

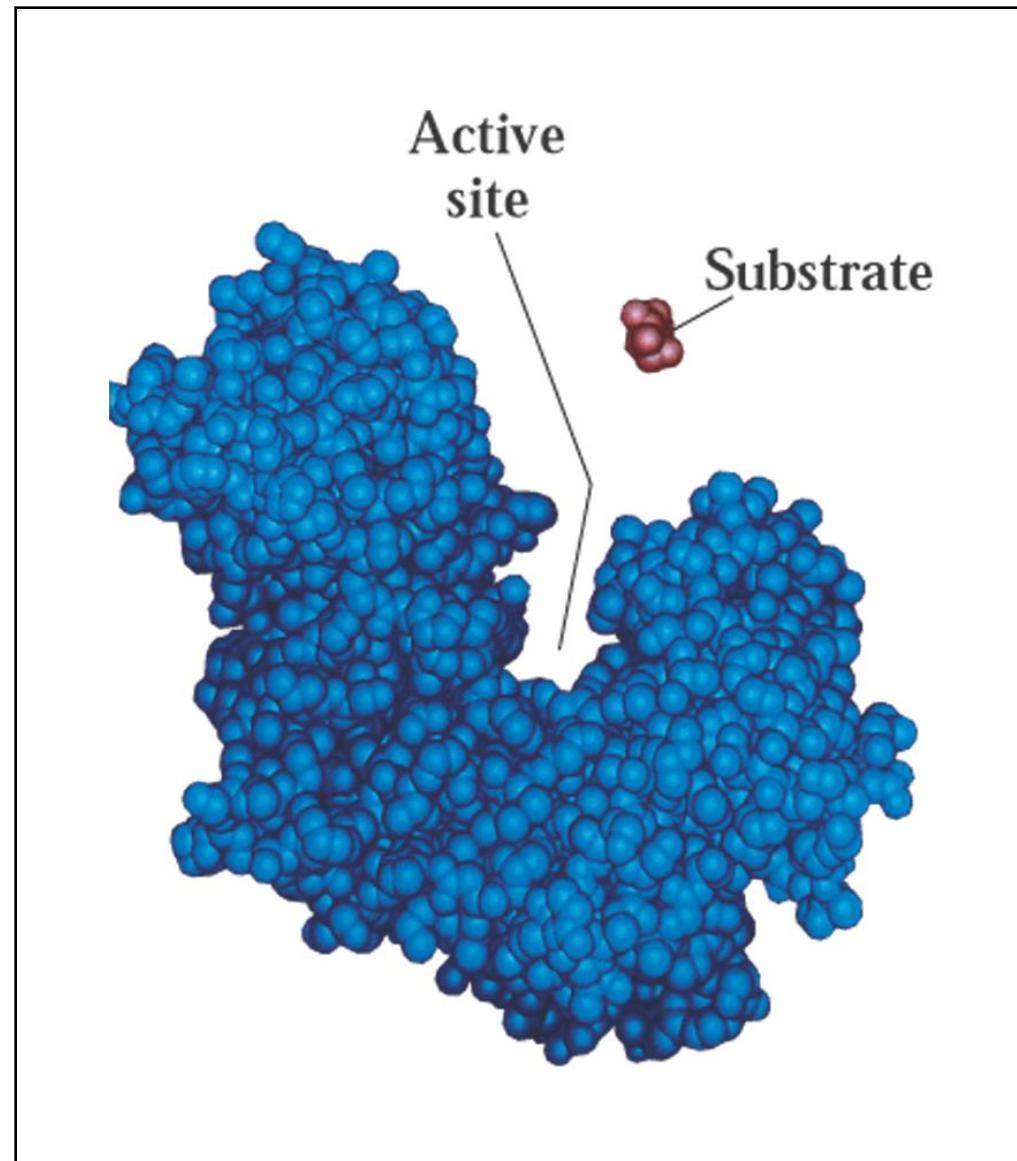
LECTURE 7:

ENZYME MECHANISMS AND CATALYSIS

- Enzyme substrate interactions
- Factors affecting enzyme activity
- Enzyme catalytic mechanisms
- Regulation of enzyme activity

Enzyme-substrate interactions

Active site of an enzyme region formed as a result of the protein's secondary and tertiary structural characteristics



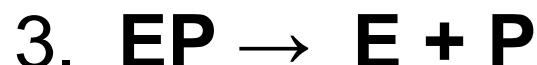
Sequence of events in enzyme catalyzed reaction



- Enzyme and Substrate collide
- Substrate bind to active site of enzyme
- A transition state forms where the structure of the substrate is altered



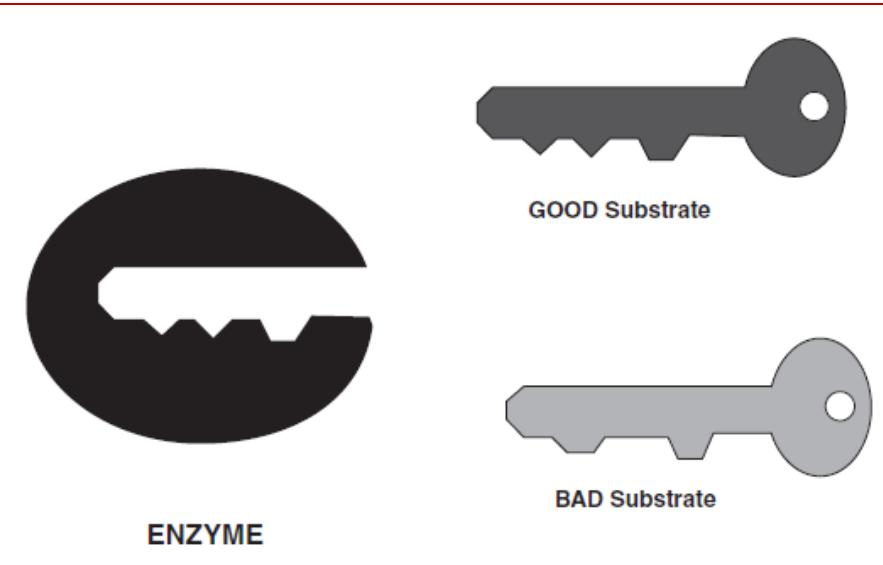
- Enzyme catalyzes the conversion of substrate to Product
- Both substrate and product remain in active site



- Product is released from active site

Enzyme-substrate interactions

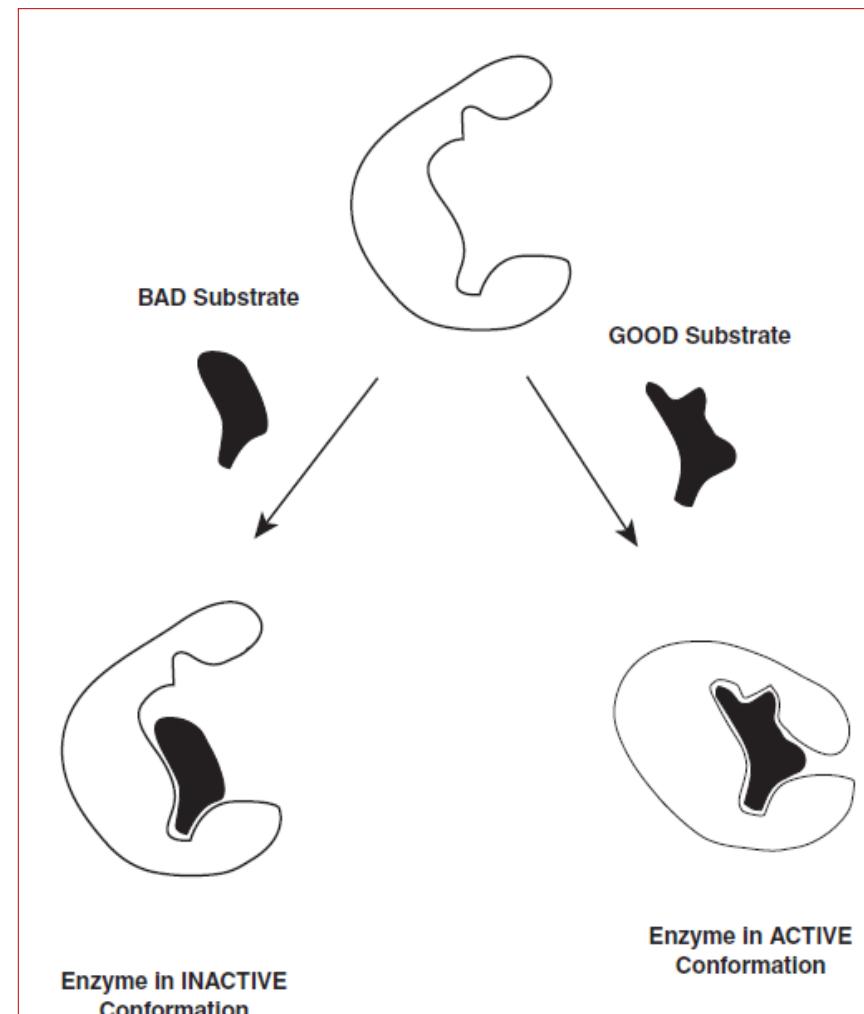
Lock and Key theory



-Active site is complementary in conformation to the substrate
(Emil Fischer, 1894)

Induced-fit theory

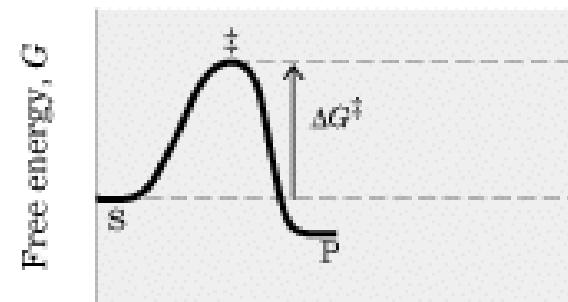
- Enzyme changes shape on binding substrate (Daniel Koshland, 1958)



Transition state stabilization

- Imaginary enzyme ("stickase") designed to catalyze "cleavage" (breaking) of a metal stick ("magnetic" interactions, red dashed lines, represent non-covalent interactions between enzyme and substrate and between enzyme and transition state)
- Metal stick must be bent, a "high energy state", before it can be broken, so "transition state" is **bent stick**.

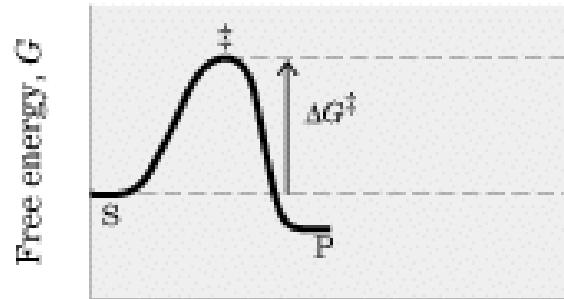
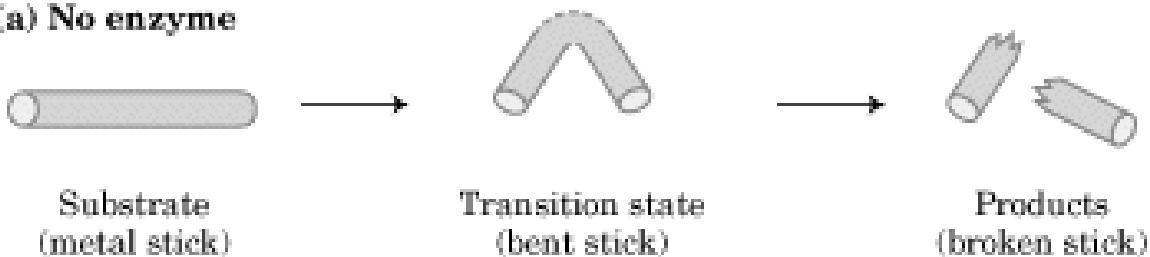
(Nelson & Cox, *Lehninger Principles of Biochemistry*, 3rd ed., 2000)



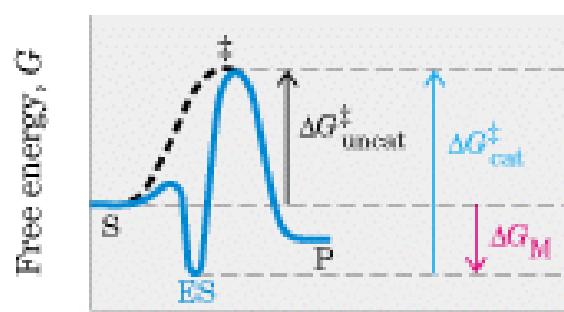
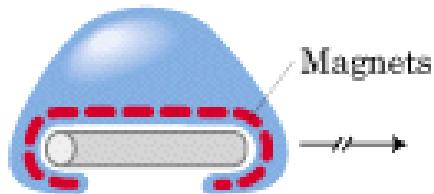
No Enzyme

The bent stick is energetically unfavorable, but must be formed for the stick to be broken.

(a) No enzyme



(b) Enzyme complementary to substrate

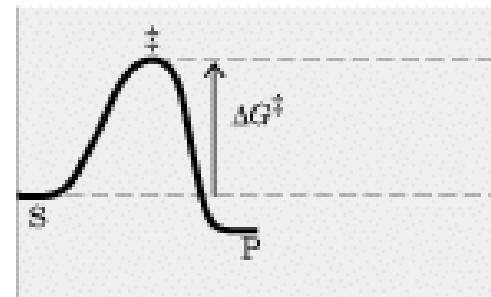


No catalysis is obtained by just binding substrate tightly!

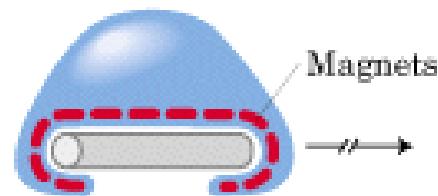
(a) No enzyme



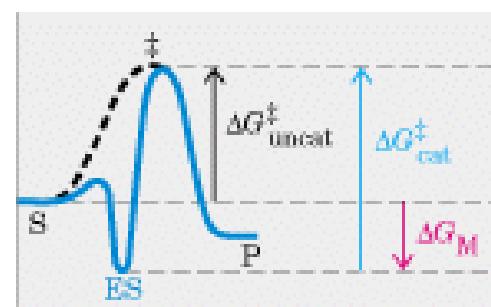
Free energy, G



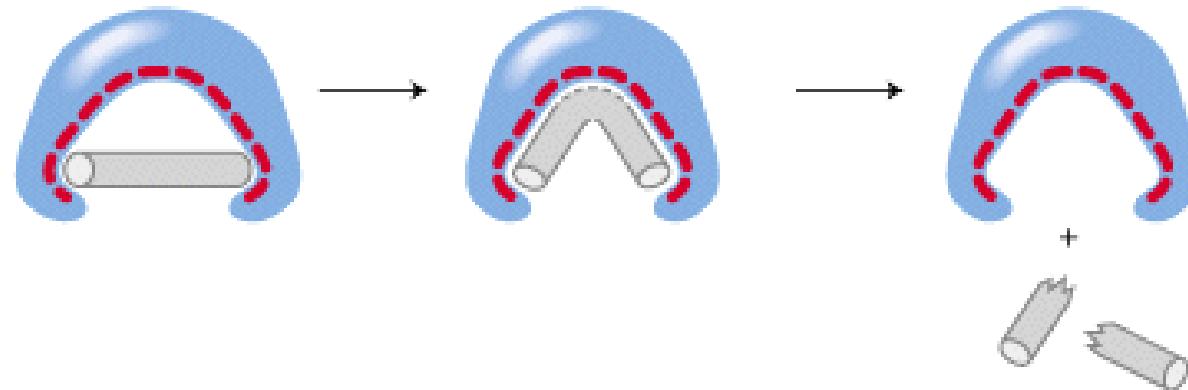
(b) Enzyme complementary to substrate



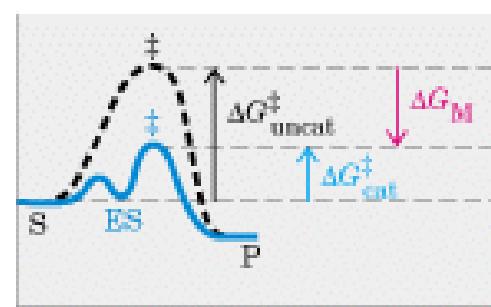
Free energy, G



(c) Enzyme complementary to transition state



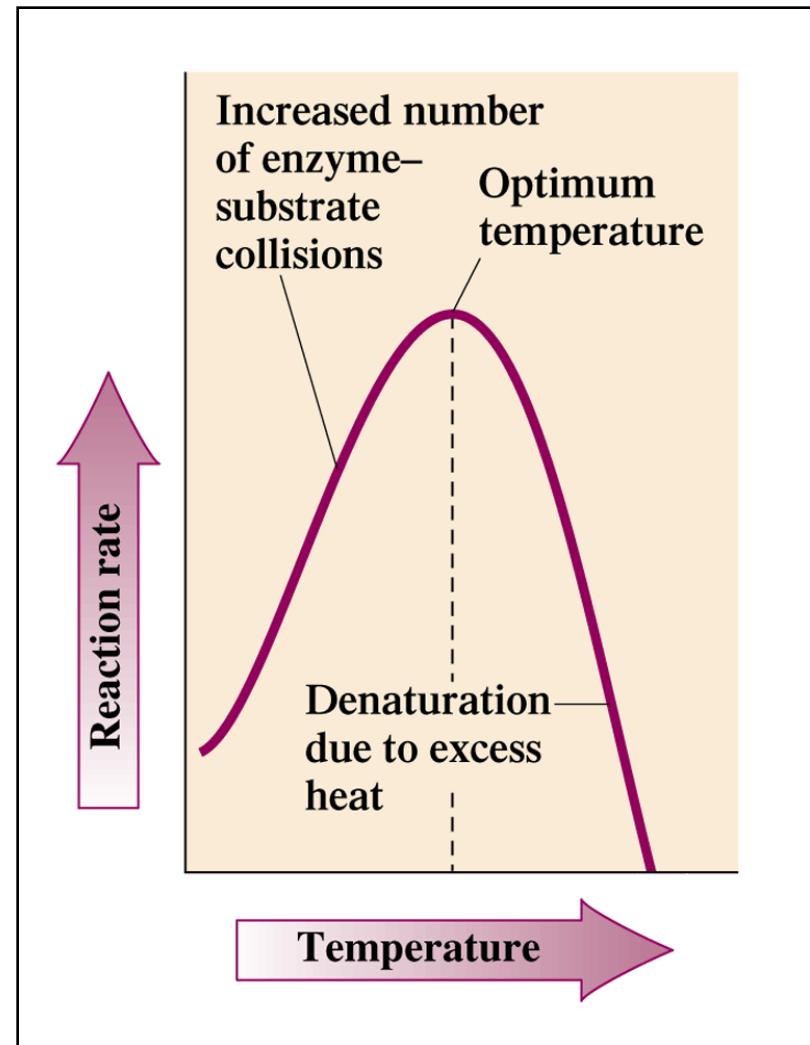
Free energy, G



Reaction coordinate

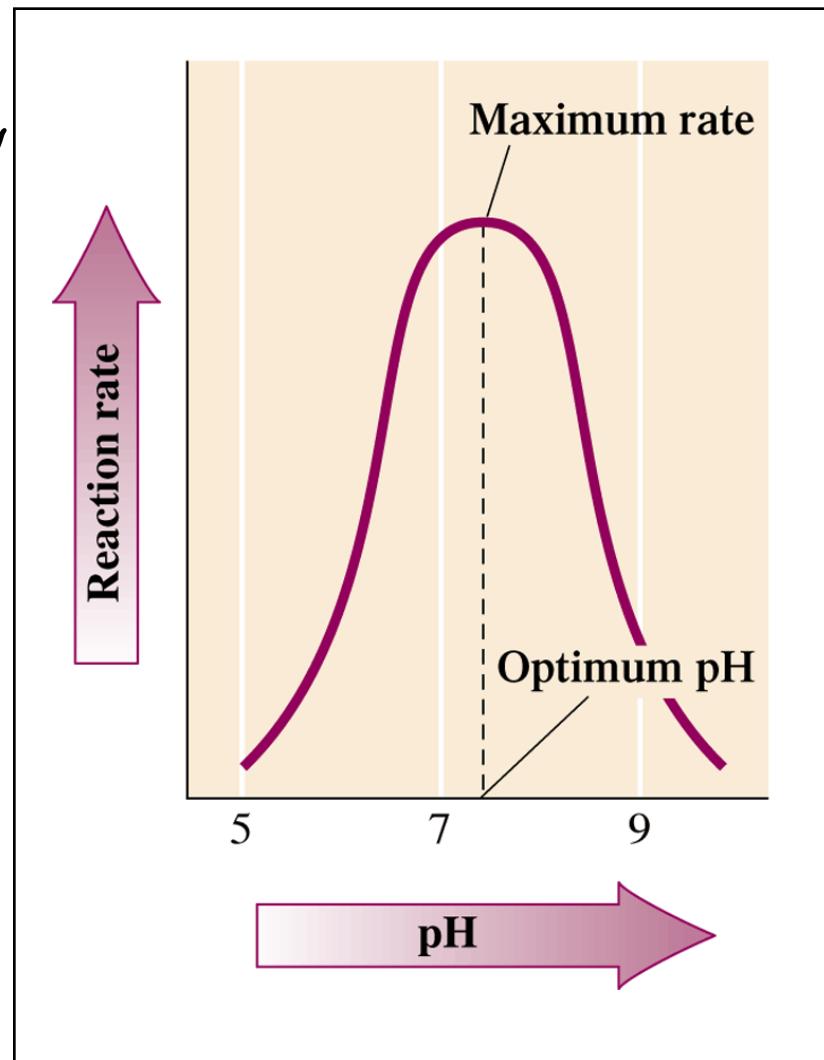
Factors Affecting Enzyme Activity: Temperature

- Little activity at low temperature
- Most active at optimum temperatures (usually 37°C in humans)



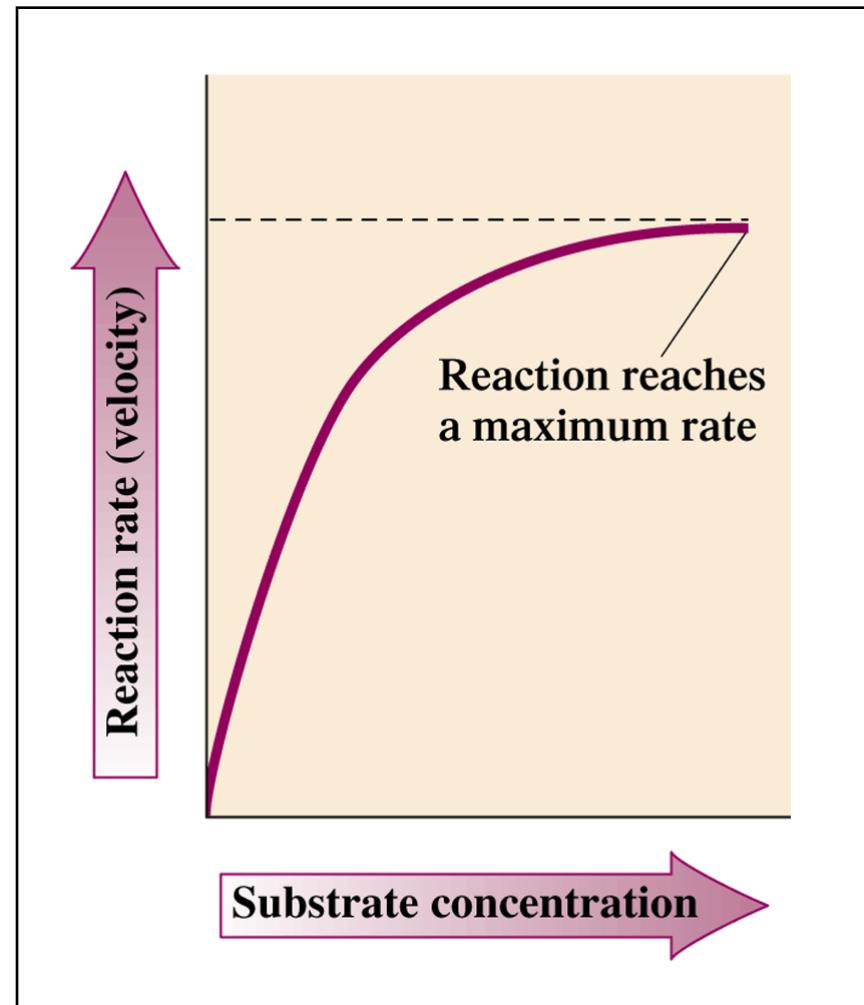
Factors Affecting Enzyme Activity: pH

- Maximum activity at *optimum pH*
- Narrow range of activity
- Most enzymes lose activity in low or high pH
- Why is one pH better than another?
 - R groups of amino acids have proper charge at certain pH values
 - Tertiary structure of enzyme is correct



Factors Affecting Enzyme Activity: Substrate Concentration

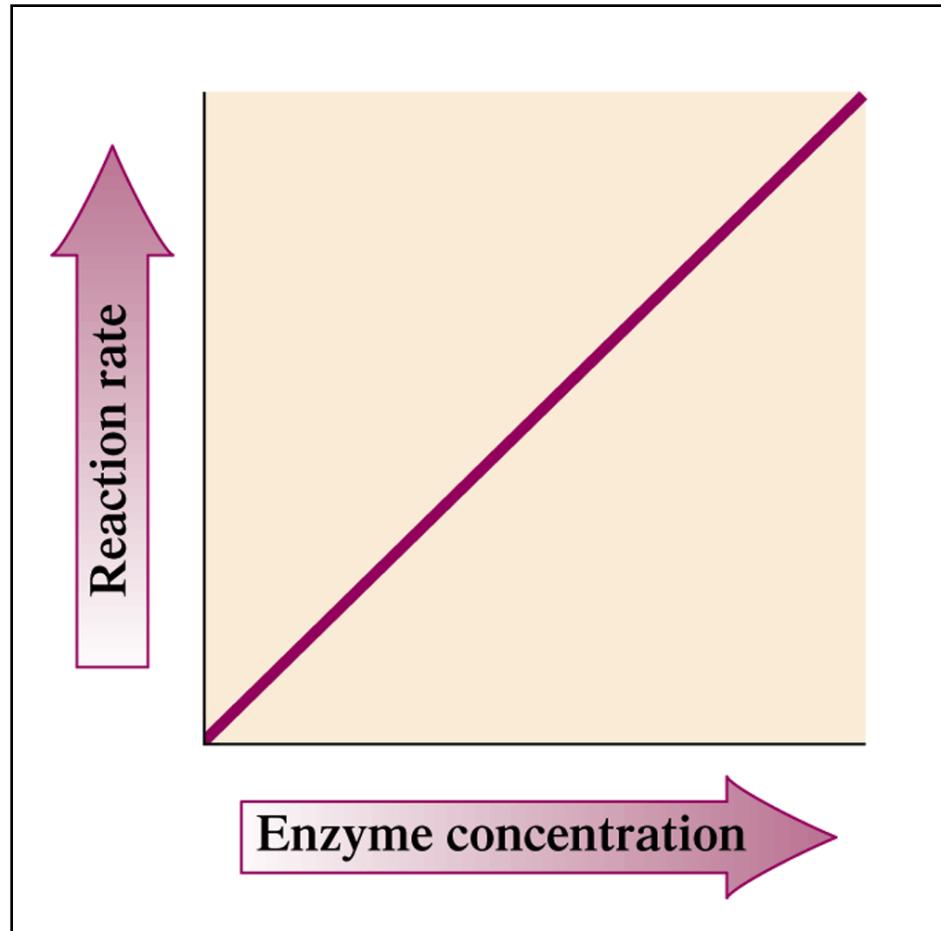
- Increasing substrate concentration increases the rate of reaction (enzyme concentration is constant)
- Maximum activity reached when all of enzyme combines with substrate



Factors Affecting Enzyme Activity:

Enzyme Concentration

- The rate of reaction increases as enzyme concentration increases (at constant substrate concentration)
- At higher enzyme concentrations, more substrate binds with enzyme

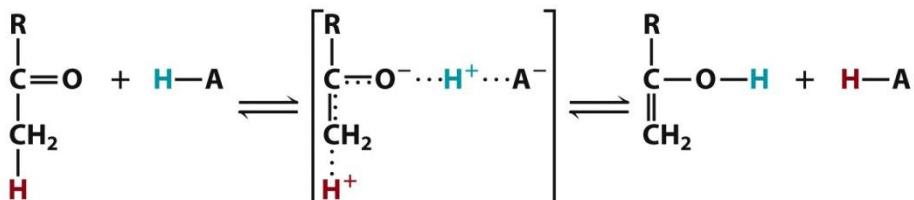


TYPES OF ENZYME CATALYTIC MECHANISMS

- Acid-base catalysis
- Covalent catalysis
- Metal ion catalysis
- Proximity and orientation effects
- Preferential binding of the transition state

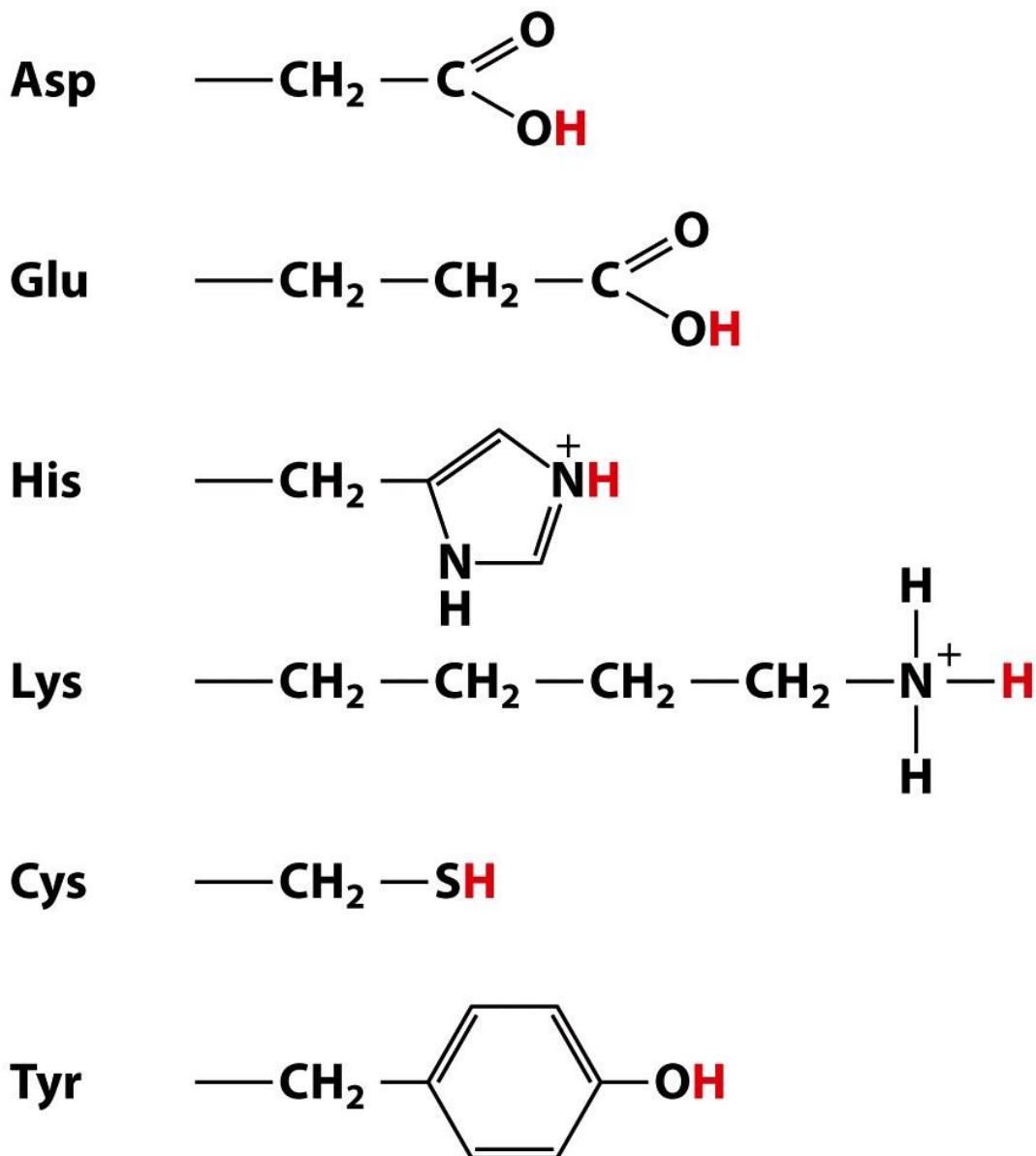
A. Acid - Base catalysis

- Involves proton transfer
- Protons can be transferred from:
 - Water (specific acid-base catalysis)
 - Side chains of amino acid functional groups (general acid-base catalysis)



