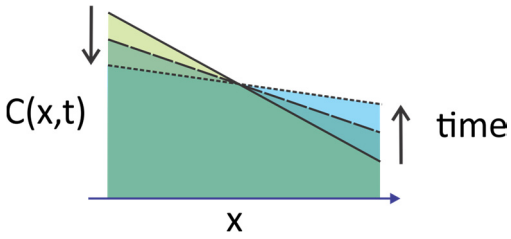
10. Diffusion

Continuum Diffusion

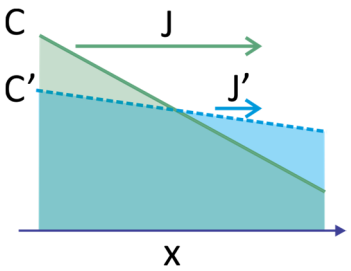
We are now going to start a new set of topics that involve the dynamics of molecular transport. A significant fraction of how molecules move spatially in biophysics is described macroscopically by "diffusion" and microscopically through its counterpart "Brownian motion". Diffusion refers to the phenomenon by which concentration and temperature gradients spontaneously disappear with time, and the properties of the system become spatially uniform. As such, diffusion refers to the transport of mass and energy in a nonequilibrium system that leads toward equilibrium. Brownian motion is also a spontaneous process observed in equilibrium and non-equilibrium systems. It refers to the random motion of molecules in fluids that arises from thermal fluctuations of the environment that rapidly randomize the velocity of particles. Much of the molecular transport in biophysics over nanometer distances arises from diffusion. This can be contrasted with directed motion, which requires the input of energy and is crucial for transporting cargo to targets over micron-scale distances. Here we will start by describing diffusion in continuum systems, and in the next section show how this is related to the Brownian motion of discrete particles.



Fick's First Law

We will describe the time evolution of spatially varying concentration distributions C(x,t) as they evolve toward equilibrium. These are formalized in two laws that were described by Adolf Fick (1855).[1] Fick's first law is the "common sense law" that is in line with everyone's physical intuition. Molecules on average will tend to diffuse from regions of higher concentration to regions of lower concentration. Therefore we say that the flux of molecules through a surface, J, is proportional to the concentration gradient across that surface.

$$J = -D\frac{\partial C}{\partial x} \tag{1}$$



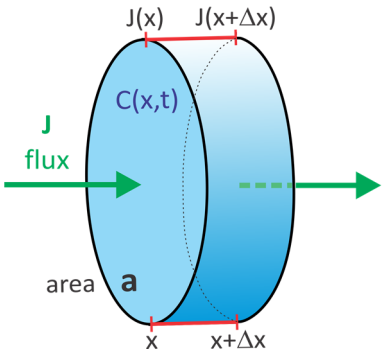
J is more accurately called a flux density, since it has units of concentration or number density per unit area and time. The proportionality constant between flux density J (mol m^{-2} s^{-1}) and concentration gradient (mol m^{-4}) which sets the timescale for the process is the diffusion constant D (m^2 s^{-1}). The negative sign

1. A. Fick, Ueber diffusion, Ann. Phys. **170**, 59–86 (1855).

assures that the flux points in the direction of decreasing concentration. This relationship follows naturally, when we look at the two concentration gradients in the figure. Both C and C' have a negative gradient that will lead to a flux in the positive direction. C will give a bigger flux than C' because there is more probability for flow to right. The gradient disappears and the concentration distribution becomes constant and time invariant at equilibrium. Note, in a general sense, $\partial C / \partial x$ can be considered the leading term in an expansion of C in x.

Fick's Second Law

Fick's second law extends the first law by adding an additional constraint based on the conservation of mass. Consider diffusive transport along x in a pipe with cross-sectional area a, and the change in the total number of particles within a disk of thickness Δx over a time period Δt. If we take this disk to be thin enough that the concentration is a constant at any moment in time, then the total number of particles in the slab at that time is obtained from the concentration times the volume:



$$N = aC(t)\Delta x$$

Within the time interval Δt the concentration can change and therefore the total number of particles within the disk changes by an amount

$$\Delta N = a\{C(t+\Delta t) - C(t)\}\Delta x$$

Now, the change in the number of particles is also dependent on the fluxes of molecules at the two surfaces of the disk. The number of molecules passing into one surface of the disk is $-aJ\Delta t$, and therefore the net change in the number of molecules during Δt is obtained from the difference of fluxes between the left and right surfaces of the disk:

