

# Engineering Principles in Bio-molecular Systems: From Retroactivity to Modularity

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**Abstract**—Modularity plays a fundamental role in the prediction of the behavior of a system from the behavior of its components, guaranteeing that the properties of individual components do not change upon interconnection. Just as electrical, hydraulic, and other physical systems often do not display modularity, nor do many biochemical systems, and specifically, genetic and signaling networks. Here, we study the effect of interconnections on the input/output dynamic characteristics of transcriptional components, focusing on a concept, which we call “retroactivity” that plays a role similar to impedance in electrical circuits. In order to attenuate the effect of retroactivity on a system dynamics, we propose to design insulation devices based on a feedback mechanism inspired by the design of amplifiers in electronics. In particular, we introduce a bio-molecular realization of an insulation device based on phosphorylation.<sup>1</sup>

## I. INTRODUCTION

A common approach to study the behavior of a complex system is to decompose it into simpler components with known functions and to then predict the behavior of the overall system by those of the components. This approach has been successfully applied in engineering disciplines such as Electrical Engineering and Control System Design. More recently, it has been argued for the recognition of functional “modules” as a critical level of biological organization [1, 10], which could be employed to decipher the complex behavior of bio-molecular networks. Examples of functional modules are signaling subsystems such as MAPK cascades, or machinery for protein synthesis or DNA replication [3, 16]. This modular approach is even more relevant in the nascent field of Synthetic Biology, in which synthetic bio-molecular “circuits” composed of genes and proteins are synthesized and then placed into living cells (through the process of transformation or transfection) to control cell behavior [2, 4, 8, 9, 22].

The modular approach to analysis and design is based on the tacit assumption that the 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). Consider for

example the oscillator of [4] as a source generator to be employed to synchronize a number of downstream transcriptional processes. The oscillator is “interconnected” with these downstream processes by having one of the proteins of the oscillator be a transcription factor for the downstream transcriptional processes. These downstream processes in turn act as a load on the oscillator by using up its output protein and by thus affecting its dynamics. 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 review a recently proposed framework for studying, characterizing, and designing systems with retroactivity [5–7]. We illustrate this framework with engineering and biological examples, and study general approaches to the reduction of retroactivity by setting a disturbance attenuation problem.

The principle of studying complex systems through decomposition and interconnection techniques is at the heart of systems and control theory. Approaches based on this general principle range from passivity and more generally dissipativity-based analysis [19, 31, 32, 34, 35], to the derivation of stability properties of large interconnected systems from the graph-theoretic properties of interconnections and stability of individual systems [18, 33], to the use of backstepping feedback approaches [15, 27] based on input to state stability [30]. Our work complements, but differs from, problems of optimally partitioning large networks into “modules” for which retroactivity-like effects are minimized, which typically employ graph theoretic and statistical approaches [2, 14, 17, 20, 23, 28]. In contrast, and similar to the work in [26], we are not concerned with network topology but with the understanding of dynamical behavior. Our ultimate goal is not top-down partitioning or to necessarily minimize retroactivity, but to formally define and characterize these effects especially in view of enabling modular assembly of synthetic bio-molecular networks.

The standard model, used in virtually every control and systems theory mathematical and engineering textbook since the 1950s, e.g. [29], 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 [21]. 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

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<sup>1</sup>This paper is a review of results that have already appeared in [5–7].

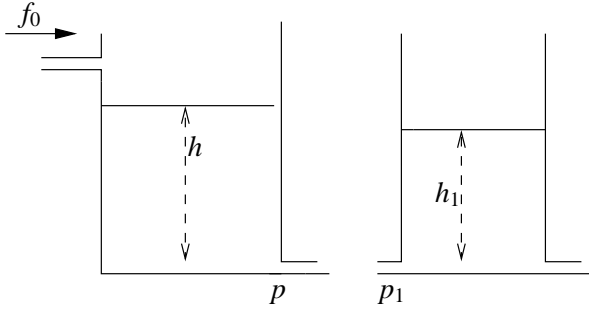


Fig. 1. On the left, we represent a tank system that takes as input the constant flow  $f_0$  and gives as output the pressure  $p$  at the output pipe. On the right, we show a downstream tank.

illustrated in this paper, it does make sense to distinguish between input and output channels. Thus, 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.

