Effective Interactions Arising from Resource Limitations in Gene Transcription Networks

by

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Abstract

Protein production in gene transcription networks relies on the availability of resources necessary for transcription and translation, such as RNA polymerase (RNAP) and ribosomes, which are found in cells in limited amounts. As various genes in a network compete for a common pool of resources, a hidden layer of interactions among genes arises. Such interactions are not reflected by standard Hill-function-based models and their interaction graphs. Recent experimental results have revealed that resource limitations can affect the behavior of gene networks, and thus impede our efforts to analyze and design gene transcription networks.

This thesis mainly addresses two problems: how to model the hidden interactions due to resource limitations, and the potential effects of these hidden interactions. A model is developed to account for the sharing of limited amounts of RNAP and ribosomes in gene networks. The model is based on deterministic reaction rate equations, and can be reduced to have the same dimension as the standard Hill-function-based models. Hidden interactions due to resource limitations are identified using this model, and transformed into simple rules to modify the interaction graph of a network. The model is applied to two common network motifs, the activation and repression cascades, where the hidden interactions dramatically change their behaviors. In particular, it is demonstrated that, as a result of resource limitations, a cascade of activators can behave like an effective repressor or a biphasic system, and that a repression cascade can become bistable. The results presented in this thesis may be helpful to mitigate the undesirable effects arising from resource limitations in the future.

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The stochastic potential is different from the deterministic potential through noise-induced bifurcation. The peaks of the stochastic distribution is identical to the deterministic steady states only if noise intensity $I \to 0$ or noise is additive. For a system with bimodal stochastic potential, if its Kramers’ escape rate is large compared to experiment time scale, we will observe bimodal distribution without any hysteresis behavior. Alternatively, if the escape rate is small compared to experiment time scale, then only metastable states can be observed, the system behaves almost deterministically. It will demonstrate hysteresis without bimodality.
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Chapter 1

Introduction

1.1 Resource Limitations in Gene Transcription Networks

The cell is an integrated biological “machine” made of several thousands of interacting proteins. It closely monitors the environment and produces different proteins at various rates in order to respond to external stimuli. This information processing capability is largely carried out by transcriptional regulation. The cell uses special proteins called transcription factors (TFs) to bind with the promoter site of a target gene to control its expression. The interactions between TFs and genes is described by gene transcription networks [1].

Context dependence, the unintended interactions among TFs and with host cell physiology, is a current challenge in analyzing and engineering gene transcription networks [9][38][54]. Such unintended interactions often hinder our ability to predict design outcomes, which often leads to lengthy and ad hoc design processes. Therefore, much research has sought to better understand and mitigate context dependence in gene transcription networks [9][13]. For example, one of the recent efforts to reduce context dependence in gene transcription networks is aimed at retroactivity. Retroactivity is a loading effect arising from connecting functional gene transcription modules. The effects of retroactivity on the dynamics of interconnected genetic mod-
ules are systematically studied in [20] by modifying the standard Hill-function-based models for protein dynamics. Characterizing the dynamics of this context dependence phenomenon laid the foundation for designing an insulation device that largely mitigate its undesirable effects [33].

In this thesis, we study another potential source of context dependence: competition for cellular transcriptional and translational resources. In a gene transcription network, genes are transcribed by RNA polymerase (RNAP) into mRNA, and mRNA is translated by ribosomes into proteins. Proteins that are TFs can affect the expression of other genes in the network by either activating or repressing its target’ ability to recruit RNAP for transcription. However, the abundance of RNAP and ribosomes in living cells is limited and all genes in the network are simultaneously competing for a common pool of these resources [6][30]. The amount of RNAP and ribosomes in cell has largely been assumed constant when modeling gene transcription networks, due to the small scale of networks considered so far. Using constant resources assumption, the resultant standard Hill-function-based models have been successful in engineering gene network such as genetic toggle switches [18], and genetic oscillators [4][17].

In large scale gene networks, however, the competition for limited resources has been shown to introduce interactions in gene expression levels even in the absence of explicit regulatory links [42][45]. In this thesis, we consider general gene transcription networks and develop a modeling framework to predict the effective interactions arising from limitations in RNAP and ribosome availability. Related theoretical works have recently appeared that study resource sharing problems in gene transcription networks. Mather et al. develop a stochastic model based on queuing theory which shows strong negative correlations between steady state protein concentrations when the total mRNA count exceeds ribosome count [32]. De Vos et al. analyze the response of network flux toward changes in total competitors (mRNAs) and common targets (ribosomes) [12]. Yeung et al. illustrate, using tools from dynamical systems, that resource sharing leads to non-minimum phase zeros in the transfer function of a linearized genetic cascade circuit [55]. Gyorgy et al. develop the notion of realizable region for steady state gene expression to account for the limitations in the
availability of RNAP and ribosomes [19]. This result is verified experimentally by the authors in [21] for a benchmark network. Hamadeh et al. analyze and compare different feedback architectures to mitigate resource competition [22].

This thesis focuses on the idea of effective interactions to account for how sharing of RNAP and ribosomes alters the dynamics of a general gene transcription network. For example, when a TF activates the production of protein \( x_1 \) in the network, more RNAP is recruited to produce a larger number of mRNA \( m_1 \). Increased \( m_1 \) further increases the demand for ribosomes to produce \( x_1 \). Both effects decrease the amount of resources available to produce other protein species (for example, an unregulated protein \( x_2 \)) in the network. This waterbed effect creates an effective inhibition of protein \( x_2 \), which is not accounted for in the standard Hill-function-based models and can be incorporated into an interaction graph, which is commonly used by biologists to represent transcription regulations (activation/repression) in gene networks. The proposed model is based on deterministic reaction rate equations and ODEs, and assuming conservation of RNAP and ribosomes. Using the time scale separation property of biochemical reactions, and the order of magnitude difference between free resources amount and their corresponding dissociation constants in bacteria, the model can be reduced to maintain the same dimension as the standard Hill-function-based models in [1][14]. Employing this model, we provide simple rules to identify the hidden interactions due to resource limitations, and the resulting effective interactions in the network. The hidden interactions arising from resource limitations can dramatically change the topology of a gene transcription network, which we will discuss in detail for the examples of activation and repression cascades in Chapter 3. The results presented in this thesis may be helpful to mitigate the undesirable effects of resource limitations in the future.

As a motivating example, we first illustrate the effects of resource limitations in Section 1.2.
1.2 A Motivating Example

Cascade circuits are one of the most common network motifs in both natural and synthetic gene networks due to their ability to amplify signals [10] and achieve “switch-like” behavior [1]. In fact, such cascade structure is an essential element of the ubiquitously used fluorescence-based biosensors [34]. In Fig. 1-1, we consider a simple two-stage activation cascade composed of gene 1 and gene 2. Protein u is the input TF that binds with promoter $p_1$ to activate the production of protein $x_1$. Protein $x_1$ is an activator for the output protein ($x_2$). The structure of this motif can be represented by the interaction graph as $u \rightarrow x_1 \rightarrow x_2$.

![Diagram of a two-stage activation cascade](image)

Figure 1-1: A simplified diagram of a two-stage activation cascade. A limited amount of RNAP and ribosomes is shared between the two stages for the transcription of mRNAs ($m_1$ and $m_2$), and translation of proteins ($x_1$ and $x_2$), respectively.

The dynamics of binding reactions and mRNA dynamics are often neglected because they are much faster than protein dynamics [1],[14]. We use $u$, $x_1$ and $x_2$ to represent the concentration of $u$, $x_1$ and $x_2$, respectively. In a standard model, we use Hill functions to describe gene activation, thus we have:

\[
\dot{x}_1 = \frac{\alpha_0 + \alpha \left( \frac{x_1}{k_1} \right)^n}{1 + \left( \frac{x_1}{k_1} \right)^n} - \gamma_1 x_1, \quad (1.1)
\]

\[
\dot{x}_2 = \frac{\beta_0 + \beta \left( \frac{x_1}{k_2} \right)^m}{1 + \left( \frac{x_1}{k_2} \right)^m} - \gamma_2 x_2, \quad (1.2)
\]
Figure 1-2: \( \bar{x}_2 \) is the steady state concentration of protein \( x_2 \). (A) According to the standard model, steady state output \( \bar{x}_2 \) increases with input \( u \). (B) However, simulation using ODEs (2.1) to (2.7) and conservation of resources shows that system response can be biphasic. Simulation parameters in the standard model in equation (1.2): \( \alpha_0 = \beta_0 = 1 \text{ (hr)}^{-1} \), \( \alpha = \beta = 100 \text{ (hr)}^{-1} \), \( k_1 = k_2 = 10 \text{ (nM)}^2 \), \( \gamma_1 = \gamma_2 = 1 \text{ (hr)}^{-1} \) and \( n = m = 2 \). Simulation parameters in the full mechanistic model are in Table A.1 and A.2.

where \( \alpha_0 \) and \( \beta_0 \) are the basal production rate constants; \( \alpha \) and \( \beta \) are the production rate constants with activation; \( k_1 \) and \( k_2 \) are the dissociation constants of activators \( u \) and \( x_1 \) binding with their respective promoters, \( \gamma_1 \) and \( \gamma_2 \) are the dilution/degradation rate of the proteins, and \( n \) and \( m \) are the cooperativity coefficients. Solving for the steady state of equation (1.2) gives a monotonically increasing I/O response (Fig. 1-2A).

To examine whether the standard model in (1.2) is a good representation of system response under resource limitations, we simulate the system with a mechanistic model that explicitly accounts for the usage of RNAP and ribosomes, and for their conservation law (listed in Section 3.1). Surprisingly, simulation of this mechanistic model reveals that the steady state I/O response can be biphasic (Fig. 1-2B).

With reference to Fig. 1-2A, decrease of steady state expression of \( x_2 \) with \( u \) at high input level in Fig. 1-2B can be explained by the following resource sharing mechanism. When promoter \( p_1 \) and mRNA \( m_1 \) have much stronger ability to sequester resources than promoter \( p_2 \) and mRNA \( m_2 \), as we increase \( u \), the production of protein \( x_1 \) sequesters resources from the production of protein \( x_2 \), decreasing the amount of free resources available to produce \( x_2 \). When this effective repression is stronger
than the activation $x_1 \rightarrow x_2$, $x_2$ decreases with $u$. Decrease in $x_2$ is particularly undesirable if the above system is a biosensor designed to monitor the level of protein $u$ in living cells. While the actual concentration of input $u$ increases monotonically, the sensor output $x_2$ can demonstrate entirely opposite response, potentially creating catastrophic effect in application.

