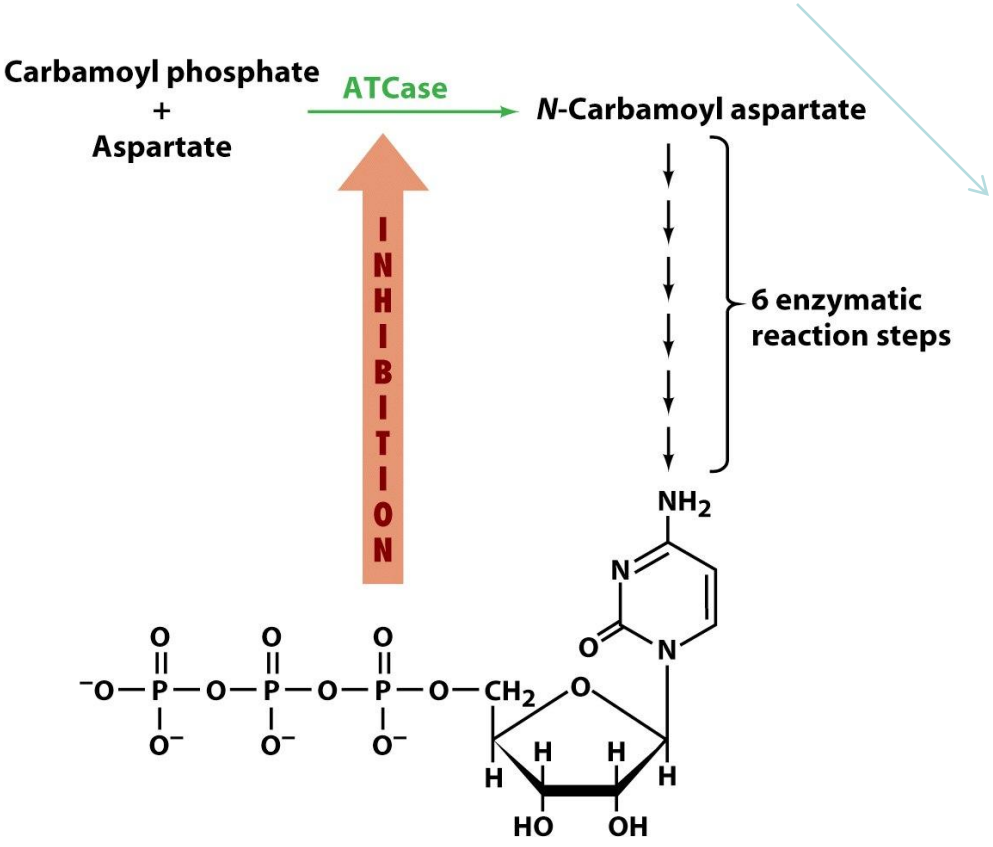
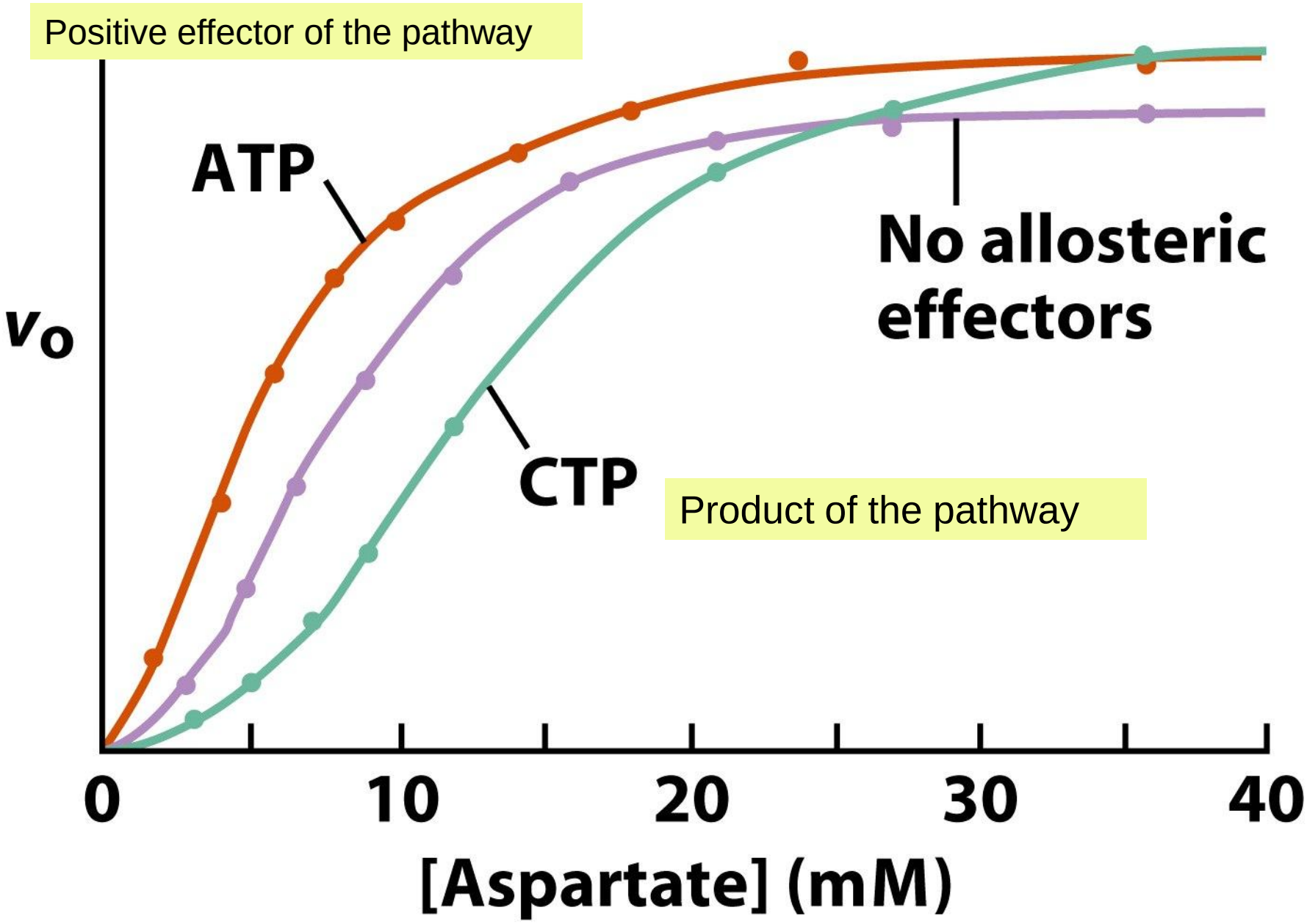


