Hierarchical Modeling and Local Stability Analysis for Repressilators Coupled by Quorum Sensing

Tomohiko Nakamura *, Yutaka Hori *, and Shinji Hara *

Abstract: This paper proposes a hierarchical system representation of large-scale multi-cellular networks coupled by the quorum-sensing mechanism and present a control theoretic approach to systematically analyzing the local stability and instability of an equilibrium state. In particular, we are concerned with the dynamics of coupled Repressilators and analytically derive conditions for the local instability of a homogeneous equilibrium. It is first shown that the dynamics of the quorum-sensing network can be formulated as a dynamical multi-agent system with a hierarchical structure having low rank interconnections. This structural property then allows us to decompose the high-order dynamics of the coupled Repressilator network into two low-order systems. Thus, the proposed approach significantly reduces the complexity of the local stability analysis and leads to the analytic necessary and sufficient local stability conditions of a homogeneous equilibrium point. The analytic conditions reveal the reaction parameters that essentially affect the existence of oscillatory dynamics in the coupled Repressilator network.

Key Words: quorum sensing, gene regulatory network, large-scale systems, stability analysis.

1. Introduction

In living cells, protein concentrations exhibit complex dynamical behaviors ranging from convergence to oscillations. Recent progress in biology has revealed that cell-to-cell communication plays an important role for the emergence of such behaviors. Thus, there is a strong demand for the systematic analysis of cell-to-cell communication networks to understand physiological processes in biological organisms. Moreover, the development of such systematic analysis is worthwhile from an engineering point of view, since it can be useful for applications such as biosensing and electronic circuit design [1],[2]. In this paper, our focus is particularly on convergent and oscillatory dynamical behavior of chemical concentrations in cell-to-cell communication.

Quorum sensing is one of cell-to-cell communication mechanisms for bacteria, and it enables bacteria to sense and respond to their surrounding environment [3]. The mechanism of quorum sensing is explained by the diffusion of signaling molecules called auto inducers (AIs). AIs are small molecules that can diffuse through cell membranes, whereas messenger RNAs (mRNAs) and proteins produced in cells cannot due to their size. Thus, AIs convey the information of internal states of a cell to others.

The use of mathematics helps us obtain qualitative insights and reveal the essence of complex biological processes as proven in recent studies in systems biology [4]–[7]. However, theoretical analysis of the cell-to-cell communication networks, or quorum-sensing networks, can suffer from the difficulty that the size of the system is very large, since many cells interact each other by quorum sensing. Thus, it is not an easy task to find essential parameters that characterize the dynamics of the quorum-sensing networks.

In simulation approach, Garcia-Ojalvo et al. [8] studied the oscillations of quorum-sensing networks with multi-cell environment, and Brown [9] investigated the effect of quorum sensing on an isolated bacterium. A similar system was also studied in mathematical approach using control theoretic frameworks [4],[5]. Their results, however, were restricted uni-cellular systems, so they cannot be directly applied to the multi-cellular systems of our interests. Arcak [10],[11] and Russo et al. [6] studied conditions for spatially uniform and non-uniform global behavior in multi-cellular environment. However, local stability analysis, which leads to an oscillation condition, remains open to explore. Regarding oscillation conditions, Hori et al. [7] recently developed a control theoretic approach and derived analytic conditions for the existence of oscillations in cyclic gene regulatory networks (CGRNWs) and revealed the essential parameters that characterize the oscillatory dynamics in a cell. This motivates us to analytically explore the dynamics of quorum-sensing networks using a control theoretic framework.

The objectives of this paper are twofold. (i) This paper proposes a novel control theoretic framework to systematically deal with large-scale multi-cellular networks, and (ii) qualitative insights of oscillatory dynamical behaviors in such systems are provided. A key idea to the systematic analysis is to utilize a characteristic hierarchical structure of the dynamics of quorum-sensing network and to formulate it as a multi-agent dynamical system with low-rank hierarchical interconnections. This formulation allows us to show that the local stability analysis of the overall system reduces to that of small subsystems. As a result, we can analytically derive local instability conditions and obtain three essential parameters that characterize the existence of oscillations in the quorum-sensing network.

