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THE EFFECT OF DYNAMIC RECEPTOR CLUSTERING ON THE SENSITIVITY OF BIOCHEMICAL SIGNALING

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Lateral clustering has emerged as a general mechanism used by many cellular receptors to control their responses to critical changes in the external environment. Here we derive a general mathematical framework to characterize the effect of receptor clustering on the sensitivity and dynamic range of biochemical signaling. In particular, we apply the theory to the bacterial chemosensory system and show that it can integrate a large body of experimental observations and provide a unified explanation to many aspects of chemotaxis. The principles of dynamic receptor clustering and signal amplification incorporated into this theory may underlie the design of many cellular networks.

1 Introduction

The swimming behavior of the bacterium *Escherichia coli* is determined by the rotation of its flagella.[1] When the flagella rotate in a counterclockwise (CCW) direction, the bacterial cell swims straight ahead; when the flagella rotate in a clockwise direction (CW), the cell tumbles. An *E. coli* cell is equipped with a family of transmembrane receptors [2] that can sense a variety of chemical stimuli. These proteins are part of a signal transducing system [3] that the cell uses to compare the current level of a specific ligand with the concentration experienced in the recent past and to adjust swimming behavior appropriately.[4]

Signal transduction in bacterial chemotaxis involves two highly integrated processes, excitation and adaptation (for a recent review see Ref. 5). The excitation process is initiated by a change in the state of ligand occupancy of chemoreceptor. Such a change generates a signal that is transmitted to the cytoplasmic portion of the receptor and modulates the autophosphorylation and phosphoryltransfer activity of the receptor-bound histidine kinase CheA. CheA activation increases the cellular content of the phosphorylated response regulator CheY (phospho-CheY) which interacts with switch proteins in the flagellar motors to induce CW rotation (CCW being the default state in the absence of phospho-CheY). The binding of attractant or repellent to the chemoreceptor downregulates or upregulates the activity of CheA to increase the relative duration of runs or tumbles, respectively.

Following a transient alteration in tumbling frequency, a bacterial cell re-

gains sensitivity to further changes in ligand concentration through an adaptation process. The cytoplasmic domain of each chemoreceptor possesses multiple glutamate residues that are subject to reversible methylation. Methylation increases the kinase activation signal of the receptor. Adaptation is a result of the kinetic competition between the activities of methyltransferase CheR and methylesterase CheB; the latter is active in its phosphorylated form and is also a substrate for phosphoryltranfer from CheA. By regulating the activity of CheA, and in turn CheB, the chemoreceptors are able to control their own methylation level, and thereby attenuates the initial response to a stimulus and returns the motor rotational bias to prestimulus level.

The bacterial chemosensory system has become one of the leading paradigms for receptor-regulated phosphorylation pathways.[5] Detailed molecular descriptions of essentially all pathway components have now been obtained, and many of the enzymatic reactions involved have been analyzed.[6] Despite this progress, the molecular mechanism controlling a key aspect of bacterial chemotaxis remains unknown. The chemosensory system is exquisite in its sensitivity: in the case of taxis towards aspartate, a change in receptor occupancy of as little as $0.2 - 0.3\%$ can trigger a detectable motor response.[4,7] Moreover, an *E. coli* cell remains sensitive at attractant concentrations two orders of magnitude higher than the dissociation constant.[8] This combination of high sensitivity and extraordinary dynamic range calls for a reexamination of the assumption that chemoreceptors function as isolated dimers,[9] since the response control at the flagellar motor alone exhibits only limited cooperativity.[10,11] Increasing evidence suggests that communications between receptor dimers might play an important role in the proper functioning of the signaling network. In fact, a large number of chemoreceptors in *E. coli* are co-localized with kinase CheA and adapter protein CheW in complexes or patches at the poles of the cell.[12] It has been shown that CheR bound to one receptor dimer catalyzes methylation of another receptor dimer.[13,14] The reports that signaling can occur through receptor dimers that have been genetically engineered so that one monomer lacks a signaling domain[15,16] are also suggestive of receptor interactions extending beyond the dimeric state. Recently, the oligomerization of the cytoplasmic domains of chemoreceptors has been characterized by *in vitro* experiments. Oligomerized complexes were found to be better folded[17] and more effective in stimulating CheA activity than the homodimers.[18,19,20] Based on these findings, it has been proposed that a signal generated at a single receptor dimer may perturb the lateral packing within an array of closely positioned receptor signaling domains,[20] thereby inducing an amplified response.

