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Traveling waves in response to a diffusing quorum sensing signal in spatially-extended bacterial colonies

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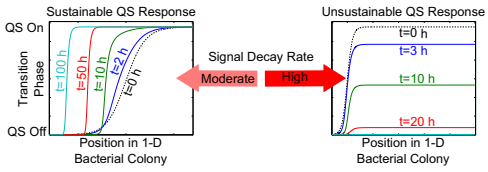
HIGHLIGHTS

- Quorum sensing (QS) is a means of bacterial communication via an autoinducer signal.
- Developed a spatially-extended model for the LuxR–LuxI QS circuit in *A. scheri*.
- Experiments show that model describes the LuxR–LuxI QS system in *A. scheri* well.
- Model exhibits a propagating wave of QS activation in simulations.
- Model has an analytic traveling wave solution.

GRAPHICAL ABSTRACT



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ABSTRACT

In the behavior known as quorum sensing (QS), bacteria release diffusible signal molecules known as autoinducers, which by accumulating in the environment induce population-wide changes in gene expression. Although QS has been extensively studied in well-mixed systems, the ability of diffusing QS signals to synchronize gene expression in spatially extended colonies is not well understood. Here we investigate the one-dimensional spatial propagation of QS-circuit activation in a simple, analytically tractable reaction-diffusion model for the LuxR–LuxI circuit, which regulates bioluminescence of the marine bacterium *Aliivibrio fischeri*. The quorum activation loop is modeled by a Hill function with a cooperativity exponent ($m=2.2$). The model is parameterized from laboratory data and captures the major empirical properties of the LuxR–LuxI system and its QS regulation of *A. fischeri* bioluminescence. Our simulations of the model show propagating waves of activation or deactivation of the QS circuit in a spatially extended colony. We further prove analytically that the model equations possess a traveling wave solution. This mathematical proof yields the rate of autoinducer degradation that is compatible with a traveling wave of gene expression as well as the critical degradation rate at which the nature of the wave switches from activation to deactivation. Our results can be used to predict the direction and activating or deactivating nature of a wave of gene expression in experimentally controlled bacterial populations subject to a diffusing autoinducer signal.

1. Introduction

It is not uncommon in the natural world for organisms to act collectively, as we see in schools of fish, flocks of birds and swarms of insects. To enact collective action, individuals make decisions

Abbreviation: QS, Quorum sensing

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based on locally acquired cues, which on the macro-scale may be gained via sight or sound (Couzin, 2009). On the micro-scale, some bacteria can also use locally acquired cues to regulate their behavior. In a behavior known as quorum sensing (QS), bacteria synthesize and secrete diffusible signal molecules called autoinducers that accumulate in the local environment. Once a critical concentration of autoinducer is reached, it triggers a population-wide shift in gene expression (Dunlap, 1999; Fuqua et al., 1996; Miyashiro and Ruby, 2012).

Traditionally, QS research focused on well-mixed systems, where this shift in gene expression appears homogeneously in space (Chiang et al., 2011; Ward et al., 2001; Nilsson et al., 2001; James et al., 2000; Garde et al., 2010). However QS often occurs in spatially-extended and inhomogeneous environments, where a colony-wide change in gene expression can appear as a propagating wave. Danino et al. (2010) experimentally exhibited such a wave by exposing a colony of engineered cells to an autoinducer flow within a microfluidic device.

Studies of spatially extended QS systems often focus on biofilms (Anguige et al., 2006; Frederick et al., 2011; Klapper and Dockery, 2010; Chopp et al., 2002; Dockery and Keener, 2001), extracellular matrices of polysaccharides that encase bacteria and protect them from their environment (Costerton et al., 1999). Ward et al. (2003) develop a spatially-extended QS model that incorporates biofilm production and demonstrates (via simulation) a propagating wave of up-regulation through the colony. Since biofilms, like many natural bacterial environments, are highly spatially heterogeneous (Stewart, 2003), one would expect diffusional signaling to be inefficient. This raises the question of how well group behavior can be synchronized by a diffusing signal. Though some authors have observed propagating waves of QS response, a more efficient and significant response would emerge as a traveling wave, a specific type of propagating wave with fixed wave front and speed that travels an infinite distance. (A precise mathematical definition is given in Section 2.2.) A traveling wave of response is indicative of perfect colony-wide synchronization: once the fixed wave front and speed have developed, each cell in the colony receives exactly the same information and enters the same activation state in exactly the same way. The feasibility of traveling waves raises the possibility that microbes can transmit and exchange significant amounts of information through the quorum sensing mechanism. Ward et al. (2003) explore certain necessary conditions for the existence of such a traveling wave solution to their model, but do not give a mathematical proof.

Here, we introduce a simple QS model intended to represent the LuxR–LuxI QS system in *Aliivibrio fischeri* (formerly *Vibrio fischeri*, Urbanczyk et al., 2007). Our model incorporates a minimal essential set of biologically relevant terms and exhibits a QS shift in gene expression, which appears as a traveling wave. Our model is sufficiently simple to be analytically tractable, and we mathematically prove the existence of this traveling wave. This proof allows us to obtain a simple, biologically interpretable condition under which such a propagating wave of QS activation can occur. We experimentally parameterize our model and show that the aforementioned analytic traveling wave exists under biologically feasible parameter values. This paper enhances the existing body of work on propagating waves in quorum sensing systems by detailing a simple, biologically interpretable model of the LuxR–LuxI QS system that not only exhibits a propagating wave of QS activation in simulations, but also has an analytic traveling wave solution. Our experimental parameterization shows that our model contains sufficient detail to describe the major qualitative behavior of the LuxR–LuxI QS system in *A. fischeri* and thus that our model contains the components necessary to describe a biologically relevant shift in QS regulation.

1.1. LuxR–LuxI system

The regulation of bioluminescence in the marine bacterium *Aliivibrio fischeri* receives input from three QS circuits, designated AinS–AinR, LuxS–LuxP/Q, and LuxR–LuxI. The LuxR–LuxI system is the more dominant and best-studied regulator of bioluminescence (Miyashiro and Ruby, 2012; Lupp et al., 2003) and is the focus of this work. The AinS–AinR and LuxS–LuxP/Q systems influence bioluminescence by regulating production of sRNA that transcriptionally represses *litR* transcript. The transcriptional regulator LitR enhances *luxR* expression without altering expression of the other *lux* genes (Miyashiro and Ruby, 2012).

