A Systems Theory with Retroactivity: Application to Transcriptional Modules

D. Del Vecchio, A. J. Ninfa, and E. D. Sontag

Abstract— In standard control systems theory, a system is modeled as an input/output (I/O) module with internal dynamics. This implicitly assumes that the dynamics of a module do not change when the module is connected to other components. However, such an assumption is not realistic in a wide number of electrical, hydraulic, mechanical, and biological systems. We thus propose a new model that incorporates signals that travel from downstream to upstream, which we broadly call retroactivity. We quantify such a retroactivity in transcriptional components and show how to attenuate its effect by the design of insulation devices.

I. Introduction

The property of modularity covers a fundamental role both for constructing synthetic systems by the composition of simple units and for predicting the behavior of natural systems by the behavior of their components. Such a desirable property guarantees that the input/output behavior of a component does not change upon interconnection. As it occurs in several engineering systems such as electrical, mechanical, and hydraulic systems, the property of modularity does not generally hold in biological systems. Upon interconnection, the behavior of an "upstream" component (the one that sends the signal) is affected by the presence of the "downstream" component (the one that receives the signal). We broadly call *retroactivity* the phenomenon by which the behavior of an upstream component changes upon interconnection. The above considerations strongly motivate the need for a novel theoretical framework to formally define and quantify retroactivity effects. In this paper, we present such a formalism, illustrate it with engineering and biological examples, and study general approaches to the reduction of retroactivity. The standard model, used in virtually every control and systems theory mathematical and engineering textbook since the 1950s, e.g. [9], is based on the view of devices described solely in terms of input channels, output channels, and state (internal, non-shared) variables. A notable exception to this standard model is found in the work of Willems [5]. Willems has emphasized the fact that, for many physical situations, directionality of signals is an artificial, and technically wrong, assumption. While agreeing with this general point of view, we argue that, in certain circumstances such as those illustrated in this paper, it is important to distinguishing between input and output channels. Thus,

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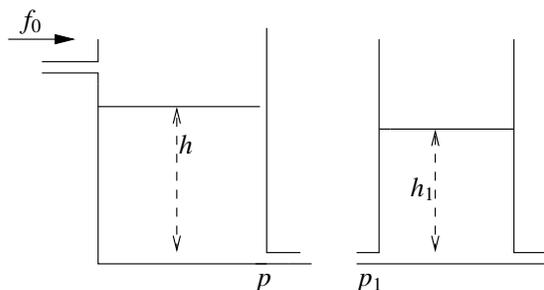
Fig. 1. On the left, we represent a tank system that takes as input the constant flow f_0 and gives as output the pressure p at the output pipe. On the right, we show a downstream tank.

instead of blurring the distinction between inputs, states, and outputs as in Willems work, we prefer to keep these three distinct entities but augment the model with two additional signals, namely the retroactivities to inputs and to outputs, respectively.

Example. As a simple example, consider the one-tank system shown on the left of Figure 1. We consider a constant input flow f_0 as input to the tank system and the pressure p at the output pipe is considered the output of the tank system. The corresponding output flow is given by $k\sqrt{p}$, in which k is a positive constant depending on the geometry of the system. The pressure p is given by (neglecting the atmospheric pressure for simplicity) $p = \rho h$, in which h is the height of the water level in the tank and ρ is water density. Let A be the cross section of the tank, then the tank system can be represented by the equation $A\frac{dp}{dt} = \rho f_0 - \rho k \sqrt{p}$. Let us now connect the output pipe of the same tank to the input pipe of a downstream tank shown on the right of Figure 1. Let $p_1 = \rho h_1$ be the pressure generated by the downstream tank at its input and output pipes. Then, the flow at the output of the upstream tank will change and will now be given by $g(p, p_1) = k\sqrt{|p - p_1|}$ if $p > p_1$ and by $g(p, p_1) = -k\sqrt{|p - p_1|}$ if $p \leq p_1$. As a consequence, the time behavior of the pressure p generated at the output pipe of the upstream tank will change to

$$A\frac{dp}{dt} = \rho f_0 - \rho g(p, p_1)$$
$$A_1\frac{dp_1}{dt} = \rho g(p, p_1) - \rho k_1 \sqrt{p_1},$$

in which A_1 is the cross section of the downstream tank and k_1 is a positive parameter depending on the geometry of the downstream tank. Thus, the input/output response of the tank measured in isolation does not stay the same when the tank

is connected through its output pipe to another tank. We will model this phenomenon by a signal that travels from downstream to upstream, which we call *retroactivity*. The amount of such a retroactivity will change depending on the features of the interconnection and of the downstream system. For example, if the aperture of the pipe connecting the two tanks is very small compared to the aperture of an output pipe of the downstream tank, the pressure p at the output of the upstream tank will not change much when the downstream tank is connected. We thus model a system

