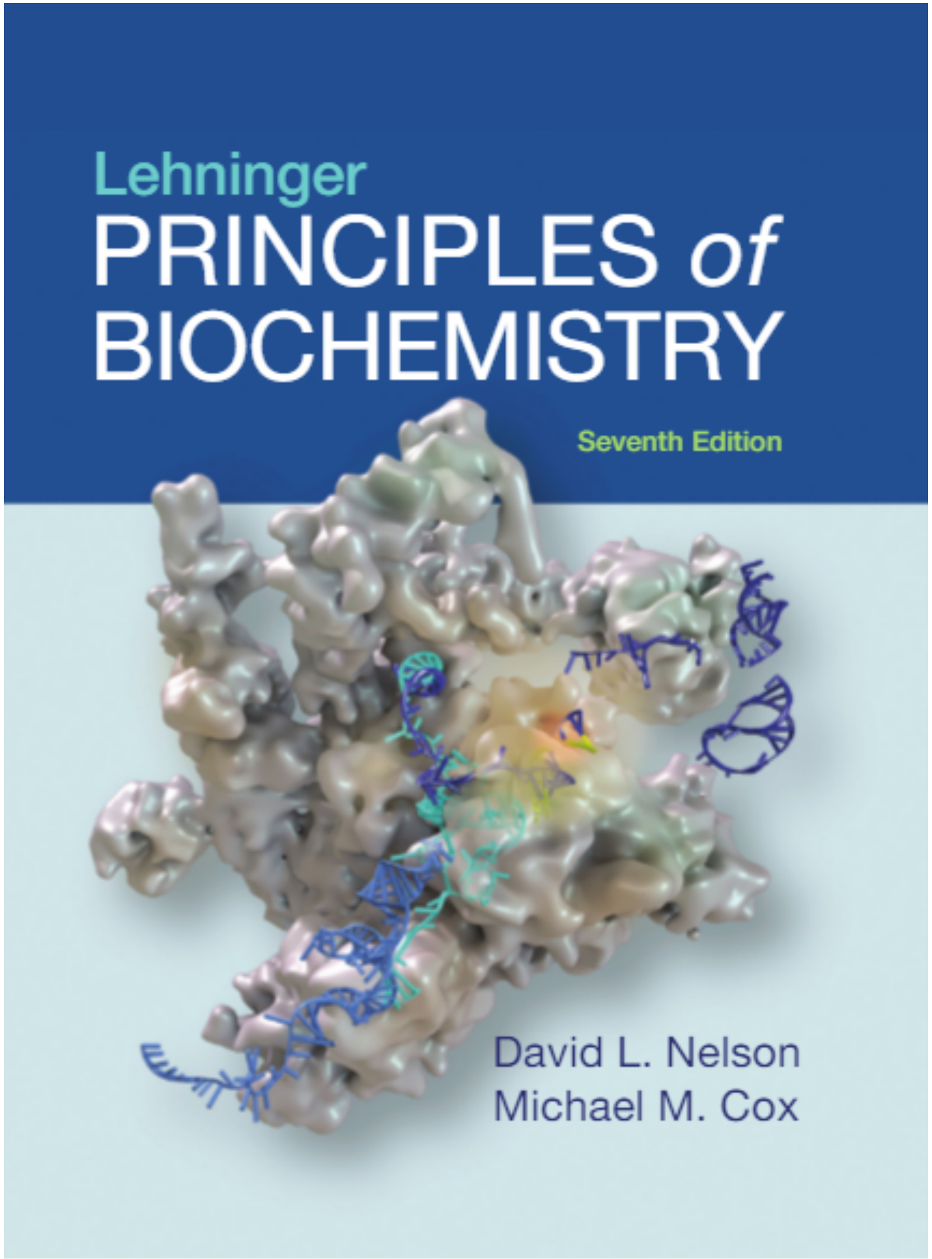
1 | The Foundations of Biochemistry

2 | Water



Lehninger
PRINCIPLES *of*
BIOCHEMISTRY

Seventh Edition

David L. Nelson
Michael M. Cox

Biochemistry is the Chemistry of Living Matter

- The basis of all life is the chemical reactions that take place within the cell.

Chemistry allows for:

- A high degree of complexity and organization
- Extraction, transformation, and systematic use of energy to create and maintain structures and to do work
- The interactions of individual components to be dynamic and coordinated.
- The ability to sense and respond to changes in surrounding
- A capacity for fairly precise self-replication while allowing enough change for evolution

The Molecular Logic of Life

We look at the chemistry that is behind:

- Accelerating reactions
- Organization of metabolism and signaling
- Storage and transfer of information

The ABCs of Life

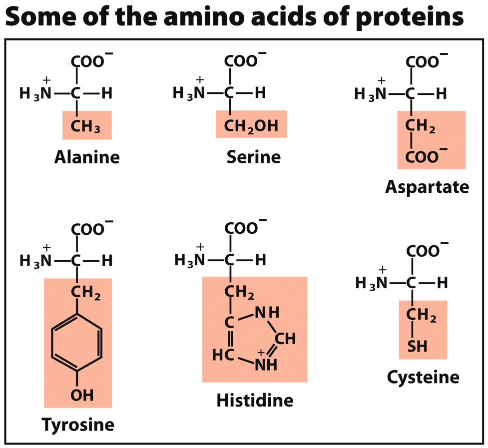


Figure 1-10a
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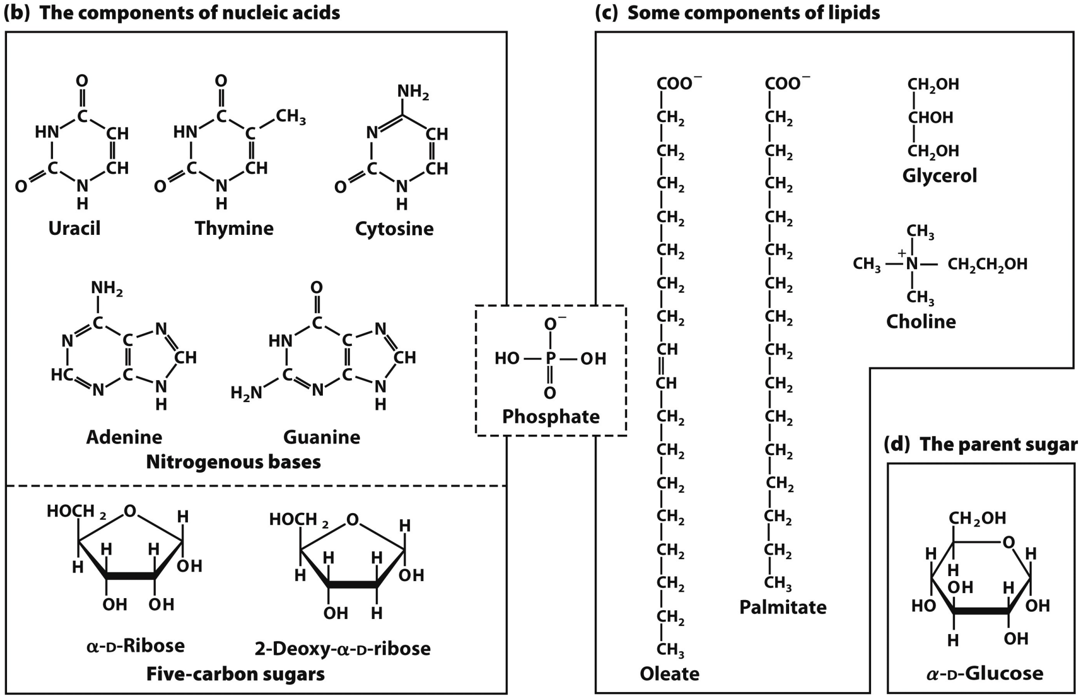

Figure 1-10bcd
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The Molecular Hierarchy of Structure

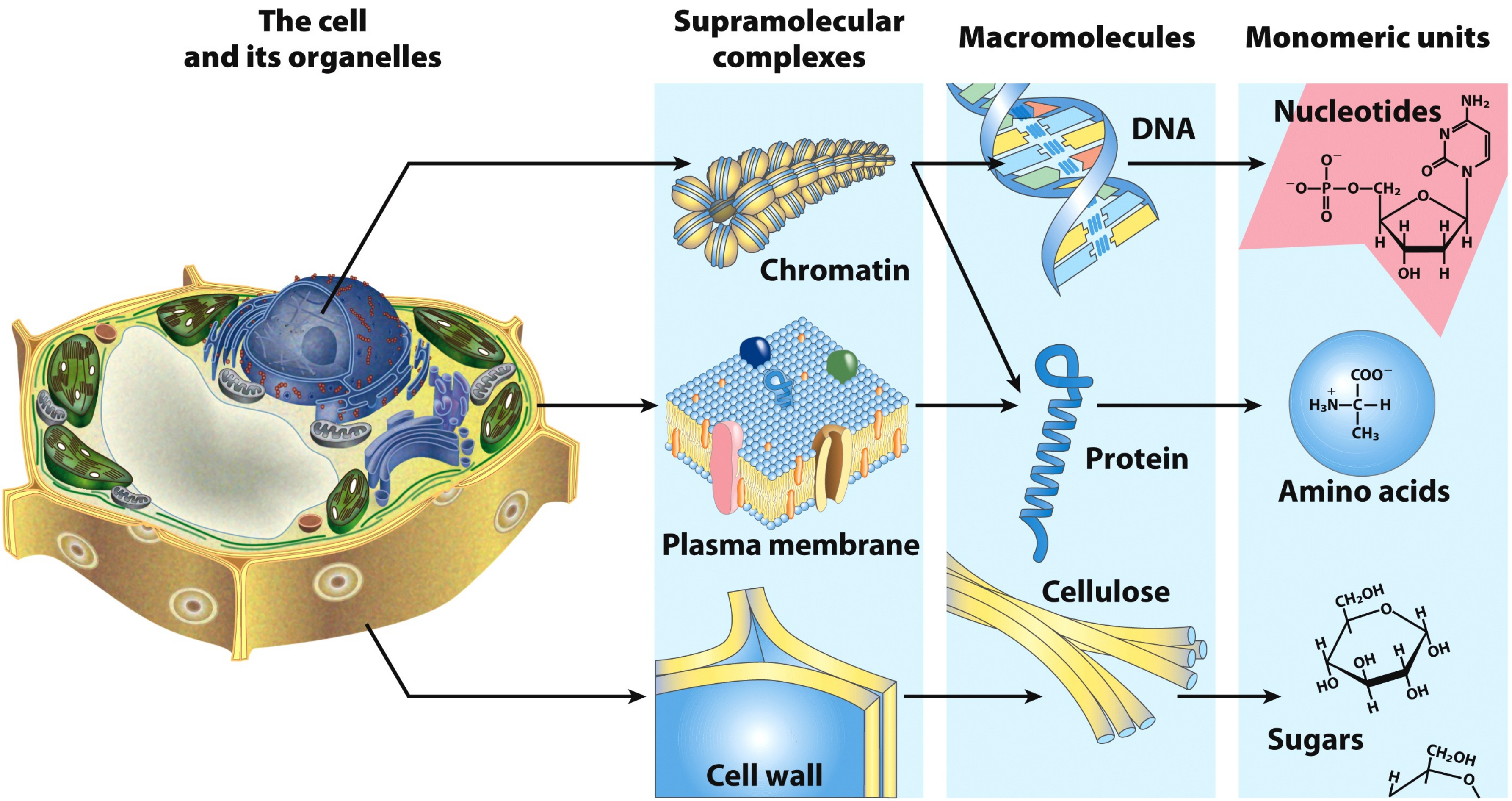

Figure 1-11
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Diameter, appx: Mito **(1000 nm);** ribosomes **(20 nm);** Hb **(5.5 nm);** Alanine **(0.5 nm)**

In vitro, in vivo, in silico

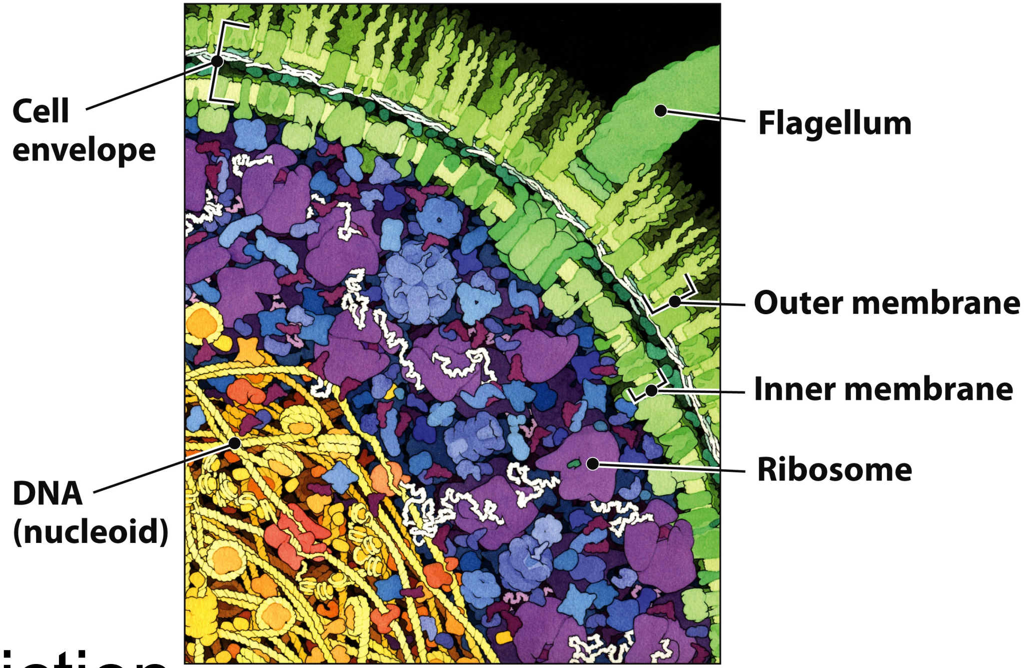
We can study biological processes either