$$\Delta N = -a\ \{J(x+\Delta x) - J(x)\}\Delta t$$

Setting these two calculations of ΔN equal to each other, we see that the flux and concentration gradients for the disk are related as

$$\{C(t+\Delta t) - C(t)\}\Delta x = -\{J(x+\Delta x) - J(x)\}\Delta t$$

or rewriting this in differential form

$$\frac{\partial C}{\partial t} = -\frac{\partial J}{\partial x} \qquad (2)$$

This important relationship is known as a continuity expression. Substituting eq. (1) into this expression leads to Fick's Second Law

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} \tag{3}$$

This is the diffusion equation in one dimension, and in three dimensions:[2]

$$\frac{\partial C}{\partial t} = D\nabla^2 C \tag{4}$$

Equation (4) can be used to solve diffusive transport problems in a variety of problems, choosing the appropriate coordinate system and applying the specific boundary conditions for the problem of interest.

Diffusion from a Point Source

As our first example of how concentration distributions evolve diffusively, we consider the time-dependent concentration profile when the concentration is initially all localized to one point in space, $x = 0$. The initial condition is

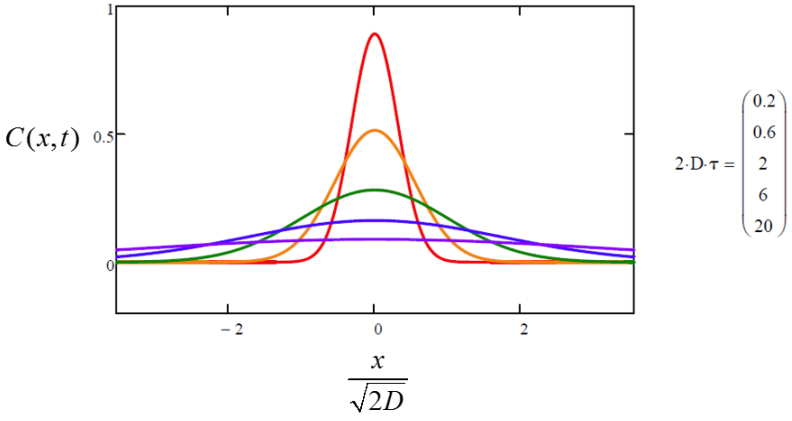
$$C(x, t = 0) = C_0\,\delta(x)$$

and the solution to eq. (3) is

$$C(x,t) = \frac{C_0}{\sqrt{4\pi Dt}} e^{-x^2/4Dt} \tag{5}$$

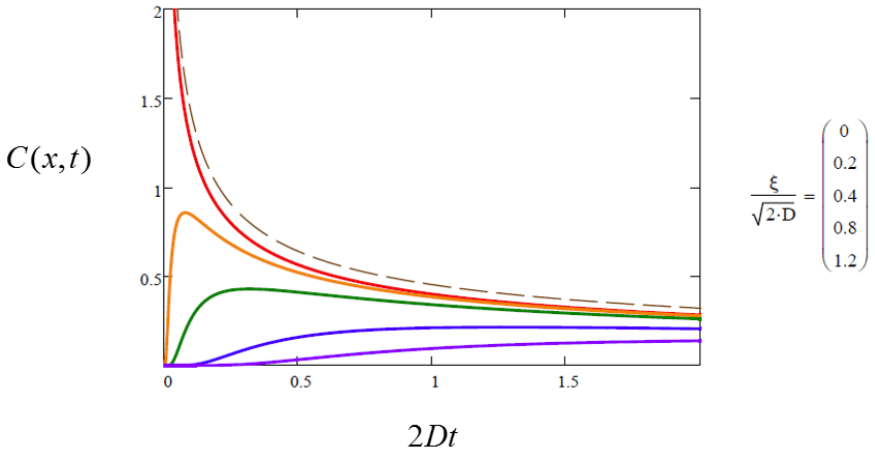
The concentration profile has a Gaussian form which is centered on the origin, $\langle x \rangle = 0$, with the mean square displacement broadening with time as:

$$\langle x^2 \rangle = 2Dt$$



2. This equation assumes that D is a constant, but if it is a function of space: $\dot{C} = \nabla\left(D\nabla C\right)$. In three dimensions, Fick's First Law and the continuity expression are: $\mathbf{J}(\mathbf{r},t) = \mathbf{v}C(\mathbf{r},t) - \mathrm{D}\nabla C(\mathbf{r},t)$ and $dC(\mathbf{r},t)/dt = -\nabla\cdot\mathbf{J}(\mathbf{r},t)$ where $\mathbf{v}$ is the velocity of the fluid. These expressions emphasize that flux density and velocity are vectors, whereas concentration field is a scalar.