In the LuxR–LuxI system (Fig. 1), the *luxR* gene encodes the transcription factor LuxR. The *luxICDABEG* operon encodes the LuxI enzyme as well as components necessary for synthesis of the luciferase, the light-producing enzyme, and production of its substrates. LuxI catalyzes the synthesis of the acyl-homoserine lactone (AHL) 3OC6-HSL, an autoinducer that interacts with LuxR to form the LuxR/3OC6-HSL complex. The LuxR/3OC6-HSL complex activates the expression of the *luxICDABEG* operon, creating a positive feedback loop (Dunlap, 1999; Fuqua et al., 1996; Miyashiro and Ruby, 2012).

This positive feedback loop acts as a switch, flipping genetic expression from an unactivated state to an activated state. In the case of the LuxR–LuxI system in *A. fischeri*, the activated state is characterized by bioluminescence, which the unactivated state lacks. Since AHLs are freely diffusible through the cell membrane, their concentrations are locally approximately equal extracellularly and intracellularly (Fuqua et al., 1996). Hence, we expect that if one bacterium is experiencing an AHL level high enough to be activated, then so are its nearest neighbors. As more bacteria become activated, we will see a switch on the colony level from the unactivated state to the activated state. In a spatially extended system, the activated region grows in the spatial dimension as the AHL signal diffuses from its source. Similarly, since LuxR binds to 3OC6-HSL reversibly (Miyashiro and Ruby, 2012) if we remove 3OC6-HSL from the system at a fast enough rate, we expect to see a colony-wide switch from the activated state to the unactivated state.

Both Danino et al. (2010) and Dilanji et al. (2012) experimentally exhibited spatially extended patterns of QS activation initiated by a diffusive signal. This raises the question of whether or not a spatially extended QS system with a diffusing AHL signal could exhibit a traveling wave of activation, where such a wave takes a specific mathematical form (described in Section 2.2). To answer this question, we developed a simple mathematical model that describes the LuxR–LuxI system in *A. fischeri* and incorporates a minimal essential set of biologically relevant components. We then proved that there exists a traveling wave solution to this model under certain parameter conditions. It then remained to show that biologically plausible parameter values satisfy these conditions. We parameterized our model using experimental data collected under spatially-homogeneous conditions (Section 6) and

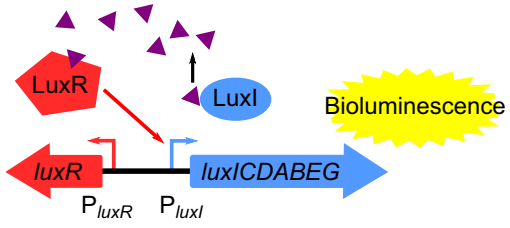

Fig. 1. The LuxR–LuxI circuit contained in wild type *A. fischeri* strain ES114. ES114 has an intact lux operon for synthesis (via LuxI) and detection (via LuxR) of AHL signal and production of bioluminescence.

used these to parameterize our model. We found that the LuxR–LuxI system can exhibit a traveling wave of QS activation in a spatially extended colony of *A. fischeri*. Lastly, we simulated our model with these experimentally determined parameter values to demonstrate the wave of QS activation and to explore the effects of varying the AHL diffusion rate and the AHL decay rate.

2. Mathematical model and theoretical results

2.1. Model

The following model is largely inspired by that in Dilanji et al. (2012), but has been simplified to allow for the mathematical characterization of a traveling wave. In Dilanji et al. (2012), we presented a model for the activation of a QS circuit in the sensor strain *E. coli* + pJBA132 in response to a diffusing AHL signal and showed good agreement between this model and experiments. As in Dilanji et al., we model a colony of bacteria (in this case, *A. fischeri*) embedded in agar in a long rectangular lane environment of fixed volume. The agar lane is narrow and homogeneous in its width and depth and therefore we describe it as one dimensional, with the coordinate x representing spatial position along the length of the lane. Here, however, we assume that the lane has infinite length, that is, that $x \in \mathbb{R}$.

To model the LuxR–LuxI system found in *A. fischeri*, we consider only AHL concentration and LuxI concentration in an infinite one-dimensional domain x. Though the concentration of LuxR plays a role in the LuxR–LuxI system, we assume that it is not a limiting factor in our system and do not model it explicitly. We also do not model the population density of *A. fischeri*, though the growth phase of bacteria plays a role in the regulation of protein synthesis (Ward et al., 2003; Dilanji et al., 2012). Thus, we expect that our model will only be valid for short time scales relative to cell growth.

As AHL is freely diffusible through the *A. fischeri* cell membrane (Kaplan and Greenberg, 1985), we describe the spatial spread of AHL (concentration $A(x,t)$, nM) by the diffusion equation:

$$\frac{\partial A}{\partial t} = D\frac{\partial^2 A}{\partial x^2}$$

where D (mm^2/h) is the diffusion constant (Kaplan and Greenberg, 1985; Fuqua et al., 1996). Since LuxI catalyzes *A. fischeri* synthesis of AHL, the production rate of AHL depends explicitly on the LuxI concentration L (measured as luminescence, counts/pixel, as will be described later). We denote the per unit LuxI production rate of AHL by λ (units of (nM pixel)/(counts h)). Finally, we assume that AHL degrades at a constant per capita rate γ (h^{-1}). Though AHL is stable on the scale of hours at neutral pH, AHL degrades at a non-negligible rate under alkaline conditions and in the presence of quorum-quenching enzymes (Horswill et al., 2007; Englmann et al., 2007; Amara et al., 2011; Wang et al., 2007). Hence, we describe the AHL concentration $A(x,t)$ by

$$\frac{\partial A}{\partial t} = D\frac{\partial^2 A}{\partial x^2} + \lambda L - \gamma A.$$

The rate of change of the concentration of LuxI per unit volume of agar is explicitly dependent on the concentration of AHL through the activation of the expression of the *luxICDABEG* operon by the LuxR/AHL complex, modeled here with a Hill function (1). The Hill function enables a cooperative switch from the synthesis-off state to the synthesis-on state via the parameters a and m:

$$f(A) = \frac{hA^m}{a^m + A^m} \tag{1}$$

where a (nM) is the half-saturation constant, m (unitless) is the Hill coefficient and h (units of counts/(pixel h)) is the maximum LuxI production rate. We denote the enzymatic per capita LuxI decay rate by β (h^{-1}). Thus, the concentration of LuxI is given by

$$\frac{\partial L}{\partial t} = f(A) - \beta L$$

where $f(A)$ is given in (1).