Acid - Base catalysis

- Amino acid side chains in acid-base catalysis
- Groups precisely positioned in **active site**
- Function as proton donors or acceptors
- Microenvironment can affect side chain pKa values



Many different amino acid side chains can act as either an acid or a base

Amino acid residues	General acid form (proton donor)	General base form (proton acceptor)
Glu, Asp	R—COOH	R—COO ⁻
Lys, Arg	$\begin{matrix} \text{H} \\ \\ \text{R}—\text{N}^+ \text{H} \\ \\ \text{H} \end{matrix}$	R—NH ₂
Cys	R—SH	R—S ⁻
His	$\begin{matrix} \text{R}—\text{C}=\text{CH} \\ \\ \text{HN} \\ \\ \text{C}=\text{N}^+ \text{H} \\ \\ \text{H} \end{matrix}$	$\begin{matrix} \text{R}—\text{C}=\text{CH} \\ \\ \text{HN} \\ \\ \text{C}=\text{N}: \\ \\ \text{H} \end{matrix}$
Ser	R—OH	R—O ⁻
Tyr	$\text{R}-\text{C}_6\text{H}_4-\text{OH}$	$\text{R}-\text{C}_6\text{H}_4-\text{O}^-$

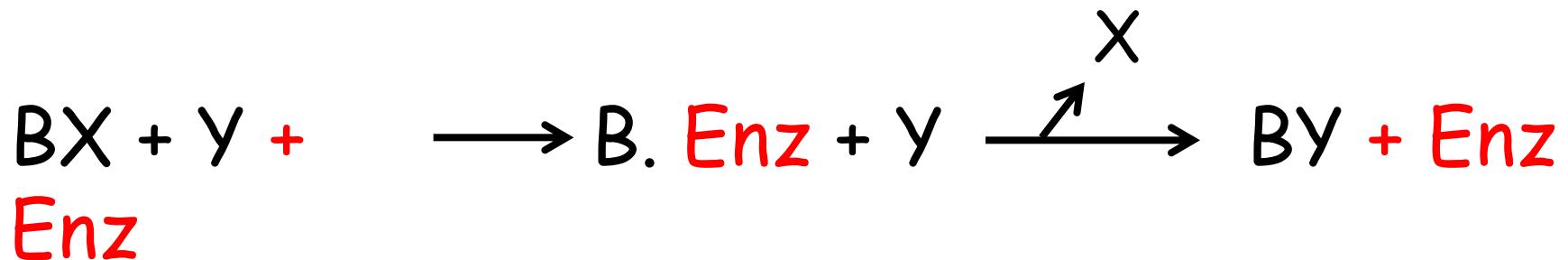
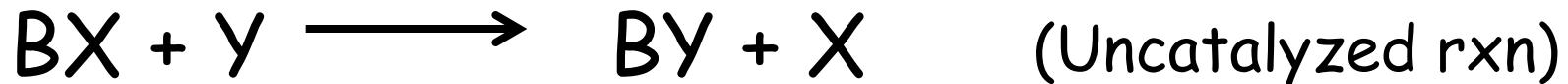
Figure 6-9

Lehninger Principles of Biochemistry, Fifth Edition

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B. Covalent catalysis

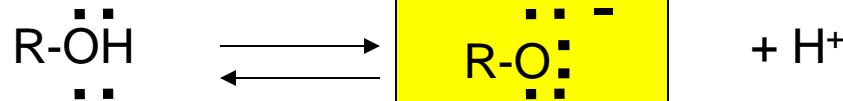
- Transient covalent bond formed between enzyme and substrate
- Is a two part reaction process
- Often called nucleophilic catalysis



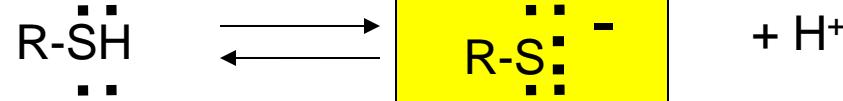
Covalent catalysis

Biologically important nucleophilic groups:

Hydroxyl group



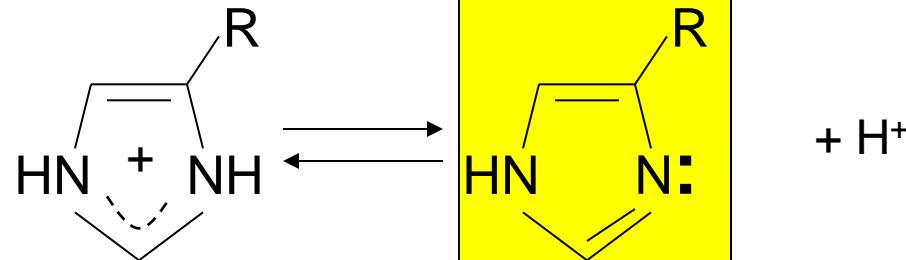
Sulphydryl group



Amino group



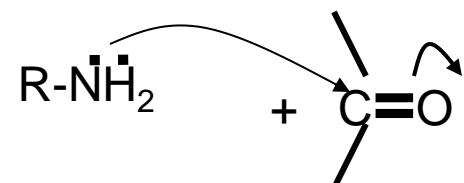
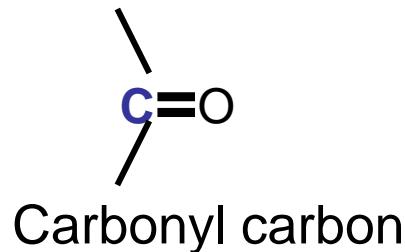
Imidazole group



Biologically important electrophiles:

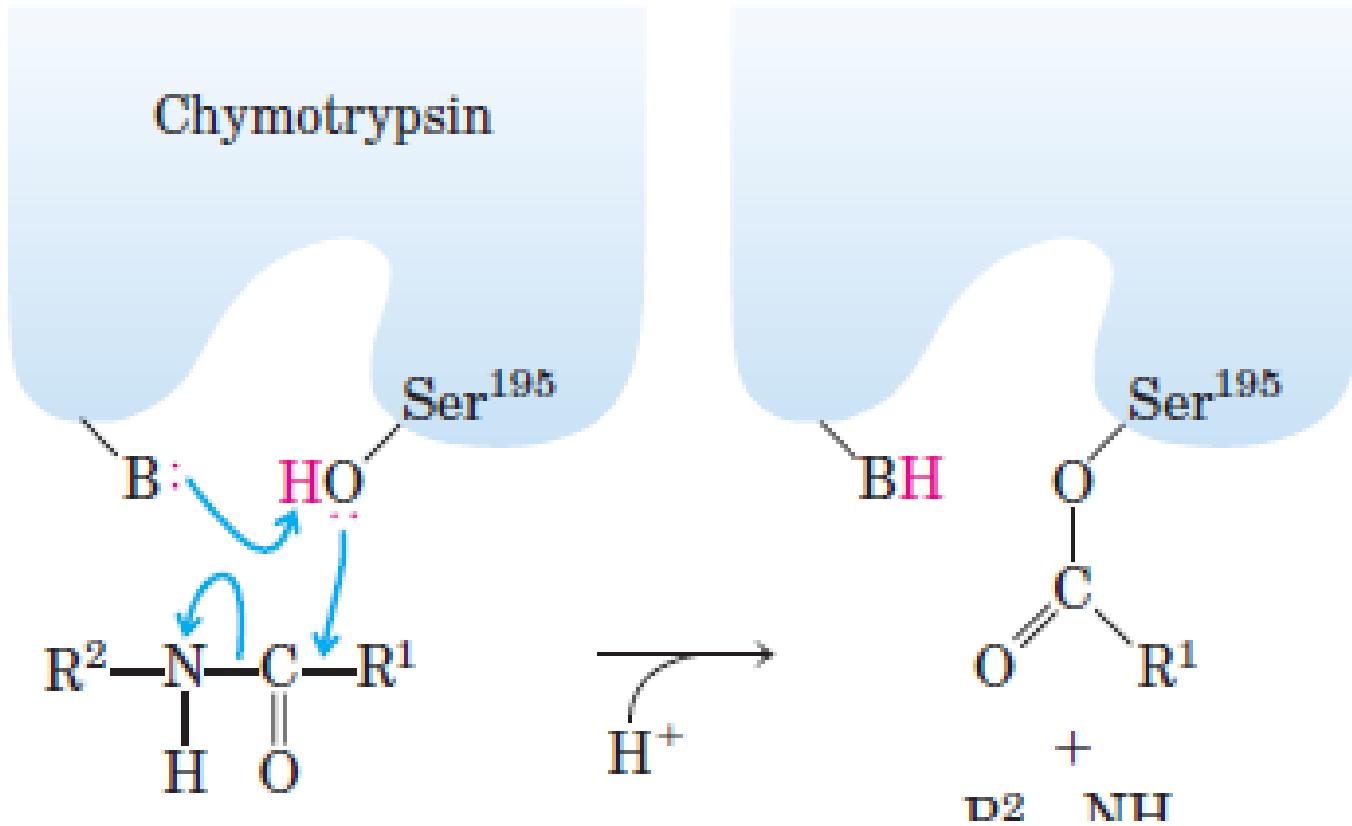
H^+
Protons

M^{n+}
Metal Ions



Adapted from Voet & Voet, *Biochemistry*

Most enzymes combine several catalytic strategies



Covalent and general acid-base catalysis. The first step in the reaction catalyzed by chymotrypsin is the acylation step. The hydroxyl group of Ser195 is the nucleophile in a reaction aided by general base catalysis

(adapted from Lehninger 4th ed page 202)

C. Metal ion catalysis

Metal ions are often used for one or more of the following:

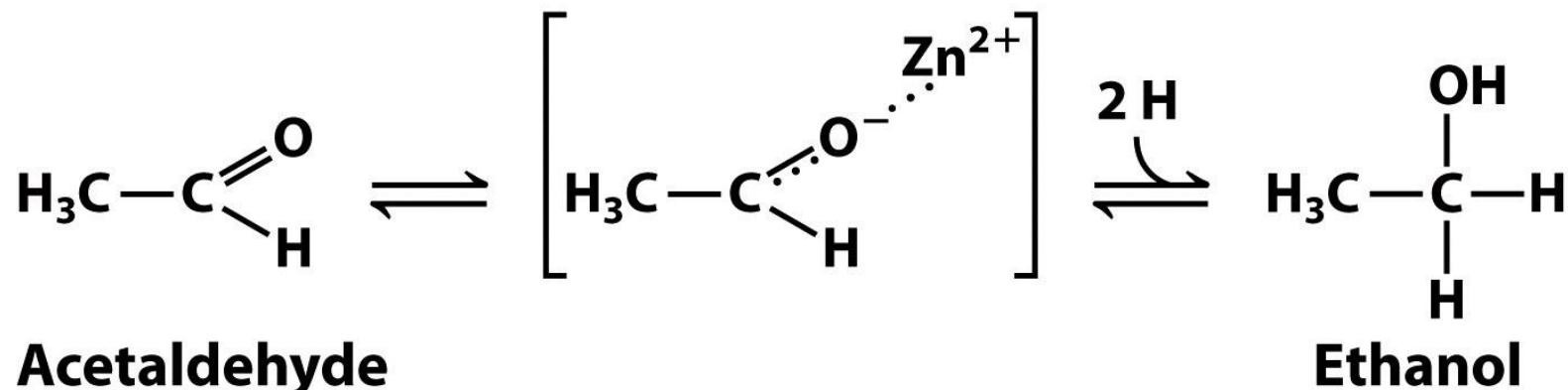
- * binding substrates in the proper orientation
- * mediating oxidation-reduction reactions
- * electrostatically stabilizing or shielding negative charges (electrostatic catalysis)

Metalloenzymes contain tightly bound metal ions:
(usually Fe^{+2} , Fe^{+3} , Cu^{+2} , Zn^{+2} , or Mn^{+2})

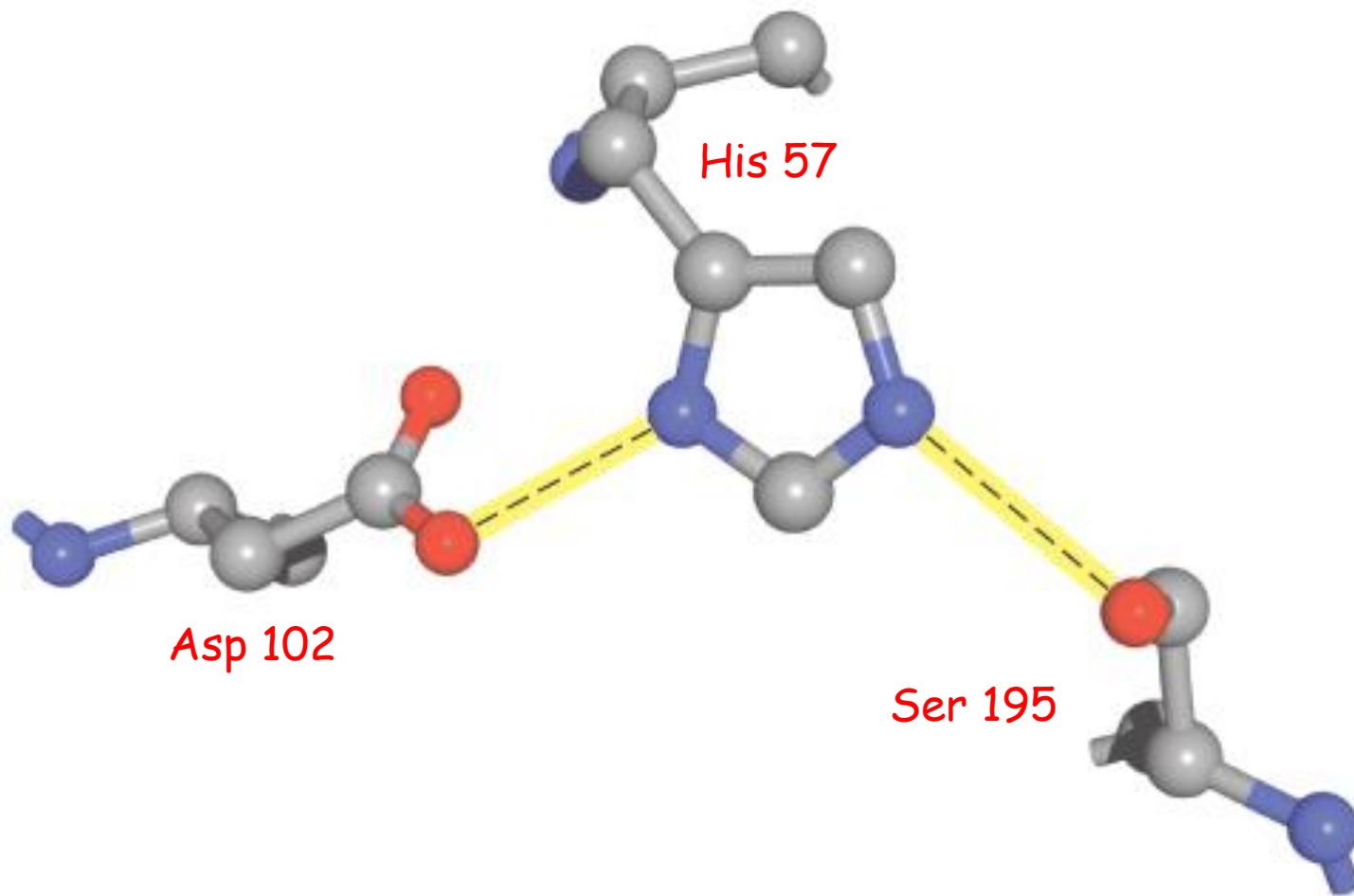
Metal-activated enzymes contain loosely bound metal ions: (usually Na^+ , K^+ , Mg^{+2} , or Ca^{+2})

Metal ion catalysis...

- Metal bound at the active site of enzymes can act as electrophilic catalysts, stabilizing the increased electron density of negative charge that can develop during reaction

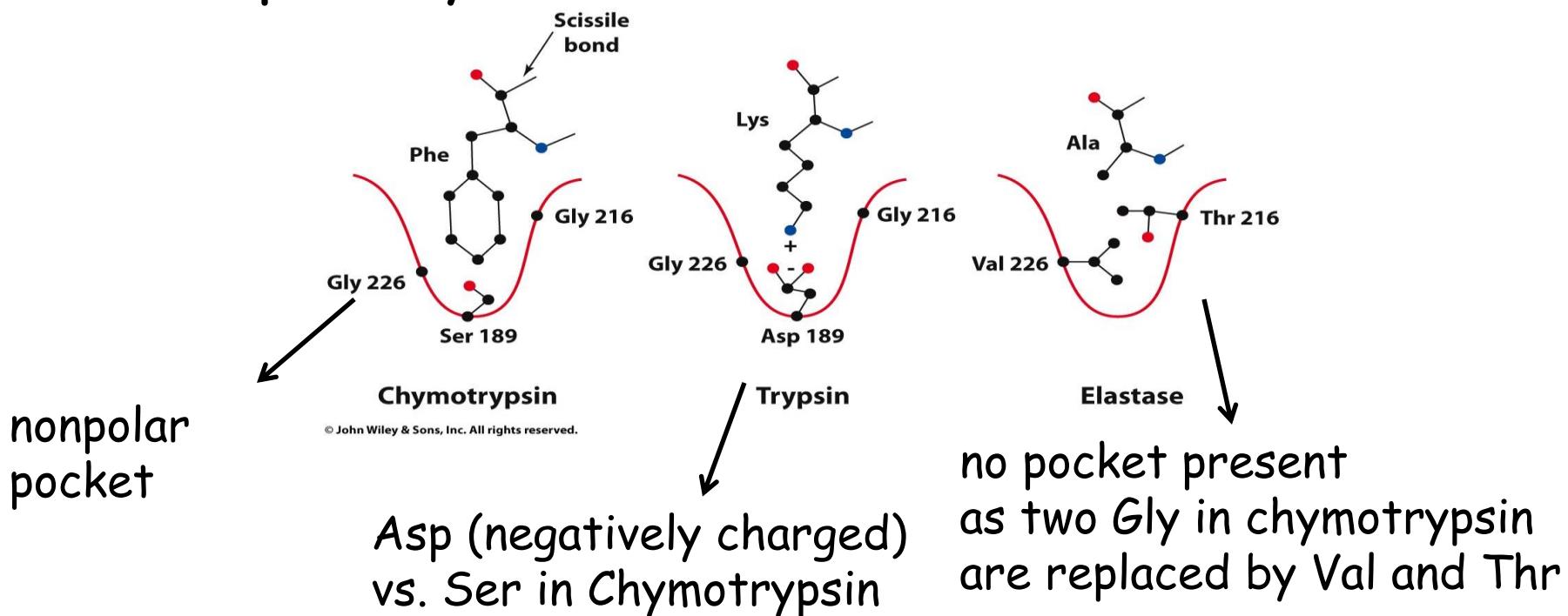


Chymotrypsin: Catalytic triad



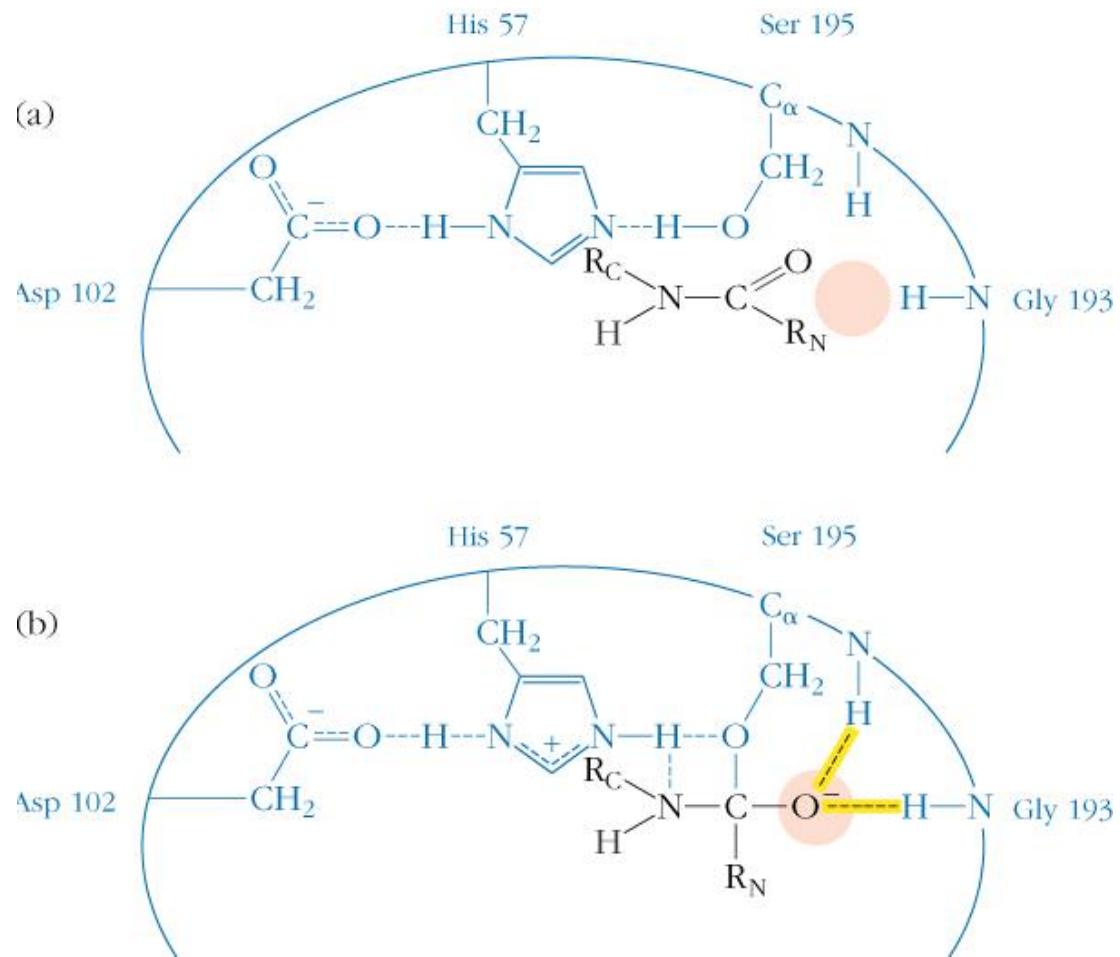
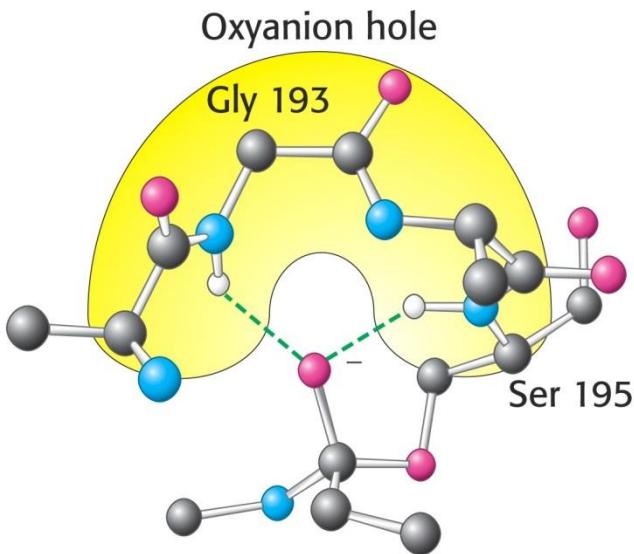
Serine proteases - Enzyme specificity

- Enzymes are substrate specific
 - Chymotrypsin: aromatic or bulky nonpolar side chain (for Trp (W), Tyr (Y), Phe (F))
 - Trypsin: Lys or Arg
 - Elastase: smaller & uncharged side chains
- Small structural difference in the binding site explains the substrate specificity



E. Preferential stabilization of the transition state

The transition state is stabilized by the oxyanion hole of chymotrypsin



How is the activity of
enzymes regulated?

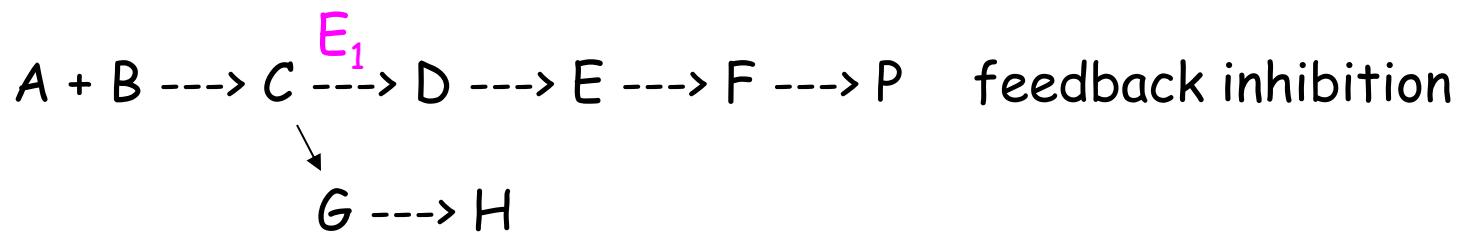
Regulation of Enzyme Activity

- Control of enzyme availability
- Covalent modification
- Allosteric
- Zymogen Regulation

1) Control of enzyme availability

Rate of synthesis/rate of degradation of enzyme

- Fairly slow (several hours), too slow to be effective in eukaryotic cells
- Need something that can occur in seconds or less
- Usually done through regulatory enzymes and occur in metabolic pathways early or at **first committed step**:



- Result is to conserve material and energy by preventing accumulation of intermediates.

2) Covalent modification

Reversible covalent modification of an important catalytic residue to make it inactive (e.g. phosphorylation of Serine)

Which type of bond is formed in this case?

