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Aggregates of Monotonic Step Response Systems: A Structural Classification

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***Abstract*—Complex dynamical networks can often be analyzed as the interconnection of subsystems: This allows us to considerably simplify the model and better understand the global behavior. Some biological networks can be conveniently analyzed as aggregates of monotone subsystems. Yet, monotonicity is a strong requirement; it relies on the knowledge of the state representation and imposes a severe restriction on the Jacobian (which must be a Metzler matrix). Systems with a monotonic step response (MSR), which include input–output monotone systems as a special case, are a broader class and still have interesting features. The property of having a monotonically increasing step response (or, equivalently, in the linear case, a positive impulse response) can be evinced from experimental data, without an explicit model of the system. We consider networks that can be decomposed as aggregates of MSR subsystems and we provide a structural (parameter-free) classification of oscillatory and multistationary behaviors. The classification is based on the exclusive or concurrent presence of negative and positive cycles in the system *aggregate graph*, whose nodes are the MSR subsystems. The result is analogous to our earlier classification for aggregates of monotone subsystems. Models of biomolecular networks are discussed to demonstrate the applicability of our classification, which helps build synthetic biomolecular circuits that, by design, are well suited to exhibit the desired dynamics.**

***Index Terms*—Bifurcations, biological networks, graph theory, positive impulse response, structural analysis.**

I. Introduction

THE THEORY of monotone systems has been one of the most successful tools for the analysis of biological systems, in particular, biomolecular circuits and gene networks [30], [32]. It is straightforward to check the monotonicity of low-order phenomenological models by inspecting their Jacobian matrix, and verification (or lack) of this property immediately provides important information about the potential dynamic behaviors of the system, without having to resort to extensive numerical studies. Large, complex networks can often be decomposed into the interconnection of input-output monotone subsystems, making it possible to employ many theoretical tools that help establish the admissible dynamics of the network: for instance, interconnected monotone modules have been shown to exhibit multistationarity [2] and oscillations [4] depending on the interconnection topology.

It remains difficult, however, to establish monotonicity of biological networks in many cases. Due to the absence of compartments, molecular circuits are often plagued by the presence of unmodeled or unknown dynamics. Also, phenomenological models often neglect the effects of the environment on a module, and further simplify several chemical reactions into few equations: these simplifications may yield monotone models that enable sophisticated theoretical analysis, whereas the mechanistic (and more realistic) state-space model does not in reality enjoy such properties; we are not aware of systematic criteria allowing us to establish monotonicity of generic chemical reaction networks.

While it can be difficult to apply the tools of monotone systems theory to realistic biological models, whose state-space model may be too complex or uncertain, we suggest an alternative route that focuses on the monotonicity of the system step response. We focus on identifying the possible instability patterns that can arise in interconnections of systems having a monotonic step response (MSR) in isolation, and we prove a structural (parameter-independent) [7] classification analogous to the classification that was previously established for interconnections of monotone subsystems [9]. A first structural classification for systems with a sign-definite Jacobian [8] relied on the Jacobian graph, where the nodes are associated with state variables and the arcs with signed Jacobian entries: *strong (weak) candidate oscillators* were identified as systems that can exclusively (possibly) transition to instability due to a complex pair of eigenvalues, while *strong (weak) candidate multistationary systems* can exclusively (possibly) transition to instability due to a real eigenvalue. Building on a vast literature (see [5], [18], [21], [28], [31], [33], [34], and the discussion in [8]), a structural classification of oscillatory and multistationary networks was proposed based on the exclusive or concurrent presence of negative and positive cycles in the Jacobian graph. These results were extended to interconnections of monotone subsystems in [9], based on cycles in the aggregate graph, whose nodes are the monotone subsystems.

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Here, conversely, we show how large networks can often be regarded as aggregates of interacting subsystems with monotonic step response (MSR). MSR systems include, and significantly generalize, input–output monotone systems. As a main result, we prove that the classification in [8] and [9] can be scaled and suitably adapted to consider interconnections of MSR subsystems. We provide a graph-based characterization of potential multistationary and oscillatory behaviors, based on the exclusive or concurrent presence of positive and negative cycles in the aggregate graph, whose nodes are the MSR subsystems. We then propose a summary of available criteria to establish whether a system has a positive impulse response (PIR), which is equivalent to MSR in the linear case. Our classification can be successfully applied to *structurally* evaluate the behavior of artificial biomolecular networks: The analysis of oscillators and bistable systems built of potentially MSR aggregates [12] reveals that their design is well suited to achieve the desired dynamics.

A. Motivating Example: Gene Expression

Consider the following elementary gene expression process with negative autoregulation [29], where x is the RNA concentration and y is the protein concentration:

$$\dot{x} = \frac{a}{A+y} - \alpha y + u \tag{1}$$

$$\dot{y} = \gamma x - \beta y \tag{2}$$

with output y. Negative autoregulation is extremely common in bacterial systems, and there is evidence that it helps reduce variability of protein expression at the population level [24]; thus, it is a very important "module" in the context of synthetic biology. The Jacobian of this system is not a Metzler matrix; therefore, the system is not monotone. However, since the degradation of RNA molecules and of proteins occurs on time scales having different orders of magnitude, this system has a monotonic step response (for values of the parameters that are compatible with physical observations), as will be shown in Section VII.

II. Monotonic Step Response (MSR) Systems

Given a dynamical system of the form

$$\dot{x}(t) = f(x(t), u(t)), \quad x \in \mathbb{R}^n \tag{3}$$

$$y(t) = g(x(t)) \tag{4}$$

where the input $u \in \mathbb{R}$ and the output $y \in \mathbb{R}$ are both scalars, consider an equilibrium pair $(\bar{x}, \bar{u})$ and the corresponding output value $\bar{y}$, so that $0 = f(\bar{x}, \bar{u})$ and $\bar{y} = g(\bar{x})$. Let us consider the following definition.

Definition 1: System (3)–(4) is an *MSR* system if, for any equilibrium pair $(\bar{x}, \bar{u})$ and any constant input $u > \bar{u}$, the output function $y(t)$ corresponding to the trajectory $x(t)$ with initial condition $x(0) = \bar{x}$ is monotonically increasing. ◇

The previous definition admits a local version.

Definition 2: System (3)–(4) is a *locally MSR (locMSR)* system with respect to the equilibrium pair $(\bar{x}, \bar{u})$ if, for sufficiently small constant $u > \bar{u}$, the output function $y(t)$ corresponding to the trajectory $x(t)$ with initial condition $x(0) = \bar{x}$ is monotonically increasing. ◇

In the linear case, the two definitions are equivalent.

The MSR property can be characterized as follows.

