CHAPTER

1

Biochemical Thermodynamics

Learning Objectives

1. Define and use correctly the terms *system, closed, open, surroundings, state, energy, temperature, thermal energy, irreversible process, entropy, free energy, electromotive force (emf), Faraday constant, equilibrium constant, acid dissociation constant, standard state,* and *biochemical standard state.*

2. State and appropriately use equations relating the free energy change of reactions, the standard-state free energy change, the equilibrium constant, and the concentrations of reactants and products.

3. Explain qualitatively and quantitatively how unfavorable reactions may occur at the expense of a favorable reaction.

4. Apply the concept of coupled reactions and the thermodynamic additivity of free energy changes to calculate overall free energy changes and shifts in the concentrations of reactants and products.

5. Construct balanced reduction–oxidation reactions, using half-reactions, and calculate the resulting changes in free energy and emf.

6. Explain differences between the standard-state convention used by chemists and that used by biochemists, and give reasons for the differences.

7. Recognize and apply correctly common biochemical conventions in writing biochemical reactions.

Basic Quantities and Concepts

Thermodynamics is a system of thinking about interconnections of heat, work, and matter in natural processes like heating and cooling materials, mixing and separation of materials, and—of particular interest here—chemical reactions. Thermodynamic concepts are freely used throughout biochemistry to explain or rationalize chains of chemical transformations, as well as their connections to physical and biological processes such as locomotion or reproduction, the generation of fever, the effects of starvation or malnutrition, and more. Thermodynamics uses a set of technical terms that may seem somewhat artificial, but that are necessary for clarity and conciseness in thinking about thermodynamic problems. Thermodynamics also relies on

three general statements about the behavior of matter—the "laws" of thermodynamics—that reflect long experience in dealing with energy, equilibria, and natural processes and their tendencies.

Terminology

Thermodynamics uses a specialized and precise vocabulary in its explanations of natural processes, to give more rigor to its deductions about these phenomena.

- A *system* is whatever part of the universe we are interested in, in terms of thermodynamics. *Closed* systems cannot exchange matter across their boundaries; *open* systems, however, can pass matter back and forth across their boundaries.
- The *surroundings* are everything else in the universe that lie outside the boundaries of the system. It can include reservoirs of heat energy or of matter, mechanical devices to perform or absorb work, and so on. The system could be, for example, a collection of biochemicals in aqueous solution in a beaker or flask, while the surroundings would be the water bath, lab bench, and other apparati around the beaker of dissolved biochemicals. **Figure 1–1** contains examples of some simple systems and their surroundings.
- The overall *state* of a system refers to its temperature, pressure, composition (e.g., how many moles of each constituent; their presence as gas, liquid, or solid), and perhaps other properties such as electrical charge or electrical potential. When matter, heat, or some other form of energy crosses from the surroundings into the system (or if it leaves the system and passes into the surroundings), the system reaches a new state. For example, chemical reactions might take place in the beaker, changing its composition, and perhaps liberating some heat that would cause the volume of solution to expand slightly. This heat might pass over to the water bath, outside the system, and warm the surroundings.
- *Pressure* (P) is defined as the force exerted per unit area. The SI unit of pressure is the Pascal (Pa). For reference, atmospheric pressure is 101,325 Pa.
- Pressure multiplied by volume (V) has the dimensions of *energy* (E), so that volume or pressure changes are often related to work done on or extracted from a system. The SI unit for energy is the joule (J).
- The common scale of *temperature* used in thermodynamics is the absolute or Kelvin scale; the unit is the Kelvin (K). 0°C equals 273.15 K. The absolute zero of temperature on the Kelvin scale is the point where all thermal motion would cease; it corresponds to -273.15°C.
- The *thermal energy* of a system is related to motions on the atomic or molecular level. For each gas particle in an ideal monatomic gas at temperature T, the energy is

$$E = \frac{3}{2} k_{\mathrm{B}} T \qquad (1\text{-}1)$$

Here k_{B} is Boltzmann's constant, equal to 1.38066×10^{-23} J/K. Per mole of ideal monatomic gas, the energy is $\frac{3}{2} RT$, where R is the gas constant, equal to 8.3144 J/mol–K.

In thermodynamics, the concepts of systems and surroundings are quite general. For example, the concept of system could be extended to include living cells or even whole organisms, along with a suitable enlargement of the notion of "boundaries" and "surroundings."

Closed System with Heat Reservoir

Thermometer

Closed System   Open System

A.   **B.**   **C.**

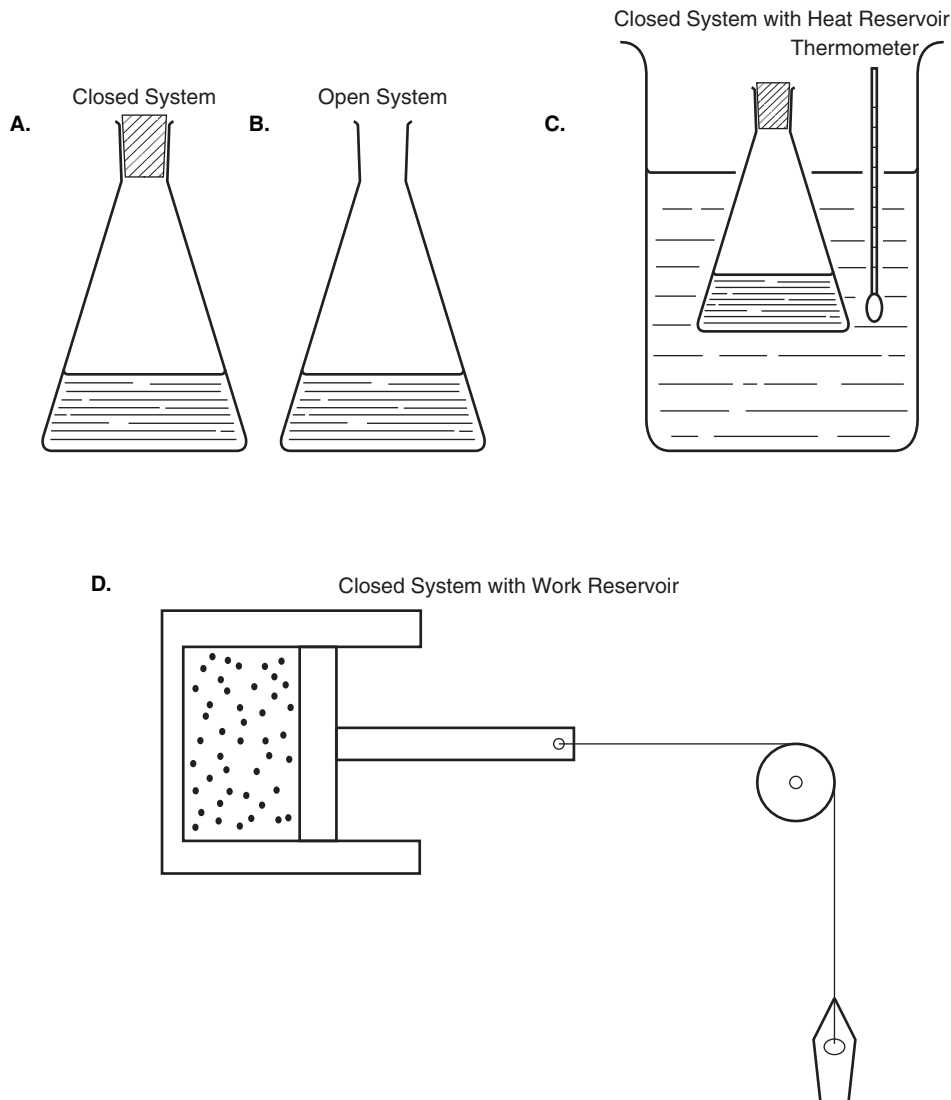**D.**   Closed System with Work Reservoir

