LECTURE 7:

ENZYME CATALYTIC MECHANISMS

- Enzyme substrate interactions
- Factors affecting enzyme activity
- Mechanisms of Enzyme catalysis
- Regulation of enzyme activity

Enzyme-substrate interactions

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

Sequence of events in enzyme catalyzed reaction

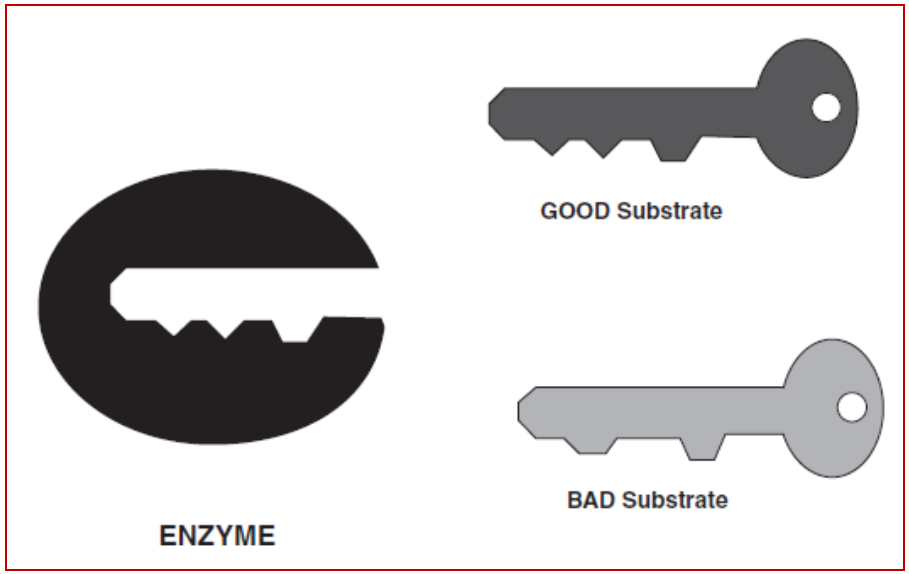
1. **E + S → ES**
   - Enzyme and Substrate collide
   - Substrate bind to active site of enzyme
   - A transition state forms where the structure of the substrate is altered

2. **ES → EP**
   - Enzyme catalyzes the conversion of substrate to Product
   - Both substrate and product remain in active site

3. **EP → E + P**
   - Product is released from active site

Enzyme-substrate interactions

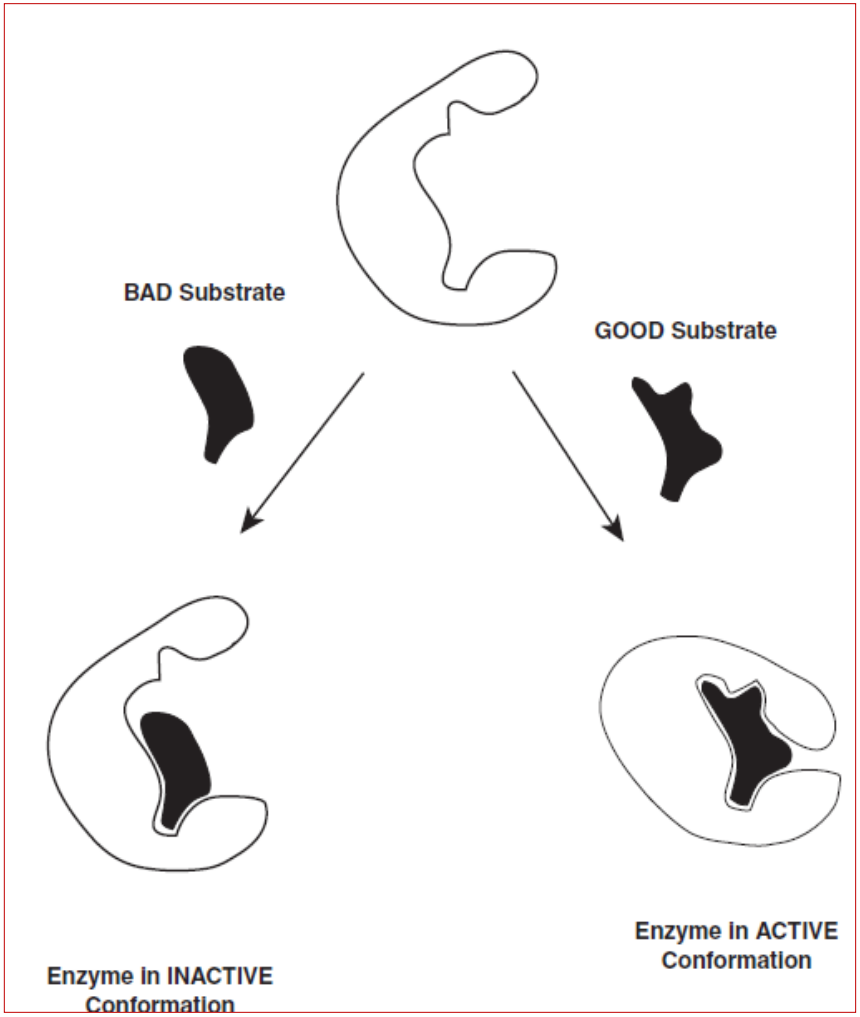
Lock and Key theory



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

Induced-fit theory

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

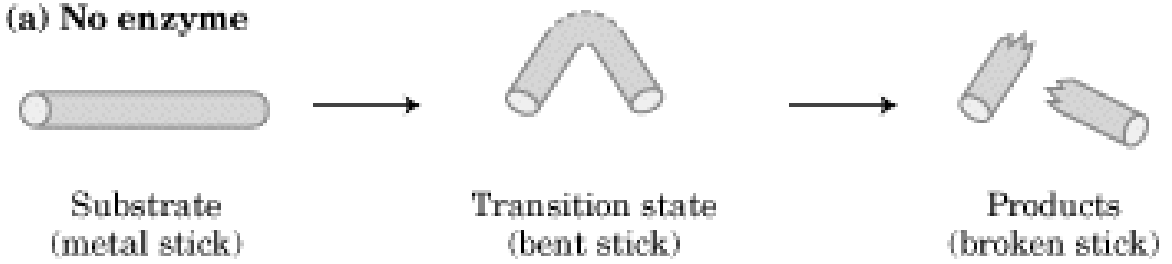


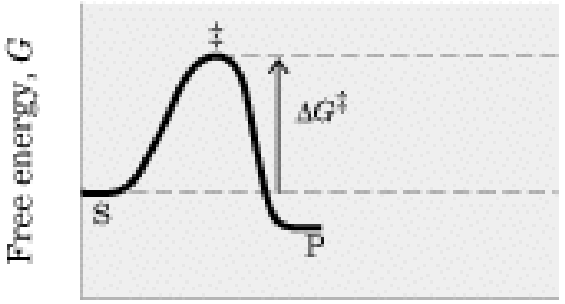
Transition state stabilization

- 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.**

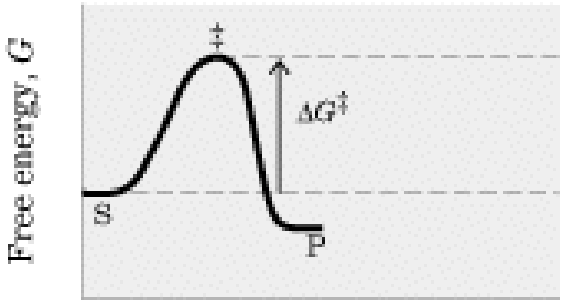
(Nelson & Cox, *Lehninger Principles of Biochemistry*, 3rd ed., 2000)

