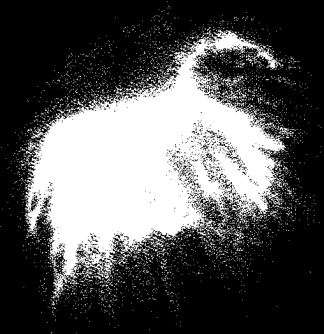
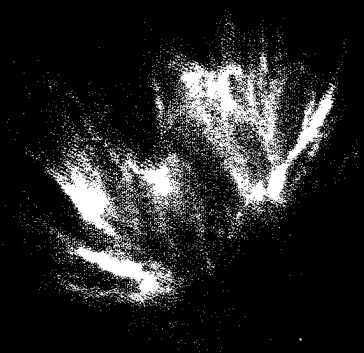
EXHIBIT C

MOLECULAR CELL BIOLOGY

SIXTH EDITION





Lodish
Berk
Kaiser
Krieger
Scott
Bretscher
Ploegh
Matsudaira

MOLECULAR CELL BIOLOGY

SIXTH EDITION

Harvey Lodish
Arnold Berk
Chris A. Kaiser
Monty Krieger
Matthew P. Scott
Anthony Bretscher
Hidde Ploegh
Paul Matsudaira

W. H. Freeman and Company
New York

PUBLISHER: Sara Tenney
EXECUTIVE EDITOR: Katherine Ahr
DEVELOPMENTAL EDITORS: Matthew Tontonoz, Erica Pantages Frost, Elizabeth Rice
ASSOCIATE PROJECT MANAGER: Hannah Thonet
ASSISTANT EDITOR: Nick Tymoczko
ASSSOCIATE DIRECTOR OF MARKETING: Debbie Clare
SENIOR PROJECT EDITOR: Mary Louise Byrd
TEXT DESIGNER: Marsha Cohen
PAGE MAKEUP: Aptara, Inc.
COVER DESIGN: Blake Logan
ILLUSTRATION COORDINATOR: Susan Timmins
ILLUSTRATIONS: Network Graphics, Erica Beade, H. Adam Steinberg
PHOTO EDITOR: Cecilia Varas
PHOTO RESEARCHER: Christina Micek
PRODUCTION COORDINATOR: Susan Wein
MEDIA AND SUPPLEMENTS EDITOR: Hannah Thonet
MEDIA DEVELOPERS: Biostudio, Inc., Sumanas, Inc.
COMPOSITION: Aptara, Inc.
MANUFACTURING: RR Donnelley & Sons Company

About the cover: Mitotic PtK2 cells in late anaphase stained blue for DNA and green for tubulin. Courtesy of Torsten Wittman.

Library of Congress Cataloging-in-Publication Data
Molecular cell biology / Harvey Lodish . . . [et al.]. —6th ed.
p. cm.
Includes bibliographical references and index.
1. Cytology. 2. Molecular biology. I. Lodish, Harvey F.
QH581.2.M655 2007
571.6—dc22 2007006188
ISBN-13: 978-0-7167-7601-7
ISBN-10: 0-7167-7601-4

Printed in the United States of America

First printing

W. H. Freeman and Company
41 Madison Avenue, New York, NY 10010
Houndmills, Basingstoke
RG21 6XS, England

www.whfreeman.com

Polarized light microscopic image of crystals of ATP, whose hydrolysis is a primary source of energy that drives many cellular chemical reactions. [Dr. Arthur M. Siegelman/Visuals Unlimited.]