Diffusive transport has no preferred direction. Concentration profiles spread evenly in the positive and negative direction, and the highest concentration observed will always be at the origin and have a value $C_{max} = C_0 / \sqrt{4\pi Dt}$. Viewing time-dependent concentrations in space reveal that they reach a peak at $t_{max} = x^2/2D$, before decaying at $t^{-1/2}$ (dashed line below).



When we solve for 3D diffusion from a point source:

$$C(x,y,z,t=0) = C_0\,\delta(x)\delta(y)\delta(z)$$

If we have an isotropic medium in which D is identical for diffusion in the x, y, and z dimensions,

$$C(x,y,z,t) = \frac{C_0}{(4\pi Dt)^{3/2}} e^{-r^2/4Dt} \tag{6}$$

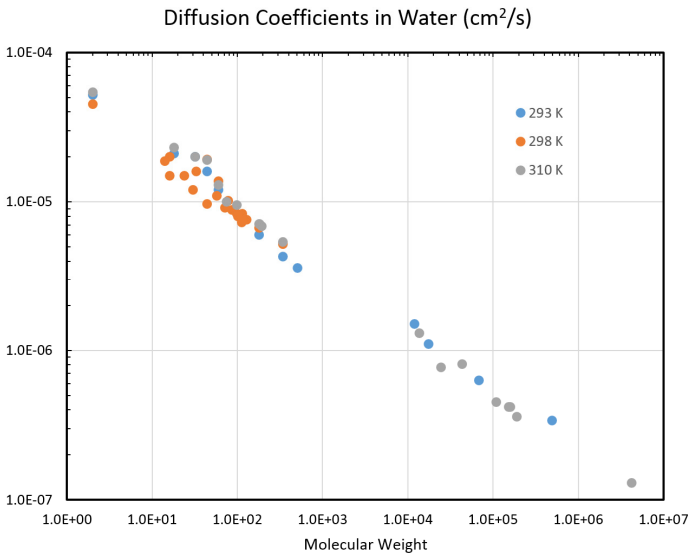
where $r^2 = x^2 + y^2 + z^2$. Calculating the mean square displacement from

$$\langle r^2 \rangle = \frac{\int_0^\infty dr\, r^2 C(r,t)}{\int_0^\infty dr\, C(r,t)}$$
$$= 6Dt$$

or in d dimensions, $\langle r^2 \rangle = d(2Dt)$.

Diffusion Constants

Typical diffusion constants for biologically relevant molecules in water are shown in the graph below, varying from small molecules such as O_2 and glucose in the upper left to proteins and viruses in the lower right.



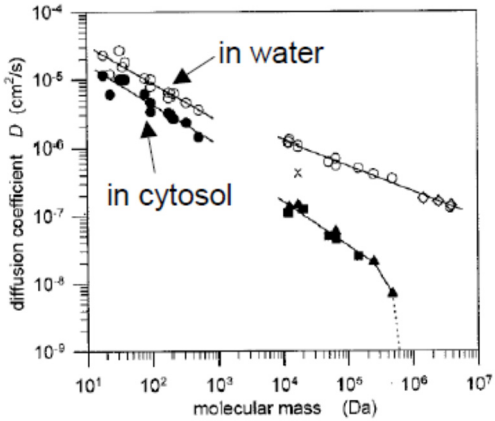
- For a typical globular protein, typically diffusion coefficients are:

$$\begin{aligned} &\text{in water} && D \sim 10^{-10}\ \text{m}^2/\text{s} \\ &\text{in cells} && D \sim 10^{-12}\ \text{m}^2/\text{s} \\ &\text{in lipids} && D \sim 10^{-14}\ \text{m}^2/\text{s} \end{aligned}$$

$$\langle r^2 \rangle^{1/2} = 1\,\mu m, \quad t \sim 0.4 \text{ sec in cells}$$

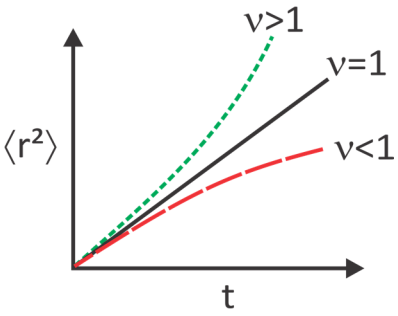
$$= 10\,\mu m, t \sim 40 \text{ sec in cells}$$

