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Quantification of loading effects in interconnections of stochastic reaction networks

Ankit Gupta[1], Patrik Dürrenberger[2] and Mustafa Khammash[3]

***Abstract*— Modular design of networks in synthetic biology is highly desirable but difficult to achieve due to loading effects that change the properties of upstream modules upon connection with downstream networks. Precise quantification of these loading effects would allow us to predict the behavior of large interconnected networks more accurately, and enable us to systematically identify insulator circuits that can help in achieving modularity. Most of the existing results on this topic apply only in the deterministic setting and hence they do not account for the stochastic nature of biomolecular interactions. In this work we propose a novel sensitivity-based metric for quantifying loading effects in the stochastic setting. We discuss how this metric can be efficiently computed for stochastic reaction dynamics and demonstrate its usefulness in rational design of insulator circuits.**

I. Introduction

In recent years it has come to light that intracellular biological networks often comprise certain recurrent motifs or modules, across different cell-types and cellular contexts [1]. This remarkable property suggests that complex networks can in principle be studied by viewing them as interconnections of simple modules whose properties are well-characterized [2]. Such an approach is highly desirable and it is known to work extremely well for engineering systems. However this modular approach often fails for biological networks because a module's behavior can change in unpredictable ways once it is connected to its context [3]. This phenomenon is called *loading effect* or *retroactivity* in the literature [4]. The main reason behind this phenomenon is that when an *upstream* module passes information to a *downstream* module, this information transfer can either consume some species of the upstream module or make them temporarily unavailable, thereby disrupting the normal dynamics of the upstream module.

The phenomenon of retroactivity has been well-studied but mainly in the deterministic setting where the network dynamics is specified by a system of ODEs [5]–[7]. In this setting, the seminal work of del Veccio et al. [5] shows that retroactivity can have a profound influence on the dynamics of the upstream module. For example, loading effects can increase the response time of the upstream module or disrupt its oscillatory behavior. Some retroactivity metrics have been proposed to quantify the loading effects for gene-expression networks [7] and using these metrics one can address the problem of rationally designing insulator components that attenuate the loading effects, thereby rendering modularity to the connected network. This methodology has been experimentally tested and found to be quite successful [8], which highlights the importance of proper insulator design in synthetic biology applications, where one typically employs a "bottom-up" approach for designing complex systems from simpler well-characterized parts.

Intracellular networks often consist of biomolecular species that are present in small copy-numbers, like DNA transcripts or transcription factors in gene-expression networks. These low abundance species ensure that the reactions they participate in fire *randomly* [9], and due to this *intrinsic* noise the ODE-based descriptions of the dynamics become highly inaccurate [10]. Hence one needs to consider stochastic models of reaction dynamics which represent the dynamics as a *continuous-time Markov chain* (CTMC) [11].

The main contribution of this paper is to propose a *sensitivity-based* metric for quantifying loading effects for such stochastic models and highlight the usefulness of this metric for studying modularity in the presence of noisy dynamics. Through a couple of illustrative examples we demonstrate how this metric can enable us to rationally design insulator modules that reduce the loading effects. Unlike other existing approaches [5] we focus on the steady-state behavior of the network which is often of interest in synthetic biology applications.

II. The Stochastic Model of a reaction network

We now describe the stochastic model of a reaction network with species $\mathbf{S}_1, \dots, \mathbf{S}_d$ and K reactions of the form

$$\sum_{i=1}^{d} \nu_{ik} \mathbf{S}_i \longrightarrow \sum_{i=1}^{d} \nu'_{ik} \mathbf{S}_i. \tag{1}$$

Here $\nu_{ik}, \nu'_{ik} \in \mathbb{N}_0 := \{0, 1, 2, \dots\}$ denote the number of molecules of $\mathbf{S}_i$ that are consumed (ν_{ik}) and produced (ν'_{ik}) by the k-th reaction. Under the classical well-stirred assumption [12], the state of the system at any time is given by the vector of copy-numbers $x = (x_1, \dots, x_d) \in \mathbb{N}_0^d$ of all the species. Let $\nu_k = (\nu_{1k}, \dots, \nu_{dk})$ and $\nu'_k = (\nu'_{1k}, \dots, \nu'_{dk})$. At state x, the k-th reaction is assumed to fire at rate $\lambda_k(x)$ and it displaces the state to $(x + \nu'_k - \nu_k)$. The function $\lambda_k : \mathbb{N}_0^d \to [0, \infty)$ is called the *propensity* function for the k-th reaction and commonly it is given by

