CHAPTER

4

Thermal Forces and Diffusion

In addition to the mechanical forces discussed in Chapter 2, proteins and cells are subject to **thermal forces** that arise from collisions with water and other molecules in the surrounding fluid. During each short-lived collision, the change in momentum of the fluid molecule imparts an impulsive force on the object that it strikes. These collisional forces are called thermal forces because their amplitudes are proportional to the temperature of the fluid molecules. The resulting movement is called **thermal motion**, and the object is said to have **thermal energy**. Because the forces are randomly directed, the motion is characterized by frequent changes in direction and is called **diffusion**. The diffusion of a free particle or molecule is called **Brownian motion**.

Understanding thermal motion is crucial for molecular and cellular mechanics because the chemical reactions that drive biological processes have energies that are only a little higher than thermal energy; as a result, diffusive motions are quite large compared to directed ones, and thermal fluctuations are necessary for proteins to reach their transition states. It is the noisy, diffusive environment in which protein machines operate that distinguishes molecular machines from the macroscopic machines that we experience in our everyday world.

This chapter begins with Boltzmann's law, the fundamental physical law that describes how the probability of a molecule having a certain energy depends on the surrounding temperature. We then discuss some of the many corollaries of Boltzmann's law. These include the Principle of Equipartition of Energy, which states how much thermal energy a molecule has at a certain temperature, and the Einstein relation, which relates the diffusion coefficient of a molecule to its drag coefficient. Next we examine the diffusion of molecules under several different circumstances. One of these, diffusion up an energy gradient (i.e., against a force), is especially interesting and important because it makes

predictions of how forces affect the rates of diffusion-limited reactions, a subject that is explored in more detail in Chapter 5. The present chapter ends with a discussion of the dynamics of a particle or molecule undergoing Brownian motion. We show that thermal motion can be exactly simulated by an applied mechanical force of a particular amplitude and time course that acts via the damping elements (the dashpots).

Boltzmann's Law

A particle or molecule always tends towards its lowest energy state. However, except at the absolute zero of temperature, the particles are agitated by molecular collisions. As a result, they do not spend all their time in the state with the lowest energy, but instead spend a fraction of their time in states with higher energy (Figure 4.1). **Boltzmann's law** says that if such a particle (or a group of particles or molecules in a larger system) is in thermal equilibrium, then the probability, p_i, of finding the particle (or group) in a state i that has energy U_i is

$$p_i = \frac{1}{Z}\exp\left[-\frac{U_i}{kT}\right] \qquad \text{where } Z = \text{constant} = \sum_i \exp\left[-\frac{U_i}{kT}\right] \quad (4.1)$$

k is the Boltzmann constant and T is the absolute temperature. The Boltzmann constant is equal to 1.381×10^{-23} J/K. At the standard temperature of 25°C, corresponding to 298.15 K, kT is therefore equal to 4.116×10^{-21} J (Table 4.1). The value of kT is compared to other biologically relevant energies in Table 4.2. Z is a constant, sometimes called the **partition function**, whose value assures that the sum of all the probabilities adds up to 1 ($\Sigma p_i = 1$). Equation 4.1 is sometimes called Boltzmann's equation, Boltzmann's distribution, or Boltzmann's formula, and the exponential term in the equation is called the Boltzmann factor.

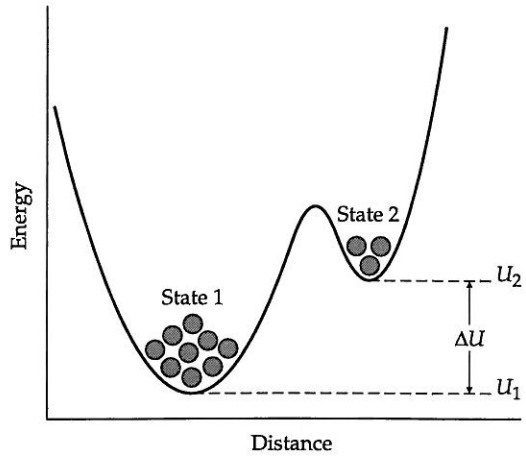


Figure 4.1 Molecules in an energy landscape
According to Boltzmann's law, the probability of finding a molecule in state 2 relative to state 1 is $\exp(-\Delta U/kT)$.

Table 4.1 Thermal energy

Quantity	25°C	37°C
kT	4.1164×10^{-21} J	4.2821×10^{-21} J
RT (= NkT)	2.4789 kJ/mol	2.5787 kJ/mol
RT	0.5921 kcal/mol	0.6159 kcal/mol
kT/e (= RT/F)	25.69 mV	26.73 mV

Note: Conversions: Temperature, 0 K = –273.15°C; Energy, 1 calorie = 4.1868 joules.

Boltzmann's law is very general. The energy could correspond to the particle's potential energy (gravitational, elastic, or electrical), its kinetic energy, or the energy associated with its phase, or electronic or chemical state. The state of a particle (or group of particles) is specified by the position and velocity of the constituent atoms as well as their electronic states. If there are just two states with energies U_1 and U_2 (and energy difference $\Delta U = U_2 - U_1$), then the ratio of the probabilities of being in the two states is

$$\frac{p_2}{p_1} = \exp\left(-\frac{\Delta U}{kT}\right) \tag{4.2}$$

Boltzmann's law is a corollary of a postulate in statistical mechanics stating that each configuration of a closed system (a system of fixed total energy) is equally likely. The derivation of Boltzmann's law from this postulate can be found in Pauling (1970) and Berg (1993). Boltzmann's law is the most important physical law in biology and chemistry because it has so many consequences. Some of these consequences are illustrated in Example 4.1, some are illustrated in the next sections, and some in the next chapter. Boltzmann's law is also nonlinear, a point that should be heeded by those who seek linear approximations.

In our statement of Boltzmann's law we defined neither equilibrium nor temperature. These are deep concepts that might take an entire course in statistical physics to explore. Luckily, there is a simple way out. So fundamental is Boltzmann's law that we can use it to define temperature and equilibrium. We will say that a system is at **equilibrium** if Boltzmann's law holds, and we will define the temperature as the corresponding constant in the exponent of Equation 4.1. This definition of equilibrium is consistent with the more usual definition of equilibrium and temperature. First, if two systems are each in

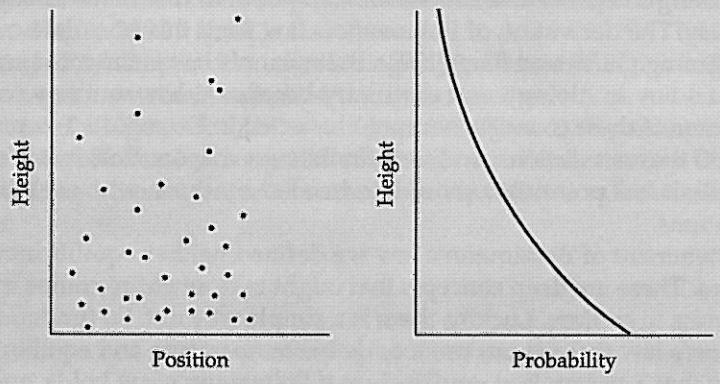
Table 4.2 A comparison of energies

Energy	Formula	Value (10^{-21} J)
Thermal energy (25°C)	kT	4.1
Photon (green, λ = 500 nm)	$h\nu = hc/\lambda$	397
ATP hydrolysis in the cell	ΔG	100
Electron transport (180 mV)	eV	28.8

equilibrium with a third system, then the two systems are in equilibrium with each other. This follows from Boltzmann's law because the exponential function satisfies $\exp[-a] \times \exp[-b] = \exp[-(a + b)]$. And second, if a system is at equilibrium then it is at **steady state**, meaning that its (average) properties do not change with time. This follows from Boltzmann's law because if the energy U does not change with time (as we are implicitly assuming in our statement of the law), then the probability p also does not change with time. The difference between steady state and equilibrium is that in the former there can be a constant net flux of particles, but in the latter the net flux is zero.

