Mathematical Models for Quorum Sensing in
*Vibrio harveyi* and *Pseudomonas aeruginosa*

Kaytlin Brinker
University of Michigan
Ann Arbor, Michigan 48109
Email: kbrink@umich.edu

Jack Waddell
University of Michigan
Ann Arbor, Michigan 48109
Email: seoc@umich.edu

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**Abstract**

Quorum sensing is a hypothesis of how bacterial cells communicate in order to control gene expression. Quorum sensing relies on the secretion, detection, and response of chemical signaling molecules typically called autoinducers. The marine bacterium *Vibrio harveyi* uses information about the size and density of colonies from quorum-sensing systems to regulate genes related to luminescence. Another bacterium, *Pseudomonas aeruginosa*, uses this information to control the production of substances such as toxins. The goal of this paper is to present mathematical models for the quorum-sensing systems in both *Vibrio harveyi* and *Pseudomonas aeruginosa*.

1 **Introduction**

Quorum sensing is a process used by bacteria to coordinate behavior. One such behavior is gene expression and it is regulated based on the population density [2]. In both *Vibrio harveyi* and *Pseudomonas aeruginosa*, autoinducers are used as signaling molecules. These molecules are then detected by a receptor. Once the molecule binds to the receptor, a signal is induced which leads to the transcription of particular genes, some of which code for yet further production of autoinducer. As the population of bacterium increases, the concentration of the autoinducer grows. When the receptor becomes fully activated, upregulation of other specific genes is induced and the population of bacteria begin transcription of specific factors [3]. Because the behavior in the bacteria is coordinated, bioluminescence
or virulence can take place at a population level. For example, individuals a virulent species bacteria may suppress virulence factors until, through quorum sensing molecules, each bacterium determines that it is a member of a large population. Each bacterium can now upregulate virulence factors, and the population may be large enough to overwhelm the host’s immune system. This paper focuses on the bacterium Vibrio harveyi and Pseudomonas aeruginosa.

The study of the quorum-sensing in Vibrio harveyi was the first published observation that bacteria could communicate with multiple quorum-sensing signals [1]. V. harveyi is comprised of three parallel quorum-sensing systems. The autoinducer AI-1, in the class of AHL (acyl homoserine lactone) autoinducers, is an intraspecies signaler sensed by LuxN [2]. AI-2 is a furanosyl borate diester, is sensed by LuxPQ, and does inter-species signaling [2]. The third system, CAI-1, is sensed by CqsS and is thought to be responsible for Vibrio-specific signaling [2]. LuxN, LuxPQ, and CqsA are known as hybrid sensor kinases. At low cell densities, the point where autoinducers are not present in high concentration, these autophosphorylate. Information from the kinases is conveyed by phosphorelay via LuxO to a gene regulatory network. At high cell density, a high concentration of autoinducer is reached and the kinases are thought to switch to phosphatases leading to reversed phosphate flow. This activates genes which allow V. harveyi to produce light [3].

Pseudomonas aeruginosa is a Gram-negative bacterium that has three hierarchically arranged signaling pathways. The first pathway is referred to as the Las system and it is at the top of the hierarchy. It has three components, LasI, LasR, and 3-oxo-C₁₂-HSL, control transcription of several virulence factors [5]. The Las system regulates Rhl and PQS signaling systems. The Rhl system also has three components, RhlI, RhlR, and C₄-HSL, which also control transcription of rhamnolipid and proteases[5]. Both the Las system and the Rhl system are AHL signaling systems. The third system, the PQS signaling system, is a non-AHL system. The PQS stands for Pseudomonas quinolone signal and it is the only known quinolone used to intercellularly signal [6]. Because the quorum-sensing systems of P. aeruginosa control many virulence factors, drugs could be specifically designed to target QS.
Due to finding experimental results for LuxN only, we focus our attention on that quorum-sensing system for *V. harveyi*. Specifically, this model deals with phosphorylation of LuxN. This subsystem can be found in Figure 1. At low cell density, LuxN autophosphorylates its histidine (H) domain, transfers to its aspartic acid (D) domain, which then transfers phosphate to protein LuxU [3]. LuxU passes the phosphate on to the response regulator, LuxO. Once LuxO is phosphorylated and active, it combines with $\sigma^{54}$ to activate gene expression for small regulatory RNAs (sRNAs) [3]. The sRNAs and Hfq destabilize the mRNA for the activator protein LuxR. Because LuxR is necessary for the expression of genes in the quorum-sensing regulon, no light is produced. However, when there is high cell density, LuxN switches from a kinase to a phosphatase, causing the phosphate flow to reverse, creating an unphosphorylated LuxO which cannot contribute to the expression of the sRNAs. Translation of luxR mRNA now occurs unimpeded, and LuxR binds to activate the gene expression to produce light.

