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ORIGINAL ARTICLE

Multiple regulation mechanisms of bacterial quorum sensing

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***Abstract*—Many bacteria have developed a possibility to recognise aspects of their environment or to communicate with each other by chemical signals. The so-called Quorum sensing (QS) is a special case of this kind of communication. Such an extracellular signalling via small diffusible compounds (called autoinducers) is known for many bacterial species, including pathogenic and beneficial bacteria. Using this mechanism allows them to regulate their behaviour, e.g. virulence. We will focus on the typical QS system of Gram negative bacteria of the so-called *lux* type, based on a gene regulatory system with a positive feedback loop.**

There is increasing evidence that autoinducer systems themselves are controlled by various factors, often reflecting the cells' nutrient or stress state. We model and analyse three possible interaction patterns. Typical aspects are e.g. the range of bistability, the activation threshold and the long term behaviour. Additionally, we aim towards understanding the differences with respect to the biological outcomes and estimating potential ecological or evolutionary consequences, respectively.

***Keywords*-Quorum Sensing, ODE system, bifurcations, nutrients, qualitative behaviour**

I. Introduction

Extracellular signalling via small diffusible compounds (autoinducers) is known for an increasing number of bacterial species, including pathogenic and human health promoting bacteria. Briefly, bacteria release autoinducers and simultaneously regulate target gene expression dependent on the environmental autoinducer concentration. Regulated behaviour often includes critical life style switches, e.g. from non-virulent to virulent. Thus mechanistic understanding of autoinducer regulation and its ecological significance is of high relevance for the development of treatment strategies. Autoinducer regulation was originally

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assumed to be a strategy enabling coordinated responses of whole bacteria populations dependent on the cell density (Quorum sensing) [15]. The later detected influence of other aspects such as mass transfer properties of the environment and cell distribution led to the alternative concept of diffusion sensing (assuming that the mass transfer properties of the environment around a cell - including diffusion conditions - are estimated by autoinducers) and of the unifying efficiency sensing [31, 19]. The autoinducer mechanism was first described in the gram-negative *Vibrio fischeri*, which possesses an autoinducer system of *lux*-type with an AHL (acylhomoserine lactone) acting as signal. The signal is produced by the synthase LuxI. It binds to a receptor molecule (LuxR). Dimers of the AHL-LuxR complex bind to the *lux* box in the *lux* operon, where the autoinducer synthase (LuxI) and luminescence genes are upregulated (Fig. 1), but also to other target genes of the regulon [15]. This AHL system, including the positive feedback loop, represents an archetypal example for the architecture of autoinducer mediated gene regulation of many gram negative bacteria. Autoinducer systems in other bacteria often follow similar design principles, although details may vary.

There is increasing evidence that autoinducer systems themselves are controlled by various factors, often reflecting the cells' nutrient or stress state [12, 27]. Recently it has been suggested that such controls allow for integrating the demand of the cells for the regulated behaviour into the signal strength, generating a kind of hybrid push/pull control [20]. Here, "demand" reflects the strength of the potential benefit a group of cells could have from this behaviour under the current environmental conditions. For example, the demand for the release of an exoprotease might be low as long as available essential amino acids abound in the environment, but increase when the amino acids deplete. Integration of the demand into signal strength can be realised by tuning Quorum sensing dependent on the environmental conditions. The factors have been shown to interfere with the autoinducer regulation pathway in various ways. The reasons for this variety remain largely unclear. We hypothesise that different ecological and/or evolutionary impacts emerge. A number of fine-tuning strategies with respect to autoinducer systems are realised or at least possible, including e.g. degradation of autoinducers, control of the availability or activity of autoinducers, control of the activity of autoinducer synthases or receptors, or a combination of these. The existence of multiple regulation systems within the same species, controlled by different environmental or cellular factors, respectively, has been reported (e.g. [27]). In this study, we focus on three basic interaction principles affecting the signal synthase and receptor by control of production and degradation:

1) Regulation of the LuxR-type signal receptor (termed LuxR)
2) Regulation of the LuxI-type signal synthase (termed LuxI)
3) Regulation of LuxI and LuxR

Different scenarios are analysed by mathematical modelling. Our aim is to understand the differences with respect to resulting regulation dynamics and the reached equilibria, and to estimate potential ecological and evolutionary consequences, respectively. Relevant aspects are the range of bistability, the activation threshold and long term behaviour. From a mathematical point of view, bifurcation analysis can help to answer these questions. We mainly study single effects on single cells using deterministic models; nevertheless combinations of effects are also possible.

However, small numbers of cellular molecules in the regulatory system or spatial inhomogeneity of environmental factors controlling the regulatory system may cause stochastic differences between cells. We therefore consider shortly the potential relevance of stochasticity in the regulation systems on a small population. Remark that we neglect any spatial structure itself, as our goal here is to understand the basic principles of the regulation system and its qualitative behaviour. For the same reason, but also due to the differences between species or even strains and the general lack of

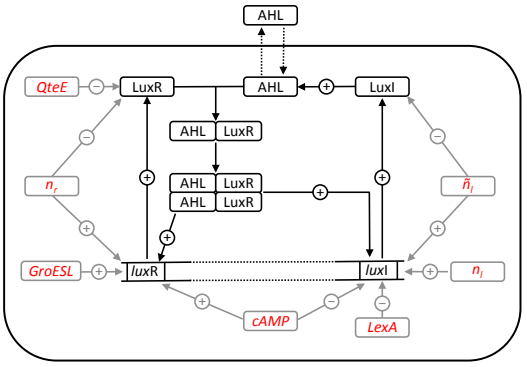


Fig. 1: Scheme of the *lux*-type Quorum sensing system with potential influences of regulators

Name	Variable
x_e	extracellular AHL concentration
x_c	intracellular AHL concentration
l	concentration of LuxI
r	concentration of LuxR
y_1	concentration of the LuxR-AHL complex
y_2	concentration of the dimer of LuxR-AHL complexes

TABLE I: Model variables of the basic Quorum sensing model

available experimentally derived quantitative data, we do not emphasize on real parameter values, which are realised in a specific species.
The paper is organised as follows: We start in section II by introducing the basic model for Quorum sensing of LuxI-LuxR type and explain the influences by nutrient-governed regulators. To focus on the signal dynamics, we assume that all other processes not involving AHL are fast and thus in equilibrium, including concentration of the regulators of Quorum sensing ([28]). The qualitative behaviour of these modified systems is examined in section III, e.g. by considering bifurcation diagrams. Some stochastic influences caused possibly by small numbers of molecules are simulated in section IV. As an example we consider coupled influences of different regulators in the stochastic case

II. The basic mathematical model and its modifications

In order to focus on the basic qualitative behaviour of our system we neglect any spatial structure and assume a homogeneous intracellular distribution of all involved regulators and substances. Also in the extracellular space, spatial structure is neglected, which is a reasonable assumption, e.g. for well stirred batch cultures or continuous cultures. For the typical Quorum sensing system of LuxI/LuxR type, basic ODE models were introduced, e.g. in [9, 28]. We start with the following ODE system for a single cell which distinguishes between intracellular and extracellular AHL (x_c resp. x_e), including equations for LuxR, LuxR-AHL complex, the corresponding dimer and LuxI:

$$\begin{aligned}
\dot{x}_e &= d_c x_c - d_e x_e - \gamma_e x_e && (1)\\
\dot{x}_c &= \beta_l l - \gamma_c x_c - d_c x_c + d_e x_e && (2)\\
&\quad -\pi_1^+ r x_c + \pi_1^- y_1 \\
\dot{r} &= \alpha_r + \pi_1^- y_1 - \pi_1^+ r x_c - \gamma_r r && (3)\\
\dot{y}_1 &= \pi_1^+ r x_c - \pi_1^- y_1 + 2\pi_2^- y_2 - 2\pi_2^+ y_1^2 && (4)\\
\dot{y}_2 &= \pi_2^+ y_1^2 - \pi_2^- y_2 && (5)\\
\dot{l} &= \alpha_l - \gamma_l l + \beta_y \frac{y_2}{1 + (\beta_y/\kappa_y) y_2}. && (6)
\end{aligned}$$

For the meaning of all variables and parameters see Tables I and II. The model assumes the typical positive feedback which leads to a Hill function in the equation for LuxI (the AHL producing enzyme, denoted by l) with Hill coefficient $n = 2$, assuming that LuxR-AHL dimers (denoted by y_2) are relevant for the increased LuxI production. Exchange of AHL between intracellular and extracellular space is described by rates d_e and d_c. For LuxR (r), a constitutive basic production is assumed. The notation of the model terms is chosen in a similar way as in previous publications (e.g. [28, 23]), to keep it comparable to the simpler models.