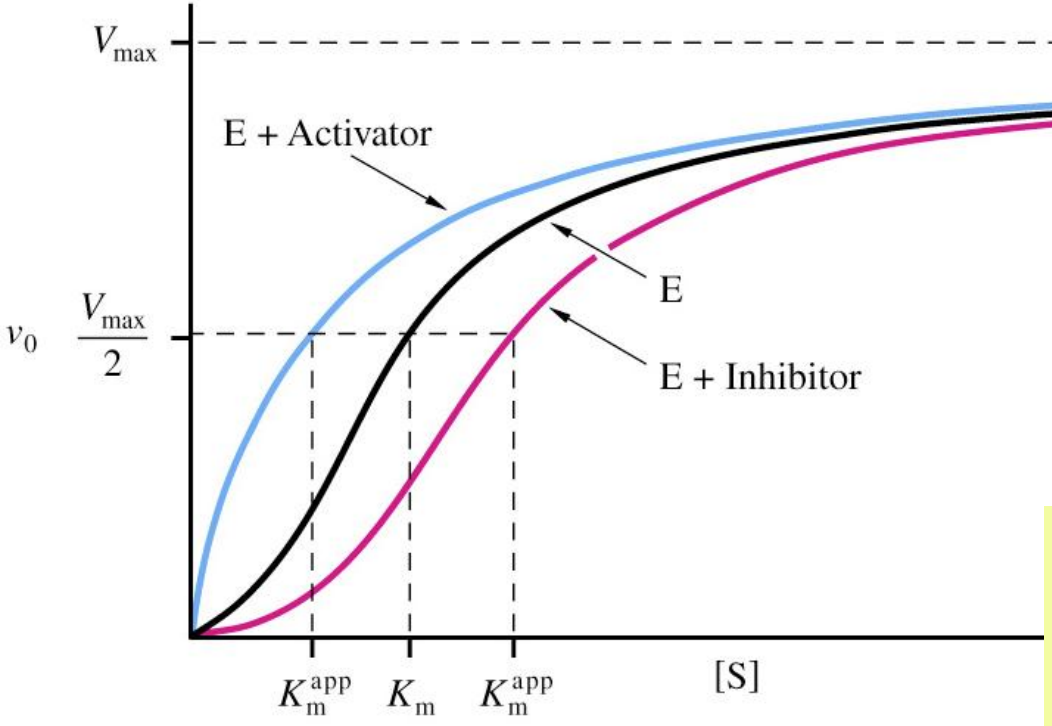
Figure 12-11 Fundamentals of Biochemistry, 2/e


Positive effector of the pathway
ATP
No allosteric effectors
v_o
CTP
Product of the pathway
0
10
20
30
40
[Aspartate] (mM)

2) Role of cooperativity of binding in regulation

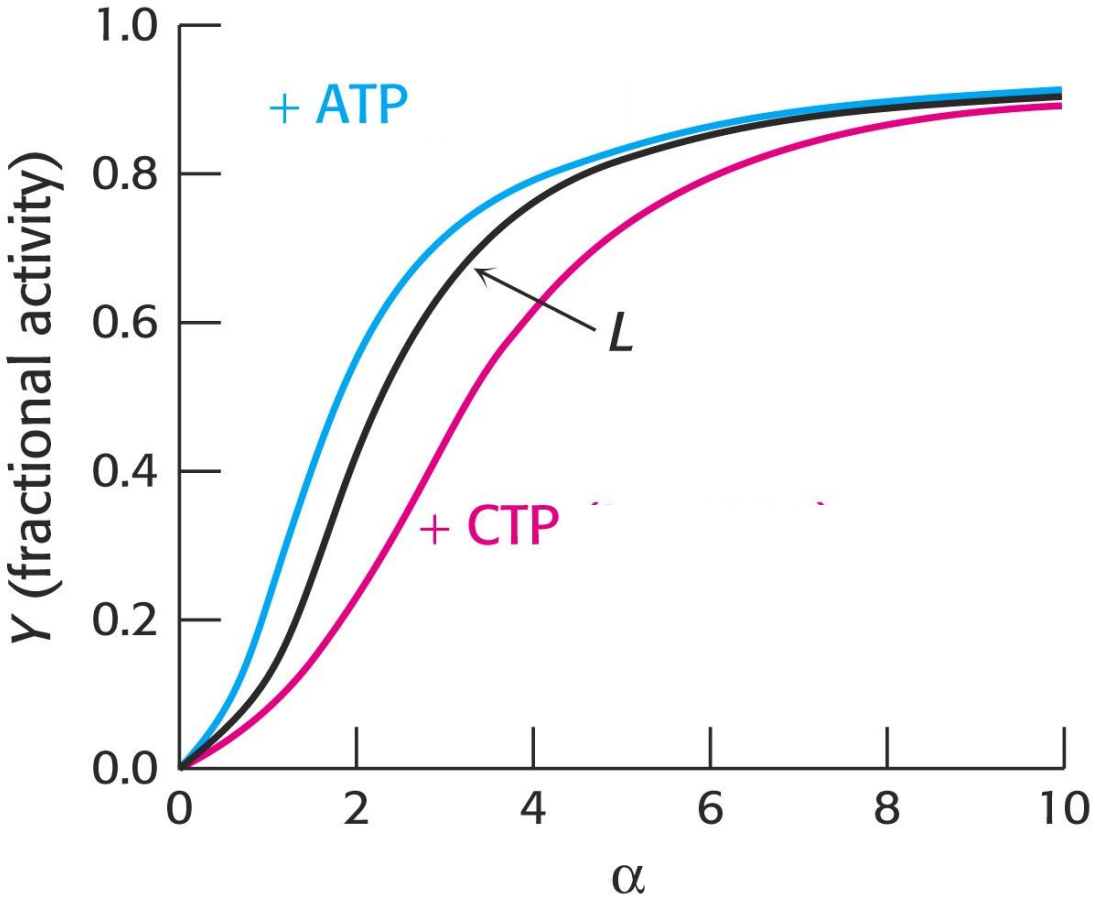
positive cooperativity of multiple substrate binding sites.

- Addition of modulators alters enzyme activity
- Activators can lower K_m, inhibitors can raise K_m



Which does the sigmoidal v_o versus [S] curve mean?

