Fold-change detection as a chemotaxis model discrimination tool

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Abstract—Fold-change detection (FCD) is the property that a dynamical system with an adapting output will exhibit identical transient output responses when its input signals are scaled. This feature was recently demonstrated in the chemotactic response of the bacterium Escherichia coli, confirming earlier theoretical predictions. The chemotaxis pathway of the bacterium Rhodobacter sphaeroides has the same modular structure as that in E. coli but is significantly more complex in that it has multiple homologues of the latter’s chemotaxis proteins and features two, rather than one, chemosensory cluster. Recent experimental results suggest that R. sphaeroides may also exhibit FCD.

In this paper, we present a set of theoretical assumptions on the dynamics of the R. sphaeroides chemosensory system, and use these to fit an integrated chemotaxis model to experimental data. We then show that the assumptions we place are sufficient to make FCD a robust property of this chemotaxis pathway, in agreement with preliminary experimental evidence. We argue that the fact that the model we present here is able to reproduce this transient dynamic property whilst earlier models cannot makes FCD a useful tool for model discrimination on the basis of transient dynamic response. This is in contrast to earlier model discrimination methods which tested the validity of models based on their ability to reproduce a finite set of experimental data. Further experiments that can provide additional validation of our theoretical assumptions are suggested.

I. INTRODUCTION

Mathematical modeling has played an important role in explaining key features of biological systems, such as their response to stimuli and their robustness properties. These advances have traditionally been realized through a cyclical process of model construction and validation, the latter typically involving a comparison of simulated outputs with experimental observation.

This paper gives an overview of a complementary general approach to model falsification, based on the use of the qualitative properties of the transient responses of dynamical systems with adapting outputs, as described in [1]. We first present a model of the chemotaxis pathway of the bacterium Rhodobacter sphaeroides. Departing from previous representations of this system, this model assumes that the bacterium’s receptor dynamics can be approximated using the Monod-Wyman-Changeaux (or, MWC) allosteric model [2], as has recently been done with Escherichia coli chemotaxis models [3], [4], [5]. It is shown that this model can reproduce previously published experimental time-series data.

We then discuss a general mathematical result given in [1] that shows that in certain concentration ranges, scaling the ligand input signals detected by the bacterium leads to the same chemotactic output response, a behavior termed fold change detection (FCD), recently observed experimentally in E. coli. Recent experimental results in [6] suggest that R. sphaeroides, like E. coli, also exhibits this behavior. Unlike previous R. sphaeroides chemotaxis models, we show, both analytically and through simulations, that the model we present here is capable of displaying FCD. In this way, we are able to invalidate previous models through testing for a characteristic transient response that does not rely on fitting to a fixed set of time-series data.

We additionally discuss how the assumptions we place on this model make FCD a feature of the chemotactic response of this bacterium that is robust to substantial variation in the pathway’s signaling structure. This is in line with experimental results also presented in [6] that FCD appears to feature in bacteria that have different cell architectures arising from different growth conditions.

II. BACKGROUND

The chemotaxis pathways of the bacteria E. coli and R. sphaeroides share homologies and a fundamental modular structure illustrated in Figure 1. In both, receptor proteins are responsible for sensing the presence of ligands in their local environment. These signals are then transduced through phosphorylation to a cascade of chemotaxis proteins, and the net effect of ligand sensing is a reduction in this kinase activity of the receptors. The protein CheY in E. coli and its homologues CheY3, CheY4 and CheY5 in R. sphaeroides, when phosphorylated, interact with the bacteria’s flagellar motors in a way that increases their stopping rates (see [7]). At the same time, protein CheB in E. coli and its homologues CheB1 and CheB2 in R. sphaeroides act as methylesterases. De-methylation of the receptors by the CheB proteins has the effect of reducing the receptors’ kinase activity, and thus the CheB proteins in the two bacteria can be viewed as negative feedback signals. Viewed holistically, this feedback system causes the bacteria to climb attractant ligand gradients: a sensed increase in attractant concentration causes the amount of phosphorylated CheY and CheB protein to fall. The fall in CheY proteins leads to a lengthening of the bacterium’s average swim length (due to a decrease in its stopping frequency). However, the fall in CheB proteins reduces the de-methylation rate at the receptors, thus causing a resurgence in their kinase activity and a restoration of the pre-stimulus steady state stopping rate of the flagellar motors [8]. This return to the steady state stopping frequency
is termed adaptation, and has been studied extensively and modeled as a phenomenon that arises from a biochemical mechanism that parallels integral control [9], [10], [7].

