Assessing Feasibility in Resource Limited Genetic Networks*

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***Abstract*— The design of biological systems is a challenging endeavor due to the lack of modularity caused by many reasons, such as sharing of a limited pool of resources by multiple gene expression modules. This work considers the problem of determining when specifications on the steady state system behavior can be met for suitable parameter choices, while accounting for resource sharing. We establish both sufficient and necessary conditions for the feasibility of a specification for a given network of subsystems that share both production and degradation resources. This extends previous work that focused only on the presence of production resource sharing. With this, this work lays the foundations for the development of co-design techniques for genetic networks with both production and degradation resources, where one may be able to mitigate the effects of one type of resource sharing by tuning the other.**

I. INTRODUCTION

The design of systems by leveraging the properties of the composing subsystems and their connectivity is a modular approach that has proven convenient in several fields of engineering to tackle the complexity of design problems. Although this modular approach to design has also been employed for engineering genetic circuits in synthetic biology, the connectivity among subsystems is often difficult to identify [1]. Indeed, effects such as retroactivity caused by connecting systems' outputs to downstream systems [2]–[4], competition for limited cellular resources needed for gene expression [5], [6] and for protein degradation [7], affect system performance in surprising ways [8].

Previous efforts to mitigate the undesired effects of resource sharing have concentrated on two approaches [9], namely, decentralized control of subsystems and centralized control of a shared resource. The decentralized control approach focuses on isolating the module from perturbations in cellular resources [10]–[12]. On the other hand, the centralized control approach aims to maintain the free resource level at a constant value [13], [14].

In this article, we utilize the I/O framework proposed in [12], where each input/output system has additional disturbance outputs and disturbance inputs. The former ones account for the load that the system applies on shared resources, while the latter ones capture the cumulative load that all other systems apply on such resources. Our goal 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. Our analysis focuses on the feasibility of designing such networks by only tuning subsystem parameters, such as ribosomes and protease [8] binding strength.

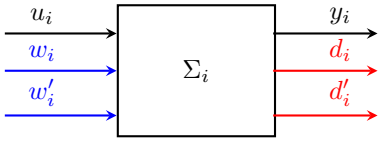

Fig. 1. Block diagram representation of subsystem Σ_i.

Previous work on tackling the resource sharing problem has determined conditions for network disturbance decoupling, which offers insights on designing feedback controllers to achieve decoupling in a network with multiple genes [12]. A co-design approach, similar to that of our paper, is presented in [15], where the ribosome binding strength is designed to achieve system specifications on the steady state output. Different from [15], which considers only production resource sharing, here we also consider degradation resource sharing and additionally provide a tightening of the results of [15].

This work is organized as follows. In Section II, we introduce the system, the specification each system has to adhere to, and state the feasibility problem. In Section III, we focus on the problem solution, proving multiple sufficient and necessary conditions for the existence of a solution to the feasibility problem. In Section IV, we present application examples.

II. PROBLEM FORMULATION

Consider the following model for a network of N subsystems, shown in Figure 1, where each subsystem Σ_i has dynamics described by

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

where $\theta_i \geq 0, \theta_i' \geq 0, i \in \{1, \ldots, N\}$ are tunable parameters. Throughout this work we assume that $u_i, \alpha_i, \alpha_i' >$

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$0, i \in \{1,\dots,N\}$ and $\delta_0, \delta > 0$. Additionally, w_i and w_i' are state-dependent disturbance inputs given by

$$\begin{cases} w_i &= \sum_{j\neq i} d_j \\ w_i' &= \sum_{j\neq i} d_j'. \end{cases} \tag{2}$$

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 [12] and has been derived and experimentally validated in [6]. 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 [16]. 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 $u_i = u_i^*, i \in \{1,\dots,N\}$, we can write the steady state equations for our subsystem as

$$\Sigma_{i,ss}: \begin{cases} m_i &= \frac{u_i^*}{\delta_0} \\ 0 &= \alpha_i \frac{d_i}{1+d_i+w_i} - \delta p_i - \alpha_i' \frac{d_i'}{1+d_i'+w_i'} \\ y_i &= p_i. \end{cases} \tag{3}$$

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

$$0 = \alpha_i \frac{\theta_i u_i^*}{\delta_0 + \sum_{j=1}^N \theta_j u_j^*} - \delta y_i - \alpha_i' \frac{\theta_i' y_i}{1 + \sum_{j=1}^N \theta_j' y_j}, \tag{4}$$

$i \in \{1,\dots,N\}$. 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 $\varepsilon_i > 0, i \in \{1,\dots,N\}$.