Figure 1-1   Simple thermodynamic systems. **A, B.** Open and closed systems: The stoppered flask cannot exchange matter with the surroundings. **C.** A closed system (stoppered flask) in contact with a heat reservoir (water bath). **D.** A closed system (gas in piston-cylinder) in contact with a work reservoir (weight-pulley).

First Law: Energy Conservation

The *first law of thermodynamics* states that energy is conserved. The forms of energy can be interconverted, but the sum of the energies must remain constant. This includes mechanical work and heat, as well as less apparent chemical or electrical changes.

- If the energy of a closed system in state A is E_A, and if the system passes to a different state B, with a different energy E_B, then the energy change for this process is
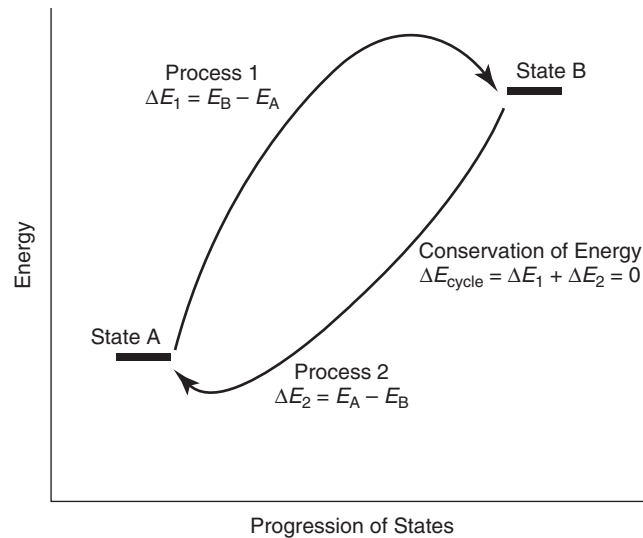
$$\Delta E = E_B - E_A \qquad (1\text{-}2)$$

Figure 1-2   The first law of thermodynamics: energy conservation in a cyclic process.

- A negative sign for ΔE implies that the system has a lower energy in state B than in state A; informally, B is energetically "downhill" from A.
- For a *cyclic process,* taking a closed system from state A to B and back to A, ΔE is zero (**Figure 1-2**).
- In terms of exchanges of heat (ΔQ) and work (ΔW), the change in energy for a closed system is

$$\Delta E = \Delta Q + \Delta W \tag{1-3}$$

- More generally, ΔE for a particular system is equal in magnitude, but opposite in sign, to the total energy change for all other systems (including the surroundings) involved in the change of the first system.

For energy exchange processes at constant pressure, thermodynamics introduces a new quantity, called *enthalpy* (H). The enthalpy function is defined by

$$H = E + PV \tag{1-4}$$

At constant pressure (the conditions under which most biochemical experiments are performed), the change in enthalpy accounts for both work and heat exchanges. The change in enthalpy is then

$$\Delta H = \Delta E + P\Delta V \tag{1-5}$$

As with ΔE, in a cyclic process that takes a closed system to another state and then back to precisely the original state, ΔH for the system is zero. For many biochemical systems and processes at constant pressure, the change in volume is small. Under these conditions the term $P\Delta V$ is often negligible compared to ΔE in Equation 1-5; then the change in enthalpy is very nearly the same as the change in energy.

Second Law: Entropy and the Direction of Spontaneous Change

Many natural processes are observed to proceed spontaneously in one direction, but never in the opposite direction; that is, they are *irreversible* (**Figure 1–3**). For example, two inert gases spontaneously mix throughout their container uniformly, but the mixture is not observed to
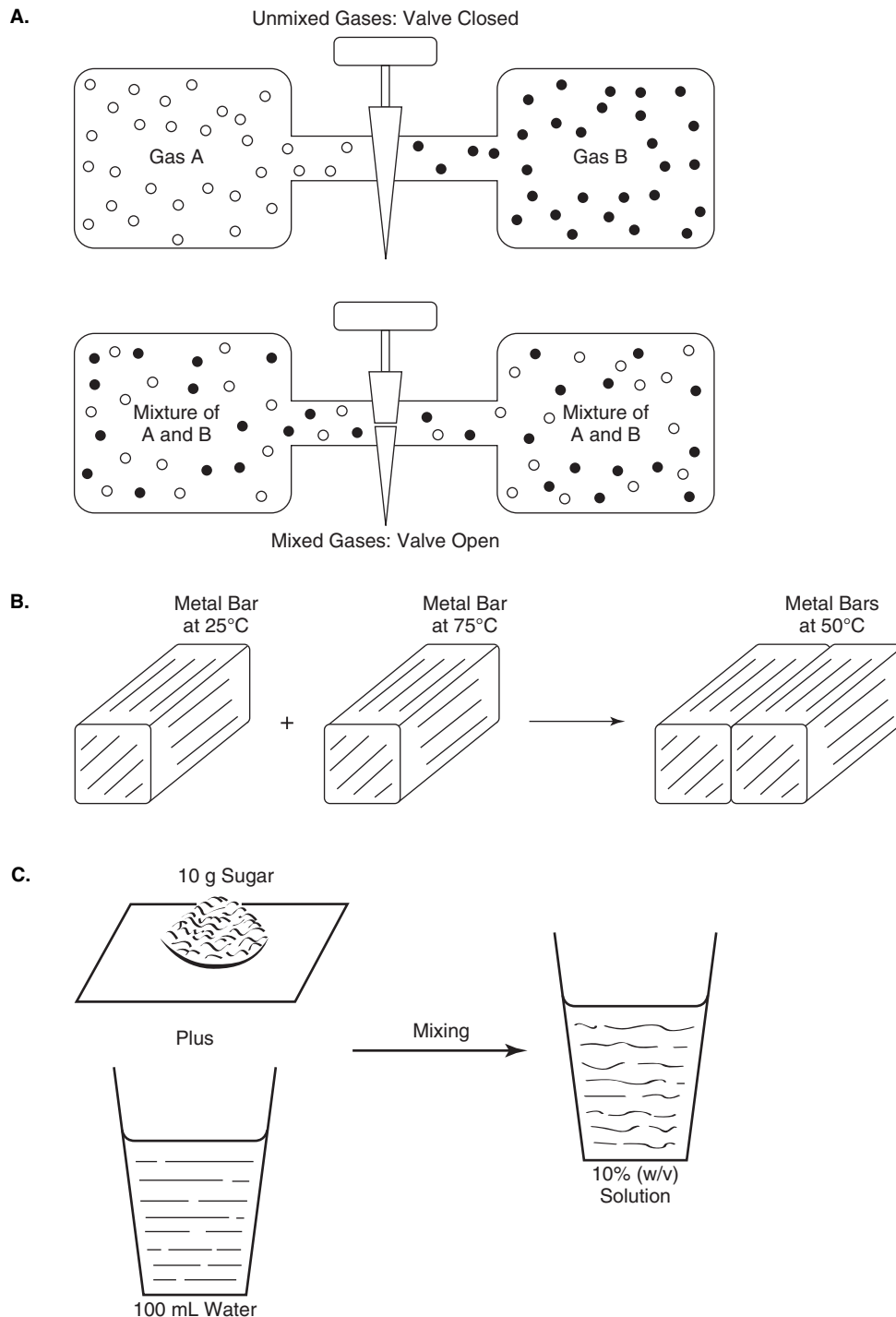
A.

