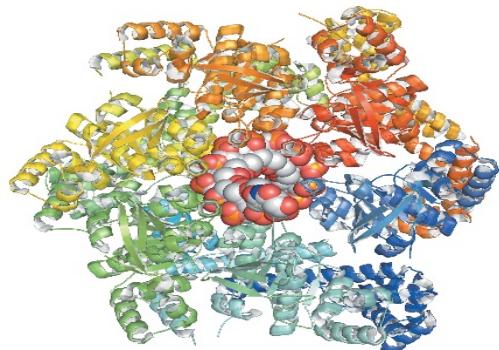


biochemistry



Reginald H. Garrett | Charles M. Grisham
Ninth Edition



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Chapter 1

The Facts of Life: Chemistry is the Logic of Biological Phenomena



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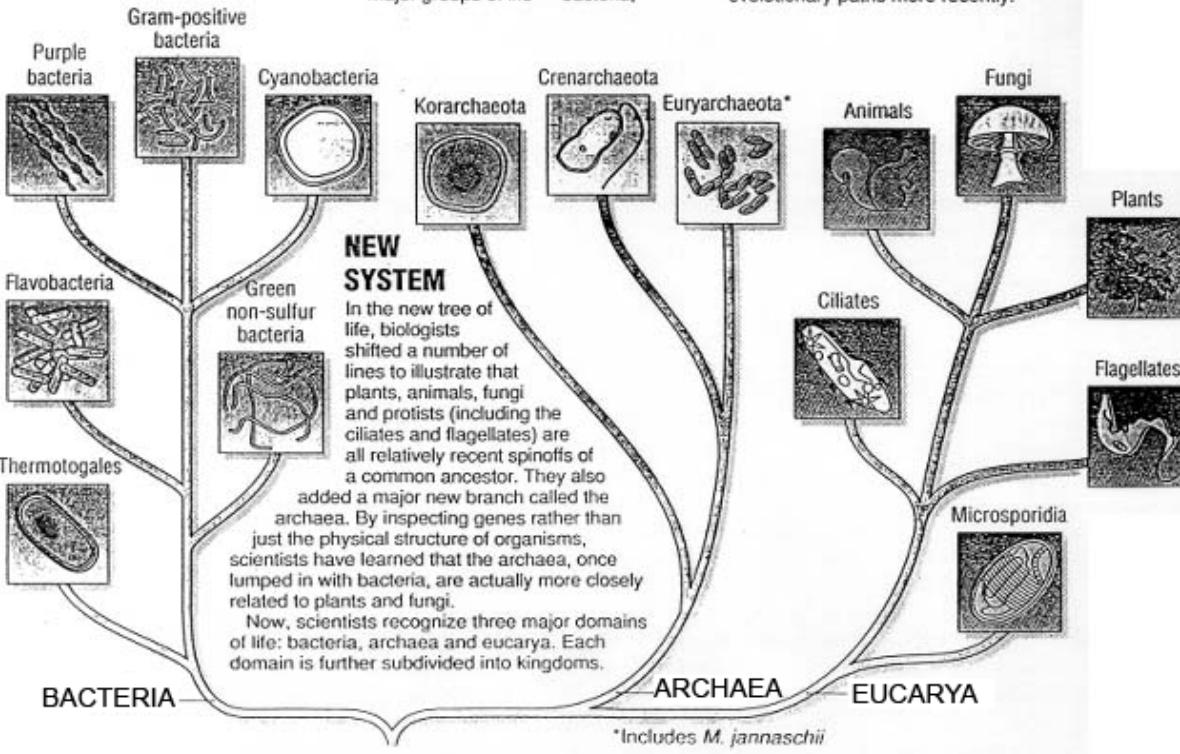


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From the Science Section of the Dallas Morning News

BRANCHING

Last month, biologists announced that they had completely sequenced the DNA — the cellular blueprints — of an organism called *Methanococcus jannaschii*. The domain of organisms to which this one belonged — the archaea — has been recognized by biologists as a distinct group for several years. New genetic evidence, however, indicates that the archaea are far more diverse than previously believed. Moreover, knowing the gene sequence of an archaea is helping biologists get a clearer picture of the evolution of life on Earth.



- What are the 3 domains of life?

- A. Archaea, Eukarya, Bacteria
- B. Archaea, Fungi, Protists
- C. Eukarya, Phylum, Shrek
- D. Kingdom, Phylum, Class

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How Many Genes Does a Cell Need?

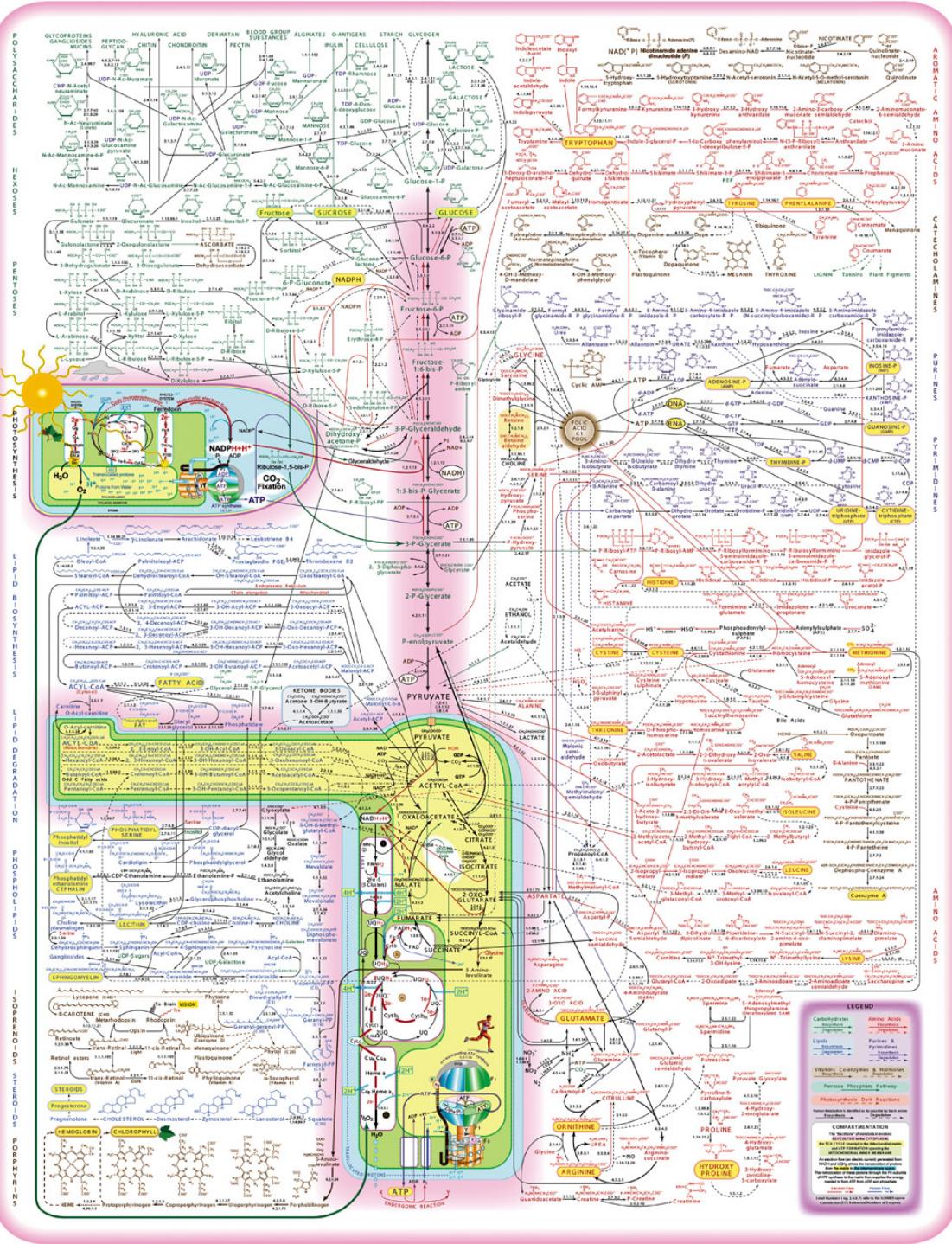
TABLE 1.6 How Many Genes Does It Take To Make An Organism?

Organism	Number of Cells in Adult*	Number of Genes
<i>Mycobacterium genitalium</i> (<i>Mycoplasma genitalium</i>)	1	523
Pathogenic bacterium		
<i>Methanococcus jannaschii</i>	1	1,800
Archaeal methanogen		
<i>Escherichia coli</i> K12	1	4,400
Intestinal bacterium		
<i>Saccharomyces cerevisiae</i>	1	6,000
Baker's yeast (eukaryote)		
<i>Caenorhabditis elegans</i>	959	19,000
Nematode worm		
<i>Drosophila melanogaster</i>	10^4	13,500
Fruit fly		
<i>Arabidopsis thaliana</i>	10^7	27,000
Flowering plant		
<i>Fugu rubripes</i>	10^{12}	26,700 (est.)
Pufferfish		
<i>Homo sapiens</i>	10^{14}	20,500 (est.)
Human		

Figure 17.2 A metabolic map, indicating the reactions of intermediary metabolism and the enzymes that catalyze them. More than 500 different chemical intermediates, or metabolites, and a greater number of enzymes are represented here.

For a detailed look go to:

http://www.sigmaaldrich.com/etc/medialib/docs/Sigma-Aldrich/General_Information/metabolicpathways_updated_02_07.Par.0001.File.tmp/metabolic_pathways_poster.pdf



1.2 What Kinds of Molecules are Biomolecules?

- H, O, C and N make up 99+% of atoms in the human body

ELEMENT	PERCENTAGE
Hydrogen	63
Oxygen	25.5
Carbon	9.5
Nitrogen	1.4

Elements highlighted in red comprise 99.9% of human body

Elements highlighted in green are essential trace elements
in human body

Elements highlighted in blue are trace elements found
in some organisms

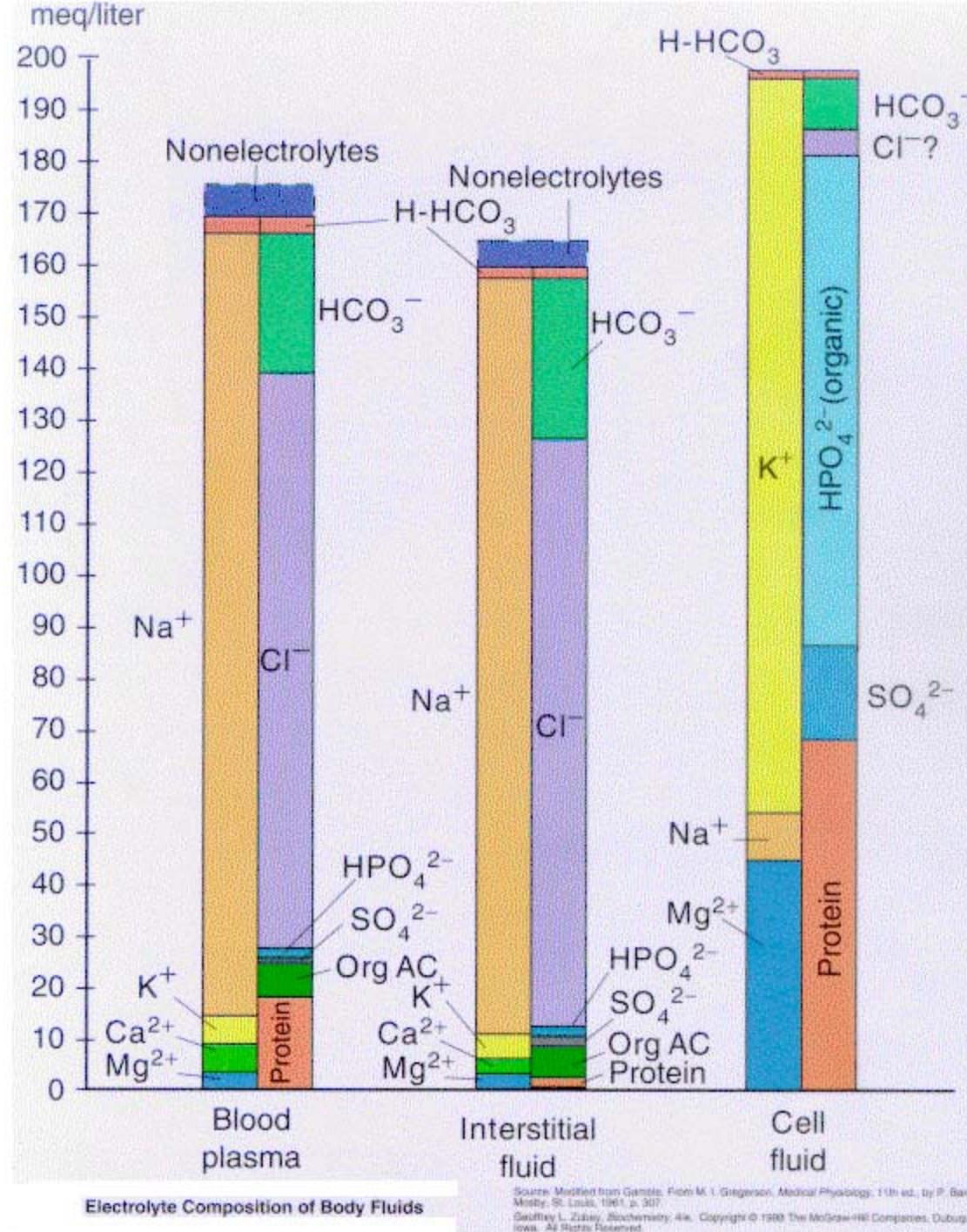
Periodic Table of the Elements

Period	Group Ia	Group IIa	Group IIIa	Group IVa	Group Va	Group VIa	Group VIIa	Group VIII	Group Ib	Group IIb	Group IIIb	Group IVb	Group Vb	Group VIb	Group VIIb	Group O			
1 1s	1 H														1 H	2 He			
2 2s2p	3 Li	4 Be												5 B	6 C	7 N	8 O	9 F	10 Ne
3 3s3p	11 Na	12 Mg											13 Al	14 Si	15 P	16 S	17 Cl	18 Ar	
4 4s3d 4p	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	
5 5s4d 5p	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	
6 6s (4f) 5d 6p	55 Cs	56 Ba	57* La	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	
7 7s (5f) 6d	87 Fr	88 Ra	89** Ac																

*Lanthanide series 4f	58 Ce	59 Pr	60 Nd	61 Pm	62 Sm	63 Eu	64 Gd	65 Tb	66 Dy	67 Ho	68 Er	69 Tm	70 Yb	71 Lu
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**Actinide series 5f	90 Th	91 Pa	92 U	93 Np	94 Pu	95 Am	96 Cm	97 Bk	98 Cf	99 Es	100 Fm	101 Md	102 No(?)	103 Lw
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What are the major ions of life?



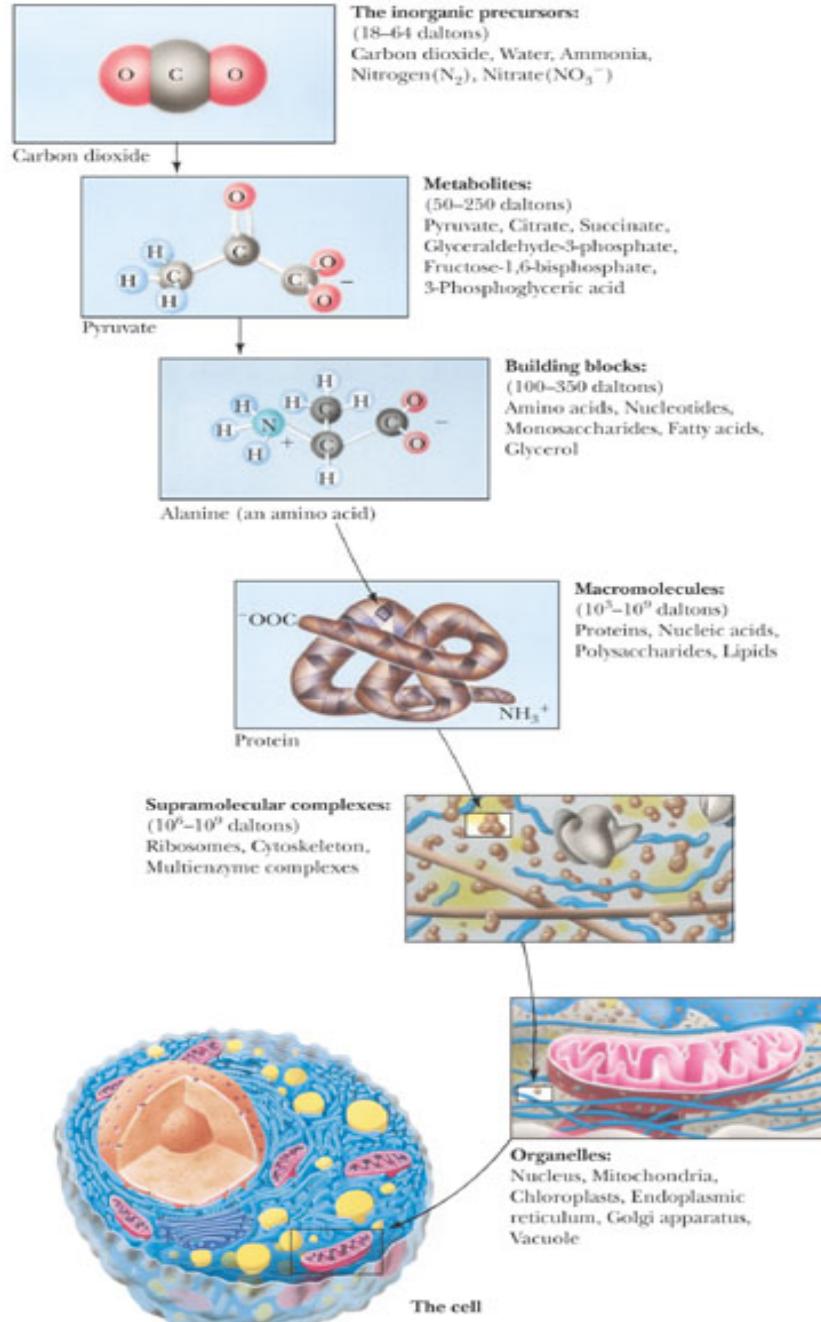
- Which of the following is false?
- a. Potassium is the principal extracellular cation
- b. Potassium is the principal intracellular cation
- c. HPO₄(2-) is the principal intracellular buffer
- d. Sodium is the principal extracellular cation
- e. Chloride is the principal extracellular anion

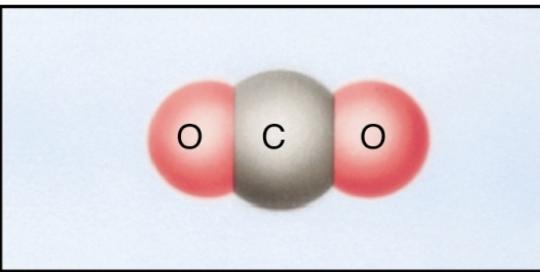
- Which of the following is false?
- a. **Potassium is the principal extracellular cation**
- b. Potassium is the principal intracellular cation
- c. HPO₄(2-) is the principal intracellular buffer
- d. Sodium is the principal extracellular cation
- e. Chloride is the principal extracellular anion

Major Functional Groups in Biomolecules

Ionized Form	Structure	Name
	$\begin{matrix} \backslash \\ \text{:C} \\ / \end{matrix} - \text{OH}$	HYDROXYL
	$\begin{matrix} \backslash \\ \text{:C} \\ / \end{matrix} = \text{O}$	CARBONYL
$-\text{C}^{\text{--}}\text{O}^-$	\rightleftharpoons	CARBOXYL
$\begin{matrix} \backslash \\ \text{:C} \\ / \end{matrix} - \text{NH}_3^+$	\rightleftharpoons	AMINO
	$\begin{matrix} \backslash \\ \text{:C} \\ / \end{matrix} = \text{NH}$	IMINO
$-\text{C}^{\text{--}}\text{S}^-$	\rightleftharpoons	THIOL
$\begin{matrix} \backslash \\ \text{:C} \\ / \end{matrix} - \text{O}-\text{P}(\text{O})=\text{O}$	\rightleftharpoons	PHOSPHATE
	$\begin{matrix} \backslash \\ \text{:C} \\ / \end{matrix} - \text{O}-\text{P}(\text{O})=\text{O} \\ \quad \quad \quad \backslash \quad \quad \quad / \\ \quad \quad \quad \text{O}^- \quad \quad \quad \text{OH}$	

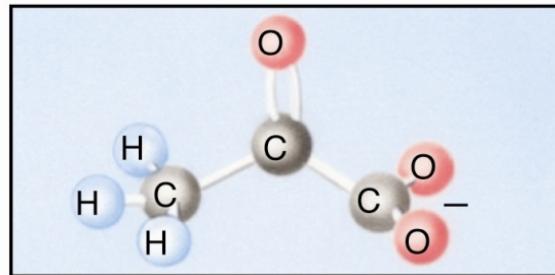
Figure 1.8
Molecular organization
in the cell is a hierarchy.