Even though *V. fischeri* possesses at least two Quorum sensing systems, we restrict ourselves to the well-known *lux* system, i.e., there is only one positive feedback via LuxI. Degradation of LuxR

Name	Parameter
α_l	Basal/Background production rate of LuxI
α_r	Basal/Background production rate of LuxR
β_c	Maximum increase of/Slope of increase of LuxR-production by cAMP
β_l	Production rate of AHL by LuxI
β_y	Maximum increase of/Slope of increase of LuxI-production by AHL-LuxR dimer
γ_c	Degradation rate of AHL in the cytoplasm
γ_e	Degradation rate of AHL outside of the cell
γ_l	Degradation rate of LuxI
γ_r	Degradation rate of LuxR
κ_r	Asymptotics of increase of LuxR-production (high cAMP concentration)
κ_y	Asymptotics of increase of LuxI-production (high AHL-LuxR dimer concentration)
μ_l	Production rate of LuxI induced by regulator n_l, $\tilde{n}_l$
μ_r	Production rate of LuxR induced by GroESL
π_1^+	Rate of AHL binding to LuxR (complex association)
π_1^-	Rate of AHL-LuxR complex dissociation
π_2^+	Rate of AHL-LuxR dimer association (binding of two AHL-LuxR complexes)
π_2^-	Rate of AHL-LuxR dimer dissociation
d_c	Diffusion rate of AHL from the cell to the extracellular space
d_e	Diffusion rate of AHL from the extracellular space into the cell
n_l, $\tilde{n}_l$	Regulator n_l, $\tilde{n}_l$ which influences the LuxI-production
n_r	GroESL, a regulator, which influences the LuxR-production
a	LexA, a regulator, which inhibits binding of the AHL-LuxR dimer to the LuxI-operon
b	Affinity of a regulator (LexA or cAMP) to the *lux* operon compared to the AHL-LuxR dimer
c	cAMP, which influences LuxI as well as LuxR
$n_{l,thr}$	Michaelis constant for destabilisation of LuxI by regulator $\tilde{n}_l$
$n_{r,thr}$	Michaelis constant for destabilisation of LuxR by GroESL
p_{n_l}	Strength of destabilisation of LuxI by regulator $\tilde{n}_l$
p_{n_r}	Strength of destabilisation of LuxR by GroESL
p_q	Strength of destabilisation of LuxR by QteE
q	QteE, which destabilises LuxR
q_{thr}	Michaelis constant for destabilisation of LuxR by QteE

TABLE II: Model parameters of the basic and the modified Quorum sensing models

is for simplicity only assumed to take place in the state of a single LuxR, not within the LuxR-AHL complex and not within the dimer.
In order to derive the model, we essentially assume that all dynamics of the more detailed model (Eq.(1) - (6)) are fast but that of x_c and x_e. E.g. complex association or dissociation is faster than the production of a larger molecule. This results in

$$\begin{aligned}
\dot{x}_e &= d_c x_c - d_e x_e - \gamma_e x_e \\
\dot{x}_c &= \beta_l l - \gamma_c x_c - d_c x_c + d_e x_e - \pi_1^+ r x_c + \pi_1^- y_1 \\
\varepsilon \dot{r} &= \alpha_r + \pi_1^- y_1 - \pi_1^+ r x_c - \gamma_r r \\
\varepsilon \dot{y}_1 &= \pi_1^+ r x_c - \pi_1^- y_1 + 2\pi_2^- y_2 - 2\pi_2^+ y_1^2 \\
\varepsilon \dot{y}_2 &= \pi_2^+ y_1^2 - \pi_2^- y_2 \\
\varepsilon \dot{l} &= \alpha_l - \gamma_l l + \beta_y \frac{y_2}{1 + (\beta_y/\kappa_y) y_2}.
\end{aligned}$$

This mathematical assumption is valid as considering the whole system shows qualitatively the same behaviour as the reduced system.
For $\varepsilon \to 0$ we obtain a function for l, only depending on x_c ,

$$l = \frac{\alpha_l}{\gamma_l} + \frac{\beta_y}{\gamma_l} \frac{x_c^2}{\frac{\pi_2^-}{\pi_2^+}\left(\frac{\pi_1^-\gamma_r}{\pi_1^+\alpha_r}\right)^2 + x_c^2\beta_y/\kappa_y}.$$

Hence we obtain the simplified model

$$\begin{aligned}\dot{x}_c &= \beta_l\left(\frac{\alpha_l}{\gamma_l} + \frac{\beta_y}{\gamma_l}\frac{x_c^2}{\frac{\pi_2^-}{\pi_2^+}\left(\frac{\pi_1^-\gamma_r}{\pi_1^+\alpha_r}\right)^2 + x_c^2\beta_y/\kappa_y}\right) \\ &\quad -(\gamma_c + d_c)x_c + d_e x_e \\ \dot{x}_e &= d_c x_c - d_e x_e - \gamma_e x_e\end{aligned}$$

or, lumping parameters together,

$$\begin{aligned}\dot{x}_c &= f(x_c) - d_c x_c + d_e x_e \\ \dot{x}_e &= d_c x_c - d_e x_e - \gamma_e x_e \\ f(x_c) &:= \alpha + \frac{\beta x_c^2}{x_{thresh}^2 + x_c^2} - \gamma_c x_c.\end{aligned} \tag{7}$$

In a further step we introduce some typical additional influences to the mathematical models.

A. Influences on the dynamics of LuxR

Increase of the LuxR production: It was reported, e.g. [1] that the protein GroESL in *V. fischeri* appears in high numbers, when there are insufficient nutrients available. Although the mechanisms behind this are not fully understood, GroESL seems to cause, besides a stabilisation of LuxR, an up-regulation of the gene expression. Production of LuxR-type autoinducers by environmental factors has been reported also for other species such as *Pseudomonas aeruginosa* [32]. Focusing on the regulation of LuxR production, we change the equation, which describes the dynamics of LuxR, to

$$\dot{r} = \mu_r n_r + \alpha_r + \pi_1^- y_1 - \pi_1^+ r x_c - \gamma_r r, \tag{8}$$

where n_r describes the available concentration of e.g. GroESL. As the copy number of the protein in the cell is low, we neglect saturation effects.

Using this equation instead of the basic equation 3 for LuxR and applying again the idea of different time scales yields

$$\begin{aligned}\dot{x}_c =& B\alpha_l + B\beta_y \frac{A_r x_c^2}{1 + (\beta_y/\kappa_y)A_r x_c^2} \\ &- \gamma_c x_c - d_c x_c + d_e x_e,\end{aligned} \tag{9}$$

where $B := \frac{\beta_l}{\gamma_l}$ and $A_r := \frac{\pi_2^+}{\pi_2^-}\left(\frac{\pi_1^+(\alpha_r+\mu_r n_r)}{\pi_1^-\gamma_r}\right)^2$.

Destabilisation of LuxR: The protein QteE destabilises the LuxR-homologue LasR in *Pseudomonas aeruginosa* resulting in a faster degradation of LasR [35]. Although the regulation of *qteE* expression yet needs to be investigated in detail, environmental factors seem to be involved [40]. This extension can be described by a slight modification of the LuxR-governing equation

$$\dot{r} = \alpha_r + \pi_1^- y_1 - \pi_1^+ r x_c - (1 + \frac{p_q q}{q + q_{thr}})\gamma_r r. \tag{10}$$

Proceeding in the same way as done for GroESL results in

$$\begin{aligned}\dot{x}_c =& B\alpha_l + B\beta_y \frac{A_q x_c^2}{1 + (\beta_y/\kappa_y)A_q x_c^2} \\ &- \gamma_c x_c - d_c x_c + d_e x_e,\end{aligned} \tag{11}$$

where

$$A_q := \frac{\pi_2^+}{\pi_2^-}\left(\frac{\pi_1^+\alpha_r(q + q_{thr})}{\pi_1^-(pq + q + q_{thr})\gamma_r}\right)^2.$$

Increase of the LuxR production and destabilisation of LuxR: Typically a number of mechanisms regulating Quorum sensing systems occur in the same species (see e.g. [3]). As a hypothetical example, we assume that both mechanisms analysed before, i.e., up-regulation of LuxR production and destabilisation of the LuxR protein, are induced at the same time by environmental triggers, in our case by a single regulator. Such a combination can be assumed to help the bacteria to react faster to environmental changes. The equation of LuxR has the following form:

$$\begin{aligned}\dot{r} =& \mu_r n_r + \alpha_r + \pi_1^- y_1 - \pi_1^+ r x_c \\ &- \left(1 + \frac{p_{n_r} n_r}{n_r + n_{r,thr}}\right)\gamma_r r.\end{aligned} \tag{12}$$

Using the same mathematical tools as in the paragraphs above yields

$$\begin{aligned}\dot{x}_c =& B\alpha_l + B\beta_y \frac{A_r^{new} x_c^2}{1+(\beta_y/\kappa_y)A_r^{new}x_c^2} \\ &- \gamma_c x_c - d_c x_c + d_e x_e,\end{aligned} \tag{13}$$

where $A_r^{new} := \frac{\pi_2^+}{\pi_2^-}\left(\frac{\pi_1^+}{\pi_1^-}\right)^2\left(\frac{n_r+n_{r,thr}}{(1+p_{n_r})n_r+n_{r,thr}}\right)^2\left(\frac{\mu_r n_r+\alpha_r}{\gamma_r}\right)^2$.