- Ions in water at room temperature usually have a diffusion coefficient of 0.6×10^{-5} to 2×10^{-5} cm^2/s.
- Lipids:
  - Self-diffusion 10^{-12} m^2/s
  - Tracer molecules in lipid bilayers $1–10\times10^{-12}$ m^2/s



Anomalous Diffusion

The characteristic of simple diffusive behavior is the linear relationship between the mean square displacement and time. Deviation from this behavior is known as anomalous diffusion, and is characterized by a scaling relationship $\langle r^2 \rangle \sim t^{\nu}$. We refer to $\nu<1$ as sub-diffusive behavior and $\nu>1$ as super-diffusive. Diffusion in crowded environments can result in sub-diffusion.[3]



Thermodynamic Perspective on Diffusion

Thermodynamically, we can consider the driving force for diffusion as a gradient in the free energy or chemical potential of the system. From this perspective, in the absence of any other interactions, the driving force for reaching uniform spatial concentration is the entropy of mixing. For a mixture with mole fraction x_A, we showed

$$\begin{aligned} \Delta S_{\text{mix}} &= -Nk_B\left(x_A \ln x_A + x_B \ln x_B\right) \qquad x_B = 1 - x_A \\ &\approx -N_A k_B \ln x_A \qquad\qquad\qquad for\, x_A << 1 \end{aligned}$$

We then use $\Delta F = -T\Delta S$ to calculate the chemical potential:

$$\mu_A = \left(\frac{\partial F}{\partial N_A}\right)_{V,T}$$

$$\mu_A \approx k_B T \ln x_A$$

We see that a concentration gradient, means that the mole fraction and therefore chemical potential is different for two positions in the system. At equilibrium $\mu_A(r_1) = \mu_A(r_2)$, which occurs when $x_A(r_1) = x_A(r_2)$.

Thermodynamics does not tell you about rate, only the direction of spontaneous change (although occasionally diffusion is discussed in terms of a time-dependent "entropy production"). The diffusion constant is the proportionality constant between gradients in concentration or chemical potential and the time-dependent flux of particles. The flux density described in Fick's first law can be related to μ_i, the chemical potential for species i:

$$J_i = \frac{-D_i C_i}{k_B T}\frac{\partial \mu_i}{\partial r_i}$$

3. J. A. Dix and A. S. Verkman, Crowding effects on diffusion in solutions and cells, Annu. Rev. Biophys. **37**, 247–263 (2008).

Solving the Diffusion Equation

Solutions to the diffusion equation, such as eq. (5) and (6), are commonly solved with the use of Fourier transforms. If we define the transformation from real space to reciprocal space as

$$\tilde{C}(k,t) = \int_{-\infty}^{\infty} C(x)\, e^{ikx}\, dx$$

one can express the diffusion equation in 1D as

$$\frac{d\tilde{C}(k,t)}{dt} = -Dk^2\tilde{C}(k,t) \tag{7}$$

[More generally one finds that the Fourier transform of a linear differential equation in x can be expressed in polynomial form: $\mathcal{F}(\partial^n f / \partial x^n) = (ik)^n \tilde{f}(k)$]. This manipulation converts a partial differential equation into an ordinary one, which has the straightforward solution $\tilde{C}(k,t) = \tilde{C}(k,0)\exp\left(-Dk^2 t\right)$. We do need to express the boundary conditions in reciprocal space, but then, this solution can be transformed back to obtain the real space solution using $C(x,t) = (2\pi)^{-1}\int_{-\infty}^{\infty} \tilde{C}(k,t)\, e^{-ikx}\, dk$.

