Escherichia coli autoinducer-2 uptake network does not display hysteretic behavior but AI-2 synthesis rate controls transient bifurcation

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\textbf{A B S T R A C T}

Analysis of different architectures of quorum sensing networks has been the center of attention in recent times. The approach employs mathematical models to uncover the factors behind the dynamics. Quorum sensing networks mostly display autoregulation such as Pseudomonas aeruginosa and Vibrio cholerae. However, Escherichia coli autoinducer-2 (AI-2) synthesis does not display autoinduction (i.e. autoregulation). This and other features have raised questions about the actual function of AI-2 inside the cell. In this paper we propose a model for \textit{lsr} operon regulation which explains or at least is consistent with AI-2 uptake in \textit{E. coli}. The model was employed to determine the main factors that control the concentration of the signal and the uptake activation. We investigated deterministic and stochastic variants of the network model and we found no states that could lead to the typical bistability in quorum sensing systems. However, stochastic simulations predict a transient bifurcation (positively regulated by AI-2 synthesis) that could provide some advantage in adapting to new environments. LsrR inactivation was found to play a crucial role in the uptake activation compared to AI-2 synthesis, that could provide some advantage in adapting to new environments. Our hypothesis is that positive regulation of the level of expression is the main factor in understanding the function of the \textit{lsr} operon. This is in contrast to the conventionally held belief that the main factor is the onset of activation.

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1. Introduction

Bacteria have the ability to adapt to different situations in order to get the most efficient response in terms of energy (Kim et al., 2004). Under special conditions, the amount of energy necessary to elicit a response would require less energy when a specific quorum is achieved (Waters and Bassler, 2005). Among these processes are biofilm formation (González Barrios et al., 2006), virulence (Passador et al., 1993) and conjugation (Fuqua et al., 1994). Quorum sensing is triggered by a diffusible substance called autoinducer, which accumulates nearby the cell when proper local cell density is present. Once the autoinducer concentration threshold is reached, signal uptake takes place and gene induction is initiated (Waters and Bassler, 2005). The most studied quorum sensing system so far is the signal synthase-signal receptor system LuxI/LuxR, which has been seen as the "paradigm" (Nealson and Hastings, 1979). This quorum sensing system displays an autocatalytic behavior, as \textit{lux} is indirectly induced by the presence of the signal. However, LuxI synthesizes a signal belonging to \textit{N}-acyl-homoserine lactones family which are completely or partly species-specific (Manefield and Turner, 2002). On the other hand, autoinducer-2 (AI-2) has been found to be synthesized in several species, thereby enabling the communication among them (Bassler et al., 1997).

AI-2 as signal regulator has been broadly studied, for example, Ren et al. (2004a) reported 150 genes AI-2-regulated in \textit{Escherichia coli} (\textit{E. coli}) DH5\textalpha{} (Ren et al., 2004a). Moreover, González Barrios et al. (2006) dilucidated an interesting biofilm regulation process mediated by AI-2 in \textit{E. coli} through \textit{mqsR}. On the other hand, Sperandio et al. (2001) reported that \textit{E. coli} is capable of synthesizing AI-2 and this signal regulates more than 400 genes in enterohemorrhagic \textit{E. coli}, but they later found AI-3 as the actual regulator (Sperandio et al., 2003). The AI-2 transport apparatus is encoded by the \textit{lsr} operon and its uptake was recently elucidated by Wang et al. (2005a). When uptaken (see Fig. 1), AI-2 is phosphorilated by the kinase encoded by \textit{lsrK}, followed by binding of phospho-AI-2 to LsrR, which is a negative regulator of the \textit{lsr} operon. Once bound, LsrR no longer represses \textit{lsr}. LsrFG appears to process phospho-AI-2 (Wang et al., 2005b). Unlike other known quorum sensing network architectures such as \textit{Pseudomonas aeruginosa} and \textit{Vibrio harvey}, LuxS has not been found directly or indirectly to be induced by the signal involved in the uptake process (Waters and Bassler, 2005). This fact has raised questions regarding the role of AI-2 as a molecular signal in \textit{E. coli} (Taga, 2007). Instead, this sig-
A standard deterministic model was developed to carry out the bifurcation analysis and understand the dynamics of the network. This model was developed based on the layout displayed in Fig. 1 (Wang et al., 2005a). Kinetic parameters of the deterministic model were estimated using published data and the Systems Biology toolbox (Schmidt and Jirstrand, 2005) in Matlab (The Mathworks Inc., Natick, MA). Moreover we carried out a sensitivity analysis for each constant a selected those which had a strong influence on the dynamics and will be analyzed throughout. We consider the rate of change in formation of two messenger RNA LsrACBDFG (R1) and LsrRK (R2), five different proteins: LsrACBDFG (P1), LsrR (P2), LsrFG (P3), and LsrK (P4), the intracellular and excreted AI-2 (S1 and S2 respectively), and its phosphorylated state (S3). The transcription process and inhibition were modeled using Michaelis–Menten approximation as it has been shown to accurately describe the saturation kinetics when the inhibition takes place on the binding site (Goryachev et al., 2006). Tables 1 and 2 list the parameters and variables respectively of the model. A thorough description of the model is presented as follows.