Theorem 1: Assume that functions f and g are continuously differentiable. Then, system (3)–(4) is an MSR system if and only if, for any equilibrium pair $(\bar{x}, \bar{u})$ and any constant $u > \bar{u}$, there exists a set $\mathcal{P}_{u,\bar{x}}$ that is positively invariant for $\dot{x} = f(x, u)$, such that $\bar{x} \in \mathcal{P}_{u,\bar{x}}$ and

$$\mathcal{P}_{u,\bar{x}} \subseteq \left\{ \frac{\partial g(x)}{\partial x} f(x,u) \geq 0 \right\}$$

where $\{\varphi(x) \geq 0\}$ generically denotes the set of all points at which function φ is non-negative. ■

Proof: Sufficiency is obvious; if $\bar{x} \in \mathcal{P}_{u,\bar{x}}$, then, for all $t > 0$, $\bar{x}(t) \in \mathcal{P}_{u,\bar{x}}$ (where $\bar{x}(t)$ denotes the trajectory with initial condition $\bar{x}$). Hence, the system is MSR because

$$\dot{y} = \frac{\partial g(x)}{\partial x} f(x,u) \geq 0.$$

As for necessity, given an MSR system, consider the set $\mathcal{P}^*_{u,\bar{x}}$ of all states for which $x(t_0) \in \mathcal{P}^*_{u,\bar{x}}$ implies $\dot{y} \geq 0$ for all $t \geq t_0$. The set $\mathcal{P}^*_{u,\bar{x}}$ is positively invariant, $\bar{x} \in \mathcal{P}^*_{u,\bar{x}}$, and $\mathcal{P}^*_{u,\bar{x}} \subseteq \{\frac{\partial g(x)}{\partial x} f(x,u) \geq 0\}$. ■

Proposition 1: Given the linear system

$$\dot{x}(t) = Ax(t) + Bu(t), \quad x \in \mathbb{R}^n \tag{5}$$

$$y(t) = Cx(t) \tag{6}$$

where $A \in \mathbb{R}^{n\times n}$, $B \in \mathbb{R}^{n\times 1}$, and $C \in \mathbb{R}^{1\times n}$, assume without restriction that $\bar{x} = 0$. The following properties are equivalent.

1) [locMSR] System (5)–(6) is a locally MSR system.
2) [MSR] System (5)–(6) is an MSR system.
3) [PIR] System (5)–(6) has a PIR. ■

Proof: The equivalence between [locMSR] and [MSR] is due to linearity. [PIR] and [MSR] are equivalent since the impulse response is the derivative of the step response. ■

Our analysis is performed on the linearized system. Hence, we always assume that the overall nonlinear system admits an equilibrium, around which it can be linearized, and is defined in a neighborhood of this equilibrium. Then, we have the following preliminary result.

Theorem 2: If system (3)–(4) is an MSR system, then its linearization about any equilibrium point is an MSR system (or, equivalently, a PIR system). ■

Proof: Let the equilibrium be $\bar{x} = 0$ and $\bar{u} = 0$, w.l.o.g., then

$$\dot{x}(t) = Ax(t) + Bu(t) + R(x(t), u(t)) \tag{7}$$

$$y(t) = Cx(t) + S(x(t)) \tag{8}$$

where R and S are infinitesimals of order greater than one.

Consider the sign-preserving coordinate transformation

$$z = kx, \quad w = ky, \quad \text{and} \quad v = ku$$

with k integer and positive. Also, let $R_k(z,v) \doteq kR(\frac{z}{k}, \frac{v}{k})$ and $S_k(z) \doteq kS(\frac{z}{k})$. Then

$$\dot{z}(t) = Az(t) + Bv(t) + R_k(z(t), v(t)) \tag{9}$$

$$w(t) = Cz(t) + S_k(z(t)) \tag{10}$$

where $R_k(z,v)$ and $S_k(z)$ converge to 0 uniformly, as $k \to \infty$, in any compact ball $\mathcal{B}$ including 0 (in the z space). Let z_k be the solution of (9) and (10) and z_∞ the solution of the associated linear system with $R_k(z,v) = S_k(z) = 0$, and let w_k and w_∞ be the corresponding outputs.

To prove that the linear system is an MSR system (that is, its step response is nondecreasing), assume by contradiction that

$$w_\infty(t_1) - w_\infty(t_2) \geq \epsilon > 0 \tag{11}$$

for $t_1 < t_2$ and for a positive input v, and let the compact ball $\mathcal{B}$ include $z_\infty(t_1)$ and $z_\infty(t_2)$.

Due to the uniform convergence of R_k and S_k, the solution z_k of the nonlinear system uniformly converges to the solution z_∞ of the linear system [19]. Hence, for ϵ small enough and for k large enough, it must be $|w_k(t_1) - w_\infty(t_1)| \leq \epsilon/2$ and $|w_k(t_2) - w_\infty(t_2)| \leq \epsilon/2$. In view of (11), this would imply $w_k(t_1) \geq w_k(t_2)$, against our assumption. ■

Henceforth, we will consider MSR systems and remember that their linearization is a PIR system.

It is fundamental to compare the properties of MSR systems and monotone systems.

Definition 3: System (3) is *input-to-state monotone* if, given $u_2(t) \geq u_1(t)$ $\forall t$ and $x_2(0) \geq x_1(0)$, the corresponding solutions satisfy $x_2(t) \geq x_1(t)$. System (3)–(4) is *input–output monotone* if it is input-to-state monotone and the output function g is sign preserving, that is, $x_1 \leq x_2$ implies

$$g(x_1) \leq g(x_2).$$

All of the inequalities are to be intended componentwise. ◇

The following well-known result holds [32].

Proposition 2: If system (3)–(4) is input–output monotone, then its linearization (5)–(6) is such that

1) the Jacobian A is Metzler: $A_{ij} \geq 0$ for $i \neq j$;
2) B and C are non-negative.

Hence, the linearization is a PIR system. ■

Proposition 2 shows that if a system is input–output monotone, then its linearization is a PIR system. The opposite is not true: having a linearization for which 1) and 2) hold is a stronger requirement. Hence, there are systems that are not monotone, but whose linearization is a PIR system, as shown next.

Example 1: The linear system

$$\dot{x} = \begin{bmatrix} -\alpha & -\beta & \gamma \\ -\alpha & -(\beta+\delta) & 0 \\ \alpha & \beta & -(\gamma+\epsilon) \end{bmatrix} x + \begin{bmatrix} 1 \\ 0 \\ 0 \end{bmatrix} u \tag{12}$$

$$y = \begin{bmatrix} 0 & 0 & 1 \end{bmatrix} x, \tag{13}$$

where the Greek letters denote positive parameters, is associated with the transfer function

$$F(s) = \frac{\alpha(s+\delta)}{s^3 + p_2 s^2 + p_1 s + p_0} \tag{14}$$

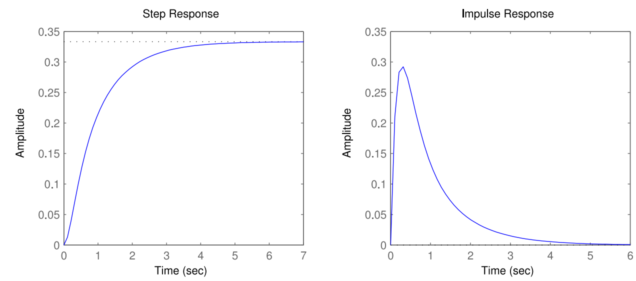

Fig. 1. MSR (left) and the PIR (right) of the system in Example 1 with $\alpha = \epsilon = 3$, $\beta = \gamma = 1$, and $\delta = 2$.