We began with a complex model for the LuxN subsystem, with no LuxU or downstream elements, containing nine differential equations describing the following reactions (see Table 1). 

$$
\begin{align*}
    HD + ATP & \xrightleftharpoons{k_{-1}}^{k_1} HD : ATP \\
    HDP + ATP & \xrightleftharpoons{k_{-3}}^{k_2} HDP : ATP \\
    HPD & \xrightarrow{k_5} HD + P \\
    HPD & \xrightarrow{k_6} HDP \\
    ADP + P & \xrightarrow{k_7} ATP
\end{align*}
$$

In order to analyze the system, we derived kinetic equations for the LuxN system by the law of mass-action. The concentrations of the reactants and corresponding variables in the equations can be found in Table 1. The rate equation gives loss of $HD$ at forward rate $k_1$, the addition of $HD$ by the backward reaction $k_{-1}$, and also by the decomposition of $HPD$.

$$
\frac{d(HD)}{dt} = -k_1 HD \cdot ATP + k_{-1} HD : ATP + k_5 HPD \quad (1)
$$

The formation of $HPD$ is given by forward reaction rate $k_2$ and it is decomposed through $k_5$, and passes to $HDP$ with rate $k_6$. 

2 *Vibrio Harveyi*

3
Figure 1: Model for *V. harveyi* quorum-sensing system. *V. harveyi* has three parallel quorum-sensing systems to regulate gene expression for bioluminescence. Figure adapted from [2] and [3]. Abbreviations: H, histidine; D, aspartate; I.M., inner membrane; O.M., outer membrane.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Chemical Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>HD</td>
<td>Unphosphorylated LuxN</td>
</tr>
<tr>
<td>HPD</td>
<td>Phosphorylated H Domain</td>
</tr>
<tr>
<td>HDP</td>
<td>Phosphorylated D Domain</td>
</tr>
<tr>
<td>HPDP</td>
<td>Phosphorylated H&amp;D Domains</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
</tr>
<tr>
<td>P</td>
<td>Phosphate</td>
</tr>
</tbody>
</table>

Table 1: Variables for Chemical Species

\[
\frac{d(HPD)}{dt} = k_2 HD \cdot ATP - k_3 HPD - k_6 HPD
\]  
(2)
Figure 2: Autophosphorylation of LuxN over time. The upper graph shown with the open circle is a LuxN mutant with no aspartic acid site (D). The lower graph shown with the open square contains the wild-type LuxN, which has a D site. Figure data from [2].

$HDP$ can be obtained from $HD$ receiving $P$ from $HPD$ at rate $k_6$, then through the backward reaction from $HDP: ATP$ with rate $k_{-3}$, but is lost in the forward reaction containing $k_3$.

$$\frac{d(HDP)}{dt} = -k_3HDP \cdot ATP + k_{-3}HDP : ATP + k_6HPD$$  \hspace{1cm} (3)

The only way to form $HPDP$ is with the forward reaction rate $k_4$.

$$\frac{d(HPDP)}{dt} = k_4HDP : ATP$$  \hspace{1cm} (4)

The complexes $HD : ATP$ and $HDP : ATP$ have similar terms for formation and decomposition in equations 5 and 6.

$$\frac{d(HD : ATP)}{dt} = k_1HD \cdot ATP - k_{-1}HD : ATP - k_2HD : ATP$$  \hspace{1cm} (5)

$$\frac{d(HDP : ATP)}{dt} = k_3HDP \cdot ATP - k_{-3}HDP : ATP - k_4HDP : ATP$$  \hspace{1cm} (6)

Derivation for the equations for $ATP, ADP$, and $P$ can be worked out from the rate equations given above and conservation of the chemical components.
Due to the concentration of ATP being so high, constants $k_1$ and $k_3$ can be solved for as Michaelis-Menten rates (See Appendix). ADP and P are neglected because of the high concentration of ATP and ATP is held constant. We are left with a system involving HD, HPD, HDP, and HPDP containing new rate constants.

\[
\frac{d(\text{ATP})}{dt} = -k_1 \text{HD ATP} + k_{-1} \text{HD} : \text{ATP} - k_3 \text{HDP ATP} + k_{-3} \text{HDP} : \text{ATP} + k_7 \text{ADP} P
\]  

\[
\frac{d(\text{ADP})}{dt} = k_2 \text{HD} : \text{ATP} + k_4 \text{HPD} : \text{ATP} - k_7 \text{ADP} P
\]  

\[
\frac{d(P)}{dt} = -k_7 \text{ADP} P
\]