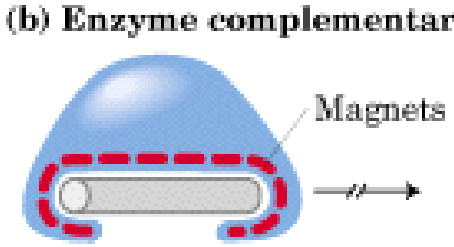


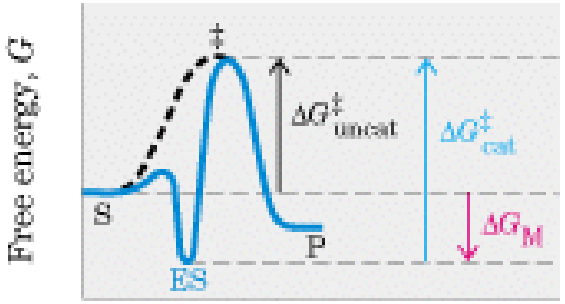


No Enzyme

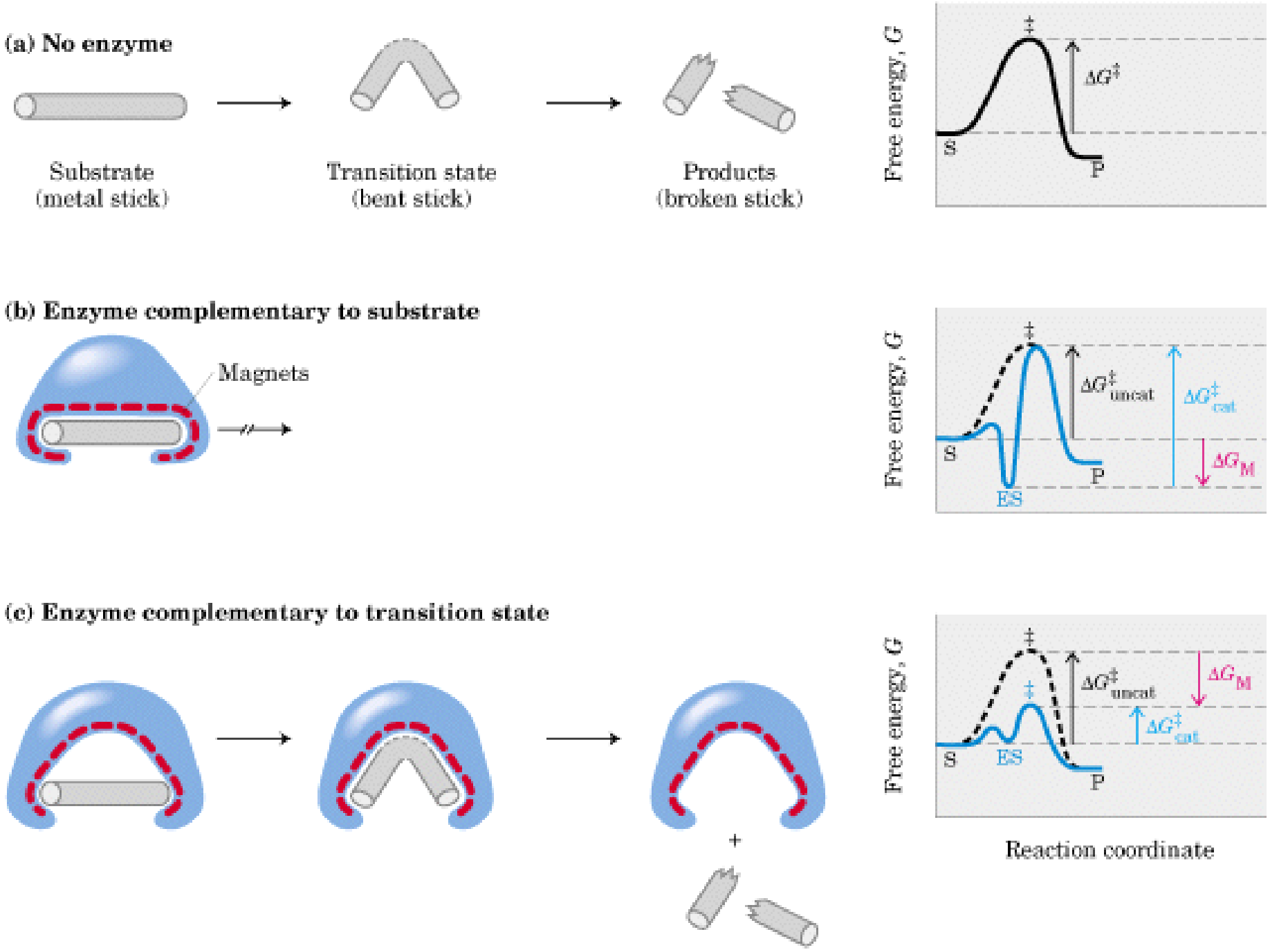
The bent stick is energetically unfavorable, but must be formed for the stick to be broken.







No catalysis is obtained by just binding substrate tightly!


(a) No enzyme
Substrate
(metal stick)
Transition state
(bent stick)
Products
(broken stick)
Free energy, G
‡
ΔG‡
S
P
(b) Enzyme complementary to substrate
Magnets
Free energy, G
‡
ΔG‡uncat
ΔG‡cat
ΔGM
S
P
ES
(c) Enzyme complementary to transition state
+
Free energy, G
‡
‡
ΔG‡uncat
ΔGM
ΔG‡cat
S
ES
P
Reaction coordinate

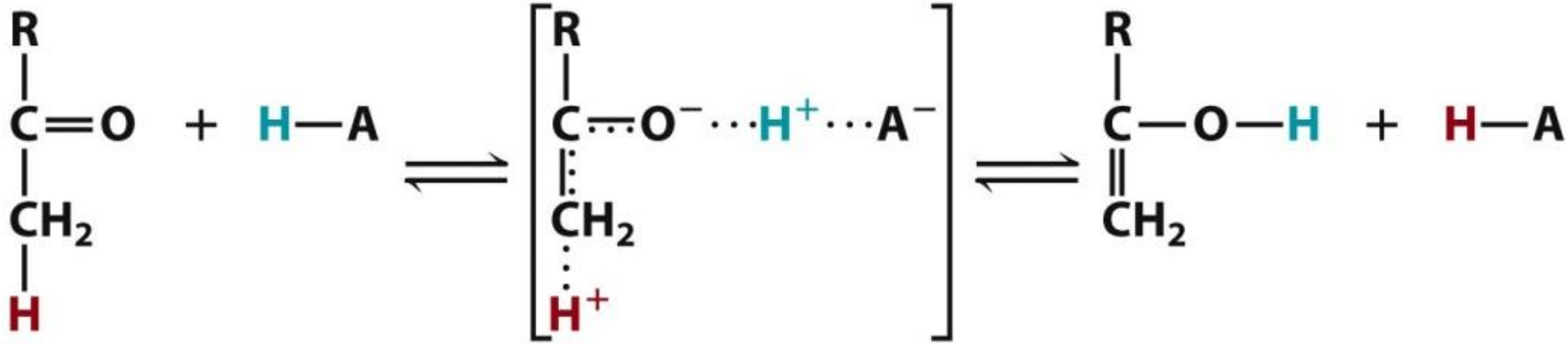
What factors affect the activity of an Enzyme?