Here we show that activity spread within receptor clusters could quantitatively account for the observed sensitivity and dynamic range of the chemotac-

tic response, assuming 1) that attractant-bound receptors can inactivate other receptors of the same cluster and 2) that the extent of receptor clustering depends on the concentration of ligand. This approach is similar to one taken by Bray et al.[21] who make the first assumption and also discuss a possible effect of ligand concentration on receptor clustering. However, the present study considers explicitly the number of clusters and the distribution of receptors within them, and derives mathematical expressions that describe the chemotactic response over the entire range of ligand concentration (whereas the early study[21] only considers isolated, extreme conditions). Furthermore, in Bray et al.[21] and other previous computer models of the chemosensory system,[22,23] a bacterial cell was considered to express a single receptor type, although interactions between receptor homodimers of different ligand specificities clearly have physiological significance.[24] In our model, receptors of mixed specificity are randomly clustered on the cell surface; we show that this treatment is not only more realistic, but also leads to better agreement with experimental observations.

2 Theory

Minimum detectable activity change $\Delta A_{\min}$. The fraction of time the flagellum spends in a CCW motor rotation (or smooth swimming) mode was defined as the rotational bias (R_{bias}).[1] We adopt the following relationship between the concentration of response regulator phospho-CheY (Y_{p}) and R_{bias}:[21]

$$R_{\text{bias}} = \frac{1}{1 + (Y_{\text{p}}/3.44)^{5.5}} \tag{1}$$

where Y_{p} is determined jointly by the activity of the phosphoryltransferase CheA (A) and the concentration of phosphatase CheZ (Z) which accelerates the decay of phospho-CheY.[5] If Z is treated as a constant, the steady-state activity of the signaling network, A, can be derived as

$$A = N\gamma \frac{Y_{\text{p}}}{Y_{\text{T}} - Y_{\text{p}}} \tag{2}$$

where Y_{T} is the total concentration of CheY ($\sim$20 μM in *E. coli*[21,22]) and N is the total number of chemoreceptors in a cell. We choose the constant γ in equation (2) so that receptors in an unstimulated cell have an activity of $A_0 = N\hat{a}_0$ where $\hat{a}_0 = 1$ is used as the activity unit. The unstimulated wild-type bacteria used in the experiments published recently[7] had a CCW rotation bias of $\sim$0.65, corresponding to $Y_{\text{p}} = 3.07$ μM (equation (1)) and $\gamma = 5.5\ \hat{a}_0$.

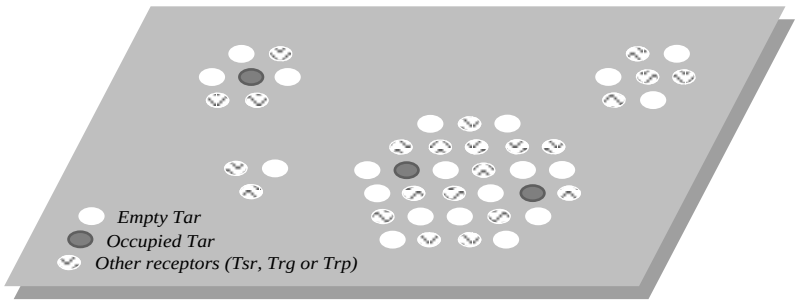

Figure 1: The receptor clustering model.

