

Cellular Thermodynamics: the molecular and macroscopic views

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by Joe Wolfe

On the macroscopic scale, biology obeys thermodynamics, and this chapter introduces the laws of thermodynamics and its macroscopic variables, including entropy. The molecular description is often more directly useful to biologists. So this chapter explains Boltzmann's distribution, which describes how the proportion of molecules having a particular energy depends on energy and temperature, and quantifies the compromise between minimising energy and maximising entropy. This simple law is then used to explain and to quantify several aspects of the function and structure of cells. These include Arrhenius' law for reaction rates, the Nernst equation for electrochemical equilibria, and hydraulic and osmotic equilibria between solutions with different compositions. It also explains the equilibrium between different phases (solid, liquid, vapour), the aggregations of molecules in self-assembly, aspects of the stability and mechanical homeostasis of membranes, and entropic forces in biomolecules.

Keywords: entropy; osmosis; Arrhenius' law, Nernst equation, molecular aggregation, oligamerisation, Gibbs free energy, chemical potential, membrane homeostasis, entropic forces.

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Introduction

Consider these variables and effects: temperature, energy, entropy, concentrations, molecular motions, electrochemistry, osmotic pressure, reaction rates, changes of phase, molecular motion, configuration and aggregation. All of them are literally vital to the structure, function, operation and health of cells. The physical laws and phenomena relating these variables are discussed in the subjects thermodynamics, which deals with the macroscopic level, and statistical mechanics, which deals with the molecular level. This article introduces the topics most essential to biology, and serves as an introduction to the material of several other articles. The approach is to concentrate on biological examples and to emphasise explanations at the molecular level rather than to follow the traditional development using macroscopic variables and the ideal gas. The macroscopic and molecular pictures are related via the chemical potential. The mathematical and formal rigour that is thus bypassed may be readily found in much longer accounts in classic texts in thermal physics (e.g. Hobbie, 1997; Landau and Lifschitz, 1969). See also [Thermodynamics in Biochemistry](#), and [Cell Biophysics](#).

Equilibrium

The concept of equilibrium is surprisingly useful in biology. Surprising because, by definition, equilibrium is the state achieved by a system when the relevant variables cease to vary with time and when no energy or matter enters or leaves. If a mammal dies in a cool, dry environment, its body, initially warmer than its surroundings, gradually comes to thermal equilibrium

with them, *i.e.* reaches the same temperature. Over a much longer time scale, its water content falls to several percent: it is approaching hydraulic equilibrium. Complete, global chemical equilibrium is rarely of direct interest to biologists: even seeds are not completely equilibrated with their environment. In many cases, however, local equilibria are achieved, to an excellent approximation. Cells and their surroundings are usually extremely close to thermal equilibrium because of their proximity: if there were a temperature difference over the small distance between them, heat would usually flow rapidly from the hotter to the colder. Similarly, water can often leave and enter cells relatively rapidly, because of the permeability of membranes to water. In this case, permeation by water allows rapid approach to osmotic equilibrium.

Steady state

In a system in steady state, the variables do not change over time, but energy and/or matter enter the system and leave the system at the same rate. The energy may leave in a different form or at a different temperature. For example, a portion of human skin approximates steady state with respect to some variables: it is losing heat via radiation and evaporation but is receiving heat via conduction from subcutaneous tissue and the flow of blood; it is losing water and some volatiles by evaporation but receiving them via bulk flow, diffusion and permeation. In steady state, most of the variables of the system do not change with time. Steady state is a more obvious approximation to the behaviours that interest biologists, although it does not include such important features as growth.

The maintenance of a steady state requires a continuous throughput of energy, which is schematised in Figure 1. Remove the source or the sink of energy, and the living system comes to equilibrium – it dies.

Heat, light, photons and 'useful energy'

The illustrations of steady state in Figure 1 introduce some important ideas about the transmission of heat, and other forms of energy, in systems approximating steady state. The temperatures are given in the thermodynamic or absolute scale (in kelvins, K); to convert to Celsius ($^{\circ}\text{C}$), subtract 273.14 from the temperature in K.

In one sense, ultraviolet (UV) and visible light and infrared (IR) radiation (radiant heat) differ only quantitatively: they are all electromagnetic waves but UV has shorter wavelengths. In biochemistry, the

difference is qualitative: visible and ultraviolet light are either useful energy for photosynthesis or photoreception, or potentially dangerous radiation for photolabile molecules. In contrast, the energy radiated by an organism at $\sim 300\text{ K}$ to its surroundings (or from the surroundings to the organism) is only useful in biochemistry indirectly, as heat. The difference is due to the quantization of energy.

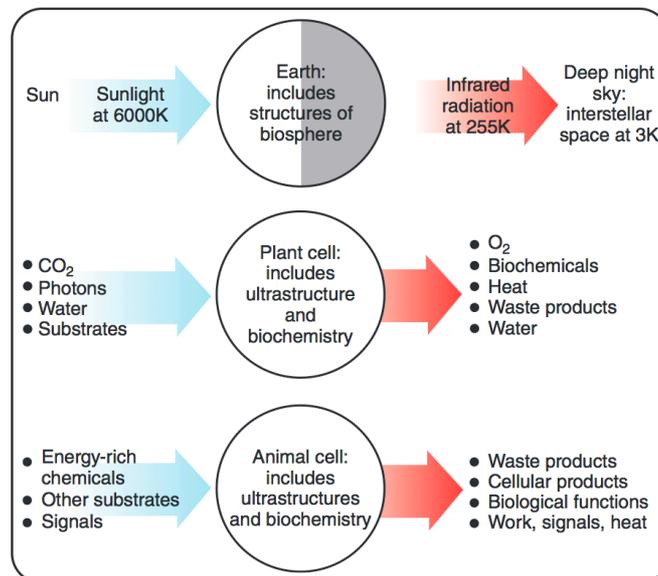


Figure 1. Thermal physics schematics of the Earth, a photosynthetic plant cell and an animal cell. Their macroscopically ordered structure – and anything else they do or produce – is ultimately maintained or 'paid for' by a flux of energy in from the sun (at $\sim 6000\text{ K}$) and out to the night sky (at $\sim 3\text{ K}$). The equal size of the arrows represents the near equality of average energy flux in and out. The entropy flux is not equal: the rate of entropy export is in all cases much greater than the entropy intake. Apart from exceptional ecosystems on the ocean floor that obtain their energy from geochemistry rather than from sunlight, all life on Earth depends on photosynthesis, and thus on this energy and entropy flux. The Earth absorbs solar energy at a rate of about $5 \times 10^{16}\text{ W}$, so the rate of entropy input is about $5 \times 10^{16}\text{ W}/6000\text{ K} \cong 8 \times 10^{12}\text{ W.K}^{-1}$. The Earth radiates heat at nearly the same rate, so its rate of entropy output is $5 \times 10^{16}\text{ W}/255\text{ K} \cong 2 \times 10^{14}\text{ W.K}^{-1}$. Thus the biosphere creates entropy at a rate of nearly $2 \times 10^{14}\text{ W.K}^{-1}$. Living things contribute only a fraction of this entropy, but that is still a huge amount. Despite the disingenuous claims of some anti-evolutionists, biological processes are in no danger of violating the Second Law of thermodynamics.

