Biomolecular Feedback Systems

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Chapter 7
Interconnecting Components

7.1 Input/Output Modeling and the Modularity Assumption

Each node y of a transcriptional circuitry is usually modeled as an input/output module taking as input the concentrations of transcription factors that regulate gene y and giving as output the concentration of protein expressed by gene y, denoted Y. This is not the only possible choice for delimiting a module: one could in fact let the messenger RNA (mRNA) or the RNA polymerase flow along the DNA (as suggested by [19]) play the role of input and output signals. The transcription factor enters as input of the transcriptional module through the binding and unbinding dynamics of the transcription factors with the DNA promoter sites upstream of gene y. The internal dynamics of the transcriptional component is determined by the transcription and translation dynamics. The processes of transcription and translation are much slower than the binding dynamics of the transcription factor to the promoter sites on the DNA [3]. Thus, the binding of the transcription factor to the DNA promoter site reaches the equilibrium in seconds, while transcription and translation of the target gene takes minutes to hours. This time scale separation, a key feature of transcriptional circuits, leads to the following central modeling simplification.

> **Modularity assumption.** The dynamics of transcription factor/DNA binding are considered at the equilibrium and each transcription factor concentration enters the input/output transcriptional module through *static* input functions that drive the transcription/translation dynamics (Figure 7.1).

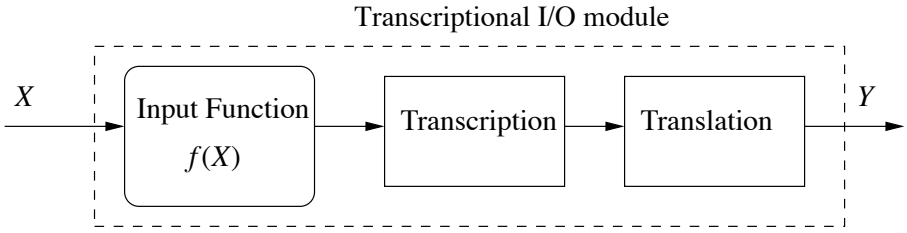

Figure 7.1: A transcriptional module is modeled as an input/output component with input function given by the transcription regulation function $f(X)$ and with internal dynamics established by the transcription and translation processes.

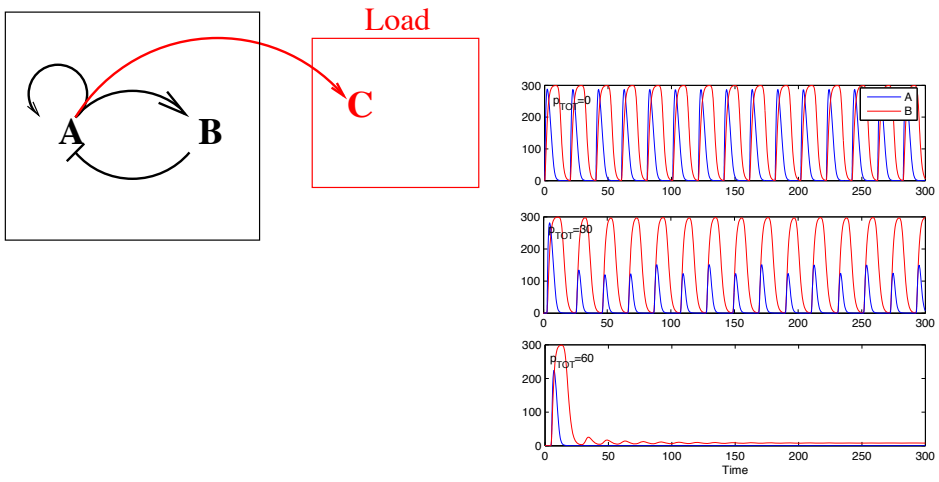

Figure 7.2: The clock behavior can be destroyed by a load. As the number of downstream binding sites for A, p_{TOT}, is increased in the load, the activator and repressor dynamics loose their synchronization and ultimately the oscillations disappear.

For engineering a system with prescribed behavior, one has to be able to change the physical features so as to change the values of the parameters of the model. This is often possible. For example, the binding affinity ($1/K$ in the Hill function model) of a transcription factor to its site on the promoter can be affected by single or multiple base pairs substitutions. The protein decay rate (constant α_2 in equation (2.16)) can be increased by adding degradation tags at the end of the gene expressing protein Y (*http://parts.mit.edu/registry/index.php/Help:Tag*). (Degradation) Tags are genetic additions to the end of a sequence which modify expressed proteins in different ways such as marking the protein for faster degradation. Promoters that can accept multiple input transcription factors (called combinatorial promoters) to implement regulation functions that take multiple inputs can be realized by combining the operator sites of several simple promoters [**?**]. For example, the operators $O_{R1} - O_{R2}$ from the λ promoter of the λ bacteriophage can be used as binding sites for the λ transcription factor [44]. Then, the pair $O_{R2} - O_{R1}$ from the 434 promoter from the 434 bacteriophage [11] can be placed at the end of the $O_{R1} - O_{R2}$ sequence from the λ promoter. Depending on the relative positions of these sites and on their distance from the RNA polymerase binding site, the 434 transcription factor may act as a repressor as when this protein is bound to its $O_{R2} - O_{R1}$ sites it prevents the polymerase to bind, while the λ transcription factor may act as an activator.

7.2 Beyond the Modularity Assumption: Retroactivity

In the previous sections, we have outlined a circuit design process, often used in synthetic biology, that relies on the interconnection of well characterized input/output transcriptional modules through suitable static input functions. Examples of designs performed through this process can be found in Chapter 9. It deeply relies on the modularity assumption, by virtue of which the behavior of the obtained circuit topology can be directly predicted by the properties of the composing units. For example, the monotonicity of the input functions of the transcriptional modules composing the repressilator have been a key feature to formally show the existence of periodic solutions. The form of the input functions in the activator-repressor clock design have been key enablers to easily predict the location and number of equilibria as the parameters are changed. 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. Indeed, after designing, testing, and characterizing the input/output behavior of an individual component in isolation, it is certainly desirable if its characteristics do not change substantially when another component is connected to its output channel. This issue, the effect of "loads" on the output of a system, is well-understood in many fields of engineering, for example in electrical circuit design. It has often been pointed out that similar issues arise for biological systems. Alon states that "modules in engineering, and presumably also in biology, have special features that make them easily embedded in almost any system. For example, output nodes should have 'low impedance,' so that adding on additional downstream clients should not drain the output to existing clients (up to some limit)." An extensive review on problems of loads and modularity in signaling networks can be found in [50, 51, 52], where the authors propose concrete analogies with similar problems arising in electrical circuits.

These questions are even more delicate in *synthetic* biology. For example, suppose that we have built a timing device, a clock made up of a network of activation and/or repression interactions among certain genes and proteins, such as the one of diagram c) of Figure 6.1. Next, we want to employ this clock (upstream system) in order to drive one or more components (downstream systems), by using as its *output* signal the oscillating concentration $A(t)$ of the activator. 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 the binding and unbinding of A to promoter sites in a downstream system competes with the biochemical interactions that constitute the upstream block (retroactivity) and may therefore disrupt the operation of the clock

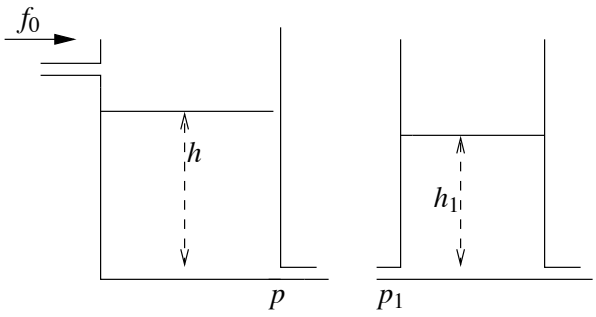

Figure 7.3: On the left, we represent a tank system that takes as input the constant flow f_0 and gives as output the pressure p at the output pipe. On the right, we show a downstream tank.

itself (Figure 7.2). One possible approach to avoid disrupting the behavior of the clock, motivated by the approach used with reporters such as GFP, 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, has still 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. The net result is still that the downstream systems are not properly timed.