Example 4.1 Applications of Boltzmann's law

EARTH'S ATMOSPHERE. The density of molecules in a gravitational field falls exponentially with the height (see figure below). The gravitational potential energy of a particle of mass m is $U = mgh$, where g is the acceleration due to gravity and h is the height above the Earth's surface. Let h_0 be the height at which this energy equals kT (i.e., $mgh_0 = kT$). According to Boltzmann's law, the probability of finding the particle at this height is 37% ($1/e$) that of finding it at zero height. For an oxygen molecule of molecular mass 32, $h_0 \cong 7.5$ km, the approximate height of Earth's atmosphere.



SETTLING OF BEADS. Instead of an oxygen molecule, consider a glass sphere of density (ρ) twice that of water (ρ_w), and radius r. The corresponding height of the "atmosphere" is

$$h_0 = kT/mg = kT/(\rho - \rho_w)Vg = 3kT/4\pi(\rho - \rho_w)gr^3$$

For a 200-nm-diameter bead, this corresponds to ~100 μm. For a 2-μm-diameter bead, the height decreases 1000-fold to 100 nm.

ANALYTIC CENTRIFUGATION. A molecule in a centrifuge experiencing a centrifugal acceleration, a_c, has potential energy $U = (m - m_w)a_c h$, where $m - m_w$ is the additional mass over that of the displaced solvent and h is

the height above the bottom of the centrifuge tube. For a 100 kDa protein in water ($m - m_w \cong 66 \times 10^{-24}$ kg) spinning at $a_c = 10{,}000\ g$, $h_0 \cong 6$ mm. In an analytic ultracentrifuge, the protein "atmosphere" is measured and h_0 estimated from the exponential decrease in the protein concentration; this allows measurement of the mass of the protein.

THE NERNST EQUATION. If molecules with charge q are free to equilibrate between two compartments at electrical potential 0 and V volts, then the concentrations in the two compartments, C_0 and C_V are related by

$$\frac{C_V}{C_0} = \frac{p_V}{p_0} = \exp\left[-\frac{U}{kT}\right] = \exp\left[-\frac{qV}{kT}\right]$$

This is known as the **Nernst equation** (Hille, 1992). At 37°C, kT/e equals 26.7 mV (see Table 4.1), where e is the charge of the electron. The Nernst equation says that for each 26.7 mV increase in voltage, the concentration of monovalent cations decreases e-fold.

Equipartition of Energy

Boltzmann's law allows one to calculate the average thermal energy of a molecule (or system of molecules). Suppose that the molecule is at equilibrium in an **energy landscape**, $U(x)$, that varies with position, x, but not with time. For example, the molecule could be connected to a spring with potential energy $U(x) = \frac{1}{2}\kappa x^2$, where x is the extension (Figure 4.2). Due to thermal agitation, the molecule is constantly changing position. Now there are two ways that we can calculate the statistical properties of the molecule's position, such as its mean or its variance. First, we could follow the molecule over a long period of time, T, and measure its time-averaged **mean** position or **mean-squared** position

$$\langle x \rangle_T \equiv \frac{1}{T}\int_0^T x(t)\cdot dx \qquad \langle x^2 \rangle_T \equiv \frac{1}{T}\int_0^T x^2(t)\cdot dx$$

Alternatively, we could use Boltzmann's law to calculate the probability, $p(x)$, of finding the molecule at position x, and then calculate the **expected value** of the position or position squared according to

$$E(x) \equiv \int_{-\infty}^{\infty} xp(x)\cdot dx \qquad E(x^2) \equiv \int_{-\infty}^{\infty} x^2 p(x)\cdot dx$$

If we measure for a long enough time, then these two estimates of the average position should agree with each other

$$\langle x \rangle \equiv \langle x \rangle_\infty = E(x) \qquad \langle x^2 \rangle \equiv \langle x^2 \rangle_\infty = E(x^2) \qquad (4.3)$$

In this way, we can relate measurements (time averages) to the expectations based on Boltzmann's law. Equation 4.3 is the link between experiment and theory! Equation 4.3 holds generally for any function of x: $E[f(x)] = \langle f(x) \rangle$. In particular it holds for the **variance** of x,

$$\sigma_x^2 \equiv \langle (x - \langle x \rangle)^2 \rangle = \langle x^2 \rangle - \langle x \rangle^2$$

Often the mean of a variable is zero (e.g., the mean extension of a spring, the mean velocity), in which case the variance is equal to the mean square.

This approach can be used to calculate the average energy of a molecule. For example, consider the spring in Figure 4.2. The average energy is

$$\langle U \rangle = \tfrac{1}{2}\kappa \langle x^2 \rangle = \tfrac{1}{2}\kappa \int_{-\infty}^{\infty} x^2 p(x) \cdot dx = \tfrac{1}{2} kT \qquad (4.4)$$

where we have used Boltzmann's law for $p(x)$ (Equation 4.1) and evaluated the integral in Appendix 4.1. This result is remarkable because the average energy does not depend on the stiffness of the spring! It only depends on the temperature. This is a special case of a general theorem known as the **Principle**

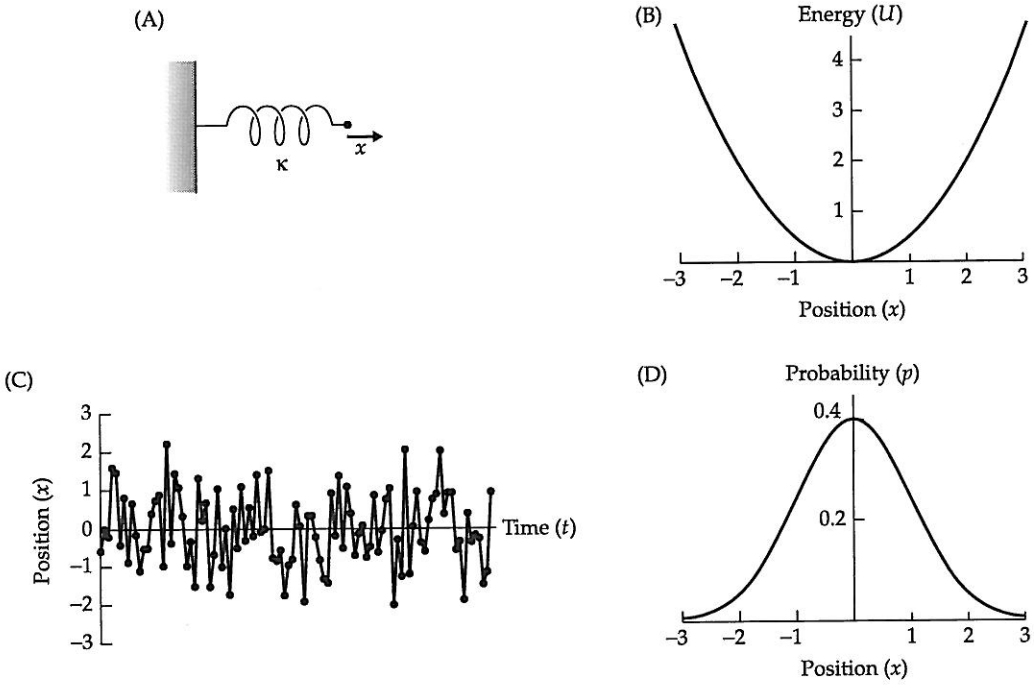


Figure 4.2 Thermal fluctuations of a molecule attached to a spring
A molecule attached to a spring (A) sits in a parabolic potential well (B). While it fluctuates (C), it spends more time near the center of the well (the deepest region) than at the periphery; its probability distribution (D) is a Gaussian (or Normal) distribution, which is peaked at the center.

of Equipartition of Energy, which states that if the energy of a molecule depends on the square of a parameter such as position or speed, then the mean energy associated with the degree of freedom measured by that parameter is $\langle U \rangle = \frac{1}{2}kT$. Another example of the principle is that the average kinetic energy of a molecule (in one direction) with mass m is $\langle K.E. \rangle = \frac{1}{2}m\langle v^2 \rangle = \frac{1}{2}kT$. If the molecule (or system of molecules) has two (or more) degrees of freedom that are independent, as defined in Appendix 4.1, then each degree of freedom contains $\frac{1}{2}kT$ of energy. An example of independent degrees of freedom are the components of the velocity of a molecule in the x-, y-,and z-directions: There is $\frac{1}{2}kT$ of kinetic energy in each, so the total kinetic energy is $\frac{3}{2}kT$. The **root-mean-square** speed, v_{rms}, of a molecule in three dimensions is therefore

$$v_{\text{rms}} = \sqrt{\langle v^2 \rangle} = \sqrt{\frac{3kT}{m}} \tag{4.5}$$

For example, the root-mean-square speed of a water molecule is 640 m/s (in air or water), that of a 100 kDa protein is 8.6 m/s at 25°C, and that of a bacterium of volume 1 μm^3 is 3.5 mm/s.