LuxR feedback: LuxR type receptors may be able to induce the expression of their own gene after binding to its autoinducer [34]. Considering the possibility of a self-induced positive feedback of LuxR leads to qualitatively similar results as the addition of GroESL into our model. We thus omit the analysis in this study for the reason of brevity.

B. Influences on the dynamics of LuxI

Increase of the LuxI production: Stress factors as starvation have been reported to up-regulate the transcription of the lux operon in *V. fischeri*, including the *luxI* gene, via $\sigma 32$ [38]. AHL synthase genes in other species also are known to be controlled in an environment dependent way (e.g. [8]). Regulation of AHL synthase can be incorporated in two different ways: Either only the basal synthase expression (and correlated with this the basal autoinducer production) is increased by the addition of a regulator n_l, or both, the basal and the induced production, are increased. Unfortunately, experimental studies usually do not allow to discriminate between both variants. However as the qualitative behaviour is the same in both approaches, we will only consider the second in this study. This modification leads to the following governing equation for LuxI:

$$\dot{l} = \left(\alpha_l + \beta_y \frac{y_2}{1+(\beta_y/\kappa_y)y_2}\right)(1+\mu_l n_l) - \gamma_l l. \tag{14}$$

Assuming again different time scales and reducing the system to a two component model changes the governing equation for the intracellular concentration of AHL accordingly (equation not shown here for the reason of brevity).

Inhibition of the LuxI production: LexA is a repressor enzyme, which usually acts on SOS response genes. In *V. fischeri*, it has been reported to act antagonistically with LuxR-AHL dimers by competing for the same binding site on the *lux* operon. LexA binding does not induce transcription of the *lux* operon, the transcription is not increased [37]. Repressors of AHL synthase genes have also been shown in other species (see e.g. [43]). Neglecting the details about the binding mechanism, we follow a non-classic approach (as used in [23]): The percentage of present molecules determines if transcription is possible and the grade of transcription is determined as usual by the Monod term. The corresponding modified equation for LuxI reads

$$\dot{l} = \alpha_l - \gamma_l l + \beta_y \frac{y_2}{1+(\beta_y/\kappa_y)y_2} \cdot \frac{by_2}{by_2+a}. \tag{15}$$

The modified equation for x_c is left out again. The influence of oxygen concentration on the expression of the *lux* operon, which is mediated via ArcA, may act similarly [5].

Increase of the LuxI production and destabilisation of LuxI: Although much more evidence exists for regulation of stability of LuxR type AHL receptors, similar behaviour was also reported for LuxI type AHL synthase. In *P. aeruginosa*, the half-life of LasI is controlled by the LON protease, which itself has been reported to be induced by environmental stress due to certain antibiotics [36, 25]. Analogue to the analysis of effects on LuxR, we thus analyse a combination of a destabilising effect on LuxI and an increased LuxI-production by a single regulator. This changes the equation for LuxI in a similar way as in the corresponding regulation of LuxR:

$$\begin{aligned}\dot{l} \;=\; & \left(\alpha_l + \beta_y \frac{y_2}{1+(\beta_y/\kappa_y)y_2}\right)(1+\mu_l \tilde{n}_l) \\ & - \left(1+\frac{p_{n_l}\tilde{n}_l}{\tilde{n}_l+n_{l,thr}}\right)\gamma_l l.\end{aligned} \tag{16}$$

The resulting governing equation for the intracellular AHL concentration reads

$$\begin{aligned}\dot{x}_c &= \frac{\tilde{n}_l + n_{l,thr}}{(1+p_{n_l})\tilde{n}_l + n_{l,thr}}(1+\mu_l\tilde{n}_l)\cdot \\ &\left(B\alpha_l + B\beta_y \frac{A_l x_c^2}{1+(\beta_y/\kappa_y)A_l x_c^2}\right) \\ &-\gamma_c x_c - d_c x_c + d_e x_e.\end{aligned} \tag{17}$$

C. Influence on the dynamics of LuxI and LuxR

Regulation factors can have pleiotropic effects on different target molecules. Starvation induces an increased occurrence of 3':5'-cyclic AMP (cAMP) in bacteria such as *V. fischeri* [12]. This molecule is able to bind to the cAMP receptor protein (CRP). The so-formed complex influences the *lux* system in *V. fischeri* on two different sites. On the one hand it amplifies the production of LuxR. On the other hand cAMP inhibits the LuxI-production using a similar mechanism as LexA. We analysed the effect of cAMP as an example for more complex regulation mechanisms. From now on for the reason of simplicity the (cAMP-CRP)-complex will be referred to as cAMP.
Adding cAMP to the model yields a change in the dynamics of LuxI and LuxR resulting in

$$\dot{r} = \alpha_r + \beta_c \frac{c}{1+(\beta_c/\kappa_r)c} + \pi_1^- y_1 - \pi_1^+ r x_c - \gamma_r r$$

$$\dot{l} = \alpha_l - \gamma_l l + \beta_y \frac{y_2}{1+(\beta_y/\kappa_y)y_2}\frac{by_2}{by_2+c}.$$

The reduction of the so modified system obviously affects the governing equation of x_c. Those changes lead to the following equation:

$$\begin{aligned}\dot{x}_c =& B\alpha_l + B\beta_y \frac{PA_c^2x_c^2}{1+(\beta_y/\kappa_y)PA_c^2x_c^2}\frac{bPA_c^2x_c^2}{bPA_c^2x_c^2+c} \\ &- \gamma_c x_c - d_c x_c + d_e x_e,\end{aligned} \tag{18}$$

where $P := \frac{\pi_2^+}{\pi_2^-}\left(\frac{\pi_1^+}{\pi_1^-}\right)^2$ and $A_c := \frac{\beta_c}{\gamma_r}\frac{c}{1+(\beta_c/\kappa_r)c}$.

III. Model analysis and results

In this section we analyse the effects of different strengths of the regulation impact on Quorum sensing signals. Therefore we take a look at simulations made with the above derived models for the different influences of regulators. The variables (listed in Table III) and parameters are used in a non-dimensional form. The values of the parameters in the simulations are shown in Table IV. We aim in this study at comparing the potential qualitative consequences of different regulators on the function of the AHL-type Quorum sensing system in a generic approach. The parameter values were chosen in a way to disclose the full complexity of such a system, including e.g. the maximum number of stationary states. We assume that evolution of a system enabling complex behaviour suggests that the bacterium at least under certain conditions exploits this complexity. Using an experimentally derived parameter set of a specific bacterium, which was gained under certain environmental conditions, was thus not meaningful, and would have been difficult due the lack of such data and variability of parameters in response to changes of the environmental conditions ([18]). Note that other parameter values might cause more simple behaviour, including absence of multistationarity. However, the qualitative messages in the results with respect to time and strength of Quorum sensing induction will hold. As the basal production rate of the autoinducer synthase, which is critical for induction dynamics, may vary between different species, we use two different parameter values. Changes due to the variation of the basal LuxR-production rate are not subject to this study and hence the same value was used throughout. In the following solid lines in the bifurcation diagrams represent stable stationary states whereas dashed lines represent unstable stationary states.

For the time courses in this section an initial condition of zero intra/extracellular AHL was assumed.
The numerical analyses were done with XPPAUT Version 5.41 [13].

A. Influences on LuxR

Increase of the LuxR production: For low basal production rates of LuxI (α_l) we observe a bistable behaviour of the *lux* system (Fig. 2(a)), when assuming the strength of the regulator (e.g. GroESL) to be the bifurcation parameter. This means that for

	Regulator	Influence	Eq.	Fig.
LuxR	n_r	Increased production	9	2(a)+(b)
LuxR	QteE	Destabilisation	11	2(c)+(d)
LuxR	$\tilde{n}_r$	Increased production & Destabilisation	13	2(e)+(f)
LuxI	n_l	Increased production	14*	3(a)+(b)
LuxI	LexA	Inhibited production	15*	3(c)+(d)
LuxI	$\tilde{n}_l$	Increased production & Destabilisation	17	3(e)+(f)
LuxI/LuxR	cAMP	Increased LuxR production & Inhibited LuxI production	18	4

TABLE III: Different scenarios. *Equation referenced has to be inserted into Eq.(2) assuming a quasi-steady state.

