Co-Design of Resource Limited Genetic Networks Tuning System Parameters to Satisfy Specifications

by

Carlos Eduardo Celeste Junior

Submitted to the Department of Mechanical Engineering
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE IN MECHANICAL ENGINEERING

at the

MASSACHUSETTS INSTITUTE OF TECHNOLOGY

May 2024

Authored by: Carlos Eduardo Celeste Junior
Department of Mechanical Engineering
May 10, 2024

Certified by: Domitilla Del Vecchio
Professor of Mechanical Engineering, Thesis Supervisor

Accepted by: Nicolas Hadjiconstantinou
Professor of Mechanical Engineering
Graduate Officer, Department of Mechanical Engineering

Co-Design of Resource Limited Genetic Networks Tuning System Parameters to Satisfy Specifications

by

Carlos Eduardo Celeste Junior

Submitted to the Department of Mechanical Engineering
on May 10, 2024 in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE IN MECHANICAL ENGINEERING

ABSTRACT

Modular composition is a very powerful and widely used tool in engineering disciplines, as it aids in maintaining the system complexity tractable. Its main idea is that parts of the systems can be encapsulated into black box models characterized only by its input to output behavior, which eliminates the need to consider the complex dynamics inside the black box. Moreover, this process can be done iteratively, allowing the design of highly complex systems, such as computer chips. But this powerful tool is not always available, like in synthetic biology, where engineered systems in cells have very complex and intricate interconnections between subsystems, which makes encapsulating parts of theses systems a very challenging endeavor. There are many reasons for this failure in modularity in biological systems, such as load effects (retroactivity), unknown interactions and resource competition, which is our focus for this work. Recent efforts to achieve modular design in systems with resource competition, have focused in adding additional machinery to the cell to either try to isolate the subsystems or control the availability of the shared resource. In this work we explore a co-design approach, where instead of adding additional machinery to the cell, we aim to tune some systems parameters to satisfy some specification. To this end we provide conditions on the systems parameters for a network of subsystems to meet a given specification, which are derived using mathematical logic and ideas on how to tackle similar problems. With this, this work lays the foundations for further development of co-design techniques for genetic networks with production and/or degradation resources, where one may be able to mitigate the effects of one type of resource sharing by tuning the other.

Thesis supervisor: Domitilla Del Vecchio
Title: Professor of Mechanical Engineering

Acknowledgments

I would like to thank the Air Force Office of Scientific Research (AFOSR) for the financial support of this work under Grant # FA9550-22-1-0316. I would also like to thank Ilaria Di Loreto and Theodore Wu Grunberg for the valuable ideas and contributions to this work. Finally I would also like to thank my family, friends and girlfriend for the continued support during the development of this work.

Contents

List of Figures

List of Tables

Chapter 1

Introduction

Modular composition is a very powerful technique in the design of complicated systems, and is commonly used in many traditional engineering disciplines, such as electrical engineering, mechanical engineering and computer systems. In this technique, systems are characterized by its input/output behavior, with the additional assumption that the systems don't interact when composed together. To this end, many engineering fields have gone to great lengths to develop tools to isolate this modules from outside influences. In synthetic biology, modular composition of systems is a very challenging endeavor because, in the cell, there are many interactions among subsystems, which go beyond what we regard as the regulatory inputs and outputs that we use for connecting systems to one another [1], [2], and the connectivity among subsystems is often difficult to identify [3]. There are many reasons for the failure of modularity in biological circuits, such as the effect of loads (retroactivity) on a system output caused by downstream circuits [4], [5], [6], unknown interactions between adjacent genetic sequences and factors [7], [8], [9], [10], as well as resource competition between systems [2], [3], [11], [12], [13], more specifically competition for limited cellular resources needed for gene expression [14], [15] and for protein degradation [16], affect system performance in surprising ways [17].

In this work, we focus on the failure of modularity due to resource sharing. Prior work on this topic experimentally demonstrated how two genetic modules become coupled when they become activated concurrently in the cell even when they are not connected through regulatory links [12]. Related work has further shown that this is the case even if one of the genetic modules is placed on the chromosome [13], highlighting even more this problem as a global perturbation to all genes in the cell. Previous efforts to mitigate the undesired effects of resource sharing have concentrated on two approaches [2], namely, centralized control of a shared resource and decentralized control of subsystems. The centralized control approach aims to maintain the free resource level at a constant value [18], [19]. On the other hand, the decentralized control approach focuses on isolating the module from perturbations in cellular resources [20], [21], [22]. More specifically, for genetic circuits, wherein more genetic modules are connected to each other through regulatory links, competition for resources among the modules leads to surprising emergent circuit behavior and mathematical models were introduced that well predict experimental outcomes [15]. These experimentally validated models were later adopted in a theoretical study aiming at designing local feedback controllers to insulate genetic modules from one another [22]. This line of work followed the general idea

of capturing resource transactions through disturbance inputs to each genetic module and to solve a disturbance attenuation problem [21], [20].

Our goal in this work is to design networks of subsystems that adhere to a specification even in the presence of undesired coupling caused by resource sharing, be it production resources, such as ribosomes or RNAPs, and/or degradation resources, such as microRNAs and proteases. Moreover, we utilize the I/O framework proposed in [22], where each black box system is characterized by its input/output behavior and also additional disturbance outputs and disturbance inputs. These additional outputs capture the cumulative load that the system applies on shared resources, while the additional inputs capture the cumulative load that all other systems apply on the shared resources. Part of this work was published in [23], where only production (ribossomes) resource sharing is considered, and another part, which considers production (ribossomes) and degradation (proteases) resource sharing is under review.

Chapter 2

Production Resource Sharing

2.1 PROBLEM FORMULATION

The system model we consider in this paper, for the process of gene expression [14], is depicted in Fig.2.1. This model describes the protein production process, while accounting for the fact that multiple such systems all share ribosomes required for gene expression [15], [12]. In what follows, we use the standard notation, in which for a species S we let *italics S* denote its concentration.

The i-th subsystem is responsible for the expression of the i-th gene, where the mRNA $\mathrm{m_i}$ is transcribed at a rate r_i, which is then translated into protein $\mathrm{p_i}$. So, we define the i-th subsystem states $x_i = [m_i \ p_i]' \in \mathbb{R}^2_+$, with input $u_i = r_i \in \mathbb{R}_+$ and output $y_i = p_i \in \mathbb{R}_+$, as well as disturbance input $w_i \in \mathbb{R}_+$ and output $d_i \in \mathbb{R}_+$. With this, the subsystem dynamics are given by [22]

$$\begin{aligned} \dot{m}_i &= u_i - \delta_0 m_i \\ \dot{p}_i &= \alpha_i \frac{(m_i/k_i)}{1 + (m_i/k_i) + w_i} - \delta p_i \\ y_i &= p_i \\ d_i &= m_i/k_i, \end{aligned} \tag{2.1}$$

for $i = \{1, \ldots, N\}$. Here, α_i is the translation rate constant, k_i is the dissociation constant of mRNA binding with ribosome, δ is the decay rate constant of the protein, δ_0 is the decay rate constant of mRNA. All parameters are strictly positive.

The disturbance input w_i and disturbance output d_i capture the unintended interactions among subsystems. Specifically, this model was derived in [22] and captures the fact that ribosomes are required in the translation step, where the mRNA binds to ribosomes to be translated to protein, which causes a "load" on the ribosome pool. In particular, the larger m_i and the smaller k_i (stronger ribosome binding site), the larger the load $d_i = m_i/k_i$ that subsystem Σ_i applies to ribosomes. Because the decrease of translation rate that system Σ_i experiences results from the overall load that all subsystems apply to ribosomes, we have that the disturbance input is given by

$$w_i = \sum_{j \neq i} d_j, \tag{2.2}$$

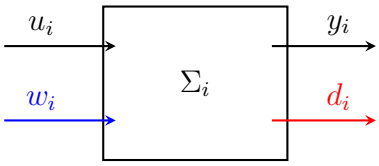

Figure 2.1: Block diagram representation of subsystem Σ_i.

which represents the effect that load on ribosomes from all other subsystems has on the i-th subsystem. The full derivation of this model can be found in [22].

Since in this paper we are interested in guarantees on the steady state behavior of N interconnected systems, we first prove uniqueness and stability of the equilibrium point.

Lemma 1. *The network of systems Σ_i as given in (2.1), with interconnection (2.2), admits a unique equilibrium point. Furthermore, this equilibrium point is locally asymptotically stable for all parameter values.*

Proof. System Σ_i equilibrium point is given by

$$\begin{aligned} m_{i,e} &= u_i^*/\delta_0 \\ p_{i,e} &= \frac{(\alpha_i/\delta)(u_i^*/k_i\delta_0)}{1+\sum_{j=1}^{N}(u_j^*/k_j\delta_0)}. \end{aligned}$$

For all parameters and fixed inputs $u_i = u_i^*$, this equilibrium point is unique. Now to conclude about its stability lets define the column vector $\xi = \big((m_1 - m_{1,e}), \ldots, (m_N - m_{N,e}), (p_1 - p_{1,e}), \ldots, (p_N - p_{N,e})\big)$ and linearize the system about its equilibrium, which yields $\dot{\xi} = A\xi$, where A is defined as

$$A = \begin{bmatrix} -\text{diag}\big(\delta_0, \ldots, \delta_0\big) & 0 \\ c & -\text{diag}\big(\delta, \ldots, \delta\big) \end{bmatrix},$$

with $c \in R^{N\times N}$ block matrix, which has entries $\{c\}_{i,j}$ given by

$$\{c\}_{i,j} = \begin{cases} \frac{\alpha_i}{k_i}\frac{1+\sum_{n\neq i}(m_{n,e}/k_n)}{(1+\sum_{n=1}^{N}(m_{n,e}/k_n))^2}, & \text{if } i = j \\ -\frac{\alpha_i}{k_j}\frac{(m_{i,e}/k_i)}{(1+\sum_{n=1}^{N}(m_{n,e}/k_n))^2}, & \text{otherwise.} \end{cases}$$

As A is a lower triangular matrix, its eigenvalues are given by its diagonal entries, which are all negative and equal to $-\delta$ and $-\delta_0$. Therefore, we conclude that the network of subsystems Σ_i as given in (2.1), with interconnection rule (2.2), is locally asymptotically stable.

□

We are interested in steady state behavior, so we consider the following input/output steady state characteristic of system Σ_i:

$$y_i = \frac{\alpha_i}{\delta}\frac{(u_i/\delta_0 k_i)}{1+(u_i/\delta_0 k_i)+w_i} \tag{2.3}$$

$$d_i = u_i/\delta_0 k_i, \tag{2.4}$$

and let y_i^* be the output of the isolated system with $u_i = r_i^* > 0$, i.e., y_i in (2.3) with $w_i = 0$, and nominal parameter values $\alpha_i = \alpha_i^*$, $\delta_i = \delta^*$, $\delta_0 = \delta_0^*$, and $k_i = k_i^* \in K_i$ with $K_i \subset \mathbb{R}^+$. Now let us define the disturbance steady state I/O maps $f_i : w_i \to d_i$ as

$$d_i = f_i(w_i) = \gamma_i w_i + \gamma_i, \quad \gamma_i = \frac{\delta y_i}{\alpha_i - \delta y_i}. \tag{2.5}$$

With this, our system specification is given as follows:

Specification: Given $u_i = r_i^*$, y_i^*, and fixed tolerances $\varepsilon_i > 0$, $i = \{1, \dots, N\}$. The specifications on the connected systems given in (2.2), (2.3), (2.4) are given in the form

$$y_i \in [y_i^* - \varepsilon_i, y_i^* + \varepsilon_i], \ i \in \{1, ..., N\}. \tag{2.6}$$

Remark 1. *The systems gains γ_i are monotonically increasing with respect to y_i, hence, the steady state I/O maps $d_i = f_i(w_i)$ are monotonically increasing with respect to y_i. In fact, if $y_i^* - \varepsilon_i \leq y_i^* + \varepsilon_i$, then $\tilde{\gamma}_i \leq \hat{\gamma}_i$ and also $f_i|_{(y_i^* - \varepsilon_i)} \leq f_i|_{(y_i^* + \varepsilon_i)}$.*

Based on this specification, we seek to tackle two problems. First, we seek to determine sufficient conditions on the systems' parameters to satisfy this specification (Problem 1). The second problem is to design the systems such that the specification is met (Problem 2). For this problem, we regard the ribosome binding site strengths, captured by parameters $1/k_i$ (see [14]) as the design parameters since they are easily and quantitatively tunable.

Problem 1 (**Feasibility**). *Given a network of N subsystems of the form (2.1) and connection rule (2.2). Determine sufficient conditions on each subsystem parameters such that the specification is met for all subsystems. That is, there exists tunable parameters $k_i \geq 0, i \in \{1, \dots, N\}$ such that (2.6) is satisfied.*

The practical relevance of this problem stands in the fact that once multiple systems are concurrently operating in the cell, they may not be able to achieve their nominal outputs as they do in isolation because of decreased availability of gene expression resources to each of them. Therefore, we investigate to what extent it is still possible to meet the specifications as the number of subsystems increases and as the tolerance is changed. Indeed, it is reasonable to expect that with more systems, one may require a larger tolerance and hence a larger degradation of the system specification.

Problem 2 (**Feasible Region**). *With all other parameters fixed, compute the region for the parameters $(k_1, \dots, k_N) \in K_1 \times \cdots \times K_N$ such that the specification is met.*

With this, we define the quantities

$$\tilde{\gamma}_i = \frac{\delta(y_i^* - \varepsilon_i)}{\alpha_i - \delta(y_i^* - \varepsilon_i)} \tag{2.7}$$

$$\hat{\gamma}_i = \frac{\delta(y_i^* + \varepsilon_i)}{\alpha_i - \delta(y_i^* + \varepsilon_i)}. \tag{2.8}$$

Lemma 2. *The following conditions*

$$\begin{cases} d_i \geq \tilde{\gamma}_i w_i + \tilde{\gamma}_i & (2.9) \\ d_i \leq \hat{\gamma}_i w_i + \hat{\gamma}_i, & (2.10) \end{cases}$$

are equivalent to those in (2.6).

Proof. We start by showing that (2.6) implies (2.9)-(2.10). The specifications given in (2.6) define lower and upper bounds on the output y_i, based on the tolerances ε_i. With this, we can substitute these bounds on γ_i as defined in (2.5), yielding

$$\tilde{\gamma}_i \leq \gamma_i \leq \hat{\gamma}_i, \tag{2.11}$$

with $\tilde{\gamma}_i$ as given in (2.7) and $\hat{\gamma}_i$ as given in (2.8). Now we substitute this into the steady state I/O map given in equation (2.5), resulting in

$$\tilde{\gamma}_i w_i + \tilde{\gamma}_i \leq \gamma_i w_i + \gamma_i \leq \hat{\gamma}_i w_i + \hat{\gamma}_i$$

in which we substitute d_i as defined in (2.5), resulting in

$$\tilde{\gamma}_i w_i + \tilde{\gamma}_i \leq \quad d_i \quad \leq \hat{\gamma}_i w_i + \hat{\gamma}_i,$$

which are the conditions presented in (2.9)-(2.10).

Now we show that (2.9)-(2.10) implies (2.6). We start by rewriting the inequalities (2.9)-(2.10) in the following form

$$\tilde{\gamma}_i w_i + \tilde{\gamma}_i \leq \quad d_i \quad \leq \hat{\gamma}_i w_i + \hat{\gamma}_i,$$

in which we substitute d_i as defined in (2.5), resulting in

$$\tilde{\gamma}_i w_i + \tilde{\gamma}_i \leq \gamma_i w_i + \gamma_i \leq \hat{\gamma}_i w_i + \hat{\gamma}_i.$$

Now we divide all terms by $(1 + w_i)$, yielding

$$\tilde{\gamma}_i \leq \gamma_i \leq \hat{\gamma}_i,$$

which from the monotonicity of γ_i with respect to y_i, implies that $y_i^* - \varepsilon_i \leq y_i \leq y_i^* + \varepsilon_i, \forall i \in \{1, \ldots, N\}$, which gives the specifications given in (2.6).

□

2.2 PROBLEM SOLUTION

We tackle Problem 1 first, that is, we want to determine if there exist parameters $(k_1, \ldots, k_n)$ such that our steady state output y_i stays in the prescribed region around y_i^*, with tolerances ε_i.

Let $w = (w_1, \ldots, w_N)$ and $d = (d_1, \ldots, d_N)$, then (2.2) implies that

$$w = Td,$$

with $T \in \mathbb{R}^{N \times N}$ the interconnection matrix defined as

$$\{T\}_{i,j} = \begin{cases} 0, & \text{if } i = j \\ 1, & \text{otherwise.} \end{cases} \tag{2.12}$$

In turn, (2.5) with $y_i = y_i^* - \varepsilon_i$ can we rewritten in vector form as

$$d = \tilde{\gamma} w + \underline{\tilde{\gamma}},$$

in which where the gain vector $\underline{\tilde{\gamma}} \in \mathbb{R}^N$ is defined as $\underline{\tilde{\gamma}} = (\tilde{\gamma}_1, \ldots, \tilde{\gamma}_N)$, the gain matrix $\tilde{\gamma} \in \mathbb{R}^{N \times N}$ is defined as

$$\{\tilde{\gamma}\}_{i,j} = \begin{cases} \tilde{\gamma}_i = \frac{\delta(y_i^* - \varepsilon_i)}{\alpha_i - \delta(y_i^* - \varepsilon_i)}, & \text{if } i = j \\ 0, & \text{otherwise.} \end{cases} \tag{2.13}$$

The following Theorem provides a sufficient condition to solve Problem 1. For a matrix A, we let $\rho(A)$ denote the spectral radius of A.

Theorem 1. *Let $\tilde{\gamma}$ be the gain matrix defined in (2.13), and let T be the interconnection matrix defined in (2.12). If $\rho(\tilde{\gamma}T) < 1$, then Problem 1 has a solution.*

Proof. By Lemma 2, satisfaction of the specification is equivalent to (2.9)-(2.10) with $d_i \geq 0$. We then focus on providing sufficient conditions for (2.9)-(2.10) to be satisfied.