Binding a substrate molecule to one of the catalytic subunits of an allosteric enzyme increases the substrate-binding affinity of the other catalytic subunits



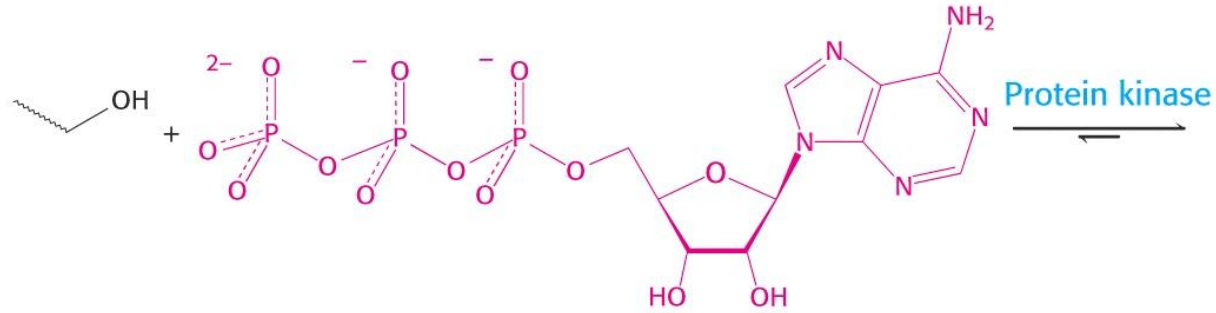
Q: Do allosteric enzymes follow traditional Michaelis-Menten kinetics?

No, They displays different kinetics. A plot of rate vs. substrate concentration is a sigmoidal curve, as opposed to the simple curve obtained by enzymes displaying Michaelis-Menten kinetics.

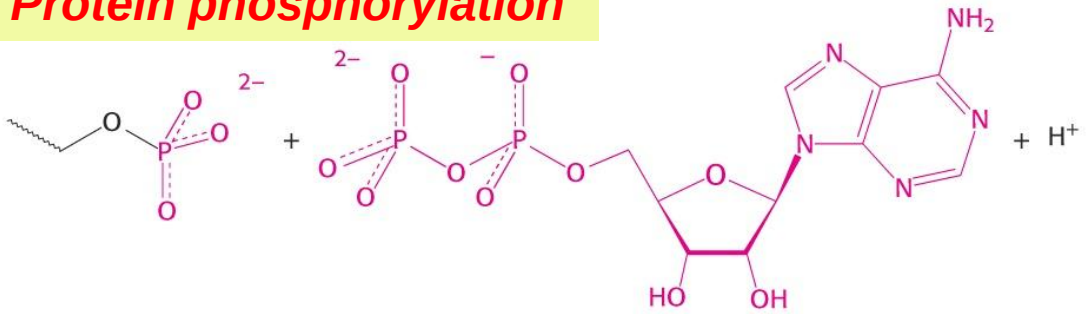
2. Enzyme Regulation by Covalent Modification

TABLE 10.1 **Common covalent modifications of protein activity**

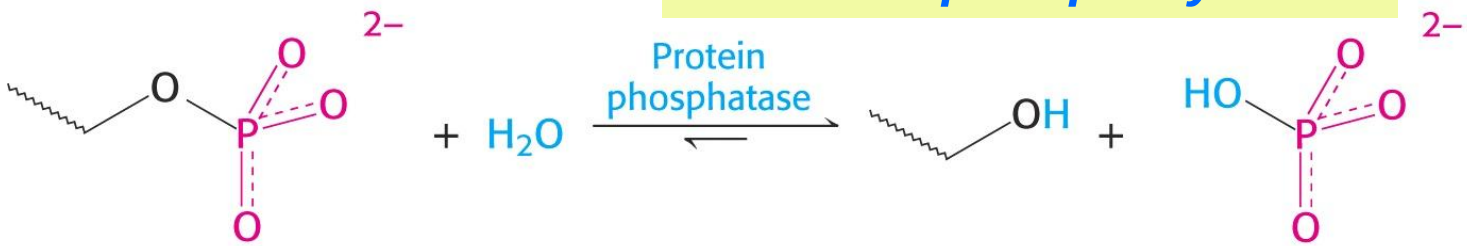
Modification	Donor molecule	Example of modified protein	Protein function
Phosphorylation	ATP	Glycogen phosphorylase	Glucose homeostasis; energy transduction
Acetylation	Acetyl CoA	Histones	DNA packing; transcription
Myristoylation	Myristoyl CoA	Src	Signal transduction
ADP-ribosylation	NAD	RNA polymerase	Transcription
Farnesylation	Farnesyl pyrophosphate	Ras	Signal transduction
γ-Carboxylation	HCO_3^-	Thrombin	Blood clotting
Sulfation	3′-Phosphoadenosine-5′-phosphosulfate	Fibrinogen	Blood-clot formation
Ubiquitination	Ubiquitin	Cyclin	Control of cell cycle