Modeling retroactivity

We broadly call retroactivity the phenomenon by which the behavior of an upstream system is changed upon interconnection to a downstream system. As a simple example, which may be more familiar to an engineering audience, consider the one-tank system shown on the left of Figure 7.3. We consider a constant input flow f_0 as input to the tank system and the pressure p at the output pipe is considered the output of the tank system. The corresponding output flow is given by $k\sqrt{p}$, in which k is a positive constant depending on the geometry of the system. The pressure p is given by (neglecting the atmospheric pressure for simplicity) $p = \rho h$, in which h is the height of the water level in the tank and ρ is water density. Let A be the cross section of the tank, then the tank system can be represented by the equation

$$A\frac{dp}{dt} = \rho f_0 - \rho k\sqrt{p}. \tag{7.1}$$

Let us now connect the output pipe of the same tank to the input pipe of a downstream tank shown on the right of Figure 7.3. 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 behav-

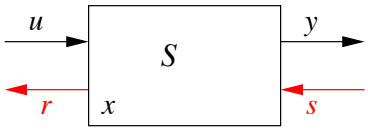

Figure 7.4: A system S input and output signals. The red signals denote signals originating by retroactivity upon interconnection.

ior of the pressure p generated at the output pipe of the upstream tank will change to

$$\begin{aligned} A\frac{dp}{dt} &= \rho f_0 - \rho g(p, p_1) \\ A_1\frac{dp_1}{dt} &= \rho g(p, p_1) - \rho k_1 \sqrt{p_1}, \end{aligned} \tag{7.2}$$

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 (7.1)) does not stay the same when the tank is connected through its output pipe to another tank (equation (7.2)). We will model this phenomenon by a signal that travels from downstream to upstream, which we call *retroactivity*. The amount of such a retroactivity will change depending on the features of the interconnection and of the downstream system. For example, if the aperture of the pipe connecting the two tanks is very small compared to the aperture of an output pipe of the downstream tank, the pressure p at the output of the upstream tank will not change much when the downstream tank is connected.

We thus model a system by adding an additional input, called s, to the system to model any change in its dynamics that may occur upon interconnection with a downstream system. Similarly, we add to a system a signal r as another output to model the fact that when such a system is connected downstream of another system, it will send upstream a signal that will alter the dynamics of the upstream system. More generally, we define a system S to have internal state x, two types of inputs (I), and two types of outputs (O): an input "u" (I), an output "y" (O), a *retroactivity to the input* "r" (O), and a *retroactivity to the output* "s" (I) (Figure 7.4). We will thus represent a system S by the equations

$$\dot{x} = f(x, u, s),\ y = Y(x, u, s),\ r = R(x, u, s), \tag{7.3}$$

in which f, Y, R are arbitrary functions and the signals x, u, s, r, y may be scalars or vectors. In such a formalism, we define the input/output model of the isolated system as the one in equations (7.3) 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

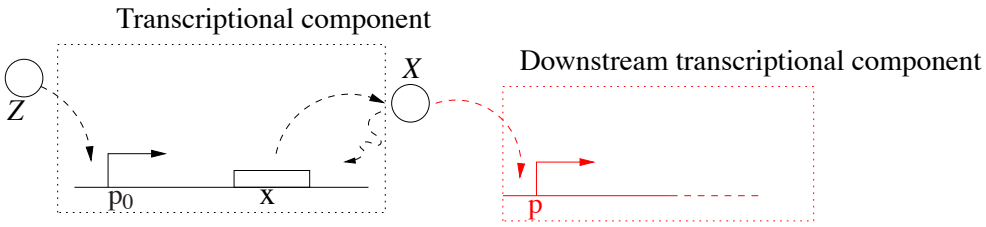

Figure 7.5: The transcriptional component takes as input u protein concentration Z and gives as output y protein concentration X. The transcription factor Z binds to operator sites on the promoter. The red part belongs to a downstream transcriptional block that takes protein concentration X as its input.

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.

Retroactivity in gene transcriptional 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 dynamic behavior can change. In this section, we focus on transcriptional circuits and show what form the retroactivity takes.

We denote by X the protein, by X (italics) the average protein concentration, and by x (lower case) the gene expressing protein X. A transcriptional component that takes as input protein Z and gives as output protein X is shown in Figure 7.5 in the dashed box. The activity of the promoter controlling gene x depends on the amount of Z bound to the promoter. If $Z = Z(t)$, such an activity changes with time. We denote it by $k(t)$. By neglecting the mRNA dynamics, which are not relevant for the current discussion, we can write the dynamics of X as

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

in which δ is the decay rate of the protein. We refer to equation (7.4) as the isolated system dynamics. For the current study, the mRNA dynamics can be neglected because we focus on how the dynamics of X changes when we add downstream systems to which X binds. As a consequence, also the specific form of $k(t)$ is not relevant. Now, assume that X drives a downstream transcriptional module by binding to a promoter p with concentration p (the red part of Figure 7.5). The reversible binding reaction of X with p is given by

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

in which C is the complex protein-promoter and k_{on} and k_{off} are the binding and dissociation rates of the protein X to the promoter site p. Since the promoter is

not subject to decay, its total concentration p_{TOT} is conserved so that we can write $p + C = p_{TOT}$. Therefore, the new dynamics of X is governed by the equations

$$\begin{aligned} \frac{dX}{dt} &= k(t) - \delta X + \boxed{k_{off}C - k_{on}(p_{TOT} - C)X}, \qquad s = k_{off}C - k_{on}(p_{TOT} - C)X \\ \frac{dC}{dt} &= -k_{off}C + k_{on}(p_{TOT} - C)X, \end{aligned} \tag{7.5}$$

in which the terms in the box represent the signal s, that is, the retroactivity to the output, while the second of equations (7.5) describes the dynamics of the input stage 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 (7.5) reduces to the dynamics of the isolated system given in equation (7.4). Here, we have assumed that X binds directly to the promoter p. The case in which a signal molecule is needed to transform X to the active form that then binds to p, can be treated in a similar way by considering the additional reversible reaction of X binding to the signal molecule. The end result of adding this reaction is the one of having similar terms in the box of equation (7.5) involving also the signaling molecule concentration.

How large is the effect of the 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 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 the retroactivity s on the behavior of X can be very large (Figure 7.6). This is undesirable in a number of situations in which we would like an upstream system to "drive" a downstream one as is the case, for example, when a biological oscillator has to time a number of downstream processes. If, due to the retroactivity, the output signal of the upstream process becomes too low and/or out of phase with the output signal of the isolated system (as in Figure 7.6), the coordination between the oscillator and the downstream processes will be lost. We next propose 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 propose a general approach for providing an operative quantification of the retroactivity to the output on the dynamics of the upstream system.

