Optimal tuning of bacterial sensing potential

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Through production and sensing of small signal molecules, quorum sensing (QS) enables bacteria to detect changes in their density and regulate their functions accordingly. QS systems are tremendously diverse in terms of their specific sensory components, the biochemical and transport properties of signaling molecules, their target functions and the context in which QS-mediated functions are activated. Cutting across this diversity, however, the central architecture of QS systems is universal; it comprises signal synthesis, secretion, degradation and detection. We are thus able to derive a general metric for QS ‘sensing potential’ based on this ‘core’ module. The sensing potential quantifies the ability of a single bacterium to sense the dimensions of its microenvironment. This simple metric captures the dominant activation properties of diverse QS systems, giving a concise description of the sensing characteristics. As such, it provides a convenient quantitative framework to study the phenotypic effects of QS characteristics. As an example, we show how QS characteristics uniquely determine the scenarios in which regulation of a typical QS-controlled function, such as exoenzyme secretion, becomes advantageous.

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Introduction

Quorum sensing (QS) is the mechanism using which many bacteria modulate gene expression depending on changes in their density. This modulation is accomplished by the production and sensing of small signal molecules that, at a sufficiently high concentration, activate specific target functions (Keller and Surette, 2006; Williams et al, 2007). A QS system can be divided into a sensor module that houses the signal synthesis, secretion and detection systems and an effector module that carries out the targeted function when induced.

For the sensors, a wide variety of signal molecules have been identified. Gram-negative bacteria often use acyl homoserine lactones (AHLs) as signals (Lazdunski et al, 2004; Williams et al, 2007). These AHLs are typically synthesized by LuxI-type enzymes from fatty acids, in which LuxI is the canonical AHL synthase from bacterium Vibrio fischeri. Gram-positive bacteria often use small peptides as QS signals (Kleerebezem et al, 1997; Sturme et al, 2002). These peptides differ in size and in complexity of post-translational modifications. In all QS sensors, signals are produced intracellularly and transported to the extracellular environment. Small AHLs diffuse freely across bacterial cell membranes (Kaplan and Greenberg, 1985), whereas large AHLs seem to be actively transported by pumps, such as the multidrug efflux (mex) pumps in Pseudomonas aeruginosa (Pearson et al, 1999). The peptides are typically too large for diffusion across membranes and are transported by dedicated ATP-binding cassette (ABC) transporters (Sturme et al, 2002; Lyon and Novick, 2004).

Different strategies are in place to detect these signals. AHL signals often lead to the activation of cytoplasmic regulator proteins, such as LuxR in V. fischeri (Kaplan and Greenberg, 1985; Lazdunski et al, 2004), which then activates target gene expression. The peptide signals and even some AHLs, are typically sensed by membrane-associated receptors to initiate a phosphorylation cascade that leads to target gene expression (Kleerebezem et al, 1997; Sturme et al, 2002). In Vibrio harveyi, an AHL (HAI-1) and a furanone (AI-2) are detected by different surface receptors (LuxN and LuxP/Q, respectively) (Henke and Bassler, 2004). An additional level of complexity arises in that bacteria often house multiple QS systems. In V. harveyi, three QS sensors work in parallel to control luminescence of which only one is confirmed to be an AHL-based system (Henke and Bassler, 2004). In V. fischeri, two AHL-based QS systems, ain and lux, are involved in the control of luminescence and symbiotic growth in the squid host (Lupp and Ruby, 2004, 2005) but in the atr system the extracellular
AHL signal is detected by a membrane-based sensor, whereas in the lux system the receptor (LuxR) is cytoplasmic.

The list of bacterial functions under QS control has expanded tremendously from its initial discovery for bioluminescence in V. harveyi (Nealson et al., 1970) and competence regulation in Streptococcus pneumoniae (Tomasz, 1965) to diverse functions, such as exoenzyme secretion in P. aeruginosa and other plant pathogens (Smith and Iglewski, 2003; Von Bodman et al., 2003), conjugation in Agrobacterium tumefaciens (Fuqua and Winans, 1994), sporulation control in Myxococcus xanthus (Kuspa et al., 1992), virulence in Staphylococcus aureus (Winzer and Williams, 2001) and eukaryote host detection in Enterococcus faecalis (Coburn et al., 2004). The QS systems behind these functions are all drastically different and as such the link between QS characteristics and the function regulated in terms of benefit to the host bacterium is unclear (Redfield, 2002; Bassler and Losick, 2006).

Here we show that despite the diversity in structure and function, the essential properties of QS can be captured by a simple generic metric, 'sensing potential'. The metric is based on a universally conserved 'core' signaling module that consists of signal synthesis, transport, detection, and degradation as the fundamental parameters; one that appears across diverse QS systems. We exploit its universality to model this core module and derive sensing potential as a general measure of QS. This sensing potential conveys the ability of a QS bacterium to measure the size of its enclosure. We validate our model using experimental observations of diverse QS bacteria reported in literature. In doing so, we also provide a comprehensive survey of the available quantitative information on the kinetics of these QS systems (see Supplementary Text 1). We find that, in addition to providing a concise, integrated description of the sensing property of a QS module, the sensing potential also captures the dominant trend of sensing characteristics of different QS systems. Thus, capitalizing on these properties, we focus on the effector modules under QS regulation and study how QS characteristics affect the effector regulation. Starting with a common QS-controlled effector, exoenzyme synthesis (Smith and Iglewski, 2003; Von Bodman et al., 2003), we study how and when QS regulation of an effector benefits the host bacterium. The analysis shows how QS characteristics may be tuned to maximize the host bacterial fitness by providing effective regulation.

