A Mathematical and Engineering Framework to
Predict the Effect of Resource Sharing on Genetic
Networks

by

Cameron D. McBride

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Signature redacted

Author .................................................. ............................................

Department of Mechanical Engineering

May 12, 2017

Signature redacted

Certified by ........

Domitilla Del Vecchio
Associate Professor
Thesis Supervisor

Signature redacted

Accepted by .........................

Rohan Abeyaratne
Chairman, Department Committee on Graduate Theses
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Abstract

In this thesis, a framework is developed to investigate the effect of resource sharing on the performance of genetic networks. A model of a genetic system with shared resources for protein degradation is developed that captures resource sharing effects and is subsequently analyzed to discover ways in which this form of resource sharing effects genetic networks. It is shown that sharing of degradation resources may cancel undesirable effects due to resource sharing of protein production resources. Next, a theoretical framework is developed to find conditions in which a genetic network may exhibit a change in its number of equilibria due to resource sharing effects. Finally, metrics and an experimental method are proposed to estimate the quantity of resources a genetic network uses and the sensitivity of the network to disturbances in resource availability. These measures may be utilized to inform design choices in genetic networks in which resource sharing plays a significant role. These effects become increasingly important in more complex genetic networks. Quantification of such resource sharing effects are an important step in increasing the predictability of genetic networks.

Thesis Supervisor: Domitilla Del Vecchio
Title: Associate Professor
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Chapter 1

Introduction and Motivation

In synthetic biological systems, it has been desirable to create genetic networks in a modular fashion, to enable coherent design of complex systems from simpler parts [2, 3]. However, genetic networks do not always behave modularly due to additional effects known as context dependence [4, 5, 6]. One aspect of context dependence is resource sharing effects. Traditional models assume that cellular resources are abundant [7]; however, in reality this assumption may not be valid [8, 1, 9] is not always the case.

Other context dependent effects such as retroactivity has been modeled and experimentally shown to be significant in biological systems [4]. Additionally, progress has been made into determining limitations of protein production in cells due to resource sharing [1]. Measures of total cellular resources have additionally been proposed to [10]. Additionally, resource sharing for enzymes has been shown to be significant experimentally [11]. However, there has been little research into ways in which resource sharing changes the structure of a genetic network such as when perturbations due to resource sharing change the number of equilibria in a general manner or quantification of the effect a synthetic network may have on other networks within the same context. In this thesis, we examine ways in which resource sharing changes the qualitative behavior of a genetic network, how resource sharing effects may be estimated to inform design choices, and ways in which undesirable resource sharing effects may be reduced.
This thesis is organized as follows. In Chapter 2, a model for sharing of proteases is developed and applied to show that competition effects from sharing of proteases may balance competition from sharing of ribosomes. In Chapter 3, a theoretical framework is developed to obtain conditions when a genetic network may undergo a change in its number of equilibria. Using results from degree theory, conditions are given dependent on the determinant of the Jacobian of the system at its equilibrium points. Examples of networks sharing different resources are presented to illustrate the results of the theory. In Chapter 4, metrics are proposed to estimate the quantity of resources used in a genetic network and the sensitivity of the output of that network to changes in resource availability. Additionally, an experimental procedure is given to measure these metrics for an arbitrary genetic network. Finally, in Chapter 5, conclusions are made reflecting on the applicability of this work and future directions.

1.1 A Motivating Example

In this section, we illustrate the counterintuitive effects that protease sharing may bring about with a motivating example. In particular, we have chosen a genetic activation cascade because cascade motifs are some of the most common motifs in natural and synthetic biomolecular networks [3]. Consider the simple two gene cascade, shown in Fig. 1-1, where protein, $x_1$ transcriptionally activates the production of protein $x_2$. For species $x$, we denote the concentration as $x$. The set of deterministic ordinary differential equations (ODEs) describing this cascade are

$$\begin{align*}
\frac{dx_1}{dt} &= U - \delta x_1 \\
\frac{dx_2}{dt} &= \frac{\alpha(x_1/K_{dna})^n}{1 + (x_1/K_{dna})^n} - \delta x_2,
\end{align*}$$

(1.1)

where $U$ is the induction of $x_1$, $\delta$ is the dilution due to cell growth, $\alpha$ is the maximal production rate of $x_2$ when the gene is fully activated by $x_1$, $K_{dna}$ is the effective dissociation constant of $x_1$ binding to the promoter of the gene of $x_2$, and $n$ is the cooperativity of $x_1$. Let us now consider the case in which both proteins are tagged
Figure 1-1: Simple activation cascade with proteins $x_1$ and $x_2$. $U$ induces the production of $x_1$, and $x_1$ then activates the production of $x_2$.

by the same protease, which is found in a limited total amount. The resulting ODE model now becomes (derived in Section 2.2)

$$\frac{dx_1}{dt} = U - \frac{\bar{P}x_1/K_p}{1 + x_1/K_p + x_2/K_p} - \delta x_1$$

$$\frac{dx_2}{dt} = \frac{\alpha(x_1/K_{dna})^n}{1 + (x_1/K_{dna})^n} - \frac{\bar{P}x_2/K_p}{1 + x_1/K_p + x_2/K_p} - \delta x_2,$$

where $\bar{P}$ is the maximal degradation rate of $x_1$ and $x_2$ by the protease and is proportional to the total concentration of protease and $K_p$ is the binding constant between the protein and the protease. Note that when $\bar{P} = 0$, (1.2) becomes (1.1). Therefore, we simulate (1.2) for $\bar{P} = 0$ and for $\bar{P} \neq 0$ to obtain the plot in Fig. 1-2. Fig. 1-2 shows that the system with the shared protease has hysteresis, which implies multistability for some input, while monostability for the system without a shared protease can be easily verified algebraically from (1.1). This indicates that non-explicit interactions arise between proteins due to protease sharing, which can have significant effects on the qualitative behavior of gene networks. In this thesis, we seek to mathematically characterize these effects, to explore the extent to which protease sharing affects global behavior of biomolecular systems, and to provide guidelines to predict these interactions.
Figure 1-2: Input–output steady state response of the cascade (1.2) with protease sharing (P ≠ 0, blue dashed line) and without protease (P = 0, red solid line) as U is varied. The yellow dashed line corresponds to unstable equilibria of the system with protease sharing. Parameters used here include \( \alpha = 13644 \text{nM h}^{-1} \), \( n = 1 \), \( \bar{P} = 54000 \text{nM h}^{-1} \), \( K_{\text{dna}} = 1000 \text{nM} \), \( K_p = 1200 \text{nM} \), \( \delta = 0.5 \text{h}^{-1} \). The maximum production rate of the protein, \( \alpha \), was calculated using the formula

\[
\alpha = \frac{(DNA)k_0k_1(RNAP)R_{\text{tot}}}{\delta_1(RNAP+K_0)(R_{\text{tot}}+K_1)}
\]

where DNA = 200 \text{nM} is the concentration of the plasmid, \( k_0 = 250 \text{h}^{-1} \) is the rate of transcription, \( k_1 = 300 \text{h}^{-1} \) is the rate of translation, RNAP = 2000 \text{nM} is the total concentration of RNA polymerase, \( R_{\text{tot}} = 6800 \text{nM} \) is the total concentration of ribosomes, \( \delta_1 = 12 \text{h}^{-1} \) is the dilution rate of mRNA, \( K_0 = 200 \text{nm} \) is the binding constant of RNAP with the promoter, and \( K_1 = 50000 \text{nM} \) is the binding constant of mRNA with the ribosome binding site. Ranges for each parameter are given in Table 2.1.
Chapter 2

Protease Sharing

2.1 Introduction

Proteases are a class of enzymes used in the cell for a variety of purposes including degradation of misfolded or aggregated proteins and disassembly of large macromolecular complexes [12, 13, 14]. In synthetic contexts, proteases may be used to decrease the response time of circuits with targeted degradation of specific proteins [15]. Because they appear in limited quantities, proteases may be considered as a limited resource for protein degradation [16].

Proteins may be tagged for degradation by enzymes, known as proteases [13], using peptide tags [17, 14, 18]. The proteins bind to the protease, and are subsequently degraded enzymatically. Limitations of protease availability may then create non-explicit coupling between different proteins that require the same protease for degradation. It has been previously shown that this coupling may have a significant effect on the function of certain networks [8, 16], may be used to tune some system behavior [19] or, may couple modules together through degradation [20]. Despite the fact that proteases are a finite cellular resource, traditional models do not usually take this into account [7]. Therefore, these models may not have satisfactory predictive ability when resources become depleted.

Previous work that considers proteases as a shared resource examines how proteases create coupling in systems [11, 21], how proteases may change the stability of
oscillatory systems [16], and how protease sharing may change the time response of a system [22]. However, previous work has not considered shared proteases in the context of a network with shared ribosomes.

2.2 Modeling of Degradation Processes

In this section, we derive a general model for systems with a shared protease, which can then be used to model arbitrary gene networks to perform further analysis. We use a deterministic ODE model and assume mass action kinetics [23]. We assume that mRNA dynamics are at their quasi-steady state and can be neglected.

We suppose that, in a well-stirred network of $n$ proteins, all proteins, $x_i$, are degraded by a single, common protease, $P$, for each $i = 1, \ldots, n$. Then, the chemical reactions modeling the system are

$$
\emptyset \xrightarrow{\delta} x_i, \quad C_i \xrightarrow{\delta} \emptyset
$$

$$
\emptyset \xrightarrow{\alpha_0} P
$$

$$
x_i + P \xrightarrow{a_i / d_i} C_i \xrightarrow{k_i} \emptyset + P,
$$

where $H_i(x)$ is the rate of production of the protein $x_i$ and depends on all other protein species $x$, $\alpha_0$ is the rate of production of the protease, $\emptyset$ is the null complex which represents the constitutive production or dilution of species and is assumed to not require resources, $a_i$ and $d_i$ are the association and dissociation rate constants of proteins binding with the protease, respectively, $C_i$ is the complex of protein $x_i$ bound to the protease, and $k_i$ is the catalytic rate constant of degradation for each $i = 1, \ldots, n$. We account for the growth of the cell by assuming that all species dilute
with rate constant \( \delta \). Then, the set of ODEs is

\[
\begin{align*}
\frac{dx_i}{dt} &= H_i(x) - a_i x_i P + d_i C_i - \delta x_i &\quad (2.1a) \\
\frac{dC_i}{dt} &= a_i x_i P - (d_i + k_i + \delta) C_i &\quad (2.1b) \\
\frac{dP}{dt} &= \alpha_0 - \sum_i a_i x_i P + \sum_i d_i C_i - \delta P, &\quad (2.1c)
\end{align*}
\]

We define the total protease concentration as

\[
P_{\text{tot}} := P + \sum_{i=1}^{n} C_i, \quad (2.2)
\]

then the ODE for the total protease concentration is

\[
\frac{dP_{\text{tot}}}{dt} = \alpha_0 - \delta P_{\text{tot}}.
\]

Now, we let the initial condition of the total protease concentration be at its equilibrium. Then for all time, the total concentration of protease is constant so we can substitute \( P_{\text{tot}} = P + \sum_{i=1}^{n} C_i \) in (2.1) and eliminate (2.1c). We now reduce the system using the fact that binding/unbinding reactions are much faster than protein production, degradation, and dilution [7]. Specifically, because \( \delta \ll d_i \), we define a small parameter \( \epsilon_i = \delta / d_i \ll 1 \) for each node, \( i \), and substitute the relation \( K_i = d_i / a_i \), into (2.1) to obtain the system

\[
\begin{align*}
\frac{dx_i}{dt} &= H_i(x) - \frac{\delta}{\epsilon_i} \left( \frac{x_i P}{K_i} - C_i \right) - \delta x_i &\quad (2.3a) \\
\frac{dC_i}{dt} &= \frac{\delta}{\epsilon_i} \left[ \frac{x_i P}{K_i} - C_i \right] - (k_i + \delta) C_i. &\quad (2.3b)
\end{align*}
\]

Observe that \( C_i \) is a fast variable, while \( x_i \) is a mixed variable since it operates at both fast and slow timescales. Then, to put the system in standard singular perturbation form, we substitute \( z_i = x_i + C_i \), which is a slow variable. Substituting and multiplying
all terms in (2.3b) by $\epsilon_i$, the system becomes

\[
\frac{dz_i}{dt} = H_i(x) - k_i C_i - \delta z_i \quad (2.4a)
\]

\[
\epsilon_i \frac{dC_i}{dt} = \delta \left[ \frac{x_i P}{K_i} - C_i \right] - \epsilon_i (k_i + \delta) C_i, \quad (2.4b)
\]

which is in standard singular perturbation form [24]. It can be easily verified that the slow manifold is always exponentially stable. Then, setting each $\epsilon_i = 0$ and solving in (2.4b), we obtain that the equation for the slow manifold is

\[
\overline{C}_i = \frac{x_i P}{K_i} =: g_i(x). \quad (2.5)
\]

