Summary

What we have learned

- **Water**
- **Amino acids**
- **Proteins**
  - **Purification**
  - **Structure**
  - **Function (myoglobin, hemoglobin, antibody)**
  - **Enzymes**

Structure of biological molecules is important for their function

- **Configuration（构型）: the fixed spatial arrangement of atoms in a molecule**
- **Geometric isomers (cis-trans isomers): different in the arrangement of their substituent groups with respect to the nonrotating double bond**
- **Optical isomers: chiral molecule vs. achiral molecule**
- **Stereoisomers cannot be interconverted without temporarily breaking one or more covalent bonds**
- **Conformation（构象）: the spatial arrangement of substituent groups that are free to assume different positions in space without breaking any bonds, because of the freedom of rotation about single bonds**

Henderson–Hasselbalch Equation

$$HA \rightleftarrows H^+ + A^-$$

$$K_a = \frac{[H^+][A^-]}{[HA]}$$

$$pH = pK_a + \log \frac{[A^-]}{[HA]}$$

The equation relates the pH of a solution of a weak acid and its salt to the relative concentrations of the acid and its salt

$pK_a = -\log K_a$ (strong acid → large K_a → small pK_a)

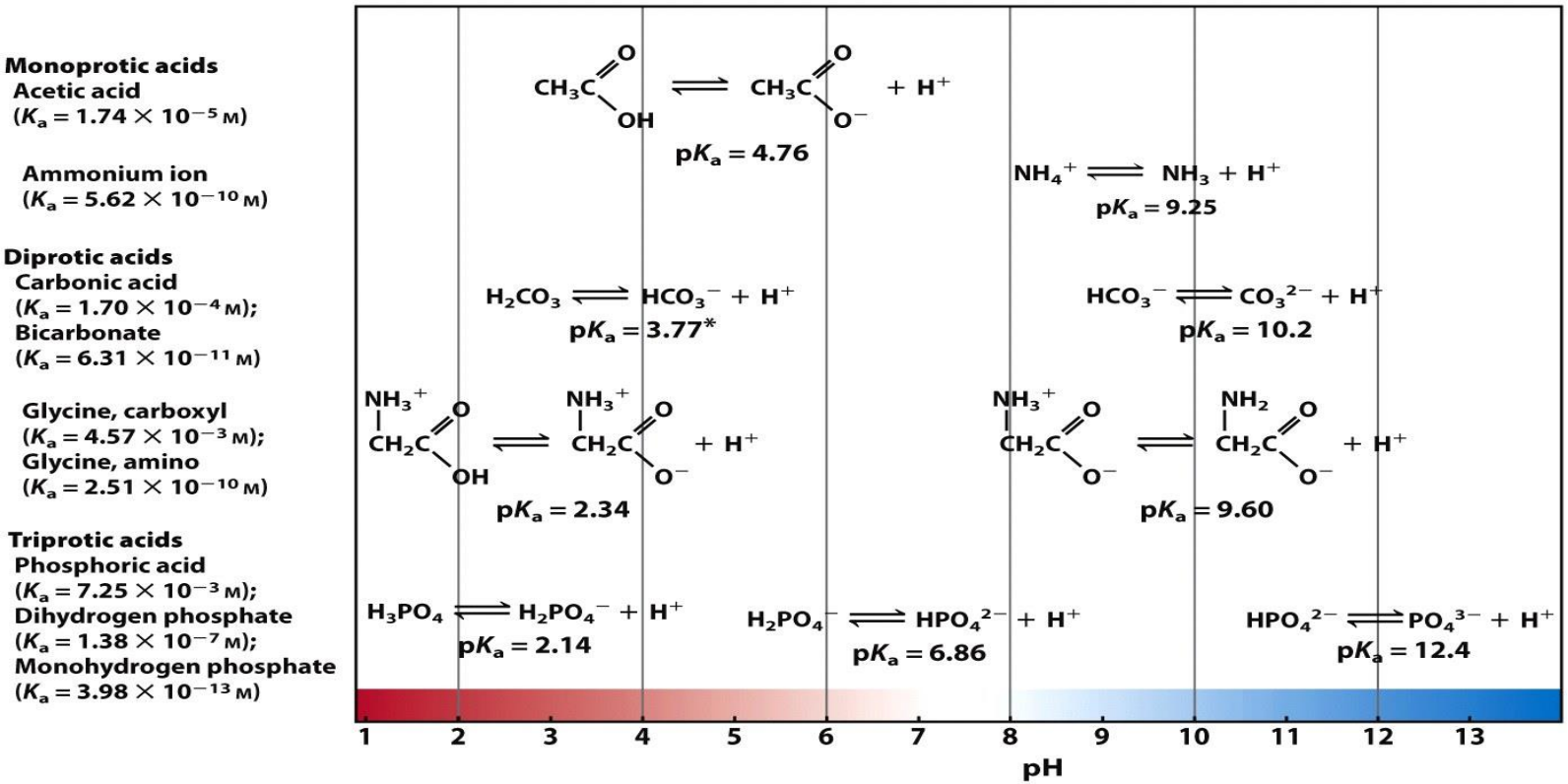

Figure 2-15
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Conjugate acid-base pairs consist of a proton donor and a proton acceptor

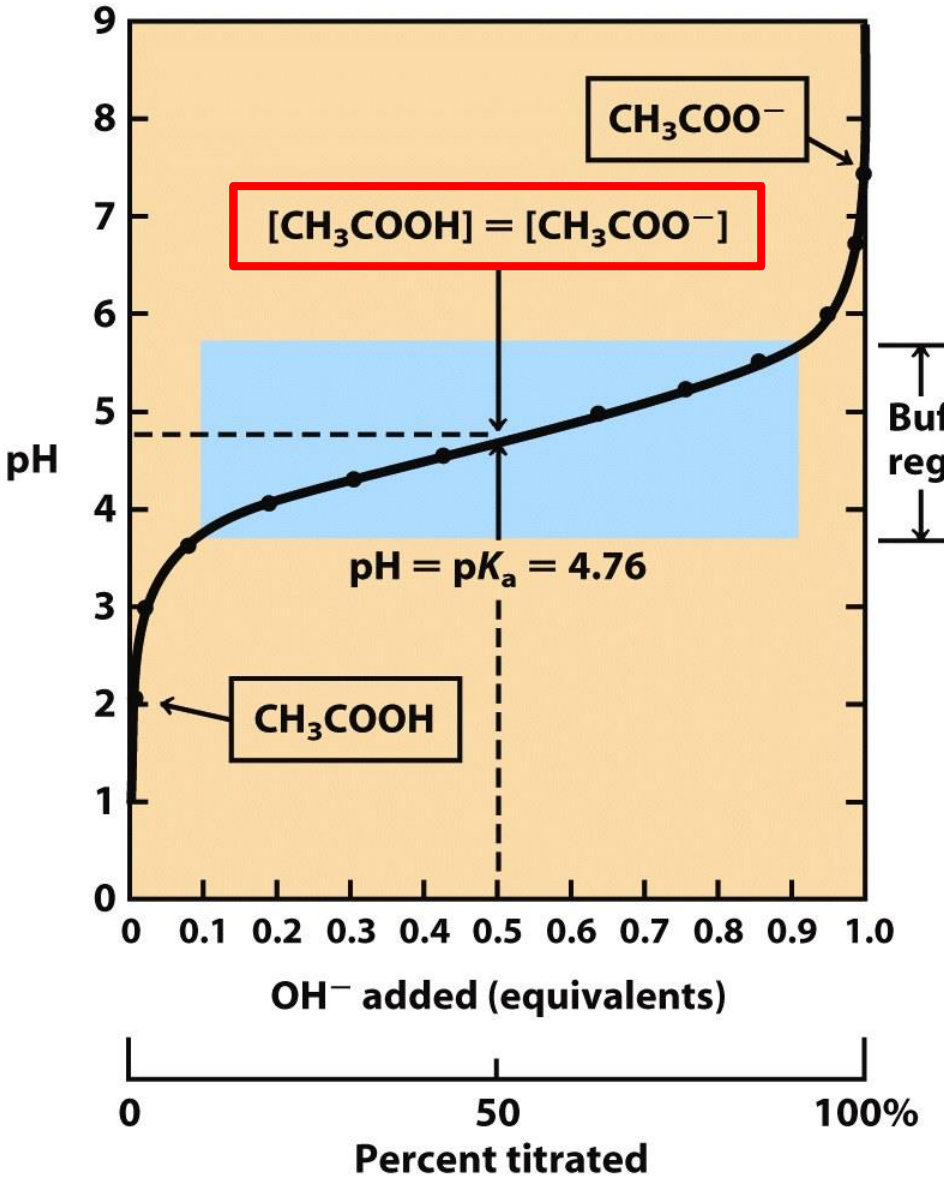

Figure 2-16
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$$HA + NaOH \rightarrow Na^+A^- + H_2O$$

At the midpoint of the titration, the concentrations of the proton donor and proton acceptor are equal, and the pH is numerically equal to the pK_a.

The shaded zone is the useful region of buffering power, generally between 10% and 90% titration of the weak acid.

The titration curve of acetic acid

Biological buffer systems

- **Maintenance of intracellular pH is vital to all cells**
  - **Enzyme-catalyzed reactions have optimal pH**
  - **Solubility of polar molecules depends on H-bond donors and acceptors**
  - **Equilibrium between CO_2 gas and dissolved HCO_3^- depends on pH**

- **Buffer systems *in vivo* are mainly based on**
  - **Phosphate ($H_2PO_4^-$ / HPO_4^{2-}), acts in the cytoplasm**
  - **Bicarbonate (H_2CO_3 /HCO_3^-), important for blood plasma (pH 7.35-7.45)**
  - **histidine, efficient buffer at neutral pH**

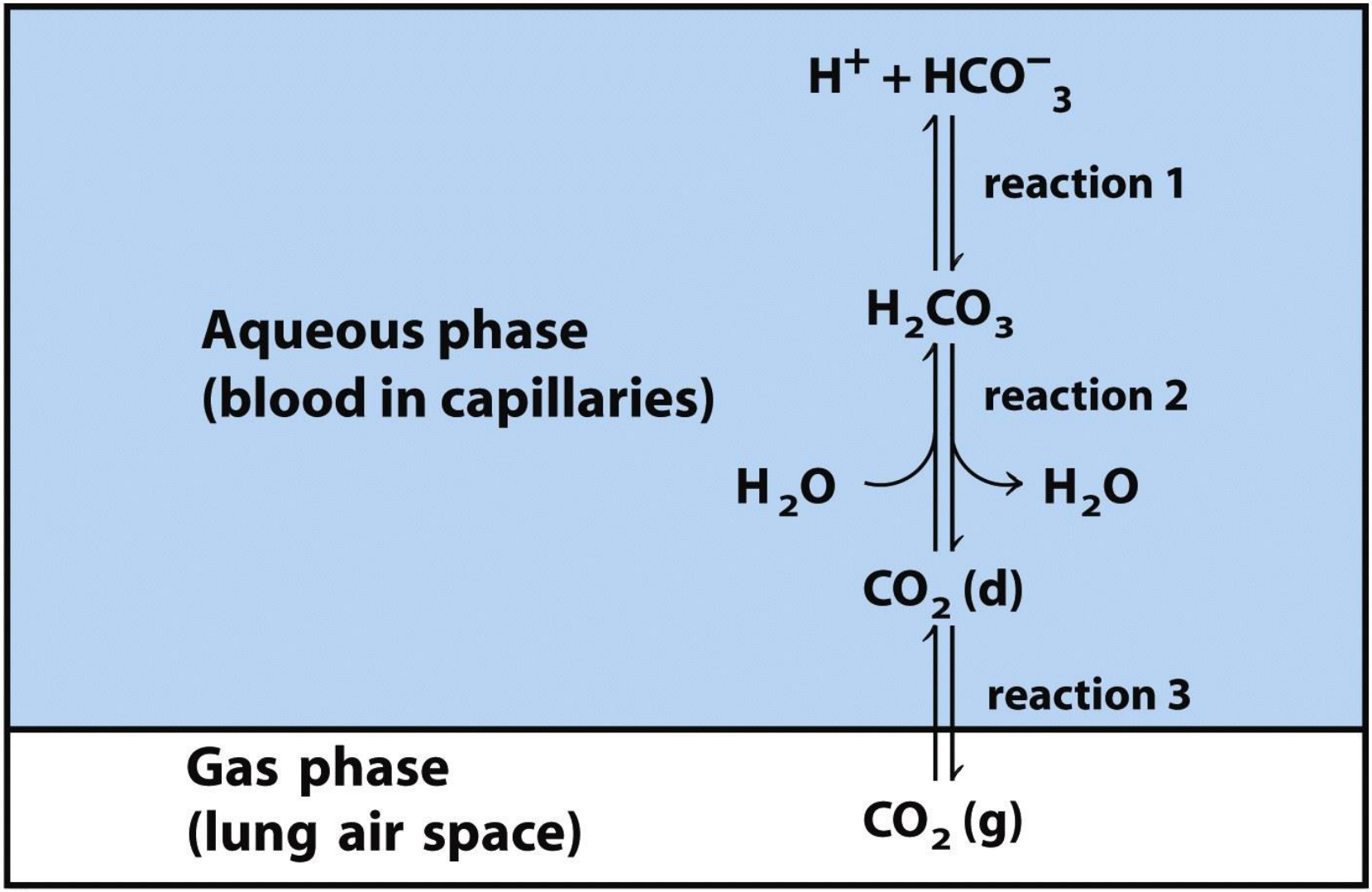

Figure 2-20
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The bicarbonate buffer system