Parameter	Value	Parameter	Value
α_l	refer to figures	α_r	0.1
β_c	1	β_l	0.1
β_y	1	γ_c	0.03
γ_e	0.03	γ_l	0.1
γ_r	0.1	κ_r	1
κ_y	1	μ_l	0.1
μ_r	0.1	π_1^+	1
π_1^-	1	π_2^+	1
π_2^-	10	d_c	0.5
d_e	0.5	b	1
$n_{l,thr}$	1	$n_{r,thr}$	1
p_{n_l}	10	p_{n_r}	10
p_q	5	q_{thr}	1

TABLE IV: Values of the dimensionless parameters for the simulations

concentrations of GroESL (or similar acting regulators) larger than a certain threshold (the bifurcation point, here at about $n_r = 1.9$) the system will always be induced in the used parameter value setting. For concentrations of regulator beneath this threshold the final AHL-concentration within the cells depends on the extra- and intracellular concentration of AHL at the beginning of the simulation. The unstable stationary state (dashed region of the black line in Fig. 2(a)) marks the threshold: a starting AHL-concentration lower than the threshold causes the system to stay non-induced, while higher AHL-concentrations lead to considerably higher stationary AHL-concentrations, i.e. an induction of the whole system.

Assuming higher basal rates α_l shifts the bifurcation diagram to the left and hence bistability is lost. In this case independent of the starting AHL-concentration the system always gets activated (Fig.2(a) red line).

Fig. 2(b) shows the time course of the extracellular AHL-concentration for different GroESL-concentrations in the low basal production case, corresponding to the black line in Fig. 2(a). In case the GroESL concentration is above the bifurcation point, increasing the GroESL concentration results in an earlier induction of the cell. The final AHL-concentration of the induced cells does not depend

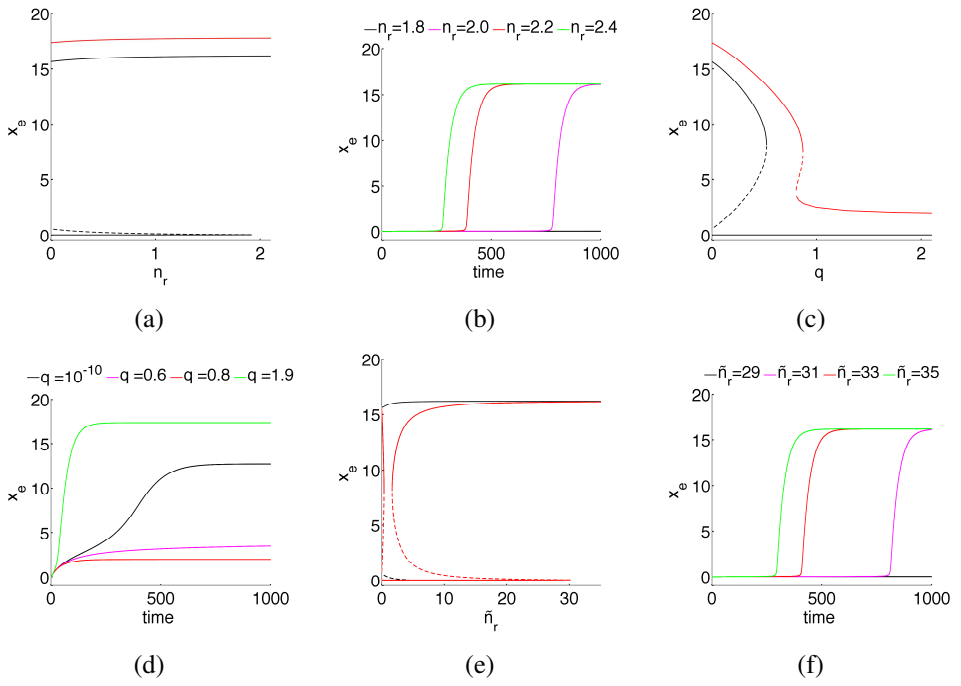


Fig. 2: Influences on LuxR. (a) Bifurcation diagram for the extracellular AHL-concentration, where only the increase in the LuxR-production by GroESL was considered. The basal LuxI-production rate α_l is 0.001 (black line) resp 0.1 (red). (b) Time courses for the extracellular AHL-concentration, which were generated by cells with different GroESL-concentrations. Those time courses correspond to the bifurcation diagram shown in (a) by the black line, i.e., $\alpha_l = 0.001$. (c) Bifurcation diagram for the extracellular AHL-concentration, where the influence of QteE on the system is examined. QteE destabilises LuxR and hence leads to a faster degradation of LuxR. Basal LuxI-production rate is assumed to be 0.001 (black) resp $\alpha_l = 0.1$ (red). (d) Time courses for the extracellular AHL-concentration, which were generated by cells with different QteE-concentrations. Those time courses correspond to the bifurcation diagram shown in the red line in (c), i.e., $\alpha_l = 0.1$. (e) Bifurcation diagram, where both, an up-regulation of LuxR-production, and a destabilising effect on the LuxR protein is assumed. Basal LuxI-production rate is 0.001. The destabilising effect is $p_{n_r} = 1$ (black) or $p_{n_r} = 10$ (red) (f) Time courses for the extracellular AHL-concentration, which were generated by cells with different concentrations of the GroESL-like regulator. Those time courses correspond to the bifurcation diagram shown in (e) with $p_{n_r} = 10$.

on the amount of GroESL.

Destabilisation of LuxR: Here (Fig. 2(c)-(d)) we choose the concentration of a QteE-like regulator as the bifurcation parameter. The bifurcation diagrams show that a high level of QteE completely prevents an activation of the Quorum sensing system. Even induced systems will switch to the non-induced state after some time, when there is a high concentration of QteE present. In addition the basic production rate of LuxI (α_l) also plays a significant role: if α_l is large, q must be large as well to prevent an induction of the *lux* system. However, if α_l is very low, a non-induced system will never, i.e., independent of the concentration of QteE, be able to activate itself. The presence of QteE may shift the potential stationary states, but typically keeps the bistable behaviour with the possibility to switch on for growing bacteria, see Fig. 2(c). Fig. 2(d) shows the time course for cells which are provided with different amounts of QteE. It is evident that the time of induction and the height of the final AHL-concentration depend on the amount of present QteE. The more QteE available the lower is the final AHL-concentration.

The moment of induction - in case the system is induced - is late if the concentration of QteE is close to the QteE-concentration at which the bifurcation occurs.

Increase of the LuxR production and destabilisation of LuxR: The results of a combined impact on LuxR, i.e., an increase of the LuxR-production and a faster degradation of LuxR, which could be interpreted as a combined effect of GroESL- and QteE-like regulators, is shown in Figures 2(e) - 2(f). As we have already discussed different basal production rates α_l, we now focus on changing the ratio between the strength of degradation of LuxR and the increase of the LuxR-production by varying the latter. As a bifurcation parameter we use GroESL concentration.
For weak effects of GroESL on LuxR stability the bifurcation diagram is similar to the one, where no influence on the degradation of LuxR was assumed (compare Figures 2(a) and 2(e) black lines). When assuming a stronger destabilisation of LuxR, an intermediate range of GroESL-concentrations exists for which the system is never able to get activated in our parameter setting (Fig. 2(e) red line). By increasing the destabilisation strength, the bistable range increases.
As already seen for Fig. 2(b) the moment of induction depends on the GroESL-concentration. The closer it is to the bifurcation point the later the system gets activated (Fig. 2(f)).

B. Influences on LuxI

Increase of the LuxI production: In contrast to a regulator which acts by increasing the production of LuxR, introducing regulator n_l into the system changes the concentration of AHL in the stationary phase (Fig. 3(a)). While the system acts bistable when a small basal LuxI-production rate α_l is assumed, this bistability is lost for high basal rates. In Fig. 3(b) the time courses of AHL concentration for different amounts of regulator n_l are shown. Increasing n_l does not only result in higher maximum concentrations of AHL, but - similar to a factor up-regulating the production of LuxR - promotes an earlier induction

Inhibition of the LuxI production: Inhibition of LuxI production by LexA results in similar effects as described for the LuxR destabilising regulator above (Figures 3(c) - 3(d)), including a decrease of maximum AHL concentration in stationary phase, and a delay of activation for higher LexA concentrations. A similar effect takes place if one considers LuxI destabilisation only, due to the "simple" production of AHL by LuxI, formulated as a linear term, no further non-trivial effects appear in that context.

Increase of the LuxI production and destabilisation of LuxI: The results are shown in Figures 3(e) - 3(f) (Please note the logarithmic axes in Fig. 3(e)). Similar to the corresponding regulation of LuxR, in our parameter setting using a stronger destabilisation effect, there is an intermediate range of concentrations of regulator $\tilde{n}_l$, in which the system cannot be activated. As a main difference between regulation of LuxR and LuxI, the maximum concentration of AHL in an activated state increases significantly with increasing concentrations of regulator $\tilde{n}_l$. Again similar to LuxR regulation, the intermediate range vanishes for small values of p_{n_l}.