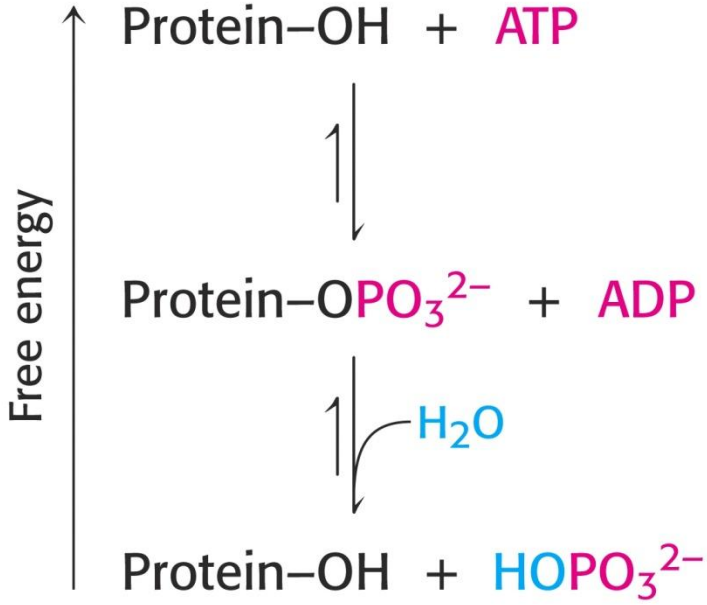
Protein phosphorylation



Protein dephosphorylation



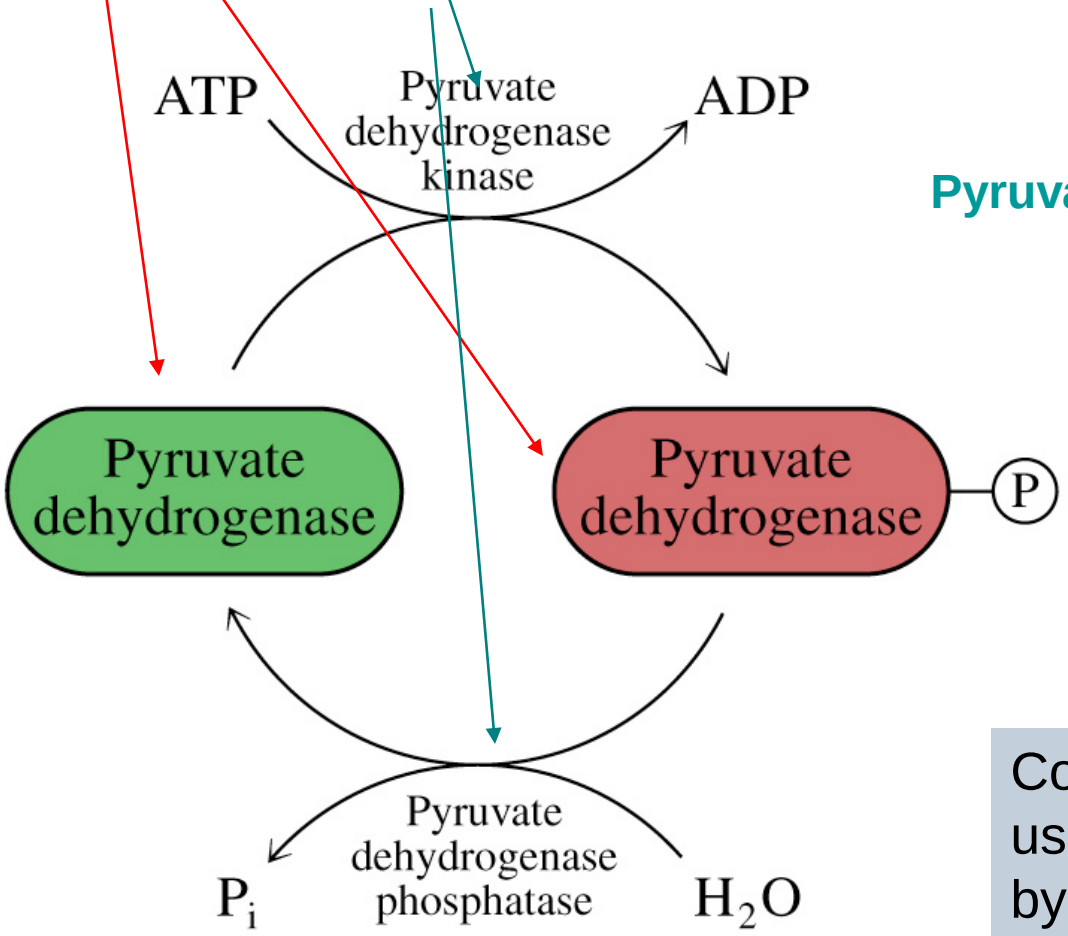
Explain the reasons why phosphorylation is an extremely effective tool for catalytic control.



- A phosphoryl group adds negative charges, allowing new electrostatic interactions, and new hydrogen bond formation.
- The free energy change of phosphorylation is large, which can affect the conformational equilibrium of different states.
- Using ATP means that the reaction is linked to the energy status of the cell.
- Phosphorylation is rapid and reversible, and can result in amplified effects.
- These factors affect structural, thermodynamic, regulatory, and kinetic properties

2. Enzyme Regulation by Covalent Modification

- **Interconvertible enzymes** are controlled by covalent modification
- **Converter enzymes** catalyze covalent modification



Pyruvate dehydrogenase regulation

- Phosphorylation stabilizes the inactive state (red)
- Dephosphorlyation stabilizes the active state (green)

Converter enzymes are usually controlled themselves by allosteric modulators

3. Activation of Zymogen (proenzyme): An active enzyme is formed by proteolytic cleavage of the inactive precursor of an enzyme (zymogen).

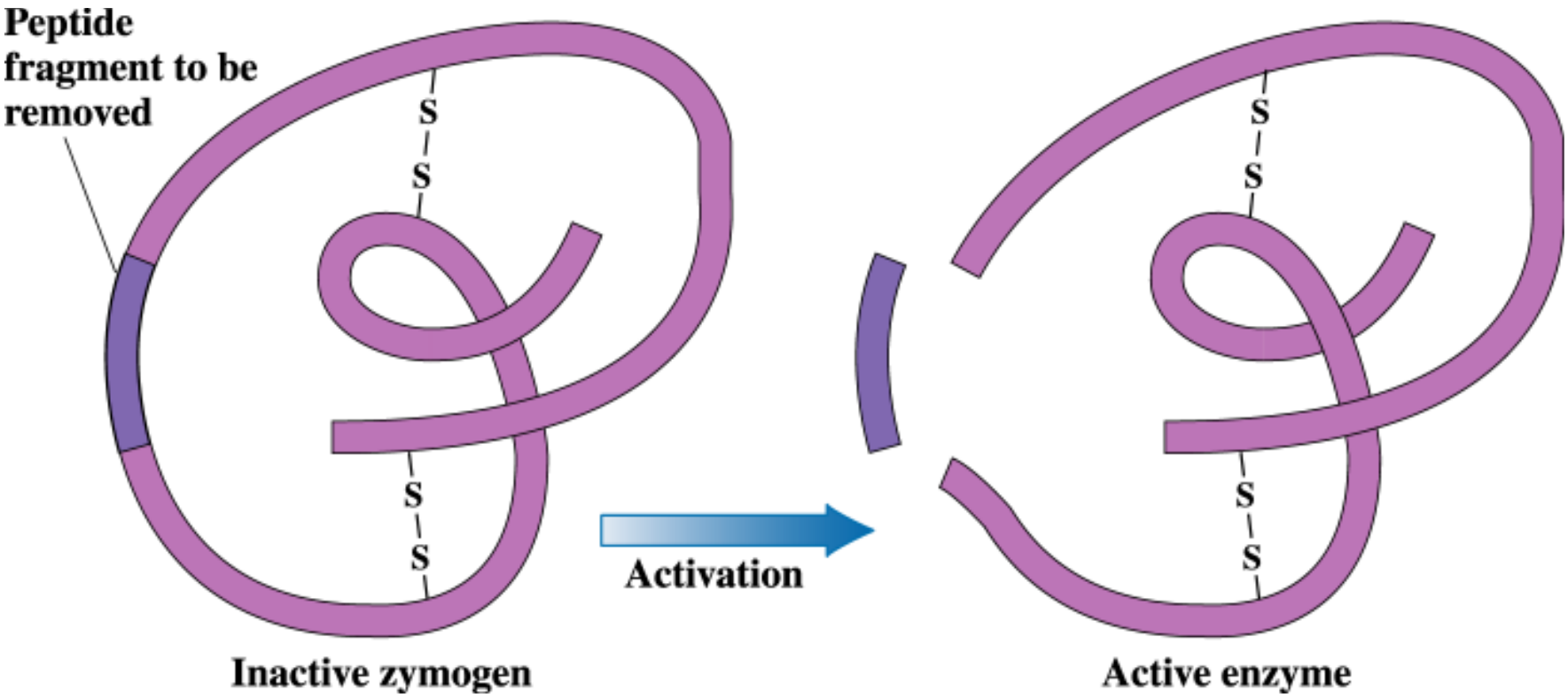
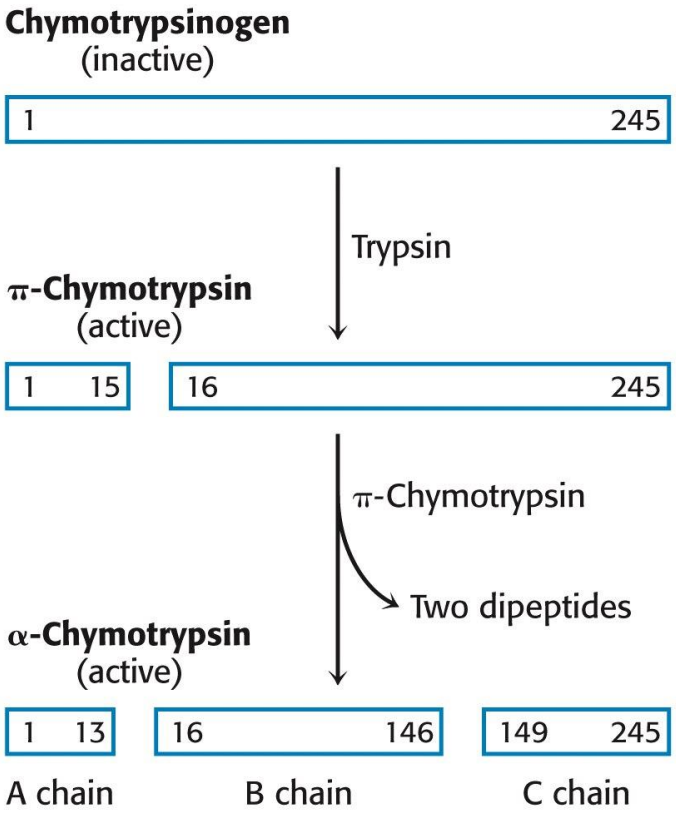


