Quorum Sensing regulation in Aeromonas hydrophila

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Abstract

We present detailed results on the C4-HSL mediated quorum sensing (QS) regulatory system of the opportunistic Gram negative bacterium Aeromonas hydrophila. This bacterium contains a particularly simple QS system allowing for detailed modeling of the kinetics. In a model system, i.e. the E. coli monitor strain MH205, the C4-HSL production of A. hydrophila is interrupted by fusion of gfp(ASV). In the present in vitro study, we measure the response of the QS regulatory ahyRI locus in the monitor strain to predetermined concentrations of C4-HSL signal molecules. A minimal kinetic model describes the data well. It can be solved analytically providing substantial insight into the QS mechanism: At high concentration of signal molecules, the slow decay of the activated regulator sets the time scale for the QS regulation loop. The slow saturation ensures that, in an A. hydrophila cell, the QS system is activated only by signal molecules produced by other A. hydrophila cells. Separate information on the ahyR and ahyI loci can be extracted thus allowing the probe to be used in identifying the target when testing QS inhibitors.

Key words: quorum sensing, Aeromonas hydrophila, Michaelis-Menten, E. coli, signal molecules

1. Introduction

Quorum sensing (QS) is an intercellular communication system by which bacterial cells are capable of indirectly monitoring their own population density through production and exchange of diffusible signal molecules. This enables bacteria to control gene expression dependent on population size and thereby perform coordinated phenotypic changes in a multicellular fashion [1, 2].

The first evidence of the social behavior of quorum sensing was observed in the marine bacterium Vibrio fischeri. V. fischeri colonizes "light organs" of certain fish and starts to produce visible light when a threshold density of bacteria has amassed. At present, QS regulatory systems have been reported for Gram positive as well as Gram negative bacteria [3, 4].

For both types of bacteria the regulation depends on the concentration of signal molecules which are small peptides for Gram positives and acyl homoserine lactones (AHL) for Gram negatives. For Gram negatives the regulatory system consists of a signal molecule synthetase and a transcriptional regulator and the constituents of the QS regulation are referred to as Lux homologues, derived from the luciferase of V. fischeri (LuxR for the regulator and LuxI for the signal molecule synthetase). At low population densities the production and secretion of quorum sensing signal molecules proceed at a basal level. As population density increases, the signal molecules accumulate above the threshold and fuse with the transcriptional regulator to induce transcription of the luxI and other target genes. This results in a positive feed-back loop which ensures a rapid amplification of the signal [5, 6, 7, 8].

Aeromonas hydrophila is a motile Gram-negative bacillus found in water sources, and it is a known pathogen for both humans and animals. The pathogenicity typically includes minor skin infections or gastroenteritis in humans. The A. hydrophila QS sensor consists of a AhyRI monitor system homologue to the LuxRI system [9, 10, 11, 12, 13, 2].

In order to investigate the kinetics which governs the quorum sensing activation loop, we broke the ahyRI locus into its constituents and measured the response to introduction of predetermined concentrations of C4-HSL signal molecules. To limit the investigation to the AhyRI sensor components, the sensor components were cloned into Escherichia coli. Knowledge about corresponding input and output levels is acquired by the construction of an E. coli monitor strain MH205 representing the ahyRI locus (Sec. 5.1). As depicted in Fig. 1 the ahyR is oriented in the reverse direction of the ahyI which has been interrupted by fusion of gfp(ASV). The E. coli strain responds to the C4-HSL through synthesis of the unstable green fluorescence protein Gfp(ASV) with a degradation rate of order 0.4 h\textsuperscript{-1} [14, 15] and maturation constant 1.5 h\textsuperscript{-1} [16]. Henceforth Gfp(ASV) will be referred to as Gfp. Thus MH205 is capable of responding to the signal molecules in a measurable way while lacking the ability to produce the signal molecules itself. This permits measurement of the response of the simulated AhyRI system to predetermined signal molecule concentrations.

