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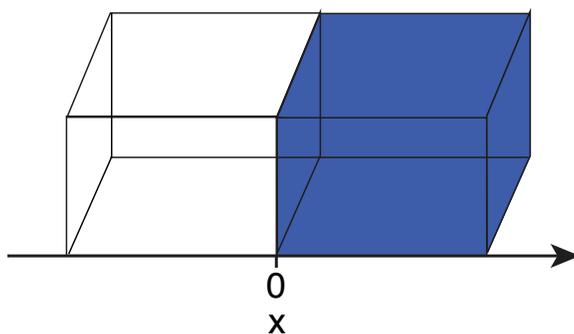
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Chapter 4: Diffusion

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Diffusion

Now, we go back to the problem introduced when we first began discussing probability, Brownian motion and diffusion. We would like some real numbers about how far a molecule or particle will diffuse in a given time, and we would like to know what factors determine this. Traditionally most experimental measurements of diffusion have been based on measuring changes in bulk concentration. An example that we will consider in some detail involves setting up two adjacent volumes, one containing a molecule of interest and the other without. At the beginning of the experiment, there is a sharp boundary between the two volumes, as diagrammed below.



Setting up an arrangement like this is technically challenging, but not impossible. Typically, the apparatus is set up vertically, but it is shown horizontally here, because we will define the x axis as the axis of diffusion, as indicated

With time, we expect the molecules to diffuse and the concentration to become more even. The rates at which the concentration changes at different points along the x -axis will depend on the rates at which the molecules move, and we should be able to deduce the parameters of the random walk from the rate of change in concentration.

What we are trying to do here is to extend the treatment of individual random walks to the bulk behavior of molecules that lead to concentration changes. This will require thinking about things a little differently.

4.1 Flux: Fick's First Law

I. The derivation

We will look at diffusion along a single dimension, x . Diffusion depends on Brownian motion, which can be described as a random walk. In each step of the walk, the direction is random, in three dimensions. If the mean-square length of the steps is $\langle l^2 \rangle$,

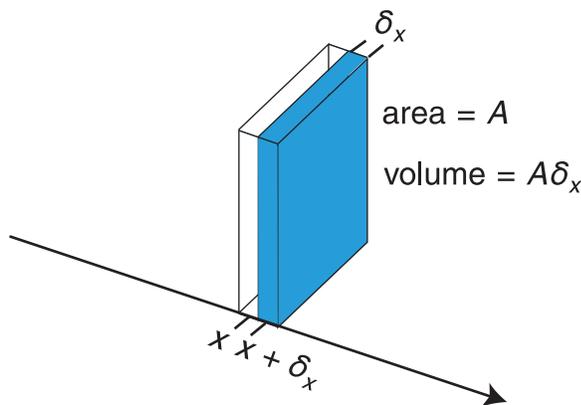
then the mean-square displacement of the step along the x -axis will be $\langle \delta_x^2 \rangle = \langle l^2 \rangle / 3$. For convenience, we will refer to the step size along the x -axis as $\delta_x = \text{RMS}(\delta_x)$.

We will also define the average time interval between steps as τ . After a time period t , the number of steps will be $n = t/\tau$. The mean-square displacement along the x -axis will be:

$$\langle x^2 \rangle = n\delta_x^2 = t\delta_x^2/\tau$$

Notice that the average (RMS) distance will increase with the square root of time.

Now, let's consider two thin slices of the volume diagrammed in in the previous figure, cut perpendicular to the direction of the concentration difference:



The thickness of each volume is set to be δ_x , the RMS length of a random-walk step along the x -axis, and the cross-section along the x -axis has an area of A . Therefore, the volume of each slice is $A\delta_x$. During the interval τ , one half of the molecules within a slice will move to the left and half will move to the right. This will happen in both slices. If the number of molecules is the same in the two slices, the number of molecules that cross in each direction will be the same. But, if there are more molecules at position $x + \delta_x$ than at position x , then there will be a net movement of molecules to the left.

Call the number of molecules in the slice centered at x N_x , and the number of molecules in the slice at $x + \delta_x$ $N_{x+\delta_x}$. The net number of molecules going to the right in time t will be:

$$\begin{aligned} dN &= \frac{1}{2}N_x - \frac{1}{2}N_{x+\delta_x} \\ &= -\frac{1}{2}(N_{x+\delta_x} - N_x) \end{aligned}$$

Notice that we have defined things so that if the number of molecules to the right is larger than to the left, the flow of molecules to the right will be negative.

The flux across a given surface area is expressed as the number of molecules (or moles) per unit time per unit area. The expression above is divided by A and the time interval,

τ , to give the flux, J :

$$J = -\frac{1}{A\tau} \frac{1}{2} (N_{x+\delta_x} - N_x)$$

We can express the number of molecules in each slice in terms of the concentrations and the volumes of each slice:

$$N_x = C_x A \delta_x$$

$$N_{x+\delta_x} = C_{x+\delta_x} A \delta_x$$

We can then re-write the flux equation as:

$$\begin{aligned} J &= -\frac{1}{A\tau} \frac{1}{2} (N_{x+\delta_x} - N_x) \\ &= -\frac{1}{A\tau} \frac{1}{2} (C_{x+\delta_x} A \delta_x - C_x A \delta_x) \\ &= -\frac{\delta_x}{\tau} \frac{1}{2} (C_{x+\delta_x} - C_x) \end{aligned}$$

Now, we can write the difference in concentrations at the two positions in terms of a derivative with respect to x

$$\frac{dC}{dx} = \lim_{\delta_x \rightarrow 0} \frac{C_{x+\delta_x} - C_x}{\delta_x}$$

In the limit of small δ_x :

$$C_{x+\delta_x} - C_x = \delta_x \frac{dC}{dx}$$

So, we now have:

$$J = -\frac{\delta_x^2}{2\tau} \frac{dC}{dx}$$

Consider the quantity $\delta_x^2/(2\tau)$. Both parameters in this ratio are properties of the diffusing particle under a particular set of conditions. For now, we won't worry about its particular significance, but we will replace it with a new parameter, which we call the diffusion constant, D . Thus:

$$J = -D \frac{dC}{dx}$$

This equation is known as Fick's first law. (Adolf Eugen Fick, German physiologist, 1829–1901.)

Important conclusion: The *net* flux of molecules per unit area and per unit time is determined by the difference in concentration, and the diffusion coefficient, which reflects the steps in a random walk.

Why do molecules “move down a concentration gradient”? It’s not because they can sense concentration! All of the molecules move randomly, but the probability for moving from a high concentration to a lower concentration is higher than the reverse simply because there are more molecules in the high-concentration region.

Let’s also look at the units in this equation:

- The flux, J , has dimensions of molecules per cross-sectional area per unit time, or, in SI units: $\text{molecules} \cdot \text{m}^{-2}\text{s}^{-1}$. Alternatively, it can be expressed in terms of moles.
- The diffusion constant, $D = \delta_x^2/(2\tau)$, has units: m^2s^{-1} .
- The derivative of concentration with respect to x has dimensions of molecules per volume per length. In SI basic units this is: $\text{molecules} \cdot \text{m}^{-3}\text{m}^{-1} = \text{molecules} \cdot \text{m}^{-4}$
- Combining these:

$$J = -D \frac{dC}{dx}$$

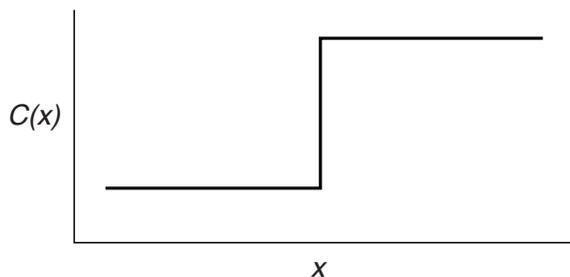
$$\text{molecules} \cdot \text{m}^{-2}\text{s}^{-1} = \text{m}^2\text{s}^{-1} \times \text{molecules} \cdot \text{m}^{-4}$$

$$\text{molecules} \cdot \text{m}^{-2}\text{s}^{-1} = \text{molecules} \cdot \text{m}^{-2}\text{s}^{-1}$$

Looks good!

II. The distribution of molecules diffusing from a single position.

Consider the case described earlier, where there is initially a sharp boundary between an area where $C = 0$ and an area with $C = 1$, in arbitrary concentration units



We might ask: For a molecule at any initial position along the x -axis, what is its most likely position after a given period of time? If we treat this as a random-walk problem, we conclude that the most likely position is the starting position, even though the random walk will have taken the molecule to many other positions during the time period. But, this must be true for all of the molecules in the sample. So, how does net diffusion ever take place?

The solution to the paradox lies in the nature of the probability distribution function. Recall that the Gaussian distribution for a random walk is given by:

$$p(x) = \sqrt{\frac{1}{2\pi\langle x^2 \rangle}} e^{-x^2/(2\langle x^2 \rangle)}$$

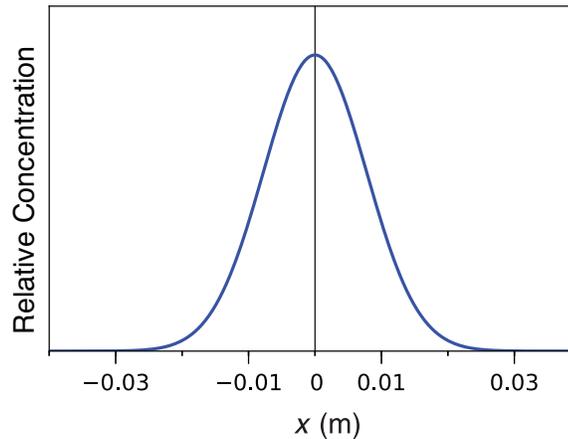
where x is the position of the endpoint and $\langle x^2 \rangle$ is the mean-squared value of x . For diffusion, we defined the random walk parameters in terms of δ_x (the step size), τ (the time interval between steps), t (the total time) and $D = \delta_x^2/(2\tau)$, so that $\langle x^2 \rangle$ is given by:

$$\begin{aligned}\langle x^2 \rangle &= n\delta_x^2 \\ &= \frac{t\delta_x^2}{\tau} \\ &= 2Dt\end{aligned}$$

So, the probability function can be expressed in terms of D and t :

$$p(x) = \sqrt{\frac{1}{4\pi Dt}} e^{-x^2/(4\pi Dt)}$$

A plot of the function looks like:



For this plot, $D = 3 \times 10^{-10} \text{ m}^2/\text{s}$, and $t = 10^5 \text{ s}$.