Like matter, light is not infinitely divisible. Light of a given colour (and thus, given frequency and wavelength) has a minimum quantity or quantum of energy. This is conveyed by a photon. A photon may be imagined as a minimum 'packet of energy', while in transit as light. The energy of a photon is hc/λ , where h is the Planck constant, c is the velocity of light and λ is the wavelength. For the sun, radiating at a temperature of $\sim 6000\text{ K}$, most of the energy is carried by photons with wavelengths of about $0.5\ \mu\text{m}$ (visible light) and energies of about $4 \times 10^{-19}\text{ J}$ per photon, which gives 250 kJ per 'mole of photons'. For living things,

radiating at ~ 300 K, the corresponding values are $10\ \mu\text{m}$ (far infrared), 2×10^{-20} J per photon or ~ 10 kJ per 'mole of photons'. One significant biological implication of quantisation is this: a quantum of energy of visible or ultraviolet light is comparable with the separation of the quantised energy levels of electrons in atoms and molecules. Photons with this energy can activate many chemical reactions directly, as they do in photosynthesis and vision. The much smaller quantum

energy of infrared radiation from a body at 300 K (~ 10 kJ per mole) is comparable with the typical kinetic energies of molecules due to their thermal motion. This energy can usually only activate a reaction indirectly: most reactions that occur at temperatures of ~ 300 K are indeed activated by the thermal motion of molecules, but, as we shall see, it is usually only a small proportion of molecules, having rather more than the average energy, that produce the reaction.

Box 1: The laws of thermodynamics

The Zeroth Law states that if one system is in thermal equilibrium with a second, and the second is in thermal equilibrium with a third, the first and the third are in thermal equilibrium. This law is what makes temperature a useful concept: temperature is defined as that quantity which is equal in two systems at thermal equilibrium.

The First Law is the statement of conservation of energy at the macroscopic, thermal level. It states that the internal energy U of a system is increased by the net amount of heat ΔQ added to it and decreased by the amount of work ΔW that the system does on its surroundings: $\Delta U = \Delta Q - \Delta W$, and that the internal energy of a system is a function of its physical variables (i.e. it is a state function). Suppose that a muscle cell does work ($\Delta W > 0$) and, in so doing, exports heat to its surroundings ($\Delta Q < 0$). The energy for both of these comes from the cell's internal (chemical) energy, which therefore decreases.

The Second Law states that the total entropy generated by a process is greater than or equal to zero. It is possible for the entropy of a system to decrease (e.g. the contents of a refrigerator during cooling), but in doing so the entropy of the surroundings is increased (the refrigerator heats the air in the kitchen).

The Third Law (not directly relevant to biology) states that no finite series of processes can attain zero absolute temperature. Different but equivalent statements of the second and third law are possible. The laws are jocularly summarised as: 1st law: you can't win. 2nd law: you always lose. 3rd law: you can't get out of the game.

Entropy

This difference between the chemically 'useful' energy (light or UV in our example), and the 'waste' energy (infrared heat) can be formalised using the concept of entropy. Here is the definition: when an amount of heat ΔQ is transferred at temperature T , the system gaining the heat has its entropy increased by $\Delta Q/T$. Thus sunlight (whose radiation temperature is ~ 6000 K) is radiation with low entropy. One joule of thermal energy radiation from the surface of an organism at ~ 300 K (~ 20 times cooler) transmits 20 times as much entropy as a joule of solar radiation. This theme is a common one in biology: energy is acquired from low-entropy sources (e.g. light and food), and is used in a variety of different biochemical, physiological and ecological mechanisms. Ultimately, the energy is lost to a 'sink' for energy at low temperature and correspondingly high entropy. Traditional thermodynamics discusses heat engines (such as the steam turbines at thermal power stations) taking heat from the high-temperature source, converting part of it into work, rejecting the rest as heat into the low-temperature sink (the cooling tower) and generating entropy in so

doing. Leaving aside deep-sea vent ecosystems, the biology of the biosphere does something similar: it receives the low entropy energy from the sun (a source at $T \sim 6000$ K) but it also expels high entropy energy into the ultimate sink for energy, the night sky (at ~ 3 K). See Figure 1.

Macroscopic and microscopic views

A cell is, for our purposes, a macroscopic object. It contains perhaps 10^{14} molecules, and we can define and measure such parameters as its temperature, the pressure inside a vacuole, the tension in a membrane and so on. This is the domain of thermodynamics. The power of this discipline derives from the fact that one can often apply thermodynamics to the overall behaviour of systems without knowing the molecular details.

For a single small molecule, on the other hand, temperature and pressure are neither well-defined nor measurable. The complementary discipline of statistical mechanics applies the laws of mechanics to individual

molecules, atoms and photons and, by considering the statistical behaviour of large numbers, deduces the behaviour of macroscopic systems. It thus relates thermodynamics to Newtonian or quantum-mechanical laws at the molecular level.

From cells to ultrastructure to molecules, we move from the macroscopic to microscopic points of view. For instance, work, kinetic energy and heat are forms of energy and, in the macroscopic world, they are clearly distinguished. This difference almost disappears at the molecular level.

The laws of thermodynamics (see box) apply to macroscopic systems, where entropy and temperature have a clear meaning, and where heat is clearly distinguished from other forms of energy. At the microscopic level, they may be applied to a large number of molecules in a statistical way, but not to individual molecules. For example, the kinetic energy of a small molecule *is* heat. If it is accelerated by interaction with another molecule, has work been done on it, or has heat been given to it? For a large collection of molecules, however, collective organised motion (say a current of air) can do work, whereas random individual motion (in a hot, stationary gas) can supply heat.

The second law can be stated thus in molecular terms: on average, systems go from improbable states (states having a small number of possible configurations) to probable states (having many possible configurations). For small numbers of molecules, however, the improbable is possible. If you toss 10 coins many times, you will occasionally (roughly 0.1% of the time) get 10 heads. If you could toss 6×10^{23} coins, however, you would never toss 6×10^{23} heads.

Distribution of molecules and energy: low energy vs high entropy

Consider a solution. At atmospheric pressure and a given temperature, it has a certain amount of heat and other energies. But how is energy distributed among molecules? How many of them have enough energy to react, or perhaps to evaporate? How are molecules distributed in states or regions having different energies? How do systems compromise between maximizing entropy and minimizing energy? At equilibrium, the answer to these questions is usually given by the Boltzmann distribution. This distribution, shown in Figure 2, explains several important phenomena (chemical and ionic equilibration, reaction rates, osmotic pressure and more). It can be viewed as a compromise or balance involving energy and entropy:

an important theme in cellular physics and biochemistry. In the state of highest entropy, all molecules or atoms would be distributed homogeneously in space. The state of lowest energy is equally uninteresting to biologists: the biosphere would consist of large crystals of nitrogen, oxygen, ice, and other substances. Equilibrium is a compromise between the two extremes. Because entropy S is heat (a form of energy) divided by temperature T , entropy appears multiplied by T in equations concerning equilibrium (the product TS having units of energy). Consequently, entropic effects tend to dominate at high temperature, i.e. molecules spread out to achieve maximum molecular disorder. At low temperature, minimizing energy is more important, so molecules distribute more strongly into the lower-energy states.

