BIOREPS Problem Set #4
Adaptation and cooperation

1 Background

Adaptation is one of the most distinctive features of our physical senses. The thermoreceptors in our skin react sharply to the change in outside temperature when we jump into a cold pool on a summer day, but rapidly adjust. Olfactory receptors in the nose can detect minute traces of chemicals in the air (a waft of perfume carried on a breeze), but will become insensitive to an odor after persistent exposure (the classic case of someone wearing too much perfume or cologne). Photoreceptors in the eye will adapt themselves to the overall intensity of light, giving us the ability to distinguish visual features of our environment in both very bright and very dim conditions. In a number of examples (certain photoreceptors, bacterial and eukaryotic chemotaxis), this adaptation approaches perfection: the sensing system essentially reacts only to changes in stimulus (contrast in an image, gradients in concentration), and returns to the same baseline behavior irrespective of the absolute level of the stimulus. Strictly speaking, this "perfect adaptation" holds only over some range of stimulus amplitudes, but that range could be many orders of magnitude of ligand concentration or light intensity.

The evolutionary reasons for adaptation in sensing are easy to grasp when we consider the alternative: imagine a detector that has evolved to amplify faint signals but has no capacity to adapt. Such a detector will generally become completely saturated and hence useless if the environment changes and the background level of the stimulus becomes very high (a photoreceptor capable of detecting single photons in the darkness is suddenly exposed to sunlight). Adaptation will solve this problem, by allowing the same sensor to operate (distinguish temporal and/or spatial variations in the stimulus) under many different background amplitudes.

E. coli chemotaxis, as we described in lecture, is perhaps the canonical example of perfect adaptation. And though we know more about the molecular details of its sensing system than any other case in biology, we still do not have a final, comprehensive picture of how it can achieve perfect adaptation. The question has intrigued theorists for over forty years [1], and continues to be an active research area. In this problem set, we will get a taste of why understanding *E. coli* adaptation is highly non-trivial: it requires going beyond the level of individual receptors, to figuring out how clusters of receptors interact with each other, coordinating their responses to ligands in a cooperative manner. The bacterium has thousands of receptors grouped together near its poles, and long-range interactions between these receptors could allow small groups (ten or fewer) to synchronize their activity. Unfortunately the detailed chemistry of these interactions has not entirely been worked out, but only indirectly inferred from experiments [2]. Our approach will be mathematical: we will argue in general terms that a model of independent, uncoordinated receptors is insufficient to explain perfect adaptation. We will then work out a simple alternative model involving an interacting cluster of receptors, and show that it can indeed approach perfection. This model of interaction has roots in a classic 1965 theory of Monod, Wyman, and Changeux (MWC) for how protein function changes with ligand binding [3]. It represents one extreme of cooperation: rather than each receptor switching between inactive and active states independently, the entire cluster does so in unison, with either all active at the same time, or all inactive at the same time. The MWC model has become a popular description of receptor

clusters in *E. coli*, and this problem set draws on the arguments laid out by Howard Berg [2], Ned Wingreen [4], and their collaborators. Because we do not yet understand the molecular basis of the long-range interactions between receptors that could lead to perfect unison, the MWC model remains for now a plausible hypothesis that can fit experimental data: it is not necessarily the "true" picture, but it certainly suggests that receptor cooperation is a key ingredient to perfect adaptation.

References

[1] Koshland Jr, D. E., Goldbeter, A., and Stock, J. B. Amplification and adaptation in regulatory and sensory systems. *Science* **217**, 220–225 (1982).

[2] Sourjik, V. and Berg, H. C. Functional interactions between receptors in bacterial chemotaxis. *Nature*, **428**, 437–441 (2004).

[3] Monod J., Wyman J., and Changeux J.-P. On the nature of allosteric transitions: a plausible model. *J. Mol. Biol.* **12**, 88–118 (1965).

[4] Hansen, C. H., Endres, R. G., and Wingreen, N. S. Chemotaxis in Escherichia coli: a molecular model for robust precise adaptation. *PLoS Comput. Biol.*, **4**, e1 (2008).

