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# A structural classification of candidate oscillatory and multistationary biochemical systems

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**Abstract** Molecular systems are uncertain: the variability of reaction parameters and the presence of unknown interactions can weaken the predictive capacity of solid mathematical models. However, strong conclusions on the admissible dynamic behaviors of a model can often be achieved without detailed knowledge of its specific parameters. In systems with a sign definite Jacobian, for instance, cycle-based criteria related to the famous Thomas' conjectures have been largely used to characterize oscillatory and multistationary dynamic outcomes.

We build on the rich literature focused on the identification of potential oscillatory and multistationary behaviors using parameter–free criteria. We propose a classification for sign–definite non–autocatalytic biochemical networks which summarizes several existing results in the literature. We call weak (strong) candidate oscillators systems which can possibly (exclusively) transition to instability due to the presence of a complex pair of eigenvalues, while we call weak (strong) candidate multistationary systems those which can possibly (exclusively) transition to instability due to the presence of a real eigenvalue. For each category we provide a characterization based on the exclusive or simultaneous presence of positive and negative cycles in the associated sign graph.

Most realistic examples of biochemical networks fall in the gray area of systems in which both positive and negative cycles are present: therefore, both oscillatory and bistable behaviors are in principle possible.

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However, many canonical example circuits exhibiting oscillations or bistability fall in the categories of strong candidate oscillators/multistationary systems, in agreement with our results.

**keywords** Feedback loops, cycles, multistationarity, stability, oscillator, structural properties

## 1 Introduction

What interaction networks can generate multistationary and periodic behaviors in molecular systems? This is a fundamental question in the area of systems and synthetic biology, as it is relevant both when *analyzing* biochemical networks naturally present in an organism, and when *building* artificial networks to achieve target dynamics. However, the variability and uncertainty characterizing molecular circuits pose significant challenges to answering such question. Mathematical models facilitate and support the analysis and interpretation of uncertain dynamic data, but models are often plagued by uncertainty as well whenever it is impractical (if not impossible) to measure their parameters. Thus, from a modeling perspective, satisfactory answers to our question may be found by identifying "structural" sources of multistationarity and periodicity, seeking parameter–free criteria to predict the capacity of a model to exhibit specific dynamic "phenotypes".

For systems with a sign definite Jacobian, parameter–free criteria to determine local admissible dynamics can be formulated by studying *Jacobian graphs*. Usually graph nodes are associated with molecular species and directed, signed edges with the interactions among them. We consider graphs where species concentrations (states of the dynamical system) are associated with nodes, and signed Jacobian entries linking different states are associated with the edges of the graph. Checking for the presence of positive or negative cycles in the Jacobian graph is a widely accepted method to explain multistationarity and oscillations in molecular/chemical systems [60]. Some of the first and best known mathematical conjectures in this area were formulated by R. Thomas [57]: given a Jacobian graph, a negative cycle is a necessary condition for stable periodic behavior, while a positive cycle is a necessary condition for multistationarity (see [15] for a very thorough survey). These conjectures were proved in [26], [24] and [51], with several further extensions and refinements [7, 31, 46, 53].

While Thomas' cycle conditions are only necessary (the dynamic phenotype of a system always depends on its specific parameters), they provide useful guidelines for analysis and design. In particular, design of feedback loops underlies the successful creation of many novel artificial biochemical networks capable of bistability [6, 21, 33, 44] or oscillations [17, 20, 32, 41, 55, 59]. In practice, however, it is extremely challenging to generate "clean" feedback loops in any experimental system. Even *in vitro* artificial networks [20, 32,

41] with a small number of components are generally affected by unknown and undesired interactions among reactants, which can create additional parasitic feedback loops of uncertain strength. This challenge motivates the search for structural criteria to discriminate dynamic outcomes of a network where several potentially unknown cycles coexist.

We contribute to the research in this direction by proposing a structural classification where we contrast *weak* and *strong candidate* oscillators and multistationary systems. Our classification depends on the exclusive or simultaneous presence of positive and negative cycles. We consider non–autocatalytic systems having a sign definite Jacobian, and we identify the *structure* of a system with the sign pattern of its Jacobian and the corresponding directed graph. A structure can be specified into a dynamic *realization* by choosing a specific set of functions along with specific parameter values. Our definition of candidate dynamic behaviors is based on the admissible types of *transition to instability*, which occurs when the eigenvalues of the Jacobian cross the imaginary axis, due to a change in parameters. Multistationarity is associated with a real eigenvalue transitioning to instability, *i.e.* changing sign from negative to positive as parameter values change, while oscillatory behaviors are associated with a complex pair of eigenvalues transitioning to instability, *i.e.* whose real part changes sign from negative to positive as parameter values change. It is well known that these phenomena are related to bifurcation theory. Typically, a transition on the real axis is related to the presence of a zero–eigenvalue bifurcation [56], while a transition with complex eigenvalues is related to a Hopf bifurcation. Formally, however, these types of bifurcation occur under a range of additional assumptions which complicate their mathematical analysis (see, for instance, [2, 38, 56]).

