Biomolecular Feedback Systems

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Chapter 7
Interconnecting Components

In Chapter 2 and Chapter 6, we studied the behavior of simple biomolecular modules, such as oscillators, toggles, self repressing circuits, signal transduction and amplification systems, based on reduced order models. One natural step forward is to create larger and more complex systems by composing these modules together. In this chapter, we illustrate problems that need to be overcome when interconnecting components and propose a number of engineering solutions based on the feedback principles introduced in Chapter 3. Specifically, we explain how impedance-like effects arise at the interconnection between modules, which change the expected circuit behavior. These impedance problems appear in several other engineering domains, including electrical, mechanical, and hydraulic systems, and have been largely addressed by the respective engineering communities. In this chapter, we explain how similar engineering solutions can be employed in biomolecular systems to defeat impedance effects and guarantee "modular" interconnection of circuits. In Chapter 8, we further study loading of the cellular environment by synthetic circuits employing the same framework developed in this chapter.

7.1 Input/Output Modeling and the Modularity Assumption

The input/output modeling introduced in Chapter 1 and further developed in Chapter 3 has been employed so far to describe the behavior of various modules and subsystems. Such an input/output description of a system allows to connect systems together by setting the input u_2 of a downstream system equal to the output

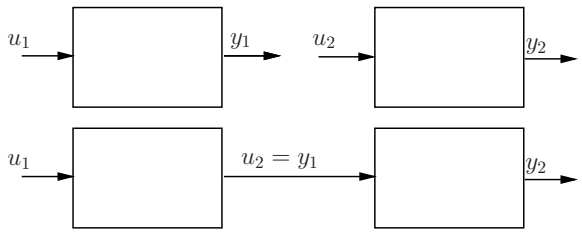

Figure 7.1: In the input/output modeling framework, systems are interconnected by statically assigning to the input of the downstream system the value of the output of the upstream system.

y_1 of the upstream system (Figure 7.1). This interconnection framework has been used extensively in the previous chapters.

Specifically, each node of a gene circuit has been modeled as an input/output module taking as input the concentrations of transcription factors that regulate a gene y and giving as output the concentration of protein Y expressed by gene y. This is of course not the only possible choice for delimiting a module. We could in fact let the mRNA or the RNA polymerase flowing along the DNA, called PoPS (polymerase per second) [29], play the role of input and output signals. Similarly, each node of a signal transduction network is usually a protein covalent modification module, which takes as input a modifying enzyme (a kinase in the case of phosphorylation) and gives as an output the modified protein.

For example, one of the models of the MAPK cascade considered in Section 2.5 was obtained by setting the value of the kinase concentration of a downstream cycle equal to the value of the concentration of the modified protein of the upstream cycle. A similar technique was employed for designing all the circuits of Chapter 6. For example, the repressilator model was obtained by setting the concentration of the input transcription factor of each gene equal to the concentration of the output transcription factor of the upstream gene.

This input/output modeling framework is extremely useful because it allows us to predict the behavior of an interconnected system based on the behavior of the isolated modules. For example, the location and number of steady states in the toggle switch of Section 6.3 were predicted by intersecting the steady state input/output characteristics of the isolated modules A and B. Similarly, the number of steady states in the repressilator was predicted by modularly composing the input/output steady state characteristics of the three modules composing the circuit.

For this input/output interconnection framework to reliably predict the behavior of connected modules, however, one must have that the input/output (dynamic) behavior of a system does not change upon interconnection to another system. We refer to the property by which a system input/output behavior does not change upon interconnection as *modularity*. Of course, all the designs and modeling described in the previous chapter assume that the modularity property holds. In this chapter, we question this assumption and investigate when modularity holds in gene and in signal transduction circuits.

7.2 Introduction to Retroactivity

The modularity assumption implies that when two modules are connected together, their behavior does not change because of the interconnection. However, a fundamental systems-engineering issue that arises when interconnecting subsystems is how the process of transmitting a signal to a "downstream" component affects the dynamic state of the sending component. This issue, the effect of "loads" on the output of a system, is well-understood in many engineering fields such as electrical

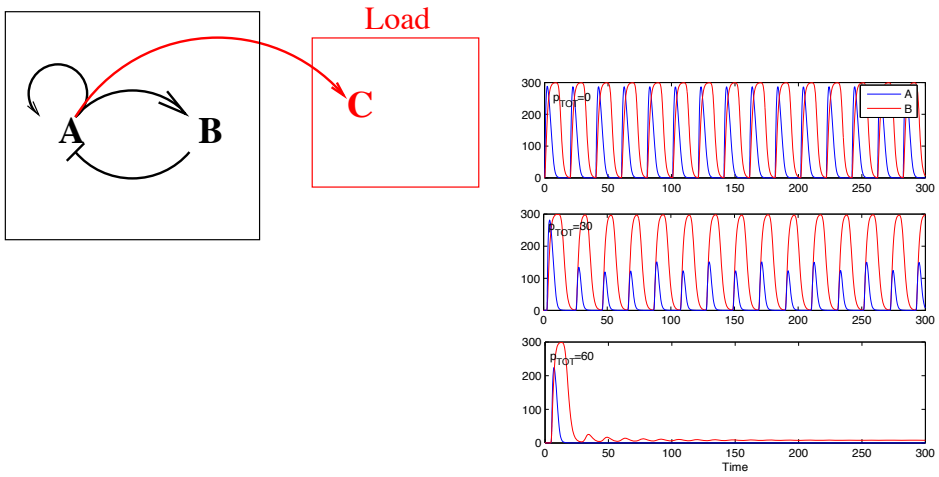

Figure 7.2: The clock behavior can be destroyed by a load. As the number of downstream binding sites for A, p_{tot}, is increased in the load, the activator and repressor dynamics loose their synchronization and ultimately the oscillations disappear.

engineering. It has often been pointed out that similar issues may arise for biological systems. These questions are especially delicate in design problems, such as those described in Chapter 6.

For example, consider a biomolecular clock, such as the activator-repressor clock introduced in Section 6.5. Assume that the activator protein concentration $A(t)$ is now used as a means to synchronize or time some downstream systems. From a systems/signals point of view, $A(t)$ becomes an *input* to the second system (Figure 7.2). The terms "upstream" and "downstream" reflect the direction in which we think of signals as traveling, *from* the clock *to* the systems being synchronized. However, this is only an idealization, because, as seen in Figure 7.2, the binding and unbinding of A to promoter sites in a downstream system competes with the biochemical interactions that constitute the upstream clock and may therefore disrupt the operation of the clock itself. We call this "back-effect" *retroactivity* to extend the notion of impedance or loading to non-electrical systems and in particular to biomolecular systems. This phenomenon, while in principle may be used in an advantageous way from natural systems, can be deleterious when designing synthetic systems.

One possible approach to avoid disrupting the behavior of the clock is to introduce a gene coding for a new protein X, placed under the control of the same promoter as the gene for A, and using the concentration of X, which presumably mirrors that of A, to drive the downstream system. This approach, however, still has the problem that the behavior of the X concentration in time may be altered and even disrupted by the addition of downstream systems that drain X, as we shall see in the next section. The net result is that the downstream systems are not prop-

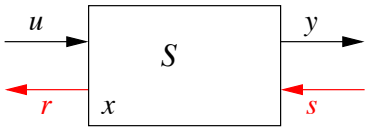

Figure 7.3: A system S input and output signals. The r and s signals denote signals originating by retroactivity upon interconnection [22].

erly timed as X does not transmit the desired signal. Methods to model and prevent retroactivity is the subject of this chapter.

To model a system with retroactivity, we add to the input/output modeling framework used so far, an additional input, called s, to model any change that may occur upon interconnection with a downstream system. That is, s models the fact that whenever y is taken as an input to a downstream system the value of y may change, because of the physics of the interconnection. This phenomenon is also called in the physics literature "the observer effect", implying that no physical quantity can be measured without being altered by the measurement device. Similarly, we add a signal r as an additional output to model the fact that when a system is connected downstream of another one, it will send a signal upstream that will alter the dynamics of that system. More generally, we define a system S to have internal state x, two types of inputs, and two types of outputs: an input "u", an output "y" (as before), a *retroactivity to the input* "r", and a *retroactivity to the output* "s" (Figure 7.3). We will thus represent a system S by the equations

$$\frac{dx}{dt} = f(x,u,s), \qquad y = h(x,u,s), \qquad r = R(x,u,s), \tag{7.1}$$

where f, g, and R are arbitrary functions and the signals x, u, s, r, and y may be scalars or vectors. In such a formalism, we define the input/output model of the isolated system as the one in equation (7.1) without r in which we have also set $s = 0$.

Let S_i be a system with inputs u_i and s_i and with outputs y_i and r_i. Let S_1 and S_2 be two systems with disjoint sets of internal states. We define the interconnection of an upstream system S_1 with a downstream system S_2 by simply setting $y_1 = u_2$ and $s_1 = r_2$. For interconnecting two systems, we require that the two systems do not have internal states in common.

Inset. As a simple example, which may be more familiar to an engineering audience, consider the hydraulic system shown in Figure 7.4. We consider a constant input flow f_0 as input to the upstream tank and the pressure p as its output. 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

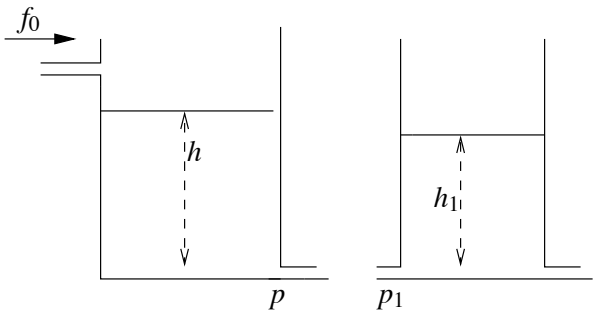

Figure 7.4: On the left, we represent a tank system that takes as input the constant flow f_0 and gives as output the pressure p at the output pipe. On the right, we show a downstream tank.

level in the tank and ρ is water density. Let A be the cross section of the tank, then the tank system can be represented by the equation

$$A\frac{dp}{dt} = \rho f_0 - \rho k\sqrt{p}. \tag{7.2}$$

Hence, the steady state value of the pressure p is given by

$$p_{eq} = (f_0/k)^2.$$

We now connect the output pipe of the same tank to the input pipe of a downstream tank shown on the right of Figure 7.4. 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

$$\begin{aligned} A\frac{dp}{dt} &= \rho f_0 - \rho g(p, p_1), \\ A_1\frac{dp_1}{dt} &= \rho g(p, p_1) - \rho k_1\sqrt{p_1}, \end{aligned} \tag{7.3}$$

in which A_1 is the cross section of the downstream tank and k_1 is a positive parameter depending on the geometry of the downstream tank. Thus, the input/output response of the tank measured in isolation (equation (7.2)) does not stay the same when the tank is connected through its output pipe to another tank (equation (7.3)). The resulting equilibrium pressure is also different and given by

$$p_{eq} = \left(\frac{f_0}{k}\right)^2\left(1 + \frac{k^2}{k_1^2}\right).$$

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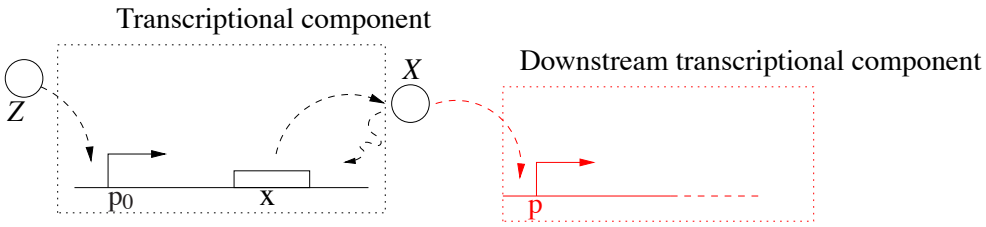


Figure 7.5: The transcriptional component takes as input u protein concentration Z and gives as output y protein concentration X. The downstream transcriptional component takes protein concentration X as its input.