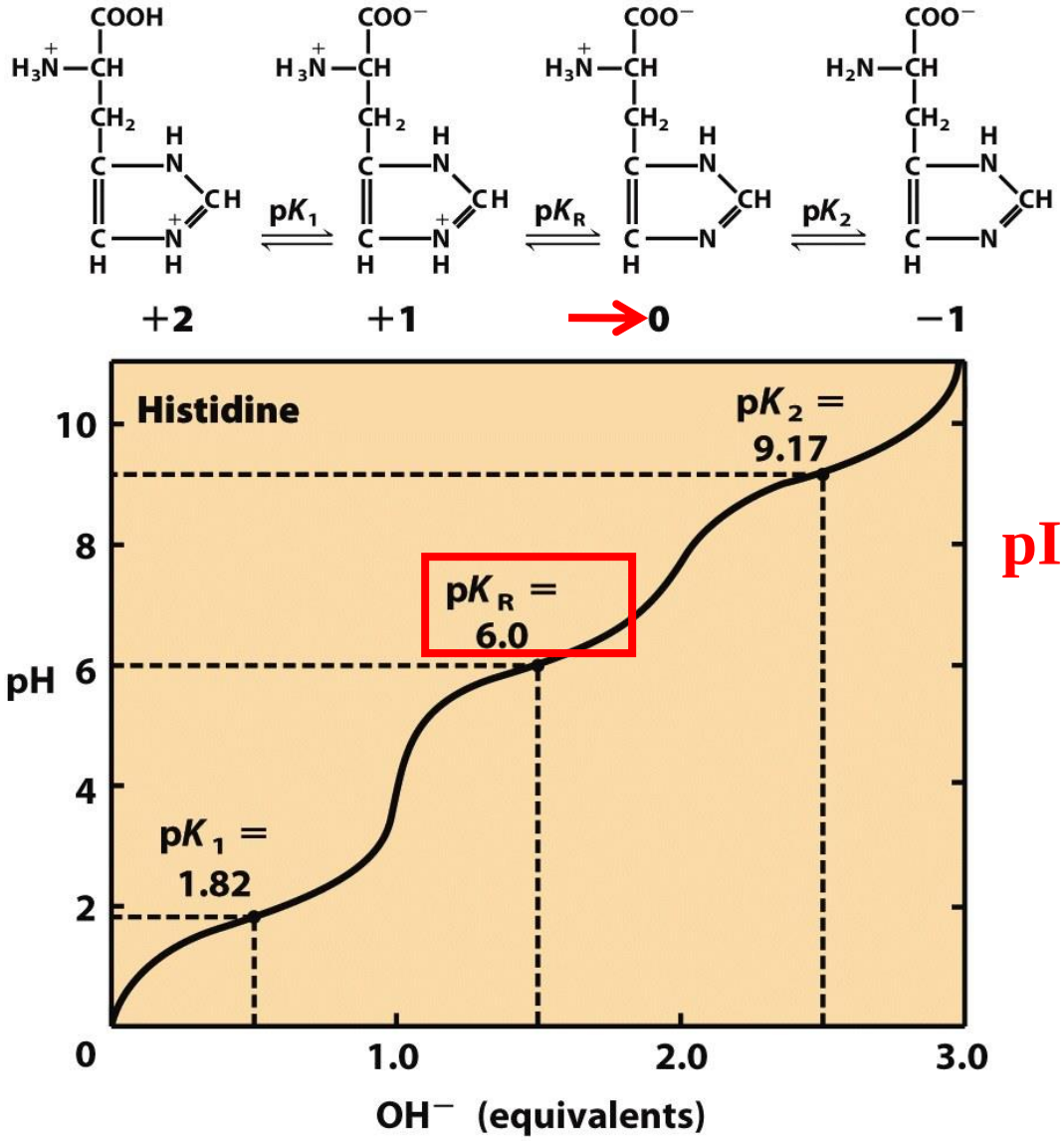
Treatment of acidosis with bicarbonate

$$pH = pK_a + \log([A^-]/[HA])$$
$$= pK_a + \log([HCO_3^-]/[CO_2(d)])$$
$$= 6.1 + \log([HCO_3^-]/0.23 \times pCO_2)$$
$$= 6.1 + \log(24/1.2)$$
$$= 6.1 + 1.3$$
$$= 7.4$$

20

Solubility coefficient for CO_2 in water

4.6-6.7kPa



pI = 7.59

Figure 3-12b
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Titration curve of histidine

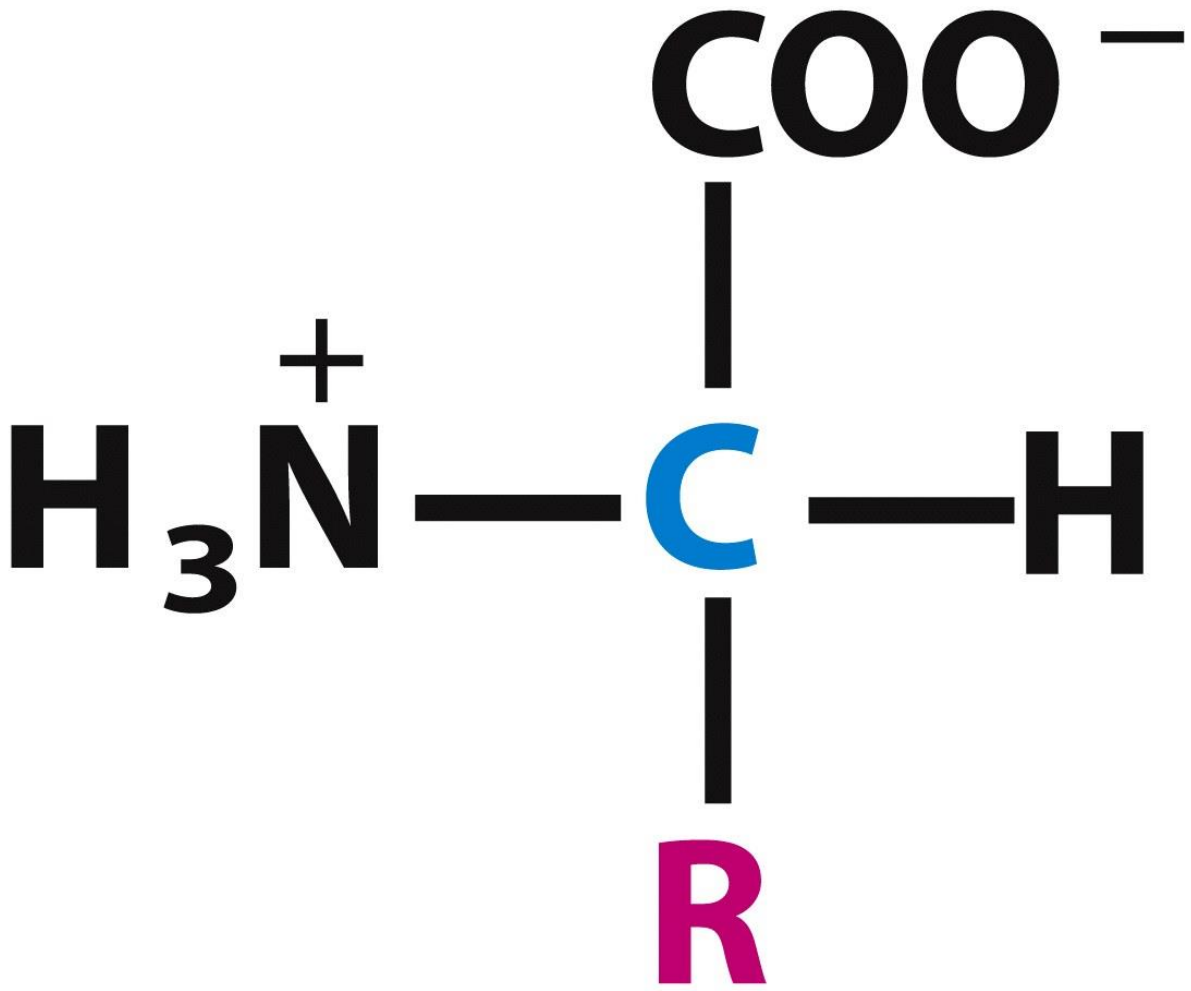

Figure 3-2
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General structure of an amino acid

*****

Groups

TABLE 3–1 Properties and Conventions Associated with the Common Amino Acids Found in Proteins

Amino acid	Abbreviation/ symbol	M_r*	pK_a values: pK_1 (—COOH)	pK_2 ($—NH_3^+$)	pK_R (R group)	pI	Hydropathy index†	Occurrence in proteins (%)‡
Nonpolar, aliphatic R groups								
Glycine	Gly G	75	2.34	9.60		5.97	−0.4	7.2
Alanine	Ala A	89	2.34	9.69		6.01	1.8	7.8
Proline	Pro P	115	1.99	10.96		6.48	1.6	5.2
Valine	Val V	117	2.32	9.62		5.97	4.2	6.6
Leucine	Leu L	131	2.36	9.60		5.98	3.8	9.1
Isoleucine	Ile I	131	2.36	9.68		6.02	4.5	5.3
Methionine	Met M	149	2.28	9.21		5.74	1.9	2.3
Aromatic R groups								
Phenylalanine	Phe F	165	1.83	9.13		5.48	2.8	3.9
Tyrosine	Tyr Y	181	2.20	9.11	10.07	5.66	−1.3	3.2
Tryptophan	Trp W	204	2.38	9.39		5.89	−0.9	1.4
Polar, uncharged R groups								
Serine	Ser S	105	2.21	9.15		5.68	−0.8	6.8
Threonine	Thr T	119	2.11	9.62		5.87	−0.7	5.9
Cysteine§	Cys C	121	1.96	10.28	8.18	5.07	2.5	1.9
Asparagine	Asn N	132	2.02	8.80		5.41	−3.5	4.3
Glutamine	Gln Q	146	2.17	9.13		5.65	−3.5	4.2
Positively charged R groups								
Lysine	Lys K	146	2.18	8.95	10.53	9.74	−3.9	5.9
Histidine	His H	155	1.82	9.17	6.00	7.59	−3.2	2.3
Arginine	Arg R	174	2.17	9.04	12.48	10.76	−4.5	5.1
Negatively charged R groups								
Aspartate	Asp D	133	1.88	9.60	3.65	2.77	−3.5	5.3
Glutamate	Glu E	147	2.19	9.67	4.25	3.22	−3.5	6.3

*M_r values reflect the structures as shown in Figure 3–5. The elements of water (M_r 18) are deleted when the amino acid is incorporated into a polypeptide.

†A scale combining hydrophobicity and hydrophilicity of R groups. The values reflect the free energy (ΔG) of transfer of the amino acid side chain from a hydrophobic solvent to water. This transfer is favorable ($\Delta G < 0$; negative value in the index) for charged or polar amino acid side chains, and unfavorable ($\Delta G > 0$; positive value in the index) for amino acids with nonpolar or more hydrophobic side chains. See Chapter 11. From Kyte, J. & Doolittle, R.F. (1982) A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol.* **157**, 105–132.

‡Average occurrence in more than 1,150 proteins. From Doolittle, R.F. (1989) Redundancies in protein sequences. In *Prediction of Protein Structure and the Principles of Protein Conformation* (Fasman, G.D., ed.), pp. 599–623, Plenum Press, New York.

§Cysteine is generally classified as polar despite having a positive hydropathy index. This reflects the ability of the sulfhydryl group to act as a weak acid and to form a weak hydrogen bond with oxygen or nitrogen.

Table 3-1

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Amino acids carry a net charge of zero at a specific pH

- **Zwitterions predominate at pH values between the p*K*a values of the amino and the carboxyl group**
- **For amino acid without ionizable side chains, the Isoelectric Point (pI, Isoelectric pH) is**

$$pI = \frac{pK_1 + pK_2}{2}$$

- **At this point, the net charge is zero**
- **Amino acid is least soluble in water**
- **Amino acid does not migrate in electric field**

Major methods in purifying a protein

Method	Principle
Salting out	Solubility
Dialysis	Size
Size-exclusion chromatography	Size
Ion-exchange chromatography	Charge
Affinity chromatography	Binding affinity

TABLE 3–7 The Specificity of Some Common Methods for Fragmenting Polypeptide Chains

*****

Reagent (biological source)*	Cleavage points†
Trypsin (bovine pancreas)	Lys, Arg (C)
Submaxillarus protease (mouse submaxillary gland)	Arg (C)
Chymotrypsin (bovine pancreas)	Phe, Trp, Tyr (C)
Staphylococcus aureus V8 protease (bacterium *S. aureus*)	Asp, Glu (C)
Asp-*N*-protease (bacterium *Pseudomonas fragi*)	Asp, Glu (N)
Pepsin (porcine stomach)	Leu, Phe, Trp, Tyr (N)
Endoproteinase Lys C (bacterium *Lysobacter enzymogenes*)	Lys (C)
Cyanogen bromide	Met (C)

*All reagents except cyanogen bromide are proteases. All are available from commercial sources.

†Residues furnishing the primary recognition point for the protease or reagent; peptide bond cleavage occurs on either the carbonyl (C) or the amino (N) side of the indicated amino acid residues.