Specification: Consider a fixed input $u_i = u_i^*$, fixed desired output value y_i^*, and fixed tolerances $\varepsilon_i \geq 0$, $i = \{1,\dots,N\}$. The specifications on the steady state of the network of subsystems Σ_i given in (1) with interconnection rule (2) are given as

$$y_i \in [y_i^* - \varepsilon_i, y_i^* + \varepsilon_i],\ i \in \{1,...,N\}. \tag{5}$$

Problem 1 (Feasibility). *Given a network of N subsystems Σ_i of the form (1) and interconnection rule (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 (4), satisfies (5).*

A. Equilibrium Point and Stability Analysis

Before we start tackling Problem 1, we analyze the number of equilibrium points of (1) and their stability.

Lemma 1. *The network of subsystems $\Sigma_i, i \in \{1,\dots,N\}$, with dynamics described by (1) and interconnection rule (2), has a unique equilibrium point in the positive orthant.*

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

$$\dot{x} = h(x,u) + \lambda g(x) - \Lambda x = f_\lambda(x,u), \tag{6}$$

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

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

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

Now we show that the system $\dot{x} = f_0(x)$ is bounded in the sense of Definition 7 in [17]. Consider the following energy like vector function E

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

and its time derivative

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

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

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

thus, our state trajectories x_i converge in finite time to the set $x_i \in [0, (u_i/\delta_0) + \Delta], i \in \{1,\dots,N\}$ and $x_i \in [0, (\alpha_{i-N}/\delta) + \Delta], i \in \{N+1,\dots,2N\}$. 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 [17].

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

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

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

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

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

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

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

The sub-matrix $-A_4$ is a Z-matrix, as all elements of the off-diagonal of $-A_4$ are nonpositive, 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 [18] 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 [17]. With this, by Theorem 10 of [17] we know that the system in (6) with $\lambda = 1$ has the same number of equilibrium points as the system with $\lambda = 0$.

System (6) with $\lambda = 0$ and fixed input $u_i = u_i^*$ gives us

$$\begin{aligned} \dot{m}_i &= u_i^* - \delta_0 m_i \\ \dot{p}_i &= \alpha_i \frac{\theta_i m_i}{1 + \sum_{j=1}^N \theta_j m_j} - \delta p_i. \end{aligned}$$

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

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

Therefore, system (6) with $\lambda = 0$ has a unique equilibrium point in the positive orthant, implying by Theorem 10 of [17] that system (6) with $\lambda = 1$, that is, system (1), also has a unique equilibrium point in the positive orthant. □

Lemma 2. *The equilibrium point of the network of subsystems $\Sigma_i, i \in \{1, \ldots, N\}$, with dynamics described by* (1) *and interconnection rule* (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

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

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

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

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

$$\begin{aligned} \{A_1\}_{i,j} &= \begin{cases} -\delta_0, & \text{if } i = j \\ 0, & \text{if } i \neq j, \end{cases} \\ \{A_2\}_{i,j} &= 0, \forall i, j, \\ \{A_3\}_{i,j} &= \begin{cases} \alpha_i \frac{\theta_i \left(1 + \sum_{n \neq i} (m_{n,e}\theta_n)\right)}{\left(1 + (m_{i,e}\theta_i) + \sum_{n \neq i} (m_{n,e}\theta_n)\right)^2}, & \text{if } i = j \\ -\alpha_i \frac{\theta_j (m_{i,e}\theta_i)}{\left(1 + (m_{i,e}\theta_i) + \sum_{n \neq i} (m_{n,e}\theta_n)\right)^2}, & \text{if } i \neq j, \end{cases} \\ \{A_4\}_{i,j} &= \begin{cases} -\delta - \alpha'_i \frac{\theta'_i \left(1 + \sum_{n \neq i} (p_{n,e}\theta'_n)\right)}{\left(1 + (p_{i,e}\theta'_i) + \sum_{n \neq i} (p_{n,e}\theta'_n)\right)^2}, & \text{if } i = j \\ \alpha'_i \frac{\theta'_j \left(p_{i,e}\theta'_i\right)}{\left(1 + (p_{i,e}\theta'_i) + \sum_{n \neq i} (p_{n,e}\theta'_n)\right)^2}, & \text{if } i \neq j. \end{cases} \end{aligned}$$