CHAPTER

2

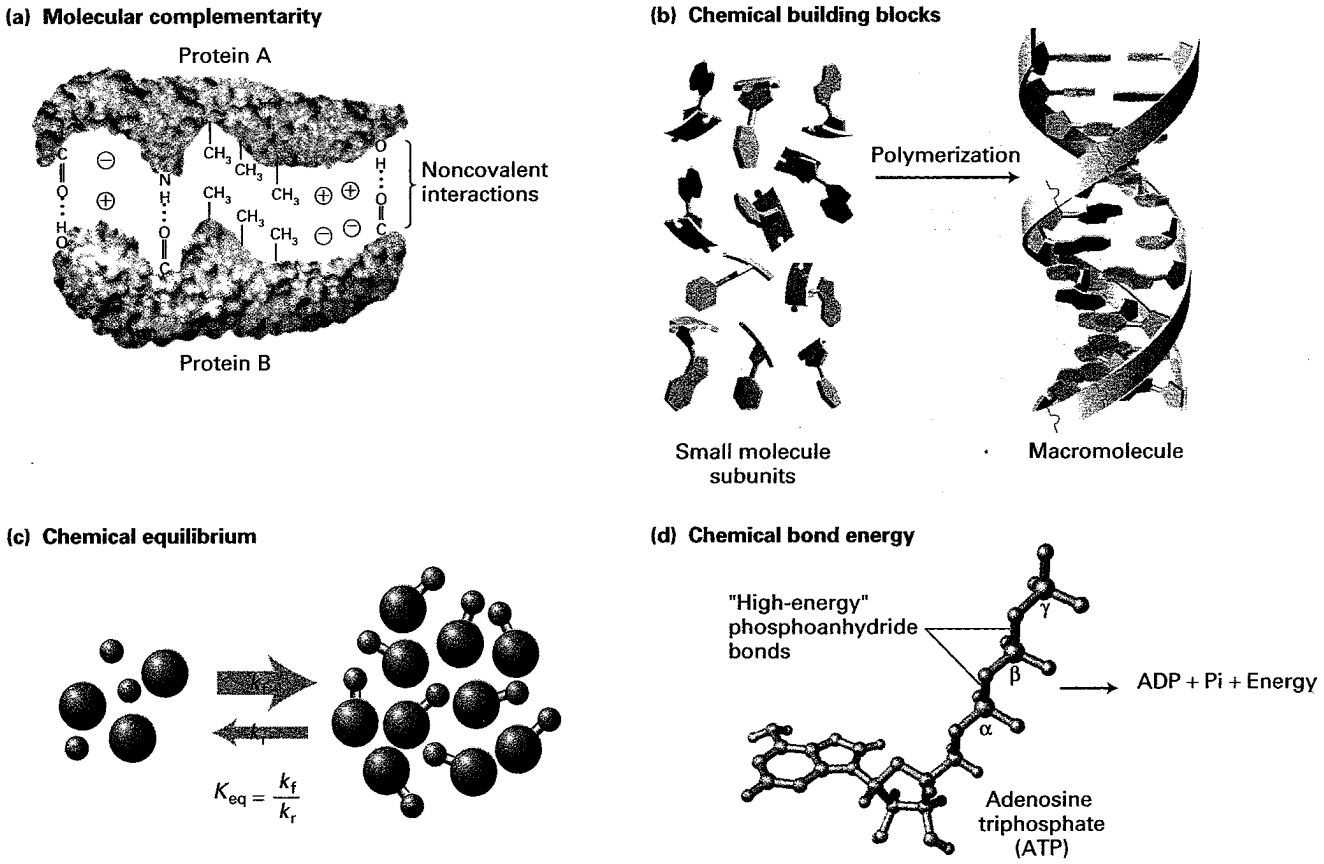
CHEMICAL FOUNDATIONS

The life of a cell depends on thousands of chemical interactions and reactions exquisitely coordinated with one another in time and space and under the influence of the cell's genetic instructions and its environment. By understanding at a molecular level these interactions and reactions, we can begin to answer fundamental questions about cellular life: How does a cell extract critical nutrients and information from its environment? How does a cell convert the energy stored in nutrients into work (movement, synthesis of critical components)? How does a cell transform nutrients into the fundamental structures required for its survival (cell wall, nucleus, nucleic acids, proteins, cytoskeleton)? How does a cell link itself to other cells to form a tissue? How do cells communicate with one another so that a complex, efficiently functioning organism can develop and thrive? One of the goals of *Molecular Cell Biology* is to provide answers to these and other questions about the structure and function of cells and organisms in terms of the properties of individual molecules and ions.

For example, the properties of one such molecule, water, have controlled and continue to control the evolution, structure, and function of cells. You cannot understand biology without appreciating how the properties of water control the chemistry of life. Life first arose in a watery environment. Constituting 70–80 percent by weight of most cells, water is the most abundant molecule in biological systems. It is within this aqueous milieu that small molecules and ions, which make up about 7 percent of the weight of living matter, assemble into the larger macromolecules and macromolecular aggregates that make up a cell's machinery and architecture and so the remaining mass of organisms. These small molecules include amino acids (the building blocks of proteins), nucleotides (the building blocks of DNA and RNA), lipids (the building blocks of biomembranes), and sugars (the building blocks of starches and cellulose).

Many biomolecules (e.g., sugars) readily dissolve in water; these molecules are called **hydrophilic** (water liking). Others (e.g., cholesterol) are oily, fatlike substances that shun water; these are said to be **hydrophobic** (water fearing). Still other biomolecules (e.g., phospholipids) are a bit schizophrenic, containing both hydrophilic and hydrophobic regions; these molecule are said to be **amphipathic.** Phospholipids are used to build the flexible membranes that form the wall-like boundaries of cells and their internal organelles. The smooth functioning of cells, tissues, and organisms depends on all these molecules, from the smallest to the largest. Indeed, the chemistry of the simple proton (H^+) can be as important to the survival of a human cell as that of each gigantic, genetic-code-carrying DNA molecule (the mass of the DNA molecule in human