This approach can be generally applied whenever there is a separation of time-scales between the dynamics of the output of the upstream module and the dynamics of the input stage of the downstream module. This separation of time-scales is always encountered in transcriptional circuits. In fact, the dynamics of the input stage of a downstream system is governed by the reversible binding reaction of the

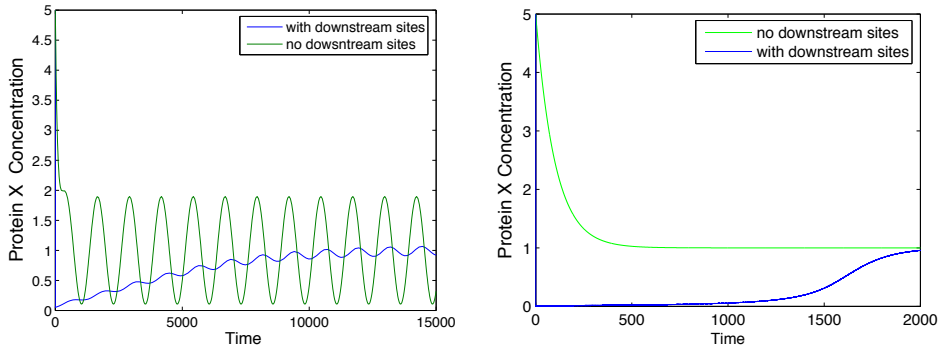

Figure 7.6: The dramatic effect of interconnection. Simulation results for the system in equations (7.5). The green plot (solid line) represents $X(t)$ originating by equations (7.4), while the blue plot (dashed line) represents $X(t)$ obtained by equation (7.5). Both transient and permanent behaviors 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 [6].

transcription factor with the operator sites. These reactions are often on the time scales of a second and thus are fast compared to the time scales of transcription and translation (often of several minutes) [3]. These determine, in turn, the dynamics of the output of a transcriptional module. Such a separation of time-scales is encountered even when we extend a transcriptional network to include as interconnection mechanisms between transcriptional modules protein-protein interactions (often with a subsecond timescale [55]), as encountered in signal transduction networks.

We quantify the difference between the dynamics of X in the isolated system (equation (7.4)) and the dynamics of X in the connected system (equations (7.5)) 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 (7.5) by a one dimensional system describing the evolution of X on the slow manifold [34]. This reduced system takes the form:

$$\frac{d\bar{X}}{dt} = k(t) - \delta\bar{X} + \bar{s},$$

where $\bar{X}$ is an approximation of X and $\bar{s}$ is an approximation of s, which can be written as $\bar{s} = -\mathcal{R}(\bar{X})(k(t) - \delta\bar{X})$. If $\mathcal{R}(\bar{X})$ is zero, then also $\bar{s} = 0$ and the dynamics

of $\bar{X}$ becomes the same as the one of the isolated system (7.4). Since $\bar{X}$ approximates X, the dynamics of X in the full system (7.5) is also close to the dynamics of the isolated system (7.4) whenever $\mathcal{R}(\bar{X}) = 0$. The factor $\mathcal{R}(\bar{X})$ provides then a measure of the retroactivity on the dynamics of X. It is also computable as a function of measurable biochemical parameters and of the signal X traveling across the interconnection, as we next illustrate.

Consider again the full system in equations (7.5), in which the binding and unbinding dynamics is much faster than protein production and decay, that is, $k_{off} \gg k(t)$, $k_{off} \gg \delta$ [3], and $k_{on} = k_{off}/k_d$ with $k_d = O(1)$. 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" [34]. To explicitly model the difference in time scales between the two equations of system (7.5), we introduce a parameter ϵ, which we define as $\epsilon = \delta/k_{off}$. Since $k_{off} \gg \delta$, we also have that $\epsilon \ll 1$. Substituting $k_{off} = \delta/\epsilon$, $k_{on} = \delta/(\epsilon k_d)$, and letting $y = X + C$ (the *total* protein concentration), we obtain the system in singular perturbation form

$$\begin{aligned} \frac{dy}{dt} &= k(t) - \delta(y - C) \\ \epsilon\frac{dC}{dt} &= -\delta C + \frac{\delta}{k_d}(p_{TOT} - C)(y - C). \end{aligned} \tag{7.6}$$

This means, as some authors proposed [**?**], that y (total concentration of protein) is the slow variable of the system (7.5) as opposed to X (concentration of free protein). We can then obtain an approximation of the dynamics of X in the limit in which ϵ is very small, by setting $\epsilon = 0$. This leads to (see [17] for details) the approximated X dynamics

$$\frac{d\bar{X}}{dt} = k(t) - \delta\bar{X} - (k(t) - \delta\bar{X})\frac{d\gamma(\bar{y})}{d\bar{y}}. \tag{7.7}$$

The smaller ϵ, the better is the approximation. Since $\bar{X}$ well approximates X for ϵ small, conditions for which the dynamics of equation (7.7) is close to the dynamics of the isolated system (7.4) also guarantee that the dynamics of X given in system (7.5) is close to the dynamics of the isolated system.

The difference between the dynamics in equation (7.7) (the connected system after a fast transient) and the dynamics in equation (7.4) (the isolated system) is zero when the term $\frac{d\gamma(\bar{y})}{d\bar{y}}$ in equation (7.7) is also zero. We thus consider the factor $\frac{d\gamma(\bar{y})}{d\bar{y}}$ 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 $\frac{d\gamma(\bar{y})}{d\bar{y}}$ 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 $\frac{d\gamma(\bar{y})}{d\bar{y}}$:

$$\frac{d\gamma(\bar{y})}{d\bar{y}} = \frac{1}{1+\frac{(1+\bar{X}/k_d)^2}{p_{TOT}/k_d}} =: \mathcal{R}(\bar{X}), \tag{7.8}$$

in which one can verify that $\mathcal{R}(\bar{X}) < 1$ (see [17] for details). The expression $\mathcal{R}(\bar{X})$ quantifies the retroactivity to the output on the dynamics of X after a fast transient, when we approximate X with $\bar{X}$ in the limit in which $\epsilon \approx 0$. The retroactivity measure is thus low if the affinity of the binding sites p is small (k_d large) or if the signal $X(t)$ is large enough compared to p_{TOT}. Thus, the expression of $\mathcal{R}(\bar{X})$ provides an operative quantification of the retroactivity: such an expression can in fact be evaluated once the association and dissociation constants of X to p are known, the concentration of the binding sites p_{TOT} is known, and the range of operation of the signal $\bar{X}(t)$ that travels across the interconnection is also known.

Therefore, the modularity assumption introduced in Section 7.1 holds if the value of $\mathcal{R}(\bar{X})$ is low enough. As a consequence, the design of a simple circuit motif such as the ones of Figure 6.1 can assume modularity if the interconnections among the composing modules can be designed so that the value of $\mathcal{R}(\bar{X})$ as given in expression (7.8) is low.

7.3 Insulation Devices to Enforce Modularity

Of course, it is not always possible to design an interconnection such that the retroactivity is low. This is, for example, the case of an oscillator that has to time a downstream load: the load cannot be in general designed and the oscillator must perform well in the face of unknown and possibly variable load properties (Figure 7.2). Therefore, in analogy to what is performed in electrical circuits, one can design a device to be placed between the oscillator and the load so that the device output is not changed by the load and the device does not affect the behavior of the upstream oscillator. Specifically, consider a system S as the one shown in Figure 7.4 that takes u as input and gives y as output. We would like to design it in such a way that (a) the retroactivity r to the input is very small; (b) the effect of the retroactivity s to the output on the internal dynamics of the system is very small independently of s itself; (c) its input/output relationship is about linear. Such a system is said to enjoy the **insulation** property and will be called an insulation component or 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. In electronics, amplifiers enjoy the insulation property by virtue of the features of the operational amplifier (op amp) that they employ [54] (Figure 7.7).