having coefficients

$$p_2 = \alpha + \beta + \gamma + \delta + \epsilon$$

$$p_1 = \alpha\delta + \alpha\epsilon + \beta\gamma + \beta\epsilon + \gamma\delta + \delta\epsilon$$

$$p_0 = \alpha\delta\epsilon.$$

The system is not monotone, since its Jacobian is not Metzler. However, its linearization is a PIR system, hence an MSR system. We can see this for the choice of parameters $\alpha = \epsilon = 3$, $\beta = \gamma = 1$, and $\delta = 2$; in this case, the transfer function becomes

$$F(s) = \frac{3(s+2)}{s^3 + 10s^2 + 27s + 18}$$

and the corresponding impulse response

$$f(t) = \mathcal{L}^{-1}[F(s)] = \frac{3}{10}e^{-t} + \frac{1}{2}e^{-3t} - \frac{4}{5}e^{-6t}$$

is positive for all $t > 0$. The MSR and the PIR are shown in Fig. 1. Actually, system (12)–(13) is *structurally* PIR, for any choice of the positive parameters α, β, γ, δ, and ϵ, as shown in Section VII. ◇

III. TRANSITION TO INSTABILITY AND STRUCTURE

Our analysis proceeds along the lines in [8] and [9]. To investigate *transitions to instability*, we consider the system

$$\dot{x}(t) = f(x(t), \mu), \qquad x \in \mathbb{R}^n \tag{15}$$

where μ is a real-valued parameter and $f(\cdot,\cdot)$ is a sufficiently smooth function, continuous in μ. We assume that the system has a structure (a sign pattern, formally defined later in Definition 6) that is invariant with respect to μ, and an equilibrium $\bar{x}_\mu$ exists as a function of μ, such that $f(\bar{x}_\mu, \mu) = 0$.

We aim at assessing which type of instability can arise [8], [9]. To this aim, we denote as *critical* a choice of parameters for which the system loses stability due to poles crossing the imaginary axis. Then, the system is a *strong* (respectively, *weak*) *candidate bistable system* if its response is monotone for all of the critical choices (respectively, for some critical choice) of the parameters. In this case, stability is typically lost due to a real pole that crosses the imaginary axis at zero. Conversely, the system is a *strong* (respectively *weak*) *candidate oscillator* if its response for critical choices of the parameters is never monotone (respectively, can be nonmonotone). This typically occurs due to a pair of complex eigenvalues that cross the imaginary axis.

Remark 1: We keep the terminology in [8] and [9], although our analysis is carried out in a linear context, while the terms *candidate oscillator* and (especially) *candidate bistable system* are meaningful, in principle, in a nonlinear context. The transition of a pair of complex eigenvalues to the right-half plane induces sustained oscillations if the overall solution is bounded; and the boundedness has to be proved in the nonlinear framework. The transition of a real eigenvalue from the negative to the positive real axis typically generates new equilibria, which are stable under additional assumptions [8], [9]; and this kind of phenomena has to be studied in the nonlinear framework. ◇

The structure of an MSR aggregate system is given by the pattern of the signed interactions in an *aggregate graph* where the arcs represent the signed interactions among the (linearized) subsystems, which, in turn, correspond to the nodes. (In our earlier work, the system structure was defined as the Jacobian sign pattern [8] or as the pattern of signed interactions in the aggregate graph associated with the system [9].) To qualitatively represent the interaction between subsystem k and subsystem h, we consider a coefficient σ_{kh}, which can be either positive or negative (equivalently, in the aggregate graph, an arc with weight σ_{kh} goes from node h to node k).

For instance, consider a system of the form

$$\begin{aligned} \dot{x}_1 &= f_1(x_1, \sigma_{12}y_2, \sigma_{14}y_4), & y_1 &= g_1(x_1) \\ \dot{x}_2 &= f_2(x_2, \sigma_{21}y_1), & y_2 &= g_2(x_2) \\ \dot{x}_3 &= f_3(x_3, \sigma_{32}y_2), & y_3 &= g_3(x_3) \\ \dot{x}_4 &= f_4(x_4, \sigma_{43}y_3), & y_4 &= g_4(x_4) \end{aligned}$$

where each subsystem is an MSR system with respect to all of its inputs $\sigma_{ij}y_j$. The system structure is given by the sign pattern, sign$[\Sigma]$, of the interaction matrix

$$\Sigma \doteq \begin{bmatrix} 0 & \sigma_{12} & 0 & \sigma_{14} \\ \sigma_{21} & 0 & 0 & 0 \\ 0 & \sigma_{32} & 0 & 0 \\ 0 & 0 & \sigma_{43} & 0 \end{bmatrix}. \tag{16}$$

After linearization, the above system corresponds to

$$\begin{aligned} y_1(s) &= F_{12}(s)\sigma_{12}y_2(s) + F_{14}(s)\sigma_{14}y_4(s) \\ y_2(s) &= F_{21}(s)\sigma_{21}y_1(s) \\ y_3(s) &= F_{32}(s)\sigma_{32}y_2(s) \\ y_4(s) &= F_{43}(s)\sigma_{43}y_3(s) \end{aligned}$$

where $F_{ij}(t) = \mathcal{L}^{-1}[F_{ij}(s)]$ are generic PIRs ($\mathcal{L}$ denotes the Laplace transform operator and $\mathcal{L}^{-1}$ its inverse). Then, the question is: which kind of instability is possible, given the sign pattern sign$[\Sigma]$?

IV. Problem Definition and Main Results

We assume that the transfer functions of the subsystems are admissible, according to the following definition.

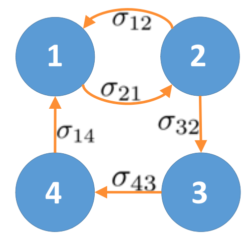

Fig. 2. Aggregate graph of the system with the interaction matrix (16).

Definition 4: If $F(t)$ is the impulse response of a linear single-input single-output system, then the Laplace transform

$$F(s) = \mathcal{L}[F(t)] = \int_0^\infty F(t)e^{-st}dt$$

is its *transfer function*[1]. The transfer function

$$F(s) = e^{-s\tau}G(s)$$

is *admissible* if G is rational, strictly proper (hence, $\lim_{s\to\infty} F(s) = 0$) and stable (namely, its poles have negative real parts), and the delay $\tau > 0$. ◇

A (possibly small) delay is always present in practice; from a technical point of view, the presence of a delay will allow us to provide clean necessary and sufficient conditions.

With a slight abuse of terminology, we call *PIR transfer function* a transfer function $F(s)$ corresponding to a PIR $F(t)$.

Let $y(s)$ be an N-dimensional vector including the Laplace-transformed outputs of the N MSR linearized subsystems that compose the overall system. Then, our model can be written as

$$y(s) = \Phi(s)y(s) \tag{17}$$

where matrix $\Phi(s)$ has entries of the form

$$\Phi_{ij}(s) = \sigma_{ij}F_{ij}(s) \tag{18}$$

with $F_{ij}(s)$ admissible PIR transfer functions.

The interaction matrix Σ, whose entries are the interaction coefficients σ_{ij}, is the weighted adjacency matrix of the oriented *aggregate graph*, where the nodes represent the MSR subsystems. In the graph, there exists an arc from node j to node i if and only if $\sigma_{ij} \neq 0$, namely, if and only if y_j affects y_i. As an example, the interaction matrix (16) is associated with the graph in Fig. 2. The arc from node j to node i can be either positive or negative, depending on the sign of σ_{ij}.