Solving for HPD and HDP, and setting HD = $H_0 - \text{HD} - \text{HDP}$, $k_3 = 0$, and neglecting HPDP, we obtain two differential equations for the V. harveyi model.
\[
\frac{d(HPD)}{dt} = -(k_1 + k_{-1} + k_2)HPD - k_1 HDP + k_1H_0 \tag{14}
\]

\[
\frac{d(HDP)}{dt} = k_2 HPD - k_3 HDP \tag{15}
\]

This final model contains two differential equations. This model, however, did not reproduce experimental results that have been obtained. These equations appear to produce only growth when sensible parameter regimes are used; however, the known experiment result has growth and decay. When modeling the LuxN upper graph from Figure 2, we used a two-variable model, even though mathematically it should have been a single variable model. The single variable model could not contain both growth and decay. We therefore believe that the reason behind this finding is that there is a missing variable not accounted for in the model, and therefore in the biochemical understanding of Vibrio harveyi.

### 3 Vibrio Harveyi Results

The system we ended up modeling contained HPD and HDP. The equations were turned into a system of ODEs with the goal of finding a general solution.

\[
HPD = \frac{H_0 k_1 k_2}{2c} (\frac{k}{\sqrt{k^2 - 4c}} - 1)(\frac{k_6}{k_2} - \frac{k + \sqrt{k^2 - 4c}}{2k_2})e^{-k + \sqrt{k^2 - 4c}} t - \frac{H_0 k_1 k_2}{2c} (\frac{k}{\sqrt{k^2 - 4c}} + 1)(\frac{k_6}{k_2} - \frac{k - \sqrt{k^2 - 4c}}{2k_2})e^{-k - \sqrt{k^2 - 4c}} t + \frac{H_0 k_1 k_2}{c k_2} - \frac{H_0 k_1 k_2}{2c} (\frac{k}{\sqrt{k^2 - 4c}} + 1)(\frac{k_6}{k_2} - \frac{k - \sqrt{k^2 - 4c}}{2k_2})e^{-k - \sqrt{k^2 - 4c}} t + \frac{H_0 k_1 k_2}{c k_2} \tag{16}
\]

We solved the system of ODEs and found a general solution.

\[
Sol = \frac{H_0 k_1 k_2}{2c} (\frac{k_6}{k_2} - \frac{k + \sqrt{k^2 - 4c}}{2k_2})e^{-k + \sqrt{k^2 - 4c}} t - \frac{H_0 k_1 k_2}{2c} (\frac{k}{\sqrt{k^2 - 4c}} + 2)(\frac{k_6}{k_2} - \frac{k - \sqrt{k^2 - 4c}}{2k_2})e^{-k - \sqrt{k^2 - 4c}} t + \frac{H_0 k_1 k_2}{c k_2} \tag{16}
\]

We set \( k = k_0 + k_1 + k_2 + k_6 \) and \( c = k_1 k_2 + k_0 k_6 + k_1 k_6 + k_2 k_6 \).

When HPD and HDP are added, it takes the general form of:

\[
ae^{-l_1t} + be^{-l_2t} + c \tag{16}
\]

Given that this is the correct model, which we have no reason to believe it is not, there are some statements that must be true. First, we assume
that $l_1 > l_2$. The reason for this is we believe this is a model of decay that initially decays to a higher value, followed by decaying down to a lower value, and it is a non-negative steady state. We also assume that $l_1$ and $l_2$ are greater than zero. Secondly, it must be true that $a < 0$. This would mean $\left( -\frac{H_0k_1k_2}{2c} \left( \frac{k}{\sqrt{k^2-4c}} + 2 \right) \left( \frac{k}{k_2} - \frac{k-\sqrt{k^2-4c}}{2k_2} \right) \right)$ is negative. This is the case because the first term is positive due to all of the constants being positive, the second term is positive because $(k^2 - 4c)$ must be greater than zero and there is a plus one even if it were not, leaving that $\frac{k}{k_2}$ must be greater than $\frac{k-\sqrt{k^2-4c}}{2k_2}$, due to the negative sign out front. Assuming that $b > 0$, the first term is already positive, it must be true that $k$ is greater than $\sqrt{k^2-4c}$, which is true for any real number, and again $\frac{k}{k_2}$ must be greater than $\frac{k-\sqrt{k^2-4c}}{2k_2}$. Finally, $c \geq 0$, otherwise the model would converge to zero.

