The effect of retroactivity on the transfer function of a phosphorylation system

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The Effect of Retroactivity on the Transfer Function of a Phosphorylation System

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***Abstract*— It was theoretically shown that impedance-like effects, called retroactivity, in biomolecular circuits can significantly impact the behavior of a system. In this paper, we quantify the effect of retroactivity on the transfer function of a phosphorylation system after linearization of its nonlinear model about a steady state. Our analysis shows that retroactivity shifts the poles of the transfer function toward the low frequency.**

I. Introduction

A modular approach to either understanding or designing the behavior of complex systems has been customary in fields such as electrical engineering and computer science. Such an approach has been more recently proposed also in systems and synthetic biology, where researchers seek to understand the behavior of existing networks and to engineer new systems from a set of building blocks [2], [3], [15], [21]. A modular approach is based on the assumption that the behavior of a system is not altered due to connection with another system. Is this assumption natural in biomolecular networks? It was theoretically shown that impedance-like effects, called retroactivity, take place at the interconnection of biomolecular systems, just as it occurs in many engineering systems [8]–[10], [24], [25]. Retroactivity can dramatically affect the behavior of a system upon interconnection and hence challenges a modular approach to understand biological complexity [8], [17]. In view of engineering complex biomolecular systems starting from a library of building blocks, it is thus necessary to quantify the effects of retroactivity on important system features and to device solutions to attenuate retroactivity effects. Toward the latter end, it was proposed to engineer insulation devices to be placed between an upstream system sending the signal and a downstream one receiving the signal to buffer them from retroactivity effects [7], [8], [18]. In this paper, we focus on the first problem and carry our analysis for a phosphorylation system, the fundamental building block of any signaling network.

Numerous cellular signaling systems consist of cycles of protein phosphorylation, and in several cases multiple cycles are linked to form cascade systems [23], [27]. The importance of these signaling systems has long been realized, and a wealth of theoretical work has established the potential behaviors of such systems and the mechanisms by which parameters and circuitry affect system behavior [1], [6], [11], [13], [14], [28]. These works described how a cycle would behave in the absence of any loading caused by interconnection with downstream systems, that is, how the cycle would behave as an *isolated* signaling module. But, of course signaling systems are usually connected to the downstream targets they regulate. These targets, in turn, cause retroactivity on the upstream system and can thus change the upstream system behavior. It is thus important to determine the effect of these targets on the response of the upstream system. Specifically, it was recently shown that the dynamic properties of signaling systems, such as bandwidth, play a key role in important cellular functions such as preventing crosstalk mechanisms, which can often lead to diseases such as cancer [4], [5]. Here, we thus focus on the transfer function of a phosphorylation cycle and show how the poles are affected by retroactivity.

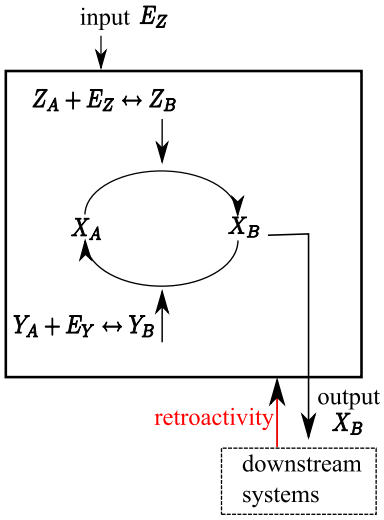

Fig. 1. Phosphorylation cycle representation. The output of the cycle X_B is taken as an input by a downstream system. Even though the information travels from upstream to downstream, the presence of a physical interconnection causes retroactivity on the upstream system.

In this paper, we report a modeling study to quantify the effects of retroactivity on the dynamic and steady state responses of a signaling component with the aim of obtaining predictions that are experimentally testable. We show that the steady state response decreases for every input stimulation when the load is applied and that the poles of the transfer function shift toward the imaginary axis.

This paper is organized as follows. In Section II, we introduce the model of the system. In Section III, we quantify the steady state and dynamic effects of retroactivity. In

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Section IV, we discuss the implications of the modeling results.