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 [18] condition (I_{29}), $(-A_4)^\top$ is a nonsingular M-matrix. Furthermore, condition (G_{20}) of Theorem 2.3 in Chapter 6 of [18] 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 (1) and interconnection rule (2), is locally asymptotically stable for all parameter values. □

Theorem 1. *The network of N subsystems Σ_i with dynamics described by* (1) *and interconnection rule* (2)*, with fixed input $u_i = u_i^*$ has steady state protein output y_i that satisfies the specification in* (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* (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 (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 (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 (4) satisfies the specification in (5). From (4) we have

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

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

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

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

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

This can be verified by substituting $\theta_i' = 0, \theta_i = \theta_i^*, \forall i$ into (4), yielding

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

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 (5) for some $\theta_i \geq 0, \theta_i' = 0$, then the same network has y_i which satisfies (5) with the same $\theta_i \geq 0, \theta_i' = 0 \geq 0$. □

B. 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 1 we have that the feasibility of a specification (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) allows us to derive the following steady state I/O map

$$d_i = \gamma_i(1 + w_i), \tag{7}$$

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

$$\gamma_i = \frac{\delta y_i}{\alpha_i - \delta y_i}.$$

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

$$\tilde{\gamma}_i = \frac{\delta(y_i^* - \varepsilon_i)}{\alpha_i - \delta(y_i^* - \varepsilon_i)}, \tag{8}$$
$$\hat{\gamma}_i = \frac{\delta(y_i^* + \varepsilon_i)}{\alpha_i - \delta(y_i^* + \varepsilon_i)}. \tag{9}$$

III. PROBLEM SOLUTION

Let $w = [w_1, \ldots, w_N]^\top$ and $d = [d_1, \ldots, d_N]^\top$, then (2) implies

$$w = Td,$$

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

$$\{T\}_{i,j} = \begin{cases} 0, & \text{if } i = j \\ 1, & \text{if } i \neq j. \end{cases} \tag{10}$$

Moreover, (7) can be written in matrix form as

$$d = \gamma + \Gamma w,$$

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

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

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

$$\{\tilde{\Gamma}\}_{i,j} = \begin{cases} \tilde{\gamma}_i, & \text{if } i = j \\ 0, & \text{if } i \neq j. \end{cases} \tag{11}$$

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 (5).

Theorem 2. *Let $\tilde{\Gamma}$ be the gain matrix defined in* (11)*, T be the interconnection matrix defined in* (10) *and $\theta_i' = 0$. There exist $\theta_i \geq 0$ such that y_i, defined as the solution to* (4)*, satisfies* (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 (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 [18], 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

$$d_i \geq \tilde{\gamma}_i(1 + w_i) \tag{12}$$
$$d_i \leq \hat{\gamma}_i(1 + w_i), \tag{13}$$

where $\tilde{\gamma}$ is defined as in (8) and $\hat{\gamma}$ is defined as in (9), along with $d_i \geq 0$ and (2). Using matrices (10) and (11), the constrains in (12) can be written as follows

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

Substituting $d = d^*$ in (12) yields

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

So inequality (12) in matrix form holds with $d = d^* \geq 0$. Now choose $d = d^*$. Consider the quantity

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

Since $w_i \geq 0$ and $\hat{\gamma}_i \geq \tilde{\gamma}_i$ by definition, then the above quantity is always nonnegative and thus (13) is satisfied by $d = d^*$. With this, by Lemma 2 in [15] we have that satisfying the specification (5) is equivalent to satisfying (12)-(13).