### 1.3 Thesis Content

The main objective of this thesis is twofold. First, an explicit mathematical model, with the same dimension as the standard Hill-function-based model, that predicts the effective interactions arising from resource limitations in cells is provided. Second, the potential effects of such effective interactions are analyzed for the activation and repression cascades. The thesis is organized as follows. In Chapter 2, the general modeling framework is established. The chemical reactions and resultant reduced ODE models for a single gene are derived in Section 2.1.1. In Section 2.1.2, the resource competition problem with multiple genes competing for a limited pool of RNAP and ribosomes is analyzed, which gives the expression of the modified model. The effective interactions arising from the modified model is studied in Section 2.4. Chapter 3 studies how resource limitations alter the behavior of activation and repression cascades, two of the most commonly seen network motifs in genetic networks. It is found that resource limitations can completely alter the steady state I/O response of an activation cascade in Section 3.1. In Section 3.2, the bistability behavior of repression cascades due to resource limitations is studied. In Chapter 4, the limitations of the current model, as well as directions for future research are discussed.
Chapter 2

A Generalized Model for Resource Limitation Problems in Gene Networks

2.1 Development of the Generalized Model

2.1.1 Gene Expression in a Transcriptional Component

In this section, we model the chemical reactions within a node of the gene transcription network. We consider a transcriptional component as a node in the gene network [20]. A transcriptional component takes a number of TFs to bind with its gene promoter \( p_i \) and triggers a series of chemical reactions to produce a TF \( x_i \) as output. The input TFs can either activate or repress the expression of gene \( i \) by changing the binding strength of \( p_i \) with RNAP. Since most gene promoters take at most two input TFs [1][14], we consider a node \( i \) taking two input TFs (\( x_1 \) and \( x_2 \)) that form complexes with \( p_i \). The reactions are:

\[
\begin{align*}
    p_i + n_1 \cdot x_1 & \xrightleftharpoons[k_i^{-1,1}]{k_i^{1,1}} c_i^1, \\
    c_i^1 + n_2 \cdot x_2 & \xrightleftharpoons[k_i^{-1,2}]{k_i^{1,2}} c_i^{12}, \\
    p_i + n_2 \cdot x_2 & \xrightleftharpoons[k_i^{-1,2}]{k_i^{2,2}} c_i^2, \\
    c_i^2 + n_1 \cdot x_1 & \xrightleftharpoons[k_i^{-1,21}]{k_i^{1,21}} c_i^{12},
\end{align*}
\]
where \( n_1 \) and \( n_2 \) are the cooperativities of \( x_1 \) and \( x_2 \) binding with \( p_i \), respectively. The promoter \( p_i \) and the promoter/TF complexes \( (c_{i1}, c_{i2}, c_{i12}) \) recruit free RNAP \( (y) \) to form an open complex for transcription. The reactions are given by:

\[
\begin{align*}
 p_i + y & \xrightleftharpoons{d_i'} a_i' C_i, \\
 c_{i1}^2 + y & \xrightleftharpoons{d_{i1}^2} a_{i1}^2 C_i^2, \\
 c_{i2}^1 + y & \xrightleftharpoons{d_{i2}^1} a_{i2}^1 C_i^1, \\
 c_{i12}^1 + y & \xrightleftharpoons{d_{i12}^1} a_{i12}^1 C_i^{12}.
\end{align*}
\]

These transcriptionally active complexes can then be transcribed into mRNA \( (m_i) \), with reactions given by:

\[
\begin{align*}
 C_i & \xrightarrow{\alpha_i^0} p_i + y + m_i, \\
 C_{i1}^2 & \xrightarrow{\alpha_{i1}^2} c_{i1}^2 + y + m_i, \\
 C_{i1}^1 & \xrightarrow{\alpha_{i1}^1} c_{i1}^1 + y + m_i, \\
 C_{i12}^1 & \xrightarrow{\alpha_{i12}^1} c_{i12}^1 + y + m_i.
\end{align*}
\]

Translation is initiated by ribosomes \( (z) \) binding with the ribosome binding site (RBS) on mRNA \( m_i \) to form a translationally active complex \( M_i \), which is then translated into protein \( x_i \). Meanwhile, mRNA and proteins are also diluted/degraded. The reactions are:

\[
\begin{align*}
 m_i + z & \xrightleftharpoons{\kappa_i^-} \kappa_i^+ M_i, \\
 M_i & \xrightarrow{\theta_i} m_i + z + x_i, \\
 m_i & \xrightarrow{\delta_i} \emptyset, \\
 M_i & \xrightarrow{\omega_i} z, \\
 x_i & \xrightarrow{\gamma_i} \emptyset.
\end{align*}
\]
Consequently, the concentration of each species \(\text{(italic)}\) in node \(i\) follows the following ODEs:

\[
\frac{d}{dt} c_i^j = k_{i,j}^+ p_i x_j^n - k_{i,j}^- c_i^j - a_i^j y c_i^j + d_i^j C_i^j + \alpha_i^j C_i^j, \tag{2.1}
\]

\[
\frac{d}{dt} c_i^{12} = k_{i,12}^+ c_i^1 x_2^n - k_{i,12}^- c_i^{12} + k_{i,21}^+ c_i^2 x_1^n - k_{i,21}^- c_i^{12} - a_{i,12}^{12} c_i^{12} y - d_{12}^i C_i^{12} + \alpha_i^{12} C_i^{12}, \tag{2.2}
\]

\[
\frac{d}{dt} C_i = a_i^0 p_i y - d_i^0 C_i - \alpha_i^0 C_i, \tag{2.3}
\]

\[
\frac{d}{dt} C_i^k = a_i^k y c_i^k - d_i^k C_i^k - \alpha_i^k C_i^k, \tag{2.4}
\]

\[
\frac{d}{dt} m_i = \alpha_i^0 C_i + \alpha_i^1 C_i^1 + \alpha_i^2 C_i^2 + \alpha_i^{12} C_i^{12} - \delta_i m_i - \kappa_i^+ m_i z + \kappa_i^- M_i + \theta_i M_i, \tag{2.5}
\]

\[
\frac{d}{dt} M_i = \kappa_i^+ m_i z - \kappa_i^- M_i - \theta_i M_i - \omega_i M_i, \tag{2.6}
\]

\[
\frac{d}{dt} x_i = \theta_i M_i - \gamma_i x_i, \tag{2.7}
\]

where indices \(j = 1, 2\) and \(k = 1, 2, 12\). Since DNA concentration is conserved \([1]\), we have

\[
p_{i,T} = p_i + C_i + \sum_{j=1,2,12} (c_i^j + C_i^j), \tag{2.8}
\]

where \(p_{i,T}\) is the total concentration of gene \(i\). Biochemical reactions described by the above ODEs are characterized by a strong time-scale separation property. TF, RNAP and ribosome binding reactions, described by equation (2.1) to (2.4), and equation (2.6), reaches equilibrium within a time-scale of about 1 seconds. mRNA dynamics described by equation (2.5) has a time-scale of about 5 mins. While protein dynamics in (2.7) has a time-scale of about an hour \([1]\). Since we are interested in the dynamics of protein production in the gene transcription network, we can set (2.1) to (2.6) to quasi-steady state (QSS) to simplify our analysis. We first obtain the QSS
concentration of complexes formed with \( p_i \):

\[
c_i^1 = \frac{p_i x_1^{n_1}}{k_i^1}, \quad c_i^2 = \frac{p_i x_2^{n_2}}{k_i^2}, \quad c_i^{12} = \frac{p_i x_1^{n_1} x_2^{n_2}}{k_i^1 k_i^{12}} + \frac{p_i x_1^{n_1} x_2^{n_2}}{k_i^2 k_i^{21}},
\]

(2.9)

\[
C_i = \frac{p_i y}{K_i'}, \quad C_i^j = \frac{c_i^j y}{K_i^j} \quad (j = 1, 2, 12),
\]

(2.10)

where dissociation constants are defined as:

\[
K_i' = \frac{d_i' + \alpha_i^0}{\alpha_i'}, \quad K_i^j = \frac{d_i^j + \alpha_i^j}{\alpha_i^j}, \quad k_i^j = \frac{k_{i,j}^-}{k_{i,j}^+} \quad (j = 1, 2, 12).
\]

Here, \( K_i' \) is the basal dissociation constant of promoter \( p_i \) with RNAP \( y \), \( K_i^j \) is the dissociation constant of promoter/TF complex \( c_i^j \) with \( y \), and \( k_i^j \) is the dissociation constant of TF \( x_j \) binding with \( p_i \). A smaller dissociation constant indicates stronger binding. When node \( i \) takes only one input, for simplicity, we write \( K_i \) for \( K_i^1 \) and \( k_i \) for \( k_i^1 \). To obtain the QSS concentration of mRNA complexes, we further assume that the transcription rates are independent of how transcriptions are initiated and thus \( \alpha_0 = \alpha_1 = \cdots = \alpha_i \). We can then substitute (2.10) into the QSS of ODEs (2.5) and (2.6) and obtain

\[
M_i = \frac{\alpha_i}{\delta_i} \frac{z}{\kappa_i} (C_i + \sum_j C_i^j) = \frac{\alpha_i p_i T}{\delta_i} \frac{z}{\kappa_i} \frac{y}{K_i'} F_i(\mathbf{u}_i),
\]

(2.11)

where vector \( \mathbf{u}_i = [x_1, x_2]^T \) and index \( j = 1, 2, 12 \). \( \kappa_i = (\kappa_i^- + \theta_i + \omega_i)/\kappa_i^+ \) is the dissociation constant of \( m_i \) binding with ribosomes \( z \). A smaller \( \kappa_i \) indicates stronger RBS strength. \( F_i(\mathbf{u}_i) : R^2 \mapsto R \) is the Hill function derived by substituting (2.10) into the DNA conservation law in (2.8) and solving for \( C_i + C_i^1 + C_i^2 + C_i^{12} \). Assuming that the free amount of RNAP and ribosomes are limited, in particular,

\[
y \ll K_i, K_i' \quad \text{and} \quad z \ll \kappa_i,
\]

(2.12)
$F_i(u_i)$ can be written as:

$$F_i(u_i) = \frac{1 + a_i^1 x_i^{n_1} + a_i^2 x_i^{n_2} + a_i^3 x_i^{n_1} x_i^{n_2}}{1 + b_i^1 x_i^{n_1} + b_i^2 x_i^{n_2} + b_i^3 x_i^{n_1} x_i^{n_2}}.$$  \quad (2.13)

where

$$a_i^1 = \frac{K_i'}{K_i^1 k_i^1}, \quad a_i^2 = \frac{K_i'}{K_i^2 k_i^2}, \quad a_i^3 = \frac{K_i'}{K_i^{12}} \left( \frac{1}{k_i^{11} k_i^{12}} + \frac{1}{k_i^{22}} \right),$$

$$b_i^1 = \frac{1}{k_i^1}, \quad b_i^2 = \frac{1}{k_i^2}, \quad b_i^3 = \frac{1}{k_i^{11} k_i^{12}} + \frac{1}{k_i^{22} k_i^{21}}.$$ \quad (2.14)

Situations in (2.12), where resources are limited, are described in detail in Section 2.2. Finally, we combine equation (2.11) and (2.7) to obtain the dynamics of $x_i$:

$$\dot{x}_i = \frac{\alpha_i \theta_i p_{i,T}}{\delta_i} \cdot \frac{y}{K_i'} \cdot \frac{z}{\kappa_i} \cdot F_i(u_i) - \gamma_i \cdot x_i.$$ \quad (2.15)

Since $y$ and $z$ are shared among all nodes in the network, their free concentrations need to be determined from the network context. This will be discussed in the next section.

