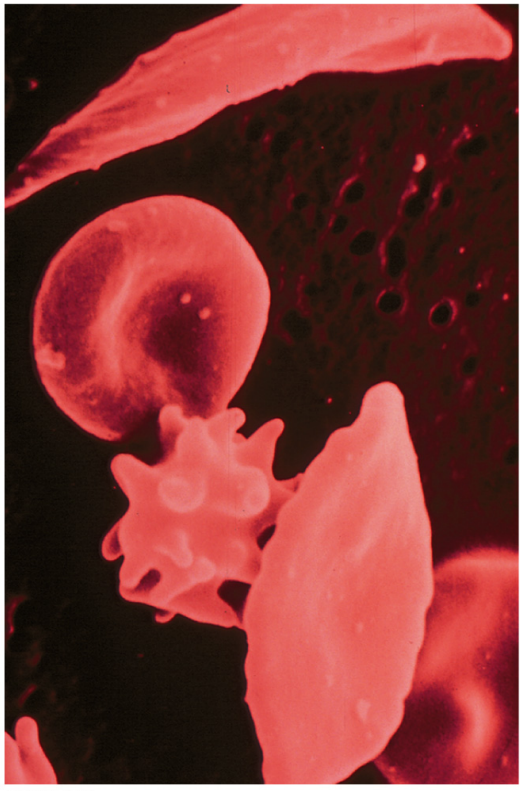
Sickle-Cell Anemia Is Due to a Mutation in Hemoglobin

- Glu6 → Val in the β chain of Hb
- The new Valine side chain can bind to a different Hb molecule to form a strand similar to the amyloidgenic proteins discussed in Chapter 4.
- This sickles the red blood cells.
- Untreated homozygous individuals generally die in childhood.
- Heterozygous individuals exhibit a resistance to malaria.



Jackie Lewin, Royal Free Hospital/Science Source

Figure 5-19b
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Formation of Hb Strands in Sickle-Cell Anemia

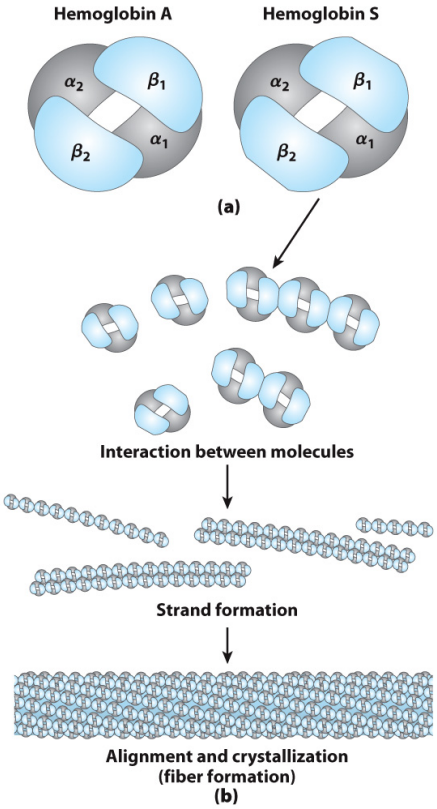

Figure 5-20
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PORPHYRIA

- Porphyria is a group of different disorders caused by abnormalities in the chemical steps leading to the production of heme
- It is characterized by extreme sensitivity to light (exposure to sunlight causes vesicular erythema), reddish-brown urine, reddish-brown teeth, and ulcers which destroy cartilage and bone, causing the deformation of the nose, ears, and fingers.
- Mental aberrations, such as hysteria, manic-depressive psychosis, and delirium, characterize this condition as well.

Cellular Immune System

- Antibodies bind to fragments displayed on the surface of invading cells.
- Phagocytes: specialized cells that eat invaders
- Macrophages: large phagocytes that ingest bacteria that are tagged by antibodies

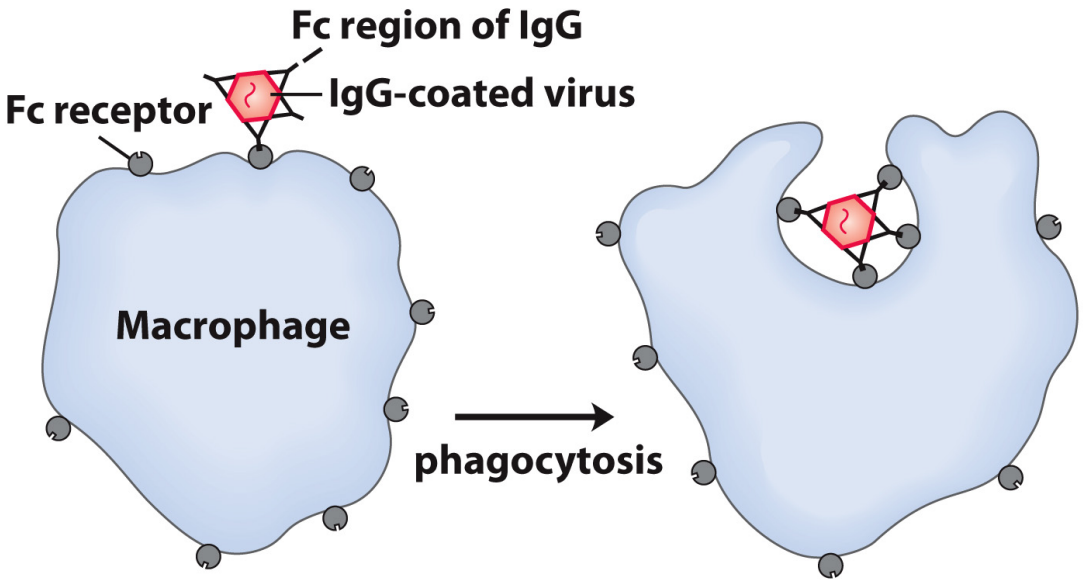

Figure 5-24
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Humoral Immune System

- Vertebrates also fight infections with soluble antibodies that specifically bind antigens.
  - Antigens are substances that stimulate production of antibodies.
    - typically macromolecular in nature
    - recognized as foreign by the immune system
    - coat proteins of bacteria and viruses
    - surface carbohydrates of cells or viruses
  - Antibodies are proteins that are produced by B cells and that specifically bind to antigens.
    - Binding will mark the antigen for destruction or interfere with its function.
    - A given antibody will bind to a small region (epitope) of the antigen.
    - One antigen can have several epitopes.

