Random walks in bacterial motility

MOTIVATION

Like higher organisms, bacteria also perform directed movement through various mechanisms. Different bacterial species exploit different physical principles to move through aqueous environments and on surfaces. At the scale of microorganisms, i.e. micrometers, Brownian motion leads to continuous agitation of free-swimming bacteria. In this experiment, you will address the question, how efficiently can bacteria explore their environment when their movement is exclusively driven by diffusion. Then making use of cellular appendages known as flagella, which can propel bacteria forward, you will use microscopy techniques and particle-tracking algorithms to explore how dramatically the space over which a bacterium travels expands by active movement.

Diffusive dynamics

The diffusion equation describes the evolution of concentration gradients over time. Here, we derive the diffusion equation from a microscopic perspective [1]. The key idea is to consider the motions of individual diffusing particles and sum over all possible micro-trajectories of the system. The net macroscopic response emerges as the average over all of these underlying micro-trajectories. We begin by describing a random trajectory using the probability density of finding a particle at a particular position and time $p(x,t)$. In particular, the probability of finding a particle in a box of width Δx centered at point x at time t is given by $p(x,t)\Delta x$. To simplify the mathematics, we simplify to one-dimensional motion and discretize space and time. Micro-trajectories and their corresponding weights are shown in Fig. 1. Over a time Δt, the diffusing particle either stays put or jumps left or right a distance a. (We assume particles can only occupy sites on a lattice with spacing a.) The probability of making a jump in either direction is $k\Delta t$, while the probability of staying put is $1-2k\Delta t$, thus ensuring that the sum of all three probabilities is one.

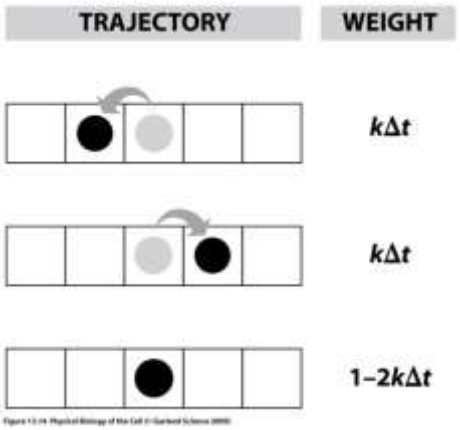

Fig. 1. Trajectories and weight for simple diffusion. A given particle can do one of three things at every time step: jump left, jump right, or stay put. Each of these micro-trajectories has an associated statistical weight. [1]

We obtain the mean displacement by summing over the three micro-trajectories that can occur during a given time step as

$$\langle \Delta x \rangle = a \times k\Delta t + (-a) \times k\Delta t + (0) \times (1 - 2k\Delta t) = 0 . \tag{1.1}$$

We can compute the variance as the average of the square of displacement, once again summing over all three possibilities at a given time.

$$\langle \Delta x^2 \rangle = a^2 \times k\Delta t + (-a)^2 \times k\Delta t + (0)^2 \times (1 - 2k\Delta t) = 2a^2 k\Delta t \tag{1.2}$$

The variance of the total displacement is $N = t/\Delta t$ times greater resulting in

$$\langle \Delta x_{tot}^2 \rangle = 2(a^2 k)\, t \tag{1.3}$$

which is the result for diffusive spreading if we define a^2k as the diffusion constant D.

This trajectories and weights approach can also be used to derive the governing equation for $p(x,t)$, the probability density of finding a particle at position x and time t. Here, we sum over all micro-trajectories starting at time t that result in the particle being at position x at time $t+\Delta t$. In order to find the particle at position x and time t, the particle must be (1) at position x and stays put during the next time step, (2) at position $x-a$ and jumps to the right during the next time step, or (3) at position $x+a$ and jumps to the left during the next time step. The associated probabilities are then $p(x,t)$, $p(x-a,t)$, and $p(x+a,t)$, respectively. Using the probability weights from Fig. 1, we can write $p(x,t+\Delta t)$ as a sum of trajectories,

$$p(x,t+\Delta t) = (1 - 2k\Delta t) \times p(x,t) + k\Delta t \times p(x-a,t) + k\Delta t \times p(x+a,t), \tag{1.4}$$

stay put jump right jump left

leading to a discrete differential (or difference) equation for $p(x,t)$.