### 2.1.2 Resource Sharing in Gene Networks

A gene network $\mathcal{N}$ is composed of $N$ nodes and $M$ external TF inputs $(v_1, \cdots, v_M)$. The concentration of the external inputs can be represented by $v = [v_1, \cdots, v_M]^T$ and the state of the network is represented by the concentrations of output proteins of each node $x = [x_1, \cdots, x_N]^T$. The set of all TFs in the network is $\mathcal{X} = \{x_1, \cdots, x_N, v_1, \cdots, v_M\}$, and we use $\xi = [x^T, v^T]^T$ to represent the vector of their concentrations. Nodes can be connected by transcriptional regulation interactions where protein $x_j$ can either activate or repress the production of $x_i$ by binding to its promoter. We call $x_i$ as a target of $x_j$ and $x_j$ as a parent of $x_i$. We denote by $\mathcal{U}_i \subseteq \mathcal{X}$ the set of all parents of $x_i$. Their concentrations are given by a vector $u_i = Q_i \cdot \xi$. 


where elements in $Q_i$ are defined as:

$$q_{jk} = \begin{cases} 
1, & \text{if } \xi_k \text{ is the } j\text{th input to node } i, \\
0, & \text{otherwise}. 
\end{cases} \quad (2.16)$$

Fig. 2-1 illustrates an example gene network. To determine the effect of RNAP and ribosome limitations on the gene network, we account for the fact that the total amount of resources available to network $\mathcal{N}$ is constant [6]:

$$y_T = y + \sum_{i=1}^{N} y_i, \quad z_T = z + \sum_{i=1}^{N} z_i, \quad (2.17)$$

where $y_T$ and $z_T$ represent the total amount of RNAP and ribosomes, respectively.

We let $y_i$ and $z_i$ denote the RNAP and ribosomes bound to (used by) node $i$, thus $y_i = C_i + C_i^{11} + C_i^2 + C_i^{12}$, and $z_i = M_i$. According to (2.11), we have:

$$y_i = p_{i,T} \frac{y}{K_i} F_i(u_i), \quad z_i = \frac{\alpha_i p_{i,T} y}{\delta_i K_i} \frac{z}{\kappa_i} F_i(u_i). \quad (2.18)$$
Combining equation (2.17) and (2.18), we obtain:

\[
y = \frac{y_T}{1 + \sum_{i=1}^{N} \left[ \frac{p_i}{K_i} F_i(u_i) \right]}, \quad z = \frac{z_T}{1 + \sum_{i=1}^{N} \left[ \frac{\alpha_i p_i}{K_i} \delta_i F_i(u_i) \right]}.
\]

Hence,

\[
y \cdot z = \frac{y_T \cdot z_T}{1 + \sum_{i=1}^{N} \frac{p_i}{K_i} \cdot \left( 1 + \frac{\alpha_i}{\kappa_i \delta_i} y_T \right) \cdot F_i(u_i)}.
\] (2.19)

Substituting (2.19) into (2.15), the dynamics of \( x_i \) are given by:

\[
\dot{x}_i = \frac{T_i F_i(u_i)}{1 + \sum_{k=1}^{N} J_k F_k(u_k)} - \gamma_i x_i, \tag{2.20}
\]

where \( J_i \) and \( T_i \) are lumped parameters defined as:

\[
J_i := \frac{p_i}{K_i} \cdot \left( 1 + \frac{\alpha_i}{\kappa_i \delta_i} y_T \right), \quad T_i := y_T z_T p_i \cdot \frac{\theta_i \alpha_i}{K_i^2 \kappa_i \delta_i}. \tag{2.21}
\]

\( F_i(u_i) \) is the only element in equation (2.20) that reflects transcriptional regulations on node \( i \). According to equation (2.13), the form of \( F_i(u_i) \) is the same as those of the standard Hill functions described in [14] and [1]. Note that \( F_i(u_i) \equiv 1 \) when \( u_i = 0 \), hence, according to equation (2.15), \( T_i \) represents the “baseline” gene expression of node \( i \), because \( T_i \) quantifies production rate of \( x_i \) when \( u_i = 0 \), \( y = y_T \) and \( z = z_T \).

## 2.2 Modeling Assumptions

In this section, we recall several major assumptions we have made in order to derive the reduced model in equation (2.20), and evaluate them in detail.

1. Chemical reactions in the cell can be modeled using reaction rate equations. Modeling chemical reactions as reaction rate equations largely increases the tractability of system behavior. Moreover, reaction rate equations have been
proven to be a valid representation of chemical reactions in E. coli in most cases [1]. Although this is a widely used assumption, it is necessary to bear in mind that chemical reactions are inherently random, and ODEs can only represent the behavior of the system when the number of molecules of each species is large enough [14]. When molecular count is low, the reactions are often modeled as discrete state Markovian processes, and deterministic ODEs often fails to capture salient system behaviors [31].

2. The binding and unbinding reactions and mRNA dynamics are sufficiently fast than the protein dynamics, and can be set to quasi-steady-state ($d_i' \gg \delta_i \gg \gamma_i$). In bacteria E. coli, the dynamics of mRNA degradation/dilution is on the scale of several minutes [43], while the dynamics of protein degradation/dilution ranges from 40 mins to several hours [3]. Binding and unbinding happens much faster, usually in seconds or milliseconds [1]. Furthermore, we are interested in the dynamics of protein concentration, which is on the slowest time scale. Therefore, it is reasonable to assume that binding reactions and mRNA levels have reached quasi-steady-states.

3. The total amount of RNAP and ribosomes available for the transcription networks of interest is constant: $y_T \equiv \text{constant}$ and $z_T \equiv \text{constant}$. Although ideally, a model including the production of RNAP and ribosomes by the endogenous circuit in cells is most favorable to fully understand the resource sharing mechanism, how house-keeping genes in cells control the total amount of transcriptional and translational resources is still largely unknown. However, researches have shown that the total amount of RNAP and ribosomes in bacteria E. coli is mainly dependent on cell growth rate. At a given growth rate, the total amount of RNAP and ribosomes is approximately constant [6] [35]. For our current interest, growth rate can be controlled using a chemostat in experiments.

4. The amount of free RNAP and ribosomes is very limited, such that their concentrations are much smaller than their corresponding dissociation constants
(\(y \ll K_i\) and \(z \ll \kappa_i\)). The dissociation constant of T7 RNAP binding with promoter is \(K = 220[\text{nM}]\) [50]. T7 RNAP has stronger binding with promoters than other RNAP species [48], therefore, \(K \gg 220[\text{nM}]\). Furthermore, since \(y < y_T \approx 100[\text{nM}]\) [6], we can assume \(y \ll K\). Physically, this corresponds to the fact that promoters are rarely occupied by RNAP, which is common in experiments. For instance, Chrchward et al. find that DNA template is in excess of free RNAP in constitutively expressing \(lac\) genes [11]. The free amount of ribosome in \(E.\ coli\) is estimated to be \(z < z_T \approx 1000[\text{nM}]\) [6] at low growth rate of 1 doubling/hr, and a typical value of RBS dissociation constant is \(\kappa \approx 5000[\text{nM}]\) [28], which suggests \(z \ll \kappa\). These assumptions are closer to reality when the network is larger in scale, and thus resources become more scarce.

2.3 A Measure of Resource Usage by each Gene Transcription Component

The lumped parameter \(J_i\) is a constant for node \(i\) that defines its “baseline” resource usage when \(u_i = 0\). We take \(J_i\) as a measure of resource usage by node \(i\) because the expression in equation (2.19) implies the “conservation law” for \(y \cdot z\):

\[
y_T \cdot z_T = \left(\frac{y \cdot z}{\text{free resources}}\right) + \sum_{i=1}^{N} \left(J_i \cdot F_i(u_i) \cdot y \cdot z\right) \cdot (2.22)
\]

Furthermore, the only difference between our modified model in equation (2.20) and the standard no-resource-sharing model in [14] and [1] is the denominator term \(D = 1 + \sum_{k=1}^{N} J_k F_k(u_k)\). The following claim shows that when resources used by every node in \(\mathcal{N}\) are negligible, the resource usage measure \(J_i \ll 1\).

**Claim 1.** For every \(u_i\), if \(y_i \ll y\) and \(z_i \ll z\) for all \(i = 1, \ldots, N\), then \(J_i \ll 1\) for all \(i = 1, \ldots, N\).

**Proof.** Using equation (2.18), \(y_i \ll y\) for every \(u_i\) is equivalent to \(p_{i,T} F_i(u_i)/K'_{i} \ll 1\) for every \(u_i\). Thus, we must have \(p_{i,T}/K'_{i} \ll 1\). Similarly, \(z_i \ll z\) for every \(u_i\) requires
\( \frac{\alpha_i p_{i,T} y_{i}}{\delta_i K''_i \kappa_i} \ll 1 \). Since \( y_i \ll y \) for all \( i \), \( y \approx y_T \). Therefore, \( \frac{\alpha_i p_{i,T} y_{i}}{\delta_i K''_i \kappa_i} \ll 1 \) and \( J_i \ll 1 \) for all \( i \).

This claim shows that when resource usage is negligible in the network, \( 0 < J_i \ll 1 \) (\( i = 1, \cdots, N \)) and the modified model reduces back to the standard model in [14] and [1]:

\[
\dot{x}_i = T_i F_i(u_i) - \gamma_i x_i,
\]

which has the same form as equation (1.2).

Equation (2.21) indicates that a node \( i \) is a strong resource sink when \( u_i = 0 \) if its (i) copy number is large; (ii) basal RNAP sequestering capability is strong (small \( K''_i \)); (iii) transcription rate constant is large; (iv) ribosome sequestering capability is strong (small \( \kappa_i \)); (v) mRNA degradation rate is low and (vi) the total amount of RNAP is large. Conditions (i) and (ii) are associated with the \( p_{i,T}/K''_i \) term in equation (2.21), and describe the node’s capability to sequester RNAP. Conditions (iii) to (vi) are the contributions from the \( \frac{\alpha_i y_T}{\kappa_i \delta_i} \) term and characterize the node’s capability to sequester free ribosomes.

### 2.4 Effective Interactions Arising From Resource Limitations

Directed edges, such as those in Fig. 2-1, have been used to represent transcriptional regulation interactions, where one TF binds with the promoters of its targets to regulate the target’s production [1]. Here, we mathematically define the standard to draw interaction graphs and illustrate that resource limitations lead to effective interactions in gene networks that do not rely on TF regulation.

**Definition 1:** Let the dynamics of \( x_i \) be given by \( \dot{x}_i = G_i(\xi) - \gamma_i \cdot x_i \). We draw the interaction graph from TF \( \xi_j \) to \( x_i \) based on the following rules:

- If \( \frac{dG_i}{d\xi_j} \equiv 0 \) for all \( \xi_j \in R^+ \), then there is no interaction from \( \xi_j \) to \( x_i \);
• If $\frac{\partial G_i}{\partial \xi_j} \geq 0$ for all $\xi_j \in R^+$ and $\frac{\partial G_i}{\partial \xi_j} \neq 0$ for some $\xi_j$, then $\xi_j$ activates $x_i$ and we draw $\xi_j \rightarrow x_i$;

• If $\frac{\partial G_i}{\partial \xi_j} \leq 0$ for all $\xi_j \in R^+$ and $\frac{\partial G_i}{\partial \xi_j} \neq 0$ for some $\xi_j$, then $\xi_j$ represses $x_i$ and we draw $\xi_j \dashv x_i$.

• If $\frac{\partial G_i}{\partial \xi_j} < 0$ for some $\xi_j \in R^+$ and $\frac{\partial G_i}{\partial \xi_j} < 0$ for some other $\xi_j$, then the regulation of $\xi_j$ on $x_i$ is undetermined and we draw $\xi_j \xrightarrow{} x_i$.

Based on Definition 1, for the standard model in equation (2.23), $G_i(\xi) = T_i F_i(\xi) = T_i F_i(u_i)$, and therefore there is a link from $\xi_j$ to $x_i$ if and only if $\xi_j \in U_i$. In our modified model in equation (2.20), instead we have

$$G_i(\xi) = \frac{T_i F_i(Q_i \cdot \xi)}{1 + \sum_{k=1}^{N} J_k F_k(Q_k \cdot \xi)} = \frac{T_i F_i(u_i)}{1 + \sum_{k=1}^{N} J_k F_k(u_k)},$$

which implies that the dynamics of $x_i$ may be influenced by TFs that do not belong to its parents $U_i$.

