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Thermodynamics

One does not understand thermodynamics, one can only know it
—Jan Hermans

As a biophysicist, you must know thermodynamics
—Barry Lentz

1.1 INTRODUCTION

Thermodynamics describes the relation between different forms of energy, their interconversion, and the exchange of energy between physical systems. Thermodynamics is applicable to energy management in all situations. It was developed in the context of the industrial revolution, with an important goal being the design of more efficient versions of newly invented machines, first the steam engine, later such devices as the internal combustion engine and the refrigerator. Thermodynamics also describes how the *total energy* of a system is partitioned between *useful energy* (available to do work) and *wasted energy* (that associated with the randomness of a system), and establishes conditions that must be met for a system to not undergo spontaneous change, that is, to be at equilibrium. The branch of thermodynamics that concerns us most deals with the energetics of chemical systems and systems containing interacting molecules. However, thermodynamics does not formally assume a molecular nature of matter, but is simply a formal description of the relationship between work, heat, and energy. Three laws, which are based on "everyday" observations, form the foundation of thermodynamics. The surprisingly profound conclusions that follow from these laws have been verified extensively.

Thermodynamics strikes many as a boring formalism, seemingly devoid of the interesting intellectual content of quantum and statistical mechanics. Indeed, one

can think of thermodynamics as a bookkeeping tool that tracks otherwise obscure relations between different forms of energy storage, and in doing so keeps the biophysicist from many an egregious error. At the same time, the very fact that a complex framework of relations can be built on a few fundamental laws should be a source of marvel, as is the insight of the scientists who developed thermodynamics in the nineteenth century. The development of thermodynamics on the basis of a few laws resembles the development of mathematics from a small number of axioms. However, the axioms of mathematics can be chosen by the mathematician, while the laws of thermodynamics are based on observations of our physical world, and these laws could be changed only on the basis of radically new experimental findings.

This chapter is not a textbook on thermodynamics; it is presumed that students using this book have had an introductory physical chemistry course that treated chemical thermodynamics in some detail. It is also presumed that many who have had such a course do not remember it very well. Thus, our goal is to review briefly the fundamental *concepts* of thermodynamics and then to give them a context in terms of solutions of macromolecules and their interactions with other molecules.

1.2 THE FUNDAMENTAL POSTULATES OR LAWS OF THERMODYNAMICS

1.2.1 Systems

A system is a part of the universe in which we have interest for a particular problem. In biology, it is often some collection of molecules. It is separated by some boundary from the rest of the universe (its *surroundings*; Fig. 1.1).

Open systems exchange energy and matter with their surroundings.

Closed systems exchange energy but not matter with their surroundings.

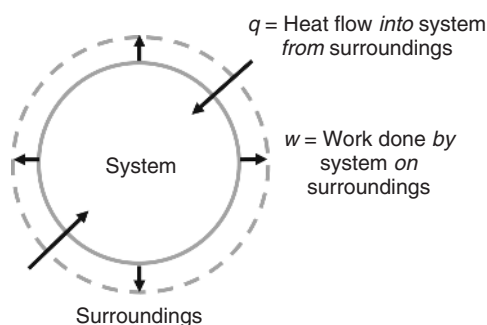*Isolated systems* exchange neither energy nor matter with their surroundings.

FIGURE 1.1 A *closed system* exchanges energy in the form of heat and work but not matter with its surroundings. If no heat is exchanged ($q = 0$), the process is *adiabatic*. An open system can also exchange matter with the surroundings.

1.2.2 States and State Functions

The *state* of a closed system can be changed by the exchange of energy with the outside (surroundings), and can also change spontaneously. *Thermodynamics is concerned with the* equilibrium *states that are the outcome of spontaneous change*, and with the processes *by which change from one equilibrium state to another occurs*. Many equilibrium states are *metastable*; for example, a mixture of oxygen and hydrogen gases is stable, but can be ignited to explode (spontaneous change) and form water vapor. A state is defined in terms of characteristic properties, such as temperature, density, pressure, and chemical composition. The energy of a state is one of its fundamental characteristics, and is therefore called a *state function*. By definition, a state function depends on certain properties of a system such as the number of molecules composing it (N), the volume (V), perhaps the pressure on the system, and a very interesting property called temperature (T).

Observation tells us that not all these properties are independent; that is, if we set the values of some, then others are fixed by these assignments. Aside from the extensive (how big the system is) property N, the thermodynamics of a closed system are defined by two additional properties, which are referred to as *independent* variables of the system. All other properties of the system, including its state functions, are *dependent* properties of the system. There is nothing holy or sacrosanct about an independent variable, these are defined by the experimental conditions we use to observe the system and are those *properties over which we exercise control*. However, once we choose these independent properties or variables, the values of the state functions for the system are defined and can be obtained by the laws of thermodynamics. Thermodynamic state functions depend only on the values of these independent properties and not on how the system reached this state.

1.2.3 The First Law and Forms of Energy—Energy a State Function

Classical mechanics introduces three forms of energy: kinetic energy, potential energy, and work. *Kinetic energy* is evident in an object's motion. The *potential energy* of an object is latent energy that allows the object to do work or to acquire kinetic energy. *Work* has associated with it a *force* and a *path*; force acting along the path changes the potential energy and/or the kinetic energy of an object. *Thermodynamics* considers an additional category of energy, *heat*, and is concerned solely with the relationships between and interconversion of heat, work, and energy. We stress that thermodynamics does not distinguish between kinetic and potential energy, nor does it bother itself with motion—these issues are totally the venue of mechanics. These two independent areas of physics came together only in the latter half of the nineteenth century through the collaboration of the Scottish mathematician James Scott Maxwell (kinetic theory of gases) and the Austrian physicist Ludwig Eduard Boltzmann (the Boltzmann distribution) to develop a statistical description of the average speed of molecules in a gas. This is the Maxwell–Boltzmann distribution, which forms the basis of *statistical mechanics* (also called *statistical thermodynamics*; see Chapter 5).

The First Law of thermodynamics states that the energy of a system and its surroundings is conserved. The "everyday" experience of doing *work* to move a mass up a hill against the force of gravity leads to the concept that the work done is converted into *potential energy*, which remains hidden, until the object is released and rolls back down. In the absence of friction, energy is conserved during the rollback down the hill, and the object acquires a new form of energy, *kinetic energy*. There are many familiar examples of converting energy into work or heat or capturing work as energy. Several are illustrated in Fig. 1.2. The invention of the steam engine stimulated the development of thermodynamics. In it and its modern-day replacement, the internal combustion energy, the energy released in the form of heat produced when hydrocarbons react with oxygen to form CO_2 and H_2O, causes this gas mixture (or water vapor in the steam engine) to expand and this produces pressure–volume work (PV work) on a piston that is captured as the work needed to increase the kinetic energy of a vehicle. Thus, by virtue of the First Law, *heat* also must be considered a form of energy. In this example, a chemical reaction liberates energy in the form of heat. By virtue of the First Law, the *chemical* (or *internal*) *energy* of the reactants must decrease by a like amount. Similarly, a charged battery possesses potential energy that is released when electrons are allowed to flow through a wire to drive an electric motor that
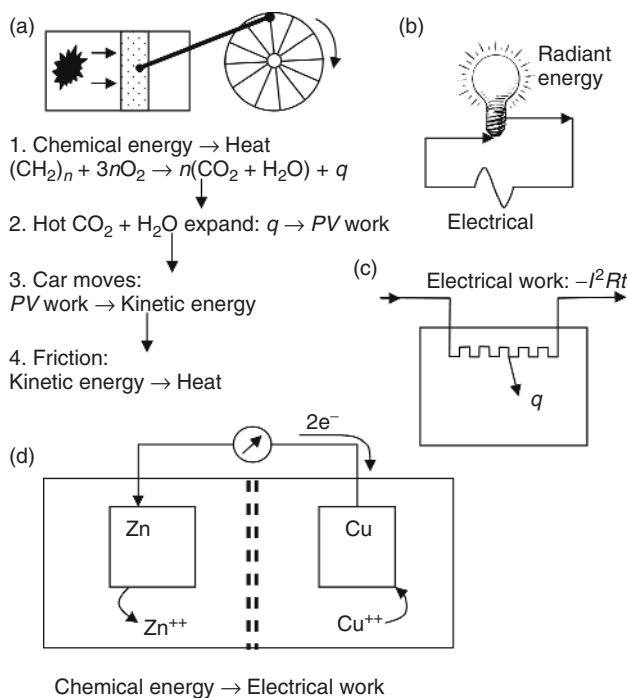

FIGURE 1.2 Examples of interconversion of different forms of energy. (a) Internal combustion engine, (b) light bulb, (c) electric water heater, and (d) flashlight battery.

performs work. This process can also be used to produce heat by running a current I through a resistor R.

Heat has been traditionally defined in terms of the amount needed to change the temperature of $1\,g$ of water by $1\,°C$, the calorie. In modern usage, heat is treated as energy and expressed in appropriate standard international units of energy. Thus, the calorie is now defined by the equation $1\,cal = 4.184\,J$. Physical scientists preferentially use the standard units, while nutritionists have adhered to the calorie.

To make a more formal definition of the First Law, we note that the total energy of a closed system can be changed by two means: by *work* (work done by the system) or by the transfer of *heat* into the system. The internal energy *decreases* as a result of work w done *by* the system, and *increases* as a result of heat q transferred *into* the system. Thus, the First Law states that the change of the internal energy is[1]

$$\Delta U_{a \rightarrow b} = U_b - U_a = q - w. \qquad (1.1)$$

The First Law requires *the internal energy*, U *to be a state function*, that is, to depend only on the internal state of a system, as determined by its characteristics such as temperature, volume, and composition

$$\Delta U_{a \rightarrow b} = -\Delta U_{b \rightarrow a}$$
$$q - w = -(q' - w'). \qquad (1.2)$$

(the primed quantities are for a process that produces state a from state b). Were the First Law not to hold, it would be possible to build a perpetual motion machine ("of the first kind"), a device that indefinitely continues to produce energy, a situation that all our experience tells us is impossible.

