Modularity in Signaling Systems

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Abstract. Modularity is a property by which the behavior of a system does not change upon interconnection. It is crucial for understanding the behavior of a complex system from the behavior of the composing subsystems. Whether modularity holds in biology is an intriguing and largely debated question. In this paper, we discuss this question taking a control systems theory view and focusing on signaling systems. In particular, we argue that, despite signaling systems are constituted of structural modules, such as covalent modification cycles, modularity does not hold in general. As in any engineering system, impedance-like effects, called retroactivity, appear at interconnections and alter the behavior of connected modules. We further argue that while signaling systems have evolved sophisticated ways to counter-act retroactivity and enforce modularity, retroactivity may also be exploited to finely control the information processing of signaling pathways. Testable predictions and experimental evidence are discussed with their implications.

1. Introduction

The idea that modularity is a general principle of biological organization and that signaling systems, in particular, are modularly assembled has been considered and discussed by several researchers [1,10,21]. In particular, a modular systems approach to understanding information transfer in signaling networks has been proposed as a way to defeat the complexity of networks [4,20,24,25]. For example, signaling pathways involved in caspase activation have been studied through a modular approach to determine the key contributors in triggering apoptosis [9]. Similarly, a study of tradeoffs in metabolic networks suggested highly structured modularity [26]. Modular structures, such as phosphorylation cycles, phosphotransfer motifs, and methylation, seem to be fundamental building blocks of signal transduction, which have been conserved through the course of evolution [19]. Whether these modular structures maintain their behavior unchanged independently of where they are connected in a network, however, is debatable [4, 15, 16].

Theoretical studies on modularity in biomolecular systems have shown that, just as in electrical, mechanical, or hydraulic engineering systems, impedance-like effects arise at the interconnection of physical modules [5,24]. These effects, called retroactivity, can be so dramatic to change the input/output response of a module upon interconnection. Basically, when a "sender" module transmits information to a "receiver" module, its (dynamic) state is changed by the physical mechanism that allows connection to the receiver module. The extent of this change depends on the physical characteristics of the interconnection and increases with increased "flow of matter" between the sender and the receiver. Even in the presence of a large flow of matter, however, it has been theoretically shown that signaling modules, such as covalent modification cycles, can be engineered to attain insulation from retroactivity and enforce modular behavior [5, 13].

These theoretical findings have been supported by recent experimental evidence. Studies performed *in vitro* on a reconstituted signal transduction system [14, 27] and *in vivo* on the MAPK cascade [17, 18] provide evidence that signaling modules do not, in general, display modularity but that they can be tuned so to enforce modular behavior. In particular, these studies revealed that the input/output behavior of a signaling

