Modeling Quorum Sensing in Sinorhizobium meliloti

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Abstract

Background: For bacteria, it is important for survival to sense their population density or quorum. We use a well-known quorum sensing system of the nitrogen-fixing bacterium Sinorhizobium meliloti in order to study and model its molecular basis. The quorum sensing system depends upon the players ExpR, SinR, SinI and AHL, the signaling molecule within the system.

Results: We present a minimal mathematical model for quorum sensing based on a system of differential equations. The quorum sensing system undergoes three stages. At low cell density, all produced AHL molecules quickly leave the cells. In the medium, the concentration of AHLs increases only slowly. If the number of cells grows, the concentration of AHLs in the medium increases and AHLs can be transported back into the cell where they regulate their own production, i.e. they are responsible for a positive feedback loop. Finally, if cells are even more abundant, production of AHLs is switched off.

Conclusions: Although our mathematical model is only qualitative it can correctly predict several empirical observations from wild-type and mutant strains.
Background

Bacteria are one of the most prolific life forms known to man, successfully inhabiting a huge variety of habitats. A major contribution to their success is the ability to coordinate their behaviour in response to the population density of the community, referred to as quorum sensing (QS). Numerous QS systems have been discovered throughout the bacterial world. These systems have been shown to enable cooperative responses that increase rates of survival, for example, through improved defence against competitors or hosts or through greater access to nutrients. Some well characterized examples are the pathogens Agrobacterium, Pseudomonas, Erwinia, and Burkholderia, where QS plays an important role in virulence via the regulation of processes such as plasmid conjugation, aggregation, exopolysaccharide production and exo-protease production. Other examples include the symbionts Vibrio fischeri and several rhizobial species Mesorhizobium loti, Rhizobium leguminosarum and Sinorhizobium meliloti, where QS is also found to either improve or to be essential for symbiosis. Yet other examples of QS regulated processes, that are beneficial for survival in the free living state of various bacteria are biofilm formation, competence, swarming motility, antibiotic production and spore formation ([1–3]).

A comparison of all the QS systems uncovered thus far reveals some generalities. For a typical Gram negative bacterium, a QS system consists of a single LuxI-type protein that synthesizes the signals (also called autoinducers, AI) and one or more LuxR-type proteins which function as transcription regulators. At low population density, bacteria produce a low basal level of signal which is constantly being released from and taken up by the cells. With population growth, the signal levels increase until a significant proportion is bound to LuxR. This LuxR/AI complex then binds to the LuxR promoter region, affecting the rate of transcription of the downstream genes that are responsible for such processes as swarming motility or biofilm formation ([1–4]). In many bacteria, QS is complicated by the presence of multiple QS systems with interconnected regulation. Furthermore, many ecosystems contain multiple bacterial species with overlapping signal recognition. But with no two QS systems alike, any one signal may produce a variety of responses between bacterial species that detect that signal. Thus QS within an ecosystem tends to appear as a ‘network of networks’, teeming with ‘eavesdropping’, competitive and cooperative processes. Given such enormous complexity and variation surrounding QS, understanding the ‘message’ associated with each signal in any bacterium requires a delineation of the molecular basis of QS in that bacterium.

S. meliloti, a model organism for the study of rhizobia-legume symbiosis, also contains a QS system called the Sin system. This system has been shown to significantly enhance symbiotic potential ([5,6]) and controls a variety of cellular processes such as cell division, cell motility, transcription, translation, metabolism and
transport ([7–9]). Most of the studies have focused on the activating role that the Sin system has on the production of the symbiotically essential exopolysaccharides succinoglycan and galactoglucan ([5,10–13]).

The Sin system is based on several long-chain N-acyl homoserine lactones (AHLs) as signal molecules or autoinducers, including oxo-C14-HL, oxo-C16:1-HL and C16:1-HL ([14, 15]). The enzyme catalyzing the synthesis of these AHLs is SinI, a homologue of LuxI-type proteins ([14]). sinI expression is regulated by SinR and ExpR ([11,13]), both homologues of the LuxR-type proteins ([16]). Some LuxR-type proteins are encoded directly upstream of their target genes. SinR belongs to this paired class of LuxR-type proteins because it is encoded upstream of sinI, separated by an intergenic region of 215 bp. SinR is essential for the expression of sinI, most likely by binding to the intergenic region. However, its activity is independent of the Sin system AHLs. The AHL receptor is ExpR, encoded distant from sinR and sinI. In addition to regulating sinI, ExpR also controls genes in the production of symbiotically important exopolysaccharides and a variety of cellular processes such as cell division, transcription, translation, metabolism and transport ([8]).