The messenger RNA that encodes for the AI-2 transport and synthesis apparatus LsrACBDFG:

$$\frac{d[R_1]}{dt} = \frac{V_1[C]}{K_m(1 + ([P_2]/[R_1]) + [C])} - \eta_1[R_1]$$

The mass balance for the messenger RNA that mediates the synthesis of the regulator LsrR and the kinase LsrK:

$$\frac{d[R_2]}{dt} = \frac{V_2[C]}{K_m(1 + ([P_2]/[R_2]) + [C])} - \eta_2[R_2]$$

The kinetic equation for the AI-2 uptake apparatus LsrACBDFG is expressed as:

$$\frac{d[P_1]}{dt} = K_1[R_1] - \rho_1[P_1]$$

Similarly, the expression for the rate of change of DNA-binding transcriptional regulator LsrR is expressed as:

$$\frac{d[P_2]}{dt} = K_2[R_2] - \rho_2[P_2] - \lambda_1[P_2][S_3]$$

Further phospo-AI-2 processing protein LsrFG is expressed as:

$$\frac{d[P_3]}{dt} = K_1[R_1] - \rho_3[P_3] - \lambda_2[P_3][S_3]$$
the uptake model in *E. coli*. Kinetic constants used for the AI-2 uptake model in *E. coli.*

