

**Covalent and Non-Covalent Bonds;  
Chemistry of Aqueous Solutions;  
Ionization of Weak Acids (Buffers); Functional Groups**

CHEM 420 – Principles of Biochemistry  
Instructor – Anthony S. Serianni

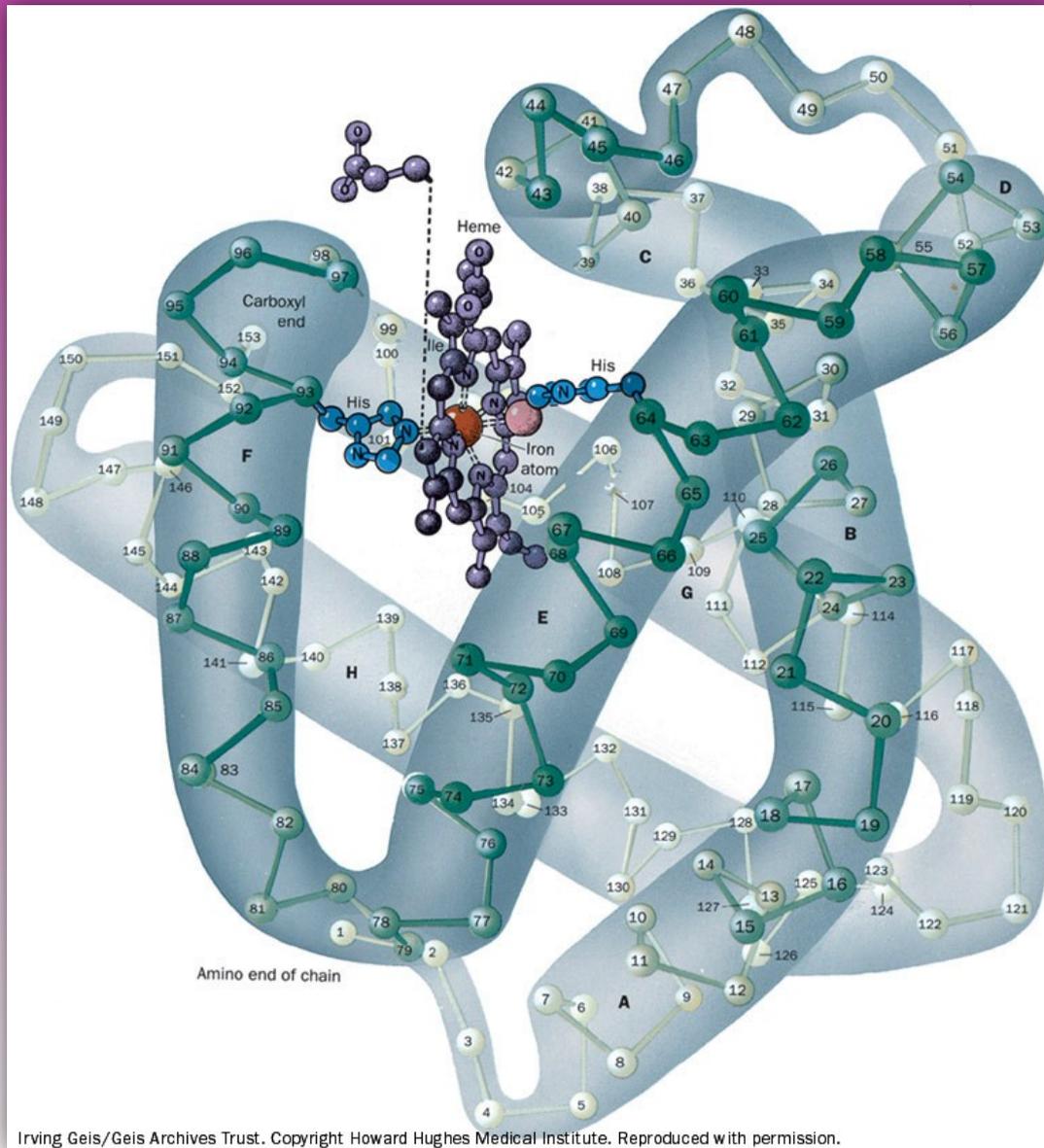
**Chapter 2**

Voet/Voet, Biochemistry, 2011

Fall 2015

August 26, 28, 31

# A "folded" protein



**Myoglobin:  
An O<sub>2</sub> binding  
protein  
(153 amino acid  
polypeptide)**

**1958 – Kendrew  
X-ray crystallography**

**TABLE 1-1** Strengths of Bonds Common in Biomolecules

<i>Type of bond</i>	<i>Bond dissociation energy* (kJ/mol)</i>	<i>Type of bond</i>	<i>Bond dissociation energy (kJ/mol)</i>
<b>Single bonds</b>		<b>Double bonds</b>	
O—H	470	C=O	712
H—H	435	C=N	615
P—O	419	C=C	611
C—H	414	P=O	502
N—H	389		
C—O	352	<b>Triple bonds</b>	
C—C	348	C≡C	816
S—H	339	N≡N	930
C—N	293		
C—S	260		
N—O	222		
S—S	214		

\*The greater the energy required for bond dissociation (breakage), the stronger the bond.

**4.18 kJ = 1 kcal**

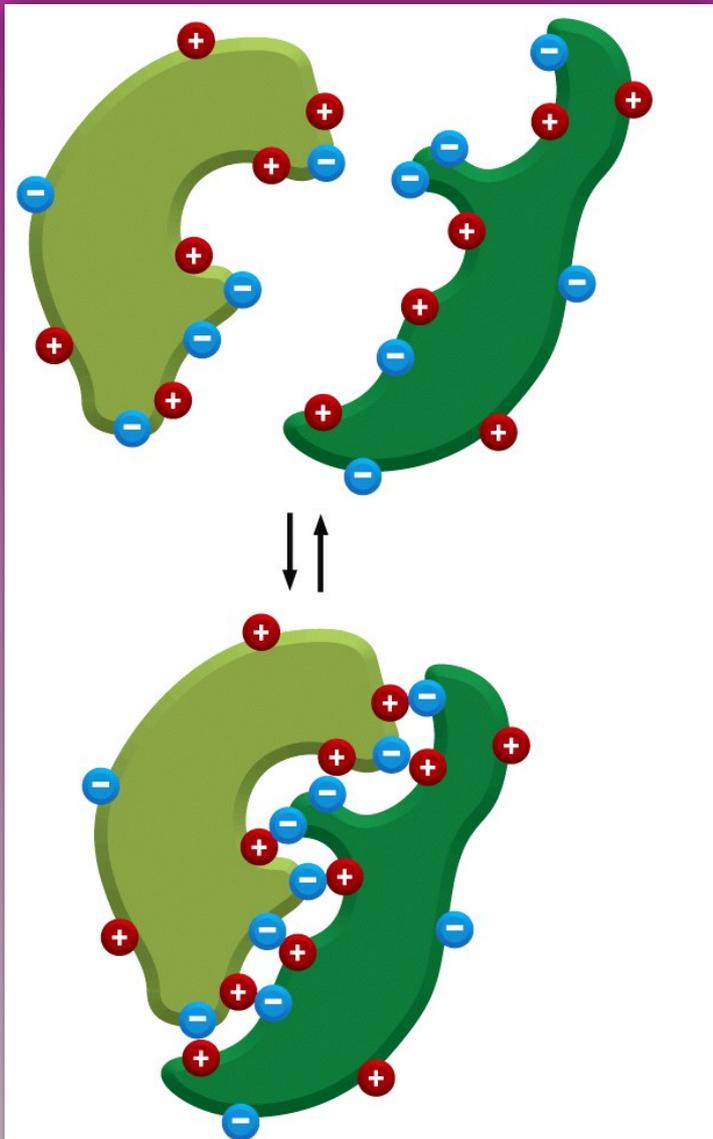
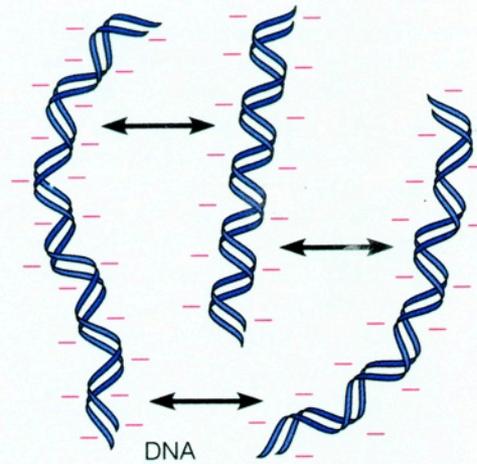
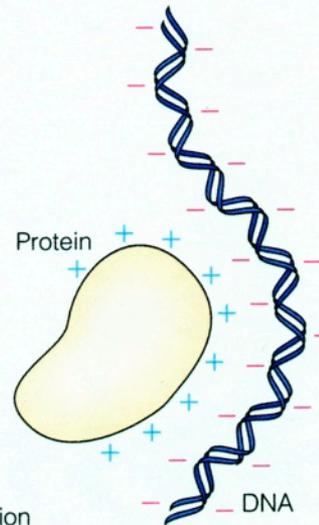


Figure 2-16 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Weak electrostatic interactions between two macromolecules with complementary surfaces; non-covalent interactions



(a) Repulsion



(b) Attraction

FIGURE 2.20

**Electrostatic interactions between macroions.** (a) Repulsion. DNA molecules, with many negative charges, strongly repel one another in solution. (b) Attraction. If DNA is mixed with a positively charged protein, these molecules have a strong tendency to associate.

**TABLE 3.15 Strength of Bonds Found in Protein Structures**

<b><i>Bond Type</i></b>	<b><i>Bond Strength (kcal mol<sup>-1</sup>)</i></b>
Covalent	>50
Noncovalent	0.6–7
Hydrophobic (i.e., two benzyl side chain groups of Phe)	2–3
Hydrogen	1–7
Ionic (low dielectric environment)	1–6
van der Waals	<1
Average energy of kinetic motion (37°C)	0.6

# Types of non-covalent (reversible) bonds in biological systems

Type of Interaction	Model	Example	Dependence of Energy on Distance
<b>(a) Charge-charge</b> Longest-range force; nondirectional		$-\overset{+}{\text{N}}\text{H}_3$ $\text{O}=\overset{-}{\text{C}}-\text{O}$	$1/r$
<b>(b) Charge-dipole</b> Depends on orientation of dipole		$-\overset{+}{\text{N}}\text{H}_3$ $\overset{q-}{\text{O}}-\overset{q+}{\text{C}}-\text{H}$	$1/r^2$
<b>(c) Dipole-dipole</b> Depends on mutual orientation of dipoles		$\overset{q-}{\text{O}}-\overset{q+}{\text{C}}-\text{H}$ $\overset{q-}{\text{O}}-\overset{q+}{\text{C}}-\text{H}$	$1/r^3$
<b>(d) Charge-induced dipole</b> Depends on polarizability of molecule in which dipole is induced		$-\overset{+}{\text{N}}\text{H}_3$ $\text{C}_6\text{H}_6$	$1/r^4$
<b>(e) Dipole-induced dipole</b> Depends on polarizability of molecule in which dipole is induced		$\overset{q-}{\text{O}}-\overset{q+}{\text{C}}-\text{H}$ $\text{C}_6\text{H}_6$	$1/r^5$
<b>(f) Dispersion</b> Involves mutual synchronization of fluctuating charges		$\text{C}_6\text{H}_6$ $\text{C}_6\text{H}_6$	$1/r^6$
<b>(g) van der Waals repulsion</b> Occurs when outer electron orbitals overlap			$1/r^{12}$
<b>(h) Hydrogen bond</b> Charge attraction + partial covalent bond		$\text{N}-\text{H}\cdots\text{O}=\text{C}$ Hydrogen bond length	Length of bond fixed

$$E_{\text{el}} \approx \frac{Z_A \cdot Z_B \cdot \epsilon^2}{D \cdot r_{\text{ab}}}$$

**Figure 3.49. Strength of electrostatic interactions.**

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$$E_{VDW} = -\frac{A}{r_{ab}^6} + \frac{B}{r_{ab}^{12}}$$

**Figure 3.50. Strength of van der Waals interactions.**

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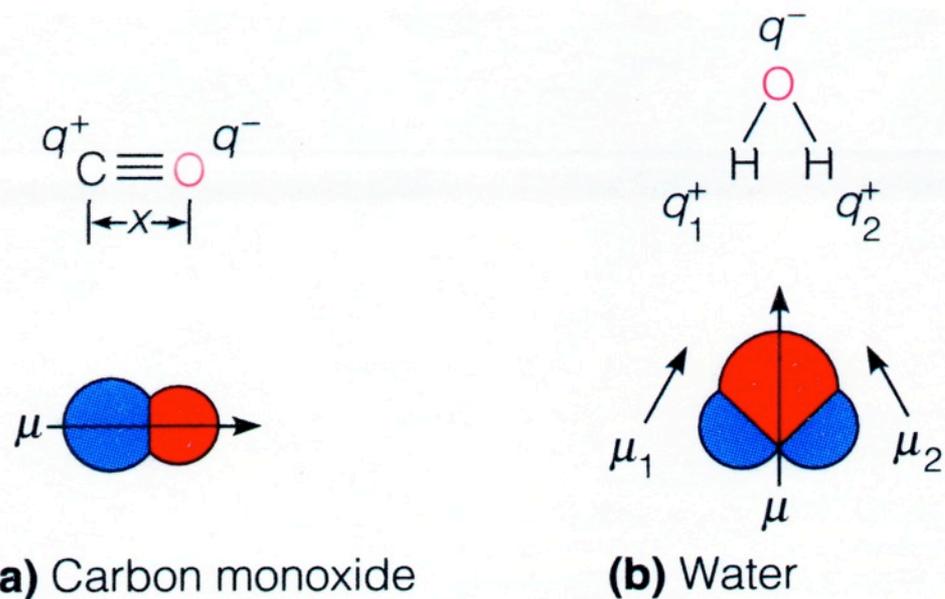
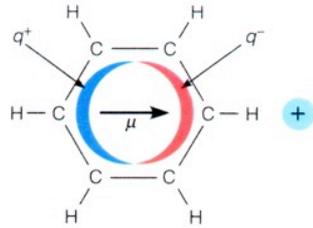


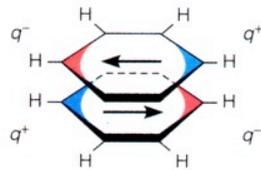
FIGURE 2.4

**Dipolar molecules.** (a) Carbon monoxide: the excess negative charge ( $q^-$ ) on the oxygen together with corresponding positive charge ( $q^+$ ) on the carbon produces a dipole moment directed along the C—O axis.

