Design of an insulation device using phosphotransfer systems

Shridhar Jayanthi and Domitilla Del Vecchio
Electrical Engineering and Computer Science
University of Michigan, Ann Arbor
Ann Arbor, MI 48109
Email: {jayanthi,ddv}@umich.edu

Abstract— As in several engineering applications, biomolecular systems present impedance-like effects at interconnections called *retroactivity*. This phenomenon presents a challenge for modular design in synthetic biology. An *insulation device* is a biological module that enables attenuation of retroactivity at interconnections. In this paper, we propose the design of an insulation device by exploiting the naturally existing time-scale separation of *phosphotranspher* systems. [1]

I. INTRODUCTION TO RETROACTIVITY

Modules are reusable components designed to have a specific function in different contexts. In digital electronics, for example, a clock module can be used to drive a counter in one application and to synchronize a register in another [1]. One fundamental requirement in designing modules is the fact their behavior is not altered upon connection to downstream clients. In digital electronics, this property, called *modularity* [10], is captured by *fan-out* specifications, which limit the number of clients that can be connected to the output of a component. Modularity has been recently advocated in systems and in synthetic biology, areas in which networks of biomolecular interactions between DNA, RNA, proteins and signaling molecules are studied. In the field of systems biology, the challenge is in understanding the behavior of naturally occurring biomolecular networks from the behavior of the composing modules [2]–[4]. In contrast, researchers in synthetic biology aim at constructing networks with the goal of controlling cell behavior. Several simple biomolecular systems have been designed, such as oscillators [5], [6] and toggles [7], with the goal of designing biomolecular circuits with more complex functionalities in a modular fashion [8], [9].

In order for this approach to work, these components must have the modularity property [10]. It has been shown theoretically, however, that output connections can be affected by large impedance-like effects that distort dramatically the dynamics of a system when facing a downstream load [11]. This effect has been called *retroactivity* to extend the notion of impedance to biomolecular systems. Retroactivity has been modeled by the addition of two signals to the standard input/output system: the retroactivity to the output s and the retroactivity to the input r, as shown in Figure 1 [11]. In this framework, achieving
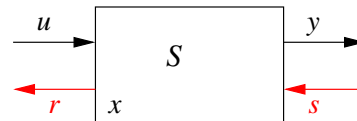
Fig. 1. System S along with its input and output signals. The retroactivity to the output s accounts for the effect of downstream systems in the system S dynamics upon connection. The retroactivity to the input r accounts for the impact that S exerts on its upstream systems when a connection to receive the information u is established.

low output impedance becomes the problem of attenuating the retroactivity to the output.

To illustrate the effect of retroactivity in biological systems we consider a simple transcriptional component, in which a *transcription factor* X is produced at rate $k(t)$ and decays at rate δ [10]. Transcription factors are proteins that regulate the expression of a gene by binding to the *promoter* region of a gene. Therefore, consider X to be an input to a downstream component through the reversible binding of X to promoter sites p, assumed to have total concentration p_{TOT}. The biochemical reactions involved are $\varnothing \underset{\delta}{\overset{u(t)}{\rightleftharpoons}} X$ and $X + p \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} C$, in which C is the complex of X bound to site p. The ordinary differential equation model is given by

$$
\begin{aligned}
\dot{X} &= u(t) - \delta X + k_{off}C - k_{on}(p_{TOT} - C)X \\
\dot{C} &= -k_{off}C + k_{on}(p_{TOT} - C)X,
\end{aligned}
\tag{1}
$$

in which the retroactivity to the output is given by $s(X, C) = k_{off}C - k_{on}(p_{TOT} - C)X$. Setting $p_{TOT} = 0$, and thus $s = 0$, one obtains the dynamics of the system without load, given by $\dot{X} = u(t) - \delta X$. The effect of retroactivity to the output on the dynamics is illustrated in Figure 2.

