

Analyzing and Exploiting the Effects of Protease Sharing in Genetic Circuits^{*}

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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.

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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

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

where U is the induction of x_1 , δ is the dilution due to

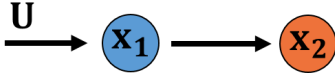


Fig. 1. Simple activation cascade with proteins x_1 and x_2 . U induces the production of x_1 , and x_1 then activates the production of x_2 .

cell growth, α 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)

$$\frac{dx_1}{dt} = U - \frac{\bar{P}x_1/K_p}{1 + x_1/K_p + x_2/K_p} - \delta x_1 \quad (2a)$$

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

where \bar{P} is the maximal degradation rate of x_1 and x_2 by the protease and is proportional to the total concentration of protease and K_p is the binding constant between the protein and the protease. Note that when $\bar{P} = 0$, (2) becomes (1). Therefore, we simulate (2) for $\bar{P} = 0$ and for $\bar{P} \neq 0$ 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

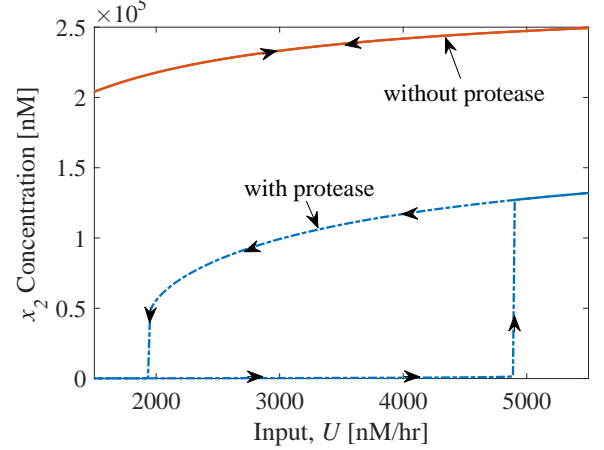
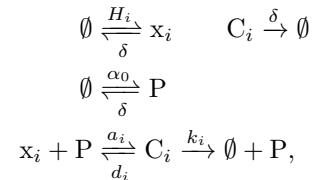


Fig. 2. Input–output steady state response of the cascade (2) with protease sharing ($\bar{P} \neq 0$, blue dashed line) and without protease ($\bar{P} = 0$, red solid line) as U is varied. Parameters used here include $\alpha = 136\,044 \text{ nM h}^{-1}$, $n = 1$, $\bar{P} = 54\,000 \text{ nM h}^{-1}$, $K_{dna} = 1000 \text{ nM}$, $K_p = 1200 \text{ nM}$, $\delta = 0.5 \text{ h}^{-1}$. The maximum production rate of the protein, α , 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 $DNA = 200 \text{ nM}$ is the concentration of the plasmid, $k_0 = 250 \text{ h}^{-1}$ is the rate of transcription, $k_1 = 300 \text{ h}^{-1}$ is the rate of translation, $RNAP = 2000 \text{ nM}$ is the total concentration of RNA polymerase, $R_{tot} = 6800 \text{ nM}$ is the total concentration of ribosomes, $\delta_1 = 12 \text{ h}^{-1}$ is the dilution rate of mRNA, $K_0 = 200 \text{ nM}$ is the binding constant of RNAP with the promoter, and $K_1 = 50 \text{ } \mu\text{M}$ 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 $i = 1, \dots, n$. Then, the reactions for modeling the system are



where $H_i(\mathbf{x})$ is the rate of production of the protein x_i and depends on all other protein species, \mathbf{x} , α_0 is the rate of production of the protease, a_i and d_i are the association and dissociation rate constants of proteins binding to 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 $i = 1, \dots, n$. We account for the growth of the cell by assuming that all species dilute with rate constant δ . Then, the set of ODEs is

$$\frac{dx_i}{dt} = H_i(\mathbf{x}) - a_i x_i P + d_i C_i - \delta x_i \quad (3a)$$

$$\frac{dC_i}{dt} = a_i x_i P - (d_i + k_i + \delta) C_i \quad (3b)$$

$$\frac{dP}{dt} = \alpha_0 - \sum_i a_i x_i P + \sum_i d_i C_i - \delta P, \quad (3c)$$