Unmixed Gases: Valve Closed

Gas A

Gas B

Mixture of
A and B

Mixture of
A and B

Mixed Gases: Valve Open

B.

Metal Bar
at 25°C

Metal Bar
at 75°C

Metal Bars
at 50°C

+

C.

10 g Sugar

Plus

Mixing

100 mL Water

10% (w/v)
Solution

Figure 1-3   Spontaneous irreversible processes. **A.** Mixing of inert gases. **B.** Temperature equilibration of two metal bars. **C.** Dissolution of sugar (sucrose) in water.

spontaneously segregate into dense clumps of one gas and the other. Hotter objects in contact with cooler ones spontaneously transfer heat to the cooler object, but the cooler object does not become cooler and the hotter object even warmer. Common sugar (sucrose) dissolves in water to form a solution, but that solution does not spontaneously separate back into pure water and solid sucrose. The direction of change in these processes is not predicted by the first law. Instead, the second law of thermodynamics introduces a new thermodynamic quantity, called *entropy* (S), to help explain such spontaneous changes, including their direction and magnitude. The units of entropy are joules per mole-Kelvin (J/mol-K).

Entropy is closely connected to notions of order and disorder, sometimes in a very general and abstract way.

- The entropy of a system is proportional to the logarithm of the number of ways of arranging the system, down to the quantum level:

$$S = k_B \ln W \qquad (1\text{-}6)$$

  where W is the number of arrangements of the system with the same overall energy. **Table 1-1** compares arrangements and the value of W for a simple quantum system of two molecules and four available states.
- The meaning of "arrangements," "order," or "mixing," in connection with entropy, can refer to positions or orientations in space, but also includes freedom of motion (i.e., rotations, translations, and vibrations) and distributions over quantum energy levels. Such motions and quantum distributions must be considered, for example, in chemical reactions.
- For a change from a state A where there are W_A arrangements available to the system, to a different state B with W_B arrangements, the change in entropy is

$$\Delta S = S_B - S_A = k_B \ln \left(\frac{W_B}{W_A} \right) \qquad (1\text{-}7)$$

- The overall entropy change ΔS for the system depends only on the initial and final states of the system. This is similar to the energy change, ΔE, which likewise depends only on the initial and final states of the system.

| Distribution 1 (W = 4; more ordered) | | | | Distribution 2 (W = 6; less ordered) | | | |
State 1	State 2	State 3	State 4	State 1	State 2	State 3	State 4
ab				a	b		
	ab			a		b	
		ab		a			b
			ab		a	b	
					a		b
						a	b

Table 1-1   Two Molecules, *a* and *b*, with Four Available States

- A simple version of the second law is that the entropy S of the system plus that of the surroundings must increase in an irreversible process, and it remains constant in a reversible process. In terms of changes in entropy for the system and surroundings, this relationship is expressed as follows:

$$\Delta S_{\text{system}} + \Delta S_{\text{surr}} \geq 0 \qquad\qquad (1\text{–}8)$$

The equality holds for reversible changes, the inequality for irreversible (and spontaneous) changes.

Spontaneous changes are associated with a positive entropy change; spontaneous processes also result in a greater overall state of "mixing" or disorder. Very disordered systems have a high entropy, whereas highly ordered systems have a low entropy. A crystal, which has long-range ordering of its atoms, is a good example of a low-entropy system, while a hot gas of the same atoms would have a much greater disorder and a much higher entropy. The mixing process in Figure 1-3, for example, clearly leads to greater disorder and a positive entropy change. The entropy change involved in the temperature equilibration process is more subtle, but can be thought of as the net result of matching the cooling and ordering of atoms in the hot metal bar against the greater thermal disorder gained by the atoms in the bar that is warmed up.

Illustrating the entropy change for a chemical reaction is more difficult yet. If we view the reaction as distributing the particles (atoms, molecules) of a system over a broader range of energy levels, then this process increases the disorder of the system. Hence, the entropy increases; ΔS is positive for such a chemical reaction (**Figure 1-4**). Conversely, a process that collects otherwise dispersed particles into a narrow set of positions, or a limited set of energy levels, would have a negative value for ΔS. Indeed, it is quite possible to have chemical reactions with negative entropy changes, when the products are more "organized" or "ordered" than the reactants, in terms of distributing them over energy levels.

If a system can exchange heat or matter with its surroundings, then the system can have a decrease in entropy (an increase in its ordering). The surroundings, however, undergo an
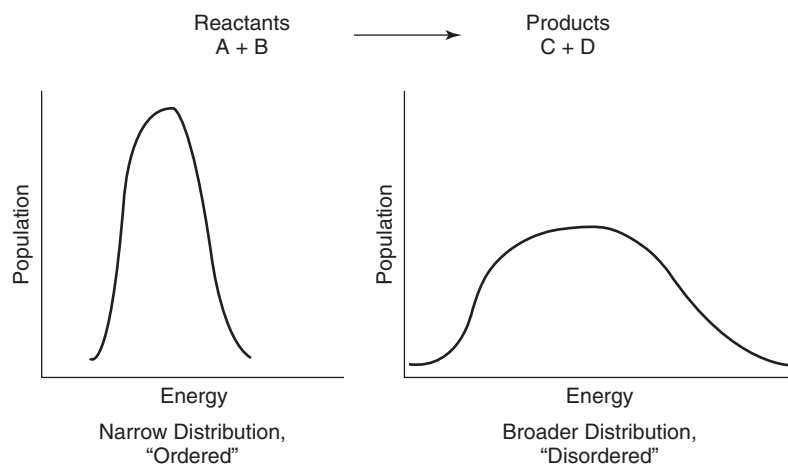