We then have for any small change in the system that

$$dU = \partial q - \partial w. \qquad (1.3)$$

Work and heat are definitely *not* state functions, as one can raise a system's temperature by transferring into it heat from a bath, but also by performing work, for instance, electrical energy applied to an electrical heating element (Fig. 1.2c), or mechanical energy applied by stirring; by writing ∂q and ∂w (rather than dq and dw), we indicate that q and w are not state functions.

We now understand heat as kinetic and potential energy that is distributed *randomly* over the atoms making up any chemical or physical system, as described in detail in chapters 5 and 6 on Statistical Thermodynamics. Note however, that this insight was unavailable when thermodynamics was first developed as a science.

1.2.4 Temperature and the Ideal Gas or Kelvin Scale

Simple "everyday" experience tells us that two systems in contact through a wall that allows the flow of heat will change until they reach *thermal equilibrium*, and if
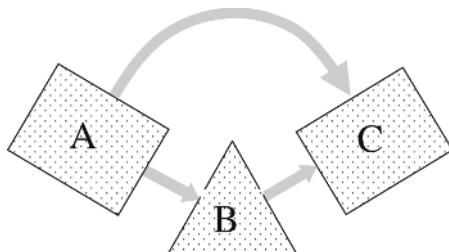
FIGURE 1.3 If heat flows from A to B and from B to C, then (i) heat will also flow from A to C, and (ii) A is said to be hottest and have the highest temperature, and C is coolest and has the lowest temperature. When heat flow ceases, the systems are said to be in thermal equilibrium with each other, are equally "hot" and have the same temperature.

two systems are in thermal equilibrium with a third, they are in thermal equilibrium with each other (Fig. 1.3). We say the two systems have a common property called *temperature*. If two systems are not in thermal equilibrium, they are at different temperatures. Heat flows from high to low temperature, and temperature orders "hotness." (This is sometimes called the *Zeroth Law of Thermodynamics*.) A rise of a system's energy content in the form of heat corresponds to a rise in the temperature.

Early scales of temperature (such as Celsius' scale) depended on two sharply defined experimental points ($0\,°C$ as the melting temperature of ice, $100\,°C$ as the boiling temperature of water at 1 atm pressure) and interpolation assuming linear expansion of liquid volume (e.g., mercury).

The Kelvin temperature scale is set by relating temperature to physical properties of an ideal gas, as follows.

Because, in the gaseous state, molecules interact only slightly, the gaseous state is a natural starting point for theories of matter. The *ideal or perfect gas* is a *hypothetical state in which the molecules do not interact at all*. One approximates an ideal gas by diluting a real gas, that is, by increasing its volume and thus lowering its pressure. Thermal motions in an ideal gas consist of internal vibrations within each molecule, and of rotational and translational motions of the gas molecules. As the molecules do not interact (except by rare collisions), the thermal energy does not depend on the volume occupied by a sample.

It is known *from observation* (Boyle's Law) that dilute gases, which we expect to be close to ideal, follow a simple relationship between pressure and volume

$$PV = \text{constant}, \tag{1.4}$$

and that this product increases with temperature. By now setting this proportional to the absolute temperature, that is,

$$PV = Nk_{\mathrm{B}}T, \tag{1.5}$$

where N is the number of molecules, one obtains the Kelvin scale; the value of the proportionality constant, k_B, called *Boltzmann's constant*,[2] is fixed by retaining the $100°$ interval of the Celsius scale.

1.2.5 The Second Law: Real and Reversible Processes

Three simple examples suffice to show that work is not a state function. A viscous liquid can absorb energy by transfer of heat from warmer surroundings, or by work in the form of agitation; in either case the end result is a resting, but warmer liquid. A gas can expand against a piston and in so doing perform work on the environment, or it can expand into a vacuum, and perform no work (Fig. 1.4b). Heat generated by combustion of fuel can be used to drive machinery (steam or internal combustion engine, steam turbine), but the heat can also be used to warm the environment directly, without any work being generated.

In the first example, *work is turned into heat and "lost,"* and everyday experience shows many such instances of friction. In the second and third examples, the *ability to perform a certain amount of work is lost*.

The Second Law reflects this experience by stating that in any real process some ability to perform work is lost. The magnitude of work performed by a system in a real process is less than the maximum possible, and, if a fixed amount of work is performed on the system, then the system's ability to perform work is increased by a smaller amount (and, perhaps, not increased at all).

The Second Law states that it is not possible to create a machine that, for example, captures heat to do an equivalent amount of work (e.g., PV work due to expansion), and then applies this work to generate a high energy state (e.g., an electrical potential) that can then be used to generate an equivalent amount of heat (I^2R heating) that can be used to do an equivalent amount of work, etc. As energy is conserved according to the First Law, if each of these processes were completely efficient, we would have a perpetual motion machine ("of the second kind"), which, by our experience, is not possible.

A quantitative statement of this law requires that we distinguish between *reversible* and *irreversible* processes.[3] In brief, if a *reversible process*, say A → B
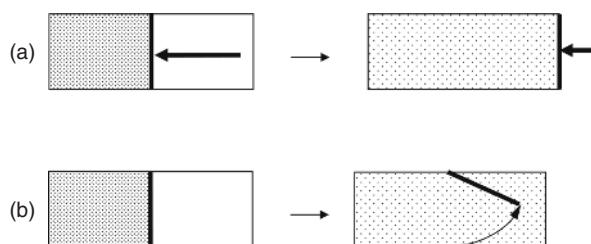

FIGURE 1.4 (a) When a gas expands against a force (indicated by the arrows) acting on a piston, the gas performs so-called *PV* work. (b) When the gas is allowed to expand into a vacuum by removal of a partition, no work is performed.

is repeated in reverse following exactly the same path, and the complete process is A → B → A, then there is no *net* exchange of work or heat with the environment, that is, any heat or work expended in running the system through the first leg is recovered during the second leg, and vice versa. However, reversible change is an idealization that we can never achieve in real processes. We approximate a reversible process by carrying out the change very slowly, that is, in infinitesimal steps. While a reversible A → B → A process (e.g., a swinging pendulum) can continue indefinitely, such a process can only be imagined and never be achieved in a real system. Thus, real processes are *irreversible* and behave according to the Second Law.

1.2.6   New State Functions: Free Energy and Entropy

We have seen that the energy of a system is defined by its current state (pressure, volume, temperature, and contents), and not by its history; on the other hand, work and heat are definitely *not* state functions. However, we *can* now ask *how much work might be performed by a system in an optimally chosen process, and thereby define a new state function, the free energy*, A, *the process to take place at constant temperature*.

Because total energy is conserved, a change in A cannot exceed the concomitant change of the internal energy, U of the system. The function $U-A$, is of course also a state function, which is written as TS, where S is our *second new state function, the entropy*

$$A = U - TS. \qquad (1.6)$$

Naturally, this will serve to determine A only if we can determine S, and this is where the (famous) Carnot cycle comes into play.[4]

We will not describe the Carnot cycle here as it involves steps that are adiabatic, making the description obscure. Instead, we describe a simple scheme that employs compression and expansion of an ideal gas in a heat pump (a machine that exchanges work and heat flow) as shown in Fig. 1.5. As mentioned, the (N) molecules in an ideal gas do not interact, the internal energy is independent of P or V, which, themselves, are related by the gas law (Eq. 1.5).

We now pick a temperature, T_1 and a volume V_1 and compress the gas from that starting point to a smaller volume V_2, by moving a piston. When the moving piston collides with the gas molecules, it increases their velocities, and thus the internal energy increases and the temperature rises. (The increase of the internal energy exactly equals the amount of work done by pushing the piston.) However, if the system is placed in a heat reservoir[5] at constant temperature T_1, then the excess heat, q_1 will flow from the gas into the heat bath, and the energy of the gas does not change. Because the internal energy of the gas does not change, the work done on the gas is equal to the amount of heat transferred. Because, according to our definition of the free energy, the work done in this process can be equated with
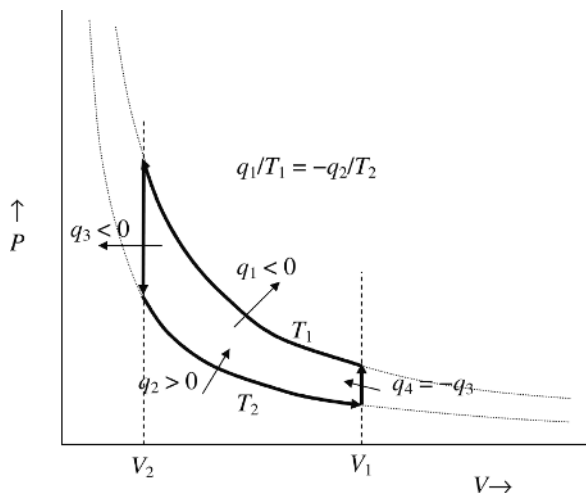
FIGURE 1.5 Four-step cycle (not a Carnot cycle) of isothermal compression, cooling by contact with a bath, isothermal expansion and heating by contact with a bath, applied to an ideal gas. Arrows indicate heat flow into or out of the system in each step.

the free energy change, we have

$$\Delta A_1 = -\int P dV = -Nk_B T_1 \ln(V_2/V_1) = -q_1$$

$$\Delta A_1 = \Delta U - q_1 = \Delta U - T_1\frac{q_1}{T_1}, \tag{1.7}$$

ΔU being zero.