Fig. 2. A system S input and output signals. The red signals denote signals originating by retroactivity upon interconnection.

by adding an additional input, called s, to the system to model any change in its dynamics that may occur upon interconnection with a downstream system. Similarly, we add to a system a signal r as another output to model the fact that when such a system is connected downstream of another system, it will send upstream a signal that will alter the dynamics of the upstream system. More generally, we define a system S to have internal state x, two types of inputs (I), and two types of outputs (O): an input "u" (I), an output "y" (O), a *retroactivity to the input* "r" (O), and a *retroactivity to the output* "s" (I) (Figure 2). We will thus represent a system S by the equations

$$\dot{x} = f(x, u, s), \; y = Y(x, u, s), \; r = R(x, u, s), \qquad (1)$$

in which f, Y, R are arbitrary functions and the signals x, u, s, r, y may be scalars or vectors. In such a formalism, we define the input/output model of the isolated system as the one in equations (1) without r in which we have also set $s = 0$. Let S_i be a system with inputs u_i and s_i and with outputs y_i and r_i. Let S_1 and S_2 be two systems with disjoint sets of internal states. We define the interconnection of an upstream system S_1 with a downstream system S_2 by simply setting $y_1 = u_2$ and $s_1 = r_2$. For interconnecting two systems, we require that the two systems do not have internal states in common.

In this paper, we focus on transcriptional components. We analyze first the dynamics of a component in isolation and then we quantify the change in its dynamics, the retroactivity, due to the interconnection with other modules (Section II). We show in Section III how insulation between an upstream component and a downstream one can be attained by connecting them through an *insulation device*. A biological realization of an insulation device is proposed in Section IV.

II. Retroactivity in transcriptional components

It has been proposed by previous authors [6], [7] that the occurrence of retroactivity, that is, having nonzero signals r and s, depends on the specific choice of input u and output

y. In the context of a gene transcriptional network, it is not clear whether choices that lead to negligible retroactivity are generally possible. We thus consider the input u and output y of the system to be fixed *a priori*. We then attenuate the effects of a nonzero s by a suitable feedback mechanisms and we decrease the value of r by specific physical component choices. For the transcriptional components considered in this paper, we let protein concentration play the role of both the input and output signals u and y. In the sequel, we denote by X the protein, by X (italics) the protein concentration, and by x (lower case) the gene expressing protein X. A transcriptional component that takes as input protein concentration Z and gives as output protein concentration X is shown in Figure 3 in the dashed box. In particular, protein Z is a transcription factor that binds to the operator sites on the promoter controlling gene x. The output protein concentration X is the concentration of the protein expressed by the gene x. The activity of the promoter controlling gene

Transcriptional component
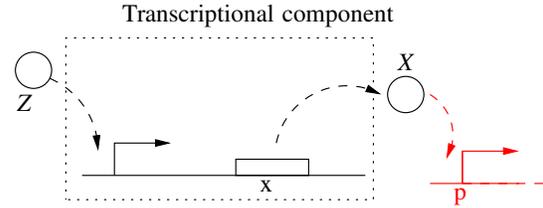
Fig. 3. The transcriptional component takes as input u protein concentration Z and gives as output y protein concentration X. The transcription factor Z binds to operator sites on the promoter. The red part belongs to a downstream transcriptional block that takes protein concentration X as its input.

x will depend on the amount of Z bound to the promoter. If $Z = Z(t)$, such an activity will also be changing in time. We denote it by $k(t)$. By neglecting the mRNA dynamics, we can write the dynamics of X as [1]

$$\frac{dX}{dt} = k(t) - \delta X, \qquad (2)$$

in which δ is the decay rate of the protein. We will refer to equation (2) as the isolated system response. Now, assume that X drives a downstream transcriptional block by binding to a promoter p with concentration p (the red part of Figure 3). The reversible binding reaction of X with p is given by X+p $\frac{k_{on}}{k_{off}}$ C, in which C is the complex protein-promoter. Since the promoter is not subject to decay, its total concentration p_{TOT} is conserved so that we can write $p + C = p_{TOT}$. Therefore, the new dynamics of X is governed by the equations

