BCHS 6229
Protein Structure and Function

Lecture 5 (Oct 25, 2011)

From Structure to Function (II): Enzyme Structure & Catalysis

Outline

- **Catalysis: Overview**
- **Active site geometry**
- **Proximity and ground-state destabilization**
- **Stabilization of transition state and exclusion of water**
- **Catalytic mechanisms**
- **Active site chemistry**

Catalytic strategies used by enzymes:

Examples:

Proteases (e.g. Serine Proteases)
Transferases (e.g. Glycosyltransferases)

Catalysis: Overview

Enzymes

- Enzymes are protein catalysts
- Enzymes act as catalysts of chemical reactions so the reactions occur at a much faster rate than uncatalyzed reactions

  Free energy is a useful thermodynamic function for understanding enzymes

- As we will see, they do so through two mechanisms
  - –By providing a protected environment
  - –By providing all of the reactive partners [acids (Asp, Glu, C-term), bases (Lys, Arg, His, N-term), and hydrogen-bonding] in the correct configuration

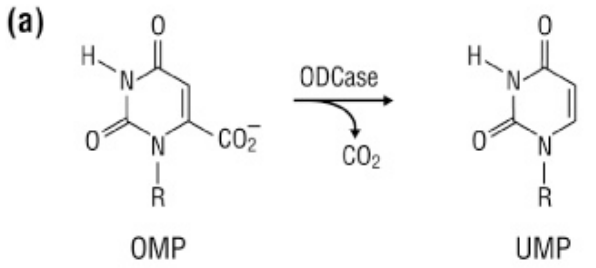
Catalysis: Overview

General properties of enzymes

- Higher reaction rates
  - –10^6-10^{12} times greater than those of uncatalyzed reactions and at least 10^7 times greater than chemically catalyzed reactions
- Milder reaction conditions
  - –Near neutral pH, < 100^{o}C, atmospheric pressure
  - –Chemical catalysis often requires high T, pressure, and pH
- Greater reaction specificity
- Capacity for regulation

Catalysis: Overview

Catalysts accelerate the rate of a chemical reaction without changing overall equilibrium.



Comparison of Uncatalyzed and Catalyzed Rates for Some Enzymatic Reactions

Enzyme	Nonenzymatic rate k_{non} (s^{-1})	Enzymatic rate k_{cat} (s^{-1})	Rate acceleration k_{cat}/k_{non}
Cyclophilin	2.8×10^{-2}	1.3×10^{4}	4.6×10^{5}
Carbonic anhydrase	1.3×10^{-1}	10^{6}	7.7×10^{6}
Chymotrypsin	4×10^{-9}	4×10^{-2}	10^{7}
Triosephosphate isomerase	6×10^{-7}	2×10^{3}	3×10^{9}
Fumarase	2×10^{-8}	2×10^{3}	10^{11}
Adenosine deaminase	1.8×10^{10}	370	2.1×10^{12}
Urease	3×10^{-10}	3×10^{4}	10^{14}
Alkaline phosphatase	10^{-15}	10^{2}	10^{17}
ODCase	2.8×10^{-16}	39	1.4×10^{17}

Orotidine 5'-monophosphate decarboxylase

~10^{17}-fold

From **Protein Structure and Function**
by Gregory A Petsko and Dagmar Ringe

Catalysis: Overview

Catalysis usually requires more than one factor.

After decades of intense research, including the determination of the atomic structures of many enzyme-substrate complexes, it has become apparent that there is no unique secret to enzyme catalysis.

A variety contributory factors:

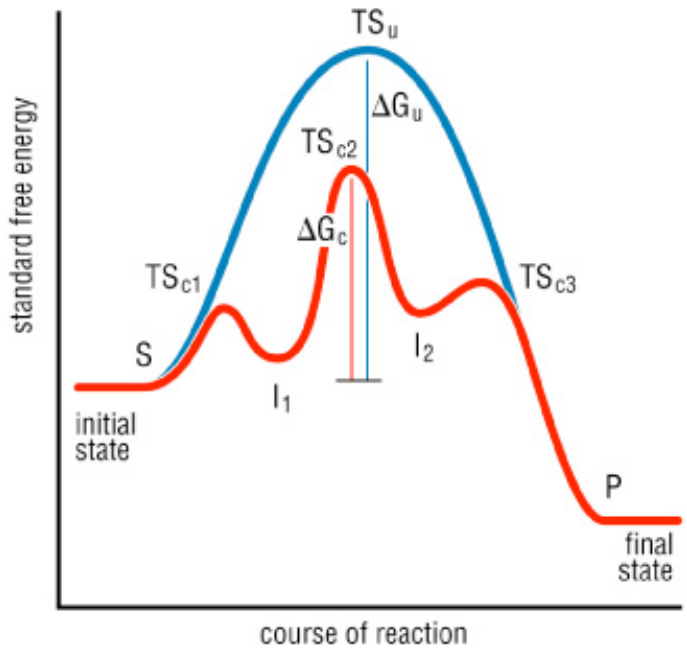
Physical: structure and physical properties of the enzyme: orientation of ligand relative to catalytic residue in the active site.

Chemical: chemical properties of enzymes' amino acids (ability to polarize bonds, ability to form covalent adducts, etc.).

The net contribution of many simple effects accounts for the extraordinary power of enzymes to speed up reactions.

Catalysis: Overview

Catalysis is reducing the activation-energy barrier to a reaction.

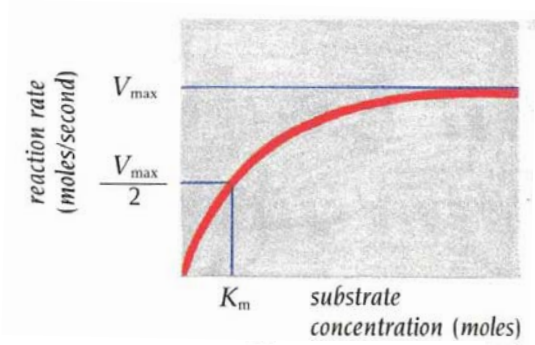


Energetics of catalysis.
Uncatalyzed vs. Catalyzed reaction

Some definitions:
- activation energy
- ground state
- transition state
- rate limiting step?

From **Protein Structure and Function**
by Gregory A Petsko and Dagmar Ringe

Some basic enzymological concepts:

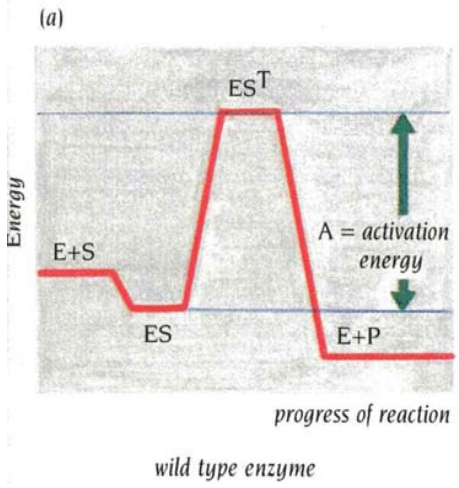


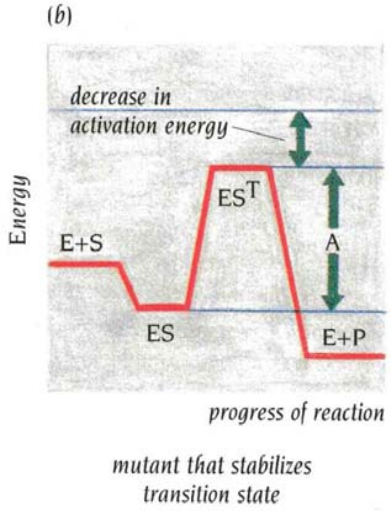
$$v = \frac{V_{max} \times [S]}{K_m + [S]}$$

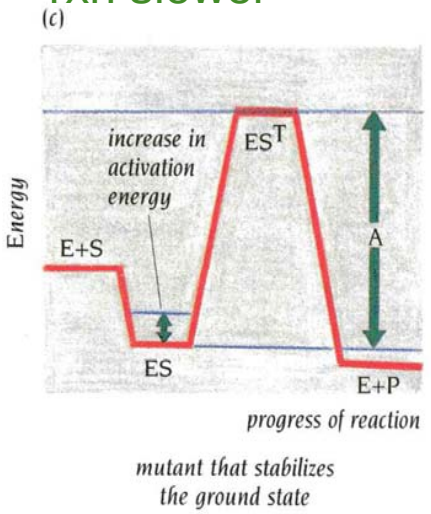
$$E + S \underset{}{\overset{K_d}{\leftrightarrows}} ES \overset{k_{cat}}{\leftrightarrows} E + P$$

Km↘, ΔG‡↗, kcat↘
rxn slower

Differential binding energy







ΔG and Km not changed, ΔG‡ decreases, kcat increases.
Reaction will be faster.

Enzyme nomenclature

Enzymes are typically named by adding –ase to the end of the name of the substrate acted on by the enzyme or a phrase describing the reaction catalyzed.

Urease; Alcohol dehydrogenase; etc.

A mammalian cell produces more than 10,000 different proteins, of which more than half are enzymes. But, many of the reactions are similar (A relatively small number of chemical reactions account for most biological transformation).

Can be described as only a few types: Recall enzyme classification

Table 11-2 Enzyme Classification According to Reaction Type

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

Table 11-2 Fundamentals of Biochemistry, 2/e

Catalytic mechanisms

1. Acid-base catalysis
2. Covalent catalysis
3. Metal ion catalysis
4. Electrostatic catalysis
5. Proximity and orientation effects
6. Preferential binding of the transition state complex

Acid-base catalysis

Mechanism of keto-enol tautomerism

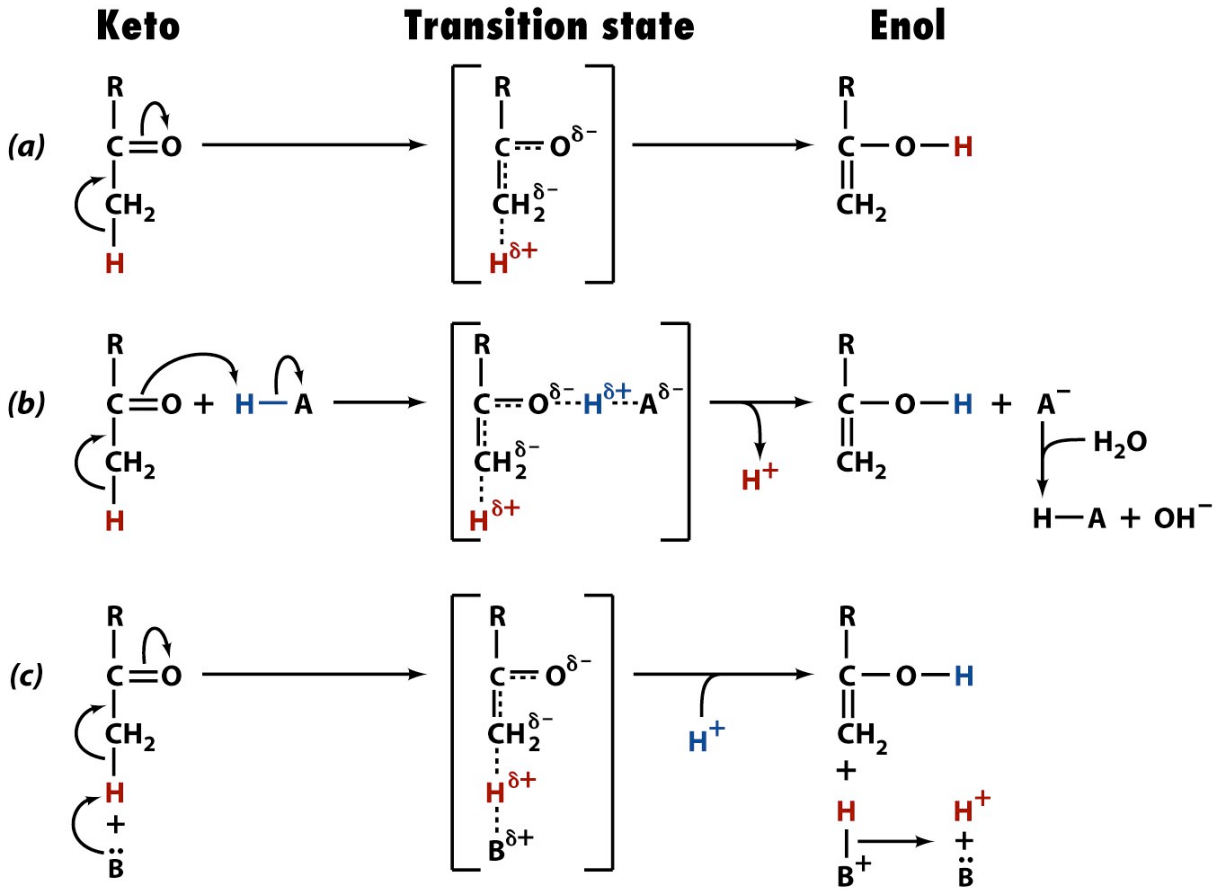


Figure 11-8 Fundamentals of Biochemistry, 2/e

Acid-base reactions are sensitive to pH since the ionization state of Asp, Glu, Lys, Arg, and His residues can be affected by pH