TYPES OF ENZYME CATALYTIC MECHANISMS

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

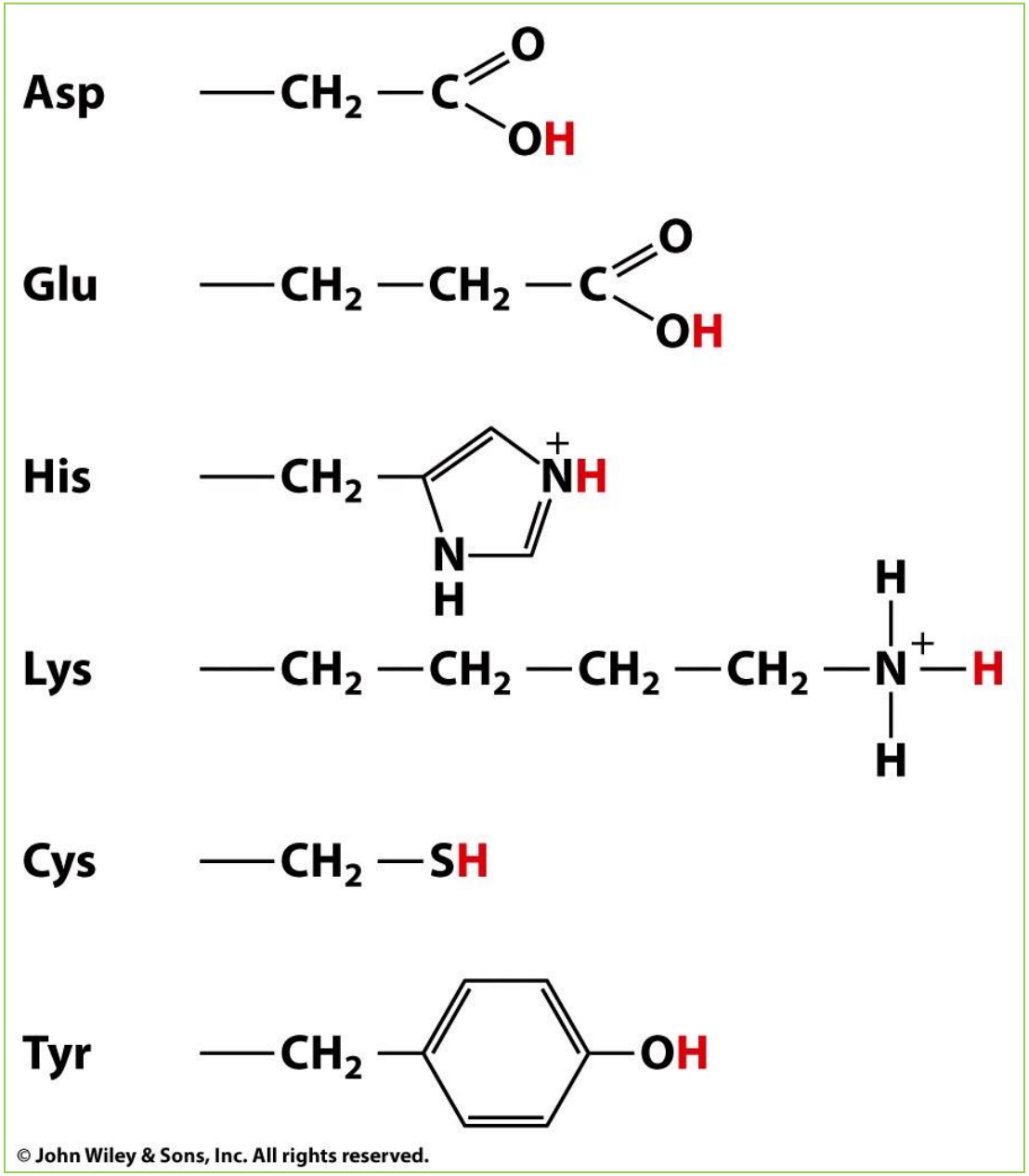
A. Acid – Base catalysis

- 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

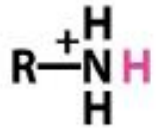
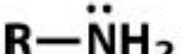
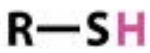
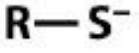
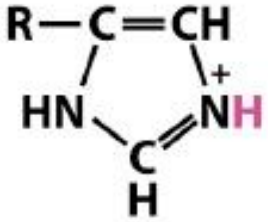
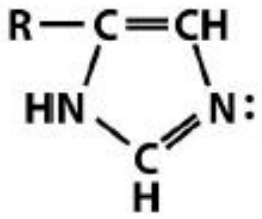
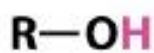
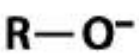
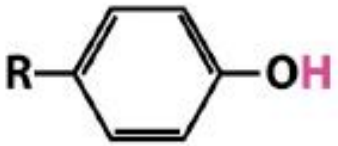
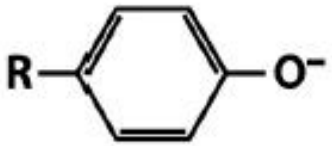
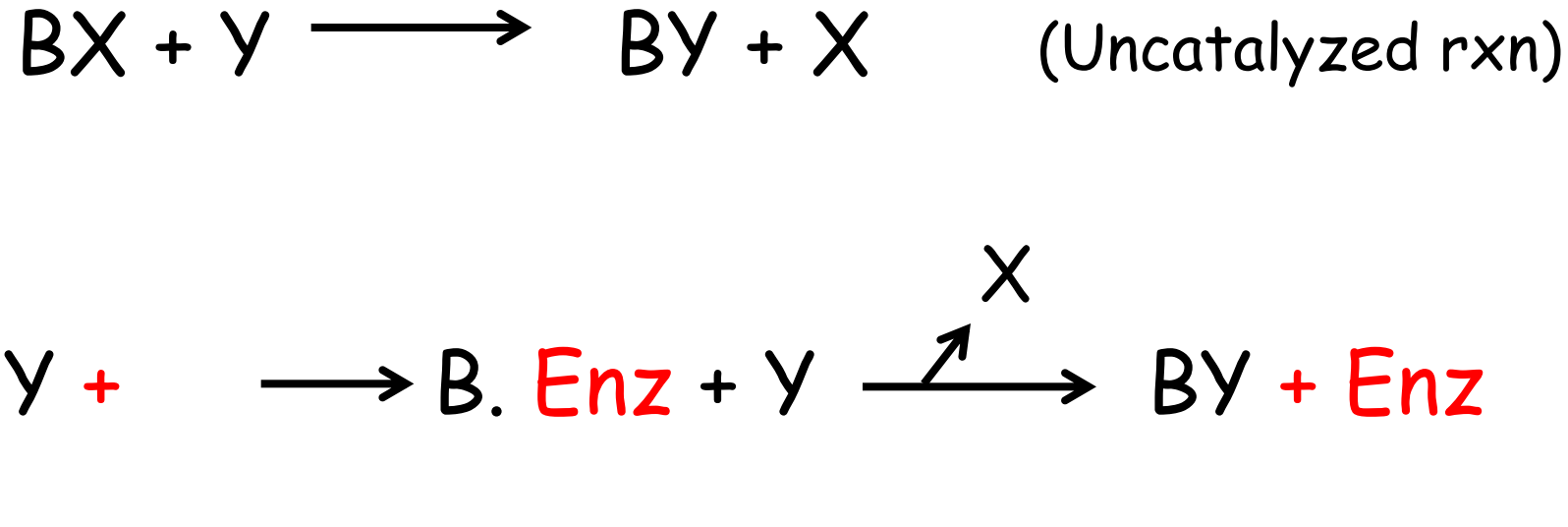
Amino acid residues	General acid form (proton donor)	General base form (proton acceptor)
Glu, Asp		
Lys, Arg		
Cys		
His		
Ser		
Tyr		

Figure 6-9
Lehninger Principles of Biochemistry, Fifth Edition

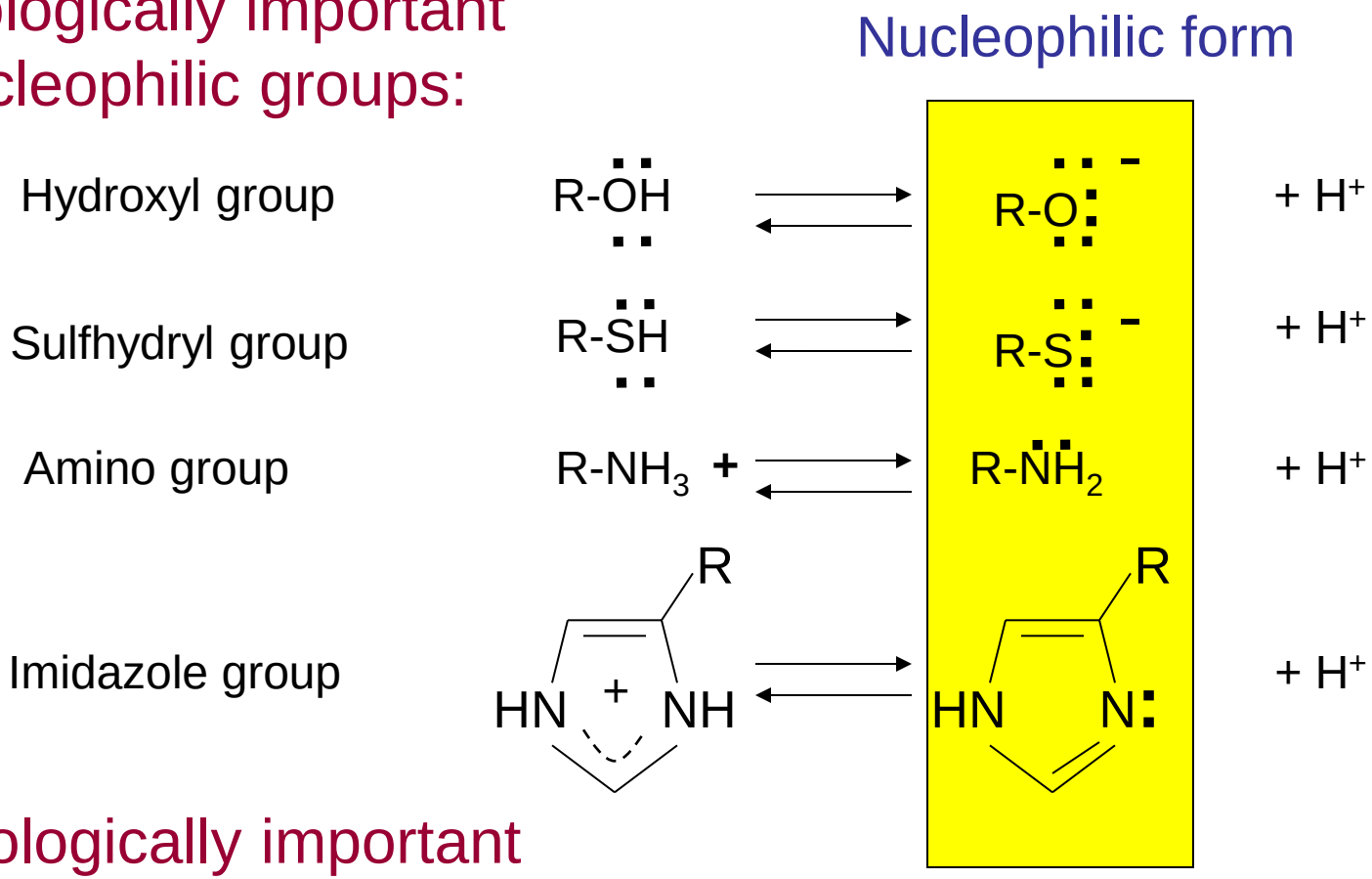
B. Covalent catalysis

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



Covalent catalysis

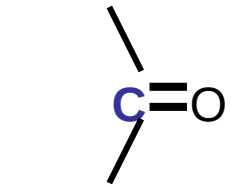
Biologically important nucleophilic groups:



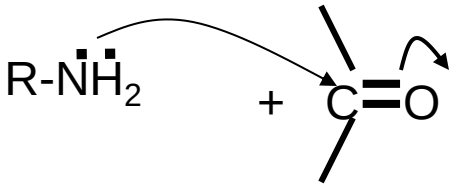
Biologically important electrophiles:

