Supporting Information

Precision sensing by two opposing gradient sensors: How does *Escherichia coli* find its preferred pH level?

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Contents

A Tractable Mean-Field Model for pH Sensing

In this supplementary section, we give details about the analytically tractable model for pH sensing. If we just focus on the system-level behaviors of the signaling pathways, the *E. coli* chemosensory machinery can be described by five dynamic variables [1]: the external stimuli $\mathrm{pH}(t)$ (the input), the average receptor kinase activity $a_q(t)$ (the output), and the average methylation level of type-q receptors $m_q(t)$ (the memory), where $q = 1$ for Tar and $q = 2$ for Tsr. Again, the separation of timescales argument allows us to apply the quasi-equilibrium approximation to the kinase activity and ligand binding. Therefore, a general coarse-grained model for bacterial pH sensing can be written as:

$$a_1 = G_1(\mathrm{pH}, m_1, a_1, a_2), \tag{S1}$$

$$a_2 = G_2(\mathrm{pH}, m_2, a_1, a_2), \tag{S2}$$

$$\frac{dm_1}{dt} = F_1(a_1, a), \tag{S3}$$

$$\frac{dm_2}{dt} = F_2(a_2, a). \tag{S4}$$

In the above, $F_{1,2}$ is a transfer function describing the feedback gain of the network depending on both the local activity $a_{1,2}$ and the global activity a, whereas the function $G_{1,2}$ integrates the pH stimuli, the methylation feedback, and the receptor-receptor coupling. Inspired by the Ising-type model described in the main text, we can assume that the receptor activities of Tar and Tsr take the following forms,

$$\frac{1}{a_1} = 1 + \frac{1 + 10^{K_1^I - \mathrm{pH}}}{1 + 10^{K_1^A - \mathrm{pH}}} \cdot \exp\left[E_{m1} + f_1 C_{11}\left(a_1 - \frac{1}{2}\right) + f_2 C_{12}\left(a_2 - \frac{1}{2}\right)\right], \tag{S5}$$

$$\frac{1}{a_2} = 1 + \frac{1 + 10^{K_2^I - \mathrm{pH}}}{1 + 10^{K_2^A - \mathrm{pH}}} \cdot \exp\left[E_{m2} + f_2 C_{22}\left(a_2 - \frac{1}{2}\right) + f_1 C_{21}\left(a_1 - \frac{1}{2}\right)\right]. \tag{S6}$$

The above expressions resemble Eq. (5) in the main text and can be viewed as a mean-field approximation of the Ising-type model by using an average methylation level for each type of receptors ($E_{m,q}$ is a function of m_q for $q = 1, 2$).

The total receptor-kinase activity is $a = f_1 a_1 + f_2 a_2$. For cells that are pre-adapted to a background pH level (denoted by the variable pH), the total activity changes to $a(\mathrm{pH}, \mathrm{pH}')$ right after the pH level changes to a new level pH' (before adaptation sets in). The response at the background to an

infinitesimal increase of pH is characterized by the sensitivity S defined as:

$$S(\mathrm{pH}) \equiv \lim_{\delta \mathrm{pH} \to 0} \frac{a(\mathrm{pH}, \mathrm{pH} + \delta \mathrm{pH}) - a(\mathrm{pH}, \mathrm{pH})}{\delta \mathrm{pH}} = \lim_{\delta \mathrm{pH} \to 0} \frac{\delta a}{\delta \mathrm{pH}}, \tag{S7}$$

where $a(\mathrm{pH}, \mathrm{pH}) = a_0$ is the pre-stimulus (adapted) activity. For precision sensing, S needs to reverse sign and thus the inversion point pH^* should satisfy:

$$S(\mathrm{pH}^*) = 0, \tag{S8}$$

which is equivalent to

$$\frac{da}{d\mathrm{pH}} = f_1 \frac{da_1}{d\mathrm{pH}} + f_2 \frac{da_2}{d\mathrm{pH}} = 0. \tag{S9}$$

