Switch-Like Regulation of Signal Transduction by Small RNA-mediated Quorum Sensing

Xi Liu
Department of Mathematics,
Institute of Systems Biology,
Shanghai University,
Shanghai 200444, China
Email: steafy5@163.com

Peipei Zhou
Institute of Systems Biology,
Shanghai University,
Shanghai 200444, China
Email: 513zpp@163.com

Ruiqi Wang
Institute of Systems Biology,
Shanghai University,
Shanghai 200444, China
Email: rqwang@shu.edu.cn
Telephone: (021) 66136130

***Abstract*—Quorum sensing (QS) is a mechanism by which bacteria produce, release, and then detect and respond to biosignals called autoinducers (AIs). There are multiple feedback loops in the QS system of *Vibrio harveyi*. However, how these feedback loops function to control signal processing remains unclear. In this paper, we present a computational model for switch-like regulation of signal transduction by small regulatory RNA-mediated QS based on intertwined network involving AIs, LuxO, LuxU, Qrr sRNAs, and LuxR. In agreement with experimental observations, the model suggests that different feedbacks play critical roles in the switch-like regulation. Our results reveal that *Vibrio harveyi* uses multiple feedbacks to precisely control signal transduction.**

I. Introduction

Cell-to-cell communication in bacteria is a process known as quorum sensing (QS) by which bacteria produce, release, and then detect and respond to biosignals. Often, bacteria use multiple autoinducers and feedback loops to obtain information and orchestrate collective behaviors. QS systems are widespread in the bacterial world and can be used to control such diverse functions as bioluminescence, virulence-factor secretion, biofilm formations, conjugation, and antibiotic production.

The wild-type bioluminescent marine bacterium *Vibrio harveyi*(*V. harveyi*) QS circuit consists of three parallel signaling pathways with three different autoinducers: AI-1, CAI-1, and AI-2. Their synthases are LuxM, CqsA, LuxS, and their transmembrane receptors are LuxN, CqsS, LuxPQ, respectively. In the absence of autoinducers (i.e. at low cell density), the receptors act predominantly as kinases and pass phosphate (designated as -P to LuxU and thence to LuxO. Phosphorylated LuxO activates transcription of genes encoding five small regulatory RNAs (sRNAs). These sRNAs inhibit the translation of LuxR. In the presence of autoinducers (i.e. at high cell density), the receptors switch to a predominantly phosphatase-active state which reverses the direction of phosphoryl transfer through the circuit, so that LuxO is dephosphorylated and becomes inactive. Therefore, the genes encoding the five sRNAs are not transcribed, luxR mRNA is translated, and LuxR protein is made.

The report by Teng *et al.* brought new insights into possible roles of sRNAs in modulating quorum-sensing, revealing that the interplay between different types of RNAs and proteins can exert precise control of QS [1]. However, the mechanism of the sRNA-mediated regulation has not been well characterized. The relationship between five sRNAs, Qrr1-5, and the QS is partially experimentally known [1] and there has no well-developed computational models on the sRNA-mediated QS, making development of a computational model involving the sRNA-mediated QS regulation important. Here we develop a model by incorporating multiple sRNA-mediated feedback loops into the QS regulation. To understand their post-transcriptional roles in regulating the QS regulation, we carry out a detailed study of the model and show that our results are in consistence with the experimental observations on the roles of the sRNAs in regulating QS regulation. Therefore, the model captures the main features of the sRNA-mediated QS regulation. Moreover, some interesting predictions are made.

II. Material and Methods

A. The sRNA-mediated quorum-sensing network

The bacterial QS systems have been extensively studied for many years and some computational models have been presented. The QS network in *V. harveyi* requires the activity of several components: autoinducers AI-1, CAI-1, and AI-2, their synthases LuxM, CqsA, and LuxS, and their transmembrane receptors LuxN, CqsS, and LuxPQ, and multiple sRNA-mediated feedback loops, as shown in Figure 1. At the molecular level, the bacterial QS is based on integration of different autoinducers and multiple sRNA-mediated feedback loops so as to permit fine-tuning of QS response.