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 (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 (4), with $\theta_i' = 0$, yields

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

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

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

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 [19]. 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

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

which yields

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

From above $\{M^{-1}\}_{i,j} \geq 0$ and by Theorem 3.11 in Chapter 6 of [18], we have that $\rho(\tilde{\Gamma}T) < 1$. Therefore, if there exists $\theta_i \geq 0$ such that y_i is the solution to (4) and satisfies the specification (5), then $\rho(\tilde{\Gamma}T) < 1$. □

Corollary 1. *Given a network of N subsystems of the form* (1) *and interconnection rule* (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* (4)*, satisfies the specification in* (5)*.*

Proof: 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 (5) if and only if $\rho(\tilde{\Gamma}T) < 1$. Additionally, by Theorem 1 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 (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 (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 (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 3. *Let $\tilde{\Gamma}$ be the gain matrix defined in* (11) *and T be the interconnection matrix defined in* (10)*. Then $\rho(\tilde{\Gamma}T) < 1$ if and only if the inequality*

$$\frac{1}{\delta} - \sum_{j=1}^{N}\frac{y_j^* - \varepsilon_j}{\alpha_j} > 0, \tag{14}$$

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 [19]

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

which yields

$$\{M^{-1}\}_{i,j} = \begin{cases} \frac{1}{1+\tilde{\gamma}_i} + \frac{1}{\frac{1}{\delta} - \sum_{k=1}^{N}\frac{y_k^* - \varepsilon_k}{\alpha_k}}\frac{\tilde{\gamma}_i}{(1+\tilde{\gamma}_i)^2}, & \text{if } i = j \\ \frac{1}{\frac{1}{\delta} - \sum_{k=1}^{N}\frac{y_k^* - \varepsilon_k}{\alpha_k}}\frac{\tilde{\gamma}_i}{(1+\tilde{\gamma}_i)(1+\tilde{\gamma}_j)}, & \text{if } i \neq j \end{cases}. \tag{15}$$

If (14) 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 (15) 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 (15) 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 (14) is satisfied. From Theorem 3.11 in Chapter 6 of [18], 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 (14) is satisfied. □

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

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

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* (4)*, satisfies the specification in* (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 (5) if and only if $\rho(\tilde{\Gamma}T) < 1$. By Theorem 3 we have that $\rho(\tilde{\Gamma}T) < 1$ if and only if (14) 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 (5) if and only if if and only if the inequality

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

is satisfied

IV. 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 (14) 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 (14), 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 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 presents the achievable for y^* region with different values of α. Notice that the achievable set presented in Figure 2 appears in the plane (y_1^*, y_2^*) when $y_3^* = 0$ in Figure 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.

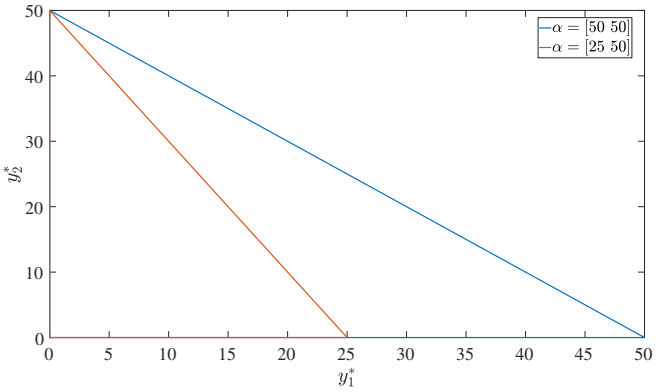

Fig. 2. Achievable region for the desired steady state output protein concentration y^* with $\delta = 1$ hr^{-1} and different values of α nM/hr.

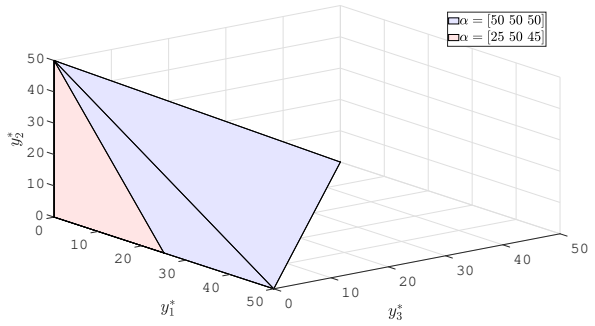

Fig. 3. Achievable region for the desired steady state output protein concentration $y^*$with $\delta = 1$ hr^{-1} and different values of α nM/hr.

