

# How Slaves Affect a Master Module in Gene Transcription Networks

Andras Gyorgy and Domitilla Del Vecchio

**Abstract**—One of the major challenges in systems and synthetic biology is the lack of modular composition. Modules change their behavior once connected, due to retroactivity. In this paper, we build upon our earlier results and provide a theorem establishing how the dynamics of a master module change once slave modules are present. We quantify the change in the dynamics of the master module due to interconnection as a function of measurable biochemical parameters. Based on this, we provide a bound on the difference between the trajectories of the connected system and those of the isolated system by employing contraction theory. Therefore, we obtain a measure of robustness, which helps evaluating the degree of modularity in a system, while providing guidelines for robust module design. We illustrate the results by considering a recurring motif in gene transcription networks: an autorepressed gene regulating the expression of several downstream targets.

## I. INTRODUCTION

Since many biological networks show modular organization [1], [2], they might be understood, just like engineering systems, in a modular fashion [3]. Unfortunately, modules often exhibit context-dependent behavior [4], so that the emergent behavior of a network cannot be predicted by simply considering the dynamics of the composing modules in isolation. This lacking ability poses a fundamental challenge in both systems biology and synthetic biology [5].

One cause of context-dependence is retroactivity, a phenomenon describing how a downstream module perturbs an upstream one due to interconnection [6], [7]. As result, for example, a two-gene network can be either stable or display oscillations depending on the addition of a downstream module [8]. Retroactivity has been experimentally demonstrated both in natural and in engineered systems *in vivo* and *in vitro*. For instance, it has been shown *in vivo* that retroactivity affects the response time of genes [9] and it can lead to unexpected interactions in the MAPK cascade [10]. Retroactivity has been shown experimentally to alter the behavior of *in vitro* transcriptional clocks [11] and to affect the dynamics of signal transduction networks [12].

In this paper, we build upon our earlier results [13] and give a more detailed description of complex gene transcription networks, providing insight regarding both the analysis and the design of large genetic networks. We introduce the internal and external retroactivity of a module, capturing how an isolated module responds to changes in the concentration of its internal and input transcription factors (TFs).

Furthermore, we define the scaling and mixing retroactivity of a module establishing how the dynamics of modules change once connected in a master-slave interconnection. The scaling retroactivity of the slave describes how the isolated dynamics of a master is “scaled”, whereas the mixing retroactivity of the slave captures the extent of the coupling between the dynamics of the slave and the master. We show that for obtaining a master module that is robust to interconnections, its internal retroactivity should be “large” compared to the scaling and mixing retroactivity of the slave. Finally, we provide bounds on the change in both the dynamics and the trajectory of the master once the slave is connected. Using contraction theory [14], we illustrate the trade-off between performance and robustness on a specific example: it is not possible to increase independently the speed of the master and its robustness to interconnections.

The concept of retroactivity connects with the notion of fan-out introduced in [15]. Our work is complementary to but different from those partitioning large transcription networks into modules by minimizing retroactivity ([16], [6] and [17]). Instead, our results connect with those of other disciplines’ of biochemical systems analysis, such as metabolic control analysis [18], [19] and metabolic supply and demand analysis [20]. However, whereas these methods are primarily interested in the steady state and near-equilibrium behavior of a system, we focus on the global nonlinear dynamics of biomolecular systems.

The paper is organized as follows: first, the system of interest is introduced in detail. In Section III, we define the key retroactivity matrices and we provide a theorem connecting the behavior of connected modules to their dynamics in isolation. Based on this, we quantify the effect of slave modules on a master in Section IV, with an application example in Section V where we investigate the trade-off between performance and robustness to interconnection. Finally, we conclude with discussion and present future research directions.

## II. SYSTEM MODEL

Transcription networks are the input/output interconnection of transcription components, which we call *nodes*. A node takes TFs as input forming complexes with promoter sites through reversible binding reactions to produce a single TF as output (detailed description of the reactions are provided in the Appendix). There is a directed edge from node  $x_j$  to  $x_i$  if  $x_j$  is a TF regulating the activity of the promoter controlling the expression of  $x_i$  [1], in which case  $x_j$  is a *parent* of  $x_i$ .

This work was supported by grant #FA9550-10-1-0242

A. Gyorgy is with the Department of Electrical Engineering and Computer Science, MIT, 77 Massachusetts Avenue, Cambridge, MA 02139, USA [gyorgy@mit.edu](mailto:gyorgy@mit.edu)

D. Del Vecchio is with the Department of Mechanical Engineering, MIT, 77 Massachusetts Avenue, Cambridge, MA 02139, USA [ddv@mit.edu](mailto:ddv@mit.edu)

We call a group of nodes a *module*, and the inputs of the module are parents not from the module. We call the nodes inside the module *internal TFs*, while inputs are considered *external TFs*. Let  $x$ ,  $u$  and  $c$  denote the concentration vectors of internal TFs, external TFs, and promoter complexes, respectively.

According to [21], we can write the dynamics of the module as

$$\begin{pmatrix} \dot{c} \\ \dot{x} \end{pmatrix} = Nv, \quad (1)$$

where  $N$  is the stoichiometry matrix and  $v$  is the reaction flux vector. The reactions are either protein production/decay or binding/unbinding reactions, therefore, we partition  $v$  into  $r^*$  and  $r$ , representing the reaction flux vectors corresponding to production/decay and binding/unbinding reactions, respectively (see the detailed system model in the Appendix). We assume that the DNA copy number is constant, therefore, we can rewrite (1) as

$$\begin{pmatrix} \dot{c} \\ \dot{x} \end{pmatrix} = \underbrace{\begin{bmatrix} 0 & L \\ K^* & K \end{bmatrix}}_N \underbrace{\begin{pmatrix} r^* \\ r \end{pmatrix}}_v,$$

where the upper left block matrix in  $N$  is the zero matrix as DNA is not produced/degraded. As a result, with  $g(x, c) := K^*r^*(x, c)$  we obtain

$$\begin{aligned} \dot{c} &= Lr(x, c, u), \\ \dot{x} &= g(x, c) + Kr(x, c, u), \end{aligned} \quad (2)$$

which we call the *isolated dynamics* of a module. Note that  $\dot{x} = g(x, c)$  describes the retroactivity-free dynamics of  $x$ , i.e., neglecting the retroactivity arising inside the module (captured by the term  $Kr$ ).