By our definition, weak (strong) candidate oscillators can possibly (exclusively) transition to instability due to a complex pair of eigenvalues, while weak (strong) candidate multistationary systems can possibly (exclusively) transition to instability due to a real eigenvalue. We prove that a system can be classified as a strong candidate oscillator when all cycles in its structure are negative; it can be classified as a weak candidate oscillator if there exists at least one negative cycle in its structure. Similarly, a system can be classified as a strong (weak) candidate multistationary system if its structure presents exclusively (at least one) positive cycles.

Our classification work builds on a rich body of literature [7, 24, 26, 31, 46, 51, 53, 57]. Our proofs rely on the so–called degree theory [27, 43] and on the properties of sign–definite systems [37]. Our approach can be used to characterize systems affected by delays, which are often present in models of gene networks [35]. While an extensive analysis of networks with delays is outside of the scope of this paper, in Appendix C we propose cycle–based sufficient conditions to identify candidate oscillators and multistationary systems when delays are present.

### 1.1 Motivating example

We begin with a simple example to introduce some mathematical notions used throughout this paper, and to clarify the relevance of our study in the context of biomolecular systems.

We consider a standard model for transcription and translation of two genes, where proteins reciprocally modulate their expression forming a feedback loop. Similar models are commonly encountered in the literature (see, for instance [17, 32]). For illustrative purposes, we use a nondimensional model:

$$\dot{r}_1 = \gamma_1 + H_1(p_2) - r_1, \qquad \dot{p}_1 = \beta_1 r_1 - p_1, \tag{1a}$$

$$\dot{r}_2 = \gamma_2 + H_2(p_1) - r_2, \qquad \dot{p}_2 = \beta_2 r_2 - p_2, \tag{1b}$$

where, for $i = 1, 2$, $r_i$ are RNA species concentrations; $p_i$ are protein concentrations; $H_i(\cdot)$ are Hill functions, and all Greek letters denote reaction rates that are positive scalars. Nondimensionalization is justified and carried out in detail in Appendix A (along with a boundedness proof).

Depending on the regulatory action of the proteins, and thus depending on the type of Hill function, the network presents a different number of equilibria and different possible dynamic behaviors. For example, suppose $H_1(p_2) = \alpha_1 \frac{p_2^n}{1+p_2^n}$ and $H_2(p_1) = \alpha_2 \frac{p_1^n}{1+p_1^n}$: this is a two–gene positive feedback loop, which is often encountered in developmental networks [1, 13]. The Jacobian of the system is:

$$J = \begin{bmatrix} -1 & 0 & 0 & \frac{\partial H_1}{\partial p_2} \\ \beta_1 & -1 & 0 & 0 \\ 0 & \frac{\partial H_2}{\partial p_1} & -1 & 0 \\ 0 & 0 & \beta_2 & -1 \end{bmatrix}, \qquad \text{where} \quad \frac{\partial H_i}{\partial p_j} = \alpha_i \frac{n p_j^{n-1}}{(1+p_j^n)^2}, \quad (i,j) \in \{(1,2),(2,1)\}. \tag{2}$$

The first remarkable feature is that the Jacobian entries, evaluated at a positive equilibrium, are sign definite, *i.e.* they do not change sign for arbitrary choices of the (positive) parameters $\alpha_i$, $\beta_i$ and $n$.

The Jacobian sign pattern is thus a "structural" property of this system, and it can be associated with a graph: nodes correspond to the concentrations of biochemical species and are interconnected by positive $(+1)$ or negative $(-1)$ arcs according to the corresponding Jacobian entries[1], as shown in Figure 1 a. We also remark that the system is non–autocatalytic, *i.e.* there is spontaneous degradation of each species, and the diagonal terms of the Jacobian are negative.

[1] we consider only the sign of each Jacobian entry as the weight of the corresponding arc in the graph