The Equipartition principle breaks down in a number of circumstances. First, it is generally true only if the energy dependence is quadratic (see, e.g., Problem 4.7). Second, it breaks down if the thermal energy kT is small compared to the energy levels between different quantum states. In this case, the degree of freedom is said to be "frozen out." But for proteins at room temperature, thermal energy is large compared to the mechanical vibrational energy levels because proteins are relatively soft materials, and so the Equipartition principle applies to elastic deformations of proteins (Appendix 4.1).

Diffusion as a Random Walk

The forces that agitate molecules cause diffusion. Diffusion is a form of random motion that is characterized by frequent, abrupt changes in direction. The randomness is the result of the collisions with surrounding molecules, which themselves are moving in random directions. Some examples of diffusive motion are shown in Figure 4.3. The aim of this section is to derive the diffusion equation, which describes how the average concentration of a collection of molecules changes over time due to the diffusive motion of the individual molecules.

Diffusion plays a crucial role in many physical and chemical processes. Einstein was cited in his Nobel prize award for elucidating the molecular mechanism of Brownian motion. His original papers (see Einstein, 1956) are still among the clearest treatments of the subject. Careful measurements of the diffusion of micron-sized particles by Perrin and Svedberg, who won Nobel prizes in 1926 in physics and chemistry, respectively, confirmed Einstein's theory and permitted the measurement of Boltzmann's constant, k. Because the ideal gas constant $R = Nk$ was already known, the measurements allowed the determination of the Avogadro number N to within a few percent. Thus the study of

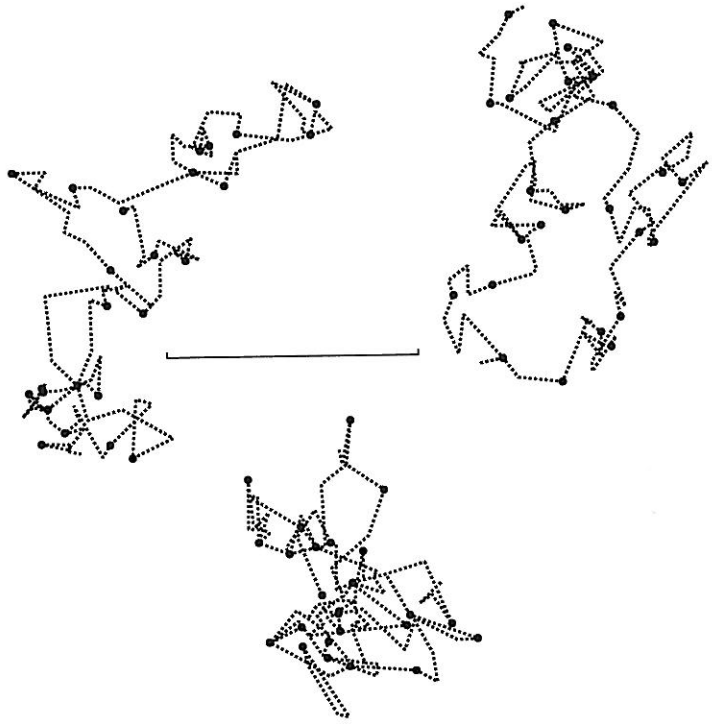


Figure 4.3 Examples of Brownian motion in two dimensions
Each simulated walk consists of 10,000 steps starting from the origin; each step has size +1 or −1 in both the horizontal and vertical directions. The positions at 100-step intervals are connected by dotted lines and every 400th position is marked by a filled circle. Note that the trajectories between the points are themselves as highly convoluted, but this is not shown. The scale bar corresponds to 100 step sizes. If the particle is a 100 kDa protein diffusing in water (diffusion coefficient 50 $\mu m^2/s$; see Table 2.2) and the step interval were 1 second (10,000 s total time), then the scale bar would equal 1 mm. If the intervals were 100 μs (1 s total time), then the scale bar would equal 10 μm.

Brownian motion confirmed the atomic theory of gases and liquids and bridged the gap between visible objects and invisible molecules. The rich history means that virtually every equation has a name!

The first result needed to derive the diffusion equation is the following interesting consequence of random motion. If molecules are moving in random directions, then, on average, they will tend to move from areas of high concentration to areas of low concentration. The proof is in Appendix 4.2. The prediction, confirmed experimentally, is that the **concentration flux**, $J(x)$, the rate of movement of molecules per unit area, is proportional to the concentration gradient, dc/dx:

$$J(x) = -D\frac{dc}{dx}(x) \tag{4.6}$$

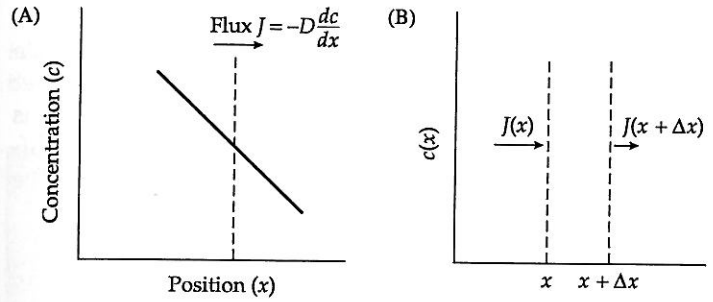


Figure 4.4 Flux and concentration change
(A) According to Fick's law, molecules diffuse from regions of high concentration to regions of low concentration. (B) Change in concentration due to change in flux. If the flux is not uniform—for example, if there is less flux out of a region than into it—then the concentration will change.

This equation is known as **Fick's law** and is shown in Figure 4.4A. The constant of proportionality, D, is called the **diffusion coefficient**. It is related to the size and frequency of the steps underlying the random motion: The larger and more frequent the steps, the greater the diffusion coefficient (Appendix 4.2). The negative sign reflects the tendency of molecules to move from regions of high concentration to regions of low concentration. The **concentration**, c, will usually be expressed in units of molecules per cubic meter, though sometimes moles per cubic meter or moles per liter will be used. The flux has units of molecules per unit area per second, so the diffusion coefficient has units of m^2/s.

To derive the diffusion equation we need to relate the flux back to the concentration. Consider Figure 4.4B. If fewer molecules leave a region to the right than enter it from the left, then there will be a net increase in the concentration in that region. In other words, provided that there are no sinks or sources of molecules, the change in concentration over time at any point equals the negative of the flux gradient at that point

$$\frac{\partial c}{\partial t}(x,t) = -\frac{\partial J}{\partial x}(x,t) \tag{4.7}$$

This is also proved in the Appendix. One application of this equation is that if the system is in the steady state—that is, if there is no change in concentration over time ($dc/dt = 0$)—then the flux is the same everywhere in the solution ($dJ/dx = 0$). Conversely, if the flux does not change from one position to another, then the concentration does not change with time.