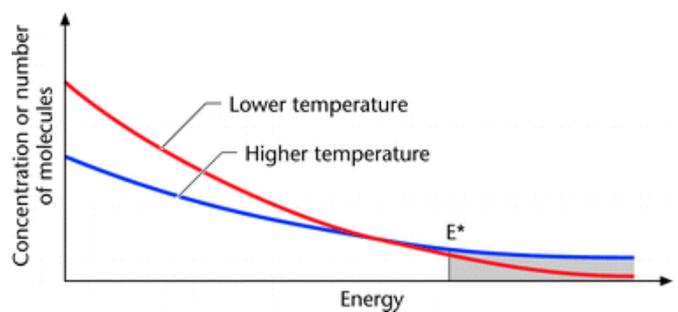


Figure 2. At equilibrium, molecules and their energies are distributed according to the Boltzmann distribution, sketched here. In states, phases or regions having different energies E , the concentrations or numbers of molecules are proportional to $\exp(-E/kT)$, where E is the energy per molecule, $k = 1.38 \times 10^{-23} \text{ J.K}^{-1}$, is the Boltzmann constant and T is the absolute temperature.

A familiar example of the variation in concentration with energy is the earth's atmosphere: at sea level, gravitational potential energy is low and the gas concentration is high. As altitude and gravitational potential energy rise, the concentration falls. Figure 3 uses a simplified version of this to derive the Boltzmann distribution.

The Boltzmann distribution gives the proportion of molecules in particular states as a function of the energy of the state. In many cases this corresponds to the concentration c in a region with energy E (eqns [1a,1b](#)).

$$c = c_0 \exp(-E/kT) \quad (1a)$$

$$C = C_0 \exp(-E_m/RT) \quad (1b)$$

Here c_0 or C_0 is, by definition, the reference concentration at which $E = 0$. The relation is given in two forms. Equation [1a] refers to molecules, so E is the energy per molecule and k is Boltzmann's constant. Equation [1b] refers to moles, so E_m here is the energy per mole and $R = N_A k$ is the gas constant, where N_A is

the Avogadro number. People who deal with or think about molecules tend to use the first form, and those who think about macroscopic quantities tend to use the latter, so it is useful to be able to convert between them. kT is called the thermal energy because the various thermal motions of molecules have energies of this order.

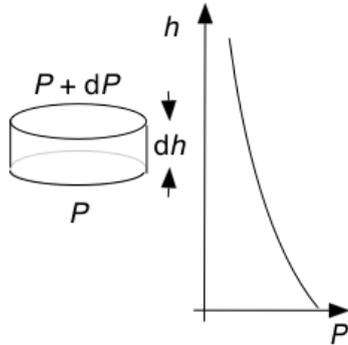


Figure 3. The variation with altitude of the concentration of gas in a gravitational field gives a simple derivation of the Boltzmann distribution, if we assume constant temperature. Concentration is high at ground level (low gravitational potential energy), and falls as the altitude h increases. The gravitational potential energy is $U_g = Mgh$ per mole, where g is gravitational acceleration and M is the molar mass. Consider the volume element sketched, with thickness dh and area A : the pressure difference dP acting on the area A supports the weight of the element ($dP < 0$). So $A dP = -\rho A dh g$, where ρ is the density of the gas. The equation of state $PV = nRT$ gives $\rho = nM/V = MP/RT$. Combining these equations an expression for dP/dh .

$$\frac{dP}{dh} = - \left(\frac{Mg}{RT} \right) P = - \frac{P}{\lambda}$$

where $\lambda = RT/Mg \approx 8$ km. Solving this equation gives $P = P_0 \exp(-Mgh/RT)$, and substitution in the equation of state gives $C = C_0 \exp(-Mgh/RT) = C_0 \exp(-U_g/RT)$, which is the Boltzmann distribution. Thus at low altitude the potential energy is low, the concentration is high and the entropy per mole is low, and conversely for high altitudes.

Equation 1 tells us that concentrations decrease exponentially with increasing energy, but it is worth giving quantitative examples. kT or RT is called the thermal energy and its value is about 4×10^{-21} J per molecule and 2 kJ mol^{-1} respectively for ordinary temperatures. Thus concentration of molecules in a state with an energy kT or $10kT$ higher than our reference is lower than the reference concentration by $e^{-1} = 0.36\dots$ and $e^{-10} = 0.000045\dots$ respectively.

Figure 2 and eqn 1 also show us that, at higher temperatures, the distribution varies less strongly with energy. At the molecular level of explanation, high temperature corresponds to high average levels of molecular energy. To increase the average energy, fewer molecules must have low energy and more must have high. Alternatively, we could say that entropy becomes more important at higher temperature. The

entropy is increased if the molecules are distributed more uniformly among the different energies. At the macroscopic level, we could say that the temperature is raised by putting in heat, and from the definition we saw above, that raises the entropy.

In some systems, the variation of concentration with energy may include other terms, although the Boltzmann factor of eqn 1 is always present. In some systems, the number of available states may vary as a function of energy; however, in such systems the Boltzmann term dominates at high energies. In concentrated systems there are other complications, discussed below under ‘Osmotic effects’. In most texts on thermodynamics and physical chemistry, the Boltzmann factor appears via the chemical potential, which we shall introduce later in this molecular-level development.

Reaction rates and equilibria

The unidirectional rates of reactions are often determined by the Boltzmann term (provided that quantum tunnelling is negligible). If the activation energy of a reaction is E^* in one direction, then inspection of Figure 2 shows that the proportion of molecules having more than that energy is proportional to the shaded portion of the area under the curve. Qualitatively, we see immediately why reaction rates increase at high temperature: more molecules (greater shaded area) have sufficient energy to react. Quantitatively, integration from E^* to ∞ gives this proportion as $\exp(-E^*/kT)$. The forward reaction rate r is proportional to this factor, as well as others, such as the rate of collisions, the fraction having appropriate orientation, etc. Taking logs gives eqn 2.

$$\ln r_m = - E_m^* / RT + \text{constant} \quad (2)$$

(for moles)

If only one direction of a reaction is considered and if all other effects are temperature-independent, then the reaction rate is given by eqn 2, which is Arrhenius's law. Thus the negative slope of an Arrhenius plot – a plot of $\ln(\text{reaction rate})$ vs $1/T$ (where T is in kelvins) gives the activation energy divided by k or R , subject to the caveats above, i.e. a unidirectional reaction and no other temperature-dependent effects. The caveats are important and are only rarely satisfied in biology. For instance, many enzymes become inactive at very low and very high temperatures, owing to such processes as denaturation, de-oligomerization and encapsulation in ice or vitrified solutions. So the slope of a typical Arrhenius plot goes from plus infinity to minus infinity over a temperature range of several tens of $^\circ\text{C}$. This does not imply that the activation energy is infinite and/or negative: rather it means that the local slope of such a plot, divided by k or R , should usually be called

an ‘apparent’ activation energy. (See also [Arrhenius](#), [Svante August](#).)