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2. Model

Below we describe a simple model of the Gfp production in the MH205 E. coli monitor strain. The variables and parameters used in the model are listed in Table 1. The AhyRI construct of MH205 and the main processes of interest are illustrated in Fig. 1.

The model separates in two: First, the production of RS monomer $r_1$. Second, the activation of ahyR promoter and production of mature Gfp. This is a valid separation since the concentration of activated ahyR operator sites ($I_a \sim nM$) is much smaller than the RS monomer concentration ($r_1 \sim \mu M$).

The production of regulator molecules is controlled by the ahyR site, i.e. proportional to the concentration of carrier plasmid indicated in the production term in Eq. (1). ($R_i$ is the density of ahyR-sites, equal to the plasmid copy number divided by the volume of the cell.)

The activation of the regulator through binding to a signal molecule is a slow process described by Eq. (2). While the free regulator $R$ decays rapidly, $\lambda_1 \sim 20$ h$^{-1}$, the RS complex is protected against proteases and has a much slower decay rate, $\lambda_1 \sim 0.6$ h$^{-1}$ [17]. In section 3.1 the details of how the different time scales come into play are discussed.

The RS complex activates a free operator site ($I_f$) for Gfp production forming an activated operator site ($I_a$) described by Eq. (3). This, in turn, initiates production of non-mature Gfp ($n$), Eq. (4), subsequently maturing into fluorescent Gfp ($g$), Eq. (5), [16, 14, 15]. In parallel there is a spontaneous production of Gfp proportional to the number of free operator sites. Estimates of the decay constants are of order $\lambda_{n,g} \sim 0.4$ h$^{-1}$ [14, 15, 16]. The introduction of such a long time scale from the production forming an activated operator site ($I_a$) described by Eq. (3), in turn, initiates production of non-mature Gfp ($n$), Eq. (4), subsequently maturing into fluorescent Gfp ($g$), Eq. (5), [16, 14, 15]. In parallel there is a spontaneous production of Gfp proportional to the number of free operator sites. Estimates of the decay constants are of order $\lambda_{n,g} \sim 0.4$ h$^{-1}$ [14, 15, 16].

By normalizing data to the spontaneous Gfp production, we directly measure the enhancement factor caused by the added signal molecules. Since, from Fig. 2, we can assume exponential growth, this leads to the simplification that all calculations can be done “per plasmid copy” by augmenting the decay rate of all intracellular concentrations by the growth rate $\lambda$.

\[
\frac{dr_0}{dt} = b_0 R_i + k_{1}^{-} r_1 - k_{1}^{+} r_0 s - (\lambda_0 + \lambda_c) r_0 \tag{1}
\]

\[
\frac{dr_1}{dt} = k_{1}^{+} r_0 s - (k_{1}^{-} + \lambda_1 + \lambda_c) r_1 \tag{2}
\]

\[
\frac{dn}{dt} = b_n I_a + (k_n^{+} - b_n) I_a - (\lambda_n + \lambda_c + k_n^{-}) n \tag{4}
\]

\[
\frac{dg}{dt} = k_g^{+} n - (\lambda_g + \lambda_c) g \tag{5}
\]

\[
I_f = I_a + I_f \tag{6}
\]

This description assumes a number of simplifications. First, the possible decay of the signal molecules has been omitted from the model since, as described in section 5.3, it has been measured to be negligible. Second, since the signal molecules diffuse rapidly through the cell membrane they are assumed to be in transmembrane equilibrium [19]. Third, the possible feedback mechanism in the regulator production indicated in Fig. 1 has been ignored, since we find no need for this mechanism in the description of our data. Finally, we limit the description to the formation of the signal molecule concentration: the response to rapid changes in the underlying processes.