Definition 5: Given a graph, a *cycle* is an oriented, closed sequence of distinct nodes connected by distinct directed arcs. A cycle is *negative* (*positive*) if the number of negative arcs involved in the cycle is odd (even). ◇

If the graph is represented by the matrix Σ, a cycle is associated with a sequence of nonzero off-diagonal entries: $\{\sigma_{k_2,k_1}\sigma_{k_3,k_2}\ldots\sigma_{k_s,k_{s-1}}\sigma_{k_1,k_s}\}$. We assume that at least a cycle exists in the aggregate graph.

Definition 6: Given an aggregate of interconnected subsystems, matrix $S = \text{sign}[\Sigma]$ is the system *structure*, while matrix Σ is a *realization* of structure S. ◇

[1] We accept the standard notation abuse of denoting $\mathcal{L}[F(t)]$ as $F(s)$

TABLE I
STRUCTURAL CLASSIFICATION IN THEOREM 3

	Candidate oscillator	Candidate bistable system
Weak	A negative cycle exists	A positive cycle exists
Strong	All cycles are negative	All cycles are positive

The overall system is stable if the interactions σ_{ij} are small, because each subsystem is assumed to be stable. Hence, potential instability can be due to the interactions only.

Definition 7: Matrix Σ^* is a *critical realization* if, in each neighborhood $\mathcal{N}_\epsilon = \{\Sigma : \|\Sigma - \Sigma^*\| \leq \epsilon\}$ of radius $\epsilon > 0$, the structure $S = \text{sign}[\Sigma^*]$ admits both asymptotically stable and exponentially unstable realizations. ◇

We consider systems with a perturbing input vector u as follows:

$$y(s) = \Phi(s)y(s) + \Delta u(s) \tag{19}$$

where Δ is a diagonal matrix with non-negative entries.

For example, if Δ has a single positive diagonal entry $\Delta_{kk} > 0$, then the step response of the system shows (roughly speaking) the reaction to a persistent "injection" of y_k from the outside, whereas the impulse response shows the reaction to the instantaneous addition of a "large" amount of y_k.

Let us introduce the following classification.

Definition 8: Given a system of the form (19) having structure $S = \text{sign}[\Sigma]$, assume that a step input is applied to a single variable y_k (namely, $\Delta_{kk} > 0$ and $\Delta_{jj} = 0$ for all $j \neq k$). Then, the system is:

1) a *strong candidate bistable system:* if, for any choice of admissible functions and any critical realization Σ^*, the step response of all variables $y_i(t)$ $(i = 1, \ldots, N)$ with zero initial conditions is either monotonically increasing, or monotonically decreasing, for all $k \in \{1, \ldots, N\}$;
2) a *strong candidate oscillator:* if, for any choice of admissible functions and any critical realization Σ^*, the step response of all variables $y_i(t)$ $(i = 1, \ldots, N)$ with zero initial conditions is not monotone, for all $k \in \{1, \ldots, N\}$;
3) a *weak candidate bistable system:* if, for some choice of admissible functions and some critical realization Σ^*, the step response of all variables $y_i(t)$ $(i = 1, \ldots, N)$ with zero initial conditions is either monotonically increasing, or monotonically decreasing, for all $k \in \{1, \ldots, N\}$;
4) a *weak candidate oscillator:* if, for some choice of admissible functions and some critical realization Σ^*, the step response of all variables $y_i(t)$ $(i = 1, \ldots, N)$ with zero initial conditions is not monotone, for all $k \in \{1, \ldots, N\}$. ◇

Remark 2: In practice, the critical configuration is achieved either due to a single eigenvalue at zero, for candidate bistable systems, or due to a single pair of purely imaginary eigenvalues, for candidate oscillators. However, for the sake of generality, we consider the case of possibly many eigenvalues on the imaginary axis. ◇

We have the following result, summarized in Table I.

Theorem 3: A system of the form (19) having the structure $S = \text{sign}[\Sigma]$, associated with an aggregate graph, is:

1) a strong candidate bistable system if and only if all of the cycles in the aggregate graph are positive;
2) a strong candidate oscillator if and only if all of the cycles in the aggregate graph are negative;
3) a weak candidate bistable system if and only if there exists at least one positive cycle in the aggregate graph;
4) a weak candidate oscillator if and only if there exists at least one negative cycle in the aggregate graph. ■

V. PROOF OF THE MAIN RESULTS

A. Preliminaries

The following results are preliminary for the proof of Theorem 3, but they are of interest *per se*.

Proposition 3: Given the PIR $F(t)$ of an asymptotically stable system, the corresponding transfer function $F(s)$ is positive for s real and non-negative. ■

Proof: From the expression of the Laplace transform, it immediately follows that $F(s) > 0$ for any real $s \geq 0$. ■

In particular, $F(s) > 0$ for $s = 0$. An immediate consequence is the following:

Proposition 4: Given the transfer function $F(s)$ corresponding to an asymptotically stable system with PIR $F(t)$, the negative loop characteristic equation $1 + F(s) = 0$ cannot have 0 roots

$$1 + F(0) \neq 0.$$

Hence, the negative loop has no zero poles. ■

The following result considers the cascade (series connection) of transfer functions.

Proposition 5: The cascade of PIR transfer functions is a PIR transfer function. ■

Proof: It follows from the convolution expression; if $y(s) = F_1(s)F_2(s)1$, where $1 = \mathcal{L}[\delta(t)]$, then

$$y(t) = \int_0^t F_1(t-\theta)F_2(\theta)d\theta$$

which is positive since F_1 and F_2 are positive. For the cascade of more transfer functions, the proof is identical. ■

Definition 9: A pole λ_1 of a transfer function $F(s)$ is *dominant* if any other pole λ of $F(s)$ has a nongreater real part: $Re(\lambda) \leq Re(\lambda_1)$. A real pole, or a pair of complex poles, is *strictly dominant* if the inequality is strict: for all other poles λ, $Re(\lambda) < Re(\lambda_1) = Re(\lambda_1^*)$.

Proposition 6: A PIR transfer function cannot have strictly dominant imaginary poles different from zero. ■

Proof: It is immediate, since a pair of dominant imaginary poles $\pm\jmath\omega$, with $\omega \neq 0$, would introduce oscillations. ■

Note that a PIR transfer function can have both zero and imaginary poles, as long as zero is dominant; for instance,

$$F(t) = \mathcal{L}^{-1}\left[\frac{2}{s} + \frac{1}{s^2+1}\right] = 2 + \sin(t) > 0.$$

Proposition 7: Given the transfer function $F(s)$ corresponding to the PIR $F(t)$ of an asymptotically stable system, the non-negative feedback loop transfer function

$$W(s) = \frac{1}{1-\mu F(s)}, \quad \mu \geq 0$$

is associated with a PIR (MSR) system. ■

Proof: The step response satisfies the equation

$$y(t) = \int_0^t \mu F(t-\theta) y(\theta) d\theta + 1.$$

Since the integrand function is non-negative, $y(t)$ is monotonically increasing. ■

Corollary 1: Given the transfer function $F(s)$ corresponding to the PIR $F(t)$ of an asymptotically stable system, the complementary sensitivity function

$$W(s)F(s) = \frac{F(s)}{1-\mu F(s)}, \quad \mu \geq 0$$

is associated with a PIR (MSR) system. ■

Proof: It follows from Proposition 5, the complementary sensitivity function is the cascade of two PIR transfer functions. ($F(s)$ is a PIR transfer function by assumption, and $W(s)$ is a PIR transfer function in view of Proposition 7.) ■

B. *Proof of Theorem 3*

1) All Cycles Positive $\Longrightarrow$ Strong Candidate Bistable System: If all of the cycles are positive, then there exists a sign-change transformation $\tilde{y}_k = \pm y_k$, such that, after changing sign to some nodes and to the arcs incident in these nodes, all of the arcs become positive [32]. Assume that the transformation has been applied, so that all of the nonzero coefficients σ_{ij} are positive.

