# *Analyzing and Exploiting the Effects of Protease Sharing in Genetic Circuits*



**Citation:** McBride, Cameron, and Domitilla Del Vecchio. "Analyzing and Exploiting the Effects of Protease Sharing in Genetic Circuit." IFAC-PapersOnLine 50, no. 1 (July 2017): 10924–10931.

**As Published:** http://dx.doi.org/10.1016/J.IFACOL.2017.08.2459

**Publisher:** Elsevier

**Persistent URL:** http://hdl.handle.net/1721.1/119166

**Version:** Author's final manuscript: final author's manuscript post peer review, without publisher's formatting or copy editing

# Analyzing and Exploiting the Effects of Protease Sharing in Genetic Circuits ⋆

**Cameron McBride** * **Domitilla Del Vecchio** *

* *Department of Mechanical Engineering, MIT, 77 Massachusetts Ave., Cambridge, MA 02139 USA (e-mail: cmcbride@mit.edu, ddv@mit.edu).*

**Abstract:** Degradation of proteins in cells plays a large role in the dynamics of gene networks. This degradation is enabled by proteases, which are found in limited quantities in the cell. Proteins that compete for protease may therefore become coupled by non-explicit interactions, which are often neglected in mathematical models. In this work, we develop a model for these non-explicit interactions in gene networks. We examine the effects of protease sharing on the number of equilibria of a system and on the steady state protein concentrations. As a consequence of this analysis, we find that protease sharing effects may be used to cancel undesirable effects due to ribosome sharing.

*Keywords:* Biomolecular systems, resource allocation, modeling, bio control, networks

## 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 (Sen et al., 2013), (Baker and Sauer, 2006) (Farrell et al., 2005). In synthetic contexts, proteases may be used to decrease the response time of circuits with targeted degradation of specific proteins (Cameron and Collins, 2014). Because they appear in limited quantities (Rondelez, 2012), proteases are a limited resource for protein degradation.

Context dependence, such as arising from resource sharing effects, in synthetic biology is a major barrier to the predictability and modular design of synthetic genetic circuits (Cardinale and Arkin, 2012). Cellular resource limitations and context dependence can play a large role in the behavior of cellular systems (Del Vecchio et al., 2008) (Gyorgy et al., 2015). Examples include sharing of transcriptional or translational resources such as ribosomes (Qian and Del Vecchio, 2015).

Limitations of proteases may create non-explicit coupling between different proteins that require the same resource. It has been previously shown this coupling may have a significant effect on the function of certain networks (Genot et al., 2012) (Rondelez, 2012), may be used to tune some system behavior (Huang et al., 2012) or, may couple modules together through degradation (Prindle et al., 2014). Despite the fact that proteases are a finite cellular resource, traditional models do not usually take this into account (Del Vecchio and Murray, 2014). 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 (Cookson et al., 2011) (Mather et al., 2010), how proteases may change the stability of oscillatory systems (Rondelez, 2012), and how protease sharing may change the time response of a system (Yeung et al., 2013). However, previous work has not considered how coupling due to protease sharing changes the number of equilibrium points in the system or considered shared proteases in the context of a network with shared ribosomes.

In this paper we consider proteases as a limited cellular resource by explicitly modeling the limited quantity of protease within a general system. Then we examine how resource limitations affect various system properties such as the number and location of equilibrium points. We show that the addition of a shared protease in a biomolecular network may change the number of equilibrium points in the network. Accordingly, we give a theorem providing sufficient conditions on the determinant of the Jacobian of the system for when the addition of a shared protease does not affect the number of equilibria. Then, we examine how the effect of non-explicit interactions due to protease sharing may compensate undesired effects from ribosome sharing in a gene network with no transcriptional regulations.

This paper is organized as follows. In Section 2, we examine a motivating example to illustrate how unexpected effects may arise due to protease sharing. In Section 3 we derive a general form for a gene network that contains a shared protease, and in Section 4, we derive a theorem that gives conditions under which a system is guaranteed to preserve the number of equilibrium points with protease sharing. In Section 5, we show how the equilibrium concentrations of multiple proteins may be coupled through protease sharing, and how this may be used to compensate for ribosome sharing effects. We discuss our approach and provide direction for future work in Section 6.

⋆ This work was supported by AFOSR grant number FA9550-14-1-0060 and NSF award number 1521925.

## 2. MOTIVATION

In this section, we illustrate the counterintuitive effects that protease sharing may bring about using 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 (Hooshangi et al., 2005). Consider the simple two gene cascade, shown in Fig. 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

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

where $U$ is the induction of $x_1$, $\delta$ is the dilution due to

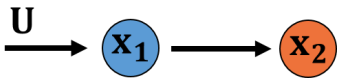


Fig. 1. Simple activation cascade with proteins $x_1$ and $x_2$. $U$ induces the production of $x_1$, and $x_1$ then activates the production of $x_2$.

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 by the same protease, which is found in a limited total amount. The resulting ODE model now becomes (derived in Section 3)

$$\frac{dx_1}{dt} = U - \frac{\overline{P}x_1/K_p}{1+x_1/K_p+x_2/K_p} - \delta x_1 \tag{2a}$$

$$\frac{dx_2}{dt} = \frac{\alpha(x_1/K_{dna})^n}{1+(x_1/K_{dna})^n} - \frac{\overline{P}x_2/K_p}{1+x_1/K_p+x_2/K_p} - \delta x_2, \tag{2b}$$

where $\overline{P}$ 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 $\overline{P} = 0$, (2) becomes (1). Therefore, we simulate (2) for $\overline{P} = 0$ and for $\overline{P} \neq 0$ to obtain the plot in Fig. 2. Fig. 2 shows that the system with the shared protease has hysteresis, which implies multi-stability for some input, while monostability for the system without a shared protease can be easily verified algebraically from (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 paper, 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 for how to predict these interactions.