B. Experimental observations

The experimental observations by Teng *et al.* brought new insights into possible roles of sRNA-mediated feedback loops in modulation of the QS response [1]. After a series of experiments, they found that there are multiple feedback loops in the *V. harveyi* QS system, i.e., LuxO autorepresses its own transcription, the Qrr sRNAs repress *luxO* translation, LuxR autorepresses its own transcription, LuxR activates expression of the *qrr*2-4 genes, which in turn, repress *luxR* translation, and the *luxMN* operon, encoding the AI-1 synthase and receptor, is repressed by the Qrr sRNAs. It has been found that disruption

2012 IEEE 6th International Conference on Systems Biology (ISB)
978-1-4673-4398-5/12/$31.00 ©2012 IEEE

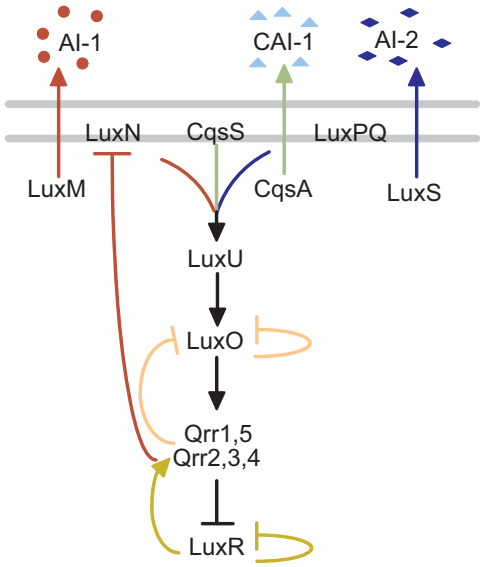

Fig. 1. The V. harveyi quorum-sensing network. The network includes five feedback loops to integrate the three AI signals to control the master quorum-sensing regulator, LuxR.

of LuxO loop or sRNA loop or both can increase LuxO-P levels. In addition, these feedback loops also affect the timing of quorum-sensing target gene expression. There have been extensive experimental studies of quorum-sensing response, but the operating mechanisms and potential implications of the multiple feedback loops are less clear and need to be further investigated.

C. Incorporation of the sRNA-mediated regulation

These experimental findings indicate that sRNAs may play crucial roles in modulating the quorum-sensing response. The interactions between sRNAs and their regulated targets have been modeled in several recent studies which shed light on how target protein expression is controlled by sRNA-mediated regulation [2]–[6]. Some computational models for the quorum-sensing response in *V. harveyi* nbased on negative feedback loops involving sRNAs and the master regulator protein, LuxR [7] have been presented. These models capture the main features of the quorum-sensing system, e.g., oscillation dynamics. However, these models focus mainly on the interaction of sRNAs and their downstream genes. Here we develop a computational model by considering the interaction of sRNAS and their upstream genes into SHEN's model [7] according to the experimental findings [8]. Incorporating sRNAs into other models can be similarly discussed.

The receptors in the QS response can be modeled as two-state systems [9], [10]. We consider a further simplification which takes the receptors to be existing either in the kinase mode, K_N , or in the phosphatase mode, P_N. In the kinase mode, the receptors can autophosphorylate and then transfer the phosphate through LuxU to LuxO, whereas in the phosphatase mode the phosphate flow is reversed. Experiments indicate that at low cell density the receptors are primarily in the kinase mode, whereas at high cell density, the receptors are primarily in the phosphatase mode. Correspondingly, we consider a simplified model wherein the free receptors correspond to the kinase mode, whereas binding of autoinducers result in a transition to the phosphatase mode.