Now, we substitute (2.5) into the conservation law for the protease from (2.2) and solve for the concentration of the free protease, $P$, to obtain

\[
P = \frac{P_{tot}}{1 + \sum_{j=1}^{n} \frac{x_j}{K_j}}. \quad (2.6)
\]

Next, we take the derivative of all terms in the definition of $z_i$ and solve for $\frac{dx_i}{dt}$ as

\[
\frac{dz_i}{dt} = \frac{dx_i}{dt} + \frac{dC_i}{dt} = \frac{dx_i}{dt} + \frac{dg_i}{dx_i} \frac{dx_i}{dt} = \frac{dx_i}{dt} \left( 1 + \frac{dg_i}{dx_i} \right),
\]

from which we obtain

\[
\frac{dx_i}{dt} = \frac{\frac{dx_i}{dt}}{1 + \frac{dg_i}{dx_i}}. \quad (2.7)
\]

Substituting (2.4a), (2.5), and (2.6) together into (2.7), we obtain the set of differential equations

\[
\frac{dx_i}{dt} = \left[ H_i(x) - \frac{P_{tot}(k_i + \delta) x_i}{1 + \sum_{k=1}^{n} \frac{x_k}{K_k}} - \delta x_i \right] \cdot \left( \frac{1}{1 + \frac{P_{tot}/K_i(1 + \sum_{j \neq i} x_j/K_j)}{(1 + \sum_{k} x_k/K_k)^2}} \right). \quad (2.8)
\]

for $i = 1, \ldots, n$. To simplify notation, we can substitute $\overline{P}_i$ for $P_{tot}(k_i + \delta)$. Then $\overline{P}_i$
is the maximal velocity of degradation by the protease. Term (2.8a) represents the dynamics of the protein, term (2.8b) represents the degradation of the protein by the protease, and term (2.8c) represents the retroactivity to the protein due to binding with the protease [4]. The term that represents degradation by the protease, (2.8b), is dependent on the concentration of every protein in the network that is degraded by the protease. This results in non-regulatory coupling among species. Note that if the protease were not considered as a finite resource, the denominator of this term would be unity and no non-regulatory coupling through competition for the protease would occur.

For the remainder of the chapter, we will examine the effect of protease sharing on the location and number of equilibrium points. The retroactivity term, (2.8c), is always less than 1, so it has the effect of slowing down the dynamics of the system due to the protein being bound to the protease. Since (2.8c) is always positive, it does not change the location, stability, or number of equilibrium points. Therefore, we can safely ignore retroactivity for our purposes.

2.3 Exploiting Protease Sharing as a Compensation Mechanism

We now consider a system of non-transcriptionally linked genes with limited amounts of both production and degradation resources, e.g. ribosomes and proteases, to investigate whether the effects of competition for these two resources balance each other. In [1], it was shown that two non-transcriptionally linked proteins show linear, negative correlation with each other due to ribosome sharing, e.g., as the concentration of one protein increases, the concentration of the other protein decreases. Additionally, it was shown in [1] that in protein production in *E. coli*, ribosomes are likely the limiting resource while competition for RNA polymerase can be neglected. Therefore, we consider a system with ribosomes and proteases as finite resources and investigate if including proteases mitigates the negative correlation observed between gene prod-
ucts due to ribosome competition. The chemical reactions for this system with no transcriptional links between proteins are

\[
\begin{align*}
\frac{\delta_1}{\beta_i} m_i & \rightarrow \emptyset \quad & C_{1i} & \rightarrow \emptyset \\
 m_i + R & \xrightarrow{a_{1i}} C_{1i} & k_{1i} & \rightarrow m_i + x_i \quad & C_{2i} & \rightarrow \emptyset \\
x_i + P & \xrightarrow{a_{2i}} C_{2i} & k_{2i} & \rightarrow P + \emptyset \\
x_i & \xrightarrow{\delta_2} \emptyset \\
\end{align*}
\]

(2.9)

for \( i = 1, 2, \ldots, n \). Here, \( m_i \) is mRNA, \( R \) is the free ribosomes, \( C_{1i} \) is the complex formed by the mRNA and ribosomes, \( x_i \) is the protein, \( P \) is the protease, \( C_{2i} \) is the complex formed by the protease bound to the protein, and \( \emptyset \) is the null complex which is assumed to provide production and degradation without the use of resources. Here \( \beta_i \) is the production rate of protein \( x_i \), \( \delta_1 \) is the dilution or mRNA, \( a_{1i} \) is the association rate constant and \( d_{1i} \) is the dissociation rate constant between \( m_i \) and ribosomes, \( k_{1i} \) is the rate of translation, \( a_{2i} \) is the association rate constant and \( d_{2i} \) is the dissociation rate constant between protein \( x_i \) and protease, \( k_{2i} \) is the catalytic rate of degradation by the protease, \( \delta_2 \) is the dilution of proteins, and \( \alpha_0 \) is the production rate of the protease, which is assumed to be independent of the free amount of ribosomes. This system is illustrated in Fig. 2-1. Then, using mass-action kinetics, this system leads

![Figure 2-1](image-url)

Figure 2-1: Visual representation of (2.9) for the case with two proteins. Shared production resources, \( R \), create non-explicit repression shown with red dashed arrows, while shared degradation resources, \( P \), create non-explicit activation of \( x_1 \) and \( x_2 \), shown with green dashed arrows.
to the set of ODEs

\[
\begin{align*}
\frac{dm_i}{dt} &= \beta_i - a_{1i}m_iR + (d_{1i} + k_{1i})C_{1i} - \delta_1m_i \\
\frac{dC_{1i}}{dt} &= a_{1i}m_iR - (d_{1i} + k_{1i} + \delta_1)C_{1i} \\
\frac{dx_i}{dt} &= k_{1i}C_{1i} - a_{2i}x_iP + d_{2i}C_{2i} - \delta_2x_i \\
\frac{dC_{2i}}{dt} &= a_{2i}x_iP - (d_{2i} + k_{2i} + \delta_2)C_{2i} \\
R_{tot} &= R + \sum_{k=1}^{n} C_{1k} \\
\frac{dP}{dt} &= \alpha_0 - \sum_{k=1}^{n} \left[a_{2k}x_kP + (d_{2k} + k_{2k})C_{2k}\right] - \delta_2P.
\end{align*}
\]

(2.10)

We reduce (2.10) by using singular perturbation and following a similar process as previously outlined in Section 2.2 and neglecting retroactivity. Then the mRNA dynamics are given as

\[
\frac{dm_i}{dt} = \beta_i - \delta_1m_i \left(1 + \frac{R}{K_{1i}}\right)
\]

(2.11)

where \(K_{1i} = d_{1i}/a_{3i}\) is the the binding constant between \(m_i\) and ribosomes. To simplify the system, we wish to set the mRNA to the steady state; however, the mRNA steady state depends on the concentration of free ribosomes, which, in turn, depends on the concentration of other mRNA in the network. The algebraic derivation can be simplified by considering that the total concentration of ribosomes is much smaller than the mRNA–ribosome binding constant, \(R_{tot} \ll K_{1i}\) [1]. We assume that mRNA is at the steady state since mRNA dynamics are faster than protein dynamics. Then the steady state of mRNA is \(m_i = \beta_i/\delta_1\). Then, neglecting retroactivity since we are only interested in the steady state response, the system becomes

\[
\frac{dx_i}{dt} = k_{1i}R\frac{\beta_i}{\delta_1K_{1i}} - (k_{2i} + \delta_2)P\frac{x_i}{K_{2i}} - \delta_2x_i
\]

(2.12)
for \( i = 1, \ldots, n \). Here, \( K_{2i} = d_{2i}/a_{2i} \) is the binding constant between the protein \( x_i \) and the protease. Solving for the equilibrium point of the system at steady state gives

\[
x_{i}^{eq} = \frac{k_{1i}R\beta_i/(\delta_1 K_{1i})}{(k_{2i} + \delta_2)P/K_{2i} + \delta_2}.
\] (2.13)

Using the conservation laws for both protease and ribosomes results in

\[
R_{tot} = R \left( 1 + \sum_{i=1}^{n} \frac{\beta_i}{\delta_1 K_{1i}} \right)
\] (2.14)

\[
P_{tot} = P \left( 1 + \sum_{i=1}^{n} \frac{x_i}{K_{2i}} \right)
\]

\[
= P \left[ 1 + R \sum_{i=1}^{n} \frac{k_{1i}\beta_i/(\delta_1 K_{1i})}{k_{2i}P + K_{2i}\delta} \right].
\] (2.15)

Now, we are interested to observe how the coupling between \( x_i \) and \( x_j \) is affected by protease and ribosome sharing. We induce the transcription of protein \( x_j \) by increasing \( \beta_j \) from 0 to some positive induction level. We define the coupling coefficient between protein \( x_i \) and \( x_j \) as

\[
M^i_j = \frac{x_{i}^{eq} - x_{i}^{eq}|_{\beta_j=0}}{x_{j}^{eq} - x_{j}^{eq}|_{\beta_j=0}}
\] (2.16)

for any \( i \neq j \). Solving (2.13)–(2.15) simultaneously for the concentration of each protein, substituting into (2.16), and assuming that \( \delta_2 \ll k_{2i}P/K_{2i} \) for each \( i = 1, \ldots, n \), we obtain that \( M^i_j \) is constant for all levels of induction of \( x_j, \beta_j \). This assumption is valid when protein decay is dominated by degradation. We will examine the range of applicability of this assumption in Section 2.3.1. Then, the coupling coefficient is given by

\[
M^i_j = \frac{k_{1i}k_{2j}K_{2i}}{k_{1j}k_{2i}K_{2j} \delta_1 K_{1i}} \left[ \frac{k_{1j}R_{tot}}{k_{2j}P_{tot}} - 1 \right]
\]

\[
= \frac{1 + \sum_{k \neq j} \frac{\beta_k}{\delta_1 K_{1k}} - \frac{R_{tot}}{P_{tot}} \sum_{k \neq j} \frac{k_{1k}\beta_k}{k_{2k}\delta_1 K_{1k}}}{1 + \sum_{k \neq j} \frac{\beta_k}{\delta_1 K_{1k}} - \frac{R_{tot}}{P_{tot}} \sum_{k \neq j} \frac{k_{1k}\beta_k}{k_{2k}\delta_1 K_{1k}}}.
\] (2.17)

Note from (2.17) that the coupling coefficient of protein \( x_i \) with respect to protein \( x_j \), \( M^i_j \), is 0 whenever

\[
k_{1i}R_{tot} = k_{2j}P_{tot}.
\] (2.18)
This corresponds to the case where the maximal speeds of production and degradation are exactly the same.

Now, we compare this to the system with shared ribosomes, but without a shared protease (i.e. $P_{tot} = 0$). In this case, the assumption that $\delta_2 \ll k_2iP/K_{2i}$ no longer holds. Therefore, the analysis must be repeated setting $P_{tot} = 0$ and using (2.13)–(2.14). After some algebra, the coupling coefficient between $x_i$ and $x_j$ is

$$M^i_j = \frac{-k_{1i} \beta_i}{k_{ij} \delta_i K_{1i}} \left[ 1 - \sum_{k \neq j} \frac{1}{\delta_k K_{1k}} \right].$$

(2.19)

Observe that $M^i_j$ is always strictly negative, while $M^j_i$ in (2.17) may be positive, negative, or zero. Additionally, observe from (2.17) and (2.19) that for systems with more species (i.e. larger networks), the magnitude of the coupling coefficient, $|M^j_i|$ and $|\overline{M}^j_i|$, becomes smaller in both cases since the terms from any additional proteins appear only in the denominator of (2.17) and (2.19). Finally, to examine the asymptotic behavior as $P_{tot}$ becomes large in (2.17), we set $K_{2i} = K_{2j}$ and $k_{2i} = k_{2j}$, then (2.19) is recovered. This shows that the coupling coefficient in (2.17) approaches the coupling coefficient in (2.19) as $P_{tot}$ becomes large. In summary, (2.17) and (2.19) indicate that competition for a shared protease may compensate for the effects of competition for ribosomes by decreasing the magnitude of the coupling coefficient.