Let $g_q(\mathrm{pH}) \equiv \ln \left[(1 + 10^{K_q^I - \mathrm{pH}}) / (1 + 10^{K_q^A - \mathrm{pH}}) \right]$ for $q = 1, 2$. Taking derivative on both sides of Eq. (S5) and Eq. (S6) yields

$$-\frac{da_1}{a_1} - \frac{da_1}{1 - a_1} - f_1 C_{11} da_1 - f_2 C_{12} da_2 = g_1'(\mathrm{pH}) d\mathrm{pH}, \tag{S10}$$

$$-\frac{da_2}{a_2} - \frac{da_2}{1 - a_2} - f_2 C_{22} da_2 - f_1 C_{21} da_1 = g_2'(\mathrm{pH}) d\mathrm{pH}, \tag{S11}$$

where $g_q'(\mathrm{pH})$ is the first derivative of $g_q(\mathrm{pH})$ for $q = 1, 2$. At the inversion point pH^*, we should have $f_1 da_1 + f_2 da_2 = 0$ so that Eqs. (S10) and (S11) can be rewritten as

$$-\frac{da_1}{a_1} - \frac{da_1}{1 - a_1} - f_1 C_{11} da_1 + f_1 C_{12} da_1 = g_1'(\mathrm{pH}^*) d\mathrm{pH}, \tag{S12}$$

$$-\frac{da_2}{a_2} - \frac{da_2}{1 - a_2} - f_2 C_{22} da_2 + f_2 C_{21} da_2 = g_2'(\mathrm{pH}^*) d\mathrm{pH}, \tag{S13}$$

which are equivalent to the following

$$\frac{da_1}{d\mathrm{pH}} = \frac{g_1'(\mathrm{pH}^*)}{f_1(C_{12} - C_{11}) - [a_1(1 - a_1)]^{-1}}, \tag{S14}$$

$$\frac{da_2}{d\mathrm{pH}} = \frac{g_2'(\mathrm{pH}^*)}{f_2(C_{21} - C_{22}) - [a_2(1 - a_2)]^{-1}}. \tag{S15}$$

Plugging the above equations into the condition Eq. (S9) for the inversion pH gives

$$\frac{f_1 g_1'(\mathrm{pH}^*)}{f_1(C_{12} - C_{11}) - [a_1(1 - a_1)]^{-1}} + \frac{f_2 g_2'(\mathrm{pH}^*)}{f_2(C_{21} - C_{22}) - [a_2(1 - a_2)]^{-1}} = 0. \tag{S16}$$

By redefining $y \equiv 10^{\mathrm{pH}}$, $y_q^A \equiv 10^{K_q^A}$ and $y_q^I \equiv 10^{K_q^I}$ for $q = 1, 2$, we have

$$g_q'(\mathrm{pH}) = \frac{10^{\mathrm{pH}}(10^{K_q^A} - 10^{K_q^I})\ln(10)}{(10^{\mathrm{pH}} + 10^{K_q^A})(10^{\mathrm{pH}} + 10^{K_q^I})} = \frac{y(y_q^A - y_q^I)\ln(10)}{(y + y_q^A)(y + y_q^I)}, \tag{S17}$$

and Eq. (S16) amounts to

$$\frac{f_1}{f_2}\cdot\frac{f_2(C_{21} - C_{22}) - [a_2(1-a_2)]^{-1}}{f_1(C_{12} - C_{11}) - [a_1(1-a_1)]^{-1}} = -\frac{g_2'(\mathrm{pH}^*)}{g_1'(\mathrm{pH}^*)} = \frac{y_2^A - y_2^I}{y_1^I - y_1^A}\cdot\frac{(y^* + y_1^A)(y^* + y_1^I)}{(y^* + y_2^A)(y^* + y_2^I)}. \tag{S18}$$

Note that the above is actually a quadratic equation of y^*. Thus it is easy to solve y^* and get the inversion point $\mathrm{pH}^* = \log_{10}(y^*)$ from Eq. (S18). For simplicity, we assume that $C_{11} = C_{12}$ and $C_{21} = C_{22}$ and suppose that both a_1 and a_2 are perfectly adapted to $a_0 \equiv k_R/(k_R + k_B)$, as can be guaranteed if we assume perfect adaptation for both Tar and Tsr: $dm_q/dt = k_R(1 - a_q) - k_B a_q$. Then, Eq. (S18) is reduced to

$$\frac{f_1}{f_2} = \frac{(y_2^A - y_2^I)(y^* + y_1^A)(y^* + y_1^I)}{(y_1^I - y_1^A)(y^* + y_2^A)(y^* + y_2^I)}. \tag{S19}$$