Table 3-7

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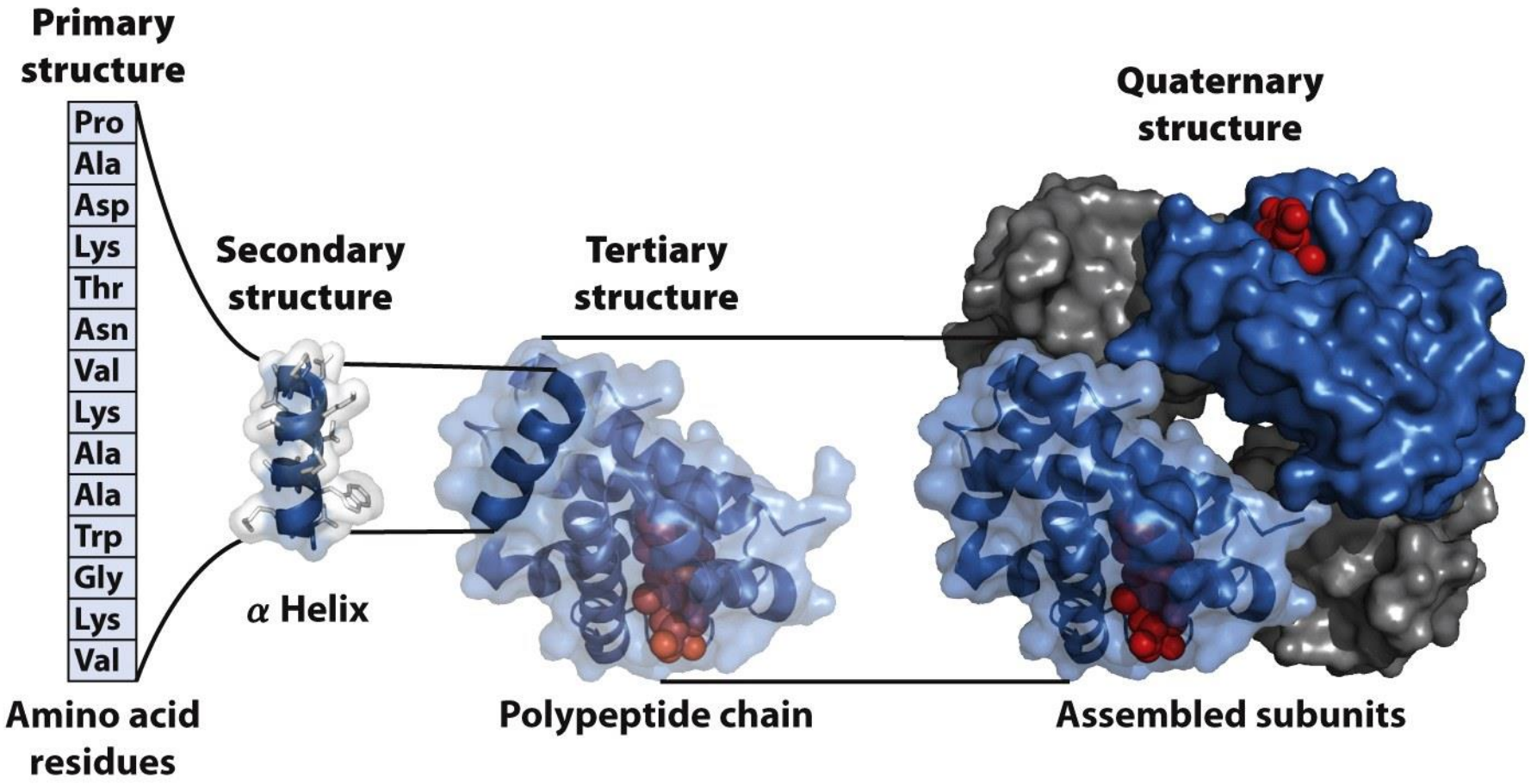

Figure 3-23
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Levels of structure in proteins

Protein structure

- **Primary structure: the amino acid sequence**
- **Secondary structure: the spatial arrangement of amino acid residues that are nearby in the sequence (α helix, β sheet)**
- **Tertiary structure: the spatial arrangement of amino acid residues that are far apart in the sequence; the pattern of disulfide bonds**
- **Quaternary structure: the spatial arrangement of subunits and the nature of their interactions**

Favorable interactions in proteins

- **Hydrophobic effect**
  - **Association or folding of nonpolar groups with each other in the aqueous system**
- **Hydrogen bonds**
  - **Interaction of N-H and C=O of the peptide bond leads to local regular structures such as α-helixes and β-sheets**
- **Van der Waals interactions**
  - **Medium-range weak attraction between all atoms contributes significantly to the stability in the interior of the protein**
- **Electrostatic interactions**
  - **Long-range strong interactions between permanently charged groups**

Common hydrogen bonds in biological systems

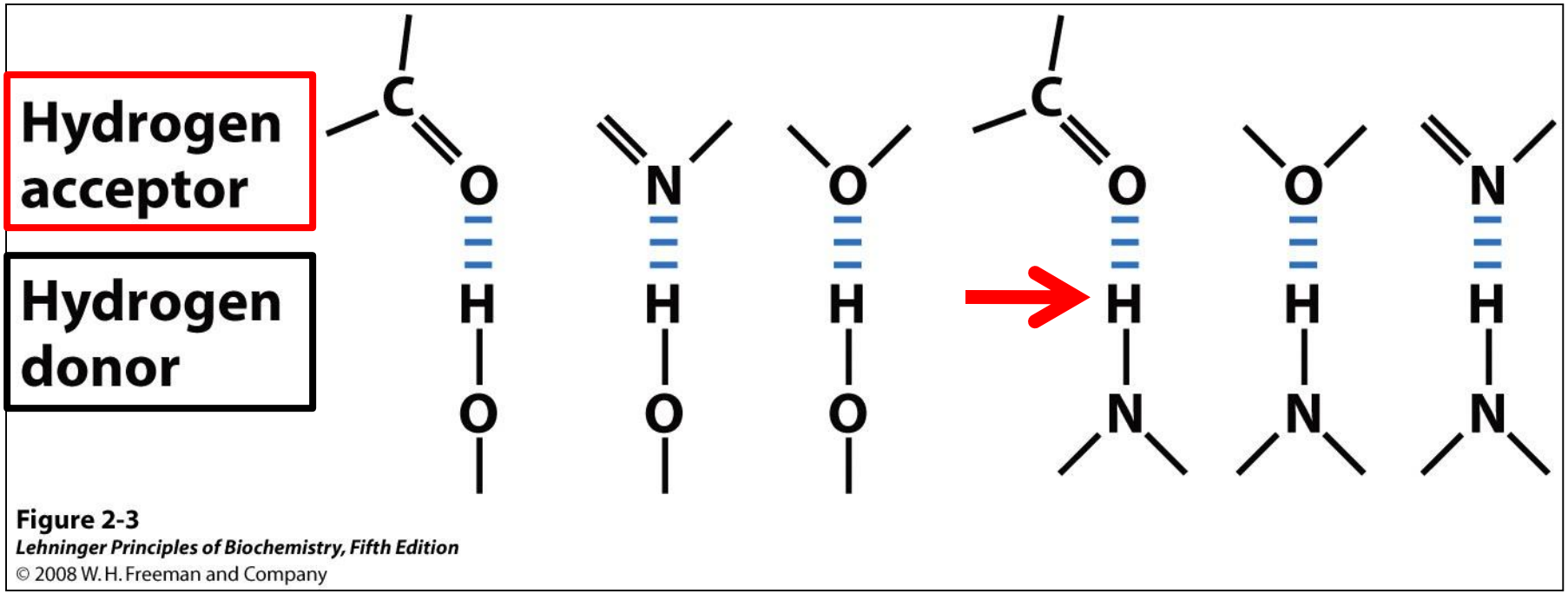

Figure 2-3
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The hydrogen acceptor is usually oxygen or nitrogen; the hydrogen donor is another electronegative atom.

Noncovalent interactions

- **Much weaker than covalent bonds**
- **Reversible --- continually forming and breaking**
- **Weak individually, but strong cumulatively**
- **Weak interactions are crucial for structure and function of DNA, RNA and proteins**
- **The most stable macromolecular conformations are those in which hydrogen bonding is maximized within the molecule and between the molecule and the solvent, and in which hydrophobic moieties cluster in the interior of the molecule away from the aqueous solvent.**

C=N double bond:1.27Å

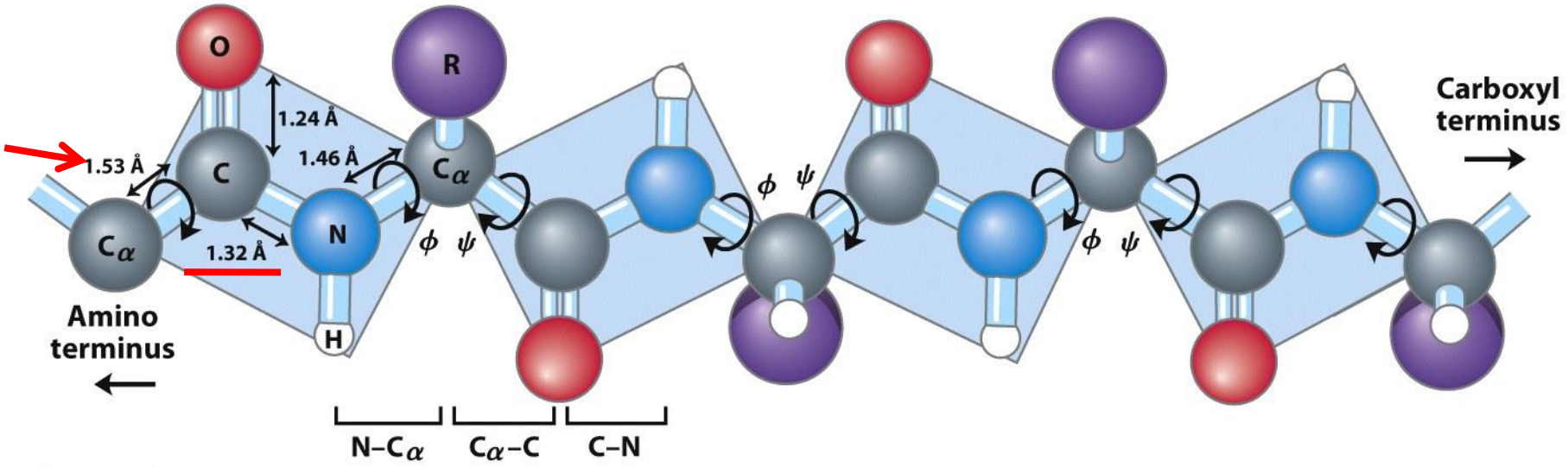

Figure 4-2b
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The planar peptide groups

Three bonds separate sequential α carbons in a polypeptide chain. The N—C_α and C_α—C bonds, designated ϕ and ψ, respectively, can rotate. The peptide C—N bond is not free to rotate. Other single bonds in the backbone may also be rotationally hindered, depending on the size and charge of the R groups.

The α helix

- **The backbone is more compact with the ψ dihedral (N–C_α—C–N) in the range (0° < ψ < –70°)**
- **Helical backbone is held together by hydrogen bonds between the nearby backbone amides**
- **Right-handed helix with 3.6 residues (5.4 Å) per turn**
- **Stabilized by the hydrogen bond between the CO and NH groups of the main chain**
- **Peptide bonds are aligned roughly parallel with the helical axis**
- **Side chains point out and are roughly perpendicular with the helical axis**

β Sheet

- **The backbone is more extended with the ψ dihedral (N–C_α—C–N) in the range (90° < ψ < 180°)**
- **The planarity of the peptide bond and tetrahedral geometry of the α-carbon create a pleated sheet-like structure**
- **Sheet-like arrangement of backbone is held together by hydrogen bonds between the more distal backbone amides**
- **Side chains protrude from the sheet alternating in up and down direction**

Protein denaturation and folding

- **A protein's function depends on its three-dimensional structure**
- **Loss of structural integrity with accompanying loss of activity is called denaturation**
- **Proteins can be denatured by**
  - **heat**
  - **pH extremes**
  - **organic solvents: alcohol, acetone**
  - **denaturing reagents: urea, guanidine hydrochloride**