### 2.3.1 Nonlinear Simulations

Here we explore how the coupling coefficient varies when we change the total protease concentration over a wide range through numerical simulation of (2.10). In this case, the assumption that $\delta_2 \ll k_2iP/K_{2i}$ may not hold. It is assumed that there are two proteins, $x_1$ and $x_2$, in the system. We induce the transcription of $x_2$ and calculate the steady state of $x_1$ and $x_2$ for different levels of induction of $x_2$, $\beta_2$. Fig. 2-2 shows example curves for the case with only ribosome competition and both protease and ribosome competition. The curve corresponding to the case with only ribosome competition, (2.19), has a constant, negative coupling coefficient for
Figure 2-2: The steady state of (2.10) with only ribosome competition \( P_{tot} = 0 \), shown by the red dashed line, and both protease and ribosome competition, shown with the blue solid line for different levels of induction of \( x_2 \). The parameters were used here and are \( k_{i1} = 300 \text{ h}^{-1} \), \( R_{tot} = 6800 \text{ nM} \), \( K_{11} = 20000 \text{ nM} \), \( K_{12} = 6000 \text{ nM} \), \( k_{2i} = 270 \text{ h}^{-1} \), \( P_{tot} = 250 \text{ nM} \), \( K_{2i} = 1200 \text{ nM} \), \( \delta_1 = 12 \text{ h}^{-1} \), \( \delta_2 = 0.5 \text{ h}^{-1} \). The maximum production rate of the mRNA, \( \beta_i \), was calculated using the formula \( \beta_i = \frac{(DNA)k_{0i}(RNAP)}{RNAP+K_{0i}} \) [1] where \( DNA = 20 \text{ nM} \) is the concentration of the plasmid, \( k_{0i} = 200 \text{ h}^{-1} \) is the rate of transcription, \( RNAP = 2000 \text{ nM} \) is the total concentration of RNA polymerase, and \( K_{0i} = 560 \text{ nM} \) is the binding constant of RNAP with the promoter. Ranges for each parameter are given in Table 2.1.

all levels of induction. For the curve corresponding to the case with both protease and ribosome competition, the assumption that the dilution is small for the coupling coefficient in (2.17) does not hold, so the curve is not a line in this case.

Additionally, we observe how the system behaves as the total amount of protease changes numerically. The coupling coefficient is calculated using (2.10) and (2.16) for various levels of the total amount of protease, \( P_{tot} \), and for three different levels of induction of the promoter of \( x_2 \): 20\%, 50\%, and 100\% compared to the induction of \( x_1 \), shown in Fig. 2-3. For small concentrations of total protease, the coupling coefficient for all levels of induction is less than zero. This corresponds to regions where the protease is not degrading enough protein to have a significant effect. For moderate concentrations of total protease, the coupling coefficient is positive which implies
that the effects from the shared protease over-compensate for the effects of ribosome sharing. Additionally, for large concentrations of protease, the coupling coefficient becomes negative, which corresponds to the case when total protease is abundant, so protease competition effects are not significant. This implies that there are two points where the coupling coefficient is 0 by continuity. Indeed, zero crossing of the coupling coefficient occurs when $P_{tot} \approx 10 \text{nM}$ and when $P_{tot} \approx 1000 \text{nM}$. At these concentrations, ribosomes sharing effects perfectly balance protease sharing effects. Thus, when designing a genetic circuit, a shared protease may perfectly balance the effects of ribosome competition at either a small or a large concentration of protease.

Note that the range of validity of the assumption that dilution is small compared to degradation can be seen from Fig. 2-3 where all three simulated curves collapse onto the curve calculated with (2.17). This occurs for total protease concentrations $P_{tot} > 800 \text{nM}$, which is feasible in synthetic systems but is significantly larger than concentrations typically present in natural systems [14].

| Table 2.1: Values for biological constants in *E. coli* used throughout this paper. |
|------------------------------------------|-----|-----------------|-----------------|
| description                              | variable      | value           | reference       |
| copy number (low, medium, high)          | DNA           | 10, 50, 100 nM  |
| mRNA decay rate                          | $\delta_1$    | 7 h$^{-1}$ to 20 h$^{-1}$ | [25] |
| protein dilution rate                    | $\delta_2$    | 0.4 h$^{-1}$ to 1 h$^{-1}$ | [26] |
| transcription rate                       | $k_{0i}$      | 195 h$^{-1}$ to 275 h$^{-1}$ | [27], [28] |
| translation rate                         | $k_{1i}$      | 180 h$^{-1}$ to 315 h$^{-1}$ | [27], [28] |
| degradation by protease rate             | $k_{2i}$      | 126 h$^{-1}$ to 360 h$^{-1}$ | [18] |
| RNAP with DNA promoter Michaelis–Menten constant mRNA with ribosome binding constant | $K_{0i}$     | 150 nM to 560 nM | [29] |
| protein with protease Michaelis–Menten constant | $K_{1i}$     | $\approx 100,000 \text{nM}$ | [1] |
| total RNAP concentration                 | $R_{NAP}$     | 1500 nM to 11,400 nM | [27] |
| total ribosome concentration             | $R_{tot}$     | 6800 nM to 72,000 nM | [27] |
| total protease concentration             | $P_{tot}$     | 75 nM to 250 nM | [14] |
| (ClpXP)                                  |               |                 |
| Transcription factor binding constant (nonspecific) | $K_{dna}$     | $10^{-3}$ M to $10^{-6}$ M | [30] |
Figure 2-3: The coupling coefficient of (2.10) for different concentrations of total protease. The purple dashed line is the coupling coefficient predicted by (2.17) and the green dashed line is the coupling coefficient predicted by (2.19). The parameters used here are $k_{1i} = 300 \text{ h}^{-1}$, $R_{tot} = 6800 \text{ nM}$, $K_{11} = 20000 \text{ nM}$, $K_{12} = 6000 \text{ nM}$, $k_{2i} = 270 \text{ h}^{-1}$, $K_{2i} = 1200 \text{ nM}$, $\delta_1 = 12 \text{ h}^{-1}$, $\delta_2 = 0.5 \text{ h}^{-1}$. The maximum production rate of the mRNA, $\beta_i$, was calculated using the formula $\beta_i = \frac{(DNA)k_0(RNAP)}{RNAP + K_0}$ [1] where $DNA = 20 \text{ nM}$ is the concentration of the plasmid, $k_0 = 200 \text{ h}^{-1}$ is the rate of transcription, $RNAP = 2000 \text{ nM}$ is the total concentration of RNA polymerase, $K_0 = 560 \text{ nM}$ is the binding constant of RNAP with the promotor. $\alpha_0$ was calculated as $P_{tot}\delta_2$ to give the desired total protease concentration at steady state. Ranges for each parameter are given in Table 2.1.
2.4 Conclusions

In this chapter, we examined how a shared protease may affect the behavior of a genetic network. A model of protease degradation with a shared protease was developed and used to show that a shared protease balance the effect of ribosome competition on a network with no transcriptional regulations. In our analysis, we assumed that the protease does not require ribosomes to be produced; however, the results are similar if this assumption is not made. The coupling coefficient is zero at a small concentration of protease as well as at a large concentration of protease.

Cells may use this phenomenon to decouple the concentrations of proteins together and mitigate effects of ribosome sharing. Since protein degradation by protease is faster than protein production, this pathway provides a faster response than transcriptional regulation. Additionally, these effects are important for consideration in the design synthetic biomolecular circuits with a protease. Unmodeled protease dynamics may cause unexpected behavior to occur if not considered in the design of the circuit. In future work, we will examine the extent to which these systems can be designed to be robust to resource sharing effects and apply these results to give simple tools for the design of synthetic circuits.
Chapter 3

Resource Sharing Leads to a Change in Number of Equilibria

3.1 Introduction

As was seen in Section 1.1, resource sharing caused a change in the number of equilibrium points of a simple, two protein network with a shared protease. Additionally, [16] examined the operation of a genetic oscillator with a shared protease through simulation where it was found that the oscillator exhibits various regimes, each with different stability or number of equilibria properties depending on the level of resource competition. These results only apply to the specific system however. Previous work in the field has focused largely on specific systems and studying the effects of resource sharing through simulation. We wish to provide more general results that may be applied to any system and are easily verifiable either analytically or computationally.

In this chapter, we prove theorems providing conditions on the determinant of the Jacobian determining when the number of equilibrium points of a biological network is invariant to disturbances due to resource sharing. The theorems presented utilize concepts from degree theory and dynamical systems theory. We conclude the chapter with examples of biological systems in which the theorems are applied to show when biological systems may or may not exhibit a change in number of equilibria due to resource competition.
3.2 Theoretical Results

To state and prove our results, our results use mathematical tools from degree theory [31]. In order to state the results, we begin with definitions and theorems that are essential for the statement of the theorem we wish to prove.

3.2.1 Mathematical Preliminaries

Definition 1. The cone with the relation $x > a$ ($x \geq a$) is defined such that $\{x \in \mathbb{R} : x_i > a \ (x_i \geq a) \ \forall i = 1, \ldots, n\}$. $<$ and $\leq$ are defined similarly. The difference between the vector and scalar relations are determined by context.

Definition 2. A point, $x_0$, of a function $f(x)$ is called degenerate if $\det \left( \frac{\partial f}{\partial x} \right) \Big|_{x=x_0} = 0$. Additionally, $x_0$ is called a degenerate zero if $f(x_0) = 0$ and $\det \left( \frac{\partial f}{\partial x} \right) \Big|_{x=x_0} = 0$.

Definition 3. Let $\Omega \subset \mathbb{R}^n$ be a bounded domain and $f$ a $C^1$ smooth function $f : \overline{\Omega} \rightarrow \mathbb{R}^n$ and $f$ has no degenerate zeros and has no zeros on the boundary of $\Omega$, then the topological degree of $f$ with respect to zero is

$$\deg(f, \Omega) = \sum_{x \in f^{-1}(0)} \text{sgn} \left( \det \left( \frac{\partial f}{\partial x} (x) \right) \right)$$

(3.1)

where $\overline{\Omega}$ is the closure of $\Omega$, $\text{sgn} : \mathbb{R} \rightarrow \{-1, 0, 1\}$ is the sign function, $f^{-1}(0)$ is the set of zeros of $f$ in $\Omega$.

Note that the degree essentially counts the number of zeros of $f$ with consideration of the sign of the determinant at each zero. We use this concept to characterize the number of equilibrium points of the system (3.4). Additionally, Definition 3 can be extended to apply to the case where $f$ does have degenerate zeros [32]. We will use Definition 3 in this chapter.

The following theorem comes directly from [33] and is one of the main theorems of degree theory.
Theorem 1. Consider a bounded domain $\Omega \subset \mathbb{R}^n$ and a continuously varying family of smooth functions $f_\lambda : \Omega \rightarrow \mathbb{R}^n$ for $\lambda \in [0, 1]$, such that $f_\lambda$ does not have any zeros on the boundary of $\Omega$ for all $\lambda \in [0, 1]$. Then $\deg(f_\lambda, \Omega)$ is constant for all $\lambda \in [0, 1]$.

This theorem essentially says that the degree is a topological constant and is true for other definitions of the topological degree. Intuitively, this means that zeros may appear or disappear as the parameter $\lambda$ is varied, but the zeros must appear in pairs with opposite signs of their determinants. Many examples of local bifurcation show this behavior, such as the pitchfork or saddle-node bifurcation [24].

We now define the index of a function, which is a local version of the degree.

Definition 4. Let $\Omega$ and $f$ as in Definition 3. Suppose $f$ has only isolated zeros, let $x_i$ be a zero of $f$ and $\Omega_i$ be a sufficiently small open and bounded neighborhood of $x_i \in \Omega_i$ such that $x_i$ is the only solution of $f(x) = 0$ in $\Omega_i$, then the index of an isolated zero of $f$ is

$$\iota(f, x_i) = \deg(f, \Omega_i).$$

Additionally, if all $\Omega = \bigcup_i \Omega_i$, then $\deg(f, \Omega) = \sum_i \iota(f, x_i)$.

The following lemma builds on this idea that the number of equilibrium points is related to the degree.

Lemma 1. Let $\Omega \subset \mathbb{R}^n$ and $f : \Omega \rightarrow \mathbb{R}^n$ be a smooth function and suppose that $\det \left( \frac{\partial f}{\partial x}(x) \right) \neq 0$ for all $x \in f^{-1}(0)$. Then the number of zeros of $f$ in $\Omega$ is equal to the sum of the absolute values of the indexes of $f$ in $\Omega$, i.e.

$$n = \sum_i |\iota(f, x_i)|.$$  \hspace{1cm} (3.3)

Proof. The proof follows by definition of index and degree. Since $\det \left( \frac{\partial f}{\partial x}(x) \right) \neq 0$ for all $x \in f^{-1}(0)$, all zeros of $f$ are isolated and the definition of index may be applied. Note that $n = \sum_{x \in f^{-1}(0)} |\sgn \left( \det \left( \frac{\partial f}{\partial x}(x) \right) \right)| = \sum_i |\iota(f, x_i)|$ since $|\sgn(z)| = 1$ for any $z \neq 0$ which is assumed in the definition of deg.