II. PHOSPHOTRANSFER-BASED INSULATION DEVICE

In order to counteract the effect of retroactivity one can employ *insulation devices*, biomolecular systems that are designed to attenuate the retroactivity to the output and to have low retroactivity to the input. An insulation device is placed between the upstream system producing the signal and the downstream system receiving the signal. In analogy to the design of non-inverting amplifiers in electronics, an insulation device can be obtained by employing a large amplification
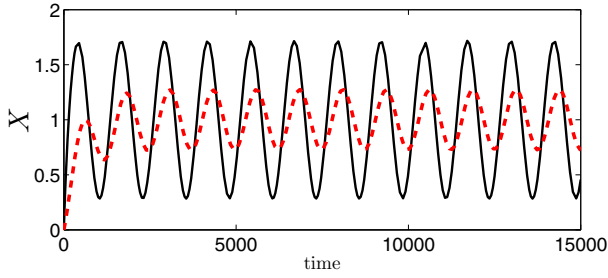
Fig. 2. Effect of the retroactivity to the output s on the transcriptional component. When $p_{TOT} = 0$, $s = 0$ and the system is isolated. The addition of load ($p_{TOT} = 100$) makes $s \neq 0$. The input signal is $u(t) = \delta(1 + 0.8 \sin(\omega t))$ with $\omega = 0.005$, $\delta = 0.01$, $k_{off} = 50$, and $k_{on} = 20$.

Fig. 3. System Σ is a phosphotransfer system. The output X* activates transcription through the reversible binding of X* to downstream DNA promoter sites p.

gain in a negative feedback loop. Examples of two insulation devices based on this design technique are shown in [11].

In this paper, we present a different design principle for insulation based on separation of time scales, realized through the *phosphotransfer* system of Figure 3. The system is based on a chain of *phosphorylation* and *dephosphorylation* reactions [12]. Ubiquitous in prokariotic signaling pathways, phosphotransfer systems are found to be reused as a signal relay module in different bacterial pathways [13]. Here, we show that the phosphotransfer system is capable of attenuating retroactivity to the output and, therefore, can be employed to design insulation devices. Let X be a transcription factor in its inactive form and let X* be the same transcription factor once it has been activated through phosphorylation. Let Z* be a phosphate donor, that is, a protein that can transfer its phosphate group to the acceptor X. The phosphotransfer reactions [12] can be modeled according to the two-step model $Z^* + X \underset{k_2}{\overset{k_1}{\rightleftharpoons}} C_1 \underset{k_4}{\overset{k_3}{\rightleftharpoons}} X^* + Z$, in which C_1 is the complex of Z bound to X bound to the phosphate group. Also, protein Z can be phosphorylated and protein X* dephosphorylated by other phosphotransfer reactions, respectively. These reactions are modeled as one step reactions, that is, $Z \xrightarrow{\pi_1} Z^*$, $X^* \xrightarrow{\pi_2} X$. Protein X is assumed to be conserved in the system, that is, $X + C_1 + X^* + C = X_{TOT}$. We assume that protein Z is produced with time-varying production rate $k(t)$ and decays with rate δ. The active form of the transcription factor X* binds to downstream binding sites p with total concentration p_{TOT} through the reversible reaction $p+X^* \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} C$. It is also assumed that the total amount of p is conserved, that is, $C + p = p_{TOT}$. The ODE model is thus given by

$$
\begin{aligned}
\dot{Z} &= k(t) - \delta Z + k_3 C_1 - k_4 X^* Z - \pi_1 Z \\
\dot{C}_1 &= k_1 (X_{TOT} - X^* - C_1 - C) Z^* - k_3 C_1 - k_2 C_1 + k_4 X^* Z \\
\dot{Z} &= \pi_1 Z + k_2 C_1 - k_1 (X_{TOT} - X^* - C_1 - C) Z^* \\
\dot{X}^* &= k_3 C_1 - k_4 X^* Z - k_{on} X^* (p_{TOT} - C) + k_{off} C - \pi_2 X^* \\
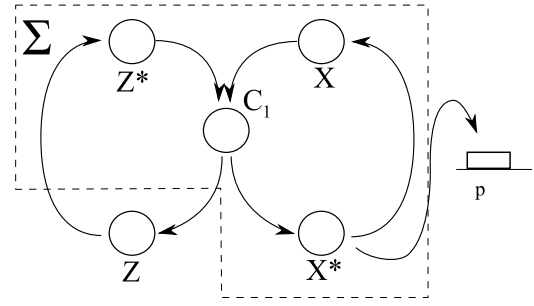\dot{C} &= k_{on} X^* (p_{TOT} - C) - k_{off} C.
\end{aligned} \tag{2}
$$