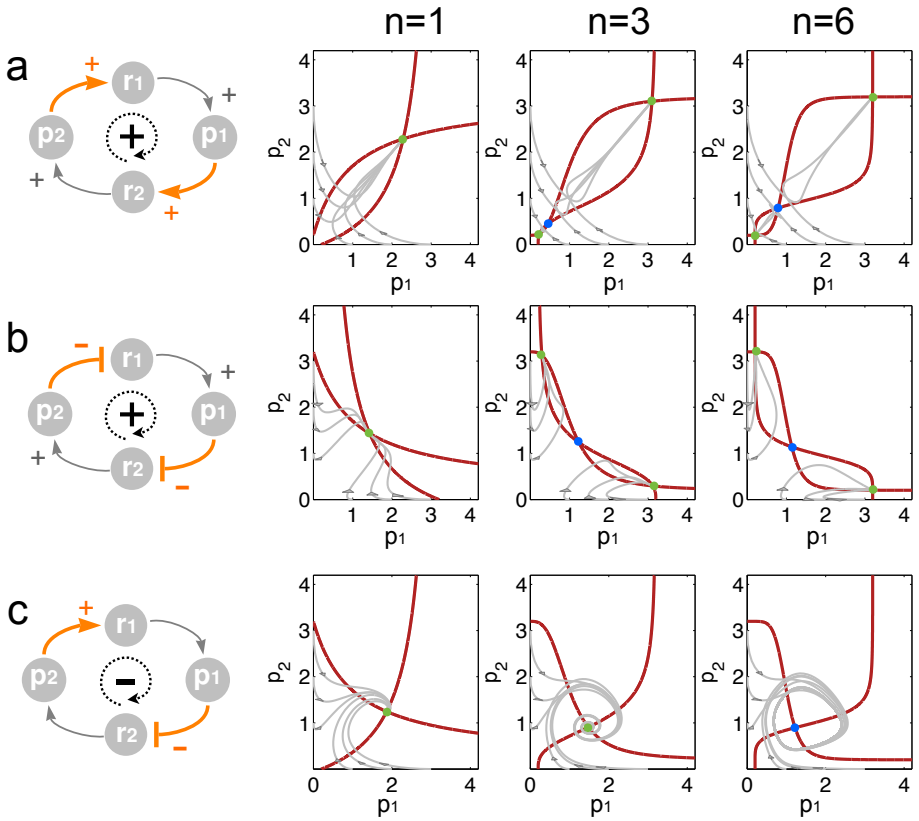


**Fig. 1** a: Two–gene system with double positive feedback loop (positive cycle). Pointed arrowheads indicate positive Jacobian interconnection entries, while hammer–arrowheads indicate negative interconnections. b: Two–gene system with double negative feedback loop, resulting in an overall positive cycle. c: Two–gene feedback interconnection with positive and negative regulation, resulting in an overall negative cycle. The nondimensional parameters are chosen as $\gamma_1 = \gamma_2 = 0.2$, $\alpha_1 = \alpha_2 = 3$, $\beta_1 = \beta_2 = 1$ and $n$ is varied. The right column shows the corresponding value of $p_2$ equilibria for varying $n$, and their different pattern of transition to instability. Green dots are stable equilibria, blue dots are unstable equilibria.

We can derive expressions for the equilibria of the system, which are given by the intersections of the two equilibrium conditions (Figure 1 a, top row):

$$p_1 = \beta_1 \left( \gamma_1 + \alpha_1 \frac{p_2^n}{1 + p_2^n} \right), \quad p_2 = \beta_2 \left( \gamma_2 + \alpha_2 \frac{p_1^n}{1 + p_1^n} \right).$$

For $n = 1$ there is an intersection with $p_1$ and $p_2$ positive. For $n > 1$, the system may admit multiple, typically three, positive equilibria. Assume that, for a certain choice of the parameters, there exists a single equilibrium. We wish to examine its stability properties by finding the eigenvalues of the Jacobian, which are the roots of its characteristic polynomial

$$(s+1)^4 - K = 0, \quad \text{where} \quad K = \beta_1 \beta_2 \frac{\partial H_1}{\partial p_2} \frac{\partial H_2}{\partial p_1} > 0. \tag{3}$$

The roots of (3) are

$$s = -1 + q, \ -1 - q, \ -1 + iq, \ -1 - iq, \quad \text{where} \quad q = K^{\frac{1}{4}}.$$

If the parameters change, then these roots change accordingly and, if instability occurs, then it is due to a single real root which becomes positive. Precisely, if $K > 1$, there is only one root having positive real part, and it is on the real axis. If $K < 1$, all of the roots have negative real part. Thus, the system can only admit real (exponential) instability, *i.e.* instability arising due to a real eigenvalue changing sign from negative to positive. The transition to instability of the equilibrium is associated with the appearance of new equilibria, a property that we will prove in general later. Figure 1 a, top row, shows equilibrium conditions and example trajectories in the $p_1 - p_2$ plane of the phase space for different values of $n$ (stable equilibria are represented as green circles, unstable equilibria as blue circles).

If $H_1 = \alpha_1 \frac{1}{1+p_2^n}$, $H_2 = \alpha_2 \frac{1}{1+p_1^n}$, network (1) specifies a two–gene double negative feedback loop, depicted in Figure 1 b, left. This circuit is also known as toggle switch, an example of which is the famous synthetic biological circuit by Gardner et al. [21]; a natural example of a toggle switch is the Cdc2–Wee1 network considered, for instance, in [3]. We can repeat the same analysis performed for the two–gene double positive feedback loop, and get similar results in terms of admissible transitions to instability, which can be only real (exponential), regardless of the considered equilibrium (Figure 1 b).