2 Review of *E. coli* chemotaxis

Before we get to the details of the model, it is useful to briefly review some of the properties of *E. coli* chemotaxis we covered in class (the full derivations are in the online lecture notes). Let us start with the top row of Fig. 1: the bacterium moves through a uniform background of ligands, at some low concentration c_L. It randomly alternates between running (mainly inactive receptors) and tumbling (a sufficient number of active receptors), carrying out a random walk through its environment. The running to tumbling ratio, or equivalently the ratio of inactive-to-active stationary probabilities p_I^s/p_A^s, is dependent on the ligand concentration. For a single receptor, we derived the general formula for this ratio at any concentration c,

$$\frac{p_I^s}{p_A^s} = \frac{r}{s} = e^{-\beta\epsilon_0} F(c), \tag{1}$$

where $F(c) = (1 + c/K_I)/(1 + c/K_A)$. Here r is the transition rate from state A to I, s is the the rate from I to A, K_I is the ligand dissociation constant for the I state, and K_A is the ligand dissociation constant for the A state. The fact that the ligand is much less likely to bind to the A state leads to $K_A \gg K_I$, which makes p_I^s/p_A^s much larger at high c than at low c. The variable ϵ_0 is the energy difference between the inactive and active receptor in the absence of ligand, and it plays a major role in the process of adaptation. The larger the ϵ_0, the less likely we are to see the inactive state, shifting the entire p_I^s/p_A^s curve to smaller values.

When the bacterium passes into a region of high concentration c_H (bottom row of Fig. 1) we initially get a much larger value of p_I^s/p_A^s. The inactive state becomes dominant, and we will see a long run, generally lasting hundreds of seconds. However if it stays in the c_H region for