Some theorems and a corollary in this paper were previously presented in the authors' conference paper [12]. This paper ex-
tends the results by adding the complete proofs of the theorems and more illustrative examples. In this paper, we use the following notations. $\mathbb{R}_+ := \{x \in \mathbb{R} | x \geq 0\}$, $I_N$ is a $N \times N$ identity matrix, and $I_N := [1, 1, \ldots, 1]^T$ with the size $N$.

2. Model of Quorum-Sensing Networks

2.1 Quorum-Sensing Networks

Repressilator was originally implemented in [13] as a gene regulatory network consisting of three genes as shown in Fig. 1, where the products of these genes inhibit transcription in a cyclic way. This gene regulatory network is known to exhibit periodic oscillations under certain parameter conditions [7],[13],[14]. Recently, Garcia-Ojalvo et al. [8] proposed Repressilator networks coupled by intercellular signaling mechanisms as an example of network having cell-to-cell communication (see Fig. 1).

The intercellular signaling mechanism is implemented with a small molecule named AI. In each cell, AI is synthesized from LuxI, of which the production is repressed by lacI protein produced in the repressor network (see Fig. 1). Then, AIs can diffuse through cell membrane to other cells. In cells, the AIs bind to the AI receptor protein, LuxR, and the resulting complex, LuxR-AI, activates the production of lacI as depicted in Fig. 1. Thereby, the repressor networks are coupled via the diffusive AI molecules.

The overall topology of quorum-sensing network is so complicated that mathematical analysis of the network is not an easy task. Nevertheless, we hereafter present rigorous stability analysis of the system using a characteristic topology of the network. Specifically, we point out that the overall network has a hierarchical structure consisting of two layers as depicted in Fig. 1. The cells communicate with all-to-all coupling topology in the upper layer, while the genes interact in a cyclic way in the lower layer. This topological feature of the system allows a systematic way of analyzing the large-scale complex network.

2.2 Mathematical Model

The dynamics of the quorum-sensing network consisting of $N$ cells can be written as

\[
\begin{aligned}
    \dot{r}_i(t) &= -\delta_j r_i(t) + \alpha_j f(p_{i,j-1}) + k_f S_i(t), \\
    \dot{p}_{i,j}(t) &= \gamma_j r_i(t) - \beta_j p_{i,j}(t), \\
    \dot{S}_i(t) &= -k_{i,0} S_i(t) + k_{i,1} p_{i,1}(t) - \eta(S_i(t) - S_e(t)), \\
    \dot{S}_e(t) &= -k_{i,e} S_e(t) + \eta_{i,e} \sum_{i} (S_i(t) - S_e(t)),
\end{aligned}
\]

where $i = 1, 2, \ldots, N$ and $j = 1, 2, 3$ [8]. The variables $r_i(t) \in \mathbb{R}_+$ and $p_{i,j}(t) \in \mathbb{R}$ respectively denote the concentrations of the mRNA and protein synthesized from the $j$-th gene ($j = 1, 2, 3$) inside the $i$-th cell ($i = 1, 2, \ldots, N$). Specifically, we define $r_{1,1}(t), r_{1,2}(t),$ and $r_{1,3}(t)$ as the concentrations of tetR, cl, and lacI mRNAs. The concentrations of the AI inside the $i$-th cell are denoted by $S_i \in \mathbb{R}_+$, and that of the AIs outside the cells is defined by $S_e \in \mathbb{R}_+$.

We define $p_{i,0}(t) := p_{i,3}(t)$ and $r_{i,0}(t) := r_{i,3}(t)$ throughout this paper for the sake of notational simplicity. Note that (1) represents the dynamics of the repressilators, which are affected by the AI concentrations $S_i$, and that (2) captures the dynamics of the AI concentrations.