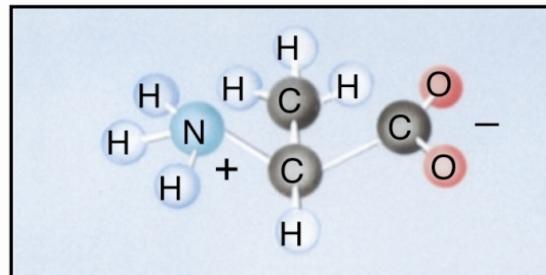
The inorganic precursors:
(18–64 daltons)
Carbon dioxide, Water, Ammonia,
Nitrogen(N_2), Nitrate(NO_3^-)

Carbon dioxide



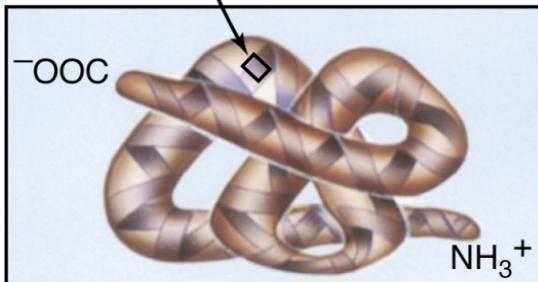
Metabolites:
(50–250 daltons)
Pyruvate, Citrate, Succinate,
Glyceraldehyde-3-phosphate,
Fructose-1,6-bisphosphate,
3-Phosphoglyceric acid

Pyruvate



Building blocks:
(100–350 daltons)
Amino acids, Nucleotides,
Monosaccharides, Fatty acids,
Glycerol

Alanine (an amino acid)



Macromolecules:
(10^3 – 10^9 daltons)
Proteins, Nucleic acids,
Polysaccharides, Lipids

Protein

Fig.1.9 Biopolymers (aka. macromolecules)

Amino acids build proteins

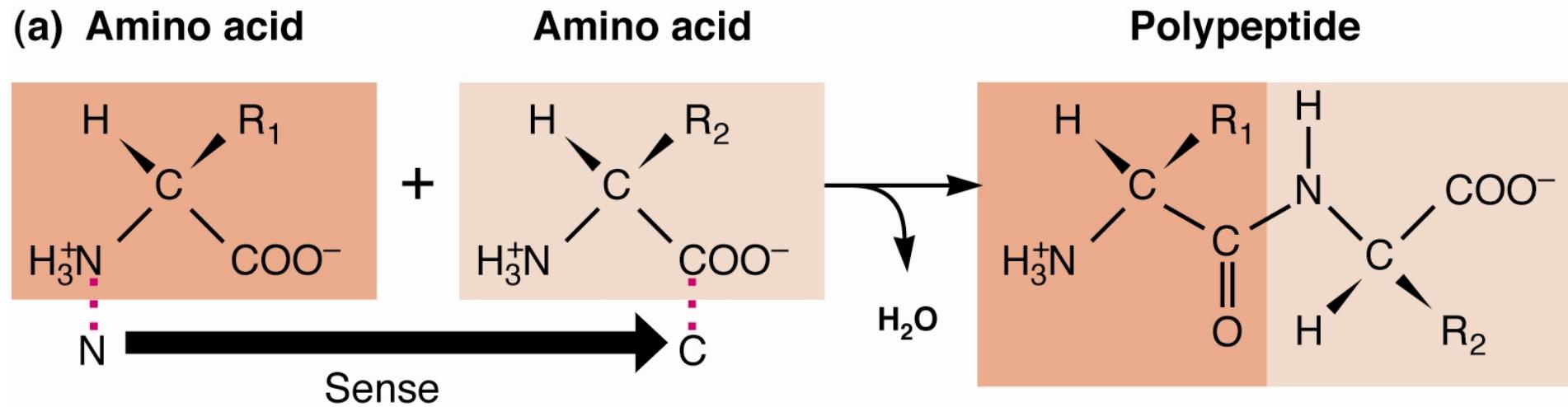


Fig.1.9 Biopolymers (aka. macromolecules)

Polysaccharides are built by joining sugars together

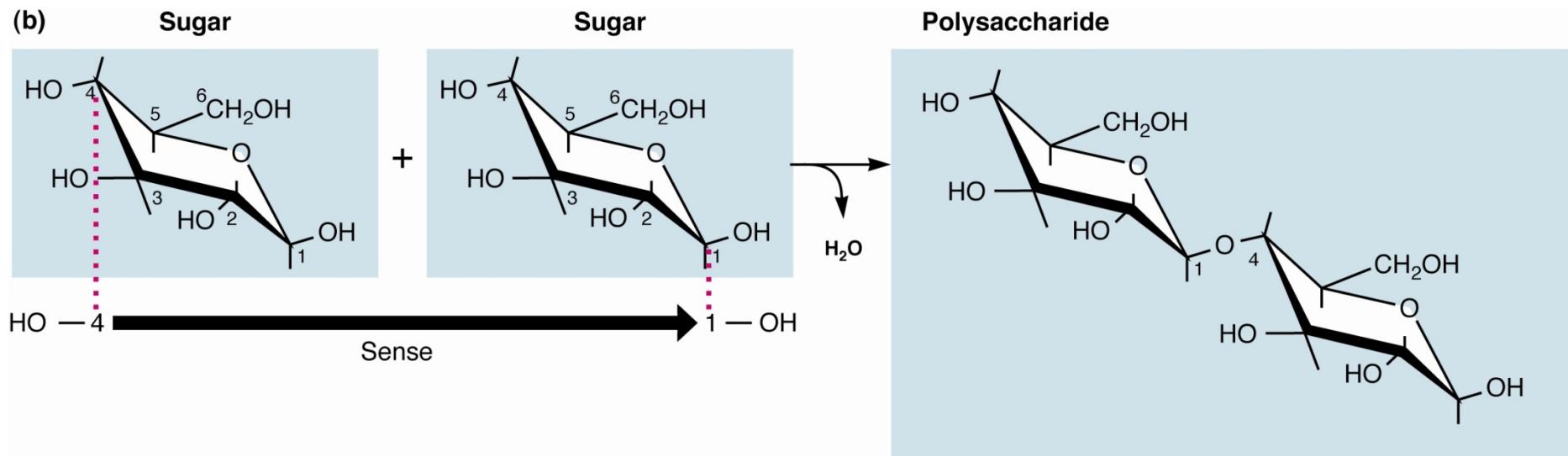
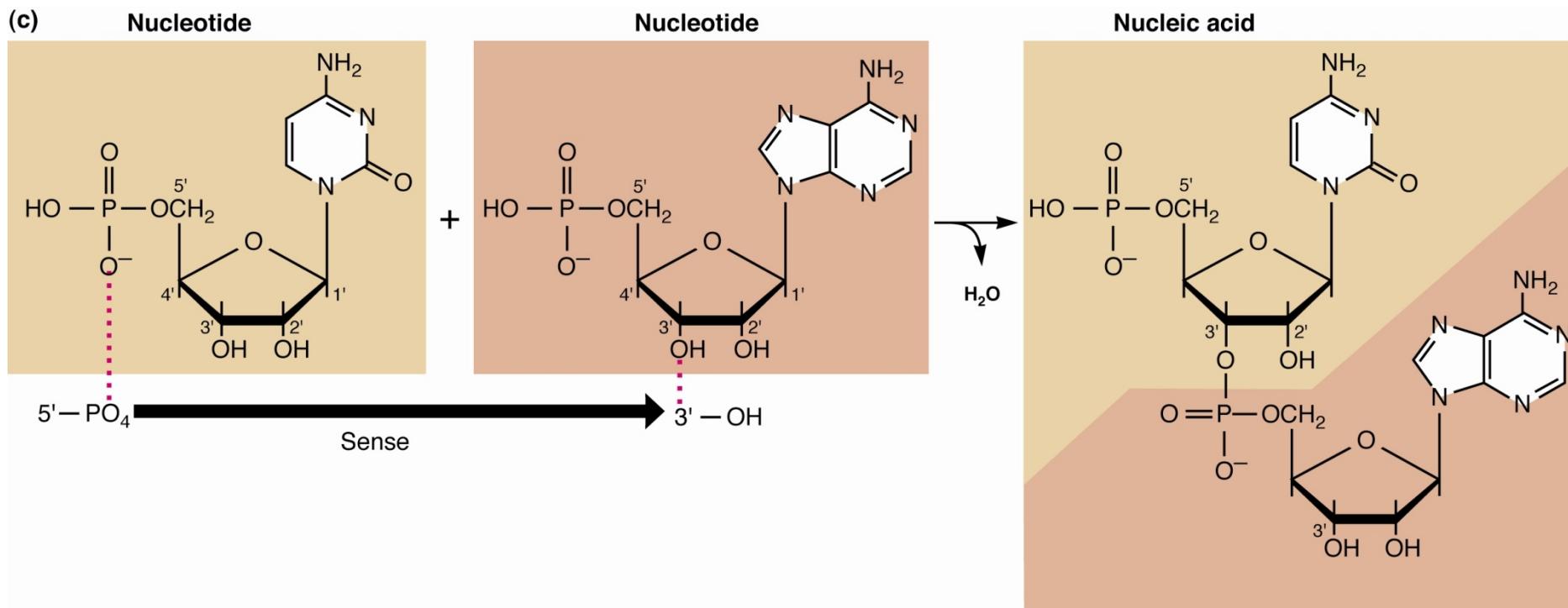
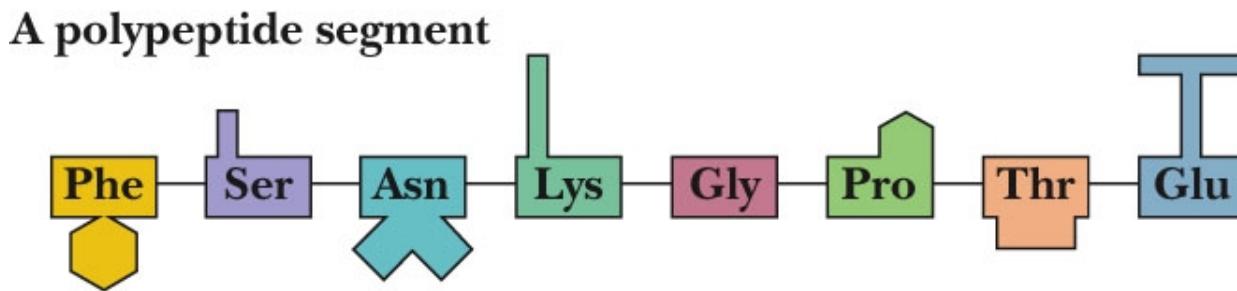
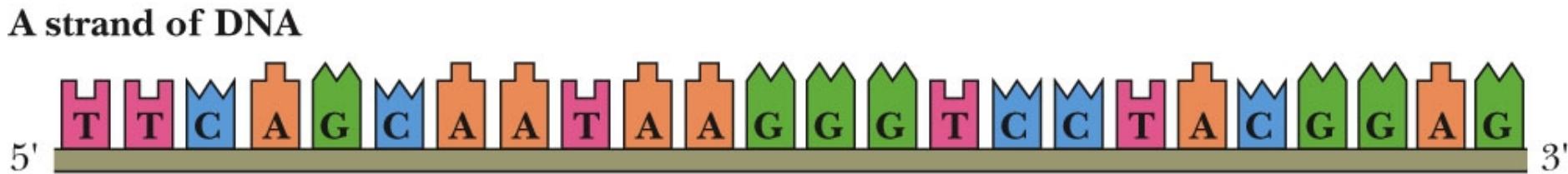


Fig.1.9 Biopolymers (aka. macromolecules)

Nucleic acids are polymers of nucleotides



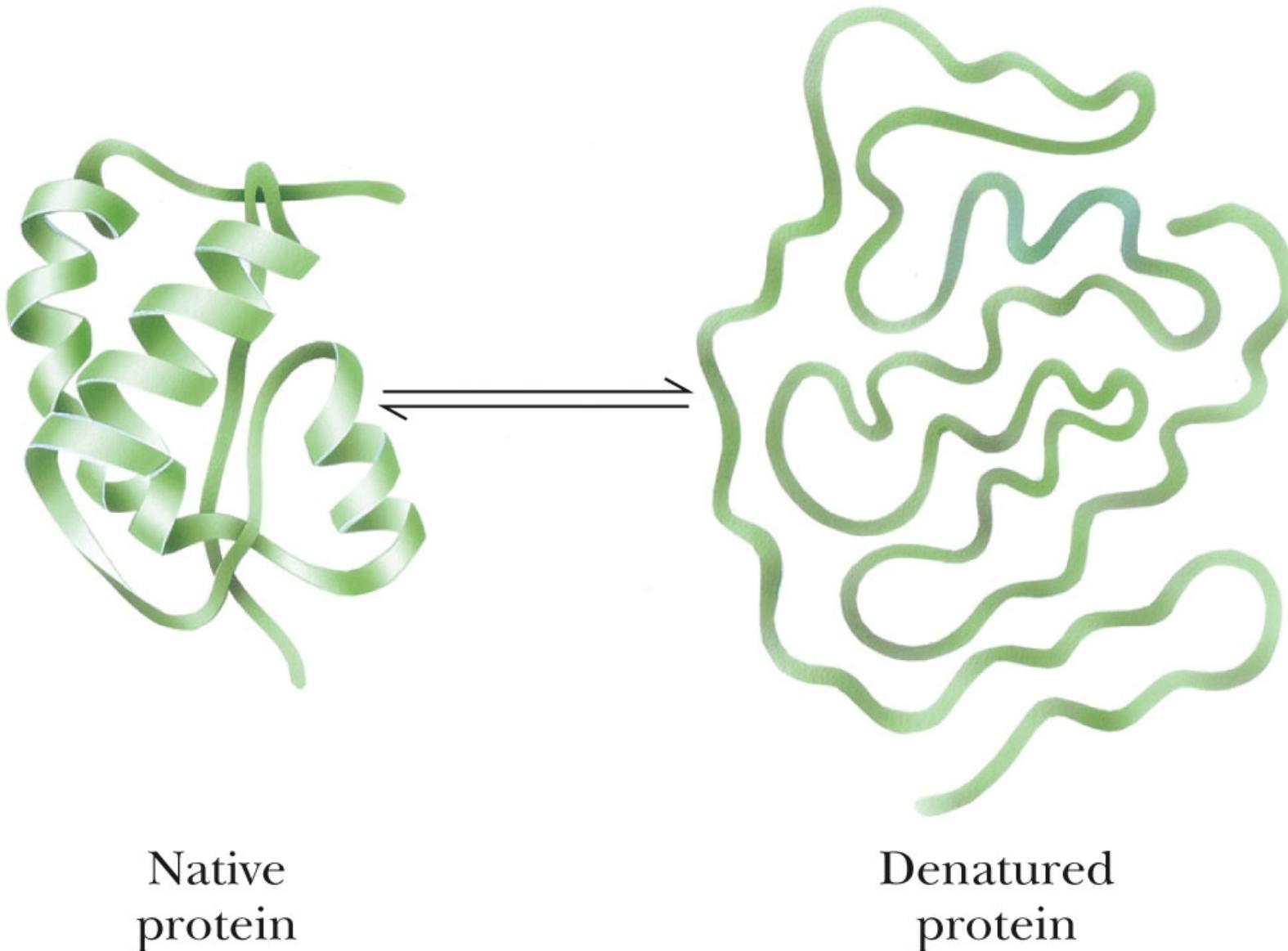
The Major Biopolymers



Sec. 1.4 Properties of Biomolecules Reflect Their Fitness to the Living Condition

- Macromolecules and their building blocks have a “sense” or directionality
- Macromolecules are informational
- Biomolecules have characteristic three-dimensional architecture

Figure 1.17 Denaturation and renaturation of the intricate structure of a protein.



- Which of the following is false?
- a. Nucleic acids contain phosphodiester bonds
- b. Sugars are joined by glycosidic bonds
- c. Peptides are joined by covalent bonds
- d. Nucleic acids, sugar, and peptides contain directionality
- e. Nucleotides contain phosphodiester bonds

- Which of the following is false?
- a. Nucleic acids contain phosphodiester bonds
- b. Sugars are joined by glycosidic bonds
- c. Peptides are joined by covalent bonds
- d. Nucleic acids, sugar, and peptides contain directionality
- e. **Nucleotides contain phosphodiester bonds**

Weak Forces

Weak Interaction	Electrostatic Model	Example	$E(r)$
charge - charge		--NH_3^+ --C=O^-	r^{-1}
charge - dipole		--NH_3^+ $\text{H}_2\text{O}^{\cdot-}$	r^{-2}
dipole - dipole		$\text{H}_2\text{O}^{\cdot-}$ $\text{H}_2\text{O}^{\cdot-}$	r^{-3}
charge - induced dipole		--NH_3^+ C_6H_5^-	r^{-4}
dipole - induced dipole		$\text{H}_2\text{O}^{\cdot-}$ C_6H_5^-	r^{-5}
dispersion		C_6H_5^-	r^{-6}
van der waals repulsion		C_6H_5^-	r^{-12}
hydrogen bond		$\text{N-H}\cdots\text{O=C}$ Hydrogen bond length	Fixed bond length

Coulomb's Law
for electrostatic interactions

$$F = \frac{k q_1 q_2}{\epsilon r^2}$$

where q = charge
 ϵ = dielectric const
 $\epsilon_{H_2O} = 80$

Energy of interaction = work needed
to completely separate charges

$$E = \frac{k q_1 q_2}{\epsilon} \int_r^\infty \frac{1}{r^2} dr$$
$$= \frac{k q_1 q_2}{\epsilon r}$$

Terms for energy:

$$1 \text{ cal} = 4.184 \text{ joules}$$

Van der Waals Interactions E(distance of separation, r)

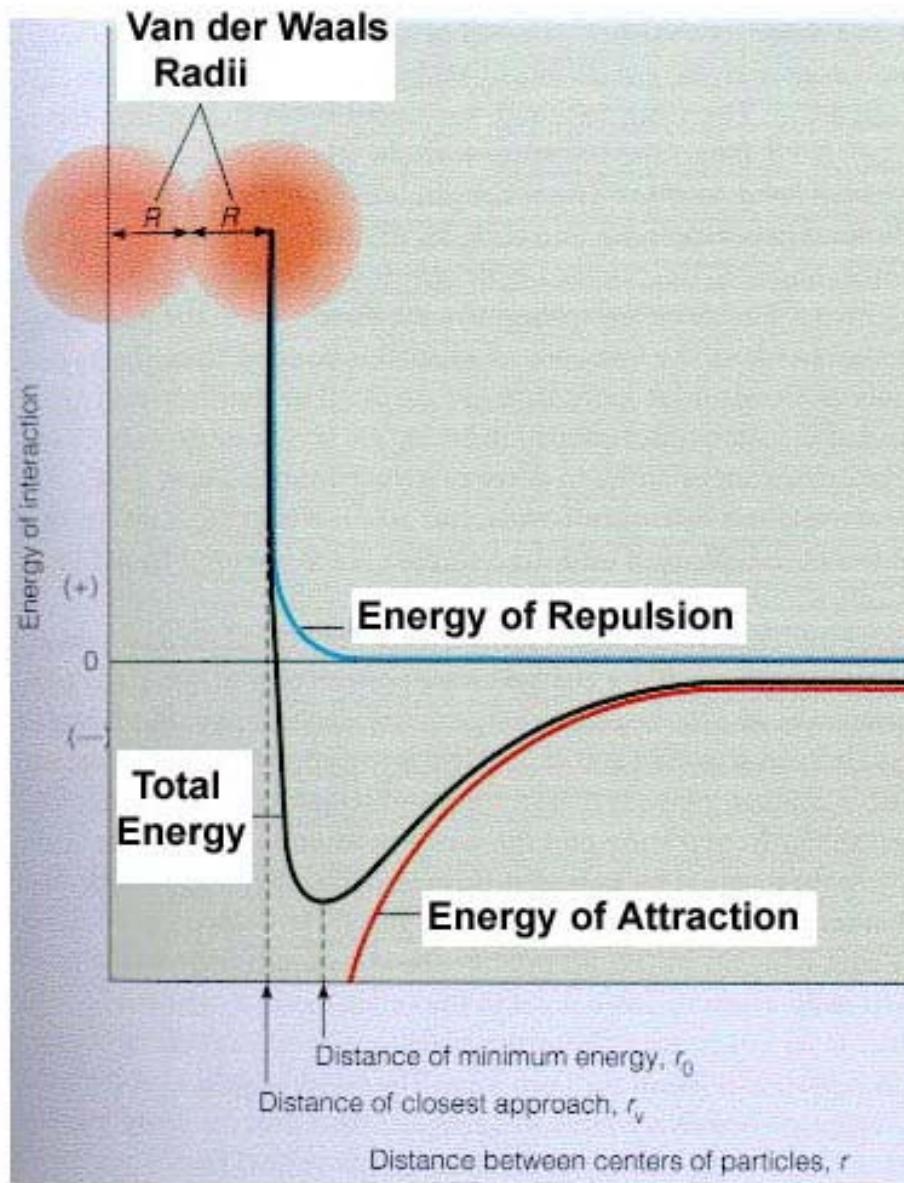


Fig. 1.12 Van der Waals Forces Are Important to Biomolecular Interactions

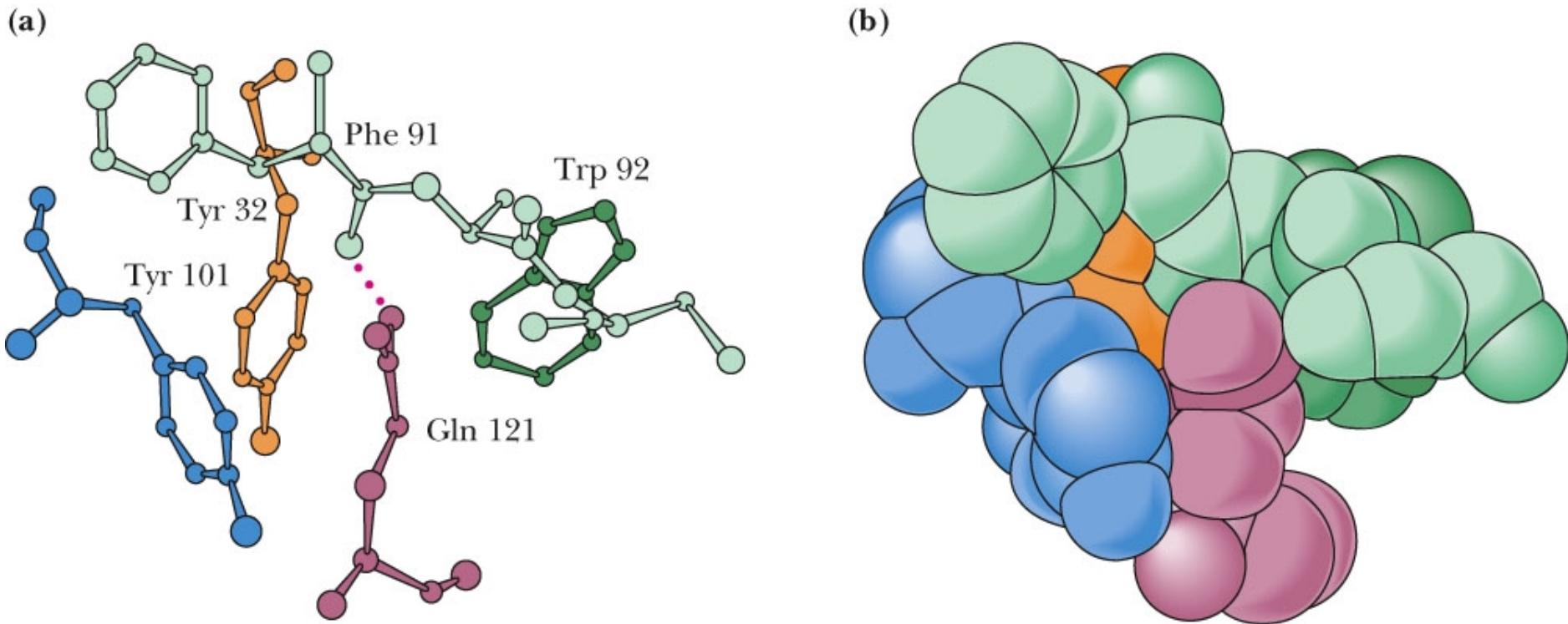


Figure 1.12

Van der Waals packing is enhanced in molecules that are structurally complementary. Gln^{121} represents a surface protuberance on the protein lysozyme. This protuberance fits nicely within a pocket (formed by Tyr^{101} , Tyr^{32} , Phe^{91} , and Trp^{92}) in the antigen-binding domain of an antibody raised against lysozyme. (See also Figure 1.16.) (a) A ball-and-stick model. (b) A space-filling representation. (From Science 233:751 (1986), figure 5.)

Fig. 1.14 Some biologically important H bonds

H bonds Bonded atoms	Approximate bond length*
O—H---O	0.27 nm
O—H---O ⁻	0.26 nm
O—H---N	0.29 nm
N—H---O	0.30 nm
⁺ N—H---O	0.29 nm
N—H---N	0.31 nm

*Lengths given are distances from the atom covalently linked to the H to the atom H bonded to the hydrogen:

$$\begin{array}{c} \text{O}—\text{H}---\text{O} \\ | \qquad \qquad | \\ \leftarrow 0.27 \text{ nm} \rightarrow \end{array}$$

Figure 1.14
Some of the
biologically important
functional groups
that serve as H bond
donors and
acceptors.