In what follows, we discuss the effective interactions from $\xi_j \in \chi$ to protein $x_i$ when (i) $x_i$ is the only target of $\xi_j$, (ii) $x_i$ is one of the multiple targets of $\xi_j$, and (iii) $x_i$ is not a target of $\xi_j$. We do not require $x_i \neq \xi_j$ and assume that a TF cannot be both an activator and a repressor. When $x_i$ is the only target of $\xi_j$, the following claim shows that resource limitations do not alter the activation/repression of $x_i$ by $\xi_j$ in the interaction graph.

**Claim 2.** If $\xi_j \in U_i$ and $\xi_j \notin U_q$ for all ($q \neq i$). Then we have $\text{sign}[\partial G_i(\xi)/\partial \xi_j] = \text{sign}[\partial F_i(Q_i \xi)/\partial \xi_j]$.

**Proof.** According to equation (2.20),

$$\frac{\partial G_i(\xi)}{\partial \xi_j} = \frac{\partial G_i}{\partial F_i} \cdot \frac{\partial F_i(Q_i \xi)}{\partial \xi_j} \Rightarrow \text{sign} \left( \frac{\partial G_i}{\partial \xi_j} \right) = \text{sign} \left( \frac{\partial F_i}{\partial \xi_j} \right).$$

$\square$

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Figure 2-2: When the input node $u$ has two targets $x_1$ and $x_2$, and $u$ represses both of its targets, the effective interactions between $u$ and $x_1$ and $x_2$ is undetermined. The effective interaction can be repression for some range of $u$, and activation for some other values of $u$. Simulation parameters are in Table A.1 and A.2.

**Remark 1:** In the case where $\xi_j \in U_1, \cdots, U_k$ ($k \geq 2$), the effective interactions from $\xi_j$ to its targets are undetermined. For example, if $\xi_j$ represses $x_1$ and $x_2$ simultaneously, the effective interaction from $\xi_j$ to $x_1$ is given by

$$\frac{\partial G_1(\xi)}{\partial \xi_j} = \frac{\partial G_1}{\partial F_1}_{positive} \cdot \frac{\partial F_1(Q_1\xi)}{\partial \xi_j}_{negative} + \frac{\partial G_1}{\partial F_2}_{negative} \cdot \frac{\partial F_2(Q_2\xi)}{\partial \xi_j}_{negative}.$$ 

As $\text{sign}(\partial G_1/\partial \xi_j)$ cannot be determined, the effective interaction from $\xi_j$ to $x_1$ is undetermined. Simulation results for this case is shown in Fig. 2-2.

When $\xi_j$ is not a parent of $x_i$, the following claim shows $\xi_j$ is an effective repressor for $x_i$ if $\xi_j$ is an activator. Conversely, $\xi_j$ is an effective activator for $x_i$ if it is a repressor.

**Claim 3.** If $\xi_j \notin U_i$ but $\xi_j \in U_k$ for some $k \neq i$, then we have $\text{sign}[\partial G_i(\xi)/\partial \xi_j] = -\text{sign}[\partial F_k(Q_k\xi)/\partial \xi_j]$. 

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Proof. Since \( \xi_j \notin U_i \), \( \partial G_i / \partial F_k < 0 \) for all \( k \).

\[
\frac{\partial G_i(\xi)}{\partial \xi_j} = \sum_k \frac{\partial G_i}{\partial F_k} \text{ negative} \cdot \frac{\partial F_k(Q_k \xi)}{\partial \xi_j}.
\]

Therefore, \( \text{sign}(\partial G_i / \partial \xi_j) = -\text{sign}(\partial F_k / \partial \xi_j) \).

The effective interactions for the above three cases are summarized in Table 2.1, with illustrative examples given in each case. For any index \( i, j \in \{1, \ldots, N\} \), a black solid line from node \( j \) to node \( i \) represents \( \partial F_i(Q_i \xi) / \partial \xi_j \), the interaction due to transcriptional regulation, while a red dashed line represents any hidden (additional) interactions arising from \( \partial G_i(\xi) / \partial \xi_j \).

### 2.5 A Conservation Property

In this section, we look into a special structure of the gene networks that give rise to a “conservation law” for the steady state expression of all nodes in the network. Such a conservation law is termed “isocost line” in [19] [21] for a benchmark resource competition problem shown in Fig. 2-3.

Using the reduced model in equation (2.20), we re-produce the result in [19], and generalize it to all gene transcription networks with at least one constitutive node.
Figure 2-3: Diagram of the benchmark resource limitation model considered in [19] [21], and the isocost line proposed. The steady state expression of the inducible node $x_1$ and the constitutive node $x_2$ falls on a straight line with negative slope.

This is shown by Claim 4 below.

**Claim 4.** Consider the steady-state of a transcription regulation network composed of $N$ nodes $x_1, x_2 \cdots x_N$, where node $x_k$ ($1 \leq k \leq N$) is expressed constitutively. Then the steady state expression of all nodes in the networks follows

$$\frac{(1 + J_k) \gamma_k}{T_k} \bar{x}_k + \sum_{i \neq k} \frac{J_i \gamma_i}{T_i} \bar{x}_i = 1,$$

where $T_i$ and $J_i$ are lumped constants defined in equation (2.21), and $\gamma_i$ is the protein dilution/degradation rate of node $i$.

**Proof.** From equation (2.20), the steady state expression of a node $x_i$ in the network can be written as

$$\bar{x}_i = \frac{T_i F_i(u_i)}{\gamma_i \left[1 + \sum_{q=1}^{N} J_q F_q(u_q)\right]}.$$  

Since node $x_k$ is constitutive, we have $F_k(u_k) \equiv 1$. Therefore,

$$\bar{x}_k = \frac{T_k}{\gamma_k \left[1 + J_k + \sum_{q \neq k} J_q F_q(u_q)\right]}.$$
and
\[
\frac{(1 + J_k)\gamma_k}{T_k} \bar{x}_k = \frac{1 + J_k}{1 + J_k + \sum_{q \neq k} J_q F_q(u_q)}.
\] (2.26)

Similarly, for \( i \neq q \), we have
\[
\frac{J_i \gamma_i}{T_i} \bar{x}_i = \frac{1 + J_i F_i(u_i)}{1 + J_k + \sum_{q \neq k} J_q F_q(u_q)}.
\] (2.27)

Combine equation (2.26) and (2.27), we have
\[
\frac{(1 + J_k)\gamma_k}{T_k} \bar{x}_k + \sum_{i \neq k} \frac{J_i \gamma_i}{T_i} \bar{x}_i = 1.
\]

\[\square\]

The conservation equation in (2.24) is applicable to any network with a constitutive node, and satisfying the basic assumptions in Section 2.2. It can be used to study the steady state expression level and relative sensitively of various nodes in the network with resource limitations. A simulation is performed for a system comprised of a two-stage activation cascade \( u \rightarrow x_1 \rightarrow x_2 \) and a constitutive node \( x_3 \) (Fig. 2-4). According to Claim 4, the steady state expression of the three genes obey the following conservation law
\[
\frac{(1 + J_3)\gamma_3}{T_3} \bar{x}_3 + \frac{J_1 \gamma_1}{T_1} \bar{x}_1 + \frac{J_2 \gamma_2}{T_2} \bar{x}_2 = 1.
\] (2.28)
Figure 2-4: (A): Network simulated is composed of a two-stage activation cascade and a constitutive node. (B): Steady state expression of $x_1$, $x_2$ and $x_3$. (C): Simulation of the full model falls onto the isocost plane predicted by equation (2.28). Simulation parameters can be found in Table A.1 and A.2.
Chapter 3

Applications to Activation and Repression Cascades

In this section, we investigate how resource limitations alter the dynamics of activation and repression cascades. For each case, we will first write the full model before directly applying the reduced model in equation (2.20), and the corresponding effective interaction graphs. The full models will be used for simulation, and the reduced models are used for analytical analysis. We first revisit the activator cascade in the motivating example in Section 1.2.

3.1 Two-stage Activation Cascade

3.1.1 ODE models and Interaction Graphs

Full Model

In the activation cascade shown in Fig. 1-1, $u$ is the input TF that activates $x_1$ with cooperativity $n$, and $x_2$ serves as an activator for the output reporter node $x_2$ with cooperativity $m$. The transcriptional regulations in the cascade is shown in Fig. 3-1A. Using the results in equation (2.1) to (2.7), a full model of the activation cascade is composed of the following ODEs:
Stage 1:

\[
\frac{d c_1}{dt} = k_{1,1} p_1 u^n - k_{1,1} c_1 - a_1^1 y c_1 + d_1^1 C_1^1 + \alpha_1^1 C_1^1, \tag{3.1}
\]

\[
\frac{d C_1}{dt} = a_1^1 p_1 y - d_1^1 C_1 - \alpha_1^0 C_1, \tag{3.2}
\]

\[
\frac{d C_1^1}{dt} = a_1^1 y c_1^1 - d_1^1 C_1^1 - \alpha_1^1 C_1^1, \tag{3.3}
\]

\[
\frac{d m_1}{dt} = \alpha_1^0 C_1 + \alpha_1^1 C_1^1 - \delta_1 m_1 - \kappa_1^+ m_1 z + \kappa_1^- M_1 + \theta_1 M_1, \tag{3.4}
\]

\[
\frac{d M_1}{dt} = \kappa_1^+ m_1 z - \kappa_1^- M_1 - \theta_1 M_1 - \omega_1 M_1, \tag{3.5}
\]

\[
\frac{d x_1}{dt} = \theta_1 M_1 - \gamma_1 x_1, \tag{3.6}
\]

Stage 2:

\[
\frac{d c_2^1}{dt} = k_{2,1}^+ p_2 x_2^m - k_{2,1}^- c_2^1 - a_2^1 y c_2^1 + d_2^1 C_2^1 + \alpha_2^1 C_2^1, \tag{3.7}
\]

\[
\frac{d C_2}{dt} = a_2^1 p_2 y - d_2^1 C_2 - \alpha_2^0 C_2, \tag{3.8}
\]

\[
\frac{d C_2^1}{dt} = a_2^1 y c_2^1 - d_2^1 C_2^1 - \alpha_2^1 C_2^1, \tag{3.9}
\]

\[
\frac{d m_2}{dt} = \alpha_2^0 C_2 + \alpha_2^1 C_2^1 - \delta_2 m_2 - \kappa_2^+ m_2 z + \kappa_2^- M_2 + \theta_2 M_2, \tag{3.10}
\]

\[
\frac{d M_2}{dt} = \kappa_2^+ m_2 z - \kappa_2^- M_2 - \theta_2 M_2 - \omega_2 M_2, \tag{3.11}
\]

\[
\frac{d x_2}{dt} = \theta_2 M_2 - \gamma_2 x_2, \tag{3.12}
\]

and the following conservation laws for DNA concentration, total RNAP and ribosomes:

\[
p_{1,T} = p_1 + C_1 + c_1^1 + C_1^1, \tag{3.13}
\]

\[
p_{2,T} = p_2 + C_2 + c_2^1 + C_2^1, \tag{3.14}
\]

\[
y_T = y + C_1 + C_1^1 + C_2 + C_2^1, \tag{3.15}
\]

\[
Z_t = z + M_1 + M_2. \tag{3.16}
\]
ODEs (3.1) to (3.12) and equations (3.13) to (3.16) constitute the full model of the two stage activator cascade with resource limitations, and are used in Section 1.2 and the sequel for simulations.