We can compress the gas from V_2 to V_1 at another temperature, T_2, which gives the same equation relating ΔA_2, T_2, V_1/V_2, q_2, and ΔS_2 and thus

$$\Delta S_2 = -\Delta S_1.$$

We can then construct the cyclic process of Fig. 1.5 by compressing from V_1 to V_2 at T_1, then cooling to T_2, expanding back to V_1 at T_2, and heating back up to T_1. Because the internal energy is independent of P and V, the heating and cooling steps produce/require exactly opposite changes of energy, $q_3 = -q_4$. In a cyclic process, the net change of any state function is zero. The change in energy for the entire cycle is obviously zero.

$$\Delta U_1 + \Delta U_2 + \Delta U_3 + \Delta U_4 = 0 + 0 + q_3 + q_4 = 0. \tag{1.8}$$

Now, by *defining the entropy as the integral of the heat exchanged divided by the temperature in a reversible (or quasi-static) process*,

$$dS = \frac{dq}{T}$$

$$S = \int \frac{dq}{T}, \tag{1.9}$$

the entropy changes in the heating and cooling steps are also equal and opposite,[6] so that for this cycle

$$\Delta S_{\text{cycle}} = \Delta S_1 + \Delta S_2 + \Delta S_3 + \Delta S_4 = 0. \tag{1.10}$$

It is easily shown that this equation holds for any cycle that combines two such cycles that share a part of their circumference, and as any closed cycle can be decomposed in smaller cycles of the form of Fig. 1.5, it follows that the *entropy as defined by* Eqs. 1.6 and 1.9 *is a state function for ideal gas systems*. This can then be generalized to any other system by an argument that invokes the Second Law for this system thermodynamically coupled to an ideal gas.[4]

The *work* done to run the cycle $\left(-\int PdV\right)$, which equals the area inside the closed curve in Fig. 1.5), is not zero, and this energy ends up as the difference between the *heat* given off in the compression leg and that taken up in the expansion leg of the cycle. When run in the indicated direction, each cycle transfers heat from the cooler heat reservoir at T_2 to the warmer heat reservoir at T_1 in the amount $q_2 = -\Delta A_2$, and thus acts as a (completely impractical) heat pump.

1.2.7 Entropy Tends to Increase

The transfer of heat between two systems at different temperatures occurs in one direction and is an irreversible process. The entropy of the colder system increases by dq/T_1, and that of the warmer decreases by dq/T_2; the net change is positive, and in the absence of performance of work

$$dS = dq\left(1/T_1 - 1/T_2\right) > 0.$$

Many processes produce a rise or fall in the temperature of the system. In order to maintain the system at a constant temperature, the necessary heat is exchanged with a heat reservoir, and one sees that this cannot be done reversibly without an increase of the entropy (of system plus reservoir) unless the temperature of the heat reservoir is at all times exactly the same as that of the system (which would require an infinitely slow process).

In a system kept at constant temperature by contact with a heat reservoir (an isothermal system), a real, irreversible process is one in which the work done by the system is less than the maximum possible, that is, is less than the decrease in free energy,

$$-dA \geq \partial w. \tag{1.11}$$

Given the relation between A, U, and S, we then have

$$-(dU - TdS) \geq \partial w$$

$$dS \geq \frac{dU + \partial w}{T} = \frac{\partial q}{T}, \tag{1.12}$$

and one sees that the entropy increases by *more* than the amount corresponding to the heat transferred into or out of the system.

The general conclusion is that in all real processes,[7] because of the Second Law, *the entropy of the universe (the system plus its reservoir) increases* ($dS_U > 0$). This is a familiar statement of the Second Law. It is also the least transparent statement, although it follows completely from the more common-sense statement that heat does not flow from a lower to a higher temperature system.[8]

1.2.8 The Second Law and Equilibrium

A fundamental concept of thermodynamics is that of *equilibrium, a state from which spontaneous change* (i.e., not induced by an exchange of work or heat) *is not possible*. The majority of applications of thermodynamics in molecular biophysics consider conditions needed to establish equilibrium and the deviations from equilibrium if these conditions are not met.

For an isolated system (no w or q), the Second Law requires that entropy of a system increases (irreversible or spontaneous change) or remains the same (reversible change). Thus, at equilibrium, the entropy of an isolated system tends to become larger, until it reaches a maximum, which can be expressed as

$$dS \geq 0 \text{ in the absence of exchange of heat or work.}$$

We can now see that the entropy plays a critical role in thermodynamics, as it allows us to *express the equilibrium condition as the maximum of a state function. The rest is algebra.*

If the system is able to exchange heat or work with a reservoir, we have

$$\partial w = \partial q - dU \leq TdS - dU$$

$$dU \leq TdS - PdV - \partial w', \tag{1.13}$$

where w has been separated into work for expansion (PV work) and other work, w'.[9] In this expression the differential of the state function U is expressed as a function of the independent variables S and V, and is the state function that is minimized when entropy and volume are constant ($dS = dV = 0$; and no other work is done). This expresses the *combined First and Second Laws*. The equal sign holds for reversible processes. However, a condition of constant entropy is not easily realized experimentally, and its meaning is difficult to grasp. In the next section, we discuss which state functions are minimized when temperature, rather than entropy, is constant, and when work is done (pressure, not volume, being constant).

1.3 OTHER USEFUL QUANTITIES AND CONCEPTS

1.3.1 Gibbs and Helmholtz Free Energies and Enthalpy

We now have the basis for the normal treatment of thermodynamics. In this treatment, it is convenient to define two new functions, in addition to U, S, and A. These functions are the enthalpy, H and the Gibbs free energy, G; the previously introduced state function A is distinguished as the Helmholtz free energy. (Older literature tends to use the symbol F for A, while some still use F for G.) We then have

$$H = U + PV$$
$$A = U - TS$$
$$G = A + PV = H - TS. \tag{1.14}$$

By combining Eqs. 1 and 1.14 we get the following expressions for dH, dA, and dG,[10]

$$dH \leq TdS + VdP - \partial w'$$
$$dA \leq -SdT - PdV - \partial w'$$
$$dG \leq -SdT + VdP - \partial w'. \tag{1.15}$$

This allows us to identify state functions that are minimized under three different sets of equilibrium conditions:

1. At equilibrium, the Helmholtz free energy, A, is a minimum at constant T and V.
2. At equilibrium, the enthalpy, H, is a minimum at constant S and P.
3. At equilibrium, the Gibbs free energy, G, is a minimum at constant T and P.

As a corollary,

1. A is the state function defined by independent variables T, V, and composition N_i.
2. H has the same form as U except that its independent variable is P instead of V.
3. G is the state function defined by independent variables T, P, and composition N_i.

Within narrow margins, biological systems operate at constant temperature. Not surprisingly, applications of thermodynamics in molecular biophysics rely on state functions A and G whose minima define the equilibrium condition at constant temperature. Specifically, spontaneous processes at constant T result in decreases

in free energy until they reach equilibrium, at which point the free energy change is zero:

$$\delta A \leq 0, \quad \text{in a system held at constant volume and temperature,} \qquad (1.16)$$

$$\delta G \leq 0, \quad \text{in a system held at constant pressure and temperature;} \qquad (1.17)$$

for each, the equal sign holds at equilibrium.

At equilibrium, A or G is at a minimum, and any perturbation of the conditions of the system causes an increase of A or G,

$$dA \geq 0 \quad \text{for perturbations from equilibrium at constant } V, \qquad (1.18)$$

$$dG \geq 0 \quad \text{for perturbations from equilibrium at constant } P. \qquad (1.19)$$

As biophysical systems are studied at constant pressure, the Gibbs free energy is normally the more useful. Note however that, in most experiments with solutions, the changes in PV are so small that a distinction between A and G has no noticeable effect.[11]

1.3.2   Chemical Potential

A fundamental concept of thermodynamics, and certainly one of the most useful in chemistry, is that of *chemical potential*, which is crucial to a description of the thermodynamics of mixtures (and hence of solutions). It is defined as the partial derivative of the Gibbs free energy with respect to the amount, N_i of component i,[12] while T, P, and the amounts, $N_{j \neq i}$ of all other components are taken as defined (i.e., constant or fixed), so that

$$\mu_i = \left(\frac{dG}{dN_i} \right)_{T,P,N_{j \neq i}} = \left(\frac{dA}{dN_i} \right)_{T,V,N_{j \neq i}}. \qquad (1.20)$$

The chemical potential describes the intrinsic or *intensive free energy* that a substance has in a mixture (or in a pure state).

If we are describing an open system, we must extend the total differentials of U, A, H, and G (Eqs. 1.13 and 1.15), which reflect the combined First and Second Laws, in order to take into account the dependence on n_i, and this gives

$$dU \leq TdS - PdV - \partial w' + \sum \mu_i dN_i$$

$$dH \leq TdS + VdP - \partial w' + \sum \mu_i dN_i$$

$$dA \leq -SdT - PdV - \partial w' + \sum \mu_i dN_i$$

$$dG \leq -SdT + VdP - \partial w' + \sum \mu_i dN_i, \qquad (1.21)$$

where ∂w is any additional work done by the system.

1.3.3 Fundamental Relationships Between State Functions

Our statement of the combined First and Second Laws (Eq. 1.13) expresses dU as an exact differential with respect to the independent variables V and S. Several properties of exact differentials are quite useful in thermodynamics. (See Section A9.7, "Useful relations between partial differential quotients.")

Single derivatives. The first is that the total differential of any function is given by the sum of the partial derivatives of that function with respect to each independent variable of the function times the differentials of the variable (Eqs. A9.16 and A9.17).

