

Mitigation of ribosome competition through distributed sRNA feedback

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Abstract—A current challenge in the robust engineering of synthetic gene networks is context dependence, the unintended interactions among genes and host factors. Ribosome competition is a specific form of context dependence, where all genes in the network compete for a limited pool of translational resources available for gene expression. Recently, theoretical and experimental studies have shown that ribosome competition creates a hidden layer of interactions among genes, which largely hinders our ability to predict design outcomes. In this work, we establish a control theoretic framework, where these hidden interactions become disturbance signals. We then propose a distributed feedback mechanism to achieve disturbance decoupling in the network. The feedback loop at each node consists of the protein product transcriptionally activating a small RNA (sRNA), which forms a translationally inactive complex with mRNA rapidly. We illustrate that with this feedback mechanism, protein production at each node is only dependent on its own transcription factor inputs, and almost independent of hidden interactions arising from ribosome competition.

I. INTRODUCTION

Context dependence is a recurrent challenge in the bottom-up design of large scale synthetic gene networks [1]. In particular, although input/output (I/O) responses of simple genetic parts can be well-characterized in isolation, their behaviors may change significantly when connected in a network [2],[3],[4]. Such behaviors, which are often referred to as lack of modularity [5], largely hinder our capability to carry out predictive design at the system level. In order to preserve modularity of circuit modules, recently there has been an increasing interest in finding methods to mitigate of various forms of context dependence [2],[6],[7].

In this paper, we focus on competition of translational resources (ribosomes) as a special form of context dependence in gene (transcription) networks. In a gene network, each node consists of a gene that is expressed to produce proteins, which serve as transcription factors (TFs) that regulate gene expression at other nodes. Gene expression relies on the availability of ribosomes, which are molecular machines that are found in limited amount in cells at constant growth rate [8]. Limited access to free ribosomes has been identified as a major bottleneck in genetic circuits [4]. As all genes in the network compete for a common pool of ribosomes, a hidden layer of interactions among nodes arises, which can significantly change network behavior [9].

In order to engineer the cells to mitigate the effects of ribosome competition, An and Chin [10] propose the use of orthogonal ribosomes (O-ribosomes) to decouple ribosome

usage of endogenous mRNAs and synthetic mRNAs. However, the problem of mitigating the coupling among synthetic mRNAs remains. Using ideas from classical control theory, in [11], the authors compare performance of three negative feedback mechanisms that increase robustness of steady state expression of a constitutive gene with respect to resource competition. The role of negative feedback in gene networks has been widely studied, with applications to noise attenuation [12] and insulation devices that mitigate TF competition (also known as retroactivity) [13].

In this paper, we propose a distributed sRNA feedback mechanism to mitigate the effects of ribosome competition on protein production in a gene network. By modeling competition-induced hidden interactions as disturbances among nodes, we formulate a static network disturbance decoupling problem, whose aim is to attenuate the static effects of disturbances on the output of each node i (y_i), so that y_i only depends on its own reference input. Attenuating external disturbances through distributed control has been widely studied in control literature (see [14], for example). However, in our case, disturbance input to each node is produced by the rest of the network. Thus, to achieve network disturbance decoupling, we require each node to possess a disturbance attenuation property, and that the network doesn't amplify the disturbances as we increase disturbance attenuation at individual nodes. The requirement on the network can be verified if an interconnection matrix, constructed by the static node I/O gains and the interconnection rule, is diagonally dominant. Such a requirement highly resembles the network small-gain criteria in [15]. We then show that the key node and network properties are satisfied by a gene network with distributed sRNA feedback.

The rest of the paper is organized as follows. In Section II, we model hidden interactions arising from ribosome competition as disturbances. In Section III, we formulate the static network disturbance decoupling problem, and provide sufficient conditions that guarantee network disturbance decoupling. In Section IV, we propose an sRNA mediated distributed feedback design, through which network disturbance decoupling can be achieved. We test our design with an activation cascade example in Section V. Discussion and conclusions are in Section VI.

Notations: Let $\mathbf{y} = [y_1, \dots, y_n]^T$ be a vector in \mathbb{R}^n , we define \mathbf{y}_{-i} as the vector $[y_1, \dots, y_{i-1}, y_{i+1}, \dots, y_n]^T$. When there is no risk of ambiguity, $\bar{\mathbf{x}}$ stands for the steady state of signal \mathbf{x} under some dynamics of interests. $\mathbf{y}_{(i)}$ represents the i -th element of vector \mathbf{y} , and $\mathbf{A}_{(j,k)}$ is the (j,k) -th element of matrix \mathbf{A} . We use \mathbb{R}_+^n to represent the non-negative orthant $\mathbb{R}_{\geq 0}^n$, and we use $\otimes_{i=1}^n \mathcal{X}_i$ to represent the set $\mathcal{X}_1 \times \dots \times \mathcal{X}_n$.

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II. DISTURBANCES ARISING FROM RIBOSOME COMPETITION

A. Gene Expression with Limited Ribosomes

A transcriptional component is a fundamental building block in gene networks. It takes a number of TFs as inputs to regulate the production of protein x_i as output. A transcriptional component is also a *node* in a gene network. Here, we consider a node i taking two inputs TFs (u_{i1} and u_{i2}) that bind with the promoter region of gene i (p_i) with cooperativity n_{i1} and n_{i2} . We use $\mathbf{u}_i = [u_{i1}, u_{i2}]^T$ to represent the concentration of TF inputs to node i . Depending on the type of TFs (activator or repressor), they can either promote or inhibit gene transcription to produce mRNAs (m_i). mRNAs are then translated by ribosomes (z) to produce protein (x_i). At a constant growth rate, the total amount of ribosomes are conserved [8]. Assuming that binding reactions are much faster than transcription and translation [16], and thus can be set to quasi-steady state, each node can be described by the concentrations of its mRNA and protein: $\mathbf{x}_i = [m_i, x_i]^T \in \mathbb{R}_+^2$.

If node i is the only node in the network, all ribosomes are available for its translation, and the ribosome conservation law is $z_t = z + z_i$, where z_t (z) is the total (free) amount of ribosomes, and z_i is the amount of ribosomes bound with m_i . Using standard reaction rate equations for transcriptional regulation [17], simplified dynamics of node i can be written as:

$$\dot{m}_i = T_i v_i - \delta m_i, \quad \dot{x}_i = R_i \frac{m_i / \kappa_i}{1 + m_i / \kappa_i} - \gamma x_i, \quad (1)$$

where T_i is the basal transcription rate of node i when $\mathbf{u}_i \equiv 0$, δ (γ) is the dilution/degradation rate of mRNA (protein), R_i is a lumped translation rate constant that is proportional to z_t , and κ_i is the dissociation constant between ribosomes and mRNA ribosome binding site (RBS). Smaller κ_i indicates stronger binding. We call $v_i = v_i(\mathbf{u}_i)$ as the *reference input* to node i . The reference input describes the combined regulation effect of TFs u_{i1} and u_{i2} on the transcription rate of node i , and is defined as

$$v_i = v_i(\mathbf{u}_i) := \frac{1 + \frac{T_{i1}}{T_i} \left(\frac{u_{i1}}{k_{i1}}\right)^{n_{i1}} + \frac{T_{i2}}{T_i} \left(\frac{u_{i2}}{k_{i2}}\right)^{n_{i2}}}{1 + \left(\frac{u_{i1}}{k_{i1}}\right)^{n_{i1}} + \left(\frac{u_{i2}}{k_{i2}}\right)^{n_{i2}}}, \quad (2)$$

where k_{ij} is the dissociation constant between u_{ij} and p_i , and T_{ij} is the transcription rate of node i when $u_{ij} \rightarrow \infty$ ($j = 1, 2$). Therefore, $T_{ij} \ll T_i$ if u_{ij} is a repressor, and $T_{ij} > T_i$ if u_{ij} is an activator. Detailed derivation of (1) can be found in [9]. Note that according to (1), the output of each node, x_i , is only dependent on v_i , and consequently only on \mathbf{u}_i .