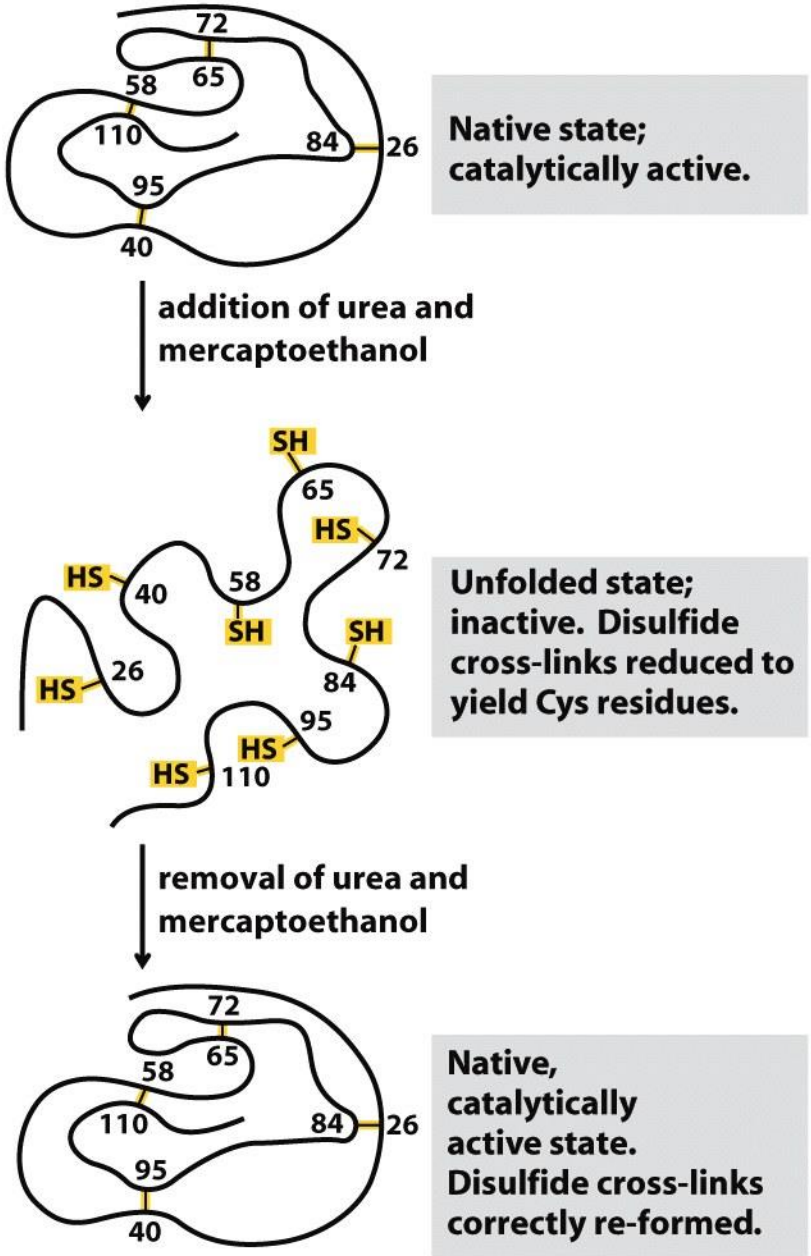

Figure 4-26
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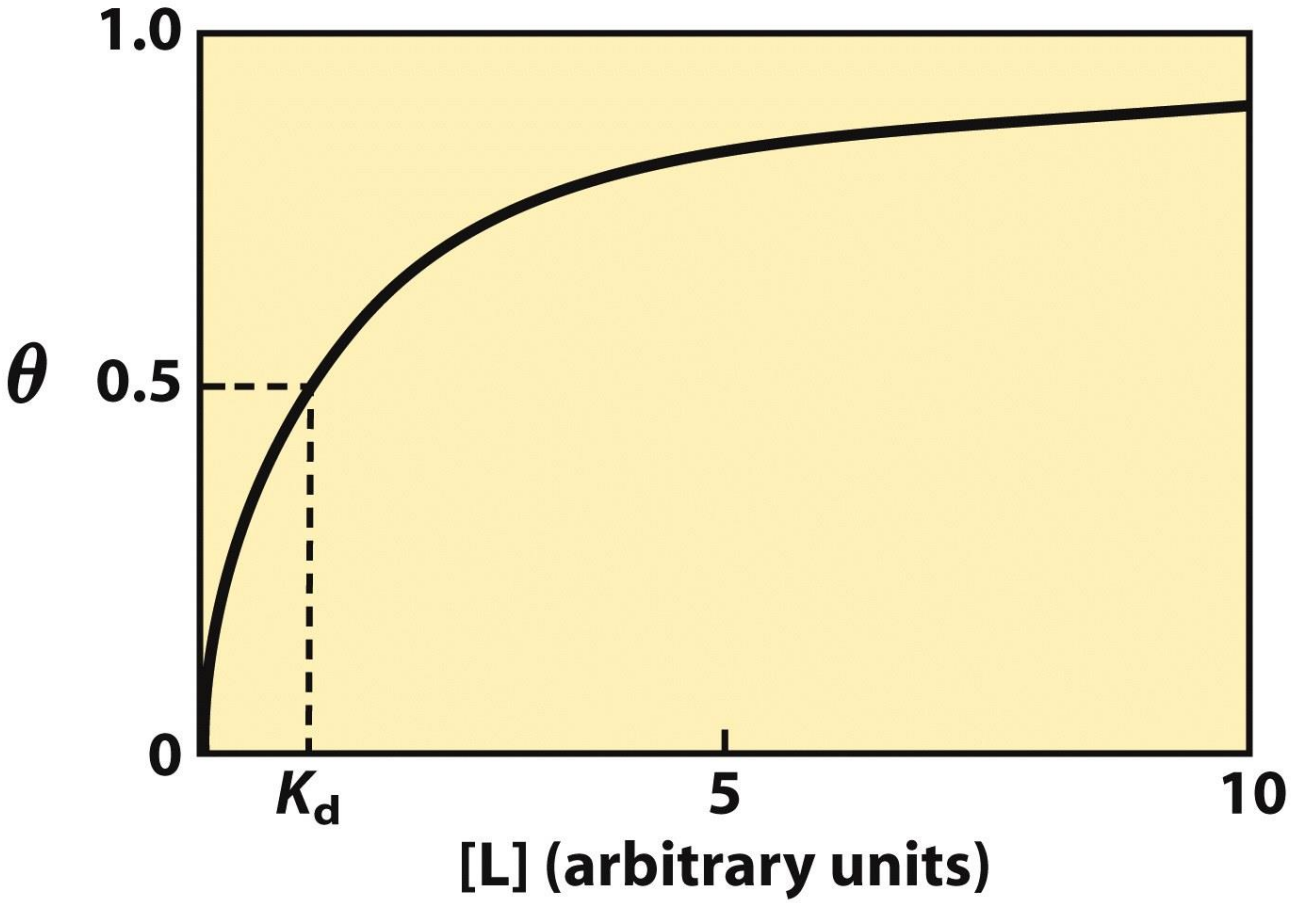
Renaturation of unfolded, denatured ribonuclease

The [L] at which half of the available ligand-binding sites are occupied is equivalent to $1/K_a$, or K_d



$$\theta = \frac{[L]}{[L] + K_d}$$

Figure 5-4a
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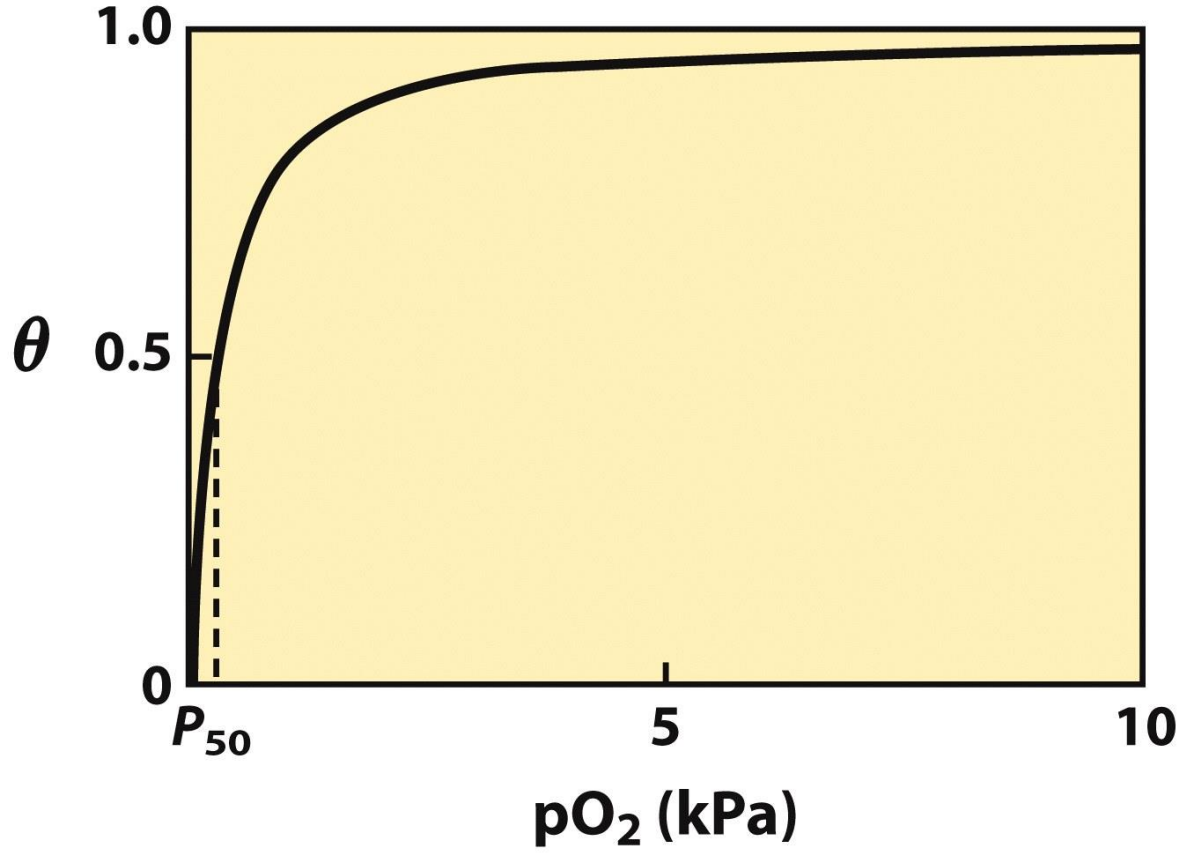
Graphical representations of ligand binding



$$\theta = \frac{pO_2}{pO_2 + P_{50}}$$

Figure 5-4b
Lehninger Principles of Biochemistry, Fifth Edition

Binding of oxygen to myoglobin

The partial pressure of O_2 in the air above the solution is expressed in kilopascals (kPa). Oxygen binds tightly to myoglobin, with a P_{50} of only 0.26 kPa.

The fraction of binding sites occupied by O_2 is a hyperbolic function of pO_2.