Substituting Equation 4.6 into Fick's law gives:

$$\frac{\partial c}{\partial t}(x,t) = D\frac{\partial^2 c}{\partial x^2}(x,t) \tag{4.8}$$

This is known as the **diffusion equation**. Usually we are thinking about single molecules and want to know the probability, $p(x,t)$, of finding a molecule at position x at time t, rather than the concentration, $c(x,t)$, of a large number of molecules. Because the probability is proportional to the concentration (it is the concentration divided by the total number of molecules) and because differentiation is a linear operation ($d[a \cdot f(x)]/dx = a \cdot df/dx$), it follows that the probability, $p(x, t)$, also satisfies the diffusion equation.

Einstein Relation

Boltzmann's law allows us to derive an expression that relates the diffusion coefficient to the drag coefficient, introduced in Chapter 2. Suppose that an external force, $F(x)$, acts on a diffusing molecule. The force could be due to gravity or to an attached spring. The force will cause the molecule to move with velocity $v(x) = F(x)/\gamma$, where γ is the drag coefficient (Chapter 2). This "drift" velocity is an average speed superimposed on the diffusive motion. It is straightforward to show that the external force increases the flux by $v(x) \cdot c(x,t)$, or by $v(x) \cdot p(x,t)$ if we are thinking of the **probability flux**, $j(x)$:

$$j(x) = -D\frac{dp}{dx}(x) + \frac{F(x)}{\gamma}p(x) \tag{4.9}$$

Thus, in the presence of a force, the probability satisfies

$$\frac{\partial p}{\partial t}(x,t) = D\frac{\partial^2 p}{\partial x^2}(x,t) - \frac{\partial}{\partial x}\left[\frac{F(x)}{\gamma}p(x,t)\right] \tag{4.10}$$

This equation, which describes diffusion with drift, is known as the forward diffusion equation, or the **Fokker–Planck equation** (Papoulis, 1991).

If the system is in equilibrium, the probability does not change with time. The Fokker–Planck equation can then be solved to obtain $p(x)$. When this solution is compared to Boltzmann's law (Equation 4.1), it is found that the flux must be equal to zero everywhere, and that the diffusion coefficient is related to the drag coefficient by

$$D = \frac{kT}{\gamma} \tag{4.11}$$

This is known as the **Einstein relation**. It is proved in Appendix 4.2. By relating a molecular parameter, the drag coefficient, to a macroscopic parameter, the diffusion coefficient, the Einstein relation provides the link between the microscopic and macroscopic theories of diffusion. With the help of Stokes' law (Equation 3.6), this equation allows one to estimate the diffusion coefficient from the size of the particle and the viscosity of the solution

$$D = \frac{kT}{6\pi\eta r} \tag{4.12}$$

where r is the radius of a spherical particle, and η is the viscosity. Conversely, knowledge of the viscosity and the diffusion coefficient permits an estimate of the size of the particle, as shown in the following example.

Example 4.2 Diffusion of ions A sodium ion has a diffusion coefficient of 1.33×10^{-9} m^2/s at 25°C (Hille, 1992). From Einstein's relation and Stokes' law, this corresponds to an apparent radius of 1.8 Å, where we used η = 0.89 mPa·s and $kT = 4.12 \times 10^{-21}$ J at this temperature. This is about two times the ionic radius of 0.95 Å measured in crystals (Hille, 1992). A useful rule of thumb is that a diffusion coefficient of 10^{-9} m^2/s corresponds to 1 μm^2/ms, so a small ion diffuses ~1 μm in 1 ms.

In this chapter we consider only the case where there is no chance of the molecule being destroyed. In other words, the total probability is unity at all times, or

$$\int_{-\infty}^{\infty} p(x,t) \cdot dx = 1 \tag{4.13}$$

In later chapters, when we consider the polymerization of cytoskeletal filaments and the movement of motor proteins, we will relax this condition by allowing chemical reactions to convert one type of molecule into another, or to destroy or create molecules. When these reactions also depend on position, the motion becomes very rich and is described by the **reaction–diffusion equation**

$$\frac{\partial p_i}{\partial t}(x,t) = D\frac{\partial^2 p_i}{\partial x^2}(x,t) - \frac{\partial}{\partial x}\left[\frac{F(x)}{\gamma} p_i(x,t)\right] + \sum_j \left[k_{ji}(x) \cdot p_j(x,t) - k_{ij}(x) \cdot p_i(x,t)\right] \tag{4.14}$$

where p_i, p_j, and so forth are the probabilities of the molecule being in various chemical states i, j, and so on, and k_{ij} is the rate constant for the transition between the i and j states.

Some Solutions to the Diffusion Equation

The utility of the diffusion equation is that it allows one to calculate how quickly, on average, it takes for a molecule to diffuse through a certain distance. This information can be used to evaluate the efficiency of diffusion as a transport process within cells. Furthermore, with the aid of the Fokker–Planck equation, we can calculate the time that it takes for a molecule to diffuse against an applied force. By turning this argument around, one can then gain insight into how forces affect chemical rates, a subject dealt with in detail in the next chapter. In this section we solve the diffusion equation in a few special cases that are relevant to cellular and molecular mechanics. Solutions for a wide variety of other cases can be found in Carslaw and Jaeger (1986).

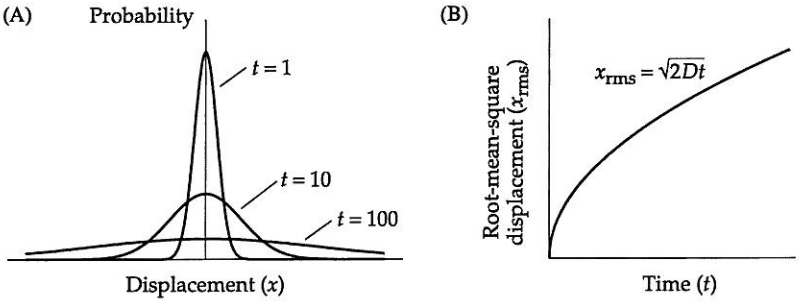


Figure 4.5 Diffusion of a molecule released at time 0 at the origin
(A) The curves show the probability of finding a molecule at increasing times.
(B) The growth in the root-mean-square displacement (x_{rms}) as a function of time.

Free Diffusion from a Point Source

If a molecule is released at the origin and allowed to diffuse in one dimension, then the probability of finding it at position x at time t later is

$$p(x,t) = \frac{1}{\sqrt{4\pi Dt}} \exp\left[-\frac{x^2}{4Dt}\right] \qquad t>0 \qquad (4.15)$$

The solution is illustrated in Figure 4.5A. Note that the total probability is unity for all times greater than 0. The probability distribution corresponds to a normal, or Gaussian, distribution whose variance, σ^2, is $2Dt$. The **root-mean-square displacement**, x_{rms} (which equals the standard deviation, σ), therefore increases in proportion to the square root of time (Figure 4.5B). This is in contrast to motion with constant velocity, v, where the displacement increases in proportion to time ($x = vt$) and is always in one direction.

Example 4.3 The efficiency of diffusion as a cellular transport mechanism
Consider a 3-nm-radius protein (corresponding to a molecular mass of 100 kDa) diffusing through water. Its diffusion coefficient is ~100 $\mu m^2/s$ (at 37°C). The table on page 61 shows how long it takes for the protein to diffuse various distances. Because the average distance a particle diffuses increases only with the square root of time, diffusion of proteins becomes slow, greater than ~1 minute, over distances greater than ~100 μm. This might explain why the diameters of eukaryotic cells are usually smaller than 100 μm. On the other hand, a 1-μm-diameter organelle (such as a mitochondrion) in water has a diffusion coefficient of only ~0.5 $\mu m^2/s$ (at 25°C). Diffusion through 100 μm in this case takes ~3 hours; in a real cell it would take much, much longer because the cytoplasm is more like a gel with a mesh size of only ~50 nm (Luby-Phelps et al., 1987), so that

organelles larger than 50 nm are almost immobile. Thus even small eukaryotic cells will require motor proteins to move organelles from one place to another. The low mobility of organelles does have a benefit, though: Large organelles will stay where they are put and the internal structure of the cell will therefore be reasonably stable. For highly elongated cells—an extreme example are the neurons of the sciatic nerve, whose processes bridge from the spinal cord to the foot—active transport is essential, even for small metabolites and proteins.