The minimum motor rotational bias change that could be detected was ∼0.05, corresponding to an activity change of $\Delta A_{\min} = 4.77 \times 10^{-2} N \hat{a}_0$.

Dynamic receptor clustering. Five chemoreceptors have been found in *E. coli*: Tar, Tsr, Tsg, Tap, and Aer, which mediate taxis toward aspartate and maltose, serine, ribose and galactose, dipeptides, and oxygen and redox potential, respectively. Here we only consider the first four types of receptors whose characteristics are well known. The folding unit of chemoreceptors is a homodimer.[9] An E. coli cell has about 600 Tar dimers and 1200 Tsr dimers.[2] Since high-abundance receptors Tar and Tsr are present in cellular amounts approximately 10-fold greater than the low-abundance receptors Tsg and Tap,[24] we assigned 100 dimers each to Tsg and Tap, and thereby placed the total number of chemoreceptor dimers N at 2000.

In our model, the N chemoreceptors are randomly mixed and form a total of B clusters. The sizes of the clusters are not uniform (Figure 1), since both free dimers and receptor oligomers with various numbers of subunits have been identified.[12,19,20,25] At the concentrations found in cells, we expect that a large fraction of the clusters contain a single dimer or a few associated dimers,[9,25] whereas a small percentage of the clusters are large complexes.[12,20] This gives rise to an exponentially decaying distribution of clusters on size (also called a geometric distribution).

Our primary objective is to model the chemotactic response to attractant aspartate, which is mediated by chemoreceptor Tar. When exposed to aspartate at a concentration C, the average number of Tar dimers bound with ligand is $\Omega = N_{\rm Tar} C/(K_{\rm d} + C)$, where $N_{\rm Tar} = 600$ is the total number of Tar dimers and $K_{\rm d}$ is the dissociation constant. At equilibrium, these Ω dimers can be considered as a random sample from the $N_{\rm Tar}$ Tar dimers, and the number of

clusters they are members of is[a]

$$B_\Omega = \frac{BN\Omega}{N\Omega + B(N-\Omega)} \tag{3}$$

(in particular, we denoted B_Ω at $\Omega = N_{\rm Tar}$ as $B_{\rm Tar}$). In general, these clusters also contain unligated Tar receptors as well as non-Tar receptors. One can show that the total number of chemoreceptors in the B_Ω clusters is

$$R(\Omega) = N - \left(\frac{NB}{NW(\Omega) + B(N - W(\Omega))}\right)^2 (N - W(\Omega)) \tag{4}$$

where $W(\Omega)$ is the number of Tar receptors included in $R(\Omega)$:

$$W(\Omega) = N_{\rm Tar} - \left(\frac{N_{\rm Tar}B_{\rm Tar}}{N_{\rm Tar}\Omega + B_{\rm Tar}(N_{\rm Tar} - \Omega)}\right)^2 (N_{\rm Tar} - \Omega). \tag{5}$$

The only free parameter in the above expressions is B which describes the extent of receptor clustering on cell surface. Because the loss of kinase stimulation function is correlated with the dissociation of receptor complex,[20] attractant binding is expected to promote the rate of complex dissociation. Here, we model the process by a simple function

$$B = B_0 + \lambda \left(\Omega/N_{\rm Tar}\right)^\tau N_{\rm Tar} \tag{6}$$

where the extent of receptor clustering is inversely correlated with the level of receptor occupancy. We assign the number of receptor clusters in an unstimulated cell $B_0 = 300$ based on a recent estimate that oligomers formed by soluble Tar cytoplasmic domains contain on average 14 Tar monomer (or 7 Tar dimers).[20] We assume that in a fully ligated state, the chemoreceptors form $B_0 + N_{\rm Tar}$ clusters, i.e. $\lambda = 1$. The remaining parameter τ specifies how many clusters would be added for every newly engaged receptor dimer at a given ligand occupancy state. As we show later, the precise value of τ has a significant impact on the dynamics of receptor clustering, and in turn, the overall behavior of chemotactic response. A choice can be made in accordance with the experimental observation.