B. Ribosome Usage as Disturbances in a Network

A gene network \mathcal{N} is an interconnection of n transcriptional components (nodes). Nodes can be connected through transcriptional regulation, where protein x_i serves as a TF input to node j ($j \neq i$). Let $\mathbf{x} = [x_1, \dots, x_n]^T$, the input to node i is given by $\mathbf{u}_i = \mathbf{Q}_i \cdot \mathbf{x}$, where \mathbf{Q}_i is an input selection matrix defined as

$$\mathbf{Q}_i(j,k) = \begin{cases} 1, & \text{if } x_k \text{ is the } j\text{-th input to node } i, \\ 0, & \text{otherwise.} \end{cases} \quad (3)$$

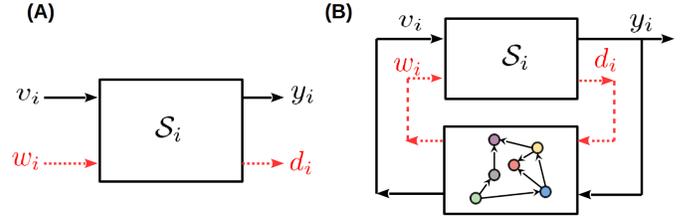


Fig. 1. (A) Node i in isolation. The black solid lines represents the reference I/O signals, and the red dashed lines represents disturbances I/O signals. (B) In a network, inputs to node i are produced by the rest of the network, whose dynamics are also affected by disturbance output of node i .

When the network has multiple nodes, due to the ribosome conservation law,

$$z_t = z + \sum_{i=1}^n z_i, \quad (4)$$

the node dynamics can be written as ([9]):

$$\dot{m}_i = T_i v_i - \delta m_i, \quad (5)$$

$$\dot{x}_i = R_i \frac{m_i / \kappa_i}{1 + m_i / \kappa_i + \sum_{j \neq i} m_j / \kappa_j} - \gamma x_i.$$

Note that in (5), dynamics of node i are not only dependent on its own reference input v_i , but also on the concentration of mRNA transcripts of other nodes in the network (m_j), which is undesirable for predictable design. In this sense, we regard

$$w_i := \sum_{j \neq i} m_j / \kappa_j \quad (6)$$

as a disturbance input to node i . This is because without w_i , dynamics of (5) are identical to those in (1), which are the dynamics of node i in the absence of other ribosome-competing nodes. According to (6), ribosome demand of node i , which we define as $d_i := m_i / \kappa_i$, is disturbance input to nodes $1, \dots, i-1, i+1, \dots, n$.

As a consequence, we can regard each node as a dynamical system with two inputs and two outputs as shown in Fig. 1. The black solid arrows are reference input (v_i) and reference output (y_i), while the red dashed arrows represent disturbance input (w_i) and output (d_i). In (5), the reference output is defined as $y_i = x_i$. Previous theoretical study [9], and experimental results [4], [18] have demonstrated significant effects of competition-induced hidden interactions on steady state gene expression. It is thus desirable to design a feedback mechanism to mitigate the effect of disturbances, so that the network behaves as if the only interactions among nodes are transcriptional regulations. In the following section, we formulate a general control theoretic framework to address this problem.

III. NETWORK DISTURBANCE DECOUPLING

Our objective is to have the steady state reference output of each node (y_i) be only dependent on its own reference input (v_i), while independent of the reference inputs to other nodes ($v_j, j \neq i$), which enter dynamics of node i through disturbances. Therefore, we expect the I/O response of each node to be as if they were the only nodes in the network. We refer to this problem as *static network disturbance*

decoupling problem.

Here, we propose sufficient conditions that guarantee static network disturbance decoupling. These conditions fall into two categories: properties of the node and of the network. In particular, when a node is viewed in isolation (Fig.1(A)), by decreasing a suitable positive parameter ϵ , y_i should become arbitrarily insensitive to w_i (node disturbance attenuation). When node i is part of a network (Fig.1(B)), w_i is determined by the network, and may depend on ϵ . We therefore require that w_i is not increasing as we decrease ϵ (network ϵ -well-posedness). Algebraic conditions are given for both conditions in what follows.

A. Disturbance Attenuation of a Node

Consider a dynamical system S_i^ϵ of the form:

$$\dot{\mathbf{x}}_i = \mathcal{F}_i(\mathbf{x}_i, v_i, w_i, \epsilon), \quad y_i = \mathcal{H}_i(\mathbf{x}_i), \quad d_i = \mathcal{G}_i(\mathbf{x}_i), \quad (7)$$

evolving on a set of states $\mathcal{X}_i \subseteq \mathbb{R}^m$. The system takes two inputs: an external reference input $v_i(t)$ taking values on a set $\mathcal{V}_i \subseteq \mathbb{R}$, and an external disturbance input $w_i(t)$ taking values on $\mathcal{W}_i \subseteq \mathbb{R}$. We call \mathcal{V}_i the *admissible reference input set*, and \mathcal{W}_i the *admissible disturbance input set*. The system produces two outputs: a reference output $y_i \in \mathbb{R}$ and a disturbance output $d_i \in \mathbb{R}$ (refer to Fig.1(A)). System S_i^ϵ is parameterized by a small positive constant ϵ , with $0 < \epsilon < \epsilon^* \ll 1$.

Definition 1: (Static I/O characteristics). System (7) admits a static input to state (I/S) characteristic $\bar{f}_i(\cdot) : \mathcal{V}_i \mapsto \mathcal{X}_i$, if for all constant inputs $v_i(t) \equiv \bar{v}_i \in \mathcal{V}_i$ and $w_i(t) \equiv \bar{w}_i \in \mathcal{W}_i$, there exists a unique locally asymptotically stable (LAS) equilibrium $\bar{\mathbf{x}}_i := \bar{f}_i(\bar{v}_i, \bar{w}_i, \epsilon)$. If system (7) admits a static I/S characteristic, then its static I/O characteristics are given by

$$\begin{aligned} \bar{y}_i &= \bar{h}_i(\bar{v}_i, \bar{w}_i, \epsilon) := \mathcal{H}_i(\bar{f}_i(\bar{v}_i, \bar{w}_i, \epsilon)), \\ \bar{d}_i &= \bar{g}_i(\bar{v}_i, \bar{w}_i, \epsilon) := \mathcal{G}_i(\bar{f}_i(\bar{v}_i, \bar{w}_i, \epsilon)), \end{aligned} \quad (8)$$

where $\bar{h}_i(\cdot), \bar{g}_i(\cdot) : \mathcal{V}_i \times \mathcal{W}_i \times (0, \epsilon^*) \mapsto \mathbb{R}$.

We assume any node i we consider here satisfy the following conditions for all $\bar{v}_i \in \mathcal{V}_i$ and $\bar{w}_i \in \mathcal{W}_i$.

- (A1) Node i has well-defined static I/O characteristics.
- (A2) The static I/O maps \bar{h}_i and \bar{g}_i are sufficiently smooth with bounded derivatives.