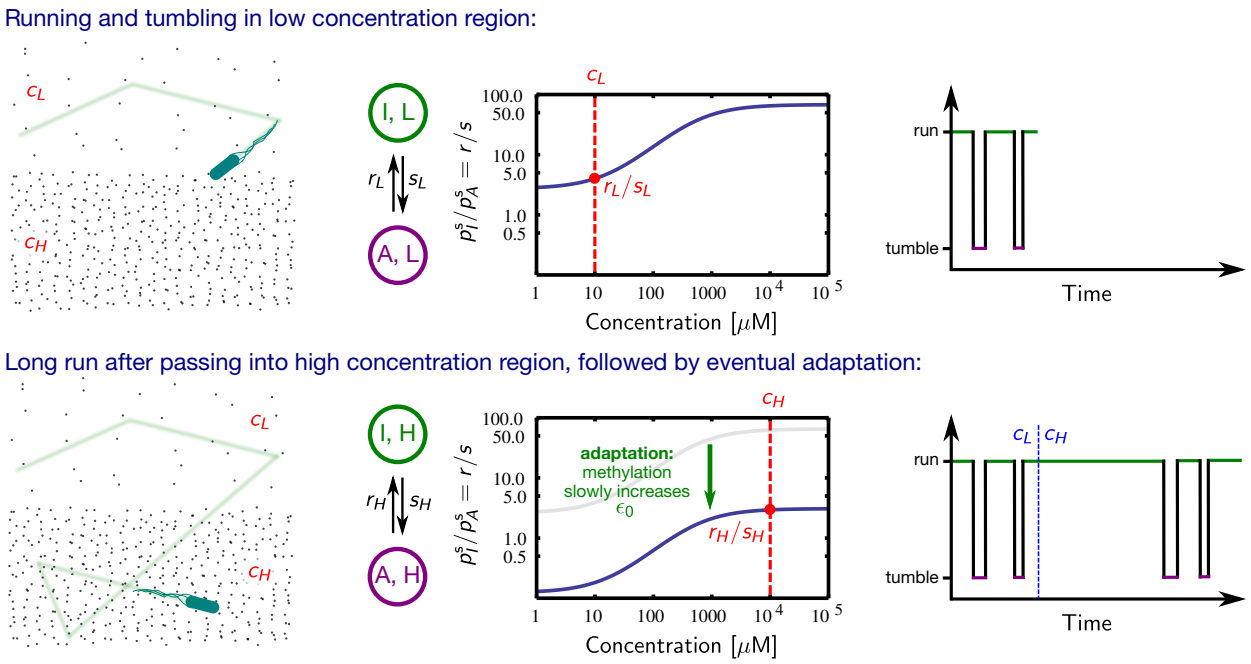

Figure 1: *E. coli* behavior before and after crossing a low-to-high concentration boundary.

an extended period of time, it makes sense for the bacterium to eventually return to its earlier strategy of mixed running and tumbling. This way it can continue its random exploration (there are perhaps even richer targets somewhere out there). The recovery occurs by a gradual increase in the value of ϵ_0, driven by methylation of the receptor. The p_I^s/p_A^s curve shifts down until its value at c_H is equal to the original curve's value at c_L. The bacterium has perfectly adapted, going back to exactly the same ratio of running and tumbling it had at the beginning. Thus the *E. coli* chemotaxis system has two key features: it can temporarily adjust itself to take advantage of sudden concentration changes (i.e the long run on passing from c_L to c_H), but always returns to the same base-line strategy in homogeneous ligand environments. Essentially all it cares about are increases (or decreases) in ligand concentration, not the overall magnitude of the concentration.

3 Questions

3.1 Independent receptor model

We will start by looking at the behavior of a model where all receptors randomly switch between I and A independently of each other, without any kind of coordination. At fixed ligand concentration c, each receptor will then have a steady-state probability of being in the inactive state p_I^s, or active state p_A^s, with a ratio given by Eq. (1). For simplicity of notation, let us define the "activity" $a(c) \equiv p_A^s$ as the probability of finding a receptor in the active state. Using Eq. (1) and the fact that $p_A^s + p_I^s = 1$, the activity is given by:

$$a(c) = \left[1 + e^{-\beta\epsilon_0} F(c)\right]^{-1}. \tag{2}$$

To model the effects of methylation, we assume the receptor can accomodate up to 8 methyl groups, and so can be in a state $m = 0, 1, \ldots, 8$, where m denotes the number of methylated sites. We now make ϵ_0 a function of m (linear for simplicity),

$$\epsilon_0(m) = \mu + \sigma m \tag{3}$$

where μ and σ are some constants. If $\sigma > 0$ then $\epsilon_0(m)$ increases as m increases, which is the observed behavior of the receptor. This makes $a(c)$ also a function of m, so we can rewrite Eq. (2) as

$$a(m, c) = \left[1 + e^{-\beta\epsilon_0(m)} F(c)\right]^{-1}. \tag{4}$$

To complete the description of the model, we need to describe the dynamics of methylation. This can be done by assigning transition rates between the m states, as shown below:

The rate of adding a methyl group to state m, and thus transitioning to state $m + 1$ is given by:

$$g_m = \gamma(1 - a(m, c)). \tag{5}$$

This represents the action of the chemotaxis enzyme CheR, which methylates the receptors, and hence the rate constant γ is proportional to the concentration of CheR. The factor $1 - a(m, c)$ is due to the assumption that CheR can only methylate receptors in the inactive conformation, and hence g_m should be proportional to the probability $1 - a(m, c)$ that the receptor is inactive.

Similarly the rate of removing a methyl group from state m, and thus transitioning to $m - 1$, is given by:

$$r_m = \rho a(m, c). \tag{6}$$

This represents the action of the chemotaxis enzyme CheB, which removes methyl groups from the receptor. Since CheB can only perform its demethylation function if it binds to the receptor and gets phosphorylated by the active receptor kinase domain, r_m is proportional to $a(m, c)$, the probability that the receptor is in the active state.

