Chapter 3

Network-level variability in bacterial chemotaxis

Most of our current knowledge about the bacterial chemotaxis network comes from studies of the model organism Escherichia coli. However, recent genomic studies have revealed that topologies of the chemotactic signaling networks demonstrate a great diversity across prokaryotes. We have studied the network-level properties of chemotactic signaling in response to the attractant α-methyl-aspartate in Salmonella typhimurium, which shares a homologous chemotactic network with E. coli. Using in vivo fluorescence resonance energy transfer (FRET) measurements with time-varying inputs, we revealed that the parameters of S. typhimurium’s receptor and adaptation transfer functions are different from those of E. coli’s. The response to α-methyl-aspartate shows three-fold lower apparent cooperativity and three-fold faster adaptation kinetics. The manner in which receptor sensitivity is modulated as a function of background concentration also differs between the two species, with S. typhimurium demonstrating a broader range over which the response is invariant to the absolute level of input (fold-change detection, FCD). We observed the migration of S. typhimurium populations in spatial gradients of attractant, created in microfluidic devices. We demonstrated differences from E. coli in the chemotactic drift velocity, which we relate to the underlying control physiology. Our study demonstrates how dynamical features of cell signaling networks can be evaluated in the context of organism behavior and evolution using a quantitative physiology approach.
Chapter 3

3.1. Introduction

The bacterial chemotaxis system, which allows bacteria to sense and respond to changes in chemical concentrations in the surrounding environment, is one of the best understood intracellular signaling networks. It is well characterized at the molecular level, and the network-level dynamics of the model species *Escherichia coli* has been unravelled by numerous experimental and theoretical studies. However, the chemotaxis system of *E. coli* represents a streamlined example of bacterial chemotaxis: recent advances in genomics and bioinformatics have revealed that the topologies of the chemotactic networks in other species, even of closely related bacteria, demonstrate a great diversity. Moreover, chemotactic performance varies even among different strains of *E. coli*, suggesting that the chemotactic signaling dynamics has been fine tuned in the recent evolutionary history.

We investigated the chemotactic signaling and behavior of *Salmonella typhimurium*: a pathogenic species that diverged from the common ancestor with *E. coli* 120 to 160 million years ago. Although *S. typhimurium* and *E. coli* coexist both in their host and non-host habitats, they have different lifestyles; therefore it is likely that their chemotactic responses have been subject to distinct evolutionary optimization. The chemotaxis genes of *E. coli* and *S. typhimurium* are similar enough to permit swapping of components on a gene-by-gene basis: deletions in one species are complemented by the homologous genes from the other. However, no systematic comparison of the dynamic features of chemotactic signaling of these two species has been performed to date.

At the molecular level, chemoeffector sensing and chemotactic signal transduction are achieved in a similar manner in the two species. Chemoeffectors are detected by clustered transmembrane chemoreceptors, which form homodimers and are coupled to a histidine kinase (CheA). The kinase phosphorylates a response regulator CheY, which transduces the signal to the flagellar motors and bias the direction of their rotation. The dephosphorylation of CheY is accelerated by a phosphatase CheZ. Adaptation is achieved by a pair of enzymes with opposing actions, CheR and CheB, which provide a negative feedback by
Network-level variability in bacterial chemotaxis

reversible methylation of the chemoreceptors at multiple sites. Differences between chemotaxis protein species of *E. coli* and *S. typhimurium* are observed at the level of the receptor-kinase complex. In addition to the receptor types common for both species: Tar, Tsr, Trg and Aer, there are five *S. typhimurium*-specific receptors Tcp, Tip, McpA, McpB and McpC and one *E. coli*-specific receptor: Tap (Table 3.1). Coupling of chemoreceptors to kinase molecules in *E. coli* is achieved by a single scaffolding protein, CheW. A second type of scaffolding protein, CheV, exists in *S. typhimurium*, although no direct evidence that CheV couples chemoreceptors to CheA in *S. typhimurium* has been reported. CheV shares regions of homology in its N-terminal domain with CheW, and in its C-terminal domain with CheY, and it likely can be phosphorylated by CheA. Although most of the chemotactic proteins can be swapped between *E. coli* and *S. typhimurium*, some functional differences exist between these proteins: for example, Tar from *E. coli* but not from *S. typhimurium* is able to sense maltose.

**Figure 3.1.** Molecular representation of chemotactic signaling network. Components that differ between *S. typhimurium* and *E. coli* are shown in color. Mixed chemoreceptor clusters regulate the activity of the kinase, CheA. Scaffolding proteins (CheW, CheV) couple the receptors and kinase molecules. The response regulator CheY that transduces the signal to the flagellar motors is phosphorylated and dephosphorylated by CheA and CheZ respectively. Receptors are methylated and demethylated / deamidated at multiple sites by the enzymes CheR and CheB respectively, and CheB activity is also feedback-regulated by phosphorylation from CheA. CheV is also likely to be phosphorylated by CheA.
Table 3.1. Chemoreceptors (MCPs) in *S. typhimurium* and *E. coli*.

*Receptor contains the conserved C-terminal pentapeptide motif (NWET/SF).

The *E. coli* chemotaxis system can be described as a modular network: a receptor module detects environmental cues and generates an intracellular response, and an adaptation module maintains the kinase activity at a steady state indifferent to the background concentration of chemoeffector 137,226 (see Chapter 2, Figure 2.4A). In a coarse-grained model 260, the essential system-level properties of *E. coli* chemotaxis signaling are described by three dynamic variables: chemoeffector concentration $[L](t)$ (input), receptor-controlled kinase activity $a(t)$ (output), and average methylation level of the receptors $m(t)$ (memory), linked by two transfer functions 260. The transition between the active and inactive state of the receptors is described by an allosteric model of the Monod-Wyman-Changeux (MWC) type 176. The transfer function, $G([L],m)$, represents the cooperative modulation of kinase activity by chemoreceptors, determined through the free energy difference, $f([L],m)$, between the active and inactive states of the receptor-kinase complex. The second transfer function, $F(a)$, represents enzyme-driven methylation kinetics (see Materials and methods).

Both transfer functions of the chemotactic signaling response to the non-metabolizable chemoattractant α-methyl-aspartate (MeAsp) have been characterized in *E. coli*, using a fluorescence resonance energy transfer (FRET) system 242 that provides a real time readout of the output kinase activity, $a(t)$. This FRET assay uses a donor-acceptor pair between the
phosphatase CheZ and the response regulator CheY, fused to yellow and cyan fluorescent proteins (YFP and CFP) respectively. The measured FRET value is proportional to the intracellular concentration of the complex formed between CheZ and phosphorylated CheY, [CheZ\cdot CheY-P], which is determined by the balance between CheY phosphorylation by CheA and its dephosphorylation by CheZ, which have equal rates at steady state. Therefore, the kinase (CheA) activity, \( a(t) \), is proportional to [CheZ\cdot CheY-P], and this FRET pair provides a real-time readout of the chemotaxis pathway output \( a(t) \) on time scales slower than the relaxation time of the CheY phosphorylation cycle.

Measurements of the amplitude of the initial rapid FRET response to step stimuli allowed characterization of the sensitivity and cooperativity of the \( E. coli \) receptor module. The methylation kinetics has been studied in \( E. coli \) by applying temporal exponential ramp stimuli matching the adaptation time scale. The frequency response of the chemotaxis system has been characterized by monitoring the FRET response to oscillatory input signals. Response dynamics has been also measured at different background concentrations of chemoeffectors, showing that \( E. coli \) chemotaxis follows the fold-change detection (FCD) strategy, that is, it responds faithfully to the shape of the input profile irrespective of its absolute intensity (see Chapter 2). The high degree of conservation between \( S. typhimurium \) and \( E. coli \) chemotaxis proteins permits the utilization of the same FRET assay to probe properties of \( S. typhimurium \) chemotaxis signaling, as exemplified in Chapter 4.

The behavioral performance of \( E. coli \) in spatial chemoeffect gradient has also been investigated. Microfluidic technology facilitated studies of the quantitative properties of bacterial chemotaxis performance by enabling the generation of precisely controlled gradients of chemoeffectors, and observations of bacterial behavior at high spatial and temporal resolution. For example, cell tracking in controlled microenvironments allowed quantification of population-scale transport parameters in \( E. coli \), and diffusion-based microfluidic devices have been developed to generate steady gradients of arbitrary shape. Such devices were used to observe \( E. coli \) distributions in linear gradients rescaled by
their mean concentration and showed that the FCD property holds also in the behavioral chemotactic response.\(^{137}\)

We examined here the chemotaxis system of \textit{S. typhimurium}, using FRET and microfluidic experiments and the modeling framework developed for \textit{E. coli} chemotaxis. We find that the quantitative parameters that characterize these two transfer functions of the chemotaxis response in \textit{E. coli} and \textit{S. typhimurium} are not conserved. The chemoreceptor response to MeAsp of \textit{S. typhimurium} is less cooperative and the sensitivity of the response has a different dependence on the background level of chemoeffector. \textit{S. typhimurium} shows faster kinetics of receptor methylation, and differences in network-level adaptation properties lead to a higher characteristic frequency below which \textit{S. typhimurium} can compute time derivatives of the input signal. We show that the differences in the control physiology affect the transient and steady-state chemotaxis performance. Using receptor-mutant strains, we demonstrate that the differences between \textit{S. typhimurium} and \textit{E. coli} chemotaxis properties can be ascribed to differences in the receptor population composition, as well as the characteristics of the individual receptors. Our comparison of the dynamical features of \textit{S. typhimurium} and \textit{E. coli} chemotactic signaling and behavior is of fundamental interest as it provides a snapshot of the evolution of bacterial chemotaxis.

\subsection*{3.2. Receptor-kinase response in \textit{S. typhimurium}}

To test \textit{S. typhimurium}'s receptor sensitivity to MeAsp, we applied sequential steps of MeAsp, $\Delta[L]$, to populations of cells adapted to a background level $[L]_0$, and measured the amplitude of the initial rapid excitatory response, $\Delta FRET$. Figure 3.2A illustrates an example of such an experiment. Like in \textit{E. coli}, the adaptation to MeAsp in \textit{S. typhimurium} is perfect: the steady-state kinase activity of adapted cells is the same as the kinase activity prior to stimulation\(^{12,241}\) (Figure 3.2B, see also FRET response time series in Figure 3.2A). However, the steady-state kinase activity, $a_0$, in \textit{S. typhimurium}, $a_0^{ST}=0.4$, is higher than $a_0$ for \textit{E. coli} measured under identical conditions ($a_0^{Ec}=0.33$)\(^{228}\). (The superscript Ec is
used for *E. coli* components and parameters and the superscript $St$ – for *S. typhimurium* components and parameters throughout the text.)