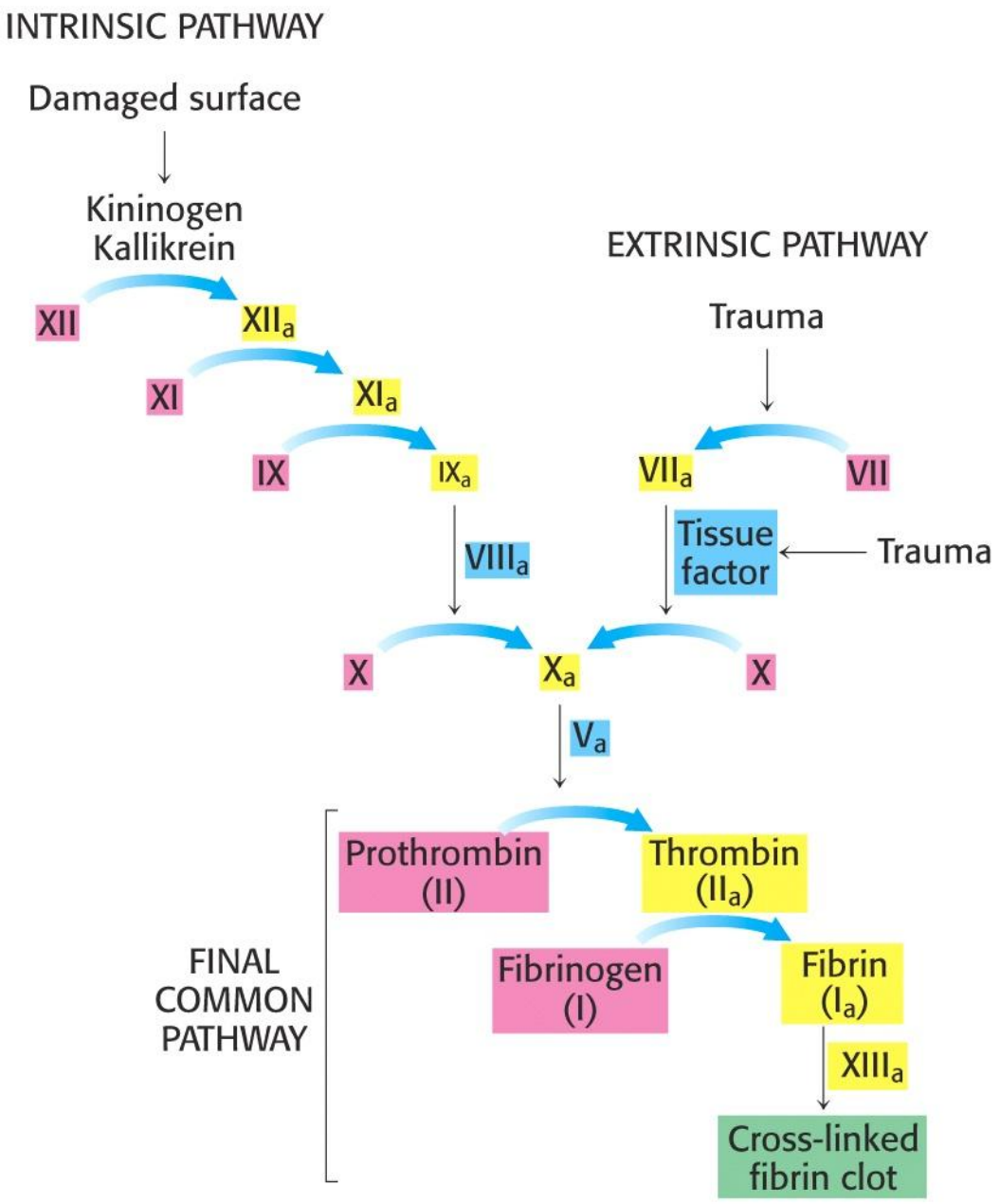
TABLE 10.3 **Gastric and pancreatic zymogens**

Site of synthesis	Zymogen	Active enzyme
Stomach	Pepsinogen	Pepsin
Pancreas	Chymotrypsinogen	Chymotrypsin
Pancreas	Trypsinogen	Trypsin
Pancreas	Procarboxypeptidase	Carboxypeptidase
Pancreas	Proelastase	Elastase



The Blood Coagulation Cascade

These enzymes are sequentially activated by proteolysis of their zymogens



Why do Onions make us cry?

It is not the strong odor of the onion that makes us cry, but the gas that the onion releases when we sever this member of the lily family.

