Abstract—Synthetic biology is a bottom-up engineering discipline: modules are systematically designed with predefined behavior and then combined to build up larger biomolecular circuits. Although the modules produce the desired behavior in isolation, they fail to operate properly when they are connected due to retroactivity, an effect which extends the notion of impedance to biomolecular systems. Despite playing a central role, retroactivity is not yet characterized in complex gene transcription networks. In this paper, we mathematically describe and quantify this effect. This result is obtained by applying singular perturbation on the finite time interval. We identify the biomolecular counterpart of impedance and introduce the effective retroactivity to the input of a gene. Furthermore, we provide a theorem similar to Thevenin’s, describing how modules affect each other when connected. We illustrate the implications of the results by investigating crosstalk in a simple genetic system.

I. INTRODUCTION

Modularity is a central concept in every engineering discipline. In lack of it, building large, complex systems by composing smaller, simple pieces together is infeasible. Digital electronics prominently illustrates this: with functional modules such as clocks, memories and arithmetic units one can design large systems by connecting these basic building blocks. What makes this approach powerful is the fact that modules behave the same as if they were in isolation.

In case of analog electronics, modules typically affect each other. However, the behavior of connected modules can be easily described by considering their models in isolation. The fundamental theorem by Thevenin makes it possible to substitute electrical circuits between any two terminals by an equivalent circuit consisting of the series connection of a single voltage source and impedance. When connecting modules, one can consider the equivalent models describing the modules’ behavior in isolation. This result heavily relies on the fact that the impedance of an electrical component remains unchanged when connected to other components.

Synthetic biology is closely related to analog electronics. The basic building blocks are transcription components producing a single transcription factor (TF) as output and taking a few TFs as input. Instead of wires, transcription components are connected via binding reactions: input TFs bind to the promoter region and as a result of transcription and translation processes the output TF is produced. Given the close relationship between synthetic biology and analog electronics, it is natural that an impedance-like effect is observable when connecting biomolecular components together: this effect is called retroactivity [1]. A key feature of retroactivity is that it enables a downstream system to affect the behavior of the upstream one [2], [3]. In spite of its central role, retroactivity is not yet defined for complex gene transcription networks.

Therefore, in this paper we investigate retroactivity in gene transcription networks with arbitrary topology. We define the effective retroactivity to the input of a transcription component and we argue that it can be interpreted as the analog of impedance. We introduce the internal retroactivity of a module capturing the effect of intramodular connections. This is followed by our main result: a theorem for complex gene transcription networks analogous to Thevenin’s describing how the dynamics of modules change upon interconnection. We introduce the effective retroactivity to the input of a module, a quantity similar to input impedance. We show how the dynamics of the interconnected modules can be determined considering their dynamics in isolation, their internal retroactivity and their retroactivity to the input. For the most common binding types (independent, cooperative and competitive) we provide the explicit expression of the effective retroactivity to the input of a transcription component. This means that having a transcription network where the binding reactions are of these basic types, one can compute the internal and input retroactivity of a module just as easily as in case of electrical circuits. In order to show the power of the framework, we investigate crosstalk between modules.

Our work is complementary to those partitioning large transcription networks into modules by minimizing retroactivity among them, see [4], [5], [6] and [7]. Our approach differs from these as we analytically characterize and quantify retroactivity using singular perturbation, exploiting the fact that binding and unbinding reactions occur on a much faster time scale than protein production and decay [8]. Our approach is based on the tools of dynamical systems analysis, hence it connects with other disciplines of biochemical systems analysis, such as metabolic control analysis [9], [10] and metabolic supply and demand analysis [11]. However, whereas these methods are primarily interested in the steady state and near-equilibrium behavior of a system, we focus on the dynamics of modules and biomolecular circuits.

Retroactivity to the Input and Thevenin’s Theorem for Complex Gene Transcription Networks

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Therefore, in this paper we investigate retroactivity in gene transcription networks with arbitrary topology. We define the effective retroactivity to the input of a transcription component and we argue that it can be interpreted as the analog of impedance. We introduce the internal retroactivity of a module capturing the effect of intramodular connections. This is followed by our main result: a theorem for complex gene transcription networks analogous to Thevenin’s describing how the dynamics of modules change upon interconnection. We introduce the effective retroactivity to the input of a module, a quantity similar to input impedance. We show how the dynamics of the interconnected modules can be determined considering their dynamics in isolation, their internal retroactivity and their retroactivity to the input. For the most common binding types (independent, cooperative and competitive) we provide the explicit expression of the effective retroactivity to the input of a transcription component. This means that having a transcription network where the binding reactions are of these basic types, one can compute the internal and input retroactivity of a module just as easily as in case of electrical circuits. In order to show the power of the framework, we investigate crosstalk between modules.

Our work is complementary to those partitioning large transcription networks into modules by minimizing retroactivity among them, see [4], [5], [6] and [7]. Our approach differs from these as we analytically characterize and quantify retroactivity using singular perturbation, exploiting the fact that binding and unbinding reactions occur on a much faster time scale than protein production and decay [8]. Our approach is based on the tools of dynamical systems analysis, hence it connects with other disciplines of biochemical systems analysis, such as metabolic control analysis [9], [10] and metabolic supply and demand analysis [11]. However, whereas these methods are primarily interested in the steady state and near-equilibrium behavior of a system, we focus on the dynamics of modules and biomolecular circuits.
The paper is organized as follows: first, the system of interest is introduced in detail. In Section III, we define the retroactivity to the input of a transcription component, the internal retroactivity and the effective retroactivity to the input of a module, furthermore, we present a theorem analog to Thevinin’s for complex gene transcription networks. The results are illustrated in Section IV: we analyze crosstalk between modules. Finally, we conclude with discussion and present future research directions.