We adapted *S. typhimurium* populations to 14 different background levels, $[L]_0$, and constructed dose-response curves of the kinase activity, $a$, as a function of the total attractant concentration $[L] = [L]_0 + \Delta[L]$ (Figure 3.2C). The sigmoid dose-response curves we fit by a Hill function,

$$a = a_l + \left( a_h - a_l \right) \frac{[L]^{n_H}}{[L]^{n_H} + [K_{1/2}]^{n_H}}$$

where $K_{1/2}$ is the ligand concentration of the half-maximum of the response, $n_H$ is the Hill coefficient, and $a_l$ and $a_h$ are the lowest and the highest kinase activity in the dose-response curve. The $K_{1/2}$ values, which provide an estimate for the receptor sensitivity, scale proportionately with the background level over a 1000-fold concentration range (0.03-30 mM $[L]_0$): $\log K_{1/2} \propto \log [L]_0$ with a slope $\approx 1$ (Figure 3.2D). In contrast to *E. coli*, where the apparent cooperativity of the response is not constant but peaks in the range $[L]_0 = (0.02 – 3) \text{mM}$, where $n_H > 2^{166,241}$, *S. typhimurium* shows a low and nearly constant apparent cooperativity of its MeAsp response: $n_H = 1.3\pm0.2$ across the entire range of tested background concentrations (Figure 3.2E). It has been previously shown that within the concentration range 0.02 - 3 mM $[L]_0$ the instantaneous chemotactic kinase response of *E. coli* follows Weber’s law$^{171,247}$ (see Chapter 2), i.e. it is proportional to the ratio of the change in ligand sensitivity, $\Delta[L]$, to the background concentration of ligand, $[L]_0$: $\Delta a([L], [L]_0) = k \Delta [L] / [L]_0$. The proportionality constant $k$ is called the Weber-Fechner constant, and in the limit of small $\Delta[L]$, is equivalent to $k = \frac{da}{d\ln[L]} |_{[L]=[L]_0}$.

From fits to the data of Figure 3.2C, we showed that $\frac{da}{d\ln[L]} |_{[L]=[L]_0} = 260$. From fits to the data of Figure 3.2C, we showed that $\frac{da}{d\ln[L]} |_{[L]=[L]_0}$ for *S. typhimurium* is nearly constant in the range $[L]_0 = (0.03 – 30) \text{mM}$ (Figure 3.2F). Thus, the *S. typhimurium* chemotaxis network also follows Weber’s law, and this property holds over a concentration range spanning three orders of magnitude.
Figure 3.2. Dose-response measurements in wild type *S. typhimurium*. (A) FRET response (top) to step changes in [L] (bottom). (B) Precision of adaptation to different MeAsp concentrations. Adaptation precision is determined as the ratio between the prestimulus value of the kinase activity and the new constant value of kinase activity, reached after adaptation. Perfect adaptation (adaptation precision of 1) is indicated by the gray line. (C) Dose-response data of the kinase activity, \( a \), to steps of MeAsp, applied to cells, adapted at different background concentrations of MeAsp, \([L]_0\). Fits using the MWC model of equations (1), (2), and (3) are shown with parameters \( N = 2 \), \( K_i = 0.026 \text{ mM} \) and \( K_A = 27.889 \text{ mM} \). (D) Concentration of half-maximum response (\( K_{1/2} \)) and (E) Hill coefficient (\( n_H \)) and at different \([L]_0\) for *S. typhimurium* (red) and *E. coli* (blue, data from 239). In (D), a linear fit with a slope \( 0.97 \pm 0.1 \) of \( K_{1/2} \) values of *S. typhimurium* is shown. Lines in (E) indicate \( n_H \) for \([L]_0=0\) for *S. typhimurium* (red, 1.1 \pm 0.1) and *E. coli* (blue, 1.2 \pm 0.1) (F) The local slope of the kinase activity with respect to the logarithm of ligand concentration for *S. typhimurium* at \( a = a_0 \) and \([L] = [L]_0\), demonstrating Weber’s law (see text).
The effect of receptor cooperativity on the transfer function $a = G([L], m)$ is well described in *E. coli* by an MWC-type allosteric model,

$$a = G([L], m) = G(f_t([L], m)) = \left(1 + e^{f_t([L], m)}\right)^{-1}$$  

where $f_t$ is the total free energy, expressed in units of $k_BT$, of the receptor-kinase complex. $f_t$ is an additive function of two linearly independent terms:

$$f_t([L], m) = Nf_L([L]) + f_m(m)$$  

where $N$ is the number of ligand-binding receptor units in a functional cluster, $f_L([L])$ represents the free energy contribution from ligand binding and $f_m(m)$ is the contribution from receptor methylation (see Materials and methods).

In *E. coli*, the ligand-dependent fraction of total free energy $f_L([L]) = \ln \frac{1+([L]/K_i)}{1+([L]/K_a)}$, where $K_i$ and $K_a$ are the dissociation constants of the inactive and active receptor states, respectively, is linear in $\log[L]$ over the concentration range $K_i \ll [L] \ll K_a$, where $K_i^{Ec} = 0.018$ mM, $K_a^{Ec} = 2.903$ mM, and the cooperativity of the main receptor for MeAsp, Tar, is high ($N^{Ec} = 6$)\(^{166}\). *S. typhimurium*’s dose-response curves are well fit by the aforementioned functional form for $a$ (equations (1), (2) and (3)), assuming fixed values for $N$, $K_i$ and $K_a$ over the whole range of background concentrations\(^{260}\), with parameters $K_i^{St} = 0.026$ mM and $K_a^{St} = 27.889$ mM and $N^{St} = 2$ (Figure 3.2C). The three-fold lower $N$ in *S. typhimurium* could be a consequence of the larger number of receptor species (see Table 3.1), decreasing the number of ligand-binding receptors per cluster, as it has been shown previously that cooperativity increases in pure receptor populations in *E. coli*\(^{240}\). Using *S. typhimurium* cells expressing as the sole receptor species Tar\(^{St}\), we found that the cooperativity of the response observed in pure Tar populations in *S. typhimurium* also increases (Figure 3.3A).

The methylation-dependent free energy in *E. coli* is well-approximated by a linear function, $f_m(m) = \alpha(m^* - m)$, where the offset methylation level $m^* = 0.5$\(^{226}\) and the free energy contribution per methylation increment $\alpha \approx 2$ $k_BT$ (see Materials and methods). Using adaptation-deficient ΔcheR ΔcheB *S. typhimurium* cells, expressing as the
sole receptor species Tar\textsuperscript{st} at a fixed modification state (see Materials and methods, Figure 3.3A) so as to fix the methylation-dependent free energy, \( f_m \), we found that the change of \( f_m \) per modification increment is linear, similarly to \( E. coli \) (Figure 3.3B).

![Figure 3.3. Effect of Tar modification in \textit{S. typhimurium}. (A) Dose-response curves for Tar\textsuperscript{st} with a different number of glutamines (Q) per monomer (a modification that mimics methylated glutamate Em), expressed in adaptation deficient \( \Delta 9T \Delta \text{cheR} \Delta \text{cheB} \textit{S. typhimurium} \) cells, in which all nine receptors are deleted from the chromosome. Tar\textsuperscript{st} expression was induced with 1.4 µM sodium salicylate. Fits using the MWC model of equations (1), (2), and (3) are shown with parameters \( N = 12, K_I = 0.026 \) mM and \( K_A = 27.889 \) mM. (B) Methylation-dependent free energy \( f_m \) of Tar\textsuperscript{st} as a function of the number of glutamines (Q) at the four modification sites per monomer, i.e. the methylation increment. The blue line is a linear fit to data from 226 for \( \Delta \text{tar} \Delta \text{tap} \Delta \text{tsr} \textit{E. coli} \), expressing Tar\textsuperscript{Ec} in different modification states.

### 3.3. Methylation-level adaptation in \textit{S. typhimurium}

The dynamics of the adaptation module of \textit{E. coli} was recently mapped by FRET measurements using temporal exponential ramp stimuli of the form \( [L](t) = [L]_0 e^{rt} \), where \( r \) is the ramp rate, \( t \) is the time, and \( [L]_0 \) is the background concentration of MeAsp, to which the cells were pre-adapted before applying a stimulus \( L(t) \) 226. Upon sustained temporal exponential ramp stimulation the methylation feedback signal eventually cancels the change in the ligand input signal exactly, leading to a kinase
Network-level variability in bacterial chemotaxis

output, $a_c$, that is constant in time \(^{226}\). The dependence of the constant output $a_c$ as a function of the ramp rate $r$ represents the sensitivity of the steady-state kinase activity to sustained gradients, and the shape of the feedback transfer function

$$\frac{dm}{dt} = F(a)$$

(4)

can be inferred from the curve $a_c(r)$, which we refer to here as the gradient-sensitivity curve. The changes in the ligand-dependent and methylation-dependent free energy cancel each other, i.e., $\frac{df_L}{dt} + \frac{df_m}{dt} = r - aF(a_c) = 0$ (see reference \(^{226}\)), where $a = \frac{df_m}{dm}$ is the free energy change per methylation increment, thus $F(a_c) = \frac{r}{a}$ can be obtained from the gradient-sensitivity curve by inverting $a_c(r) = F^{-1}(r/a)$, i.e. rescaling the abscissa and inverting the axes about $(r = 0, a_c = a_0)$ \(^{226}\).

We probed the gradient sensitivity of \textit{S. typhimurium}, applying up ($r > 0$) and down ($r < 0$) exponential ramp stimuli to cells, adapted to $[L]_0 = 0.229$ mM MeAsp (a concentration that belongs to the region of maximal sensitivity for \textit{E. coli}, and was used for analogous experiments in \textit{E. coli} \(^{226}\), using a computer-controlled fluid mixer \(^{35,226}\). Figure 3.4A shows an example of up- and down-ramps ($r = \pm0.01$), in which constant kinase activities $a_c$ are reached. We plotted $a_c$ as a function of the ramp rate to obtain a gradient-sensitivity curve (Figure 3.4B). The slope $\Delta a_c/\Delta r$ near $r = 0$ is less steep than that of \textit{E. coli} by a factor of approximately three: $\Delta a_c/\Delta r \approx -11$ s for \textit{S. typhimurium}, whereas for \textit{E. coli} under identical conditions $\Delta a_c/\Delta r \approx -30$ s \(^{226}\). Thus, $a_c$ responds more sensitively to shallow gradients in \textit{E. coli} than in \textit{S. typhimurium}.

From the gradient-sensitivity curve, we obtained the feedback transfer function $F(a)$, which reveals the methylation kinetics for \textit{S. typhimurium} (Figure 3.4C). The shape of $F(a)$ in \textit{S. typhimurium} is similar to that in \textit{E. coli}. It is a non-linear monotonically decreasing function, which has a single fixed point at $a = a_0$ with $a_0$ given by $F(a_0) = 0$. The conditions (i) $F'(a_0) < 0$ and (ii) $da/dm > 0$ show that this fixed point is stable \(^{226}\). Thus, regardless of the background ligand concentration $[L]_0$, the kinase activity always returns to the same steady-state level $a_0$. The slope near $a = a_0$ determines how fast the system relaxes to the steady state after small changes in $a$. Thus, this slope provides a measure for the strength of
the methylation-dependent negative feedback in the chemotaxis system. A shallow slope $F'(a_0) \approx -0.01 \text{ (s)}^{-1}$ was measured in \textit{E. coli} \textsuperscript{226}. The three-fold steeper slope $F'(a_0) \approx -0.03 \text{ (s)}^{-1}$ that we measured in \textit{S. typhimurium} under identical conditions indicates that the methylation-dependent negative feedback in \textit{S. typhimurium} is three-fold stronger.