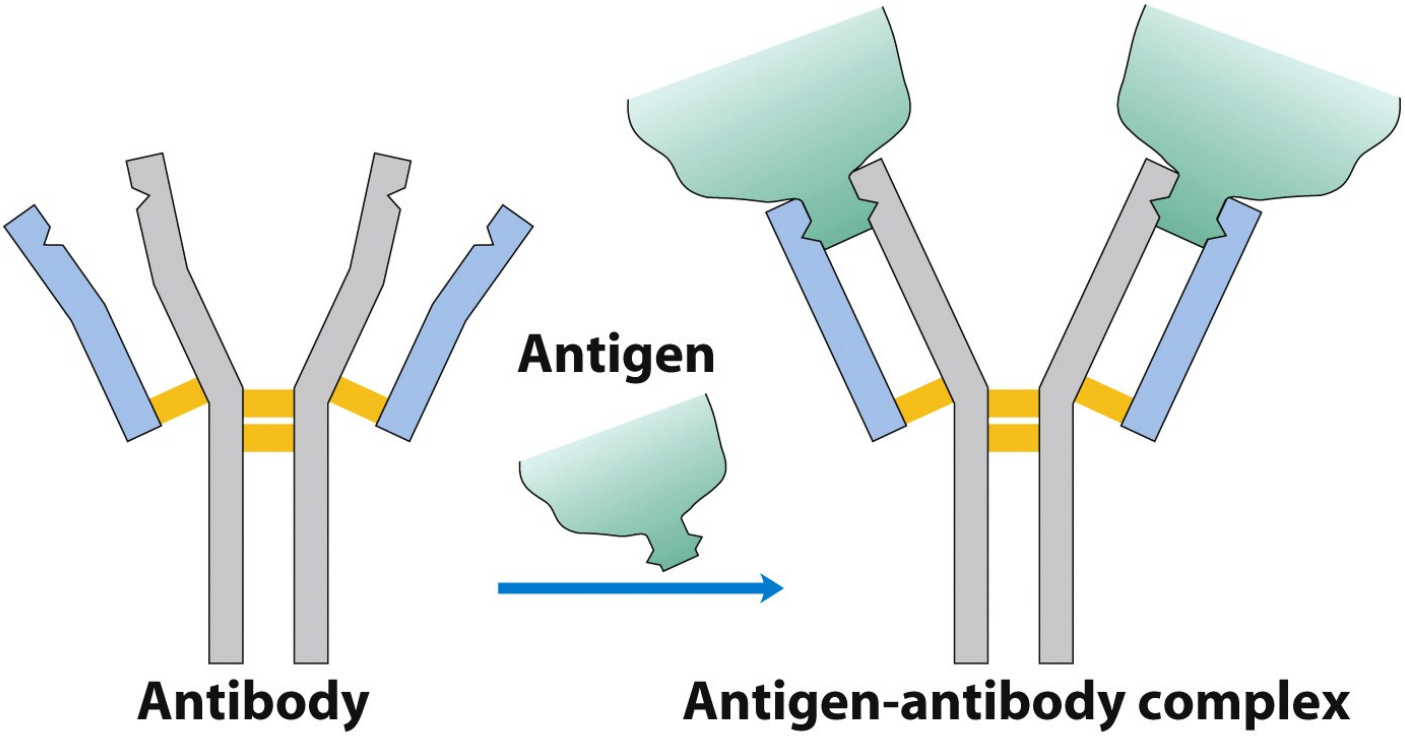

Figure 5-22
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Antibodies: Immunoglobulin G

Two **heavy chains** and two **light chains**

- composed of constant domains and variable domains

Light chains: one constant and one variable domain

Heavy chains: three constant and one variable domain

Variable domains of each chain make up the antigen-binding site (two per antibody) and are hypervariable, which confers antigen specificity.

