

## Modules and Retroactivity: Theoretical Framework, Analysis, and Design

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In their influential paper [1], Hartwell et. al. argued for the recognition of functional “modules” as a critical level of biological organization, contending that modularity is a key feature that makes biology close to synthetic disciplines such as computer science and engineering. Lauffenberger [2] further elaborates that biology could be understood in a hierarchical or nested manner, analogous to engineering design, where components are studied first in isolation, tested and individually characterized, prior to their incorporation into larger systems.

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 A in isolation, it is certainly desirable if its characteristics do not change substantially when another component B 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. In [3], 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).”

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 in [4]. 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 one of the proteins A involved in its implementation. Typically, A will be an activator or a repressor of a gene involved in the network constituting a downstream system. From a systems/signals point of view,  $A(t)$  becomes an *input* to the second system. 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 itself.

The above considerations strongly motivate the need for a novel theoretical framework to formally define and quantify retroactivity effects. In this paper, we present such a formalism, illustrate it with engineering and biological examples, and study general approaches to the reduction of retroactivity by means of feedback. Our work complements, but is different from, questions of optimally partitioning large networks into “modules” for which

retroactivity-like effects are minimized and the identification of possible functional modules from co-expression and other data, which typically employ graph, information theoretic, and statistical approaches [5, 6, 7]. In contrast, our ultimate goal is not top-down partitioning, nor necessarily to ignore, or even to necessarily minimize, retroactivity, but to formally define and characterize these effects, thus making the problem amenable to theoretical analysis and concrete in vivo solutions, in the context of gene and protein networks.

One of our key theoretical contributions is *a new paradigm for input/output systems analysis* that allows us to characterize the equivalents of “impedance” for biochemical networks. The standard model, used in virtually every control and systems theory mathematical and engineering textbook since the 1950s, e.g. [8], is based on the view of devices described solely in terms of input channels, output channels, and state (internal, non-shared) variables. (A notable exception is found in the work of Willems.) However, in many natural physical and biological systems, the hypothesis of unidirectional signaling may be unrealistic. To address this shortcoming of the standard theory, we extend the basic setup, by considering components (“modules” or “subsystems”)  $\Sigma$ , described by equations as follows:  $\dot{x}=f(x, u, s)$ ,  $y=Y(x, u)$ ,  $r=R(x, u)$ . The new aspect compared to the standard paradigm is the introduction of the variables  $r=r(t)$  and  $s=s(t)$ , which we refer to respectively as the *retroactivities to the input* and *output*, in addition to the classical state, input, and output variables  $x, u, y$ .

The signal  $r$  specifies the manner in which the given system  $\Sigma$ , upon receiving an input  $u$  (which is typically the output  $y$  of an upstream component) imposes a load on the sender. Physically, this load might be a flux representing a drawn current or the temporary sequestration of a chemical species through binding. Conversely, the signal  $s$  specifies the back effect received from a downstream component  $\Sigma'$  to which the output signal  $y$  is sent, and coincides with  $r'$  upon interconnection. We make the convention that  $s(t) = 0$  if there is no downstream component connected to the output  $y$ . In general, the actual form of the signal  $s$  will depend on the specific component  $\Sigma'$  to be connected to the output  $y$ ; thus, in the absence of further information,  $s$  must be viewed as a potential *unknown disturbance* when designing  $\Sigma$ . In our formalism, achieving low output impedance becomes the control-theoretic problem of *disturbance attenuation*.