Figure 3.4. Gradient-sensitivity curve and feedback transfer function $F(a)$ in \textit{S. typhimurium}. (A) Kinase activity response of \textit{S. typhimurium} (top) to exponential ramp stimuli with ramp rate $r = \pm 0.01 \text{ s}^{-1}$ (bottom). (B) Gradient-sensitivity curve of wild type \textit{S. typhimurium}, evaluated by measuring the constant kinase activity $a_c$ reached during exponential ramps with rate $r$. The slope of near $r = 0$ is $\Delta a_c/\Delta r \approx -11\text{s}$. The slope for \textit{E. coli} ($\approx -30 \text{s}$) is shown by a blue line (data from reference \textsuperscript{226}). Dashed lines show the steady-state kinase activity ($a_0$) in \textit{E. coli} (blue) and \textit{S. typhimurium} (red) (C) The feedback transfer function $F(a)$ of \textit{S. typhimurium} is mapped using the data from Figure 3.4B (see text). The slope near steady state $F'(a_0)$ in \textit{S. typhimurium} is $\approx -0.03 \text{ (s)}^{-1}$, whereas in \textit{E. coli} the slope $F'(a_0) \approx -0.01 \text{ (s)}^{-1}$ (blue line, \textit{E. coli} data is taken from reference \textsuperscript{226}).
Network-level variability in bacterial chemotaxis

3.4. Network-level adaptation in *S. typhimurium*

Calibration of the response functions of the receptor and adaptation modules allows an estimate of the characteristic frequency, $\nu_m$, which sets an upper bound on the frequency range over which the chemotaxis network is able to take time derivatives of the chemoeffector input:

$$\nu_m = \frac{-\alpha F'(a_0)Na_0(1-a_0)}{2\pi}$$  \hspace{1cm} (5)

For *E. coli* $N = 6$, $F'(a_0) = -0.01$ (s)$^{-1}$, $a_0 = 0.33$, and $\alpha = 2$, yielding $\nu_m \approx 0.004$ Hz, which is in good agreement with the value experimentally determined using FRET measurements with exponential sinewave inputs, $\nu_m \approx 0.006$ Hz. This characteristic frequency is a network-level adaptation property of the chemotaxis system, as it involves parameters of both modules of the network, and is inversely proportional to the adaptation timescale, $\tau_m$, of the chemotaxis response:

$$\tau_m = \frac{1}{2\pi \nu_m}$$  \hspace{1cm} (6)

From the calibration of the transfer functions $G([L], m)$ and $F(a)$ (Figures 3.2, 3.3 and 3.4), we estimated the values for the transfer function parameters for *S. typhimurium*: $N = 2$, $F'(a_0) = -0.03$ (s)$^{-1}$, $a_0 = 0.4$, and $\alpha = 2$. Using equation (5), these values lead to a predicted value of the characteristic frequency $\nu_m \approx 0.005$ Hz.

We tested experimentally the frequency response of populations of *S. typhimurium* adapted to 0.229 mM MeAsp using temporal exponential sinusoid inputs $[L](t) = [L]_0\exp(A_L \sin(2\pi t))$ with a fixed input amplitude $A_L = 0.2$, $[L]_0 = 0.229$ mM, and frequencies $\nu$ ranging between 0.002 and 0.05 Hz. For stimulus variation within the range $K_i << [L]_0 << K_a$, the change in ligand-dependent free energy $\Delta f_L = f_L([L](t)) - f_L([L]_0)$ follows the logarithm of the ligand concentration, and the kinase outputs to exponential sinewave stimuli are pure sinusoids with frequencies $\nu$ matching the frequency of input modulation, and amplitudes $|A|$ and phase delays $\phi_D$ dependent on the frequency of input modulation (Figure 3.5A). Both $|A|$ and $\phi_D$ can be solved for by linearizing equations (1) and (4) around $a = a_0$, which gives $\phi_D(\nu) = \pi - \tan^{-1}(\nu_m/\nu)$ and $|A| = \frac{Na_0(1-a_0)}{\sqrt{1+(\nu_m/\nu)^2}}A_L$. 

63
Figure 3.5B shows the frequency dependence of the phase delay, $\varphi_D/\pi$, and normalized amplitude of the FRET response, $|A|/|A_{\text{max}}|$ for *S. typhimurium*, where $A_{\text{max}}$ is the amplitude of the output sinewave with maximal amplitude. Fits to the both amplitude and phase response data revealed for *S. typhimurium* a characteristic frequency $\nu_m = 0.017\pm0.002$ Hz, nearly three times higher than that of *E. coli*. Interestingly, the measured value for $\nu_m^{St}$ differs from that predicted ($\nu_m \approx 0.005$ Hz, see above) from measurements of the transfer functions of receptor and adaptation modules of *S. typhimurium*.

**Figure 3.5. Frequency response of *S. typhimurium*.** (A) Normalized FRET response (top) to exponential sinewave stimuli with frequencies $\nu = 0.015$ Hz and 0.024 Hz and amplitude $A_L = 0.2$ and $[L]_0 = 0.229$ mM (bottom). (B) Bode plot for wild type *S. typhimurium*, showing the phase delay (top) and the normalized amplitudes (bottom) of the output sinusoids as a function of the driving frequency $\nu$. The red curve is a fit to *S. typhimurium* data and the blue curve is a fit to *E. coli* data from 226. The characteristic frequencies for *S. typhimurium* (red) and *E. coli* (blue) are indicated with dashed lines.
3.5 Role of CheV on network-level properties of *S. typhimurium* chemotaxis

The hybrid protein CheV, which has an N-terminal domain homologous to the scaffolding protein CheW, and C-terminal regulatory domain homologous to CheY and hence is likely to be phosphorylated, exists in *S. typhimurium* but not in *E. coli* \(^8\) (see Chapter 5). Thus CheV might be involved in the differences in the transfer functions \(F(a)\) and \(G([L],m)\), as well as the frequency response we observed between the two species. For example, CheV phosphorylation could provide a mechanism for methylation-independent feedback in *S. typhimurium*, leading to faster or slower network-level adaptation.

We measured the dependence of the rapid response to step changes in [MeAsp] for different background concentrations of MeAsp in \(\Delta\text{cheV} S. typhimurium\) (Figure 3.6A), in the same manner by which we measured this response in wild type *S. typhimurium* (Figure 3.2C). We fit these dose-response data of the \(\Delta\text{cheV}\) strain using the MWC model of equations (1), (2), and (3), and obtained parameters \(N = 2, K_i = 0.015 \text{ mM} \) and \(K_A = 20.207 \text{ mM}\), which are very similar to those obtained from the data for the wild type strain.

We also applied exponential sinewave inputs of different frequencies to determine the characteristic frequency \(\nu_m\) of the \(\Delta\text{cheV} S. typhimurium\) frequency response. Fits of both amplitude and phase delay data (Figure 3.6B) gave \(\nu_m \approx 0.016 \text{ Hz}\), which is very similar to the \(\nu_m\) measured for wild type *S. typhimurium*. Thus neither the higher characteristic frequency of the response, nor the lower cooperativity of the response of *S. typhimurium* compared to *E. coli* can be explained by the presence of the *S. typhimurium*-specific protein CheV (see Chapter 5 for further experiments on the effects of CheV in the chemotactic signaling of *S. typhimurium*).
Figure 3.6. Kinase response of ΔcheV S. typhimurium. (A) Dose-response data of the kinase activity (a) to steps of MeAsp, applied to ΔcheV S. typhimurium cells, adapted at different background concentrations of MeAsp, [L]. Fits using the MWC model of equations (1), (2), and (3) are shown with parameters $N = 2$, $K_I = 0.015$ mM and $K_A = 20.207$ mM. (B) Bode plot for ΔcheV S. typhimurium (black points), showing the phase delay (top) and the normalized amplitudes (bottom) of the output sinusoids as a function of the driving frequency $\nu$ (see text). Red and blue curves are fits to data for wild type S. typhimurium and wild type E. coli, respectively. The characteristic frequencies for ΔcheV S. typhimurium (black), wild type S. typhimurium (red) and wild type E. coli (blue) are indicated with dashed lines.
3.6. Differences in chemotactic performance of *S. typhimurium* and *E. coli* in spatial gradients

The transfer functions of chemotactic signaling determine the organism’s chemotactic performance, thus it is of interest to ask how the observed differences in receptor-kinase response and adaptation kinetics of *S. typhimurium* and *E. coli* affect their chemotactic performance in identical microenvironments. The behavioral response of *E. coli* was recently probed in steady linear gradients of MeAsp with the magnitude of the gradient \( \nabla [L] \) rescaled by the mean concentration \([L]_0\), i.e \( \nabla [L]/[L]_0 = \text{const} \). We tested the *S. typhimurium* response in steady linear gradients of MeAsp created in a similar manner using a microfluidic platform of a design described in reference 7 (Figure 3.7A). The platform consisted of a polydimethylsiloxane (PDMS) layer, in which “source”, “sink”, and “test” channels were patterned, and one face opened towards an agarose hydrogel. MeAsp solutions with different concentrations were flowed through the source and the sink channels, such that \( \nabla [L]/[L]_0 = 0.7 \text{ mm}^{-1} \), and \([L]_0\) was varied over the range 0.005 - 36.871 mM. Diffusion of MeAsp within the agarose layer, sandwiched between a glass slide and the PDMS layer, lead to establishment of a stable linear gradient in the agarose hydrogel, which is rapidly mirrored in the test channel after injection of the bacterial suspension 7 (Materials and methods).

We recorded the time evolution of the distribution of *S. typhimurium* populations within the test channel using time-lapse video microscopy. We determined the positions of the bacteria in the channel to yield the distribution of the bacteria along the spatial coordinate \( x \) in time \( t \), \( B(x, t) \). (For simplicity we consider a one-dimensional system along coordinate \( x \) throughout this study.) To estimate the strength of the chemotactic response in different gradients, we computed the chemotaxis migration coefficient (CMC), a dimensionless metric, for one hundred equally spaced time frames for each experiment. CMC represents the mean displacement of the population from the center of the channel, normalized by the channel half-width:

\[
CMC(t) \equiv \frac{\langle x(t) - W/2 \rangle}{W/2} 
\]

(7)
where $W$ is the width of the test channel (600 µm) and $\langle x \rangle(t)$ is the population-averaged spatial coordinate of the bacteria along the chemoeffector gradient. We used the absolute value $|CMC|(t)$ to quantify the dynamics of the chemotaxis response. Figure 3.7B shows the time evolution of $|CMC|$ for each of the eight tested gradients of MeAsp. For each gradient, $|CMC|$ increases until it reaches a steady state, $|CMC|_{SS}$.