**Reduced Model**

If we assume that all the assumptions in Section 2.2 are satisfied, the full model can be reduced to the following reduced model

\[
\dot{x}_1 = \frac{T_1 F_1(u)}{1 + J_1 F_1(u) + J_2 F_2(x_1)} - \gamma_1 x_1, \quad (3.17)
\]

\[
\dot{x}_2 = \frac{T_2 F_2(x_1)}{1 + J_1 F_1(u) + J_2 F_2(x_1)} - \gamma_2 x_2. \quad (3.18)
\]

Both nodes take a single input: \( U_1 = u \) and \( U_2 = x_1 \). Thus, equations (2.14) yield, \( a_1^1 = \frac{K_1'}{k_1}, b_1^1 = \frac{1}{k_1}, a_2^1 = \frac{K_2'}{k_2}, \) and \( b_2^1 = \frac{1}{k_2} \), with the remaining parameters in (2.14) being 0. Denote by \( n \) and \( m \) the cooperativity coefficients for \( u \) and \( x_1 \) binding with genes 1 and 2, respectively. From (2.13) we have:

\[
F_1(u) = \frac{1 + a_1^1 u^n}{1 + b_1^1 u^n}, \quad F_2(x_1) = \frac{1 + a_2^1 x_1^m}{1 + b_2^1 x_1^m}.
\]

From Claim 3, since \( u \) is an activator, there is a hidden repression from \( u \) to \( x_2 \). Similarly, there is a hidden negative auto-regulation on \( x_1 \). These hidden interactions are represented by dashed lines in Fig. 3-1B. From Claim 2, since \( u \) and \( x_1 \) both have only one target, we draw \( u \rightarrow x_1 \) and \( x_1 \rightarrow x_2 \) in Fig. 3-1B.

### 3.1.2 Incoherent Feed-Forward Loop due to Resource Limitations

The effective interaction graph of the activation cascade becomes that of an incoherent feed-forward loop (IFFL) [1]. The steady state I/O response of an IFFL can, depending on parameters, be qualitatively characterized by monotonically increasing, monotonically decreasing or biphasic functions [1] [29]. We can predict which of these
function classes the steady state I/O response of the activation cascade falls into by linearizing the model in equations (3.17) and (3.18), as shown by the following claim.

**Claim 5.** Consider a monostable nonlinear time-invariant SISO system: \( \dot{x} = f(x, u) \), \( y = g(x, u) \), where \( f(x, u) \) and \( g(x, u) \) are analytic functions with respect to their arguments. Let the linearized system at input \( \bar{u} \) and corresponding locally asymptotically stable equilibrium \( \bar{x} \) be \( \dot{\bar{x}} = A\bar{x} + B\bar{u} \), \( \bar{y} = C\bar{x} + D\bar{u} \). Let the steady state output of the nonlinear system be \( \bar{y} = g(\bar{x}, \bar{u}) \), then \( \frac{d\bar{y}}{du} \bigg|_{\bar{x}, \bar{u}} = H = -CA^{-1}B + D \).

**Proof.** In the linearized model, \( A = \frac{\partial f}{\partial x} \bigg|_{\bar{x}, \bar{u}} \), \( B = \frac{\partial f}{\partial u} \bigg|_{\bar{x}, \bar{u}} \), \( C = \frac{\partial g}{\partial x} \bigg|_{\bar{x}, \bar{u}} \) and \( D = \frac{\partial g}{\partial u} \bigg|_{\bar{x}, \bar{u}} \). Therefore,

\[
\frac{d\bar{y}}{du} \bigg|_{\bar{x}, \bar{u}} = \frac{\partial g(\bar{x}, \bar{u})}{\partial x} \frac{d\bar{x}}{du} + \frac{\partial g(\bar{x}, \bar{u})}{\partial u}. \tag{3.19}
\]

Since \( \bar{x} \) satisfies \( f(\bar{x}, \bar{u}) = 0 \), using the implicit function theorem, we have,

\[
\frac{d\bar{x}}{du} = - \left[ \frac{\partial f(\bar{x}, \bar{u})}{\partial x} \right]^{-1} \frac{\partial f(\bar{x}, \bar{u})}{\partial u} = -A^{-1}B. \tag{3.20}
\]

Matrix \( A \) is invertible because the equilibrium \( \bar{x} \) is asymptotically stable. Combining equations (3.19) and (3.20), we obtain \( \frac{d\bar{y}}{du} \bigg|_{\bar{x}, \bar{u}} = H = -CA^{-1}B + D \).

To apply Claim 5 to the two-stage activation cascade, we first linearize (3.17) and
(3.18) at input $\bar{u}$ and equilibrium $\bar{x}$ to obtain:

$$A = \begin{bmatrix} \frac{\partial G_1}{\partial x_1} - \gamma_1 & 0 \\ \frac{\partial G_2}{\partial x_1} & -\gamma_2 \end{bmatrix}, \quad B = \begin{bmatrix} \frac{\partial G_1}{\partial u} \\ \frac{\partial G_2}{\partial u} \end{bmatrix}, \quad C = \begin{bmatrix} 0 & 1 \end{bmatrix}, \quad D = 0. \quad (3.21)$$

The transfer function $H(s)$ of the linearized activation cascade is

$$H(s) = C(sI - A)^{-1}B = \begin{bmatrix} 0 & 1 \end{bmatrix} \cdot \left[ \frac{s + \gamma_y}{s - \frac{\partial G_1}{\partial x_1} + \gamma_1} \right] \cdot \begin{bmatrix} \frac{\partial G_1}{\partial u} \\ \frac{\partial G_2}{\partial u} \end{bmatrix}$$

where $I$ is the identity matrix. From equation (3.21),

$$\det(sI - A) = (s + \gamma_2)(s + \gamma_1 - \frac{\partial G_1}{\partial x_1}),$$

since

$$\frac{\partial G_1}{\partial x_1} = \frac{\partial G_1}{\partial F_2} \cdot \frac{dF_2}{dx_1} < 0,$$

and $\gamma_1, \gamma_2 > 0$, the two poles of the linearized model $H(s)$ are

$$s_1 = -\gamma_2 < 0, \quad s_2 = -\gamma_2 + \frac{\partial G_1}{\partial x_1} < 0. \quad (3.23)$$

Therefore, the linearized system $H(s)$ is always stable, and satisfies the requirement in Claim 5. The slope of the steady state response ($d\bar{x}_2/du$) can be found by

$$H(0) = \frac{\frac{\partial G_2}{\partial x_1} \cdot \frac{\partial G_2}{\partial u} + (-\frac{\partial G_1}{\partial x_1} + \gamma_1) \cdot \frac{\partial G_2}{\partial u}}{\det(-A)} \quad (3.24)$$

We use equation (3.24) to numerically find the slope of the steady state response ($d\bar{x}_2/du$) for all linearization points to obtain parameter conditions that admit different steady state responses. Fig. 3-2 shows that a decreasing steady state response occurs
Figure 3-2: Numerical simulation of $d\tilde{x}_2/du$ for different DNA copy numbers and relative ribosome binding strengths ($\kappa_2/\kappa_1$). The uncolored region indicates $d\tilde{x}_2/du > 0$ for all simulated $u$ (monotonically increasing response). The region with red dots represent parameters where $d\tilde{x}_2/du$ becomes negative at high input levels (biphasic response), and the region with blue dots represent parameters that give $d\tilde{x}_2/du < 0$ for all input levels (monotonically decreasing response). Simulation parameters are shown in Table A.1 and A.2 when (a) resources are limited (large DNA copy number) and (b) $x_1$ has stronger resource sequestering capability than $x_2$ (stronger RBS).

The numerical result in the last section is in agreement with the following analytical results providing sufficient conditions for different steady state I/O responses.

**Claim 6.** If node 1 and 2 have the same DNA copy numbers $p_{1,T} = p_{2,T} = p_T$, and transcription rate constants $\alpha_1 = \alpha_2 = \alpha$, then in a two-stage activation cascade the slope of the steady state I/O response $d\tilde{x}_2/du$ satisfies:

1. $d\tilde{x}_2/du > 0$ for all $u > 0$ if (a) $K_1 \gg p_T$ and (b) $\kappa_1 \cdot \delta_1 \gg \alpha \cdot y_T$;

2. $d\tilde{x}_2/du < 0$ for all $u > 0$ if (a) $p_T \gg K'_2 > K_2 \gg K'_1 > K_1$ and (b) $\alpha \cdot y_T \gg \delta_2 \cdot \kappa_2 \gg \delta_1 \cdot \kappa_1$;
3. \( \frac{d\bar{x}_2}{du} > 0 \) when \( u \to 0 \) and \( \frac{d\bar{x}_2}{du} < 0 \) when \( u \to \infty \) if (a) \( K'_1 \gg p_T \geq K_2 \gg K_1 \) and (b) \( \kappa_2 \cdot \delta_2 > \kappa_1 \cdot \delta_1 \gg \alpha \cdot \gamma_T \).

**Proof.** Using equation (3.18), the steady state concentration of the output \( (\bar{x}_2) \) can be written as:

\[
\bar{x}_1(u, \bar{x}_1) = \frac{1}{\gamma_1} \cdot \frac{T_1 F_1(u)}{1 + J_1 F_1(u) + J_2 F_2(\bar{x}_1)}, \tag{3.25}
\]

\[
\bar{x}_2(u, \bar{x}_1) = \frac{1}{\gamma_2} \cdot \frac{T_2 F_2(\bar{x}_1)}{1 + J_1 F_1(u) + J_2 F_2(\bar{x}_1)}. \tag{3.26}
\]

From Claim 2, \( \bar{x}_1 \) increases monotonically with \( u \). When conditions in 1) are satisfied, we have \( J_1 F_1(u) < \frac{p_T}{K'_1} (1 + \frac{\alpha \gamma_T}{\kappa_1}) \frac{K'_1}{K_1} \ll 1 \). Equation (3.26) becomes \( \bar{x}_2(u, \bar{x}_1) = \frac{1}{\gamma_2} \cdot \frac{T_2 F_2(\bar{x}_1)}{1 + J_1 F_1(u) + J_2 F_2(\bar{x}_1)} \), and therefore \( \bar{x}_2 \) increases with \( u \). When conditions in 2) are satisfied, we have \( J_1 F_1(u) \gg J_2 F_2(\bar{x}_1) \gg 1 \). Combining with (3.25), we have \( \bar{x}_1 = T_1 / J_1 = \text{constant} \), and (3.26) becomes a single variable decreasing function of \( u \). To prove 3), note that when \( u \to 0 \), \( F_1(u) \to 1 \) and when \( u \to \infty \), \( F_1(u) \to \frac{K'_1}{K_1} \). The conditions give \( J_1 F_1(u) \ll 1 \) when \( u \to 0 \), and \( J_1 F_1(u) \gg J_2 F_2(\bar{x}_2) \gg 1 \) when \( u \to \infty \). The rest of the proof follows from case 1) and 2).