Our general exponential fit was an excellent match to the data (See Figures 4 and 5). [The only problem that arose was when the rate constants were solved for, there would always be one that would come out negative. This is just another indication that there is some variable unbeknownst to us and biochemists.]

![Graph](image)

Figure 3: A fit of the LuxN mutant data using a general exponential solution (eqn 16). The values we obtained are as follows: $a = 0.00277968$, $b = -0.00274226$, $l_1 = 1.99576$, $l_2 = 0.999451$, $c = 0.0000731046$
Figure 4: A fit of the wild-type LuxN data using a general exponential solution (eqn 16). The values we obtained are as follows: $a = -0.000270684$, $b = 0.000154927$, $l_1 = 0.625742$, $l_2 = 48.424$, $c = 0.00011576$

4 Pseudomonas aeruginosa Model

Many models currently exist for *P. aeruginosa*; however, none of these proposed models contain the complete picture. Our goal is to model *P. aeruginosa* as accurately as possible with all of the current theories on the mechanisms involved in the quorum-sensing system. We consider an ordinary differential equation model, in which the cells are assumed to be in a well-stirred container.

We began modeling with the *Las* system seen in Figure 3. New concentrations and variables can be found in Table 2. *P* is the *LasR* : $C_{12} - HSL$ complex and is produced by mass action at rate $k_{RA}$ and degrades at rate $k_P$.

$$\frac{dP}{dt} = k_{RA} R A - k_P P$$  \hspace{1cm} (17)

We assume that the process of translation is much faster than that of transcription. The mRNA degrades, is produced at a basal rate, and is activated at a Michaelis-Menten rate. Enzyme LasR degrades at $k_{RA}$, is produced at $k_P$, and is also produced following Michaelis-Menten.

$$\frac{dR}{dt} = -k_{RA} R A + k_P P - k_R R + k_1 \frac{V_r \frac{P}{K_r + P} + r_0}{k_r}$$  \hspace{1cm} (18)
Figure 5: Model of the *P. aeruginosa* quorum-sensing system. *P. aeruginosa* has three hierarchically arranged signaling pathways. The three systems include the Las system, Rhl system, and PQS signaling system. Figure adapted from [5] and [6].

For this ODE model, the autoinducer has a concentration $A$ inside the cells and $E$ in the extracellular fluid, which is assumed to be well stirred. Autoinducer is produced inside the cell from LasI, and can complex with LasR. It can also diffuse outside the intracellular space at rate $\delta$. $\rho$ is the volume density of the cells in the environment.

$$\frac{dA}{dt} = -k_{R\text{A}}R A + k_{P}P + k_{2}L - k_{A}A - \frac{\delta(A - E)}{\rho}$$  \hspace{1cm} (19)

The extracellular autoinducer is allowed to degrade at a rate $k_{E}$. The
<table>
<thead>
<tr>
<th>Variable</th>
<th>ChemicalSpecies</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>LasR:C_{12}-HSL</td>
</tr>
<tr>
<td>R</td>
<td>LasR</td>
</tr>
<tr>
<td>A</td>
<td>C_{12}-HSL internal</td>
</tr>
<tr>
<td>E</td>
<td>C_{12}-HSL external</td>
</tr>
<tr>
<td>L</td>
<td>LasI</td>
</tr>
<tr>
<td>S</td>
<td>RsaL</td>
</tr>
<tr>
<td>P2</td>
<td>RhrR:C_{4}-HSL</td>
</tr>
<tr>
<td>RR</td>
<td>RhlR</td>
</tr>
<tr>
<td>A2</td>
<td>C_{4}-HSL</td>
</tr>
<tr>
<td>RI</td>
<td>RhlII</td>
</tr>
</tbody>
</table>

Table 2: Variables for Chemical Species

term \((1-\rho)\) conserves the number of autoinducer molecules diffusing across the cellular membrane into a new volume.

\[
(1 - \rho) \left( \frac{dE}{dt} + k_E E \right) = \delta(A - E) \quad (20)
\]

LasR and LasL last much longer than lasR mRNA and lasI mRNA. Because of our assumption that translation is much faster than transcription, \(k_r\) and \(k_l\) are much larger than \(k_R\) and \(k_L\). Thus, we set \(k_3l = k_LL\). LasI is an enzyme formed at basal rate \(k_3\) and degraded at rate \(k_L\), in addition to being activated at a Michaelis-Menten rate.

\[
\frac{dL}{dt} = k_3 \frac{V_l P}{K_p + P} \frac{1}{k_l + S} + l_0 - k_L L \quad (21)
\]

