RANDOM WALKS IN BACTERIAL MOTILITY

I. MOTIVATION

Like higher organisms, bacteria developed mechanisms for movement. Interestingly, different bacterial species exploit different physical principles for movement through aqueous environment and on surfaces.

At the scale of microorganisms, i.e. at the length scale of micrometers, Brownian motion leads to continuous agitation of free-swimming bacteria in aqueous solution. Here, we will address the question of how efficiently bacteria can explore their environment when their movement is exclusively driven by diffusion. Using cellular appendages, so-called flagella, bacteria can propel themselves forward. Using microscopic techniques in combination with particle-tracking algorithms, we will explore how strongly the range expands by active movement.

A. DIFFUSIVE DYNAMICS

The diffusion equation describes the evolution of concentration gradients with time. Here, we will derive the diffusion equation from a microscopic perspective [1]. The key idea is to consider the motions of individual diffusing particles and to sum over all of the possible micro-trajectories of the system. The overall macroscopic response emerges as the average over all of these underlying micro-trajectories. The key idea is to describe a random trajectory by the probability density for finding a particle at a particular position at a given instant in time $p(x,t)$. In particular, the probability of finding the 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 math we specialize to one-dimensional motion, and discretize space and time. Micro-trajectories and their corresponding weights are shown in Figure.1. The diffusing particle, over time Δt, stays put, or jumps to the left of right a distance a, where we imagine that the particles can only occupy lattice 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 $1-2k\Delta t$, ensuring that the probabilities for all three possible outcomes add up to 1.

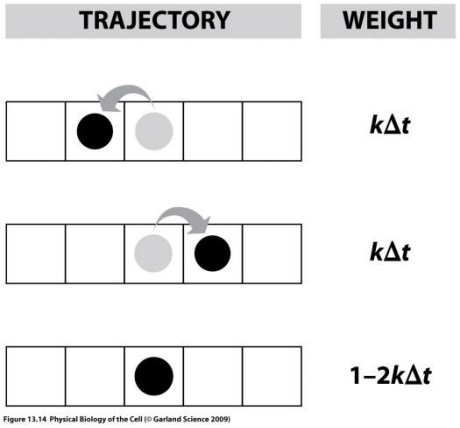

Fig. 1. Trajectories and weight for simple diffusion. A given particle can do one of three things at every time step: jump left, jump right, or stay put. Each of these microtrajectories has an associated statistical weight. [1]

We obtain the mean 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 by summing over all the eventualities at a given instant as

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

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 identify a^2k with the diffusion constant D.

The trajectories and weights approach can also be used to derive the governing equation for $p(x,t)$, the probability density that the particle is at position x at time t. The idea is to sum over all the micro-trajectories starting at time instant t that result in the particle being at position x at time $t+\Delta t$. For this to happen the particle needs to be at position x (if it is stay put on the next time step), $x-a$ (if it is to jump to the right at the next time step), or $x+a$ (if it is to jump to the left at the next time step) at time t, and the associated probabilities are $p(x,t)$, $p(x-a,t)$ and $p(x+a,t)$, respectively. Using the probabilities in Figure 1, we can write $p(x,t+\Delta t)$ as a sum over trajectories,

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

stay put jump right jump left

which leads to a discrete differential (or difference) equation for *p(x,t)*.

In writing the above equation so-called Markov property of the process was used, namely the fact that the probability of a micro-trajectory at time *t* is independent of the previous history of the particle; this is what allows us to express the probability of each outcome as a product of probabilities. To arrive at the more familiar, continuous diffusion equation we can use the Taylor expansion

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

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

Substituting these formulas into equation 1.4 gives

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

Again $D = a^2k$. In terms of concentration *c* we can write the diffusion equation

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2}, \qquad (1.8)$$

Solution of the diffusion equation

In order to examine biological consequences of diffusion equation one might use tool which corresponds to knowing how to solve the diffusion equation for a spike of concentration at the origin at time=0. 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 in the following way

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

Further, by dividing by *N*, this equation can then be interpreted as giving the probability density for finding a particle between *x* and *x*+*dx*. The solution quoted above is often denoted as the Green's function of the diffusion equation and its evolution can be seen in Figure 2. This equation for the concentration tells us that the profile has the form of a Gaussian. The width of the Gaussian is 4*Dt* and hence, it increases linearly with time.

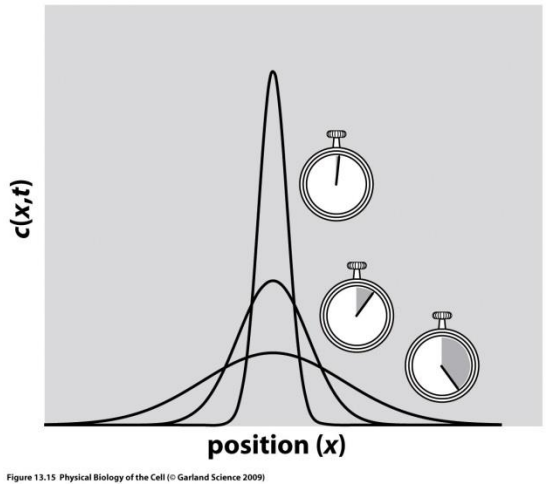

Figure 2. Time evolution of the concentration field. The plot shows the solution for the diffusion equation at different times for an initial concentration profile that is a spike at *x*=0. [1]

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

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

where we made use of the probability distribution for finding a particle at position *x* at time *t*, which is related to the concentration distribution, eqn 1.16, by *c(x,t)/N*.