H^+ Protons

M^{n+} Metal Ions

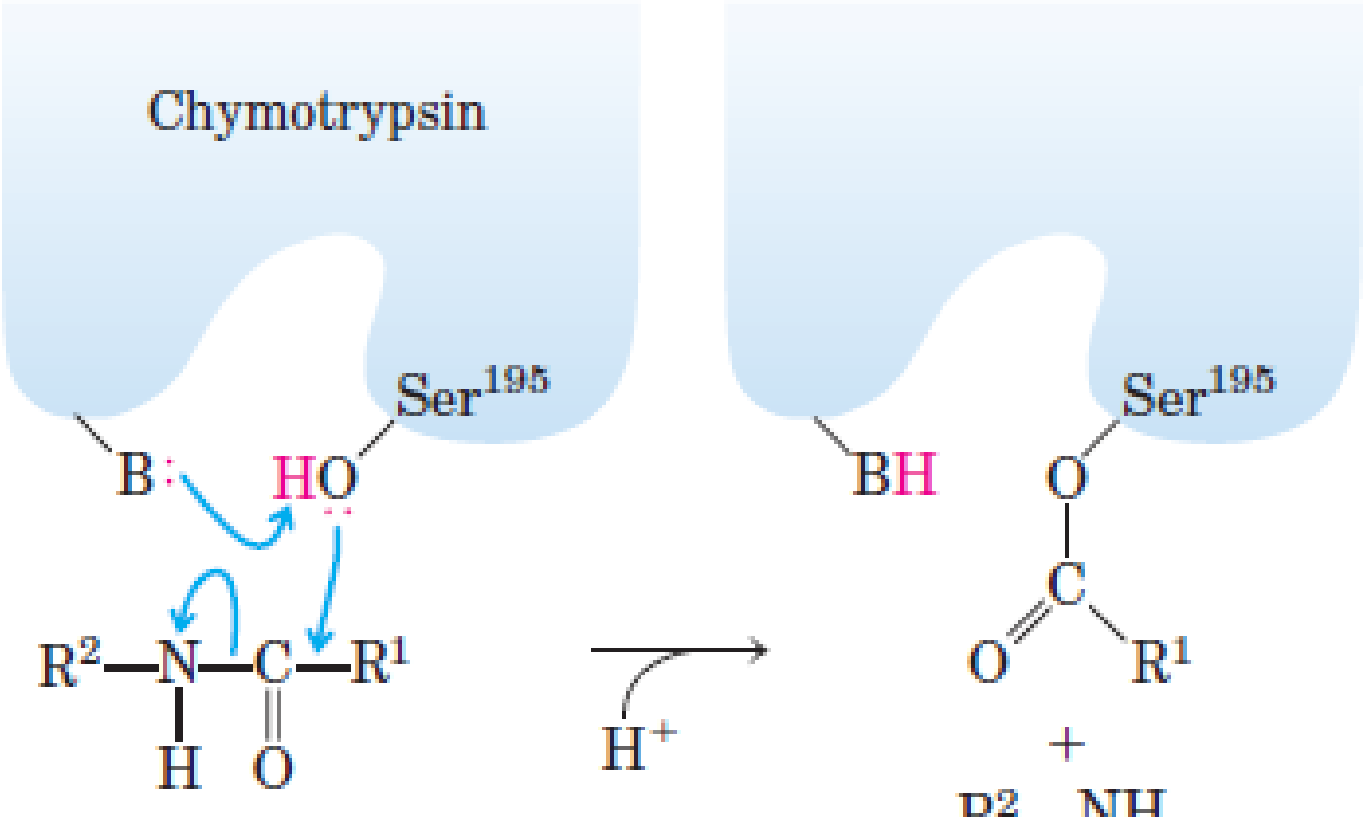


Carbonyl carbon



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:

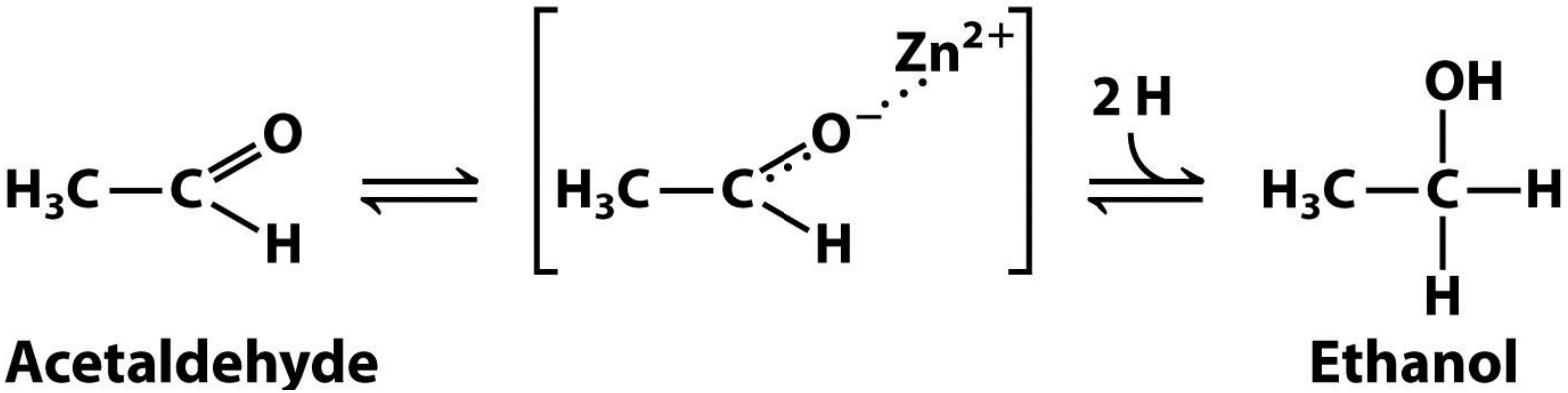
* **binding substrates** in the proper orientation
* mediating **oxidation-reduction reactions**
* **electrostatically stabilizing or shielding negative charges** (electrostatic catalysis)

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})

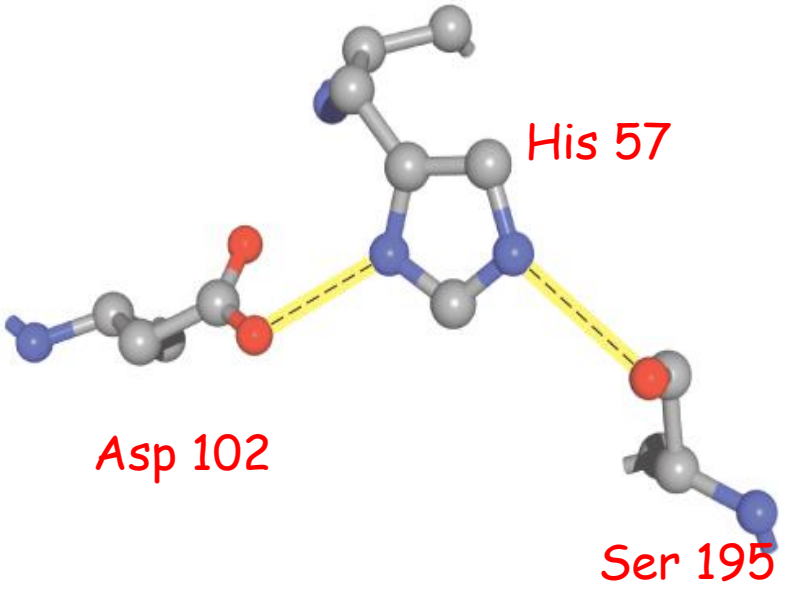
Metal ion catalysis…

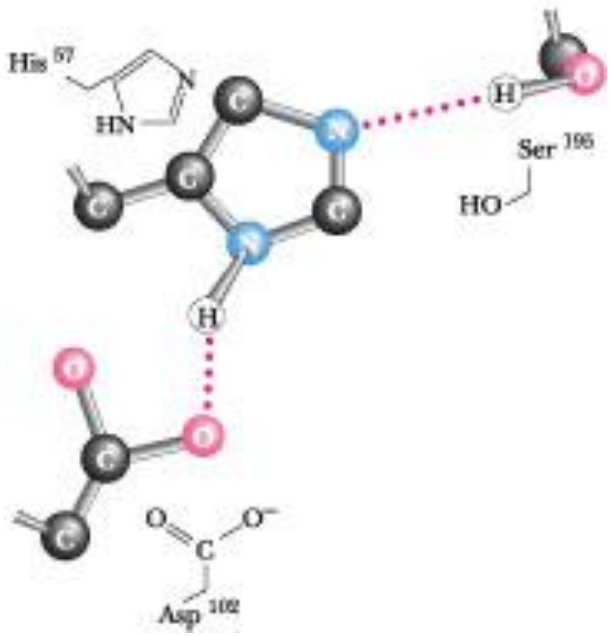
- Metal bound at the active site of enzymes can act as electrophilic catalysts, stabilizing the increased electron density of negative charge that can develop during reaction



D. Proximity and orientation effects

- In this type of catalysis, the enzyme holds the various players in a reaction next to each other and in an orientation that is suitable in order to increase the rate of reaction
- e.g. the catalytic triad in chymotrypsin



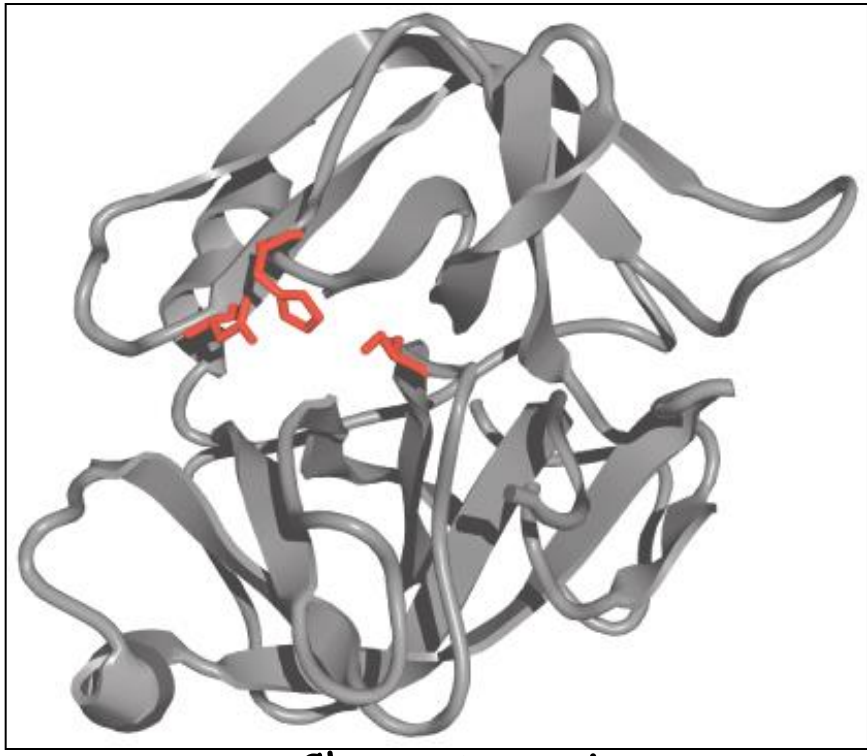


Serine proteases

- Are a class of proteolytic enzymes whose catalytic mechanism is based on an active-site serine residue
- e.g. *trypsin, chymotrypsin, elastase*