The positive constants $\delta_j, \beta_j, \alpha_j, \gamma_j, \kappa, k_{i,0}, k_{i,1}, k_{i,e}, \eta$ and $\eta_{i,e}$ denote the rates of the corresponding reactions, and the definitions are summarized in Table 1. The protein concentrations and parameters are normalized by the Michaelis-Menten constants of the corresponding promoter-polymerase binding, respectively, which are the concentrations at a half maximal repression. The AI concentration is also scaled by its Michaelis-Menten state that is particularly important for understanding the degree of cooperative binding.

3. Local Stability Analysis of the Quorum-Sensing Networks

3.1 Local Stability Condition

In what follows, we analyze the local stability of an equilibrium state that is particularly important for understanding the
predict the existence of oscillations for the initial values of our interest. (see Remark 1 in Sec. 3.2.)

For the local stability analysis, we derive the linearized system around the equilibrium. Let

\[
\begin{align*}
\hat{r}_{ij}(t) := r_{ij}(t) - r^*_r, \\
\hat{p}_{ij}(t) := p_{ij}(t) - p^*_p,
\end{align*}
\]

\[
\hat{S}_i(t) := S_i(t) - S^*_S, \quad \hat{S}_j(t) := S_j(t) - S^*_S,
\]

for \(i = 1, 2, \ldots, N \) and \( j = 1, 2, 3 \). Under Assumption 1, the linearized system is expressed as

\[
\frac{d}{dt} \begin{bmatrix}
\hat{r}_{ij}(t) \\
\hat{p}_{ij}(t) \\
\hat{S}_i(t) \\
\hat{S}_j(t)
\end{bmatrix} = \begin{bmatrix}
-\delta_j & 0 & 0 & 0 \\
-\beta_j & -\eta_j & 0 & 0 \\
0 & 0 & -\eta_{\alpha S} & 0 \\
0 & 0 & 0 & -\eta_{\alpha S}
\end{bmatrix} \begin{bmatrix}
\hat{r}_{ij}(t) \\
\hat{p}_{ij}(t) \\
\hat{S}_i(t) \\
\hat{S}_j(t)
\end{bmatrix} + \begin{bmatrix}
k e \\
0
\end{bmatrix} \hat{u}_{ij}(t),
\]

(7)

\[
\frac{d\hat{S}_i(t)}{dt} = -(k_{\alpha S} + \eta_j)\hat{S}_i(t) + k_{\alpha S}\hat{p}_{1i}(t) + \hat{v}_i(t),
\]

(8)

\[
\frac{d\hat{S}_j(t)}{dt} = -k_{\alpha S}\hat{S}_j(t) + \eta_{\alpha S}\sum_{i=1}^{N} (\hat{S}_i(t) - \hat{S}_j(t)),
\]

(9)

where \(\hat{u}_{ij}(t)\), \(\hat{v}_i(t)\), \(\xi_j\), \(\eta_j\), \(\hat{S}_i(t)\) and \(\hat{p}_{ij}(t)\) for \(j = 1, 2, 3\) are defined as

\[
\begin{align*}
\hat{u}_{ij}(t) &:= \begin{bmatrix} 0 \\ S_i(t) \\ S_j(t) \end{bmatrix} \quad (j = 1, 2), \\
&:= \begin{bmatrix} 0 \\ S_i(t) \\ S_j(t) \end{bmatrix} \quad (j = 3), \\
\hat{v}_i(t) &:= \eta_S\hat{S}_i(t),
\end{align*}
\]

(10)

\[
\begin{align*}
\xi_j &:= \frac{df(p^*_p)}{dp}, \\
\eta_j &:= \frac{dg_S(S^*_S)}{dS}, \\
\hat{S}(t) &:= [\hat{S}_1(t), \hat{S}_2(t), \ldots, \hat{S}_N(t)]^\top,
\end{align*}
\]

(11)

\[
\hat{p}(t) := [\hat{p}_{11}(t), \hat{p}_{21}(t), \ldots, \hat{p}_{N1}(t)]^\top.
\]

(13)

Equation (7) describes the linearized dynamics of mRNA and protein concentrations in CGRNWs in each cell. The input and the output of this system are AI concentration \(\hat{u}_{ij}(t)\) and \(\hat{p}_{1i}(t)\), respectively. We can regard the CGRWN in each cell as a subsystem, and all the subsystems are connected by AI, whose dynamics is described by (8) and (9). We see that the linearized system as the hierarchical system with two layers. The upper layer describes the cell-to-cell communication mediated by the AI concentration, where the systems with the input \(\hat{p}_{1i}(t)\) and the output \(\hat{S}_i(t)\) are connected with equal weight. The lower layer consists of gene regulatory networks, where mRNA and protein molecules are main constituents.

