Chemosensing in *Escherichia coli*: Two regimes of two-state receptors

Juan E. Keymer†‡, Robert G. Endres†‡, Monica Skoge*, Yigal Meir§, and Ned S. Wingreen*†

Departments of *Molecular Biology and §Physics, Princeton University, Princeton, NJ 08540-1014; †NEC Laboratories America, Inc., 4 Independence Way, Princeton, NJ 08540; and ‡Department of Physics, Ben Gurion University, Beer Sheva 84105, Israel

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The chemotaxis network in *Escherichia coli* is remarkable for its sensitivity to small relative changes in the concentrations of multiple chemical signals. We present a model for signal integration by mixed clusters of interacting two-state chemoreceptors. Our model results compare favorably to the results obtained by Sourjik and Berg with *in vivo* fluorescence resonance energy transfer. Importantly, we identify two distinct regimes of behavior, depending on the relative energies of the two states of the receptors. In regime I, coupling of receptors leads to high sensitivity, while in regime II, coupling of receptors leads to high cooperativity, i.e., high Hill coefficient. For homogeneous receptors, we predict an observable transition between regime I and regime II with increasing receptor methylation or amidation.

chemotaxis | Monod, Wyman, and Changeux model | receptor clustering

The chemotaxis network in *Escherichia coli* is the best studied signal-transduction network of any living organism. The function of the network is to allow *E. coli* to swim toward attractants, such as amino acids or sugars, and away from repellents. The cells perform chemotaxis by detecting temporal changes in their chemical environment and transducing this information into a decision to swim straight or change direction (tumble). The chemotaxis system is remarkable for its high sensitivity to small relative changes in ambient chemoeffector levels (1). The latter property relies on an adaptation system in which receptors are methylated/demethylated by CheR/CheB at four specific residues (modification sites) (2, 3). Adaptation in chemotaxis is precise, i.e., cells return precisely to the same rate of tumbles if chemoeffector levels stop changing. The adaptation system is also robust in that precise adaptation occurs for a range of levels of chemotaxis proteins (4). Another remarkable property of the system is its ability to integrate signals from different chemical cues, allowing chemotaxis toward any of multiple attractants (5).

In *E. coli*, there are five chemotaxis receptors: two high-abundance receptors, Tar and Tsr, and three low-abundance receptors Tap, Trg, and Aer. These receptors are highly similar in their cytoplasmic signaling domains, with differences primarily in the periplasmic ligand-binding domains. All five chemoreceptors associate as homodimers. In living cells, these homodimers are observed to cluster near one or both poles of the cell (6). *In vitro* crystallographic studies of cytoplasmic domains of the receptors reveal a complex of three homodimers (a “trimer of dimers”) (7). *In vivo* crosslinking studies demonstrate that trimers of dimers can be composed of mixtures of homodimers of different types (8, 9). Clustering of trimers of dimers is mediated by the linker protein CheW and by the kinase CheA (6, 8), both of which are essential for phosphorylation of the response regulator CheY (10). In its phosphorylated form, CheY interacts with the flagellar motors to induce tumbling (11).

Recently, Sourjik and Berg (12–14) introduced a new tool to study signaling in chemotaxis: *in vivo* fluorescence resonance energy transfer (FRET). They constructed fluorescent protein fusions to CheY and to its phosphatase CheZ, thereby creating a FRET pair that they used to monitor the stimulus-dependent activity of the receptor–kinase complex (Fig. 1a). Receptors were engineered with specific patterns of glutamates (E) and glutamines (Q) at the modification sites. Higher numbers of glutamines favor increased CheA kinase activity. In the absence of the adaptation system (cheReB strains), the glutamates and glutamines are not modified. In the presence of the adaptation system, glutamates are methylated and demethylated by CheR and CheB, respectively, and glutamines are also demethylated to glutamates by CheB. Adaptation compensates for the effects of ligand binding on CheA kinase activity; for example, a net increase in methylation (CheA kinase enhancement) follows addition of attractant (CheA kinase inhibition). Sourjik and Berg observed that the inhibition constant $K_i$ of the response to the attractant $\omega$-methylaspartate (MeAsp) varied over almost five orders of magnitude depending on the modification states of the Tar and Tsr receptors (12). Moreover, in strains expressing both Tar and Tsr receptors, the higher the fraction of a given receptor, the lower was the $K_i$ and the higher the cooperativity of the response to its ligand (see Fig. 3c). Cells expressing only Tsr receptors showed an extremely cooperative (i.e., steep) response to serine, with a Hill coefficient of $\approx 10$.

