THE IMPACT OF RETROACTIVITY ON THE INPUT/OUTPUT STATIC CHARACTERISTICS OF A SIGNALING COMPONENT

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ABSTRACT

In prior work, we have shown that just as in many engineering systems, impedance-like effects appear at the interconnection of biomolecular systems. These effects are called retroactivity, to extend the notion of impedance to biological systems. Signaling components, such as covalent modification cycles, play a central role in the transmission of signals within a cell and from outside the cell. They are typically found in highly interconnected architectures in which a component has several downstream clients. In this paper, we investigate how retroactivity due to downstream clients affects the input/output steady state characteristics of a covalent modification cycle.

1 Introduction

Signal transduction systems cover a central role in a cell ability to respond to external or internal input stimuli and their malfunction can often result in pathological conditions including cancer [1–3]. Numerous cellular signal transduction systems consist of cycles of reversible protein modification, wherein a protein is reversibly converted between two forms [4]. In several cases, multiple cycles of covalent modification are linked to form cascade systems [5, 6]. 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 response [7–11]. These milestone works described how covalent modification cycles 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. Therefore, it is important to determine whether and how the response of an upstream system is influenced by the presence of its downstream targets. Ideally, since the information propagates from upstream to downstream, the presence of a downstream client receiving the signal should not affect the system that sends the signal.

However, this is only an idealization. Just as in many engineering systems, such as electrical, mechanical, and hydraulic systems, impedance-like effects appear at interconnections in biomolecular systems and in particular in signaling networks [12–20]. These effects have been called *retroactivity* to extend the notion of impedance to non-electrical systems and in particular to biomolecular systems [12, 13, 16]. Specifically, it was theoretically shown that the presence of downstream signaling targets can have a dramatic impact both on the dynamics and the steady state of signaling components [12, 18].

In tasks such as sensing and in the regulation of metabolism, in which signaling systems play a cardinal role, it is important

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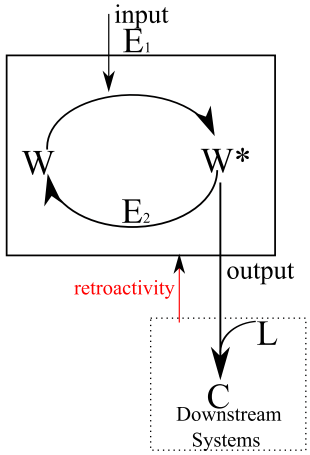

Figure 1. Covalent modification cycle. The output of the cycle W* is taken as an input by a downstream system through a binding reaction with target sites L to form a complex C. Even though the information travels from upstream to downstream, the presence of a physical interconnection causes retroactivity on the upstream system.

that the "turning on" of one signaling pathway and the "turning off" of another one is sensitive to relatively small changes in the input stimulation. Factors that impact this sensitivity and therefore the shape if the input/output characteristics of covalent modification cycles have been extensively studied by a number of theoretical and experimental works [7–10, 21, 22]. In this paper, we explicitly quantify the effect of retroactivity on the shape of the input-output static response of a covalent modification cycle.

This paper is organized as follows. In Section 2, we describe the system under study and its model. In Section 3, we characterize the effects of retroactivity on the shape of the input/output characteristics of the system. In Section 4, we conclude with a discussion of the results.

2 Model

A covalent modification cycle can be depicted according to the general diagram of Figure 1, in which a protein is reversibly converted between two different forms by converting enzymes. Specifically, a protein W (called the substrate) is converted to a form denoted W* by enzyme E_1 and converted back to form W by enzyme E_2. In the case of phosphorylation, for example, in which W* represents the phosphorylated version of protein W, enzyme E_1 is called a kinase while enzyme E_2 is called a phosphatase. We model this system through two coupled two-step enzymatic reactions [1, 9, 10], in which we denote by C_1 the complex of E_1 with W and by C_2 the complex of E_2 with W*. The reaction equations are thus given by