III. RETROACTIVITY ATTENUATION

In order to make the multiple time scales and the structural properties of the system explicit, we rewrite system (2) as follows. Since phosphotransfer reactions are faster than protein production and decay [12], define the constant $G_1 \gg 1$ and write $\bar{k}_1 := X_{TOT} k_1 / G_1$, $\bar{k}_2 := k_2 / G_1$, $\bar{k}_3 := k_3 / G_1$, $\bar{k}_4 := X_{TOT} k_4 / G_1$, $\bar{\pi}_1 := \pi_1 / G_1$ and $\bar{\pi}_2 := \pi_2 / G_1$, so that $\bar{k}_1 / X_{TOT}, \bar{k}_2, \bar{k}_3, \bar{k}_4 / X_{TOT}, \bar{\pi}_1, \bar{\pi}_2$ are of the same order as $k(t)$ and δ. Since the process of protein binding to promoter sites is much faster than phosphorylation [10], define the constant $G_2 \gg 1$ with $G_2 \gg G_1$ as $G_2 := k_{off} / \delta$, $k_d := k_{off} / k_{on}$. Relabel the variables as $u := Z$, $x := [\; C_1 \quad Z^* \quad X^* \;]^T$ and $v := C$. Define also the functions $g(u, t) := k(t) - \delta u$, $r(x, u) :=$

$$
\begin{bmatrix} \bar{k}_3 x_1 - \bar{k}_4 X_{TOT} u x_3 \\ -\bar{\pi}_1 u \\ 0 \end{bmatrix}, \quad s(x, v) := \frac{\delta}{k_d} x_3 (p_{TOT} - v) - \delta v \text{ and}
$$

$$
f(u, x) := \begin{bmatrix} \bar{k}_1 \left(1 - \frac{x_3}{X_{TOT}} - \frac{x_1}{X_{TOT}}\right) x_2 - \bar{k}_2 x_1 \\ \bar{k}_2 x_1 - \bar{k}_1 \left(1 - \frac{x_3}{X_{TOT}} - \frac{x_1}{X_{TOT}}\right) x_2 \\ \bar{k}_3 x_1 - \bar{k}_4 X_{TOT} x_3 (z - x_1 - x_2) - \bar{\pi}_2 x_3 \end{bmatrix}. \text{ Define fi-}
$$

nally the matrices $A := [\; 1 \quad 1 \quad 0 \;]$ and $C := [\; 0 \quad 0 \quad 1 \;]^T$. System (2) can be rewritten as

$$
\begin{aligned}
\dot{u} &= g(u, t) + G_1 A r(x, u) \\
\dot{x} &= -G_1 r(x, u) + G_1 f(u, x) - G_2 C s(x, v) \\
\dot{v} &= G_2 s(x, v).
\end{aligned} \tag{3}
$$

in which the retroactivity to the output is given by $-G_2 C s(x, v)$

Since we assume $G_1 \gg 1$ and $G_2 \gg G_1$, system (3) presents three different time scales. The isolated system, obtained when $s(x, v) = 0$ is given by

$$
\begin{aligned}
\dot{u} &= g(u, t) + G_1 A r(x, u) \\
\dot{x} &= -G_1 r(x, u) + G_1 f(u, x).
\end{aligned} \tag{4}
$$

Employing singular perturbation [14], we show that as $G_1 \to \infty$ and $G_2 \to \infty$, both the isolated and connected system solutions tend to the same signals. This is only possible due to the structural characteristics of the system and in particular to the fact that $AC = 0$, which allows clear separation of the time scales by employing linear coordinate transformations.