Let $p_m(t)$ be the probability that a receptor is in state m at time t. Then the mean activity of a receptor at time t, for ligand concentration c,

$$\bar{a}(c, t) \equiv \sum_{m=0}^{8} a(m, c) p_m(t) \tag{7}$$

is the crucial physical variable of interest. This will be correspond to the mean activity of the entire population of receptors, since in this model each receptor is independent. Hence $\bar{a}(c, t)$ will control the running vs. tumbling ratio (lower $\bar{a}$ means more running).

We can now pose the question of perfect adaptation in a mathematically rigorous way: imagine that at time $t = 0$ the bacterium enters a region of concentration c. Its receptors have some methylation probability distribution $p_m(0)$, and hence mean activity $\bar{a}(c, 0)$. Assuming it stays in the same concentration region for an arbitrarily long time, the probability $p_m(t) \to p_m^s$ as $t \to \infty$,

a steady state solution, and hence $\bar{a}(c,t) \to \bar{a}^s(c) = \sum_{m=0}^{8} a(m,c) p_m^s$. The long-time limit is the baseline behavior towards which the system tends. If $\bar{a}^s(c)$ is a constant independent of c, then we have perfect adaptation. If $\bar{a}^s(c)$ varies depending on c, then we have imperfect adaptation. Let us now see which one occurs in the independent receptor model.

a) Write down an expression for the current $J_{m+1,m}(t)$ from state m to $m+1$. The stationary solution p_m^s corresponds to the case where all currents $J_{m+1,m}^s$ in the system are equal to zero. Use this fact to write down a recursion relation, $p_{m+1}^s = \nu_m p_m^s$. Find the factor ν_m.

b) Each current being zero in the stationary state also means that $\sum_{m=0}^{7} J_{m+1,m}^s = 0$. Show that this implies the following equation for $\bar{a}^s(c)$:

$$\bar{a}^s(c) = \frac{\gamma}{\gamma+\rho} + p_0^s \frac{a(0,c)\rho}{\gamma+\rho} + p_8^s \frac{\gamma(a(8,c)-1)}{\gamma+\rho} \tag{8}$$

This is a potentially interesting result: if both p_0^s and p_8^s were zero, then $\bar{a}^s(c)$ would be independent of c, leading to perfect adaptation. Is this what actually occurs? We will find out in the next part.

c) Write a program that numerically solves the recursion relation from part a) for a given set of parameters. Your program should initially set p_0^s to an arbitrary guess (like $p_0^s = 0.1$) and then solve for p_1^s, p_2^s, through p_8^s. To ensure normalization, at the end of the procedure all the p_m^s values should be divided by the sum $Z = \sum_{m=0}^{8} p_m^s$. Use the following set of parameters:

$$K_I = 20\,\mu\text{M}, \quad K_A = 500\,\mu\text{M}, \quad \gamma = 0.1\,\text{s}^{-1}, \quad \rho = 0.2\,\text{s}^{-1}, \quad \mu = -1\,k_BT, \quad \sigma = 0.5\,k_BT$$

Plot the distributions p_m^s, $m = 1, \ldots, 8$, for the following values of concentration c, which covers the range of ligand environments the bacterium is likely to encounter: $c = 0.1$, 1, 10, 100, and 1000 μM. Note the general trend of the distribution, with its peak shifting to higher m values as c increases. However also note that given the limited set of possible m states, the scenario $p_s^0 = p_s^8 \approx 0$ does not occur. (There is always a non-negligible probability that either all the receptor sites are methylated, or all are empty.) Use Eq. (8) to calculate $\bar{a}^s(c)$, and plot it versus $\log_{10} c$ for the values of c listed above. How much larger is $\bar{a}^s(0.1\,\mu\text{M})$ compared to $\bar{a}^s(1000\,\mu\text{M})$? Clearly the independent receptor model does not achieve perfect adaptation.