Times for one-dimensional diffusion in aqueous solution

	Distance diffused			
Object	**1 μm**	**100 μm**	**10 mm**	**1 m**
K^+	0.25 ms	2.5 s	2.5×10^4 s (7 hrs)	2.5×10^8 s (8 years)
Protein	5 ms	50 s (~1 min.)	5×10^5 s (6 days)	5×10^9 s (150 years)
Organelle	1 s	10^4 s (~3 hr.)	10^8 s (3 years)	10^{12} s (30 millennia)

Note: K^+: Radius ~0.1 nm, $T = 25°C$, $D \cong 2000\ \mu m^2/s$.
Protein: Radius = 3 nm, viscosity = 0.6915 mPa·s^{-1}, $T = 37°C$, $D \cong 100\ \mu m^2/s$.
Organelle: Radius = 500 nm, viscosity = 0.8904 mPa·s^{-1}, $T = 25°C$, $D \cong 0.5\ \mu m^2/s$.

First-Passage Times

In the previous section we answered the question: How far, on average, does a molecule diffuse in a given time? However, a more relevant question is: How long, on average, does it take a molecule to diffuse through a given distance? We call this time the **first-passage time**. The rephrased question is more relevant because it allows us to calculate the rate of a process that is limited by diffusion. The **diffusion-limited** rate is the reciprocal of the first-passage time.

The first-passage time can be calculated by solving the diffusion equation for the particular geometry of the problem. In the absence of an external force, the first-passage time for one-dimensional diffusion through a distance x_0 is

$$t = \frac{x_0^2}{2D} \tag{4.16}$$

(Appendix 4.2). Not coincidentally, this is the same answer that we got when we approached the problem the other way round by considering the average distance diffused!

Example 4.4 Diffusion over molecular dimensions The first-passage time is very small when the distances are small. For example, consider a 1-μm-diameter spherical organelle diffusing in aqueous solution near a microtubule. If the sphere were kept from diffusing away from the microtubule but free to diffuse along it, then diffusion through 8 nm, the distance between adjacent tubulin dimers along the microtubule lattice, would only take 64 μs! Thus it is an appealing idea that motor proteins exploit this rapid motion by somehow allowing diffusion to occur in only one direction. But as we will see in later chapters, such a model for the motor mechanism is ruled out by several types of experiments.

The first-passage time becomes more interesting from a biological point of view when we consider diffusion in the presence of an external force. For example: How long does it take for a molecule to diffuse over an energy barrier at $x = x_0$? When the force is constant—that is, when the potential energy is $U(x) = -Fx$—the first-passage time is

$$t = 2\left(\frac{x_0^2}{2D}\right)\left(\frac{kT}{Fx_0}\right)^2\left\{\exp\left(-\frac{Fx_0}{kT}\right) - 1 + \frac{Fx_0}{kT}\right\} \tag{4.17}$$

This equation is plotted in Figure 4.6. When the diffusion is steeply downhill—that is, the force is large and positive—the first-passage time approaches x_0/v, where v is the drift velocity ($v = F/\gamma$), as expected for a molecule drifting at constant speed. When the diffusion is steeply uphill—that is, the force is large and negative—the first-passage time increases approximately exponentially as the opposing force is increased. In this case, the diffusion rate, the inverse of the first-passage time, decreases approximately exponentially as the force

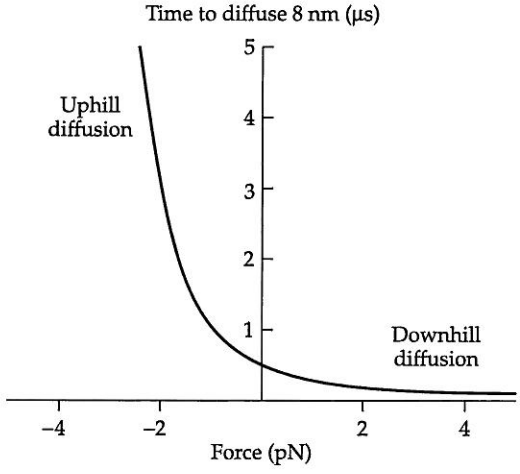


Figure 4.6 Time for a 100 kDa protein to diffuse 8 nm
When diffusion is uphill (against an opposing force) the first-passage time is long (left-hand side). When diffusion is downhill (in the direction of the force), the first-passage time is short (right-hand side).

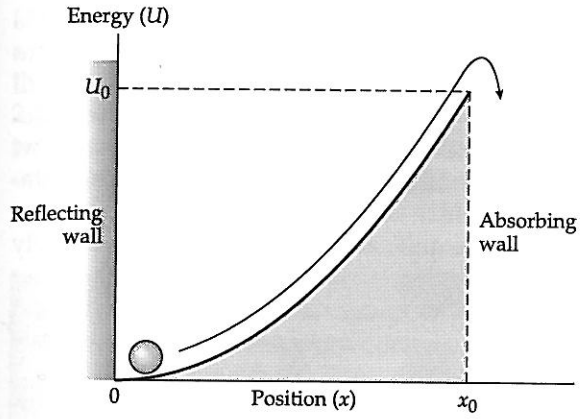


Figure 4.7 Diffusion of a molecule out of a hemiparabolic energy well

times the distance increases. In other words, the diffusive process behaves as though there is a barrier with energy $U_0 = |F| \cdot x_0$ (Chapter 5).

If the force opposes the motion and has amplitude proportional to position (Figure 4.7)—that is, the molecule is attached to an elastic element so that $F = -\kappa x$—the first-passage time is

$$t_K = \tau \sqrt{\frac{\pi}{4}} \sqrt{\frac{kT}{U_0}} \exp\left(\frac{U_0}{kT}\right) \tag{4.18}$$

where $\tau = \gamma/\kappa$ is the drag coefficient divided by the spring constant. This equation is derived in Appendix 4.2 and assumes that the height of the energy barrier is high—that is, $U_0 \equiv U(x_0) \equiv \frac{1}{2}\kappa x_0^2 >> kT$. t_K is called the Kramers time, after Kramers, who first derived it (Kramers, 1940). This equation forms the basis of the Kramers rate theory, which postulates that the rate of reactions is limited by diffusion over a high-energy transition state (Chapter 5).

Correlation Times*

So far I have been vague about the nature of the thermal forces that drive diffusion and Brownian motion. Indeed, we didn't need any information about the thermal forces to derive the Principle of Equipartition of Energy, only that Boltzmann's law was satisfied, and we only needed to assume that the thermal forces were randomly directed to derive the diffusion equation and to relate the diffusion coefficient to the drag coefficient. However, there are several "microscopic" details of diffusive motion that are important. For example, how long, on average, will a free molecule keep moving in one direction before

*An asterisk next to a heading denotes a more advanced section.

the thermal forces randomize its direction of motion? In other words, what is the **persistence time** (or **correlation time**) of the velocity? Another important question is: How long, on average, will it take for a molecule in a potential well to explore the different energy levels? In particular, how long will the molecule spend at each energy level? In other words, what is the persistence time, or correlation time, of the position? Finally, what are the amplitudes and statistical properties of the thermal forces? In this chapter, we will answer these questions. Because the answers require quite advanced mathematics, namely concepts from Fourier analysis, this section is more advanced than the other sections in this book. For those without this mathematical background, the next three sections can be skipped, except for Examples 4.5 and 4.6, which illustrate the main results.