7.3 Retroactivity in Gene Circuits

In the previous section, we have defined retroactivity as a general concept modeling the fact that when an upstream system is input/output connected to a downstream one, its behavior can change. In this section, we focus on gene circuits and show what form retroactivity takes and what its net effects are.

Consider the transcriptional system of Figure 7.5 in the dashed box. It is an input/output system that takes as input the transcription factor concentration Z and gives as output the transcription factor concentration $X(t)$. 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. To simplify notation, we denote it by $k(t)$. We assume here that the mRNA dynamics are at their quasi-steady state. The reader can verify that all the results hold unchanged when the mRNA dynamics are included (see exercises). We write the dynamics of X as

$$\frac{dX}{dt} = k(t) - \delta X, \tag{7.4}$$

in which δ is the decay rate constant of the protein. We refer to equation (7.4) as the *isolated system dynamics*.

Now, assume that X drives a downstream transcriptional module by binding to a promoter p with concentration p (Figure 7.5). The reversible binding reaction of X with p is given by

$$\mathrm{X} + \mathrm{p} \underset{k_{\mathrm{on}}}{\overset{k_{\mathrm{off}}}{\rightleftharpoons}} \mathrm{C}$$

in which C is the complex protein-promoter and k_{on} and k_{off} are the association and dissociation rate constants of protein X to promoter site p. Since the promoter is not subject to decay, its total concentration p_{tot} is conserved so that we can write $p + C = p_{\mathrm{tot}}$. Therefore, the new dynamics of X are governed by the equations

$$\frac{dX}{dt} = k(t) - \delta X + [k_{\mathrm{off}} C - k_{\mathrm{on}}(p_{\mathrm{tot}} - C)X], \tag{7.5}$$

$$\frac{dC}{dt} = -k_{\mathrm{off}} C + k_{\mathrm{on}}(p_{\mathrm{tot}} - C)X, \tag{7.6}$$

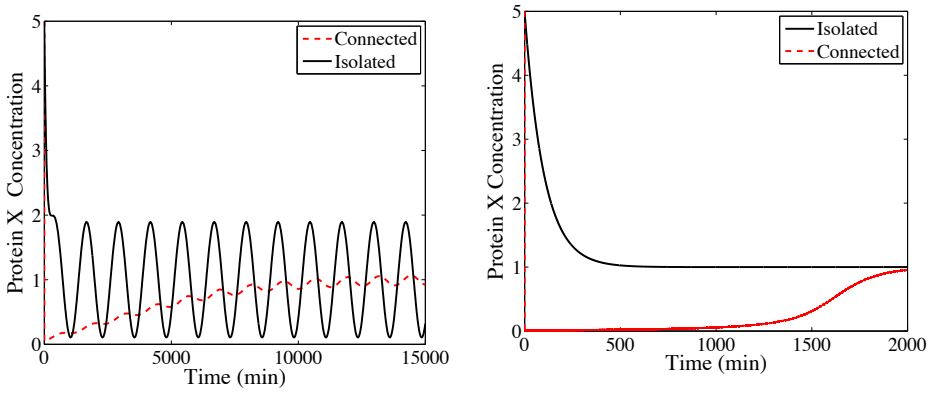


Figure 7.6: The effect of interconnection. Simulation results for the system in equations (7.6). The solid line represents $X(t)$ originating by equations (7.4), while the dashed line represents $X(t)$ obtained by equation (7.6). Both transient and permanent behaviors are different. Here, $k(t) = 0.01(1 + sin(\omega t))$ 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 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 [5].

in which

$$s = k_{off}C - k_{on}(p_{tot} - C)X.$$

We refer to this system as *connected* system. The terms in the brackets represent the signal s, that is, the retroactivity to the output, while the second of equation (7.6) describes the dynamics of the downstream system driven by X. Then, we can interpret s as being a mass flow between the upstream and the downstream system. When $s = 0$, the first of equations (7.6) reduces to the dynamics of the isolated system given in equation (7.4).

How large is the effect of retroactivity s on the dynamics of X and what are the biological parameters that affect it? 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, here modeled by $k(t)$, in the presence of its binding sites p_0 in Figure 7.5 in a way similar to how we analyze the dynamics of X in the presence of the downstream binding sites p.

The effect of retroactivity s on the behavior of X can be very large (Figure 7.6). By looking at Figure 7.6, we notice that the effect of retroactivity is to "slow down" the dynamics of $X(t)$ as the response time to a step input increases and the response to a periodic signal appears attenuated and phase-shifted. We will come back to this more precisely in the next section.

These effects are 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 7.6), the coordination between the oscillator and the downstream processes will be lost. We next provide a procedure to obtain an operative quantification of the effect of the retroactivity on the dynamics of the upstream system.

Quantification of the retroactivity to the output

In this section, we provide a general approach to quantify the retroactivity to the output. To do so, we quantify the difference between the dynamics of X in the isolated system (7.4) and the dynamics of X in the connected system (7.6) 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 (7.6) by a one dimensional system describing the evolution of X on the slow manifold (see Section 3.6).

Consider again the full system in equations (7.6), in which the binding and unbinding dynamics are much faster than protein production and decay, that is, $k_{\text{off}}, k_{\text{on}} \gg k(t), \delta$ and define $K_{\text{d}} = k_{\text{off}}/k_{\text{on}}$ as before. Even if the second equation goes to equilibrium very fast compared to the first one, the above system is not in standard singular perturbation form. In fact, while C clearly is a fast variable, X is neither fast nor slow since its differential equation displays both fast and slow terms. To explicitly model the difference of time scales, we introduce a parameter ϵ, which we define as $\epsilon = \delta/k_{\text{off}}$. Since $k_{\text{off}} \gg \delta$, we also have that $\epsilon \ll 1$. Substituting $k_{\text{off}} = \delta/\epsilon$, $k_{\text{on}} = \delta/(\epsilon K_{\text{d}})$, and letting $y = X + C$ (the *total* protein concentration), we obtain the system in standard singular perturbation form

$$\frac{dy}{dt} = k(t) - \delta(y - C), \qquad \epsilon\frac{dC}{dt} = -\delta C + \frac{\delta}{K_{\text{d}}}(p_{\text{tot}} - C)(y - C), \tag{7.7}$$

in which y is the slow variable. The reader can check as an exercise that the slow manifold of system (7.7) is locally exponentially stable (see Exercises).

We can obtain an approximation of the dynamics of X in the limit in which ϵ is very small, by setting $\epsilon = 0$. This leads to

$$-\delta C + \frac{\delta}{K_{\text{d}}}(p_{\text{tot}} - C)X = 0 \rightarrow C = \gamma(X) \text{ with } \gamma(X) = \frac{p_{\text{tot}}X}{X + K_{\text{d}}}.$$

Since $dy/dt = dX/dt + dC/dt$, we have that $dy/dt = dX/dt + (d\gamma/dX)dX/dt$. This along with $dy/dt = k(t) - \delta X$ lead to

$$\frac{dX}{dt} = (k(t) - \delta X)\left(\frac{1}{1 + d\gamma/dX}\right). \tag{7.8}$$

The difference between the dynamics in equation (7.8) (the connected system after a fast transient) and the dynamics in equation (7.4) (the isolated system) is zero when the term $\frac{d\gamma(X)}{dX}$ in equation (7.8) is zero. We thus consider the factor $\frac{d\gamma(X)}{dX}$ as a quantification of the retroactivity s after a fast transient in the approximation in which $\epsilon \approx 0$. We can also interpret the factor $\frac{d\gamma(X)}{dX}$ as a percentage variation of the dynamics of the connected system with respect to the dynamics of the isolated system at the quasi steady state. We next determine the physical meaning of such a factor by calculating a more useful expression that is a function of key biochemical parameters.

By using the implicit function theorem, one can compute the following expression for $d\gamma(X)/dX$:

$$\frac{d\gamma(X)}{dX} = \frac{p_{\text{tot}}/K_{\text{d}}}{(X/K_{\text{d}}+1)^2} =: \mathcal{R}(X). \tag{7.9}$$

The retroactivity measure $\mathcal{R}$ is low basically whenever the ratio $p_{\text{tot}}/K_{\text{d}}$, which can be seen as an effective load, is low. This is the case if the affinity of the binding sites p is small (K_{d} large) or if p_{tot} is low. Also, the retroactivity measure is dependent on X in a nonlinear fashion and it is such that it is maximal when X is the smallest. The expression of $\mathcal{R}(X)$ provides an operative quantification of the retroactivity: such an expression can in fact be evaluated once the dissociation constant of X to p is known, the concentration of the binding sites p_{tot} is known, and X is also known.

Summarizing, the modularity assumption introduced in Section 7.1 holds only when the value of $\mathcal{R}(X)$ is small enough. As a consequence, the design of a simple circuit can assume modularity if the interconnections among the composing modules can be designed so that the value of $\mathcal{R}(X)$ is low. From a design point of view, low retroactivity can be obtained by either choosing low-affinity binding sites p or making sure that the amounts of p is not too high. This can be guaranteed by placing the promoter sites p on low copy number plasmids or even on the chromosome (with copy number equal to 1). High copy number plasmids are expected to lead to non-negligible retroactivity effects on X.

However, in the presence of very low affinity and/or very low amount of promoter sites, the amount of complex C will be very low. As a consequence, the amplitude of the transmitted signal to downstream may be also very small. Hence, there will be a design compromise between guaranteeing a sufficiently high signal while minimizing retroactivity. A better approach is to design insulation devices (as opposed to designing the interconnection for low retroactivity) to buffer systems from retroactivity as explained later in the chapter.

