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**Abstract:** Delays appear in the dynamics of many systems due to non-vanishing reaction times of control systems. In biochemical systems, long sequences of repeated steps, especially in biopolymerization processes, can be modeled by delays. However, modelling systems with delays is often complicated by physical constraints, such as the requirement that solutions representing concentrations of chemical species remain positive. In this work, we consider a model for a detoxifying enzyme whose synthesis is controlled by its substrate. The model includes binding-site clearance delays, caused by the time required for an RNA polymerase or ribosome to clear its binding site before another such machine can bind. The existence of a positive equilibrium and the positivity and boundedness of solutions of the corresponding delay-differential equations are proven. In addition, the stability of the model is studied using the "small-gain" theorem.

*Keywords:* Delay systems, biochemical systems, gene-regulatory systems.

## 1. INTRODUCTION

Delays appear in a wide variety of models, from engineering to biological systems, due to transport and propagation phenomena (see Niculescu (2001) and references therein). In addition to "physical" delays, delays may be introduced into a model to reduce the number of variables required to describe the system, sometimes leading to insights regarding the dynamical roles of some of the variables (Epstein and Luo, 1991; Roussel, 1996; Ünal et al., 2017). However, since systems with time delays are in the class of infinite-dimensional systems (Curtain and Zwart, 1995), their analysis is not an easy task. For the sake of simplicity, "sufficiently small" delays are often neglected. Since delays may however induce unexpected behaviours, neglecting small delays is not without risk (Niculescu, 2001).

The law of mass-action, which states that the rate of an elementary reaction is proportional to the product of the reactant concentrations (Waage and Guldberg, 1986), is the foundation for modeling biochemical systems. This leads to models expressed by polynomial ordinary differential equations (ODEs) with positive solutions for positive initial conditions (Horn and Jackson, 1972; Vol'pert, 1972). However, when modeling gene expression, delay-differential equations (DDEs) are often used to take into account the significant time required for transcription and translation. It is well known that delays can have important qualitative and quantitative effects on the dynamics. However, it remains to be seen if it is necessary to include some of the smaller delays associated with the clearance of binding sites for the molecular machines that carry out biopolymerizations (RNA polymerase, ribosome) found in some models (Roussel and Zhu, 2006; Zhu and Salahub, 2008). In a recent study, it was found that small binding-site delays can have dramatic effects on bifurcation diagrams of gene expression models that cannot be compensated for by, e.g., adjusting the rate constant for initiation (Trofimenkoff and Roussel, 2020). As these delays are almost always neglected in gene expression models, it seems worthwhile to explore these effects further, using a different model with a different bifurcation structure.

*Delayed mass-action kinetics* provides a convenient framework for modeling gene expression delays (Roussel, 1996) and forms the basis for this work. This formalism was originally formulated to avoid the unphysical negative solutions that plague some DDE models. A formal proof of

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the positivity of solutions of delayed mass-action models was however only recently obtained (Lipták et al., 2018).

In this work, a model for an inducible gene is considered to understand the effects of both standard synthesis (transcription and translation) and binding-site clearance delays. First, the occurrence of a single positive equilibrium for the model will be proven. Then, positivity and boundedness of the solutions of the corresponding DDEs will be discussed. Using methods of asymptotic stability analysis, a complete stability picture of the model will be presented. Numerical examples will illustrate the results, which suggest a stabilizing effect of binding-site clearance.

### *1.1 Notation and Preliminaries*

Throughout the paper, $\mathbb{C}$, $\mathbb{R}_+$, and $\mathbb{Z}_+$ are, respectively, the sets of complex numbers, of positive real numbers, and of the positive integers. $\mathbb{R}^n_{\geq 0}$ represents the non-negative orthant of $\mathbb{R}^n$, $n \in \mathbb{Z}_+$. For $\boldsymbol{x} \in \mathbb{R}^n$, $\boldsymbol{x}$ is called positive (non-negative) and bounded if $x_l > 0 (\geq 0)$ and $x_l \leq M$, for some $M$, $l = 1, 2, \ldots, n$. $\mathbb{C}_o := \{\lambda \in \mathbb{C} \mid R(\lambda) > 0\}$, where $R(\lambda)$ is the real part of $\lambda$, and $\mathbb{C}_+ := \{\lambda \in \mathbb{C} \mid R(\lambda) \geq 0\}$ is called the rhp (right half-plane), for short. $\mathcal{H}_\infty$ is the space of analytic and bounded functions in $\mathbb{C}_o$. A function $\Phi(\lambda; \tau) \in \mathcal{H}_\infty$, $\tau \in \mathbb{R}^n_{\geq 0}$, called *strictly proper in* $\mathbb{C}_o$, if $\lim_{\omega \to \infty} |\Phi(i\omega; \tau)| = 0$.

