11. Brownian Motion

Brownian motion refers to the random motions of small particles under thermal excitation in solution first described by Robert Brown (1827), who with his microscope observed the random, jittery spatial motion of pollen grains in water. This phenomenon is intrinsically linked with diffusion. Diffusion is the macroscopic realization of the Brownian motion of molecules within concentration gradients. The theoretical basis for this relationship was described by Einstein in 1905, and Jean Perrin provided the detailed experiments that confirmed his predictions.

Since the motion of any one particle is unique, the Brownian motion must be described statistically. We observe that the mean-squared displacement of a particle averaged over many measurements grows linearly with time, just as with diffusion.

\[ \langle r^2 \rangle \propto t \]

The proportionality factor between mean-squared displacement and time is the diffusion constant in Fick’s Second Law. As for diffusion, the proportionality factor depends on dimensionality. In 1D, if \( \langle x^2(t) \rangle / t = 2D \) then in 3D \( \langle r^2(t) \rangle / t = 6D \), where \( D \) is the diffusion constant.

Brownian motion is a property of molecules at thermal equilibrium. It applies to a larger particle (i.e., a protein) experiencing an imbalance of many microscopic forces exerted by many much small molecules of the surroundings (i.e., water). The thermal agitation originates by partitioning the kinetic energy of the system on average as \( k_B T / 2 \) per degree of freedom. Free diffusion implies motion which is only limited by kinetic energy.

Brownian motion applies to a specific range of forces and masses where thermal energy \( (k_BT(300\, \text{K}) = 4.1\, \text{pN nm}) \) can have a significant influence on a particle. Let’s look at the average translational kinetic energy:

\[
\langle \frac{mv^2}{2} \rangle = \frac{1}{2} k_B T
\]

For a ~10 kDa protein with mass \( \sim 10^{-23} \, \text{kg} \), the root mean squared velocity due to thermal energy is \( v_{\text{rms}} = \langle v^2 \rangle^{1/2} = 20 \, \text{m/s} \). For water at 300 K, \( D \sim 10^{-5} \, \text{cm}^2/\text{s} \). The same protein has a net displacement in one second of \( x_{\text{rms}} = \langle x^2 \rangle^{1/2} = \sqrt{2D}\tau \approx 50 \, \mu\text{m} \). The large difference in these values indicates the large number of randomizing collisions that this particle experiences during one second of evolution: \( (v_{\text{rms}} \cdot 1\, \text{sec})/x_{\text{rms}} \approx 4 \times 10^5 \). For the protein, the velocities and displacements are a dominant force on the molecular scale. In comparison, a 1 kg mass with \( k_BT \) of energy will have \( v_{\text{rms}} \sim 10^{-11} \, \text{m/s} \), and an equally insignificant displacement!

**Ergodic Hypothesis**

A system is known as ergodic when time average and ensemble averages for a time-dependent variable are equal.

\[
\text{Ensemble average: } \langle x \rangle = \frac{1}{N} \sum_i x_i = \int_0^\infty P(x) dx
\]

\[
\text{Time-average: } \overline{x(t)} = \lim_{T \to \infty} \frac{1}{T} \int_0^T x(t) dt
\]

In practice, the time average can be calculated using a single particle trajectory by averaging over the displacement observed for all time intervals within the trajectory such that \( t=(t_{\text{final}}-t_{\text{initial}}) \).

In the case of Brownian motion and diffusion:

\[
\langle |\mathbf{r}(t)-\mathbf{r}_0|^2 \rangle = |\mathbf{r}(t)-\mathbf{r}_0|^2.
\]
Random Walk and Diffusion

We want to describe the correspondence between a microscopic picture for the random walk of particles and macroscopic diffusion of particle concentration gradients. We will describe the statistics for the location of a random walker in one dimension \( x \), which is allowed to step a distance \( \Delta x \) to the right (+) or left (−) during each time interval \( \Delta t \). At each time point a step must be taken left or right, and steps to left and right are equally probable.

Let’s begin by describing where the system is at after taking \( n \) steps qualitatively. We can relate the position of the system to where it was before taking a step by writing:

\[
x(n) = x(n-1) \pm \Delta x
\]

This expression can be averaged over many steps:

\[
\langle x(n) \rangle = \langle x(n-1) \pm \Delta x \rangle
\]
\[
= \langle x(n-1) \rangle = \langle x(n-2) \rangle = \cdots = \langle x(0) \rangle
\]

Since there is equal probability of moving left or right with each step, the \( \pm \Delta x \) term averages to zero, and \( \langle x \rangle \) does not change with time. The most probable position for any time will always be the starting point.