After evaluation of this integral we find for 1D diffusion

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

2D diffusion $\left\langle x^2 \right\rangle = 4Dt$

3D diffusion $\left\langle x^2 \right\rangle = 6Dt$

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

$$D = \frac{k_B T}{\gamma} \qquad (1.12)$$

It gives an important connection between the microscopic diffusion constant D and the macroscopic friction coefficient γ.

B. BACTERIAL SWIMMING BEHAVIOUR

Swimming is a common strategy for motility, driven by rotating flagella, which extend from the cell body. Each flagellum consists of a long (~10µm), thin (~20 nm), helical filament, turned like a screw by a rotary motor at its base (Fig. 3) [2].

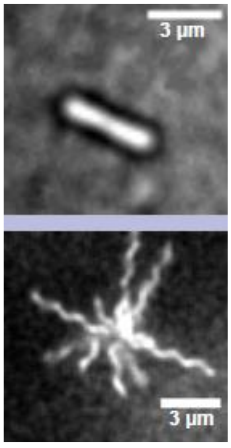

Fig. 3. Microscopic images of the bacterium *Bacillus subtilis*. Top: The brightfield image shows the rod-like cell body. Bottom: The fluorescence image shows flagella emanating from the cell body.

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 filaments form a bundle that pushes the cell steadily forward. The hydrodynamic interactions between the flagella and the surrounding fluid leading to movement of the bacterial cell body shall not be discussed here. Each run is interrupted by an erratic rotation ("tumble") of the cell in place, which caused by a change to a clockwise rotation of the flagella, and then swims steadily again in a new direction [3].

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 the memory of direction. 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 characteristic of 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 to increase the effective diffusion constant of the bacterium [1].

Tumbling randomizes the direction and via modulating the tendency to tumble, a biased random walk is achieved in order to achieve net migration along a concentration gradient. The ability to move directed towards or away from substances is essential for bacteria such as *Bacillus subtilis* to survive in its versatile habitat where it stands in permanent competition for nutrients, and is exposed to environmental toxins and harmful compounds [4].

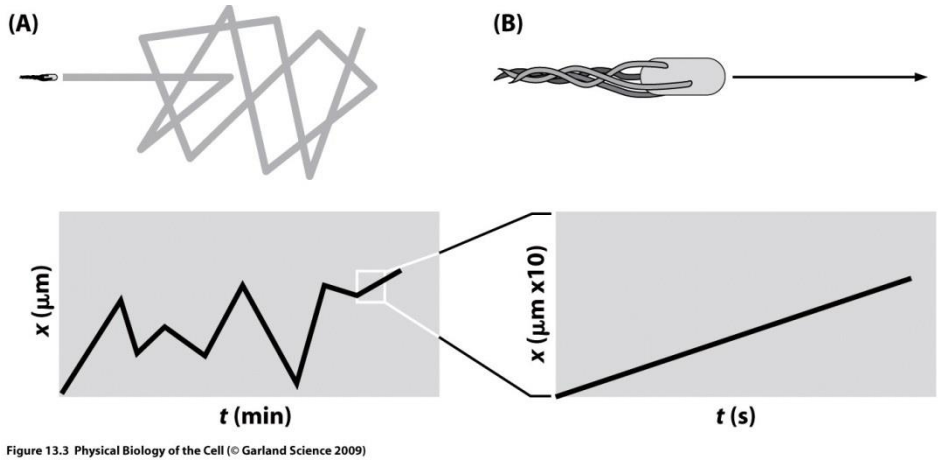

Fig. 5 Flagella-driven bacterial movement at different time scales. a) At time scales longer than minutes, the movement can be described as a random walk. b) At time scales shorter than seconds, the movement is ballistic.

II. EXPERIMENTAL PROCEDURE

A. QUESTIONS

1. Estimate how far a single bacterium would move on average from its starting position after 10s, 1 h, 1 day. For estimating 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 *Bacillus subtilis* cells (addition of CCCP - Carbonyl Cyanide m-Chlorophenyl Hydrazine) by analyzing the paths of individual bacteria. For this, plot the mean squared displacements of individual bacteria and average the data. Use eq. (2.2) for calculating the diffusion coefficient. Compare your data at 10s with your prediction and discuss the discrepancy.