The signaling properties of the chemotaxis network have been the subject of numerous modeling studies. Notably, Barkai and Leibler (15) were able to account for the adaptation properties of the network by using a two-activity-state model for receptor complexes (16). In one state, a receptor is both active as a kinase and susceptible to demethylation by CheB; in the other state, the receptor is inactive and not susceptible to demethylation. This direct coupling between kinase activity and rate of demethylation provides a mechanism for integral feedback (17) and leads to precise and robust adaptation. However, this elegant model for adaptation does not directly account for the sensitivity or signal-integration properties of the network. To account for enhanced sensitivity, several studies have invoked interactions among receptors (18–24). In particular, Bray et al. (18) proposed a model of “conformational spread” among receptors. Along these lines, and in light of the *in vivo* FRET results, Shimizu et al. (22) reported an Ising-type lattice model for receptors, and Sourjik and Berg (14) studied the related allosteric model of Monod, Wyman, and Changeux (MWC) (25). However, these studies were limited to receptors of a single type. To study a mixed array of receptors, Mello and Tu (21) used a mean-field version of an Ising-type model, later generalized by Mello et al. (24) to include stochastic simulations. They achieved excellent agreement with the FRET data but at the cost of a very large number of parameters. Moreover, different parameter sets had

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Abbreviations: FRET, fluorescence resonance energy transfer; MeAsp, $\omega$-methylaspartate; MWC, Monod, Wyman, and Changeux.

†J.E.K. and R.G.E. contributed equally to this work.

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to be used for wild-type and nonadapting cells (21). The model of Albert et al. (23) also produced excellent agreement with the FRET data but relied on dynamic receptor-complex formation, which is not supported by experiment (26).

**FRET Studies Suggest Two Regimes of Receptor Response**

In Fig. 1a, we reproduce kinase-activity dose–response curves to steps of MeAsp measured by using in vivo FRET by Sourjik and Berg (12). The two curves at the lower left have approximately the same inhibition constant $K_i \approx 3$ μM for half-maximal activity. However, what is not seen in Fig. 1a because the response curves are normalized is that the initial activity in the absence of attractant is $\approx 16$ times higher for wild-type cells than for cheR mutant cells. In cheR mutant cells, the receptors are presumably mostly demethylated. The remaining curves, for engineered cheRcheB mutant cells, show two distinct declines in kinase activity. For the first decline, the value of $K_i$ increases (and the amplitude decreases) with increasing glutamine content of the Tar receptors, whereas for the second decline, the value of $K_i$ remains approximately constant. Also, for these four cheRcheB curves, the initial activity, in the absence of attractant, is higher than for wild-type, and changes by a factor of $<1.5$ among the four.

Overall, the six dose–response curves suggest two regimes of receptor response. Encompassed in the first regime are the wild-type and cheR cells, which have low initial activity and a single low $K_i$. The second regime includes the four cheRcheB cells, which have high initial activity, a high and variable $K_i$, and a distinct and even higher $K_{ii}$. One should note that, for the cheRcheB cells, the receptors do not undergo methylation/demethylation, so that the Tar receptors remain as engineered (e.g., EEEE and QEEE), whereas the other receptors, mainly Tsr receptors, remain “wild type,” i.e., QEEE.

In what follows, we show that a model of coupled two-state receptors (25) can account for the full range of FRET data (12–14), including receptors of multiple types both with and without a functioning adaptation system. An essential observation is that there are two regimes of behavior of two-state receptors and that both regimes are present in the FRET data. Interestingly, in one regime, receptor coupling leads to enhanced sensitivity to ligand (lower apparent $K_i$), whereas, in the other regime, receptor coupling leads to an increased Hill coefficient. Homogeneous receptors are predicted to display a transition between these two regimes as a function of increasing receptor methylation or glutamine content, which favors the active state of receptors.

**Model**

**Two Regimes of a Single Two-State Receptor.** To explain precise adaptation in chemotaxis, Barkai and Leibler (15) used a two-activity-state model for receptor complexes (16). Presumably, the two activity states correspond to two distinct configurations of each receptor homodimer, one leading to high kinase activity (on) and one leading to low or zero kinase activity (off).

We consider a model receptor with two activity states implying a total of four free-energy states (Fig. 2 Inset): (i) on without ligand bound $E_{on}$(i) on with ligand bound $E_{on}–\log[\text{L}] / K_i^{on}$, (ii) off without ligand bound $E_{off}$, and (iii) off with ligand bound $E_{off}–\log[\text{L}] / K_i^{off}$, where $[\text{L}]$ is the ligand concentration and $K_i^{on}$ and $K_i^{off}$ are the dissociation constants in the on and off states (27) (all energies are in units of the thermal energy $k_B T$). The
terms proportional to log([L]) represent the loss of ligand volume entropy upon binding to the receptor. Within this model, the probability for a receptor to be on at equilibrium is the sum of Boltzmann factors for the two on states, with and without ligand, divided by the sum of the Boltzmann factors for all four states.