### 3.1.3 Error Analysis

Here, we compare the performance of the reduced model with full model simulation. We define modeling error \( E \) as:

\[
E := \max_{u_i \in R^+} \left[ \frac{Y(u_i) - \bar{Y}(u_i)}{Y(u_i)} \right] \times 100\% \tag{3.27}
\]

where \( Y(u_i) \) is the output of the full system using equation (3.1) to (3.16), and \( \bar{Y}(u_i) \) is the steady state output of our model in equation (2.20). We calculate the modeling error for systems with different \( y_t \) and \( z_y \) and find that modeling error is most significant when \( y_t \approx K_i (i = 1, 2) \) and \( z_t \approx \kappa_i \). RNAP has a more dominant role in determine modeling error in our simulation, particularly, when \( y_t \leq 0.1 K_i \), the modeling error becomes less than 10% (Fig. 3-3).
Figure 3-3: Using the two-stage activator cascade as an example, we calculate the modeling error for systems with different RNAP and ribosome amount. Modeling error is most significant when the total amount of resources becomes as large as their dissociation constants. The error becomes very insignificant when $y_t \leq 0.1K_i$. Simulation parameters are shown in Table A.1 and A.2.
3.1.4 Discussion

Claim 6, together with the numerical results in Fig. 3-2, serves as a good guidance for experimental implementation to achieve all three possible shapes of steady state I/O responses in a two-stage activation cascade. In general, if resource competition is weak and node $x_1$ is not a strong resource sequester, then the feed-forward hidden repression loop is not strong enough to inhibit $x_2$, and the resultant cascade shows positive I/O response. Alternatively, if resource competition is fierce, for example, when circuit copy number is large, and node $x_1$ is a much stronger resource sequester than $x_2$, then the resultant cascade will show negative I/O response. This phenomenon can be explained physically as follows. When $u = 0$, due to the promoter leakage in $x_1$ and its large copy number and RBS strength, $x_2$ is not completely off. Instead, if $x_2$ is very sensitive to its activator $x_2$, it can be almost fully activated. When more input $u$ is added, more resources are needed to express $x_1$. Since $x_1$ is a much stronger resource sequester than $x_2$, a portion of the resources dedicated to $x_2$ production is reallocated to produce $x_1$. Meanwhile, the activation from $x_1$ to $x_2$ has almost been saturated. The relatively stronger repression due to resource competition can lead to negative I/O response. If the parameter conditions are such that they fill between these two extreme cases (positive and negative), the cascade can demonstrate biphasic response.

In the experiment, we can tune the relative RBS of the two stages and their plasmid copy number to obtain all three possible I/O responses. The following simulation results (Fig. 3-4) shows the various steady state I/O responses that can be achieved by this two-stage activation cascade by tuning the plasmid copy numbers $p_{1,T} = p_{2,T}$ and the relative RBS strengths $\kappa_1/\kappa_2$.

3.2 Two-stage Repression Cascade

3.2.1 ODE Models and Interaction Graphs

A two-stage repression cascade consists of two repressors: TF $u$ is the repressor for protein $x_1$, and $x_1$ is a repressor for output protein $x_2$ (Fig. 3-5A). A repressor inhibits
Figure 3-4: The two-stage activation cascade can have positive, negative or biphasic steady state I/O responses. In (A), we only the RBS strength of node \( x_2 \). In (B), we tune both \( x_2 \) RBS strength and plasmid copy number to achieve more dramatic negative response. In each case, the steady state expression of \( x_2 \) is normalized against its maximum value. Simulation parameters are listed in Table A.1 and A.2.
the production of its target by binding with its promoter region, thus inhibiting RNAP (y) recruitment. A repression cascade is expected to have a unique steady state and a monotone I/O response [1][24]. For simplicity, we assume that the repressors are not leaky such that when u or x\(_1\) are bound to the promoters of their targets, y can not bind with the promoters. We have the following ODEs and conservation laws for a full model of this two-stage repression cascade.

### Full Model

#### Stage 1:

\[
\frac{dc_1}{dt} = k_{1,1}^+ p_1 u^n - k_{1,1}^- c_1 - a_1^1 y c_1 + d_1^1 C_1 + \alpha_1^1 C_1^1, \quad (3.28)
\]

\[
\frac{dC_1}{dt} = a_1^1 p_1 y - d_1^1 C_1 - \alpha_1^0 C_1, \quad (3.29)
\]

\[
\frac{dm_1}{dt} = \alpha_1^0 C_1 - \delta_1 m_1 - \kappa_1^+ m_1 z + \kappa_1^- M_1 + \theta_1 M_1, \quad (3.30)
\]

\[
\frac{dM_1}{dt} = \kappa_1^+ m_1 z - \kappa_1^- M_1 - \theta_1 M_1 - \omega_1 M_1, \quad (3.31)
\]

\[
\frac{dx_1}{dt} = \theta_1 M_1 - \gamma_1 x_1, \quad (3.32)
\]

#### Stage 2:

\[
\frac{dc_2}{dt} = k_{2,1}^+ p_2 x_1^n - k_{2,1}^- c_2 - a_2^1 y c_2 + d_2^1 C_2 + \alpha_2^1 C_2^1, \quad (3.33)
\]

\[
\frac{dC_2}{dt} = a_2^1 p_2 y - d_2^1 C_2 - \alpha_2^0 C_2, \quad (3.34)
\]

\[
\frac{dm_2}{dt} = \alpha_2^0 C_2 + \alpha_2^1 C_2^1 - \delta_2 m_2 - \kappa_2^+ m_2 z + \kappa_2^- M_2 + \theta_2 M_2, \quad (3.35)
\]

\[
\frac{dM_2}{dt} = \kappa_2^+ m_2 z - \kappa_2^- M_2 - \theta_2 M_2 - \omega_2 M_2, \quad (3.36)
\]

\[
\frac{dx_2}{dt} = \theta_2 M_2 - \gamma_2 x_2, \quad (3.37)
\]

and the following conservation laws for DNA concentration, total RNAP and ribo-
Figure 3-5: In addition to transcriptional repressions (solid lines) in (A), resource limitations introduce two hidden activations (dashed lines) into the system (B): activation from u to x₂ and positive auto-regulation of x₁.

\[ p_{1,T} = p_1 + C_1 + c_1^l + C_1^l, \]  
\[ p_{2,T} = p_2 + C_2 + c_2^l + C_2^l, \]  
\[ y_T = y + C_1 + C_1^l + C_2 + C_2^l, \]  
\[ Z_t = z + M_1 + M_2. \]

Equations (3.28) to (3.41) will be used in the sequel to derive simulation results.

**Reduced Model**

If we assume that all the conditions in Section 2.2 are satisfied, the two-stage repression cascade can be modeled as:

\[ \dot{x}_1 = \frac{T_1 F_1(u)}{1 + J_1 F_1(u) + J_2 F_2(x_1)} - \gamma_1 x_1, \]
\[ \dot{x}_2 = \frac{T_2 F_2(x_1)}{1 + J_1 F_1(u) + J_2 F_2(x_1)} - \gamma_2 x_2. \]

Using (2.14), we have \( b_1^l = \frac{1}{k_1} \) and \( b_2^l = \frac{1}{k_2} \), with the remaining parameters being 0.

We denote \( n \) and \( m \) as the cooperativity coefficients of \( u \) and \( x_1 \) binding with \( p_1 \) and
We obtain from (2.13):
\[ F_1(u) = \frac{1}{1 + \frac{1}{k_1} u^n}, \quad F_2(x_1) = \frac{1}{1 + \frac{1}{k_2} x_1^m}. \]

Using Claim 2, we find that resource limitations do not affect \( u \upharpoonright x_1 \) and \( x_1 \upharpoonright x_2 \) in the interaction graph. From Claim 3, we find that there is a hidden activation of \( x_2 \) by \( u \) and a hidden positive auto-regulation on \( x_1 \).

### 3.2.2 Bistability Arising from Resource Limitations

Positive feedback loops like the one in Fig. 3-5B have been closely related to bistable behaviors theoretically [49], and bimodal reporter gene distributions experimentally [37]. In fact, a positive circle in system interaction graph is a necessary condition for a dynamical system to admit more than one steady-states.

In order to determine whether the repression cascade can display bistability because of this positive auto-regulation, we perform nullcline analysis. The two nullcline equations of the nonlinear system in equations (3.42) and (3.43) at equilibrium \( \bar{x} = [\bar{x}_1, \bar{x}_2]^T \) and constant input \( \bar{u} \) (and thus, constant \( F_1(\bar{u}) \)) are given by:

\[ \frac{T_1 F_1(\bar{u})}{1 + J_1 F_1(\bar{u}) + J_2 F_2(\bar{x}_1)} - \gamma_1 \bar{x}_1 = 0, \tag{3.44} \]
\[ \frac{T_2 F_2(\bar{x}_1)}{1 + J_1 F_1(\bar{u}) + J_2 F_2(\bar{x}_1)} - \gamma_2 \bar{x}_2 = 0. \tag{3.45} \]

Equation (3.44) is a single variable equation of \( \bar{x}_1 \), and equation (3.45) defines a unique \( \bar{x}_2 \) for every \( \bar{x}_1 \). Therefore, the number of equilibria of this nonlinear system is solely determined by equation (3.44) which can be re-written as:

\[ h_1(\bar{x}_1) = \frac{T_1 F_1(\bar{u})}{1 + J_1 F_1(\bar{u}) + J_2 F_2(\bar{x}_1)} = h_2(\bar{x}_1) = \gamma_1 \bar{x}_1. \tag{3.46} \]

Since \( h_1(\bar{x}_1) \) is an increasing Hill function and \( h_2(\bar{x}_1) \) is an increasing linear function, they can have either 1 or 3 intersections when the cooperativity \( m > 1 \). Particularly, when there exists \( \bar{x}^1_1 < \bar{x}^2_1 < \bar{x}^3_1 \) satisfying \( h_1(\bar{x}^k_1) = h_2(\bar{x}^k_1) \) (\( k = 1, 2, 3 \), \( \bar{x}^1_1 \) and \( \bar{x}^3_1 \) are
locally stable nodes and $x_1^2$ is a saddle point.

Resource-limitation-induced bistability can potentially explain the experimental results in [16] and [36]. Both works observed bimodal distribution of protein concentrations at the output of a repression cascade, which disappears when negative auto-regulation is added to the cascade. However, bimodal distribution can stem from a number of other sources in addition to deterministic bistability, such as transcriptional and translational bursts [27]. A brief summary of the relationship between deterministic bistable system and stochastic bimodal distribution is provided in Appendix B. Further theoretical and experimental work is required to verify the source of bimodality in these experiments.

**Negative Auto-Regulation Removes Bistability Arising from Resource Limitations**

Now we seek to obtain parameter conditions that give rise to a bistable repression cascade. To do this, we utilize the following claim showing that the nonlinear repression cascade is bistable if and only if its linearized system is unstable at some equilibrium.

**Claim 7.** For a given input $u^*$, let $x^*$ be one of the corresponding equilibria. The nonlinear system in equation (3.42) and (3.43) is bistable if and only if $-\gamma_2 + \frac{\partial G_1}{\partial x_1} \bigg|_{x^*, u^*} > 0$ for some $(x^*, u^*)$.

**Proof.** Note that $\lambda_1 = -\gamma_2 + \frac{\partial G_1}{\partial x_1} \bigg|_{x^*, u^*}$ and $\lambda_2 = -\gamma_1 < 0$ are the two eigenvalues of the linearization of nonlinear system in (3.42) and (3.43) at $(x^*, u^*)$. The linearized system is unstable if and only if $\lambda_1 > 0$.

$(\Rightarrow)$ When the nonlinear system is bistable at input $u^*$, according to our nullcline analysis, there are 3 equilibria: 2 stable nodes and a saddle point. Linearizing the system around the saddle point yields an unstable linearized system.