For ϵ sufficiently small, the I/O characteristics (8) can be written as series expansions in ϵ :

$$\begin{aligned} \bar{y}_i &= \bar{h}_i(\bar{v}_i, \bar{w}_i, \epsilon) = \bar{h}_i(\bar{v}_i, \bar{w}_i, 0) + \epsilon \tilde{h}_i(\bar{v}_i, \bar{w}_i, 0) + \mathcal{O}(\epsilon^2), \\ \bar{d}_i &= \bar{g}_i(\bar{v}_i, \bar{w}_i, \epsilon) = \bar{g}_i(\bar{v}_i, \bar{w}_i, 0) + \epsilon \tilde{g}_i(\bar{v}_i, \bar{w}_i, 0) + \mathcal{O}(\epsilon^2), \end{aligned}$$

where

$$\tilde{h}_i(\bar{v}_i, \bar{w}_i, 0) := \left. \frac{\partial \bar{h}_i}{\partial \epsilon} \right|_{\bar{v}_i, \bar{w}_i, 0}, \quad \tilde{g}_i(\bar{v}_i, \bar{w}_i, 0) := \left. \frac{\partial \bar{g}_i}{\partial \epsilon} \right|_{\bar{v}_i, \bar{w}_i, 0}.$$

Definition 2: (Node disturbance attenuation). Node i is said to have the ϵ -static disturbance attenuation property in \mathcal{V}_i if $\bar{h}_i(\bar{v}_i, \bar{w}_i, 0) \equiv \bar{h}_i(\bar{v}_i, 0, 0)$ for all $\bar{v}_i \in \mathcal{V}_i$ and $\bar{w}_i \in \mathcal{W}_i$.

For a node with ϵ -static disturbance attenuation property, any contribution from the disturbance input to the reference output is attenuated by a factor of ϵ . However, in a network setting, disturbance input w_i is generated by other nodes in the network, and in principle, it may increase as we decrease

ϵ . Therefore, the next requirement is that the disturbance signals are not increased when ϵ is decreased, which we refer to as the network ϵ -well-posedness property.

B. Network ϵ -well-posedness

Consider a network \mathcal{N}^ϵ composed of n nodes with dynamics in (7). We denote by \mathcal{I} the index set $\{1, \dots, n\}$, and by $\mathbf{x} = [\mathbf{x}_1^T, \dots, \mathbf{x}_n^T]^T \subseteq \mathbb{R}^{nm}$ the state of the network. Let $\mathbf{v} = [v_1, \dots, v_n]^T$, $\mathbf{y} = [y_1, \dots, y_n]^T$, $\mathbf{w} = [w_1, \dots, w_n]^T$, and $\mathbf{d} = [d_1, \dots, d_n]^T$ be concatenations of reference input, reference output, disturbance input and disturbance output signals at all nodes. We assume disturbance coupling takes the following form.

$$(A3) \text{ For all } i, j \in \mathcal{I}, w_i = \sum_{j \neq i} d_j.$$

We assume all networks we study have well-defined I/S characteristic.

$$(A4) \text{ Network } \mathcal{N}^\epsilon \text{ has a unique LAS equilibrium } \bar{\mathbf{x}} = \bar{\mathbf{x}}(\epsilon) \text{ for all } \epsilon \in (0, \epsilon^*) \text{ and } \bar{\mathbf{v}} \in \otimes_{i=1}^n \mathcal{V}_i.$$

Definition 3: (Network ϵ -well-posedness): Let $\mathcal{V}_{\mathcal{N}} \subseteq \otimes_{i=1}^n \mathcal{V}_i$, we say that \mathcal{N}^ϵ is ϵ -well-posed in $\mathcal{V}_{\mathcal{N}}$ if for all $i \in \mathcal{I}$, and $\bar{\mathbf{v}} \in \mathcal{V}_{\mathcal{N}}$, $\lim_{\epsilon \rightarrow 0} \bar{w}_i(\epsilon)$ is finite. Furthermore, $w_i(\epsilon) \in \mathcal{W}_i$ for all $\epsilon \in (0, \epsilon^*)$.

An ϵ -well-posed network has the property that if static reference input $\bar{\mathbf{v}}$ falls in the set $\mathcal{V}_{\mathcal{N}}$, then $\bar{\mathbf{w}} \in \mathcal{W}_i$. In addition, it stays bounded as we decrease ϵ . To find sufficient conditions for ϵ -well-posedness, we restrict our attention to networks with the following properties.

- (A5) Disturbance signals are non-negative: $\mathcal{W}_i = \mathbb{R}_+$.
- (A6) There exists a positive constant μ_i , independent of ϵ , such that $|\bar{v}_i| \leq \mu_i$ for all $\epsilon \in (0, \epsilon^*)$.

(A5) is common for biological systems where all signals are non-negative. (A6) implies that the regulation signals are bounded, which is also the case in a gene network due to (2). At each node, we assume the static disturbance output \bar{d}_i is an affine function of disturbance input \bar{w}_i as $\epsilon = 0$,

$$(A7) \text{ For all } \bar{\mathbf{v}} \in \mathcal{V}_{\mathcal{N}} \text{ and } i \in \mathcal{I}, \bar{g}_i(\bar{v}_i, \bar{w}_i, 0) = g_i(\bar{v}_i) + \hat{g}_i(\bar{v}_i) \bar{w}_i, \text{ where } g_i(\bar{v}_i), \hat{g}_i(\bar{v}_i) > 0.$$

Due to (A3) and (A7), disturbance signals can be solved from

$$\bar{w}_i = \sum_{j \neq i} [g_j(\bar{v}_j) + \hat{g}_j(\bar{v}_j) \bar{w}_j + \epsilon \tilde{g}_j(\bar{v}_j, \bar{w}_j, 0) + \mathcal{O}(\epsilon^2)]. \quad (9)$$

With abuse of notation, we write $\bar{\mathbf{w}} = \bar{\mathbf{w}}(\bar{\mathbf{v}}, \epsilon)$ as the solution of (9) for a fixed $\bar{\mathbf{v}}$ and ϵ . To put (9) into a compact form, we introduce the interconnection matrix $\mathbf{A}(\bar{\mathbf{v}})$ and a positive vector $\Phi(\bar{\mathbf{v}})$. Elements in $\mathbf{A}(\bar{\mathbf{v}})$ are:

$$\mathbf{A}_{(j,k)}(\bar{\mathbf{v}}) := \begin{cases} 1, & \text{if } j = k, \\ -\hat{g}_k(\bar{v}_k), & \text{if } j \neq k, \end{cases} \quad (10)$$

and the i -th element in $\Phi(\bar{\mathbf{v}})$ is:

$$\Phi_{(i)}(\bar{\mathbf{v}}) = \sum_{j \neq i} g_j(\bar{v}_j). \quad (11)$$

We shall introduce the following lemma [19], which gives sufficient conditions for a class of matrices to be inverse-positive.

Lemma 1: If $\mathbf{B} \in \mathbb{R}^{p \times p}$ is a strictly diagonally dominant matrix, where $|\mathbf{B}_{(i,i)}| > \sum_{j \neq i} |\mathbf{B}_{(i,j)}|$ for all $i, j \in \{1, \dots, p\}$, then \mathbf{B} is non-singular. Furthermore, let $\zeta \in \mathbb{R}^p$, and $\zeta > 0$. If $\mathbf{B}_{(i,i)} > 0$ and $\mathbf{B}_{(i,j)} < 0$ for all $i \neq j$, then $\mathbf{B}^{-1}\zeta > 0$.