II. System Model

Phosphorylation cycles can be depicted according to the general scheme of Figure 1, in which an effector (a small signaling molecule) E_Z converts the enzyme Z from its inactive form Z_A to its active form Z_B through an allosteric modification [12]. The activated enzyme Z_B in turn converts the signaling protein X from its form X_A to its form X_B, which is in turn converted back by enzyme Y once it is in its active form Y_B. The effector E_Y brings about the activation of the enzyme Y, taking it from an inactive form Y_A to an active form Y_B through another allosteric modification. Protein X when in form X_B can transmit the signal to downstream systems (for example, other signaling targets or DNA binding sites) by binding with targets denoted p [2], [19], [20].

In signaling systems, it is usually the active form of the protein (i.e., the phosphorylated form) to carry information to downstream systems and to thus bind to downstream targets. In this case, referring to the diagram of Figure 1, X_B would be the phosphorylated protein and X_A would be the unphosphorylated one. In other cases, however, the inactive protein can carry information and bind to downstream signaling targets [22]. In this case, protein X_B would be the inactive (unphosphorylated) protein. In either case, the protein that can be usually experimentally detected (directly or indirectly) is the active protein. Therefore, it is relevant in the configuration of Figure 1 to characterize the effects of retroactivity not only on X_B but also on X_A.

For any species W, we denote in italics W its concentration. The cycle can be modeled by the following one-step reactions [16], in which we have neglected the complexes formed between X_A and Z_B and between X_B and Y_B, as these are not relevant for the result that we seek to show here: $Z_A + E_Z \underset{f_2}{\overset{f_1}{\rightleftharpoons}} Z_B$, with $Z_A + Z_B = Z_{tot}$, $Y_A + E_Y \underset{\bar{f}_2}{\overset{\bar{f}_1}{\rightleftharpoons}} Y_B$, with $Y_A + Y_B = Y_{tot}$, $X_A + Z_B \xrightarrow{k_1} X_B + Z_B$, $X_B + Y_B \xrightarrow{k_2} X_A + Y_B$, with $X_A + X_B = X_{tot}$. Since the allosteric modification reactions are much faster than phosphorylation reactions [12], we employ the quasi-steady state approximation (QSSA) with $k_D := f_2/f_1$ and $\bar{k}_D := \bar{f}_2/\bar{f}_1$ to obtain $Z_B = \frac{Z_{tot}E_Z}{k_D+E_Z}$, and $Y_B = \frac{Y_{tot}E_Y}{\bar{k}_D+E_Y}$ [26]. Therefore, the ODE model describing the phosphorylation system is given by

$$\frac{dX_B}{dt} = k_1(X_{tot} - X_B)\frac{Z_{tot}E_Z(t)}{k_D + E_Z(t)} - k_2 X_B \frac{Y_{tot}E_Y}{\bar{k}_D + E_Y}, \quad (1)$$

in which we view E_Y as constant, while $E_Z(t)$ is a time-varying input for our study. However, note that in practice also E_Y can be a time-varying input as it is an effector just like E_Z [1]. We will refer to the ODE system model (1) as the *isolated system*. For shortening notation, we denote $V(t) := \frac{Z_{tot}E_Z(t)}{k_D+E_Z(t)}$ and $V' := \frac{Y_{tot}E_Y}{\bar{k}_D+E_Y}$.