Let's consider the case that $y_1^A \approx y_2^A$ (i.e., $K_1^A \approx K_2^A$), $y_2^A \gg y_2^I$, $y_1^I \gg y_1^A$, and $y_1^I \gg y^* \gg y_2^I$. In this scenario, Eq. (S19) implies

$$\frac{f_1}{f_2} = \frac{(y_2^A - y_2^I)(y^* + y_1^I)}{(y_1^I - y_1^A)(y^* + y_2^I)} \approx \frac{y_2^A}{y_1^I}\cdot\frac{y_I^1}{y^*} = \frac{y_2^A}{y^*}. \tag{S20}$$

Thus we have $y^* \approx y_2^A f_2/f_1$ and the inversion point is

$$\mathrm{pH}^* \approx K_2^A - \log_{10}(f_1/f_2), \tag{S21}$$

which is a decreasing function of the Tar/Tsr ratio f_1/f_2. Given a change of Tar/Tsr ratio from 0.5 to 1.5, one can estimate that the shift of inversion pH point is roughly: $\log_{10}(1.5) - \log_{10}(0.5) \approx 0.48$, which is close to the experimental observation $8.0 - 7.5 = 0.5$. Thus, despite the simplicity of this model, it can give a simple quantitative prediction about the dependence of the inversion point on the Tar/Tsr ratio.

Of course, one can relax the assumption of $C_{11} = C_{12}$ and $C_{22} = C_{21}$ by allowing that $C_{21} = C_{22} + \Delta C$ and $C_{12} = C_{11} + \Delta C$. Here, we take $\Delta C \geq 0$ which means that the coupling between homogeneous receptors is at least stronger than the coupling between heterogeneous receptors. We define $h \equiv (k_R + k_B)^2/(k_R k_B) = [a_0(1 - a_0)]^{-1}$. Then Eq. (S18) suggests

$$\frac{f_1}{f_2}\cdot\frac{f_2\Delta C - h}{f_1\Delta C - h} \approx \frac{y_2^A}{y^*}, \text{ such that } \mathrm{pH}^* \approx K_2^A + \log_{10}\left(\frac{h - f_1\Delta C}{h - f_2\Delta C}\right) - \log_{10}\left(\frac{f_1}{f_2}\right). \tag{S22}$$

As tested by various numerical examples, the second term is dominated by the last term in Eq. (S22). This suggests that the coupling between different types of receptors does not affect the inversion point significantly. For this reason, we will assume $C_{11} = C_{12}$ and $C_{22} = C_{21}$ for simplicity in the rest of this Supporting Information. Under this condition, Eqs. (S14) and (S15) at the inversion point pH^* will reduce to (for $q = 1, 2$):

$$da_q/d\mathrm{pH} = a_q(a_q - 1)g_q'(\mathrm{pH}^*). \tag{S23}$$

As the second condition required for precision sensing, the inversion point pH^* needs to be "attractive", which is ensured only if

$$S'(\mathrm{pH}^*) \equiv \frac{dS}{d\mathrm{pH}}|_{\mathrm{pH}^*} > 0. \tag{S24}$$

By Eq. (S23), we can calculate that

$$S'(\mathrm{pH}^*) = \frac{d^2a_q}{d\mathrm{pH}^2} = (2a_q - 1)a_q(a_q - 1)[g_q'(\mathrm{pH}^*)]^2 + a_q(a_q - 1)g_q''(\mathrm{pH}^*). \tag{S25}$$

Suppose that both a_1 and a_2 are perfectly adapted to $a_0 \equiv k_R/(k_R + k_B)$. If $a_0 \leq 1/2$ (which is the case for the wild-type *E. coli*), then the first term in Eq. (S25) is obviously nonnegative. Thus, our main interest is the sign of the second term there. For this reason, we just need to examine the particular case that $a_0 = 1/2$ (i.e., $k_R = k_B$) which makes the first term vanish. Direct calculation of $g_q''(\mathrm{pH})$ yields

$$S'(\mathrm{pH}) = \frac{d^2a_q}{d\mathrm{pH}^2} = \frac{\ln(10)^2}{4} \times \sum_{q=1}^{2} f_q y \left[\frac{y_q^A}{(y + y_q^A)^2} - \frac{y_q^I}{(y + y_q^I)^2}\right]. \tag{S26}$$