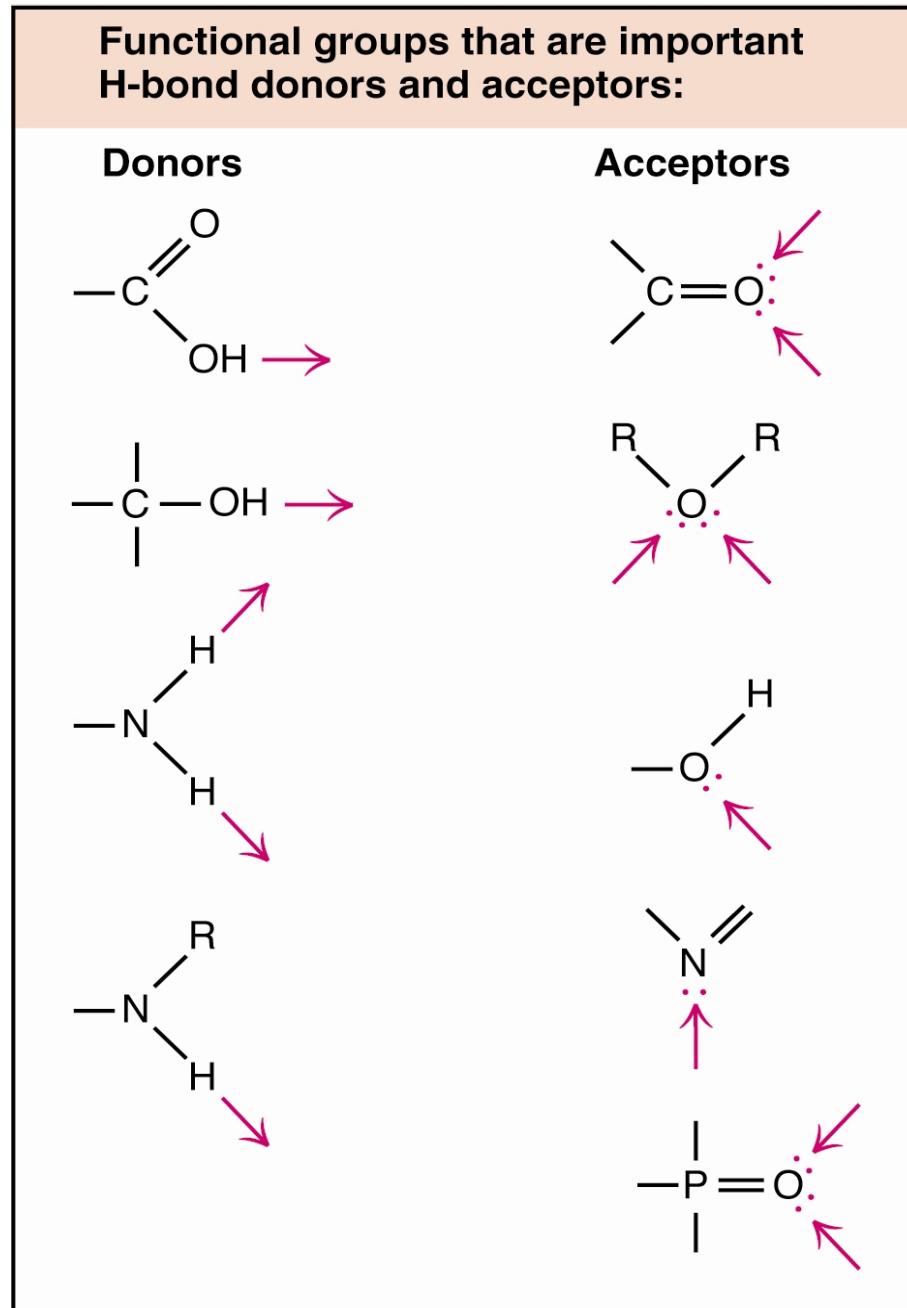


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Covalent and Noncovalent Bond Energies

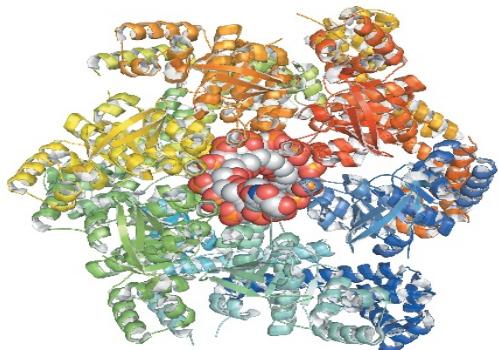
Bond	Strength (kJ/mol)
C – H	414
C – OH	351
C – C	343
C – NH	292
Van der Waals and dipole interactions	0.4 - 4.0
H-bonds	12 - 30
Ionic (aka. Charge - Charge)	20
Hydrophobic Interactions (not a specific bond, but energy derived from changes in H-bonding in the solvent H ₂ O)	<40

Two Important Points About Weak Forces

- If an enzyme is covalently modified at its active site by an inhibitor by a single bond, how much energy would you expect to put in in order to relieve inhibition?
- a. >300kJ/mol
- b. <40kJ/mol
- c. 0.4-4kJ
- d. 20kJ/mol

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Chapter 2

Water: the Medium of Life

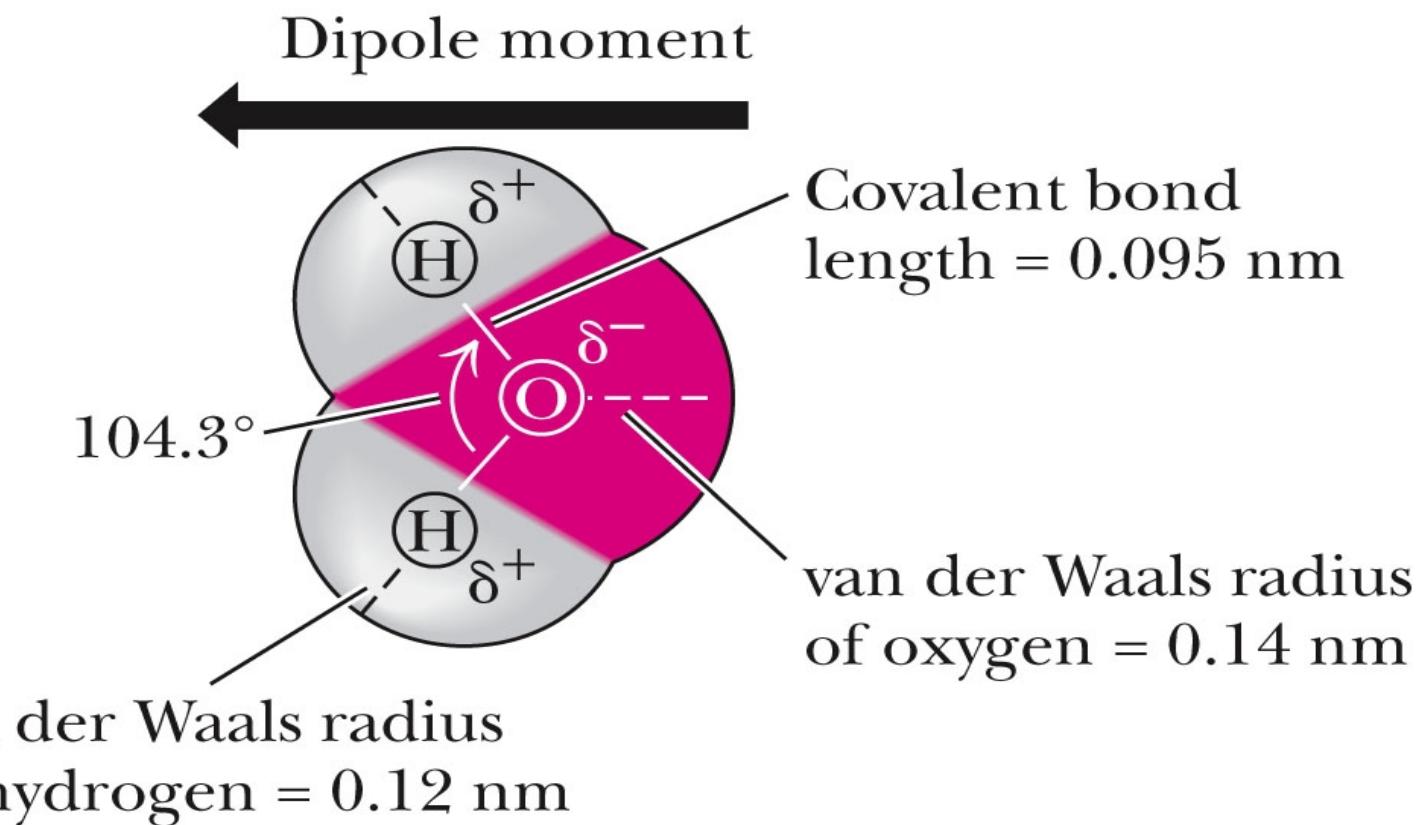
Outline

- Properties of water
- Osmotic Pressure
- pH
- Buffers, and how they work

2.1 What Are the Properties of Water?

- Water has unusual properties:
 - High b.p., m.p., heat of vaporization, heat capacity, surface tension
 - Polar due to separation of centers of + and – charge
 - High dielectric constant
 - H-bond donor and acceptor
 - Potential to form four H-bonds per water molecule
 - Can ionize into H^+ and OH^-
 - Can accept and donate H^+

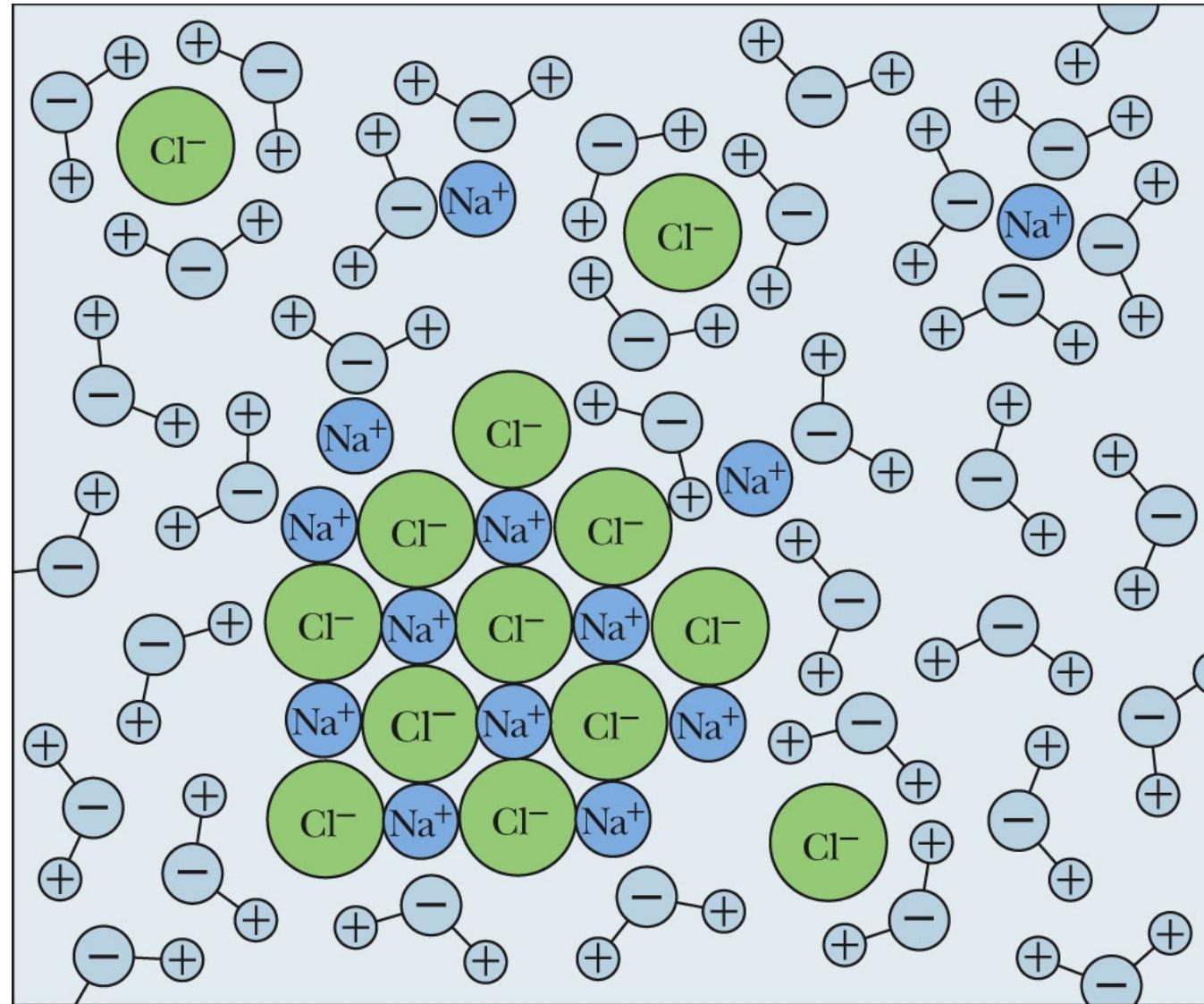
Fig. 2.1 The Structure of Water



Two lobes of negative charge formed by the lone-pair electrons of the oxygen atom lie above and below the plane of the diagram. This electron density contributes substantially to the large dipole moment. Note that the H—O—H angle is 104.3°, *not* 109°, the angular value found in molecules with tetrahedral symmetry, such as CH₄. (The dipole moment in this figure points in the direction from negative to positive, the convention used by physicists and physical chemists; organic chemists draw it pointing in the opposite direction.)

The Solvent Properties of Water Derive from Its Polar Nature

Figure 2.4
Hydration shells surrounding ions in solution.



The Solvent Properties of Water Derive from Its Polar Nature

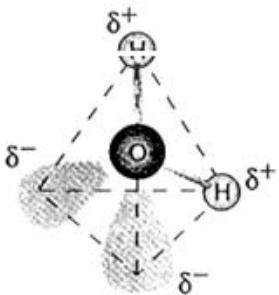
TABLE 2.1

Dielectric Constants* of Some Common Solvents at 25°C

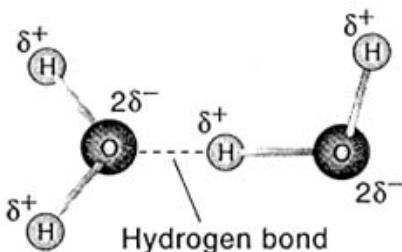
Solvent	Dielectric Constant (<i>D</i>)
Formamide	109
Water	78.5
Methyl alcohol	32.6
Ethyl alcohol	24.3
Acetone	20.7
Acetic acid	6.2
Chloroform	5.0
Benzene	2.3
Hexane	1.9

*The dielectric constant is also referred to as *relative permittivity* by physical chemists.

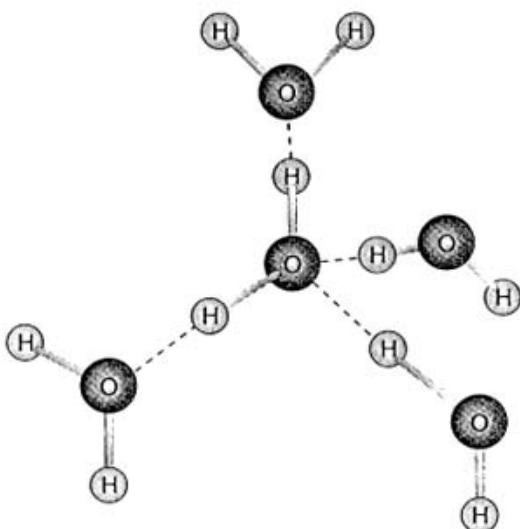
H₂O Structure and H-bonding



(a) Single water molecule



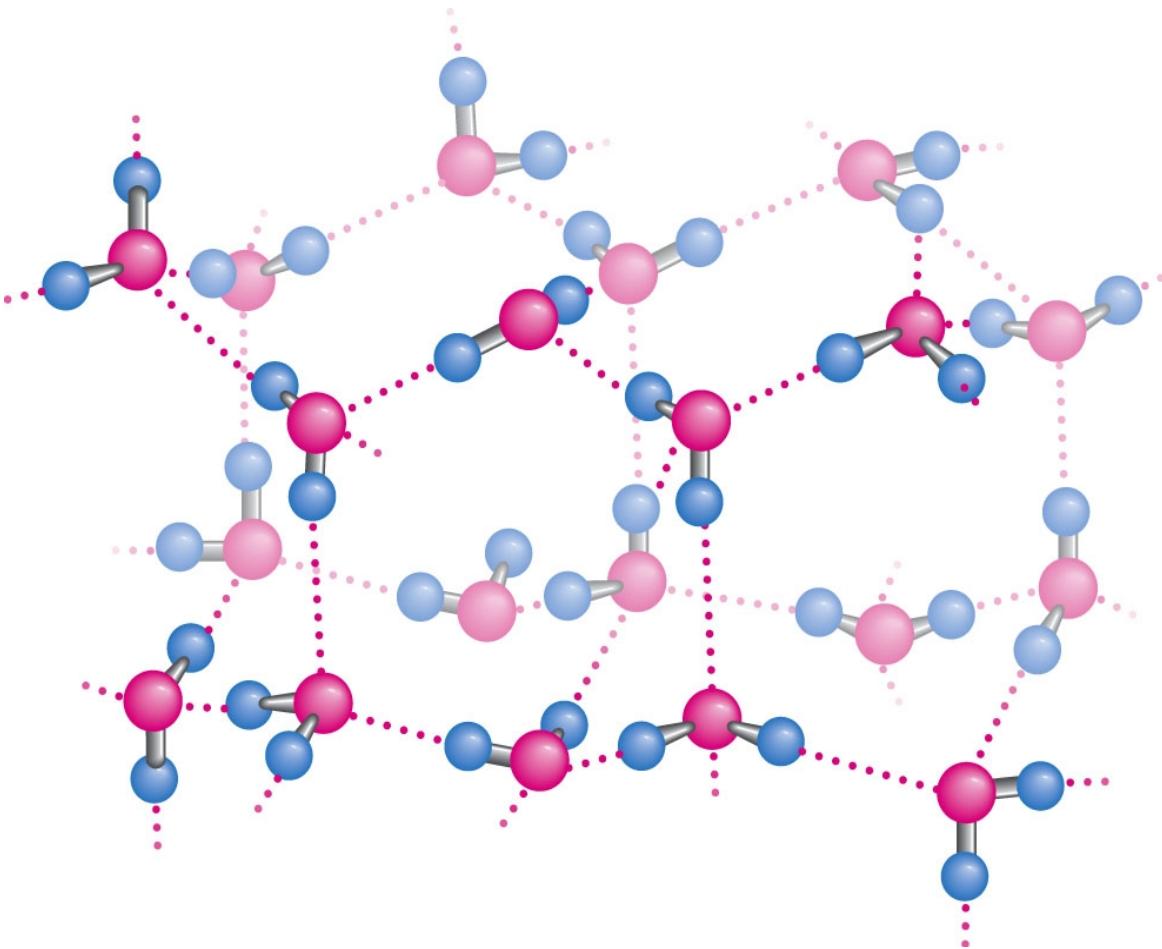
(b) Two interacting water molecules



(c) Cluster of interacting water molecules

Figure 2.2 The structure of normal ice.

The hydrogen bonds in ice form a three-dimensional network. The smallest number of H_2O molecules in any closed circuit of H-bonded molecules is six, so this structure bears the name hexagonal ice. Covalent bonds are represented as solid lines, whereas hydrogen bonds are shown as dashed lines. The directional preference of H bonds leads to a rather open lattice structure for crystalline water and, consequently, a low density for the solid state. The distance between neighboring oxygen atoms linked by a hydrogen bond is 0.274nm. Since the covalent H-O bond is 0.095nm, the H-O hydrogen bond length in ice is 0.18 nm.



Comparison of Ice and Water

Issues: H-bonds and Motion

- Ice: 4 H-bonds per water molecule
- Water: 2.3 H-bonds per water molecule at 10°C
- Ice: H-bond lifetime - about 10 microsec
- Water: H-bond lifetime - about 10 psec