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  $\rho$  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

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

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. It is therefore not the case that the input/output response of the tank measured in isolation stays the same when the tank is connected through its output pipe to another tank. The dynamics of the pressure  $p$  changes upon interconnection. In this example, the interconnection mechanism between an upstream system and a downstream system affects the dynamics of the internal state and thus of the output of the upstream system. 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 propose to directly model a system by

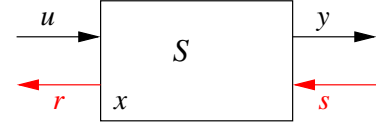


Fig. 2. A system  $S$  input and output signals. The red signals denote signals originating by retroactivity upon interconnection.

taking into account the interconnection mechanism. That is, we can add 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 will 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

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

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  $s = 0$ . In practice, it is simpler to model the isolated system first, and only later model the interconnection mechanism to obtain model (1). 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. For example, in the case of transcriptional components, this would mean that the two transcriptional components express different protein species; in the case of electrical circuits, this would mean that the two circuits do not share common electrical parts except for the ones that establish the interconnection mechanism.

## II. RETROACTIVITY IN A TRANSCRIPTIONAL SYSTEM

Transcriptional networks are usually viewed as the input/output interconnection of fundamental modules, transcriptional components, that take a transcription factor as an input and produce a transcription factor as an output [1].

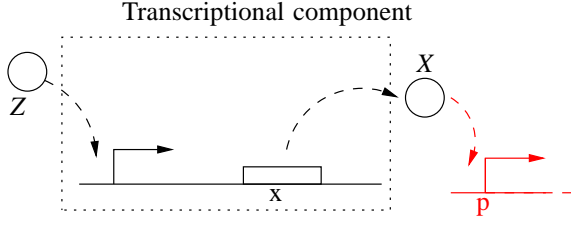


Fig. 3. The transcriptional component takes as input  $u$  protein concentration  $Z$  and gives as output  $y$  protein concentration  $X$ .

However, we showed in [6] that the behavior of a transcriptional component in isolation differs from that of the same component when connected in the network. To illustrate this point, consider a transcriptional component, whose output is connected to downstream processes, which can be, for example, other transcriptional components (Figure 3). The activity of the promoter controlling gene  $x$  depends on the amount of  $Z$  bound to the promoter. If  $Z = Z(t)$ , such an activity changes with time. We denote it by  $k(t)$ . By neglecting the mRNA dynamics, which are not relevant for the current discussion, we can write the dynamics of  $X$  as

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

in which  $\delta$  is the decay rate of the protein. We refer to equation (2) as the isolated system dynamics. Now, assume that  $X$  drives a downstream transcriptional module by binding to a promoter  $p$  with concentration  $p$  (3). The reversible binding reaction of  $X$  with  $p$  is given by  $X + p \xrightleftharpoons[k_{off}]{k_{on}} C$ , in which  $C$  is the complex protein-promoter and  $k_{on}$  and  $k_{off}$  are the binding and dissociation rates of the protein  $X$  to the promoter site  $p$ . 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

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

in which  $s = k_{off}C - k_{on}(p_{TOT} - C)X$  is the retroactivity to the output. Then, we can interpret  $s$  as being a mass flow between the upstream and the downstream system. When  $s = 0$ , the first of equations (3) reduces 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. We focus on the retroactivity to the output  $s$ . We can analyze the effect of the retroactivity to the input  $r$  on the upstream system by simply analyzing the

dynamics of  $Z$  in the presence of its binding sites  $p_0$  in Figure 3 in a way similar to how we analyze the dynamics of  $X$  in the presence of the downstream binding sites  $p$ .