Remember that a continuous probability distribution function is interpreted in terms of its integral. In this case, the probability that the molecule lies between two positions, a and b is given by the integral:

$$\int_a^b \sqrt{\frac{1}{4\pi Dt}} e^{-x^2/(4Dt)} dx$$

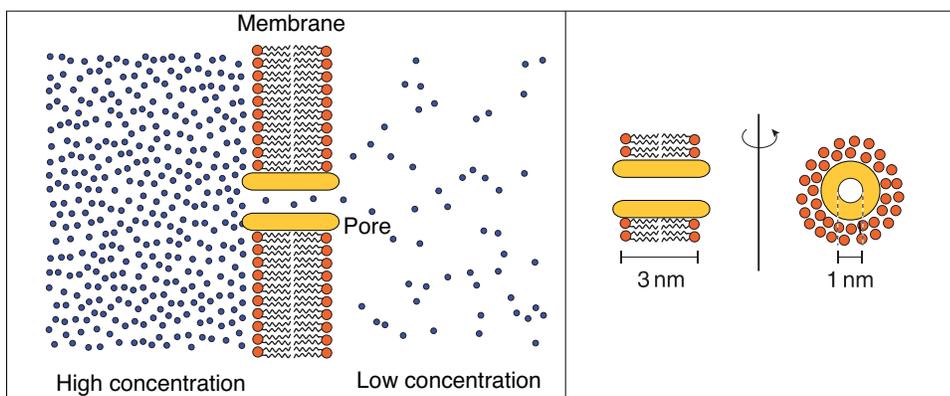
If we divide up the range of x values into thin slices, the slice with the largest probability is the one centered at $x = 0$. *But* the *total* probability that the molecule will be in one of the other slices is much larger than the probability that x will be close to zero.

So, we really do expect virtually all of the molecules to be somewhere else after the time period. The lesson here is that it is not enough to ask what the most likely outcome is! The most likely may represent only a tiny fraction of the total.

III. A (simplified) biological example

In biology, we don't really have examples where we start with a perfectly sharp boundary, with nothing separating the two sides. But, we do have lots of cases where there is a sharp change in concentration across a membrane. Biological membranes are composed of bilayers of phospholipids, along with proteins that are embedded in the bilayer. For now, the structural details are not very important, except that most molecules diffuse across lipid bilayers extremely slowly, and proteins can act as pores that allow much faster selective diffusion of molecules.

Suppose that we have a membrane with a thickness of about 3 nm, a typical value, and a small-molecule compound that has a concentration of 50 mM on one side of the membrane and 5 mM on the other.



Across the width of the membrane, we can estimate the concentration gradient as:

$$\begin{aligned}\frac{dC}{dx} &= \frac{50 \text{ mM} - 5 \text{ mM}}{3 \text{ nm}} = \frac{0.045 \text{ M}}{3 \times 10^{-9} \text{ m}} \\ &= 1.5 \times 10^7 \text{ M/m}\end{aligned}$$

To go further, we need to convert the concentration gradient to units with consistent units of length:

$$\begin{aligned}\frac{dC}{dx} &= 1.5 \times 10^7 \text{ M/m} \\ &= 1.5 \times 10^7 \frac{\text{moles}}{\text{L} \times \text{m}} \times \frac{1 \text{ L}}{10^{-3} \text{ m}^3} \\ &= 1.5 \times 10^{10} \text{ moles/m}^4\end{aligned}$$

From Fick's first law, we can calculate the flux, J :

$$J = -D \frac{dC}{dx}$$

A typical diffusion coefficient for small molecules is $10^{-10} \text{ m}^2/\text{s}$.

$$\begin{aligned} J &= -10^{-10} \text{ m}^2/\text{s} \times 1.5 \times 10^{10} \text{ moles}/\text{m}^4 \\ &= -1.5 \text{ moles}/(\text{m}^2\text{s}) \end{aligned}$$

This looks like a lot of molecules per second, but remember that the flux is expressed per unit of area, in this case 1 m^2 . The negative sign simply indicates that the flux is in the opposite direction of the concentration gradient.

The pores in membranes vary greatly in size and shape, and many of them have very small and specialized structures for which a general treatment of diffusion is probably not appropriate. But, there are examples of pores with diameters of a few nm. For a pore diameter of 1 nm, the area is:

$$A = \pi r^2 = \pi(0.5 \times 10^{-9} \text{ m})^2 = 7.8 \times 10^{-19} \text{ m}^2$$

The flow through this pore is then:

$$1.5 \text{ moles}/(\text{m}^2\text{s}) \times 7.8 \times 10^{-19} \text{ m}^2 = 1.2 \times 10^{-18} \text{ moles}/\text{s}$$

The number of molecules per second is:

$$1.2 \times 10^{-18} \text{ moles}/\text{s} \times 6.02 \times 10^{23} \text{ molecules}/\text{mole} \approx 7 \times 10^5 \text{ molecules}/\text{s}$$

How many molecules would be in the volume of the pore at any instant? The volume is:

$$\begin{aligned} V &= A \times L = \pi r^2 \times L = \pi(0.5 \times 10^{-9} \text{ m})^2 \times 3 \times 10^{-9} \text{ m} \\ &= 7.8 \times 10^{-19} \text{ m}^2 \times 3 \times 10^{-9} \text{ m} = 2.4 \times 10^{-27} \text{ m}^3 \\ &= 2.4 \times 10^{-24} \text{ L} \end{aligned}$$

If the concentration within the pore is the average of that on the two sides of the membrane, 20 mM, the average number of molecules in the pore is calculated as:

$$2.4 \times 10^{-24} \text{ L} \times 0.02 \text{ moles}/\text{L} = 5 \times 10^{-26} \text{ moles} \approx 0.03 \text{ molecules}$$

This result means that the pore is empty nearly all of the time, even though about 10^6 molecules are passing through every second. So, each is there for a very short time.

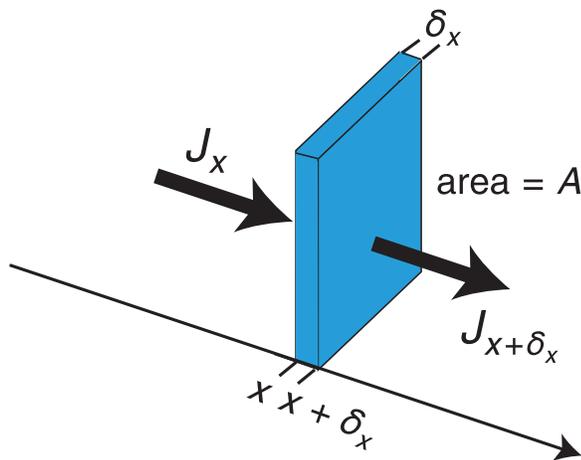
Can a flow of 10^6 molecules/s be detected through a single pore? It can be if the molecule is charged, by measuring electric current. A current of 1 ampere (A) corresponds to 1 coulomb per s, or about 6×10^{18} charges per s. So 10^6 charges/s corresponds to about 2×10^{-13} A, or 0.2 pA. This is a very small current, but currents of this magnitude are routinely measured by electrophysiologists studying single channels in membranes in (or removed from) neurons and muscle cells.

4.2 Fick's second law

As soon as there is a net flux between regions of a sample, the concentrations will change. Fick's second law describes the change in concentration with time.

I. The derivation

Consider, again, a thin slice cut perpendicular to the x -axis, with area A and thickness δ_x :



The net number of molecules moving to the right at the two sides of the slice during an interval dt will be:

$$N_x = AJ_x dt$$

$$N_{x+\delta_x} = AJ_{x+\delta_x} dt$$

where J_x and $J_{x+\delta_x}$ are the values of the flux at positions x and $x + \delta_x$ respectively. The change in the number of molecules in the slice will be:

$$\begin{aligned} dN &= AJ_x dt - AJ_{x+\delta_x} dt \\ &= A dt (J_x - J_{x+\delta_x}) \end{aligned}$$

The change in concentration will be:

$$\begin{aligned} dC &= \frac{dN}{A\delta_x} \\ &= \frac{A dt (J_x - J_{x+\delta_x})}{A\delta_x} \\ &= -dt \frac{J_{x+\delta_x} - J_x}{\delta_x} \end{aligned}$$

In the limit of small dt and small δ_x :

$$\frac{dC}{dt} = -\frac{J_{x+\delta_x} - J_x}{\delta_x} = -\frac{dJ}{dx}$$

From Fick's first law, we know how J depends on the change of C with respect to x :

$$J = -D\frac{dC}{dx}$$

Differentiating J with respect to x gives:

$$\frac{dJ}{dx} = -D\frac{d^2C}{dx^2}$$

Substituting:

$$\frac{dC}{dt} = D\frac{d^2C}{dx^2}$$

This is the usual form of Fick's second law. It is also referred to as the "diffusion equation", and it provides the basis for calculating how concentration will change with time, provided that we know how concentration depends on position, x , which, of course, changes continuously.

The good news is that diffusion is described by this very simple equation. The bad news is that it's not at all simple to solve this equation for real problems. What is required is to find a function, C , of both x and t , that satisfied this differential equation *and* describes the particular physical arrangement at when $t = 0$.

Historically, problems of this type were first solved in the context of heat flow through materials, which follows the same mathematical laws as diffusion. Consideration of problems of this type led the French mathematician Joseph Fourier to develop the methods now known by his name, Fourier analysis. This can be, and is, the subject of entire courses.

The two laws of Fick describe different, but closely related, features of the dynamics:

1. Fick's first law states that the net flux of diffusing molecules is proportional to the change in concentration with respect to distance, *i.e.*, the "concentration gradient".
2. Fick's second law states that the change in concentration with respect to time, at a given position, is proportional to the derivative of the concentration gradient, *i.e.*, how rapidly the concentration gradient changes with position.

To see how these relationships work, we will look at the solution to the case of diffusion from a sharp boundary.

4.3 Diffusion from a Sharp Boundary

The case introduced earlier, diffusion from a sharp boundary, is one for which a solution to the diffusion can be found relatively easily. Though this case is highly simplified and restricted to diffusion in only a single dimension, its solution reflects general properties of diffusion and provides considerable insight.

I. A solution to the diffusion equation

In looking for a solution to the diffusion equation, we are looking for a function, $C(x, t)$, that describes the concentration of the diffusing molecules as a function of both x and t such that the derivatives of the function satisfy the differential equation:

$$\frac{dC}{dt} = D \frac{d^2C}{dx^2}$$

For each value of x , the concentration at time t will represent all of the molecules that have diffused to that point, from all of the points at which molecules were initially present (including x itself). For the case of diffusion from a sharp boundary, we can say the following:

- For $x < 0$, the initial concentration is 0.
- For $x \geq 0$ the concentration is initially the same, and we can call this value 1, in arbitrary units.

These constitute the boundary conditions for the problem. Any solution must satisfy these conditions, as well as the differential equation.