For the scheme $K_2^I < K_1^A \approx K_2^A < K_1^I$ (such that $y_1^I \gg y_1^A \approx y_2^A \gg y_2^I$ and $y_1^I \gg y \gg y_2^I$) considered in our main text, Eq. (S26) can be simplified:

$$S'(\mathrm{pH}) \approx \frac{\ln^2(10)}{4}\left[\frac{yy_2^A}{(y + y_2^A)^2} - \frac{f_1 y}{y_1^I} - \frac{f_2 y_2^I}{y}\right] \approx \frac{\ln^2(10)}{4} \times \frac{yy_2^A}{(y + y_2^A)^2} > 0. \tag{S27}$$

The positive sign above indicates that the inversion point is indeed an attractive fixed point.

In the main text, we have considered another scheme for pH sensing: $K_2^I \approx K_1^A < K_2^A < K_1^I$ (i.e., $y_2^I \approx y_1^A \ll y_2^A \ll y_1^I$ and $y_2^I \approx y_1^A \ll y \ll y_1^I$). Then Eq. (S19) reduces to:

$$\frac{f_1}{f_2} = \frac{(y_2^A - y_2^I)(y^* + y_1^I)}{(y_1^I - y_1^A)(y^* + y_2^A)} \approx \frac{y_2^A}{y_1^I} \times \frac{y_1^I}{(y^* + y_2^A)} = \frac{y_2^A}{y^* + y_2^A}, \tag{S28}$$

which leads to

$$y^* \approx (f_2/f_1 - 1)y_2^A \quad \text{or} \quad \text{pH}^* \approx K_2^A + \log_{10}(f_2/f_1 - 1). \tag{S29}$$

The inversion point pH^* decreases with the Tar/Tsr ratio f_1/f_2 and exists only if $f_1 < f_2$. The second condition Eq. (S24) is also satisfied in this case:

$$\begin{aligned} S'(\text{pH}) &\approx \frac{\ln^2(10)}{4}\left[\frac{f_2 y y_2^A}{(y+y_2^A)^2} - \frac{f_1 y}{y_1^I} + \frac{(f_1-f_2)y_1^A}{y}\right] \\ &\approx f_2 \times \frac{\ln^2(10)}{4} \times \frac{y y_2^A}{(y+y_2^A)^2} > 0, \end{aligned} \tag{S30}$$

which is roughly proportional to f_2, the fraction of Tsr.

2D Simulation for Bacterial pH Taxis

In this supplementary section, we provide details about the 2D simulation algorithm for bacterial pH taxis. This model is based on the *Signaling Pathway-based E. coli Chemotaxis Simulator* (SPECS) proposed in Ref. [2]. This simulator allows us to study the chemotaxis behaviors in an environment with spatiotemporal complexity. In this 2D model for pH taxis, the state of Tar or Tsr is represented by its average kinase activity $a_q(t)$ and average methylation level $m_q(t)$ at time t for $q = 1, 2$. The external environment is defined by $\text{pH}(x, t)$ at the physical point x and time t. Since we consider a stable gradient here, the pH level only depends on the spatial variable. At each time step, each individual cell will sense its local pH level which leads to the changes of its kinase activities and methylation levels, $\{a_{1,2}(t), m_{1,2}(t)\}$. The total kinase activity $a(t) = f_1 a_1(t) + f_2 a_2(t)$ regulates the switching probability $P(a(t))$ of the flagellar motor between CCW and CW states. This switching behavior finally leads to the tumble and run motion of the cell. When the cell moves to a new position in the next time step, the algorithm repeats itself as the cell senses a new pH value.