Our complete model is

$$\frac{\partial A}{\partial t} = D\frac{\partial^2 A}{\partial x^2} + \lambda L - \gamma A \tag{2a}$$

$$\frac{\partial L}{\partial t} = f(A) - \beta L \tag{2b}$$

where

$$f(A) = \frac{hA^m}{a^m + A^m}. \tag{3}$$

Table 1
A summary of model variables and parameters. Values listed were obtained via fitting as described in Appendix C, SI. The value of h listed is derived from those that give good qualitative agreement in bulk culture experiments and is not a fit value. A value for γ is not listed because γ must be artificially induced.

Variable/ parameter	Definition	Value
A	AHL concentration	nM
D	AHL diffusion constant	1.98 mm^2/h
λ	AHL production rate per unit of LuxI	0.43 (nM pixel)/ (counts h)
γ	Per capita AHL degradation rate	h^{-1}
L	LuxI concentration measured as luminescence	counts/pixel
f	Cooperative switch function	
h	Maximum LuxI production rate	970 counts/(pixel h)
a	Half-activation coefficient	450 nM
m	Hill coefficient	2.2 (unitless)
β	Per capita decay rate of luminescence (LuxI)	0.32 h^{-1}

Table 1 gives a summary of parameter and variable definitions as well as parameter fit values. Our fit values were derived via a series of spatially homogeneous experiments and subsequent optimizations-of-fit. (See Section 6, Appendix C, Supplemental Information (SI), Abdulkarim et al., 2009; Chicone, 2006; Ignowski and Schaffer, 2004; Logemann and Ryan, 2004; Lundin et al., 1976; Matlab, 2009; Nealson et al., 1970; Rudin, 1976; Schaefer et al., 1996; Thompson et al., 1991.)

To determine the concentration of LuxI, which we do not explicitly detect, we first measure the luminescence of the system. Since the transcription of *luxICDABEG* is necessary to synthesize both LuxI and components necessary for bioluminescence, the production rate of LuxI (due to the AHL concentration A) is proportional to the rate of increase of luminescence of the system. Since the system begins devoid of both LuxI and luminescent compounds, the concentration of LuxI is then proportional to the measured luminescence of the system. Thus, we describe the concentration of LuxI using arbitrary luminescence units (counts/pixel).

2.2. The existence of a traveling wave solution

As the proof of the general theorem (Theorem 2) is somewhat technical, we will discuss the existence of a traveling wave solution to our particular example system (2) to give insight as to the outline of the proof. The complete proof of Theorem 2 can be found in the SI. This theorem and its proof are extensions of those found in Jin and Zhao (2008). Our extension allows for a wider class of functional forms to describe the rate of change of LuxI concentration per unit volume of agar. In the context of our

system and model, this extension is necessary to allow for the use of the cooperative switch function $f(A)$ (3) to describe the dependence of the change of the concentration of LuxI per unit volume agar on the concentration of AHL. We seek a traveling wave solution to system (2). We let the domains of x and t be infinite. Mathematically, a traveling wave solution to (2) is a solution of the form $(A(\tau), L(\tau))$ where $\tau = x + ct$ for some real number c, called the wave speed, and there exist some finite real numbers $A_0 < A_2$ and $L_0 < L_2$ such that

$$\lim_{\tau \to -\infty} (A(\tau), L(\tau)) = (A_0, L_0) \tag{4}$$

and $$\lim_{\tau \to \infty} (A(\tau), L(\tau)) = (A_2, L_2). \tag{5}$$

Suppose that a traveling wave solution to (2) exists and that the wave speed $c \neq 0$. Substituting $(A(\tau), L(\tau))$ into system (2), we have

$$A' = W \tag{6a}$$

$$L' = \frac{f(A) - \beta L}{c} \tag{6b}$$

$$W' = \frac{cW - \lambda L + \gamma A}{D} \tag{6c}$$

where prime ($'$) denotes $d/d\tau$ and we have introduced $W = A'$. (See Appendix D) If there exists a solution $(A(\tau), L(\tau), W(\tau))$ of (6) such that

$$\lim_{\tau \to -\infty} (A(\tau), L(\tau), W(\tau)) = (A_0, L_0, 0) \tag{7}$$

and $$\lim_{\tau \to \infty} (A(\tau), L(\tau), W(\tau)) = (A_2, L_2, 0) \tag{8}$$

then $(A(\tau), L(\tau))$ is a solution of (2) and clearly the limits (4) and (5) are satisfied. We seek this solution.

In A, L, W-space, such a solution appears as a trajectory connecting the steady states $(A_0, L_0, 0)$ and $(A_2, L_2, 0)$. To find such a value of c, and a corresponding connection of steady states, we first show that for all positive c-values there is always a solution of (6) that satisfies (7), but not necessarily (8). Under a suitable integral condition (see Theorem 1 below), and for certain choices of positive c's, this solution overshoots $(A_2, L_2, 0)$, whereas for other choices of c, it undershoots. Finally, we show that there exists some value of c such that the solution neither overshoots nor undershoots, and in fact, satisfies (8). This value of c and the corresponding solution yield the sought-after wave speed and traveling wave solution for our original model. We conclude our discussion by stating the existence of a traveling wave solution to (2) as a theorem:

Theorem 1. *Suppose (2) satisfies the conditions*

(i) *$f(A)$ is continuously differentiable for $A \in [0, \infty)$.*
(ii) *There exist three points $E_0 = (0, 0)$, $E_1 = (A_1, L_1)$ and $E_2 = (A_2, L_2)$ with $0 < A_1 < A_2$ and $0 < L_1 < L_2$ such that E_0, E_1 and E_2 are the only zeros of the reaction system*

$$\frac{dA}{dt} = \lambda L - \gamma A \tag{9a}$$

$$\frac{dL}{dt} = f(A) - \beta L \tag{9b}$$

in the order interval $[E_0, E_2]$.
(iii) *E_0 and E_2 are stable and E_1 is a saddle.*

Then there exists some $c \in \mathbb{R}$ such that $(A(x+ct), L(x+ct)) = (A(\tau), L(\tau))$ is a traveling wave solution to (2) with $\lim_{\tau \to -\infty}(A(\tau), L(\tau)) = E_0$ and $\lim_{\tau \to \infty}(A(\tau), L(\tau)) = E_2$. Furthermore, the wave speed c has the same sign as the integral $\int_0^{A_2} (1/\beta) f(A) - (\gamma/\lambda) A \, dA$.

The proof of the general theorem (Theorem 2) is conceptually identical to that of Theorem 1 above. The statement and complete proof of the general theorem can be found in Appendix A (SI). The following two sections discuss the conditions and wave speed result of Theorem 1. A similar analysis could be conducted for any model that satisfies the conditions of the general theorem.