Previously, mathematical models have been used to study QS. Simple approaches distinguish between two types of cells, depending on their contribution to autoinducer (AI) production, without taking the molecular mechanisms of this contribution into account ([17]). Several models consider the molecular mechanisms within a single cell, leading to a bi-stable behaviour depending on a high or low AI concentration ([18–20]) or the density of cells within the medium ([18]). Moreover, in V. fischeri, it is known that the regulator LuxR forms a complex with the autoinducer AHL and then forms a dimer before it binds to the promoter region of the regulator, an effect taken into account in [19] and [20]. All above-mentioned modeling papers only explain the positive feedback which is responsible for distinguishing the basic state and cells which sense their quorum. In S. meliloti, we have to introduce a negative feedback which is responsible for switching off production of AHLs once the population has grown large enough. In addition, we take a practical approach and treat a spatial dimension in a mean-field way, i.e. we model a single cell and AI molecules in the surrounding medium. This is in contrast to using partial differential equations for transport of AIs, which are hard to fit to empirical data ([19]).

QS poses several conceptual questions: (i) It has been argued that bacteria not only sense their quorum, but the diffusion coefficient of their environment, which differs between liquid or a biofilm ([21]). These two assumptions have different consequences for the interpretation of QS. (ii) Since individual cells benefit from production of AIs by other cells, one might ask whether QS can be conserved during evolution. One way
out is that only contributors to AI production can use the AI for downstream processes ([22]). In order to study such issues, an extendible quantitative model is needed.

In this study we present a mathematical model for QS in *S. meliloti*. It is based on molecular mechanisms that have been discovered by comparisons between wild-type and mutant strains and fits qualitatively with all features we measured using different genetic backgrounds. The most important foci of the model are a positive feedback which increases AHL production if the number of cells exceeds a certain threshold and a negative feedback for an even higher number of cells.

**Results and Discussion**

Our goal is to provide a validated mathematical model for the Sin QS system in *S. meliloti*. After introducing the model, we give empirical evidence which shows how we came up with it. Afterwards we analyse the model and compare simulations with measurements.

**The model**

We introduce a minimal model for the molecular basis of quorum sensing, illustrated in Figure 1. It consists of two ingredients: (i) a network of chemical reactions within each cell and (ii) the interaction between cells. The interaction between these two spatial scales is necessary for understanding the molecular mechanisms of quorum sensing. An essential ingredient is the signaling molecule which diffuses between cells. In our system the signal is an AHL. These are produced with small variations in the chemical structure in nature but are modeled as a single chemical species here. The AHL has two functions: firstly, it transports information between cells and secondly, it regulates the production of certain proteins within their host cells.

We describe the mechanisms of the two spatial scales in our system:

(i) The full network consists of the genes *sinR, sinI*, the corresponding proteins SinR and SinI, the protein ExpR, the signal AHL and the complex ExpR/AHL. Here, AHL is in either of two classes, within the cell (denoted AHL) and outside the cell (denoted AHL$_o$). The basic mechanisms of the full model are as follows (parameters $\alpha_j$ are rate for production, $\beta_j$ are binding affinities to promoters and $\gamma_j$ are degradation rates):

1. ExpR is a stable protein (no degradation is assumed in the model) and builds a complex with AHL at rate $\alpha_4$. 

[Figure 1 about here.]
2. The complex ExpR/AHL binds and unbinds to and from the binding site 2 upstream of sinR at rates proportional to $\beta_3$ and 1, respectively, and can inhibit the expression of sinR. The complex is degraded at rate $\gamma_4$. The maximal production rate of SinR is $\alpha_1$ and $\gamma_1$ is its degradation rate.