Important Colligative Property of Solutes: Osmotic Pressure

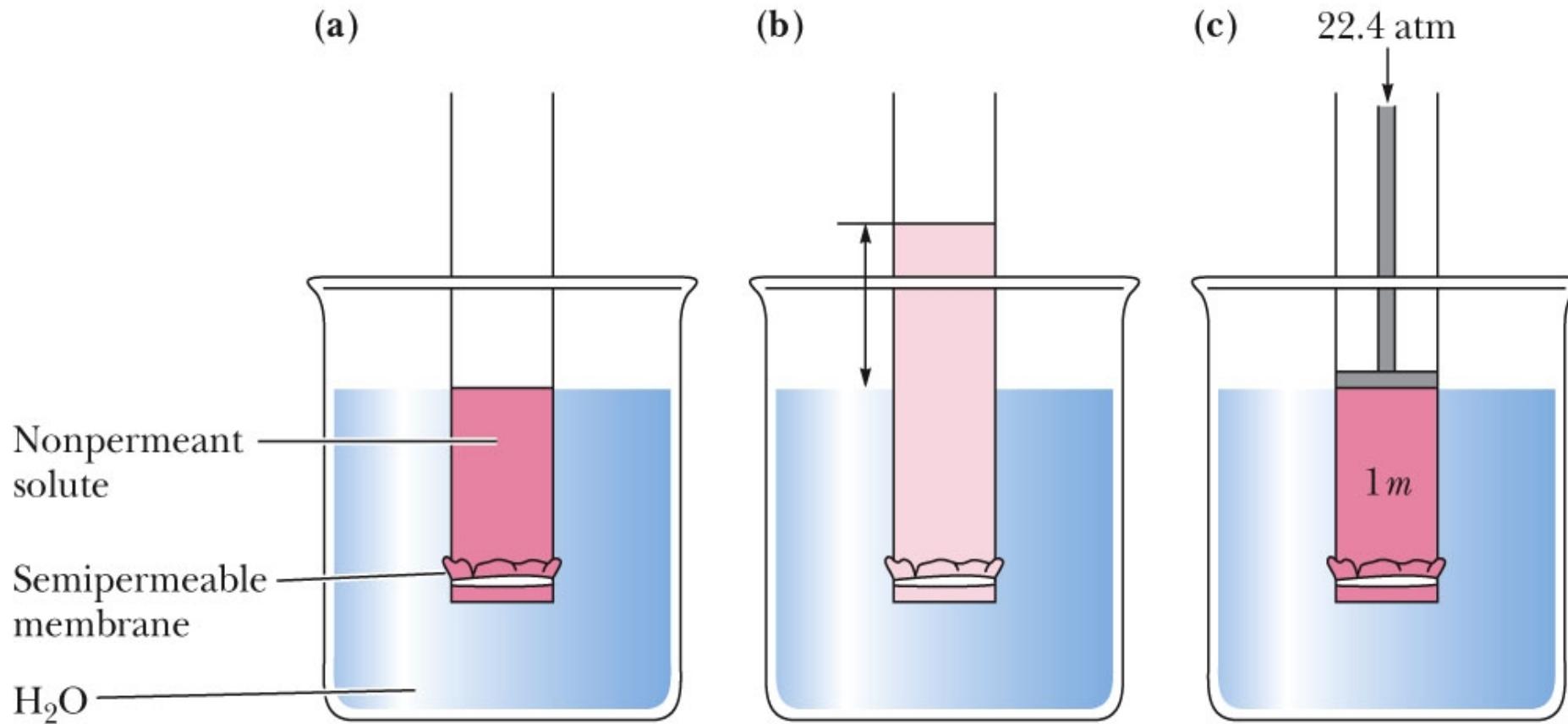
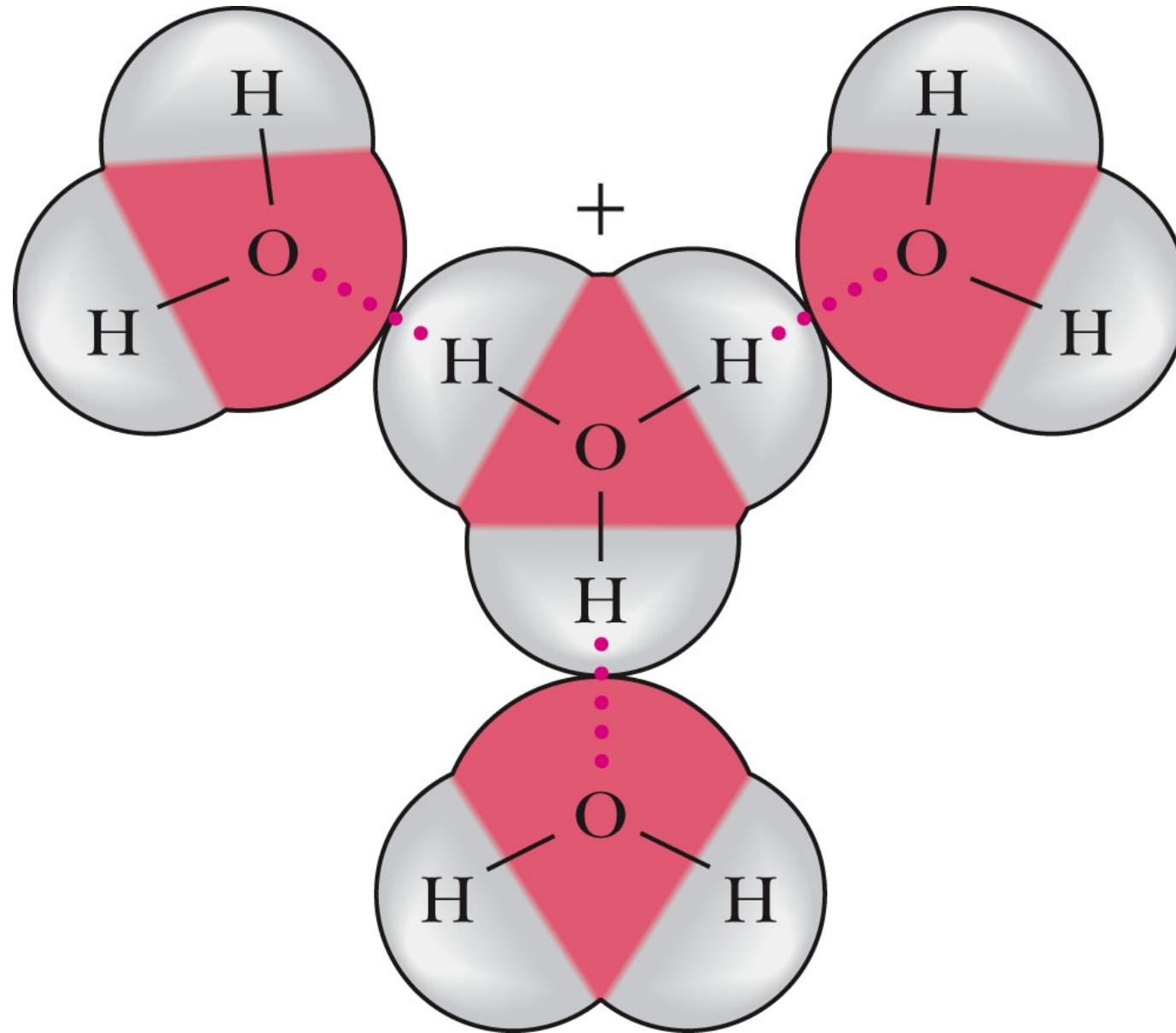


Figure 2.8 The osmotic pressure of a 1 molal (m) solution is equal to 22.4 atmospheres. Osmotic pressure is directly proportional to the concentration of the nonpermeant solute.

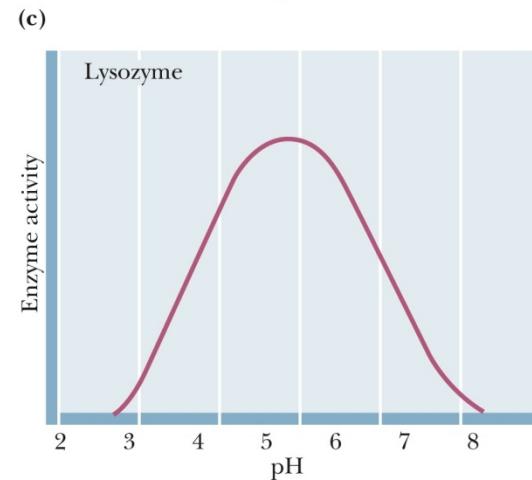
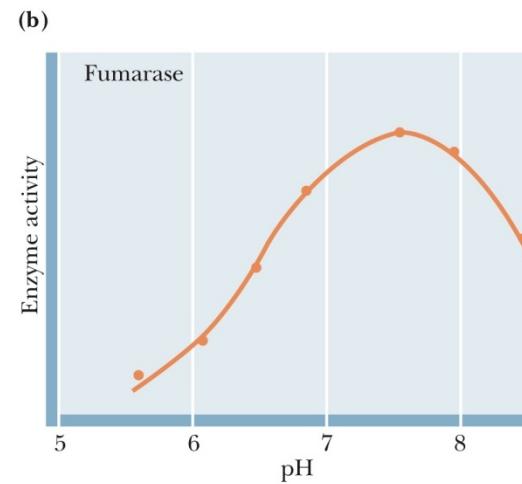
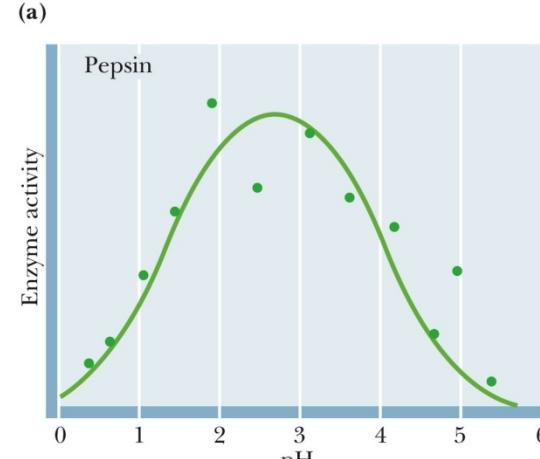
Water Can Ionize to Form H⁺ and OH⁻

Figure 2.10
The hydration
of H₃O⁺.

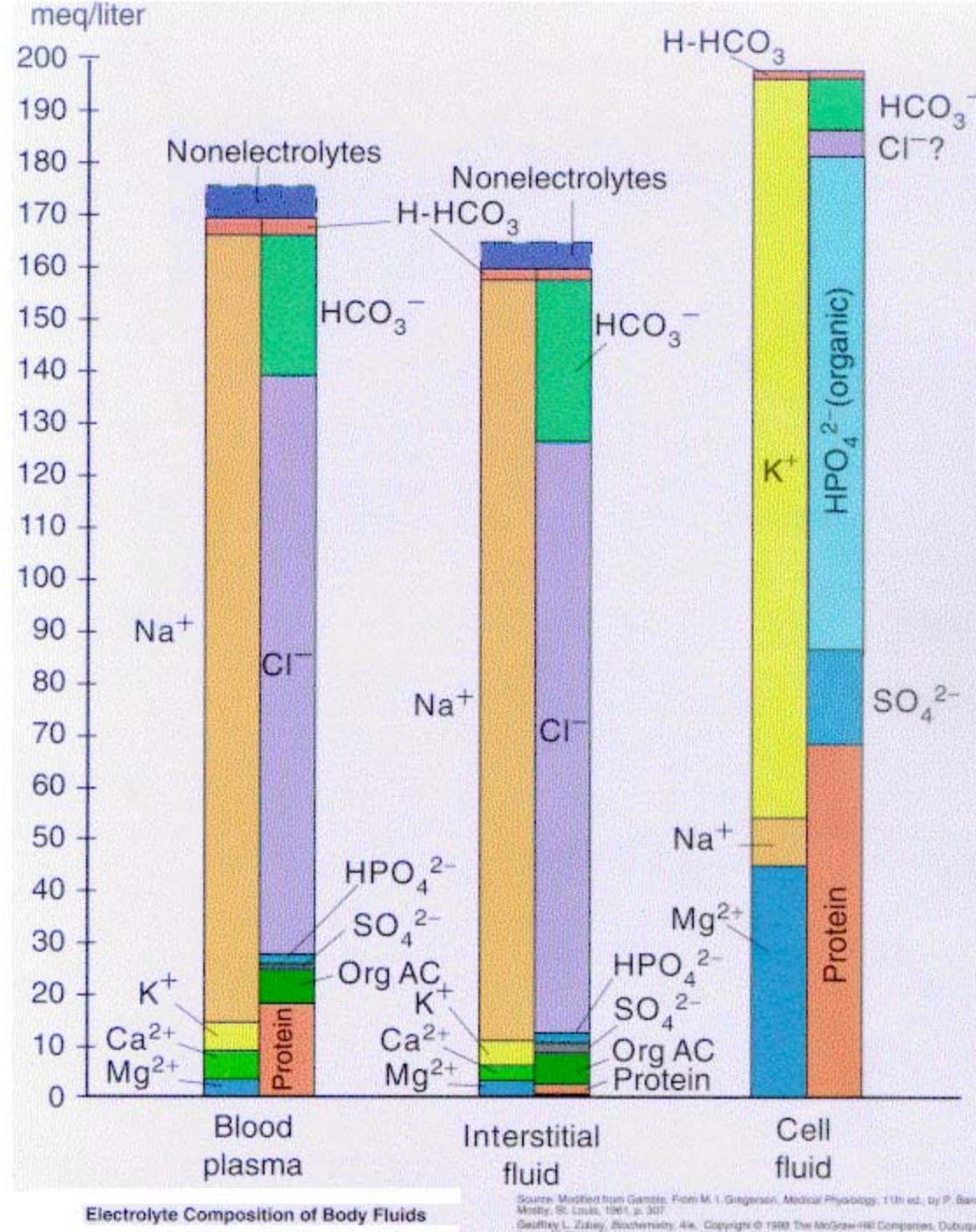


2.2 Enzyme Activity is Influenced by pH

Figure 2.15 pH versus enzymatic activity.
Pepsin is a protein-digesting enzyme active in gastric fluid.
Fumarase is a metabolic enzyme found in mitochondria.
Lysozyme digests the cell walls of bacteria. It is found in tears.



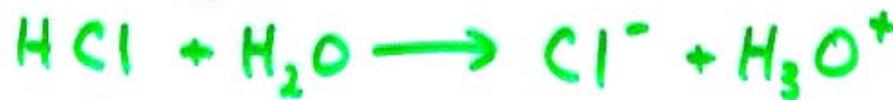
What are the pH Buffers of Life?



pH Equations

pH vs. [acid]

For strong acid



$$[H^+] \equiv [H_3O^+] = [HA]_0$$

$$pH = -\log [H^+] = -\log [HA]_0$$

For weak acid



i.e.



$$K_{\text{eq}} = \frac{[A^-][H^+]}{[HA^{(+)})][H_2O]}$$

$$K_a = K_{\text{eq}} [H_2O] = \frac{[A^-][H^+]}{[HA^{(+)})]}$$

Since $[A^-] = [H^+]$

and $[HA^{(+)})] = [HA^{(+)})_0 - [H^+]$

$$K_a = \frac{[H^+]^2}{[HA^{(+)})_0 - [H^+]}$$

$$[H^+]^2 + K_a[H^+] - K_a[HA^{(+)})_0] = 0$$

Solve quadratic eq. for $[H^+]$
and convert to pH

$$ax^2 + bx + c = 0$$
$$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

where

$$x = [H^+]$$

$$a = 1$$

$$b = K_a$$

$$c = -K_a [HA^+]_0$$

TABLE 2.4 Acid Dissociation Constants and pK_a Values for Some Weak Electrolytes (at 25°C)

Acid	$K_a (M)$	pK_a
HCOOH (formic acid)	1.78×10^{-4}	3.75
CH ₃ COOH (acetic acid)	1.74×10^{-5}	4.76
CH ₃ CH ₂ COOH (propionic acid)	1.35×10^{-5}	4.87
CH ₃ CHOHCOOH (lactic acid)	1.38×10^{-4}	3.86
HOOCH ₂ CH ₂ COOH (succinic acid) p K_1^*	6.16×10^{-5}	4.21
HOOCH ₂ CH ₂ COO ⁻ (succinic acid) p K_2	2.34×10^{-6}	5.63
H ₃ PO ₄ (phosphoric acid) p K_1	7.08×10^{-3}	2.15
H ₂ PO ₄ ⁻ (phosphoric acid) p K_2	6.31×10^{-8}	7.20
HPO ₄ ²⁻ (phosphoric acid) p K_3	3.98×10^{-13}	12.40
C ₃ N ₂ H ₅ ⁺ (imidazole)	1.02×10^{-7}	6.99
C ₆ O ₂ N ₃ H ₁₁ ⁺ (histidine-imidazole group) p K_R^\dagger	9.12×10^{-7}	6.04
H ₂ CO ₃ (carbonic acid) p K_1	1.70×10^{-4}	3.77
HCO ₃ ⁻ (bicarbonate) p K_2	5.75×10^{-11}	10.24
(HOCH ₂) ₃ CNH ₃ ⁺ (tris-hydroxymethyl aminomethane)	8.32×10^{-9}	8.07
NH ₄ ⁺ (ammonium)	5.62×10^{-10}	9.25
CH ₃ NH ₃ ⁺ (methylammonium)	2.46×10^{-11}	10.62

*The p K values listed as p K_1 , p K_2 , or p K_3 are in actuality p K_a values for the respective dissociations. This simplification in notation is used throughout this book.

[†]p K_R refers to the imidazole ionization of histidine.

$$\text{pH vs. } \frac{[\text{A}^-]}{[\text{HA}^{/\!+}]}$$

For most applications in biochemistry
this is the relationship used and is
easily seen in the Henderson-Hassel-
bach rearrangement of the K_a
equality:

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

$$\log K_a = \log [\text{H}^+] + \log \frac{[\text{A}^-]}{[\text{HA}^{/\!+}]}$$

$$-\log [\text{H}^+] = -\log K_a + \log \frac{[\text{A}^-]}{[\text{HA}^{/\!+}]}$$

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

Figure 2.11 The titration curve for acetic acid. Note that the titration curve is relatively flat at pH values near the pK_a . In other words, the pH changes relatively little as OH^- is added in this region of the titration curve.

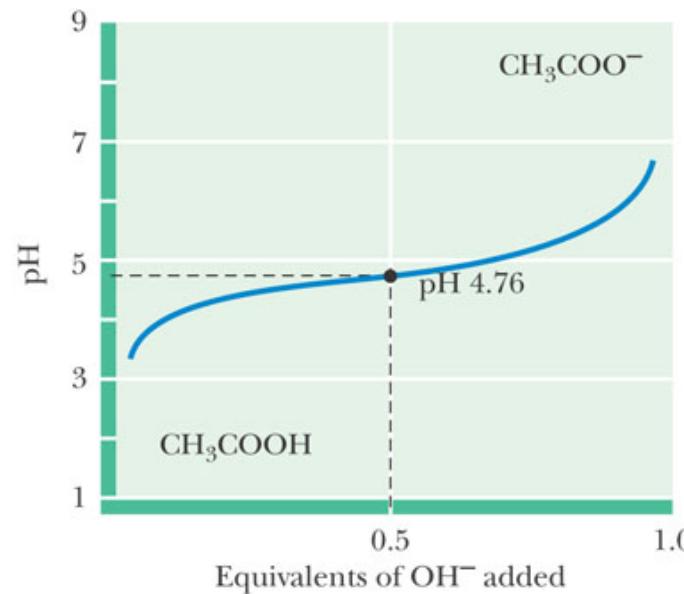
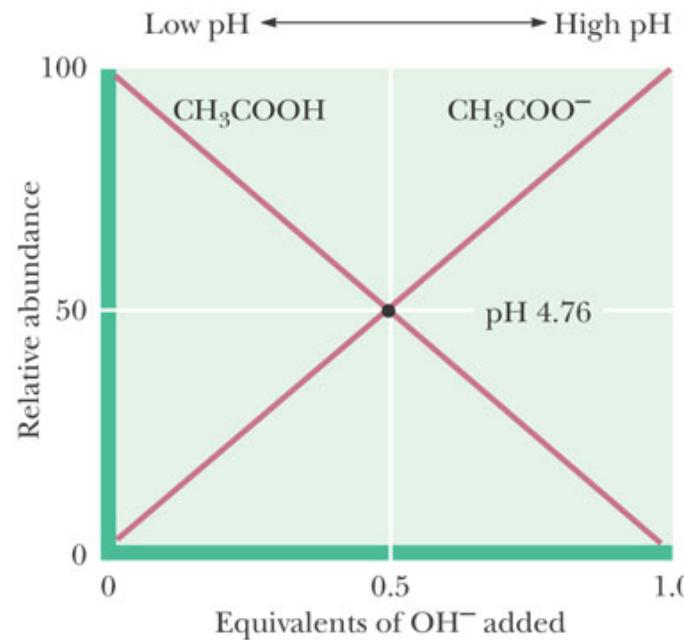
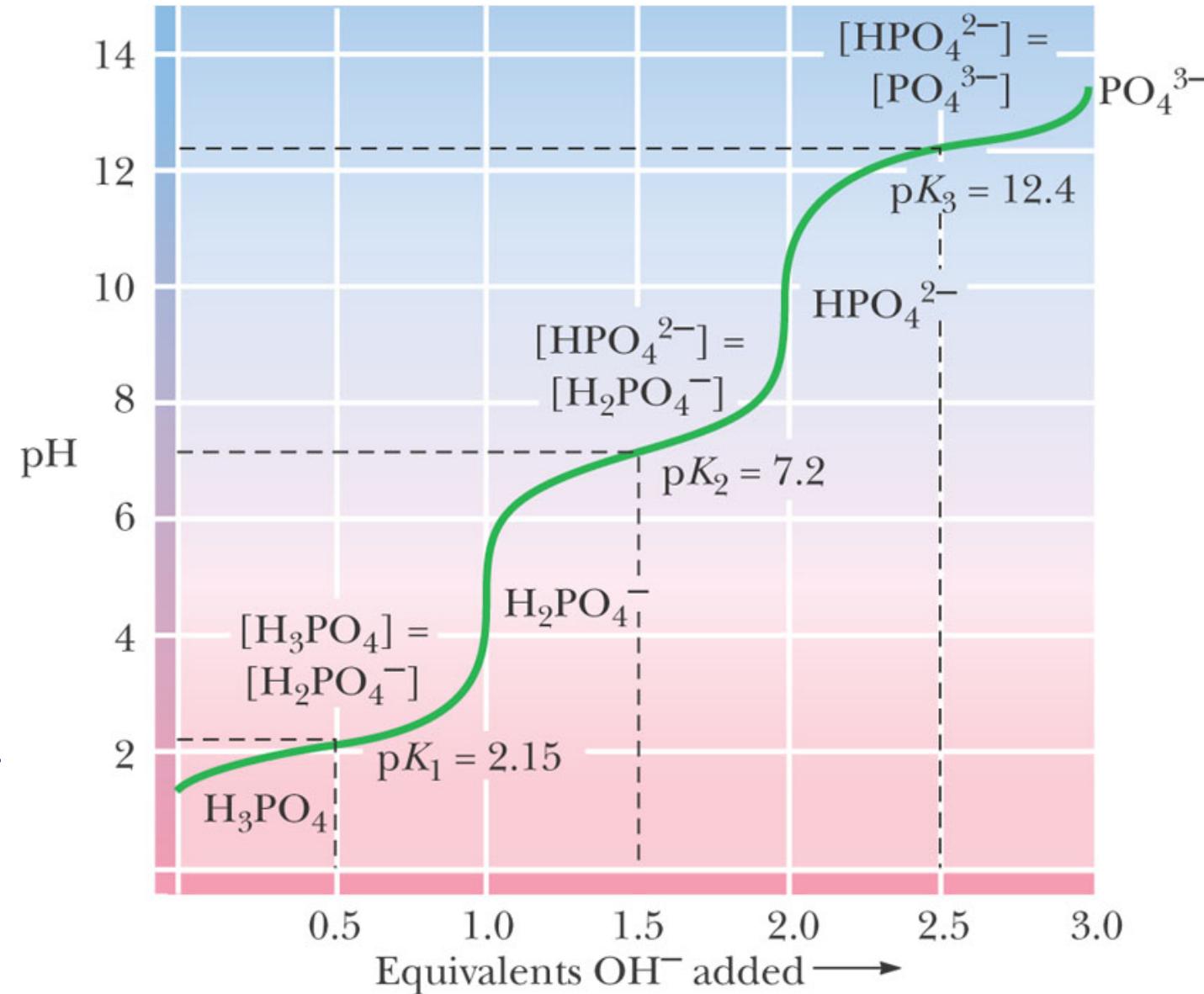


Figure 2.13

The titration curve for phosphoric acid. The chemical formulas show the prevailing ionic species present at various pH values. Phosphoric acid (H_3PO_4) has three titratable hydrogens and therefore three midpoints are seen: at pH 2.15 (pK_1), pH 7.20 (pK_2), and pH 12.4 (pK_3).