Characterizing the effects of retroactivity

How do we explain the amplitude attenuation and phase shift due to retroactivity observed in Figure 7.6? In order to answer this question, we can linearize the system about its steady state and determine the effect of retroactivity on the

frequency response. Let the input be $k(t) = k + A_0 \sin(\omega t)$ and let $X = k/\delta$ and $C = p_{\text{tot}} X/(X + K_{\text{d}})$ be the equilibrium values corresponding to k. The isolated system is already linear, so there is no need to perform linearization and the transfer function from k to X is given by

$$G^I_{Zk}(s) = \frac{1}{s+\delta}.$$

For the connected system, denote the displacements with respect to the steady state (k, X, C) by $\tilde{k} = k - k$, $x = X - X$, and $c = C - C$. Then, the linearized dynamics are given by

$$\begin{aligned}
\frac{dx}{dt} &= \tilde{k}(t) - \delta x - \frac{\delta}{\epsilon K_{\text{d}}} x(p_{\text{tot}} - C) + \frac{\delta}{\epsilon} Xc + \frac{\delta}{\epsilon} c \\
\frac{dc}{dt} &= \frac{\delta}{\epsilon K_{\text{d}}} x(p_{\text{tot}} - C) - \frac{\delta}{\epsilon} Xc - \frac{\delta}{\epsilon} c
\end{aligned}$$

Letting $y := c + x$, these can be taken to standard singular perturbation form:

$$\begin{aligned}
\frac{dy}{dt} &= \tilde{k}(t) - \delta(y - c), \\
\epsilon \frac{dc}{dt} &= \frac{\delta}{\epsilon K_{\text{d}}} x(p_{\text{tot}} - C) - \frac{\delta}{\epsilon} Xc - \frac{\delta}{\epsilon} c \quad .
\end{aligned}$$

Setting $\epsilon = 0$, gives the expression of the slow manifold as $c = x(p_{\text{tot}} - C)/(X/K_{\text{d}} + 1) =: \gamma(x)$. Using the expression of C, the fact that $dx/dt + dc/dt = dy/dt = \tilde{k}(t) - \delta x$ and that $dc/dt = (d\gamma/dx)dx/dt$, we finally obtain the expression of the x dynamics on the slow manifold as

$$\frac{dx}{dt} = (\tilde{k}(t) - \delta x) \frac{1}{1 + (p_{\text{tot}}/K_{\text{d}})/(X/K_{\text{d}} + 1)^2}.$$

Denoting $R := (p_{\text{tot}}/K_{\text{d}})/(X/K_{\text{d}} + 1)^2$, we obtain the transfer function from $\tilde{k}$ to x of the approximated connected system linearization as

$$G^C_{Zk} = \frac{1}{1+R} \frac{1}{s + \delta/(1+R)}.$$

Hence, we have the following result for the frequency response amplitude and phase shift:

$$\begin{aligned}
M^I_{Zk}(\omega) &= \frac{1}{\sqrt{\omega^2 + \delta^2}}, \ \phi^I_{Zk}(\omega) = \tan^{-1}(-\omega/\delta), \\
M^C_{Zk}(\omega) &= \frac{1}{1+R} \frac{1}{\sqrt{\omega^2 + \delta^2/(1+R)^2}}, \ \phi^C_{Zk}(\omega) = \tan^{-1}(-\omega(1+R)/\delta),
\end{aligned}$$

from which one obtains that $M^I_{Zk}(0) = M^C_{Zk}(0)$ and, since $R > 0$, the bandwidth of the connected system is lower than that of the isolated system. Also, the phase shift of the connected system will be larger than that of the isolated system.

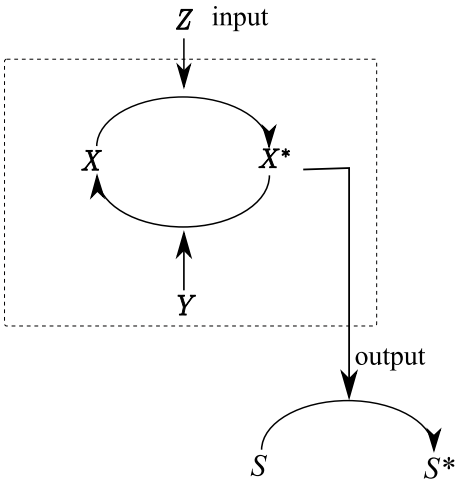


Figure 7.7: Covalent modification cycle (in the box) with its downstream system.

7.4 Retroactivity in Signaling Systems

Signaling systems are circuits that take external stimuli and through a sequence of biolmolecular reactions transform them to useful signals that establish how cells respond to their environment. These systems are usually composed of covalent modification cycles (phosphorylation, methylation, urydylilation, etc.) connected in cascade fashion, in which each cycle has multiple downstream targets (or substrates). An example is that of the MAPK cascade, which we have analyzed in Section 2.5. Since covalent modification cycles always have downstream targets, such as DNA binding sites or other substrates, it is particularly important to understand whether and how retroactivity from these downstream systems affect the response of the upstream cycles to input stimulation. In this section, we study this question both for the steady state and dynamic response of a covalent modification cycle to its input (refer to Figure 7.7).

Steady state effects of retroactivity

One important characteristic of signaling systems and, in particular, of covalent modification cycles, is the steady state characteristics (also called dose response). This describes the steady state output value in response to a constant input stimulation. For a single covalent modification cycle, this has been extensively studied as a function of important cycle parameters, such as the Michaelis-Menten constants and the total amount of protein. In particular, it was found that when the Michaelis-Menten constants are sufficiently small compared to the total protein amount, the cycle characteristic becomes ultrasensitive, a condition called zero-order ultrasen-

sitivity (Section 2.4).

However, when the cycle is interconnected to its downstream targets, this characteristic may change shape. In order to understand how this may change, we rewrite the reaction rates and corresponding differential equation model for the covalent modification cycle incorporating the binding of X* to its downstream targets. Referring to Figure 7.7, we have the following reactions:

$$\mathrm{Z} + \mathrm{X} \underset{d_1}{\overset{a_1}{\rightleftharpoons}} \mathrm{C}_1 \xrightarrow{k_1} \mathrm{X}^* + \mathrm{Z}, \qquad \mathrm{X}^* + \mathrm{Y} \underset{d_2}{\overset{a_2}{\rightleftharpoons}} \mathrm{C}_2 \xrightarrow{k_2} \mathrm{X} + \mathrm{Y},$$

to which we add the binding reaction of X* with its substrates S:

$$\mathrm{X}^* + \mathrm{S} \underset{k_{\mathrm{off}}}{\overset{k_{\mathrm{on}}}{\rightleftharpoons}} \mathrm{C},$$

in which C is the complex of X* with S. In addition to this, we have the conservation laws $X_{\mathrm{tot}} = X^* + X + C_1 + C_2 + C$, $Z + C_1 = Z_{\mathrm{tot}}$, and $Y + C_2 = Y_{\mathrm{tot}}$.

The rate equations governing the system are given by

$$\begin{aligned}
\frac{dC_1}{dt} &= a_1 XZ - (d_1 + k_1)C_1 \\
\frac{dX^*}{dt} &= -a_2 X^* Y + d_2 C_2 + k_1 C_1 - k_{\mathrm{on}} S X^* + k_{\mathrm{off}} C \\
\frac{dC_2}{dt} &= a_2 X^* Y - (d_2 + k_2)C_2 \\
\frac{dC}{dt} &= k_{\mathrm{on}} X^* S - k_{\mathrm{off}} C.
\end{aligned}$$

The input/output characteristics are found by solving this system for the equilibrium. In particular, by setting $dC_1/dt = 0$, $dC_2/dt = 0$, using that $Z = Z_{\mathrm{tot}} - C_1$ and that $Y = Y_{\mathrm{tot}} - C_2$, we obtain the familiar expressions for the complexes:

$$C_1 = \frac{Z_{\mathrm{tot}} X}{K_1 + X}, \qquad C_2 = \frac{Y_{\mathrm{tot}} X^*}{K_2 + X^*}, \qquad \text{with} \qquad K_1 = \frac{d_1 + k_1}{a_1} \qquad \text{and} \qquad K_2 = \frac{d_2 + k_2}{a_2}.$$

By setting $dX^*/dt + dC_2/dt + dC/dt = 0$, we obtain $k_1 C_2 = k_2 C_2$, which leads to

$$V_1 \frac{X}{K_1 + X} = V_2 \frac{X^*}{K_2 + X^*}, \qquad V_1 = k_1 Z_{\mathrm{tot}} \qquad \text{and} \qquad V_2 = k_2 Y_{\mathrm{tot}}. \tag{7.10}$$

By assuming that the substrate X_{tot} is in excess compared to the enzymes, we have that $C_1, C_2 \ll X_{\mathrm{tot}}$ so that $X \approx X_{\mathrm{tot}} - X^* - C$, in which (from setting $dC/dt = 0$) $C = X^* S / K_{\mathrm{d}}$ with $K_{\mathrm{d}} = k_{\mathrm{off}}/k_{\mathrm{on}}$, leading to $X \approx X_{\mathrm{tot}} - X^*(1 + S/K_{\mathrm{d}})$. Calling $\lambda = S/K_{\mathrm{d}}$, equation (7.10) finally leads to

$$y := \frac{V_1}{V_2} = \frac{X^* \left(\frac{K_1}{1+\lambda} + \left(\frac{X_{\mathrm{tot}}}{1+\lambda} - X^* \right) \right)}{(K_2 + X^*) \left(\frac{X_{\mathrm{tot}}}{1+\lambda} - X^* \right)}. \tag{7.11}$$

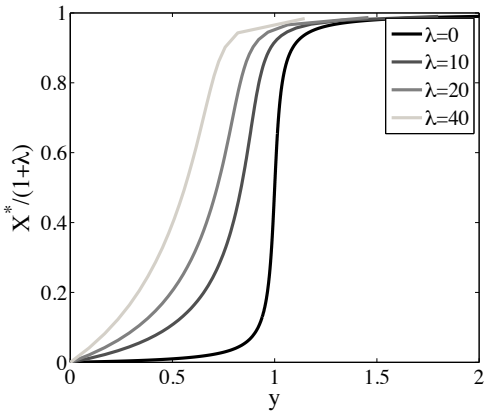


Figure 7.8: The addition of downstream target sites make the input/output characteristic more linear-like, that is, retroactivity makes a switch-like response into a more graded response.

Here, we can interpret λ as an effective load, which increases with the amount of targets of X^* but also with the affinity of these targets ($1/K_d$). The ratio $V_1/V_2 = y$ is a normalized input stimulation as it linearly increases with the input Z_{tot}.

We are interested in how the shape of the steady state curve of X^* as function of y changes when the effective load λ is changed. As seen in Section 2.4, a way to characterize the shape of the steady state characteristic is to calculate the response coefficient

$$R = \frac{y_{90}}{y_{10}}.$$

In the case of the current system, we have that the maximal value of X^* obtained as $y \to \infty$ is given by $X_{tot}/(1+\lambda)$. Hence, from equation (7.11), we have that

$$y_{90} = \frac{(K_1 + 0.1)0.9}{(K_2(1+\lambda) + 0.9)0.1}, \qquad y_{10} = \frac{(K_1 + 0.9)0.1}{(K_2(1+\lambda) + 0.1)0.9},$$

$$K_1 := \frac{K_1}{X_{tot}}, \qquad K_2 = \frac{K_2}{X_{tot}},$$

so that

$$R = 81\frac{(K_1 + 0.1)(K_2(1+\lambda) + 0.1}{(K_2(1+\lambda) + 0.9)(K_1 + 0.9)}.$$

This expression clearly indicates that the net effect of the load is to increase the Michaelis-Menten constant K_2 of the backward enzymatic reaction.

One can check that R is a monotonically increasing function of λ. In particular, as λ increases, the value of R tends to $81(K_1 + 0.1)/(K_2 + 0.9)$, which, in turn, tends

to 81 for $K_1, K_2 \to \infty$. When $\lambda = 0$, we recover the results of Section 2.4, according to which R approaches 81 (Michaelis-Menten type of response) for K_1, K_2 large, while R decreases for decreasing values of K_1, K_2, corresponding to an ultrasensitive response. Independently of the values of K_1 and K_2, the addition of the load makes any characteristic more linear-like (see Figure 7.8). This finding has been experimentally confirmed employing signal transduction circuits reconstituted *in vitro* [95].