Now consider the variance in the displacement:

\[
\langle x^2(n) \rangle = \langle x^2(n-1) \pm 2 \Delta x \times x(n-1) + (\Delta x)^2 \rangle
\]
\[
= \langle x^2(n-1) \rangle + (\Delta x)^2
\]

In the first line, the middle term averages to zero, and the variance gains a factor of \( (\Delta x)^2 \). Repeating this process for each successive step back shows that the mean square displacement grows linearly in the number of steps.

\[
\langle x^2(0) \rangle = 0
\]
\[
\langle x^2(1) \rangle = (\Delta x)^2
\]
\[
\langle x^2(2) \rangle = 2(\Delta x)^2
\]
\[
\vdots
\]
\[
\langle x^2(n) \rangle = n(\Delta x)^2
\]  \hspace{1cm} (1)

Qualitatively, these arguments indicate that the statistics of a random walker should have the same mean and variance as the concentration distribution for diffusion of particles from an initial position.
Random Walk Step Distribution Function

Now let’s look at this a little more carefully and describe the probability distribution for the position of particles after \( n \) steps, which we equate with the number of possible random walk trajectories that can lead to a particular displacement. What is the probability of starting at \( x_0 = 0 \) and reaching point \( x \) after \( n \) jumps separated by the time interval \( \Delta t \)?

![Diagram](image)

Similar to our discussion of the random walk polymer, we can express the displacement of a random jumper to the total number of jumps in the positive direction \( n_+ \) and in the negative direction \( n_- \). If we make \( n \) total jumps, then

\[
 n = n_+ + n_- \quad \rightarrow \quad t = n \Delta t
\]

The total number of steps \( n \) is also our proxy for the length of time for a given trajectory, \( t \). The distance between the initial and final position is related to the difference in \( + \) and \( - \) steps:

\[
 m = n_+ - n_- \quad \rightarrow \quad x = m \Delta x
\]

Here \( m \) is our proxy for the total displacement \( x \). Note from these definitions we can express \( n_+ \) and \( n_- \) as

\[
 n_\pm = \frac{n \pm m}{2}
\]

The number of different ways of making \( n \) jumps with the constraint of \( n_+ \) positive and \( n_- \) negative jumps is

\[
 \Omega = \frac{n!}{n_+!n_-!}
\]

The probability of observing a particular sequence of \( n \) “+” and “−” jumps is

\[
 P(n) = (P_+)^{n_+} (P_-)^{n_-} = \left(\frac{1}{2}\right)^n.
\]

The total number of trajectories that are possible with \( n \) equally probably “+” and “−” jumps is \( 2^n \), so the probability that any one sequence of \( n \) steps will end up at position \( m \) is given by \( \Omega/2^n \) or
This is the binomial probability distribution function. Looking at the example below for twenty steps, we see \( \langle m \rangle = 0 \) and for a discrete probability distribution which has a Gaussian envelope.

\[
P(m,n) = \left( \frac{1}{2} \right)^n \frac{n!}{n_+!n_-!}
\]

\[
= \left( \frac{1}{2} \right)^n \frac{n!}{\left( \frac{n+m}{2} \right)!\left( \frac{n-m}{2} \right)!}
\]

For very large \( n \), the distribution function becomes continuous. To see this, let’s apply Stirling’s approximation, \( n! \approx (n/e)^n \sqrt{2\pi n} \), and after a bit of manipulation we find

\[
P(m,n) = \sqrt{\frac{2}{\pi n}} e^{-m^2/2n}
\]

(3)

Note, this distribution has an envelope that follows a normal Gaussian distribution for a continuous variable where the variance \( \sigma^2 \) is proportional to the number of steps \( n \).

To express this with a time variable, we instead insert \( n = t/\Delta t \) and \( m = x/\Delta x \) in eq. (3) to obtain the discrete probability distribution function:

\[
P(x,t) = \sqrt{\frac{\Delta t}{2\pi}} \exp \left[ -\frac{\Delta t x^2}{2t(\Delta x)^2} \right]
\]

Note that we can re-write this discrete probability distribution similar to the continuum diffusion solution

\[
P(x,t) = \sqrt{\frac{(\Delta x)^2}{4\pi Dt}} e^{-x^2/4Dt}
\]

(4)

if we equate the variance and diffusion constant as

---

\[ D = \frac{(\Delta x)^2}{2\Delta t} \]

Equation (4) is slightly different because \( P \) is a unitless probability for finding the particle between \( x \) and \( x + \Delta x \), rather than a continuous probability density \( \rho \) with units of \( m^{-1} \): \( \rho(x,t) \, dx = P(x,t) \). Even so, eq. (4) suggests that the time-dependent probability distribution function for the random walk obeys a diffusion equation

\[ \frac{\partial P}{\partial t} = \Delta x D \frac{\partial^2 P}{\partial x^2} \quad \text{or} \quad \frac{\partial \rho}{\partial t} = D \frac{\partial^2 \rho}{\partial x^2} \quad (5) \]