Cutting an onion arouses a gas contained within the onion, **propanethiol** S-oxide, which then couples with the **enzymes** in the onion to emit a passive sulfur compound. When this upwardly mobile gas encounters the water produced by the tear ducts in our eyelids, it produces sulfuric acid.

http://www.sciencedaily.com/releases/2008/02/080202115345.htm

What types of the enzymes catalyzing the following reactions:

1. Formation of disulfide bonds

Oxidoreductases (dehydrogenases)

2. Proteolytic cleavage of proteins

Hydrolases

3. Phosphorylation of a protein

Transferases

IV. Mechanism of enzyme action

1. An enzyme accelerates the rate of a reaction by what?

   decreasing the reaction **activation energy.**

2. How does an enzyme lower the reaction activation energy?

   through the formation of the **transition state intermediate, ES***

3. True or false:

Enzymes allow equilibrium to be reached more rapidly by speeding up the forward reaction rates.

False, enzymes do not change the reaction equilibrium.

An enzyme increase the reaction rate for both forward and reverse reactions.

V. Enzyme-Substrate Interaction

3. Substrate Specificity

What substrate specificity do we call or expect for the following enzymes?

a. Hexokinase catalyzes the addition of a phosphate moiety to all hexoses

b. Methionyl tRNA synthetase that links methionine to the tRNA involved in the addition of the first amino acid in protein synthesis?

c. Digestive enzymes

a. Protein synthesis enzymes that only can incorporate L-amino acid

a. group specificity

b. absolute specificity

c. linkage specificity

d. stereochemical specificity

Understand the meaning and use of K_m

2. With [S] = K_M for a simple enzymatic reaction, when [S] is doubled, the rate becomes _____Vmax.
   a. 1
   b. 0.67
   c. 0.5
   d. 0.75

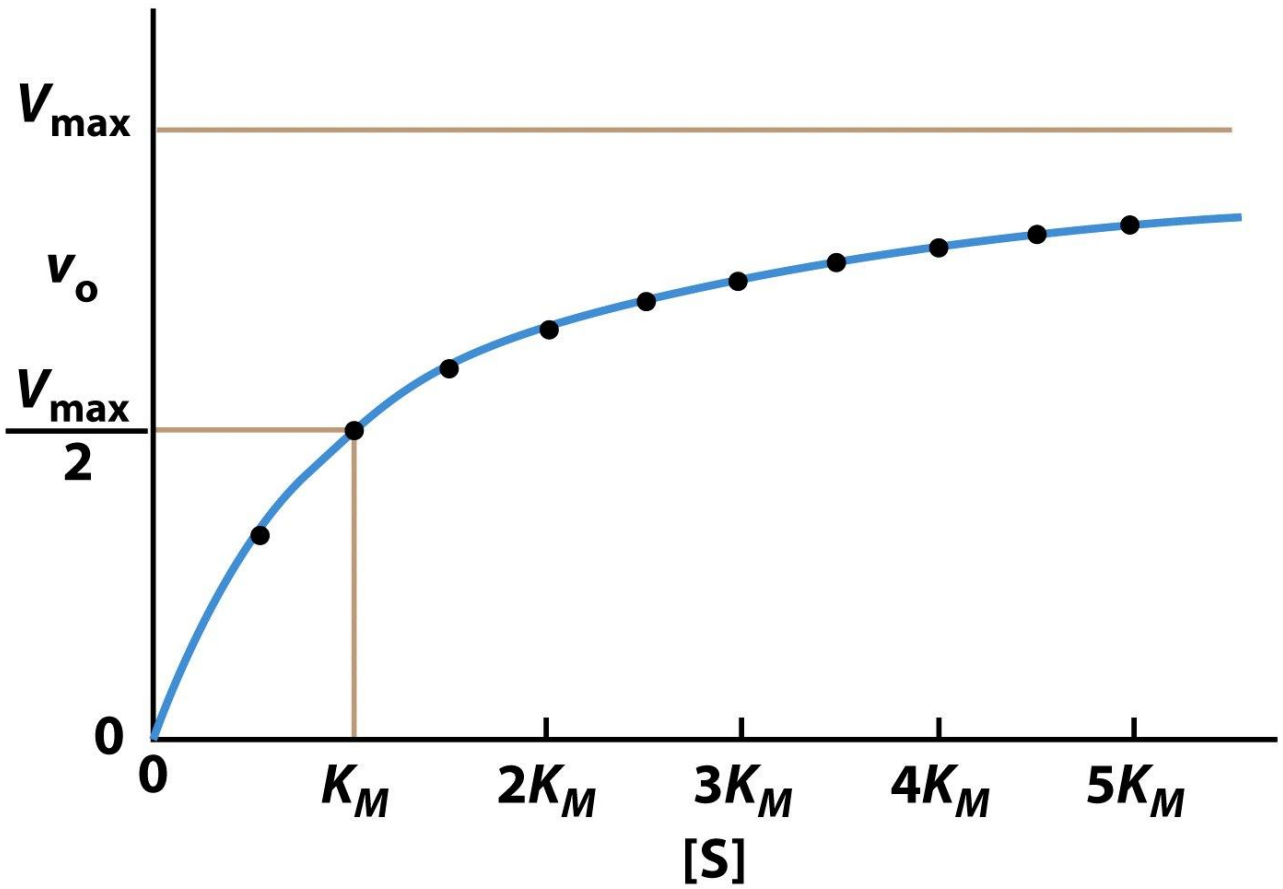


Figure 12-3 Fundamentals of Biochemistry, 2/e

RECAP

The Km for the reaction of an enzyme with N-acetylvaline ethyl ester is 8.8 x 10^{-2} M, and the Km of the reaction of the saem enzyme with N-acetyltyrosine ethyl ester is 6 x 10^{-4} M.

a)Which substrate has the higher affinity for the enzyme?
b)Which substrate is likely to give a higher value for Vmax?

In order for an enzymatic reaction obeying the Michaelis–Menten equation to reach 0.75 of its maximum velocity, [S] would need to be ?

A. not enough information is given to make this calculation
B. [S] would need to be 50% greater than K_M
C. [S] would need to be $3K_M$
D. [S] would need to be $3/4K_M$