- ***In vivo*** – in the living, study of molecules within cells
- ***In vitro*** – in glass, study of molecules without interference from other molecules
- ***In silico*** – simulations using computers

- Keeping in mind that the environment in the cell is much different from inside the test tube

The cytosol is very crowded

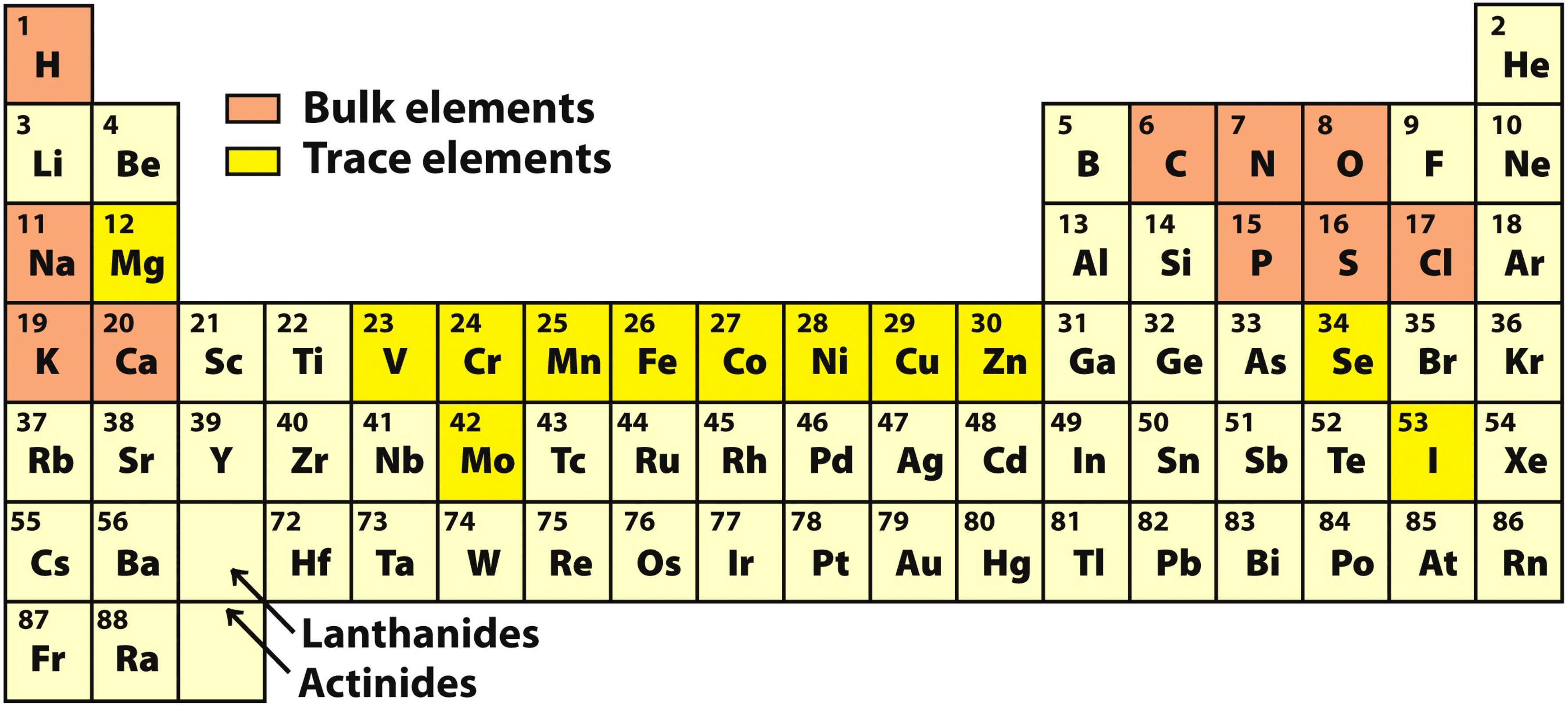
A certain molecule can behave differently in vitro than in vivo



- Crowding of cytosol → less diffusion of molecules and more friction
- An enzyme activity in vitro may be very different from that in vivo

Biochemistry: 30 Elements Essential for Life

- Other than carbon, elements H, O, N, P, S are also common
- Metal ions (e.g., K^+, Na^+, Ca^{++}, Mg^{++}, Zn^{++}, Fe^{++}) play important roles in metabolism



1 H																	2 He
3 Li	4 Be											5 B	6 C	7 N	8 O	9 F	10 Ne
11 Na	12 Mg											13 Al	14 Si	15 P	16 S	17 Cl	18 Ar
19 K	20 Ca	21 Sc	22 Ti	23 V	24 Cr	25 Mn	26 Fe	27 Co	28 Ni	29 Cu	30 Zn	31 Ga	32 Ge	33 As	34 Se	35 Br	36 Kr
37 Rb	38 Sr	39 Y	40 Zr	41 Nb	42 Mo	43 Tc	44 Ru	45 Rh	46 Pd	47 Ag	48 Cd	49 In	50 Sn	51 Sb	52 Te	53 I	54 Xe
55 Cs	56 Ba		72 Hf	73 Ta	74 W	75 Re	76 Os	77 Ir	78 Pt	79 Au	80 Hg	81 Tl	82 Pb	83 Bi	84 Po	85 At	86 Rn
87 Fr	88 Ra																

Figure 1-13
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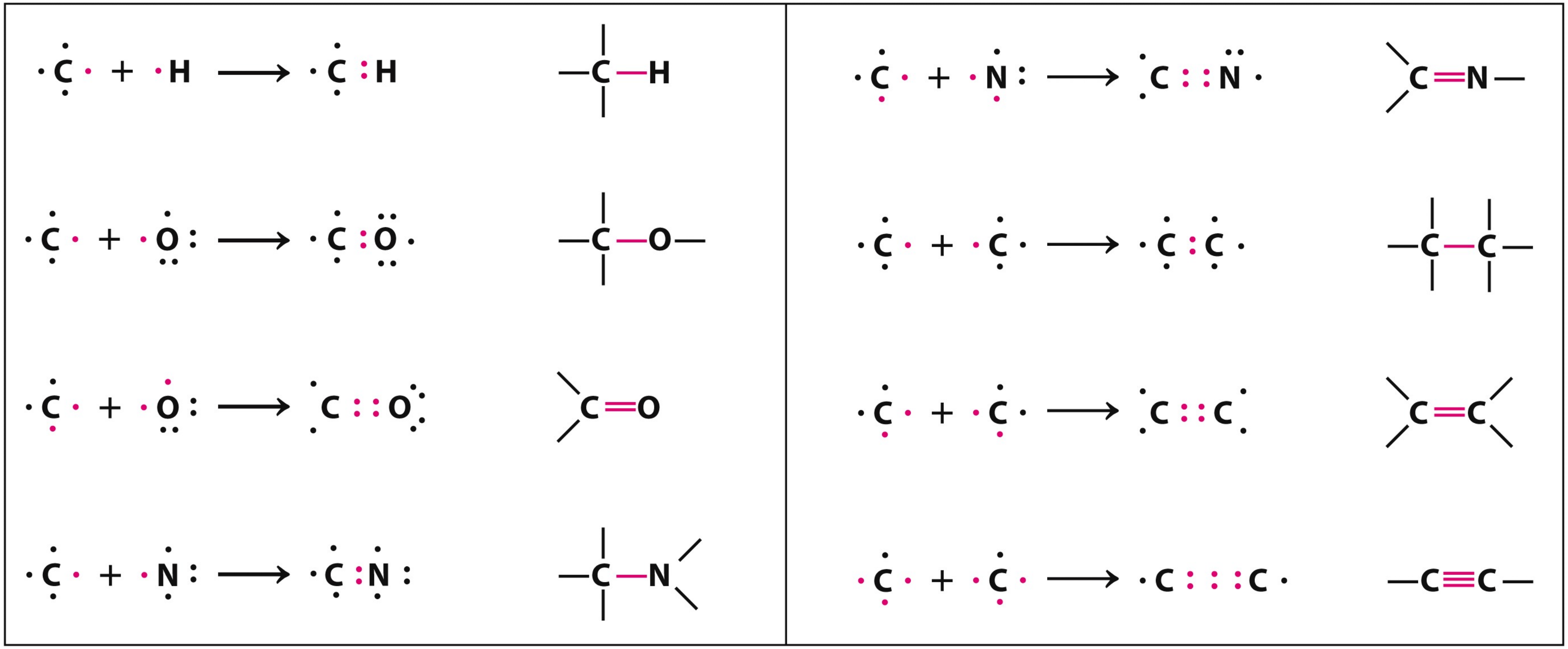
Biochemistry: Unique Role of Carbon

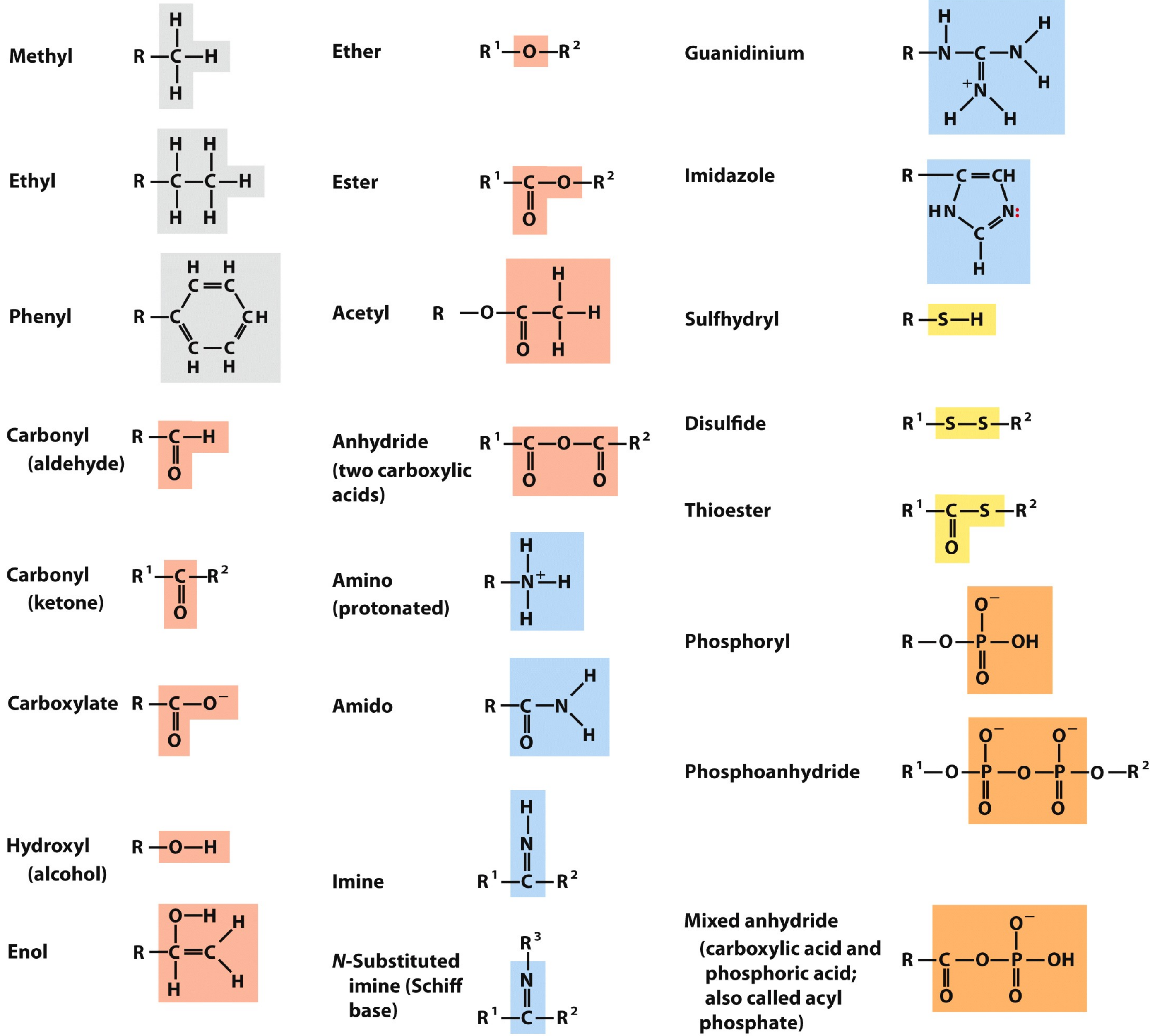
- Ability of C to form very stable bond with up to 4 other C atoms