C. Influence on the dynamics of LuxI and LuxR

Increasing the affinity of cAMP to the *lux* operon (parameter b) stretches the bifurcation diagram, but keeps its shape (compare the black with the red lines in Figures 4(a),(c) and (e), respectively).
In Figures 4(a) - 4(b) a low basal LuxI-production rate was assumed. With this assumption and our parameter setting a system which starts in a non-activated state is not able to get induced (Fig. 4(b)). The four different curves are all close to zero (thus indistinguishable from each other).
Using our set of parameters and an intermediate basal production rate α_l the bacteria will always get activated as long as they are neither starving nor drowned with nutrients, i.e., an intermediate amount of cAMP is present (Fig. 4(c)). Contrarily, the system is never activated with very low or very high amounts of cAMP. Regions of bistability exist, i.e., dependent on the initial concentration,

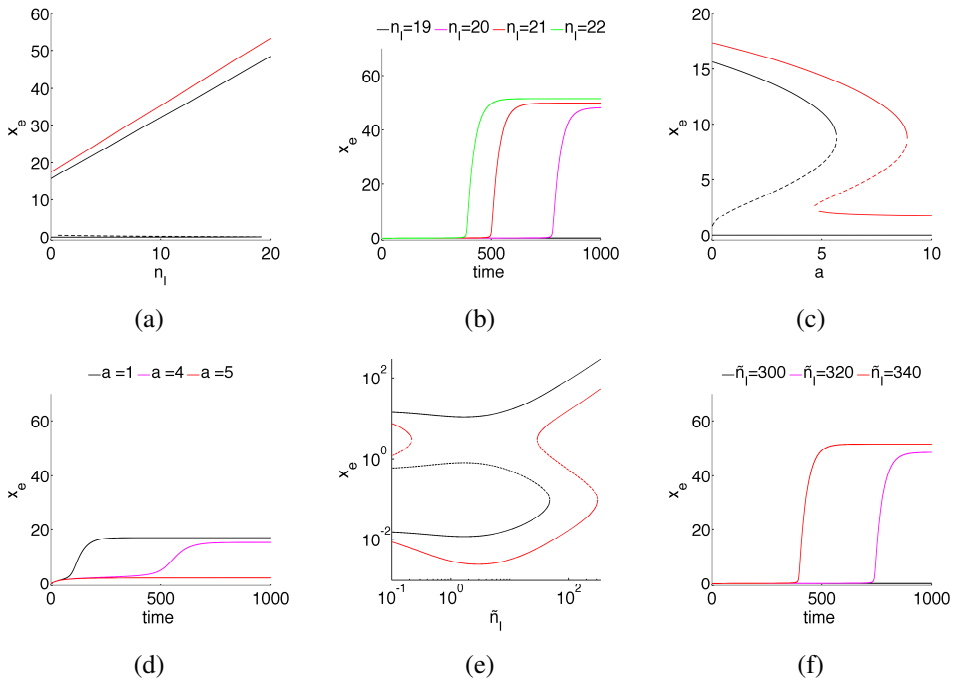


Fig. 3: Influences on LuxI. (a) Bifurcation diagram for the extracellular AHL-concentration, where only the increase in the LuxI-production by regulator n_l was considered. The basal LuxI-production rate α_l is 0.001 (black line) or 0.1 (red line), respectively. (b) Time courses for the extracellular AHL-concentration, which were generated by cells with different concentrations of regulator n_l. Those time courses correspond to the bifurcation diagram shown in (a), with $\alpha_l = 0.001$. (c) Bifurcation diagram for the extracellular AHL-concentration, where the influence of LexA on the system is examined. LexA destabilises LuxI and hence leads to a faster degradation of LuxI. Basal LuxI-production rate is assumed to be $\alpha_l = 0.001$ (black line) or $\alpha_l = 0.1$ (red line), respectively. (d) Time courses for the extracellular AHL-concentration, which were generated by cells with different concentrations of regulator n_l. Those time courses correspond to the bifurcation diagram shown in (c), with $\alpha_l = 0.1$. (e) Bifurcation diagram, where in addition to the increased LuxI-production by regulator $\tilde{n}_l$, a destabilising effect of regulator $\tilde{n}_l$ on LuxI is assumed. Basal LuxI-production rate is 0.1. The destabilising effect is $p_{n_l} = 1$ (black line) or $p_{n_l} = 10$ (red line). (f) Time courses for the extracellular AHL-concentration, which were generated by cells with different concentrations of regulator $\tilde{n}_l$. Those time courses correspond to the bifurcation diagram shown in (e), with $p_{n_l} = 10$.

the system will either be activated or not. The time course, which is shown in Fig. 4(d), displays similar effects as already seen for LexA-, QteE- and n_l-type regulators. Depending on the proximity of the cAMP-concentration to the bifurcation point the *lux* system is induced at different time points. The final AHL-concentration in an activated system changes with different concentrations of cAMP.
The bistability behaviour of the previous figures is lost, when assuming a high basal production rate α_l. The system is induced independently of the added cAMP-concentration (Fig. 4(e)). All systems are induced at about the same time (Fig. 4(f)). They only differ in the final AHL-concentrations.

IV. Stochastic influences

So far, any stochasticity was neglected in our modelling approach. Nevertheless, as e.g. some parts of the intracellular regulation system may consist only of few molecules, and the regulation system acts non-linearly, the behaviour of individual cells might significantly differ from the

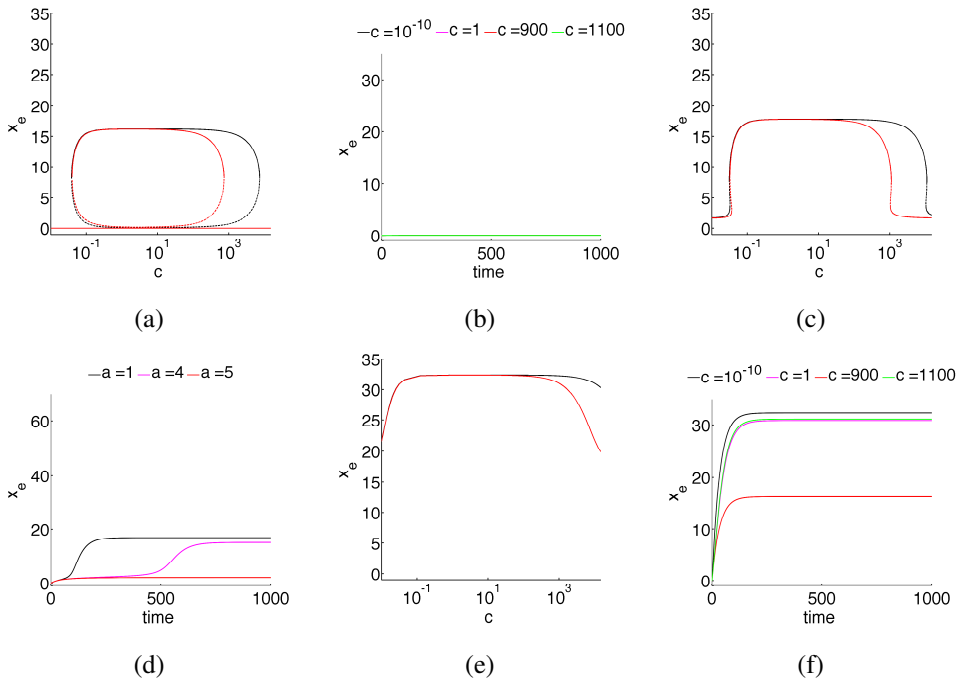


Fig. 4: Influences on LuxI and LuxR simultaneously. (a) Bifurcation diagram for the extracellular AHL-concentration, where the effect of cAMP on the competitive inhibition of the LuxI-production and the increase of the LuxR-production is considered. The basal LuxI-production rate α_l is 0.001. Affinity of cAMP to the *lux* operon compared to the AHL-LuxR dimer is assumed to be equal. This is achieved by setting $b = 1$ (black line) or by assuming the affinity of cAMP to the *lux* operon to be stronger compared to the AHL-LuxR dimer by setting $b = 10$ (red line). (b) Time courses for the extracellular AHL-concentration, which were generated by cells with different cAMP-concentrations. Those time courses correspond to the bifurcation diagram shown in (a), with $b = 1$ and $\alpha_l = 0.001$. (c) Same figure as seen in (a), only the basal LuxI-production rate is increased to 0.1. (d) Time courses for the extracellular AHL-concentration, which were generated by cells with different cAMP-concentrations. Those time courses correspond to the bifurcation diagram shown in (c), with $b = 1$ and $\alpha_l = 0.1$. (e) Same figure as seen in (a), only $\alpha_l = 1$. (f) Time courses for the extracellular AHL-concentration, which were generated by cells with different concentrations of regulator n_l. Those time courses correspond to the bifurcation diagram shown in (e), with $b = 1$ and $\alpha_l = 1$

bulk behaviour. This is also the case for nutrient-dependent regulators, as nutrients often are heterogeneously distributed under natural conditions. As an example we will consider nutrient-dependent influences in this section. Of course, the dynamic behaviour itself is the same as in the deterministic setting. But this stochastic approach allows us to track a number of cells with typical variations in molecule numbers and hence, leads to a better understanding of how realistic cell populations could behave.