<table>
<thead>
<tr>
<th>Constant</th>
<th>Legend</th>
<th>Value</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_1$</td>
<td>Maximum transcription rate of LsrACBDFG</td>
<td>0.02 m s$^{-1}$</td>
<td>Kierzek et al. (2001) and Neidhardt (1996)</td>
</tr>
<tr>
<td>$V_2$</td>
<td>Maximum transcription rate of LsrK</td>
<td>0.02 m s$^{-1}$</td>
<td>Kierzek et al. (2001) and Neidhardt (1996)</td>
</tr>
<tr>
<td>$Km_1$</td>
<td>Affinity constant between LsrR and LsrACBDFG</td>
<td>1 m</td>
<td>Estimated from Wang et al. (2005a)</td>
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<tr>
<td>$Km_2$</td>
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<td>2 m</td>
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<td>$Kl_1$</td>
<td>Inhibition constant between LsrR and LsrACBDFG</td>
<td>$1 \times 10^{-3}$ m</td>
<td>Estimated from Wang et al. (2005a)</td>
</tr>
<tr>
<td>$Kl_2$</td>
<td>Inhibition constant on LsrK</td>
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<tr>
<td>$\eta_1$, $\eta_2$</td>
<td>Degradation of mRNA LsrACBDFG and LsrK</td>
<td>6 $\times 10^{-3}$ s$^{-1}$</td>
<td>Hamsbraeus et al. (2003) and Wang et al. (2005a)</td>
</tr>
<tr>
<td>$\rho_1$</td>
<td>Degradation of LsrACB</td>
<td>1 $\times 10^{-5}$ s$^{-1}$</td>
<td>Goryachev et al. (2006) and Pratt et al. (2002)</td>
</tr>
<tr>
<td>$\rho_2$, $\rho_3$, $\rho_4$</td>
<td>Degradation of LsrR, LsrFG, and LsrK</td>
<td>1 $\times 10^{-4}$ s$^{-1}$</td>
<td>Goryachev et al. (2006) and Pratt et al. (2002)</td>
</tr>
<tr>
<td>$P_1$</td>
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<td>1.6 $\times 10^{-3}$ s$^{-1}$</td>
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</tr>
<tr>
<td>$P_2$</td>
<td>Translation of LsrK</td>
<td>1.2 $\times 10^{-3}$ s$^{-1}$</td>
<td>Wang et al. (2005b)</td>
</tr>
<tr>
<td>$P_3$</td>
<td>Translation of LsrFG</td>
<td>7 $\times 10^{-5}$ m$^{-1}$ s$^{-1}$</td>
<td>Estimated from Wang et al. (2005a)</td>
</tr>
<tr>
<td>$P_4$</td>
<td>Translation of LsrACB and extracellular AI-2</td>
<td>7 $\times 10^{-7}$ m$^{-1}$ s$^{-1}$</td>
<td>Estimated from Wang et al. (2005a)</td>
</tr>
<tr>
<td>$S_5$</td>
<td>AI-2 synthesis rate</td>
<td>0.2 m s$^{-1}$</td>
<td>Estimated from Wang et al. (2005a)</td>
</tr>
<tr>
<td>$S_6$</td>
<td>Excretion constant</td>
<td>0.2 s$^{-1}$</td>
<td>Parsek et al. (1999)</td>
</tr>
<tr>
<td>$D_b$</td>
<td>Dimerization rate (active LsrR $\rightarrow$ inactive LsrR)</td>
<td>0.0001 s$^{-1}$</td>
<td>Estimated from Wang et al. (2005a)</td>
</tr>
</tbody>
</table>

**The change of kinase LsrK is described as:**

$$\frac{d[P_4]}{dt} = K_2[P_2] - \rho_4[P_4] - \lambda_3[P_4][S_1]$$  \hspace{1cm} (6)

AI-2 synthesis model has been published elsewhere and highlights synthesis and regulation of AI-2 (Li et al., 2006). Therefore we emphasized the uptake of the signal with the intention of elucidating key factors in the signal intensity and uptake activation. We aggregated the AI-2 synthesis metabolic network into one parameter $S_0$ which corresponds to the synthesis rate of AI-2. Moreover, previous studies reported (Herzberg et al., 2006) that the AI-2 excretion rate does not rely on the diffusion mechanism but it is actively transported out of the cell through a mechanism that is not yet well known but controlled by the AI-2 transporter TqsA.

The AI-2 excretion constant $E_x$ is incorporated into the intracellular AI-2 balance so we finally obtain the following:

$$\frac{d[S_1]}{dt} = S_0 - \rho_4[P_4][S_1] - E_x[S_1] + \lambda_4[P_4][S_2]$$  \hspace{1cm} (7)

By balancing the AI-2 signal during the uptake and excretion we obtain the following rate of change for excreted AI-2:

$$\frac{d[S_2]}{dt} = E_x[S_1] - \lambda_4[P_4][S_2]$$  \hspace{1cm} (8)

The phosphorylated AI-2 signal rate of change is modeled as:

$$\frac{d[S_3]}{dt} = \lambda_3[P_4][S_1] - \lambda_2[P_3][S_3]$$  \hspace{1cm} (9)

Considering the nonlinear behavior of the dynamic system and the potential bistable response of the cell we employed bifurcation analysis in order to determine the presence of hysteretic behavior upon modifying what we consider several key network parameters. All deterministic simulations were carried out using the Matlab ODE solver for deterministic and Dizzy for stochastic modeling (Ramsey et al., 2005). Bifurcation analysis was performed on MATCONT (Dhooge et al., 2003).