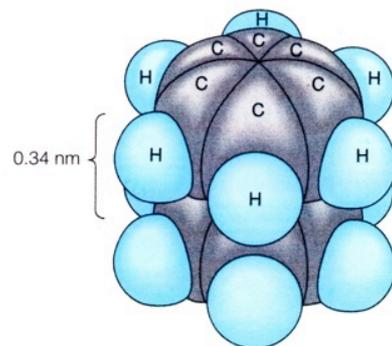
(b) Water: the excess negative charge on O together with the excess positive charge on each H produces two moments,  $\mu_1$  and  $\mu_2$ , directed along the H—O bonds. Their vector sum ( $\mu$ ) represents the dipole moment of the molecule.



(a) Induction of a dipole in benzene



(b) Dispersion forces between benzene molecules



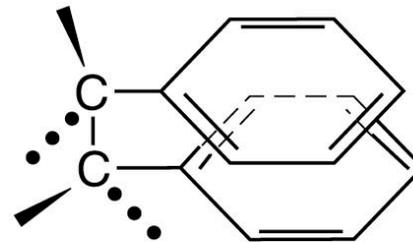
(c) Space-filling model of molecules in (b)

FIGURE 2.5

**Induced dipoles and dispersion forces.**

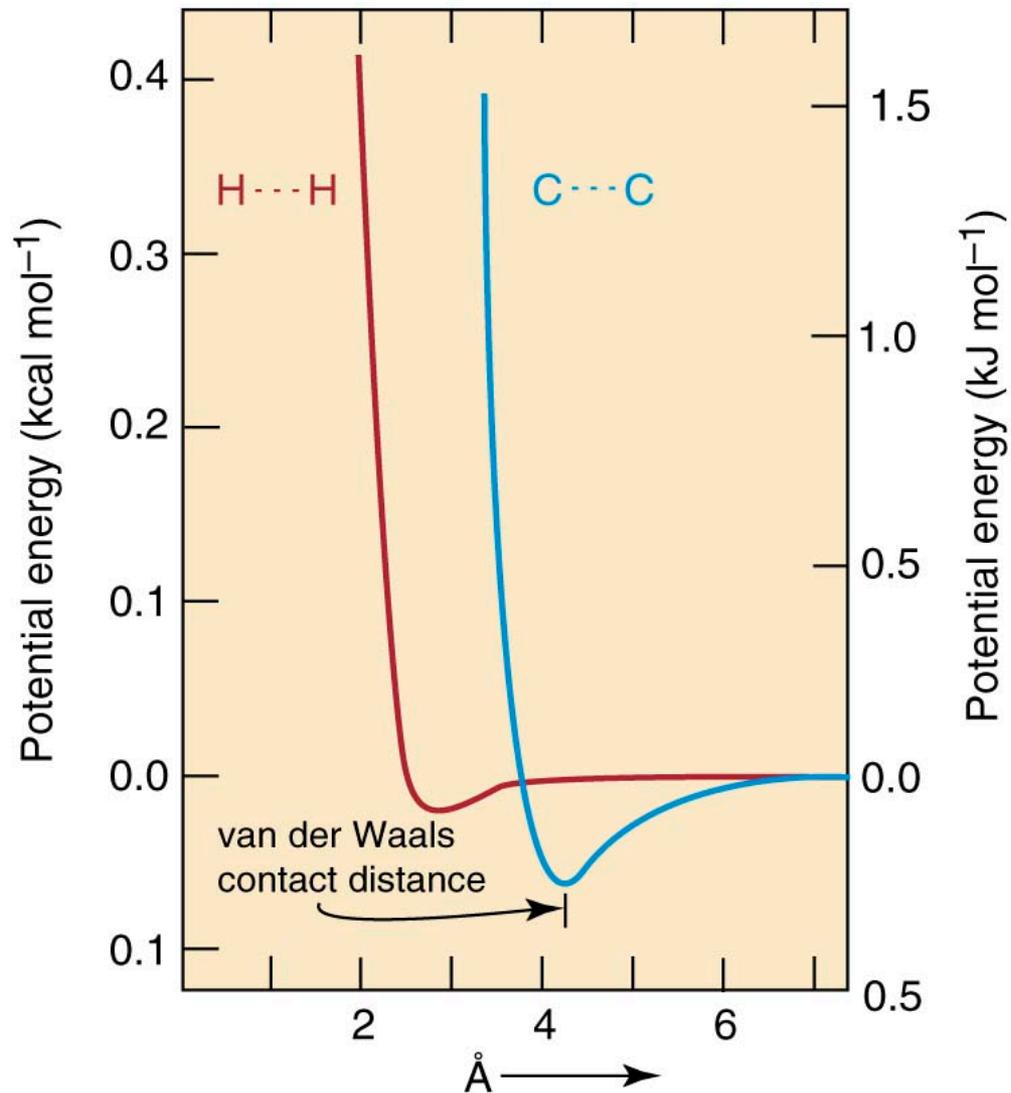
(a) Benzene has neither a net charge nor a permanent dipole moment, but a nearby charge can induce a redistribution of electrons within the benzene ring, producing an induced moment ( $\mu$ ). (b) Planar molecules like benzene have a strong tendency to stack, because fluctuations in the electron clouds of the stacked rings interact with one another, producing a dispersion force. (c) Although the molecules approach closely, they do not interpenetrate.

(c) © Irving Geis.



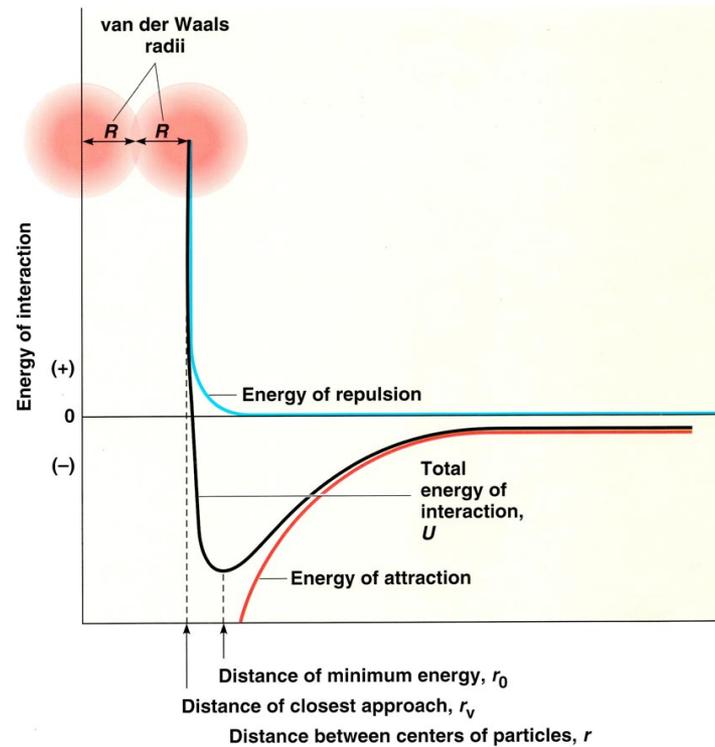
**Figure 3.52.  $\pi$ -Electron- $\pi$ -electron interactions between two aromatic rings.**

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**Figure 3.51.** van der Waals–London dispersion interaction energies between two hydrogen atoms and two (tetrahedral) carbon atoms. Redrawn from Fersht, A. *Enzyme Structure and Mechanism*. San Francisco: Freeman, 1977, p. 228.

Figure 2.6 Noncovalent interaction energy of two approaching particles

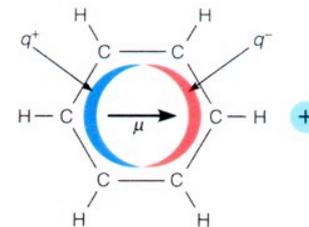


$$E_{\text{VDW}} = -\frac{A}{6r_{\text{ab}}} + \frac{B}{r_{\text{ab}}^{12}}$$

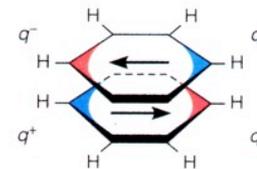
Figure 3.50. Strength of van der Waals interactions.

**TABLE 2.2 van der Waals radii of some atoms and groups of atoms**

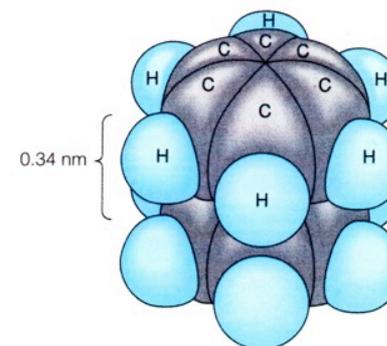
	$R$ (nm)
<b>Atoms</b>	
H	0.12
O	0.14
N	0.15
C	0.17
S	0.18
P	0.19
<b>Groups</b>	
—OH	0.14
—NH <sub>2</sub>	0.15
—CH <sub>2</sub> —	0.20
—CH <sub>3</sub>	0.20
Half-thickness of aromatic ring	0.17



(a) Induction of a dipole in benzene



(b) Dispersion forces between benzene molecules



(c) Space-filling model of molecules in (b)

**FIGURE 2.5**

**Induced dipoles and dispersion forces.**

(a) Benzene has neither a net charge nor a permanent dipole moment, but a nearby charge can induce a redistribution of electrons within the benzene ring, producing an induced moment ( $\mu$ ). (b) Planar molecules like benzene have a strong tendency to stack, because fluctuations in the electron clouds of the stacked rings interact with one another, producing a dispersion force. (c) Although the molecules approach closely, they do not interpenetrate.

**Table 2–1 Covalent and Noncovalent Chemical Bonds**

BOND TYPE	LENGTH (nm)	STRENGTH (kcal/mole)	
		IN VACUUM	IN WATER
Covalent	0.15	90	90
Noncovalent: ionic*	0.25	80	3
hydrogen	0.30	4	1
van der Waals attraction (per atom)	0.35	0.1	0.1

\*An ionic bond is an electrostatic attraction between two fully charged atoms.

Table 2-1 Molecular Biology of the Cell 5/e (© Garland Science 2008)

$$E_{\text{el}} \approx \frac{Z_A \cdot Z_B \cdot \epsilon^2}{D \cdot r_{\text{ab}}}$$

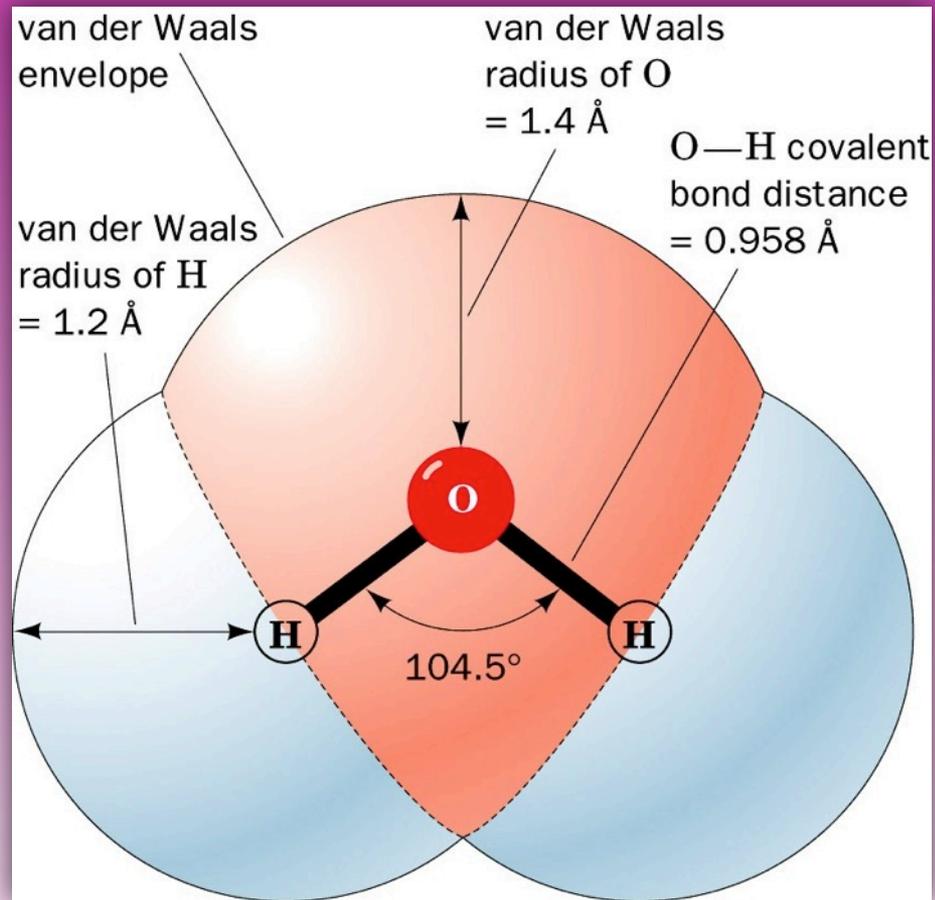
**Figure 3.49. Strength of electrostatic interactions.**

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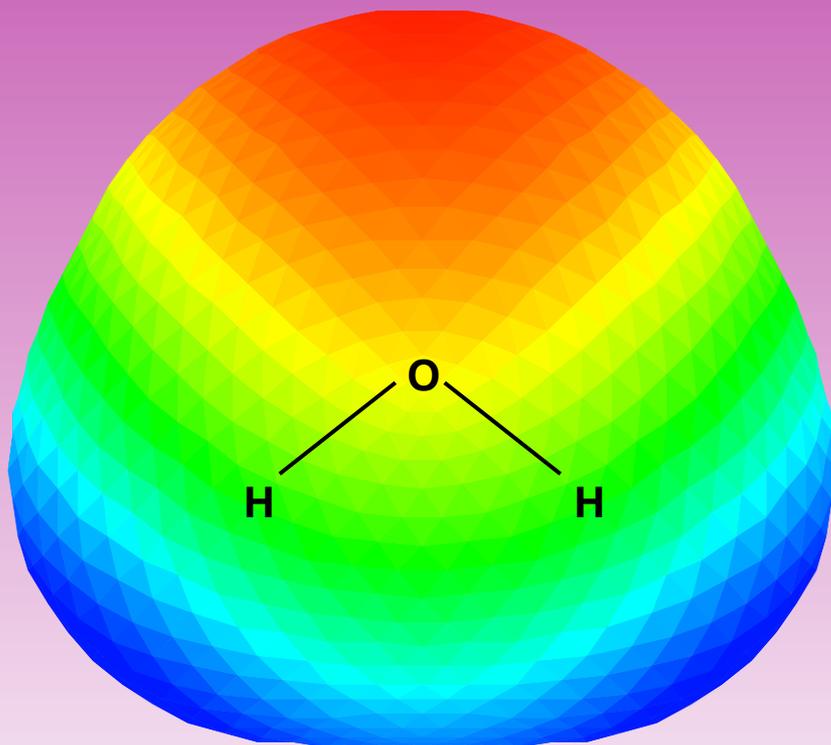
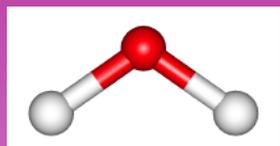
Substance	Dielectric Constant	Dipole Moment (debye)
Formamide	110.0	3.37
Water	78.5	1.85
Dimethyl sulfoxide	48.9	3.96
Methanol	32.6	1.66
Ethanol	24.3	1.68
Acetone	20.7	2.72
Ammonia	16.9	1.47
Chloroform	4.8	1.15
Diethyl ether	4.3	1.15
Benzene	2.3	0.00
Carbon tetrachloride	2.2	0.00
Hexane	1.9	0.00

Source: Brey, W.S., *Physical Chemistry and Its Biological Applications*, p. 26, Academic Press (1978).