**Results**

**Modeling framework and definitions**

We note that every QS sensor falls into one of the two categories (which we refer to as Type I or Type II) of the core module depending on where the signal concentration \( A \) is detected. In a Type I system (Figure 1A), the extracellular signal concentration is sensed whereas in a Type II system, the intracellular signal concentration is sensed (Figure 1B). To describe the dynamics of each Type of sensing, we assume that the signal \( A \) is synthesized at a constant rate \( k \) inside the bacterium (of volume \( V_e \)) and is lost by degradation and transport to its microenvironment (of volume \( V_o \)). The signal concentration inside the cell \( (A_t) \) and that outside \( (A_o) \) is assumed to be uniform and transport across the bacterial membrane is assumed to be rate limiting (see Materials and methods and Supplementary Text 1). In the microenvironment, the exported signal is diluted by a factor of \( V_o/V_e \) and is again subject to degradation. With these assumptions, we can derive the dependence of \( A_t \) and \( A_o \) on \( V_e \) at steady state (see Materials and methods). For both Type I and Type II systems, \( A_t \) and \( A_o \) increase with a decreasing \( V_e \) (Figure 1C). For a sufficiently small \( V_e \) \((<V_{e,c})\), the signal concentration would exceed a threshold \( (K) \) required for phenotypic expression (Figure 1C).

We define the dimensionless ratio \( V_{e,c}/V_e \) as the sensing potential \( (v) \) of the sensor for the host bacterium. The value for \( v \) is determined using the key QS parameters (Figure 1D), such as signal synthesis rate \( (k) \), activation threshold \( (n^0) \), and rate constants for degradation \( (d_e) \) and transport \( (D) \). These parameters can be rearranged into two dimensionless variables by appropriately scaling with respect to \( d_e \): \( \beta=k/Kd_e \) and \( \delta=D/d_e \). These dimensionless forms were chosen such that \( \delta \) conveys how fast a signal is transported from a cell once it is made at a rate conveyed in \( \beta \). As the ratio of signal synthesis rate to the activation threshold and signal degradation rate constant, \( \beta \) quantifies the efficiency of signal synthesis. \( \delta \) is the ratio of the transport rate constant and degradation rate constant. As such, \( 1/\delta \) is analogous to the Thiele modulus seen in reaction–diffusion processes (Bird et al., 1960; Truskey et al., 2004) and developmental processes, involving diffusion (Goentoro et al., 2006), where it measures the relative rates of reactive and diffusive processes. The above analysis can be readily generalized to account for variations, such as feedback, two-way signal transport in the Type I case (Type Ia) and the use of specialized pumps for signal transport (Type Ila) (see Supplementary Text 1).

By definition \( v \) conveys the size of the microenvironment required for effector activation, in multiples of the bacterium volume. To interpret sensing potential, we note that \( v \) is 0 for a QS system that is never activated. In contrast, \( v \) approaches infinity if target function is always active, as under a constitutive promoter. Note that \( v \) is drawn from the simple core-module depiction of QS, which, in reality, can have additional complex regulation. Despite this (as we shall show later), it is still applicable and provides a convenient integrated measure for the more complex cases.

As an analogy, consider the following; under appropriate assumptions, the kinetic theory of gases gives rise to a simple gas law that relates different gas properties (state variables), pressure \( (P) \), volume \( (V) \) and temperature \( (T) \) (Bird et al., 1960). A ‘real’ gas would still have the pressure property \( P \), as signal synthesis rate \( k \), activation threshold \( n^0 \), and rate constants for degradation \( d_e \) and transport \( D \). These parameters can be rearranged into two dimensionless variables by appropriately scaling with respect to \( d_e \): \( \beta=k/Kd_e \) and \( \delta=D/d_e \). These dimensionless forms were chosen such that \( \delta \) conveys how fast a signal is transported from a cell once it is made at a rate conveyed in \( \beta \). As the ratio of signal synthesis rate to the activation threshold and signal degradation rate constant, \( \beta \) quantifies the efficiency of signal synthesis. \( \delta \) is the ratio of the transport rate constant and degradation rate constant. As such, \( 1/\delta \) is analogous to the Thiele modulus seen in reaction–diffusion processes (Bird et al., 1960; Truskey et al., 2004) and developmental processes, involving diffusion (Goentoro et al., 2006), where it measures the relative rates of reactive and diffusive processes. The above analysis can be readily generalized to account for variations, such as feedback, two-way signal transport in the Type I case (Type Ia) and the use of specialized pumps for signal transport (Type Ila) (see Supplementary Text 1).

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Measuring the sensing potential of QS bacteria

In population-level experiments, \( V_{ec} \) can be approximately calculated as \( 1/d_{crit} \), where \( d_{crit} \) is the population density observed to trigger a QS phenotype (Henke and Bassler, 2004). Thus, the observed sensing potential \( v_{observed} \) is \( 1/(d_{crit} V_c) \). This \( v_{observed} \) reflects the phenotype (potential) of the actual QS system, comprising of all its regulatory interactions in addition to the core module whereas \( v_{calculated} \) represents the estimated potential based only on the core module. To test the applicability of our framework, we compare the sensing potential calculated \( v_{calculated} \) using our framework with \( v_{observed} \) (Figure 2).