The chemotaxis pathway in R. sphaeroides differs from that in E. coli in one key respect however: in addition to the set of chemosensory receptors that span the cell membrane in the two bacteria, R. sphaeroides features an additional set of receptors found within its cytoplasm, and it is believed that, in addition to integrating metabolic information from within the cell, information about the external ligand concentration is conveyed to this internal set of receptors [7].

As in [11], [12], [13], the dynamics of a single receptor cluster can be modeled as a first-order system. Since R. sphaeroides has two such clusters, its sensing dynamics can be modeled as two such systems that are coupled. The average degree of methylation of the membrane receptors is denoted by the state \( m \), and its kinase activity, \( a \) (the ‘output’ of this system in control theoretic terminology) is a static function of \( m \) and the ligand concentration sensed by the membrane receptors, \( L \) (the ‘input’). Analogously, at the cytoplasmic cluster, we have methylation state \( \tilde{m} \), a kinase activity output \( \tilde{a} \) and cytoplasmic sensed ligand input \( \tilde{L} \). For simplicity, we assume that \( \tilde{L} = L \), although this restriction is relaxed in [1] (in the remainder of this paper, signals associated with the cytoplasmic cluster will be similarly denoted with a tilde). A simple model of the receptor dynamics is then given by

\[
\begin{align*}
\dot{m} &= F(a, x) \quad a = G(m, L) \\
\dot{\tilde{m}} &= \tilde{F}(\tilde{a}, \tilde{x}) \quad \tilde{a} = \tilde{G}(\tilde{m}, \tilde{L})
\end{align*}
\]  
(1)

where \( x \) is a vector of signals that generally represents the levels of phosphorylated chemotaxis proteins. It features in the methylation dynamics due to the role of the CheB, CheB\(_2\) proteins in de-methylating the receptors. As in [8], [11], the condition for adaptation, that only inactive receptors undergo methylation and only active receptors undergo de-methylation, leads to the functions \( F \) and \( \tilde{F} \) being monotonically decreasing functions of \( a \) and \( \tilde{a} \) respectively.

The kinase activities of the two receptors are transduced to the CheB and CheY chemotaxis proteins via the proteins CheA\(_2\) (at the membrane cluster) and CheA\(_3\) (at the cytoplasmic cluster): the auto-phosphorylation rates of these two proteins are modeled as being proportional to \( a, \tilde{a} \) respectively. The CheY and CheB proteins then compete for phosphoryl groups from CheA\(_2\), CheA\(_3\): CheA\(_2\) phosphorylates CheY\(_3\), CheY\(_4\), CheY\(_6\), CheB\(_1\) and CheB\(_2\) whilst CheA\(_3\) phosphorylates CheB\(_2\) and CheY\(_6\). The reaction rates for all of these phosphorylations are given in [14], [12]. As in [12], this phosphotransfer network is represented as a nonlinear system, with a state vector that gives the concentration of phosphorylated chemotaxis proteins:

\[
x = \begin{bmatrix} A_2 \ Y_3 \ Y_4 \ A_3 \ Y_6 \ B_1 \ B_2 \end{bmatrix}^T
\]

and dynamics governed by

\[
\dot{x} = H(x, a, \tilde{a})
\]

where \( H(x, a, \tilde{a}) \) in [12]. The equations (1) and (2) then fully represent the sensing and signal transduction dynamics of the R. sphaeroides chemotaxis system.

