Supporting Text

Nonuniqueness of the parameters. As described in the main text, parameters for our model cannot be uniquely determined from the existing mutant data (the same is true for the WT model). In other words, there are many parameter sets that can be used in our model to fit the experimental MeAsp response data for all the mutant strains within the experimental error. For example, if the local energy differences for the occupied Tar receptors E_{1m1} are decreased, the effects can be absorbed by an increase of the local energy differences E_{2m0} for the unoccupied Tsr receptors. In Table 3, we list such a new set of parameters that are different from those given in Table 2. The fit of our model with this new set of parameters is shown in Figure 4, where the fits are as good as in Fig. 1, and the differences between the fit and the experimental data are well within the experimental error. Such ambiguity in parameters can be fixed only by having more mutant experiments, in particular where the Tsr receptor is also engineered to be in different methylation states (or in the presence of serine), resulting in different activities. However, some parameters of the model seem to be more tightly constrained by the existing data, e.g., the coupling strength C_{12} is always large because the large influence of Tsr on Tar activity/sensitivity is needed to explain the large difference between the cheR$^-$ and cheRcheB(EEEE) strains; the interaction strength C_{11} between Tar receptors themselves, is found to be small (practically zero in table 3). This may be related to the fact that all response curves are found to have a rather modest Hill coefficient, which could be true if the Tar receptor interacts preferably with Tsr in the presence of the Tsr receptors.

The gain (in response to MeAsp) due to Tsr. Although Tsr does not respond (bind) directly to MeAsp except at extremely high concentrations of MeAsp, Tsr can contribute to the system's gain through its coupling to the Tar receptors. Within our model, we can estimate the contribution of gain from the Tsr receptors as compared to that from the Tar receptors. For our WT model, the total energies and therefore the activities from the Tar and Tsr receptors can be determined from the following coupled equations:

$$\Delta E_{1m\lambda} = E_{1m\lambda} + C_{11}(A_1 - \frac{1}{6}) + C_{12}(A_2 - \frac{1}{3}) \quad (7)$$

$$\Delta E_2 = E_2 + C_{21}(A_1 - \frac{1}{6}) + C_{22}(A_2 - \frac{1}{3}) \quad (8)$$

$$A_2 = \frac{2}{3}(1 + \exp(\Delta E_2))^{-1} \quad (9)$$

$$A_1 = \sum_{m\lambda} f_{1m\lambda} a_{1m\lambda}. \quad (10)$$

Summing the methylation balance equations (Eq. **5**) over m, and assuming that the Tar receptor population in the $m = 4$ state is small, we obtain the total activity due to the Tar receptors:

$$A_1 \approx \frac{1}{3} \times \frac{k_{RB}}{1 + k_{RB}}. \quad (11)$$

The total activity due to Tsr receptors, A_2, can then be obtained by Eqs. **8**, **9** and **11**.

For a small change in ligand concentration $\Delta[L]$: $[L] \rightarrow [L] + \Delta[L]$, the corresponding (fast) change (before methylation takes place) in receptor occupancy is represented by ΔL, and activity changes for the two receptor types are ΔA_1 and ΔA_2, respectively. From Eqs. **8** and **9**, we can relate the activity changes:

$$\Delta A_2 = -\frac{2}{3}\chi \Delta\Delta E_2 \quad (12)$$

$$\Delta\Delta E_2 = C_{21}\Delta A_1 + C_{22}\Delta A_2, \quad (13)$$

where $\Delta\Delta E_2$ is the change in total energy difference for the Tsr receptor caused by the change in ligand concentration, and

$$\chi = \exp(\Delta E_2)/(1 + \exp(\Delta E_2))^2 \quad (14)$$

is the measure of susceptibility of Tsr activity with respect to change in its total energy difference, and ΔE_2 is the Tsr energy difference before the stimulus.

Solving Eqs. **12** and **13**, we obtain the ratio between the changes in activities due to Tsr and Tar, respectively:

$$\frac{\Delta A_2}{\Delta A_1} \equiv g_{21} = -\frac{2\chi C_{21}}{3 + 2\chi C_{22}}. \quad (15)$$

The total gain of the system (as defined by Sourjik and Berg[1]) is then:

$$G = \frac{1}{A}\frac{\Delta A_1 + \Delta A_2}{\Delta L} = \frac{1}{A}\frac{\Delta A_1}{\Delta L}(1 + g_{21}), \quad (16)$$

where it is clear that Tsr's contribution to the total gain is g_{21} times that of the contribution from Tar.

It is also clear from Eq. **15** that g_{21}, i.e., the relative contribution to gain from Tsr is larger when either $|C_{21}|$ or $|C_{22}|$ or χ is large. The dependence on C_{21} makes perfect sense, because Tsr contributes indirectly to the gain through its coupling with Tar. Quantitatively, the gain from Tsr also depends on the coupling between Tsr receptors themselves, and how susceptible Tsr activity is to changes in its interaction energy with Tar, measured by χ.