## Dielectric constants and permanent molecular dipole moments of some common solvents



**Structure of the water molecule**



The polar  
water molecule  
red =  $\delta^-$  region  
blue =  $\delta^+$  region

View: HOH atoms  
in viewing plane; both  
oxygen lone-pairs  
eclipsed and  
perpendicular to the  
viewing plane.

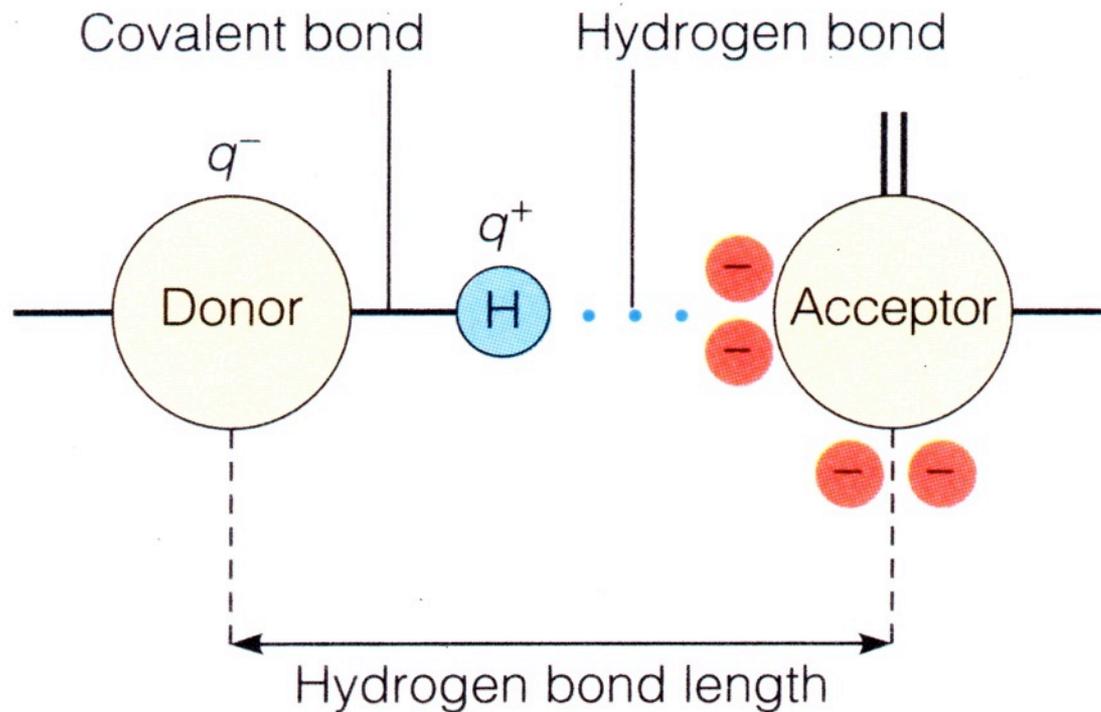
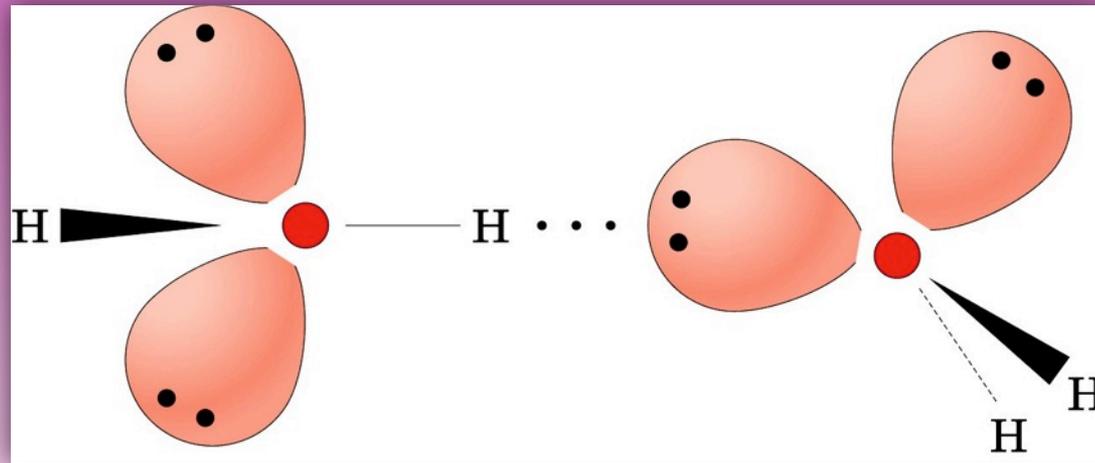
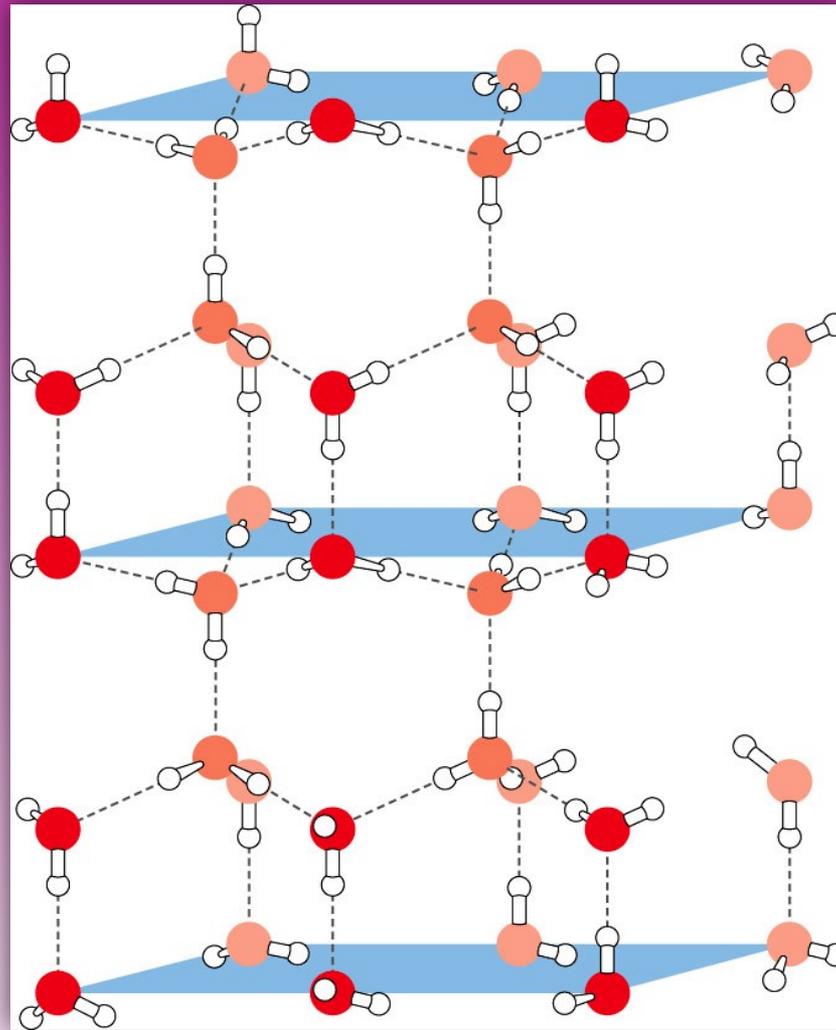


FIGURE 2.7

**The hydrogen bond.** The figure shows an idealized H bond that might exist, for example, in  $\text{—O—H}\cdots\text{O=}$ . Although the H bond is between H and the acceptor, the H-bond length is defined as the distance between donor and acceptor.



Hydrogen bonding between two water molecules

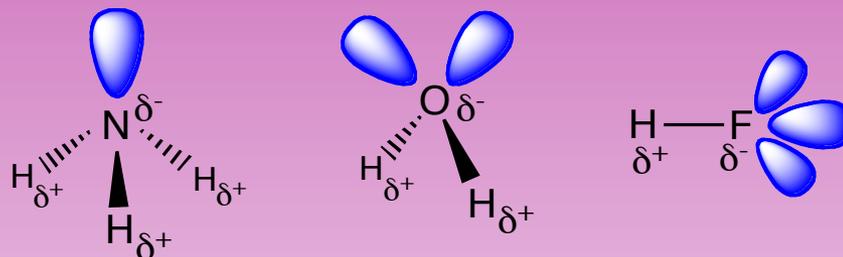


**Structure of ice: 4 H-bonds per water molecule  
(two as donor, two as acceptor)**

# General characteristics of H-bonds

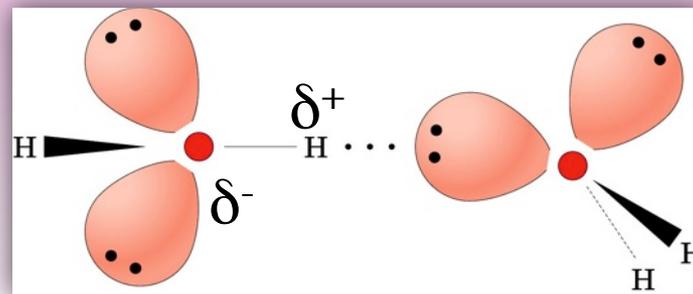
Hydrogen covalently attached to an electronegative atom

- Partial positive (+) charge of hydrogen on donor
- Partial negative (-) charge on the electronegative donor atom
- At least one lone-pair of electrons on the acceptor atom
- The partial positive hydrogen is strongly attracted to the lone-pair electrons on the acceptor

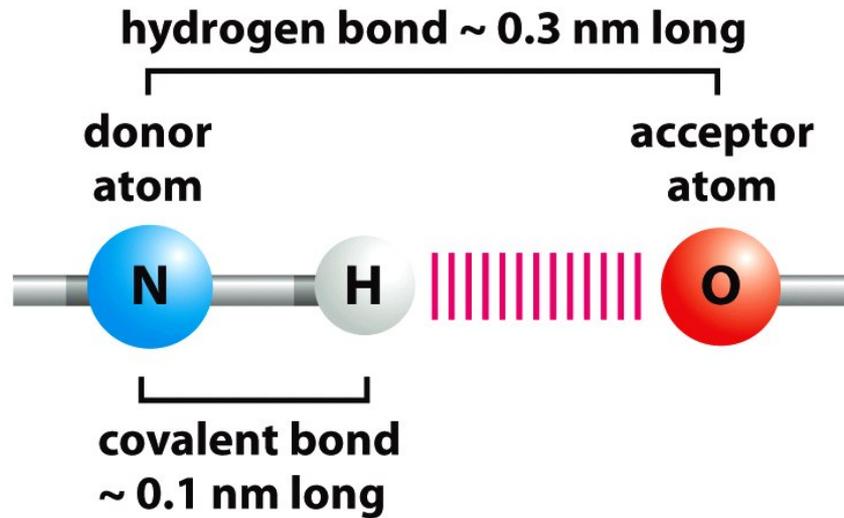


Interaction much stronger than dipole-dipole interaction

- ethanol (bp = 78.5 °C)
- methoxymethane (bp = -24.8 °C)  
~100° elevation of bp



(A)



(B)

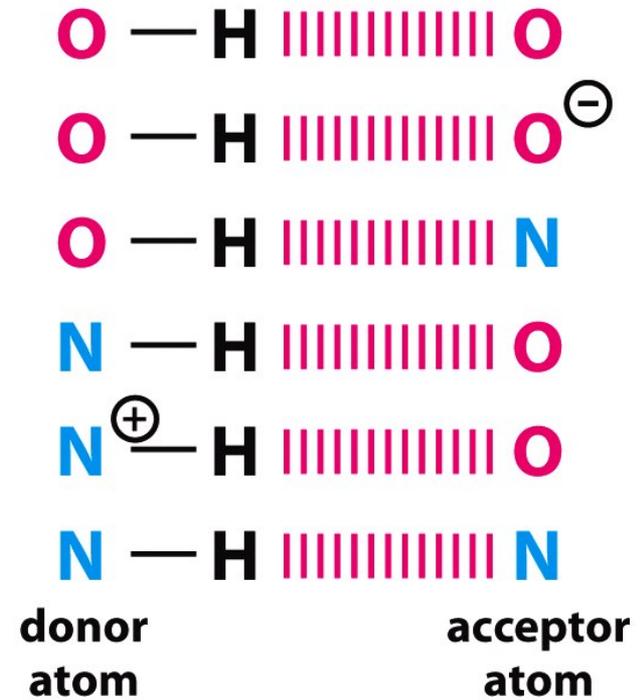
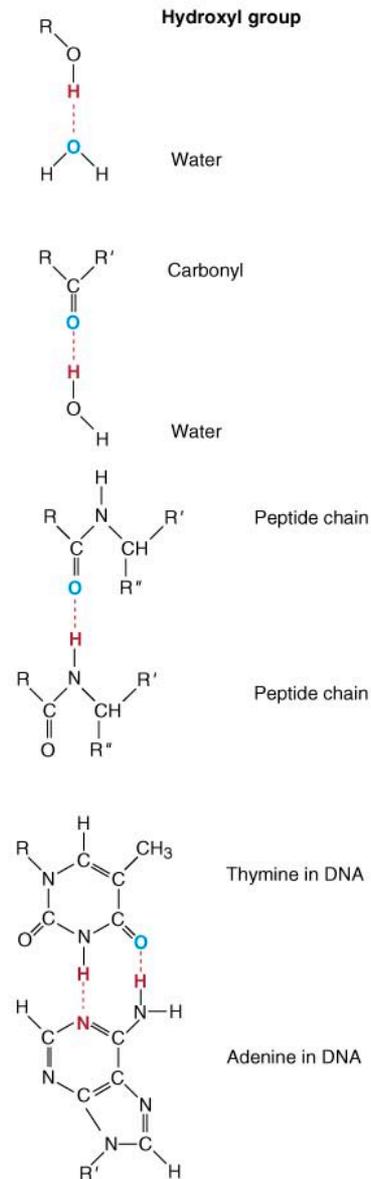