$(\Leftarrow)$ We let $H(x_1, u) = G_1(x_1, u) - \gamma_1 x_1$, at fixed $u = u^*$, with abuse of notation, we write $H(x_1) = H(x_1, u^*)$. $H(x_1)$ is continuously differentiable and solution to $H(x_1) = 0$ entirely determines the number of equilibria. When $\lambda_2(x_1^*, u^*) = H'(x_1^*) > 0$. 

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0, since $H(x_1^*) = 0$, by continuity, there exists $\epsilon > 0$ such that $H(x_1 - \epsilon) < 0$ and $H(x_1 + \epsilon) > 0$. Also, when $x_1 = 0$, $H(0) = G_1(0) > 0$, and when $x_1 \to \infty$, $H(x_1) \to -\infty$. According to the intermediate value theorem, there exist a $x_1^{*-}$ such that $0 < x_1^{*-} < x_1 - \epsilon$ and satisfies $H(x_1^{*-}) = 0$. Similarly, there exists a $x_1^{*+}$ such that $x_1 + \epsilon < x_1^{*+}$ and satisfies $H(x_1^{*+}) = 0$. Since there are at most three zeros to the equation $H(x_1) = 0$, $H'(x_1^{*-})$ and $H'(x_1^{*+})$ are negative, and thus they are stable. □

Remark 2: To obtain a bistable cascade, we need

$$\lambda_1 = -\gamma_2 + \frac{\partial G_1}{\partial x_1} = -\gamma_2 - \frac{T_1 J_2 F_1(u^*) \frac{\partial F_2(x_1^*)}{\partial x_1}}{[1 + J_1 F_1(x_1^*) + J_2 F_2(x_2^*)]^2} > 0. \quad (3.47)$$

Partial differentiation of $\lambda_1$ with respect to $J_2$ shows that $\lambda_1$ monotonically increases with $J_2$ when $J_2 F_2(x^*) > 1 + J_1 F_1(u^*)$. Therefore, we can observe a bistable repression cascade if we increase the resource sequestering capability of node 2 ($J_2 F_2(x^*)$) and decrease that of node 1 ($J_1 F_1(u^*)$). Physically, these conditions increase the amount of resources released by node 2 upon repression from $x_1$, which effectively “activates” the production of $x_1$, promoting the hidden positive auto-regulation. Simulation results in Fig. 3-6A using (3.46) is consistent with these analysis. Full mechanistic model simulation using ODEs and resource conservations in Section 2 confirms that this deterministic system is bistable in some parameter and input ranges (Fig. 3-6B). Conversely, from (3.47), we can remove bistability by adding a sufficiently strong negative auto-regulation to node 1 such that $\partial G_1/\partial x_1 < \gamma_2$, which ensures monostability. Note that when a two-stage repression cascade is monostable, both the transcriptional activation loop and the hidden interaction loop from $u$ to $x_2$ are positive. The only resultant steady state I/O response is thus positive.

3.2.3 Discussion

A physical explanation of the positive auto-regulation arising from resource limitations is as follows. When a repressor inhibits the expression of its downstream gene,
Figure 3-6: (A): Dots indicate parameters that admit three solutions to equation (3.46), and thus lead to bistability in some input ranges. Bistability occurs when node 2 has strong capability to sequester resources (high copy number and ribosome binding strength). (B): When simulation starts from no induction ($u_0 = 0$) and full induction ($u_0 = 1(\mu M)$), system steady state response show hysteresis. Simulation parameters for both cases are shown in Table A.1 and A.2.
a portion of the resources originally allocated to express the downstream gene is released. The newly released resources can be used to produce more repressor, creating a positive auto-regulation loop on the repressor gene. Strength of such auto-regulation loop becomes stronger if the downstream gene is a strong resource sequester, and is sensitive to change in repressor concentration. In both repression circuits in [16] and [36], the copy number of the downstream reporter gene is much larger than the upstream repressor, creating a favorable environment for resource-limitation-induced bistability to occur. From an application point of view, a monostable repression cascade always have positive steady state I/O response, and is thus a more rational design than the activation cascade. When the repression cascade is bistable, we can render it monostable by adding a negative auto-regulation to counteract the hidden positive auto-regulation.
Chapter 4

Conclusions and Future Works

Resource limitations are physical bottle-necks for both natural and synthetic gene transcription networks. From an engineering perspective, unlike electronic circuits, where transmission of a signal between two elements can be made almost independent of the rest of the circuit, transmitting information in a gene transcription network pays a price. The proper function of every element (node) in the network requires cellular resources (here, we focus on RNAP and ribosomes) for transcription and translation. Inevitably, all nodes in the network are forced to compete for a limited amount of resources, introducing hidden interactions in addition to the transcriptional regulation interactions. Such context dependence has largely impeded our capability to understand and engineer biological systems. In this thesis, a general modeling framework to describe the dynamics of gene networks in a resource-limited environment has been developed. The model reveals a hidden layer of interactions among nodes in the network, which have been largely neglected so far but will become more relevant when resources are limited and the scale of the system enlarges. The results presented in this thesis could serve as a preliminary step towards understanding the roles resource limitations play in gene transcription networks. This is especially crucial for researches in synthetic biology, as resource limitations have been shown to limit the scale and complexity of synthetic circuits we can design. Therefore, it is essential to understand their effects and then engineer the network topology or the host cell to mitigate such undesirable effects.
One important limitation of the model presented in this thesis is the over-simplification of cell metabolism by assuming constant total amount of resources and their homogeneous spatial distribution for the network of interest. A cell system has a number of additional complications. Firstly, recent evidence suggests that resources are not distributed evenly in cells [5][8][26]. In particular, a considerable amount of RNAP is trapped near the ribosomal protein production sites through chromosome supercoiling, while another significant amount of resources are dedicated to the production of membrane proteins. Future work is required to understand how does spatial distribution of resources changes our current results. Secondly, cellular resources, such as RNAP and ribosomes, are produced by house-keeping genes in the cell. The exact mechanisms for living cells to regulate resource concentrations, and respond to external disturbances are still largely unknown. It has been observed that when exogenous circuits are overly activated, living cells tend to reduce the production of ribosomal proteins and produce heat shock proteins [15][26][44][46]. A simple but insightful model of the house-keeping genes’ activity is necessary to refine our understanding of resource limitation issues without over complication of their mathematical representations. Moreover, although the key limiting factors appeared to be RNAP and ribosome [53][11], resource sharing occurs at all levels of protein production. For instance, it is well known that RNAP compete for $\sigma$-factors [12] and mRNA transcripts compete for degradation resources [47]. In future work, to what extent these additional considerations need to be factored into the model will be analyzed. While our current analysis is restricted to prokaryotic transcription and translation, the situation will be further complicated when we want to analyze resource limitations in eukaryotic cells. This is due to fact that transcription initiation requires a number of co-factors in eukaryotic cells, which are also limited in amounts, and it becomes increasingly difficult to identify the sources of resource limitations.

How to engineer the cells to avoid undesirable hidden interactions due to resource limitations is a problem that need to be addressed in the near future for synthetic biology research. In [42], the authors use RNAP extracted from phage T7 to build up a library of orthogonal sources of RNAP that completely decoupled exogenous
resource usage from the host, and achieve satisfactory cross-talk mitigation performance. Similarly, An and Chin use orthogonal ribosomes (O-ribosomes) which target specific O-mRNAs to decouple the ribosome usage of the target synthetic network from that of the endogenous house-keeping networks [2]. However, these techniques do have their limitations, as we have only a very limited number of orthogonal sources of RNAP and ribosomes. Especially when the number of synthetic genes enlarges, the current number of orthogonal resources becomes far from enough [13]. Alternatively, the theoretical analysis in [22] shows that, in the case of the benchmark network composed of a constitutive node and an inducible node, the robustness of the constitutive node to resource fluctuations is increased with negative auto-regulations. While it provides some promising insights to mitigate the problem systematically through distributed, local feedback, a generalization is required for networks with more nodes. This may also be helpful to understand the functions of negative auto-regulations, which are found in more than 40% of known TFs in E. coli [40].
Appendix A

Simulation Parameters
### Table A.1: Simulation parameters - Part 1 ($i = 1, 2$)

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Appendix B

Relationship between Bistability and Bimodality

The concepts of bistability and bimodality are often correlated and confused. Here, we give a brief review of these two concepts. Although a deterministically bistable biomolecular system is often accompanied by bimodal distribution in flow cytometry measurements, a bimodal distribution is not necessarily a result of deterministic bistability. Inversely, a deterministically bistable system can demonstrate unimodal distribution.

B.1 Basic Concepts

Biomolecular system are often measured by flow cytometry. A simplified diagram of this measurement device is shown in Fig. B-1. Due to the inherently stochastic nature of biological processes, cell-to-cell variability is inevitable even in very carefully designed experiments. Randomness in gene expression has many sources, including transcriptional and translational bursting [27] and genetic mutations. Therefore, experimental results are often in the form of a distribution of florescence level vs. cell counts.

From a modeling perspective, in fact, the core of many reactions that take place in cells are stochastic, and is most accurately captured by a discrete state, continuous
Figure B-1: A simplified diagram of the flow cytometry device used to measure fluorescence.
time Markov process. The corresponding models are called Chemical Master Equations (CME). When the cell volume and molecular count are large enough such that system states evolves in an approximately smooth fashion, the CME can be replaced by a more tractable stochastic differential equation (SDE), the Chemical Langevin Equation (CLE), which describes a continuous state, continuous time Markov process. While CLE describes system states’ time history, Fokker-Planck Equation (FPE) describes the evolution of system states’ probability distributions. The Reaction Rate Equations that we uses in the earlier chapters are only applicable when the protein counts are large enough, and noise effects become negligible. Relationship between different modeling techniques is shown in Fig. B-2.

To obtain some insight into the relationship between bistability and bimodality. We will focus on chemical reactions that can be described by a 1-D CLE (and thus FPE), and study the relationship between the distribution obtained by the FPE and the deterministic solution obtained from its reaction rate equation. Analytical solutions of CMEs and higher order CLEs (FPEs) become highly intractable, and as we will see, the 1-D case is sufficient for us to differentiate the two concepts.
Using CLE (FPE) to describe the stochasticity in gene expression means that we have neglected all possible stochastic effects arising from low molecular numbers, which is an inevitable trade-off between the more accurate CME and the more tractable FPE.

A biological system is bimodal if the response of this system to a given input or condition has two peaks (local maxima) [14]. When system volume is large enough, and we can use CLE to describe system behavior, the probability distribution of the long-time CLE solution has two peaks. A biological system is bistable if the reaction rate equations describing its dynamics have two stable equilibria.