Let $u(t, 1/G_1, 1/G_2)$, $x(t, 1/G_1, 1/G_2)$, $v(t, 1/G_1, 1/G_2)$ and $u_{is}(t, 1/G_1)$, $x_{is}(t, 1/G_1)$ be the unique solutions for $t \in [t_0, \bar{t}_f]$

of the connected system (3) and the isolated system (4), respectively. In what follows, we assume that $G_2/G_1 \to \infty$ as $G_1 \to \infty$.

Definition 1: System (2) has the *retroactivity to the output attenuation property* if there is a time $t_f > t_0$, constants $G_1^* > 0$, $G_2^* > 0$ and $t_b \in (t_0, t_f)$ such that $u(t, 1/G_1, 1/G_2) - u_{is}(t, 1/G_1) = O(G_1/G_2)$ and $x(t, 1/G_1, 1/G_2) - x_{is}(t, 1/G_1) = O(G_1/G_2)$ for all $t \in [t_b, t_f]$ when $G_1 > G_1^*$ and $G_2 > G_2^*$.

Let $x = \gamma_x(u)$ be the locally unique solution to the algebraic equation $r(x, u) + f(x, u) = 0$. We show the dynamics of the input for both systems (3) and (4) approximate $\bar{u}(t)$ which is the unique solution of the system

$$\dot{u} = \left(1 + A\frac{d}{d\bar{u}}\gamma_x(\bar{u})\right)^{-1} g(\bar{u}, t) \tag{5}$$

and the internal dynamics of the device for systems (3) and (4) is well approximated by $\gamma_x(\bar{u}(t))$.

Proposition 1: *Isolated System.* There is a time $t_f > t_0$ such that for all $t_b \in (t_0, t_f)$, there is a $G_1^* > 0$ such that $u_{is}(t, 1/G_1) - \bar{u}(t) = O(1/G_1)$ and $x_{is}(t, 1/G_1) - \gamma_x(\bar{u}(t)) = O(1/G_1)$ uniformly for $t \in [t_b, t_f]$ whenever $G_1 > G_1^*$.

Proof: This proposition can be shown by writing system (4) in the standard singular perturbation form and employing Tikhonov theorem [14]. This can be achieved by employing the change of coordinates $z = u + Ax$. Let $\epsilon = 1/G_1$ be the small parameter. System (4) becomes

$$\begin{aligned}\dot{z} &= g(z - Ax, t) \\ \epsilon\dot{x} &= -r(x, z - Ax) + f(z - Ax, x).\end{aligned} \tag{6}$$

Define $\tilde{f}(z, x) := -r(x, z - Ax) + f(z - Ax, x)$. Let the locally unique solution of $\tilde{f}(z, x) = 0$ be $x = \phi_x(z)$. Let also $\bar{z}_{is}(t)$ be the unique solution for $t \in [t_0, t_f]$ of the reduced system

$$\dot{\bar{z}} = g(\bar{z} - A\phi_x(\bar{z}), t), \tag{7}$$

with initial conditions $\bar{z}(t_0) = u_0 + Ax_0$. It is possible to show using the Routh-Hurwitz criterion that $\frac{\partial}{\partial x}\tilde{f}(z, x)\big|_{x=\phi_x(z)}$ is Hurwitz. This guarantees that the trajectories of the system (6) are attracted to the slow manifold [14]. Then, from Tikhonov theorem, for all $t_b \in (t_0, t_f)$ there exists $\epsilon^* > 0$ such that if $\epsilon < \epsilon^*$,

$$\begin{aligned}z_{is}(t, \epsilon) - \bar{z}_{is}(t) &= O(\epsilon) \text{ uniformly for } t \in [t_0, t_f] \\ x_{is}(t, \epsilon) - \phi_x(\bar{z}_{is}(t)) &= O(\epsilon) \text{ uniformly for } t \in [t_b, t_f].\end{aligned} \tag{8}$$