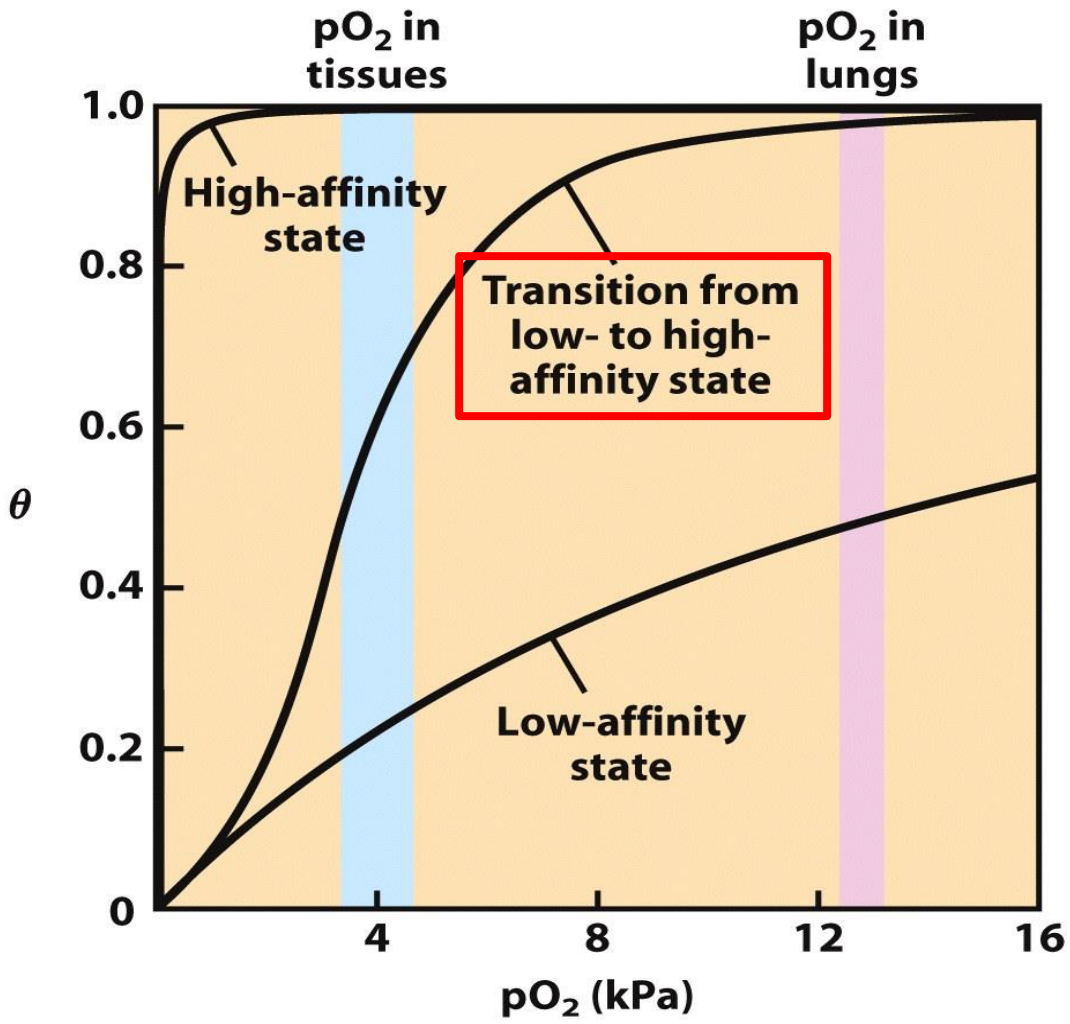


Figure 5-12
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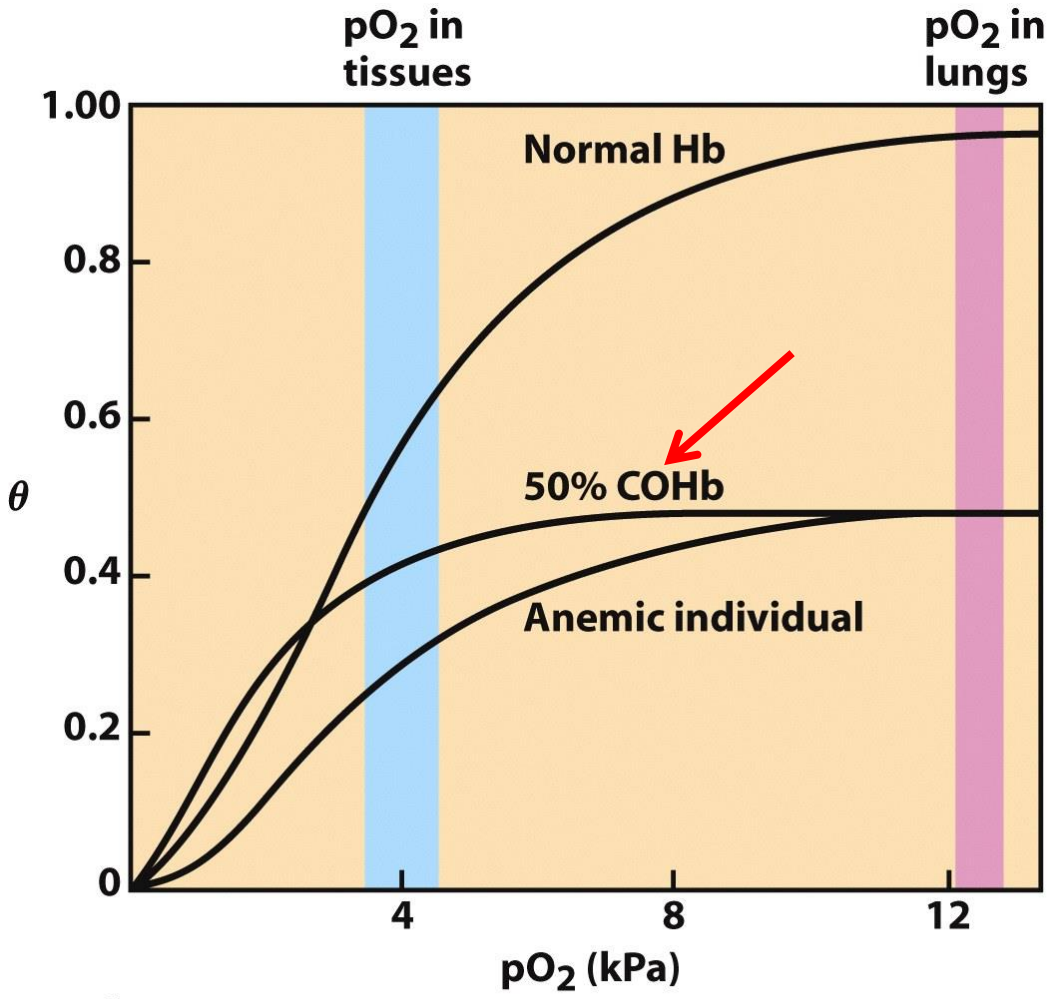
A sigmoid (cooperative) binding curve

A sigmoid binding curve can be viewed as a hybrid curve reflecting a transition from a low-affinity to a high-affinity state. Because of its cooperative binding, hemoglobin is more sensitive to the small differences in O_2 concentration between the tissues and the lungs, allowing it to bind oxygen in the lungs (where pO_2 is high) and release it in the tissues (where pO_2 is low).



Box 5-1 figure 2
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Oxygen-binding curves for normal hemoglobin, hemoglobin from an anemic individual with only 50% of her hemoglobin functional, and hemoglobin from an individual with 50% of his hemoglobin subunits complexed with CO

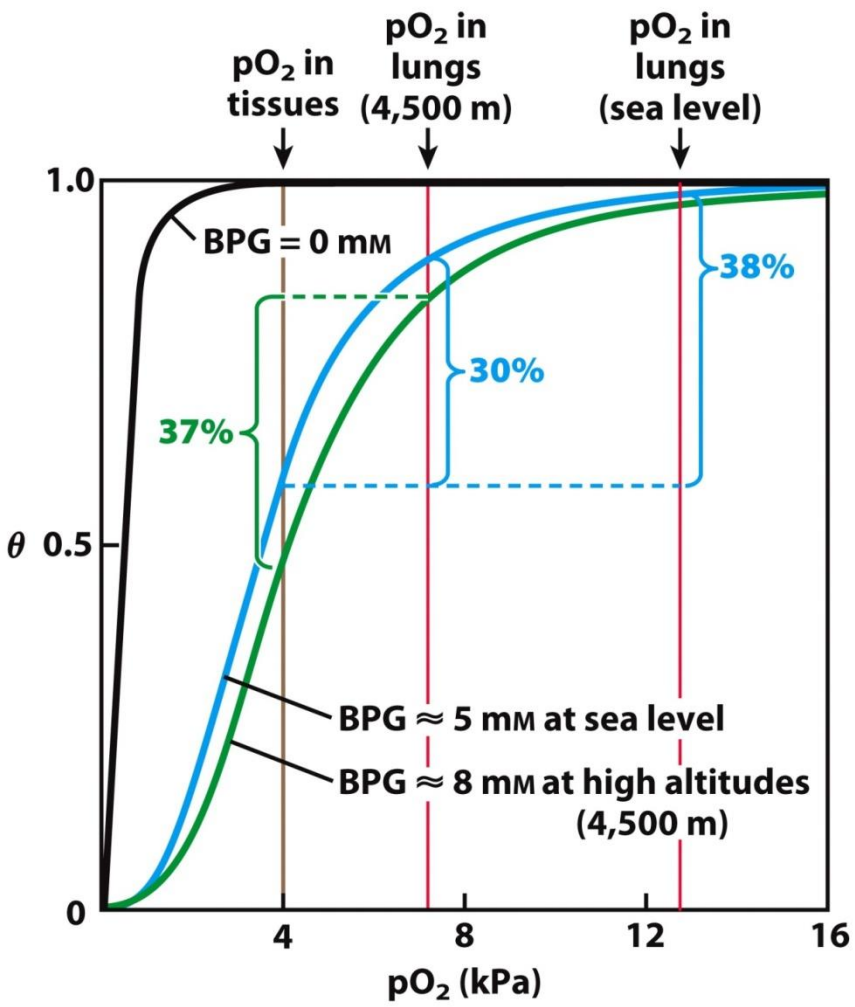

Figure 5-17
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2,3-bisphosphoglycerate (BPG) binds in the central cavity between the four subunits by interactions between negatively charged groups on BPG and positively charged amino acid residues that line the central cavity. and stabilizes the deoxy form of hemoglobin.

Effect of BPG on oxygen binding to hemoglobin

The BPG concentration in normal human blood is about 5 mM at sea level and about 8 mM at high altitudes. At sea level, hemoglobin is nearly saturated with O_2 in the lungs, but just over 60% saturated in the tissues. At high altitudes, O_2 delivery declines by about one-fourth. An increase in BPG concentration decreases the affinity of hemoglobin for O_2, so approximately 37% of what can be carried is again delivered to the tissues.

Effects of ligand-binding on the affinity of O_2 to hemoglobin

Ligand	Binding site	Effect on affinity
CO	Same as O_2	increase
CO_2	N-terminal end of hemoglobin	decrease
H^+	His HC3 and other amino acids	decrease
BPG	Central cavity	decrease

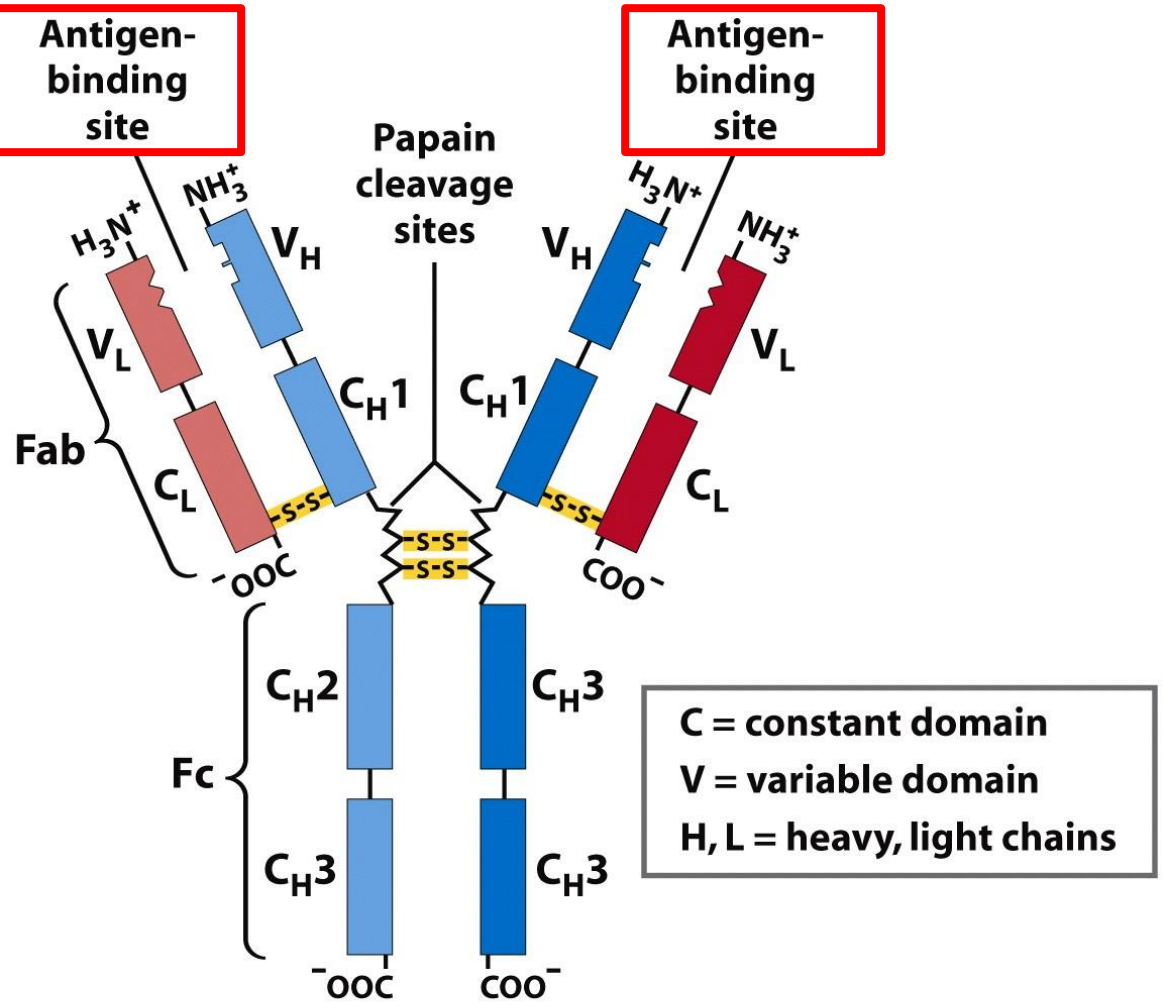

Figure 5-21a
Lehninger Principles of Biochemistry, Fifth Edition

Structure of immunoglobulin G

Pairs of heavy and light chains combine to form a Y-shaped molecule. Two antigen-binding sites are formed by the combination of variable domains from one light (V_L) and one heavy (V_H) chain. Cleavage with papain separates the Fab and Fc portions of the protein in the hinge region. The Fc portion of the molecule also contains bound carbohydrate.