The concept of amplifier in the context of a biochemical network has been considered before in relation to its robustness and insulation property from ex-

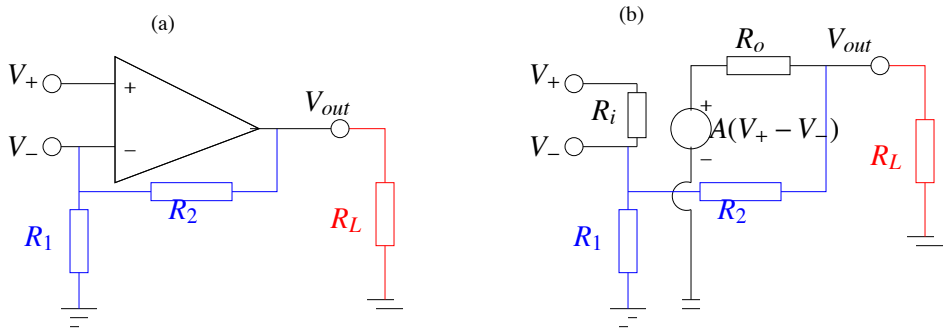

Figure 7.7: In diagram (a), we show the basic non-inverting amplifier circuit that is composed of the op amp plus a feedback circuit. The op amp is the triangular shape that takes as input the differential voltage $V_+ - V_-$ and gives as (open) output $V_{out} = A(V_+ - V_-)$, in which the gain A is infinity in the ideal op amp. The blue circuit components represent the feedback circuit, while the red component is the load. Letting $K = R_1/(R_1 + R_2)$, direct computation leads to $V_{out} \to V_+/K$ as $A \to \infty$. That is, the output voltage does not depend on the load: the retroactivity to the output is almost completely attenuated. In diagram (b), we zoom inside the op amp to show the abstraction of its internal structure. In an ideal op amp, $R_i = \infty$ so that it absorbs almost zero current and any upstream voltage generator will not experience a voltage drop at its output terminals upon interconnection with the amplifier. That is, the retroactivity to the input of the amplifier is almost zero.

ternal disturbances ([52] and [51]). Here, we revisit the amplifier mechanism in the context of gene transcriptional networks with the objective of mathematically and computationally proving how suitable biochemical realizations of such a mechanism can attain properties (a), (b), and (c).

Retroactivity to the input

In electronic amplifiers, r is very small because the input stage of an op amp absorbs almost zero current (Figure 7.7). This way, there is no voltage drop across the output impedance of an upstream voltage source. Equation (7.8) quantifies the effect of retroactivity on the dynamics of X as a function of biochemical parameters that characterize the interconnection mechanism with a downstream system. These parameters are the affinity of the binding site $1/k_d$, the total concentration of such binding site p_{TOT}, and the level of the signal $X(t)$. Therefore, to reduce the retroactivity, we can choose parameters such that (7.8) 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 is a small "flow" of protein X toward its target sites. Thus, we can say that a low retroactivity to the input is obtained when the "input flow" to the system is small. This interpretation establishes a nice analogy to the electrical case, in which low retroactivity to the

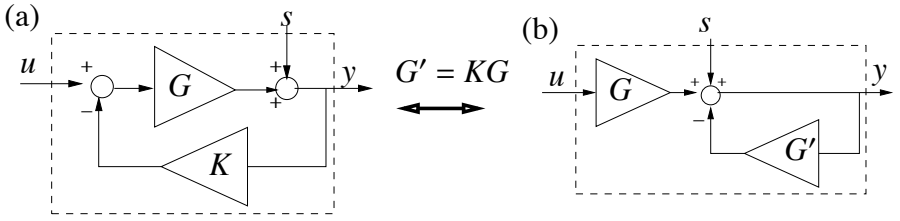

Figure 7.8: Diagram (a) shows the basic feedback/amplification mechanism by which amplifiers attenuate the effect of the retroactivity to the output s. Diagram (b) shows an alternative representation of the same mechanism of diagram (a), which will be employed to design biological insulation devices.

input is obtained, as explained above, by a low input current. Such an interpretation can be further carried to the hydraulic example. In such an example, if the input flow to the downstream tank is small compared, for example, to the output flow of the downstream tank, the output pressure of the upstream tank will not be affected by the connection. Therefore, the retroactivity to the input of the downstream tank will be small.

Retroactivity to the output

In electronic amplifiers, the effect of the retroactivity to the output s on the amplifier behavior is reduced to almost zero by virtue of a large (theoretically infinite) amplification gain of the op amp and an equally large negative feedback mechanism that regulates the output voltage (Figure 7.7). Genetic realization of amplifiers have been previously proposed (see [47], for example). However, such realizations focus mainly on trying to reproduce the layout of the device instead of implementing the fundamental mechanism that allows it to properly work as an insulator. Such a mechanism can be illustrated in its simplest form by diagram (a) of Figure 7.8, which is very well known to control engineers. For simplicity, we have assumed in such a diagram that the retroactivity s is just an additive disturbance. The reason why for large gains G the effect of the retroactivity s to the output is negligible can be verified through the following simple computation. The output y is given by

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

which leads to

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

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 through a large gain the input of the component and (2) to apply a large negative output feedback. We next illustrate this general idea in

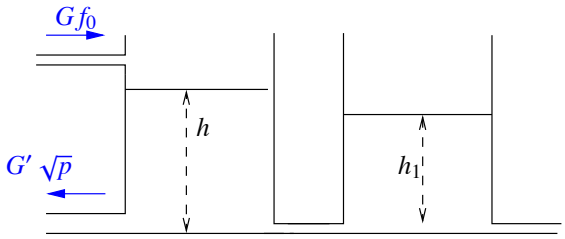

Figure 7.9: We amplify the input flow f_0 through a large gain G and we apply a large negative feedback by employing a large output pipe with output flow $G'\sqrt{p}$.

the context of a simple hydraulic system.

Hydraulic example. Consider the academic hydraulic example consisting of two connected tanks shown in Figure 7.9. 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 pressure p at the output pipe of the upstream tank is obtained for $p > p_1$ and it is given by

$$p_{eq} = \left(\frac{Gf_0}{G' + (kk_1)/\sqrt{k_1^2 + k^2}} \right)^2 .$$

If we let G' be sufficiently larger than k_1 and k and we let $G' = KG$ for some positive $K = O(1)$, then for G sufficiently large $p_{eq} \approx (f_0/K)^2$, which does not depend on the presence of the downstream system. In fact, it is the same as the equilibrium value of the isolated upstream system $A\frac{dp}{dt} = \rho G f_0 - \rho G'\sqrt{p} - \rho k\sqrt{p}$ for G sufficiently large and for $G' = KG$ with $K = O(1)$.

Coming back to the transcriptional example, consider the approximated dynamics of equation (7.7) 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 (7.7) given by

$$\frac{dX}{dt} = (Gk(t) - (G' + \delta)X)(1 - d(t)), \tag{7.9}$$

in which we have defined $d(t) := \frac{d\gamma(y)}{dy}$, where $y(t)$ is given by the reduced system $\frac{dy}{dt} = Gk(t) - (G' + \delta)(y - \gamma(y))$. It can be shown (see [56] for details) that as G and

thus as G' grow, the signal $X(t)$ generated by the connected system (7.9) becomes close to the solution $X(t)$ of the isolated system

$$\frac{dX}{dt} = Gk(t) - (G' + \delta)X, \tag{7.10}$$

that is, 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 the retroactivity effect on the dynamics of X, such an effect is thus attenuated by employing large gains G and G'. *How can we obtain a large amplification gain G and a large negative feedback G′ in a biological insulation component?* This question is addressed in the following chapter, in which we show two possible realizations of insulation devices.

7.4 Design of genetic circuits under the modularity assumption

Based on the modeling assumptions introduced in Chapter 2 and on the tools for studying the dynamics of a nonlinear system introduced in Chapter 3, a number of synthetic genetic circuits have been designed and fabricated by composing transcriptional modules through input/output connection (Figure 6.1). Through such a design procedure one seeks to predict the behavior of a circuit by the behavior of the composing units, once these have been well characterized in isolation. This approach is standard also in the design and fabrication of electronic circuitry.