3. SinI is fully responsible for production of AHL at rate $\alpha_3$. The promoter region of sinI has two binding sites, one for ExpR/AHL and one for SinR. SinR binds (to binding site 1 in Figure 1) at a rate proportional to $\beta_1$ (relative to dissociation) and only when it is bound can production of SinI at rate $\alpha_2$ begin. In addition, the complex ExpR/AHL can bind (to the binding site 2 in Figure 1) at rate $\beta_2$ (relative to dissociation) and stimulate expression of sinI, leading to a maximal production rate $\alpha_2(1 + \alpha_5)$ for SinI. However, the latter is only possible if SinR occupies binding site 1.

(ii) For the spatial component, AHLs are transported outside at rate $\delta_1$ and to the inside of the cell at rate $\delta_2$. We note that $\delta_2/\delta_1$ scales with the ratio of the volume of a single cell as compared to the volume of the medium. In addition, AHLs degrade at (small) rate $\gamma_3$.

In order to simplify our analysis, we assume that binding and unbinding rates of proteins to their DNA binding sites are rapid. This means that we only get effective rates for production of proteins, where down- and up-regulation of genes appears proportional to the probability that the promoter region is bound. E.g., if binding of ExpR/AHL to the promoter region of sinR is $\beta_3$ relative to dissociation, this means that the chance that the promoter region is bound at any given time is $\beta_3C/(\beta_3C + 1)$, if $C$ is the number of available ExpR/AHL complexes. The resulting simplified model (illustrated in Figure 2) comes with the variables $R_i$ (SinR in cell $i$), $I_i$ (SinI in cell $i$), $C_i$ (ExpR/AHL complex in cell $i$), $A_{ii}$ (AHL in cell $i$) and $A_o$ (AHL in the medium). Here, $B$ is the total number of cells in the population. Since we assume that ExpR is not degraded, there is a constant $\bar{E}$ such that the number of molecules of ExpR is $\bar{E} - C_i$. The equations read

\[
\begin{align*}
\frac{dR_i}{dt} &= \alpha_1 \frac{1}{\beta_3 C_i + 1} - \gamma_1 R_i, \\
\frac{dI_i}{dt} &= \alpha_2 \frac{\beta_1 R_i}{\beta_1 R_i + 1} \left( 1 + \alpha_5 \frac{\beta_2 C_i}{\beta_2 C_i + 1} \right) - \gamma_2 I_i, \\
\frac{dA_{ii}}{dt} &= \alpha_3 I_i - \delta_1 A_{ii} + \delta_2 A_o - \alpha_4 (\bar{E} - C_i) - \gamma_4 A_{ii} + \gamma_4 C_i, \\
\frac{dA_o}{dt} &= B (\delta_1 A_{ii} - \delta_2 A_o) - \gamma_3 A_o, \\
\frac{dC_i}{dt} &= \alpha_4 A_{ii} (\bar{E} - C_i) - \gamma_4 C_i.
\end{align*}
\]
Empirical justification of the Sin system model

The advantages of modeling the Sin system include a relatively well understood molecular basis for transcriptional control and a relatively simple system coded by (only) three genes on one hand, and yet capable of responding appropriately to its environment and contributing significantly to the survival of the bacterium on the other. The measurements we used to justify the equations (1) come from fluorescence data of the \( \text{sinI} \) promoter activity as given in Figure 3.

QS requires a molecular basis which provides both a positive feedback loop and a negative feedback loop. The Sin system appears to be remarkably efficient at self-regulation by such feedbacks which are achieved when an AHL molecule forms a complex with ExpR. The positive feedback is switched on when a cell contains high amounts of the signaling molecule AHL. When the population reaches a high enough density, production of signaling molecules is no longer necessary and the system is switched off by a negative feedback. These two forms of regulation are both implemented in the system from Figure 1. The positive feedback is activated when the ExpR/AHL binds to binding site 1 of the promoter region of \( \text{sinI} \) and increases expression of \( \text{sinI} \) which in turn increases production of AHLs. The negative feedback is seen when ExpR/AHL binds to the binding site 3 of the promoter region of \( \text{sinR} \) and inhibits expression of \( \text{sinR} \), which leads to inhibition of \( \text{sinI} \) expression and thus a decrease of AHL production.