We now compare the previous two examples to the case when Hill functions have opposite regulatory roles, *i.e.* $H_1 = \alpha_1 \frac{p_2^n}{1+p_2^n}$ an $H_2 = \alpha_2 \frac{1}{1+p_1^n}$: the network can behave as a two–gene oscillator [32]. First, we observe that the Jacobian is still a sign definite matrix. However, the "interconnection" terms $\frac{\partial H_1}{\partial p_2}$ and $\frac{\partial H_2}{\partial p_1}$, the derivatives of the Hill functions, now have opposite signs, due to the different slopes of such functions, and thus generate an overall *negative* feedback loop (Figure 1 c). The equilibrium conditions are now

$$p_1 = \beta_1 \left( \gamma_1 + \alpha_1 \frac{p_2^n}{1+p_2^n} \right), \quad p_2 = \beta_2 \left( \gamma_2 + \alpha_2 \frac{1}{1+p_1^n} \right),$$

and admit a single intersection regardless of the value of $\alpha_i$, $\beta_i$, and $n$ (panels in Figure 1 c show the equilibrium conditions for increasing values of $n$). The characteristic polynomial is

$$(s+1)^4 - K = 0, \quad \text{where} \quad K = \beta_1 \beta_2 \frac{\partial H_1}{\partial p_2} \frac{\partial H_2}{\partial p_1} < 0. \tag{4}$$

Since now $K < 0$, all of the roots of (4) are complex:

$$s = (-1+q) + iq, \ (-1-q) - iq, \ (-1-q) + iq, \ (-1+q) - iq, \quad \text{where} \quad q = \frac{(-K)^{\frac{1}{4}}}{\sqrt{2}}.$$

As a consequence, only oscillatory unstable dynamics can arise, rather than real (exponential). Precisely, unstable oscillations do arise when $K < -4$. As we can see by studying the original nonlinear system, for

any given value of $n$ there is only one equilibrium, whose stability properties can change depending on the values of $\alpha_i$ and $\beta_i$.

To summarize, the analysis of this simple two–gene system has shown that, without a precise knowledge of the functions $H_1$ and $H_2$, we can reach very strong conclusions regarding the possible dynamic behaviors of the system. Such conclusions do not depend on specific functions or parameter choices, but rather on the positive or negative feedback interconnection, thus on the presence of a positive or a negative cycle in the graph associated with the system. In particular, this example clearly highlights that there is a relationship between cycles and admissible transitions to instability. A qualitatively similar study was carried out and validated by building synthetic bacterial circuits in [6], where analysis relied on the S–systems formalism [49].

In the next sections, we propose a general framework to categorize oscillatory and multistationary systems based on the exclusive or coexisting presence of positive and negative cycles.

## 2 Assumptions and definitions

We consider the dynamical system

$$\dot{x} = f(x), \quad x \in \mathbb{R}^n, \tag{5}$$

where $f(\cdot)$ is continuously differentiable in all its components $f_i(\cdot)$, $i = 1, ..., n$, and may be any function which satisfies the following assumptions.

**Assumption 1** *We assume that all the solutions of system (5) are globally uniformly asymptotically bounded in a ball $\mathcal{S}$: formally, we say that for any compact set $\mathcal{S}_0 \subseteq \mathbb{R}^n$ including $\mathcal{S}$, there exists $T > 0$ (depending on $\mathcal{S}_0$) such that $x(t) \in \mathcal{S}$ for any $x(0) \in \mathcal{S}_0$ and any $t \geq T$.*

As a consequence, the system admits an equilibrium point $\bar{x}$ in $\mathcal{S}$ ([54], see also [47, 48]).

**Assumption 2** *We assume that, for each component $f_i(\cdot)$, $\partial f_i/\partial x_j$ is either always positive, always negative, or always null in all the considered domain.*

**Assumption 3** *We assume that the system is non–autocatalytic,* i.e. *for all $i$, $\partial f_i/\partial x_i < 0$.*

Since $f_i(\cdot)$ is monotone (either non–increasing or non–decreasing) with respect to each argument, the Jacobian of system (5) is sign definite and can be immediately associated with a sign pattern matrix $\Sigma$. Zero elements in the Jacobian correspond to zero elements in $\Sigma$. We refer to $\Sigma$ as the *system structure*. This structure is associated with a directed, $n$–node graph, whose arcs are positive $(+1)$, negative $(-1)$, or zero depending on the sign of the corresponding entries in $\Sigma$. We provided several graph examples within the two–gene network considered in Figure 1.