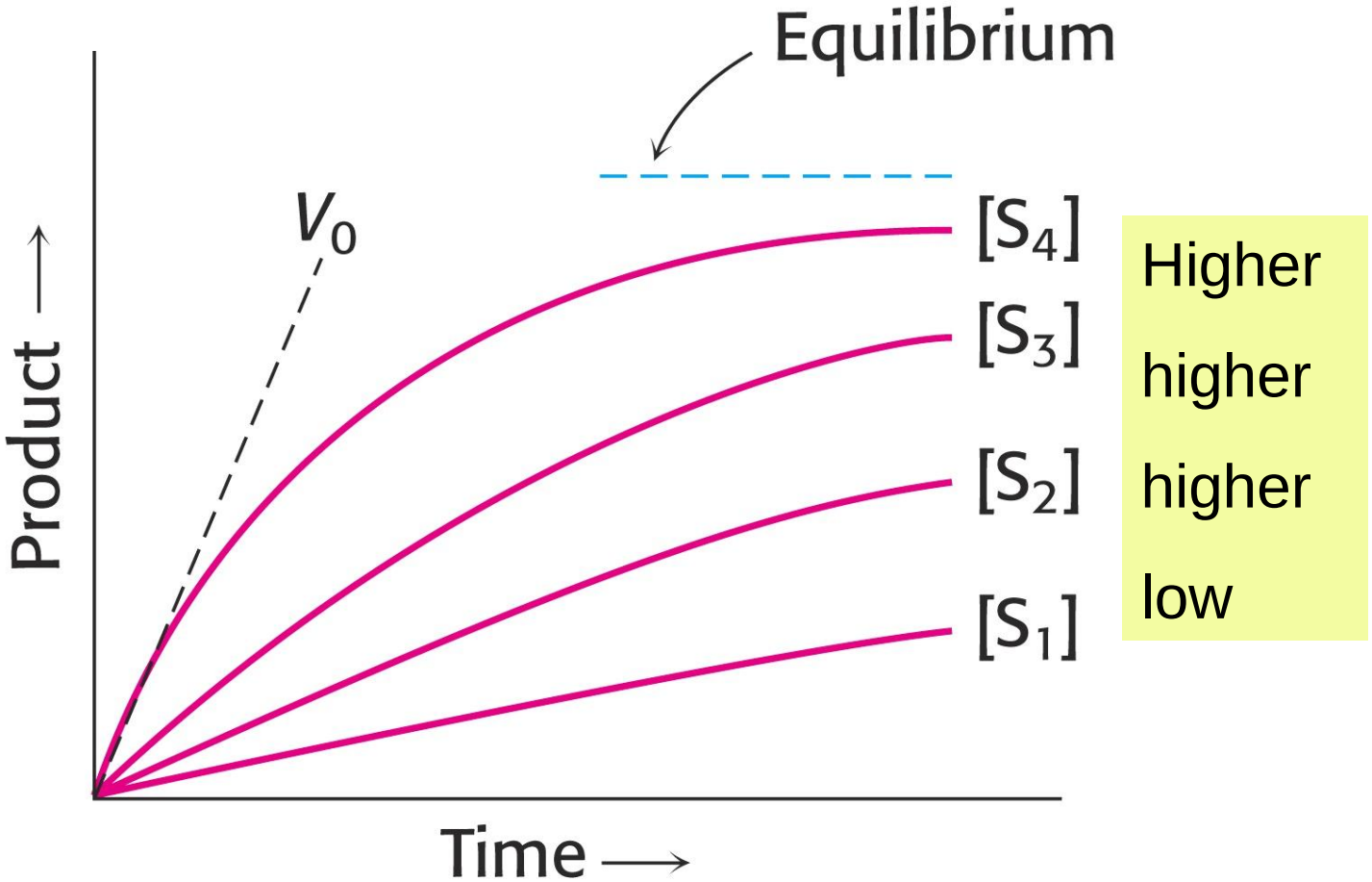
When an enzyme is saturated with substrates, the rate of catalysis is equal to

a. K_d b. K_{cat} c. V_0 d. k_{cat}/K_m E. K_m

Effect enzyme activity in response to Substrate concentration

Initially, the rate of an enzyme reaction is responsive to increase in substrate concentration. But, at a certain concentration, the rate levels out and remains constant.