## 3. MODELING FRAMEWORK

In this section, we will derive a general form of systems with a shared protease, which can then be used to model

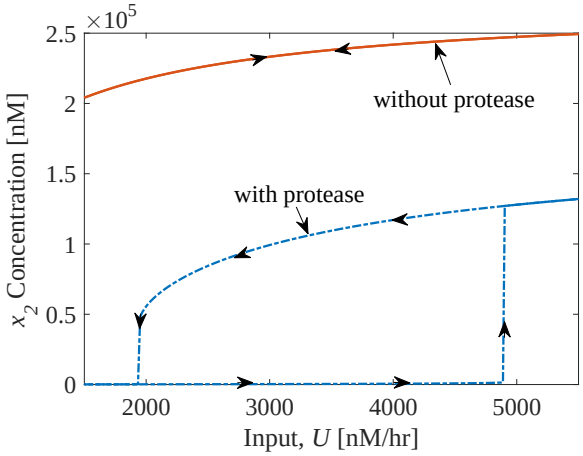


Fig. 2. Input–output steady state response of the cascade (2) with protease sharing ($\overline{P} \neq 0$, blue dashed line) and without protease ($\overline{P} = 0$, red solid line) as $U$ is varied. Parameters used here include $\alpha = 136\,044\,\mathrm{nM\,h^{-1}}$, $n = 1$, $\overline{P} = 54\,000\,\mathrm{nM\,h^{-1}}$, $K_{dna} = 1000\,\mathrm{nM}$, $K_p = 1200\,\mathrm{nM}$, $\delta = 0.5\,\mathrm{h^{-1}}$. The maximum production rate of the protein, $\alpha$, was calculated using the formula $\alpha = \frac{(DNA)k_0k_1(RNAP)R_{tot}}{\delta_1(RNAP+K_0)(R_{tot}+K_1)}$ (Gyorgy et al., 2015) where $DNA = 200\,\mathrm{nM}$ is the concentration of the plasmid, $k_0 = 250\,\mathrm{h^{-1}}$ is the rate of transcription, $k_1 = 300\,\mathrm{h^{-1}}$ is the rate of translation, $RNAP = 2000\,\mathrm{nM}$ is the total concentration of RNA polymerase, $R_{tot} = 6800\,\mathrm{nM}$ is the total concentration of ribosomes, $\delta_1 = 12\,\mathrm{h^{-1}}$ is the dilution rate of mRNA, $K_0 = 200\,\mathrm{nM}$ is the binding constant of RNAP with the promoter, and $K_1 = 50\,\mu\mathrm{M}$ is the binding constant of mRNA with the ribosome binding site. Ranges for each parameter are given in Table 1.

arbitrary gene networks to perform further analysis. We use a deterministic model and assume mass action kinetics (Érdi and Tóth, 1989). We assume that mRNA dynamics are at quasi-steady state without loss of generality.

Proteins may be tagged for degradation by enzymes, known as proteases (Baker and Sauer, 2006), using peptide tags (Keiler et al., 1996), (Farrell et al., 2005) (Farrell et al., 2007). The proteins bind to the protease, and are subsequently degraded enzymatically. We now 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 reactions for modeling the system are

$$\begin{aligned}\emptyset \underset{\delta}{\overset{H_i}{\rightleftharpoons}} \mathrm{x}_i \qquad & \mathrm{C}_i \xrightarrow{\delta} \emptyset \\ \emptyset \underset{\delta}{\overset{\alpha_0}{\rightleftharpoons}} \mathrm{P} \\ \mathrm{x}_i + \mathrm{P} \underset{d_i}{\overset{a_i}{\rightleftharpoons}} \mathrm{C}_i & \xrightarrow{k_i} \emptyset + \mathrm{P},\end{aligned}$$

where $H_i(\boldsymbol{x})$ is the rate of production of the protein $x_i$ and depends on all other protein species, $\boldsymbol{x}$, $\alpha_0$ is the rate of production of the protease, $a_i$ 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

$$\frac{dx_i}{dt} = H_i(\boldsymbol{x}) - a_i x_i P + d_i C_i - \delta x_i \tag{3a}$$
$$\frac{dC_i}{dt} = a_i x_i P - (d_i + k_i + \delta) C_i \tag{3b}$$
$$\frac{dP}{dt} = \alpha_0 - \sum_i a_i x_i P + \sum_i d_i C_i - \delta P, \tag{3c}$$

We define the total protease concentration as

$$P_{tot} := P + \sum_{i=1}^{n} C_i, \tag{4}$$

then the ODE for the total protease concentration is

$$\frac{dP_{tot}}{dt} = \alpha_0 - \delta P_{tot}.$$

Here, for simplicity, we let the initial condition of the protease be at the equilibrium. Then for all time, the total concentration of protease is constant so we can substitute $P = P_{tot} - \sum_{i=1}^{n} C_i$ in (3) and eliminate (3c).