For the case of autoinducers binding to their receptors, we have the kinetic scheme

$$K_N + nAI \underset{k_{-i}}{\overset{k_i}{\rightleftharpoons}} P_N \tag{1}$$

from which the mean steady state concentrations of the receptors in either the kinase or phosphatase mode can be obtained. More generally, to account for cooperative effects in binding, we take the kinase/phosphatase fractions to be

$$[K_N] = (1 - g_i)[S_T] \quad \text{and} \quad [P_N] = g_i[S_T], \tag{2}$$

where

$$[K_N] + [P_N] = [S_T] = \text{const}, \tag{3}$$

$$g_i = \frac{a_i}{(1 + a_i)}, \quad a_i = [AI]^n/\kappa_i, \quad \kappa_i = k_{-i}/k_i. \tag{4}$$

Typically in bacterial signal transduction, the receptors in the kinase/phosphase modes serve as enzymes which transfer the phosphate to/from a response regulator protein or a phosphorelay protein [12]–[15]. In *V. harveyi*, this step involves phosphotransfer to the phosphorelay protein LuxU (U). Phosphorylated LuxU (U_P) can then transfer the phosphate to the downstream regulator LuxO (O); similarly, unphosphorylated LuxU serves as a receiver for removing the phosphate group from phosphorylated LuxO (O_P). We represent these processes by the following reactions:

$$K_N + U \xrightarrow{k'_n} U_P, \tag{5}$$

$$P_N + U_P \xrightarrow{p'_n} U, \tag{6}$$

$$U_P + O \xrightarrow{k_+} O_P + U, \tag{7}$$

$$O_P + U \xrightarrow{k_-} U_P + O. \tag{8}$$

In the above reactions, *AI* represents autoinducer, U represents LuxU, U_P represents phosphorylated LuxU, O represents LuxO and O_P represents phosphorylated LuxO, k_i, k'_n, p'_n, k_+ represent the reaction rate and k_{-i}, k_- represent the dissociation constant.

By using the Mass Action Law and Michaelis-Menten

978-1-4673-4398-5/12/$31.00 ©2012 IEEE

Kinetics, we can obtain the mathematical model as follows

$$\frac{dM_O}{dt} = v_{so} - v_{dmo}\frac{M_O}{K_{dmo}+M_O}, \quad (9)$$

$$\frac{dO}{dt} = k_{so}M_O - k_+U_P\frac{O}{K_1+O} + k_-U\frac{O_P}{K_2+O_P} - v_{do}\frac{O}{K_{do}+O}, \quad (10)$$

$$\frac{dO_P}{dt} = -k_-U\frac{O_P}{K_2+O_P} + k_+U_P\frac{O}{K_1+O} - v_{dop}\frac{O_P}{K_{dop}+O_P}, \quad (11)$$

$$\frac{dU_P}{dt} = k'_nK_N\frac{U}{K_3+U} - p'_nP_N\frac{U_P}{K_4+U_P} - k_+U_P\frac{O}{K_1+O} + k_-U\frac{O_P}{K_2+O_P} - v_{dup}\frac{U_P}{K_{dup}+U_P}. \quad (12)$$

where M_O denotes *luxO* mRNA, v_{so} denotes the production rate of *luxO* mRNA, k_{so} denotes the production rate of LuxO protein, v_{dmo}, v_{do}, v_{dop}, v_{dup} denote the degration rates. The degradation rates of all the components and the phosphorylation–dephosphorylation processes are assumed to obey the Michaelis–Menten (MM) kinetics. The translation from *luxO* mRNA to the LuxO protein in the cytoplasm is assumed to be linear. The conservation law for LuxU protein is

$$U + U_P = U_T = \text{const.} \quad (13)$$

To understand the post-transcriptional roles of sRNAs in regulating the *V. harveyi* quorum-sensing response, we now incorporate the sRNA-mediated regulation into the SHEN's model according to the regulation mechanisms revealed by experimental observations. The experiments suggested that the *V. harveyi* Qrr sRNAs repress *luxO* translation and that LuxO represses its own transcription independent of its phosphorylation state [8]. Based on these regulation mechanisms, when the Michaelis-Menten kinetics is assumed for the transcriptional rates, the new equation about *luxO* mRNA can be written as