The dynamics of the signaling pathway for pH sensing is governed by the Ising-type model outlined in the main text. We use the same parameter set given in Table 1 for the signaling module which produces the total kinase activity $a(t)$ over time for each cell and drives its tumble or run motion in space. A phenomenological model is used here to model the bacterial motion. Let $r = 0, 1$ represent the tumble and run state of the cell. For the time period $t \to t + \Delta t$, a cell switches from state r to state $1 - r$ with probability $P_r([CheYp](t))\Delta t$, where $[CheYp](t)$ is assumed to be linearly proportional to the kinase activity $a(t)$. According to the measurements by

Cluzel *et al.* [3], the ratio between the two probability rates for one flagellar motor can be described as:

$$\frac{P_1([CheYp])}{P_0([CheYp])} = \frac{[CheYp]^H}{K_{1/2}^H}, \tag{S31}$$

with the Hill coefficient $H \approx 10$ and the constant $K_{1/2} \approx 3\mu M$. We assume that the tumble time is constant $P_0([CheYp]) = \tau_0^{-1}$ where $\tau_0 \approx 0.2$sec is the average duration of the tumble state. Then, the average run time is $\tau_1 \approx 0.8$sec in steady state, and the probability rate to switch from the run state to the tumble state is given by:

$$P_1([CheYp]) = \tau_0^{-1} \frac{[CheYp]^H}{K_{1/2}^H}. \tag{S32}$$

After a tumbling episode, a new run direction is chosen randomly with the run velocity $v_0 = 16.5\mu m/$sec. A small time step $\Delta t = 0.1$sec is chosen in our simulations to resolve the average tumbling time.

Due to the Brownian fluctuation of the medium, the rotational diffusion of the chemotactic cell can be captured by adding a small Gaussian random angle $\delta\theta$ to the direction of the velocity in every run time step [2]: $\theta \to \theta + \delta\theta$. The amplitude of this rotational diffusion angle $\Delta\theta \equiv \sqrt{\langle \delta\theta^2 \rangle}$ is estimated to be about 10 degrees. We also implement appropriate boundary condition to ensure the cells swim in the specified region. The following table summarizes other parameters used in our 2D simulator for bacterial pH taxis.

Table S1: **Other parameters used in the 2D Monte Carlo simulation.**

Parameter	Value
Total Simulation Time	2000 sec
Time Step, Δt	0.1 sec
Number of Cells	100
Channel Length	600 μm
Channel Width	300 μm
Hill Coeff., H	10.3
Ave. Run Velocity, v_0	16.5 μm/sec
Ave. Run Time, τ_1	0.8 sec
Const. Tumble Time, τ_0	0.2 sec
Ave. Directional Change	30 per sec
pH Gradient (ΔpH)	1 per 200μm

An Extended Model Integrating both pH and Chemical Sensing

In this supplementary section, we describe an extended Ising-type model which integrates both pH and chemical signals. We start by assuming that the external pH signal modulates the receptor-kinase activity primarily by affecting the periplasmic domain, a process independent of the ligand binding to chemoreceptors. Then, each single receptor can be characterized by five state variables (q, l_c, l_p, s, m) which are labeled as subscripts: q defines the type of receptor with $q = 1$ for Tar and $q = 2$ for Tsr; $l_c = 0, 1$ denotes the chemical ligand binding state; $l_p = 0, 1$ indicates the proton "binding" state of the receptor; $s = 0, 1$ represents the inactive or active conformation of the receptor; and $m \in [0, 4]$ records the receptor's methylation level. Thus, the free energy of an individual receptor is given by

$$H_{q,l_c,l_p,s,m} = \mu_q^c \cdot l_c + \mu_q^p \cdot l_p + (E_q^{L,c} \cdot l_c + E_q^{L,p} \cdot l_p + E_{q,m}^M + E_q^C) \cdot s, \quad \text{(S33)}$$

where $\mu_q^c = \ln(K_q^{I,c}/[L]_q)$ and $\mu_q^c + E_q^{L,c} = \ln(K_q^{A,c}/[L]_q)$ are the chemical potentials of the inactive and active ligand-bound receptors, respectively. Here, $[L]_q$ is the concentration of ligand that specifically binds to the type-q receptor. We use $K_1^{I,c} = 18.1\mu M$, $E_1^{L,c} = 8$ for Tar and $K_2^{I,c} = 6\mu M$, $E_2^{L,c} = 3$ for Tsr [4, 5]. Other parameters including μ_q^p, $E_q^{L,p}$, $E_{q,m}^M$, and E_q^C were defined by Eqs. (2-4) in the main text.