## 2. INDUCIBLE-GENE MODEL AND RELATED ASSUMPTIONS

We study a model of a gene induced by its own substrate. This is a common regulatory relationship in gene expression systems. Examples include: the *lac* operon, in which several genes related to the metabolism of lactose are induced, albeit indirectly, by lactose (Beckwith, 1987); and Hmp, the enzyme chiefly responsible for detoxifying nitric oxide (NO) in bacteria (Robinson and Brynildsen, 2013), whose synthesis is induced is a fairly direct way by the presence of NO in the cell (Poole and Hughes, 2000; Cruz-Ramos et al., 2002). The model described here is based on an earlier, highly simplified model (Roussel, 1998) of a gene induced by its own substrate. Here we formulate a delayed-mass action version of the model in order to study the effects of the binding-site clearance delays for both the promoter and ribosome binding site (RBS):

$$\xrightarrow{\kappa_{\text{in}}} \mathrm{S}(t) \tag{C1}$$

$$\mathrm{E}(t) + \mathrm{S}(t) \underset{\kappa_{-1}}{\overset{\kappa_1}{\rightleftharpoons}} \mathrm{C}(t) \xrightarrow{\kappa_{-2}} \mathrm{E}(t) + \mathrm{Q}(t) \tag{C2}$$

$$\mathrm{E}(t) \xrightarrow{\kappa_d} \tag{C3}$$

$$n\,\mathrm{S}(t) + \mathrm{A}(t) \underset{\kappa_{-3}}{\overset{\kappa_3}{\rightleftharpoons}} \mathrm{P}(t) \tag{C4}$$

$$\mathrm{P}(t) \xrightarrow{\kappa_4} \mathrm{P}(t+\tau_1) + \mathrm{R}(t+\tau_2) \tag{C5}$$

$$\mathrm{R}(t) \xrightarrow{\kappa_5} \mathrm{R}(t+\tau_3) + \mathrm{E}(t+\tau_4) \tag{C6}$$

$$\mathrm{R}(t) \xrightarrow{\kappa_6} \tag{C7}$$

In this model, $t$ denotes time. S is a substrate to be metabolized to a product Q by the enzyme E. E is synthesized by the usual gene expression pathway, namely transcription (C5) and translation (C6). The active gene promoter, P, is cleared and the RNA, R, appears $\tau_1$ and $\tau_2$ time units after transcription initiation respectively. Similarly, the RBS is cleared and E appears $\tau_3$ and $\tau_4$ time units after translation initiation, respectively. The delays $\tau_1$ and $\tau_3$ are associated with the clearance of, respectively, the RNA polymerase binding site in the promoter region of the gene, and the RBS of the messenger RNA by the corresponding biomolecular machines. These binding-site clearance delays would usually be short, for transcription in prokaryotes of the order of a few seconds (Lutz et al., 2001; Lloyd-Price et al., 2016) but sometimes up to tens of minutes (Hsu, 2002; Aye-Han and Hsu, unpublished), and in eukaryotes of the order of ten minutes (Kugel and Goodrich, 2000; Darzacq et al., 2007), which is still a short time compared to the overall transcription time, particularly in mammals due to the very long genes (Venter, 2001) and slow splicing (Singh and Padgett, 2009).

The activation of the gene by the substrate would normally involve binding or modification of a regulatory protein by the substrate, although we do not represent this binding explicitly, nor do we differentiate between the case in which binding of S to the regulatory protein derepresses the gene and the case in which S binds to a protein that activates the promoter, resulting in the effective activation reaction (C4). The cooperativity implied by reaction (C4) likewise can be thought of as a simple model of any of a variety of processes that result in sigmoidal activation kinetics (Ferrell, 1996, 1997, 1998).

For simplicity, and because we want to particularly focus on the control system, we treat the transformation of S to Q as a simple Michaelis-Menten reaction (C2), although it will usually be the case that metabolizing S will involve other substrates.