Then, let us consider the time-domain response to a step input, weighted by the diagonal matrix Δ.

We first temporarily assume that all Δ_{kk} are strictly positive. Then

$$y(t) = \int_0^t \Phi(t-\theta) y(\theta) d\theta + \Delta \mathbf{1} \tag{20}$$

where $\mathbf{1}$ is the $N \times 1$ vector of all ones. For t_1 small enough, $y(t)$ is componentwise positive for $0 \leq t \leq t_1$, because the integral is small. Also, in a right neighborhood of t_1, the contribution of the integral is positive ($\Phi \geq 0$ componentwise, because $\sigma_{ij} \geq 0$ for all i, j in view of the transformation). Hence, when we extend the integral interval, $y(t)$ increases.

Now apply the positive step to a single variable, say y_1. So, $\Delta_{11} > 0$ only (and $\Delta_{jj} = 0$ for all $j \neq 1$), as per Definition 8. Then, $y_1(t)$ is positive in an interval $0 \leq t \leq t_1$, with t_1 arbitrarily small. For all of the variables y_k such that node k is directly connected with node 1 in the aggregate graph

$$y_k(t) = \int_0^t \sum_{j=1}^N \Phi_{kj}(t-\theta) y_j(\theta) d\theta. \tag{21}$$

Then, all of these variables become positive at a time instant $0 < t_2 \leq t_1$, because all of the terms in the integral are non-negative and at least that depending on y_1 is positive. By iterating the reasoning, if the graph is connected, we have that all of the variables become positive at some time $0 < \bar{t} \leq \cdots \leq t_2 \leq t_1$. Hence, since the integrals always provide a positive contribution (for the same argument presented before), $y_i(t)$ is monotonically increasing for all i. If the graph is not connected, then the response is monotonically increasing for all of the nodes in the same connected component as node 1, while the response is 0 for the other nodes. ■

2) All Cycles Positive $\Longleftarrow$ Strong Candidate Bistable System: Assume, by contradiction, that there exists a negative cycle of length l, involving the variables y_1, y_2, ..., y_l. Then, we can set all of the coefficients σ_{ij} to "virtually" zero (cf. [8] and [9]), except for those pertaining to the negative cycle. If y_1 is taken as an output, the resulting loop is

$$\begin{aligned} y_1(s) &= -\prod_{k=1}^{l} |\sigma_{k,k-1}| F_{k,k-1}(s) e^{-\left(\sum_{k=1}^{l} \tau_{k,k-1}\right)s} y_1(s) \\ &\doteq -\sigma_c F_c(s) e^{-\tau_c s} y_1(s) \end{aligned}$$

where the subscript 0 corresponds to l, $\sigma_c = \prod_{k=1}^{l} |\sigma_{k,k-1}|$, $F_c(s) = \prod_{k=1}^{l} F_{k,k-1}(s)$, and $\tau_c = \sum_{k=1}^{l} \tau_{k,k-1}$. Since $F_c(0) > 0$, in view of Proposition 3, there are no poles at $s = 0$, because the characteristic equation

$$1 + \sigma_c F_c(s) e^{-\tau_c s} = 0$$

is not satisfied by $s = 0$. For $\sigma_c > 0$ small, the loop is asymptotically stable by assumption, since all elements $F_{k,k-1}(s)$ are stable. However, as we can see via Nyquist plot analysis, if we increase $\sigma_c > 0$, there is necessarily a critical value σ_c^* for which a pair of imaginary roots $\pm j\omega^*$ (associated with an undamped oscillatory mode [25]) appear, with all other roots having nonpositive real part. At $s = 0$, we have $\sigma_c^* F_c(0) > 0$. For the sign conservation theorem, $F_c(\lambda)$ must be positive in an interval $\lambda \in (-\zeta, 0]$, where $1 + \sigma_c F_c(\lambda) e^{-\tau_c \lambda} > \sigma_c F_c(\lambda) e^{-\tau_c \lambda} > 0$. Then, there cannot exist real poles of the closed-loop transfer function (associated with nonoscillatory modes) that are larger than $-\zeta$: real modes, if any, are converging exponentially, faster than $e^{-\zeta t}$. The presence of persistent oscillatory modes implies that, if we apply an impulse to y_1, the response of the loop is oscillatory; hence, it has both positive and negative values: the system is not a strong candidate bistable system. ■

3) All Cycles Negative $\Longrightarrow$ Strong Candidate Oscillator: We show that if all cycles are negative, then no critical configuration (at the stability boundary) can have zero eigenvalues. The loop equation corresponding to (17) is

$$\det[-I + \Phi(s)] = 0. \tag{22}$$

We now invoke the following result, from [23, Theor. 3.1].

Theorem 4: Given a real matrix M with negative diagonal entries, such that all of the cycles in it are nonpositive, each leading minor of M having order k has sign $(-1)^k$. ■

As a corollary, the determinant of M is nonzero. If we take $s = 0$, then (22) becomes the real equation $\det[-I + \Phi(0)] = 0$, which is false (because the matrix satisfies the assumptions of Theorem 4, hence it must be nonsingular). Therefore, any critical configuration must have purely imaginary dominant eigenvalues. Hence, there are undamped oscillatory modes and the step response cannot be monotone [25]. ■

4) All Cycles Negative $\Longleftarrow$ Strong Candidate Oscillator: Assume, by contradiction, that there is a positive cycle of length l, involving the variables $y_1, y_2, ..., y_l$. Then, we set to "virtually" zero all of the coefficients σ_{ij} that are not involved in this cycle. Considering the resulting loop, with y_1 taken as an output, $\Delta_{11} > 0$ and a constant unitary step input, we obtain

$$y_1(s) = \sigma_c F_c(s) e^{-\tau_c s} y_1(s) + \Delta_{11} \frac{1}{s}$$

where $\sigma_c = \prod_{k=1}^{l} \sigma_{k,k-1} > 0$, $F_c(s) = \prod_{k=1}^{l} F_{k,k-1}(s)$, and $\tau_c = \sum_{k=1}^{l} \tau_{k,k-1}$, with the subscript 0 corresponding to l. In the time domain, we have the convolution

$$y_1(t) = \int_0^t \sigma_c F_c(t-\theta) y_1(t-\tau_c) d\theta + \Delta_{11}.$$

Since the integrand function is positive, $y_1(t)$ is monotonically increasing, so this is not a strong candidate oscillator. ■

The proof of the two last statements follows immediately from the fact that, by definition, a system with structure S is not a strong candidate oscillator if and only if it is a weak candidate bistable system and is not a strong candidate bistable system if and only if it is a weak candidate oscillator. ■

Our classification is stated in terms of monotonicity and nonmonotonicity of the step response for all/some critical configurations. We have seen that when the dominant eigenvalues are *purely imaginary*, then the step response cannot be monotonic, because an MSR requires a *real* dominant eigenvalue.