Assuming that there are initially a very large number of molecules in the vicinity of each value of $x \geq 0$, we know that the final distribution of molecules *from that position* will be described by a Gaussian probability distribution function that is centered at the initial position. Recall that the Gaussian function, for molecules beginning at $x = 0$, can be written as:

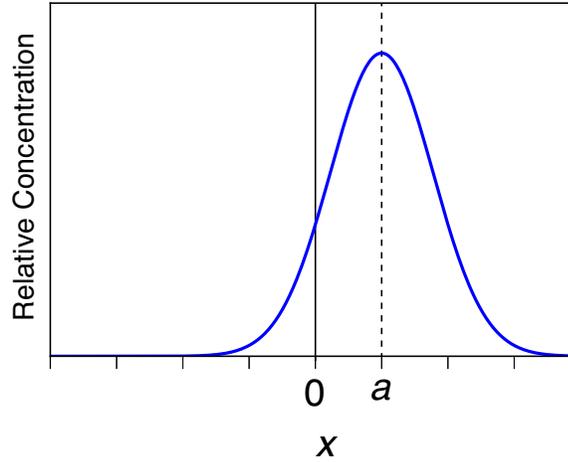
$$p(x) = \sqrt{\frac{1}{4\pi Dt}} e^{-x^2/(4Dt)}$$

More generally, if we consider molecules that begin at position $x = a$, we can replace x with $(a - x)$ in the distribution to give

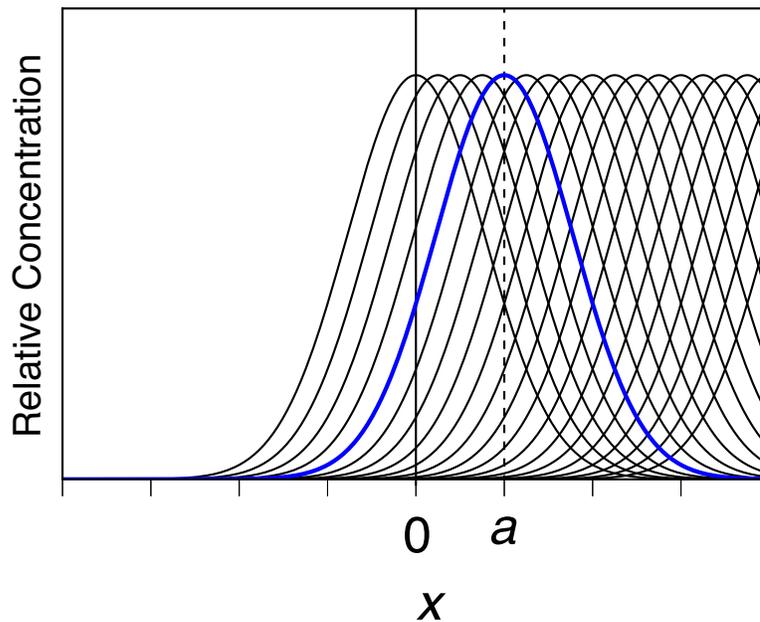
$$P(x, a) = \sqrt{\frac{1}{4\pi Dt}} e^{-(a-x)^2/(4Dt)}$$

A plot of the function looks like:

4.3. DIFFUSION FROM A SHARP BOUNDARY



For a given position x , the final concentration will be the total of molecules from all values of $a > 0$. So, we add together the value of all of the probability functions for all values of $a > 0$, as represented in the figure below:



In other words, we integrate:

$$C(x, t) = \int_0^{\infty} \sqrt{\frac{1}{4\pi Dt}} e^{-(a-x)^2/(4Dt)} da$$

Notice that the variable a doesn't appear in the final result, it simply represents all of the possible starting positions of the molecules. This integral cannot be evaluated analytically, but it can be estimated numerically.

In most textbooks, this result is presented somewhat differently, as:

$$C(x, t) = \frac{1}{2} \left[1 + \operatorname{erf} \left(\frac{x}{\sqrt{4Dt}} \right) \right]$$

where erf is called the “error function” (because it arises in the statistical analysis of measurement errors) and is defined as:

$$\operatorname{erf}(z) = \frac{2}{\sqrt{\pi}} \int_0^z e^{-u^2} du$$

We can check that our solution satisfies Fick’s second law by calculating the appropriate derivatives. For this purpose, it is convenient to use the form using the error function, substituting $x/\sqrt{4Dt}$ for z :

$$C(x, t) = \frac{1}{2} + \frac{1}{\sqrt{\pi}} \int_0^{x/\sqrt{4Dt}} e^{-u^2} du$$

Since the function $C(x, t)$ is an integral of the Gaussian function, it should not be surprising that the derivatives of $C(x, t)$ are Gaussian functions. The fundamental theorem of calculus stipulates that if a function $F(X)$ is defined as:

$$F(X) = \int_a^X f(x) dx$$

then the derivative of $F(X)$ is simply $f(X)$.

Using this relationship and some substitutions, differentiating $C(x, t)$ with respect to x gives:

$$\begin{aligned} \frac{dC}{dx} &= \frac{d}{dx} \left[\frac{1}{2} + \frac{1}{\sqrt{\pi}} \int_0^{x/\sqrt{4Dt}} e^{-u^2} du \right] \\ &= \sqrt{\frac{1}{4\pi Dt}} e^{-x^2/(4Dt)} \end{aligned}$$

The second derivative of C with respect to x is:

$$\frac{d^2C}{dx^2} = -\frac{x}{4\sqrt{\pi D^3 t^3}} e^{-x^2/(4Dt)}$$

With some effort (or the assistance of a computer program such as Mathematica or Maxima), the derivative of C with respect to t can be shown to be:

$$\frac{dC}{dt} = -\frac{x}{4\sqrt{\pi D^3 t^3}} e^{-x^2/(4Dt)}$$

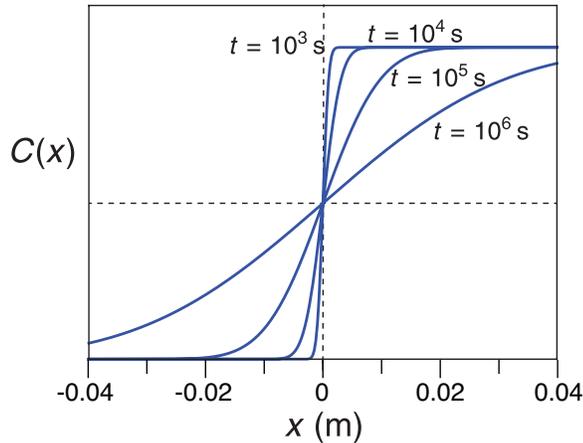
Thus, Fick’s second law is satisfied by this solution:

$$\begin{aligned} \frac{dC}{dt} &= D \frac{d^2C}{dx^2} \\ -\frac{x}{4\sqrt{\pi D t^3}} e^{-x^2/(4Dt)} &= -D \frac{x}{4\sqrt{\pi D^3 t^3}} e^{-x^2/(4Dt)} \end{aligned}$$

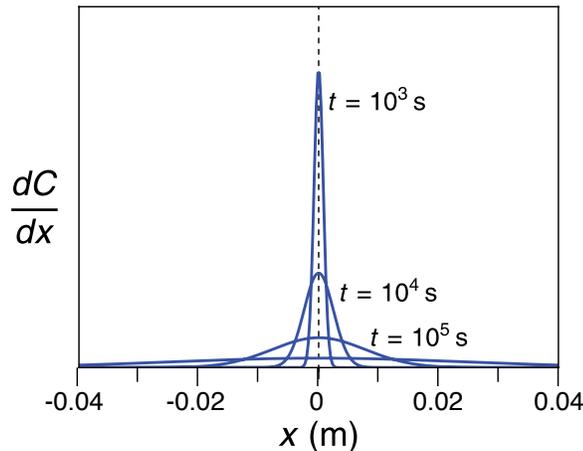
II. Graphical representations of the solution

The best way to get a feel for all of this is to look at graphs representing the solution and its derivatives.

The profiles predicted by the solution to the diffusion equation (for this particular starting state) are shown in the graph below, for the case where $D = 3 \times 10^{-10} \text{ m}^2/\text{s}$ and the time after creation of the sharp boundary, t , is 10^3 , 10^4 , 10^5 or 10^6 s, as indicated. (Note that the function is not defined for $t = 0$.)



The first derivative of C with respect to x is plotted below for the same value of D and the indicated times.

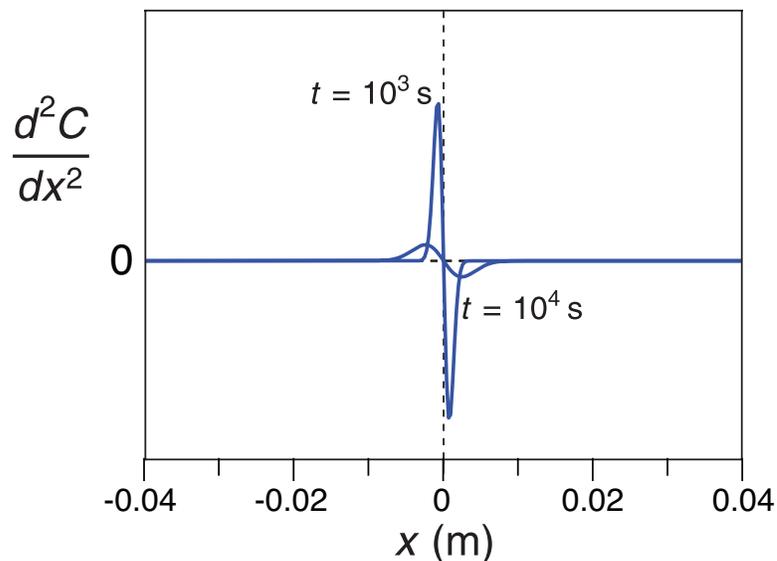


Notice that the derivative has the form of a Gaussian function, which reflects the fact that $C(x, t)$ has the form of an integral of the Gaussian. The peak of the concentration gradient remains at $x = 0$, but decreases in steepness with time as the gradient covers a wider range of x values.

The *net* rate at which molecules pass a particular point is proportional to the concentration gradient. Thus, the flux, J , is always maximal at $x = 0$ but decreases with

time at this point. At other points, however, the flux increases and then decreases with time.

Finally, we look at the second derivative of C with respect to x :



Notice that there are two peaks, one positive and one negative. At $t = 0$, these are extremely sharp and represent the two edges of the sharp concentration gradient. With time, these peaks move apart and become less pronounced as the concentration gradient becomes more gentle.

The positive peak on the left represents the region where the concentration is beginning to increase and where the gradient increases most rapidly. This is where the concentration is increasing most rapidly. But, it's not where the flux, J , is maximal! The flux is always maximal at $x = 0$.

Why is the region where the flux is greatest *not* where the concentration changes most rapidly?

At $x = 0$, the flux is maximal, but the molecules are constantly being replaced by the “reservoir” to the right and are being drawn off to the left. So, the concentration stays constant.

Where the second derivative is maximal, the flux changes most rapidly with position. This means that the flux going into a volume element is greater than that leaving, so that the concentration changes most rapidly.

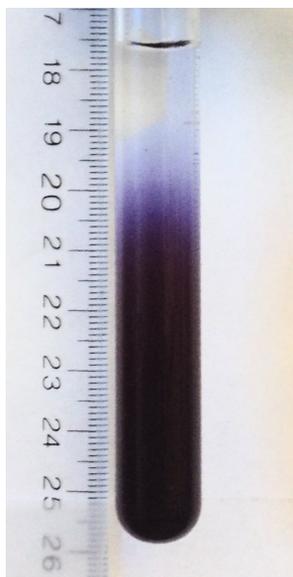
As time increases, the absolute values of both the first and second derivatives decrease, so that both the flux and the rate of change in concentration decrease.

Notice, also that the concentration at positions close to $x = 0$ change very rapidly, but even a millimeter away, the change is quite slow.