#### A. Quantification of the retroactivity to the output

An operative quantification of the retroactivity to the output can be obtained by exploiting the difference of time-scales between the dynamics of the output of the upstream module and the dynamics of the input stage of the downstream module. This separation of time-scales is always encountered in transcriptional circuits [1]. We quantify the difference between the dynamics of  $X$  in the isolated system (equation (2)) and the dynamics of  $X$  in the connected system (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 approximate system (3) by a one dimensional system describing the evolution of  $X$  on the slow manifold [13]. This reduced system takes the form  $\frac{d\bar{X}}{dt} = k(t) - \delta\bar{X} + \bar{s}$ , where  $\bar{X}$  is an approximation of  $X$  and  $\bar{s}$  is an approximation of  $s$ , which can be written as  $\bar{s} = -\mathcal{R}(\bar{X})(k(t) - \delta\bar{X})$  with (see [6, 7] for details)

$$\mathcal{R}(\bar{X}) = \frac{1}{1 + \frac{(1 + \bar{X}/k_d)^2}{p_{TOT}/k_d}}. \quad (4)$$

The expression  $\mathcal{R}(\bar{X})$  quantifies the retroactivity to the output on the dynamics of  $X$  after a fast transient, when we approximate  $X$  with  $\bar{X}$  in the limit in which  $\epsilon \approx 0$ . The retroactivity measure is thus low if the affinity of the binding sites  $p$  is small ( $k_d$  large) or if the signal  $X(t)$  is large enough compared to  $p_{TOT}$ . Thus, the expression of  $\mathcal{R}(\bar{X})$  provides an operative quantification of the retroactivity: such an expression can in fact be evaluated once the association and dissociation constants of  $X$  to  $p$  are known, the concentration of the binding sites  $p_{TOT}$  is known, and the range of operation of the signal  $\bar{X}(t)$  that travels across the interconnection is also known.

### III. DESIGN OF INSULATION DEVICES TO ATTENUATE RETROACTIVITY

Of course, it is not always possible to design an interconnection such that the retroactivity is low. This is, for example, the case of an oscillator that has to time a downstream load: the load cannot be in general designed and the oscillator must perform well in the face of unknown and possibly variable load properties. Therefore, in analogy to what is performed in electrical circuits, one can design a device to be placed between the oscillator and the load so that the device output is not changed by the load and the device does not affect the behavior of the upstream oscillator. Specifically, 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; (c) its input/output

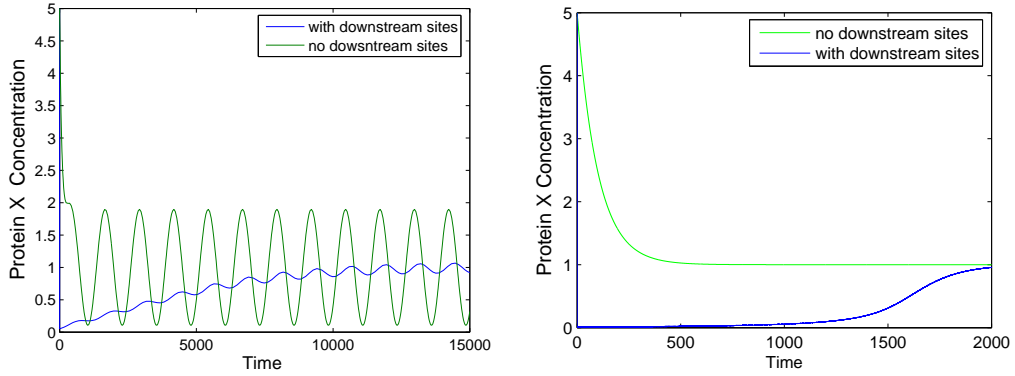


Fig. 4. The dramatic effect of interconnection. Simulation results for the system in equations (3). The green line represents  $X(t)$  originating by equations (2), while the blue line represents  $X(t)$  obtained by equation (3). Both transient and permanent behaviors are different. Here,  $k(t) = 0.01(1 + \sin(\omega t))$  with  $\omega = 0.005$  in the left side plots and  $\omega = 0$  in the right side plots,  $k_{on} = 10$ ,  $k_{off} = 10$ ,  $\delta = 0.01$ ,  $p_{TOT} = 100$ ,  $X(0) = 5$ . The choice of protein decay rate (in  $\text{min}^{-1}$ ) corresponds to a half life of about one hour. The frequency of oscillations is chosen to have a period of about 12 times the protein half life in accordance to what is experimentally observed in the synthetic clock of [4].