Several factors affect this comparison, in addition to the uncertainty in measurements of biological parameters. The first is the estimation of a threshold \( K \). The experimental measurement of \( K \) as \( A \) at half maximal activation is an approximation for when phenotypic expression can be considered ON (Figure 1C, inset). Second, owing to lack of reliable data to quantify positive feedback, we do not include this effect while estimating \( v_{calculated} \). The positive feedback increases \( v \) (see Materials and methods) and many, but not all, of the QS systems considered here show this regulatory phenomenon. Thus, \( v_{calculated} \) will probably under predict \( v_{observed} \). Third, \( v_{observed} \) is calculated from continuously growing cultures, in which species concentrations may differ from steady-state values.

Despite these issues, we find a strong correlation between \( v_{calculated} \) and \( v_{observed} \) \((n=15, P<0.05)\), indicating that \( v_{calculated} \) captures the dominant characteristics of the diverse QS systems listed (see additional notes in Supplementary information and Supplementary Figure S10). A linear regression of \( v_{calculated} \) against \( v_{observed} \) gives a slope slightly greater than one \((1.1 \pm 0.05)\), which is consistent with our expectation that \( v_{calculated} \) would tend to under predict \( v_{observed} \). In addition, six of the sensor modules shown in Figure 2 occur together in pairs in QS bacteria, where they form a hierarchical structure of phenotypic activation. For each of the three pairs from \( V. fischeri \) \((ain, lux)\) (Lupp et al, 2003), \( V. harveyi \) \((HAI-I, luxS)\) (Henke and Bassler, 2004) and \( P. aeruginosa \) \((las, rhl)\) (Latifi et al, 1996), \( v_{calculated} \) correctly predicts the order of activation (see Supplementary Text 2).

Modulation of sensing potential

We use the model to study the interplay between signal syntheses, its transport and sensing and its effect on activation by looking at the effect of \( \beta \) and \( \delta \) on \( v \). In Type I sensing, in which the extracellular signal is detected, an increase in \( \delta \) helps speed up the extracellular-signal accumulation leading to an increase in \( v \) (Figure 3A). This increase, however, is limited by \( \beta \), representing the amount of signal being made (Figure 3A). Thus, in Type I sensing, \( v \) increases with both
β and δ but the increase in v with δ saturates at a level depending on β.

In Type II sensing, gene expression is triggered by the intracellular signal. Although increasing β increases v, faster export (larger δ) tends to remove the intracellular signal and reduce v (Figure 3B). The dependence of the two Types of sensing on δ is hence opposite. Importantly, as the signal is both produced and detected intracellularly in Type II systems, for a given δ, transport across the bacterial membrane places an upper limit on β (Figure 3B). An increase in β beyond this limit results in a discontinuity in v (v → ∞). To restate, if signal synthesis is fast (large β) and its export rate is sufficiently small (small δ), its intracellular concentration would always exceed the activation threshold (K), regardless of the microenvironment size. This can also be seen mathematically by considering the case, in which v → ∞ so that the extracellular signal is infinitely diluted, Aν → 0. Putting Aν=0 in equation (1.3) shows that Aν can still exceed the threshold K if synthesis k is sufficiently large, and/or D + da is sufficiently small. Hence, fast signal synthesis or slow signal turnover, or both, could lead to ‘self-activation’ of the effector (irrespective of v). This predicted Type II ‘self-activation’ seems to occur in nature under appropriate conditions. In P. aeruginosa, starvation can cause increased signal synthesis leading to effector activation irrespective of cell density (van Delden et al., 2001). In A. tumefaciens, TraM sequesters TraR from the TraR–3OC6-HSL complex (Hwang et al., 1995; Swiderska et al., 2001). As TraR induces the QS phenotype on binding with 3OC6-HSL, deletion of TraM can give rise to a lower K (higher β) such that v → ∞ (see Supplementary information) leading to constitutive activation (Hwang et al., 1995; Swiderska et al., 2001). As in Type I, v becomes insensitive to δ in Type II systems for sufficiently fast transport, and is limited by β (Figure 3B).

Cost and benefit of QS regulation for exoenzyme secretion

As an integrated measure of QS characteristics, v represents a collective QS phenotype, irrespective of the parameters that lead to it. For example, two QS systems could have the same potential v but result from different synthesis and transport-rate parameters. This framework can then be conveniently applied to study the phenotypic consequences of differing QS characteristics. As one such application, we study the potential benefit that QS regulation offers its host and how this benefit depends on the sensor’s characteristics.

Figure 2  Calculated and observed sensing potentials for 15 well-characterized QS systems. Squares represent Type I sensing; triangles Type II sensing. Each dot represents a different module. Details of the parameters and equations to estimate ν_calculated as well as the calculation of ν_observed from experimental observations are provided in Supplementary Text 2. See Supplementary Tables S1 and S2 for a synopsis of the data used to plot this figure. For each system, the equation for the base model (either Type I or Type II) shown in Figure 1D or the variation that best represents the system (Supplementary Figure S2 and Supplementary information) is used to estimate ν_calculated.