Claim 1: Under assumptions (A3)-(A7), \mathcal{N}^ϵ is ϵ -well-posed in $\mathcal{V}_\mathcal{N}$ if the interconnection matrix $\mathbf{A}(\bar{\mathbf{v}})$ is strictly diagonally dominant for all $\bar{\mathbf{v}} \in \mathcal{V}_\mathcal{N}$. Furthermore, static disturbance signals are

$$\bar{\mathbf{w}}(\bar{\mathbf{v}}, \epsilon) = \mathbf{A}(\bar{\mathbf{v}})^{-1} \Phi(\bar{\mathbf{v}}) + \mathcal{O}(\epsilon). \quad (12)$$

Proof: To claim ϵ -well-posedness, we need to show $\lim_{\epsilon \rightarrow 0} \bar{w}_i < \infty$ and \bar{w}_i positive for ϵ small. It is thus sufficient to find a positive $\mathcal{O}(1)$ solution $\bar{\mathbf{w}}$ to (9). In this case, $\epsilon \tilde{g}_i(\bar{v}_i, \bar{w}_i, 0) = \mathcal{O}(\epsilon)$. Equation (9) can be re-written as

$$\mathbf{A}(\bar{\mathbf{v}}) \bar{\mathbf{w}} = \Phi(\bar{\mathbf{v}}) + \mathcal{O}(\epsilon). \quad (13)$$

Due to Lemma 1, we can write $\bar{\mathbf{w}} = \bar{\mathbf{w}}(\bar{\mathbf{v}}, \epsilon) = \mathbf{A}(\bar{\mathbf{v}})^{-1} \Phi(\bar{\mathbf{v}}) + \mathcal{O}(\epsilon)$, with $\lim_{\epsilon \rightarrow 0} \bar{\mathbf{w}}(\bar{\mathbf{v}}, \epsilon) = \mathbf{A}(\bar{\mathbf{v}})^{-1} \Phi(\bar{\mathbf{v}}) < \infty$, and $\bar{\mathbf{w}} > 0$ for ϵ sufficiently small. ■

Note that the interconnection matrix \mathbf{A} functions similarly as the test matrix used for network small-gain theorem in [15], our result can thus be regarded as a static generalization in the same spirit.

C. Network Disturbance Decoupling

Given the interconnection signals $\bar{\mathbf{w}}(\bar{\mathbf{v}}, \epsilon)$ for a fixed $\bar{\mathbf{v}} \in \mathcal{V}_\mathcal{N}$, the reference output at each node is given by:

$$\bar{y}_i = \bar{H}_i(\bar{v}_i, \bar{\mathbf{v}}_{-i}, \epsilon) := \bar{h}_i(\bar{v}_i, \bar{\mathbf{w}}_{(i)}(\bar{\mathbf{v}}, \epsilon), \epsilon). \quad (14)$$

Equation (14) can be written as a series expansion of ϵ for ϵ sufficiently small:

$$\bar{y}_i = \bar{H}_i(\bar{v}_i, \bar{\mathbf{v}}_{-i}, 0) + \mathcal{O}(\epsilon). \quad (15)$$

Similar to the single node case, we define an ϵ -disturbance decoupling property for the network.

Definition 4: (Network disturbance decoupling). Network \mathcal{N}^ϵ is said to have ϵ -static network disturbance decoupling property in $\mathcal{V}_\mathcal{N}$ if $\bar{H}_i(\bar{v}_i, \bar{\mathbf{v}}_{-i}, 0) \equiv \bar{H}_i(v_i, \mathbf{0}, 0)$ for all $\bar{\mathbf{v}} \in \mathcal{V}_\mathcal{N}$ and $i \in \mathcal{I}$.

For a network with such property, static reference output of each node is practically independent of the reference input to other nodes ($\bar{\mathbf{v}}_{-i}$).

Claim 2: Under (A4), \mathcal{N}^ϵ has the ϵ -static network disturbance decoupling property in $\mathcal{V}_\mathcal{N}$ for ϵ sufficiently small if

- 1) each node has ϵ -static disturbance attenuation property for all $\bar{v}_i \in \mathcal{V}_i$, and
- 2) the network is ϵ -well-posed in $\mathcal{V}_\mathcal{N}$.

Proof: Let $\bar{\mathbf{w}}(\bar{\mathbf{v}}, \epsilon)$ be the static disturbance input of \mathcal{N}^ϵ with $\lim_{\epsilon \rightarrow 0} \bar{w}_i(\bar{\mathbf{v}}, \epsilon) \in \mathcal{W}_i$ for all $i \in \mathcal{I}$. Therefore, at each node with ϵ -static disturbance attenuation property, we have

$$\bar{y}_i = \bar{h}_i(\bar{v}_i, 0, 0) + \epsilon \tilde{h}_i(\bar{v}_i, \bar{\mathbf{w}}_{(i)}(\bar{\mathbf{v}}, \epsilon), 0) + \mathcal{O}(\epsilon^2).$$

Since \mathcal{N}^ϵ is ϵ -well-posed, $\lim_{\epsilon \rightarrow 0} \bar{\mathbf{w}}_{(i)}(\bar{\mathbf{v}}, \epsilon)$ is finite. Due to boundedness of \bar{v}_i , we have $\lim_{\epsilon \rightarrow 0} \epsilon \tilde{h}_i(\bar{v}_i, \bar{\mathbf{w}}_{(i)}(\bar{\mathbf{v}}, \epsilon), 0) =$

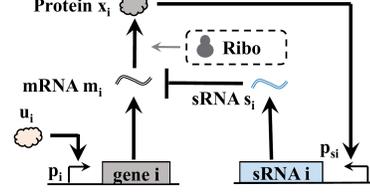


Fig. 2. A schematic of the sRNA feedback acting on node i .

0. Therefore, for ϵ sufficiently small, we can write $\bar{y}_i = \bar{h}_i(\bar{v}_i, 0, 0) + \mathcal{O}(\epsilon)$. ■

IV. DISTURBANCE DECOUPLING REALIZED THROUGH DISTRIBUTED sRNA FEEDBACK

Small RNAs have been recognized as critical regulators in gene expression [20]. In this section, we propose a distributed sRNA feedback design that achieves the static network disturbance decoupling described in Section II.

A. sRNA Feedback Setup

A diagram of the sRNA feedback mechanism for node i is shown in Fig.2. To attenuate disturbances arising from ribosome competition, sRNA-enabled mRNA inhibition creates an effective negative feedback loop around the translation process: the output protein (x_i) transcriptionally activates the production of sRNA (s_i), which forms a translationally inactive complex with mRNA. The complex then degrades rapidly. Recent experimental results suggest that sRNA is a potent repressor for target gene expression, inhibiting target gene expression by up to 150 folds [21].

When ribosome availability decreases, for instance, x_i production decreases, down-regulating sRNA production, which in turn up-regulates m_i , and consequently x_i , compensating for the loss in x_i production due to ribosome limitation. To compensate for the decrease in gene (sRNA) expression due to the addition of the feedback, we increase the concentration of p_i and p_{si} . Assuming binding reactions have reached quasi-steady state (QSS), state of node i can be represented by $\mathbf{x}_i = [m_i, s_i, x_i]^T$. Based on reaction rate equations and ribosome conservation law (4), we derive an ODE model for a node with sRNA feedback:

$$\begin{aligned} \dot{m}_i &= GT_i v_i - G m_i s_i - \delta m_i, \\ \dot{s}_i &= GT_{si} \frac{x_i / k_{si}}{1 + x_i / k_{si}} - G m_i s_i - \delta s_i, \\ \dot{x}_i &= R_i \frac{m_i / \kappa_i}{1 + m_i / \kappa_i + w_i} - \gamma x_i. \end{aligned} \quad (16)$$

Detailed derivation of this model can be found in Appendix A. In equations (16), $G := \beta / k_*$ is defined as the effective repression of translation, where β is the degradation rate of the mRNA-sRNA complex, and k_* is the dissociation constant between sRNA and mRNA. Magnitude of G can be tuned by rational design of the sRNA target-binding sequence [20]. Parameter k_{si} is the dissociation constant between activator x_i and sRNA promoter p_{si} . Parameters κ_i , δ and γ are defined identically as in (1). Other lumped parameters are defined as follows:

$$T_i := \frac{p_i^\dagger \pi_{i0}}{G}, \quad T_{si} := \frac{p_{si}^\dagger \pi_{si}}{G}, \quad R_i := \theta_i z_t,$$

where p_i^t (p_{si}^t) is the gene (sRNA gene) copy number, π_{i0} (π_{si}) is the mRNA (sRNA) basal transcription rate constant, θ_i is the mRNA translation rate constant, and z_t is the total number of ribosome available. T_i (T_{si}) can be made constant as we tune G by changing p_i^t (p_{si}^t).