We can also study the behavior of the point of half maximal induction

$$y_{50} = \frac{K_1 + 0.5}{K_2(1+\lambda) + 0.5},$$

to find that as λ increases, y_{50} decreases. That is, as more downstream load is applied, a smaller stimulus is required to obtain a significant response of the output (see exercises).

Dynamic effects of retroactivity

In order to understand the dynamic effects of retroactivity on the signaling module, we seek a one dimensional approximation of the X^* dynamics, which can be easily analyzed. To do so, we exploit time scale separation and apply singular perturbation analysis.

Specifically, we have that $a_i, d_i, k_{\text{on}}, k_{\text{off}} \gg k_1, k_2$, so we can choose as a small parameter $\epsilon = k_1/k_{\text{off}}$ and slow variable $y = X^* + C + C_2$. By setting $\epsilon = 0$, we obtain that $C_1 = Z_{\text{tot}} X/(K_1 + X)$, $C_2 = Y_{\text{tot}} X^*/(K_2 + X^*) =: \gamma(X^*)$, and $C = \lambda X^*$, in which Z_{tot} is now a time-varying signal. Hence, the dynamics of the slow variable y on the slow manifold is given by

$$\frac{dy}{dt} = k_1 \frac{Z_{\text{tot}}(t) X}{K_1 + X} - k_2 Y_{\text{tot}} \frac{X^*}{X^* + K_2}.$$

Using $dy/dt = dX^*/dt + dC/dt + dC_2/dt, dC/dt = \lambda dX^*/dt, dC_2/dt = \partial\gamma/\partial X^* dX^*/dt$, and the conservation law $X = X_{\text{tot}} - X^*(1+\lambda)$, we finally obtain the approximated X^* dynamics as

$$\frac{dX^*}{dt} = \frac{1}{1+\lambda}\left(k_1 \frac{Z_{\text{tot}}(t)(X_{\text{tot}} - X^*(1+\lambda))}{K_1 + (X_{\text{tot}} - X^*(1+\lambda))} - k_2 Y_{\text{tot}} \frac{X^*}{X^* + K_2}\right), \tag{7.12}$$

where we have assumed that that $Y_{\text{tot}}/K_2 \ll S/K_{\text{d}}$, so that the effect of the binding dynamics of X* with Y (modeled by $\partial\gamma/\partial X^*$) is negligible with respect to λ. The reader can verify this derivation as an exercise (see exercises).

From this expression, one can understand immediately the effect of the load λ on the rise time and decay time in response to extreme input stimuli. For the decay

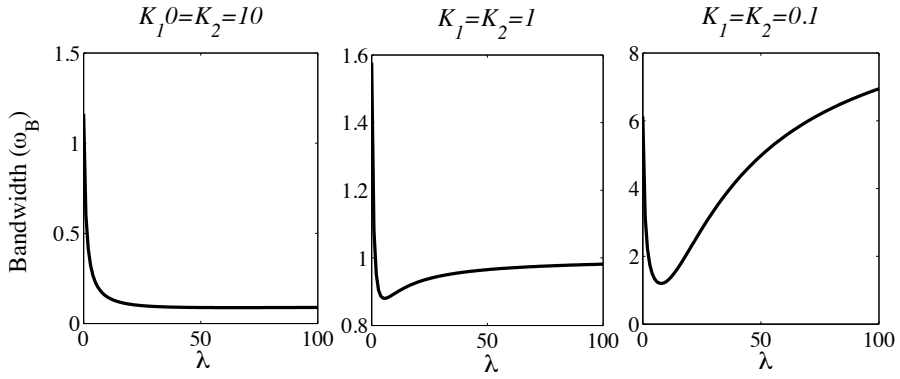


Figure 7.9: Behavior of the bandwidth as a function of the load for different values of the Michaelis-Menten constants K_1, K_2. Here $X_{\text{tot}} = 1$.

time, one has to assume an initial condition $X^*(0) \neq 0$ and $Z_{\text{tot}}(t) = 0$ for all t. In this case, we have that

$$\frac{dX^*}{dt} = -k_2 Y_{\text{tot}} \frac{X^*}{X^* + K_2} \frac{1}{1+\lambda},$$

from which, since $\lambda > 0$, it is apparent that the transient will be slower and hence that the system will have an increased decay time due to retroactivity. For the rise time, one can assume $Z_{\text{tot}} \approx \infty$ and $X^*(0) = 0$. Hence, we have that

$$(1+\lambda)\frac{dX^*}{dt} = \left(k_1 \frac{Z_{\text{tot}}(t)(X_{\text{tot}} - X^*(1+\lambda))}{K_1 + (X_{\text{tot}} - X^*(1+\lambda))} \right),$$

which is the same expression for the isolated system in which X^* is scaled by $(1+\lambda)$. So, the rise time is not affected.

In order to understand how the bandwidth of the system is affected by retroactivity, we consider $Z_{\text{tot}}(t) = Z + A_0 \sin(\omega t)$. Let X be the equilibrium of X^* corresponding to Z and denote the displacements $z = Z_{\text{tot}} - Z$ and $x = X^* - X$. The linearized dynamics are given by

$$\frac{dx}{dt} = -a(\lambda)x + b(\lambda)z(t),$$

in which

$$a(\lambda) = \frac{1}{1+\lambda} \left(k_1 Z \frac{K_1(1+\lambda)}{(K_1 + (X_{\text{tot}} - X(1+\lambda)))^2} + k_2 Y_{\text{tot}} \frac{K_2}{(K_2 + X)^2} \right)$$

and

$$b(\lambda) = \frac{k_1}{1+\lambda} \left(\frac{X_{\text{tot}} - X(1+\lambda)}{K_1 + (X_{\text{tot}} - X(1+\lambda))} \right),$$

so that the bandwidth of the system is given by $\omega_B = a(\lambda)$.

Figure 7.9 shows the behavior of the bandwidth as a function of the load. When the isolated system static characteristics are linear-like ($K_1, K_2 \gg X_{\text{tot}}$), the bandwidth monotonically decreases with the load. Hence applying any load decreases system bandwidth. When the isolated system static characteristics are ultrasensitive ($K_1, K_2 \ll X_{\text{tot}}$), the bandwidth of the connected system can be larger than that of the isolated system for sufficiently large amounts of loads. In these conditions, one should expect that the response of the connected system becomes faster than that of the isolated system.

7.5 Insulation Devices: Retroactivity Attenuation

As explained earlier, it is not always possible or advantageous to design the downstream system so that it applies low retroactivity. This is because the downstream system may have already been designed and optimized for other purposes. A better approach, in analogy to what is performed in electrical circuits, is to design a device to be placed between the upstream system (the oscillator, for example) and the downstream load so that the device output is not changed by the load and the device does not affect the behavior of the upstream system. That is, the output of the device should follow the prescribed behavior independently of any loading applied by a downstream system.

Specifically, consider a system S such as the one shown in Figure 7.3 that takes u as input and gives y as output. We would like to design such a system so that

(a) the retroactivity r to the input is very small;

(b) the effect of the retroactivity s to the output on the internal dynamics of the system is very small independently of s itself.

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.

Retroactivity to the input

Equation (7.9) 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 $1/K_{\text{d}}$, the total concentration of such binding site p_{tot}, and the level of the signal $X(t)$. Therefore, to reduce the retroactivity, we can choose parameters such that (7.9) is small. A sufficient condition is to 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

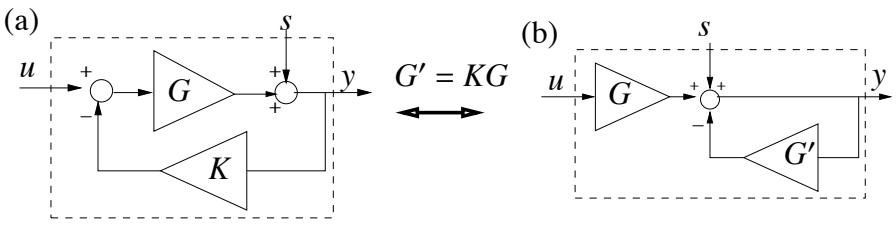


Figure 7.10: Diagram (a) shows the basic feedback/amplification mechanism by which amplifiers attenuate the effect of the retroactivity to the output s. Diagram (b) shows an alternative representation of the same mechanism of diagram (a), which will be employed to design biological insulation devices.

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.

Attenuation of retroactivity to the output: Principle 1

The basic mechanism for retroactivity attenuation is based on the concept of disturbance attenuation presented in Section 3.2. In its simplest form, it can be illustrated by diagram (a) of Figure 7.10, in which the retroactivity to the output s plays the same role as an additive disturbance. For large gains G, the effect of the retroactivity s to the output is negligible as the following simple computation shows. The output y is given by

$$y = G(u - Ky) + s,$$

which leads to

$$y = u\frac{G}{1+KG} + \frac{s}{1+KG}.$$

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 the input of the component through a large gain and (2) apply a large negative output feedback. The inset illustrates this general idea in the context of a simple hydraulic system.

Inset. Consider the academic hydraulic example consisting of two connected tanks shown in Figure 7.11. 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 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

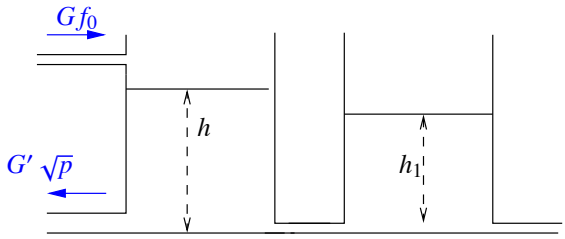


Figure 7.11: We amplify the input flow f_0 through a large gain G and we apply a large negative feedback by employing a large output pipe with output flow $G'\sqrt{p}$.

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, 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 described by

$$A\frac{dp}{dt} = \rho G f_0 - \rho G' \sqrt{p} - \rho k \sqrt{p}$$

for G sufficiently large and for $G' = KG$.

◊

Going back to the transcriptional example, consider the approximated dynamics of equation (7.8) for X. Let us thus 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 (7.8) given by

$$\frac{dX}{dt} = (Gk(t) - (G' + \delta)X)(1 - d(t)), \tag{7.13}$$

in which we have defined $d(t) = (d\gamma/dX)/(1 + d\gamma/dX)$. Since $d(t) < 1$, letting $G' = KG$, we can verify (see exercises) that as G grows $X(t)$ tends to $k(t)/K$ for both the connected system in the form of equation (7.13) and the isolated system

$$\frac{dX}{dt} = Gk(t) - (G' + \delta)X. \tag{7.14}$$

That is, the solutions $X(t)$ of the connected and isolated system tend to each other as G increases. As a consequence, the presence of the disturbance term $d(t)$ will not significantly affect the time behavior of $X(t)$. Since $d(t)$ is a measure of retroactivity, its effect on the behavior of $X(t)$ is attenuated by employing large gains G and G'.

The next questions we address is how we can implement such amplification and feedback gains in a biomolecular system.