$$\frac{dX}{dt} = k(t) - \delta X + \boxed{k_{off}C - k_{on}(p_{TOT} - C)X}$$
$$\frac{dC}{dt} = -k_{off}C + k_{on}(p_{TOT} - C)X, \qquad (3)$$

in which the terms in the box represent the signal s, that is, the retroactivity to the output, while the second of equations (3) describes the dynamics of the interconnection mechanism. When $s = 0$, the first of equations (3) reduces
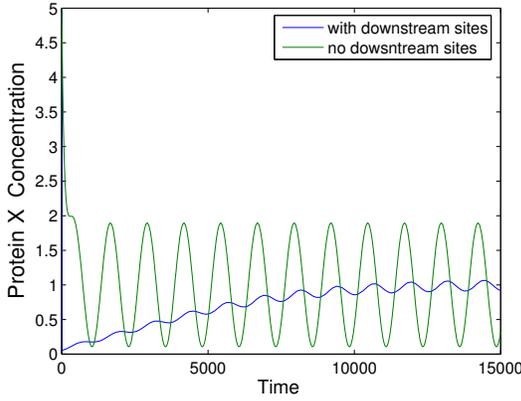
Fig. 4. Simulation results for the system in equations (3). In the plot, $k(t) = 0.01(1 + sin(\omega t))$ with $\omega = 0.005$, $k_{on} = 10$, $k_{off} = 10$, $\delta = 0.01$, $p_{TOT} = 100$, $X(0) = 5$. The amount of downstream binding sites is large compared to $X(0)$ as it occurs in synthetic systems in which these sites are present in high copy-number plasmids. Time is in minutes. The green plot represents $X(t)$ originating by the isolated system in equations (2), while the blue plot represents $X(t)$ obtained by the interconnected system of equation (3). Both transient and permanent behaviors are different.

to the dynamics of the isolated system given in equation (2). The effect of the retroactivity s on the behavior of X can be very large (Figure 4). This is undesirable in a number of situations in which we would like an upstream system to "drive" a downstream one as is the case, for example, when a biological oscillator has to time a number of downstream processes. If, due to the retroactivity, the output signal of the upstream process becomes too low and/or out of phase with the output signal of the isolated system (as in Figure 4), the coordination between the oscillator and the downstream processes will be lost. In order to counteract the effect of the retroactivity to the output, we quantify it and determine the biological parameters that affect its value.

A. Quantification of the retroactivity to the output

In this section, we quantify the difference between the dynamics of X in equation (2) and the dynamics of X in equations (3) by establishing conditions on the biological parameters that make the two dynamics close to each other. This is achieved by exploiting the difference of time scales between the protein production and decay processes and its binding and unbinding process to the promoter p. By virtue of this separation of time scales, we can reduce system (3) to a one dimensional system describing the evolution of X on the slow manifold [4]. This way, we can simply compare two one-dimensional systems. Consider again the full system in equations (3), in which $k_{off} \gg \delta$ [1] and $k_{on} = k_{off}/k_d$ with $k_d = O(1)$. To explicitly model the difference in time scales between the two equations of system (3), we introduce a parameter ϵ, which we define as $\epsilon = \delta/k_{off}$. Since $k_{off} \gg \delta$, $\epsilon \ll 1$. Substituting $k_{off} = \delta/\epsilon$ and $k_{on} = \delta/(\epsilon k_d)$ in system (3), we obtain the system $\frac{dX}{dt} = k(t) - \delta X + \frac{\delta}{\epsilon}C - \frac{\delta}{\epsilon k_d}(p_{TOT} - $

$C)X$ and $\frac{dC}{dt} = -\frac{\delta}{\epsilon}C + \frac{\delta}{\epsilon k_d}(p_{TOT} - C)X$, in which the singular perturbation parameter ϵ appears in both equations. We can take the above system to standard singular perturbation form by performing the change of variable $y = X + C$, in which y physically corresponds to the *total* concentration of protein X. Then, the system in the new variables becomes

$$\begin{aligned} \frac{dy}{dt} &= k(t) - \delta(y - C) \\ \epsilon\frac{dC}{dt} &= -\delta C + \frac{\delta}{k_d}(p_{TOT} - C)(y - C), \end{aligned} \quad (4)$$