When the phosphorylation cycle transmits its signal through X_B to the downstream system, we add to the isolated system model the reversible binding reaction of X_B with downstream target sites denoted p. These sites can either belong to a substrate that is modified by X_B through another phosphorylation cycle as it occurs in the MAPK cascades [23], [27], or they can belong to promoter regions on the DNA if X_B is an active transcription factor [2]. We model this additional binding reaction as $X_B + p \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} C$, with $p+C = p_{tot}$, in which C denotes the complex of X_B with p. The conservation law for X thus modifies to $X_A + X_B + C = X_{tot}$. Note that if the phosphorylation cycle is not the last stage in a signaling cascade, the binding reaction to downstream targets would be another phosphorylation reaction. One can show that even in this case the impact of retroactivity on the upstream phosphorylation system is significant [30]. The new ODE model describing the phosphorylation system with its downstream system is thus given by

$$\begin{aligned} \frac{dX_B}{dt} &= k_1(X_{tot} - X_B - \boxed{C})\frac{Z_{tot}E_Z}{k_D + E_Z} - k_2 X_B \frac{Y_{tot}E_Y}{\bar{k}_D + E_Y} \\ &\quad \boxed{-k_{on}X_B(p_{tot} - C) + k_{off}C} \\ \frac{dC}{dt} &= k_{on}X_B(p_{tot} - C) - k_{off}C, \end{aligned} \quad (2)$$

which we refer to as the *connected system*. Retroactivity enters the dynamics of the phosphorylation cycle in two places indicated by the boxes. Specifically, the term in the small box causes an effect on the steady state response of the system, while the term in the large box does not have any effect on the steady state and it affects the dynamics only.

III. Effect of Retroactivity on System Response

In this section, we study in detail the effect of retroactivity on the steady state response of the system and on the dynamic response of the system.

A. Steady State Effect of Retroactivity

The effect of retroactivity on the steady state of the system in correspondence to a constant input stimulus $E_Z(t) = \bar{E}_Z$ is measured by the difference of the two steady states for the isolated and connected systems, that is, we have:

$$\text{isolated system steady state: } \bar{X}_B = \frac{k_1 X_{tot}\frac{Z_{tot}\bar{E}_Z}{k_D+\bar{E}_Z}}{k_2\frac{Y_{tot}E_Y}{\bar{k}_D+E_Y} + k_1\frac{Z_{tot}\bar{E}_Z}{k_D+\bar{E}_Z}}, \quad (3)$$

$$\text{connected system steady state: } \bar{X}_{B,c} = \frac{k_1(X_{tot} - \bar{C})\frac{Z_{tot}\bar{E}_Z}{k_D+\bar{E}_Z}}{k_2\frac{Y_{tot}E_Y}{\bar{k}_D+E_Y} + k_1\frac{Z_{tot}\bar{E}_Z}{k_D+\bar{E}_Z}}, \quad (4)$$

in which $\bar{C} > 0$ is the equilibrium value of the complex C, which is given by

$$\bar{C} = \gamma(\bar{X}_{B,c}) := \frac{p_{tot}\bar{X}_{B,c}}{\bar{X}_{B,c} + k_M}. \quad (5)$$

By substituting equation (5) into equation (4), we obtain the expression of the equilibrium of the connected system as a

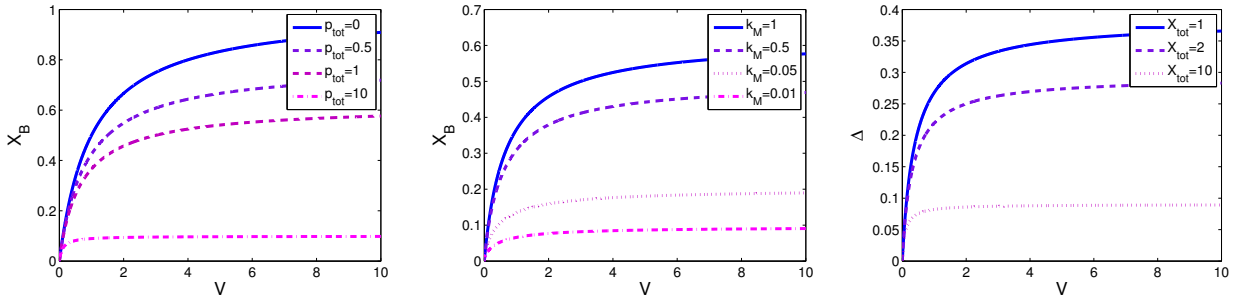

Fig. 2. (Left) Increasing the load amount decreases monotonically the steady state value of X_B for each value of the input stimulation. (Center) Decreasing the dissociation constant k_M, for constant amount of load, decreases the steady state value of X_B for every value of the input stimulation. (Right) The percentage difference Δ between the isolated and connected system steady states can be decreased by increasing the amounts of total protein X_{tot}.