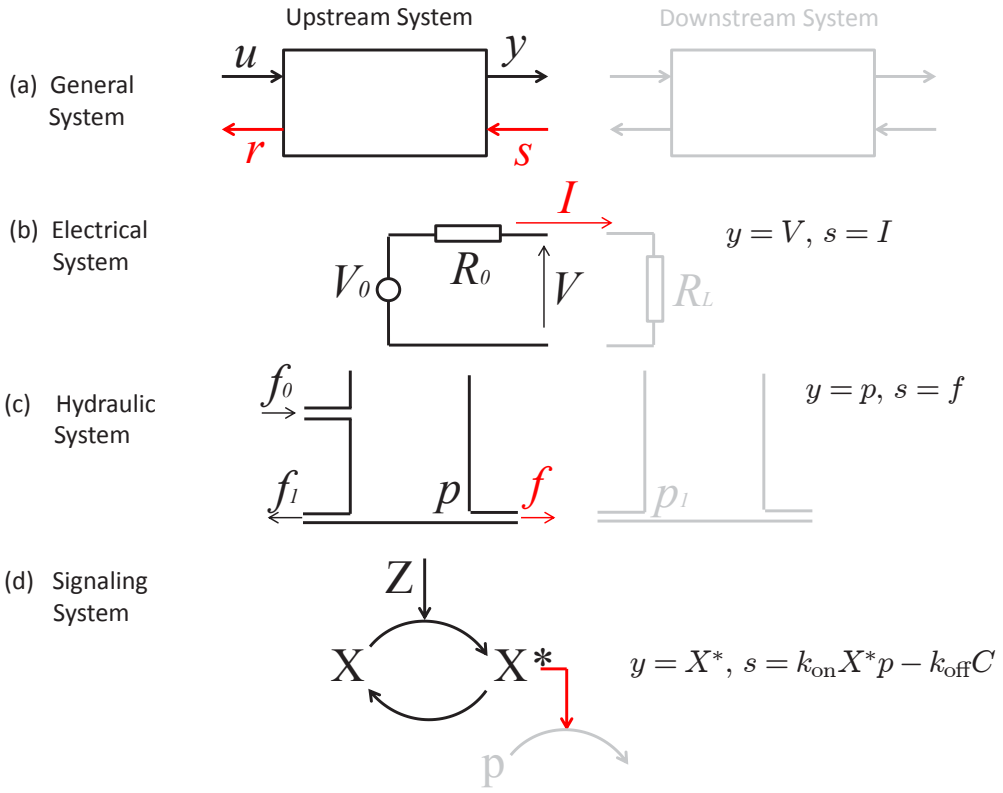

Figure 1. Interconnection of two systems. (a) The upstream system is sending the signal y and the downstream system is receiving it. Information travels from upstream to downstream. The r and s arrows represent retroactivity to the input and retroactivity to the output, respectively. (b) V and I in the electrical system represent voltage and current, respectively; (c) f and p in the hydraulic system represent flow and pressure, respectively; (d) Z, X, X* in the signaling system represent a kinase, a substrate, and its phosphorylated form, respectively, k_{on} and k_{off} represent association and dissociation rates of X* with downstream binding sites p to form complex C. Retroactivity takes different forms depending on the specific physical domain, but it always has the physical meaning of a *flow*.

module can be largely dependent on the downstream clients to which it transmits information. At the same time, they showed that the impact of downstream clients can be reduced by increasing suitable gains within the module.

In this paper, we discuss modularity and retroactivity by taking a control theory point of view and illustrating tight analogies with engineering systems. We argue how this point of view offers useful insights both on predicting the behavior of a signaling module after interconnection and on uncovering general design principles for modularity.

2. A control systems view on modularity

In this paper, a systems and control theory view is taken to discuss modularity in signaling systems. In particular, the system concept adopted is depicted in Figure 1 (a) [5]. A system takes as input a signal denoted u and provides a signal denoted y as

an output. Upon interconnection with a downstream system, the information in y is transmitted to downstream. Here, upstream and downstream denote the direction in which we think the information is being transmitted: from upstream to downstream. Interconnection usually occurs through some physical mechanism, such as connecting wires in electrical circuits, pipes in hydraulic circuits, or placing two reagents in the same medium in biomolecular circuits. This physical interconnection often results in a change of the information being transmitted, an effect also known in physics as the observer effect. A typical example is checking the pressure of a tire: it is difficult to measure it without letting some of the air out. This fact, in turn, changes the pressure being measured. In the context of a signaling system, for example, phospho-specific antibodies can be employed to measure the amount of a phosphorylated protein in a phosphorylation cycle. However, to do so, antibodies bind to the phosphorylated protein impeding it to take place in the reactions of the phosphorylation cycle. This will unavoidably change the behavior of the cycle and hence the amount of phosphorylated protein being measured.

We call these effects *retroactivity* to extend the notion of "loading" to non-electrical systems and, in particular, to biomolecular systems. We model the retroactivity phenomenon by adding two arrows to our system model: a retroactivity to the input r and a retroactivity to the output s. The first one models the fact that when the system receives signal u, because of the physics of the interconnection, it will change the behavior of the system sending u. Similarly, the retroactivity to the output s models the fact that when the upstream system sends signal y to a downstream system, the latter, in order to measure/use signal y, will change the behavior of the upstream system generating signal y. We call the upstream system *isolated* when $s = 0$, corresponding to no interconnection. We call the upstream system *connected* when $s \neq 0$, corresponding to an interconnection with a downstream system.

2.1. Retroactivity across different physical domains

There are plenty of engineering examples of retroactivity. Here, we briefly discuss retroactivity in electrical and hydraulic systems to draw the analogies with retroactivity in signaling systems.

Electrical Systems. Figure 1(b) depicts a voltage generator with internal resistor R_0 (upstream system) and the downstream system to which it gets connected, a load resistor with value R_L. The output of the isolated system is $y = V_0$ while the output of the connected system is given by $y = V_0 - R_0 I$. The two outputs are the same when the current drawn by the resistor is $I = 0$. Hence, we can take $s = I$. Note that for the connected system, I can be rendered arbitrarily small by taking R_L very large, corresponding to a downstream system with high input impedance (a downstream system with low retroactivity to the input).