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Lemma 2. If \( \det \left( \frac{\partial f_\lambda(x_0)}{\partial x} \right) \neq 0 \) for all \( x_0 \in \{ x^* > 0 : f_\lambda(x^*) = 0 \text{ for } \lambda \in [0,1] \} \), then the cardinality of the set \( f_\lambda^{-1}(0) \) is does not depend on \( \lambda \). The cardinality of the set \( f_\lambda^{-1}(0) \) is does not depend on \( \lambda \) only if \( \det \left( \frac{\partial f_\lambda(x_0)}{\partial x} \right) \neq 0 \) for all \( x_0 \in \{ x^* > 0 : f_\lambda(x^*) = 0 \text{ for } \lambda \in [0,1] \} \).

Proof. Suppose \( \det \left( \frac{\partial f_\lambda}{\partial x} \right) \neq 0 \) for \( \lambda \in [0,1] \). Then, by the Inverse Function Theorem, there exists a ball (tube in \( \mathbb{R}^n \times \mathbb{R} \) for \( \lambda \in [0,1] \) space) \( \Omega_i = B_i(\varepsilon, x_i) \) such that \( x_i \) is the unique solution of \( f_\lambda(x) = 0 \) for \( x \in \Omega_i \) so all equilibrium points are isolated for all \( \lambda \in [0,1] \). Then the degree over \( \Omega_i \) is constant for all \( \lambda \in [0,1] \) by Theorem 1. Since all equilibrium points are isolated and the degree is non-zero, the cardinality of \( f^{-1}(0) \) is constant. 

This lemma provides a sufficient condition for when the cardinality of the set \( f_\lambda^{-1}(0) \) is constant which will be used to prove further sufficient conditions when the number of equilibria of a dynamical system is guaranteed to be constant.

3.2.2 Dynamical Systems Preliminaries

Many of these definitions are standard in dynamical systems or come from [24] Consider the system of ordinary differential equations

\[ \dot{x} = f(x) \] (3.4)

where \( \dot{x} \) is the time derivative of the vector \( x \in \mathbb{R}^n \), and \( f(x) \) is a continuous function.

Definition 5. A function \( f(x) \) is positive invariant if \( f(x) \geq 0 \) whenever \( x \geq 0 \), \( x_j = 0 \) for some \( j \in \{1, \ldots, n\} \).

Definition 6. A system in the form of (3.4) is bounded if there exists an \( M \in \mathbb{R} \) such that if \( \|x(0)\| < M \), then \( \|x(t)\| < M \) for all \( t \geq 0 \).
Proposition 1. Consider a system of the form

\[
\begin{align*}
  x_1 &= h_1(x_1, x_2) - \Lambda_{01}x_1 \\
  x_2 &= h_2(x_1) - \Lambda_{02}x_2
\end{align*}
\] (3.5a)

where \( h_1 \) and \( h_2 \) are continuous positive functions for all positive inputs and \( h_1 \) is a globally bounded function, and \( \Lambda_{01}, \Lambda_{02} \) are diagonal matrices with strictly positive terms on the diagonal. Then, the states of the system, \( x_1(t) \) and \( x_2(t) \), are bounded for all \( t > 0 \).

Proof. Note that (3.5) is positive invariant. Write the system in the form of (3.5). Then \( x_1 \) is bounded since \( x_1 \leq \sup_x \Lambda_{01}^{-1} h_1(x) \) and \( h_1 \) is bounded. Then, since \( h_2 \) is continuous and only depends on \( x_1 \), \( \|h_2(x_1)\| \) is bounded for bounded \( x_1 \). Therefore, \( x_2 \leq \Lambda_{02}^{-1} h_2(x_1) \) and the states \( x_1(t) \) and \( x_2(t) \) remain bounded for all time. \( \blacksquare \)

Proposition 1 illustrates one situation where the states of a system are bounded even when \( f \) in (3.4) is not bounded and is encountered in many applications of biological systems, as will be shown in Example 3.

Definition 7. A function \( g(x) : \mathbb{R}^n \rightarrow \mathbb{R}^n \) is mass dissipating if there exists some \( m \in \mathbb{R}_{\geq 0}^n \) such that \( m \cdot g(x) \leq 0 \) for all \( x \in \mathbb{R}_{\geq 0}^n \).

Definition 8. Two functions \( \alpha(x) : \mathbb{R}^n \rightarrow \mathbb{R}^n \) and \( g(x) : \mathbb{R}^n \rightarrow \mathbb{R}^n \) are jointly mass dissipating if there exists an \( m \in \mathbb{R}_{\geq 0}^n \) such that \( m \cdot \alpha(x) \leq 0 \) and \( m \cdot g(x) \leq 0 \) for all \( x \in \mathbb{R}_{\geq 0}^n \).

The idea of mass dissipating functions are that they provide some damping to the system which can guarantee they remain bounded when additional mass dissipating continuous functions are added to a system’s dynamics.

3.2.3 Main Results

We now present the main theoretical results of the paper which guarantee that systems of a particular form do not undergo a change in number of equilibria as resource sharing is increased under some conditions. Corollaries 1 – 5 follow from Theorem 2.
Theorem 2. Consider the continuous-time system

\[ \dot{x} = h(x) \odot [\alpha(x)\mu + 1(1 - \mu)] + \lambda g(x) - \Lambda_0 x \overset{\Delta}{=} f_{\mu,\lambda}(x) \quad (3.6) \]

for \( x \in \mathbb{R}_{\geq 0}^n \), where \( h(x) \) and \( g(x) \) are all positive invariant \( C^1 \) functions for all positive \( x \), \( h(x) \geq 0 \) \( \forall x \geq 0 \), \( \odot \) is the Hadamard product, \( \alpha(x) \geq 0 \) \( \forall x \geq 0 \) is a \( C^1 \) function for all positive \( x \), \( \Lambda_0 \) is a diagonal matrix with strictly positive terms on the diagonal, and \( \alpha(x) - 1 \) and \( g \) are jointly mass dissipating where \( 1 \) represents a vector of 1’s. Assume that \( x(t) \) is bounded. Then, if \( \det \left( \frac{\partial f_{\mu,\lambda}(x_0)}{\partial x} \right) \neq 0 \) for all \( x_0 \in \{ x^* > 0 : f_{\mu,\lambda}(x^*) = 0 \text{ for } (\mu, \lambda) \in [0,1] \times [0,1] \} \), then \( \dot{x} = f_{\mu,0}(x) \) and \( \dot{x} = f_{1,1}(x) \) have the same number of equilibria.

Proof. This proof is structured as follows: first, we find a bounded domain, \( \Omega_M \subset \mathbb{R}_{\geq 0}^n \), that contains all equilibrium points within the positive orthant, and we prove that the system in (3.6) has no zeros on the boundary of \( \Omega_M \). Then, Lemma 1 and Lemma 2 are applied to show that the number of equilibrium points of \( f_{\mu,\lambda} = h(x) \odot [\alpha(x)\mu + 1(1 - \mu)] + \lambda g(x) - \Lambda_0 x \) is constant for all \( \mu \in [0,1], \lambda \in [0,1] \) and therefore the number of equilibrium points of \( f_{0,0} \) is equal to the number of equilibrium points of \( f_{1,1} \).

First note that \( f_{\mu,\lambda} \) is a positive invariant family of \( C^1 \) functions with continuous dependence on \( \mu \in [0,1] \) and \( \lambda \in [0,1] \) since it is composed of a combination of positive invariant \( C^1 \) functions. Now, to construct a bounded domain, select an \( m > 0 \) such that \( m \cdot g(x) \leq 0 \) and \( \alpha(x) - 1 \leq 0 \) which can be done since \( \alpha - 1 \) and \( g \) are jointly mass dissipating. Since \( x(t) \) is bounded and \( h \) is continuous, there exists a \( C \in \mathbb{R} \) such that whenever \( \|x^*\| \leq A \), \( m \cdot h(x) \leq C \) since the image of a compact set under a continuous mapping is compact. Now, choose \( M > C \) and define a set \( \Omega_M = \{ x \in \mathbb{R}_{\geq 0}^n : m \cdot (\Lambda_0 x) < M \} \). Then \( \Omega_M \) is an (open) bounded domain and \( \{ f_{\mu,\lambda} : (\mu, \lambda) \in [0,1] \times [0,1] \} \) is a continuously varying family of smooth functions on \( \overline{\Omega}_M \). \( f_{\mu,\lambda}(x) \) has no zeros on the sides of \( \Omega_M \) by assumption, i.e. whenever \( x_i = 0 \) for each \( i = 1, \ldots, n \). Now we prove that \( f_{\mu,\lambda} \) has no zeros on the outer boundary of \( \Omega_M \), i.e., when \( M = m \cdot (\Lambda_0 x) \) and no zeros in the positive orthant outside of \( \Omega_M \). To this
end, consider

\[ m \cdot f_{\mu,\lambda} = m \cdot h(x) \odot [\alpha(x)\mu + 1(1 - \mu)] + m \cdot \lambda g(x) - m \cdot (\Lambda_0 x) \]  
(3.7a)

\[ = m \cdot h(x) \odot [(\alpha(x) - 1)\mu + 1] + m \cdot \lambda g(x) - m \cdot (\Lambda_0 x) \]  
(3.7b)

\[ \leq m \cdot (h(x) \odot 1) + m \cdot \lambda g(x) - m \cdot (\Lambda_0 x) \]  
(3.7c)

\[ \leq m \cdot h(x) - m \cdot (\Lambda_0 x) \]  
(3.7d)

since \( m \cdot (\alpha(x) - 1) \leq 0 \) so \( m \cdot [\alpha(x)\mu + 1(1 - \mu)] < 1 \) and \( m \cdot g \leq 0 \). Now, since \( M = m \cdot (\Lambda_0 x) \) and \( M > C \geq m \cdot h(x(t)) \) for \( t > t^* \), then \( m \cdot f_{\mu,\lambda} \leq m \cdot h(x) - m \cdot (\Lambda_0 x) < 0 \) for all \( x \) such that \( m \cdot (\Lambda_0 x) \geq M \). So it follows that \( f_{\mu,\lambda} \) has no zeros on the outer boundary of \( \Omega_M \) for all \( (\mu, \lambda) \in [0, 1] \times [0, 1] \) and \( \Omega_M \) contains all zeros in the positive orthant. Next, Lemma 2 is applied twice, once for \( \mu \in [0, 1] \) with \( \lambda = 0 \) fixed and once for \( \lambda \in [0, 1] \) with \( \mu = 1 \) fixed. Then by Lemma 1, if \( \det \left( \frac{\partial f_{\mu,\lambda}(x)}{\partial x} \right) \neq 0 \) for all \( x \in \{x^* > 0 : f_{\mu,\lambda}(x^*) = 0 \text{ for } (\mu, \lambda) \in [0, 1] \times [0, 1]\} \), the number of zeros of \( f_{\mu,\lambda}|_{(\mu, \lambda) = (0, 0)} \) is equal to the absolute value of the sum of the indexes of \( f_{\mu,\lambda}|_{(\mu, \lambda) = (0, 0)} \) which is constant over all \( \Omega_M \) by Lemma 2. This proves the Theorem.  

\[ \square \]

**Remark 1.** If \( g(x) = [g_1(x), \ldots, g_n(x)] \) where \( g_i(x) \leq 0 \) for each \( i \in \{1, \ldots, n\} \), then \( g \) is mass dissipating and any \( m > 0 \) can be chosen such that \( m \cdot g \leq 0 \).

**Remark 2.** If \( \alpha(x) = [\alpha_1(x), \ldots, \alpha_n(x)] \) where \( 0 < \alpha_i(x) \leq 1 \) for each \( i \in \{0, \ldots, n\} \), then \( \alpha(x) - 1 \) is mass dissipating and any \( m > 0 \) can be chosen such that \( \alpha(x) - 1 \leq 0 \).

**Remark 3.** If \( g(x) \) satisfies Remark 1 and \( \alpha(x) - 1 \) is mass dissipating, then \( \alpha - 1 \) and \( g \) are jointly mass dissipating. Equivalently, if \( \alpha(x) \) satisfies Remark 2 and \( g \) is mass dissipating, then \( \alpha - 1 \) and \( g \) are jointly mass dissipating.

Remarks 1–3 are special cases of functions that satisfy the conditions in Theorem 2.

We now consider special cases of Theorem 2.