However, a transfer function with a (even strictly) dominant zero pole is not necessarily associated with an MSR (or with a sign-definite impulse response). The transfer function

$$F(s) = \frac{1-s}{s(s+1)} = \mathcal{L}\left[1 - 2e^{-t}\right]$$

for instance, is not PIR. However, this impulse response is positive for large values of t.

To consider this point, we can call the step response *eventually monotonic* if there is $\bar{t} \geq 0$ such that the step response is monotonically increasing or decreasing for $t > \bar{t}$. Definition 8 can be then restated in terms of eventually monotonic (instead of monotonic) step responses just by replacing "monotonically increasing/decreasing" with "eventually monotonically increasing/decreasing." With this new definition, the ambiguity associated with the presence of a strictly dominant zero pole disappears: If all other poles have a negative real part, then the step response is eventually monotonic.

It is worth stressing that with the new definition, the proposed classification would hold without changes. Indeed, going back to the proof of Theorem 3: all cycles being positive implies that the step responses are monotonic, hence eventually monotonic. Conversely, in the presence of a negative cycle, the system admits a critical configuration with dominant imaginary poles, corresponding to step responses that are not eventually monotonic. Furthermore, if all cycles are negative, any critical configuration has imaginary dominant poles; therefore, there are persistently oscillatory modes and the step responses are not eventually monotonic. On the other hand, the presence of a positive cycle implies that, by "virtually eliminating" all other cycles, we get a monotonic (hence eventually monotonic) step response.

VI. Consequences of the Classification

The results in the previous section have some interesting consequences.

For instance, a *positive interconnection of PIR subsystems* (an interconnection such that all of the cycles in the aggregate graph are positive; namely, a strong candidate bistable system) has some properties in common with monotone systems. In particular, if we increase one variable by adding a persistent positive input, all of the others increase as well.

Corollary 2: A positive interconnection of PIR subsystems is a PIR system, regardless of which variable y_k is chosen as an output and to which y_h the positive input is applied. ■

Remark 3: To build the *structural influence matrix* M [17], which is a sign matrix, we apply a step input to the jth system variable and we consider the sign of the ensuing steady-state variation of the ith variable. The structural steady-state influence is determined if M_{ij} is sign definite; if the sign depends on the parameters, the influence is indeterminate and in this case we write $M_{ij} =$ "?." For a positive interconnection of PIR subsystems, matrix M is a sign definite and has all "+" entries. This particular property had been shown to hold, as a special case, for monotone systems [17]. ◇

Another property concerns the worst case input signal, namely, the signal $|u| \leq 1$ that produces the largest output deviation from a nominal condition. It is well known that for an input–output monotone system, the worst case input is a constant signal. For a PIR system, the same property holds. Assuming a zero initial condition, the worst case deviation is

$$\sup_{|u(\cdot)| \leq 1, t \geq 0} |y(t)| = \int_0^\infty F(t) dt$$

and the worst case input is a step.

For a strong candidate bistable system, we also have the following results.

Proposition 8: A strong candidate bistable system always has a real dominant eigenvalue for any configuration Σ. ■

Proof: Since the system is a strong candidate bistable system, if Σ is critical, then it must have a zero dominant eigenvalue. Let Σ be noncritical and let λ^* be the largest real part of the eigenvalues. If we artificially replace $F_{kh}(s)$ by $F_{kh}(s - \lambda^*)$, then we achieve a critical configuration for the same system, in which all of the impulse responses are replaced as follows:

$$F_{kh}(t) \longrightarrow e^{-\lambda^* t} F_{kh}(t).$$

This operation does not alter positivity of the impulse responses. However, all eigenvalues are translated of $-\lambda^*$. Thus, there cannot be dominant complex eigenvalues, because the translation would lead to a critical configuration with nonzero imaginary eigenvalues (hence, to oscillations). ■

Corollary 3: Consider a configuration Σ corresponding to a strong candidate bistable system. If Σ is critical, then it remains critical for any possible value of the delays. ■

Proof: For any critical configuration of a strong candidate bistable system, 0 is the dominant eigenvalue. For $s = 0$,

$e^{-\tau_k 0} = 1$. Hence, 0 remains the dominant eigenvalue for any possible choice of the delays τ_k. ■

VII. Review of Criteria for Establishing PIR

Does a given linear system have a PIR transfer function? This problem has been considered for a long time [20], [22] and is not fully solved. Sufficient conditions, as well as necessary conditions, are available in terms of zeros and poles.

Also, the link between PIR systems and monotone (positive, in the linear case) systems is worth investigating. Any input–output monotone linear system is a PIR system. The opposite question is: does a PIR transfer function admit a positive realization? This is the positive realization problem [15]. Under proper assumptions, any PIR transfer function admits a positive realization, but this realization is nonminimal: to find a state-space representation that is input–output monotone, the state needs to be artificially augmented [27]. This augmentation can be avoided, under some assumptions, by considering eventually positive minimal realizations [1].

We summarize a set of properties concerning PIR systems.

The rational transfer function $F(s)$ is a PIR transfer function:

1) iff its step response is monotonically nondecreasing;
2) only if it has no complex strictly dominant poles;
3) if it is the positive feedback of a PIR system;
4) if it is the cascade of PIR systems;
5) if it has n real poles and no zeros;
6) if it has n real poles and $m < n$ real zeros with the ordering property $-p_1 > -z_1,\ -p_2 > -z_2,\ \ldots -p_m > -z_m$, whereas the other real poles are arbitrary [20], [22].

Criterion (6) can be proved by noting that $F(s)$ can be written as the product of terms of the type $\frac{\mu_k}{s+p_k}$, which are PIR due to criterion (5), and terms of the type $\frac{s+z_k}{s+p_k}$, which have a PIR if $-p_k > -z_k$ [20], [22]. Hence, the whole transfer function is PIR, in view of criterion (4).

Example 2: To demonstrate the application of the criteria, consider the chemical reaction network

$$X_1 + X_2 \overset{g_{12}}{\rightharpoonup} X_3,\ X_3 \overset{g_3}{\rightharpoonup} X_1,\ X_2 \overset{g_2}{\rightharpoonup} \emptyset,\ X_3 \overset{\tilde{g}_3}{\rightharpoonup} \emptyset$$
$$\emptyset \overset{x_{1,0}}{\rightharpoonup} X_1,\ \emptyset \overset{x_{2,0}}{\rightharpoonup} X_2.$$

Chemical species are denoted with uppercase letters and their concentrations with the corresponding lowercase letter. In the presence of an additive input u affecting x_1, and taking x_3 as an output, the concentrations evolve according to the equations

$$\begin{aligned}\dot{x}_1 &= -g_{12}(x_1,x_2) + g_3(x_3) + x_{1,0} + u\\ \dot{x}_2 &= -g_{12}(x_1,x_2) - g_2(x_2) + x_{2,0}\\ \dot{x}_3 &= +g_{12}(x_1,x_2) - g_3(x_3) - \tilde{g}_3(x_3)\\ y &= x_3\end{aligned}$$

where all reaction rate functions (gs and $\tilde{g}_3$) are increasing and $x_{1,0}$, $x_{2,0}$ are positive terms. If we denote the positive partial derivatives by $\alpha = \partial g_{12}/\partial x_1$, $\beta = \partial g_{12}/\partial x_2$, $\gamma = \partial g_3/\partial x_3$, $\delta = \partial g_2/\partial x_2$ and $\epsilon = \partial \tilde{g}_3/\partial x_3$, and $x = [x_1 \quad x_2 \quad x_3]^\top$, the linearized system can be written as system (12)–(13) in Example 1, which has transfer function (14).