- Recall, single bonds are 0.154 nm, double bonds are 0.134 nm
- Bonding versatility of C was a major factor in selecting C for molecular machinery during evolution. *No other chemical element can form molecules of such widely different sizes, shapes and composition!*

Common Functional Groups of Biological Molecules

- **Biomolecules** are derivatives of hydrocarbons
- H-atom is replaced by functional groups
- *Recall the naming of groups, and groups without H from organic chemistry*



Biological molecules typically have several functional groups (polyfunctional)

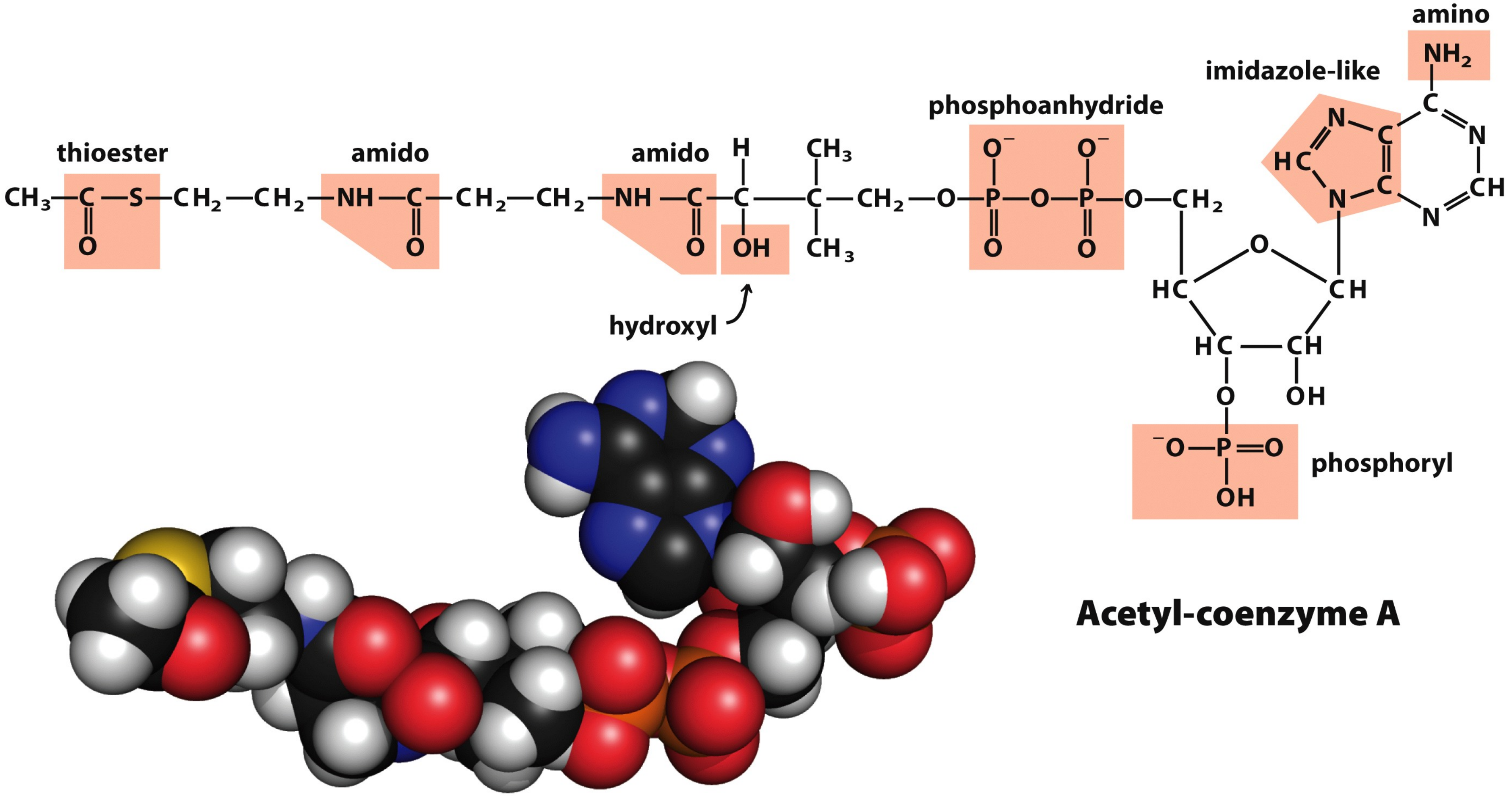


Figure 1-17
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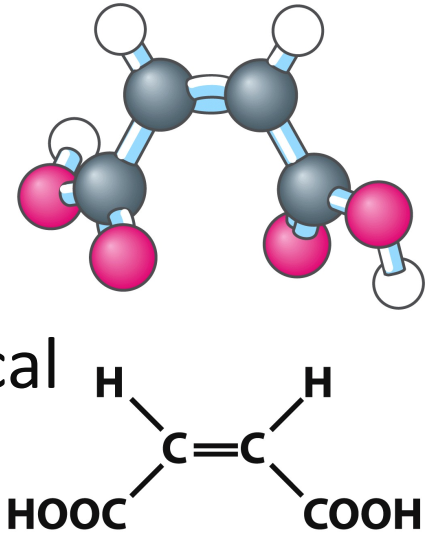
The function of molecules strongly depends on three-dimensional structure

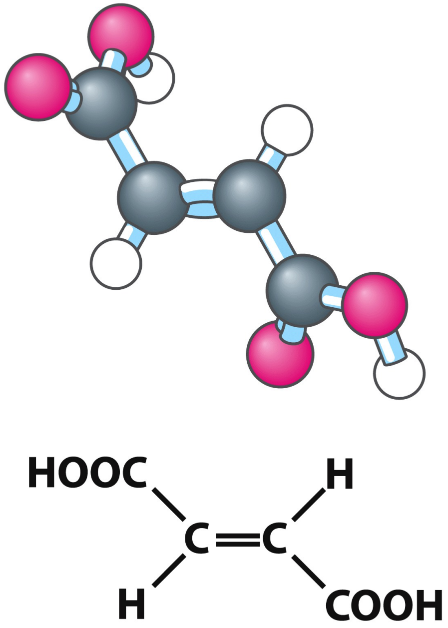
- Stereoisomers
  - have different physical properties
  - have same chemical bonds, different **configuration** around the bonds
  - Cannot be interconverted without temporarily breaking one or more covalent bonds
  - Configuration is due to:
    (1) double bonds
    (2) chiral centers
  - Interactions between biomolecules are **stereospecific**

Cis-Trans isomers (geometrical isomers)

- Geometric Isomers
  - have different physical and chemical properties
  - differ in the arrangement about a double bond



Maleic acid (cis)



Fumaric acid (trans)

  - An enzyme binding site that fits maleic acid will not fit fumaric acid and vice versa

Why we see is because of a cis-trans configurational change

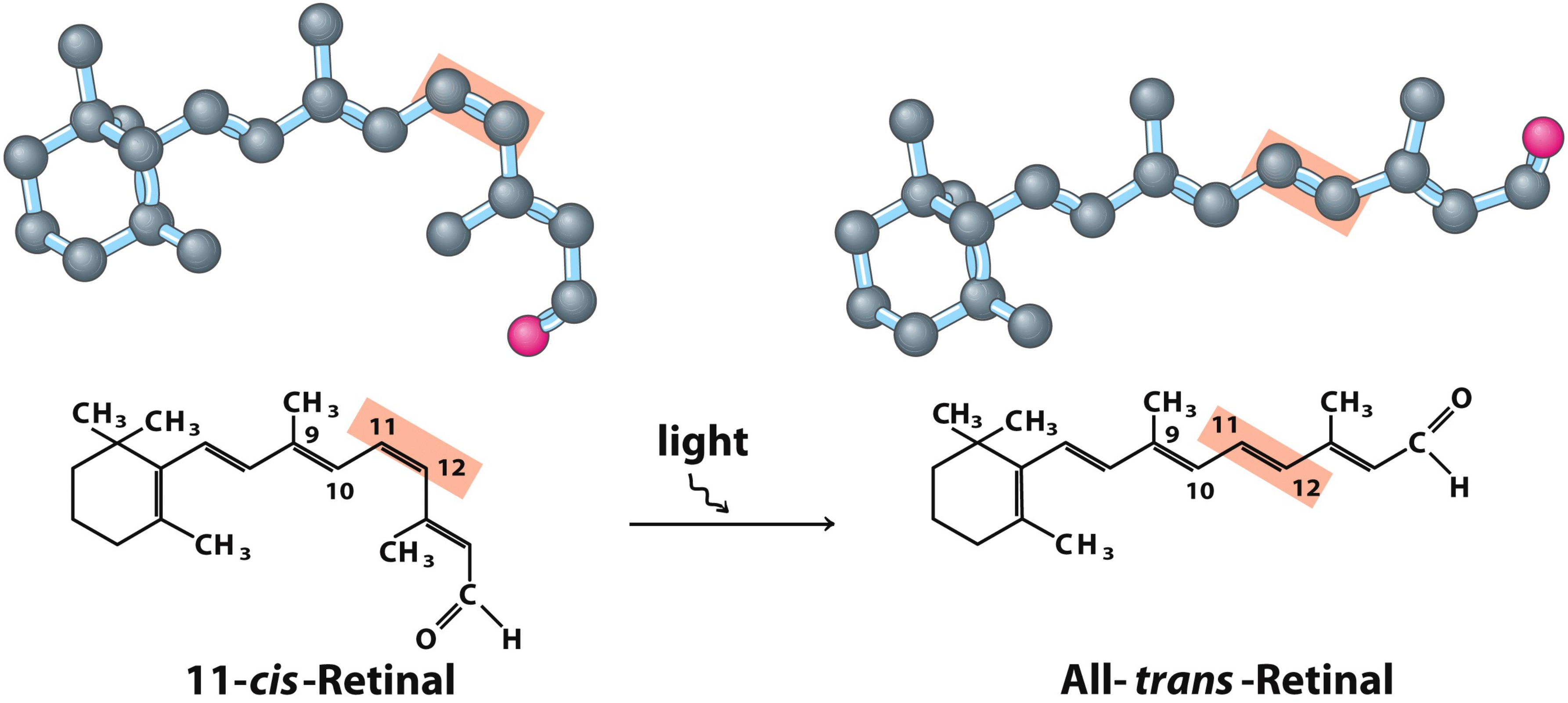


Figure 1-19b
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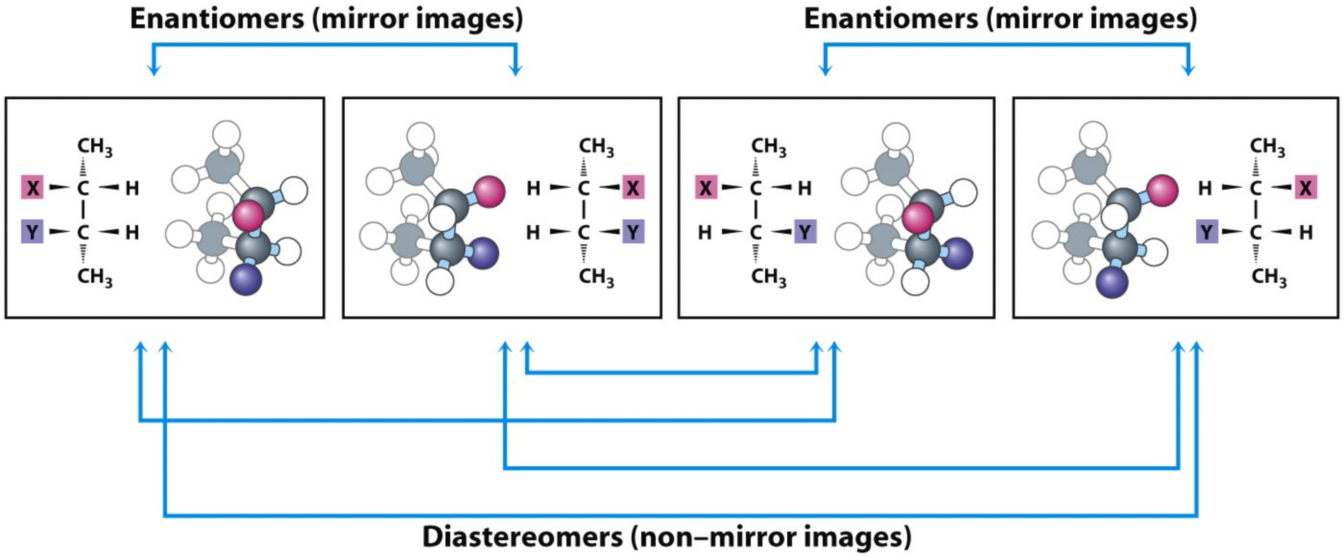
Enantiomers and Diastereomers



- Enantiomers (mirror images)
  - have identical physical properties (except with regard to polarized light) and react identically with achiral reagents
- Diastereomers
  - have different physical and chemical properties