Exact adaptation. For simplicity, here we assume all receptors within the same cluster are turned on or off together. The occupation of Ω receptors by attractant molecules should inactivate all $R(\Omega)$ receptors falling in the same clusters

[a] The mathematical framework used to derive equations (3-5) were developed previously[26] in the context of an unrelated problem which yet shared the same underlying distribution.

(equation (4)). The activity of the entire chemoreceptor array is dependent on both $R(\Omega)$ and the methylation state of the receptors (represented by M):

$$A = \alpha_M(N - R(\Omega)) + \beta_M R(\Omega) \tag{7}$$

where α_M and β_M represent the average activities of active and inactive receptors, respectively.

The adaptation in chemotaxis has been shown to be remarkably robust,[27] although the underlying mechanism remains to be determined. In an exact adaptation,[23,27] the effect of methylation should completely balance the signals generated by the bound attractant. For a constant stimulus Ω (or C), the activity of the receptor array should maintain its resting value A_0:

$$\alpha_M(N - R(\Omega)) + \beta_M R(\Omega) = A_0. \tag{8}$$

Here we assume that the methyltransferase and methylesterase enzymes act globally on all the chemotaxis receptors in the cell[28] and thus all receptors are methylated at the same level M. Because the activity of ligand-bound receptors is about 20 fold less than the unoccupied receptors,[29] for simplicity we assign β_M=0. Therefore, the adaptation to attractant is primarily mediated by increasing the activity of those receptors that are not affected by ligand binding (α_M).

Excitation and gain. A transient rise in aspartate concentration (ΔC) from a constant background shifts more Tar receptors ($\Delta\Omega$) to the occupied state. The signal is spread to turn off more chemoreceptors ($\Delta R(\Omega)$) and cause a net reduction in the activity of CheA:

$$\Delta A = (\alpha_M - \beta_M)\Delta R(\Omega). \tag{9}$$

Because receptor-sensing and activity spread are rapid relative to the adaptation reaction and the stimulus-induced receptor reorganization process, we assume that the methylation level M (and therefore α_M and β_M) and the number of the receptor clusters B remain the same during the early phase of the excitation response. We define the gain of the signaling network as

$$G = \Delta A/\Delta\Omega. \tag{10}$$

Based on equation (9), G is a function of both Ω and $\Delta\Omega$. If $\Delta\Omega$ is taken as the minimum occupancy change $\Delta\Omega_{\min}$ for a detectable activity reduction $\Delta A_{\min}$, the gain $G^* = \Delta A_{\min}/\Delta\Omega_{\min}$ depends solely on Ω. Using the following relationship between ligand concentration and receptor occupancy

$$\Delta C_{\min} = \left(\frac{\Delta\Omega_{\min}}{N_{\mathrm{Tar}} - \Delta\Omega_{\min}}\right) K_{\mathrm{d}}, \tag{11}$$

we can also deduce the minimum detectable concentration change $\Delta C_{\min}$ for any given background concentration C. The sensitivity of the excitation response is defined as

$$S = C/\Delta C_{\min}. \tag{12}$$

Threshold concentration and saturation concentration. The dynamic range of a receptor signaling network is defined by two values, the threshold concentration ($C_{\min}$) and the saturation concentration ($C_{\max}$).[8] $C_{\min}$ describes the concentration of attractant that gives a just experimentally measurable change in R_{bias} (or equivalently, an activity change of $\Delta A_{\min}$). The minimum number of Tar dimers ($\Omega_{\min}$) that have to be ligated to produce $\Delta A_{\min}$ can be estimated from

$$G(0, \Omega_{\min})\Omega_{\min} = \Delta A_{\min}, \tag{13}$$

and $C_{\min}$ can be obtained from $C_{\min} = K_{\text{d}}\Omega_{\min}/(N_{\text{Tar}} - \Omega_{\min})$.