Covalent catalysis

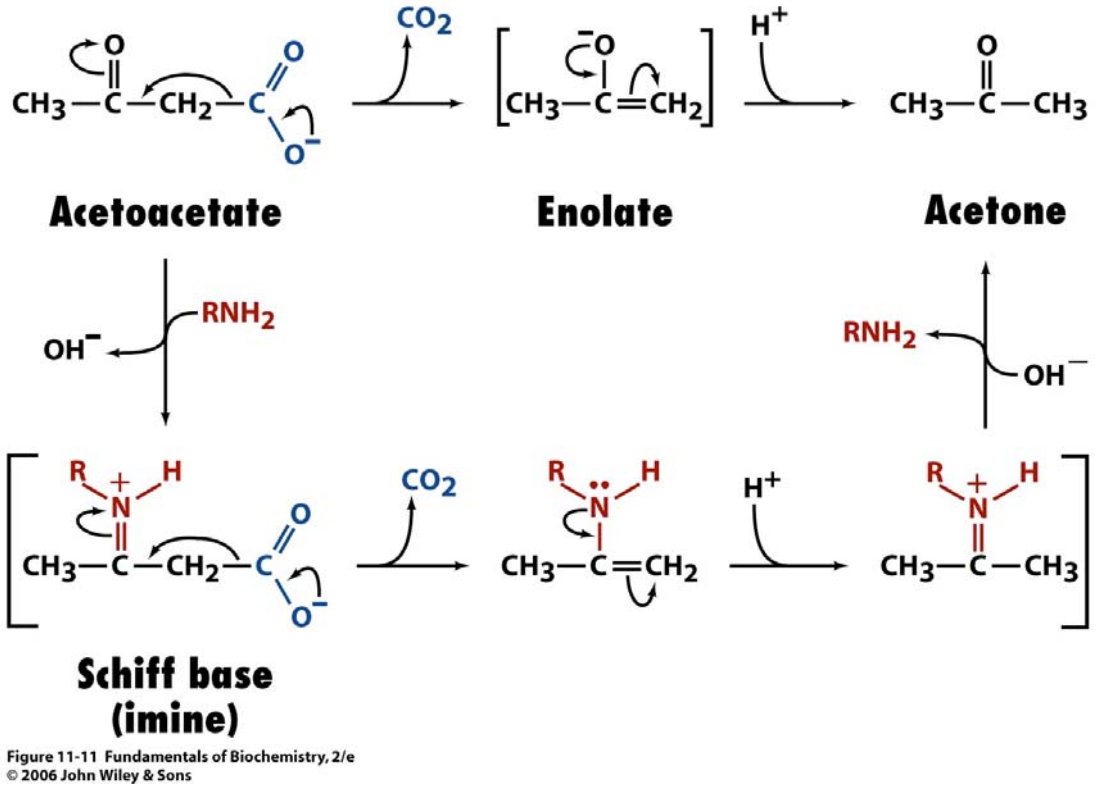


Figure 11-11 Fundamentals of Biochemistry, 2/e
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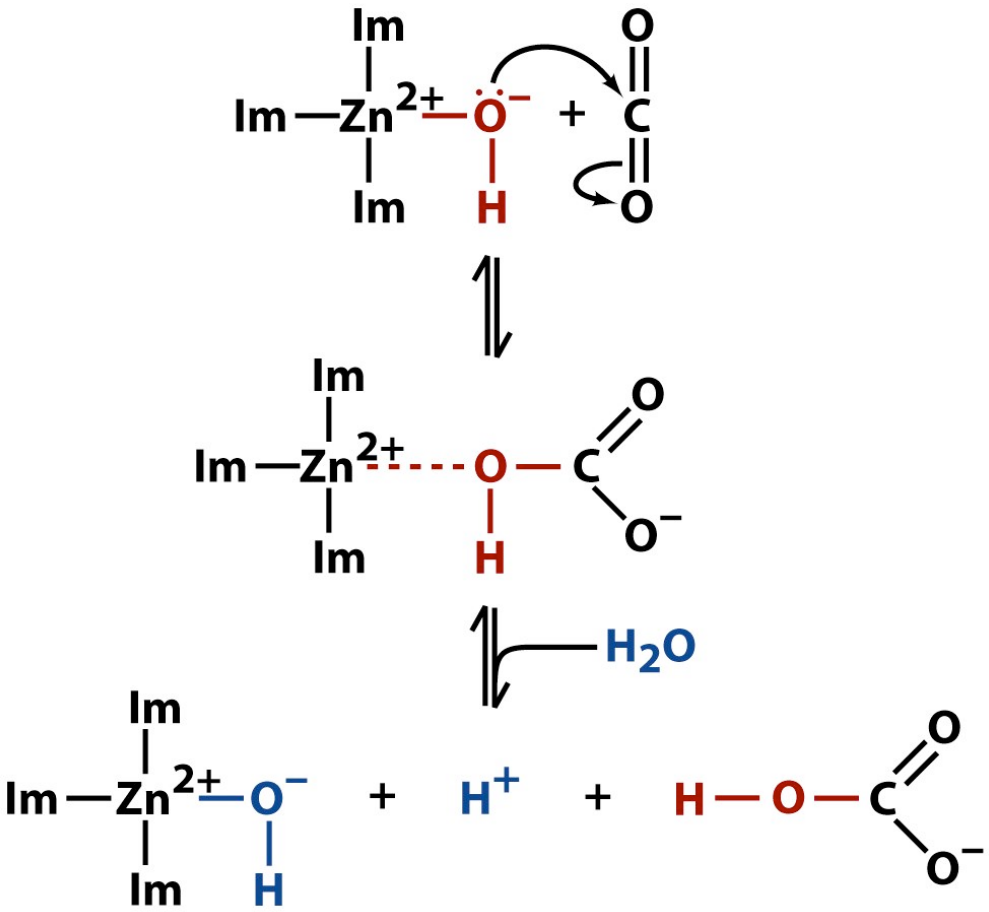
- Decarboxylation of acetoacetate – uncatalyzed at top and catalyzed at bottom
- Nucleophilic catalysis – substrate forms a bond with enzyme/coenzyme
- RNH_2 is from the enzyme or cofactor
- Covalent bond is transiently formed, accelerating the reaction

Metal ion catalysis

Carbonic anhydrase reaction

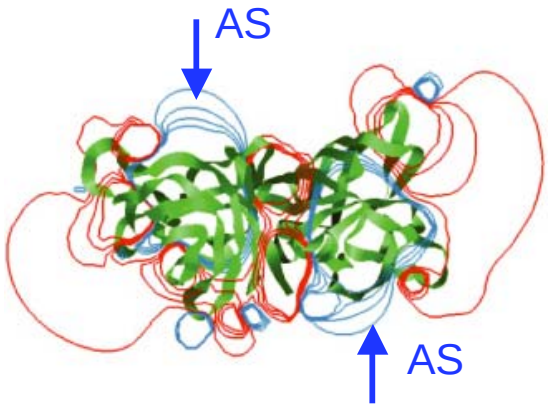


Unnumbered figure pg 327 Fundamentals of Biochemistry, 2/e

- It converts between CO_2 and HCO_3^-
- HO- is stabilized by Zn^{2+} where it nucleophilically attacks CO_2
- A water molecule completes the process, regenerating the cofactor and liberating the product

Active-Site Geometry

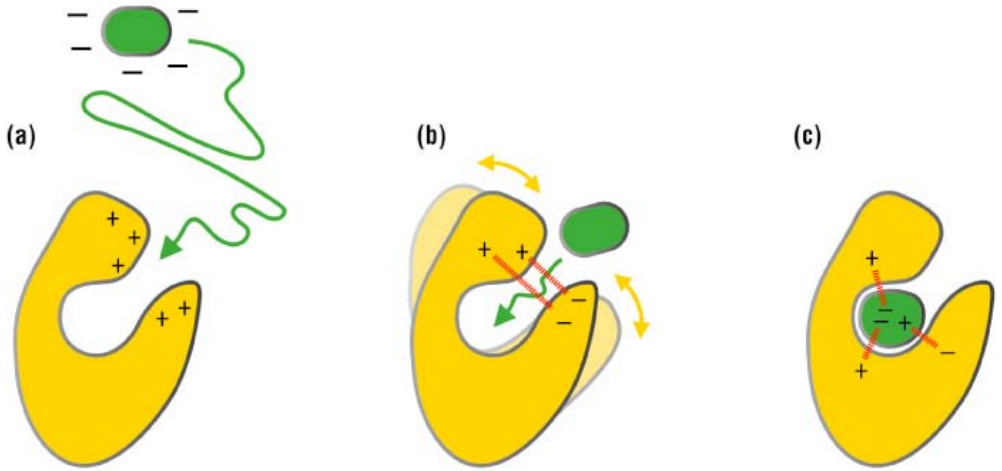
Reaction groups in enzyme active sites are optimally positioned to interact with the substrate.



Enzyme Cu,Zn-SOD

Although active sites have exposed hydrophobic patches, the overall electrostatic field produced by the protein with all its charged and polar groups can yield an electrostatic potential with a net charge in the active-site region.

Some of ways in which electrostatic interactions can influence the binding of a ligand to a protein



Productive binding

'Gated' binding

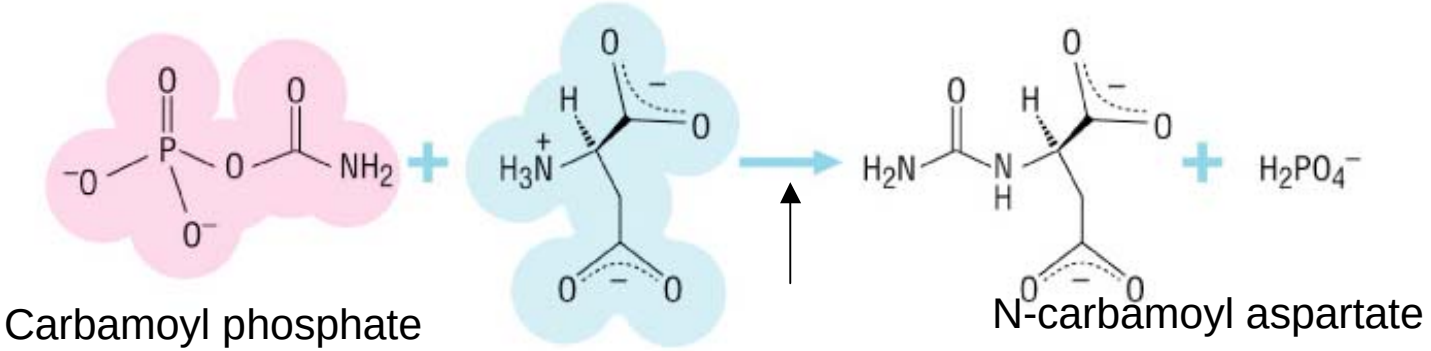
electrostatic interactions between L+P.

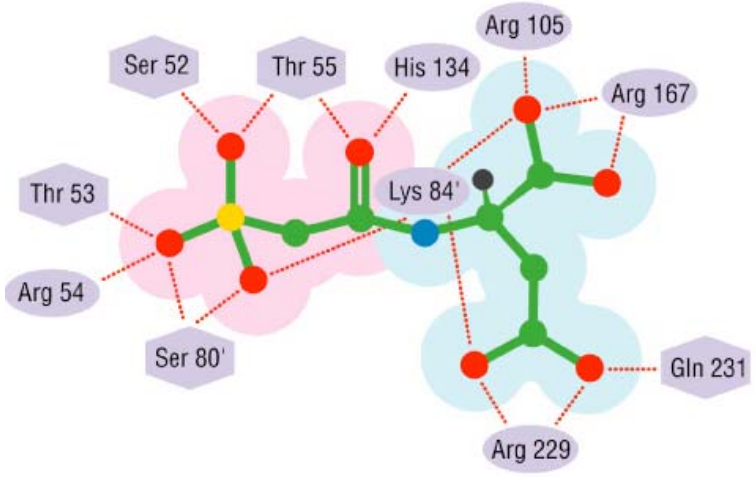
Wade RC, *et al.*: PNAS 1998, 95:5942-5949

Proximity and Ground-State Destabilization

Some active sites chiefly promote proximity.

Proximity factor: the concept that a reaction will be facilitated if the reacting species are brought close together in an orientation appropriate for chemistry to occur.





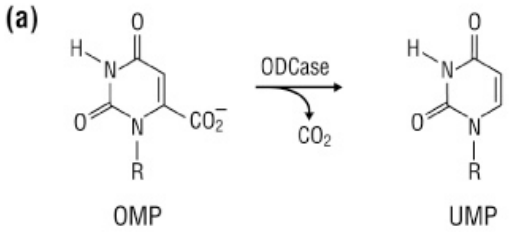
PALA (N-phosphonoacetyl-L-aspartate)

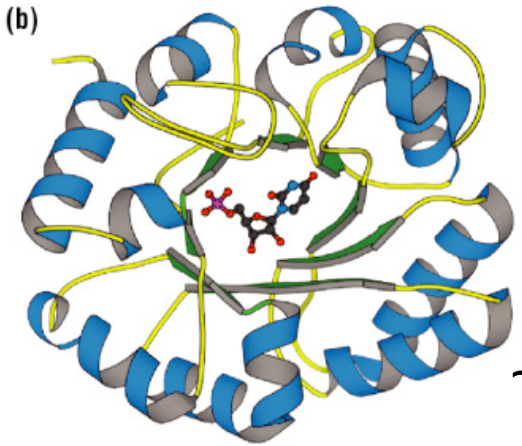
Proximity and Ground-State Destabilization

Some active sites destabilize ground states.

Raising the ΔG (relative to some reference state), of the ground state, usually referring bound substrate in the active site before any chemical change has occurred.

Geometric or electronic strains.

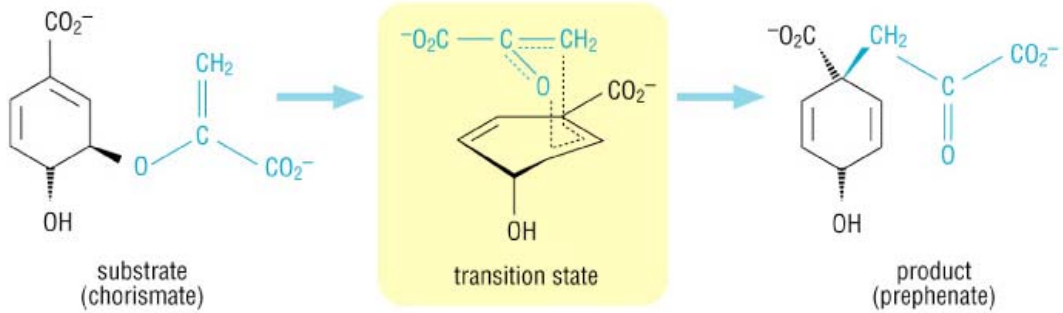




~10^{17}-fold

Orotidine 5'-monophosphate decarboxylase

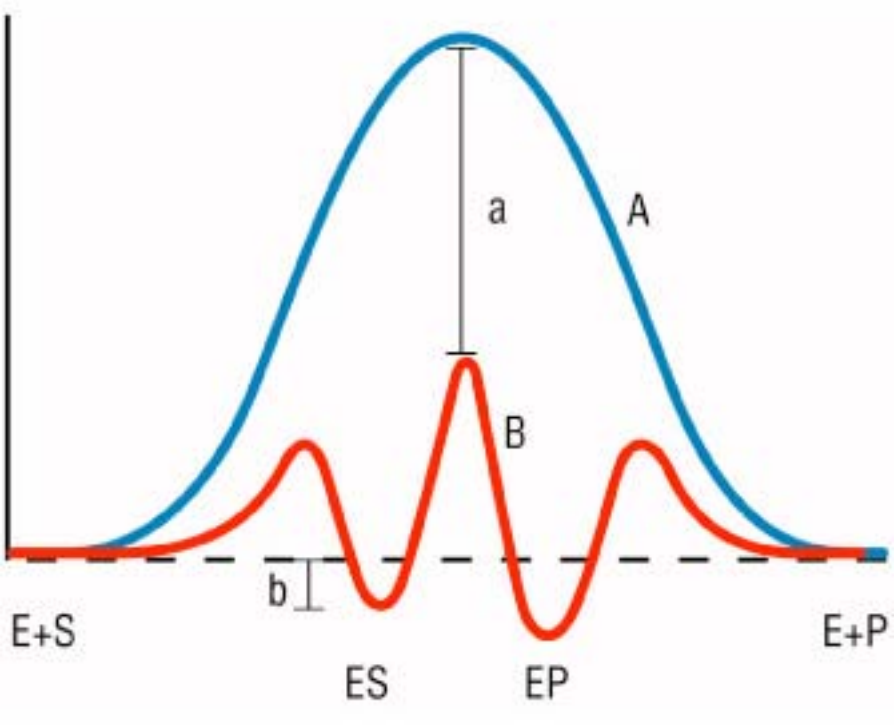
Chorismate mutase



pericyclic rearrangement

Stabilization of Transition States and Exclusion of H_2O

Some active sites primarily stabilize transition states.