$$\begin{aligned} &\mathrm{W} + \mathrm{E}_1 \underset{d_1}{\overset{a_1}{\rightleftharpoons}} \mathrm{C}_1 \xrightarrow{k_1} \mathrm{W}^* + \mathrm{E}_1 \\ &\mathrm{W}^* + \mathrm{E}_2 \underset{d_2}{\overset{a_2}{\rightleftharpoons}} \mathrm{C}_2 \xrightarrow{k_2} \mathrm{W} + \mathrm{E}_2, \end{aligned} \tag{1}$$

in which k_1 and k_2 are called the catalytic rates, a_i are the association rates, and d_i are the dissociation rates. Protein W when in form W* can transmit the signal to downstream systems (for example, other signaling targets or DNA binding sites) by binding with targets denoted L [5, 6, 23–25]. This physical "connection" can be modeled by the additional binding reaction of W* with downstream sites L:

$$\mathrm{W}^* + \mathrm{L} \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} \mathrm{C}, \tag{2}$$

in which the value of the dissociation constant $k_D := k_{off}/k_{on}$ determines how high is the "flux" between the upstream system and the downstream load. Low values of k_D correspond to high values of flux as the binding reaction with the load is highly shifted toward forming the complex C.

A common assumption when modeling signaling systems is that both the total protein and the total enzymes amounts are not subject to change in the time scales typical of covalent modification [4]. Therefore, we have the following conservation laws, in which for a species X we denote in italics X its concentration:

$$\begin{aligned} &E_1 + C_1 = E_{1T},\ E_2 + C_2 = E_{2T},\ W + W^* + C_1 + C_2 + C = W_T, \\ &W^* + L = L_T. \end{aligned} \tag{3}$$

The converter enzyme E_1 can be viewed as an input to the system while the protein in form W* can be viewed as an output. In this modeling study, we seek to quantify the effect of the downstream targets L on the input/output steady state characteristics of the covalent modification cycle. That is, we are interested in characterizing the effects of retroactivity due to downstream loading on the static response of the system.

In signaling systems, it is usually the active form of the protein to carry information to downstream systems and to thus bind to downstream targets. In this case, referring to the diagram of Figure 1, W* would be the active protein and W would be the inactive one. In other cases, however, the inactive protein can carry information and bind to downstream signaling targets [26–28]. In this case, protein W* would be the inactive protein. In either case, the protein that can be usually experimentally detected and measured (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 W^* but also on W. The kinetic equations corresponding to the reaction equations (1,2) are given

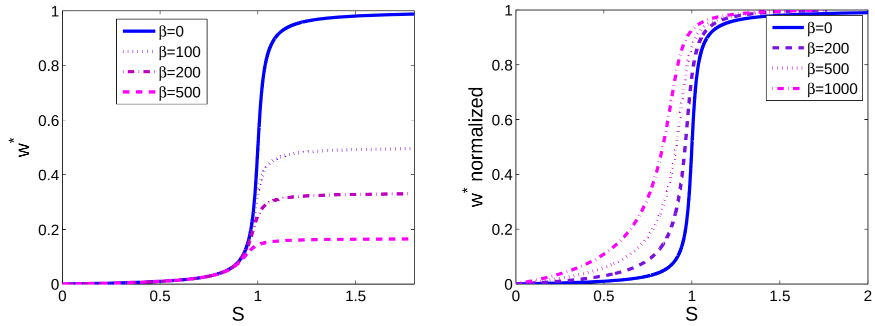

Figure 2. (Left) Steady state response of w^* as a function of the input stimulus S for different values of the load amount β. (Right) Same plots but with the value of w^* normalized by the maximum value achieved for $S = \infty$. Here, we have set $c = 100$ and $K_1 = K_2 = 0.01$.

by

$$\begin{aligned}\frac{dW}{dt} &= -a_1 W E_1 + d_1 C_1 + k_2 C_2\\ \frac{dC}{dt} &= a_1 W E_1 - (d_1 + k_1) C_1\\ \frac{dW^*}{dt} &= -a_2 W^* E_2 + d_2 C_2 + k_1 C_1 - k_{on} W^* L + k_{off} C\\ \frac{dC_2}{dt} &= a_2 W^* E_2 - (d_2 + k_2) C_2\\ \frac{dC}{dt} &= k_{on} W^* L - k_{off} C. \end{aligned} \tag{4}$$

In the next section, we solve the above system for the steady state to characterize the effect of the load L on the system static response to E_{1T}.

