Chem 352 - Lecture 4
Part II: Enzyme Catalysis

Enzymes are biological catalysts; nearly every reaction that takes place in a living cell is catalyzed by an enzyme. Most enzymes are proteins, with some requiring non-protein components called coenzymes in order to function. The control of enzymatic activity plays a central roll in controlling the activities and proper functioning of a living cell.

Introduction to Enzyme Catalysis

Enzymes can be amazingly proficient.

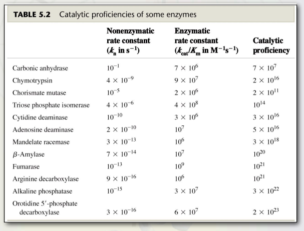

TABLE 5.2 Catalytic proficiencies of some enzymes

	Nonenzymatic rate constant (k_n in s^{-1})	Enzymatic rate constant (k_{cat}/K_m in $M^{-1}s^{-1}$)	Catalytic proficiency
Carbonic anhydrase	10^{-1}	7×10^{6}	7×10^{7}
Chymotrypsin	4×10^{-9}	9×10^{7}	2×10^{16}
Chorismate mutase	10^{-5}	2×10^{6}	2×10^{11}
Triose phosphate isomerase	4×10^{-6}	4×10^{8}	10^{14}
Cytidine deaminase	10^{-10}	3×10^{6}	3×10^{16}
Adenosine deaminase	2×10^{-10}	10^{7}	5×10^{16}
Mandelate racemase	3×10^{-13}	10^{6}	3×10^{18}
β-Amylase	7×10^{-14}	10^{7}	10^{20}
Fumarase	10^{-13}	10^{9}	10^{21}
Arginine decarboxylase	9×10^{-16}	10^{6}	10^{21}
Alkaline phosphatase	10^{-15}	3×10^{7}	3×10^{22}
Orotidine 5′-phosphate decarboxylase	3×10^{-16}	6×10^{7}	2×10^{23}

Introduction to Enzyme Catalysis

Overview

- Review of chemical reactions mechanisms
- Discussion of catalysis in general terms
- Examination of some major modes of enzymatic catalysis
  - acid/base catalysis
  - covalent catalysis
  - substrate binding
  - transition state stabilization

Chemical Reaction Mechanisms

A chemical mechanism lays out in detail the steps in a chemical reaction.

- With a focus on
  - the making and breaking of covalent bonds.
  - the movement of electrons at each step in a reaction.

Chemical Reaction Mechanisms

We will focus on three possible aspects to a reaction mechanism:

- Nucleophilic substitution
- Covalent bond cleavage
- Oxidation/Reduction

These are not necessarily independent of one another.

- e.g. Oxidation/Reduction can also involve covalent bond cleavage.

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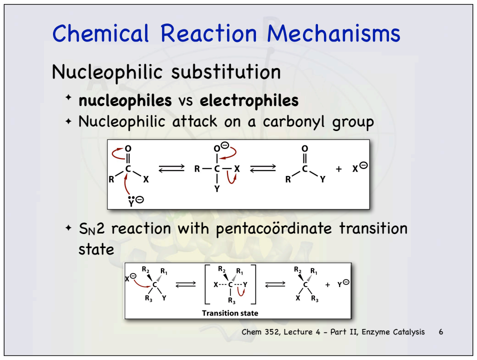

Chemical Reaction Mechanisms

Nucleophilic substitution

- **nucleophiles** vs **electrophiles**
- Nucleophilic attack on a carbonyl group

- S_N2 reaction with pentacoördinate transition state

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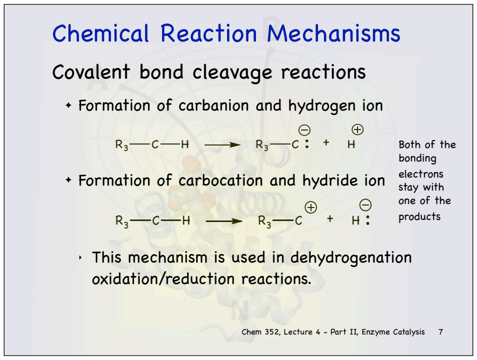

Chemical Reaction Mechanisms

Covalent bond cleavage reactions

- Formation of carbanion and hydrogen ion

$$R_3—C—H \longrightarrow R_3—C{:}^{\ominus} + H^{\oplus}$$

- Formation of carbocation and hydride ion

$$R_3—C—H \longrightarrow R_3—C^{\oplus} + H{:}^{\ominus}$$

Both of the bonding electrons stay with one of the products

- This mechanism is used in dehydrogenation oxidation/reduction reactions.

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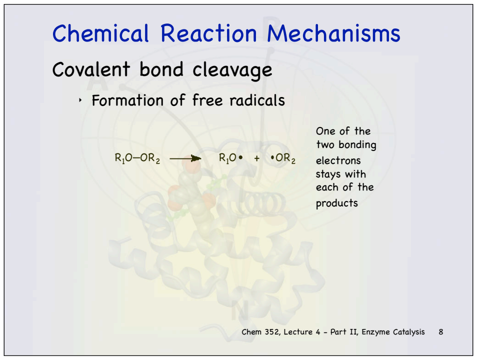

Chemical Reaction Mechanisms

Covalent bond cleavage

- Formation of free radicals

$$R_1O—OR_2 \longrightarrow R_1O\bullet + \bullet OR_2$$

One of the two bonding electrons stays with each of the products

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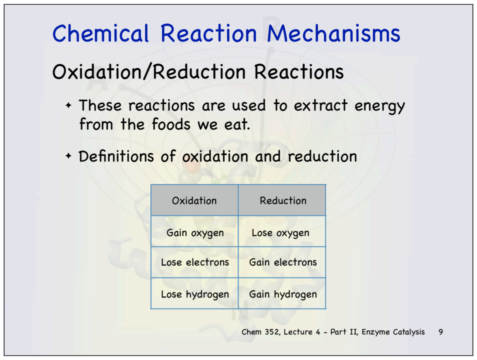

Chemical Reaction Mechanisms

Oxidation/Reduction Reactions

- These reactions are used to extract energy from the foods we eat.
- Definitions of oxidation and reduction

Oxidation	Reduction
Gain oxygen	Lose oxygen
Lose electrons	Gain electrons
Lose hydrogen	Gain hydrogen

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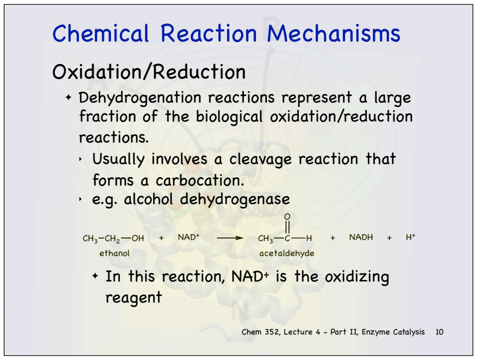


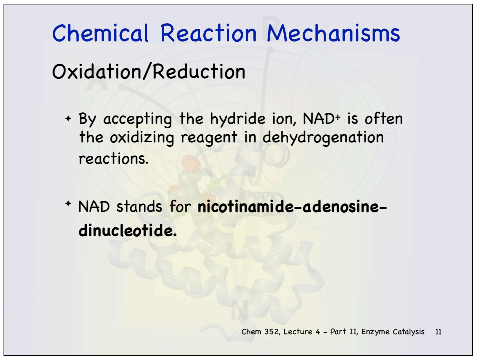
Chemical Reaction Mechanisms

Oxidation/Reduction

- Dehydrogenation reactions represent a large fraction of the biological oxidation/reduction reactions.
  - Usually involves a cleavage reaction that forms a carbocation.
  - e.g. alcohol dehydrogenase

$$\underset{\text{ethanol}}{CH_3—CH_2—OH} + NAD^+ \longrightarrow \underset{\text{acetaldehyde}}{CH_3—\overset{\overset{\Large O}{\|}}{C}—H} + NADH + H^+$$

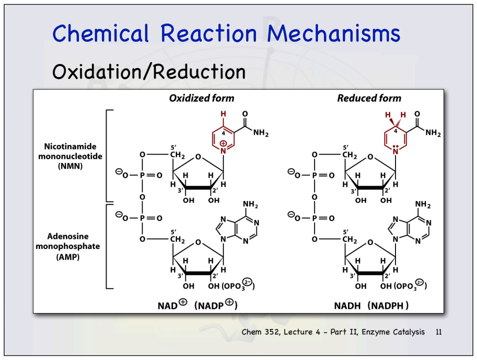
  - In this reaction, NAD^+ is the oxidizing reagent