$$\frac{dM_O}{dt} = v_{so}\frac{K_O^m}{K_O^m + K_7O^m + \alpha M_Q^n} - v_{dmo}\frac{M_O}{K_{dmo}+M_O} \quad (14)$$

where K_7 and α denote the efficiency of LuxO and sRNA inhibition of protein expression, respectively. This equation means that the production of *luxO* mRNA is negatively regulated by both LuxO and Qrr sRNAs. In addition, the kinetic equations for the sRNA and LuxR are

$$\frac{dM_Q}{dt} = v_{sq} + v_o\frac{O_P}{K_5+O_P} + v_r\frac{R}{K_6+R} - v_{dmq}\frac{M_Q}{K_{dmq}+M_Q} - \gamma M_QM_R, \quad (15)$$

$$\frac{dM_R}{dt} = v_{sr}\frac{K_R^m}{K_R^m+K_8R^m} - v_{dmr}\frac{M_R}{K_{dmr}+M_R} - \gamma M_QM_R, \quad (16)$$

$$\frac{dR}{dt} = k_{sr}M_R - v_{dr}\frac{R}{K_{dr}+R}. \quad (17)$$

where M_Q, M_R, R denote *qrr* mRNA, *luxR* mRNA and LuxR protein respectively, v_{sq}, v_{sr}, k_{sr} denote the basal rates of transcription in the absence of transcription. v_o and v_r are the production rate of Qrr sRNAs under the function of LuxO-P and LuxR respectively. v_{dmq}, v_{dmr}, v_{dr} represent the degradation rate. The degradation rate of Qrr sRNA and the LuxO activation of Qrr sRNA are assumed to obey the Michaelis–Menten (MM) kinetics. The sRNAs base pair with the target *luxR* mRNA at a rate γ.

III. RESULTS

In this section we first list the character caused by the phosphorylation/dephosphorylation process, then we report the comparison between the results of experimental luminescence curves presented in Tu's paper and those provided by our system in order to verify that our model is able to replicate the system qualitative behavior. The model is subsequently applied to study the dynamics of the QS system in *V. harveyi*.

A. Phosphorylation/dephosphorylation induced switch-like regulation

The phosphorylation/dephosphorylation process can induce switch-like regulation, as shown in Fig. 2. At negligible concentration of AIs, i.e., at low cell density (LCD), the sensors act as kinases that transfer phosphate through LuxU to LuxO. LuxO-P activates the expression of genes encoding five highly conserved small regulatory RNAs (sRNAs) called Qrr1-5. The Qrrs pair with the 5' UTR of the *luxR* mRNA and destabilize it, a process that requires the RNA chaperone Hfq. LuxR is the master transcriptional regulator of QS genes in *V. harveyi*. Thus, at LCD, when little LuxR is present, there is no QS and V. harveyi cells act as individuals. At high cell density (HCD), AIs accumulate and bind to their cognate sensors. This event causes the sensors to act as phosphatases, leading to dephosphorylation of LuxO. Unphosphorylated LuxO is inactive. Transcription of the sRNA-encoding genes is terminated, causing *luxR* mRNA to accumulate. Newly produced LuxR protein activates and represses numerous genes. Thus, at HCD, QS is initiated and *V. harveyi* cells act as a group. Solving these equations graphically (Fig. 2), we can easily find that higher n values correspond to sharper switching from kinase to phosphatase mode which mimics cooperative effects in binding. Take the parameter values as follows (see Table1).

978-1-4673-4398-5/12/$31.00 ©2012 IEEE

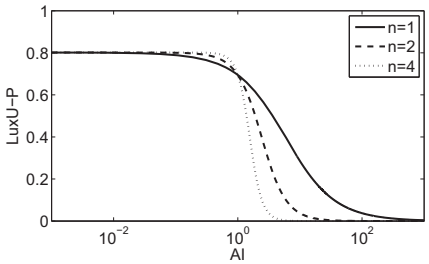

Fig. 2. Dose-response curves for AI.