We define the total protease concentration as

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

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

$$\frac{dx_i}{dt} = H_i(\mathbf{x}) - \frac{\delta}{\epsilon_i} \left(\frac{x_i P}{K_i} - C_i \right) - \delta x_i \quad (5a)$$

$$\frac{dC_i}{dt} = \frac{\delta}{\epsilon_i} \left[\frac{x_i P}{K_i} - C_i \right] - (k_i + \delta) C_i. \quad (5b)$$

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 ϵ_i , the system becomes

$$\frac{dz_i}{dt} = H_i(\mathbf{x}) - k_i C_i - \delta z_i \quad (6a)$$

$$\epsilon_i \frac{dC_i}{dt} = \delta \left[\frac{x_i P}{K_i} - C_i \right] - \epsilon_i (k_i + \delta) C_i, \quad (6b)$$

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

$$\bar{C}_i = \frac{x_i P}{K_i} =: g_i(\mathbf{x}). \quad (7)$$

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

$$P = \frac{P_{tot}}{\left(1 + \sum_{j=1}^n \frac{x_j}{K_j}\right)}. \quad (8)$$

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

$$\frac{dx_i}{dt} = \frac{\frac{dz_i}{dt}}{1 + \frac{dg_i}{dx_i}}. \quad (9)$$

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

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

for $i = 1, \dots, n$. To simplify notation, we can substitute \bar{P}_i for $P_{tot}(k_i + \delta)$. Then \bar{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 $f : \bar{\Omega} \rightarrow \mathbb{R}^n$ such that f has no degenerate zeros and has no zeros on the boundary of Ω , then the *topological degree with respect to zero of f* equals:

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

where $\bar{\Omega}$ is the closure of Ω , $\operatorname{sgn} : \mathbb{R} \rightarrow \{-1, 0, 1\}$ is the sign function, Z_f is the set of zeros of f in Ω , 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 $f_\lambda : \bar{\Omega} \rightarrow \mathbb{R}^n$ for $\lambda \in [0, 1]$, such that f_λ does not have any zeros on the boundary of Ω for all $\lambda \in [0, 1]$. Then $\deg(f_\lambda)$ is constant for all $\lambda \in [0, 1]$.

Theorem 2. Let Ω , 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_λ in Ω must equal the absolute value of the degree of f_λ in Ω , 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 $j \in \{1, \dots, n\}$, the j th coordinate of $g(x)$ is non-negative whenever the j th coordinate of $x \in \mathbb{R}_{\geq 0}^n$ is zero.

Definition 3. A dynamical system $\frac{dx}{dt} = g(x)$ is *mass dissipating* if there exists some $m \in \mathbb{R}_{> 0}^n$ such that $m \cdot g(x) \leq 0$ for all $x \in \mathbb{R}_{\geq 0}^n$ (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 $x \in \mathbb{R}_{\geq 0}^n$, and Λ_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 $x \in \mathbb{R}_{\geq 0}^n$. For the systems under consideration, $h(x)$, $g(x)$, and Λ_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 $\Omega = \{x \in \mathbb{R}_{\geq 0}^n\}$, and define $f_0(x) = h(x) - \Lambda_0 x$ and $f(x) = h(x) + g(x) - \Lambda_0 x$ where $x \in \mathbb{R}_{\geq 0}^n$ and $g : \Omega \rightarrow \mathbb{R}^n$ 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 Ω . If g is mass dissipating, h is bounded and *strictly positive* on $\mathbb{R}_{\geq 0}^n$, 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 Ω . 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 $m \in \mathbb{R}_{> 0}^n$ 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 $x \in \mathbb{R}_{\geq 0}^n$. 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 $\Omega_M = \{x \in \mathbb{R}_{\geq 0}^n : m \cdot (\Lambda_0 x) < M\}$. Then Ω_M is a bounded domain and $\{f_\lambda : \lambda \in [0, 1]\}$ is a continuously varying family of smooth functions on $\bar{\Omega}_M$. We will now prove that f_λ has no zeros on the boundary of Ω_M (both the sides and outer boundary). For $j = 1, \dots, n$, consider $x_j \in \bar{\Omega}_M$ 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_λ has no zeros on the sides of $\Omega_M (\bar{\Omega}_M \cap \partial \mathbb{R}_{\geq 0}^n)$. Now we will prove that f_λ has no zeros on the outer boundary of Ω_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_λ has no zeros on the outer boundary of Ω_M for all $\lambda \in [0, 1]$.