In what follows, we verify that a gene network \mathcal{N}_s^ϵ consisting of nodes with distributed sRNA feedback has network disturbance decoupling property defined in Definition 4. Following Claim 2, in the next two subsections, we first verify the node disturbance attenuation property, and then ϵ -well-posedness of the network.

B. Node Disturbance Attenuation

Here, we view node i in isolation, and treat v_i and w_i as external inputs. By studying static I/O characteristics of (16), we show that it has the desired node disturbance attenuation property within a suitable admissible input set. We let $\epsilon := \delta/G \ll 1$ be a small parameter that can be decreased by increasing G . Setting the time derivatives in (16) to zero, we can find its steady state $\bar{x}_i = [\bar{m}_i, \bar{s}_i, \bar{x}_i]^T$. Note that independent of \bar{w}_i , $\bar{x}_i = \mathbf{0}$ if and only if $\bar{v}_i = 0$. Therefore, in the sequel, we only consider the nontrivial case where \bar{v}_i is strictly positive. To verify that node i has well-defined I/O characteristics, the steady state of (16) can be obtained from:

$$T_{si} \frac{R_i \bar{m}_i}{\gamma k_{si} \kappa_i (1 + \bar{w}_i) + (\gamma k_{si} + R_i) \bar{m}_i} - \epsilon \frac{T_i \bar{v}_i}{\bar{m}_i} + \epsilon \bar{m}_i = T_i \bar{v}_i - \epsilon^2. \quad (17)$$

$$\bar{x}_i = \frac{R_i}{\gamma} \cdot \frac{\bar{m}_i / [\kappa_i (1 + \bar{w}_i)]}{1 + \bar{m}_i / [\kappa_i (1 + \bar{w}_i)]}, \quad \bar{s}_i = \frac{T_i \bar{v}_i}{\bar{m}_i} - \epsilon. \quad (18)$$

Lemma 2: System (16) has a unique steady state \bar{x}_i for all $\bar{v}_i > 0$ and $\bar{w}_i \geq 0$.

Proof: According to (18), \bar{s}_i, \bar{x}_i are bijective functions of \bar{m}_i , hence, we only need to show uniqueness of \bar{m}_i . The left hand side of (17) increases monotonically with \bar{m}_i , and ranges \mathbb{R} , while the right hand side of (17) is a constant. Therefore, the solution \bar{m}_i to (17) is unique. ■

According to [22], for ϵ sufficiently small and $\bar{v}_i \in \mathcal{V}_i$, the unique steady state of (16) can be written as

$$\begin{aligned} \bar{m}_i &= \frac{T_i \kappa_i k_{si} \bar{v}_i (1 + \bar{w}_i)}{T_{si} R_i - (\gamma k_{si} + R_i) T_i \bar{v}_i} + \mathcal{O}(\epsilon), \\ \bar{s}_i &= \frac{T_{si} R_i - (\gamma k_{si} + R_i) T_i \bar{v}_i}{\kappa_i k_{si} \bar{v}_i (1 + \bar{w}_i)} + \mathcal{O}(\epsilon), \\ \bar{x}_i &= \frac{T_i k_{si} \bar{v}_i}{T_{si} - T_i \bar{v}_i} + \mathcal{O}(\epsilon), \end{aligned} \quad (19)$$

where \mathcal{V}_i is the set in which the approximation in (19) is valid. In particular, we have

$$\mathcal{V}_i = \left\{ 0 \leq v_i < \frac{T_{si} R_i}{T_i (\gamma k_{si} + R_i)} \right\}. \quad (20)$$

Lemma 3: The steady state \bar{x}_i of (16) is LAS for ϵ sufficiently small.

Proof: Substituting the $\mathcal{O}(1)$ approximation of \bar{x}_i derived in (19) into the Jacobian of (16), local stability of \bar{x}_i can be verified by Routh-Hurwitz condition. ■

Given Lemma 2 and 3, node i has well-defined I/O characteristics. Note that in (19), the zeroth order approximation

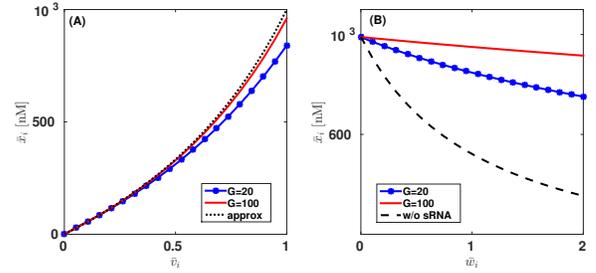


Fig. 3. Simulation of static I/O characteristics of node i in isolation with sRNA feedback using (16). (A) Static I/O characteristic from reference input (\bar{v}_i) to reference output (\bar{x}_i). (B) Static I/O characteristic from disturbance input (\bar{w}_i) to reference output (\bar{x}_i). Simulation parameters: $T_{si} = 1000[\text{nM}]^2$, $\gamma = 1[\text{hr}]^{-1}$, $\delta = 10[\text{hr}]^{-1}$, $\kappa_i = 1000[\text{nM}]$, $R_i = 10^4[\text{nM/hr}]$. In (A), $T_i = 500[\text{nM}]^2$, $w_i = 0$. In (B), for comparison purpose, value of T_i is taken such that \bar{x}_i is the same at $\bar{w}_i = 0$ for all three cases.

of reference output \bar{x}_i is independent of \bar{w}_i . Choosing \mathcal{V}_i as the admissible reference input set, according to Definition 2, we verify the desired node disturbance attenuation property in \mathcal{V}_i .

Lemma 4: For all $\bar{v}_i \in \mathcal{V}_i$, system (16) has the ϵ -static disturbance attenuation property.

In Fig. 3, we simulate the static I/O characteristics of (16). As G increases (and therefore ϵ decreases), static I/O characteristic from \bar{v}_i to \bar{x}_i becomes closer to the zeroth order approximation in (19) (Fig.3(A)). In addition, static output \bar{x}_i becomes insensitive to disturbance \bar{w}_i as G increases (Fig.3(B)).

C. Network Disturbance Coupling with sRNA Feedback

Now we consider a gene network \mathcal{N}_s^ϵ consisting of n nodes. Each node has a local sRNA feedback in the form of (16). In order to study the ϵ -well-posedness property of \mathcal{N}_s^ϵ , we first verify (A3)-(A7), and then find a network admissible input set \mathcal{V}_N , where Claim 1 can be applied. The following Lemma justifies the uniqueness of steady state in (A4).