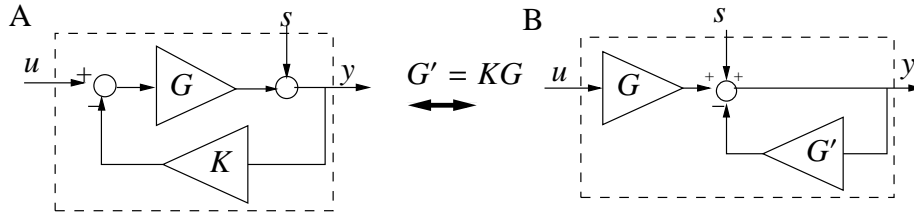


Fig. 5. Diagram A shows the basic feedback/amplification mechanism by which amplifiers attenuate the effect of the retroactivity to the output  $s$ . Diagram B shows an alternative representation of the same mechanism of diagram A, which will be employed to design biological insulation devices.

relationship is about linear. Such a system is said to enjoy the **insulation** property and will be called an insulation device. Indeed, such a system will not affect an upstream system because  $r \approx 0$  and it will keep the same output signal  $y$  *independently* of any connected downstream system. The concept of amplifier in the context of a biochemical network has been considered before in relation to its robustness and insulation property from external disturbances ([25] and [24]). Here, we revisit the amplifier mechanism in the context of gene transcriptional networks with the objective of mathematically and computationally proving how suitable biochemical realizations of such a mechanism can attain properties (a), (b), and (c).

In electronic amplifiers,  $r$  is very small because the input stage of an operational amplifier (OPAMP) absorbs almost zero current. This way, there is no voltage drop across the output impedance of an upstream voltage source. Equation (4) quantifies the effect of retroactivity on the dynamics of  $X$  as a function of biochemical parameters that characterize the interconnection mechanism with a downstream system. These parameters are the affinity of the binding site  $1/k_d$ , the total concentration of such binding site  $p_{TOT}$ , and the level of the signal  $X(t)$ . Therefore, to reduce retroactivity, we can choose  $k_d$  large (low affinity) and  $p_{TOT}$  small, for example. Having small value of  $p_{TOT}$  and/or low affinity implies that there is a small “flow” of protein X toward

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. This interpretation establishes a nice analogy to the electrical case, in which low retroactivity to the input is obtained by a low input current. In electronic amplifiers, the effect of the retroactivity to the output  $s$  on the amplifier behavior is reduced to almost zero by virtue of a large (theoretically infinite) input amplification gain and a negative output feedback. Such a mechanism can be illustrated in its simplest form by Figure 5A, which is very well known to control engineers. For simplicity, we have assumed in such a diagram that the retroactivity  $s$  is just an additive disturbance. The reason why for large gains  $G$  the effect of the retroactivity  $s$  to the output is negligible can be verified through the following simple computation. The output  $y$  is given by  $y = G(u - Ky) + s$ , which leads to  $y = \frac{G}{1+KG}u + \frac{s}{1+KG}$ . As  $G$  grows,  $y$  tends to  $u/K$ , which is independent of the retroactivity  $s$ .

Therefore, a central enabler to attenuate the retroactivity effect at the output of a component is to (1) amplify through a large gain the input of the component and (2) to apply a large negative output feedback (Figure 5B).

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 6. 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 the

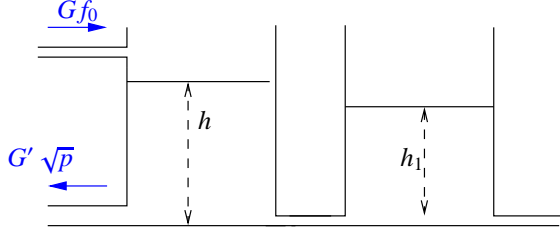


Fig. 6. We amplify the input flow  $f_0$  through a large gain  $G$  and we apply a large negative feedback by employing a large output pipe with output flow  $G' \sqrt{p}$ .

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  $A \frac{dp}{dt} = \rho G f_0 - \rho G' \sqrt{p} - \rho k \sqrt{p}$  for  $G$  sufficiently large and for  $G' = KG$  with  $K = O(1)$ .