For any possible choice of the positive parameters, the application of the Routh–Hurwitz criterion shows that this system is asymptotically stable. To prove that it is also a PIR system for all possible choices of $\alpha, \beta, \gamma, \delta, \epsilon > 0$, note that it can be viewed as the feedback loop

$$y(s) = \frac{\alpha(s+\delta)}{(s+\alpha)(s+\delta)+s\beta}\ \frac{1}{s+\gamma+\epsilon}(u(s)+y(s)). \quad (23)$$

According to criterion (3) (see also Proposition 7), the positive feedback of a PIR system yields a PIR transfer function, so we just need to show that the transfer function in (23) is PIR. This function is the cascade connection of

$$F_2(s) \doteq \frac{1}{s+\gamma+\epsilon}$$

which is a PIR transfer function due to criterion (5), and of

$$F_1(s) \doteq \frac{\alpha(s+\delta)}{(s+\alpha)(s+\delta)+s\beta}.$$

Since, according to criterion (4), the cascade connection of PIR transfer functions is a PIR transfer function, we need only to show that $F_1(s)$ is a PIR transfer function.

$F_1(s)$ has two real negative poles $-\lambda_1 > -\lambda_2$ and one real negative zero $-\delta$. Moreover, the dominant pole $-\lambda_1$ is strictly greater than the zero ($\lambda_1 < \delta$). The denominator of the transfer function evaluated at $s = -\delta$ is

$$(s+\alpha)(s+\delta)+s\beta|_{s=-\delta} = -\delta\beta < 0.$$

For s real, this second-order polynomial is a parabola having positive limits at $s = \pm\infty$. Hence, its roots are, respectively, to the right and to the left of $-\delta$. In view of criterion (6), $F_1(s)$ is a PIR transfer function, and our proof is over. ◇

VIII. Examples

A. Negative Autoregulation Yields an MSR Module

Reconsider the gene expression (transcription–translation) system with negative autoregulation discussed in Section I-A. After linearization around the equilibrium $(\bar{x}, \bar{y})$, we can notice that $a\gamma/(A+\bar{y})^2 = \alpha\beta\bar{y}/(A+\bar{y})$ in view of the equilibrium conditions and then we can write the transfer function as

$$F(s) = \frac{n(s)}{d(s)} = \frac{\gamma}{s^2 + (\alpha+\beta)s + \alpha\beta\left(1 + \frac{\bar{y}}{A+\bar{y}}\right)}. \quad (24)$$

Since $0 < \frac{\bar{y}}{A+\bar{y}} < 1$, this system does not have complex poles if the roots of the polynomial $s^2 + (\alpha+\beta)s + 2\alpha\beta$, obtained by replacing $\bar{y}/(A+\bar{y})$ with 1, are real. This happens when

$$\frac{(\alpha-\beta)^2}{\alpha\beta} > 4 \quad (25)$$

a condition that is normally verified by typical degradation rates α and β in bacteria. Since there are no zeros and all of the poles are real, the linearized system is a PIR system in view of criterion (5).

Therefore, this fundamental module in both systems and synthetic biology is indeed an MSR module.

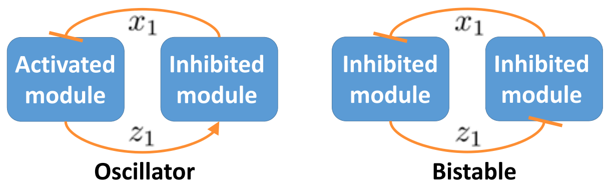

Fig. 3. Left: biomolecular oscillator in [12], built as the overall negative feedback interconnection of an activated and an inhibited module. Right: biomolecular bistable system in [12], built as the overall positive feedback interconnection of two mutually inhibiting modules.

B. Monomeric Activator–Inhibitor Loop: An Oscillator

While some synthetic biomolecular oscillators have been shown to be the negative feedback interconnection of input–output monotone modules [6], [11], this is not the case for the system considered in [12].

The biomolecular oscillator in [12] is the interconnection of an activated module, having equations

$$\begin{aligned} \dot{z}_1 &= \alpha_z(z_1^{tot} - z_1)x_3 - \delta_z z_1 z_2 \\ \dot{z}_2 &= \kappa_z(z_2^{tot} - z_2 - z_1^{tot} + z_1) - \delta_z z_1 z_2 - \nu_z x_3 z_2 \\ \dot{x}_3 &= \beta_x x_1 - \alpha_z(z_1^{tot} - z_1)x_3 - \nu_z x_3 z_2 - \phi_z x_3 \end{aligned} \tag{26}$$

where x_1 is the input and z_1 is the output, and of an inhibited module, having equations

$$\begin{aligned} \dot{x}_1 &= \alpha_x(x_1^{tot} - x_1)x_2 - \delta_x x_1 z_3 \\ \dot{x}_2 &= \kappa_x(x_2^{tot} - x_2 - x_1) - \alpha_x(x_1^{tot} - x_1)x_2 - \nu_x x_2 z_3 \\ \dot{z}_3 &= \beta_z z_1 - \delta_x x_1 z_3 - \nu_x x_2 z_3 - \phi_x z_3 \end{aligned} \tag{27}$$

where z_1 is the input and x_1 is the output. This results in an overall negative feedback loop: the only cycle in the aggregate graph is negative, as shown in Fig. 3 (left). For these two modules, no monotonicity property can be proved, unless we neglect the titration reactions by assuming $\nu_x = \nu_z = 0$. (See [12] for details.) However, for the nominal value of the parameters, both modules are MSR systems; to be precise, the activated module has a monotonically increasing step response, whereas the inhibited module has a monotonically decreasing step response: the overall interconnection can be seen as the negative feedback of two MSR modules. Also, it can be numerically shown that for large ranges of the parameters, this property is very likely to be preserved, as discussed below. Then, whenever the MSR property holds, the overall system can be classified as a *strong candidate oscillator*.

Following the approach in [13], we generated random parameter values in the range from 10^{-a} to 10^{a} times the nominal values listed in Table II. Next, we used MATLAB to integrate the ordinary differential equations with zero initial conditions: The response is considered monotone if the numerical derivative of z_1 (respectively, x_1) is always positive (respectively, negative). We considered 10 000 samples: in the range with $a = 1$, the fraction of MSR occurrences was 59.44% for the first module, 83.61% for the second. With a larger sampling range $a = 3$, the fraction of MSR occurrences was 62.57% for the first module and 70.83% for the second; the plots in Fig. 4 show some projections in the parameter space.