In writing Eqn. 1.4, we make use of the so-called Markov property, namely that the probability of a micro-trajectory at time *t* is independent of the previous history of the particle. This allows us to express the probability of each outcome as a product of probabilities. To arrive at the more familiar, continuous diffusion equation we use the Taylor expansions

$$p(x,t+\Delta t) \approx p(x,t) + \Delta t \frac{\partial p(x,t)}{\partial t}, \tag{1.5}$$

$$p(x \pm a,t) \approx p(x,t) \pm a \frac{\partial p(x,t)}{\partial x} + \frac{a^2}{2} \frac{\partial^2 p(x,t)}{\partial x^2}. \tag{1.6}$$

Substituting these formulas into Eqn 1.4 gives

$$\frac{\partial p(x,t)}{\partial t} = \left(a^2 k\right) \frac{\partial p(x,t)}{\partial x^2}. \tag{1.7}$$

Substituting D for a^2k and writing the micro-trajectories in terms of concentration, c we arrive at

$$\frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial x^2}. \tag{1.8}$$

Solution to the diffusion equation

In order to examine the biological consequences of diffusion, we could measure how a concentration spike diffuses over time. In particular, if at time $t = 0$ we start with N molecules in an infinitesimally small region around $x = 0$, the concentration profile will evolve as

$$c(x,t) = \frac{N}{\sqrt{4\pi D t}} e^{-x^2/4Dt}. \tag{1.9}$$

This solution is often denoted as the Green's function of the diffusion equation and has a Gaussian form (Eqn. 1.9) with a linearly increasing width ($4Dt$) (Fig. 2). Dividing Eqn. 1.9 by N gives us the probability density of finding a particle between x and $x+dx$.

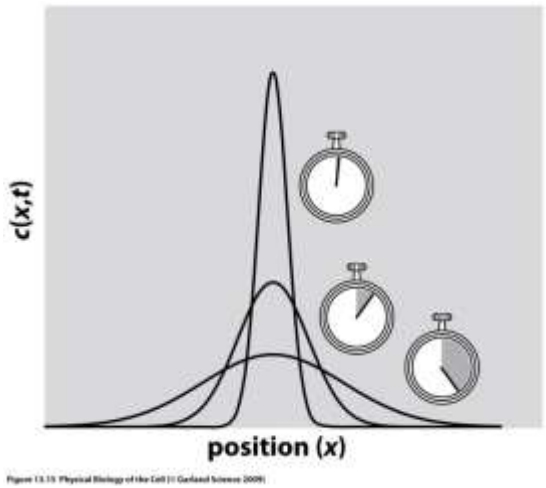

Fig. 2. Time evolution of the concentration field. Diffusion equation solutions are shown at various time points for an initial concentration profile of an infinitesimally wide spike at $t = 0$. [1]

One of the most interesting quantities among diffusive dynamics is the width of the distribution, $\langle x^2 \rangle$, which broadens over time. To compute this broadening, we need to evaluate $\langle x^2 \rangle$ as

$$\langle x^2 \rangle = \frac{\int_{-\infty}^{+\infty} x^2 \frac{N}{\sqrt{4\pi D t}} e^{-x^2/4Dt} dx}{N} = \frac{1}{\sqrt{4\pi D t}} \int_{-\infty}^{+\infty} x^2 e^{-x^2/4Dt} dx, \tag{1.10}$$

where we make use of the probability distribution for finding a particle at position x and time t. (This is also related to Eqn. 1.9 by $c(x,t)/N$.) After evaluation of this integral, we find the mean squared displacement of the distribution for 1-D diffusion is

$$\langle x^2 \rangle = \frac{1}{\sqrt{4\pi D t}} \int_{-\infty}^{+\infty} x^2 e^{-x^2/4Dt} dx = \frac{1}{\sqrt{4\pi D t}} \frac{\sqrt{\pi}}{2} (4Dt)^{3/2} = 2Dt \tag{1.11}$$

and further, for 2-D diffusion: $\langle x^2 \rangle = 4Dt$ and 3-D diffusion: $\langle x^2 \rangle = 6Dt$.