Define the coordinate transformation $\bar{u}_{is} = \bar{z}_{is} - A\phi_x(\bar{z}_{is})$. Since $x = \phi_x(z)$ is the locally unique solution of $\tilde{f}(z, x) = 0$, we have that $\tilde{f}(\bar{z}, \phi_x(\bar{z})) = 0$. But note that $\tilde{f}(z, x) = -r(x, z - Ax) + f(z - Ax, x)$ and hence $-r(\phi_x(\bar{z}_{is}), z - A\phi_x(\bar{z}_{is})) + f(\bar{z}_{is} - A\phi_x(\bar{z}_{is}), \phi_x(\bar{z}_{is})) = 0$. Note also that $x = \gamma_x(u)$ is the locally unique solution of $-r(x, u) + f(u, x) = 0$, therefore

$$\phi_x(\bar{z}_{is}) = \gamma_x(\bar{z}_{is} - A\phi_x(\bar{z}_{is})) = \gamma_x(\bar{u}_{is}). \tag{9}$$

Thus, $\bar{z}_{is} = \bar{u}_{is} + A\gamma_x(\bar{u}_{is})$. Using the chain rule we obtain that $\dot{\bar{z}}_{is} = \dot{\bar{u}}_{is}\left(1 + A\frac{d\gamma_x(\bar{z}_{is})}{d\bar{u}_{is}}\right)$. Rearranging this expression and substituting (7), we conclude that $\bar{u}_{is}(t)$ satisfies the differential equation (5).

Finally, from the coordinate change, we have that $u_{is}(t, \epsilon) = z_{is}(t, \epsilon) - Ax_{is}(t, \epsilon)$ and $\bar{u}_{is}(t) = \bar{z}_{is} - A\phi_x(z_{is})$. From identity (9) and relations (8) we obtain the desired result. ∎

Proposition 2: *Connected System.* There is a time $t_f > t_0$ such that for all $t_b \in (t_0, t_f)$, there are $G_1^* > 0$, $G_2^* > 0$ such that $x(t, 1/G_1, 1/G_2) - \gamma_x(\bar{u}(t)) = O(G_1/G_2)$ and $u(t, 1/G_1, 1/G_2) - \bar{u}(t) = O(G_1/G_2)$ uniformly for $t \in [t_b, \bar{t}_f]$ when $G_1 > G_1^*$ and $G_2 > G_2^*$.

Proof: The connected system (3) presents three different time scales. We proceed by employing nested applications of the Tikhonov theorem [15]. Employ the change of coordinates $z = u + Ax$ and $y = x + Cv$, which brings (3) to standard singular perturbation form

$$\begin{aligned}\dot{z} &= g(z - A(y - Cv), t) \\ \epsilon\dot{y} &= -r(y - Cv, z - A(y - Cv)) + f(z - A(y - Cv), y - Cv) \\ \epsilon\mu\dot{v} &= s(y - Cv, v).\end{aligned} \tag{10}$$

Define $\bar{f}(z, y, v) := -r(y - Cv, z - A(y - Cv)) + f(z - A(y - Cv), y - Cv)$. Let the locally unique solution of $s(y - Cv, v) = 0$ be $v = \phi_1(y)$. Let also $\bar{z}(t, \epsilon)$ and $\bar{y}(t, \epsilon)$ be the unique solution for $t \in (t_b, t_f)$ of the reduced system obtained setting $\mu = 0$,

$$\begin{aligned}\dot{z} &= g(z - A(y - C\phi_1(y)), t) \\ \epsilon\dot{y} &= \bar{f}(z, y, \phi_1(y)).\end{aligned} \tag{11}$$

It is possible to show that $\frac{\partial}{\partial v}s(y - Cv, v)\big|_{v=\phi_1(y)}$ is Hurwitz. This guarantees that the trajectories of system (10) are attracted to the slow manifold. Then, from Tikhonov theorem, for all $t_b \in (t_0, t_f)$, there exists μ^* such that if $\mu < \mu^*$,

$$\begin{aligned}z(t, \epsilon, \mu) - \bar{z}(t, \epsilon) &= O(\mu) \text{ uniformly for } t \in [t_0, t_f] \\ y(t, \epsilon, \mu) - \bar{y}(t, \epsilon) &= O(\mu) \text{ uniformly for } t \in [t_0, t_f] \\ v(t, \epsilon, \mu) - \phi_1(y) &= O(\mu) \text{ uniformly for } t \in [t_b, t_f].\end{aligned} \tag{12}$$