The steady-state $|CMC|$ profile, $|CMC|_{SS}$, as a function of the mean concentration of the gradient, showed a characteristic inverted U-shape for *S. typhimurium* (Figure 3.7C). $|CMC|_{SS}$ was high and nearly invariant ($|CMC|=0.37\pm0.04$) in the range $[L]_0=(0.064 - 10.345)$ mM, whereas for smaller or larger $[L]_0$, $|CMC|_{SS}$ decreases monotonically. In contrast, the *E. coli* $|CMC|_{SS}$ profile showed two nearly flat plateaus, in the ranges $[L]_0=(0.12 - 0.43)$ mM and $(0.815 - 10.345)$ mM: these two plateaus correspond to the concentration regimes in which the response of pre-adapted bacterial populations to fold-changes in the input are invariant to changes in the background, described earlier as “FCD regimes” (Chapter 2). Our results show that the fold-change detection (FCD) property, i.e. invariance of the response to relative gradients $\nabla[L]/[L]_0$, irrespective of the absolute magnitude of $\nabla[L]$ or $[L]_0$, holds in *S. typhimurium* within a single FCD regime of background concentrations, $[L]_0=(0.064 - 10.345)$ mM.

At steady state, the distribution of the bacteria is well described by an exponential distribution $B(x)_{SS} = B_0 e^{-x/\lambda_{SS}}$, where $B_0$ is the cell density at $x = 0$, and $\lambda_{SS} = \langle x \rangle_{SS}$ is the mean position of the bacterial population (Figure 3.7D; see Materials and methods). $\lambda_{SS}$ can be expressed as $\lambda = D_B / \nu_D$, where $\nu_D$ is the drift velocity of the bacterial population, and $D_B$ is the random motility coefficient of the cells in an absence of attractant. From equation (7) it follows that

$$ |CMC|_{SS} \equiv |2\lambda_{SS}/W - 1| = |2D_B/(\nu_D W) - 1| \quad (8) $$

Thus $(1/\lambda)_{SS}$ is proportional to $\nu_D$ and has a hyperbolic dependence on $|CMC|_{SS}$, i.e. $(1/\lambda)_{SS} = 2/(2W - W|CMC|_{SS}) = \nu_D/D_B$. We plotted $(1/\lambda)_{SS}$ as a function of the background level $[L]_0$, for both *S. typhimurium* and *E. coli* (Figure 3.7C Inset), and this also revealed a single plateau shape for *S. typhimurium* and two plateaus for *E. coli*.
In order to compare the transient chemotactic response between
*S. typhimurium* and *E. coli*, we calculated the times $\tau_{50}$ and $\tau_{90}$, in which the
swimming populations of cells reached respectively 50% and 90% of the
steady-state $|\text{CMC}|$ levels, $|\text{CMC}|_{\text{SS}}$ (Figure 3.7E and Figure 3.7E Inset
respectively). We calculated also $\tau_{50}$ and $\tau_{90}$ for *E. coli*, using the data of
reference 138. The two species show a large difference in the mean time to
reach steady state: $\langle \tau_{50} \rangle = 105 \pm 38 \, s$ for *S. typhimurium*, whereas $\langle \tau_{50} \rangle = 489 \pm 248 \, s$ for *E. coli*. The respective $\langle \tau_{90} \rangle$ values are 322 ± 68 s
and 1157 ± 335 s.

Recent theoretical studies by Yuhai Tu’s group have shown that the
drift velocity, $v_D$, of numerically simulated bacterial populations is
phenomenologically well described by a simple relation, i.e
$v_D = CG/(1 + G/G_c)$: it depends linearly on the gradient $G$, until it reaches
a maximal value $v_D^{\text{max}}$ beyond a critical gradient $G_c$ 114,232. $v_D^{\text{max}}$ and $G_c$ are
proportional to the square root of the methylation rate constant $K_R$ 114
(corresponding to $F'(a_0)$ in our notation 260), which could provide an
explanation of the smaller values of $\tau_{50}$ and $\tau_{90}$ of *S. typhimurium* compared
to that of *E. coli* (we determined that the methylation rate in *S. typhimurium
is three-fold lower than that of *E. coli*, see Figure 3.4). The dependence of
$|\text{CMC}|_{\text{SS}}$ on $[L]_0$ that reflects the dependence of $v_D$ on $[L]_0$ suggests that the
gradients that we used in our experiments have steepness smaller or
comparable to $G_c$. The reason why $\tau_{50}$ and $\tau_{90}$, reflecting the transient drift
velocity, are nearly constant in *S. typhimurium* requires further
investigation. The larger spread in the $\tau_{50}$ and $\tau_{90}$ values for *E. coli* might
reflect the larger variation in the drift velocities at different $[L]_0$, expected
from the data shown on Figure 3.7C. Note that the *E. coli* data come from
single experiments per gradient 137 whereas 3-5 repeats per gradient were
performed for *S. typhimurium*, which is also likely to explain the greater
spread in the *E. coli* data.
Figure 3.7. Chemotactic behavior of *S. typhimurium* in steady linear gradients of MeAsp. (A) Schematic cross-section of the diffusion-based microfluidics gradient generator (see text). (B) 3D plot of the chemotaxis migration coefficient \(|\text{CMC}(t, [L])|\). The projections (gray) show the time evolution of the chemotactic response. (C) Steady-state levels of \(|\text{CMC}|, |\text{CMC}_{ss}|, for *S. typhimurium* (red) and *E. coli* (blue) in rescaled gradients of MeAsp. Inset: inverse value of the decay constant \(\lambda_{ss}\) taken from exponential fits to steady-state distributions \(B(x)_{ss}\) of both species (see (D)). (D) Examples of \(B(x)_{ss}\) of *S. typhimurium* and *E. coli* for different gradients (see legends). Exponential fits to \(B(x)_{ss}\) from which \(\lambda_{ss}\) values are obtained are shown in gray (note that not all the fits are well constrained). (E) Times, \(\tau_50\), in which the swimming populations of *S. typhimurium* (red) and *E. coli* (blue) populations reached 50% of \(|\text{CMC}_{ss}|\) in the rescaled gradients of MeAsp with different mean concentration, \([L]_0\). Inset: Times, \(\tau_90\), in which the same populations reached 90% of \(|\text{CMC}_{ss}|\). The lines indicate the mean values of \(\tau_50\) and \(\tau_90\). Error bars represent standard deviation from 3-5 repeats.
3.7. Sensitivity modulation of *S. typhimurium* and *E. coli* chemotactic response

We sought to explain the differences in the behavioral performance of *S. typhimurium* and *E. coli* in terms of the differences in the underlying control physiology. We define $\psi([L]_0) \equiv \frac{\partial f_L}{\partial \ln[L]}|_{[L]=[L]_0}$ as the ‘sensitivity modulation profile’ of the kinase response. The dependence of both the change of kinase activity upon temporal ligand stimulation and drift velocity of cells in spatial gradients is proportional to $\psi([L]_0)$, i.e. $\Delta a \propto \psi([L]_0)$ and $v_D \propto \psi([L]_0)$ (see Materials and methods):

$\Delta a = -a_0(1 - a_0) \psi([L]_0) \frac{\Delta [L]}{[L]_0}$ (9),

$v_D \approx \frac{\nu^2 H(1-a_0) \psi([L]_0) \ln[L]}{24 \frac{1}{\tau_m} dx}$ (10),

where $a_0$ is the steady-state kinase activity, $v$ is the mean run speed, $r$ is the average run length, $H$ is the Hill coefficient of the flagellar motor response and $\tau_m$ is the adaptation time scale of the chemotactic response.$^{114,115,260}$

Our FRET setup allowed us to determine experimentally the shape of $\psi([L]_0)$. We pre-adapted immobilized bacterial populations to different background levels of MeAsp within the range $[L]_0 = (0.001 - 36.871)$ mM, and probed $\psi([L]_0)$ for both *S. typhimurium* and *E. coli* using exponential sinewave stimuli with an amplitude $A_L = 0.2$ and frequency close to characteristic frequency $\nu_m$ of the respective species (Figure 3.8A; $\nu = 0.015$ Hz for *S. typhimurium* and $\nu = 0.006$ Hz for *E. coli*, see section 3.4). The absolute amplitude $|A|$ of the sinusoidal response can be expressed as a function of $\psi([L]_0)$ by linearizing equations (1) and (4) around the steady state $a = a_0$ and solving for $|A|$:

$|A|([L]_0) = \frac{\psi([L]_0)a_0(1-a_0)^2}{\sqrt{1+\nu_m/\nu^2}} A_L$ (11)

In the experiments shown on Figure 3.8, $\nu \approx \nu_m$, thus $\psi([L]_0) = \frac{|A|([L]_0)}{a_0(1-a_0)^2 \frac{A_L}{\nu^2}}$

For both species the phase delay $\varphi_D$ does not change over the tested range of background concentrations (Figure 3.8B): it has a mean value of $(0.72\pm0.02)\pi$ for *S. typhimurium* and $(0.78\pm0.02)\pi$ for *E. coli*, showing that the adaptation timescale $\tau_m$ (see equation (6)) is invariant with the background...
level over the entire range of tested concentrations. However, in both species the amplitudes of the kinase response depend on the background concentration, $[L]_0$, leading to different shapes of $\psi([L]_0)$. For *S. typhimurium*, $\psi([L]_0)$ forms a single peak, where a plateau of nearly constant amplitudes is observed in the range $[L]_0 = (0.064 - 2.903) \text{ mM}$ (Figure 3.8C left). In contrast, two adjacent plateaus of nearly invariant $\psi([L]_0)$, in the ranges $[L]_0 = (0.018 - 0.229) \text{ mM}$ and $(0.815 - 10.345) \text{ mM}$, are observed for *E. coli* (Figure 3.8C right). The plateaus correspond to the FCD regimes of invariant responses described in Chapter 2, although the transition between the plateaus is somewhat smoother than in the data reported in Chapter 2.

The sensitivity modulation $\psi([L]_0)$ is defined as the change of the free energy $f_t$ with the logarithm of ligand concentration $[L]_0$:

$$\psi([L]_0) = \frac{\partial f_t}{\partial \ln [L]} = N \left( \frac{[L]_0}{[L]_0 + K_i} - \frac{[L]_0}{[L]_0 + K_A} \right)$$

Thus, the profile of $\psi([L]_0)$ for ligand binding to a single receptor species has the shape of a single peak, where the width of the peak depends on the separation between the dissociation constants of the inactive and active receptor states, $K_i$ and $K_A$, and the number of ligand-binding receptor units per cluster, $N$, determines the height of the peak. We found that $\psi([L]_0)$ for *S. typhimurium* could be well fit using equation (12) with parameters $N = 4$, $K_i = 0.014 \text{ mM}$, $K_A = 23 \text{ mM}$ (Figure 3.8C left, dotted line). Surprisingly, $N$ is two-fold higher than that predicted from the measurements of the receptor sensitivity using step stimuli (Figure 3.2). Using $N = 4$, and the other parameters obtained from the characterization of the chemotactic transfer functions, the predicted characteristic frequency of *S. typhimurium* from equation (5) is ~0.010 Hz. This value is still lower but closer to that measured experimentally (~0.017 Hz).