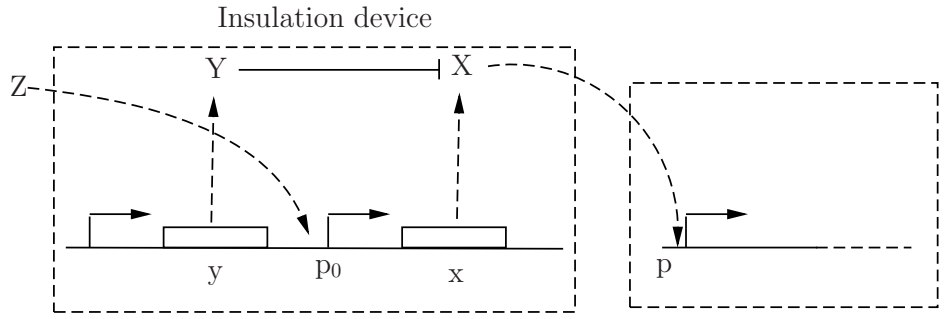


Figure 7.12: In this design, the input Z is amplifed through a strong promoter p_0. The negative feedback on the output X is obtained by enhancing its degradation through the protease Y.

Biomolecular realizations of Principle 1

In the previous section, we have proposed a general principle to attenuate the retroactivity to the output. Such a principle consists of a large amplification of the input and a large negative output feedback. In this section, we determine two possible biomolecular implementations to obtain a large amplification gain to the input Z of the insulation component and a large negative feedback on the output X. Both mechanisms realize the negative feedback through enhanced degradation. The first design realizes amplification through transcriptional activation, while the second design through phosphorylation.

Design 1: Amplification through transcriptional activation

In this design, we obtain a large amplification of the input signal $Z(t)$ by having promoter p_0 (to which Z binds) be a strong, non-leaky promoter. The negative feedback mechanism on X relies on enhanced degradation of X. Since this must be large, one possible way to obtain an enhanced degradation for X is to have a protease, called Y, be expressed by a strong constitutive promoter. The protease Y will cause a degradation rate for X, which is larger if Y is more abundant in the system. This design is schematically shown in Figure 7.12.

In order to investigate whether such a design realizes a large amplification and a large negative feedback on X as needed, we analyze the model for the system of Figure 7.12. The reaction of the protease Y with protein X is modeled as the two-step reaction

$$\mathrm{X} + \mathrm{Y} \underset{\eta_2}{\overset{\eta_1}{\rightleftharpoons}} \mathrm{W} \xrightarrow{\beta} \mathrm{Y},$$

which can be found in Section 2.3.

The input/output system model of the insulation component that takes Z as an

input and gives X as an output is given by the following equations

$$\frac{dZ}{dt} = k(t) - \delta Z + \left[k_- Z_p - k_+ Z(p_{0,\text{tot}} - Z_p)\right] \tag{7.15}$$

$$\frac{dZ_p}{dt} = k_+ Z(p_{0,\text{tot}} - Z_p) - k_- Z_p \tag{7.16}$$

$$\frac{dm_X}{dt} = GZ_p - \delta_1 m_X \tag{7.17}$$

$$\frac{dW}{dt} = \eta_1 XY - \eta_2 W - \beta W \tag{7.18}$$

$$\frac{dY}{dt} = -\eta_1 YX + \beta W + \alpha G - \gamma Y + \eta_2 W \tag{7.19}$$

$$\frac{dX}{dt} = \nu m_X - \eta_1 YX + \eta_2 W - \delta_2 X + \left[k_{\text{off}} C - k_{\text{on}} X(p_{\text{tot}} - C)\right] \tag{7.20}$$

$$\frac{dC}{dt} = -k_{\text{off}} C + k_{\text{on}} X(p_{\text{tot}} - C), \tag{7.21}$$

in which we have assumed that the expression of gene z is controlled by a promoter with activity $k(t)$. In this system, we have denoted by k_+ and k_- the association and dissociation rates of Z with its promoter site p_0 in total concentration $p_{0,\text{tot}}$ is the total concentration of the promoter p_0. Also, Z_p denotes the complex of Z with such a promoter site. m_X is the concentration of mRNA of X, C is the concentration of X bound to the downstream binding sites with total concentration p_{tot}, and γ is the decay rate of the protease Y. The promoter controlling gene y has strength αG, for some constant α, and it has the same order of magnitude strength as the promoter controlling x.

The terms in the square brackets in equation (7.15) represent the retroactivity r to the input of the insulation component in Figure 7.12. The terms in the square brackets in equation (7.20) represent the retroactivity s to the output of the insulation component of Figure 7.12. The dynamics of equations (7.15)–(7.21) without s (the elements in the box in equation (7.20)) describe the dynamics of X with no downstream system.

Equations (7.15) and (7.16) simply determine the signal $Z_p(t)$ that is the input to equations (7.17)–(7.21). For the discussion regarding the attenuation of the effect of s, it is not relevant what the specific form of signal $Z_p(t)$ is. Let then $Z_p(t)$ be any bounded signal $v(t)$. Since equation (7.17) takes $v(t)$ as an input, we will have that $m_X = Gv(t)$, for a suitable signal $v(t)$. Let us assume for the sake of simplifying the analysis that the protease reaction is a one step reaction, that is,

$$\text{X} + \text{Y} \xrightarrow{\beta} \text{Y}.$$

Therefore, equation (7.19) simplifies to

$$\frac{dY}{dt} = \alpha G - \gamma Y$$

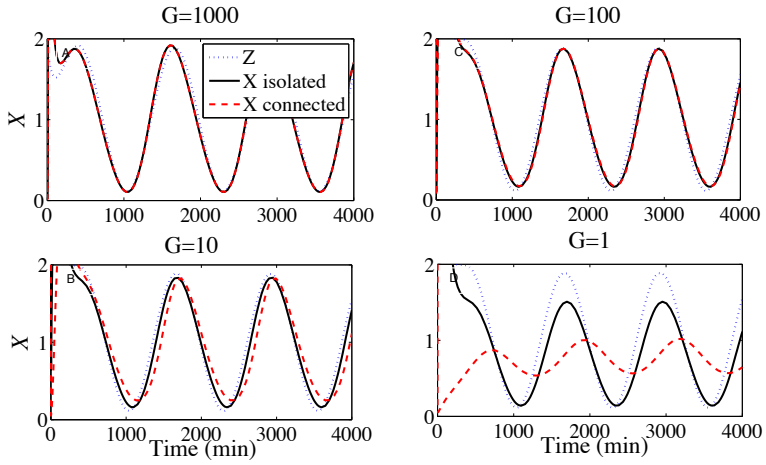


Figure 7.13: Design 1: results for different gains G. In all plots, $k(t) = 0.01(1 + sin(\omega t))$, $p_{\text{tot}} = 100$, $k_{\text{off}} = k_{\text{on}} = 10$, $\delta = 0.01$, and $\omega = 0.005$. The parameter values are $\delta_1 = 0.01$, $p_{0,\text{tot}} = 1$, $\eta_1 = \eta_2 = \beta = \gamma = 0.01$, $k_- = 200$, $k_+ = 10$, $\alpha = 0.1$, $\delta_2 = 0.1$, $\nu = 0.1$, and $G = 1000, 100, 10, 1$. The retroactivity to the output is not well attenuated for values of the gain $G = 1$ and the attenuation capability begins to worsen for $G = 10$.

and equation (7.20) simplifies to

$$\frac{dX}{dt} = \nu m_X - \beta YX - \delta_2 X + k_{\text{off}} C - k_{\text{on}} X(p_{\text{tot}} - C).$$

If we consider the protease to be at its equilibrium, we have that $Y(t) = \alpha G/\gamma$.

As a consequence, the X dynamics become

$$\frac{dX}{dt} = \nu G v(t) - (\beta\alpha G/\gamma + \delta_2)X + k_{\text{off}} C - k_{\text{on}} X(p_{\text{tot}} - C),$$

with C determined by equation (7.21). By using the same singular perturbation argument employed in the previous section, we obtain that the dynamics of X will be (after a fast transient) approximatively given by

$$\frac{dX}{dt} = (\nu G v(t) - (\beta\alpha G/\gamma + \delta_2)X)(1 - d(t)), \tag{7.22}$$

in which $0 < d(t) < 1$ is the retroactivity measure. Then, as G increases, $X(t)$ becomes closer to the solution of the isolated system

$$\frac{dX}{dt} = \nu G v(t) - (\beta\alpha G/\gamma + \delta_2)X,$$

as explained in the previous section.

We now turn to the question of minimizing the retroactivity to the input r because its effect can alter the input signal $Z(t)$. In order to decrease r, we guarantee that the retroactivity measure given in equation (7.9) in which we substitute Z in place of X and $p_{0,\text{tot}}$ in place of p_{tot}, is small. This is seen to be true if $(K_\text{d} + Z)^2/(p_{0,\text{tot}}K_\text{d})$ is very large, in which $1/K_\text{d} = k_+/k_-$ is the affinity of the binding site p_0 to Z. Since after a short transient, $Z_p = (p_{0,\text{tot}}Z)/(K_\text{d} + Z)$, for Z_p not to be a distorted version of Z, it is enough to ask that $K_\text{d} \gg Z$. This, combined with the requirement that $(K_\text{d} + Z)^2/(p_{0,\text{tot}}K_\text{d})$ is very large, leads to the requirement $p_{0,\text{tot}}/K_\text{d} \ll 1$. Summarizing, for not having distortion effects between Z and Z_p and small retroactivity r, we need that

$$K_\text{d} \gg Z \text{ and } p_{0,\text{tot}}/K_\text{d} \ll 1. \tag{7.23}$$

Simulation results are presented for the insulation system of equations (7.15)–(7.21) as the mathematical analysis of such a system is only valid under the approximation that the protease reaction is a one step reaction. In all simulations, we consider protein decay rates to be 0.01min^{-1} to obtain a protein half life of about one hour. We consider always a periodic forcing $k(t) = 0.01(1 + \sin(\omega t))$, in which we assume that such a periodic signal has been generated by a synthetic biological oscillator. Therefore, the oscillating signals are chosen to have a period that is about 12 times the protein half life in accordance to what is experimentally observed in the synthetic clock of [5].

For large gains ($G = 1000$, $G = 100$), the performance considerably improves compared to the case in which X was generated by a plain transcriptional component accepting Z as an input (Figure 7.6). For lower gains ($G = 10$, $G = 1$), the performance starts to degrade for $G = 10$ and becomes not acceptable for $G = 1$ (Figure 7.13). Since we can view G as the number of transcripts produced per unit time (one minute) per complex of protein Z bound to promoter p_0, values $G = 100, 1000$ may be difficult to realize *in vivo*, while the values $G = 10, 1$ could be more easily realized. The values of the parameters chosen in Figure 7.13 are such that $K_\text{d} \gg Z$ and $p_{0,\text{tot}} \ll K_\text{d}$. This is enough to guarantee that there is small retroactivity r to the input of the insulation device independently of the value of the gain G, according to relations (7.23). The poorer performance of the device for $G = 1$ is therefore entirely due to poor attenuation of the retroactivity s to the output.

To obtain a large gain, we need to guarantee high expression of the protease. This may be difficult to do because in general proteases are not specific and target for degradations all proteins. Hence, global undesired effects on the cell behavior may result. The next design avoids this problem by using dephosphorylation as the mechanism for enhanced degradation.