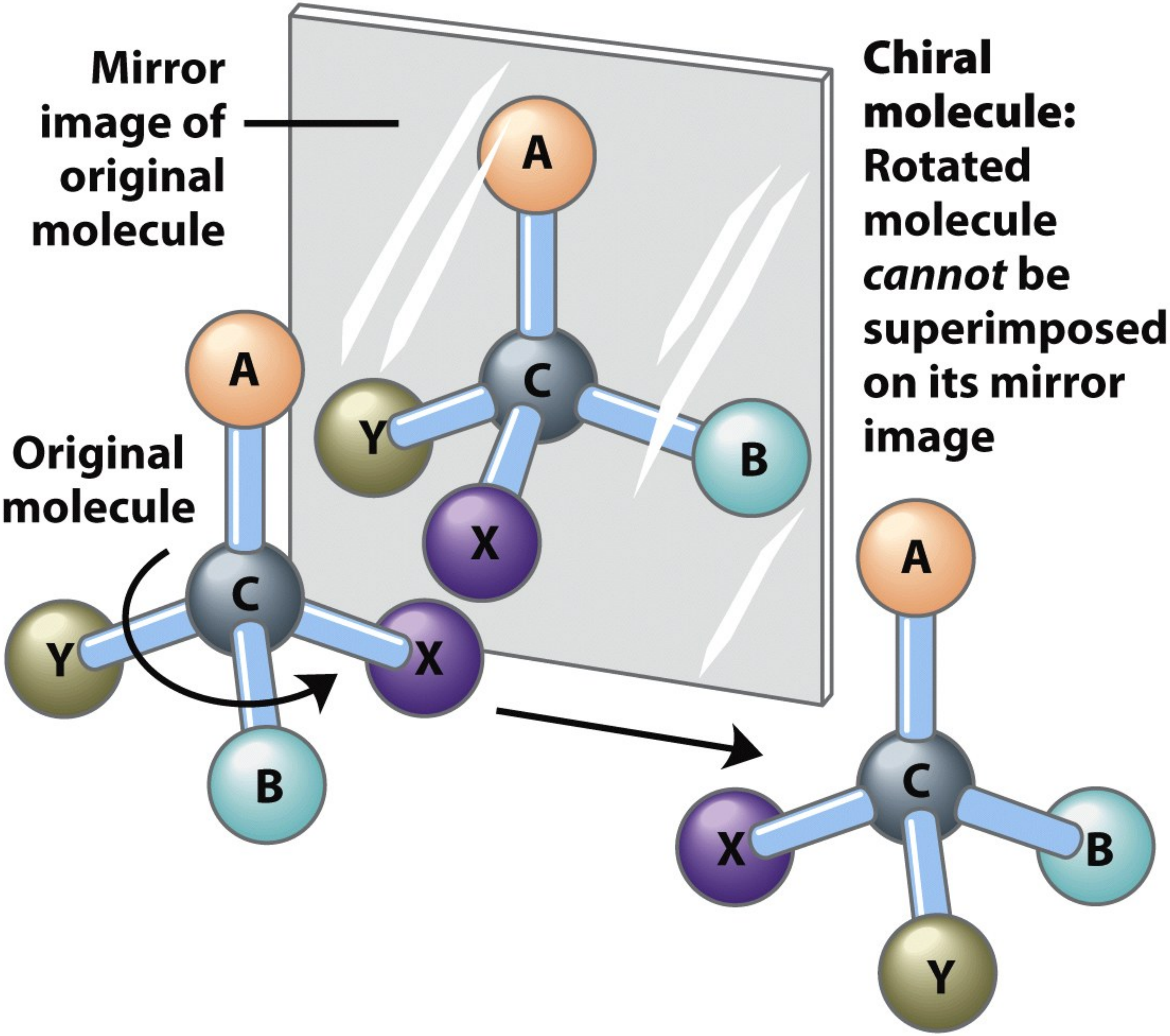


Figure 1-19a
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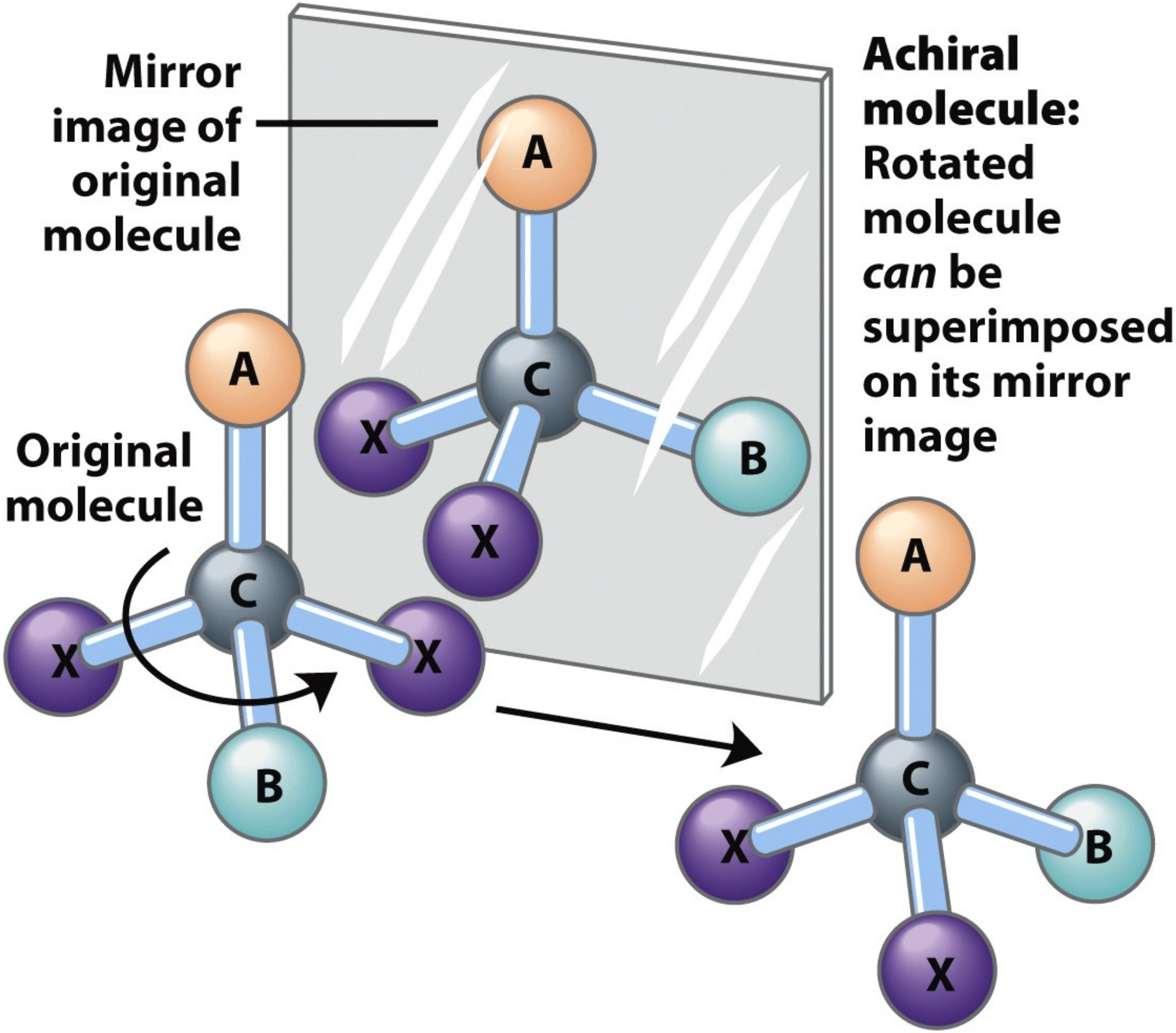

Achiral molecule: Rotated molecule *can* be superimposed on its mirror image

Figure 1-19b

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The function of molecules strongly depends on three-dimensional structure

- All molecules can assume different **conformations**
  - Spatial arrangement of groups that are free to take different positions *without breaking any bonds*
  - Because of freedom of rotation about single bonds
  - Some conformations are more favorable than others (e.g. staggered versus eclipsed in ethane)

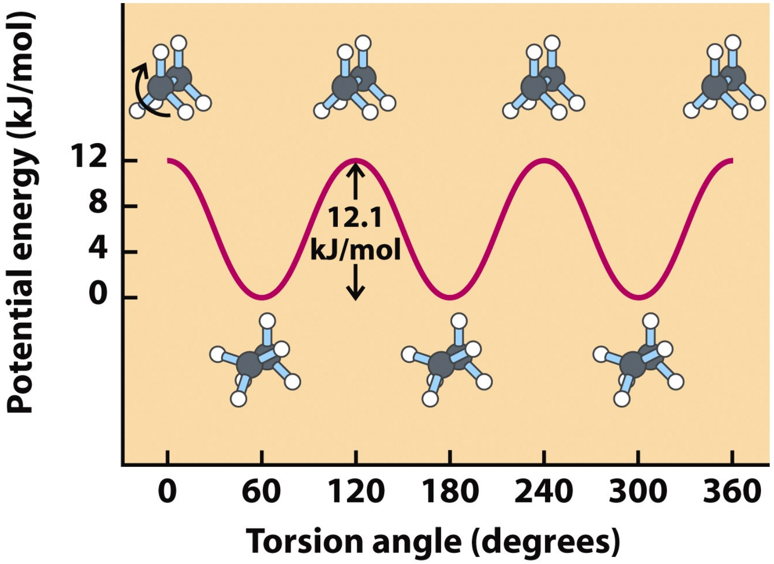

Figure 1-21
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Interactions between biomolecules are specific

- Macromolecules have unique binding pockets
- Only certain molecules fit in well and can bind
- Binding of chiral biomolecules is stereospecific (if a binding site on a protein is complementary to the L stereoisomer, it will not fit the D sterioisomer)

Interactions Between Biomolecules Are Specific

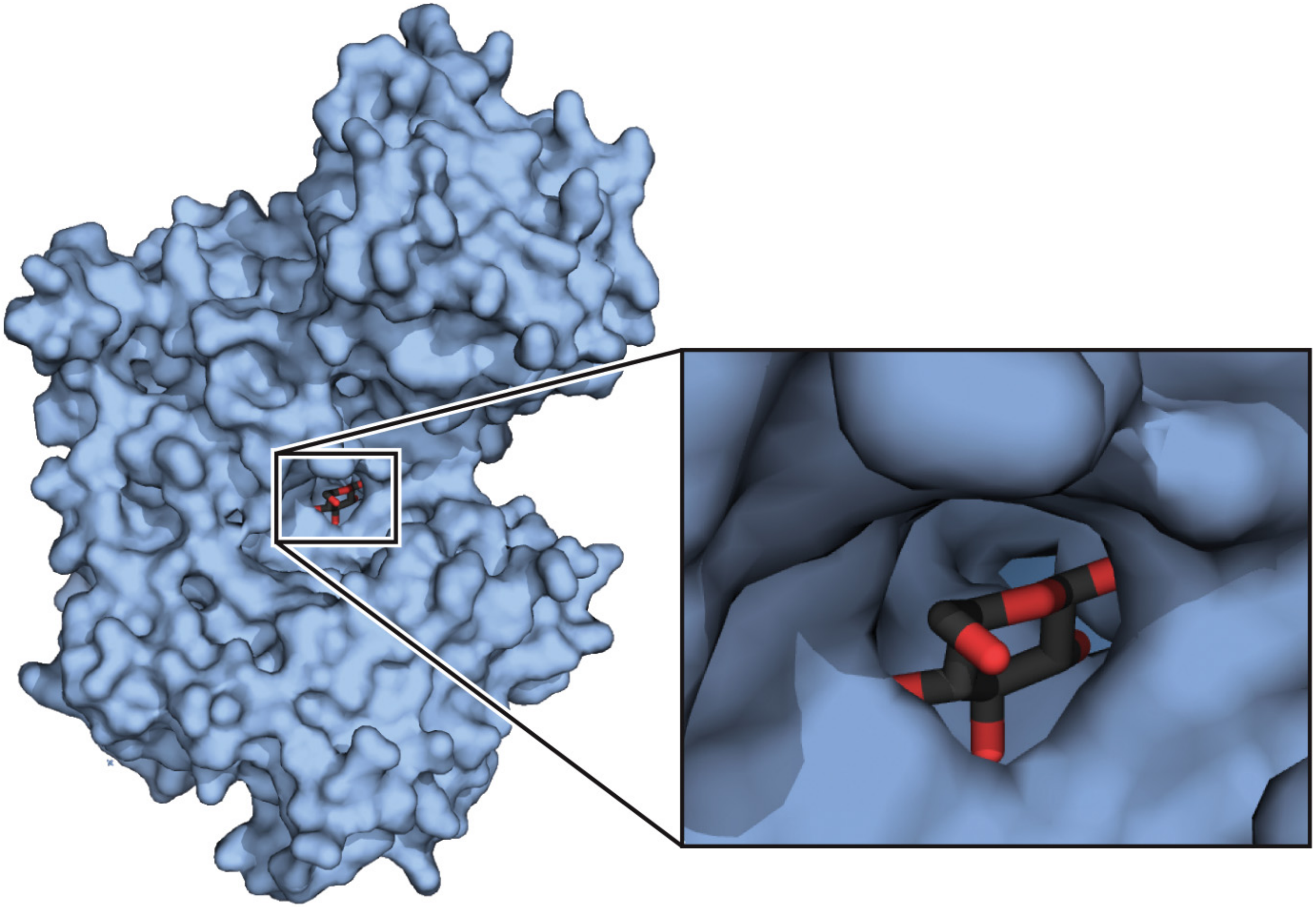

Figure 1-24
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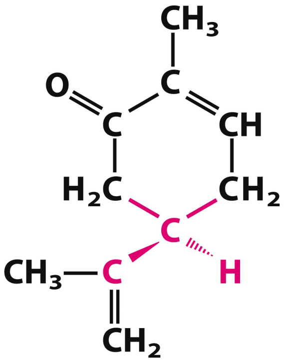
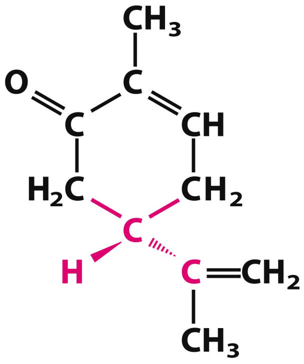
Interactions between biomolecules are stereospecific