Figure 1-4   Chemical reactions that spread the system over more states will have positive entropy changes.
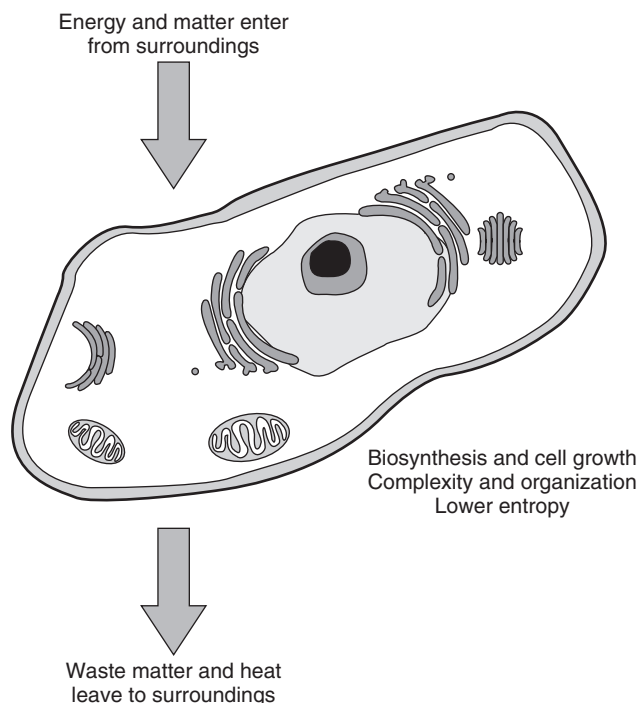
Figure 1-5 Living systems are open systems and order themselves by using energy and matter from the surroundings.

increase in their entropy. In this way, an influx of energy to a system can produce more order in that system. Living systems exploit such energy fluxes to order or organize themselves; however, their surroundings must become ever more disordered or disorganized as a result of this activity (**Figure 1-5**).

Third Law: An Absolute Scale for Entropies

There are several ways to state the third law of thermodynamics. One version says that it is not possible to reach absolute zero in temperature through any finite series of processes. Another states that as the temperature approaches absolute zero, the magnitude of the entropy change in a reversible process also approaches zero. While these formulations may be helpful to physicists and material scientists, a more chemically relevant version of the third law is that the entropy of a pure substance is zero when that substance is in a physical state such that there is no contribution to the entropy from translation, rotation, vibration, configuration, or electronic terms. The substance must be perfectly ordered, with no disorder from any motions or spatial disarrangements. As a mathematical equation, this version of the third law can be written as

$$\lim_{T \to 0} S = 0 \ (\text{perfectly ordered substance}) \tag{1-9}$$

A (hypothetical) physical state that matches these criteria is that of a perfectly ordered crystalline chemical, at the absolute zero of temperature. Note that a gas or liquid would still have some disorder as the temperature approached absolute zero (in making this statement, we set aside quantum physics anomalies such as liquid helium).

With a perfect crystal, there is of course, no contribution from alternative configurations of the molecules (there is only one "arrangement" in a perfectly ordered material); also, there are no rotational or translational contributions to the entropy. As the temperature drops, lattice vibrations and electron distributions in the crystal drops to the lowest permitted quantum level, so that vibrational and electronic contributions to the entropy also approach zero. Thus chemists use the convention that S is zero for pure compounds in the most stable crystalline form, at the absolute zero of temperature. This sets the scale for entropies of chemical compounds, so-called absolute entropies, with zero entropy attained at the zero of temperature, and more positive entropy values occurring as the temperature rises.

The entropy of a substance does depend on the temperature. For a reversible change in temperature from T_1 to T_2, the change in entropy is given by

$$S_2 - S_1 = \int_{T_1}^{T_2} \frac{C\, dT}{T} \tag{1-10}$$

where C is the heat capacity of the substance. With the convention that S for a pure substance is zero at absolute zero temperature, this last equation allows calculation of the entropy at any higher temperature, provided that the heat capacity function C is known. Because C is always a positive quantity, any increase in temperature increases the entropy; rising temperature leads to more disorder in the system.

The third law of thermodynamics sets a zero point for entropy values and allows the calculation of values of the entropy for chemical compounds. With these entropy values, along with measurements of other thermal properties of those compounds, one can predict the equilibrium constant for a chemical processes using a quantity called the free energy.

Free Energy Changes

The ΔS criterion given in the second law of thermodynamics is insufficient to determine the spontaneity of a process for a system that is connected to its surroundings. What complicates things here is the exchange of heat, work, and matter with the surroundings. Supplying energy or matter to the system can overcome unfavorable changes in ordering the system, thereby making the overall process (for system and surroundings) spontaneous even though, from the system's point of view, the entropy change is not at all favorable. To determine the spontaneity of such processes, a broader, more inclusive quantity that takes such changes into account is needed. For processes taking place at constant pressure, the Gibbs free energy G is just the quantity needed.

- G is defined as a composite of enthalpy and the entropy:

$$G = H - TS \tag{1-11}$$

- Changes in G depend only on the initial and final states of the system; for a process going from state A to state B,

$$\Delta G = G_B - G_A \tag{1-12}$$

- Spontaneous changes have a negative value for ΔG, while nonspontaneous processes have a positive ΔG. If ΔG is zero, then the system is at equilibrium.

- In terms of changes in enthalpy and entropy, for processes at constant temperature:

$$\Delta G = \Delta H - T\Delta S \tag{1-13}$$

Formally, ΔG measures the available work in a spontaneous process—that is, how much work can be extracted by doing the process. This includes chemical "work," or the conversion of reactants to products, which is our main interest. In particular, the use of ΔG enables quantitative predictions for biochemical reactions, the energetic bases for life processes. Evaluation of ΔG allows prediction of whether a process will tend to occur naturally (spontaneously) or whether it is at equilibrium. The connection of ΔG to conditions for equilibrium allows prediction of the way an equilibrium will shift, and how far.

The free energy criterion for a spontaneous process, $\Delta G < 0$, says nothing about the *rate* at which the process occurs. Although many spontaneous processes occur at a moderate to rapid rate, this is not universally true. Many thermodynamic processes occur at extremely slow rates, even though the free energy change is quite negative. A familiar example is the kinetic stability of diamond relative to graphite. Here ΔG at room temperature is -2.9 kJ/mol for the diamond-to-graphite conversion, so diamonds are thermodynamically unstable with respect to graphite. The rate of conversion, however, is so slow that, for all practical purposes, it does not occur; thus diamond can be described as kinetically stable but thermodynamically unstable. This distinction will turn out to be an important one when we discuss biochemical reaction pathways later in this book.

