Supporting Text

Precise Adaptation in Bacterial Chemotaxis Through "Assistance Neighborhoods"

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Comparison Between Barkai-Leibler (BL) and Assistance-Neighborhood (AN) Adaptation Models

Here we highlight the difference between the BL (1) and the AN adaptation models by using mean-field equations for the rate of change of the receptor methylation $[m]$ in the cell. Both models have in common methylation of receptors by enzyme CheR (with rate constant a) and demethylation of receptors by enzyme CheB (with rate constant b). The main difference is that in the BL model, CheR and CheB are assumed to bind and act on single receptors, whereas in the AN model, CheR and CheB are assumed to bind to the flexible tethers at the C termini of the receptors, independent of receptor activity, and to act on all receptors in a surrounding AN. Since in the AN model a bound CheR (CheB) can act on any inactive (active) receptor within the AN, the number of available modification sites is increased compared with the BL model.

The kinetics in the BL model may be described by

$$\left(\frac{d[m]}{dt}\right)_{BL} = a[\mathrm{CheR}]\frac{[T]}{K_R+[T]}\cdot(1-<\delta_{m,8}>) - b[\mathrm{CheB}]\frac{[T]A}{K_B+[T]A}\cdot(1-<\delta_{m,0}>_A) \quad (4)$$

$$\approx a[\mathrm{CheR}] - b[\mathrm{CheB}]\frac{[T]A}{K_B+[T]A}, \quad (5)$$

where [CheR], [CheB], and $[T]$ are the CheR, CheB, and total receptor concentrations, respectively; A is the time-averaged probability for a receptor to be active; and the K's are Michaelis constants. The first term on the right of Eq. **4** describes methylation by CheR, which is assumed to bind to receptors independent of their activity. A bound CheR methylates available modification sites on its associated receptor at a rate a unless the receptor is fully methylated with $m = 8$ methyl groups. The second term on the right of Eq. **4** describes demethylation by CheB, which is assumed to only bind to active receptors.

A bound CheB demethylates available modification sites on its associated receptor at a rate b unless the receptor is fully demethylated with $m = 0$ methyl groups. Eq. **5** applies under the assumptions, as in the original BL model (1), that CheR works at saturation and that CheR and CheB act at rates independent of the receptor methylation level, neglecting the (rare) instances that CheR (CheB) encounters a fully methylated (fully demethylated) receptor: Within this approximation, the steady-state solution of Eq. **5** corresponds to precise adaptation,

$$A = \frac{K_B}{[T]} \frac{a[\mathrm{CheR}]}{b[\mathrm{CheB}] - a[\mathrm{CheR}]}. \tag{6}$$

In Eq. **4**, the factor of $(1 - <\delta_{m,0}>_A)$ that can lead to imprecise adaptation at low methylation levels can be eliminated by assuming that a fully demethylated receptor is always inactive, so that $<\delta_{m,0}>_A = 0$. Similarly, the factor of $(1 - <\delta_{m,8}>)$ that can lead to imprecise adaptation at high methylation levels can be effectively eliminated by further assuming that CheR only binds inactive receptors and that fully methylated receptors are always active (2-4).

The AN model produces precise adaptation without any additional assumptions on receptor activity. The kinetics in the AN model are given by

$$\begin{aligned} \left(\frac{d[m]}{dt}\right)_{AN} = \; & a[\mathrm{CheR}]\frac{[T]}{K_R + [T]}\,(1 - A)\,(1 - <\delta_{m_{AN},48}>) \\ & - b[\mathrm{CheB}]\frac{[T]}{K_B + [T]}\,A\,(1 - <\delta_{m_{AN},0}>) \qquad (7) \\ \approx \; & a[\mathrm{CheR}](1 - A) - b[\mathrm{CheB}]A. \qquad (8) \end{aligned}$$

Eq. **7** describes methylation by CheR and demethylation by CheB, which are assumed to bind receptors independent of their activity. A bound CheR can methylate available modification sites of any inactive receptor within the AN unless all the receptors within the AN are fully methylated, i.e., $m_{AN} = 48$, whereas a bound CheB can demethylate available modification sites of any active receptor within the AN unless all the receptors within the AN are fully demethylated, i.e., $m_{AN} = 0$. The resulting rates of methylation and demethylation are practically independent of the receptor methylation level due to the large number (48) of modification sites within the AN, leading naturally to precise adaptation. Eq. **8** applies within the additional simplifying assumption, made in this work, that both CheR and CheB work at saturation.