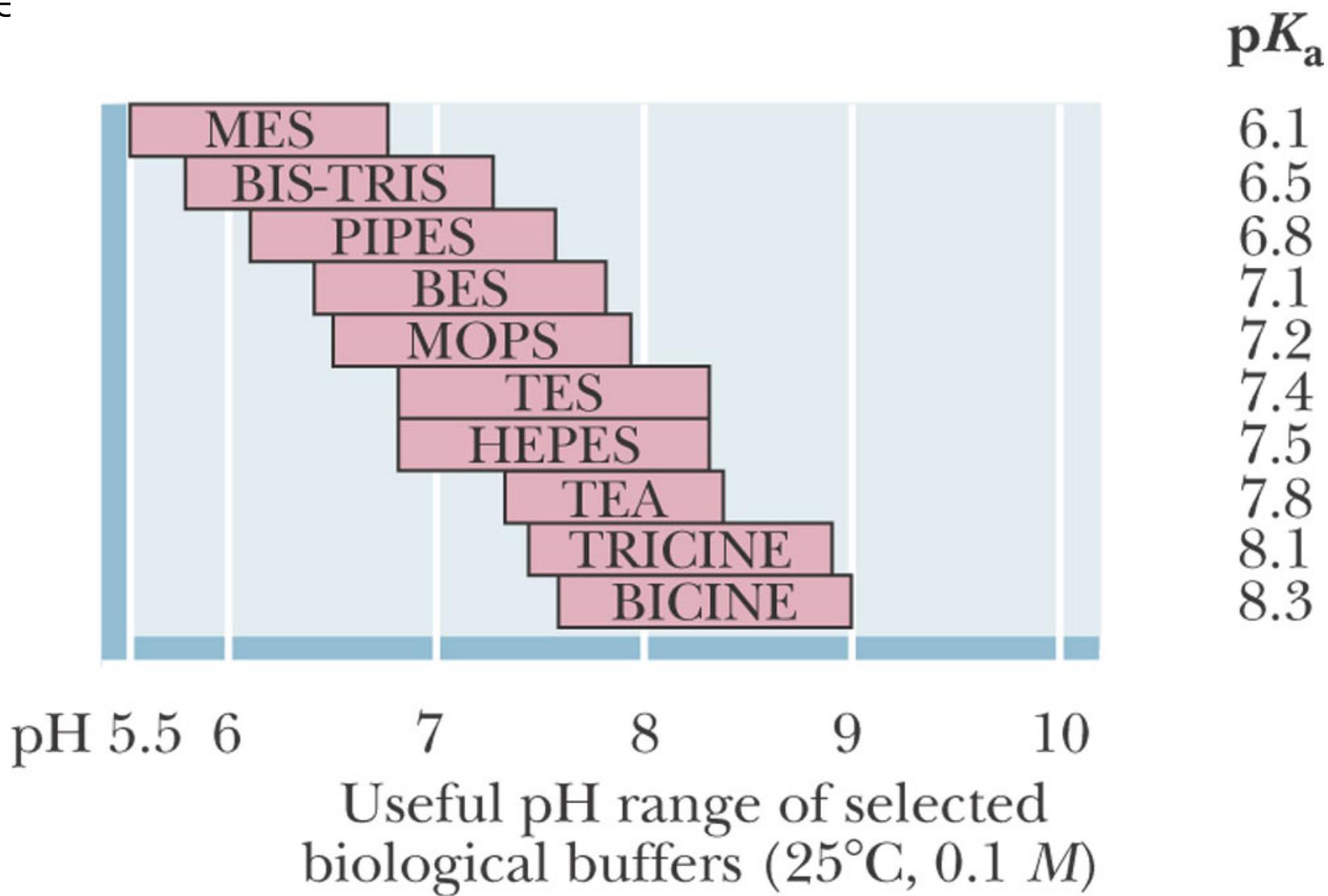
- What is the pH of 50mM Phosphate? $pK_a1 = 2$, $pK_a2 = 7$, $pK_a3 = 12$
- A. 5.4
- B. 6.5
- C. 3.4
- D. 1.7

- What is the pH of 50mM Phosphate? $pK_a1 = 2$, $pK_a2 = 7$, $pK_a3 = 12$
- A. 5.4
- B. 6.5
- C. 3.4
- D. 1.7

2.3 What Are Buffers, and What Do They Do?

- Buffers are solutions that resist changes in pH as acid and base are added.
- Most buffers consist of a weak acid and its conjugate base.
- Buffer range is $pK_a \pm 1.0$. This is the range in which a buffer can be used reliably.

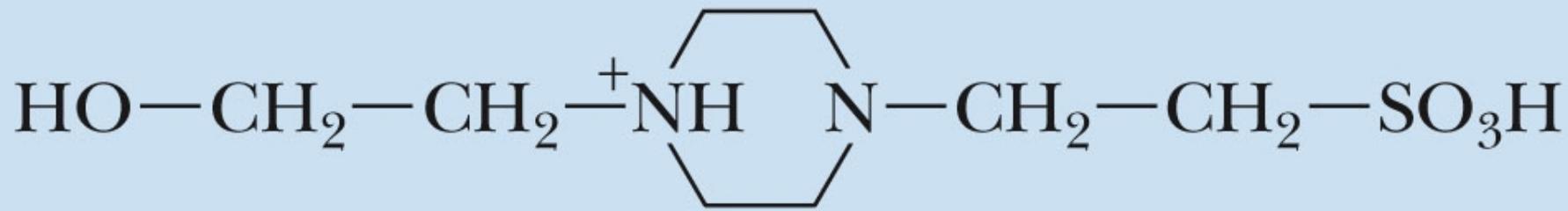
What are Buffers and What Do They Do?



- Which is not a suitable buffer for a solution at pH = 7.6?
- A. Bicine ($pka = 8.3$)
- B. Tricine ($pka = 8.1$)
- C. TES ($pka = 7.4$)
- D. BIS-TRIS ($pka = 6.5$)

- Which is not a suitable buffer for a solution at pH = 7.6?
- A. Bicine (pka = 8.3)
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- D. BIS-TRIS (pka = 6.5)

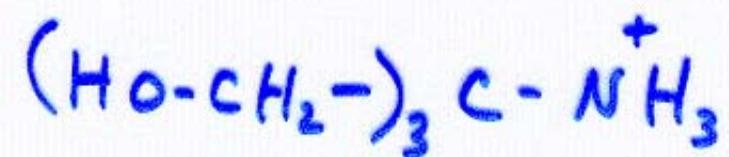
What are Buffers and What Do They Do?



HEPES

Figure 2.17 The structure of HEPES, in its fully protonated form.

Tris (hydroxymethyl)aminomethane



$$\text{p}K_a = 8.1$$

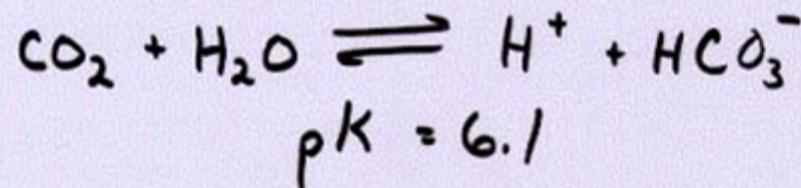
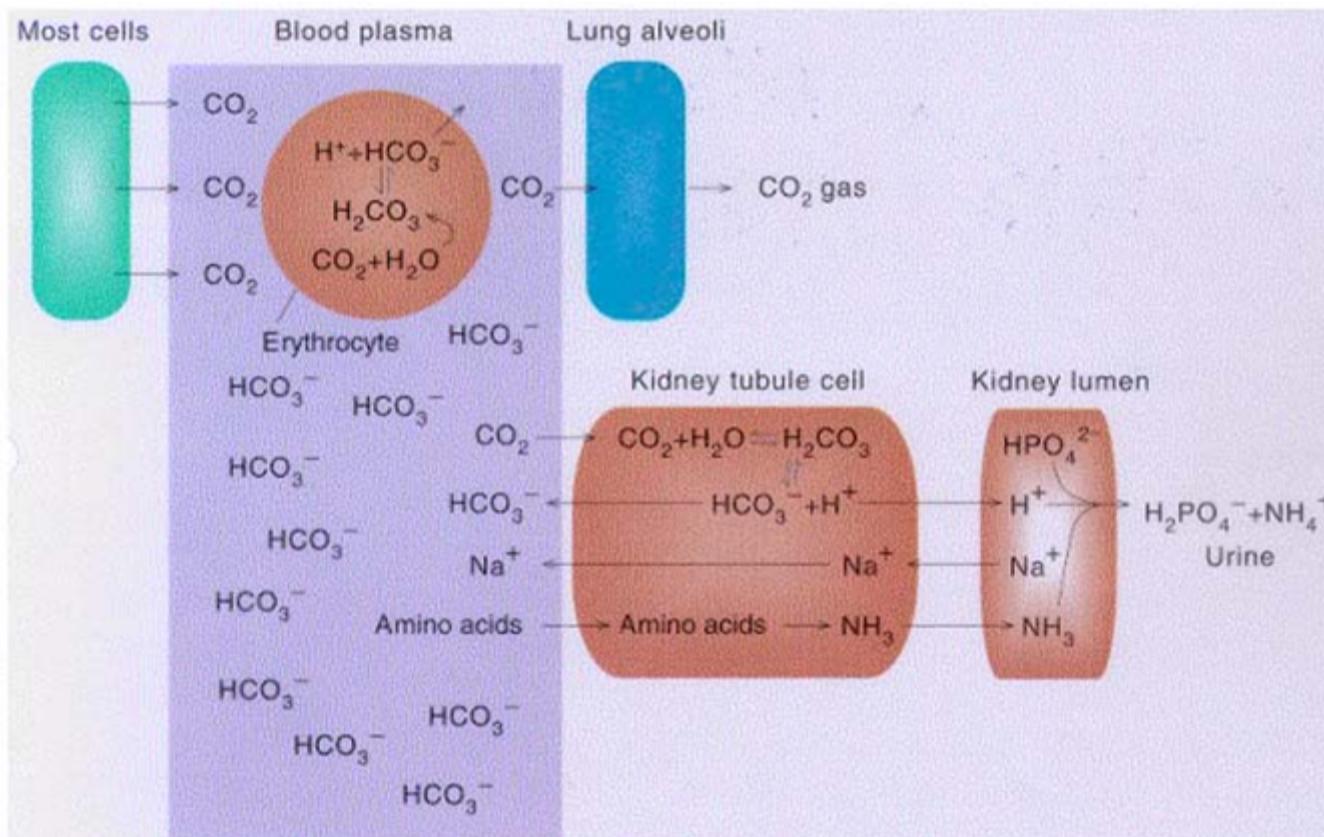
TABLE 2.4 Acid Dissociation Constants and pK_a Values for Some Weak Electrolytes (at 25°C)

Acid	$K_a (M)$	pK_a
HCOOH (formic acid)	1.78×10^{-4}	3.75
CH ₃ COOH (acetic acid)	1.74×10^{-5}	4.76
CH ₃ CH ₂ COOH (propionic acid)	1.35×10^{-5}	4.87
CH ₃ CHOHCOOH (lactic acid)	1.38×10^{-4}	3.86
HOOCH ₂ CH ₂ COOH (succinic acid) p K_1^*	6.16×10^{-5}	4.21
HOOCH ₂ CH ₂ COO ⁻ (succinic acid) p K_2	2.34×10^{-6}	5.63
H ₃ PO ₄ (phosphoric acid) p K_1	7.08×10^{-3}	2.15
H ₂ PO ₄ ⁻ (phosphoric acid) p K_2	6.31×10^{-8}	7.20
HPO ₄ ²⁻ (phosphoric acid) p K_3	3.98×10^{-13}	12.40
C ₃ N ₂ H ₅ ⁺ (imidazole)	1.02×10^{-7}	6.99
C ₆ O ₂ N ₃ H ₁₁ ⁺ (histidine-imidazole group) p K_R^\dagger	9.12×10^{-7}	6.04
H ₂ CO ₃ (carbonic acid) p K_1	1.70×10^{-4}	3.77
HCO ₃ ⁻ (bicarbonate) p K_2	5.75×10^{-11}	10.24
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*The p K values listed as p K_1 , p K_2 , or p K_3 are in actuality p K_a values for the respective dissociations. This simplification in notation is used throughout this book.

[†]p K_R refers to the imidazole ionization of histidine.

Blood Buffering; CO₂ and H⁺ Removal



$$\text{pH} = 6.1 + \log \frac{[\text{HCO}_3^-]}{[\text{CO}_2]}$$

NB: If you are using the G&G 4th ed.,
in the text box ‘The Bicarbonate Buffer
System of Blood Plasma’ on p.43, middle of
right column, there is a typo.

The overall equilibrium for the ionization of
 H_2CO_3 in equilibrium with $\text{CO}_2(\text{d})$ should be

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

i.e., K_h should not be present on the right
side of the equation

Thermodynamics

Compiled by Jaime Cruz

What is thermodynamics

- Thermodynamics is simply the **study of energy**
- It's the science that deals with energy in its various forms and the **conversion of one form of energy into another**
- Energy
 - **Required by all organism**
 - May be kinetic or potential

- 1st Law of Thermodynamics
- 2nd Law of Thermodynamics
- What drives a reaction to process ?
 - Entropy
 - Enthalpy
 - Gibbs Free Energy Equations
 - Coupled Reactions
- Van't Hoff Plots

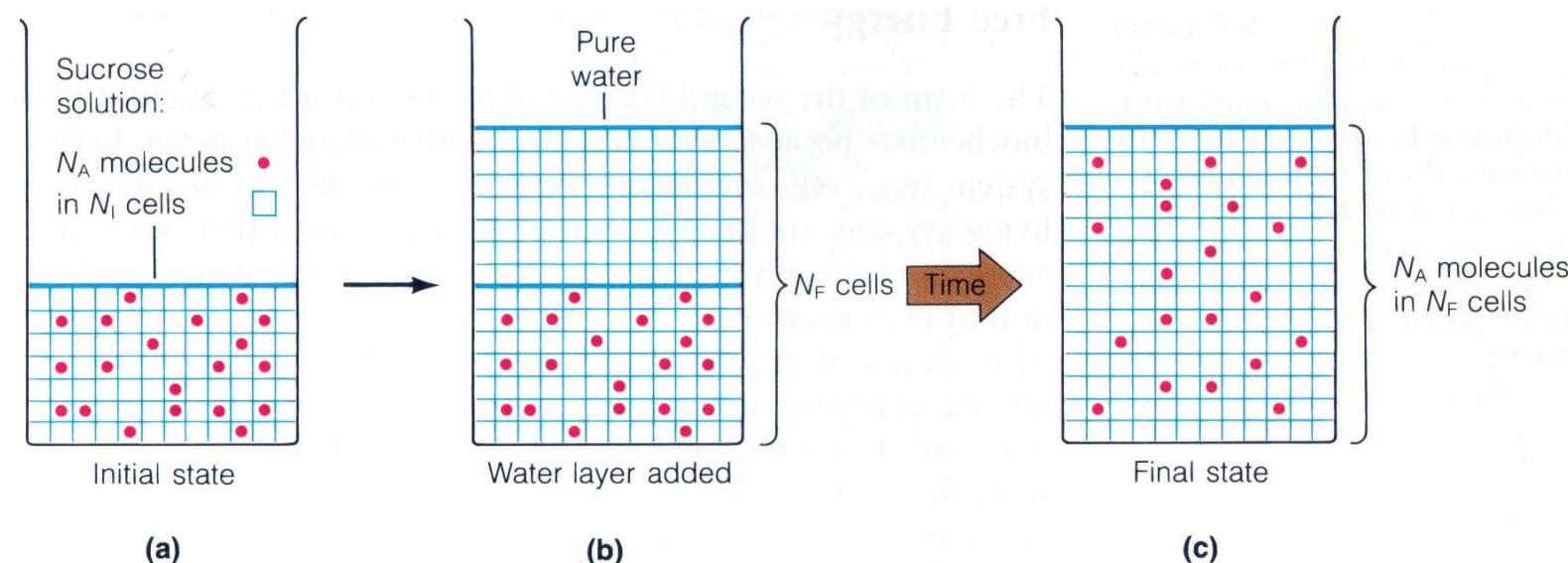
First Law of Thermodynamics

- Energy cannot be created or destroyed, but can only be converted into other forms
- This means that the total amount of energy in the universe is constant.

Second Law of Thermodynamics

- The Disorder in the universe is always increasing
 - Entropy: the amount of disorder in a system
 - $\Delta S_{\text{universe}} = S_f - S_i = +$
 - Living organisms preserve their internal order by taking free energy from their surroundings in the form of nutrients or sunlight and returning to their surroundings and equal amount of energy as heat and entropy.
- Types of Entropy:**
- Translational** – molecules move around
 - Rotational** – molecules spin/rotate
 - Vibrational** – molecules vibrate (INSIGNIFICANT)
 - Electronic** – entropy attributed to electrons' occupation of energy states (INSIGNIFICANT)

- Entropy (S) is a quantitative expression for the randomness or disorder in a system.
 - The unit of ΔS is joules/mole * Kelvin
- $S = k \ln W$
- $K = \text{boltzmann constant} (1.38E-23 \text{ J/K})$
- $W = \text{number of equal energy states a system can arrange itself in.}$
 - W rises with disorder, so S increases
- $S \text{ per moles} = kN \ln W$
 - $N = \text{Avogadro's number}$
- $kN \ln W \rightarrow R \ln W \rightarrow 2.303R \log W$



Example

Entropy is a

- A) linear function of the number of states that can be attained by a system, all at equivalent energy
- B) log function of the number of states that can be attained by a system, all at equivalent energy
- C) log function of the work done by or to a system
- D) property of a state defined by the first law of thermodynamics

Example

- Entropy is a
- A) linear function of the number of states that can be attained by a system, all at equivalent energy
- **B) log function of the number of states that can be attained by a system, all at equivalent energy**
- C) log function of the work done by or to a system
- D) property of a state defined by the first law of thermodynamics

- Enthalpy (H) is the heat content of the reacting system (at constant pressure)
 - H reflects the number and kinds of chemical bonds in the reactants and products
- **When a chemical rxn releases heat, it is said to be exothermic, the heat content of the products is less than that of the reactants, and the ΔH has a negative value**
- Reacting systems that take up heat from their surroundings are **endothermic and have a positive values of ΔH**
- The unit of ΔH is Joules/mole

Gibbs Free Energy

- When a reacting system **is not at equilibrium**, the tendency to move toward equilibrium represents a driving force.
 - The magnitude of this driving force is expressed as free energy change (ΔG)
- **When ΔG is negative**, the products contain less free energy than the reactants, and the reaction will proceed **spontaneously in the forward direction**
- **When ΔG is positive**, the products contain more free energy than the reactants, and the reaction will tend to proceed **spontaneously in the reverse direction**

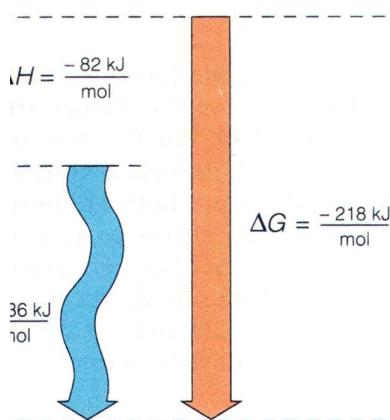
- Changes in free energy, enthalpy and entropy in biological systems are related to each other by the equation

$$\Delta G = \Delta H - T\Delta S \Rightarrow \Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$$

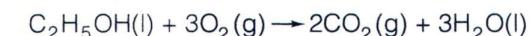
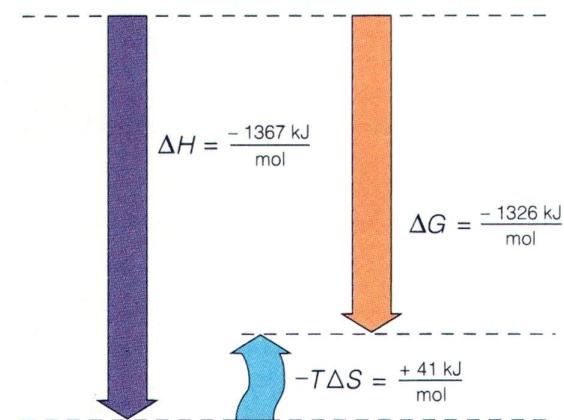
- $^\circ$ indicates standard conditions and ' indicates pH=7
- All reactions proceed in the direction of:
 - Increasing entropy
 - A release of free energy ($- \Delta G$)

The more negative the ΔG , the greater the release of free energy during a chemical reaction

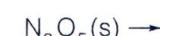
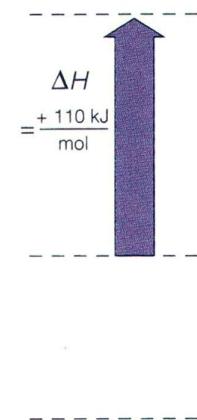
3.4



Enthalpy and entropy changes favor reaction.



Enthalpy favors this reaction, but entropy opposes it. We could call this an "enthalpy-driven" reaction. If water vapor were the product, an entropy increase would favor the reaction as well.

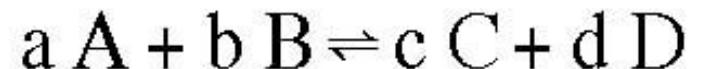


This is a somewhat unusual reaction in that the reaction is favored by the resulting products.

Gibbs Free Energy

in terms of Reactant and Product concentrations

- $\Delta G = \Delta G^\circ + RT\ln Q$
- Hence, ΔG relies primarily on:
 1. Intrinsic properties of the species themselves (ΔG°_{RX})
 2. $\Delta G^\circ_{RX} = -RT\ln K_{eq}$
 3. Ratio of []'s of species given (Q)
 4. Temperature is in Kelvin and must match the temperature at which ΔG°_{RX} is determined.



$$\Delta G = \Delta G^\circ_{RX} + RT \ln \frac{[C]^c [D]^d}{[A]^a [B]^b}$$

Gibbs Free Energy

- Under **standard conditions** (25°, 1 atm), when reactants and products are initially at 1M concentrations,
 - The force driving the system toward equilibrium is defined as the **standard free energy change (ΔG°)**
 - By this definition, standard state for reactions involves $[H^+]=1M$ or $pH=0$
- However, most biochemical reactions occur in well-buffered aqueous solutions near $pH=7$
 - For the convenience of calculations, biochemists define a **different standard state** in which the concentration of $[H^+]$ is 10^{-7}
- Physical constants based on this **biochemical standard state** are called transformed constants
 - They are written as $\Delta G^\circ'$ and K_{eq}'
 - This serves to distinguish them from the untransformed constants which are used by chemists

Gibbs Free Energy

Actual free energy change

- $\Delta G = \Delta G^\circ + RT\ln Q$

Actual free energy change (ΔG) is a function of reactant and product concentrations and of the temperature prevailing during the reaction

Q will not necessarily match the standard conditions

The T at which ΔG° was determined must match the temperature prevailing during the reaction

Example

- The, $\Delta G^\circ'$ for the reaction below is -15 kJ/mol at 37 C. What is the Gibbs free energy change for the reaction at 37 C if $[A] = 1 \times 10^{-4}$ M and each of the products are 1mM?

R is 8.31 J/K*mol



- a. -20 kJ/mol
- b. -17 kJ/mol
- c. -54 kJ/mol
- d. -27 kJ/mol

Example

- The, $\Delta G^\circ'$ for the reaction below is -15 kJ/mol at 37 C. What is the Gibbs free energy change for the reaction at 37 C if $[A] = 1 \times 10^{-4}$ M and each of the products are 1mM?