The resulting solution is

\[
r_1 = b_0 R_i \left( \frac{1}{\lambda_0 + \lambda_c + K_1 + s} \left( \frac{s}{K_1 + s} e^{-\lambda t} \right) \right) \tag{7}
\]

with

\[
K_1 = \frac{k_1^{-} + \lambda_1 + \lambda_c}{k_1^{+}} \tag{8}
\]

\[
\tilde{K}_1 = \frac{\lambda_0 + \lambda_c}{\lambda_1 + \lambda_c} \tag{9}
\]

\[
\lambda = \frac{K_1}{\tilde{K}_1 + s} \left( \lambda_0 + \lambda_c \right) + \frac{s}{K_1 + s} (\lambda_1 + \lambda_c)
\]

3. Results

In order to investigate the kinetics of the QS activation loop we measured the response to predetermined concentrations of signal molecules, $s = 12 \mu M / 2^i$, $i = 0, 1, ..., 8$. The culture was in exponential growth phase when signal molecules were added (Fig. 2 and sections 5.2 and 5.5).

After subtraction of the spontaneous Gfp production the concentration of Gfp per plasmid copy is the induced response (see section 5.4 for details). The data are shown in Fig. 3 and compared with the minimal model of the AhyRI system to be discussed below.

At this point we note that the response saturates as a function of the signal molecule concentration: the response to $s = 6 \mu M$ does not differ from the response to $s = 12 \mu M$. Furthermore, the maximal response seems to be reached earlier at lower signal molecule concentrations. Finally, the response is a higher-order response, i.e. the underlying kinetics is at least 2nd order in time. These observations will be illuminated in the discussion of results in the light of the analytical solution of the model.

3.1. RS formation

The signal molecules do not decay (section 5.3) and the on/off rates for RS formation are large, Table 1, the RS formation may be solved in the quasi-static limit. This is done by adding up Eq. (1) and Eq. (2) and setting $dr_1/dt = 0$ in Eq. (2). The resulting solution is

\[
r_1 = b_0 R_i \left( \frac{1}{\lambda_0 + \lambda_c + K_1 + s} \left( \frac{s}{K_1 + s} e^{-\lambda t} \right) \right) \tag{7}
\]

with

\[
K_1 = \frac{k_1^{-} + \lambda_1 + \lambda_c}{k_1^{+}} \tag{8}
\]

\[
\tilde{K}_1 = \frac{\lambda_0 + \lambda_c}{\lambda_1 + \lambda_c} \tag{9}
\]

\[
\lambda = \frac{K_1}{\tilde{K}_1 + s} \left( \lambda_0 + \lambda_c \right) + \frac{s}{K_1 + s} (\lambda_1 + \lambda_c)
\]
\[
\begin{aligned}
    \lambda_0 + \lambda_c, \quad s \ll K_1 \\
    \lambda_1 + \lambda_c, \quad s \gg K_1
\end{aligned}
\]  

(10)

From these equations we read two important features. First, \( \lambda \) in Eq. (10) sets the time scale for reaching steady state of \( r_1 \). At \( s \gg K_1 \) this time scale is \( 1/(\lambda_1 + \lambda_c) \). With rapid diffusion (~ 5 s) out of the cell, this ensures that at high concentrations of signal molecules only intercellular activation is permitted in \( A. hydrophila \), whereas autoinduction is suppressed. With diffusion constant \( D \sim 10^4 \text{mm}^2/\text{s} \) and typical \( \lambda_1 + \lambda_c \sim 1\text{h}^{-1} \) the signal molecules activating the QS system have traveled \( \gtrless 1 \) mm when binding to a regulator. Second, the rescaled dissociation constant \( K_1 \sim 3 \mu M \) acts as the cut-off in the equilibrium \( r_1 \) concentration at \( t \gtrsim 1/(\lambda_1 + \lambda_c) \), thus enhancing the dynamic range of sensitivity to \( s \). To our knowledge, this rescaling of the dissociation constant has not been reported in the literature. Thus, while fitting, it is convenient to measure \( r_1 \) and \( K_I \) in units of the saturated \( r_1 \)-level, \( r_{1,\text{sat}} = b_0 r_0/(\lambda_1 + \lambda_c) \). Choosing the independent parameters as \( \lambda_1, K_1, \tilde{K}_1, \Lambda_n, \Lambda_g, K_I, \), and \( A \) we express the induced response as

\[
g(t) - g_0 = A \left( h_g * \frac{r_1}{K_I + r_1} \right)(t)
\]