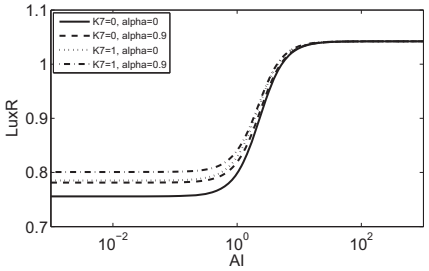

Fig. 3. Dose-response curves of luxR for AI in four cases.

TABLE I
THE VALUE OF THE PARAMETERS

Parameter	Basal value	Parameter	Basal value
St(nM)	1.0	Ut(nM)	1.0
$k_i(h^{-1})$	0.6	$k_{-i}(h^{-1})$	1.0
$k'_n(h^{-1})$	1.0	$p'_n(h^{-1})$	0.1
$k_+(h^{-1})$	3.2	$k_-(h^{-1})$	1.58
$K_1(nM)$	2	$K_2(nM)$	2
$K_3(nM)$	2	$K_4(nM)$	2
$K_5(nM)$	1	$K_6(nM)$	1
$K_7(nM)$	1	$K_8(nM)$	1
$\gamma(nM^{-1}\cdot h^{-1})$	0.2	$\alpha(nM)$	0.9
$v_{so}(nM\cdot h^{-1})$	0.2	$v_{dmo}(nM\cdot h^{-1})$	0.65
$v_{do}(nM\cdot h^{-1})$	0.65	$k_{so}(h^{-1})$	0.18
$v_{dop}(nM\cdot h^{-1})$	0.12	$v_{dup}(nM\cdot h^{-1})$	0.12
$K_{dmo}(nM)$	2	$K_{do}(nM)$	2
$K_{dop}(nM)$	2	$K_{dop}(nM)$	2
$K_O(nM)$	1	$K_R(nM)$	2
$v_{sq}(nM\cdot h^{-1})$	0.01	$v_{dmq}(nM\cdot h^{-1})$	0.65
$v_o(nM\cdot h^{-1})$	0.1	$v_r(nM\cdot h^{-1})$	0.1
$v_{sr}(nM\cdot h^{-1})$	0.1	$v_{dmr}(nM\cdot h^{-1})$	0.32
$k_{sr}(h^{-1})$	0.38	$v_{dr}(nM\cdot h^{-1})$	0.2
$K_{dmq}(nM)$	1	$K_{dmr}(nM)$	2
$K_{dr}(nM)$	0.2		

B. The feedback loops make V. harveyi more sensitive to AIs

Experiments in Tu's paper show that the feedback loops involving LuxO affect the timing of QS target gene expression. To test and verify our model, we run our model by using the suitable parameter set that is based on experimental data or extracted from scientific literature and compare with the results of experimental luminescence curves presented in Tu's paper. Since we are interested in the effect of feedback loops on AIs, we obtain dose-response of LuxR curves in four cases: $K_7 = 0$, $\alpha = 0$; $K_7 = 0$, $\alpha = 0.9$; $K_7 = 1$, $\alpha = 0$; $K_7 = 1$, $\alpha = 0.9$ to simulate wild-type, LuxO Loop-, sRNA Loop-, and Double Loop- strains. Similarly the EC_{80} values (the concentration of AIs at the point of eighty percent of the maximal LuxR) for the wild-type, LuxO Loop-, sRNA Loop-, and Double Loop- strains are , 0.90, 1.06, 1.23, 1.44 nM, respectively (Figure 3). Thus, in the absence of either the LuxO or sRNA feedback loop, *V. harveyi* becomes less sensitive to AIs, whereas in the absence of both feedback loops, *V. harveyi* becomes the least sensitive to AIs. Hence we can conclude that the feedback loops make *V. harveyi* more sensitive to AIs, which is in consistent with the experimental findings in Tu's paper.