The numerical analyses were done with MATLAB Version R2010a [26], using the solver ode45 with its standard precision.

A. *Influence of a single regulator on the system*

We start by considering the influence of stochasticity of a single regulator on the whole Quorum sensing system. For the number of regulator molecules per cell we assume a normal distribution with a fixed expected value and variance. This can be interpreted as a normal distributed nutrient availability under natural conditions, which then transfers to the nutrient-dependent regulator.

For the simulations, we set the number of cells to ten. For higher numbers of cells the results are qualitatively the same (not shown). A fixed cell number can be realised experimentally e.g. in a chemostat-like setting. Above, a deterministic single cell model was introduced. Now we slightly alter this model in order to obtain a model with n cells and a random distribution of regulators, i.e., we focus on the influence of stochasticity by the regulators but neglect other stochastic effects on the Quorum sensing system. This means that we still assume AHL production in each cell to be deterministic but dependent on the random number of regulator molecules in each cell. Assuming once again different time scales, we reduce the model to a two component hybrid model. While the basic equation for the intracellular AHL-concentration in the reduced model (Eq. (7)) stays the same, the governing equation for x_e changes to be

$$\dot{x}_e = \sum_{k=1}^{n} d_c x_c^{(k)} - n d_e x_e - \gamma_e x_e, \qquad (19)$$

where the superscript describes the k-th cell, as now, each cell may have an individual intracellular AHL-concentration, dependent on its available regulators. When regarding the above mentioned assumptions, the governing equations for x_c are modified only slightly. As an example we show how the equation for the intracellular AHL-concentration under the influence of a regulator controlling LuxR production in a way as reported for GroESL (Eq. (9)) changes:

$$\begin{aligned} \dot{x}_c^{(k)} &= B\alpha_l + B\beta_y \frac{A_r \left(x_c^{(k)}\right)^2}{1 + (\beta_y/\kappa_y) A_r \left(x_c^{(k)}\right)^2} \\ &\quad -\gamma_c x_c^{(k)} - d_c x_c^{(k)} + d_e x_e, \qquad (20) \end{aligned}$$

for $k = 1, ..., n$ and $A_r := \frac{\pi_2^+}{\pi_2^-} \cdot \left(\frac{\pi_1^+}{\pi_1^-}\right)^2 \cdot \left(\frac{\alpha_r + \mu_r N_r}{\gamma_r}\right)^2$.

Note that the only difference to the non-stochastic equation concerns the superscript k, which describes the k-th cell, and the random variable N_r instead of the fixed n_r. This random variable N_r is, as stated above, normally distributed with an expected value $\mathrm{E}[N_r]$ and a variance $\mathrm{Var}[N_r]$. In the following we choose the variances relatively high such that the effects due to the randomness in the regulator distribution become visible. The realisations of N_r will be different concentrations of GroESL-like regulators in different cells. All the other equations are altered in a similar way but omitted here for the reason of brevity. For the hybrid model, which includes a higher number of cells, the diffusion constants d_c and d_e are changed. This helps to identify the studied effects better. In the simulations the diffusion constants are set to $d_c = d_e = 0.05$ in contrast to 0.5 in the simulations without a stochastic distribution of regulators in order to keep the extracellular concentration of AHL comparable to the single cell scenario, i.e., we implicitly assume that the extracellular volume of n cells is n times the extracellular volume of one cell.

Taking these changes into account, the bifurcation points in the simulations with multiple cells are considerably lower, i.e., lower regulator-concentrations - in the case of regulator n_l and GroESL - are bifurcation points than the ones identified in the single cell simulations (results not shown). Introducing LexA- or QteE-like regulators into the equations and assuming that the basal production rate of LuxI (α_l) to be 0.001 obviously never leads to an activation of the bacteria in the ten cell setting, under the given conditions, when starting with zero AHL and an arbitrary concentration of LexA or QteE (Figures 2(d) and 3(d)).

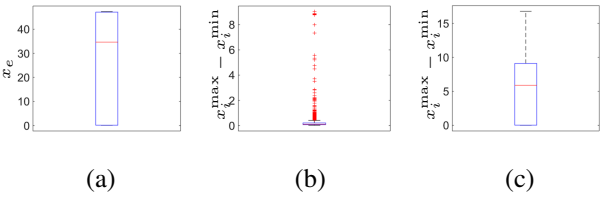
From here on it is important to keep the differences between the following figures - especially Figures 6, 8, 9 and 10 - and the time courses in Figures 2, 3 and 4 in mind. While the single cells were not able to influence each other in the previous sections, there is now an influence between the different cells within one colony.

Running 1000 simulations with the amount of a regulator near the bifurcation point in each run, results in large differences of the final intra- and extracellular AHL concentrations due to non-

linearity (Fig.5(a) for extracellular AHL concentration). We use the same amount of regulators for each run as we only want to examine the effect of the distribution of the regulator on the final AHL-concentration. Each data point in the box plot can be interpreted as one colony, where each colony has the same size and the same amount of regulator available. The only difference between the runs is the distribution of the regulator over the cells. This result gives rise to the idea that the distribution of a regulator is to some extent responsible for the activation of the system, neglecting the time course for a moment which also might be influenced by the stochastic regulatory effects. The same result was attained for the other effects of regulators on the system, but they are omitted here.

Effects due to GroESL-variation: When running a simulation with one colony, one can compare the cell with the highest intracellular AHL-concentration at the end of a simulation ($t_{end} = 1000$) within the colony with the one having the lowest final intracellular AHL-concentration. Subtracting those concentrations from each other gives information about variation between cells within a colony. Doing this for one thousand colonies - again assuming the same size of the colonies - leads to the box plot shown in Figure 5(b). Most cells within a colony - when assuming an inhomogeneous distribution of GroESL-like regulators - have a similar final intracellular AHL-concentration as the difference between the cells is low compared to the relative deviation of regulator n_l of approximately 40% (Fig. 5(c)). However, some outliers occur in Figure 5(b) (red crosses). A possible interpretation for those is that the distribution of regulators within one colony might influence the time of activation as some cells are already activated while others are not yet. This idea will be supported in section IV-B (see below).

Effects due to n_l-variation: Proceeding with regulator n_l in the same way as with GroESL leads to the box plot shown in Fig. 5(c). There, one can see that on the one hand the system as a whole is always either induced or non-induced as the difference of the AHL-concentrations of the



(a) (b) (c)

Fig. 5: Box plots of AHL-concentrations under different conditions. The colours mean the following: red line is the median, blue box is the $25-75\%$-quantile, black limiters (whiskers) extend to the most extreme values which are no more than $1.57/\sqrt{1000} \cdot (75\%-\text{quantile} - 25\%-\text{quantile})$ away from the box and red crosses show outliers not belonging to the region limited by the whiskers. (a) Extracellular AHL-concentration within one colony. Each colony had the same amount of regulator n_l available and the same number of cells. However, the concrete distribution of regulator n_l amongst the individuals is different in each colony. The AHL-concentration is measured at the end of the simulation at time $t_{end} = 1000$. The values of regulator n_l are simulated with $E[N_l] = 6.5$ and $Var[N_l] \approx 5.2$. (b) Box plot, where each data point is obtained by subtracting the cell with the lowest intracellular AHL-concentration at the end of the simulation ($t_{end} = 1000$) from the cell with the highest intracellular AHL-concentration within one colony. The difference of the intracellular AHL-concentration between the cells is - in this subfigure - due to the influence of GroESL on the LuxR-production. One thousand colonies were simulated to create this box plot. $E[N_r] = 6$ and $Var[N_r] \approx 0.055$. (c) Box plot was created in the same way as in (b), only the influence of regulator n_l on the LuxI-production is varying this time. $E[N_l] = 6.5$ and $Var[N_l] \approx 5.2$. End of simulation at time $t_{end} = 1000$.

two cells is considerably lower than between an activated and a non-activated state (compare to Fig. 5(a)). This means that regulator n_l has no relevant effect on the time of activation within one colony in our parameter setting. On the other hand the AHL-concentration level at the end of the simulation ($t_{end} = 1000$) depends on the amount of n_l (Fig. 5(c)), which is different compared to the influence of GroESL where the distance between the cells containing most and fewest AHL-molecules is considerably lower than here (Fig. 5(b)).

