BE/APh161: Physical Biology of the Cell
Homework 3
Due Date: Wednesday, January 28, 2015

"It is better to debate a question without settling it than to settle a question without debating it." - Joseph Joubert

1. Kinetics of ligand-receptor binding

In class we discussed three different ways of working out the probability of ligand-receptor binding. I did two of those methods through to completion and in this problem, you will work out the details of the third of those methods. We are interested in the quantities $p_{bound}([L], t)$ and $p_{unbound}([L], t)$. I claim that we can write the equation for $p_{unbound}([L], t)$ as

$$\frac{dp_{unbound}([L], t)}{dt} = -k_{on}[L]p_{unbound} + k_{off}p_{bound}. \tag{1}$$

Explain why this makes sense. Using the constraint that $p_{bound}([L], t) + p_{unbound}([L], t) = 1$ (why is that true?), eliminate p_{bound} from the differential equation and obtain an equation strictly involving $p_{unbound}$. Find the analytic solution to that problem and determine the long time limit for p_{bound} and demonstrate that it can be written in the same form considered in class, namely,

$$p_{bound}([L]) = \frac{[L]/K_d}{1 + [L]/K_d}. \tag{2}$$

Given this result, how does K_d depend upon k_{on} and k_{off}?

2. Diffusive speed limits: It's not just a good idea, it's the law

In order for a chemical reaction to take place, the reactants must be at the same place at the same time. A very interesting calculation explores the way in which diffusion can control the on rate for reactions. Imagine some reaction in which A and B come together to form the complex AB. To simplify the problem, we are going to imagine B as a sphere of radius a that is fixed at the origin of our coordinate system. Further, we are going to imagine that very far away the concentration of A is held at c_0. What I really mean by this is that $lim_{r\to\infty}c(r) = c_0$, where $c(r)$ is the concentration of reactant

A as a function of distance from the origin. Our goal is to compute the so-called "diffusion-limited on rate" for the reaction. We begin by working out the steady-state solution to the diffusion equation with the boundary condition that $c(a) = 0$, which corresponds to the physical statement that the sphere is a "perfect absorber". What this really means is that every time a molecule of A arrives at the sphere, the reaction occurs. (Note that this tells us that the diffusion-limited on rate is the fastest that a reaction could occur. It could be true that after the molecule arrives, it has to wait for some favorable orientation to occur, for example, which would make the rate of the reaction even slower).

(a) Solve the diffusion equation in steady state and find the concentration profile $c(r)$ as a function of c_0 and a.

(b) Use that result to compute the diffusive flux $J(a)$ at the surface of the sphere.

(c) Use the result of part (b) to write an equation for dn/dt, the rate at which A molecules arrive at the sphere and thus the rate of production of AB. The function $n(t)$ simply tells me how many molecules have arrived at the "perfect absorber" during the time between $t = 0$ and the time t.

(d) Now, use the result of part (c) to write an equation of the form

$$\frac{dn}{dt} = k_{on} c_0, \tag{3}$$

and hence write an expression for k_{on}. This is the so-called Smoluchowski rate.

(e) Find a numerical value for this diffusion limited on rate, k_{on}. Justify the units it has and provide an actual numerical value by estimating the relevant parameters that determine k_{on}.

3. Chemotaxis and Receptor Binding

As described in class, bacterial chemotaxis is claimed to be the best studied signal transduction problem in biology. In this problem, we work through some of the statements and results in a few of the classic papers I presented

in class. We develop a feeling for the numbers by examining direct quotations from the experimental papers that have really driven the field recently as well as a commentary on this work by Dennis Bray. Begin by reading both of these papers which are attached on the website.

(a) Write a one-paragraph summary of each of the two papers.

In their 2002 paper in PNAS entitled "Receptor sensitivity in bacterial chemotaxis", Sourjik and Berg say: "The changes in receptor occupancy encountered by bacteria swimming in spatial gradients (e.g., near the mouth of a capillary tube in the capillary assay) are very small. For example, in the tracking experiments, cells about 0.6 mm from the tip of a capillary tube (consider a pipette with a radius of 5.0 μm) containing 1 mM aspartate moved in a gradient of steepness 0.02 μM/μm at a mean concentration of about 8 μM. A 10-μm run straight up such a gradient would change the concentration from 8 to 8.2 μM, i.e., by 2.5 %. Assuming K_d values for aspartate of 7.1 μM and 62 mM (see above), this step gives a fractional change in receptor occupancy of about 0.003". **RP to class:** the two K_d values correspond to the fact that two of the different chemotactic receptors (Tar and Tsr) will bind aspartate, but with quite different affinities. For your estimates, only consider the smaller K_d since the larger one will be irrelevant at the concentrations of interest here.

(b) Carry out calculations that exploit the numbers given above and using what you know about the definitions of concentration, the size of *E. coli* cells and about the meaning of K_d and simple binding curves (i.e. $p_{bound} = (L/K_d)/(1+(L/K_d))$, corresponding to the simplest model in which there is only a single binding site per receptor and there is no cooperativity between receptors). First, use the steady-state diffusion equation for a spherically symmetric source to estimate the concentration at 0.6 mm from the pipette. The idea is to solve the 3D diffusion equation in spherical coordinates, given that the concentration at the source (i.e. the pipette) is 1 mM and that the concentration in the far field is zero. (NOTE: we work this out in chap. 13 of PBoC in a different context, but the ideas are all the same.) Do you agree with them about the concentration being 8 μM at a distance of 0.6 mm? Next, examine the statement about the consequences of a 10-μm run and also about the fractional change in occupancy. Do you agree with their numbers? Do you agree with the qualitative thrust of their

statements? Now, to be more realistic, consider the MWC treatment of the $p_{active}(c)$ curve we discussed in class. Assume that $K_d^I = 7.1\mu M$ and that $K_d^A = 100K_d^I$. Use $\epsilon = 5\ k_BT$ for the difference between the inactive and active states. Assume the cluster size is $n = 10$ and work out the change in occupancy using this model rather than the simple binding function we started with at the beginning of the problem. How does this change our estimate for $\Delta p/p$ compared to the simple binding function?

In his commentary on the paper of Sourjik and Berg, Dennis Bray says: "The mystery can be expressed in a different way. Estimates of the binding affinity of aspartate to the membrane receptor of wild-type *E. coli* typically give a dissociation constant in the range 15 μM. A bacterium responding to a change in occupancy of 0.1% is therefore sensing concentrations of aspartate of a few nanomolar. And yet we know from decades of observations that the same bacterium is also capable of responding to gradients of aspartate that extend up to 1 mM. Somehow, *E. coli* is able to sense aspartate over a range of at least 5 orders of magnitude in concentration by using just one molecular species of receptor!"

(c) Do the estimate/calculation that supports the claim made by Bray. In particular, examine a 0.1% change in occupancy and see what that means about the change in concentration given that the K_d has the value claimed. Also, if there is a change of concentration of order a few nanomolar, how many fewer molecules are there in a box of size 1 μm^3 due to such a concentration difference at the front and back of a cell?