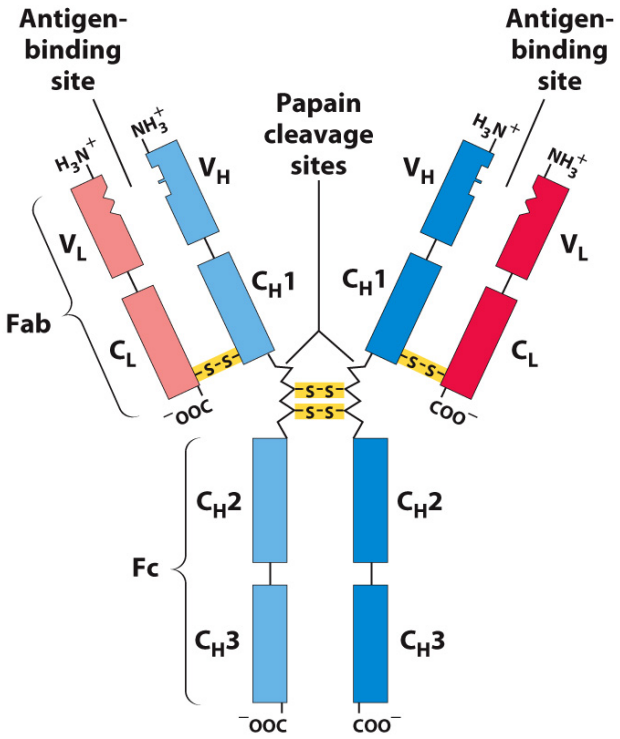

Figure 5-21a
Lehninger Principles of Biochemistry, Seventh Edition

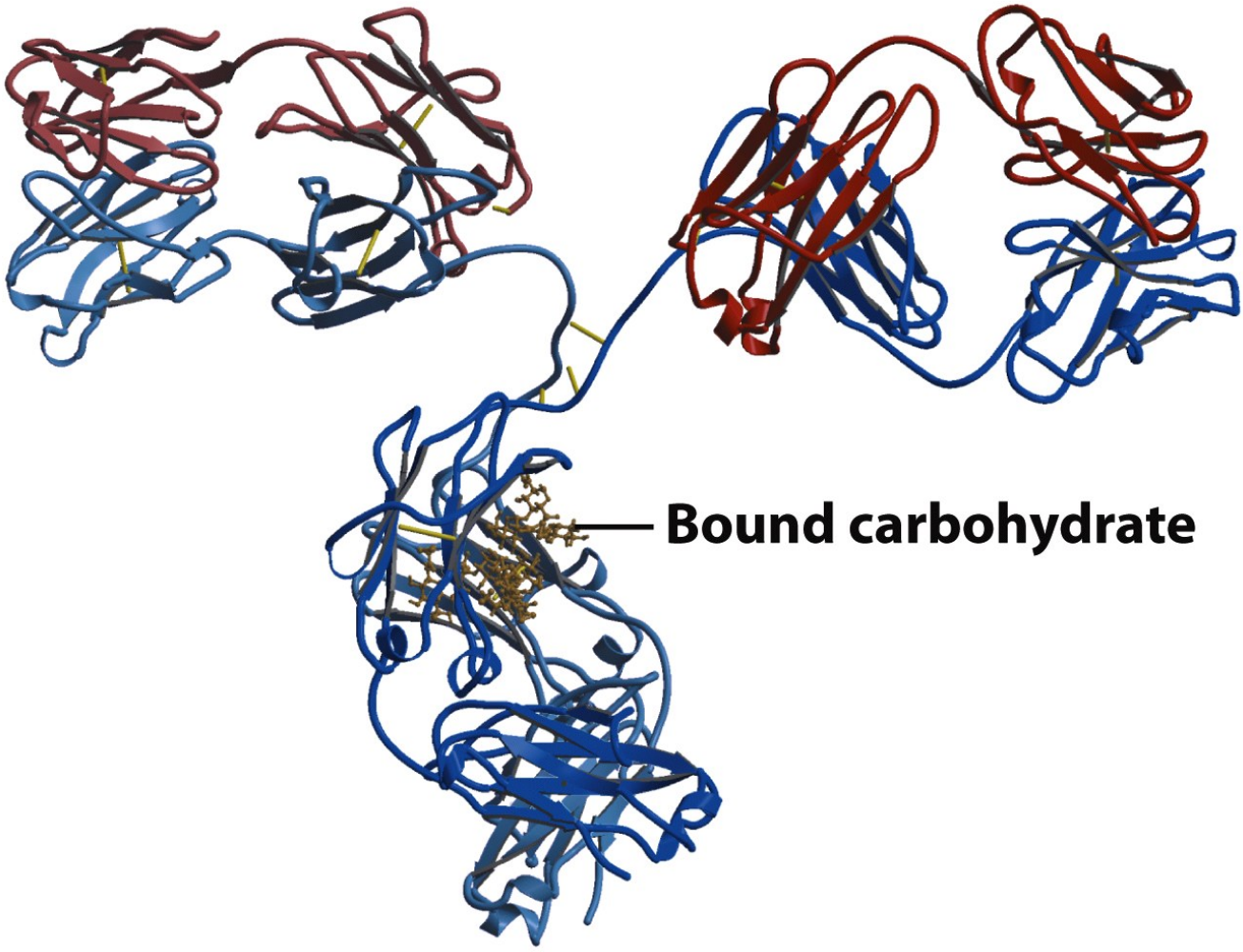

Figure 5-21b
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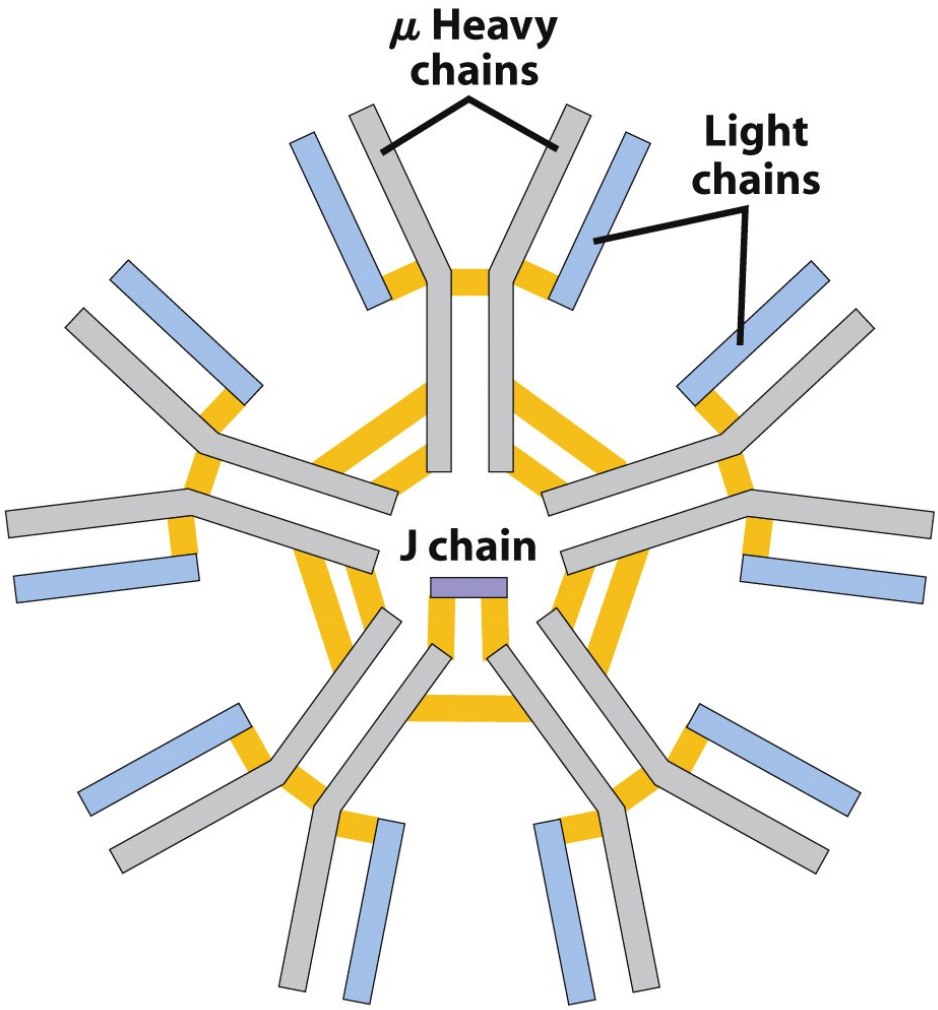

Figure 5-23
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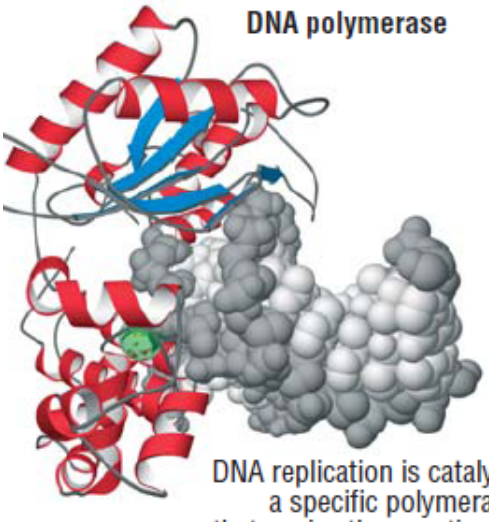
There Are Five Classes of Heavy Chains

- IgM, which has μ heavy chains, is always the first class of antibody made by a developing B cell;
- After leaving the bone marrow, the B cell starts to produce cell-surface IgD molecules as well, with the same antigen-binding site as the IgM molecules.
- The major class of immunoglobulin in the blood is IgG, which is a four-chain monomer produced in large quantities during secondary immune responses;
- IgA is the principal class of antibody in secretions, including saliva, tears, milk, and respiratory and intestinal secretions;
- The tail region of IgE molecules, which are four-chain monomers, binds with unusually high affinity (Ka ~ 1010 liters/mole) to yet another class of Fc receptors;

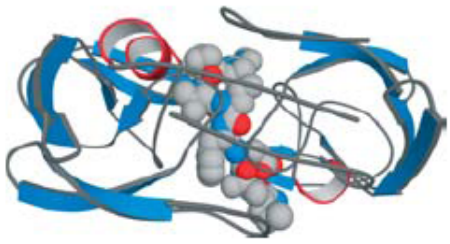
CATALYSIS

DNA polymerase



DNA replication is catalyzed by a specific polymerase that copies the genetic material and edits the product for errors in the copy. (PDB 1pbx)