**Corollary 1.** Consider the continuous-time system

\[ \dot{x} = h(x) \odot [\alpha(x)\mu + (1 - \mu)] - \Lambda_0 x \triangleq f_{\mu}(x) \]  
(3.8)
where $x(t)$, $h$, $\mathcal{O}$, and $\Lambda_0$ are as in Theorem 2 and $\alpha(x) \geq 0 \ \forall x \geq 0$ is such that $\alpha(x) - 1$ is mass dissipating where $1$ represents a vector of 1’s. Then, if
\[
\det \left( \frac{\partial f_\mu(x_0)}{\partial x} \right) \neq 0 \ \text{for all } x_0 \in \{x^* > 0 : f_\mu(x^*) = 0 \ \text{for } \mu \in [0,1] \}, \text{then } \dot{x} = f_0(x) \text{ and } \dot{x} = f_1(x) \text{ have the same number of equilibria.}
\]

**Proof.** The Corollary follows from Theorem 2 by setting $g(x) = 0$, which is mass dissipating and positive invariant. 

**Corollary 2.** Consider the continuous-time system
\[\dot{x} = h(x) + \lambda g(x) - \Lambda_0 x \triangleq f_\lambda(x) \quad (3.9)\]

where $x(t)$, $h$, $\Lambda_0$, are as in Theorem 2 and $g$ is mass dissipating. Then, if
\[
\det \left( \frac{\partial f_\lambda(x_0)}{\partial x} \right) \neq 0 \ \text{for all } x_0 \in \{x^* > 0 : f_\lambda(x^*) = 0 \ \text{for } \lambda \in [0,1] \}, \text{then } \dot{x} = f_0(x) \text{ and } \dot{x} = f_1(x) \text{ have the same number of equilibria.}
\]

**Proof.** The theorem follows from Theorem 2 by setting $\alpha(x) = 1$ which implies that $\alpha(x) - 1$ is mass dissipating.

**Global Results**

Theorem 2 and Corollaries 1 and 2 have each provided a sufficient condition on when a change in the number of equilibrium points is guaranteed not to occur if one can check that there exist no points at which satisfy $f_{\mu, \lambda}(x_0) = 0$ and $\det \left( \frac{\partial f_{\mu, \lambda}(x_0)}{\partial x} \right) = 0$. In practice this is difficult to do analytically since the but may be done computationally. Additionally, we can more easily verify our conditions by checking the condition that the $\det \left( \frac{\partial f_{\mu, \lambda}(x_0)}{\partial x} \right)$ is non-zero everywhere in the positive orthant which is a weakening of Theorem 2. The following corollaries state exactly this.

**Corollary 3.** Consider the system in Theorem 2. If $\det \left( \frac{\partial f_{\mu, \lambda}(x_0)}{\partial x} \right) \neq 0$ for all $x_0 > 0$, then $\dot{x} = f_{0,0}(x)$ and $\dot{x} = f_{1,1}(x)$ have the same number of equilibria.

**Proof.** The theorem follows from Theorem 2, since if $\det \left( \frac{\partial f_{\mu, \lambda}(x_0)}{\partial x} \right) \neq 0$ is satisfied for all $x_0 > 0$, it must also be satisfied for all $x_0 \in \{x^* > 0 : f_{\mu, \lambda}(x^*) = 0 \ \text{for } (\mu, \lambda) \in [0,1] \times [0,1] \}$. 

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**Corollary 4.** Consider the system in Corollary 1. Then, if \( \det \left( \frac{\partial f_\mu(x_0)}{\partial x} \right) \neq 0 \) for all \( x_0 > 0 \), then \( \dot{x} = f_0(x) \) and \( \dot{x} = f_1(x) \) have the same number of equilibria.

**Proof.** The corollary follows from Corollary 3 by setting \( g(x) = 0 \) which is a positive invariant \( C^1 \) function. Additionally, by Remarks 1 and 3, \( \alpha - 1 \) and \( g \) are jointly mass dissipating.

**Corollary 5.** Consider the system in Corollary 2. Then, if \( \det \left( \frac{\partial f_\Lambda(x_0)}{\partial x} \right) \neq 0 \) for all \( x_0 > 0 \), then \( \dot{x} = f_0(x) \) and \( \dot{x} = f_1(x) \) have the same number of equilibria

**Proof.** The corollary follows from Corollary 3 by setting \( \alpha(x) = \mathbb{1} \) which is a non-negative function. Additionally, by Remarks 2 and 3, \( \alpha - 1 \) and \( g \) are jointly mass dissipating.

Note that in general, \( f_{\mu, \Lambda}^{-1}(0) \) is a multi-valued function. In order to state further results, we require some concepts regarding continuity of multi-valued functions.

**Continuity of Multivalued Functions**

Concepts about upper and lower semi-continuous Note that the map \( x_0 \in f_{\Lambda}^{-1}(0) \) may be a multivalued function if \( f_\Lambda \) has multiple equilibrium points. We use definitions from [34] in order to present the remainder of the results.

**Definition 9.** Let \( d \) denote the distance in the metric space \( X \) and let \( K \) be a subset of \( X \). The **ball of radius** \( r > 0 \) **around** \( K \) **in** \( X \) is denoted by

\[
B_X(K, r) \triangleq \{ x \in X | d(x, K) \leq r \}. \quad (3.10)
\]

**Definition 10.** A set valued map \( F : X \rightarrow Y \) is called **upper semicontinuous at** \( x \in X \) if and only if for any neighborhood \( \mathcal{U} \) of \( F(x) \),

\[
\exists \eta > 0 \text{ such that } \forall x' \in B_X(x, \eta), \quad F(x') \subset \mathcal{U}. \quad (3.11)
\]

It is said to be **upper semicontinuous** if and only if it is upper semicontinuous at any point in \( X \).
Notation: Translating the notation given in Definitions 10 to our application, \( F(x = \lambda) \triangleq f_{\lambda}^{-1}(0) \). Note that \( x_0(\mu, \lambda) = f_{\mu, \lambda}^{-1}(0) \) may be a multi-valued function.

The converse of Theorem 2 and Corollaries 1 – 5 are not true in general; however, the following theorem provides a necessary and sufficient condition when the index of an equilibrium changes as a parameter is varied under the assumption that the equilibrium point always exists.

**Theorem 3.** Let \( f_{\lambda} \) be as in Theorem 1 and suppose that an equilibrium point \( x_0(\lambda) \in f_{\lambda}^{-1}(0) \) has upper semicontinuous dependence on \( \lambda \) and \( f_{\lambda}^{-1}(0) \) is strict for all \( \lambda \in [0, 1] \). Then the index of \( f_{\lambda} \) about \( x_0(\lambda) \) is constant for all \( \lambda \in [0, 1] \) if and only if \( \text{det} \left( \frac{\partial f_{\lambda}(x_0)}{\partial x} \right) \) never changes sign or becomes 0 for all \( \lambda \in [0, 1] \).

**Proof.** \( \Rightarrow \) We assume the opposite and prove by contradiction. Suppose that \( \text{det} \left( \frac{\partial f_{\lambda}}{\partial x} \right) \neq 0 \) for all \( x_0(\lambda) \in f_{\lambda}^{-1}(0) \). Choose an \( x_0(\lambda) \in f_{\lambda}^{-1}(0) \). Now, suppose for some \( \lambda_1, \lambda_2 \) that \( \text{sgn(\text{det}(f_{\lambda_1}(x_0))) = 1} \) and \( \text{sgn(\text{det}(f_{\lambda_2}(x_0))) = -1} \). Then, since the determinant is a real valued upper semicontinuous function in \( \lambda \), there exists a \( \lambda_3 \in (\lambda_1, \lambda_2) \) such that \( \text{det} \left( \frac{\partial f_{\lambda_3}(x_0)}{\partial x} \right) = 0 \) by the Intermediate Value Theorem (which can be done since the graph is upper semicontinuous and therefore closed by the closed graph theorem), resulting in a contradiction.

\( \Leftarrow \) The converse is proved by definition. Since the index is defined as \( \text{sgn} \left( \text{det} \left( \frac{\partial f_{\lambda}}{\partial x} \right) \right) \), if the determinant of the Jacobian changes sign, then it become zero by upper semicontinuity and the Intermediate Value Theorem resulting in a change in the index. 

A necessary condition for the appearance or disappearance of equilibrium points as \( \mu \) or \( \lambda \) is varied is now presented.

**Theorem 4.** Suppose a system in the form of (3.6) that satisfies the assumptions in Theorem 2 on \( h, g, \) and \( A_0 \), and that \( \text{det} \left( \frac{\partial f_{x, \lambda}(x)}{\partial x} \right) \) at all equilibrium points have the same sign. Assume that \( x_0(\mu, \lambda) \in \{f_{\mu, \lambda}^{-1}(0)\} \) is upper semicontinuous. Then, if \( \text{det} \left( \frac{\partial f_{x, \lambda}(x)}{\partial x} \right) \) changes sign as \( \mu \) and \( \lambda \) are varied from \( (\mu^*, \lambda^*) \in [0, 1] \times [0, 1] \) to \( (\mu^* + \epsilon, \lambda^* + \epsilon) \in [0, 1] \times [0, 1] \) for some \( \epsilon \) at at least one equilibrium point, then the two systems \( \dot{x} = f_{\mu^*, \lambda^*}(x) \) and \( \dot{x} = f_{\mu^* + \epsilon, \lambda^* + \epsilon}(x) \) do not have the same number of equilibrium points.
Proof. Suppose that the number of equilibrium points is constant for all $\lambda, \mu \in [0, 1] \times [0, 1]$. Without loss of generality, we assume that for some $\mu^*, \lambda^*$ that the $\det \left( \frac{\partial f_{\mu^*, \lambda^*}}{\partial x} \right) > 0$ at all equilibrium points. Choose an $\Omega \subset \mathbb{R}^n_{\geq 0}$ that contains all equilibrium points in the positive orthant and that has no zeros on the boundary of $\Omega$, as in the proof of Theorem 2. Then, $|\deg(f_{\mu, \lambda}, \Omega)| = n$ since all equilibrium points of the system have the same sign. Now, suppose that the determinant at at least one equilibrium point becomes negative when $(\mu, \lambda) = (\mu^* + \epsilon, \lambda^* + \epsilon)$, i.e. $\iota(f_{\mu^*+\epsilon, \lambda^*+\epsilon}(x_0)) = -1$. Then the degree is $|\deg(f_{\mu^*+\epsilon, \lambda^*+\epsilon})| \leq n - 2$. This contradicts Theorem 1 that the degree of the system is constant. Therefore, must be a change in the number of equilibrium points for Theorem 1 to hold.

Remark 4. Suppose the conditions of Theorem 4 hold and suppose further that the system has one equilibrium point. Then the results of Theorem 4 hold.

We note that the degree reveals some information about the stability of an equilibrium point.

Theorem 5. Suppose the degree of a non-degenerate equilibrium point of $\dot{x} = f(x)$ is $(-1)^{n+1}$, where $n$ is the dimension of the system. Then the equilibrium point has at least one eigenvalue strictly in the right half plane and is unstable.

Proof. Using the fact that the $\det \left( \frac{\partial f}{\partial x} \right)$ is the product of the eigenvalues, if all eigenvalues are in the left half plane, then the sign of the determinant is $(-1)^n$. The contrapositive is what we wished to prove.

This theorem provides a sufficient condition on the stability of an equilibrium point if the determinant of the Jacobian is known. Typically, biological systems are designed while neglecting any context-dependent effects such as resource sharing. This corresponds to designing $h(x)$ while neglecting $\alpha(x)$ and $g(x)$ in (3.6). The theorems presented in this section provide tools to aid in determining when resource sharing may cause a change in the number of equilibria of a system. We will now examine a few examples to show the application of the theoretical results.
3.3 Application of Theory

In this section, we examine a variety of systems where multiple equilibria may occur due to resource sharing. First, however, we provide a brief commentary on some considerations when modeling biological systems in order for the theoretical results to apply and provide an intuitive explanation to aid in visualizing what the conditions exactly mean for the examples.

For the systems under consideration, \( h(x) \), \( \Lambda_0 \), \( \alpha(x) \), and \( g(x) \) in (3.6) may represent protein production, dilution, terms from sharing of protein production resources, and sharing of protein degradation resources, respectively. These terms match those in general models including resource sharing terms for production resources [35] and degradation resources, derived in Section 2.2.

For the assumptions of Theorem 2 and 4, and Corollaries 1 – 5 to hold, it must be ensured that the system does not have any zeros on the boundary of the positive orthant. This can be done by assuming that production always has some small amount of leakiness. For example, \( h_i(x) \) represents activation, it has the form

\[
\frac{\alpha(x/K_{dna})^n + \alpha_0}{1 + (x/K_{dna})^n},
\]

where \( \alpha_0 > 0 \) represents the leakiness.