\[
P_{on} = \frac{e^{-E_{on} + e^{-[E_{on} - \log([L]/K_d^{on})]}}}{e^{-E_{on} + e^{-[E_{on} - \log([L]/K_d^{on})]}} + e^{-E_{off} + e^{-[E_{off} - \log([L]/K_d^{off})]}}} = \frac{e^{-E_{on}} (1 + \frac{[L]}{K_d^{on}})}{e^{-E_{on}} (1 + \frac{[L]}{K_d^{on}}) + e^{-E_{off}} (1 + \frac{[L]}{K_d^{off}})}. \tag{1}
\]

For attractants, we assume that the binding of ligand favors the off state, i.e., \( K_d^{off} \ll K_d^{on} \).

Eq. 1 predicts two regimes of behavior depending on the relative energies \( E_{on} \) and \( E_{off} \). As shown schematically in Fig. 2, regime I occurs when \( E_{on} > E_{off} \), and regime II occurs when \( E_{on} < E_{off} \) (a crossover occurs when \( E_{on} \approx E_{off} \)). In regime I, in the absence of ligand, the off-state predominates, so most receptors are already off \( (p_{on} \ll 1) \). Adding a ligand causes \( p_{on} \) to decrease further. Specifically, \( p_{on} \) is reduced to approximately half-maximum when the denominator of Eq. 1 doubles, i.e., when \( 1 + \frac{[L]}{K_d^{on}} = 2 \), or, equivalently, when the off state with a ligand becomes copredominant with the off state without a ligand. Thus the \( K_i \) for half-maximal activity in regime I is constant and is set by the dissociation constant in the off state, \( [L] = K_i = K_d^{off} \).

In contrast, in regime II in the absence of a ligand, the on state predominates, so most receptors are on \( (p_{on} \approx 1) \). In this case, to reduce \( p_{on} \) to half-maximum requires that the off state with ligand becomes copredominant with the on state without a ligand. Half-maximum \( p_{on} \) corresponds to setting \( \exp(-E_{on}) [L]/K_d^{on} \) equal to \( \exp(-E_{on}) \) in the denominator of Eq. 1. Compared to regime I, the result is a larger ligand concentration \([L]\) for half-maximal activity, \( K_i \approx K_d^{off} \exp(E_{off} - E_{on}) \), which increases as \( E_{on} \) decreases.

This simple two-activity-state model accounts qualitatively for a number of features of the response curves in Fig. 1a. The very low activity of the cheR mutant is natural if the receptors in this strain are in regime I. Similarly, the approximately constant value of \( K_i \) for the cheR and wild-type cells is expected if both are in regime I. For the engineered cheRcheB cells, the high and nearly constant initial kinase activities correspond to regime II. Moreover, the increase of the \( K_i \) values follows automatically from Fig. 2 (or Eq. 1) if the replacement of glutamates (E) by glutamines (Q) lowers the on-state energy of the Tar receptors (3, 28).

However, the two-state model for a single receptor does not account for many other features of the data, including the high sensitivity to ligand (28), the integration of multiple chemical signals, or the increase of cooperativity with receptor homogeneity (Fig. 3a; ref. 14). To account for these features, we must consider interactions among receptors.

Two Regimes of Coupled Two-State Receptors. We first consider the MWC model (25), in which \( n \) identical two-state receptors are so strongly coupled that all \( n \) receptors are either off or on together. The probability for the cluster of \( n \) receptors to be on at equilibrium is

\[
P_{on} = \frac{e^{-nE_{on}} (1 + \frac{[L]}{K_d^{on}})^n}{e^{-nE_{on}} (1 + \frac{[L]}{K_d^{on}})^n + e^{-nE_{off}} (1 + \frac{[L]}{K_d^{off}})^n}. \tag{2}
\]

If the individual receptors are in regime I \( (E_{on} > E_{off}) \), the \( K_i \) for half-maximal activity is given by the concentration at which \( (1 + \frac{[L]}{K_d^{on}})^n = 2 \), which means \( K_i \approx (\log 2/n)K_d^{off} \). In other words, the apparent \( K_i \) of a cluster of \( n \) receptors is smaller than the dissociation constant \( K_d^{off} \) of a single receptor by a factor \( \approx n \). Therefore, the larger the cluster, the smaller is the apparent \( K_i \). In contrast, if the individual receptors are in regime II \( (E_{on} < E_{off}) \), the \( K_i \) for half-maximal activity is \( K_d^{off} \exp[E_{off} - E_{on}] \), the same as for a single receptor, but now the cooperativity of the transition, i.e., the Hill coefficient, is equal to \( n \) because

\[
P_{on} \approx \frac{1}{1 + \left(\frac{[L]}{K_d^{off} \exp(E_{off} - E_{on})}\right)^n}. \tag{3}
\]

Thus, the coupling of \( n \) identical receptors leads to qualitatively different effects in the two regimes: In regime I, the sensitivity to ligand is increased by a factor of \( n \), with the Hill coefficient remaining equal to 1, whereas in regime II, the sensitivity to ligand is unchanged, but the Hill coefficient (cooperativity) increases to \( n \). (In the next section, we will show how these results are modified if the receptors are not identical.)