function of the input stimulation $V = k_1 \frac{Z_{tot}\bar{E}_Z}{k_D+\bar{E}_Z}$ and of the reverse reaction speed V' as

$$\bar{X}_{B,c} = \frac{-((V+V')k_M + V(p_{tot}-X_{tot}))}{2(V+V')} + \frac{\sqrt{((V+V')k_M + V(p_{tot}-X_{tot}))^2 + 4(V+V')VX_{tot}k_M)}}{2(V+V')}.$$

Figure 2 shows the behavior of this steady state response to V for different values of the load p_{tot} and for different values of the affinity $1/k_M$ of the load to protein X_B. The equilibrium value of the output X_B of the connected system is lower than that of the isolated system and it monotonically decreases as p_{tot} increases and/or as k_M decreases. That is, the larger the load amount (p_{tot}) or the "flux" amount given by the affinity of the binding sites $1/k_M$, the more the steady state value is drained. In the same figure, we show the effect of increasing the total protein concentration X_{tot} on the percentage difference between the connected and isolated systems steady states $\Delta := \frac{\bar{X}_B - \bar{X}_{B,c}}{\bar{X}_B}$. As the amount of total protein is increased, the percentage difference decreases and therefore the effects of retroactivity on the steady state response decrease. Note that having $X_{tot} \gg p_{tot}$ does not imply that the value of X_B is large compared to the load amount p_{tot}, as it can be still much smaller than the load (in such a case the value of X_A would be much larger than the load p_{tot}). The effects of the downstream load on the steady state response of X_A are qualitatively the same as those obtained for X_B. Therefore, we do not include them here.

Note that if a two-step reaction model for the phosphorylation system was considered, the steady state calculations here performed would be affected. This case has been addressed in detail in [29], which shows that the apparent Hill coefficient of the characteristics decrease due to the addition of the downstream target.

B. Effects on the Transfer Function

While retroactivity has similar effects on X_A and X_B steady state responses, it has qualitatively different consequences on the dynamic response of these two variables. In this section, we first analyze these differences on the nonlinear ODE models and then we perform linearization about the steady state and compute the frequency response of the system.

By exploiting the time-scale difference between the phosphorylation reactions and the binding and unbinding reactions, that is, $k_{off} \gg V'$ and $k_{on} \gg V$, we can apply singular perturbation with small parameter $\epsilon := V'/k_{off}$ and obtain (see, for example [7], [8]) the dynamics of X_B from system (2) on the slow manifold as

$$\dot{X}_B = ((X_{tot} - X_B - \gamma(X_B))V - V'X_B)\left(\frac{1}{1 + d\gamma(X_B)/dX_B}\right). \quad (6)$$

It follows that (by comparison to equation (1)) the dynamic response of X_B to input stimulations V or V' is affected by the presence of the load even when $X_{tot} \gg p_{tot}$ (so that $\gamma(X_B) \ll X_{tot}$), that is, even when the load does not affect the steady state.

We now turn to the dynamics of X_A. From the conservation law $X_A = X_{tot} - X_B - C$ with $C = \gamma(X_B)$, we have that $\dot{X}_A = -\dot{X}_B(1 + \frac{d\gamma(X_B)}{dX_B})$. As a consequence, the dynamics of X_A on the slow manifold for the connected system are given by