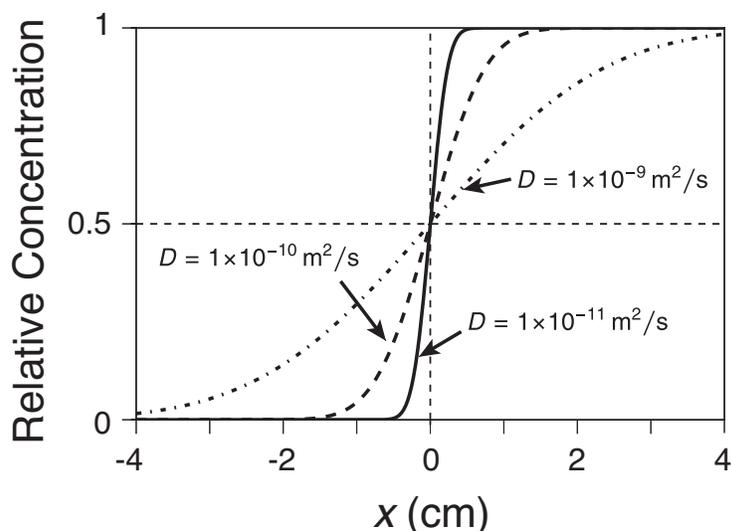
4.4 Estimating a Diffusion Constant from a Simple Experiment

An approximation to the ideal sharp boundary experiment can be realized in practice by overlaying two solutions, one containing a dye. In order to form the (relatively) sharp boundary easily, it is also necessary to make the lower solution slightly more dense than the upper one, for instance by adding a few percent of glycerol. The increased viscosity of the lower solution slightly complicates the situation, but not so much as to obscure the essential features of the experiment

The photograph below shows the result of such an experiment 48 h after establishing the boundary.



Even after 48 h, very little, if any, of the dye has reached the top of the solution, about 2 cm from the initial boundary. We can use the result of this experiment to make a rough estimate of the diffusion coefficient, D , by comparing the observation to those predicted by the solution for diffusion from a boundary. The plot below shows the expected concentration, after 48 h, as a function of position, x , with different values assumed for the diffusion coefficient, as indicated.



As a rough estimate, it appears that the experimental result lies somewhere between the curves calculated for $D = 10^{-10}$ and $10^{-9} \text{ m}^2/\text{s}$. Later, we will see how to calculate the diffusion coefficient from the size of a molecule and the viscosity of the solution, and we'll see how close the two estimates match.

4.5 Molecular Motion and Kinetic Energy

So far, we have considered diffusion from a macroscopic point of view, focusing on the net flux of molecules and changes in concentration, without considering the nature of the molecular motions. To take a more microscopic view, we need to consider the motions of individual molecules, which reflect their kinetic energy.

I. Kinetic energy

We can begin this discussion by asking, in the most general way, what is energy? The standard textbook definition is that energy is the ability to do work. But what, then, is work?

For mechanical motion, work, and therefore energy, represents an integral of force with respect to distance:

$$w = \int_a^b F dx$$

The units of energy are those of force times distance. In SI units $\text{N}\cdot\text{m} = \text{joule (J)}$.

The unit of force, N, is defined from Newton's second law:

$$F = m \cdot a = \text{kg} \cdot \text{m} \cdot \text{s}^{-2}$$

Therefore, in the basic SI units, energy has units of $1 \text{ J} = 1 \text{ kg} \cdot \text{m}^2 \cdot \text{s}^{-2}$.

The kinetic energy of an object moving along a given direction, x , from classical mechanics is:

$$E_{k,x} = m \cdot v^2/2$$

This represents the work required to accelerate an object of mass m from rest to velocity v , in the absence of friction. It doesn't matter how rapidly or slowly the acceleration is, the final energy depends only on mass and velocity. If the object slows down, it loses some of that energy.

Note that kinetic energy has the correct units, $= \text{kg} \cdot \text{m}^2 \cdot \text{s}^{-2}$

Also note that doubling the velocity increases the kinetic energy four fold. This is why car accidents become so much more dangerous at higher speeds.

II. Thermal energy

Fundamentally, temperature is a measurement of the motion of molecules. The simplest thermometer is a container of gas that expands or contracts when the temperature changes (at constant pressure). Alternatively, we can measure temperature by measuring the pressure of a gas at constant volume. Though this was not always understood, we now know that the pressure represents the collisions of molecules against the side of the container.

For an ideal gas, we have the relationship:

$$PV = nRT$$

where P is pressure, V is volume, n is the number of moles of gas, T is temperature and R is the gas constant. An ideal gas is one that is made up of particles that do not interact at all with one another. At moderate temperatures and pressures, real gases are well approximated by the ideal gas law.

What are the units of the gas constant?

Pressure has the units of force per unit area, or $\text{N} \cdot \text{m}^{-2}$, and volume has the units of m^3 . T has units of K, and n has units of moles. Therefore, R has units of:

$$R = \frac{PV}{nT} = \frac{\text{N} \cdot \text{m}^{-2} \text{m}^3}{\text{K} \cdot \text{mol}} = \text{N} \cdot \text{m} / (\text{K} \cdot \text{mol})$$

In the basic SI units, R has the units of:

$$\text{N} \cdot \text{m} / (\text{K} \cdot \text{mol}) = \text{kg} \cdot \text{m}^2 \text{s}^{-2} \text{K}^{-1} \text{mol}^{-1}$$

Notice, though, that we just showed that $\text{kg} \cdot \text{m}^2 \cdot \text{s}^{-2}$ is a unit of energy, the Joule. So, R can be expressed in units of J/K, and the product RT must, in some sense be a measure of the energy that one mole of moving molecules have at a given temperature, irrespective of pressure and volume.

To discuss the energy of individual molecules, it is convenient to divide the gas constant by Avogadro's number. ($\approx 6.02 \times 10^{23}$). This is the Boltzmann constant, which, in SI units, is:

$$k = 1.3806 \times 10^{-23} \text{ kg} \cdot \text{m}^2 \text{s}^{-2} \text{K}^{-1} = 1.3806 \times 10^{-23} \text{ J/K}$$

In a given volume of gas, not all of the molecules will have the same energy (or velocity) at a given instant. In fact, they will have a broad distribution of energies as they collide with one another and the walls and exchange energy. So, we have to express the kinetic energy as an average. Without going through the derivation, the RMS translational kinetic energy in one direction is:

$$\text{RMS}(E_{k,x}) = kT/2$$

And the total translational energy, summed over all three directions is:

$$\text{RMS}(E_k) = 3kT/2$$

Remember, though that the kinetic energy is also expressed in terms of the velocity and mass of a particle:

$$E_k = m \cdot v^2/2$$

Combining these equations gives:

$$v = \sqrt{kT/m}$$

where v is understood to be an RMS average velocity. With this equation, we can calculate the RMS velocity knowing only the mass of a molecule and the temperature.

III. Steps in the random walk

The equations for kinetic energy in a gas also apply to molecules in a liquid, at the same temperature. The instantaneous velocities are the same, it's just that the molecules collide with one another much more frequently in a liquid, and we can now calculate just how frequent that is.

For a single (average) step in the random walk, the displacement is δ_x and the time is τ . Therefore, the velocity during this period is:

$$v = \delta_x/\tau$$

If we have measured the diffusion coefficient, D , we can now calculate δ_x :

$$\begin{aligned} D &= \frac{\delta_x^2}{2\tau} \\ &= \frac{\delta_x}{2} v \\ &= \frac{\delta_x}{2} \sqrt{kT/m} \\ \delta_x &= \frac{2D}{\sqrt{kT/m}} \end{aligned}$$

The average time between collisions is given by:

$$\begin{aligned} \tau &= \delta_x/v \\ &= \frac{2D}{\sqrt{kT/m}} \frac{1}{\sqrt{kT/m}} \\ &= \frac{2D}{kT/m} \end{aligned}$$

What is implied by these equations?

- The average velocity of a molecule depends on the temperature and mass, irrespective of the surrounding environment.
- But, the average distance that a molecule goes before colliding and bouncing off in different directions does depend on the environment.
- All molecules at a given temperature have the same average kinetic energy. (This is implied by the ideal gas law.) However, the average velocity is inversely related to the square root of the molecular mass. Big molecules move more slowly.

IV. Some typical values of D , δ_x and τ .

From the simple diffusion experiment, we estimated that the diffusion coefficient for the bromophenol blue dye is about $10^{-10} \text{ m}^2/\text{s}$. The molecular weight of this dye is 670 g/mol. Thus, the mass of a single molecule is $1.1 \times 10^{-21} \text{ g}$, or $1.1 \times 10^{-24} \text{ kg}$. At room temperature ($\approx 300 \text{ K}$), the expected velocity of the molecule is then:

$$\begin{aligned} v &= \sqrt{kT/m} \\ &= \sqrt{\frac{1.38 \times 10^{-23} \text{ kg} \cdot \text{m}^2 \cdot \text{s}^{-2} \cdot \text{K}^{-1} \cdot 300 \text{ K}}{1.1 \times 10^{-24} \text{ kg}}} \\ &\approx 60 \text{ m/s} \end{aligned}$$

This seems very fast, especially considering how slowly the molecules diffused. But, we know that they only move in a given direction for a short time before colliding with another molecule in the solution. From the relationships derived earlier, and the estimate of the diffusion coefficient, we can calculate the distance between collisions as:

$$\begin{aligned}\delta_x &= 2D/v \\ &\approx 2 \times 10^{-10} \text{ m}^2/\text{s} \div 60 \text{ m/s} \\ &\approx 3 \times 10^{-12} \text{ m}\end{aligned}$$

Thus, the average displacement is extremely small: A hydrogen atom is about 10^{-10} m in diameter. The time interval between collisions is correspondingly small:

$$\begin{aligned}\tau &= \delta_x/v \\ &\approx 3 \times 10^{-12} \text{ m} \div 60 \text{ m/s} \\ &\approx 5 \times 10^{-14} \text{ s}\end{aligned}$$

The RMS displacement along one axis as a function of time is given by:

$$\begin{aligned}\text{RMS}(x) &= \sqrt{2Dt} \\ &\approx \sqrt{2 \times 10^{-10} \text{ m}^2/\text{s} \cdot t(\text{s})} \\ &\approx 1.4 \times 10^{-5} \text{ m} \sqrt{t(\text{s})}\end{aligned}$$

V. The relationship between molecular size and diffusion coefficient

In general, we expect the diffusion coefficient to depend on the molecule and its environment. More specifically, we might expect D to depend on the size of the molecule, the temperature and the viscosity of the solution. Indeed, this is the key relationship that Einstein formulated in his classic 1905 paper on Brownian motion:

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

where η is the viscosity of the solution and r is the radius of a spherical particle. This is usually referred to as the Stokes-Einstein equation, showing that even Einstein built on the work of others! Strictly, this applies only to spherical particles, but it is common to refer to an “effective radius” for particles that are less symmetrical.

We will ignore the question of how viscosity is defined and measured except to note that it is most commonly expressed in units of centipoise, which is equivalent to $10^{-3} \text{ N} \cdot \text{s} \cdot \text{m}^{-2}$. The factor of 10^{-3} has obscure historical origins, but the unit of centipoise has been retained, probably because water at room temperature has a viscosity very close to 1 centipoise. It is left to the student to demonstrate that the units in the Stokes-Einstein equation are consistent.