Enzyme Regulation by Covalent Modification

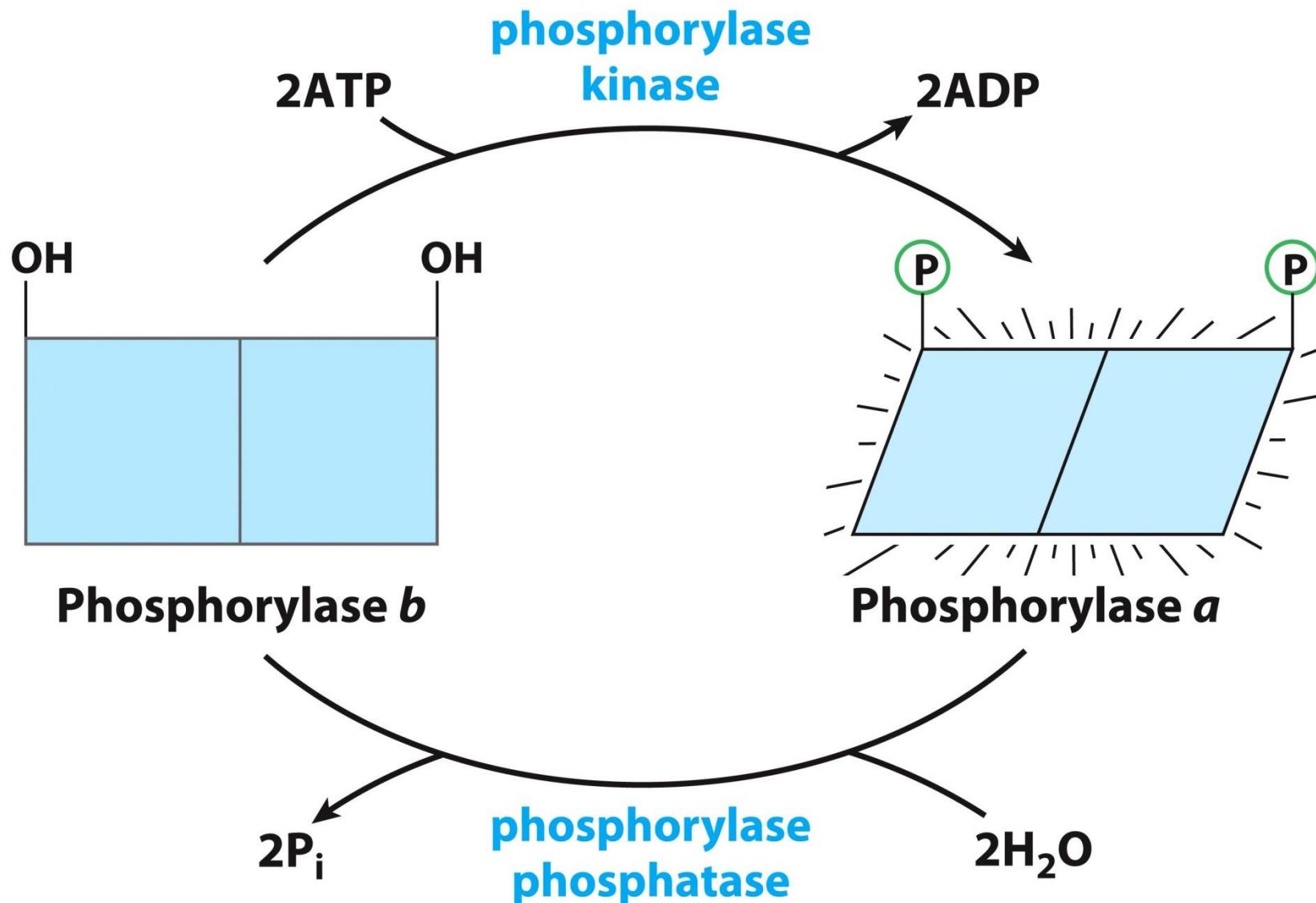


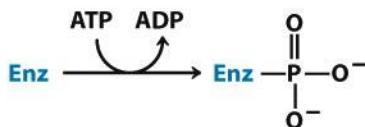
Figure 6-36

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Covalent modification (target residues)

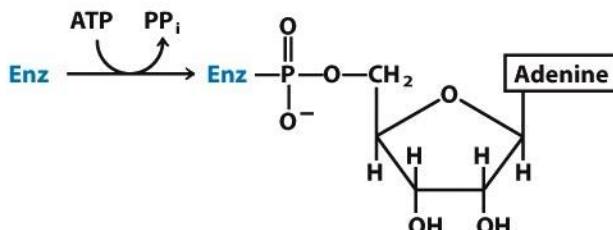
Phosphorylation

(Tyr, Ser, Thr, His)



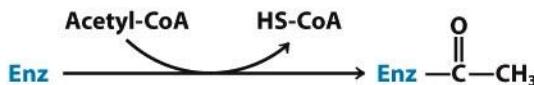
Adenylylation

(Tyr)



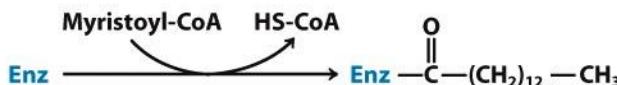
Acetylation

(Lys, α -amino (amino terminus))



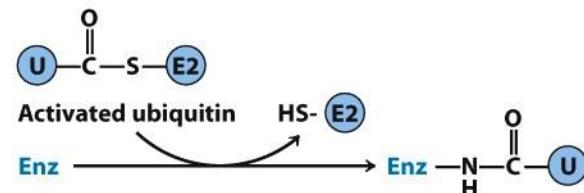
Myristoylation

(α -amino (amino terminus))



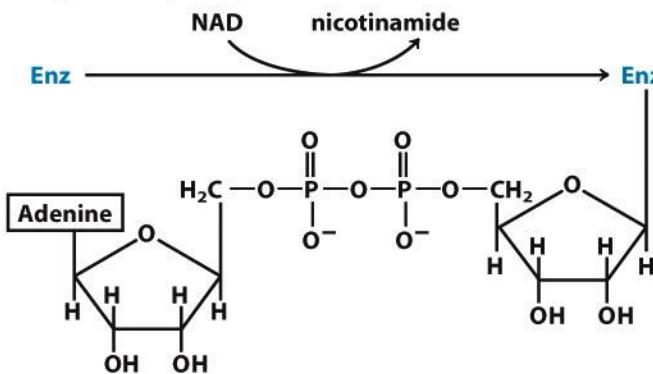
Ubiquitination

(Lys)



ADP-ribosylation

(Arg, Gln, Cys, diphthamide—a modified His)



Methylation

(Glu)

S-adenosyl-methionine + *S*-adenosyl-homocysteine



Figure 6-35

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3) Allosteric regulation

- Done through allosteric sites or regulatory sites on the enzyme - site other than active site where inhibitor or activator can bind

Properties of allosteric enzymes

- a) sensitive to metabolic inhibitors and activators
- b) binding is non-covalent; not chemically altered by enzyme
- c) regulatory enzymes possess quaternary structure - individual polypeptide chains may or may not be identical
- d) enzyme has at least one substrate that gives **sigmoidal** curve due to positive cooperativity because of multiple substrate binding sites

Theories of allosteric regulation

i) concerted theory or symmetry-driven theory

- Assumes 1 binding site/subunit for each ligand
- Enzyme can assume either R or T conformation
- Assumes that all subunits are in R or T state, and all switch at same time when the first substrate is bound
- Also called the Monod-Wyman-Changeux (MWC) model

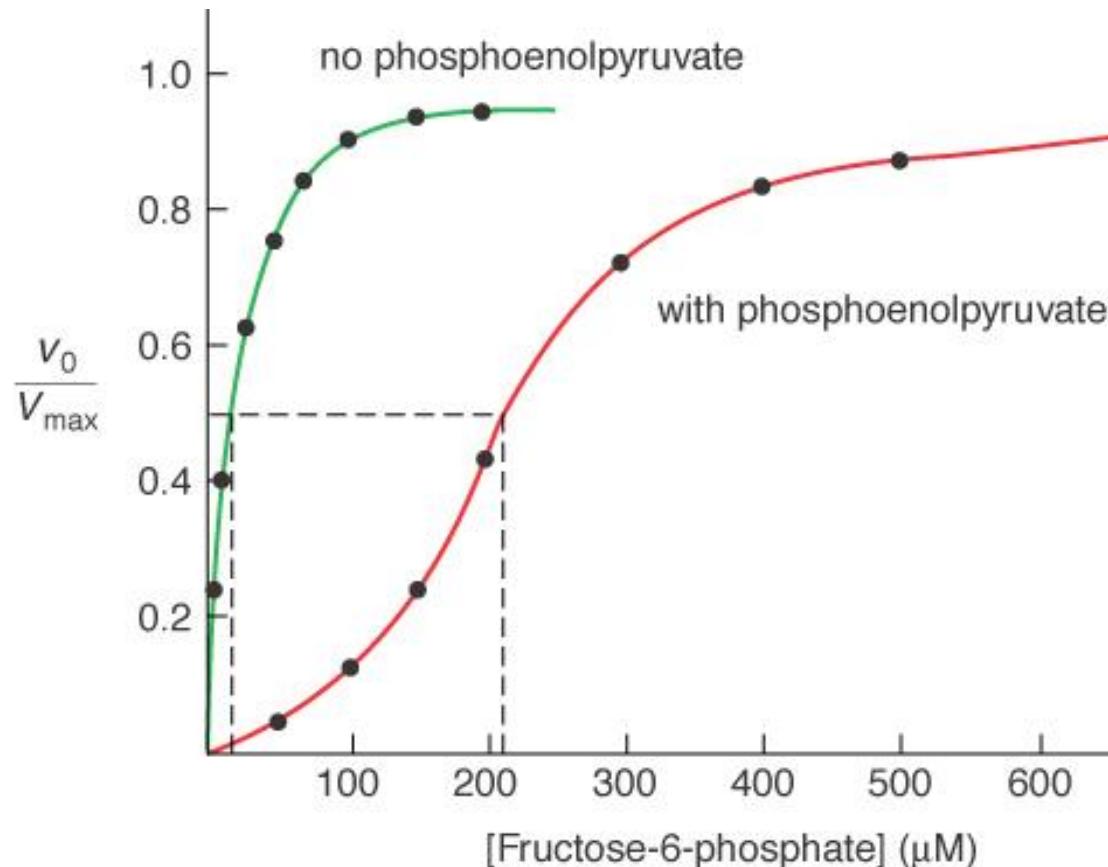
ii) sequential theory

- Ligand introduces a change in the tertiary structure of a subunit. Only that subunit is converted to R conformation

Allosteric regulation cont'd

When the ligand binds to one subunit of an enzyme thereby decreasing the catalytic activity of sites

- Remember hemoglobin? What are its allosteric regulators?



The kinetics of allosteric regulators differ from Michaelis-Menten kinetics. **HOW?**

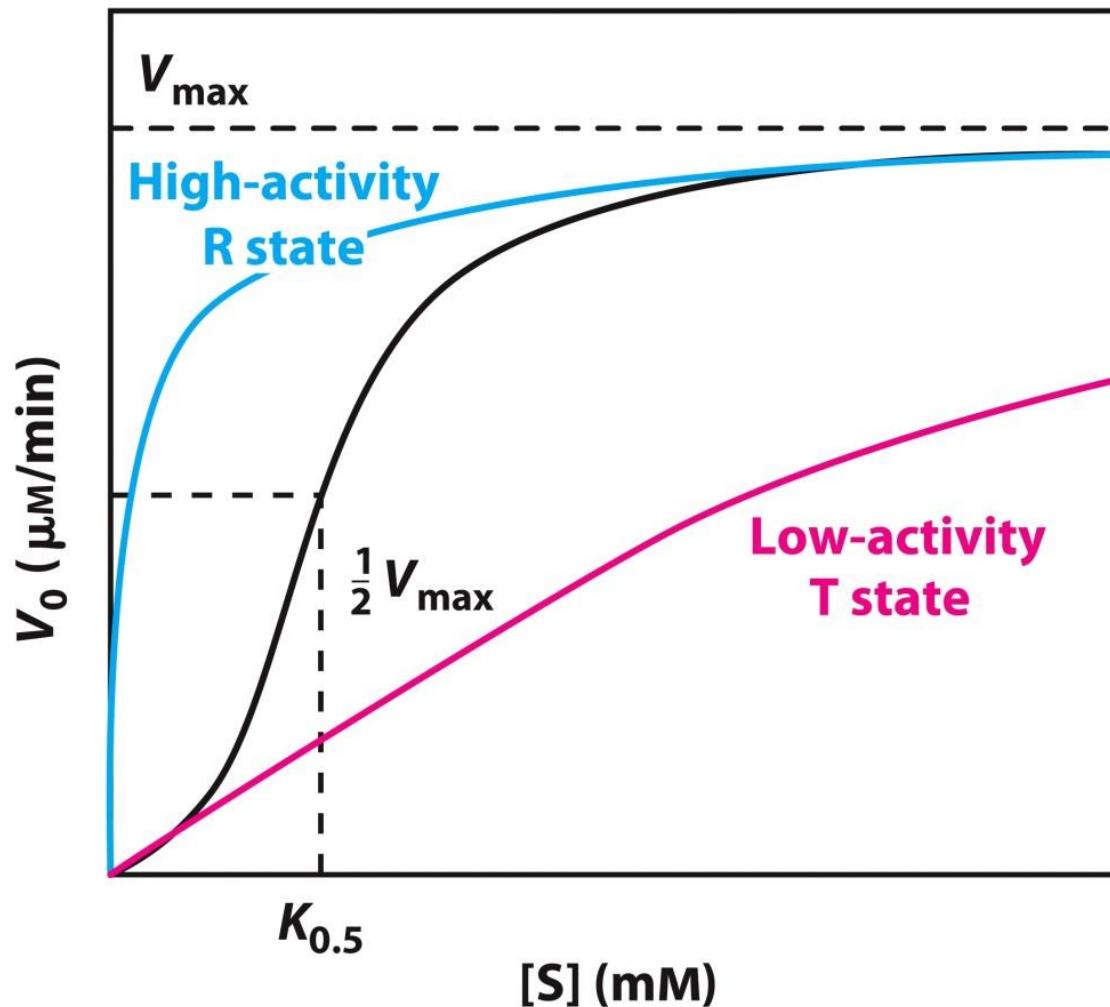
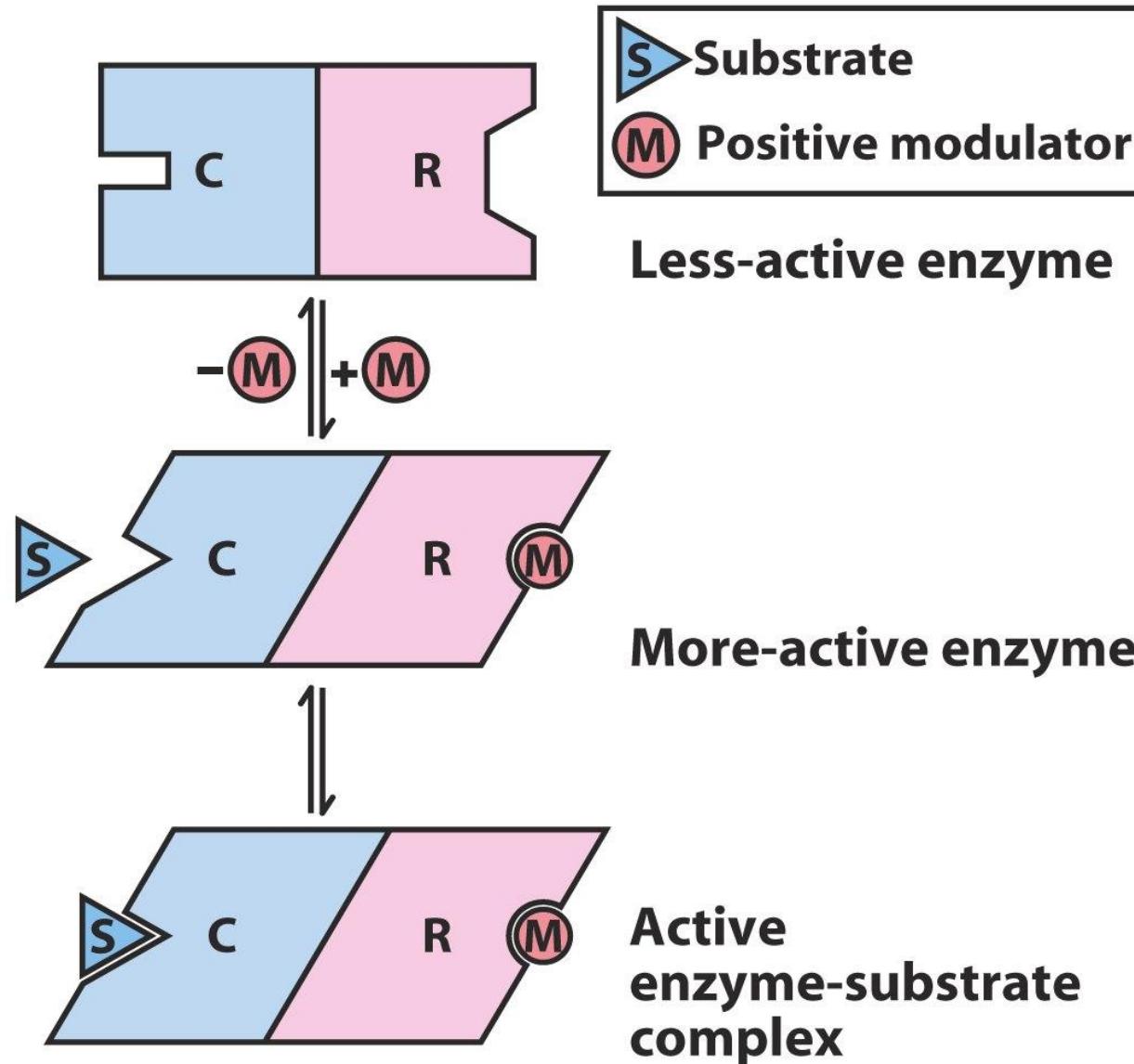


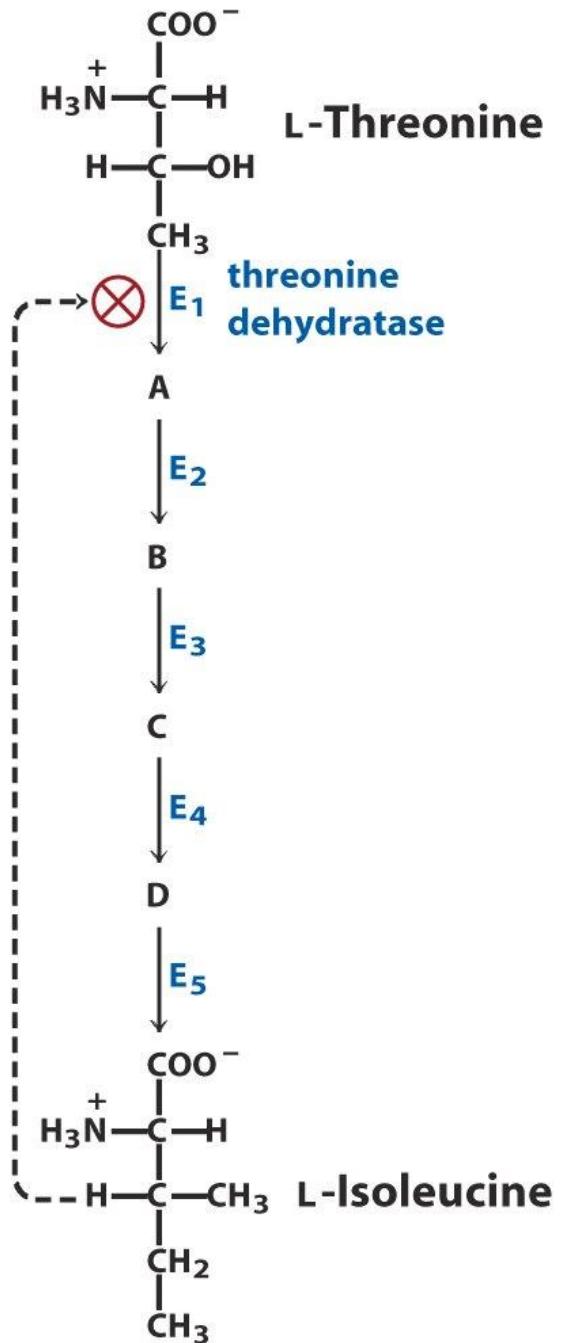
Figure 6-34a

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Allosteric Effectors - Bind to Allosteric Site



Feedback Inhibition is the Classic Form of Allosteric Inhibition



Allosteric Positive & Negative Regulators: Affecting V_{max}

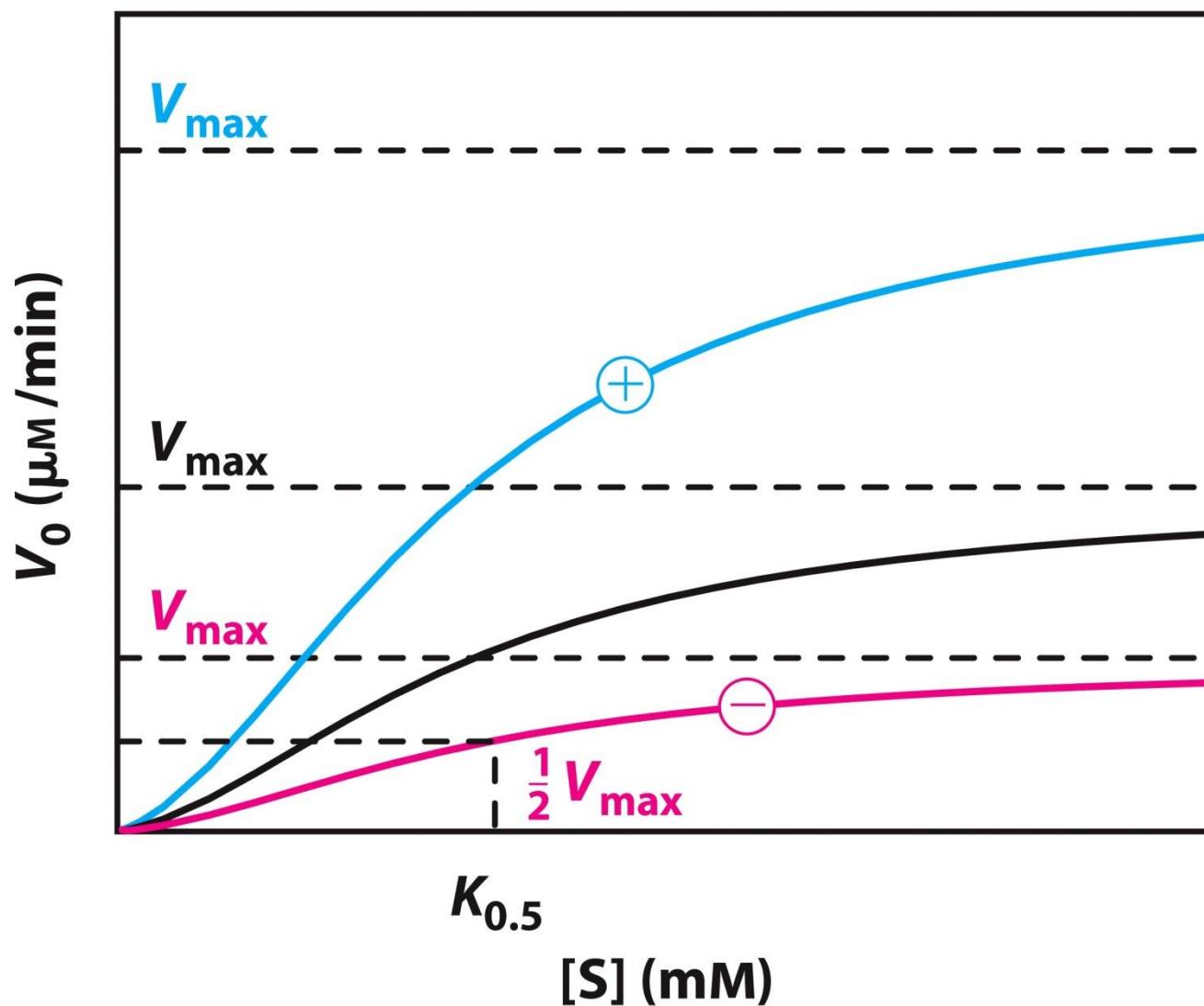


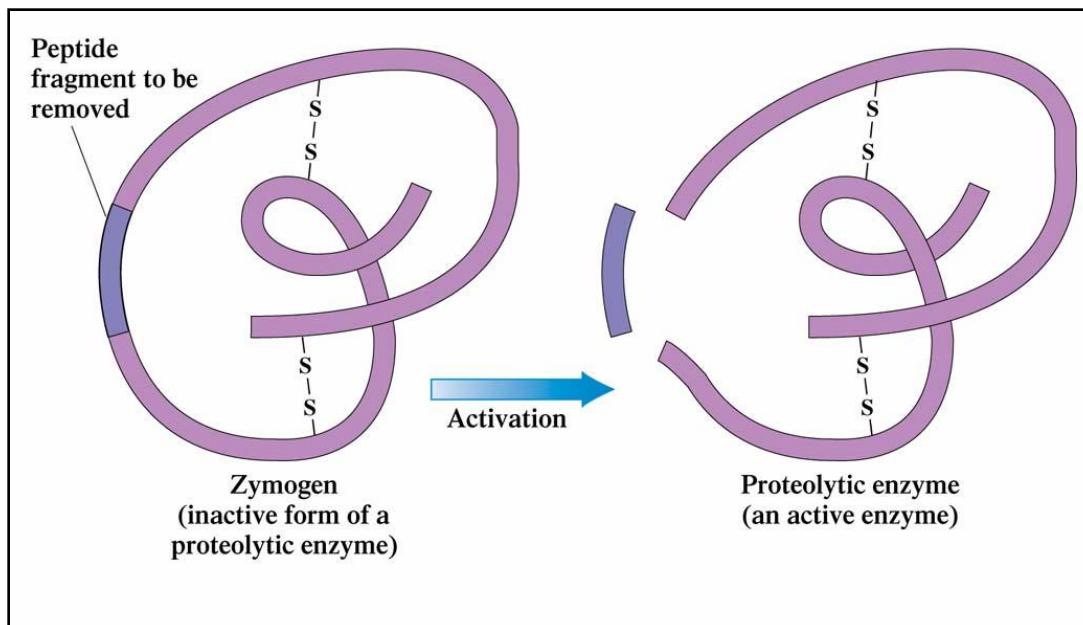
Figure 6-34c

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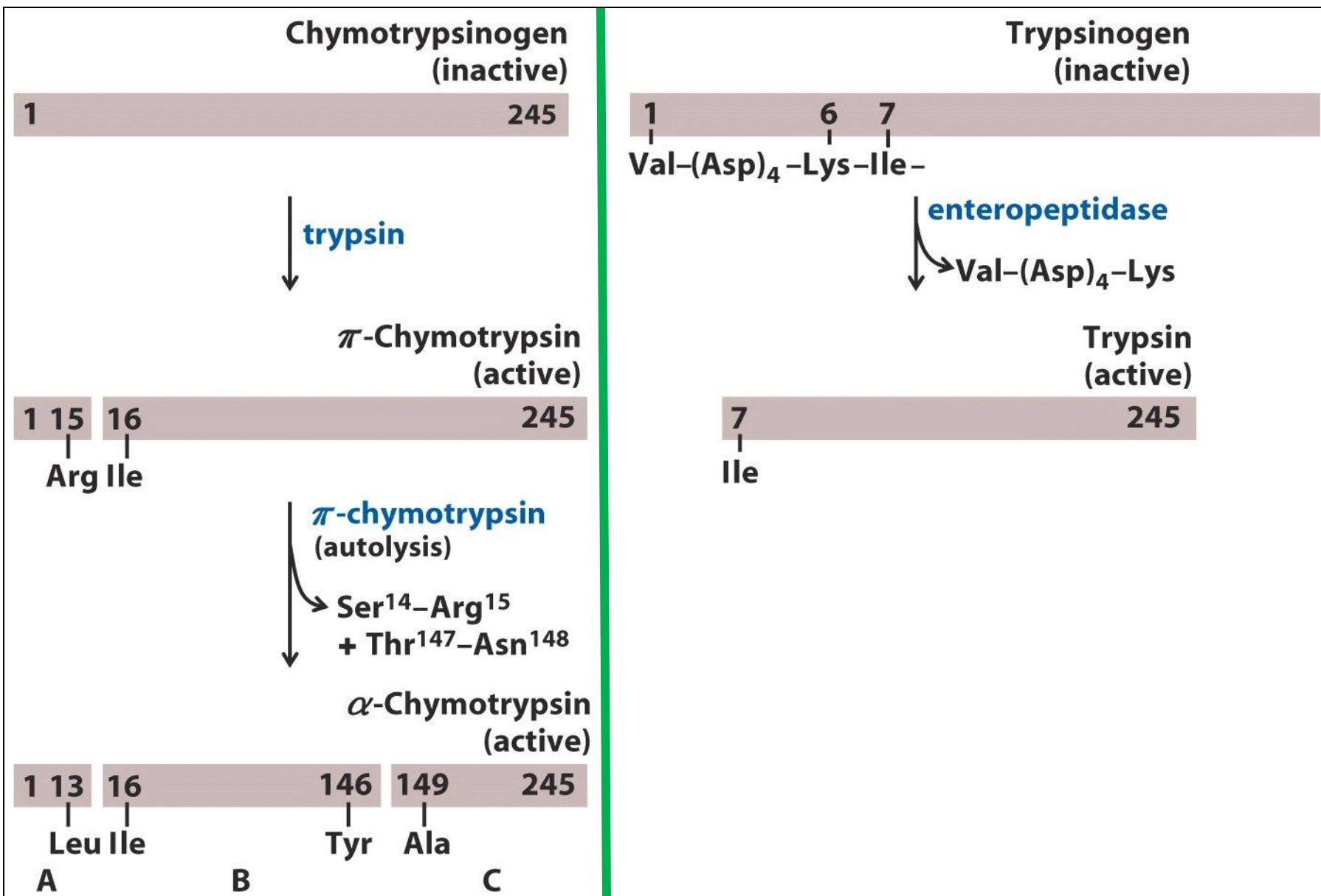
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4) Zymogen Regulation

- Inactive forms of enzymes
- Activated when one or more peptides are cleaved
- E.g. Proinsulin is converted to insulin by removing a small peptide chain
- Digestive enzymes are produced in one organ as zymogens, but not activated until they are needed; Ex. trypsinogen / trypsin



Zymogen Regulation



Next lecture

Enzyme kinetics

LECTURE 8: ENZYMES KINETICS

- Kinetics of single substrate enzyme catalysed reactions
- Michaelis- Menten (M-M) equation and its transformations
- Mechanism of Bi-substrate and Multi-substrate
- Mechanism Co-operativity and the Hill Plot

Why enzyme kinetics is important?

1. Substrate binding constants can be measured as well as **inhibitor strengths** and maximum catalytic rates
2. Kinetics alone will not give a chemical mechanism but combined with chemical and structural data **mechanisms can be elucidated**
3. Kinetics help understand the enzymes role in **metabolic pathways**
4. Under “proper” conditions rates are proportional to enzyme concentrations and these can be used to determine “ **metabolic problems**”

Chemical kinetics and Elementary Reactions

A simple reaction like $A \rightarrow B$ may proceed through several elementary reactions like $A \rightarrow I_1 \rightarrow I_2 \rightarrow B$ Where I_1 and I_2 are intermediates

The characterization of elementary reactions comprising an overall reaction process constitutes its mechanistic description

Rate Equations

Consider $aA + bB + \dots + zZ$. The rate of a reaction is proportional to the frequency with which the reacting molecules simultaneously bump into each other

$$\text{Rate} = k [A]^a [B]^b \cdots [Z]^z$$

The order of a reaction = the sum of exponents

The order means how many molecules have to bump into each other at one time for a reaction to occur

A first order reaction one molecule changes to another



A second order reaction two molecules react



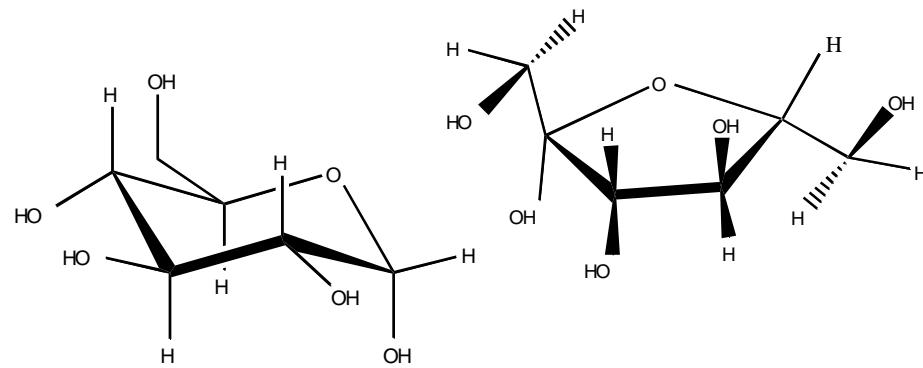
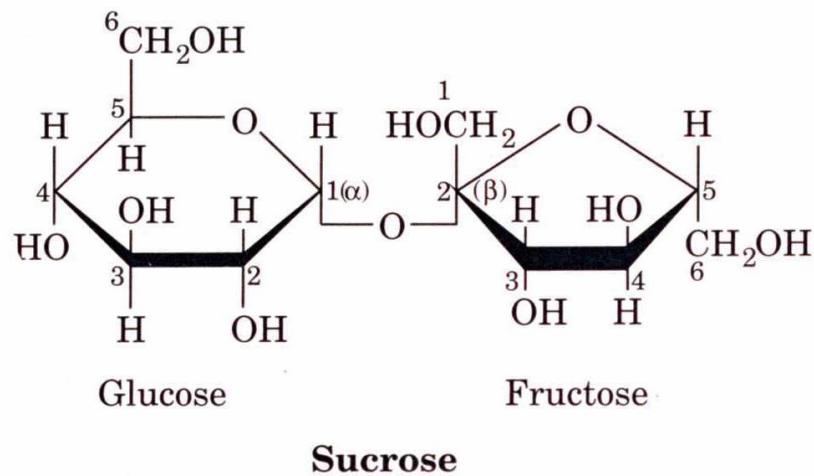
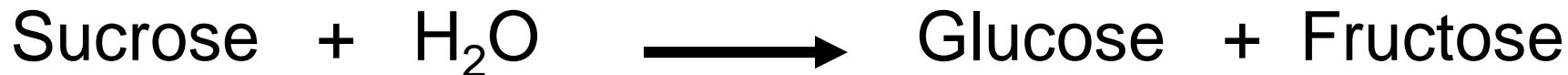
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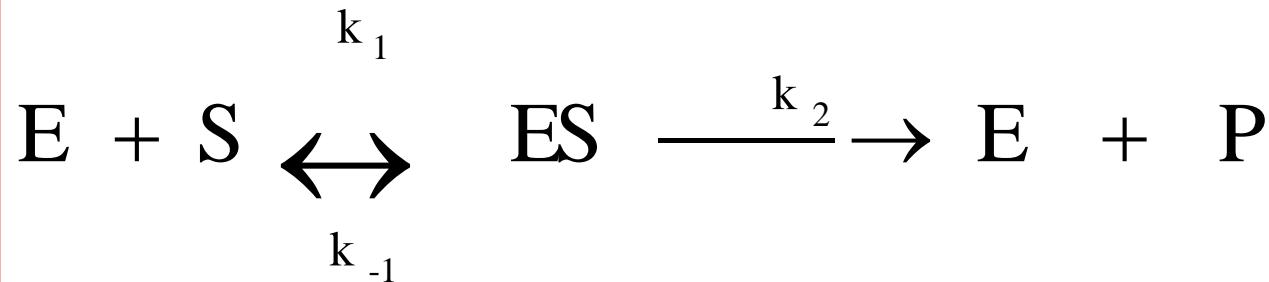


Kinetics of Enzymes

Enzymes follow zero order kinetics when substrate concentrations are high. Zero order means there is no increase in the rate of the reaction when more substrate is added

Given the following breakdown of sucrose to glucose and fructose





E = Enzyme S = Substrate P = Product

ES = Enzyme-Substrate complex

k_1 rate constant for the forward reaction

**k_{-1} = rate constant for the breakdown of the
ES to substrate**

**k_2 = rate constant for the formation of the
products**

Enzymes kinetics.....