$$\dot{X}_A = -VX_A + V'(X_{tot} - X_A - \gamma(X_B)), \quad (7)$$

which is the same equation as in the isolated system except for the presence of the term $\gamma(X_B)$. If $X_{tot} \gg p_{tot}$ so that the load does not affect the steady state of the system, in equation (7) the term $\gamma(X_B)$ can be neglected with respect to X_{tot} and therefore there is no effect of retroactivity on the dynamic response of X_A to the inputs V and V'. This is a first important difference between the ways retroactivity affects the dynamics of X_A and X_B. While retroactivity has effects on the dynamics of X_B even when it has no steady state effects, retroactivity affects the dynamics of X_A if and only if it has steady state effects. This fact implies that the best way to experimentally measure the dynamic effects of retroactivity is to measure the behavior of X_B (as opposed to the behavior of X_A) employing $X_{tot} \gg p_{tot}$ so that no discernible steady state effects are observed. Another consequence of equation (7) is that even when $\gamma(X_B)$ cannot be neglected compared to X_{tot}, retroactivity has no effect on the response of X_A when the system operates with $V' = 0$. Therefore, if one can measure X_A only, to measure dynamic effects of retroactivity,

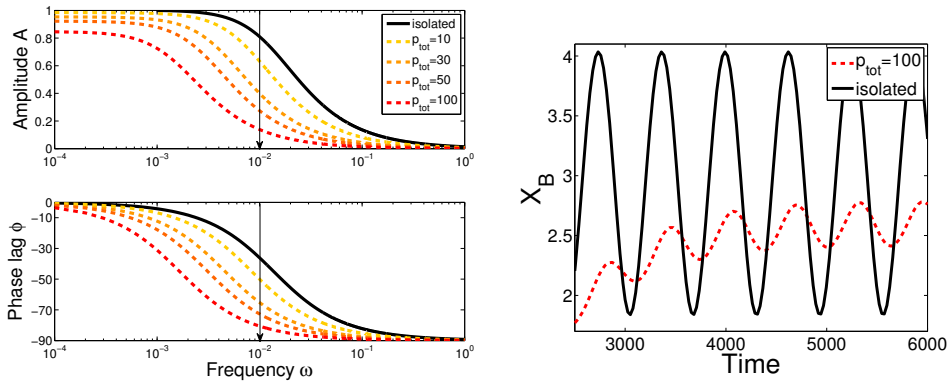

Fig. 3. (Left) Effect of increasing the amount of p_{tot} on the frequency response of the system. The parameters are $k_1 = k_2 = 0.01$, $Z_{tot} = 0.075$, $X_{tot} = 600$, $E_y = 1$, $Y_{tot} = 1.5$, $k_D = 100$, $\bar{k}_D = 0.1$, $k_{on} = 50$, and $k_{off} = 50$. (Right) Simulation for the input frequency as indicated by the arrow in the left plots for the value $p_{tot} = 100$.

the system should operate with sufficiently high values of V' and for values of X_{tot} sufficiently smaller than p_{tot}.

In order to more precisely quantify how the dynamic response of the system is affected by retroactivity, we next linearize the system about its steady state and compute the transfer function for both the isolated and connected systems. Linearization is a good approximation of the system dynamics for sufficiently small amplitudes of the input stimulus. A detailed study on how large the amplitude of the input can be for maintaining a good approximation can be found in [14]. For the isolated system, let $(\bar{E}_Z, \bar{X}_B)$ be the equilibrium point and let $\tilde{E}_Z(t) = E_Z(t) - \bar{E}_Z$ and $\tilde{X}_B(t) = X_B(t) - \bar{X}_B$ denote the variations about the equilibrium value. The linearized dynamics are thus given by

$$\dot{\tilde{X}}_B = \beta\tilde{E}_Z - \alpha\tilde{X}_B, \tag{8}$$

in which we have defined

$$\beta := k_1(X_{tot} - \bar{X}_B)\frac{Z_{tot}k_D}{(k_D + \bar{E}_Z)^2}, \quad \alpha := \left(k_1\frac{Z_{tot}\bar{E}_Z}{k_D + \bar{E}_Z} + k_2\frac{Y_{tot}E_Y}{\bar{k}_D + E_Y}\right). \tag{9}$$