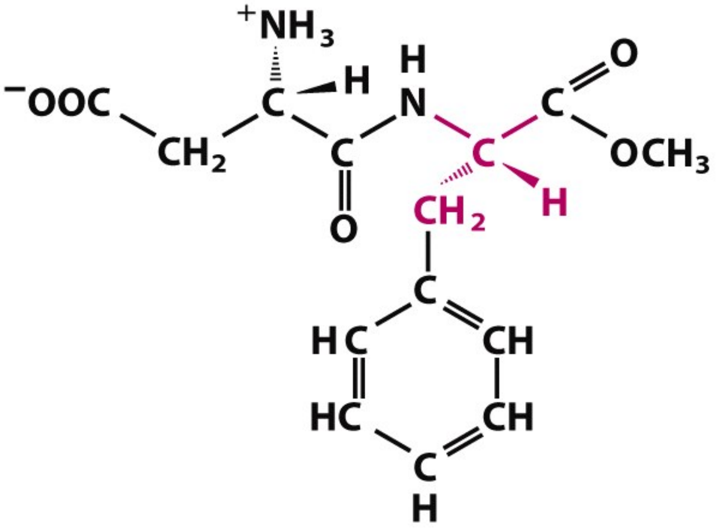
Stereoisomers have different effects in humans



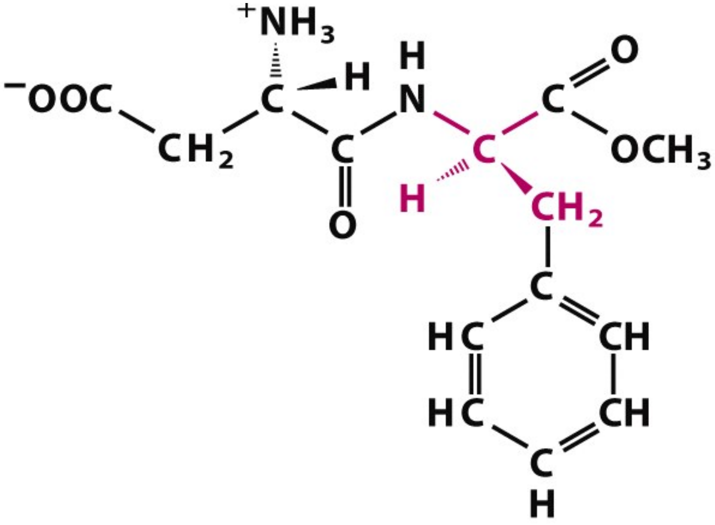
(*R*)-Carvone
(spearmint)



(*S*)-Carvone
(caraway)



L-Aspartyl-L-phenylalanine methyl ester
(aspartame) (sweet)



L-Aspartyl-D-phenylalanine methyl ester
(bitter)

Water is the medium for life

- Organisms typically contain 70–90% water
- Chemical reactions occur in aqueous milieu (water environment)
- Water is a critical determinant of the structure and function of proteins, nucleic acids, and membranes

Hydrogen Bonds

- Hydrogen bonds are strong dipole-dipole or charge-dipole interactions that arise between a covalently bound hydrogen and lone pair of electrons.
- They typically involve two electronegative atoms (frequently nitrogen and oxygen).
- Hydrogen bonds are strongest when the bonded molecules allow for linear bonding patterns.
- Ideally, the three atoms involved are in a line.

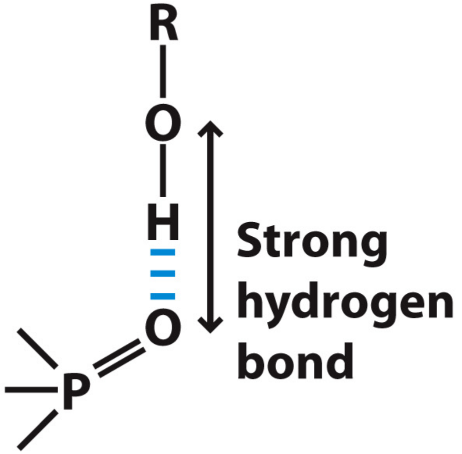
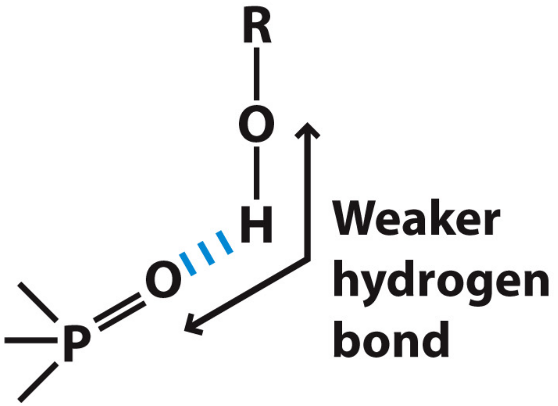


Figure 2-5



Hydrogen Bonding in Water

- Water can serve as both:
  - an H donor
  - an H acceptor
- Up to four H-bonds per water molecule gives water its:
  - anomalously high boiling point
  - anomalously high melting point
  - unusually large surface tension
- Hydrogen bonding in water is cooperative.
- Hydrogen bonds between neighboring molecules are weak (20 kJ/mol) relative to the H–O covalent bonds (420 kJ/mol).

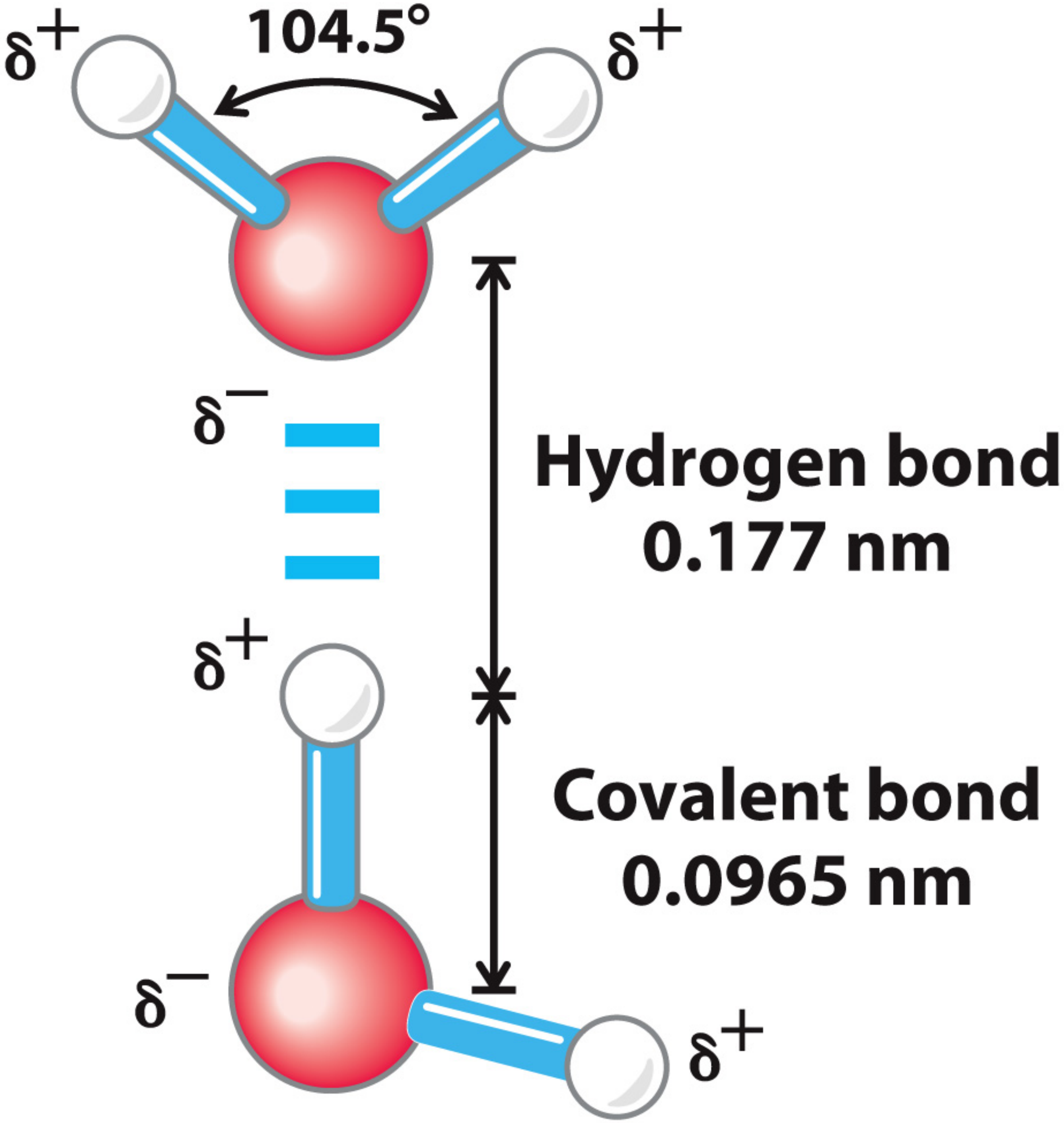


Figure 2-1b
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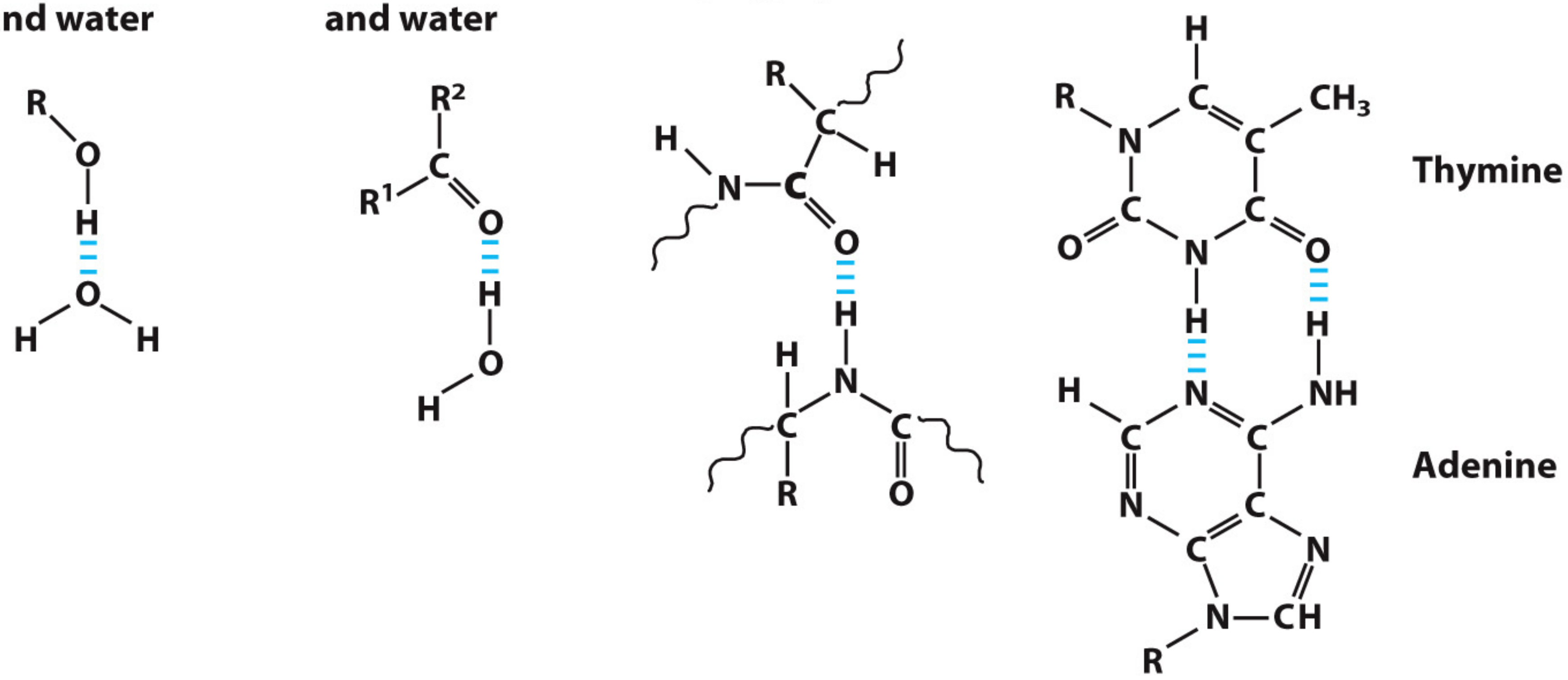
Importance of Hydrogen Bonds

- Source of unique properties of water
- Structure and function of proteins
- Structure and function of DNA
- Structure and function of polysaccharides
- Binding of substrates to enzymes
- Binding of hormones to receptors
- Matching of mRNA and tRNA

"I believe that as the methods of structural chemistry are further applied to physiological problems, it will be found that the significance of the hydrogen bond for physiology is greater than that of any other single structural feature."