7.5 Biological realizations of an insulation component

In the previous section, we have proposed a general mechanism in order to create an insulation component. In particular, we have specified how one can alter the biological features of the interconnection mechanism in order to have low retroactivity to the input r and we have shown a general method to attenuate the retroactivity to the output s. Such a method consists of a large amplification of the input and a large negative output feedback. The insulation component will be inserted in place of the transcriptional component of Figure 7.5. This will guarantee that the system generating Z, an oscillator, for example, will maintain the same behavior as in isolation and also that the downstream system that accepts X as its input will not alter the behavior of X. The net result of this is that the oscillator generating signal Z will be able to time downstream systems with the desired phase and amplitude independently of the number and the features of downstream systems. In this section, we determine two possible biological mechanisms that can be exploited to obtain a large amplification gain to the input Z of the insulation component and a large negative feedback on the output X of the insulation component. Both mechanisms realize the negative feedback through enhanced degradation. The first design realizes amplification through transcriptional activation, while the second design through phosphorylation of a protein that is in abundance in the system.

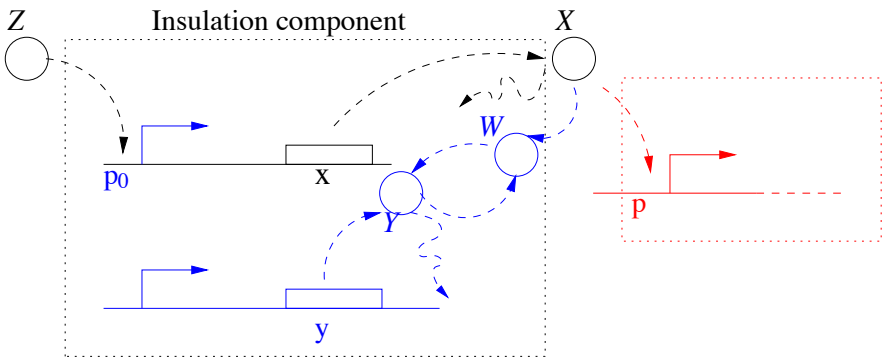

Figure 7.10: We highlight in blue the parts that Design 1 affects. In particular, a negative feedback occurring through post-translational regulation and a promoter that produces a large signal amplification are the central parts of this design. The red part indicates the downstream component that takes as input the concentration of protein X.

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 7.10.

In order to investigate whether such a design realizes a large amplification and a large negative feedback on X as needed, we analyze the full input/output model for the block in the dashed box of Figure 7.10. In particular, the expression of gene x is assumed to be a two-step process, which incorporates also the mRNA dynamics. Incorporating these dynamics in the model is relevant for the current study because they may contribute to an undesired delay between the Z and X signals. The reaction of the protease Y with protein X is modeled as the two-step reaction

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

which can be found in standard references (see [**?**], for example). 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

$$\frac{dZ}{dt} = k(t) - \delta Z + \boxed{k_- Z_p - k_+ Z(p_{0,TOT} - Z_p)} \tag{7.11}$$

$$\frac{dZ_p}{dt} = k_+ Z(p_{0,TOT} - Z_p) - k_- Z_p \tag{7.12}$$

$$\frac{dm_X}{dt} = GZ_p - \delta_1 m_X \tag{7.13}$$

$$\frac{dX}{dt} = \nu m_X - \eta_1 YX + \eta_2 W - \delta_2 X + \boxed{k_{off} C - k_{on} X(p_{TOT} - C)} \tag{7.14}$$

$$\frac{dW}{dt} = \eta_1 XY - \eta_2 W - \beta W \tag{7.15}$$

$$\frac{dY}{dt} = -\eta_1 YX + \beta W + \alpha G - \gamma Y + \eta_2 W \tag{7.16}$$

$$\frac{dC}{dt} = -k_{off} C + k_{on} X(p_{TOT} - C), \tag{7.17}$$

in which we have assumed that the expression of gene z is controlled by a promoter with activity $k(t)$. These equations will be studied numerically and analyzed mathematically in a simplified form. The variable Z_p is the concentration of protein Z bound to the promoter controlling gene x, $p_{0,TOT}$ is the total concentration of the promoter p_0 controlling gene x, m_X is the concentration of messenger RNA of X, C is the concentration of X bound to the downstream binding sites with total concentration p_{TOT}, γ is the decay rate of the protease Y. The value of G is the production rate of X mRNA per unit concentration of Z bound to the promoter controlling x; 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 box in equation (7.11) represent the retroactivity r to the input of the insulation component in Figure 7.10. The terms in the box in equation (7.14) represent the retroactivity s to the output of the insulation component of Figure 7.10. The dynamics of equations (7.11)–(7.17) without s (the elements in the box in equation (7.14)) describe the dynamics of X with no downstream system.

We mathematically explain why system (7.11)–(7.17) allows to attenuate the effect of s on the X dynamics. Equations (7.11) and (7.12) simply determine the signal $Z_p(t)$ that is the input to equations (7.13)–(7.17). 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 (7.13) takes $v(t)$ as an input, we will have that $m_X = G\bar{v}(t)$, for a suitable signal $\bar{v}(t)$. Let us assume for the sake of simplifying the analysis that the protease reaction is a one step reaction, that is, $X + Y \xrightarrow{\beta} Y$. Therefore, equation (7.16) simplifies to $\frac{dY}{dt} = \alpha G - \gamma Y$ and equation (7.14) simplifies to $\frac{dX}{dt} = \nu m_X - \beta YX - \delta_2 X + k_{off} C - k_{on} X(p_{TOT} - C)$. If we consider the protease to be at its equilibrium, we have that $Y(t) = \alpha G/\gamma$. As a

consequence, the X dynamics becomes

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

with C determined by equation (7.17). 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

$$\frac{dX}{dt} = (\nu G\bar{v}(t) - (\beta\alpha G/\gamma + \delta_2)X)(1 - d(t)), \tag{7.18}$$

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

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

as explained in Section 7.3[1].

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 (**??**) is small. This is seen to be true if $(\bar{k}_d + Z)^2/(p_{0,TOT}\bar{k}_d)$ is very large, in which $1/\bar{k}_d = k_+/k_-$ is the affinity of the binding site p_0 to Z. Since after a short transient, $Z_p = (p_{0,TOT}Z)/(\bar{k}_d + Z)$, for Z_p not to be a distorted version of Z, it is enough to ask that $\bar{k}_d \gg Z$. This, combined with the requirement that $(\bar{k}_d + Z)^2/(p_{0,TOT}\bar{k}_d)$ is very large, leads to the requirement $p_{0,TOT}/\bar{k}_d \ll 1$. Summarizing, for not having distortion effects between Z and Z_p and small retroactivity r, we need that

$$\bar{k}_d \gg Z \text{ and } p_{0,TOT}/\bar{k}_d \ll 1. \tag{7.19}$$

Simulation results. Simulation results are presented for the insulation system of equations (7.11)–(7.17) 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 [6]. All simulation results were obtained by using MATLAB (Simulink), with variable step ODE solver ODE23s. 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

[1]See the supplementary material for the mathematical details.