The block diagram of the linearized system is depicted in Fig. 2. The transfer function \(H(s)\) from \(\hat{S}(t)\) to \(\hat{p}_{1i}(t)\) is obtained as

\[
H(s) = \frac{k}{\alpha_3} \frac{R_2^2}{\prod_{j=1}^{3}(T_{a2s} + 1)(T_{b2s} + 1) - \prod_{j=1}^{3} R_{j2}^2 s},
\]

(14)

where \(T_{a2j}\), \(T_{b2j}\), and \(R_{lj}\) for \(j = 1, 2, 3\) are defined as

\[
T_{a2j} := 1/\delta_{jj}, \quad T_{b2j} := 1/\beta_j, \quad R_{lj} := \sqrt{\frac{\alpha_{lj}\gamma_j}{\delta_l\beta_j}}.
\]

(15)

Note that \(R_{lj}\) represents the geometric mean of the ratio of production and degradation rates.

The block \(H(s)\) in Fig. 2 represents the CGRNWs of all cells. The other blocks in Fig. 2 describe the dynamics of a quorum-sensing network whose input is the protein concentrations \(\hat{p}_{ij}(t)\) and output is the AI concentrations \(\hat{S}(t)\).
posed into two small systems shown in Fig. 3 by diagonalizing
λ
are Hurwitz polynomials, where

\[ N_H(s) := \frac{k}{\alpha_3} R^2 \xi_T (T_{\alpha_2} s + 1) (T_{\alpha_2} s + 1), \]  
\[ D_H(s) := \prod_{j=1}^3 (T_{\beta_j} s + 1) (T_{\beta_j} s + 1) - \prod_{j=1}^3 R^2 \xi_{j}, \]  

Given the parameters, we can easily verify the local stability using this theorem. In particular, the orders of (16) and (17) are independent of the number of cells (N), which means that this result is applicable to a broad class of systems with a large number of cells. It is worth noting that (16) and (17) rely not on N but on \( \eta_{ext} N \) which reflects the density of cells. Thus, the local stability is not determined by the number of cells, but by cell density. This result is consistent with prior works (see [3] and the references therein).

### 3.2 Numerical Simulation

In order to illustrate our result, we provide two examples that satisfy and dissatisfy the condition in Theorem 1. Let the quorum-sensing network composed of \( N = 3 \) cells. Suppose \( \delta_j = 1, \alpha_j = 216, \beta_1 = 1.0, \beta_2 = 0.2, \beta_3 = 0.4, \gamma_j = \beta_j, (j = 1, 2, 3), \eta = 2.0, \eta_{ext} = 0.9, k_{\alpha} = 0.08, \nu = 2.6 \) and \( \kappa = 10 \).

We first consider the case where the AI degradation rates inside and outside cells are given by \( k_{\alpha 0} = 0.01 \) and \( k_{\alpha e} = 0.005 \), respectively. Two polynomials \( \varphi_1(s) \) and \( \varphi_2(s) \) with \( k_{\alpha 0} = 0.01 \) and \( k_{\alpha e} = 0.005 \) are Hurwitz polynomials, as seen from their poles location depicted in Fig. 4 (a). Figure 5 (a) illustrates the evolution of \( p_{i,j}(t) \). We see that the concentrations converge to a constant value.

The other example is the case where the AI degradation rates inside and outside cells are \( k_{\alpha 0} = 1 \) and \( k_{\alpha e} = 0.5 \). Two polynomials \( \varphi_1(s) \) and \( \varphi_2(s) \) with \( k_{\alpha 0} = 1 \) and \( k_{\alpha e} = 0.5 \) are not Hurwitz polynomials, since each polynomial has two unstable roots as seen in Fig. 4 (b). In this case, we can see periodic oscillations of the CI level inside each cell as illustrated in Fig. 5 (b).