(19)

where

\[
r_1(t) = \frac{K_1}{K_I + s} \frac{s}{K_I} e^{-\lambda_I t} + \frac{s}{K_I + s} (1 - e^{-\lambda_I t})
\]

(20)

and

\[
A = \frac{\tilde{K}_1}{K_I + s} (\lambda_1 + \lambda_c). \tag{21}
\]

3.4. Variable reduction

While fitting, it is convenient to measure \( r_1 \) and \( K_I \) in units of the saturated \( r_1 \)-level, \( r_{1,\text{sat}} = b_0 r_0/(\lambda_1 + \lambda_c) \). Choosing the independent parameters as \( \lambda_1, K_1, \tilde{K}_1, \Lambda_n, \Lambda_g, K_I, \), and \( A \) we express the induced response as

\[
g(t) - g_0 = A \left( h_g * \frac{r_1}{K_I + r_1} \right)(t)
\]

(19)

where

\[
r_1(t) = \frac{K_1}{K_I + s} \frac{s}{K_I} e^{-\lambda_I t} + \frac{s}{K_I + s} (1 - e^{-\lambda_I t})
\]

(20)

and

\[
A = \frac{\tilde{K}_1}{K_I + s} (\lambda_1 + \lambda_c). \tag{21}
\]

3.5. Scaling relations

After a time of order \( 1/(\lambda_1 + \lambda_c) \), the system reaches steady state. Still in units of the saturated level, \( r_1 \) in Eq. (20) may then be expressed

\[
r_1 \approx \frac{s}{K_I + s}. \tag{22}
\]

At steady state, the convolution in Eq. (19) reduces to the identity and we get

\[
g(t) - g_0 \approx A \frac{r_1}{K_I + r_1}
\]

(23)

Thus, we expect \( A/(K_I+1) \) and \( K_I \tilde{K}_1/(K_I+1) \) to be well defined constants.
3.6. Numerical results

A comparison of the model with the measured response to introduction of signal molecules is shown in Fig. 3. With the parameters listed in Table 2 the model reproduces the data well. The parameter estimation is detailed in section 3.6. Given the model, we are able to follow the response for each step in the QS regulatory system.

As expected, fits to data reveal narrow bounds on the steady state relations

\[
\frac{A}{K_f + 1} = 19.3 \pm 0.3
\]

\[
\frac{K_fK_i}{K_i + 1} = (0.80 \pm 0.05) \mu M
\]

between the parameters from Eq. (23).

As the signal molecule concentration is lowered, the maximal response occurs earlier. This reflects the change towards large \( \lambda \) in Eq. (10): the QS system is sensitive to the transition from fast (\( \lambda \approx 12 \text{h}^{-1} \)) RS saturation at low signal molecule concentration to slow (\( \lambda \approx 0.9 \text{h}^{-1} \)) RS saturation at high signal molecule concentration.

In Fig. 4a-c the concentrations of free regulator \( r_0 \), activated regulator \( r_1 \), and activation of \( ahyl \)-operator site \( I_0 \) are shown. The concentration of free regulator, \( r_0 \), starts at a high equilibrium level. The addition of signal molecules results in a rapid drop of \( r_0 \) expressing that the RS+RS = RS is in static equilibrium (\( r_0s = K_s(r_1) \)). Hereafter \( r_1 \) builds up at a time scale given by \( 1/\lambda \) in Eq. (10). At high signal molecule concentration, where \( \lambda \) is small, the equilibrium concentration of \( r_0 \) and \( r_1 \) is reached only slowly. The plots of \( r_0 \) and \( r_1 \) confirm the understanding, that this time scale is of order 1 h when the signal molecule concentration is much larger than \( K_s \). As noted earlier, this serves to suppress autoinduction of the QS system in the \textit{in vivo} \textit{A. hydrophila}.