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the mass-action form

$$\lambda_k(x_1,\ldots,x_d)=\gamma_k\prod_{i=1}^{d}\frac{x_i(x_i-1)\ldots(x_i-\nu_{ik}+1)}{\nu_{ik}!}, \quad (2)$$

where $\gamma_k>0$ is the rate constant.

In the *continuous-time Markov chain* (CTMC) model of a reaction network the propensity functions specify the transition rates [11]. Hence at state x, the next jump time of the CTMC is exponentially distributed with rate $\lambda_0(x)=\sum_{k=1}^{K}\lambda_k(x)$, and unless $\lambda_0(x)=0$ (i.e. x is an *absorbing* state) the k-th reaction fires at the next jump time with probability $\lambda_k(x)/\lambda_0(x)$.

Let $(X(t))_{t\geq 0}$ be the Markov process representing the stochastic reaction dynamics. For any two states x and y let

$$p_x(t,y)=\mathbb{P}\left(X(t)=y|X(0)=x\right),$$

be the probability that the dynamics starts at x and is at y at time t. The dynamics of $p_x(t,\cdot)$ is given by an infinite system of ODEs, called the *Chemical Master Equation* (CME) which is given by

$$\frac{dp_x(t,y)}{dt}=\sum_{k=1}^{K}(p_x(t,y-\zeta_k)\lambda_k(y-\zeta_k)-p_x(t,y)\lambda_k(y)),$$

where $\zeta_k=\nu_k'-\nu_k$ and the initial condition is $p_x(0,y)=1$ if $x=y$ and $p_x(0,y)=0$ for all $y\neq x$. The CTMC is called *ergodic* if $p_x(t)$ converges to a unique stationary distribution π, as $t\to\infty$, irrespective of the initial state x. In other words, for any x we have

$$\lim_{t\to\infty}\|p_x(t)-\pi\|_{\ell_1}=0, \quad (3)$$

where $\|\mu\|_{\ell_1}=\sum_x\|\mu(x)\|$ denotes the ℓ_1 norm of a signed measure μ. A stronger form of ergodicity, called *exponential* ergodicity holds when the convergence in (3) is exponentially fast at a rate which is independent of the initial state x. Checking exponential ergodicity of the CTMC model of a reaction network is a difficult problem, but recently computational approaches have been developed for this purpose that work well for a wide variety of biological networks (see [13] and [14]).

Now suppose that the propensity functions (λ_k-s) can depend on a scalar parameter θ (like a rate constant or cell volume) in addition to the state x. Let $(X_\theta(t))_{t\geq 0}$ be the corresponding θ-dependent CTMC which we assume is ergodic with π_θ as the stationary distribution. Suppose $f:\mathbb{N}_0^d\to\mathbb{R}$ is some function specifying the *output* of the system at time t as the expectation $\mathbb{E}(f(X_\theta(t)))$. The effect of parameter θ on this output can be quantified using the infinitesimal sensitivity value defined as

$$S_\theta(f,t):=\frac{\partial}{\partial\theta}\mathbb{E}(f(X_\theta(t))).$$

Estimating this quantity is complicated but many simulation-based methods have been developed for this task [15]–[19]. In this paper, we are interested in the steady-state output