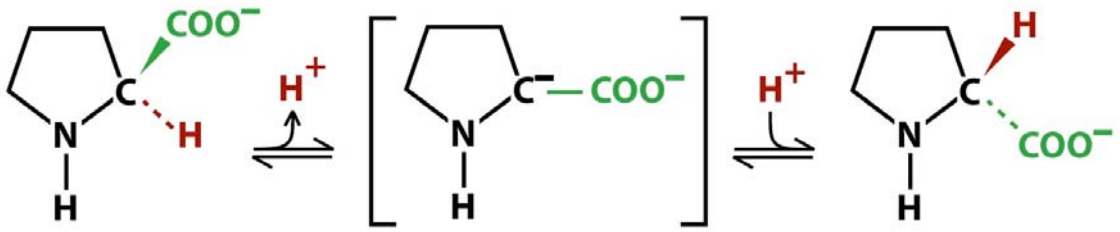


a > b

- Uncatalyzed (blue) and enzymatically catalyzed (red) reaction
- Note the minimum for the enzyme-substrate (ES) complex – this is called the Michaelis complex
- Note that the substrates and product have the same energy in the catalyzed verses uncatalyzed

Effect of binding energy on enzyme catalysis

Transition state binding



Unnumbered figure pg 331a Fundamentals of Biochemistry, 2/e

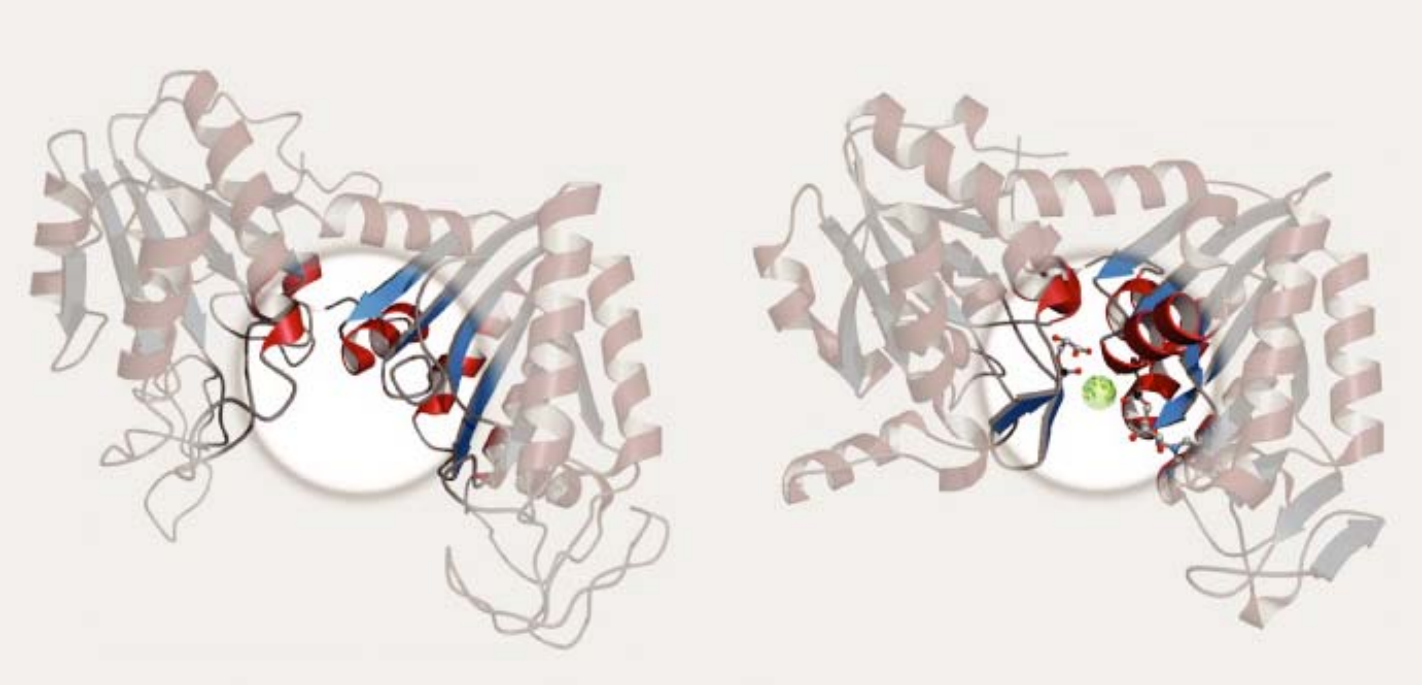
Unnumbered figure pg 331b Fundamentals of Biochemistry, 2/e

- Enzymes typically stabilize the transition state of a reaction more than that of the reactants or the products

  This leads to the idea of small TS-like molecules that might act as "inhibitors" of the enzymatic process by more strongly binding to the active site than the substrate
- This example is proline racemase

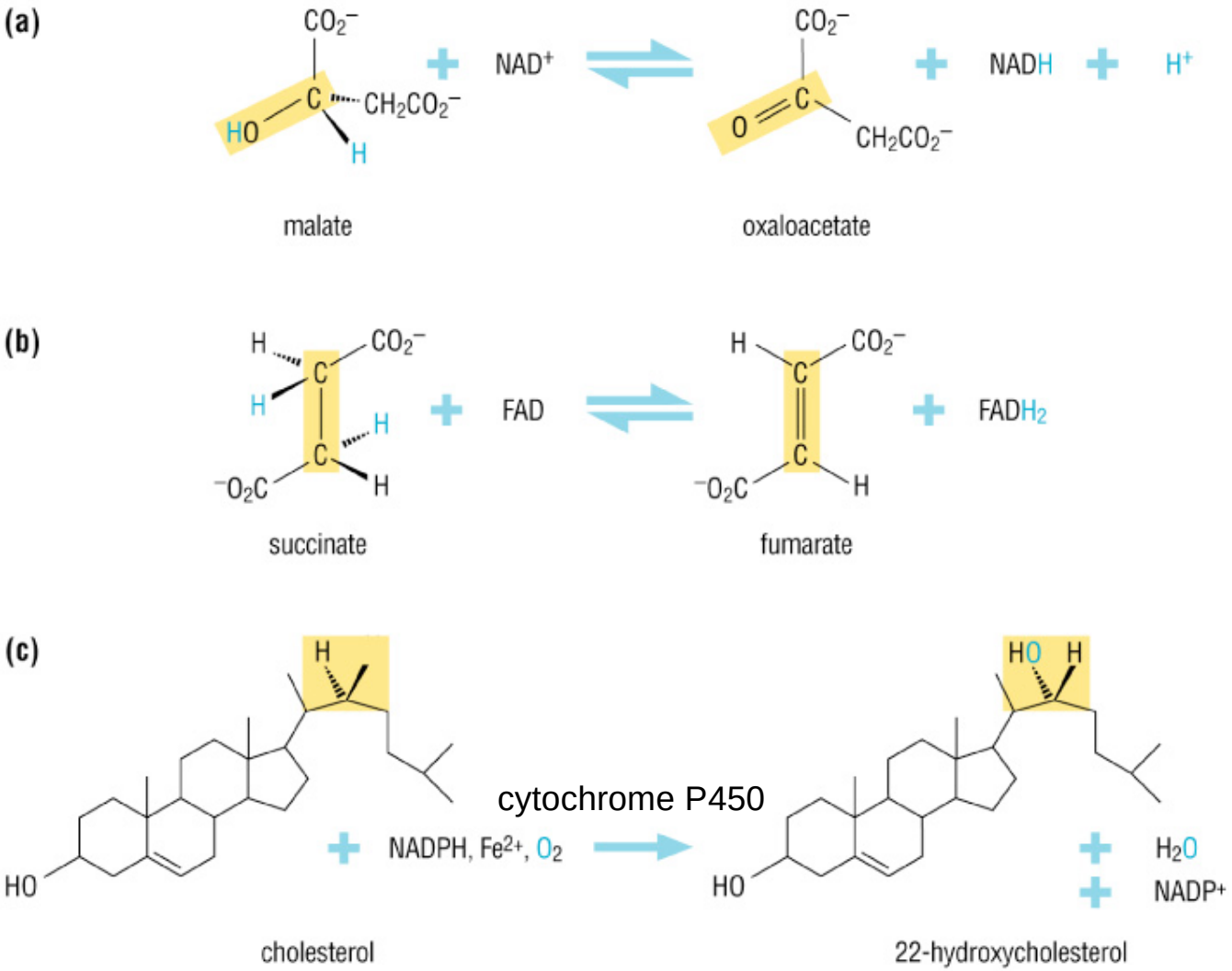
Statbilization of Transition States and Exclusion of H_2O

Many active sites must protect their substrates from water, but must be accessible at the same time



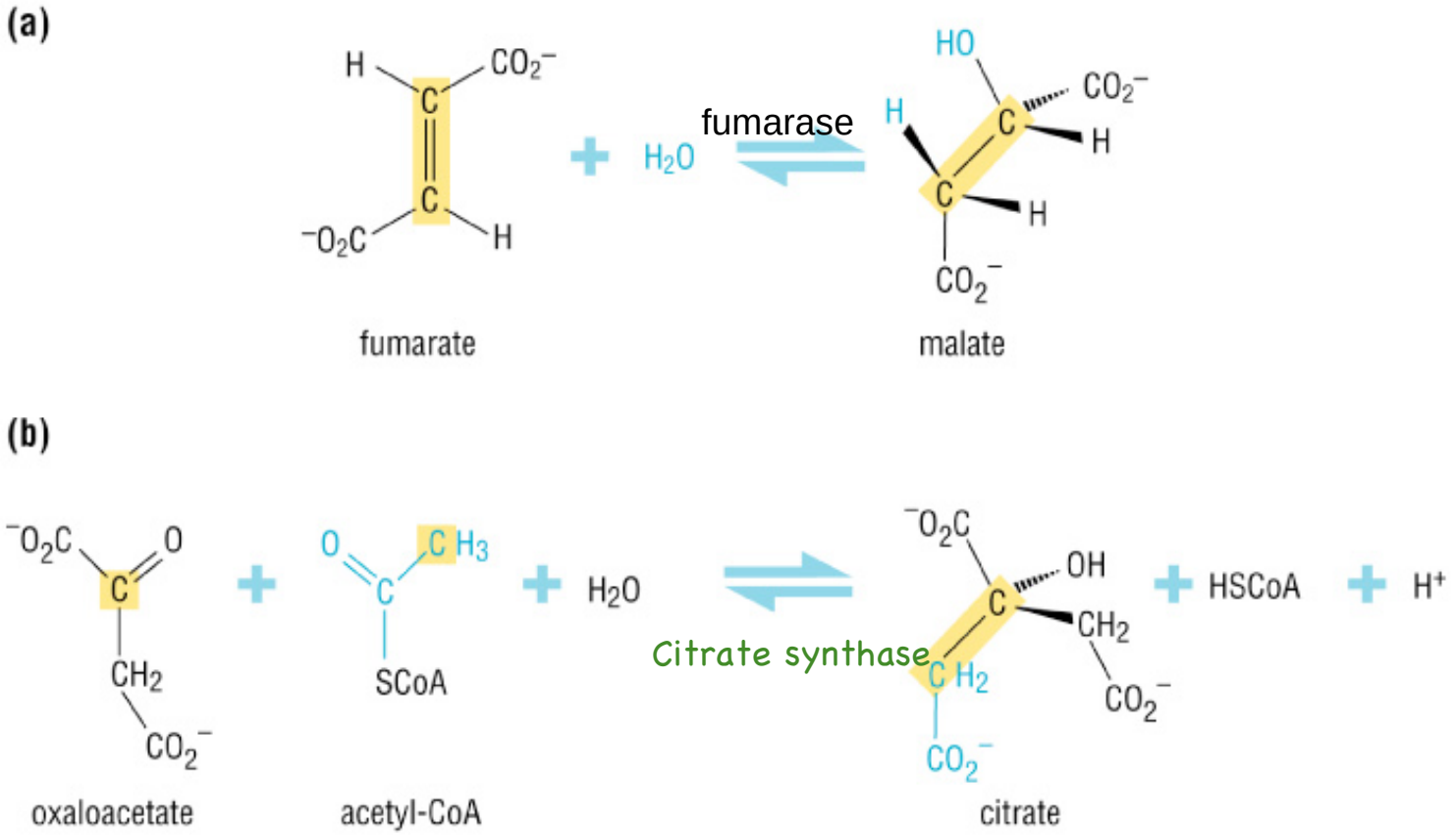
Redox reactions

Oxidation/reduction reactions involve the transfer of electrons and often require specific cofactors.



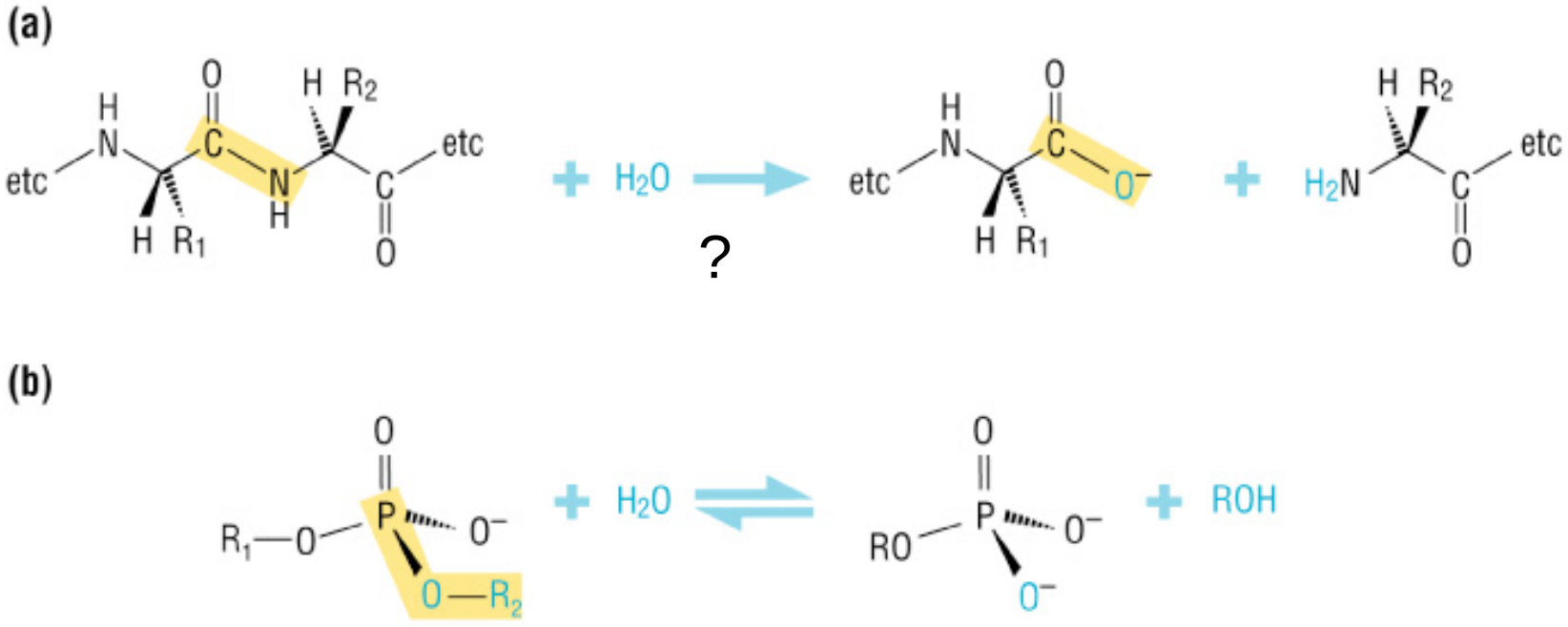
Addition/Elimination, Hydrolysis & Decarboxylation

Addition reactions add atoms or chemical groups to double bonds, while elimination reactions remove them to form double bonds.