Since eq. (7) is a linear differential equation, sums of solutions to the diffusion equation are also solutions. We can use this superposition principle to solve problems for complex initial conditions. Similarly, when the diffusion constant is independent of x and t, the general solution to the diffusion equation can also be expressed as a Fourier series. If we separate the time and space variables, so that the form of the solution is $C(x,t) = X(x)T(t)$ we find that we can write

$$\frac{1}{DT}\frac{\partial T}{\partial t} = \frac{1}{x}\frac{\partial^2 x}{\partial x^2} = -\alpha^2$$

Where α is a constant. Then $T = e^{-\alpha^2 Dt}$ and $x = A\cos\alpha x + B\sin\alpha x$. This leads to the general form:

$$C(x,t) = \sum_{n=0}^{\infty}\left(A_n \cos\alpha_n x + B_n \sin\alpha_n x\right)e^{-\alpha_n^2 Dt} \tag{8}$$

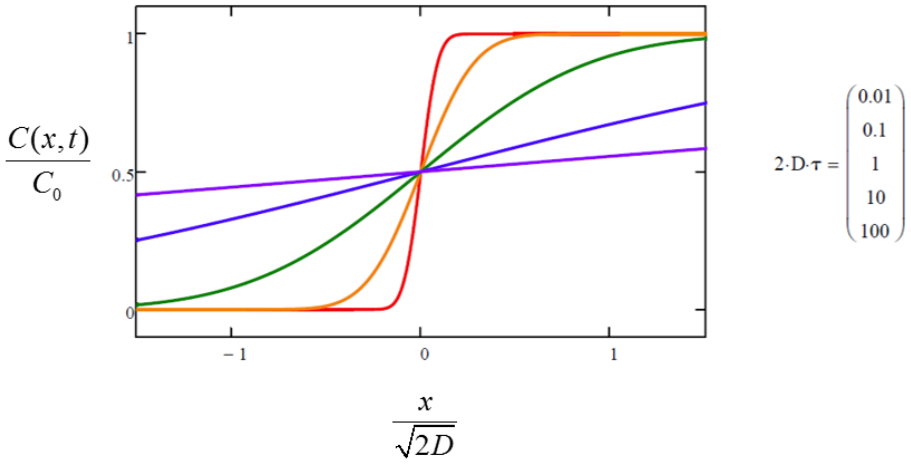
Here A_n and B_n are constants determined by the boundary conditions.

Examples

Diffusion across boundary

At time $t = 0$, the concentration is uniform at a value C_0 for $x \geq 0$, and zero for $x < 0$, similar to removing a barrier between two homogeneous media. Using the superposition principle, the solution is obtained by integrating the point source solution, eq. (5), over all initial point sources $\delta(x - x_0)$ such that $x_0 = 0 \to \infty$. Defining $y^2 = (x - x_0)^2 / 4Dt$,

$$C(x,t) = \frac{C_0}{\sqrt{\pi}} \int_{\frac{(x-x_0)}{\sqrt{4Dt}}}^{\infty} dy\, e^{-y^2} = \frac{C_0}{2} erfc\left(\frac{-(x-x_0)}{\sqrt{4Dt}}\right)$$

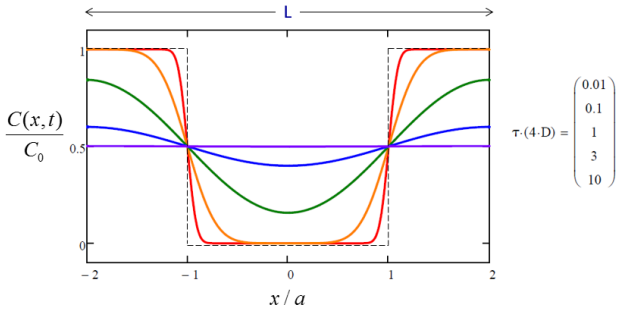


Diffusion into "hole"

A concentration "hole" of width *2a* is inserted into a box of length *2L* with an initial concentration of C_0. Let's take $L = 2a$. Concentration profile solution:

$$C(x,t) = C_0\left[\left(\frac{L-a}{L}\right) - \sum_{n=1}^{\infty} A_n \cos(\alpha_n x) e^{-\alpha_n^2 Dt}\right]$$