Using the definition of a total differential, we get the following expressions for T, P, and μ_i in terms of partial derivatives of U

$$T = \left(\frac{\partial U}{\partial S}\right)_{V,N_i}; \quad P = -\left(\frac{\partial U}{\partial V}\right)_{S,N_i}; \quad \mu_i = \left(\frac{\partial U}{\partial N_i}\right)_{S,V,N_j}. \qquad (1.22)$$

Application to the total differentials of the other state functions we have defined (G, H, A; see Eq. 1.15) gives the following additional relations between state variables and state functions

$$S = -\left(\frac{\partial G}{\partial T}\right)_{P,N_i}; \quad V = -\left(\frac{\partial G}{\partial P}\right)_{T,N_i}; \quad \mu_i = \left(\frac{\partial G}{\partial N_i}\right)_{T,P,N_j}$$

$$T = \left(\frac{\partial H}{\partial S}\right)_{P,N_i}; \quad V = \left(\frac{\partial H}{\partial P}\right)_{S,N_i}; \quad \mu_i = \left(\frac{\partial H}{\partial N_i}\right)_{S,V,N_j}$$

$$S = -\left(\frac{\partial A}{\partial T}\right)_{V,N_i}; \quad P = -\left(\frac{\partial A}{\partial V}\right)_{T,N_i}; \quad \mu_i = \left(\frac{\partial A}{\partial N_i}\right)_{T,V,N_j}. \qquad (1.23)$$

Double derivatives. The second property that we can exploit is that the order of partial differentiation can be switched according to the Euler chain rule (Eq. A9.13). For example,

$$\left(\frac{\partial}{\partial V}\left(\frac{\partial A}{\partial T}\right)_V\right)_T = \left(\frac{\partial}{\partial T}\left(\frac{\partial A}{\partial V}\right)_T\right)_V$$

$$\text{i.e., } \left(\frac{\partial S}{\partial V}\right)_T = -\left(\frac{\partial P}{\partial T}\right)_V. \qquad (1.24)$$

This is a so-called Maxwell relation. The following Maxwell relations (in which S does not appear) are particularly useful,

$$\left(\frac{\partial V}{\partial N_i}\right)_{P,V} = -\left(\frac{\partial \mu_i}{\partial P}\right)_{V,T}$$

$$\left(\frac{\partial P}{\partial N_i}\right)_{V,T} = -\left(\frac{\partial \mu_i}{\partial V}\right)_{P,T}$$

$$\left(\frac{\partial \mu_i}{\partial N_j}\right)_{N_i,T} = \left(\frac{\partial \mu_j}{\partial N_i}\right)_{N_j,T}. \tag{1.25}$$

Multiple variables. As noted, what we call "independent variables" are not really independent but are what we choose to control, and control does not always mean that we keep the independent variable constant. For example, P and V might both vary, while we control how V varies, and we must then consider how state functions vary when both P and V vary. This is again accomplished using additional rules about differentials.

As an example of application of Eq. A9.15, consider the internal energy, U, which is a function of both V and P. For example, we may ask how U depends on P when V also varies, which is formally expressed by

$$\left(\frac{\partial U}{\partial P}\right)_T = \left(\frac{\partial U}{\partial P}\right)_V + \left(\frac{\partial U}{\partial V}\right)_P \left(\frac{\partial V}{\partial P}\right)_T. \tag{1.26}$$

The first term on the right is zero because no PV work is done at constant V. Substituting the expression for $(\partial U/\partial V)_P$ derived earlier (Eq. 1.22), we get

$$\left(\frac{\partial U}{\partial P}\right)_S = P\left(\frac{\partial V}{\partial P}\right)_T = P\kappa, \tag{1.27}$$

where κ is the isothermal compressibility.

Temperature dependence of energy and free energy. The temperature dependence of both the energy and of the enthalpy is called a *specific heat*

$$\left(\frac{\partial U}{\partial T}\right)_{V,N_i} = C_V \quad \text{and} \quad \left(\frac{\partial H}{\partial T}\right)_{P,N_i} = C_P; \tag{1.28}$$

C_V is the specific heat at constant volume, and C_P the specific heat at constant pressure. The temperature dependence of the free energy can be expressed as

$$\left(\frac{\partial A}{\partial T}\right)_V = -S \quad \text{and} \quad \left(\frac{\partial G}{\partial T}\right)_P = -S, \tag{1.29}$$

or instead as[13]

$$\frac{\partial(A/T)}{\partial(1/T)} = U \quad \text{and} \quad \frac{\partial(G/T)}{\partial(1/T)} = H. \tag{1.30}$$

Thus, a complete knowledge of A or G implies a knowledge of all thermodynamic functions.

1.3.4   The Gibbs–Duhem Equation and Equilibrium

At fixed intensive variables T, P, and μ_i,[14] we can integrate the exact differential for dG

$$dG = -SdT + VdP + \sum \mu_i dN_i \tag{1.31}$$

to obtain

$$G = \sum N_i \mu_i. \tag{1.32}$$

Note that this relationship between the free energy and the chemical potential of all species applies only for the Gibbs free energy at fixed T and P. If we take the total differential of this, we obtain

$$dG = \sum N_i d\mu_i + \sum \mu_i dN_i. \tag{1.33}$$

If we equate the total differential of G from this equation with the total differential form of dG given in Eq. 1.21 we obtain the *Gibbs–Duhem equation*[15]

$$\sum N_i d\mu_i = VdP - SdT, \tag{1.34}$$

which, at constant T and P, becomes

$$\sum N_i d\mu_i = 0. \tag{1.35}$$

The usefulness of this expression will become clear as we apply it in a variety of situations. We shall see that Gibbs–Duhem relates the chemical potential of solvent to that of solutes (Eq. 1.49), defines the condition of chemical equilibrium and leads to the definition of the equilibrium constant (Eq. 1.67), and is again used in a derivation of linkage relations in Chapter 9.

1.3.5   Relation Between Heat Capacity and Other Functions

We said earlier that if A is completely known, then other thermodynamic functions are also known. But we can just as well base a knowledge of A, U, and S on a knowledge of the specific heat, C_V. The reason for doing this is that specific heat of very many systems can be measured accurately with a calorimeter.

To begin with, the energy U and the entropy S are integrals of the specific heat (heat is transferred slowly, at constant volume)

$$\Delta U = \int dq = \int dT \frac{dq}{dT} = \int dT\, C_V$$

$$\Delta S = \int \frac{dq}{T} = \int \frac{dT}{T} \frac{dq}{dT} = \int dT \frac{C_V}{T}. \tag{1.36}$$

The entropy S of all systems is set equal to zero when the absolute temperature, T, is zero. (This is the so-called *Third Law*.) Classical chemical thermodynamics

by convention sets the energy of each pure chemical element to zero when T is zero; current practice sets the energy at $T = 0$ to the quantum-mechanical ground state energy, ε_0.

The free energy is then given by these two equations

$$\Delta A = -\int S \, dT$$

$$\frac{\Delta A}{T} = \int U \, d(1/T),$$
(1.37)

where $\Delta A = A(T) - \varepsilon_0$. If the calorimetric measurements are done at constant pressure, then equivalent expressions relate C_P, H, and G.

1.4 THERMODYNAMICS OF THE IDEAL GAS

Pressure and volume of an ideal gas are related by Boyle's ideal gas law, $PV = Nk_{\mathrm{B}}T$ (Eq. 1.5). Boyle's law is an empirical relation, which we now understand to apply only when each gas molecule behaves *independently* of all the others.

Accordingly, the free energy depends on the volume according to

$$A = -\int P dV = -\int \frac{Nk_{\mathrm{B}}T}{V} dV = \mathrm{C} - Nk_{\mathrm{B}}T \ln V = Nk_{\mathrm{B}}T \ln(V/V^{\bullet})$$

$$\mathrm{C} = -Nk_{\mathrm{B}}T \ln V^{\bullet},$$
(1.38)

where the integration constant C is set by choosing a fixed reference volume $V^{\bullet}$. The volume $V^{\bullet}$ represents a *standard state*, that is, a state relative to which we can define the chemical potential of the gas at any other experimentally defined volume, V (or pressure P, related to V by the ideal gas law). In principle, the choice of standard state is arbitrary, but, in practice, convention sets the pressure of the standard state at 1 bar, and $V^{\bullet}$ then depends on T according to the ideal gas law.[16] The equation for A states that if we change either P or V away from *standard conditions*, A varies as the natural logarithm of the ratio of the new volume to the volume at standard conditions.

By differentiating the free energy A of the ideal gas in Eq. 1.38 with respect to N at constant T and V, one obtains according to Eq. 1.23 an expression for the chemical potential

$$\mu = k_{\mathrm{B}}T \ln V^{\bullet} - k_{\mathrm{B}}T \ln V$$

$$\mu = k_{\mathrm{B}}T \ln\left(\frac{V^{\bullet}}{N}\right) + k_{\mathrm{B}}T \ln \frac{N}{V} = \mu^{\bullet} + k_{\mathrm{B}}T \ln \frac{N}{V},$$
(1.39)

where the constant term $\mu^{\bullet}$ is the chemical potential of the gas at a standard state, which is here taken as $N/V = 1$; $\mu^{\bullet}$ is still a function of T. One can also express

μ as a function of T and $P = Nk_BT/V$,

$$\mu = \mu^{\bullet} + k_BT \ln(N/V) = \mu^{\circ} + k_BT \ln P. \tag{1.40}$$

The constant terms $\mu^{\bullet}$ and μ° both represent the chemical potential of the gas at the conventional standard state of a gas at 1 bar, but their values differ according to the different choice of either volume or pressure as state function.

The terms indicated with $\mu^{\bullet}$ and μ° are termed the *unitary* or *standard chemical potential* and are independent of concentration; however, their values depend on the choice of standard state. The terms in $\ln(N/V)$ and $\ln P$ (the *cratic terms*) contain the concentration dependence and are related to the entropy of the gas, which becomes greater the more the gas is dilute.