OUTLINE



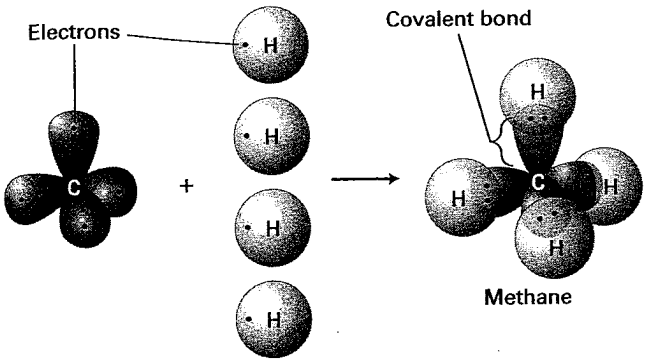
▲ **FIGURE 2-1 Chemistry of life: four key concepts.** (a) Molecular complementarity lies at the heart of all biomolecular interactions, as when two proteins with complementary shapes and chemical properties come together to form a tightly bound complex. (b) Small molecules serve as building blocks for larger structures. For example, to generate the information-carrying macromolecule DNA, four small nucleotide building blocks are covalently linked into long strings (polymers), which then wrap around each other to form the double helix. (c) Chemical reactions are reversible, and the distribution of the chemicals between starting reagents (*left*) and the products of the reactions (*right*) depends on the rate constants of the forward (k_f, upper arrow) and reverse (k_r, lower arrow) reactions. The ratio of these, K_{eq}, provides an informative measure of the relative amounts of products and reactants that will be present at equilibrium. (d) In many cases, the source of energy for chemical reactions in cells is the hydrolysis of the molecule ATP. This energy is released when a high-energy phosphoanhydride bond linking the β and γ phosphates in the ATP molecule (red) is broken by the addition of a water molecule, forming ADP and P_i.

chromosome 1 is 8.6×10^{10} times that of a proton!). The chemical interactions of all of these molecules, large and small, with water and with one another, define the nature of life.

Luckily, although many types of biomolecules interact and react in numerous and complex pathways to form functional cells and organisms, a relatively small number of chemical principles are necessary to understand cellular processes at the molecular level (Figure 2-1). In this chapter we review these key principles, some of which you already know well. We begin with the covalent bonds that connect atoms into a molecule and the noncovalent forces that stabilize groups of atoms into functional structures within and between molecules. We then consider the key properties of the basic chemical building blocks of macromolecules and macromolecular assemblies. After reviewing those aspects of chemical equilibrium that are most relevant to biological systems, we end the chapter with basic principles of biochemical energetics, including the central role of ATP (adenosine triphosphate) in capturing and transferring energy in cellular metabolism.

2.1 Covalent Bonds and Noncovalent Interactions

Strong and weak attractive forces between atoms are the "glue" that holds them together in individual molecules and permits interactions between different biomolecules. Strong forces form a **covalent bond** when two atoms share one pair of electrons ("single" bond) or multiple pairs of electrons ("double" bond, "triple" bond, etc.). The weak attractive forces of **noncovalent interactions** are equally important in determining the properties and functions of biomolecules such as proteins, nucleic acids, carbohydrates, and lipids. We will first review covalent bonds and then discuss the four



▲ **FIGURE 2-2 Covalent bonds form by the sharing of electrons.** Covalent bonds, the strong forces that hold atoms together into molecules, form when atoms share electrons from their outermost electron orbitals. Each atom forms a defined number and geometry of covalent bonds.

major types of noncovalent interactions: ionic bonds, hydrogen bonds, van der Waals interactions, and the hydrophobic effect.

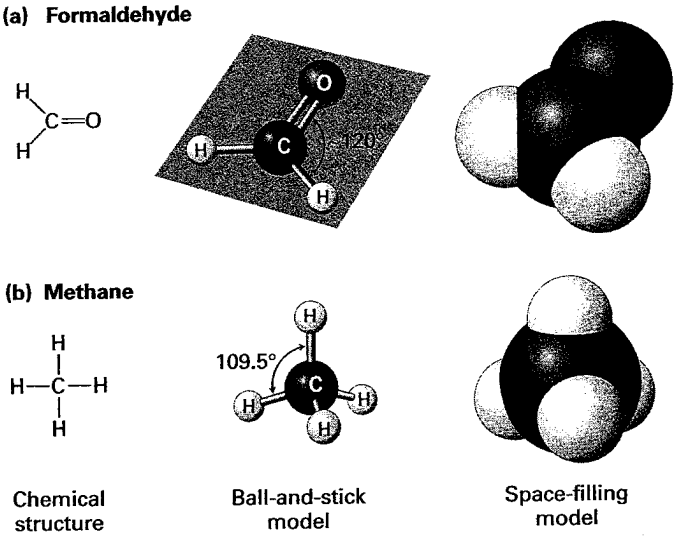
The Electronic Structure of an Atom Determines the Number and Geometry of Covalent Bonds It Can Make

Hydrogen, oxygen, carbon, nitrogen, phosphorus, and sulfur are the most abundant elements in biological molecules. These atoms, which rarely exist as isolated entities, readily form covalent bonds, using electrons in the outermost electron orbitals surrounding their nuclei (Figure 2-2). As a rule, each type of atom forms a characteristic number of covalent bonds with other atoms, with a well-defined geometry determined by the atom's size and by both the distribution of electrons around the nucleus and the number of electrons that it can share. In some cases (e.g., carbon), the number of stable covalent bonds formed is fixed; in other cases (e.g., sulfur), different numbers of stable covalent bonds are possible.