For a type-q receptor at methylation state m, it can be in any of the following $2^3 = 8$ states in the (l_c, l_p, s) subspace: $(0, 0, 0)$, $(0, 1, 0)$, $(1, 0, 0)$, $(1, 1, 0)$, $(0, 0, 1)$, $(0, 1, 1)$, $(1, 0, 1)$, and $(1, 1, 1)$, with the corresponding energies (in the units of the thermal energy k_BT) given by:

$$\begin{aligned}
H_{q,0,0,0,m} &= 0, && \text{(S34)}\\
H_{q,0,1,0,m} &= \mu_q^p, && \text{(S35)}\\
H_{q,1,0,0,m} &= \mu_q^c, && \text{(S36)}\\
H_{q,1,1,0,m} &= \mu_q^c + \mu_q^p, && \text{(S37)}\\
H_{q,0,0,1,m} &= E_{q,m}^M + E_q^C, && \text{(S38)}\\
H_{q,0,1,1,m} &= \mu_q^p + E_q^{L,p} + E_{q,m}^M + E_q^C, && \text{(S39)}\\
H_{q,1,0,1,m} &= \mu_q^c + E_q^{L,c} + E_{q,m}^M + E_q^C, && \text{(S40)}\\
H_{q,1,1,1,m} &= \mu_q^p + \mu_q^c + E_q^{L,p} + E_q^{L,c} + E_{q,m}^M + E_q^C. && \text{(S41)}
\end{aligned}$$

Under the quasi-equilibrium approximation, the probability for the receptor to be in each of the 8 states follows the Boltzmann distribution which is proportional to $\exp(-H_{q,l_c,l_p,s,m})$. So the average activity of the type-q receptor

at methylation state m is given by:

$$\langle a \rangle_{q,m} = \frac{e^{-H_{q,0,0,1,m}} + e^{-H_{q,0,1,1,m}} + e^{-H_{q,1,0,1,m}} + e^{-H_{q,1,1,1,m}}}{\sum_{l_c} \sum_{l_p} \sum_{s} \exp\left(-H_{q,l_c,l_p,s,m}\right)}. \tag{S42}$$

The extended model is completed by including Eqs. (6) and (7) for the methylation kinetics in the main text. We have presented in the *Discussion* section the simulation result of this model for Tar-only mutant which was pre-adapted to $\mathrm{pH}_0 = 7.0$ and $[\mathrm{MeAsp}]_0 = 10^{-1} K_1^{I,c}$ prior to stimulation/changes of both pH and [MeAsp]. Since Tar elicits an attractant response to [MeAsp] yet a repellent response to an increase of pH, the model predicts a "neutral" response curve along which the effects of changing pH and [MeAsp] cancel out with each other. This prediction can be easily tested by experiments and will tell us, for example, whether the proton "binding" process is relatively independent of the (chemical) ligand binding process.

This extended model also allows us to study how the presence of chemical attractants affects the pH responses. In Fig. S1, we plot the pH responses of Tar-only and Tsr-only mutants in the absence (solid lines) or presence (dashed lines) of attractant ($100\mu M$ MeAsp for Tar and $100\mu M$ serine for Tsr). These mutants were pre-adapted to their respective attractant prior to stimulation of pH changes (increasing pH steps from pH=6.5 to pH=9.2 with step size ΔpH=0.3). Fig. S1 shows the amplitude of the adaptive pH responses right after the stimulation versus the ambient pH prior to each stimulation. One can see that the presence of the ligands (MeAsp and serine) weakens the pH responses of Tar and Tsr, respectively. This is in qualitative agreement with the experimental data [6].

A Model Variant with Methylation Level Dependence

In the Ising-type model we described in the main text, the dissociation constants $K_q^{I,A}$ are assumed to be constant for simplicity. In principle, these parameters may depend on the receptor methylation level, i.e. $K_q^{I,A} = K_q^{I,A}(m)$, as suggested by pH sensing experiments in Ref. [6]. However, our simulations demonstrate that, regardless of the methylation level dependence, the push-pull mechanisms works for pH sensing as long as the opposing sensors (Tar and Tsr) dominate different pH regimes.