The ideal gas law applies also when the molecules in the gas are not all of the same kind, and the gas can contain a mixture of different components. We say that in an ideal gas mixture each component contributes to the total pressure as if it were the only gas occupying the volume, that is, $P = P_1 + P_2 + P_3 + \cdots$, where P_j is the partial pressure of gas j, which is X_jP, where X_j is the mole fraction of gas j in the mixture $\left(X_j = N_j/\sum_i N_i\right)$.

It is then easy to show that the equivalent expression for the chemical potential for a component of a gas mixture is

$$\mu_i = \mu_i^{\bullet} + k_BT \ln(N_i/V)$$
$$\mu_i = \mu_i^{\circ} + k_BT \ln P_i. \tag{1.41}$$

Alternatively, if we make such a mixture at constant pressure and temperature, then the volume of the mixture will be the sum of the volumes of all the gases that we mix $\left(V = \sum_i V_i = \sum_i k_BT N_i/P_i\right)$ and the total number of molecules will be $N = \sum_i N_i$. The free energy change for creating this mixture is the sum of the free energies for expanding each of the component gases from volume V_i to volume V.

$$\Delta A = -k_BT \sum_i N_i \ln(V/V_i) = k_BT \sum_i N_i \ln X_i \tag{1.42}$$

This is the *free energy of mixing* the gases at constant T and P. We note here that this same expression describes the free energy of mixing of an ideal mixture. This is a mixture in which all molecular species interact in an identical manner; thus, in a two-component system 1–1, 2–2, and 1–2 interactions are equivalent in this model system.

1.5 THERMODYNAMICS OF SOLUTIONS

Most biophysical experiments are done in solution. Fortunately, the thermodynamics of dilute solutions are relatively easy to describe on the basis of experiments that allow one to relate these to the thermodynamics of gases.

1.5.1 "Ideal" Dilute Solutions

The thermodynamics of solutions can be related to those of dilute gases by experiments that consist of equilibrating the solution with its vapor and measuring the concentration of the solute in both phases. At low concentration of solute in the liquid, the ratio between the concentration of a particular solute,[17] component 1, in solution $c_{1,s}$ and the concentration in the vapor $c_{1,v}$ is a constant whose value is found to be specific for that solute and that solvent:

$$\frac{c_{1,s}}{c_{1,v}} = \frac{(N_1/V)_s}{(N_1/V)_v} = K_{v\to s}. \tag{1.43}$$

Here, $K_{v\to s}$ is the *partition coefficient* or equilibrium constant for transferring solute from vapor to solution phase. This relation (called *Henry's Law*) holds only in the limit as the concentration of solute approaches zero, but in practice it holds over a sufficient range of concentration that accurate values of transfer equilibrium constants can be determined. In an ideal gas, the molecules are assumed not to interact with each other. In a dilute solution, we assume that solute molecules interact only with the surrounding solvent molecules but not with other solute molecules. This model is called the *ideal solution*.

We now make use of the fact that G is a minimum for the equilibrated system; consequently, transfer of a *small* amount of solute from solution to vapor or vice versa causes balancing changes in G of the gas and solution according to

$$dG = dN_1(\mu_{1,s} - \mu_{1,v}) = 0, \tag{1.44}$$

so that the two chemical potentials are equal,

$$\mu_{1,s} = \mu_{1,v}. \tag{1.45}$$

(This is true for all components in all phase equilibria.) First, substituting the expression for $\mu_{1,v}$ of the ideal gas, Eq. 1.39, and second using the proportionality of $c_{1,s}$ and $c_{1,v}$, Eq. 1.43, one obtains the following expression for the chemical potential of the solute in an ideal solution,

$$\mu_{1,s} = \mu_{1,v}^{\bullet} + k_B T \ln c_{1,v}$$
$$\mu_{1,s} = \mu_{1,v}^{\bullet} + k_B T \ln(c_{1,s}/K_{v\to s}) = \mu_{1,s}^{\circ} + k_B T \ln c_{1,s}. \tag{1.46}$$

The standard or unitary chemical potential in the vapor and solution differ; $\mu_{1,v}^{\circ}$ is the chemical potential of pure component 1 in the vapor at a concentration (particle density) $c_{1,v} = (N_1/V)_v = 1$, and $\mu_{1,s}^{\circ}$ is the chemical potential of component 1 in solution at a concentration $c_{1,s} = (N_1/V)_s = 1$.

The standard chemical potential of solute in an ideal solution is the standard chemical potential of solute in the ideal gas phase plus the free energy of transferring a molecule of solute from the vapor to the solution phase,

$$\mu_{1,s}^{\circ} = \mu_{1,v}^{\bullet} - k_B T \ln(K_{v\to s}). \tag{1.47}$$

The *value* of the standard chemical potential depends not only (as expected) on the state for which it is defined but also on the units in which the concentration is expressed.[16] Unless it is explicitly stated otherwise, one should assume that concentration units are in moles per liter, and, if we use any concentration scale other than the molarity scale, then the value of $\mu_{i,s}^{\circ}$ must be altered by subtracting $k_B T$ times the natural log of the factor that converts molarity to the new concentration unit, and, if we wish to compare the standard states of a solute in two different solutions, we must use the same units of concentration for both.

Using units of mole fraction, the chemical potential of an ideal solution is

$$\mu_{1,s} = \mu_{1,s}^{*} + k_B T \ln X_{1,s}, \tag{1.48}$$

where the standard chemical potential, $\mu_{i,s}^{*}$, is the chemical potential of pure component $i(X_i = 1)$ surrounded by solvent. The observant reader will note that this seems nonsensical, as for $X_i = 1$ no solvent is present. Indeed $\mu_{i,s}^{*}$ is the value required to obtain $\mu_{i,s}$ equal to $\mu_{1,s}^{*} + k_B T \ln X_{1,s}$ for proper dilute solutions, that is, for $X_{1,s} \ll 1$; $\mu_{i,s}^{*}$ represents the (imaginary) state of pure compound 1 interacting only with the solvent.

The last step is to derive an equation for the *chemical potential of the solvent* in an ideal solution. We start with the Gibbs–Duhem equation (Eq. 1.35), which becomes for just two components

$$N_1 d\mu_1 + N_0 d\mu_0 = 0. \tag{1.49}$$

If we wish to focus on how the chemical potentials change with N_1, we can divide both sides of Eq. 1.49 by dN_1,

$$N_1 \frac{d\mu_1}{dN_1} + N_0 \frac{d\mu_0}{dN_1} = 0$$

$$\frac{d\mu_0}{dN_1} = -\frac{N_1}{N_0} \frac{d\mu_1}{dN_1}. \tag{1.50}$$

With this expression for the chemical potential of the dilute solute ($N_1 \ll N_0$), Eq. 1.46, one has with the mole fraction of solute, $X_1 = N_1/(N_1 + N_0)$

$$\frac{d\mu_0}{dN_1} = -\frac{N_1}{N_0} \frac{d(-k_B T \ln X_1)}{dN_1} = -\frac{k_B T}{N_0} \left(1 - \frac{N_1}{N_1 + N_0}\right) \approx -k_B T \frac{1}{N_0}, \tag{1.51}$$

and integration gives

$$\mu_{0,s} = \text{constant} - k_B T \frac{N_1}{N_0} \approx \mu_{0,s}^{\circ} - k_B T \, X_1 = \mu_{0,s}^{\circ} - k_B T \, (1 - X_0). \tag{1.52}$$

At low total solute concentrations, the expression for μ_0 can also be written as

$$\mu_{0,s} = \mu_{0,s}^{\circ} + k_B T \ln(1 - X_1) = \mu_{0,s}^{\circ} + k_B T \ln X_0. \tag{1.53}$$

If one equates $\mu_{0,s}$ with the chemical potential of an ideal gas of Eq. 1.39, one sees that the (partial) pressure of solvent in the vapor is proportional to the mole fraction of solvent in dilute solution, always assuming that both vapor and solution behave ideally. This is the classical form of *Raoult's Law*.

In summary, the chemical potentials of components of a dilute solution are

$$\mu_0 = \mu_{0,s}^\circ - k_B T \sum_{i>0} X_i$$

$$\mu_{i>0} = \mu_{i,s}^* + k_B T \ln X_i = \mu_{i,s}^\circ + k_B T \ln c_i, \tag{1.54}$$

where $\mu_{0,s}^\circ$ is the chemical potential of pure solvent at T, P, but $\mu_{i,s}^*$ of the dilute solute is not the chemical potential of pure solute, but rather the value needed to give the *actual* value of $\mu_{i,s}$ of a dilute solution ($X_i \ll 1$), when substituted in Eq. 1.46, and similarly for $\mu_{i,s}^\circ$.

1.5.2 Nonideal Solutions

If the proportionality between $c_{1,v}$ and $c_{1,s}$ does not hold in Eq. 1.46, the solution is said to be "*nonideal*" and a correction is needed. This deviation from ideal behavior is due to interactions between solute molecules. However, at equilibrium the chemical potential of solute in solution and vapor are equal whether or not either phase is ideal. Thus, we can develop a description of a nonideal solution just as we did for an ideal solution and, in this way, hold on to the simplified functional forms for the concentration dependence of ideal solutions by replacing concentration by *activity*, a,

$$\mu_i = \mu_{i,s}^\circ + k_B T \ln a_i = \mu_{i,s}^\circ + k_B T \ln \gamma_i c_i, \tag{1.55}$$

where γ_i is the activity coefficient of component i in the real solution. As can be seen from this discussion, deviations from $\gamma_i = 1$ imply the existence of significant interactions between solutes in a mixture, and deviations are most severe for strongly interacting solutes at high concentrations. Of course, this is all easy to write down, and even easier to say, but it is rarely easy to measure the activities of solutes in a multicomponent solution. The reality is, then, that we usually end up using the thermodynamic expressions for ideal solutions, even though these probably do not hold in a cell. We shall use activities wherever this is appropriate (e.g., we shall express equilibrium constants as ratios of activities, not concentrations), but in practical applications ideal behavior is assumed and activities are nearly always equated with the corresponding concentrations.