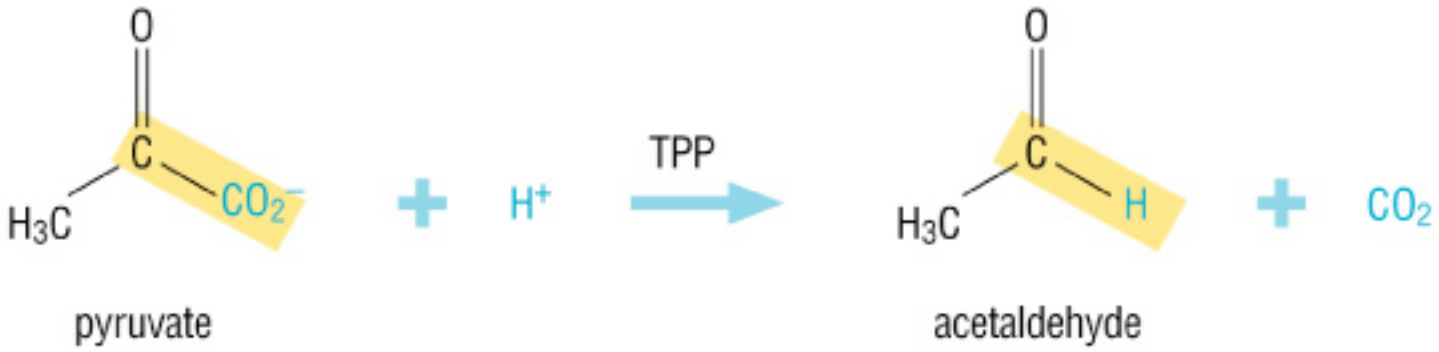
Addition/Elimination, Hydrolysis & Decarboxylation

Esters, amides and acetals are cleaved by reaction w/ H_2O; their formation requires removal of H_2O



Addition/Elimination, Hydrolysis & Decarboxylation

Loss of carbon dioxide is a common strategy for removing a single C atom from a molecule.



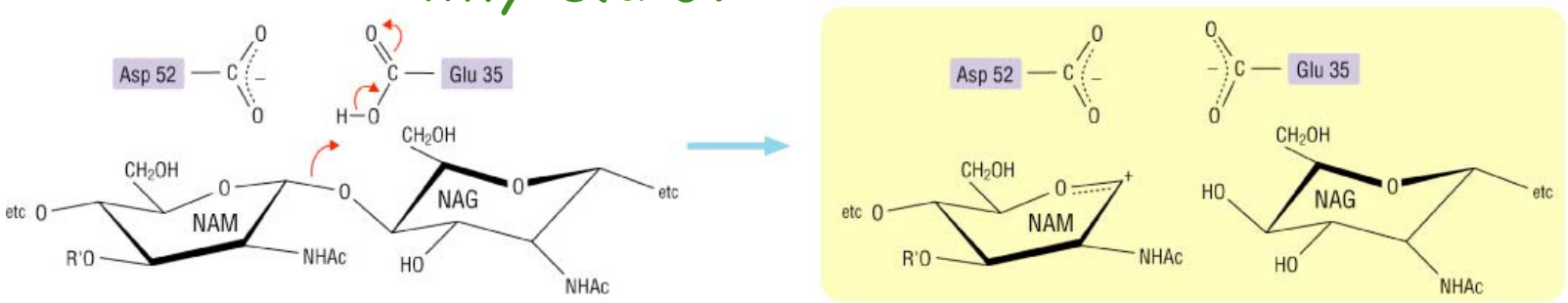
Active-Site Chemistry

Active-Site promote acid-base catalysis:

One step that involves the transfer of a proton from one group to another.

why Asp -1?

why Glu 0?



Active site of lysozyme

Cofactors and coenzymes

- Cofactors: essential non-protein elements of some enzymes
  - Cu^{2+}, Fe^{3+}, Zn^{2+}, etc.
- Coenzymes: organic/biological cofactors
  - NAD+, heme, pyridoxal phosphate, etc.
  - Cosubstrates: transiently enzyme-associated coenzymes
  - Prosthetic groups: permanently enzyme-associated coenzymes
- Holoenzyme: catalytically active enzyme-cofactor complex
- Apoenzyme: inactive protein resulting from removal of cofactor
- Coenzymes are generally reduced/oxidized during a reaction and must be returned to their initial state – this can be done by the same enzyme or a separate one

Apoenzyme *(inactive)* **+ cofactor** $\rightleftharpoons$ **holoenzyme** *(active)*

Cofactors

Many active sites use cofactors to assist catalysis

Some Common Coenzymes

Coenzyme [vitamin from which it is derived]	Entity transferred	Representative enzymes that use coenzyme	Deficiency disease
thiamine pyrophosphate (TPP or TDP) [vitamin B_1, thiamin]	aldehydes	pyruvate dehydrogenase	beri beri
flavin adenine dinucleotide (FAD) [vitamin B_2, riboflavin]	hydrogen atoms	succinate dehydrogenase	(a)
nicotinamide adenine dinucleotide (NAD^+) [niacin]	hydride ion	lactate dehydrogenase	pellagra
nicotinamide adenine dinucleotide phosphate ($NADP^+$) [niacin]	hydride ion	isocitrate dehydrogenase	pellagra
pyridoxal phosphate (PLP) [vitamin B_6, pyridoxal]	amine groups	aspartate aminotransferase	(a)
coenzyme A (CoA) [pantothenic acid]	acyl groups	acetyl-CoA carboxylase	(a)
biotin (biocytin) [biotin]	CO_2	propionyl-CoA carboxylase	(a)
5'-deoxyadenosylcobalamin [vitamin B_{12}]	H atoms and alkyl groups	methylmalonyl-CoA mutase	pernicious anemia
tetrahydrofolate (THF) [folate]	one-carbon units	thymidylate synthase	megaloblastic anemia
lipoamide [lipoic acid]	two-carbon units; R-SH	pyruvate dehydrogenase	(a)
heme [no vitamin]	e^-, O_2, NO, CO_2	cytochrome oxidase	anemia, leukemia

(a) no specific name

Metal Ions and Some Enzymes Requiring Them

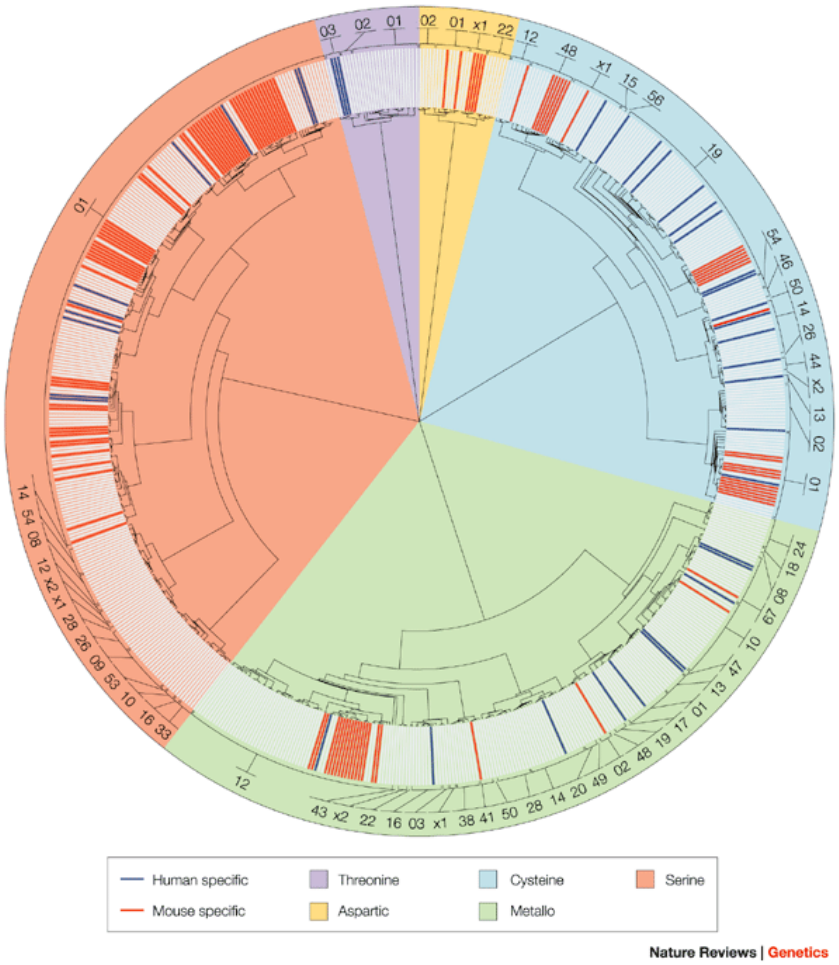
Metal ion	Enzyme
Fe^{2+} or Fe^{3+}	cytochrome oxidase catalase peroxidase
Cu^{2+}	cytochrome oxidase
Zn^{2+}	DNA polymerase carbonic anhydrase alcohol dehydrogenase
Mg^{2+}	hexokinase glucose-6-phosphatase pyruvate kinase
Mn^{2+}	arginase
K^+	pyruvate kinase
Ni^{2+}	urease
Mo	nitrate reductase
Se	glutathione peroxidase

Cofactor: a small, non-protein molecule or ion that is bound in the functional site of a protein and assits in ligand binding or catalysis or both. Some cofactors are bound covalently.

Coenzyme: a cofactor that is an organic or organometallic molecule and that assist catalysis.

Example 1 **Proteases**

common and widespread enzymes



The protease wheel

Unrooted phylogenetic tree of human and mouse proteases (distributed in 5 catalytic classes and 63 different families).

The figure shows the non-redundant set of proteases. Orthologous proteases are shown in light grey, mouse-specific proteases are shown in red and human-specific proteases in blue. Metalloproteases are the most abundant class of enzymes in both organisms, but most lineage-specific differences are in the serine protease class, making this sector wider. The 01 family of serine proteases can be divided into 22 smaller subgroups on the basis of involvement in different physiological processes, to facilitate the interpretation of differences.

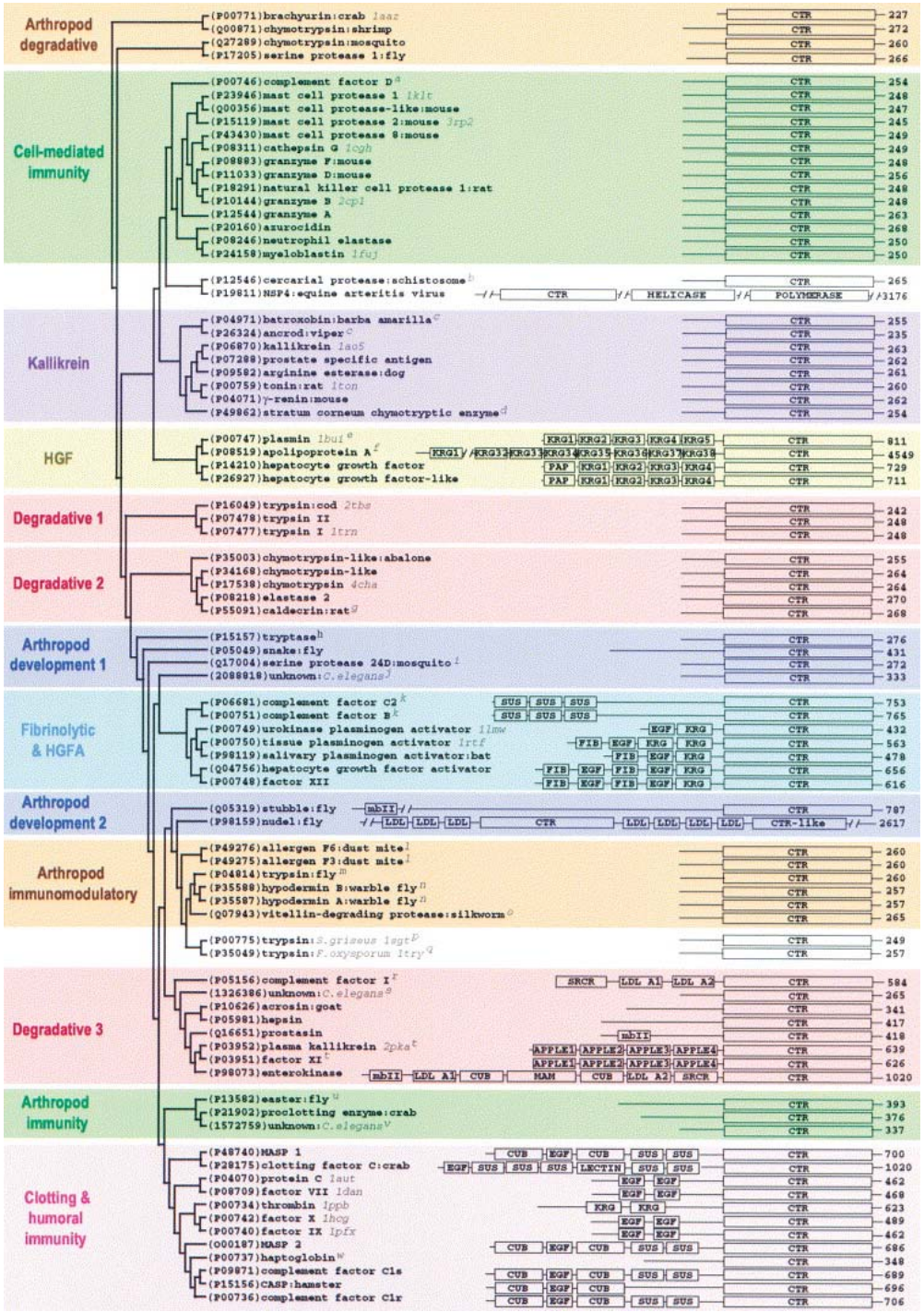
Nat Rev Genet. 2003 Jul;4(7):544-58.

Example 1 **Proteases**

Serine proteases

- Chymotrypsin as a model enzyme
- How do we know Serine is involved?
- A covalent intermediate is formed in the reaction of chymotrypsin.
- Crystals structures clarify the picture and suggest a mechanism.
- What common structural features are found in these enzymes?
- Substrate specificity among these enzymes
- Planned mutations with surprising results
- How might an enzyme mechanism like this arise?
- Not all proteases are serine proteases…

Serine proteases



Evolutionary Trees Indicate Serine Protease Lineage and Function

Phylogenetic tree of serine proteases constructed from residues 16–245 of the protease domain.