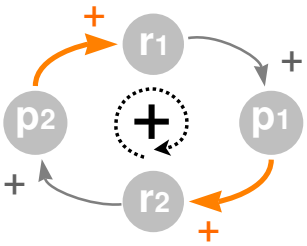


**Fig. 2** Graph corresponding to the structure at Equation 6.

Given a system structure $\Sigma$, we call a *realization* of such a structure any choice of functions $f_i(\cdot)$ along with specific parameter values, and thus of the Jacobian entries $J = [\partial f_i/\partial x_j]$, which is compatible with $\Sigma$.

**Definition 1** We say a property $\mathcal{P}$ is *structural* if it is satisfied by any system with a given structure, independently of the specific realization [8].

For example, the structure corresponding to the two–node double positive feedback loop is

$$\Sigma = \begin{bmatrix} - & 0 & 0 & + \\ + & - & 0 & 0 \\ 0 & + & - & 0 \\ 0 & 0 & + & - \end{bmatrix}, \tag{6}$$

as shown in Figure 2. The specific model we considered and its Jacobian matrix (2) are a possible realization of this structure.

**Definition 2** A *cycle* is an oriented, closed sequence of distinct nodes connected by distinct directed arcs in the graph of a structure. A cycle is *negative* if the number of negative arcs is odd, *positive* otherwise. The number of arcs forming a cycle is called the *order* of the cycle.

Referring to our two–genes example network, all three cases have a single cycle. However, the double positive feedback system and the toggle switch exhibit an overall positive cycle, while the two–nodes oscillator presents an overall negative cycle.

The order of cycles in a system can be an important factor in determining its dynamic behavior. To provide a unified view of our results, we introduce the following definition.

**Definition 3** System (5) with structure $\Sigma$ is *critical* iff all the negative cycles of its structure (if any) are of order two.

In the remainder of this paper, we consider non–critical systems and we discuss the validity of our results for possibly critical structures in Appendix B.

We now introduce general definitions of *transition to instability*. We consider the parameter–dependent dynamical system

$$\dot{x}(t) = g(x(t), \mu), \qquad x \in \mathbb{R}^n, \tag{7}$$

where $\mu$ is a real–valued parameter and $g(\cdot, \cdot)$ is a sufficiently smooth function, continuous in $\mu$, satisfying all of the previously stated assumptions for every value of $\mu$. We always assume that the structure $\Sigma$ is invariant with respect to the parameter $\mu$. Recalling Assumption 1, we have that an equilibrium exists; all the following definitions refer to such an equilibrium point, which is, in general, a function of the parameter $\mu$: $g(\bar{x}_\mu, \mu) = 0$. We assume that $\bar{x}_\mu$ depends continuously on $\mu$. Note that a suitable change of coordinates always allows us to shift the equilibrium to the origin, without affecting our analysis. Indeed, if we define $z = x - \bar{x}_\mu$, we have that $\dot{z}(t) = g(z(t) + \bar{x}_\mu, \mu)$ and $g(\bar{z} + \bar{x}_\mu, \mu) = 0$ for $\bar{z} = 0$. The Jacobian matrix is unchanged, since $\left.\frac{\partial g}{\partial z}\right|_{z=0} = \left.\frac{\partial g}{\partial x}\right|_{x=\bar{x}_\mu}$. This equilibrium translation will be used in the following to simplify our notation.

We say that a matrix is asymptotically stable if all its eigenvalues have a negative real part, while it is asymptotically unstable if at least one of its eigenvalues has a positive real part.

**Definition 4 Transition to Instability (TI)**: System (7) undergoes a *Transition to Instability* at $\mu = \mu^*$ iff its Jacobian matrix $J(\bar{x}_\mu)$, evaluated in $\bar{x}_\mu$, is asymptotically stable in a left neighborhood of $\mu^*$, and asymptotically unstable in a right neighborhood[2]. A TI is *simple* if at most a single real eigenvalue or a single complex pair of eigenvalues crosses the imaginary axis.

Note that, in principle, many eigenvalues could leave the stability region simultaneously. However, this is not likely to occur in realistic situations. Typically, most systems have a *dominant* eigenvalue, *i.e.* an eigenvalue (or a complex pair of eigenvalues) having real part larger than any other. The dynamic behavior of a system is substantially characterized by its dominant eigenvalue.

In this paper we will always consider simple TIs. We now specify two types of simple TIs that are related to oscillatory and multistationary dynamic behaviors.