which is in standard singular perturbation form. This means, as some authors recently proposed [3], that y (total concentration of protein) is the slow variable of the system (3) as opposed to X (concentration of free protein). By setting $\epsilon = 0$ in the second one of system (4), we obtain the slow manifold. The dynamics of (4) restricted to the slow manifold are a good approximation of the dynamics of system (4) only if the slow manifold is asymptotically stable. Before approximating system (4) by its dynamics on the slow manifold, we thus verify first that the slow manifold is asymptotically stable. For a variable v involved in system (4), we denote by $\bar{v}$ the value of the variable v once we have set $\epsilon = 0$ in system (4). Let $g(C, y) := -\delta C + \frac{\delta}{k_d}(p_{TOT} - C)(y - C)$ and let $\gamma(\bar{y})$ be such that $g(\gamma(\bar{y}), \bar{y}) = 0$. Then, $\bar{C} = \gamma(\bar{y})$ defines the slow manifold. Model (4) reduced to the slow manifold leads to the reduced model

$$\frac{d\bar{y}}{dt} = k(t) - \delta(\bar{y} - \gamma(\bar{y})). \quad (5)$$

Let $\tau = t/\epsilon$ and let $e_C = C - \bar{C}$ be the error between C and its approximation $\bar{C}$. The dynamics of such an error, called the boundary layer system, is given by

$$\frac{de_C}{d\tau} = -\delta(e_C + \bar{C}) + \frac{\delta}{k_d}(p_{TOT} - e_C - \bar{C})(\bar{y} - e_C - \bar{C}), \quad (6)$$

and describes the dynamics of the error of C with respect to $\bar{C}$, in which $\bar{y}$ and thus $\bar{C}$ are considered frozen at the initial condition. Since we desire C to tend to $\bar{C}$, we study the stability of the equilibrium point $e_C = 0$ of equation (6).

Proposition 1: The equilibrium $e_C = 0$ of the boundary layer system (6) is asymptotically stable uniformly in $\bar{y}$ and $\partial g/\partial C|_{\bar{C}(t),\bar{y}(t)}$ has real part smaller than a fixed negative number.

Proof: One can easily verify that $\partial g/\partial C|_{\bar{C}(t),\bar{y}(t)} \leq -\delta$ and that $\frac{de_C}{d\tau} = -K(\bar{y})e_C + \frac{\delta}{k_d}e_C^2$, in which $K(\bar{y}) \geq K_0$ with K_0 independent of $\bar{y}$. Therefore, the asymptotic stability is uniform in $\bar{y}$. ∎

It thus follows that (Theorem 3.1 [4]) if $e_C(0)$ is in the region of attraction of the equilibrium $e_C = 0$, then there are positive constants ϵ^*, t_1, t_2 with $0 < t_1 < t_2 < \infty$ such that for all $\epsilon < \epsilon^*$, we have $y(t) = \bar{y}(t) + O(\epsilon)$, for all $t \in [0, t_2]$ and $C(t) = \bar{C}(t) + O(\epsilon)$, for all $t \in [t_1, t_2]$. As a consequence, we also have that $X(t) = \bar{X}(t) + O(\epsilon)$, for all $t \in [t_1, t_2]$. Since $\bar{X}(t) = \bar{y}(t) - \bar{C}(t)$, the differential equation that $\bar{X}$ satisfies is given by $\frac{d\bar{X}}{dt} = \frac{d\bar{y}(t)}{dt} - \frac{d\gamma(\bar{y})}{d\bar{y}}\frac{d\bar{y}(t)}{dt}$, which finally leads to

$$\frac{d\bar{X}}{dt} = (k(t) - \delta\bar{X})(1 - \frac{d\gamma(\bar{y})}{d\bar{y}}). \quad (7)$$

After a fast transient, $X(t)$ will follow $\bar{X}(t)$ solution of equation (7). We thus assume that for $t \in [t_1, t_2]$ we have that $X(t) \approx \bar{X}(t)$ and we quantify the retroactivity to the output s after a fast transient by quantifying the difference between the dynamics in equation (7) (the connected system) and the dynamics in equation (2) (the isolated system). Such dynamics are the same when $\frac{d\gamma(\bar{y})}{d\bar{y}} = 0$. We thus take as a measure of the retroactivity at interconnection between transcriptional modules the quantity $\mathcal{R}(\bar{X}, t) = |\frac{d\gamma(\bar{y})}{d\bar{y}}|$, in which $\frac{d\gamma(\bar{y})}{d\bar{y}}$ may be viewed as depending only on time as γ is a function of $\bar{y}$ and $\bar{y}$ is the solution of equation (5). The retroactivity measure $\mathcal{R}$ can also be interpreted as a percentage variation of the dynamics of the connected system with respect to the dynamics of the isolated system. We next determine the physical meaning of the retroactivity measure defined above by determining what the key physical parameters are that regulate the value of $\mathcal{R}$. In the sequel, we will omit the bar from the variables on the slow manifold to simplify notation.