The two feedbacks

The ExpR/AHL complex is central for regulating the QS system. A short DNA region (\( \approx 40 \) nucleotides) within the 159 nucleotide \( \text{sinR-sinI} \) intergenic region was identified to be responsible for the regulation by ExpR ([23]). Moreover, the specific DNA binding site sequence for ExpR/AHL was found by systematically exchanging each nucleotide within the 40 nucleotide region, narrowing the recognition sequence to 18 nucleotides (Figure 5). The effect of ExpR/AHL binding to the \( \text{sinI} \) promoter region was a significant (3-fold) increase in \( \text{sinI} \) promoter activity, providing a positive feedback ([11]). However, the activity of the \( \text{sinI} \) promoter (and hence the positive feedback) is absolutely dependent on the presence of SinR, since disruption of \( \text{sinR} \) renders the \( \text{sinI} \) promoter inactive ([11,24]). SinR belongs to a class of regulators called the LuxR-type regulators, and a DNA sequence resembling a LuxR recognition site (tatgtcatgt) in the promoter region of \( \text{sinI} \) has been suggested ([23]). In many AHL quorum sensing systems, the LuxR-type protein binds to and
regulates the promoter of the gene coding for the AHL synthase. To test this, we fused various lengths of the 159 nucleotide \textit{sinR-sinI} intergenic region (Figure 5) to the promoterless reporter gene \textit{egfp}. The variation in region length was due to a sequential removal of nucleotides from the promoter region, beginning with the nucleotides most distant from the \textit{sinI} gene. Promoter activity was dependent upon the presence of various parts of the promoter region. For example, the loss of the ExpR binding site (94 nucleotides) corresponded with the loss of \(\approx 70\%\) activity, as was expected. Reducing the length of the region to 83 nucleotides did not further reduce activity, but the reduction to only 70 nucleotides resulted in the loss of \(> 95\%\) activity. We note that the DNA sequence surrounding this site forms a perfect palindrome separated by 6 nucleotides, as indicated in Figure 5. Also significant is that the second half of the palindrome matches the predicted LuxR recognition site. Furthermore, the transcription start of \textit{sinI} has been identified at 27 nucleotides upstream of the start of \textit{sinI} ([11]). This places the palindrome immediately upstream of a classical -35 promoter. Because this site is very close to the -35 region of a classical promoter, we cannot be sure that the removal of half the palindrome has not somehow destroyed the promoter. However, taken together, these observations fit well with the tentative conclusion that the palindrome is the SinR recognition site, and strengthens the conclusion that the presence of bound SinR is necessary for the activity of the \textit{sinI} promoter.

The ExpR/AHL complex can also inhibit \textit{sinR}, i.e. the complex negatively regulates \textit{sinR} promoter activity through another specific DNA sequence in the DNA region upstream of \textit{sinR}, providing a negative feedback loop ([24]). Both ExpR binding sites require AHLs for the full effect, and alterations of the DNA sequence within the binding sites resulted in corresponding loss of function. This finding was based on both, a gel shift assay approach which measures the activity of purified components and by introducing the altered DNA into the cell and measuring its effect at a population level. The critical feature that determines which feedback loop dominates AHL production is the concentration of AHLs in the growth medium. At low AHL concentrations of 1-20 nM, the positive feedback loop dominates, resulting in the increased production of AHLs. As the AHLs accumulate to 50-100 nM, the negative feedback begins to decrease the rate of AHL production. Production continues, however, until the AHL concentration reaches \(\approx 1000\) nM, at which point \textit{sinI} promoter activity is completely inhibited ([24]). Thus the positive and negative feedback mechanisms act in concert not only to quickly raise AHL levels, but also to prevent excess of AHL production. Extremely high AHL concentrations may drain energy sources or saturate the environment, rendering the quorum sensing signal obsolete.
Effects of alteration of expR expression on the sinI promoter