4.5. MOLECULAR MOTION AND KINETIC ENERGY

Here are a few examples of particles with a range of sizes and calculated diffusion coefficients:

- Small molecule (1 nm): $2 \times 10^{-10} \text{ m}^2\text{s}^{-1}$
- Protein (10 nm): $2 \times 10^{-11} \text{ m}^2\text{s}^{-1}$
- Bacterium (1 μm): $2 \times 10^{-13} \text{ m}^2\text{s}^{-1}$
- 1 mm sphere: $2 \times 10^{-16} \text{ m}^2\text{s}^{-1}$

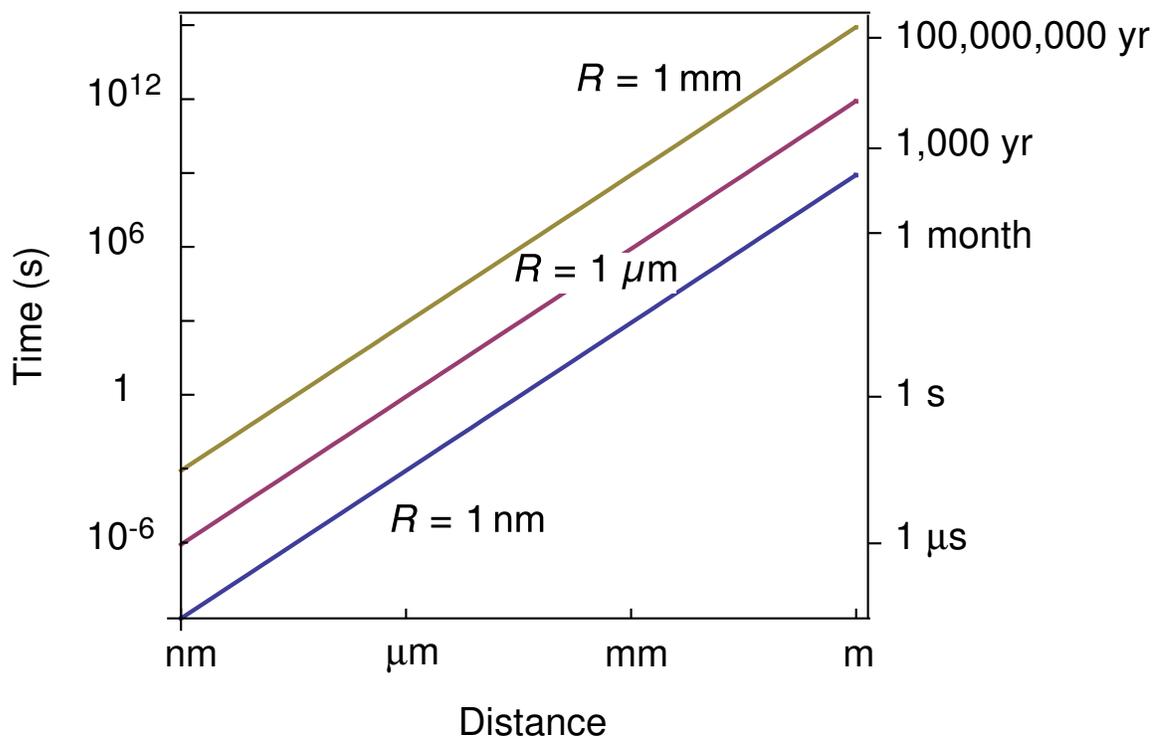
To place these diffusion coefficients into some context, it is helpful to calculate the time required for a particle to diffuse until the RMS distance from the starting point reaches a given value. Earlier, the relationship between the RMS distance and time was given as:

$$\text{RMS}(x) = \sqrt{2Dt}$$

Rearranging this equation gives:

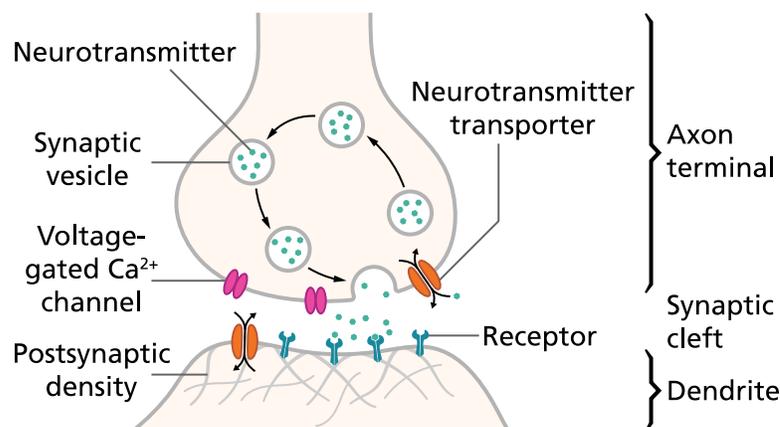
$$t = \frac{\text{RMS}(x)^2}{2D}$$

The graph below shows how the time required to diffuse a given RMS distance depends on time for different size molecules:



This graph shows that diffusion leads to quite fast net movements of molecules over short distances, but the times required for movement over longer distances can greatly exceed what is necessary in many biological contexts.

A biological example highlighting the differences in diffusion times over different distances is neural transmission. Individual neurons communicate with adjacent neurons and muscle cells via chemical synapses, as diagrammed below¹:



When stimulated, the axon terminus of a neuron (the pre-synaptic cell) releases neurotransmitter molecules, such as acetylcholine, glutamate or dopamine. These small molecules diffuse across the synaptic cleft, which has a width of about 20 nm, and bind to receptors on the adjacent neuron or muscle cell (the post-synaptic cell). Binding to these receptors then generates a signal within the post-synaptic cell. The time required for the transmitter to diffuse an RMS distance of 20 nm is calculated (assuming a diffusion coefficient of $2 \times 10^{-10} \text{ m}^2\text{s}^{-1}$) as:

$$\begin{aligned} t &= \frac{\text{RMS}(x)^2}{2D} \\ &= \frac{(2 \times 10^{-8} \text{ m})^2}{2 \times 2 \times 10^{-10} \text{ m}^2\text{s}^{-1}} \\ &= 10^{-6} \text{ s} = 1 \mu\text{s} \end{aligned}$$

Signals also must travel along the lengths of neurons, which can be up meters in length. To travel by diffusion a distance this long, would take:

$$\begin{aligned} t &= \frac{(1 \text{ m})^2}{2 \times 2 \times 10^{-10} \text{ m}^2\text{s}^{-1}} \\ &= 2.5 \times 10^{10} \text{ s} \approx 80 \text{ yr} \end{aligned}$$

The obvious conclusion from this calculation is that some other mechanism must be employed to transmit signals over the length of the neuron, and this mechanism is the propagation of an electrical potential across the membrane. Within neurons and other eukaryotic cells, the components of various structures must be transported over distances that are similarly too long for diffusion to be effective. Molecular motors that move along structural tracks in the cell facilitate this kind of motion.

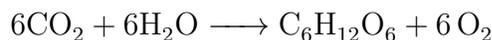
¹Figure from https://en.wikipedia.org/wiki/Chemical_synapse, Thomas Spletstoesser (www.scistyle.com).

4.6 A Plant Faces Diffusion

Diffusion plays a role in the physiology of all organisms, as they exchange nutrients and other compounds with their environments. Here we consider diffusion at the surface of plant leaves, a process that dictates many aspects of plant physiology, structure and ecology.

I. A plant's demand for CO₂

Consider the growth of a seed to a plant. Where does all of the mass, especially the carbon, come from? Nearly all of the carbon comes, literally, from thin air. The net chemical reaction is:



This is an extremely unfavorable chemical reaction, except when it is coupled to the absorption of energy provided by sunlight.

A back of the envelope calculation: How much CO₂ must cross the leaf surface per second to support a plant's growth? Suppose that a plant incorporates 1 kg of carbon a year. How much leaf area does such a plant have? A rough estimate might be that the plant has 1,000 leaves with an area of 1 cm² each, for 0.1 m² total.

Total moles of carbon per year:

$$1 \text{ kg} \div 12 \text{ g/mol} \approx 80 \text{ mol}$$

Total seconds per year:

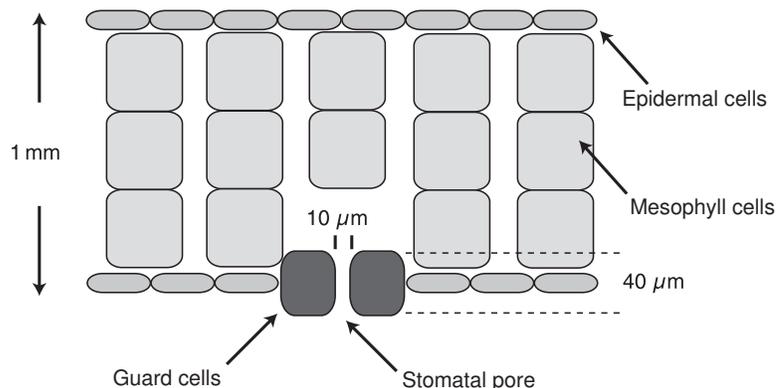
$$1 \text{ yr} \times 365 \text{ days/yr} \times 24 \text{ hr/day} \times 60 \text{ min/hr} \times 60 \text{ s/min} \approx 3 \times 10^7 \text{ s}$$

But, CO₂ is incorporated only during daylight, so the total time available is only about half of this. The number of moles per second is:

$$80 \text{ mol} \div 1.5 \times 10^7 \text{ s} \approx 5 \times 10^{-6} \text{ mol/s}$$

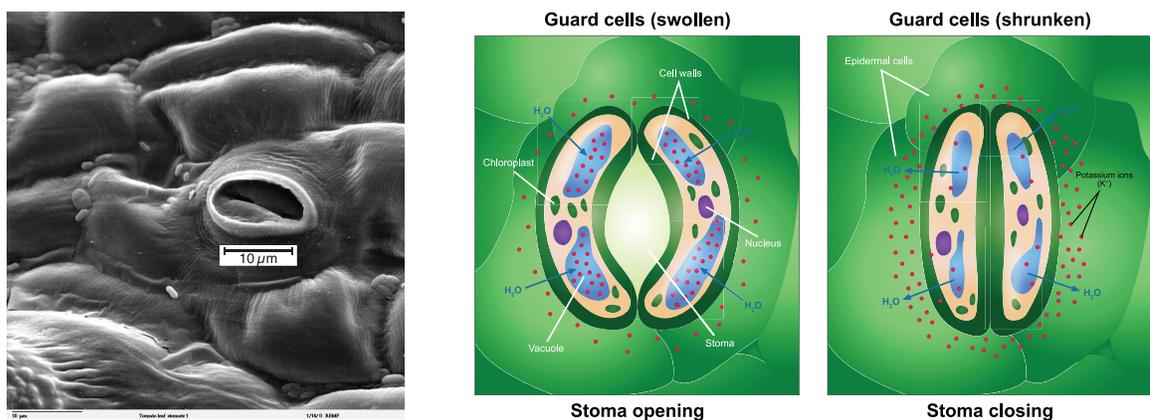
II. Leaf structure and stomata

CO₂ enters leaves only through special openings, called stomata, which can be regulated depending on physiological state. A rough cross-sectional drawing of a typical plant leaf is shown below.