**Three-Dimensional Random Walk**

We can extend this treatment to diffusion from a point source in three dimensions, by using a random walk of \( n \) steps of length \( \Delta x \) on a 3D cubic lattice. The steps are divided into those taken in the \( x \), \( y \), and \( z \) directions:

\[ n = n_x + n_y + n_z \]

and distance of the walker from the origin is obtained from the net displacement along the \( x \), \( y \), and \( z \) axes:

\[ r = (x^2 + y^2 + z^2)^{1/2} = m \Delta x \]

\[ m = \sqrt{m_x^2 + m_y^2 + m_z^2} \]

For each time-interval the walker takes a step choosing the positive or negative direction along the \( x \), \( y \), and \( z \) axes with equal probability. Since each dimension is independent of the others

\[ P(r,n) = P(m_x,n_x)P(m_y,n_y)P(m_z,n_z) \]

Looking at the radial displacement from the origin, we find

\[ \sigma_x^2 + \sigma_y^2 + \sigma_z^2 = \sigma_r^2 \]

\[ \sigma_x^2 = \frac{(\Delta x)^2 t}{\Delta t} \rightarrow 2D \Delta t \]

where

but since each dimension is equally probable \( \sigma_r^2 = 3\sigma_x^2 \). Then using eq. (3)

\[ P(r,t) = \left( \frac{3 \Delta x^2}{2 \pi \sigma_r^2} \right)^{3/2} e^{-3r^2/2\sigma_r^2} \]

where \( \sigma_r^2 = 6Dt \).
Markov Chain and Stochastic Processes\(^5\)

Working again with the same problem in one dimension, let’s try and write an equation of motion for the random walk probability distribution: \(P(x,t)\).

- This is an example of a stochastic process, in which the evolution of a system in time and space has a random variable that needs to be treated statistically.
- As above, the movement of a walker only depends on the position where it is at, and not on any preceding steps. When the system has no memory of where it was earlier, we call it a Markovian system.
- Generally speaking, there are many flavors of a stochastic problem in which you describe the probability of being at a position \(x\) at time \(t\), and these can be categorized by whether \(x\) and \(t\) are treated as continuous or discrete variables. The class of problem we are discussing with discrete \(x\) and \(t\) points is known as a Markov Chain. The case where space is treated discretely and time continuously results in a Master Equation, whereas a Langevin equation or Fokker–Planck equation describes the case of continuous \(x\) and \(t\).
- To describe the walkers time-dependence, we relate the probability distribution at one point in time, \(P(x,t+\Delta t)\), to the probability distribution for the preceding time step, \(P(x,t)\) in terms of the probabilities of a walker making a step to the right (\(P_+\)) or to the left (\(P_-\)) during the interval \(\Delta t\). Note, when \(P_+ \neq P_-\), there is a stepping bias in the system. If \(P_+ + P_- < 1\), there is a resistance to stepping either as a result of an energy barrier or excluded volume on the chain.
- In addition to the loss of probability by stepping away from \(x\) to the left or right, we need to account for the steps from adjacent sites that end at \(x\).

\[
\begin{align*}
P(x,t+\Delta t) &= P(x,t) - P_- \cdot P(x,t) - P_+ \cdot P(x,t) + P_+ \cdot P(x-\Delta x,t) + P_- \cdot P(x+\Delta x,t) \\
&= (1 - P_+ - P_-) \cdot P(x,t) + P_+ \cdot P(x-\Delta x,t) + P_- \cdot P(x+\Delta x,t) \\
&= P(x,t) + P_+[P(x-\Delta x,t) - P(x,t)] + P_-[P(x+\Delta x,t) - P(x,t)]
\end{align*}
\]

and the net change probability is
\[ P(x,t + \Delta t) - P(x,t) = P_x [P(x - \Delta x,t) - P(x,t)] + P_r [P(x + \Delta x,t) - P(x,t)] \]

We can cast this as a time-derivative if we divide the change of probability by the time-step \( \Delta t \):
\[
\frac{\partial P}{\partial t} = \frac{P(x,t + \Delta t) - P(x,t)}{\Delta t}
= \dot{P}_r [P(x - \Delta x,t) - P(x,t)] + \dot{P}_r [P(x + \Delta x,t) - P(x,t)]
= \dot{P}_r \Delta P_r (x,t) + \dot{P}_r \Delta P_r (x,t)
\]

Where \( \dot{P}_r = P_r / \Delta t \) is the right and left stepping rate, and \( \Delta P_r (x,t) = P(x \pm \Delta x,t) - P(x,t) \)