Trends Cardiovasc Med. (2000) 10(4):171-6.

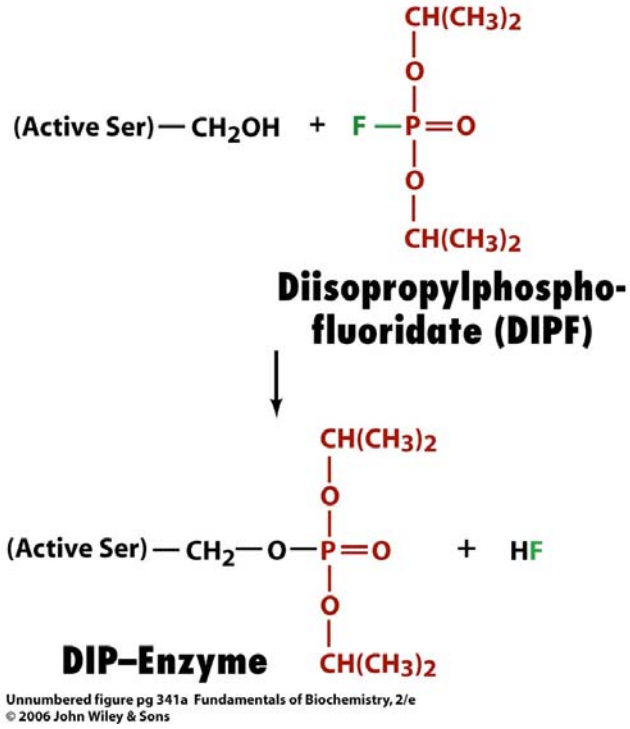
Ser proteases have an unusually reactive Ser residue at the active site

TABLE 14-4. A SELECTION OF SERINE PROTEASES

Enzyme	Source	Function
Trypsin	Pancreas	Digestion of proteins
Chymotrypsin	Pancreas	Digestion of proteins
Elastase	Pancreas	Digestion of proteins
Thrombin	Vertebrate serum	Blood clotting
Plasmin	Vertebrate serum	Dissolution of blood clots
Kallikrein	Blood and tissues	Control of blood flow
Complement C1	Serum	Cell lysis in the immune response
Acrosomal protease	Sperm acrosome	Penetration of ovum
Lysosomal protease	Animal cells	Cell protein turnover
Cocoonase	Moth larvae	Dissolution of cocoon after metamorphosis
α-Lytic protease	*Bacillus sorangium*	Possibly digestion
Proteases A and B	*Streptomyces griseus*	Possibly digestion
Subtilisin	*Bacillus subtilus*	Possibly digestion

Source: Stroud, R.M., *Sci. Am.* **231**(1): 86 (1974).

DIPF reacts only with the active site Ser residue in Ser proteases and so serves as a diagnostic for Ser proteases



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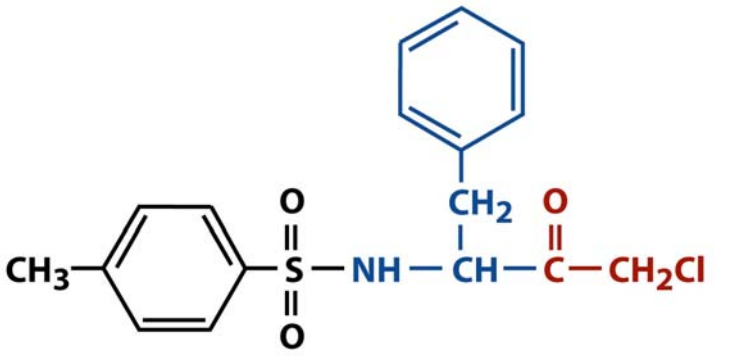
Second catalytic residue



Unnumbered figure pg 341b Fundamentals of Biochemistry, 2/e
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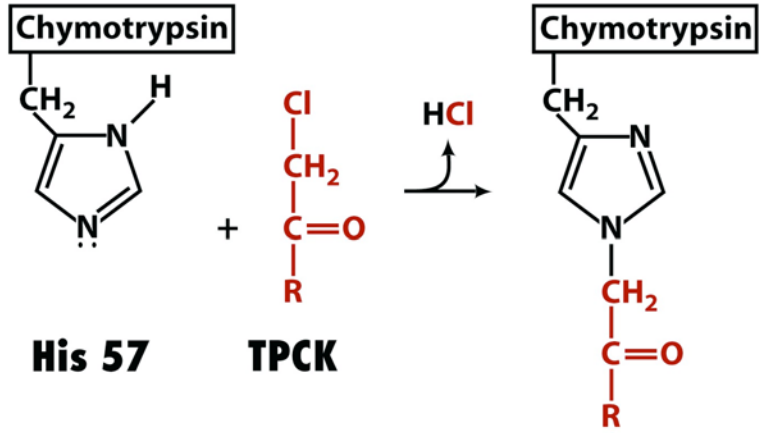

Figure 11-24 Fundamentals of Biochemistry, 2/e
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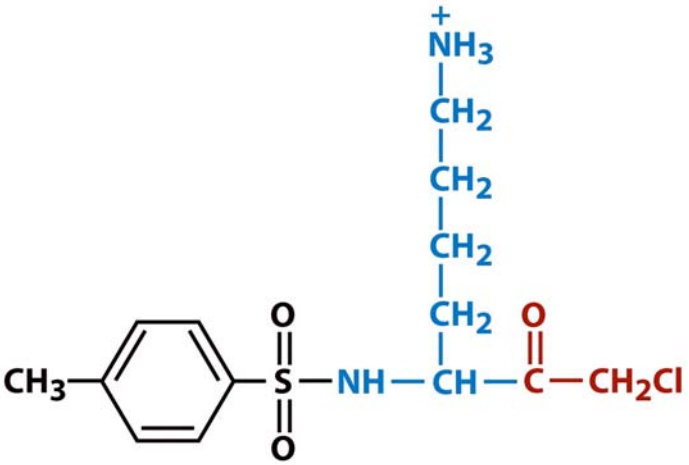

Unnumbered figure pg 341c Fundamentals of Biochemistry, 2/e
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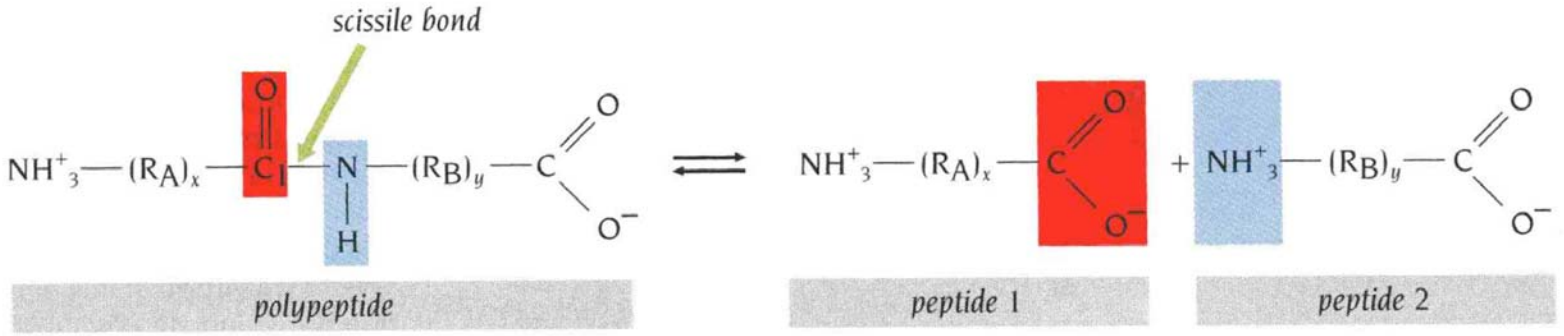
- A His residue is a second catalytic residue in Ser proteases
- Chymotrypsin has a preference for cleaving next to Phe
- Substrate analog TPCK binds to the chymotrypsin active site – the catalytic His residue reacts with the acid chloride
- Similarly, Trypsin binds TLCK…

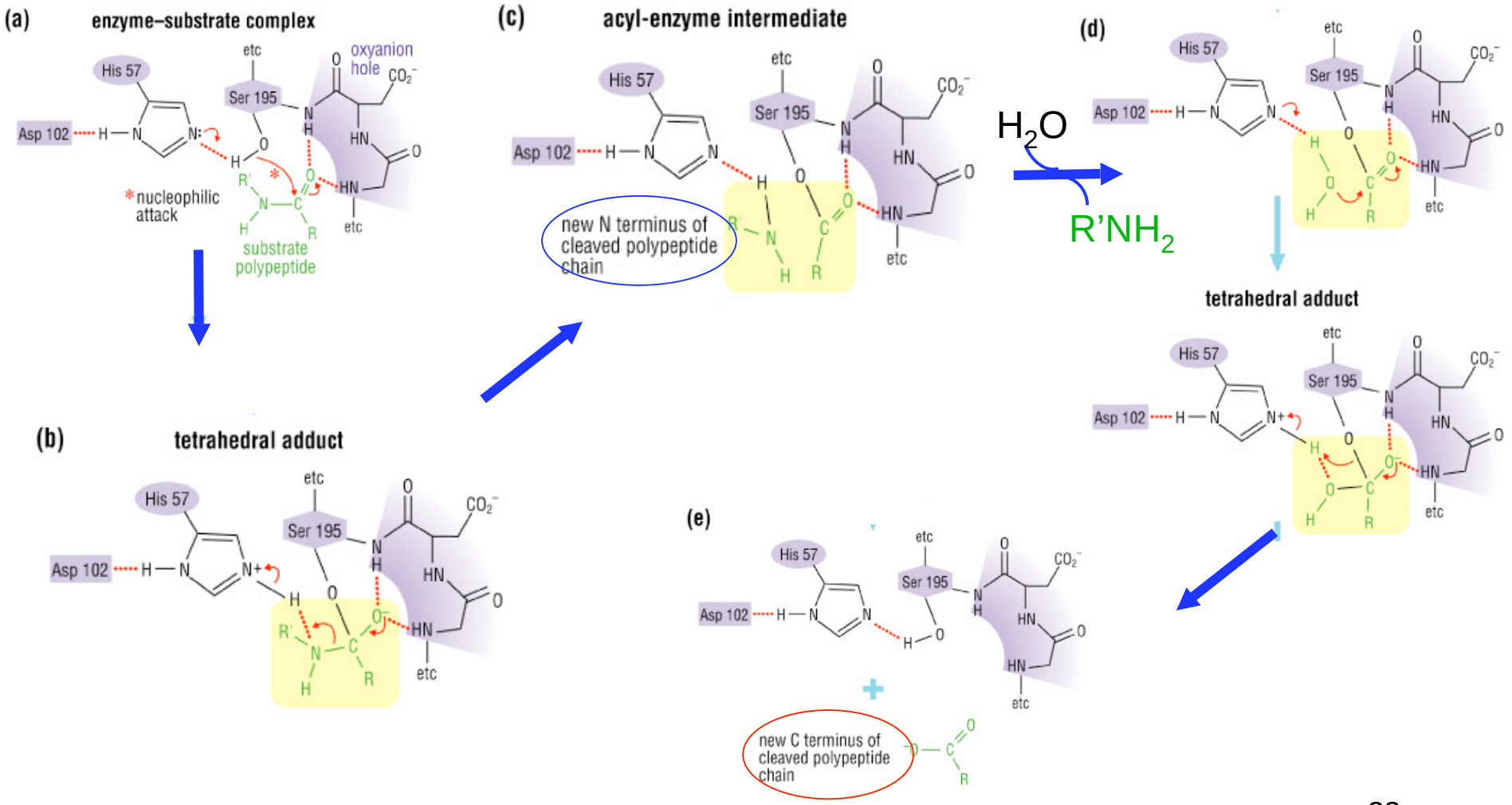
Multi-step Reactions: Serine proteases

Well characterized proteases: Serine, Cystein, Aspartic, and Metallo proteinases
(the most prominent functional group in the active site)



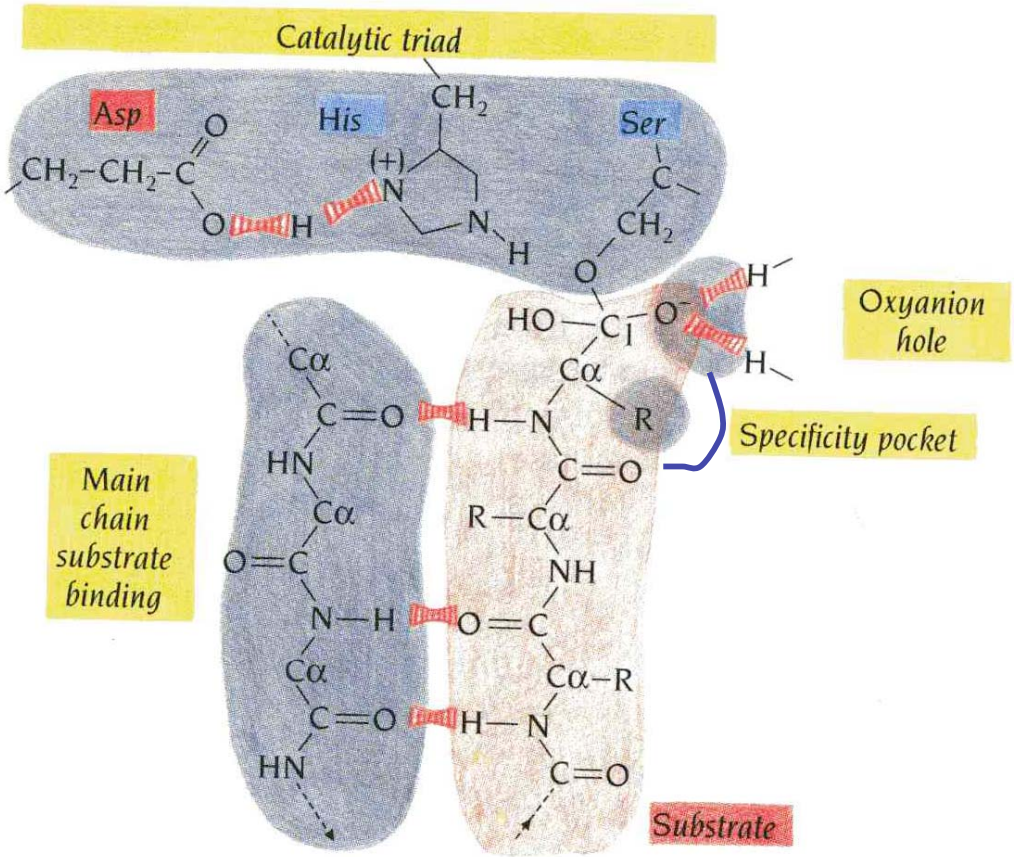
Multi-step Reactions: Serine proteases

Serine proteases cleave the peptide bonds by forming tetrahedral transition states