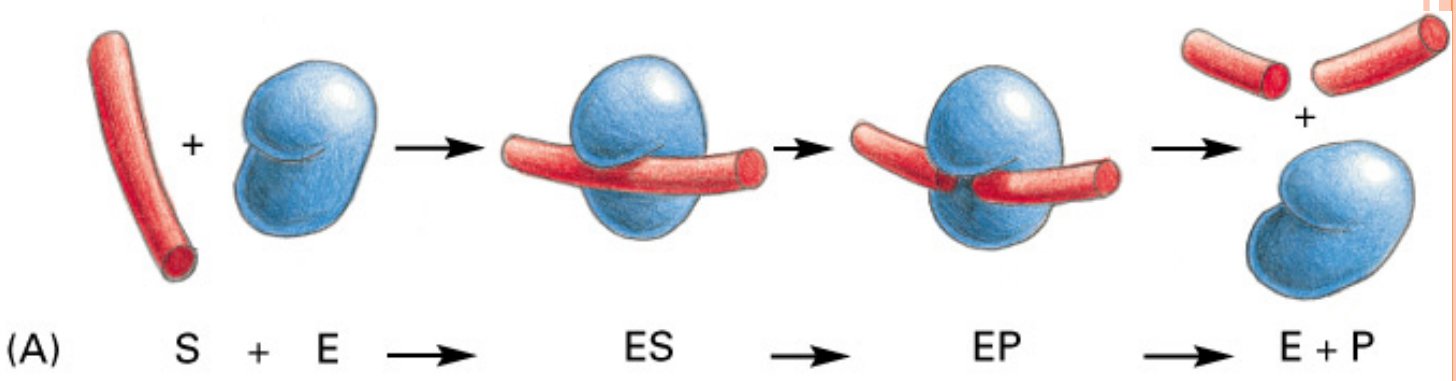
HIV protease

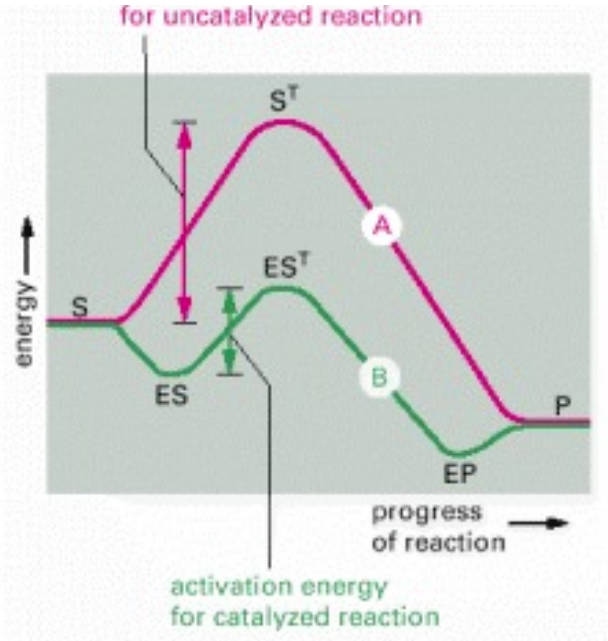


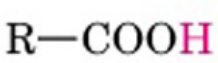
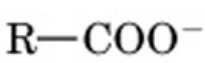
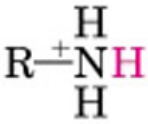
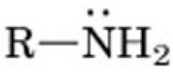
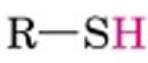
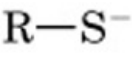
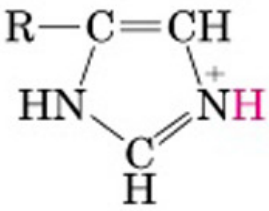
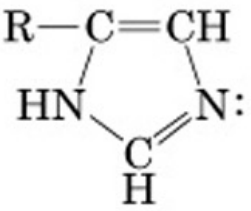
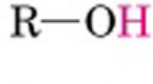
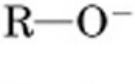
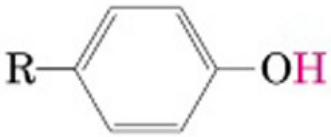
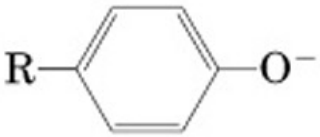
Replication of the AIDS virus HIV depends on the action of a protein-cleaving enzyme called HIV protease. This enzyme is the target for protease-inhibitor drugs (shown in grey). (PDB 1a8k)

Catalysis

- Some protein bind to one or more ligands, called substrates, and convert them into one or more chemically modified products – Enzymes
- Enzymes speed up reactions, often by a factor of a million or more, without themselves being changed
- hey act as catalysts that permit cells to make or break covalent bonds in a controlled way
- Enzymes can be grouped into functional classes that perform similar chemical reactions


(A)
S + E
ES
EP
E + P


for uncatalyzed reaction
S^T
A
ES^T
S
energy
ES
B
P
EP
progress
of reaction
activation energy
for catalyzed reaction

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

What Are Enzymes?

An Enzyme:

- is a biopolymer (protein, sometimes RNA), that
- increases the rate of (catalyzes) a chemical reaction,
- regulates the rate of a chemical reaction (sometimes),
- is not consumed/produced in the chemical reaction,
- does not alter the equilibrium condition of the reaction,
- converts specific substrates to specific products.

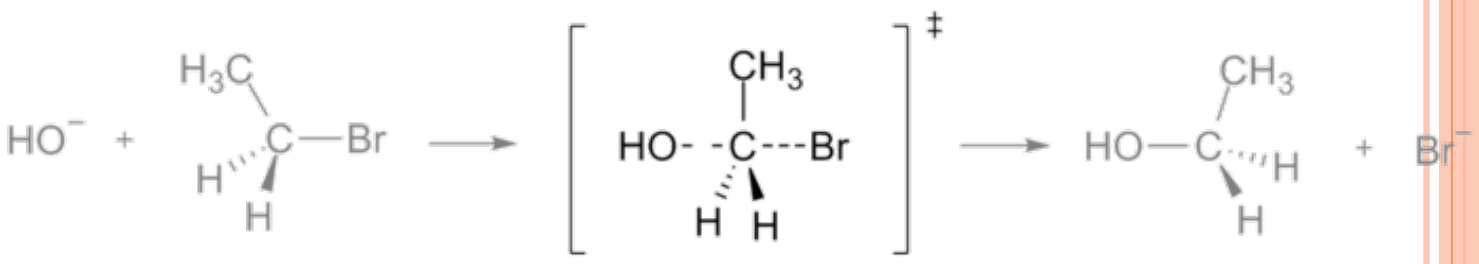
What Are Enzymes?