**Definition 5** System (7) undergoes an *Oscillatory Transition to Instability (OTI)* at $\mu = \mu^*$ iff its Jacobian matrix $J(\bar{x}_{\mu^*})$ has a single pair of pure imaginary eigenvalues, while all the other eigenvalues have negative

[2] This definition holds for systems transitioning to instability from the right to the left neighborhood of $\mu^*$: in the above definition, it suffices to take $\hat{\mu} = \mu^* - \mu$ as the bifurcation parameter.

real part:

$$\sigma\left(J(\bar{x}_{\mu^*})\right) = \{\lambda_1, \lambda_2, \ldots, \lambda_n\}, \quad \text{where} \quad \lambda_{1,2} = \pm i\omega,$$

with $Re(\lambda_k) < 0$ for $k > 2$ and $Re(\lambda_k) > 0$ for $k = 1, 2$ in a right neighborhood of $\mu^*$.

System (7) undergoes a *Real Transition to Instability (RTI)* at $\mu = \mu^*$ iff its Jacobian matrix $J(\bar{x}_{\mu^*})$ has a single zero eigenvalue, while all the other eigenvalues have negative real part:

$$\sigma\left(J(\bar{x}_{\mu^*})\right) = \{\lambda_1, \ldots, \lambda_n\}, \quad \text{where} \quad \lambda_1 = 0,$$

with $Re(\lambda_k) < 0$ for $k > 1$ and $Re(\lambda_1) > 0$ in a right neighborhood of $\mu^*$.

When an RTI occurs, the determinant of the Jacobian changes sign as the parameter crosses the stability limit. Indeed the characteristic polynomial can be factored out as $p_\mu(\lambda) = [\lambda - z(\mu)]\tilde{p}_\mu(\lambda)$, with $\tilde{p}_\mu(\lambda)$ having roots with negative real part in a neighborhood of $\mu = \mu^*$. We have that $z(\mu) < 0$ for $\mu < \mu^*$, in view of the stability assumption, and $z(\mu) > 0$ for $\mu > \mu^*$. Since the constant term $p_\mu(0) = -z(\mu)\tilde{p}_\mu(0) = -\det[J(\bar{x}_\mu)]$, the determinant changes sign at $\mu^*$. As we will see later (Corollary 2), under overall boundedness assumptions, this implies that an RTI causes the appearance of new equilibrium points. This is not the case for OTIs. OTIs are generally related to Andronov–Hopf bifurcations, while RTIs are associated with zero–eigenvalue bifurcations [56]. However, these types of bifurcation require additional assumptions [2, 38, 56].

## 3 Structural classification

We consider a system *structure*, defined as the sign pattern matrix $\Sigma$ associated with the system Jacobian matrix, and we examine the sign of the cycles in the corresponding directed graph. We begin by stating general definitions for candidate oscillatory and multistationary systems. Based on these definitions, we then propose necessary and sufficient conditions that link the presence of negative and positive cycles in a structure to the oscillatory or multistationary nature of the system.

*Remark 1* In the definitions below, we consider systems of the form (5), with a given structure $\Sigma$ and under Assumptions 1, 2 and 3. We call *alteration of* (5) a system of the form (7), such that for $\mu = \mu_0, \quad g(x, \mu_0) = f(x)$ and for $\mu \neq \mu_0$ the structure $\Sigma$ is preserved, yielding equilibrium $\bar{x}_\mu$.

**Definition 6** A system of the form (5) with sign pattern $\Sigma$ is structurally a *candidate oscillator in the weak sense* iff there exists an alteration (7) which admits an OTI.

**Definition 7** A system of the form (5) with sign pattern $\Sigma$ is structurally a *candidate oscillator in the strong sense* iff, for any alteration (7), every simple transition to instability (if any) is an OTI.

As a dual case, we consider candidate multistationary systems.

**Definition 8** A system of the form (5) with sign pattern $\Sigma$ is structurally a *multistationary system in the weak sense* iff there exists an alteration (7) which admits an RTI.

**Definition 9** A system of the form (5) with sign pattern $\Sigma$ is structurally a *multistationary system in the strong sense* iff, for any alteration (7), every simple transition to instability (if any) is an RTI.

A weak candidate oscillator admits an unstable equilibrium point where trajectories spiral out of the equilibrium with oscillatory dynamics. A strong candidate oscillator structure admits transitions to instability exclusively of oscillatory nature. The two–gene oscillator system described in the previous section is a strong candidate oscillator, since, as we have seen, unstable dynamics can arise only in association with complex conjugate eigenvalues, hence must be oscillatory. Conversely, a weak candidate multistationary structure admits unstable behaviors due to a real unstable eigenvalue. A strong candidate multistationary system can admit solely unstable dynamics due to a real unstable eigenvalue. Going back to our examples, both the two–gene double positive feedback loop and the toggle switch are strong candidate multistationary systems, since unstable dynamics can arise only due to a real unstable eigenvalue.