Let $F(t)$ be the thermal force acting on a molecule due to collisions with surrounding solvent molecules: It comprises very brief impulses with random direction, occurring at random times. The equation of motion of the molecule in response to this force is

$$m\frac{d^2x}{dt^2}(t)+\gamma\frac{dx}{dt}(t)+\kappa x(t)=F(t) \tag{4.19}$$

and is known as the **Langevin equation** (Langevin, 1908). Because the thermal force is a random one, the most we can hope for is a description of the statistical properties of the resulting motion. These properties are described by the **autocorrelation function**, $R_x(\tau)$, of the position $x(t)$ of a molecule, which is defined by

$$R_x(\tau)=\langle x(t)\cdot x(t-\tau)\rangle \equiv \lim_{T\to\infty}\left\{\frac{1}{T}\int_{-T/2}^{T/2} x(t)x(t-\tau)\cdot dt\right\} \tag{4.20}$$

The autocorrelation at delay τ is calculated by multiplying the position at a given time by the position at time τ earlier, and averaging over all times.

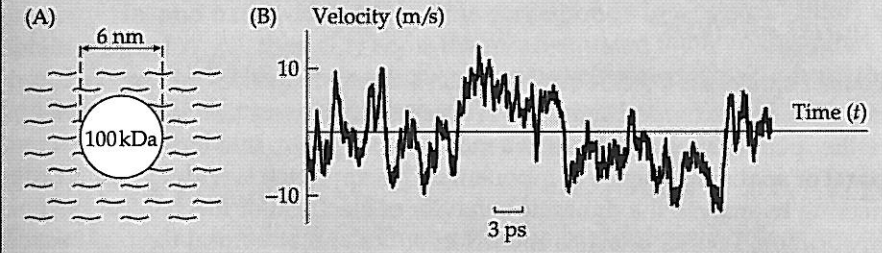
The autocorrelation function $R_x(\tau)$ has the properties that coincide with our intuitive notion of temporal correlation or persistence time. The autocorrelation function has its maximum value (equal to the variance of the signal $\langle x^2\rangle = \sigma^2$) when $\tau = 0$ and it falls to zero when $\tau = \infty$ (we are assuming that the signal has mean $\langle x\rangle = 0$). This accords with the positions at two closely spaced times being highly correlated, but the positions at two widely separated times being uncorrelated. In addition, the autocorrelation is symmetric, $R_x(\tau) = R_x(-\tau)$. This relationship means that the correlation drops equally quickly whether we compare the signal with itself at earlier or later times.

The crucial additional property is that the autocorrelation function satisfies the equation of motion!

$$m\frac{d^2R_x}{d\tau^2}(\tau)+\gamma\frac{dR_x}{d\tau}(\tau)+\kappa R_x(\tau)=0 \qquad \tau>0 \tag{4.21}$$

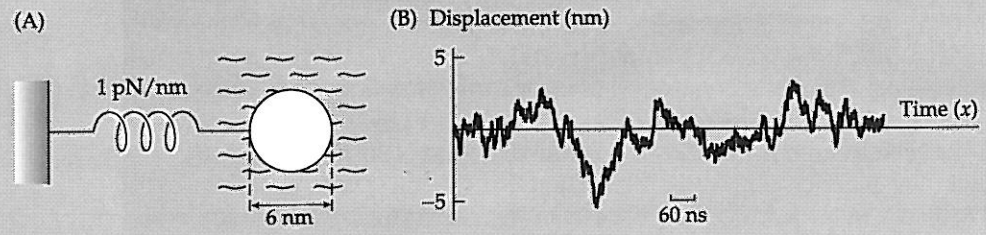
(Appendix 4.3). This means that the autocorrelation function has the same form as the response of the molecule to an impulsive external force. For example, in the case of overdamped motion appropriate for protein dynamics (Chapters 2 and 3), Equation 4.21 yields an autocorrelation function that is the sum of two exponentials, a fast, small-amplitude one with time constant m/γ (on the order of ps) and a slow, large-amplitude one with a time constant equal to γ/κ. This result is elaborated in Examples 4.5 and 4.6.

Example 4.5 Diffusion of a free protein Consider a protein of molecular mass 100 kDa whose velocity (in one dimension) is simulated in the figure below. It has a mass of 166×10^{-24} kg (see Table 2.2) and a root-mean-square speed, $v_{rms} = (3kT/m)^{0.5} \cong 8.6$ m/s. The time constant is $\tau = m/\gamma$, where γ is the drag. If the protein is globular with a radius of 3 nm, then the damping is $6\pi\eta r \cong 60$ pN·s/m (see Table 2.2) and the time constant is only ~3 ps! This is the persistence time, or correlation time, of the velocity. Over this time the protein will move 0.24 Å, only ~1/300th its diameter! Thus even though the speeds of molecules are large, the high damping that they experience in water means that their inertia carries them for extremely small distances, so that after a fraction of an angstrom, they are likely to be moving in a different direction. Thus the model of diffusion as a random walk is a good one, provided that the time between the postulated steps is much longer than the 3 ps inertial time.



An important consequence of Equation 4.21 is that it suggests a strategy for estimating a molecule's molecular properties, such as stiffness and damping. Measure the thermal motion, calculate the autocorrelation function via Equation 4.20, and compare it to the theoretical autocorrelation function predicted from a particular model (via Equation 4.21) to obtain the molecular parameters that give the best fit. However, rather than calculating the autocorrelation of a fluctuating signal, it is more common to perform a Fourier analysis on the signal, and compare that to the predicted behavior, as described in the next section.

Example 4.6 Diffusion of a tethered protein Suppose that the protein of the previous example is attached to a spring. Its position is simulated in the figure below. If the stiffness is 1 pN/nm, it will have a root-mean-square displacement, $x_{rms} = \sqrt{\langle x^2 \rangle} = \sqrt{kT/\kappa}$ $\cong 2$ nm. This is independent of its molecular mass! How long will it take such a protein to relax to a new position? The time constant is $\tau = \gamma/\kappa \approx 60$ ns, which is ~20,000 times greater than the inertial time constant (see the figure in Example 4.5). This is the persistence time, or correlation time, of the protein's position. The protein's position will be correlated on the nanosecond time scale: After times less than 60 ns, the protein will still be "quite near" to where it was. But over much longer times (>> 60 ns) the protein's position will be uncorrelated, and the probability of finding the protein in a certain position will depend only on its potential energy and not on time.



*Fourier Analysis**

Fourier analysis is a technique by which a signal that varies in time, such as an electrical or mechanical signal, or in space, such as an optical signal (an image) or the spatial pattern of atoms in a molecule, is split up into its constituent temporal or spatial frequency components. This approach is widely used in engineering to analyze the dynamic behavior of electrical or mechanical systems. In addition, Fourier analysis is used in optics and structural biology because when light waves or X rays pass through and are diffracted by a material, the light or X rays that exit the specimen in a particular direction correspond to the scattering due to a particular spatial frequency of structures in the material. In other words, diffraction is a physical way of separating the different spatial frequency components. Likewise, the graphic equalizer on a stereo system is an analogue circuit that separates electrical signals into different temporal frequency components. The ear is another example of an analogue Fourier analyzer: Different frequencies of sound are separated into mechanical vibrations at different spatial locations along the cochlea. We are going to apply Fourier analysis to the dynamics of a molecule undergoing thermal motion. But the underlying principles are generally applicable and can be used to interpret shape fluctuations of polymers, to understand various contrast techniques used in light microscopy, and to see how diffraction patterns are used to deduce the structures of proteins.

Fourier analysis is based on the following mathematical property: A signal $x(t)$ whose total duration is T can be expressed as a **Fourier series**

$$x(t) = \sum_{n=1}^{\infty} a_n \cos\frac{2\pi nt}{T} + b_n \sin\frac{2\pi nt}{T} \qquad (4.22)$$

where the amplitudes of the cosine and sine components, a_n and b_n, are calculated from the time averages

$$a_n = 2\left\langle x(t)\cos\frac{2\pi nt}{T}\right\rangle = \frac{2}{T}\int_{-T/2}^{T/2} x(t)\cos\frac{2\pi nt}{T}\cdot dt \qquad n\geq 1$$

$$b_n = 2\left\langle x(t)\sin\frac{2\pi nt}{T}\right\rangle = \frac{2}{T}\int_{-T/2}^{T/2} x(t)\sin\frac{2\pi nt}{T}\cdot dt \qquad n\geq 1$$

As in the last section, we are assuming that the mean of the signal is zero (if not, the mean can simply be subtracted). There are a number of fast algorithms for calculating the Fourier series from digitized time traces (e.g., the Cooley–Tukey algorithm; Bendat and Piersol, 1986; Press, 1997).