- The **VELOCITY** (reaction rate) of an enzyme catalysed reaction is dependent upon the substrate concentration [S]

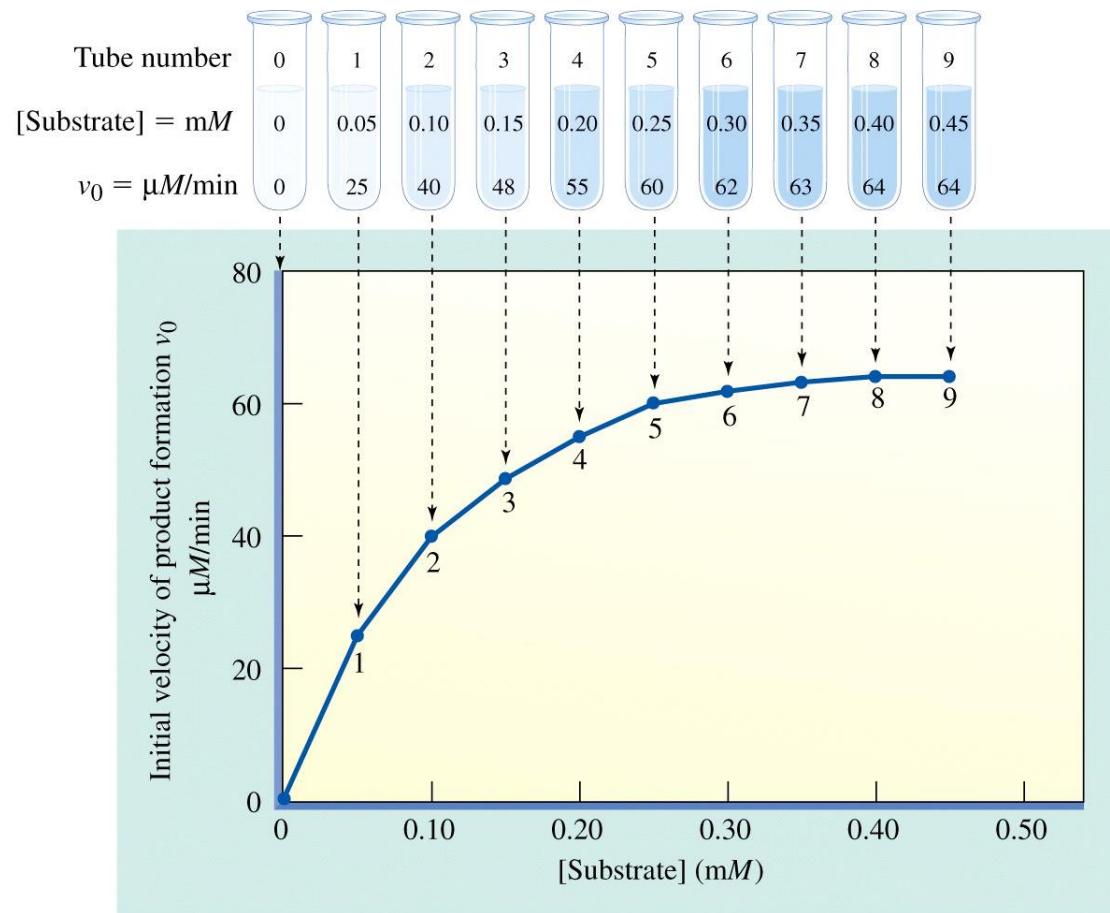


Figure 5-3 Concepts in Biochemistry, 3/e
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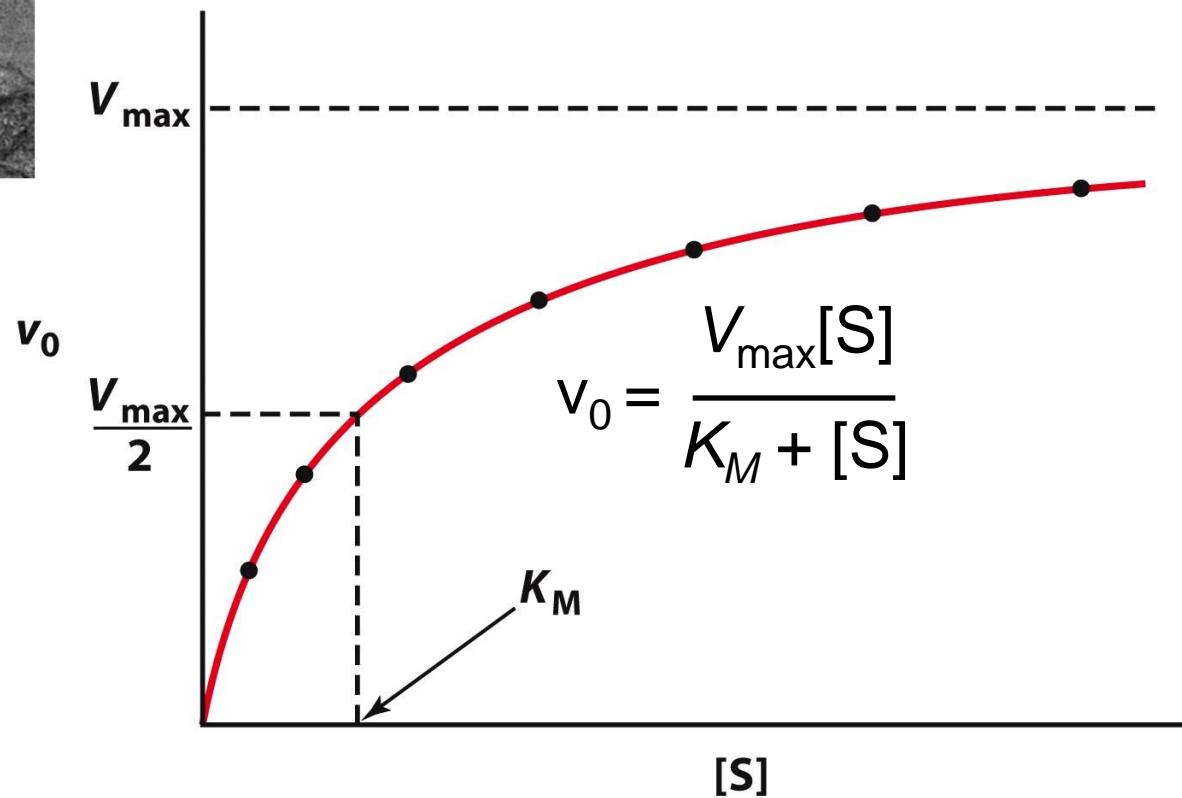
Enzymes kinetics: Michaelis- Menten equation



Leonor Michaelis
1875–1949



Maud Menten
1879–1960



Significance of Michaelis- Menten equation

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

- K_M - substrate concentration where v_o equals one-half V_{\max}
- Used to evaluate the specificity of an enzyme for a substrate
- K_M – is usually a little higher than the physiological $[S]$

Small K_M → tight binding

High K_M → weak binding

Hexose Kinase



Glucose

Allose

Mannose

$K_M = 8 \times 10^{-6}$

$K_M = 8 \times 10^{-3}$

$K_M = 5 \times 10^{-6}$

Turnover number

Kcat – enzyme turn over number defined as the number of reactions a molecule of enzyme can catalyze per second under optimal condition

$$k_{\text{cat}} = \frac{V_{\text{max}}}{[E]_T} = k_2$$

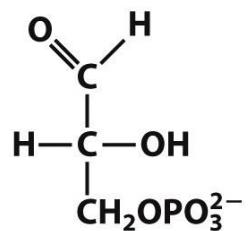
 **TABLE 7-1 | Catalytic Constants of Some Enzymes**

Enzyme	$k_{\text{cat}} (\text{s}^{-1})$
Staphylococcal nuclease	95
Cytidine deaminase	299
Triose phosphate isomerase	4300
Cyclophilin	13,000
Ketosteroid isomerase	66,000
Carbonic anhydrase	1,000,000

Catalytic efficiency

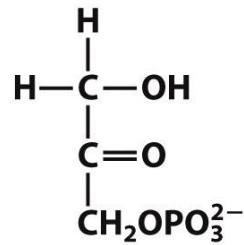
- Defined as:

$$\frac{k_{cat}}{K_M}$$

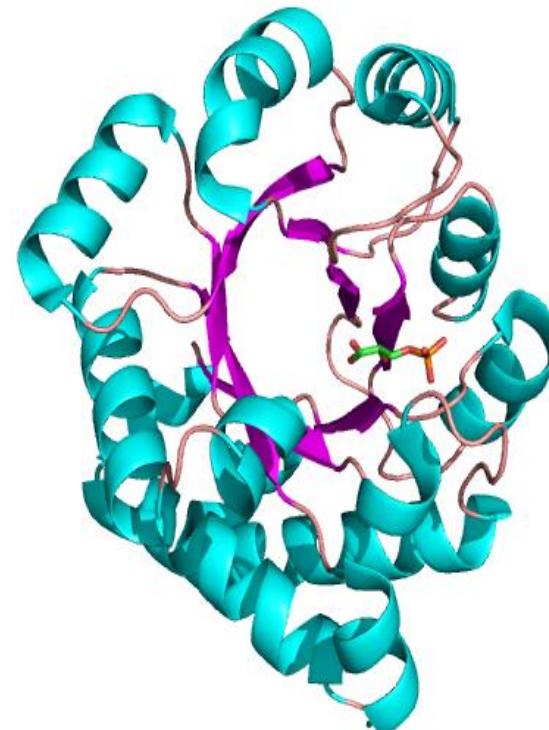


Glyceraldehyde-3-phosphate

triose phosphate isomerase



Dihydroxyacetone phosphate



$$K_{cat}/K_M = 2.4 \times 10^8 \text{ M}^{-1}\text{s}^{-1}$$

Triose Phosphate Isomerase

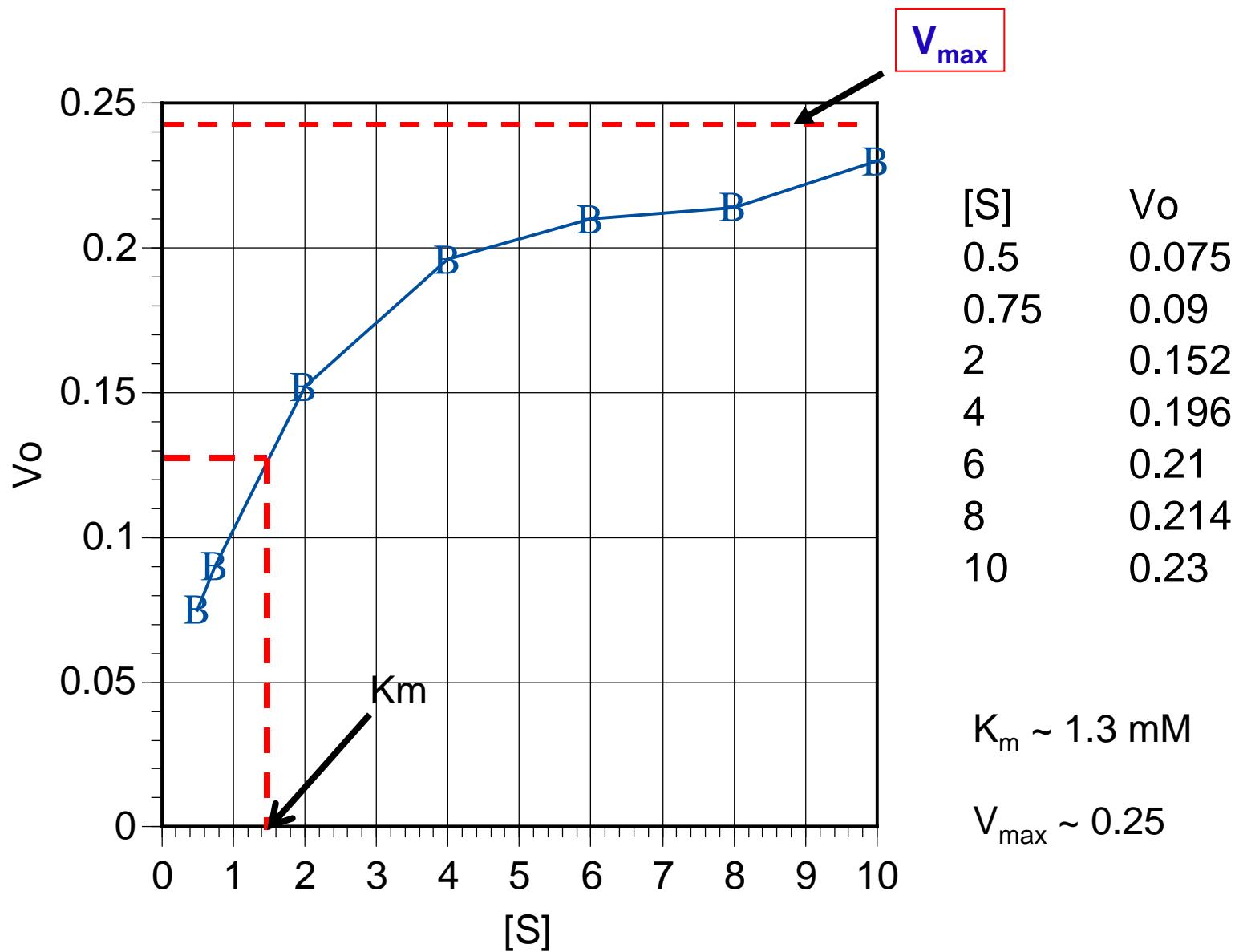
Limitations of M-M

1. Some enzyme catalyzed rxns show more complex behavior $E + S \rightleftharpoons ES \rightleftharpoons EZ \rightleftharpoons EP \rightleftharpoons E + P$
With M-M can look only at rate limiting step
2. Often more than one substrate
 $E + S_1 \rightleftharpoons ES_1 + S_2 \rightleftharpoons ES_1S_2 \rightleftharpoons EP_1P_2 \rightleftharpoons EP_2 + P_1 \rightleftharpoons E + P_2$
Must optimize one substrate then calculate kinetic parameters for the other
3. Assumes $k_{-2} = 0$
4. Assume steady state conditions (see next slide)

Steady State Assumption

- The M-M equation was derived in part by making several assumptions. An important one was: **the concentration of substrate must be much greater than the enzyme concentration.**
- In the situation where $[S] \gg [E]$ and at initial velocity rates, it is assumed that the changes in the concentration of the intermediate ES complex are very small over time (v_o). This condition is termed a **steady-state rate**, and is referred to as **steady-state kinetics**. **Therefore, it follows that the rate of ES formation will be equal to the rate ES breakdown.**

Difficult to determine V_{max} experimentally



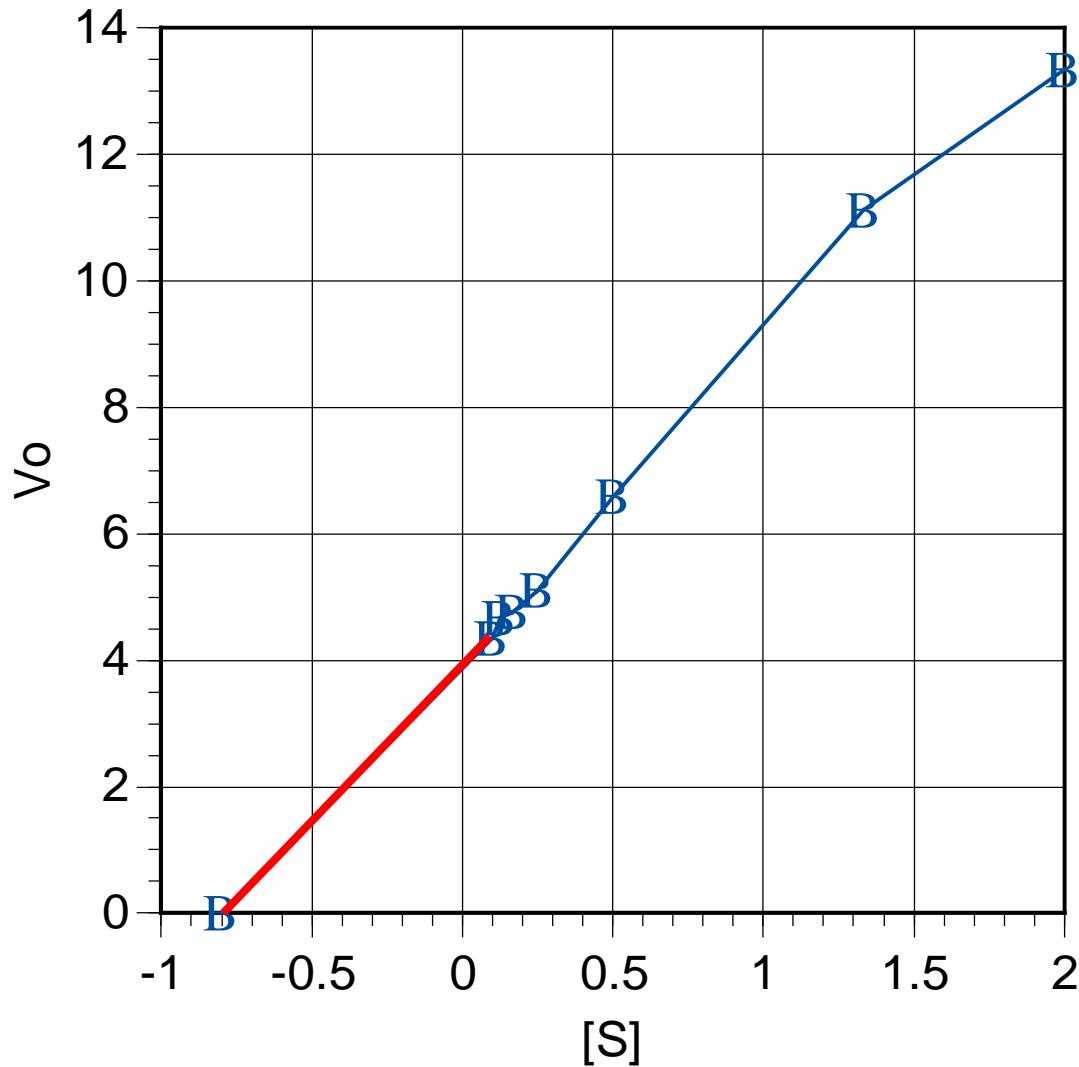
Lineweaver-Burke plot (double reciprocal plot)

- it is difficult to determine V_{max} experimentally
- hyperbola equation can be transformed into the equation for a straight line by taking the reciprocal of each side
- the formula for a straight line is $y = mx + b$

$$\frac{1}{V} = \frac{K_M}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}}$$
$$y = m \cdot x + b$$

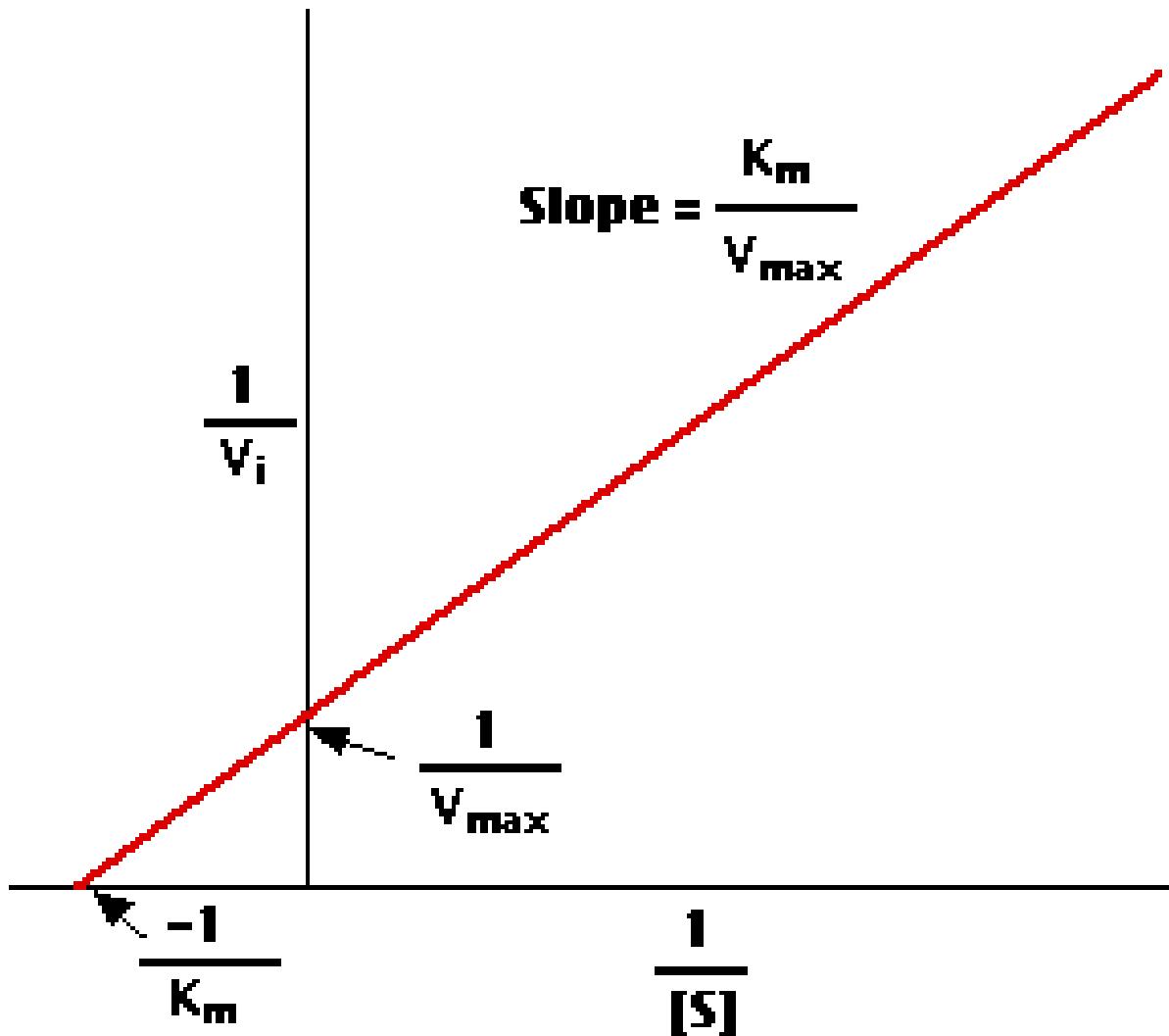
$$\frac{1}{v_o} = \left(\frac{K_M}{V_{max}} \right) \frac{1}{[S]} + \frac{1}{V_{max}}$$

Difficult to determine V_{max} experimentally



[S]	V_o
2.000	13.333
1.333	11.111
0.500	6.579
0.250	5.102
0.167	4.762
0.125	4.673
0.100	4.348

$$\begin{aligned}-1/K_m &= -0.8 \\ K_m &= 1.23 \text{ mM} \\ 1/V_{max} &= 4.0 \\ V_{max} &= 0.25\end{aligned}$$



Provides a more precise way to determine V_{max} and K_M

Other enzymatic kinetics plots

The Hanes plot

Makes use of the Lineweaver-Burk equation by multiplying with $[S]$ on both sides

Plot $[S]/V_0$ vs $[S]$

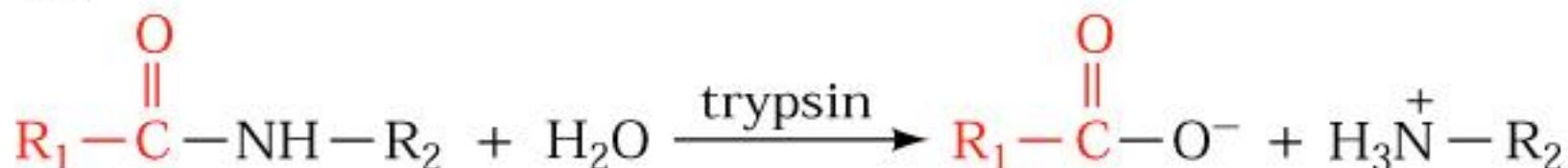
The Eadie-Hofstee plot

Multiply both sides of the Lineweaver-Burk equation by the factor $V_0 V_{\max}$

Plot V_0 vs $V_0/[S]$

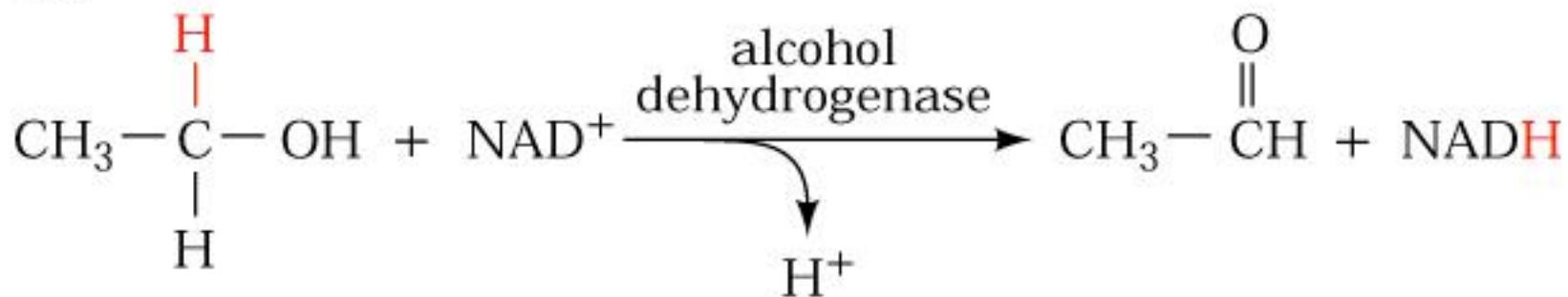
Bisubstrate reactions

(a)



Polypeptide

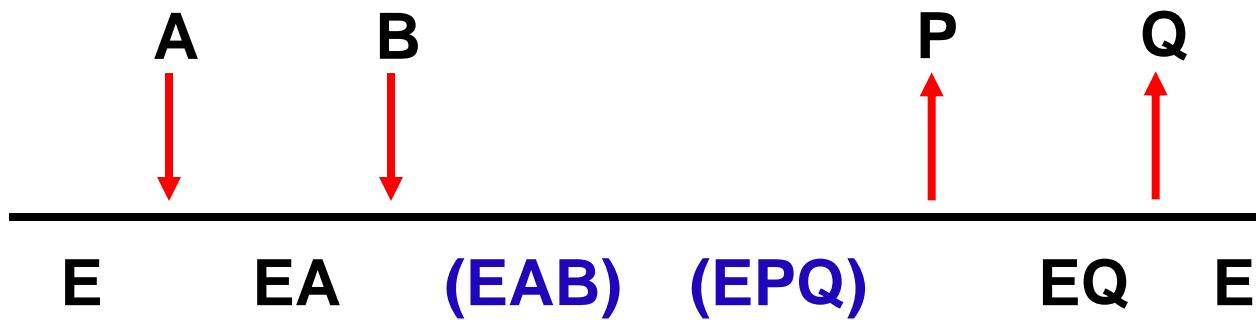
(b)



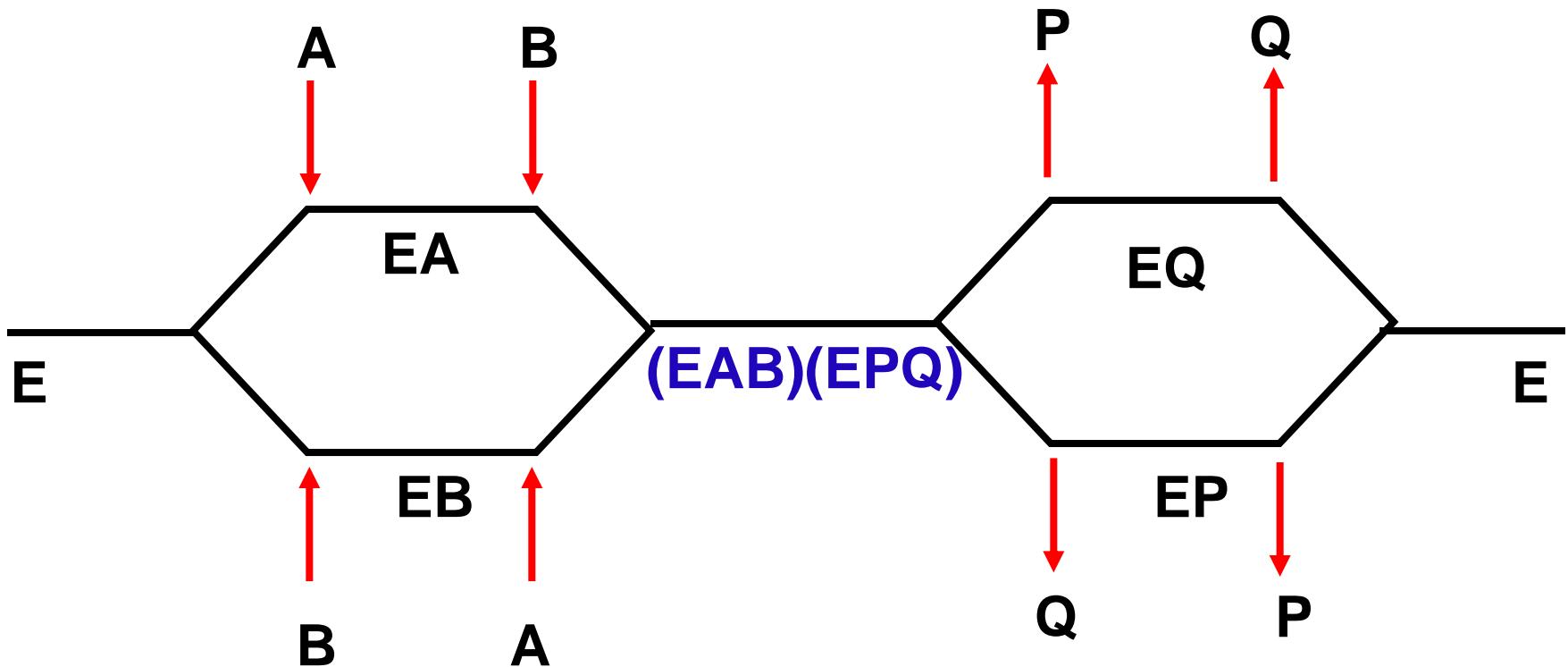
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a) Sequential Reactions