Chemical Reaction Mechanisms

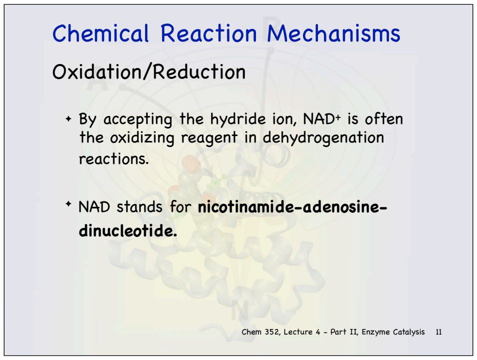
Oxidation/Reduction

- By accepting the hydride ion, NAD+ is often the oxidizing reagent in dehydrogenation reactions.
- NAD stands for **nicotinamide-adenosine-dinucleotide.**



Chemical Reaction Mechanisms

Oxidation/Reduction



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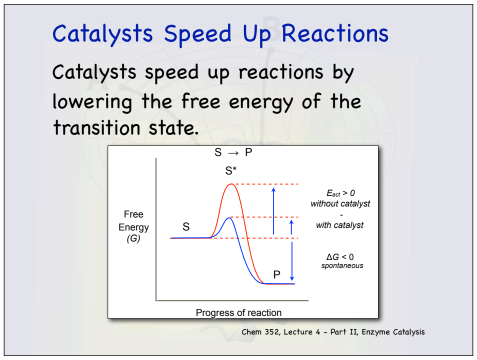
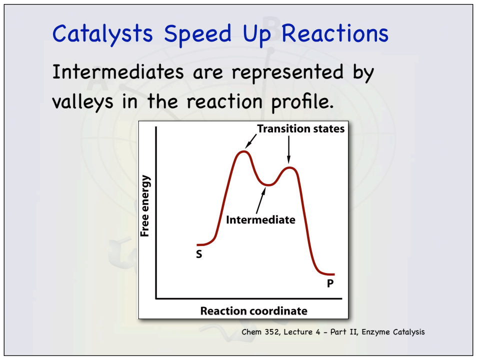


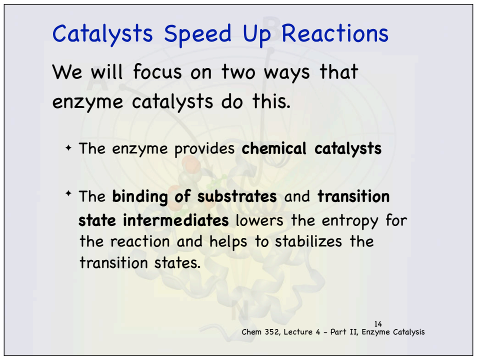
Catalysts Speed Up Reactions

Catalysts speed up reactions by lowering the free energy of the transition state.



Catalysts Speed Up Reactions

Intermediates are represented by valleys in the reaction profile.



Catalysts Speed Up Reactions

We will focus on two ways that enzyme catalysts do this.

- The enzyme provides **chemical catalysts**
- The **binding of substrates** and **transition state intermediates** lowers the entropy for the reaction and helps to stabilizes the transition states.

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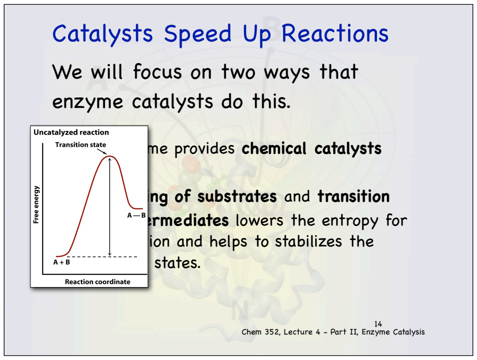

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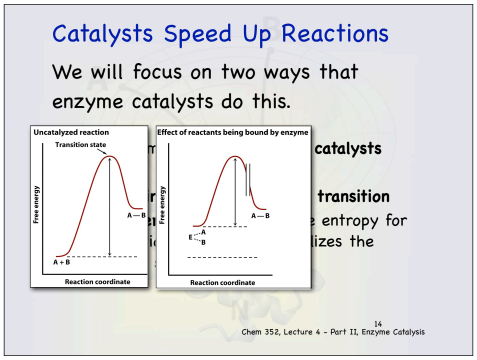

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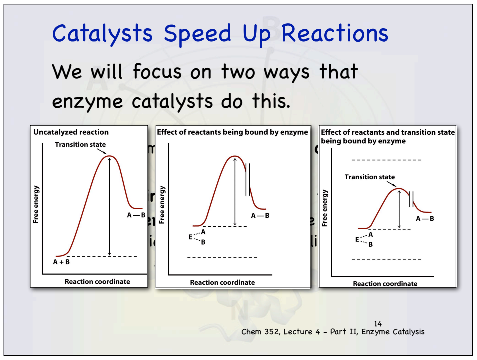

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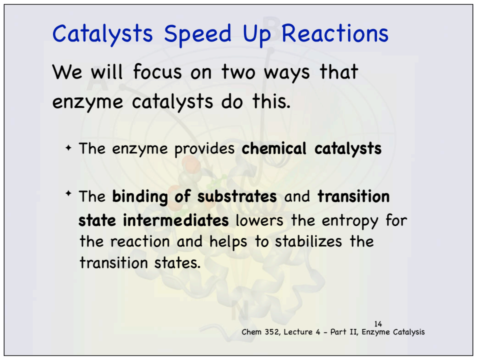

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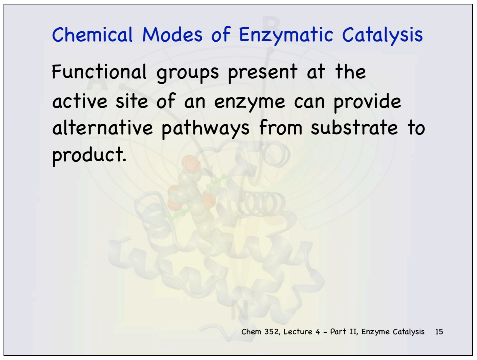

Chemical Modes of Enzymatic Catalysis

Functional groups present at the active site of an enzyme can provide alternative pathways from substrate to product.

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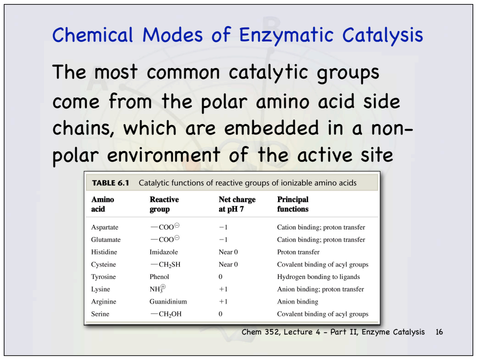

Chemical Modes of Enzymatic Catalysis

The most common catalytic groups come from the polar amino acid side chains, which are embedded in a non-polar environment of the active site

TABLE 6.1 Catalytic functions of reactive groups of ionizable amino acids

Amino acid	Reactive group	Net charge at pH 7	Principal functions
Aspartate	$-COO^{\ominus}$	−1	Cation binding; proton transfer
Glutamate	$-COO^{\ominus}$	−1	Cation binding; proton transfer
Histidine	Imidazole	Near 0	Proton transfer
Cysteine	$-CH_2SH$	Near 0	Covalent binding of acyl groups
Tyrosine	Phenol	0	Hydrogen bonding to ligands
Lysine	$NH_3^{\oplus}$	+1	Anion binding; proton transfer
Arginine	Guanidinium	+1	Anion binding
Serine	$-CH_2OH$	0	Covalent binding of acyl groups

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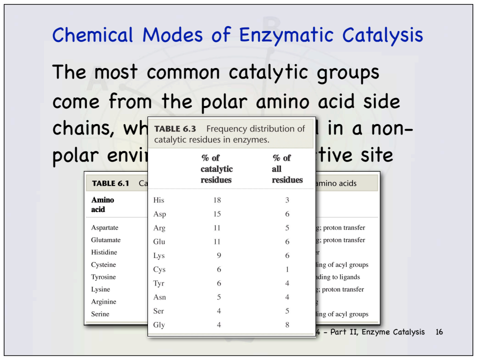

Chemical Modes of Enzymatic Catalysis

The most common catalytic groups come from the polar amino acid side chains, wh l in a non-polar envi tive site

TABLE 6.3 Frequency distribution of catalytic residues in enzymes.