II. SYSTEM MODEL

We view a gene transcription network as a hierarchical structure with three levels: nodes represent transcription components, a group of connected nodes forms a module, whereas a system consists of several modules. Similar to [12], we believe that modules are a critical level of biological organization, and understanding the extent of modularity in biological systems is a key step required to move closer to engineering disciplines. Throughout the paper, species are denoted by capital letters, whereas the corresponding small letter stands for their concentration, e.g., the concentration of free TF is denoted by \( x_p \). The concentration of complexes at node \( p \) and define \( A_p \) as the concentration vector of complexes at node \( p \) except for the free promoter, i.e.,

\[
A_p = [c_{p,1} \ c_{p,2} \ \ldots \ c_{p,T_p}]^T.
\]

The reactions we consider for node \( p \) are as follows. We denote by \( v_p \) and \( \delta_p \) the external input and protein decay, respectively, that is, \( \varphi = \frac{v_p}{\delta_p}X_p \). Reversible binding reactions are characterized by rate constants \( a_{p,i,j} \) and \( a_{p,j,i} \) such that \( c_{p,i} + Z_{p,k} \frac{a_{p,i,j}}{a_{p,j,i}} C_{p,j} \) for \( Z_{p,k} \in Z_p \). Finally, we denote by \( \pi_{p,i} \) the rate constants describing protein production: \( C_{p,i} \frac{a_{p,i}}{a_{p,j}} C_{p,i} + X_p \). Moreover, we assume that the total concentration of promoter \( \eta_p \) is conserved:

\[
\eta_p = \sum_{i=0}^{T_p} c_{p,i}. \tag{1}
\]

Define the set \( \Omega_p \), as follows: \( \{i,j,k\} \in \Omega_p \) if TF \( Z_{p,k} \) can bind to complex \( C_{p,i} \) forming complex \( C_{p,j} \), i.e.,

\[
C_{p,i} + Z_{p,k} \frac{a_{p,i,j}}{a_{p,j,i}} C_{p,j} \quad \text{with} \quad Z_{p,k} \in Z_p.
\]

For instance, consider node \( p \) having two parents \( Z_{p,1} \) and \( Z_{p,2} \) binding cooperatively, that is, first \( Z_{p,1} \) has to bind to the free promoter \( C_{p,0} \) forming complex \( C_{p,1} \) and only after that can \( Z_{p,2} \) bind resulting in complex \( C_{p,2} \). In this case \( \Omega_p = \{(0,1,1),(1,2,2)\} \).

Considering the reactions for node \( p \), we have

\[
\dot{c}_{p,i} = \frac{g_{p,i}(z_p,c_p,c_0)}{v_p} \quad \text{for} \quad i = 0,1,\ldots,T_p
\]

with

\[
g_{p,i}(z_p,c_p,c_0) \triangleq \sum_{\{j,k\}\in \Omega_p} \left( a_{p,j,i} c_p z_{p,k} - a_{p,j,i} c_{p,i} \right)
- \sum_{\{j,k\}\in \Omega_p} \left( a_{p,i,j} c_i z_{p,k} - a_{p,i,j} c_{p,i} \right).
\]

Conservation law (1) implies \( \sum_{i=0}^{T_p} c_{p,i} = \eta_p \). Substituting it back to \( g_{p,i}(z_p,c_p,c_0) \), we obtain

\[
g_{p,i}(z_p,c_p) \quad \text{for} \quad i = 0,1,\ldots,T_p.
\]

In addition, it follows from (1) that \( \sum_{i=0}^{T_p} \dot{c}_{p,i} = 0 \). Consequently, we can disregard one of the equations \( \dot{c}_{p,i} = g_{p,i}(z_p,c_p) \), for instance the one standing for the free promoter \( i = 0 \). Introducing \( g(z_p,c_p) \)

\[
g(z_p,c_p) \triangleq [g_{p,1}(z_p,c_p) \ g_{p,2}(z_p,c_p) \ \ldots \ g_{p,T_p}(z_p,c_p)]^T
\]

the evolution of complexes of node \( p \) is described by

\[
\dot{c}_p = g(z_p,c_p). \quad \tag{2}
\]

Let \( \gamma_p(z_p) \) be the solution of

\[
\begin{bmatrix}
\gamma_{p,1}(z_p) \\
\gamma_{p,2}(z_p) \\
\vdots \\
\gamma_{p,T_p}(z_p)
\end{bmatrix} = \left[ \gamma_{p,1}(z_p) \ \gamma_{p,2}(z_p) \ \ldots \ \gamma_{p,T_p}(z_p) \right]^T
\]

of the linear system \( \dot{c}_p = g(z_p,c_p) \). By (1) we can define \( y_p = \gamma_p(z_p) \) as the concentration of complexes at node \( p \) when \( \dot{c}_p = 0 \).
Definition 1. The isolated dynamics of node $p$ are defined as $\dot{x}_p = \varphi_p(x_p, z_p)$ with

$$\varphi_p(x_p, z_p) \triangleq v_p - \delta_p x_p + \sum_{i=0}^{x_p} \pi_p \cdot \gamma_p \cdot (z_{\alpha^p})_{i}.$$ \hspace{1cm} (3)

As for the physical interpretation of (3), $\dot{x}_p = \varphi_p(x_p, z_p)$ describes the dynamics of $x_p$ as if $X_p$ was not an input to any node, that is, if node $p$ was in isolation.

Assume now that $X_p$ is taken as input to other nodes. Having a total of $n$ nodes, define $c \triangleq \begin{bmatrix} c_1^T & c_2^T & \cdots & c_n^T \end{bmatrix}^T$, the concentration vector of all complexes (except for the free promoters). Denote by $b_p(c)$ the concentration of bound $X_p$, or equivalently, the concentration of complexes having $X_p$ bound:

$$b_p(c) \triangleq \sum_{q=1}^{n} \sum_{i(j,i,k)\in \Omega_q} \sum_{c_{q, i}} e_{q, i}.$$ \hspace{1cm} (4)

Note that $b_p(c)$ represents the rate of change of bound $X_p$. Consequently, the rate of change of free $X_p$ due to binding reactions is $-b_p(c)$. Combining this with the reactions considered in the system, we obtain $\dot{x}_p = f_p(x_p, c)$ with

$$f_p(x_p, c) \triangleq v_p - \delta_p x_p + \sum_{i=0}^{x_p} \pi_p \cdot c_p \cdot (z_{\alpha^p})_{i} - b_p(c).$$ \hspace{1cm} (5)

Definition 2. The connected dynamics of node $p$ are defined as $\dot{x}_p = f_p(x_p, c)$ with $f_p(x_p, c)$ given by (5), where the evolution of complexes is described by (2).

B. Second Hierarchical Level: Modules

Modules are considered to be a group of connected nodes with some functionality (including the case with only one node).