Hydraulic Systems. Figure 1(c) depicts the interconnection of two tanks. In the case the upstream tank is isolated, we have that the valve at the output pipe is closed

and hence $f = 0$. When the tank is connected to the downstream tank, we have that $f = \rho k\sqrt{(p - p_1)}$ for $p > p_1$, in which ρ is the fluid density and k is a parameter that depends on the output valve. In this case, the retroactivity to the output can be taken as $s = f$. This additional flow will cause a change in the pressure p of the upstream tank. Note that for the connected system this can be rendered small by decreasing k, which corresponds to decreasing the aperture of the valve.

Signaling Systems. Figure 1(d) depicts the module of any signaling pathway: a covalent modification cycle, such as phosphorylation. In the case in which the cycle is connected, X* is not taken as an input to any downstream system. When it is connected, X* serves as an input to a downstream system, such as another covalent modification cycle. This interconnection takes place by having X* bind to downstream substrates p through a reversible binding reaction. The corresponding reaction rate is given by $-k_{\text{on}}X^*p + k_{\text{off}}C$. Hence, we can take $s = -k_{\text{on}}X^*p + k_{\text{off}}C$. This "usage" of X* can in principle change the behavior of the cycle and hence the value of the X* concentration as discussed in Section 3.

Retroactivity physically has the form of a flow of matter that takes place between systems upon interconnection: it is a current in the case of the electrical example, a flow in the case of the hydraulic system, and the rate of a chemical reaction in the signaling system. In all cases, an interconnection can be associated with a power flow P between the upstream and the downstream system. Such a power is positive when power flows from the upstream system to the downstream one. For the electrical system, we have that the power exchange is given by $P = V \cdot I$ and for the hydraulic system $P = p \cdot f$. Also the interconnection in the signaling system can be associated with a power flow. Specifically, the chemical power spent by the binding reaction is given by the affinity of the reaction A multiplied by the reaction rate $-k_{\text{on}}X^*p+k_{\text{off}}C$ [23]. In this case, we have that $A = RT \log\left(\frac{CK_{eq}}{X^*p}\right)$, in which R is the Boltzmann constant, T is the temperature in Kelvin, and $K_{eq} = k_{\text{off}}/k_{\text{on}}$. Since the affinity has the dimension of Joule/mole and the reaction rate has the dimension of mole/seconds, their product has the dimension of Joule/second, which has the unit of a power. This can be interpreted as the chemical power flowing from the upstream system to the downstream one.

When $s \approx 0$, there is no power transaction between the systems, only information is being transmitted. For the case of the signaling system, this corresponds to having a low affinity binding or/and having an excess of X^* compared to the substrate p (load). Hence, when the power transactions between the upstream and downstream system are very small, the interconnection is basically modular and signals are transmitted faithfully from one system to the next. Note, however, that there are cases in which the internal structure of the upstream system is such that even a large retroactivity to the output s minimally affects the output y. This robustness property, also known as *insulation*, allows to tolerate large power transactions between systems while faithfully transmitting the signal y across the interconnection. This property will be discussed in Section 4, in which we will illustrate sophisticated mechanisms that natural signaling systems have evolved to attain insulation.