Now consider the case when the a module is inserted into a network, which we call the *context* of the module. Let  $x_c$  and  $c_c$  denote the concentration vectors of TFs and promoter complexes of the context, respectively. Furthermore, denote by  $r_c^*$  and  $r_c$  the reaction flux vectors corresponding to production/decay and binding/unbinding reactions in the context of the module, respectively. Then, the dynamics of the species in the module ( $c$  and  $x$ ) and in the context ( $c_c$  and  $x_c$ ) can be written as

$$\begin{pmatrix} \dot{c} \\ \dot{c}_c \\ \dot{x} \\ \dot{x}_c \end{pmatrix} = \begin{bmatrix} 0 & 0 & L & 0 \\ 0 & 0 & 0 & L_c \\ K^* & 0 & K & H_c \\ 0 & K_c^* & H & K_c \end{bmatrix} \begin{pmatrix} r^* \\ r_c^* \\ r \\ r_c \end{pmatrix}, \quad (3)$$

where the upper left block matrix is zero as DNA is assumed to be a conserved species. Furthermore, the off-diagonal matrices in the upper right and lower left blocks can be shown to be zero by the definition of  $r$ ,  $r_c$ ,  $r^*$  and  $r_c^*$ . Finally, the stoichiometry matrix  $H_c$  represents how internal TFs of the module participate in binding/unbinding reactions in its context: for instance,  $H_c = 0$  if internal TFs of the module do not regulate the expression of TFs in the context ( $H$  can be interpreted similarly). With  $s := H_c r_c$ , we obtain

$$\begin{aligned} \dot{c} &= Lr(x, c, u), \\ \dot{x} &= g(x, c, u) + Kr(x, c, u) + s, \end{aligned} \quad (4)$$

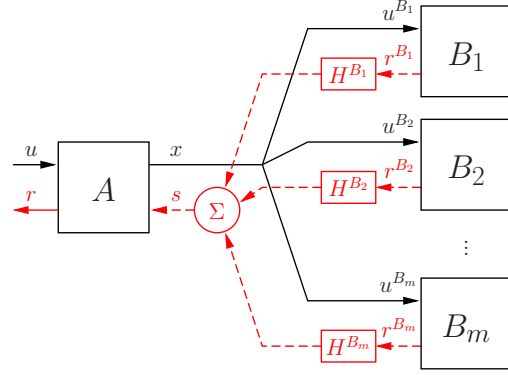


Fig. 1: Block diagram representation of interconnections explicitly accounting for retroactivity

which we call the *connected dynamics* of a module. We refer to  $s$  as the *retroactivity to the output* of the module, encompassing retroactivity arising from the context of the module. Similarly, we call  $r$  the *retroactivity to the input* of the module, representing retroactivity originating inside the module. The general interconnection of a group of modules (Fig. 1) can be treated similarly: the retroactivity to the input  $r^{B_i}$  of module  $B_i$  is multiplied by the stoichiometry matrix  $H^{B_i}$ , so that  $s = \sum_{i=1}^m H^{B_i} r^{B_i}$  yields the retroactivity to the output of the upstream module. If TFs of the upstream system do not take part in reactions of module  $B_i$ , module  $B_i$  does not contribute to  $s$  (represented by  $H^{B_i} = 0$ ).

### III. INTERCONNECTION OF MODULES

In order to understand how downstream modules affect upstream ones, we first focus on a single module and describe how internal connections affect the behavior of this module. Then, we describe how retroactivity between modules affect their dynamics, and finally, we consider the special case of master-slave hierarchy.

#### A. Internal and External Retroactivity

Consider node  $x_i$  and let  $p_i$  and  $c_i$  denote the concentration vectors of its parents and complexes formed with its promoter, respectively. Define the binary matrix  $\Psi_i$  as follows: the  $(j, k)$  element of  $\Psi_i$  is 1 if the  $k^{\text{th}}$  promoter complex has the  $j^{\text{th}}$  parent bound, otherwise the entry is zero. Consequently,  $b_i(c_i) := \Psi_i c_i$  represents the concentration vector of bound parents at node  $x_i$ , i.e., its  $j^{\text{th}}$  entry is the concentration of the  $j^{\text{th}}$  parent bound at node  $x_i$ . Parents can be either nodes in the module (internal TFs) or inputs to the module (external TFs). To represent the interconnection topology, we define the binary matrices  $V_i$  and  $D_i$  as follows. The  $(j, k)$  element of  $V_i$  (or  $D_i$ ) is 1 if the  $j^{\text{th}}$  parent is  $x_k$  (or  $u_k$ ), otherwise the entry is zero. For instance,  $V_i = 0$  if all the parents of  $x_i$  are external TFs, and similarly,  $D_i = 0$  if all parents are internal TFs. Finally, let  $\Phi$  denote the set of nodes having parents in the module, and similarly,  $\Omega$  denotes the set of nodes having parents outside the module (so that  $V_i = 0$  for  $x_i \notin \Phi$  and  $D_i = 0$  if  $x_i \notin \Omega$ ). With this, we

obtain

$$p_i(x, u) = [V_i \quad D_i] \begin{pmatrix} x \\ u \end{pmatrix}, \quad (5)$$

and it follows that

$$y(c) := \sum_{\{i | x_i \in \Phi\}} V_i' b_i(c) \quad (6)$$

represents the total concentration of bound internal TFs in the module ( $y_j$  is the concentration of bound  $x_j$ ).

Binding/unbinding reactions are much faster than protein production/decay [1], thus all protein decay rates are much smaller than all dissociation rate constants. As a result, we can define  $0 < \epsilon := \delta/\alpha \ll 1$  as a fraction of any nonzero protein decay rate  $\delta$  and dissociation rate constant  $\alpha$ .

**Theorem 1.** *Let  $(c(t), x(t))$  be the solution of the isolated module dynamics (2) for  $t \in [0, t_f]$  with initial condition  $(c_0, x_0)$  and with input  $u(t)$  continuous in  $t$ . Let  $y(c)$  be given by (6) and introduce  $z := x + y(c)$ . Let  $c = \tilde{\gamma}(z, u)$  be an isolated root of  $0 = Lr(z - y(c), c, u)$ , and define  $\hat{\gamma}(z, t) := \tilde{\gamma}(z, u)$ ,  $w := c - \hat{\gamma}(z, t)$ ,  $h(x, c, t, \epsilon) := \epsilon Lr(x, c, u)$ , and  $\tau := t/\epsilon$ . Assume that the origin is an exponentially stable equilibrium point of  $\frac{dw}{d\tau} = h(z - y(w + \hat{\gamma}(z, t)), w + \hat{\gamma}(z, t), t, \epsilon)$  uniformly in  $(z, t)$  and let  $\mathcal{C}$  be a compact subset of its region of attraction. Define  $\hat{r}(z, c, t) := r(z - y(c), c, u)$  and assume that  $\frac{\partial[\hat{L}\hat{r}(z, c, t)]}{\partial c}$  and  $\hat{\gamma}(z, t)$  have continuous first partial derivatives with respect to their arguments, moreover,  $\frac{\partial[\hat{L}\hat{r}(z, c, t)]}{\partial t}$  is also continuous. Let  $\bar{c} = \gamma(\bar{x}, u)$  be the locally unique solution of  $0 = Lr(\bar{x}, \bar{c}, u)$  and define  $\bar{y}(\bar{x}, u) := y(\gamma(\bar{x}, u))$ . Introduce  $R(\bar{x}, u) := \frac{\partial \bar{y}}{\partial \bar{x}}$ ,  $Q(\bar{x}, u) := \frac{\partial \bar{y}}{\partial u}$  and the dynamics*

$$\dot{\bar{x}} = (I + R)^{-1} [g(\bar{x}, \gamma(\bar{x}, u)) - Q\dot{u}] =: f(\bar{x}, u, \dot{u}). \quad (7)$$

Let  $\bar{x}(t)$  be the solution of (7) for  $t \in [0, t_f]$  with initial condition  $\bar{x}(0) = \bar{x}_0$  such that  $\bar{x}_0 + \bar{y}(\bar{x}_0, u(0)) = x_0 + y(c_0)$ . If  $c_0 \in \mathcal{C}$ , then there exist a constant  $\epsilon^* > 0$  such that for  $0 < \epsilon < \epsilon^*$  we have  $\|x(t) - \bar{x}(t)\|_2 = O(\epsilon)$  for  $t \in [0, t_f]$ .