Proposition 2: The value of the retroactivity measure is given by

$$\mathcal{R}(X, t) = \frac{1}{1 + \frac{(1+X/k_d)^2}{p_{TOT}/k_d}} \tag{8}$$

and $\mathcal{R}(X, t) < 1$.

Proof: suppose that $\gamma(y)$ satisfies that $g(\gamma(y), y) = 0$, where $g(C, y) = \delta\left[-C + \frac{1}{k_d}(p_{TOT} - C)(y - c)\right]$. We want to calculate $d\gamma/dy$.

$$d\gamma/dy = -\frac{\partial g/\partial y}{\partial g/\partial C} = \frac{\frac{1}{k_d}(p_{TOT} - C)}{1 + \frac{1}{k_d}(p_{TOT} - C) + \frac{1}{k_d}(y - C)}$$

so substituting $\frac{1}{k_d}(y - C) = \frac{C}{p_{TOT}-C}$ this equals

$$\frac{1}{1 + \frac{k_d p_{TOT}}{(p_{TOT}-C)^2}} = \frac{1}{1 + \frac{k_d}{p_{TOT}}\left(1 + \frac{C}{p_{TOT}-C}\right)^2}$$

and now substituting $\frac{C}{p_{TOT}-C} = \frac{1}{k_d}(y - C)$ we conclude that this equals $\frac{1}{1 + \frac{k_d}{p_{TOT}}\left(1 + \frac{1}{k_d}(y-C)\right)^2}$, in which $y - C = X$. ∎

Retroactivity is small if $p_{TOT}/k_d \ll 1$. If this condition is not satisfied, the only way to make $\mathcal{R}$ small is to have X large enough compared to p_{TOT}. A similar measure of the retroactivity to the input r of a transcriptional module can be obtained as

$$\mathcal{R}(Z, t) = \frac{1}{1 + \frac{(1+\frac{Z}{\bar{k}_d})^2}{\frac{p_{0,TOT}}{\bar{k}_d}}}, \tag{9}$$

in which $\bar{k}_d = k_-/k_+$ and $1/\bar{k}_d$ is the affinity of Z to its target sites p_0 (with total concentration $p_{0,TOT}$) on the promoter controlling gene x.

III. Attenuation of the retroactivity to the output by feedback

Consider a system S as the one shown in Figure 2 that takes u as input and gives y as output. We would like to design it in such a way that (a) the retroactivity r to the input is very small; (b) the effect of the retroactivity s to the output on the internal dynamics of the system is very small independently of s itself; (c) the u to y response is about linear. Such a system is said to enjoy the **insulation** property and will be called an insulation component. In electronics, amplifiers enjoy the insulation property by virtue of the features of the operational amplifier (OPAMP) that they employ [8]. In such electronic components, r is very small because the input stage of an OPAMP absorbs almost zero current. This way, there is no voltage drop across the output impedance of an upstream voltage source. Similarly, equation (9) provides a measure of the retroactivity to the input r of a transcriptional system after a fast transient as a function of biological parameters that characterize the interconnection mechanism. To reduce the amount of r, we can choose $\bar{k}_d$ large (low affinity) and $p_{0,TOT}$ small, for example. Having low affinity means that there is a low "flow" of protein Z that goes to bind its target sites. Thus, we can say that a low retroactivity to the input is obtained when the "input flow" to the system is small. Such an interpretation can be further carried to the hydraulic example. If the input flow to the downstream tank is small compared, for example, to the output flow of the downstream tank, the output pressure of the upstream tank will not be affected by the connection. Therefore, the retroactivity to the input of the downstream tank will be small.

In electronic amplifiers, the effect of s on the amplifier behavior is reduced to almost zero by virtue of a large (theoretically infinite) amplification gain of the OPAMP and an equally large negative feedback mechanism that regulates the output voltage. In order to show the generality of such a mechanism, we show how it can be applied to the academic hydraulic example consisting of two connected tanks shown in Figure 5. The objective is to attenuate the effect of the pressure applied from the downstream tank to the upstream tank, so that the output pressure of the upstream system does not change when the downstream tank is connected. We let
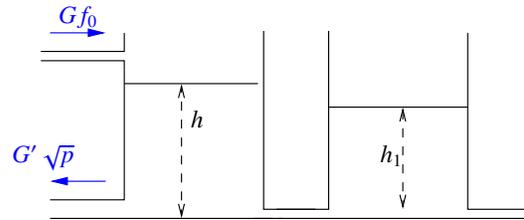
Fig. 5. We amplify the input flow f_0 through a large gain G and we apply a large negative feedback by employing a large output pipe with output flow $G' \sqrt{p}$.