The large value of \( K_1 \) in Eq. (7) and Eq. (9) ensures a large dynamic range in the sensitivity of \( r_1 \) to \( s \). Even at the highest tested concentrations of signal molecules, \( r_1 \) is still sensitive to \( s \) thus allowing for virulence factors at other sites in \textit{A. hydrophila} to turn on at higher \( s \) than the cut-off for signal molecule production. In \textit{in vivo} \textit{A. hydrophila}, this ensures a large dynamic range in sensitivity to colony size for the QS controlled virulence factors.

In Fig. 4d the concentration of mature Gfp (g) is shown. The slow production and maturation of the Gfp is a limiting factor in the time resolution of the experiment preventing direct access to the activation of the \( ahyl \) locus. We have considered an unfolding of the linear production and maturation of Gfp. However, this involves effectively differentiating twice the data and is not possible given the experimental uncertainties.

4. Discussion

We have presented a detailed experimental study of the C4-HSL mediated QS regulatory system from \textit{A. hydrophila} in the \textit{E. coli} monitor strain MH205. The experimental data were presented in conjunction with a minimal mathematical model providing remarkable insight into the QS mechanism reaching beyond the description of the MH205 monitor system.

The analytical solution of the formation of the AhyRI/C4-HSL complex demonstrates how the dramatic difference in decay rate of free regulator and activated regulator reported in the literature [17, 21] plays its role: First, it ensures that only intercellular activation of the QS process is allowed. Second, it ensures an enhanced dynamic range of sensitivity to the signal molecule concentration.

The large time scale is only activated at high signal molecule concentrations. In the experiment this shows up as a delay in the maximal response, making our experiment sensitive to the \( ahyl \) locus. Furthermore, the overall saturation in the response to signal molecule concentration is predominantly determined by the dissociation constant \( K_f \) controlling the dissociation of the AhyR/C4-HSL complex from the \( ahyl \) site and less sensitive to the cutoff \( K_i \) for the binding of the regulator to the signal molecule. This makes our experiment sensitive to the \( ahyl \) locus. (In \textit{A. hydrophila} this ensures that, at signal molecule concentration beyond the saturation of the regulatory loop, the concentration of AhyR/C4-HSL complexes is a measure of the size of the \textit{A. hydrophila} colony.) Together, this makes the experiment sensitive to \( ahyl \) and the \( ahyl \) loci individually thus making the monitor strain a sensitive probe for identifying targets for QS inhibitors.

5. Materials and Methods

5.1. Construction of the AhyRI sensor system in MH205

The \( ahyl \) and \( Pahy \) (promoter region) from \textit{A. hydrophila} was isolated as a 2601 bp BamHI - EcoRI fragment and inserted into pMH391 [15]. The vector pMH391 contains an unstable version of the green fluorescent protein Gfp(ASV) inserted between the NotI sites of pUCP22Not (Ap\(^{\beta}\) Gm\(^{\beta}\)). The fusion point between PahyI and gfp(ASV) is located between the BamHI terminal point of the \( ahyl \) sequence and XbaI (originating from pBA113 [14]). The resulting plasmid termed pMHRHL contains a divergent transcribed PahyR-ahylR and PahyI-gfp(ASV) transcriptional fusion flanked by NotI restriction sites (Fig. 5a). The plasmid was introduced by electroporation into \textit{E. coli} MT102 [22]. The resulting monitor strain is named MH205.

