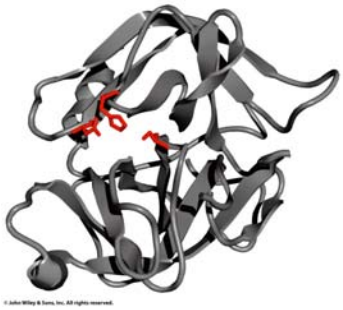
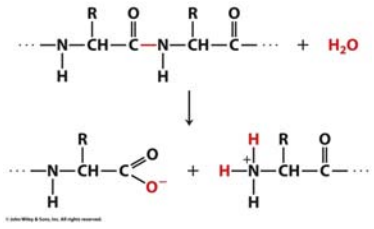
How Enzymes Work

Pratt & Cornely Ch 6

Enzymes

- Biocatalyst
- Active site
- Substrate/Product
- Reaction specificity
- Stereospecificity
- Coupled reactions
- Regulation





Rate Enhancement

[TABLE 6-1] Rate Enhancements of Enzymes

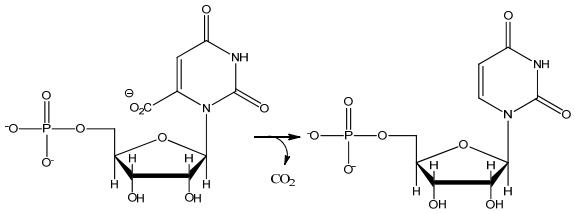
Enzyme	Half-Time (uncatalyzed)*	Uncatalyzed Rate (s^{-1})	Catalyzed Rate (s^{-1})	Rate Enhancement (catalyzed rate/ uncatalyzed rate)
Orotidine-5′-monophosphate decarboxylase	78,000,000 years	2.8×10^{-16}	39	1.4×10^{17}
Staphylococcal nuclease	130,000 years	1.7×10^{-13}	95	5.6×10^{14}
Adenosine deaminase	120 years	1.8×10^{-10}	370	2.1×10^{12}
Chymotrypsin	20 years	1.0×10^{-9}	190	1.7×10^{11}
Triose phosphate isomerase	1.9 years	4.3×10^{-6}	4,300	1.0×10^{9}
Chorismate mutase	7.4 hours	2.6×10^{-5}	50	1.9×10^{6}
Carbonic anhydrase	5 seconds	1.3×10^{-1}	1,000,000	7.7×10^{6}

*The half-times of very slow reactions were estimated by extrapolating from measurements made at very high temperatures.

[Data mostly from Radzicka, R., and Wolfenden, R., *Science* **267**, 90–93 (1995).]

Orotidine Decarboxylase

- Key enzyme in production of nucleotides for DNA
- $T_{1/2}$ = 14 ms
- But what makes it a great enzyme?



The Speed of the Uncatalyzed Rxn

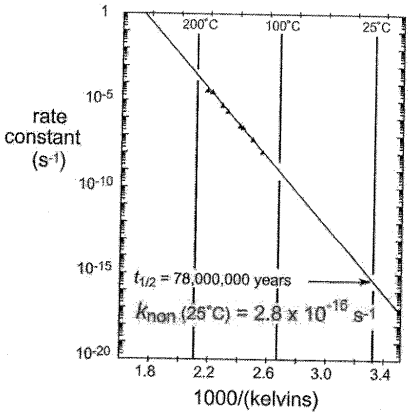
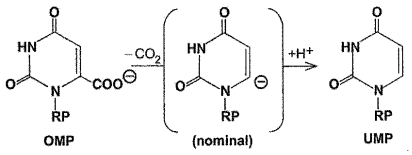


Figure 4 Temperature dependence of the rate of decarboxylation of 1-methyloro-tate in 0.1 M potassium phosphate buffer, pH 6.8 (6).