–Linus Pauling, *The Nature of the Chemical Bond*, 1939

Biological Relevance of Hydrogen Bonds



Between the hydroxyl group of an alcohol and water

Between the carbonyl group of a ketone and water

Between peptide groups in polypeptides

Between complementary bases of DNA

Figure 2-4
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Physics of Noncovalent Interactions

Noncovalent interactions do not involve sharing a pair of electrons. Based on their physical origin, one can distinguish between:

- Ionic (Coulombic) Interactions
  - Electrostatic interactions between permanently charged species, or between the ion and a permanent dipole
- Dipole Interactions
  - Electrostatic interactions between uncharged, but polar molecules
- van der Waals Interactions
  - Weak interactions between all atoms, regardless of polarity
  - Attractive (dispersion) and repulsive (steric) components
- Hydrophobic Effect
  - Complex phenomenon associated with the ordering of water molecules around nonpolar substances

Examples of Noncovalent Interactions

TABLE 2–5 **Four Types of Noncovalent (“Weak”) Interactions among Biomolecules in Aqueous Solvent**

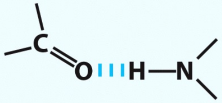
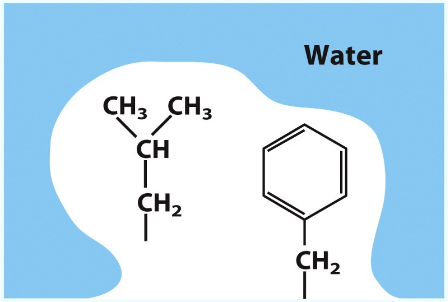
Hydrogen bonds **Between neutral groups**	
Between peptide bonds	
Ionic interactions **Attraction**	 $—{}^{+}NH_3 \rightarrow\leftarrow {}^{-}O—\overset{\overset{O}{\|}}{C}—$
Repulsion	$—{}^{+}NH_3 \longleftrightarrow H_3N^{+}—$
Hydrophobic interactions	
van der Waals interactions	**Any two atoms in close proximity**

Table 2-5
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van der Waals Interactions

- van der Waals interactions have two components:
  - Attractive force (London dispersion) depends on the interaction of electron clouds together
  - Repulsive force (Steric repulsion) depends on the size of atoms
- Attraction dominates at longer distances (typically 0.4–0.7 nm)
- Repulsion dominates at very short distances
- There is a minimum energy distance for every atom (**van der Waals radius**)

TABLE 2–4 van der Waals Radii and Covalent (Single-Bond) Radii of Some Elements

Element	van der Waals radius (nm)	Covalent radius for single bond (nm)
H	0.11	0.030
O	0.15	0.066
N	0.15	0.070
C	0.17	0.077
S	0.18	0.104
P	0.19	0.110
I	0.21	0.133

Sources: For van der Waals radii, Chauvin, R. (1992) Explicit periodic trend of van der Waals radii. *J. Phys. Chem.* 96, 9194–9197. For covalent radii, Pauling, L. (1960) *Nature of the Chemical Bond,* 3rd edn, Cornell University Press, Ithaca, NY.

Note: van der Waals radii describe the space-filling dimensions of atoms. When two atoms are joined covalently, the atomic radii at the point of bonding are less than the van der Waals radii, because the joined atoms are pulled together by the shared electron pair. The distance between nuclei in a van der Waals interaction or a covalent bond is about equal to the sum of the van der Waals or covalent radii, respectively, for the two atoms. Thus the length of a carbon–carbon single bond is about 0.077 nm + 0.077 nm = 0.154 nm.

Table 2-4
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Biochemical Significance of Weak Interactions

- Weak individually
  - easily broken, reversible
  - much weaker than covalent bonds
  - much more in amount than covalent bonds; cumulative effects are very significant
- Universal
  - occur between any two atoms that are near each other (van der Waal)
  - occur between positive and negative ions (ionic)
  - occur between H-bond donor and acceptor (H-bond)
- Importance
  - stabilizes biological macromolecules
  - facilitates binding of ligands

Affects of Solutes on Properties of Water

- Colligative Properties
  - Boiling point, melting point, and osmolarity
  - *Do not depend on the nature of the solute, just the <u>number of solute particles</u>*
  - The concentration of water is lower in solutions than in pure water
- Noncolligative Properties
  - Viscosity, surface tension, taste, and color
  - Depend on the chemical nature of the solute

- Cytoplasm of cells are highly concentrated solutions and have high osmotic pressure

Osmotic Pressure

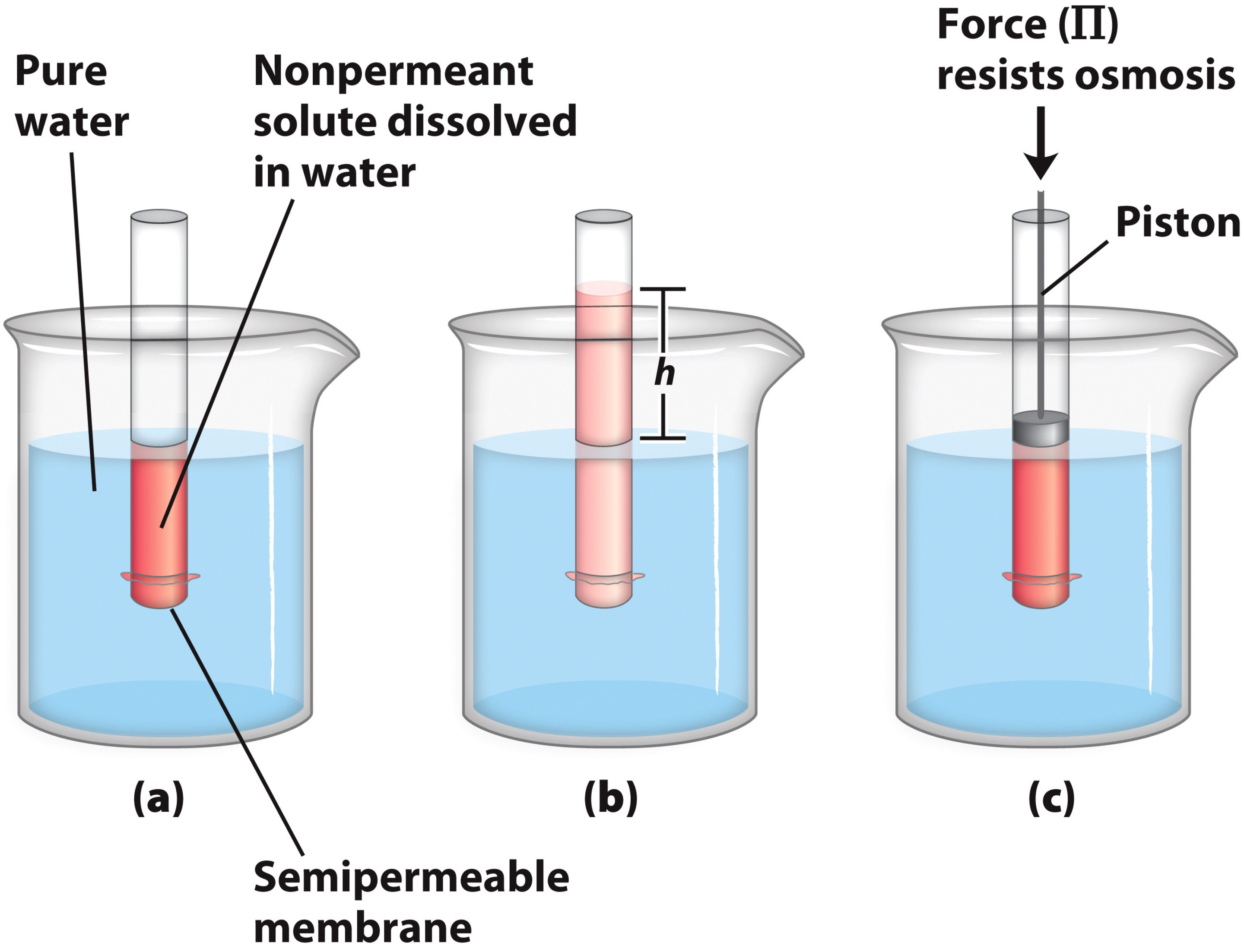

Figure 2-12
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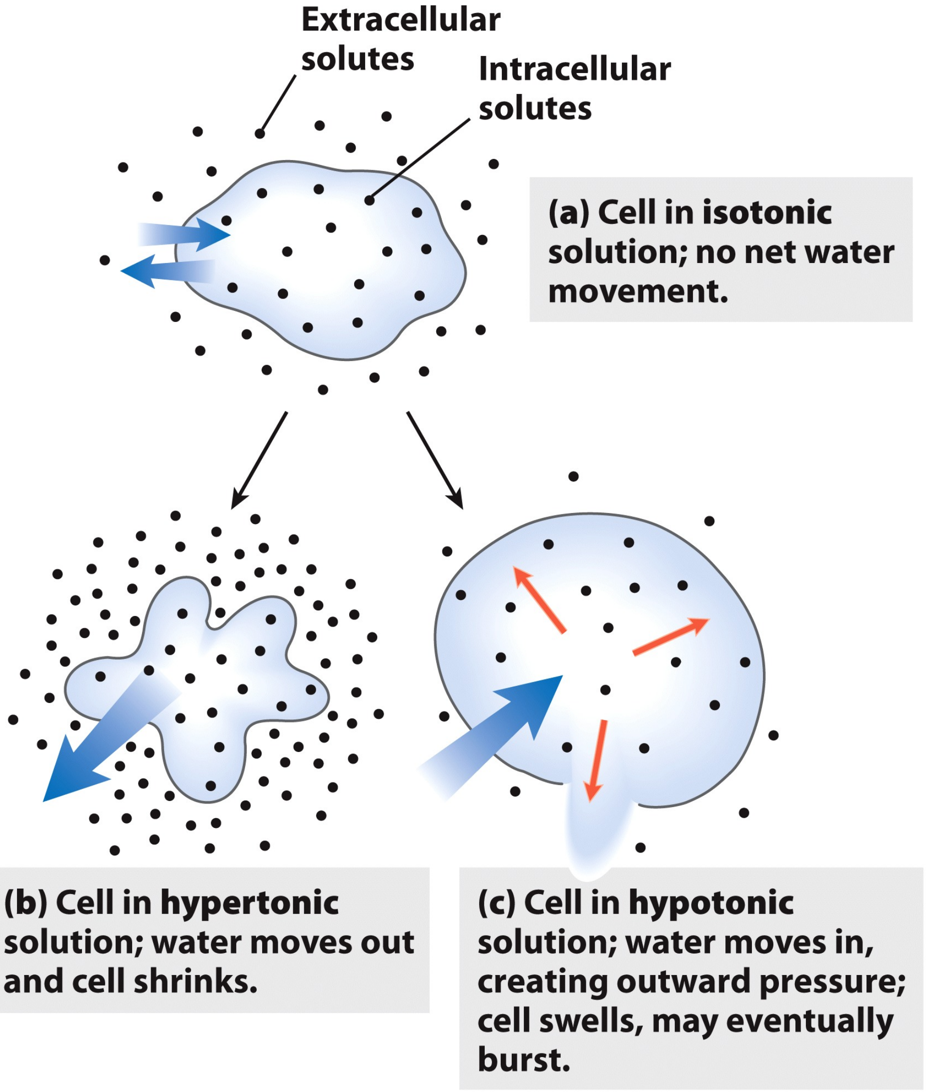
Osmotic pressure

- Osmotic pressure, $\Pi = icRT$
  - $i = 1$ for all nonionizing solutes (e.g. glucose)
  - $i = 2$ for completely ionizing salts with 1:1 ratio (dilute NaCl solution)
  - ic is the **osmolarity** of the solution
- For solutions with several solutes, $\Pi = RT(i_1c_1 + \dots\ i_nc_n)$
- The effects of solutes on osmolarity depends on the ***number*** of particles, not their mass

  – macromolecules have much less effect on osmolarity than an equal mass of monomeric components

  – **1 g** of polysaccharide of 1000 glucose monomers has the SAME effect on osmolarity of **1 mg** of glucose!

Effect of Extracellular Osmolarity



(a) Cell in isotonic solution; no net water movement.

(b) Cell in hypertonic solution; water moves out and cell shrinks.

(c) Cell in hypotonic solution; water moves in, creating outward pressure; cell swells, may eventually burst.