Figure 3  Sensing potential plotted as a function of β and δ from equations in Figure 1D. (A) Type I sensing, in which the extracellular signal is detected (Figure 1A). Thus an increase in δ increases the extracellular signal accumulation leading to an increase in ν. The increase is limited by the amount of signal being made given by β. (B) Type II sensing, in which the intracellular signal is sensed (Figure 1B). Faster export (larger δ) removes the intracellular signal leading to a reduction in ν. As the signal is produced intracellularly, low signal transport, δ in comparison to the signal production, β could lead to intracellular signal accumulation to above threshold levels, irrespective of ν. This appears as a steep rise in ν (v → ∞) for particular combinations of β and δ and represents ‘self-activation’ of the QS host. The interplay of β and δ leading to self-activation can be seen as follows; for β=10, the vertical line Q marks a critical δ (= β−1), below which v approaches infinity (effector self-activation). Line R does the same for β=100. Consider point M on β=10 and low v. If β is increased to 100 while keeping δ constant, the change results in self-activation.
For this, we first define a QS-associated change in host fitness ($\Delta f$) as the benefit gained minus the cost incurred upon effector activation. Assuming the cost of sensor operation to be negligible compared with effector cost (Haas, 2006), we examine $\Delta f$ due to effector activation by different sensors with varying $v$ values. We note that effector activation ($E$) by QS for a bacterium in an enclosure of size $V_e$ can be approximately modeled with a Hill equation in terms of $v$: $E = \frac{E_{\text{max}}v^a}{V_e/V_c + v^a}$ where $a$ is the Hill coefficient and $E_{\text{max}}$ represents maximal activation (see Supplementary Text 1). The cost and benefit of the effector (which are functions of $E$) then determine whether QS regulation is beneficial to the host bacterium in a given scenario and, if so, how tuning $v$ affects the host fitness.

To elucidate this, we model a typical biological target function regulated by QS: the secretion of exoenzymes (Redfield, 2002; Von Bodman et al., 2003; Diggie et al., 2007). Here, QS controls the synthesis of the enzyme ($P$), which is secreted to the extracellular microenvironment (Figure 4A). In this context, $E$ represents the synthesis rate of $P$. In the microenvironment, $P$ degrades a substrate ($S$) to produce a nutrient $N$ (Figure 4A). We assume that diffusion of enzymes across the cell membrane is negligible because of their large size and that they are actively secreted by pumps. Furthermore, we assume that diffusion and mixing in the environment are much faster than cell growth so that all species (enzyme, nutrient and generated nutrient) are uniformly distributed in the environment.

Consider a batch culture, in which, diffusion and mixing in the environment are much faster than cell growth so that all species (enzyme, nutrient and generated nutrient) are uniformly distributed in the environment. For this, we first define a QS-associated change in host fitness ($\Delta f$) as the benefit gained minus the cost incurred upon effector activation. Assuming the cost of sensor operation to be negligible compared with effector cost (Haas, 2006), we examine $\Delta f$ due to effector activation by different sensors with varying $v$ values. We note that effector activation ($E$) by QS for a bacterium in an enclosure of size $V_e$ can be approximately modeled with a Hill equation in terms of $v$: $E = \frac{E_{\text{max}}v^a}{V_e/V_c + v^a}$ where $a$ is the Hill coefficient and $E_{\text{max}}$ represents maximal activation (see Supplementary Text 1). The cost and benefit of the effector (which are functions of $E$) then determine whether QS regulation is beneficial to the host bacterium in a given scenario and, if so, how tuning $v$ affects the host fitness.

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By further assuming that the bacterial growth rate \( g \) is an increasing function of \( Af \) (see Materials and methods) (Koch, 1983; Dekel and Alon, 2005), we can analyze the overall benefit of QS-mediated effector regulation during \( T \).

First, two scenarios emerge in which regulation of exoenzyme synthesis is unnecessary. If the cell density during \( T \) is never sufficient for benefit to outweigh the cost of synthesis and secretion \((\Delta f < 0)\), the best strategy is not to activate the effector \((v = 0)\). However, if the benefit always outweighs the cost during \( T \), the best strategy is constitutive enzyme synthesis at the maximal rate \((v \to \infty)\).

Excluding these two scenarios, QS regulation of exoenzyme synthesis is advantageous and needs to be optimally tuned to maximize bacterial fitness. As bacteria grow in the culture, there is a gradual reduction in the average enclosure volume \( V_e \) per bacterium (Supplementary Figure S9B) from \((V/(n_0V_c))\) to \((V/(n_tV_c))\), where \(n_0\) and \(n_t\) are the numbers of bacteria at \( t=0\) and \( t=T\), respectively. This leads to an increase in \( E \) by QS-controlled activation. Overall, this results in a continual change in \( \Delta f \) and its path depends on the value of sensor’s \( v \) (Figure 4B). The best QS strategy, such as early activation (with large \( v \)) or late activation (with small \( v \)) of enzyme production during growth can be determined by the accrued \( \Delta f \) during \( T \). This ‘collective’ fitness can be measured by \( n_T \), the final number of bacteria at the end of \( T \). The numerical calculation indicates that \( n_T \) is a biphasic function of \( v \) (Figure 4C) with a distinct potential \((v_{opt})\) at which \( n_T \) is maximal. \( v_{opt} \) represents the optimal QS sensor characteristics for each set of physical and biological parameters that define this cost–benefit scenario.

Studying the properties of the function itself shows the underlying mechanism for QS regulation to be advantageous. The available benefit from enzyme secretion depends on \( V_c \) as the secreted enzyme gets diluted in the microenvironment. Thus, for maximum fitness during bacterial growth (changing \( V_e \)), enzyme secretion needs to be continually changed with \( V_e \) (see Supplementary Text 1 and Supplementary Figure S4). QS provides just such \( V_c \)-dependent regulation that is critical for it to become beneficial (see Supplementary Text 1).