The diffusion constant is a microscopic quantity. In this experiment, you will measure the diffusion constant by analyzing the mean squared displacement $\langle x^2 \rangle$ of individual particles. To estimate the diffusion constant, it is often convenient to use the Einstein relation

$$D = \frac{k_B T}{\gamma}. \tag{1.12}$$

It provides an important link between the microscopic diffusion constant D and the macroscopic friction coefficient γ.

Bacterial Swimming Behavior

Swimming is a common strategy for motility, driven by rotating flagella that extend from the cell body. Each flagellum consists of a long (~10µm) and thin (~20 nm) helical filament which is turned like a screw by a rotary motor at its base (Fig. 3).

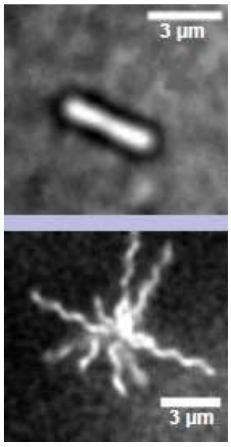

Fig. 3. Microscopic images of the bacterium *Bacillus subtilis*. Top: The bright-field image shows the rod-like cell body. Bottom: The fluorescence image shows flagella emanating from the cell body.

In the absence of external stimuli, the flagella switch between counter-clockwise (ccw) and clockwise (cw) rotation. During ccw rotation bacteria swim in nearly linear trajectories ("runs") (Fig. 4). The flagella bundle, pushing the cell steadily forward. (Hydrodynamic interactions between the flagella and the surrounding fluid, leading to movement of the bacterial cell body, will not be discussed here.) Each run is interrupted by an erratic rotation ("tumble") of the cell in place, caused by flagella rotating cw. Once the bacterium begins rotating ccw, it swims off steadily again, in a new direction [5].

Fig. 4 Time lapse of a swimming bacterium. When flagella rotate ccw, they bundle together and propel the bacterium through aqueous solution. When the flagella rotate cw, they fly apart and induce tumbling motion.

During the tumbling period, bacteria nearly lose directional memory. Thus, at short time scales, the bacterial movement is ballistic (i.e. bacteria swim straight) with a characteristic velocity v (Fig. 5b) that is determined by the flagella motor and the hydrodynamic interaction of the flagella with the fluid. At long time scales, they perform a random walk (Fig. 5a). In other words, flagella driven movement can be thought of as increasing the effective diffusion constant of the bacterium.

Tumbling randomizes the subsequent swim direction and via modulating the tendency to tumble, a biased random walk is achieved, yielding net migration along a concentration gradient. The ability to perform directed motion towards or away from substances is essential for bacteria, such as *Bacillus subtilis*, to survive in versatile habitats, with constant competition for nutrients and exposure to toxins [6].