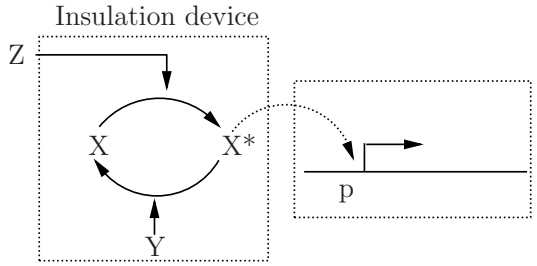


Figure 7.14: In this design, negative feedback occurs through a phosphatase Y that converts the active form X* back to its inactive form X. Amplification occurs through Z activating the phosphorylation of X.

Design 2: Amplification through phosphorylation

In this design, the amplification gain G of Z is obtained 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 negative feedback gain G' on X^* is obtained by having a phosphatase Y activate the dephosphorylation of active protein X^*. Protein Y is also available in abundance in the system. This mechanism is depicted in Figure 7.14. A similar design has been proposed by [83, 84], in which a MAPK cascade plus a negative feedback loop that spans the length of the MAPK cascade is considered as a feedback amplifier. The design presented here is simpler as it involves only one phosphorylation cycle and does not require any explicit feedback loop. In fact, a strong negative feedback can be realized by the action of the phosphatase that converts the active protein form X^* back to its inactive form X.

We consider a simplified model for the phosphorylation and dephosphorylation processes, which will help in obtaining a conceptual understanding of what reactions realize the desired gains G and G'. The one step model that we consider is the same as considered in Chapter 2 (Exercise 2.6):

$$Z + X \xrightarrow{k_1} Z + X^*,$$

and

$$Y + X^* \xrightarrow{k_2} Y + X.$$

We assume that there is an abundance of protein X and of phosphatase Y in the system and that these quantities are conserved. The conservation of X gives $X + X^* + C = X_{\text{tot}}$, in which X is the inactive protein, X^* is the phosphorylated protein that binds to the downstream sites p, and C is the complex of the phosphorylated protein X^* bound to the promoter p. The X^* dynamics can be described by the first

equation in the following model

$$\frac{dX^*}{dt} = k_1 X_{\text{tot}} Z(t)\left(1 - \frac{X^*}{X_{\text{tot}}} - \left[\frac{C}{X_{\text{tot}}}\right]\right) - k_2 Y X^* + [k_{\text{off}} C - k_{\text{on}} X^*(p_{\text{tot}} - C)] \quad (7.24)$$

$$\frac{dC}{dt} = -k_{\text{off}} C + k_{\text{on}} X^*(p_{\text{tot}} - C). \quad (7.25)$$

The terms in the square brackets represent the retroactivity s to the output of the insulation system of Figure 7.14. For a weakly activated pathway [42], $X^* \ll X_{\text{tot}}$. Also, if we assume that the concentration of total X is large compared to the concentration of the downstream binding sites, that is, $X_{\text{tot}} \gg p_{\text{tot}}$, equation (7.24) is approximatively equal to

$$\frac{dX^*}{dt} = k_1 X_{\text{tot}} Z(t) - k_2 Y X^* + k_{\text{off}} C - k_{\text{on}} X^*(p_{\text{tot}} - C).$$

Let $G = k_1 X_{\text{tot}}$ and $G' = k_2 Y$. Exploiting again the difference of time scales between the X^* dynamics and the C dynamics, after a fast initial transient the dynamics of X^* can be well approximated by

$$\frac{dX^*}{dt} = (GZ(t) - G'X^*)(1 - d(t)), \quad (7.26)$$

in which $0 < d(t) < 1$ is the retroactivity contribution. Therefore, for G and G' large enough, $X^*(t)$ tends to the solution $X^*(t)$ of the isolated system $\frac{dX^*}{dt} = GZ(t) - G'X^*$, as explained before. As a consequence, the effect of the retroactivity to the output s is attenuated by increasing $k_1 X_{\text{tot}}$ and $k_2 Y$ 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 the values of the phosphorylation/dephosphorylation rates cover an important role toward the realization of the insulation property of the module of Figure 7.14.

From a practical point of view, the effective rates can be increased by increasing the total amounts of X and Y, which can be done by placing the corresponding genes under the control of inducible promoters. Experiments performed on a covalent modification cycle reconstituted *in vitro*, showed that increasing these protein amounts is an effective means to attain insulation [51].

Attenuation of retroactivity to the output: Principle 2

In this section, we present a more general mechanism for insulation, that is not inspired by the design of electrical circuits and is naturally implemented by the structure of biomolecular systems. For this purpose, consider Figure 7.15. We illustrate how the system can achieve insulation from s whenever its internal dynamics are much faster compared to the dynamics of the input u. To this end, we consider the

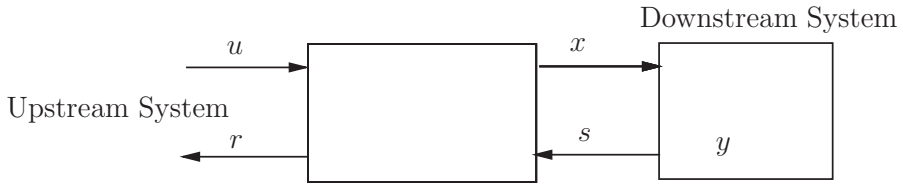


Figure 7.15: Interconnection of a device with input u and output x to a downstream system with internal state y applying retroactivity s.

following simple structure in which (for simplicity) we assume that all variables are scalar:

$$\begin{aligned}\frac{du}{dt} &= f_0(u,t) + r(u,x)\\ \frac{dx}{dt} &= Gf_1(x,u) + Gs(x,u)\\ \frac{dy}{dt} &= -Gs(x,y).\end{aligned} \tag{7.27}$$

Here $G \gg 1$ models the fact that the internal dynamics of the device is much faster than that of the input; similarly, $G \gg 1$ models the fact that the dynamics of the interconnection with downstream systems is also fast (as it is usually the case, being it due to binding mechanisms). The claim that we make about this system is the following.

> If $G \gg 1$ and the Jacobian of f_1 has eigenvalues with negative real part, then $x(t)$ is not affected by retroactivity s after a short initial transient, independently of the value of G.

This result states that independently of the characteristics of the downstream system, the device can be tuned (by making G large enough) so to function as an insulation device. To clarify why this would be the case, it is useful to rewrite the above system in standard singular perturbation form by employing $\epsilon := 1/G$ as a small parameter and $\tilde{x} := x + y$ as the slow variable. Hence, it can be re-written as

$$\begin{aligned}\frac{du}{dt} &= f_0(u,t) + r(u,x)\\ \epsilon\frac{d\tilde{x}}{dt} &= f_1(\tilde{x}-y,u)\\ \frac{dy}{dt} &= -Gs(\tilde{x}-y,y).\end{aligned} \tag{7.28}$$

Since $\partial f_1/\partial \tilde{x}$ has eigenvalues with negative real part, one can apply standard singular perturbation to show that after a very fast transient, the trajectories are attracted to the slow manifold given by $f_1(\tilde{x}-y,u) = 0$. This is locally given by $x = \gamma(u)$

solving $f_1(x,u)=0$. Hence, on the slow manifold we have that $x(t)=\gamma(u(t))$, which is independent of the downstream system, that is, it is not affected by retroactivity.

The same result holds for a more general class of systems in which the variables u, x, y are vectors:

$$\begin{aligned}\frac{du}{dt} &= f_0(u,t)+r(u,x)\\ \frac{dx}{dt} &= Gf_1(x,u)+GAs(x,u)\\ \frac{dy}{dt} &= -GBs(x,y)\end{aligned} \tag{7.29}$$

as long as there are matrices T and M such that $TA+MB=0$ and T is invertible. In fact, one can take the system to new coordinates $u, \tilde{x}, y$ with $\tilde{x}=Tx+My$, in which the system will have the form (7.28).

Biomolecular realizations of Principle 2

We next consider possible biomolecular structures that realize Principle 2. Since this principle is based on a fast time scale of the device dynamics when compared to that of the device input, we focus on signaling systems, which are known to evolve on faster time scales than those of protein production and decay.

Design 1: Implementation through phosphorylation

We consider a more complex model for the phosphorylation and dephosphorylation reactions in a phosphorylation cycle and perform a parametric analysis to highlight the roles of the various parameters for attaining the insulation properties. In particular, we consider a two-step reaction model as seen in Section 2.4. According to this model, we have the following two reactions for phosphorylation and dephosphorylation:

$$\mathrm{X}+\mathrm{Z} \underset{d_1}{\overset{a_1}{\rightleftharpoons}} \mathrm{C}_1 \xrightarrow{k_1} \mathrm{X}^*+\mathrm{Z}, \qquad \mathrm{Y}+\mathrm{X}^* \underset{d_2}{\overset{a_2}{\rightleftharpoons}} \mathrm{C}_2 \xrightarrow{k_2} \mathrm{X}+\mathrm{Y}. \tag{7.30}$$

Additionally, we have the conservation equations $Y_{\mathrm{tot}}=Y+C_2$, $X_{\mathrm{tot}}=X+X^*+C_1+C_2+C$, because proteins X and Y are not degraded. Therefore, the differential

equations modeling the insulation system of Figure 7.14 become

$$\frac{dZ}{dt} = k(t) - \delta Z\left[-a_1 Z X_{\text{tot}}(1 - \frac{X^*}{X_{\text{tot}}} - \frac{C_1}{X_{\text{tot}}} - \frac{C_2}{X_{\text{tot}}} - \left[\frac{C}{X_{\text{tot}}}\right]) + (d_1 + k_1)C_1\right] \quad (7.31)$$

$$\frac{dC_1}{dt} = -(d_1 + k_1)C_1 + a_1 Z X_{\text{tot}}(1 - \frac{X^*}{X_{\text{tot}}} - \frac{C_1}{X_{\text{tot}}} - \frac{C_2}{X_{\text{tot}}} - \left[\frac{C}{X_{\text{tot}}}\right]) \quad (7.32)$$

$$\frac{dC_2}{dt} = -(k_2 + d_2)C_2 + a_2 Y_{\text{tot}} X^*(1 - \frac{C_2}{Y_{\text{tot}}}) \quad (7.33)$$

$$\frac{dX^*}{dt} = k_1 C_1 + d_2 C_2 - a_2 Y_{\text{tot}} X^*(1 - \frac{C_2}{Y_{\text{tot}}}) + [k_{\text{off}} C - k_{\text{on}} X^*(p_{\text{tot}} - C)] \quad (7.34)$$

$$\frac{dC}{dt} = -k_{\text{off}} C + k_{\text{on}} X^*(p_{\text{tot}} - C), \quad (7.35)$$

in which the expression of gene z is controlled by a promoter with activity $k(t)$. The terms in the large square bracket in equation (7.31) represent the retroactivity r to the input, while the terms in the square brackets of equations (7.32) and (7.34) represent the retroactivity s to the output.