*Proof:* Since we can write  $\dot{z} = g(x, c)$  with  $z = x + y(c)$  (Lemma 1 in the Appendix), we can transform (2) into standard singular perturbation form [22] with  $z_0 := x_0 + y(\gamma(x_0, u(0)))$ :

$$\dot{z} = g(z - y(c), c) \quad z(0) = z_0, \quad (8)$$

$$\epsilon \dot{c} = h(z - y(c), c, t, \epsilon) \quad c(0) = c_0. \quad (9)$$

To obtain the reduced order model, we set  $\epsilon = 0$  in (9). Since  $0 = h(\bar{z} - y(\bar{c}), \bar{c}, t, 0)$  implies  $0 = Lr(\bar{z} - y(\bar{c}), \bar{c}, u) = Lr(\bar{x}, \bar{c}, u)$ , we conclude that the slow manifold [22] is given by  $\bar{c} = \tilde{\gamma}(\bar{z}, u) = \gamma(\bar{x}, u)$ . Therefore, the reduced system becomes  $\dot{\bar{z}} = g(\bar{x}, \gamma(\bar{x}, u))$  with  $\bar{z}(0) = z_0$ . As  $\bar{z} = \bar{x} + \bar{y}$ , we have  $\dot{\bar{z}} = \dot{\bar{x}} + \frac{\partial \bar{y}}{\partial \bar{x}} \dot{\bar{x}} + \frac{\partial \bar{y}}{\partial u} \dot{u} = (I + R)\dot{\bar{x}} + Q\dot{u}$ , equivalent to (7) as  $\dot{\bar{z}} = g(\bar{x}, \gamma(\bar{x}, u))$ . Applying Theorem 11.1 in [22] concludes the proof. ■

According to Lemma 1 in the Appendix,  $\dot{z} = g(x, \gamma(x, u))$  where  $z = x + y$  represents the total concentration of internal TFs (free and bound). We call  $R$  the *internal retroactivity* of the module. When  $Q\dot{u} = 0$ , (7)

reduces to  $\dot{x} = (I + R)^{-1} \dot{z}$ . That is, the ‘‘greater’’  $R$ , the ‘‘harder’’ to change the concentration of free internal TFs by changing their total concentration. We refer to  $Q$  as the *external retroactivity* of the module. If the total concentration of internal TFs is constant ( $\dot{z} = 0$ ), then (7) reduces to  $\dot{x} = -(I + R)^{-1} Q\dot{u}$ , therefore,  $Q$  represents the fact that one can change the free concentration of internal TFs without changing their total concentration (by forcing internal TFs to bind/unbind as a result of changes in  $u$ ).

Recall that  $p_i$  and  $c_i$  denote the concentration vectors of parents of node  $x_i$  and complexes with the promoter of  $x_i$ , respectively. Introduce  $r_i$  as the reaction flux of binding/unbinding reactions at node  $x_i$ , so that  $\dot{c}_i = L_i r_i(p_i, c_i)$  where  $L_i$  is the corresponding stoichiometry matrix. Let  $\gamma_i(p_i)$  be defined such that  $0 = L_i(p_i \gamma_i(p_i))$  and define  $R_i(p_i) := \frac{db_i(\gamma_i(p_i))}{dp_i}$ , which we call the *retroactivity of node*  $x_i$  [13]. Combining the definition of  $R$  and  $Q$  with (6) and the fact that  $\frac{\partial p_i}{\partial x} = V_i$  and  $\frac{\partial p_i}{\partial u} = D_i$  by (5), we can write the internal retroactivity of a module as  $R = \sum_{\{i | x_i \in \Phi\}} V_i' R_i V_i$  using the chain rule. Similarly, the external retroactivity of a module becomes  $Q = \sum_{\{i | x_i \in (\Phi \cap \Omega)\}} V_i' R_i D_i$ .

### B. Scaling and Mixing Retroactivity

Take modules  $A$  and  $B$ , and for simplicity, assume that the modules have inputs only from each other, that is,  $B$  encompasses the context of  $A$  (see (3)), and vice versa (this assumption can be relaxed using the results from the previous subsection). Using the superscript to denote to which module a quantity belongs, the isolated dynamics of  $A$  given by (2) become  $\dot{c}^A = L^A r^A(x^A, c^A, u^A)$  and  $\dot{x}^A = g^A(x^A, c^A) + K^A r^A(x^A, c^A, u^A)$ , and similarly, the isolated dynamics of  $B$  are  $\dot{c}^B = L^B r^B(x^B, c^B, u^B)$  and  $\dot{x}^B = g^B(x^B, c^B) + K^B r^B(x^B, c^B, u^B)$ .

As the inputs of module  $B$  are nodes in module  $A$ , and vice versa, we need to establish the relationship between the inputs of  $B$  and the nodes of  $A$ . Therefore, we define the binary matrix  $T^B$  as follows: the  $(j, k)$  element of  $T^B$  is 1 if the  $j^{\text{th}}$  input of  $B$  is the  $k^{\text{th}}$  node in  $A$  ( $u_j^B = x_k^A$ ), otherwise the entry is zero. As a result, we have  $u^B = T^B x^A$  (we define the matrix  $T^A$  similarly for module  $A$ , resulting in  $u^A = T^A x^B$ ). Once we connect  $A$  with  $B$ , the behavior is captured by the connected dynamics (4) for  $A$ :

$$\begin{aligned} \dot{c}^A &= L^A r^A(x^A, c^A, T^A x^B), \\ \dot{x}^A &= g^A(x^A, c^A) + K^A r^A(x^A, c^A, T^A x^B) + s^A \end{aligned} \quad (10)$$

with  $s^A = H^B r^B(x^B, c^B, T^B x^A)$ , and similarly, for  $B$ :

$$\begin{aligned} \dot{c}^B &= L^B r^B(x^B, c^B, T^B x^A), \\ \dot{x}^B &= g^B(x^B, c^B) + K^B r^B(x^B, c^B, T^B x^A) + s^B \end{aligned} \quad (11)$$

with  $s^B = H^A r^A(x^A, c^A, T^A x^B)$ . In order to predict how the dynamics of  $A$  and  $B$  change upon interconnection, it is useful to treat the interconnected  $A$  and  $B$  modules as one module in isolation. To this end, define  $x := ((x^A)' \quad (x^B)')'$ ,  $c := ((c^A)' \quad (c^B)')'$ ,  $g(x, c) := ((g^A(x^A, c^A))' \quad (g^B(x^B, c^B))')'$ ,  $r(x, c, u) := ((r^A(x^A, c^A, T^A x^B))' \quad (r^B(x^B, c^B, T^B x^A))')'$ , and

since  $u$  is vacuous (modules  $A$  and  $B$  have no inputs but from each other), we write  $r(x, c)$ . Furthermore, let

$$L := \begin{bmatrix} L^A & 0 \\ 0 & L^B \end{bmatrix}, \quad K := \begin{bmatrix} K^A & H^B \\ H^A & K^B \end{bmatrix}. \quad (12)$$