- All substrates must combine with enzyme before reaction can occur

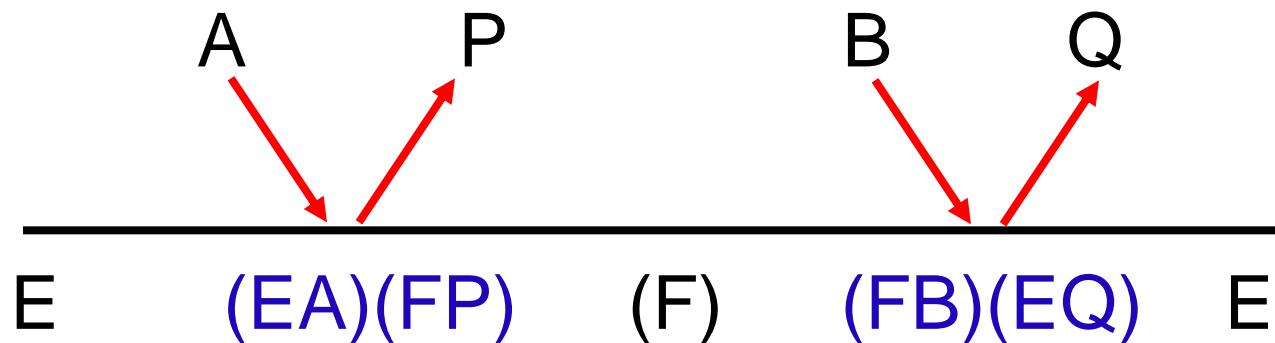


b) Random bisubstrate reactions



c) Ping-Pong Reactions

- Group transfer reactions
- In **Ping-Pong** reactions first product released before second substrate binds
- When E binds A, E changes to F
- When F binds B, F changes back to E



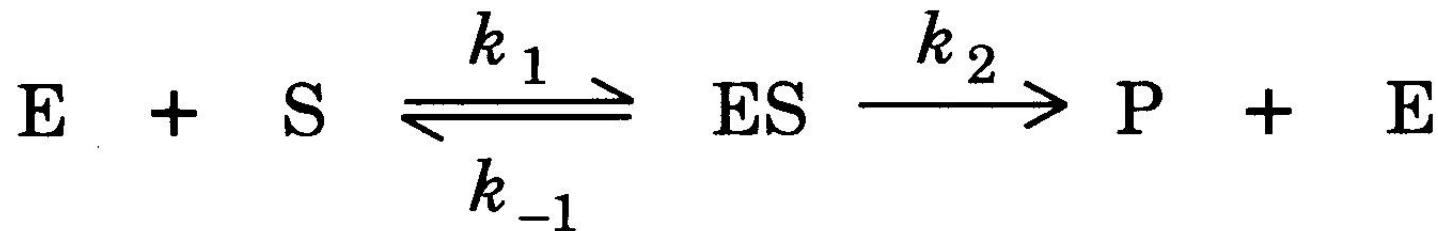
Types of enzyme inhibition

Types of enzyme inhibition

- **Inhibitor**- substance that binds to an enzyme and interferes with its activity
- Inhibitor can prevent **ES** complex formation or prevent the **ES** complex breakdown to **E + P**
 - A. Irreversible – covalent
 - B. Reversible – non-covalent binding
 - i. Competitive
 - ii. Uncompetitive
 - iii. Non-competitive

Competitive Inhibition

Inhibitor molecule binds to the same site on the enzyme as the substrate preventing the substrate from binding



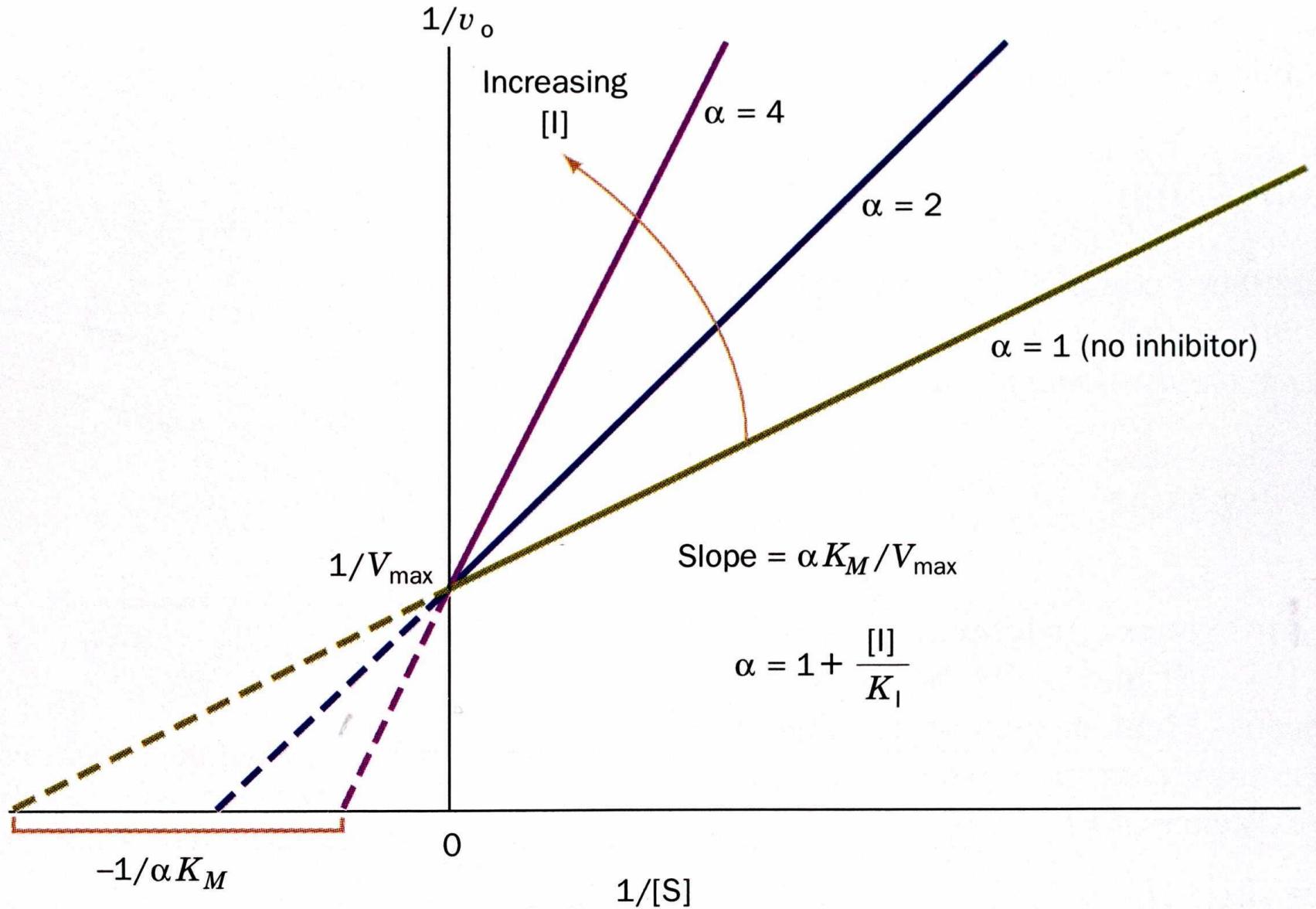
+

I

$$K_I \downarrow$$

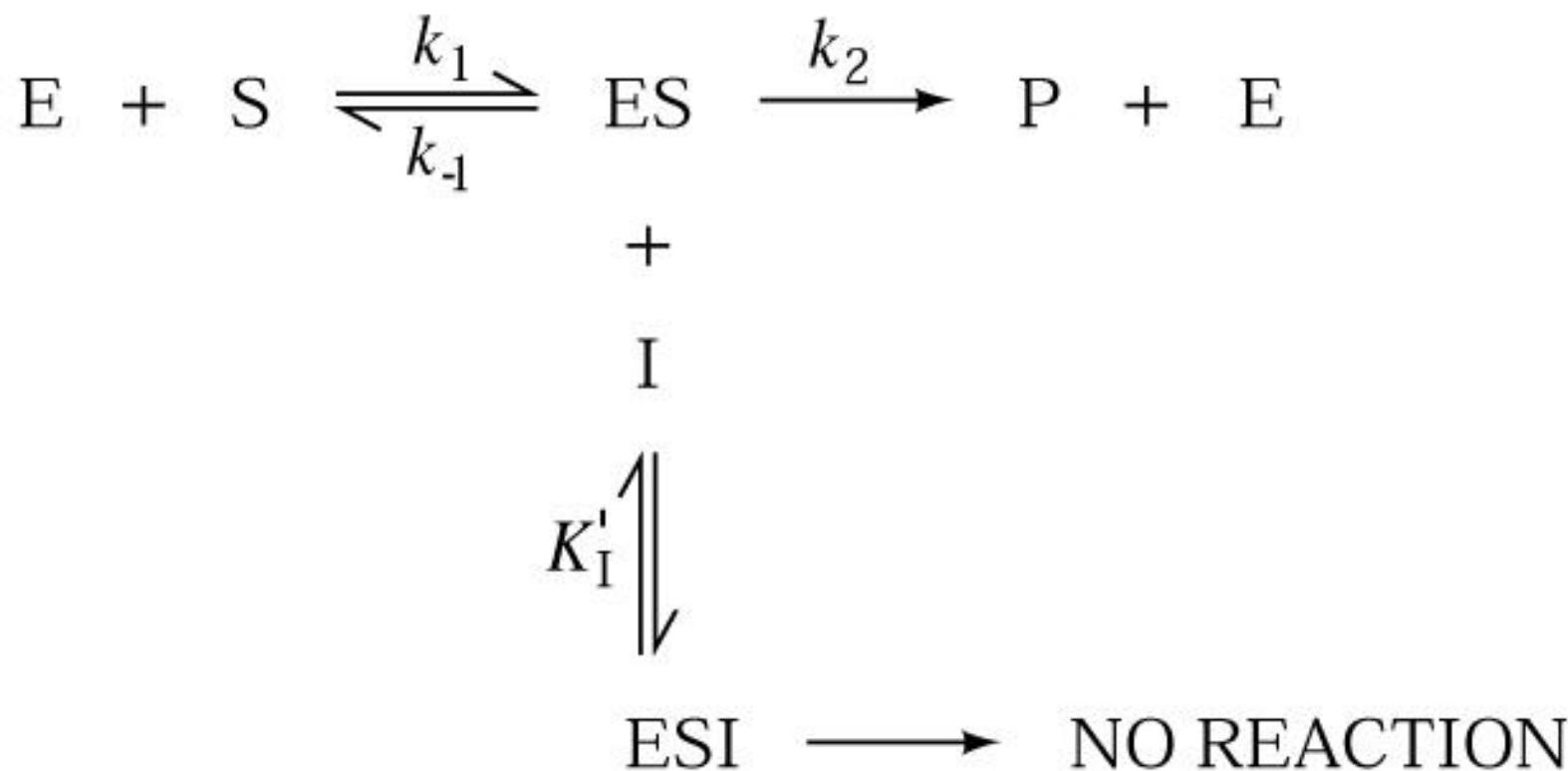


Competitive Inhibition: Lineweaver-Burk Plot

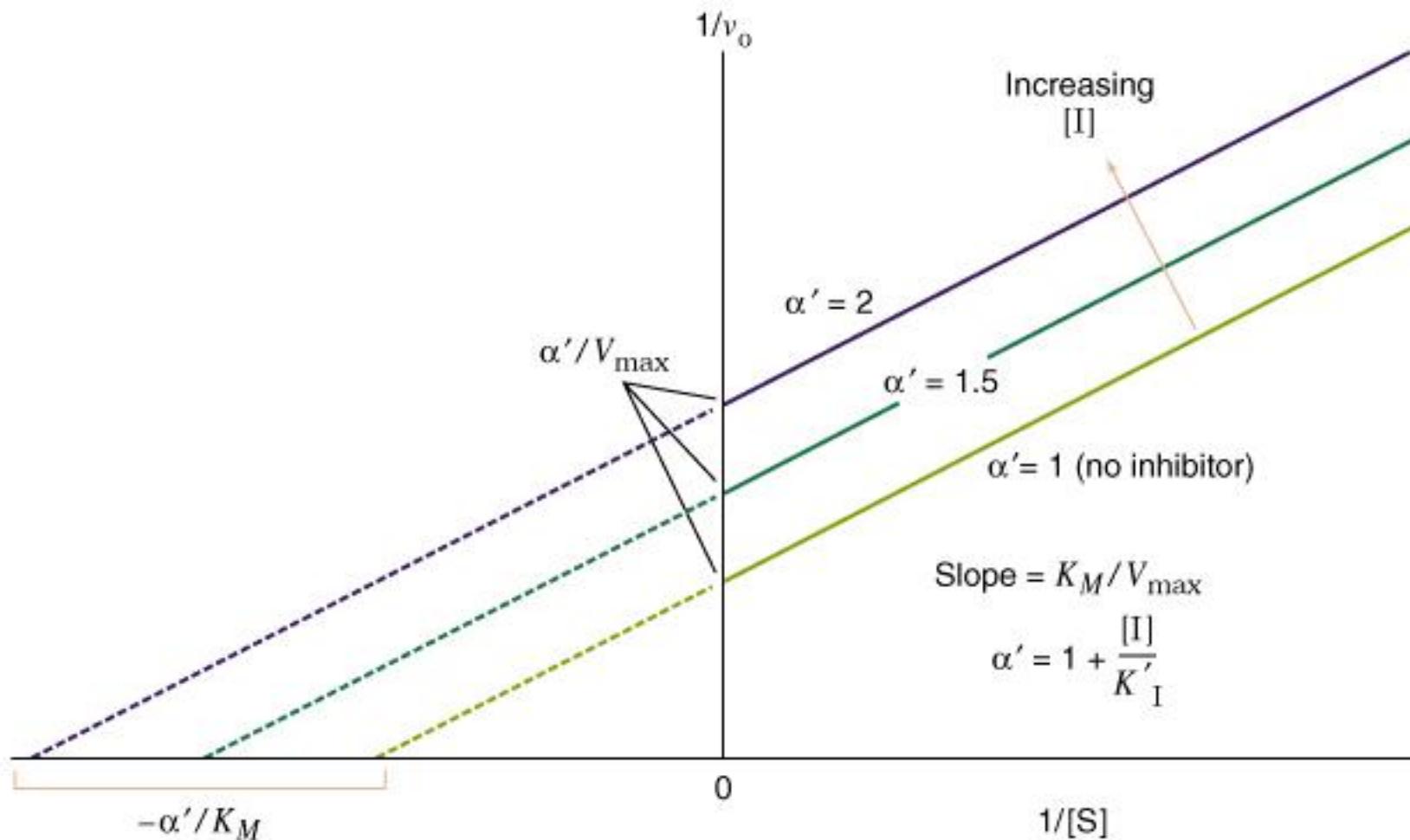


Uncompetitive Inhibition

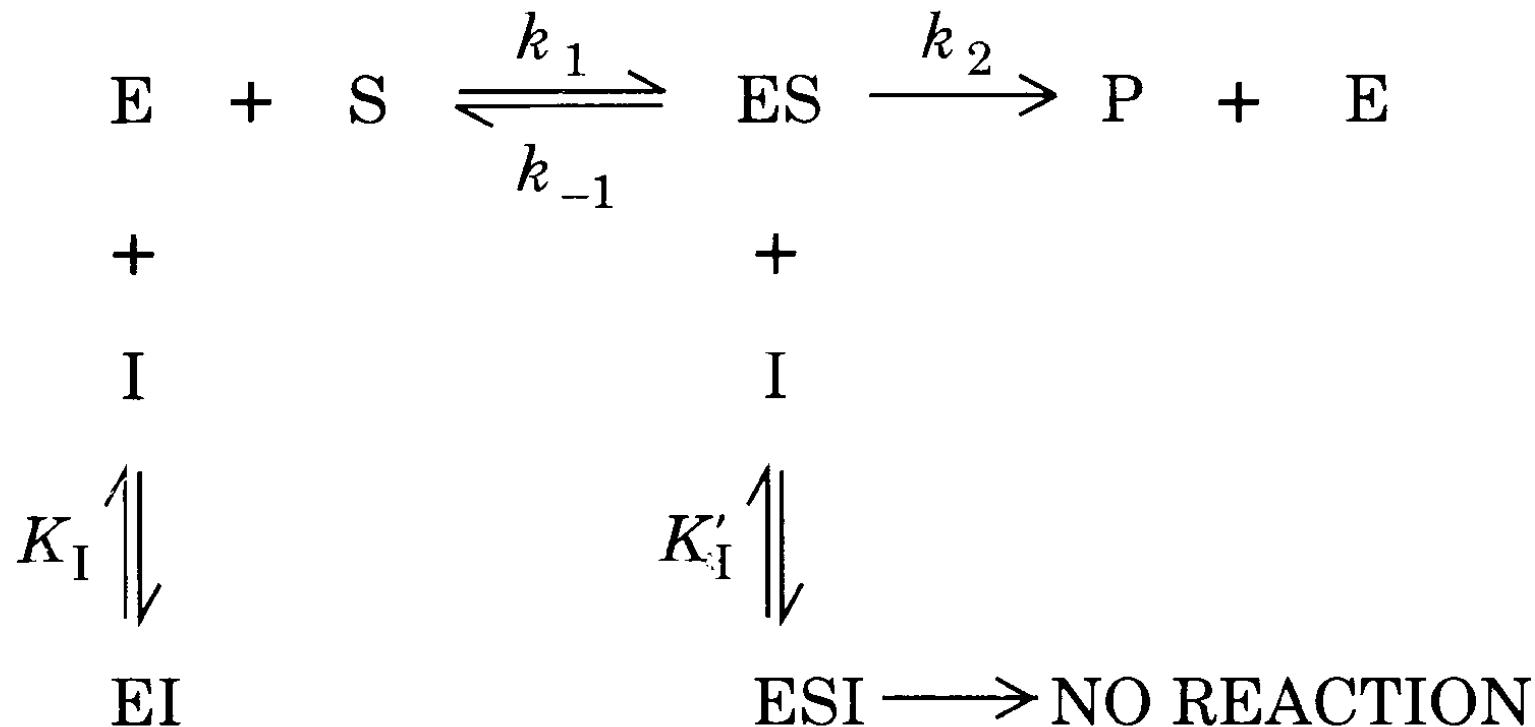
Inhibitor molecule bind to some other site on the enzyme other than the active site reducing its catalytic power



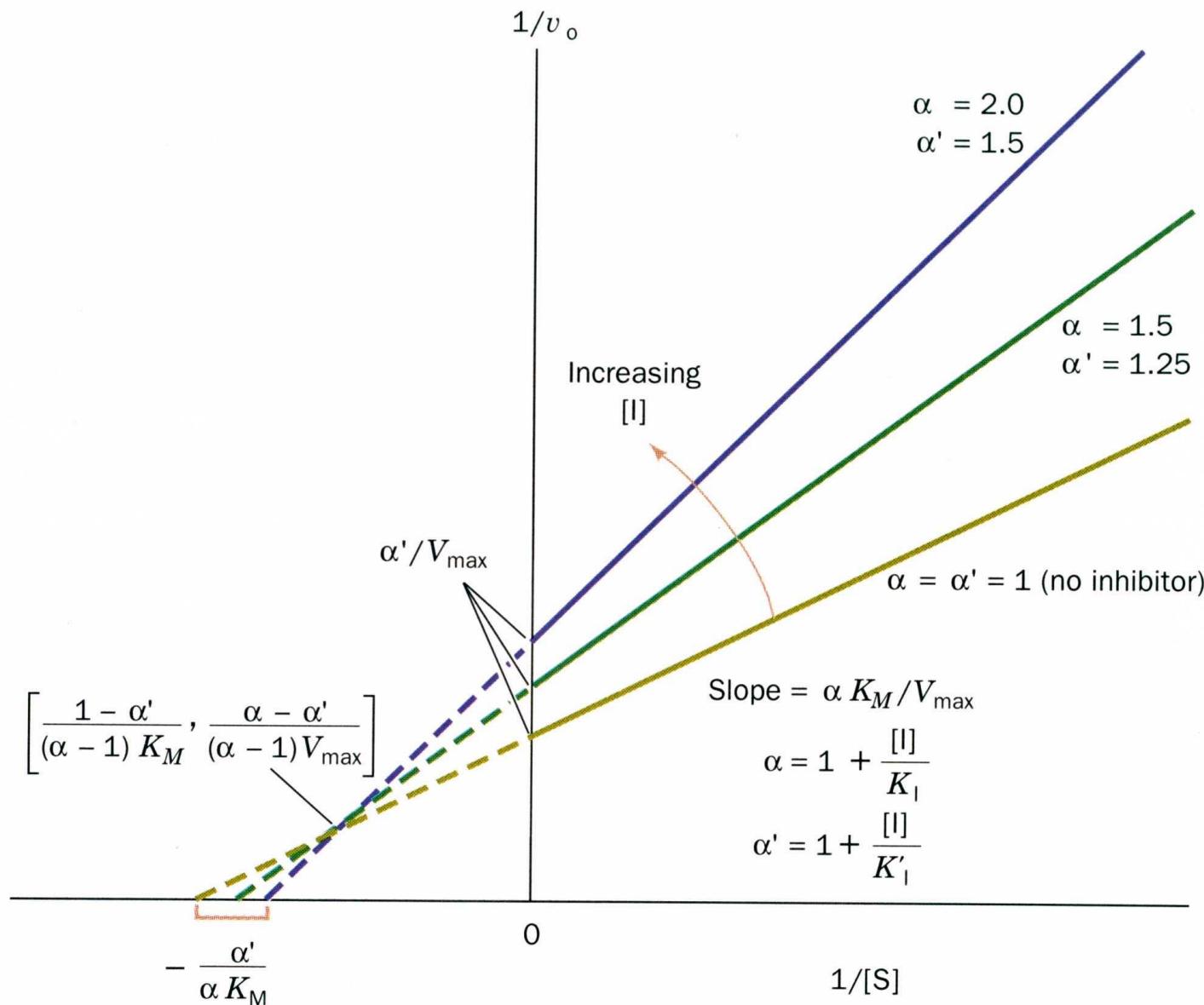
Uncompetitive Inhibition: Lineweaver-Burk Plot



Non-competitive inhibition (Mixed)



Non-competitive Inhibition: Lineweaver-Burk Plot



Mixed inhibition is when the inhibitor binds to the enzyme at a location distinct from the substrate binding site. The binding of the inhibitor will either alter the K_M or V_{max} or both

$$K_I = \frac{[E][I]}{[EI]} \quad K'_I = \frac{[ES][I]}{[ESI]}$$

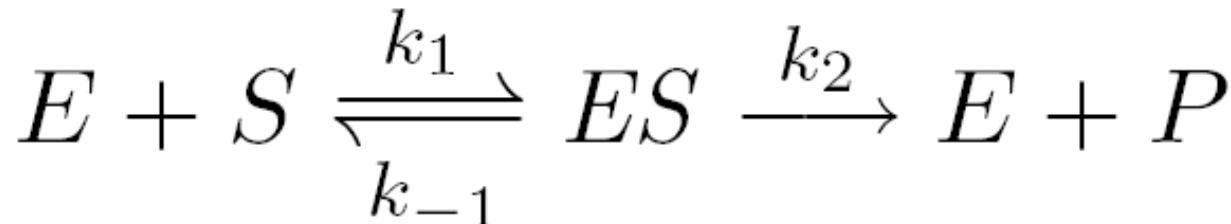
$$v_o = \frac{V_{max} [S]}{\alpha K_M + \alpha' [S]} \quad \alpha' = \left(1 + \frac{[I]}{K'_I} \right)$$

Enzyme Cooperativity

Unfortunately the kinetic constants that describe enzyme catalysis are very difficult to measure and as a result researchers do not tend to use the explicit mechanism, instead they use certain approximations

The two most popular approximations are:

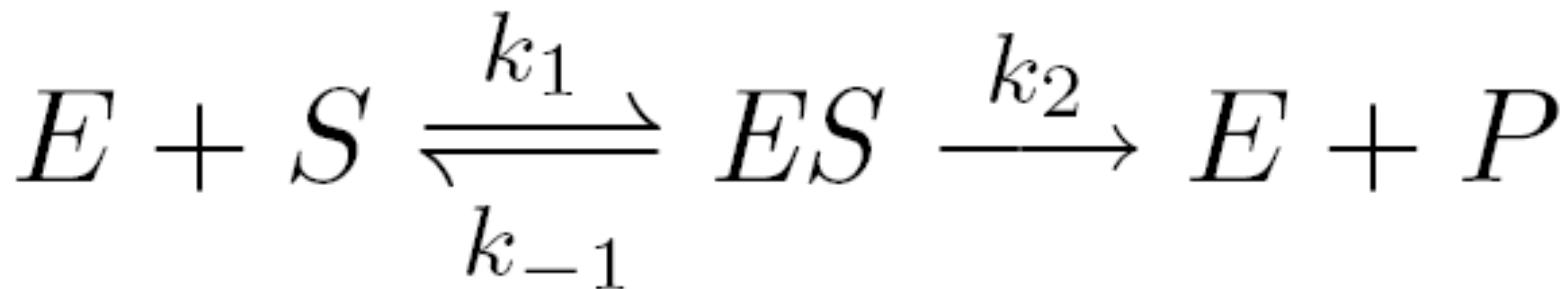
1. Rapid Equilibrium
2. Steady State



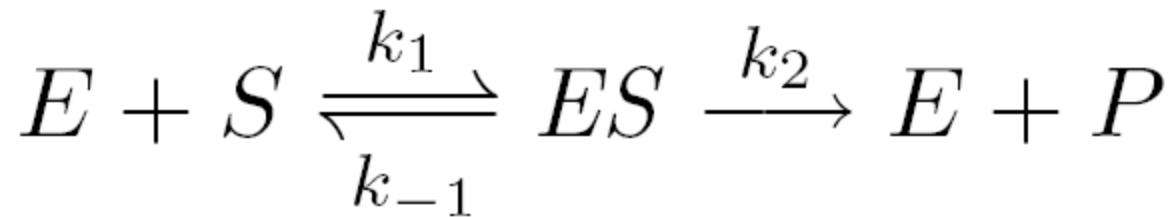
The rapid equilibrium approximation assumes that the binding and unbinding of substrate to enzyme is much faster than the release of product. As a result, one can assume that the binding of substrate to enzyme is in equilibrium

That is, the following relation is true at all times (Kd=dissociation constant):

$$K_d = \frac{E \cdot S}{ES}$$



Steady State Assumption



It is possible to relax the constraints that ES should be in equilibrium with E and S by assuming that ES has a relatively steady value of a **wide range of substrate concentrations**

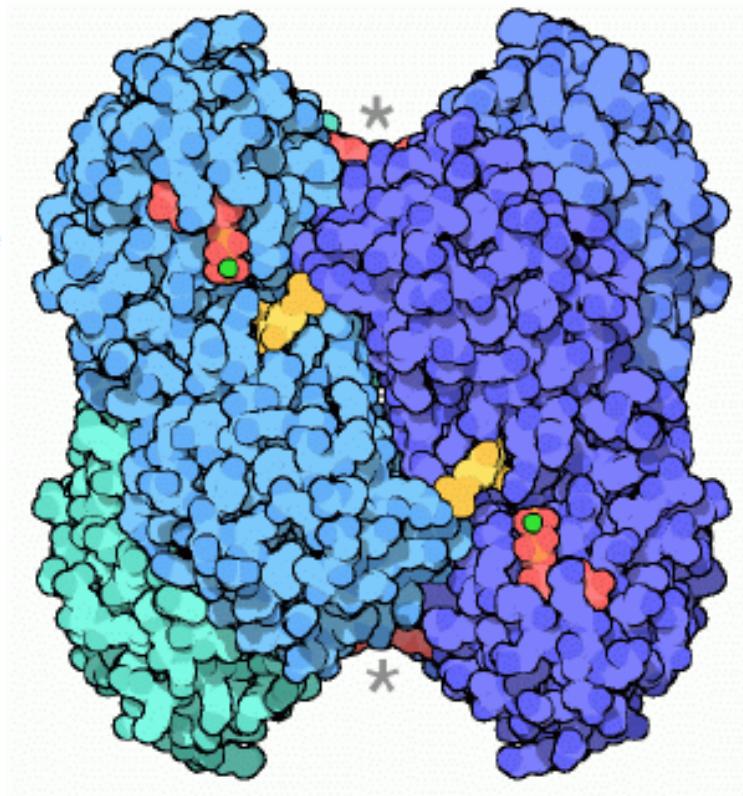
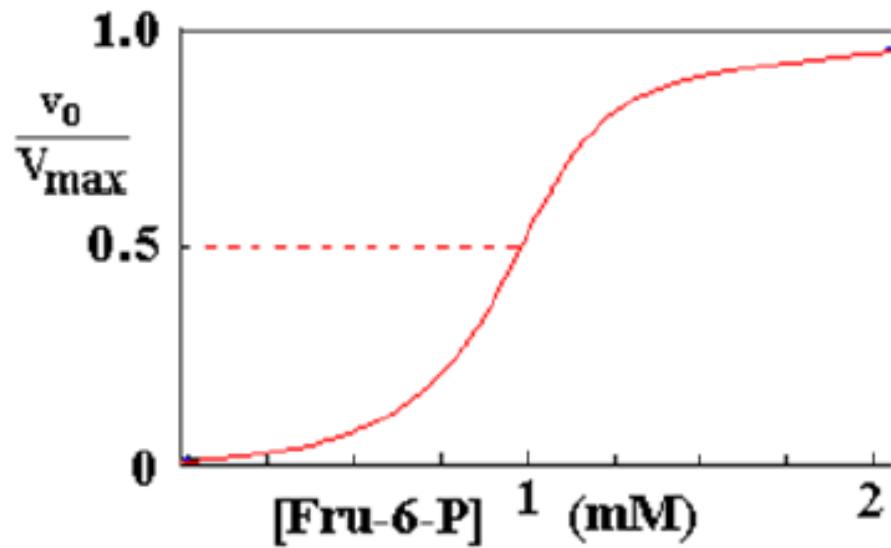
$$v = \frac{V_{max} \cdot S}{K_m + S}$$

An example is the M-M equation

Sigmoid responses are generally seen in multimeric systems

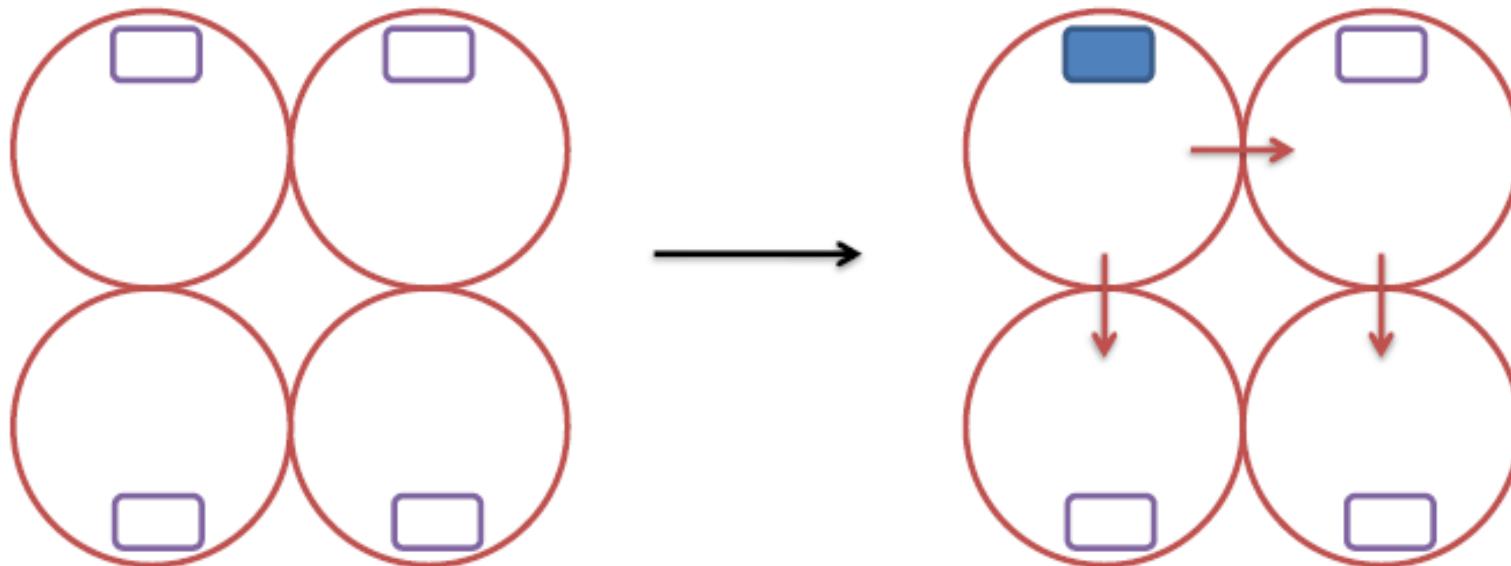
Phosphofructokinase

Tetramer of identical subunits



Sigmoid responses arise from cooperative interactions

Binding at one site results in changes in the binding affinities at the remaining sites.