To investigate the effect of noise on the stability and dynamics we simulated the uptake process with the exact Gillespie algorithm (Gillespie, 1976). The algorithm is initiated with the selection of two random numbers. The first is used to determine which reaction in the system will take place and the second number determines the time this reaction will start. The probability that a reaction will be selected is proportional to the product of its stochastic rate constant and the number of substrate molecules. The distribution of the waiting times is given by the following equation:

$$P(\tau, \mu) = a_\mu \exp(-a_0 \tau)$$  \hspace{1cm} (10)

where

$$a_\mu = h_\mu c_\mu$$  \hspace{1cm} (11)

$$a_0 = \sum a_\mu$$  \hspace{1cm} (12)

$P(\tau, \mu)$ is the probability for the reaction $\mu$ occurring at time $\tau$; $h_\mu$ is the number of substrate molecules in reaction $\mu$; and $\mu$ is the stochastic rate constant of this reaction. After the reaction and the time are selected, the reaction is executed, the time of the reaction is updated by $\tau$, and the next simulation step is executed. For the stochastic kinetic constants the volume of a generic bacteria was assumed to be $5.65 \times 10^{-13}$ cm$^3$ (Goryachev et al., 2006). All constants were adjusted to molecules per cell in both deterministic and stochastic simulations such that the results are comparable.

### 3. Results and Discussion

Here, we proposed an alternative way to conceive the AI-2 network; orderly AI-2 synthesis does not control de uptake activation but regulates the concentration of the signal inside the cell. Moreover, our stochastic model predicts the presence of a transient bimodal distribution which is induced by AI-2 synthesis rate that could facilitate the cell during adaptation. We also found that either the affinity of the phosphorylated signal to LsrR or its dimerization rate to be decisive on the uptake activation. Finally, we believed that cell density could strengthen the response of the cell and this could be an alternative way to perceive this special architecture regarding its relation with the quorum.

### 3.1. *E. coli* AI-2 Uptake Network Displays No Bifurcation Points

The appearance of a response when the “quorum” has been reached in the cell has been associated to hysteretic or bistable

### Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_1$</td>
<td>mRNA LsrACBDFG</td>
</tr>
<tr>
<td>$R_2$</td>
<td>mRNA LsrK</td>
</tr>
<tr>
<td>$P_1$</td>
<td>LsrACBDFG</td>
</tr>
<tr>
<td>$P_2$</td>
<td>LsrK</td>
</tr>
<tr>
<td>$P_3$</td>
<td>LsrFG</td>
</tr>
<tr>
<td>$P_4$</td>
<td>LsrR</td>
</tr>
<tr>
<td>$S_1$</td>
<td>Intracellular AI-2</td>
</tr>
<tr>
<td>$S_2$</td>
<td>Extracellular AI-2</td>
</tr>
<tr>
<td>$S_3$</td>
<td>Phosphorylated AI-2</td>
</tr>
</tbody>
</table>
points (Goryachev et al., 2006); the system becomes unstable and can exhibit several environmental responses at the same concentration of the signal. Bifurcation analysis is a powerful tool that allows us to identify hysteresis when network parameters are varied. In other words we used bifurcation analysis to detect any parameter that could lead to the appearance of bifurcation points. Among the parameters evaluated were $S_R$, $V_1$, $V_2$, and $C$. These parameters were selected since they covered the environmental and endogenous factors that affect the dynamics of the network such as presence of glucose (catabolic repression), synthesis of the signal, synthesis of the uptake apparatus, and signal processing. Interestingly, we found no bifurcation points in the network with any parameter evaluated, clearly indicating that this network inherently pursues just one state regardless of the environment that the cell cope with. The majority of the quorum sensing models reported to date base their conclusions on steady-state behavior (Fagerlind et al., 2003; Goryachev et al., 2006; Ward et al., 2001). However, we believe that considering the singularities in the *E. coli* AI-2 uptake network such as its lack of bistability, it is necessary to analyze the network’s transient state behavior. This is the subject for discussion in the next section.