3 Effect of Retroactivity on the Cycle Characteristics

In order to quantify the effect of retroactivity on the static input/output characteristics of the system, we solve system (4) for the steady state and determine the values of W^* and W as functions of the input E_{1T}, the amount of downstream load L_T, and the dissociation constant k_D of the binding of W* to L.

By equating the last equation of system (4) to zero with $L = L_T - C$, we obtain that $C = \frac{W^* L_T}{W^* + K_D}$. Assuming that the enzymes are in amounts much smaller than the amounts of substrate, that is, $E_{1T}, E_{2T} \ll W_T$ (a common assumption when studying covalent modification cycles [9]), we have that $W_T = W^* + W + C$, so that

$$W = W_T - W^* - \frac{W^* L_T}{W^* + k_D}. \tag{5}$$

By summing up the first and second equations of system (4) and equating the result to zero, we obtain the equilibrium condition $k_1 C_1 = k_2 C_2$. This can be solved for $w^* := W^*/W_T$ using the conservation equations (5) to obtain the implicit equation that w^* satisfies as

$$S(w^*) = \frac{w^*[(w^*)^2 - w^*(\bar{\beta} + K_1) - c(K_1 + 1)]}{(w^*)^3 - (w^*)^2(\beta - K_2) - (w^*)[K_2(\beta) + c] - cK_2}, \tag{6}$$

in which we have denoted $S := \frac{E_{1T} k_1}{E_{2T} k_2}$ the input stimulus, $\bar{\beta} := 1 - \beta - c$ with $\beta := \frac{L_T}{W_T}$ the normalized amount of load and $c := \frac{k_D}{W_T}$ the normalized dissociation constant, and $K_1 := \frac{d_1 + k_1}{a_1 W_T}$, $K_2 := \frac{d_2 + k_2}{a_2 W_T}$ the Michaelis-Menten constants divided by the total protein concentration W_T.

In Figure 2, we plot relation (6) for different values of the load. The presence of the load decreases for every input stimulus the value of w^* as W* is "drained" by the binding to downstream targets. More interestingly, the *shape* of the input/output characteristics change: the response becomes less steep and the point of half maximal induction decreases. The steepness of the characteristics and the point of half maximal induction are physiologically relevant quantities in signaling systems as they determine how linear versus ultrasensitive, i.e., switch-like, the response to input stimuli is [9, 10]. We thus mathematically define the steepness and the point of half maximal induction and analytically determine how they are affected by the addition of the load.

A standard way in signaling and transcriptional systems to characterize the shape of a static input/output characteristic is to compare the characteristic under study to one of the Hill function form

$$w^* = w^*_{MAX} \frac{S^n}{K^n + S^n} \tag{7}$$

and determine estimates of the values of K and of n [9, 23]. Parameter n is the Hill coefficient and determines how sensitive the response is. High values of n correspond to almost switch-like response, referred to as ultrasensitive response, while low values of n (close to 1) correspond to almost linear response, referred to