Figure 2-15 Molecular Biology of the Cell 5/e (© Garland Science 2008)



**Figure 1.4. Representative hydrogen bonds of importance in biological systems.**

## Different types of H-bonds

- Common elements that form H-bonds: S, O, N, F
- H-bonds involving C-H donors (proteins)

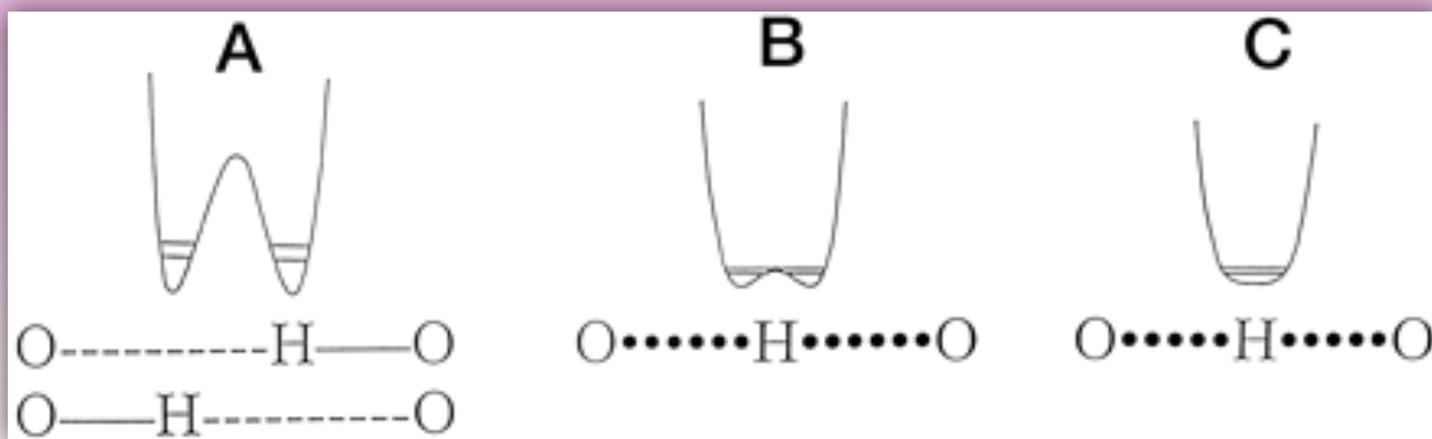
## Summary of H-bond properties

	Strong	Moderate	Weak
interaction type	strongly covalent	mostly electrostatic	electrostat./dispers.
bond lengths [Å]			
H...A	1.2–1.5	1.5–2.2	> 2.2
lengthening of X–H [Å]	0.08–0.25	0.02–0.08	< 0.02
X–H versus H...A	X–H ≈ H...A	X–H < H...A	X–H ≪ H...A
X...A [Å]	2.2–2.5	2.5–3.2	> 3.2
directionality	strong	moderate	weak
bond angles [°]	170–180	> 130	> 90
bond energy [kcal mol <sup>-1</sup> ]	15–40	4–15	< 4
relat. IR shift $\Delta\tilde{\nu}_{\text{XH}}$ [cm <sup>-1</sup> ]	25 %	10–25 %	< 10 %
<sup>1</sup> H downfield shift	14–22	< 14	

For an **X-H...A** H-bond

## Low-barrier hydrogen bonds (LBHB)

- H-bond strength depends on its length, linearity, microenvironment and  $pK_a$  values of the H-sharing components.
- H-bonds in water are relatively weak because of the  $pK_a$  mismatch between  $H_3O^+$  (-1.7) and  $H_2O$  (15.7).
  - The proton in the structure is tightly associated with  $OH^-$  as a water molecule.
- In the gas phase, the dielectric constant is low.
  - Hydrogen bonds between heteroatoms with **matched**  $pK_a$  values can be  $\cong 2.5 \text{ \AA}$  and very strong (25-30 kcal/mol)



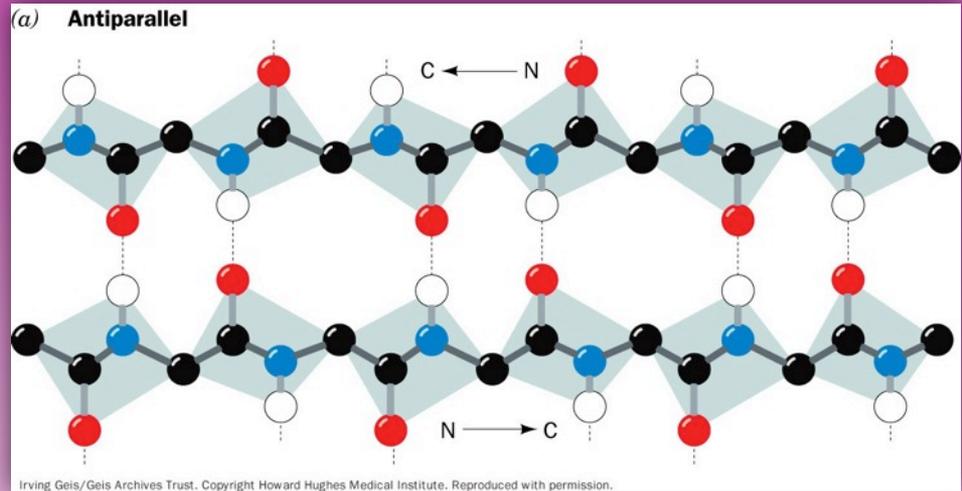
## H-bonds in proteins

- I. H-bonds contribute to **structure and folding**.
- II. H-bonds contribute to **catalysis**.

# Structure/folding - Protein $\beta$ -sheets

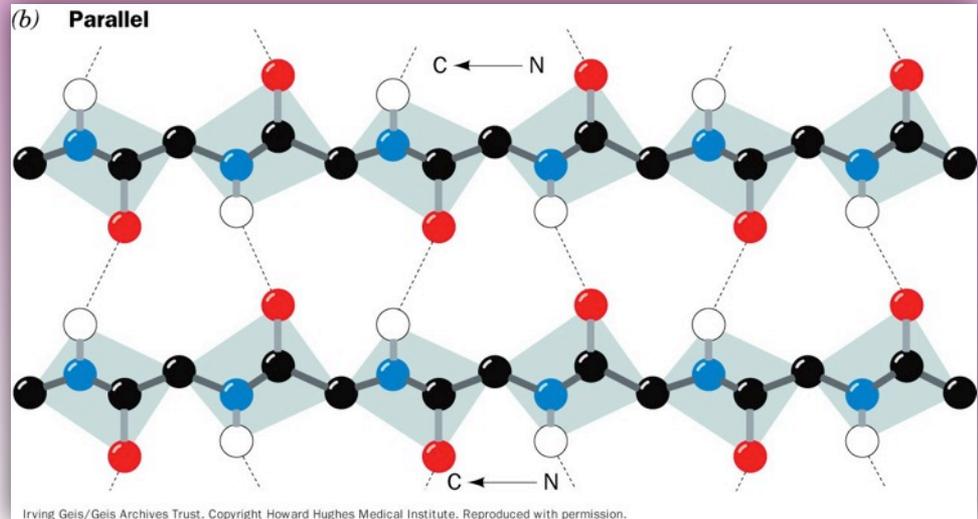
## Anti-parallel $\beta$ -sheets

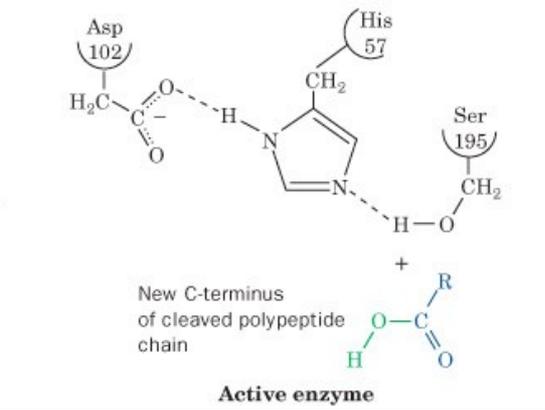
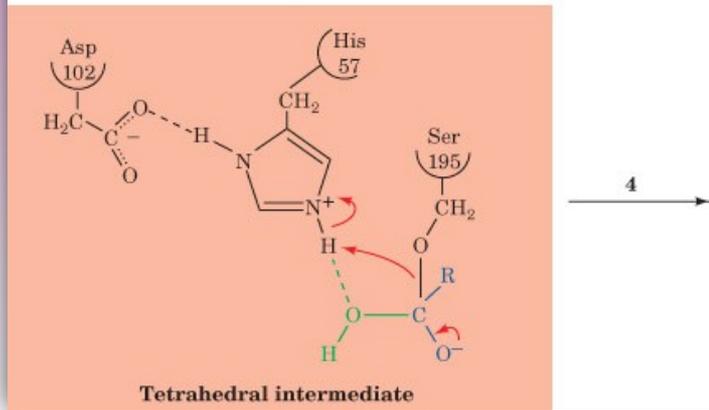
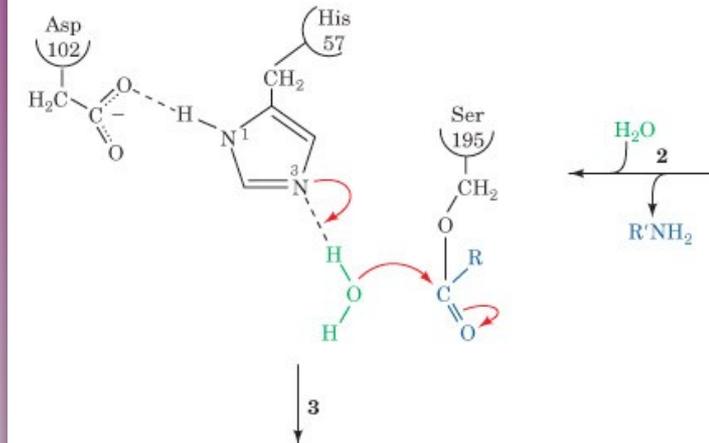
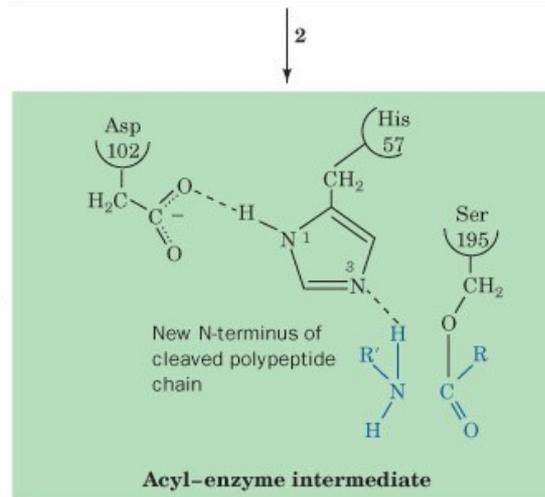
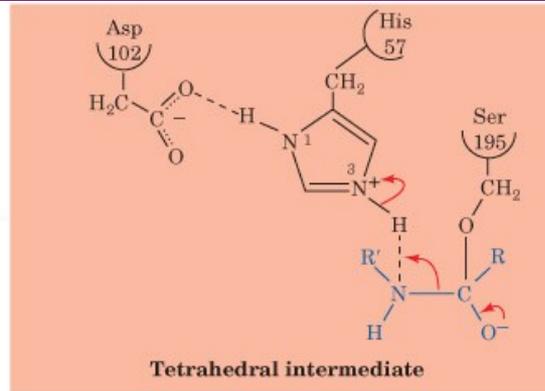
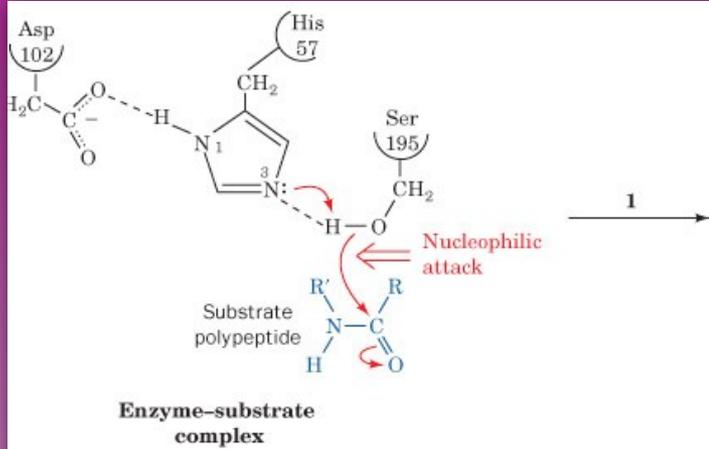
- 10-14 atoms in a ring
- H-bonds linear
- Stability?



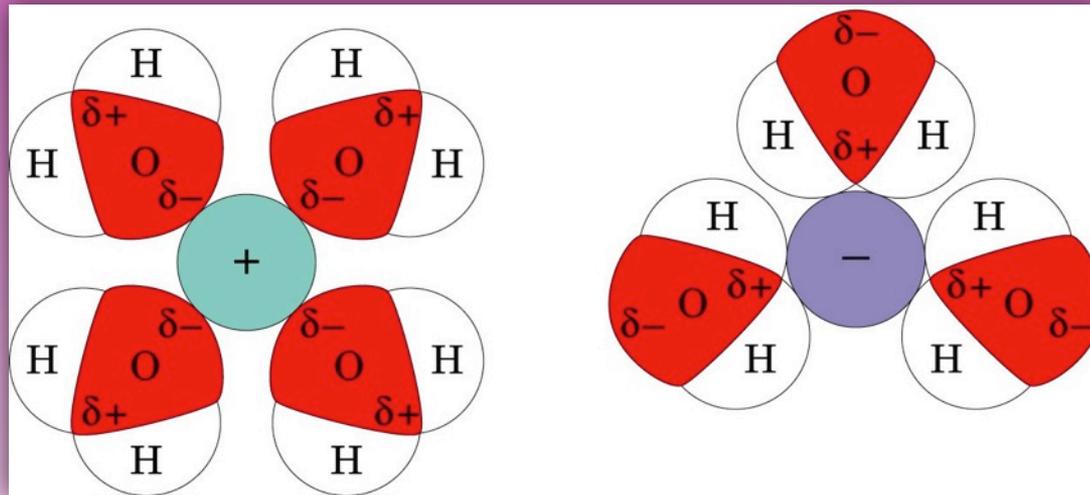
## Parallel $\beta$ -sheets

- 12 atoms in a ring
- H-bonds not  $180^\circ$
- Stability?