The rate \(k_4\) produces RsaL, and \(k_S\) diminishes it.

\[
\frac{dS}{dt} = k_4 \frac{V_s \frac{P}{K_s + P}}{k_s} - k_S S \quad (22)
\]

Following the Las system, we modeled the Rhl system. Because these two systems function in a similar way, each equation in the Las system has a corresponding equation in the Rhl system.

\[
\frac{dP_2}{dt} = k_R R A2 - k_{P_2} P_2 \quad (23)
\]
\[
\frac{dRR}{dt} = -k_{RR} A^2 + k_{P} P^2 - k_{RR} R^2 + k_1 \frac{V_{rR} \frac{P^2}{K_r + P^2} + rR_0}{k_r} \quad (24)
\]

\[
\frac{dA}{dt} = -k_{RR} R^2 A^2 + k_{P} P^2 + k_{A2} A^2 - k_{A2} A^2 - \frac{\delta(A^2 - E)}{\rho} \quad (25)
\]

\[
\frac{dR}{dt} = k_{3RR} \frac{V_{rI} \frac{P^2}{K_r + P^2} + rI_0}{k_r I} - k_{RI} R I \quad (26)
\]

\[
\frac{dE}{dt} = \frac{\delta(A^2 - E)}{1 - \rho} - k_E E \quad (27)
\]

It remains to model the PQS signaling system. However, little is known about its interactions in the \textit{P. aeruginosa} QS system.

5 Discussion

We have presented both a model for the \textit{V. harveyi} quorum-sensing system and the \textit{P. aeruginosa} quorum-sensing system. These models are based on both the biochemical information in the scientific literature, and modification of other models that have been produced for the two systems.

Our model for \textit{V. harveyi} demonstrates that there is still unknown information about the system. This model took the shape of the sum of two exponentials plus a constant. Considering the fit that was produced, we believe this mathematical model is a plausible explanation for the quorum-sensing system of \textit{V. harveyi}. However, one problem with this model is that a negative rate constant is produced. Further research of the \textit{V. harveyi} quorum-sensing system by biochemists would be very valuable in solving this problem. It would be promising to see research that can elucidate or prove that there is a missing element in our current understanding of quorum sensing in \textit{V. harveyi}.

Our model for \textit{P. aeruginosa} takes into account all of the elements of the quorum-sensing system known to date. This model is meant to be accurate in the depiction of what occurs during quorum sensing. Given that we have included all three systems that are a part of gene regulation for \textit{P. aeruginosa}, this model depicts the bacterial communication very accurately.

More experiments are necessary to verify what we believe to be the missing pieces of these quorum-sensing systems.
6 Acknowledgements

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References


7 Appendix

Derivation of Michaelis-Menten

Michaelis-Menten kinetics are the kinetics used to describe enzymes. A general starting reaction sequence looks like this [7]:

\[ E + S \xrightleftharpoons[k_{-1}]{k_1} ES \xrightleftharpoons[k_{-2}]{k_2} EP \xrightleftharpoons[k_{-3}]{k_3} E + P \]
To simplify the equation, we assume there is only one central complex, ES. The equation becomes:

$$E + S \xrightleftharpoons[k_{-1}]{k_1} ES \xrightarrow{k_p} E + P$$

To determine the velocity, we assume rapid equilibrium. The instantaneous velocity becomes:

$$v = k_p[ES]$$

$k_p$ is the catalytic rate constant. The equation for the total amount of enzyme is:

$$[E]_t = [E] + [ES]$$

Now divide the velocity equation by the total enzyme equation:

$$\frac{v}{[E]_t} = \frac{k_p[ES]}{[E] + [ES]}$$

Because we are assuming equilibrium, $[ES]$ can be written in terms of $[S]$, $K_s$ is the dissociation constant of ES:

$$\frac{v}{[E]_t} = \frac{k_p \frac{[S]}{K_s}[E]}{[E] + \frac{[S]}{K_s}[E]}$$

The equation can be rewritten as:

$$\frac{v}{k_p[E]_t} = \frac{\frac{[S]}{K_s}}{1 + \frac{[S]}{K_s}}$$

Since $v = k_p[ES]$, $k_p[E]_t = V_{max}$, which is the maximum velocity observed when the system goes to all ES. Yielding:

$$\frac{v}{V_{max}} = \frac{\frac{[S]}{K_s}}{1 + \frac{[S]}{K_s}}$$

Or more commonly:

$$\frac{v}{V_{max}} = \frac{[S]}{K_s + [S]}$$