### 3.2. Unsteady-State Analysis

AI-2 uptake in *E. coli* is catabolic repression controlled through the induction of *lsr* operon and repression of *luxS* (Wang et al., 2005a). The addition of glucose then affects the uptake of the signal by altering the amount of molecules that are excreted and uptaken afterwards and the moment when the uptake takes place. Wang et al. (2005a) measured AI-2 activity during exponential and stationary phase and the addition of glucose hurried the uptake activation from 6 to 4 h. At the same time, the presence of glucose increased the peak of extracellular AI-2 activity around two-fold. The stochastic model developed by Li et al. (2006) indicates that the uptake is purely controlled by the metabolic state of the cells when they reach the stationary state. However, even though the growth curve in both cases (with and without glucose) seems to display that the cells reach the stationary state at equal times the uptake is delayed when glucose is absent (Wang et al., 2005a). Hence, we performed different simulations modifying several parameters we believe have a significant role on the uptake of AI-2 in order to determine if this phenomenon is actually just metabolic controlled or if there exist another factors related with the “quorum”. First, we carried out a non-steady analysis modifying key parameters and modeled the effect on the external amount of AI-2 (Fig. 2). Following the analysis of Li et al. (2006) we analyzed the effect of AI-2 synthesis rate $S_R$ on the variation in time of extracellular AI-2 (Fig. 2A). Increasing of signal synthase transcription rate does have an impact on the peak intensity; however, we did not find a relevant effect on the AI-2 activation. Our results then seems to agree with the positive effect of AI-2 synthesis on the peak size as previously reported (Li et al., 2006) but yet does not explain the signal uptake activation mechanism.

The operon *lsr* is positively regulated by the complex cAMP-CRP; as such we carried out simulations at several *lsr* transcription rates (Fig. 2B). Again, the variation in the transcription of *lsr* does not significantly affect the uptake activation as when $V_1$ is modified the effect on the uptake activation is not significant. Moreover, our model prediction disagrees with published experimental data (Wang et al., 2005a), as the presence of glucose should induce

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**Fig. 2.** Deterministic simulations results at different values of AI-2 synthesis rate ($S_R$) (A), *lsr* maximum transcription rate ($V_1$) (B), AI-2 excretion constant ($E_R$) (C), and binding constant between LsrR and phosphorylated AI-2 ($k_{NAK}$) (D).
sooner the uptake the signal but Fig. 2B displays a delay when \( V_1 \) diminishes which is a direct consequence of the presence of glucose. Nevertheless, our model simply demonstrates what should be expected when \( lsr \) transcription rate decreases: an uptake delay due to the reduction of LsrACBDFG, therefore this result suggests the presence of an additional transporter which expression is triggered at high signal concentration as already suggested by Wang et al. (2005a).

Quorum sensing signal excretion has often been associated with passive transport where the difference between the signal concentration outside and inside of the cell constitutes the driving force for exporting the signal (Fagerlind et al., 2003). Li et al. (2006) reported to be negligible intracellular AI-2 accumulation. Nevertheless, Herzberg et al. (2006) found that TqsA plays a role in AI-2 excretion and accumulates inside the cell, moreover \( tqsA \) is differentially expressed in biofilm \( E. coli \) cells and is induced when cells reach stationary phase (Domka et al., 2007). Hence we performed several simulations by varying the AI-2 excretion constant \( E_R \) in order to determine the effects on uptake activation. Analogous results were obtained compared to \( S_R \) and \( V_1 \) as the AI-2 excretion rate has a positive effect on the peak intensity but does not cause any effect on the uptake activation (Fig. 2C).

Li et al. (2006) demonstrate that the transcription rate of \( luxS \) and \( pfs \) do not explain the enhancement of AI-2 production. Then, they hypothesized that the rate synthesis from 4,5-dihydroxy-2,3-pentanediol (DPD) explained the variation in AI-2 synthesis. Different from their model, we intend to reproduce the AI-2 uptake network so DPD rate could not indirectly explain the variations in uptake activation as AI-2 synthesis rate has previously been found to have a weak role on the cell. There exist several environmental factors such as temperature, pH, osmolarity, activator proteins binding, and protein inhibition (Xavier et al., 2007) that could control this reaction. We performed several simulations at different LsrR-phospoAI-2 binding rate constant \( \lambda_1 \). Interestingly, our model predicts a significant effect on the uptake activation (Fig. 2D). We therefore propose LsrR deactivation through phosphorylated AI-2 binding as a crucial step in the dynamics of the signal uptake in \( E. coli \).