The cells that carry out photosynthesis, the mesophylls, are enclosed in a space bounded by the leaf epidermis on both sides. In some plants, the stomata are found only on the lower leaf surface, but in others they are on both surfaces. Photosynthesis takes place within the chloroplasts of the mesophylls, and the CO_2 is converted into sugars by the enzyme ribulose-1,5-bisphosphate carboxylase (Rubisco). This is the most abundant enzyme on earth, and is arguably the most important for life. Essentially every carbon atom in our bodies passes through this enzyme.

The left-most panel in the figure² below is a scanning electron micrograph of a tomato-plant leaf. The large gaping opening is a single open stoma, with a diameter of about $10\ \mu\text{m}$ and a depth of about $40\ \mu\text{m}$.



The two other panels in the figure are diagrams of a stoma in the open and closed states. The opening is controlled by two large cells, called guard cells on either side, which expand and change shape when their water content increases to open the stoma. When the water content decreases, the guard cells contract, and the opening closes.

III. Diffusion of CO_2 through stomata

In the air, CO_2 is a trace gas, making up about 580 parts per million of the atmosphere by mass. This number has increased with the burning of fossil fuels, which is, of course, a very important issue right now. At sea level, the concentration of CO_2 is about $1.5 \times 10^{-2} \text{ mol} \cdot \text{m}^{-3} = 15\ \mu\text{M}$.

Within the leaf, the consumption of CO_2 by the chloroplasts depletes the concentration in the leaf airspace. The flux through the leaf involves many steps and concentration gradients, but the most significant barrier to diffusion is in the stomata. In the airspace, the CO_2 concentration is about half of what it is in the atmosphere, *i.e.*, about $7.5 \times 10^{-3} \text{ mol} \cdot \text{m}^{-3}$. So, the concentration difference across the stomata is about $7.5 \times 10^{-3} \text{ mol} \cdot \text{m}^{-3}$.

Recall Fick's first law:

$$J = -D \frac{dC}{dx}$$

²Scanning electron micrograph of tomato-leaf surface by Louisa Howard, <http://remf.dartmouth.edu/images/botanicalLeafSEM/source/16.html>

Diagrams of open and closed stomata by Ali Zifa <https://en.wikipedia.org/wiki/Stoma>

where J is the flux, D is the diffusion coefficient, C is concentration and x is distance along the direction of diffusion.

For the stomata:

$$\frac{dC}{dx} = \frac{1.5 \times 10^{-2} \text{ mol} \cdot \text{m}^{-3} - 0.75 \times 10^{-2} \text{ mol} \cdot \text{m}^{-3}}{40 \times 10^{-6} \text{ m}} = 190 \text{ mol} \cdot \text{m}^{-4}$$

The diffusion coefficient for CO_2 at atmospheric pressure is $1.5 \times 10^{-5} \text{ m}^2\text{s}^{-1}$. This is much larger than the numbers we discussed for molecules in water. Why?

The flux, per unit of surface area, is then:

$$\begin{aligned} J &= -D \frac{dC}{dx} = -1.5 \times 10^{-5} \text{ m}^2\text{s}^{-1} \times 190 \text{ mol} \cdot \text{m}^{-4} \\ &= -2.8 \times 10^{-3} \text{ mol} \cdot \text{m}^{-2}\text{s}^{-1} \end{aligned}$$

How much surface area do we need in order to fix 1 kg of CO_2 per year?

$$5 \times 10^{-6} \text{ mol} \cdot \text{s}^{-1} \div 2.8 \times 10^{-3} \text{ mol} \cdot \text{m}^{-2}\text{s}^{-1} \approx 0.002 \text{ m}^2$$

We can then calculate the minimal number of stomata required to allow this transfer of CO_2 into the leaves. The area of each stomatal pore is:

$$\pi(5 \times 10^{-6} \text{ m})^2 \approx 10^{-10} \text{ m}^2$$

and the number of pores required is:

$$0.002 \text{ m}^2 \div 10^{-10} \text{ m}^2 = 2 \times 10^7$$

If the plant has a total of 0.1 m^2 of leaf area, then about 2% of that must be devoted to stomatal pores. A plant that grows by 1 kg/yr might have about 1,000 leaves of $1 \text{ cm}^2 = 10^{-4} \text{ m}^2$ each, so that there would be about 20,000 stomata per leaf, or 200 stomata per mm^2 . Actual densities of stomata on plant leaves range from 100 to 1,000 per mm^2 , depending on plant species and environmental conditions.

IV. The big problem: Water diffusion

Because the stomata are just open holes in the leaf surface, other gasses can also diffuse in and out of the airspace. The diffusion of water out of leaves, *transpiration*, is a major factor that limits the ability of plants to fix CO_2 .

Within the airspace of the leaf, water reaches saturation concentration, *i.e.*, close to 100% humidity. For a leaf at 25°C , this is about $1.3 \text{ mol} \cdot \text{m}^{-3}$. Outside the leaf, the water vapor concentration is about half this.

So, we can calculate the H_2O vapor gradient across the stomata:

$$\frac{dC}{dx} = \frac{0.6 \text{ mol} \cdot \text{m}^{-3}}{40 \times 10^{-6} \text{ m}} = 1.5 \times 10^4 \text{ mol} \cdot \text{m}^{-4}$$

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The diffusion coefficient is slightly larger for H₂O than for CO₂. Why? $D = 2.4 \times 10^{-5} \text{ m}^2\text{s}^{-1}$.

The flux per unit area of stomata is:

$$\begin{aligned} J &= -D \frac{dC}{dx} = -2.4 \times 10^{-5} \text{ m}^2\text{s}^{-1} \times 1.5 \times 10^4 \text{ mol} \cdot \text{m}^{-4} \\ &= -0.36 \text{ mol} \cdot \text{m}^{-2}\text{s}^{-1} \end{aligned}$$

For our plant with 0.1 m² of leaf surface area and 0.002 m² of stomatal surface area:

$$0.36 \text{ mol} \cdot \text{m}^{-2}\text{s}^{-1} \times 0.002 \text{ m}^2 = 7 \times 10^{-4} \text{ mol/s}$$

In one year:

$$\begin{aligned} 1.5 \times 10^7 \text{ s} \times 7 \times 10^{-4} \text{ mol/s} &= 10^4 \text{ mol} \\ 10^4 \text{ mol} \times 18 \text{ g/mol} &= 18 \times 10^4 \text{ g} \\ &= 180 \text{ kg} \\ &\approx 45 \text{ gal} \end{aligned}$$

The plant needs about 180 kg of water for each kg of carbon it fixes, just because of evaporation from the leaves.

Note: This is a very rough approximation!

This dwarfs the amount of H₂O directly used in the photosynthesis reactions ($\approx 80 \text{ mol} \approx 1.4 \text{ kg}$).

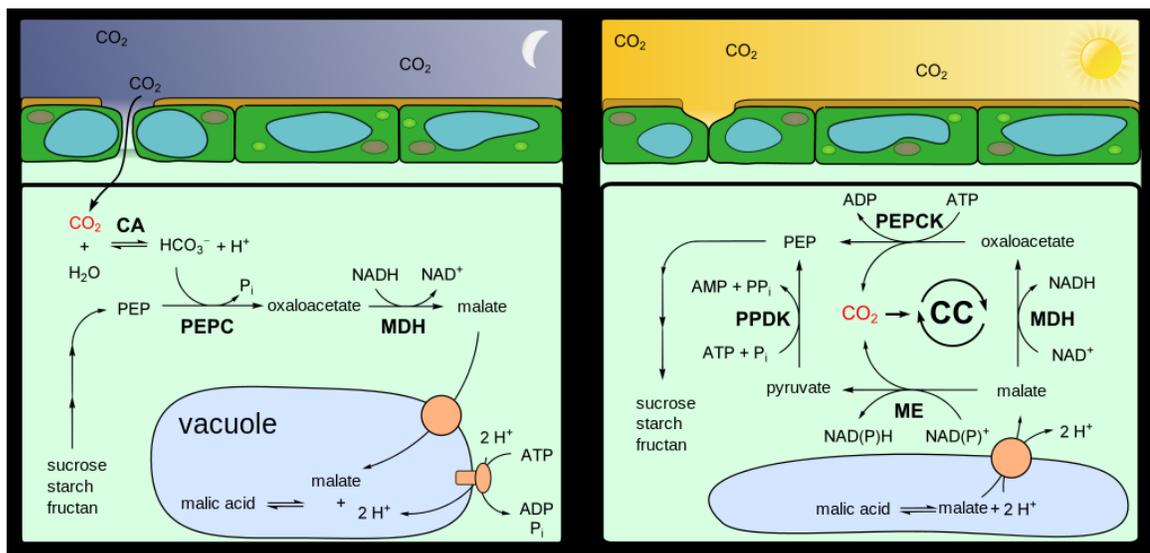
Consequences of huge water losses:

- Stomata are closed when photosynthesis rates are low (*e.g.*, at night). This is probably why plants evolved stomata, rather than allowing diffusion across the entire leaf area.
- The tradeoff between photosynthesis and water loss is the major physiological challenge to plants. Plants in different environments evolve to optimize this trade-off.
- All of this water has to pass through roots and stems of the plant.
- For tall trees, there is a huge pressure difference from the bottom to the top of the trunk of the tree. Conduction depends on unbroken flow of liquid. If bubbles form, conduction stops. Tree trunks have small parallel tubes to conduct water, the xylem. If one develops a cavity, it is sealed off.
- For trees, each year there is a discontinuity in the flow, and new tissue has to be grown, leading to rings.

V. The Crassulacean Acid Metabolism Cycle

In some plant lineages, special adaptations have evolved to minimize the loss of water through stomata, particularly in species that live in arid environments. One of

these adaptations is based on a metabolic pathway, the crassulacean acid metabolism (CAM) cycle, which allows CO_2 to be captured at night and then incorporated into carbohydrates during the day. The name, crassulacean, comes from the plant family *Crassulaceae* (which includes the pineapple and jade plants), where the pathway was first studied in detail. The overall cycle is illustrated in the figure³ below:



During the night, when water transpiration is minimal because of lower temperatures, the stomata are open to allow CO_2 into the leaves. Because, there is no sunlight, however, the plants are not able to convert the CO_2 into carbohydrate via photosynthesis. Instead, the CO_2 is used to convert phosphoenolpyruvate (PEP) into oxaloacetate, which is then converted to malate. The resulting malate is then stored in vacuoles. During the daytime, the stomata are closed, to prevent transpiration, and the malate that accumulated overnight is metabolized to regenerate CO_2 , which is used for photosynthesis.