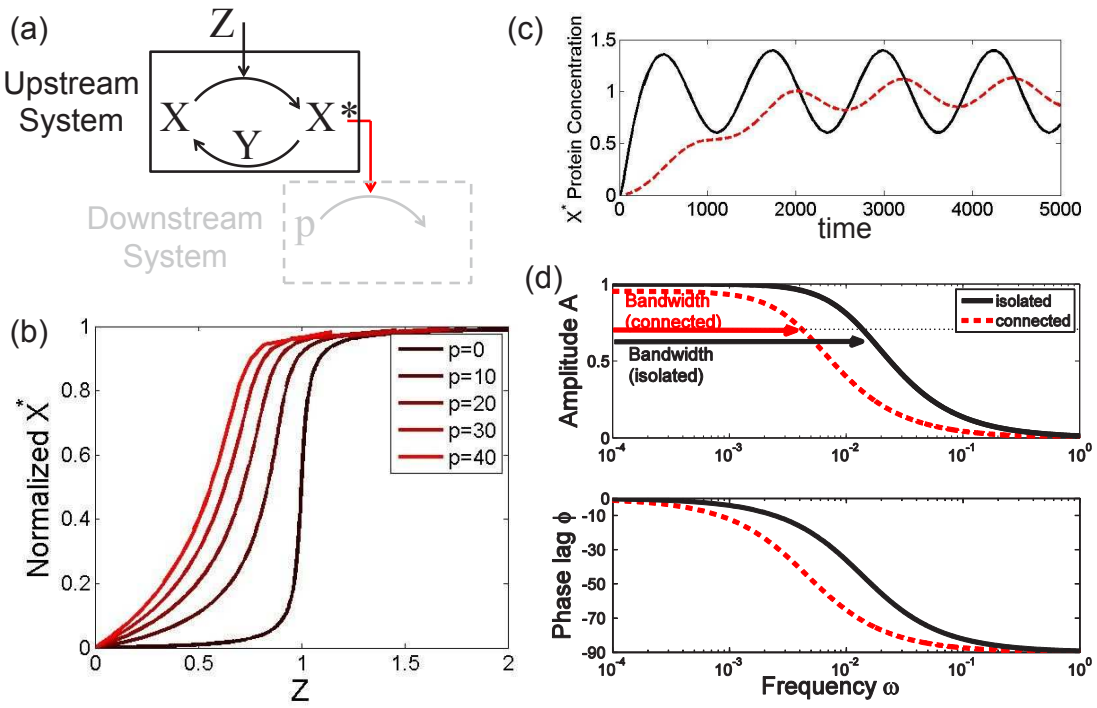

Figure 2. Effects of retroactivity on signaling systems. (a) Signaling module (upstream system) with a downstream client. (b) Retroactivity turns a switch-like dose response curve into a linear-like dose response curve. The plot shows on the x-axis the constant value of the input stimulus Z and on the y-axis the corresponding steady state value of the output X* normalized by its maximum. The different curves correspond to different amounts of the downstream targets p. As these amounts are increased, the sensitivity of the response of X* to Z decreases. (c) When $Z(t)$ is time varying as coming from a biological clock, retroactivity delays and attenuates the signal. That is, the connected system transmits less of the signal than the isolated system. (d) Frequency response showing that retroactivity decreases system bandwidth.

3. Controlling information transfer in signaling pathways through retroactivity

What is the effect of retroactivity from downstream systems on a covalent modification cycle? One should expect that if the loading from downstream targets is high (high affinity or large amounts of sites), the behavior of the cycle will be affected because the active protein X* will be busy binding with the targets and will be partially "sequestered" from the reactions internal to the cycle. One should expect two main types of effects: static effects and dynamic effects. Static effects are those that alter the steady state response of the system output to constant input stimuli u, that is, those effects that alter the dose response curve of the cycle. Dynamic effects are those that alter the speed of response of the output to time-varying input stimuli. These effects may not imply each other, that is, retroactivity may have dynamic effects while not having static effects.

3.1. Static effects of retroactivity.

The sensitivity of the input/output static characteristic, or dose response curve, of a signaling system is a crucial feature and a wealth of work has studied mechanisms by which it can be controlled [7, 12]. In particular, zero-order ultrasensitivity as studied by Goldbeter and Koshland [7] is known to be a key responsible of high sensitivity in natural signaling cascades, such as the MAPK cascade [12]. Sensitivity is quantified by the response coefficient R given by the ratio between the input stimulus corresponding to 90% response and the input stimulus corresponding to 10% response. For a Goldbeter-Koshland covalent modification cycle, the expression of R is given by

$$R = 81\frac{(K_2 + 0.1)(K_1 + 0.1)}{(K_1 + 0.9)(K_2 + 0.9)},$$

in which K_1 and K_2 are the Michaelis-Menten constants of the forward and backward modification reactions, respectively, divided by the total amount of substrate X. When K_1 and K_2 decrease, the value of R approaches 1. In this case, the input(Z)/output(X*) characteristic of a covalent modification cycle becomes highly sensitive (switch-like in the case in which $R \approx 1$), a condition that is usually referred to as zero-order ultrasensitivity [7].