$C_{\max}$ represents the highest background concentration at which bacteria can still generate a detectable response to higher concentration stimuli.[8] In analogy with $C_{\min}$, $C_{\max}$ can be estimated from $C_{\max} = K_{\text{d}}\Omega_{\max}/(N_{\text{Tar}} - \Omega_{\max})$, where $\Omega_{\max}$ is the solution to

$$G\left(\Omega_{\max}, N_{\text{Tar}} - \Omega_{\max}\right)\left(N_{\text{Tar}} - \Omega_{\max}\right) = \Delta A_{\min}. \tag{14}$$

3 Results and Discussion

The model presented here provides a direct estimate on the minimum concentration change $\Delta C_{\min}$ required to produce a detectable motor bias change at any initial concentration C. A plot of $\Delta C_{\min}$ vs. C is called a sensitivity curve whose essential features can be described by five values. The concentration at which sensitivity S (equation (12)) reaches maximum ($S_{\max}$), is defined as the maximally sensitive concentration (denoted here as C^*). Earlier studies have shown that the C^* of an attractant approximately equals the apparent dissociation constant (K_{D}) of its chemoreceptors.[8,30,31] The region surrounding C^* with sensitivity deviating less than 20% from the maximum ($S \geq 0.8S_{\max}$) is defined as the maximal sensitivity region (MSR). The lower and upper bounds of MSR, $C^l_{20\%}$ and $C^u_{20\%}$, correspond to the two concentration values on either side of C^* that have $S = 0.8S_{\max}$.

The only adjustable parameter in our model is τ (equation (6)). For τ ranging from 0.1 to 0.7, the values of C^*, $C^l_{20\%}$ and $C^u_{20\%}$ are listed in Table 1 (note that when $\tau \geq 0.8$, α_M peaks before receptors reach saturation). $C^* = 1.08\ \mu$M at $\tau = 0.6$ matches the maximally sensitive concentration observed in experiments;[8,30] it is also comparable with the apparent dissociation constant

Table 1: The maximally sensitive concentration (C^*) and the maximal sensitivity region (MSR, $[C^l_{20\%}, C^u_{20\%}]$) as functions of τ.

τ	0.1	0.2	0.3	0.4	0.5	0.6	0.7
C^* (μM)	5.26	4.30	3.34	2.43	1.64	1.08	0.78
$C^l_{20\%}$ (μM)	1.068	0.742	0.488	0.313	0.208	0.158	0.129
$C^u_{20\%}$ (μM)	18.8	17.7	16.2	14.5	12.5	10.3	8.1

of aspartate binding to Tar, $K_{\rm D} = 1.2$ μM.[32] The fact that the optimal value τ is less than 1 is consistent with the observation that receptors with higher levels of methylation are more stable.[20] The sensitivity curve corresponding to $\tau = 0.6$ is shown in Figure 2. The MSR extends from 0.158 μM to 10.3 μM, over a range of about two orders of magnitude in concentration. Within MSR, $\Delta C_{\rm min}$ can be considered as a linear function of C, a type of behavior specified by the Weber-Fechner law.[33] The MSR predicted here is much broader than previously predicted by using law of mass action ($[0.31K_{\rm D}, 3.2K_{\rm D}]$),[8,31] and agrees better with the observed sensitivity behavior.[8]

The other two values that can be read out from the sensitivity curve include the threshold concentration $C_{\rm min}$ and saturation concentration $C_{\rm max}$. Based on equation (13), $C_{\rm min}$ corresponds to $\Delta C_{\rm min}$ when no aspartate is initially present ($C = 0$). Unlike the three aforementioned values, $C_{\rm min}$ is independent of parameter τ. The lowest concentration to produce a detectable rotational bias change in our model is $C_{\rm min} = 3$ nM, in close agreement with the previously published values.[4,8] Furthermore, the $\Delta C_{\rm min}$ at $C = 60$ nM predicted by our model, 10 nM, is remarkably close to the experimental value (11 nM) reported recently.[7] These results indicate that the extent of known lateral interactions in the receptor array[20] could fully account for the signal amplification observed in experimental systems.