Definition 3. Module $M$ is defined as $M = \{X^M, C^M, U^M, P^M\}$ where

- $X^M = \{X_1^M, X_2^M, \ldots, X_n^M\}$ is the set of TFs in $M$;
- $C^M = \{C_1^M, C_2^M, \ldots, C_n^M\}$ is the set of complexes in $M$, where $C_p^M = \{C_{p,0}^M, C_{p,1}^M, \ldots, C_{p,n_p^M}^M\}$ is the set of complexes formed at node $p$ in $M$;
- $U^M = \{U_1^M, U_2^M, \ldots, U_{n_p^M}^M\}$ is the set of inputs to $M$ where $U_j$ is a TF from a module different from $M$;
- $P^M = \{p_1^M, p_2^M, \ldots, p_{n_p^M}^M\}$ is the set of parameters describing the reactions governing the behavior of $M$, where $P_p^M$ is the set of parameters associated with node $p$ in $M$.

Since a module is an ensemble of connected nodes, the ODE model of a module is simply $\dot{x}_M = f_p(M, x^M, c^M)$ and $\dot{c}_M = b_p(M, z_p, c^M)$ for $p = 1, 2, \ldots, n_p^M$ by (5) and (2), where vector $c_p^M$ is the stack of vectors $c_p^M$ for $p = 1, 2, \ldots, n_p^M$, that is, the concentration vector of all complexes in $M$ (except for the free promoters). Furthermore, introduce $x^M \triangleq [x_1^M, x_2^M, \ldots, x_{n_p^M}]^T$ and

$$f^M(x^M, c^M) \triangleq \begin{bmatrix} f_1^M(x_1^M, c_1^M) \\ f_2^M(x_2^M, c_2^M) \\ \vdots \\ f_n^M(x_{n_p^M}, c_{n_p^M}) \end{bmatrix},$$ \hspace{1cm} (6)

$$g^M(x^M, c^M, u^M) \triangleq \begin{bmatrix} g_1^M(z_1^M, c_1^M) \\ g_2^M(z_2^M, c_2^M) \\ \vdots \\ g_n^M(z_{n_p^M}, c_{n_p^M}) \end{bmatrix}. \hspace{1cm} (7)

Note that in (7), the argument on the right hand side is $z_p^M$ and $c_p^M$ for $p = 1, 2, \ldots, n_p^M$, whereas on the left hand side it is $x^M$, $u^M$, and $c^M$. This is because $u^M_{p=1} = z_{p=1}^M = X^M \cup U^M$, i.e., parents in $M$ are either nodes in $M$ or inputs to $M$.

Definition 4. The isolated dynamics of module $M$ are defined as $\dot{x}_M = f^M(x^M, c^M)$ and $\dot{c}_M = g^M(x^M, c^M, u^M)$ with (6)–(7).

The above definition describes the case when TFs of $M$ are not taken as input to any other module, that is, module $M$ is in isolation.

Example 1. Consider the system in Fig. 2, which will serve as a running example. Focus now on $X_1^N$. Given that $X_1^N$ has two parents, we choose $Z_{1,1}^N = X_1^M$ and $Z_{1,2}^N = X_1^N$. There are four complexes associated with node 1 in $N$: the free promoter at this node is denoted by $C_{1,0}^N$, whereas $C_{1,1}^N$ and $C_{1,2}^N$ stand for the complexes of promoter with $X_1^M$ and $X_1^N$, respectively. Finally, $C_{1,3}^N$ denotes the complex of promoter with both TFs bound.

Considering the three basic binding patterns:

(i) independent binding: the binding of $X_1^M$ is independent of the binding of $X_1^N$, i.e., $a_{1,1,0} = a_{1,2,0}$, $a_{1,1,0} = a_{1,3,0}$, $a_{1,2,0} = a_{1,3,0}$, and $a_{1,2,0} = a_{1,3,0}$;

(ii) cooperative binding: $X_1^N$ can only bind after $X_1^M$, i.e., $a_{1,2,0} = a_{1,3,0} = a_{1,3,0}$ = 0;

(iii) competitive binding: $X_1^M$ and $X_1^N$ can not be both bound, i.e., $a_{1,1,0} = a_{1,2,0} = a_{1,3,0} = 0$.

---

**Fig. 2:** Module $M$ consists of a single node with no inputs, whereas module $N$ comprises of two nodes with one input ($U_1^N = X_1^M$). Their interconnection forms module $MN$ with no input and three nodes such that $X_1^{MN} = X_1^M$, $X_2^{MN} = X_1^N$, and $X_3^{MN} = X_2^N$. sidenote: (1) $x^M = [x_1^M, x_2^M, \ldots, x_n^M]$, $\varphi_p = \gamma_p \cdot (X_p)_{i}$, and $\delta_p = \sum_{i=0}^{x_p} \pi_p \cdot \gamma_p \cdot (X_p)_{i}$, (2) $\varphi = \gamma \cdot (X)_{i}$, and $\delta = \sum_{i=0}^{X} \pi \cdot \gamma \cdot (X)_{i}$.
C. Third Hierarchical Level: Systems

Definition 5. We say modules \( M \) and \( N \) are *composable* if \( X^M \cap X^N = \emptyset \), that is, the modules do not share nodes.

Definition 6. The *interconnection* of composable modules \( M \) and \( N \) is module \( MN = (X^{MN}, C^{MN}, U^{MN}, p^{MN}) \) such that \( X^{MN} = X^M \cup X^N \), \( C^{MN} = C^M \cup C^N \), \( U^{MN} = U^M \cup U^N \), \( p^{MN} = p^M \cup p^N \), and for all \( i, j \) such that \( i_{p^M} = x^N_j \) set \( u^i = x^N_j \), and similarly, if \( i_{p^N} = x^M_j \) set \( u^i = x^M_j \).

III. Effect of Interconnections

Our first question relates to connecting nodes: what is the relation between the isolated and connected dynamics of a node? The second question focuses on connecting modules: how do the dynamics of composable modules change upon interconnection?