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 (5) with $\varepsilon_i = 0, i \in \{1, \ldots, N\}$ is satisfied. Since the specification can be satisfied, we can validate our feasibility checks from Corollary 1

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

and from Corollary 2

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

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 com-

putationally difficult task, which we simplify by fixing the value of $\theta'_i, i \in \{1,\dots,N\}$. To this end, we state a method to calculate θ_i, $i \in \{1,\dots,N\}$ as a function of θ'_i and y_i, $i \in \{1,\dots,N\}$, 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

$$\beta'_i = \frac{\alpha'_i \theta'_i y_i}{\delta\left(1+\theta'_i y_i + \sum_{j\neq i}\theta'_j y_j\right)}.$$

Fixing the values of $y_i, i \in \{1,\dots,N\}$ fixes the values of $\beta'_i, i \in \{1,\dots,N\}$, and thus one can use (3) to derive the modified steady state I/O map

$$d_i = \gamma_i^{\dagger}(1+w_i), \tag{16}$$

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

$$\gamma_i^{\dagger} = \frac{\delta(y_i+\beta'_i)}{\alpha_i - \delta(y_i+\beta'_i)}.$$

With this, (16) can be rewritten in matrix form as

$$(I-\Gamma^{\dagger}T)d = \gamma^{\dagger}, \tag{17}$$

where $\gamma^{\dagger} = [\gamma_1^{\dagger},\dots,\gamma_N^{\dagger}]$, T is as defined in (10) and $\Gamma^{\dagger} = \mathrm{diag}(\gamma^{\dagger})$. Let $A = I+\Gamma^{\dagger}$, $v = [-1,\dots,-1]^{\top}$, 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 [19]

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

This yields

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

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

$$\theta_i = \frac{\delta_0\,(y_i+\beta'_i)}{\alpha_i u_i^*\left(\frac{1}{\delta}-\sum_{j=1}^{N}\left(\frac{y_j+\beta'_j}{\alpha_j}\right)\right)}. \tag{18}$$

Note that $\theta_i \geq 0$ since $(1/\delta)-\sum_{i=1}^{N}(y_i+\beta'_i)/\alpha_i > 0$. We note that (18) was derived using the modified I/O map given in (17). It can be verified that substituting the θ_i values obtained from (18) into (3) yields the fixed values y_i as the system's steady state, which justifies (18). With this, computing the θ feasible region can be numerically done by utilizing the map (18) from the protein y_i space to the θ_i space, for $y_i \in [y_i^*-\varepsilon_i, y_i^*+\varepsilon_i], i \in \{1,\dots,N\}$ and y_i such that $(1/\delta)-\sum_{i=1}^{N}(y_i+\beta'_i)/\alpha_i > 0$.

Figure 4 presents the boundary of the θ parameter feasible region for multiple values of $\theta'_i = \theta'^*, i \in \{1,\dots,N\}$, computed using (18). To achieve this, we have sampled the specification in the y space, then numerically computed (θ_1,θ_2) using (18) 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.

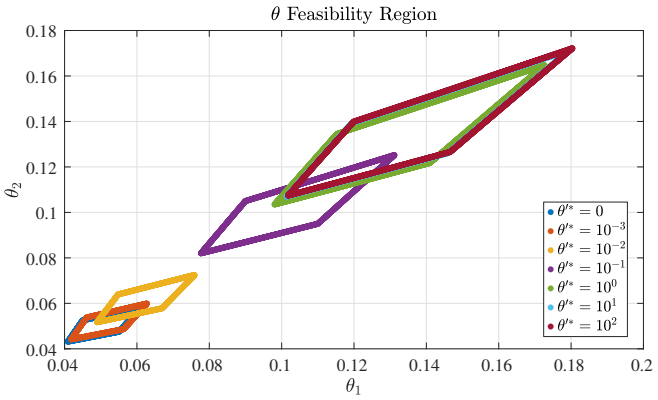

Fig. 4. Feasible region for θ tunable parameters with different values of θ'^*.

V. Conclusion and Future Work

In this paper, we have expanded the work previously carried in [15] to also include degradation resource sharing, such as protease sharing, and improved the feasibility check to a sufficient and necessary condition, as shown in Theorem 2. Finally, we also provide a simpler sufficient and necessary condition on the problem feasibility shown in Corollary 2. Our results also demonstrate that the feasibility of the system with production and degradation resource sharing is tied to the feasibility of the system with only production resource sharing. Future work on this topic will focus on expanding this framework to sequential systems and on handling the choice of the tunable parameters for multiple simultaneous specifications.

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