A constant passage of matter and energy through living systems occurs such that living systems are not at thermodynamic equilibrium; in fact, they are generally very far from equilibrium. Living organisms exploit the flux of matter and energy to promote their internal organization, a higher state of order, or a state of lower entropy in the thermodynamic sense. Clearly, certain nonspontaneous processes (usually involving chemistry) takes place in achieving such organization. It is also obvious that counterbalancing spontaneous processes must occur for the living system to maintain its life processes overall. Thermodynamics, especially the use of ΔG, can help us make sense of the combination of favorable and unfavorable processes that permit life to continue.

Chemical Equilibria

There is a very close connection between the free energy change for a chemical process and the equilibrium constant for that process. To predict which way a reaction might spontaneously proceed, or the amounts of chemicals formed or depleted by setting up a chemical reaction, one needs to study the free energy change and the associated equilibrium constant for that process. This can be particularly important in biochemistry, where many reactions share products and reactants. The products of one reaction may be the reactants for another, and careful dissection of the various free energy changes is needed to understand the direction and magnitude of the overall change in the biochemical system. This line of study will be very useful in later chapters in understanding how metabolic pathways such as glycolysis or the citric acid cycle function.

Basic Equations

For the reaction

$$a\text{A} + b\text{B} \rightleftharpoons c\text{C} + d\text{D} \tag{1-14}$$

the *equilibrium constant* K_{eq} is given by

$$K_{eq} = \frac{[C]^c[D]^d}{[A]^a[B]^b} \tag{1-15}$$

where [A], [B], and so on are the equilibrium concentrations of the reactants and products.

The Gibbs free energy change ΔG for a chemical reaction is given by

$$\Delta G = G(\text{products}) - G(\text{reactants}) \tag{1-16}$$

The free energy of j moles of a species I can be written as

$$G_I = j \times \overline{G_I^\circ} + j \times RT \ln [I] \tag{1-17}$$

where $\overline{G_I^\circ}$ is the free energy per mole of that substance under standard conditions of temperature, pressure, and concentration.

Setting up conditions that define a "standard state" gives a reference point for free energy changes relative to this state. Recall that the third law of thermodynamics sets a "zero point" for entropy changes, but that there is no corresponding zero point for energy (or enthalpy) changes; thus we need a way to set a scale for free energy changes. Using standard states for substances (e.g., gases, liquids, or solutions) is the chemist's way of setting that scale. In biochemistry, the standard concentration for a solution is typically one molar, unit concentration. Other standard state conditions are one atmosphere of pressure and a temperature of 298 K.

For the reaction shown in Equation 1-14, the free energy change is

$$\Delta G = c\overline{G_C} + d\overline{G_D} - a\overline{G_A} - b\overline{G_B} \tag{1-18}$$

Notice the inclusion in this relation of the stoichiometric coefficients $a, b, c,$ and d. This equation can be expanded to

$$\Delta G = c \times G_C^\circ + c \times RT \ln[C] + d \times G_D^\circ + d \times RT \ln[D]$$
$$- a \times G_A^\circ + a \times RT \ln[A] - b \times G_B^\circ + b \times RT \ln[B] \tag{1-19}$$

Grouping terms then leads to

$$\Delta G = \Delta G^\circ + RT \ln\left(\frac{[C]^c[D]^d}{[A]^a[B]^b}\right) \tag{1-20}$$

The quantity ΔG° is the *standard state free energy change,* the free energy change for the reaction if all components are in their *standard state* (a temperature of 298 K, atmospheric pressure, and unit concentration). This is different from the free energy change ΔG when the components are at arbitrary and non–unit concentrations [A], [B], [C], and [D]. The quantity ΔG but not ΔG° determines whether the system is at equilibrium.

At equilibrium ΔG is zero, by definition. This gives the connection to the equilibrium constant for "standard chemical conditions":
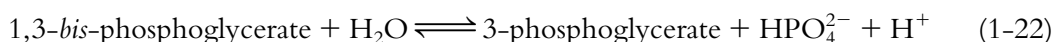
$$\Delta G^\circ = -RT \ln K_{eq} \quad \text{or} \quad K_{eq} = e^{-\Delta G^\circ/RT} \tag{1-21}$$

The second (exponential) relation shows that even small changes in the (standard) free energy can lead to large values of the equilibrium constant.

Biochemical Standard States

For reactions involving the release or uptake of protons, the species H^+ appears as a reactant or product. It is clear that the concentration of protons influences the equilibrium. But biological systems are typically buffered at around pH 7, where the hydrogen ion concentration is smaller by a factor of 10^7 than for the standard state of one molar $(pH = 0)$. Many hydrolysis reactions also exist in which water appears as a reactant or product. Reactions of biochemical interest almost always are studied in dilute aqueous solution, where the concentration of water remains essentially constant at 55.6 molar.

As an example, consider the following hydrolysis reaction:

$$1,3\text{-}bis\text{-phosphoglycerate} + H_2O \rightleftharpoons 3\text{-phosphoglycerate} + HPO_4^{2-} + H^+ \qquad (1\text{-}22)$$

Strictly speaking, the free energy change for this reaction should be written as

$$\Delta G = \Delta G^\circ + RT \ln \left(\frac{[3\text{-phosphoglycerate}][HPO_4^{2-}][H^+]}{[1,3\text{-}bis\text{-phosphoglycerate}][H_2O]} \right) \qquad (1\text{-}23)$$

Because we expect neither $[H^+]$ nor $[H_2O]$ to change much, we can separate out the logarithmic terms in $[H^+]$ and $[H_2O]$ from the other concentrations:

$$\Delta G = \Delta G^\circ + RT \ln \left(\frac{[3\text{-phosphoglycerate}][HPO_4^{2-}]}{[1,3\text{-}bis\text{-phosphoglycerate}]} \right) + RT \ln \left(\frac{[H^+]}{[H_2O]} \right) \qquad (1\text{-}24)$$

Because the last logarithmic term is essentially constant, we can combine it with the ΔG° term and write this as a combined $\Delta G'^\circ$. In essence we have defined a new standard state for the 1,3-*bis*-phosphoglycerate, the phosphate, and the 3-phosphoglycerate. This *biochemical standard state* is defined as pH 7, 298 K, 1 atmosphere pressure, and unit concentrations for chemicals other than water or protons.

Notice that we have a new symbol for the standard free energy change: $\Delta G'^\circ$. However, just as ΔG° is not the criterion for spontaneity for regular chemical reactions (under nonstandard conditions, we should use ΔG), neither is $\Delta G'^\circ$ the appropriate criterion for biochemical reactions. Instead, we should (for biochemical reactions, at pH 7) use $\Delta G'$.