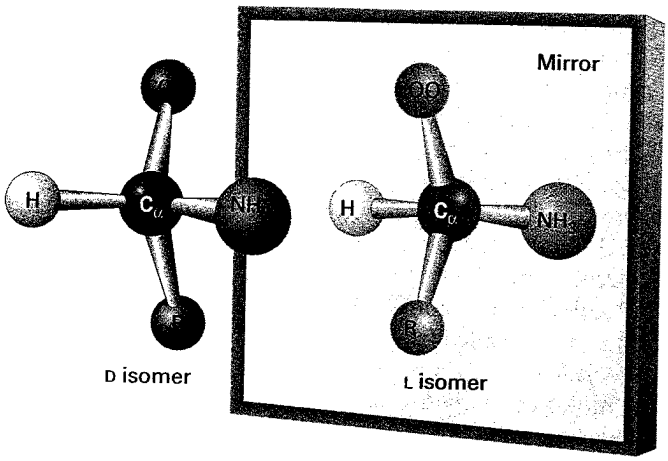
All the biological building blocks are organized around the carbon atom, which normally forms four covalent bonds with three or four other atoms. As illustrated in Figure 2-3a for formaldehyde, carbon can bond to three atoms, all in a common plane. The carbon atom forms two typical single bonds with two atoms and a double bond (two shared electron pairs) with the third atom. In the absence of other constraints, atoms joined by a single bond generally can rotate freely about the bond axis, whereas those connected by a double bond cannot. The rigid planarity imposed by double bonds has enormous significance for the shapes and flexibility of biomolecules such as phospholipids, proteins, and nucleic acids.

Carbon can also bond to four rather than three atoms. As illustrated by the methane (CH_4) molecule, when carbon is bonded to four other atoms, the angle between any two bonds is 109.5° and the positions of bonded atoms define the four points of a tetrahedron (Figure 2-3b). This geometry defines the structures of many biomolecules. A carbon (or any other) atom bonded to four dissimilar atoms or groups in a nonplanar configuration is said to be asymmetric. The tetrahedral orientation of bonds formed by an **asymmetric carbon atom** can be arranged in three-dimensional space in two different ways, producing molecules that are mirror images of each other, a property called *chirality* (from the Greek word *cheir*, meaning "hand") (Figure 2-4). Such molecules are called *optical isomers*, or **stereoisomers.** Many molecules in cells contain at least one asymmetric carbon atom, often called a *chiral carbon* atom. The different stereoisomers of a molecule usually have completely different biological activities because the arrangement of atoms within their structures differs, yielding their unique abilities to interact and chemically react with other molecules.

Some drugs are mixtures of the stereoisomers of small molecules in which only one stereoisomer has the biological activity of interest. The use of a pure single stereoisomer of the chemical in place of the mixture can result in a more potent drug with reduced side effects. For example, one stereoisomer of the antidepressant drug citalopram (Celexa) is



▲ **FIGURE 2-3 Geometry of bonds when carbon is covalently linked to three or four other atoms.** (a) A carbon atom can be bonded to three atoms, as in formaldehyde (CH_2O). In this case, the carbon-bonding electrons participate in two single bonds and one double bond, which all lie in the same plane. Unlike atoms connected by a single bond, which usually can rotate freely about the bond axis, those connected by a double bond cannot. (b) When a carbon atom forms four single bonds, as in methane (CH_4), the bonded atoms (all H in this case) are oriented in space in the form of a tetrahedron. The letter representation on the left clearly indicates the atomic composition of the molecule and the bonding pattern. The ball-and-stick model in the center illustrates the geometric arrangement of the atoms and bonds, but the diameters of the balls representing the atoms and their nonbonding electrons are unrealistically small compared with the bond lengths. The sizes of the electron clouds in the space-filling model on the right more accurately represent the structure in three dimensions.



▲ **FIGURE 2-4 Stereoisomers.** Many molecules in cells contain at least one asymmetric carbon atom. The tetrahedral orientation of bonds formed by an asymmetric carbon atom can be arranged in three-dimensional space in two different ways, producing molecules that are mirror images, or stereoisomers, of each other. Shown here is the common structure of an amino acid, with its central asymmetric carbon and four attached groups, including the R group, discussed in Section 2.2. Amino acids can exist in two mirror-image forms, designated L and D. Although the chemical properties of such stereoisomers are identical, their biological activities are distinct. Only L amino acids are found in proteins.