We define the following dimensionless variables: $c(\widetilde{t}) := C(t)/K_M$, $e(\widetilde{t}) := E(t)/K_M$, $s(\widetilde{t}) := S(t)/K_M$, $a(\widetilde{t}) := A(t)/K_M$, $p(\widetilde{t}) := P(t)/K_M$, $r(\widetilde{t}) := R(t)/K_M$, $\widetilde{t} := \gamma t$, where $K_M := \gamma/\kappa_1$ is the Michaelis constant, and

$$\gamma := (\kappa_{-1} + \kappa_{-2}). \tag{1}$$

We also define the dimensionless parameters $k_l = \kappa_l/\gamma$, where $l \in \{-1, -3, 4, 5, 6\}$, $k_{\text{in}} = \kappa_{\text{in}}/(K_M\gamma)$, $k_d = \kappa_d/\gamma$, $k_3 = \kappa_3 K_M^n/\gamma$, $\widetilde{\tau}_i := \gamma\tau_i$, $i \in \{1, 2, 3, 4\}$. Note that, physically, all of the dimensionless parameters must be positive quantities. Applying the delayed mass-action construction yields DDEs for the concentrations which, using the dimensionless variables and parameters defined above, become

$$\frac{d}{dt}c(t) = e(t)s(t) - c(t), \tag{2a}$$

$$\frac{d}{dt}e(t) = -e(t)s(t) - k_d e(t) + c(t) + k_5 r(t-\tau_4), \tag{2b}$$

$$\frac{d}{dt}s(t) = k_{in} - e(t)s(t) + k_{-1}c(t) - nk_3 s(t)^n a(t) + nk_{-3}p(t), \tag{2c}$$

$$\frac{d}{dt}a(t) = -k_3 s(t)^n a(t) + k_{-3}p(t), \tag{2d}$$

$$\frac{d}{dt}p(t) = k_3 s(t)^n a(t) - (k_{-3} + k_4)p(t) + k_4 p(t-\tau_1), \tag{2e}$$

$$\frac{d}{dt}r(t) = -(k_5+k_6)r(t) + k_4 p(t-\tau_2) + k_5 r(t-\tau_3), \tag{2f}$$

where the tildes have been dropped for notational simplicity. The solutions of these equations obey the following dimensionless conservation equation

$$z = a(t) + p(t) + k_4 \int_{t-\tau_1}^{t} p(\theta)\, d\theta. \tag{3}$$

We can see that $z$ is a conserved quantity by differentiating equation (3) with respect to time and applying the fundamental theorem of calculus, which gives

$$\frac{dz}{dt} = \frac{da}{dt} + \frac{dp}{dt} + k_4 p(t) - k_4 p(t-\tau_1).$$

Subtitution of equations (2d) and (2e) into the latter gives $dz/dt = 0$, thus proving that $z$ is a conserved quantity, namely the dimensionless equivalent of the total concentration of promoter. Note that for any physically sensible initial function, since concentrations must be positive, $z$ must be a positive parameter.

## 3. QUALITATIVE ANALYSIS

In this section, first, dynamic constraints such as presence of a positive equilibrium, positivity and boundedness of solutions under the positive and bounded initial functions will be considered. Then, asymptotic stability properties of the model will be presented.

### *3.1 Physical Constraints*

In open systems governed by the law of mass-action, there are no guarantees with respect to the existence or uniqueness of positive equilibria. Indeed, it is easy to write down models that have runaway solutions. These issues are even trickier in delay systems. Let $\boldsymbol{x}(t) := [\, c(t)\ e(t)\ s(t)\ a(t)\ p(t)\ r(t)\,]^T$ and $\boldsymbol{x}^*$ be the corresponding equilibrium point of the model. Then, from (2) and (3),

$$\boldsymbol{x}^* = \left[\, \beta\ \ \beta/S^*\ \ S^*\ \ K_3\beta\alpha/(S^*)^{n+1}\ \ \beta\alpha/S^*\ \ k_d\beta/(k_5 S^*)\,\right]^T, \tag{4}$$

where $\alpha := k_6 k_d/(k_4 k_5)$, $\beta := k_{\text{in}}/(1-k_{-1})$, $K_3 = k_{-3}/k_3$, and $S^*$ is the positive solution of

$$(z/(\alpha\beta))\,(S^*)^{n+1} - (k_4\tau_1+1)(S^*)^n - K_3 = 0. \tag{5}$$

*Remark 1.* Note that the equilibrium point depends on the value of the promoter clearance delay $\tau_1$. Thus, even the position of the equilibrium point depends on this small delay.

*Remark 2.* Since the coefficients of the polynomial (5) in variable $S^*$ are real and positive, and the number of sign changes is 1, by Descartes' rule, the positive solution of (5) is unique.