### B.2 1-D Noise Induced Bifurcation

In the 1-D case, relationship between bimodality and bistability can be investigated by studying the following two equations,

\[
\text{ODE} : \quad \dot{x} = f(x) \quad , \quad (B.1)
\]
\[
\text{CLE} : \quad \dot{x} = f(x) + I \cdot g(x) \Gamma(t) \quad . \quad (B.2)
\]

In the CLE, \( f(x) \) describes the deterministic dynamics (drift term), and \( I \cdot g(x) \Gamma(t) \) describes the stochastic contribution (diffusion term). \( I \) is a measure of noise intensity in the cell, which is often inversely related to system volume \( \Omega \) [51]. \( \Gamma(t) \) is a zero mean, unit variance white noise process. Alternative, we can describe the evolution of the probability distribution of \( x, P(x,t) \), by the following FPE:

\[
\frac{\partial P(x,t)}{\partial t} = -\frac{\partial}{\partial x} [f(x)P(x,t)] + \frac{1}{2} I^2 \cdot \frac{\partial^2}{\partial x^2} [g^2(x)P(x,t)] . \quad (B.3)
\]

Let’s assume that our experiment lasts long enough such that the obtained fluorescence distribution has reached a steady state, and can thus be modeled by the stationary distribution of the FPE. According to [39], stationary distribution of a FPE is unique given that there is no run-away solution (to infinity), and no singularity in the drift term (infinitely high potential barrier). In general, the stationary solution of a 1-D
FPE can be written as:

\[ P_s(x) = \mathcal{N} \exp(-\Phi(x)), \]

where \( \mathcal{N} \) is a normalization factor and \( \Phi(x) \) is the equivalent stochastic potential defined as

\[ \Phi(x) = \ln \left[ \frac{I^2}{2} g^2(x) \right] - \frac{2}{I^2} \int_0^x \frac{f(u)}{g^2(u)} du. \]  \hspace{1cm} (B.4)

Note that the stationary solution of FPE is independent of initial conditions. Thus, hysteresis behavior common to bistable deterministic systems is theoretically not possible in any system described by an SDE, except for the cases where \( \Phi(x) \) has singularities. In addition, the shape of the stationary distribution \( P_s(x) \) is completely determined by the equivalent stochastic potential \( \Phi(x) \). Now, let’s compare the stochastic potential in (B.4) with the deterministic potential given by

\[ V(x) = -\int_0^x f(u) du. \]  \hspace{1cm} (B.5)

The extremes \( x^* \) of the stochastic potential can be found by solving

\[ f(x^*) - I^2 \cdot g(x^*) g'(x^*) = 0, \]  \hspace{1cm} (B.6)

while the extremes of the deterministic potential are found by

\[ f(x^*) = 0. \]

Not surprisingly, the extremes of the deterministic potential, which are the positions of the deterministic steady states, are exactly the zeros of the drift term \( f(x) \). However, the position of the extremes in the stochastic case, which are the positions of the distribution peaks, are determined by both the drift and the diffusion term, as well as noise intensity. Note that when noise is additive, \( i.e. \) when \( g(x) = \text{const.} \), the zeros of (B.6) coincides with the zeros of the deterministic dynamics \( f(x) = 0 \). However,
for biological systems, the noise is usually multiplicative instead of additive. Using noise intensity $I$ as a bifurcation parameter, we can study how does the peaks of the stochastic distribution deviate from the deterministic equilibria as noise intensity increases. This is shown in the example below.

**Noise Induced Bifurcation in Schlögl Model**

Consider the following toy chemical reaction model proposed by Schlögl [41],

$$
2x + A \xrightarrow{k_1} 3x, \quad x \xrightarrow{k_3} B,
$$

where the concentration of species A and B are kept constant at $a$ and $b$. The CLE for this reaction is

$$
\dot{x} = \left[ -k_2 x^3 + k_1 ax^2 - k_3 x + k_4 b \right] f(x) + \left[ I \cdot \sqrt{k_2 x^3 + k_1 ax^2 + k_3 x + k_4 b} \right] g(x) \Gamma(t). \quad (B.7)
$$

One sample path and its histogram of this system is shown in Fig. B-3. Although we have chosen system parameters such that the deterministic equilibria are 8, 10 and 12, there is only 1 peak in the histogram, and the state variable $x$ stays near its low state near 8 much more frequently than the high state near 12.

In fact, if we compare the equivalent stochastic potential $\Phi(x)$ and the deterministic potential $V(x)$ in this case (Fig. B-4), we find that there is only one peak near 8 in the stochastic potential. This can be explained by the fact that the local noise intensity characterized by $I \cdot g(x)$ near $x = 12$ is much larger than that of $x = 8$ because $g(x)$ is monotonically increasing. Thus, a phase point in the right well of the deterministic potential is much more likely to escape to the left well by jumping over the potential barrier between them.

The $I \cdot g(x)$ terms in the CLE (B.7) serves as a perturbation to the original deterministic dynamics $f(x)$. If we regard noise intensity $I$ as bifurcation, we can have different shapes of bifurcation diagrams for different system parameters as shown in Fig. B-5. The y-axis shows the peak of the distributions. The deterministic equilibria can be found when $I = 0$. For different system parameters, increasing noise
Figure B-3: A sample path of the CLE in B.7 and its histogram. For this simulation we have chosen $a = b = 1$, $k_1 = 30$, $k_2 = 1$, $k_3 = 296$ and $k_4 = 960$. The three resultant zeros of the deterministic ODE are $x^*_{1} = 8$, $x^*_{2} = 10$ and $x^*_{3} = 12$. By taking a noise intensity $I = 0.1$, the distribution in the histogram is unimodal although the deterministic dynamic is bistable.
intensity $I$ can either make a deterministically bistable system unimodal or make a deterministically monostable system bimodal. Creation and destruction of the peaks of a stationary distribution purely due to noise is termed noise-induced transition in physics literature [25]. Looking at the bifurcation diagram in Fig. B-5, the only link we can impose between stochastic bimodality and deterministic bistability is that they imply each other when $I \to 0$.

\section*{B.3 Metastable States in Double Well Potentials}

Our previous analysis has assumed that the stationary distribution of the FPE is fully reflected in the experimental data (fluorescence measurement from flow cytometry). However, this is not always true, and we have to take into account that the time required to reach steady state in FPE (and thus is stationary distribution) can be so long that it becomes infeasible to take a measurement. Therefore, the distribution data we obtain from an experiment may well be a metastable state instead of the true stationary solution.

Let’s carry out a thought experiment of the formation of a stationary distribution in a double well potential. Given a double well equivalent stochastic potential $\Phi(x)$
Figure B-5: A deterministically bistable (monostable) system can be unimodal (bimodal) when noise intensity is large enough. (A): Simulation parameters are $k_1 = 45$, $k_2 = 1$, $k_3 = 506$ and $k_4 = 840$. A deterministically bistable system is bimodal only at low noise intensity level and loses bimodality as $I$ increases. (B): Simulation parameters are $k_1 = 35$, $k_2 = 1$, $k_3 = 322$ and $k_4 = 840$. A deterministically monostable system is unimodal at low $I$ level, as $I$ increases, the system becomes bimodal. In all cases $a = b = 1$.

with two local minima $x_1^*$ and $x_2^*$, if all phase points are initiated near $x_1^*$, they have to jump over the potential barrier between $x_1^*$ and $x_2^*$ to reach the other local minimum. Stationary distribution will have two peaks at $x_1^*$ and $x_2^*$, but if the potential barrier is high enough, and noise is not strong enough, then the diffusion over the potential barrier might take infinitely long. Although as $t \to \infty$, they do reach the stationary distribution. Therefore, the time scale to reach stationary distribution is affected by the potential barrier height $H$ and noise intensity $I$. Such intuition is actually consistent with a classical mathematical approximation by Kramers [23]. For a 1-D CLE with additive noise, and bistable drift potential $V(s)$:

$$
\dot{x} = -V'(x) + I \cdot \Gamma, \quad \text{(B.8)}
$$

the approximate passage rate from one potential well to the other is

$$
\rho_k = (2\pi)^{-1} \sqrt{V''(x_{\min})} \|V''(x_{\max})\| \exp \left\{ -\frac{(V(x_{\max}) - V(x_{\min}))/I}{2} \right\}. \quad \text{(B.9)}
$$
$r_k$ is called Kramers’ escape rate. It is in fact the first order approximate of the lowest non-vanishing eigenvalue of the corresponding FPE [39]. Note that the escape rate $r_k$ decrease exponentially with potential barrier height $H = V(x_{\text{max}}) - V(x_{\text{min}})$, and increases exponentially with noise intensity $I$. For general CLEs with multiplicative noise

$$\dot{x} = f(x) + I \cdot g(x) \Gamma,$$

there is a change of coordinate $y = y(x) = \int_{0}^{x} \frac{\sqrt{2}}{g^{2}(\xi)}d\xi$ that transforms the system into

$$\dot{y} = -\Phi'(y) + \Gamma,$$  \hspace{1cm} (B.10)

and we can then calculate Kramers’ escape rate from (B.10) [39].

We can define two different time scales:

- $\tau_d \sim \tau_e$: The time scale of the deterministic dynamics, which usually coincides with the time window of our experimental measurements.

- $\tau_s \sim 1/r_k$: The diffusion time scale characterized by Kramers’ escape rate that describes how fast the continuous state Markov process reaches stationary distribution.

When $\tau_d \gg \tau_s$, stochastic behavior (diffusion) happens very fast, and the true stationary distribution can be reached. If the stochastic potential is bimodal, we can also observe bimodality in the experiment. This is independent of the initial conditions of the states, as the stationary distribution is unique. When $\tau_d \ll \tau_s$, diffusion reaches steady state very slowly, and experimental data only reflects the meta-stable distribution. System trajectory behaves almost deterministically, except for Brownian motion around deterministic steady states, and jump between them becomes almost impossible. In this case, if we initiate the cells homogeneously, the output will be unimodal and depends on initial conditions. We will be able to observe hysteresis like in entirely deterministic systems. This idea is illustrated through the simulation
of the following fictitious CLE with additive noise:

\[ \dot{x} = -(x - 6)(x - 6.5)(x - 7) + I \cdot \Gamma. \]  

(B.11)

The deterministic part of this CLE has two equal depth potential wells. Using different \( I \) value without changing the deterministic dynamics, we can observe very different histogram after 2000 secs of simulation. As shown in Fig. B-6, in the case of \( H/I = 1 \), transition between two local minima in the potential well is very rare. Using either the high or the low state as the initiation state, the resultant histogram has a sharp peak near the initiation state, indicating that the distribution obtained after 2000 secs of simulation remembers its initial condition. This is similar to the behaviors of bistable deterministic systems. In the case of \( H/I = 0.1 \), noise intensity increases, which consequently increases diffusion effects. Jump between the high and low states becomes possible. After running the simulation for 2000 secs, the resultant histograms have almost entirely “forgotten” the initial condition information, and become identical.

For more detailed and specialized treatment of this topic, the readers are suggested to refer to [7], [31] and [52]. A diagram summarizing our discussion for the 1-D system is shown in Fig. B-7.
Figure B-6: Simulation of the CLE in (B.11) with additive noise using different initial conditions and noise intensities. The blue histogram is for the case where $x_0 \approx 6$, and the red histogram is for the case where $x_0 \approx 7$. When noise intensity is low, the system behavior within 2000 secs is largely dependent on the initial conditions, and demonstrates hysteresis like bistable deterministic systems (left). When noise is intensified, Kramers’ rate increases, and the distributions have almost reached their steady state, which is independent of the initial conditions (right). In this example, we can observe hysteresis without bimodality when noise is low, and observe bimodality without hysteresis when noise is high.
Figure B-7: The stochastic potential is different from the deterministic potential through noise-induced bifurcation. The peaks of the stochastic distribution is identical to the deterministic steady states only if noise intensity $I \to 0$ or noise is additive. For a system with bimodal stochastic potential, if its Kramers’ escape rate is large compared to experiment time scale, we will observe bimodal distribution without any hysteresis behavior. Alternatively, if the escape rate is small compared to experiment time scale, then only metastable states can be observed, the system behaves almost deterministically. It will demonstrate hysteresis without bimodality.
Bibliography