3. Now use wild type bacteria that use 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 (2.3), and compare with the values obtained by performing an exponential fit of the form $a\ e^{bx}$ to the obtained velocity autocorrelation function (2.4). Consider the limit t >> τ_c and calculate the effective diffusion constant in comparison to (2.2). For this calculate the average run length $L = \tau_r\ v_r$ and set $L^2 = 4\ D_{eff}\ \tau_r$. Estimate how far a single bacterium would move on average from its starting position after 1s, 1 h, 1 day. Fit the data of $wt + \mathrm{CCCP}$ with (2.3) and discuss the differences to the wild type w/o CCCP.

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

B. STOCHASTIC DESCRIPTION OF CORRELATED RANDOM WALKS

To examine the bacterial movement trajectories of a single motile bacterium can be recorded (in a video sequence) and analyzed using mean-square displacement (MSD) as a function of time that describes the average distance explored by the particle:

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

where $r_i(t)$ is the distance by which particle i moved within time t, and N is the number of particles analyzed.

In the simplest case of a Brownian particle moving freely in three dimensions over longer time scales (the so-called diffusive regime) the MSD will increase linearly with time (t):

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

where D is the diffusion constant and t denotes the time since start. In general, the form of the MSD over time may deviate from a linear pattern due to movement constraints or external forces. In particular, a molecular motor like the flagellum generates directional persistence at short time scales. The trajectory of the bacterium can then be described by a correlated random walk [7].

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

with a characteristic velocity of v and a correlation time τ_c [5]. At short time scales t << τ_c movement is dominated by the motor movement that drives the bacterium with a velocity v. At long time scales t >> τ_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

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

where $v(t)$ is the instantaneous velocity of the cell at the moment t, $v(t + \Delta t)$ is the velocity at the later moment $t + \Delta t$, both obtained from the first order derivative of its 2D position, with the average < > taken over different initial times t.

C. CULTIVATION AND SAMPLE PREPARATION OF BACILLUS SUBTILIS

1. Thaw the aliquots of the *Bacillus subtilis* strain *(wt,* with and without CCCP) at room temperature and centrifuged at 5000 rpm for 3 minutes in order to get rid of glycerol.
2. Re-suspend cell pellets in 500 µl fresh competence buffer (will be prepared) and diluted them in this buffer to optical densities (at 600 nm wavelength) between 0.02 and 0.05.
3. Drop ~ 9-10 µL of bacterial culture re-suspended in competence buffer on a clean cover slip and cover it with a clean microscope slide slightly pressing down the latter. Seal the sample with hot wax mixture VALEPP.

BACTERIAL STRAINS AND BUFFER

***B. subtilis* strain**	**Genotype**	**Comments**
WT	*hisH2 metC typF7*	Wild type

Competence buffer will be prepared with the supervisor.

D. DATA ACQUSITION

- For imaging bacteria, use a student's homebuilt microscope and 20x air objective (Nikon) that supports phase contrast microscopy.
- The temperature of the microscope stage should be set to 37°C. Samples should not be used on the microscope for longer than 15 minutes due to heating upon bright-field illumination.
- Record a video of swimming bacteria (*wt* with and without CCCP) for 5 minutes with 10 fps at 5 different locations. Choose areas where bacteria move (no crowding and collisions). Save movie as a sequence of .tif files.
- Cells need to be diluted to a density of 0.02.

E. DATA ANALYSIS

- Before analyzing the tracks should be processed in order to increase contrast. For this open the image-analysis program ImageJ. Import image sequence to the program (File>Import>Image Sequence). Process image with Process/Filter/Variance/ Set radius for 5 pixels. Save a movie as a .tif in a separate folder.
- Open MATLAB program and choose a tracking program: GC_tracking. Set the following parameters for the tracking:

  Bright object on dark background;
  Object Diameter 30 pixel;
  Minimal Datapoints: 50;
  Number of datapoints for recovery: 5;
  Maximum Displacement: 15 pixel;
  Intensity Treshold 20%;
  File Format: 8 bit Multipage Tiff;
  Camera Settings: "Laser Trap";
  Objective: 20x;
  Binning 1x;
  Framerate 10.

- Click Run and choose the .tif file of the recorded movie. After tracking is finished you can find the folders "results" with tracks of individual bacteria.
- Next, evaluate tracks with Evaluate Button, open a folder "results", choose a txt-file, containing tracks (track.txt), next choose a tif-file, containing the corresponding movie. It is advised to check cells for tracking defects (cell crossovers) and stuck cells and discard those.

- Track and evaluate recorded movies and collect the txt-files of all tracks in the separate folder.
- In order to calculate the MSD, τ_c and v_r from the tracks, choose the correct file in MATLAB and follow the code cells. Try to write a small code snippet that will compute the velocity auto-correlation function and fit it to its exponential form. The graphs with plot and single track can be saved as File>Save as>TIFF image.
- In order to compare the individual tracks, scale the axes X and Y of the graph appropriately. For this, go to the very right button called Show Plot Tool and Dock figure and by clicking at the X or Y axis set the limits.

III. LITERATURE

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6. Codling EA, Plank MJ, Benhamou S. Random walk models in biology. *J.R. Soc interface* (2008)
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The first item will be relevant for the oral exam.