The Michaelis-Menten constant K_2 is inversely proportional to the association rate of X* with the backward modification enzyme Y (refer to Figure 2(a)). When the cycle protein X* is used as an input to a downstream system, X* is busy with the binding reactions with downstream substrates p. As a consequence, less of X* will take place in the backward enzymatic reaction of the cycle, decreasing the effective association rate of X* with Y. This causes an increase of the effective value of K_2 to $K_2(1 + \lambda)$, in which λ is a positive number and is given by the ratio p/k_D with k_D the dissociation constant of X* with sites p. The parameter λ can be interpreted as the effective load that the downstream sites apply to X*. As a consequence, the mathematical expression for the response coefficient R should be modified by replacing K_2 with $K_2(1 + \lambda)$. The precise derivation of the effective Michaelis-Menten constants of a connected cycle can be found in [27]. As a result, zero-order ultrasensitivity effects decrease because the effective value of the Michaelis-Menten constant K_2 increases. This leads to a more linear-like input/output characteristic (Figure 2(b)). Experiments on a reconstituted signaling system *in vitro* support this phenomenon showing that the shape of the dose response curve can be precisely tuned by retroactivity from downstream targets. In particular, by playing with targets for both the active and inactive cycle proteins, one can independently tune the sensitivity and the point of half-maximal induction [27].

Signaling modules have several downstream targets, even outside the pathway they belong to. Our discussion suggests that these targets may be used as a mechanism to finely control the shape of the input/output characteristic of a cycle. At the same time, these facts also indicate that signaling cascades, such as the MAPK cascades, have optimized the amounts of substrate and the values of the Michaelis-Menten constants

to obtain an acceptable tradeoff between zero-order ultrasensitivity effects and the (consequent) decrease of sensitivity due to retroactivity.

3.2. Dynamic effects of retroactivity.

Signaling systems have to also respond to a variety of time-varying input stimuli, such as the transient occurrence of nutrients, hormones, toxins, and pathogens, or the periodic stimulation of circadian clocks. How signaling systems filter and transmit these signals to the nucleus is crucial for the healthy functioning of the cell. For example, the transient over-activation of a pathway can lead to gene over-expression and contribute to several forms of cancer [3, 11]. Weather a pathway is capable of filtering out such undesired stimuli depends on the pathway *bandwidth* [6,8]. The bandwidth is the largest frequency of the input stimulation that a system can transmit. Most physical systems are low-pass filters, that is, they can respond only to input stimuli that change slower than a given frequency (the bandwidth). Input stimuli that change faster than the bandwidth are filtered out and not transmitted to the output.

By appropriately cascading signaling modules with different bandwidths, it is possible to selectively transmit stimuli at desired frequencies [2]. This is a powerful mechanism for a signal transmission system to transmit desired information while filtering out potentially harmful signals. How do signaling modules, such as covalent modification cycles, tune their bandwidths? Basically, the faster the cycle reactions are, the largest the bandwidth. Hence, large bandwidths are usually associated with large kinetic constants and large amounts of modifying enzymes and/or substrates. This intuitive explanation can be precisely formulated by calculating the frequency response of a cycle and analytically calculating the bandwidth [8, 14]. Specifically, the frequency response of a system quantifies how the amplitude and lag of the output response changes when the frequency of a periodic input stimulation increases. The bandwidth is mathematically defined as the frequency of the stimulation at which the amplitude drops below $1/\sqrt{2}$ of the amplitude at low frequency (corresponding to a 50% drop in the signal power). For a covalent modification cycle the frequency response is well approximated (see [8, 14] for details) by

$$A(\omega) = \frac{a}{\sqrt{\omega^2 + b^2}} \text{ and } \phi(\omega) = \tan^{-1}(-\omega/b),$$

in which $A(\omega)$ is the amplitude of the output response and $\phi(\omega)$ is the lag between the input and the output for an input signal $Z(t)$ with unit amplitude and frequency ω. Here, a and b are positive constants and their values depend on the biochemical parameters of the cycle. For example, for a cycle where the input/output static characteristic is linear-like, that is, the normalized Michaelis-Menten constants K_1, K_2 are sufficiently large, and the cycle is weakly activated, that is, X^* is much smaller than X and Z is much smaller than Y, a and b are well approximated by

$$b \approx \frac{k_2 Y_{TOT}}{K_2 X_{TOT}} \text{ and } a \approx \frac{k_1}{K_1},$$

in which k_1 and k_2 are the catalytic rates of the forward and backward modification reactions, respectively, and X_{TOT} and Y_{TOT} are the total amounts of substrate X and enzyme Y, respectively. Here, b is exactly the bandwidth of the cycle. Hence, as rate k_2 is increased and/or the total amount of enzyme Y is increased, the bandwidth increases.