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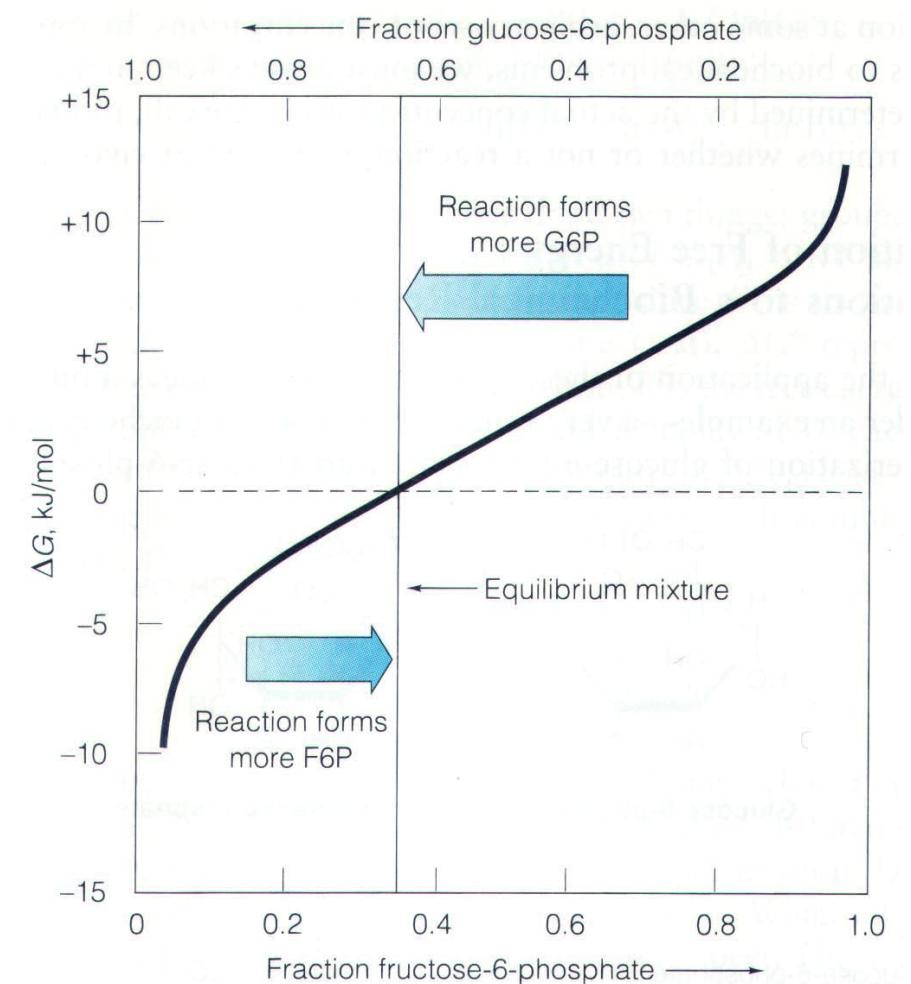
$$\Delta G = \Delta G^\circ' + RT\ln Q$$

$$\bullet Q = [B][C]/[A]$$

$$\Delta G = -15 \text{ kJ/mol} + (8.314 \times 10^{-3} \text{ kJ/mol*K}) (273.15 + 37) \text{ K} \ln ([1 \times 10^{-3} \text{ M}][1 \times 10^{-3} \text{ M}])$$

$$\Delta G = -26.87 \text{ kJ/mol}$$

- Reactions that have **no net change in substrate or product** are termed **equilibrium reactions**, and have no change in free energy ($\Delta G = 0$).
- All reactions are **potentially reversible**.
- The **directionality and amount of free energy release** of a chemical reaction can be modified by altering substrate and product concentrations.
- - **↑ products** may **reverse the direction** of the reaction
- - **↑ substrates** can make the ΔG **more negative**



What happens at Equilibrium?

- At equilibrium, $\Delta G = 0$, therefore:
- $\Delta G = \Delta G^\circ + RT\ln Q = 0$
 - Where **Q= Reaction Quotient** which describes the concentrations of reactants and products at any point during the reaction
- $0 = \Delta G^\circ + RT\ln Q$
- $\Delta G^\circ = -RT\ln K_{eq}$
 - Where **K_{eq}= Equilibrium Constant**, which describes the molar concentrations of reactants and products **at equilibrium**.

Example

- If the standard state free energy for a folding of a protein is 11.4kJ/mol at 25 C, what is the equilibrium ratio of folded to unfolded molecules of the protein at this temperature? (R is 8.31 J/K*mol)
- A. 10:1
- B. 100:1
- C. 1000:1
- D. 10,000:1

Example

- If the standard state free energy for a **folding of a protein** is -11.4kJ/mol at 25 C, what is the equilibrium ratio of folded to unfolded molecules of the protein at this temperature? (R is 8.31 J/K*mol)
- A. 10:1
- B. 100:1
- C. 1000:1
- D. 10,000:1

$$\Delta G^\circ = -RT\ln K_{eq}$$

$$K_{eq} = [\text{products}]/[\text{reactants}]$$

Unfolded Folded

$$K_{eq} = [\text{folded}]/[\text{unfolded}]$$

$$-11.4 \text{ kJ/mol} = - (8.31 \times 10^{-3} \text{ kJ/mol*K})$$

$$(273.15 + 25) \text{ K} \times \ln([\text{folded}]/[\text{unfolded}])$$

$$\ln[\text{folded}]/[\text{unfolded}] = 4.6 \quad [\text{folded}]/[\text{unfolded}] = \\ 99.6/1 = 100/1$$

Standard Free Energy Changes are Additive

- Many reactions that take place in the human body are, by themselves, thermodynamically unfavorable (positive ΔG°)
- A reaction can be driven in the forward direction by coupling it to a highly exergonic reaction (large negative ΔG°) through a common intermediate.
- **The ΔG° values of the sequential reactions are additive:**
$$\Delta G^\circ_{\text{total}} = \Delta G^\circ_1 + \Delta G^\circ_2$$

Coupled Reactions

- Many crucial reaction in our body that have a $\Delta G^\circ > 0$ are able to proceed typically by being **coupled to the hydrolysis of a high energy phosphate compound such as ATP**
- **The overall ΔG° for a coupled reaction is the sum of the two ΔG° values for the individual reactions**

Coupled Reactions

An example is the first step in glycolysis

- Glucose + Pi \rightleftharpoons Glucose 6-phosphate + H₂O
 - $\Delta G^\circ' = 13.8 \text{ kJ/mol}$
 - * $\Delta G_0' > 0$
*reaction is not spontaneous
- ATP + H₂O \rightleftharpoons ADP + Pi
 $\Delta G^\circ' = -30.5 \text{ kJ/mol}$
- Large negative $\Delta G^\circ'$
- Highly exergonic



+

***Overall free energy change of the coupled reactions = sum of $\Delta G^{\circ'}$ rxn1 + $\Delta G^{\circ'}$ rxn2 :**

$$\Delta G^{\circ'} = -16.7 \text{ kJ/mol}$$

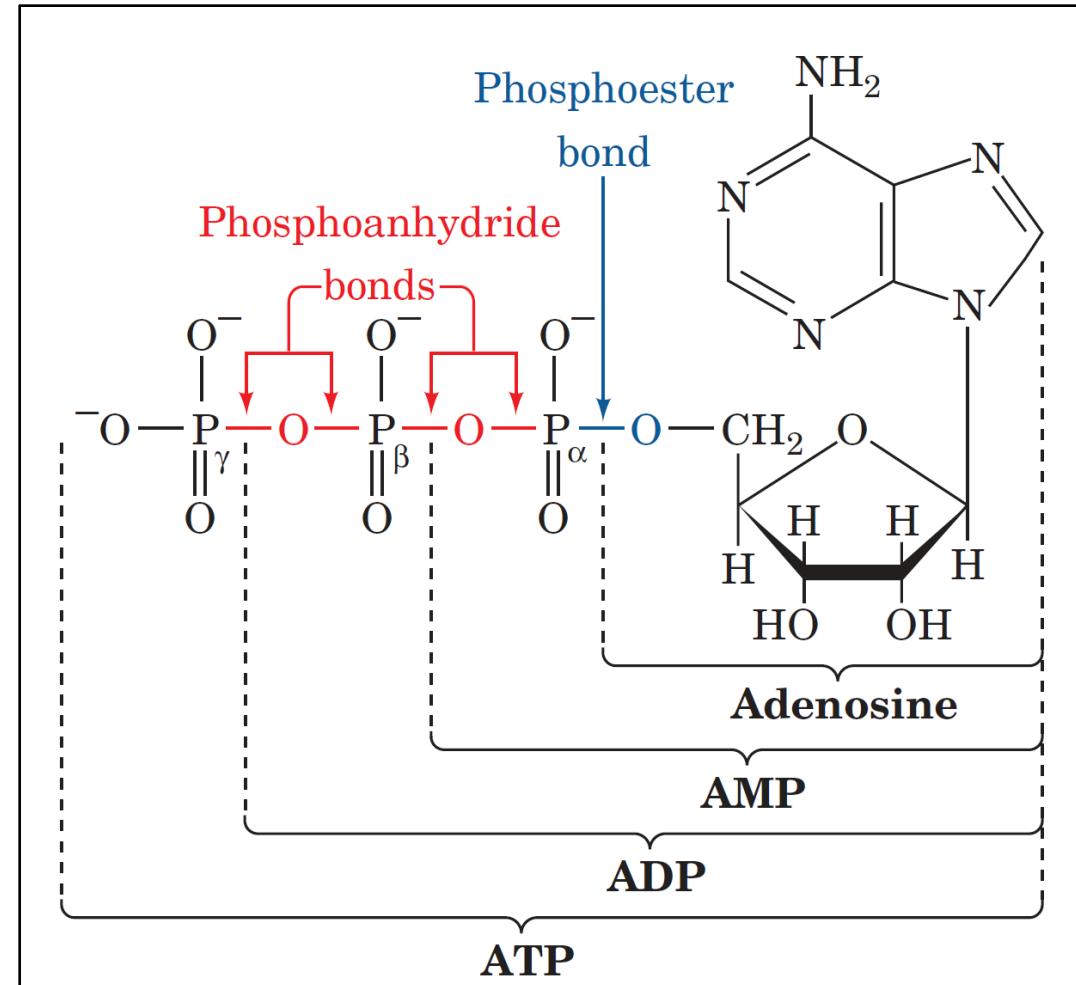
***overall reaction is favorable**

Coupled Reactions: ATP

- ATP is the most commonly used high energy compound. It can be considered the energy currency of the cell
- As we saw, energy is stored in ATP is used to drive the synthesis of glucose 6 phosphate, even though its formation from glucose and Pi is endergonic.
- This strategy works only if compounds such as ATP are continuously available.

ATP Contains Two Pyrophosphate Linkages

- ATP is composed of
Adenine ring
Ribose sugar
Three phosphate groups.
- The bonds between phosphates are
high energy phosphoanhydride bonds.
When are broken, ATP becomes ADP+P,
energy is released (favorable ΔH)
- $1 \text{ ATP} \rightarrow 1 \text{ pi} + 1 \text{ ADP}$ (2 molecules), so
 ΔS is also favorable.
- **ATP transfers energy** to many different
chemical reactions; almost all metabolic
pathways directly or indirectly run on
energy supplied by ATP.



Van't Hoff Plot

Determination of ΔH° and ΔS°

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$$

$$-\text{RTlnK}_{\text{eq}} = \Delta H^\circ - T\Delta S^\circ$$

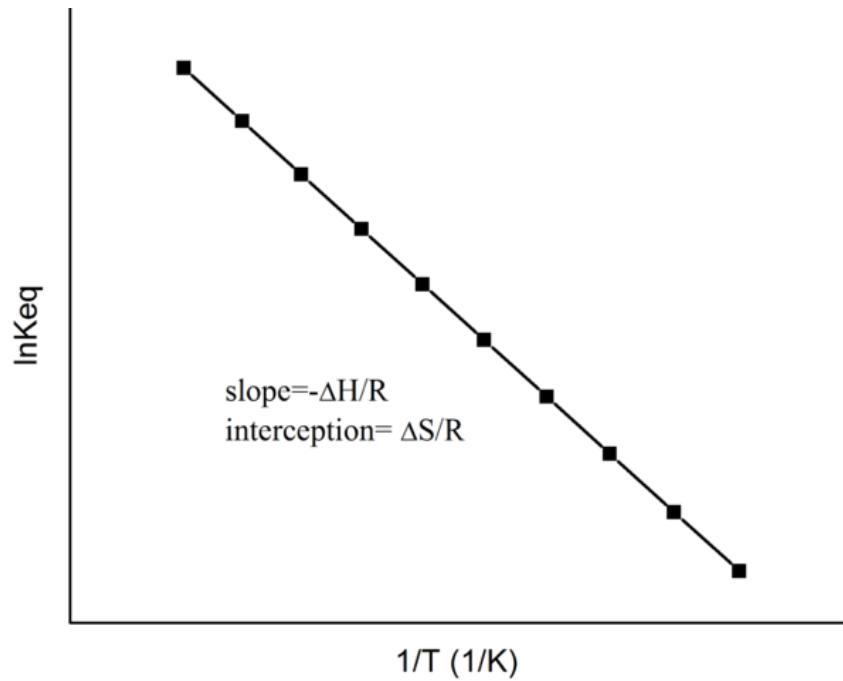
$$\ln K_{\text{eq}} = -\frac{\Delta H^\circ}{R} \frac{1}{T} + \frac{\Delta S^\circ}{R}$$

$y = mx + b$

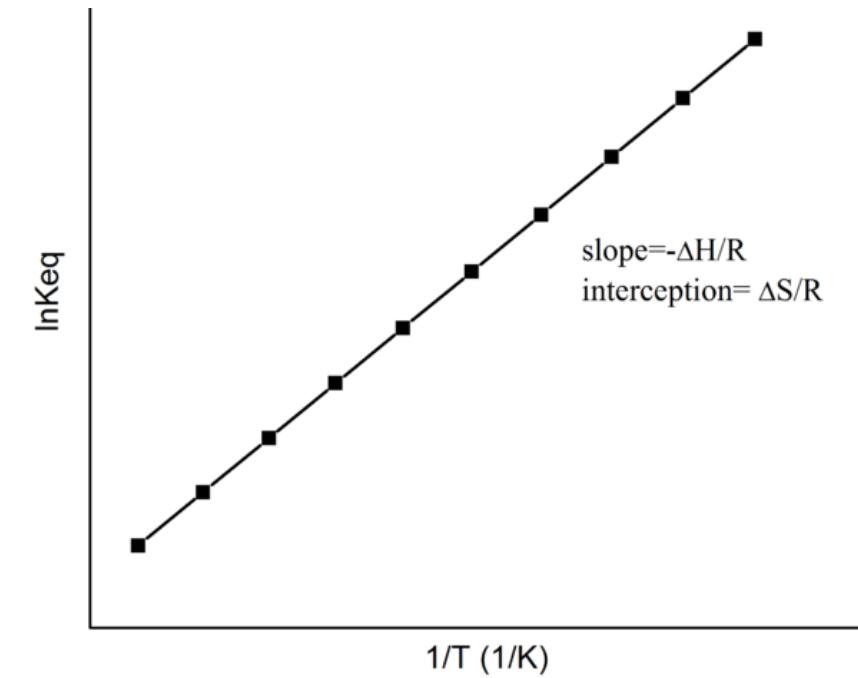
Assuming that enthalpy and entropy are invariant with temperature, you can plot $\ln K_{\text{eq}}$ vs. $1/T$.

$$\text{Slope} = -\frac{\Delta H^\circ}{R}$$

$$\text{y-intercept} = \frac{\Delta S^\circ}{R}$$



Endothermic

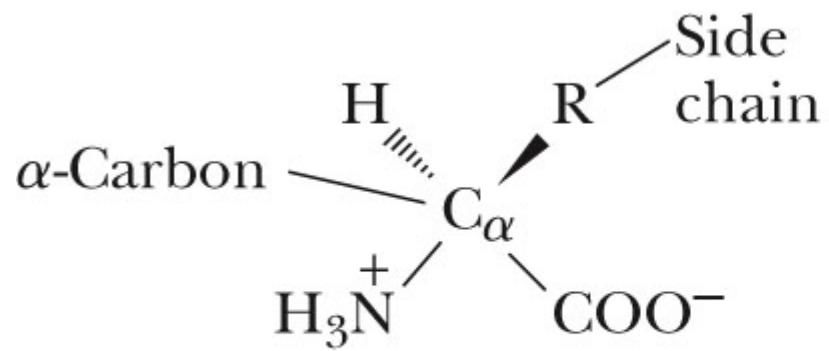


Exothermic

Amino Acids

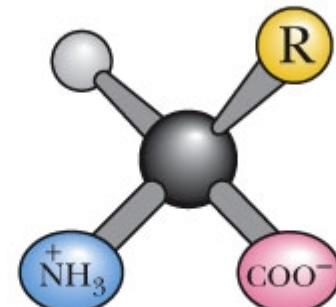
Sanjay Venugopal

Amino Acid Structure

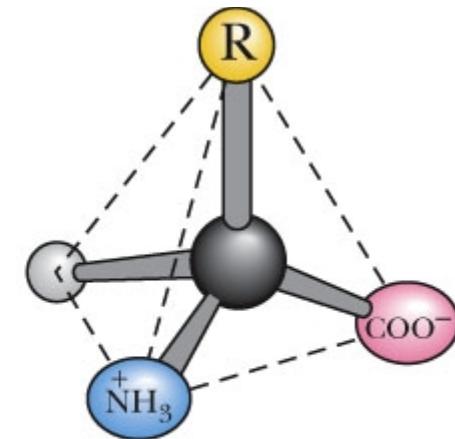


Amino group

Carboxyl group



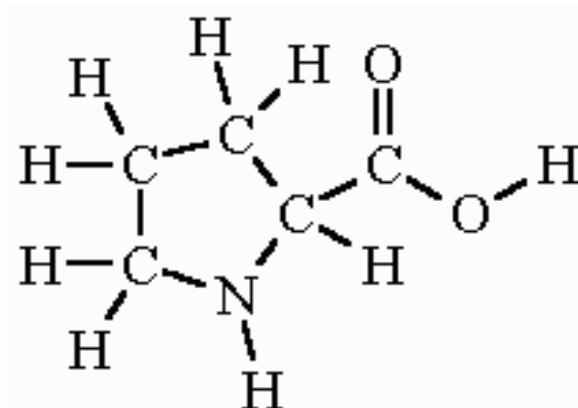
Ball-and-stick model



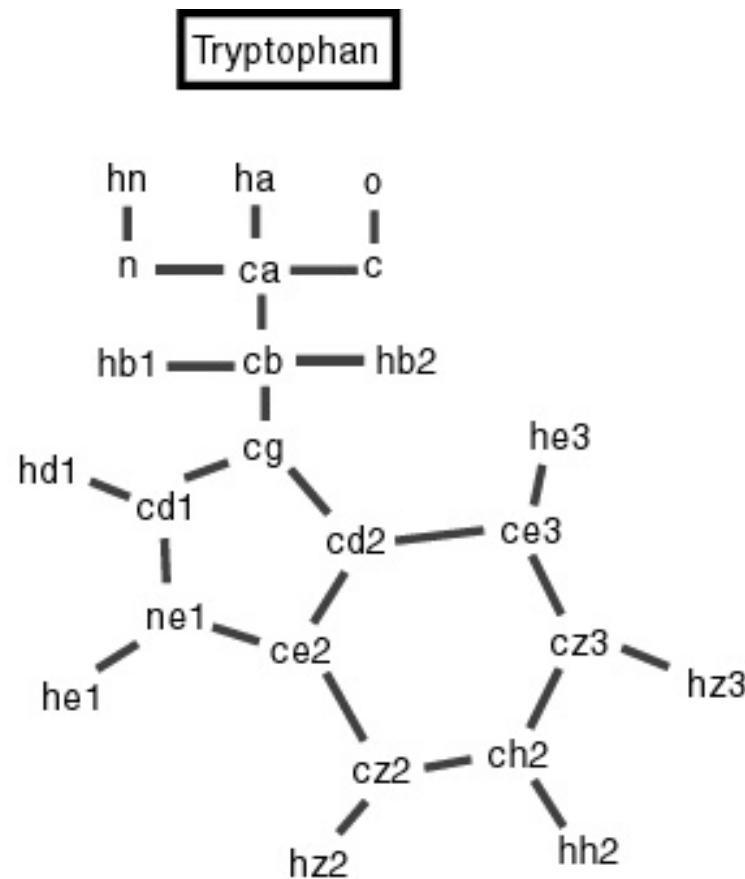
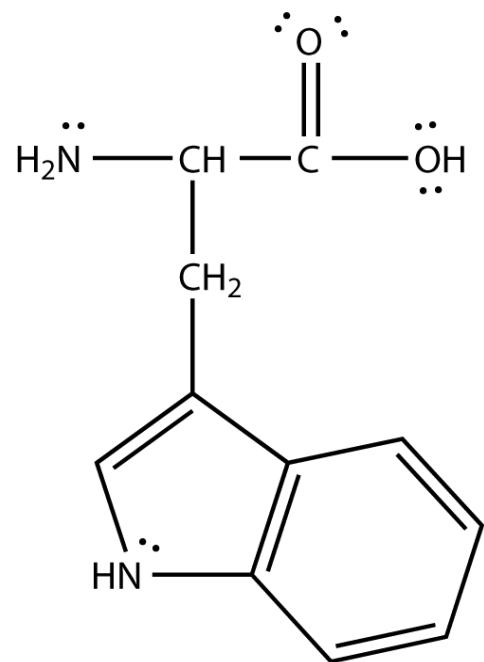
Amino acids are tetrahedral structures

Exceptions for proline

- Proline is actually an imino acid
- R-group is connected to amine nitrogen as well as alpha carbon
- Causes kinks when found in proteins

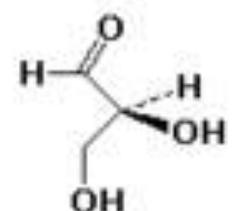


Naming of carbons in Amino Acids

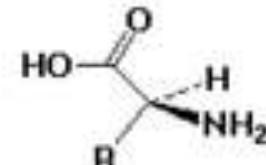


Chirality of Amino acids

- All amino acids are chiral except for glycine, which has a hydrogen as a side chain.
- Two forms are D and L, based on glyceraldehyde structure
- L is the form that is found in nature, and the form incorporated into proteins
- Can also be characterized by R and S
- Most amino acids are S, but cysteine is R



L-glyceraldehyde

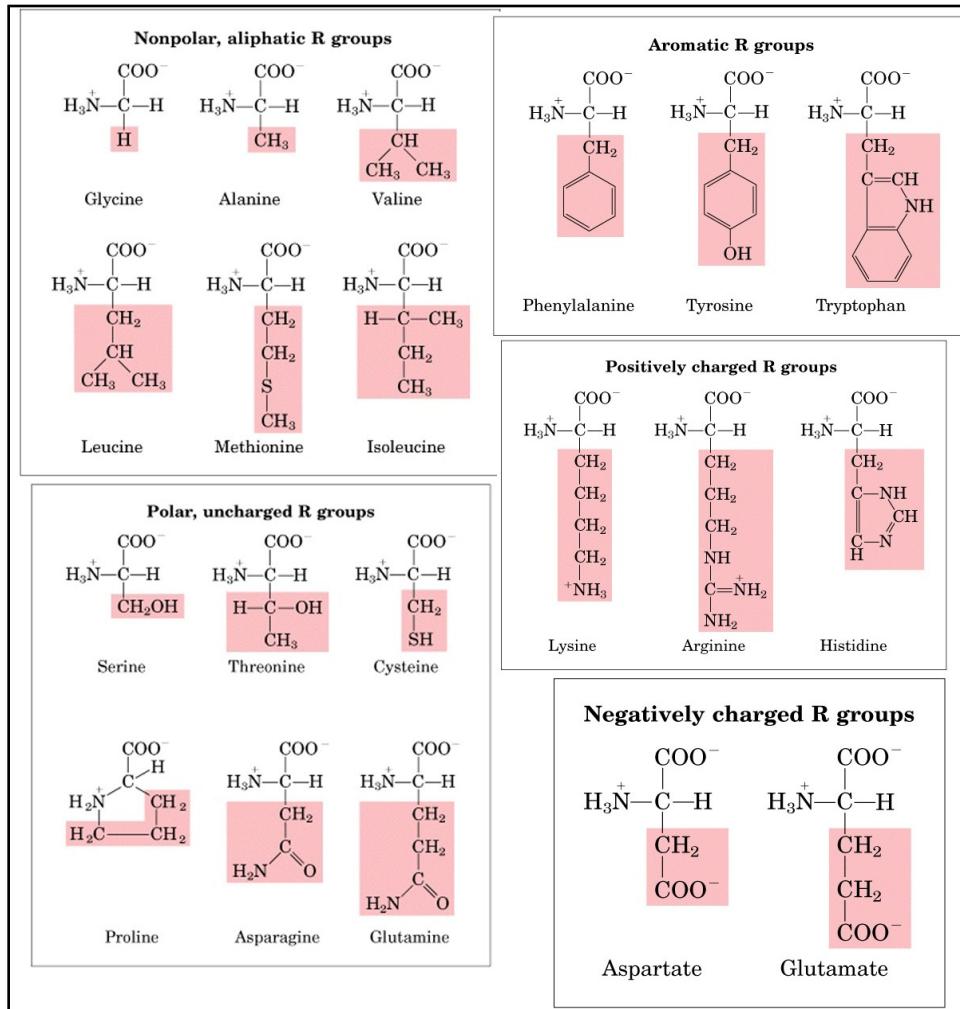


L-amino acids

1-letter and 3-letter Amino acid codes

<u>Amino Acid Residue</u>	<u>3-Letter Code</u>	<u>1-Letter Code</u>
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic Acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic Acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

Structures of the 20 AMINO ACIDS



In this figure,
Proline is incorrectly placed among the polar R groups.
It is nonpolar aliphatic.