We assume that $X_{\text{tot}} \gg p_{\text{tot}}$ so that in equations (7.31) and (7.32) we can neglect the term C/X_{tot} because $C < p_{\text{tot}}$. Also, phosphorylation and dephosphorylation reactions in equations (7.30) can occur at a much faster rate than protein production and decay processes (see Chapter 2). Choosing X_{tot} and Y_{tot} sufficiently large, let $G = k_1 X_{\text{tot}}/\delta$ and $G = k_{\text{off}}/\delta$, then we can re-write the system with $k_{\text{on}} = k_{\text{off}}/K_{\text{d}}$, $b_1 = a_1 X_{\text{tot}}/(\delta G)$, $a_1 = a_2 Y_{\text{tot}}/(\delta G)$, $b_2 = d_1/(\delta G)$, $a_2 = d_2/(\delta G)$, $c_i = k_i/(\delta G)$, and $k_{\text{on}} = G\delta/K_{\text{d}}$. Letting $z = Z + C_1$ we obtain the system in the form

$$\begin{aligned}
\frac{dz}{dt} &= k(t) - \delta(z - C_1) \\
\frac{dC_1}{dt} &= G\left(-\delta(b_2 + c_1)C_1 + \delta b_1(z - C_1)\left(1 - \frac{X^*}{X_{\text{tot}}} - \frac{C_1}{X_{\text{tot}}} - \frac{C_2}{X_{\text{tot}}})\right)\right) \\
\frac{dC_2}{dt} &= G\left(-\delta(c_2 + a_2)C_2 + \delta a_1 X^*\left(1 - \frac{C_2}{Y_{\text{tot}}}\right)\right) \\
\frac{dX^*}{dt} &= G\left(\delta c_1 C_1 + \delta a_2 C_2 - \delta a_1 X^*\left(1 - \frac{C_2}{Y_{\text{tot}}}\right)\right) + G\left(\delta C - \delta/K_{\text{d}}(p_{\text{tot}} - C)X^*\right) \\
\frac{dC}{dt} &= -G\left(\delta C - \delta/K_{\text{d}}(p_{\text{tot}} - C)X^*\right),
\end{aligned} \quad (7.36)$$

which is in the form of system (7.29) with $u = z$, $x = (C_1, C_2, X^*)$, and $y = C$, in which one can choose T as the 3 by 3 identity matrix and $M = (0\ 0\ 1)'$. Hence, this system, for G sufficiently larger than 1 attenuates the effect of the retroactivity to the output s. For G to be large, one has to require that $k_1 X_{\text{tot}}$ is sufficiently large and that $a_2 Y_{\text{tot}}$ is also comparatively large. These are the same design requirements obtained in the previous section based on the one-step reaction model of the enzymatic reactions.

In order to understand the effect of retroactivity to the input on the Z dynamics, one can perform the following calculations. Letting $K_m = (d_1 + k_1)/a_1$ and $K_m = (d_2 + k_2)/a_2$ represent the Michaelis-Menten constants of the forward and backward enzymatic reactions and setting $\epsilon = 0$ in the third and fourth equations of (7.36) the following relationships can be obtained:

$$C_1 = F_1(X^*) = \frac{\frac{X^* Y_{\text{tot}} k_2}{K_m k_1}}{1 + X^*/K_m}, \quad C_2 = F_2(X^*) = \frac{\frac{X^* Y_{\text{tot}}}{K_m}}{1 + X^*/K_m}. \tag{7.37}$$

Using expressions (7.37) in the second of equations (7.36) with $\epsilon = 0$ leads to

$$F_1(X^*)(b_2 + c_1 + \frac{b_1 Z}{X_{\text{tot}}}) = b_1 Z(1 - \frac{X^*}{X_{\text{tot}}} - \frac{F_2(X^*)}{X_{\text{tot}}}). \tag{7.38}$$

Assuming for simplicity that $X^* \ll K_m$, we obtain that $F_1(X^*) \approx X^* Y_{\text{tot}} k_2 / K_m k_1$ and that $F_2(X^*) \approx X^*/K_m Y_{\text{tot}}$. As a consequence of these simplifications, equation (7.38) leads to

$$X^* = \frac{b_1 Z}{\frac{b_1 Z}{X_{\text{tot}}}(1 + Y_{\text{tot}}/K_m + (Y_{\text{tot}} k_2)/(K_m k_1)) + \frac{Y_{\text{tot}} k_2}{K_m k_1}(b_2 + c_1)} := m(Z).$$

In order not to have distortion from Z to X^*, we require that

$$Z \ll \frac{Y_{\text{tot}} \frac{k_2}{k_1} \frac{K_m}{K_m}}{1 + \frac{Y_{\text{tot}}}{K_m} + \frac{Y_{\text{tot}}}{K_m} \frac{k_2}{k_1}}, \tag{7.39}$$

so that $m(Z) \approx Z X_{\text{tot}} K_m k_1 / Y_{\text{tot}} K_m k_2$ and therefore we have a linear relationship between X^* and Z with gain from Z to X^* given by $X_{\text{tot}} K_m k_1 / Y_{\text{tot}} K_m k_2$. In order not to have attenuation from Z to X^* we require that the gain is greater than or equal to one, that is,

$$\text{input/output gain} \approx \frac{X_{\text{tot}} K_m k_1}{Y_{\text{tot}} K_m k_2} \geq 1. \tag{7.40}$$

Requirements (7.39), (7.40) and $X^* \ll K_m$ are enough to guarantee that we do not have nonlinear distortion between Z and X^* and that X^* is not attenuated with respect to Z. In order to guarantee that the retroactivity r to the input is sufficiently small, we need to quantify the retroactivity effect on the Z dynamics due to the binding of Z with X. To achieve this, we proceed as in Section 7.3 by computing the Z dynamics on the slow manifold, which gives a good approximation of the dynamics of Z if $\epsilon \approx 0$. Such a dynamics are given by

$$\frac{dZ}{dt} = (k(t) - \delta Z)\left(1 - \frac{dF_1}{dX^*}\frac{dX^*}{dz}\right),$$

in which $\frac{dF_1}{dX^*}\frac{dX^*}{dz}$ measures the effect of the retroactivity r to the input on the Z dynamics. Direct computation of $\frac{dF_1}{dX^*}$ and of $\frac{dX^*}{dz}$ along with $X^* \ll K_m$ and with

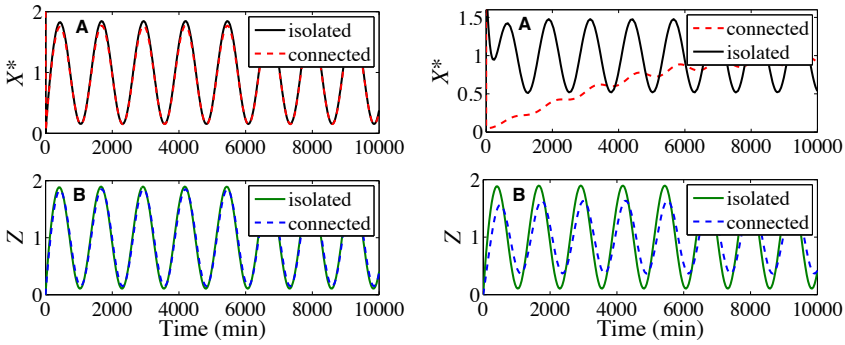


Figure 7.16: (a) Performance with fast time scales. Simulation results for system in equations (7.31–7.35). In all plots, $p_{\text{tot}} = 100$, $k_{\text{off}} = k_{\text{on}} = 10$, $\delta = 0.01$, $k(t) = 0.01(1 + sin(\omega t))$, and $\omega = 0.005$. In subplots A and B, $k_1 = k_2 = 50$, $a_2 = a_1 = 0.01$, $d_1 = d_2 = 10$, and $Y_{\text{tot}} = X_{\text{tot}} = 1500$. In plot A, the isolated system is without downstream binding sites p and the connected system is with binding sites p. The small error shows that the effect of the retroactivity to the output s is attenuated very well. In subplot B, the isolated system stands for the case in which Z does not have X to bind to, while the connected system stands for the case in which Z binds to substrate X ($X_{\text{tot}} = 1500$). The small error confirms a small retroactivity to the input r. (b) Performance with slow time scale. Phosphorylation and dephosphorylation rates are slower than the ones in (a), that is, $k_1 = k_2 = 0.01$, while the other parameters are left the same, that is, $d_2 = d_1 = 10$, $a_2 = a_1 = 0.01$, and $Y_{\text{tot}} = X_{\text{tot}} = 1500$.

(7.39) leads to $\frac{dF_1}{dX^*}\frac{dX^*}{dz} \approx X_{\text{tot}}/K_m$, so that in order to have small retroactivity to the input, we require that

$$\frac{X_{\text{tot}}}{K_m} \ll 1. \tag{7.41}$$

Hence, a design trade-off appears: X_{tot} should be sufficiently large to provide a gain G large enough to attenuate the retroactivity to the output. Yet, X_{tot} should be small enough compared to K_m so to apply minimal retroactivity to the input.

Concluding, for having attenuation of the effect of the retroactivity to the output s, we require that the time scale of the phosphorylation/dephosphorylation reactions is much faster than the production and decay processes of Z (the input to the insulation device) and that $X_{\text{tot}} \gg p_{\text{tot}}$, that is, the total amount of protein X is in abundance compared to the downstream binding sites p. To obtain also a small effect of the retroactivity to the input, we require that $K_m \gg X_{\text{tot}}$. This is satisfied if, for example, kinase Z has low affinity to binding with X. To keep the input/output gain between Z and X^* close to one (from equation (7.40)), one can choose $X_{\text{tot}} = Y_{\text{tot}}$, and equal coefficients for the phosphorylation and dephosphorylation reactions, that is, $K_m = K_m$ and $k_1 = k_2$.

System in equations (7.31–7.35) was simulated with and without the down-

stream binding sites p, that is, with and without, respectively, the terms in the small box of equation (7.31) and in the boxes in equations (7.34) and (7.32). This is performed to highlight the effect of the retroactivity to the output s on the dynamics of X^*. The simulations validate our theoretical study that indicates that when $X_{\text{tot}} \gg p_{\text{tot}}$ 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 7.16(a), plot A). Similarly, the time behavior of Z was simulated with and without the terms in the large box in equation (7.31), 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 7.16(a), plot B), indicates that there is no substantial retroactivity to the input r generated by the insulation device. This is obtained because $X_{\text{tot}} \ll K_m$ as indicated in equation (7.41), in which $1/K_m$ can be interpreted as the affinity of the binding of X to Z.

Our simulation study also indicates that a faster time scale of the phosphorylation/dephosphorylation reactions is necessary, even for high values of X_{tot} and Y_{tot}, to maintain perfect attenuation of the retroactivity to the output s and small retroactivity to the output r. In fact, slowing down the time scale of phosphorylation and dephosphorylation, the system looses its insulation property (Figure 7.16(b)). In particular, the attenuation of the effect of the retroactivity to the output s is lost because there is not enough separation of time scales between the Z dynamics and the internal device dynamics. The device also displays a non negligible amount of retroactivity to the input because the condition $K_m \ll X_{\text{tot}}$ is not satisfied anymore.

Design 2: Realization through phosphotransfer

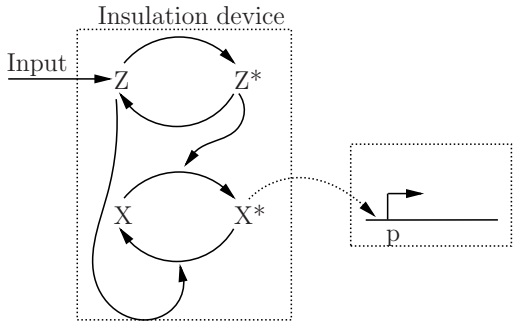


Figure 7.17: System S is a phosphotransfer system. The output X* activates transcription through the reversible binding of X* to downstream DNA promoter sites p.