	% of catalytic residues	% of all residues
His	18	3
Asp	15	6
Arg	11	5
Glu	11	6
Lys	9	6
Cys	6	1
Tyr	6	4
Asn	5	4
Ser	4	5
Gly	4	8

TABLE 6.1 Ca amino acids

Amino acid	
Aspartate	g; proton transfer
Glutamate	g; proton transfer
Histidine	r
Cysteine	ing of acyl groups
Tyrosine	ding to ligands
Lysine	g; proton transfer
Arginine	g
Serine	ing of acyl groups

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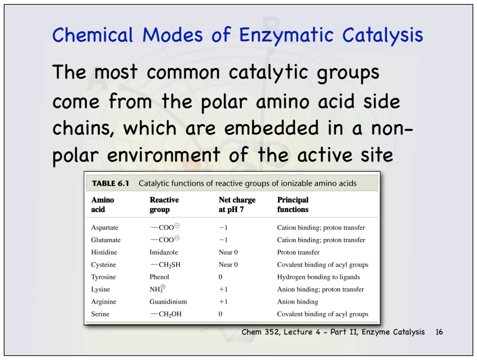

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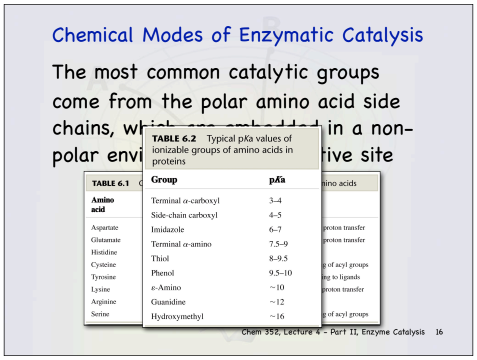

Chemical Modes of Enzymatic Catalysis

The most common catalytic groups come from the polar amino acid side chains, w in a non-polar envi tive site

TABLE 6.2 Typical p*K*a values of ionizable groups of amino acids in proteins

Group	p*K*a
Terminal α-carboxyl	3–4
Side-chain carboxyl	4–5
Imidazole	6–7
Terminal α-amino	7.5–9
Thiol	8–9.5
Phenol	9.5–10
ε-Amino	~10
Guanidine	~12
Hydroxymethyl	~16

TABLE 6.1 C nino acids

Amino acid	
Aspartate	proton transfer
Glutamate	proton transfer
Histidine	
Cysteine	g of acyl groups
Tyrosine	ing to ligands
Lysine	proton transfer
Arginine	
Serine	g of acyl groups

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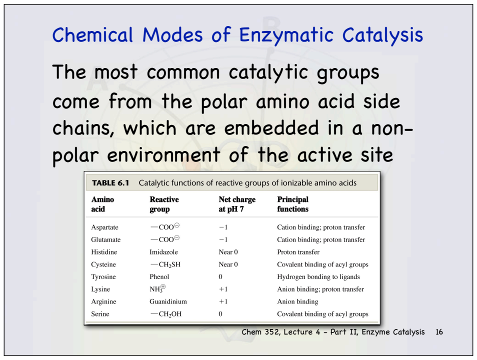
16-5



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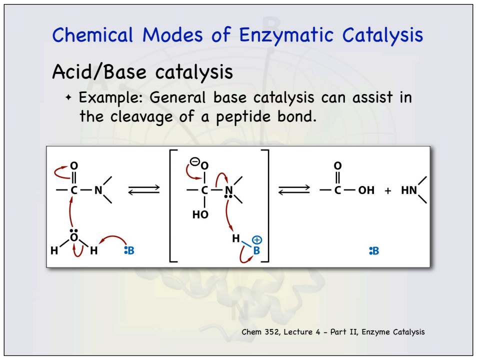

Chemical Modes of Enzymatic Catalysis

Acid/Base catalysis

- Example: General base catalysis can assist in the cleavage of a peptide bond.

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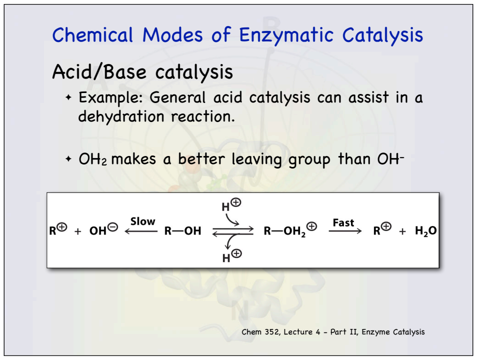

Chemical Modes of Enzymatic Catalysis

Acid/Base catalysis

- Example: General acid catalysis can assist in a dehydration reaction.
- OH_2 makes a better leaving group than OH^-

$$R^{\oplus} + OH^{\ominus} \xleftarrow{\text{Slow}} R{-}OH \rightleftharpoons R{-}OH_2^{\oplus} \xrightarrow{\text{Fast}} R^{\oplus} + H_2O$$

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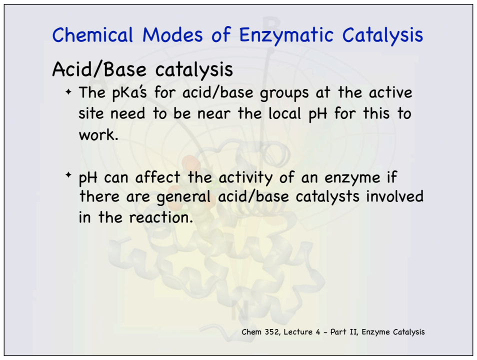
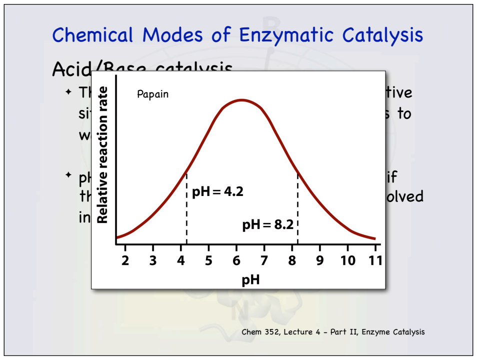


Chemical Modes of Enzymatic Catalysis

Acid/Base catalysis

- The pKa's for acid/base groups at the active site need to be near the local pH for this to work.
- pH can affect the activity of an enzyme if there are general acid/base catalysts involved in the reaction.

19-2



Chemical Modes of Enzymatic Catalysis

Acid/Base catalysis

- Th … tive si … s to w…
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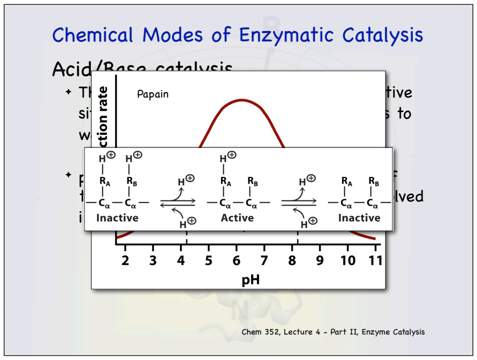

Chemical Modes of Enzymatic Catalysis

Acid/Base catalysis

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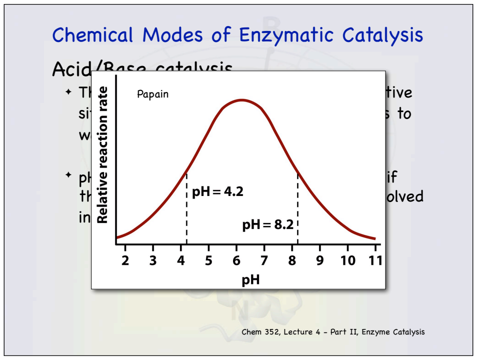

Chemical Modes of Enzymatic Catalysis

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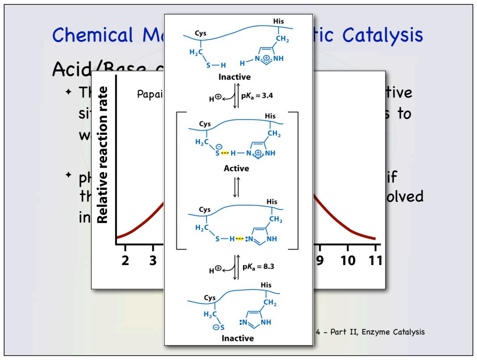