In Theorem 2, and Corollaries 1 – 5, a condition was given on the determinant of the Jacobian being non-zero at any equilibrium point. One can visualize these conditions as a boundary between regions in the positive orthant with positive determinant and with negative determinant, shown in Figure 3-1. If an equilibrium point crosses one of these boundaries as \( \lambda \) is varied, then the number of equilibrium points may change. This boundary moves within the positive orthant as well since it depends on \( \lambda \). Additionally, the existence of this boundary does not, however, guarantee that the path of any equilibrium point may cross this boundary. We now present a few examples of biological systems with resource sharing and determine if the number of equilibria may change due to resource sharing.
Figure 3-1: Visualization of change of sign of determinant in the plane as $\lambda$ is varied. When $\lambda = 0$, $\det \left( \frac{\partial f}{\partial x} \right) > 0$ everywhere; however, at some $\lambda^*$ there exists one point where $\det \left( \frac{\partial f}{\partial x} \right) = 0$ which then becomes an area where $\det \left( \frac{\partial f}{\partial x} \right) < 0$.

Example 1. Ribosome Sharing Cascade In biological systems, the cascade motif is one of the most common motifs [3]. Additionally, it can easily be shown that cascades without shared resources may exhibit only one equilibrium point. We consider a two-protein cascade with shared ribosomes. The dynamics, given in the form of (3.6) and derived in [35], are

\begin{align*}
\dot{x}_1 &= T_1 F_1(u) \left[ \frac{\mu}{1 + J_1 F_1(u) + J_2 F_2(x_1)} + 1 - \mu \right] - \delta x_1 \quad (3.12) \\
\dot{x}_2 &= T_2 F_2(x_1) \left[ \frac{\mu}{1 + J_1 F_1(u) + J_2 F_2(x_1)} + 1 - \mu \right] - \delta x_2 \quad (3.13)
\end{align*}

where $T_i$ is the maximum production rate of protein $x_i$, $F_i$ is a normalized Hill function, $J_i$ represents the resources used by protein $x_i$ in production, and $\delta$ represents dilution for $i = 1, 2$. $\mu \in [0, 1]$ is a parameter that can be chosen to determine the magnitude of resource sharing. Since both $F_1$ and $F_2$ are monotonic functions, and the system has a single equilibrium point when $\mu = 0$, this implies that if the system changes number of equilibrium for some $\mu \in [0, 1]$, then the system will also show that change for $\mu = 1$. Thus, for simplicity in analysis, we set $\mu = 1$. Then, the
Jacobian is
\[
J = \begin{bmatrix}
-\delta - \frac{T_1 F_1(u) T_2 F_2'(x_1)}{[1 + J_1 F_1(u) + J_2 F_2(x_1)]^2} & 0 \\
* & -\delta
\end{bmatrix}
\]  
(3.14)
and the determinant of the Jacobian is
\[
\det(J) = \delta^2 + \delta \frac{T_1 F_1(u) T_2 F_2'(x_1)}{[1 + J_1 F_1(u) + J_2 F_2(x_1)]^2}. 
\]  
(3.15)
To verify the condition from Corollary 1, we find solutions when \( \det(J) = 0 \). If, additionally, that fact that the determinant of the Jacobian must cross zero at an equilibrium point is included, as required in Corollary 1, then the condition from the corollary is satisfied if
\[
-F_2'(x_1) \geq \frac{\delta}{J_2 T_1 F_1(u)} (1 + J_1 F_1(u) + J_2 F_2(x_1))^2 
\]  
(3.16a)
\[
0 = \frac{T_1 F_1(u)}{1 + J_1 F_1(u) + J_2 F_2(x_1)} - \delta x_1 
\]  
(3.16b)
\[
0 = \frac{T_2 F_2(x_1)}{1 + J_1 F_1(u) + J_2 F_2(x_1)} - \delta x_2. 
\]  
(3.16c)
Let \( x_1^* \) and \( x_2^* \) be solutions to (3.16b) and (3.16c). Then, rearranging (3.16b) and (3.16c) is
\[
\frac{1}{\delta x_1^*} = \frac{(1 + J_1 F_1(u) + J_2 F_2(x_1))}{T_1 F_1(u)} 
\]  
(3.17)
\[
\frac{T_2 F_2(x_1^*)}{x_2^*} = \delta(1 + J_1 F_1(u) + J_2 F_2(x_1)). 
\]  
(3.18)
Substituting the right hand side of (3.18) and (3.17) into (3.16a) results in the condition
\[
\frac{-F_2'(x_1^*)}{F_2(x_1^*)} \geq \frac{T_2}{\delta J_2 x_1^* x_2^*}, 
\]  
(3.19)
which, if satisfied guarantees a change in number of equilibria of a cascade with ribosome sharing. This shows that if the repression curve of \( x_2 \) is steep enough at the equilibrium point, then the system with resource competition will have multiple equilibrium points while the system without has only one equilibrium point by Remark
4.

Figure 3-2 shows the right and left-hand sides of (3.19) for one set of parameters. When the left-hand side is larger than the right hand side, the system does have more than one equilibrium points. Practically speaking, in order for (3.19) to be satisfied requires a repression cascade with high cooperativity. Note that in Figure 3-2, \( n \) must be larger than approximately 2.3 for this behavior to be possible.

![Figure 3-2: Graphical representation of the right (blue) and left (red) hand sides of (3.19) vs \( x^*_1 \). \( F_1 \) is assumed to be of the form \( \frac{1+ax^*_1}{1+bx^*_1} \) with constants assumed to be \( a = 1, b = 10, n = 3 \), and \( \frac{T_x}{K_x} = \frac{1}{2} \). Points at which the red curve is larger than the blue curve correspond to values of \( x_1 \) with multiple equilibria due to ribosome sharing.](image)

**Example 2. Two Protein Cascade with Protease** Consider a two node protein cascade, shown in Figure 3-3 where \( x_1 \) either activates or represses \( x_2 \) through transcriptional regulation and one protease degrades both proteins \( x_1 \) and \( x_2 \). Then the

![Figure 3-3: Two protein cascade with a shared protease, where \( x_1 \) is inducible, and \( x_2 \) is either transcriptionally activated or repressed by \( x_1 \). The red arrows represent non-explicit interactions through the shared protease.](image)
set of ODEs is given by

\[
\begin{align*}
\dot{x}_1 &= U - \frac{Px_1}{1 + x_1/K_1 + x_2/K_2} - \delta x_1 \\
\dot{x}_2 &= H(x_1) - \frac{Px_2}{1 + x_1/K_1 + x_2/K_2} - \delta x_2
\end{align*}
\] (3.20a, 3.20b)

where \(H(x_1)\) is a Hill function, and is equal to either \(\frac{\alpha(x_1/K_{DNA})^n}{1+(x_1/K_{DNA})^n}\) or \(\frac{\alpha}{1+(x_1/K_{DNA})^n}\), and \(U\) is a strictly positive constant. Since \(U > 0\), then \(H(x_1) > 0\), so there exists an \(\Omega \subset \mathbb{R}^n_{\geq 0}\) such that this system has no zeros on the boundary of \(\Omega\) and \(\Omega\) is positively invariant. It has previously been shown that this system has one equilibrium point when there is no protease present, i.e. \(P = 0\) [7]. Then to show when (3.20) has the same number of equilibrium points as when \(P = 0\), we verify the condition of Theorem 2 by calculating the Jacobian for this system. This is given as

\[
\frac{\partial f}{\partial x} = \begin{bmatrix}
-\frac{(P/K_1)(1+x_2/K_2)}{(1+x_1/K_1+x_2/K_2)^2} - \delta \\
\frac{Px_2/(K_1K_2)}{(1+x_1/K_1+x_2/K_2)^2}
\end{bmatrix}
\] (3.21)

The red arrows in Fig. 3-3 represent non-explicit interactions through the sharing of the common protease, which can be seen from the off-diagonal terms of the Jacobian, (3.21), which are always positive. This indicates that protease sharing creates non-explicit activation between \(x_1\) and \(x_2\). Then the determinant of the Jacobian is

\[
\text{det} \left( \frac{\partial f}{\partial x} \right) = \delta^2 + \delta \frac{P(K_2 + x_2) + P(K_1 + x_1)}{K_1K_2(1 + x_1/K_1 + x_2/K_2)^2} + \frac{P^2/(K_1K_2)}{(1 + x_1/K_1 + x_2/K_2)^3} - H'(x_1)\frac{Px_1/(K_1K_2)}{(1 + x_1/K_1 + x_2/K_2)^2}. \] (3.22)

It can be seen that (3.22) is never 0 whenever \(H'(x_1) < 0\) since all terms are then strictly positive. This satisfies the conditions of Corollary 2, and thus, (3.20) is guaranteed to have one equilibrium point for all \(x \in \mathbb{R}^2_{\geq 0}\). This corresponds to the case where \(x_1\) represses \(x_2\). Therefore, in a two-protein repression cascade, it is not possible to have bistability due to protease sharing. If, instead \(x_1\) activates \(x_2\), then \(H'(x_1) > 0\) and the determinant may change sign for some parameter values. Then
the system fails the sufficient condition in Corollary 2, so by Remark 4, the system has multiple equilibrium points whenever the sign of the determinant of the Jacobian is negative at an equilibrium point. Indeed, the activation cascade with protease sharing shown in Figure 3-4 has three equilibrium points—two stable and one unstable for a range of inputs, $U$.

![Figure 3-4: saddle-node bifurcation of the activation cascade with only protease sharing and neglected ribosome sharing terms.](image)

**Example 3. microRNA Cascade** We now consider a similar system where microRNA (miRNA) degrade the mRNAs of two proteins in a cascade, shown in Figure 3-5. MicroRNA bind to mRNA and degrade the target while preserving the microRNA in much the same way that proteases degrade proteins [36, 37, 38, 39]. Then,
the set of equations governing this system are given as

\[ \dot{m}_1 = U + \lambda g_1(m_1, m_2) - \delta_1 m_1 \]  
\[ \dot{x}_1 = \alpha m_1 - \delta_2 x_1 \]  
\[ \dot{m}_2 = H(x_1) + \lambda g_2(m_1, m_2) - \delta_1 m_2 \]  
\[ \dot{x}_2 = \alpha m_2 - \delta_2 x_2 \]  

where \( U \) is the activation of the system, \( g_1, g_2 \) are the degradation of \( m_1 \) and \( m_2 \) by microRNA, respectively, and are negative, \( \delta_1 \) is dilution and degradation of mRNA, \( \alpha \) is the rate of translation, \( \delta_2 \) is protein dilution, and \( H(x_1) \) is the transcriptional activation or repression of gene 2 by protein \( x_1 \) and is positive. It is typically assumed that \( H(x_1) \) is in the form of a Hill function and therefore bounded. Note that the states of the system are bounded by Proposition 1 since the system can be written in the form of 3.5. Additionally, it is additionally assumed that \( \frac{\partial g_1}{\partial x_1}, \frac{\partial g_2}{\partial x_2} < 0 \) and \( \frac{\partial g_1}{\partial x_2}, \frac{\partial g_2}{\partial x_1} > 0 \). The Jacobian is given by

\[
J = \begin{bmatrix}
\lambda \frac{\partial g_1}{\partial m_1} - \delta_1 & 0 & \lambda \frac{\partial g_1}{\partial m_2} & 0 \\
\alpha & -\delta_2 & 0 & 0 \\
\lambda \frac{\partial g_2}{\partial m_1} & H'(x_1) & \lambda \frac{\partial g_2}{\partial m_2} - \delta_1 & 0 \\
0 & 0 & \alpha & -\delta_2 \\
\end{bmatrix}
\]  

Then, by Corollary 1 and Remark 4, this system exhibits multiple equilibria if and only if the Jacobian changes sign at an equilibrium point for some \( \lambda \in [0, 1] \). The determinant of the Jacobian is given as

\[
\text{det}(J) = \delta_2 \left[ \delta_2 \left( \delta_1 - \lambda \frac{\partial g_1}{\partial m_1} \right) \left( \delta_2 - \lambda \frac{\partial g_2}{\partial m_2} \right) - \lambda \frac{\partial g_1}{\partial m_2} \left( \alpha H'(x_1) + \lambda \frac{\partial g_2}{\partial m_1} + \delta_2 \right) \right] > 0
\]  

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Note that when $\lambda = 0$, $\det(J) > 0$. Then, the condition in Remark 4 is satisfied if

$$H'(x_1) \geq \delta_1 \delta_2 \left( \delta_1 + \lambda \left| \frac{\partial g_1}{\partial m_1} \right| + \lambda \left| \frac{\partial g_2}{\partial m_2} \right| \right) \left( \lambda \frac{\partial g_1}{\partial m_2} \right)^{-1} > 0,$$  

(3.26)

then there exists a region in the positive orthant where the determinant changes sign. Further, if this condition is satisfied at an equilibrium point, the system has three equilibrium points. (3.26) may be satisfied only if $H'(x_1) > 0$, which corresponds to the activation of $x_2$ by $x_1$. We have not checked that this occurs at an equilibrium point, however, because it is computationally difficult to do so. Therefore, we are left with the result that we can guarantee the uniqueness of the equilibrium point in this system if $x_1$ represses $x_2$, but are unable to say definitively whether the activation cascade will cause multiple equilibria without further analysis.