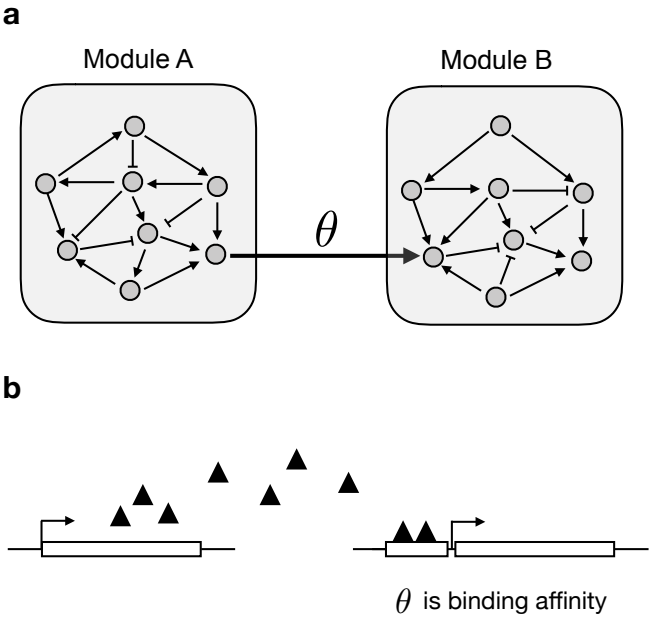

Fig. 1. a) Conceptual figure depicting an upstream module (Module A) connected to a downstream module (Module B). The connector reaction strength is parametrized by parameter θ. b) Depiction of an example where Module A is a simple gene-expression network which expresses a transcription factor that can go and bind to the promoter sites of another gene-expression network (Module B). Here the connection strength parameter θ represents the binding affinity.

given by the expectation of function f at the stationary distribution π_θ, i.e.

$$\lim_{t\to\infty}\mathbb{E}(f(X_\theta(t)))=\mathbb{E}_{\pi_\theta}(f(X)):=\sum_{x\in\mathbb{N}_0^d}f(x)\pi_\theta(x).$$

To assess the influence of parameter θ on this output we need to evaluate the steady-state sensitivity

$$S_\theta(f):=\frac{\partial}{\partial\theta}\mathbb{E}_{\pi_\theta}(f(X))=\lim_{t\to\infty}S_\theta(f,t).$$

The second equality is non-trivial as the limit $t\to\infty$ and the derivative $\partial/\partial\theta$ may not commute. However under some mild conditions on the propensity functions this equality can be justified [20]. The methods available for estimating finite-time sensitivity $S_\theta(f,t)$ do not work well for estimating the steady-state sensitivity $S_\theta(f)$. Therefore we have recently developed a simulation-free approach [21] for estimating $S_\theta(f)$ that is based on using the *stationary Finite State Projection* (sFSP) method for obtaining an approximation of the stationary distribution π_θ and computing the solution of a certain Poisson equation for the CTMC (see [22]). We call this method the *Poisson Estimator* (PE) and we show that it applies successfully to many examples of biological interest (see [21] for more details).

III. Quantification of loading effects

We now come to the main contribution of this paper which is to propose a sensitivity-based metric for quantifying loading effects for networks whose dynamics may be intrinsically noisy. Consider the scenario depicted in Figure 1(a) where

the upstream module (Module A) is connected to another downstream module (Module B) via a reaction with rate constant θ. For example, θ can represent the binding affinity of transcription factors produced by Module A to the promoter sites that are part of another gene-expression network given by Module B (see Figure 1(b)). Let $(X_\theta(t), Y_\theta(t))_{t\geq 0}$ be the joint CTMC dynamics of both the modules (i.e. $X_\theta(t)$ and $Y_\theta(t)$ denote the state at time t of Modules A and B respectively). Suppose this CTMC is ergodic with stationary distribution π_θ.

We assume that the *output* of Module A is measured by some real-valued function f of the state of this module. In particular, the output is the steady-state expectation $\mathbb{E}_{\pi_\theta}(f(X))$. This output does not depend on the state of Module B. Notice that when $\theta = 0$, the reaction connecting the two modules is absent, and hence Module A is *isolated* and its output is given by $\mathbb{E}_{\pi_0}(f(X))$. To quantify loading effects, we would like to measure the relative change in the output of Module A when θ is positively perturbed. Therefore we propose the following sensitivity-based measure of loading effects