**Remark 1** It is worth noting that Theorem 1 is a local stability condition, thus global behaviors of the protein concentrations are not guaranteed. We conducted 100,000 additional simulations with randomly sampled initial values in order to assess the relation between the local stability condition in Theorem 1 and global behaviors. The initial values were taken within \( \pm 100 \% \) of the homogeneous equilibrium. In all simulations, the protein concentrations converged to the equilibrium (oscillated) when the condition in Theorem 1 was satisfied (dissatisfied, respectively). Therefore, the existence of oscillations can be empirically determined from the local stability condition in Theorem 1.

### 4. Local Stability Analysis Based on Simplified Model for Qualitative Insights

#### 4.1 Simplified Model

We have derived a condition for local stability of the quorum-sensing network in Section 3. However, the condition is not simple enough to induce qualitative insights of the quorum-sensing network. To obtain qualitative insights and to understand underlying principles of the quorum-sensing network, we here investigate local stability conditions based on a simplified model that captures the essence of the dynamics and provide analytical conditions on local stability. We assume that parameters of a gene network in all cells are identical to each other in order to reduce the number of parameters while preserving the essence of dynamics. That is, \( \alpha_1 = \alpha_2 = \alpha_3, \beta_1 = \)
\[ \beta_2 = \beta_3, \delta_1 = \delta_2 = \delta_3, \gamma_1 = \gamma_2 = \gamma_3. \] Note that this is a standard assumption for theoretical analysis of gene regulatory networks [7, 8, 13]. We hereafter omit the subscript \( j \) of the above parameters (\( \alpha_j, \beta_j, \delta_j, \gamma_j \)) for notational simplicity.

The simplified model is derived based on the following facts. The degradation rates of mRNAs are often much larger than those of proteins, which yields that the mRNA concentrations reach an equilibrium state rapidly. The time scale between intracellular and intercellular dynamics is quite large, and the intercellular dynamics of AI is negligible around a quasi-steady state. Then, we can impose the following assumption to the model described by (1) and (2).

**Assumption 2** \( \dot{r}_i(t) = 0 \) for \( j = 1, 2, 3, i = 1, 2, \cdots, N \) and \( \dot{S}_s(t) = 0 \).

Substituting \( \dot{r}_i(t) = 0 \) into (7), we obtain

\[
0 \begin{bmatrix} \dot{p}_{i,1}(t) \\ \dot{p}_{i,2}(t) \end{bmatrix} = \begin{bmatrix} -\delta & 0 \\ -\gamma & -\beta \end{bmatrix} \begin{bmatrix} r_{i,1}(t) \\ r_{i,2}(t) \end{bmatrix} + \alpha f(p_{i,j-1}) + \kappa g_j(S_i). \tag{20}
\]

\[ \dot{S}_s(t) = 0 \] means that the extracellular AI concentration is at a quasi-steady state, and it implies

\[ S_s(t) = W \bar{S}(t), \tag{21} \]

where \( \bar{S}(t) \) is an average of \( S_1(t), S_2(t), \cdots, S_N(t) \),

\[ W := \frac{\bar{\eta}_{ext}N}{k_{ext} + \bar{\eta}_{ext}N}. \tag{22} \]

Consequently, (1) and (2) are simplified to the following equations.

\[
\begin{align*}
\dot{p}_{i,1}(t) &= -\frac{1}{T_b} p_{i,1}(t) + \frac{1}{T_b} R^2 f(p_{i,1}) + \frac{k_1}{T_b} R^2 g_j(S_i), \tag{23} \\
\dot{S}_s(t) &= -k_{d0} S_s(t) + k_{d1} p_{i,1}(t) - \eta(S_s(t) - W \bar{S}(t)). \tag{24}
\end{align*}
\]

4.2 Local Stability Analysis

In this subsection, we perform local stability analysis for the simplified model (23) and (24) obtained under Assumption 2. We can first derive the following lemma on the uniqueness of the homogeneous equilibrium point.