The amount of the complex ExpR/AHL and thus the regulation of AHL concentration via SinI depends on concentrations of both ExpR and AHL. While AHL levels can be controlled externally, we also checked for the dependence of the activity of the sinI promoter and the production of AHLs on the level of ExpR; see also Figure 3. We used an inducible E. coli lac promoter in plasmid pBSexpR, which also expresses the LacIQ protein. Suppression of the lac promoter by LacIQ is not complete, which means that a small amount of ExpR protein is produced in the absence of the inducer, isopropyl β-D-1-thiogalactopyranoside (IPTG). Addition of IPTG releases the lac promoter, resulting in the production of significantly higher levels of ExpR. When this plasmid was placed in a S. meliloti strain lacking a functional expR, leaky expression of expR (this mutant is denoted ↓ExpR) in the absence of IPTG resulted in a strong activation of sinI promoter activity. This activity was about 4-fold higher compared to the sinI promoter activity in the wild-type. However, when IPTG was added, releasing a strong expression of expR (↑ExpR), the sinI promoter activity responded by a 4-fold decrease compared to that in the wild-type. Strains lacking either expR or sinI exhibited a basal level of sinI promoter activity. This was about 3-fold lower than in the wild-type during earlier time points, but which eventually almost matched the wild-type activity at the last time point (30-40h). Taken together, these results indicate that lower levels of ExpR in the cell stimulate AHL production, while higher levels of ExpR repress AHL production. In particular, these experiments are in line with the presence of both a positive and a negative feedback in the wild-type strain. In the absence of either ExpR or AHLs, sinI expression is not regulated by quorum sensing. When expR expression is reduced, only few ExpR/AHL complexes are built. These are sufficient to bind to the sinI promoter region and enhance expression of sinI, but insufficient to inhibit sinR expression. When expR is over-expressed, many complexes are built. This results in the binding of the complex to the sinR promoter region and inhibition of sinR expression follows, resulting in less SinI and AHL production.

AHL extraction and detection

Fluorescence measured from the sinI promoter indicated that (i) a basal level of AHLs is produced if ExpR was missing, (ii) production of AHLs is up-regulated if the level of ExpR is low and (iii) production of AHLs is down-regulated if the level of ExpR is high. To confirm these predictions, AHLs were extracted from the supernatants of stationary phase cultures. Extracted AHLs from 0.5 mL of culture from the different strains were spotted (5 µL) on an agar surface containing the indicator strain Agrobacterium tumefaciens; see Figure 6. Colour development by the indicator strain is an indication of the amount of AHLs in the extract.
Extract from the $\textit{sinI}^-$ mutant contained no AHLs, while extract from the $\textit{expR}^-$ mutant contained levels slightly less than that of the wild-type. However, the ↓ExpR mutant with leaky expression from pBSexpR produced an obviously higher (\(\geq 4\)-fold) amount of AHLs than the wild-type.

Once again, the AHL extraction and spot test indicates that high levels of ExpR activate AHL production, while higher levels inhibit AHL production. This mirrors the effect of AHL levels previously observed with $\textit{sinI}$ promoter activity ([24], where lower levels of supplemented AHLs to a $\textit{sinI}^-$ mutant strongly activated $\textit{sinI}$ promoter activity (by binding of ExpR/AHL to the promoter) and higher levels repressed $\textit{sinI}$ promoter activity (by inhibition of expression of $\textit{sinR}$ by binding to the promoter of $\textit{sinR}$). Note that this explanation requires that the ExpR/AHL complex has a higher affinity for the Sin-system activating DNA binding site upstream of $\textit{sinI}$ than the Sin-system repressor site upstream of $\textit{sinR}$. In particular, alterations of levels of ExpR provides another line of evidence that confirms the model which involves self-regulation based upon positive and negative feedback loops.

[Figure 6 about here.]

**Qualitative fit to the mathematical model**

The model as given by the equations (1) is only qualitative, since we have not yet specified any parameters. The reason is that we cannot directly compare outcomes from the model with empirical measurements. However, we can in fact estimate orders of reaction rates such that the system shows all features of a functioning QS system.

We assume that the number of cells is given as an external parameter. Equivalently, reactions within each cell is in equilibrium at the time the cell divides. When the cell replication rate is approximately constant – which is a reasonable assumption in the early phase of QS – the x-axes of Figure 4 are a proxy for the linear evolution of time.