Acid–Base Equilibria

Many biochemicals contain acidic or basic functional groups, and the state of titration of these groups plays an important role in their biological function (e.g., stability of conformation, enzymatic catalysis, binding of other molecules). Examples of such groups include the following:

- Acidic carboxyl groups of amino acids and acidic metabolites such as citric and lactic acids
- Basic amino groups of amino acids, amino sugars, and biological amines such as spermidine
- Side chains of several amino acids containing groups that can act as weak acids or bases, such as the phenolic hydroxyl group in tyrosine, the thiol group of cysteine, and the imidazole ring of histidine

The Henderson-Hasselbach equation connects the *acid dissociation constant* with the solution pH. For an acid dissociation reaction of a simple acid HA to its conjugate base A⁻,

$$HA \rightleftharpoons H^+ + A^- \tag{1-25}$$

the acid dissociation constant K_a is given by

$$K_a = \frac{[H^+][A^-]}{[HA]} \tag{1-26}$$

This rearranges to

$$[H^+] = K_a \cdot \left(\frac{[HA]}{[A^-]} \right) \tag{1-27}$$

Taking negative logarithms gives

$$-\log[H^+] = -\log K_a - \log\left(\frac{[HA]}{[A^-]} \right) \tag{1-28}$$

With the usual definitions of pH and pK_a, this gives

$$pH = pK_a + \log\left(\frac{[A^-]}{[HA]} \right) \tag{1-29}$$

If the concentrations of the undissociated acid HA and the conjugate base A⁻ are equal, as when the acid is half-dissociated, then the logarithmic term on the right is cancelled, and the pH then equals the pK_a. Such a situation occurs at the midpoint of the acid's titration curve (**Figure 1-6**). At this pH, the solution has its maximum buffering capacity (buffers are solutions that minimize changes in pH upon addition of either acid or base).

Coupling of Reactions

Many individual biochemical processes are nonspontaneous by themselves and have positive ΔG values. However, by adding a second, spontaneous reaction with a (sufficiently) negative
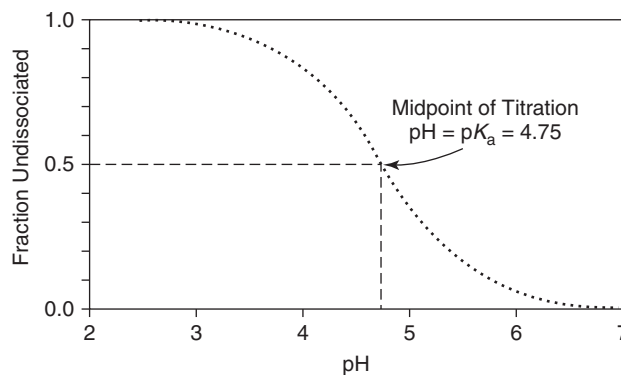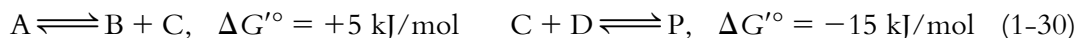

Figure 1-6   Monoprotic acid titration curve. The midpoint pH corresponds to the acid's pK_a.
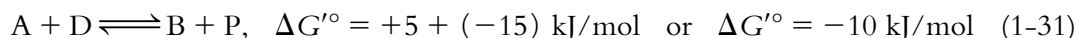
ΔG, the sum of the two reactions may result in an overall negative ΔG and, therefore, become overall spontaneous. This practice is referred to as *coupling* the reactions.

For example, consider two reactions with their associated free energy changes under standard conditions:

$$A \rightleftharpoons B + C, \quad \Delta G'^{\circ} = +5 \text{ kJ/mol} \qquad C + D \rightleftharpoons P, \quad \Delta G'^{\circ} = -15 \text{ kJ/mol} \quad (1\text{-}30)$$

The first reaction is not spontaneous under standard conditions, but the second is; B (and C) will *not* form spontaneously from A under standard conditions.

Now suppose that, for biological reasons, it would be desirable to drive the conversion of A over to B. This can be done by combining or coupling the unfavorable first reaction with the favorable second reaction, thereby taking advantage of the tendency of C to react with D. The net result is

$$A + D \rightleftharpoons B + P, \quad \Delta G'^{\circ} = +5 + (-15) \text{ kJ/mol} \quad \text{or} \quad \Delta G'^{\circ} = -10 \text{ kJ/mol} \quad (1\text{-}31)$$

The coupled reaction is now spontaneous under standard conditions. Thus feeding in A and D leads spontaneously to the formation of B and P, at standard concentrations all around. The coupling of unfavorable reactions with favorable ones appears widely throughout biochemistry, and we will see several examples of this phenomenon in later chapters.

Reduction–Oxidation Reactions (Redox)

Reduction–oxidation (*redox*) reactions occur when one chemical species loses electrons and another gains electrons. The species losing electrons is *oxidized,* and the species gaining electrons is *reduced*. Redox reactions involve free energy changes just like any other chemical reaction, and the tendency for a redox reaction to proceed depends on whether there is a favorable free energy change for that reaction.

Organisms exploit the free energy released by the oxidation of fuel molecules to do biological "work," including biosynthesis, transport, and mechanical work. The passage of electrons from the fuel molecules down to the ultimate electron acceptor, molecular oxygen, is overall a spontaneous process. Coupling to unfavorable (but desirable) reactions occurs at several steps in this process. The reactions are often complex, and many different intermediates carry electrons in these pathways. Redox reactions will appear throughout later chapters of this book as we discuss the breakdown of foodstuffs and the biosynthesis of complex biological molecules.

Redox reactions can be analyzed using *redox couples.*

- Redox couples are the pair of reactions that together add up to the overall reaction but that individually show which species is losing or gaining electrons.
- The individual reaction in one of these redox couples is called a *half-reaction*.

A simple, familiar example is the transfer of a pair of electrons from metallic zinc to copper ions, as might occur in an electrochemical cell (**Figure 1-7**):