**Generality of unique optimal sensing potential**

The above analysis shows that: (a) the cost and benefit of exoenzyme secretion determines whether QS regulation is advantageous to the host in a given scenario and (b) when advantageous, a unique tuning of the QS characteristics \((v_{opt})\) provides the maximum \( n_T \) (see Figure 4C). How applicable are these results for other QS-regulated target functions?

To address this question, we extend the analysis to a general effector that is costly but beneficial. Moreover, \( n_T \) depends on the specific cost \((C)\) and benefit \((B)\) functions for the effector. \( C \) used in exoenzyme analysis is based on the measurements of the effect of gene expression on growth rate (Dekel and Alon, 2005) and can be assumed to remain qualitatively unchanged for different effectors. Learning from the exoenzyme study, \( B \) will depend on the extent of effector activation \( E \) and enclosure volume \( V_c \). In particular, we note that \( B \) can be assumed to be an increasing but saturating function in terms of \( E \) and \( 1/V_c \) (equation (2.7)), which can capture the effects of a wide range of effectors.

Similar to exoenzyme regulation, QS regulation is unnecessary when either the cost or benefit of effector activation overwhelms the other (Supplementary Figures S6A and B). In case the cost of effector activation is much larger than its benefit, the best strategy is to keep the cost minimal by shutting off the effector \((v=0)\). However, if benefit from effector activation is overwhelming, the best strategy is to simply maximize possible benefit (and hence \( \Delta f \)) by always operating the effector at full activation \((v \to \infty)\). Both scenarios require no regulation. Otherwise, QS regulation is advantageous over constitutive effector control and its characteristics need to be uniquely tuned \((v_{opt})\) to maximize \( n_T \) (Figure 4D).

A large \( v_{opt} \) indicates that early activation during growth is optimal, whereas a small \( v_{opt} \) indicates that activation at a high density is optimal. \( v_{opt} \), in each case, is uniquely determined by the parameters of the effector (Figure 4D, inset). This result shows that distinct functions require QS systems with appropriately tuned characteristics for optimal regulation.

**Discussion**

Here we develop a simple metric ‘sensing potential’ to quantify the ability of a bacterium to sense the confinement of its microenvironment. The metric emerges from a ‘core module’ seen in all QS systems so that it is a generic measure; \( v \) can be measured for any given QS system. We have made a number of simplifying assumptions in our analysis to derive \( v \) from the core module properties, such as homogeneous distribution of reactive species in a cell and in its microenvironment and negligible impact of a periplasm. These assumptions are based on experimental observations of QS signal diffusion and mathematical analysis (see Supplementary information). They allow us to reduce the complex nature of QS-regulated activation, which typically involves multiple steps and many regulatory species (Hwang et al., 1995; Tu and Bassler, 2007), down to four fundamental measurable parameters governing signal synthesis, transport, degradation and detection.

Despite the simplicity of the metric, our analysis indicates that \( v \) of the core module can capture the ‘dominant’ trend of sensing properties across the highly diverse QS systems (Figure 2). The same analysis also shows cases of deviations between the actual potential of a QS system \((v_{observed})\) and the estimated potential \((v_{calculated})\) that is based on the simple core module. This deviation indicates additional regulatory interactions that act over and above that captured by the minimal core module. For example, positive feedback on signal synthesis—positive feedback was not included in the estimations in Figure 2—would increase \( v_{calculated} \) (see Materials and methods) and could account for many of the deviations (see additional notes in Supplementary Text 2).

In addition to providing an intuitive classification of QS modules (Figure 1), the framework also helps show the commonality and difference between Type I and Type II sensing. For sufficiently fast signal transport \((\delta \to \infty)\), the sensing potential for both types is uniquely determined by and approximately proportional to \( B \), suggesting a common strategy to modulate sensing potential. We see several examples of this strategy: in the plant pathogen *A. tumefaciens*, plant-produced compounds called opines act as primary
regulators of the tra QS (Type II) system by controlling signal synthesis (Piper et al., 1999; Farrand et al., 2002). Without opines, transcription of the signal synthase tral is repressed to a low basal level (low $v$ as $\beta$ is low), whereas the presence of opines, indicating the presence of the plant host, relieves the repression and leads to normal expression of tral and virulence at high density (high $v$, see Figure 2). The staphylococcal accessory regulator SarA has a similar function for the agr QS (Type I) system in S. aureus (Heinrichs et al., 1996; Novick, 2003). SarA acts as a global virulence-factor regulator in S. aureus and, under certain conditions, is shown to modulate QS-controlled phenotypic induction by directly controlling transcription at the agr locus.