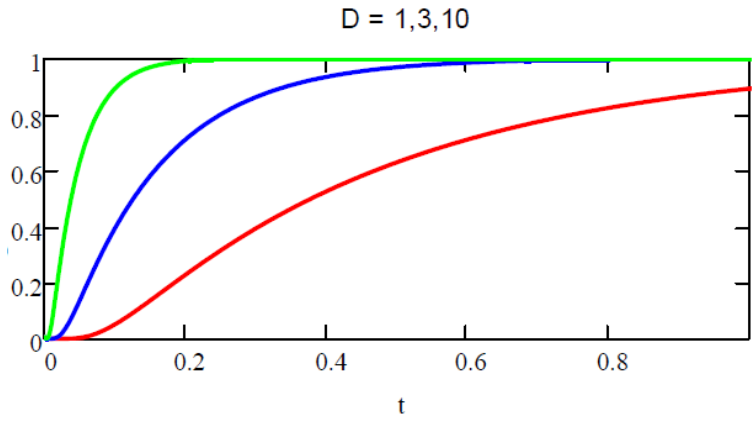
$$A_n = \frac{2\sin(\alpha_n a)}{n\pi} \qquad \alpha_n = \frac{n\pi}{L}$$



- **Fluorescence Recovery after Photobleaching (FRAP)**: We can use this solution to describe the diffusion of fluorescently labeled molecules into a photobleached spot. Usually observe the increase of fluorescence with time from this spot. We integrate concentration over initial hole:

$$N_{FRAP}(t) = \int_{-a}^{+a} C(x,t)dx$$

$$= C_0\left[\frac{2a}{L}(L-1) - L\sum_{n=1}^{\infty} A_n^2 e^{-\alpha_n Dt}\right]$$



Reflecting and Absorbing Boundary Conditions

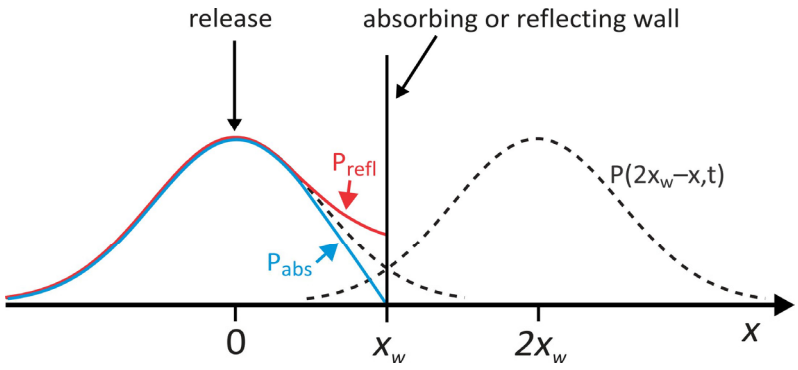
We will be interested in describing the time-dependent probability distribution for the case in which particles are releases at $x = 0$, subject to encountering an impenetrable wall at $x = x_w$, which can either absorb or reflect particles.

Consider the case of a reflecting wall, where the boundary condition requires that the flux at x_w is zero. This boundary condition and the resulting pile-up near the wall can be described by making use of the fact that any $P(x > x_w, t)$ can be reflected about x_w, which is equivalent to removing the boundary and adding a second source term to $P(x,t)$ for particles released at $x = 2x_w$

$$P_{\text{refl}}(x,t) = P(x,t) + P(2x_w - x, t) \qquad (x < x_w)$$

This is also known as a wrap-around solution, since any component with any population from $P(x,t)$ that passes the position of the wall is reflected about x_w. Similarly, an absorbing wall, $P(x = x_w, t) = 0$, means that we remove any population that reached x_w, which is obtained from the difference of the two mirrored probability distributions:

$$P_{\text{abs}}(x,t) = P(x,t) - P(2x_w - x, t) \qquad (x < x_w)$$



Steady-State Solutions

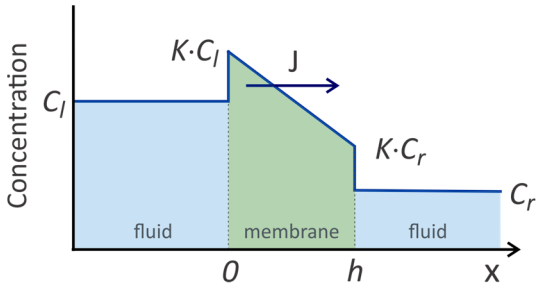
Steady state solutions can be applied when the concentration gradient may vary in space but does not change with time, $\partial C / \partial t = 0$. Under those conditions, the diffusion eq. (4) simplifies to Laplace's equation

$$\nabla^2 C = 0 \tag{9}$$

For certain conditions this can be integrated directly by applying the proper boundary conditions, and then the steady state flux at a target position is obtained from Fick's first law, eq. (1).