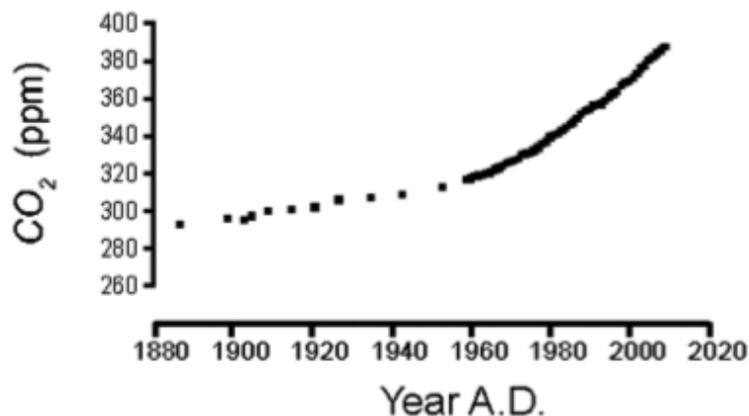
By temporally separating the processes of CO_2 diffusion and photosynthesis, plants using this cycle minimize the loss of water. But, this does come with a cost in metabolic energy, including the hydrolysis of ATP to drive the reformation of PEP and for transport of malate into vacuoles.

VI. Changes in atmospheric CO_2 concentration

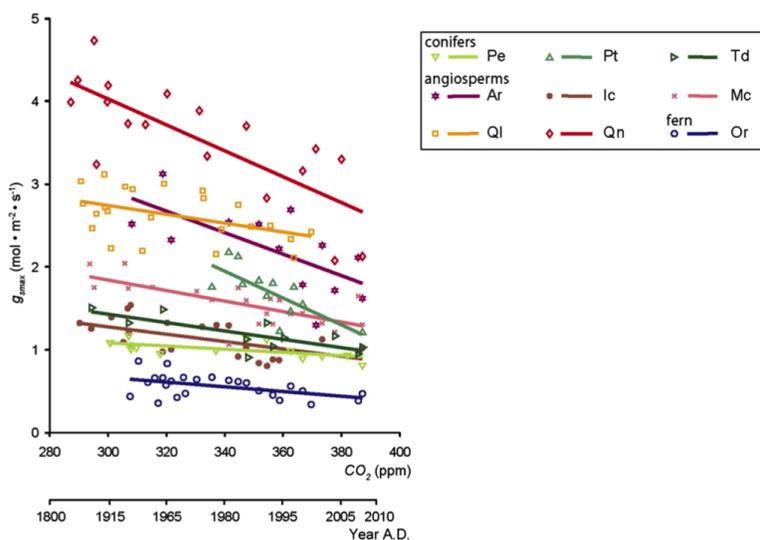
It is now well established that the atmospheric concentration of CO_2 has increased markedly over the past century, as shown in the graph below⁴:

³Figure from https://en.wikipedia.org/wiki/Crassulacean_acid_metabolism

⁴The figures in this section are from Lammersma, E. I., de Boer, H. J., Dekker, S. C., Ditcher, D. L., Lotter, A. F. & Wagner-Cremer, F. (2011). Global CO_2 rise leads to reduced maximum stomatal conductance in Florida vegetation. *Proc. Natl. Acad. Sci., USA*, 108, 4035–4040. <http://dx.doi.org/10.1073/pnas.1100371108>



In molar units, the increase in CO_2 concentration from 300 to 400 ppm is an increase from 12 to 16 μM . This increase in CO_2 concentration could, in principle, be beneficial for plants, both by increasing the efficiency of CO_2 fixation and by minimizing the loss of water through stomata. It appears that plants have responded to the relatively recent increase in CO_2 concentration by reducing the total area of open stomata on their leaves. The results of a study examining the nature of this change in nine plant species found in Florida is shown below:



The quantity plotted on the vertical axis of this plot is termed the *anatomical maximal stomatal conductance to water*, g_{smax} and has the units of $\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. Note that these are the same units as used for flux, J , but g_{smax} is made up of several terms, as defined by the equation:

$$g_{\text{smax}} = \frac{d_{\text{wat}} D \cdot a_{\text{max}} / v}{l + \sqrt{\pi a_x} / 2}$$

where d_{wat} is the diffusion coefficient of water, D is the density of stomata on a leaf, a_{max} is the maximum open area of an individual stoma, l is the length of the stomatal

pore and v is the molar volume of air (the inverse of molar concentration, with units m^3/mol). g_{max} represents the maximum conductivity of water per unit of leaf area and reflects both the dimensions of the stomata and their density on the leaves of a plant.

The important message from the figure shown above is that conductance per unit of leaf area has decreased over the time period when the atmospheric CO_2 concentration has increased. Analogous changes have been demonstrated over geological timescales, when atmospheric CO_2 concentrations have both increased or decreased, and stomatal conductance has decreased or increased, respectively.

In different plant species, the reduction in overall stomatal surface area is due to a reduction in stomatal density or in pore size, or both. For the species of Florida plants examined in this study, the major change appears to be in the density of stomata on the leaf surface, rather than changes in the size of the stomata.

4.7 Bacterial Chemotaxis: Overcoming the Limits of Diffusion

Microorganisms are subject to Brownian motion and can diffuse over short distances, but the requirements for traveling longer distances has led to the evolution of special mechanisms, referred to as chemotaxis. Among the best studied examples of chemotactic microorganisms are the closely related gram-negative bacteria *Escherichia coli* and *Salmonella enterica*⁵.

I. Diffusion from a bacterial perspective

As an approximation, we will treat a bacterial cell as a sphere of $1\ \mu\text{m}$ radius. First, we calculate the diffusion coefficient from the Stokes-Einstein equation:

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

η is viscosity and r is the radius of the particle.

$$k = 1.38 \times 10^{-23} \text{ kg} \cdot \text{m}^2 \text{s}^{-2} \text{K}^{-1}$$

$$T = 300 \text{ K}$$

$$kT = 4.1 \times 10^{-21} \text{ kg} \cdot \text{m}^2 \text{s}^{-2}$$

$$\eta = 10^{-3} \text{ N} \cdot \text{s} \cdot \text{m}^{-2} = 10^{-3} \text{ kg} \cdot \text{m}^{-1} \text{s}^{-1}$$

$$\begin{aligned} D &= \frac{4.1 \times 10^{-21} \text{ kg} \cdot \text{m}^2 \text{s}^{-2}}{6\pi 10^{-3} \text{ kg} \cdot \text{m}^{-1} \text{s}^{-1} \cdot 10^{-6} \text{ m}} \\ &= 2.2 \times 10^{-13} \text{ m}^2 \text{s}^{-1} \end{aligned}$$

⁵The species *S. enterica* is classified into smaller groups, called serovars, and the serovar that has been most extensively studied with respect to chemotaxis is Typhimurium. Until recently this serovar was identified as a species, *Salmonella typhimurium*. It's very confusing.

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This is about 1/1,000 of the value for a small molecule.

A short cut: Remember a few key facts:

- A "small molecule" (≈ 100 Daltons) has a radius of $r \approx 1$ nm.
- A molecule of this size has a diffusion coefficient of about $10^{-10} \text{ m}^2\text{s}^{-1}$.
- The diffusion coefficient is inversely proportional to the radius of a particle.

A bacterium with a radius of $1 \mu\text{m}$ should have a diffusion coefficient of about 1,000th that for a small molecule, or about $10^{-13} \text{ m}^2\text{s}^{-1}$.

Next, we calculate the velocity of the bacterial cell during its random-walk steps, using the relationship:

$$\text{RMS}(v) = \sqrt{kT/m}$$

We need to know the mass. Bacteria (and the great majority of all organisms) have a density that is about the same as water. (Because they are mostly made up of water!) The density is about $1 \text{ g/mL} = 1 \text{ kg/L}$. So if we know the volume we should be able to make a reasonable estimate of the mass.

$$\begin{aligned} V &= \frac{4}{3}\pi r^3 = \frac{4}{3}\pi(10^{-6} \text{ m})^3 \\ &= 4.2 \times 10^{-18} \text{ m}^3 \end{aligned}$$

$1 \text{ m}^3 = 10^3 \text{ L}$, so we can calculate the mass as:

$$\begin{aligned} m &= 4.2 \times 10^{-18} \text{ m}^3 \times \frac{10^3 \text{ L}}{1 \text{ m}^3} \times \frac{1 \text{ kg}}{1 \text{ L}} \\ &= 4.2 \times 10^{-15} \text{ kg} \end{aligned}$$

The average velocity is:

$$\begin{aligned} \text{RMS}(v) &= \sqrt{kT/m} = \sqrt{\frac{4.1 \times 10^{-21} \text{ kg} \cdot \text{m}^2\text{s}^{-2}}{4.2 \times 10^{-15} \text{ kg}}} \\ &= \sqrt{10^{-6} \text{ m}^2\text{s}^{-2}} \\ &= 10^{-3} \text{ m/s} \end{aligned}$$

About 1 mm/s : Much slower than the small molecules, which have a velocity of about 100 m/s .

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The random-walk step size is then calculated as:

$$D = \frac{\delta_x^2}{2\tau} = \frac{v}{2}\delta_x$$

$$\delta_x = \frac{2D}{v}$$

$$\delta_x = \frac{2 \times 2.2 \times 10^{-13} \text{ m}^2\text{s}^{-1}}{10^{-3} \text{ m/s}}$$

$$\delta_x = 4.4 \times 10^{-10} \text{ m}$$

Compare this with $3 \times 10^{-12} \text{ m}$ for a small molecule. The average step size increases with particle size, but the velocity decreases much more rapidly

For a three-dimensional random walk, the mean-square end-to-end distance is calculated as:

$$\langle r^2 \rangle = 6Dt$$

where r is the distance, in three-dimensions, from the start to end of a walk, and t is time.

Note that this expressions differs from the one for one-dimensional diffusion, with the factor of 6 replacing 2. The reason for this has to do with the definition of the diffusion coefficient in terms of the random-walk step size, as given above. The parameter δ_x is the average projection of the steps onto the x -axis (or any arbitrary axis, for that matter). If we only consider the net diffusion in one-dimension, the δ_x corresponds to the average step size in that direction. But, if we are considering the diffusion away from the starting point and are calculating the distance through three dimensions, then the projections along all three axes contribute to the distance:

$$\langle r^2 \rangle = \langle x^2 \rangle + \langle y^2 \rangle + \langle z^2 \rangle = 6Dt$$

How long does it take for an average walk to reach 1 mm?

$$\langle r^2 \rangle = (10^{-3} \text{ m})^2 = 6Dt$$

$$t = \frac{(10^{-3} \text{ m})^2}{6 \times 2.2 \times 10^{-13} \text{ m}^2\text{s}^{-1}}$$

$$t = 7.6 \times 10^5 \text{ s}$$

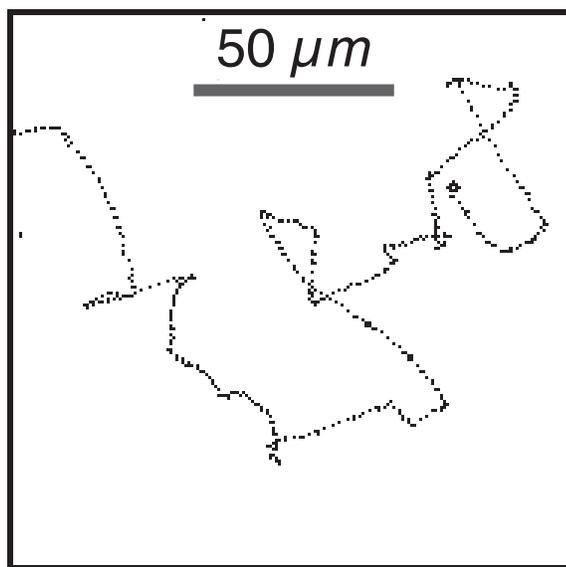
$$t \approx 9 \text{ days}$$

Since an *E. coli* bacterium can divide in as little as 20 min, this is obviously a long time for such an organism!