2.3. A discussion and reduction of Conditions (i)–(iii) of Theorem 1

Theorem 1 gives three easy-to-check conditions that guarantee the existence of a traveling wave solution to (2). Condition (i) requires that the function $f(A)$, which describes the production of LuxI due to the concentration of AHL, is continuous, differentiable and that its derivative $((d/dA)f(A))$ is continuous. This means that as the concentration of AHL changes smoothly, the rate at which LuxI is produced by AHL changes smoothly.

The second and third conditions of Theorem 1 can be interpreted as conditions for a well-mixed (spatially homogeneous) batch culture model given by (9). These conditions require that system (9) has one lower stable steady state E_0 (that represents the down-regulated QS state) and one upper stable steady state E_2 (that represents the up-regulated QS state). Since both E_0 and E_2 are stable, if the system starts in a down-regulated (or up-regulated) state, it will remain in that state. That is, a QS signal cannot appear spontaneously in a down-regulated system and a QS signal is sustainable in an up-regulated system. Bistability in the pure reaction system (i.e., ignoring diffusion) is an important mechanism underlying traveling waves in many biological systems (Chang et al., 2010; Hadeler and Rothe, 1975; Ward et al., 2003).

The three conditions of Theorem 1 can be reduced to a biologically interpretable lemma (proof in Appendix B, SI):

Lemma 1. *System (2) satisfies Conditions (i)–(iii) of Theorem 1 if and only if $\lambda > 0$, $h > 0$, $m > 1$, $a > 0$, $\beta > 0$ and*

$$1 < \sup_{A \in [0, A_2]} \frac{f(A)}{A\gamma} \frac{\lambda}{\beta} \tag{10}$$

or equivalently,

$$0 < \gamma < \frac{\lambda h (m-1)^{(m-1)/m}}{\beta a m}.$$

We now give a biological interpretation of the inequality (10). First, note that $f(A)/A$ is the LuxI production rate per nM AHL and that $1/\gamma$ is the mean lifetime of one nM AHL. Then $f(A)/A\gamma$ gives the mean units of LuxI produced by one nM AHL over its lifetime. Second, since λ is the AHL production rate per unit of LuxI and $1/\beta$ is the expected lifetime of one unit LuxI, λ/β is the mean units of AHL produced by one unit of LuxI over its lifetime. Thus, the product $(f(A)/A\gamma)\lambda/\beta$ is the mean nM AHL produced by one nM AHL over its lifetime by way of the LuxI pathway and $\sup_{A \in (0, A_2)}(f(A)/A\gamma)\lambda/\beta$ is the supremum (least upper bound) of this production for positive AHL concentrations that are less than A_2, the AHL concentration associated with the up-regulated steady state E_2. If (10) holds, then, at least for particular environmental concentrations of AHL, one nM AHL produces more than one nM AHL over its lifetime. Thus, the AHL signal is amplified and QS up-regulation is sustainable. Furthermore, since $1 = (f(A_2)/A_2\gamma)\lambda/\beta$ (by examination of the nullclines of (9)) and since (for $m > 1$) $(d/dA)(f(A)/A\gamma)\lambda/\beta|_{A = A_2} < 0$, there exists some $A^* > 0$ such that $(f(A)/A\gamma)\lambda/\beta > 1$ for all AHL concentrations A between A^* and A_2. Then for these concentrations of AHL, the AHL signal is amplified and approaches the up-regulated steady state value A_2.

Conversely, if (10) does not hold, then either $1 > \sup_{A \in (0, A_2)}(f(A)/A\gamma)\lambda/\beta$ or $1 = \sup_{A \in (0, A_2)}(f(A)/A\gamma)\lambda/\beta$. In the former case, one

nM AHL produces less than one nM AHL over its lifetime via the LuxI pathway and an up-regulated system cannot be maintained. In the latter case, it is easy to show that $\sup_{A\in(0,A_2)}(f(A)/A\gamma)\lambda/\beta$ is unique and attained by some $0<A^*<A_2$. Then for all $A\in(0,A_2)$ such that $A\neq A^*$, one nM AHL produces less than one nM AHL over its lifetime via the LuxI pathway. Thus, the maximum sustainable AHL signal occurs for $A=A^*<A_2$ and an up-regulated system cannot be maintained.

2.4. The sign of the wave speed c

Lastly, we examine the integral condition given in Theorem 1. The condition states that if a traveling wave solution to (2) exists, then the wave speed c has the same sign as the integral

$$\int_0^{A_2}\frac{1}{\beta}f(A)-\frac{\gamma}{\lambda}A\,dA.$$

We first discuss the implications of the sign of the wave speed c, then give a biological interpretation of the above integral condition.

If c is positive, then for a fixed x and increasing t, the argument of the traveling wave solution $\tau=x+ct$ increases. Thus, in a fixed x-coordinate frame, the solution $A(x+ct)$ (or $L(x+ct)$) shifts to the left. Similarly, if c is negative, then for a fixed x and increasing t, $\tau=x+ct$ decreases and the solution $A(x+ct)$ (or $L(x+ct)$) shifts to the right. If $c=0$, then the solution is constant through time. The limit conditions (4) and (5) imply that if the wave has positive speed (and is therefore shifting to the left) then the system is switching from the down-regulated state to the up-regulated state. Similarly, a negative wave speed (and shift to the right) corresponds to a switch from the up-regulated state to the down-regulated state.

Now we consider the integral

$$\int_0^{A_2}\frac{1}{\beta}f(A)-\frac{\gamma}{\lambda}A\,dA=\frac{\gamma}{\lambda}\int_0^{A_2}A\left(\frac{f(A)}{A\gamma}\frac{\lambda}{\beta}-1\right)dA.$$

Since both γ and λ are positive, this integral has the same sign as

$$\int_0^{A_2}A\left(\frac{f(A)}{A\gamma}\frac{\lambda}{\beta}-1\right)dA. \tag{11}$$

As in the above discussion, $(f(A)/A\gamma)\lambda/\beta$ gives the mean nM AHL produced by one nM AHL over its lifetime when the environmental AHL concentration is equal to A. Then $(f(A)/A\gamma)\lambda/\beta-1$ is the mean change in nM AHL induced by one nM AHL over its lifetime and $A((f(A)/A\gamma)\lambda/\beta-1)$ is the total change in AHL concentration when the environmental AHL concentration is equal to A. Recall that since a traveling wave solution exists, $A(\tau)$ satisfies $\lim_{\tau\to-\infty}A(\tau)=0$ and $\lim_{\tau\to\infty}A(\tau)=A_2$ and that $A(\tau)$ is increasing (and therefore $A(\tau)\in(0,A_2)$ for all τ finite). Then (11) gives the total change in AHL concentration for all AHL present in the environment. Thus, if (11) is positive, the QS signal is amplified and the system shifts to an up-regulated state, corresponding to a positive wave speed c. Similarly, if (11) is negative, the QS signal decays and the system shifts to a down-regulated state, corresponding to a negative wave speed c.