Mechanism and RDS



EC Nomenclature

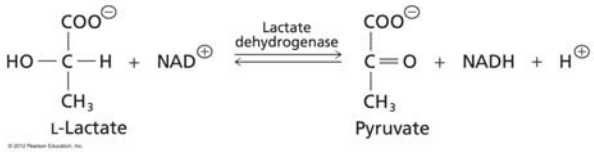
[TABLE 6-2]

Enzyme Classification

Class of Enzyme	Type of Reaction Catalyzed
1. Oxidoreductases	Oxidation–reduction reactions
2. Transferases	Transfer of functional groups
3. Hydrolases	Hydrolysis reactions
4. Lyases	Group elimination to form double bonds
5. Isomerases	Isomerization reactions
6. Ligases	Bond formation coupled with ATP hydrolysis

Enzyme Classes

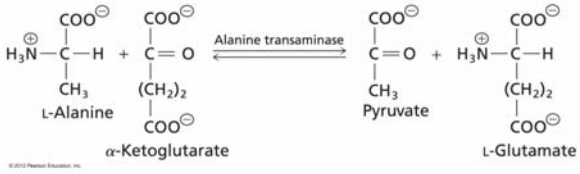
1. Oxidoreductase



- Recognize Redox reactions
- Redox cofactors: NAD^+/NADH, $FAD/FADH_2$, Q/QH_2
- Dehydrogenases, oxidases, peroxidases, reductase

Enzyme Classes

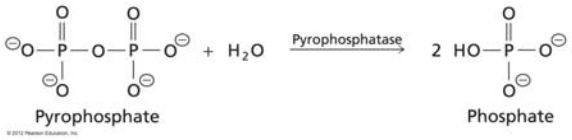
2. Transferase



- 2 substrates
- Coenzymes—PLP
- Transferase, kinase

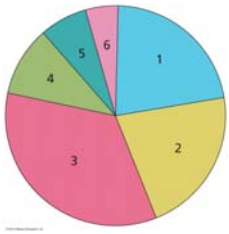
Enzyme Classes

3. Hydrolase

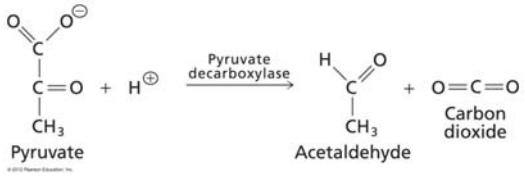


- Water nucleophile
- Phosphatase, nuclease, protease, peptidase

Enzyme Classes



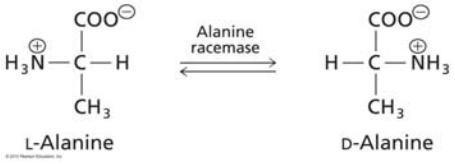
4. Lyase



- Hardest to recognize—not redox, hydrolysis
- Elimination of a group to give double bond
- Reversible
- Hydratase, decarboxylase, (formerly synthases)

Enzyme Classes

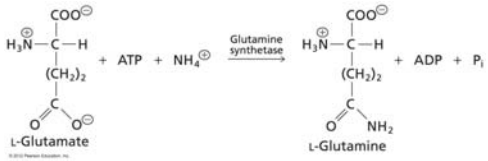
5. Isomerase



- Rearrangement without loss/add
- Racemase, isomerase, mutase (phosphate)

Enzyme Classes

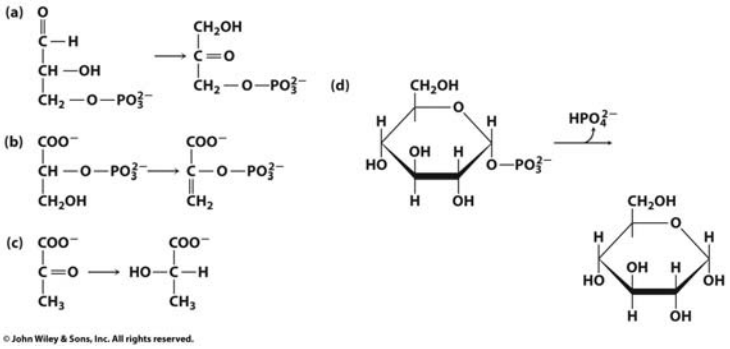
6. Ligase



- Joining together with ATP input
- Irreversible
- Synthetase

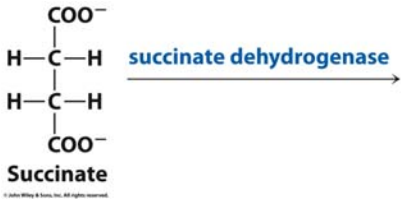
Problem 10

- To which class do the enzymes that catalyze the following reactions belong?