Thus, the model for identical receptors helps explain both the observed high sensitivity to ligand (cheR and wild-type cells in Fig. 1a) and the observed high cooperativity for homogeneous receptors (Fig. 2a) as consequences of receptor-receptor coupling in regimes I and II, respectively. The model further indicates how the wild-type strain can achieve simultaneous low...
$K_1$ and high kinase activity. If adaptation tunes the receptors in wild-type cells to the crossover regime, $E_{on} \sim E_{off}$, then $K_1 \sim K_{off}^{eff}$ (high sensitivity) and $p_{on} \sim 1/2$ (high activity), consistent with the wild-type dose–response curve shown in Fig. 1a.

Mixed Clusters of Two-State Receptors. To compare theory to experiment in detail, we must take into account the presence of receptors of different types. We study a variant of the MWC model in which clusters are composed of random mixtures of receptors of two types, Tar and Tsr (details in supporting information, which is published on the PNAS web site). Receptors of each type are characterized by an offset energy $e_i = E_{on} - E_{off}$ and by dissociation constants $K_{on}^{eff}$ and $K_{off}^{eff}$ for MeAsp, where $r = a, s$ for Tar, Tsr receptors ($K_{on}^{eff}$ is taken to be arbitrarily large). In terms of the offset energies, $e_i > 0$ corresponds to regime I, and $e_i < 0$ corresponds to regime II. Methylation of glutamates, or replacement of glutamates by glutamines, affects receptors only by decreasing $e_i$, i.e., favoring the on state. As in the usual MWC model, all receptors in a cluster are assumed to be off or on together.

Results

Response of Mixed Clusters of Two-State Receptors. In Fig. 1b, we show dose–response curves to MeAsp for equally weighted 14, 15, and 16 receptor clusters with an average Tar:Tsr ratio of 1:2, which is nominally the in vivo ratio, for different values of $e_a$ and $e_s$, but with no other changes of parameters. The curves reproduce well a number of features of the experimental data. The “cheR” curve is in regime I and has low initial activity ($0.05$) and high sensitivity ($K_1 \approx 3.5 \mu M$). The wild-type curve is in the crossover regime and achieves both high initial activity (0.5) and high sensitivity ($K_1 \approx 5.4 \mu M$). For the cheR curve, the value of $K_1$ is $\approx 5$ times smaller than $K_{off}^{eff} = 0.02$ mM; this 5-fold increase of sensitivity to MeAsp corresponds to the average number of Tar receptors in the clusters. The remaining “cheRcheB” curves, which have high initial activity ($\approx 1$), are generated for a series of offset energies $e_a$ for the engineered Tar receptors, with a single offset energy $e_s$ for the Tsr(QEQE) receptors. For these cheRcheB curves, the effect of mixed clusters becomes apparent. First, there are two comparable declines in activity, at $K_1$ and $K_{off}$, corresponding to MeAsp saturation of the Tar and Tsr receptors, respectively. Second, the value of $K_1$ is always larger than $K_{off}^{eff}$ and increases with Tar glutamine content (decreasing $e_s$). The large initial activity and large and increasing value of $K_1$ are characteristic of receptors in regime II but occur even for Tar{EEEE} receptors that have offset energies in regime I ($e_a = 1.0$). The explanation is that, in a cluster, the Tar{EEEE} receptors (≈1/3) are likely outnumbered by the Tsr{QEQE} receptors (≈2/3) that are biased to be on ($e_s = -1.5$), resulting in the cluster as a whole being strongly in regime II. Third, the plateaus in activity between $K_1$ and $K_{off}$ reflect a competition between Tar receptors, which are saturated with MeAsp and individually favor being off, and Tsr receptors, which have little MeAsp bound ($K_{off}^{eff} = 100$ mM) and which individually favor being on. The heights of plateaus increase with the number of Tar glutamines because the associated decrease of Tar offset energies $e_a$ translates directly into higher cluster activities.