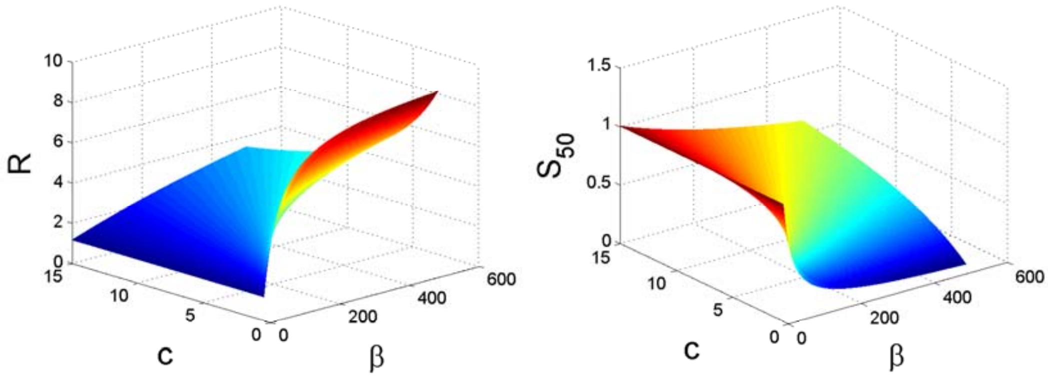

Figure 3. Response coefficient R (Left) and S_{50} (right) as a function of the normalized load amount β and the normalized dissociation constant c. Here, we set $K_1 = K_2 = 0.01$ and $c \in [1,15]$.

as hyperbolic response. The value of K corresponds to the value of S for which half maximal response is obtained. Consider the function under study $S(w^*)$ given in equation (6) and let w^*_{MAX} be the maximal value of w^* corresponding to $S = +\infty$. For $\alpha \in [0,1]$ we denote $S_{100\alpha} := S(\alpha\, w^*_{MAX})$. Thus, the value of S for which half maximal response is obtained (corresponding to the value of K in the Hill function of equation (7)) is given by the value of S_{50}. In order to estimate the value of the Hill coefficient, it is common to use the *response coefficient* defined as

$$R := \frac{S_{90}}{S_{10}}, \tag{8}$$

which for a Hill function satisfies the relationship $R = (81)^{1/n}$, so that R tends to 81 when n tends to 1 (hyperbolic response) and R monotonically decreases when n increases. We next analytically determine how the value of R and of S_{50} are affected by the value of the normalized load β.

3.1 Effect of retroactivity on the response of w^*

As a first step, we compute the maximal value w^*_{MAX} as a function of the load β. This can be obtained from equation (5) when we set $W = 0$:

$$w^*_{MAX} = \frac{1}{2}\left((1-\beta-c) + \sqrt{(1-\beta-c)^2 + 4c}\right).$$

The response coefficient R can be calculated by evaluating $S(w^*)$ from equation (6) for $w^* = 0.1 w^*_{MAX}$ and $w^* = 0.9 w^*_{MAX}$. This gives a function of the load-related parameters β and c for every value of the constants K_1 and K_2. This function is depicted in Figure 3 (left). From this, we deduce immediately that the response coefficient is a monotonically increasing function of the load amount β and a monotonically decreasing function of the dissociation constant c. Therefore, as the amount β and/or affinity $1/c$ of downstream binding sites increases, the response coefficient increases and as a consequence the Hill coefficient n decreases. The function S_{50} is depicted in the same figure (right). As the amount of load β increases and/or c decreases, the value of S_{50} decreases. As a consequence, increasing the amount and/or affinity of downstream binding sites decreases the value of S_{50}. These results are consistent with what observed in Figure 2.

We next seek to obtain analytical expression for R and S_{50} as function of c and β. To this end, we approximate the value of w^*_{MAX} in the limits of large values of load ($\beta \gg 1$) and of low values of load ($\beta \ll 1$):

$$w^*_{MAX} = \begin{cases} (1-\beta) & \text{if } \beta \ll 1 \text{ and } c \ll 1 \\ \frac{c}{\beta + c} & \text{if } \beta \gg 1. \end{cases} \tag{9}$$

The values of S_{10} and S_{90} can be computed by evaluating the right-hand side of equation (6) with $w^* = 0.1 w^*_{MAX}$ and $w^* = 0.9 w^*_{MAX}$. We perform this for the two different cases of equation (9).