Chemical Mo … tic Catalysis

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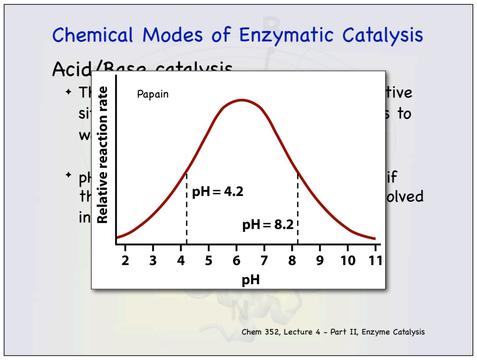

Chemical Modes of Enzymatic Catalysis

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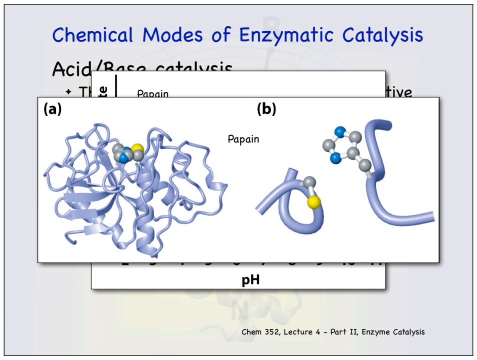

Chemical Modes of Enzymatic Catalysis

Acid/Base catalysis

- Th … tive

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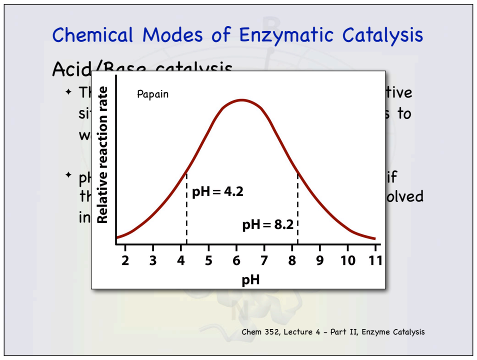

Chemical Modes of Enzymatic Catalysis

Acid/Base catalysis

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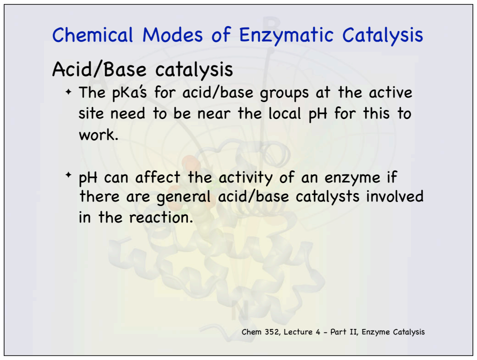

Chemical Modes of Enzymatic Catalysis

Acid/Base catalysis

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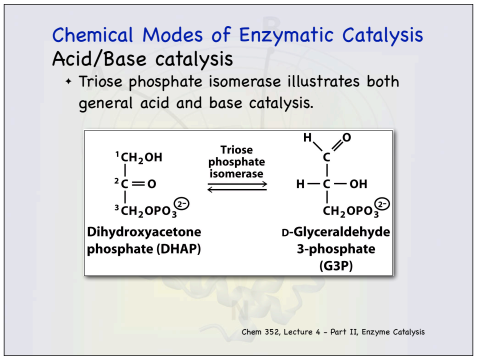


Chemical Modes of Enzymatic Catalysis

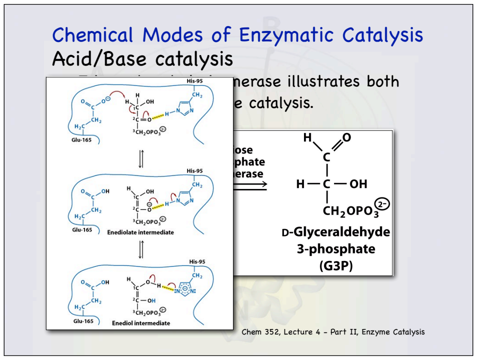
Acid/Base catalysis

- Triose phosphate isomerase illustrates both general acid and base catalysis.

Dihydroxyacetone phosphate (DHAP)

D-Glyceraldehyde 3-phosphate (G3P)

20-2



Chemical Modes of Enzymatic Catalysis

Acid/Base catalysis

D-Glyceraldehyde 3-phosphate (G3P)

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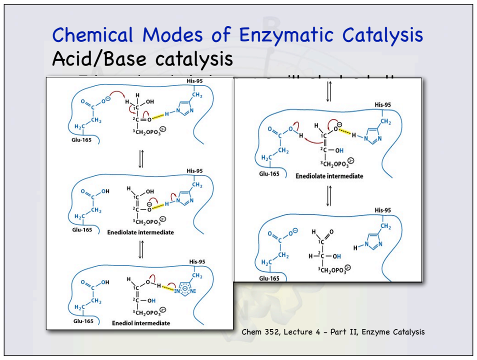
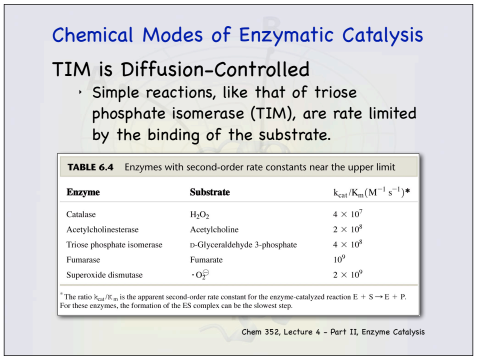


Chemical Modes of Enzymatic Catalysis

Acid/Base catalysis



Chemical Modes of Enzymatic Catalysis

TIM is Diffusion-Controlled

- Simple reactions, like that of triose phosphate isomerase (TIM), are rate limited by the binding of the substrate.

TABLE 6.4 Enzymes with second-order rate constants near the upper limit

Enzyme	Substrate	$k_{cat}/K_m (M^{-1} s^{-1})$*
Catalase	H_2O_2	4×10^7
Acetylcholinesterase	Acetylcholine	2×10^8
Triose phosphate isomerase	D-Glyceraldehyde 3-phosphate	4×10^8
Fumarase	Fumarate	10^9
Superoxide dismutase	$\cdot O_2^{\ominus}$	2×10^9

*The ratio k_{cat}/K_m is the apparent second-order rate constant for the enzyme-catalyzed reaction E + S → E + P. For these enzymes, the formation of the ES complex can be the slowest step.

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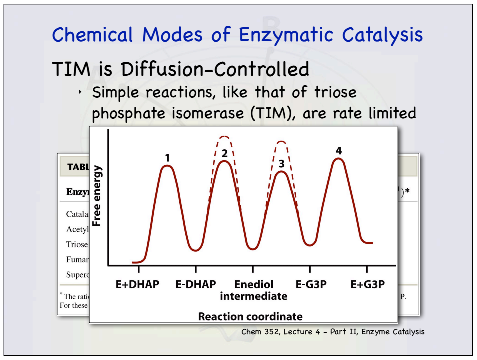

Chemical Modes of Enzymatic Catalysis

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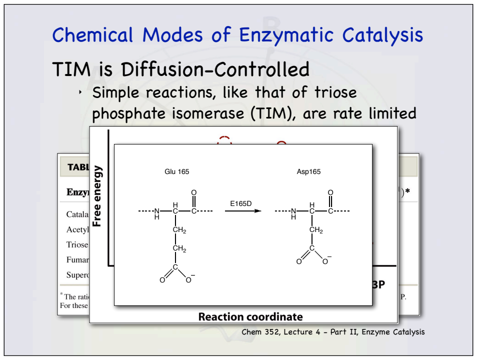

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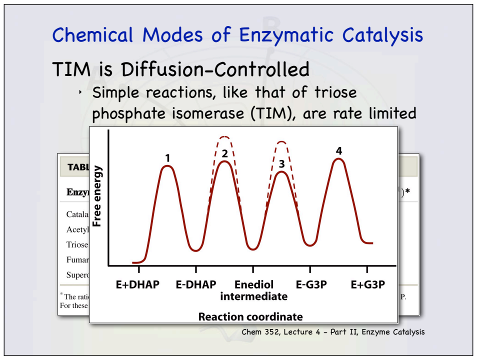
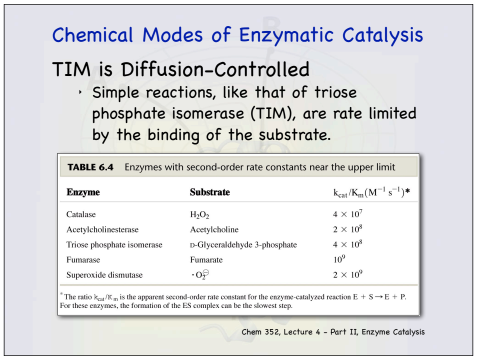


Chemical Modes of Enzymatic Catalysis

TIM is Diffusion-Controlled

- Simple reactions, like that of triose phosphate isomerase (TIM), are rate limited

21-4



Chemical Modes of Enzymatic Catalysis

TIM is Diffusion-Controlled

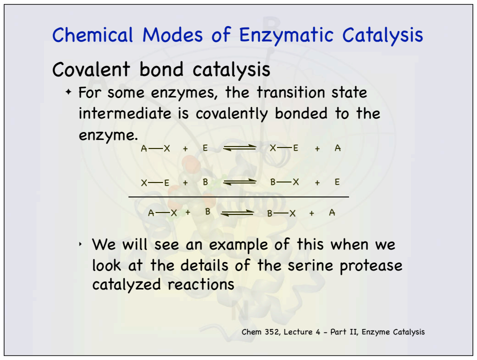
- Simple reactions, like that of triose phosphate isomerase (TIM), are rate limited by the binding of the substrate.