A. Effective Retroactivity to the Input of a Node

Define the parent matrix of node \( p \) in \( M \) as \( \Psi(p)_k = \begin{bmatrix} \Psi^M(p) & I_p \end{bmatrix} \) where \( \Psi^M(p)_{k,j} = 1 \) if there exists \( i \) such that \((i, j) \in \Omega^p \) otherwise it is zero. Since \( \Psi^M(p)_{k,j} = 1 \) means that complex \( C_{p,j}^M \) has TF \( Z^M_{p,k} \) bound, we can calculate the concentration of bound parents at each node by defining

\[
u^M_p(c^M_p) = \Psi^M(p)^{c^M_p},
\]

\[
u^M_p(c^M_p) = \left[ u^M_p(1) \right].
\]

Note that \( u^M_p(c^M_p) \) denotes the total concentration of complexes at node \( p \) in \( M \) having TF \( Z^M_{p,k} \) bound, or equivalently, the total concentration of bound \( Z^M_{p,k} \) at node \( p \) in \( M \) \((p=1, \ldots, n^M \text{ and } k=1, \ldots, c_{p}^M) \).

Example 2. Continuing Example 1, since \( C_{2}^M \) and \( C_{1}^N \) have \( Z^1_1 \) bound, whereas \( C_{1}^M \) and \( C_{1}^N \) have \( Z^1_2 \) bound, we have \( \Psi^N_1 = \begin{bmatrix} 1 & 0 & 1 \\ 0 & 0 & 1 \end{bmatrix} \).

Denote by \( \Phi^M \) the set of nodes in \( M \) having parents, that is, \( \Phi^M = \{ p | Z^M_p \neq \emptyset \} \).

Definition 7. Define the *effective retroactivity* to the input of node \( p \) in module \( M \) as

\[
R^M_p(z^M_p) = \frac{\partial u^M_p(c^M_p)}{\partial z^M_p} \mid_{c^M_p = \gamma^M_p(z^M_p)} p \in \Phi^M.
\]

In other words, \( R^M_p(z^M_p) \) denotes the sensitivity of the total concentration of bound parents to the concentration of free of parents at node \( p \) in \( M \) when \( c^M_p = \gamma^M_p(z^M_p) \). Furthermore, by (8)–(9) we obtain

\[
R^M_p(z^M_p) = \Psi^M(p)^{\gamma^M_p(z^M_p)}.
\]

Since \( \gamma^M_p(z^M_p) \) only depends on parameters of node \( p \) in \( M \), \( R^M_p(z^M_p) \) also depends only on the parameters of node \( p \) by (10). Therefore, \( R^M_p(z^M_p) \) is the property of the node and it is independent of network topology, that is, it does not change upon interconnection.

Example 3. Consider the system in Fig. 2. Defining dissociation constants \( k_1 = \frac{a_{1,1}^N}{a_{1,0}^N} \) and \( k_2 = \frac{a_{2,1}^N}{a_{2,0}^N} \), one can calculate the effective retroactivities to the input of nodes in \( M \) and \( N \) using (10) and obtain:

\[
R^M_1(z^M_1) = \eta^M_1 \frac{1}{\left(1 + \frac{z^M_1}{k_1^M}\right)^2}, \quad R^N_2(z^N_2) = \eta^N_2 \frac{1}{\left(1 + \frac{z^N_2}{k_2^N}\right)^2},
\]

and considering the regulation patterns in Example 1:

(i) independent binding with \( k_M = \frac{a_{1,1}^M}{a_{1,0,1}} = \frac{a_{1,2}^N}{a_{1,0,2}} \) and \( k_N = \frac{a_{1,1}^N}{a_{1,0,1}} \)

\[
R^M_1(z^M_1) = \left[ \begin{array}{c} \frac{\eta^M_1}{k_1^M} \left(1 + \frac{z^M_1}{k_1^M}\right)^{-2} \\ 0 \end{array} \right], \quad R^N_2(z^N_2) = \left[ \begin{array}{c} \frac{\eta^N_2}{k_2^N} \left(1 + \frac{z^N_2}{k_2^N}\right)^{-2} \\ 0 \end{array} \right];
\]

(ii) cooperative binding with \( k_M = \frac{a_{1,1}^M}{a_{1,0,1}} \) and \( k_N = \frac{a_{1,1}^N}{a_{1,0,1}} \) and \( r = \frac{\eta^M_1}{k_1^M} \)

\[
R^M_1(z^M_1) = \left[ \begin{array}{c} \frac{1}{k_1^M} \left(1 + \frac{z^M_1}{k_1^M}\right)^{-2} \\ \frac{1}{k_1^M} \frac{z^M_1}{k_1^M} \end{array} \right], \quad R^N_2(z^N_2) = \left[ \begin{array}{c} \frac{1}{k_2^N} \left(1 + \frac{z^N_2}{k_2^N}\right)^{-2} \\ \frac{1}{k_2^N} \frac{z^N_2}{k_2^N} \end{array} \right];
\]

(iii) competitive binding with \( k_M = \frac{a_{1,1}^M}{a_{1,0,1}} \) and \( k_N = \frac{a_{1,1}^N}{a_{1,0,1}} \) and \( r = \frac{\eta^M_1}{k_1^M} \)

\[
R^M_1(z^M_1) = \left[ \begin{array}{c} \frac{1}{k_1^M} \left(1 + \frac{z^M_1}{k_1^M}\right)^{-2} \\ -\frac{1}{k_1^M} \frac{z^M_1}{k_1^M} \end{array} \right], \quad R^N_2(z^N_2) = \left[ \begin{array}{c} \frac{1}{k_2^N} \left(1 + \frac{z^N_2}{k_2^N}\right)^{-2} \\ -\frac{1}{k_2^N} \frac{z^N_2}{k_2^N} \end{array} \right].
\]

Making later computations simpler, let us write

\[
R^M_1(z^M_1) = a \quad R^M_1(z^N_1) = \left[ \begin{array}{c} b \\ c \end{array} \right], \quad R^N_2(z^N_2) = f,
\]

where \( a, b, \ldots, f \) are implicitly defined in (11)–(14).

B. Effect of Intramodular Connections

Here, we show that the isolated dynamics of module \( M \) given in Definition 4 can be well approximated by considering only the isolated dynamics of nodes in \( M \) and the effective retroactivity to the input of node \( p \) for \( p \in \Phi^M \).