5.2. Growth assays

An overnight culture of MH205 was diluted in fresh ABT minimal medium (B medium (Clark & Maaløe, 1967) plus 2.5 mg/l thiamine and 10% A10 (Clark & Maaløe, 1967)) supplemented with 0.5% glucose and 0.5% casa amino acids and grown to exponential phase at 37°C and 200 rpm. The growing culture was diluted to OD\(_{540\text{nm}}\) = 0.1 measured on Genesys 10 uv, USA and distributed to a heated (37°C) 96 welled microtiter plate (Nunc, black PolyBase, USA) containing two-fold dilution rows of C4-HSL (Sigma Aldrich, BioChemika Fluka, 09945) starting at 12\,\mu M. The microtiter plate was placed in a
Wallac 1420 VICTOR² (Perkin Elmer, MA, USA) set to measure OD₅₆₀nm and fluorescence (excitation: 485 nm, emission: 535 nm) every fifth minute, 99 repetitions in total. Additionally, corresponding measurements of OD₅₆₀nm and fluorescence offsets were performed and subtracted from the data to obtain the growth curve (Fig. 2) and the active response of the monitor strain to the exogenous C4-HSL. Throughout data are normalized to spontaneous Gfp production deduced from an exponential fit (Fig. 2) to the fluorescence offset experiments after subtraction of Gfp from a nutrient-only offset measurement.

5.3. Decay of signal molecules

The decay of exogenous C4-HSL was established by measuring the Gfp response of the MH205 culture to a 1 μM C4-HSL solution having already aged 1, 2, 4, and 8 hours in ABT minimal medium. Finding identical responses at all ages, the decay of C4-HSL is negligible (λs < 0.051 h⁻¹).

5.4. Background subtraction

The spontaneous Gfp production shown in Fig. 2 is extracted from data by subtraction of an ABT-medium-only from a sample with MH205 cells (C) growing exponentially in medium (M), g(t) = gC(t) - gd(t). The signal (induced + spontaneous) at a finite concentration of signal molecules (S) is g(t) = gS(t) + gd(t). The induced signal is then g(t) - gd(t) = gS(t) + gd(t). The interpretation of this as an induced signal relies on the linearity of the production and maturation of Gfp with respect to IA and IF.

5.5. Normalization

In order to simplify the comparison with the mathematical model the Gfp is normalized "per plasmid". Traditionally, this is done by normalization of the Gfp signal to the optical density. However, we use the fluorescent response from the spontaneous Gfp production for normalization. This has the advantage that the light path for excitation as well as emission is the same in the normalization measurement and the induced excitation measurements thus ensuring correct normalization "per plasmid" of the response. In Fig. 2 the exponential normalization divisor is shown. Exponential growth is observed up till t = 5 h. Beyond this time we refrain from interpreting the data since knowledge of the growth rate λs entering in the model description is lost.

5.6. Estimation of parameters

The estimation of the model parameters is obtained by minimizing a chi-square measure \( \chi^2 = \sum_{i=1}^{n}(g(s,t) - gd(t))/g0(t) - \text{model}(s,t)^2/\sigma^2(s,t) \), where summations are over signal concentrations and time. The variance \( \sigma^2(s,t) \) consists of two contributions, the variance between 6 repetitions of the experiment and the variance of the rapid fluctuations in the data. Only data for \( t < 4 \text{ h and } s > 12/2^9 \mu M \) and included in the fit. The uncertainties of the estimates in Table 2 reflect the variations obtained in the simplex minimization procedure from random initial parameter seeds [23]. The resulting chi-square is of order \( \chi^2 = 0.9-1.2 \). During the parameter search we have restricted ourselves to \( K_1 > 0.15 \), to obtain values in reasonable agreement with the literature [19]. We find that, when restricting the search to values of \( \lambda_s \) in accordance with Leveau et al. [16], only values of \( K_1 \) smaller than 0.4 are acceptable. The scaling relations discussed in section 3.5 are excellently reproduced by all possible parameter sets.

6. Acknowledgments

We are grateful to Anne Kathrine Nielsen for assistance in the laboratory.