The saturation concentration predicted by our model is 70.8 μM, approaching the reported 100 μM$-$1 mM range.[8] A number of factors may contribute to the discrepancy. Most importantly, in this study we only considered the high-affinity aspartate binding site on a Tar dimer. Based on an analysis of the concentration dependence of chemotactic recovery times, Jasuja *et al.*[7] suggested that the effect of the low-affinity binding site of the Tar dimer, which had an apparent dissociation constant of $\sim$70 μM, became significant for taxis towards aspartate at high concentrations. These low-affinity binding sites could act as aspartate "sinks" to deter the onset of saturation. Furthermore, in addition to lateral control at the receptor level and response control at

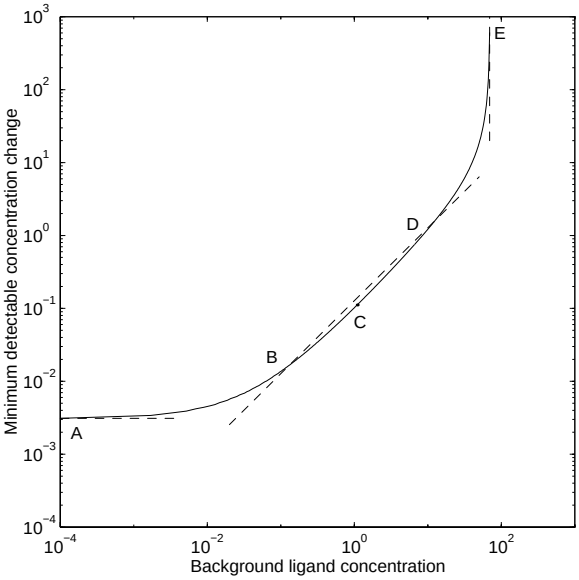

Figure 2: The sensitivity curve for taxis towards aspartate. The background concentration of aspartate (C) is plotted on the abscissa, and the minimum concentration change $\Delta C_{\min}$ required to produce a detectable flagellar rotational bias change is plotted on the ordinate, both in logarithmical scale. Five values that define the characteristics of the sensitivity curve are: A. the threshold concentration $C_{\min}$; B. the lower bound of the maximal sensitivity region $C^l_{20\%}$; C. the maximally sensitive concentration C^*; D. the upper bound of the maximal sensitivity region $C^u_{20\%}$; and E. the saturation concentration $C_{\max}$.

the flagellar motor level, additional control sites might be involved in bacterial chemotaxis,[5] which may have an impact on the near-saturation response of the receptor array.

Figure 3 provides more details about the operation of the chemosensory system at different aspartate concentrations. In an unstimulated cell ($C = 0$), the 600 Tar dimers, none of which is occupied (i.e. $\Omega = 0$), are present in 222 of the 300 clusters. A detectable rotational bias change is obtained when as little as 0.3% of receptors change their occupation states (Figure 3(b)). The activity is spread to 95 chemoreceptors. The signaling pathway has a net gain of 52 (activity unit per receptor binding) at $\Omega = 0$ (Figure 3(c)). As the background ligand concentration (or Ω) increases, the gain of the system decreases monotonically, and as a result, increasingly larger $\Delta\Omega$ is required to induce a detectable motor response. At saturation concentration $C = 70.8$