Diffusion through a Membrane[4]

The steady-state solution to the diffusion equation in one dimension can be used to describe the diffusion of a small molecule through a cell plasma membrane that resists the diffusion of the molecule. In this model, the membrane thickness is h, and the concentrations of the diffusing small molecule in the fluid on left and right side of membrane are C_l and C_r. Within the membrane resists diffusion of the small molecule, which is reflected in the small molecule's partition coefficient between membrane and fluid:



$$K_p = \frac{C_{\text{membrane}}}{C_{\text{fluid}}}$$

K_p can vary between 10^3 and 10^{-7} depending on the nature of the small molecules and membrane composition.

For the steady-state diffusion equation $\partial^2 C / \partial x^2 = 0$, solutions take the form $C(x) = A_1 x + A_2$. Applying boundary conditions for the concentration of small molecule in the membrane at the two boundaries, we find

$$A_1 = \frac{K_p (C_r - C_l)}{h} \qquad A_2 = K_p C_l$$

Then we can write the transmembrane flux density of the small molecule across the membrane as

$$J = -D_{mol} \frac{\partial C}{\partial x} = \frac{K_p D_{mol}}{h} (C_\ell - C_r) = \frac{K_p D_{mol} \Delta C}{h}$$

The membrane permeability is equivalent to the volume of small molecule solution that diffuses across a given area of the membrane per unit time, and is defined as

4. A. Walter and J. Gutknecht, Permeability of small nonelectrolytes through lipid bilayer membranes, J. Membr. Biol. **90**, 207–217 (1986).

$$P_m \equiv \frac{J}{\Delta C} = \frac{K_p D_{mol}}{h} (\text{m s}^{-1}) \tag{10}$$

The membrane resistance to flow is $R = 1/P_m$, and the rate of transport across the membrane is $dn/dt = J A$, where A is area.

This linear relationship in eq. (10) between P_m and K_p, also known as the Overton relation, has been verified for thousands of molecules. For small molecules with molecular weight <50, P_m can vary from 10^1 to 10^{-6} cm s^{-1}. It varies considerably even for water across different membrane systems, but its typical value for a phospholipid vesicle is 10^{-3} cm s^{-1}. Some of the highest values (>50 cm s^{-1}) are observed for O_2. Cations such as Na^+ and K^+ have permeabilities of ~5×10^{-14} cm s^{-1}, and small peptides have values of 10^{-9}–10^{-6} cm s^{-1}.

Diffusion to Capture

What is the flux of a diffusing species onto a spherical surface from a solution with a bulk concentration C_0? This problem appears often for diffusion limited reaction rates. To find this, we calculate the steady-state radial concentration profile $C(r)$ around a perfectly absorbing sphere with radius a, i.e. $C(a) = 0$. At steady state, we solve eq. (9) by taking the diffusion to depend only on the radial coordinate r and not the angular ones.

$$\frac{1}{r^2}\frac{\partial}{\partial r}\left(r^2 \frac{\partial C}{\partial r}\right) = 0$$

Let's look for the simplest solution. We begin by assuming that the quantity in parenthesis is a constant and integrate twice to give

$$C(r) = -\frac{A_1}{r} + A_2 \tag{11}$$

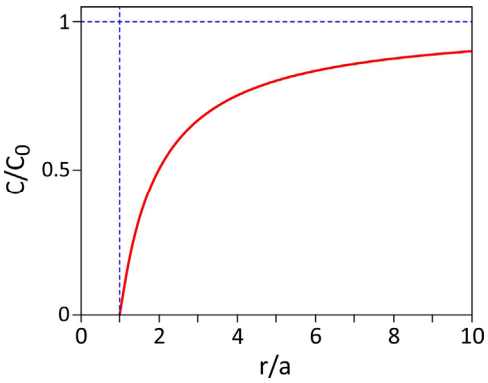
Where A_1 and A_2 are constants of integration. Now, using the boundary conditions $C(a) = 0$ and $C(\infty) = C_0$ we find:

$$C(r) = C_0\left(1 - \frac{a}{r}\right)$$

Next, we use this expression to calculate the flux of molecules incident on the surface of the sphere ($r = a$).