Let us consider just the constraints of the form (2.9), which, given the matrices T and $\tilde{\gamma}$, defined in (2.12) and (2.13), can be rewritten as

$$d \geq \tilde{\gamma} T d + \underline{\tilde{\gamma}} \iff (I - \tilde{\gamma} T) d \geq \underline{\tilde{\gamma}},$$

where $d = (d_1, \ldots, d_N)$ and $\underline{\tilde{\gamma}} = (\tilde{\gamma}_1, \ldots, \tilde{\gamma}_N)$. Since $\underline{\tilde{\gamma}} \geq 0$ and $d_i \geq 0$, for all i, must hold from the definition of the models, we have that if $(I - \tilde{\gamma}T)^{-1} \geq 0$, i.e., $(I - \tilde{\gamma}T)^{-1}$ has non-negative entries, then

$$d \geq (I - \tilde{\gamma}T)^{-1} \underline{\tilde{\gamma}} \geq 0 \Rightarrow d \geq 0. \tag{2.14}$$

A sufficient condition to prove that $(I - \tilde{\gamma}T)^{-1} \geq 0$ is given by checking that $(I - \tilde{\gamma}T)$ is an M-matrix ([24]). Now, given that $M = (I - \tilde{\gamma}T)$ is such that $\{M\}_{i,j} \leq 0$ for all $i \neq j$, and given that $\tilde{\gamma}T \geq 0$, we can exploit the result stated in Lemma 2.5.2.1 in [24], picking $\alpha = 1$, and finally obtaining that

$$(I - \tilde{\gamma}T) \quad \text{is an } M\text{-matrix} \quad \iff 1 > \rho(\tilde{\gamma}T).$$

We can conclude that if $1 > \rho(\tilde{\gamma}T)$, then $(I - \tilde{\gamma}T)^{-1} \geq 0$ and hence (2.9) is satisfied for $d_i \geq 0$. Now, we prove the satisfaction of the conditions in (2.10). Let us consider values for d_i, such that, $d_i = \tilde{\gamma}_i w_i + \tilde{\gamma}_i$. By plugging these expressions for d_i into (2.10), we obtain

$$w_i(\hat{\gamma}_i - \tilde{\gamma}_i) + (\hat{\gamma}_i - \tilde{\gamma}_i) \geq 0,$$

which is always true for $(k_1, \ldots, k_N)$ when (2.14) is satisfied, given that $\tilde{\gamma}_i \leq \hat{\gamma}_i$. To conclude, we have shown that if $\rho(\tilde{\gamma}T) < 1$, then (2.6) is satisfied. Therefore, Problem 1 has a solution. □

With this we can move on to Problem 2, where we want to find the feasible region for the systems parameters $(k_1, \ldots, k_N)$. We consider first $N = 2$ as an illustrative example and then propose a general algorithm for arbitrary N.

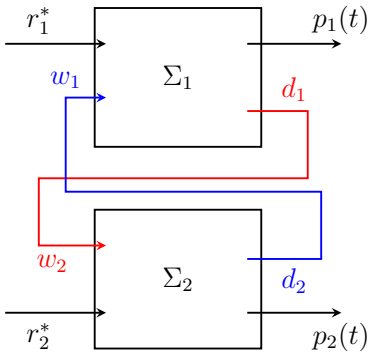

Figure 2.2: Example $N = 2$ subsystem network block diagram.

2.2.1 Illustrative Example

In the case in which $N = 2$, the system network takes the simple form shown in Fig. 2.2. In this case, we have $w_1 = d_2$ and $w_2 = d_1$. The gains of the subsystems, for $y_1 = y_1^* - \varepsilon_1$, $y_2 = y_2^* - \varepsilon_2$ are given by $\tilde{\gamma}_1 = \delta(y_1^* - \varepsilon_1)/(\alpha_1 - \delta(y_1^* - \varepsilon_1))$ and $\tilde{\gamma}_2 = \delta(y_2^* - \varepsilon_2)/(\alpha_2 - \delta(y_2^* - \varepsilon_2))$. The gain matrix $\tilde{\gamma} = \text{diag}\big((\tilde{\gamma}_1, \tilde{\gamma}_2)\big)$ and the interconnection matrix $T = U - I$, where U is the unitary matrix, with ones in all elements and I is the identity matrix. The eigenvalues of $\tilde{\gamma}T$ are given by $\lambda_1 = \sqrt{\tilde{\gamma}_1\tilde{\gamma}_2}$ and $\lambda_2 = -\sqrt{\tilde{\gamma}_1\tilde{\gamma}_2}$. Then, $\rho(\tilde{\gamma}T) = \sqrt{\tilde{\gamma}_1\tilde{\gamma}_2}$. As a consequence, for a solution to Problem 1 to exist, it is sufficient that $\sqrt{\tilde{\gamma}_1\tilde{\gamma}_2} < 1$. We next compute the region of $(1/k_1, 1/k_2)$ that ensures $\sqrt{\tilde{\gamma}_1\tilde{\gamma}_2} < 1$.

To compute the feasible region, we first substitute (2.2) and (2.4) in (2.9)-(2.10), to obtain these inequalities in terms of $(1/k_1, 1/k_2)$

$$\tilde{\gamma}_1\left(\frac{u_2}{\delta_0}\cdot\frac{1}{k_2}\right) + \tilde{\gamma}_1 \leq \left(\frac{u_1}{\delta_0}\cdot\frac{1}{k_1}\right) \leq \hat{\gamma}_1\left(\frac{u_2}{\delta_0}\cdot\frac{1}{k_2}\right) + \hat{\gamma}_1 \tag{2.15}$$

$$\tilde{\gamma}_2\left(\frac{u_1}{\delta_0}\cdot\frac{1}{k_1}\right) + \tilde{\gamma}_2 \leq \left(\frac{u_2}{\delta_0}\cdot\frac{1}{k_2}\right) \leq \hat{\gamma}_2\left(\frac{u_1}{\delta_0}\cdot\frac{1}{k_1}\right) + \hat{\gamma}_2, \tag{2.16}$$

where $\tilde{\gamma}_i$ is as defined in (2.7) and $\hat{\gamma}_i$ is as defined in (2.8). Then, if $\tilde{\gamma}_1$ and $\tilde{\gamma}_2$ satisfy $\sqrt{\tilde{\gamma}_1\tilde{\gamma}_2} < 1$, we can compute the $(1/k_1, 1/k_2)$ feasible region directly from the inequalities (2.15)-(2.16), which is a linear program in the variables $(1/k_1, 1/k_2)$.

One possible solution is shown in Fig.2.3 in terms of $1/k_1$ and $1/k_2$, that is, the polygon in cyan contain all the points $(1/k_1, 1/k_2)$ for which the specification given in (2.6) holds. What we obtain is that inside the feasible region, we can decrease concurrently both k_1 and k_2, so that d_1 and d_2 also will increase. This, in turn, implies that also p_1, p_2 will increase, keeping on satisfying the specifications. On the other hand, on the boundaries of the feasible region, we can decrease either k_1 or k_2, in order to preserve the satisfaction of the specifications.

2.2.2 General Solution to Problem 2

Now we consider the general case in which we have N subsystems and provide an algorithm to determine the feasible region while allowing to change the tolerance ε_i. Suppose we have a network composed of N subsystems, with prescribed outputs $y_i^* = p_i^*$, with fixed input

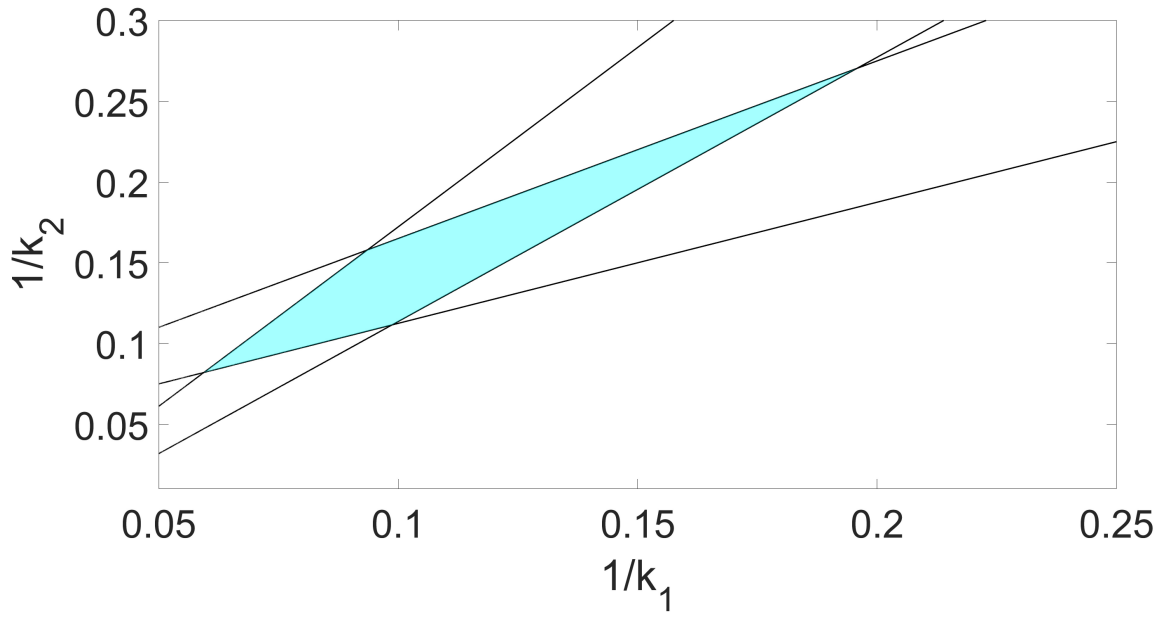

Figure 2.3: Feasible region for $1/k_1$ and $1/k_2$, with $r_i^* = (1\ 1)$ $[nM]$, $y_i^* = (2\ 2)$ $[nM]$, $\varepsilon_i = 0.1y_i^* = (0.2\ 0.2)$ $[nM]$, $\alpha_i = (5.8\ 4.2)$ $[nM/hr]$, $\delta = 1$ $[1/hr]$, $\delta_0 = 0.05$ $[1/hr]$, which yields $\tilde{\gamma}_i = (0.45\ 0.75)$ and $\hat{\gamma}_i = (0.61\ 1.10)$.

$u_i = r_i^* > 0$ and tolerances ε_i, with $\tilde{\gamma}_i$ and $\hat{\gamma}_i$ defined in (2.7) and (2.8), respectively, for fixed parameter values α_i, δ and δ_0. Our goal is to find the feasible region for $1/k_i$, $i \in \{1, ..., N\}$.

In order to achieve this, we consider the inequalities in (2.9)-(2.10), as they describe the feasible region. These inequalities are linear with respect to d_i since $w_i = \sum_{j \neq i} d_j$, so we will first compute the polygon that describes the feasible region for d_i, by computing its vertices, then we use the linear relationship between d_i and $1/k_i$ given in (2.4) to obtain the vertices for the polygon that describes the $1/k_i$ feasible region.

To do this, we solve the following linear system of equations

$$d = \beta T d + \underline{\beta} \iff d = (I - \beta T)^{-1}\underline{\beta}, \tag{2.17}$$

where $\underline{\beta} = (\beta_1, \ldots, \beta_N)$, $d = (d_1, \ldots, d_N)$, $\beta = \text{diag}(\underline{\beta})$ and T is as defined in (2.12). It is important to note that the conditions from Theorem 1 guarantee that the matrix $(I - \beta T)$ is invertible. We define β_i as having two possible values, $\tilde{\gamma}_i$, as given in (2.7), or $\hat{\gamma}_i$, as given in (2.8) for $i \in \{1, \ldots, N\}$. We then solve (2.17) for all possible $(\beta_1, ..., \beta_N)$ tuples such that $\beta_i = \tilde{\gamma}_i$ or $\beta_i = \hat{\gamma}_i$. Then, to find the vertices for the $1/k_i$ feasible region we use the relationship $1/k_i = \delta_0 d_i/u_i$ that comes from (2.4).

Next, to aid in the choice of the tolerance ε_i, we introduce a minimization problem that returns suitable values ε_i, $\tilde{\gamma}_i$ and $\hat{\gamma}_i$, for fixed parameters α_i, δ and δ_0.

Tolerance Minimization Problem

$$\begin{aligned} \min \quad & \sum_{i=1}^{N} \varepsilon_i \\ \text{s.t.} \quad & \varepsilon_{min} \leq \varepsilon_i \leq \varepsilon_{max} \\ & \tilde{\gamma}_i = \frac{\delta(p_i^* - \varepsilon_i)}{\alpha_i - \delta(p_i^* - \varepsilon_i)} \\ & \rho(\tilde{\gamma}T) < 1 \end{aligned}$$

To solve this minimization problem we use the YALMIP toolbox for MATLAB [25]. The bounds on the tolerance ε_{min} and ε_{max} affect the size of the feasible region, which is useful in practice as it is challenging to experimentally set the values of k_i with precision. So, with this in mind, we have introduced a lower bound on the tolerance ε_i, which, in turn, makes the feasible region larger, i.e., provides a trade-off between performance and implementability of the design.

2.3 APPLICATION EXAMPLES

Let us consider an example scenario, in which we have a network composed of $N = 2$ subsystems and show the effect of the minimum tolerance ε_{min} on the feasible region. For this, we will use the following parameters, the subsystem input and output $r^* = p^* = (9,\ 1)\ [nM]$, the translation rate constant $\alpha_i = (2,\ 0.5)\ [nM/hr]$, the decay rate constant for the protein is $\delta = 0.0770\ [1/hr]$ and for the mRNA $\delta_0 = 0.0693\ [1/hr]$. Moreover, we set the maximum tolerance $\varepsilon_{max} = 0.3p^* = (2.7,\ 0.3)\ [nM]$ and for the minimum tolerance we test two different values, the first $\varepsilon_{min} = 0.1p^* = (0.9,\ 0.1)\ [nM]$ and the second $\varepsilon_{min} = 0.02p^* = (0.18,\ 0.02)\ [nM]$.

Fig.2.4 presents the $(1/k_1, 1/k_2)$ feasible region for the two values of ε_{min}. From the figure, we see that changing this variable affects the size of the feasible region, but not its shape. This occurs because as we increase ε_i we also decrease $\tilde{\gamma}_i$ and increase $\hat{\gamma}_i$. Decreasing $\tilde{\gamma}_i$ will make the $1/k_i$ coordinates of some of the vertices smaller (the ones closest to the origin in the $1/k_i$ axis). Increasing $\hat{\gamma}_i$ will make the $1/k_i$ coordinates of the remaining vertices larger (the ones furthest from the origin in the $1/k_i$ axis). Taken together, these result into the observed increase in the size of the feasible region. We conclude that ε_{min} is the parameter to be adjusted if the feasible region is too small.

Now we consider another example scenario, where we have a network composed of $N = 3$ subsystems and we wish to maintain the outputs of all subsystems around the same value of $p^* = (100,\ 100,\ 100)\ [nM]$ with a minimum tolerance of $\varepsilon_{min,i} = 0.2p_i^* = 20\ [nM]$ and a maximum tolerance of $\varepsilon_{max,i} = 0.3p_i^* = 30\ [nM]$. Moreover, the inputs $r^* = (100,\ 100,\ 100)\ [nM]$, the translation rate constant $\alpha_i = (43,\ 89,\ 62)\ [nM/hr]$, the decay rate constant for the protein is $\delta = 0.0770\ [1/hr]$ and for the mRNA $\delta_0 = 0.0693\ [1/hr]$.

Solving the minimization problem, we obtain values for the tolerance ε_i, and the gains $\tilde{\gamma}_i$ and $\hat{\gamma}_i$. Table 2.1 presents the values for these variables. Note that in this case the tolerance is the same as the minimum tolerance specified, that is, the feasible region we will obtain can be made smaller if the designer wishes and is able to implement the k_i with greater precision.

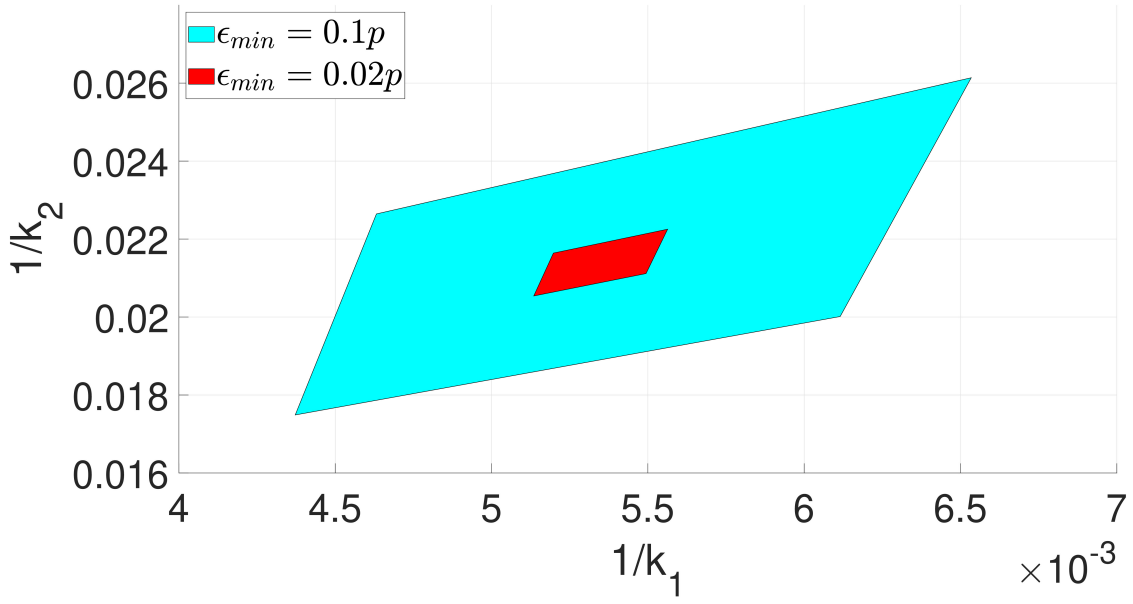

Figure 2.4: Feasible region for $1/k_i$ with different values of ε_{min}.

i	1	2	3
ε_i $[nM]$	20	20	20
$\tilde{\gamma}_i$	0.1673	0.0744	0.1103
$\hat{\gamma}_i$	0.2738	0.1159	0.1752

Table 2.1: ε_i tolerances, $\tilde{\gamma}_i$ and $\hat{\gamma}_i$ gains for the case $N = 3$ subsystems example.