170 times more potent than the other. Some stereoisomers have very different activities. Darvon is a pain reliever, whereas its stereoisomer, Novrad (*Darvon* spelled backward), is a cough suppressant. One stereoisomer of ketamine is an anesthetic, whereas the other causes hallucinations. ▮

The number of covalent bonds formed by other common atoms is shown in Table 2-1. A hydrogen atom forms only one covalent bond. An atom of oxygen usually forms only two covalent bonds but has two additional pairs of electrons that can participate in noncovalent interactions. Sulfur forms two covalent bonds in hydrogen sulfide (H_2S) but also can accommodate six covalent bonds, as in sulfuric acid (H_2SO_4) and its sulfate derivatives. Nitrogen and phosphorus each have five electrons to share. In ammonia (NH_3), the nitrogen atom forms three covalent bonds; the pair of electrons around the atom not involved in a covalent bond can take part in noncovalent interactions. In the ammonium ion (NH_4^+), nitrogen forms four covalent bonds, which have a tetrahedral geometry. Phosphorus commonly forms five covalent bonds, as in phosphoric acid (H_3PO_4) and its phosphate derivatives, which form the backbone of nucleic acids. Phosphate groups covalently attached to proteins play a key role in regulating the activity of many proteins, and the central molecule in cellular energetics, ATP, contains three phosphate groups (see Section 2.4). A summary of common covalent linkages and functional groups (portions of molecules that confer distinctive chemical properties) is provided in Table 2-2.

TABLE 2-1 Bonding Properties of Atoms Most Abundant in Biomolecules

ATOM AND OUTER ELECTRONS	USUAL NUMBER OF COVALENT BONDS	TYPICAL BOND GEOMETRY
H	1	H
O	2	O
S	2, 4, or 6	S
N	3 or 4	N
P	5	P
C	4	C

Electrons May Be Shared Equally or Unequally in Covalent Bonds

The extent of an atom's ability to attract an electron is called its *electronegativity.* In a bond between atoms with identical or similar electronegativities, the bonding electrons are essentially shared equally between the two atoms, as is the case for most C—C and C—H bonds. Such bonds are called **nonpolar.** In many molecules, the bonded atoms have different electronegativities, resulting in unequal sharing of the electrons. The bond between them is said to be **polar.**

One end of a polar bond has a partial negative charge (δ^-), and the other end has a partial positive charge (δ^+). In an O—H bond, for example, the greater electronegativity of the oxygen atom relative to hydrogen results in the electrons spending more time around the oxygen atom than the hydrogen. Thus the O—H bond possesses an *electric dipole,* a positive charge separated from an equal but opposite negative charge. The amount of δ^- charge on the oxygen atom of a O—H dipole is approximately 25 percent of that of an electron, with an equivalent δ^+ charge on the H atom. Because of its two O—H bonds that are not on exact opposite sides of the O atom, water molecules (H_2O) are dipoles (Figure 2-5) that form electrostatic, noncovalent interactions with one another and with other molecules. These interactions play a critical role in almost every biochemical interaction and so are fundamental to cell biology.

The polarity of the O=P double bond in H_3PO_4 results in a *resonance hybrid,* a structure between the two forms shown below in which nonbonding electrons are shown as pairs of dots:

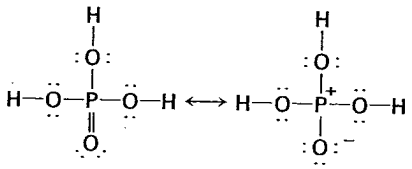


TABLE 2-2 Common Functional Groups and Linkages in Biomolecules

FUNCTIONAL GROUPS

—OH Hydroxyl (alcohol)	O ‖ —C—R Acyl (triacylglycerol)	O ‖ —C— Carbonyl (ketone)	O ‖ —C—O⁻ Carboxyl (carboxylic acid)
—SH Sulfhydryl (Thiol)	—NH_2 or —$\overset{+}{N}H_3$ Amino (amines)	O ‖ —O—P—O⁻ | O⁻ Phosphate (phosphorylated molecule)	O O ‖ ‖ —O—P—O—P— | | O⁻ O⁻ Pyrophosphate (diphosphate)

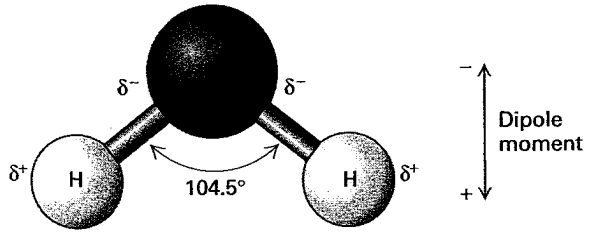
LINKAGES

O ‖ —C—O—C— Ester	—C—O—C— Ether	O ‖ —N—C— Amide

In the resonance hybrid on the right, one of the electrons from the P=O double bond has accumulated around the O atom, giving it a negative charge and leaving the P atom with a positive charge. These charges are important in noncovalent interactions.