Case 1: $\beta \ll 1$. *Small loads.* Let $\alpha = 0.1, 0.9$ and denote $v := (1-\beta)$, then for $c \ll 1$ we have that

$$S_{100\alpha} = \left(\frac{\alpha}{1-\alpha}\right)\left(\frac{v(1-\alpha) + K_1}{v\alpha + K_2}\right), \tag{10}$$

so that the response coefficient becomes

$$R = 81\frac{(0.1 + K_1/v)(0.1 + K_2/v)}{(0.9 + K_2/v)(0.9 + K_1/v)}.$$

From this expression, we notice the following facts:

(i) If $v = 1$ ($\beta = 0$, i.e., no load), the expression of R is the same as the one of a covalent modification cycle with no load obtained in standard references such as [9];

(ii) If $v < 1$ ($\beta > 0$, i.e., we add load), the expression of R is the same as the one for a covalent modification cycle obtained by [9] in which the values of K_1 and K_2 have been both increased by a factor of $1/v$. Therefore, when v decreases (β increases) the value of the response coefficient R increases and as a consequence the Hill coefficient n decreases.

The value of S_{50} is given by

$$S_{50} = \left(\frac{0.5v + K_1}{0.5v + K_2}\right),$$

in which, computing the derivative with respect to v, we obtain that $\frac{dS_{50}}{dv} = \frac{0.5(K_2-K_1)}{(0.5v+K_2)^2}$, so that if $K_2 > K_1$ the value of S_{50} decreases with β, while if $K_2 < K_1$ the value of S_{50} increases with β. In the case in which $K_1 = K_2$, the value of S_{50} does not change with the β.

Case 2: $\beta \gg 1$. *Large loads.* Let $\alpha = 0.1, 0.9$, assume that $(\alpha c)/(\beta + c) \ll 1$ and that $(1 + K_1) \ll (\beta + c)$, then the value of S can be well approximated by

$$S_{100\alpha} = \frac{c(\alpha(1-\alpha) + \alpha K_1)}{(\beta + c)K_2(1-\alpha) + \alpha(K_2 + c) - \alpha^2 c}, \tag{11}$$

which is clearly a decreasing function of β. Therefore, The value of S_{50} monotonically decreases with β. The value of the response coefficient is given by

$$R = \left(\frac{0.09 + 0.9K_1}{0.09 + 0.1K_1}\right)\left(\frac{0.9(\beta + c)K_2 + 0.1(K_2 + c) - 0.01c}{0.1(\beta + c)K_2 + 0.9(K_2 + c) - 0.81c}\right),$$

which is represented in Figure 4 in comparison with the actual value of R. These plots show that the approximation is good for sufficiently high values of c and that the approximation improves as c becomes larger (as expected from the conditions under which Case 2 holds). From this expression, the following facts emerge:

(i) The response coefficient R is a monotonically increasing function of β as the derivative of R with respect to β is always positive. Therefore, R increases with the load β and as a consequence the Hill coefficient n decreases with the load.
(ii) For large loads (i.e., $\beta \to \infty$), the value of R tends to a value that increases with K_1. When K_1 is small, the maximal value of R with increasing amounts of load is 9, which corresponds to a Hill coefficient equal to 2. When K_1 increases to values greater than 1, the value of R tends to 81, which corresponds to a Hill coefficient equal to 1. Therefore, unless K_1 is large enough, even large amounts of load will not bring the Hill coefficient down to 1.

Summarizing the conclusions of Case 1 (small loads) and Case 2 (large loads), we have that the Hill coefficient n decreases with increasing amount of loads, that for small loads, the response coefficient tends to the standard one obtained by standard references [9], and that for large loads the Hill coefficient tends to a value (strictly greater than 1), which tends to 1 only for increasing values of K_1.