The transfer function from $\tilde{E}_Z$ to $\tilde{X}_B$ is given by

$$T(s) = \frac{\beta}{s + \alpha},$$

in which $T(s) := \tilde{X}_B(s)/\tilde{E}_Z(s)$, so that amplitude and phase lag are given by

$$\begin{aligned} A(\omega) &= \sqrt{T(j\omega)T(-j\omega)} = \frac{\beta}{\sqrt{\omega^2 + \alpha^2}} \\ \phi(\omega) &= \arctan\left(\frac{\mathrm{Im}(T(j\omega))}{\mathrm{Re}(T(j\omega))}\right) = \arctan(-\omega/\alpha). \end{aligned} \tag{10}$$

The bandwidth is thus given by

$$\omega_{bandwidth} = \alpha.$$

For the connected system, let the equilibrium point be given by $(\bar{E}_Z, \bar{X}_{B,c}, \bar{C})$ and the variations about this equilibrium be denoted by $\tilde{E}_Z(t) = E_Z(t) - \bar{E}_Z$, $\tilde{X}_{B,c}(t) = X_{B,c} - \bar{X}_{B,c}$, and $\tilde{C}(t) = C(t) - \bar{C}$. The linearized system is thus given by

$$\begin{aligned} \dot{\tilde{X}}_B &= \bar{\beta}\tilde{E}_Z - (\alpha + \gamma)\tilde{X}_B - (\sigma + \eta)\,\tilde{C} \\ \dot{\tilde{C}} &= \gamma\tilde{X}_B - \eta\tilde{C}, \end{aligned} \tag{11}$$

in which we have denoted

$$\bar{\beta} := k_1(X_{tot} - \bar{X}_{B,c} - \bar{C})\frac{Z_{tot}k_D}{(k_D + \bar{E}_Z)^2}, \ \sigma := k_1\frac{Z_{tot}\bar{E}_Z}{k_D + \bar{E}_Z},$$

$$\gamma := k_{on}(p_{tot} - \bar{C}), \quad \eta := k_{on}\bar{X}_{B,c} + k_{off}.$$

The transfer function $T_c(s) := \tilde{X}_B(s)/\tilde{E}_Z(s)$ is given by

$$T_c(s) = \frac{\bar{\beta}(s + \eta)}{s^2 + s(\eta + \alpha + \gamma) + \eta\alpha + \sigma\gamma}.$$

Exploiting the fact that the binding and unbinding process of a protein to binding sites is usually much faster than phosphorylation reactions [12], we set $\eta = \bar{\eta}/\epsilon$ and $\gamma = \bar{\gamma}/\epsilon$, in which $\epsilon \ll 1$ and $\bar{\gamma}$ and $\bar{\eta}$ are of the same order as k_1 and k_2. By using the expressions of $\bar{\eta}$ and $\bar{\gamma}$ and setting $\epsilon = 0$, we obtain the reduced transfer function for the connected system as

$$T_c(s) = \frac{\bar{\beta}}{s(1 + \mu) + \alpha + \sigma\mu}, \text{ with } \mu = \frac{p_{tot}k_M}{(\bar{X}_{B,c} + k_M)^2}.$$

The amplitude and phase lag corresponding to $T_c(s)$ are given by

$$\begin{aligned} A_c(\omega) &= \sqrt{T_c(j\omega)T_c(-j\omega)} = \frac{\bar{\beta}}{\sqrt{\omega^2(1 + \mu)^2 + (\alpha + \sigma\mu)^2}} \\ \phi_c(\omega) &= \arctan\left(\frac{\mathrm{Im}(T_c(j\omega))}{\mathrm{Re}(T_c(j\omega))}\right) = \arctan\left(\frac{-\omega(1 + \mu)}{\alpha + \sigma\mu}\right), \end{aligned} \tag{12}$$

so that the bandwidth of the connected system is given by

$$\omega_{bandwidth,c} = \alpha\frac{1 + \mu(\sigma/\alpha)}{1 + \mu}.$$

Therefore, $\omega_{bandwidth,c} < \omega_{bandwidth}$, that is, the bandwidth of the connected system is strictly smaller than the bandwidth of the isolated system and the connected system displays a phase lag with respect to the isolated system. This is