$$\mathcal{L} = \left| \frac{\partial}{\partial \theta} \log \left(\mathbb{E}_{\pi_\theta}(f(X)) \right) \right|_{\theta=0} = \frac{|S_0(f)|}{|\mathbb{E}_{\pi_0}(f(X))|}, \qquad (4)$$

where we implicitly assume that $\mathbb{E}_{\pi_0}(f(X)) \neq 0$. In many examples the output is measured by the expected copy-number of some species $\mathbf{S}_i$ in Module A and hence $f(x) = x_i$ and we refer to $\mathbf{S}_i$ as the *output* species. The loading metric $\mathcal{L}$ can be computed by estimating the expectation $\mathbb{E}_{\pi_0}(f(X))$ and the sensitivity $S_0(f)$ using sFSP and the PE method respectively (see Section II). We have developed a software library to carry out these tasks (see [21] for more details) and we shall use this computational tool to compute $\mathcal{L}$ for the examples in Section V.

In typical applications the interconnection strength parameter θ can vary from 0 to some maximum value θ_{max}. Since normalizing all the network reaction-rate constants by θ_{max} does not change the steady-state, we can assume without loss of generality that θ is always between 0 and 1. Note that if $\mathcal{L} \approx 0$ then for $\theta \approx 0$ we can expect the output of Module A to remain "close" to its output in the isolated case (i.e. $\theta = 0$). Our examples suggest that this remains true even when θ approaches 1, which suggests that $\mathcal{L}$ can be a useful metric to quantify the loading effects.

IV. Rational design of insulators

Now that we have a metric for quantifying loading effects in the stochastic setting, we can employ this metric for rational design of *insulator* modules that can attenuate loading effects, and make the interconnected network more modular in its behavior. Two distinct approaches for designing such insulators have been proposed in the literature. The first approach is based on a feedback strategy that adds robustness to the output of the upstream module w.r.t. perturbations caused by the downstream module [5], while the second approach is based on introducing a *buffering* device that relays the output of Module A to the input of Module B in such a way that loading effects caused by the connection are minimized [6]. A conceptual representation of both these approaches is given in Figure 2.

The task of rationally designing insulators can be viewed as designing a biomolecular reaction network which, when introduced into the connected network, significantly decreases the loading metric $\mathcal{L}$ given by (4). We illustrate this approach with two examples in the next section. These two examples demonstrate the two insulator design approaches depicted in Figure 2.

V. Illustrative Examples

In this section we provide a couple of examples to illustrate our method for quantifying loading effects and using it for rational design of insulator modules. We shall employ CTMC descriptions (see Section II) of the reaction dynamics and all propensity functions are assumed to follow mass-action kinetics (2) unless otherwise stated.

A. Two-step gene-expression with a feedback insulator

Suppose that the upstream module (Module A) is the standard two-step gene-expression network in [23], where mRNA (M) molecules are first transcribed by a constitutively expressing gene at rate γ_1, and then these mRNA molecules translate Protein (P) molecules at rate γ_2. Both mRNA and Protein molecules degrade spontaneously at rates γ_3 and γ_4 respectively. Module A can be represented as a reaction network as

$$\textbf{Module A:} \qquad \emptyset \xrightarrow{\gamma_1} M, \quad M \xrightarrow{\gamma_2} M + P, \quad M \xrightarrow{\gamma_3} \emptyset \quad \text{and} \quad P \xrightarrow{\gamma_4} \emptyset.$$

We assume that Module A is connected with the downstream Module B by the reaction

$$\textbf{Connector Reaction:} \qquad P \xrightarrow{\theta} P^*,$$

where P^* refers to a modified form of the Protein P (e.g. phosphorylated protein). The species P^* drives downstream processes that are part of Module B. Note that from the perspective of Module A, the connector reaction serves as an extra degradation reaction for the protein molecules.