Further, it is rare to have a reaction proceed in one direction only. If the concentration of reaction products is initially zero, then the initial reaction rate is that of the forward reaction. If no reagents or products are supplied or removed, the rate of the reverse reaction increases until chemical equilibrium is achieved, when the net reaction rate is zero. Applying the Boltzmann factor leads to the equations of chemical equilibrium, for which see also [Thermodynamics in Biochemistry](#).

To deal with chemical and biochemical processes, we need an energy ‘accounting system’ that includes the possibility that a reaction involves doing work on the environment and/or receiving heat from the environment. For this purpose, the quantities enthalpy and Gibbs free energy are introduced.

Enthalpy

The enthalpy is defined as $H = U + PV$, where U is the internal energy, P is the pressure and V is the molecular or molar volume. This quantity appears because, under usual conditions such as constant (atmospheric or other) pressure, molecular volumes may change during biochemical processes. A change in volume ΔV at pressure P requires the performance of work $P\Delta V$, which is the work required to push the surroundings away to allow the expansion ΔV . In a reaction, the energy for this work must be provided by the reacting molecules, and so an increase in V of the activated state will make a reaction a little slower, and a positive ΔV of reaction will lead to a smaller equilibrium constant. For instance, in the process of evaporation of water at

atmospheric pressure ($P \cong 10^5$ Pa), a mole of liquid water (0.018 litres = 1.8×10^{-5} m³) expands to become a mole of water vapour (~20–30 litres, depending on the temperature), so the work done in ‘pushing the atmosphere out of the way’ is ~2–3 kJ.mol⁻¹. At lower pressures, less work is required and consequently the boiling temperature is lower.

In biochemical processes at atmospheric pressure that do not involve the gas phase, however, the value of $P\Delta V$ is usually negligible. A molecular volume change of one cubic nanometre (the volume of thirty water molecules) would be very large in most biochemical contexts, yet here $P\Delta V$ is only 10^{-22} J per molecule, which is ~40 times smaller than kT , the characteristic thermal energy of molecules.

Gibbs free energy

Biochemical processes often occur at constant temperature: this means that any heat that is generated is transferred elsewhere (usually by conduction), and that heat absorbed in a process is likewise provided from neighbouring regions. For such cases, it is useful to use the Gibbs free energy, defined as $G = H - TS = U + PV - TS$, where S is the entropy. Now $T\Delta S$ is the heat gained at constant temperature (from the definition of entropy), so the Gibbs free energy adds to the internal energy the work done in volume changes and also the heat that the process loses to its environment. In the spirit of this article, however, we need to explain why G is the appropriate energy accounting at the molecular level, where the distinction between heat and other energy disappears. We can do this by quantifying entropy at the molecular level: see Box 2.

Box 2: Entropy and molecular disorder

Boltzmann's microscopic definition of entropy is $S = k \ln w$, where w is the number of different possible configurations of a system. This expression gives rise to the idea that entropy quantifies disorder at the molecular level. For example, when a solid melts, it goes from an ordered state (each molecule in a particular place) to a state in which the molecules may move, so the number of possible configurations increases enormously. The entropy produced by equilibrium melting is just the latent heat divided by the temperature. For 1 g of ice at 273 K, this gives 1.2 J.K⁻¹.

Note that this is disorder at the molecular level: *macroscopic disorder is unimportant to entropy*. If one shuffled an initially ordered pack of cards, the entropy associated with the disordered sequence is about 3×10^{-21} J K⁻¹. The entropy produced by one's muscles during the shuffling would be roughly 10^{20} times larger. Another example to stress the irrelevance of macroscopic order is to consider the cellular order of a human. If we took the $\sim 10^{14}$ cells in a human and ‘shuffled’ them, we would lose not only a person, but a great deal of macroscopic order. However, the shuffling would produce an entropy of only $k \ln(10^{14}) \cong 4 \times 10^{-8}$ J K⁻¹, 30 million times smaller than that produced by melting the 1 g ice cube. Again, the heat of the ‘shuffling’ process would contribute a vastly larger entropy.

Consider this example: if a reaction has (for every initial state) w different activated states, all with approximately equal energies, then, all else equal, the reaction is w times more likely. The forward reaction rate would be proportional to $w \exp(-E^*/kT) = \exp(\ln w - E^*/kT)$. Using the result from the box, this gives a reaction rate proportional to $\exp[-(E^* - TS)/kT]$. If we include the possibility of volume change, so that the activation energy $E^* = \Delta U^* + P \Delta V$, we have the forward reaction rate proportional to $\exp(-G^*/kT)$, where G^* is the free energy of activation.

An important conclusion for biology is that the TS term in the Gibbs free energy allows for entropic forces. The forces discussed in mechanics usually involve the internal or potential energy U of a system. For example, the gravitational potential energy of a system comprising the Earth and a mass m is mgh . Here the force is $-\partial U/\partial h = -mg$, which is the weight of the mass. In biological systems, displacements (in a direction x , say) often change the Gibbs free energy, and so the force is $-\partial G/\partial x$. Thus the forces depend on changes both in energy (often electric potential) and entropy. An important example is given in Figure 4.

Other equilibria

From eqn 1 we can simply obtain expressions for electrochemical equilibria (the Nernst equation), for maximal reaction rates, for osmotic pressure, for phase equilibria and for the chemical potential. These are derived from the macroscopic view in classical texts, but here we sketch molecular explanations.

Electrochemical effects

Positively charged ions tend to accumulate in regions of low electrical potential Φ , where their electrical potential energy is low. Entropy, on the other hand, tends to distribute them with equal concentration everywhere. For an ion of charge q , the electrical potential energy is $q\Phi$. At equilibrium, positive ions (cations) accumulate preferentially in regions of low electric potential, while negative ions (anions) have higher concentrations at high Φ . From the Boltzmann distribution, the equilibrium concentration is $c = c_0 \exp(-q\Phi/kT)$. (In mole terms, $C = C_0 \exp(-ZF\Phi/RT)$, where Z is the valence and F is the charge on a mole of monovalent ions, called the faraday.) This is called the Nernst equation, and it is usually written taking logs of both sides: $\ln(c/c_0) = -q\Phi/kT = -ZF\Phi/RT$. Note that the characteristic voltage $kT/e = RT/F \cong 25$ mV at ordinary temperatures, so local biological potential differences are typically of this order.