We next illustrate this idea in the context of the transcriptional example. Consider the approximated dynamics of  $X$ . Let us assume that we can apply a gain  $G$  to the input  $k(t)$  and a negative feedback gain  $G'$  to  $X$  with  $G' = KG$ . This leads to the new differential equation for the connected system given by

$$\frac{dX}{dt} = (Gk(t) - (G' + \delta)X)(1 - \mathcal{R}(X)). \quad (5)$$

It can be shown (see [7] for details) that as  $G$  and thus as  $G'$  grow, the signal  $X(t)$  generated by the connected system (5) becomes close to the solution  $X(t)$  of the isolated system

$$\frac{dX}{dt} = Gk(t) - (G' + \delta)X, \quad (6)$$

that is, the presence of the disturbance term  $\mathcal{R}(X)$  will not significantly affect the time behavior of  $X(t)$ . *How can we obtain a large amplification gain  $G$  and a large negative feedback  $G'$  in a biological insulation component?* This question is addressed in the following section, in which we show that a simple phosphorylation/dephosphorylation cycle has remarkable insulation properties (for additional designs of bio-molecular insulation devices, the reader is referred to [6]).

#### A. A bio-molecular realization of an insulation device through protein phosphorylation

In this design, we propose to obtain input amplification through a fast phosphorylation reaction and negative feed-

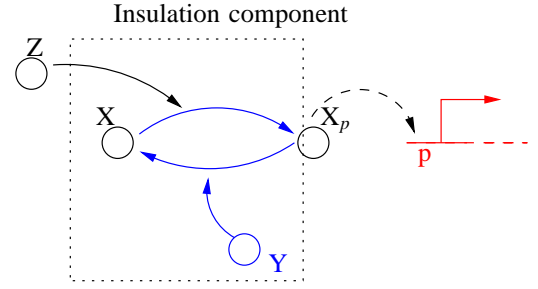


Fig. 7. The dashed box contains the insulation device.

back through a fast dephosphorylation reaction. In particular, this is realized by having  $Z$  activate the phosphorylation of a protein  $X$ , which is available in the system in abundance. That is,  $Z$  is a kinase for a protein  $X$ . The phosphorylated form of  $X$ , called  $X_p$ , binds to the downstream sites, while  $X$  does not. A negative feedback on  $X_p$  is obtained by having a phosphatase  $Y$  activate the dephosphorylation of protein  $X_p$ . Protein  $Y$  is also available in abundance in the system. This mechanism is depicted in Figure 7. A similar design has been proposed by [24, 25], in which a MAPK cascade plus a negative feedback loop that spans the length of the MAPK cascade is considered as a feedback amplifier. Our design is much simpler as it involves only one phosphorylation cycle and does not require the additional feedback loop.

We consider a one-step reaction model for the phosphorylation reactions to convey the idea of how this device realizes the insulation function. The one step model that we consider is the one of [11]  $Z + X \xrightarrow{k_1} Z + X_p$  and  $Y + X_p \xrightarrow{k_2} Y + X$ . We assume that there is plenty of protein  $X$  and of phosphatase  $Y$  in the system and that these quantities are conserved. The conservation of  $X$  gives  $X + X_p + C = X_{TOT}$ , in which  $X$  is the inactive protein,  $X_p$  is the phosphorylated protein that binds to the downstream sites  $p$ , and  $C$  is the complex of the phosphorylated protein  $X_p$  bound to the promoter  $p$ . The  $X_p$  dynamics can be described by the first equation in the following model

$$\frac{dX_p}{dt} = k_1 X_{TOT} Z(t) \left( 1 - \frac{X_p}{X_{TOT}} - \frac{C}{X_{TOT}} \right) - k_2 Y X_p + \frac{k_{off} C - k_{on} X_p (p_{TOT} - C)}{X_{TOT}} \quad (7)$$

$$\frac{dC}{dt} = -k_{off} C + k_{on} X_p (p_{TOT} - C). \quad (8)$$

The boxed terms represent the retroactivity  $s$  to the output of the insulation system of Figure 7. For a weakly activated pathway ([11]),  $X_p \ll X_{TOT}$ . Also, if we assume that the concentration of total  $X$  is large compared to the concentration of the downstream binding sites, that is,  $X_{TOT} \gg p_{TOT}$ , equation (7) is approximately equal to  $\frac{dX_p}{dt} = k_1 X_{TOT} Z(t) - k_2 Y X_p + k_{off} C - k_{on} X_p (p_{TOT} - C)$ .