- We would like to show that this random walk model results in a diffusion equation for the probability density \( \rho(x,t) \) we deduced in eq. (5). To simplify, we assume that the left and right stepping probabilities \( P_r = P_- = \frac{1}{2} \), and substitute
\[ P(x,t) = \rho(x,t) \, dx \]
into eq. (6):
\[
\frac{\partial \rho}{\partial t} = \dot{P} [\rho(x - \Delta x,t) - 2 \rho(x,t) + \rho(x + \Delta x,t)]
\]

where \( \dot{P} = 1 / 2 \Delta t \). We then expand these probability density terms in \( x \) as
\[ \rho(x,t) = \rho(0,t) + \frac{\partial \rho}{\partial x} x + \frac{1}{2} \frac{\partial^2 \rho}{\partial x^2} x^2 \]
and find that the probability density follows a diffusion equation
\[
\frac{\partial \rho}{\partial t} = D \frac{\partial^2 \rho}{\partial x^2}
\]
where \( D = \Delta x^2 / 2 \Delta t \).
Fluorescence Correlation Spectroscopy

Fluorescence correlation spectroscopy (FCS) allows one to measure diffusive properties of fluorescent molecules, and is closely related to FRAP. Instead of measuring time-dependent concentration profiles and modeling the kinetics as continuum diffusion, FCS follows the steady state fluctuations in number density of a very dilute fluorescent probe molecule in the small volume observed in a confocal microscope. We measure the fluctuating changes in fluorescence intensity emitted from probe molecules as they diffuse into and out of the focal volume.

- Average concentration of sample: \( C_0 = <10^{-9} \text{ M} - 10^{-7} \text{ M} \).
  
  This corresponds to an average of ~0.1-100 molecules in the focal volume, although this number varies with diffusion into and out of the volume.

- The fluctuating fluorescence trajectory is proportional to the time-dependent number density or concentration:

\[
F(t) \propto C(t)
\]

- How big are the fluctuations? For a Gaussian random process, we expect \( \frac{\delta N_{\text{rms}}}{N} \sim \frac{1}{\sqrt{N}} \).

- The observed concentration at any point in time can be expressed as time-dependent fluctuations about an average value: \( C(t) = \bar{C} + \delta C(t) \).

To describe the experimental observable, we model the time-dependence of \( \delta C(t) \) from the diffusion equation:

---

\[
\frac{\partial \delta C}{\partial t} = D \nabla^2 \delta C
\]
\[
\langle \delta C(r,0) \delta C(r',t) \rangle = \frac{C_0}{(4\pi Dt)^{3/2}} e^{-|r-r'|^2/4Dt}
\]

The concentration fluctuations can be related to the fluorescence intensity fluctuations as

\[ F(t) = A \, W(r) \, C(r,t) \]

\textit{W}(r): Spatial optical profile of excitation and detection

\textit{A}: Other experimental excitation and detection parameters

Calculate FCS correlation function for fluorescence intensity fluctuations. \( F(t) = \langle F \rangle - \delta F(t) \)

\[
G(t) = \frac{\langle \delta F(0) \delta F(t) \rangle}{\langle \delta F \rangle^2}
\]

For the case of a Gaussian beam with a waist size \( w_0 \):

\[
G(t) \sim \frac{B}{1 + t/\tau_{\text{FCS}}}
\]

Where the amplitude is \( B = 4\pi A^2 T_0^2 \overline{\delta C^2} w_0^2 \), and the correlation time is related to the diffusion constant by:

\[
\tau_{\text{FCS}} = \frac{w_0^2}{4D}
\]
Orientational Diffusion

The concepts we developed for translation diffusion and Brownian motion are readily extended to rotational diffusion. For continuum diffusion, if one often assumes that one can separate the particle probability density into a radial and angular part: \( P(r, \theta, \phi) = P(r)P(\theta, \phi) \). Then one also separate the diffusion equation into two parts for which the orientational diffusion follows a small-angle diffusion equation

\[
\frac{\partial P(\Omega,t)}{\partial t} = D_{or} \nabla^2 P(\Omega,t)
\]

(7)

where \( \Omega \) refers to the spherical coordinates \((\theta,\phi)\). \( D_{or} \) is the orientational diffusion constant with units of \( \text{rad}^2 \text{s}^{-1} \). Microscopically, one can consider orientational diffusion as a random walk on the surface of a sphere, with steps being small angular displacements in \( \theta \) and \( \phi \). Equation (7) allows us to obtain the time-dependent probability distribution function \( P(\Omega,t|\Omega_0) \) that describes the distribution of directions \( \Omega \) at time \( t \), given that the vector had the orientation \( \Omega_0 \) at time \( t = 0 \). This can be expressed as an expansion in spherical harmonics

\[
P(\Omega,t|\Omega_0) = \sum_{\ell=0}^{\infty} \sum_{m=-\ell}^{\ell} c^m_\ell(t) \left[ Y^m_\ell(\Omega_0) \right]^* Y^m_\ell(\Omega)
\]

The expansion coefficients are given by

\[
c^m_\ell(t) = \exp\left[-\ell(\ell+1)D_{or}t\right]
\]

Readings