$$J(a) = -D\left.\frac{\partial C}{\partial r}\right|_{r=a} = -\frac{DC_0}{a} \tag{12}$$

Here J is the flux density in units of (molecules area^{-1} sec^{-1}) or [(mol/L) area^{-1} sec^{-1}]. The sign of the flux density is negative reflecting that it is a vector quantity directed toward $r = 0$. We then calculate the rate of collisions of molecules with the sphere (the flux, j) by multiplying the magnitude of J by the surface area of the sphere ($A = 4\pi a^2$):



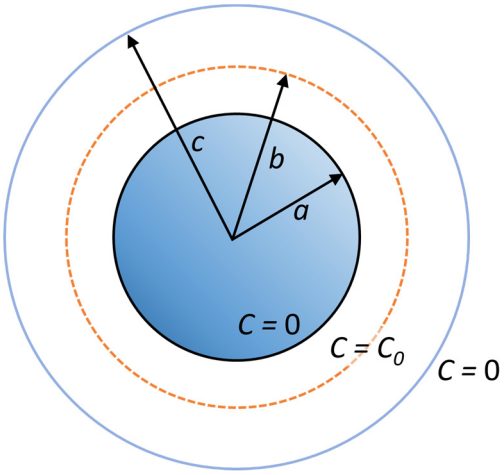
$$j = JA = 4\pi D a C_0$$

This shows that the rate constant, which expresses the proportionality between rate of collisions and concentration is $k = 4\pi Da$.

Probability of capture

In an extension of this problem useful to ligand binding simulations, we can ask what the probability is that a molecule released near an absorbing sphere will reach the sphere rather than diffuse away?

Suppose a particle is released near a spherical absorber of radius a at a point $r = b$. What is the probability that the particle will be absorbed at $r = a$ rather than wandering off beyond an outer perimeter at $r = c$?



To solve this problem we solve for the steady-state flux at the surfaces a and c subject to the boundary conditions $C(a) = 0$, $C(b) = C_0$, and $C(c) = 0$. That is, the inner and outer surfaces are perfectly absorbing, but the concentration has a maximum value C(b) = C_0 at $r = b$.

We separate the problem into two zones, a-to-b and b-to-c, and apply the general solution eq. (11) to these zones with the appropriate boundary conditions to yield:

$$C(r) = \frac{C_0}{(1 - a/b)}\left(1 - \frac{a}{r}\right) \qquad a \le r \le b$$

$$C(r) = \frac{C_0}{(c/b - 1)}\left(1 - \frac{a}{r}\right) \qquad b \le r \le c$$

Then the radial flux density is:

$$J_r(r) = -\frac{DC_0}{(1-a/b)}\frac{a}{r^2} \qquad a \le r \le b$$

$$J_r(r) = \frac{DC_0}{(c/b-1)}\frac{c}{r^2} \qquad b \le r \le c$$

Calculating the areas of the two absorbing surfaces and multiplying the flux densities by the areas gives the flux. The flux from the spherical shell source to the inner absorber is

$$j_{\text{in}} = 4\pi DC_0 \frac{a}{(1-a/b)}$$

and the flux from the spherical shell source to the outer absorber is

$$j_{\text{out}} = 4\pi DC_0 \frac{c}{(c/b-1)}$$

We obtain the probability that a particle released at $r = b$ and absorbed at $r = a$ from the ratio

$$P_{capture} = \frac{j_{\text{in}}}{j_{\text{in}} + j_{\text{out}}} = \frac{a(c-b)}{b(c-a)}$$

In the limit $c \to \infty$, this probability is just *a/b*. This is the probability of capture for the sphere of radius *a* immersed in an infinite medium. Note that this probability decreases only inversely with the radial distance b^{-1}, rather than the surface area of the sphere.

Readings

1. H. C. Berg, *Random Walks in Biology*. (Princeton University Press, Princeton, N.J., 1993).
2. K. Dill and S. Bromberg, *Molecular Driving Forces: Statistical Thermodynamics in Biology, Chemistry, Physics, and Nanoscience*. (Taylor & Francis Group, New York, 2010).