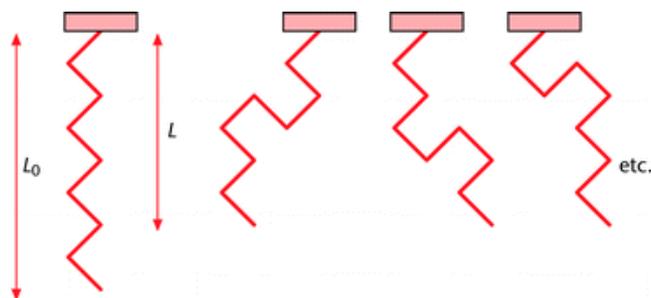


Figure 4. This highly simplified diagram of a polymer, fixed at one end, illustrates how the entropy term in the Gibbs free energy contributes to a force. Force times change in length gives the work done in stretching the polymer, so we examine states with different lengths. In the all-*trans* configuration, the polymer has a length L_0 , but there is only one configuration corresponding to that length, so the entropy associated with the internal bending is $k \ln 1 = 0$. At a shorter length L , there are N possible configurations, of which only a few are shown. The entropy associated with this particular length is here $k \ln N > 0$. Suppose that the extra potential energy associated with the *cis* bonds is ΔU , and neglect changes in the molecule's partial volume. The change in G is $\Delta G = \Delta U - TS_{\text{bend}}$. We consider the case where $TS_{\text{bend}} > \Delta U$, where the shorter state is stable (has a lower G) with respect to the longer. In molecular terms, we would say that the Boltzmann distribution indicates that the ratio of the number of molecules with length L to that with length L_0 is $N \exp(-\Delta U/kT)$, which for this case is > 1 . Note that, when we increase the temperature, the average length decreases – which also happens when one heats a rubber band, and many other polymers. We could also calculate the (average) force exerted by this molecule, $\partial G/\partial L$, and, from a collection of such molecules, we can find the elastic properties of this (idealised) polymer. Finally, note that the entropic term is more important as T increases, because T multiplies S . (In Boltzmann terms, more molecules are in the higher energy state.) This increasing effect with temperature is important for entropic forces, including for the hydrophobic interaction, discussed later in the text and in the article [The Hydrophobic Effect](#).

Ionic distributions across membranes

Because the cytoplasm has a lower electric potential than the external solution, the cytoplasmic concentration of Cl^- ions is lower at equilibrium. In contrast, permeating cations usually have higher cytoplasmic concentration at equilibrium, as shown in Figure 5. Note, however, that many ions and other charged molecules do not equilibrate across membranes over ordinary time scales. Some do not permeate (proteins and other large molecules), some cross the membrane under certain conditions, while others are continuously maintained in a non-equilibrium state. The negative potential in the cytoplasm is in part maintained by ‘pumps’, i.e. mechanisms that, by expending energy, produce a flux of specific ions in a particular direction. See also [Membrane Potential, Ion Channels, Sodium, Calcium and Potassium Channels, Cell Membranes: Intracellular pH and Electrochemical](#)

[Potential, Ion Transport at the Plant Plasma Membrane and Ion Transport across Nonexcitable Membranes.](#)

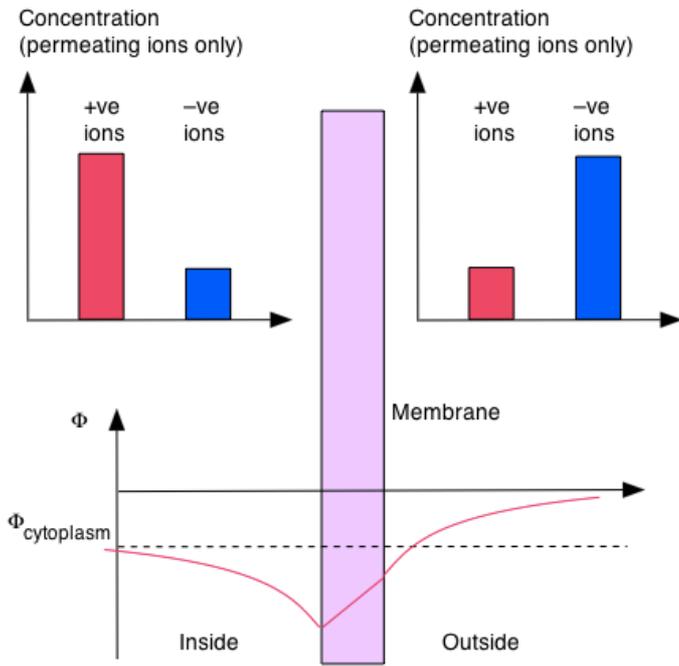


Figure 5. The Boltzmann distribution explains the equilibrium distribution across a membrane of permeating positive (+ve) and negative ions. The electric potential energy per unit charge is called the electric potential or voltage (Φ). For a permeating cation, the left (low Φ) has lower energy, while for permeating anions it is on the right. The highest-entropy state would have equal concentrations of the permeating ions everywhere. Note that this distribution applies only to freely permeating molecules that are not actively pumped. In practice, the total number of positive charges inside a cell differs only by a very tiny fraction from the total number of negative charges. In terms of this diagram, many of the extra intracellular anions required to approach electroneutrality are found on non-permeating species, including macromolecules, while the extracellular solution will also contain many cations that are expelled from the cell by active (energy-using) processes.

Ionic distributions near charged membranes and macromolecules

Similarly, in the vicinity of the negatively charged surface of a membrane or a macromolecule, the concentration of cations in the solution is higher than in the bulk solution. The surfaces of many membranes are, on average, negatively charged, so there is usually an excess of cations and a depletion of anions near the membrane, as shown in Figure 6. Many macromolecules have a small excess of negative charge and so are surrounded by a region of similarly perturbed ion concentration. See also [Membrane Potential](#).

Figure 6 shows the mean field approximation: the assumption that the distribution of an individual ion is determined by the average distribution of all other ions. Locally, this is a severe simplification: near an anion

one is more likely to find a cation than another anion, so there are local correlations in the distribution. These are important in the electrical interaction between macromolecules or membranes and can even lead to counterion-mediated attractions between like-charged macromolecules (Grønbech-Jensen *et al.*, 1997).

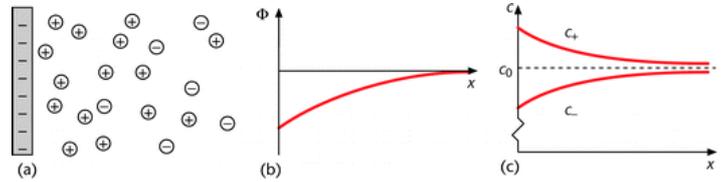


Figure 6. A negatively charged surface in an ionic solution gives a non-uniform ion distribution over a scale of nanometres (a). Positive ions are attracted towards the interface, negative ions are repelled. The layer of excess positive ions (called a double layer) counteracts the electrical effect of the charged surface at sufficiently large distance. (b) and (c) show how the electrical potential Φ and the ionic concentrations c_+ and c_- vary near the surface, but approach their bulk concentration c_0 at large distance x from the charged surface. The electric charge of ions has another effect that contributes to their distribution. The self-energy or Born energy of an ion is the energy associated with the field of the ion itself. This depends upon the medium, and is lower in polar media such as water than in nonpolar media such as the hydrophobic regions of membranes or macromolecules. Thus ions are found in only negligible concentration in the hydrocarbon interior of membranes, where their Born energy is very high. This energy is also thought to affect the distribution of ions inside aqueous pores or near protein molecules: if the ion is sufficiently close (\sim nm) to a nonpolar region of membrane or macromolecule, it still has a high Born energy because its field penetrates into the nonpolar regions. The Born energy and the Boltzmann distribution mean that the concentration of ions of either sign is lower than would be otherwise expected, not only in nonpolar regions, but also in the aqueous solution very near such regions. Again, the equilibrium distribution is a compromise between minimizing the Born energy (ions keep away from nonpolar regions) and maximizing entropy (ions distribute uniformly everywhere).