Consequently, (10)–(11) can be rewritten as the isolated module dynamics (2) with (12). Furthermore, introduce

$$\begin{aligned} S^A(x) &:= \sum_{\{i | x_i^A \in \Omega^A\}} (D_i^A T^A)' R_i^A D_i^A T^A, \\ M^A(x) &:= \sum_{\{i | x_i^A \in (\Phi^A \cap \Omega^A)\}} (D_i^A T^A)' R_i^A V_i^A, \\ S^B(x) &:= \sum_{\{i | x_i^B \in \Omega^B\}} (D_i^B T^B)' R_i^B D_i^B T^B, \\ M^B(x) &:= \sum_{\{i | x_i^B \in (\Phi^B \cap \Omega^B)\}} (D_i^B T^B)' R_i^B V_i^B. \end{aligned} \quad (13)$$

Note that  $M^B = 0$  if  $\Phi^B \cap \Omega^B = \emptyset$ , that is, if nodes in  $B$  do not have parents from both modules. Moreover, considering the master-slave hierarchy where only  $B$  takes inputs from  $A$ , we also obtain  $M^A = 0$  and  $S^A = 0$  as  $\Omega^A = \emptyset$ . Finally, let  $R^A$  and  $R^B$  denote the internal retroactivity of  $A$  and  $B$ , respectively, and define

$$\Delta(x) := \begin{bmatrix} S^B & M^B \\ M^A & S^A \end{bmatrix}, \quad (14)$$

$$G(x) := \begin{bmatrix} (I + R^A)^{-1} & 0 \\ 0 & (I + R^B)^{-1} \end{bmatrix}, \quad (15)$$

together with the dynamics

$$\dot{\hat{x}} = (I + G\Delta)^{-1} \underbrace{\begin{pmatrix} f^A(\bar{x}^A, T^A \bar{x}^B, T^A \dot{\hat{x}}^B) \\ f^B(\bar{x}^B, T^B \bar{x}^A, T^B \dot{\hat{x}}^A) \end{pmatrix}}_{\text{isolated dynamics of A and B}}. \quad (16)$$

Pick any nonzero decay rate  $\delta$ , dissociation rate constant  $\alpha$ , and define the dimensionless parameter  $0 < \epsilon := \delta/\alpha \ll 1$  [1]. Then, we have the following result.

**Theorem 2.** *Let  $(c(t), x(t))$  be the solution of the isolated module dynamics (2) with (12) for  $A$  and  $B$  for  $t \in [0, t_f]$  with initial condition  $(c_0, x_0)$ . Let  $y(c)$  be given by (6) and introduce  $z := x + y(c)$ . Let  $c = \tilde{\gamma}(z)$  be an isolated root of  $0 = Lr(z - y(c), c)$ , and define  $w := c - \gamma(z)$ ,  $h(x, c, \epsilon) := \epsilon Lr(x, c)$ , and  $\tau := t/\epsilon$ . Assume that the origin is an exponentially stable equilibrium point of  $\frac{dw}{d\tau} = h(z - y(w + \gamma(z)), w + \gamma(z), \epsilon)$  uniformly in  $z$  and let  $\mathcal{C}$  be a compact subset of its region of attraction. Furthermore, define  $\hat{r}(z, c) := r(z - y(c), c)$  and assume that  $\frac{\partial[L\hat{r}(z, c)]}{\partial c}$  and  $\hat{\gamma}(z)$  have continuous first partial derivatives with respect to their arguments. Let  $\bar{c} = \gamma(\bar{x})$  be the locally unique solution of  $0 = Lr(\bar{x}, \bar{c})$  and define  $\bar{y}(\bar{x}) := y(\gamma(\bar{x}))$ . Let  $\bar{x}(t)$  be the solution of (16) for  $t \in [0, t_f]$  with initial condition  $\bar{x}_0$ , such that  $\bar{x}_0 + \bar{y}(\bar{x}_0) = x_0 + y(c_0)$ . If  $c_0 \in \mathcal{C}$ , then there exist a constant  $\epsilon^* > 0$  such that for  $0 < \epsilon < \epsilon^*$  we have  $\|x(t) - \bar{x}(t)\|_2 = O(\epsilon)$  for  $t \in [0, t_f]$ .*

*Proof:* Theorem 1 applied to the network (2) with (12) yields that the solution of

$$\dot{\bar{x}} = (I + R)^{-1} g(\bar{x}, \gamma(\bar{x})) \quad (17)$$

with  $\bar{x}(0) = \bar{x}_0$  satisfies the claim. Therefore, it is sufficient to show that (16) and (17) are equivalent. Let  $\bar{c}^A = \gamma^A(\bar{x}^A, u^A)$  and  $\bar{c}^B = \gamma^B(\bar{x}^B, u^B)$  denote the locally unique solution of  $0 = L^A r^A(\bar{x}^A, \bar{c}^A, u^A)$  and  $0 = L^B r^B(\bar{x}^B, \bar{c}^B, u^B)$ , respectively. Given the block diagonal structure of  $L$  in (12), we obtain  $\gamma(\bar{x}) = ((\gamma^A(\bar{x}^A, T^A \bar{x}^B))' (\gamma^B(\bar{x}^B, T^B \bar{x}^A))')'$ . Since  $g(x, c) = ((g^A(x^A, c^A))' (g^B(x^B, c^B))')'$ , we can substitute  $g^A(\bar{x}^A, \gamma^A(\bar{x}^A, u^A))$  and  $g^B(\bar{x}^B, \gamma^B(\bar{x}^B, u^B))$  from (7) with superscripts “A” and “B”, respectively, into (17). We can show that with  $Q^A$  and  $Q^B$  denoting the external retroactivity of  $A$  and  $B$ , respectively, by defining

$$\hat{Q} := \begin{bmatrix} 0 & Q^A T^A \\ Q^B T^B & 0 \end{bmatrix}, \quad (18)$$

the internal retroactivity  $R$  of the connected modules in (2) can be written as  $R = G^{-1} - I + \Delta + \hat{Q}$ . For details, see Lemma 2 in the Appendix. Substituting  $R$  and  $g(\bar{x}, \gamma(\bar{x}))$  into (17) yields (16). ■

Note that (16) reduces to  $\dot{\hat{x}}^A = f^A(\bar{x}^A, T^A \bar{x}^B, T^A \dot{\hat{x}}^B)$  and  $\dot{\hat{x}}^B = f^B(\bar{x}^B, T^B \bar{x}^A, T^B \dot{\hat{x}}^A)$  if  $\Delta = 0$ , that is, the modules behave as if they were in isolation if  $S^A = 0$ ,  $M^A = 0$ ,  $S^B = 0$  and  $M^B = 0$ .