Receptor Free-Energy for Multiple Ligands

The receptor free energy in Eq. **2** can be extended to allow for multiple types of ligand. For example, when a receptor is in equilibrium with both an attractant (A) and a repellent (R) (see Fig. 2*b*), the free-energy difference between the on and off states for competitive and exclusive binding is

$$f_{r(m)} = \epsilon_{r(m)} + \log\left(\frac{1 + [L_A]/K^{\rm off}_{A,r} + [L_R]/K^{\rm off}_{R,r}}{1 + [L_A]/K^{\rm on}_{A,r} + [L_R]/K^{\rm on}_{R,r}}\right), \tag{9}$$

where r is the receptor type $r = a$ for Tar and $r = s$ for Tsr, $\epsilon_{r(m)}$ is the methylation-dependent offset energy, and $K^{\rm on/off}_{A/R}$ are the ligand dissociation constants, with $K^{\rm off} < K^{\rm on}$ for attractant and $K^{\rm off} > K^{\rm on}$ for repellent.

Adaptation Error

Precision of Adaptation With and Without ANs.

As shown in Fig. 2, adaptation is precise with AN, whereas adaptation is imprecise without AN at physiological ligand concentrations. Imprecise adaptation in the presence of repellent results from CheB encountering fully demethylated receptors, which, of course, cannot be further demethylated. The result is a lower than ideal demethylation rate, leading to an activity that is too high. Imprecise adaptation at large attractant concentrations results from CheR encountering fully methylated receptors, which cannot be further methylated, leading to a lower than ideal methylation rate and an activity that is too low. Fig. 8*a* shows the fraction of abortive methylation attempts, i.e., (unsuccessful) attempts to methylate a fully methylated receptor versus total number of methylation attempts. Conditions correspond to Fig. 2 after steady state was reached following addition of attractant. Without AN, these unsuccessful methylation attempts are quite frequent because receptors are relatively highly methylated, and the result is visibly imprecise adaptation (Fig. 2*b* *Inset*). Analogously, Fig. 8*b* shows the fraction of abortive demethylation attempts at zero ambient ligand concentration and at the repellent concentration from Fig. 2, after reaching steady state. The fraction of these abortive demethylation attempts is significant since receptors are mostly demethylated, leading to imprecise adaptation. In the presence of repellent, some abortive

demethylation attempts occur even with AN.

To further illustrate the difference between adaptation with and without AN, Fig. 9 shows time-averaged histograms of methylation levels at zero ambient, in the presence of attractant, and in the presence of repellent at ligand concentrations from Fig. 2 after reaching steady state. In Fig. 9*a*, the broad distribution of methylation levels arises because the methylation level of an individual receptor has little effect on the cluster activity and, hence, on the methylation and demethylation rates. In contrast, in Fig. 9*b*, the distribution of methylation levels is sharper with AN, because within an AN, CheR is more likely to methylate the receptor with the least number of methyl groups and CheB is more likely to demethylate the receptor with the largest number of methyl groups.

Dependence of Adaptation Precision on Cluster Sizes and ANs. Fig. 10 shows that clusters of various sizes without AN have much larger adaptation errors than a mixed cluster of 18 receptors with AN. (The activity of a single receptor does not vanish completely at large ligand concentrations because the free-energy difference between on and off states of a single receptor is comparable with the thermal energy kT.) The adaptation error of a mixed cluster without AN becomes worse the larger the size of the cluster because individual methylation and demethylation events have increasingly less effect on the cluster activity, and the distribution of the average methylation level becomes increasingly broader. Hence, the methylating enzyme CheR more frequently encounters fully methylated receptors, and the demethylating enzyme CheB more frequently encounters fully demethylated receptors leading to imprecise adaptation.