We will now reduce the system using the fact that binding/unbinding reactions are much faster than protein production, degradation, and dilution (Del Vecchio and Murray, 2014). 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 (3) to obtain the system

$$\frac{dx_i}{dt} = H_i(\boldsymbol{x}) - \frac{\delta}{\epsilon_i}\left(\frac{x_i P}{K_i} - C_i\right) - \delta x_i \tag{5a}$$
$$\frac{dC_i}{dt} = \frac{\delta}{\epsilon_i}\left[\frac{x_i P}{K_i} - C_i\right] - (k_i + \delta) C_i. \tag{5b}$$

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 use $z_i = x_i + C_i$, which is a slow variable. Substituting and multiplying all terms in (5b) by $\epsilon_i$, the system becomes

$$\frac{dz_i}{dt} = H_i(\boldsymbol{x}) - k_i C_i - \delta z_i \tag{6a}$$
$$\epsilon_i \frac{dC_i}{dt} = \delta\left[\frac{x_i P}{K_i} - C_i\right] - \epsilon_i (k_i + \delta) C_i, \tag{6b}$$

which is in standard singular perturbation form (Khalil, 2001). It can be easily verified that the slow manifold is always exponentially stable. Then, setting each $\epsilon_i = 0$ and solving in (6b), we obtain that the equation for the slow manifold is

$$\overline{C}_i = \frac{x_i P}{K_i} =: g_i(\boldsymbol{x}). \tag{7}$$

Now, we substitute (7) into the conservation law for the protease from (4) and solve for the concentration of the free protease, $P$, to obtain

$$P = \frac{P_{tot}}{\left(1 + \sum_{j=1}^{n} \frac{x_i}{K_i}\right)}. \tag{8}$$

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

$$\frac{dx_i}{dt} = \frac{\frac{dz_i}{dt}}{1 + \frac{dg_i}{dx_i}}. \tag{9}$$

Substituting (6a), (7), and (8) together into (9), we obtain the set of differential equations

$$\frac{dx_i}{dt} = \underbrace{\left[H_i(\boldsymbol{x}) - \overbrace{\frac{P_{tot}(k_i + \delta)\frac{x_i}{K_i}}{1 + \sum_{k=1}^{n} \frac{x_k}{K_k}}}^{(10b)} - \delta x_i\right]}_{(10a)} \cdot \underbrace{\left(\frac{1}{1 + \frac{P_{tot}/K_i(1 + \sum_{j\neq i} x_j/K_j)}{(1 + \sum_k x_k/K_k)^2}}\right)}_{(10c)}, \tag{10}$$

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 (10a) represents the dynamics of the protein, term (10b) represents the degradation of the protein by the protease, and term (10c) represents the retroactivity to the protein due to binding with the protease (Del Vecchio et al., 2008). The term that represents degradation by the protease, (10b), 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 paper, we will examine the effect of protease sharing on the location and number of equilibrium points. The retroactivity term, (10c), 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 (10c) is always positive, it does not change the location, stability, or number of equilibrium points. Therefore, we can safely ignore retroactivity for our purposes.

## 4. MULTIPLE EQUILIBRIA DUE TO PROTEASE SHARING

As seen in the Section 2, the presence of a shared protease may lead to a change in the stability of a system. In this section, we prove a theorem providing a sufficient condition on the determinant of the Jacobian when the number of equilibrium points is invariant with the addition of a shared protease.

### *4.1 Preliminaries*

We use mathematical tools from degree theory, as presented by Craciun et al. (2008). We begin with definitions and two theorems that are essential for the statement of the theorem we want to prove. The first two theorems are presented without proof since they come directly from Craciun et al. (2008) without modification.

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

$$\deg(f) = \deg(f, \Omega) = \sum_{x \in Z_f} sgn\left(\det\left(\frac{\partial f}{\partial x}(x)\right)\right) \quad (11)$$

where $\overline{\Omega}$ is the closure of $\Omega$, $sgn : \mathbb{R} \to \{-1, 0, 1\}$ is the sign function, $Z_f$ is the set of zeros of $f$ in $\Omega$, and $x^*$ is a degenerate point if $\det\left(\frac{\partial f}{\partial x}(x^*)\right) = 0$, i.e., $f$ has an equilibrium point where at least one eigenvalue is 0.

*Theorem 1.* Consider a bounded domain $\Omega \subset \mathbb{R}^n$ and a continuously varying family of smooth functions $f_\lambda : \overline{\Omega} \to \mathbb{R}^n$ 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 $\deg(f_\lambda)$ is constant for all $\lambda \in [0, 1]$.

*Theorem 2.* Let $\Omega$, and $f_\lambda, \lambda \in [0, 1]$ be defined as in Theorem 1. Then, for any $\lambda \in [0, 1]$ such that $\det\left(\frac{\partial f_\lambda}{\partial x}(x)\right) \neq 0$ for all $x \in \Omega$, the number of zeros of $f_\lambda$ in $\Omega$ must equal the absolute value of the degree of $f_\lambda$ in $\Omega$, which equals the absolute value of the degree of $f_{\lambda'}$ for any $\lambda' \in [0, 1]$.

*Definition 2.* A system, $\frac{dx}{dt} = g(x)$ is *positive invariant* if it has the property that for each $j \in \{1, \ldots, n\}$, the $j$th coordinate of $g(x)$ is non-negative whenever the $j$th coordinate of $x \in \mathbb{R}^n_{\geq 0}$ is zero.

*Definition 3.* A dynamical system $\frac{dx}{dt} = g(x)$ is *mass dissipating* if there exists some $m \in \mathbb{R}^n_{>0}$ such that $m \cdot g(x) \leq 0$ for all $x \in \mathbb{R}^n_{\geq 0}$ (using the standard dot product).

Now, consider the system

$$\frac{dx}{dt} = h(x) - \Lambda_0 x := f_0(x) \quad (12)$$

where $h(x) > 0$ is a smooth function for all $x \in \mathbb{R}^n_{\geq 0}$, and $\Lambda_0$ is a diagonal matrix with strictly positive entries on the diagonal. We consider the system augmented with $g(x)$:

$$\frac{dx}{dt} = h(x) + g(x) - \Lambda_0 x =: f(x) \quad (13)$$

where $g(x)$ is a smooth, mass dissipating function for all $x \in \mathbb{R}^n_{\geq 0}$. For the systems under consideration, $h(x)$, $g(x)$, and $\Lambda_0$ represent protein production, degradation and dilution, respectively. Then the following theorem gives a sufficient condition when the number of equilibrium points of (12) is guaranteed to be the same as the number of equilibrium points of (13).