An Enzyme:

- can change the mechanism of a reaction,
- binds to substrate(s) by non-covalent interactions,
- is complementary to the substrate(s),
- binds by ‘lock and key’ (some enzymes), or by
- ‘Induced fit’ (other enzymes),
- is stereospecific and chemically specific (usually), and
- stabilizes one or more transition states.

A Transition State

A transition state is the highest energy species (most unstable species) along the reaction coordinate.

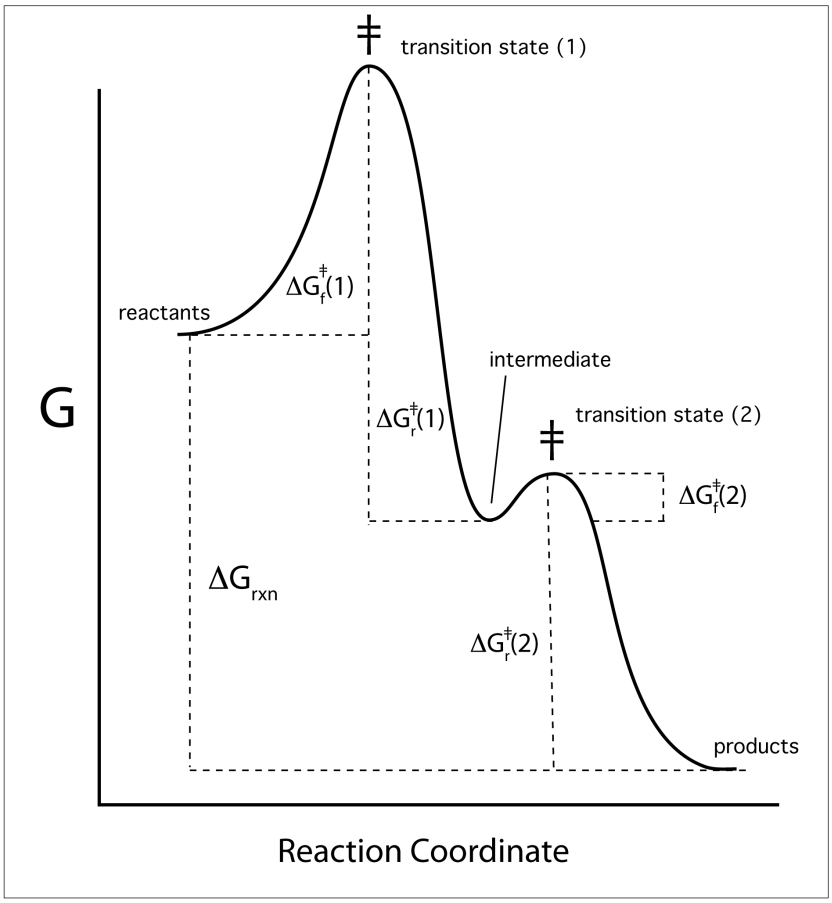


An intermediate is at a local minima along the reaction coordinate.

Reactions with intermediates (all enzymatic reactions) have multiple transition states.

Enzymatically Catalyzed Reaction Coordinate

All enzymatic reactions have multiple steps.



What do Enzymes do?

Enzymes:

- break down molecules,
- build molecules,
- transduce energy,
- do work or generate light (luciferase),
- signal (phosphatases, kinases, E3 ligase),
- replicate information (DNA & RNA polymerases),
- transduce information (ribosome),
- pump ions.

- The study of enzymatic processes is the oldest field of biochemistry, dating back to late 1700s.
- The study of enzymes has dominated biochemistry in the past and continues to do so.

Three notable enzymologists (dead white guys)

Science Museum/Science & Society Picture Library

Eduard Buchner, 1860–1917

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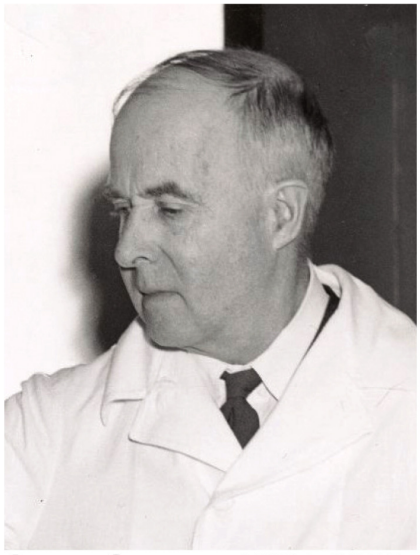

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James Sumner, 1887–1955

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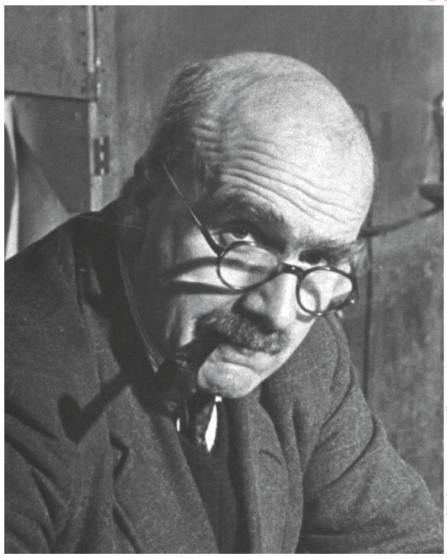

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J. B. S. Haldane, 1892–1964

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Eduard Buchner, 1860–1917

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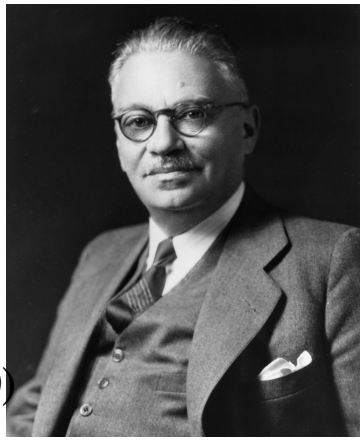
Eduard Buchner. Nobel Prize 1907, Voluntered for the German army and was killed in 1917 (WWI) when he was hit by a shell fragment.

Biological catalysis was first recognized and described in the early 1800s, in studies of the digestion of meat by secretions of the stomach and the conversion of starch into sugar by saliva and plant extracts. In the 1850s Louis Pasteur concluded that fermentation of sugar into alcohol by yeast is catalyzed by "ferments." He postulated (incorrectly) that these ferments, later named **enzymes**, are inseparable from the structure of living cells, a view that prevailed for many years. The discovery by Eduard Buchner in 1897 that **yeast extracts can ferment sugar to alcohol** proved that the **enzymes involved in fermentation can function outside of living cells**. This encouraged biochemists to attempt the isolation of many different enzymes and to examine their catalytic properties.

Maud Menten (1879-1960)
Woman in science!