The local correlations in ion distribution also lead to electrostatic screening: electrical interactions are weaker in solution than they would be in pure water because ions of the opposite sign (counterions) tend to accumulate near charges and screen their effects. Further, electrical effects in pure water are much weaker than they would be in vacuum because water molecules are dipolar, which means they have a tendency to rotate so that the positive charge at the hydrogen side of the molecule and the negative charge on the oxygen side act to reduce any electrical effects. Once again, the highest entropy would yield a random orientation of water molecules, but this is compromised because the energy is lower in the arrangement just described. (An empirical way of describing this effect for large volumes is to say that water has a dielectric constant of about 80: electrical effects in water are about 80 times weaker than those produced by the same charge distribution in vacuum.)

Osmotic effects

A difference in hydrostatic pressure produces a difference in energy: to move a molecule whose partial molecular volume is V from a reference pressure of zero to a higher pressure P requires an energy PV . Substituting this energy in the Boltzmann distribution (eqn 1a) gives the equilibrium osmotic distribution. Usually we are concerned with the equilibration of water, because water permeates many biological barriers more easily than most solutes. Pressures are usually measured with respect to atmospheric pressure.

Many membranes are permeable to water but impermeable to macromolecules, sugars and some ions. See also [Water Channels](#), [Water Channels: Aquaporins](#) and [Water Transport across Cell Membranes](#). (Some small organic molecules such as ethanol and dimethyl sulphoxide pass relatively easily through membranes, so their osmotic effects are usually transient.) Figure 7 shows a semipermeable membrane with pure water on the left and a solution of an impermeant solute on the right. Suppose that initially the pressure on both sides is zero (Figure 7a). We notice that the solutes are not in equilibrium because their concentration is higher on the right, so they would diffuse to the left if there were no membrane. The equilibrium of the solute does not interest us, however, because the semipermeable membrane prevents it from being achieved. Water can permeate through the membrane, however. Further, water is not in equilibrium in this situation, because the concentration of water is higher on the left: if there is no pressure difference, water will move from the pure water on the left into the solution on the right where the water is, one might say, diluted by the solutes. So water moves through the membrane from pure water (low solute concentration) into the side with higher solute concentration. If we put, say, a blood cell of any sort into pure water, water would flow into the cell, which would expand until the membrane burst. If the membrane is supported by something stronger, such as a plant cell wall, the expansion continues only until the mechanical tension in the wall is large enough to resist a substantial difference in hydrostatic pressure. Hydraulic equilibrium is achieved when the lower concentration of water in the high-pressure side is balanced by the higher pressure and therefore higher PV energy on that side. Large osmotic pressure differences (typically 1 MPa or 10 atmospheres) are commonly found in plants, where water is close to equilibrium between the extracellular solution (with low solute concentrations) and the much more concentrated intracellular solution. The plant cell wall resists the difference in hydrostatic pressure (Slatyer, 1967; see also [Plant Water Relations](#)). In animal cells, there is no comparably strong structure, so the

hydrostatic pressure differences are much smaller. See also [Cell Membrane Features](#).

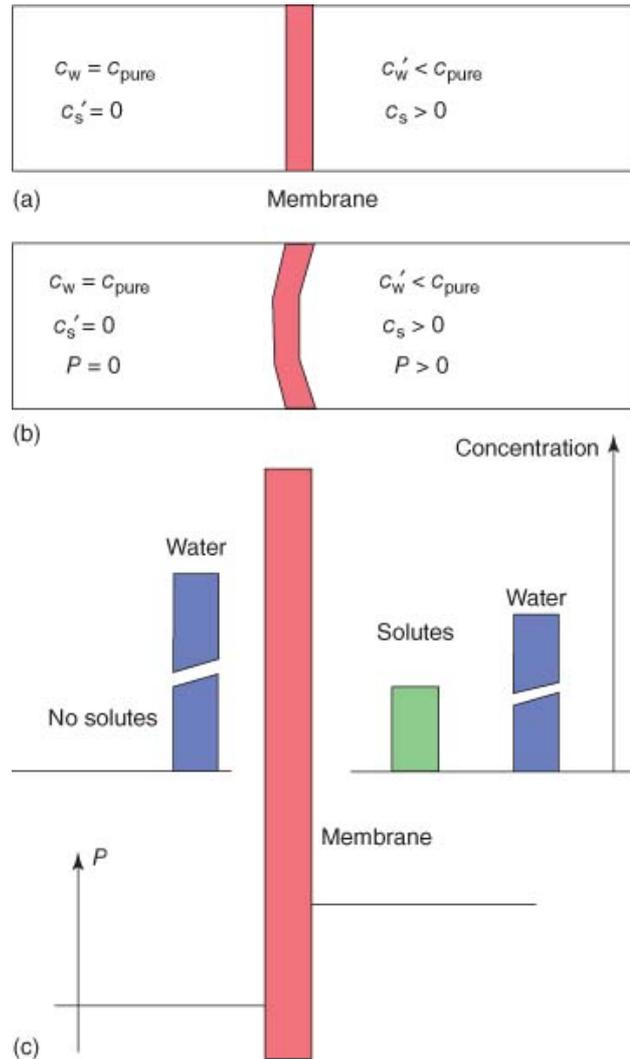


Figure 7. The membrane shown in (a) is permeable to water but not to solutes. Water flows towards the more concentrated solution (which has a lower water concentration). This flow stops and hydraulic equilibrium is achieved when the pressure P in the concentrated solution is sufficiently high. The system in (b) has a higher energy than (a), but it also has higher entropy. Entropy will be maximised if the membrane bursts and the solutions mix freely. Using the Boltzmann distribution with PV as the energy difference and making the approximations that solute volume and concentrations are small, we obtain the condition for osmotic equilibrium – the value of the hydrostatic pressure difference required to stop further flow of water into the solution side. In the case of a solution, the number fraction of water molecules, rather than their concentration, must be used in eqn 1a. Using the approximation that solute volume and concentration are small, one readily obtains an approximate expression for the equilibrium pressure difference, $P_{osm} \cong kTc_s = RTC_s$, where the solute concentration is c_s in molecules m^{-3} or C_s in $kmol m^{-3}$ respectively. Note that water concentration is usually much higher ($\sim 50 kmol.m^{-3}$) than solute concentrations, and so the proportional difference in water concentration is small. However, rates of diffusion and permeation depend on the absolute difference. A solution of $1 kmol.m^{-3}$ or $1 mol.litre^{-1}$ of non-dissociating solute gives an osmotic pressure of approximately 2 MPa, or 20 atmospheres, the exact value depending on temperature and on some corrections due to solute–solvent interactions.