System (11) is also in standard singular perturbation form with small parameter ϵ. Let then $y = \phi_2(z)$ be the locally unique solution of $\bar{f}(z, y, \phi_1(y)) = 0$. Let also $\tilde{z}(t)$ be the solution for $t \in [t_0, t_f]$ of the reduced system obtained setting $\epsilon = 0$,

$$\dot{\tilde{z}} = g(\tilde{z} - A(\phi_2(\tilde{z}) - C\phi_1 \circ \phi_2(\tilde{z})), t), \tag{13}$$

It is possible to show, with the Routh-Hurwitz criterion, that $\frac{\partial}{\partial y}\bar{f}(z, y, \phi_1(y))\big|_{y=\phi_2(z)}$ is Hurwitz. This guarantees that the trajectories of the system (11) are attracted to the slow manifold. Then, from the application of Tikhonov theorem on system (11), for all $t_b \in (t_0, t_f)$, there exists $\epsilon^* > 0$ such that if $\epsilon < \epsilon^*$,

$$\begin{aligned}\bar{z}(t, \epsilon) - \tilde{z}(t) &= O(\epsilon) \text{ uniformly for } t \in [t_0, t_f] \\ \bar{y}(t, \epsilon) - \phi_2(\tilde{z}(t)) &= O(\epsilon) \text{ uniformly for } t \in [t_b, t_f].\end{aligned} \tag{14}$$

By combining results (12) and (14), we have that for all $t_b \in (t_0, t_f)$, and all $\epsilon < \epsilon^*$ and $\mu < \mu^*$

$$\begin{aligned}z(t, \epsilon, \mu) - \tilde{z}(t) &= O(\epsilon) + O(\mu) \\ y(t, \epsilon, \mu) - \phi_2(\tilde{z}(t)) &= O(\epsilon) + O(\mu) \\ v(t, \epsilon, \mu) - \phi_1 \circ \phi_2(\tilde{z}(t)) &= O(\epsilon) + O(\mu),\end{aligned} \tag{15}$$

uniformly in $t \in [t_b, t_f]$, in which we use $\phi_1(\phi_2(z) + O(\epsilon)) = \phi_1 \circ \phi_2(z) + O(\epsilon)$ due to continuity of ϕ_1.

Define the coordinate transformation $\bar{u} = \tilde{z} - A(\phi_2(\tilde{z}) - C\phi_1 \circ \phi_2(\tilde{z}))$. Since $y = \phi_2(z)$ is the locally unique solution of $\bar{f}(z, y, \phi_1(y)) = 0$, we have that $\bar{f}(\tilde{z}, \phi_1(\tilde{z}), \phi_1 \circ \phi_2(\tilde{z})) = 0$. But from the definition of $\bar{f}(z, y, v)$ one concludes that $-r(\phi_1(\tilde{z}) - C\phi_1 \circ \phi_2(\tilde{z}), \bar{u}) + f(\bar{u}, \phi_1(\tilde{z}) - C\phi_1 \circ \phi_2(\tilde{z})) = 0$. Note also that $x = \gamma_x(u)$ is the locally unique solution of $-r(x, u) + f(x, u) = 0$, therefore we can write

$$\gamma(\bar{u}) = \phi_1(\tilde{z}) - C\phi_1 \circ \phi_2(\tilde{z}). \tag{16}$$

We can thus write $\bar{u} = \tilde{z} - A\gamma(\bar{u})$. Rearranging the terms of the expression and using the chain rule, we obtain the time derivative expression $\dot{\tilde{z}} = \dot{\bar{u}}\left(1 + A\frac{d}{d\bar{u}}\gamma_x(\bar{u})\right)$. Rearranging this expression and substituting (13), we conclude that $\bar{u}(t)$ satisfies the differential equation (5).