Hill Equation – Simplest Model

We assume that the ligands bind simultaneously (unrealistic!):



Assuming Rapid Equilibrium

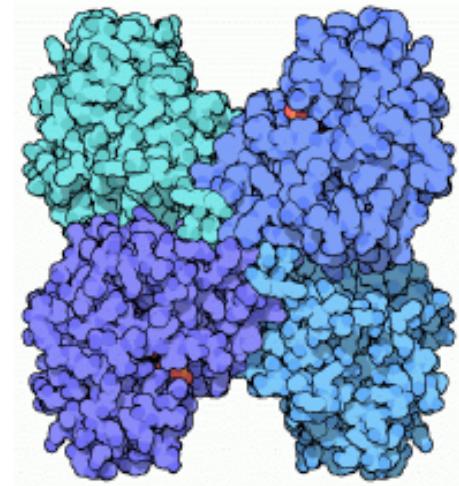
$$K = \frac{ES}{E \cdot S^n}$$

$$E_t = E + ES$$

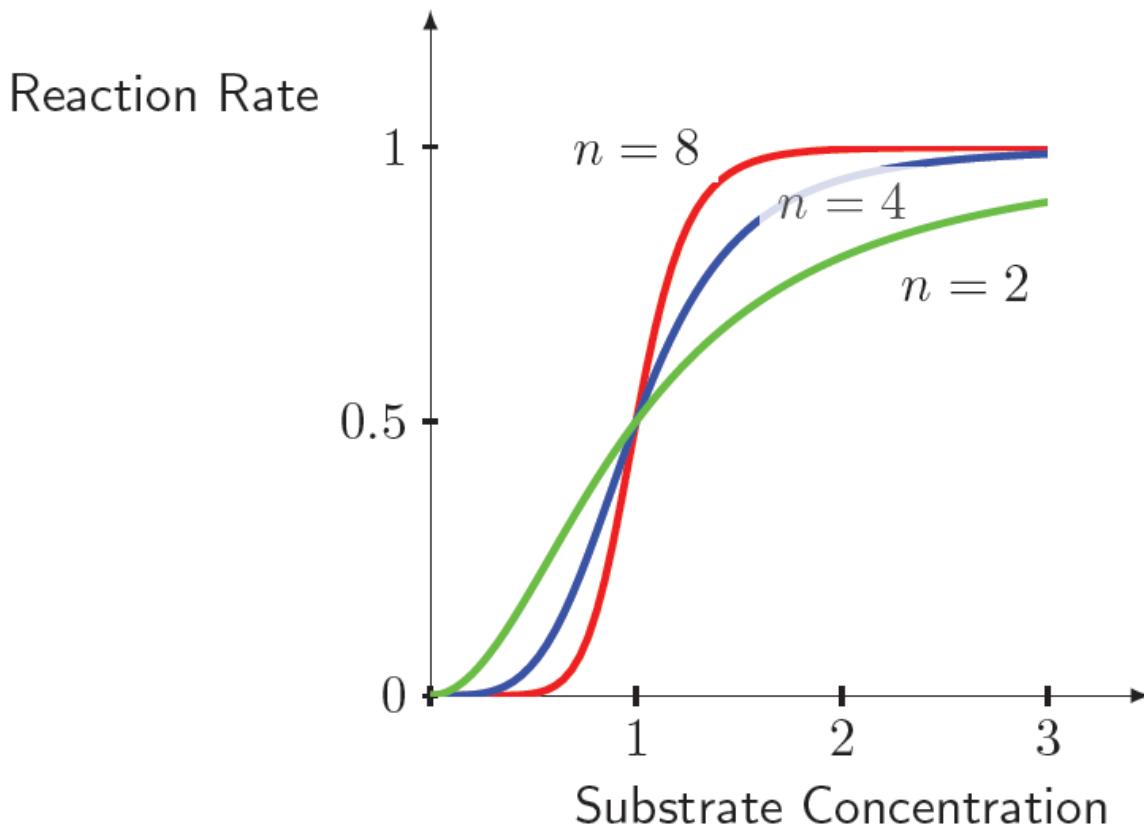
$$\frac{ES}{E_t} = \frac{S^n}{1/K + S^n}$$



$$v = \frac{V_{max} S^h}{K_H + S^h}$$



Hill equation



The Hill Coefficient, **n**, describes the degree of cooperativity.

If $n = 1$, the equation reverts to a simple hyperbola

$n > 1$: Positive Cooperativity

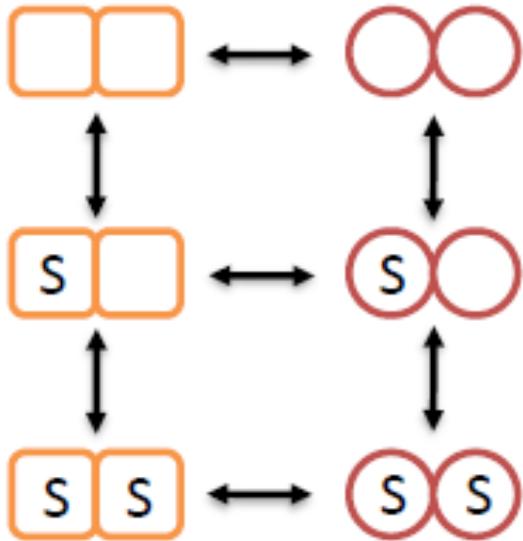
$n = 1$: No Cooperativity

$n < 1$: Negative Cooperativity

$$v = \frac{V_{\max} S^n}{K + S^n}$$

S^n ← Hill coefficient

Other Models –MWC Model MWC Model or concerted model (Monod, Wyman, Changeux)



Is disallowed

1. Subunits exist in two conformations, relaxed (R) and taut (T)
2. One conformation has a higher binding affinity than the other (R)
3. Conformations within a multimer are the same
4. Conformations are shifted by binding of ligand



Taut (T) – less active

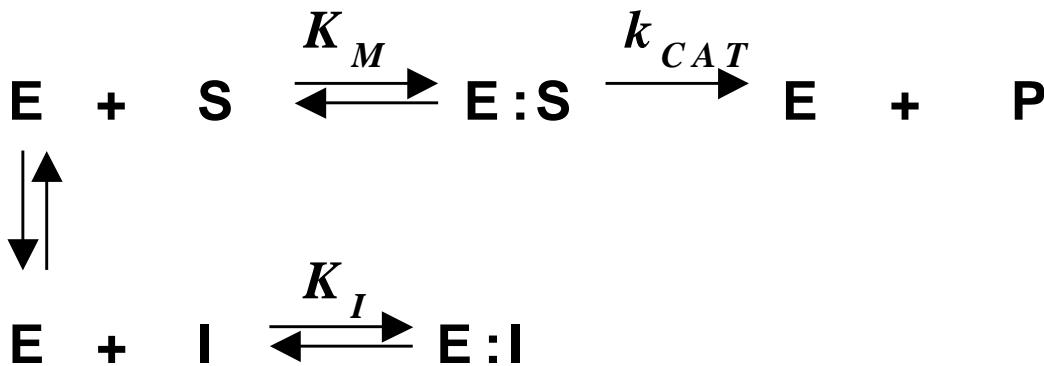


Relaxed (R) – more active

LECTURE 9:

ENZYME INHIBITION AND APPLICATIONS

ENZYME INHIBITION AND APPLICATIONS



$$K_I$$

$$K_I = \frac{[E][I]}{[E:I]}$$

Units of K_I and IC_{50} = M

Why inhibit enzymes?

- Certain **disease states** may be caused by the product of an enzyme catalysis
- **Drugs** can target enzymes which are present in pathogens but not in the host
- Understanding the nature of enzyme catalysis AND the mechanism of a biochemical reaction can lead to the **design of effective drugs**

Enzymes as Drug Targets - a Closer Look

Transition state analogs and “suicide substrates”

Table 1: Database of Enzyme Targets for Marketed Drugs

entry	enzyme common name	EC no. ^a	example drug	indication	no. of drugs	organism ^b	ref ^c
1	1,3- β -glucan synthase	2.4.1.34	caspofungin	antifungal	1	F	87
2	3(or 17)- β -hydroxysteroid dehydrogenase	1.1.1.51	trilostane	breast cancer	1	H	88
3	3',5'-cyclic-GMP phosphodiesterase	3.1.4.35	sildenafil	ED ^d	3	H	89
4	3',5'-cyclic-nucleotide phosphodiesterase	3.1.4.17	theophylline	asthma	11	H	90
5	4-hydroxyphenylpyruvate dioxygenase	1.13.11.27	nitisinone	tyrosinemia	1	H	91
6	3-oxo-5- α -steroid 4-dehydrogenase	1.3.99.5	finasteride	BPH ^e	2	H	92
7	acetylcholinesterase	3.1.1.7	pyridostigmine	MG ^f	11	H	93
8	adenosine deaminase	3.5.4.4	pentostatin	HCL ^g	2	H	94
9	alanine racemase	5.1.1.1	cycloserine	tuberculosis	1	B	95
10	alcohol dehydrogenase	1.1.1.1	fomepizole	alcoholism	1	H	96
11	aldehyde dehydrogenase (NAD)	1.2.1.3	disulfiram	alcoholism	1	H	97
12	α -amylase	3.2.1.1	acarbose	diabetes	1	H	98
13	α -glucosidase	3.2.1.20	miglitol	diabetes	1	H	99
14	amine oxidase (flavin-containing)	1.4.3.4	tranylcypromine	depression	5	H	100
15	arabinosyltransferase	2.4.2.34	ethambutol	antibacterial	1	B	101
16	arachidonate 5-lipoxygenase	1.13.11.34	zileuton	inflammation	4	H	102
17	aromatic L-amino acid decarboxylase	4.1.1.28	carbidopa	Parkinson's	1	H	103
18	β -lactamase	3.5.2.6	tazobactam	antibacterial	3	B	104
19	carbonate dehydratase ^h	4.2.1.1	acetazolamide	glaucoma	6	H	105
20	catechol O-methyltransferase	2.1.1.6	entacapone	Parkinson's	2	H	106
21	ceramide glucosyltransferase	2.4.1.80	miglustat	Gaucher's	1	H	107
22	D-alanine-D-alanine ligase	6.3.2.4	cycloserine	tuberculosis	1	B	108
23	dihydrofolate reductase	1.5.1.3	methotrexate	leukemia	7	H	109
24	dihydroorotate dehydrogenase	1.3.99.11	leflunomide	inflammation	1	H	110
25	dihydropteroate synthase	2.5.1.15	dapsone	antifungal	17	F	111
26	DNA topoisomerase	5.99.1.2	topotecan	ovarian cancer	2	H	112
27	DNA topoisomerase (ATP-hydrolyzing)	5.99.1.3	ciprofloxacin	antibacterial	18	B	113
28	DNA-directed DNA polymerase	2.7.7.7	acyclovir	herpes	11	V	114
29	DNA-directed RNA polymerase	2.7.7.6	rifapentine	antibacterial	3	B	115
30	dolichyl phosphatase	3.1.3.51	bacitracin	antibacterial	1	B	116
31	enoyl-[acyl carrier protein] reductase	1.3.1.9	isoniazid	tuberculosis	1	B	117
32	exo- α -sialidase	3.2.1.18	oseltamivir	influenza	2	V	118
33	factor Xa	3.4.21.6	fondaparinux	thrombosis	2	H	119
34	farnesyl-diphosphate farnesyltransferase	2.5.1.21	alendronate	osteoporosis	6	H	120
35	fatty acid synthase	2.3.1.85	pyrazinamide	tuberculosis	1	B	121

Enzymes as Drug Targets - a Closer Look

Transition state analogs and “suicide substrates”

36	glucan 1,4- α -glucosidase	3.2.1.3	miglitol	diabetes	1	H	122
37	histone acetyltransferase	2.3.1.48	valproic	seizures	1	H	123
38	HIV-1 retropepsin	3.4.23.16	nelfinavir	AIDS ^h	8	V	124
39	hydrogen/potassium-exchanging ATPase	3.6.3.10	esomeprazole	GERD ⁱ	5	H	125
40	HMG-CoA reductase (NADPH2)	1.1.1.34	atorvastatin	hyperlipidemia	6	H	126
41	IMP dehydrogenase	1.1.1.205	mycophenolate	IS ^j	2	H	127
42	iodide peroxidase	1.1.1.18	propylthiouracil	hyperthyroid	2	H	128
43	isoleucine tRNA ligase	6.1.1.5	mupirocin	antibacterial	1	B	129
44	membrane dipeptidase	3.4.13.19	cilastatin	resistance	1	H	130
45	ornithine decarboxylase	4.1.1.17	eflornithine	trypanosomes	1	P	131
46	orotidine-5'-phosphate decarboxylase	4.1.1.23	allopurinol	gout	1	H	132
47	peptidyl-dipeptidase A	3.4.15.1	captopril	hypertension	12	H	133
48	phosphoribosylglycinamide formyltransferase	2.1.2.2	pemetrexed	cancer	1	H	134
49	plasma kallikrein	3.4.21.34	aprotinin	thrombosis	1	H	135
50	plasmin	3.4.21.7	aminocaproic	thrombosis	3	H	136
51	prostaglandin-endoperoxide synthase	1.14.99.1	etodolac	inflammation	30	H	137
52	proteasome endopeptidase complex	3.4.25.1	bortezomib	myeloma	1	H	138
53	protein-tyrosine kinase	2.7.1.112	imatinib	leukemia	3	H	139
54	ribonucleoside-diphosphate reductase	1.17.4.1	gemcitabine	cancer	4	H	140
55	RNA-directed RNA polymerase	2.7.7.48	ribavirin	pneumonia	1	V	141
56	RNA-directed DNA polymerase	2.7.7.49	abacavir	AIDS	13	V	142
57	serine-type D-Ala-D-Ala carboxypeptidase	3.4.16.4	cefonicid	antibacterial	52	B	143
58	sodium/potassium-exchanging ATPase	3.6.3.9	digitoxin	CHF ^k	3	H	144
59	squalene monooxygenase	1.14.99.7	butenafine	antifungal	3	F	145
60	sterol 14-demethylase	1.14.13.70	itraconazole	antifungal	11	F	146
61	sucrose α -glucosidase	3.2.1.48	miglitol	diabetes	1	H	147
62	thrombin	3.4.21.5	lepirudin	thrombosis	10	H	148
63	thymidylate synthase	2.1.1.45	floxuridine	cancer	6	H	149
64	thyroxine 5'-deiodinase	1.97.1.10	propylthiouracil	hyperthyroid	1	H	150
65	triacylglycerol lipase	3.1.1.3	orlistat	obesity	1	H	151
66	tyrosine 3-monooxygenase	1.14.16.2	metyrosine	PC ^l	1	H	152
67	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	2.5.1.7	fosfomycin	antibacterial	1	B	153
68	unspecific monooxygenase	1.14.14.1	aminoglutethimide	breast cancer	5	H	154
69	urease	3.5.1.5	acetohydroxamic	gastritis	1	B	155
70	vitamin-K-epoxide reductase (warfarin-sens.)	1.14.1	dicumarol	thrombosis	5	H	156
71	xanthine oxidase	1.17.3.2	allopurinol	gout	1	H	157

^a EC numbers are from IUBMB Enzyme Nomenclature (www.chem.qmw.ac.uk/iubmb/enzyme). ^b Organism is the target organism: H, human; B, bacterial; V, viral; F, fungal; P, protozoal. ^c The reference is a general reference for the target. ^d Erectile dysfunction. ^e Benign prostatic hyperplasia.

^f Myasthenia gravis. ^g Hairy cell leukemia. ^h Acquired immunodeficiency syndrome. ⁱ Gastroesophageal reflux disease. ^j Immunosuppression.

^k Congestive heart failure. ^l Pheochromocytoma. ^m Several enzymes are better known by their more popular names: 3-oxo-5- α -steroid 4-dehydrogenase as 5- α -reductase, carbonate dehydratase as carbonic anhydrase, DNA topoisomerase as DNA gyrase, DNA-directed DNA polymerase as DNA polymerase, DNA-directed RNA polymerase as RNA polymerase, HIV-1 retropepsin as HIV protease, peptidyl-dipeptidase A as angiotensin-converting enzyme, RNA-directed DNA polymerase as reverse transcriptase, ribonucleoside-diphosphate reductase as ribonucleotide reductase, serine-type D-Ala-D-Ala carboxypeptidase as DD transpeptidase, and unspecific monooxygenase as microsomal P₄₅₀ or aromatase.

Drugs that act as enzyme inhibitors:

A. Reversible inhibitors

- Competitive
- Non-competitive

B. Irreversible inhibitors

Poisonous snake bite, plant alkaloids, Nerve gas, Malathion, Parathion, etc...

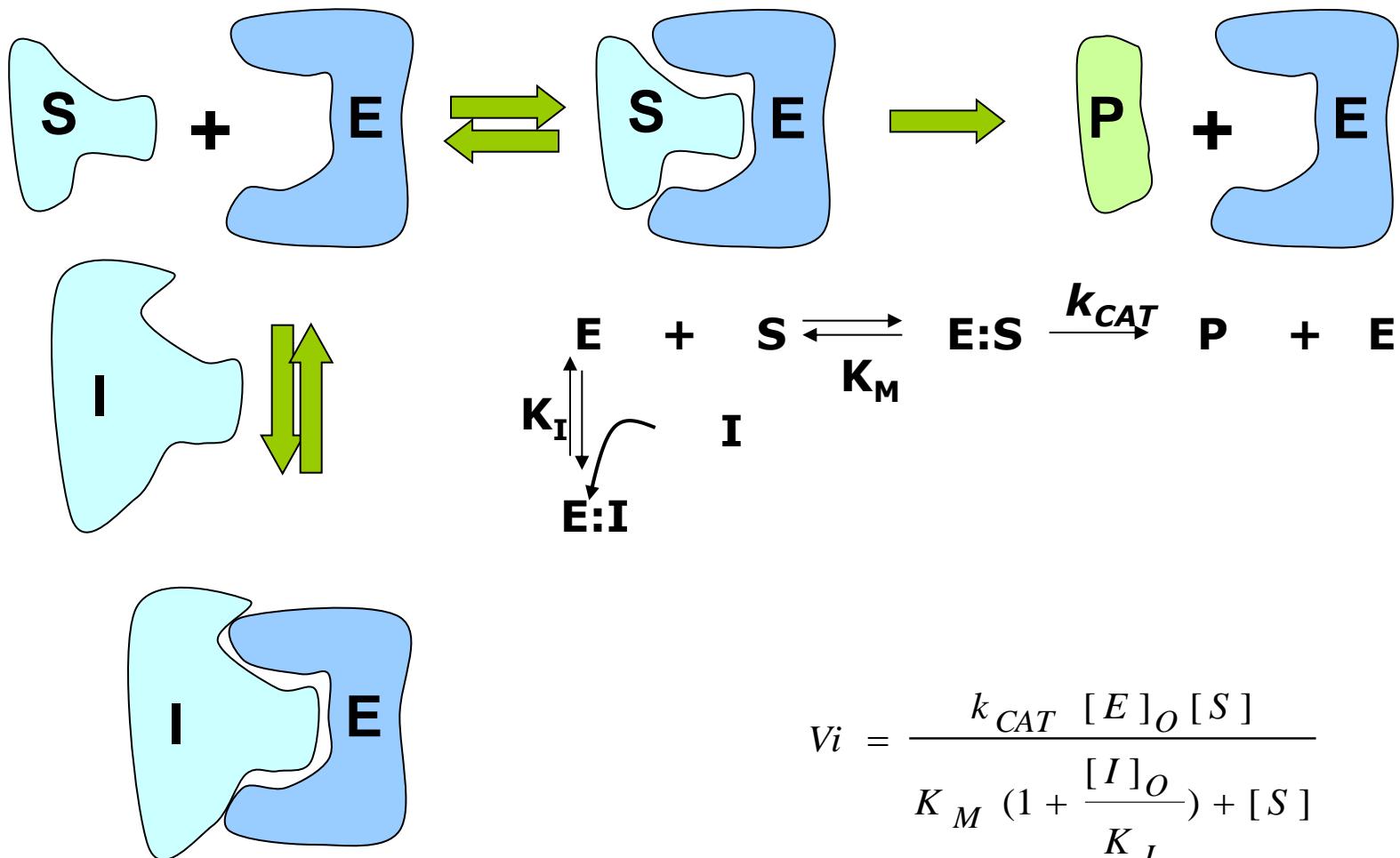
Reversible Inhibitors

To design a reversible competitive inhibitor as a drug, design a mimic of the substrate or the transition state

1. Transition state mimic for adenosine deaminase (enzyme which degrades anticancer drugs)
2. Substrate mimic for dihydropteroate synthase (dihydrofolate synthesis)
3. Transition state mimic for HMG-CoA reductase (cholesterol synthesis)

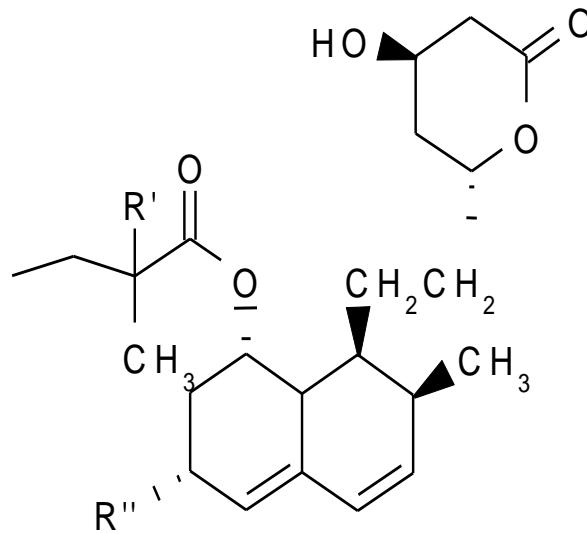
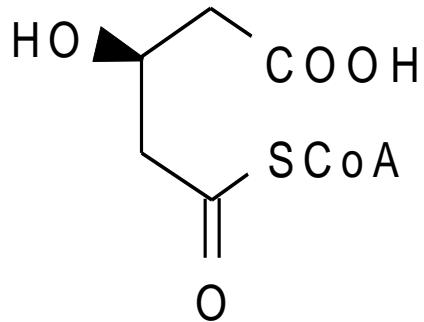
Substrate versus Transition-state analogs: Which approach should result in the highest affinity drug? Why?

a) Competitive Inhibition



❑ Competitive Inhibition Example

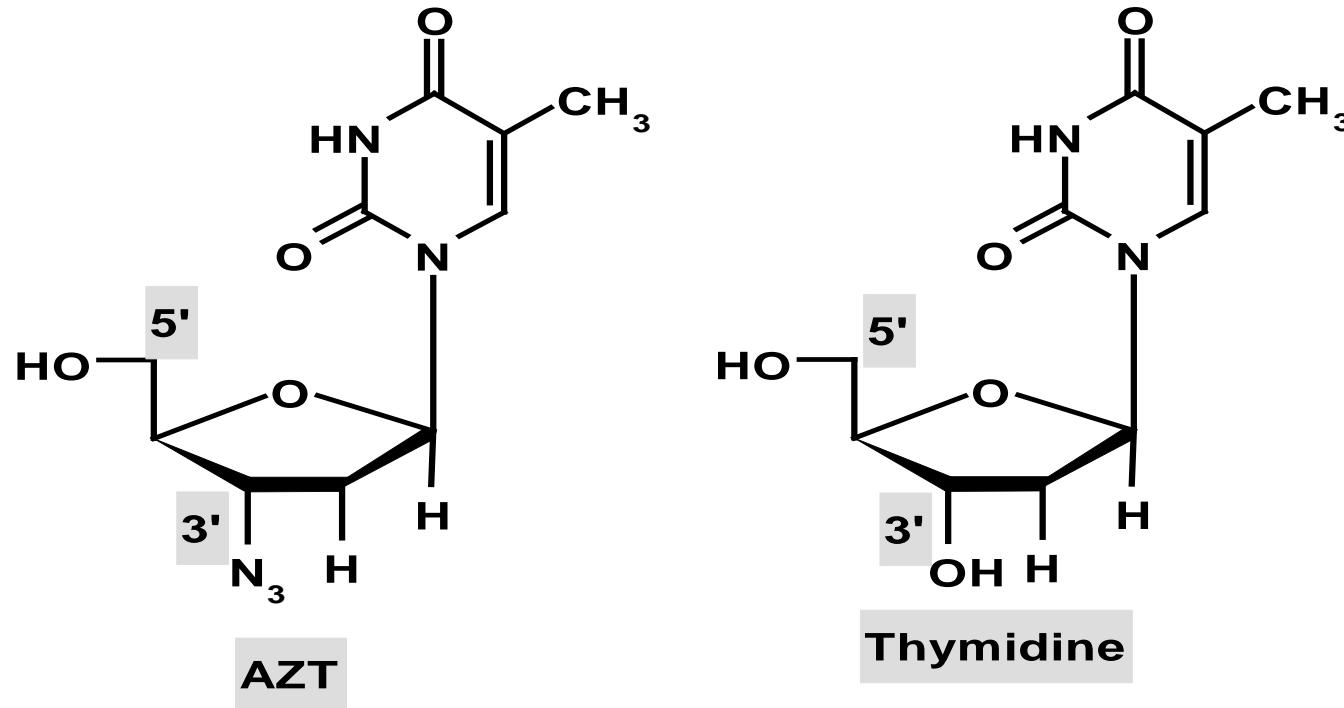
Design of Statins



Substrate

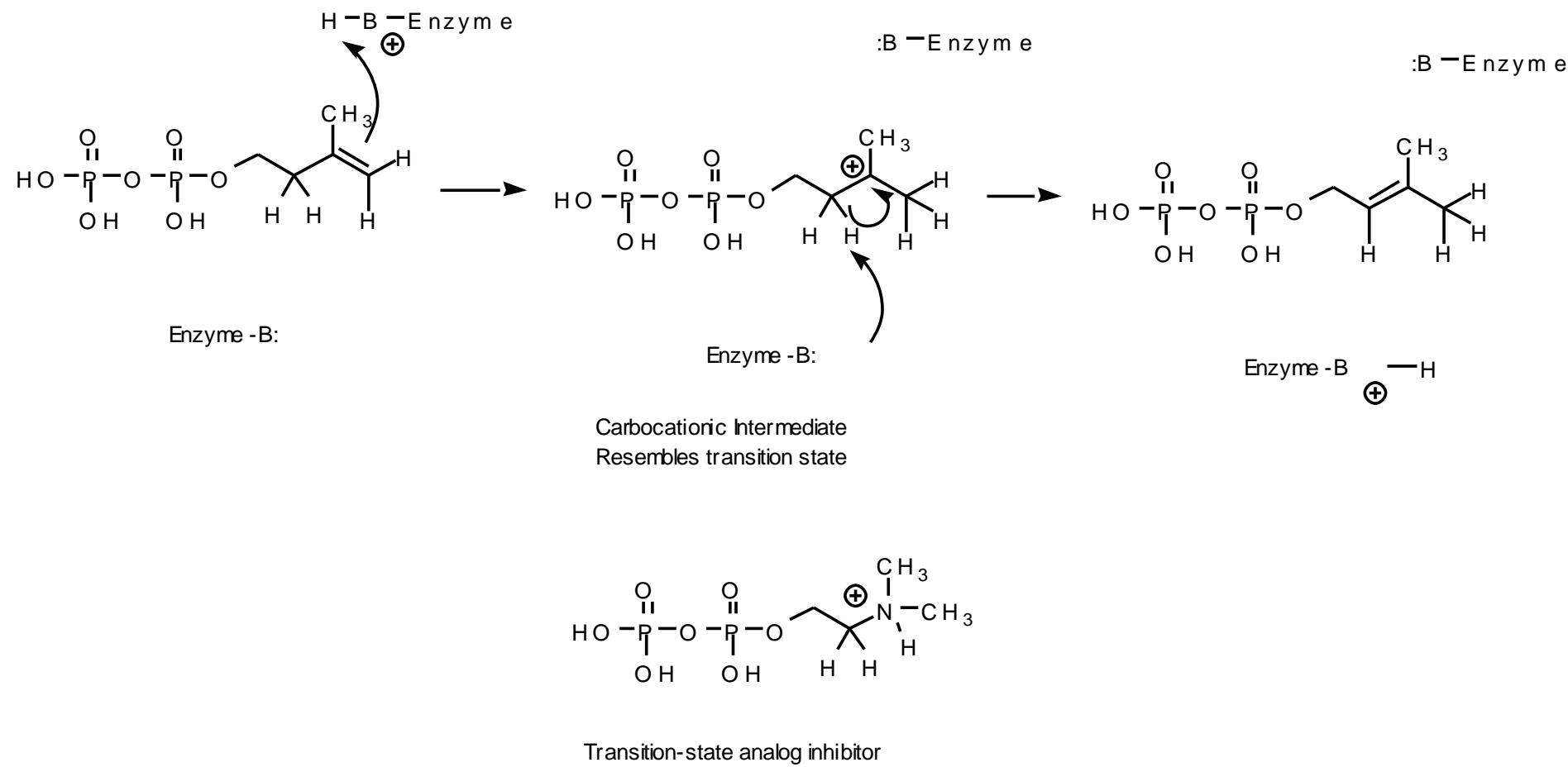
Inhibitor

□ Competitive Inhibition Example



AZT → potent inhibitor of HIV-RT, the retroviral polymerase which catalyzes the formation of proviral DNA from viral RNA

The transition state is stabilized *more* than the substrate



Example 1: Isopentyl Diphosphate isomerase, a key enzyme in isoprenoids synthesis

Example 2: Purine nucleoside phosphorylase. Lower activity causes T-cell immunodeficiency. Potential therapy for T-cell cancer and T-cell autoimmune disorders

Transition state structure was determined with analogs of substrates:

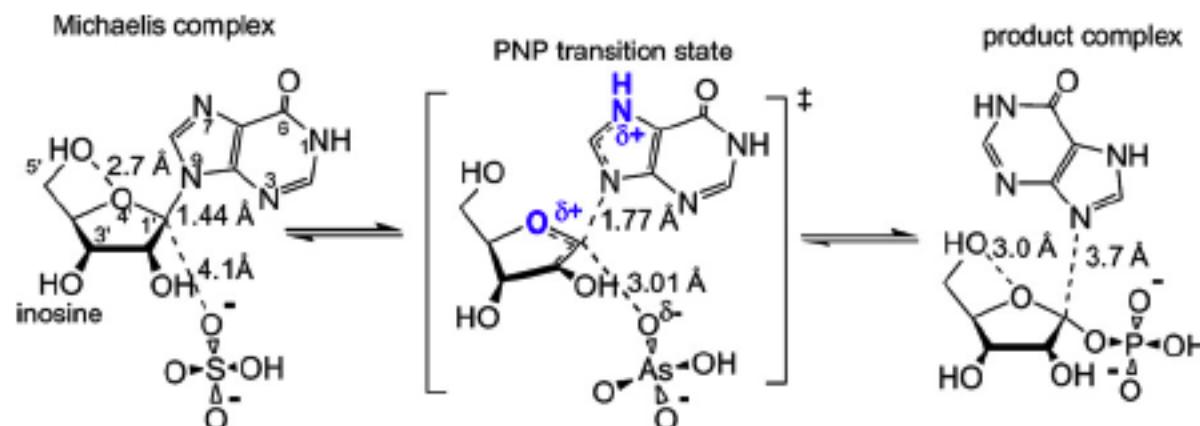


Fig. 2. Crystal structure distances of complexes representing the reaction catalyzed by PNP. The transition state parameters are for arsenolysis of inosine by bovine PNP. Distances in substrate and product complexes are from crystallography studies with the complexes shown in the figure [25,59].