Let X be a transcription factor in its inactive form and let X^* be the same transcription factor once it has been activated by the addition of a phosphate group.

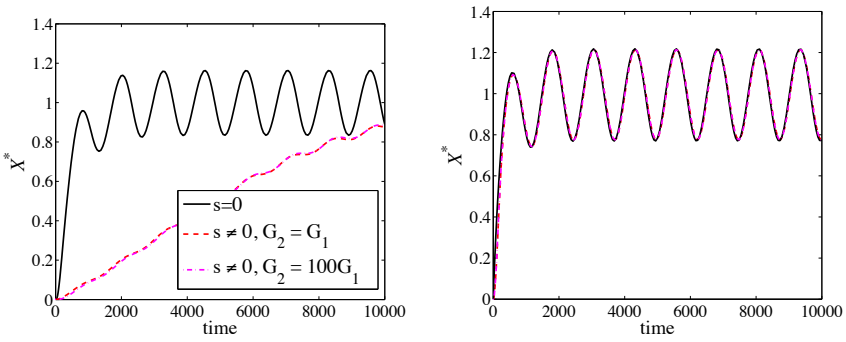
Let Z^* be a phosphate donor, that is, a protein that can transfer its phosphate group to the acceptor X. The standard phosphotransfer reactions (see Chapter 2, Section 2.4) can be modeled according to the two-step reaction model

$$Z^* + X \underset{k_2}{\overset{k_1}{\rightleftharpoons}} C_1 \underset{k_4}{\overset{k_3}{\rightleftharpoons}} X^* + Z,$$

in which C_1 is the complex of Z bound to X bound to the phosphate group. Additionally, protein Z can be phosphorylated and protein X^* dephosphorylated by other phosphotransfer interactions. These reactions are modeled as one step reactions depending only on the concentrations of Z and X^*, that is, $Z \xrightarrow{\pi_1} Z^*$, $X^* \xrightarrow{\pi_2} X$. Protein X is assumed to be conserved in the system, that is, $X_{\text{tot}} = X + C_1 + X^* + C$. We assume that protein Z is produced with time-varying production rate $k(t)$ and decays with rate δ. The active transcription factor X^* binds to downstream DNA binding sites p with total concentration p_{tot} to activate transcription through the reversible reaction $p + X^* \underset{k_{\text{off}}}{\overset{k_{\text{on}}}{\rightleftharpoons}} C$. Since the total amount of p is conserved, we also have that $C + p = p_{\text{tot}}$. The ODE model corresponding to this system is thus given by the equations

$$\begin{aligned}
\frac{dZ}{dt} &= k(t) - \delta Z + k_3 C_1 - k_4 X^* Z - \pi_1 Z \\
\frac{dC_1}{dt} &= k_1 X_{\text{tot}} \left(1 - \frac{X^*}{X_{\text{tot}}} - \frac{C_1}{X_{\text{tot}}} - \left[\frac{C}{X_{\text{tot}}}\right]\right) Z^* - k_3 C_1 - k_2 C_1 + k_4 X^* Z \\
\frac{dZ^*}{dt} &= \pi_1 Z + k_2 C_1 - k_1 X_{\text{tot}} \left(1 - \frac{X^*}{X_{\text{tot}}} - \frac{C_1}{X_{\text{tot}}} - \left[\frac{C}{X_{\text{tot}}}\right]\right) Z^* \\
\frac{dX^*}{dt} &= k_3 C_1 - k_4 X^* Z + [k_{\text{off}} C - k_{\text{on}} X^* (p_{\text{tot}} - C)] - \pi_2 X^* \\
\frac{dC}{dt} &= k_{\text{on}} X^* (p_{\text{tot}} - C) - k_{\text{off}} C.
\end{aligned} \tag{7.42}$$

Since phosphotransfer reaction are faster than protein production and decay, define $G_1 := X_{\text{tot}} k_1 / \delta$ so that $k_1 := X_{\text{tot}} k_1 / G_1 = \delta$, $k_2 := k_2 / G_1$, $k_3 := k_3 / G_1$, $k_4 := k_4 / G_1$, $\pi_1 := \pi_1 / G_1$, $\pi_2 := \pi_2 / G_1$ are of the same order of $k(t)$ and δ. Similarly, the process of protein binding and unbinding to promoter sites is much faster than protein production and decay. Let $G := k_{\text{off}} / \delta$ and $K_{\text{d}} := k_{\text{off}} / k_{\text{on}}$. Assuming also



(a) $G = 1$ (b) $G = 100$

Figure 7.18: Output response of the phosphotransfer system with a periodic signal $k(t) = \delta(1 + 0.5\sin\omega t)$. The parameters are given by $\delta = 0.01$, $X_{\text{tot}} = 5000$, $k_1 = k_2 = k_3 = k_4 = \pi_1 = \pi_2 = 0.01G$ in which $G = 1$ (left-side panel), and $G = 100$ (right-side panel). The downstream system parameters are given by $K_{\text{d}} = 1$ and $k_{\text{off}} = 0.01G_2$, in which G assumes the values indicated on the legend. The isolated system ($s = 0$) corresponds to $p_{\text{tot}} = 0$ while the connected system ($s \neq 0$) corresponds to $p_{\text{tot}} = 100$.

that $p_{\text{tot}} \ll X_{\text{tot}}$, we have that $C \ll X_{\text{tot}}$ so that system (7.42) can be rewritten as

$$\begin{aligned}
\frac{dZ}{dt} &= k(t) - \delta Z + G\left(k_3 C_1 - k_4 Z X^* - \pi_1 Z\right)\\
\frac{dC_1}{dt} &= G\left(k_1\left(1 - \frac{X^*}{X_{\text{tot}}} - \frac{C_1}{X_{\text{tot}}}\right)Z^* - k_3 C_1 - k_2 C_1 + k_4 X^* Z\right)\\
\frac{dZ^*}{dt} &= G\left(\pi_1 Z + k_2 C_1 - k_1\left(1 - \frac{X^*}{X_{\text{tot}}} - \frac{C_1}{X_{\text{tot}}}\right)Z^*\right)\\
\frac{dX^*}{dt} &= G\left(k_3 C_1 - k_4 X^* Z - \pi_2 X^*\right) - G\left(\frac{\delta}{K_{\text{d}}} X^*(p_{\text{tot}} - C) + \delta C\right)\\
\frac{dC}{dt} &= G(\frac{\delta}{K_{\text{d}}} X^*(p_{\text{tot}} - C) - \delta C).
\end{aligned} \tag{7.43}$$

Taking $T = \mathbb{I}_{3\times 3}$, the 3 by 3 identity matrix, and $M = (0,0,1)^T$, the coordinate transformation $\tilde{x} = Tx + My$ brings the system to the form of system (7.29) with $u = Z$, $x = (C_1, Z^*, X^*)$, and $y = C$.

We illustrate the retroactivity to the output attenuation property of this system using simulations for the cases in which $G \gg G$, $G = G$, and $G \ll G$. Figure 7.18 shows that, for a periodic input $k(t)$, the system with low value for G suffers the impact of retroactivity to the output. However, for a large value of G, the permanent behavior of the connected system becomes similar to that of the isolated system, whether $G \gg G$, $G = G$ or $G \ll G$. Notice that, in the bottom panel of Figure 7.18, when $G \gg G$, the impact of the retroactivity to the output is not as dramatic as it is when $G = G$ or $G \ll G$. This is due to the fact that s is scaled by G and it is

not related to the retroactivity to the output attenuation property. This confirms the theoretical result that, independently of the order of magnitude of G, the system can arbitrarily attenuate retroactivity for large enough G.

Exercises

7.1 Include in the study of retroactivity in transcriptional systems the mRNA dynamics and demonstrate how/whether the results change. Specifically, consider the following model of a connected transcriptional component

$$\begin{aligned}\frac{m_X}{dt} &= k(t) - \gamma m_X \\ \frac{dX}{dt} &= \beta m_X - \delta X + [k_{\text{off}} C - k_{\text{on}}(p_{TOT} - C)X], \\ \frac{dC}{dt} &= -k_{\text{off}} C + k_{\text{on}}(p_{TOT} - C)X,\end{aligned}$$

7.2 Consider the system in standard singular perturbation form, in which $\epsilon \ll 1$. Demonstrate that the slow manifold is locally exponentially stable.

$$\frac{dy}{dt} = k(t) - \delta(y - C), \qquad \epsilon \frac{dC}{dt} = -\delta C + \frac{\delta}{k_d}(p_{TOT} - C)(y - C).$$

7.3 The characterization of retroactivity effects in a transcriptional module was based on the following model of the interconnection:

$$\begin{aligned}\frac{dX}{dt} &= k(t) - \delta X + [k_{\text{off}} C - k_{\text{on}}(p_{\text{tot}} - C)X], \\ \frac{dC}{dt} &= -k_{\text{off}} C + k_{\text{on}}(p_{\text{tot}} - C)X,\end{aligned}$$

in which it was implicitly assumed that the complex C does not dilute. This is often a fair assumption. However, depending on the experimental conditions, a more appropriate model may include dilution for the complex C. In this case, the model modified to

$$\begin{aligned}\frac{dX}{dt} &= k(t) - (\mu + \delta)X + [k_{\text{off}} C - k_{\text{on}}(p_{\text{tot}} - C)X], \\ \frac{dC}{dt} &= -k_{\text{off}} C + k_{\text{on}}(p_{\text{tot}} - C)X - \mu C,\end{aligned}$$

in which μ represents decay due to dilution and δ represents protein degradation. Employ singular perturbation to determine the reduced X dynamics and the effects of retroactivity in this case. Is the steady state characteristic of the transcriptional module affected by retroactivity? How?

7.4 We have illustrated that the expression of the point of half-maximal induction in a covalent modification cycle is affected by the effective load λ as follows:

$$y_{50} = \frac{K_1 + 0.5}{K_2(1+\lambda) + 0.5}.$$

Study the behavior of this quantity when the effective load λ is changed.

7.5 Show how equation (7.12) is derived in Section 7.4.

7.6 Demonstrate that in the following system

$$\frac{dX}{dt} = G\,(k(t) - KX)\,(1 - d(t)),$$

$X(t) - k(t)/K$ becomes smaller as G is increased.

7.7 Consider the activator-repressor clock from Atkinson *et al*. (Cell 2003), described in Section 6.5. Take the same simulation model derived for that exercise and pick parameter values to obtain a stable limit cycle. Then, assume that the activator A connects to another transcriptional circuit through the reversible binding of A with operator sites p to form activator-operator complex C: $\mathrm{A} + \mathrm{p} \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} \mathrm{C}$. This occurs, for example, if you want to use this clock as a source generator for some downstream system. Answer the following questions:

- Simulate the system with this new binding phenomenon and vary the total amount of p, that is, p_T. Explore how this affects the behavior of the clock.
- Give a mathematical explanation of the phenomenon you saw in (i). To do so, use singular perturbation to approximate the dynamics of the clock with downstream binding on the slow manifold (here, $k_{\mathrm{on}}, k_{\mathrm{off}} \gg \delta_A, \delta_B$). You can follow the process we used in class when we studied retroactivity for the transcriptional component with downstream binding.