the input flow f_0 be amplified by a large factor G. Also, we consider a large pipe in the upstream tank with output flow $G' \sqrt{p}$, with $G' \gg k$ and $G' \gg k_1$. Let p be the pressure at the output pipe of the upstream tank and p_1 the pressure at the bottom of the downstream tank. One can verify that the only equilibrium value for the pressure p at the output pipe

of the upstream tank is obtained for $p > p_1$ and it is given by

$$p_{eq} = \left(\frac{Gf_0}{G' + (kk_1)/\sqrt{k_1^2 + k^2}} \right)^2.$$

If we let G' be sufficiently larger than k_1 and k and we let $G' = KG$ for some positive $K = O(1)$, then for G sufficiently large $p_{eq} \approx (f_0/K)^2$, which does not depend on the presence of the downstream system. In fact, it is the same as the equilibrium value of the isolated upstream system $A\frac{dp}{dt} = \rho Gf_0 - \rho G'\sqrt{p} - \rho k\sqrt{p}$ for G sufficiently large and for $G' = KG$ with $K = O(1)$. *How do we attenuate the effect of the retroactivity to the output for protein and gene systems?* This mechanism is summarized by the following lemma and corollary in a form that is directly applicable to the isolated and connected systems (systems (2) and (7)), as they appear after a short transient, once a large amplification gain G and an equally large feedback are employed.

Lemma 1: Consider the system $\frac{dX}{dt} = G(t)(u(t) - KX)$ in which $G(t) \geq G_0 > 0$ and $|u'(t)| \leq V$ uniformly in t. Then,

$$|X(t) - \frac{u(t)}{K}| \leq \exp(-tG_0K)|X(0) - \frac{u(0)}{K}| + \frac{V}{G_0K^2}.$$

Proof: Let $e = X - u/K$. The error dynamics is given by $\dot{e} = -G(t)Ke - \frac{\dot{u}(t)}{K}$. The solution of such a differential equation is provided by $e(t) = e(0)\exp(-\int_0^t KG(\tau)d\tau) + \int_0^t \exp(-\int_\tau^t KG(\sigma)d\sigma)\frac{u'(t)}{K}d\tau$. Since $|u'(t)| \leq V$ and $G(t) \geq G_0 > 0$ for all t, we have that $|X(t) - \frac{u(t)}{K}| \leq \exp(-tG_0K)|X(0) - \frac{u(0)}{K}| + (1 - \exp(-tG_0K))V/(G_0K^2)$. ∎

Corollary 1: Consider the two systems

$$\frac{dX_r}{dt} = G(u(t) - KX_r) \text{ and } \frac{dX}{dt} = \bar{G}(t)(u(t) - KX), \quad (10)$$

in which $|u'(t)| \leq V$, $\bar{G}(t) \geq G_0$, and $G \geq G_0$ for $G_0 > 0$. Then, for a suitable nonnegative constant C_0

$$|X(t) - X_r(t)| \leq \exp(-tG_0K)C_0 + 2\frac{V}{G_0K^2}.$$

Proof: We can apply Lemma 1 to the two systems in equation (10), separately. This along with the triangular inequality $|X(t) - X_r(t)| \leq |X(t) - u(t)/K| + |X_r(t) - u(t)/K|$ leads to $|X(t) - X_r(t)| \leq \exp(-tG_0K)C_0 + 2\frac{V}{G_0K^2}$, for a suitable nonnegative constant C_0 depending on the initial conditions. ∎

Let us now consider the isolated system (2) and the connected system (7) and assume that we can amplify with gain G the input $k(t)$ and apply an additional negative feedback $-G'X$, in which $G' = \alpha G$ for some $\alpha = O(1)$. Then, we obtain the two systems (isolated an connected) as

$$\frac{dX_r}{dt} = G(k(t) - (\alpha + \delta/G)X_r) \quad (11)$$

and

$$\frac{dX}{dt} = G(k(t) - (\alpha + \delta/G)X)(1 - d(t)) \quad (12)$$

respectively, in which $d(t) = \frac{d\gamma(y)}{dy}$ and $y(t)$ given by the reduced system $\frac{dy}{dt} = Gk(t) - (G' + \delta)(y - \gamma(y))$. We can apply Corollary 1 to the two systems (11) and (12) with

$\bar{G}(t) = G(1 - d(t))$, $K = (\alpha + \delta/G)$, and $k(t) = u(t)$, to obtain that $X(t)$ can be made close to $X_r(t)$ by increasing the gain G. *How can we obtain a large amplification gain and a large negative feedback in a biological insulation component?* This realization question is addressed in the following section.