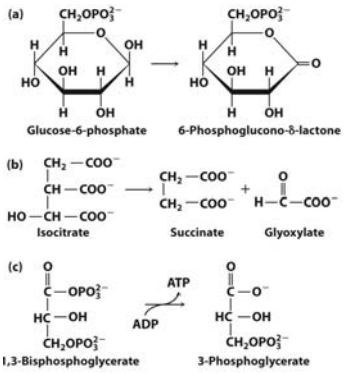
Problems 11-12

- Draw the structures of the products



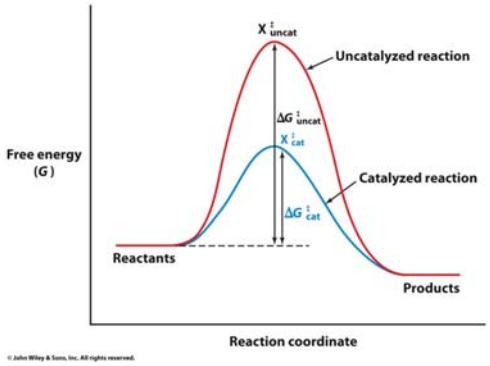
Problem 14

- Propose a name for each enzyme.



Catalysis

- Thermodynamics
- Kinetics
- Rxn coordinate
- Transition state
- 5.7 kJ ~ 10x change



Mechanisms

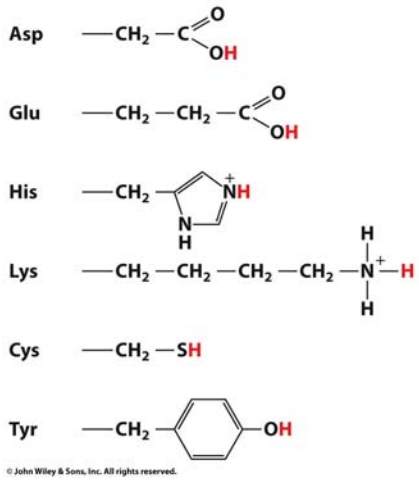
- Two major mechanisms—any or all may be used in a given enzyme
  - Chemical Mechanisms (Changes in pathway)
    - Acid-base catalysis
    - Covalent catalysis
    - Metal ion catalysis
  - Binding Mechanisms (Lowering of E_A)
    - Proximity/orientation effect
    - Transition State Stabilization
    - Electrostatic catalysis

1. Acid/Base Catalysis

- Sidechains affect most proton transfers

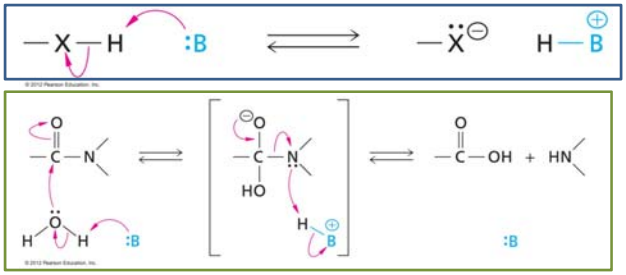
Table 6.2 Typical pK_a values of ionizable groups of amino acids in proteins

Group	pK_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

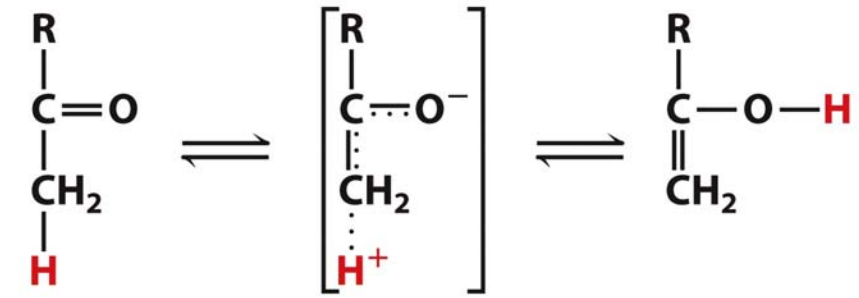


General Acid-Base Catalysis

- H^+ and HO^- are "specific acid/base" and depend on pH
- Amino acid sidechains are general acid-base, and can conduct reactions inside active site pocket that aren't possible in solution

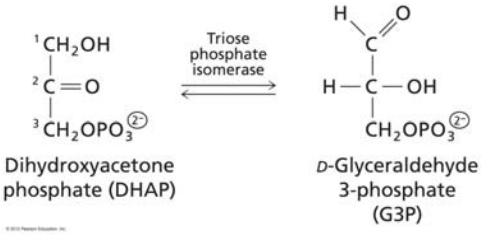


What's Wrong with This?