We will provide necessary and sufficient conditions characterizing candidate oscillators and multistationary systems based on the sign of the cycles present in their structure $\Sigma$. Precisely, we will see that a system is a candidate oscillator in the strong sense iff all the cycles are negative, in the weak sense iff there is at least one negative cycle; conversely, a system is a candidate multistationary system in the strong sense iff all the cycles are positive, in the weak sense iff there is at least one positive cycle. A table summarizing our classification based on cycles in the system structure is in Figure 3.

| | Candidate oscillator | Candidate multistationary system |
|---|---|---|
| **Weak** | A negative cycle exists | A positive cycle exists |
| **Strong** | All cycles are negative | All cycles are positive |

**Fig. 3** Table summarizing our structural cycle–based classification of candidate oscillators and multistationary systems.

*Remark 2* Some of our proofs are built with the following argument. Suppose we are given a condition $\mathcal{C}$ on a structure associated with a system $\dot{x}(t) = f(x(t))$, and we want to prove that the condition is necessary

for a certain structural property $\mathcal{P}$. We reason by contradiction, supposing condition $\mathcal{C}$ is not satisfied by the structure. Then, we show that there always exists a realization of the structure for which $\mathcal{P}$ fails.

Before formally stating our main results, we introduce a vector field alteration (7) of systems of the form (5), by $\nu_{\kappa,\epsilon}$ functions, where $\kappa,\ \epsilon > 0$ are real parameters. This alteration, which leaves $\Sigma$ unchanged, will be employed throughout the proofs to find, given a structure, a realization which satisfies a property of interest. To simplify the notation, we henceforth assume that the equilibrium is at zero.

**Definition 10** A $\nu_{\kappa,\epsilon}$ *function* (see for example Figure 4) is a strictly increasing, continuously differentiable, odd function (*i.e.* $\nu_{\kappa,\epsilon}(x) = -\nu_{\kappa,\epsilon}(-x)$), such that:

$$\begin{cases} \nu_{\kappa,\epsilon} = x \quad \text{for} \quad |x| \geq \epsilon, \\ \frac{d\nu_{\kappa,\epsilon}(0)}{dx} = \kappa. \end{cases}$$

A $\nu_{\kappa,\epsilon}$ function has a scalable derivative at the origin and is the identity function outside the $\epsilon$–ball.

**Example** Among the infinite possible $\nu_{\kappa,\epsilon}$ functions, we consider this example for illustrative purposes:

$$\nu_{\kappa,\epsilon}(x) = \begin{cases} x + \arctan((\kappa-1)x)\left(1 - \left(\frac{x}{\epsilon}\right)^2\right)^2 & \text{for} \quad |x| < \epsilon \\ x & \text{for} \quad |x| \geq \epsilon \end{cases} \tag{8}$$

The derivative of function (8) inside the interval $[-\epsilon, \epsilon]$ is

$$\nu'_{\kappa,\epsilon}(x) = 1 + \frac{(\kappa-1)\left(1 - \left(\frac{x}{\epsilon}\right)^2\right)^2}{1 + (\kappa-1)^2x^2} - \frac{4}{\epsilon^2}x\arctan((\kappa-1)x)\left(1 - \left(\frac{x}{\epsilon}\right)^2\right), \quad \text{for} \quad |x| < \epsilon,$$

while $\nu'_{\kappa,\epsilon}(x) = 1$ for $|x| \geq \epsilon$. Its derivative $\nu'_{\kappa,\epsilon}(x) > 0$ is continuous and positive for all $\kappa > 0$, and in $x = 0$ it is equal to $\kappa$ as desired. This example function is plotted in Figure 4.

**Transformation by $\nu_{\kappa,\epsilon}$ function:** Let us compose the vector field $f(x)$ and a $\nu_{\kappa,\epsilon}$ function, introducing a transformation:

$$\dot{x} = f(\dots, x_i, \dots) \quad \rightarrow \quad \dot{x} = f(\dots, \nu_{\kappa,\epsilon}(x_i), \dots).$$

This operation:

a) does not change the equilibrium $x_i = 0$;

b) does not change the structure $\Sigma$ of the system (does not alter the sign of the Jacobian entries);

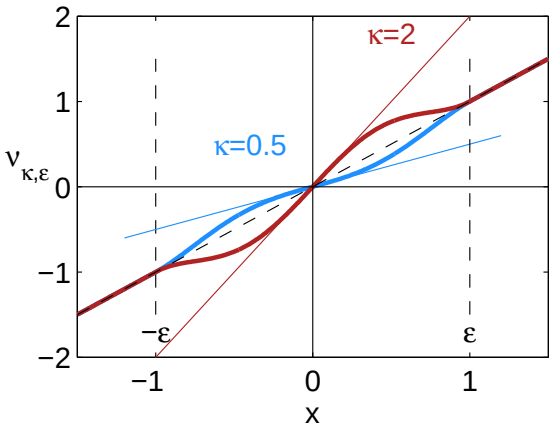


**Fig. 4** Plot of the example $\nu_{\kappa,\epsilon}(x)$ function in equation (8), with $\epsilon = 1$, $\kappa = 2$ (red) and $\kappa = 0.5$ (blue).