Additionally note that since this condition requires the slope of $H(x_1)$, which is bounded and continuous, to be larger than some value. This cannot be satisfied the determinant may change sign from positive to negative this cannot hold true for all $U$ larger than a fixed value.

3.4 Conclusions

In this chapter, we have developed a theoretical framework to determine conditions under which a system is guaranteed to maintain its number of equilibrium points when additional functions in the dynamics are introduced. In Theorem 2, we presented a general result stating a sufficient condition for any system in the form of (3.6). Additionally we stated a necessary condition for a smaller class of systems in Theorem 4, which when combined with the previous result gives necessary and sufficient conditions for determining a change in the number of equilibrium points. We applied these results in a few examples of biological systems with resource sharing of ribosomes, proteases, and microRNA.

Using Theorem 2 and Theorem 4, for certain classes of systems, it is possible to determine exact conditions when the number of equilibrium points in a system with
resource sharing changes as a parameter $\mu$ or $\lambda$ is varied. The only drawback to this framework is that it may be difficult to analytically verify the condition that the Jacobian must be non-zero at an equilibrium point. These checks can instead be easily implemented via simulation. Using this framework, however, we are able to say very little about whether an equilibrium point may change stability as resource sharing is introduced since the theorems only comment on changes in the number of equilibrium points and not their stability. Changes in stability of equilibrium points may still pose issues in the design of biological networks; however, capturing changes in the number of equilibrium points is an important step in characterizing the effects of resource sharing on the qualitative behavior of a genetic network with resource sharing. The examples presented were all of biological systems; however, the theoretical results presented in this chapter are applicable to general systems.
Chapter 4

A Method for Estimating the Effect of Resource Usage on Genetic Circuits

4.1 Introduction

The behavior of genetic circuits depends on the context in which they operate [5]. One subset of context dependence effects is resource sharing, in which the sequestration of a finite pool of cellular resources causes a coupling between otherwise uncoupled nodes [1]. This effect limits the predictability of the behavior of genetic circuits when composed due to changes in the availability of shared resources. As additional genes are introduced to the host cell, each requires resources and disturbs the function of all other genes through the demand of resources. These resources may be free ribosomes, RNAP, amino acids, et. cetera.

In this chapter, we propose a method to estimate the effects of resource sharing on synthetic circuits in two ways. The first is the quantity of free resources used by a circuit which characterizes the magnitude of disturbance it places upon other circuits in the same context. The second is the sensitivity of the output of the circuit to changes in resource availability and characterizes the effect other circuits have on
its function. These two measures capture resource context dependence as the effect of the module on the environment and the effect of the environment on the module, which allow for complete quantification of resource sharing effects.

We then propose a set of experiments to measure the proposed metrics and show how the proposed metrics can be measured from the experimental outputs. We consider only the steady state behavior of the system. We assume that all resource sharing only occurs for production resources and degradation resources are either not present or not under significant competition.

4.2 Overview and Setup

To design a complex circuit, the design process is typically broken up into the design of separate parts, known as modules. These modules are then combined to perform the desired function. We define a module as a set of genes in a genetic circuit with a given input–output behavior. It is assumed that each module is a black box, so no knowledge is available about the nature of the interactions within the module.

Consider the situation where, given a set of modules each with some input–output behavior, we wish to choose the modules to give the desired behavior and to maximize robustness of the overall circuit. One of the major limits to robustness is resource sharing. If we can predict resource sharing effects as modules are combined, we can increase the robustness of the overall circuit. Therefore, in this situation, we wish to devise a method to achieve this. We will design a system that measures the output of the module through a fluorescent protein reporter and a synthetic system that we will call the sensor.

In order to achieve this, we assume that the concentration of a protein in a network is of the generic form

\[
\frac{dx}{dt} = \frac{T_x F_x(u)}{1 + J_0 + \sum_i J_i F_i + J_x F_x(u)} - \delta x
\]

where \(x\) is the concentration of a protein in the system, \(T_x\) is the rate of production
of \( x \), \( F_x(u) \) is a normalized Hill function, \( J_0 \) is the resource load from nodes external to the module in which \( x \) resides, \( \sum J_i F_i \) is the resource load from nodes within the module. This model assumes all complexes are at their quasi-steady state, linear binding between mRNA and ribosomes, and accounts for resource sharing. Additionally, we assume that growth rate is constant, independent of the synthetic circuit and protein concentrations.

Using the terms in the general model in (4.1), we propose that the metrics useful to measure resource sharing are the quantity of resources used

\[
Q = \sum J_i F_i
\]  

(4.2)

and the sensitivity of the output of the module with respect to changes in resource availability

\[
S = \frac{dx}{dJ_0} \frac{1}{x_0} \bigg|_{J_0=0}.
\]

(4.3)

In this case, \( x \) is the concentration of the output of the module. We normalize the derivative by the concentration of \( x \) in the unperturbed state since it is desirable to for the measure to be independent of the equilibrium point. Both \( Q \) and \( S \) are non-dimensional numbers. \( Q \) is a positive real number and \( S \) may be any real number, but is typically negative since concentrations tend to decrease when resource competition is increased.

### 4.3 Integrating Resource Information into the Decision Process

Now that the two measures, \( Q \) and \( S \), have been proposed, we will illustrate how they may be used to inform design and allow prediction of resource effects without the need to test each module with every other combination of modules to determine which performs optimally. This means that the number of experiments required scales linearly with the number of modules and not combinatorially. For this analysis, we
will assume that each module can be characterized by a single pair of numbers, \( Q \) and \( S \) as measured Sections 4.4. Then, each module may be characterized into four categories:

1. both \( Q \) and \( S \) are small
2. \( Q \) is small, but \( S \) is large
3. \( S \) is small, but \( Q \) is large
4. both \( Q \) and \( S \) are large

If the module of interest falls into category 1, it is clear that this module will behave robustly under most circumstances and will not cause significant changes in other modules. If the module of interest falls into category 4, it is clear that there are few circumstances in which this module will behave properly when connected in a larger network. This may be acceptable only when few other modules are present within the cell and they all have small values of \( Q \) and \( S \). However, the situation is less clear if the module happens to fall in categories 2 or 3.

To investigate these situations, we consider the situation where multiple modules are present. As can be seen from (4.1), the total resource usage from each all modules is the sum of the resource usage from all individual modules within the same host cell. Therefore, the effective resource disturbance on module \( i \) from all other modules in a system is

\[
J_{0}^{\text{eff}} = \sum_{j \neq i} Q_{j}
\]  

(4.4)

where \( J_{0}^{\text{eff}} \) is the effective disturbance due to resources used by other modules, \( Q_{j} \) is the measured resource usage \( (Q) \) from each module \( j \). Suppose some allowable tolerance of the output of each module \( i \), \( \beta_{i} \), is required by the designer. This can be expressed as

\[
\frac{x_{i}^{d} - x_{i}}{x_{i}} \leq \beta_{i},
\]  

(4.5)

where \( x_{i} \) is the output of the module when measured in isolation and \( x_{i}^{d} \) is the output of the module when measured with other modules disturbing the resource pool. Now,
using an approximation of the derivative in the definition of sensitivity, then, the
sensitivity of module $i$, $S_i$ is given as

$$ S_i = \frac{x_i^d - x_i}{\frac{1}{x_i} + x_i^0} $$  \hspace{1cm} (4.6) $$

so, solving for the tolerance in terms of $S_i$ and $Q_j$ is given by

$$ S_i(u_i) \cdot \sum_{j \neq i} Q_j(u_j) = \frac{x_i^d - x_i}{x_i} \leq \beta_i. $$ \hspace{1cm} (4.7) $$

Then the tolerance specification is met for all inputs only if

$$ \sup_{u_i, u_j} \left\{ S_i(u_i) \cdot \sum_{j \neq i} Q_j(u_j) \right\} \leq \beta_i. $$ \hspace{1cm} (4.8) $$

Then given tolerances for the output of each module, one can run an optimization
process with constraints for each module in the form of (4.8). Additionally, we can
predict the output of each module when combined with the other modules sharing
the same resource pool by rearranging (4.7), which is given by

$$ x_i^d = x_i \left( 1 + S_i \cdot \sum_{j \neq i} Q_j \right) $$ \hspace{1cm} (4.9) $$

which allows for the prediction of the output of each module given information of
the input-output curve $x_i(u_i)$, resource usage of all other modules, $\sum_{j \neq i} Q_j(u_j)$, and
sensitivity $S_i(u_i)$.

**4.4 Experiments**

We now propose a series of five experiments used to estimate the metrics $Q$ and $S$
for any module. These experiments can be separated into two distinct steps. The
first three experiments is used to characterize the measurement sensor. The final two
experiments then characterizes the module given that the measurement sensor is well
characterized.
The proposed measurement device consists of combinations of the module, a fluorescent reporter protein to measure the output of the module (GFP), and an additional fluorescent reporter (RFP) representing an external demand for resources, which we will call the measurement sensor. The measurement sensor provides a perturbation to the available resource pool. By observing the change in module output concentration (GFP) and change in measurement sensor concentration (RFP) in response to this perturbation allows the quantity of resources the module uses and the sensitivity of the module to disturbances in resources to be characterized.

No specific properties are assumed about these reporters other than that one is able to measure their concentrations with some arbitrary units. Any reporter protein may be substituted, but for clarity we will refer to the output of the module as GFP and the external reporter as RFP.

In experiments 1, 2, and 3, we first characterize the constitutive GFP and RFP reporters by running experiments without a module. Then in experiments 4 and 5, we characterize the module.

Experiment 1. First, measure the steady state output of a constitutive GFP reporter alone. Using the form of (4.1), the steady state is given by

\[ G_{1}^{ss} = \frac{T_G F_G / \delta}{1 + J_G F_G} \]  \hspace{1cm} (4.10)

Experiment 2. Next, measure the steady state output of a constitutive RFP reporter alone. Using the form of (4.1), the steady state is given by

\[ R_{2}^{ss} = \frac{T_0 / \delta}{1 + J_0} \]  \hspace{1cm} (4.11)


**Experiment 3.** Then, measure the steady state output of the RFP reporter and GFP module, both constitutively expressed in the same host cell. Using the form of (4.1), the steady state is given by

\[
G_3^{ss} = \frac{T_G F_G / \delta}{1 + J_0 + J_G F_G} \quad (4.12a)
\]

\[
R_3^{ss} = \frac{T_0 / \delta}{1 + J_0 + J_G F_G}. \quad (4.12b)
\]

The data from the previous three experiments will be used to characterize the RFP measurement sensor in Section 4.5.

**Experiment 4.** Next measure the output of the module (GFP) at steady state while varying the input to the module, \( u \). Using the form of (4.1), the steady state is given by

\[
G_4^{ss} = \frac{T_G F_G(u) / \delta}{1 + \sum J_i F_i + J_G F_G(u)}. \quad (4.13)
\]
**Experiment 5.** Finally, measure the output of the module at steady state (GFP) with the RFP measurement sensor in the same host cell. Using the form of (4.1), the steady state is given by

\[
G_{5}^{ss} = \frac{T_G \tilde{F}_G(\bar{w})/\delta}{1 + J_0 + \sum J_i F_i + J_G \tilde{F}_G(\bar{w})} \quad (4.14a)
\]

\[
R_{5}^{ss} = \frac{T_0/\delta}{1 + J_0 + \sum J_i F_i + J_G \tilde{F}_G(\bar{w})}. \quad (4.14b)
\]

In this case, the RFP reporter acts as a perturbation to the module, which allows for the measurement of the module's resource usage and sensitivity when compared with data from 4.

With these 5 experiments, it is possible to obtain a complete representation of the relative resource usage by solving for \( Q \) and \( S \) using the equations for steady state of each measurement.