# Enzyme Catalysis: Catalytic mechanism of a Serine protease



**Solvation of ions (salts) by oriented solvent water molecules**



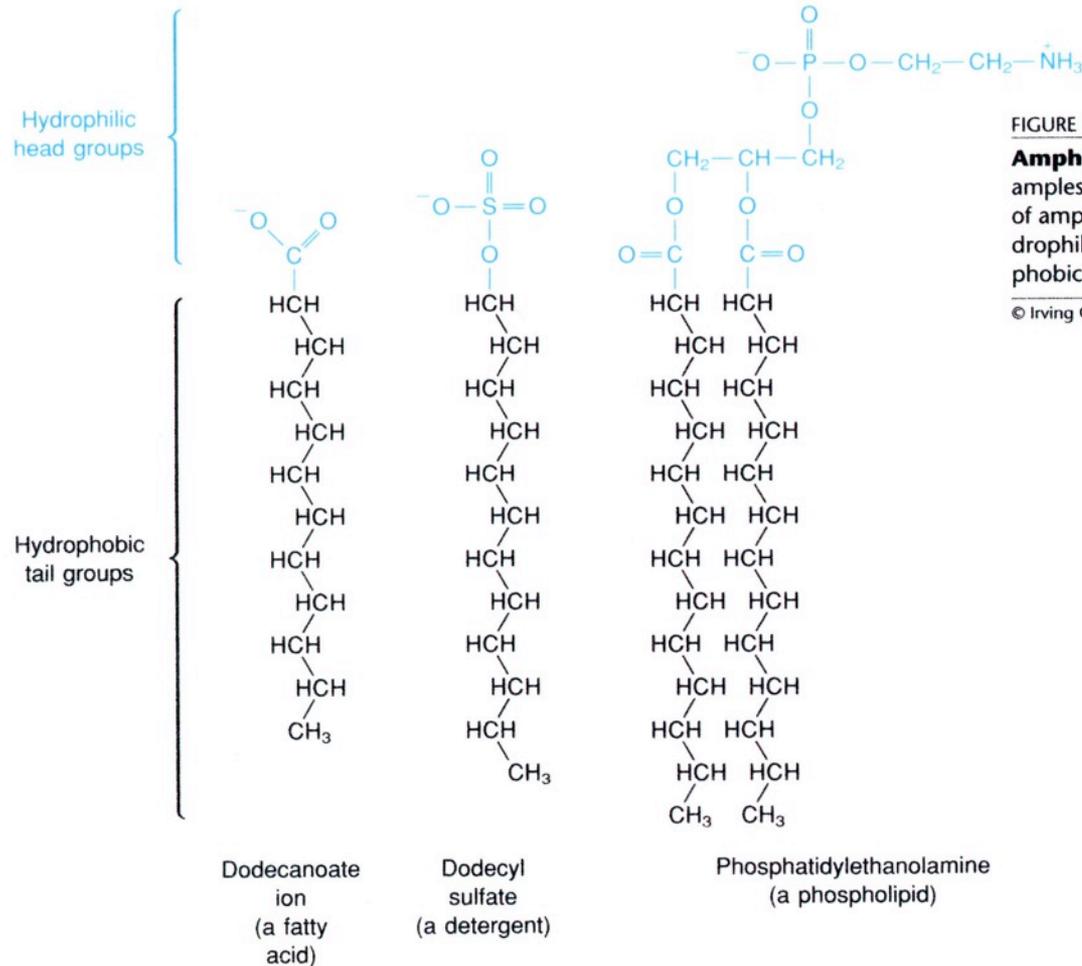
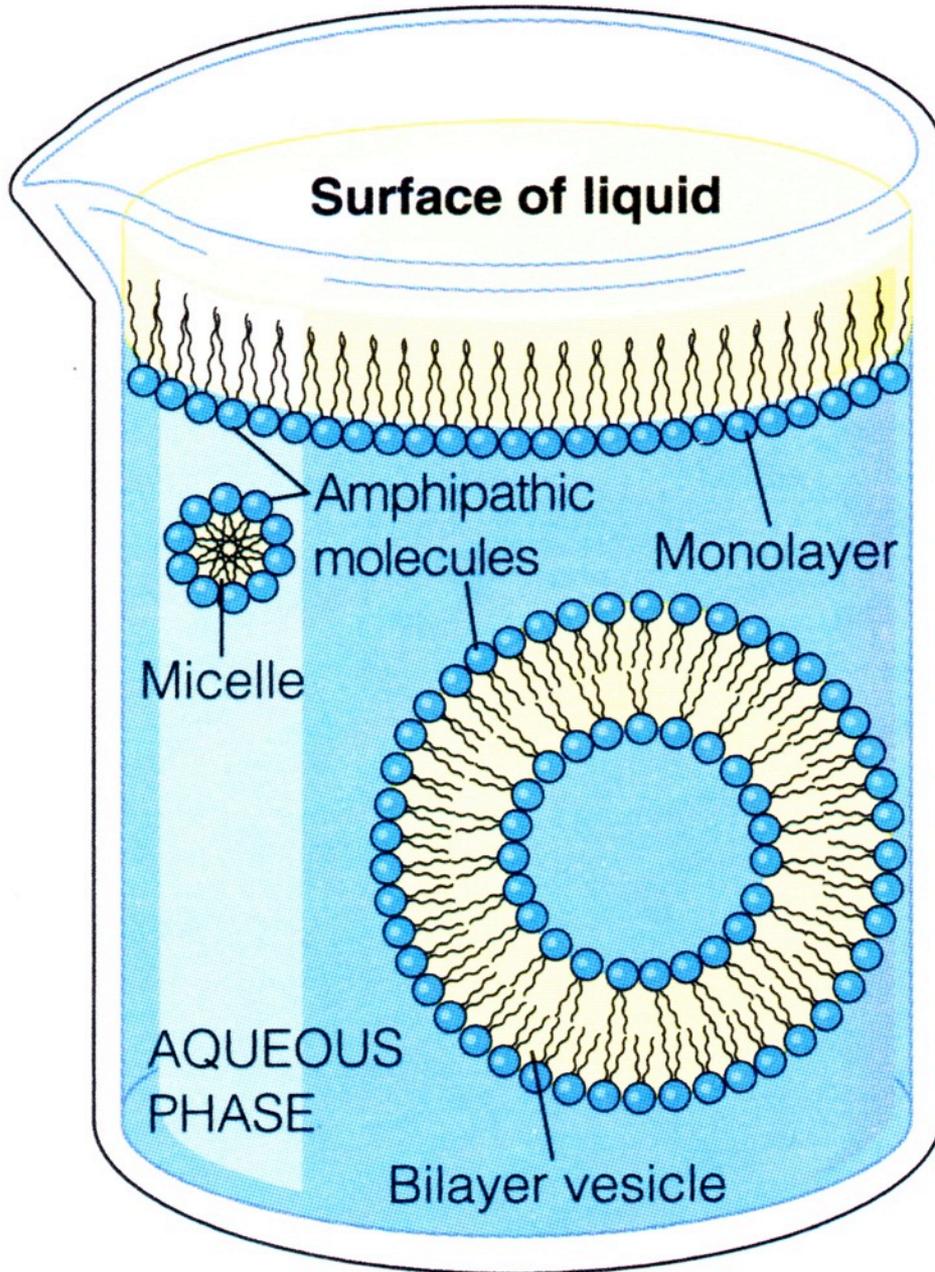


FIGURE 2.14

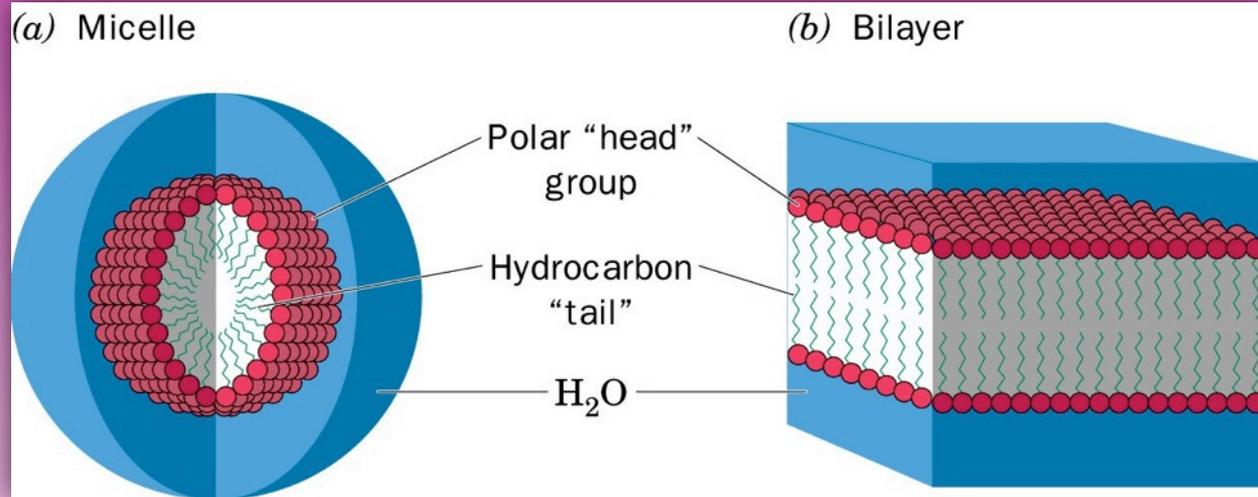
**Amphipathic molecules.** These three examples illustrate the “schizophrenic” structure of amphipathic molecules, which have a hydrophilic head group attached to a hydrophobic tail.

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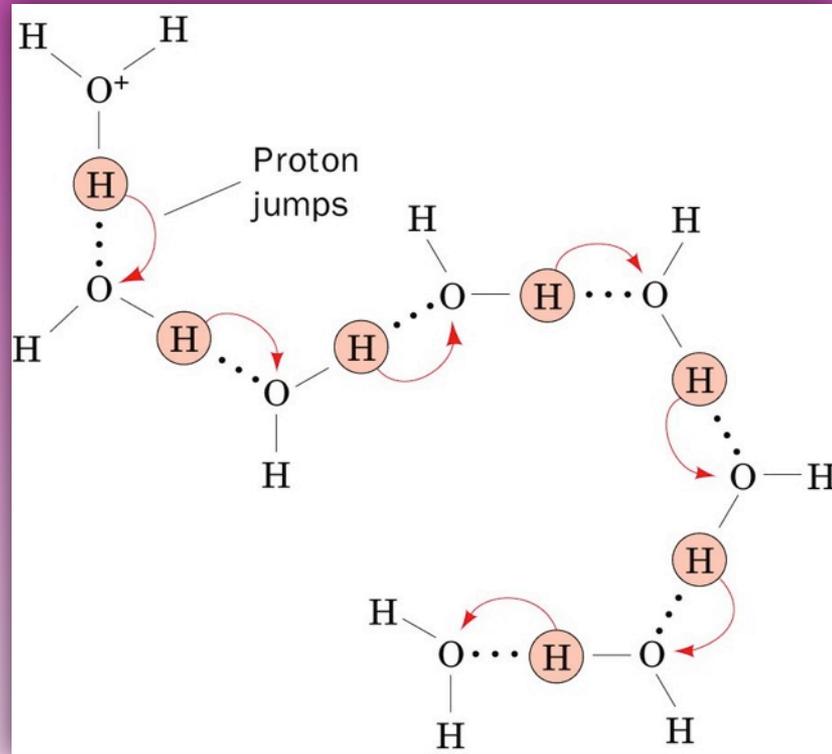
Examples of amphipathic (amphiphilic) compounds



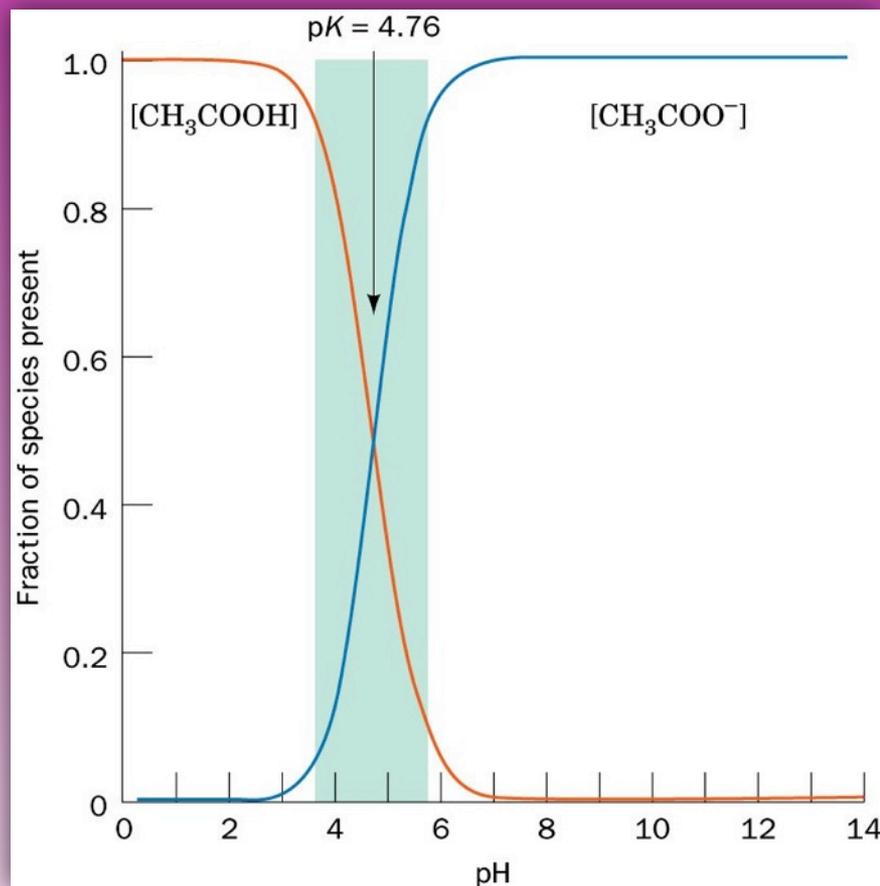
**Spontaneous  
self-assembly of  
amphipathic  
compounds  
in aqueous  
solution**



Spontaneous self-association of amphipathic molecules in aqueous solutions



Hydronium ion migration in aqueous solution via proton jumps



Relative concentrations of acetic acid and acetate ion in aqueous solution as a function of solution pH