II. Bacteria under the microscope

When bacteria such as *E. Coli* are examined under a microscope it is often observed that they move *much* faster than the calculated rates of brownian motion. For many of these motile bacteria, the motion appears to be a random walk, but with much larger steps than expected for Brownian motion.

A pioneer in the biophysical study of bacterial swimming is Prof. Howard Berg. In 1972 he built a very fancy microscope, especially for the time, that could track the motion of individual bacteria in three dimensions⁶. The figure below shows an example of one of the paths, projected onto two dimensions, as traced by Berg and his colleagues:



This looks like a random walk with variable step length, and detailed analyses showed that the typical parameters for the random walks were:

- Velocity $\approx 2 \times 10^{-5} \text{ ms}^{-1}$
- Average time of forward motion $\approx 3 \text{ s}$
- Average step length $\approx 6 \times 10^{-5} \text{ m}$

Note that the velocity is much lower than the instantaneous velocity from thermal motion. But, the length of the steps is vastly longer, about 60 bacterial body lengths.

The number of steps is $n = t/(3 \text{ s/step})$

⁶Berg, H. C. & Brown, D. A. (1972). Chemotaxis in *Esherichia coli* analyzed by three-dimensional tracking. *Nature*, 239, 500–504. <http://dx.doi.org/10.1038/239500a0>

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What is the average time to move 1 mm? First calculate the number of steps:

$$\begin{aligned}n &= \frac{\langle r^2 \rangle}{\delta^2} \\ &= \frac{(10^{-3} \text{ m})^2}{(6 \times 10^{-5} \text{ m})^2} \\ &\approx 280 \text{ steps}\end{aligned}$$

The total time, then, is:

$$\begin{aligned}t &= 280 \text{ steps} \times 3 \text{ s/step} \\ &\approx 840 \text{ s} \approx 15 \text{ min}\end{aligned}$$

This is almost 1,000 times shorter than the time required for diffusion over the same distance.

This is an important feature of random walks: For a given period of time, the *average* distance from the starting point will be larger if the steps are longer, even if there are fewer of them.

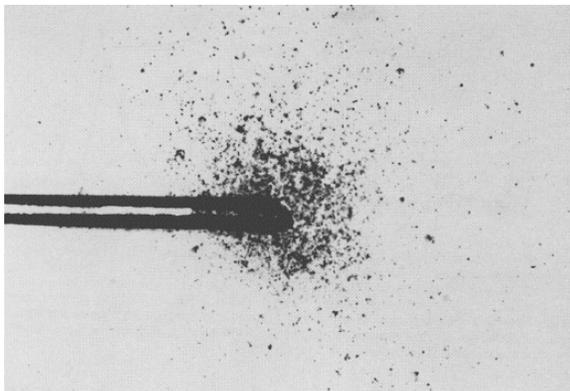
But, why doesn't the bacterium take longer steps? Because it wouldn't help! Notice the tracks in the microscope. They are curved, because Brownian motion is moving them off of a straight path. After a few seconds, the bacterium essentially forgets what direction it is going.

But, it is suddenly changing direction. Why do this if it is going to be randomly altering direction anyway?

Because, it is doing something much smarter!

III. Chemotaxis: Movement to or from specific chemicals

The ability of bacteria to systematically move towards or away from certain compounds was first demonstrated by Wilhelm Pfeffer in 1884 in a very simple experiment. Pfeffer placed a solution of sugar in a small capillary tube and then placed the end of this tube in a liquid culture of bacteria, as illustrated in the figure below⁷:



⁷Figure from: Adler, J. (1969). Chemoreceptors in bacteria. *Science*, 166, 1588–1597. <http://dx.doi.org/10.1126/science.166.3913.1588>

As shown in the microphotograph, the bacteria quickly cluster around the open end of the capillary tube. This simple experiment demonstrated that bacteria have the capability to detect specific compounds and move in a directed fashion.

The first question these observation raise is, how do they know which way to go? Somehow, they need to detect a concentration gradient and then move in the direction of increased concentration, if they want to use the compound as a nutrient, or decreased concentration, if the compound is toxic. One might imagine that the bacteria could somehow sense concentrations at the two ends of the cell and compare these to determine the concentration gradient. But, our earlier calculation show that the time required for a small molecule to diffuse over the the length of a typical bacterial cell, about 1-2 μm , is less than a second, so that concentration gradients are insignificant over these distance.

Instead, bacteria use a modified random walk strategy that involves the following steps:

1. Choose a random direction.
2. Swim for a while.
3. Is life getting better? (more food, less poison)
 - Yes: keep going.
 - No: Stop and choose a new *random* direction.

Steps in the good direction are still limited to a few seconds, but steps in the wrong direction can be much shorter.

This requires that the bacteria have a concentration sensor and a “memory”, so that they can compare concentrations as they move in a particular direction. How do the bacteria actually do all of this?

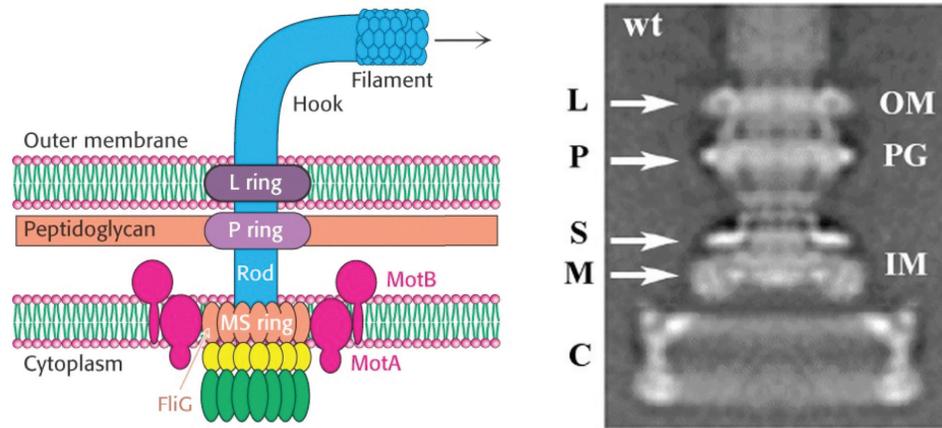
- The bacteria swim using a propeller and a rotary motor.
- Bacteria change direction by stopping the flagella and tumbling for an instant.
- Sensors on cell surface detect concentration changes and transmit that information to the rotary motor.

IV. The rotary motor

E. coli and many other bacterial species are propelled through liquid media by long helical flagella. Each cell contains multiple flagella, each with a rotary motor embedded in the cell membranes and cell wall, as shown in the diagram in the left panel below⁸. The right panel shows an image of the motor reconstructed from electron micrographs.

⁸The diagram of the bacterial motor is from Berg, J. M., Tymoczko, J. L. & Stryer, L. (2002). *Biochemistry*. W. H. Freeman, 5th edition. <https://www.ncbi.nlm.nih.gov/books/NBK22489/>
 The electron microscopy reconstruction is from Thomas, D., Morgan, D. G. & DeRosier, D. J. (2001). Structures of bacterial flagellar motors from two FliF-FliG gene fusion mutants. *J. Bacteriol.*, 183, 6404–6412. <http://dx.doi.org/10.1128/JB.183.21.6404-6412.2001>

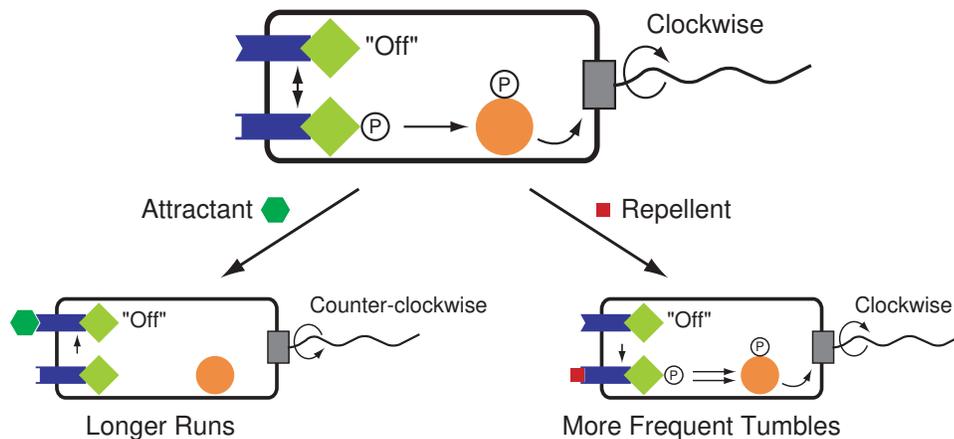
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The motor, which we will discuss in more detail later in the course, is driven by the flow of H^+ ions from outside of the cell inward and rotates the flagella at up to 10000 RPM. When the motor rotates in the counter-clockwise direction, the individual flagella of a cell bundle together and act as a propeller. The bacterium then swims in a (relatively) straight direction. When the motors reverse direction, the flagella unbundle, and the bacterium tumbles randomly. After a few seconds, the motor reverses again, and the bacterium swims in a new direction. By this process, the bacteria carry out their random walk. The reversals of the motor are controlled by a signalling system that detects changes in the concentrations of specific compounds in the surrounding liquid.

V. The sensory and signalling system

The system for sensing specific molecules and signalling the rotary motor is quite complex, but a simplified diagram is shown below:



Molecules in the extracellular environment are detected when they bind to receptors that cross the cellular membrane. These receptors are bound to an enzyme and can exist in two conformations. In one conformation (“on”), the enzyme, a kinase, is activated and phosphorylates another protein, which, in turns binds to the rotary motor. When this phosphorylated protein is bound to the motor, the clockwise rotation is favored, leading to tumbling. When the receptors are in the other conformation (“off”),

the kinase is inactivated, the signalling molecule is less likely to be phosphorylated, and counter-clockwise rotation is favored.

The equilibrium between the two conformations of the receptors is controlled by multiple factors, including the presence of attractant and repellant molecules. When attractants are bound to the receptors, the off conformation is favored, promoting counter-clockwise rotation and forward swimming. In the absence of attractants or the presence of repellants, the on conformation is favored, leading to tumbling and shorter steps in the random walk.

In addition to sensing the concentrations of attractants and repellants, the bacterium has to do one other very important thing: It has to remember what the concentrations were a short time ago! In order to bias the random walk in the direction of a concentration gradient, the bacterium has to compare the concentrations at different times as it swims. This memory is established by another set of enzymes that covalently modify the receptors by methylating specific glutamate residues. These modifications are reversible and adjust the sensitivity of the receptors to attractants or repellents. As the concentration of an attractant increases, the receptors are modified so that higher concentrations of attractant are required to keep the receptor in the off conformation. In a real sense, the cell becomes addicted to the attractant and require more of it to keep swimming in the same direction. In this way, the random walk is biased in a way that leads it up the concentration gradient. The system adjusts to repellent concentrations in the opposite way, to favor moving down the concentration gradient.