3. Results

3.1. Existence of a traveling wave

Using the parameter values listed in Table 1 (found in Appendix C, SI) and by Theorem 1, we have that there exists a traveling wave solution to system (2) if Conditions (i)–(iii) of Theorem 1 hold. (For demonstrative purposes, we check the conditions of Theorem 1 instead of those given in Lemma 1.) In this section, we determine the range of γ values that admit a traveling wave solution and give a numerical representation of a traveling wave solution for a chosen AHL decay rate γ.

First, note that the function

$$f(A)=\frac{970A^{2.2}}{450^{2.2}+A^{2.2}}$$

is continuously differentiable for $A\in[0,\infty)$. Next, we examine the steady states of the reaction system

$$\frac{dA}{dt}=0.43L-\gamma A \tag{12a}$$

$$\frac{dL}{dt}=f(A)-0.32L. \tag{12b}$$

The nullclines of this system are

$$L=\frac{\gamma}{0.43}A \quad\text{and}\quad L=\frac{1}{0.32}f(A)$$

and the steady states' system (12) is given by the intersection of the nullclines. (See, for example, Fig. 2A and D.) It can be shown that (12) has exactly three nonnegative steady states if and only if $0<\gamma\lesssim1.45$. (For a general proof of this fact, see Appendix B, SI.) As an example, we let $\gamma=0.7\ \mathrm{h}^{-1}$. Then the steady states of (12) are $(0,0)$, $(A_1,L_1)=(148,240)$, and $(A_2,L_2)=(1775,2890)$. By examination of the Jacobian matrix, it is easy to see that $(0,0)$ and $(1775,2890)$ are stable, and that $(148,240)$ is a saddle. Then, by Theorem 1, there exists a traveling wave solution to (2). (In fact, this is true for any $0<\gamma\lesssim1.45$, as shown in Appendix B, SI.)

Fig. 2 shows two simulations of system (2) with the parameter values given in Table 1. For Fig. 2(A–C), $\gamma=0.7\ \mathrm{h}^{-1}$ and for Fig. 2 (D–F), $\gamma=2\ \mathrm{h}^{-1}$. In our simulations, the initial condition ($t=0$) is a continuous approximation to a step function with height equal to the upper stable steady state value when $\gamma=0.7\ \mathrm{h}^{-1}$ (1775 for $A(x,0)$ and 2890 for $L(x,0)$). The initial condition attains its maximum derivative at the midpoint of the simulated (effectively infinite) spatial domain. As expected, Fig. 2(A–C) displays a traveling wave while Fig. 2(D–F) does not. For $\gamma=0.7\ \mathrm{h}^{-1}$ (Fig. 2B, C), $A(x,t)$ and $L(x,t)$ approach their wave front profiles during the first ≈35 h. Then the A and L profiles do not change and these profiles shift to the left. It is during this time period that we can examine the speed of the exhibited wave. For $\gamma=2\ \mathrm{h}^{-1}$ (Fig. 2E, F), $A(x,t)$ and $L(x,t)$ decay to approximately zero by $t=30$ h. In this case, the rate of autoinducer decay is too large for the system to maintain a QS signal.

3.2. Wave speed

By Theorem 1, if a traveling wave solution to system (2) exists, then the wave speed c has the same sign as the integral

$$\int_0^{A_2}\frac{1}{\beta}f(A)-\frac{\gamma}{\lambda}A\,dA.$$

For the parameter values listed in Table 1, the above integral and the corresponding wave speed are positive for $0<\gamma<1.318$ and negative for $1.318<\gamma\lesssim1.45$. This implies that our system can establish a wave of QS activation through the entire colony only if the per capita AHL decay rate is less than $1.318\ \mathrm{h}^{-1}$ (equivalently if the AHL half-life is greater than $t_{1/2}\approx0.53$ h). Much work has been done to determine the rate of AHL decay under many conditions (for a review, see Wang et al., 2007), which allows us to infer experimental conditions under which our QS system will become completely up-regulated. For example, Schaefer et al. (2000) give AHL half-life as a function of pH: $t_{1/2}=10^{(7-\mathrm{pH})}$ days. Thus, we expect that our QS system can become completely up-regulated only if pH $\lesssim8.7$. Since the pH of seawater is less than 8.7 (Marion et al., 2011), our model implies that a wave of QS activation in *A. fischeri* can occur in the open ocean.