Lemma 5: Network \mathcal{N}_s^ϵ has a unique steady state $\bar{x} = [\bar{x}_1^T, \dots, \bar{x}_n^T]^T$ for all positive integer n .

Proof: See Appendix B. ■

We adopt the notations for interconnection signals defined in Section II for \mathcal{N} when there is no ambiguity. In particular, reference input acts on each node according to (2), where \bar{v}_i has an upper bound that is independent of ϵ . We thus have (A6) verified. We defined before in (6) that $w_i = \sum_{j \neq i} d_j$, therefore, (A3) is satisfied. According to (19), when $\bar{\mathbf{v}} \in \otimes_{i=1}^n \mathcal{V}_i$, for all $i \in \mathcal{I}$, we have

$$\bar{d}_i = \frac{\bar{m}_i}{\kappa_i} = \frac{T_i k_{si} \bar{v}_i (1 + \bar{w}_i)}{T_{si} R_i - (\gamma k_{si} + R_i) T_i \bar{v}_i} + \mathcal{O}(\epsilon), \quad (21)$$

which satisfies (A7) with

$$\hat{g}_i(\bar{v}_i) = \frac{T_i k_{si} \bar{v}_i}{T_{si} R_i - (\gamma k_{si} + R_i) T_i \bar{v}_i}.$$

In order to find the network admissible reference input set \mathcal{V}_N , according to Claim 1, we need to satisfy the strictly diagonally dominant requirement of the interconnection matrix

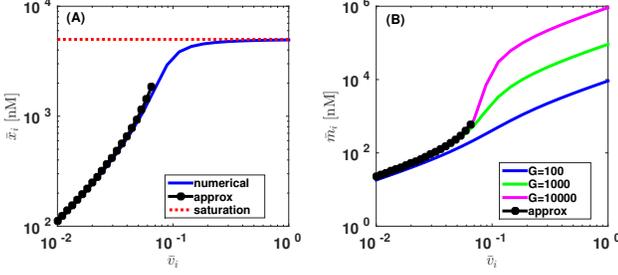


Fig. 4. Static I/O characteristics of a node with $\mathcal{V}_i = [0, 0.08]$. Approximate analytical solution within \mathcal{V}_i and numerical solution for $\bar{v}_i \leq 1$ are given in (A) and (B) for protein and mRNA concentrations, respectively.

defined in (10). To ensure \mathbf{A} is strictly diagonally dominant, we define $\mathcal{V}_{\mathcal{N}}$ as:

$$\mathcal{V}_{\mathcal{N}} := \left\{ \bar{\mathbf{v}} \in \times_{i=1}^n \mathcal{V}_i : \sum_{j \neq i} \hat{g}_j(\bar{v}_j) < 1, \forall i, j \in \mathcal{I} \right\}. \quad (22)$$

According to Claim 1, network \mathcal{N}_s^ϵ is ϵ -well-posed in $\mathcal{V}_{\mathcal{N}}$. Since disturbance attenuation property of each node has been shown in Lemma 4. As an immediate application of Claim 2, \mathcal{N}_s^ϵ has ϵ -static network disturbance disturbance decoupling property in $\mathcal{V}_{\mathcal{N}}$ for ϵ sufficiently small.

D. Admissible Reference Input Set

We have picked \mathcal{V}_i defined in (20) as the admissible input set for each node throughout our analysis. Here, we first emphasize the necessity of $\bar{v}_i \in \mathcal{V}_i$, by studying the undesirable consequences of $\bar{v}_i \notin \mathcal{V}_i$. We then discuss what physical parameters enlarge the size of \mathcal{V}_i .

When $\bar{v}_i \notin \mathcal{V}_i$, solution of (17) in series expansion of ϵ becomes

$$\bar{m}_i = \frac{T_i \bar{v}_i (\gamma k_{si} + R_i) - T_{si} R_i}{T_i \bar{v}_i \epsilon} + \mathcal{O}(1), \quad \bar{x}_i = \frac{R_i}{\gamma} + \mathcal{O}(\epsilon). \quad (23)$$

In (23), static reference output \bar{x}_i becomes independent of the reference input \bar{v}_i , and mRNA concentration is on the scale of $\mathcal{O}(1/\epsilon)$ (see Fig. 4 (A), (B)). In this scenario, target protein production specified by \bar{v}_i is beyond the maximum gene expression capability of the node: although a large amount of m_i (control input) has been produced, target protein production still couldn't be reached due to limitation of ribosomes (actuator saturation). This is a biological analogy to integrator windup in the control literature [23].

Similarly, in a network setting, according to (22), a fundamental trade-off in our design is that increasing the number of nodes n shrinks the size $\mathcal{V}_{\mathcal{N}}$. This is due to the fact that free ribosomes become more scarce as we increase the number of nodes.

According to (20), the size of \mathcal{V}_i increases with the maximum transcription rate of sRNA (T_{si}), while decreases with the basal transcription rate of gene i (T_i). Both parameters (T_i and T_{si}) can be tuned by gene (sRNA) copy number and promoter strength. The size of \mathcal{V}_i also increases with the total amount of ribosomes ($\propto R_i$), and the binding strength of x_i with p_{si} ($1/k_{si}$).

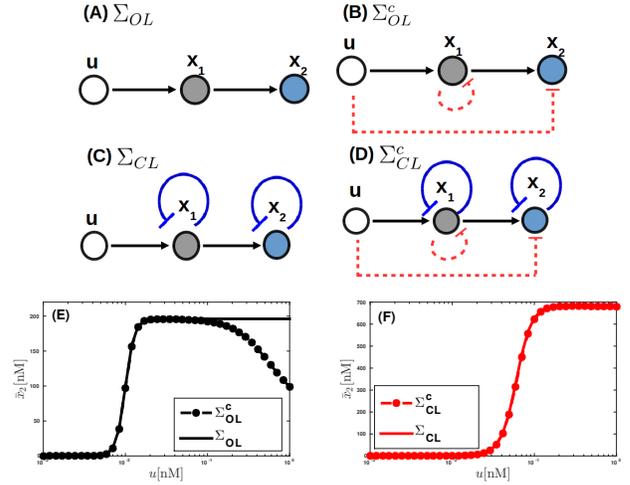


Fig. 5. (A)-(D) Interaction graph of the four networks we simulated. Black arrows represent transcriptional regulations, red dashed arrows are the hidden interactions arising from ribosome limitations, and blue arrows represent the feedback loops through sRNA. (E) Static I/O characteristic of systems Σ_{OL} and Σ_{OL}^c . (F) Static I/O characteristics of systems Σ_{CL} , Σ_{CL}^c . Simulation parameters: $T_1 = 1000[\text{nM}]^2$, $T_2 = 100[\text{nM}]^2$, $T_{s1} = 1200[\text{nM}]^2$, $T_{s2} = 120[\text{nM}]^2$, $R_1 = R_2 = 10^4[\text{nM}/\text{hr}]$, $k_{s1} = k_{s2} = 200[\text{nM}]$, $\kappa_1 = 100[\text{nM}]$, $\kappa_2 = 10^3[\text{nM}]$, $\delta = 5[\text{hr}]^{-1}$, $\gamma = 1[\text{hr}]^{-1}$, $k_1 = 1[\text{nM}]$, $k_2 = 2[\text{nM}]$, $n_1 = 2$, $n_2 = 4$.

V. APPLICATION TO AN ACTIVATION CASCADE

A two-stage activation cascade is composed of a TF input (u) activating node x_1 , which serves as a transcriptional activator for the output node x_2 . With only transcriptional regulations, an activation cascade is expected to have positive I/O response from u to x_2 [16]. However, in [9], we showed that hidden interactions arising from resource limitations can make the response of a two-stage activation cascade to become biphasic.