*Theorem 3.* Let $\Omega = \{x \in \mathbb{R}^n_{\geq 0}\}$, and define $f_0(x) = h(x) - \Lambda_0 x$ and $f(x) = h(x) + g(x) - \Lambda_0 x$ where $x \in \mathbb{R}^n_{\geq 0}$ and $g : \Omega \to \mathbb{R}^n$ is positive invariant on $\Omega \subset \mathbb{R}^n$. Additionally, assume that $f_0, f$ have no degenerate zeros or zeros on the boundary of $\Omega$. If $g$ is mass dissipating, $h$ is bounded and *strictly* positive on $\mathbb{R}^n_{\geq 0}$, and

$$\det\left(\frac{\partial f}{\partial x}(x)\right) \neq 0$$

for all $x \in \Omega$, then the number of equilibria of the system $\frac{dx}{dt} = f_0(x)$ is the same as that of $\frac{dx}{dt} = f(x)$.

**Proof.** This proof is structured as follows: first, we will prove that the system in (13) has no zeros on the sides of the positive orthant and then will prove that (13) has no equilibrium points on the outer boundary of $\Omega$. Finally, we will apply Theorems 1 and 2 to show that the number of equilibrium points of $f_\lambda = h(x) - \Lambda_0 x + \lambda g(x)$ is constant for all $\lambda \in [0, 1]$ and therefore the the number of equilibrium points of $f_0$ is equal to the number of equilibrium points of $f$.

First, select an $m \in \mathbb{R}^n_{>0}$ such that $m \cdot g(x) \leq 0$ (which can be done since $g$ is mass dissipating). Now, let $A \in \mathbb{R}_{>0}$ be a finite upper bound of $m \cdot h(x) \leq A$ for all $x \in \mathbb{R}^n_{\geq 0}$. Define $f_\lambda(x) = h(x) - \Lambda_0 x + \lambda g(x)$ for $\lambda \in [0, 1]$ and fix $M > A$. Now, define a set $\Omega_M = \{x \in \mathbb{R}^n_{\geq 0} : m \cdot (\Lambda_0 x) < M\}$. Then $\Omega_M$ is a bounded domain and $\{f_\lambda : \lambda \in [0, 1]\}$ is a continuously varying family of smooth functions on $\overline{\Omega}_M$. We will now prove that $f_\lambda$ has no zeros on the boundary of $\Omega_M$ (both the sides and outer boundary). For $j = 1, \ldots, n$, consider $x_j \in \overline{\Omega}_M$ such that the $j$th coordinate of $x$ is zero. Since $h(x) > 0$, then $f_\lambda > 0$ since $g$ is positive invariant. Therefore, $f_\lambda$ has no zeros on the sides of $\Omega_M (\overline{\Omega}_M \cap \partial \mathbb{R}^n_{\geq 0})$. Now we will prove that $f_\lambda$ has no zeros on the outer boundary of $\Omega_M$, i.e., when $M = m \cdot (\Lambda_0 x)$. To this end, consider that

$$\begin{aligned} m \cdot f_\lambda &= m \cdot h(x) - m \cdot (\Lambda_0 x) + \lambda m \cdot g(x) \\ &\leq m \cdot h(x) - m \cdot (\Lambda_0 x) \end{aligned}$$

since $m \cdot g(x)$ is mass dissipating. Now, since $M = m \cdot (\Lambda_0 x)$ and $M > A = m \cdot h(x)$, then $m \cdot h(x) - m \cdot (\Lambda_0 x) < 0$. So it follows that $f_\lambda$ has no zeros on the outer boundary of $\Omega_M$ for all $\lambda \in [0, 1]$.

Then by Theorem 2, if $\det(\frac{\partial f}{\partial x}) \neq 0$ in $\Omega$, the number of zeros of $f_\lambda\big|_{\lambda=0}$ is equal to the absolute value of the sum of the local degrees of $f_\lambda\big|_{\lambda=0}$, which is equal to the absolute value of the degree of $f_\lambda\big|_{\lambda=1}$ and equal to the number of zeros of $f_\lambda\big|_{\lambda=0}$. Then by Theorem 1, this quantity is constant over all $\Omega$. Thus, $f(x) = h(x) - \Lambda_0 x$, and $f(x) = h(x) + g(x) - \Lambda_0 x$ have the same number of equilibrium points. □

Observe that the form of the system we derived in Section 3 matches the form of (13), i.e., if $g_i(x) = \frac{-\overline{P}_i x_i / K_i}{1 + \sum_j x_j / K_j}$ for all $i = 1, \ldots, n$ (as derived in Section 3), then $g(x)$ is positive invariant and mass dissipating for any $m \in \mathbb{R}^n_{>0}$. For the assumptions of the theorem to hold, $h(x)$ must be *strictly* positive to ensure 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. So if $h_i(x)$ is an activation, it has the form: $\frac{\alpha (x/K_{dna})^n + \alpha_0}{1 + (x/K_{dna})^n}$, where $\alpha_0 > 0$ is the leakiness.