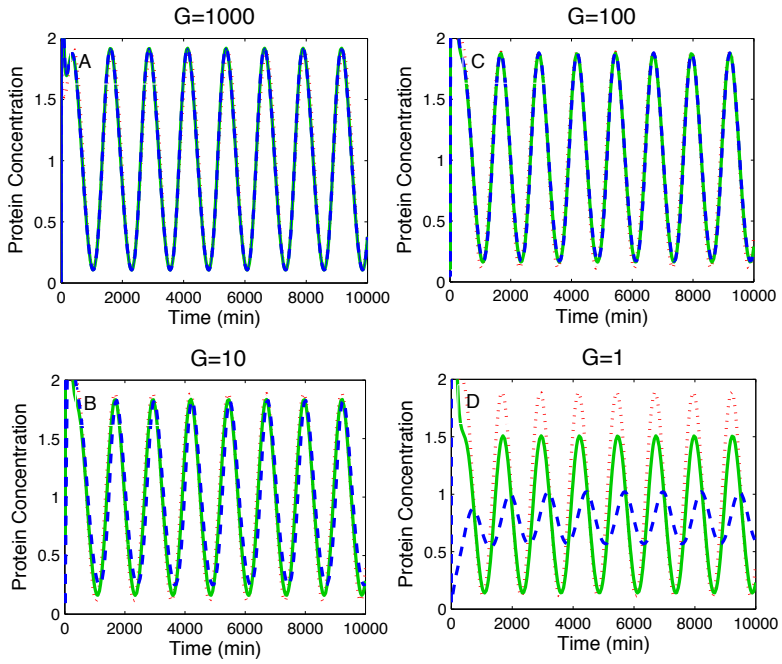

Figure 7.11: Design 1: results for different gains G. In all plots, red (dotted line) is the input Z to the insulation device, green (solid line) is the output X of the insulation device in isolation (without the downstream binding sites p), blue (dashed line) is the output X of the insulation device when downstream sites p are present. In all plots, $k(t) = 0.01(1 + sin(\omega t))$, $p_{TOT} = 100$, $k_{off} = k_{on} = 10$, $\delta = 0.01$, and $\omega = 0.005$. The parameter values are $\delta_1 = 0.01$, $p_{0,TOT} = 1$, $\eta_1 = \eta_2 = \beta = \gamma = 0.01$, $k_- = 200$, $k_+ = 10$, $\alpha = 0.1$, $\delta_2 = 0.1$, $\nu = 0.1$, 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 $G = 10$.

7.11). 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 7.11 are such that $\bar{k}_d \gg Z$ and $p_{0,TOT} \ll \bar{k}_d$. 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 (7.19). The poorer performance of the device for $G = 1$ is therefore entirely due to poor attenuation of the retroactivity s to the output.

Design 2: Amplification through phosphorylation

In this design, the amplification of Z is obtained by having Z activate the phosphorylation of a protein X, which is available in the system in abundance. That is,

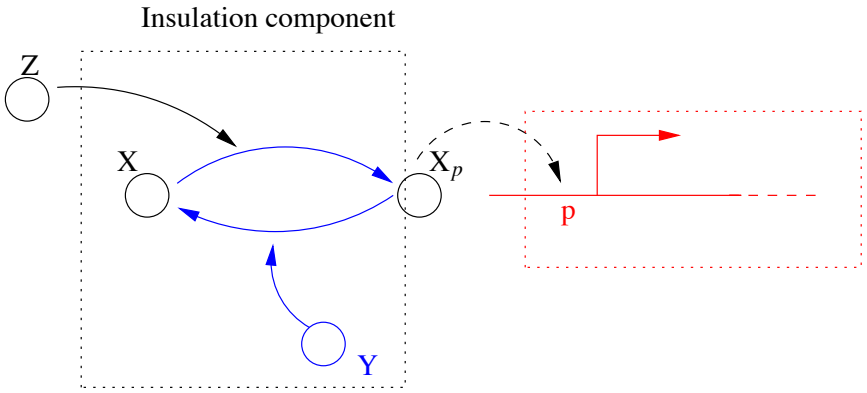

Figure 7.12: The dashed box contains the insulation device. The blue parts highlight the mechanism that provides negative feedback and amplification. Negative feedback occurs through a phosphatase Y that converts the active form X_p back to its inactive form X. Amplification occurs through Z activating the phosphorylation of X.

Z is a kinase for a protein X. The phosphorylated form of X, called X_p, binds to the downstream sites, while X does not. A negative feedback on X_p is obtained by having a phosphatase Y activate the dephosphorylation of protein X_p. Protein Y is also available in abundance in the system. This mechanism is depicted in Figure 7.12. A similar design has been proposed by [52, 51], in which a MAPK cascade plus a negative feedback loop that spans the length of the MAPK cascade is considered as a feedback amplifier. Our design is much simpler as it involves only one phosphorylation cycle and does not require the additional feedback loop. In fact, we realize a strong negative feedback by the action of the phosphatase that converts the active protein form X_p to its inactive form X. This negative feedback, whose strength can be tuned by varying the amount of phosphatase in the system, is enough to mathematically and computationally show that the desired insulation properties are satisfied.

We consider two different models for the phosphorylation and dephosphorylation processes. A one step reaction model is initially considered to illustrate what biochemical parameters realize the input gain G and the negative feedback G'. Then, we turn to a more realistic two step model to perform a parametric analysis and numerical simulation. The one step model that we consider is the one of [28]:

$$Z + X \xrightarrow{k_1} Z + X_p,$$

and

$$Y + X_p \xrightarrow{k_2} Y + X.$$

We assume that there is plenty of protein X and of phosphatase Y in the system and that these quantities are conserved. The conservation of X gives $X + X_p + C = X_{TOT}$, in which X is the inactive protein, X_p is the phosphorylated protein that binds to

the downstream sites p, and C is the complex of the phosphorylated protein X_p bound to the promoter p. The X_p dynamics can be described by the first equation in the following model

$$\frac{dX_p}{dt} = k_1 X_{TOT} Z(t)\left(1 - \frac{X_p}{X_{TOT}} - \boxed{\frac{C}{X_{TOT}}}\right) - k_2 Y X_p + \boxed{k_{off} C - k_{on} X_p (p_{TOT} - C)} \tag{7.20}$$

$$\frac{dC}{dt} = -k_{off} C + k_{on} X_p (p_{TOT} - C). \tag{7.21}$$

The boxed terms represent the retroactivity s to the output of the insulation system of Figure 7.12. For a weakly activated pathway ([28]), $X_p \ll X_{TOT}$. Also, if we assume that the concentration of total X is large compared to the concentration of the downstream binding sites, that is, $X_{TOT} \gg p_{TOT}$, equation (7.20) is approximatively equal to

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

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

$$\frac{dX_p}{dt} = (GZ(t) - G' X_p)(1 - d(t)), \tag{7.22}$$

in which $0 < d(t) < 1$ is the effect of the retroactivity s to the output after a short transient. Therefore, for G and G' large enough, $X_p(t)$ tends to the solution $X_p(t)$ of the isolated system $\frac{dX_p}{dt} = GZ(t) - G' X_p$, as explained in Section 7.3[2]. As a consequence, the effect of the retroactivity to the output s is attenuated by increasing $k_1 X_{TOT}$ 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 7.12.