Further osmotic effects

The semipermeability of membranes is not the only thing that prevents solutes and water from mixing completely. Sufficiently large solutes are prevented from entering the narrow spaces between closely packed macromolecules or membranes. Further, the centre of a large solute cannot usually approach more closely than about one radius from an ultrastructural surface (called the excluded volume effect). These effects complicate the osmotic forces acting on macromolecules and membranes (Parsegian, 2000). When cells are dehydrated by extracellular freezing or air drying, the highly concentrated solutes produce extremely large osmotic effects. In these cases, studies of the phase equilibria show how the mechanical stresses and strains associated with freezing and dehydration damage in cells can be substantially reduced by osmotic effects (Wolfe and Bryant, 1999).

The chemical potential

The chemical potential presents another way of writing the Boltzmann equation $C = C_0 \exp(-E/RT)$. Taking logs and rearranging gives eqn 3.

$$RT \ln C_0 = RT \ln C + E \quad (3)$$

E , the difference in energy between the state considered and the reference state, may include the work done against pressure, the electrical potential energy and other terms if necessary. The utility of this formulation is that, at equilibrium at a given temperature T , one sees that the quantity $(RT \ln C + E)$ does not vary with position in a given phase. For the moment, let PV_m be the only energy term, as is the case for an electrically neutral molecule, where V_m is the partial molar volume. The chemical potential μ is defined in eqn 4.

$$\mu = \mu^\circ + RT \ln C + PV_m = \mu^\circ + RT \ln C_0 \quad (4)$$

where μ° , called the standard chemical potential, is the value of μ in some reference state with concentration C_0 . (The reference state is often the pure substance). Thus the Boltzmann distribution translates as the statement that the chemical potential is uniform at equilibrium. The significance of μ° is seen when we consider the distribution of molecules among different phases. See also [Cell Biophysics](#).

Effects of different molecular environments and phases

Energy is required to move a molecule from a solid, where it is usually strongly bound to its neighbours, into a liquid, where it is less strongly bound. Further energy is required to take it out of the liquid into a gas. Here is an approximate guide for small molecules: in a

solid, the average intermolecular energy of attraction is $\gg kT$; in a gas it is $\ll kT$, in a liquid it is comparable with kT . The entropy in a solid is lowest because the molecules have least motion and therefore relatively few different configurations, and it is greatest in a gas. The energy also increases from solid to liquid to gas, as we supply the latent heats of fusion and evaporation. Thus the coexistence of two phases in equilibrium, such as ice in equilibrium with an aqueous solution, and of that solution in equilibrium with water vapour, gives another example of the entropy–energy compromise.

Similarly, the energy of a solute molecule is different in different solvents such as water, alcohol and oil. The energy of a molecule (e.g. a nonpolar anaesthetic) is different when it is in an aqueous solution from its energy when it is partitioned into the interior of a membrane. These differences in what we might call the energy of the molecular environment may similarly be included in the standard chemical potential μ° .

If molecules in phase 1 and phase 2 equilibrate at temperature T and pressure P , then substituting the difference in environmental energy ($\mu_1^\circ - \mu_2^\circ$) into equation 1, taking logs and rearranging gives:

$$\mu_1^\circ + kT \ln c_1 + PV_1 = \mu_2^\circ + kT \ln c_2 + PV_2 = \mu \quad (5a)$$

$$\mu_{m1}^\circ + kT \ln C_1 + PV_{m1} = \mu_{m2}^\circ + RT \ln C_2 + PV_{m2} = \mu_m$$

where the chemical potential is μ_m per mole and μ per molecule. If we include in the energy terms the electrostatic potential energy discussed above ($Ze\Phi$ or $ZF\Phi$), we obtain the electrochemical potential $\mu = \mu^\circ + kT \ln c + PV + Ze\Phi$, which should be used when discussing the equilibrium of ions. In principle, one could also include the gravitational potential energy $mg\Delta h$ in the chemical potential. This is rarely necessary. Because diffusion over large distances is slow, one usually only applies the chemical potential in cases for which $mg\Delta h$ is much smaller than the other terms. In the example of Figure 3 the term is important because we consider heights in kilometres.

In eqn 5a, $(k \ln c)$ represents the entropy associated with a solute/gas molecule's freedom to translate: to sample the volume available. We return to this under 'Molecular Aggregation'. We can now rearrange eqn 5a to retrieve the Boltzmann distribution: (eqn 1a).

Now, using the standard chemical potentials to quantify the energy and entropy changes between different phases or molecular environments, we relax the constraint concerning a single phase in the section above. The chemical potential is a quantity that is equal in any regions in which molecules are distributed according to the Boltzmann distribution, so we have an important result: chemical potentials are equal

throughout a region in which molecules are in equilibrium.

Further, in the absence of active processes, molecules diffuse so as to achieve equilibrium, which yields another important result: molecules diffuse from high μ to low μ , at a rate that increases with the gradient in μ . It is important to note this distinction, however: Molecular diffusion (individual molecular motion) follows the negative gradient in μ , but this does not apply to bulk flow (collective molecular motion). If a jug of milk is held above a cup of water, the milk (water plus solutes) will *flow* from jug (high mgh) to cup (low mgh): macroscopic volumes of fluid behave according to the mechanics of the macroscopic world. However, if you sealed the cup and jug in a box, whatever their heights, water would very slowly diffuse, via the vapour phase, from the cup of water (water at high μ) to the jug of milk (low μ).

Surface energies and surface tensions

A further important free energy difference in cellular biology is that between water molecules at the surface and water molecules in the interior of the water/solution volume. This is introduced in Figure 8. In surfaces between two pure fluids having a surface tension γ , the extra work done in introducing a molecule of area a to the interface is γa : the surface tension equals the free energy per unit area. Surface tension is by definition the force acting per unit length in the plane of the interface, so it scales with the linear dimension of objects. Contrast this with weight, which scales with volume and thus with the cube of linear dimension. It follows that surface tension is much more important in the microscopic world than in the macroscopic. Surface tension is negligible for a human swimmer, but it is the force that supports those insects that walk on water. On the molecular scale, weight is negligible but surface tension can be dominant: on the ultrastructural scale, the surface tension of water is what holds us together. For that reason a surface term must often be introduced into the Boltzmann distribution and thus to the chemical potential.

Molecular aggregation

A single molecule has an entropy of translation, which is the entropy associated with its freedom to diffuse throughout the available volume. When molecules aggregate to form oligomers or macroscopic ultrastructure, that entropy of translation is shared among N participating molecules, so the entropy per molecule is divided by the number N in the aggregate.

Thus, if the energy of aggregation is independent of T , the proportion of monomers will increase with T as this entropy becomes more important. Molecular aggregation is important in various processes, including the aggregation of several proteins to produce an oligomeric enzyme and the assembly of cellular ultrastructure.