In *E. coli* we observed two adjacent plateaus in both behavioral (Figure 3.7C) and signaling (Figure 3.8C) data suggesting that $\psi([L]_0)$ has a more complex shape. FRET measurements of receptor sensitivity in *E. coli* using step stimuli showed that the serine receptor, Tsr$^{Ec}$, likely also binds MeAsp at high concentrations, with dissociation constants $K_i^2 \approx 100 \text{ mM}$ and $K_A^2 >> 100 \text{ mM}$. Thus the second plateau could correspond to the transition regime between Tar-dependent and Tsr-dependent peaks. Tsr-
Network-level variability in bacterial chemotaxis

dependent responses are hard to measure experimentally because concentrations of MeAsp > 10 mM were reported to mechanically perturb receptors in \textit{E. coli} via the osmotic pressure they induce \cite{26}. However, a two receptor species MWC model (see Materials and methods) fits well the experimental data for \textit{E. coli}'s \(\psi([L]_0)\) with parameters \(a_0 = 0.33, N_1 = N_2 = 7, K_{I1} = 0.005\) mM, \(K_{A1} = 0.7\) mM, \(K_{I2} = 10\) mM, \(K_{A2} = 10^4\) mM (Figure 3.8C right).

![Figure 3.8](image)

**Figure 3.8. Sensitivity modulation profile \(\psi([L]_0)\) in wild type and mutant strains.** (A) An example of exponential sinusoid inputs with amplitude rescaled with \([L]_0\) (\(\nu = 0.015\) Hz, \([L]_0 = 0.005, 0.229,\) and \(10.345\) mM) (left). For each input, the normalized FRET output for WT \textit{S. typhimurium} is shown (right). (B) Phase delays of the output sinusoids for stimuli of the type shown in (A) with a driving frequency of \(\nu = 0.015\) Hz for \textit{S. typhimurium} (WT – red, \(\Delta\text{tar}\) - open points), and \(\nu = 0.006\) Hz for WT \textit{E. coli} (blue). The solid lines represent the mean values for each strain. (C-E) \(\psi([L]_0)\) in response to inputs of the type shown in (A) for (C) WT \textit{S. typhimurium} (red) and WT \textit{E. coli} (blue), (D) \(\Delta\text{tar} \textit{S. typhimurium}\) (open points), (E) \(\Delta\text{tar} \textit{S. typhimurium}\), complemented with Tar\textsuperscript{Ec} (purple, NaSal induction level of 1.4 \(\mu\)M) or Tar\textsuperscript{St} (violet, 1.4 \(\mu\)M NaSal). The plateaus of invariant responses are indicated by the shaded rectangles. Error bars represent standard deviation from 3-5 repeats. The fitted parameters are indicated within the text.
3.8. Tar-independent MeAsp response in *S. typhimurium*

To test whether the shape of the sensitivity modulation profile \( \psi([L]_0) \) of wild type *S. typhimurium* is determined by a single chemoreceptor, we probed the response of \( \Delta \text{tar} \) *S. typhimurium* cells that lack the main chemoreceptor for MeAsp, \( \text{Tar} \) (see Table 3.1). We used exponential sinewave stimuli of the type shown on Figure 3.8A. Responses of \( \Delta \text{tar} \) *S. typhimurium* to MeAsp were detected starting at relatively low background concentrations of MeAsp, \([L]_0\), with a threshold \( \sim 0.229 \text{ mM} \) (Figure 3.8D). The steady-state kinase activity \( a_0 \) was \( \sim 0.4 \), similar to that of wild type *S. typhimurium*. We have not tested background concentrations higher than 36.871 mM because the osmotic shock they induce is likely to mechanically perturb receptors in *S. typhimurium* as has been observed in *E. coli* 263. A fit using equation (12) with parameters \( N = 3, K_I = 0.743 \text{ mM}, K_A = 36.388 \text{ mM} \) is shown on Figure 3.8D (see section 3.9).

The phase delays of the output sinewaves remained nearly constant, with a mean \( \sim 0.84 \pm 0.02 \), which is \( \sim 14\% \) higher than the phase delay observed for wild type *S. typhimurium* (Figure 3.9B). Since \( \varphi_D = \pi - \tan^{-1}(\nu_m / \nu) \) 226, we calculated that the characteristic frequency of the chemotactic response of \( \Delta \text{tar} \) *S. typhimurium* is \( \nu_m = 0.004 \text{ Hz} \), \( \sim 4 \)-fold lower than that of wild type *S. typhimurium* (note that both strains have \( a_0 \approx 0.4 \)). Thus, the Tar-independent response has a longer adaptation timescale, *i.e.* smaller characteristic frequency, than that for Tar\(^{\text{sc}}\).

The Tar-independent response to MeAsp could be mediated either via chemoreceptors or alternative pathways 185. In the presence of the methylation enzymes CheR and CheB, \( \Delta \text{tar} \) *S. typhimurium* cells do adapt perfectly to MeAsp steps (Figure 3.9A), whereas minor partial adaptation is observed in \( \Delta \text{cheR} \Delta \text{cheB} \) knockout cells (Figure 3.9B; see also Chapter 5 that discusses possible methylation-independent mechanisms of partial adaptation in *S. typhimurium*). These results indicate that adaptation in the Tar-independent response depends on the activities of CheR and CheB, presumably by methylation / demethylation of chemoreceptors.
3.9. Differences between the Tar receptors of *E. coli* and *S. typhimurium* contribute to the different shape of the sensitivity modulation profiles

Differences in the population of chemoreceptor species (see Table 3.1), or in the properties of the Tar receptors of *E. coli* and *S. typhimurium* could affect the shape of the sensitivity modulation profile $\psi([L]_0)$, which determines the background dependence of the kinase response upon temporal modulation of ligand concentration, and drift velocity of cells in spatial gradients. At the receptor-population level, high concentrations of MeAsp are thought to be sensed not only by Tar, but also by Tsr in *E. coli* [34], and we have found evidence for a pathway independent of Tar in *S. typhimurium* (Figure 3.8D). The Tar-independent receptor response in *S. typhimurium* does not manifest itself as second plateau in the receptor-sensitivity profile $\psi([L]_0)$, as observed in *E. coli*. This reflects the fact that the responsive concentration range of the Tar-independent pathway in *S. typhimurium* is not well separated from that of the Tar-mediated pathway, leading to partially overlapping sensitivity peaks for the two pathways.

To test whether differences in the Tar receptor itself contribute to differences in the sensitivity modulation profiles, we expressed in Δtar *S. typhimurium* cells either TarSt or TarEc from a plasmid with sodium salicylate (NaSal) inducible promoter (Figure 3.8E; for both strains $\omega_0 \approx 0.2$).
Δtar S. typhimurium, complemented with TarSt, showed a singly-peaked sensitivity profile similar to that of wild type S. typhimurium. We obtained fits of the data for wild type S. typhimurium, Δtar S. typhimurium complemented with TarSt, and Δtar S. typhimurium with the two-species MWC model, using the same parameters $N_2 = 3$, $K_{I2} = 0.74$ mM, and $K_{A2} = 36.39$ mM for species 2. For species 1, i.e. Tar, the parameters obtained for wild type S. typhimurium are $N_1 = 5$, $K_{I1} = 0.020$ mM, and $K_{A1} = 1.20$ mM, and for the strain, expressing TarSt from a plasmid, $N_1 = 7$, $K_{I1} = 0.008$ mM, and $K_{A1} = 1.34$ mM ($N_1 = 0$ for Δtar S. typhimurium). The lower $N_1$ in wild type is likely to be a consequence of lower number of TarSt.

Δtar S. typhimurium, complemented with TarEc, showed a sensitivity profile with two plateaus (at (0.064-0.229) mM and (0.815-10.345) mM [L0]) resembling more closely the response of E. coli. The data could be fit by a two-species MWC model, using for species 2 the same parameters that we used to fit the Δtar S. typhimurium data (Figure 3.8D), i.e. $N_1 = 7$, $K_{I1} = 0.031$ mM, $K_{A1} = 0.29$ mM, $N_2 = 3$, $K_{I2} = 0.74$ mM, $K_{A2} = 36.39$ mM (Figure 3.8E left). Note that the dissociation constants for species 1 differ from those for wild type E. coli ($N_1 = 7$, $K_{I1} = 0.005$ mM, $K_{A1} = 0.7$ mM).

3.10. Discussion

We have provided the first, to our knowledge, systematic comparison of chemotaxis signaling dynamics and behavior between S. typhimurium and E. coli. Despite the similarity of their chemotaxis systems at the molecular level, the two species show substantial quantitative differences in their network-level chemotactic signaling characteristics and behavioral responses.

We used in vivo FRET combined with time-varying stimuli to characterize both transfer functions of the modular network: $G([L], m)$, which determines how the receptor module detects chemoafferctor signals and generates the excitatory response, and $F(a)$, which describes the dynamics of the adaptation module that maintain the steady-state kinase activity invariant to the background chemoafferctor concentration. We determined conserved features of the response between E. coli and S. typhimurium: perfect adaptation to MeAsp, invariant adaptation time
Network-level variability in bacterial chemotaxis

scale over a broad range of background concentrations, and linear dependence of the modification-dependent free energy on the number of modified glutamates. However, many quantitative features of the signal detection and processing and behavioral responses differ between the two species. We discuss below the mechanistic origin of each of these features.

The apparent cooperativity of the response to MeAsp is low and constant in *S. typhimurium* over more than four orders of magnitude of background concentrations (Figure 3.2), whereas the apparent cooperativity in *E. coli* is not constant and nearly three-fold higher in the background concentration range centered at the geometric mean of the dissociation constants of active and inactive chemoreceptor Tar 166,241. This weaker signal amplification at the level of the receptor-kinase complex in *S. typhimurium* could be a consequence of a smaller number of ligand-binding receptor units associated with each kinase molecule, e.g. because of the larger number of receptor species in *S. typhimurium*, leading to a smaller relative number of MeAsp binding receptors in the overall receptor population. Indeed we showed that for pure Tar populations in cells that express Tar as the sole chemoreceptor, the cooperativity of the response is augmented (see Figure 3.3).

In *S. typhimurium* the time scales of adaptation, both evaluated at the level of methylation kinetics by mapping *F*(α) using exponential ramp stimuli (Figure 3.4), and at the level of the network by measuring the frequency response (Figure 3.5), are three-fold shorter than those observed in *E. coli*. The mechanistic origin of the faster adaptation to MeAsp of *S. typhimurium* likely lies in the properties of the populations of adaptation enzymes, CheR and CheB. However, using the parameters of the two transfer functions obtained for *S. typhimurium*, we were not able to predict the experimentally measured network-level adaptation timescale: according to equations (5) and (6), the three-fold lower cooperativity factor *N* of the response in *S. typhimurium* should cancel out the three-fold faster methylation kinetics. Thus, additional factors not represented in the model might contribute to the faster network-level adaptation timescale (i.e. higher characteristic frequency of the response) in wild type *S. typhimurium*. We ruled out the involvement of the *S. typhimurium* protein CheV in this
discrepancy, by showing that it does not affect either the cooperativity or the characteristic frequency of the response (Figure 3.6).