References


<table>
<thead>
<tr>
<th>Parameter/variable</th>
<th>Magnitude</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$s$, $[S]$, [C4-HSL]</td>
<td>Signal molecule concentration.</td>
<td>$&lt; 10 \mu M$</td>
</tr>
<tr>
<td>$r_0$, $[R]$, [AhyR]</td>
<td>Free regulator concentration.</td>
<td>$&lt; 1 \mu M$</td>
</tr>
<tr>
<td>$r_1$, $[RS]$</td>
<td>Concentration of RS complex.</td>
<td>$&lt; 10 \mu M$</td>
</tr>
<tr>
<td>$r_{1\text{sat}}$</td>
<td>Saturated concentration of RS complex.</td>
<td>$\sim 10 \mu M$</td>
</tr>
<tr>
<td>$n$, $[N]$</td>
<td>Concentration of non-mature Gfp in cell.</td>
<td>(1-100) $\mu M$</td>
</tr>
<tr>
<td>$g$, $[G]$</td>
<td>Concentration of mature Gfp in cell</td>
<td>(1-100) $\mu M$</td>
</tr>
<tr>
<td>$R_a$, [ahyR]</td>
<td>Concentration of ahyR sites in cell</td>
<td>$\sim 10 \text{nM}$</td>
</tr>
<tr>
<td>$I_a$, [ahyI]</td>
<td>Concentration of ahyI sites in cell</td>
<td>$\sim 10 \text{nM}$</td>
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<tr>
<td>$I_a$</td>
<td>Concentration of activated ahyI sites in cell</td>
<td>$&lt; I_a$</td>
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<tr>
<td>$\lambda_1$</td>
<td>Decay of C4-HSL</td>
<td>$&lt; 0.05 \text{h}^{-1}$</td>
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<tr>
<td>$\lambda_c$</td>
<td>Growth rate for cells.</td>
<td>$(0.58 \pm 0.02) \text{h}^{-1}$</td>
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<tr>
<td>$b_0$</td>
<td>AhyR transcription rate per plasmid.</td>
<td>$\sim 1000 \text{h}^{-1}$</td>
</tr>
<tr>
<td>$\lambda_0$</td>
<td>Degradation rate for AhyR.</td>
<td>$\sim 20 \text{h}^{-1}$</td>
</tr>
<tr>
<td>$k^+_c$</td>
<td>Production rate for RS complex.</td>
<td>$\sim 600 \mu M^{-1}\text{h}^{-1}$</td>
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<tr>
<td>$k^-_c$</td>
<td>Dissociation rate for RS complex.</td>
<td>$\sim 1000 \text{h}^{-1}$</td>
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<tr>
<td>$\lambda_1$</td>
<td>Degradation rate for RS complex</td>
<td>$\sim 0.6 \text{h}^{-1}$</td>
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<tr>
<td>$k^+_l$</td>
<td>On-rate for RS complex binding to ahyI</td>
<td>$\sim 200 \mu M^{-1}\text{h}^{-1}$</td>
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<tr>
<td>$k^-_l$</td>
<td>Off-rate for RS complex binding to ahyI</td>
<td>$\sim 300 \text{h}^{-1}$</td>
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<tr>
<td>$b_n$</td>
<td>Spontaneous Gfp transcription rate</td>
<td>$\sim 1000 \text{h}^{-1}$</td>
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<tr>
<td>$k^+_n$</td>
<td>Induced non-mature Gfp production rate.</td>
<td>$\sim 20 b_n$</td>
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<tr>
<td>$\lambda_n$</td>
<td>Degradation rate for non-mature Gfp.</td>
<td>$\sim 0.4 \text{h}^{-1}$</td>
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<td>$k_g$</td>
<td>Maturation rate for Gfp.</td>
<td>$\sim 1.5 \text{h}^{-1}$</td>
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<tr>
<td>$\lambda_g$</td>
<td>Degradation rate for mature Gfp.</td>
<td>$\sim 0.4 \text{h}^{-1}$</td>
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</table>