Binding mode of the tetrahedral transition state intermediate for the deacylation step

Four important structural features:



Specificity pockets of three serine proteases

(located near the catalytic groups)

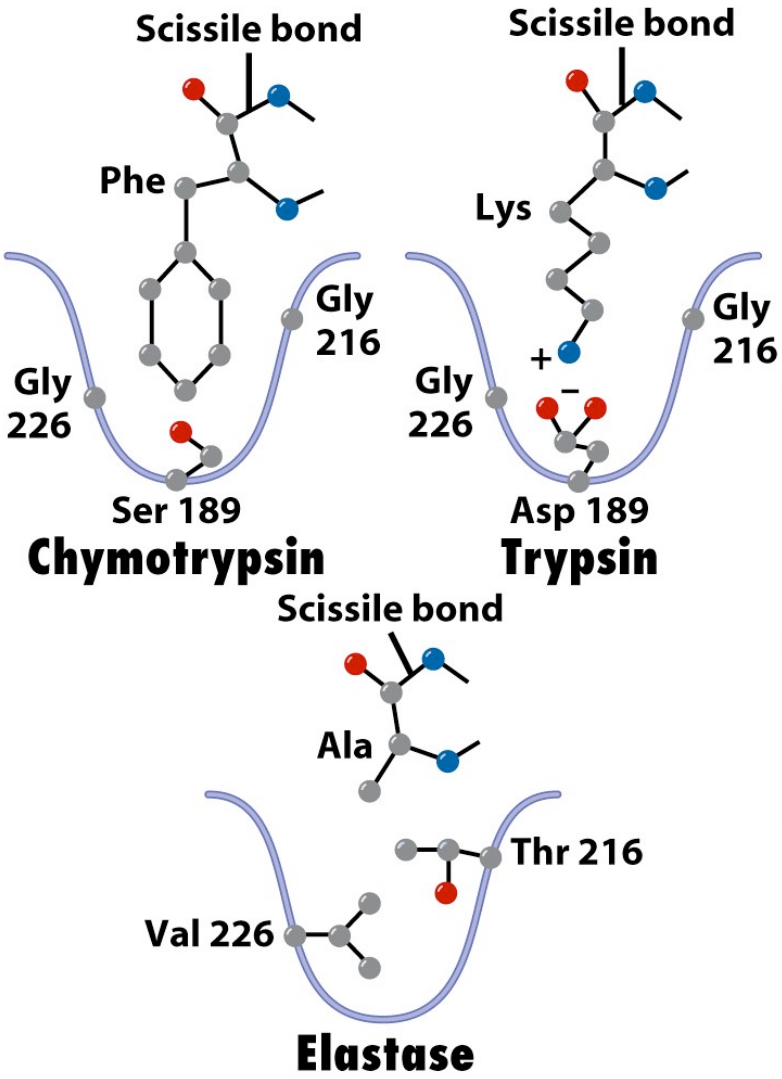


Figure 11-27 Fundamentals of Biochemistry, 2/e
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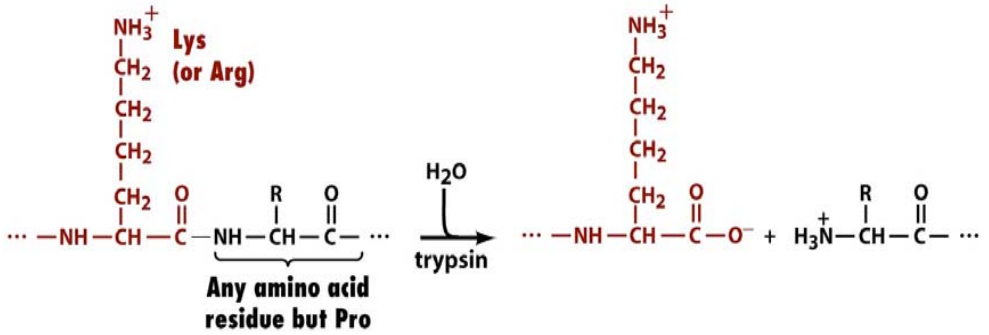
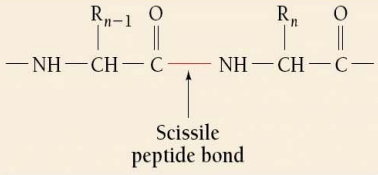


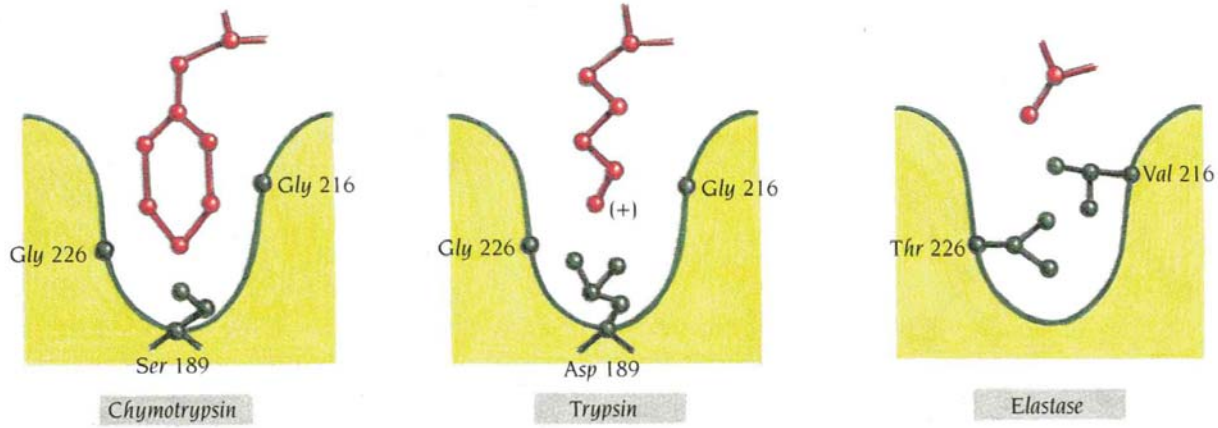
Table 5-3 Specificities of Various Endopeptidases

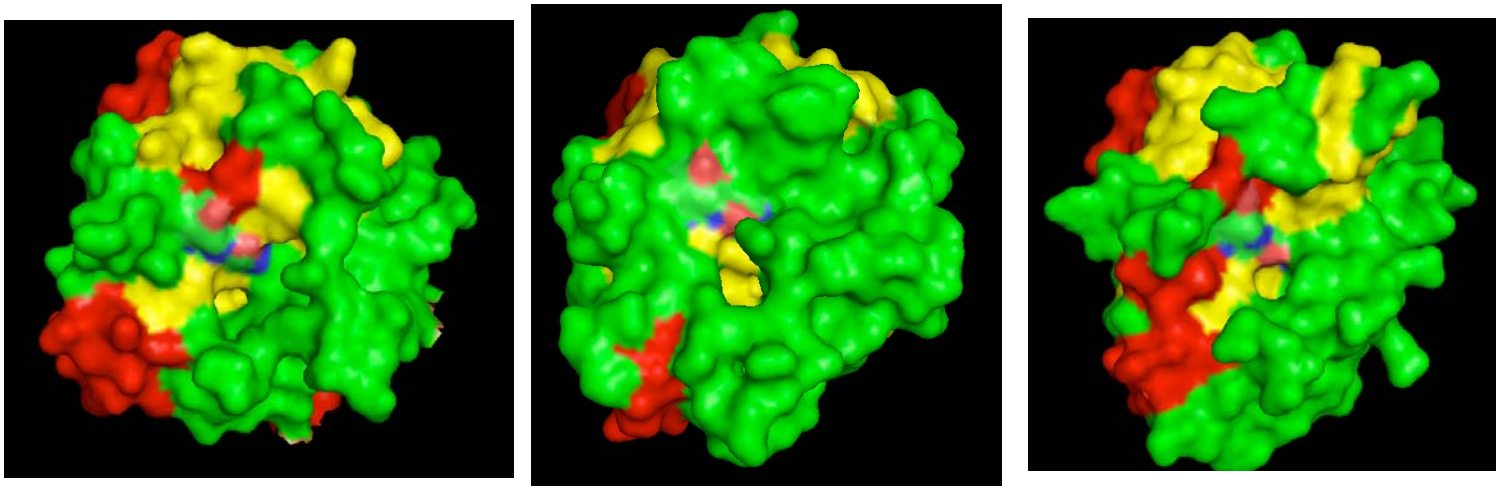


Enzyme	Source	Specificity	Comments
Trypsin	Bovine pancreas	R_{n-1} = positively charged residues: Arg, Lys; $R_n \neq$ Pro	Highly specific
Chymotrypsin	Bovine pancreas	R_{n-1} = bulky hydrophobic residues: Phe, Trp, Tyr; $R_n \neq$ Pro	Cleaves more slowly for R_{n-1} = Asn, His, Met, Leu
Elastase	Bovine pancreas	R_{n-1} = small neutral residues: Ala, Gly, Ser, Val; $R_n \neq$ Pro	

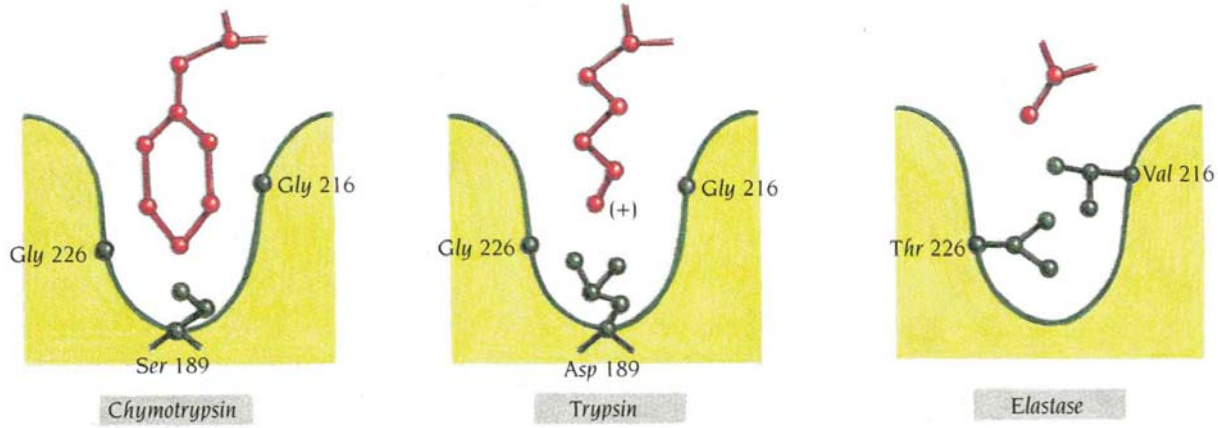
See: Table 5-3. Enzyme Specificity

Specificity of the binding pockets:





Specificity of the binding pockets:



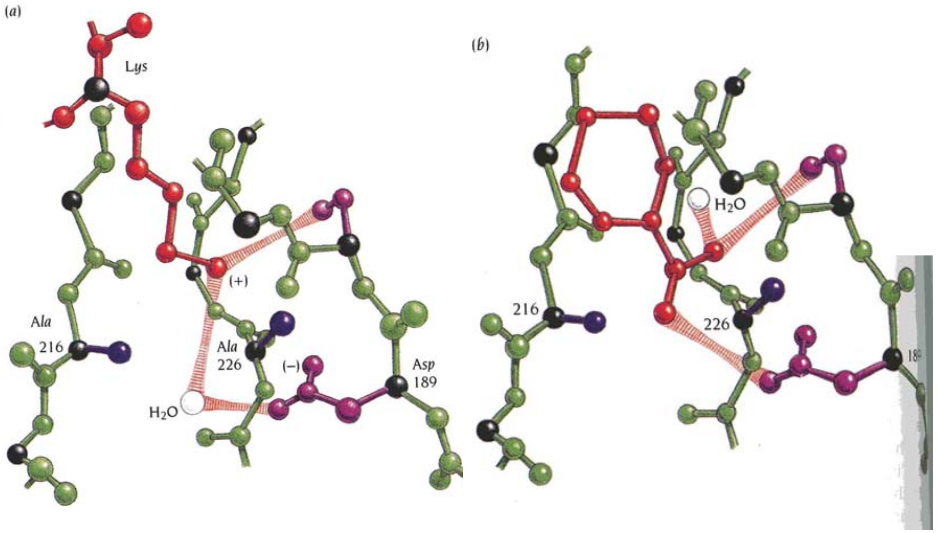


Table 11.1 Kinetic data for wild-type and mutant trypsins

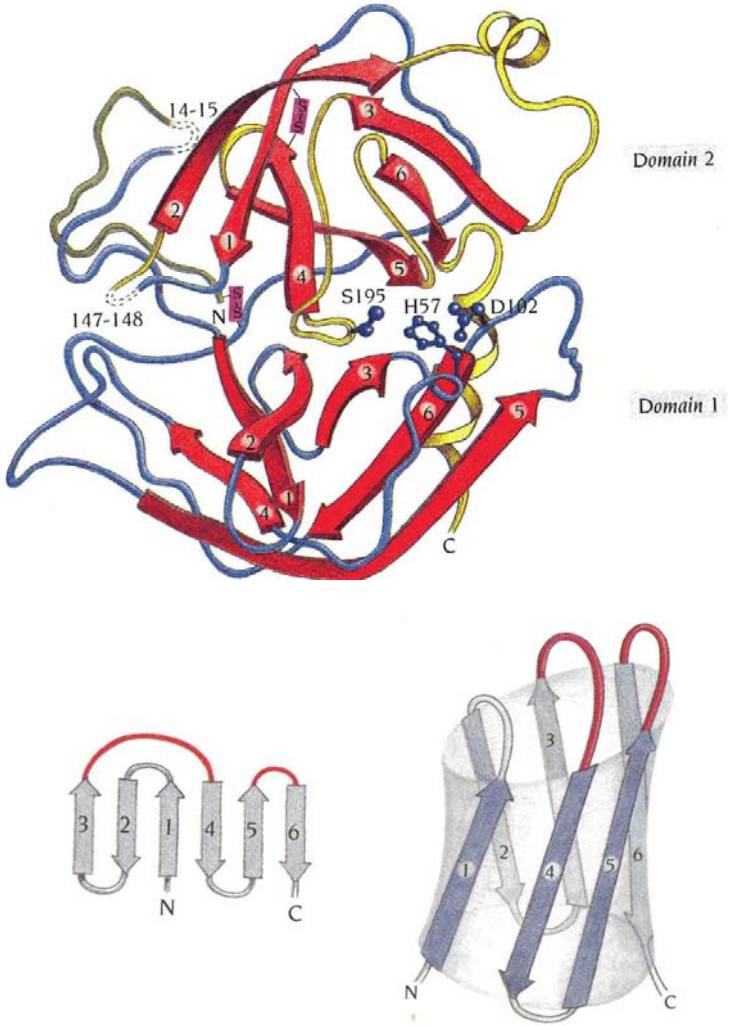
Enzyme	Arg			Lys			(k_{cat}/K_m) Arg / (k_{cat}/K_m) Lys
	k_{cat}	K_m	k_{cat}/K_m	k_{cat}	K_m	k_{cat}/K_m	
Wild type	1	1	1	0.9	10	0.1	10
Gly 216, Gly 226→Ala	0.001	15	0.0001	0.0005	25	0.00002	25
Gly 226→Ala	0.01	35	0.0003	0.1	250	0.0005	0.5
Gly 216→Ala	0.7	30	0.02	0.2	280	0.001	20

The substrates used were D-Val-Leu-Arg-amino fluorocoumarin (Arg) and D-Val-Leu-Lys-amino fluorocoumarin (Lys). For clarity the K_m and k_{cat} values have been normalized to those of the wild-type enzyme for the Arg substrate.