Then by Theorem 2, if $\det \left(\frac{\partial f}{\partial x} \right) \neq 0$ in Ω , the number of zeros of $f_\lambda|_{\lambda=0}$ is equal to the absolute value of the sum of the local degrees of $f_\lambda|_{\lambda=0}$, which is equal to the absolute value of the degree of $f_\lambda|_{\lambda=1}$ and equal to the number of zeros of $f_\lambda|_{\lambda=0}$. Then by Theorem 1, this quantity is constant over all Ω . 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. \square

Observe that the form of the system we derived in Section 3 matches the form of (13), i.e., if $g_i(x) = \frac{-\bar{P}_i x_i / K_i}{1 + \sum_j x_j / K_j}$ for all $i = 1, \dots, n$ (as derived in Section 3), then $g(x)$ is positive invariant and mass dissipating for any $m \in \mathbb{R}_{> 0}^n$. 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

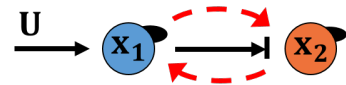


Fig. 3. Two protein cascade with a shared protease, where x_1 is inducible, and x_2 is either transcriptionally activated or repressed by x_1 . The red arrows represent non-explicit interactions through the shared protease.

$$\dot{x}_1 = U - \frac{\bar{P}x_1/K_1}{1 + x_1/K_1 + x_2/K_2} - \delta x_1 \quad (14a)$$

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

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 $\Omega \subset \mathbb{R}_{\geq 0}^n$ such that this system has no zeros on the boundary of Ω and Ω is positively invariant. It has previously been shown that this system has one equilibrium point when there is no protease present, i.e. $\bar{P} = 0$ (Del Vecchio and Murray, 2014). Then to show when (14) has the same number of equilibrium points as when $\bar{P} = 0$, we verify the condition of Theorem 3 by calculating the Jacobian for this system. This is given as

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

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

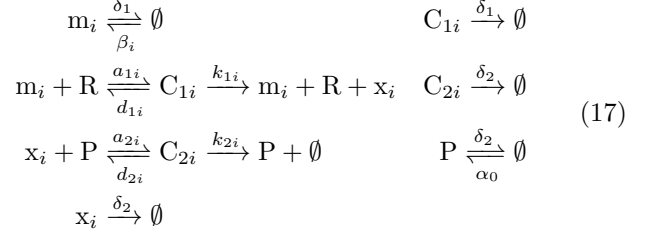
$$\det\left(\frac{\partial f}{\partial x}\right) = \delta^2 + \delta \frac{\bar{P}(K_2+x_2) + \bar{P}(K_1+x_1)}{K_1K_2(1+x_1/K_1+x_2/K_2)^2} + \frac{\bar{P}^2/(K_1K_2)}{(1+x_1/K_1+x_2/K_2)^3} - H'(x_1) \frac{\bar{P}x_1/(K_1K_2)}{(1+x_1/K_1+x_2/K_2)^2}. \quad (16)$$