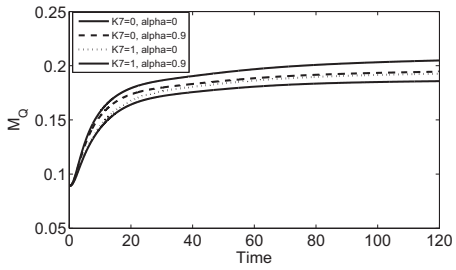

Fig. 4. Qrr sRNA level during the HCD-to-LCD Transition.

C. The feedback loops have little affect on the kinetics of the HCD-to-LCD transition

In the foregoing subsection we show that our model is able to replicate some qualitative behavior when compared with the results presented in Tu's paper, here we verify the model again to assure its applicability in other aspects.

Previous experimental studies have demonstrated that the feedback loops involving LuxO do not affect the kinetics of the HCD-to-LCD transition [8]. So we run our model to observe the change of *qrr* mRNA concentration and obtain the kinetics of the HCD-to-LCD transition. We use the equilibrium point at saturate AI concentration as the initial points, and subsequently simulate the variation of cellular sRNA levels over time when there are no AIs (Figure 4). We find that *qrr* levels increasing to maximal values within short time in all four *V. harveyi* cases. There do not appear to be significant differences in the rates at which Qrr sRNAs increase or the maximun values reached in the feedback loop mutants, suggesting that the feedback loops have little affect on the kinetics of the HCD-to-LCD transition, which also have qualitative behavior when compared with the results presented in Tu's paper.

IV. DISCUSSION

Bacteria often use a cell-to-cell communication process called quorum sensing (QS) to regulate biological behaviors in response to changes in the cell-population density and species-composition of the surrounding microbial community. The *V. harveyi* QS circuit induces switch-like regulation by small

978-1-4673-4398-5/12/$31.00 ©2012 IEEE

non-coding RNAs (sRNAs) and uses multiple feedbacks to precisely control signal transduction.

In this paper, we present a computational model for the *V. harveyi* QS system with incorporation of two feedback loops involving LuxO into a previous studied model. According to the analogous effect of *luxO* mRNA affected by LuxO Loop- and sRNA Loop-, we set the repression coefficient to be comparable. In agreement with experimental observations, the model indicates that LuxO represses its own transcription, whereas the feedback loops involving LuxO make *V. harveyi* more sensitive to AIs and have little affect on the kinetics of the HCD-to-LCD transition. The potential mechanism by which sRNAs exert their effects on affecting the timing of QS target gene expression is that sRNAs repress *luxO* translation so as to shorten the time to reach a quorum. We also find that the model can give rise to switch-like behavior by small RNAs and the more AIs, the sharper switching happens. There are so many experiments studied about the *V. harveyi* QS system, nevertheless lacking well-developed computational models on the sRNA- mediated quorum-sensing, this makes development of a computational model involving the sRNA-mediated quorum-sensing regulation very important. So we develop a model by incorporating multiple sRNA-mediated feedback loops into the quorum-sensing regulation.

There are several factors with regard to the *V. harveyi* QS system which are not taken into account in our model. Firstly, experiments show that LuxR directly binds to the promoters of *qrr2*, *qrr3* and *qrr4* but not to those of *qrr1* or *qrr5* [17], yet we establish the model by assuming that LuxR, activates Qrr1-5 and use Qrrs to replace them. Secondly, we integrate CAI-1, HAI-1 and AI-2 to one form AIs. Thirdly, we ignore the effort of Hfq and σ^{54} in the network. In the end, we leave out of the consideration of stochastic noises on some mRNAs. The complexity and nonlinearity of biological networks may make our model not quite comprehensive, and have some disunity with experimental results. But our work still capture the main characteristics of the *V. harveyi* QS system. So we believe that our work is instructive and meaningful to the study of *V. harveyi* QS system and some other relative systems.

ACKNOWLEDGMENT

This research is supported by the National Natural Science Foundation of China (Grants No. 11171206 and 10832006), Innovation Program of Shanghai Municipal Education Commission (No. 12YZ030).