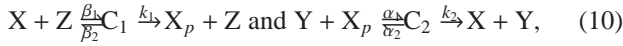
Denote  $G = k_1 X_{TOT}$  and  $G' = k_2 Y$ . Exploiting again the difference of time scales between the  $X_p$  dynamics and the  $C$  dynamics, after a fast initial transient, the dynamics of  $X_p$

can be well approximated by

$$\frac{dX_p}{dt} = (GZ(t) - G'X_p)(1 - \mathcal{R}(X_p)), \quad (9)$$

in which  $\mathcal{R}(X_p)$  is the measure of the retroactivity  $s$  to the output after a short transient. Therefore, for  $G$  and  $G'$  large enough,  $X_p(t)$  tends to the solution  $X_p(t)$  of the isolated system  $\frac{dX_p}{dt} = GZ(t) - G'X_p$ . As a consequence, the effect of the retroactivity to the output  $s$  is attenuated by increasing  $k_1X_{TOT}$  and  $k_2Y$  enough. That is, to obtain large input and feedback gains, one should have large phosphorylation/dephosphorylation rates and/or a large amount of protein X and phosphatase Y in the system. This reveals that  $G \propto X_{TOT}k_1$  and  $G' \propto Yk_2$ .

A more complex model for the phosphorylation and dephosphorylation reactions can be considered and parametric analysis can be performed to highlight the roles of the various parameters for attaining the insulation properties. In particular, we consider a two-step reaction model such as those in [12]. According to this model, we have the following two reactions for phosphorylation and dephosphorylation, respectively:



in which  $C_1$  is the [protein X/kinase Z] complex and  $C_2$  is the [phosphatase Y/protein  $X_p$ ] complex. Additionally, we have the conservation equations  $Y_{TOT} = Y + C_2$ ,  $X_{TOT} = X + X_p + C_1 + C_2 + C$ , because proteins X and Y are not degraded. Therefore, the differential equations modeling the insulation system of Figure 7 become

$$\frac{dZ}{dt} = \frac{k(t) - \delta Z \left[ -\beta_1 Z X_{TOT} \left( 1 - \frac{X_p}{X_{TOT}} - \frac{C_1}{X_{TOT}} - \frac{C_2}{X_{TOT}} \right) - \frac{C}{X_{TOT}} \right] + (\beta_2 + k_1) C_1}{1} \quad (11)$$

$$\frac{dC_1}{dt} = \frac{-(\beta_2 + k_1) C_1 + \beta_1 Z X_{TOT} \left( 1 - \frac{X_p}{X_{TOT}} - \frac{C_1}{X_{TOT}} \right) - \frac{C_2}{X_{TOT}} - \frac{C}{X_{TOT}}}{1} \quad (12)$$

$$\frac{dC_2}{dt} = -(k_2 + \alpha_2) C_2 + \alpha_1 Y_{TOT} X_p \left( 1 - \frac{C_2}{Y_{TOT}} \right) \quad (13)$$

$$\frac{dX_p}{dt} = \frac{k_1 C_1 + \alpha_2 C_2 - \alpha_1 Y_{TOT} X_p \left( 1 - \frac{C_2}{Y_{TOT}} \right) + k_{off} C - k_{on} X_p (p_{TOT} - C)}{1} \quad (14)$$

$$\frac{dC}{dt} = -k_{off} C + k_{on} X_p (p_{TOT} - C), \quad (15)$$

in which the expression of gene  $z$  is controlled by a promoter with activity  $k(t)$ . The terms in the large box in equation (11) represent the retroactivity  $r$  to the input, while the terms in the small box in equation (11) and in the boxes of equations (12) and (14) represent the retroactivity  $s$  to the output. A detailed analysis of the system in equations (11–15) also provides analytical relationships among the parameters for obtaining small retroactivity to the input  $r$  and linear input/output relationship (see [6] for details). It was