Finally, consider the case when only module  $B$  (slave) takes inputs from module  $A$  (master), that is,  $u^A$  is vacuous. We wish to understand how module  $B$  affects the dynamics of module  $A$ . Since module  $A$  does not take inputs from module  $B$ , we have  $\Omega^A = \emptyset$ , thus  $S^A = 0$  and  $M^A = 0$ . As a result, with  $E := [I + (I + R^A)^{-1} S^B]^{-1}$  and  $F := (I + R^A + S^B)^{-1} M^B$ , (16) reduces to

$$\begin{aligned} \dot{x}^A &= E f^A(x^A, T^A x^B, T^A \dot{x}^B) - F f^B(x^B, T^B x^A, T^B \dot{x}^A), \\ \dot{x}^B &= f^B(x^B, T^B x^A, T^B \dot{x}^A), \end{aligned}$$

that is, the isolated dynamics of  $B$  appear in the dynamics of  $A$  through the matrix  $F$ . In the special case when  $M^B = 0$ , we have  $F = 0$ , so that the dynamics of  $A$  reduce to

$$\dot{x}^A = E f^A(x^A, T^A x^B, T^A \dot{x}^B), \quad (19)$$

that is, the connected dynamics of the master are the “scaled” version of its dynamics in isolation. Therefore, we call  $S^B$  the *scaling retroactivity* of the slave. Since the isolated dynamics of the slave enter through  $M^B$  in the dynamics of the master,  $M^B$  establishes how the dynamics of the master and the slave are “mixed”.

#### IV. QUANTIFYING THE EFFECT OF SLAVES ON THE MASTER

According to Theorem 1, the isolated dynamics of the master can be well approximated by its reduced order model  $\dot{x}^A = f^A(x^A, u^A, \dot{u}^A)$  defined in (7) with superscript “A”. As  $u^A$  is vacuous, we write  $f^A(x^A)$  instead

of  $f^A(x^A, u^A, \dot{u}^A)$  to simplify notation. Consequently, the dynamics of the master module in isolation are given by

$$\dot{x}^A = f^A(x^A). \quad (20)$$

We denote by  $R^A$  the internal retroactivity of the master, and since the master does not have inputs,  $R^A$  is only a function of  $x^A$ . Furthermore, assuming that there are no mixed parents in the slave ( $\Phi^B \cap \Omega^B = \emptyset$ ), we have that  $S^B$  given by (13) is also only a function of  $x^A$ , moreover,  $M^B = 0$ .

Once we connect the master module to the slave, the dynamics of the master change according to (19). Define  $G^A(x^A) := [I + R^A(x^A)]^{-1}$ , so that (19) yields

$$\dot{x}^A = (I + G^A S^B)^{-1} f^A(x^A). \quad (21)$$

We define the percentage difference in the dynamics of the master upon interconnection with the slave as

$$d(x^A) := \frac{\|(I + G^A S^B)^{-1} f^A(x^A) - f^A(x^A)\|_2}{\|f^A(x^A)\|_2}.$$

Using the sub-multiplicative property of the induced 2-norm, we have  $d(x^A) \leq \|[I + G^A(x^A)S^B(x^A)]^{-1} - I\|_2 =: \mu(x^A)$ , so that  $\mu(x^A)$  is an upper bound of the percentage change in the dynamics of the master once connected to the slave.

**Proposition 1.** *We can bound  $\mu$  from above as  $\mu \leq \hat{\mu}$  with  $\hat{\mu} := \|G^A\|_2 \|S^B\|_2 / (1 - \|G^A\|_2 \|S^B\|_2)$  if  $\|G^A\|_2 \|S^B\|_2 < 1$ .*

*Proof:* Consider the eigenvalue  $\lambda$  of  $G^A S^B$  with the corresponding eigenvector  $v$ , so that

$$|\lambda| \|v\|_2 = \|G^A S^B v\|_2 \leq \|G^A\|_2 \|S^B\|_2 \|v\|_2 < \|v\|_2,$$

yielding that  $|\lambda| < 1$ . Consequently, the spectral radius  $\rho$  of  $G^A S^B$  satisfies  $\rho < 1$ . Using the result on the convergence of geometric series of matrices in [23], we write  $(I + G^A S^B)^{-1} = I + \sum_{k=1}^{\infty} (-1)^k (G^A S^B)^k$ , so that  $\mu \leq \sum_{k=1}^{\infty} \|G^A S^B\|_2^k = \|G^A S^B\|_2 / (1 - \|G^A S^B\|_2)$ , and we conclude the proof by using  $\|G^A S^B\|_2 \leq \|G^A\|_2 \|S^B\|_2 < 1$ . ■

A system  $\dot{x} = f(x, t)$  is called contracting [14] with respect to the metric transformation  $\Theta(x, t)$  if (i)  $\Theta' \Theta$  is uniformly positive definite and (ii) the symmetric part of the generalized Jacobian

$$J(x, t) := \left( \dot{\Theta} + \Theta \frac{\partial f}{\partial x} \right) \Theta^{-1} \quad (22)$$

is uniformly negative definite. The absolute value of the largest eigenvalue of the symmetric part of  $J$  is called the system's contraction rate with respect to  $\Theta$ .

**Theorem 3.** *Let  $x^A(t)$  and  $\hat{x}^A(t)$  denote the solution of (20) and (21), respectively, with identical initial conditions. Assume that (20) is contracting with rate  $\lambda > 0$  and metric transformation  $\Theta$ . Denote by  $\chi$  the condition number of  $\Theta$ . Assume that  $\|f^A(x)\|_2 \leq \hat{f}$  and  $\mu(x) \leq \hat{\mu}$  for  $x \in \mathcal{D}$ . If  $x^A(t), \hat{x}^A(t) \in \mathcal{D}$  for  $t \in [0, \infty)$ , then we have  $\|x^A(t) - \hat{x}^A(t)\|_2 \leq \hat{\mu} \hat{f} \chi / \lambda$  for  $t \in [0, \infty)$ .*

*Proof:* Rewrite (21) as

$$\dot{x}^A = f^A(x^A) + [(I + G^A S^B)^{-1} - I] f^A(x^A), \quad (23)$$

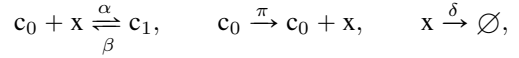
in which, using the sub-multiplicative property of the induced 2-norm,  $\|[I + G^A S^B]^{-1} - I\|_2 \leq \hat{\mu} \hat{f}$  for  $x^A \in \mathcal{D}$ . Since  $x^A \in \mathcal{D}$  for all  $t$ , we apply Lemma 1 in [24] with (20) as the nominal system and (23) as the perturbed system. ■

According to Proposition 1,  $\mu$  decreases with  $\|G^A\|_2 \|S^B\|_2$  (more precisely, the upper bound of  $\mu$  decreases), making the master more robust to interconnection. Denote by  $\sigma_{\min}(F)$  and  $\sigma_{\max}(F)$  the smallest and largest singular value of  $F$ , respectively, and note that  $\|F\|_2 = \sigma_{\max}(F)$  and  $\|F^{-1}\|_2 = \sigma_{\min}^{-1}(F)$  from Lemma 2.12 in [25]. As a result, we can make the master more robust to interconnection (decrease the upper bound of the change in the master's behavior) by increasing  $\sigma_{\min}(I + R^A)$  or by decreasing  $\sigma_{\max}(S^B)$ .