**Lemma 2** Consider the quorum-sensing network modeled by (23) and (24). Suppose Assumption 1 holds. Then, the equilibrium point \((p_{1,1}^*, p_{2,1}^*, p_{3,1}^*, S^*)\) is unique for \( j = 1, 2, 3 \).

This lemma shows that the homogeneous equilibrium point of the system is unique under Assumption 1.

We consider the linearization around the homogeneous equilibrium of the system represented by (23) and (24). When \( \dot{r}_i(t) = 0 \), the block \( H(s)I_N \) in Fig. 2 is simplified to \( H(s)I_N \), where

\[ H(s) := \frac{k_1 R^2 \xi_3(T_b s + 1)}{\eta_1 N}. \tag{25} \]

with \( L := R^2 (-\xi_1 \xi_2 \xi_3)^{1/3} \) which represents the cubic root of the sub-system’s gain. The blocks \( \eta_{ext}(s + k_{d0}) + \eta_1 N \) and \( 1_N I_N / N \) in Fig. 2 are reduced to the block \( (W \eta_1 N)I_N I_N / N \), because of \( \dot{S}_s(t) = 0 \) in Assumption 2. Then, the overall block diagram can be depicted as Fig. 6.

Since the order of the overall system is reduced in comparison to that of the original system, we can explicitly derive the local stability condition from Theorem 1 as follows:

**Theorem 2** Consider the quorum-sensing network modeled by (23) and (24). Suppose Assumption 1 holds, then the quorum-sensing network is locally stable, if and only if the following inequality holds.

\[
L^6 d_M^2 - 2L^3 (T_b^2 - L^2 - 1)d_M
+ (L^3 - 8)(T_b^3 + 1 + L^3) < 0, \tag{26}
\]

for \( i = 1, 2, \) and \( T_1, T_2, \) and \( d_M \) are defined as

\[ T_1 := T_5(k_{d0} + \eta), \quad T_2 := T_5(k_{d0} + (1 - W)\eta). \tag{27} \]

\[ d_M := k_{d1} T_b \frac{\xi_1}{\alpha R^2 \xi_3 \xi_2}. \tag{28} \]

where \( W \) is defined by (22).

It is interesting to observe that the local stability condition in Theorem 2 is dependent not on the number of cell \( N \) but the parameter related to the density of cells \( \eta_{ext}N \), as it is so in Theorem 1. Note that this is consistent with the well-known fact that quorum-sensing is a mechanism for sensing cell density [3]. Moreover, (26) implies that the local stability is not significantly affected by the number of cells when \( N \) is sufficiently large, since \( W \) approaches to unity as \( N \) becomes large.

It should be emphasized that only three dimensionless parameters normalized by the protein lifetime \( T_b \), namely \( T_b, d_M \) and \( L \), appear in (26) and that the left hand side is a second-order polynomial with respect to \( d_M \). Hence, Theorem 2 leads to the following sufficient condition for local instability.

**Corollary 1** Consider the quorum-sensing network modeled by (23) and (24). Suppose Assumption 1 holds. Then, the quorum-sensing network is locally unstable if the following condition is satisfied:

\[
\begin{cases}
L \geq 2 T_2 \leq 3, \\
L \geq \sqrt{(T_2 + 1)^2} - 1, \\
T_2 > 3.
\end{cases} \tag{29}
\]

In Section 5, we will discuss biological insights obtained from Theorem 2 and Corollary 1. We remark that the effect of cell-to-cell communication disappears when \( d_M = 0 \), and the conditions in Theorem 2 reduce to \( L < 2 \), which is consistent with the one derived [7].

4.3 Numerical Examples

This section provides two numerical simulations in order to confirm our result for the simplified model. Consider the quorum-sensing network composed of \( N = 8 \) cells. Suppose \( k_{d0} = 0.001, k_{d1} = 0.001, \kappa = 5, \alpha = 20, W = 0.07 \) and \( \nu = 2.6 \).