In Figure 4, we used the following parameters for the wild-type and $N = 10$:

\[
\begin{align*}
\alpha_1 &= N^{3/2}, & \alpha_2 &= N^{1/2}, & \alpha_3 &= N^{3/2}, & \alpha_4 &= N^{-3}, & \alpha_5 &= N^{3/2} \\
\beta_1 &= N^{-1/4}, & \beta_2 &= N^{-1/2}, & \beta_3 &= N^{-1}, \\
\gamma_1 &= N, & \gamma_2 &= N, & \gamma_3 &= N^{-1}, & \gamma_4 &= N^{-1}, \\
\delta_1 &= N^3, & \delta_2 &= N^{-2}, & \bar{E} &= N^3.
\end{align*}
\] (2)

The absolute numbers are less important for our simulations than their orders of magnitude. We explain the qualitative choice of the parameters as follows:
• **The QS system must be able to quickly react to an external signal:** If QS helps the population to sense the environment and therefore react to limited nutrition or other signals, the QS system must be able to react quickly to an external stimulus. For this reason, the degradation rate $\gamma_2$ of SinI must be high. Otherwise, SinI would continue producing AHLs although the environment of the cell changed. The same argument applies to the degradation rate $\gamma_1$ of SinR, because SinR regulates the transcription of $\text{sinI}$. (Interestingly, in our group, extremely high degradation rates of SinR and SinI have recently been observed, Stefan Meyer, personal communication.) The production and degradation rates of SinR and SinI must be appropriate to allow for both positive and negative feedback mechanisms.

• **The positive feedback must be a reaction to the presence of more cells:** In other words, a single cell in a large volume is not able to enter a positive feedback loop. To see how this works in our system, look at a single AHL molecule which is just created within cell $i$. Either it builds a complex (at maximal rate $\alpha_4 E$, if no complex has been built yet) or it is transported out of the cell (at rate $\delta_1$). We assume that $\delta_1 \gg \alpha_4 E$. In that case, the AHLs produced by a single cell in a large volume exit the cell and are lost from the system without forming any ExpR/AHL complexes which would lead to the positive feedback. When many cells are present and AHLs also enter the cell by the rate $\delta_2$ (which is much lower than $\delta_1$ since the volume inside a cell is relatively small), the positive feedback can start.

• **Both, the positive and the negative feedback loop must be reached:** When an ExpR/AHL complex is built, it can either bind to the promoter region of $\text{sinR}$ or to the promoter region of $\text{sinI}$. We assume that it is more likely to bind to the promoter region of $\text{sinI}$. Then, if the promoter region of $\text{sinI}$ is bound, but the promoter region of $\text{sinR}$ is still unbound, expression of $\text{sinI}$ is increased and a positive feedback is activated. This means we assume that $\beta_2 \gg \beta_3$. See Figure 7 to see the expression of $\text{sinI}$, which is proportional to AHL production for a cell in all three states, i.e., the basic, positive and negative feedback states.

• **Both AHL and the complex ExpR/AHL are stable, but still degrade at a low rate:** In order to reach a proper equilibrium, we assume a small degradation rate of AHLs and a small disassembly rate of the complex ExpR/AHL into ExpR and AHL.
**Behaviour of mutants**

Compare Figure 3 and Figure 4(D). Since Figure 3 gives observations for the activity of the promoter of *sinI*, and SinI is fully responsible for AHL production, these two figures should show the same behaviour. As seen in both figures, the ↓ExpR mutant produces most AHLs, and the wild-type amounts to a higher AHL concentration than the ↑ExpR mutant. Only the *sinI*− mutant shows a different behaviour; the reason is that the measurements indicate activity of the promoter rather than the AHL production which is null if SinI is not present. We give a more detailed view of the mutants:

- **Behaviour of the *sinR*− mutant** (In the simulations we used $\alpha_1 = 0$): In the absence of SinR, binding site 1 of *sinI* cannot be occupied and so, *sinI* cannot be expressed, leading to no AHL production. This behaviour was experimentally seen previously in a *sinR*− strain ([11, 24]).