### *4.2 Two Protein Cascade*

We now consider an example to illustrate the use of this result. Consider a two node protein cascade, shown in Fig. 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 set of ODEs is given by

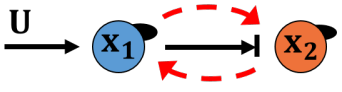


Fig. 3. Two protein cascade with a shared protease, where $x_1$ is inducible, and $x_2$ is either transcriptionally activated or repressed by $x_1$. The red arrows represent non-explicit interactions through the shared protease.

$$\dot{x}_1 = U - \frac{\overline{P}x_1/K_1}{1 + x_1/K_1 + x_2/K_2} - \delta x_1 \tag{14a}$$

$$\dot{x}_2 = H(x_1) - \frac{\overline{P}x_2/K_2}{1 + x_1/K_1 + x_2/K_2} - \delta x_2 \tag{14b}$$

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. $\overline{P} = 0$ (Del Vecchio and Murray, 2014). Then to show when (14) has the same number of equilibrium points as when $\overline{P} = 0$, we verify the condition of Theorem 3 by calculating the Jacobian for this system. This is given as

$$\frac{\partial f}{\partial x} = \begin{bmatrix} \frac{-(\overline{P}/K_1)(1+x_2/K_2)}{(1+x_1/K_1+x_2/K_2)^2} - \delta & \frac{\overline{P}x_1/(K_1K_2)}{(1+x_1/K_1+x_2/K_2)^2} \\ H'(x_1) + \frac{\overline{P}x_2/(K_1K_2)}{(1+x_1/K_1+x_2/K_2)^2} & \frac{-(\overline{P}/K_2)(1+x_1/K_1)}{(1+x_1/K_1+x_2/K_2)^2} - \delta \end{bmatrix} \tag{15}$$

The red arrows in Fig. 3 represent non-explicit interactions through the sharing of the common protease, which can be seen from the off-diagonal terms of the Jacobian, (15), 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

$$\det\left(\frac{\partial f}{\partial x}\right) = \delta^2 + \delta\frac{\overline{P}(K_2+x_2)+\overline{P}(K_1+x_1)}{K_1K_2(1+x_1/K_1+x_2/K_2)^2} + \frac{\overline{P}^2/(K_1K_2)}{(1+x_1/K_1+x_2/K_2)^3} - H'(x_1)\frac{\overline{P}x_1/(K_1K_2)}{(1+x_1/K_1+x_2/K_2)^2}. \tag{16}$$

It can be seen that (16) is never 0 whenever $H'(x_1) < 0$ since all terms are then strictly positive. This satisfies the conditions of Theorem 3, and thus, (14) 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 Theorem 3, so the number of equilibrium points for the system with a shared protease is not guaranteed to be the same as the number of equilibrium points without a shared protease. Indeed, we have seen that the activation cascade in (2) has three equilibrium points–two stable and one unstable.

## 5. 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. Gyorgy et al. (2015) showed 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 by Gyorgy et al. (2015) 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 products due to ribosome competition. The chemical reactions for this system with no transcriptional links between proteins are

$$\begin{aligned} &\mathrm{m}_i \underset{\beta_i}{\overset{\delta_1}{\rightleftharpoons}} \emptyset && \mathrm{C}_{1i} \xrightarrow{\delta_1} \emptyset \\ &\mathrm{m}_i + \mathrm{R} \underset{d_{1i}}{\overset{a_{1i}}{\rightleftharpoons}} \mathrm{C}_{1i} \xrightarrow{k_{1i}} \mathrm{m}_i + \mathrm{R} + \mathrm{x}_i && \mathrm{C}_{2i} \xrightarrow{\delta_2} \emptyset \\ &\mathrm{x}_i + \mathrm{P} \underset{d_{2i}}{\overset{a_{2i}}{\rightleftharpoons}} \mathrm{C}_{2i} \xrightarrow{k_{2i}} \mathrm{P} + \emptyset && \mathrm{P} \underset{\alpha_0}{\overset{\delta_2}{\rightleftharpoons}} \emptyset \\ &\mathrm{x}_i \xrightarrow{\delta_2} \emptyset \end{aligned} \tag{17}$$

for $i = 1, 2, \ldots, n$. Here, $\mathrm{m}_i$ is mRNA, R is the free ribosomes, $\mathrm{C}_{1i}$ is the complex formed by the mRNA and ribosomes, $\mathrm{x}_i$ is the protein, P is the protease, and $\mathrm{C}_{2i}$ is the complex formed by the protease bound to the protein. Here $\beta_i$ is the production rate of protein $\mathrm{x}_i$, $\delta_1$ is the dilution or mRNA, $a_{1i}$ is the association rate constant and $d_{1i}$ is the dissociation rate constant between $\mathrm{m}_i$ 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 $\mathrm{x}_i$ 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. 4. Then, using mass-action kinetics, this system leads to the

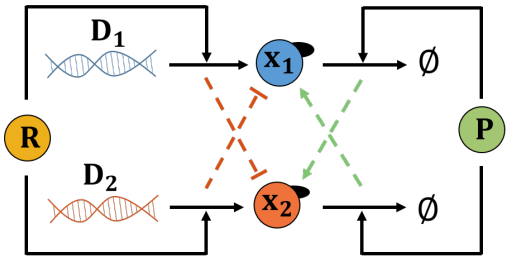


Fig. 4. Visual representation of (17) for the case with two proteins. Shared production resources, R, create non-explicit repression shown with red dashed arrows, while shared degradation resources, P, create non-explicit activation of $x_1$ and $x_2$, shown with green dashed arrows.

set of ODEs

$$\begin{aligned} \frac{dm_i}{dt} &= \beta_i - a_{1i}m_iR + (d_{1i} + k_{1i})C_{1i} - \delta_1 m_i \\ \frac{dC_{1i}}{dt} &= a_{1i}m_iR - (d_{1i} + k_{1i} + \delta_1)C_{1i} \\ \frac{dx_i}{dt} &= k_{1i}C_{1i} - a_{2i}x_iP + d_{2i}C_{2i} - \delta_2 x_i \\ \frac{dC_{2i}}{dt} &= a_{2i}x_iP - (d_{2i} + k_{2i} + \delta_2)C_{2i} \\ R_{tot} &= R + \sum_{k=1}^{n} C_{1k} \\ \frac{dP}{dt} &= \alpha_0 - \sum_{k=1}^{n} [a_{2k}x_kP + (d_{2k} + k_{2k})C_{2k}] - \delta_2 P. \end{aligned} \tag{18}$$