Neither the data in Fig. 1a nor the mixed-cluster-model results in Fig. 1b show enhanced Hill coefficients, even for the cheRcheB curves. In the theoretical model, a single cluster in regime II has a Hill coefficient determined by the number of receptors that bind a ligand, i.e., for MeAsp, the Hill coefficient is given by the number of Tar receptors. However, clusters of different sizes and different numbers of Tar and Tsr receptors are inhibited at different ligand concentrations. The resulting spread in $K_1$ values results in an ensemble Hill coefficient close to 1. According to this analysis, the Hill coefficient should increase with increasing receptor homogeneity. Such an increase is observed in Fig. 3b, where theoretical dose–response curves are shown for increasingly homogeneous clusters of Tar receptors. Indeed, an identical effect was observed experimentally by Sourjik and Berg (14), who found the Hill coefficient to increase to ≈4 with increasing homogeneity of Tar{QEQE} receptors (Fig. 3a).

One prediction of our model is that for homogeneous receptors, there will be a transition between regime I and regime II behavior with increasing receptor methylation or glutamine content. In Fig. 4, we show theoretical results for homogeneous clusters of Tar receptors. Note in regime I the enhanced sensitivity, $K_i/K_{off}^{eff} = 0.05$ for the Tar{EEEE} curve, and in regime II, the high Hill coefficient is ≈9 for the Tar{QEQQ} curve. The Hill coefficient for the Tar{QEQQ} receptors remains high despite our use of three different cluster sizes (14, 15, and 16) because $K_1$ is the same for all cluster sizes of homogeneous receptors in regime II (see Eq. 2). In Fig. 4, we also show the fraction of receptors with bound MeAsp. In regime I, binding of a ligand to a small fraction of receptors results in a large decline in activity. In contrast, in regime II, ligand binding and loss of activity are exactly correlated, and both are highly cooperative.

Free-Energy Model for Scaling of Wild-Type Response Data. Sourjik and Berg made the striking observation that the dose–response curves for wild-type cells, adapted at different ambient concentrations of MeAsp, could be collapsed onto a single curve (figure 3c in ref. 12). They proposed that the response to the addition of MeAsp might be solely a function of change in receptor occupancy, and they inferred occupancy versus total MeAsp from a particular nonadapting mutant. Our model suggests an alternative interpretation, namely that the response to MeAsp is solely a function of change in receptor free energy. Specifically, in our model, the only effect of adding MeAsp is to lower the free energy of receptor off states relative to on states. Assuming that adaptation always returns this free-energy difference to some fixed value, then the response to the addition of MeAsp should depend solely on the induced change in free-energy difference. In Fig. 5, we show a collapse of Sourjik and Berg’s data by using this free-energy difference as a scaling variable (details in supporting information). The data collapse is roughly as good as the collapse found initially by Sourjik and Berg and, importantly, includes the response for adaptation at zero ambient MeAsp, which their approach could not.

Precision of Adaptation and Assistance Neighborhoods. In Fig. 4 Inset, we show adaptation results for model Tar receptors (details in Table 1, which is published as supporting information on the PNAS web site). Similar to the model of Barkai and Leibler (15), we assume that the demethylation rate is proportional to receptor activity and that the methylation rate is proportional to receptor “inactivity.” An important difference from previous adaptation models is that we assume that CheB and CheR act on groups of receptors (assistance neighborhoods). Our use of assistance neighborhoods follows the recent observation by Li and Hazelbauer (29) that single CheR and CheB proteins have a range of, respectively, seven and five observation by Li and Hazelbauer (29) that single CheR and CheB proteins have a range of, respectively, seven and five
fully demethylated or fully methylated conditions. Indeed, the “assistance neighborhood” model does lead to precise adaptation (Fig. 4 Inset). For homogeneous Tar clusters, the range of adaptation is limited by \( K_a \) because above this concentration, the Tar receptors become saturated and stop responding. However, the range of adaptation increases for mixed clusters because the Tsr receptors with \( K_s \) continue to respond to MeAsp.

Discussion

The chemosensing system of *E. coli* is notable for its exquisite sensitivity, over a wide range of concentrations, to small relative changes in multiple attractants and repellents. Quantification of these properties has been greatly enhanced recently by the *in vivo* FRET studies of Sourjik and Berg (Figs. 1a and 3a; refs. 12–14). These studies suggested to us two regimes of receptor behavior: one regime characterized by low to moderate kinase activity and a low, constant \( K_i \) (high sensitivity) and the other regime characterized by high kinase activity and a high \( K_i \), which increased with receptor glutamine content (nominally equivalent to increased receptor methylation). We showed that similar regimes occur automatically in the model for two-state receptors (16) used by Barkai and Leibler (15) to account for precise

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**Fig. 4.** Transition from regime I to regime II for homogeneous receptors. Response of homogeneous clusters of Tar receptors to steps of MeAsp within the MWC model. Dose–response curves (solid) and receptor-occupancy curves (dashed) are shown for Tar receptor methylation states EEEE, QEEE, QEQE, and QEQQ (left to right), where the Tar-receptor parameters and cluster sizes are those used in Fig. 1b. (Inset Upper) Adaptation of averaged activity is shown of a cluster Tar receptors exposed to two steps of MeAsp from 0 mM up to 1 mM at \( t = 30 \) s and then down to 0.01 mM at \( t = 90 \) s. (Inset Lower) Average methylation level of receptors. Averages are taken over 100 independent clusters of six Tar receptors. Details of the Barkai-Leibler-type adaptation model (15) are given in supporting information.