Remarkably, retroactivity can dramatically reduce system bandwidth. This can be easily predicted recalling that retroactivity increases the effective Michaelis-Menten constant of the backward enzymatic reaction by the factor $(1+\lambda)$. Hence, the expression of the bandwidth b modifies to

$$b \approx \frac{k_2 Y_{TOT}}{K_2 X_{TOT}(1+\lambda)},$$

showing a decrease of bandwidth as the loading λ from downstream sites increases. For a detailed derivation for several operating regimes of the cycle, the reader is referred to [14]. Figure 2(c) shows the time response of the output $X^*(t)$ when the input $Z(t)$ is a periodic signal, such as coming from a biological oscillator. The black plot shows the output response of the isolated system (downstream targets p are absent). The red plot shows the output response when the system is connected to downstream targets p. This connection causes the response to be delayed in time and the amplitude of response is attenuated. That is, the system, when connected, has a reduced ability to transmit time-varying information. This is confirmed by Figure 2(d), which shows the frequency response of the module. Precisely, the upper plots of Figure 2(d) show the amplitude A of the output response $X^*(t)$ as a function of the frequency of the input stimulation. As expected, when the input frequency increases, the amplitude of response decreases as the system internal dynamics provide some "inertia" to follow the input. The connected system displays lower bandwidth than that of the isolated system. Hence, for a given frequency, for example for $\omega = 10^{-2}$, the connected system presents a substantially attenuated response compared to that of the isolated system (confirmed by the time response of panel (c)). The lower plots of Figure 2(d) show the phase lag ϕ of the output $X^*(t)$ with respect to the input $Z(t)$ for every frequency ω of the input stimulation. The connected system appears substantially delayed compared to the isolated system (confirmed by the time response of panel (c)). This is still a consequence of the decrease of bandwidth b due to retroactivity. Qualitatively, these effects are due to retroactivity slowing down the effective kinetic rates of the cycle. This is intuitive since the rate at which X* reacts with the converter enzyme Y will be smaller when the sites p are present because less X* will be available to react with Y. Interestingly, this finding has been validated experimentally in a reconstituted signal transduction system *in vitro* [14].

Hence, retroactivity provides one more degree of freedom to tune the bandwidth of a signaling module. It follows that the number of targets of signaling proteins is likely optimized to attain the desired bandwidth. Given the impact of retroactivity from downstream targets on bandwidth, signaling pathways and off-pathway targets may have evolved to optimize tradeoffs between bandwidth and zero-order ultrasensitivity as retroactivity decreases the first but, up to some limit, enables the second. If