This highest enzyme rate is called V_{max}

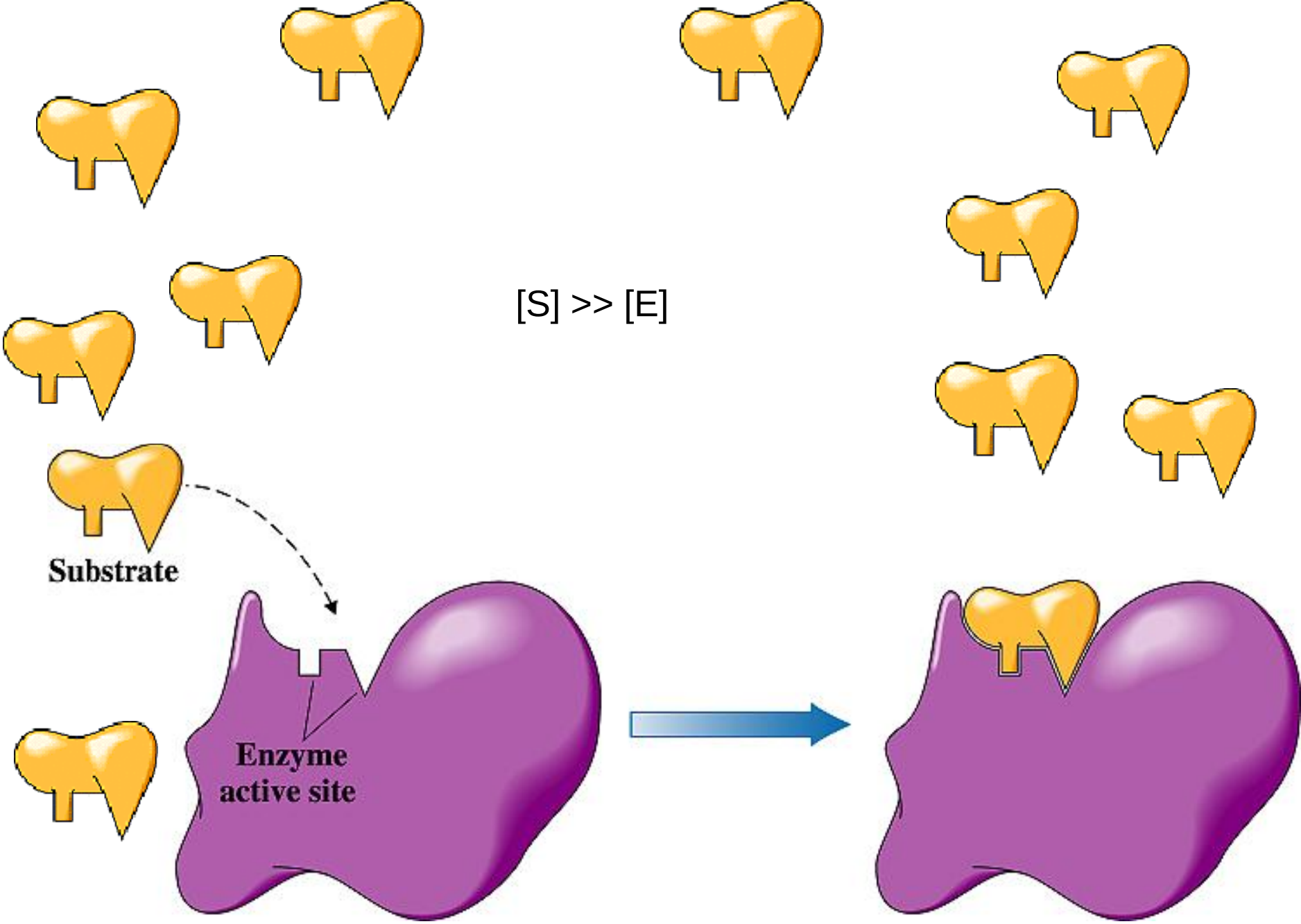


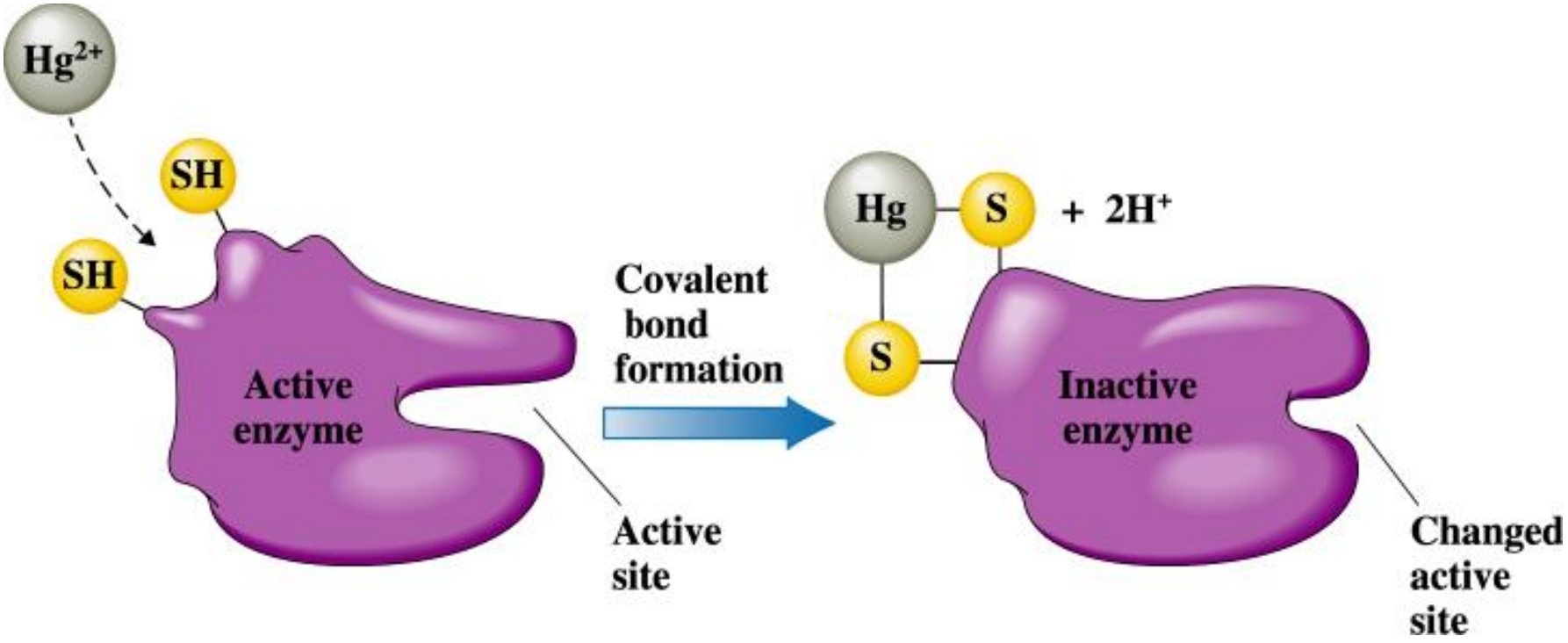
- When [S] >> [E], every enzyme binds a molecule of substrate (enzyme is **saturated** with substrate)
- Under these conditions the rate depends only upon [E].

So the reaction rate is limited by the level of the enzyme (i.e. by the number of active sites).

- **Enzyme-substrate complex** (**ES**) - complex formed when specific substrates fit into the enzyme active site

[S] >> [E]





Thiols react with heavy metals ions (lead Pb^{2+}, mercury Hg^{2+}) to form insoluble salt.

BAL as an antidote $CH_2(OH)\text{-}CH(SH)\text{-}CH_2(SH) + Hg^{2+} \longrightarrow$ $CH_2(OH)\text{-}CH(S)\text{-}CH_2(S)\text{-}Hg$

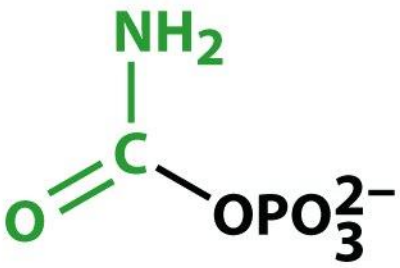
Why does an organism need to control enzyme activities?

1. coordinate numerous metabolic processes
2. respond to changes in its environments
3. Grow and differentiate in an orderly manner

Two types of regulation:

1. Long-term control: control of enzyme availability.
   Effects on the amount of enzymes, determined by enzyme synthesis & degradation, gene expression

2. Short-term control: control of enzyme activity
   Effects on enzyme's catalytic activity by activation or inhibition via structural alterations that influence enzyme substrate binding/affinity and/or turnover.



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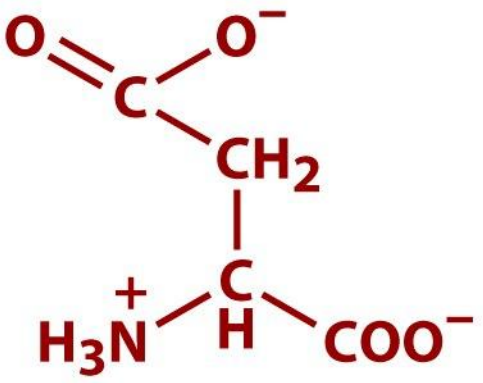
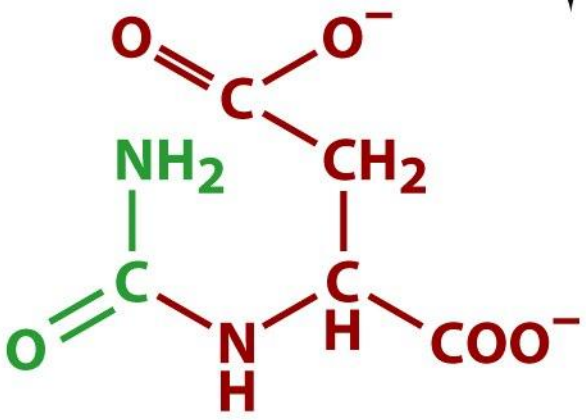


Carbamoyl phosphate **Aspartate**

aspartate
transcarbamoylase



+ $H_2PO_4^-$

***N*-Carbamoylaspartate**