Furthermore, using (2.17) we can find the vertices of the $(1/k_1, 1/k_2, 1/k_3)$ feasible region shown in Fig.2.5, where a plot of the $(1/k_1, 1/k_2, 1/k_3)$ feasible region is displayed.

Now we consider two modifications to this scenario, in the first one we want Σ_3 to increase its production to $p_3^* = 300$ $[nM]$ and in addition to this, in the second modification, we want Σ_2 to also increase its production to $p_2^* = 275$ $[nM]$. Moreover, we perform these modifications while maintaining all other parameters at their nominal values, but the tolerances ε_{min} and ε_{max} depend on the desired output values p_i^*, so the relationships remain the same, but the actual values change.

Fig.2.6 shows the effects of the modifications to the desired output levels in the feasible region, where we can see that as we demand more protein production from the systems we stretch the feasible region. This is due to the increase in p_i^*, which makes $\tilde{\gamma}_i$ and $\hat{\gamma}_i$ also increase, causing an increase in the $1/k_i$ coordinates of the vertices. This is especially true for $\hat{\gamma}_i$, which sees the largest increase due to the fact that it depends on the sum of two variables that have increased in value p_i^* and ε_i.

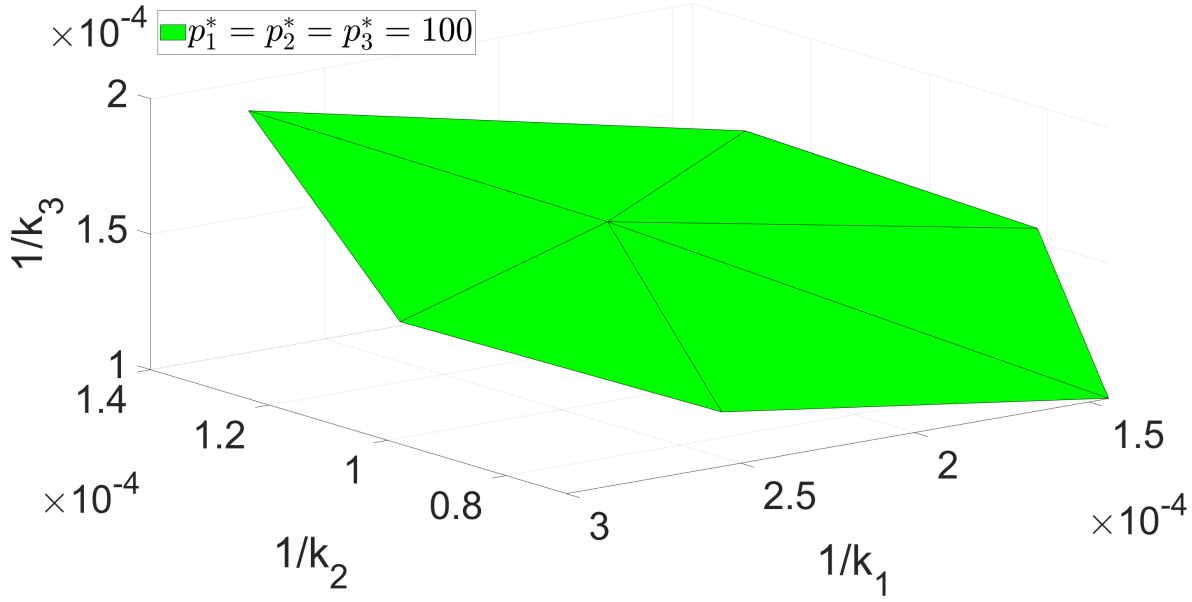

Figure 2.5: Feasible region for $1/k_1$, $1/k_2$ and $1/k_3$.

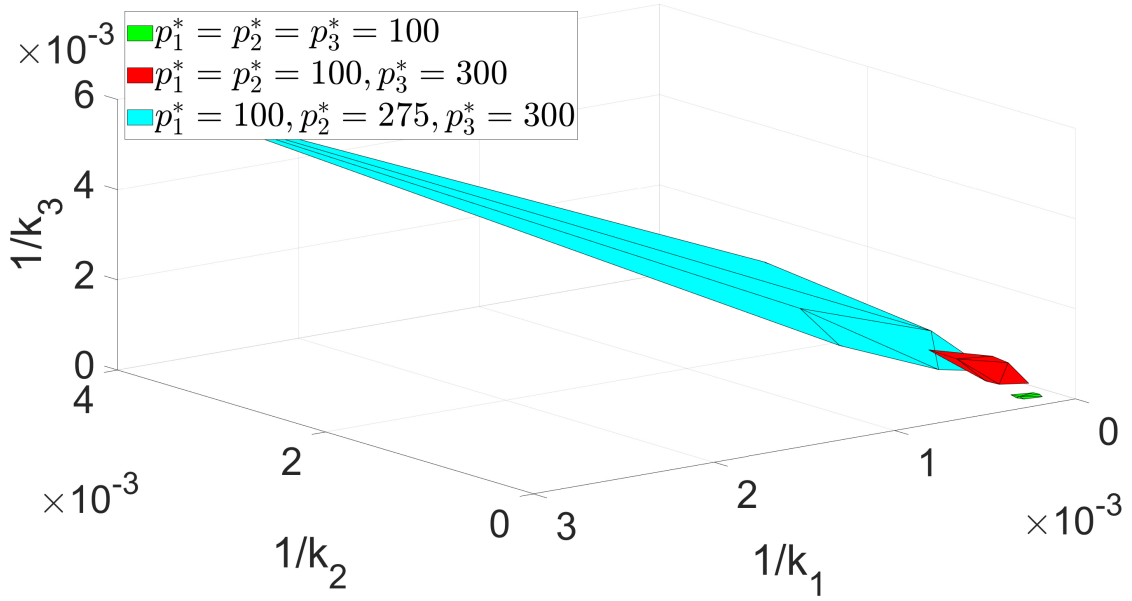

Figure 2.6: Feasible region for $1/k_1$, $1/k_2$ and $1/k_3$ for different desired output values p^*.

Chapter 3

Production Degradation Resource Sharing

3.1 PROBLEM FORMULATION

Consider the following model for a network of N subsystems, shown in Figure 3.1, where each subsystem Σ_i has dynamics described by

$$\begin{cases} \dot{m}_i = u_i - \delta_0 m_i \\ \dot{p}_i = \alpha_i \frac{\theta_i m_i}{1+\theta_i m_i + w_i} - \delta p_i - \alpha_i' \frac{\theta_i' p_i}{1+\theta_i' p_i + w_i'} \\ d_i = \theta_i m_i \\ d_i' = \theta_i' p_i \\ y_i = p_i, \end{cases} \tag{3.1}$$

where $\theta_i \geq 0, \theta_i' \geq 0, i \in \{1, \ldots, N\}$ are tunable parameters. Throughout this work we assume that $u_i, \alpha_i, \alpha_i' > 0, i \in \{1, \ldots, N\}$ and $\delta_0, \delta > 0$. Additionally, w_i and w_i' are state-dependent disturbance inputs given by

$$\begin{cases} w_i &= \sum_{j \neq i} d_j \\ w_i' &= \sum_{j \neq i} d_j'. \end{cases} \tag{3.2}$$

Here, each system Σ_i represents a genetic module, which transcribes mRNA m_i and translates protein p_i. The translation rate of the protein p_i depends also on the level of mRNAs m_j with $j \neq i$ due to ribosome sharing [22] and has been derived and experimentally validated in [15]. The decay rate of the protein, in addition to the dilution term δp_i, includes a degradation term, which arises from a protease, which is being shared by all modules. This model of protease sharing was derived before in [26]. From an input/output system representation, we can regard as (d_i, d_i') the "load" that system Σ_i is applying on the production and degradation resources (ribosomes and proteases), while (w_i, w_i') is the cumulative load on these resource due to all systems except for Σ_i.

With this, for a fixed input $u_i = u_i^*, i \in \{1, \ldots, N\}$, we can write the steady state equations for our subsystem as

$$\Sigma_{i,ss} : \begin{cases} m_i &= \frac{u_i^*}{\delta_0} \\ 0 &= \alpha_i \frac{d_i}{1+d_i+w_i} - \delta p_i - \alpha_i' \frac{d_i'}{1+d_i'+w_i'} \\ y_i &= p_i. \end{cases} \tag{3.3}$$

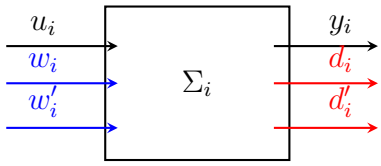

Figure 3.1: Block diagram representation of subsystem Σ_i.

From this, we obtain that the steady state output concentration y_i is the solution to the following system of equations

$$0 = \alpha_i \frac{\theta_i u_i^*}{\delta_0 + \sum_{j=1}^N \theta_j u_j^*} - \delta y_i - \alpha_i' \frac{\theta_i' y_i}{1 + \sum_{j=1}^N \theta_j' y_j}, \tag{3.4}$$

$i \in \{1, \ldots, N\}$. Our goal is to choose parameters θ, θ', such that, the steady state output y_i for each subsystem is close to a desired output concentration y_i^* with tolerances $\varepsilon_i > 0, i \in \{1, \ldots, N\}$.

Specification: Consider a fixed input $u_i = u_i^*$, fixed desired output value y_i^*, and fixed tolerances $\varepsilon_i \geq 0$, $i = \{1, \ldots, N\}$. The specifications on the steady state of the network of subsystems Σ_i given in (3.1) with interconnection rule (3.2) are given as

$$y_i \in [y_i^* - \varepsilon_i, y_i^* + \varepsilon_i], \ i \in \{1, ..., N\}. \tag{3.5}$$

Problem 3 (Feasibility). *Given a network of N subsystems Σ_i of the form (3.1) and interconnection rule (3.2), with fixed input $u_i = u_i^*$ and a set $S = \Theta \times \Theta'$, with $\Theta, \Theta' \subseteq \mathbb{R}^N_{\geq 0}$, for the nonnegative tunable parameters θ_i, θ_i'. Determine if there exists $(\theta_i, \theta_i') \in S, \forall i$, such that y_i, defined as the solution to (3.4), satisfies (3.5).*

3.1.1 Equilibrium Point and Stability Analysis

Before we start tackling Problem 3, we analyze the number of equilibrium points of (3.1) and their stability.

Lemma 3. *The network of subsystems $\Sigma_i, i \in \{1, \ldots, N\}$, with dynamics described by (3.1) and interconnection rule (3.2), has a unique equilibrium point in the positive orthant.*

Proof. Let $x = [m_1, \ldots, m_N, p_1, \ldots, p_N]$, which allows us to rewrite our system in the following form

$$\dot{x} = h(x, u) + \lambda g(x) - \Lambda x = f_\lambda(x, u), \tag{3.6}$$

where $\Lambda = \text{diag}(\delta_0, \ldots, \delta_0, \delta, \ldots, \delta)$, $\lambda \in [0, 1]$ and the vectors $h(x, u) \in \mathbb{R}^{2N}$ and $g(x) \in \mathbb{R}^{2N}$ are defined as follows

$$\{h(x,u)\}_i = \begin{cases} u_i, & \text{if } 1 \leq i \leq N \\ \alpha_{i-N} \frac{\theta_{i-N} x_{i-N}}{1 + \sum_{j=1}^N \theta_j x_j}, & \text{otherwise} \end{cases}, \tag{3.7}$$

$$\{g(x)\}_i = \begin{cases} 0, & \text{if } 1 \leq i \leq N \\ -\alpha_{i-N}' \frac{\theta_{i-N}' x_i}{1 + \sum_{j=1}^N \theta_j' x_{j+N}}, & \text{otherwise} \end{cases}. \tag{3.8}$$

Now we show that the system $\dot{x} = f_0(x)$ is bounded in the sense of Definition 7 in [27]. Consider the following energy like vector function E

$$\{E\}_i = \begin{cases} \frac{1}{2}\left(x_i - \frac{u_i}{\delta_0}\right)^2, & \text{if } 1 \leq i \leq N \\ \frac{1}{2}\left(x_i - \frac{\alpha_{i-N}}{\delta}\right)^2, & \text{otherwise} \end{cases}, \tag{3.9}$$

and its time derivative

$$\{\dot{E}\}_i = \begin{cases} \left(x_i - \frac{u_i}{\delta_0}\right)\dot{x}_i, & \text{if } 1 \leq i \leq N \\ \left(x_i - \frac{\alpha_{i-N}}{\delta}\right)\dot{x}_i, & \text{otherwise} \end{cases}. \tag{3.10}$$

Notice that for $x_i \geq (u_i/\delta_0) + \Delta, i \in \{1, \ldots, N\}$ and $x_i \geq (\alpha_i/\delta) + \Delta, i \in \{N+1, \ldots, 2N\}$, with $\Delta > 0$, we have

$$\{\dot{E}\}_i \leq \begin{cases} -\delta_0\Delta^2, & \text{if } 1 \leq i \leq N \\ -\delta\Delta^2, & \text{otherwise} \end{cases}, \tag{3.11}$$

thus, our state trajectories x_i converge in finite time to the set $x_i \in [0, (u_i/\delta_0) + \Delta], i \in \{1, \ldots, N\}$ and $x_i \in [0, (\alpha_{i-N}/\delta) + \Delta], i \in \{N+1, \ldots, 2N\}$. Therefore, for each initial condition, there exist M and T such that $\|x(t)\| < M = \max((u_i/\delta_0) + \Delta, (\alpha_i/\delta) + \Delta)$ for all $t > T$, so $\dot{x} = f_0(x)$ is bounded in the sense of Definition 7 of [27].

Now fix the input $u_i = u_i^*$, define the set $\mathcal{A}_\lambda = \mathbb{R}^{2N}_{\geq 0}$ and compute the derivative of $f_\lambda(x)$ with respect to x, which yields a matrix A composed of four sub-matrices $A_1, A_2, A_3, A_4 \in \mathbb{R}^{N\times N}$ as follows

$$A = \begin{bmatrix} A_1 & A_2 \\ A_3 & A_4 \end{bmatrix}, \tag{3.12}$$

where the sub-matrices $A_1, A_2, A_3, A_4 \in \mathbb{R}^{N\times N}$ are defined as follows

$$\{A_1\}_{i,j} = \begin{cases} -\delta_0, & \text{if } i = j \\ 0, & \text{if } i \neq j, \end{cases} \tag{3.13}$$

$$\{A_2\}_{i,j} = 0, \forall i, j, \tag{3.14}$$

$$\{A_3\}_{i,j} = \begin{cases} \alpha_i \frac{\theta_i\left(1+\sum_{n\neq i}(x_n\theta_n)\right)}{\left(1+\sum_{n=1}^{N}(x_n\theta_n)\right)^2}, & \text{if } i = j \\ -\alpha_i \frac{\theta_j(x_i\theta_i)}{\left(1+\sum_{n=1}^{N}(x_n\theta_n)\right)^2}, & \text{if } i \neq j, \end{cases} \tag{3.15}$$

$$\{A_4\}_{i,j} = \begin{cases} -\delta - \lambda\alpha_i' \frac{\theta_i'\left(1+\sum_{n\neq i}(x_{n+N}\theta_n')\right)}{\left(1+\sum_{n=1}^{N}(x_{n+N}\theta_n')\right)^2}, & \text{if } i = j \\ \lambda\alpha_i' \frac{\theta_j'\left(x_{i+N}\theta_i'\right)}{\left(1+\sum_{n=1}^{N}(x_{n+N}\theta_n')\right)^2}, & \text{if } i \neq j. \end{cases} \tag{3.16}$$

The sub-matrix $-A_4$ is a Z-matrix, as all elements of the off-diagonal of $-A_4$ are non-positive, that is, $\{-A_4\} \leq 0, \forall i \neq j$. Further, $(-A_4)^\top D$, with $D = \text{diag}(1/\alpha_1', \ldots, 1/\alpha_N')$, is strictly diagonally dominant, that is, the row sum, for all rows of $(-A_4)^\top D$, is positive. With this, by Theorem 2.3 in Chapter 6 of [28] condition (I_{29}), $(-A_4)^\top$ is a nonsingular M-matrix for any $\lambda \in [0, 1]$ and $x \in \mathcal{A}_\lambda$.

Since A is a block lower triangular matrix, its determinant $\det(A) = \det(A_1)\det(A_4) \neq 0$ for any $\lambda \in [0,1]$ and $x \in \mathcal{A}_\lambda$, as $\det(A_1) = (-\delta_0)^N$ and A_4 is a nonsingular M-matrix. Also observe that $h(x)$ has no zeros on the boundary of the positive orthant and $g(x)$ is mass dissipating in the sense of Definition 8 in [27]. With this, by Theorem 10 of [27] we know that the system in (3.6) with $\lambda = 1$ has the same number of equilibrium points as the system with $\lambda = 0$.