## V. APPLICATION EXAMPLE

Consider the case when the master module is an autorepressed gene, that is, it inhibits its own production [1], and the slave module consists of  $n$  nodes regulated by the TF of the master, which is a frequently recurring motif in transcription networks. Here, we seek to understand how the slaves affect the master's robustness to interconnection and performance. In particular, we investigate how the response time of the master changes upon interconnection.

The reactions governing the behavior of the isolated master are



such that  $\eta = c_0 + c_1$  denotes the total concentration of the promoter in the master module with dissociation constant  $K_d := \beta/\alpha$ . The dynamics of the isolated master are given

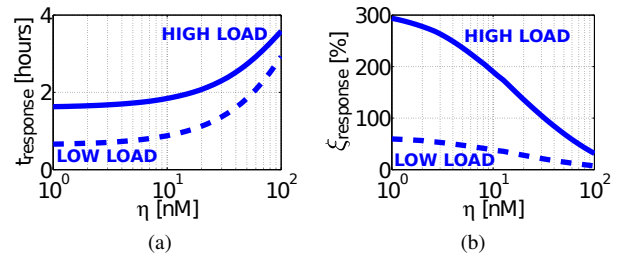


Fig. 2: (a) The response time  $t_{\text{response}}$  of the master increases with its internal retroactivity (which is proportional to  $\eta$ ). Furthermore, the greater the load (the higher the copy number  $\eta_1$  of the slave), the slower the response. (b) The percentage change  $\xi_{\text{response}}$  in the response time of the master upon interconnection with the slave decreases with its internal retroactivity. Furthermore, the greater the load, the greater this percentage change. Simulation parameters:  $\delta = 1 \text{ hr}^{-1}$ ,  $K_d = 10 \text{ nM}$  and  $\pi$  is chosen such that it yields  $x_{ss} = 10 \text{ nM}$ ,  $K_{d,1} = 10 \text{ nM}$ ,  $\eta_1 = 50 \text{ nM}$  (solid, HIGH LOAD) and  $\eta_1 = 10 \text{ nM}$  (dashed, LOW LOAD).

by

$$\dot{x} = \frac{1}{1 + R^A} \left( -\delta x + \pi \frac{\eta K_d}{x + K_d} \right) =: f(x) \quad (24)$$

according to (7), in which the internal retroactivity of the master is given by  $R^A = \eta K_d (x + K_d)^{-2}$  [13].

Upon interconnection with the slave, the dynamics of the master change due to the reactions

$$c_{i,0} + x \xrightleftharpoons[\beta_i]{\alpha_i} c_{i,1}, \quad \text{for } i = 1, 2, \dots, n.$$

Let  $\eta_i = c_{i,0} + c_{i,1}$  denote the total concentration of the promoter of slave nodes, and define  $K_{d,i} := \beta_i/\alpha_i$ , the dissociation constant of  $x$  and the promoter of the  $i^{\text{th}}$  slave node ( $i = 1, 2, \dots, n$ ). According to (16), the dynamics of the master once connected to the slave become

$$\dot{x} = \frac{1 + R^A}{1 + R^A + S^B} f(x), \quad (25)$$

where the scaling retroactivity  $S^B$  of the slave can be shown to be  $S^B = \sum_{i=1}^n \eta_i K_{d,i} (x + K_{d,i})^{-2}$  by (13). In order to apply Theorem 3 to the isolated system (24) and the connected system (25), we need to find  $\mathcal{D}$ ,  $\hat{f}$ ,  $\hat{\mu}$  and the contraction rate  $\lambda > 0$  (here, we provide a sufficient condition for contraction).

Since  $R^A > 0$  for  $x \geq 0$ , (24) has a unique positive steady state  $x_{ss}$ . Given that  $f(x) > 0$  for  $0 \leq x < x_{ss}$  and  $f(x) < 0$  for  $x > x_{ss}$ , we conclude that  $x_{ss}$  is stable. Consider a trajectory  $x(t)$  with initial condition  $x_0 \geq 0$  and define  $m := \max(x_0, x_{ss})$ . The set  $\mathcal{D} := [0, m]$  is positively invariant, as it can be easily verified. We can use this to provide a bound on  $f(x)$  and on  $\mu(x)$  for  $x \in \mathcal{D}$ : in particular, with  $\hat{f} := \delta m + \pi \eta$  we obtain  $|f(x)| \leq \hat{f}$  for all  $x \in \mathcal{D}$ . Furthermore, given that  $\mu = \|(I + G^A S^B)^{-1} - I\|_2 = S^B/(1 + R^A + S^B)$ , we have  $\mu(x) \leq \hat{\mu}$  for all  $x \in \mathcal{D}$  with  $\hat{\mu} := \sum_{i=1}^n \frac{\eta_i}{K_{d,i}} \left( 1 + \frac{\eta_i K_{d,i}}{(K_{d,i} + m)^2} + \sum_{i=1}^n \frac{\eta_i K_{d,i}}{(K_{d,i} + m)^2} \right)^{-1}$ .

Finally, choosing the metric transformation  $\Theta = I$ , the generalized Jacobian in (22) reduces to  $J = \frac{df}{dx}$ , so that we obtain  $J \leq [-\delta + \pi(R^A)^2](1 + R^A)^{-2}$ . The master is contracting with rate  $\lambda > 0$  if  $J \leq -\lambda < 0$ , which is satisfied if  $\delta$  is large enough (e.g., if  $\delta \geq \pi(\eta/K_d)^2 + \lambda(1 + \eta/K_d)^2$ ). Furthermore, we have that  $\lambda \leq \frac{\delta - \pi(R^A)^2}{(1 + R^A)^2}$ , which means that the bound on  $\lambda$  decreases with  $R^A$ . The trajectories of a contracting system converge exponentially to each other [14]: the greater  $\lambda$ , the faster the convergence. The greater  $R^A$ , the smaller (the upper bound of)  $\lambda$ , thus the slower the master. The change in the response time of the master once a single slave is added can be seen in Fig. 2a. As expected by Proposition 1, as we increase  $R^A$  or decrease  $S^B$ , the master becomes more robust to interconnection with the slave since it is less affected by the slave, as it can be appreciated in Fig. 2b. That is, a trade-off between performance and robustness appears: increasing the internal retroactivity of the master makes it more robust to interconnection at the expense of slower response.