We reduce (18) by using singular perturbation and following a similar process as previously outlined in Section 3. Then the mRNA dynamics are given as

$$\frac{dm_i}{dt} = \beta_i - \delta_1 m_i \left(1 + \frac{R}{K_{1i}}\right) \tag{19}$$

where $K_{1i} = d_{1i}/a_{1i}$ 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, $R_{tot} \ll K_{1i}$ (Gyorgy et al., 2015). Then the steady state of mRNA is $m_i = \beta_i/\delta_1$. We assume that mRNA is at the steady state since mRNA dynamics are faster than protein dynamics. Then, neglecting retroactivity since we are only interested in the steady state response, the system becomes

$$\frac{dx_i}{dt} = k_{1i} R \frac{\beta_i}{\delta_1 K_{1i}} - (k_{2i} + \delta_2) P \frac{x_i}{K_{2i}} - \delta_2 x_i \tag{20}$$

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

$$x_i^{eq} = \frac{k_{1i} R \beta_i/(\delta_1 K_{1i})}{(k_{2i} + \delta_2) P/K_{2i} + \delta_2}. \tag{21}$$

Using the conservation laws for both protease and ribosomes, we obtain

$$R_{tot} = R \left(1 + \sum_{i=1}^{n} \frac{\beta_i}{\delta_1 K_{1i}}\right) \tag{22}$$

$$\begin{aligned} P_{tot} &= P \left(1 + \sum_{i=1}^{n} \frac{x_i}{K_{2i}}\right) \\ &= P \left[1 + R \sum_{i=1}^{n} \frac{k_{1i}\beta_i/(\delta_1 K_{1i})}{k_{2i} P + K_{2i}\delta}\right]. \end{aligned} \tag{23}$$

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

$$M_j^i = \frac{x_i^{eq} - x_i^{eq}|_{\beta_j = 0}}{x_j^{eq} - x_j^{eq}|_{\beta_j = 0}} \tag{24}$$

for any $i \neq j$. Solving (21)–(23) simultaneously for the concentration of each protein and substituting into (24). By assuming that $\delta_2 \ll k_{2i} P/K_{2i}$ for each $i = 1, \ldots, n$, we obtain that $M_j^i$ 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 5.1. Then, the coupling coefficient is given by

$$M_j^i = \frac{k_{1i} k_{2j} K_{2i}}{k_{1j} k_{2i} K_{2j}} \frac{\beta_i}{\delta_1 K_{1i}} \left[\frac{\frac{k_{1j} R_{tot}}{k_{2j} P_{tot}} - 1}{(1 + \sum_{k \neq j} \frac{\beta_k}{\delta_1 K_{1k}}) - \frac{R_{tot}}{P_{tot}} \sum_{k \neq j} \frac{k_{1k}\beta_k}{k_{2k}\delta_1 K_{1k}}}\right]. \tag{25}$$

Note from (25) that the coupling coefficient of protein $x_i$ with respect to protein $x_j$, $M_j^i$, is 0 whenever

$$k_{1i} R_{tot} = k_{2j} P_{tot}. \tag{26}$$

This means that 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 $\delta_2 \ll k_{2i} P/K_{2i}$ no longer holds. Therefore, we must repeat the analysis setting $P_{tot} = 0$ and using (21)–(22). Then, we obtain the coupling coefficient between $x_i$ and $x_j$ to be

$$\overline{M}_j^i = \frac{-k_{1i}\beta_i}{k_{1j}\delta_1 K_{1i}} \left[\frac{1}{1 + \sum_{k \neq j} \frac{1}{\delta_1} \frac{\beta_k}{K_{1k}}}\right]. \tag{27}$$

Observe that $\overline{M}_j^i$ is always strictly negative, while $M_j^i$ in (25) may be positive, negative, or zero. Additionally, observe from (25) and (27) 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 (25) and (27). Finally, to examine the asymptotic behavior as we let $P_{tot}$ become large in (25) and set $K_{2i} = K_{2j}$ and $k_{2i} = k_{2j}$, then we recover (27), which shows that the coupling coefficient in (25) approaches the coupling coefficient in (27) as $P_{tot}$ becomes large. In summary, (25) and (27) indicate that competition for a shared protease may compensate for the effects of competition for ribosomes by decreasing the magnitude of the coupling coefficient.

### *5.1 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 (18). In this case, the assumption that $\delta_2 \ll k_{2i} P/K_{2i}$ may not hold. We now assume 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. 5 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, (27), has a constant, negative coupling coefficient for 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 (25) 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. We calculate the coupling coefficient using (18) and (24) 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. 6. 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