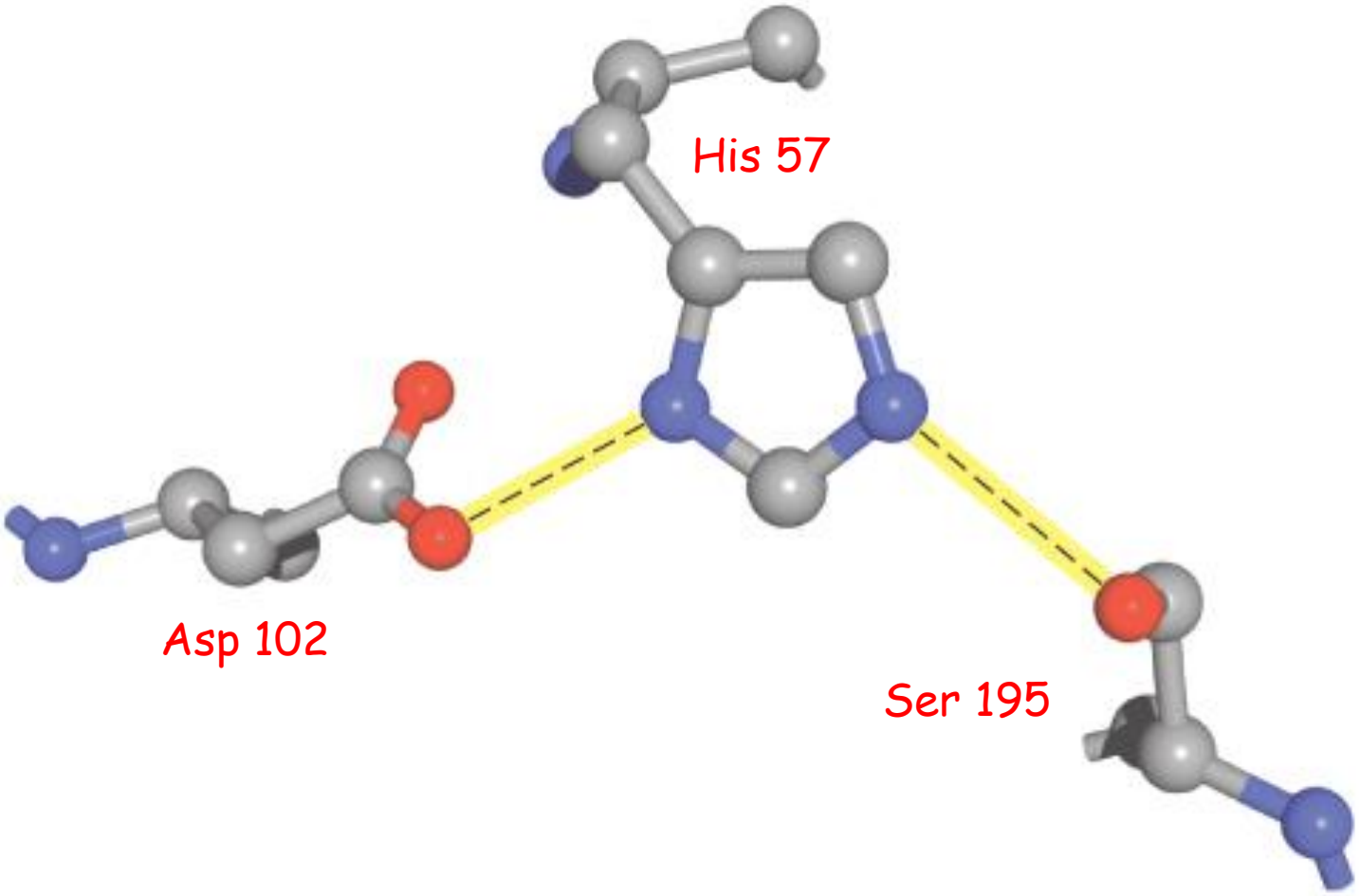
Chymotrypsin:

- Digestive enzyme produced in pancreas, secreted into small intestine
- Two domains
- Active site in cleft
- Catalyses hydrolytic cleavage of peptide bond



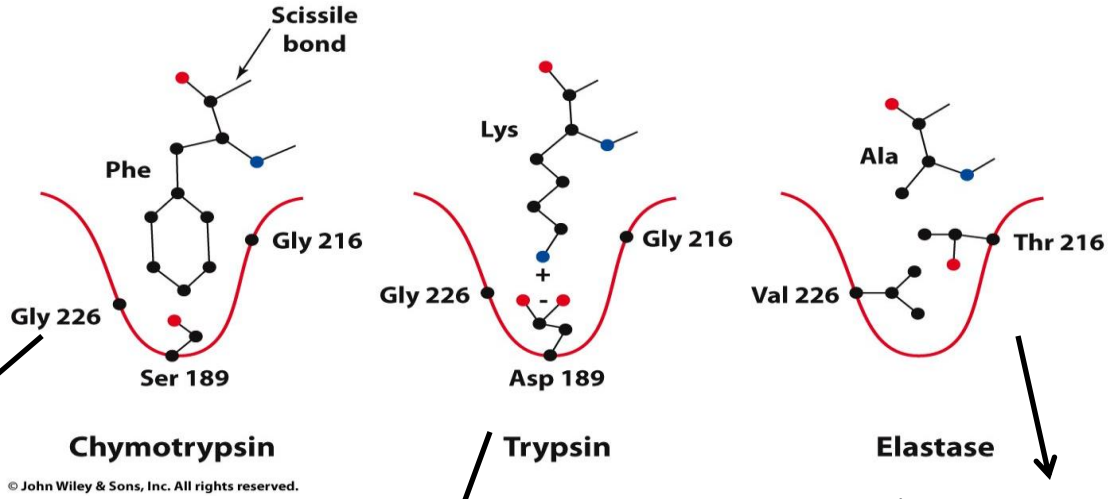
Chymotrypsin:

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



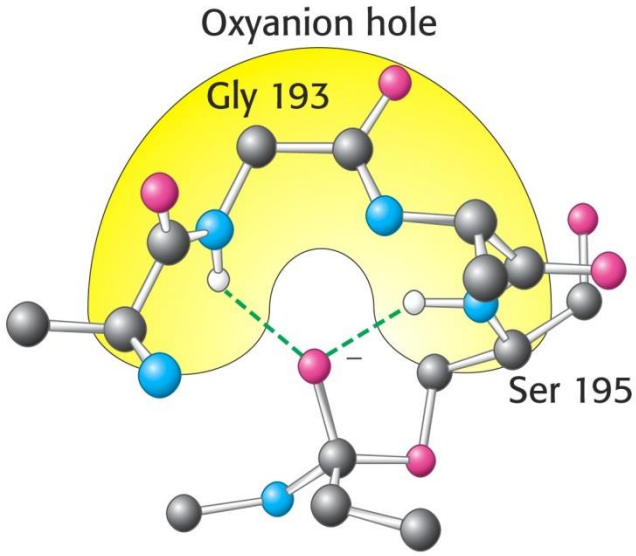
nonpolar pocket

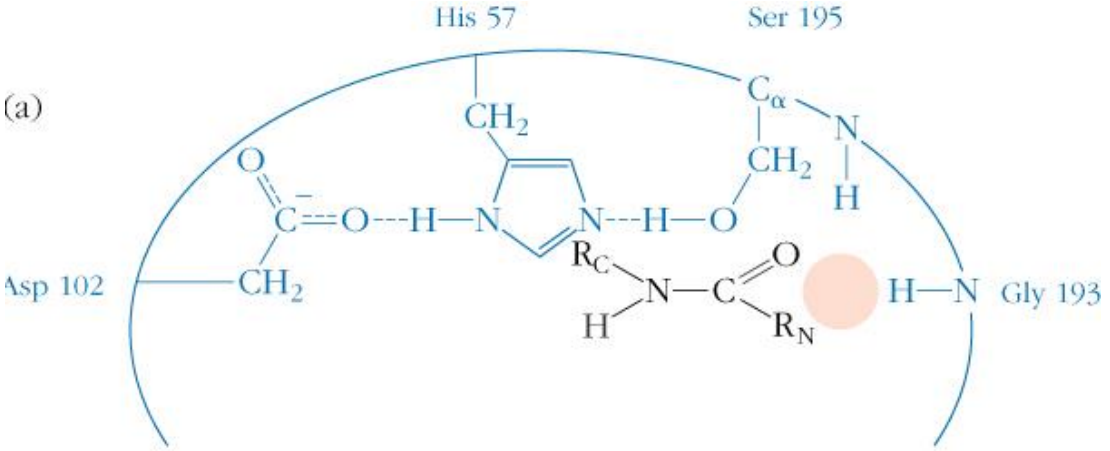
Asp (negatively charged) vs. Ser in Chymotrypsin

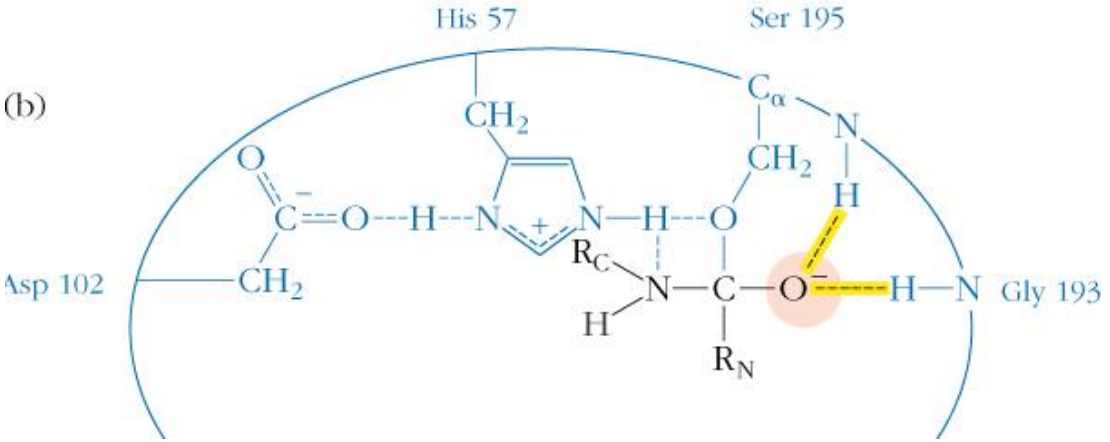
no pocket present as two Gly in chymotrypsin are replaced by Val and Thr