Adaptation to Serine and Aspartate

As described in *Results* and *Discussion*, the two limits of adaptation can be achieved by varying the fraction of ligand-binding versus non-ligand-binding receptors in a cluster. The higher the fraction of ligand-binding receptors, the lower the free energy of the off state of the cluster at high attractant concentration. If the free energy of the fully methylated on state is not sufficiently low to compensate, adaptation stops as the cluster activity is

reduced to zero at high ligand concentration. In contrast, if the free energy of the fully methylated on state is low enough, then the on state binds ligand before the receptors reach full methylation; consequently, response to attractant stops when the receptors become fully saturated. These two limits of adaptation can be observed for a single mixed cluster responding to different ligands. Fig. 11 shows adaptation of a mixed-receptor cluster with receptor ratio Tsr:Tar = 2:1 to step increases of aspartate and serine. There are twice as many Tsrs available to bind serine than there are Tars available to bind aspartate. This leads to lack of adaptation at high concentrations of serine because full methylation is reached, but, in contrast, to lack of response at high concentrations of aspartate. Our model predictions are in line with observations by Berg and Brown (5) who measured the run length between consecutive tumbles of adapted cells to various concentrations of serine or aspartate.The run lengths increased for increasing serine concentration (corresponding to decrease in activity), whereas the run lengths stayed constant for increasing aspartate concentration (corresponding to constant activity).

Activity in Mean-Field Approximation

We introduce a mean-field model for the activity $A = 1/(1 + \exp(F))$ where the free-energy difference for a cluster of n_a Tars and n_s Tsrs is approximated by $F = n_a < f_a > + n_s < f_s >$. Approximating F as a function of the average receptor free-energy differences $< f_a >$ and $< f_s >$ is equivalent to neglecting fluctuations in the receptor methylation levels (See *Model*). Upon a change in ligand concentration, adaptation returns F and A back to the steady-state level F^* and A^* through methylation-induced changes in the average offset energy $<\Delta\epsilon_r> = <E_r^{\rm on}> - <E_r^{\rm off}>$.

Linear Response of Activity. Assuming receptors are adapted to ambient ligand concentration $[L]$ (corresponding to free-energy difference F^* and activity A^*), a change of ligand concentration $\Delta[L]$ leads to a change ΔF in the free-energy difference between the on and off states of the cluster. For small changes ΔF, the initial change of activity ΔA is given in

linear response by

$$\Delta A = A - A^* \approx \left.\frac{dA}{dF}\right|_{F^*} \Delta F, \tag{10}$$

where the first derivative of activity A with respect to F is given by

$$\frac{dA}{dF} = -\frac{e^F}{(1+e^F)^2}. \tag{11}$$

Alternatively, in terms of a change in ligand concentration $\Delta[L]$, we can write

$$\begin{aligned} \Delta A &\approx \left.\frac{dA}{dF}\right|_{F^*} \left.\frac{dF}{d\log([L])}\right|_{[L]} \left.\frac{d\log([L])}{d[L]}\right|_{[L]} \Delta[L] \\ &= \left.\frac{dA}{dF}\right|_{F^*} \left.\frac{dF}{d\log([L])}\right|_{[L]} \frac{\Delta[L]}{[L]}. \end{aligned} \tag{12}$$

For a cluster with n_a Tars and n_s Tsrs, the methylation-independent derivative is given by

$$\frac{dF}{d\log([L])} = n_a \left(\frac{[L]}{K_a^{\rm off}+[L]} - \frac{[L]}{K_a^{\rm on}+[L]}\right) + n_s \left(\frac{[L]}{K_s^{\rm off}+[L]} - \frac{[L]}{K_s^{\rm on}+[L]}\right). \tag{13}$$

This formula is used to generate the curve in Fig. 4*b*.