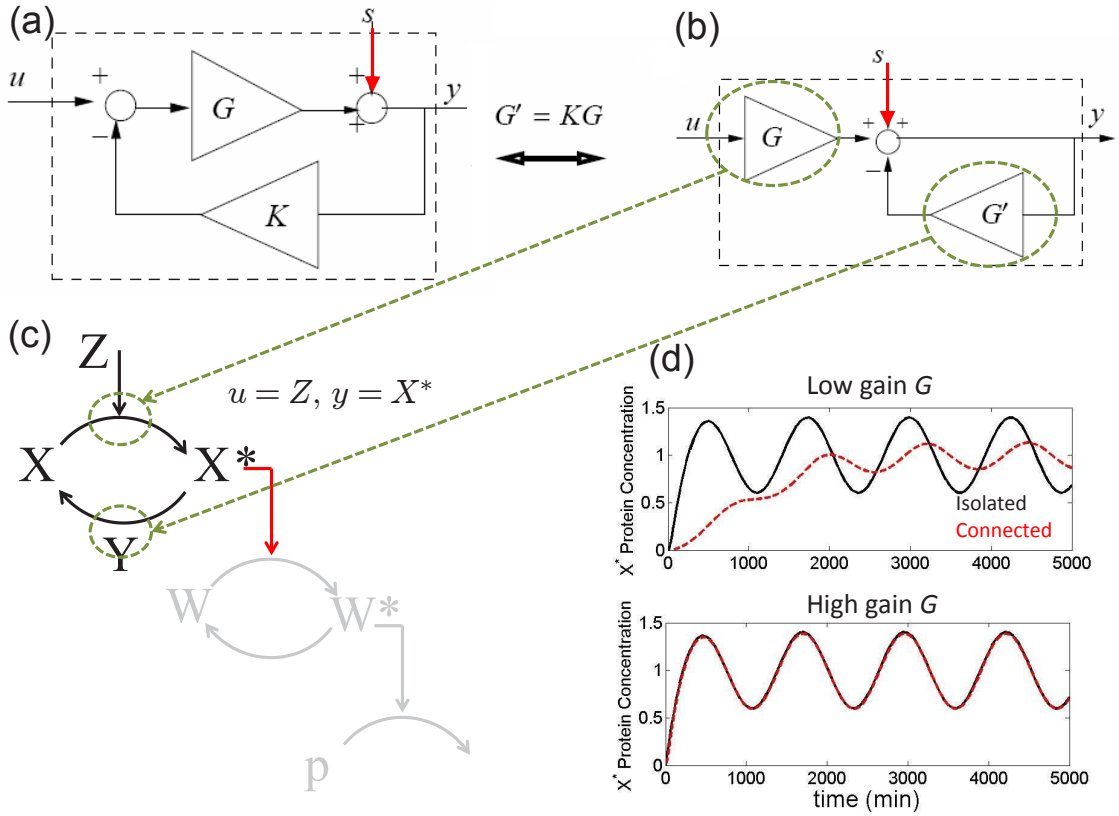

Figure 3. Robustness to retroactivity through high-gain feedback. (a) Basic block diagram of a high-gain feedback system. (b) Re-arranging the block diagram in a form that brings a clear analogy to how covalent modification cycles implement high gain feedback. (c) A covalent modification cycle implements high-gain feedback when the modification rates are very fast. The picture shows also other downstream stages in the signaling cascade. (d) High gains make the system output be unaffected by retroactivity from downstream targets.

signaling systems were modular, covalent modification cycles would have a pre-defined behavior independent on the way they are interconnected. That is, retroactivity at interconnections would be zero and the illustrated mechanisms to tune the behavior of a cycle would not be possible. This seems suboptimal as it would remove a powerful means to control the performance of a signaling system.

4. Enforcing modularity through insulation design

Even if in general retroactivity in signaling systems enables a powerful mechanism to tune system performance, modularity may still be required for other functions. In particular, if a signaling system is highly affected by retroactivity, it can, in principle, transmit some information backward, i.e., from downstream to upstream (through the red arrows of Figure 1(a)), opposite to the desired direction of signal propagation. This could be detrimental in a number of cases. For example, consider two signaling pathways

sharing a common component, such as a substrate. Because of retroactivity from the common substrate, the stages upstream of it in one pathway will receive some of the signal traveling in the other pathway. As a result, aberrant signaling can arise in the stages upstream of the common substrate with potentially serious consequences [11].

This suggests that natural signaling systems should have evolved the ability to enforce modularity when needed despite the presence of retroactivity. That is, a system may be subject to high retroactivity from its downstream clients, yet its output response should be only slightly affected. In this case, we say that the system is *robust* to retroactivity. Robustness to undesired input stimuli, also called disturbances, is one of the central properties that must be engineered in any human-made control system [6]. Control theory has been addressing this problem for several decades and a number of techniques have been devised.

The simplest way to engineer robustness to undesired stimuli, while keeping a sensitive response to selected inputs u, is *high-gain feedback* (Figure 3)(a)). Specifically, suppose that we would like to faithfully transmit the signal u to y, that is, we would like y to be a scaled version of u. The idea of high-gain feedback is to (i) calculate the error $e = u - Ky$ between an output measurement Ky and the desired output value u and (ii) to feedback such an error through a high gain G in the production of y itself. This way, if $e > 0$ ($u > Ky$) the value of y is increased while when $e < 0$ ($u < Ky$) the value of y is decreased. Ideally, this process should take y to reach the value u/K. Indeed, the block diagram of Figure 3(a) leads to the relation

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

which can be solved for y to obtain

$$y = \frac{Gu}{1 + GK} + \frac{s}{1 + GK},$$

so that when the feedback gain G is high, the contribution of s becomes negligible and we reach $y \approx u/K$, which is a scaled version of the input.