E. Preferential stabilization of the transition state

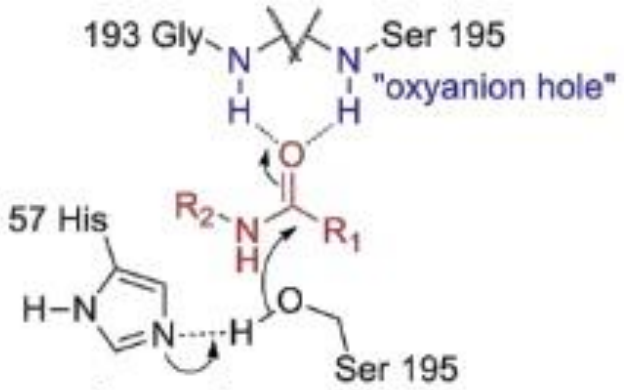
The transition state is stabilized by the oxyanion hole of chymotrypsin







Oxyanion Hole



- An **oxyanion hole** is a pocket in the active site of an enzyme that stabilizes transition state negative charge on a deprotonated oxygen or alkoxide.
- The pocket typically consists of backbone amides or positively charged residues. Stabilizing the transition state lowers the activation energy necessary for the reaction, and so promotes catalysis.
- In chymotrypsin, the amide hydrogens (-N-H) of Ser195 and Gly193 form an oxyanion hole which,

Regulation of Enzyme Activity

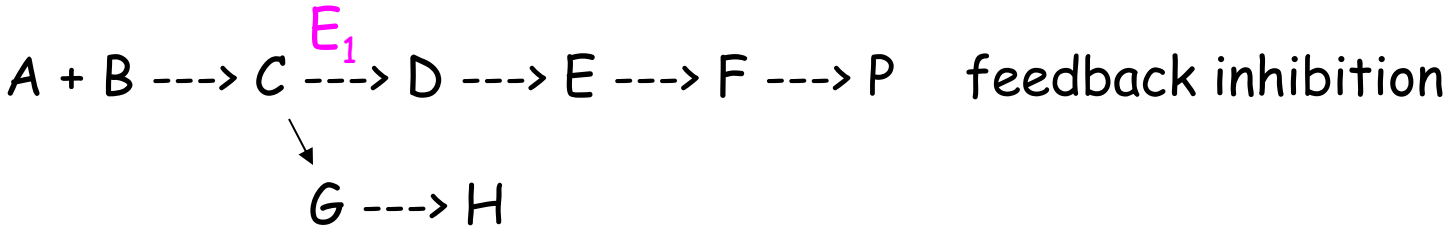
- Control of enzyme availability
- Covalent modification
- Allosteric
- Zymogen Regulation

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

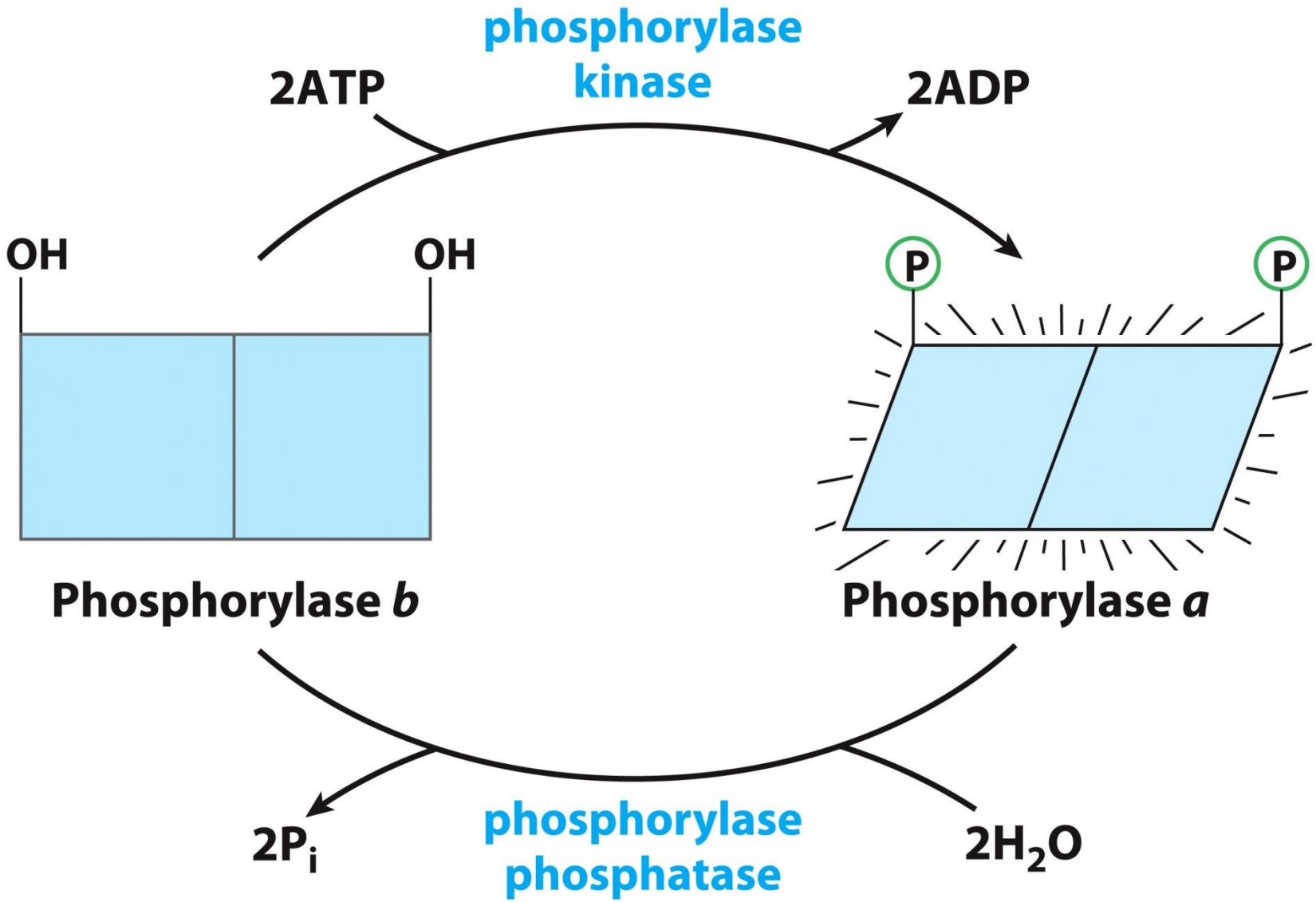
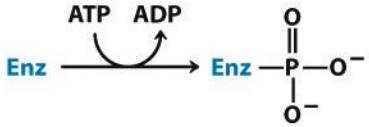


Figure 6-36
Lehninger Principles of Biochemistry, Sixth Edition

Covalent modification (target residues)

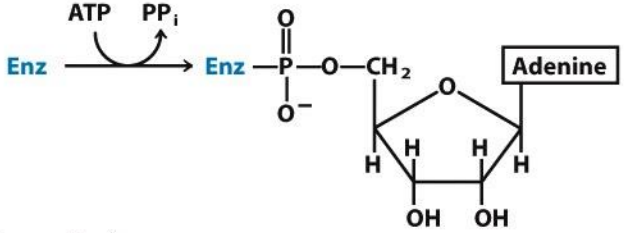
Phosphorylation

(Tyr, Ser, Thr, His)



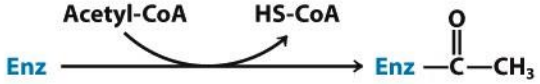
Adenylylation

(Tyr)



Acetylation

(Lys, α-amino (amino terminus))

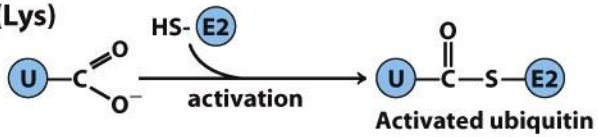


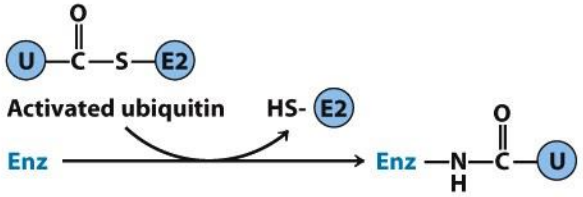
Myristoylation

(α-amino (amino terminus))

Ubiquitination

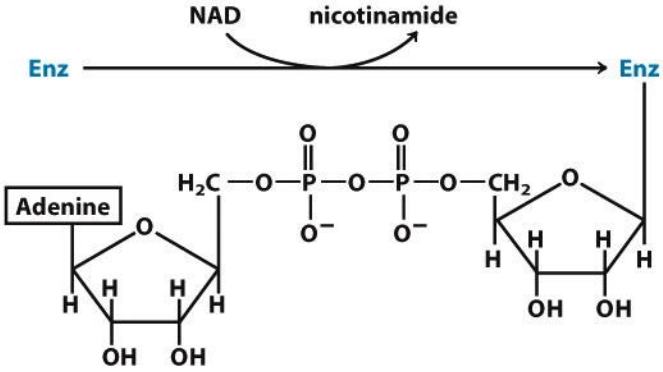
(Lys)





ADP-ribosylation

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



Methylation

(Glu)

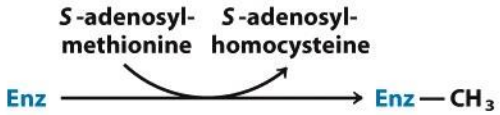


Figure 6-35

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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