System (3.6) with $\lambda = 0$ and fixed input $u_i = u_i^*$ gives us

$$\dot{m}_i = u_i^* - \delta_0 m_i \tag{3.17}$$

$$\dot{p}_i = \alpha_i \frac{\theta_i m_i}{1 + \sum_{j=1}^N \theta_j m_j} - \delta p_i. \tag{3.18}$$

Computing the equilibrium point for this system yields equilibrium mRNA concentration $m_{i,eq} = u_i^*/\delta_0$, which we substitute on the second equation yielding the unique solution

$$p_{i,eq} = \frac{\alpha_i}{\delta} \frac{\theta_i u_i^*}{\delta_0 + \sum_{j=1}^N \theta_j u_j^*}. \tag{3.19}$$

Therefore, system (3.6) with $\lambda = 0$ has a unique equilibrium point in the positive orthant, implying by Theorem 10 of [27] that system (3.6) with $\lambda = 1$, that is, system (3.1), also has a unique equilibrium point in the positive orthant. □

Lemma 4. *The equilibrium point of the network of subsystems $\Sigma_i, i \in \{1, \ldots, N\}$, with dynamics described by (3.1) and interconnection rule (3.2), is locally asymptotically stable for all parameter values.*

Proof. We first define the state $\xi = [(m_1 - m_{1,e}), \ldots, (m_N - m_{N,e}), (p_1 - p_{1,e}), \ldots, (p_N - p_{N,e})]$, where $m_{i,e}$ is the mRNA concentration equilibrium point and $p_{i,e}$ is the protein concentration equilibrium point. Then we linearize the system at its equilibrium, yielding

$$\dot{\xi} = A\xi, \tag{3.20}$$

where the matrix A is composed of four sub-matrices $A_1, A_2, A_3, A_4 \in \mathbb{R}^{N \times N}$ as follows

$$A = \begin{bmatrix} A_1 & A_2 \\ A_3 & A_4 \end{bmatrix}, \tag{3.21}$$

where the sub-matrices $A_1, A_2, A_3, A_4 \in \mathbb{R}^{N \times N}$ are defined as follows

$$\{A_1\}_{i,j} = \begin{cases} -\delta_0, & \text{if } i = j \\ 0, & \text{if } i \neq j, \end{cases} \tag{3.22}$$

$$\{A_2\}_{i,j} = 0, \forall i, j, \tag{3.23}$$

$$\{A_3\}_{i,j} = \begin{cases} \alpha_i \frac{\theta_i\left(1 + \sum_{n \neq i}(m_{n,e}\theta_n)\right)}{\left(1 + (m_{i,e}\theta_i) + \sum_{n \neq i}(m_{n,e}\theta_n)\right)^2}, & \text{if } i = j \\ -\alpha_i \frac{\theta_j(m_{i,e}\theta_i)}{\left(1 + (m_{i,e}\theta_i) + \sum_{n \neq i}(m_{n,e}\theta_n)\right)^2}, & \text{if } i \neq j, \end{cases} \tag{3.24}$$

$$\{A_4\}_{i,j} = \begin{cases} -\delta - \alpha_i' \frac{\theta_i'\left(1 + \sum_{n \neq i}(p_{n,e}\theta_n')\right)}{\left(1 + (p_{i,e}\theta_i') + \sum_{n \neq i}(p_{n,e}\theta_n')\right)^2}, & \text{if } i = j \\ \alpha_i' \frac{\theta_j'\left(p_{i,e}\theta_i'\right)}{\left(1 + (p_{i,e}\theta_i') + \sum_{n \neq i}(p_{n,e}\theta_n')\right)^2}, & \text{if } i \neq j. \end{cases} \tag{3.25}$$

Moreover, the sub-matrix $-A_4$ is a Z-matrix, as all the off-diagonal elements of $-A_4$ are nonpositive, that is, $\{-A_4\} \leq 0, \forall i \neq j$, and additionally, $(-A_4)^\top D$, with $D = \text{diag}(1/\alpha'_1, \ldots, 1/\alpha'_N)$, is strictly diagonally dominant. With this, by Theorem 2.3 in Chapter 6 of [28] condition (I_{29}), $(-A_4)^\top$ is a nonsingular M-matrix. Furthermore, condition (G_{20}) of Theorem 2.3 in Chapter 6 of [28] states that the eigenvalues of $(-A_4)^\top$ have positive real part. We know that $-A_4$ has the same eigenvalues as $(-A_4)^\top$, which implies that all the eigenvalues of A_4 have negative real part. Since A is a lower block triangular matrix due to A_2 having all entries equal to zero, its eigenvalues are the union of the eigenvalues of A_1 and A_4. The eigenvalues of A_1 are all equal to $-\delta_0$ and all of the eigenvalues of A_4 have negative real part, so we can conclude that all the eigenvalues of A have negative real part. Therefore, the equilibrium point of the network of subsystems $\Sigma_i, i \in \{1, \ldots, N\}$, with dynamics described by (3.1) and interconnection rule (3.2), is locally asymptotically stable for all parameter values. □

Theorem 2. *The network of N subsystems Σ_i with dynamics described by (3.1) and interconnection rule (3.2), with fixed input $u_i = u_i^*$ has steady state protein output y_i that satisfies the specification in (3.5) for some $\theta_i \geq 0, \theta'_i \geq 0$, if and only if, the same system has steady state protein output y_i that satisfies the specification in (3.5) for some $\theta_i \geq 0, \theta'_i = 0$.*

Proof. First we show that if there exists a network with N subsystems and steady state protein output y_i which satisfies (3.5) for some $\theta_i \geq 0, \theta'_i \geq 0$, then the same systems with some $\theta_i \geq 0, \theta'_i = 0$ have y_i which satisfies (3.5). Suppose there exists $\theta_i \geq 0$ and $\theta'_i \geq 0, \forall i$, such that, the steady state protein concentration y_i, defined as the solution to (3.4) satisfies the specification in (3.5). From (3.4) we have

$$\frac{y_i}{\alpha_i} = \frac{1}{\delta}\left(\frac{\theta_i u_i^*}{\delta_0 + \sum_{k=1}^N \theta_k u_k^*} - \frac{(\alpha_i \theta'_i y_i/\alpha'_i)}{1 + \sum_{k=1}^N \theta'_k y_k}\right), \tag{3.26}$$

which substituted into $(1/\delta) - \sum_{k=1}^N (y_k/\alpha_k)$, results in

$$\frac{1}{\delta}\left(1 - \sum_{i=1}^N \left(\frac{\theta_i u_i^*}{\delta_0 + \sum_{k=1}^N \theta_k u_k^*} - \frac{(\alpha_i \theta'_i y_i/\alpha'_i)}{1 + \sum_{k=1}^N \theta'_k y_k}\right)\right) = \tag{3.27}$$

$$\frac{\delta_0 + \sum_{k=1}^N \theta_k u_k^* - \sum_{i=1}^N \theta_i u_i^*}{\delta\left(\delta_0 + \sum_{k=1}^N \theta_k u_k^*\right)} + \frac{\sum_{i=1}^N (\alpha_i \theta'_i y_i/\alpha'_i)}{\delta\left(1 + \sum_{k=1}^N \theta'_k y_k\right)} = \tag{3.28}$$

$$\frac{\delta_0}{\delta\left(\delta_0 + \sum_{k=1}^N \theta_k u_k^*\right)} + \frac{\sum_{i=1}^N (\alpha_i \theta'_i y_i/\alpha'_i)}{\delta\left(1 + \sum_{k=1}^N \theta'_k y_k\right)} > 0. \tag{3.29}$$

So $(1/\delta) - \sum_{k=1}^N (y_k/\alpha_k) > 0$. Then the same value of y_i can be achieved for $\theta'_i = 0$ with $\theta_i = \theta_i^* \geq 0, \forall i$ defined as follows

$$\theta_i^* = \frac{\delta_0 y_i}{\alpha_i u_i^* \left(\frac{1}{\delta} - \sum_{j=1}^N \frac{y_j}{\alpha_j}\right)}, \forall i. \tag{3.30}$$

This can be verified by substituting $\theta_i' = 0, \theta_i = \theta_i^*, \forall i$ into (3.4), yielding

$$\alpha_i \frac{\theta_i^* u_i^*}{\delta_0 + \sum_{j=1}^N \theta_j^* u_j^*} - \delta y_i = \frac{\delta_0 y_i}{\frac{\delta_0}{\delta} - \sum_{k=1}^N \frac{\delta_0 y_k}{\alpha_j} + \sum_{j=1}^N \frac{\delta_0 y_j}{\alpha_j}} - \delta y_i = \delta y_i - \delta y_i = 0. \quad (3.31)$$

Therefore, if the network of N subsystems Σ_i has steady state protein output y_i with $\theta_i' \geq 0$, then the same network can achieve steady state protein output y_i with $\theta_i' = 0$ and $\theta_i = \theta_i^*$.

We conclude the proof by noting that if there exists a network with N subsystems has steady state protein output y_i which satisfies (3.5) for some $\theta_i \geq 0, \theta_i' = 0$, then the same network has y_i which satisfies (3.5) with the same $\theta_i \geq 0, \theta_i' = 0 \geq 0$. □

3.1.2 Input-Output Characteristics

Since our network of N subsystems Σ_i has a unique and stable equilibrium point for a fixed input $u_i = u_i^*, i \in \{1, \ldots, N\}$, we can define the input-output steady state characteristics for this network. Moreover, with Theorem 2 we have that the feasibility of a specification (3.5) for a network with $\theta, \theta' \geq 0$ is tied to the feasibility of that specification for the same network but with $\theta \geq 0, \theta' = 0$. So, we define the input-output characteristics for the system with $\theta_i' = 0, i \in \{1, \ldots, N\}$. For a fixed value of $y_i, i \in \{1, \ldots, N\}$, (3.3) allows us to derive the following steady state I/O map

$$d_i = \gamma_i(1 + w_i), \quad (3.32)$$

where γ_i are the w_i to d_i system's gains defined as follows

$$\gamma_i = \frac{\delta y_i}{\alpha_i - \delta y_i}. \quad (3.33)$$

This steady state I/O map describes how a change in the disturbance inputs w_i affects the disturbance outputs d_i when y_i is held constant. With this we define the constant gains $\tilde{\gamma}_i$ and $\hat{\gamma}_i$ as follows

$$\tilde{\gamma}_i = \frac{\delta(y_i^* - \varepsilon_i)}{\alpha_i - \delta(y_i^* - \varepsilon_i)}, \quad (3.34)$$

$$\hat{\gamma}_i = \frac{\delta(y_i^* + \varepsilon_i)}{\alpha_i - \delta(y_i^* + \varepsilon_i)}. \quad (3.35)$$

3.2 PROBLEM SOLUTION

Let $w = [w_1, \ldots, w_N]^\top$ and $d = [d_1, \ldots, d_N]^\top$, then (3.2) implies

$$w = Td, \quad (3.36)$$

with the interconnection matrix $T \in \mathbb{R}^{N\times N}$ defined as

$$\{T\}_{i,j} = \begin{cases} 0, & \text{if } i = j \\ 1, & \text{if } i \neq j. \end{cases} \tag{3.37}$$

Moreover, (3.32) can be written in matrix form as

$$d = \gamma + \Gamma w, \tag{3.38}$$

where $\gamma = [\gamma_1, \ldots, \gamma_N]^\top$ and the matrix $\Gamma \in \mathbb{R}^{N\times N}$ is defined as follows

$$\{\Gamma\}_{i,j} = \begin{cases} \gamma_i, & \text{if } i = j \\ 0, & \text{if } i \neq j. \end{cases} \tag{3.39}$$

Now let $y_i = y_i^* - \varepsilon_i, i \in \{1, \ldots, N\}$, and define the gain vector $\tilde{\gamma} = [\tilde{\gamma}_1, \ldots, \tilde{\gamma}_N]^\top$ and matrix $\tilde{\Gamma} \in \mathbb{R}^{N\times N}$ as follows

$$\{\tilde{\Gamma}\}_{i,j} = \begin{cases} \tilde{\gamma}_i, & \text{if } i = j \\ 0, & \text{if } i \neq j. \end{cases} \tag{3.40}$$

The following Theorem provides sufficient and necessary conditions for the existence of $\theta_i \geq 0, \theta_i' = 0$ such that a network of N subsystems Σ_i has steady state output protein concentration y_i that satisfies the specification given in (3.5).

Theorem 3. *Let $\tilde{\Gamma}$ be the gain matrix defined in (3.40), T be the interconnection matrix defined in (3.37) and $\theta_i' = 0$. There exist $\theta_i \geq 0$ such that y_i, defined as the solution to (3.4), satisfies (3.5) if and only if $\rho(\tilde{\Gamma}T) < 1$.*

Proof. We start by showing that $\rho(\tilde{\Gamma}T) < 1$ implies that there exists $\theta_i \geq 0, i \in \{1, \ldots, N\}$ such that the steady state protein output y_i satisfies the specification (3.5). Let $M = (I - \tilde{\Gamma}T)$ and note that $\{\tilde{\Gamma}T\}_{i,j} \geq 0, \forall i \neq j$. With this, from Theorem 3.11 in Chapter 6 of [28], M is nonsingular and $\{M^{-1}\}_{i,j} \geq 0$ if and only if $\rho(\tilde{\Gamma}T) < 1$. Now let $d^* = (I - \tilde{\Gamma}T)^{-1}\tilde{\gamma}$ and since both $(I - \tilde{\Gamma}T)^{-1}$ and $\tilde{\gamma}$ are element wise nonnegative from its definition, then $d^* \geq 0$. Consider the following system of inequalities

$$d_i \geq \tilde{\gamma}_i(1 + w_i) \tag{3.41}$$
$$d_i \leq \hat{\gamma}_i(1 + w_i), \tag{3.42}$$

where $\tilde{\gamma}$ is defined as in (3.34) and $\hat{\gamma}$ is defined as in (3.35), along with $d_i \geq 0$ and (3.2). Using matrices (3.37) and (3.40), the constrains in (3.41) can be written as follows

$$(I - \tilde{\Gamma}T)d \geq \tilde{\gamma}. \tag{3.43}$$

Substituting $d = d^*$ in (3.41) yields

$$(I - \tilde{\Gamma}T)d^* = (I - \tilde{\Gamma}T)(I - \tilde{\Gamma}T)^{-1}\tilde{\gamma} = \tilde{\gamma}. \tag{3.44}$$

So inequality (3.41) in matrix form holds with $d = d^* \geq 0$. Now choose $d = d^*$. Consider the quantity

$$(1 + w_i)(\hat{\gamma}_i - \tilde{\gamma}_i). \tag{3.45}$$

Since $w_i \geq 0$ and $\hat{\gamma}_i \geq \tilde{\gamma}_i$ by definition, then the above quantity is always nonnegative and thus (3.42) is satisfied by $d = d^*$. With this, by Lemma 2 in [23] we have that satisfying the specification (3.5) is equivalent to satisfying (3.41)-(3.42).

Now we show that the existence of $\theta_i \geq 0, \forall i$, such that, the steady state protein output y_i that satisfies the specification (3.5), implies that $\rho(\tilde{\Gamma}T) < 1$. We first show that $(1/\delta) - \sum_{k=1}^{N}(y_k^* - \varepsilon_k)/\alpha_k > 0$. Substituting $y_i = y_i^* - \varepsilon_i$ in (3.4), with $\theta_i' = 0$, yields

$$\frac{y_i^* - \varepsilon_i}{\alpha_i} = \frac{1}{\delta}\frac{\theta_i u_i^*}{\delta_0 + \sum_{k=1}^{N}\theta_k u_k^*}, \tag{3.46}$$

and substituting this expression into $(1/\delta) - \sum_{k=1}^{N}(y_k^* - \varepsilon_k)/\alpha_k$ results in

$$\frac{1}{\delta}\left(1 - \sum_{i=1}^{N}\frac{\theta_i u_i^*}{\delta_0 + \sum_{k=1}^{N}\theta_k u_k^*}\right) = \frac{\delta_0 + \sum_{k=1}^{N}\theta_k u_k^* - \sum_{i=1}^{N}\theta_i u_i^*}{\delta\left(\delta_0 + \sum_{k=1}^{N}\theta_k u_k^*\right)} = \frac{\delta_0}{\delta\left(\delta_0 + \sum_{k=1}^{N}\theta_k u_k^*\right)} > 0. \tag{3.47}$$

Let $A = I + \tilde{\Gamma}$, $v = [-1, \ldots, -1]^\top$, so $M = (A + \tilde{\gamma}v^\top)$, where if $1 + v^\top A^{-1}\tilde{\gamma} \neq 0$ we can use the Sherman-Morrison formula to compute the inverse [29]. We have that $1 + v^\top A^{-1}\tilde{\gamma} = (1/\delta) - \sum_{k=1}^{N}(y_k^* - \varepsilon_k)/\alpha_k > 0$, so the inverse of M exists and is given by

$$(A + \tilde{\gamma}v^\top)^{-1} = A^{-1} - \frac{A^{-1}\tilde{\gamma}vA^{-1}}{1 + v^\top A^{-1}\tilde{\gamma}}, \tag{3.48}$$

which yields

$$\{M^{-1}\}_{i,j} = \begin{cases} \frac{1}{1+\tilde{\gamma}_i} + \frac{1}{\frac{1}{\delta} - \sum_{k=1}^{N}\frac{y_k^* - \varepsilon_k}{\alpha_k}}\frac{\tilde{\gamma}_i}{(1+\tilde{\gamma}_i)^2}, & \text{if } i = j \\ \frac{1}{\frac{1}{\delta} - \sum_{k=1}^{N}\frac{y_k^* - \varepsilon_k}{\alpha_k}}\frac{\tilde{\gamma}_i}{(1+\tilde{\gamma}_i)(1+\tilde{\gamma}_j)}, & \text{if } i \neq j \end{cases}. \tag{3.49}$$

From above $\{M^{-1}\}_{i,j} \geq 0$ and by Theorem 3.11 in Chapter 6 of [28], we have that $\rho(\tilde{\Gamma}T) < 1$. Therefore, if there exists $\theta_i \geq 0$ such that y_i is the solution to (3.4) and satisfies the specification (3.5), then $\rho(\tilde{\Gamma}T) < 1$. □

Corollary 1. *Given a network of N subsystems of the form (3.1) and interconnection rule (3.2), with fixed input $u_i = u_i^*, i \in \{1, \ldots, N\}$. Then $\rho(\tilde{\Gamma}T) < 1$ if and only if there exists $\theta_i \geq 0, \theta_i' \geq 0, i \in \{1, \ldots, N\}$, such that, the steady state protein concentration y_i, defined as the solution to (3.4), satisfies the specification in (3.5).*

Proof. By Theorem 3 we have that there exists $\theta_i \geq 0, \theta_i' = 0, i \in \{1, \ldots, N\}$ such that a network of N subsystems Σ_i has steady state output protein concentration y_i which satisfies the specification in (3.5) if and only if $\rho(\tilde{\Gamma}T) < 1$. Additionally, by Theorem 2 we have that there exists $\theta_i \geq 0, \theta_i' = 0, i \in \{1, \ldots, N\}$ such that a network of N subsystems Σ_i has

steady state output protein concentration y_i which satisfies the specification in (3.5) if and only if there exists $\theta_i \geq 0, \theta_i' \geq 0, i \in \{1, \ldots, N\}$ such that the same network has steady state output protein concentration y_i which satisfies the specification in (3.5). Therefore, there exists $\theta_i \geq 0, \theta_i' \geq 0, i \in \{1, \ldots, N\}$ such that a network of N subsystems Σ_i has steady state output protein concentration y_i which satisfies the specification in (3.5) if and only if $\rho(\tilde{\Gamma}T) < 1$ is satisfied. □

We will now present a result that relates the spectral radius of $\tilde{\Gamma}T$ to an inequality that is easy to check.