Characteristics of enzymes

- **Higher reaction rates**
- **Greater reaction specificity**
- **Milder reaction conditions**
- **Capacity for regulation**
- **Enzymes affect the rate of a reaction, not equilibrium**
- **Enzymes lower the activation energy**
- **Enzymes use binding energy to lower the activation energy**
- **Enzymes are not used up in the reaction**

2. Free energy is a useful thermodynamic function for understanding enzymes

- **The free energy difference between the products and reactants. (It determines whether the reaction will be spontaneous.)**
- **The energy required to initiate the conversion of reactants to products. (It determines the rate of the reaction.)**

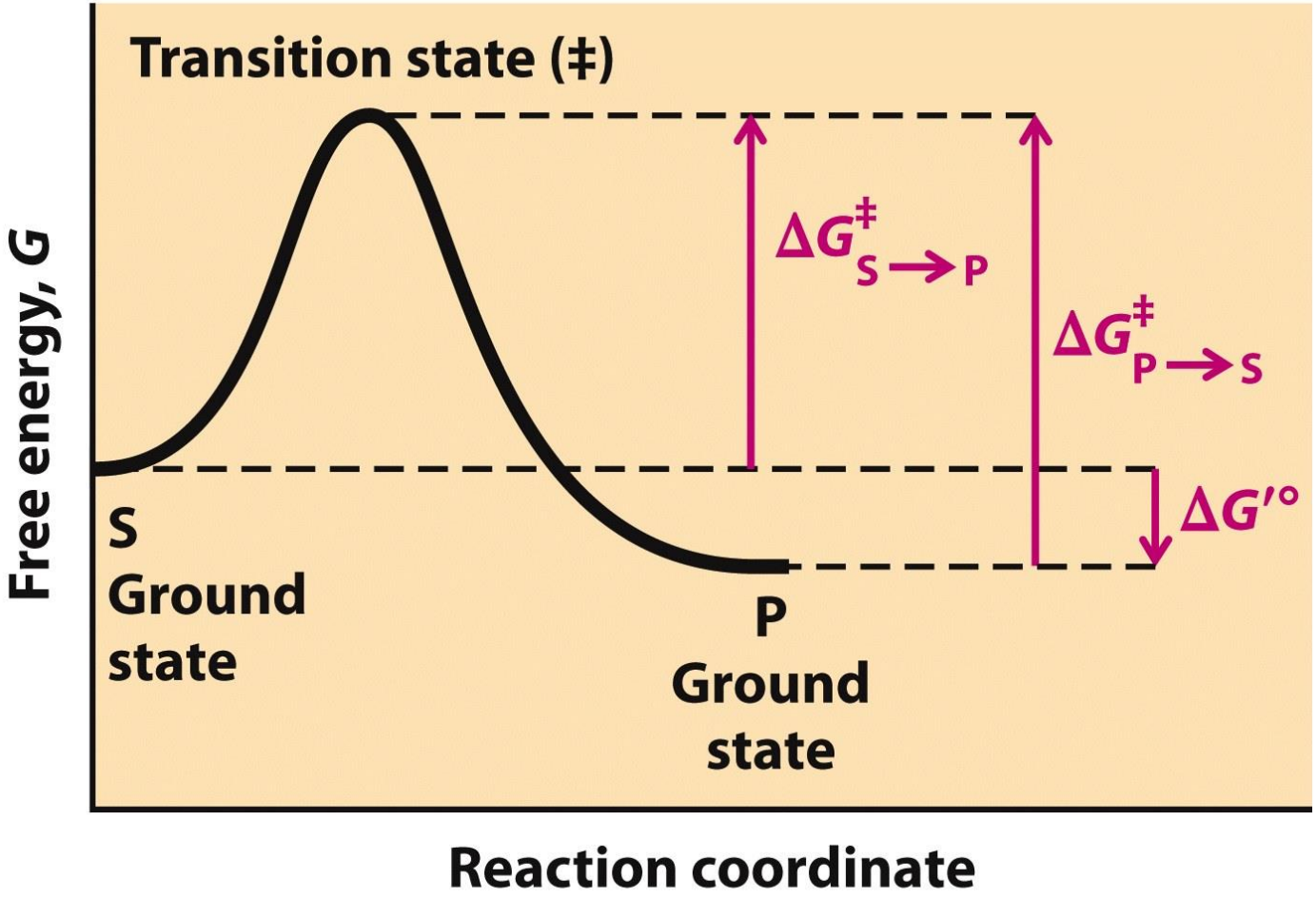

Figure 6-2
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The free energy of the system is plotted against the progress of the reaction S → P
A diagram of this kind is a description of the energy changes during the reaction, and the horizontal axis (reaction coordinate) reflects the progressive chemical changes (e.g., bond breakage or formation) as S is converted to P.
The activation energies, ΔG°, for the S → P and P → S reactions are indicated.
$\Delta G'^{\circ}$ is the overall standard free-energy change in the direction S → P.

The Free-Energy Change Provides Information About the Spontaneity but Not the Rate of a Reaction

ΔG = G (products) – G (substrates)

- ΔG depends only on the free energy of the products and the free energy of the reactants
- ΔG is independent of the molecular mechanism of the transformation
- ΔG provides no information about the rate of a reaction

$\Delta G < 0$ --- the reaction can occur spontaneously
$\Delta G = 0$ --- the reaction is in equilibrium
$\Delta G > 0$ --- the reaction cannot occur spontaneously

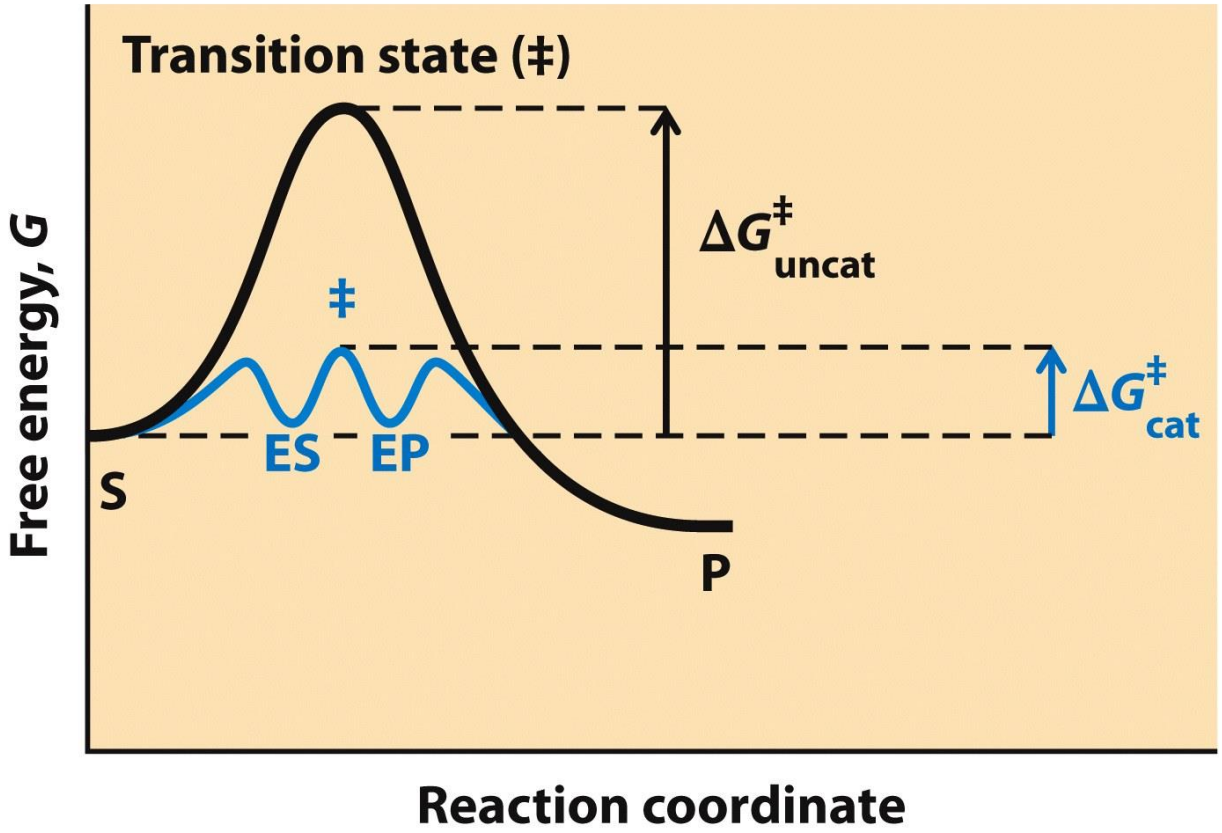
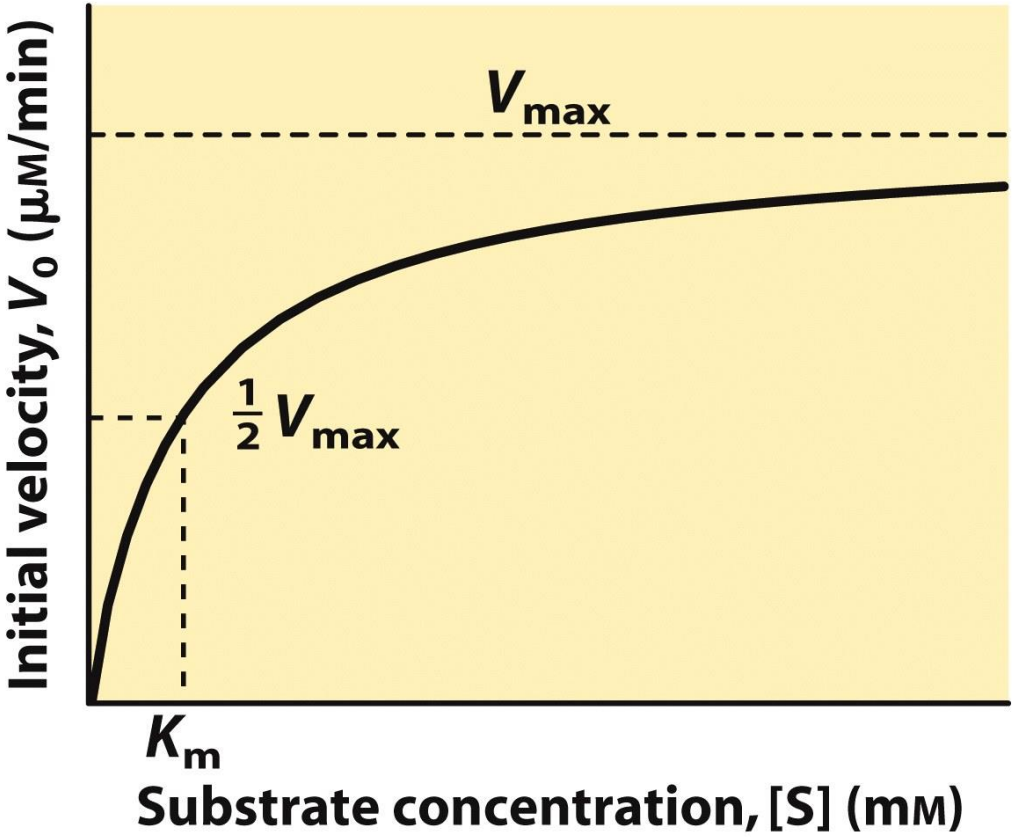


Figure 6-3
Lehninger Principles of Biochemistry, Fifth Edition

Reaction coordinate diagram comparing enzyme-catalyzed and uncatalyzed reactions

The terms $\Delta G^{\ddagger}_{uncat}$ and $\Delta G^{\ddagger}_{cat}$ correspond to the activation energy for the uncatalyzed reaction and the overall activation energy for the catalyzed reaction.

The activation energy is lower when the enzyme catalyzes the reaction.



Michaelis-Menten equation

$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

Figure 6-11
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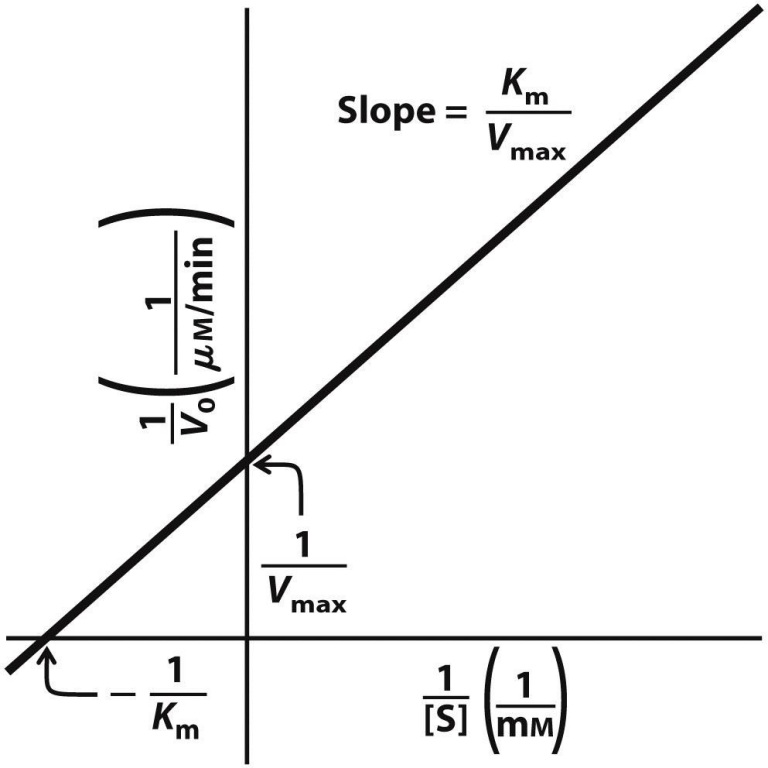
Effect of substrate concentration on the initial velocity of an enzyme-catalyzed reaction

The Michaelis constant (K_m) is the substrate concentration yielding a velocity of $V_{max}/2$.

Determine K_m and V_{max} by double-reciprocal plots

Lineweaver-Burk equation

$$V_0 = \frac{V_{max}[S]}{K_m + [S]} \longrightarrow \frac{1}{V_0} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$$



Box 6-1 figure 1
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Double-reciprocal plot of enzyme kinetics is generated by plotting $1/V_0$ as a function 1/[S]. The slope is the K_m/V_{max}, the intercept on the vertical axis is $1/V_{max}$, and the intercept on the horizontal axis is $-1/K_m$.