Leonor Michaelis (1875-1949)

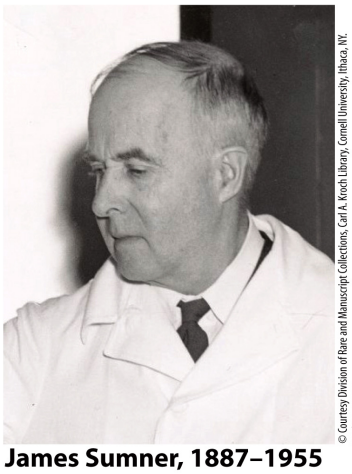


Michaelis and Menten were able to express mathematically the relationship they were investigating, which demonstrated that each enzyme not only has its own substrate but also that at sufficient concentrations of substrate it has its own rate of causing that substrate to change chemically.

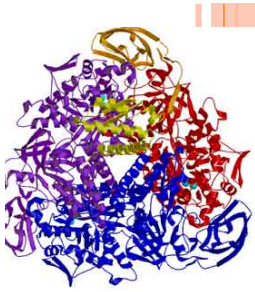
Urease: an enzyme found in bacteria, yeast, and several higher plants that catalyzes the hydrolysis of urea into carbon dioxide and ammonia.

$(NH_2)_2CO$ (urea) + $H_2O \rightarrow CO_2 + 2NH_3$

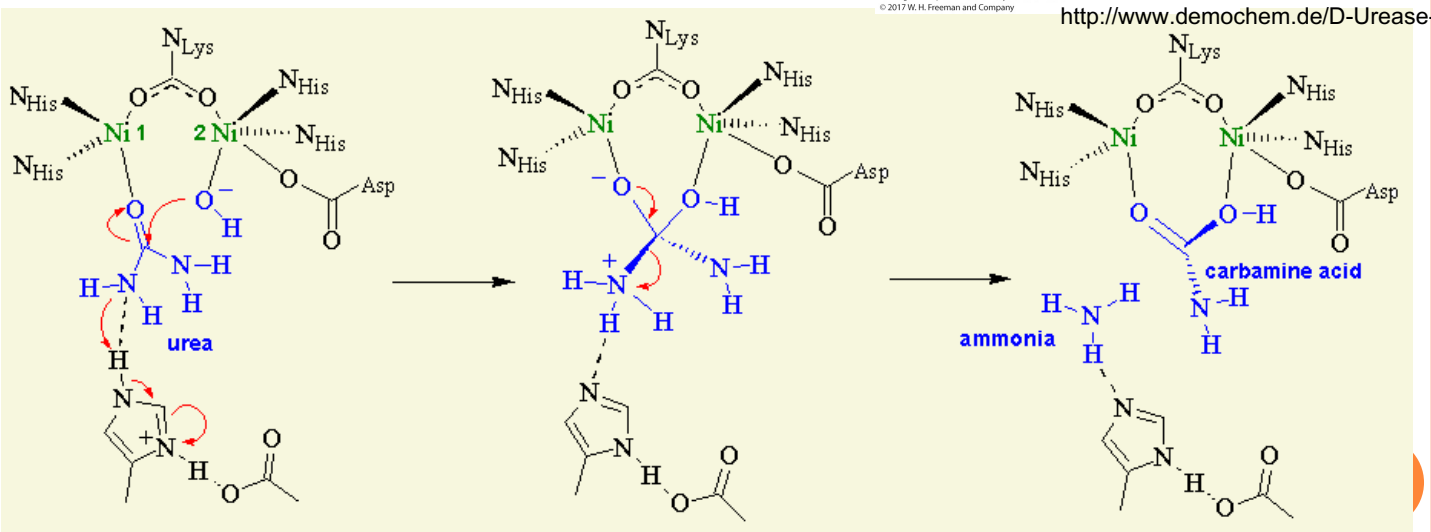
<u>James Sumner in 1926 crystallized urease and showed it is a protein. First definitive proof that catalytic activity was due to a protein.</u> The structure of urease was not solved until 1995 by P.A. Karplus!



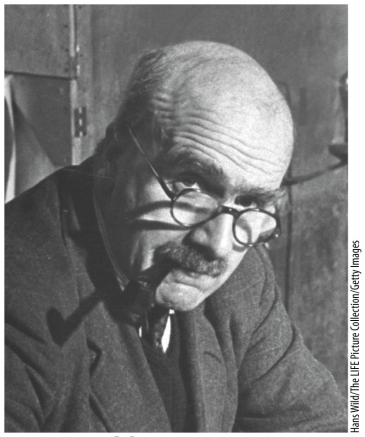
James Sumner, 1887–1955



http://www.demochem.de/D-Urease-e.htm



Sumner postulated that all enzymes are proteins.
(We now know that RNA can be enzymatic)



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J. B. S. Haldane, 1892–1964

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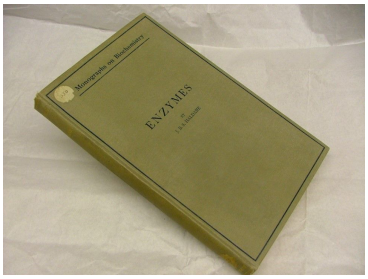
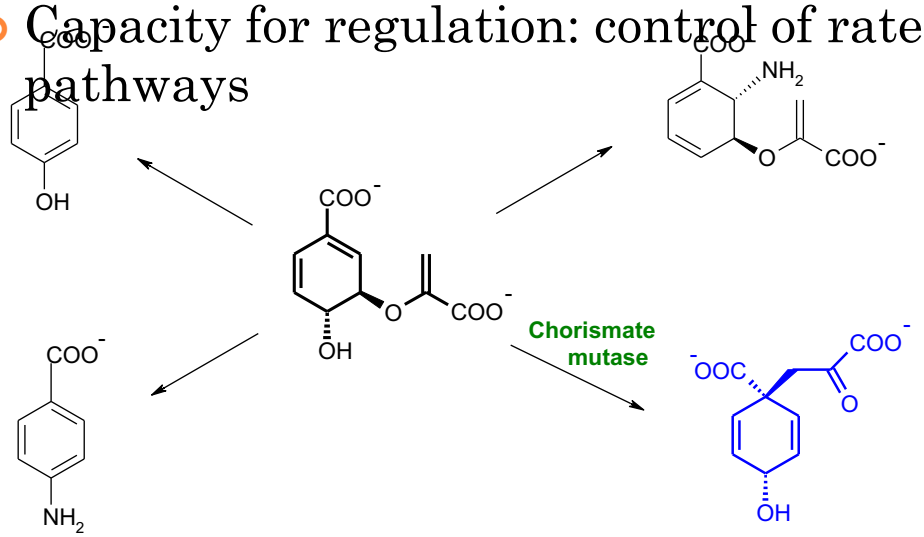


J.B.S. Haldane wrote a treatise entitled "Enzymes." Even though the molecular nature of enzymes was not yet fully appreciated, this book contained the remarkable suggestion that **weak-bonding interactions between an enzyme and its substrate might be used to distort the substrate and catalyze the reaction.**

Biocatalysis versus Inorganic Catalysts?