Theorem 4. *Let $\tilde{\Gamma}$ be the gain matrix defined in (3.40) and T be the interconnection matrix defined in (3.37). Then $\rho(\tilde{\Gamma}T) < 1$ if and only if the inequality*

$$\frac{1}{\delta} - \sum_{j=1}^{N} \frac{y_j^* - \varepsilon_j}{\alpha_j} > 0, \tag{3.50}$$

is satisfied

Proof. Let $A = I + \tilde{\Gamma}$, $v = [-1, \ldots, -1]^\top$, so $M = (A + \tilde{\gamma} v^\top)$ and from the Sherman-Morrison formula, if $1 + v^\top A^{-1} \tilde{\gamma} \neq 0$, M is invertible and the inverse is given by [29]

$$(A + \tilde{\gamma} v^\top) = A^{-1} - \frac{A^{-1} \tilde{\gamma} v A^{-1}}{1 + v^\top A^{-1} \tilde{\gamma}}, \tag{3.51}$$

which yields

$$\{M^{-1}\}_{i,j} = \begin{cases} \frac{1}{1+\tilde{\gamma}_i} + \frac{1}{\frac{1}{\delta} - \sum_{k=1}^{N} \frac{y_k^* - \varepsilon_k}{\alpha_k}} \frac{\tilde{\gamma}_i}{(1+\tilde{\gamma}_i)^2}, & \text{if } i = j \\ \frac{1}{\frac{1}{\delta} - \sum_{k=1}^{N} \frac{y_k^* - \varepsilon_k}{\alpha_k}} \frac{\tilde{\gamma}_i}{(1+\tilde{\gamma}_i)(1+\tilde{\gamma}_j)}, & \text{if } i \neq j \end{cases}. \tag{3.52}$$

If (3.50) is satisfied, then $1 + v^\top A^{-1} \tilde{\gamma} = \frac{1}{\delta} - \sum_{j=1}^{N} \frac{y_j^* - \varepsilon_j}{\alpha_j} \neq 0$, and so M is nonsingular and $\{M^{-1}\}_{i,j} \geq 0$ by (3.52) and the fact that $\tilde{\gamma}_i \geq 0$. On the other hand, if M is nonsingular and $\{M^{-1}\}_{i,j} \geq 0$, then from (3.52) we have that $\frac{1}{\delta} - \sum_{j=1}^{N} \frac{y_j^* - \varepsilon_j}{\alpha_j} > 0$. Therefore, M is nonsingular and $\{M^{-1}\}_{i,j} \geq 0$ if and only if (3.50) is satisfied. From Theorem 3.11 in Chapter 6 of [28], M is nonsingular and $\{M^{-1}\}_{i,j} \geq 0$ if and only if $\rho(\tilde{\Gamma}T) < 1$. Thus, $\rho(\tilde{\Gamma}T) < 1$ if and only if (3.50) is satisfied. □

Corollary 2. *Consider a network of N subsystems of the form (3.1) and interconnection rule (3.2), with fixed input $u_i = u_i^*, i \in \{1, \ldots, N\}$. We have that the inequality*

$$\frac{1}{\delta} - \sum_{j=1}^{N} \frac{y_j^* - \varepsilon_j}{\alpha_j} > 0 \tag{3.53}$$

is satisfied if and only if there exists $\theta_i \geq 0, \theta_i' \geq 0, i \in \{1, \ldots, N\}$, such that, the steady state protein concentration y_i, defined as the solution to (3.4), satisfies the specification in (3.5).

Proof. By Corollary 1 there exists $\theta_i \geq 0, \theta_i' \geq 0, i \in \{1, \ldots, N\}$ such that a network of N subsystems Σ_i has steady state output protein concentration y_i which satisfies the specification in (3.5) if and only if $\rho(\tilde{\Gamma}T) < 1$. By Theorem 4 we have that $\rho(\tilde{\Gamma}T) < 1$ if and only if (3.50) is satisfied. Therefore, there exists $\theta_i \geq 0, \theta_i' \geq 0, i \in \{1, \ldots, N\}$ such that a network of N subsystems Σ_i has steady state output protein concentration y_i which satisfies the specification in (3.5) if and only if if and only if the inequality

$$\frac{1}{\delta} - \sum_{j=1}^{N} \frac{y_j^* - \varepsilon_j}{\alpha_j} > 0, \tag{3.54}$$

is satisfied. □

3.3 APPLICATION EXAMPLE

In this section, we consider two different examples. In the first example we use Corollary 2 to obtain the achievable region for the steady state protein output concentration y_i^* for two systems, one with $N = 2$ and the other with $N = 3$ subsystems Σ_i, both with fixed tolerance $\varepsilon_i = 0, i \in \{1, \ldots, N\}$ and different values for α. Then in the second example, we choose the tunable parameters $\theta_i > 0$ and $\theta_i' = 0$ and compute the steady state protein output concentration y_i for some fixed input $u_i = u_i^*$ in a network with $N = 2$ subsystems Σ_i. Then we use this y_i value and the system parameters to numerically verify that $\rho(\tilde{\Gamma}T) < 1$ as established by Corollary 1 and that inequality (3.50) is satisfied as established by Corollary 2. We then expand this example by computing the feasible region for the θ parameters for fixed θ'.

In this first example, we can use inequality (3.50), as established by Corollary 2, to obtain the achievable set of desired steady state protein concentrations. That is, the region of values of y^* that can be achieved with fixed tolerance $\varepsilon_i = 0, i \in \{1, \ldots, N\}$, for systems with different number of subsystems and different values for α. Consider a network of $N = 2$ subsystems, Figure 3.2 presents the achievable region for the desired steady state protein output y^* with different values of α. Now considering a network of $N = 3$ subsystems, Figure 3.3 presents the achievable for y^* region with different values of α. Notice that the achievable set presented in Figure 3.2 appears in the plane (y_1^*, y_2^*) when $y_3^* = 0$ in Figure 3.3, due to subsystems Σ_1 and Σ_2 having the same α_1 and α_2. Moreover, as we increase y_3^*, the achievable set in the y_1^*, y_2^* plane reduces in size, showing that increasing the number of subsystems Σ_i or demanding more output from one of these subsystems, reduces the achievable set for the other system outputs.

For the second example, we consider the case where we have a network of $N = 2$ subsystems Σ_i. To this end, we consider the following parameter values for our subsystems. We let the fixed input and desired output $u^* = y^* = [10,\ 20]^\top$ nM, the tolerance $\varepsilon = [1,\ 1]^\top$ nM, the translation rate constant $\alpha = [50,\ 50]^\top$ nM/hr, the degradation rate constant $\alpha' = [10,\ 10]^\top$ nM/hr, the dilution rate constant for the protein $\delta = 1$ hr^{-1} and for the mRNA $\delta_0 = 1$ hr^{-1}. With these values, if we choose $\theta = [0.05,\ 0.05]^\top$ nM^{-1} and $\theta' = [0,\ 0]^\top$ nM^{-1} we obtain exactly the desired output y^* using the specified input u^*. So, the specification (3.5) with $\varepsilon_i = 0, i \in \{1, \ldots, N\}$ is satisfied. Since the specification can be satisfied, we can validate

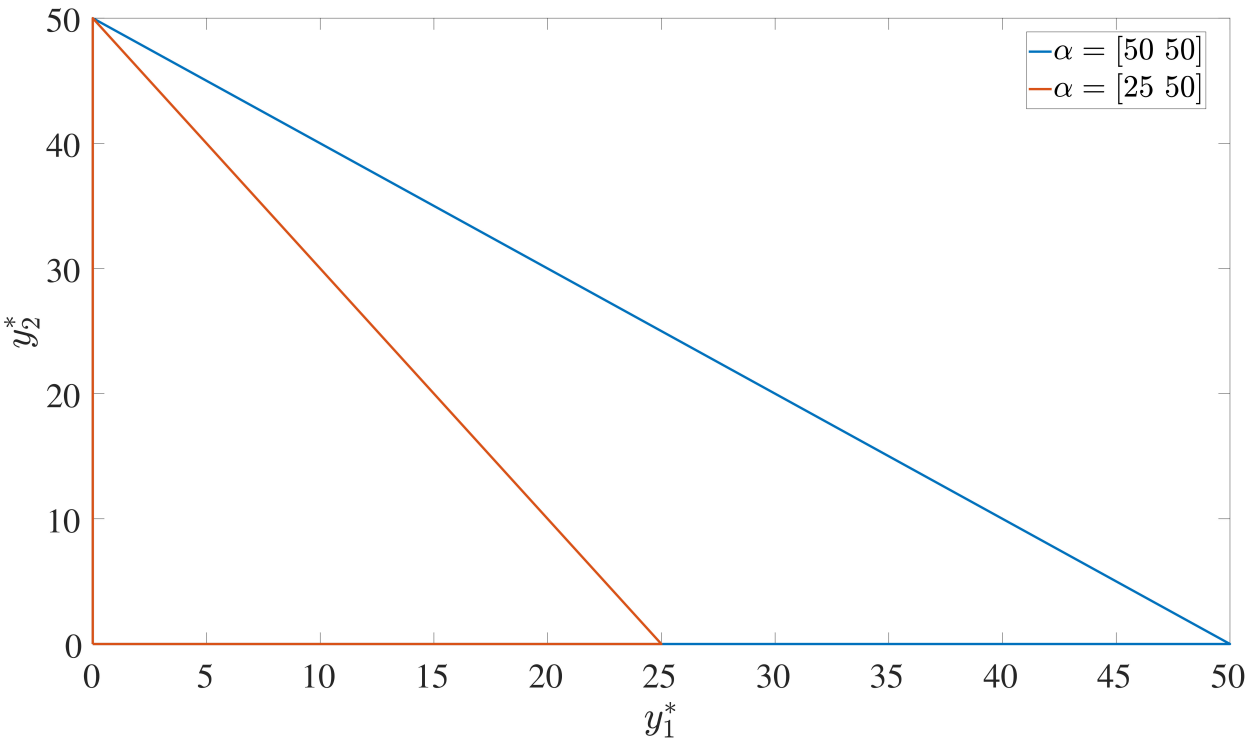

Figure 3.2: Achievable region for the desired steady state output protein concentration y^* with $\delta = 1$ hr^{-1} and different values of α nM/hr.

our feasibility checks from Corollary 1

$$\rho\left(\tilde{\Gamma}T\right) = \rho\begin{pmatrix} 0.0000 & 0.0220 \\ 0.0306 & 0.0000 \end{pmatrix} = 0.0259 < 1, \tag{3.55}$$

and from Corollary 2

$$\frac{1}{\delta} - \sum_{j=1}^{2} \frac{y_j^* - \varepsilon_j}{\alpha_j} = 1 - \frac{1}{5} - \frac{2}{5} = \frac{2}{5} > 0. \tag{3.56}$$

Observe that both feasibility checks show that the specification is feasible.

Now we are interested in designing the θ, θ' tunable parameters to meet a given specification, which is a computationally difficult task, which we simplify by fixing the value of $\theta_i', i \in \{1, \ldots, N\}$. To this end, we state a method to calculate θ_i, $i \in \{1, \ldots, N\}$ as a function of θ_i' and y_i, $i \in \{1, \ldots, N\}$, where we assume that y_i is such that $(1/\delta) - \sum_{i=1}^{N}(y_i + \beta_i')/\alpha_i > 0$. We define the quantities β_i' as follows

$$\beta_i' = \frac{\alpha_i' \theta_i' y_i}{\delta\left(1 + \theta_i' y_i + \sum_{j \neq i} \theta_j' y_j\right)}. \tag{3.57}$$

Fixing the values of $y_i, i \in \{1, \ldots, N\}$ fixes the values of $\beta_i', i \in \{1, \ldots, N\}$, and thus one can use (3.3) to derive the modified steady state I/O map

$$d_i = \gamma_i^{\dagger}(1 + w_i), \tag{3.58}$$

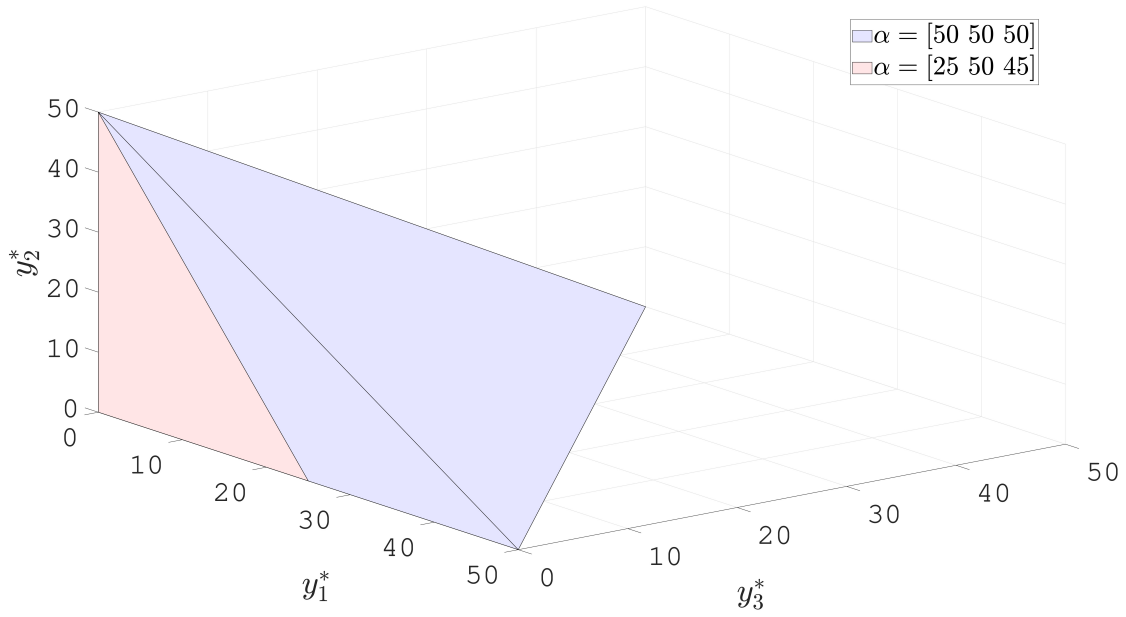

Figure 3.3: Achievable region for the desired steady state output protein concentration $y^*$with $\delta = 1$ hr^{-1} and different values of α nM/hr.

where $\gamma_i^\dagger$ is defined as follows

$$\gamma_i^\dagger = \frac{\delta(y_i + \beta_i')}{\alpha_i - \delta(y_i + \beta_i')}. \tag{3.59}$$

With this, (3.58) can be rewritten in matrix form as

$$(I - \Gamma^\dagger T)d = \gamma^\dagger, \tag{3.60}$$

where $\gamma^\dagger = [\gamma_1^\dagger, \ldots, \gamma_N^\dagger]$, T is as defined in (3.37) and $\Gamma^\dagger = \text{diag}(\gamma^\dagger)$. Let $A = I + \Gamma^\dagger$, $v = [-1, \ldots, -1]^\top$, so $M = (I - \Gamma^\dagger T) = (A + \gamma^\dagger v^\top)$. Since this procedure only consider y_i, such that $(1 + v^\top A^{-1}\gamma^\dagger) = (1/\delta) - \sum_{i=1}^N (y_i + \beta_i')/\alpha_i > 0$, then the inverse of $(I - \Gamma^\dagger T)$ exists and is given by [29]

$$(A + \gamma^\dagger v^\top)^{-1} = A^{-1} - \frac{A^{-1}\gamma^\dagger v A^{-1}}{1 + v^\top A^{-1}\gamma^\dagger}. \tag{3.61}$$

This yields

$$\{M^{-1}\}_{i,j} = \begin{cases} \frac{1}{1+\gamma_i^\dagger} + \frac{1}{\frac{1}{\delta} - \sum_{k=1}^N \frac{y_k \beta_k'}{\alpha_k}} \frac{\gamma_i^\dagger}{(1+\gamma_i^\dagger)^2}, & \text{if } i = j \\ \frac{1}{\frac{1}{\delta} - \sum_{k=1}^N \frac{y_k \beta_k'}{\alpha_k}} \frac{\gamma_i^\dagger}{(1+\gamma_i^\dagger)(1+\gamma_j^\dagger)}, & \text{if } i \neq j. \end{cases} \tag{3.62}$$

Calculating d_i using $d = (I - \Gamma^\dagger T)^{-1}\gamma^\dagger$ and recalling that $d_i = u_i^* \theta_i / \delta_0$, we finally obtain

$$\theta_i = \frac{\delta_0 \left(y_i + \beta_i'\right)}{\alpha_i u_i^* \left(\frac{1}{\delta} - \sum_{j=1}^N \left(\frac{y_j + \beta_j'}{\alpha_j}\right)\right)}. \tag{3.63}$$