Goryachev et al. (2006) demonstrated the importance of the dimerization of the transcription factor in a generic quorum sensing network and although the \( E. coli \) quorum sensing network does not display a typical architecture, we believe that LsrR dimerization when binding phosphorylated AI-2 could also affect its dynamics. We added an additional reaction in the model (see Table 1) in order to consider the conformational change of LsrR when unbinding \( lsr \) promoter. Fig. 3A shows that the effect of the dimerization rate \( D_R \) is similar to the effect of \( \lambda_1 \). It would be necessary to clarify which mechanism (protein binding or dimerization) plays a stronger role using quantum mechanics modeling and complemented with some experimental observations. External concentration of the sig-
nal is the main factor for typical population sensing networks, nevertheless, *E. coli* network, has been questioned regarding its function because of some differences in the architecture compared to these prototypes. Alternative functions such as interception of the communication between bacteria that use analogous signals (Xavier and Bassler, 2005a) or DPD scavenging (Taga, 2007) have been proposed. Fig. 3B displays the effect of the initial amount of extracellular AI-2 on its dynamics. Our model predicts that the accumulation of the signal on the outside positively impacts the intracellular signal intensity (results not shown) but it does not alter the uptake activation. Considering that it is not clear what is the function of the *lsr* operon we proposed here an alternative hypothesis that allows us to perceive this network with the capability of sensing the quorum where signal accumulation does not regulate the activation of the uptake but the intracellular signal intensity and this could explain the role of the control of AI-2 on different phenotypes in *E. coli* such as biofilm (González Barrios et al., 2006), motility (Sperandio et al., 2002), or virulence (Sperandio et al., 2001).

3.3. Parameter Sensitivity Analysis

The steady-state has been claimed to be the inherent state of biological systems in the environment. Therefore the analysis of bacteria during this state has been the basis for the majority of conclusions regarding bacterial network modeling. *E. coli* AI-2 uptake network is influenced by several environmental and endogenous factors such as stress, physiological state and carbon source (Wang et al., 2005a); as such an analysis of the importance of these factors and the parameters that represent them is, therefore, in order. Sensitivity analysis permits to weight the effect of several factors in steady-state. We selected the parameters for the analysis such that they could cover environmental and endogenous factors. The Matlab systems biology tool was used to evaluate the effect of such parameters at steady-state (27). Steady-state sensitivity for the *i* state to the parameter *J* is defined as (Varma et al., 1999)

\[
S_{ij} = \frac{X_{ii}(J_1, J_2, ..., J_n) - X_{ii}(J_1)}{\Delta J}
\]

Table 3

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Mean (AI-2 at 6 h) (mol)</th>
<th>t-Test</th>
<th>S.D.</th>
<th>F-test</th>
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<tr>
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<td>21.66</td>
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<td></td>
<td>0.0007</td>
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<tr>
<td><em>λ</em></td>
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<td>395.9</td>
<td>p-value</td>
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<td>61.0</td>
</tr>
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</table>
Fig. 6. Histograms that represent the distribution of the population regarding intracellular AI-2 (results are shown normalized for AI-2) obtained from 1000 stochastic simulations. (A) Corresponds to the constants displayed in Table 3, (B) binding kinetic constant between LsrR and phosphorylated AI-2 (\(K_{\text{NAK}1}\)) is ten-fold increased (0.007 m\(^{-1}\) s\(^{-1}\)), (C) maximum \(\text{lsrACBDFG}\) transcription rate is two-fold increased (0.04 m s\(^{-1}\)) and (D) AI-2 excretion constant is two-fold increased (0.4 s\(^{-1}\)).