Considering modules \( M \) and \( N \), define transformation matrix \( T^M_{p,N} = \begin{bmatrix} T^M_{p,N} \end{bmatrix} \) such that \( T^M_{p,N} = 1 \) if \( X^N_i = Z^M_{p,j} \), otherwise it is zero. Therefore, \( T^M_{p,N} \) provides us with a mapping between parents of node \( p \) and nodes in \( N \).
Example 4. Considering Fig. 2, $T^M_{1,1} = 1$ and

$$
T^M_{1,MN} = \begin{bmatrix}
1 \\
0 \\
0
\end{bmatrix} \\
T^N_{1,N} = \begin{bmatrix}
0 & 1 \\
0 & 0
\end{bmatrix} \\
T^N_{1,1M} = \begin{bmatrix}
1 & 0 \\
0 & 0
\end{bmatrix}.
$$

Given modules $M$ and $N$ (not necessarily composable), the restriction of $R_p^M(z_p^M)$ to $N$ is defined by

$$R_p^M(z_p^M)|_N \triangleq T^M_{p,N} R_p^M(z_p^M) T^M_{p,N}^T. \tag{16}$$

Based on (9), every row and column in $R_p^M(z_p^M)$ correspond to a parent $Z^M_p \in Z^M_p$ of node $p$. Every parent $Z^M_p \in Z^M_p$ is either a node in $N$ or not. With the restriction operator we select the rows and columns of $R_p^M(z_p^M)$ corresponding to parents belonging to $N$, and rearrange them according to the order of nodes in $N$.

Example 5. Take $R^N_1(z^N_1)$ from Example 3 with (15). Its first row and column belong to $Z^N_1 = X^M_1 \in X^M$ (parent from $M$), whereas the second row and column are associated with $Z^N_{1,2} = X^N_2 \in X^N$ (parent from $N$).

Therefore, when considering the restriction of $R^N_1(z^N_1)$ to $M$, we select $b$. Since $X^M_1$ is the only node in $M$, we have $R^N_1(z^N_1)_{|M} = b$. In case of $R^N_1(z^N_1)_{|N}$, we select $e$ and since it belongs to parent $X^N_1$ which is the first node in $N$, we obtain $R^N_1(z^N_1)_{|N} = \begin{bmatrix} e & 0 & 0 \\ 0 & 0 & 0 \end{bmatrix}$. Similarly, when considering the restriction of $R^N_1(z^N_1)$ to $MN$, we select the whole matrix and rearrange its rows and columns according to the the order of nodes in $M$ and obtain

$$R^N_1(z^N_1)_{|MN} = \begin{bmatrix} b & c & 0 \\ d & e & 0 \\ 0 & 0 & 0 \end{bmatrix}. \tag{17}$$

Definition 8. The internal retroactivity of module $M$ is

$$R^M(x^M, u^M) \triangleq \begin{cases} \sum_{p \in M} R^M_p(z^M_p) & \text{if } \Phi^M \neq \emptyset \\ 0_{n^M \times n^M} & \text{otherwise} \end{cases}$$

where $\Phi^M$ is the set of nodes in $M$ having parents.

According to (8), the binding reactions are much faster than protein production and decay, specifically:

$$\alpha_{p,i,j} \gg v_{p,r,s,k} \quad \forall p, q, r, s, i, j, k. \tag{18}$$

Consequently, for $\delta^M_r \neq 0$ and $\alpha^M_{s,k,l} \neq 0$ we define the small parameter

$$\epsilon \triangleq \frac{\delta^M_r}{\alpha^M_{s,k,l}}. \tag{19}$$

Furthermore, consider module $M$ with isolated node dynamics $\dot{x}^M_p = \phi^M_p(x^M_p, z^M_p)$ (see (3)) and initial conditions $x^M_p(0)$ for $p = 1, 2, \ldots, n^M$. Denote by $\phi^M(x^M, u^M)$ the column vector containing $\phi^M_p(x^M_p, z^M_p)$ stacked on each other ($p = 1, 2, \ldots, n^M$). Moreover, define

$$\tilde{f}^M(x^M, u^M) \triangleq \left[ I_{n^M} + R^M(x^M, u^M) \right]^{-1} \phi^M(x^M, u^M). \tag{20}$$

Recall that $c^M_p = \gamma^M_p(z^M_p)$ when $\epsilon^M_p = 0$, and define $\gamma^M(x^M, u^M)$ as the column vector containing the vectors $\gamma^M_p(z^M_p)$ for $p = 1, 2, \ldots, n^M$. Furthermore, define $b^M(c^M) \triangleq b_1^M(c^M) b_2^M(c^M) \ldots b_n^M(c^M)^T$ where $b_n^M(c^M)$ is defined by (4), hence $b^M(c^M)$ is the concentration vector of TFs of $M$ bound in $M$.

Let $\tilde{x}^M(t)$ be the solution of $\dot{\tilde{x}}^M = \tilde{f}^M(\tilde{x}^M, u^M)$ with initial condition $\tilde{x}^M(0)$ being the solution of

$$0 = g^M(\tilde{x}^M, y^M(\tilde{x}^M, u^M)) \quad \text{at } t = 0 \text{ such that } \tilde{x}^M + b^M(y^M(\tilde{x}^M, u^M)) = x^M + b^M(c^M). \tag{21}$$

Theorem 1. Let $(x^M(t), c^M(t))$ be the solution of $\dot{x}^M = f^M(x^M, c^M)$ and $c^M = g^M(x^M, u^M)$ with (6)–(7) for $t \in [0, t_f]$ with initial conditions $(x^M(0), c^M(0))$. Let $\tilde{x}^M(t)$ be the solution of $\dot{\tilde{x}}^M = \tilde{f}^M(\tilde{x}^M, u^M)$ for $t \in [0, t_f]$ with initial condition $\tilde{x}^M(0)$ being the solution of (21). Then, there exist constants $\epsilon^*, t_0, T > 0$ such that for $0 < \epsilon < \epsilon^*$

$$||x^M(t) - \tilde{x}^M(t)||_2 = O(\epsilon) \quad t \in [t_0, T)$$

provided that the matrix $\frac{\partial g^M}{\partial c^M}(c^M = y^M(x^M, u^M))$ is Hurwitz.