Determination of Kinetic Parameters

Nonlinear Michaelis-Menten plot should be used to calculate parameters K_m and V_{max}

$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

Linearized double-reciprocal plot is good for analysis of two-substrate data or inhibition

$$\frac{1}{V_0} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$$

Competitive inhibition

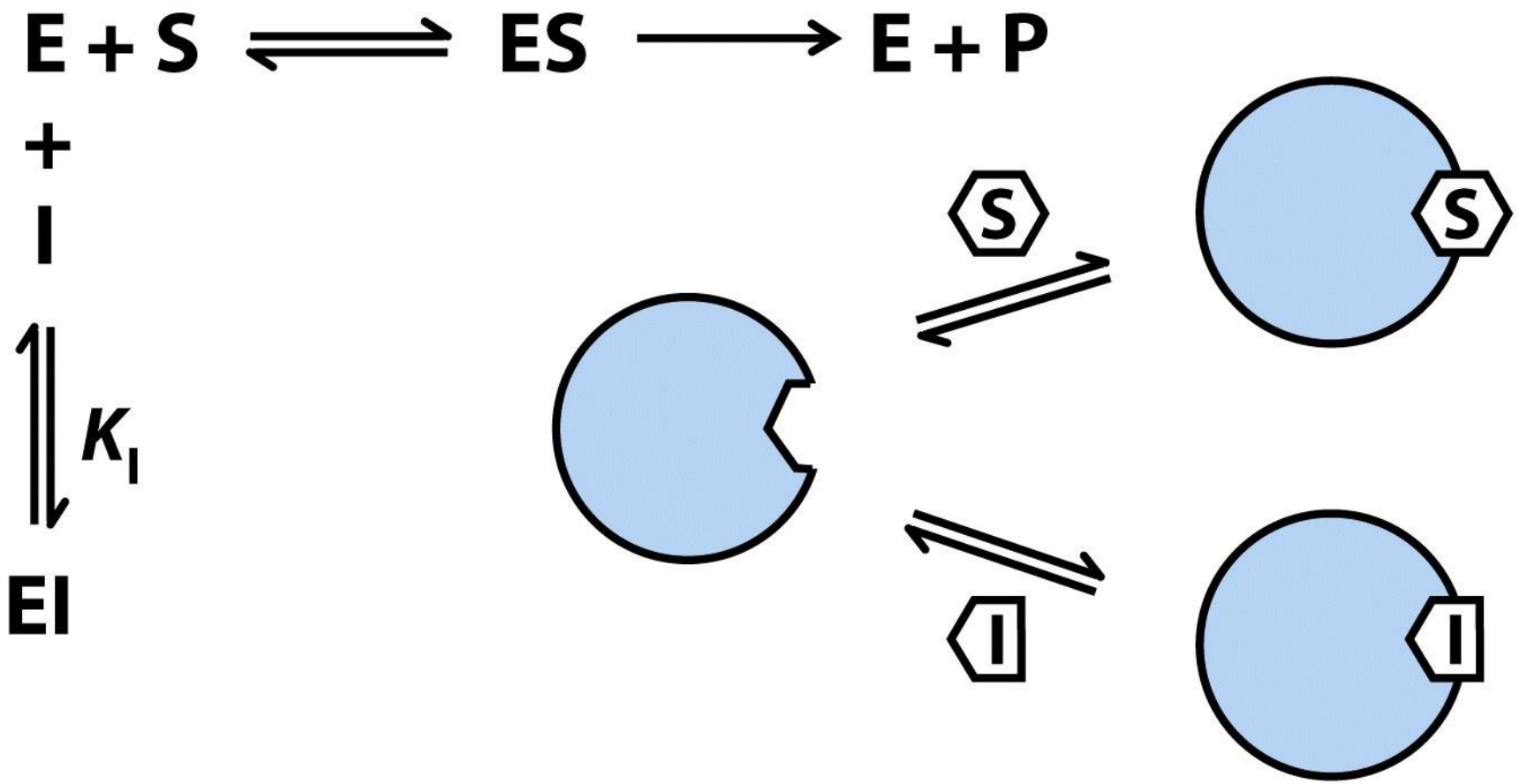
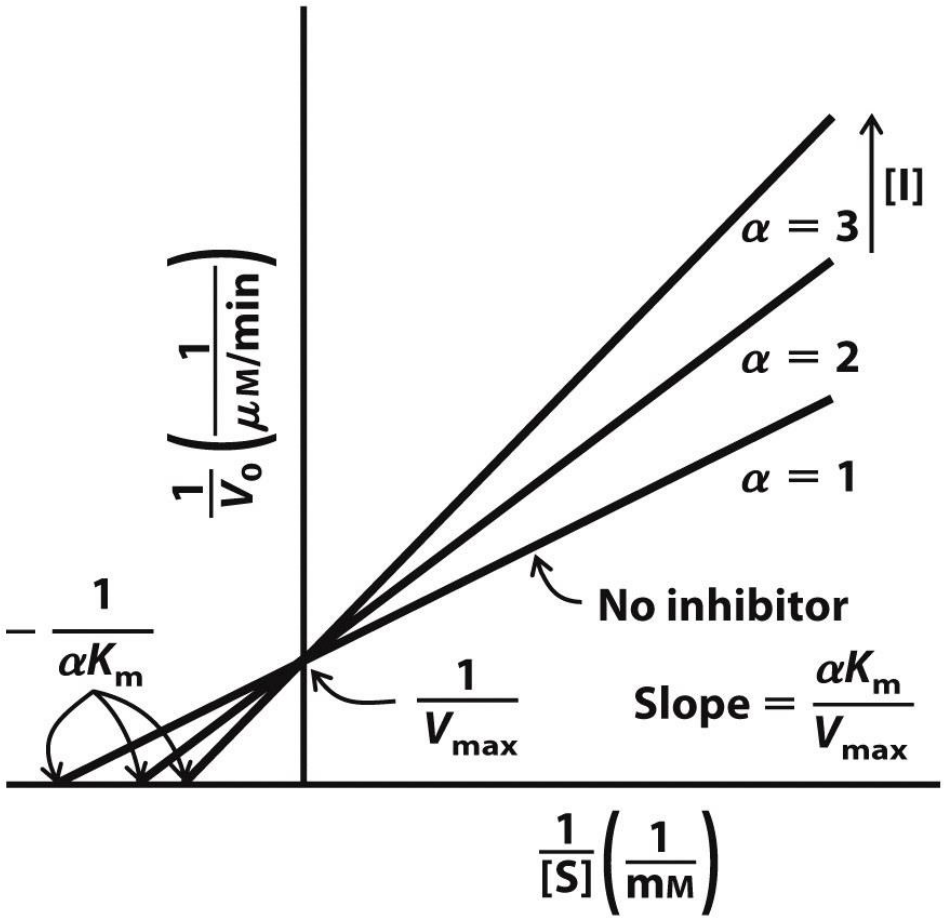


Figure 6-15a
Lehninger Principles of Biochemistry, Fifth Edition

$$\frac{1}{V_0} = \left(\frac{\alpha K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$

$$V_0 = \frac{V_{max}\,[S]}{\alpha K_m + [S]}$$



Box 6-2 figure 1
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The term α is greater than 1.0 when a competitive inhibitor is present

Competitive inhibitors bind to the enzyme's active site; K_I is the equilibrium constant for inhibitor binding to E.

Lines intersect at the y-axis

*****

TABLE 6–9 Effects of Reversible Inhibitors on Apparent V_{max} and Apparent K_m

Inhibitor type	Apparent V_{max}	Apparent K_m
None	V_{max}	K_m
Competitive	V_{max}	αK_m ↑
Uncompetitive	V_{max}/α' ↓	K_m/α' ↓
Mixed	V_{max}/α' ↓	$\alpha K_m/\alpha'$

Table 6-9
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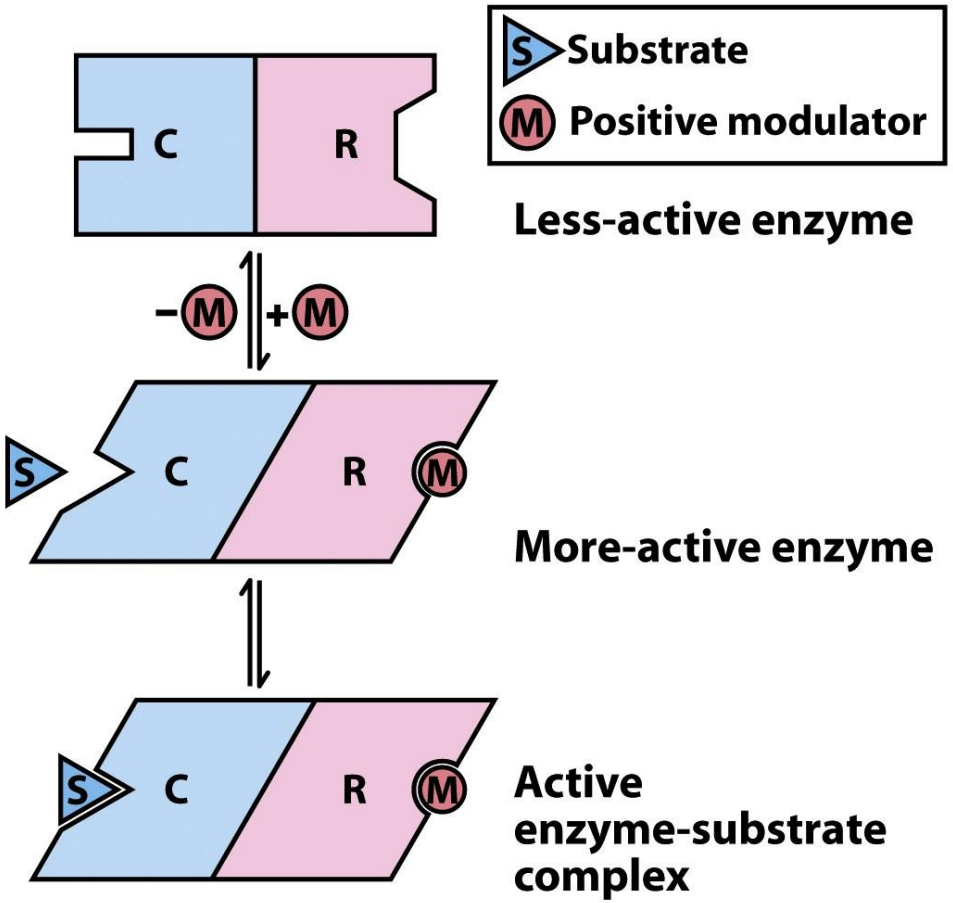

Figure 6-31
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Subunit interactions in an allosteric enzyme, and interactions with inhibitors and activators.

In many allosteric enzymes the substrate binding site and the modulator binding site(s) are on different subunits, the catalytic (C) and regulatory (R) subunits, respectively. Binding of the positive (stimulatory) modulator (M) to its specific site on the regulatory subunit is communicated to the catalytic subunit through a conformational change. This change renders the catalytic subunit active and capable of binding the substrate (S) with higher affinity. On dissociation of the modulator from the regulatory subunit, the enzyme reverts to its inactive or less active form.

Allosteric enzymes do not obey Michaelis-Menten kinetics

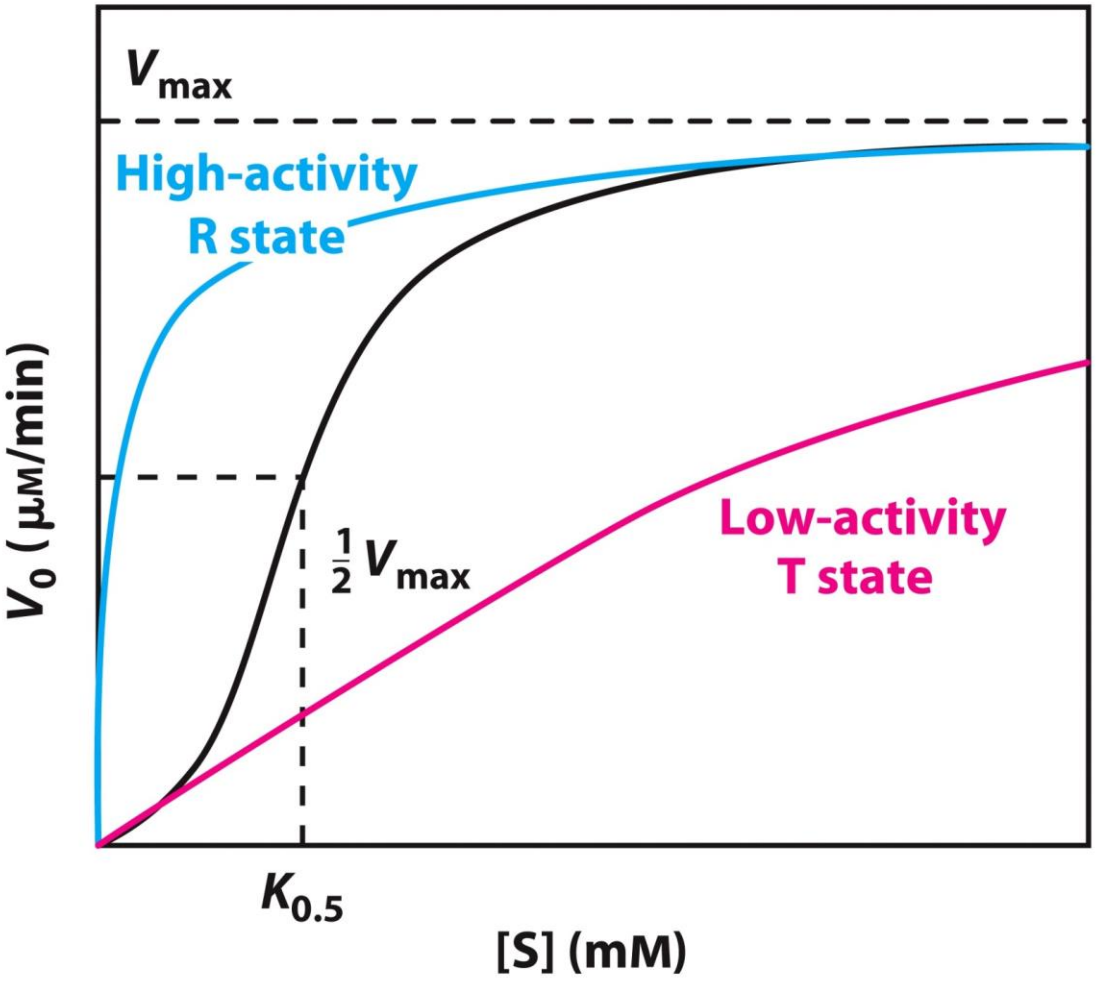

Figure 6-34a
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A homotropic enzyme: the substrate also serves as a positive (stimulatory) modulator, or activator

Substrate-activity curves for representative allosteric enzymes

Allosteric enzymes display a sigmoidal dependence of reaction velocity on substrate concentration.