- **Behaviour of the *expR*− mutant** (In the simulations we used $\bar{E} = 0$): In the absence of ExpR, the complex ExpR/AHL cannot be built. Therefore, SinR and SinI production only occur at a basal rate, and neither positive feedback (the amount of SinI does not change with a growing number of cells) nor negative feedback (SinR does not change with a growing number of cells) can lead to a change in the production of AHL.

- **Behaviour of the *sinI*− mutant** (In the simulations we used $\alpha_2 = 0$): In the absence of SinI, no AHLs can be produced, and thus no ExpR/AHL complex can be built. Only SinR molecules are generated.

- **Behaviour of the ↓ExpR mutant** (We lowered $\bar{E}$ by a factor of 50): While the *expR*− mutant shows only a basal promoter activity of *sinI* (or production of AHLs), *sinI* promoter activity of the ↓ExpR mutant is increased relative to the wild-type. The reason in our mathematical model is that the amount of ExpR protein is large enough to produce ExpR/AHL complexes such that the positive feedback can operate, but is too small for a production of ExpR/AHL molecules which bind to the *sinR* promoter and lead to the negative feedback.

- **Behaviour of the ↑ExpR mutant** (We increased $\bar{E}$ by a factor of 4): If ExpR levels are increased, the rate of production of the ExpR/AHL complex is increased. For this reason, the negative feedback is entered quicker, i.e. at a lower cell density.
Comparison with other mathematical models for QS

Our mathematical model from equation (1) differs from other approaches for a quantitative understanding of QS in several respects. In the models from [18], [19], and [20], cells host bi-stable chemical systems: if initial production of AHL is low, the complex is built not often enough for entering the positive feedback. However, if many AHLs are within the cell initially, the AHLs are more probable to build the complex rather than to exit the cell. Consequently, the positive feedback can be entered. While this bi-stable behaviour is in principle also possible for the system presented here, it does not appear with the parameters from equations (2) (numerical results not shown). For a bi-stable system, [20] report that the population consists of a bi-modal distribution, centered around cells which are in the basal state and those in the positive feedback state. However, we use a well-shaken liquid cultures in our experiments, and it is unlikely that AHLs are distributed unevenly leading to a bi-modal distribution.

In \textit{V. fischeri}, the QS system consists of two (called LuxI and LuxR) rather than three proteins (SinR, SinI and ExpR). Most models for QS in \textit{V. fischeri} concentrate on the positive feedback loop, and do not model a negative feedback loop, e.g. [19]. However, our results indicate that three players are essential in the QS system of \textit{S. meliloti}, which requires a more complex model.

Conclusion

Based on empirical evidence, we present a mathematical model for quorum sensing in the nitrogen fixing bacterium \textit{S. meliloti}. The system comes with the players, SinR, SinI and ExpR, as well as the signaling molecule AHL.

Our model does not require a bi-stable molecular basis within each cell, which is in contrast to earlier models for QS. Rather, the total number of cells controls the biochemical system within single cells. If the density of cells is low, most signaling molecules are lost to the environment. At higher cell density, the signaling molecules may re-enter the cell, build a complex with ExpR, and bind to the sinI promoter region which results in a positive feedback. At even higher cell density, even more AHLs are available in the medium, so that AHL levels within cells also increase. A negative feedback is obtained when sufficient levels of the ExpR/AHL complex are reached, and this complex binds to the sinR promoter and inhibits transcription of sinR.

Although we could not estimate model parameters within the system, the mathematical model correctly predicts the behaviour for mutants lacking ExpR or SinI, and the response to a down- or upregulation of expR expression.
Materials and Methods