Cysteine could be placed among the negatively charged R groups
Histidine could be placed among the aromatic R groups

Amino acid classification

- Can be nonpolar, uncharged polar, charged polar, amphipathic, small, large, aromatic, sulfur-containing, etc.
- Classification exists on a spectrum

Hydrophobic vs. Hydrophilic vs. Amphipathic

- Hydrophobic amino acids include the amino acids with hydrocarbon side chains (Leucine, Isoleucine, Valine, Alanine, Proline), the aromatic amino acid Phenylalanine, the S-containing Methionine, and, to a lesser extent, Glycine.
- Hydrophilic amino acids include acidic and basic amino acids (Aspartic acid, Glutamic acid, Histidine, Arginine, Lysine), uncharged polar amino acids (Asparagine, Glutamine, Cysteine, Serine, Threonine, and the aromatic Tyrosine and Tryptophan).
- All of these hydrophilic amino acids are Amphipathic amino acids, aka Amphipathic.

Question 1

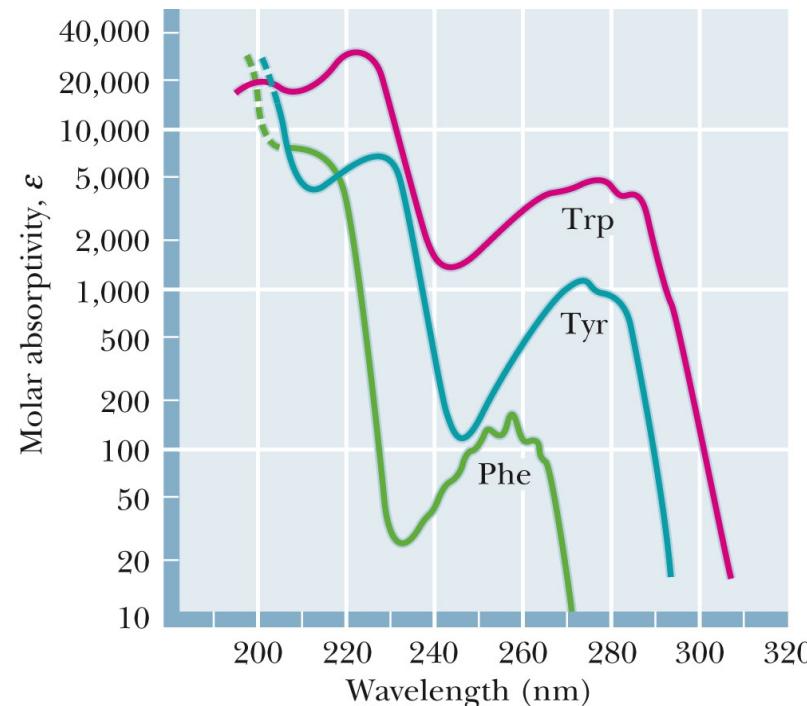
- Which of the following amino acids would most likely be found on the surface of a cell, exposed to extracellular fluid?
 - a) V
 - b) A
 - c) Y
 - d) D

Question 1

- Which of the following amino acids would most likely be found on the surface of a cell, exposed to extracellular fluid?
 - a) V
 - b) A
 - c) Y
 - d) D

Aromatic Absorbance of UV light

- Trp, Tyr, and Phe absorb UV light at wavelengths greater than 250 nm, due to the nature of the aromatic ring in their side chain.
- Trp has the largest absorbance, followed by Tyr and Phe



Amino Acid Frequencies

- Essential amino acids take more steps to synthesize them, and are obtained by humans in their diet.
- Nonessential amino acids take fewer steps, and humans have maintained the ability to synthesize them
- Has evolutionary implications
- Percent of basic residues equals the percent of acidic residues

Nonessential Amino Acids Require Fewer Reactions for Synthesis		
	Amino Acid	Reaction Steps
1	Alanine	1
2	Aspartic acid	1
3	Glutamic acid	1
4	Asparagine	2
5	Glutamine	2
6	Serine	5
7	Glycine	6
8	Proline	6
9	Cysteine	7
10	Threonine	6
11	Valine	9
12	Isoleucine	13
13	Leucine	14
14	Lysine	14
15	Methionine	17
16	Arginine	24
17	Histidine	27
18	Phenylalanine	29
19	Tyrosine‡	30
20	Tryptophan	33

Average molecular weight of amino acid residues

- The weighted average of a free amino acid is 128 da
- Within a polypeptide, however, the average weight of an amino acid residue is 110 da
- Subtract 18 da for the removal of water in the formation of a peptide bond

Question 2

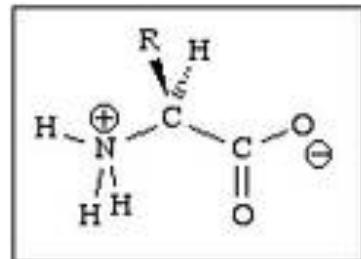
- Approximately how many amino acid residues would be found in a protein whose molecular weight is 75,000 da?
 - a) 682
 - b) 586
 - c) 543
 - d) 510

Question 2

- Approximately how many amino acid residues would be found in a protein whose molecular weight is 75,000 da?
 - a) 682
 - b) 586
 - c) 543
 - d) 510

Acid-base properties of amino acids

- Amino acids are amphoteric (can act as an acid or a base) due to their acidic carboxyl group and basic amine group.
- Carboxyl group pka (in a free amino acid) is about 2
- Amine group pka is about 9
- When pH is above 2, carboxyl group will be predominantly deprotonated
- When pH is below 9, amine group will be predominantly protonated



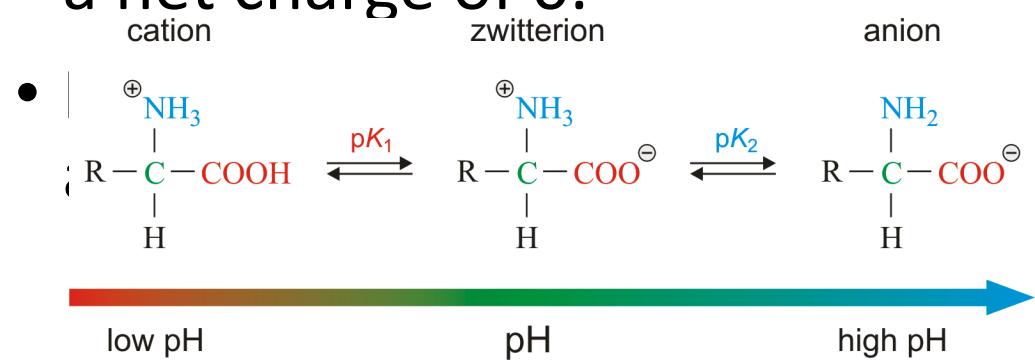
Acid-Base properties of amino acids

TABLE 4.1 pK_a Values of Common Amino Acids

Amino Acid	$\alpha\text{-COOH } pK_a$	$\alpha\text{-NH}_3^+ pK_a$	R group pK_a
Alanine	2.4	9.7	
Arginine	2.2	9.0	12.5
Asparagine	2.0	8.8	
Aspartic acid	2.1	9.8	3.9
Cysteine	1.7	10.8	8.3
Glutamic acid	2.2	9.7	4.3
Glutamine	2.2	9.1	
Glycine	2.3	9.6	
Histidine	1.8	9.2	6.0
Isoleucine	2.4	9.7	
Leucine	2.4	9.6	
Lysine	2.2	9.0	10.5
Methionine	2.3	9.2	
Phenylalanine	1.8	9.1	
Proline	2.1	10.6	
Serine	2.2	9.2	~13
Threonine	2.6	10.4	~13
Tryptophan	2.4	9.4	
Tyrosine	2.2	9.1	10.1
Valine	2.3	9.6	

Isoelectric point

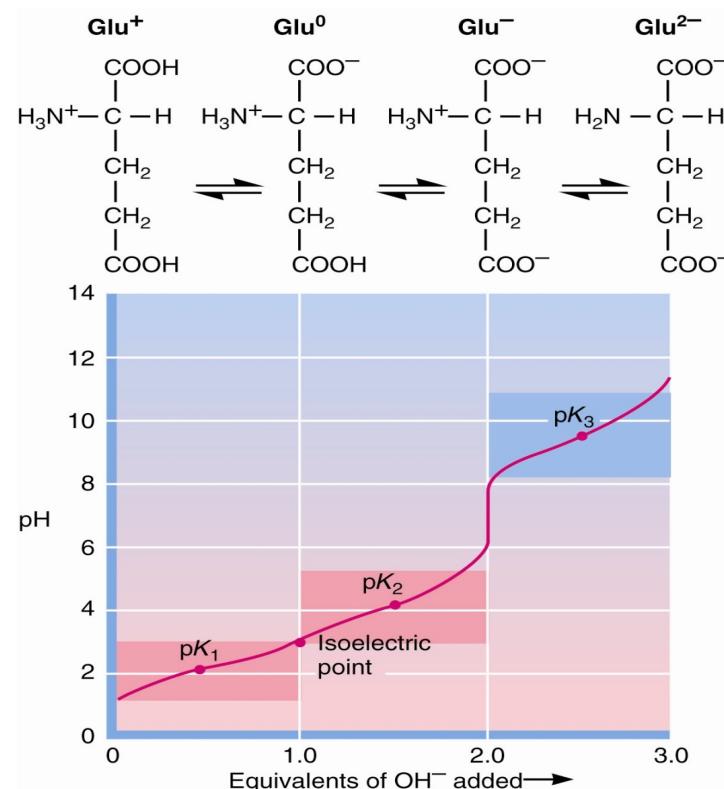
- When $\text{pH} > \text{pKa}$, side chains/functional groups will be predominantly deprotonated
 - When $\text{pH} < \text{pKa}$, side chains/functional groups will be predominantly protonated
 - When amino acids are neutral overall, exist in a “zwitterionic” form
 - Isoelectric point (pI) is the pH at which the amino acid or protein has a net charge of 0.



tein is (-) charged. If pH<pl, the amino

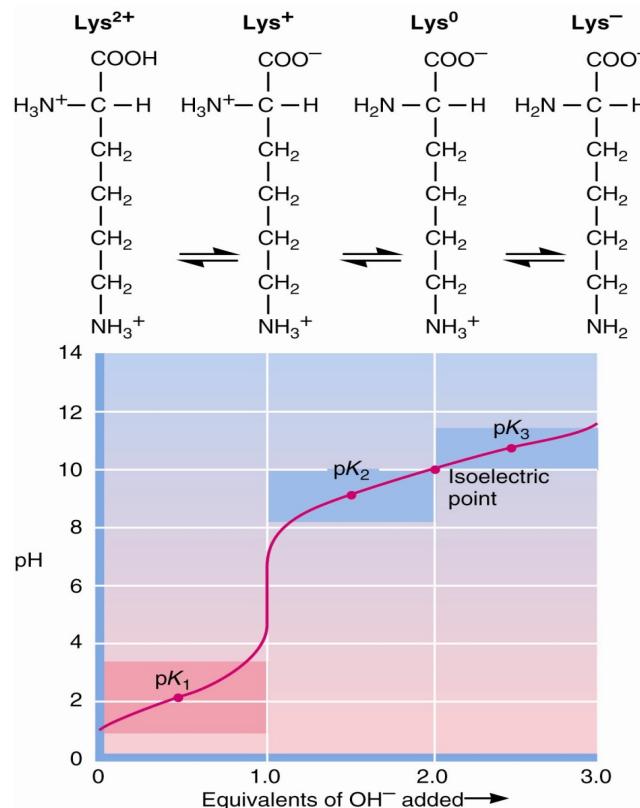
Approaches to solving for Isoelectric Point

- For acidic amino acids, average the carboxyl pKa with the side chain pKa



Approaches to solving for Isoelectric Point

- For basic amino acids, average the amine group pKa with the side chain pKa



Question 3

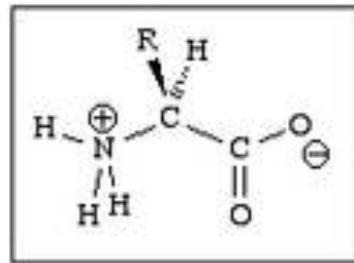
- Which of the following would be closest to the pI of lysine?
- 12
 - 10
 - 9
 - 8

Approaches to solving for Isoelectric point

- In a polypeptide, identify ionizable groups:
- Ile-Val-Asn-Arg
- Because arginine is the only ionizable group within the polypeptide, can calculate pI as if it is a basic amino acid by averaging $pKas$ of side chain and N-terminal amino group
- Try Ile-Val-Cys-Arg

Effects of Proximal groups on pKa

- Normal, aliphatic carboxylic acids have a carboxyl pKa of about 4.75
- The carboxyl group of a free amino acid has a pKa of about 2.3. Why?
- The nearby NH₃⁺ group influences the pKa by repelling the proton attached to the carboxyl group, making it held less tightly and more acidic



Effects of proximal groups on pKa

- Normal amine group pKa is around 8
- Amine group pKa of a free amino acid is around 9.6
- The carboxyl group attracts protons towards the amine group, causing them to be more tightly held

Carboxyl group pKa in a polypeptide

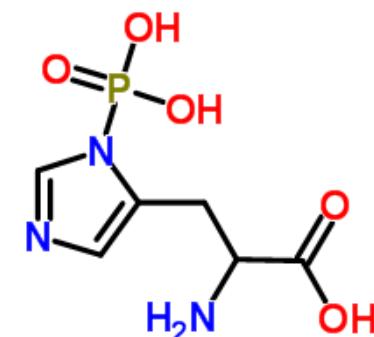
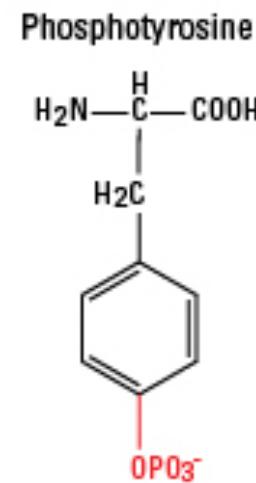
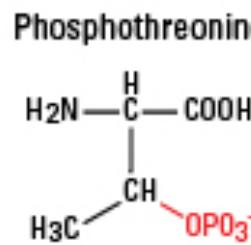
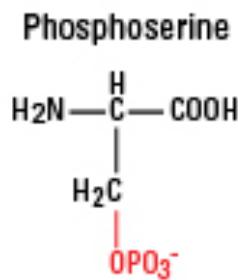
- Effects of nearby groups diminishes in a polypeptide
- More distance, aren't proximal anymore
- pKa of carboxyl group in a polypeptide (3.8) approaches that of an aliphatic acid

Post-translational modifications

- Covalent modifications made to a protein after its synthesis
- Phosphorylation, acetylation, methylation are among the most common, but >500 are known
- Can serve as an on/off switch and function in modulating protein activity

Phosphorylation

- Kinases phosphorylate hydroxyl groups of serine, threonine, tyrosine, and the amine nitrogen in the side chain of histidine.

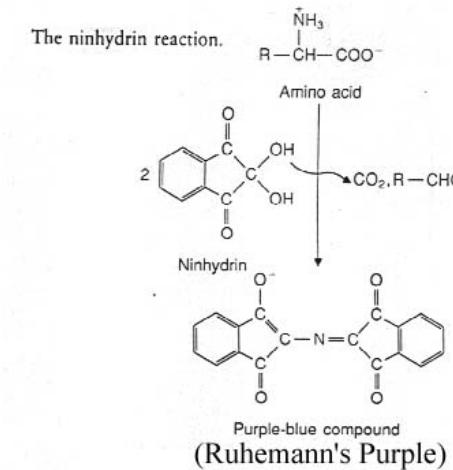
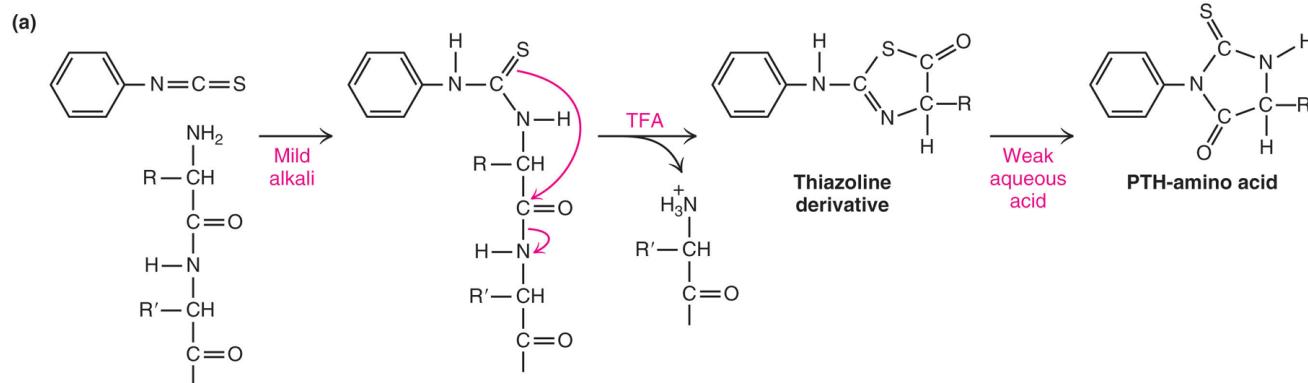


Acetylation and methylation

- Acetylases transfer an acetyl group to the side chain of lysine and the N-terminus of polypeptides
- Methylases transfer a methyl group to the side chains of lysine and arginine

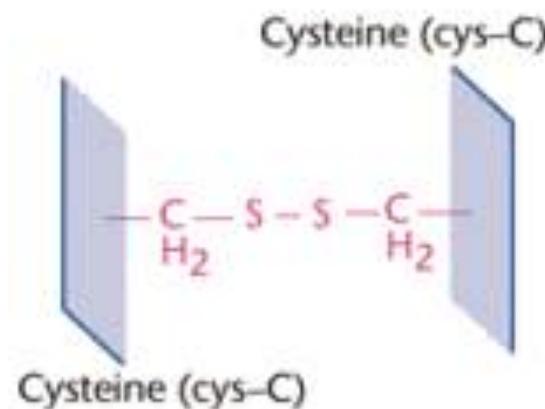
Reactivity of Amino acids

- Amino acids react with phenylisothiocyanate in Edman degradation as a method for amino acid quantification
- Ninhydrin also reacts with amino acids to produce aldehydes, CO₂, and ammonia



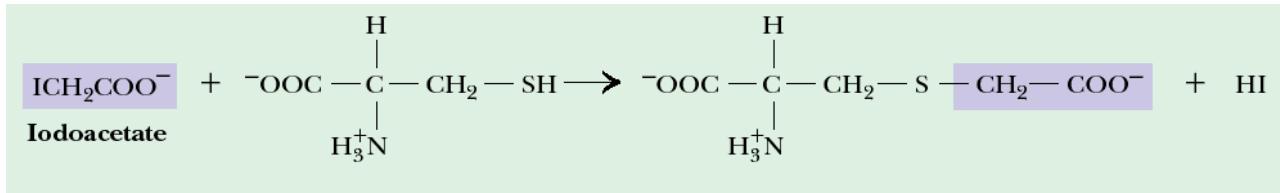
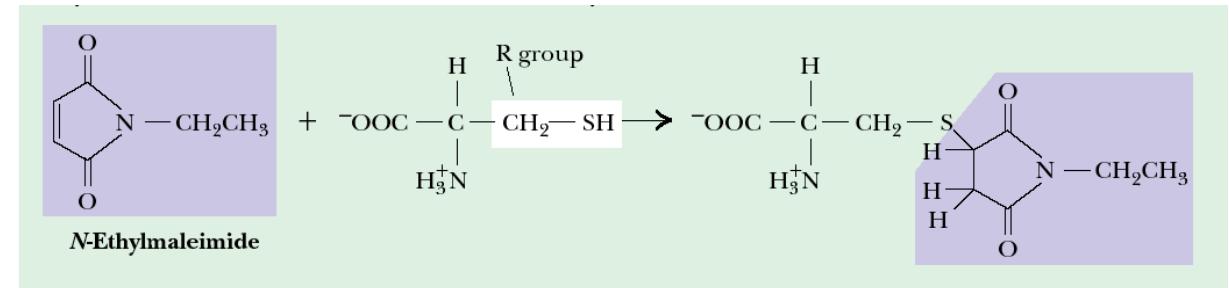
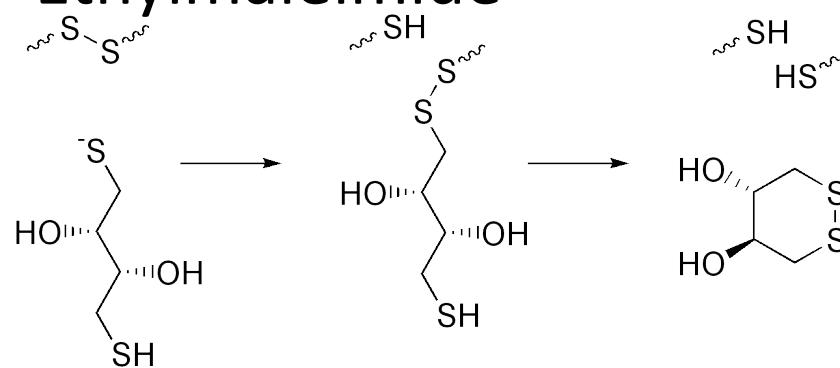
Reactivity of Amino Acids

- Cysteine residues are reactive under oxidizing conditions
- Under oxidizing conditions, cysteine residues within a protein can form disulfide bridges
- Provide additional primary structure to proteins, and stabilize tertiary structure