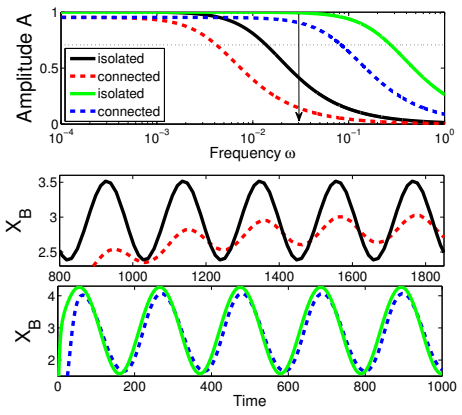

Fig. 4. Increasing the values of the enzymes Y_{tot} and Z_{tot} increases the bandwidth of the phosphorylation cycle. As a result, the response of the connected system becomes closer to the one of the isolated system. The solid black and dashed red plots correspond to the isolated and connected system behavior, respectively, while the solid green and dashed blue plots correspond to the isolated and connected system behavior, respectively, when the modification rates are increased by setting $Y_{tot} = 30$ and $Z_{tot} = 1.5$.

illustrated in Figure 3. Also, the bandwidth decreases with μ: for $\mu = 0$ it is equal to that of the isolated system while for $\mu \to \infty$ it tends to σ. In turn, μ monotonically increases with p_{tot} and (for k_M sufficiently large) it also increases with $1/k_M$ (the affinity of X_B to sites p). For values of k_M close to zero, the value of μ is not informative as the linear approximation does not hold. We thus conclude that the larger the value of μ the larger the effect of retroactivity on the dynamical properties of the cycle, that is, the smaller the frequency bandwidth and hence the slower the system response.

The bandwidth $\omega_{bandwidth,c}$ of the connected system can be increased by increasing α. One way to increase α is to equally (so not to alter the equilibrium of the system) increase the values of both Z_{tot} and Y_{tot}. The result is that the behavior of the connected system becomes closer to the one of the isolated system (Figure 4). In the limit in which $A_c(0) = A(0)$, the behavior of the connected system approaches the one of the isolated system when both Z_{tot} and Y_{tot} are increased. That is, the system becomes *insulated* from retroactivity. Note that if $\bar{\beta}$ is much smaller than β, that is, $A_c(0) \ll A(0)$, the dominant effect of retroactivity is on the steady state. In fact, increasing the frequency of the input stimulation will not result in a dramatic decrease of the connected system response compared to the isolated system response as these two responses are apart from each other already at zero frequency.

The frequency response analysis was performed for X_B only as similar qualitative results would be obtained for X_A assuming that the linearization is performed at values of X_B different from X_{tot} or 0.

IV. Discussion

In this modeling study, we have quantified the effect of retroactivity on both the steady state and transfer function of a phosphorylation system. Our study indicates that to obtain discernible effects of retroactivity on the dynamic response of the measurable quantity X_A, we should examine the system in conditions in which the steady state effects of retroactivity are also significant (that is, $X_{tot} \leq p_{tot}$). In these conditions, however, the analysis in the frequency domain indicates that if the steady state effects are too dramatic, then the difference between the frequency responses of the isolated and connected systems are mostly due to the difference in the steady state as opposed to being due to the difference in the bandwidth. Therefore, the amount of load p_{tot} should not be too low compared to X_{tot} otherwise steady state effects are not observed (and thus dynamic effects on X_A would not be observed either), but it should not be too high compared to X_{tot}, otherwise the difference between the bandwidths cannot be appreciated.

V. Conclusions

We have proposed a modeling study to quantify the effects of retroactivity on the steady state and transfer function of a phosphorylation cycle. Our results indicate that the steady state response to input allosteric effectors becomes less sensitive and that the poles of the transfer function move toward the imaginary axis. The steady state effects of retroactivity can be reduced by increasing the *total* protein amounts while the dynamic effects can be reduced by increasing the amounts of converting enzymes.

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