We measure the output of Module A by the steady-state expectation $\mathbb{E}_{\pi_\theta}(P)$ of the number of protein molecules. As the propensity functions are linear, we can compute the output $\mathbb{E}_{\pi_\theta}(P)$ exactly along with its sensitivity w.r.t. θ (see [24]) to obtain

$$\mathbb{E}_{\pi_\theta}(P) = \frac{\gamma_1 \gamma_2}{\gamma_3(\gamma_4 + \theta)} \qquad \text{and} \qquad \mathcal{L} = \frac{1}{\gamma_4}.$$

When we set the rate constants as $\gamma_1 = 47.63$ min^{-1}, $\gamma_2 = 4$ min^{-1}, $\gamma_3 = 0.5$ min^{-1} and $\gamma_4 = 0.2$ min^{-1}, the output is $\mathbb{E}_{\pi_0}(P) = 1905$ when Module A is isolated ($\theta = 0$) and the loading metric is $\mathcal{L} = 5$. The high value of this metric suggests that the output of Module A will be significantly affected as the connector reaction rate θ becomes positive. This is illustrated in Figure 3.

Now suppose that we are able to design an insulator module that senses the Protein copy-number P and implements

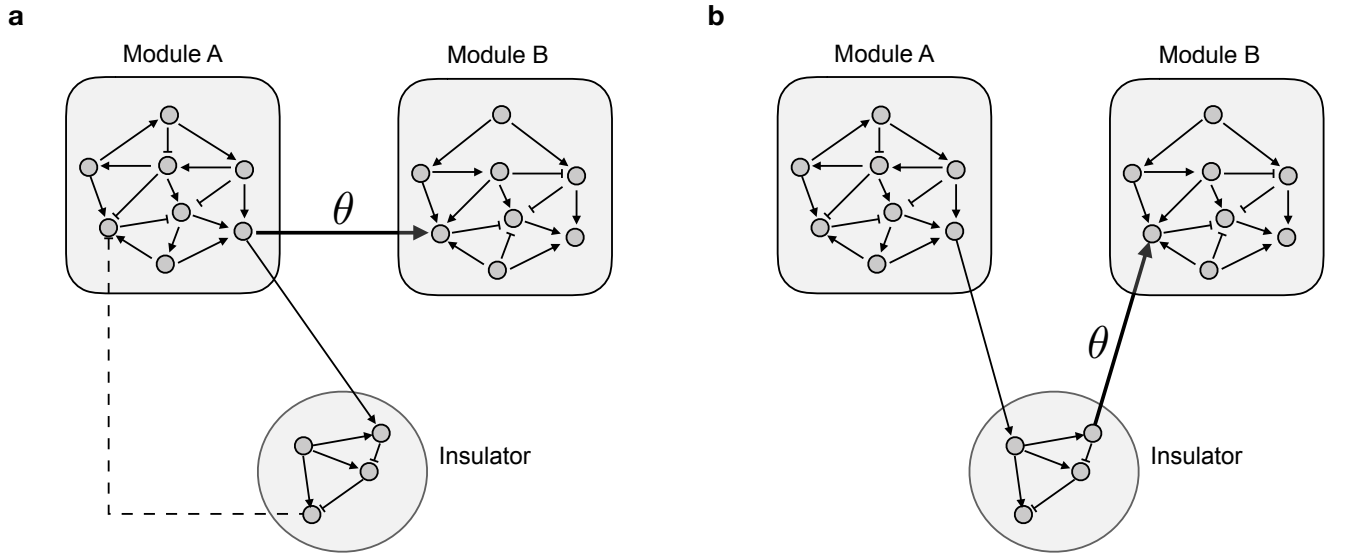

Fig. 2. This figure depicts how the loading effects can be minimized using insulator circuits. There exist two approaches for designing such circuits. In the first approach, shown in (a), the insulator senses the output species in Module A and then computes a signal that is then passed back to Module A in a feedback fashion. In the second approach, shown in (b), the output species in Module A is sensed and this information is processed by a *buffering device* (insulator circuit) which contains a *new* output species that connects to Module B.