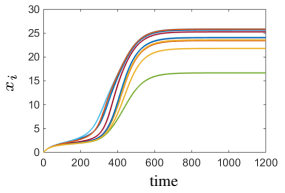


Fig. 6: Time course of the intracellular AHL-concentration of ten cells within one colony with a inhomogeneous distribution of cAMP (i.e. $E[c] = 0.03, Var[c] = 0.00005$; $\alpha_l = 0.05$)

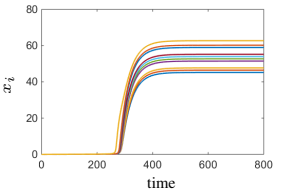


Fig. 7: Time course of the intracellular AHL-concentration of ten cells which all belong to the same colony. The distribution of regulator n_l fixed and given by the vector $(1, 2, 3, 6, 7, 8, 9, 12, 13, 15)$.

Effects due to cAMP-variation: The simulations on cAMP are done with $\alpha_l = 0.05$ since otherwise the system never gets activated in our parameter setting. In this case the lower bifurcation point of cAMP is lower than in the (deterministic) one cell setting (see Fig. 4(d)), whereas the upper bifurcation point is even higher (results not shown). The effect which cAMP has on the system is a combination of the effects of GroESL and regulator n_l, similar as in the deterministic model system. On the one hand cells with low cAMP-concentration will get activated later than cells with intermediate cAMP concentration. A low concentration of cAMP on the other hand leads to a lower final intracellular concentration of AHL than an intermediate concentration (Fig. 6). In Fig. 6 the expected value for cAMP is $c = 0.03$ and the variance is 0.00005, i.e., quite small.

Here, as well as below, one can see that the system is quite stable with respect to the variation of the different regulators, i.e., the resulting relative deviation in the AHL-concentration was below 10% even though the coefficient of variation ($\sqrt{\mathrm{Var}}/\mathrm{E}$) of c was 24% approximately, in our parameter setting. Nevertheless the figures are included to see the possible effects of the different regulators on the Quorum sensing system. In contrast to this, Fig. 7 was included, in which a range of concentrations of regulator n_l was distributed over the different cells within one colony. This means that the different regulators may yield different resulting variability in the system, due to their non-linear influences.

B. Combining several regulators

So far only the influence of a single regulator on the system has been studied. In the following we investigate the effect of several regulators influencing the Quorum sensing system at the same time. We show the governing equation for x_c in the reduced model for an influence of the exemplarily chosen regulators GroESL, LexA and n_l on the Quorum sensing system:

$$\begin{aligned}\dot{x}_c^{(k)} = & \quad (1+\mu_l N_l)\cdot \\ & \left(B\alpha_l + B\beta_y \frac{A_r\left(x_c^{(k)}\right)^2}{1+(\beta_y/\kappa_y)A_r\left(x_c^{(k)}\right)^2}\frac{bA_r x_c^{(k)}}{bA_r x_c^{(k)}+A}\right) \\ & -\gamma_c x_c^{(k)} - d_c x_c^{(k)} + d_e x_e, \end{aligned} \tag{21}$$

where $A_r := \frac{\pi_2^+}{\pi_2^-}\left(\frac{\pi_1^+}{\pi_1^-}\right)^2\left(\frac{\alpha_r+\mu_r N_r}{\gamma_r}\right)^2$.

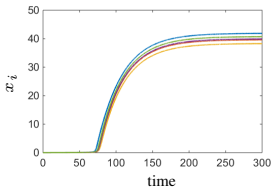
Again N_l, N_r and A are random variables representing the different amounts of regulator n_l, GroESL and LexA in the cells. To get a deeper understanding of the functionality of the respective regulators, we only examine the influence of two regulators on the system at a time, one with a fixed value, the other one with the usual variation. The expected values and variances of the simulations in this section are given in Table V. The values of expectation and variance are chosen such that all regulators have the same coefficient of variation (0.5). Note however that we had to increase the values of N_l significantly in Figure 9. Elsewise the system would not activate which is due to the inhibition of LexA even though it seems negligible.

In the deterministic model approach, we guessed that GroESL affects the time of activation of our

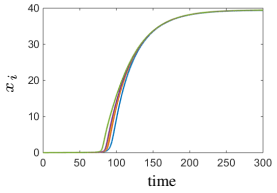
	N_l		N_r		LexA	
	Expected value	Variance	Expected value	Variance	Expected value	Variance
Fig. 8 (a)	3	2.25	1.5	0	0	0
Fig. 8 (b)	3	0	1.5	0.5625	0	0
Fig. 9 (a)	20	100	0	0	0.01	0
Fig. 9 (b)	20	0	0	0	0.01	0.000025
Fig. 10 (a)	0	0	1.5	0.5625	0.01	0
Fig. 10 (b)	0	0	1.5	0	0.01	0.000025

TABLE V: Expected values and variances for the different regulators in the Figures 8 (a) - 10 (b)

system. This behaviour can also be found in the stochastic approach under the additional presence of n_l, see Fig. 8(b). Additionally, note that the final concentrations are basically indistinguishable. The earlier expressed assertion that n_l changes the final concentration, but has no impact on the time-point of activation, is visible in Fig. 8(a).



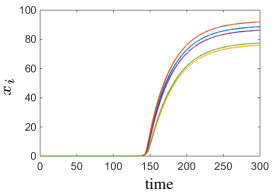
(a) constant GroESL, varying N_l



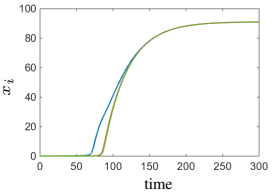
(b) constant N_l, varying GroESL

Fig. 8: Time course of the intracellular AHL-concentration of ten cells within one colony influenced by GroESL and Regulator n_l

Regarding the inhibitor LexA, a connection between the time of activation and the LexA-concentration becomes visible now. The lower the concentration of LexA, the earlier the cell is activated (Fig. 9(b)). Fig. 9(a) shows qualitatively the same behaviour as Fig. 8(a) suggesting that the qualitative impact of LexA and N_r is similar, at least once the colony gets activated.
This fact is confirmed by Figures 10(a) and (b) which show the effect of the coupled influence of LexA and GroESL. The pictures are similar, the only slight difference being that the time-point of activation with varying GroESL leads to a rather homogeneous distribution of activation



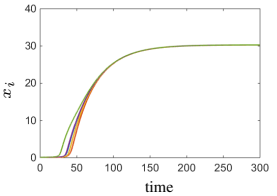
(a) constant LexA, varying N_l



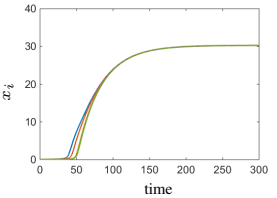
(b) constant N_l, varying LexA

Fig. 9: Time course of the intracellular AHL-concentration of ten cells within one colony influenced by LexA and Regulator n_l

time-points, whereas varying LexA only favours a single cell to activate and afterwards the bulk is induced. This means: some regulators, especially inhibitors may affect first mainly single cells and later the whole colony.



(a) constant LexA, varying GroESL



(b) constant GroESL, varying LexA

Fig. 10: Time course of the intracellular AHL-concentration of ten cells within one colony influenced by GroESL and LexA

V. Discussion and Conclusion

Although a number of studies use mathematical models to investigate traits of different Quorum

sensing systems, little is known about the impact of external regulation factors (see e.g. [22, 41, 39]). To our knowledge, we present the first comparison of different mechanisms affecting the typical basic motif of AHL based communication systems.

Modelling the full gene regulatory system for Quorum sensing of *lux* type, including all mentioned influencing regulators and mechanisms, leads to a large system of ODEs in the classical deterministic approach, containing a vast amount of (quantitatively unknown) parameters. The application of singular perturbation on the resulting mathematical model can shrink down the system essentially, and allows for a clearer analysis of the system, e.g. concerning bifurcations. Especially the possibility of bistable regions is of great interest in this context, as it allows (via a kind of hysteretic behaviour) the stabilisation of the system against perturbations [28].

Our results indicate that depending on the mode of action some regulators mainly affect the time of induction (e.g. Fig. 2(b),(f), Fig. 3(b)), which is connected with a critical cell density (plankton) or cell number (colonies). Others change the maximum signal concentration (e.g. Fig. 2(d), Fig. 3(d)) or both (e.g. 4(d)). The potential ecological and/or evolutionary benefit of these different regulator effects depends on the context, in which the population lives. For example, under spatially structured conditions such as populations living in microcolonies which support development of heterogeneity between cells, synchronicity of responses on a population level could be supported by higher induced AHL production. Hense and Schuster [18] argue that the fitness benefit of Quorum sensing regulated activity typically is not only a function of its potential strength, influenced by the cell density and some other factors, but also of the cells which demand it. Furthermore, it is highly desirable to control the timing of induction as a function of environmental conditions. Bistability can be interpreted as a simple kind of memory. The underlying positive autoregulation of components of Quorum sensing often seems to be heritable and can thus be understood as an epigenetic control [30]. It supports stability of the population e.g. against environmental fluctuations. When a Quorum sensing controlled switch between two cellular states is costly, such stability helps to minimise costs, however, at the expense of adaptation rate. Shifts of range of stability enable the cells to optimise trade-off between these opposing aspects. By combinations of various regulators, or multiple effects of one regulator on Quorum sensing via different mechanisms the cell can realise complex reaction patterns such as maximum or minimum Quorum sensing at an intermediate strength of the environmental control factor.