However, when $\delta$ is small, it has opposite, significant effects on the two types: increasing $\delta$ decreases $v$ in Type II systems but increases $v$ in Type I systems. In this case, manipulation of $\delta$ is an effective strategy to modulate $v$. Consistent with this idea, Type I systems typically use peptide signals that are generally too large to diffuse freely across bacterial membranes, and the use of specialized pumps (e.g. ABC transporters) for signal export (Havarstein et al., 1995; Lazazzera and Grossman, 1998; Dunny and Winans, 1999) will effectively modulate the sensing potential. The dependence of $v$ on $\delta$ (for small $\delta$) is more complex in Type II sensing. As $\delta$ approaches a critical threshold ($\beta - 1$), $v$ drastically increases to approach infinity. Below the threshold, a Type II system can activate its effector irrespective of cell density (Figure 3B), which is impossible in Type I systems. This control strategy seems to be adopted by some bacteria. In P. aeruginosa (van Delden et al., 2001), starvation causes faster signal synthesis in both the las and rhl QS systems. In A. tumefaciens (Hwang et al., 1995), deletion of a repressor element in the tra system lowers the signal-sensing threshold. In both cases, the change in biochemical parameters causes a large increase in $\beta$ driving $v$ to infinity (Figure 3B), leading to the activation of the QS-regulated effector independent of cell density. Thus, Type II systems seem to have an additional layer of effector control over QS so as to subvert it under certain scenarios. On the same note, we observe that Type II systems commonly control exoenzyme secretion in free-living bacteria, such as Pseudomonas, Erwinia and in Rhizobia, such as Rhizobium leguminosarum and Sinorhizobium meliloti (Gonzalez and Marketon, 2003; Von Bodman et al., 2003). These bacteria may frequently encounter nutrient exhaustion, not necessarily caused by their own growth; in such cases density-independent secretion of exoenzymes enables nutrient foraging at low densities (van Delden et al., 2001).

Overall, sensing potential provides a concise, integrated description of the sensing characteristics of a QS module, even if its underlying mechanism is more complex than the core module. Thus, $v$ can be used as a single modulated (reduced) variable to study how QS characteristics affect the downstream regulation. To illustrate its application, we use our framework to study the scenarios in which QS regulation of functions proves advantageous. This additionally provides an insight into the evolution of QS as a regulation strategy, as seen in the analysis of other evolutionary strategies (Kussell and Leibler, 2005; Wolf et al., 2005a, b).

By modeling exoenzyme control and then generalizing the conclusion to other effectors, we show that QS regulation is advantageous when the cost of effector synthesis is comparable to its resulting benefit (Figure 4). A closer look shows that an underlying requirement is that the benefit from such an effector’s activation depends on the environment size. For enzyme secretion, benefit decreases with increased dilution of the enzyme in the microenvironment (increasing $V_e$). In this case, QS regulation allows effector synthesis to be kept low for large $V_e$, in which benefit is low compared with cost, and increases it gradually with $V_e$ (Figure 4B), hence providing optimal control (see Supplementary Figure S4 and related text in Supplementary information). In the absence of such benefit dependence on $V_e$, QS regulation, regardless of any cost–benefit parameter combination, will probably not be advantageous (see Supplementary Text 1 for more details). This conclusion is quite general as many QS-controlled effectors show similar ($V_e$ dependent) benefit function as described in equation (2.7), wherein benefit is an increasing but saturating function of effector activation and density (Figure 4D).

Consider QS-controlled colonization and luminescence by V. fischeri in its symbiotic host squid (Lupp et al., 2003; Lupp and Ruby, 2005). The colonization requires synthesis of several costly aggregation factors (Visick and Ruby, 2006), whose effects (and thus benefit) increase with concentration (Yip et al., 2005) and saturate at sufficiently high concentrations. Similarly, luminescence is costly but is suggested to benefit the bacterium by consuming the O$_2$ in the crypt region after colonization takes place (Ruby and McFall-Ngai, 1999; Visick et al., 2000; Stabb, 2005). This benefit will be limited to the total amount of O$_2$ present in the region.

We further show that when QS-mediated control is advantageous, each effector will require a QS sensor with a unique $v_{\text{safe}}$ that maximizes host fitness (Figure 4D, inset). This is consistent with observations in bacteria with multiple QS systems, each controlling a distinct effector. Furthermore, consider the typical V. fischeri life cycle that starts with colonization of a juvenile host squid’s crypt regions followed by growth and light production at high cell densities (Lupp and Ruby, 2005). Colonization and luminescence thus represent distinct effectors to be sequentially induced. Consistent with our analysis, colonization is regulated by the ain sensor with a larger $V_{\text{calculated}}$ (early activation) than the lux sensor (late activation), which controls luminescence (Figure 2 and see Supplementary Text 2). A similar situation is seen in the pathogenesis of P. aeruginosa, in which virulence involves secretion of exoenzymes (Smith and Iglewski, 2003; Von Bodman et al., 2003) as well as the formation of biofilms (Kirisits and Parsek, 2006). The exoenzyme secretion is controlled by the las sensor whereas secretion of the rhamnolipid involved in biofilm formation is largely controlled by the rhl sensor (Pearson et al., 1997). The las and rhl sensors show distinct $V_{\text{calculated}}$ values (Figure 2) and are activated hierarchically during growth (Latifi et al., 1996). Lastly, we note that Bacillus subtilis uses two distinct QS sensors (Grossman, 1995; Schauder and Bassler, 2001) with vastly different potentials (Figure 2) to tightly regulate competence development and sporulation (Grossman, 1995).
expression by a population of cells. In contrast, the hypothesis of ‘diffusion sensing’ (DS) (Redfield, 2002) proposes that QS measures the mass-transfer characteristics of the environment surrounding an individual bacterium. In DS, the accumulation or dispersal of a QS signal reflects how a secreted effector would also be distributed. The DS can then avoid costly exoenzyme secretion under conditions in which it would be lost by dispersal. To reconcile DS with the traditional QS definition, the ‘efficiency sensing’ (ES) hypothesis was recently proposed (Hense et al., 2007). The ES argues that QS cells measure the combined effects of density, mass-transfer properties and their own spatial distribution. It also suggests that the benefit of QS may lie in conveying the efficiency of secreting extracellular effectors. Our analysis is based on the signaling dynamics of a single QS cell and is analogous to the approach suggested by DS, but here we do not consider diffusion limitations in the environment. Instead, we model the environment as an enclosure within which the signal concentration is uniform. This simple framework allows a quantitative analysis of QS that can be understood in terms of the different hypotheses. In particular, sensing potential provides an intuitive and measurable connection between an individual QS cell and the population-level phenotype (Supplementary Figure S9A). In addition, the benefit of QS regulation emerges naturally by analyzing the effector controlled by sensors of different potentials (Figure 4C). Taken together, our analysis combines sensing with regulation so that it can be understood and quantified in terms of both a single QS cell and a population of QS cells (Supplementary Figure S9B).