In this supplementary section, we discuss model variants considering the methylation level dependence. For example, we can fix $K_1^I = 9.0$ and $K_1^A = 7.0$ for Tar, and assume that $K_2^A = 8.0$ and $K_2^I(m) = 6.0 + 0.5m$ for Tsr. It

follows that $\mu_2(m) = \ln(10)\cdot[\mathrm{pH}-K_2^I(m)]$ and $E_2^L(m) = \ln(10)\cdot[K_2^I(m)-K_2^A]$, both depending on the Tsr methylation level m. Fig. S1 shows that this methylation dependence does not change the sign of the Tsr response to pH stimuli (ambient pH: 5.0 $\rightarrow$ 9.8). As a result (Fig. S2), this model still contains an inversion pH point around pH 7.0 for the wild-type strain (with $f_1 = f_2 = 1/2$). We have tried other forms of methylation level dependence: for example, fixing $K_2^A = 8.0$ and $K_2^I = 6.0$ for Tsr, and assuming that $K_1^I(m) = 9.0 - 0.5m$ and $K_1^A = 7.0$ for Tar. Similar to the result in Fig. S2, we found an inversion pH point around pH= 8.0 for the wild-type strain with $f_1 = f_2 = 1/2$. As long as the methylation changes do not change the order of K_q^I and K_q^A, there could be an inversion pH point in our model. Simulation results for different model variants do not alter our main conclusion that the existence of an inversion pH point requires the opposite responses of Tar and Tsr which should dominate in different pH regimes.

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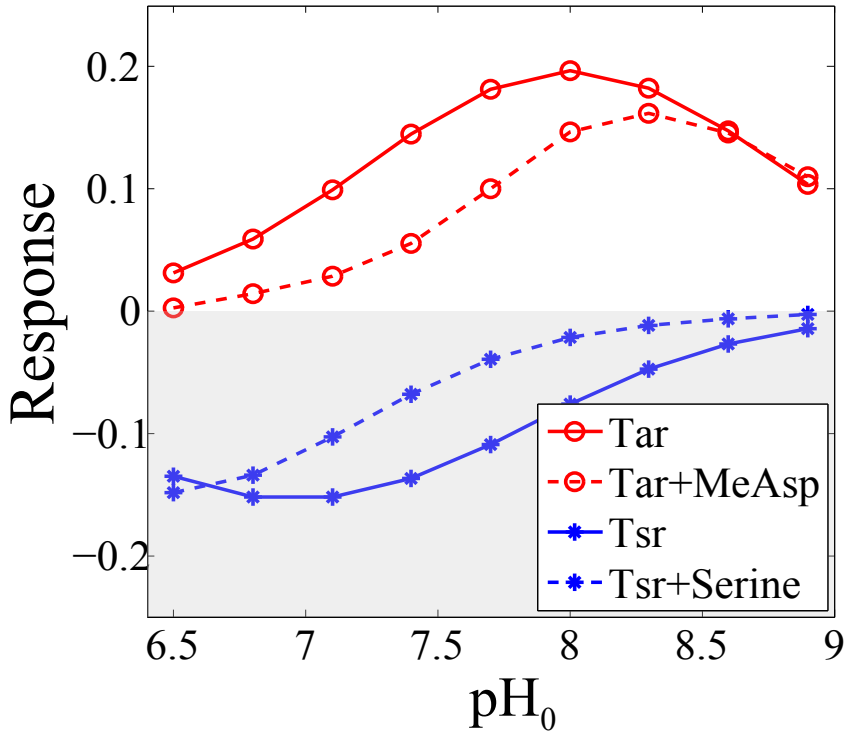

Figure S1: Responses of the Tar-only (red symbols) and Tsr-only (blue symbols) mutants to steps of increasing pH in the absence (solid lines) or presence (dashed lines) of their respective attractant: $100\mu M$ MeAsp for Tar and $100\mu M$ serine for Tsr. The ambient pH_0 ranges from 6.5 to 8.9 with the step size ΔpH=0.3)