Only quite small molecules are sufficiently volatile to allow one to establish the ideality of their solutions by measurements of vapor pressure. However, the concentration dependence of the chemical potential of macromolecules can be determined via the technique of light scattering (see Section 6.7), and these measurements give us confidence, for example, that dilute protein solutions follow close to ideal thermodynamics. Molecules of nucleic acids are highly charged and interact strongly in

water; however, the interaction is greatly reduced in solutions of reasonable ionic strength.

The activity of many inorganic ions can be measured with potentiometric methods (which are otherwise not discussed in this book). A very important application of potentiometric measurement is the pH meter. Thus, pH is defined as

$$pH = -^{10}\log a_{H^+}. \tag{1.56}$$

The pH of 0.1 N HCl is 0.11, not 0.10, which indicates that this moderately concentrated acid solution is significantly nonideal.

1.5.3   Osmotic Effects

Equation 1.51 shows that solutes change the activity of water in a manner that is roughly proportional to their concentration. For relatively inert solutes (i.e., those that interact weakly with other solutes and even with the solvent), this expression can hold up to significant concentrations. Some such solutes, called *osmolytes*, occur in cells; by lowering the activity of cellular water, they serve to maintain cellular water content under drought conditions. As biological macromolecules interact strongly with water, a change in the activity of water brought about by osmolytes such as glycerol or sucrose can have significant effects on, for example, equilibria between conformation states or equilibria for forming macromolecular complexes. This will be taken up in Chapter 9 and again in Chapter 16.

Osmotic pressure reflects a tendency of solutions to become more dilute. We can think of the free energy as a potential with a corresponding "force" that drives the system to lower values of the potential by making the solution more dilute, similar to the pressure that tends to expand the volume of a gas. This thermodynamic "force" is due to entropy and manifests itself clearly in a situation where compartments can be separated by a wall containing passages through which the solvent can pass, but the solute cannot; such a wall is called a *semipermeable wall* or membrane. Dialysis membranes pass solvent and solutes of low molecular weight, but not macromolecules; membranes used in desalination pass water, but not NaCl; lipid bilayer membranes are impermeable to many compounds, while others can pass across the membrane by dissolving in the membrane, by taking advantage of fluctuations in membrane structure, by forming hydrogen-bonded chains (in the case of water), or, in the case of cell membranes, by diffusing through specific protein channels.

If a solution and a pure solvent are equilibrated in two compartments (A containing solution; B containing solvent; Fig. 1.6) separated by a semipermeable membrane, the solvent passes from compartment B into A in order to maximize the entropy of the system. Equilibrium is reached when the pressure in compartment A is higher than that in compartment B by a certain amount, namely, when the work required to move the solvent from B to A against this pressure is equal to the decrease in free energy (increase in entropy) associated with solvent moving
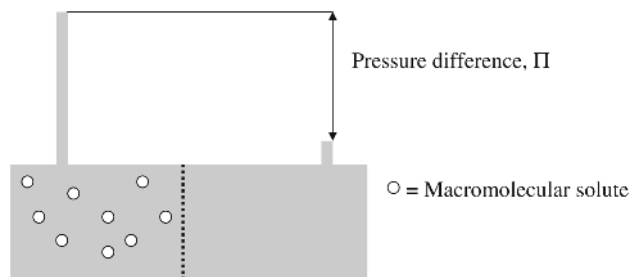
FIGURE 1.6 Osmotic pressure resulting from equilibration of solvent across a semipermeable membrane.

into compartment A. The free energy to transfer a solvent molecule from pure solvent into a solution can be written as a sum of three terms, the first for removing the solvent molecule from the solvent at constant pressure, the second for moving it to a system with a larger pressure, and the third for adding it to the solution at constant pressure,

$$\Delta G = (-\mu_0^\circ) + \Pi V_0 + \mu_0, \tag{1.57}$$

where V_0 is the volume per solvent molecule, and the difference in pressure between the two compartments is called the *osmotic pressure*, denoted with the symbol Π ($\Pi = P_A - P_B$).

In an ideal solution, the chemical potential of the solvent is given by Eq. 1.54. Setting ΔG in this equation to zero gives for the osmotic pressure that

$$-\mu_0^\circ + \Pi V_0 + \mu_0^\circ - k_B T X_1 = 0$$

$$\Pi_{\text{ideal}} = k_B T \frac{X_1}{V_0} \approx \frac{k_B T N_1}{V} = k_B T \frac{c_1}{M_1}, \tag{1.58}$$

where N_1 is the number of moles of solute and c_1 the concentration in units of mass/volume, M_1 being the solute's molecular mass. Osmotic pressure reflects solution entropy; it is one of several *colligative properties*,[18] which all depend on the number of particles in a solution (and hence offer opportunities for measurement of molecular weight). If the solute is not a single particle, we must multiply c by the number of moles of particles per mole of solute (e.g., 2 for NaCl).

1.6 PHASE EQUILIBRIA

1.6.1 Equality of Chemical Potential

Melting, boiling, and sublimation are familiar transitions between different phases of the same pure material, brought about by changes in temperature and pressure. Less familiar are the transitions between different solid phases; for example, the

relatively open structure of ice I (ice-cube ice) collapses to more compact forms of ice at high pressures.

When two phases of a pure compound are in equilibrium, the chemical potential of the compound is the same in each phase; one can prove this easily by considering the transfer of a small amount, dN, of compound from one phase (B) to the other phase (A), for which the free energy change is $dG = dN(\mu_A - \mu_B)$; this must be zero at equilibrium according to Eq. 1.19. Equality of chemical potential in the two phases holds only at specific combinations of pressure and temperature.

If the system contains more than one compound, the equilibrium condition at constant P and T

$$dG = -dG_A + dG_B = - \left(\sum \mu_{i,A} dN_i \right) + \left(\sum \mu_{i,B} dN_i \right) = 0, \qquad (1.59)$$

for *any* choice of the dN_i, which gives as the *essential condition of phase equilibrium* that the chemical potential of each component is the same in every phase (or state)

$$\mu_{i,A} = \mu_{i,B}. \qquad (1.60)$$

1.6.2 Transfer Free Energy

The chemical potential of a compound in solution can be related experimentally to the chemical potential of the pure compound, if one is able to establish equilibrium with a phase containing the same compound in pure form (crystalline solid or dilute gas) for which the chemical potential is known by standard methods described in any thermodynamics text. One then has

$$\mu_{1,c} = \mu_{1,s} = \mu_{1,s}^{\circ} + k_B T \ln a_{1,s} \text{ (for a crystalline phase)}$$

or

$$\mu_{1,v} = \mu_{1,v}^{\circ} + k_B T \ln c_{1,v} = \mu_{1,v}^{\circ} + k_B T \ln a_{1,s} \text{ (for a vapor phase).} \quad (1.61)$$

If the pure phase is a vapor, one may determine the standard chemical potential in the solution, $\mu_{1,s}^{\circ}$, by extrapolating to low concentration, where activity equals concentration. If the pure phase is a crystal, and the solution is not dilute, then the extrapolation to dilute solution can be done by measuring the chemical potential of the solvent (from equilibrium with its vapor) and applying the Gibbs–Duhem relation to relate its change upon dilution to the corresponding change in the chemical potential of the solute.

Such a difference between standard chemical potentials is called a *transfer free energy*. Measurement presupposes measurable solubility in the chosen solvent and either a measurable volatility or existence of a crystalline phase. While the *concept* of a transfer free energy applies also to solutions of a macromolecule, its measurement is usually impossible because neither the gaseous form nor a pure crystalline form of the macromolecule can be realized experimentally. Transfer free energies of small molecules related to the structure of proteins have been measured and

play an important role in understanding the effect of the solvent on the stability of native conformations of proteins and nucleic acids. These include values based on solubility of amino acids and peptides, and on transfer equilibrium to the dilute gas phase of a series of more volatile molecules related to amino acid side chains. This will be discussed in detail in Chapter 16.

1.6.3 Phase Diagram of a Pure Compound

For a system containing only a single component, the chemical potential of each phase is a different function of T and P; consequently, two phases can coexist in equilibrium only at certain combinations of T and P, at which the chemical potentials happen to have the same value. If, say, T is changed, two phases no longer coexist and the system exists as a single-phase region of its phase diagram. Indeed, for most combinations of T and P, only one phase is stable. However, P can be adjusted at the new value of T to reestablish phase equilibrium. Thus, for each T, there is a unique P for which two phases coexist. These T and P pairs define *coexistence curves* separating regions of stability of single phases. A coexistence curve establishes a *relation* between T and P, that is, P is a function of T or *vice versa*. A *triple point* is where two coexistence curves intersect, that is, where three phases (A, B, and C, which can be a crystalline form, liquid, and vapor) are at equilibrium. At this triple point, $\mu_A(T, P) = \mu_B(T, P) = \mu_C(T, P)$, and we have two equilibrium constraints.

The results can be represented in the form of a *phase diagram*. A simple example illustrates how such a diagram is interpreted (Fig. 1.7). The slope of the solid–liquid coexistence curve, AB, is negative because liquid water has higher density than ice. (This is atypical; most solids have higher density than their liquid forms.) The triple point occurs when ice and liquid water are in equilibrium at near-zero atmospheric pressure; the pressure of the water vapor in equilibrium with these two phases is only 0.006 atm (4.6 mmHg). The phase diagram also displays a so-called *critical point*: distinction between liquid and vapor phases usually becomes less marked at high temperature and pressure, as the vapor becomes denser as a result of the increase in pressure and the liquid expands because of the increase in temperature; finally, liquid and vapor phases become indistinguishable and the coexistence curve between these two phases ends.