Finally, from the coordinate changes we have that $x(t, \epsilon, \mu) = y(t, \epsilon, \mu) - Cv(t, \epsilon, \mu)$ and $u(t, \epsilon, \mu) = z(t, \epsilon, \mu) - Ax(t, \epsilon, \mu)$. Applying (15), (16) and recalling $\bar{u}(t) = \tilde{z}(t) - A\gamma_x(\bar{u})$, we have that for all $t_b \in (t_0, t_f]$, there are $\epsilon^* > 0$, $\mu^* > 0$ such that

$$
\begin{aligned}
u(t, \epsilon, \mu) - \bar{u}(t) &= O(\epsilon) + O(\mu) \\
x(t, \epsilon, \mu) - \gamma_x(\bar{u}(t)) &= O(\epsilon) + O(\mu), \tag{17}
\end{aligned}
$$

uniformly in $t \in [t_b, t_f]$ provided $\epsilon < \epsilon^*$ and $\mu < \mu^*$. ∎

Proposition 3: System (2) has the retroactivity to the output property.

Proof: Let $t_f^1 > t_0$, $G_1^{1*} > 0$ be constants such that Proposition 1 holds and let $t_f^2 > t_0, G_1^2 > 0, G_2^* > 0$ be constants such that Proposition 2 holds. Pick then $t_f = \min(t_f^1, t_f^2)$ and $G_1^* = \max(G_1^{1*}, G_1^{*2})$. Then, by combining the results of Propositions 1 and 2, the desired result is obtained. ∎

Figure 4 shows the behavior of the system as a function of G_1 when $G_2 \gg G_1$. It can be seen that when $G_1 = 1$, the output suffers from retroactivity to the output. However, when $G_1 \gg 1$, the output dynamics of the connected and the isolated system are close to each other.

IV. Conclusion

In this paper we propose a biomolecular device capable of attenuating the retroactivity to the output based on naturally occurring phosphotransfer systems. We show that this insulation property is due to the special structure of the system that allows for separation of the multiple time scales. Furthermore, we show that this property holds even when the output disturbance enters the system through reaction rates much larger than the input or internal dynamic rates. As a result, a phosphotransfer system can be used to design an insulation device, which in its turn can be employed for modular design in synthetic biology. Aditionally, the finding that phosphotransfer systems attenuate retroactivity to the output suggests that these components enforce unidirectional signal propagation, a capability that may be linked to the ubiquity of this signaling pattern in prokaryotic pathways.
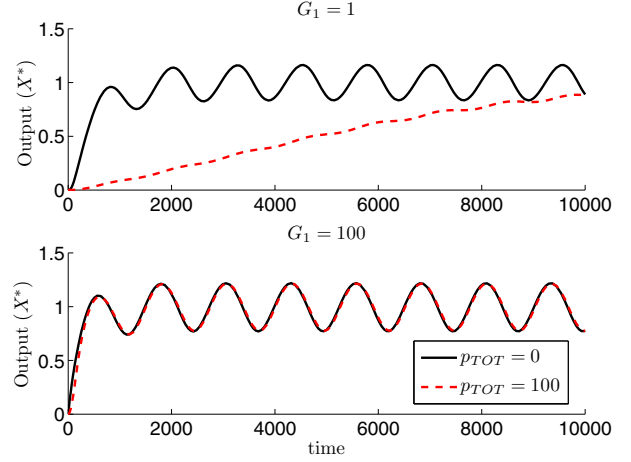

Fig. 4. Output response of the phosphotransfer system with a step signal $k(t) = \delta(1 + \sin(\omega t))$. The parameters are given by $\delta = 0.01$, $X_{TOT} = 1000$, $\bar{k}_2 = \bar{k}_3 = 0.01 = \bar{\pi}_1 = \bar{\pi}_2 = 0.01$, $\bar{k}_1 = \bar{k}_4 = 10^{-5}$, $k_d = 1$, $G_1 = 1$ (top panel), and $G_1 = 100$ (bottom panel), $G_2 = 1000$. The isolated system corresponds to $p_{TOT} = 0$ while the connected system corresponds to $p_{TOT} = 100$.

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