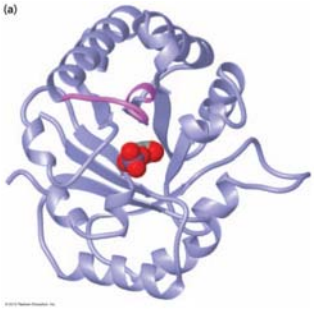
Ketone **Transition state** **Enol**

Triose Phosphate Isomerase

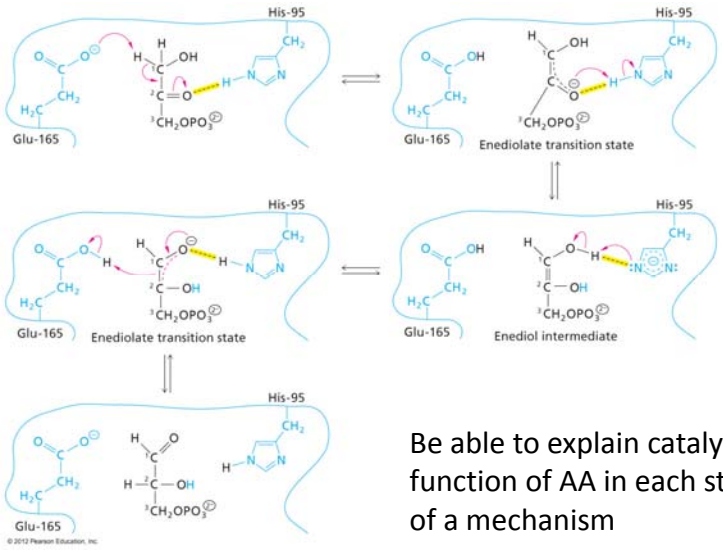


Dihydroxyacetone phosphate (DHAP)

D-Glyceraldehyde 3-phosphate (G3P)



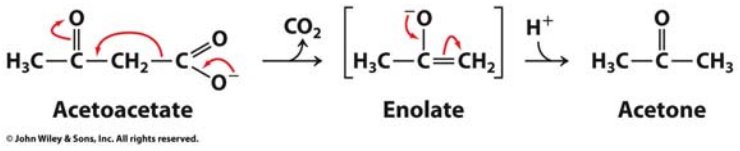
Mechanism



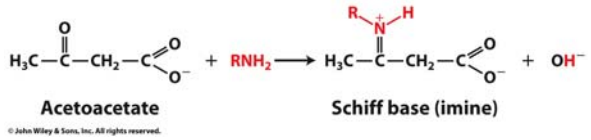
Be able to explain catalytic function of AA in each step of a mechanism

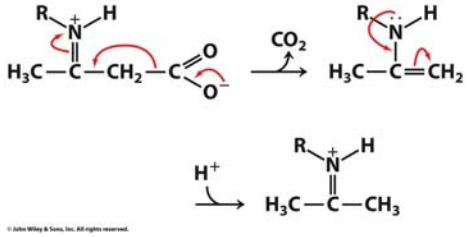
2. Covalent Catalysis

- Can act as active site nucleophile
- Can produce a more reactive electrophile
- Example:



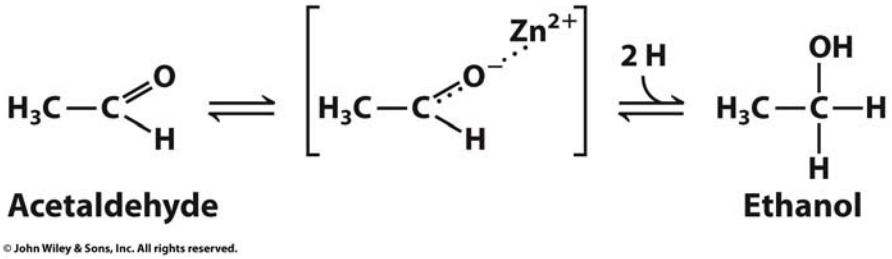
Covalent Intermediate





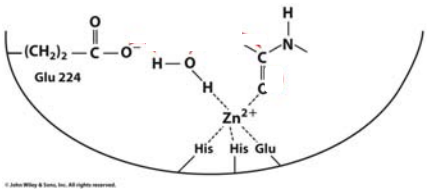
3. Metal Ion Catalysis

- Redox reactions
- Stabilization of charges



Metalloprotease

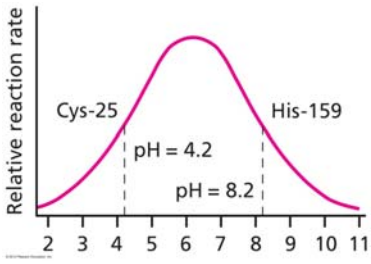
- Problem 47: Propose a mechanism for this protease:



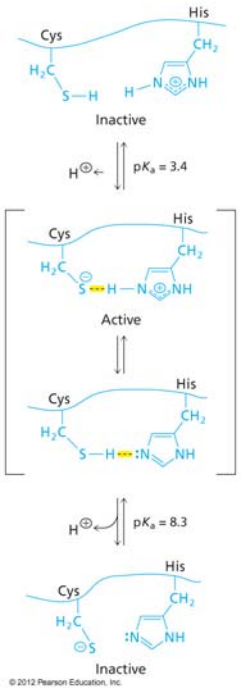
pH affects Enzyme Catalysis

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

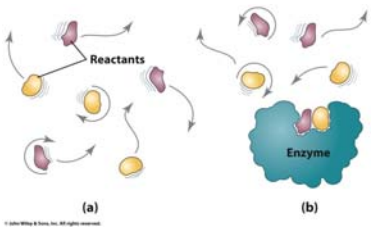


Propose possible explanations of pH profile



Binding Energy

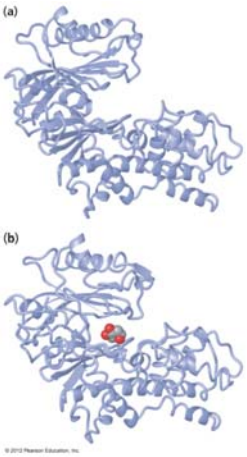
- Binding based on intermolecular forces
- "Lock and Key"
- Selectivity
- Rate Enhancement
  - Effective concentration
  - Entropy trap



Productive orientation of two molecules in the active site

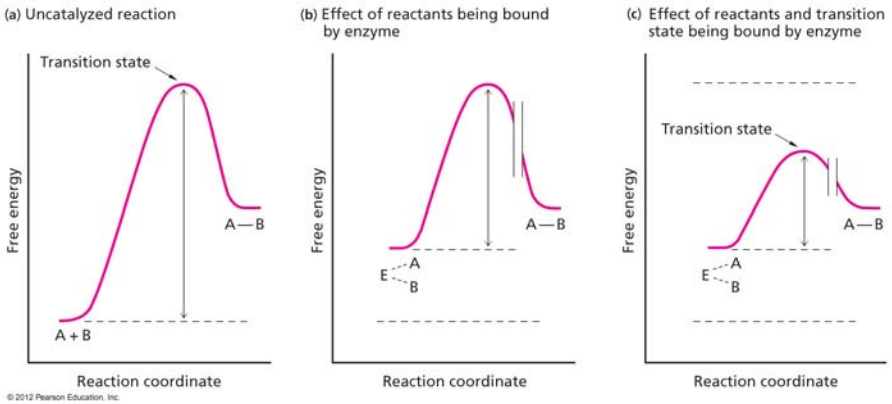
Induced Fit

- "Lock and Key" too simplistic
- Enzymes are actually somewhat flexible
- Substrate specificity comes at catalytic price
- $k_{cat} = 10^3$ per second, but worth cost