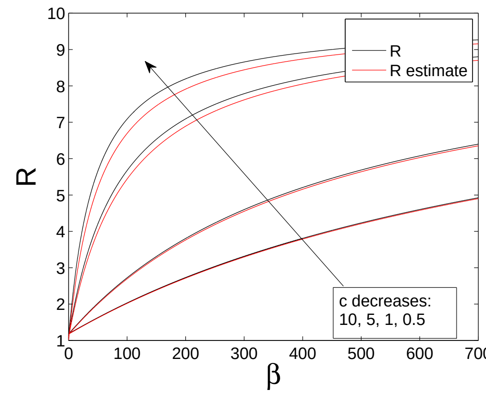

Figure 4. Response coefficient (in black) and its approximation (in red) for the case of large loads (here $K_1 = K_2 = 0.01$).

3.2 Effect of retroactivity on the response of w

In this case, we replace w^* in the expression of $S(w^*)$ by the function of w obtained by solving the conservation equation $w = 1 - w^* - \frac{w^*\beta}{w^*+c}$ for w^*:

$$w^* = \begin{cases} (1-\beta-w) & \text{if } \beta \ll 1 \text{ and } c \ll 1 \\ \frac{c(1-w)}{\beta+c} & \text{if } \beta \gg 1. \end{cases} \tag{12}$$

The maximal value of w is equal to 1 and it is obtained when $w^* = 0$. The resulting input/output characteristic is depicted in Figure 5 for different values of the load. As the load increases, the steepness of response and the value of S_{50} decrease.

In order to analytically quantify how the sensitivity and the value of S_{50} are affected by the load, we follow a similar procedure as followed in the previous section. Since w is a decreasing function of S, the response coefficient for the response of w to S is now defined as

$$R := \frac{S_{10}}{S_{90}},$$

in which S_{10} and S_{90} are calculated from equation (10), in which we have substituted expressions (12) in place of w^* for $w = 0.1$ and $w = 0.9$, respectively. As performed before, we consider two limit cases, depending on whether the load is small or large.

Case 1: $\beta \ll 1$. *Small loads.* Let $\alpha = 0.1, 0.9$ and $v = 1 - \beta$, then assuming $c \ll 1$ we obtain the new expression for the $S_{100\alpha}$

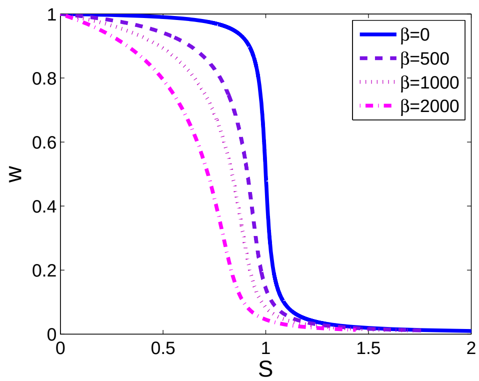

Figure 5. Steady state value of w as a function of the input stimulation S for different values of the load β (here $K_1 = K_2 = 0.01$, $c = 100$).

for the response of w to S as:

$$S_{100\alpha} = \left(\frac{\alpha + K_1}{\alpha}\right)\left(\frac{(v-\alpha)}{(v+K_2-\alpha)}\right),$$

which is valid only when $v - \alpha > 0$. This is an increasing function of v. As a consequence, the value of S_{50} decreases when the load β is increased. The response coefficient is given by

$$R = \left(\frac{9(0.1+K_1)}{(0.9+K_1)}\right)\left(\frac{(v-0.1)(v+K_2-0.9)}{(v+K_2-0.1)(v-0.9)}\right),$$

which is valid only for $v - 0.9 > 0$, that is, for $\beta < 0.1$. By computing the derivative of this expression with respect to v, we obtain that such a derivative is always negative when $(1-2\beta + K_2) > 0$. Since $v = 1-\beta$, this implies that increasing the amounts of load increases the response coefficient and as a consequence decreases the Hill coefficient n. From the expression of the response coefficient R, one can verify that when the load tends to zero, that is $v \to 1$, the response coefficient expression becomes equal to that obtained by [9] in the absence of any load.