IV. A BIOLOGICAL REALIZATION OF THE INSULATION COMPONENT

We propose a design that realizes a large amplification of the input signal $Z(t)$ by having promoter p_0 (to which Z binds) be a strong, non leaky, promoter. The negative feedback mechanism on X relies on enhanced degradation of X. Since this must be large, one possible way to obtain an enhanced degradation for X is to have a protease, called Y, be expressed by a strong constitutive promoter. The protease Y will cause a degradation rate for X, which is larger if Y is more abundant in the system. This design is schematically shown in Figure 6. The expression of gene x is assumed to
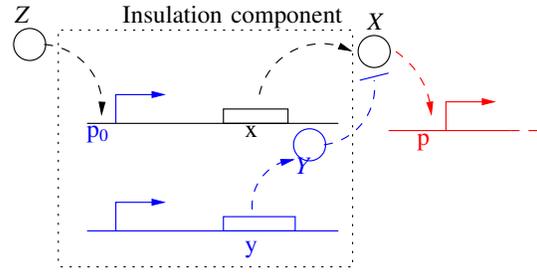
Fig. 6. Insulation device realization. Post-translational regulation of X realizes an enhanced degradation. The promoter p_0 enables a large signal amplification. The red part indicates the downstream component that takes as input the concentration of protein X.

be a two-step process, which incorporates also the mRNA dynamics. Incorporating these dynamics in the model is relevant for the current study because they may contribute to an undesired delay between the Z and X signals. The reaction of the protease Y with protein X is modeled as $X + Y \underset{\eta_2}{\overset{\eta_1}{\rightleftharpoons}} W \overset{\beta}{\rightarrow} Y$, which can be found in standard references (see [2], for example). The input/output system model of the insulation component that takes Z as an input and gives X as an output is given by the following equations

$$\frac{dZ}{dt} = k(t) - \delta Z + \boxed{k_- Z_p - k_+ Z(p_{0,TOT} - Z_p)} \quad (13)$$

$$\frac{dZ_p}{dt} = k_+ Z(p_{0,TOT} - Z_p) - k_- Z_p \quad (14)$$

$$\frac{dm_X}{dt} = GZ_p - \delta_1 m_X \quad (15)$$

$$\frac{dX}{dt} = \nu m_X - \eta_1 YX + \eta_2 W - \delta_2 X + \boxed{k_{off}C - k_{on}X(p_{TOT} - C)} \quad (16)$$

$$\frac{dW}{dt} = \eta_1 XY - \eta_2 W - \beta W \quad (17)$$

$$\frac{dY}{dt} = -\eta_1 YX + \beta W + \alpha G - \gamma Y + \eta_2 W \quad (18)$$

$$\frac{dC}{dt} = -k_{off}C + k_{on}X(p_{TOT} - C), \quad (19)$$

in which the expression of gene z is controlled by a promoter with activity $k(t)$. These equations will be studied numerically and analyzed mathematically in a simplified form. The variable Z_p is the concentration of protein Z bound to the promoter controlling gene x, $p_{0,TOT}$ is the total concentration of the promoter p_0 controlling gene x, m_X is the concentration of messenger RNA of X, C is the concentration of X bound to the downstream binding sites with total concentration p_{TOT}, γ is the decay rate of the protease Y. The value of G is the production rate of X mRNA per unit concentration of Z bound to the promoter controlling x; the promoter controlling gene y has strength αG, for some constant α, and it has the same order of magnitude strength as the promoter controlling x. The terms in the box in equation (13) represent the retroactivity r to the input of the insulation component in Figure 6. The terms in the box in equation (16) represent the retroactivity s to the output of the insulation component of Figure 6. For the discussion regarding the attenuation of the effect of s, it is not relevant what the specific form of signal $Z_p(t)$ is. Let then $Z_p(t)$ be any bounded signal $v(t)$. Since equation (15) takes $v(t)$ as an input, we will have that $m_X = G\bar{v}(t)$, for a suitable signal $\bar{v}(t)$. Let us assume for the sake of simplifying the analysis that the protease reaction is a one step reaction, that is, $X + Y \xrightarrow{\beta} Y$. Therefore, equation (18) simplifies to $\frac{dY}{dt} = \alpha G - \gamma Y$ and equation (16) simplifies to $\frac{dX}{dt} = vm_X - \beta Y X - \delta_2 X + k_{off}C - k_{on}X(p_{TOT} - C)$. If we consider the protease to be at its equilibrium, we have that $Y(t) = \alpha G/\gamma$. As a consequence, the X dynamics becomes