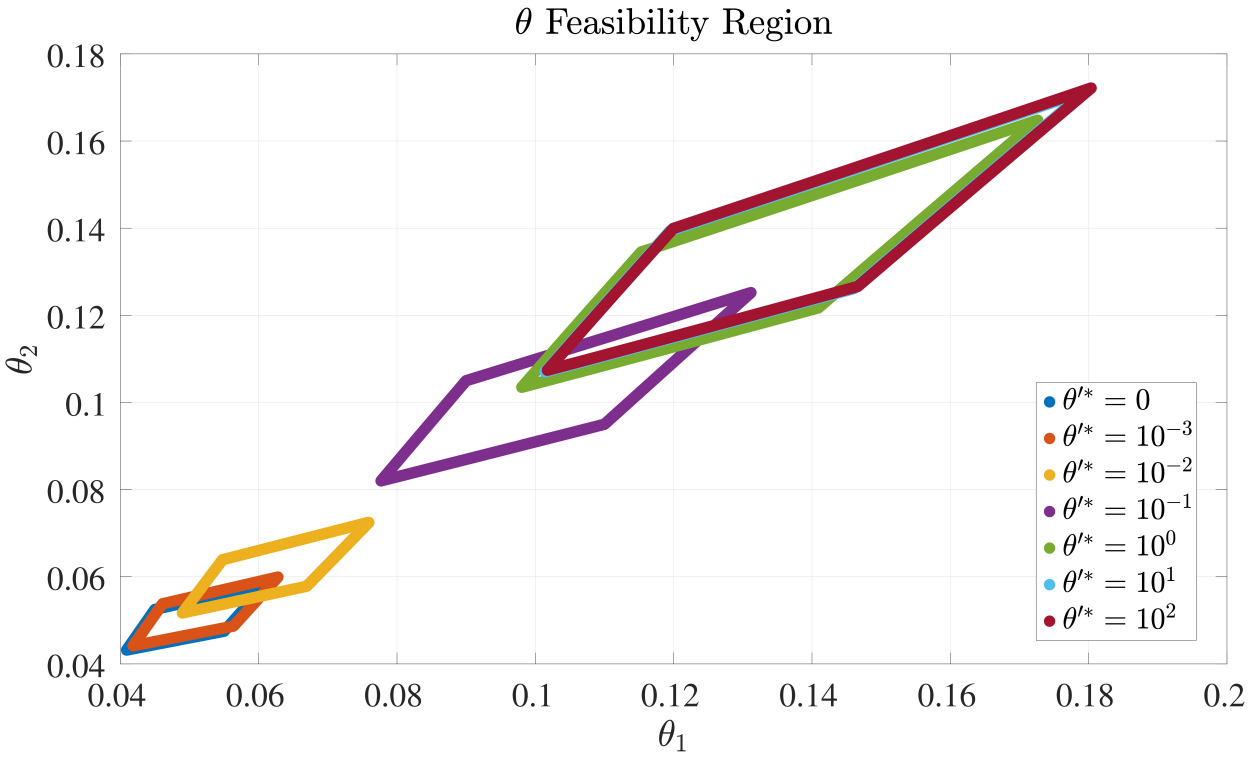

Figure 3.4: Feasible region for θ tunable parameters with different values of θ'^*.

Note that $\theta_i \geq 0$ since $(1/\delta) - \sum_{i=1}^{N}(y_i + \beta_i')/\alpha_i > 0$. We note that (3.63) was derived using the modified I/O map given in (3.60). It can be verified that substituting the θ_i values obtained from (3.63) into (3.3) yields the fixed values y_i as the system's steady state, which justifies (3.63). With this, computing the θ feasible region can be numerically done by utilizing the map (3.63) from the protein y_i space to the θ_i space, for $y_i \in [y_i^* - \varepsilon_i, y_i^* + \varepsilon_i], i \in \{1, \ldots, N\}$ and y_i such that $(1/\delta) - \sum_{i=1}^{N}(y_i + \beta_i')/\alpha_i > 0$.

Figure 3.4 presents the boundary of the θ parameter feasible region for multiple values of $\theta_i' = \theta'^*, i \in \{1, \ldots, N\}$, computed using (3.63). To achieve this, we have sampled the specification in the y space, then numerically computed (θ_1, θ_2) using (3.63) and finally plotted just the boundary obtained in the θ space. This shows that including degradation affects the θ tunable parameter feasible region, moving it towards larger values and also increasing its area.

Chapter 4

Multiplexed Bio-sensing

4.1 PROBLEM FORMULATION

Lets start by considering a network of subsystems Σ_i with the following form

$$\Sigma_i : \begin{cases} \dot{m}_i &= u_i - \delta_0 m_i - \alpha'_i \frac{\theta'_i m_i}{1+\sum_{j=1}^{N} \theta'_j m_j} \\ \dot{p}_i &= \alpha_i \frac{\theta_i m_i}{1+\sum_{j=1}^{N} \theta_j m_j} - \delta p_i \\ y_i &= p_i. \end{cases} \tag{4.1}$$

Here, each subsystem Σ_i represents again a genetic module, which transcribes mRNA m_i and translates protein p_i. The transcription rate of the mRNA m_i is equal to the input promoter concentration u_i. Now we separate dilution and degradation, where the term $\delta_0 m_i$ represents the dilution and the remaining term is the degradation, where the amount degraded is dependent also on the level of mRNAs m_j with $j \neq i$ due to RNase sharing. Moreover, we model RNase degradation as an enzymatic reaction, similarly to the protease degradation, which was introduced in the previous chapter. The translation rate of the protein p_i depends also on the level of mRNAs m_j with $j \neq i$ due to ribossome sharing. The decay rate of the protein lumps together degradation and dilution into the term δp_i.

With this, for a fixed input $u_i = u_i^*$ the system steady state is given by

$$\Sigma_{i,ss} : \begin{cases} 0 &= u_i - \delta_0 m_i - \alpha'_i \frac{\theta'_i m_i}{1+\sum_{j=1}^{N} \theta'_j m_j} \\ 0 &= \alpha_i \frac{\theta_i m_i}{1+\sum_{j=1}^{N} \theta_j m_j} - \delta p_i \\ y_i &= p_i. \end{cases} \tag{4.2}$$

4.1.1 Equilibrium Point and Stability Analysis

We start by analyzing the number of equilibrium points of (4.1) and their stability.

Lemma 5. *The network of subsystems $\Sigma_i, i \in \{1, \ldots, N\}$, with dynamics described by (4.1) has a unique equilibrium point in the positive orthant.*

Proof. Let $x = [m_1, \ldots, m_N, p_1, \ldots, p_N]$, which allows us to rewrite our system in the following form

$$\dot{x} = h(x,u) + \lambda g(x) - \Lambda x = f_\lambda(x,u), \tag{4.3}$$

where $\Lambda = \text{diag}(\delta_0, \ldots, \delta_0, \delta, \ldots, \delta)$, $\lambda \in [0,1]$ and the vectors $h(x,u) \in \mathbb{R}^{2N}$ and $g(x) \in \mathbb{R}^{2N}$ are defined as follows

$$\{h(x,u)\}_i = \begin{cases} u_i, & \text{if } 1 \leq i \leq N \\ \alpha_{i-N} \frac{\theta_{i-N} x_{i-N}}{1+\sum_{j=1}^{N} \theta_j x_j}, & \text{otherwise} \end{cases}, \tag{4.4}$$

$$\{g(x)\}_i = \begin{cases} -\alpha'_i \frac{\theta'_i x_i}{1+\sum_{j=1}^{N} \theta'_j x_j}, & \text{if } 1 \leq i \leq N \\ 0, & \text{otherwise} \end{cases}. \tag{4.5}$$

Now we show that the system $\dot{x} = f_0(x)$ is bounded in the sense of Definition 7 in [27]. Consider the following energy like vector function E

$$\{E\}_i = \begin{cases} \frac{1}{2}\left(x_i - \frac{u_i}{\delta_0}\right)^2, & \text{if } 1 \leq i \leq N \\ \frac{1}{2}\left(x_i - \frac{\alpha_{i-N}}{\delta}\right)^2, & \text{otherwise} \end{cases}, \tag{4.6}$$

and its time derivative

$$\{\dot{E}\}_i = \begin{cases} \left(x_i - \frac{u_i}{\delta_0}\right)\dot{x}_i, & \text{if } 1 \leq i \leq N \\ \left(x_i - \frac{\alpha_{i-N}}{\delta}\right)\dot{x}_i, & \text{otherwise} \end{cases}. \tag{4.7}$$

Notice that for $x_i \geq (u_i/\delta_0) + \Delta, i \in \{1, \ldots, N\}$ and $x_i \geq (\alpha_i/\delta) + \Delta, i \in \{N+1, \ldots, 2N\}$, with $\Delta > 0$, we have

$$\{\dot{E}\}_i \leq \begin{cases} -\delta_0 \Delta^2, & \text{if } 1 \leq i \leq N \\ -\delta \Delta^2, & \text{otherwise} \end{cases}, \tag{4.8}$$

thus, our state trajectories x_i converge in finite time to the set $x_i \in [0, (u_i/\delta_0) + \Delta], i \in \{1, \ldots, N\}$ and $x_i \in [0, (\alpha_{i-N}/\delta) + \Delta], i \in \{N+1, \ldots, 2N\}$. Therefore, for each initial condition, there exist M and T such that $\|x(t)\| < M = \max((u_i/\delta_0) + \Delta, (\alpha_i/\delta) + \Delta)$ for all $t > T$, so $\dot{x} = f_0(x)$ is bounded in the sense of Definition 7 of [27].

Now fix the input $u_i = u_i^*$, define the set $\mathcal{A}_\lambda = \mathbb{R}^{2N}_{\geq 0}$ and compute the derivative of $f_\lambda(x)$ with respect to x, which yields a matrix A composed of four sub-matrices $A_1, A_2, A_3, A_4 \in \mathbb{R}^{N \times N}$ as follows

$$A = \begin{bmatrix} A_1 & A_2 \\ A_3 & A_4 \end{bmatrix}, \tag{4.9}$$

where the sub-matrices $A_1, A_2, A_3, A_4 \in \mathbb{R}^{N\times N}$ are defined as follows

$$\{A_1\}_{i,j} = \begin{cases} -\delta_0 - \lambda\alpha_i' \frac{\theta_i'\left(1+\sum_{n\neq i}(x_n\theta_n')\right)}{\left(1+\sum_{n=1}^{N}(x_n\theta_n')\right)^2}, & \text{if } i = j \\ \lambda\alpha_i' \frac{\theta_j'\left(x_i\theta_i'\right)}{\left(1+\sum_{n=1}^{N}(x_n\theta_n')\right)^2}, & \text{if } i \neq j, \end{cases} \tag{4.10}$$

$$\{A_2\}_{i,j} = 0, \forall i, j, \tag{4.11}$$

$$\{A_3\}_{i,j} = \begin{cases} \alpha_i \frac{\theta_i\left(1+\sum_{n\neq i}(x_n\theta_n)\right)}{\left(1+\sum_{n=1}^{N}(x_n\theta_n)\right)^2}, & \text{if } i = j \\ -\alpha_i \frac{\theta_j(x_i\theta_i)}{\left(1+\sum_{n=1}^{N}(x_n\theta_n)\right)^2}, & \text{if } i \neq j, \end{cases} \tag{4.12}$$

$$\{A_4\}_{i,j} = \begin{cases} -\delta, & \text{if } i = j \\ 0, & \text{if } i \neq j. \end{cases} \tag{4.13}$$

The sub-matrix $-A_1$ is a Z-matrix, as all elements of the off-diagonal of $-A_1$ are non-positive, that is, $\{-A_1\} \leq 0, \forall i \neq j$. Further, $(-A_1)^\top D$, with $D = \text{diag}(1/\alpha_1', \ldots, 1/\alpha_N')$, is strictly diagonally dominant, that is, the row sum, for all rows of $(-A_1)^\top D$, is positive. With this, by Theorem 2.3 in Chapter 6 of [28] condition (I_{29}), $(-A_1)^\top$ is a nonsingular M-matrix for any $\lambda \in [0, 1]$ and $x \in \mathcal{A}_\lambda$.

Since A is a block lower triangular matrix, its determinant $\det(A) = \det(A_1)\det(A_4) \neq 0$ for any $\lambda \in [0, 1]$ and $x \in \mathcal{A}_\lambda$, as $\det(A_4) = (-\delta)^N$ and A_1 is a nonsingular M-matrix. Also observe that $h(x)$ has no zeros on the boundary of the positive orthant and $g(x)$ is mass dissipating in the sense of Definition 8 in [27]. With this, by Theorem 10 of [27] we know that the system in (4.3) with $\lambda = 1$ has the same number of equilibrium points as the system with $\lambda = 0$.

System (4.3) with $\lambda = 0$ and fixed input $u_i = u_i^*$ gives us

$$\dot{m}_i = u_i^* - \delta_0 m_i \tag{4.14}$$

$$\dot{p}_i = \alpha_i \frac{\theta_i m_i}{1 + \sum_{j=1}^{N} \theta_j m_j} - \delta p_i. \tag{4.15}$$

Computing the equilibrium point for this system yields equilibrium mRNA concentration $m_{i,eq} = u_i^*/\delta_0$, which we substitute on the second equation yielding the unique solution

$$p_{i,eq} = \frac{\alpha_i}{\delta} \frac{\theta_i u_i^*}{\delta_0 + \sum_{j=1}^{N} \theta_j u_j^*}. \tag{4.16}$$

Therefore, system (4.3) with $\lambda = 0$ has a unique equilibrium point in the positive orthant, implying by Theorem 10 of [27] that system (4.3) with $\lambda = 1$, that is, system (4.1), also has a unique equilibrium point in the positive orthant. □

Lemma 6. *The equilibrium point of the network of subsystems $\Sigma_i, i \in \{1, \ldots, N\}$, with dynamics described by (4.1) is locally asymptotically stable for all parameter values.*

Proof. We first define the state $\xi = [(m_1 - m_{1,e}), \ldots, (m_N - m_{N,e}), (p_1 - p_{1,e}), \ldots, (p_N - p_{N,e})]$, where $m_{i,e}$ is the mRNA concentration equilibrium point and $p_{i,e}$ is the protein concentration equilibrium point. Then we linearize the system at its equilibrium, yielding

$$\dot{\xi} = A\xi, \tag{4.17}$$

where the matrix A is composed of four sub-matrices $A_1, A_2, A_3, A_4 \in \mathbb{R}^{N\times N}$ as follows

$$A = \begin{bmatrix} A_1 & A_2 \\ A_3 & A_4 \end{bmatrix}, \tag{4.18}$$

where the sub-matrices $A_1, A_2, A_3, A_4 \in \mathbb{R}^{N\times N}$ are defined as follows

$$\{A_1\}_{i,j} = \begin{cases} -\delta_0 - \alpha_i' \frac{\theta_i'\left(1+\sum_{n\neq i}(m_{n,e}\theta_n')\right)}{\left(1+(m_{i,e}\theta_i')+\sum_{n\neq i}(m_{n,e}\theta_n')\right)^2}, & \text{if } i = j \\ \alpha_i' \frac{\theta_j'\left(m_{i,e}\theta_i'\right)}{\left(1+(m_{i,e}\theta_i')+\sum_{n\neq i}(m_{n,e}\theta_n')\right)^2}, & \text{if } i \neq j, \end{cases} \tag{4.19}$$

$$\{A_2\}_{i,j} = 0, \forall i, j, \tag{4.20}$$

$$\{A_3\}_{i,j} = \begin{cases} \alpha_i \frac{\theta_i\left(1+\sum_{n\neq i}(m_{n,e}\theta_n)\right)}{\left(1+(m_{i,e}\theta_i)+\sum_{n\neq i}(m_{n,e}\theta_n)\right)^2}, & \text{if } i = j \\ -\alpha_i \frac{\theta_j(m_{i,e}\theta_i)}{\left(1+(m_{i,e}\theta_i)+\sum_{n\neq i}(m_{n,e}\theta_n)\right)^2}, & \text{if } i \neq j, \end{cases} \tag{4.21}$$

$$\{A_4\}_{i,j} = \begin{cases} -\delta, & \text{if } i = j \\ 0, & \text{if } i \neq j. \end{cases} \tag{4.22}$$

Moreover, the sub-matrix $-A_1$ is a Z-matrix, as all the off-diagonal elements of $-A_1$ are nonpositive, that is, $\{-A_1\} \leq 0, \forall i \neq j$, and additionally, $(-A_1)^\top D$, with $D = \text{diag}(1/\alpha_1', \ldots, 1/\alpha_N')$, is strictly diagonally dominant. With this, by Theorem 2.3 in Chapter 6 of [28] condition (I_{29}), $(-A_1)^\top$ is a nonsingular M-matrix. Furthermore, condition (G_{20}) of Theorem 2.3 in Chapter 6 of [28] states that the eigenvalues of $(-A_1)^\top$ have positive real part. We know that $-A_1$ has the same eigenvalues as $(-A_1)^\top$, which implies that all the eigenvalues of A_1 have negative real part. Since A is a lower block triangular matrix due to A_2 having all entries equal to zero, its eigenvalues are the union of the eigenvalues of A_1 and A_4. The eigenvalues of A_4 are all equal to $-\delta$ and all of the eigenvalues of A_1 have negative real part, so we can conclude that all the eigenvalues of A have negative real part. Therefore, the equilibrium point of the network of subsystems $\Sigma_i, i \in \{1, \ldots, N\}$, with dynamics described by (4.1), is locally asymptotically stable for all parameter values. □

Theorem 5 (Achievable Point)**.** *Given a network of subsystems Σ_i of the form (4.1), with fixed input $u_i = u_i^*$. Then*

$$\frac{1}{\delta} - \sum_{j=1}^{N} \frac{y_i^*}{\alpha_i} > 0, \tag{4.23}$$

if and only if $y_i^*, \forall i$ is achievable, that is, there exists $\theta_i, \theta_i' \geq 0, \forall i$ such that steady state output protein concentration $y_i = y_i^*, \forall i$.