The precise mechanism through which the CheY proteins interact with the flagellum is unknown. Experiments show that CheY\(_6\) and either one of CheY\(_3\) and CheY\(_4\) are required for chemotaxis [7]. A range of possible connectivities between the CheY proteins and the flagellum were studied in [12], where it was even suggested that CheY\(_3\) and CheY\(_4\) could interact with the cytoplasmic cluster, affecting its kinase activity, whilst CheY\(_6\) stops the flagellar motors. There is additional uncertainty about the connectivity of the CheB proteins with the two sensing clusters. In [13] it is suggested that the protein CheB\(_1\) de-methylates the membrane cluster whilst CheB\(_2\) de-methylates both clusters, and this connectivity is used in the model presented here. It is shown in [1] that under some theoretical assumptions, which we will summarize in the next section, FCD can be displayed by the bacterium despite this uncertainty in the precise structure of the biochemical reaction network.

III. MODELING CHEMOTAXIS IN R. SPHAEROIDES

A. An MWC model of receptor dynamics

In several works such as [15], [16], [17], [11], the Monod-Wyman-Changeaux allostERIC model [2] has been employed to approximate chemoreceptor activity. The model starts with the assumptions that receptors can be in either an active state or an inactive state, and that ligands bind more strongly with active receptors than with active receptors. This latter quality is quantified by the dissociation constants between ligands and inactive receptors, \( K_j, \tilde{K}_j \) being lower than the dissociation constants between ligands and active receptors \( K_A, \tilde{K}_A \). The quantities \( a(t) \) and \( \tilde{a}(t) \) given in the previous section respectively represent the probabilities of activity of the membrane and cytoplasmic clusters at any given time. An approximation of this probability for the membrane receptors is obtained by first denoting the free energy of active receptors by \( E_A \) and of inactive receptors by \( E_I \). From [18], [16], [11] the probability of activity is approximated by the ratio of the Boltzman factor of the active state to the sum of the Boltzman factors of the active and the inactive states:

\[
a(t) = \frac{\exp(-E_A)}{\exp(-E_I) + \exp(-E_A)} = \frac{1}{1 + \exp[-E_\Delta]}
\]

where \( E_\Delta = E_I - E_A \) is the free energy difference between the active and inactive states. Similarly, for the cytoplasmic
cluster we have the approximation
\[ \tilde{a}(t) = \frac{\exp(-\tilde{E}_A)}{\exp(-E_I) + \exp(-\tilde{E}_A)} = \frac{1}{1 + \exp(-\tilde{E}_A)} \] (4)

with \( \tilde{E}_A = E_I - L_A \). As discussed in [19], we assume there are two contributions to the free energy differences \( E_{\Delta}, \tilde{E}_{\Delta} \), one from the degree of methylation of receptors (quantified by functions \( g_m(m) \) and \( \tilde{g}_m(\tilde{m}) \)) and the other from the sensed ligand concentration (quantified by functions \( g_L(L) \) and \( \tilde{g}_L(\tilde{L}) \)). We further assume that these two contributions can be combined additively, so that
\[ E_{\Delta} = -N[g_m(m) + g_L(L)] \quad \text{and} \quad \tilde{E}_{\Delta} = -\tilde{N}[\tilde{g}_m(\tilde{m}) + \tilde{g}_L(\tilde{L})] \]

with \( N, \tilde{N} \) representing the degree of cooperativity arising from the clustering of chemoreceptors [18].

The contributions to the free energy differences from receptor methylation, \( g_m(m) \) and \( \tilde{g}_m(\tilde{m}) \), are taken to be affine, decreasing functions of \( m, \tilde{m} \) respectively as was shown to be the case for \( E. coli \) in [19], with
\[ g_m(m) = \alpha (m_0 - m) \quad \text{and} \quad \tilde{g}_m(\tilde{m}) = \tilde{\alpha} (\tilde{m}_0 - \tilde{m}) \]

where \( \alpha = \tilde{\alpha} = 2 \) and \( m_0 = \tilde{m}_0 = 5 \).