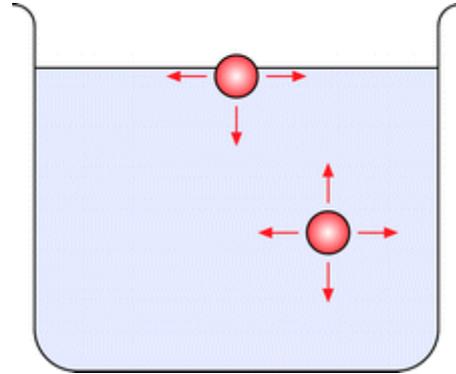


Figure 8. The surface free energy of water. In bulk solution, water molecules are transiently hydrogen-bonded to their neighbours. A water molecule at an air–water surface or a hydrocarbon–air interface has fewer neighbours with which to form hydrogen bonds, so its energy is higher. Because molecules will tend to rotate to form bonds with their neighbours, the surface is more ordered and therefore has a lower entropy per molecule. Together, these effects give the water surface a relatively large free energy per unit area, which gives rise to the hydrophobic effect. The work done per unit area of new surface created is numerically equal to the surface tension γ , which is the force per unit length acting in the surface. This result is simply derived: if one expands the surface by drawing a length L a perpendicular distance dx , the force acting along the length L is γL , so the work done is $\gamma L dx = \gamma dA$, where dA is the increased area.

We shall consider simple lipid aggregations and membranes as an example, because it involves another important energy term. Consider a single lipid molecule, in water, with a standard chemical potential μ_1° in equilibrium with a micelle or vesicle comprising N molecules (Figure 9a and b). The standard chemical potential μ_N° in the aggregate is much lower, because the aggregate has little hydrocarbon–water interface per molecule, while the monomer has much. (Note that, because this is a single molecule, the surface energy between the hydrocarbon and water has been incorporated into μ° , rather than considered as a separate term. See Israelachvili *et al.* 1980 for details. See also [Hydrophobic Effect](#), [Lipid Bilayers](#), [Water: structure and Properties](#), and [Cell Membrane Features](#).)

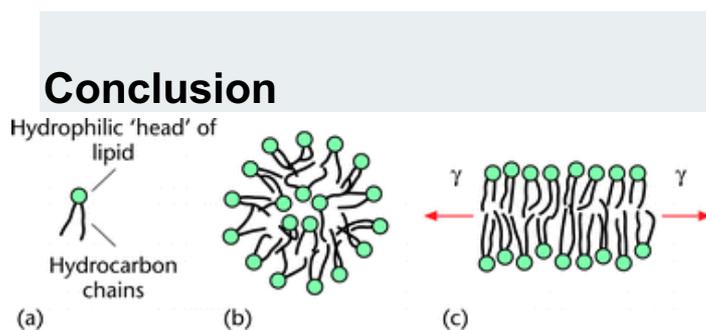
The membrane may be subject to a tensile force per unit length γ , and this may do work by changing the area. The equilibrium here is given by:

$$\mu_1^\circ + kT \ln X_1 = \mu_N^\circ + \frac{kT}{N} \ln \frac{X_N}{N} = \mu_m^\circ - \gamma \frac{a}{2}$$

where μ_m° is the standard chemical potential of lipids in the membrane and X_1 and X_N are the number fraction of lipids in the monomer and vesicle state, respectively. Because of the large number of molecules in the membrane, the translational entropy per molecule is

negligible. The factor $\frac{1}{2}$ in the last term arises because each lipid molecule has an area a in only one face of the membrane. Notice the effect of the various terms: if the lipid has more or longer hydrocarbon tails, then the extra 'environmental energy' difference due to the hydrophobic effect will increase ($\mu^{\circ}_1 - \mu^{\circ}_N$) giving rise to a lower monomer concentration. Increasing the tensile stress in the membrane (larger γ) makes the last term more negative, so monomers (and also vesicles, if a suitable mechanism exists) will move into the membrane, increasing the area and perhaps relaxing the stress. Alternatively, changing the area of a membrane causes a possibly transient mechanical change in γ , and thus a transfer of membrane material between membrane and a reservoir of material. Although the exchange mechanisms are not understood, it is possible that such processes occur in some cell membranes, where regulation or homeostasis of membrane tension and cell area have been reported (Wolfe and Steponkus, 1983; Morris and Homann, 2001).

Figure 9. The equilibria among a lipid monomer (a), a vesicle containing N molecules (b) and a macroscopic lipid bilayer membrane (c) shows the effect of molecular aggregation and mechanical stress.



The ubiquitous Boltzmann distribution explains, at a molecular level, a range of effects and processes important to biochemistry and cell biology. This chapter may also be considered as an introduction to further reading in other articles including those dealing with aspects of thermodynamics in biochemistry, cell biophysics, membranes, macromolecules, water, hydrophobic effect, plant stress, and in other works in these fields.

Acknowledgments

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Glossary

Temperature (T)

That quantity that is equal in two objects in thermal equilibrium. A measure of how hot a body is, or how much energy is associated with each type of random motion of and in molecules. Of itself, heat flows from higher to lower temperature.

Heat (Q)

Energy that is transmitted only by a difference in temperature. Adding heat may increase the energy associated with molecular motion (and thus increase the temperature), or it may increase intermolecular and/or intramolecular potential energies (in a change of phase or in endothermic reactions).

Work (W)

Energy transmitted when the point of application of a force is displaced. (When the point of application of a force \mathbf{F} is displaced a distance $d\mathbf{s}$, the work done, $d\mathbf{W}$, is the scalar product $\mathbf{F} \cdot d\mathbf{s}$, i.e. the magnitude of \mathbf{F} times the component of $d\mathbf{s}$ parallel to \mathbf{F} .)

Entropy (S)

The ratio of the heat energy transferred to the temperature at which it is transferred. It is also a measure of disorder at the molecular level only.

Equilibrium

In a system into which no matter or energy enters and from which none leaves (a closed system), equilibrium is the state achieved when parameters no longer change over time.

Steady state

A state of a system in which parameters are not changing with time, but in which energy and /or matter may be entering and leaving the system (an open system).

Gibbs free energy (G)

G is a measure of the work that may be extracted from a system at a constant pressure and temperature.
 $G \equiv U + PV - TS$.

Osmotic pressure (Π)

If a solute equilibrates between two bulk solutions with different composition, the solution with the lower mole fraction of solvent (i.e. the more concentrated solution) must have a higher (hydrostatic) pressure. This difference in pressure is called the difference in osmotic pressure.

Chemical potential (μ)

μ is a quantity of a particular component that has the same value at all places where that compound is in equilibrium. μ for a component equals the variation in a thermodynamic potential with respect to change in the amount of that component.

Surface tension (γ)

The force per unit length required to increase the area of a surface. γ equals the change in surface energy per unit area.

Photon

The carrier of the minimum quantity of electromagnetic radiation of a given wavelength. Just as one molecule is the smallest possible quantity of water, a photon is the smallest possible quantity of light.

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Key Concepts:

- Cells and components that are physically close together are often approximately in thermal and chemical equilibrium, which allows some powerful observations about their properties.
- Cells and tissues sometimes approximate steady state: they input and output energy and matter at approximately equal rates, and vary little over time.
- Entropy measures the 'uselessness' of energy: the biosphere survives by converting energy with low entropy (light from the sun) into energy with high entropy (waste heat lost to the atmosphere and to space).
- Entropy also quantifies disorder – but only disorder at a molecular level.
- The Boltzmann energy distribution leads directly to a number of important laws, quantities and effects relevant to cell biology:
 - Arrhenius' law of reaction rates,
 - ion equilibria,
 - osmotic pressure,
 - the chemical potential,
 - phase equilibria, and
 - molecular oligamerisation and aggregation.