For our purposes, the crucial function obtained from the Fourier analysis of a signal is the **power spectrum**. The power spectrum, $G_x(f)$, of a signal, $x(t)$, is defined so that $G_x(f)\cdot\Delta f$ is the mean-square displacement, or variance, of the signal in the frequency range $(f, f+\Delta f)$. The power spectrum has the following physical meaning. If a signal $x(t)$ is passed through a filter with center frequency f Hz, and bandwidth Δf Hz (this corresponds to a "notch" filter) to obtain a signal $x_{f,\Delta f}(t)$, then $G_x(f)\cdot\Delta f$ is the mean-squared value of this filtered signal (Figure 4.8). The power spectrum can be calculated directly from the Fourier series. If we pass $x(t)$ through a filter of bandwidth $\Delta f = 1/T$, and take the variance we obtain

$$G_x(f)\cdot\Delta f \equiv \langle x^2_{f,\Delta f}(t)\rangle = \tfrac{1}{2}\left(a_n^2 + b_n^2\right) \qquad f = n/T > 0,\ \Delta f = 1/T \qquad (4.23)$$

This is derived in Appendix 4.3. There are a number of slightly different ways of defining the power spectrum (Bendat and Piersol, 1986; Bracewell, 1986; Papoulis, 1991): The definition of Equation 4.23 corresponds to the "one-sided

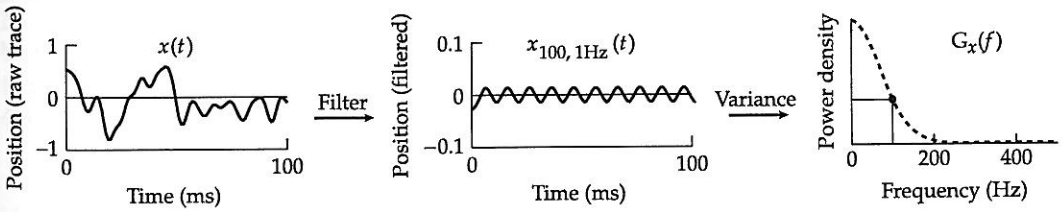


Figure 4.8 Definition of the power spectrum of a signal x(t)

power spectrum," for which only positive frequencies are considered. If the signal corresponds to a displacement, then the units of $G_x(f)\cdot\Delta f$ are m^2. The unit of $G_x(f)$ is therefore m^2/Hz. If the signal were a force or a voltage, the units would be N^2/Hz or V^2/Hz. Thus the power spectrum is not a true distribution of power, which would have units of W/Hz. The misnomer arose because the power spectrum was first used for electrical signals, in which case the square of the voltage is proportional to the power ($V^2 = V\cdot iR = P\cdot R$ by Ohms law). The power spectrum is analogous to the intensity distribution of a diffraction pattern.

The reason why the power spectrum is so useful for analyzing thermal motion is that the power spectrum is the Fourier transform of the autocorrelation function

$$G_x(f) = 2\int_{-\infty}^{\infty} R_x(\tau)e^{-2\pi i f\tau}\cdot d\tau = 4\int_{0}^{\infty} R_x(\tau)\cos(2\pi f\tau)\cdot d\tau \qquad f>0 \tag{4.24}$$

where we have a factor of two in the middle expression because we are using the one-sided power spectrum, and the right-hand expression follows because the autocorrelation function is symmetrical (Appendix 4.4). This relationship is significant because it allows one to compare the power spectrum, computed directly from the experimental recordings via the Fourier series, with a theoretical expression for the power spectrum, which is calculated with the help of Equation 4.24 from the predicted autocorrelation function (Equation 4.21). Figure 4.9 summarizes this approach. First, we measure the thermal motion of a molecule and calculate the power spectrum. Then we compare this power spectrum with the power spectrum predicted by a model equation of motion, which has variables of mass, damping, and stiffness. The model is refined by adjusting the variables until the best fit is found. In this way, the molecular parameters—the mass, damping and stiffness—can be deduced from the thermal motion. This process is shown in the following example. An analogous approach is used in X-ray crystallography; the power spectrum corresponds to the intensity of the diffracted pattern, which is compared to theoretical diffraction patterns predicted by structural models.

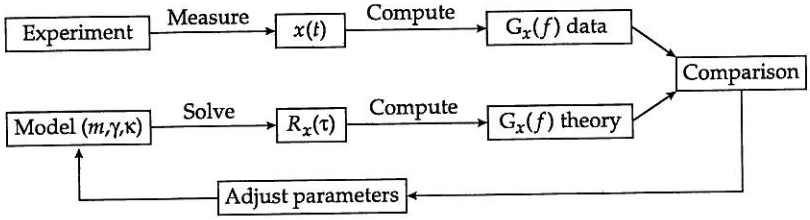


Figure 4.9 Using Fourier analysis to measure molecular parameters

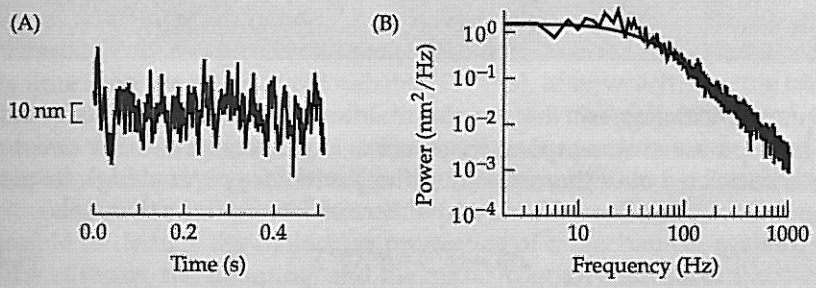
Example 4.7 Power spectrum of a damped spring Figure A shows the position of the tip of a flexible glass fiber undergoing thermal motion. The noisy curve in Figure B shows the power spectrum of this motion calculated via the Fourier series (Equation 4.23). The smooth curve is the theoretical curve obtained as follows. First, the autocorrelation function is found as the solution to Equation 4.21 with zero mass

$$R_x(\tau) = \frac{kT}{\kappa}\exp(-|\tau|/\tau_0) \qquad \tau_0 = \gamma/\kappa$$

where we have used $R_x(0) = \langle x^2 \rangle > = kT/\kappa$ from the Equipartition principle. The associated power spectrum is calculated via Equation 4.24 to be

$$G_x(f) = \frac{4kT\gamma}{\kappa^2}\frac{1}{1+(2\pi f\tau_0)^2}$$

This curve is called a **Lorenzian** and corresponds to the smooth curve in Figure B with parameters $\kappa = 0.043$ mN/m and $\gamma = 0.16$ μN·s/m. By this means, the stiffness and damping are deduced from the thermal motion. There are other approaches to estimating the stiffness and the drag coefficient. For example, measuring the mean-square displacement, $\langle x^2 \rangle = kT/\kappa$, and the correlation time, $\tau = \gamma/\kappa$ (or cutoff frequency $f_0 = 1/2\pi\tau$), allows one to solve for $\kappa = kT/\langle x^2 \rangle$ and $\gamma = \tau\kappa$. In this example, $\tau = 3.7$ ms.



(After Meyhöfer and Howard, 1995.)