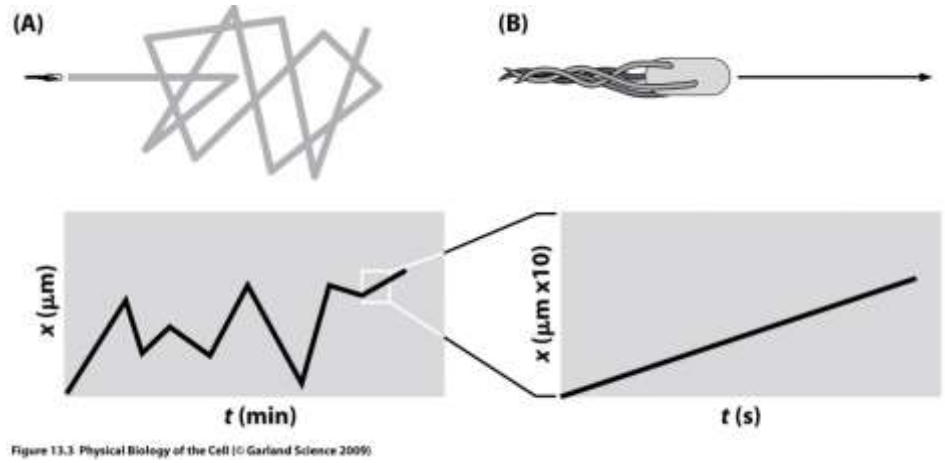

Fig. 5 Flagella-driven bacterial movement at different time scales. a) At time scale of minutes, the movement can be described as a random walk. b) At time scales shorter than seconds, the movement is ballistic.

EXPERIMENTAL PROCEDURE

Questions

1. Estimate how far a single bacterium would move on average from its starting position after 10s, 1 h, 1 day. To estimate the diffusion constant of a bacterium you can use the Einstein relation. For simplicity, assume that bacteria are spheres.
2. Determine the diffusion constant of a non-motile mutant (*Δhag*) of *B. subtilis* by analyzing the paths of individual bacteria. Do this by plotting the mean squared displacements of individual bacteria and average over all bacteria. Use Eqn. 2.2 to calculate the diffusion coefficient. Compare your data at 10 s with your prediction and discuss discrepancies.
3. Repeat (2) using wild type bacteria that can perform flagella-driven motility. Again, plot the mean squared displacements of individual bacteria and average the data. Determine the correlation time τ_c and the average speed v of a running bacterium using Eqn. 2.3. Also, determine τ_c and v by fitting an exponential function of the form $a\, e^{bx}$ to the velocity

autocorrelation function (Eqn. 2.4); compare values. Consider the limit $t >> \tau_c$ and calculate the effective diffusion constant and compare to Eqn. 2.2. Estimate how far a single bacterium moves on average from its starting position after 1 s, 1 h, 1 day.

4. Perform a Fourier transform and obtain the power spectral density of the mean-square displacement of *Δhag* and the motile wild type. Discuss the frequency dependency.

Stochastic Description of Correlated Random walks

To examine bacterial movement, the trajectory of a motile bacterium can be recorded over time (time-lapse microscopy). The average distance explored by that particle, as a function of time, can be described by the mean-squared displacement (MSD)

$$MSD = \langle r^2(t) \rangle = \langle \frac{1}{N} \sum_{i=0}^{N} \big(r_i(t) - r_i(0) \big)^2 \rangle, \tag{2.1}$$

where $r_i(t)$ is the distance particle i moved in time t, and N is the number of particles analyzed.
In the simplest case, a Brownian particle moving freely in three dimensions over long time scales (the so-called diffusive regime), the MSD increases linearly with time (t):

$$\langle r^2(t) \rangle = 6Dt, \tag{2.2}$$

where D is the diffusion constant. The shape of the MSD curve may deviate from a linear due to movement constraints or external forces. For example, molecular motors such as flagella generate directional persistence at short time scales. In such a case, the trajectory of the bacterium can then be described by a correlated random walk [11]

$$\left\langle r^2(t) \right\rangle = 2\tau_c v^2 \left(t - \tau_c \left(1 - e^{-\frac{t}{\tau_c}} \right) \right), \tag{2.3}$$

with a characteristic velocity of v and correlation time τ_c. At short time scales $t << \tau_c$, movement is dominated by the flagellum motor driving the bacterium with a velocity v. At long time scales $t >> \tau_c$, movement resembles a random walk with increased step length. The characteristic parameters τ_c and v may also be extracted from the velocity autocorrelation function

$$< v(t) \cdot v(t + \Delta t) >= v^2 \, e^{-\frac{\Delta t}{\tau_c}}, \tag{2.4}$$

where $v(t)$ is the instantaneous velocity of the cell at time t and $v(t + \Delta t)$ is the velocity at time $t + \Delta t$ (both obtained from the first order derivative of its 2-D position). The average is taken over different initial times t.