Proof sketch: Define $\xi^M \triangleq x^M + b^M(c^M)$. Replace constants $\alpha^M_{p,i,j}$ in $g^M(c^M - b^M(c^M), c^M, u^M)$ with $\tilde{\alpha}^M_{p,i,j} \triangleq \frac{\partial g^M}{\partial c^M}(c^M = y^M(x^M, u^M))$, resulting in $\tilde{g}^M(c^M - b^M(c^M), c^M, u^M)$, where $\epsilon$ is the small parameter from (19). Consequently, (6)–(7) becomes

$$\dot{\epsilon}^M = f^M(c^M - b^M(c^M), c^M), \quad \epsilon \dot{c}^M = \tilde{g}^M(c^M - b^M(c^M), c^M, u^M), \tag{22}$$

which is in the standard singular perturbation form, where $\xi^M$ is the slow variable, whereas $\epsilon^M$ is the fast variable. By setting $\epsilon = 0$ in (23), we obtain the slow manifold [13] on which the dynamics of the system are governed by the slow variable dynamics. It can be shown that in this case $c^M = y^M(x^M, u^M)$ and $\dot{\epsilon}^M = \dot{\phi}^M(x^M, u^M)$. Furthermore, applying the chain rule to $\dot{\epsilon}^M = \dot{\phi}^M(x^M, u^M)$ results in

$$\dot{\tilde{x}}^M = \left[ I_{n^M} + R^M(x^M, u^M) \right]^{-1} \phi^M(x^M, u^M). \tag{20}$$

One can verify that $\frac{\partial b^M(c^M, u^M)}{\partial c^M}(c^M = y^M(x^M, u^M))$ describes the dynamics of (6)–(7) on the slow manifold. Since we assume that $\frac{\partial b^M(c^M, u^M)}{\partial c^M}(c^M = y^M(x^M, u^M))$ is Hurwitz, the slow manifold is locally exponentially stable, hence the dynamics restricted to the slow manifold are a good approximation [13], which completes the proof. □

Theorem 1 states that $\tilde{x}^M(t)$ well approximates $x^M(t)$ if $\epsilon \ll 1$, thus we refer to $\tilde{x}^M = \tilde{f}^M(x^M, u^M)$ with (20) as the reduced order model of module $M$ in isolation.

Looking at (20), $R^M(x^M, u^M)$ relates the dynamics of the connected and isolated nodes in $M$. In other words,
\( R^M(x^M, u^M) \) captures the retroactive effects due to intramodular binding reactions, hence the notion internal retroactivity.

Regarding that \( R^M_p(z^M_p) \) does not change upon interconnection, and that it captures the loading effect of being connected to downstream nodes, it can be interpreted as the biomolecular analog of impedance.

**Example 6.** The internal retroactivity of module \( N \) in Fig. 2 by (17) with (15) is given by \( R^N(x^N, u^N) = \begin{bmatrix} e + f & 0 \\ 0 & 0 \end{bmatrix} \), thus the reduced order model of \( N \) in isolation by (20) is

\[
\begin{bmatrix}
\hat{x}^N_1 \\
\hat{x}^N_2
\end{bmatrix} = \begin{bmatrix}
\frac{1}{1+e+f} & 0 \\
0 & 1
\end{bmatrix} \begin{bmatrix}
\phi^N_1(x^N_1, z^N) \\
\phi^N_1(x^N_2, z^N)
\end{bmatrix}.
\]

(24)

From (24) it follows that the behavior of \( x^N_2 \) remains unchanged when considering intramodular retroactivity (since \( X^N_2 \) is not an input to any node). Given the fact that \( X^N_1 \) is taken as input by both nodes in \( N \), its dynamics are changed due to intramodular connections; since \( e, f > 0 \) by (11)–(14) we obtain \( 0 < \frac{1}{1+e+f} < 1 \). This implies that once connected, the dynamics of \( x^N_1 \) slow down.

**C. Effect of Intermodular Connections**

Throughout this section, we consider composable modules \( M \) and \( N \), whereas their interconnection is denoted by \( MN \). For simplicity, assume that the ordering of nodes in \( MN \) is the following: first the nodes of \( M \), then the nodes of \( N \), that is, \( X^M_{iN} = X^M_i \) for \( i = 1, 2, \ldots, n^M \) and \( X^{X^M_{iN}}_{jN} = X^N_j \) for \( j = 1, 2, \ldots, n^N \).

The embedding of \( R^M_p(z^M_p) \) to \( MN \) is defined as

\[
R^M_p(z^M_p)_{MN}^M \triangleq \begin{bmatrix}
R^M_p(z^M_p)_{M}^M & 0_{n^M \times n^N} \\
0_{n^N \times n^M} & R^N_p(z^N_p)_{n^N \times n^N}
\end{bmatrix},
\]

(25)

and similarly, the embedding of \( R^N_q(z^N_q) \) to \( MN \) is

\[
R^N_q(z^N_q)_{MN}^N \triangleq \begin{bmatrix}
0_{n^P \times n^M} & 0_{n^N \times n^M} \\
0_{n^N \times n^N} & R^N_q(z^N_q)_{n^N \times n^N}
\end{bmatrix}.
\]

(26)

We define the input layer \( \Phi^M_N \) of \( M \) with respect to \( N \) as the set of nodes in \( M \) having parents in \( N \), that is, \( \Phi^M_N \triangleq \{ p \mid Z^M_p \cap X^N \neq \emptyset \} \).

Considering the interpretation of restriction, if node \( p \) does not have parents in \( N \), that is, \( p \) does not belong to the input layer of \( N \), then \( R^M_p(z^M_p)_{MN}^M \) takes the form (25), that is, \( R^M_p(z^M_p)_{MN}^M = R^M_p(z^M_p)_{M}^M \) for \( p \in \Phi^M \setminus \Phi^M_N \). Similarly, \( R^N_q(z^N_q)_{MN}^N = R^N_q(z^N_q)_{N}^N \) for \( q \in \Phi^N \setminus \Phi^M_N \).

**Definition 9.** The effective retroactivity to the input of \( M \) to \( N \) is defined as

\[
\Delta R^M_N(x^M, u^M) \triangleq \sum_{p \in \Phi^M_N} \left( R^M_p(z^M_p)_{MN}^M - R^M_p(z^M_p)_{M}^M \right)
\]

(27)

if \( \Phi^M_N \neq \emptyset \), otherwise \( \Delta R^M_N(x^M, u^M) \triangleq 0_{n^N \times n^M} \).