We argue here that this high-gain feedback mechanism is built in any covalent modification cycle. To illustrate this point, consider the block diagram of Figure 3(b), which is equivalent to that of Figure 3(a). According to this equivalent diagram, robustness to s is reached by applying a large input amplification G and a similarly large negative feedback on the output y. Referring to Figure 3(c), we consider, for simplicity, the one-step reaction model for the enzymatic reactions

$$\mathrm{X} + \mathrm{Z} \xrightarrow{k_1} \mathrm{X}^* + \mathrm{Z}, \;\; \mathrm{X}^* + \mathrm{Y} \xrightarrow{k_2} \mathrm{X} + \mathrm{Y}, \;\; X + X^* = X_{TOT},$$

in which X_{TOT} is the total amount of substrate. Then, the rate of change of X^* (the concentration of X*), assuming the cycle is weakly activated ($X^* \ll X_{TOT}$), is well approximated by

$$\frac{dX^*}{dt} = k_1 X_{TOT} Z(t) - k_2 Y X^* + s,$$

in which s is the binding/unbinding rate of X* with substrate W in the connected system and $s = 0$ in the isolated system. Since $Z = u$ and $X^* = y$, we can single out the input amplification gain as $G = k_1 X_{TOT}$ and the negative feedback gain on the output as $G' = k_2 Y$. As a consequence, one should expect that when G and G' are high the behavior of $X^*(t)$ should be minimally impacted by the presence of s in the connected system. Figure 3(d) confirms this finding. This reasoning, based on a very simple model of covalent modification, can be applied and extended to arbitrarily complex models of covalent modification, phosphotransfer, and double phosphorylations [13]. Indeed, the plots of Figure 3(d) were obtained from simulations on a mechanistic model of covalent modification in which enzyme reactions were modeled as two-step processes and complexes of enzymes and substrates were included. This principle for insulation from retroactivity was also experimentally verified on a reconstituted system *in vitro* [14]. We conclude that modularity can be enforced if the amounts of the cycle substrate and converter enzyme for the backward reaction are high enough.

How do natural signaling systems tradeoff between enforcing modularity and using retroactivity as an effective tuning parameter? A numerical study performed on mechanistic models of signaling cascades, such as the MAPK cascade, has provided some insight [22]. Specifically, Monte Carlo simulations were performed by sampling parameters from biologically meaningful intervals taken from the literature. The study revealed that a perturbation applied at the bottom of the cascade could propagate upstream but that the amplitude of the perturbation would be attenuated more after every stage. More precisely, refer to the cascade of Figure 3(c). A perturbation on p, called Δp, can propagate upstream through retroactivity to produce a perturbation $\Delta W^* < \Delta p$ on W*, which, in turn, can propagate upstream to result into a perturbation $\Delta X^* < \Delta W^*$ on X*. This fact suggests that signaling pathways may exploit the cascading of multiple stages to obtain a satisfactory robustness to downstream perturbations, while keeping sufficient plasticity to retroactivity at every stage so to allow the desired adjustments of bandwidth and sensitivity.

5. Implications and Outlook

The system concept with retroactivity (Figure 1(a)) places a clear analogy between signaling systems and engineered systems and provides a framework to quantitatively study modularity. Specifically, we have argued that a sound approach to address modularity is to view retroactivity as a "disturbance" in Figure 1(a) and study the robustness to this disturbance in a control theory sense.

According to our study, retroactivity by downstream clients shapes the input/output behavior of signaling modules, indicating that modularity does not hold in general in signaling networks, despite their apparent modular structure. By contrast, retroactivity is a powerful mechanism that allows a signaling module to tune its static and dynamic characteristics without changing the biochemical parameters internal to the module itself. In fact, retroactivity by downstream targets finely controls the sensitivity

and point of half-maximal induction of the dose response curve and the bandwidth, the most important dynamical parameter of a system. At the same time, signaling modules can operate in regimes where robustness to retroactivity is achieved. The system of Figure 1(a), in which retroactivity s is viewed as a disturbance, translates the question of modularity enforcement to a disturbance attenuation problem, which is widely studied in control theory and for which plenty of solutions exist. This revealed that covalent modification cycles have the built-in ability to attenuate retroactivity by working in regimes where the amounts of substrate and backward converter enzyme are sufficiently high. In summary, signal transduction networks implement a smart design tradeoff between allowing plasticity to retroactivity to improve performance and rejecting retroactivity when it brings undesired effects.

Retroactivity and insulation cover a crucial role also and especially in synthetic biomolecular circuits. The success of a bottom-up design approach to engineer biomolecular circuits is contingent upon predicting whether and how interconnection changes system behavior. The study of retroactivity and insulation within the presented control theoretic framework is particularly suited to address this question. This framework allows to both predict the effects of retroactivity on the input/output response of a system and to design insulation devices that can be placed between any two systems to buffer them from retroactivity effects.

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