*Proposition 3.* Let $M := (K_3 + k_4\tau_1 + 1)\alpha\beta/z$. Then, the positive solution of (5) is bounded as follows:

$$\text{For } M > 1, \quad 1 < S^* < M; \tag{6a}$$
$$\text{for } M < 1, \quad M < S^* < 1; \tag{6b}$$
$$\text{for } M = 1, \quad S^* = 1. \tag{6c}$$

**Proof.** Equation (5) can be rearranged to

$$(S^*)^n(S^* - M) = K_3(\alpha\beta/z)(1 - (S^*)^n). \tag{7}$$

Since (5) has a positive solution $S^*$ and $M > 0$, then the left-hand side of (7) is negative iff $S^* < M$, and the right-hand side is negative iff $S^* > 1$, which proves (6a). Similarly, the left-hand side of (7) is positive iff $S^* > M$, and the right-hand side is positive iff $S^* < 1$, which proves (6b). Finally, the left-hand side passes through zero when $S^* = M$ and the right-hand side passes through zero when $S^* = 1$, which proves (6c).

*Theorem 4.* Let us consider the following DDE

$$\frac{d}{dt}x(t) = -\alpha_0 x(t) + \sum_{l=1}^{N} \alpha_l x(t-\tau_l) + Q(t), \tag{8}$$

which is supposed to have non-negative solutions for non-negative initial functions defined on $(-\max_{l=1,\dots,N}\tau_l, 0]$, with $\alpha_0 > 0$, $\alpha_l \geq 0$ and $\tau_l \geq 0$, $l = 1,\dots,N$, and $Q(t)$ non-negative and bounded. Then, the solution of (8) is bounded if $\alpha_0 > \sum_{l=1}^{N}\alpha_l$.

**Proof.** Note that (8) can be written as

$$\frac{d}{dt}\left[x(t) + \sum_{l=1}^{N}\alpha_l \int_{t-\tau_l}^{t} x(s)ds\right] = \left(-\alpha_0 + \sum_{l=1}^{N}\alpha_l\right)x(t) + Q(t). \tag{9}$$

Now, suppose that $\alpha_0 > \sum_{l=1}^{N}\alpha_l$ and that the solution is unbounded. Then, there exists a time-sequence $\{t_n\} \to \infty$ as $n \to \infty$ such that

$$\left(\frac{d}{dt}\left[x(t) + \sum_{l=1}^{N}\alpha_l \int_{t-\tau_l}^{t} x(s)ds\right]\right)_{t=t_n} > 0,$$

which implies by (9),

$$Q(t_n) > \left(\alpha_0 - \sum_{l=1}^{N}\alpha_l\right)x(t_n). \tag{10}$$

However, (10) holds only if the solutions are non-negative. Thus, since $Q(t)$ is assumed to be non-negative and bounded, non-negative solutions cannot be unbounded.

*Proposition 5.* The solutions of the DDEs (2) are non-negative and bounded whenever the initial history functions are non-negatively valued and bounded.

**Proof.** Non-negativity follows from the proof of Lipták et al. (2018) that the solutions of delayed mass-action systems are non-negative when the initial functions are non-negative. By (3), since $z$ is constant, $a(t)$ and $p(t)$ are bounded. Then, by Theorem 4, $r(t)$ is bounded. Moreover, from (2e) it can be shown that

$$p(t) = -k_4\int_{t-\tau_1}^{t} p(\nu)d\nu - k_{-3}\int_{t_o}^{t} p(\nu)d\nu + p(t_o) + k_4\int_{t_o-\tau_1}^{t_o} p(\nu)d\nu + \int_{t_o}^{t} k_3 s(\nu)^n a(\nu)d\nu.$$

Then, since $p(t)$ is bounded, $s(t)$ cannot be unbounded. Now, assume that $c(t)$ is unbounded. Then, there exist a sequence $\{t_k\} \to \infty$ as $k \to \infty$ such that

$$c(t_k) < c(t_{k+1}), \quad k \in \mathbb{Z}_+. \tag{11}$$

Then, since $p(t)$, $s(t)$ and $a(t)$ are bounded and non-negative, and $e(t)$ is also non-negative, (11) and (2c) together would imply that $\frac{d}{dt}s(t_k) > 0$ for $k > N$, where $N$ is sufficiently large. However, this contradicts the boundedness of $s(t)$, so $c(t)$ must be bounded. Now,

assume that $e(t)$ is unbounded. Then, there exists a sequence $\{t_k\} \to \infty$ as $k \to \infty$ such that $\frac{d}{dt}e(t_k) > 0$. Then, from (2b), $c(t_k) + k_5 r(t_k - \tau_4) > e(t_k)(k_d + s(t_k))$, $k \in \mathbb{Z}_+$. However, since $c(t)$ and $r(t)$ are non-negative and bounded, by the above inequality, $e(t_k)$, $k \in \mathbb{Z}_+$, cannot be unbounded.