**Proposition 1.** Take composable modules \( M \) and \( N \) with internal retroactivities \( R^M(x^M, u^M) \) and \( R^N(x^N, u^N) \), respectively. Then, the internal retroactivity of the interconnected module \( MN \) satisfies

\[
R^{MN}(x^MN, u^MN) = R^M_0(x^MN, u^MN) + \Delta R^M_N(x^M, u^M) + \Delta R^N_M(x^N, u^N) \tag{28}
\]

with \( R^M_0(x^MN, u^MN) = \text{diag}(R^M(x^M, u^M), R^N(x^N, u^N)) \).

**Proof sketch:** The internal retroactivity of \( M \) and \( MN \) can be calculated by using (17). Furthermore, the nodes can be grouped whether they belong to the input layer or not. Using \( R^M_p(z^M_p)_{MN}^M = R^M_p(z^M_p)_{M}^M \) for \( p \in \Phi^M \setminus \Phi^M_N \) and \( R^N_q(z^N_q)_{MN}^N = R^N_q(z^N_q)_{N}^N \) for \( q \in \Phi^N \setminus \Phi^M_N \), one obtains (28) with (27) and \( R^M_0(x^MN, u^MN) = \text{diag}(R^M(x^M, u^M), R^N(x^N, u^N)) \).

**Theorem 2.** Consider composable modules \( M \) and \( N \). Let their reduced order models be given by \( \hat{f}^M(x^M, u^M) \) and \( \hat{f}^N(x^N, u^N) \) with internal retroactivities \( R^M(x^M, u^M) \) and \( R^N(x^N, u^N) \), respectively. Then the reduced order model of their interconnection \( MN \) is given by \( \hat{f}^{MN}(x^MN, u^MN) \) where

\[
\hat{f}^{MN}(x^MN, u^MN) = \begin{bmatrix}
\hat{f}^M(x^M, u^M) \\
\hat{f}^N(x^N, u^N)
\end{bmatrix}
\]

(29)

with

\[
Q^{MN}(x^MN, u^MN) = \begin{bmatrix} I_{n^M + n^N} + R^M(x^MN, u^MN) & 0 \\ 0 & I_{n^M + n^N} + R^M_0(x^MN, u^MN) \end{bmatrix}^{-1}
\]

(30)

where \( R^M(x^MN, u^MN) \) is the internal retroactivity of \( MN \) and \( R^M_0(x^MN, u^MN) \) is the internal retroactivity of \( MN \).

**Proof sketch:** Calculate the reduced order models of \( M \) and \( N \) by (20) and write them as

\[
\begin{bmatrix}
\hat{f}^M(x^M, u^M) \\
\hat{f}^N(x^N, u^N)
\end{bmatrix} = \begin{bmatrix} I_{n^M} + R^M_0(x^MN, u^MN) & 0 \\ 0 & I_{n^N} \end{bmatrix}^{-1} \begin{bmatrix}
\phi^M(x^M, u^M) \\
\phi^N(x^N, u^N)
\end{bmatrix}.
\]

Furthermore, the reduced order model of \( MN \) by (20) is

\[
\hat{f}^{MN} = \begin{bmatrix} I_{n^M + n^N} + R^{MN}(x^MN, u^MN) & 0 \\ 0 & I_{n^M + n^N} \end{bmatrix}^{-1} \begin{bmatrix}
\phi^M(x^M, u^M) \\
\phi^N(x^N, u^N)
\end{bmatrix}.
\]

Combining these two completes the proof.

In order to describe the interconnected modules’ dynamics we need: (i) the isolated modules’ dynamics generated by \( x^M = \hat{f}^M(x^M, u^M) \) and \( x^N = \hat{f}^N(x^N, u^N) \) (see (20)); (ii) the internal retroactivities \( R^M(x^M, u^M) \) and \( R^N(x^N, u^N) \) (see (17)); (iii) and the effective retroactivities to the input to each other \( \Delta R^M_N(x^M, u^N) \) and \( \Delta R^N_M(x^N, u^N) \) (see (27)). Consequently, Theorem 2 can be interpreted as being conceptually analogous to Thevenin’s theorem: it relates the modules’ dynamics in isolation and in interconnection.
Internal retroactivity describes the effect of intramodular connections (Theorem 1). When connecting modules, we have to take intermodular connections into account, that is, retroactivity between modules. According to (27), \( \Delta R_N^M(x^M, u^M) \) depends only on parameters of module \( M \) since \( R_p^M(z_p^M) \) depends only on parameters associated with node \( p \) in \( M \). Furthermore, \( \Delta R_N^M(x^M, u^M) \) captures the retroactive effect of connections from \( M \) to \( N \). Therefore, it can be interpreted as similar to the input impedance of \( M \) to \( N \). Moreover, the effective retroactivity to the input of a module to another can be calculated by combining \( R_p^M(z_p^M) \) for \( p \in \Phi_M^N \) according to the embedding and restriction operators. That is, \( \Delta R_N^M(x^M, u^M) \) can be determined by considering the topology-independent \( R_p^M(z_p^M) \) for nodes in the input layer of \( M \) to \( N \) \( (p \in \Phi_M^N) \) and combine them according to the network structure via restriction and embedding.

One can apply Theorem 1 to the interconnected module \( MN \) to conclude that the reduced order model of \( MN \) approximates well the behavior of \( MN \) if \( \epsilon \approx 1 \) (see (19)).

### IV. Example and Simulation Results

We apply our results to the system in Fig. 2 with independent, cooperative and competitive binding introduced in Example 3 with (15).

Consider the isolated modules \( M \) and \( N \) first. By Example 6, the dynamics of \( N \) take the form (24) due to intramodular connections, hence internal retroactivity slows down the dynamics of \( x_1^N \), whereas the behavior of \( x_2^N \) remains the same as in isolation.

The internal retroactivity of \( M \) by (17) is \( R^M(x^M, u^M) = a \). Consequently, by (20) we have \( \dot{x}^M = \frac{1}{1 - \rho M^N}(x^M, u^M) \) where \( \dot{x}^M = \rho M^N(x^M, u^M) \) describes the dynamics of module \( M \) without considering internal retroactivity due to intramodular connections. By (11) we have \( a > 0 \), thus \( 0 < \frac{1}{1 - \rho M^N} < 1 \), hence the dynamics of \( x_1^M \) slow down (Fig. 3) due to the binding of \( x_1^M \) to its own promoter.