REFERENCES

[1] S.W. Teng, J.N. Schaffer, K.C. Tu, P. Mehta, W. Lu, N.P. Ong, B.L. Bassler, N.S. Wingreen. Active regulation of receptor ratios controls integration of quorum-sensing signals in Vibrio harveyi, *Mol. Syst. Biol.* 7: 491, 2011.

[2] D.H. Lenz, K.C. Mok, B.N. Lilley, R.V. Kulkarni, N.S. Wingreen, B.L. Bassler. The small RNA chaperone Hfq and multiple small RNAs control quorum sensing in Vibrio harveyi and Vibrio cholerae, *Cell*, 118: 69-82, 2004.

[3] E. Levine, T. Hwa. Small RNAs establish gene expression thresholds, *Curr. Opin. Microbiol*, 11: 574-579, 2008.

[4] E. Levine, Z. Zhang, T. Kuhlman, T. Hwa. Quantitative characteristics of gene regulation by small RNA, *PLoS Biol.* 5: e229, 2007.

[5] P. Mehta, S. Goyal, N.S. Wingreen. A quantitative comparison of sRNA-based and protein-based gene regulation, *Mol. Syst. Biol.* 4: 221, 2008.

[6] N. Mitarai, A.M. Andersson, S. Krishna, S. Semsey, K. Sneppen. Efficient degradation and expression prioritization with small RNAs, *Phys. Biol.* 164, 2007.

[7] J.W. SHEN. Dynamics and Mechanism of A Quorum Sensing Network Regulated by SmallRNAs in $VibrioHarveyi$, *Commun. Theor. Phys.*, 55: 465-472, 2010.

[8] K.C. Tu, T. Long, S.L. Svenningsen, N.S. Wingreen, B.L. Bassler. Negative feedback loops involving small regulatory RNAs precisely control the Vibrio harveyi quorum-sensing response, *Mol. Cell*, 37: 567-579, 2010.

[9] M.B. Neiditch, M.J. Federle, A.J.Pompeani, R.C. Kelly, D.L. Swem, P.D. Jeffrey, B.L. Bassler, F.M. Hughson. Ligand-induced asymmetry in histidine sensor kinase complex regulates quorum sensing, *Cell*, 126: 1095-1108, 2006.

[10] L.R. Swem, D.L. Swem, N.S. Wingreen, B.L. Bassler. Deducing receptor signaling parameters from in vivo analysis: LuxN/AI-1 quorum sensing in Vibrio harveyi, *Cell*, 134: 461-473, 2008.

[11] T. Long, K.C. Tu, Y. Wang, P. Mehta, N.P. Ong, B.L. Bassler, N.S. Wingreen. Quantifying the integration of quorum-sensing signals with single-cell resolution, *PLoS Biol.* 7: e68, 2009.

[12] J.L. Appleby, J.S. Parkinson, R.B. Bourret. Signal transduction via the multi-step phosphorelay: not necessarily a road less traveled, *Cell*, 86: 845-848, 1996.

[13] J.A. Hoch. Two-component and phosphorelay signal transduction, *Curr. Opin. Microbiol.*, 3: 165-170, 2000.

[14] A.M. Stock , V.L. Robinson, P.N. Goudreau. Two-component signal transduction, *Annu. Rev. Biochem.*, 69: 183-215, 2000.

[15] M.T. Laub and M. Goulian. Specificity in two-component signal transduction pathways, *Annu. Rev. Genet.*, 41: 121-145, 2007.

[16] S.L. Bumgarner, R.D. Dowell, P. Grisafi, D.K. Gifford, G.R. Fink. Toggle involving cis-interfering noncoding RNAs controls variegated gene expression in yeast, *Proc. Natl. Acad. Sci.*, 106: 18321-18326, 2009.

[17] K.C. Tu, C.M. Waters, S.L. Svenningsen, B.L. Bassler. A small-RNA-mediated negative feedback loop controls quorum-sensing dynamics in the Vibrio harveyi, *Mol. Microbio.*, 70: 896-907, 2008.