We observed differences between the sensitivity modulation profiles of *E. coli* and *S. typhimurium*, which determine the kinase-activity and drift-velocity dependence on the background level (see equations (9) and (10)). We have demonstrated that both a Tar-independent response and the properties of the Tar receptor itself contribute to the differences between the sensitivity-modulation profiles of the two species (Figure 3.8D, E). The Tar-independent response in *S. typhimurium* could be receptor-independent or mediated by one or more chemoreceptors. The fact that only minor partial adaptation is observed in Δtar ΔcheR ΔcheB cells (Figure 3.9B) suggests that the Tar-independent response does involve methylation / demethylation of chemoreceptors.

The model described in reference 260 assumes that each receptor dimer binds a single ligand molecule. Bieman and Koshland 32 indeed demonstrated that aspartate binds to *E. coli* Tar dimers with half-of-sites negative cooperativity, i.e. if one of the binding sites in the dimer is occupied, the second cannot bind aspartate. However, these authors also showed that the negative cooperativity of *S. typhimurium* Tar dimers is weaker, i.e. if one of the binding sites in the dimer is occupied, the second binds aspartate with lower affinity. If the two sets of dissociation constants, \( K_{I1}, K_{A1} \) and \( K_{I2}, K_{A2} \), of the two binding sites are separated sufficiently, the actual cooperativity of the response could be higher than the apparent one \((N=2)\), determined using the standard MWC model, which assumes a single binding site in each of \( N \) dimers in a complex.

Differences in the sensitivity profile between *S. typhimurium* and *E. coli* can explain differences in the behavioral response between the two species (Figure 3.7). Both the steady state chemotactic migration coefficients, \( |\text{CMC}|_{SS} \), and the inverse values of the exponential decay constants \( \lambda_{SS} \) of the steady-state distributions \( B(x)_{SS} \), \( (1/\lambda)_{SS} \) serve as a proxy for the drift velocity \( v_D \). Thus, as expected from the measured sensitivity profiles for *S. typhimurium* and *E. coli* and the \( v_D \) dependence on the sensitivity profile (equation (10)), \( |\text{CMC}|_{SS} \) and \( (1/\lambda)_{SS} \) for *S. typhimurium* show a single broad peak, whereas \( |\text{CMC}|_{SS} \) and \( (1/\lambda)_{SS} \) for *E. coli* – two adjacent plateaus (Figure 3.7C and Figure 3.7C Inset, note that the small difference in shape
Network-level variability in bacterial chemotaxis can be explained with the non-linear dependence of $|\text{CMC}|_{SS}$ on $v_0$). These profiles are in very good agreement with the profiles of kinase activity measured with FRET (Figure 3.8C).

The single plateau in *S. typhimurium* and two plateaus in *E. coli* observed at both the signaling (Figure 3.8C) and behavioral (Figure 3.7C) levels can be considered as fold-change detection (FCD) regimes: concentration ranges in which the entire time evolution of the response is invariant upon multiplication of both the stimulus and the background by a scalar constant $^{137,231}$ (see Chapter 2). The conditions for FCD regarding the main MeAsp receptor Tar, namely perfect adaptation (i), logarithmic sensing (ii) and linearity of the methylation-dependent free energy of the receptor-kinase complex (iii) $^{137}$, were also shown to hold in both species. Thus, the FCD property has been conserved in the two species, suggesting that it might provide a selective advantage to the bacteria.

What are the ecological implications of the observed differences in *S. typhimurium* and *E. coli* chemotactic signaling and migration behavior? The two species descended from a common ancestor, so the observed differences must reflect the consequences of natural selection in the relatively recent evolutionary history of the two species. Both species have two habitats: host (animals, primary habitat) and non-host (water, soil etc, secondary habitat) $^{274}$. *S. typhimurium* actively cycles between host and non-host environments, whereas *E. coli* has a low rate of survival in non-host environments and lower probability of colonizing a new host $^{106,274}$. Although such natural environments are not well characterized and therefore difficult to reproduce in experiments, differences in chemotactic characteristics might reflect the optimization of chemotaxis in enteric bacteria to different conditions. Future ecological studies in enteric bacteria could shed light on the ecological significance of the differences in chemotaxis that we have observed between *S. typhimurium* and *E. coli*.
3.11. Materials and methods

Bacterial strains and plasmids

The strains and plasmids used in this study are listed in Table 3.2.

In-frame chromosomal gene deletions were created using an allele replacement procedure, based on a modification of Datsenko and Wanner’s method 62, that does not leave a scar. It is based on an insertion cassette that contains the lethal ccdB gene under the control of a L-rhamnose-inducible promoter. This cassette is later removed by selection on rhamnose-minimal plates 288. Salmonella’s resident plasmid pSLT contains ccdA and ccdB genes and thus interfere with the positive selection strategy described above. Therefore it was displaced prior to allele replacements using Kit10 from Salmonella Genetics Stock Collection (SGSC): a plasmid pLL6, which is from the same compatibility group as pSLT, is transformed into the strain of interest, pSLT is cured, and pLL6 is subsequently removed using temperature selection 118.

Salmonella Tar (TarSt) constructs were designed by PCR amplification of genomic tarSt using primers that include AseI and BamHI restriction sites for ligating into NdeI and BamHI restriction sites on the sodium salicylate-inducible plasmid pKG116 (AseI and NdeI create compatible ends; this strategy was selected because of the presence of an NdeI site within tarSt sequence). TarSt mutants, in which glutamates in the methylation sites are replaced with glutamines, are created using site-directed mutagenesis using overlap PCR.

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Gene(s)</th>
<th>Resistance</th>
<th>Induction</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>pVS88</td>
<td>cheZ-ecfp / cheY-eyfp</td>
<td>ampicillin</td>
<td>IPTG</td>
<td>240</td>
</tr>
<tr>
<td>pKG116</td>
<td>Cloning vector</td>
<td>chloramphenicol</td>
<td>Sodium salicylate</td>
<td>J.S. Parkinson</td>
</tr>
<tr>
<td>pVS123</td>
<td>tarSt [QEQE]</td>
<td>chloramphenicol</td>
<td>Sodium salicylate</td>
<td>240</td>
</tr>
<tr>
<td>pML18</td>
<td>tarSt [QEQE]</td>
<td>chloramphenicol</td>
<td>Sodium salicylate</td>
<td>This work</td>
</tr>
<tr>
<td>pML20</td>
<td>tarSt [QEQQ]</td>
<td>chloramphenicol</td>
<td>Sodium salicylate</td>
<td>This work</td>
</tr>
<tr>
<td>pML21</td>
<td>tarSt [QQQQ]</td>
<td>chloramphenicol</td>
<td>Sodium salicylate</td>
<td>This work</td>
</tr>
<tr>
<td>pML22</td>
<td>tarSt [QQQQ]</td>
<td>chloramphenicol</td>
<td>Sodium salicylate</td>
<td>This work</td>
</tr>
<tr>
<td>pML23</td>
<td>tarSt [EEEE]</td>
<td>chloramphenicol</td>
<td>Sodium salicylate</td>
<td>This work</td>
</tr>
</tbody>
</table>
Network-level variability in bacterial chemotaxis

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>LT2</td>
<td>wild type <em>S. typhimurium</em> (official designation, <em>Salmonella enterica</em> serovar Typhimurium)</td>
<td><em>Salmonella</em> Genetic Stock Center</td>
</tr>
<tr>
<td>TSS500</td>
<td>LT2 $\Delta$cheY $\Delta$cheZ</td>
<td>This work</td>
</tr>
<tr>
<td>TSS515</td>
<td>LT2 $\Delta$cheY $\Delta$cheZ</td>
<td>This work</td>
</tr>
<tr>
<td>TSS878</td>
<td>LT2 $\Delta$cheY $\Delta$cheZ</td>
<td>This work</td>
</tr>
<tr>
<td>TSS1038</td>
<td>LT2 $\Delta$cheR $\Delta$cheB $\Delta$cheY $\Delta$cheZ</td>
<td>This work</td>
</tr>
<tr>
<td>14028</td>
<td>wild type <em>S. typhimurium</em> ATCC strain</td>
<td>R.M. Harshey</td>
</tr>
<tr>
<td>TSS863</td>
<td>14028 $\Delta$9T, i.e. $\Delta$cheR $\Delta$cheB $\Delta$cheY $\Delta$cheZ $\Delta$cheZ::Kan</td>
<td>This work</td>
</tr>
<tr>
<td>RP437</td>
<td><em>E. coli</em> wild type for chemotaxis</td>
<td>195</td>
</tr>
<tr>
<td>VS104</td>
<td>RP437 $\Delta$cheY $\Delta$cheZ</td>
<td>241</td>
</tr>
</tbody>
</table>

Table 3.2. Strains and plasmids used in this work. $\Delta$ and :: refer to deletion of, or deletion / substitution within the indicated gene, respectively. Kan refer to substitution with kanamycin-resistance cassette.

Growth conditions

Cells were grown at 250 rpm at 33.5°C in a rotary shaker to mid-exponential phase (OD$_{600}$ ~ 0.5) in tryptone broth (TB; 1% tryptone, 0.5% NaCl, pH 7.0) supplemented with appropriate antibiotics (100 µg/ml ampicillin, 34 µg/ml chloramphenicol) and inducers (150 µM IPTG, sodium salicylate, NaSal, with concentrations, indicated in the text). Cells were harvested by centrifugation, washed twice and resuspended in motility buffer (10 mM potassium phosphate, 0.1 mM EDTA, 1 µM methionine, 10 mM lactic acid, pH 7), and stored at 4°C for 1-5 h prior to the experiment.

In vivo FRET measurements and data analysis

FRET microscopy of immobilized bacterial populations was performed as described earlier 242. The FRET donor–acceptor pair - CheZ-CFP and CheY-YFP – was expressed from a plasmid pVS88 240. FRET fusion proteins were expressed in $\Delta$cheY $\Delta$cheZ strains.
Cells were attached to a poly-L-lysine–coated microscope coverslip, seated at the top face of a flow cell \(^{27}\), and kept under constant flow of motility buffer, generated by either a peristaltic pump (Rainin Dynamax RP1) or syringe pump (Harvard Apparatus, PHD2000). The flow cell was mounted on an upright microscope (Nikon FN1), equipped with an oil immersion objective (Nikon CFI Plan Fluor, 40x/1.3). The sample was illuminated by a metal halide arc lamp with closed-loop feedback (EXFO X-Cite exacte) through an excitation bandpass filter (Semrock, FF01-438/24-25) and a dichroic mirror (Semrock, FF458-Di01). The epifluorescent emission was split by a second dichroic mirror (Semrock, FF509-FDii01) into donor (cyan, C) and acceptor (yellow, Y) channels and collected through emission bandpass filters (Semrock FF01-483/32 and FF01-542/27 for the C and Y channels, respectively) by two photon-counting photomultipliers (Hamamatsu H7422P-40).