Glucose + ATP ⟹ Glucose 6-phosphate + ADP

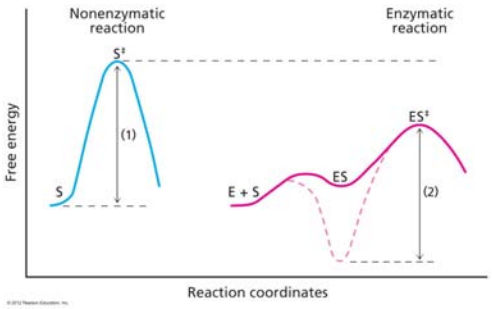
Lowering Activation Energy



- Transition state stabilization is half the story

Weak Binding of Substrate

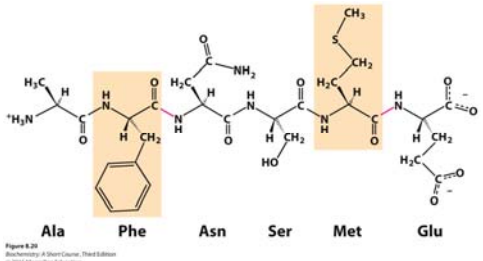
- Substrate binding: too much of a good thing
- Thermodynamic pit
- K_M ~ 10^{-4} M
- Can be 10^{-6} M for cofactors



Case Study: Chymotrypsin

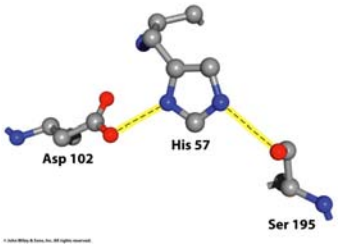
- Well studied enzyme
- Example of how enzyme mechanism studied
- Example of types of mechanism

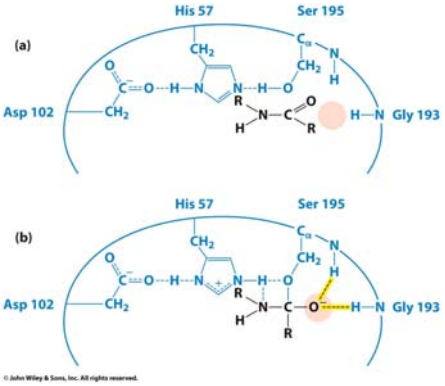
Catalyzes hydrolysis of amide

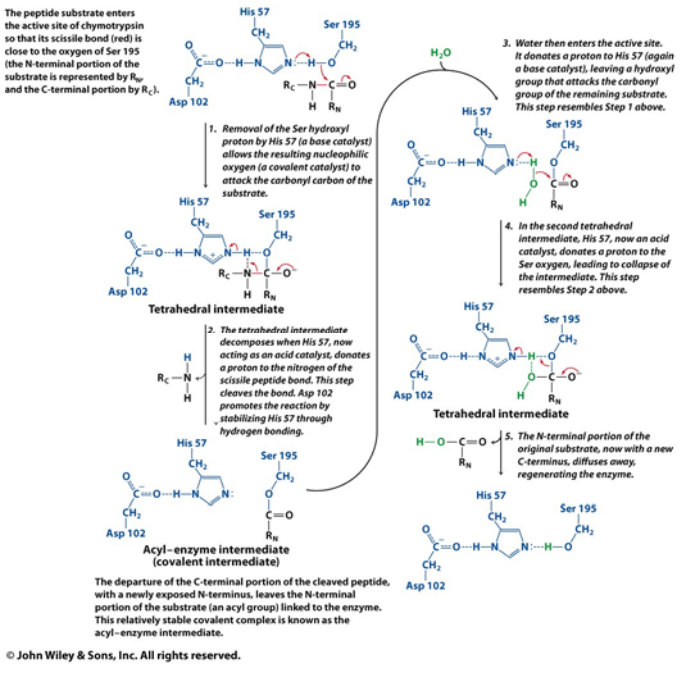


Serine Protease

- Catalytic triad
  - Low barrier H-bond (electrostatic catalysis)
- Oxyanion hole