a high-gain negative proportional feedback (see Figure 2(a)), by changing the transcription rate of mRNA from γ_1 to

$$\gamma_1(P) = \alpha + \max\{\kappa_1 - \kappa_2 P, 0\}.$$

With this insulator module in place, we can no longer compute the output $\mathbb{E}_{\pi_\theta}(P)$ and the loading metric $\mathcal{L}$ exactly because of the non-linearity of the propensity function $\gamma_1(P)$. However if we assume that at stationary $\kappa_2 P < \kappa_1$ holds with probability close to 1, then replacing $\gamma_1(P)$ with the linear propensity function $\tilde{\gamma}_1(P) = (\alpha + \kappa_1) - \kappa_2 P$ we can compute accurate approximations for the output $\mathbb{E}_{\pi_\theta}(P)$ and the loading metric $\mathcal{L}$ as

$$\mathbb{E}_{\pi_\theta}(P) = \frac{(\alpha+\kappa_1)\gamma_2}{\kappa_2\gamma_2 + \gamma_3(\gamma_4+\theta)} \quad \text{and} \quad \mathcal{L} = \frac{\gamma_3}{\kappa_2\gamma_2 + \gamma_3\gamma_4}$$

respectively. Observe that if $\kappa_2\gamma_2 \gg \gamma_3$ then $\mathcal{L}$ is small, indicating that negative proportional feedback can indeed decrease the loading effects. To see this numerically we set the feedback parameters as $\alpha = 0.1$ min^{-1}, $\kappa_1 = 1000$ min^{-1} and $\kappa_2 = 0.5$ min^{-1}. With these parameters the output of Module A in the isolated case ($\theta = 0$) is the same as before (i.e. $\mathbb{E}_{\pi_0}(P) = 1905$) but now the estimated loading metric reduces by 95% to $\mathcal{L} = 0.24$, in comparison to the earlier case with no insulator. Therefore we can expect that due to the insulator action, the loading effects will be sharply attenuated and the output of Module A will be close to the output in the isolated case when the connector reaction rate θ is positive. This is indeed the case as Figure 3 shows.

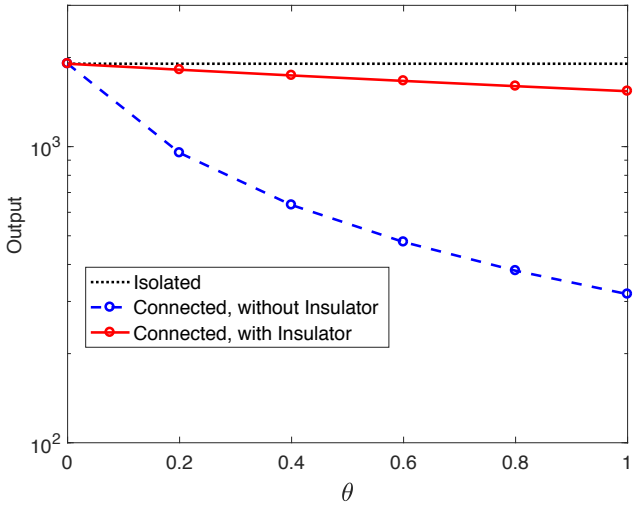

Fig. 3. This figure shows how the output of the two-step gene-expression network (see Section V-A) varies with the connector reaction rate θ. The loading effects are much higher in the absence of the feedback insulator module, while these effects are sharply attenuated in the presence of the insulator module.

B. Single-step gene-expression with a buffering insulator

We now consider an example from [5] with minor changes. Here we simplify the two-step gene-expression network (see Section V-A) by eliminating the mRNA dynamics. This elimination can be justified in some cases using a time-scale separation argument, and the reduced network (Module A) simply consists of birth and death reactions for the Protein (Z) molecules:

$$\textbf{Module A:} \qquad \emptyset \xrightarrow{\gamma_1} Z \quad \text{and} \quad Z \xrightarrow{\gamma_2} \emptyset.$$