TABLE II
NOMINAL PARAMETERS FOR THE OSCILLATOR IN (26) AND (27) [12]

Rate	Value	Rate	Value
α_z (/M/s)	$75 \cdot 10^3$	α_x (/M/s)	$3 \cdot 10^5$
δ_z (/M/s)	$3 \cdot 10^5$	δ_x (/M/s)	$3 \cdot 10^5$
ν_z (/M/s)	$3 \cdot 10^5$	ν_x (/M/s)	$3 \cdot 10^5$
β_z (/s)	$5 \cdot 10^{-3}$	β_x (/s)	$2 \cdot 10^{-2}$
κ_z (/s)	$1 \cdot 10^{-3}$	κ_x (/s)	$1 \cdot 10^{-3}$
ϕ_z (/s)	$1 \cdot 10^{-3}$	ϕ_x (/s)	$1 \cdot 10^{-3}$
z_1^{tot} (nM)	250	x_1^{tot} (nM)	120
z_2^{tot} (nM)	700	x_2^{tot} (nM)	300

C. Monomeric Inhibitor–Inhibitor Loop: A Bistable System

Also, a biomolecular bistable system is proposed in [12], built as the interconnection of two mutually inhibiting modules, having equations

$$\begin{aligned} \dot{z}_1 &= \alpha_z(z_1^{tot} - z_1)z_2 - \delta_z z_1 x_3 \\ \dot{z}_2 &= \kappa_z(z_2^{tot} - z_2 - z_1) - \alpha_z(z_1^{tot} - z_1)z_2 - \nu_z x_3 z_2 \\ \dot{x}_3 &= \beta_x x_1 - \delta_z z_1 x_3 - \nu_z z_2 x_3 - \phi_z x_3 \end{aligned} \tag{28}$$

where x_1 is the input and z_1 is the output, and

$$\begin{aligned} \dot{x}_1 &= \alpha_x(x_1^{tot} - x_1)x_2 - \delta_x x_1 z_3 \\ \dot{x}_2 &= \kappa_x(x_2^{tot} - x_2 - x_1) - \alpha_x(x_1^{tot} - x_1)x_2 - \nu_x x_2 z_3 \\ \dot{z}_3 &= \beta_z z_1 - \delta_x x_1 z_3 - \nu_x x_2 z_3 - \phi_x z_3 \end{aligned} \tag{29}$$

where z_1 is the input and x_1 is the output. This results in an overall positive feedback loop: the only cycle in the aggregate graph is positive, as shown in Fig. 3 (right).

For each of these two inhibited modules, the same analysis applies as for the inhibited module of the biomolecular oscillator in Section VIII-B. No monotonicity property can be proved, unless titration reactions are neglected (namely, $\nu_x = \nu_z = 0$, see [12] for details). However, for the nominal value of the parameters, both modules are MSR systems; precisely, they both have a monotonically decreasing step response, so that the overall interconnection can be seen as the positive feedback of two MSR modules. Again, this property is very likely to be preserved for large ranges of the parameters, as can be numerically shown. Then, whenever the MSR property holds, the overall system can be classified as a *strong candidate bistable system*.

IX. Concluding Discussion

Many biochemical systems are monotone [30], [32], or can be regarded as the interconnection of monotone subsystems (the Cds-Wee1 network [3], the MAPK pathway [32], the Goldbeter oscillator [4] in *Drosophila*, etc). However, to asses monotonicity, we need a state-space model, which is not always easy to provide for complex biomolecular networks.

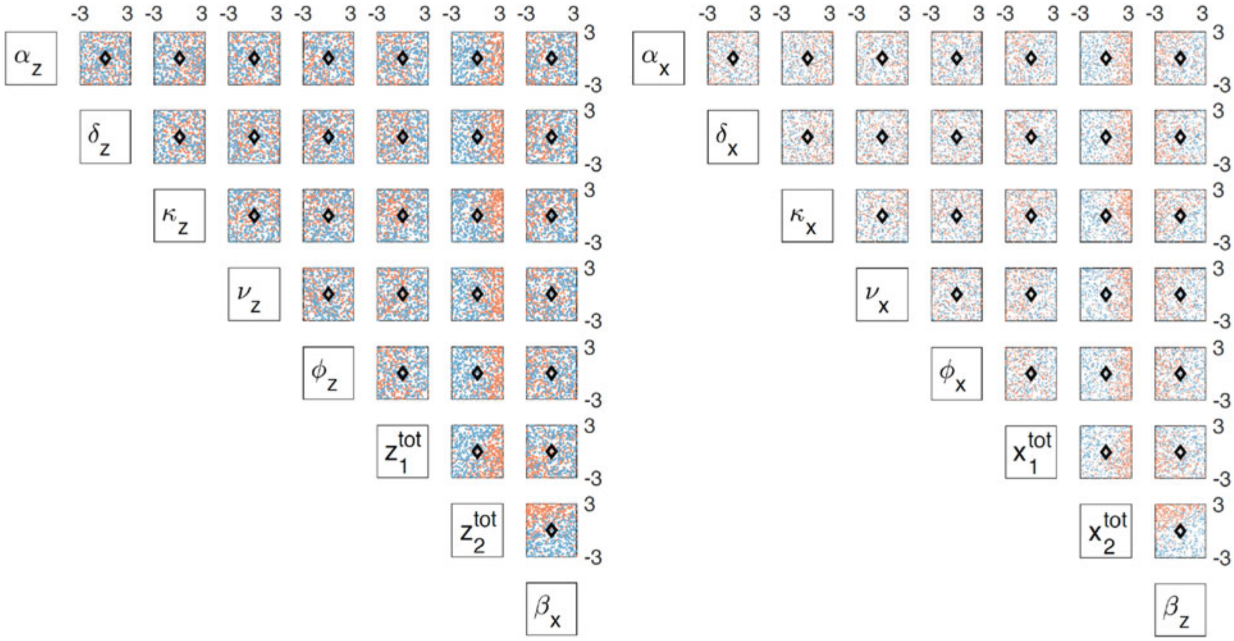

Fig. 4. Projections for random parameter choices in the range from 10^{-3} to 10^{3} times the nominal values (for the activated module on the left, for the inhibited module on the right). Points corresponding to MSR modules are cyan, whereas points corresponding to non-MSR modules are orange; the black diamond indicates the nominal parameter set. Note that, for the sake of clarity, just 350 of the 10 000 analyzed samples are actually shown in the plots.

A natural and much more general system decomposition can be achieved by considering aggregates of MSR systems. In this paper, we have shown that the *structural* classification of oscillatory and multistationary systems proposed in [8] for sign-definite systems and in [9] for *aggregate monotone systems* can be adapted to MSR aggregates. The classification is based on the exclusive presence of negative or positive cycles in the system aggregate graph, whose nodes are the MSR subsystems.

For significant biochemical examples, our classification provides a parameter-free method to assess or rule out potential dynamic behaviors. This approach can then be useful to design artificial biomolecular circuits that are structurally well suited to achieve the desired dynamics: bistable and oscillatory behaviors can be enforced by design in synthetic biomolecular circuits, by properly interconnecting MSR modules.

There are several directions for future work. First, it would be interesting to consider trajectories, rather than single equilibria: in this sense, the variational approach in [10] is very promising. Also, the connection between PIR systems and eventually monotone systems [1], [26] (and differentially positive systems [16]) has not been completely explored here and deserves further investigation. Finally, structural conditions on sign patterns related to eventual positivity [14] could be applied to provide further insight into the problem.

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