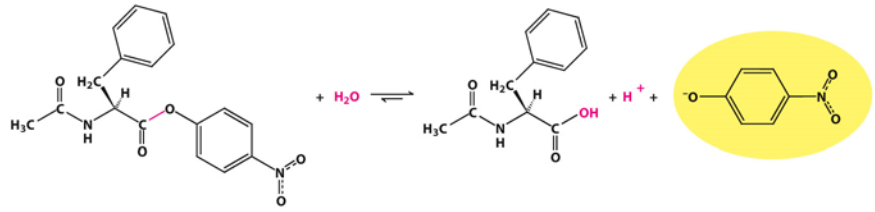






Studying Enzyme Mechanisms

- Enzyme assay
- Make artificial substrate
- Product formation may be followed with UV-vis spectroscopy

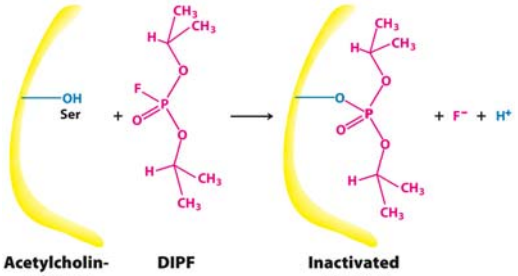


***N*-Acetyl-L-phenylalanine *p*-nitrophenyl ester** **+ H_2O** **+ H^+ +** ***p*-Nitrophenolate**

Figure 8.21
Biochemistry: A Short Course, Third Edition

Group Specific Reagents

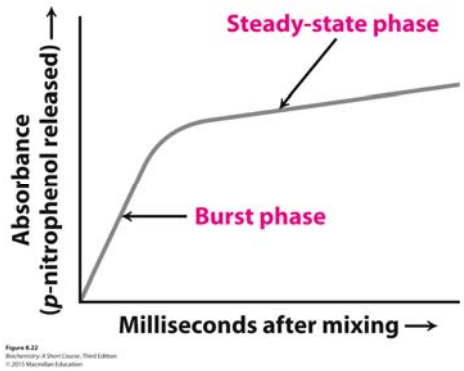
- Chemical that reacts with a particular residue
- Covalent modification
- Can be used to determine especially reactive serine-195



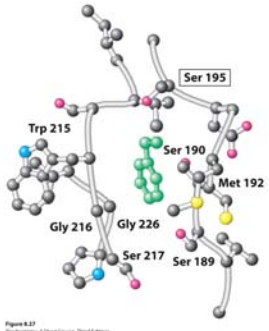
Acetylcholin-esterase **DIPF** **Inactivated enzyme**

Pre-Steady State Kinetics

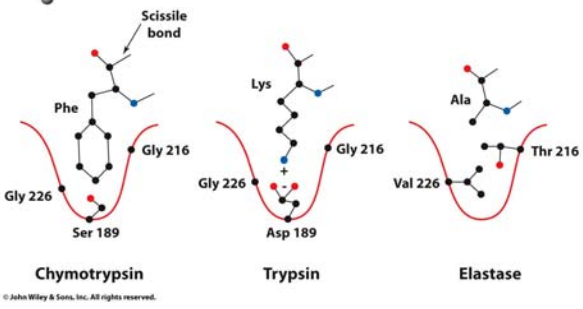
- "Burst" kinetics
- Small initial product formation followed by steady rate of product formation
- Suggests a two step mechanism



Substrate Specificity

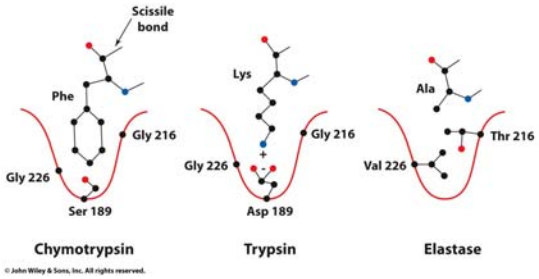


- Specificity pocket
- Binding affinity
- Promiscuity



Question

- What effects would the following mutations have on enzyme specificity?
  - Trypsin D189E
  - Elastin V226A, T216A
  - Trypsin D189K



Pancreatic Proteases

- Released from pancrease as zymogens
- Typsin activated by enteropeptidase
- Chymotrypsin activated by trypsin