The contribution from ligand binding is proportional to the logarithm of the ligand concentrations \( L, \tilde{L} \), which arises from the fact that there is a loss in ligand translational entropy when ligands bind to the receptors [16], [11]. The loss in entropy is greater in the case of ligands binding to active receptors than to inactive receptors due to the lower affinity to the former, and this is approximated by \(-\ln(L/K_A)\) and \(-\ln(\tilde{L}/K_{A})\) for the case of active receptors and \(-\ln(L/K_I)\) and \(-\ln(\tilde{L}/K_{I})\) for inactive receptors. As in [16], [11], the contribution of ligand binding to the free energy differences of the receptors between their active an inactive states is then given by
\[ g_L(L) = \ln \left( 1 + \frac{L}{K_I} \right) - \ln \left( 1 + \frac{L}{K_A} \right) \]
\[ \tilde{g}_L(\tilde{L}) = \ln \left( 1 + \frac{\tilde{L}}{K_I} \right) - \ln \left( 1 + \frac{\tilde{L}}{K_A} \right) \]

for the membrane and cytoplasmic clusters respectively. Note that since \( K_I < K_A \) and \( K_I < K_{\tilde{A}} \), the greater affinity of ligands for inactive receptors makes \( a(t) \) and \( \tilde{a}(t) \) decreasing functions of \( L \) and \( \tilde{L} \) respectively. From the [11], we adopt the values \( K_I = K_{\tilde{A}} = 18 \mu M, K_A = K_{\tilde{A}} = 3 \mu M \).

With this framework, we now make the important observation, analogous to that in [3], that in the ligand concentration range \( K_I \ll L \ll K_A \) and \( K_I \ll \tilde{L} \ll K_{\tilde{A}} \), the chemoreceptor activities can be approximated by
\[ a = \frac{1}{1 + \left[ \exp(\alpha[m_0 - m]) \frac{L}{K_I} \right]^N} \quad \text{and} \quad \tilde{a} = \frac{1}{1 + \left[ \exp(\tilde{\alpha}[\tilde{m}_0 - \tilde{m}]) \frac{\tilde{L}}{K_I} \right]^N} \] (5)

This observation will be used in Section IV in determining the ligand concentration range in which FCD can be observed.

B. An integrated \textit{R. sphaeroides} chemotaxis model

In Section III-A we outlined the physical basis for the MWC model of the chemotaxis receptors which leads to the approximation (5). Here, we combine this approximation with the sensing and signal transduction dynamics of the \textit{R. sphaeroides} chemotaxis pathway in (1) and (2).

Following the models in [13], mass action kinetics give the following general form for \( F, \tilde{F} \) in (1)
\[ \dot{m} = F(a, \tilde{a}, w(x)) = k_R(1 - a) - k_{B_1}B_{1p}a - k_{B_2}B_{2p}a \]
\[ \dot{\tilde{m}} = \tilde{F}(a, \tilde{a}, \tilde{w}(x)) = \tilde{k}_R(1 - \tilde{a}) - \tilde{k}_{B_2}B_{2p}\tilde{a} \] (6)

where \( k_R, \tilde{k}_R > 0 \) are methylation and \( k_{B_1}, k_{B_2}, \tilde{k}_{B_2} > 0 \) de-methylation rate constants. From Section III-A, the membrane and receptor cluster activities are given by
\[ a = \frac{1}{1 + \left[ \exp(\alpha[m_0 - m]) \frac{L}{K_I} \right]^N} \quad \text{and} \quad \tilde{a} = \frac{1}{1 + \left[ \exp(\tilde{\alpha}[\tilde{m}_0 - \tilde{m}]) \frac{\tilde{L}}{K_I} \right]^N} \] (7)

and the dynamics of the phosphorylation cascade is given by
\[ \dot{x} = H(x, a, \tilde{a}) \] (8)

as described in Section II, with \( H(x, a, \tilde{a}) \) given in [12].