Our analysis has limitations, which arise naturally from the assumptions made to provide a simple, yet generic framework. When the distribution of species in the environment is not homogenous—this could happen due to diffusion limitations or other mass-transfer phenomena—sensing potential may not accurately predict activation characteristics (see Supplementary Text 1). Moreover, our framework is based on an individual QS cell or, equivalently, a population of identical QS cells and does not capture the properties of a heterogeneous population. For example, we do not account for the presence of cheaters in a population that do not signal but respond to it (Diggles et al., 2007). The sensing potential measured from such a heterogeneous population containing cheaters would underestimate an individual QS cell’s (non-cheater) potential.

Lastly, we do not explicitly account for cross talk between different sensors when multiple QS systems are present in the same host. Here, signals from one QS system could weakly activate the effector of another system (Holden et al., 1999; Collins et al., 2005), as well as other regulatory parameters and hence affect the sensing potential. Such cross talk could be accounted for by simultaneously modeling the effects of all the functional QS systems present in the host. Nevertheless, sensing potential does provide a standard measure for QS systems in scenarios, in which the model assumptions are justified, and it can readily be extended. On the effector side, we note that the ‘one-effector-per-sensor’ model used is a simplification. A QS sensor usually controls multiple effectors (Antunes et al., 2007), and multiple sensors may coordinate to control a common effector (Henke and Bassler, 2004). The analysis of these systems will follow the same method but will require the estimation of a combined fitness contribution from each effector under each sensor. Similarly, if the QS sensor itself is found to be significantly costly, this cost needs to be included in the fitness calculation. Taken together, our work provides the theoretical framework as well as an experimental method to study QS regulation, its benefit and hence its evolution. The analysis presented may also help guide experimental efforts in engineering new synthetic gene circuits (Sprinzak and Elowitz, 2005; Marguet et al., 2007; Keasling, 2008; Tanouchi et al., 2009).

Materials and methods

The sensor

We model the signaling dynamics by accounting for the signal synthesis, transport and degradation. We assume that: (1) signal transport ($D$, hr$^{-1}$) and degradation ($d_a$, hr$^{-1}$) are proportional to the signal concentration ($A$, nM); (2) the signal is synthesized at a constant rate ($k$, nM hr$^{-1}$). For a Type I system (Figure 1A), the rate of change of the intracellular and extracellular signal respectively is given by

$$\frac{dA_i}{dt} = k - DA_i - d_A A_i$$  \hspace{1cm} (1.1)$$

$$\frac{dA_e}{dt} = DA_i \left( V_c \frac{V_A}{V_c} \right) - d_A A_e$$  \hspace{1cm} (1.2)$$

For Type II systems (Figure 1B), we have:

$$\frac{dA_i}{dt} = k - D(A_i - A_e) - d_A A_i$$  \hspace{1cm} (1.3)$$

$$\frac{dA_e}{dt} = D(A_i - A_e) \left( V_c \frac{V_A}{V_c} \right) - d_A A_e$$  \hspace{1cm} (1.4)$$

where $D$ ($A_i - A_e$) now accounts for the two-way transport. Here, $V_c$ represents the volume of the bacterial microenvironment and $V_e$ represents the cell volume of an average bacterium.

For Type I system, equations (1.1) and (1.2) are solved simultaneously to get the steady-state $A_e$ as a function of $V_c$:

$$A_e = \frac{DK}{\left( \frac{2k}{D} \right) \left( D_d + D_d e + \frac{\beta e}{V_c} \right)}$$  \hspace{1cm} (1.5)$$

For Type II system, equations (1.3) and (1.4) give:

$$A_i = \frac{k \left( D + \frac{\beta e}{V_c} \right) A_e}{Dd_d + D_d e + \frac{\beta e}{V_c} d_d e}$$  \hspace{1cm} (1.6)$$

According to equations (1.5) and (1.6), both $A_e$ and $A_i$ increase with decreasing $V_c$ (Figure 1C). The critical $V_c$ and hence the sensing potential $v$, for which $A_e$ (Type I) and $A_i$ (Type II) cross the threshold $K$, is calculated by solving equations (1.5) and (1.6) for $v$ at $A_e = A_i = K$ respectively. For Type 1 system, we get: $v = \frac{\beta e - k D_d e}{\beta e + D_d e + \frac{\beta e}{V_c}}$. For Type II system, we get: $v = \frac{\frac{\beta e}{V_c} - k D_d}{\frac{\beta e}{V_c} + D_d e + \frac{\beta e}{V_c} d_d e}$