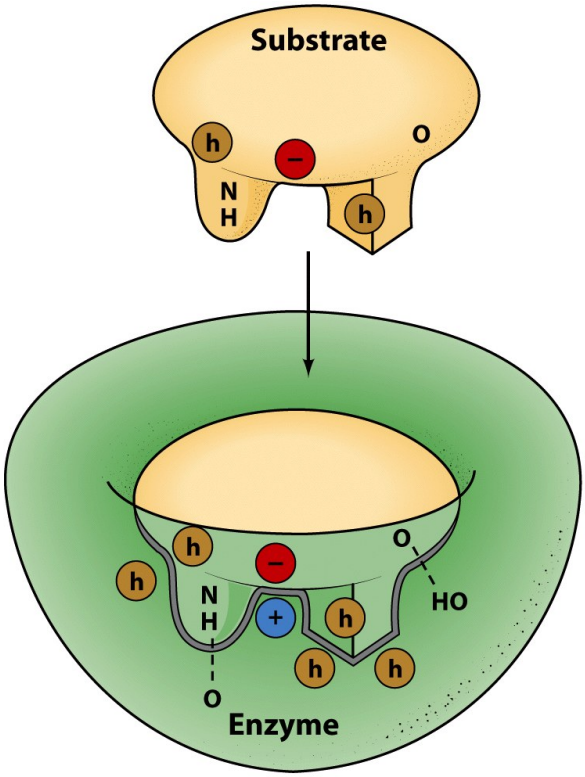
- Greater reaction specificity: avoids side products,
- Works in aqueous media,
- Milder reaction conditions: conductive to conditions in cells,

  pH ~ 7, 37°C
- Higher reaction rates: in a biologically useful timeframe
- Capacity for regulation: control of rates and biological pathways



- Metabolites have many potential pathways of decomposition.
- Enzymes make the desired one most favorable.

Lock and Key
(rigid enzyme)



Induce Fit
(flexible enzyme)

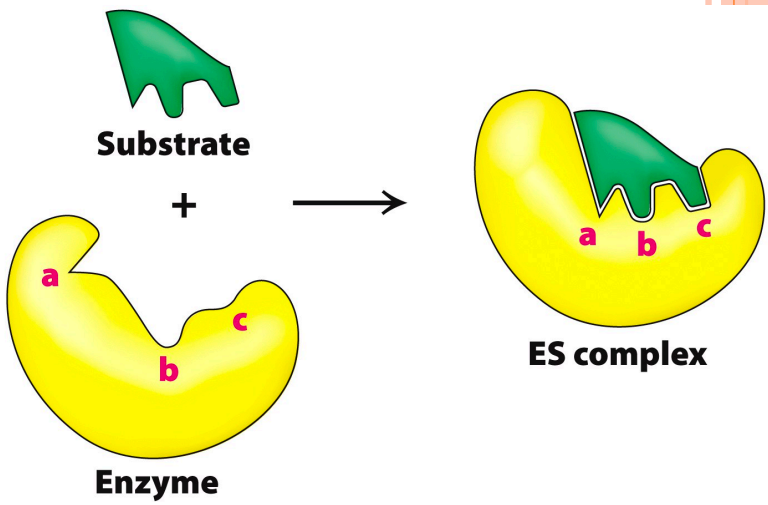


Figure 8.9
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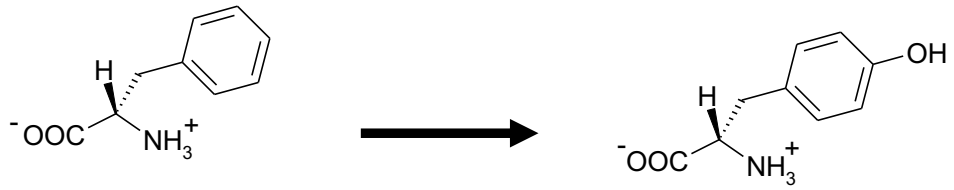
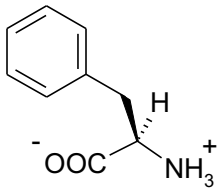
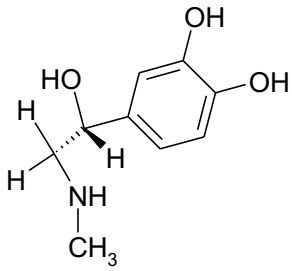
Enzymatic Substrate Selectivity





No binding



Binding but no reaction

Example: phenylalanine hydroxylase

Enzyme-Substrate Complex (ES) Drives Selectivity

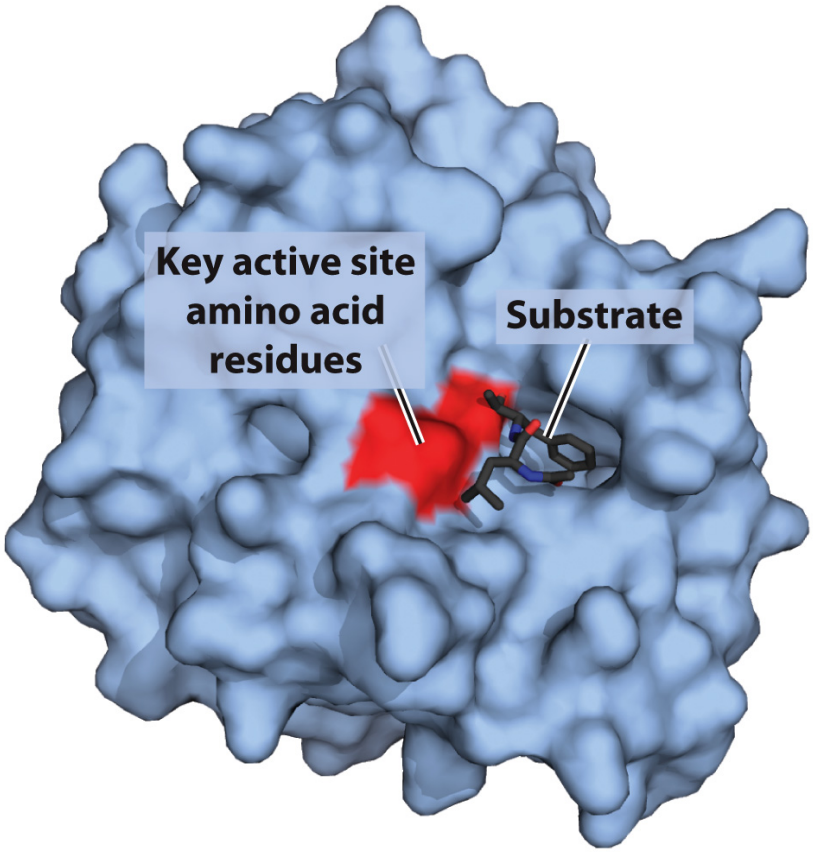


Figure 6-1
Lehninger Principles of Biochemistry, Seventh Edition

ES is stabilized by molecular interactions (non-covalent interactions)

Many enzymes "harness" the catalytic properties of metal ions (cofactors):