### *3.2 Asymptotic Stability Properties*

Let $\delta\boldsymbol{x}(t) := \boldsymbol{x}(t) - \boldsymbol{x^*}$, where $\boldsymbol{x^*}$ is given in (4). Then, linearization of (2a)–(2f) around $x^*$ results in

$$\dot{\delta x}(t) = A\delta x(t) + \sum_{i=1}^{4} A_i \delta x(t - \tau_i), \tag{12}$$

where

$$A = \begin{bmatrix} -1 & S^* & \beta/S^* & 0 & 0 & 0 \\ 1 & -k_d - S^* & -\beta/S^* & 0 & 0 & 0 \\ k_{-1} & -S^* & -\beta/S^* - ng & -nk_3 S^{*n} & nk_{-3} & 0 \\ 0 & 0 & -g & -k_3 S^{*n} & k_{-3} & 0 \\ 0 & 0 & g & k_3 S^{*n} & -k_4 - k_{-3} & 0 \\ 0 & 0 & 0 & 0 & 0 & -k_5 - k_6 \end{bmatrix},$$

$A_1 = \begin{pmatrix} \mathbf{0}_{4\times 4} & \mathbf{0} \\ \mathbf{0} & k_4 \\ & 0 \end{pmatrix}$, $A_2 = \begin{pmatrix} \mathbf{0}_{5\times 4} & \mathbf{0} \\ \mathbf{0} & k_4 \; 0 \end{pmatrix}$, $A_3 = \begin{pmatrix} \mathbf{0}_{5\times 5} & 0 \\ \mathbf{0} & k_5 \end{pmatrix}$, $A_4 = \begin{pmatrix} \mathbf{0} & 0 \\ \mathbf{0}_{4\times 5} & k_5 \\ & \mathbf{0} \end{pmatrix}$, and $g = \alpha k_{-3} n\beta/S^*$.

Then, the characteristic function of (12) can be written as

$$\begin{aligned}\Delta(\lambda;\tau) = \lambda\left(\Delta_1(\lambda)Q_2(\lambda;\tau_1) + k_{-3}\Delta_{1s}(\lambda)\right)Q_3(\lambda;\tau_3) \\ + (k_6 k_d k_{-3}\beta n/S^*)\lambda\left(\lambda + 1 - k_{-1}\right)e^{-\tau_T\lambda}\end{aligned} \tag{13}$$

where $\tau = (\tau_1, \tau_3, \tau_T) \in \mathbb{R}^3_{\geq 0}$ with

$$\tau_T = \tau_2 + \tau_4, \tag{14}$$

and

$$\Delta_1(\lambda) = (\lambda + S^{*n}k_3)\Delta_{1s}(\lambda) + (gn/S^*)\lambda Q_1(\lambda), \tag{15a}$$
$$\Delta_{1s}(\lambda) = \lambda Q_1(\lambda) + \beta(\lambda + k_d)(\lambda + 1 - k_{-1})/S^*, \tag{15b}$$
$$Q_1(\lambda) = k_d + \lambda(1 + S^* + k_d) + \lambda^2, \tag{15c}$$
$$Q_2(\lambda;\tau_1) = 1 + (k_4/\lambda)\left(1 - e^{-\lambda\tau_1}\right), \tag{15d}$$
$$Q_3(\lambda;\tau_3) = \lambda + k_6 + k_5(1 - e^{-\tau_3\lambda}). \tag{15e}$$

*Remark 6.* Note that the stability of the equilibrium point depends on the values of the clearance delays $\tau_1$ and $\tau_3$ as well as on the total expression delay $\tau_T$.

*Theorem 7.* Let $f(\lambda;\tau) \in \mathcal{H}_\infty$, where $\tau \in \mathbb{R}^n_{\geq 0}$ and $\sup_{\omega\in\mathbb{R}} |f(i\omega;\tau)| < 1$ for all $\tau \in \mathbb{R}^n_{\geq 0}$. Then $(1 + f(\lambda;\tau))$ has no zeros in $\mathbb{C}_+$.