TABLE 6.4 Enzymes with second-order rate constants near the upper limit

Enzyme	Substrate	$k_{cat}/K_m (M^{-1} s^{-1})$*
Catalase	H_2O_2	4×10^7
Acetylcholinesterase	Acetylcholine	2×10^8
Triose phosphate isomerase	D-Glyceraldehyde 3-phosphate	4×10^8
Fumarase	Fumarate	10^9
Superoxide dismutase	$\cdot O_2^{\ominus}$	2×10^9

*The ratio k_{cat}/K_m is the apparent second-order rate constant for the enzyme-catalyzed reaction E + S → E + P. For these enzymes, the formation of the ES complex can be the slowest step.

21-5



Chemical Modes of Enzymatic Catalysis

Covalent bond catalysis

- For some enzymes, the transition state intermediate is covalently bonded to the enzyme.

$$A{-}X + E \rightleftharpoons X{-}E + A$$

$$X{-}E + B \rightleftharpoons B{-}X + E$$

$$A{-}X + B \rightleftharpoons B{-}X + A$$

  - We will see an example of this when we look at the details of the serine protease catalyzed reactions

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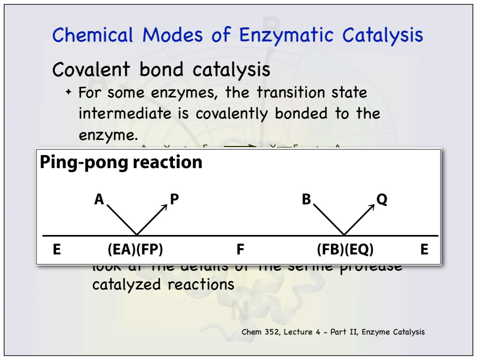

Chemical Modes of Enzymatic Catalysis

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- For some enzymes, the transition state intermediate is covalently bonded to the enzyme.

Ping-pong reaction

look at the details of the serine protease catalyzed reactions

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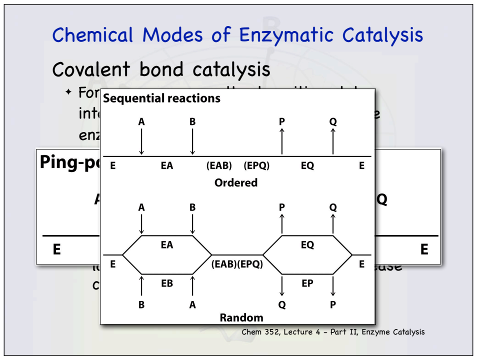
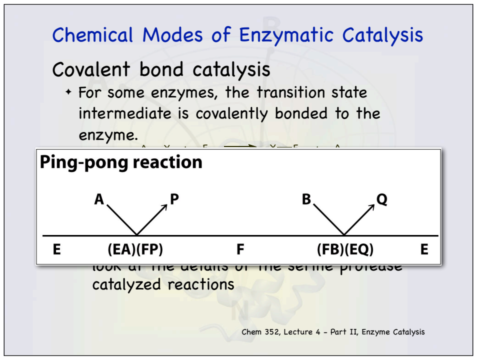


Chemical Modes of Enzymatic Catalysis

Covalent bond catalysis

Sequential reactions



Chemical Modes of Enzymatic Catalysis

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look at the details of the serine protease catalyzed reactions

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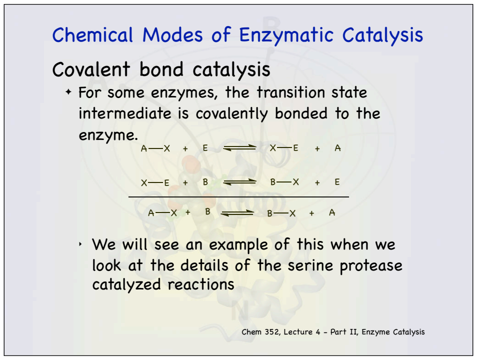

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Binding Modes of Enzymatic Catalysis

- Acid/Base catalysis and covalent bond catalysis can account for an approximately 10 to 100 fold increase in the reaction rates
- However, 10^8 fold increases are observed

23

Binding Modes of Enzymatic Catalysis

- Enzymes also bind of substrates and orient them relative to one another and to catalytic groups on the enzyme.

24

Binding Modes of Enzymatic

The Proximity Effect

- The binding of substrates creates a high effective local concentration of substrates.
- It also decreases the entropy of the substrates.

25-1

Binding Modes of Enzymatic

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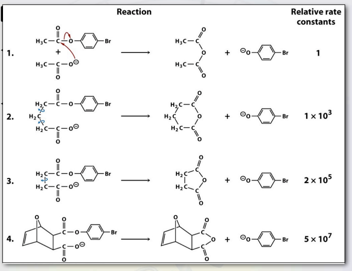


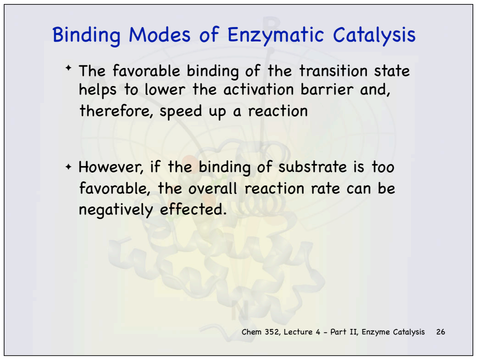
25-2

Binding Modes of Enzymatic

The Proximity Effect

- The binding of substrates creates a high effective local concentration of substrates.
- It also decreases the entropy of the substrates.

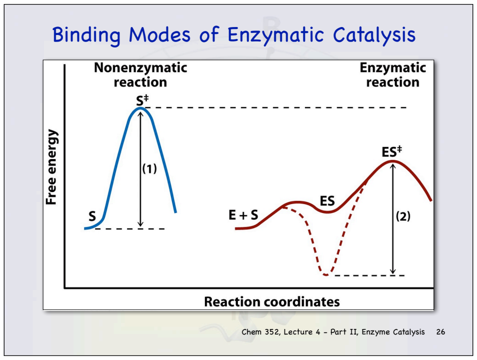
25-3



Binding Modes of Enzymatic Catalysis

- The favorable binding of the transition state helps to lower the activation barrier and, therefore, speed up a reaction
- However, if the binding of substrate is too favorable, the overall reaction rate can be negatively effected.