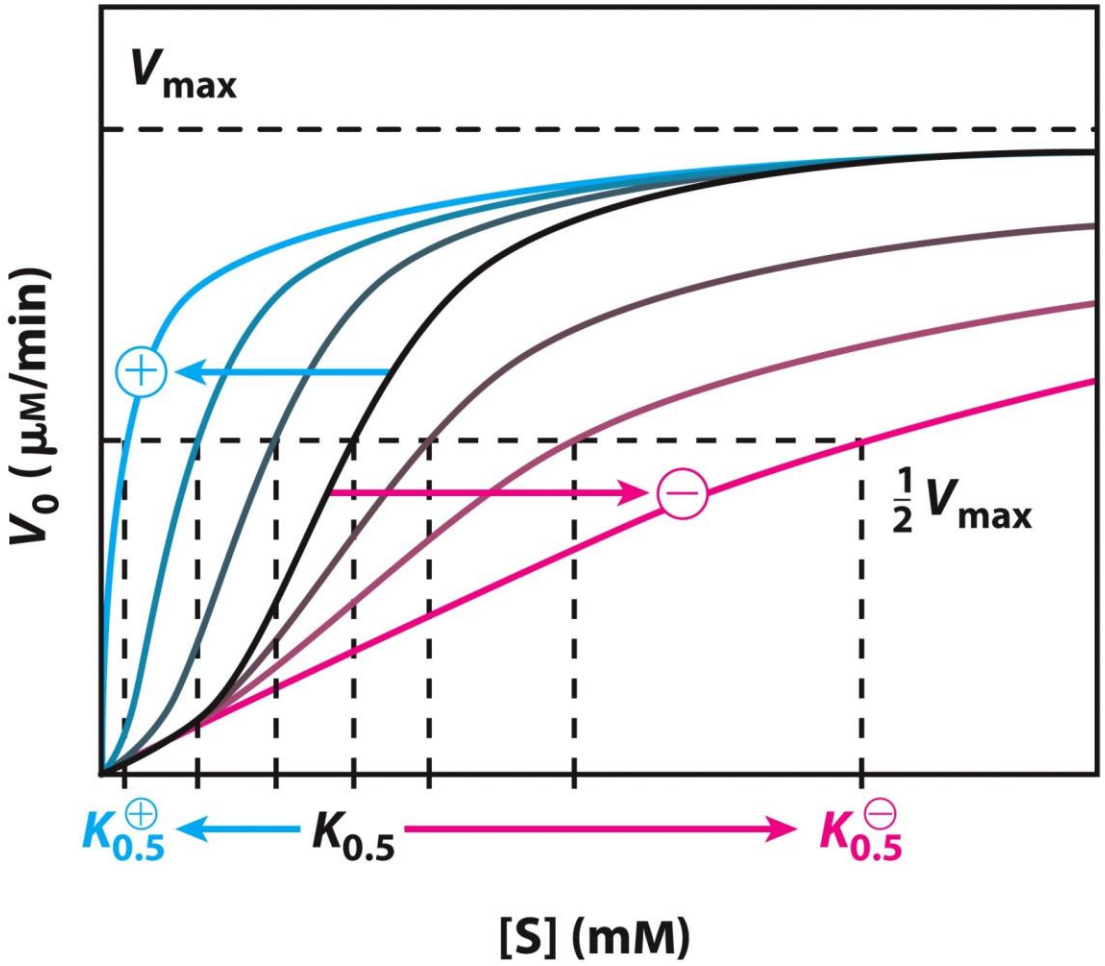

Figure 6-34b
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The kinetic behavior of allosteric enzymes reflects cooperative interactions among enzyme subunits.

Substrate-activity curves for representative allosteric enzymes

The effects of a positive modulator (+) and a negative modulator (–) on an allosteric enzyme in which $K_{0.5}$ is altered without a change in V_{max}. The central curve shows the substrate-activity relationship without a modulator.

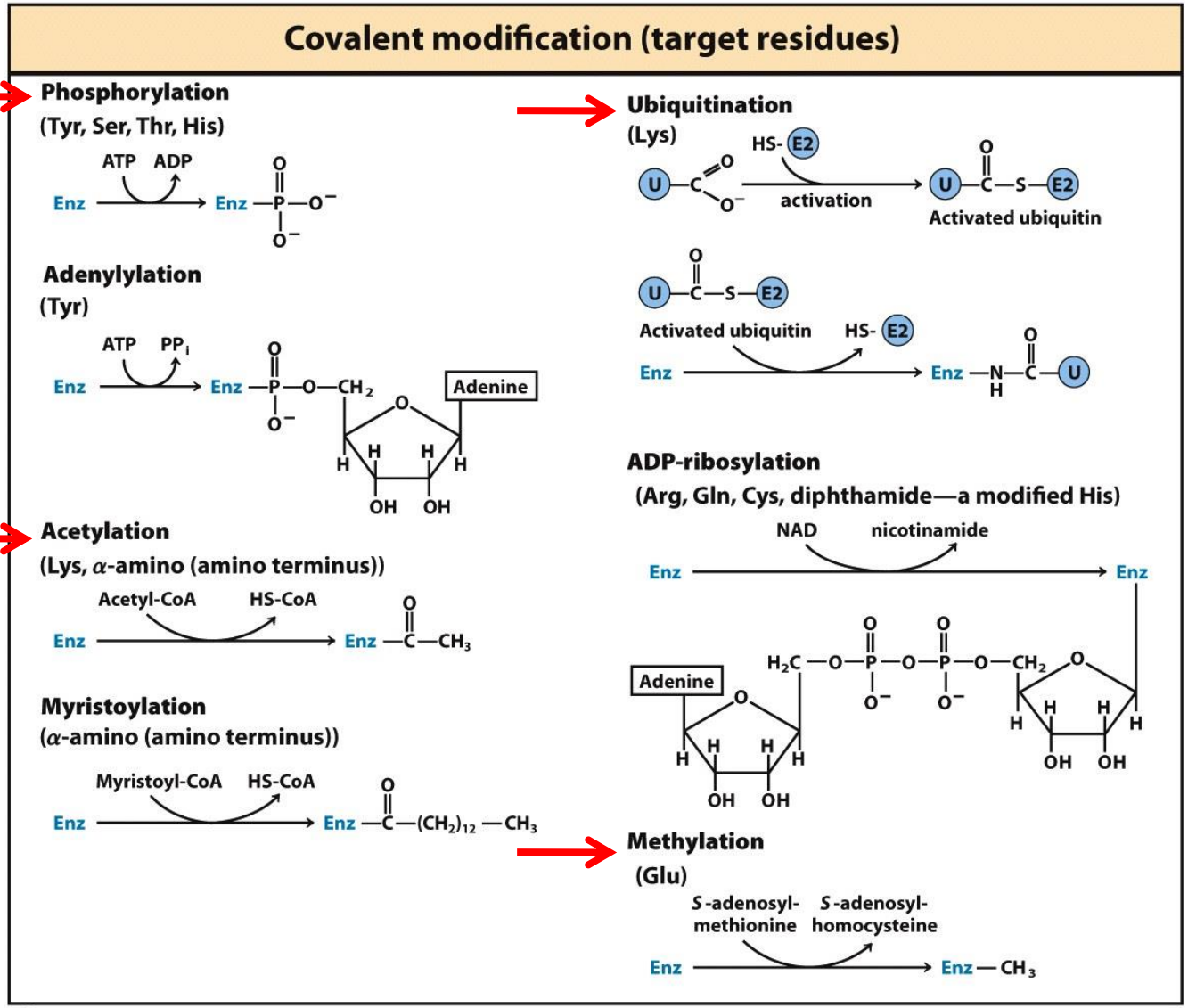

Figure 6-35

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Some enzyme modification reactions

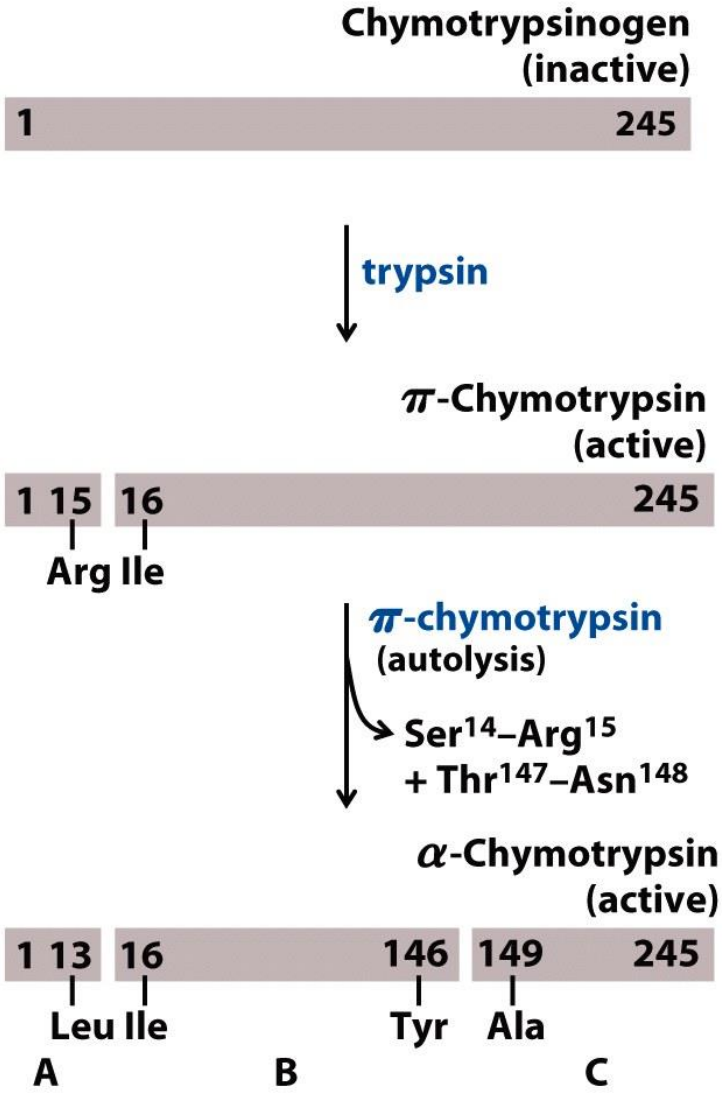

Figure 6-38
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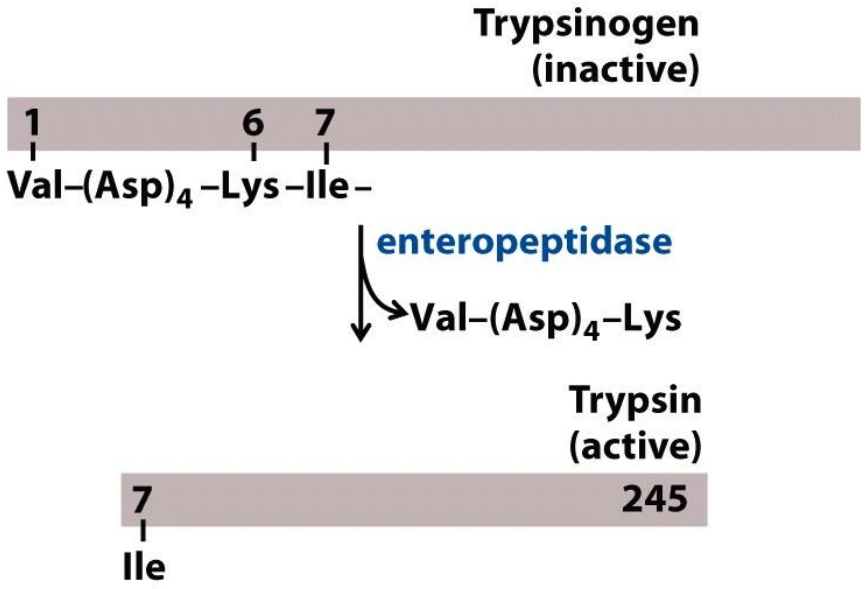

Many proteolytic enzymes are synthesized as inactive precursors called zymogens, which are activated by cleavage of small peptide fragments.

Activation of zymogens by proteolytic cleavage

Keywords

- **pH**
- **pK_a**
- **pI**
- **K_d**
- **K_m**
- **V_{max}**

Keywords

- **configuration vs. conformation**
- **motif vs. domain**
- **covalent vs. noncovalent**
- **structure vs. function**