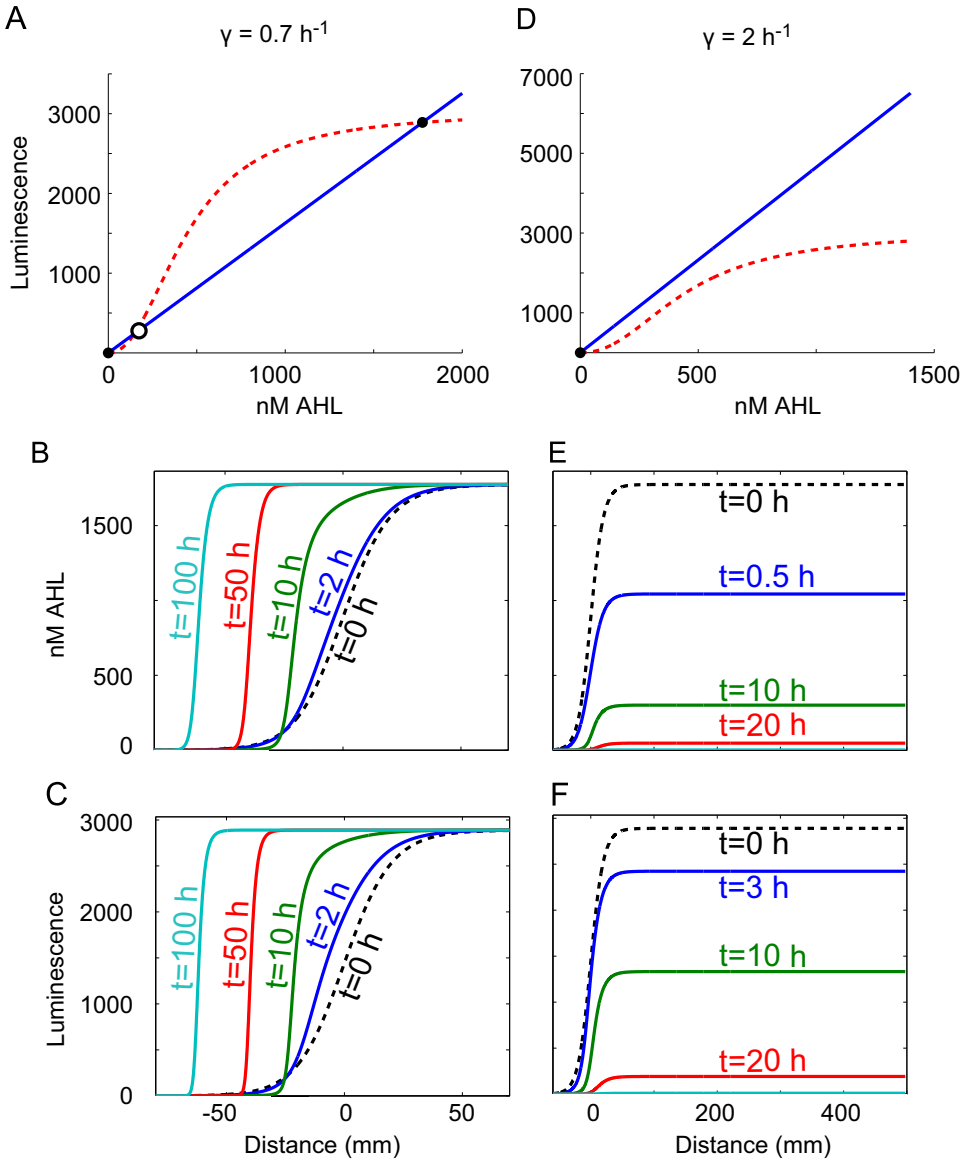

Fig. 2. A and C show the nullclines of the reaction system (9). The A-nullcline $L=(\gamma/\lambda)A$ (blue solid) and the L-nullcline $L=(1/\beta)f(A)$ (red dashed) intersect at the steady states (black dots, open circle) of (9). B, C, E and F display the evolution of AHL concentration (B, E) and LuxI concentration (C, F) (measured as luminescence) through time. Position is given as distance to the right of the center of the simulated domain. Parameter values used are listed in Table 1; $\lambda=0.7\ \mathrm{h}^{-1}$ (A–C) and $\lambda=2\ \mathrm{h}^{-1}$ (D–F). In A–C, the AHL decay rate λ is small enough for a sustained QS response to be possible. A shows that the reaction system (9) is a bistable system: it has three steady states, the extreme two of which are stable (black dots) and represent the QS activation (positive steady state) and deactivation (zero steady state) states. B and C show the time-evolution of the (AHL, LuxI concentration/luminescence) initial condition ($t=0$) given by the black dashed curve. B and C show that (for all positions x) the system approaches the positive stable steady state (positive black dot, A) asymptotically in time. In D–F, the AHL decay rate λ is too large for a QS response to be sustainable. D shows that (9) is a monostable system: it has only one, globally stable steady state at zero. E and F show that for the given initial condition (black dashed curve), the system approaches this zero steady state for all positions x asymptotically in time. (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this paper.)

Though we could find no reference to QS activation of *A. fischeri* in the open ocean, luminescent clouds of the bioluminescent QS bacteria *Vibrio harveyi* have been observed in the waters off the horn of Africa (Nealson and Hastings, 2006; Miller et al., 2005). This suggests that QS activation of *A. fischeri* in the open ocean is feasible under the correct conditions.

In addition to the sign of the wave speed, the proof of Theorem 1 gives a bound on the magnitude of the wave speed. By the Remark following Lemma 11 (Appendix A, SI), a positive wave speed c must satisfy

$$c \leq \sqrt{\frac{2D\lambda L_2(1+\epsilon)}{A_1(2+\epsilon)}}$$

where $\epsilon>0$ is chosen to be very small. Letting ϵ tend to zero, we have that

$$c \lesssim \sqrt{\frac{D\lambda L_2}{A_1}} \tag{13}$$

where A_1 and L_2 depend on the choice of space-independent parameters. We numerically estimated wave speeds for a range of diffusion constants D and AHL decay rates γ. As shown in Fig. 3, the upper bound (13) is approximately one order of magnitude larger than these numerically estimated wave speeds. Thus, this upper bound is of limited use in answering finer-scale questions. However, our numerically estimated wave speeds warrant some discussion.

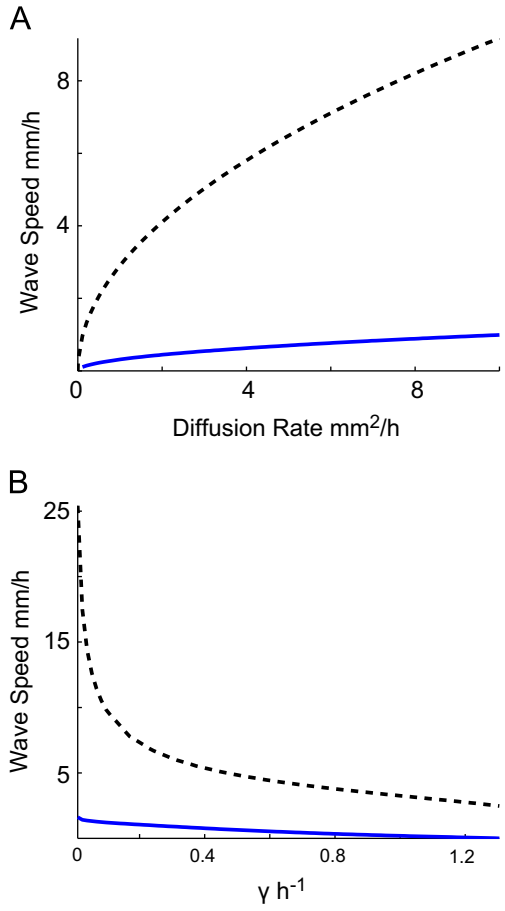

Fig. 3. The numerically estimated wave speed (blue solid) and the upper bound (13) (black dashed) as the diffusion rate D (A) or the AHL decay rate γ (B) vary. Parameter values used are listed in Table 1; $\lambda = 0.7\ \mathrm{h}^{-1}$ in A. (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this paper.)