26-1



Binding Modes of Enzymatic Catalysis

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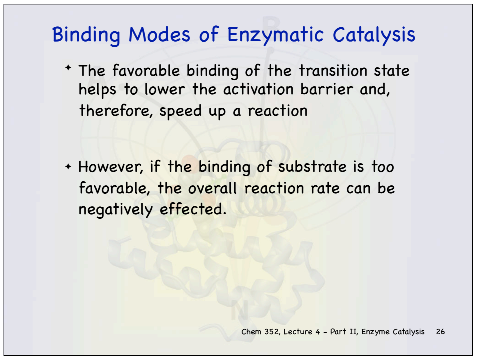

Binding Modes of Enzymatic Catalysis

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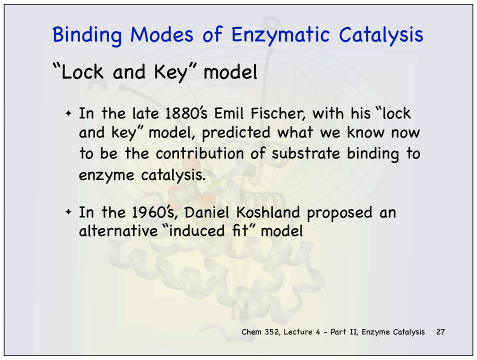
26-3

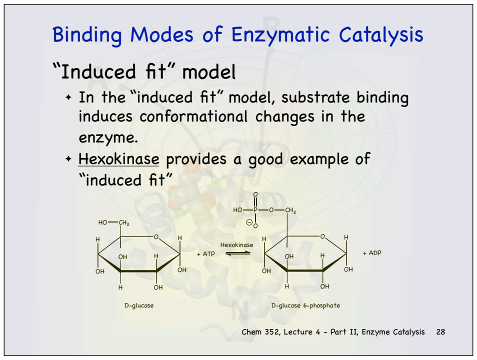


Binding Modes of Enzymatic Catalysis

“Lock and Key” model

- In the late 1880’s Emil Fischer, with his “lock and key” model, predicted what we know now to be the contribution of substrate binding to enzyme catalysis.
- In the 1960’s, Daniel Koshland proposed an alternative “induced fit” model

27



Binding Modes of Enzymatic Catalysis

“Induced fit” model

- In the “induced fit” model, substrate binding induces conformational changes in the enzyme.
- Hexokinase provides a good example of “induced fit”

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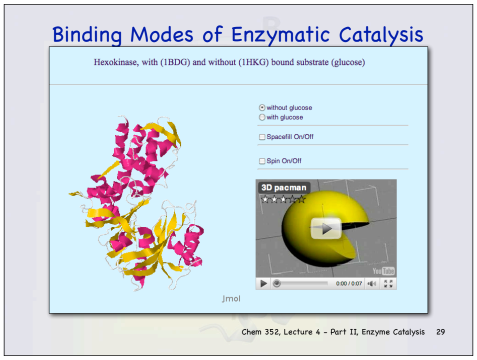
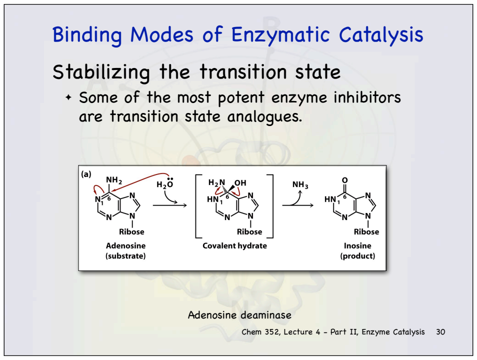


Binding Modes of Enzymatic Catalysis

Hexokinase, with (1BDG) and without (1HKG) bound substrate (glucose)

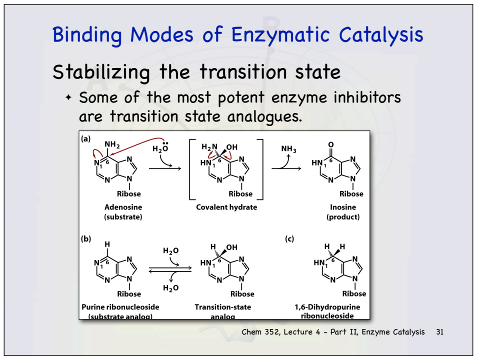


Binding Modes of Enzymatic Catalysis

Stabilizing the transition state

- Some of the most potent enzyme inhibitors are transition state analogues.

Adenosine deaminase



Binding Modes of Enzymatic Catalysis

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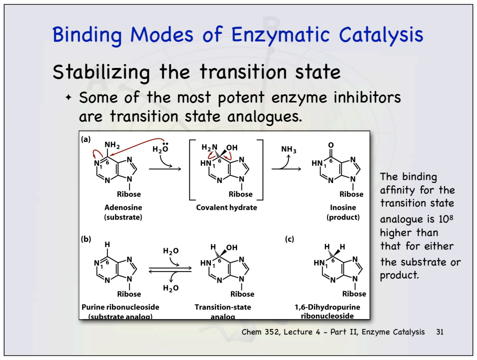
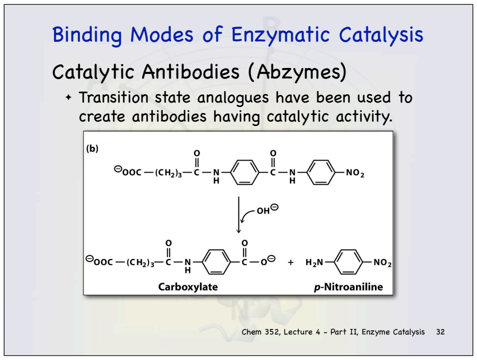


Binding Modes of Enzymatic Catalysis

Stabilizing the transition state

- Some of the most potent enzyme inhibitors are transition state analogues.

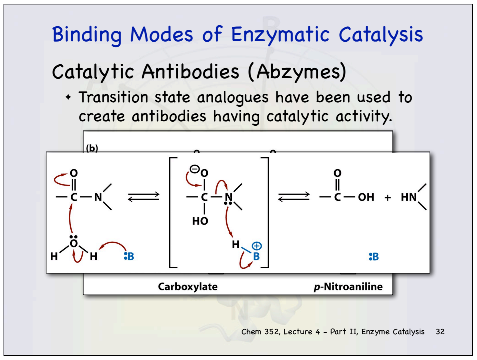
The binding affinity for the transition state analogue is 10^8 higher than that for either the substrate or product.



Binding Modes of Enzymatic Catalysis

Catalytic Antibodies (Abzymes)

- Transition state analogues have been used to create antibodies having catalytic activity.



Binding Modes of Enzymatic Catalysis

Catalytic Antibodies (Abzymes)

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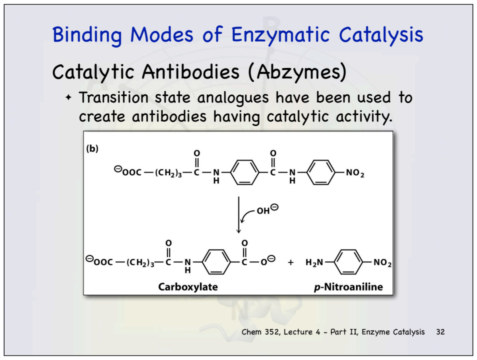
32-2



Binding Modes of Enzymatic Catalysis

Catalytic Antibodies (Abzymes)

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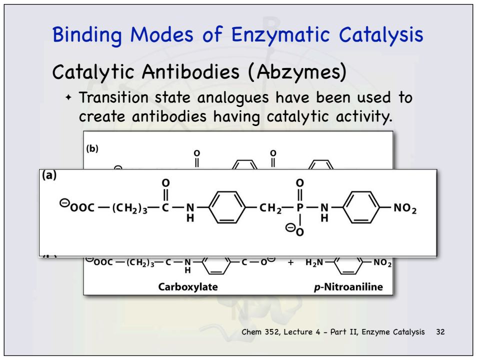
32-3



Binding Modes of Enzymatic Catalysis

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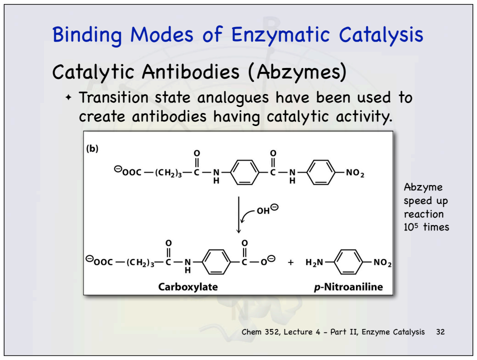
32-4



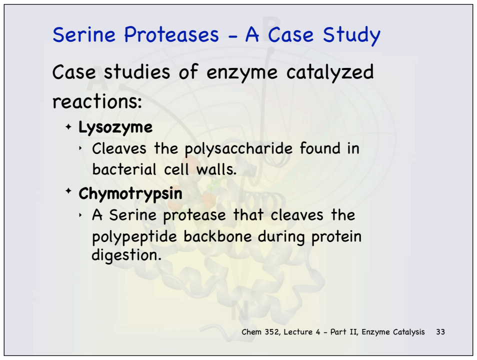
Binding Modes of Enzymatic Catalysis

Catalytic Antibodies (Abzymes)

- Transition state analogues have been used to create antibodies having catalytic activity.