Together with the simplifying assumption (which is relaxed in [11] that \( \tilde{L} = L \) and with the model of flagellar rotation inhibition by the CheY proteins given in [11], we can arrive at an integrated ODE description of the \textit{R. sphaeroides} chemotaxis network by fitting the constants \( k_R, \tilde{k}_R, k_{B_1}, k_{B_2}, \tilde{k}_{B_2} \) in (6) to experimental time-series data from [13]. A schematic is illustrated in Figure 2, and a simulation of the model together with the tethered cell assay from [13] to which the model was fitted is shown in Figure 3. The fitted parameters of the model are as follows: \( k_R = \tilde{k}_R = 0.0057, k_{B_1} = \tilde{k}_{B_2} = 2.376, k_{B_2} = 2.970, N = \bar{N} = 1 \).

![Fig. 2. Schematic of the \textit{R. sphaeroides} chemotaxis mode](image-url)
IV. Fold change detection in *R. sphaeroides*

The following definition of fold change detection (FCD) closely follows that given in the earlier works [3], [5].

**Definition 1:** The *R. sphaeroides* chemotaxis system (6), (7) and (8) exhibits FCD in response to a sensed ligand input signal $L(t)$ if its receptor activities $a(t), \tilde{a}(t)$, initially at a steady state corresponding to $L(0)$, are independent of linear scalings $p > 0$ of the input $L(t)$.

The following theorem gives conditions for FCD for the model (6), (7) and (8).

**Theorem 1:** Under approximation (5) the system (6), (7), (8) will exhibit FCD in the sense of Definition 1 if it has a unique steady state for any constant ligand input $L$.

**Proof:** Under approximation (5), the system (6), (7), (8), can be written as

$$
\dot{n} = F(a, x), \quad a = G(\exp(-m)L)
$$

$$
\dot{\tilde{n}} = \tilde{F}(\tilde{a}, x), \quad \tilde{a} = \tilde{G}(\exp(-\tilde{m})\tilde{L}), \quad \tilde{L} = L
$$

(9)

$$
\dot{x} = H(x, a, \tilde{a})
$$

Consider a ligand input signal $L(t)$, defined on $[0, \infty)$. Suppose that (9) is initially at the unique steady state pre-adapted to the constant input $L(0)$, and exhibits the solution $m_1(t) = [m(t)_1 \ n_1(t) \ x_1(t)^T]^T$ in response to the input $L_1(t) = L(t)$, yielding outputs $a_1(t)$ and $\tilde{a}_1(t)$. Now consider the solution of (9) to the input $pL(t)$ when it is initially at the unique steady state pre-adapted to the constant input $pL(0)$, $p > 0$. We denote this solution by $m_2(t) = [m(t)_2 \ n_2(t) \ x_2(t)^T]^T$ and the corresponding outputs by $a_2(t)$ and $\tilde{a}_2(t)$. The solution $m_2(t)$ and outputs $a_2(t)$ and $\tilde{a}_2(t)$ are equivalent to those of the system

$$
\dot{m} = F(a, x), \quad a = G(\exp(-m)pL)
$$

$$
\dot{\tilde{m}} = \tilde{F}(\tilde{a}, x), \quad \tilde{a} = \tilde{G}(\exp(-\tilde{m})p\tilde{L}), \quad \tilde{L} = L
$$

(10)

$$
\dot{x} = H(x, a, \tilde{a})
$$

in response to the input $L(t)$ when its initial state is the unique steady state pre-adapted to the constant input $L(0)$. Consider now the changes of variables $\exp(-w) = \exp(-m)p$ and $\exp(-\tilde{w}) = \exp(-\tilde{m})p$ so that (10) becomes

$$
\dot{w} = F(a, x), \quad a = G(\exp(-w)L)
$$

$$
\dot{\tilde{w}} = \tilde{F}(\tilde{a}, x), \quad \tilde{a} = \tilde{G}(\exp(-\tilde{w})\tilde{L}), \quad \tilde{L} = L
$$

(11)

$$
\dot{x} = H(x, a, \tilde{a})
$$

Note that in response to the input $L(t)$, when its initial state is the unique steady state pre-adapted to the constant input $L(0)$, (11) will exhibit the solution $w(t) = [w(t) \ \tilde{w}(t) \ x(t)^T]^T = m_1(t)$.