## VI. DISCUSSION AND FUTURE WORK

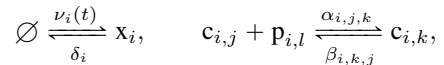
In this paper, we applied singular perturbation theory and contraction analysis to quantify how slave modules affect a master in complex gene transcription networks.

We built upon our earlier results and introduced four key quantities ( $R$ ,  $Q$ ,  $S$  and  $M$ ) establishing a module's dynamic properties in the presence of interconnections. These characteristic quantities can be computed by considering the retroactivity  $R_i$  of each node and the network structure encoded by the binary matrices  $V_i$ ,  $D_i$  and  $T$ . We also provided a formula linking the behavior of connected modules to their dynamics in isolation. As a consequence, it becomes possible to quantify the effect of slave modules on a master. First, we provided an upper bound  $\mu$  on the percentage change in the dynamics of the master once connected to the slave. We showed that this bound decreases by increasing the internal retroactivity of the master, or alternatively, by decreasing the scaling retroactivity of the slave. Second, we presented an upper bound on the difference in the trajectories of the master once connected to the slave using contraction theory. As a result, we were able to demonstrate the trade-off between performance and robustness to interconnection when the master module is an autorepressed gene: increasing the internal retroactivity of the master makes it more robust to interconnection at the expense of slower response.

The objective of this paper was to understand how modules affect each others' dynamics upon interconnection. Consequently, we chose a standard model of transcription networks that allowed us to focus on the effect of interconnections without the details of biochemical reactions obscuring them. Although this framework captures most of the relevant processes in transcription networks, we plan on extending our approach by including mRNA dynamics and multimerization.

## VII. APPENDIX

**Detailed system model.** Here, we present the reactions governing the dynamics of a transcription network considered in this paper. We introduce the alias  $p_{i,l}$  for the parents of node  $x_i$ . Parents of node  $x_i$  regulate the production of TF  $x_i$  by binding to its promoter forming promoter complexes  $c_{i,j}$ . The dynamics of the network are governed by transcription, translation and degradation processes. The reactions we consider for node  $x_i$  are



which represent the following physical phenomena. We denote by  $\nu_i(t)$  the production rate that may be due to external inputs (inducer, noise or disturbance), and let  $\delta_i$  denote protein decay. Furthermore, we consider the reversible binding reaction of parent  $p_{i,l}$  with complex  $c_{i,j}$  with the association and dissociation rate constants  $\alpha_{i,j,k}$  and  $\beta_{i,k,j}$ , respectively. Furthermore, we model gene expression as a one-step reaction process. Specifically, each promoter complex  $c_{i,j}$  will contribute to the production of  $x_i$  through a production rate constant  $\pi_{i,j}$ , modeled as

$c_{i,j} \xrightarrow{\pi_{i,j}} c_{i,j} + x_i$ . Therefore, the reaction flux vector  $r$  is partitioned such that  $\nu_i$ ,  $\delta_i x_i$  and  $\pi_{i,j} c_{i,j}$  belong to  $r^*$  (production/decay), whereas  $\alpha_{i,j,k} c_{i,j} p_{i,l}$  and  $\beta_{i,k,j} c_{i,k}$  are in  $r$  (binding/unbinding). Finally, we assume that the total concentration of promoter, denoted by  $\eta_i$ , for each node is conserved, so that  $\eta_i = \sum_{j=0}^{|C_i|} c_{i,j}$ , where  $|C_i|$  denotes the number of promoter complexes at node  $x_i$ .

**Lemma 1.** *With  $z = x + y(c)$  we obtain  $\dot{z} = g(x, c)$ .*

*Proof:* Without loss of generality, we can assume that the nodes are labeled such that the first  $|\Phi|$  are the ones having parents in the module (if not, we relabel them). Note that  $c_{i,j}$  is not involved in reactions at nodes other than  $x_i$ , consequently,  $L$  in (2) is block diagonal with blocks  $L_i$ , and we partition  $r$  accordingly:

$$L = \begin{bmatrix} L_1 & 0 & \dots & 0 \\ 0 & L_2 & \dots & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & \dots & L_{|\Phi|} \end{bmatrix}, \quad r = \begin{pmatrix} r_1 \\ r_2 \\ \vdots \\ r_{|\Phi|} \end{pmatrix},$$

yielding  $\dot{c}_i = L_i r_i$  by (2), where  $c_i$  denotes the concentration vector of promoter complexes at node  $x_i$ . Since  $b_i = \Psi_i c_i$ , by (6) we have  $\dot{y} = \sum_{i=1}^{|\Phi|} V_i' \Psi_i L_i r_i$ . Define  $N_i := V_i' \Psi_i$  and note that  $[N_i]_{j,k} = 1$  if  $c_{i,k}$  has  $x_j$  bound, otherwise  $[N_i]_{j,k} = 0$ . Let  $F_i := N_i L_i$  and  $F := [F_1 \ F_2 \ \dots \ F_{|\Phi|}]$ , so that we obtain  $\dot{y} = F r$ .

We now show that  $[K]_{m,n} = -[F_1]_{m,n}$ . To do so, consider  $[F_1]_{m,n} = \sum_{h=1}^{|C_1|} [N_1]_{m,h} [L_1]_{h,n}$ . The entries in  $r_1$  correspond to the reaction rates of the reactions of the form  $c_{1,j} + p_{1,l} \xrightleftharpoons[\alpha_{1,k,j}]{\alpha_{1,j,k}} c_{1,k}$ , so that  $[r_1]_n = \alpha_{1,j,k} c_{1,j} p_{1,l}$  or  $[r_1]_n = \alpha_{1,j,k} c_{1,k}$ , furthermore, we either have  $p_{1,l} = x_m$  or  $p_{1,l} \neq x_m$  (4 cases in total). Note that there are exactly two nonzero entries in each column of  $L_i$ . If  $[r_1]_n = \alpha_{1,j,k} c_{1,j} p_{1,l}$  and  $p_{1,l} = x_m$  then  $[K]_{m,n} = -1$ . In this case we have  $[L_1]_{j,n} = -1$ ,  $[L_1]_{k,n} = 1$ ,  $[N_1]_{m,j} = 0$  and  $[N_1]_{m,k} = 1$ , thus  $[F_1]_{m,n} = 1 = -[K]_{m,n}$ . The other three cases can be handled similarly, all yielding  $[F_1]_{m,n} = 1 = -[K]_{m,n}$ . Therefore, the columns of  $K$  multiplying  $r_1$  are the columns of  $H_1$ . We can similarly show that the columns of  $K$  multiplying  $r_i$  are the columns of  $F_i$ : to do so, just reorder the nodes so that  $x_i$  becomes the first. This implies that  $K = -F$ , yielding  $\dot{y} = -K r$ , and together with (2) it results in  $\dot{z} = g(x, c)$ . ■