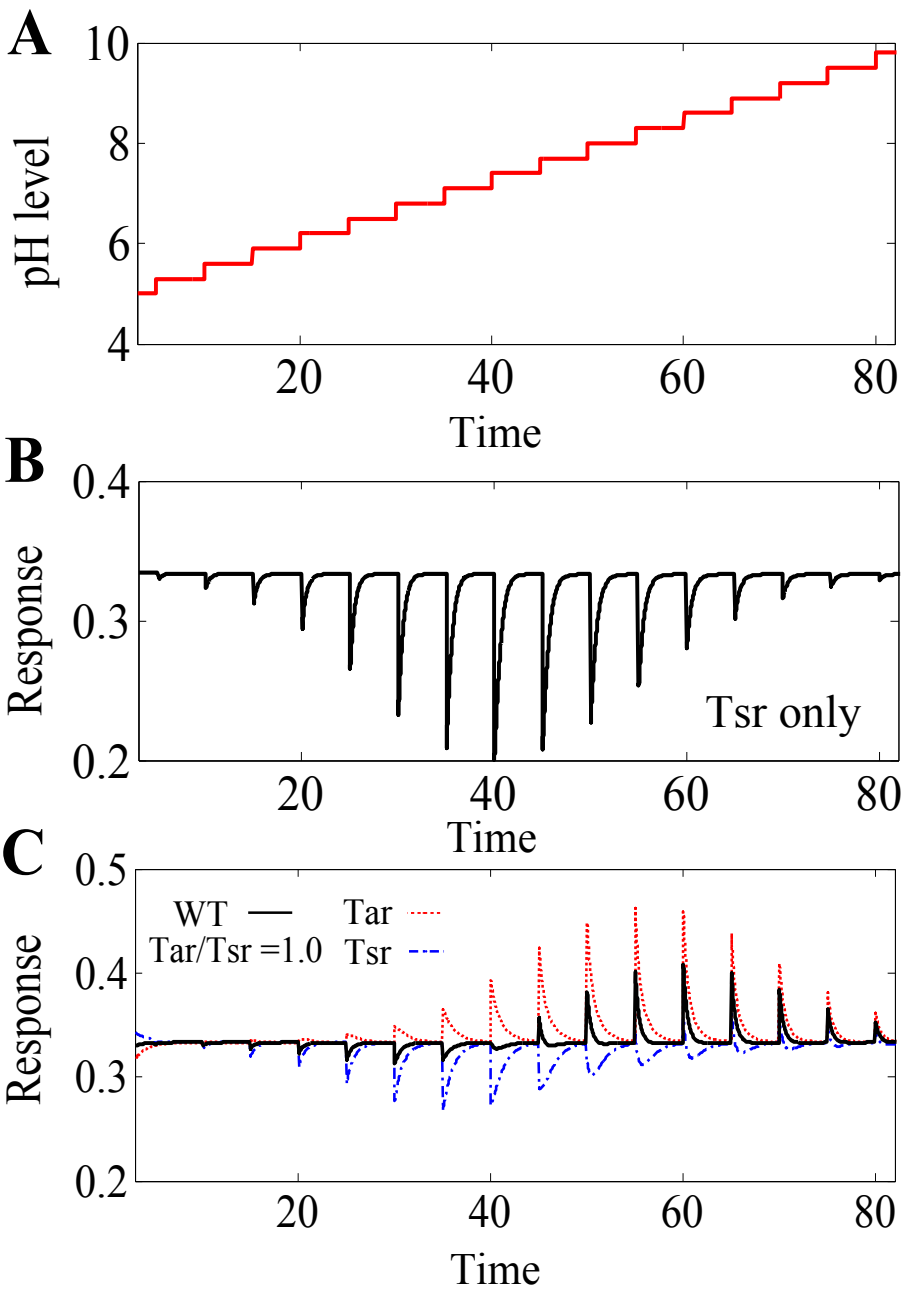

Figure S2: Responses of the Tsr-only mutant and the wild-type strain to steps of increasing pH. In this simulation, we have chosen $K_2^A = 8.0$ and $K_2^I(m) = 6.0 + 0.5m$ for Tsr. (A) Steps of increasing pH levels, with ambient pH_0: 5.0 $\rightarrow$ 9.8 with ΔpH=0.3. (B) Response of the Tsr-only mutant. (C) Response of the wild-type cell (the Tar/Tsr ratio $f_1 = f_2 = 1/2$), together with the average activities contributed by Tar (red dotted line) and Tsr (blue dashed line).