For the parameters shown in Table 2, the gain from Tar can be easily estimated. Because the self energies dominate the total energy differences in this case, i.e., $|E_{1m\lambda|} \gg 1$ for all methylation levels except $m = 0$, for the Tar receptor, the activity is either 0 or 1 depending on whether the Tar receptor is ligand bound. Therefore, for the case where the Tar receptor population in $m = 0$ is small, which is true for most of ambient MeAsp concentrations, we have:

$$\Delta A_1 \approx \Delta L. \tag{17}$$

For the parameters in Table 2, the steady-state total activity is nearly constant (near perfect adaptation): $A \approx 0.4$ (see Fig. 2a). Therefore, the gain from Tar receptor:

$$G_1 \equiv \frac{1}{A}\frac{\Delta A_1}{\Delta L} \approx 2.5, \tag{18}$$

which is much smaller than the total gain. The main contribution to the total gain comes from Tsr. The large contribution from Tsr, i.e., the large value of $g_{21} \approx 7$, is a result of large coupling constants $C_{21} = -17.9$. More interestingly, a large value of susceptibility χ, which is at its maximum value of $\frac{1}{4}$ (see Eq. **14**). Such maximum susceptibility of the Tsr receptor is maintained through all ambient MeAsp concentrations by having the Tsr total energy difference ΔE_2 fixed at approximately 0, which is possible only because the system adapts (nearly) perfectly. If we take into account all possible methylation states of Tsr, the average susceptibility would be smaller, but not by much if the distribution is centered around the methylation state with the maximal susceptibility. For example, a 50% decrease in susceptibility for half of the Tsr population will still result to only 25% reduction in the average susceptibility. More importantly, this slightly reduced, yet high gain will still be maintained through all ambient MeAsp concentrations by perfect adaptation. Because of this large value of g_{21}, the total gain, estimated as $G = G_1 \times (1 + g_{21}) \approx 20$, is also very large, which is consistent with our numerical result, shown in Fig. 2c. Our estimate of the

gain and values of its different sources G_1 and G_2 is confirmed by direct simulation of our model, as shown in Fig. 4.

Eq. **15** also gives us a sense of the strength of the coupling constants in terms neighboring interactions. Without Tsr-Tsr coupling, the total amount of Tsr activity suppressed by the binding of a MeAsp molecule to a single Tar receptor can be estimated as $-2\chi C_{21}/3 \sim 3$. Considering the maximal change of activity for Tsr is 1/2, this means about six Tsr receptors are directly affected by a single Tar receptor. The rest of the gain come from the secondary Tsr-Tsr interaction, with a total of ≈ 14 Tsr receptors being affected.

It is quite clear from our analysis that for parameters such as those listed in Table 2, the total gain depends strongly on C_{21} and C_{22} and is insensitive to the other coupling constants C_{11} and C_{12}. Indeed, we can find other parameter sets that have different values of C_{11} and C_{12} from that of table 2, and our model still retains the same high gain and fits the experimental data as well as our model with parameters in Table 2 (data not shown). This, however, does not exclude the possibility that there exist other types of parameter sets, where most gain in response to MeAsp comes from other sources, such as interaction between the Tar receptors alone, so far, however, we have not found any alternative types of parameter sets through our fitting program by using different initial starting parameters.

The two main ingredients for persistent high G_L. G_L can be written as a sum over the contributions from different methylation states:

$$G_L = \sum_{m\in[1,4]} f_{1m}([L])G_L^{(m)}([L]), \tag{19}$$

where $G_L^{(m)}([L]) = [L]\frac{\Delta L_m}{\Delta[L]}$, L_m is receptor occupancy for Tar receptor in the methylation state m. In Fig. 5, we show the dependence of $G_L^{(m)}$ for $m \in [1,4]$ and G_L on the ambient ligand concentration $[L]$ with the parameters in Table 2. In Fig. 6, we have plotted the Tar receptor distribution in different methylation states, i.e., $f_{1m}([L])$ for $m \in [0,4]$, together with the total and individual ligand occupancy. As can be seen from Fig. 5, as $[L]$ increases, the dominating $G_L^{(m)}$ shifts toward larger m, with the same trend as the receptor population as shown in Fig. 6. This tracking mechanism optimizes the product in Eq. **19** and therefore keeps G_L at high values. However, the matching (tracking) is not exact, because the positions of the peaks for the population and G_L^m are not at the same $[L]$.

On top of the "tracking" mechanism, another important ingredient in keeping G_L large is the fact that ligand binding is affected by activity. If one assumes simple ligand-binding

kinetics described by just a dissociation constant K_d^m for each methylation level m, then the maximum of $G_L^{(m)}$ will be $\frac{1}{4}$ reached at $[L]_0 = K_d^m$. However, for $G_L^{(m)}$ shown in Fig. 5, they are much bigger than $\frac{1}{4}$, which is caused by the fact that the effective K_d in our model depends on the activity.

---

[1] Sourjik, V & Berg, H. C. (2002) *Proc. Nat. Acad. Sci.* **99**, 123–127.