Table 1: Table of variables and constants used in the model. Numbers are given as order-of-magnitude estimates. CS = Current study. * Current study based on values from [19, 16].
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A/(1 + K_I)$</td>
<td>$19.3 \pm 0.3$ μM</td>
<td>Saturated response, Eq. (24).</td>
</tr>
<tr>
<td>$K_1/(1 + 1/K_I)$</td>
<td>$(0.80 \pm 0.05) \mu M$</td>
<td>Effective cut off, Eq. (25).</td>
</tr>
<tr>
<td>$K_I$</td>
<td>$0.4 \pm 0.2$</td>
<td>Dissociation constant for binding of RS complex to Ahyl site (in units of $r_{1\text{sat}}$).</td>
</tr>
<tr>
<td>$\lambda_1$</td>
<td>$(0.3 \pm 0.2) \text{h}^{-1}$</td>
<td>Degradation rate for RS complex.</td>
</tr>
<tr>
<td>$K_1$</td>
<td>$(0.20 \pm 0.20) \mu M$</td>
<td>Dissociation constant for RS complex.</td>
</tr>
<tr>
<td>$\Lambda_n$</td>
<td>$(2.5 \pm 0.3) \text{h}^{-1}$</td>
<td>Effective decay for non-mature Gfp, Eq. (16).</td>
</tr>
<tr>
<td>$\Lambda_g$</td>
<td>$(2.0 \pm 0.3) \text{h}^{-1}$</td>
<td>Effective decay for mature Gfp, Eq. (17).</td>
</tr>
<tr>
<td><strong>Fixed parameters during fit:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\lambda_c$</td>
<td>$(0.58 \pm 0.02) \text{h}^{-1}$</td>
<td>Cell growth rate.</td>
</tr>
<tr>
<td>$\lambda_s$</td>
<td>$0 \text{h}^{-1}$</td>
<td>Decay of signal molecules.</td>
</tr>
</tbody>
</table>

Table 2: Table of estimated model parameters as described in sections 3.4, 3.5, and 5.6. This set of parameters have been used in the comparison between model and data in Fig. 3.
Figure 1: Diagram of the AhyRI sensor system in the E. coli monitor strain MH205. The QS regulatory system, termed the AhyRI-system, consists of a signal molecule synthetase (AhyI) and a transcriptional regulator (AhyR).
Figure 2: Spontaneous Gfp production for MH205 measured on a Wallac 1420 VICTOR. The growth is exponential for 5 hours with a growth rate of $\lambda_c = 0.58h^{-1}$. As a control, the optical density is shown and follows the same exponential for about 3 hours, whereafter rescattering causes the OD measurement to underestimate the number of cells [18].
Figure 3: The measured Gfp level expressing the activation of the AhyRI-system for selected initial C4-HSL concentrations. The maximal initial C4-HSL concentration used is 12 μM in the datacurve labeled $i = 0$. For each $i = 1, ..., 8$ the signal molecule concentration is divided by 2. The spontaneous Gfp production, $g_0$, i.e. the Gfp production without signal molecules, has been subtracted and divided away to obtain the normalized $\frac{(g - g_0)}{g_0}$. The model curves are fits with parameters given in Table 2. Only $t < 4$ h and $s > 12/2^8$ μM and included in the fit.
Figure 4: Concentration of (a) regulator [AhyR] = r_0 and (b) activated regulator complex in units of r_1sat. (c) Activated ahyI operator (I_a) in units of I_t. (d) Mature Gfp(ASV) (g - g_0)/g_0 as a function of time after the introduction of signal molecules. The index at the end of each curve indicates the concentration of signal molecules s = 12µM/2^i, i = 0, ..., 8.
Figure 5: (a) Schematic drawing of the AhyRI sensor system and the nucleotide sequence of the fusion point between ahyRI and unstable green fluorescence protein, gfp (ASV). Relevant restriction sites are shown and primers used to sequence the insertion are indicated by arrows. On the nucleotide sequence the direction of gene transcription is indicated by arrows and start codons are shown in bold letters. RBSII refers to the synthetic ribosome binding site carried by pJBA113, 593445 and 590844 refer to nucleotide numbers of the A. hydrophila genome. (b) Primers used to generate sequence information.

Primer I: Standard pUC fwd primer
Primer II: 5'-CCTCTCCAC TGACAGAAAATTTGT-3'
Primer III: Standard pUC rev primer