$$Zn(s) + Cu^{2+}(aq) \rightleftharpoons Zn^{2+}(aq) + Cu(s) \qquad (1\text{-}32)$$
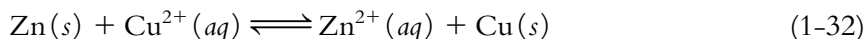
For clarity we have by the notation (s) that the native metals are in the solid state; the notation (aq) indicates that the metal ions are dissolved in aqueous solution. The metallic zinc $Zn(s)$
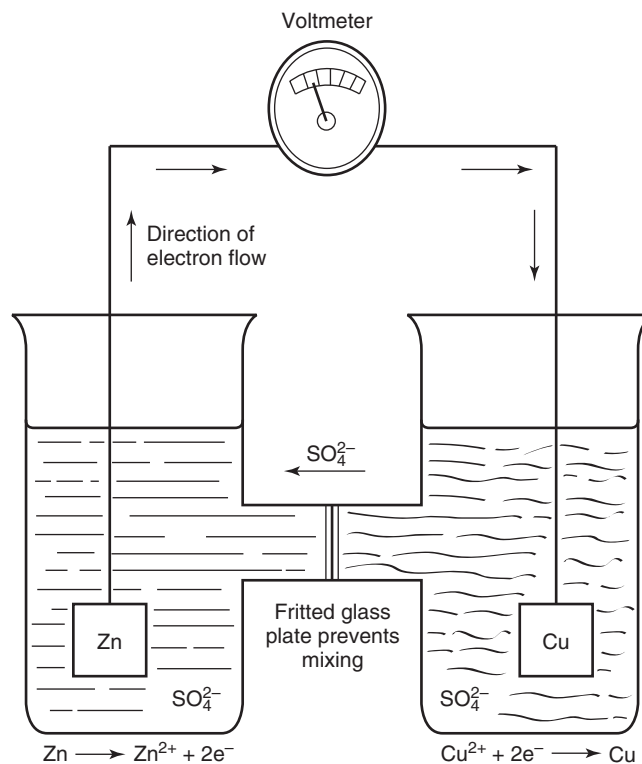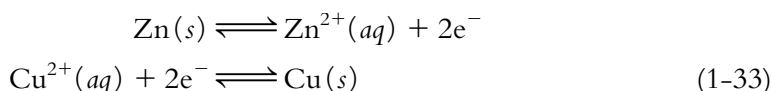
Voltmeter

Figure 1-7   Electrochemical cell showing the oxidation of zinc and the reduction of copper.

is oxidized to $Zn^{2+}(aq)$, while the copper ions, written as $Cu^{2+}(aq)$ in Equation 1-32, are reduced to metallic copper or $Cu(s)$. The individual half-reactions for the zinc and copper are

$$Zn(s) \rightleftharpoons Zn^{2+}(aq) + 2e^-$$
$$Cu^{2+}(aq) + 2e^- \rightleftharpoons Cu(s) \tag{1-33}$$

Each of these half-reactions has a certain tendency to occur—that is, a certain standard–state free–energy change can be associated with it. Historically, the energy changes for these electron transfer reactions were determined by using electrical circuits and electrochemical cells and by measuring the voltage generated by the cell with standard concentrations of reactants. The electrical current that flowed was attributed to an *electromotive force (emf)*, which was measured in volts.

By convention, these voltages are now tabulated for reactions written as reductions, and the result for a given half-reaction is expressed as a standard half-cell reduction potential, or $E°$. Another convention has set the emf for the reduction of the hydrogen ion to H_2 at precisely zero volts (under certain highly specific conditions, including a pH equal to zero; this is the so-called standard hydrogen electrode).

For biochemical reactions, a pH of 7.0 makes more sense, so biochemists use a set of standard biochemical emf values $E'°$ for biochemical half-reactions at pH 7 but are coupled to the standard hydrogen electrode at pH 0. **Table 1–2** collects a number of such half-reactions and their reduction potentials. Because the solvent is assumed to be water, the notation (aq) can be dropped.
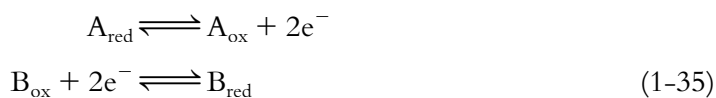
Half-reaction	E'°(volts)
$\frac{1}{2}O_2 + 2H^+ + 2e^- \rightarrow H_2O$	0.816
$Fe^{3+} + e^- \rightarrow Fe^{2+}$	0.771
$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2$	0.295
Ubiquinone $+ 2H^+ + 2e^- \rightarrow$ ubiquinol	0.045
Fumarate $+ 2H^+ + 2e^- \rightarrow$ succinate	0.031
$2H^+ + 2e^- \rightarrow H_2$ (standard conditions, pH 0)	0.000
Oxaloacetate $+ 2H^+ + 2e^- \rightarrow$ malate	-0.166
Pyruvate $+ 2H^+ + 2e^- \rightarrow$ lactate	-0.185
FAD $+ 2H^+ + 2e^- \rightarrow FADH_2$ (free solution)	-0.219
Glutathione dimer $+ 2H^+ + 2e^- \rightarrow$ 2 Reduced glutathione	-0.23
Lipoic acid $+ 2H^+ + 2e^- \rightarrow$ dihydrolipoic acid	-0.29
$NAD^+ + H^+ + 2e^- \rightarrow NADH$	-0.320
$NADP^+ + H^+ + 2e^- \rightarrow NADPH$	-0.324
Acetoacetate $+ 2H^+ + 2e^- \rightarrow \beta$-hydroxybutyrate	-0.346
α-Ketoglutarate $+ CO_2 + 2H^+ + 2e^- \rightarrow$ isocitrate	-0.38
$2H^+ + 2e^- \rightarrow H_2$ (pH 7.0)	-0.414

Table 1-2   Standard Reduction Potentials for Selected Biochemical Half-reactions

A complete redox reaction, with two half-reactions, can be written as follows:

$$A_{red} + B_{ox} \rightleftharpoons A_{ox} + B_{red} \tag{1-34}$$

This can be split into the two individual half-reactions, one written as a reduction and the other as an oxidation (subscripts indicate the state of the species, oxidized or reduced):

$$A_{red} \rightleftharpoons A_{ox} + 2e^-$$
$$B_{ox} + 2e^- \rightleftharpoons B_{red} \tag{1-35}$$

If we write a half-reaction as an oxidation, we must reverse the sign of the voltage associated with it. The change in emf, $\Delta E'^{\circ}$, for the overall reaction is simply the algebraic sum of the individual emf values for the half-reactions written so as to produce the overall balanced reaction equation:

$$\Delta E'^{\circ} = E'^{\circ}(B_{ox} \rightarrow B_{red}) - E'^{\circ}(A_{ox} \rightarrow A_{red}) \tag{1-36}$$

Note that if we write a half-reaction as an oxidation (as we did for compound A), we must reverse the sign of the voltage associated with it; this puts a minus sign in front of the term for compound A in this last equation.

The quantity $\Delta E'^\circ$ measures the overall tendency for the reaction to proceed as written under biochemical standard conditions, and it is closely connected to the standard biochemical state free energy change $\Delta G'^\circ$ for the reaction by the following relation:

$$\Delta G'^\circ = -nF\Delta E'^\circ \tag{1-37}$$

where n is the number of electrons transferred in the reaction, and F is the *Faraday constant,* equal to 96,480 Joules/volt–mole. For reactions that occur under nonstandard state conditions (mainly when the concentrations are not those of the standard state) we have
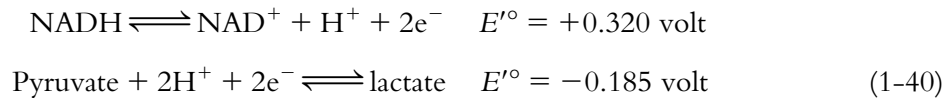
$$\Delta E' = \Delta E'^\circ - \frac{RT}{nF} \ln\left(\frac{[A_{ox}][B_{red}]}{[A_{red}][B_{ox}]}\right) \tag{1-38}$$

Also, for a single half-cell emf, we have

$$E' = E'^\circ - \frac{RT}{nF} \ln\left(\frac{[A_{ox}]}{[A_{red}]}\right) \tag{1-39}$$

Notice that the signs of $\Delta E'^\circ$ and of $\Delta G'^\circ$ are opposite to each other. The more positive the emf or voltage difference, the greater the tendency for the reaction to proceed.