**Fig. 6** The linearized block diagram of the simplified model around the homogeneous equilibrium.
Table 2 Biological interpretations of three essential parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_i$</td>
<td>Degradation rate of AI normalized by protein lifetime. AI in a cell reduces faster when $T_i$ becomes larger.</td>
</tr>
<tr>
<td>$L$</td>
<td>Linearized gain of the CGRNW.</td>
</tr>
<tr>
<td>$d_{AI}$</td>
<td>The relative gain of cell-to-cell communication to the CGRNWs. Cell-to-cell communication becomes stronger when $d_{AI}$ gets larger.</td>
</tr>
</tbody>
</table>

Next, we verify the result of Corollary 1 by setting $T_2 = 4.68$ and $L^2 = 12.26$ so that (29) is satisfied. Fig. 7 (b) verifies the claim of Corollary 1.

5. Biological Insights

5.1 Relation between Parameters and Local Stability/Instability

(26) of Theorem 2 depends only on the three parameters, $T_i$, $d_{AI}$ and $L$. These three parameters have biological meanings as listed in Table 2. The stability/instability of the quorum-sensing network is essentially determined by these parameters. In particular, the parameter $L$ was shown to play an important role to determine the stability of the lower layer network, or the gene regulatory network [7]. Thus, $T_i$ and $d_{AI}$ essentially reflect the effect of the upper layer, or cell-to-cell communication.

The parameter regions for stability are shown in Fig. 8 in terms of $T_i$, $d_{AI}$ and $L$. These graphs show that the stability regions (shaded regions) shrink as $L$ increases, thus the system tends to be unstable. We can also see that the excessive AI production makes the quorum-sensing network unstable as $L$ gets larger.

5.2 Relationship between CGRNWs and Quorum-Sensing Networks

Instability of an equilibrium often leads to oscillations in biochemical networks. In this subsection, we consider the design problem of lower-layer biological networks that robustly guarantee the instability of the quorum-sensing network for given upper-layer dynamics. Figure 8 illustrates that it is necessary to increase the gain $L$ of the lower-layer network to destabilize the system as $d_{AI}$ decreases and $T_i$ increases. This fact poses the question that how much $L$ should be large to maintain the instability. Corollary 1 is useful to obtain a clear answer to this question. Figure 9 illustrates the parameter region of $L$ that guarantees the instability for any $d_{AI}$. We can see that the destabilizing gain $L$ increases as the degradation rate of AI normalized by protein lifetime $T_2$ increases. In particular, $L \geq 2$ is sufficient.

Fig. 7 Time response of the protein CI levels in the quorum-sensing network with $N = 8$ cells.

(a) Stable case.

(b) Unstable case.

Fig. 8 Parameter region for stability in terms of $T_i$, $d_{AI}$ and $L$.
to destabilize the system when $T_2 \leq 3$, and this condition is equivalent to the one derived for the isolated CGRNW [7].

6. Conclusion

This paper has proposed a control theoretic framework to analyze large-scale biological networks with multi-cellular environment, and we have obtained qualitative insights of the quorum-sensing network. The key idea of the presented systematic analysis is to use the characteristic hierarchical structure of the mRNA, protein and AI dynamics and to formulate it as a multi-agent dynamical system with hierarchical interconnections. This formulation allows us to reduce the local stability analysis of the overall system to that of small subsystems.

We have analytically derived the necessary and sufficient conditions for local stability of the quorum-sensing networks. The conditions are applicable to systems with a large number of cells because they are independent of the number of cells. The conditions for the simplified model have revealed that three essential parameters ($T_i$, $d_{ai}$ and $L$) characterize the existence of oscillations in the quorum-sensing network. These parameters can be interpreted from a biological viewpoint.

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References


\( p_1 \) since \( f(\cdot) \) is monotonic. In contrast, the left-hand side of (B.3) strictly monotonically increases with respect to \( p_1 \), and thus \( p_1 \) is unique. With ease, we can verify that other variables are uniquely determined whenever \( p_1 \) is determined.