Cultivation and sample preparation of *Bacillus subtilis*

1. The evening before the experiment, the two strains are inoculated in 5 mL LB medium and incubated overnight at 37˚C, 250 rpm.
2. The day of the experiment, 1 mL of the overnight liquid culture is washed by centrifuging at 5 G for 2 min, decanting, and resuspending in 1 mL of competence medium. The washed solution is diluted in competence medium to OD = 0.05 (end volume of 5 mL) and incubated in a shaker for 3 h.
3. After the incubation period, solutions are, again, diluted to OD = 0.02, and 50 - 100 µL are pipetted onto a microscope slide. A clean coverslip is placed on top, sandwiching the sample in between. The slide and coverslip are then sealed with a hot wax mixture (valepp).

Table 1. Bacterial strains

***B. subtilis* strain**	**Genotype**	**Comments**
WT	*hisH2 metC typF7*	Wild type
Δhag	*hag::Tn917-lac hisA1 metB5 leu8*	Immotile, no flagella

Data Acqusition

- To image the bacteria, we will use an inverted microscope equipped with a high-speed camera and 20x air objective.
- The temperature of the microscope stage should be set a 37°C. Samples should not be imaged for longer than 15 minutes, as they will overheat from bright-field illumination.
- Record a video of swimming bacteria. Cells can exceed velocities of 50µm/s, thus a reasonable frame rate of 10 fps is recommended. No image binning is required.

Data Analysis

- Tracking will be done with a self-built tracking program (MATLAB), provided by your supervisor.
- Tracking parameters must be adjusted in the program to accurately track the various strains.
- Check the individual tracks and discard any with defects, such as cell crossovers and cells that have adhered to microscope slide surface.

LITERATURE

1. **Schuergers, N., Lenn, T., Kampmann, R., Meissner, M., Esteves, T., Temerinac-Ott, M., Korvink, J.G., Lowe, A.R., Mullineaux, C.W. and Wilde, A. *Cyanobacteria use micro-optics to sense light direction*. eLIFE (2016)**
2. Rob Phillips, Jane Kondev, Julie Theriot Physical Biology of the Cell. New York: Garland Science, 2009.
3. Eisenbach M. Bacterial chemotaxis. *Encyclopedia of life sciences*. *(2001)*
4. Sowa Y. and Berry R.M. Bacterial flagellar motor. *Quarterly Reviews of Biophysics* 41, 2, pp. 103–132, (2008)
5. Mirel D.B., Chamberlin M.J. The Bacillus subtilis flagellin gene (hag) is transcribed by theσ28 form of RNA polymerase. *J Bacteriol* 191:3095-3101 (1989)
6. Biondi S. A., Quinn J. A. and Goldfine H. Random motility of swimming bacteria in restricted geometries. *AIChE J.* 44(8), 1923–1929 (1998)
7. Aizawa S.I., Zhulin I.B., Márquez-Magana L., Ordal G.W. Chemotaxis and motility. In: Sonenshein AL, Hoch JA, Losick R (eds.) Bacillus subtilis and its closest relatives. *ASM Press*, Washington DC, pp. 437-452 (2002)
8. Berg H. C. The rotary motor of bacterial flagella. *Annu Rev Biochem.* 72:19-54 (2003)
9. Josenhans C., Suerbaum S. The role of motility as a virulence factor in bacteria. *Int J Med Microbiol* 291:605–614(2002)
10. Krukonis E.S., DiRita V.J. From motility to virulence: sensing and responding to environmental signals in Vibrio cholerae. *Curr Opin Microbiol* 6:186–190(2003)
11. Börger L. Quantifying individual differences in dispersal using net squared displacement. *Oxford University Press* (2012)
12. Kneller G.R. Stochastic dynamics and relaxation in molecular systems. *University of Orleans lecture course*

The first item will be relevant for the oral exam.