3.3. Effect of diffusion rate and AHL decay rate on wave speed

We numerically estimated the speed of signal propagation for several choices of diffusion rate D and several choices of AHL decay rate γ. Only one parameter was varied at a time and all other parameter values were held constant at those values listed in Table 1. We found that as the diffusion rate D increases, the wave speed c increases. (Fig. 3A) This implies that shorter-chain AHLs, which diffuse more quickly than longer-chain AHLs, can elicit a faster colony-wide QS response than longer-chain AHLs.

By varying the AHL decay rate γ, we found that as γ increases the wave speed decreases. (Fig. 3B) Dilanji et al. (2012) showed that a diffusing signal (with diffusion constant $D = 1.98\ \mathrm{mm}^2\ \mathrm{h}^{-1}$) can activate a QS reporter over distances of ~ 1 m on time scales of ~ 10 h. In our model, a similar QS response would be induced by a per capita AHL decay rate of $\gamma \simeq 0.28$ h ($t_{1/2} \simeq 2.5$ h).

4. Discussion

We presented a simple, analytically tractable model for QS behavior regulated by the LuxR–LuxI circuit. We proved the existence of a traveling wave solution to this model, which corresponds to a wave of QS activation (or deactivation) advancing through a spatially extended bacterial colony. We then experimentally parameterized this system and showed that a traveling wave of QS activation is plausible in a spatially extended colony of *A. fischeri*. Lastly, we explored how the AHL diffusion constant D and the per capita AHL decay rate γ affect the speed of a wave of QS activation.

Our work is related to, but is distinct from previous work on quorum sensing-regulated shifts in gene expression in spatially-extended bacterial colonies. To demonstrate this point, we discuss two previous studies: as we do in this text, both Danino et al. (2010) and Ward et al. (2003) explicitly incorporate the diffusion of autoinducer molecules in their models. Danino et al. constructed microfluidic devices in which a modified *E. coli* strain exhibited an oscillating fluorescence response under an autoinducer flow. Under low flow rates, they observed spatially propagating waves of fluorescence. Danino et al. formulate a complimentary system of delay differential equations to describe their experiments, and though the complexity of this model deters analytic exploration, they give several computational simulations. One important disparity between this work and our own is that Danino et al. construct and study a bacterium that both promotes and represses its own fluorescence response. The addition of a repressor to the system generates an oscillatory, rather than constant, response.

Ward et al. model a spatially extended QS system that incorporates biofilm production. They approximate the order of magnitude of their parameters by referencing their own (previously published Ward et al., 2001) experimentally parameterized spatially homogeneous quorum sensing model. In their 2003 paper, Ward et al. perform simulations that demonstrate a propagating wave of up-regulation through the colony. They further investigate this wave by examining the scenario where an up-regulated biofilm is artificially introduced to a significantly larger down-regulated biofilm. The scenario is additionally restricted by the assumption that bacteria do not produce any autoinducer until they are up-regulated. Though Ward et al. do not mathematically prove the existence of a traveling wave solution to this simplified model, certain necessary conditions are explored (Ward et al., 2003). Our model is substantially simpler than those in Danino et al. and Ward et al., which allows us to analytically prove the existence of a traveling wave solution to our model.

One significant difference between our model and that proposed by Ward et al. is that our model allows a section of the spatially-extended bacterial colony to transition continuously from being down-regulated to being up-regulated (or vice versa). This is reflected in our model by the continuous cooperative switch function $f(A)$ (3), which allows for intermediate levels of LuxI production given intermediate AHL concentrations. The discontinuous analog to $f(A)$, akin to the assumptions in Ward et al., is a step function that takes zero for small values of AHL concentration A and some constant value h for large values of A. Thus our model generalizes an assumption and a result of Ward et al.: Ward et al. find that a true traveling wave exists only when up-regulated bacteria produce AHL at a fixed rate and down-regulated bacteria do not produce any AHL. We proved that a traveling wave solution exists even when a section of a bacterial colony produces AHL during the transition from begin down-regulated to being up-regulated.

The simple model presented here can be used to determine a starting point for finding experimental conditions under which a colony of spatially extended bacteria will exhibit a colony-wide shift in gene expression when the activating signal propagates only via diffusion. Biofilms are one example of such a circumstance (Stewart, 2003). However, our model neglects the spatially heterogeneous structure of biofilms and the flow of compounds into and out of the biofilm induced by the surrounding liquid medium (Chopp et al., 2002; Frederick et al., 2011). The traveling wave solution we explore generates perfect colony-wide synchronization. After an initial burn-in time, all bacteria in an infinitely long colony transition between QS activation states in exactly the same way. This transition is determined by the wave front profile and speed. That our simple QS model exhibits such a homogeneous response is indicative of the

ability of the QS circuit to synchronize bacterial gene expression within a colony. Though a perfect traveling wave of QS activation (or deactivation) may or may not occur in nature, our results show that the LuxR–LuxI circuit is an effective mechanism for colony-wide QS activation. Dilanji et al. experimentally showed that the LuxR–LuxI circuit can generate a qualitatively homogeneous QS response over distances of about a centimeter. Our work shows that under ideal conditions, the LuxR–LuxI circuit can sustain such a response over infinite distances. In two-dimensional space, we expect that given an initial (filled) circle of QS-activated bacteria and an appropriate QS signal decay rate, a wave of QS activation will spread radially outward, eventually activating the infinite two-dimensional colony.

The realism of our model is limited by the assumption that the wave of QS activation is unaffected by bacterial growth. (This assumption is made implicitly by neglecting bacterial growth in our model.) In reality, QS activation occurs only during a specific bacterial growth phase (Ward et al., 2003; Dilanji et al., 2012). Consider an experiment wherein a spatially extended colony of bacteria is in the lag phase of growth and there is some exogenous AHL supplied at one location in the colony. We expect that in this experiment, QS activation would not begin until the bacteria entered the correct growth phase. At this time, all bacteria experiencing sufficiently high environmental AHL concentrations would become up-regulated, increasing production of AHL in this area. As the AHL signal diffuses, bacteria adjacent to the initially up-regulated region experience higher environmental concentrations of AHL and become up-regulated themselves. This trend continues, resulting in a traveling wave of activation through the colony, until the bacteria are no longer in the phase of growth necessary for QS activation. Then, the wave of activation decays into nonexistence and the colony becomes down-regulated again. This entire process would result in the creation of a traveling wave of activation that would propagate for some time before dispersing. The amount of time that the bacterial colony is in the appropriate growth phase for QS activation and the speed of the traveling wave of activation determines the distance that such a wave will travel, and thus, the proportion of the colony that will become up-regulated.