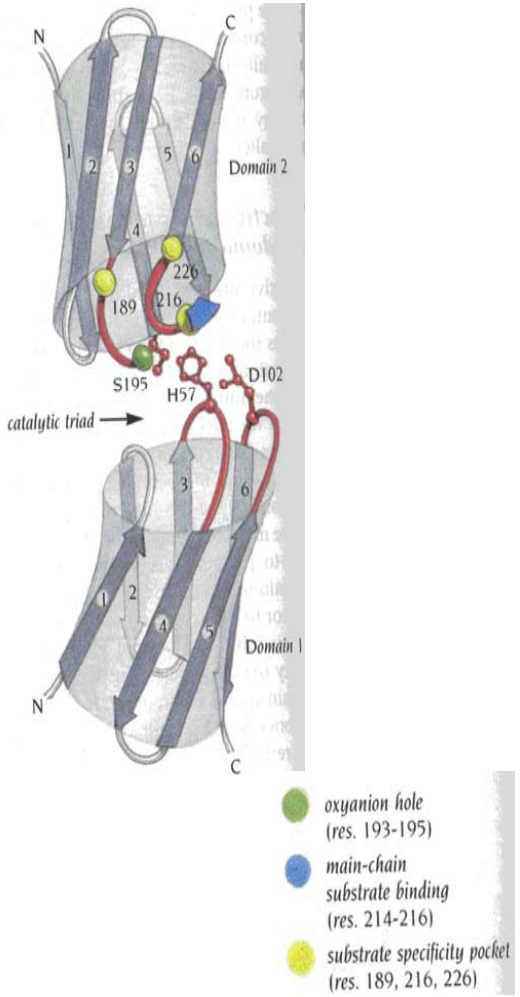
Pleiotropic roles of critical residues --> protein structure & function

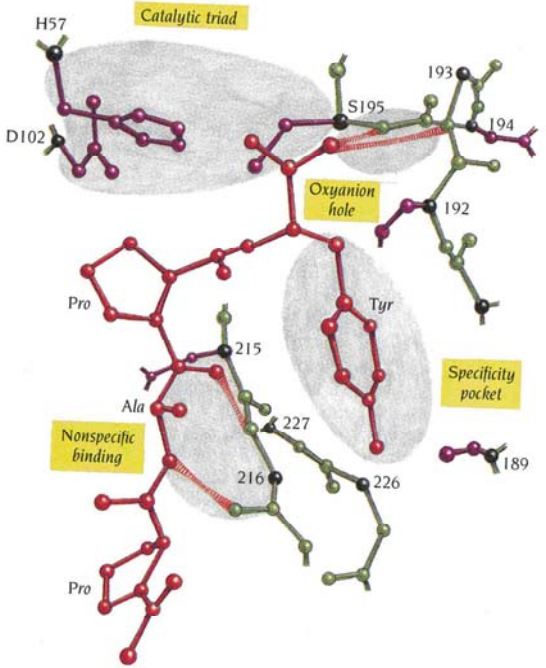
Convergent evolution has produced two different serine proteases: Chymotrypsin superfamily / Subtilisin

Chymotrypsin

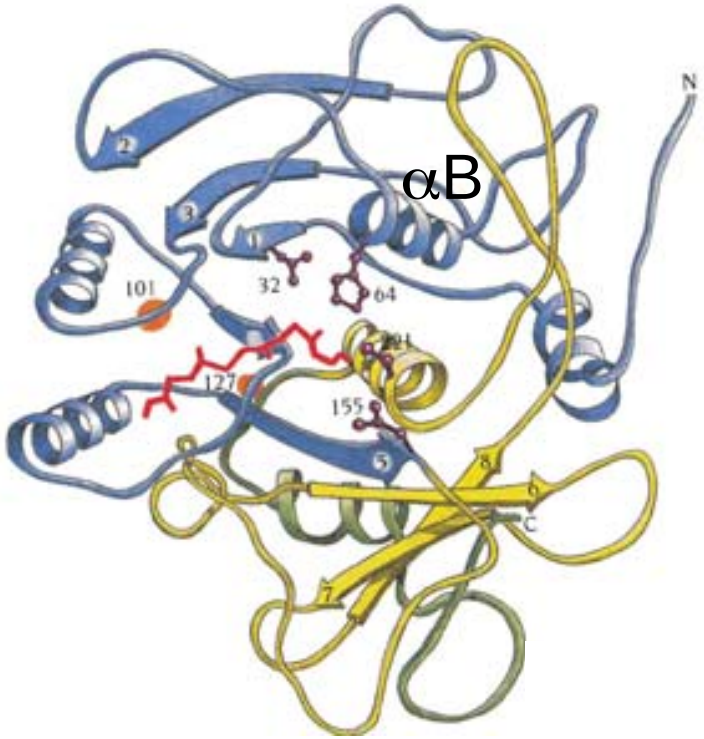


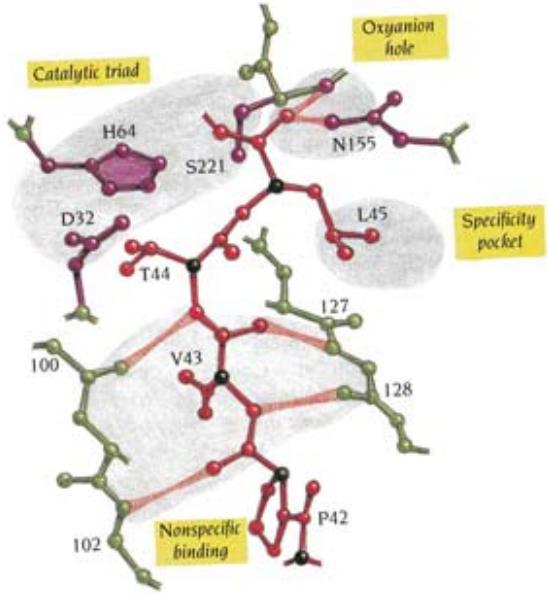
Topological diagram of the two domains:

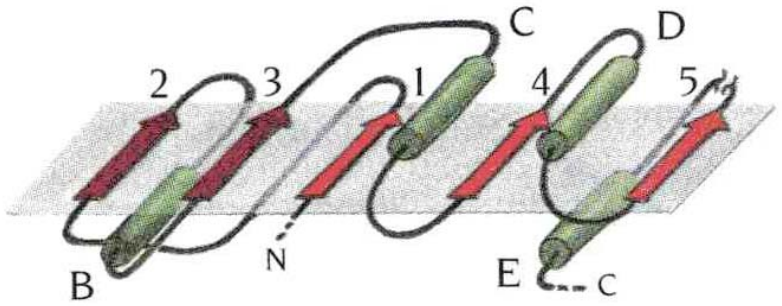




Subtilisin: α/β type







Convergent evolution

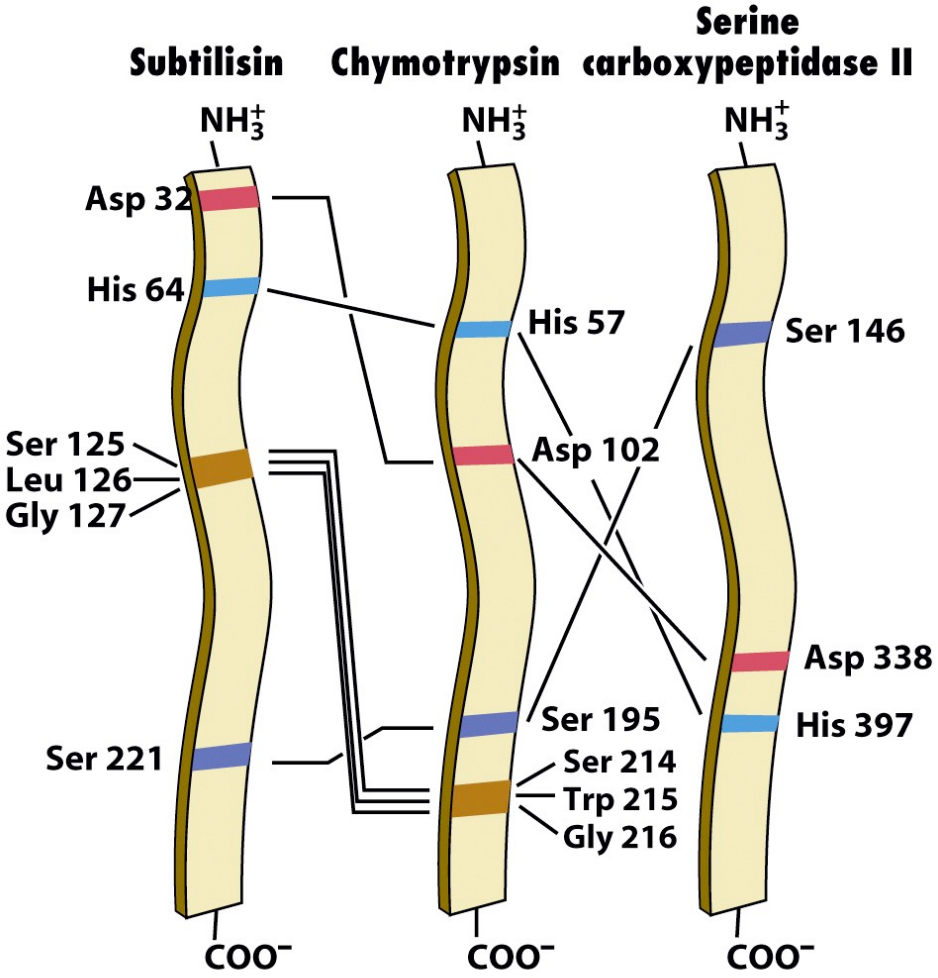


Figure 11-28 Fundamentals of Biochemistry, 2/e
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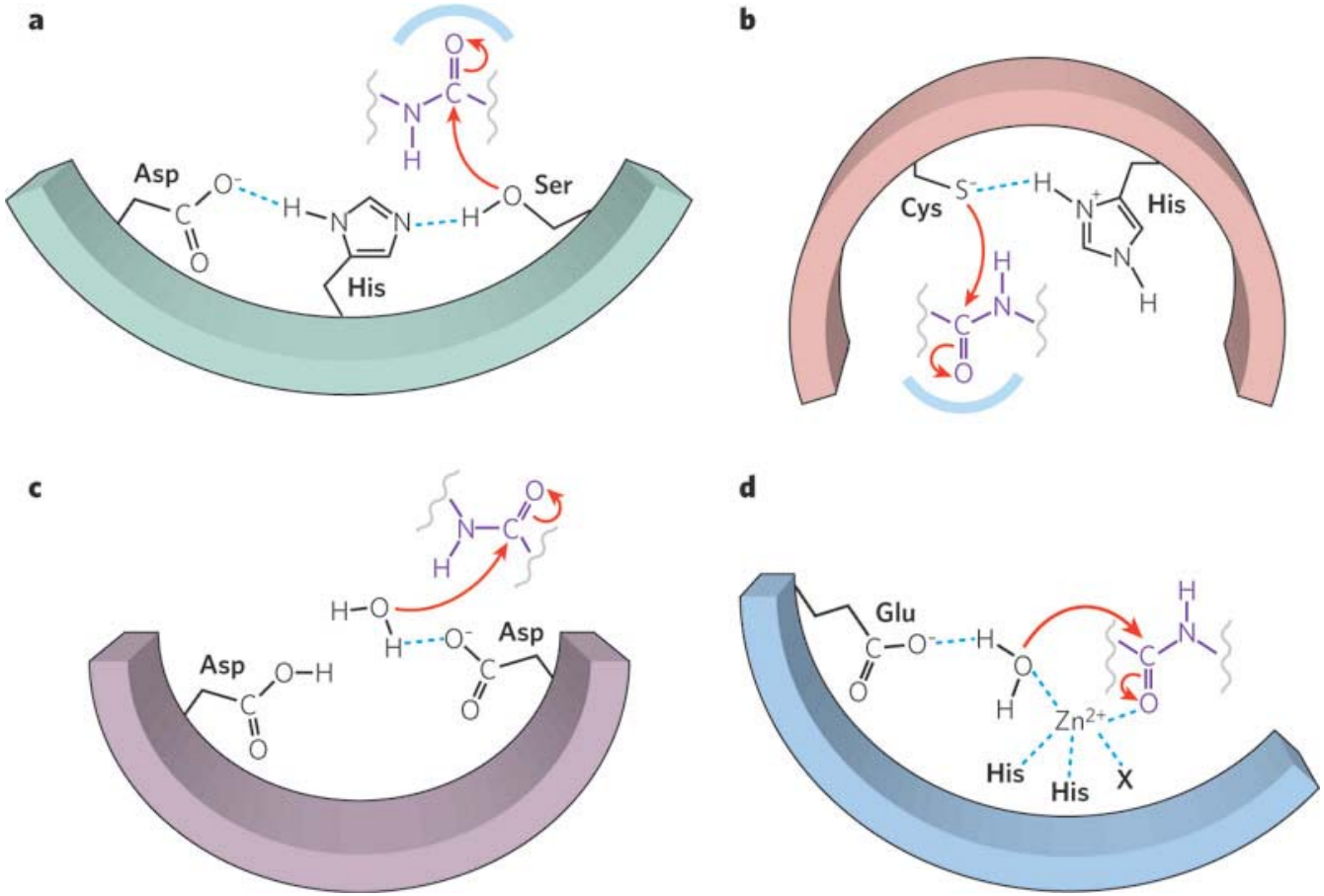
- Chymotrypsin, trypsin, and elastase are very similar in their sequences and functions – they have clearly arisen from a common ancestor
- However, subtilisin, chymotrypsin, and serine carboxypeptidase II have same function but clearly do not have a common ancestor
- This is a remarkable example of convergent evolution – i.e. the same catalytic mechanism arrived at from different starting points

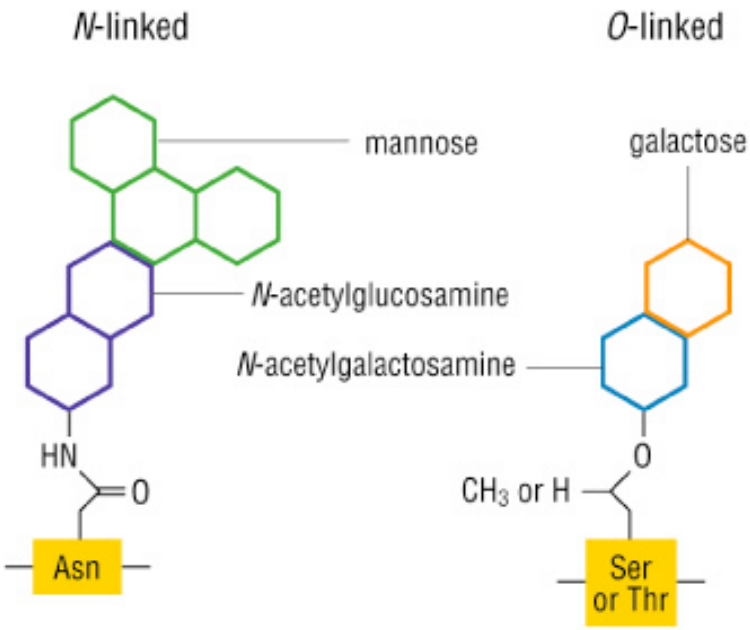


Protease mechanisms: Proteases fall into four main mechanistic classes: serine, cysteine, aspartyl and metalloproteases. In the active sites of serine and cysteine proteases, the eponymous residue is usually paired with a proton-withdrawing group to promote nucleophilic attack on the peptide bond. Aspartyl proteases and metalloproteases activate a water molecule to serve as the nucleophile, rather than using a functional group of the enzyme itself. However, the overall process of peptide bond scission is essentially the same for all protease classes. Soluble serine proteases (a); cysteine proteases (b); aspartyl proteases (c); and metalloproteases (d).