To demonstrate the effects of sRNA distributed feedback, we compare the static I/O characteristics of four activation cascades: Σ_{OL} , Σ_{OL}^c , Σ_{CL} and Σ_{CL}^c , shown in Fig. 5 (A)-(D), respectively. Σ_{OL} is a fictitious activation cascade where nodes are not competing for ribosomes. Σ_{OL}^c is the cascade where ribosomes are shared among nodes. Dynamics of node i ($i = 1, 2$) in Σ_{OL} and Σ_{OL}^c are in the form of (1) and (5), respectively. Similarly, Σ_{CL} (Σ_{CL}^c) represents a cascade with distributed sRNA feedback without (with) ribosome competition. Assuming that activation is not leaky (no protein production without the activator), for all four systems, the reference inputs are specified by

$$v_1 = v_1(u) = \frac{\left(\frac{u}{k_1}\right)^{n_1}}{1 + \left(\frac{u}{k_1}\right)^{n_1}}, \quad v_2 = v_2(x_1) = \frac{\left(\frac{x_1}{k_2}\right)^{n_2}}{1 + \left(\frac{x_1}{k_2}\right)^{n_2}},$$

where k_i is the dissociation constant of activator with DNA promoter region, and n_i is the cooperativity of activation at stage i . In Fig. 5 (E)-(F), we simulate the static I/O responses of the four systems. Due to the presence of ribosome competition, response of Σ_{OL}^c becomes significantly different from that of Σ_{OL} , whose model is usually used to guide design. On the contrary, responses of systems Σ_{CL} and Σ_{CL}^c are highly similar, implying that with the feedback, ribosome competition plays an almost negligible role in the static I/O response of the cascade. The benefit of distributed sRNA feedback thus lies in the fact that it preserve modularity

with respect to ribosome competition. Namely, they can be connected together in a “plug-and-play” fashion through transcriptional regulation, and hidden interactions generated by ribosome competition can be neglected.

VI. DISCUSSION AND CONCLUSIONS

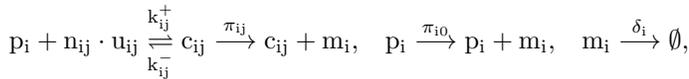
Gene expression relies on the availability of ribosomes, which are shared among all nodes in a gene network. While hidden interactions arising from ribosome limitations are often neglected in standard gene network models, they can significantly change network behaviors. In this paper, we model each node as a system with two inputs and two outputs. In addition to reference input and protein production output, ribosome demand by the rest of the network is modeled as a disturbance input to node i , and ribosome usage of node i is its disturbance output. We view the mitigation of ribosome competition effects as a static network disturbance decoupling problem, where static output of node i needs to be practically independent of the reference input to other nodes in the network. By studying the static I/O maps of each node, and the interconnection rule, we show that sRNA feedback can achieve static network disturbance decoupling, given that the reference inputs stay within an admissible input set $\mathcal{V}_{\mathcal{N}}$. Through an activation cascade example, we show that unpredictable network behaviors emerging from ribosome limitations can be eliminated through sRNA feedback. We are currently realizing the feedback system in our lab. This paper only considers static I/O responses, in future work, we plan to study the dynamical behavior of the sRNA feedback system and its performance in a gene network.

Acknowledgement: We thank Aaron Dy, Abdullah Hamadeh, Mohammad Naghnaeian and Eduardo D. Sontag for helpful discussions and suggestions.

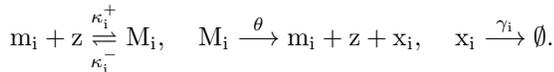
APPENDIX

A. sRNA feedback model derivation

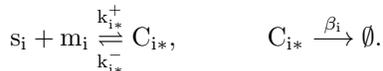
We consider a node i taking two TF inputs (u_{i1}, u_{i2}) that form complexes c_{ij} ($j = 1, 2$) with p_i . The complexes are then transcribed at a rate π_{ij} to produce mRNA (m_i). mRNA can also be transcribed at a basal rate π_{i0} , and it is diluted and degraded by RNase at a rate δ :



where $j = 1, 2$. The mRNA then binds with free ribosome (z) to produce a translationally active complex M_i , which is translated at rate θ_i to produce the protein x_i . The protein is diluted and degraded by protease at a rate γ :

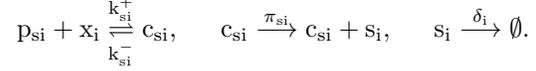


sRNA in node i (s_i) binds with mRNA to form a translationally inactive complex C_{i*} that degrades rapidly:



sRNA is activated by protein x_i to produce a complex c_{si} , which is transcribed to produce an sRNA (s_i) at rate π_{si} . We assume that the activation has cooperativity 1. s_i is diluted

and degraded by RNase at a rate δ_i :



Consequently, the concentration of each species can be described by the following ODEs:

$$\dot{c}_{ij} = k_{ij}^+ p_i u_{ij}^{n_{ij}} - k_{ij}^- c_{ij}, \quad (j = 1, 2) \quad (24a)$$

$$\dot{M}_i = \kappa_i^+ m_i z - \kappa_i^- M_i - \theta_i M_i, \quad (24b)$$

$$\dot{c}_{si} = k_{si}^+ p_{si} x_i - k_{si}^- c_{si}, \quad (24c)$$

$$\dot{C}_{i*} = k_{i*}^+ s_i m_i - k_{i*}^- C_{i*} - \beta_i C_{i*}, \quad (24d)$$

$$\begin{aligned} \dot{m}_i = & \sum_{j=1,2} \pi_{ij} c_{ij} + \pi_{i0} p_i - \delta m_i - \kappa_i^+ m_i z + \kappa_i^- M_i \\ & + \theta_i M_i - k_{i*}^+ s_i m_i + k_{i*}^- C_{i*}, \end{aligned} \quad (24e)$$

$$\dot{s}_i = \pi_{si} c_{si} - \delta s_i - k_{i*}^+ s_i m_i + k_{i*}^- C_{i*}, \quad (24f)$$

$$\dot{x}_i = \theta_i M_i - \gamma x_i - k_{si}^+ p_{si} x_i + k_{si}^- c_{si}. \quad (24g)$$

Assuming p_i^t and p_{si}^t are constants [16], we have

$$p_i^t = p_i + \sum_{j=1,2} c_{ij}, \quad \text{and} \quad p_{si}^t = p_{si} + c_{si}. \quad (25)$$

Setting equations (24a) to (24d) to QSS, complex concentrations can be obtained as follows:

$$c_{ij} = \frac{p_i u_{ij}^{n_{ij}}}{k_{ij}}, \quad M_i = \frac{m_i z}{\kappa_i}, \quad c_{si} = \frac{p_{si} x_i}{k_{si}}, \quad C_{i*} = \frac{s_i m_i}{k_{i*}}, \quad (26)$$

where $j = 1, 2$, and we have defined the following effective dissociation constants:

$$k_{ij} := \frac{k_{ij}^-}{k_{ij}^+}, \quad \kappa_i := \frac{\kappa_i^- + \theta_i}{\kappa_i^+}, \quad k_{si} := \frac{k_{si}^-}{k_{si}^+}, \quad k_{i*} := \frac{k_{i*}^- + \beta_i}{k_{i*}^+}.$$

Using equations (26) and (25), the dynamics of our target node can be re-written as:

$$\begin{aligned} \dot{m}_i = & p_i^t \frac{\pi_{i0} + \sum_{j=1,2} \pi_{ij} (u_{ij}/k_{ij})^{n_{ij}}}{1 + \sum_{j=1,2} (u_{ij}/k_{ij})^{n_{ij}}} - \delta m_i - \frac{\beta_i}{k_{i*}} m_i s_i, \\ \dot{s}_i = & p_{si}^t \pi_{si} \frac{x_i/k_{si}}{1 + x_i/k_{si}} - \delta s_i - \frac{\beta_i}{k_{i*}} m_i s_i, \\ \dot{x}_i = & \frac{\theta_i}{\kappa_i} m_i z - \gamma x_i. \end{aligned} \quad (27)$$

Due to ribosome competition, the free amount of ribosome z can be written as

$$z = \frac{z_t}{1 + m_i/\kappa_i + \sum_{j \neq i} m_j/\kappa_j}. \quad (28)$$

Substitute (28) into (27), let $G_i := \beta_i/k_{i*}$ be the effective sRNA repression rate, and define $T_{ij} := p_i^t \pi_{ij}/G_i$, we obtain the model in (16). For simplicity of analysis, we assume that $G_i = G$ for all i , and the dilution rates of mRNA and protein are the same for all nodes.