It will therefore exhibit outputs $a_1(t)$ and $\tilde{a}_1(t)$.

However, since the outputs of (10) are $a_2(t)$ and $\tilde{a}_2(t)$, it follows that $a_1(t) = a_2(t)$ and $\tilde{a}_1(t) = \tilde{a}_2(t)$.

This means that the outputs arising from the scaled input will be identical to the outputs arising from the unscaled input, and hence the system shows FCD in the sense of Definition 1.

A more general version of Theorem 1, which applies to a much wider class of chemotaxis systems structures, is given in [1]. For example, with the assumptions on the methylation dynamics given in Section III-A, FCD can be shown for models where the connectivity between the CheB proteins and the two sensing clusters is different to that presented in this model, and where there is an interaction between the CheY proteins and the cytoplasmic cluster that alters $\tilde{a}$, as in [13]. It also shown FCD will hold when the relationship between the externally sensed ligands $L$ and those detected by the cytoplasmic cluster, $\tilde{L}$ can be generalized to a linear, dynamic one. Such a relation could be a more realistic representation of the actual biochemical relationship between these two signals, modeling the likely propagation delays arising from internalization of the externally sensed ligand.

An interesting feature to note about (6), (7), (8) is that, under the conditions of Theorem 1, if the system exhibits FCD in its outputs $a, \tilde{a}$, then it also shows FCD in the concentrations of its phosphorylated chemotaxis proteins. This arises from the following argument: the outputs $a, \tilde{a}$ form the only inputs to (8). Regardless of the value of any constant input $L(0)$ to which the system (6), (7), (8) is adapted, the outputs $a(0), \tilde{a}(0)$ adapt to the same steady state value. At steady state, the phosphorylation cascade (8) is adapted to the unique equilibrium corresponding to its steady state input $a(0), \tilde{a}(0)$. By Theorem 1, the inputs to (8), $a(t)$ and $\tilde{a}(t)$ do not change with a scaling of the ligand input $L(t)$. Therefore, regardless of any scaling of the ligand input $L(t)$, the phosphorylation cascade (8) has the same initial state and is excited by the same input. Its states must therefore exhibit the same output response. Figure 4 shows that in the ligand range $K_I \ll K_A, K_I \ll K_A$, where approximation (5) applies, the model (6), (7), (8) shows very approximate FCD in its phosphorylated CheY$_6$ concentration.

Since the flagellar activity is also a function of the CheY proteins, this means that FCD should also be observed at the flagellar rotation level. The fact that FCD can be observed in both the concentration of phosphorylated chemotaxis proteins and in the flagellar activity is useful because it suggests that a variety of different experimental methods can
be used to test for this dynamic behavior, including FRET measurements and tethered cell assays. In [6], preliminary experimental evidence for FCD in \textit{R. sphaeroides} is presented in the form of tethered cell assays that show constant adaptation times in response to five-fold step changes in ligand concentration. More conclusive evidence for FCD, such as that in [4], would show that the entire shape of the flagellar response would not change as a result of ligand input scalings.

V. DISCUSSION

Unlike previous models, such as those in [12], [13], the \textit{R. sphaeroides} model we have presented features methylation dynamics that are based on the MWC approximation. This allosteric model of the receptor kinase activity follows that dynamics which are based on the MWC approximation. This \textit{Rhodobacter sphaeroides} model we have presented features methylation in [12], [13] is that a transient dynamic property in response to [L](t) is that a transient dynamic property, such as that in [4], would show that the entire shape of the flagellar response would not change as a result of ligand input scalings.

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