$$\frac{dX}{dt} = vG\bar{v}(t) - (\beta\alpha G/\gamma + \delta_2)X + \boxed{k_{off}C - k_{on}X(p_{TOT} - C)},$$

with C determined by equation (19). By using the same singular perturbation argument employed in the previous section, we obtain that the dynamics of X will be after a fast transient approximatively given by

$$\frac{dX}{dt} = (vG\bar{v}(t) - (\beta\alpha G/\gamma + \delta_2)X)(1 - d(t)), \qquad (20)$$

in which $0 < d(t) < 1$ is the effect of the retroactivity s. Corollary 1 can be applied to systems (20) and $\frac{dX_r}{dt} = vG\bar{v}(t) - (\beta\alpha G/\gamma + \delta_2)X_r$ (the isolated system dynamics) to conclude that as G grows $X(t)$ and $X_r(t)$ become close to each other.

Finally, to decrease r we have to guarantee that the retroactivity measure given in equation (9) is small. This is seen to be true if $(\bar{k}_d + Z)^2/(p_{0,TOT}\bar{k}_d)$ is very large, in which $1/\bar{k}_d = k_+/k_-$ is the affinity of the binding site p_0 to Z. Since after a short transient, $Z_p = (p_{0,TOT}Z)/(\bar{k}_d + Z)$, for Z_p not to be a distorted version of Z, it is enough to ask that $\bar{k}_d \gg Z$. This, combined with the requirement that $(\bar{k}_d + Z)^2/(p_{0,TOT}\bar{k}_d)$ is very large, leads to the requirement $p_{0,TOT}/\bar{k}_d \ll 1$. Summarizing, for not having distortion effects between Z and Z_p and small retroactivity r, we need that $\bar{k}_d \gg Z$ and $p_{0,TOT}/\bar{k}_d \ll 1$. Simulation results are shown in Figure 7.

V. Conclusions and Future Work

A modeling framework for systems with retroactivity was proposed. In the context of transcriptional networks, a
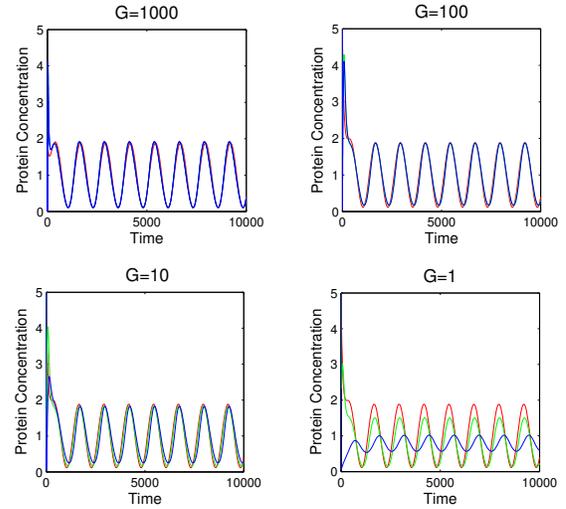
Fig. 7. Simulation results for different gains G for system in equations (13-19). In all plots, red (dotted line) is the input Z to the insulation device, green (solid line) is the output X of the insulation device in isolation (without the downstream binding sites p), blue (dashed line) is the output X of the insulation device when downstream sites p are present. In all plots, $k(t) = 0.01(1 + sin(\omega t))$, $p_{TOT} = 100$, $k_{off} = k_{on} = 10$, $\delta = 0.01$, and $\omega = 0.005$. The parameter values are $\delta_1 = 0.01$, $p_{0,TOT} = 1$, $\eta_1 = \eta_2 = \beta = \gamma = 0.01$, $k_- = 200$, $k_+ = 10$, $\alpha = 0.1$, $\delta_2 = 0.1$, $v = 0.1$, and $G = 1000, 100, 10, 1$. The retroactivity to the output is not well attenuated for values of the gain $G = 1$ and the attenuation capability begins to worsen for $G = 10$.

retroactivity measure was computed. A mechanism based on large input amplification and large negative feedback was shown to attenuate the retroactivity effects. In our future work, we will experimentally validate in synthetic biomolecular systems both the proposed retroactivity measure and the insulation device mechanism.

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