B. Sketch of the Proof of Lemma 5

Proof: Let $F_i(m_1, \dots, m_n) := -\epsilon \frac{T_i \bar{v}_i}{m_i} + \epsilon m_i - T_i \bar{v}_i + \epsilon^2 + \frac{T_{si} R_i m_i}{\gamma k_{si} \kappa_i (1 + \sum_{j \neq i} m_j/\kappa_j) + (\gamma k_{si} + R_i) m_i}$, steady state mRNA concentration of each node i , \bar{m}_i , can be found from the following n equations:

$$F_i(\bar{m}_1, \bar{m}_2, \dots, \bar{m}_n) = 0, \quad \forall i \in \mathcal{I}. \quad (29)$$

To show (29) has a unique solution for any positive integer n , we use induction. The idea is to use the first k ($1 \leq k \leq n$) equations, to uniquely find \bar{m}_k as a function of $\bar{m}_{k+1}, \dots, \bar{m}_n$. When we continue the induction to

$k = n$, we have a unique solution of \bar{m}_n , and consequently $\bar{m}_1, \dots, \bar{m}_{n-1}$, using results from previous induction steps.

When $k = 1$, regarding $\bar{m}_2, \dots, \bar{m}_n$ as positive parameters, according to Lemma 2, there exists function $f_1^1(\cdot) : \mathbb{R}^{n-1} \rightarrow \mathbb{R}_+$ such that $\bar{m}_1 = f_1^1(\bar{m}_2, \dots, \bar{m}_n)$. Now suppose that using the first $(k-1)$ equations in (29), we can find functions $f_j^{k-1} : \mathbb{R}^{n-k+1} \mapsto \mathbb{R}_+$ such that

$$\bar{m}_j = f_j^{k-1}(\bar{m}_k, \bar{m}_{k+1}, \dots, \bar{m}_n), \quad \forall j = 1, \dots, (k-1).$$

Regarding $\bar{m}_{k+1}, \dots, \bar{m}_n$ as positive parameters, with abuse of notation, we write $\bar{m}_j = f_j^{k-1}(\bar{m}_k)$, and $F_j(\bar{m}_1, \dots, \bar{m}_n) = F_j(\bar{m}_1, \dots, \bar{m}_k)$. To continue the induction, we need to show that there exists f_k^k such that

$$\bar{m}_k = f_k^k(\bar{m}_{k+1}, \dots, \bar{m}_n).$$

For this purpose, if we can show

$$\frac{d}{d\bar{m}_k} F_k(\bar{m}_1, \dots, \bar{m}_k) > 0 \quad (30)$$

for all positive \bar{m}_k , since $\text{range}(F_k) = \mathbb{R}$, there exists a unique positive \bar{m}_k such that $F_k(\bar{m}_1, \dots, \bar{m}_k) = F_k(f_1^{k-1}(\bar{m}_k), \dots, \bar{m}_k) = 0$. Note that according to the definition of F_i , if we have $P_j^k(\bar{m}_k) := \bar{m}_j / \bar{m}_k = f_j^k(\bar{m}_k) / \bar{m}_k$ decreasing monotonically with \bar{m}_k for all $j = 1, \dots, (k-1)$, then F_j increases monotonically with \bar{m}_k . Differentiating P_j^k with respect to \bar{m}_k , it is sufficient to show

$$X_j^k := \frac{d\bar{m}_j}{d\bar{m}_k} \cdot \frac{\bar{m}_k}{\bar{m}_j} < 1, \quad \forall j = 1, \dots, (k-1),$$

to guarantee that (30) holds. Applying implicit function theorem for the first $(k-1)$ equations in (29), $F_1 = 0, \dots, F_{k-1} = 0$, we obtain

$$\begin{bmatrix} \frac{\partial F_1}{\partial \bar{m}_1} \bar{m}_1 & \dots & \frac{\partial F_1}{\partial \bar{m}_{k-1}} \bar{m}_{k-1} \\ \vdots & \ddots & \vdots \\ \frac{\partial F_{k-1}}{\partial \bar{m}_1} \bar{m}_1 & \dots & \frac{\partial F_{k-1}}{\partial \bar{m}_{k-1}} \bar{m}_{k-1} \end{bmatrix} \begin{bmatrix} X_1^k \\ \vdots \\ X_{k-1}^k \end{bmatrix} = - \begin{bmatrix} \frac{\partial F_1}{\partial \bar{m}_k} \bar{m}_k \\ \vdots \\ \frac{\partial F_{k-1}}{\partial \bar{m}_k} \bar{m}_k \end{bmatrix}. \quad (31)$$

We define the following positive constants,

$$D_i := [(\gamma k_{si} + R_i) \bar{m}_i + \gamma k_{si} \kappa_i + \sum_{j \neq i} \bar{m}_j / \kappa_j]^2,$$

$$\Gamma_{ij} := \frac{T_{si} R_i \gamma k_{sj} \bar{m}_i \bar{m}_j}{D_i}, \Delta_i := \frac{T_{si} R_i \gamma k_{si} \kappa_i \bar{m}_i}{D_i} + \epsilon + \epsilon \frac{T_i \bar{v}_i}{\bar{m}_i^2}.$$

Equation (31) can be written as

$$(\mathbf{\Gamma} + \mathbf{\Delta}) \mathbf{X} = \boldsymbol{\eta}, \quad (32)$$

where $\mathbf{X}_{(i)} := X_i^k$, $\boldsymbol{\eta}_{(i)} := \Gamma_{ik}$, and

$$\mathbf{\Gamma}_{(i,j)} = \begin{cases} \sum_{q \neq i} \Gamma_{iq}, & i = j, \\ -\Gamma_{ij}, & i \neq j, \end{cases} \quad \mathbf{\Delta} := \text{diag}(\Delta_i).$$

Note that since $(\mathbf{\Gamma} + \mathbf{\Delta})$ is strictly diagonally dominant, we have $\mathbf{X} > 0$. Furthermore, $\mathbf{\Gamma} \mathbf{e} = \boldsymbol{\eta}$, where $\mathbf{e} = [1, \dots, 1]^T$. Therefore the solution satisfies,

$$\mathbf{X} = \mathbf{\Gamma}^{-1} \boldsymbol{\eta} - \mathbf{\Delta} \mathbf{X} < \mathbf{e}.$$

Therefore, we have shown (30) holds, and we have a unique positive $\bar{m}_k = f_k^k(\bar{m}_{k+1}, \dots, \bar{m}_n)$. ■

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