For example our model predicts that under certain conditions environmental factors acting via cAMP show such an intermediate maximum. cAMP is connected with starvation strength. [37] showed experimentally an intermediate maximum in a dilution series of culture medium for *V. fischeri*. Although the biochemical mechanisms behind were not fully clarified and their experimental design did not exactly reflect our model, their experiments show that such complex regulation patterns are relevant in vivo. This intermediate peak is interesting, as usually a more monotone relation between environmental factors and Quorum sensing systems has been reported (see e.g. Hense and Schuster, and citations therein.). Unfortunately, quantitative information about dose-response relations over a larger range of the strength of these factors are largely lacking, which impedes statements about the prevalence of such intermediate peaks. An exception is the well-studied *Bacillus subtilis*, in which mild starvation induce sacrifice of a fraction of the population ([24]). The purpose of this highly cooperative activity seems to be to supply nutrients for the remaining cells which might help to delay a costly sporulation. However, if starvation increases even more, the population induces sporulation. Induction of sacrifice thus peaks at intermediate starvation levels. Similarly, *B. subtilis* induces competence in a certain window of environmental intermediate stress conditions ([33]). Quorum sensing is involved in the control

of these processes. However the architecture of Quorum sensing in *B. subtilis* differs strongly from that of the AHL-type. The Com Quorum sensing system of *B. subtilis* and the influence of stress act rather in parallel in regulation of their target competence in *B. subtilis*. In contrast, in the scenario analysed in our study, cAMP impacts the Quorum sensing system directly. In more abstract terms, Quorum sensing usually induces cooperative behaviour, often as a stress response ([18]). Stress as a promoter of cooperation is a well-established concept also in other areas of ecology ([21]). [21] state that extreme stress does no longer support cooperation, but other aspects like competition tend to become dominant. As a consequence, under very severe stress conditions cells may induce other phenotypes like persistence or motility to escape from stress. Intermediate stress levels as optimal activator conditions for Quorum sensing fits to this concept. Based on these hints we speculate that such a regulation strategy may occur more frequently. More experimental dose-response studies investigating the relation between environmental conditions and regulation of Quorum sensing systems are thus desirable. Although a number of external regulators have been experimentally identified for an increasing number of Quorum sensing systems, the effect of most of these regulators on dynamics of Quorum sensing is usually unclear. RsaL in *P. aeruginosa* acts by suppressing the expression of the LuxI homologue and thereby delays the induction in experiments, which fits to our results for LexA-like regulators [11, 7]. LitR promotes the expression of LuxR in *V. fischeri*. In accordance to what we predict for GroESL-like regulators, *litR* mutants show delayed expression of Quorum sensing regulated phenotypes (Lupp and Ruby, 2005). However, for both, RsaL and LitR, effects on maximum AHL production and the potential ecological relevance of it have not been determined experimentally yet. In a second step, we combined the deterministic behaviour of a single cell with a stochastic distribution of regulators in a number of cells, allowing for simulations of more realistic populations with some individual variations. Our study indicates that, depending on how a regulator of Quorum sensing systems acts on the molecular level, such a stochastic distribution may have effects on timing of induction and/or strength of induction, due to the non-linearity and the interaction of the single cells via the signalling molecule AHL.

As Quorum sensing regulation has been regarded as a source of synchronous responses of cell populations, the existence of stochastic heterogeneity on Quorum sensing systems of isogenic populations has only recently been recognised ([16]). Underlying mechanisms, as well as ecological effects and potential benefits are far from being understood. Generally, Quorum sensing systems are thought to be prone to fluctuations due to often low numbers of receptors and signals. However, mechanisms to suppress dominance of stochasticity and hence making the system more reliable have been described (e.g. [29, 42]). There are hints that heterogeneity of expression in QS genes and/or QS regulated target genes may be a common phenomenon even in isogenic populations [2, 6]. Stochastic differences between cells play a stronger role if only a few cells are involved in the autoinducer based decision making process, e.g. in extreme if a single cell is induced by highly limited mass transfer in a pore (diffusion sensing) [17]. Our study investigates, how regulators of Quorum sensing can cause heterogeneity in Quorum sensing dynamics.

Such a heterogeneity can be an unavoidable side effect. However, if it causes significant phenotypic differences, it might have an ecological purpose, as it is often interpreted in terms of division of work [6]. The benefit of division of work strongly depends on the environmental conditions. It thus seems probable that stochastic heterogeneity of environment-dependent regulators are involved in the emergence of molecular heterogeneities between cells. Therefore, cells may not just suppress noise in their Quorum sensing systems, but rather control its level or its impact on the Quorum sensing regulation [10].

Our results indicate how stochastic variations

in the concentration of factors regulating Quorum sensing influence inter-cell heterogeneity of Quorum sensing response. Dependent on the mode of action of the regulator respectively the combination of different regulators, both timing and/or strength of the response can vary. Stochastic differences in timing of Quorum sensing induced mobility resulting in a removal of single cells from colonies has been reported for *Pseudomonas putida* ([6]). In other bacteria rather the expression levels of Quorum sensing regulated genes seem to vary ([16]), although the design of the experimental studies often impedes a clear discrimination. In almost all cases both the causes of the heterogeneity and the ecological or evolutionary benefit of heterogeneity are unknown yet. By investigating the potential impact of regulators on heterogeneity, our study aims to shed some light on these questions. The differences of the Quorum sensing response between the cells caused by the regulators, i.e., the strength of heterogeneity, was limited in our simulations. However, they might be larger in the real world, as they depend on the variability of the regulator concentration, and on the degree of coupling between cells. The latter, which is mediated by the Quorum sensing signal, has been predicted to be controlled by the cells dependent on the environmental conditions ([14]). Interestingly, [14] predicted in a mathematical model that fluctuations on the molecular level, which are regulated by environmental factors, cause a switch between all-or-none and graded responses of Quorum sensing systems on a population level. Stochastic heterogeneities between cells can also impact the functionality of Quorum sensing systems, e.g. on the induction threshold on a population level [42]. It is thus highly desirable to get a deeper understanding of sources and outcome of Quorum sensing associated stochastic heterogeneity.

Our analysis focuses on typical AHL based Quorum sensing systems, but also Quorum sensing systems with other architectures exist. The exact net effect of different regulation mechanisms depends on the design of the complete cellular regulation network (see e.g. [3]). As most pathogens and many other bacteria relevant from a human perspective use Quorum sensing to regulate virulence or factors beneficial for human health, the qualitative and quantitative understanding of the underlying mechanisms are critical for the development of adequate treatment strategies. Furthermore, knowledge of the behaviour of such motifs is required in the growing field of synthetic biology (see e.g. [4]). Thus, the qualitative and quantitative impact of regulators in QS systems should be investigated in more depth, both experimentally and theoretically.

VI. Supplementary Information

Figures 11 and 12 allow for the comparison of the qualitative behaviour of the full basic model system with the basic system with quasi-steady state assumption. Please note that the large initial differences are due to the fact that we continued to take our "standard initial values", which are not close to the quasi-steady state and needs some adaptation first.

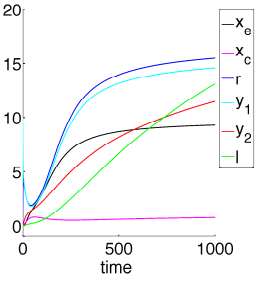


Fig. 11: Simulation of the basic model (Eq.(1)-(6)) with parameters from Table IV, $\alpha_l = 0.001$ and initial conditions $x_e = 10$ and $x_c = r = y_1 = y_2 = l = 0$.

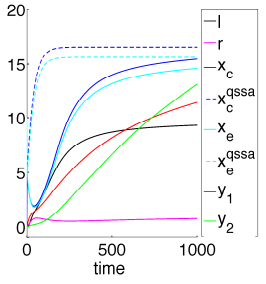


Fig. 12: Simulation of the basic model (Eq.(1)-(6)) with parameters from Table IV, $\alpha_l = 0.001$ and initial conditions $x_e = 10$ and $x_c = r = y_1 = y_2 = l = 0$. The variables x_c^{qssa} and x_e^{qssa} correspond to the basic system with quasi-steady state assumption.

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