**Fig. 5.** Free-energy scaling of wild-type response. Response measured by FRET to steps of MeAsp in ref. 12 for wild-type adapted cells. (Left) Data are shown for addition (Lower) and subsequent removal (Upper) of MeAsp (with curves to guide the eye) for cells adapted at various ambient MeAsp concentrations (see Inset, units are in mM). (Right) Response curves are rescaled according to a free-energy model as described in supporting information. The parameters are the same as in Fig. 1b, \( K_a^\text{off} = 0.2 \) mM, \( K_a^\text{on} = 0.5 \) mM, \( K_s^\text{off} = 100 \) mM.
adaption. The first regime occurs when the receptor’s kinase-active state is higher in energy than the inactive state in the absence of ligand ($E_{\text{on}} > E_{\text{off}}$), and the second regime occurs in the opposite case (Fig. 2). Interestingly, the effects of receptor-receptor coupling (20) differ markedly between these two regimes (Fig. 4). In regime I, coupling leads to enhanced sensitivity, whereas in regime II, coupling leads to high cooperativity (i.e., high Hill coefficient).

Most of the in vivo FRET studies used cells expressing multiple types of receptors, and there is strong evidence from crosslinking studies (8) that homodimers of receptors form well mixed arrays. We therefore studied a variant of the MWC model in which clusters are composed of random mixtures of two-state receptors of two types, Tar and Tsr. This mixed-cluster MWC model reproduced the central features of the experimental dose–response curves (Fig. 1), including the variable activity and low, constant $K_i$ in regime I, and the high activity and high, glutamine-dependent $K_i$, variable plateau heights, and constant $K_d$ in regime II. Within our model, the sole effect of receptor modification is to shift the receptor offset energy, $\varepsilon = E_{\text{on}} - E_{\text{off}}$. For example, the series of $cheRcheB$-mutant curves in Fig. 1b depends only on shifts of $\varepsilon_0$ for the Tar receptors. Our model also reproduces the increase of cooperativity (Hill coefficient) with receptor homogeneity (Fig. 3). Importantly, the MWC model predicts that homogeneous receptor clusters will display a transition from regime I to regime II behavior with increasing receptor methylation or glutamine content (Fig. 4). This transition has been observed experimentally in vivo by Li and Weis (30) for engineered Tsr receptors, and in vivo by Sourjik and Berg (unpublished results) in cells expressing engineered Tar receptors without Tsr receptors.

Similar models for coupled two-state receptors have been described in refs. 15, 19–21, 23–25, and Sourjik and Berg (14) used the MWC model to model homogeneous receptors. What is previously undescribed in our approach is that we used the two regimes of receptor activity to explain the FRET data for mixtures of receptors without recourse to a large number of parameters. Specifically, we used fixed $K_i$s for each type of receptor, with methylation affecting only receptor offset energies. In this regard, our work follows the elegant Ising-model study of Shimizu et al. (22). However, their choice of $K_i$ and offset energies precluded consideration of regime II, which is essential to understanding the behavior of $cheRcheB$ mutants (Figs. 1 and 3).

Finally, we generalized our mixed-cluster MWC model to include adaptation within the framework proposed by Barkai and Leibler (15). Namely, methylation and demethylation rates respond to receptor activity to return receptor to a total free-energy difference near zero. This crossover range between regimes I and II, receptor clustering leads to increased sensitivity to ligand while maintaining a high signaling activity of receptors.

Signaling by receptors that modulates their kinase and/or phosphatase activities is ubiquitous in bacteria (31). As in chemotaxis, the responses of these receptors to a ligand will depend on the relative on- and off-state free energies. Moreover, there is no such thing as “the affinity” of a two-state receptor for a particular ligand, because each of the two states has its own ligand-binding affinity, $K_0$ and $K_d$, and the total response involves an interplay of the two. Our estimated ratio $K_0^{\text{off}}/K_0^{\text{on}} = 25$ for McAsp binding to Tar is larger than found by biochemical assays for L-aspartate binding to Tar, including approximate ratios 2 (32), 7 (3), and 10 (33). Although the origin of this variability is not clear, $K_d$ values may depend on the presence and stoichiometry of the receptor-binding proteins CheW and CheA and on physiological conditions (34). Our estimated in vivo values for $K_0^{\text{on}}$ and $K_0^{\text{off}}$ compare favorably with in vivo values obtained from fits to the adaptation-time data of Berg and Tedesco (35) (see supporting information). The best way to measure in vivo $K_d$ values may be to combine systematic activity studies of cells expressing a single receptor type with scaling analysis as in Fig. 5.