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 $x \in \mathbb{R}_{\geq 0}^n$. 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



for $i = 1, 2, \dots, n$. Here, m_i is mRNA, R is the free ribosomes, C_{1i} is the complex formed by the mRNA and ribosomes, x_i is the protein, P is the protease, and C_{2i} is the complex formed by the protease bound to the protein. Here β_i is the production rate of protein x_i , δ_1 is the dilution or mRNA, a_{1i} is the association rate constant and d_{1i} is the dissociation rate constant between m_i and ribosomes, k_{1i} is the rate of translation, a_{2i} is the association rate constant and d_{2i} is the dissociation rate constant between protein x_i and protease, k_{2i} is the catalytic rate of degradation by the protease, δ_2 is the dilution of proteins, and α_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

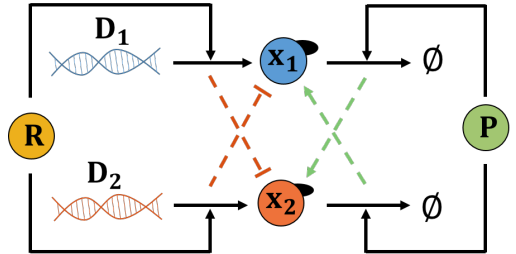


Fig. 4. Visual representation of (17) for the case with two proteins. Shared production resources, R , create non-explicit repression shown with red dashed arrows, while shared degradation resources, P , create non-explicit activation of x_1 and x_2 , shown with green dashed arrows.

set of ODEs

$$\begin{aligned} \frac{dm_i}{dt} &= \beta_i - a_{1i}m_iR + (d_{1i} + k_{1i})C_{1i} - \delta_1m_i \\ \frac{dC_{1i}}{dt} &= a_{1i}m_iR - (d_{1i} + k_{1i} + \delta_1)C_{1i} \\ \frac{dx_i}{dt} &= k_{1i}C_{1i} - a_{2i}x_iP + d_{2i}C_{2i} - \delta_2x_i \\ \frac{dC_{2i}}{dt} &= a_{2i}x_iP - (d_{2i} + k_{2i} + \delta_2)C_{2i} \\ R_{tot} &= R + \sum_{k=1}^n C_{1k} \\ \frac{dP}{dt} &= \alpha_0 - \sum_{k=1}^n [a_{2k}x_kP + (d_{2k} + k_{2k})C_{2k}] - \delta_2P. \end{aligned} \quad (18)$$

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

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

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

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

for $i = 1, \dots, n$. 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

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

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

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

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

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 β_j from 0 to some positive induction level. We define the coupling coefficient between protein x_i and x_j as

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

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 $i = 1, \dots, n$, we obtain that M_j^i is constant for all levels of induction of x_j , β_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

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

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

$$k_{1i}R_{tot} = k_{2j}P_{tot}. \quad (26)$$

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

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

Observe that \bar{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 $|\bar{M}_j^i|$, 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 , β_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