Here, \(X_{ss}\) is the value of the state at steady-state. Several reports indicate AI-2 controls different phenotypes (González Barrios et al., 2006; Sharp and Sperandio, 2007; Sperandio et al., 2001, 2002). Unfortunately the mechanisms that explain such control are not yet well understood, since it is not clear if the activator corresponds to AI-2 or the phosphorylated signal in all cases (De Lisa et al., 2001; Ren et al., 2004b; Wang et al., 2005a). Therefore we analyze the sensitivity of our model of the “naked”, phosphorylated signal as activators. We also analyzed the sensitivity of the extracellular signal. As obtained in the non-steady analysis, intracellular AI-2 displays the highest sensitivity to its synthesis rate \(SR\) (Fig. 4A). Even though we did not find a strong connection between the activation time and \(lsr\) transcription on unsteady-state, the transcription of the uptake apparatus \(V_1\) seems to play an important role on the sensitivity of intracellular AI-2 concentration (Fig. 4A).

Considering another scenario (phosphorylated signal as the activator) we found that the phosphorylated signal displays the highest sensitivity to \(\lambda_3\) (results not shown) Even though this parameter does not only play a role on the timing it does have on the phosphorylated signal concentration. On the other hand, the sensitivity analysis on external AI-2 concentration indicates that the affinity of LsrR on the \(\text{lsrACBDFG}\) promoter \(K_{i2}\) has a strong impact (Fig. 4B). Notice that intracellular concentration of the signal either in its phosphorylated and non-phosphorylated state does not exhibits a significant sensitivity to \(K_{i2}\), indicating the existence of parameters that could control the external concentration of the signal but they are not relevant inside the cell. This reveals the importance of measuring intracellular AI-2 in order to determine the factors that could perturb the dynamics of the network since extracellular measurements could lead to erroneous conclusions.

3.4. Unsteady-state Analysis Under Stochastic Modeling

Mostly, quorum-sensing networks have been evaluated based on deterministic approaches (Fagerlind et al., 2005, 2003). However, these approaches do not contemplate the influence of molecular noise (Li et al., 2006) which has significant impact on quorum sensing systems (Goryachev et al., 2006). To study the impact of molecular noise we carried out several simulations using the exact Gillespie algorithm (Gillespie, 1976). Specifically we performed unsteady-state analysis by evaluating the effects of key parameters such as \(SR, V_1, ER, K_{\text{NAK}1}\), and initial concentration of extracellular AI-2 on the number of molecules of intracellular AI-2 and the dispersion on the population. One thousand simulations were carried out per each set of parameter values; the average and standard deviation as a measure of the dispersion were then calculated. The effect of each parameter was statistically evaluated by hypothesis testing (Table 3). First, we evaluated the effect of \(SR\) on the distribution of intracellular AI-2 in the population (Fig. 5). As AI-2 synthesis increases, the separation between the peaks of the bimodal distribution is more evident. The right peak (associated with cells on the “on” state), displays a normal distribution (Fig. 5E and F); it shows stronger response regarding the number of molecules of AI-2, and less dispersion compared to the flat response.
Fig. 7. Histograms that represent the distribution of the population regarding phosphorylated AI-2 (results are shown normalized for AI-2) obtained from 1000 stochastic simulations. (A) Corresponds to the constants displayed on Table 3, (B) binding kinetic constant between LsrR and phosphorylated AI-2 ($K_{NAK}$) is ten-fold increased ($0.007 \text{ m}^{-1} \text{s}^{-1}$), (C) maximum $lsrACBDG$ transcription rate is two-fold increased ($0.04 \text{ m s}^{-1}$) and (D) AI-2 excretion constant is two-fold increased ($0.4 \text{s}^{-1}$).

at $S_R$ of $0.01 \text{ mol s}^{-1}$ (Table 3). The AI-2 synthesis rate also affects cell segregation in “on” and “off” states.

For example the “off” state population decreases from 47% to 13% when the AI-2 synthesis rate is increased two-fold at 6 h (Fig. 5B and E). Notice the left peak fades when time is increased and the population moves towards a unimodal distribution (Fig. 5F). The presence of bistability in cells has been used to explain why bacteria bifurcate into two different populations with characteristic phenotypes and therefore different roles for adaptation (Dubnau and Losick, 2006). However that such an explanation was made under the steady-state assumption. Here, we propose that in some cases, though the network may not display bifurcation points, cells

Fig. 8. Histograms that represent the distribution of the population regarding intracellular AI-2 (results are shown normalized for AI-2) obtained from 1000 stochastic simulations considering an additional LsrR dimerization represented in dimerization rate $DR$. (A) $DR = 1 \times 10^{-4}$ and (B) $DR = 1 \times 10^{-3}$.

could exhibit a transient bifurcation controlled by the synthesis of the signal that could be advantageous for adaptation.