**Proof.** It is a consequence of the "small-gain" theorem, see Zhou et al. (1996).

*Proposition 8.* Let $\Phi(\lambda;\tau)$ be a strictly proper function in $\mathbb{C}_o$ and suppose that $|\Phi(0;\tau)| > 1$, $\tau \in \mathbb{R}^m_{\geq 0}$. Then, there exists some positive $\tau_T$ such that

$$\Delta(\lambda;\tau,\tau_T) := 1 + e^{-\lambda\tau_T}\Phi(\lambda;\tau)$$

has a zero at $i\omega_o$, where $\omega_o > 0$.

**Proof.** Note, $\Delta(i\omega;\tau,\tau_T) = 0$ for some positive $\omega$ iff $e^{-i\omega\tau_T}\Phi(i\omega;\tau) = -1$. Then, since $|\Phi(j\omega;\tau)| \to 0$ as $\omega \to \infty$ and it is supposed that $|\Phi(0;\tau)| > 1$, $\tau \in \mathbb{R}^m_{\geq 0}$, there always exists a finite positive $\omega_o$ such that $|\Phi(i\omega_o;\tau)| = 1$. Then, $\Delta(i\omega_o;\tau,\tau_T) = 0$ for $\tau_T = ((2l-1)\pi - \arg(\Phi(i\omega_o;\tau)))/\omega_o)$, where $l$ is a some positive integer ensuring the positivity of $\tau_T$.

*Proposition 9.* The rightmost real zero of the characteristic function is $\lambda = 0$ and it is simple.

**Proof.** Note, since $\lambda = 0$ is removable singularity of $(1 - e^{-\tau_1\lambda})/\lambda$, from (13), it is obvious that $\Delta(0;\tau) = 0$, $\tau \in \mathbb{R}^m_{\geq 0}$. In addition, since $\left(\frac{d}{d\rho}\Delta(\rho;\tau)\right)$ is strictly positive for $\rho \geq 0$, 0 is simple and the right-most real zero of $\Delta(\lambda;\tau)$, $\tau \in \mathbb{R}^m_{\geq 0}$.

*Proposition 10.* Assume that $2k_4\tau_1 < 1$ and

$$k_{-3}/\left(k_3 S^{*n}\sqrt{1 - 2k_4\tau_1}\right) < 1. \tag{16}$$

Then,

$$G(\lambda;\tau_1) = \Delta_1(\lambda)Q_2(\lambda;\tau_1) + k_{-3}\Delta_{1s}(\lambda) \tag{17}$$

has no zeros in the rhp.

**Proof.** From (15a) and (15b), $\Delta_1(\lambda)$ and $\Delta_{1s}(\lambda)$ are respectively 4th and 3rd order polynomials with positive real coefficients. Then, by the Hurwitz-criterion, it can be shown that $\Delta_1(\lambda)$ has no zeros in $\mathbb{C}_+$, therefore $H(\lambda) := \Delta_{1s}(\lambda)/\Delta_1(\lambda)$ is analytic in $\mathbb{C}_o$. In addition, since it can be shown that $\frac{\partial}{\partial\omega}|H(i\omega)|^2 < 0$, $\omega \in \mathbb{R}$, then, $\sup_{\omega\in\mathbb{R}}|H(i\omega)| = |H(0)| = (S^{*n}k_3)^{-1}$. Thus, $H(\lambda) \in \mathcal{H}_\infty$. As discussed above, $\widehat{Q}_2(\lambda;\tau_1) := (1 - e^{-\tau_1\lambda})/\lambda$ is analytic in $\mathbb{C}_o$. In addition, since $\sup_{\omega\in\mathbb{R}}\left|\widehat{Q}_2(i\omega;\tau_1)\right| = \tau_1$ and $k_4\tau_1 < 1/2$, by Theorem 7 and from (15d), $Q_2(\lambda;\tau_1)$ has no zeros in $\mathbb{C}_o$. Therefore, $G_1(\lambda;\tau_1) := H(\lambda)/Q_2(\lambda;\tau_1)$ is analytic in $\mathbb{C}_o$. Since

$$\begin{aligned}|Q_2(i\omega;\tau_1)|^2 = 1 + \left(k_4\tau_1\frac{\sin(\omega\tau_1)}{(\omega\tau_1)}\right)^2 + 2k_4\tau_1\frac{\sin(\omega\tau_1)}{(\omega\tau_1)} \\ + \left(k_4\tau_1\left(\frac{1 - \cos(\omega\tau_1)}{\omega\tau_1}\right)\right)^2 \geq 1 - 2k_4\tau_1,\end{aligned} \tag{18}$$