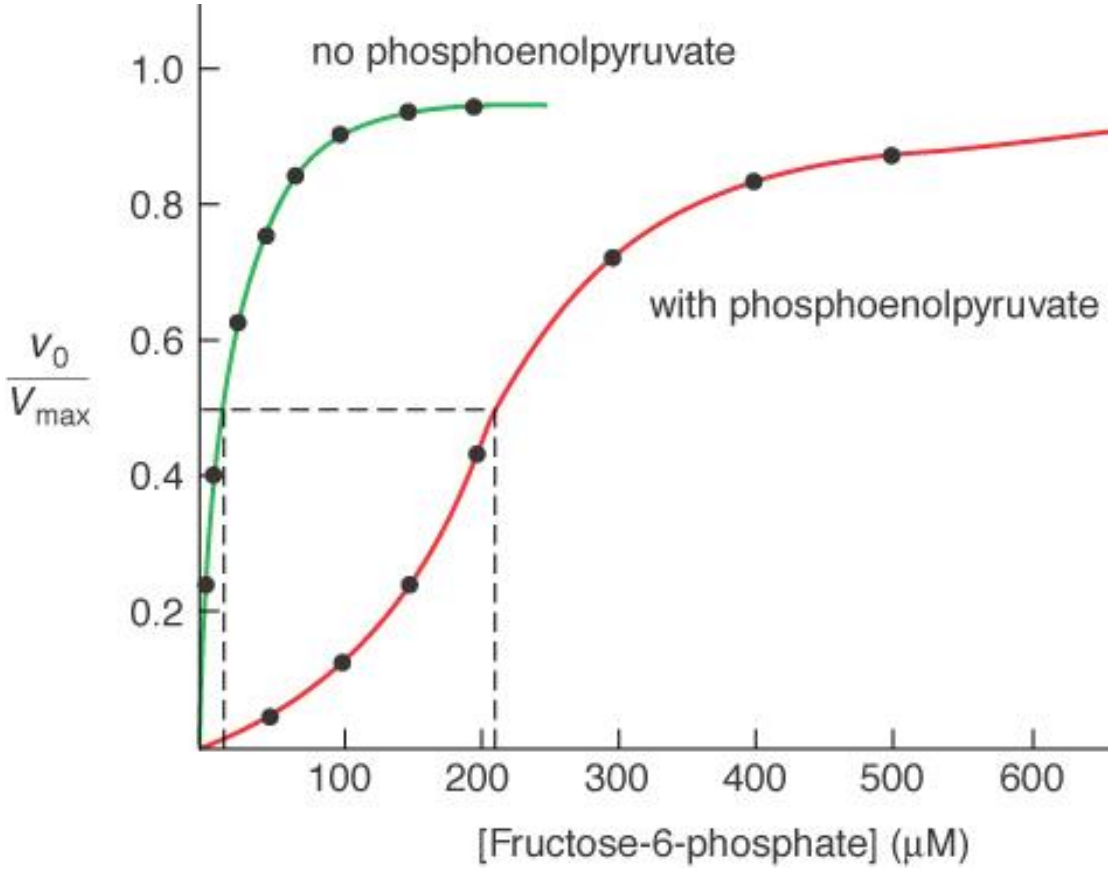
ii) sequential theory

- 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?