### 4.5 Solving for Resource Measures

We now use information obtained from experiments 1, 2, and 3 to characterize the sensor and reporter. Then, we will combine this with measurements from experiments 4 and 5 to characterize the module and obtain the desired metrics. For the remainder of this analysis, we will only consider steady state, so, for clarity, the \( ss \) will be dropped from all terms.
4.5.1 Characterization of Measurement Device

First, we define the dimensionless quantities

\[
\hat{G}_0 = \frac{G_3}{G_1} < 1 \quad (4.15a)
\]
\[
\hat{R}_0 = \frac{R_3}{R_2} < 1 \quad (4.15b)
\]

which represent the relative change in output of the constitutive GFP and RFP genes, respectively. Using the steady state concentrations of GFP and RFP from experiments 1, 2, and 3, we solve for \( J_0 \) and \( J_G F_G \), to characterize the resource usage from the reporter and sensor.

\[
\frac{G_3}{G_1} = \frac{\hat{G}_0}{1} = 1 + \frac{J_0}{1 + J_G F_G} \quad (4.16a)
\]
\[
\frac{R_3}{R_2} = \frac{\hat{R}_0}{1} = 1 + \frac{J_G F_G}{1 + J_0} \quad (4.16b)
\]

This results in the system of equations

\[
J_0 = (1 + J_G F_G(u)) \left( \frac{1}{\hat{G}_0} - 1 \right) \quad (4.17a)
\]
\[
J_G F_G(u) = (1 + J_0) \left( \frac{1}{\hat{R}_0} - 1 \right). \quad (4.17b)
\]

Then, solving simultaneously, we find

\[
J_0 = \frac{1 - \hat{G}_0}{\hat{R}_0 + \hat{G}_0 - 1} \quad (4.18a)
\]
\[
J_G F_G(u) = \frac{1 - \hat{R}_0}{\hat{R}_0 + \hat{G}_0 - 1} \quad (4.18b)
\]

Both these solutions are guaranteed to be positive, finite real numbers since \( \hat{R}_0 + \hat{G}_0 > 1 \) by (4.10), (4.11), and (4.12). Note: we only use \( J_G F_G(u) \) as a check to make sure it it much smaller than \( J_0 \). For the next analysis, we will assume that \( J_G F_G(u) \ll J_0 \).
by design and can be safely neglected. This assumption is met if the condition

\[
\frac{1 - \hat{G}_0}{1 - \hat{R}_0} \gg 1
\]  

(4.19)

is met, which corresponds to the situation where the copy number of RFP is much larger than the copy number of GFP.

### 4.5.2 Characterization of Module

Now, we combine this information with that obtained from experiments 4 and 5 to characterize the module. We define the dimensionless quantities

\[
\hat{G} = \frac{G_5}{G_4} \quad \tag{4.20a}
\]

\[
\hat{R} = \frac{R_5}{R_2} \quad \tag{4.20b}
\]

\[
\hat{F}_G = \frac{F_5}{F_4} \quad \tag{4.20c}
\]

Then, using (4.13) and (4.14) we obtain

\[
\frac{G_4}{G_5} = \frac{1}{\hat{G}} = \frac{F_4}{F_5} \left( \frac{1 + J_0 + \sum_i J_i F_i + J_G F_G}{1 + \sum_i J_i F_i + J_G F_G} \right) \quad \tag{4.21a}
\]

\[
\frac{R_2}{R_5} = \frac{1}{\hat{R}} = \frac{1 + J_0 + J_G F_G(u) + \sum_i J_i F_i}{1 + J_0} \quad \tag{4.21b}
\]

We can neglect \( J_G F_G \) since it should be significantly smaller than \( J_0 \) and \( \sum J_i F_i \) by design. Then (4.21b) becomes

\[
\frac{1}{\hat{G}} = \frac{1}{\hat{F}_G} \left( 1 + \frac{J_0}{1 + \sum_i J_i F_i} \right) \quad \tag{4.22a}
\]

\[
\frac{1}{\hat{R}} = 1 + \frac{\sum_i J_i F_i}{1 + J_0}. \quad \tag{4.22b}
\]
We solve (4.21b) and (4.21a) for the unknowns $\sum J_i F_i$ and $\hat{F}_G$, which becomes

\[ \sum_i J_i F_i = \left( \frac{1}{R} - 1 \right) (1 + J_0) \]  
\[ \hat{F}_G = \frac{\hat{G}(1 + J_0)}{1 + J_0(1 - \hat{R})} \]

where $J_0$ was given in (4.18a). These equations simplify to

\[ \sum_i J_i F_i = \frac{\hat{R}_0(1 - \hat{R})}{\hat{R}(\hat{R}_0 + \hat{G}_0 - 1)} \]  
\[ \hat{F}_G = \frac{\hat{G}\hat{R}_0}{\hat{R}(\hat{G}_0 - 1) + \hat{R}_0} \]

This gives us the information required to find $Q$ and $S$ for each module.

### 4.5.3 Finding Resource Measures $Q$ and $S$

Recall the definitions of $Q$ and $S$. $Q$ is

\[ Q = \sum_i J_i F_i \]  

which is given in terms of measured quantities from (4.24a) which is

\[ Q = \frac{\hat{R}_0(1 - \hat{R})}{\hat{R}(\hat{R}_0 + \hat{G}_0 - 1)}. \]

It should be noted that if exactly only one resource is limiting and all others are abundant, then the percent of resources that are unbound to total amount of resources is given as

\[ \frac{R_{free}}{R_{tot}} = \frac{1}{1 + Q} \]

where $R_{free}$ is the amount of available limiting resources, and $R_{tot}$ is the total amount of the limiting resource, so $Q$ is related to the relative amount of free resources available is related to $Q$. Additionally, the sensitivity of the module to disturbances
in resource availability is defined as

\[ S = \frac{dx}{dJ_0} \frac{1}{x|_{J_0=0}} \] (4.28)

where \( x \) the concentration of RFP at steady state and \( J_0 \) is the disturbance due to resource variability. This is applied to the results of experiments 1 – 5 by approximating the derivative in (4.28) by a finite difference as

\[ S \approx \frac{G_5 - G_4}{G_4 J_0} \] (4.29)

which becomes

\[ S \approx \frac{(G - 1)(\hat{R}_0 + \hat{G}_0 - 1)}{1 - \hat{G}_0} \] (4.30)

This gives the sensitivity of the output of the module to resource fluctuations, measured by the GFP reporter in terms of only measured quantities. Note that \( S \) is a function of the perturbation, \( J_0 \). This is due to the nonlinear nature of the system and is to be expected. Thus, experiments 1 – 5 give us the information needed to measure both \( Q \) and \( S \).

4.5.4 Assumptions

To be clear about when our results hold, we state all assumptions necessary in our model. We assume that the cell is well stirred so spacial effects are negligible. We assume that the measurements are taken only at steady state, and that retroactivity does not affect the steady state behavior. Therefore retroactivity effects can be neglected. We assume that dilution and degradation of proteins is the same in all experiments. Additionally, we assume that the fluorescent reporter for a module does not significantly change the amount of available resources compared to the module itself or the sensor protein.

We assume that a model of the system can be put in the form of (4.1) and any assumptions necessary to put the model into this form are consistent with our set of assumptions. For example, from [35], in modeling a system with ribosome competi-
tion, the reaction rate equations are used, and the process of DNA transcription and mRNA translation are modeled as one-step processes. It is additionally assumed that the complexes formed in each of these processes reaches the quasi-steady state and that the free amounts of RNA polymerase and ribosomes are much less than their respective Michaelis-Menten constants (linear binding). Finally, we assumed that the function of the module is not disturbed by the addition of resource perturbations.

4.6 Simulation Results

To validate the performance of the resource sensor at predicting changes in genetic circuit behavior when combined with other modules in a host cell, we simulated three different modules, A, B, and C, and ran experiments 1-5 to determine $Q$ and $S$ for each module as a function of the input, $u$. The modules are shown in Figures 4-6, 4-7, and 4-8 and are all three protein activation or repression cascades.

![Figure 4-6: Module A](image)

**Figure 4-6:** Module A—a genetic two-protein activation cascade. The input $u$ activates the protein $x_1$ which activates $x_2$ which then activates $G$.

![Figure 4-7: Module B](image)

**Figure 4-7:** Module B—a genetic two-protein repression cascade. The input $u$ represses the protein $x_1$ which activates $x_2$ which then activates $G$.

![Figure 4-8: Module C](image)

**Figure 4-8:** Module C—a genetic two-protein activation cascade. The input $u$ represses the protein $x_1$ which represses $x_2$ which then activates $G$.

Using the procedure in Sections 4.4 and 4.5, we find values for $Q$ and $S$ for each module, shown in Figure 4-9.
Figure 4-9: Quantity of resources used by each module, $Q$, and sensitivity, $S$, of each module to resource perturbations for each module A, B, and C. These values were calculated by simulating experiments 3 - 5 and calculating with (4.26) and (4.28).

Then, we predict the output of each module when combined with the other modules sharing the same resource pool using the fact that

$$\frac{G_i^d(u) - G_i(u)}{G_i(u)} = S_i(u)Q_j(u) \quad \text{for } j \neq i \quad (4.31)$$

for $i, j \in \{A, B, C\}$ where $G_i^d$ is the steady state output of module $i$ as measured in isolation and $G_i^j$ is the output of module $i$ sharing the resource pool with module $j$. Then this becomes

$$G_i^j(u) = G_i^0(u)(1 + S_i(u)Q_j(u)) \quad (4.32)$$

for $i, j \in \{A, B, C\}$ which allows for the prediction of the output of each module given information of the input-output curve $G_0(u)$, resource usage $Q(u)$, and sensitivity $S(u)$.

Thus, we simulated all combinations of pairs of modules that share one pool of
resources and compared the predictions made using the measures $Q$ and $S$, which were measured with the modules in isolation, with the actual input–output behavior. All parameters used in the simulation are given in Table 4.1. Figures 4-10, 4-11, and 4-12 show the input–output behavior of each module simulated in isolation and with other modules sharing the pool of RNAP and ribosomes and compares this behavior with that predicted using (4.32). All predictions are accurate to within 10% of the actual behavior for each of these modules simulated which is an improvement over neglecting resource sharing altogether.

Figure 4-10: Module A performance alone vs with Modules B and C (predicted vs actual)

4.7 Conclusions

In this chapter, we proposed metrics for measuring the effect of resource sharing on a genetic circuits. These metrics are the quantity of cellular resources a genetic circuit uses and the sensitivity of the output of that circuit to changes in resource availability. These two metrics then allow us to predict the effect additional circuits within the
Figure 4-11: Module B performance alone vs with Modules A and C (predicted vs actual)

Figure 4-12: Module C performance alone vs with Modules A and B (predicted vs actual)
Table 4.1: Values used in simulations of resource sensor in this Chapter

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<th>description</th>
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<tr>
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</tr>
<tr>
<td>Copy number of GFP</td>
<td>DNA₃</td>
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</tr>
<tr>
<td>Copy number of RFP</td>
<td>DNA₄</td>
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</tr>
<tr>
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</tr>
<tr>
<td>total concentration of ribosomes</td>
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</tr>
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<td>10³ nM to 10⁵ nM</td>
</tr>
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<td>RNAP–DNA dissociation constant</td>
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</tr>
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same host cell will have on the original circuit in a straightforward manner. We showed that these metrics may be measured through an experimental process that requires three experiments to characterize the measurement sensor and two experiments per module.

Then, using these metrics, it is possible to predict the output of each module with any combination of other modules present in the same context. This allows modules to be characterized individually and, using Q and S, design decisions may be made how best to design the genetic circuit. Results were verified through simulation. Simulations show that using Q and S data improves the predicted output over neglecting resource sharing. In the future, we wish to experimentally verify these metrics and analyze their performance with noisy measurements.
Chapter 5

Conclusions

In this thesis, we have shown that resource sharing within genetic networks may cause significant issues in predictability if not accounted for. Resource sharing may cause a change in the steady state concentrations of proteins within cells. In Chapter 2, we demonstrated that sharing of enzymes may successfully cancel undesirable effects due to ribosome sharing.

Additionally, resource sharing causes non-explicit interactions between proteins that may not be directly linked [11, 35]. These non-explicit interactions may cause a change in the qualitative behavior of the system resulting in a change in the number of steady states. In Chapter 3, we have derived and proven necessary and sufficient conditions for system in a specific form on when this may or may not happen.

Finally, in Chapter 4, we have developed a method to measure the effects of resource sharing on a genetic circuit. We have proposed two measures that can be estimated in a few simple experiments. These measures quantify the response of the circuit to changes in resource sharing and the amount of resources the circuit uses. Then these measures can be used to predict the output of the circuit when combined with other circuits in the same context. One can then optimize large genetic circuits by characterizing each module individually instead of testing all combinations of modules. In the future, we plan to test these results experimentally to verify their accuracy.
Bibliography