We next consider a more complex model for the phosphorylation and dephosphorylation reactions 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 such as those in [29]. According to this model, we have the following two reactions for phosphorylation and dephosphorylation, respectively:

$$\mathrm{X} + \mathrm{Z} \underset{\beta_2}{\overset{\beta_1}{\rightleftarrows}} \mathrm{C}_1 \xrightarrow{k_1} \mathrm{X}_p + \mathrm{Z}, \tag{7.23}$$

[2]See the supplementary material for the mathematical details.

and

$$\mathrm{Y} + \mathrm{X}_p \underset{\alpha_2}{\overset{\alpha_1}{\rightleftarrows}} \mathrm{C}_2 \xrightarrow{k_2} \mathrm{X} + \mathrm{Y}, \tag{7.24}$$

in which C_1 is the [protein X/kinase Z] complex and C_2 is the [phosphatase Y/protein X_p] complex. Additionally, we have the conservation equations $Y_{TOT} = Y + C_2$, $X_{TOT} = X + X_p + C_1 + C_2 + C$, because proteins X and Y are not degraded. Therefore, the differential equations modeling the insulation system of Figure 7.12 become

$$\frac{dZ}{dt} = k(t) - \delta Z \boxed{-\beta_1 Z X_{TOT}(1 - \frac{X_p}{X_{TOT}} - \frac{C_1}{X_{TOT}} - \frac{C_2}{X_{TOT}} - \boxed{\frac{C}{X_{TOT}}}) + (\beta_2 + k_1)C_1} \tag{7.25}$$

$$\frac{dC_1}{dt} = -(\beta_2 + k_1)C_1 + \beta_1 Z X_{TOT}(1 - \frac{X_p}{X_{TOT}} - \frac{C_1}{X_{TOT}} - \frac{C_2}{X_{TOT}} - \boxed{\frac{C}{X_{TOT}}}) \tag{7.26}$$

$$\frac{dC_2}{dt} = -(k_2 + \alpha_2)C_2 + \alpha_1 Y_{TOT} X_p (1 - \frac{C_2}{Y_{TOT}}) \tag{7.27}$$

$$\frac{dX_p}{dt} = k_1 C_1 + \alpha_2 C_2 - \alpha_1 Y_{TOT} X_p (1 - \frac{C_2}{Y_{TOT}}) + \boxed{k_{off} C - k_{on} X_p (p_{TOT} - C)} \tag{7.28}$$

$$\frac{dC}{dt} = -k_{off} C + k_{on} X_p (p_{TOT} - C), \tag{7.29}$$

in which the expression of gene z is controlled by a promoter with activity $k(t)$. The terms in the large box in equation (7.25) represent the retroactivity r to the input, while the terms in the small box in equation (7.25) and in the boxes of equations (7.26) and (7.28) represent the retroactivity s to the output. We assume that $X_{TOT} \gg p_{TOT}$ so that in equations (7.25) and (7.26) we can neglect the term C/X_{TOT} because $C < p_{TOT}$. Also, phosphorylation and dephosphorylation reactions in equations (7.23) and (7.24) can occur at a much faster rate (on the time scale of a second [33]) than protein production and decay processes (on the time scale of minutes [3]). Choosing X_{TOT} and Y_{TOT} sufficiently large, the separation of time-scales between equation (7.25) and equations (7.26–7.29) can be explicitly modeled by letting $\epsilon = \delta/k_{off}$, $k_{on} = k_{off}/k_d$, and by defining the new rate constants $b_1 = \beta_1 X_{TOT}\epsilon/\delta$, $a_1 = \alpha_1 Y_{TOT}\epsilon/\delta$, $b_2 = \beta_2\epsilon/\delta$, $a_2 = \alpha_2\epsilon/\delta$, $c_i = \epsilon k_i/\delta$. Letting $z = Z + C_1$ (the total amount of kinase) be the slow variable, we obtain the system in the standard singular perturbation form

$$\begin{aligned}
\frac{dz}{dt} &= k(t) - \delta(z - C_1) \\
\epsilon\frac{dC_1}{dt} &= -\delta(b_2 + c_1)C_1 + \delta b_1(z - C_1)(1 - \frac{X_p}{X_{TOT}} - \frac{C_1}{X_{TOT}} - \frac{C_2}{X_{TOT}}) \\
\epsilon\frac{dC_2}{dt} &= -\delta(c_2 + a_2)C_2 + \delta a_1 X_p (1 - \frac{C_2}{Y_{TOT}}) \\
\epsilon\frac{dX_p}{dt} &= \delta c_1 C_1 + \delta a_2 C_2 - \delta a_1 X_p (1 - \frac{C_2}{Y_{TOT}}) + \boxed{\delta C - \delta/k_d (p_{TOT} - C)X_p} \\
\epsilon\frac{dC}{dt} &= -\delta C + \delta/k_d (p_{TOT} - C)X_p,
\end{aligned} \tag{7.30}$$

in which the boxed terms represent the retroactivity to the output s. We then compute the dynamics on the slow manifold by letting $\epsilon = 0$. When we set $\epsilon = 0$, the terms due to the retroactivity s vanish. This means that if the internal dynamics of the insulation device evolve on a time scale that is much faster than the dynamics of the input signal Z, then (provided we also have $X_{TOT} \gg p_{TOT}$) the retroactivity s to the output has no effect on the dynamics of X_p at the quasi steady state. This is a crucial feature of this design. Letting $\gamma = (\beta_2 + k_1)/\beta_1$ and $\bar{\gamma} = (\alpha_2 + k_2)/\alpha_1$, setting $\epsilon = 0$ in the third and fourth equations of (7.30) the following relationships can be obtained:

$$C_1 = F_1(X_p) = \frac{\frac{X_p Y_{TOT} k_2}{\bar{\gamma} k_1}}{1 + X_p/\bar{\gamma}}, \quad C_2 = F_2(X_p) = \frac{\frac{X_p Y_{TOT}}{\bar{\gamma}}}{1 + X_p/\bar{\gamma}}. \tag{7.31}$$

Using expressions (7.31) in the second of equations (7.30) with $\epsilon = 0$ leads to

$$F_1(X_p)(b_2 + c_1 + \frac{b_1 Z}{X_{TOT}}) = b_1 Z(1 - \frac{X_p}{X_{TOT}} - \frac{F_2(X_p)}{X_{TOT}}). \tag{7.32}$$

Assuming for simplicity that $X_p \ll \bar{\gamma}$, we obtain that $F_1(X_p) \approx \frac{X_p Y_{TOT} k_2}{\bar{\gamma} k_1}$ and that $F_2(X_p) \approx \frac{X_p}{\bar{\gamma}} Y_{TOT}$. As a consequence of these simplifications, equation (7.32) leads to

$$X_p = \frac{b_1 Z}{\frac{b_1 Z}{X_{TOT}}(1 + Y_{TOT}/\bar{\gamma} + (Y_{TOT} k_2)/(\bar{\gamma} k_1)) + \frac{Y_{TOT} k_2}{\bar{\gamma} k_1}(b_2 + c_1)} := m(Z).$$

In order not to have distortion from Z to X_p, we require that

$$Z \ll \frac{Y_{TOT} \frac{k_2}{k_1} \frac{\gamma}{\bar{\gamma}}}{1 + \frac{Y_{TOT}}{\bar{\gamma}} + \frac{Y_{TOT}}{\bar{\gamma}} \frac{k_2}{k_1}}, \tag{7.33}$$

so that $m(Z) \approx Z \frac{X_{TOT} \bar{\gamma} k_1}{Y_{TOT} \gamma k_2}$ and therefore we have a linear relationship between X_p and Z with gain from Z to X_p given by $\frac{X_{TOT} \bar{\gamma} k_1}{Y_{TOT} \gamma k_2}$. In order not to have attenuation from Z to X_p we require that the gain is greater than or equal to one, that is,

$$\text{input/output gain} \approx \frac{X_{TOT} \bar{\gamma} k_1}{Y_{TOT} \gamma k_2} \geq 1. \tag{7.34}$$

Requirements (7.33), (7.34), and $X_p \ll \bar{\gamma}$ are enough to guarantee that we do not have nonlinear distortion between Z and X_p and that X_p 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 7.2 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 is given by

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

in which $\frac{dF_1}{dX_p}\frac{dX_p}{dz}$ measures the effect of the retroactivity r to the input on the Z dynamics. Direct computation of $\frac{dF_1}{dX_p}$ and of $\frac{dX_p}{dz}$ along with $X_p \ll \bar{\gamma}$ and with (7.33) leads to $\frac{dF_1}{dX_p}\frac{dX_p}{dz} \approx X_{TOT}/\gamma$, so that in order to have small retroactivity to the input, we require that

$$\frac{X_{TOT}}{\gamma} \ll 1. \tag{7.35}$$

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 $X_{TOT} \gg p_{TOT}$, 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 $\gamma \gg X_{TOT}$ as established by relation (7.35). 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_p close to one (from equation (7.34)), one can choose $X_{TOT} = Y_{TOT}$, and equal coefficients for the phosphorylation and dephosphorylation reactions, that is, $\gamma = \bar{\gamma}$ and $k_1 = k_2$.