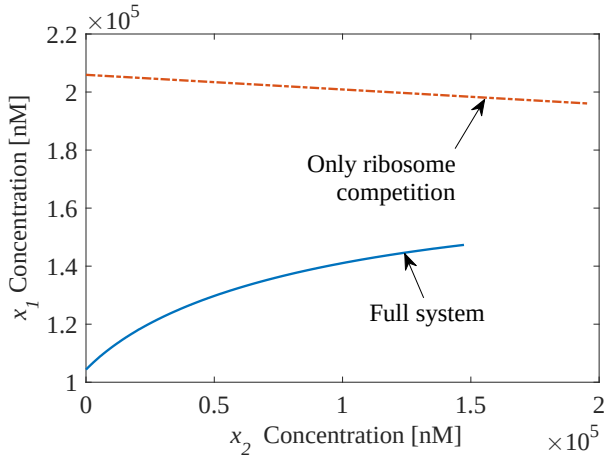


Fig. 5. The steady state of (18) with only ribosome competition ($P_{tot} = 0$), shown by the red dashed line, and both protease and ribosome competition, shown with the blue solid line for different levels of induction of $x_2$. The parameters were used here and are $k_{1i} = 300\,\mathrm{h}^{-1}$, $R_{tot} = 6800\,\mathrm{nM}$, $K_{1i} = 20\,\mu\mathrm{M}$, $k_{2i} = 270\,\mathrm{h}^{-1}$, $P_{tot} = 200\,\mathrm{nM}$, $K_{2i} = 1200\,\mathrm{nM}$, $\delta_1 = 12\,\mathrm{h}^{-1}$, $\delta_2 = 0.5\,\mathrm{h}^{-1}$. The maximum production rate of the mRNA, $\beta_i$, was calculated using the formula $\beta_i = \frac{(DNA)k_{0i}(RNAP)}{RNAP+K_{0i}}$ (Gyorgy et al., 2015) where $DNA = 70\,\mathrm{nM}$ is the concentration of the plasmid, $k_{0i} = 200\,\mathrm{h}^{-1}$ is the rate of transcription, $RNAP = 2000\,\mathrm{nM}$ is the total concentration of RNA polymerase, and $K_{0i} = 200\,\mathrm{nM}$ is the binding constant of RNAP with the promoter. Ranges for each parameter are given in Table 1.

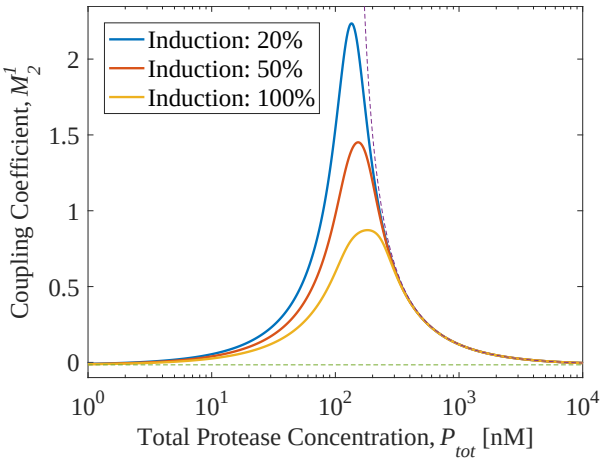


Fig. 6. The coupling coefficient of (18) for different concentrations of total protease. The purple dashed line is the coupling coefficient predicted by (25) and the green dashed line is the coupling coefficient predicted by (27). The parameters used here are $k_{1i} = 300\,\mathrm{h}^{-1}$, $R_{tot} = 6800\,\mathrm{nM}$, $K_{1i} = 63\,\mu\mathrm{M}$, $k_{2i} = 270\,\mathrm{h}^{-1}$, $K_{2i} = 1200\,\mathrm{nM}$, $\delta_1 = 12\,\mathrm{h}^{-1}$, $\delta_2 = 0.5\,\mathrm{h}^{-1}$. The maximum production rate of the mRNA, $\beta_i$, was calculated using the formula $\beta_i = \frac{(DNA)k_{0i}(RNAP)}{RNAP+K_{0i}}$ (Gyorgy et al., 2015) where $DNA = 70\,\mathrm{nM}$ is the concentration of the plasmid, $k_{0i} = 200\,\mathrm{h}^{-1}$ is the rate of transcription, $RNAP = 2000\,\mathrm{nM}$ is the total concentration of RNA polymerase, $K_{0i} = 200\,\mathrm{nM}$ is the binding constant of RNAP with the promoter. $\alpha_0$ 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 1.

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 $P_{tot} \approx 10\,\mathrm{nM}$ and when $P_{tot} \approx 7400\,\mathrm{nM}$. 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. 6 where all three simulated curves collapse onto the curve calculated with (25). This occurs for total protease concentrations $P_{tot} > 300\,\mathrm{nM}$, which is feasible in synthetic systems but is on the upper range for the concentrations typically present in natural systems (Farrell et al., 2005).

## 6. CONCLUSIONS

In this paper, we examined how a shared protease may affect the behavior of a genetic network. In particular, we showed that coupling between proteins due to a shared protease may change the number of equilibria of the system as well as balancing 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 couple the concentrations of proteins together, cause multistability in gene networks, or 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.

## REFERENCES

Andersen, J.B., Sternberg, C., Poulsen, L.K., Bjrn, S.P., Givskov, M., and Molin, S. (1998). New unstable variants of green fluorescent protein for studies of transient gene expression in bacteria. *Appl Environ Microbiol*, 64(6), 2240–2246.

Baker, T.A. and Sauer, R.T. (2006). ATP-dependent proteases of bacteria: recognition logic and operating principles. *Trends in Biochemical Sciences*, 31(12), 647–653.

Bernstein, J.A., Lin, P.H., Cohen, S.N., and Lin-Chao, S. (2004). Global analysis of Escherichia coli RNA degradosome function using DNA microarrays. *Proc Natl Acad Sci U S A*, 101(9), 2758–2763.

Cameron, D.E. and Collins, J.J. (2014). Tunable protein degradation in bacteria. *Nature Biotechnology*, 32(12), 1276–1281.