Analysis of Sensitivity. Following Sourjik and Berg (6), we define the sensitivity as the ratio of the fractional change in activity (output) to the fractional change in ligand concentration (input), i.e.,

$$\text{Sensitivity} \;=\; \frac{\Delta A/A}{\Delta[L]/[L]}. \tag{14}$$

As shown experimentally (6), the sensitivity is nearly independent of the magnitude of $\Delta[L]/[L]$ at least up to 20%. We find the same to be true in our model since $\Delta A \sim \Delta[L]/[L]$ in linear response (Eq. **12**) and $A = A^*$ is constant. For larger $\Delta[L]/[L]$ we find sublinear nonlinearity, as shown in Fig. 3*b* by the difference between the curves with $\Delta[L]/[L] =$ 10% and 50%. The decrease in sensitivity for increasing $\Delta[L]/[L]$ can easily be understood in terms of dA/dF (Eq. **11**), which is maximum for $F = 0$, or, equivalently at $A = 1/2$. For adapted receptors with $A^* \leq 1/2$, dA/dF is largest for small increases in attractant concentration and becomes smaller for larger changes.

Analysis of Response to Fractional Changes in Ligand Concentration. In Fig. 4*a*, we plot the response to fractional changes in ligand concentration $\Delta A/(\Delta[L]/[L])$ instead of the sensitivity $(\Delta A/A)/(\Delta[L]/[L])$ because the former stays well defined even

when the activity A vanishes due to failure of adaptation at large ligand concentrations. To understand the origin of the two peaks in the response of adapting receptors, and the narrow peaks of the nonadapting receptors, found in the simulations, we turn to a simple model for a single mixed-receptor cluster. Using the mean-field approximation, we can analyze the response to fractional changes in ligand concentration analytically using Eq. **3**. The second factor in Eq. **3**, given explicitly in Eq. **13**, is independent of methylation and applies to adapting and non-adapting receptors alike. As shown in Fig. 4*b*, this factor has a characteristic two peak structure. The first peak is due to the Tar affinity for MeAsp, while the second peak is due the Tsr affinity for MeAsp. The first factor in Eq. **3**, dA/dF, given explicitly in Eq. **11**, describes how susceptible the cluster activity is to changes in the cluster free energy. As shown in Fig. 4*c*, this factor is constant for perfectly adapting receptors (setting $F = F^*$), but exhibits narrow regions of susceptibility for nonadapting receptors.

Free-Energy Scaling of Receptor Response. At ligand concentration $[L]$, we assume that the receptor free-energy difference is adapted to the steady-state value F^*. For receptor type r, addition of ligand concentration $\Delta[L]$ causes an initial rise in free-energy difference

$$\Delta f_r^{\rm add} = \log\left(1 + \frac{\Delta[L]}{[L] + K_r^{\rm off}}\right) - \log\left(1 + \frac{\Delta[L]}{[L] + K_r^{\rm on}}\right), \tag{15}$$

and, after adaptation to attractant $[L + \Delta L]$, the subsequent removal of $\Delta[L]$ causes an initial drop of free-energy difference

$$\Delta f_r^{\rm rem} = \log\left(1 - \frac{\Delta[L]}{[L + \Delta L] + K_r^{\rm off}}\right) - \log\left(1 - \frac{\Delta[L]}{[L + \Delta L] + K_r^{\rm on}}\right). \tag{16}$$

In Fig. 3*c*, we scale the response of an adapted mixed cluster of n_a Tars and n_s Tsrs to addition of ligand, $\Delta[L]$, as a function of the total free-energy change

$$\Delta F^{\rm add} = n_a \Delta f_a^{\rm add} + n_s \Delta f_s^{\rm add}, \tag{17}$$

and the response of adapted receptors to subsequent removal of ligand, $-\Delta[L]$, as a function of the total free-energy change

$$\Delta F^{\rm rem} = n_a \Delta f_a^{\rm rem} + n_s \Delta f_s^{\rm rem}. \tag{18}$$

For the continuous curve in Fig. 3*c*, we used the activity function (Eq. **3**) written as

$$A(\Delta F) = \frac{1}{1 + e^{F^* + \Delta F}}, \tag{19}$$

where $F^* = \ln(2)$ is the steady-state free-energy difference for our assumed steady-state activity of $A^* = 1/3$, and $|\Delta F|$ is the abscissa of plotting.

For small changes ΔF, we can calculate $A(\Delta F)$ in linear response using Eqs. **10** and **11**. The slopes of the continuous curves in Fig. 3*c Inset* are consequently given by $\pm dA/dF = \mp 2/9 \approx \mp 0.22$.

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