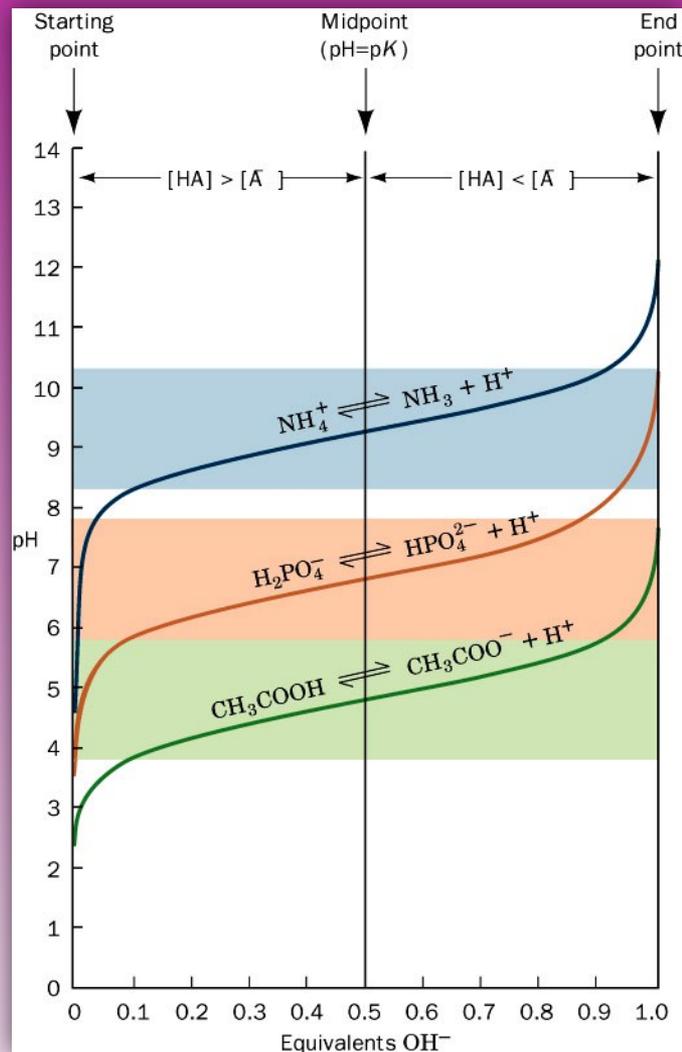
**TABLE 2.6 Some weak acids and their conjugate bases**

Acid (Proton Donor)		Conjugate Base (Proton Acceptor)		$pK_a$	$K_a$ (M)
HCOOH Formic acid	$\rightleftharpoons$	HCOO <sup>-</sup> Formate ion	+H <sup>+</sup>	3.75	$1.78 \times 10^{-4}$
CH <sub>3</sub> COOH Acetic acid	$\rightleftharpoons$	CH <sub>3</sub> COO <sup>-</sup> Acetate ion	+H <sup>+</sup>	4.76	$1.74 \times 10^{-5}$
$\begin{array}{c} \text{OH} \\   \\ \text{CH}_3\text{CH}-\text{COOH} \end{array}$ Lactic acid	$\rightleftharpoons$	$\begin{array}{c} \text{OH} \\   \\ \text{CH}_3\text{CH}-\text{COO}^- \end{array}$ Lactate ion	+H <sup>+</sup>	3.86	$1.38 \times 10^{-4}$
H <sub>3</sub> PO <sub>4</sub> Phosphoric acid	$\rightleftharpoons$	H <sub>2</sub> PO <sub>4</sub> <sup>-</sup> Dihydrogen phosphate ion	+H <sup>+</sup>	2.14	$7.24 \times 10^{-3}$
H <sub>2</sub> PO <sub>4</sub> <sup>-</sup> Dihydrogen phosphate ion	$\rightleftharpoons$	HPO <sub>4</sub> <sup>2-</sup> Monohydrogen phosphate ion	+H <sup>+</sup>	6.86	$1.38 \times 10^{-7}$
HPO <sub>4</sub> <sup>2-</sup> Monohydrogen phosphate ion	$\rightleftharpoons$	PO <sub>4</sub> <sup>3-</sup> Phosphate ion	+H <sup>+</sup>	12.4	$3.98 \times 10^{-13}$
H <sub>2</sub> CO <sub>3</sub> Carbonic acid	$\rightleftharpoons$	HCO <sub>3</sub> <sup>-</sup> Bicarbonate ion	+H <sup>+</sup>	6.37	$4.27 \times 10^{-7}$
HCO <sub>3</sub> <sup>-</sup> Bicarbonate ion	$\rightleftharpoons$	CO <sub>3</sub> <sup>2-</sup> Carbonate ion	+H <sup>+</sup>	10.25	$5.62 \times 10^{-11}$
C <sub>6</sub> H <sub>5</sub> OH Phenol	$\rightleftharpoons$	C <sub>6</sub> H <sub>5</sub> O <sup>-</sup> Phenolate ion	+H <sup>+</sup>	9.89	$1.29 \times 10^{-10}$
NH <sub>4</sub> <sup>+</sup> Ammonium ion	$\rightleftharpoons$	NH <sub>3</sub> Ammonia	+H <sup>+</sup>	9.25	$5.62 \times 10^{-10}$

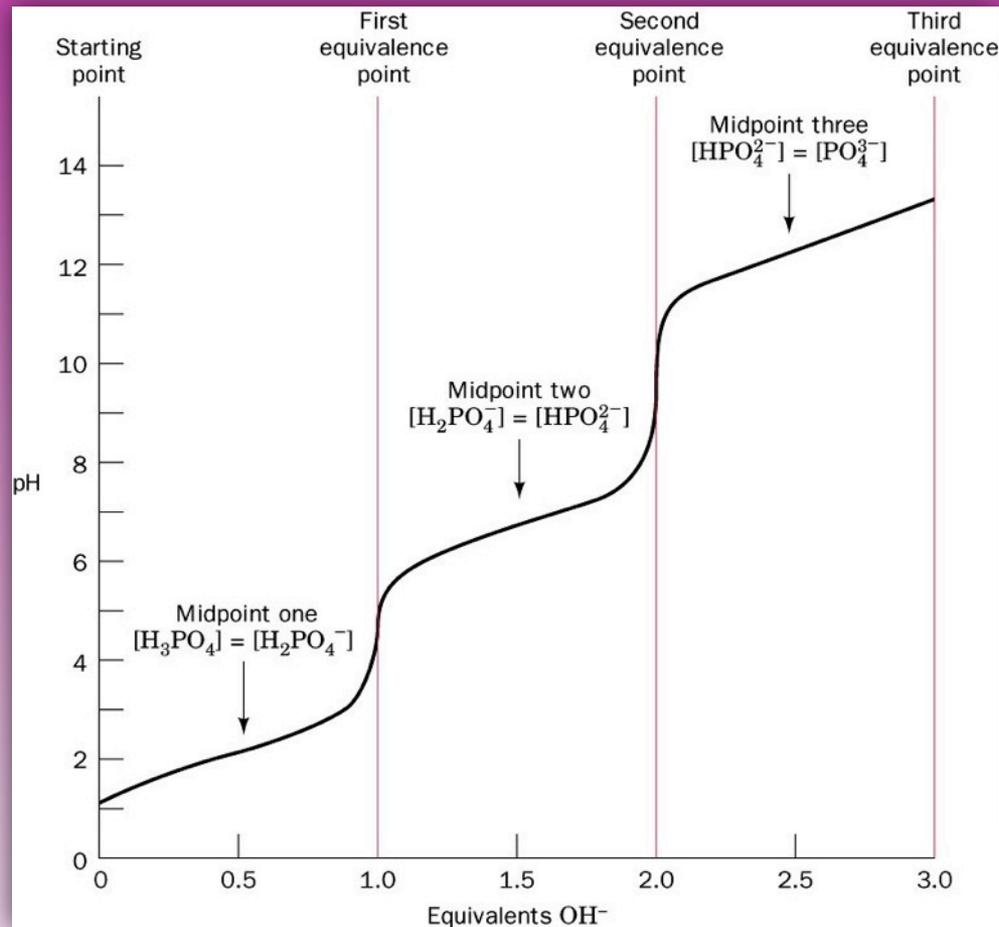
■ Phosphoric acid series

■ Carbonic acid series

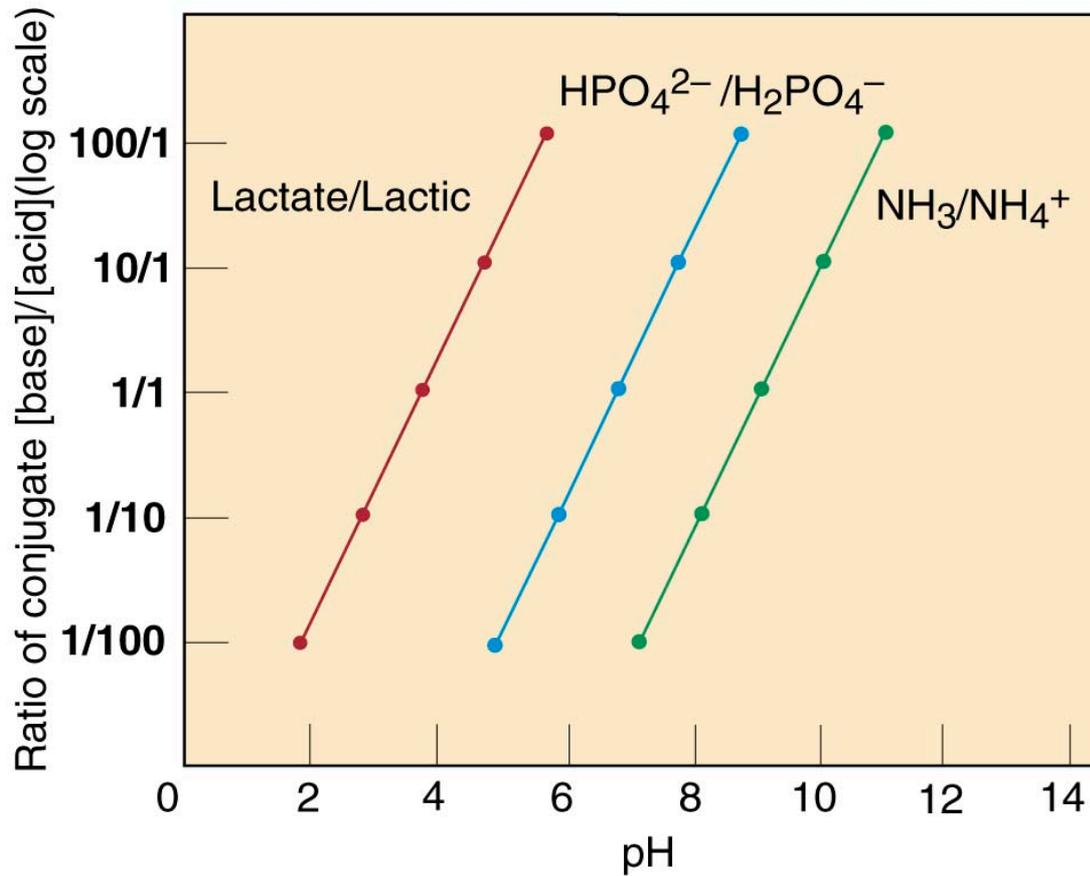
**Acid dissociation constants and  $pK_a$ s for some weak acids commonly used as biochemical buffers**



Acid-base titration curves of 1 liter solutions of 1M acetic acid,  $\text{H}_2\text{PO}_4^-$ , and  $\text{NH}_4^+$  by a strong base



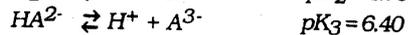
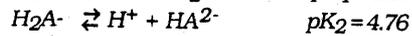
Titration curve of a 1 liter aqueous solution of 1M  $\text{H}_3\text{PO}_4$  (phosphoric acid; a triprotic weak acid with three buffering ranges)



Ratio of  
conjugate  
[base]/[acid]  
as a function  
of pH

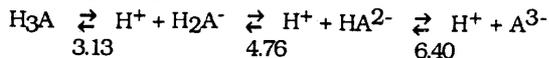
Note specifically how  
this ratio changes for  
each unit of pH  
change in either  
direction from the  
 $\text{p}K_a$ .

10. Citric acid, a tricarboxylic acid important in intermediary metabolism, can be symbolized as  $H_3A$ . Its dissociation reactions are

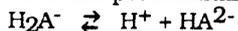


If the total concentration of the acid and its anion forms is 0.2 M, what are the individual concentrations of  $H_3A$ ,  $H_2A^-$ ,  $HA^{2-}$ , and  $A^{3-}$  at pH 5.2?

**Answer:** For citric acid



At pH = 5.2 the predominant equilibrium will involve



The ratio of their concentrations is:

$$\frac{[HA^{2-}]}{[H_2A^-]} = 2.754 \text{ (from Henderson-Hasselbach equation)} \quad (1)$$

Likewise for the other two equilibria we can write:

$$\frac{[H_2A^-]}{[H_3A]} = 117.5 \quad (2)$$

and,

$$\frac{[A^{3-}]}{[HA^{2-}]} = 0.063 \quad (3)$$

Each of the terms are related as follows

$$[A^{3-}] + [HA^{2-}] + [H_2A^-] + [H_3A] = 0.2 \quad (4)$$

Using equations (1), (2) and (3) we can relate the concentration of any one species to any other.