A very simple biological illustration of the formalism is as follows. Suppose that the concentration  $x(t)$  of protein X is the output of system  $\Sigma_1$ . If X is not involved in any other processes in  $\Sigma_1$ , then the rate of change of its concentration is given by the equation  $\dot{x} = k - \delta x$ , where  $k$  is the formation rate of X (which may be constant or time-varying). Suppose now that a second system  $\Sigma_2$  is “connected” to this output, using  $y_1 = x$  as its input. Let us say that X acts by reversibly binding to a protein Z in this second system. Modeling with ideal mass-action kinetics, the connection will introduce a flux  $-k_+xz + k_-c$  into the rate equation for  $x$ , where  $c$  is the concentration of the complex. This flux, which may or may not be present depending on whether  $\Sigma_2$  is connected, and which depends on the particulars of Z and C, is modeled as the retroactivity signal  $s_1$  in the first system. In other words, the equation for  $x$  is  $\dot{x} = k - \delta x + s_1$ . In the second system, there will be an equation  $\dot{z} = -k_+u_2z + k_-c$  for the concentration of Z (in addition to terms for production and decay as well as other possible interactions), where  $u_2$  denotes an input signal. The retroactivity to the input  $u_2$ , in this second system, is given by  $r_2 = R((z, c), u_2) = -k_+u_2z + k_-c$ . Upon interconnection,

$y_1 = u_2$ ,  $r_2 = s_1$ , and the full equation is obtained. The important point is that each system can be separately built and analyzed, with a performance goal that the behavior of  $\Sigma_1$  have low sensitivity to  $s$  and/or  $\Sigma_2$  produce a small  $r$ . An entirely similar discussion applies when  $X$  is a transcription factor that regulates the expression of a certain gene, and  $Z$  denotes the concentration of unbound (free) promoter for this gene.

**Summary of results.** We analyzed retroactivity to the output in the above simple protein binding example, using tools from singular perturbation theory. This led to the identification of a key retroactivity measure, which can be interpreted as the sensitivity of the quasi-steady state dynamics of the concentration of protein  $X$ , with respect to its dynamics if the downstream system were not present. As a corollary,  $s$  has a negligible influence on the dynamics of  $\Sigma_1$  if either (1)  $z_T \ll k_d = k_-/k_+$  or (2)  $x(t) \gg z_T$  for all  $t$ , if  $z_T$  (bound plus unbound concentration of  $Z$ ) and  $k_d$  are of comparable orders of magnitude. In general, we do not require the modification of physical features of downstream systems. We thus solve a disturbance attenuation problem with respect to  $s$ , suggesting a mechanism similar to that used to design non-inverting amplifiers employing OPAMPs. This mechanism employs a large (theoretically infinite) input gain and a similarly large negative feedback. We then propose and analyze two biological instances of this construction, for gene and protein networks. The first one involves a strong, non leaky promoter to implement a large input gain, combined with an abundant protease that degrades the protein product and hence implements a high-gain negative feedback. The second one involves a post-translational modification mechanism through a phosphorylation/dephosphorylation futile cycle, such as found in MAPK cascades. In both cases, estimates are given on rate constants that lead to low retroactivity, through the analysis of a high dimensional model using singular perturbation and control-theoretic techniques. Prototypes based on these ideas are being designed in Ninfa's lab.

## References

- [1] L.H. Hartwell, J.J. Hopfield, S. Leibler, and A.W. Murray. From molecular to modular cell biology. *Nature*, 402(6761 Suppl):47–52, Dec 1999.
- [2] D.A. Lauffenburger. Cell signaling pathways as control modules: complexity for simplicity? *Proc Natl Acad Sci U S A*, 97(10):5031–5033, May 2000.
- [3] U. Alon. *An introduction to systems biology. Design principles of biological circuits*. Chapman-Hall, Boca Raton, 2007.
- [4] M.R. Atkinson, M.A. Savageau, J.T. Meyers, and A.J. Ninfa. Development of genetic circuitry exhibiting toggle switch or oscillatory behavior in *Escherichia coli*. *Cell*, 113:597–607, 2003.
- [5] J.A. Papin, J.L. Reed, and B.O. Palsson. Hierarchical thinking in network biology: the unbiased modularization of biochemical networks. *Trends Biochem. Sci.*, 29:641–647, 2004.
- [6] Oliver Mason and Mark Verwoerd. Graph theory and networks in biology. Technical report, <http://arxiv.org/abs/q-bio.MN/0604006>, Apr 2006.
- [7] A. Kremling and J. Saez-Rodriguez. Systems biology - An engineering perspective. *Journal of Biotechnology*, 129:329–351, 2007.
- [8] E.D. Sontag. *Mathematical Control Theory*. Springer-Verlag, New York, 1998.