Proof. First we show that (4.23) implies that there exists $\theta_i, \theta_i' \geq 0, \forall i$ such that steady state output protein concentration $y_i = y_i^*, \forall i$. Let $\theta_i' = 0$ and $\theta_i = \theta_i^*, \forall i$, with θ_i^* defined as follows

$$\theta_i^* = \frac{\delta_0 y_i^*}{\alpha_i u_i^* \left(\frac{1}{\delta} - \sum_{j=1}^{N} \frac{y_j^*}{\alpha_j}\right)}. \tag{4.24}$$

Observe that $\theta_i^* \geq 0$ if and only if (4.23) is satisfied. Moreover, the steady state of (4.1), with $\theta_i' = 0, \theta_i = \theta_i^*, \forall i$, yields

$$m_i = (u_i^*/\delta_0) \tag{4.25}$$

$$p_i = \frac{\alpha_i \theta_i^* m_i}{\delta\left(1 + \sum_{j=1}^N \theta_j^* m_j\right)} \tag{4.26}$$

$$y_i = p_i. \tag{4.27}$$

And solving for the steady state output protein concentration y_i yields

$$y_i = \frac{\alpha_i \theta_i^* u_i^*}{\delta\left(\delta_0 + \sum_{j=1}^N \theta_j^* u_j^*\right)} = \frac{\delta_0 y_i^*}{\delta_0 - \delta\delta_0 \sum_{j=1}^N \frac{y_j^*}{\alpha_j} + \delta\delta_0 \sum_{j=1}^N \frac{y_j^*}{\alpha_j}} = \frac{\delta_0 y_i^*}{\delta_0} = y_i^*. \tag{4.28}$$

Now we show that $\exists \theta_i, \theta_i' \geq 0, \forall i$ such that steady state output protein concentration $y_i = y_i^*, \forall i$ implies (4.23). Let $\theta_i = \theta_i^\dagger \geq 0, \theta_i' = \theta_i'^\dagger \geq 0, \forall i$, such that $y_i = y_i^*$. From the system steady state (4.2) we have

$$y_i^* = \frac{\alpha_i \theta_i^\dagger m_i^\dagger}{\delta\left(1 + \sum_{j=1}^N \theta_j^\dagger m_j^\dagger\right)}, \tag{4.29}$$

where $m_i^\dagger \geq 0$ is the solution to

$$0 = u_i^* - \delta_0 m_i^\dagger - \alpha_i' \frac{\theta_i'^\dagger m_i^\dagger}{1 + \sum_{j=1}^N \theta_j'^\dagger m_j^\dagger}. \tag{4.30}$$

Substituting y_i^* from (4.29) into $(1/\delta) - \sum_{k=1}^N (y_k^*/\alpha_k)$ yields

$$\frac{1}{\delta} - \sum_{k=1}^N \frac{y_k^*}{\alpha_k} = \frac{1}{\delta}\left(1 - \sum_{k=1}^N \frac{\theta_k^\dagger m_k^\dagger}{1 + \sum_{j=1}^N \theta_j^\dagger m_j^\dagger}\right) = \frac{1}{\delta\left(1 + \sum_{j=1}^N \theta_j^\dagger m_j^\dagger\right)}\left(1 + \sum_{j=1}^N \theta_j^\dagger m_j^\dagger - \sum_{k=1}^N \theta_k^\dagger m_k^\dagger\right) = \frac{1}{\delta\left(1 + \sum_{j=1}^N \theta_j^\dagger m_j^\dagger\right)} > 0. \tag{4.31}$$

Therefore, (4.23) is satisfied, if and only if there exists $\theta_i, \theta_i' \geq 0, \forall i$ such that steady state output protein concentration $y_i = y_i^*, \forall i$. □

Corollary 3. *Given a network of subsystems Σ_i of the form (4.1). If $y_i^\dagger, \forall i$ is achievable, then any point $y_i \leq y_i^\dagger, \forall i$ is also achievable.*

Proof. We have that $y_i^\dagger, \forall i$ is achievable, so by Theorem 5 we have

$$\frac{1}{\delta} - \sum_{j=1}^N \frac{y_j^\dagger}{\alpha_j} > 0. \tag{4.32}$$

Moreover, we have that

$$\frac{1}{\delta} - \sum_{j=1}^{N} \frac{y_j}{\alpha_j} \geq \frac{1}{\delta} - \sum_{j=1}^{N} \frac{y_j^\dagger}{\alpha_j} > 0, \tag{4.33}$$

as $y_i \leq y_i^\dagger, \forall i$. Therefore, if $y_i^\dagger, \forall i$ is achievable, then any point $y_i \leq y_i^\dagger, \forall i$ is also achievable. □

Specification: The specifications on the steady state of the network of subsystems Σ_i given in (4.1) are given as

$$y_i \in \begin{cases} [0, y_L], & \text{if } u_i = u_L \\ [y_H, +\infty), & \text{if } u_i = u_H, \end{cases} \tag{4.34}$$

where $y_H \geq y_L$ and $y_i = y_H, \forall i$ is a achievable point in the sense of Theorem 5. Moreover, $y_i = y_L$ is also achievable by Corollary 3.

Problem 4 (Feasibility)**.** *Given a network of N subsystems Σ_i of the form (4.1), with a set $S = \theta \times \theta'$, with $\theta, \theta' \subseteq \mathbb{R}^N_{\geq 0}$, for the nonnegative tunable parameters θ_i, θ_i'. Determine if there exists $(\theta_i, \theta_i') \in S, \forall i$, such that y_i satisfies (4.34).*

4.2 PROBLEM SOLUTION

Lets start looking at the case where $\theta_i' = 0, \forall i$, that is, we have a system without the RNase sharing.

Theorem 6. *Given a system of the form (4.1), with $\theta_i \geq 0, \theta_i' = 0, \forall i$, and a specification of the form (4.34). Then*

$$y_i = \frac{\alpha_i \theta_i^* u_L}{\delta \left(\delta_0 + \sum_{j=1}^{N} \theta_j^* u_L \right)} \leq y_L, \tag{4.35}$$

with

$$\theta_i^* = \frac{\delta_0 y_H}{\alpha_i u_H \left(\frac{1}{\delta} - \sum_{j=1}^{N} \frac{y_H}{\alpha_j} \right)} \geq 0, \forall i, \tag{4.36}$$

if and only if $\exists \theta_i \geq 0, \forall i$ such that y_i satisfies the specification given in (4.34).

Proof. First we show that (4.35) with (4.36) implies that $\exists \theta_i \geq 0, \forall i$ such that y_i satisfies the specification given in (4.34). Let $\theta_i = \theta_i^* \geq 0, \forall i$. With this, for $u_i = u_H, \forall i$, then the steady state protein concentration y_i yields

$$y_i = \frac{\alpha_i \theta_i^* u_H}{\delta \left(\delta_0 + \sum_{j=1}^{N} \theta_j^* u_H \right)} = \frac{\frac{\delta_0 y_H}{\frac{1}{\delta} - \sum_{k=1}^{N} \frac{y_H}{\alpha_k}}}{\delta \left(\delta_0 + \sum_{j=1}^{N} \frac{\delta_0 y_H}{\alpha_j \left(\frac{1}{\delta} - \sum_{k=1}^{N} \frac{y_H}{\alpha_k} \right)} \right)} = \frac{y_H}{\delta \left(\frac{1}{\delta} - \sum_{k=1}^{N} \frac{y_H}{\alpha_k} + \sum_{j=1}^{N} \frac{y_H}{\alpha_k} \right)} = y_H. \tag{4.37}$$

With this, for $u_i = u_L, \forall i$, then the steady state protein concentration y_i yields

$$y_i = \frac{\alpha_i \theta_i^* u_L}{\delta\left(\delta_0 + \sum_{j=1}^N \theta_j^* u_L\right)} = \frac{\frac{u_L}{u_H}\frac{\delta_0 y_H}{\frac{1}{\delta} - \sum_{k=1}^N \frac{y_H}{\alpha_k}}}{\delta\left(\delta_0 + \sum_{j=1}^N \frac{u_L}{u_H}\frac{\delta_0 y_H}{\alpha_j\left(\frac{1}{\delta} - \sum_{k=1}^N \frac{y_H}{\alpha_k}\right)}\right)} = \frac{u_L y_H}{u_H - \delta\sum_{j=1}^N (u_H - u_L)\frac{y_H}{\alpha_j}}, \quad (4.38)$$

which from (4.35) with (4.36) we have that $y_i \leq y_L$. Now, for u_i, then the steady state protein concentration y_i yields

$$y_i = \frac{\alpha_i \theta_i^* u_L}{\delta\left(\delta_0 + \sum_{j=1}^N \theta_j^* u_L\right)} = \frac{u_i y_H}{u_H - \delta(u_H - u_i)\frac{y_H}{\alpha_i} - \delta\sum_{j\neq i}(u_H - u_j)\frac{y_H}{\alpha_j}}, \quad (4.39)$$

then for $u_i = u_H$ we have

$$y_i = \frac{u_H y_H}{u_H - \delta\sum_{j\neq i}(u_H - u_j)\frac{y_H}{\alpha_j}} \geq y_H. \quad (4.40)$$

on the other hand, for $u_i = u_L$ we have

$$y_i = \frac{u_L y_H}{u_H - \delta(u_H - u_L)\frac{y_H}{\alpha_i} - \delta\sum_{j\neq i}(u_H - u_j)\frac{y_H}{\alpha_j}} \leq \frac{u_L y_H}{u_H - \delta\sum_{j=1}^N (u_H - u_L)\frac{y_H}{\alpha_j}} \leq y_L. \quad (4.41)$$

Now we show that $\exists \theta_i \geq 0, \forall i$ such that y_i satisfies the specification (4.34) implies that (4.35) with (4.36). That is, $\exists \theta_i \geq 0, \forall i$ such that

$$\begin{cases} \frac{\alpha_i \theta_i u_L}{\delta\left(\delta_0 \sum_{j=1}^N \theta_j u_L\right)} & \leq y_L \\ \frac{\alpha_i \theta_i u_H}{\delta\left(\delta_0 \sum_{j=1}^N \theta_j u_H\right)} & \geq y_H, \end{cases} \quad (4.42)$$

which can be rewritten as

$$\begin{cases} \theta_i & \leq \hat{\gamma}_i \left(\frac{\delta_0}{u_L} + \sum_{j\neq i} \theta_j\right) \\ \theta_i & \geq \tilde{\gamma}_i \left(\frac{\delta_0}{u_H} + \sum_{j\neq i} \theta_j\right), \end{cases} \quad (4.43)$$

where the gains $\tilde{\gamma}_i$ and $\hat{\gamma}_i$ are defined as follows

$$\hat{\gamma}_i = \frac{\delta y_L}{\alpha_i - \delta y_L} \quad (4.44)$$

$$\tilde{\gamma}_i = \frac{\delta y_H}{\alpha_i - \delta y_H}. \quad (4.45)$$

Moreover, we can write in matrix form

$$\begin{cases} \theta \leq \frac{\delta_0}{u_L}\hat{\gamma} + \hat{\Gamma} T \theta \\ \theta \geq \frac{\delta_0}{u_H}\tilde{\gamma} + \tilde{\Gamma} T \theta, \end{cases} \quad (4.46)$$

where the vectors $\tilde{\gamma} = [\tilde{\gamma}_1, \ldots, \tilde{\gamma}_N]^\top, \hat{\gamma} = [\hat{\gamma}_1, \ldots, \hat{\gamma}_N]^\top$ and the matrices $\tilde{\Gamma}, \hat{\Gamma}$ and T are defined as follows

$$\{\tilde{\Gamma}\}_{i,j} = \begin{cases} \tilde{\gamma}_i, & \text{if } i = j \\ 0, & \text{if } i \neq j \end{cases} \tag{4.47}$$

$$\{\hat{\Gamma}\}_{i,j} = \begin{cases} \hat{\gamma}_i, & \text{if } i = j \\ 0, & \text{if } i \neq j \end{cases} \tag{4.48}$$

$$\{T\}_{i,j} = \begin{cases} 0, & \text{if } i = j \\ 1, & \text{if } i \neq j. \end{cases} \tag{4.49}$$

Which yield the following inequalities

$$\begin{cases} \left(I - \hat{\Gamma}T\right)\theta \leq \frac{\delta_0}{u_L}\hat{\gamma} \\ \left(I - \tilde{\Gamma}T\right)\theta \geq \frac{\delta_0}{u_H}\tilde{\gamma}. \end{cases} \tag{4.50}$$

Let $\hat{A} = I + \hat{\Gamma}, \tilde{A} = I + \tilde{\Gamma}$ and $v = [-1, \ldots, -1]^\top$, so we have $\hat{M} = \left(I - \hat{\Gamma}T\right) = \left(\hat{A} - \hat{\gamma}v^\top\right)$ and $\tilde{M} = \left(I - \tilde{\Gamma}T\right) = \left(\tilde{A} - \tilde{\gamma}v^\top\right)$. We know that $1 + v^\top \hat{A}^{-1}\hat{\gamma} = (1/\delta) - \sum_{j=1}^{N}(y_L/\alpha_j) > 0$ and $1 + v^\top \tilde{A}^{-1}\tilde{\gamma} = (1/\delta) - \sum_{j=1}^{N}(y_H/\alpha_j) > 0$, so the inverse of $\hat{M}$ and $\tilde{M}$ exists and are given by

$$\left(\hat{A} - \hat{\gamma}v^\top\right)^\top = \hat{A}^{-1} - \frac{\hat{A}^{-1}\hat{\gamma}v\hat{A}^{-1}}{1 + v^\top \hat{A}^{-1}\hat{\gamma}} \tag{4.51}$$

$$\left(\tilde{A} - \tilde{\gamma}v^\top\right)^\top = \tilde{A}^{-1} - \frac{\tilde{A}^{-1}\tilde{\gamma}v\tilde{A}^{-1}}{1 + v^\top \tilde{A}^{-1}\tilde{\gamma}}, \tag{4.52}$$

which yields

$$\{\hat{M}^{-1}\}_{i,j} = \begin{cases} \frac{1}{1+\hat{\gamma}_i} + \frac{1}{\frac{1}{\delta} - \sum_{k=1}^{N}\frac{y_L}{\alpha_k}} \frac{\hat{\gamma}_i}{(1+\hat{\gamma}_i)^2}, & \text{if } i = j \\ \frac{1}{\frac{1}{\delta} - \sum_{k=1}^{N}\frac{y_L}{\alpha_k}} \frac{\hat{\gamma}_i}{(1+\hat{\gamma}_i)(1+\hat{\gamma}_j)}, & \text{if } i \neq j \end{cases} \tag{4.53}$$

$$\{\tilde{M}^{-1}\}_{i,j} = \begin{cases} \frac{1}{1+\tilde{\gamma}_i} + \frac{1}{\frac{1}{\delta} - \sum_{k=1}^{N}\frac{y_H}{\alpha_k}} \frac{\tilde{\gamma}_i}{(1+\tilde{\gamma}_i)^2}, & \text{if } i = j \\ \frac{1}{\frac{1}{\delta} - \sum_{k=1}^{N}\frac{y_H}{\alpha_k}} \frac{\tilde{\gamma}_i}{(1+\tilde{\gamma}_i)(1+\tilde{\gamma}_j)}, & \text{if } i \neq j \end{cases} \tag{4.54}$$

Calculating bounds on θ_i using (4.50) yields

$$\begin{cases} \theta_i \leq \frac{\delta_0 y_L}{\alpha_i u_L \left(\frac{1}{\delta} - \sum_{j=1}^{N}\frac{y_L}{\alpha_j}\right)} \\ \theta_i \geq \frac{\delta_0 y_H}{\alpha_i u_H \left(\frac{1}{\delta} - \sum_{j=1}^{N}\frac{y_H}{\alpha_j}\right)} \end{cases} \tag{4.55}$$

So if there $\exists \theta_i, \forall i$ such that y_i satisfies the specification (4.34), then it has to satisfies the bounds in (4.55). Now observe that the steady state protein concentration y_i is a monotonically increasing function of θ_i, which implies that if any θ_i that satisfies the bounds (4.55), then $\theta_i = \theta_i^*$ from (4.36), that is, the lower bound in (4.55) also satisfies the specification.

Therefore, (4.35) with (4.36) if and only if $\exists \theta_i \geq 0, \forall i$ such that y_i satisfies the specification given in (4.34). □

Now lets consider the case where $\theta'_i \geq 0, \forall i$ and let the solution for the steady state of network of N subsystems Σ_i of the form (4.1), be given by

$$\begin{cases} y_i & = f_i(m_i, m_j), \forall j \neq i \\ m_i & = g_i(u_i, u_j), \forall j \neq i. \end{cases} \tag{4.56}$$

Numerically determine $\theta'^*_i = \max(\theta'_i), \forall i$ such that

$$\begin{cases} \frac{\partial y_i}{\partial u_i} & = \sum_{k=1}^{N} \frac{\partial f_i}{\partial m_k} \frac{\partial g_k}{\partial u_i} > 0 \\ \frac{\partial y_i}{\partial u_j} & = \sum_{k=1}^{N} \frac{\partial f_i}{\partial m_k} \frac{\partial g_k}{\partial u_j} \leq 0, \forall j \neq i, \end{cases} \tag{4.57}$$

for all possible combinations of the input present in the specification (4.34). Moreover, define m_i^L and m_i^H as the solutions to the system of equations

$$0 = u_L - \delta_0 m_i^L - \alpha'_i \frac{\theta'^*_i m_i^L}{1 + \sum_{j=1}^{N} \theta'^*_j m_j^L}, \forall i \tag{4.58}$$

$$0 = u_H - \delta_0 m_i^H - \alpha'_i \frac{\theta'^*_i m_i^H}{1 + \sum_{j=1}^{N} \theta'^*_j m_j^H}, \forall i. \tag{4.59}$$

Theorem 7. *Given a system of the form (4.1), with $\theta_i, \theta'_i \geq 0, \forall i$, a specification of the form (4.34) and $\theta'^*_i \geq 0, \forall i$. If*

$$y_i = \frac{\alpha_i \theta^*_i m_i^L}{\delta \left(1 + \sum_{j=1}^{N} \theta^*_j m_i^L\right)} \leq y_L, \tag{4.60}$$

with

$$\theta^*_i = \frac{y_H}{\alpha_i m_i^H \left(\frac{1}{\delta} - \sum_{j=1}^{N} \frac{y_H}{\alpha_j}\right)} \geq 0, \forall i, \tag{4.61}$$

then $\exists \theta_i \geq 0, 0 \leq \theta'_i \leq \theta'^*_i, \forall i$ such that y_i satisfies the specification given in (4.34).