3.2 Interacting receptor model

Let us now consider a model where N receptors form a cluster, and the methylation state of the jth receptor is labeled m_j. The stationary inactive-to-active probability ratio for the jth receptor is $p_{Ij}^s / p_{Aj}^s = e^{-\beta\epsilon_0(m_j)} F(c)$. The key assumption of this interacting model (inspired by the MWC theory discussed above) is that receptors have long-range interactions that perfectly synchronize their activity: all the receptors in the cluster are either simultaneously active (stationary probability p_A^s), or simultaneously inactive (stationary probability p_I^s). The ratio of the cluster probabilities is just the product of the ratios for the individual receptors:

$$\frac{p_I^s}{p_A^s} = \prod_{j=1}^{N} \frac{p_{Ij}^s}{p_{Aj}^s} = e^{-\beta(N\mu+\sigma m)} F^N(c), \tag{9}$$

where $m = \sum_{j=1}^{N} m_j$ is the total methylation state of the cluster. Using the fact that $p_I^s + p_A^s = 1$, the activity function for the entire cluster, $a(m, c) \equiv p_A^s$, is given by:

$$a(m, c) = \left[1 + e^{-\beta(N\mu + \sigma m)} F^N(c)\right]^{-1}. \tag{10}$$

Everything else in the model description from the previous section stays the same, except that m now runs between 0 and $8N$, since that is the maximum total methylation state of the cluster.

d) Redo the numerical calculation of part c) for $N = 4$. Remember that you must change the ν_m factor in the recursion relation to reflect the new definition of $a(m, c)$ in Eq. (10), and carry out the recursion up until $m = 8N$, normalizing the probabilities p_m^s afterwards. Eq. (8) remains valid, but with 8 replaced by $8N$. If everything works correctly, you should see that the system is now much closer to perfect adaptation across the range $c = 0.1$ μM to 1000 μM.

Why does the interacting receptor model work so much better? Numerically, you should have noticed that p_0^s and p_{8N}^s are much closer to zero across the investigated c range, and hence Eq. (8) predicts that $\bar{a}^s(c)$ is approximately a constant independent of c. The synchronized receptor cluster is in some ways like a giant receptor, with a much broader range of possible methylation states. It becomes statistically much less likely that you can completely demethylate every receptor in the cluster (state $m = 0$ occupied) or completely methylate every receptor (state $m = 8N$ occupied). To put this on a more rigorous footing, let us mathematically check if $p_0^s \to 0$ and $p_{8N}^s \to 0$ as $N \to \infty$ for arbitrary parameters. You can assume that $\mu < 0$, $\sigma > 0$, $\mu + 8\sigma > 0$, and that $K_I < K_A$. (Note by definition K_I, K_A are positive, being dissociation constants, and γ and ρ are positive, being rates.)

e) Let us first start with p_0^s. From the recursion relation we know that $p_0^s = p_1^s/\nu_0$. Prove that $\nu_0 \to \infty$ as $N \to \infty$. Since $0 \leq p_1^s \leq 1$, this means that p_0^s must go to zero as $N \to \infty$.

f) Now consider p_{8N}^s. We know that $p_{8N}^s = \nu_{8N-1} p_{8N-1}^s$. Show that $\nu_{8N-1} \to 0$ as $N \to \infty$ only if $c < c^*$, where c^* is a certain threshold concentration. Find c^*. Thus if $c < c^*$, then $p_{8N}^s \to 0$ as $N \to \infty$, since $0 \leq p_{8N-1}^s \leq 1$.

g) The result of part f), together with part e), implies that the interacting receptor model will approach perfect adaptation as $N \to \infty$, but only for concentrations less than c^*. Even perfection has its limits! To see this numerically, find the value of c^*, and using your code from part d) to calculate $\bar{a}^s(c)$ for a concentration $c \gg c^*$. You should find that it is noticeably different than the value at $c \ll c^*$. So while perfect adaptation holds over many orders of magnitude of c, it cannot hold for an arbitrarily large concentration. This is in agreement with our physical intuition: biological sensors cannot adapt to absolutely *all* levels of stimulation. (This one of the reasons it is not wise to stare at the sun.)