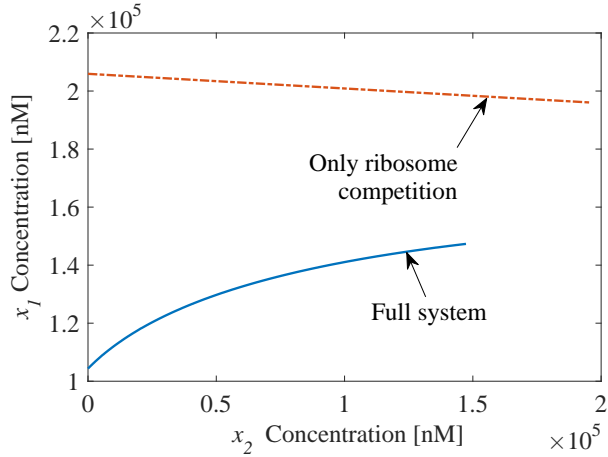


Fig. 5. The steady state of (18) with only ribosome competition ($P_{tot} = 0$), shown by the red dashed line, and both protease and ribosome competition, shown with the blue solid line for different levels of induction of x_2 . The parameters used here are $k_{1i} = 300 \text{ h}^{-1}$, $R_{tot} = 6800 \text{ nM}$, $K_{1i} = 20 \text{ }\mu\text{M}$, $k_{2i} = 270 \text{ h}^{-1}$, $P_{tot} = 200 \text{ nM}$, $K_{2i} = 1200 \text{ nM}$, $\delta_1 = 12 \text{ h}^{-1}$, $\delta_2 = 0.5 \text{ h}^{-1}$. The maximum production rate of the mRNA, β_i , was calculated using the formula $\beta_i = \frac{(DNA)k_{0i}(RNAP)}{RNAP+K_{0i}}$ (Gyorgy et al., 2015) where $DNA = 70 \text{ nM}$ is the concentration of the plasmid, $k_{0i} = 200 \text{ h}^{-1}$ is the rate of transcription, $RNAP = 2000 \text{ nM}$ is the total concentration of RNA polymerase, and $K_{0i} = 200 \text{ nM}$ is the binding constant of RNAP with the promoter. 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 $P_{tot} \approx 10 \text{ nM}$ and when $P_{tot} \approx 7400 \text{ nM}$. At these concentrations, ribosomes sharing effects perfectly balance protease sharing effects. Thus, when designing a genetic circuit, a shared protease may perfectly balance the effects of ribosome competition at either a small or a large concentration of protease.

Note that the range of validity of the assumption that dilution is small compared to degradation can be seen from Fig. 6 where all three simulated curves collapse onto the curve calculated with (25). This occurs for total protease concentrations $P_{tot} > 300 \text{ nM}$, 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.

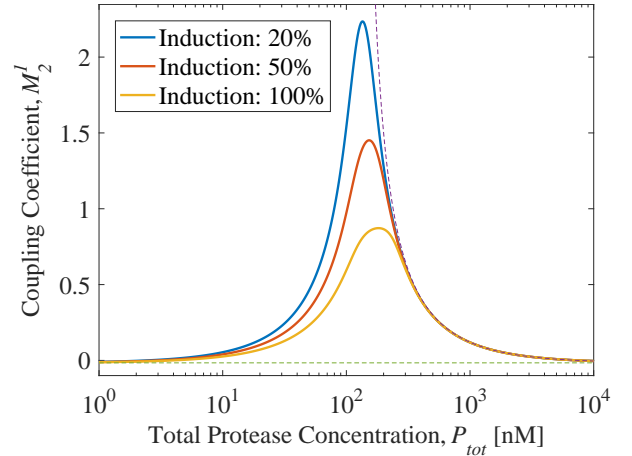


Fig. 6. The coupling coefficient of (18) for different concentrations of total protease. The purple dashed line is the coupling coefficient predicted by (25) and the green dashed line is the coupling coefficient predicted by (27). The parameters used here are $k_{1i} = 300 \text{ h}^{-1}$, $R_{tot} =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

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.

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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	δ_1	7 h^{-1} to 20 h^{-1}	Bernstein et al. (2004)
protein dilution rate	δ_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}	$\approx 100 \mu\text{M}$	estimated in Gyorgy et al. (2015)
protein with protease Michaelis-Menten constant	K_{2i}	$0.5 \mu\text{M}$ to $41 \mu\text{M}$	Farrell et al. (2007)
total RNAP concentration	$RNAP$	$1.5 \mu\text{M}$ to $11.4 \mu\text{M}$	Dennis and Bremer (2008)
total ribosome concentration	R_{tot}	$6.8 \mu\text{M}$ to $72.0 \mu\text{M}$	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)

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