1.6.4 The Gibbs Phase Rule

More complicated phase diagrams result in systems containing more than one component. These can be constructed using a relation known as the *Gibbs phase rule*. In general, we have seen that the number of independent intensive variables (those not dependent on N, the number of molecules), required to define the thermodynamic state of a system is 2 (e.g., T and P or T and V/N). This is called the *number of degrees of freedom* (n_F) of the system. Gibbs recognized that, if more than one phase was present, each equality of chemical potentials across all phases (Eq. 1.60) imposed an additional constraint on the system that reduced n_F, while
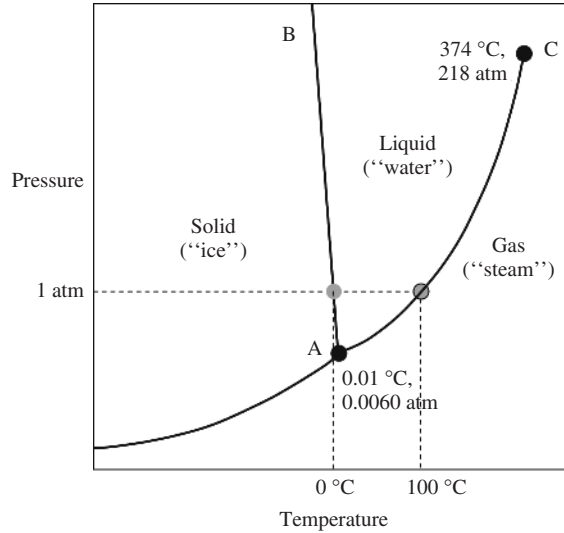
FIGURE 1.7   Schematic phase diagram separating solid, liquid, and vapor phases of water, showing triple point (A), critical point (C), and melting and boiling points at atmospheric pressure.

conversely the variable composition of each phase produced additional degrees of freedom. The phase rule states that n_F is two plus the number of components present beyond one ($n_C - 1$), and reduced by the number of constraints, which is equal to the number of phases present, less one ($n_P - 1$),

$$n_F = 2 + (n_C - 1) - (n_P - 1) = 2 + n_C - n_P.$$

We will not go into the phase rule or the process of constructing phase diagrams here in detail (any text on Physical Chemistry will treat this).

Typically, in biophysics, the phases contain *mixtures of components*. If two phases are in equilibrium, the chemical potentials of each component, μ_j, must be the same in each phase, and, of course, these now also depend on the concentrations of all components,

$$\mu_{j,A}(T, P, \{N_{i,A}\}) = \mu_{j,B}(T, P, \{N_{i,B}\}) \tag{1.62}$$

For a two-component system, three independent variables define the state of the system (T, P, $X_1 = 1-X_2$). If we fix P, the condition of phase equilibrium (Eq. 1.62) establishes a relationship between T, $X_{1,A}$, and $X_{1,B}$ that defines coexistence lines of a *temperature-composition phase diagram*.

The most commonly encountered phases in biophysics are vapor and dilute aqueous solution; with the exception of ice crystals, pure solid phases are uncommon. Of great importance is the phospholipid lamellar (bilayer) phase that provides the essential structure of biological membranes that delimit and compartmentalize the

cell. This phase self-assembles in water because water is largely excluded from the lipid, and the lipid is very poorly soluble in water. However, solutes of medium polarity can and do partition between the aqueous and lipid lamellar phases; the ability to equilibrate between bilayers and aqueous phases is thought to be the basis of activity of some anesthetics. Equilibration of immiscible liquids produces two liquid phases, each of which consists predominantly of one of the components, with a small admixture of the other (e.g., ether and water). Typical for macromolecular solutes is coexistence of a *solvent-rich phase* with a *solute-rich phase*. Examples include precipitation of proteins by addition of a poor solvent component and formation of protein crystals from supersaturated mother liquor.

1.7 CHEMICAL EQUILIBRIA

When a chemical reaction takes place, the amounts of the reactants decrease and the amounts of the products increase. For a reaction having reactants with *stoichiometries* v_i, a small change in the extent of reaction (represented by δs) leads to changes in the amounts of each of the components present in the reaction mixture

$$\delta N_i = v_i \delta s \tag{1.63}$$

For example,

$$A + 2B \leftrightarrow 2C$$

$$v_A = -1, v_B = -2, v_C = +2. \tag{1.64}$$
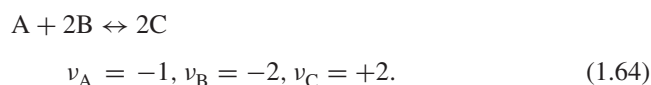
Whenever a reaction has proceeded to equilibrium (at constant T and P), a further backward or forward reaction must produce an increase in the Gibbs free energy, that is, G is at a minimum with respect to s.

$$\frac{\partial G}{\partial s} = \frac{\partial \sum N_i \mu_i}{\partial s} = 0$$

$$\Rightarrow \sum v_i \mu_i = 0 \tag{1.65}$$

This relationship can also be obtained from the Gibbs–Duhem equation.

Recalling that the chemical potential of any substance in solution is related to the activity via Eq. 1.55, we have

$$\sum v_i \mu_i = \sum v_i \mu_i^\circ + k_B T \sum (v_i \ln a_i) = 0 \tag{1.66}$$

This condition for equilibrium can be rewritten as

$$\prod_i a_i^{v_i} = K_P$$

$$-k_B T \ln K_P = \sum_i v_i \mu_i^\circ. \tag{1.67}$$

This defines K_P, the *equilibrium constant* at constant P in terms of the standard chemical potentials of the reactants and the stoichiometry of the reaction. In the case of the above-mentioned simple example this becomes

$$K_P = \exp[-(2\mu_C^\circ - \mu_A^\circ - 2\mu_B^\circ)/k_B T] = \left(\frac{a_C^2}{a_A a_B^2}\right)_{eq}. \qquad (1.68)$$

For an ideal solution, the activities can be replaced by concentrations.

It is common to write

$$-k_B T \ln K_P = \sum_i v_i \mu_i^\circ = \Delta G^\circ, \qquad (1.69)$$

where ΔG° is the *standard free energy change for the reaction*. Formally, this represents the free energy change when each of the reactants is taken from, and each of the products is placed in a solution in which its activity is equal to 1. For an ideal solution, these solutions have concentration equal to 1. As noted previously, the standard state represented by μ_i^o is not pure reactant i, but is an imaginary state in which each component is surrounded only by the solvent (see discussion of Henry's Law). In many biophysics publications, standard free energy changes are (alas) reported as ΔG, with omission of the superscript.[19]

When a chemical equilibrium is written as in Eq. 1.63, there is no clear distinction between reactants and products. By virtue of the Second Law, a chemical equilibrium will favor that side of the equation having lower free energy, and in a chemical reaction this would be the product side, with the other side, of higher free energy, then containing the reactants. Nevertheless, many important metabolic reactions seemingly proceed from reactants to products of *higher* free energy. However, upon closer study this is found to never be the case, as such an unfavorable reaction is always *coupled* to another reaction that is very favorable. Say, we have a synthesis of a compound AB, from reactants A and B,

$$A + B \overset{\longrightarrow}{\longleftarrow} AB \qquad (1.70)$$
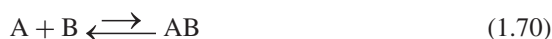
that is unfavorable (as indicated by the longer arrow pointing to the left). Then, either this reaction can proceed in two (or more) favorable steps, in which first one (or more) reactants is converted to a different form

$$A + XY \overset{\longrightarrow}{\longleftarrow} AX + Y$$
$$AX + B \overset{\longrightarrow}{\longleftarrow} AB + X, \qquad (1.71)$$
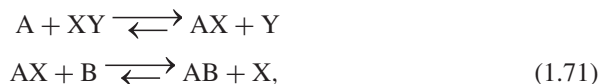
or, the favorable and unfavorable reactions may be directly coupled in a single reaction, as in

$$A + XY + B \overset{\longrightarrow}{\longleftarrow} AB + X + Y. \qquad (1.72)$$
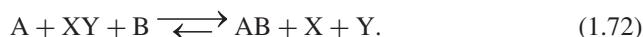
In both cases, the breakdown of one compound (XY) "produces" the free energy (and more) needed for the biosynthesis of the other (AB). Compounds such as

XY are called *high energy compounds*; a much encountered example is adenosine triphosphate (breaking down to adenosine diphosphate and phosphate ion, or to adenosine monophosphate and pyrophosphate ion).

1.8 TEMPERATURE DEPENDENCE OF CHEMICAL EQUILIBRIA: THE VAN'T HOFF EQUATION

As the standard free energy of a reaction can be found from the equilibrium constant, the enthalpy, which is related to the temperature dependence of the free energy by Eq. 1.30, can be found from the temperature dependence of the equilibrium constant as

$$\Delta H^{\circ} = \frac{\partial[\Delta G^{\circ}/T]}{\partial[1/T]} = -k_{\mathrm{B}}\frac{\partial \ln K_P}{\partial[1/T]} = k_{\mathrm{B}}T^2\frac{\partial \ln K_P}{\partial T} \tag{1.73}$$

This is the van 't Hoff equation. It states quantitatively the common observation that "an endothermic equilibrium ($\Delta H^{\circ} > 0$) shifts to the right" when the temperature is raised.