By (27), the effective retroactivity to the input of \( N \) to \( M \) is \( \Delta R_N^M(x^M, u^M) = 0 \) representing that \( M \) has no inputs from \( N \), and similarly \( \Delta R_N^M(x^N, u^N) = \begin{bmatrix} 0 & c & 0 \\ d & e & 0 \\ 0 & 0 & 0 \end{bmatrix} \). The internal retroactivity of module \( MN \) can be calculated by Proposition 1 (or equivalently by (17)) resulting in

\[
R^{MN}(x^{MN}, u^{MN}) = \begin{bmatrix} a + b & c & 0 \\ d & e + f & 0 \\ 0 & 0 & 0 \end{bmatrix}.
\]

Recall (29)–(30), the behavior of \( MN \) is given by

\[
\begin{bmatrix} \dot{x}^M \\ \dot{x}^N \end{bmatrix} = Q^{MN}(x^{MN}, u^{MN}) \begin{bmatrix} J^M(x^M, u^M) \\ J^N(x^N, u^N) \end{bmatrix},
\]

where

\[
Q^{MN} = \begin{bmatrix}
\frac{1 + a + b}{1 + a + b + 1 \cdot (1 + e + f) - cd} & -\frac{c(1 + e + f)}{1 + a + b + 1 \cdot (1 + e + f) - cd} & 0 \\
\frac{d(1 + a + b)}{1 + a + b + 1 \cdot (1 + e + f) - cd} & -\frac{e(1 + a + b)}{1 + a + b + 1 \cdot (1 + e + f) - cd} & 0 \\
0 & 0 & 1
\end{bmatrix}.
\]

Since \( \dot{x}^M = J^M(x^M, u^M) \) and \( \dot{x}^N = J^N(x^N, u^N) \) describe the behavior of the isolated modules, \( Q^{MN}(x^{MN}, u^{MN}) \) captures the effect of interconnecting \( M \) and \( N \). Considering the last row of \( Q^{MN}(x^{MN}, u^{MN}) \), we can see that \( x_2^N \) behaves the same as in isolation.

In case of independent binding we have \( c = d = 0 \) by (12), thus the off-diagonal terms in \( Q^{MN}(x^{MN}, u^{MN}) \) are zero. This implies that \( \dot{x}^M = \frac{1}{1 + a + b} J^M(x^M, u^M) \), that is, the dynamics of \( x_1^M \) slow down since \( a, b > 0 \) by (11)–(14). On the other hand, the behavior of \( x_1^N \) remains unchanged when connected to \( M \) since the second diagonal term is one. Consequently, the downstream system behaves the same as in isolation, whereas the upstream system slows down due to the extra load presented by \( N \) (Fig. 4a).

In case of cooperative and competitive binding we have \( c, d \neq 0 \) by (13)–(14). Therefore, the off-diagonal terms in \( Q^{MN}(x^{MN}, u^{MN}) \) are nonzero. It follows that we have to consider two effects. First, the isolated module dynamics of \( x_1^M \) and \( x_1^N \) are scaled by the first two diagonal terms when connected. Second, an additive crosstalk occurs between \( x_1^M \) and \( x_1^N \) through the off-diagonal terms. One might think that the latter effect from \( M \) to \( N \) is because \( M \) is upstream whereas \( N \) is downstream, but this is clearly incorrect: in case of independent binding we do not have this phenomenon. The crosstalk here is purely due to the nonindependent binding and its extent is determined by the magnitude of the off-diagonal terms.

Simulation results for cooperative (Fig. 4b) and competitive binding (Fig. 4c) confirm that the isolated behavior of the downstream module \( N \) distorts the periodic output signal of the upstream system \( M \). This will always occur whenever \( c \neq 0 \), that is, the binding of \( x_1^M \) is not independent of the binding of \( x_1^N \). The larger \( c \) is, the greater this effect. Taking (13)–(14), one can see that \( c \approx 0 \), for instance, if \( \eta_1^M \approx 0 \) or \( \eta_1^N \approx 0 \), that is, if the total concentration of the downstream system or the output

---

**Fig. 3:** Internal retroactivity slows down module \( M \): the blue plot represents the isolated node dynamics, the red line stands for the connected node dynamics, whereas the dashed green plot denotes the reduced order model. Simulation parameters: \( a_{1,0}^M = a_{1,1}^M = 10, \; \delta_1^M = 0.1, \; \delta_{1,0}^M = \delta_{1,1}^M = 0, \; \eta_1^M = 10 \), and \( v_1^M(t) = 0.1 + 0.05 \sin(0.1t) \).
signal of the upstream system is small. Furthermore, we have $c \approx 0$ if the dissociation constant $k_M$ is large. All these conditions represent that the connection between $M$ and $N$ is weak, thus the crosstalk from $N$ to $M$ is small.

V. CONCLUSION AND FUTURE WORK

In this paper, we applied singular perturbation theory to study retroactivity and modularity in complex gene transcription networks.

First, we introduced the effective retroactivity to the input of a node $R^M_{p}(z^M_p)$ and we argued that it can be interpreted as input impedance. It only depends on parameters associated with the node, that is, it remains unchanged when the transcription component is part of a larger network. Furthermore, it describes the loading effect when a downstream component is connected. In addition to providing a formula for calculating this key quantity, we presented the expression of $R^M_{p}(z^M_p)$ for the most common regulation types (independent, cooperative and competitive).

Second, we defined the internal retroactivity of a module capturing the retroactive effects due to intramodular connections. Moreover, we introduced a module’s effective retroactivity to the input to another module describing the load presented by intermodular binding reactions when connecting two modules.

Finally, we presented a theorem for complex gene transcription networks analogous to thevenin’s. It allows us to determine the behavior of connected modules by considering their model in isolation, their internal retroactivity and their effective retroactivity to the input to each other.

Although the current framework is capable of modeling the most relevant processes, such as protein production and decay, as well as binding and unbinding reactions, we will extend our approach by including mRNA dynamics and dimerization. In addition, we propose to investigate the effect of retroactivity for complex systems from a qualitative point of view.

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