According to the deterministic model $\lambda_1$ plays an important role in uptake timing. We therefore wanted to determine, based on our stochastic model, if this parameter also plays a role in the distribution of the population. Interestingly, the population moves towards a unimodal distribution when $\lambda_1$ is increased 100-fold (Fig. 6A and B) so it seems that when signal uptake is activated sooner, cells sac- rifice the advantage in adaptation when two different populations are present. $\lambda_1$ was the only parameter which was found to affect the dispersion of the cells in the “on” state as the standard deviation obtained from both in silico experiments are statistically different (Table 3).

We evaluated the effect of the excretion rate on the distribution of the population; the stochastic model disagrees with the deter- ministic approach since the excretion rate effect was not possible to elucidate (Fig. 6C and Table 1) as the effect on mean and standard deviation is not significantly different (Table 3). The effect of the extracellular concentration was evaluated by running the stochastic model at different initial amount of extracellular AI-2 molecules. Again, dispersion was not influenced by the initial amounts of extracellular AI-2 so it seems that the quorum does not affect the distribution of cells (Table 3).

We also analyzed the effect of $V_1$ since catabolic repression nega- tively regulates its expression (Wang et al., 2005a). Our model does not find a significant effect on either the mean or dispersion (Table 3) of AI-2. However, it positively regulates the on-off splitting as well (Fig. 6C). This result seems to contradict our original results with the deterministic approach where we found an effect on the AI-2 profile when varying $V_1$ (Fig. 2B); here, the stochas- tic model predicts that the catabolic repression effect should be stochastically modeled in order to find a concentration of glucose that significantly affect the behavior of the population considering the intrinsic noise modeled by the Gillespie algorithm.

It is known that LuxR indirectly controls the expression of 5.4% of the chromosone in $E. coli$ (De Lisa et al., 2001), and 10% in enterohemorrhagic $E. coli$ (EHEC), however the mechanism of acti-vation for each gene is yet not clear. For AI-2 uptake network, we know the phosphorylated signal inactivates the protein repressor LsrR (Wang et al., 2005a) so a study of the phosphorylated signal would also be helpful in order to understand the dynamics on the activation. Two factors were found to induce an unimodal distri- bution on the population regarding the signal phosphorylated: $\lambda_1$ and $V_1$; indicating again the important role of the binding constant rate between the repressor on the cells response. Even though the expression of the uptake apparatus $\mathbf{b}$ does not homogenize the population regarding AI-2 signal it has a significant effect on the dispersion (Fig. 7).

LsrR deactivation could also be modeled considering an additional dimerization step once the regulator protein binds phosphorylated AI-2. Goryachev et al. (2006) demonstrated the importance of the dimerization of the transcription factor for a generic quorum sensing network. Here $D_2$ positively controls on-off population splitting as the population in the “on” state increased from 52% to 58%. However, in contrast to the results found for $\lambda_1$, $D_2$ does not affect dispersion of the cells in the “on” state (Fig. 8 and Table 3).

4. Conclusion

In summary, we proposed an alternative way to interpret the quorum sensing uptake network in $E. coli$ in addition to the sev- eral hypotheses already proposed (Taga, 2007). First, AI-2 synthesis positively regulates the concentration of the signal inside the cell but it does not control de uptake activation nor displays a bifur- cation point when it is varied. Nevertheless our stochastic model predicts the presence of a transient bimodal distribution which is induced by AI-2 synthesis rate that could facilitate the cell during adaptation. Secondly, we proposed either the affinity of the phos- phorylated signal to LsrR or its dimerization rate to be decisive on the uptake activation. Finally, we believed that cell density could strength the response of the cell and this could be an alternative way to perceive this special architecture regarding its relation with the quorum.