Proof. Let $\theta_i = \theta^*_i, \theta'_i = \theta'^*_i, \forall i$. With this, for u_i the steady state protein concentration y_i yields

$$y_i = \frac{\alpha_i \theta^*_i m_i}{\delta \left(1 + \sum_{j=1}^{N} \theta^*_j m_i\right)} = \frac{m_i y_H}{m_i^H - \delta(m_i^H - m_i)\frac{y_H}{\alpha_i} - \delta \sum_{j \neq i}(m_j^H - m_j)\frac{y_H}{\alpha_j}}, \tag{4.62}$$

which for $u_i = u_L$ we have $m_i = m_i^L$ and

$$y_i = \frac{m_i^L y_H}{m_i^H - \delta(m_i^H - m_i^L)\frac{y_H}{\alpha_i} - \delta \sum_{j \neq i}(m_j^H - m_j)\frac{y_H}{\alpha_j}} \leq \frac{m_i^L y_H}{m_i^H - \delta \sum_{j=1}^{N}(m_j^H - m_j^L)\frac{y_H}{\alpha_j}}, \tag{4.63}$$

which from (4.60) with (4.61) we have $y_i \leq y_L$. Moreover, for $u_i = u_H$ we have $m_i = m_i^H$ and

$$y_i = \frac{m_i^H y_H}{m_i^H - \delta \sum_{j \neq i}(m_j^H - m_j)\frac{y_H}{\alpha_j}} \geq y_H. \tag{4.64}$$

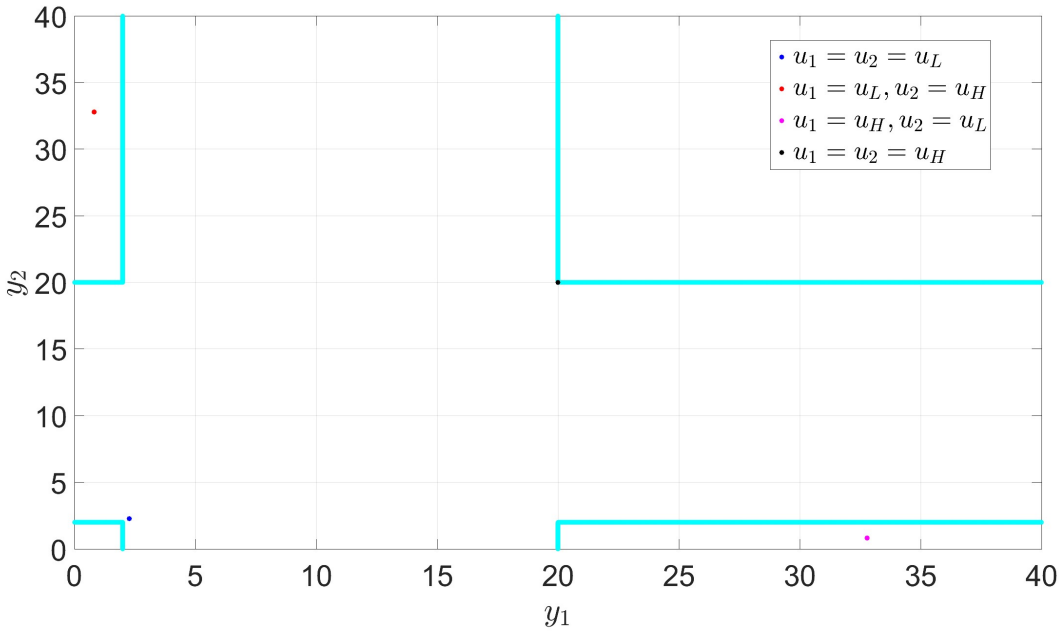

Figure 4.1: Specification boundaries in cyan and steady state output protein concentration y_i for different inputs, with no RNase degradation ($\theta'_i = 0$ [nM^{-1}]).

Therefore, if (4.60) with (4.61), then $\exists \theta_i \geq 0, 0 \leq \theta'_i \leq \theta'^*_i, \forall i$ such that y_i satisfies the specification given in (4.34). □

Illustrative Example

Consider a system with $N = 2$ subsystems Σ_i, with model parameters $\alpha = [50,\ 50]^\top$ [nM/hr], $\alpha' = [50,\ 50]^\top$ [nM/hr], $\delta = 1^\top$ [hr^{-1}], $\delta_0 = 10^\top$ [hr^{-1}]. Moreover, we set the low input $u_L = 1$ [nM], the high input $u_H = 40$ [nM], the low output $y_L = 2$ [nM] and the high output $y_H = 20$ [nM]. Computing θ^*_i as defined in (4.36) yields $\theta^*_i = [0.5, 0.5]^\top$ [nM^{-1}]. Now using this value to compute the steady state output protein concentration y_i for the low input, that is, $u_i = u_L, \forall i$, yields $y = [2.2727, 2.2727]^\top$ [nM], which does not satisfies the specification. Figure 4.1 show the specification boundaries in cyan and steady state output protein concentration y_i for different inputs, with no RNase degradation ($\theta'_i = 0$ [nM^{-1}]). Note that we verify that the specification is not satisfied.

Adding RNase degradation to our system, we have that $\theta'^* = [1.2141, 1.2141]^\top$ [nM^{-1}]

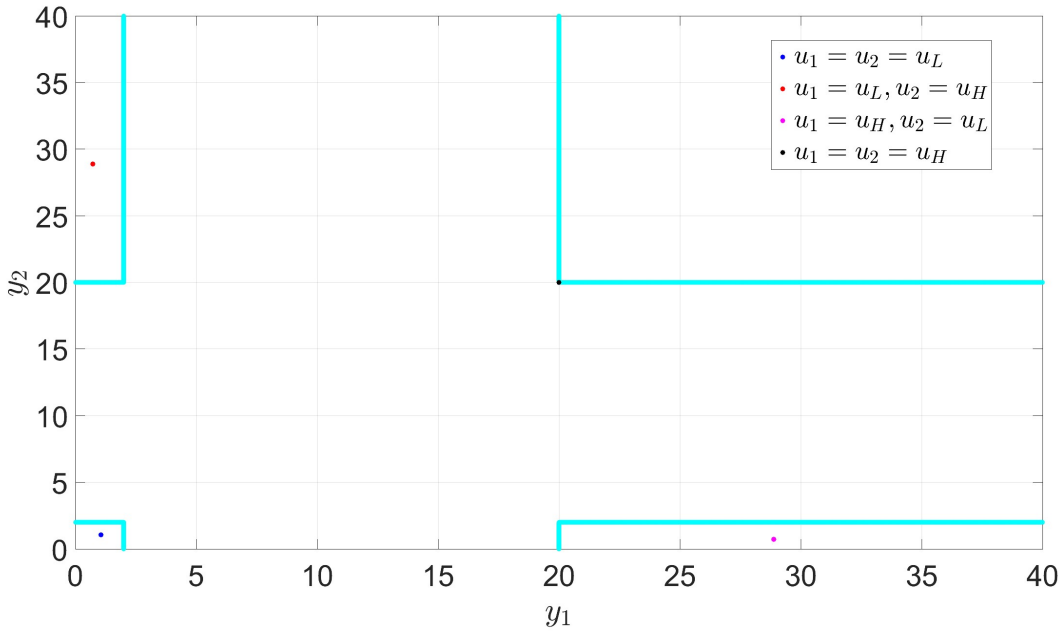

Figure 4.2: Specification and steady state output protein concentration y_i for different inputs, with RNase degradation ($\theta'_i = 1.2141$ [nM^{-1}]).

satisfies (4.57) as shown bellow

$$\left.\frac{\partial y_i}{\partial u_i}\right|_{u=[u_L,u_L]} = \begin{bmatrix} 0.7299 & -7.9615 \times 10^{-7} \\ -7.9615 \times 10^{-7} & 0.7299 \end{bmatrix} \tag{4.65}$$

$$\left.\frac{\partial y_i}{\partial u_i}\right|_{u=[u_L,u_H]} = \begin{bmatrix} 0.6629 & -0.0036 \\ -0.1424 & 0.5241 \end{bmatrix} \tag{4.66}$$

$$\left.\frac{\partial y_i}{\partial u_i}\right|_{u=[u_H,u_L]} = \begin{bmatrix} 0.5241 & -0.1424 \\ -0.0036 & 0.6629 \end{bmatrix} \tag{4.67}$$

$$\left.\frac{\partial y_i}{\partial u_i}\right|_{u=[u_H,u_H]} = \begin{bmatrix} 0.3370 & -0.1630 \\ -0.1630 & 0.3370 \end{bmatrix}. \tag{4.68}$$

With this, computing θ_i^* as defined in (4.61) yields $\theta^* = [1.0319, 1.0319]^\top$ [nM^{-1}]. Now using this value to compute the steady state output protein concentration y_i for the low input, that is, $u_i = u_L, \forall i$, yields $y = [0.7298, 0.7298]^\top$ [nM] and by Theorem 7 we have that $\exists \theta_i \geq 0, 0 \leq \theta'_i \leq \theta'^*_i, \forall i$ such that y_i satisfies the specification given in (4.34). Figure 4.2 show the specification boundaries in cyan and steady state output protein concentration y_i for different inputs, with RNase degradation ($\theta'_i = 0.6609$ [nM^{-1}]). Figure 4.2 illustrates that by adding RNase degradation to the mRNA enables the network to satisfies the specification, where it was not able to satisfie without it.

4.3 PRACTICAL APPLICATION

As shown in the illustrative example, adding RNase degradation to our network of subsystems may aid in meeting the specification, on the other hand we lose the monotonic decreasing behavior between input $u_j, j \neq i$ and output y_i, which makes it harder to analytically prove conditions about system output. But we can utilize our knowledge of the system to numerically compute the feasible region for θ or θ' for each part of the specification by fixing the other tunable parameter.

θ Feasible Region

Let the tunable parameter $\theta'_i = \theta'^*_i \geq 0, \forall i$ be fixed, with this we can obtain the feasible region using the map from y_i to θ_i defined as follows

$$\theta_i = \frac{y_i}{\alpha_i m_i \left(\frac{1}{\delta} - \sum_{j=1}^{N} \frac{y_j}{\alpha_j}\right)}, \tag{4.69}$$

where m_i is defined as the solution to

$$0 = u_i - \delta_0 m_i - \alpha'_i \frac{\theta'^*_i m_i}{1 + \sum_{j=1}^{N} \theta'^*_j m_j}, \forall i. \tag{4.70}$$

θ' Feasible Region

Let the tunable parameter $\theta_i = \theta^*_i \geq 0, \forall i$ be fixed, with this we first translate the specification from the steady state output protein y_i to the steady state mRNA concentration m_i using the following map

$$m_i = \frac{y_i}{\alpha_i \theta^*_i \left(\frac{1}{\delta} - \sum_{j=1}^{N} \frac{y_j}{\alpha_j}\right)}. \tag{4.71}$$

Now using the mRNA specification we can obtain the feasible region for the θ' tunable parameter using the map from m_i to θ'_i defined as follows

$$\theta'_i = \frac{u_i - \delta_0 m_i}{\alpha'_i m_i \left(1 - \sum_{j=1}^{N} \frac{u_j - \delta_0 m_j}{\alpha'_j}\right)}. \tag{4.72}$$

Chapter 5

Conclusion and Future Work

In this work we presented a co-design approach to deal with the resource sharing problem inherent in biological system design, due to the limited availability of certain shared resources in the cell. Moreover, this novel approach relies on tuning the system parameters to ensure that the network of subsystems adheres to a specification. In contrast to the usual approaches, namely, centralized control of a shared resource and decentralized control of subsystems, which add additional machinery to the cell in order to mitigate the coupling between the subsystems.

Three different models with ribossome sharing with either no other shared resource or protease sharing or RNase sharing were considered. In addition, we consider two different kinds of specifications, in the first we have a single input value for each subsystem and the goal is to maintain the ouput around a desired value with a fixed tolerance. In the second, we have a low and a high value for the input and our goal is to maintain the output of each subsystem bellow a low output level for that subsystem low input and above a high output value for that subsystem high input. Conditions for the feasibility of each specification were derived through rigorous mathematical logic. Moreover, illustrative and application examples were provided to demonstrate the feasibility conditions and how to compute the tunable parameter region where the specifications are met.

Further work on this topic may focus on explore additional types of specification and other network models. As for specifications, one may expand the fixed input points to input ranges where a certain output specification needs to be met. Additionally, here we consider only parallel systems, so a interesting next step would be to consider sequential networks of subsystems, which have a very desirable application in the design of logic gates.

References

[1] S. Cardinale and A. P. Arkin, "Contextualizing context for synthetic biology–identifying causes of failure of synthetic biological systems," *Biotechnology Journal*, 2012.

[2] C. D. McBride, T. W. Grunberg, and D. Del Vecchio, "Design of genetic circuits that are robust to resource competition," *Current Opinion in Systems Biology*, 2021.

[3] T. W. Grunberg and D. Del Vecchio, "Modular analysis and design of biological circuits," *Current Opinion in Biotechnology*, 2019.

[4] D. Del Vecchio, A. J. Ninfa, and E. D. Sontag, "Modular cell biology: Retroactivity and insulation," *Molecular Systems Biology*, 2008.

[5] S. Jayanthi, K. S. Nilgiriwala, and D. Del Vecchio, "Retroactivity controls the temporal dynamics of gene transcription," *ACS Synthetic Biology*, 2013.

[6] D. Mishra, P. M. Rivera, A. Lin, D. Del Vecchio, and R. Weiss, "A load driver device for engineering modularity in biological networks," *Nature Biotechnology*, 2014.

[7] D. Del Vecchio, "Modularity, context-dependence, and insulation in engineered biological circuits," *Trends in Biotechnology*, 2015.

[8] L. E. Macdonald, R. K. Durbin, J. J. Dunn, and W. T. McAllister, "Characterization of two types of termination signal for bacteriophage t7 rna polymerase," *Journal of Molecular Biology*, 1994.

[9] V. A. Rhodius, V. K. Mutalik, and C. A. Gross, "Predicting the strength of up-elements and full-length e. coli σe promoters," *Nucleic Acids Research*, 2012.

[10] S. Kosuri, D. B. Goodman, G. Cambray, V. K. Mutalik, Y. Gao, A. P. Arkin, D. Endy, and G. M. Church, "Composability of regulatory sequences controlling transcription and translation in escherichia coli," *Proceedings of the National Academy of Sciences of the United States of America*, 2013.

[11] D. Del Vecchio, Y. Qian, R. M. Murray, and E. D. Sontag, "Future systems and control research in synthetic biology," *Annual Reviews in Control*, 2018.

[12] A. Gyorgy, J. I. Jiménez, J. Yazbek, H. H. Huang, H. Chung, R. Weiss, and D. Del Vecchio, "Isocost lines describe the cellular economy of genetic circuits," *Biophysical Journal*, 2015.

[13] F. Ceroni, R. Algar, G. B. Stan, and T. Ellis, "Quantifying cellular capacity identifies gene expression designs with reduced burden," *Nature Methods*, 2015.

[14] D. Del Vecchio and R. M. Murray, *Biomolecular Feedback Systems*. Princeton University Press, 2014.

[15] Y. Qian, H. Huang, J. I. Jiménez, and D. Del Vecchio, "Resource competition shapes the response of genetic circuits," *ACS Synthetic Biology*, 2017.

[16] D. Cameron and J. Collins, "Tunable protein degradation in bacteria," *Nature Biotechnology*, 2014.

[17] N. A. Cookson, W. H. Mather, T. Danino, O. Mondragón-Palomino, R. J. Williams, L. S. Tsimring, and J. Hasty, "Queueing up for enzymatic processing: Correlated signaling through coupled degradation," *Molecular Systems Biology*, 2011.

[18] A. Darlington, J. Kim, J. Jiménez, and G. D. Bates, "Dynamic allocation of orthogonal ribosomes facilitates uncoupling of co-expressed genes," *Nature Communications*, 2018.

[19] C. Barajas, H. Huang, J. Gibson, L. Sandoval, and D. Del Vecchio, "Feedforward growth rate control mitigates gene activation burden," *Nature Communications*, 2022.

[20] T. Shopera, L. He, T. Oyetunde, Y. J. Tang, and T. S. Moon, "Decoupling resource-coupled gene expression in living cells," *ACS Synthetic Biology*, 2017.

[21] H. Huang, Y. Qian, and D. Del Vecchio, "A quasi-integral controller for adaptation of genetic modules to variable ribosome demand," *Nature Communications*, 2018.

[22] Y. Qian and D. Del Vecchio, "Robustness of networked systems to unintended interactions with application to engineered genetic circuits," *IEEE Transactions on Control of Network Systems*, 2021.

[23] C. E. Celeste Junior, I. Di Loreto, T. W. Grunberg, M. D. Di Benedetto, A. Borri, and D. Del Vecchio, "Co-design of resource limited genetic modules," *62nd IEEE Conference on Decision and Control (CDC)*, 2023.

[24] R. A. Horn and C. R. Johnson, *Topics in Matrix Analysis*. Cambridge University Press, 1991.

[25] J. Löfberg, "Yalmip : A toolbox for modeling and optimization in matlab," *In Proceedings of the CACSD Conference*, 2004.

[26] C. McBride and D. Del Vecchio, "Analyzing and exploiting the effects of protease sharing in genetic circuits," *20th IFAC World Congress*, 2017.

[27] C. McBride and D. Del Vecchio, "The number of equilibrium points of perturbed nonlinear positive dynamical systems," *Automatica*, 2020.

[28] A. Bermon and R. J. Plemmons, *Nonnegative Matrices in the Mathematical Sciences*. Society for Industrial and Applied Mathematics, 1994.

[29] J. Sherman and W. J. Morrison, "Adjustment of an inverse matrix corresponding to a change in one element of a given matrix," *Annals of Mathematical Statistics*, 1950.