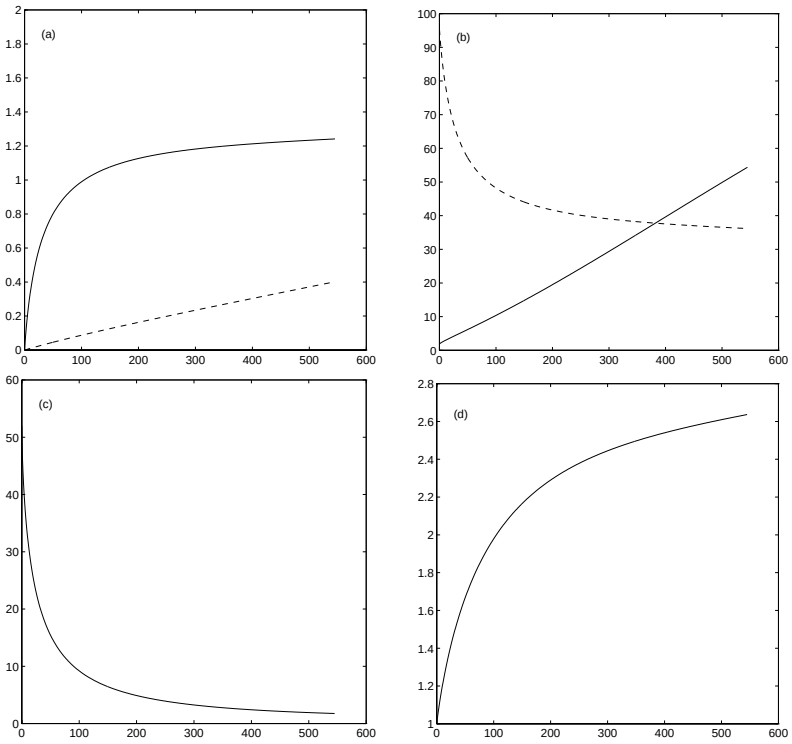

Figure 3: (a) The number of receptors (solid line, $\times 1000$) and the number of receptor clusters (dashed line, $\times 1000$) affected by ligand binding of Ω Tar dimers (abscissa); (b) $\Delta\Omega_{\min}$ (solid line) and $\Delta R(\Omega)$ (dashed line), (c) gain G^*, and (d) α_M as functions of Ω (abscissa).

μM, $\Omega = 546$, or 91% of the Tar dimers are ligated, and they affect a total of 1241 receptors in 402 clusters (Figure 3(a)). The system has a gain of 1.45 (activity unit per receptor binding) (Figure 3(c)). Theoretically, a detectable rotational bias change can still be induced, but to do so, an infinite amount of aspartate has to be administered.

The activity of the receptors unaffected by activity spread, α_M, which is determined by the receptor methylation level M, also shows a dependence on the strength of the stimulus (Figure 3(d)). At the unstimulated state, about one methylation site per receptor monomer is esterified, [34] and we have used the average activity of such receptor dimers to define the activity unit $\hat{a}_0$. In

the fully methylated state, all methylatable sites are esterified; in the case of Tar, the three additional esters raise the activity to about four times the resting level.[29] However, the fully methylated state is unlikely to be reached by adaptation to aspartate stimulus alone, because methylation acts globally on all chemoreceptors including those that do not bind aspartate.[13,28] Based on a recent report,[35] when one type of chemoreceptors has reached saturation, attractant or repellent binding to another set of receptors can still induce a motor response followed by adaptation. Therefore, we expect that the chemoreceptors are only partially methylated when Tar dimers are saturated at $C_{\max}$, and therefore, the maximum value of α_M should be less than 4 $\hat{a}_0$. From Figure 3(d), α_M increases from 1 $\hat{a}_0$ to 2.65 $\hat{a}_0$ as Ω changes from 0 to $\Omega_{\max}$. In comparison, Bray *et al.*[21] assumed that the saturated receptors were 19 times more active than the resting receptors in order to explain the dynamic range using their raindrop model on a cell expressing only a single receptor type.

In this work, we have explored the possible role of lateral interactions within a receptor array in controlling the sensitivity of chemotactic response. The proposed model provides a good quantitative account of several important aspects of bacterial chemotaxis. The mathematical framework developed should be generally applicable to the characterization of many biochemical signaling networks.

Acknowledgments

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