Table 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 $20\,h^{-1}$ | Bernstein et al. (2004) |
| protein dilution rate | $\delta_2$ | $0.4\,h^{-1}$ to $1\,h^{-1}$ | Andersen et al. (1998) |
| transcription rate | $k_{0i}$ | $195\,h^{-1}$ to $275\,h^{-1}$ | Dennis and Bremer (2008), Tsien (1998) |
| translation rate | $k_{1i}$ | $180\,h^{-1}$ to $315\,h^{-1}$ | Dennis and Bremer (2008), Tsien (1998) |
| degradation by protease rate | $k_{2i}$ | $126\,h^{-1}$ to $360\,h^{-1}$ | Farrell et al. (2007) |
| RNAP with DNA promoter Michaelis-Menten constant | $K_{0i}$ | 150 nM to 560 nM | Liu et al. (2003) |
| mRNA with ribosome binding constant | $K_{1i}$ | $\approx 100\,\mu M$ | estimated in Gyorgy et al. (2015) |
| protein with protease Michaelis-Menten constant | $K_{2i}$ | $0.5\,\mu M$ to $41\,\mu M$ | Farrell et al. (2007) |
| total RNAP concentration | $RNAP$ | $1.5\,\mu M$ to $11.4\,\mu M$ | Dennis and Bremer (2008) |
| total ribosome concentration | $R_{tot}$ | $6.8\,\mu M$ to $72.0\,\mu M$ | Dennis and Bremer (2008) |
| total protease concentration (ClpXP) | $P_{tot}$ | 75 nM to 250 nM | Farrell et al. (2005) |
| Transcription factor binding constant (nonspecific) | $K_{dna}$ | $10^{-3}$ M to $10^{-6}$ M | Revzin (1990) |


Cardinale, S. and Arkin, A.P. (2012). Contextualizing context for synthetic biology–identifying causes of failure of synthetic biological systems. *Biotechnol J*, 7(7), 856–866.

Cookson, N.A., Mather, W.H., Danino, T., Mondragn-Palomino, O., Williams, R.J., Tsimring, L.S., and Hasty, J. (2011). Queueing up for enzymatic processing: correlated signaling through coupled degradation. *Mol. Syst. Biol.*, 7, 561.

Craciun, G., Helton, J.W., and Williams, R.J. (2008). Homotopy methods for counting reaction network equilibria. *Mathematical Biosciences*, 216(2), 140–149.

Del Vecchio, D. and Murray, R.M. (2014). *Biomolecular feedback systems*. Princeton University Press, Princeton.

Del Vecchio, D., Ninfa, A.J., and Sontag, E.D. (2008). Modular cell biology: retroactivity and insulation. *Mol Syst Biol*, 4, 161.

Dennis, P.P. and Bremer, H. (2008). Modulation of chemical composition and other parameters of the cell at different exponential growth rates. *EcoSal Plus*, 3(1).

Érdi, P. and Tóth, J. (1989). *Mathematical models of chemical reactions: theory and applications of deterministic and stochastic models*. Manchester University Press. Google-Books-ID: iDu8AAAAIAAJ.

Farrell, C.M., Baker, T.A., and Sauer, R.T. (2007). Altered specificity of a AAA+ protease. *Mol. Cell*, 25(1), 161–166.

Farrell, C.M., Grossman, A.D., and Sauer, R.T. (2005). Cytoplasmic degradation of ssrA-tagged proteins. *Mol. Microbiol.*, 57(6), 1750–1761.

Genot, A.J., Fujii, T., and Rondelez, Y. (2012). Computing with competition in biochemical networks. *Phys. Rev. Lett.*, 109(20), 208102.

Gyorgy, A., Jimnez, J., Yazbek, J., Huang, H.H., Chung, H., Weiss, R., and DelVecchio, D. (2015). Isocost lines describe the cellular economy of genetic circuits. *Biophysical Journal*, 109(3), 639–646.

Hooshangi, S., Thiberge, S., and Weiss, R. (2005). Ultrasensitivity and noise propagation in a synthetic transcriptional cascade. *PNAS*, 102(10), 3581–3586.

Huang, D., Holtz, W.J., and Maharbiz, M.M. (2012). A genetic bistable switch utilizing nonlinear protein degradation. *Journal of Biological Engineering*, 6(1), 9.

Keiler, K.C., Waller, P.R., and Sauer, R.T. (1996). Role of a peptide tagging system in degradation of proteins synthesized from damaged messenger RNA. *Science*, 271(5251), 990–993.

Khalil, H.K. (2001). *Nonlinear systems*. Pearson, Upper Saddle River, N.J, 3 edition edition.

Liu, M., Gupte, G., Roy, S., Bandwar, R.P., Patel, S.S., and Garges, S. (2003). Kinetics of transcription initiation at lacP1. Multiple roles of cyclic AMP receptor protein. *J. Biol. Chem.*, 278(41), 39755–39761.

Mather, W.H., Cookson, N.A., Hasty, J., Tsimring, L.S., and Williams, R.J. (2010). Correlation resonance generated by coupled enzymatic processing. *Biophys J*, 99(10), 3172–3181.

Prindle, A., Selimkhanov, J., Li, H., Razinkov, I., Tsimring, L.S., and Hasty, J. (2014). Rapid and tunable post-translational coupling of genetic circuits. *Nature*, 508(7496), 387–391.

Qian, Y. and Del Vecchio, D. (2015). Effective interactions arising from resource limitations in gene transcription networks. In *American Control Conference (ACC), 2015*. Massachusetts Institute of Technology.

Revzin, A. (1990). *The biology of nonspecific DNA protein interactions*. CRC Press.

Rondelez, Y. (2012). Competition for catalytic resources alters biological network dynamics. *Phys. Rev. Lett.*, 108(1), 018102.

Sen, M., Maillard, R.A., Nyquist, K., Rodriguez-Aliaga, P., Press, S., Martin, A., and Bustamante, C. (2013). The ClpXP protease functions as a motor with constant rpm but different gears. *Cell*, 155(3), 636–646.

Tsien, R.Y. (1998). The green fluorescent protein. *Annual Review of Biochemistry*, 67(1), 509–544.

Yeung, E., Kim, J., and Murray, R.M. (2013). Resource competition as a source of non-minimum phase behavior in transcription-translation systems. In *52nd IEEE Conference on Decision and Control*, 4060–4067.