Case 2: $\beta \gg 1$. *Large loads.* In this case, we substitute for w^* in expression (10) the value $w^* = \frac{c(1-w)}{\beta-1}$ and then we substitute α in place of w. This gives the same expression of $S_{100\alpha}$ obtained in equation (11), in which α needs to be replaced by $1-\alpha$, that is,

$$S_{100\alpha} = \frac{c(\alpha(1-\alpha)+(1-\alpha)K_1)}{(\beta+c)K_2\alpha + (1-\alpha)(K_2+c) - (1-\alpha)^2 c}.$$

From this expression, we conclude that the value of S_{50} monotonically decreases with the load β. The expression of the response coefficient becomes the same as the one for the S to w^* response, that is,

$$R = \left(\frac{0.09+0.9K_1}{0.09+0.1K_1}\right)\left(\frac{0.9(\beta+c)K_2 + 0.1(K_2+c) - 0.01c}{0.1(\beta+c)K_2 + 0.9(K_2+c) - 0.81c}\right).$$

Since this is a monotonically decreasing function of the load β, the Hill coefficient n decreases with increasing amounts of load. When the load grows to very large values ($\beta \to \infty$), the response coefficient tends to 81 only for values of K_1 sufficiently larger than 1, while for smaller values of K_1, the Hill coefficient tends to values between 1 and 2. As a consequence, unless K_1 is sufficiently large, the value of the Hill coefficient will not be reduced to 1 by large amounts of load.

Summarizing the results of Case 1 and Case 2 for the steady state response of w to S, we obtain that the value of S_{50} decreases as the load increases and that the Hill coefficient n decreases as the load β increases. Furthermore, when $\beta \to 0$ (Case 1), the expression of the response coefficient tends to the expression obtained by [9] for a covalent modification cycle with no load. When $\beta \to \infty$ (Case 2), we obtain that R tends to 81, which corresponds to Michaelis-Menten kinetics with Hill coefficient $n = 1$, only for sufficiently large values of K_1, while for smaller values of K_1 the Hill coefficient will be reduced to values between 1 and 2 for large amounts of load.

4 Discussion

In this modeling study, we have characterized the effect of downstream loading on the input/output static characteristic of a covalent modification cycle. Retroactivity due to loading decreases the sensitivity of response to input stimuli by decreasing the apparent Hill coefficient. Specifically, as the amount β of load relative to the total amount of signaling protein increases and/or the normalized value of the dissociation constant c decreases, the apparent Hill coefficient decreases. It decreases up to a limit that approaches $n = 1$ for sufficiently high values of the normalized Michaelis-Menten constant K_1 of the forward reaction. Therefore, for a fixed value of c, the effect of retroactivity is less dramatic when the total amount W_T of the upstream signaling protein is large compared to the total amount of load L_T.

In natural systems, covalent modification cycles are often found in cascade architectures, in which a cycle has several downstream targets [5, 6, 24]. Nevertheless, these cycles are capable of highly ultrasensitive responses to their input stimuli [21, 22]. This fact suggests that in natural systems the total amounts of a signaling protein may be finely tuned based on the amounts and affinity of downstream targets so that the desired response sensitivity is maintained. That is, signaling systems may have naturally evolved mechanisms for insulation from retroactivity. Alternatively, retroactivity may be used in signaling networks, in addition to well known mechanisms, as an effective means for tuning the shape of the static response to input stimuli.

5 Conclusions

Retroactivity is an impedance-like effect that appears at the interconnection of any biomolecular system with its downstream clients. Covalent modification cycles are fundamental building blocks of signaling networks, in which they appear connected to a potentially large number of downstream targets. These cycles may thus be subject to potentially large retroactivity effects. In this modeling study, we characterized these effects on the static input/output characteristics of a covalent modification cycle and showed that retroactivity makes an ultrasensitive response into a graded response. This study was performed with the aim of guiding experimental work on a signaling system extracted from the nitrogen regulation system of *E. coli* [4, 28, 29] and reconstituted *in vitro* to quantify retroactivity. This experimental work is currently under completion.

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