Covalent Bonds Are Much Stronger and More Stable Than Noncovalent Interactions

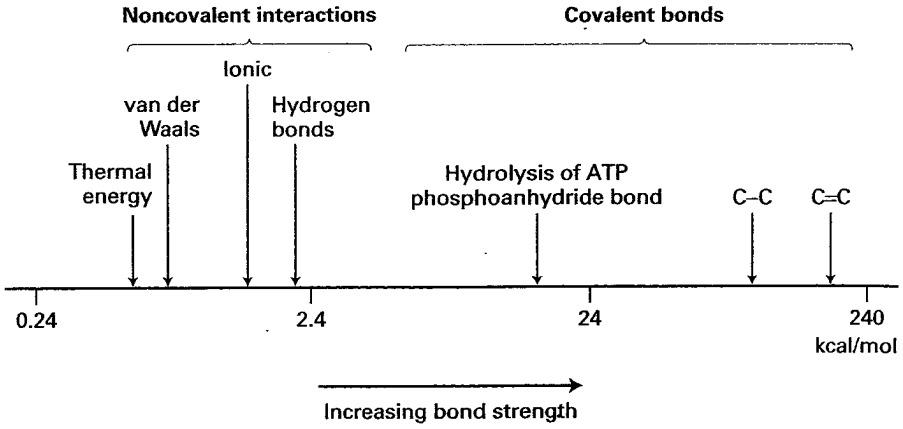
Covalent bonds are very stable (i.e., considered to be strong) because the energies required to break them are much greater than the thermal energy available at room temperature (25 °C) or body temperature (37 °C). For example, the thermal energy at 25 °C is approximately 0.6 kilocalorie per mole (kcal/mol), whereas the energy required to break the carbon-carbon single bond (C—C) in ethane is about 140 times larger (Figure 2-6). Consequently, at room temperature (25 °C), fewer than 1 in 10^{12} ethane molecules is broken into a pair of $\cdot CH_3$ molecules, each containing an unpaired, nonbonding electron (called a radical).



▲ **FIGURE 2-5 The dipole nature of a water molecule.** The symbol δ represents a partial charge (a weaker charge than the one on an electron or a proton). Because of the difference in the electronegativities of H and O, each of the polar H—O bonds in water is a dipole. The sizes and directions of the dipoles of each of the bonds determine the net distance and amount of charge separation, or **dipole moment,** of the molecule.

Covalent single bonds in biological molecules have energies similar to the energy of the C—C bond in ethane. Because more electrons are shared between atoms in double bonds, they require more energy to break than single bonds. For instance, it takes 84 kcal/mol to break a single C—O bond but 170 kcal/mol to break a C=O double bond. The most common double bonds in biological molecules are C=O, C=N, C=C, and P=O.

In contrast, the energy required to break noncovalent interactions is only 1–5 kcal/mol, much less than the bond energies of covalent bonds (see Figure 2-6). Indeed, noncovalent interactions are weak enough that they are constantly being formed and broken at room temperature. Although these interactions are weak and have a transient existence at physiological temperatures (25–37 °C), multiple noncovalent interactions can, as we will see, act together to produce highly stable and specific associations between different parts of a large molecule or between different macromolecules. Below, we review the four main types of noncovalent interactions and then consider their roles in the binding of biomolecules to one another and to other molecules.