Coupling of receptors may also prove to be a general mechanism for enhancing sensitivity to weak signals. Although our results highlight the importance of receptor-receptor coupling in the chemotaxis system and suggest an effective cluster size of $\sim 15$ receptors, little is known about possible domains or structures larger than trimers of dimers formed by chemotaxis receptors.

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Monod-Wyman-Changeux (MWC) Model for a Mixed Cluster of Two-State Receptors.

We study a variant of the MWC model (1) in which clusters may be composed of mixtures of two-state receptors of different types, in particular Tar and Tsr. Clusters may be of any specified size and receptor composition, but, as in the usual MWC model, all receptors in a cluster must be off or on together. As described in Results, a single receptor of type $r = a, s$ for Tar and Tsr, respectively, can be in any of four free-energy states (Fig. 2b Inset): (i) on without ligand-bound $E_r^{\text{on}}$, (ii) on with ligand-bound $E_r^{\text{on}} - \log([L] / K_r^{\text{on}})$, (iii) off without ligand-bound $E_r^{\text{off}}$, and (iv) off with ligand bound $E_r^{\text{off}} - \log([L] / K_r^{\text{off}})$, where $[L]$ is the ligand concentration in the medium and $K_r^{\text{on}}$ and $K_r^{\text{off}}$ are the dissociation constants for a ligand in the on and off states. All energies are in units of the thermal energy $k_B T$. Within this model, the combined free energy of the two on states is $E_r^{\text{on}} - \log(1 + [L] / K_r^{\text{on}})$, and, similarly, the combined free energy of the two off states is $E_r^{\text{off}} - \log(1 + [L] / K_r^{\text{off}})$. The relevant quantity at equilibrium is the difference between these two combined free energies,

$$f_r = [E_r^{\text{on}} - \log(1 + [L] / K_r^{\text{on}})] - [E_r^{\text{off}} - \log(1 + [L] / K_r^{\text{off}})] = \epsilon_r + \log \left( \frac{1 + [L] / K_r^{\text{off}}}{1 + [L] / K_r^{\text{on}}} \right), \quad [4]$$

where we have defined an “offset energy” $\epsilon_r = E_r^{\text{on}} - E_r^{\text{off}}$.

For a cluster of size $n$ with $m$ Tars, at a particular ligand concentration, the activity is given by

$$p_{\text{on}} = \frac{1}{1 + e^{mf_r + (n-m)f_r}} \cdot [5]$$

To model in vivo activity, we calculate an ensemble-average receptor activity $\langle p_{\text{on}} \rangle$, where the average is taken over clusters of different sizes and different receptor
compositions. For a given Tar:Tsr ratio, cluster compositions are assumed to follow the binomial distribution.

For comparison to data, we assume that the offset energy $\varepsilon_r = E_r^{on} - E_r^{off}$ depends only on the glutamine content of the receptor. In general, the addition of glutamines favors the on state; specific values of $\varepsilon_r$ are indicated in the text and figure captions. We consider only the case in which the ligand is $\alpha$-methylaspartate (MeAsp), for which we take the dissociation constants to be $K_a^{off} = 0.02$ mM, $K_a^{on} = 0.5$ mM, $K_s^{off} = 100$ mM, and $K_s^{on} = 10^6$ mM (i.e., within the range of concentrations considered, MeAsp does not bind to on-state Tsr receptors).

**Adaptation Model.** We model adaptation along the lines of Barkai and Leibler, with the one significant difference that “assistance neighborhoods” (2) form our basic units of methylation/demethylation. We assume that each CheR or CheB can act on an assistance neighborhood of 6 receptors, in line with *in vitro* experiments (2). Because each receptor is a homodimer with 8 modification sites for methylation/demethylation, there are a total of 48 modification sites for the 6 receptors in an assistance neighborhood. We assume saturated kinetics of CheR and CheB. Specifically, CheR adds a methyl group to one of the six receptors in its neighborhood at a rate independent of the number of available (unmethylated) sites. Similarly, CheB removes a methyl group at a rate independent of the number of methylated modification sites. Methyl groups are added or removed with equal probability at any available modification site in the assistance neighborhood. (CheR does nothing if all six receptors in its neighborhood are fully methylated, similarly CheB does nothing if all six receptors are fully demethylated).