Figure 2-13
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Ionization of Water

$$H_2O \rightleftarrows H^+ + OH^-$$

- O-H bonds are polar and can dissociate
- Products are a proton (H^+) and a hydroxide ion (OH^-)
- Dissociation of water is a rapid reversible process
- Most water molecules remain un-ionized, thus pure water has very low electrical conductivity (resistance: 18 MΩ•cm)
- The equilibrium is strongly to the ***left***
- Extent of dissociation depends on the temperature

Ionization of Water: Quantitative Treatment

Concentrations of participating species in an equilibrium process are not independent but are related via the equilibrium constant:

$$H_2O \rightleftarrows H^+ + OH^- \qquad K_{eq} = \frac{[H^+]\bullet[OH^-]}{[H_2O]}$$

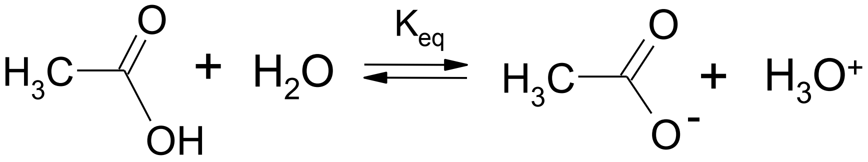
K_{eq} can be determined experimentally, it is $1.8\bullet10^{-16}$ M at 25°C.
$[H_2O]$ can be determined from water density, it is 55.5 M.

- Ionic product of water:

$$K_w = K_{eq} \cdot [H_2O] = [H^+][OH^-] = 1 \cdot 10^{-14}\,M^2$$

- In pure water $[H^+] = [OH^-] = 10^{-7}$ M

Dissociation of Weak Electrolytes: Principle



- Weak electrolytes dissociate only partially in water.

$$K_a = K_{eq} \cdot [\mathrm{H_2O}]$$

$$K_a = \frac{[\mathrm{H^+}][\mathrm{CH_3COO^-}]}{[\mathrm{CH_3COOH}]} = 1.74 \cdot 10^{-5}\,\mathrm{M}$$

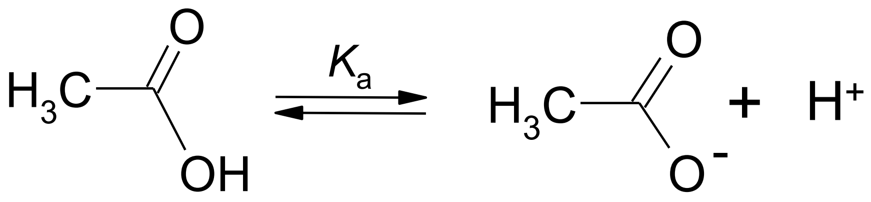
- Extent of dissociation is determined by the acid dissociation constant K_a.

$$[H^+] = K_a \cdot \frac{[\mathrm{CH_3COOH}]}{[\mathrm{CH_3COO^-}]}$$

- We can calculate the pH if the K_a is known. But some algebra is needed!

Dissociation of Weak Electrolytes: Example

What is the final pH of a solution when 0.1 moles of acetic acid is added to water to a final volume of 1L?



$0.1 - x$ $\quad x \quad x$

$$K_a = \frac{[x][x]}{[0.1 - x]} = 1.74 \cdot 10^{-5}\,\mathrm{M}$$

$$x^2 = 1.74 \cdot 10^{-6} - 1.74 \cdot 10^{-5}\,x$$

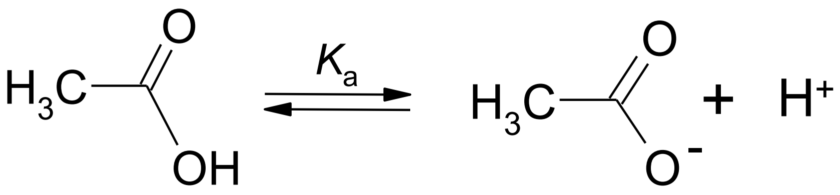
$$x^2 + 1.74 \cdot 10^{-5}\,x - 1.74 \cdot 10^{-6} = 0$$

$x = 0.00131, \quad \mathrm{pH} = -\log\,[\mathrm{H}^+] = 2.88$

- We assume that the only source of H^+ is the weak acid

- To find the $[H^+]$, a quadratic equation must be solved

Dissociation of Weak Electrolytes: Simplification



$0.1 - x \quad\quad x \quad\quad x$

$0.1 \quad\quad x \quad\quad x$

$$K_a = \frac{[x][x]}{[0.1]} = 1.74 \cdot 10^{-5}\,\mathrm{M}$$

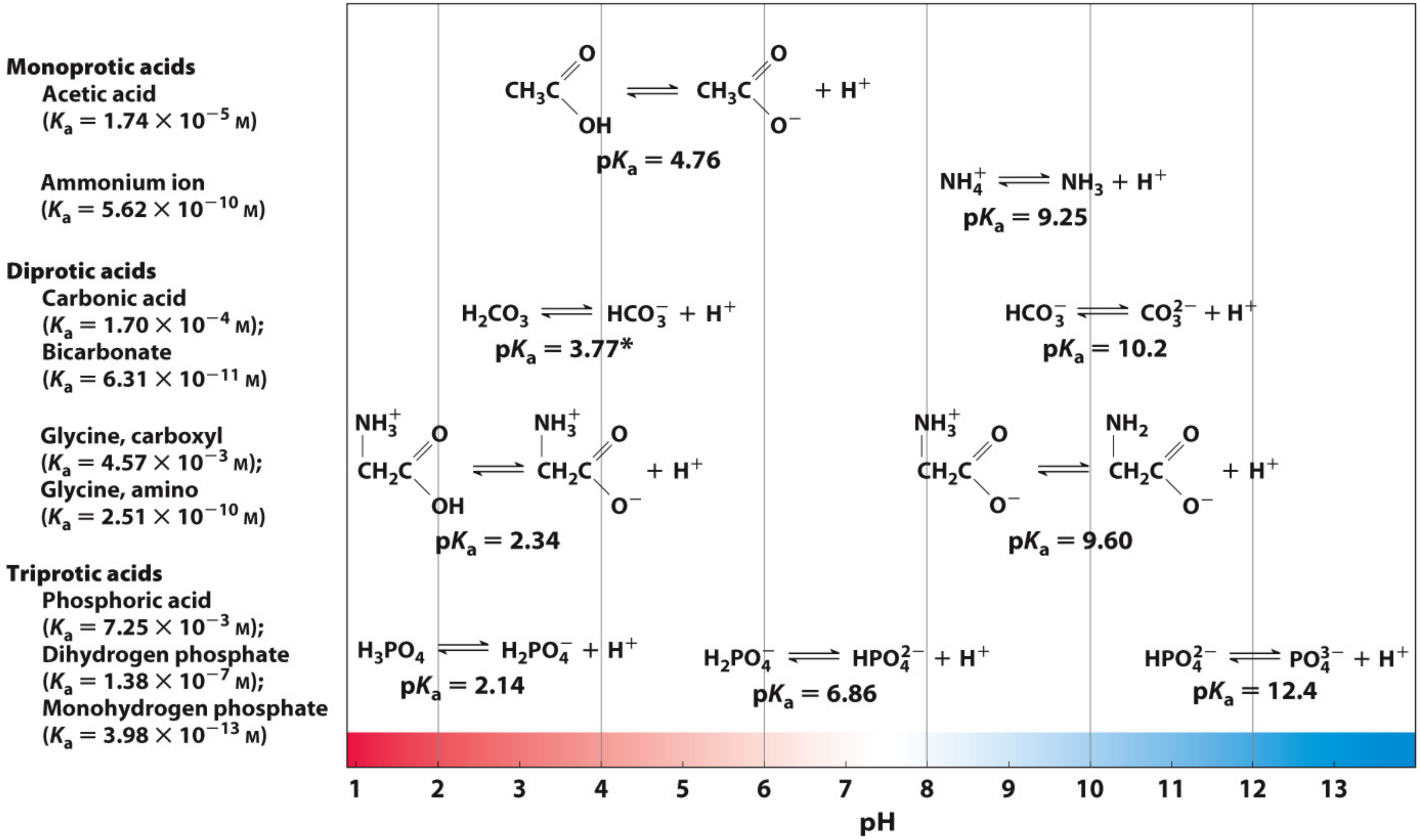
$$x^2 = 1.74 \cdot 10^{-6}$$

$x = 0.00132, \quad pH = 2.88$

- The equation can be simplified if the amount of dissociated species is much less than the amount of undissociated acid
- Approximation works for sufficiently weak acids and bases
- Check that x < [total acid]

pK_a Measures Acidity

$$pK_a = -\log K_a$$



Monoprotic acids
Acetic acid
($K_a = 1.74 \times 10^{-5}$ M)

Ammonium ion
($K_a = 5.62 \times 10^{-10}$ M)

Diprotic acids
Carbonic acid
($K_a = 1.70 \times 10^{-4}$ M);
Bicarbonate
($K_a = 6.31 \times 10^{-11}$ M)

Glycine, carboxyl
($K_a = 4.57 \times 10^{-3}$ M);
Glycine, amino
($K_a = 2.51 \times 10^{-10}$ M)

Triprotic acids
Phosphoric acid
($K_a = 7.25 \times 10^{-3}$ M);
Dihydrogen phosphate
($K_a = 1.38 \times 10^{-7}$ M);
Monohydrogen phosphate
($K_a = 3.98 \times 10^{-13}$ M)

Figure 2-16
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Buffers are mixtures of weak acids and their anions (conjugate base)

- Buffers resist change in pH
- At pH = pK_a, there is a 50:50 mixture of acid and anion forms of the compound
- Buffering capacity of acid/anion system is greatest at pH = pK_a
- Buffering capacity **is lost** when the pH differs from pK_a **by more than 1 pH unit**

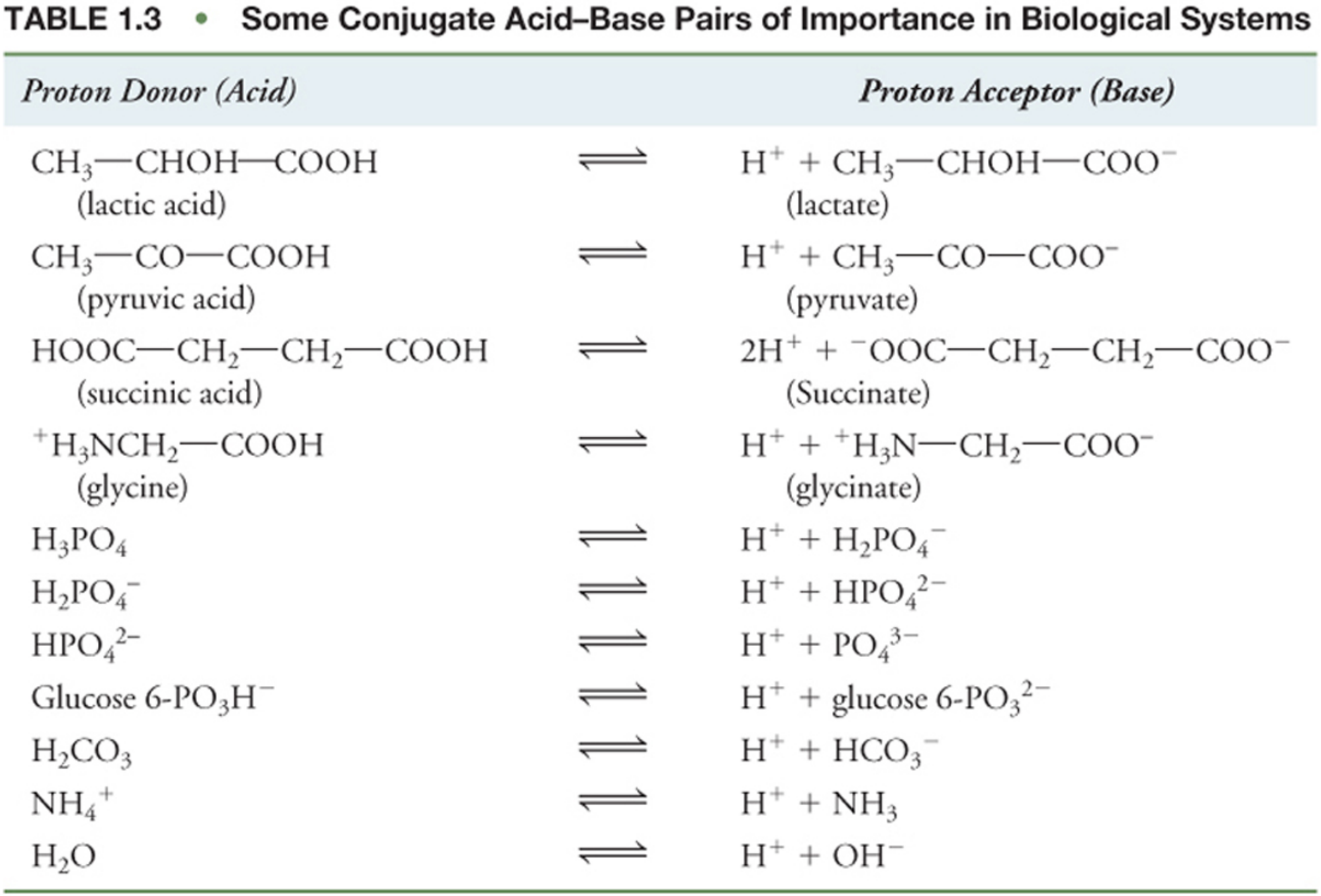

TABLE 1.3 • Some Conjugate Acid–Base Pairs of Importance in Biological Systems

Proton Donor (Acid)		*Proton Acceptor (Base)*
$CH_3—CHOH—COOH$ (lactic acid)	$\rightleftharpoons$	$H^+ + CH_3—CHOH—COO^-$ (lactate)
$CH_3—CO—COOH$ (pyruvic acid)	$\rightleftharpoons$	$H^+ + CH_3—CO—COO^-$ (pyruvate)
$HOOC—CH_2—CH_2—COOH$ (succinic acid)	$\rightleftharpoons$	$2H^+ + {}^-OOC—CH_2—CH_2—COO^-$ (Succinate)
${}^+H_3NCH_2—COOH$ (glycine)	$\rightleftharpoons$	$H^+ + {}^+H_3N—CH_2—COO^-$ (glycinate)
H_3PO_4	$\rightleftharpoons$	$H^+ + H_2PO_4^-$
$H_2PO_4^-$	$\rightleftharpoons$	$H^+ + HPO_4^{2-}$
HPO_4^{2-}	$\rightleftharpoons$	$H^+ + PO_4^{3-}$
Glucose 6-PO_3H^-	$\rightleftharpoons$	H^+ + glucose 6-PO_3^{2-}
H_2CO_3	$\rightleftharpoons$	$H^+ + HCO_3^-$
NH_4^+	$\rightleftharpoons$	$H^+ + NH_3$
H_2O	$\rightleftharpoons$	$H^+ + OH^-$