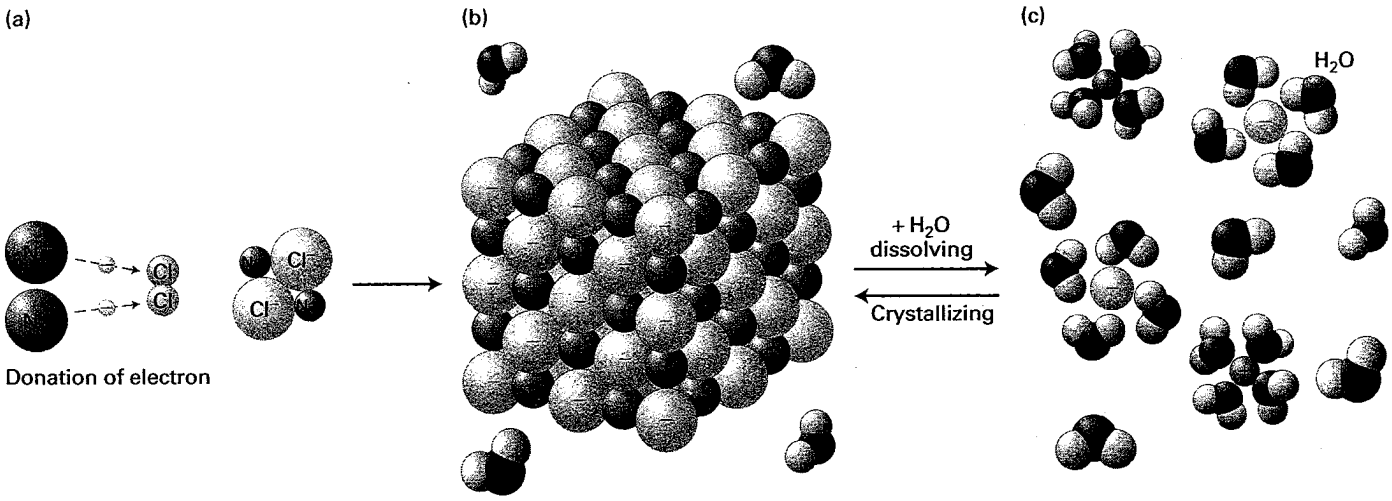
► **FIGURE 2-6 Relative energies of covalent bonds and noncovalent interactions.** Bond energies are defined as the energy required to break a particular type of linkage. Covalent bonds, including those for single (C—C) and double (C=C) carbon-carbon bonds, are one to two powers of 10 stronger than noncovalent interactions. The latter are somewhat greater than the thermal energy of the environment at normal room temperature (25 °C). Many biological processes are coupled to the energy released during hydrolysis of a phosphoanhydride bond in ATP.

Ionic Interactions Are Attractions between Oppositely Charged Ions

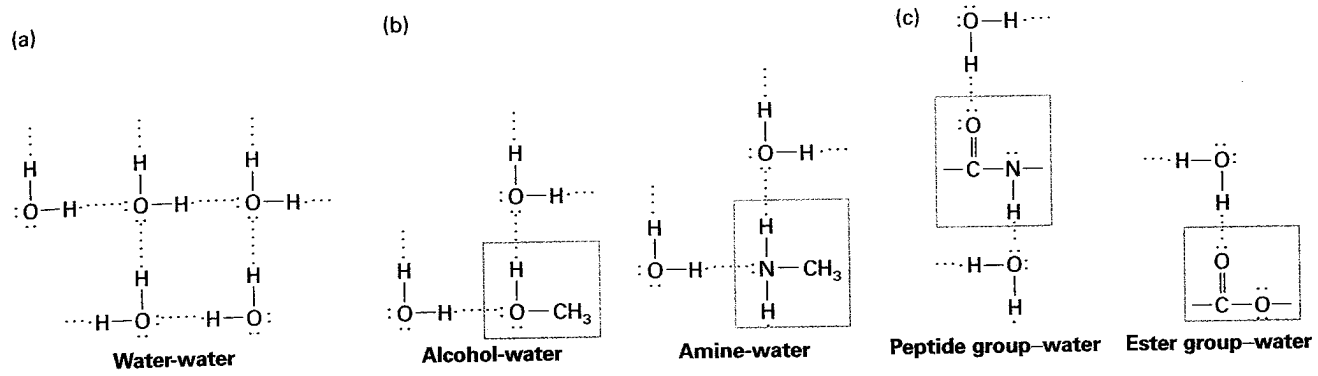
Ionic interactions result from the attraction of a positively charged ion—a **cation**—for a negatively charged ion—an **anion.** In sodium chloride (NaCl), for example, the bonding electron contributed by the sodium atom is completely transferred to the chlorine atom. (Figure 2-7a). Unlike covalent bonds, ionic interactions do not have fixed or specific geometric orientations because the electrostatic field around an ion—its attraction for an opposite charge—is uniform in all directions. In solid NaCl, many ions pack tightly together in an alternating pattern to permit opposite charges to align and thus form a highly ordered crystalline array (salt crystals) (Figure 2-7b).

When solid salts dissolve in water, the ions separate from one another and are stabilized by their interactions with water molecules. In aqueous solutions, simple ions of biological significance, such as Na^+, K^+, Ca^{2+}, Mg^{2+}, and Cl^-, are hydrated, surrounded by a stable shell of water molecules held in place by ionic interactions between the central ion and the oppositely charged end of the water dipole (Figure 2-7c). Most ionic compounds dissolve readily in water because the energy of hydration, the energy released when ions tightly bind water molecules, is greater than the lattice energy that stabilizes the crystal structure. Parts or all of the aqueous *hydration shell* must be removed from ions when they directly interact with proteins. For example, water of hydration is lost when ions pass through protein pores in the cell membrane during nerve conduction.