5. Conclusion

The ability of diffusing QS signals to synchronize gene expression in spatially extended colonies is not well understood. In order to investigate this synchronization, we developed an analytically tractable model of spatial QS in the LuxR–LuxI circuit. We proved the existence of a traveling wave solution to this model, which implies that the LuxR–LuxI circuit enables highly efficient QS synchronization, likely limited by cell growth. We showed that when omitting cell growth there is a maximum QS signal decay rate above which a traveling wave is no longer sustainable. For QS signal decay rates less than this maximum, we additionally found a critical rate that determines whether a QS system becomes completely up-regulated or completely down-regulated. As expected, we find that the speed of the traveling wave of QS activation increases with the diffusion rate of the QS signal and decreases with the decay rate of the QS signal. Our findings give insight to how quickly a colony will undergo a QS-mediated shift in gene expression and, when limited by cell growth, how much of a colony will become activated (or deactivated) before entering quiescent phase.

6. Materials and methods

6.1. Bacterial cultures

Fig. 4 shows the QS bacterial strains used in this work. The quorum sensor strain is *Escherichia coli* MT102 harboring plasmid pJBA132, constructed by Andersen et al. (2001) and containing the sequence *luxR*-P_{luxI}-*gfp*(ASV). The strain has a *gfp* reporter under control of the *luxI* promoter but lacks *luxI*. Cultures in exponential phase were prepared by growing the *E. coli* to $OD_{600}=0.3$ in Luria–Bertani (LB) medium, approximately pH 7, at 37 °C.

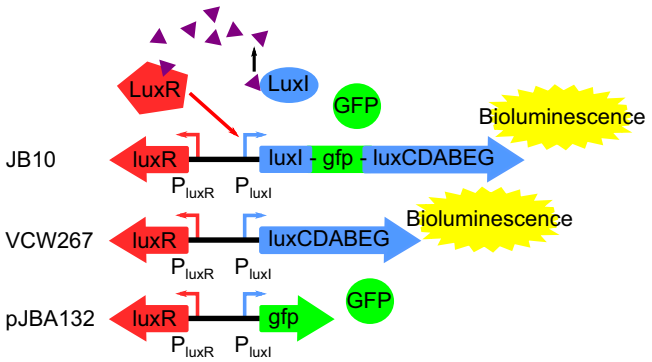

Fig. 4. Strains used in this study. JB10 is a mutant *A. fischeri* with an intact lux operon for synthesis (via LuxI) and detection (via LuxR) of AHL signal and production of bioluminescence. JB10 also contains a *gfp* reporter between *luxI* and *luxC*. VCW267 is a mutant *V. fischeri* lacking the AHL synthase (LuxI). The pJBA132 sensor strain of *E. coli* has a *gfp* reporter under control of the *luxI* promoter but lacks *luxI*.

Aliivibrio fischeri strain JB10 is a mutant produced from an ES114 wild type background in which a *gfp* reporter is inserted between *luxI* and *luxC*, producing the sequence *luxI-gfp-luxCDABEG* (Bose et al., 2007). *A. fischeri* strain VCW267 is a synthase-deficient (-*luxI*) mutant produced from an ES114 wild type background. Both strains were grown to $OD_{600}=0.3$ in commercial photobacterium medium (No. 786230, Carolina Biological), approximately pH 6.9, at room temperature. The photobacterium medium is a rich medium composed of yeast extract, tryptone, phosphate buffer, and glycerol in artificial seawater (Dilanji et al., 2012).

6.2. Well plate measurements

To obtain parameters for our mathematical model of a wave of excitation through a colony of *A. fischeri*, we performed two sets of well plate measurements. These data were collected in a multiwell plate using an automated plate reader (Biotek Synergy 2). We measured the optical density (OD) and luminescence of *A. fischeri* strain VCW267 (-luxI) in the presence of AHL concentrations ranging from 0 to 400 nM for the first set of well plate measurements and ranging from 300 to 3000 nM for the second set of well plate measurements. An exponential phase culture ($OD_{600}=0.1$) was diluted 100× into photobacterium (PB) broth (no. 786230, Carolina Biological). The photobacterium broth is composed of yeast extract, tryptone, phosphate buffer, and glycerol in artificial seawater. This inoculated medium was then loaded into individual wells containing N-(3-oxohexanoyl)-$_L$-homoserine lactone [3OC6-HSL, CAS no. 143537-62-6, Sigma Chemical], which is the natural AHL of the LuxI/LuxR system. The luminescence and OD of each well were measured over a period of ≈25 h at room temperature.

6.3. Bulk culture

To obtain the production rate of AHL per unit of LuxI (λ), we performed two trials of the following experiment, denoted Trial 1 and Trial 2. A stationary phase *A. fischeri* strain JB10 culture ($OD_{600}=2$) was washed twice in a centrifuge. We then inoculated two flasks of 30 mL PB broth. The cultures grew overnight on a shaker at room temperature.

We removed 1.3 mL samples every 30 min for the first 6 h, then every hour for the next 3 h and one sample 2 h later (for a total of

16 samples over 11.5 h). For each sample we measured the OD, luminescence and fluorescence using an automated plate reader (2 × 150 mL culture in wells) and took two measurements over the course of 5 min. For each of OD, luminescence and fluorescence, the four measurements were averaged to yield a single data value. One mL of each sample was spun and the supernatant removed, then frozen.

An exponential phase *E. coli* + pJBA132 culture (Trial 1: OD_{600} = 0.67, Trial 2: OD_{600} = 1.7) was diluted 500 × (Trial 1) or 1000 × (Trial 2) into Luria–Bertani (LB) medium. We performed a 2-fold serial dilution of each supernatant sample with this inoculated medium, resulting in at most a 2^5-fold dilution. These dilutions were loaded into individual wells. The remaining 16 wells contained *E. coli* + pJBA132 with known concentrations of AHL (0–100 nM) as a reference. The GFP fluorescence and OD of each well were measured over a period of ≈25 h at room temperature.

As described in Appendix C (SI), we then fit the resulting multidimensional datasets (OD, Luminescence × [AHL] × time and GFP fluorescence × [AHL] × time) to the model detailed in Section 2.1.

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We thank Dr. E. Stabb for *A. fischeri* strains JB10 and VCW267. Portions of this work appeared in J. Langebrake's PhD dissertation.

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jtbi.2014.07.033.

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