Signal intensities of the donor and acceptor channels were recorded through a data acquisition card (National Instruments) installed on a PC running custom-written software. After subtraction of coverslip background, the ratio \( R \) between the two channels, \( R = Y/C \) provided an indicator of FRET activity robust to fluctuations in excitation intensity. The change in FRET efficiency upon stimulation, \( \Delta \text{FRET} \), was computed at every time point from the donor and acceptor fluorescence

\[
\Delta \text{FRET} = \frac{R_{\text{pre}} + \Delta R - R_0}{R_{\text{pre}} + |\Delta Y/\Delta C|} - \frac{R_{\text{pre}} - R_0}{R_{\text{pre}} + |\Delta Y/\Delta C|},
\]

where \( \Delta R = R - R_{\text{pre}} \) is the ratio change, \( R_0 \) is the acceptor to donor ratio in absence of FRET, \( R_{\text{pre}} \) is the pre-stimulus acceptor to donor ratio, and \( |\Delta Y/\Delta C| \approx 0.6 \) for our setup is the constant absolute ratio between the changes in the acceptor and donor signals per FRET pair \(^{242}\). However, under our FRET measurement conditions, \( R_{\text{pre}} + |\Delta Y/\Delta C| \gg \Delta R \); thus \( \Delta \text{FRET} \sim \Delta R \). For simplicity we expressed \( \Delta \text{FRET} \) in arbitrary units of \( \Delta R \) throughout the study.

\( \Delta \text{FRET}(t) \) was normalized to the absolute magnitude of the response to addition of a saturating attractant step, \( |\Delta \text{FRET}_{\text{sat}}^{\text{add}}| \), to compensate for variations between different experiments. The steady-state kinase activity, \( a_0 \), was calculated as

\[
a_0 = \frac{|\Delta \text{FRET}_{\text{sat}}^{\text{add}}|}{|\Delta \text{FRET}_{\text{sat}}^{\text{add}}| + |\Delta \text{FRET}_{\text{sat}}^{\text{remove}}|},
\]

where \( \Delta \text{FRET}_{\text{sat}}^{\text{remove}} \) is the response to removal of a saturating attractant step after the cells have been
Network-level variability in bacterial chemotaxis

completely adapted. The kinase activity \( a = a_0 + \Delta a \), where \( \Delta a = \frac{\Delta \text{FRET}}{[\Delta \text{FRET}^\text{sat}] + [\Delta \text{FRET}^\text{remove}]} \) is the kinase activity change in every point in time.

In dose-response measurements to the non-metabolizable chemoeffector \( \alpha \)-methyl-DL-aspartic acid (MeAsp; Sigma Aldrich) bacteria were adapted to a background level of MeAsp, \([L]_0\), and by using a fluidic switch (Hamilton, valve HV 3-2) that could rapidly select between input flow channels with different concentrations \([L]\), cells were exposed to a sequence of steps of addition and removal of MeAsp of increasing magnitude. A Hill function, \( a = a_l + (a_h - a_l) \frac{[L]^n}{[L]^n + [K_{1/2}]^n} \), where \( K_{1/2} \) is the ligand concentration of the half-maximum of the response, \( n_H \) is the Hill coefficient, and \( a_l \) and \( a_h \) are the lowest and the highest kinase activity in the dose-response curve, was fit to each dose-response curve.

To subject the cells to input modulations, smoothly varying in time, the control of the temporal profile of chemoeffector stimulus was achieved by mixing a concentrated solution of chemoeffector and motility buffer in a fluid mixer of a type described before 226. The mixing chamber was a cylinder with internal volume \( V_{\text{mix}} \approx 100 \mu l \), with two input channels and two output channels. The in-flow rate, \( \beta_1 \), of the concentrated MeAsp was modulated using a computer-controlled syringe pump (Harvard Apparatus, PHD2000). The in-flow rate of the buffer, \( \beta_2 (\beta_2 \gg \beta_1) \), was kept constant (~2 ml/min) throughout the experiment. Thus, the output chemoeffector concentration \([L](t) = [L]^* \frac{\beta_1(t)}{\beta_1(t)+\beta_2} \), where \([L]^*\) is the MeAsp concentration in the syringe. \([L](t)\) is proportional to \( \beta_1 \) as long as the latter is varied smoothly over time scales much greater than the mixing time, \( \tau_{\text{mix}} = \frac{V_{\text{mix}}}{\beta_1+\beta_2} \).

Microfluidics experiments and data analysis

Microfluidics experiments were performed in a hydrogel-based gradient generator device with a design identical to “Design 1” described in reference 7. The device consisted of three parallel channels, each 600 µm wide, with 200 µm spacing between the channels. Both the flanking channels (“source” and “sink”) and the “test” channel (150 µm deep) were
patterned in a layer of a polydimethylsiloxane (PDMS) (Sylgard 184). The PDMS layer was placed on top of a 1-mm-thick agarose hydrogel layer (3% wt/vol agarose in motility buffer), which was positioned on top of a glass slide. Outlets of the source and sink channels were connected by metal connectors and flexible polyethylene tubing to two plastic syringes of 3 ml, driven by a syringe pump (Harvard Apparatus, PHD 2000). A constant flow rate of 1 µl/min was maintained with two different concentrations of MeAsp in the source and the sink. Diffusion between the two feeder channels, separated edge to edge by a distance $L = 1$ mm, leads to an establishment of a gradient across the agarose in ~ 20 min.

Before injection, bacteria were pre-adapted for at least 15 min to the mean concentration of the source and the sink channels (i.e. the mean concentration in the test channel, $[L]_{\text{mean}}$). After injection of bacterial suspension and immediate termination of the residual flow using T-shaped switch (Hamilton, valve HV 3-2), a gradient mirroring that in the underlying agarose layer is rapidly established in the test channel (the height of the test channel is $H = 150$ µm, and the diffusion coefficient for MeAsp is assumed to be the typical one for small molecules in water, $D = 5 \times 10^{-10}$ m² s⁻¹).

The linearity of the gradient in the test channel of this devise was previously verified. In each gradient the ratio between the concentrations of MeAsp in the source, $[L]_{\text{source}}$, and in the sink, $[L]_{\text{sink}}$ was kept constant (Table 3.3), and therefore the steepness of the expected linear gradients also remained constant. The magnitude of the actual gradient was estimated to be ~ 40% of the predicted gradient.

The distribution of the bacteria within the test channel was observed using phase-contrast microscopy. Bacteria were observed close to the agarose layer with an inverted microscope (Nikon Eclipse), using a 20x objective (phase-contrast, ELWD). Movies were recorded using an Andor Neo sCMOS camera, controlled by Nikon NIS-Elements control software at 10 frames per second for 15 min, and the image acquisition started 10-30 s after injection of the bacteria. Image analysis was performed using MATLAB. Immotile cells were removed by subtracting a mean image from each frame, followed by locating the positions of the motile cells.
Subsequent binning yielded the cell concentration profile, \( B(x, t) \), along the direction of the gradient \( x \) at each time point \( t \).

\[
\text{Table 3.3. Concentrations of MeAsp and gradients used in the microfluidics gradient generator.}
\]

<table>
<thead>
<tr>
<th>( [L]_{\text{sink}} ) mM</th>
<th>( [L]_{\text{mean}} ) mM</th>
<th>( [L]_{\text{source}} ) mM</th>
<th>Predicted gradient ( \frac{d[L]}{dx} ) mM/mm</th>
<th>Actual gradient ( \frac{d[L]}{dx} ) mM/mm</th>
<th>Relative gradient ( \frac{1}{[L]_{\text{mean}}} \frac{d[L]}{dx}, 1/mm )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.002</td>
<td>0.005</td>
<td>0.008</td>
<td>0.007</td>
<td>0.003</td>
<td>0.5</td>
</tr>
<tr>
<td>0.006</td>
<td>0.018</td>
<td>0.030</td>
<td>0.024</td>
<td>0.010</td>
<td>0.5</td>
</tr>
<tr>
<td>0.021</td>
<td>0.064</td>
<td>0.107</td>
<td>0.085</td>
<td>0.034</td>
<td>0.5</td>
</tr>
<tr>
<td>0.076</td>
<td>0.228</td>
<td>0.380</td>
<td>0.304</td>
<td>0.122</td>
<td>0.5</td>
</tr>
<tr>
<td>0.271</td>
<td>0.814</td>
<td>1.357</td>
<td>1.085</td>
<td>0.434</td>
<td>0.5</td>
</tr>
<tr>
<td>0.968</td>
<td>2.903</td>
<td>4.838</td>
<td>3.871</td>
<td>1.548</td>
<td>0.5</td>
</tr>
<tr>
<td>3.449</td>
<td>10.35</td>
<td>17.24</td>
<td>13.795</td>
<td>5.518</td>
<td>0.5</td>
</tr>
<tr>
<td>12.29</td>
<td>36.87</td>
<td>61.45</td>
<td>49.161</td>
<td>19.665</td>
<td>0.5</td>
</tr>
</tbody>
</table>

The strength of the chemotaxis response was evaluated by the absolute value of the dimensionless chemotactic migration coefficient (CMC): \( |CMC| \equiv \frac{|(\langle x \rangle(t) - W/2)/W|}{1+W/2} \), where \( W = 600 \, \mu\text{m} \) is the width of the test channel, and \( \langle x \rangle(t) \) is the population-averaged spatial coordinate, \( \int_{-W}^{W} x B(x, t) dx \), of the bacteria along the chemoeffector gradient \(^{156}\).

The distribution of the bacteria is characterized by advection-diffusion equation \( \frac{\partial B(x, t)}{\partial t} = D_B \nabla^2 B - \nabla (v_D B) \), where \( D_B \) is the random motility coefficient measuring the diffusivity of a population of bacteria resulting from their random walk behavior, and \( v_D \) is the drift velocity of the bacterial population \(^{115}\). At steady state, \( \frac{\partial B(x, t)}{\partial t} = 0 \); thus \( B(x)_{SS} = B_0 e^{-x/\lambda_{SS}} \), where \( B_0 \) is the cell density in the channel edge \( (x = 0) \) and \( \lambda_{SS} = \langle x \rangle_{SS} \), where \( \langle x \rangle_{SS} \) is the mean steady-state position of the bacterial population in the channel. \( \lambda_{SS} \) can be expressed as \( \lambda_{SS} = \frac{2}{v_D} \), therefore \( |CMC|_{SS} \equiv \left| 2 \frac{\lambda_{SS}}{W} - 1 \right| = \left| 2 \frac{D_B}{v_D W} - 1 \right| \).
The dynamics of the *E. coli* chemotaxis pathway is captured by two equations, which link the three dynamic variables: input $[L](t)$, output $a(t)$ and memory $m(t)$:

$$\frac{dm}{dt} = F(a),$$  \hspace{1cm} (13)

$$a = G([L], m) = G(f_t([L], m)) = \left(1 + e^{f([L], m)}\right)^{-1},$$  \hspace{1cm} (14)

The differential equation (13) expresses the rate of change of the feedback signal, $m$, as a function $F$ of the current signal output, $a$. Perfect adaptation follows as the function $F(a)$ has a single stable fixed point at $a = a_0$ with steady-state kinase activity $a_0$ given by $F(a_0) = 0$. The receptor-kinase activity, $a$, relaxes much rapidly, so its value at each moment in time is given by the algebraic equation (14). A variant of the Monod-Wyman-Changeux (MWC) allosteric model \cite{Allosteric} has been used to prescribe a specific form of the function $G$, which denotes the dependence of the kinase activity, $a$, on the current level of ligand input, $[L]$, and methylation feedback signal, $m$, via the free energy difference between the active and inactive states of the receptor-kinase complex,

$$f_t([L], m) = N(f_t([L]) + f_m(m)),$$  \hspace{1cm} (15)

where $N$ is the number of ligand-binding receptor units cooperatively associated with each kinase molecule. As in the classical MWC model \cite{Allosteric}, the effect of ligand depends on the active and inactive states for the receptor having distinct affinities for ligand, leading to the expression

$$f_t([L]) = \ln \frac{1+([L]/K_i)}{1+([L]/K_A)},$$  \hspace{1cm} (16)

where $K_i$ and $K_A$ are the dissociation constants for the inactive and active receptor states, respectively. From equation (16), it follows that $f_t$ is linear in $\log[L]$ within the range $K_i \ll [L] \ll K_A$ when the ratio between $K_A$ and $K_i$ values is large \cite{Linarity, Allosteric}.