The Magnitude of the Thermal Force*

Now that we know the power spectrum of the molecule's position, it should be possible to calculate the power spectrum of the equivalent force necessary to produce the motion. This is the thermal force, and it is shown in Appendix 4.3 to have a power spectrum equal to

$$G_F(f) = 4kT\gamma \qquad (4.25)$$

This equation has a number of important implications. First, it says that to account for the thermal motion, we simply redraw the mechanical circuit to include a force generator that has a variance $4kT\gamma$ N^2/Hz in each frequency interval (Figure 4.10). Second, the variance of the thermal force is independent of the frequency: This is equivalent to the force being due to impulsive collisions, as we have claimed (see also Appendix 4.3). The third implication is that the amplitude of the force depends only on the drag coefficient, and not on the stiffness (or on the mass if it had been included). This finding is quite general and is known as the **Fluctuation–Dissipation theorem** (Landau et al., 1980). Another way of stating this result is that the fluctuations come from the dissipative elements, namely the dashpots. In general, to simulate the thermal motion of any linear mechanical circuit, we place a random force generator with power spectrum $4kT\gamma_i$ *in parallel* with each damping element γ_i.

Example 4.8. Thermal noise also occurs in electrical circuits The analogue of the thermal forces that originate in the dashpots of mechanical circuits is the **Johnson noise** that originates in the resistors of electrical circuits. Johnson noise is due to thermally driven voltage fluctuations. Its power spectrum is $G_V(f) = 4kTR$, where R is the resistance. In an electrical circuit with a feedback resistor of 1 GΩ, typical of the feedback resistors in patch-clamp headstages, the root-mean-square voltage noise over a 1000 Hz bandwidth is 126 μV. Using Ohm's law ($V = iR$), the root-mean-square current noise is therefore 0.13 pA (Sigworth, 1995).

Equation 4.25 suggests that the thermal forces are instantaneous because they have power at all temporal frequencies. However, the velocity of a molecule is correlated over times $\tau = m/\gamma$. This limits the power at high frequencies and, as a result, the variance of the thermal force is finite. It equals

$$\sigma_F^2 = \langle F^2 \rangle = \gamma^2 \langle v^2 \rangle \tag{4.26}$$

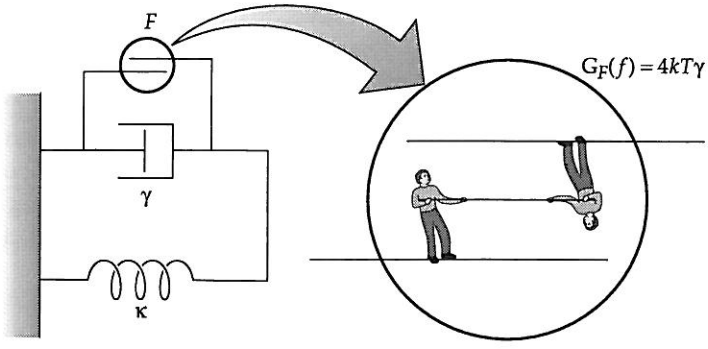


Figure 4.10 Representation of the thermal force
The thermal force is represented as a random force in parallel with the dashpot.

(Appendix 4.3). This equation shows that the root-mean-square thermal force is proportional to the root-mean-square velocity times the damping, in accordance with the notion that the thermal force has the same physical origin as the damping force.

Summary

Because molecules are agitated by collisions with other molecules, they are not always found in their lowest energy state. The probability of a molecule being in a higher energy state is given by Boltzmann's law. Boltzmann's law has several corollaries. One corollary is the Principle of Equipartition of Energy, which says that each degree of freedom of the molecule (which meets certain criteria) has $\frac{1}{2}kT$ of energy associated with it. k is the Boltzmann constant and T is the absolute temperature, so $kT \cong 4 \times 10^{-21}$ J at room temperature. An example of this principle is that the average potential energy of a spring is $\frac{1}{2}kT$. Another example is that the average kinetic energy of a molecule is $\frac{3}{2}kT$ (the 3 appears because there are three components of velocity and therefore 3 degrees of freedom). The randomly directed collisions with the surrounding molecules cause particles to diffuse, and the statistical properties of diffusing particles can be determined by solving the diffusion equation. A second corollary of Boltzmann's law is that the diffusion coefficient (D) is related to the drag coefficient (γ) via the equation $D = kT/\gamma$, known as the Einstein relation. A detailed analysis of diffusion of a particle shows that the velocity persists (or is correlated) over times shorter than m/γ, where m is the mass. For a protein, this time is on the order of picoseconds (10^{-12} s), after which time the protein is likely to be moving in a different direction and with a different speed. If the protein is tethered by a spring of stiffness κ, then the position is correlated over times shorter than γ/κ, which ranges from 1 nanosecond to 1 microsecond for spring constants between 0.016 and 16 pN/nm. Using Fourier analysis, it is possible to deduce the molecular properties of a mechanical system, such as the stiffness, the damping, and the mass, from an analysis of the thermal motion.

Problems

4.1 Consider a stack of ten shelves and suppose that the spacing of the shelves is such that the potential energy on each shelf is 1 kT higher energy than that on the shelf below. Suppose that 1000 books are placed on the shelves according to Boltzmann's law. How many books are there on each shelf? [Answer: From the bottom there are 632, 233, 85, 31, 12, 4, 2, 1, 0 and 0 books on each shelf.]

4.2 A solution of gold spheres is stored on a shelf. After a week or so, it is noticed that the spheres have settled stably in the container and that the

density (as estimated from the color) decreases *e*-fold every 20 mm from bottom to top. Given that the density of gold is 19.3 times that of water, what is the diameter of particles?

4.3 A protein solution was placed in an analytic centrifuge and spun until equilibrium was reached. The measurement was then repeated. But it was realized, too late, that prior to the second run, the protein had been denatured by the rotating graduate student. Remarkably, it was found that the distribution was the same for both runs. Why is this?

4.4 The Stokes' radius of a sodium ion, the apparent radius obtained from the diffusion coefficient, is about twice the ionic radius, found in crystals (see Example 4.2). Why might this be?

4.5 Calculate the root-mean-square velocity of a bacterium (assume it is spherical of radius 1 μm and that it is 10% heavier than water). What is the correlation time of the velocity? How far will the bacterium move during this correlation time?

4.6 Consider our canonical 100 kDa protein (see Table 2.2). How long will it take to diffuse 40 nm? Suppose there is a force of 1 pN. How long will it take to diffuse 40 nm in the direction of the force? How long will it take to diffuse 40 nm against the force? [Answers: 24 μs, 4 μs, 11 ms.]

4.7* If $U(x) = F|x|$, that is, a particle is trapped between two linear inclines, show that $\langle U \rangle = kT$ (and not $\frac{1}{2}kT$).

4.8* If a particle is released at the origin and there are absorbing walls at $x = -a$ and $x = b$, show that the probability of being absorbed at $-a$ divided by the probability of being absorbed at b is b/a (Goel and Richter-Dyn, 1974).

4.9* The flow of heat also satisfies Fick's law and the diffusion equation. The flux of heat is proportional to the thermal gradient, where the constant of proportionality is the thermal conductivity, K. The flow of heat then changes the temperature of a unit volume of solid by $c\rho$, where c is the heat capacity and ρ is the density. For the case of heat flow in solids, the equivalent to the diffusion coefficient is

$$D_{\text{thermal}} = K/c\rho$$

For water at 25°C, $K = 0.606$ W/m·K, $c = 4.18$ kJ/kg·K, and $\rho = 997$ kg/m^3. (a) What is D_{thermal} for water? (Check that the units are m^2/s.) How does this compare with the diffusion coefficient for an ion? [Answer: 0.15 mm^2/s.]

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* The asterisk denotes more advanced problems.

(b) Suppose, hypothetically, that heat were carried by little particles (called calorics). Use Stokes' law to calculate the radius of the heat particle.

(c) How long does it take heat to diffuse 3 nm, roughly the distance from the center of a globular protein to the surrounding fluid? [Answer: ~10 ps for diffusion in 3 dimensions.]