TABLE 6-1 Some Inorganic Ions That Serve as Cofactors for Enzymes

Ions	Enzymes
Cu^{2+}	Cytochrome oxidase
Fe^{2+} or Fe^{3+}	Cytochrome oxidase, catalase, peroxidase
K^{+}	Pyruvate kinase
Mg^{2+}	Hexokinase, glucose 6-phosphatase, pyruvate kinase
Mn^{2+}	Arginase, ribonucleotide reductase
Mo	Dinitrogenase
Ni^{2+}	Urease
Zn^{2+}	Carbonic anhydrase, alcohol dehydrogenase, carboxypeptidases A and B

Many enzymes use Co-enzymes

TABLE 6-2 Some Coenzymes That Serve as Transient Carriers of Specific Atoms or Functional Groups

Coenzyme	Examples of chemical groups transferred	Dietary precursor in mammals
Biocytin	CO_2	Biotin
Coenzyme A	Acyl groups	Pantothenic acid and other compounds
5′-Deoxyadenosylcobalamin (coenzyme B_{12})	H atoms and alkyl groups	Vitamin B_{12}
Flavin adenine dinucleotide	Electrons	Riboflavin (vitamin B_2)
Lipoate	Electrons and acyl groups	Not required in diet
Nicotinamide adenine dinucleotide	Hydride ion ($:H^-$)	Nicotinic acid (niacin)
Pyridoxal phosphate	Amino groups	Pyridoxine (vitamin B_6)
Tetrahydrofolate	One-carbon groups	Folate
Thiamine pyrophosphate	Aldehydes	Thiamine (vitamin B_1)

Note: The structures and modes of action of these coenzymes are described in Part II.

Enzymes are classified, and often named, according to the reaction they catalyze

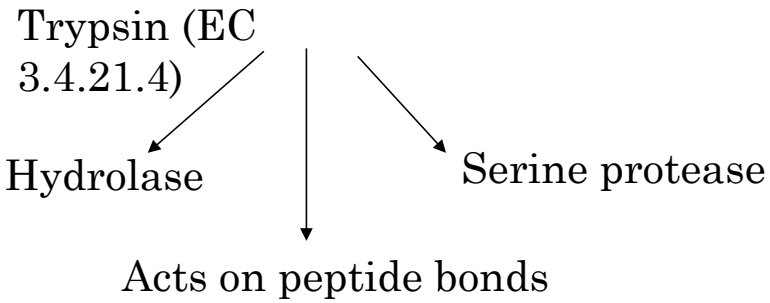
TABLE 6-3 International Classification of Enzymes

Class no.	Class name	Type of reaction catalyzed
1	Oxidoreductases	Transfer of electrons (hydride ions or H atoms)
2	Transferases	Group transfer reactions
3	Hydrolases	Hydrolysis reactions (transfer of functional groups to water)
4	Lyases	Cleavage of C—C, C—O, C—N, or other bonds by elimination, leaving double bonds or rings, or addition of groups to double bonds
5	Isomerases	Transfer of groups within molecules to yield isomeric forms
6	Ligases	Formation of C—C, C—S, C—O, and C—N bonds by condensation reactions coupled to cleavage of ATP or similar cofactor

Protein Data Bank

http://www.rcsb.org

148827 Biological Macromolecular Structures



How enzymes work

Thermodynamics and kinetics

Enzymatic Catalysis

- Enzymes do not change equilibrium constants (K_{eq})
- Enzymes do not change the free energies of reactions (ΔG).
- Enzymes increase reaction rate constants (k_f and k_r) by decreasing $\Delta G_f^{\ddagger}$ and $\Delta G_r^{\ddagger}$. [Slow reactions have large activation energies ($\Delta G^{\ddagger}$)].

How to Lower $\Delta G^{\neq}$

Entropy Trapping: Enzymes organize reactive groups into close proximity and proper orientation.

- Uncatalyzed bimolecular reactions

  Two free reactants → single restricted transition state conversion is entropically unfavorable.

- Uncatalyzed unimolecular reactions

  Flexible reactant → rigid transition state conversion is entropically unfavorable for flexible reactants.

- Catalyzed reactions

  The enzyme uses the binding energy of substrate binding and the folding energy of the enzyme to organize the reactants to a rigid ES complex.

  The entropy cost is paid during folding/binding.

  Rigid reactant complex → transition state conversion is entropically neutral.

How to Lower $\Delta G^{\neq}$

Enzymes preferentially bind transition states.

- The idea was proposed by Linus Pauling in 1946.
  - Enzyme active sites are complimentary to the transition state of the reaction.
  - Enzymes bind transition states better than substrates.
  - Stronger/additional interactions with the transition state as compared with the ground state lower the activation barrier.

Largely $\Delta H^{\ddagger}$ effect

Illustration of TS Stabilization: Imaginary Stickase

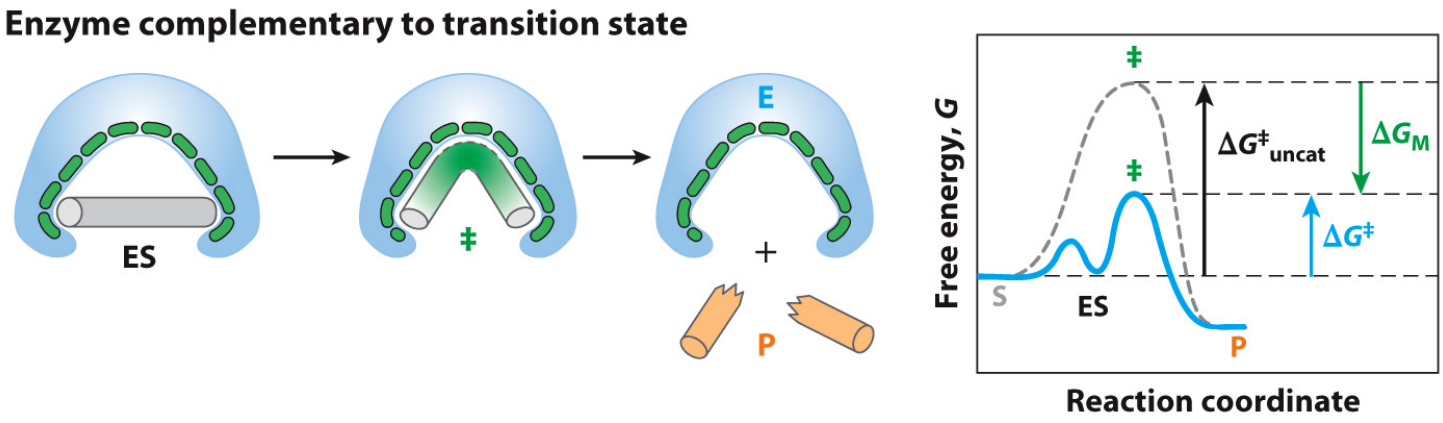


Figure 6-5c
Lehninger Principles of Biochemistry, Seventh Edition