For example,

$$[A^{3-}] = 0.063 [HA^{2-}] \text{ (from 3)}$$

$$[H_2A^-] = \frac{[HA^{2-}]}{2.754} \text{ (from 1)}$$

and,

$$[H_3A] = \frac{[H_2A^-]}{117.5} = \frac{[HA^{2-}]}{2.754 \times 117.5} \text{ (from 1 and 2)}$$

Substituting these expressions into (4), we find:

$$[HA^{2-}] = 0.140$$

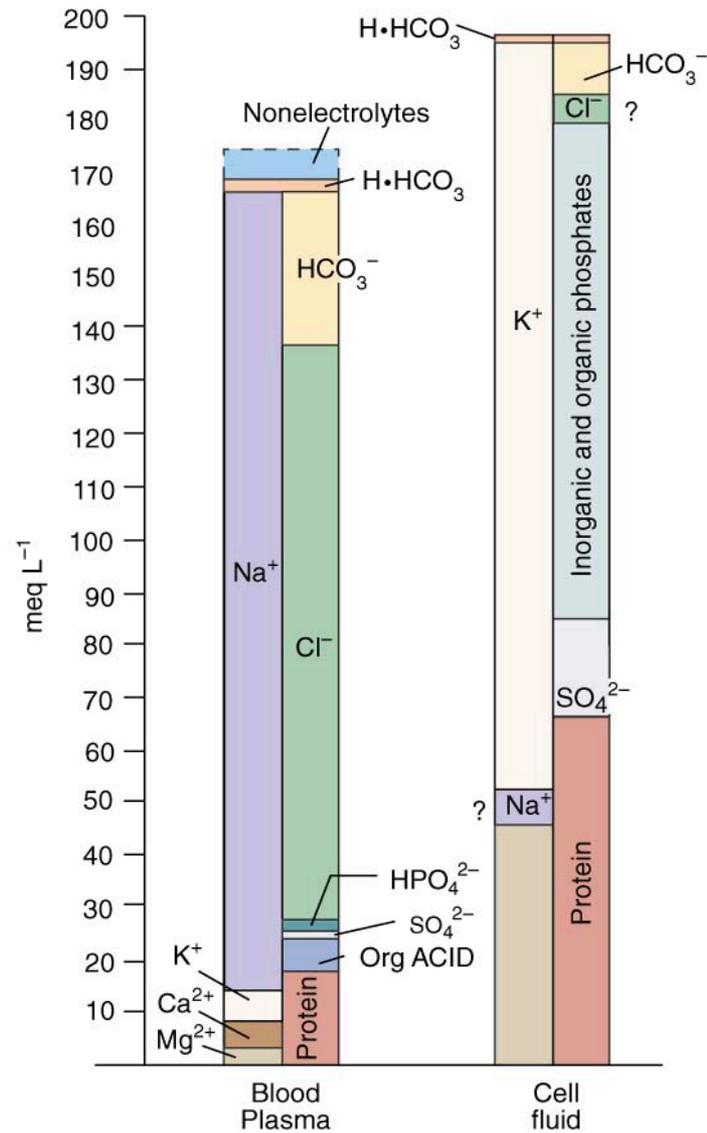
$$\text{From (1)} \quad [H_2A^-] = [HA^{2-}]/2.754 = 0.140/2.754 = 0.051$$

$$\text{From (2)} \quad [H_3A] = [HA^{2-}]/117.5 = 0.001$$

$$\text{From (3)} \quad [A^{3-}] = 0.063 \times 0.140 = 0.009$$

We could have anticipated these results because the pH is far from two of the  $pK_a$ s. Only the equilibrium between  $H_2A^-$  and  $HA^{2-}$  with a  $pK_a = 4.76$  will be significant at pH = 5.2.

An example of a buffer problem involving a tricarboxylic acid with three  $pK_a$  values that are very similar in magnitude (unlike inorganic phosphate,  $H_3PO_4$ ).



**Figure 1.10. Major chemical constituents of blood plasma and cell fluid.** Adapted from Gregersen, M. I. In: P. Bard (Ed.), *Medical Physiology*, 11th ed. St. Louis: Mosby, 1961, p. 307.

# Human Biochemistry

## The Bicarbonate Buffer System of Blood Plasma

The important buffer system of blood plasma is the bicarbonate/carbonic acid couple:



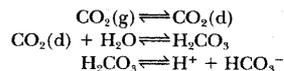
The relevant  $pK_a$ ,  $pK_1$  for carbonic acid, has a value far removed from the normal pH of blood plasma (pH 7.4). (The  $pK_1$  for  $\text{H}_2\text{CO}_3$  at 25°C is 3.77 [Table 2.4], but at 37°C,  $pK_1$  is 3.57.) At pH 7.4, the concentration of  $\text{H}_2\text{CO}_3$  is a minuscule fraction of the  $\text{HCO}_3^-$  concentration; thus the plasma appears to be poorly protected against an influx of  $\text{OH}^-$  ions.

$$\text{pH} = 7.4 = 3.57 + \log_{10} \frac{[\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3]}$$

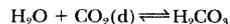
$$\frac{[\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3]} = 6761$$

For example, if  $[\text{HCO}_3^-] = 24 \text{ mM}$ , then  $[\text{H}_2\text{CO}_3]$  is only  $3.55 \mu\text{M}$  ( $3.55 \times 10^{-6} \text{ M}$ ), and an equivalent amount of  $\text{OH}^-$  (its usual concentration in plasma) would swamp the buffer system, causing a dangerous rise in the plasma pH. How, then, can this bicarbonate system function effectively? The bicarbonate buffer system works well because the critical concentration of  $\text{H}_2\text{CO}_3$  is maintained relatively constant through equilibrium with dissolved  $\text{CO}_2$  produced in the tissues and available as a gaseous  $\text{CO}_2$  reservoir in the lungs.\*

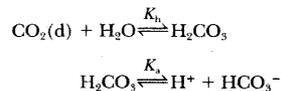
Gaseous  $\text{CO}_2$  from the lungs and tissues is dissolved in the blood plasma, symbolized as  $\text{CO}_2(\text{d})$ , and hydrated to form  $\text{H}_2\text{CO}_3$ :



Thus, the concentration of  $\text{H}_2\text{CO}_3$  is itself buffered by the available pools of  $\text{CO}_2$ . The hydration of  $\text{CO}_2$  is actually mediated by an enzyme, *carbonic anhydrase*, which facilitates the equilibrium by rapidly catalyzing the reaction



Under the conditions of temperature and ionic strength prevailing in mammalian body fluids, the equilibrium for this reaction lies far to the left, such that more than 300  $\text{CO}_2$  molecules are present in solution for every molecule of  $\text{H}_2\text{CO}_3$ . Because dissolved  $\text{CO}_2$  and  $\text{H}_2\text{CO}_3$  are in equilibrium, the proper expression for  $\text{H}_2\text{CO}_3$  availability is  $[\text{CO}_2(\text{d})] + [\text{H}_2\text{CO}_3]$ , the so-called total carbonic acid pool, consisting primarily of  $\text{CO}_2(\text{d})$ . The overall equilibrium for the bicarbonate buffer system then is



An expression for the ionization of  $\text{H}_2\text{CO}_3$  under such conditions (that is, in the presence of dissolved  $\text{CO}_2$ ) can be obtained from

$K_h$ , the equilibrium constant for the hydration of  $\text{CO}_2$ , and from  $K_a$ , the first acid dissociation constant for  $\text{H}_2\text{CO}_3$ :

$$K_h = \frac{[\text{H}_2\text{CO}_3]}{[\text{CO}_2(\text{d})]}$$

Thus,

$$[\text{H}_2\text{CO}_3] = K_h[\text{CO}_2(\text{d})]$$

Putting this value for  $[\text{H}_2\text{CO}_3]$  into the expression for the first dissociation of  $\text{H}_2\text{CO}_3$  gives

$$\begin{aligned} K_a &= \frac{[\text{H}^+][\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3]} \\ &= \frac{[\text{H}^+][\text{HCO}_3^-]}{K_h[\text{CO}_2(\text{d})]} \end{aligned}$$

Therefore, the overall equilibrium constant for the ionization of  $\text{H}_2\text{CO}_3$  in equilibrium with  $\text{CO}_2(\text{d})$  is given by

$$K_a K_h = \frac{[\text{H}^+][\text{HCO}_3^-]}{[\text{CO}_2(\text{d})]}$$

and  $K_a K_h$ , the product of two constants, can be defined as a new equilibrium constant,  $K_{\text{overall}}$ . The value of  $K_h$  is 0.003 at 37°C and  $K_a$ , the ionization constant for  $\text{H}_2\text{CO}_3$ , is  $10^{-3.57} = 0.000269$ . Therefore,

$$\begin{aligned} K_{\text{overall}} &= (0.000269)(0.003) \\ &= 8.07 \times 10^{-7} \\ pK_{\text{overall}} &= 6.1 \end{aligned}$$

which yields the following Henderson-Hasselbalch relationship:

$$\text{pH} = pK_{\text{overall}} + \log_{10} \frac{[\text{HCO}_3^-]}{[\text{CO}_2(\text{d})]}$$

Although the prevailing blood pH of 7.4 is more than 1 pH unit away from  $pK_{\text{overall}}$ , the bicarbonate system is still an effective buffer. Note that, at blood pH, the concentration of the acid component of the buffer will be less than 10% of the conjugate base component. One might imagine that this buffer component could be overwhelmed by relatively small amounts of alkali, with consequent disastrous rises in blood pH. However, the acid component is the total carbonic acid pool, that is,  $[\text{CO}_2(\text{d})] + [\text{H}_2\text{CO}_3]$ , which is stabilized by its equilibrium with  $\text{CO}_2(\text{g})$ . Gaseous  $\text{CO}_2$  serves to buffer any losses from the total carbonic acid pool by entering solution as  $\text{CO}_2(\text{d})$ , and blood pH is effectively maintained. Thus, the bicarbonate buffer system is an *open system*. The natural presence of  $\text{CO}_2$  gas at a partial pressure of 40 mm Hg in the alveoli of the lungs and the equilibrium



keep the concentration of  $\text{CO}_2(\text{d})$  (the principal component of the total carbonic acid pool in blood plasma) in the neighborhood of 1.2 mM. Plasma  $[\text{HCO}_3^-]$  is about 24 mM under such conditions.

\*Well-fed humans exhale about 1 kg of  $\text{CO}_2$  daily. Imagine the excretory problem if  $\text{CO}_2$  were not a volatile gas.

# Definitions of Buffer Capacity

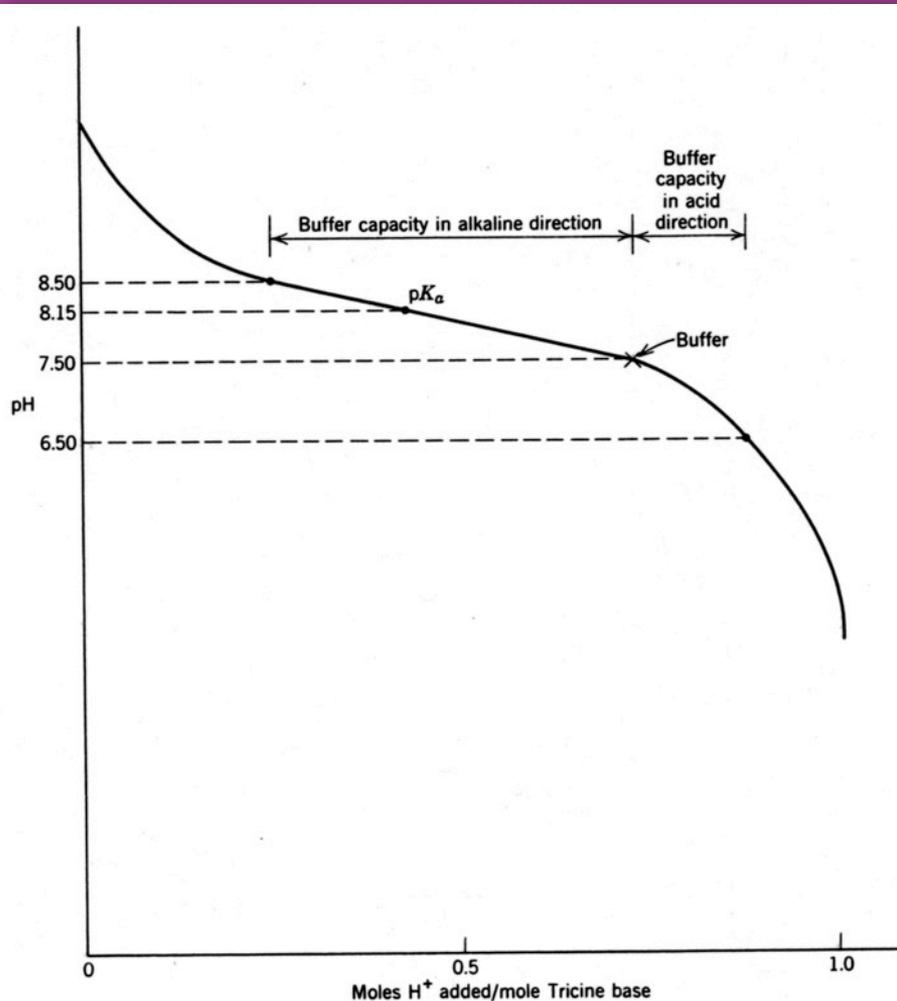


Figure 1-3 Titration of a weak monoprotic base (e.g., Tricine) with a strong acid (e.g., HCl).  $pK_a$  of Tricine = 8.15.

The number of moles of  $H^+$  that must be added to one liter of buffer in order to decrease the pH by 1 unit = the buffer capacity in the acid direction

and

The number of moles of  $OH^-$  that must be added to one liter of buffer in order to increase the pH by one unit = the buffer capacity in the alkaline direction.

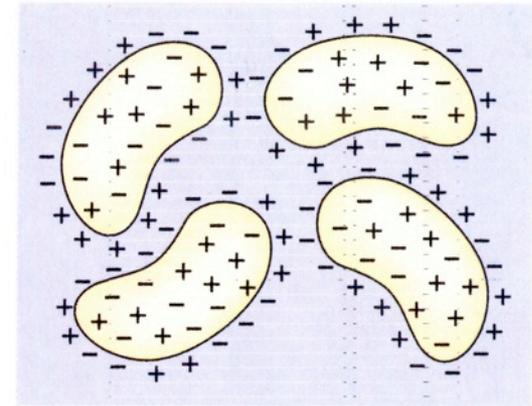
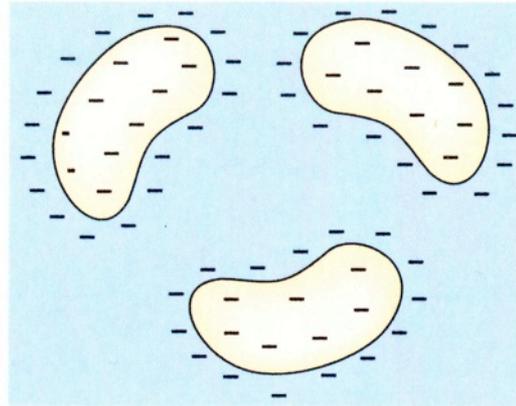
## Characteristics important to buffers used in biochemical experiments

- ❑  $pK_a$  value
- ❑ Variation of  $pK_a$  with temperature and ionic strength
- ❑ Anionic, cationic, or multiple charges on buffer species
- ❑ Interaction with other components (*e.g.*, metal ions)
- ❑ Solubility
- ❑ Expense
- ❑ UV absorption

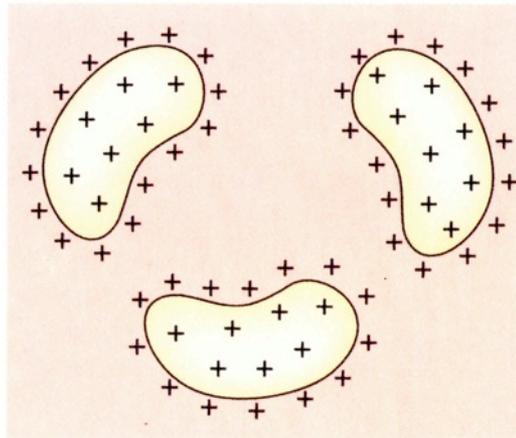
FIGURE 2.21

**Dependence of protein solubility on pH.**

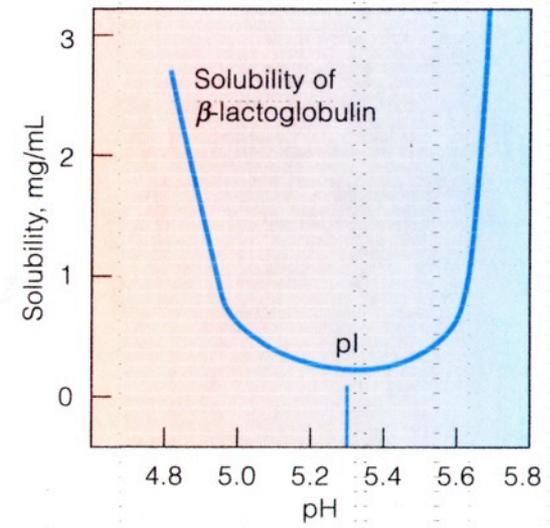
**(a)** Most proteins are very soluble at high pH, where all of their molecules are negatively charged. **(b)** At the isoelectric point, where a protein has no *net* charge, its molecules retain regions of positive and negative charge on their surfaces, resulting in aggregation and precipitation. **(c)** At low pH the proteins are soluble because of their positive charge. **(d)** The solubility of  $\beta$ -lactoglobulin with varying pH; the lowest solubility occurs at the isoelectric point.