The relative strength of the interaction between two ions, A^- and C^+, depends on the concentration of other ions in a solution. The higher the concentration of other ions (e.g., Na^+ and Cl^-), the more opportunities A^- and C^+ have to



▲ **FIGURE 2-7 Electrostatic interactions of oppositely charged ions of salt (NaCl) in crystals and in aqueous solution.** (a) In crystalline table salt, sodium atoms are positively charged ions (Na^+) due to the loss of one electron each, whereas chloride atoms are correspondingly negatively charged (Cl^-) by gaining one electron each. (b) In solid form, ionic compounds form neatly ordered arrays, or crystals, of tightly packed ions in which the positive and negatively charged ions counterbalance each other. (c) When the crystals are dissolved in water, the ions separate and their charges, no longer balanced by immediately adjacent ions of opposite charge, are stabilized by interactions with polar water. Water molecules and the ions are held together by electrostatic interactions between the charges on the ion and the partial charges on the water's oxygen and hydrogen atoms. In aqueous solutions, all ions are surrounded by a hydration shell of water molecules.



▲ **FIGURE 2-8 Hydrogen bonding of water with itself and with other compounds.** Each pair of nonbonding outer electrons in an oxygen or a nitrogen atom can accept a hydrogen atom in a hydrogen bond. The hydroxyl and the amino groups can also form hydrogen bonds with water. (a) In liquid water, each water molecule forms transient hydrogen bonds with several others, creating a dynamic network of hydrogen-bonded molecules. (b) Water also can form hydrogen bonds with alcohols and amines, accounting for the high solubility of these compounds. (c) The peptide group and ester group, which are present in many biomolecules, commonly participate in hydrogen bonds with water or polar groups in other molecules.

interact ionically with these other ions and thus the lower the energy required to break the interaction between A^- and C^+. As a result, increasing the concentrations of salts such as NaCl in a solution of biological molecules can weaken and even disrupt the ionic interactions holding the biomolecules together.

Hydrogen Bonds Determine the Water Solubility of Uncharged Molecules

A **hydrogen bond** is the interaction of a partially positively charged hydrogen atom in a molecular dipole (e.g., water) with unpaired electrons from another atom, either in the same (*intramolecular*) or different (*intermolecular*) molecule. Normally, a hydrogen atom forms a covalent bond with only one other atom. However, a hydrogen atom covalently bonded to an electronegative donor atom D may form an additional weak association, the hydrogen bond, with an acceptor atom A, which must have a nonbonding pair of electrons available for the interaction:

$$D^{\delta-}—H^{\delta+} + :A^{\delta-} \rightleftharpoons D^{\delta-}—H^{\delta+}\cdots\cdots:A^{\delta-}$$

Hydrogen bond

The length of the covalent D—H bond is a bit longer than it would be if there were no hydrogen bond because the acceptor "pulls" the hydrogen away from the donor. An important feature of all hydrogen bonds is directionality. In the strongest hydrogen bonds, the donor atom, the hydrogen atom, and the acceptor atom all lie in a straight line. Nonlinear hydrogen bonds are weaker than linear ones; still, multiple nonlinear hydrogen bonds help to stabilize the three-dimensional structures of many proteins.

Hydrogen bonds are both longer and weaker than covalent bonds between the same atoms. In water, for example, the distance between the nuclei of the hydrogen and oxygen atoms of adjacent, hydrogen-bonded molecules is about 0.27 nm, about twice the length of the covalent O—H bonds within a single water molecule (Figure 2-8a). The strength of a hydrogen bond between water molecules (approximately 5 kcal/mol) is much weaker than a covalent O—H bond (roughly 110 kcal/mol), although it is greater than that for many other hydrogen bonds in biological molecules (1–2 kcal/ mol). The extensive hydrogen bonding between water molecules accounts for many of the key properties of this compound, including its unusually high melting and boiling points and its ability to interact with (e.g., dissolve) many other molecules.

The solubility of uncharged substances in an aqueous environment depends largely on their ability to form hydrogen bonds with water. For instance, the hydroxyl group (—OH) in an alcohol (XCH_2OH) and the amino group ($—NH_2$) in amines (XCH_2NH_2) can form several hydrogen bonds with water, enabling these molecules to dissolve in water to high concentrations (Figure 2-8b). In general, molecules with polar bonds that easily form hydrogen bonds with water, as well as charged molecules and ions that interact with the dipole in water, can readily dissolve in water; that is, they are hydrophilic (water liking). Many biological molecules contain, in addition to hydroxyl and amino groups, peptide and ester groups, which form hydrogen bonds with water via otherwise nonbonded electrons on their carbonyl oxygens (Figure 2-8c). X-ray crystallography combined with computational analysis permits an accurate depiction of the distribution of the outermost unbonded electrons of atoms as well as the electrons in covalent bonds, as illustrated in Figure 2-9.

Van der Waals Interactions Are Caused by Transient Dipoles

When any two atoms approach each other closely, they create a weak, nonspecific attractive force called a **van der Waals interaction.** These nonspecific interactions result from the momentary random fluctuations in the distribution of the electrons of any atom, which give rise to a transient