In practice, we calculate the equilibrium probability for a cluster to be active and assume that CheR (CheB) methylates (demethylates) at a rate proportional to the cluster’s inactivity (activity). Because methylation and demethylation rates are assumed to be slow compared to ligand binding and unbinding, we model methylation/demethylation kinetics explicitly.
In Fig. 4 Inset, we show results of our adaptation model for 100 independent clusters of six Tar receptors. For simplicity, we have chosen MWC clusters with the same size as the assistance neighborhood, although this equal sizes are is not a necessary assumption. As time progresses, discrete methylation/demethylation events occur. After each event, the equilibrium activity $p_{on}$ is recalculated from Eq. 5. For that purpose, we use the individual receptor free energies in Eq. 4, with the offset energy of each receptor determined by its methylation level (Table 1), in accord with the values used in Fig. 1b.

The kinetics of methylation/demethylation is simulated with the stochastic but exact Gillespie algorithm. For that purpose, three random numbers are needed. The first determines whether the assistance neighborhood gets methylated with rate $\gamma_R(1 - p_{on})$ or demethylated with rate $\gamma_B p_{on}$. The second determines which modification site is methylated/demethylated. The third, $r$, is needed to correctly increment the simulation time. It is chosen with uniform probability on the interval $[0, 1]$, and the time is increased according to $\delta t = 1 / [(\gamma_R(1 - p_{on}) + \gamma_B p_{on}) \cdot \ln(1 / r)]$. We used methylation/demethylation rates $\gamma_B = 2\gamma_R$, with $\gamma_R = 3.96/s$ chosen arbitrarily to set a convenient time scale.

**Free-Energy Scaling of Wild-Type Response Data.** Our model suggests that the scaling of wild-type response data observed by Sourjik and Berg (3) can be attributed to changes in receptor free energy. For a receptor of type $r$, the on-state free energy minus the off-state free energy is given by $f_r$, Eq. 4.

We assume that at any steady ligand concentration $[L]_0$, adaptation returns $f_r$ to some fixed value $f_r^*$ via methylation-induced changes in $\varepsilon_r = E_r^{on} - E_r^{off}$. Then, following adaptation, a change in attractant $\delta[L]$ causes a change in relative free energy

$$
\delta f_r = \log \left( 1 + \frac{\delta[L]}{[L]_0 + K_r^{on}} \right) - \log \left( 1 + \frac{\delta[L]}{[L]_0 + K_r^{off}} \right). \ [6]
$$
In Fig. 5, we attempt to scale the wild-type response data as a function of the average $\delta f$ for a mixed cluster of Tar and Tsr receptors, $\langle \delta f \rangle = \sum_{r=a,s} n_r \frac{\delta f_r}{\sum_{r=a,s} n_r}$, where $a, s$ refer to Tar and Tsr receptors, respectively, and $n_r$ gives the relative number of each type of receptor ($n_a = 1, n_s = 2$). We use the same parameters as in Fig. 1b, $K_{a\text{off}} = 0.02$ mM, $K_{a\text{on}} = 0.5$ mM, and $K_{s\text{off}} = 100$ mM.

**Comparison to Adaptation-Time Data of Berg and Tedesco.** Based on measurements of adaptation times, Berg and Tedesco (4) inferred a $K_d$ value of 160 $\mu$M for MeAsp-binding to Tar receptors. The adaptation times were obtained by measuring the rotational behavior of tethered cells after a step increase of MeAsp starting from zero ambient. Berg and Tedesco (4) defined the adaptation time (“transition time”) as the time a cell needed to recover from the fully inactive state (clockwise rotation) induced by saturating amounts of MeAsp to the cell’s first onset of activity (counterclockwise rotation). Their $K_d$ value was obtained by fitting to a model in which the adaptation time is proportional to the change in ligand occupancy of the receptors. Our model makes the specific prediction that the adaptation time is proportional to the free-energy change upon addition of attractant (assuming that the subsequent increase of receptor methylation is linear in time). Because binding occurs mainly to Tar receptors, we predict that the adaptation time will obey $t \propto \delta f_a$, where the free-energy change of the Tar receptors is given by

$$\delta f_a = \log \left(1 + \frac{\delta [L]}{K_{a\text{off}}}ight) - \log \left(1 + \frac{\delta [L]}{K_{a\text{on}}}ight).$$ [7]

In this equation, $\delta [L]$ is the added MeAsp concentration, and $K_{a\text{off}}$ and $K_{a\text{on}}$ are the dissociation constants for MeAsp binding to Tar receptors in the off and on states, respectively. In Fig. 6, we treat these dissociation constants as fitting parameters and find that the resulting theoretical adaptation times compare favorably to the data. Importantly, the dissociation constants obtained from the best fit, $K_{a\text{off}} = 0.027$ mM and $K_{a\text{on}} = 0.958$
mM, compare well to the values obtained from fluorescence resonance energy transfer (FRET) data, $K_a^{\text{off}} = 0.02$ mM and $K_a^{\text{on}} = 0.5$ mM.


Final concentration of MeAsp (M)

Adaptation time (sec)

- Berg and Tedesco (1975)
- Free-energy Model