Simulation results. System in equations (7.25–7.29) was simulated with and without the downstream binding sites p, that is, with and without, respectively, the terms in the small box of equation (7.25) and in the boxes in equations (7.28) and (7.26). This is performed to highlight the effect of the retroactivity to the output s on the dynamics of X_p. The simulations validate our theoretical study that indicates that when $X_{TOT} \gg p_{TOT}$ and the time scales of phosphorylation/dephosphorylation are much faster than the time scale of decay and production of the protein Z, the retroactivity to the output s is very well attenuated (Figure 7.13, plot A). Similarly, the time behavior of Z was simulated with and without the terms in the large box in equation (7.25), 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 7.13, plot B), indicates that there is no substantial retroactivity to the input r generated by the insulation device. This is obtained because $X_{TOT} \ll \gamma$ as indicated in equation (7.35), in which $1/\gamma$ 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 7.14). 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 $\gamma \ll X_{TOT}$ is not satisfied anymore.

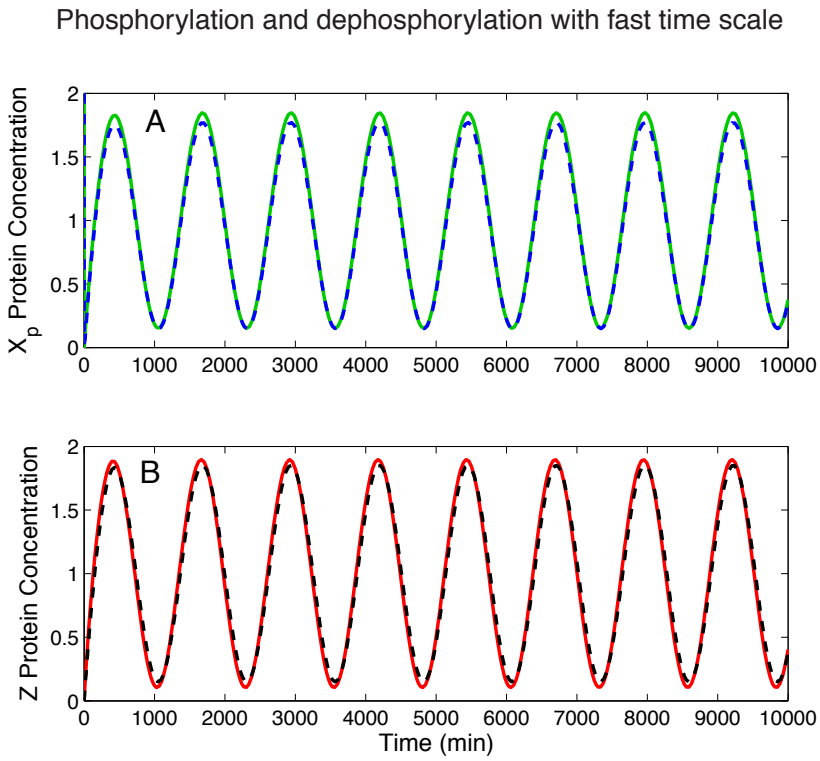

Figure 7.13: Simulation results for system in equations (7.25–7.29). In all plots, $p_{TOT} = 100$, $k_{off} = k_{on} = 10$, $\delta = 0.01$, $k(t) = 0.01(1 + sin(\omega t))$, and $\omega = 0.005$. In subplots A and B, $k_1 = k_2 = 50$, $\alpha_1 = \beta_1 = 0.01$, $\beta_2 = \alpha_2 = 10$, and $Y_{TOT} = X_{TOT} = 1500$. In subplot A, the signal $X_p(t)$ without the downstream binding sites p is in green (solid line), while the same signal with the downstream binding sites p is in blue (dashed line). The small error shows that the effect of the retroactivity to the output s is attenuated very well. In subplot B, the signal $Z(t)$ without X to which Z binds is in red (solid), while the same signal $Z(t)$ with X present in the system ($X_{TOT} = 1500$) is in black (dashed line). The small error confirms a small retroactivity to the input. The values of the complexes concentrations C_1 and C_2 oscillate about 0.4, so they are comparable to the values of X_p.

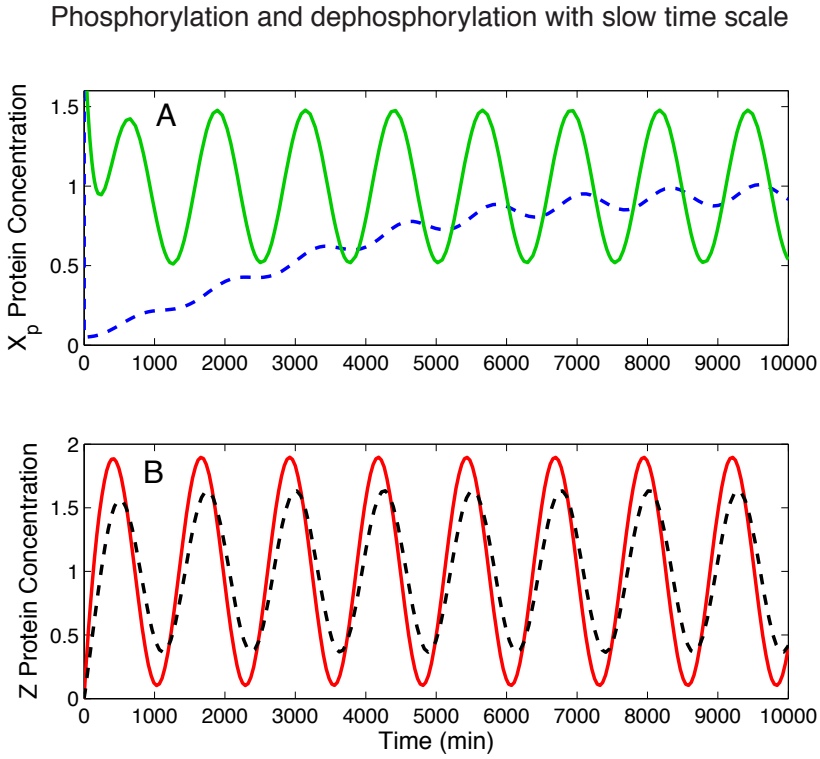

Figure 7.14: In all plots, $p_{TOT} = 100$ and $k_{off} = k_{on} = 10$, $\delta = 0.01$, $k(t) = 0.01(1 + sin(\omega t))$, and $\omega = 0.005$. Phosphorylation and dephosphorylation rates are slower than the ones in Figure 7.13, that is, $k_1 = k_2 = 0.01$, while the other parameters are left the same, that is, $\alpha_2 = \beta_2 = 10$, $\alpha_1 = \beta_1 = 0.01$, and $Y_{TOT} = X_{TOT} = 1500$. In subplot A, the signal $X_p(t)$ without the downstream binding sites p is in green (solid line), while the same signal with the downstream binding sites p is in blue (dashed line). The effect of the retroactivity to the output s is dramatic. In subplot B, the signal $Z(t)$ without X in the system is in red (solid line), while the same signal $Z(t)$ with X in the system is in black (dashed line). The device thus also displays a large retroactivity to the input r.

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