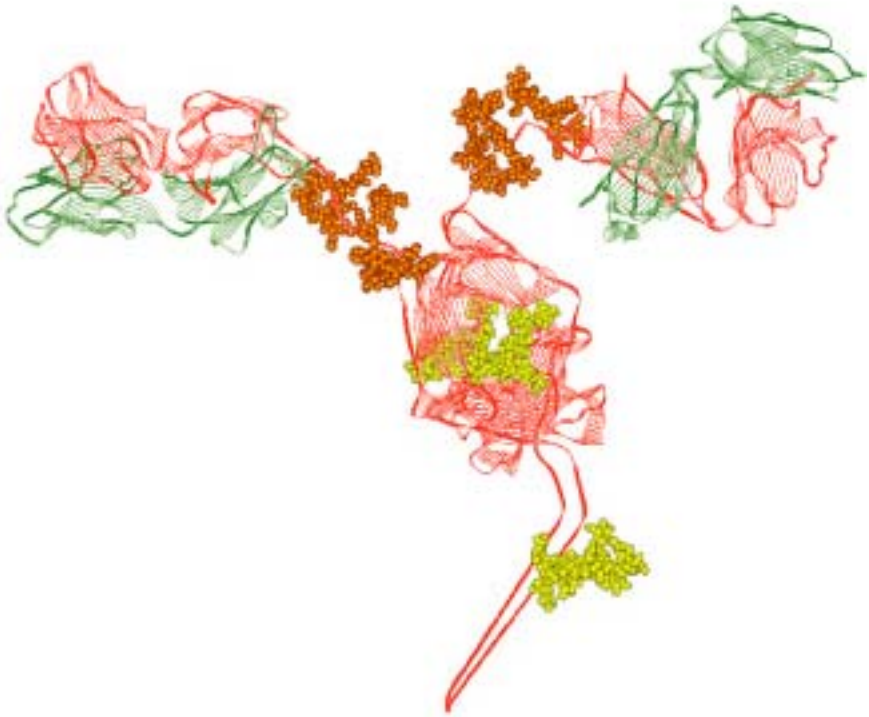
Neitzel JJ (2010) Nature Education 3(9):21

Example 2 **Transferases**

Glycosyltransferases: Glycosylation and Glycoproteins



Schematic representation of the core **N-linked** oligosaccharide and a representative **O-linked** core oligosaccharide



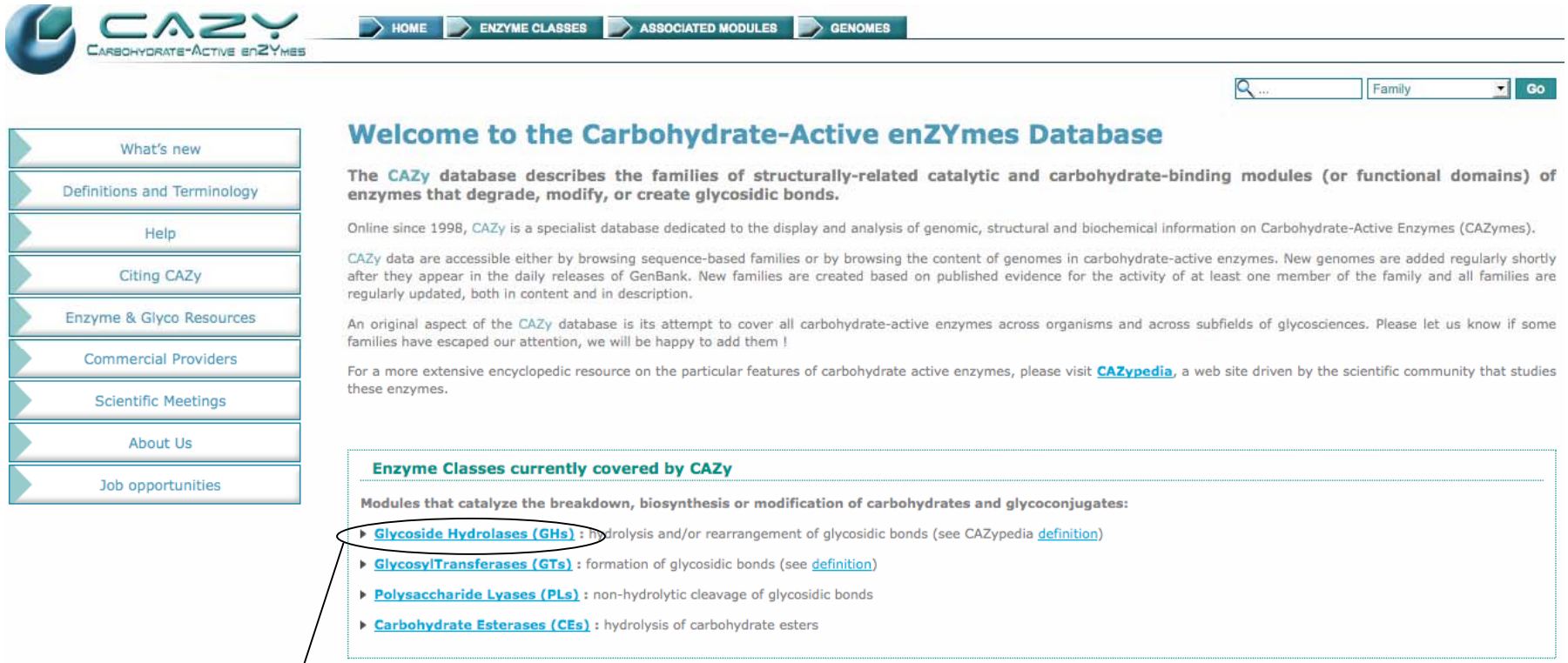
IgA protects mucosal surfaces from pathogenic organisms

From **Protein Structure and Function**
by Gregory A Petsko and Dagmar Ringe

Example 2 **Transferases**

Glycosyltransferases: Glycosylation and Glycoproteins

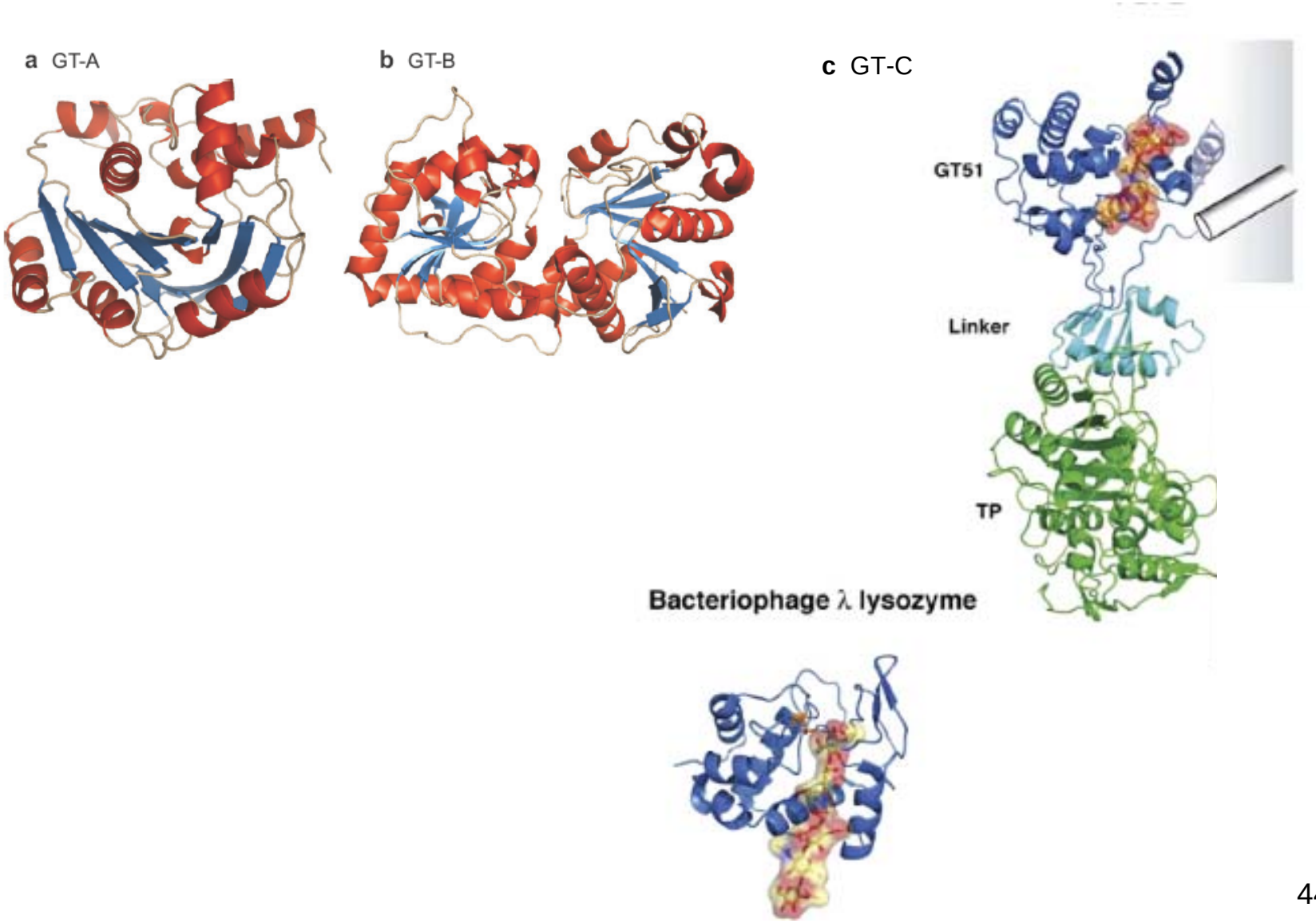
http://www.cazy.org/



Currently 94 GT families

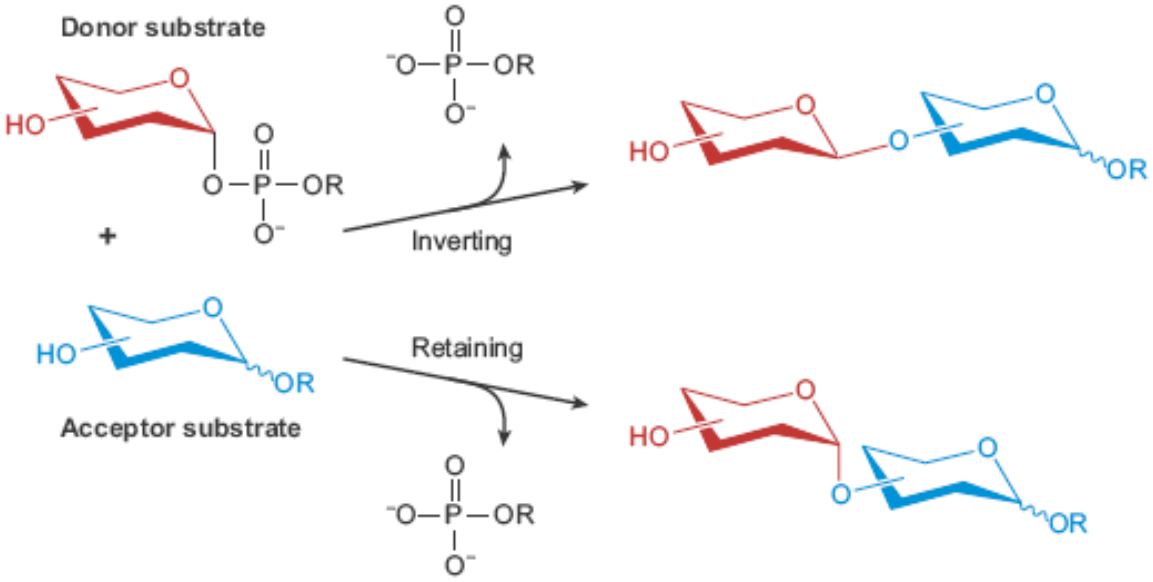
Example 2 **Transferases**

Glycosyltransferases: Glycosylation and Glycoproteins

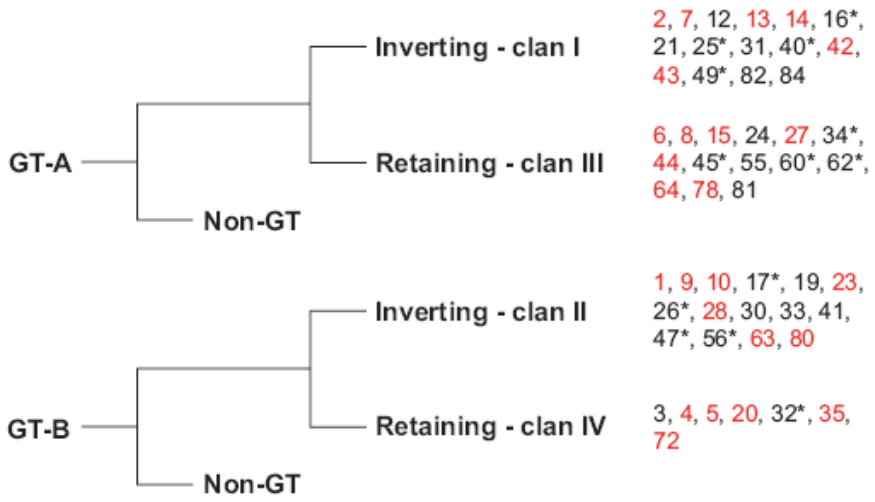


Example 2 **Transferases**

Glycosyltransferases: Glycosylation and Glycoproteins



Glycosyl group transfer with inversion or retention of the anomeric stereochemistry with respect to the donor sugar.



Project papers # 4,5