(Ex. 2 continued)....

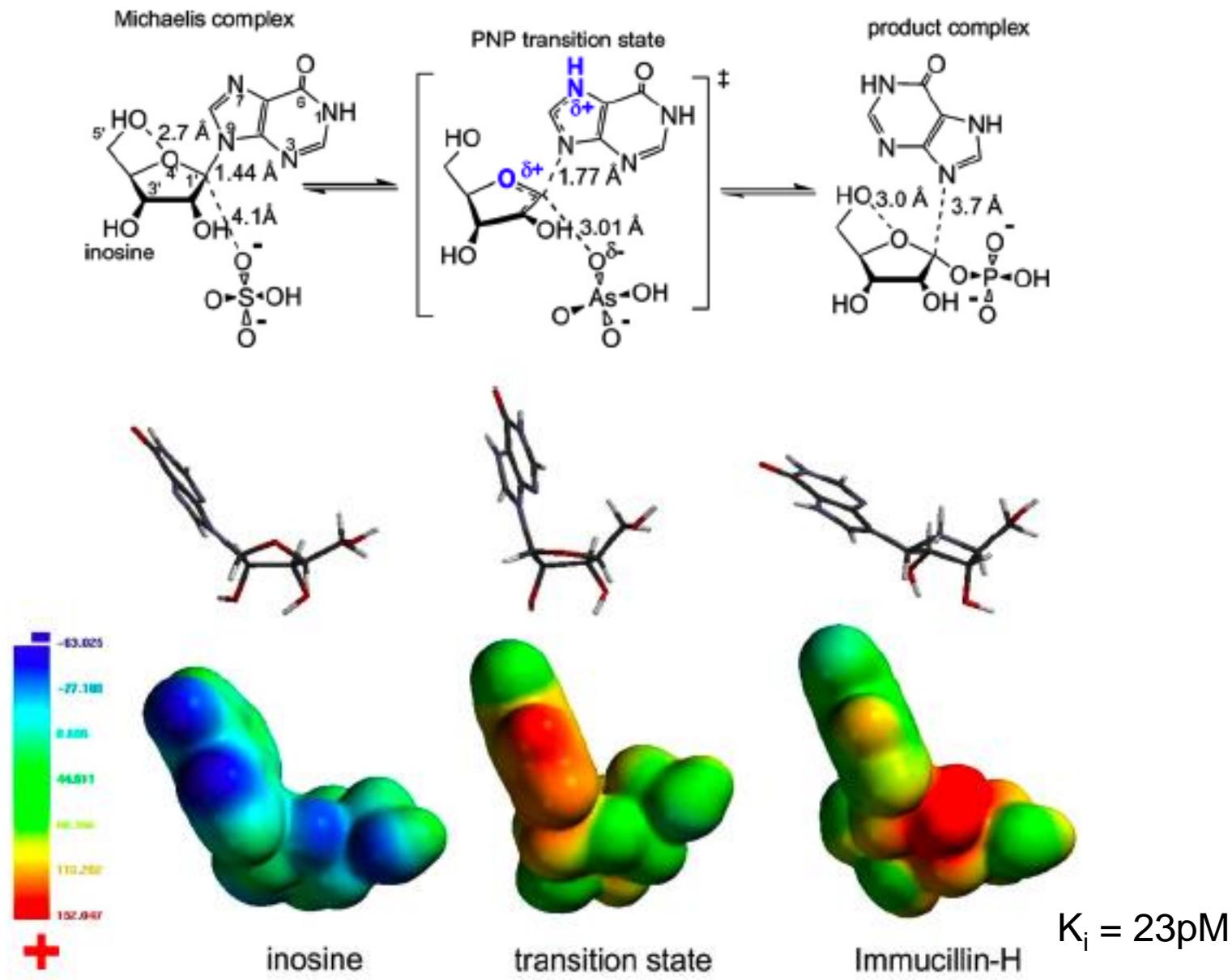
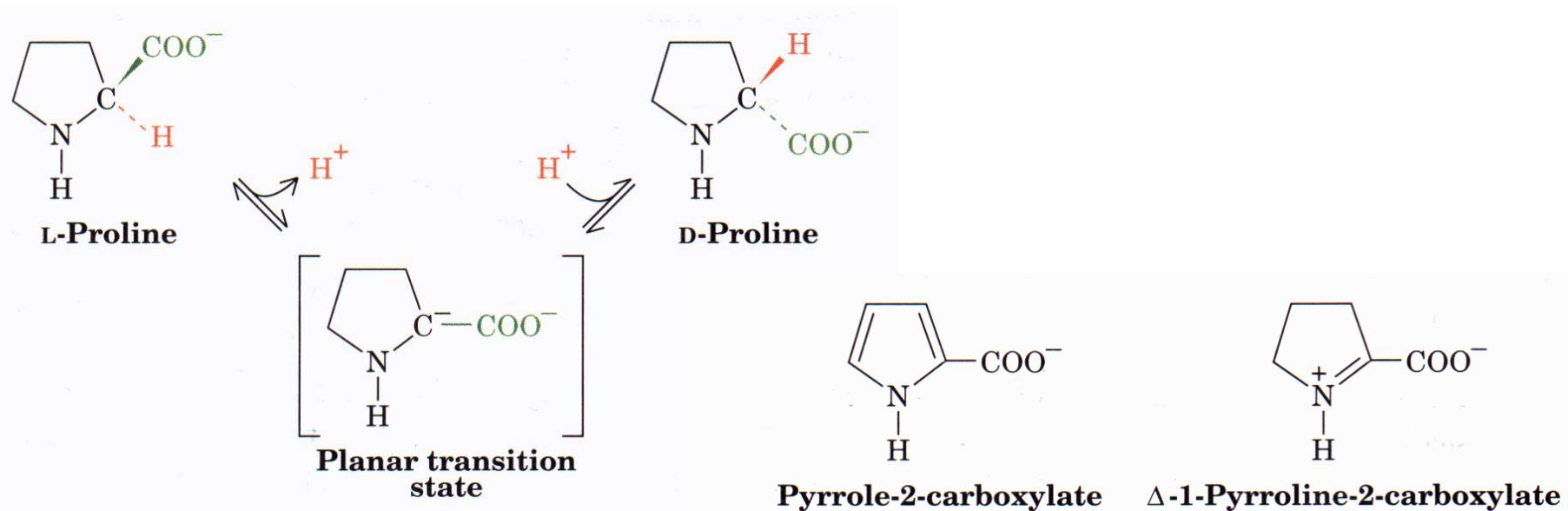
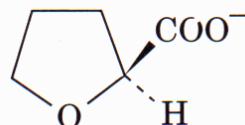


Fig. 3. Molecular electrostatic potential surfaces of inosine, transition state and Immucillin-H as the cation. The transition state is for bovine PNP. The figure is from [34] with permission of the publisher.

Example 3: Transition state analogues.....

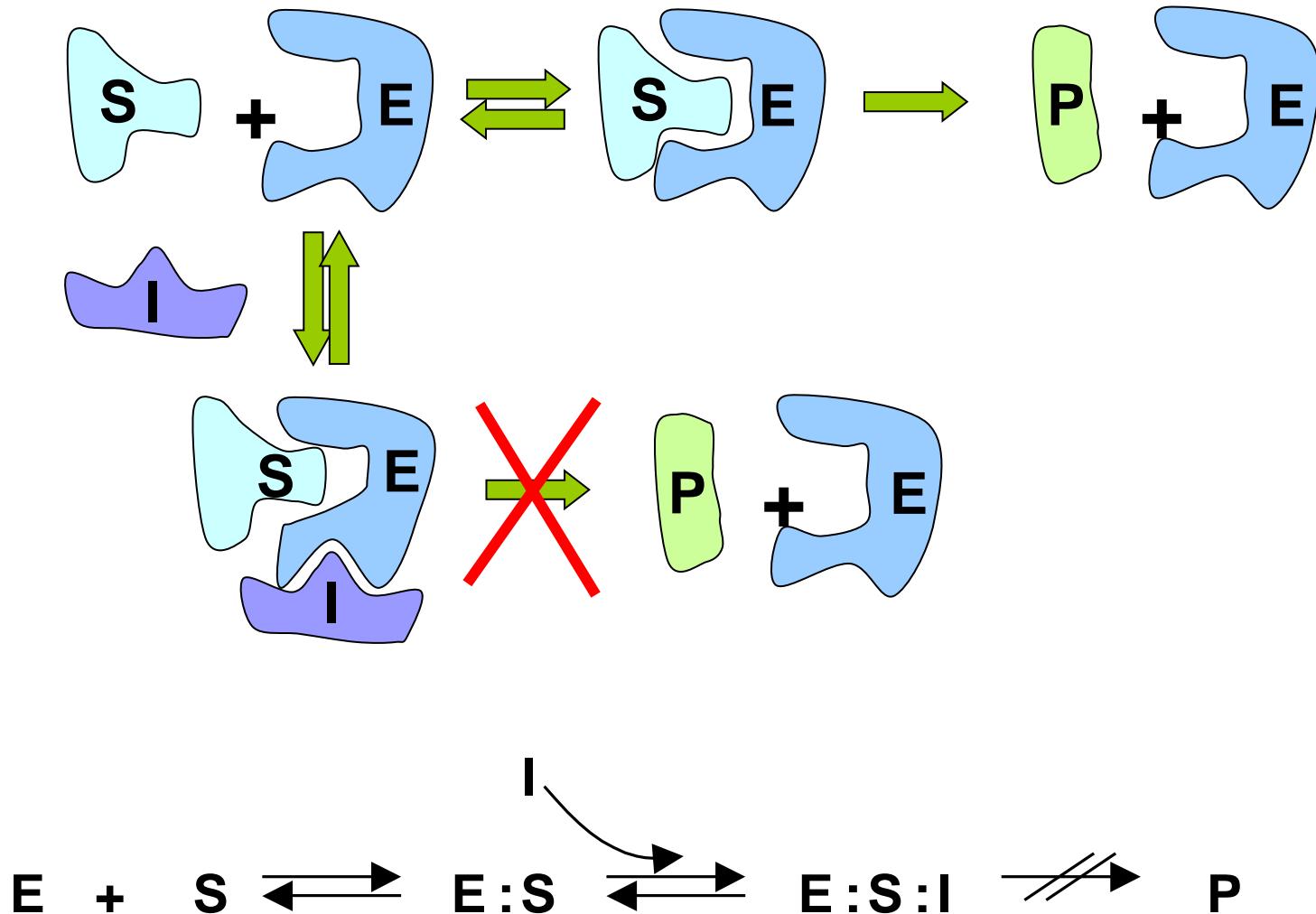


both of which bind to the enzyme with 160-fold greater affinity than does proline. These compounds are therefore thought to be analogs of the transition state in the proline racemase reaction. In contrast, **tetrahydrofuran-2-carboxylate**,

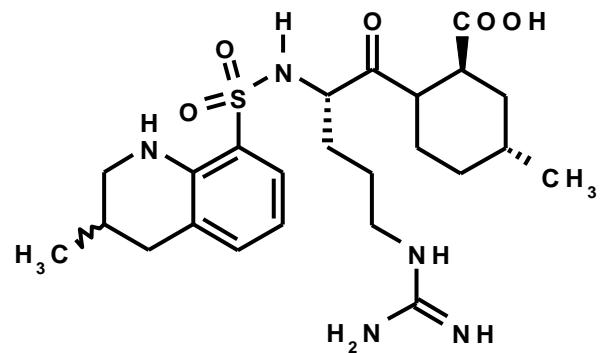


Tetrahydrofuran-2-carboxylate

b) Non-Competitive Inhibition



□ Non-Competitive Inhibition ... Example

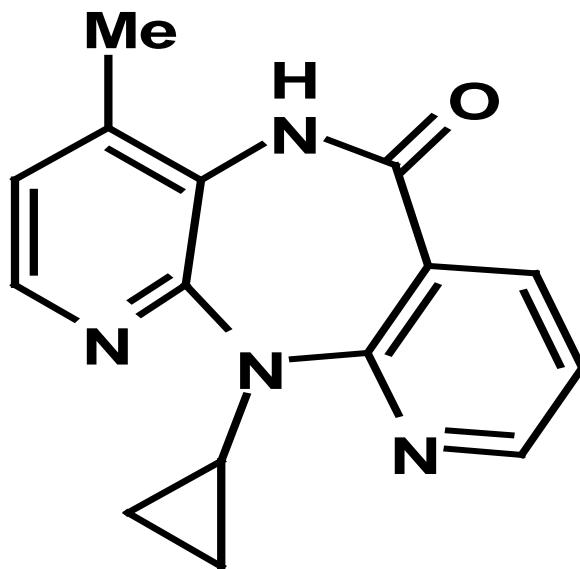


Argatroban

H₂N-D-Phe-Pro-Arg-Pro-Gly-Gly-Gly-Gly-Asn-Gly
HOOC-Leu-Tyr-Glu-Glu-Pro-Ile-Glu-Glu-Phe-Asp

Bivalirudin

❑ Non-Competitive Inhibition ... Example



Nevirapine

Nevirapine → potent inhibitor of HIV-RT ... $K_I \sim \text{nM}$...

- no resemblance to any of the natural nucleotide substrates ...
- binds in a hydrophobic binding pocket adjacent to the substrate-binding pocket and modifies the rate of polymerization

Recap:

- Reversible enzyme inhibitors bind *reversibly*!
- Competitive inhibitors’ structure should be more similar to that of the transition state for stronger binding
- Non-competitive and uncompetitive inhibitors can’t be “designed”, because they don’t resemble the substrate or transition state

Irreversible Inhibition

Can be grouped as:

- (a) coenzyme inhibitors
- (b) inhibitors of specific ion cofactor
- (c) prosthetic group inhibitors
- (d) apoenzyme inhibitors
- (e) Physiological modulators of the reaction,
such as the pH and temperature that
denature the enzyme catalytic site

- Most **irreversible** inhibitors interact with functional groups on the enzyme and **destroy enzyme** activity
- These interactions are covalent in nature
- These inhibitors are highly useful in studying enzyme reaction mechanisms



Suicide inhibitors

Time-dependent inhibitors

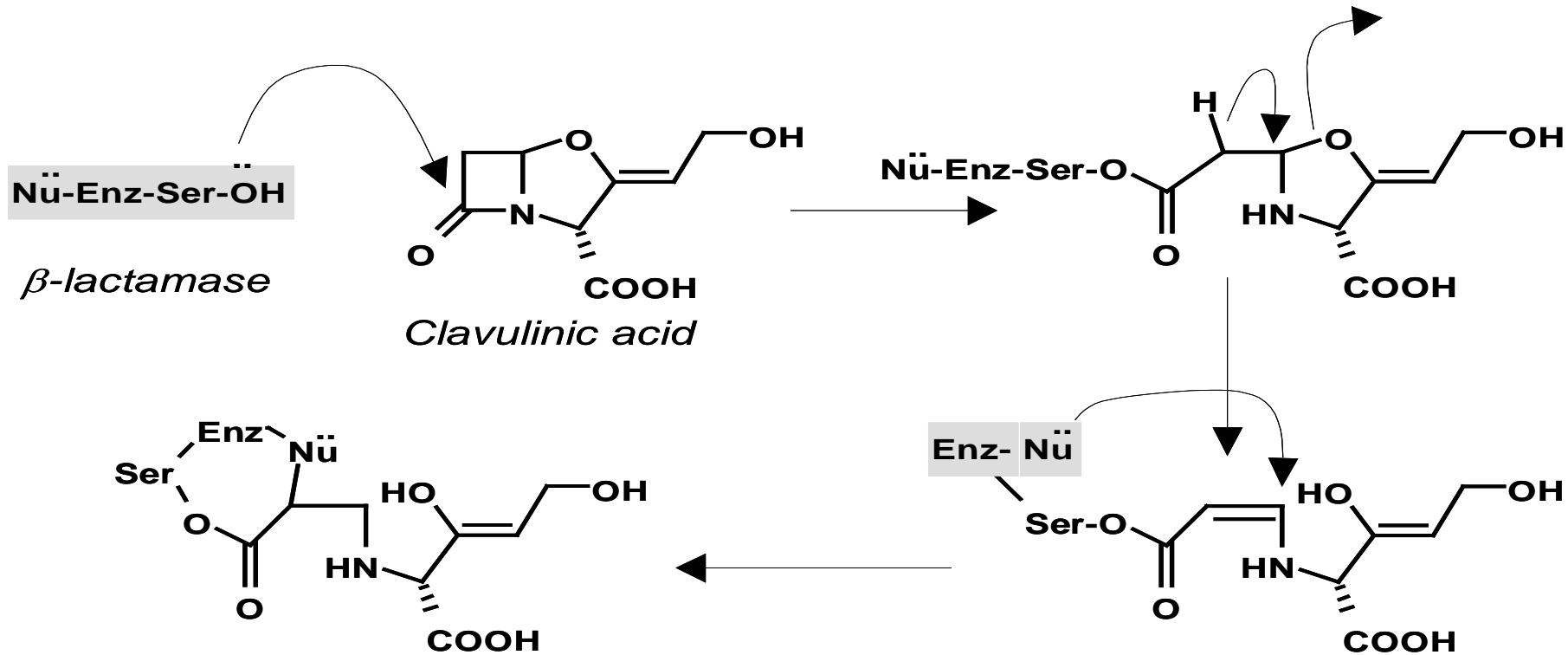
Heavy metal ion inhibitors

Irreversible Inhibition

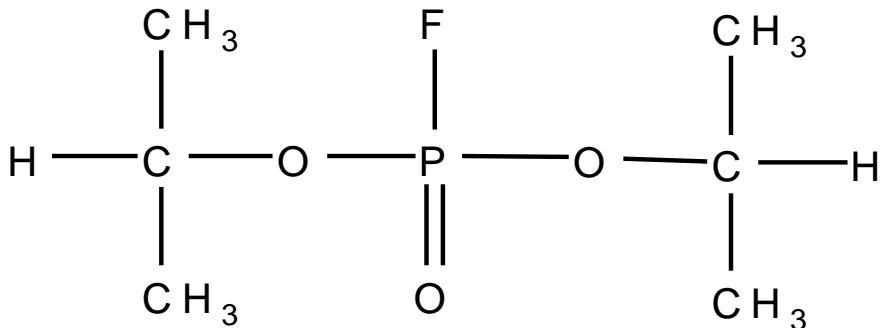
$E + S \rightleftharpoons E:S \longrightarrow E-S \longrightarrow \text{No further rxn.}$

Substrate behaves as an inhibitor!

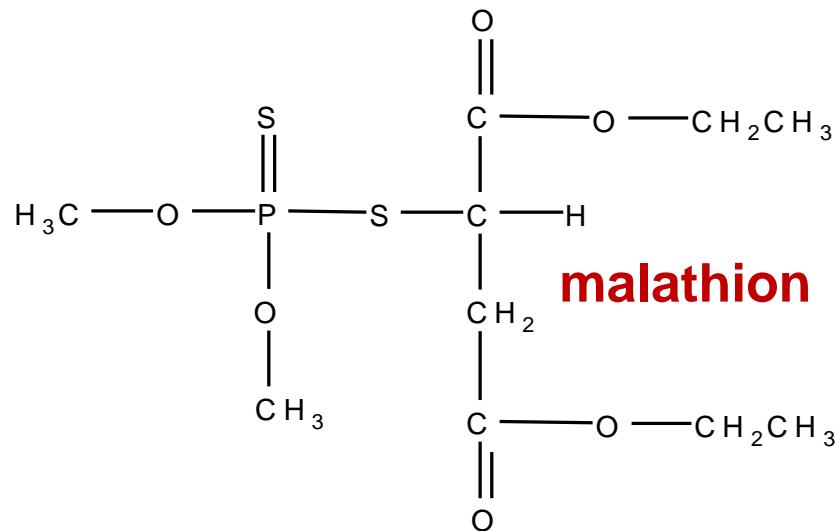
aka Suicide substrate inhibitors or Mouse trap inhibitors or Trojan horse inhibitors



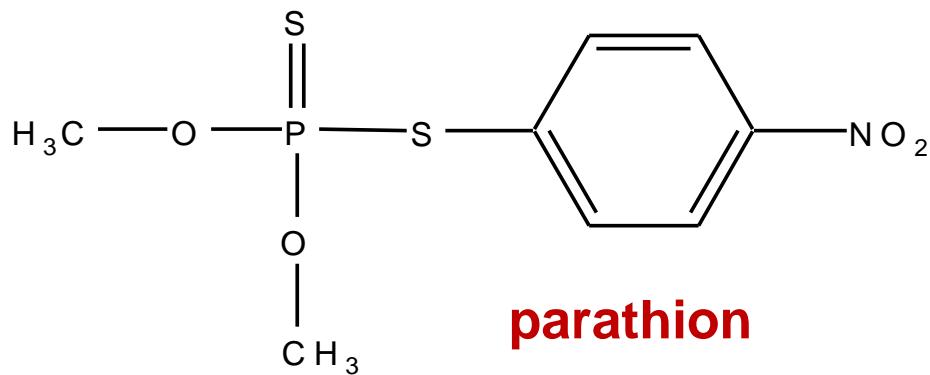
Irreversible Inhibitors



**Diisopropyl fluorophosphate
(nerve gas)**

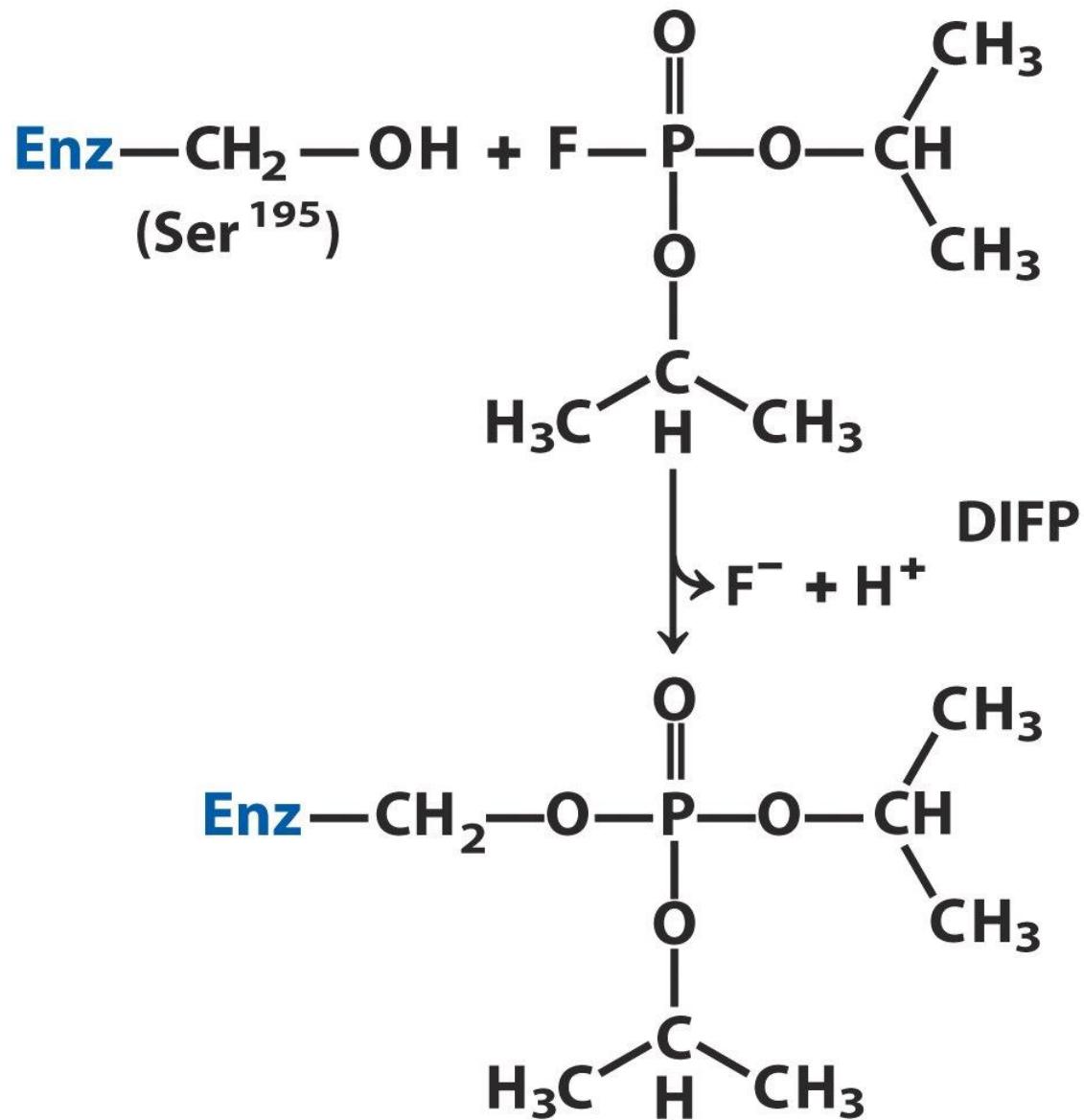


malathion



parathion

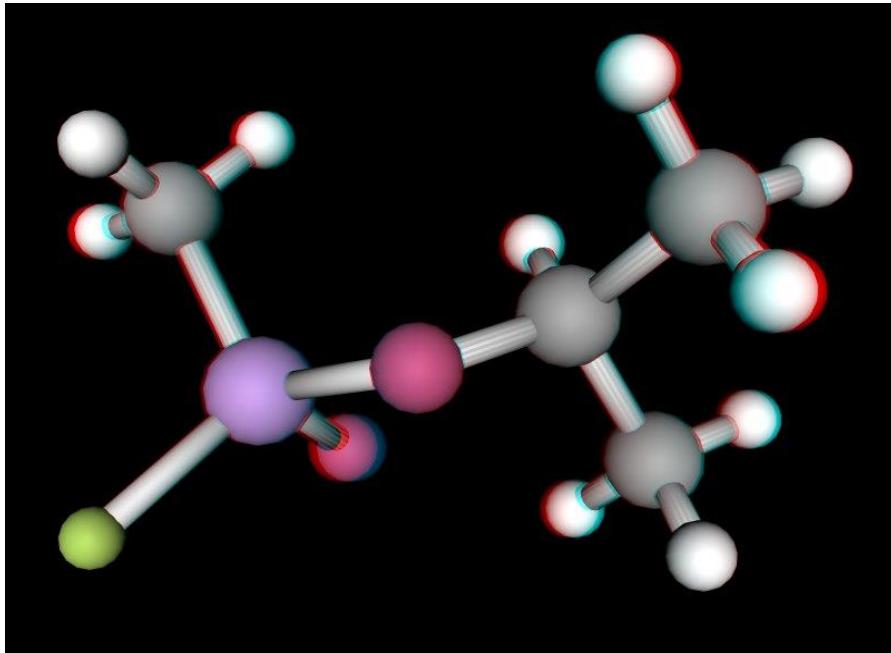
- Organophosphates
- Inhibit serine hydrolases
- Acetylcholinesterase inhibitors



The organophosphofluoride DIFP inactivates the enzyme by forming a permanent **P-O** covalent bond (suicide inhibitor)

More examples...

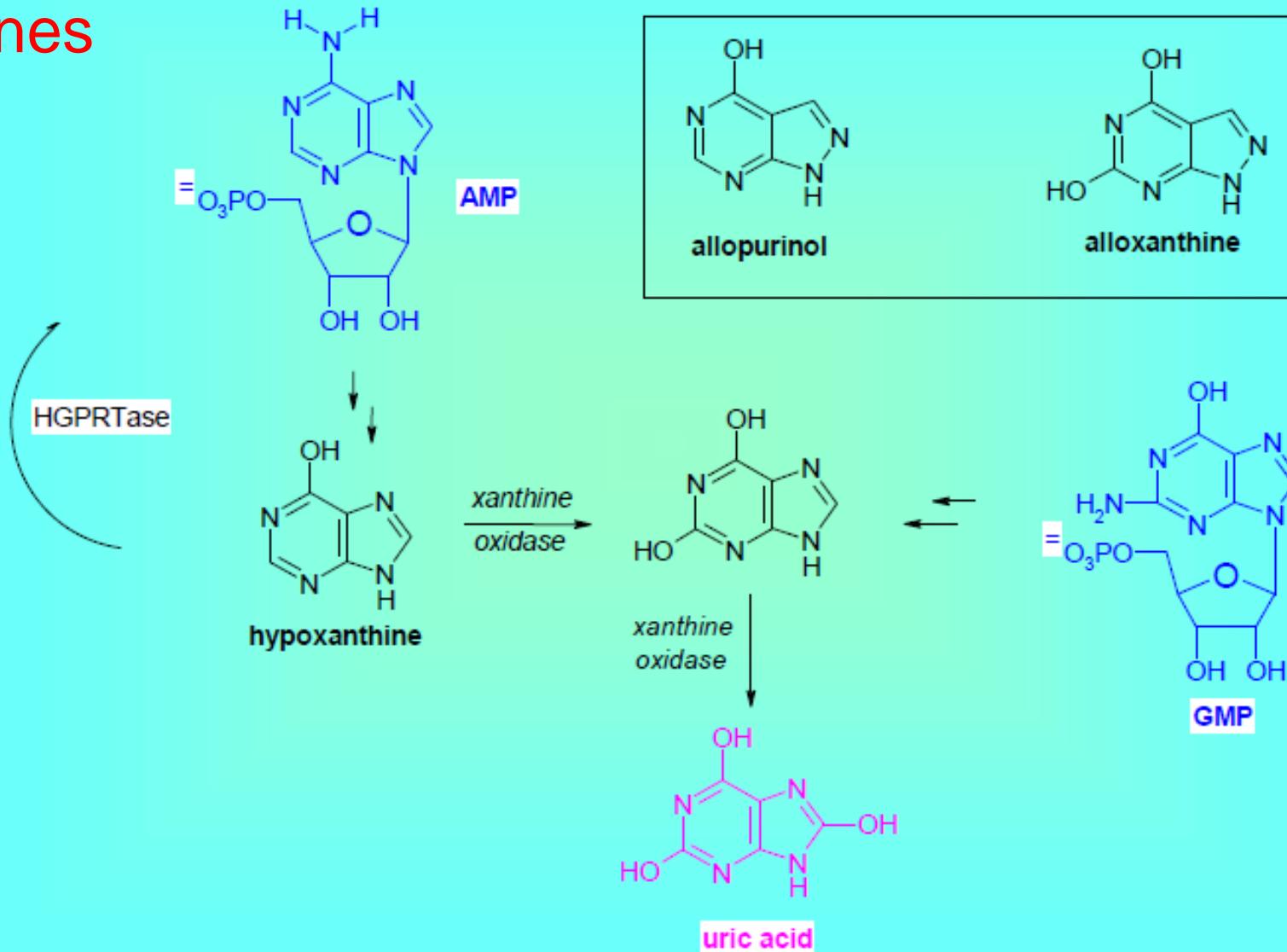
- Poisonous snake bite
- Plant alkaloids – **milkweed**, more examples?