then

$$\begin{aligned}\sup_{\omega\in\mathbb{R}}|G_1(i\omega;\tau_1)| &\leq \sup_{\omega\in\mathbb{R}}|H(i\omega)|\frac{1}{\inf_{\omega\in\mathbb{R}}|Q_2(i\omega;\tau_1)|} \\ &\leq \frac{k_{-3}}{\left(\sqrt{1 - 2k_4\tau_1}\right)S^{*n}k_3},\end{aligned} \tag{19}$$

which ensures that $G_1(\lambda;\tau_1) \in \mathcal{H}_\infty$. Furthermore, since it is assumed that $k_{-3}/\left(k_3 S^{*n}\sqrt{1 - 2k_4\tau_1}\right) < 1$, then, by Theorem 7, $1 + G_1(\lambda;\tau_1)$ has no zeros in the rhp for all non-negative $\tau_1$. Then, since (17) can be written as

$$G(\lambda;\tau_1) = Q_2(\lambda;\tau_1)\Delta_1(\lambda)\left(1 + G_1(\lambda;\tau_1)\right),$$

$G(\lambda;\tau_1)$ has no zeros in the rhp for all non-negative $\tau_1$.

*Proposition 11.* Suppose that $k_{-3}/\left(k_3 S^{*n}\sqrt{1 - 2k_4\tau_1}\right) < 1$ and $2k_4\tau_1 < 1$. Then, $\Delta(\lambda;\tau)$ has a zero on the imaginary axis, except $\lambda = 0$, for some $\tau_T$, if

$$k_{-3}n\alpha\beta > zk_3 S^{*(n+1)}. \tag{20}$$

**Proof.** Note, since $k_5$ and $k_6$ are positive, $\widehat{Q}_3(\lambda;\tau_3) := k_5 e^{-\lambda\tau_3}/(\lambda + k_5 + k_6)$ is analytic in $\mathbb{C}_o$, $\tau_3 \geq 0$. In addition, since $\sup_{\omega\in\mathbb{R}}\left|\widehat{Q}_3(i\omega;\tau_3)\right| = k_5/(k_6 + k_5) < 1$, $\widehat{Q}_3(\lambda;\tau_3) \in \mathcal{H}_\infty$. In addition, by Theorem 7 and (15e)

$$(\lambda + k_5 + k_6)\left(1 - \widehat{Q}_3(\lambda;\tau_3)\right),$$

has no zeros in $\mathbb{C}_+$. Note, by Proposition 10, $G_d(\lambda;\tau_1,\tau_3) = G(\lambda;\tau_1)Q_3(\lambda;\tau_3)$, where $G(\lambda;\tau_1)$ is defined as in (17), has no zero in the rhp. Then,

$$\Phi(\lambda;\tau_1,\tau_3) = \frac{k_6 k_d k_{-3}\beta n}{S^*}\frac{\lambda+1-k_{-1}}{G_d(\lambda;\tau_1,\tau_3)}$$

is analytic in $\mathbb{C}_o$. Furthermore, since

$$\sup_{\omega\in\mathbb{R}}|\Phi(j\omega;\tau_1,\tau_3)| \le \frac{k_6 k_d k_{-3}\beta n}{S^*}\sup_{\omega\in\mathbb{R}}\left|\frac{i\omega+1-k_{-1}}{Q_3(i\omega;\tau_3)}\right| \times \frac{1}{\inf_{\omega\in\mathbb{R}}|G(i\omega;\tau_1)|} < \infty, \quad (21)$$

$\Phi(\lambda;\tau_1,\tau_3) \in \mathcal{H}_\infty$. Then, by (5), since

$$|\Phi(0;\tau_1,\tau_3)| = \frac{k_{-3}n}{S^{*n}k_3(1+k_4\tau_1)+k_{-3}} = \frac{k_{-3}n\alpha\beta}{k_3 z S^{*n+1}}$$

which is assumed to be strictly greater than 1, by Proposition 8, there exists a positive $\tau_T$ such that

$$\widehat{\Delta}(\lambda;\tau,\tau_T) = 1 + e^{-\tau_T\lambda}\Phi(\lambda;\tau_1,\tau_3) \quad (22)$$

has a zero at some $i\omega_o$, $\omega_o > 0$. Note, from (13), since $\Delta(\lambda;\tau) = \lambda G_d(\lambda;\tau_1)\widehat{\Delta}(\lambda;\tau,\tau_T)$, $\Delta(i\omega_o;\tau) = 0$.