Positive feedback

We model positive feedback in signal synthesis by assuming that the signal synthesis rate increases linearly with its own concentration with rate constant $k_b$ (see Supplementary Text 1 for more details). This leads to the addition of a term $k_b A_i$ in equations (1.1) and (1.3). By solving these modified equations for steady-state signal concentration and then explicitly for $v$ at which $A_e = K$ as done earlier, we get

$$\text{Type I} \quad v = \frac{\delta (\alpha + \beta)}{\delta + 1}$$  \hspace{1cm} (1.7)$$


\[ v = \left( \frac{1}{z + \beta - 1} - \frac{1}{5} \right)^{-1} \]  

(1.8)

where \( z = k_Y/d_s \) is the dimensionless parameter for feedback scaled using \( d_s \). From these equations we see that for both Type I and Type II systems, positive feedback (as \( z \)) acts to effectively increase \( \beta \), which corresponds to increased signal synthesis. The effect of \( z \) on \( v \) can thus be studied equivalently as the effect of \( \beta \) on \( v \).

### The effector

The effector activation \( E \) under QS control is given by

\[ E = \frac{E_{\text{max}}v^a}{v^a + u^a} \]  

(2.1)

where \( E_{\text{max}} \) is the maximal synthesis rate and \( a \) the hill coefficient depending on the cooperativity of the signal-induced activation (see Supplementary Text 1).

For the exoenzyme case, \( E \) represents the enzyme synthesis rate. We model the exoenzyme dynamics using the following equations:

\[ \frac{dP_i}{dt} = E - D_iP_i - d_iP_i \]  

(2.2)

\[ \frac{dP_e}{dt} = \frac{D_iP_i}{V_e/V_c} - d_eP_e \]  

(2.3)

where \( i \) and \( e \) are the concentrations inside the cell and in the microenvironment, respectively; \( D_i \) and \( d_i \) the transport rate constant and the degradation rate constant of \( P_i \), respectively.

### Enzyme–substrate kinetics and nutrient

Following a model of bacterial foraging (Vetter et al, 1998), in which the enzyme absorbed to the substrate catalyzes the production of nutrient (Rubinov, 1975), the rate of production of nutrient in the environment \( (dN/dt) \) is given by \( k_o P_e/\gamma e \), where \( k_o \) and \( \gamma e \) are appropriate reaction rate and binding constants, respectively. Using nutrient transport and degradation rate constants, \( D_n \) and \( d_n \), respectively, the mass balance equations for \( N \) are:

\[ \frac{dN_e}{dt} = k_o \frac{P_e}{K_e + P_e} - D_n (N_e - N_i)/V_c - d_i N_e \]  

(2.4)

\[ \frac{dN_i}{dt} = D_n (N_e - N_i) - d_n N_i \]  

(2.5)

### Cost, benefit and fitness

For any enzyme synthesis rate \( E \) and enclosure size \( V_e \), equations (2.2)–(2.5) are solved simultaneously for steady-state concentrations of enzyme and nutrient. The benefit provided by \( N \) is then calculated (Dekel and Alon, 2005) as \( B = b_n N_i/(b_{\text{dim}} + N_i) \), where \( N_i \) is intracellular nutrient concentration. The cost of effector activation can be modeled (Monod, 1949; Dekel and Alon, 2005) as \( C = c_f E/(1 - \gamma_m E) \), \( b_n \), \( b_{\text{dim}} \) (nM), \( c_f \), \( \gamma_m \) (nm\(^{-1} \) hr\(^{-1} \)), are benefit and cost function parameters such that \( B \) and \( C \) unitless. Fitness

\[ \Delta f = B - C \]  

(2.6)

Growth rate \( g \) (hr\(^{-1} \)) is modeled as a linear combination of the growth seen in the absence of an effector (\( g_0 \) (hr\(^{-1} \)) and in its presence. Without any loss of generality of our conclusions, we assume:

\[ g = g_0 + \Delta f. \]  

The collective fitness \( n_f \) is given by \( n_f = f_T (\Delta f) dT \), where cell growth during \( T \) is modeled by a logistic equation \( (\Delta f) = g_0 (1 - e^{-t}) \), with \( n_f \) as carrying capacity. \( n_f \) from a QS sensor of given potential \( v \) is obtained by numerical integration of the logistic growth equation where growth rate at each time point is calculated based on \( \Delta f \). To obtain \( v_{\text{opt}} \) the procedure is repeated for a range of \( v \)’s and a \( n_f \) versus \( v \) graph is plotted to find the \( v \) at which \( n_f \) is maximal (Figure 4C and D).

We use the following equation to represent the benefit function for a general QS-controlled effector:

\[ B = \frac{b_n E^x}{b_{\text{dim}} + (E^x + b_{\text{dim}})^{\frac{1}{y}} V_e} \]  

(2.7)

This equation captures the characteristics of a wide range of beneficial effectors depending on choice of parameters \( (b_n, b_{\text{dim}} \) and \( b_{\text{lim}} \) and hill coefficients \( (x, y) \). Note that \( B \) increases with \( 1/V_c \) and \( E \), but saturates eventually. The calculation of \( n_f \) for the general function is repeated as above with equation (2.7) being used in equation (2.6) to calculate \( \Delta f \). All equations were solved analytically using Mathematica (Wolfram Research) whereas simulations and plots were done using MATLAB 7.1 (MathWorks).

### Supplementary information

Supplementary information is available at the Molecular Systems Biology website (www.nature.com/msb).

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### Conflict of interest

The authors declare that they have no conflict of interest.

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