A feature that is not present in the classical MWC model \cite{Allosteric} is the methylation-dependent free energy, $f_m(m)$. FRET-based studies in *E. coli* \cite{FRET} showed that it is well-approximated by a linear function,

$$f_m(m) = \alpha(m^* - m),$$  \hspace{1cm} (17)
Network-level variability in bacterial chemotaxis

where $\alpha$ is the slope of $f_m(m)$, and $m^*$ is the offset methylation level at which $f_m(m) = 0$. $\alpha \approx 2$ and $m^* \approx 0.5$ yielded good fits to *E. coli* experimental data.

Data from wild type *E. coli* taken at room temperature was well fit by the following parameters: $N=6$, $a_0=0.33$, $K_I = 0.018 \text{ mM}$, $K_A = 2.903 \text{ mM}$.

The function $F(a)$ was described by a Michaelis-Menten equation with a variable gain

$$F(a) = V_R \frac{1-a}{K_R+1-a} - V_B(a) \frac{a}{K_B+a}$$

(18)

where

$$V_B(a) = V_B(0) \left(1 + \theta(a - a_B) \frac{a-a_B}{1-a_B} r_B \right).$$

(19)

$K_R$ and $K_B$ are the Michaelis constants, and $V_R$ and $V_B(a)$ - the maximal velocities of the methylation and demethylation reactions (catalyzed by CheR and CheB respectively), and $V_B(a)$ is a piecewise linear function, the value of which remains $V_B(0)$ for values of $a$ below $a_B$, and above $a_B$ increases with a slope $\frac{r_B}{1-a_B}$ up to a maximal value $(1+r_B)V_B(0)$, implemented by use of a step function $\theta(a - a_B)$, defined as $\theta(a - a_B) = 1$ for $a > a_B$, $\theta(a - a_B) = 0$ otherwise. For *E. coli*, the fit parameters are $V_R = 0.010 \text{ s}^{-1}$, $V_B(0) = 0.013 \text{ s}^{-1}$, $K_R = 0.32$, $K_B = 0.30$, $a_B = 0.74$, $r_B = 4.0$ (all concentrations are normalized, in units of the CheA concentration: $K_R$ and $K_B$ are dimensionless).

For *S. typhimurium*, we fitted the data with $V_R = 0.030 \text{ s}^{-1}$, $V_B(0) = 0.030 \text{ s}^{-1}$, $K_R = 0.45$, $K_B = 0.30$, $a_B = 0.74$.

Chemotactic drift velocity in shallow gradients

*S. typhimurium* and *E. coli* in liquid media either run smoothly, or tumble, changing their swimming direction. We consider a bacterial population executing run-and-tumble motility with a mean run speed $v$ and average run length $\tau_0$ in a shallow gradient, i.e. a gradient $\nabla[L]$ that is small enough that a change in the kinase activity, $\Delta a$, caused by a run of length $v\tau_0$ along the gradient is much smaller than the initial kinase activity $a_0$ ($\Delta a/a_0 \ll 1$). The chemotactic drift velocity

$$v_d = v \frac{\tau_+ - \tau_-}{\tau_+ + \tau_-}$$

(20)

where $\tau_+$ and $\tau_-$ are the average run times for bacteria swimming up and down the gradient respectively. These run durations are determined by the rotational bias of the flagellar motors.
\[ \langle CW \rangle = \frac{\tau_{CW}}{\tau_{CW} + \tau_{CCW}} \]  

where \( \tau_{CW} \) and \( \tau_{CCW} \) are the average clockwise (CW) and counter clockwise (CCW) intervals, respectively. The rotational bias is controlled by the kinase activity, \( a \), and is well-fit by a Hill equation:

\[ \langle CW \rangle = \frac{a^H}{a^H + a_{1/2}^H} \]  

where \( a_{1/2} \) is the kinase activity at which \( \langle CW \rangle = \frac{1}{2} \), and \( H \) is the motor Hill coefficient. From equations (21) and (22) follows that:

\[ \tau_{CCW} = \gamma a^{-H} \]  

where \( \gamma = \tau_{CW} a_{1/2}^H \) is a constant, assuming that \( \tau_{CW} \) and \( a_{1/2} \) are not affected by changes in \( a \).

During each run, the kinase activity changes from \( a_0 \) to \( a_0 + \Delta a \) if the run is in the direction up the gradient, and from \( a_0 \) to \( a_0 - \Delta a \) if the run is in the direction down the gradient. Thus, using equations (20) and (23) we can write

\[ v_d = v \left( \frac{(1 - \Delta a/a_0)^H - (1 + \Delta a/a_0)^H}{(1 - \Delta a/a_0)^H + (1 + \Delta a/a_0)^H} \right) \]  

Considering our shallow gradient condition \( \Delta a/a_0 \ll 1 \), we have

\[ (1 \pm \Delta a/a_0)^H \approx 1 \pm H (\Delta a/a_0) \], which leads to a simple expression for the drift velocity:

\[ v_d \approx -vH \frac{\Delta a}{a_0} \]  

Note that the simple derivation for \( v_d \) given above neglects the fact that \( \Delta a \) is not constant in time. Nevertheless, we can approximate \( \Delta a \) in equation (25) by a time-averaged value \( \overline{\Delta a} \), expanding \( \Delta a(t) = \left( \frac{\Delta a}{dt} \right) t + O(t^2) \) and averaging over the run interval \( \tau \),

\[ \overline{\Delta a} = \frac{1}{\tau} \int_0^\tau \Delta a dt + \frac{1}{2} \left( \frac{\Delta a}{dt} \right) (t^2 + \ldots) \]  

where the last approximate equality is valid if \( \frac{\Delta a}{dt} \) is approximately constant over the time interval \( \tau \). To obtain \( \frac{\Delta a}{dt} \) explicitly, we appeal once again to the shallow gradient condition and linearize the two-state MWC model (equations (1) and (4)) about \( a = a_0 \) to obtain its rate of change in time,

\[ \frac{da}{dt} = -\Delta a(t)/\tau_m + \frac{\partial a}{\partial f_L} \frac{\partial f_L}{\partial \ln[L]} \frac{d}{dx} \ln[L] \]  

Chapter 3
where $f_t$ is the total free energy of the receptor-kinase complex, and

$$\tau_m = \left(\frac{\partial f_t}{\partial m} F'(a_0)\right)^{-1}$$

identifies with the adaptation timescale defined earlier in equations (5) and (6). Further differentiation of equation (26) yields for higher time derivatives

$$\frac{d^{n+1}a}{dt^{n+1}} = \tau_m^{-n} \frac{d^n a}{dt^n}$$

for all $n \geq 1$, so $\Delta a(\tau) = \left(\frac{da}{dt}\right) \tau + \mathcal{O}((\tau/\tau_m)^2)$. Thus, the truncation of the series upon averaging over the run interval $\tau$ is valid whenever $\tau \ll \tau_m$.

Combining equations (26) and (27), and considering that $\tau = \tau_0 (1 + \Delta a/a_0)^{-H}$ and $(1 \pm \Delta a/a_0)^H \approx 1 \pm H (\Delta a/a_0)$ for $\Delta a/a_0 \ll 1$, we obtain

$$\Delta a \approx -\frac{a_0}{H} (1 - a_0) \psi([L]_0) \frac{d\ln[L]}{dx}$$

(28)

which is a quadratic equation for $\Delta a$ and has solutions $\Delta a = -\beta/2 \pm \sqrt{(\beta/2)^2 - \delta}$ with $\beta = \frac{a_0}{H} (1 + \frac{\tau_0}{\tau_m})$ and $\delta = \frac{a_0}{H} (1 - a_0) v(\tau_0/2) \frac{d\ln[L]}{dx}$. Further, if $\frac{d\ln[L]}{dx} \ll (1 + \frac{\tau_0}{\tau_m})^2 / (4HN(1 - a_0) v\tau_0/2)$, then $(\beta/2)^2 \gg \delta$ and the nonnegative solution reduces to $\Delta a \approx -\delta/\beta$, yielding

$$\Delta a \approx -\frac{a_0 (1 - a_0) \psi([L]_0) e\tau_0}{2 \tau_m} \frac{d\ln[L]}{dx}$$

(29)

where $\psi([L]_0)$ is the sensitivity-modulation profile of the receptor module, defined in equation (12).

Finally, using $\Delta a$ from equation (29) for $\Delta a$ in equation (25), we obtain an expression for the drift velocity in the shallow-gradient limit:

$$v_D \approx \frac{v_0^2 H (1 - a_0) \psi([L]_0) \frac{d\ln[L]}{dx}}{2 + \frac{\tau_0}{\tau_m}}$$

(30)

Two receptor species MWC model for chemotaxis signaling

If the ligand is sensed by two different receptor species within the same MWC cluster, $f_t$ can be written as

$$f_t = N_1 \left( \ln \left[ \frac{1 + [L]/K_{I1}}{1 + [L]/K_{A1}} \right] + f_{m1} \right) + N_2 \left( \ln \left[ \frac{1 + [L]/K_{I2}}{1 + [L]/K_{A2}} \right] + f_{m2} \right)$$

(31)

where $(N_1, K_{I1}, K_{A1}, f_{m1})$ and $(N_2, K_{I2}, K_{A2}, f_{m2})$ are the number of ligand-binding receptor units, the dissociation constants for the inactive and active receptor, and the methylation-dependent free energy for the first and the
second receptor species respectively. This leads to a sensitivity modulation profile

$$\psi([L]_0) = N_1 \left( \frac{[L]_0}{[L]_0 + K_{i1}} - \frac{[L]_0}{[L]_0 + K_{A1}} \right) + N_2 \left( \frac{[L]_0}{[L]_0 + K_{i2}} - \frac{[L]_0}{[L]_0 + K_{A2}} \right)$$

(32)

3.12. Acknowledgements

I have preformed the microfluidics work presented in this chapter in the lab of Prof. Dr. Roman Stocker (Massachusetts Institute of Technology), together with Dr. Filippo Menolascina from Stocker group. Eduardo Sontag (Rutgers Institute) and Roman Stocker provided helpful comments. Simone Boskamp provided help with the cloning of the different Tar$^{st}$ modifications on plasmids.