**(a)** High pH: protein soluble (deprotonated) **(b)** Isoelectric point: protein aggregates



**(c)** Low pH: protein soluble (protonated)



**(d)** Solubility of  $\beta$ -lactoglobulin

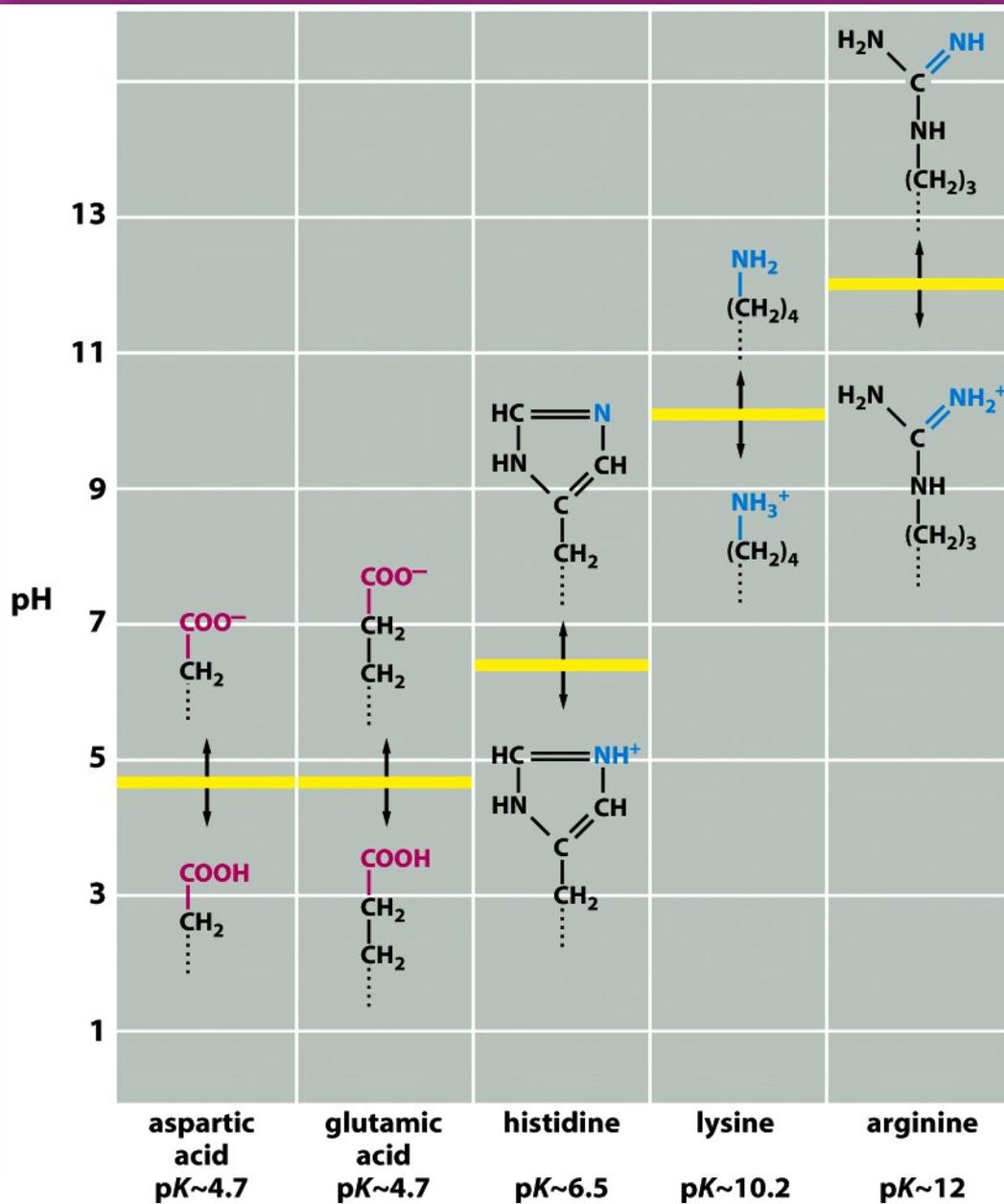
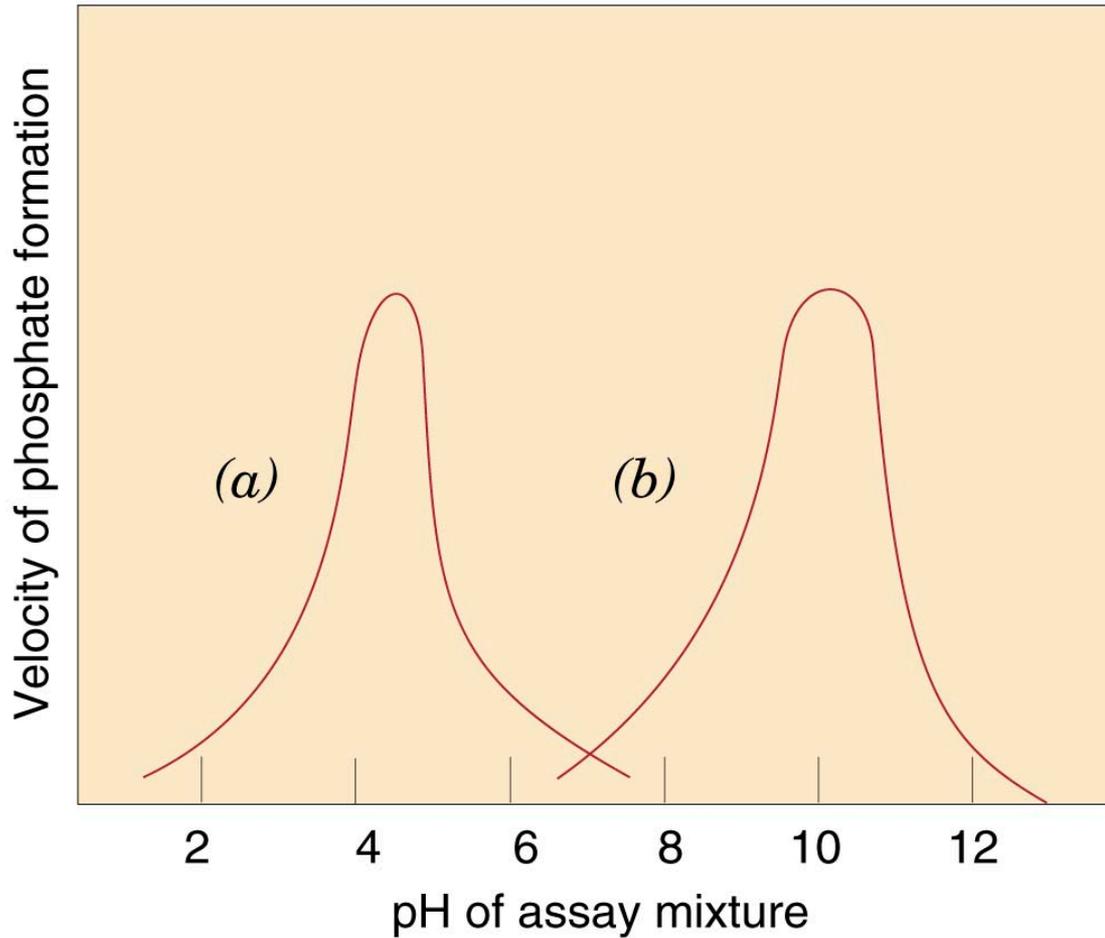


Figure 2-25 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Some ionizable amino acid sidechains found in proteins

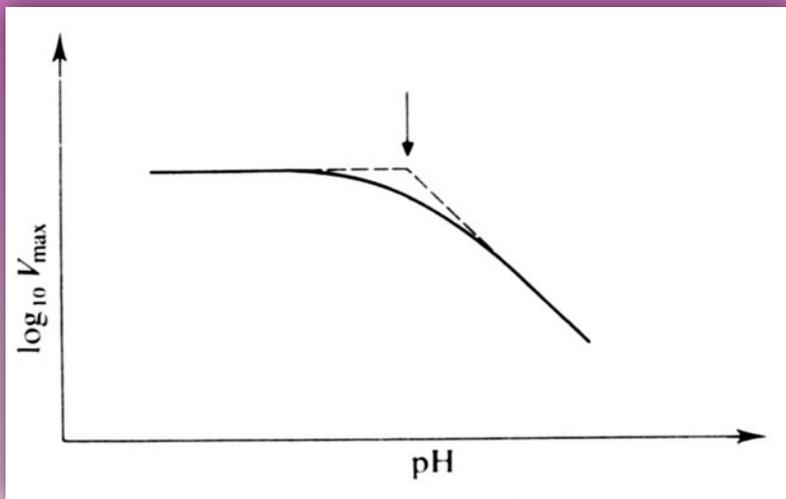
One or more of these groups can be found in the active site of an enzyme.



**Figure 10.52. The pH dependence of (a) acid and (b) alkaline phosphatase reactions.**

*Textbook of Biochemistry With Clinical Correlations, Sixth Edition, Edited by Thomas M. Devlin. Copyright © 2006 John Wiley & Sons, Inc.*

## Effect of pH on enzyme catalytic activity



Schematic representation of the effect of pH on the velocity of an enzyme-catalyzed reaction if only the protonated form of a single ionizing group is catalytically active. The solid curve represents the experimental data.

The  $pK_a$  of this group can be estimated by extrapolating the linear portions of the curve as shown.

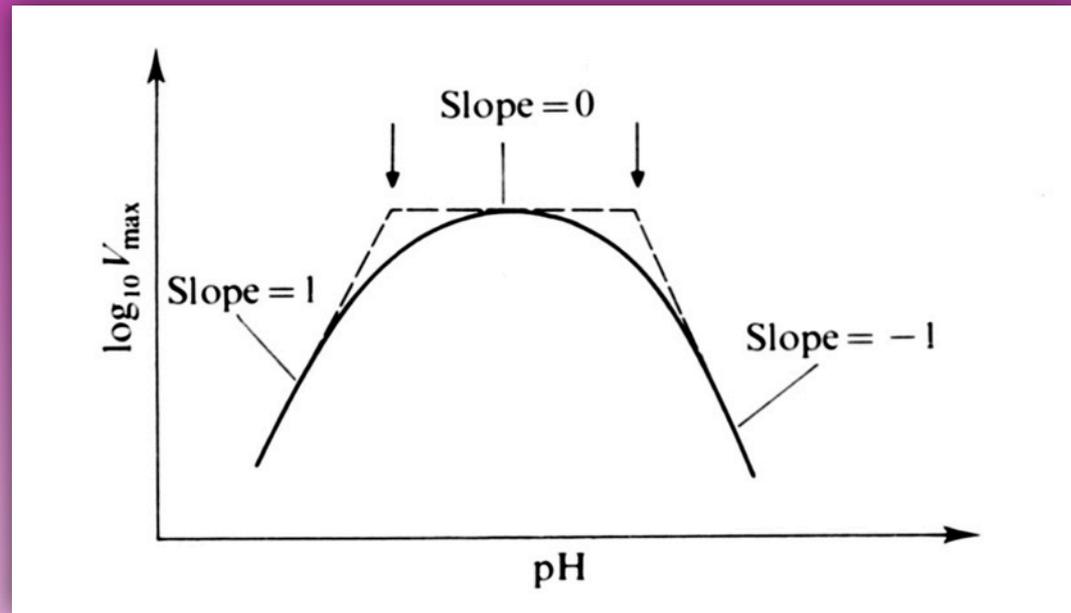
## *Ionization properties of some amino acid side chains*

Group	$pK_a(298\text{ K})$	$\Delta H_i(\text{kJ mol}^{-1})$
$\beta$ -Carboxyl (Asp)} $\gamma$ -Carboxyl (Glu) }	$\sim 4$	$\sim \pm 4$
Imidazole (His)	$\sim 6$	$\sim 29$
$\epsilon$ -Amino (Lys)	$\sim 10$	$\sim 46$
Phenolic OH (Tyr)	$\sim 10$	$\sim 25$

The assignment of  $pK_a$ s to specific amino acid sidechains is not straightforward since these  $pK_a$ s are affected by local protein structure. For example, in pepsin, an Asp residue involved in the mechanism (pepsin is an aspartic protease) has a  $pK_a$  of 1.1, whereas this sidechain has a  $pK_a$  of  $\sim 4$  in the free amino acid.

If the rate dependence on pH is known as a function of temperature, then enthalpies of ionization ( $\Delta H_i$ ) can sometimes help to make the correlation between the measured  $pK_a$  and the functional group.





The effect of pH on the velocity of an enzyme-catalyzed reaction when two ionizing groups are involved. The solid line is the experimental data.

Extrapolation of the appropriate linear portions of the plot yield values of  $pK_{a1}$  and  $pK_{a2}$  as shown.

# Review of key functional groups in biochemistry: Structure and properties

alcohol

aldehyde

carboxyl

amine

ketone

amide

oxyester

thioester

thiol

disulfide

imine

phosphoanhydride

mixed anhydride