1.9 MICROCALORIMETRY

A classical approach to measuring thermodynamic functions is to use a calorimeter (a "heat meter"). It is easy to accurately supply energy in the form of heat input to a sample by passing an electric current through a heating element embedded in the sample, and thereby measure the specific heat, C_V, by monitoring the resultant change in temperature. Two types of sensitive microcalorimeters are available, a scanning type, in which the temperature is varied over an interval by continuous heating, and a mixing type, in which successive aliquots of reactant are added to the sample. Scanning microcalorimetric studies of proteins were pioneered by J. Sturtevant; subsequently, much work has been done with instruments developed by J. Brandts in the United States. and by P. Privalov in the (then) Soviet Union; elements of both these instruments have been incorporated in commercially available instruments. We describe here both scanning and mixing type instruments, according to one particular design strategy.

A *differential scanning calorimeter* is one in which a sample (e.g., protein plus buffer) and a control (only buffer) are isolated as much as possible from the environment and from each other. The instrument is surrounded by a water bath that tracks as closely as possible the temperature of the samples, in order to suppress heat flow between reservoir and sample ("pseudo-adiabatic"). The reservoir is gradually heated and the temperature differences between the sample (ΔT_1) and the buffer (ΔT_2) ampoules and the reservoir are measured (Fig. 1.8). A feedback circuit causes electrical heaters in contact with each ampoule to provide enough heat so as to keep $\Delta T_1 \approx \Delta T_2 \approx 0$. The block is designed so that heat cannot flow
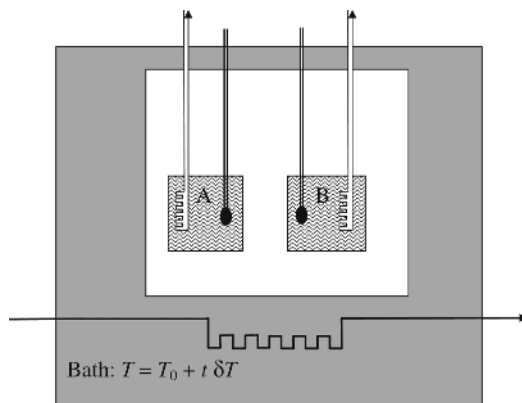
FIGURE 1.8 Diagram of a pseudo-adiabatic differential scanning calorimeter. The bath is heated at a steady rate. The cells are isolated from the bath; the temperature in each cell is sensed separately, and feedback mechanisms supply current to maintain each cell at the temperature of the bath. The difference in current supplied to the two cell heaters is recorded.

between the sample and reference, so that any heat that is produced (exothermic change) or absorbed (endothermic change) by the sample relative to the buffer reference is recorded as a difference in electrical heat energy supplied to the sample and reference ampoules ($[I^2Rt]_1 - [I^2Rt]_2$), as measured with a sensitive ammeter and recorded as a function of temperature. A temperature-induced transition or change in the sample ampoule is thus recorded as a difference in heat flow into the sample versus the reference. Sophisticated modern calorimeters can measure very small differences in heat flow ($\sim$10 ncal/s).

By measuring the heat required to take a sample relative to buffer from (nearly) 0 K to higher temperature by heating one can obtain values of C_V or C_P as a function of temperature. This can be integrated to give both internal energy and entropy changes; see Eqs. 1.36 and 1.37.

If the volume of the system is held constant during the heating process so that no PV work is done on or by the system, the procedure yields C_V, S, U, and A. If the pressure is held constant, and the volume changes during heating, the system will do work ($P\Delta V$) on its surroundings, thus reducing the change in system internal energy as a result of input of the same amount of heat. In this case, C_P, S, H, and G are the state functions obtained from the calorimetric measurement.

The enthalpy of a temperature-induced transition can be measured with such an instrument, provided that the enthalpy of the transition, and therefore the temperature dependence of the equilibrium constant (Eq. 1.73), is sufficiently large. This will be discussed in Chapter 16 where we describe how microcalorimetry is applied to study the folding–unfolding transitions of proteins.

A calorimeter can also be set up as a mixing calorimeter to measure reaction enthalpies. Figure 1.9 shows an *isothermal titration calorimeter* used for this
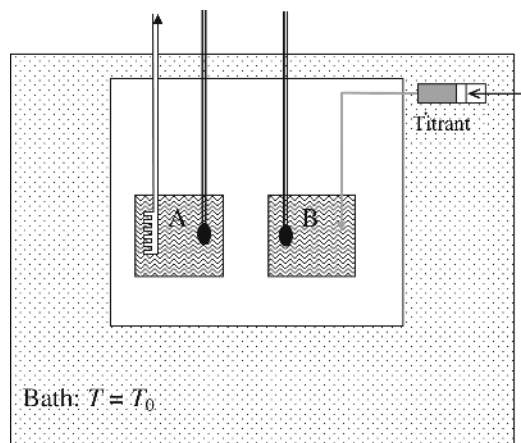
FIGURE 1.9   Diagram of an isothermal titration calorimeter (design principle similar to Fig. 1.8). At the start, sample (B) and reference (A) have identical contents. Titrant is added in small aliquots to the sample cell, and current is supplied to the reference to maintain the same temperatures in the two cells. (If the reaction is endothermic, heating is applied to cell B.) If necessary, the temperature of the bath can also be adjusted.

purpose. A sample solution is placed in an ampoule and stirred continually with a very precise stirring device so that the heat of stirring can be carefully recorded. Another solution (titrant) is added from a chamber at the same temperature as the sample. With each addition of titrant, one adds I^2Rt heat to the reference ampoule (also containing sample solution) to keep the temperatures of the sample and reference identical (if the reaction is exothermic. In the case of an endothermic reaction, the compensating heat is supplied to the sample). An example of data from such an experiment is discussed in Section 8.3 (Fig. 8.7).

Note that calorimetric enthalpy and heat capacity are intrinsically determined per unit of sample mass (e.g., gram), while enthalpy and heat capacity derived from the temperature dependence of equilibrium constants via the van't Hoff equation are determined per mole.

NOTES

1. As a convention in this book, a change of a thermodynamic function, such as $U_b - U_a$, is denoted either as $\Delta U_{a \to b}$ or as ΔU_{ab}. The corresponding notation for equilibrium constants is $K_{ab} = K_{a \to b} = c_b/c_a$, and similarly for rate constants k_{ab} or $k_{a \to b}$ represents the forward rate constant in the reaction $a \to b$.

2. Boltzmann's constant, k_B is expressed in *units* of energy/(temperature $\times$ amount of material). The *value* of k_B depends on the choice of units for any of these three quantities. If the amount of material, N, is expressed in number of molecules versus number of moles, the values of k_B differ by a factor of Avogadro's number, N_A. At one time, we distinguished between these two cases by assigning the name Boltzmann's constant (k_B)

to the value per molecule, and the name gas constant ($R = N_A k_B$) to the value per mol. In modern physics, the convention is to refer only to k_B, with the units being understood from the context in which it is used.

3. Also called, respectively, quasi-static and real processes.

4. Complete discussions of the Carnot cycle and how it leads to the conclusion that S is a state function can be found in advanced texts on thermodynamics.

5. A "heat reservoir" is a body at constant volume that does no work but simply provides or absorbs heat. The heat taken up or given off is thus equal to its change in internal energy (ΔU) and is thus a state function of the reservoir.

6. Because the dependence of U on T is the same at both pressures.

7. A (real) adiabatic process can be accomplished as an isothermal real process followed by exchange of heat with a bath at a different temperature. The entropy of the universe rises in both steps.

8. If an ideal gas expands without performing work (when the volume is suddenly increased), the ability to perform work decreases, so that A decreases. But no work is done, so the energy U is constant. In this *irreversible* process the entropy has decreased but no heat has been exchanged.

9. Other types of work include electrical work ($e \Delta E_{el}$), volume change of a gel ($\Pi \Delta V$), and mechanical work including elastic extension ($F \Delta L$), where e = charge, E_{el} = electrostatic potential, Π = osmotic pressure, F = force, L = distance or length.

10. For example, to derive the expression for dA, the definition $A = U - TS$ gives $dA = dU - TdS - SdT$. Then, substitution for dU according to the fundamental statement of the first and second laws, Eq. (1.13) gives $dA = dU - TdS - SdT \leq TdS - PdV - \partial w - TdS - SdT$, or $dA \leq -SdT - PdV - \partial w$.

11. However, for an ideal gas $G = A + PV = A + N k_B T$.

12. In this chapter, the symbol N represents number of molecules, but can equally well be taken to represent number of mols.

13. This is derived as $\partial(A/T)/\partial(1/T) = A + (1/T)\partial A/\partial(1/T) = A - T\partial A/\partial T = A + TS = U$, where we have substituted $\partial A/\partial T$ from Eq. (1.23).

14. The *proportions* of different components and P and T are held constant, so that the μ_i are constant during the integration.

15. J. Willard Gibbs was a great American scientist, professor at Yale, whose name is immortalized in the term Gibbs free energy, G.

16. Unless specifically stated otherwise, the choice of standard state is the pure gaseous, liquid, or crystalline solid state of a compound at $T = 25°C$ and pressure $P = 1$ bar $= 100$ kPa $= 10^5$ N M$^{-2} \approx 1.013$ atm.

17. It is usual to number the components of a mixture $1, 2, \ldots, i$; if one of the components is in great excess, that is, a solvent, then it is given index 0.

18. Colligative properties include depression of freezing point and elevation of boiling point.

19. Note that $\ln K_P$ is properly defined only if K_P is unitless, which is the special case in which the number of molecules on each side of the reaction is equal (i.e., if $\sum v_i = 0$), or, in the general case, if the activities are expressed in mole fraction units. When an equilibrium constant is given in concentration units other than mole fraction, and $\sum v_i \neq 0$, it is ideally first converted to mole fraction units before one calculates $\Delta G°$ (something that admittedly is not always done).