**Lemma 2.** *Consider  $\Delta$  and  $G$  defined in (14)–(15), and let*

$$\hat{Q} := \begin{bmatrix} 0 & Q^A T^A \\ Q^B T^B & 0 \end{bmatrix}. \quad (26)$$

*Then, the internal retroactivity  $R$  of the connected modules in (2) with (12) can be written as  $R = G^{-1} - I + \Delta + \hat{Q}$ .*

*Proof:* Let  $X^A$  and  $X^B$  denote the set of nodes in  $A$  and  $B$ , respectively. Given that  $x_i^A = x_i$  for  $i \leq |X^A|$  and  $x_j^B = x_{|X^A|+j}$  for  $j \leq |X^B|$  in the connected modules  $A$  and  $B$  given by (2) with (12), we can write  $V_i = [V_i^A \ D_i^A T^A]$  if  $x_i \in X^A$ , and  $V_{|X^A|+j} =$

$[D_j^B T^B \ V_j^B]$  if  $x_j \in X^B$ . Since  $\Phi = \Phi^A \cup \Omega^A \cup \Phi^B \cup \Omega^B$ , the internal retroactivity  $R$  of the network given by (2) with (12) can be written with  $\mathcal{S}_1 := \{i \mid x_i^A \in (\Phi^A \cup \Omega^A)\}$  and  $\mathcal{S}_2 := \{j \mid x_j^B \in (\Phi^B \cup \Omega^B)\}$  as  $R = \sum_{i \in \mathcal{S}_1} V_i' R_i^A V_i + \sum_{j \in \mathcal{S}_2} V_{|X^A|+j}' R_j^B V_{|X^A|+j}$ . Substituting  $V_i$  and  $V_{|X^A|+j}$  yields the sought expression. ■

## REFERENCES

- [1] U. Alon, *An Introduction to Systems Biology: Design Principles of Biological Circuits*, CRC Press; 2007.
- [2] L.H. Hartwell, J.J. Hopfield, S. Leibler and A.W. Murray, "From Molecular to Modular Cell Biology", *Nature*, vol. 402, 1999, pp. 47–52.
- [3] D.A. Lauffenburger, "Cell Signaling Pathways as Control Modules: Complexity for Simplicity?", *PNAS*, vol. 97, no. 10, 2000, pp. 5031–5033.
- [4] S. Cardinale and A.P. Arkin, "Contextualizing Context for Synthetic Biology – Identifying Causes of Failure of Synthetic Biological Systems", *Biotechnology Journal*, vol. 7, no. 7, 2012, pp. 856–866.
- [5] P.E.M. Purnick and R. Weiss, "The Second Wave of Synthetic Biology: from Modules to Systems", *Nature Reviews Molecular Cell Biology*, vol. 10, no. 6, 2009, pp. 410–422.
- [6] J. Saez-Rodriguez, A. Kremling and E.D. Gilles, "Dissecting the Puzzle of Life: Modularization of Signal Transduction Networks", *Computers and Chemical Engineering*, vol. 29, 2005, pp. 619–629.
- [7] D. Del Vecchio, A.J. Ninfa and E.D. Sontag, "Modular Cell Biology: Retroactivity and Insulation", *Molecular Systems Biology*, vol. 4, 2008, no. 161.
- [8] S. Jayanthi and D. Del Vecchio, "Tuning Genetic Clocks Employing DNA Binding Sites", *PLoS One*, vol. 7, no. 7, 2012.
- [9] S. Jayanthi, K.S. Nilgiriwala and D. Del Vecchio, "Retroactivity Controls the Temporal Dynamics of Gene Transcription", *ACS Synthetic Biology*, vol. 2, no. 8, 2013, pp. 431–441.
- [10] Y. Kim, Z. Paroush, K. Nairz, E. Hafen, G. Jimenez and S.Y. Shvartsman, "Substrate-Dependent Control of MAPK Phosphorylation *in vivo*", *Molecular Systems Biology*, vol. 7, no. 467, 2011.
- [11] E. Franco, E. Friedrichs, J. Kim, R. Jungmann, R. Murray, E. Winfree and F.C. Simmel, "Timing Molecular Motion and Production with a Synthetic Transcriptional Clock", *PNAS*, vol. 108, no. 40, 2011, E787.
- [12] P. Jiang, A. C. Ventura, S. D. Merajver, E. D. Sontag, A. J. Ninfa and D. Del Vecchio, "Load-induced Modulation of Signal Transduction Networks", *Science Signaling*, vol. 4(194), 2011, ra. 67.
- [13] A. Gyorgy and D. Del Vecchio, "Retroactivity to the Input for Complex Gene Transcription Networks", in *Proc. IEEE Conf. on Decision and Control*, 2012.
- [14] W. Lohmiller and J.-J.E. Slotine, "On Contraction Analysis for Non-Linear Systems", *Automatica*, vol. 34, issue 6, 1998, pp. 683–696.
- [15] K.H. Kim and H.M. Sauro, "Fan-Out in Gene Regulatory Networks", *Journal of Biological Engineering*, vol. 4, 2010.
- [16] B. Snel, P. Bork and M.A. Huynen, "The Identification of Functional Modules from the Genomic Association of Genes", *Proc Natl Acad Sci USA*, vol. 99, 2002, pp. 5890–5895.
- [17] E. Andrianantoandro, S. Basu, D.K. Karig and R. Weiss, "Synthetic Biology: New Engineering Rules for an Emerging Discipline", *Molecular Systems Biology*, vol. 2, 2006, pp. 1–14.
- [18] D. Fell, "Metabolic Control Analysis: a Survey of its Theoretical and Experimental Development", *Biochemical Journal*, vol. 286, 1992, pp. 313–330.
- [19] R. Heinrich and S. Schuster, "The Regulation of Cellular Systems", *Chapman & Hall*; 1996.
- [20] S.J. Hoymeyr and A. Cornish-Bowden, "Regulating the Cellular Economy of Supply and Demand", *FEBS Letters*, vol. 476, 2000, pp. 47–51.
- [21] E. Klipp, W. Liebermeister, C. Wierling, A. Kowald, H. Lehrach and R. Herwig, *Systems Biology: A Textbook*, John Wiley & Sons; 2009.
- [22] H.K. Khalil, *Nonlinear Systems*, Prentice Hall; 2002.
- [23] G. Strang, *Introduction to Linear Algebra*, SIAM; 2003.
- [24] D. Del Vecchio and J.-J.E. Slotine, "A Contraction Theory Approach to Singularly Perturbed Systems", *IEEE Transactions on Automatic Control*, vol. 58, no. 3, 2012, pp. 752–757.
- [25] K. Zhou, J.C. Doyle and K. Glover, *Robust and Optimal Control*, Prentice Hall, Englewood Cliffs, New Jersey; 1996.