Abzyme speed up reaction 10^5 times

32-5



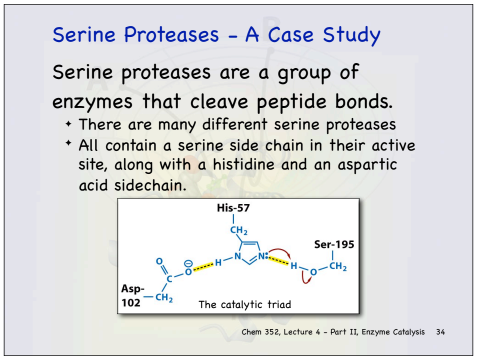
Serine Proteases – A Case Study

Case studies of enzyme catalyzed reactions:

- **Lysozyme**
  - Cleaves the polysaccharide found in bacterial cell walls.
- **Chymotrypsin**
  - A Serine protease that cleaves the polypeptide backbone during protein digestion.

33

34



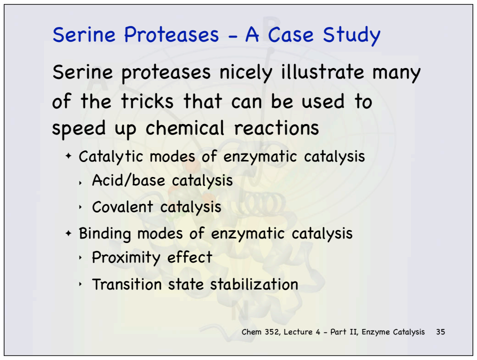
Serine Proteases - A Case Study

Serine proteases are a group of enzymes that cleave peptide bonds.

- There are many different serine proteases
- All contain a serine side chain in their active site, along with a histidine and an aspartic acid sidechain.

The catalytic triad

35

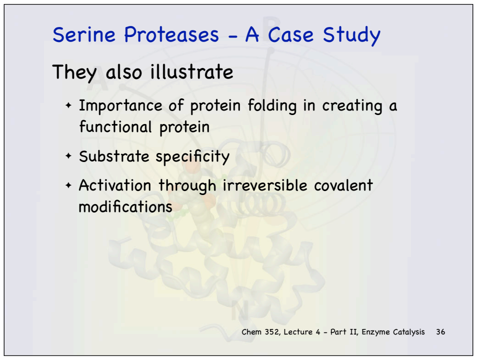


Serine Proteases - A Case Study

Serine proteases nicely illustrate many of the tricks that can be used to speed up chemical reactions

- Catalytic modes of enzymatic catalysis
  - Acid/base catalysis
  - Covalent catalysis
- Binding modes of enzymatic catalysis
  - Proximity effect
  - Transition state stabilization

36

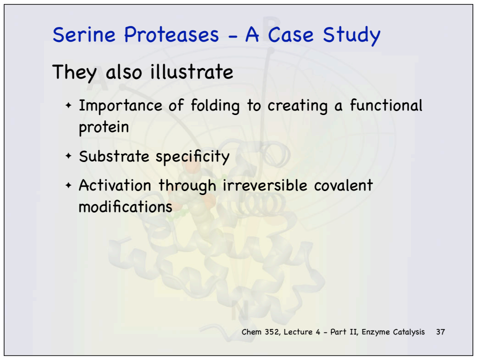


Serine Proteases - A Case Study

They also illustrate

- Importance of protein folding in creating a functional protein
- Substrate specificity
- Activation through irreversible covalent modifications

37-1



Serine Proteases - A Case Study

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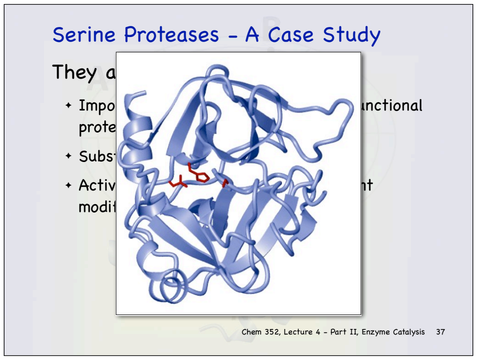
- Activation through irreversible covalent modifications

37-2



Serine Proteases - A Case Study

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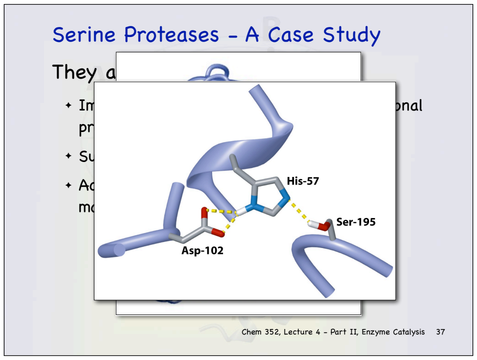

Serine Proteases - A Case Study

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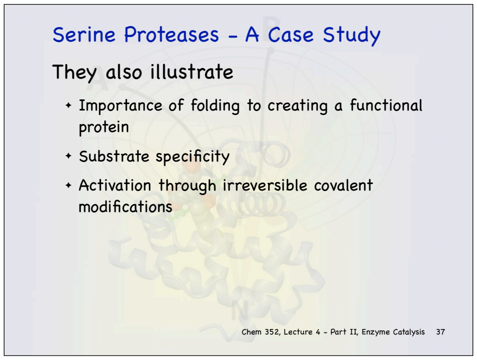

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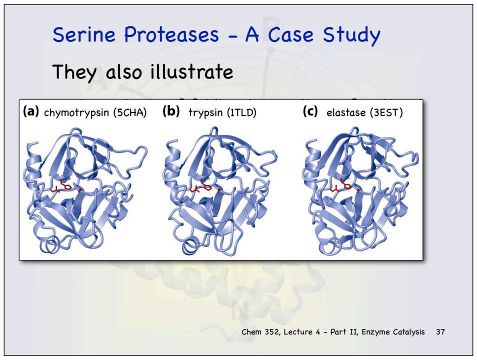
37-4



Serine Proteases - A Case Study

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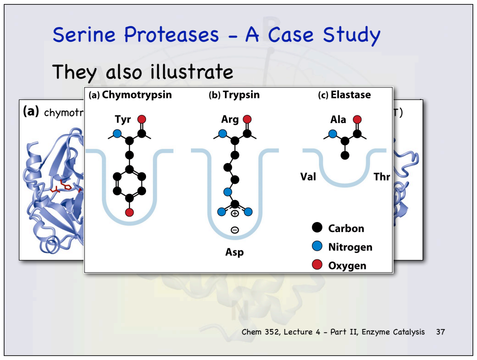
37-5



Serine Proteases - A Case Study

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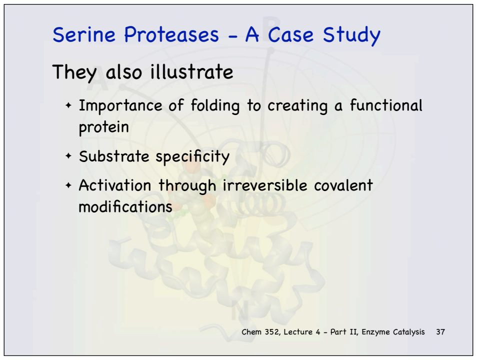
37-6



Serine Proteases - A Case Study

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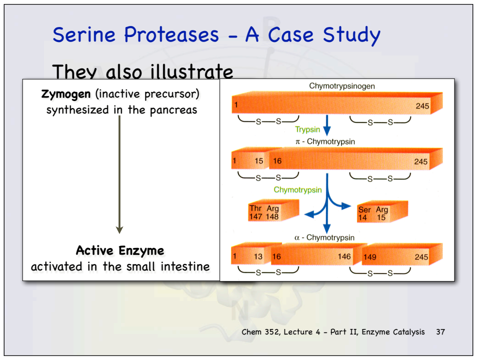
37-7



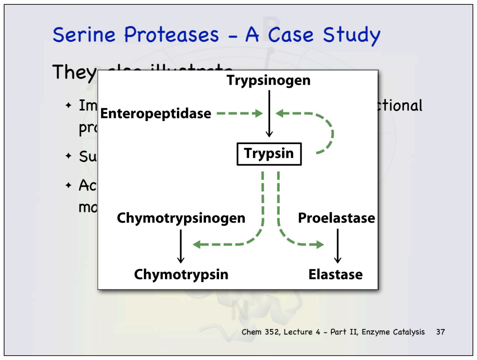
Serine Proteases – A Case Study

They also illustrate

Zymogen (inactive precursor) synthesized in the pancreas

Active Enzyme activated in the small intestine

37-8



Serine Proteases – A Case Study

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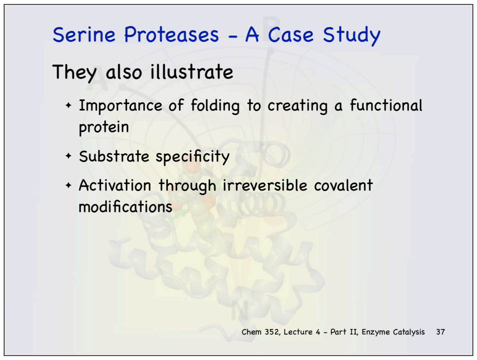