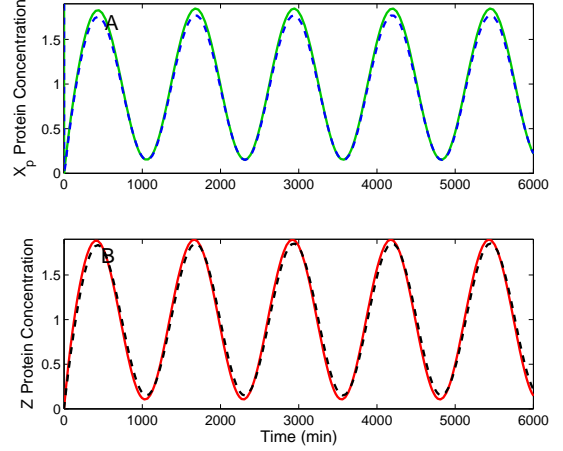


Fig. 8. Simulation results for system in equations (11–15). In all plots,  $p_{TOT} = 100$ ,  $k_{off} = k_{on} = 10$ ,  $\delta = 0.01$ ,  $k(t) = 0.01(1 + \sin(\omega t))$ , and  $\omega = 0.005$ . In subplots A and B,  $k_1 = k_2 = 50$ ,  $\alpha_1 = \beta_1 = 0.01$ ,  $\beta_2 = \alpha_2 = 10$ , and  $Y_{TOT} = X_{TOT} = 1500$ . In subplot A, the signal  $X_p(t)$  without the downstream binding sites  $p$  is the solid line, while the same signal with the downstream binding sites  $p$  is in the dashed line. The small error shows that the effect of the retroactivity to the output  $s$  is attenuated very well. In subplot B, the signal  $Z(t)$  without X to which Z binds is in the solid line, while the same signal  $Z(t)$  with X present in the system is in the dashed line. The small error confirms a small retroactivity to the input.

shown in [5] that the fast time-scale of the phosphorylation and dephosphorylation reactions with respect to the input dynamics are the fundamental feature that allows this system to reach attenuation of the retroactivity to the output.

System in equations (11–15) was simulated with and without the downstream binding sites  $p$ , that is, with and without, respectively, the terms in the small box of equation (11) and in the boxes in equations (14) and (12). This is performed to highlight the effect of the retroactivity to the output  $s$  on the dynamics of  $X_p$ . The simulations validate our theoretical study that indicates that when  $X_{TOT} \gg p_{TOT}$  and the time scales of phosphorylation/dephosphorylation are much faster than the time scale of decay and production of the protein Z, the retroactivity to the output  $s$  is very well attenuated (Figure 8A). Similarly, the time behavior of Z was simulated with and without the terms in the large box in equation (11), that is, with and without X to which Z binds, to verify whether the insulation component exhibits retroactivity to the input  $r$ . In particular, the accordance of the behaviors of  $Z(t)$  with and without its downstream binding sites on X (Figure 8B), indicates that there is no substantial retroactivity to the input  $r$  generated by the insulation device.

#### IV. CONCLUSIONS AND FUTURE WORKS

We have presented a review of recent results on retroactivity, modularity, and insulation concepts in the context of bio-molecular systems. We have illustrated that the modularity assumption does not usually hold in bio-molecular systems and that it can cause dramatic effects on the system dynamics. Such effects need to be modeled and characterized in

order to reach the correct conclusions about the behavior of a composed system. In view of modularly building synthetic bio-molecular circuits, we have illustrated the design of insulation devices. In particular, we have shown that a simple phosphorylation/dephosphorylation cycle can work in parameter ranges so as to work as an insulation device. This fact suggests that another reason why these cycles are ubiquitous in natural signal transmission systems is because they can enforce unidirectional signal propagation.

A number of future challenges need to be addressed. These include the experimental characterization of the proposed bio-molecular insulation device, the development of a frequency domain analysis that accounts for retroactivity, and the understanding of the effects of the high gains of the insulation device on biological noise.

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