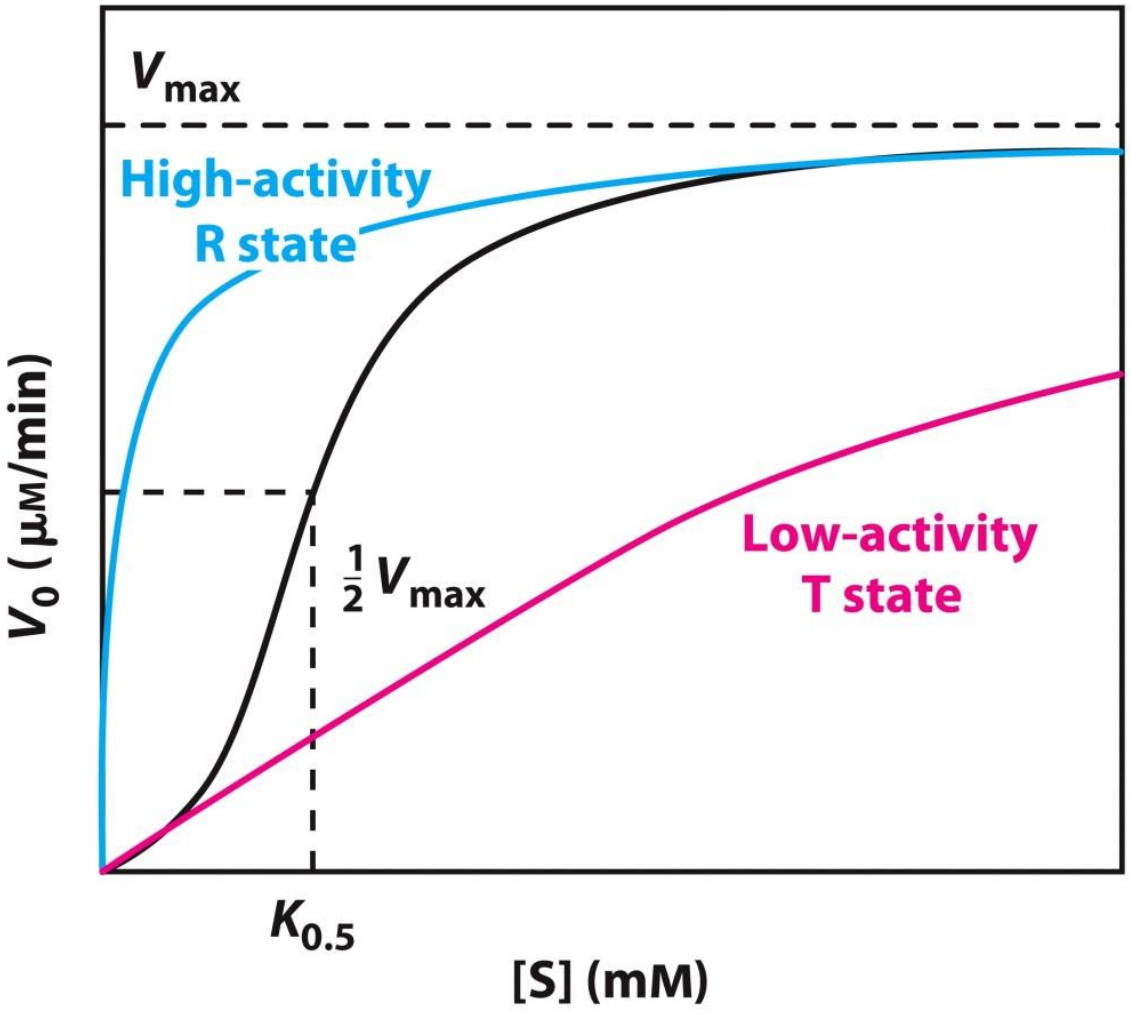


Figure 6-34a
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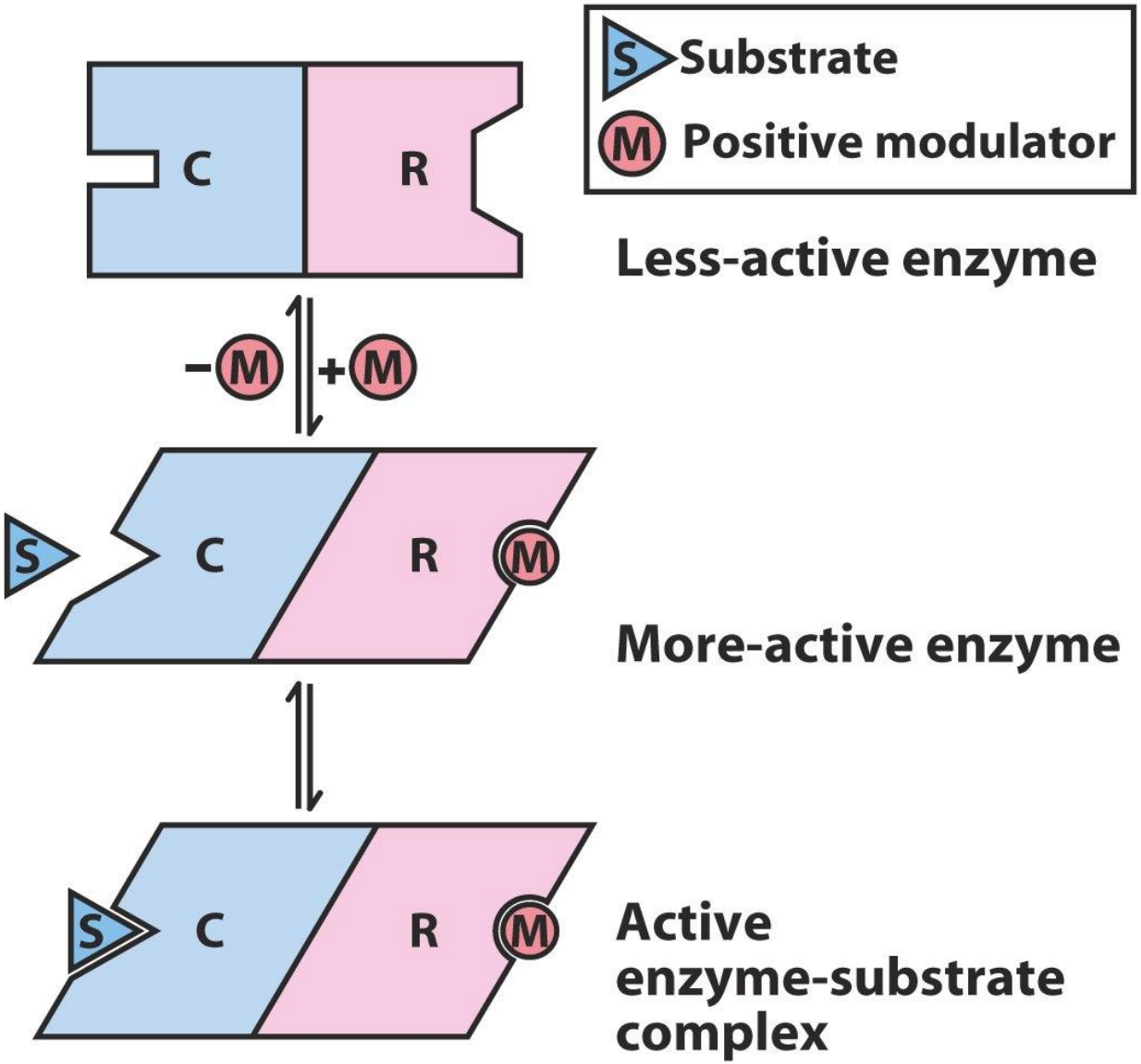
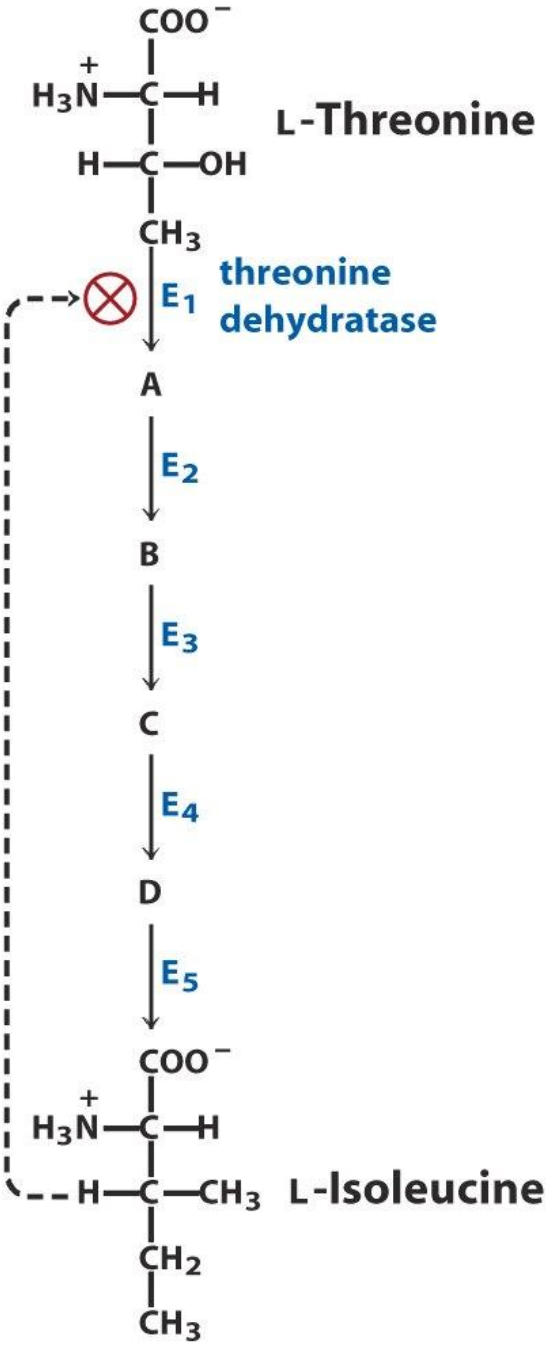
Allosteric Effectors – Bind to Allosteric Site



Feedback Inhibition is the Classic Form of Allosteric Inhibition



Allosteric Positive & Negative Regulators: Affecting V_{max}

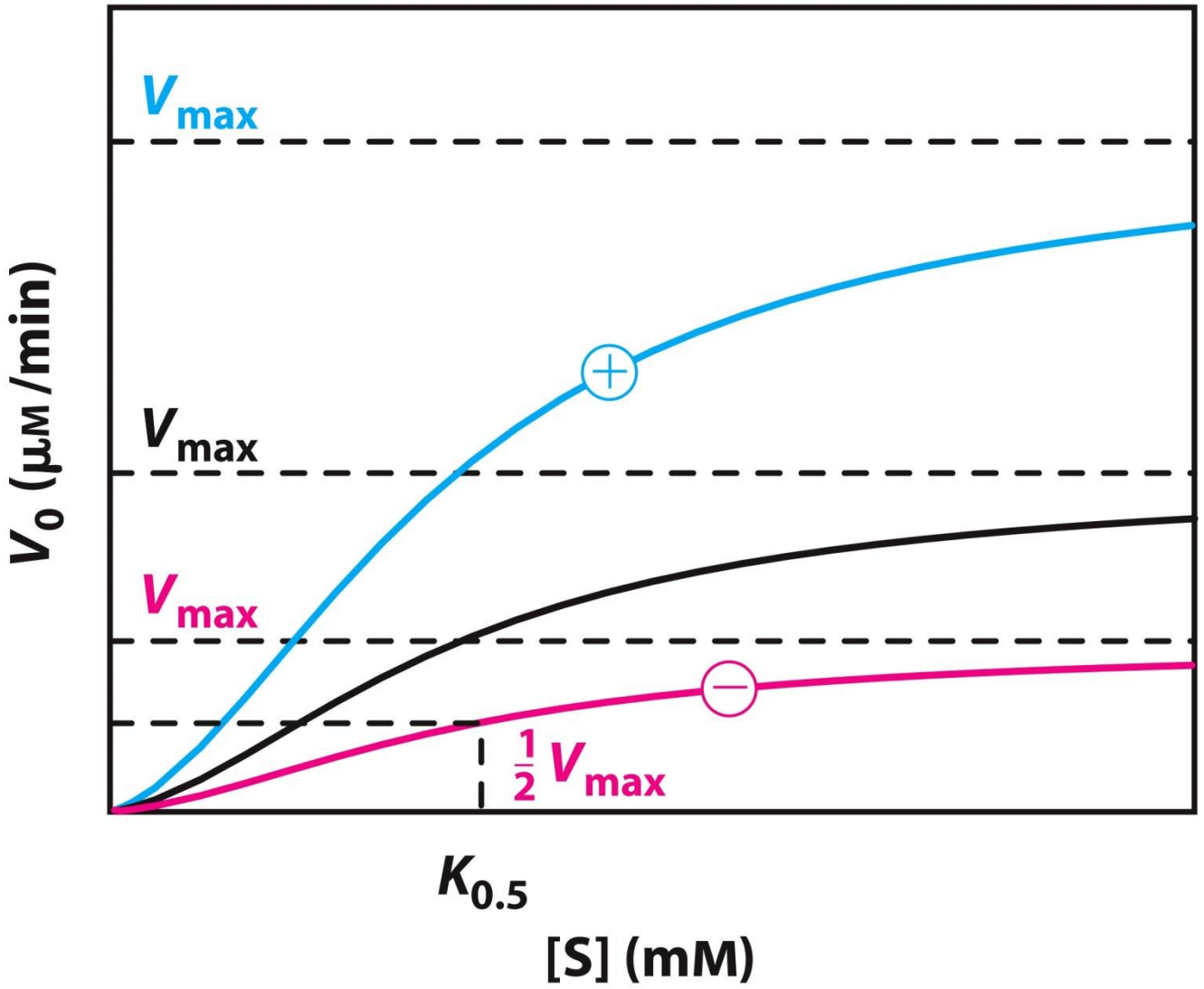


Figure 6-34c
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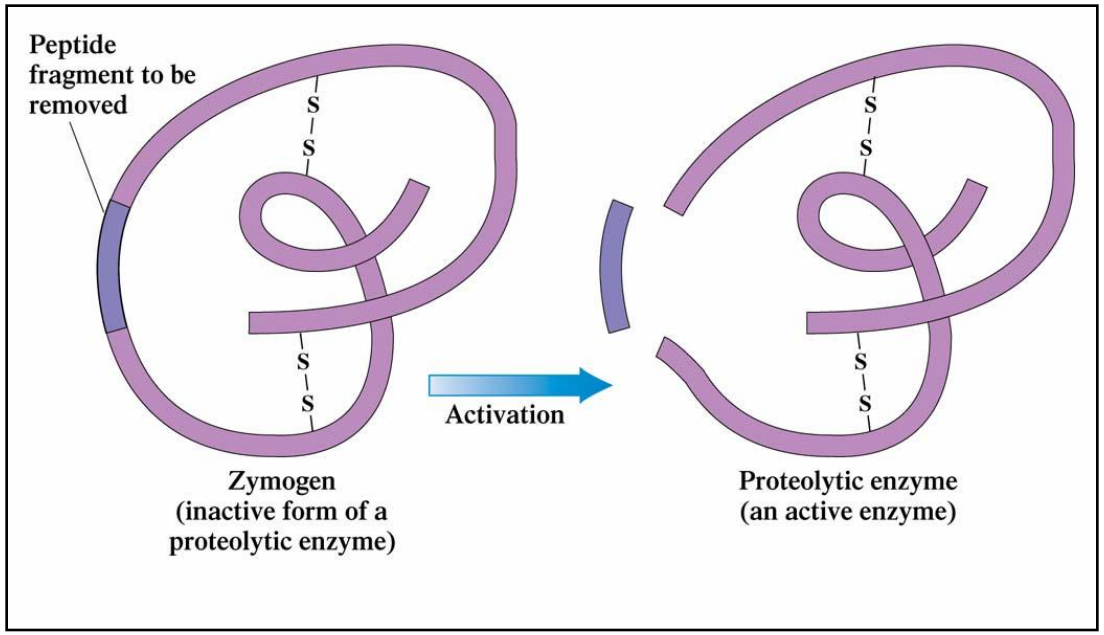
4) Zymogen Regulation

- 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