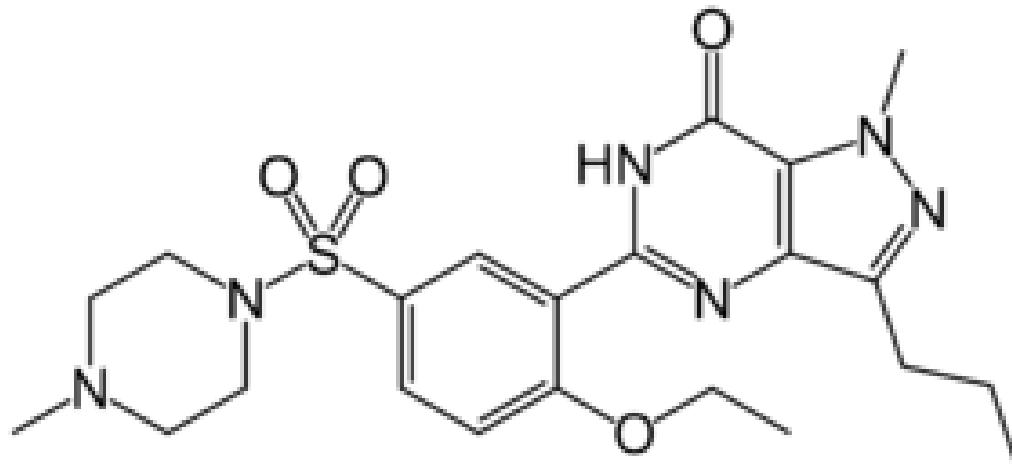
- Nerve gas

Sarin $\text{C}_4\text{H}_{10}\text{FO}_2\text{P}$

Allopurinol is an suicide inhibitor of xanthine oxidase; prevents the formation of uric acid from precursorial purines



The molecule below inhibits the enzyme PDE-5



Identify the drug and describe its mechanism of inhibition

Next lecture

Clinical applications of enzymes

LECTURE 10: CLINICAL APPLICATIONS OF ENZYMES AND ISOENZYMES

- Enzymes and Medicine
- Isoenzymes
- Marker Enzymes of Tissue Damage used in diagnosis
- Therapeutic enzymes

Enzymes and Medicine

- **Diagnostic indicators** – the activities of many enzymes are routinely determined in plasma (rarely in tissue biopsies) for diagnostic purposes in diseases of the heart, liver, skeletal muscle, pancreas and other tissues - **enzyme diagnostics**
- **Diagnostic tools** – use as chemicals in clinical laboratory assays
- **Therapeutic agents** – several enzymes are used as drugs; new approach - **enzymotherapy**

Why Enzymes are important in Medicine

1. Key to understanding inborn errors of metabolism.
2. Important in detoxification reactions.
3. Targets of chemotherapy.
4. Essential to rationale drug design.
5. Aid in diagnosis and monitoring therapy.
6. Key to many therapeutic and treatment strategies.
7. Key to metabolic control and balance.

Isoenzymes

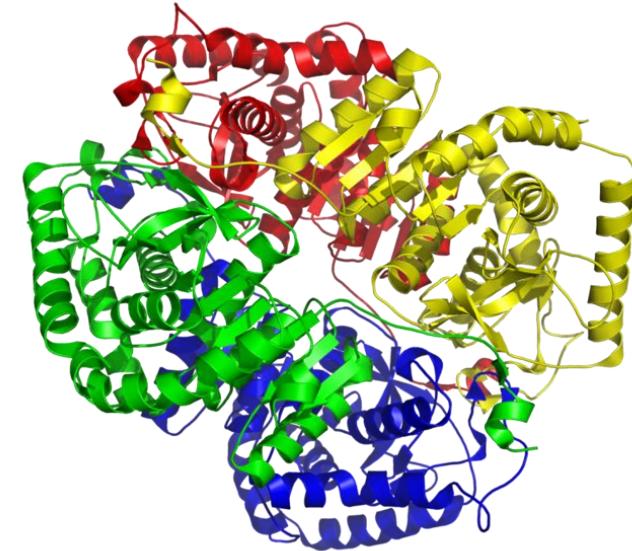
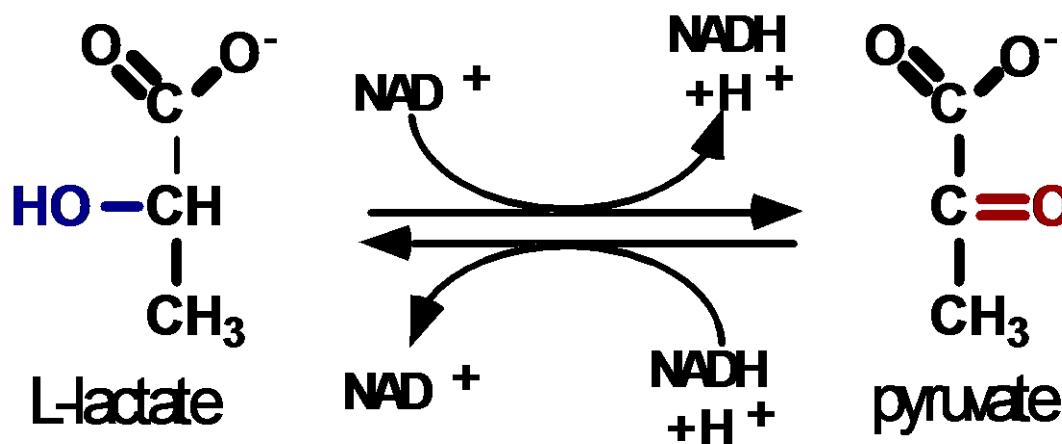
- Enzymes that have similar but not identical amino acid sequences but catalysing the same biochemical reaction

Results from gene duplication

- They differ in kinetics - different K_M and V_{max} values
- Use different effectors and forms of coenzymes
- Cellular distribution of each form will vary

Examples: Hexokinase – Muscle
Glucokinase - Liver

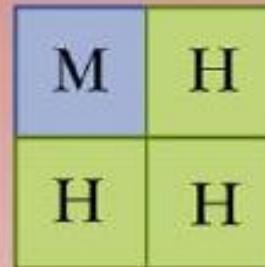
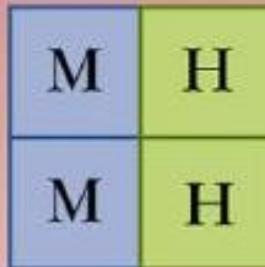
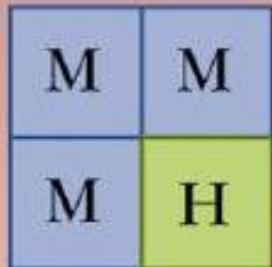
Lactate dehydrogenase (LDH)



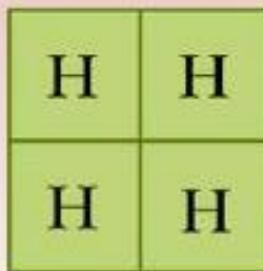
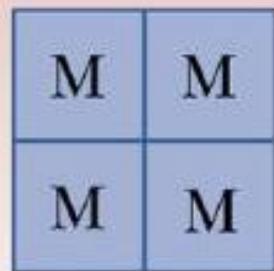
- LDH is a **tetramer** of two different types of subunits, called **H** and **M**, which have small differences in amino acid sequence.
- The two subunits can combine randomly with each other, forming **5 isoenzymes** that have the compositions H₄, H₃M, H₂M₂, HM₃, M₄.

Lactate dehydrogenase

?



Heterogeneous forms



Homogeneous forms

LDH 1

LDH 2

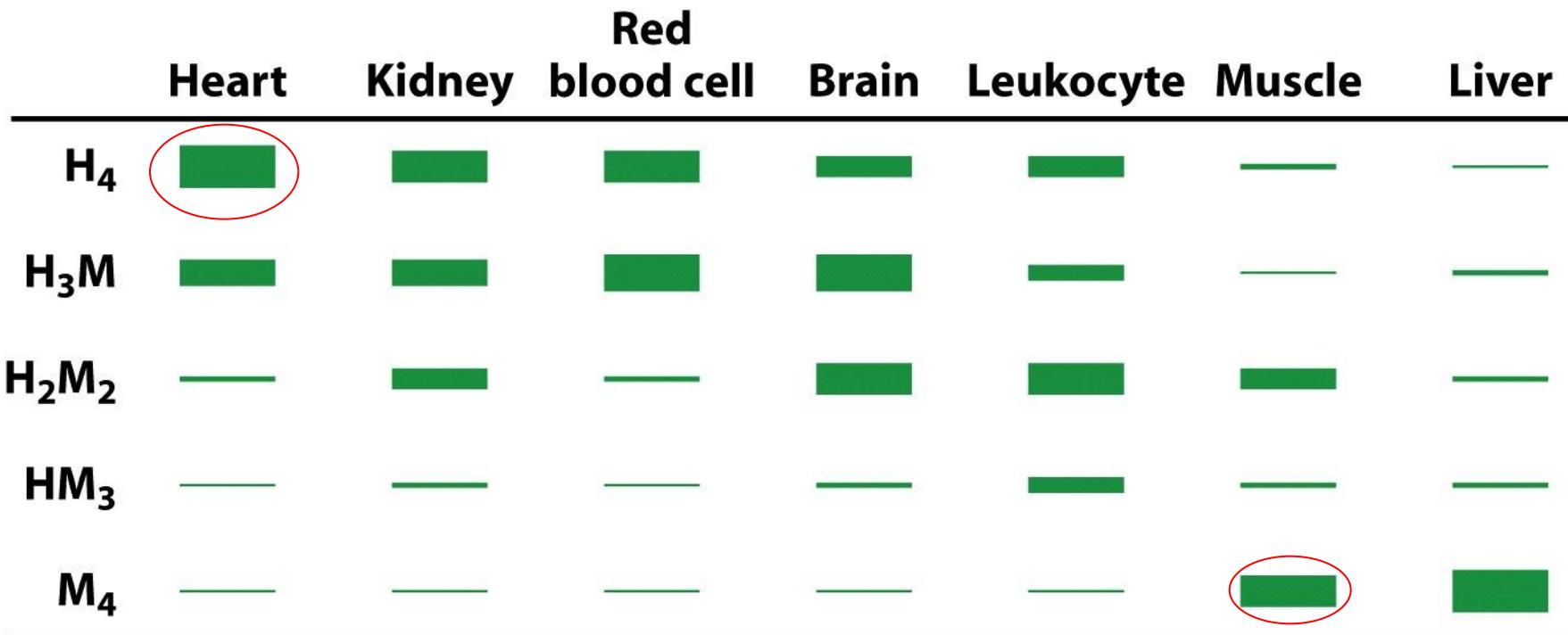
LDH 3

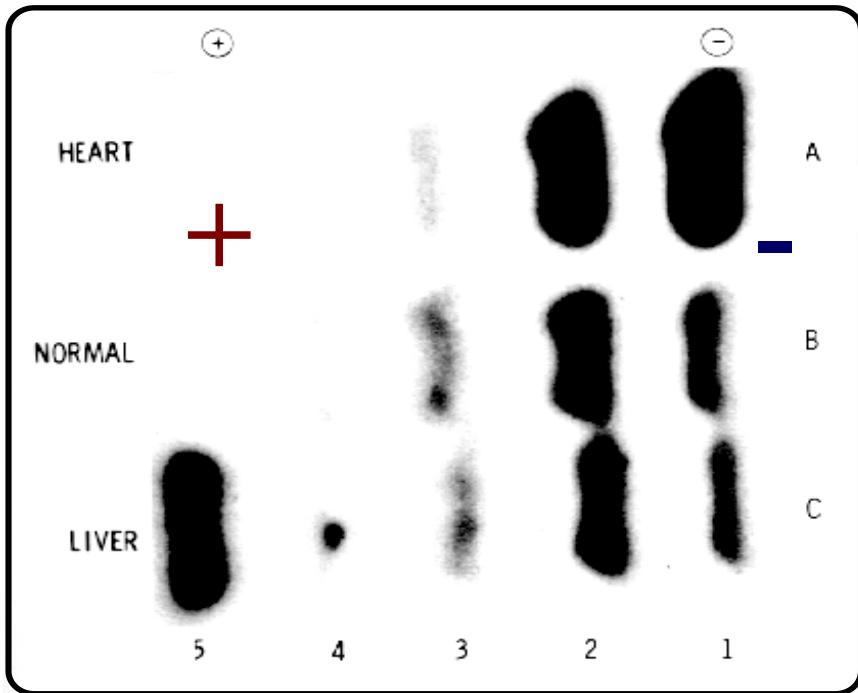
LDH 4

LDH 5

Isoenzymes of LDH

- LDH exists in 5 forms
 - LDH-1(H_4), LDH-2(H_3M): heart and red blood cells
 - LDH-3(H_2M_2): brain and kidney
 - LDH-4(HM_3), LDH-5(M_4): liver and skeletal muscle
- They can be resolved electrophoretically.





- Measurement of LDH isoenzymes helps determine the location of tissue damage.

- “**HEART**”: the serum of a patient with a myocardial infarction (heart attack)
- “**NORMAL**”: normal serum
- “**LIVER**”: the serum of a patient with liver disease.

Identification of Isoenzymes

1. In Agar gel or polyacrylamide gel **electrophoresis**, the Isoenzymes have different mobility. LDH, CK and ALP Isoenzymes can be separated by electrophoresis.
2. **Heat stability**: one of the Isoenzymes may be easily denatured by heat, e.g. bone Isoenzyme of ALP (BALP).
3. **Inhibitors**: one of the Isoenzymes may be sensitive to one inhibitor, e.g. tartrate labile ACP.
4. K_M value or **substrate specificity** may be different for Isoenzymes, e.g. glucokinase has high Km and hexokinase has low Km for glucose.
5. **Cofactor** requirements may be different for isoenzymes. Mitochondrial isocitrate dehydrogenase is NAD⁺ dependent and the cytoplasmic Isoenzyme is NADP⁺ dependent.
6. Tissue **localization** may be different for Isoenzymes. H4 form of LDH is present in heart, while M4 variety is seen in skeletal muscle.
7. Specific **antibodies** may identify different types of isoenzymes. For example, CK Isoenzymes are separated by antibodies.

List of isoenzymes

1. Lactate dehydrogenase (LDH)
2. Creatine kinase (CK or CPK)
3. Cytochrome P450s
4. Phosphodiesterases
5. Add more examples to this list

Marker Enzymes of Tissue Damage used in diagnosis

Pancreatic enzymes:

α -Amylase

- Amylases normally occurring in human plasma are small molecules with molecular weights varying from 54 to 62 kDa. The enzyme is thus small enough to pass the glomeruli of the kidneys making it the only plasma enzyme physiologically found in urine
- Marked increase (five to 10 times the upper reference limit): is a pointer to acute pancreatitis or severe glomerular impairment

Lipase

Lipase is a small molecule and is filtered through the glomerulus. It is totally reabsorbed by the renal tubules, and it is not normally detected in urine

Plasma lipase levels are elevated in acute pancreatitis and carcinoma of the pancreas

- **Trypsin**
- **Chymotrypsin**
- **Elastase**

Liver enzymes

1. Markers of hepatocellular damage

Aspartate aminotransferase (AST) is present in high concentrations in cells of cardiac and skeletal muscle, liver, kidney and erythrocytes. Damage to any of these tissues may increase plasma AST levels. Half-life = 17 hours.

Aminotransferases (ALT) is present in high concentrations in liver and to a lesser extent, in skeletal muscle, kidney and heart. Half-life = 47 hours

NOTE: In liver damage, both enzymes are increased but ALT increases more. In myocardial infarction AST is increased with little or no increase in ALT

2. Markers of cholestasis

Alkaline phosphatase (ALP) elevated in the osteoblasts of bone and the cells of the hepatobiliary tract, intestinal wall, renal tubules and placenta

- Causes of increased ALP: Aging, rickets and osteomalacia, liver disease, malignancy

Gamma-glutamyl-transferase (GGT): catalyzes the transfer of the γ -glutamyl group from peptides and compounds that contain it to an acceptor

Causes of raised plasma GGT activity

- Induction of enzyme synthesis, without cell damage, by drugs or alcohol
- Hepatocellular damage, such as that due to infectious hepatitis

Other liver enzymes of clinical significance

Cholinesterase

Glutamate dehydrogenase

Muscle enzymes

Creatine Kinase (CK)

- Serum CK activity is greatly elevated in all types of muscular dystrophy
- CK consists of two protein subunits, M (for muscle) and B (for brain), which combine to form three isoenzymes. BB (CK-1), MB (CK-2) and MM (CK-3). CK-MM is the predominant isoenzyme in skeletal and cardiac muscle and is detectable in the plasma of normal persons

Lactate Dehydrogenase (LDH)

Other clinically important enzymes

Acid Phosphatase (ACP)

Glucose -6-phosphate Dehydrogenase

What are the functions of ACP and G6PD above?

Clinically Important Enzymes

<i>Enzyme</i>	<i>Principle Sources of Enzyme in blood</i>	<i>Clinical applications</i>
<i>Alanine aminotransferase</i>	<i>Liver</i>	<i>Hepatic parenchymal diseases</i>
<i>Alkaline phosphatase</i>	<i>Liver, bone, intestinal mucosa, placenta</i>	<i>Bone diseases, hepatobiliary diseases</i>
<i>Amylase</i>	<i>Salivary glands, pancreae</i>	<i>Pancreatic diseases</i>
<i>Aspartate aminotrasferase</i>	<i>Liver, skeletal muscle, heart erythrocytes</i>	<i>Hepatic parenchymal disease, muscle disease</i>
<i>Cholinesterase</i>	<i>Liver</i>	<i>Organophosphorus insecticide poisoning, hepatic parenchymal disease</i>
<i>Creatine kinase</i>	<i>Skeletal muscle, heart</i>	<i>Muscle diseases(M.I.)</i>

<i>Enzyme</i>	<i>Principle Sources of Enzyme in blood</i>	<i>Clinical applications</i>
<i>γ-glutamyl transferase</i>	<i>Liver</i>	<i>Hepatobiliary diseases, marker of alcohol abuse</i>
<i>Lactate dehydrogenase</i>	<i>Heart, liver, skeletal muscle, erythrocytes, platelets, lymph nodes</i>	<i>Hemolysis, hepatic parenchymal diseases, tumor marker</i>
<i>lipase</i>	<i>Pancreas</i>	<i>Pancreatic diseases</i>
<i>5'-nucleotidase</i>	<i>Liver</i>	<i>Hepatobiliary diseases</i>
<i>Trypsin</i>	<i>pancreas</i>	<i>Pancreatic diseases</i>

This list is not conclusive

Which enzymes have been
used as **drug molecules**
(therapeutic enzymes)?

Therapeutic enzymes have a broad variety of specific uses

- Oncolytics
- Anticoagulants
- Thrombolytics
- Replacements for metabolic deficiencies
 - Digestive aids
 - Metabolic storage disorders, etc
- Miscellaneous enzymes of diverse function

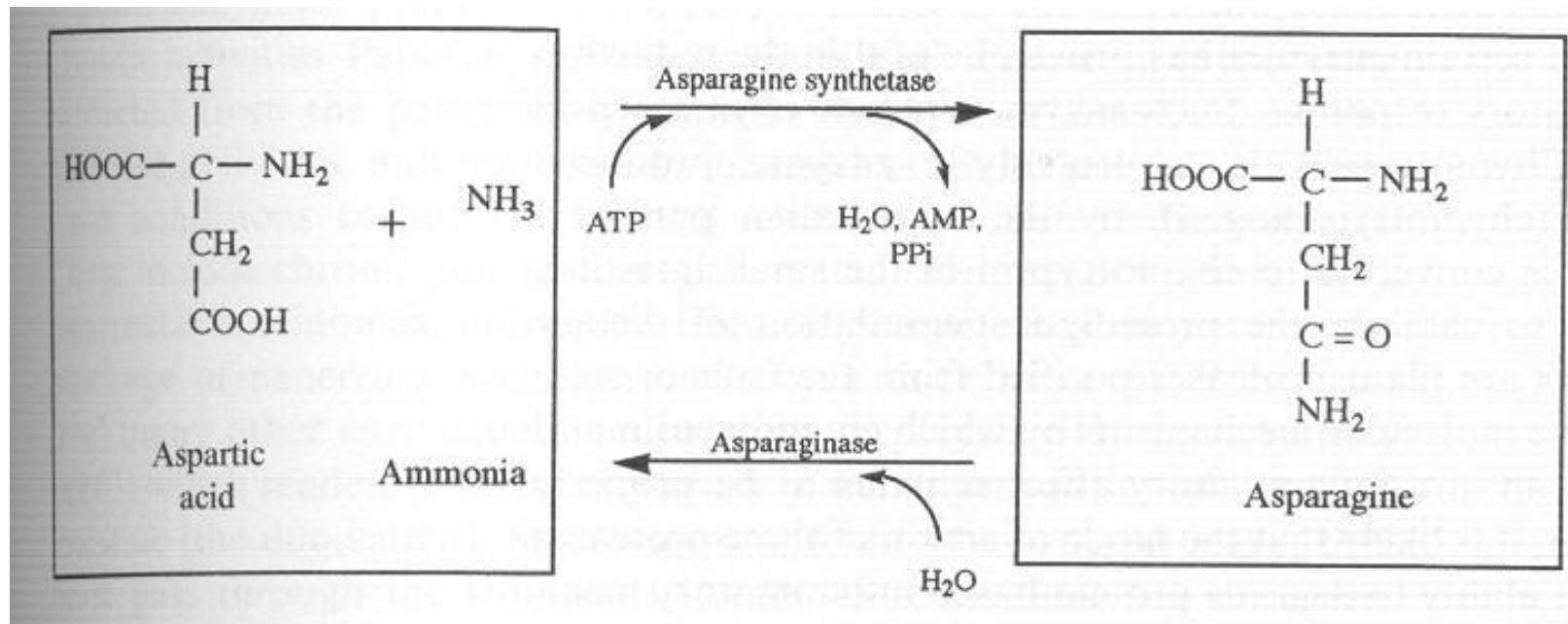
Table 6.5 Some enzymes that may be used for therapeutic purposes

Enzyme	Therapeutic application
Ancrod (serine protease)	Anticoagulant
Tissue plasminogen activator	Thrombolytic agent
Urokinase	Thrombolytic agent
(Activated) Factors II V and IX	Treatment of clotting disorders
Asparaginase	Treatment of some types of cancer
DNase	Treatment of cystic fibrosis
Glucocerebrosidase	Treatment of Gaucher's disease
Trypsin	Debriding/anti-inflammatory agents
Papain	
Collagenase	
Lactase	Digestive aids
Pepsin	
Pancrelipase	
Papain	
Superoxide dismutase	Prevention of oxygen toxicity

Oncolytic enzymes

Asparaginase

- A tetrameric enzyme that catalyses the hydrolysis of the amino acid asparagine



Asparaginase...

- It may be purified from a wide variety of microorganisms (yeast, fungi, bacteria such as *E. coli*)
 - Asn is required for normal metabolic activity
 - Most human cells are capable of synthesizing Asn but certain malignant cells are not
- This can be used in the destruction of malignant cells....

Asparaginase...

- Source of clinically used asparaginase:
 - *E. coli*: two isozymes of which only one is effective
 - *Erwinia chrysanthemi*
- Treatment of childhood leukaemia
 - Side effects: severe allergic reaction, nausea, vomiting, fever, compromised kidney and liver function
 - Allergic reaction is greatly reduced by coupling the asparaginase with PEG
- Asparaginase production by a recombinant *Pichia pastoris* strain harbouring *S. cerevisiae* ASP3 gene
(Ferrera et al, Enzyme and Microbial Technology 39(7) 2006)

Other oncolytic enzymes

- **Diphtheria toxin** (an oncolytic enzyme still in the experimental stage), catalyzes transfer of the adenosine diphosphate ribose (ADP-ribose) moiety of nicotinamide adenine dinucleotide (NAD) to elongation factor 2
- This enzyme halts protein synthesis
- The protein synthesis in tumor cells is 100 to 10,000 time more sensitive to this toxin than the analogous process in normal cells
- Enzymes that degrade macromolecules: neuraminidase, ribonuclease, and a diverse group of proteases
 - **Neuraminidase** removes sialic acid residues from the surface of (neoplastic) cells, thereby altering their immunogenicity, and rendering them sensitive to immune response
 - 2000 -- The FDA has approved the Orphan Drug application of Wobe-Mugos as an adjunct therapy for multiple myeloma. Wobe-Mugos (vitamins + proteolytic enzymes), used successfully in Europe in conjunction with chemotherapy since 1977

Debriding agents

- Debriding agents effectively clean open wounds by removal of foreign matter and any surrounding dead tissue
- Trypsin, papain and collagenase (all proteolytic enzymes) have often be used
 - **Trypsin**: from mammalian pancreas, hydrolyse peptide bonds involving arg and lys
 - **Papain**: from the leaves and the unripe fruit of the papaya tree, hydrolyse peptide bonds involving basic amino acids (e.g. lys, arg, his)
 - **Collagenase**: from culture extracts of various animal cells or normally from various *Clostridium* species (pathogenic)

Anti-inflammatory agents

- Administration of some enzymes is shown to be effective in the reduction of various inflammatory responses
 - **Chymotrypsin**: chymotrypsinogen (the zymogen form produced in pancreas) is converted to active form in small intestine
 - **Bromelains**: plant proteases purified from the stem or the fruit of pineapple
- Their anti-inflammatory action is not known in detail. Probably their ability to degrade protein-based inflammatory mediators play a role in their action

Enzymes as digestive aids-1

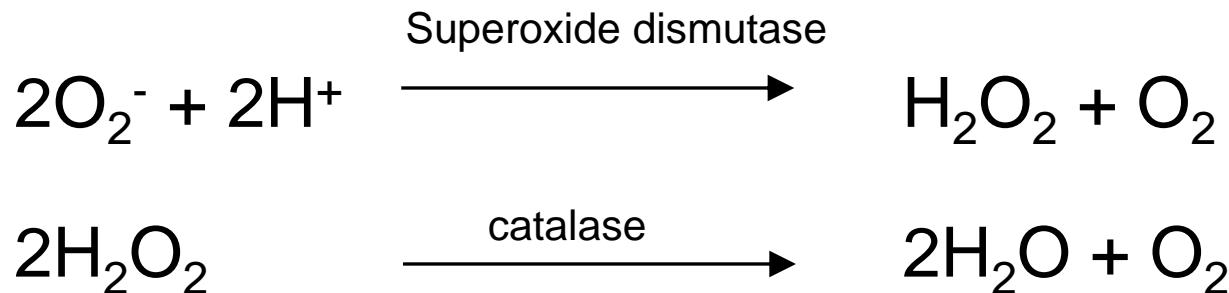
- Most digestive aid preparations are based on polymerases responsible for breakdown of polysaccharides, proteins and lipids
- Such preparations may include
 - a single enzyme or
 - multiple enzymes
- **α -amylase**: hydrolyse α 1-4 glycosidic bonds
 - Amylase from *B. subtilis* or species of *Aspergillus* have various industrial applications
 - Oral amylase administration is used to aid digestion
- **Lactase**: hydrolysis of lactose
 - In many geographical regions, adults has greatly reduced lactase activity

Enzymes as digestive aids-2

- Various proteolytic enzymes, e.g. papain, pepsin
- **Pancreatin**: a preparation extracted from pancreas containing various enzymes
 - Used in deficiencies related with secretion of pancreatic enzymes (e.g. chronic pancreatitis, pancreatic carcinomas, cystic fibrosis)
- One problem associated with oral administration is gastric inactivation
 - Co-administration of inhibitors of garstric acid secretion
 - Enteric coated tablet or capsules
 - Use of microbial proteases, amylases and lipases

Superoxide dismutase

- It is an important enzyme in all aerobic organisms



- Two forms are found in eukaryotes: cytoplasmic (zinc and copper) and mitochondrial (manganese)
- Isolates from bovine liver and erythrocytes clinically used as anti-inflammatory agent (injection into patients with osteoarthritis of the knee)

Nuclease treatment of cistic fibrosis

- Cystic fibrosis (CF) is one of the most commonly occurring genetic diseases (1 in 2500 in northern Europe)
- Underlying cause is identified to the malfunction of ion transport
- Major clinical symptom is the production of viscous mucus in the respiratory track
- Change in lung physiology \Rightarrow bacterial infections \Rightarrow immune response \Rightarrow bacterial destruction \Rightarrow liberation of DNA \Rightarrow highly viscous mucus
- Therapy:
 - Percussion therapy is used to help the ejection of mucus
 - **Bovine DNase** treatment was approved in USA in 1950s but prolonged usage caused adverse reactions
 - DNase I produced by expression of cDNA in CHO cell lines (Pulmozyme) has been approved for medical use.

Enzyme-replacement therapy (ERT)

Brady and Schiffmann, The Lancet Neurology, 2004

- Metabolic storage disorders → insufficient activity of housekeeping enzymes
 - Gaucher's disease (\$40 000–320 000/year)
 - **Glucocerebrosidase** absence (glycolipid accumulation in cells, especially in macrophages)
 - Enzyme from human placentae
 - Recombinant enzyme in CHO cell line (Cerezyme, 1994)
 - Fabry's disease, in which the heart, kidney, gastrointestinal tract, and peripheral nerves are damaged (\$160 000/year)
 - Pompe's disease, in which the heart, skeletal muscles, and brain are involved
 - Hurler's disease and Maroteaux-Lamy syndrome in which the eyes, liver, joints, and skeleton are usually affected

Nerve agent scavengers

Rochu et al., Toxicology, 2006

- The requirements:
 - (a) a high reaction rate with organophosphate molecules
 - (b) a long half-life *in vivo* to be effective over a prolonged time
 - (c) immunotolerance
 - (d) no adverse effects on physiological processes
- Stoichiometric scavengers or
- Catalytic scavengers → **paraoxanase**

Topical enzyme therapy for skin diseases

Klein et al, The Lancet, 357(9260), 2001

- Xeroderma pigmentosum → the frequency of all forms of skin cancer is higher (a genetic defect in DNA repair)
 - Bacterial DNA repair enzyme, **T4 endonuclease V**, delivered intracellularly, increases the rate of repair of sunlight-induced DNA damage in human cells
 - Topical administration of this enzyme in a liposomal delivery vehicle was tested
- Promising results with no adverse effect.

More therapeutic enzymes...

- 1. Glutaminase
- 2. Hyaluronidase
- 3. Lysozyme
- 4. Rhodanase
- 5. Ribonuclease
- 6. Streptokinase
- 7. Uricase
- 8. Urokinase

What are the practical challenges of using enzymes as drug molecules?

Challenges of Enzyme Therapy

- A. Too large molecules to be distributed in body cells
- B. Foreign/ antigenic - can elicit immune response causing allergic reactions
- C. Lifetime – minutes in circulation (too short!)

Some of these challenges can be overcome by entrapment or covalent modification of the enzyme molecules.

In contrast to industrial use of enzymes, therapeutically useful enzymes are required in relatively tiny amounts but at very high degree of **purity** and **specificity**.

- Small K_M
- High V_{max}

i.e. you maximize at both **low enzyme** and low substrate concentrations