Figure

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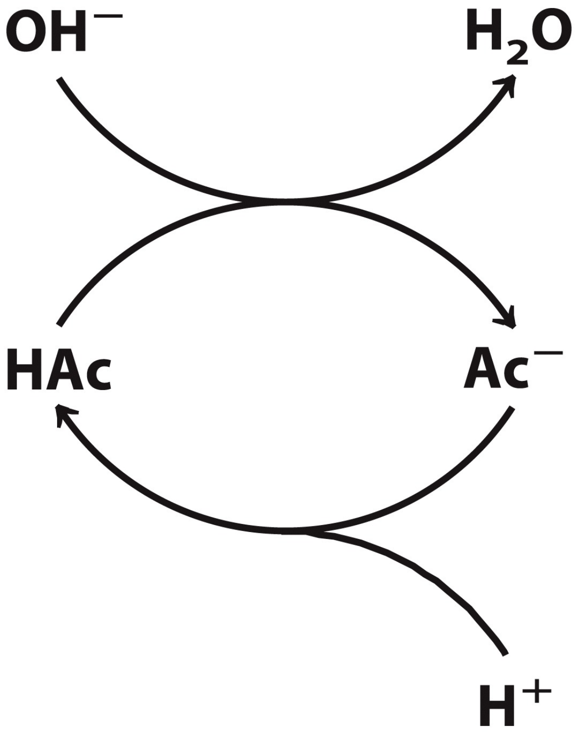
Acetic Acid-Acetate as a Buffer System

$$K_w = [H^+][OH^-]$$



Acetic acid
(CH_3COOH)

Acetate
(CH_3COO^-)

$$K_a = \frac{[H^+][Ac^-]}{[HAc]}$$

Figure 2-19
Lehninger Principles of Biochemistry, Sixth Edition

The titration curve of acetic acid. After addition of each increment of NaOH to the acetic acid solution, the pH of the mixture is measured. This value is plotted against the amount of NaOH added.

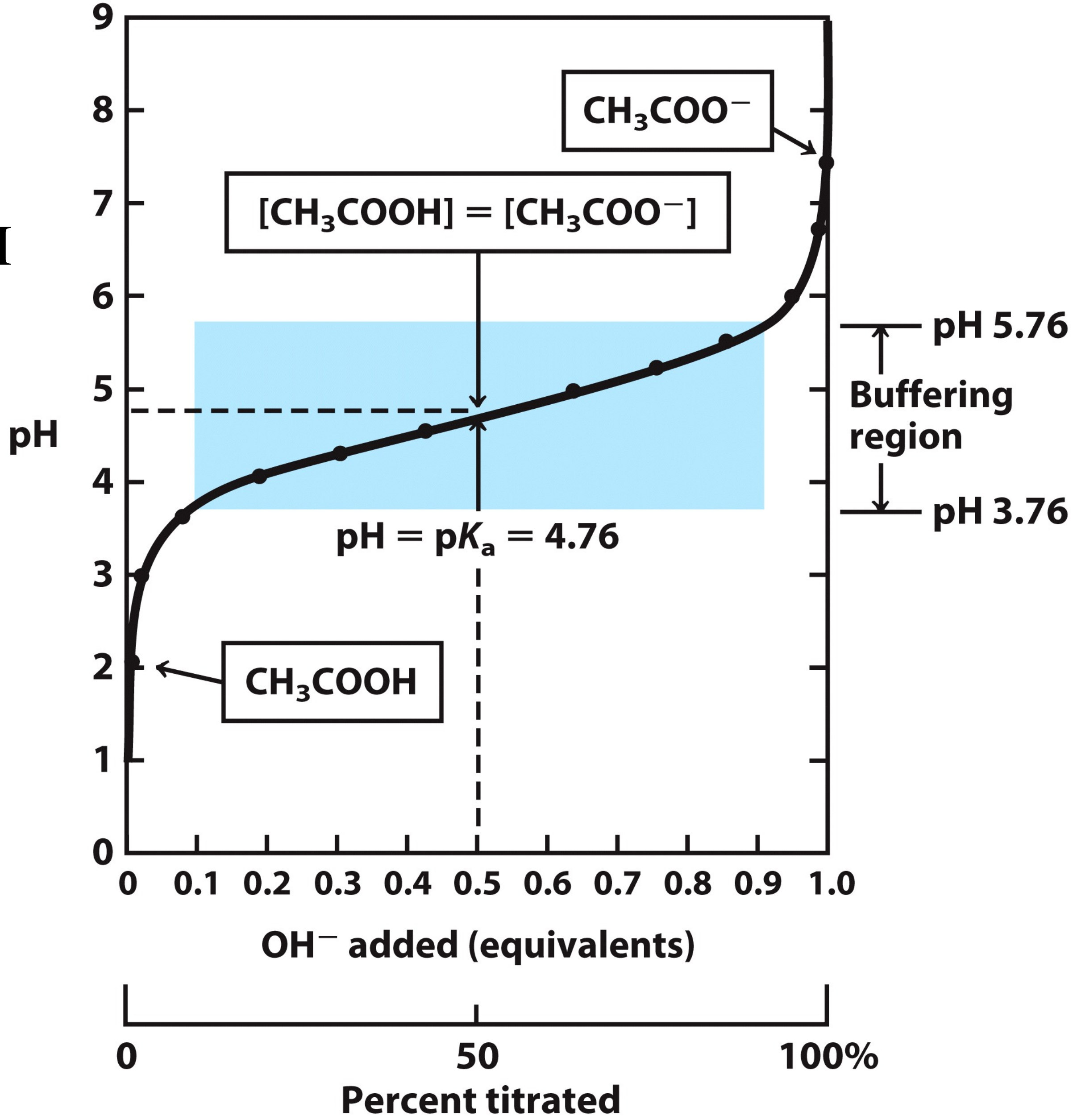


Figure 2-17
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Weak acids have different pK_as

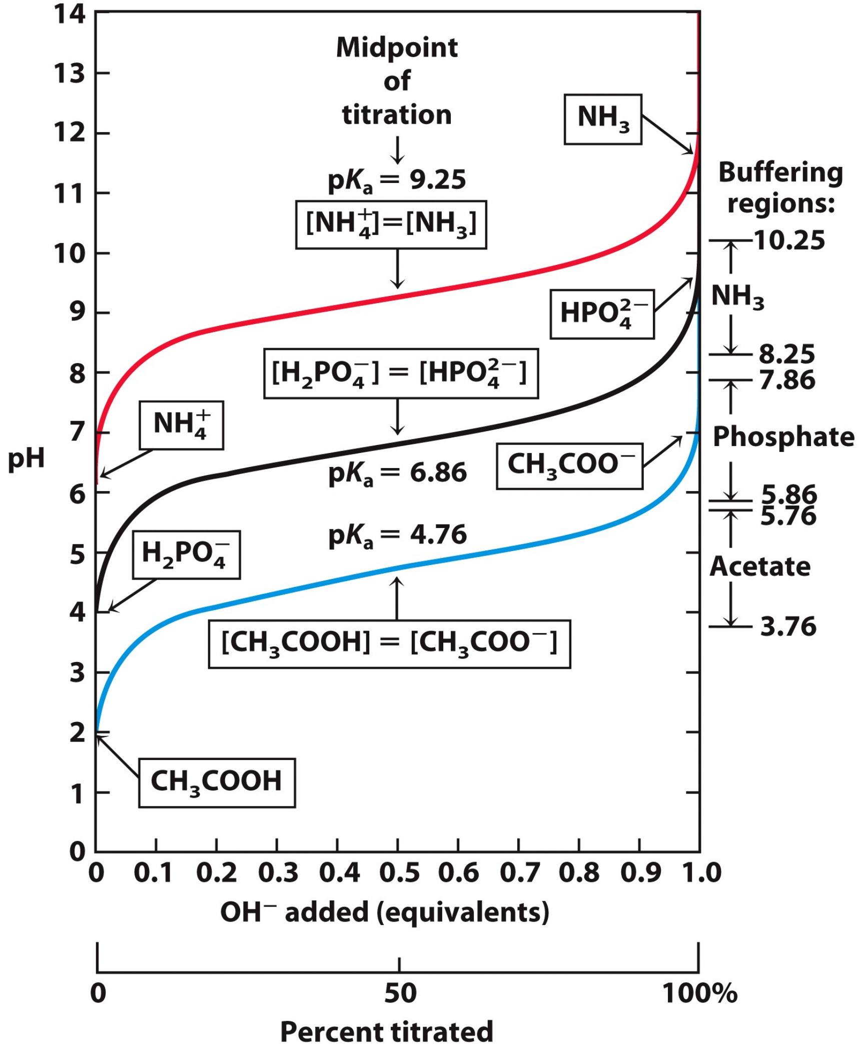

Figure 2-18

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Henderson–Hasselbalch Equation: Derivation

- The equation fits the titration curves
- Explains why the pKa of a weak acid is equal to the pH of the solution at the midpoint of the titration (you can do it!)

$$\mathrm{HA} \leftrightarrows \mathrm{H^+} + \mathrm{A^-}$$

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

$$\begin{array}{c}\downarrow\\ \downarrow\end{array}$$

$$\mathrm{pH} = \mathrm{p}K_a + \log\frac{[\mathrm{A^-}]}{[\mathrm{HA}]}$$

Henderson–Hasselbalch Equation: Example

A buffer is comprised of 0.1 M Acetic acid (CH_3COOH, pKa=4.76) and 0.05 M Sodium acetate (CH_3COONa). What is the final pH of the buffer?

$$\text{pH}= 4.76 + log \frac{[0.05]}{[0.1]}$$

Final buffer pH = 4.45

Untreated diabetes produces life threatening acidosis

- 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, concentration in millimolar range
  - bicarbonate, important for blood plasma
  - histidine, efficient buffer at neutral pH

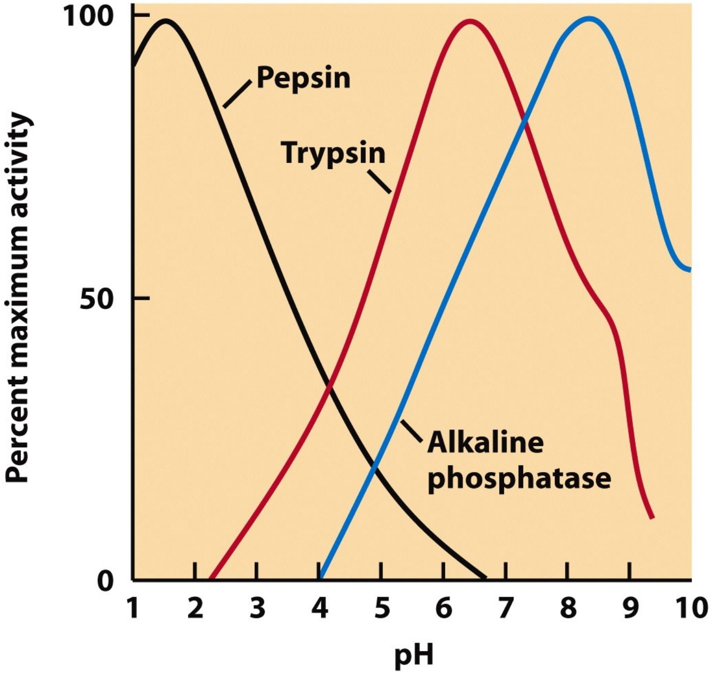

Figure 2-21
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