**Appendix C  Proof of Theorem 2**

Under Assumption 2, Theorem 1 can be restated as follows: Suppose Assumption 1 holds. Quorum-sensing networks described by (23) and (24) are locally stable if and only if

\[
T_i^4 A_i + T_i^3 (T_i + 3) s^3 + 3 T_i^2 (T_i + 1) s^2 \\
+ T_i [3 T_i + 1 + (d_{AI} + 1) L_i^3] s + T_i (L_i^3 + 1) + d_{AI} L_i^3
\]

(C.1)

is a Hurwitz polynomial for both \( i = 1 \) and \( i = 2 \). We can more specifically write this condition by applying the Routh criterion to (C.1) as follows,

\[
L_i^3 < \frac{3 T_i^2 + 9 T_i + 8}{d_{AI} + 1}, \quad (C.2)
\]

\[
(d_{AI} + 1)^2 L_i^6 + (T_i - 1)(T_i^2 + 4 T_i + 4) \sum_{i=1}^3 T_i + 7 - 2 d_{AI} (T_i + 1) L_i^3 - 8 (T_i + 1)^3 < 0. \quad (C.3)
\]

In particular, we can confirm that (C.2) is always met if (C.3) is satisfied as shown below. Letting \( x := L_i^3 \), we see that the left-hand side of (C.3) becomes a second order polynomial of \( x \), and the left-hand side of (C.3) changes from negative to positive as \( x = L_i^3 \) grows larger. Thus, if the right-hand side of (C.2) is larger than the positive solution of the polynomial (C.3), (C.3) determines the stability condition. In fact, the right-hand side of (C.3) is obtained as

\[
\frac{(T_i + 3)^2}{d_{AI} + 1} \left[ 3 T_i^2 + 3 (d_{AI} + 3) T_i^2 \right.
\]

\[
+ (10 d_{AI} + 9) T_i + 8 d_{AI} \left] > 0, \quad (C.4)
\]

when \( L_i^3 = \left(3 T_i^2 + 9 T_i + 8\right) / (d_{AI} + 1) \).

**Appendix D  Proof of Corollary 1**

We derive Corollary 1 from Theorem 2, which implies that local instability for the system is equivalent to that (26) is not satisfied for neither \( T_1 \) nor \( T_2 \).

The left-hand side of (26) can be seen as a second order polynomial of \( d_{AI} \) and is hereafter named (D.1). We can see that the following condition (i) or (ii) are sufficient conditions for (D.1) \( \geq 0 \) for all \( d_{AI} \ \geq 0 \). (i) Discriminant of (D.1) is zero or negative. (ii) Both the first order and the constant terms of (D.1) are zero or positive for all \( d_{AI} \ \geq 0 \). The discriminant is calculated as \((T_i + 1)^2 - L_i^3(T_i - 1)\), and the first order and the constant terms are expressed as \(-2 L_i^3(T_i^2 + L_i^3 - 1)\) and \((L_i^3 - 8)(T_i + 1)^3 + L_i^3\), respectively. Thus, a sufficient condition is obtained as

(i) \( (T_i - 1) L_i^3 \leq (T_i + 1)^2 \quad \text{or} \quad (D.1) \)

(ii) \( L_i^3 + 1 - T_i^2 \geq 0 \quad \text{and} \quad L_i^3 - 8 \geq 0 \quad (D.2) \)

for either \( i = 1 \) or \( i = 2 \).

We can see that for \( 0 \leq T_i \leq 1 \), (D.1) is always dissatisfied and (D.2) is satisfied if \( L \geq 2 \). For 1 < \( T_i \leq 3 \), (D.1) or (D.2) is satisfied if \( L \geq 2 \). For \( T_i > 3 \), (D.1) or (D.2) is satisfied if \( L \geq (T_i + 1)^2 / (T_i - 1) \). Therefore, a sufficient condition is obtained as \( L \geq 2 \) for \( T_i \geq 3 \) or \( L = \sqrt{(T_i + 1)^2 / (T_i - 1)} \) for \( T_i > 3 \), where \( i = 1 \) or \( i = 2 \).