A good biochemical example is the reduction of pyruvate to lactate, as NADH (nicotinamide adenine dinucleotide; see Chapter 8) is oxidized to NAD^+. The two half-reactions and their standard emfs are

$$NADH \rightleftharpoons NAD^+ + H^+ + 2e^- \quad E'^\circ = +0.320 \text{ volt}$$

$$\text{Pyruvate} + 2H^+ + 2e^- \rightleftharpoons \text{lactate} \quad E'^\circ = -0.185 \text{ volt} \tag{1-40}$$

(note the sign of the emf for oxidation of NADH) and the overall reaction is

$$\text{Pyruvate} + NADH + H^+ \rightleftharpoons \text{lactate} + NAD^+ \quad \Delta E'^\circ = +0.135 \text{ volt} \tag{1-41}$$

The standard biochemical reduction potential change here is $+0.135$ volt. The sign indicates that under standard conditions the reaction will be spontaneous. Using Equation 1-37, the standard biochemical free energy change for this reaction is -26.9 kJ/mol, which indicates a substantial tendency for the reaction to occur spontaneously under standard conditions.

Common Biochemical Conventions

Biochemists use a number of conventions in writing reaction equations, mainly for convenience and to emphasize points that are important in understanding the biochemistry. These conventions can differ from what regular chemists would write, because of the different views on what is important to represent in the equation.

- Biochemical equations (especially in diagrams) are often not balanced with respect to charge, and electrical charges on ionic species are often ignored.
- The complexation of certain species with metal ions (such as Mg^{2+}, Ca^{2+}, and Cl^-) is typically not shown, although it certainly occurs to an appreciable extent inside living systems.

Figure 1-8   Curved arrows show the participation of auxiliary species in a biochemical reaction.

- The state of titration of phosphate groups is often ignored; at pH values near 7, a mixture of different phosphate species occurs (e.g., PO_4^{3-}, HPO_4^{2-}, $H_2PO_4^-$), and the species is simply written as P_i (inorganic phosphate). The state of titration of phosphoryl moieties attached to organic compounds is likewise often ignored.

- Reactions often show a curved arrow to indicate the uptake and release of some auxiliary species. An example is the phosphorylation of the sugar glucose by the enzyme hexokinase, shown in **Figure 1-8**. Here adenosine triphosphate (ATP) enters the reaction and is converted to the diphosphate form ADP as its terminal phosphoryl group is transferred onto the glucose molecule.

- Because of the long names of many biochemicals, abbreviations are very often used (e.g., F for fructose, as long as it will not be confused with fluorine). These can be cryptic at times, but the context should help in deciphering what is meant by a particular abbreviation.

QUESTIONS FOR DISCUSSION

1. **Table 1-3** contains a list of chemical and physical processes for systems undergoing a change from an initial state to a final state. Decide whether the changes in the system are spontaneous. If they are spontaneous, explain the spontaneity in terms of entropy and order/disorder changes in the system. Also consider the questions contained in the table.

2. The formula for the free energy change in chemical equilibria, Equation 1-20, shows how the quantity ΔG depends on the concentrations of reactants and products. This formula can be adapted to physical processes, such as the transport of a solute over a difference in concentration between two regions:

$$\Delta G = RT \ln\left(\frac{C_f}{C_i}\right) \tag{1-42}$$

   where C_f is the concentration in the final state, and C_i is the concentration in the initial state. Calculate the value of ΔG for glucose transport into a cell across the plasma membrane, where the glucose concentration is 5 mM outside (initial state) and 1 mM inside (final state). Why isn't there a term for $\Delta G°$ in this formula?

3. A total of 30.5 kJ/mol of free energy is needed to synthesize ATP from ADP and P_i under biochemical standard state conditions. The actual physiological concentrations of reactants and products are, however, not at 1 M. Calculate the free energy needed to synthesize ATP if the physiological concentrations are [ATP] = 3.5 mM, [ADP] = 1.50 mM, and [P_i] = 5.0 mM.

4. Pyruvate can be reduced to lactate at the expense of oxidation of $FADH_2$ to FAD. Combine the two half-reactions to give a balanced spontaneous overall reaction, and then compute the biochemical standard–state free energy change for this reaction.

Initial State of System	Process	Final State of System
One gram of sucrose and a glass of 100 milliliters of water at room temperature	The sugar is poured into the glass of water.	A 1% solution of sucrose
An ice cube, weighing 20 grams, at $-10°C$; a benchtop at room temperature (25°C)	The ice cube is placed on the benchtop.	A puddle of 20 milliliters of water on the benchtop, at 25°C
A puddle of 20 milliliters of water on a lab benchtop, at 25°C	The puddle is allowed to stand.	A dry benchtop, and a slightly more humid lab atmosphere (Why?)
A fragile china cup on a benchtop, at room temperature (25°C)	The cup is pushed off the benchtop.	The cup hits the floor and breaks. The temperature of the cup fragments and the floor rises very slightly. (Why?)

Table 1-3   Chemical and Physical Processes for Question 1

5. The oxidation of malate to oxaloacetate can be coupled to the reduction of NAD^+ to NADH:

$$Malate + NAD^+ \rightarrow oxaloacetate + NADH + H^+$$

a. Use Table 1-1 to verify that the biochemical standard-state free energy change here is approximately +29 kJ/mol.

b. Mitochondrial concentrations of the reactants are as follows:

Oxaloacetate	5.0×10^{-6} mol/L
Malate	1.1×10^{-3} mol/L
NAD^+	7.5×10^{-6} mol/L
NADH	9.2×10^{-7} mol/L

What is the free energy change in the mitochondrion for the reaction given earlier in this question?

6. The diet of a typical 70-kg adult human male in the United States may include a caloric intake of approximately 2500 Calories per day. The dietary Calorie (note the capitalization; 1 Cal is equal to 1000 calories) is equivalent to 4.185 kJ. Assume the efficiency of converting food energy to ATP is 50%. Calculate the weight of ATP synthesized by this adult per day (see Question 3 for the free energy needed to synthesize ATP). Compare this figure to the body weight and comment.

REFERENCE

G. G. Hammes. (2000). *Thermodynamics and Kinetics for the Biological Sciences*, Wiley–Interscience, New York.