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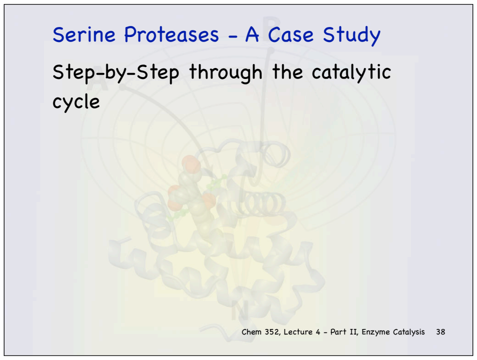
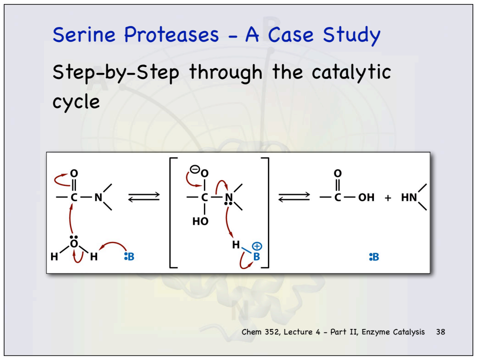
37-10



Serine Proteases – A Case Study

Step-by-Step through the catalytic cycle

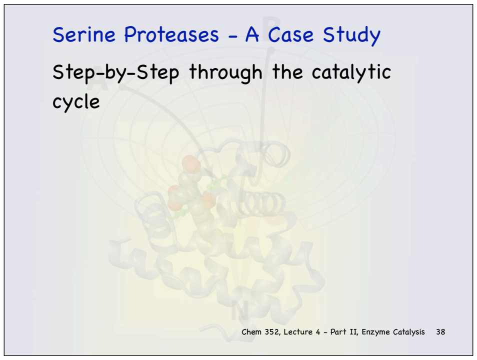
38-1



Serine Proteases – A Case Study

Step-by-Step through the catalytic cycle

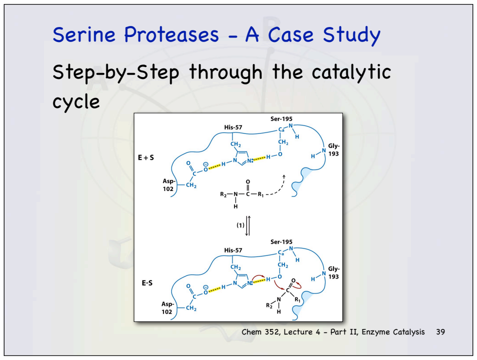
38-2



Serine Proteases - A Case Study

Step-by-Step through the catalytic cycle

38-3



Serine Proteases - A Case Study

Step-by-Step through the catalytic cycle

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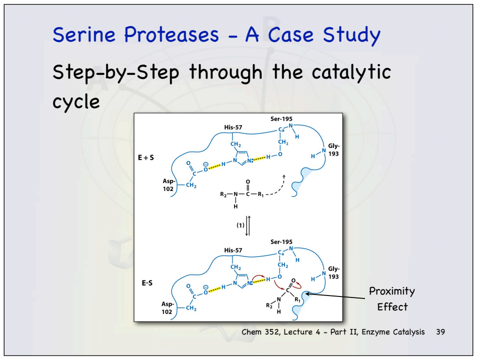
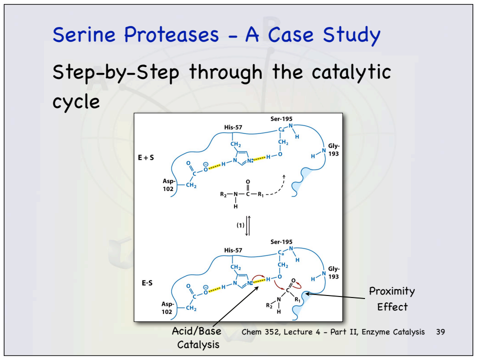


Serine Proteases - A Case Study

Step-by-Step through the catalytic cycle

Proximity Effect

39-2



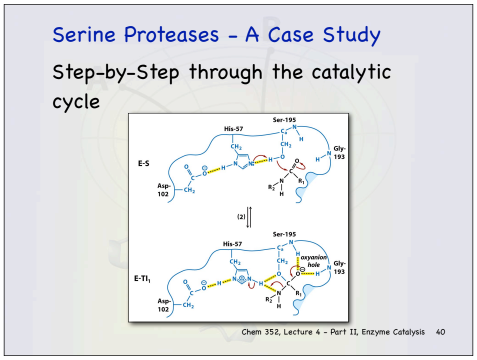
Serine Proteases - A Case Study

Step-by-Step through the catalytic cycle

Proximity Effect

Acid/Base Catalysis

39-3



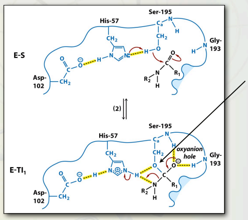
Serine Proteases - A Case Study

Step-by-Step through the catalytic cycle

40-1

Serine Proteases - A Case Study

Step-by-Step through the catalytic cycle

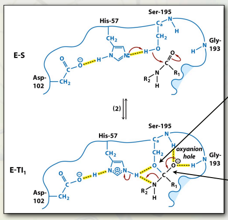


Covalent Bond Catalysis

40-2

Serine Proteases - A Case Study

Step-by-Step through the catalytic cycle



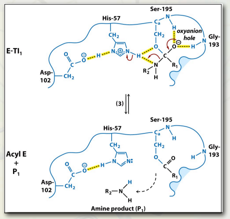
Covalent Bond Catalysis

Transition State Stabilization

40-3

Serine Proteases - A Case Study

Step-by-Step through the catalytic cycle



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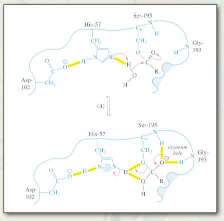
Serine Proteases - A Case Study

Step-by-Step through the catalytic cycle



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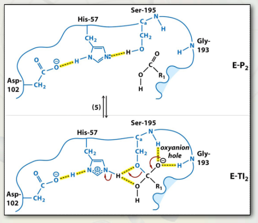
Serine Proteases - A Case Study

Step-by-Step through the catalytic cycle



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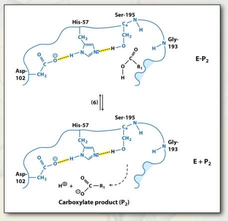
Serine Proteases - A Case Study

Step-by-Step through the catalytic cycle



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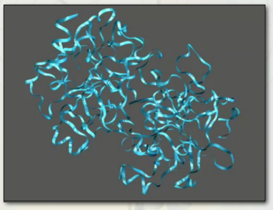
Serine Proteases - A Case Study

Step-by-Step through the catalytic cycle



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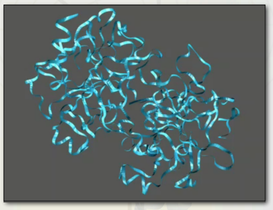
Serine Proteases - A Case Study

Step-by-Step through the catalytic cycle



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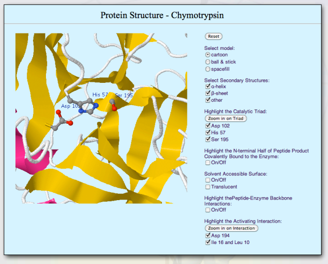
Serine Proteases - A Case Study



Protein Structure - Chymotrypsin

Select model:
● cartoon
○ ball & stick
○ spacefill

Select Secondary Structures:
☑ α-helix
☑ β-sheet
☑ other

Highlight the Catalytic Triad:
☑ Asp 102
☑ His 57
☑ Ser 195

Highlight the N-terminal Half of Peptide Product Covalently Bound to the Enzyme:
☐ On/Off

Solvent Accessible Surface:
☐ On/Off
☐ Translucent

Highlight thePeptide-Enzyme Backbone Interactions:
☐ On/Off

Highlight the Activating Interaction:
☑ Asp 194
☑ Ile 16 and Leu 10

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Next Up

- At this time we will skip over Chapter 7 (Cofactors and Vitamins)
  - We will discuss cofactors and vitamins as we encounter them throughout the rest of the semester.
- Carbohydrates (Chapter 8)

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