Bacterial strains, plasmids and growth conditions

*S. meliloti* strains were incubated at 30°C in a modified MOPS buffered minimal medium as described previously (McIntosh et al., 2009), which contained 48 mM MOPS (adjusted to pH 7.2 with KOH), 55 mM mannitol, 21 mM sodium glutamate, 1 mM MgSO$_4$, 250 µM CaCl$_2$, 37 µM FeCl$_3$, 48 µM H$_3$BO$_3$, 10 µM MnSO$_4$, 1.0 µM ZnSO$_4$, 0.6 µM NaMoO$_4$, 0.3 µM CoCl$_2$, 4.1 µM biotin and 0.15 µM K$_2$HPO$_4$. Cultures were grown in 100 µl volumes in 96 well plates, with at least 6 replicates, continuously shaken at about 1000 rpm. Where appropriate, antibiotics were added at the following concentrations: 120 µg ml$^{-1}$ neomycin, 10 µg ml$^{-1}$ nalidixic acid, and 10 µg ml$^{-1}$ tetracycline. For the monitoring of activity of the *sinI* promoter, we used a plasmid (pLK64) carrying the promoter region of *sinI* fused to the gene for green fluorescence protein (*egfp*) ([11]). Strains used in this study were also previously described, and include Rm2011 (*expR$^-$*), Sm2B3001 (*expR$^+$, derived from Rm2011 as described in [25]), and Sm2B4001 (*expR$^+$, *sinI$^-$*, derived from Sm2B3001 as described in [24]). For the controlled expression of *expR*, we used the vector pBSexpR (McIntosh et al, 2009). In this vector construct, *expR* was fused the isopropyl β-D-1-thiogalactopyranoside (IPTG) inducible lac promoter using the vector pSRK-Km ([26]). Induced expression of *expR* was via the inclusion of IPTG (0.5 mM) at inoculation of fresh medium, while leaky expression of *expR* was observed in the absence of IPTG.

EGFP fluorescence assay

Fresh minimal medium was inoculated from a preculture to an OD$_{600}$ of about 0.004. Time is indicated in number of hours after inoculation. Background fluorescence (∼700–800 F/OD) was determined from strains carrying the pLK vector with a promoterless *egfp*, and has not been subtracted from values in Figure 3, but was taken into account for the values presented in Figure 5. The population density (OD$_{600}$) and EGFP fluorescence (excitation at 488 nm and emission at 522 nm) were measured using a Tecan Infinite M200 reader (Tecan Trading AG, Switzerland).

AHL extraction and spot test

For the extraction of AHLs, *S. meliloti* strains were grown until stationary phase in minimal medium. Supernatants were obtained after centrifugation and mixed 1:1 with ethyl acetate. The polar phase was removed and discarded, and the organic phase removed by rotary evaporation. The dried residue was dissolved in acetonitrile, at 1% of the primary volume.
The acetonitrile solutions of extracted AHLs were spotted (5 µl) on agar containing the indicator strain A. tumefaciens NTL4 (pZLR4) ([27]) and X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) and the bioassay was incubated at 30°C for 12 hours.

Simulations

In order to obtain a quantitative picture of the model from equation (1), we relied on simulations using the software R. For a series of different cell numbers B, we used the package rootSolve to find the steady state of the system. We checked numerically that low and high AHL initial concentration led to the same stationary point.

Author’s Contributions

MM collected all data and wrote the manuscript; PC analyzed and interpreted the mathematical model; KB critically revised the manuscript; AB initiated and designed the biological part of the project, had the overall responsibility and wrote the manuscript; PP ran simulations, designed the mathematical part and wrote the manuscript.

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\[ \text{sinI} \]

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Figure 1: A summary of the molecular basis for the Sin quorum sensing system. Shaded boxes denote genes, other boxes denote proteins or AHLs, and \( \emptyset \) is a symbol for nothing. Each gene has its promoter and each promoter plus surrounding DNA sequence contains regulatory elements such as protein binding sites, which are denoted 1, 2, and 3. Unoccupied binding sites are denoted by \( \circ \) and bound ones by \( \bullet \). Both promoters have different possibilities of being bound, indicated by the different horizontal lines. The thickness of the lines indicates promoter activity which is dependent upon the respective binding configuration. For example, the promoter of \( \text{sinI} \) is most active when both \( \text{SinR} \) and \( \text{ExpR/AHL} \) occupy their binding sites upstream of \( \text{sinI} \). Solid arrows are reaction arrows, dashed arrows indicate binding to a promoter region, and Greek symbols denote reaction rates and binding affinities. For example, \( \text{SinR} \) is lost from the system (i.e. it degrades) at rate \( \gamma_1 \). The cell envelope is indicated by the rectangle with a dotted line, and AHLs are transported across the cell envelope.
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