We assume that these Z molecules serve as transcription factors to some downstream gene-expression network (Module B). Hence the Z molecules can reversibly bind to the *free* promoter sites (as in Figure 1(b)) to form an active gene-

complex C, and so the connector reaction is given by

$$\textbf{Connector Reaction:} \qquad Z + p \underset{k_{\text{off}}}{\overset{\theta k_{\text{on}}}{\rightleftharpoons}} C,$$

where p is the number of free promoter sites in the downstream gene-expression module. We set $\gamma_1 = 5$ min^{-1}, $\gamma_2 = 0.5$ min^{-1}, $k_{\text{on}} = 100$ min^{-1} and $k_{\text{off}} = 0.01$ min^{-1}. To account for binding by transcription factors other than Z we add reactions

$$p \underset{0.01}{\overset{0.0001}{\rightleftharpoons}} C.$$

We choose the total number of promoter sites as $p_{\text{tot}} = 50$, and hence we have the conservation relation $p + C = p_{\text{tot}}$. We measure the output of Module A by the steady-state expectation $\mathbb{E}_{\pi_\theta}(Z)$ of the number of Z molecules.

Note that when $\theta = 0$, the forward connection reaction $Z + p \longrightarrow C$ stops, and it can be checked that the backward reaction $C \longrightarrow Z + p$ also approximately ceases at steady-state as the expected copy-number of C is nearly zero. Therefore Module A becomes isolated for $\theta = 0$ and its output is $\mathbb{E}_{\pi_0}(Z) \approx 10$. We can estimate the loading metric (4) as $\mathcal{L} = 4999.9$ via the PE estimator (see Section II). As θ becomes positive, the effect on the output of Module A can be seen from Figure 4.

We now add a *buffering* insulator (see Figure 2(b)) that employs phosphorylation-dephosphorylation cycles as proposed in [5]. Here Z acts like a kinase for another protein X. Hence it catalyzes the reaction $X \longrightarrow X_p$, where X_p denotes the phosphorylated form X. There also exists a phosphatase Y that catalyzes the dephosphorylation reaction $X_p \longrightarrow X$. The connection with the downstream gene-expression module is made in the same way as before via the phosphorylated protein X_p (instead of Z) whose steady-state expectation $\mathbb{E}_{\pi_\theta}(X_p)$ is like the *buffered* output of Module A. In summary the insulator module and the new connector reaction are given by:

$$\textbf{Insulator Module:} \quad X + Z \xrightarrow{k_1} X_p + Z$$
$$\text{and} \quad X_p + Y \xrightarrow{k_2} X + Y.$$
$$\textbf{New Connector Reaction:} \quad X_p + p \underset{k_{\text{off}}}{\overset{\theta k_{\text{on}}}{\rightleftharpoons}} C.$$

We set $k_1 = k_2 = 100$, and the total number of both X and Y molecules to 5000. Once we include the insulator the buffered output of Module A is again $\mathbb{E}_{\pi_0}(X_p) \approx 10$ in the isolated case ($\theta = 0$) and we estimate the loading metric as $\mathcal{L} = 494.82$. Hence we have 90% reduction in loading strength, bringing the output of Module A upon connection closer to the isolated case, which can be seen in Figure 4.

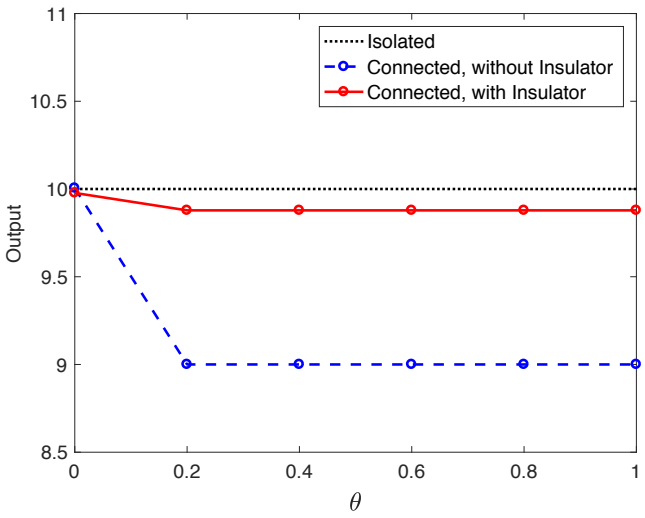

Fig. 4. This figure shows how the output of the single-step gene-expression network (see Section V-B) varies with the connector reaction rate θ. The loading effects are much higher in the absence of the buffering insulator module while these effects are sharply reduced in the presence of the insulator module.

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