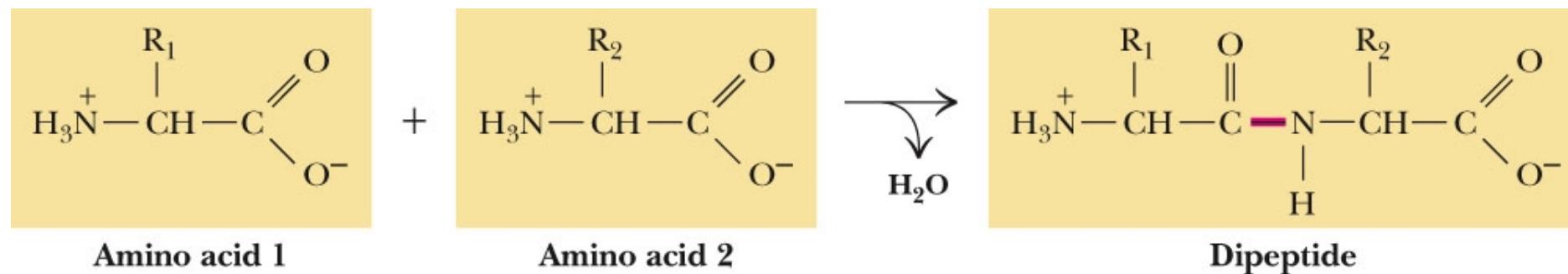
Reactivity of Amino acids

- Disulfide bridges can be cleaved by the action of beta-mercaptoproethanol, TCEP, or dithiothreitol
- Cysteine reactivity can be blocked entirely by iodoacetate and N-Ethylmaleimide



Drawing the peptide backbone

- Peptide bonds are formed from a loss of water (dehydration reaction)
- Proteins are read from N-terminus to C-terminus
- Repeating motif of N-C(alpha)-C(O)
- “N” is the amide nitrogen, “C(alpha)” is the alpha carbon of the amino acid, and “C(O)” is the carbonyl-bearing carbon



Question 4

- Draw the tripeptide MYD at pH 8, including charges

Protein Structure/Function/Purification

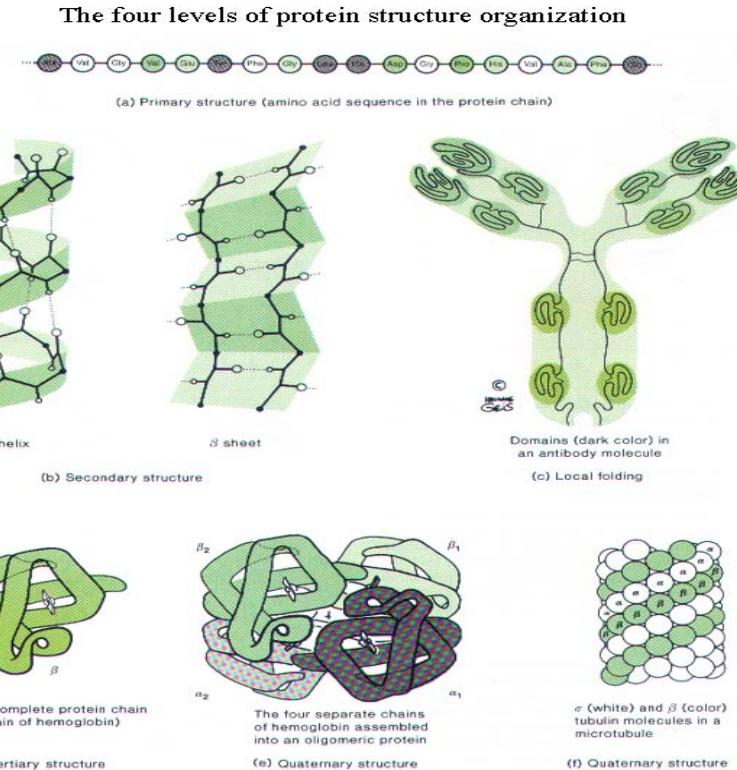
Compiled By Christina Suikkari

9/10/17

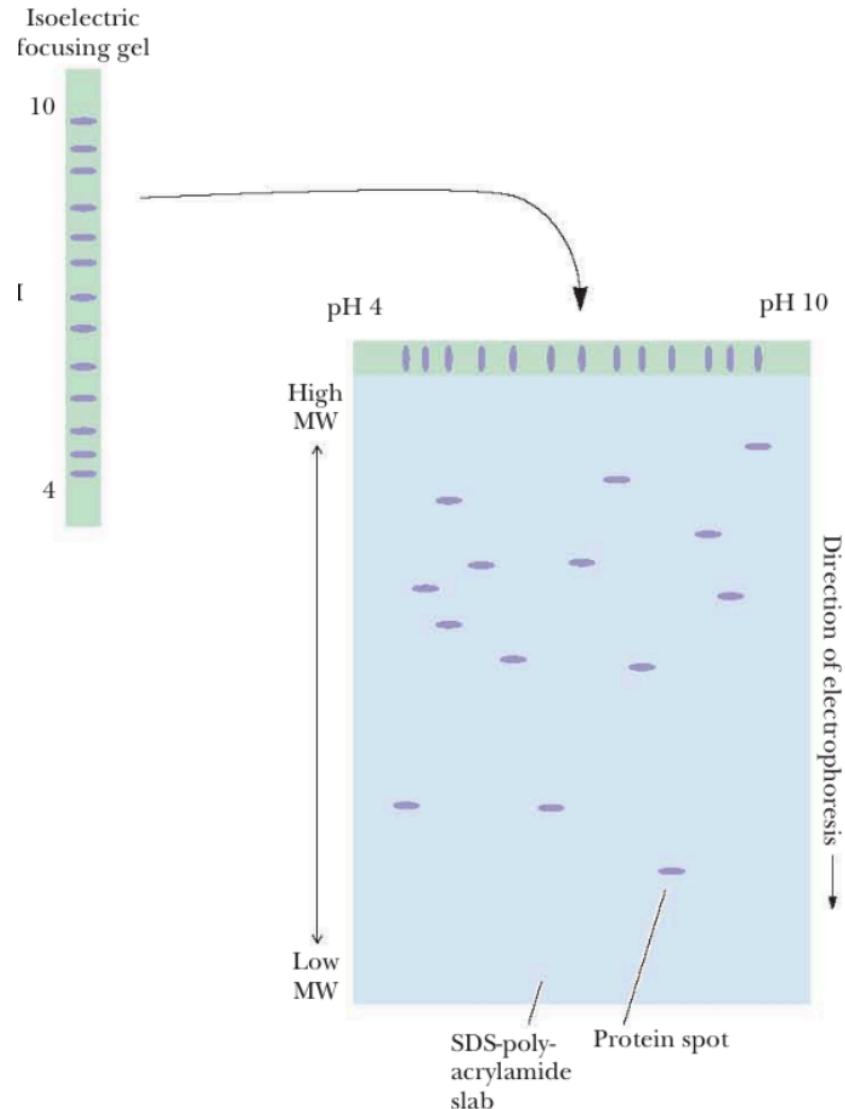
4 Levels of Protein Structure

- Primary
 - Sequence of amino acids + posttranslational/covalent modifications
- Secondary
 - Organization of polymer (alpha helices and beta sheets)
- Tertiary
 - Folding of 2⁰ structures
- Quaternary
 - Multiple protein subunits

- From Ch. 5A Lecture Notes:



From Ch. 5 in textbook:



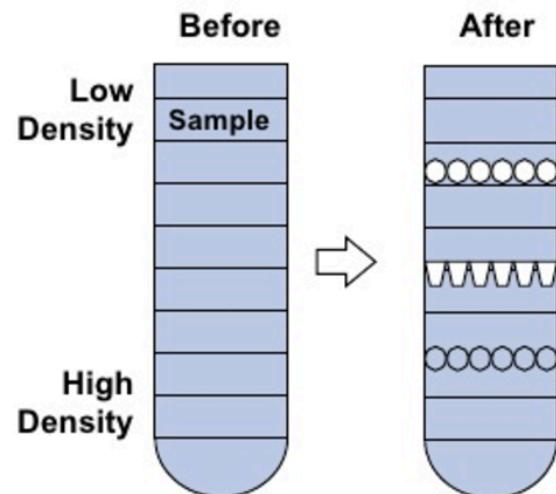
Electrophoresis

- SDS-PAGE (Sodium Dodecylsulfate Polyacrylamide Gel Electrophoresis)
 - SDS interacts with polypeptides, forming a linear, net (-) charged peptide
 - Reducing agent added to break disulfide bridges between polypeptide chains
 - Lowest M_r moves fastest
- Isoelectric Focusing
 - Separation based on charge
 - $pI = pH$ at which amino acid has a net charge of 0
 - Depends on pK_a of each amino acid
 - If $pH > pK_a$, then amino acid is deprotonated
 - Cathode = (-), attracts cations
 - Anode = (+), attracts anions

Non-equilibrium Centrifugation

➤ Zonal (Velocity)

- Solution to be separated is placed onto a shallow gradient (e.g. sucrose)
- Larger, denser molecules will migrate to bottom of the tube



<https://www.slideshare.net/attilacsordas/ultracentrifugation-basic-training>

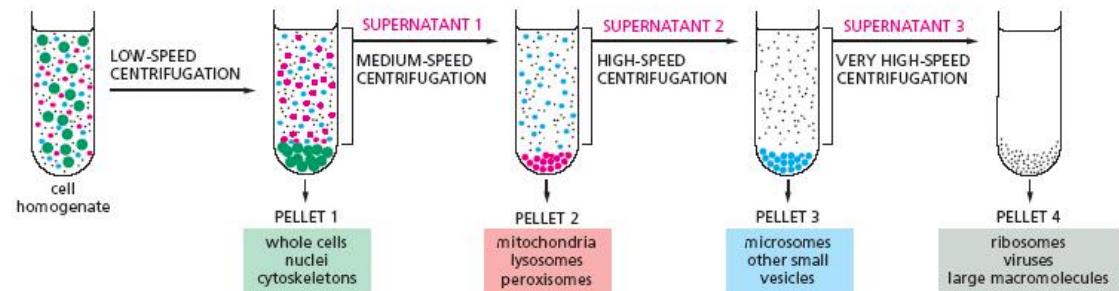
➤ Differential

- Separated cellular organelles based on size/density
- Solution is centrifuged at different speeds
 - Lower density and mass molecules require higher speed
 - Stronger intermolecular forces between molecule and solution, so need more force to sediment the molecule of interest

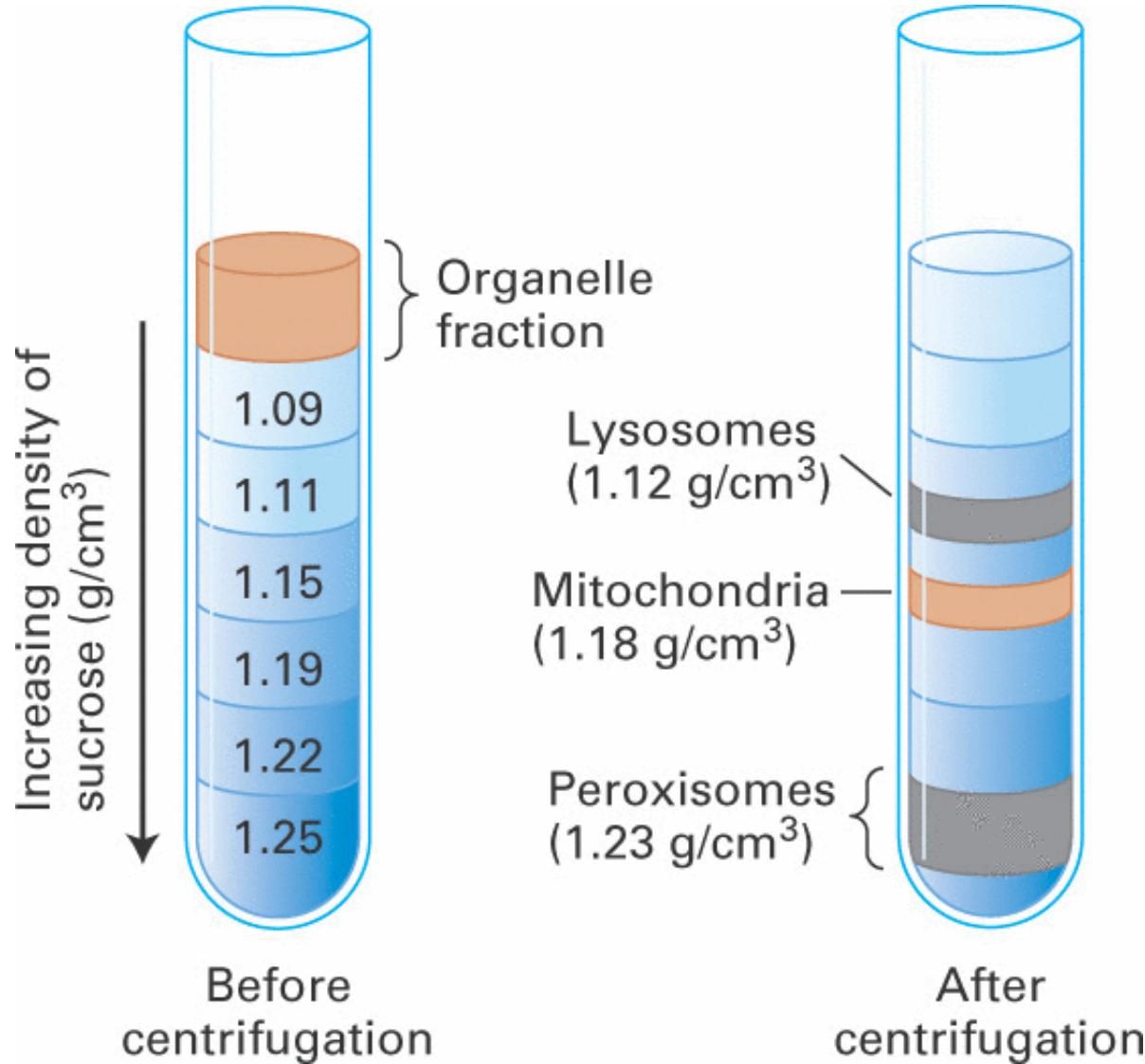
DIFFERENTIAL CENTRIFUGATION

Repeated centrifugation at progressively higher speeds will fractionate cell homogenates into their components.

Centrifugation separates cell components on the basis of size and density. The larger and denser components experience the greatest centrifugal force and move most rapidly. They sediment to form a pellet at the bottom of the tube, while smaller, less dense components remain in suspension above, a portion called the supernatant.



<http://cellbiologyolm.stevegallik.org/node/74>



Equilibrium Density Gradient Centrifugation (isopycnic)

- Separation based on density
- Molecule equilibrates to location of steep sucrose gradient that equals its density
- Can be used to separate organelles of different densities

Ion Exchange Chromatography

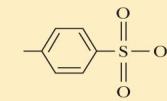
- Separation of charged molecules
- Anion Exchange
 - (+) charged resin (i.e. DEAE)
 - (-) charged proteins bind to column
- Cation Exchange (i.e. CM)
 - (-) charged resin
 - (+) charged proteins bind to column
- Elute proteins with **increasing** [salt] gradient
- pH should be between pKa of exchanger and pl of protein
 - Why?
 - Exchanger and protein must have opposite charges for protein binding
 - Protein will have less net charge and thus elutes faster

From Ch. 5 in textbook:

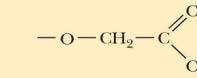
(a) Cation Exchange Media

Structure

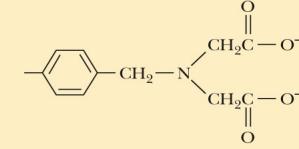
Strongly acidic, polystyrene resin (Dowex-50)



Weakly acidic, carboxymethyl (CM) cellulose



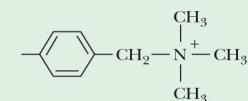
Weakly acidic, chelating, polystyrene resin (Chelex-100)



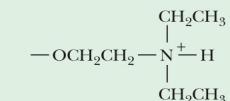
(b) Anion Exchange Media

Structure

Strongly basic, polystyrene resin (Dowex-1)

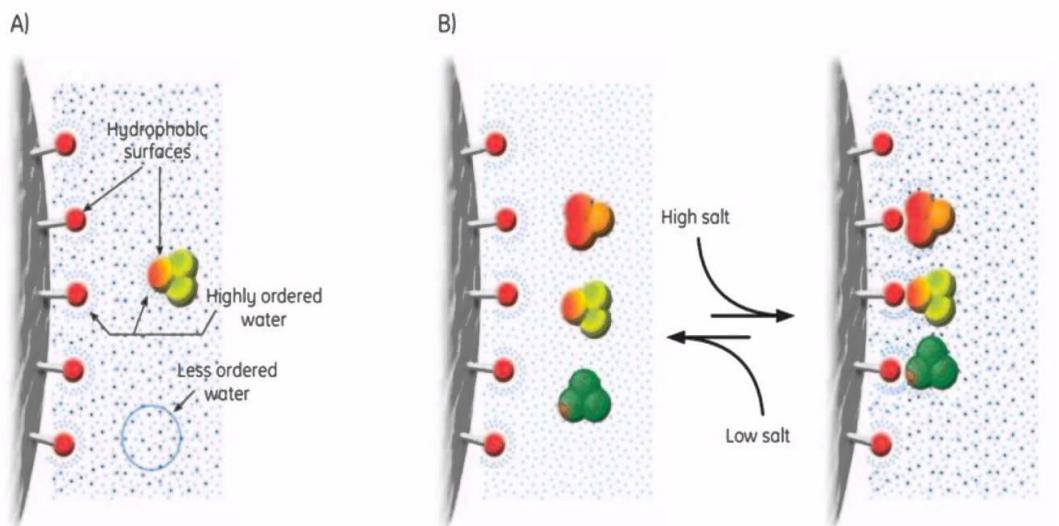


Weakly basic, diethylaminoethyl (DEAE) cellulose



Hydrophobic (Reverse-Phase) Chromatography

HIC – Salting out



A) Highly ordered water shells surround the hydrophobic surfaces of ligands and proteins. Hydrophobic substances are forced to merge to minimize the total area of such shells (maximize entropy). Salts enhance the hydrophobic interaction. B) The equilibrium of the hydrophobic interaction is controlled predominantly by the salt concentration.

www.technologyinscience.blogspot.com

<https://i.ytimg.com/vi/Hg5JTly6le8/maxresdefault.jpg>

- Hydrophobic resin in column
 - Separation of polar molecules
- **Decreasing salt gradient used to elute proteins**
 - High [salt]: proteins bind to column
 - Salt ions increase hydrophobic interactions between protein and resin
 - Low [salt]: proteins elute out of column
 - Most hydrophobic proteins elute last

Affinity Chromatography

- Separation based on specific interaction between resin and molecule of interest
 - Protein binds to resin bead with high affinity
 - Other molecules/proteins in solution pass through the column while protein of interest remains bound
 - Protein of interest elutes out with elution buffer
 - Elution buffer molecules compete for binding site of resin beads, thus releasing the protein of interest

From Ch. 5 in textbook:

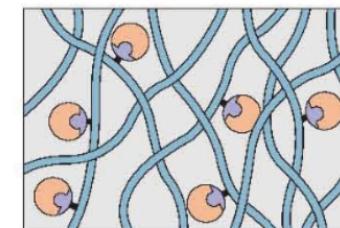
A protein interacts with a metabolite. The metabolite is thus a ligand that binds specifically to this protein



The metabolite can be immobilized by covalently coupling it to an insoluble matrix such as an agarose polymer. Cell extracts containing many individual proteins may be passed through the matrix.

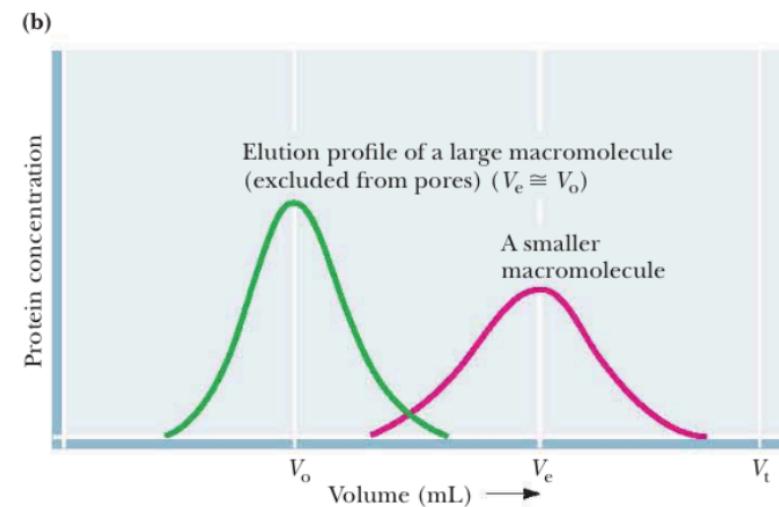
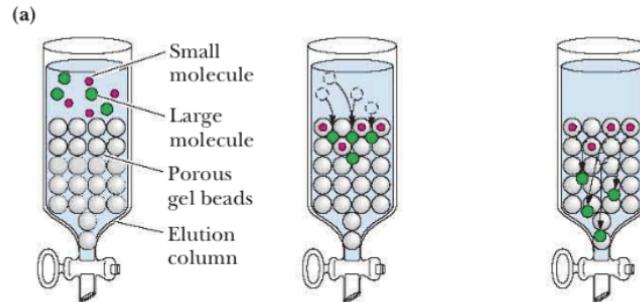


Specific protein binds to ligand. All other unbound material is washed out of the matrix.



Gel Filtration (Size Exclusion)

- Separation based on size (Protein elution)
 - Resin beads = insoluble
 - Larger proteins elute faster
 - Do not get trapped by gel beads
 - Small proteins flow in the pores of the beads
- From Ch. 5 in textbook:



▲ **Figure 4** (a) A gel filtration chromatography column. Larger molecules are excluded from the gel beads and emerge from the column sooner than smaller molecules, whose migration is retarded because they can enter the beads. (b) An elution profile.

Dialysis and Ultrafiltration

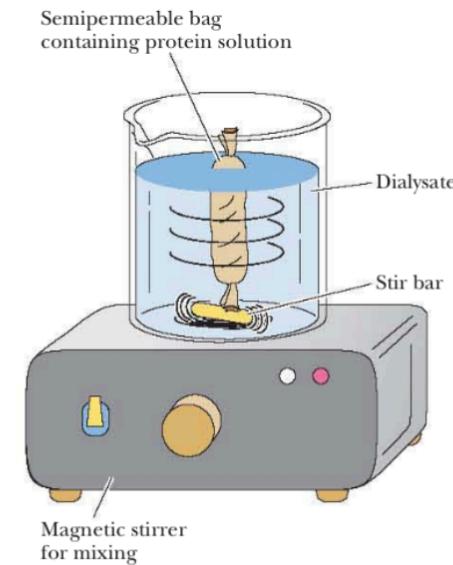
From Ch. 5 in textbook:

➤ Dialysis

- Semipermeable membrane/bag with solution (molecule of interest) and buffer
 - Pores in membrane < molecule of interest
 - Solution flows out of bag and molecule of interest stays inside

➤ Ultrafiltration

- Pores are microscopic
- High pressure gradient forces solution out of semipermeable bag
- Separation of large macromolecules (e.g. insulin)



▲ **Figure 1** A dialysis experiment. The solution of macromolecules to be dialyzed is placed in a semipermeable membrane bag, and the bag is immersed in a bathing solution. A magnetic stirrer gently mixes the solution to facilitate equilibrium of diffusible solutes between the dialysate and the solution contained in the bag.

Salting In/Out

➤ Salting-In

- As ionic strength increases in the mM range, proteins generally become more soluble
- Salt ions disrupt electrostatic attraction between protein molecules

➤ Salting-Out

- As ionic strength increases in the M range, proteins generally precipitate out of solution