## 4. NUMERICAL RESULTS

By using the QPmR software package (Vyhlídal and Zítek, 2014), zeros of $\Delta(\lambda;\tau)$ were computed as shown in Fig. 1(a). As seen in the figure, $\Delta(\lambda;\tau)$ has imaginary axis zeros (in addition to the zero at the origin) for $\tau_T \approx 40$, using parameters that satisfy both (16) and (20). A limit-cycle solution of equations (2) for the same parameter set and a value of $\tau_T$ above the Andronov-Hopf bifurcation value is shown in Fig. 1(b) [computed using the stiff integrator in XPPAUT (Ermentrout, 2002), and reducing the step size until solutions of consistent amplitude were obtained]. The model displays sustained oscillations, as was the case for the simpler single-delay model (Roussel, 1998). When the value of the promoter clearance delay $\tau_1$ is halved, from 0.04 to 0.02, the limit cycle becomes substantially larger [Fig. 1(c)]. Note that, relative to $\tau_T$, $\tau_1$ is a small delay that might have been thought to have been negligible. We see here however that it has a dramatic effect on the oscillations. In fact, since the limit cycle shrinks as $\tau_1$ increases, we see that the promoter clearance process has a stabilizing effect. The RBS clearance delay $\tau_3$ has a similar effect (not shown). The stabilizing effect of the binding-site clearance delays is likely related to what MacDonald (1987) called an "interference effect" of independent delays.

## 5. CONCLUSION

In this work, a model for a gene regulatory system incorporating both conventional production delays and binding-site clearance delays was presented. We analyzed these equations to show that they have bounded solutions for all positive values of the parameters provided the initial functions are non-negative. We also studied the stability properties of the model, developing the conditions for an Andronov-Hopf bifurcation. We found that the binding-site clearance delays, usually neglected in gene expression models, in fact have a significant effect on the dynamics. In both this and a previous paper (Trofimenkoff and Roussel, 2020), binding-site clearance delays were found to have a

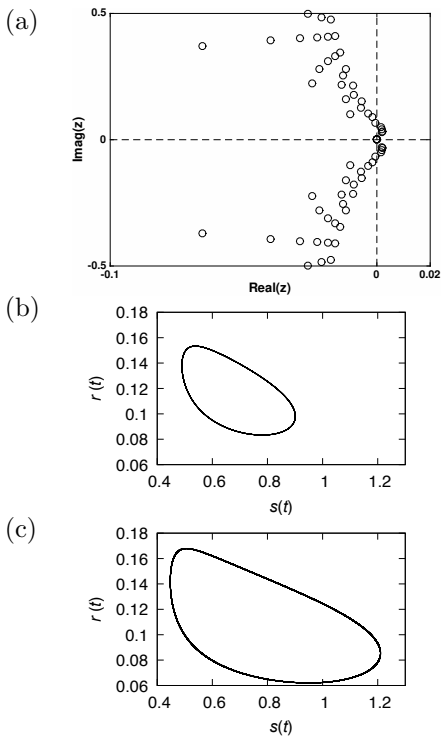


Fig. 1. Characteristic function zeros and numerical solutions of the model for the parameter set $S_1 = \{k_{in} = 2, k_{-1} = 0.001, k_3 = 3, k_{-3} = 1, k_4 = 20, k_5 = 80, k_6 = 0.1, k_d = 3, K_M = 16\times10^{-7}, \tau_1 = 0.04, \tau_3 = 0.005, n = 3\}$. (a) Zeros of the characteristic function while $5 \le \tau_T \le 100$. (b) Limit cycle for $\tau_2 = 35$, $\tau_4 = 20$ ($\tau_T = 55$) and initial conditions $c = e = s = m = a = 0$, $p = z = 0.001$. (c) Same as (b), except $\tau_1 = 0.02$.

stabilizing effect. It is an interesting question whether this will generally be the case. If so, one wonders if the lengthy clearance times observed in some systems (Hsu, 2002; Aye-Han and Hsu, unpublished) are an evolved mechanism for stabilizing steady states.

These studies also suggest that it would be worth revisiting some classic gene expression models with delays to see if similarly large effects are seen with modest clearance delays in a wider range of models than we have so far examined. It would also be interesting to examine the effects of clearance delays in models implementing the network motifs with delays recently studied by Glass et al. (2021). The authors of this study suggested, for example, that the response delays encoded in biological networks may have evolved to suppress chaos. If so, the stabilizing tendency of binding-site clearance delays may play an important role in avoiding dynamical regimes that imperil a cell.

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