c) changes the partial derivatives in $x_i = 0$ as:

$$\left.\frac{\partial f(\dots,\nu_{\kappa,\epsilon}(x_i),\dots)}{\partial x_i}\right|_{x_i=0} = \left.\frac{\partial f(\dots,\nu_{\kappa,\epsilon}(x_i),\dots)}{\partial \nu_{\kappa,\epsilon}}\frac{\partial \nu_{\kappa,\epsilon}(x_i)}{\partial x_i}\right|_{x_i=0} = \kappa \left.\frac{\partial f(\dots,x_i,\dots)}{\partial x_i}\right|_{x_i=0};$$

d) $\dot{x} = f(x)$ is unchanged outside the $\epsilon$–ball after the transformation. In particular, boundedness properties of the solution are not affected.

If we apply this alteration to a vector field $f(x)$ in a neighborhood of the origin as an equilibrium point, the elements of the Jacobian of $f$ at $x = 0$ can be arbitrarily scaled.

*Remark 3* We are going to use $\nu_{\kappa,\epsilon}$ alterations in our proofs. Given a structure, we will exploit a $\nu_{\kappa,\epsilon}$ alteration to find a realization that satisfies the property of interest: such a special transformation allows us to independently scale the magnitude of desired cycles in a realization. Note that $\nu_{\kappa,\epsilon}$ alterations preserve the boundedness of the system solution. Finally, we stress that we will use different parameters $\kappa_i$ to scale different arcs and we may always assume that $\kappa_i(\mu)$ are functions of a single parameter $\mu$, consistently with (7).

We now present our main results. The following proposition provides a characterization of weak candidate oscillators, exemplified in the literature by the amplified negative feedback oscillators and incoherent oscillators described in [42] and by the toggle–switch/oscillator circuit in [6].

**Proposition 1** *A non–critical system is a candidate oscillator in the weak sense if and only if its structure has at least one negative cycle (necessarily of order greater than two).*

*Proof* **Necessity**. We want to show that, if there are no negative cycles, OTIs cannot occur.

First, we consider structures corresponding to strongly connected graphs. We say that a graph is strongly connected if each node is connected to any other by an oriented path. In this case, as it is pointed out in [52], p. 68, the absence of directed negative cycles is equivalent to the absence of undirected negative cycles, which in turn guarantees monotonicity with respect to some orthant cone[3] [50, 52]. Since the Jacobian of a monotone system is a Metzler matrix (up to a change of coordinates), it has a real dominant eigenvalue and any simple transition to instability is an RTI (thus the system does not admit undamped oscillations).

If instead the graph is not strongly connected, it can be partitioned into *strongly–connected components*. A node belongs to one (and only one) strongly–connected component iff it is connected to all the nodes in that component by an oriented path, and vice versa. If we collapse each strongly–connected component in a single hyper–node, this implies that the arcs connecting hyper–nodes (*i.e.* connecting two nodes belonging to two different strongly–connected components) cannot form cycles (see, for instance, Figure 5). Therefore, the graph composed by the $M$ hyper–nodes corresponds to a block–triangular matrix

$$G = \begin{bmatrix} N_{11} & \mathbf{0} & \dots & \mathbf{0} \\ L_{21} & N_{22} & \dots & \mathbf{0} \\ \vdots & \vdots & \ddots & \vdots \\ L_{M1} & L_{M2} & \dots & N_{MM} \end{bmatrix},$$

where $N_{ii}$ is the Jacobian matrix associated with the $i$–th strongly–connected component (and is therefore equivalent to a Metzler matrix), while $L_{ij}$ denotes the interconnection between the $i$–th and the $j$–th strongly–connected components.

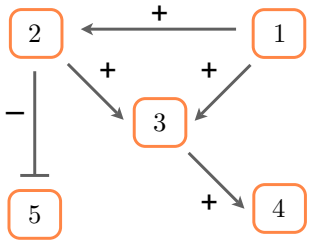


**Fig. 5** Graph formed by the hyper–nodes associated with strongly–connected components, corresponding to matrix (9).

$$G = \begin{bmatrix} N_{11} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ L_{21} & N_{22} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ L_{31} & L_{32} & N_{33} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & L_{43} & N_{44} & \mathbf{0} \\ \mathbf{0} & L_{52} & \mathbf{0} & \mathbf{0} & N_{55} \end{bmatrix} \tag{9}$$

Since matrix $G$ is block–triangular, its spectrum is $\sigma_G = \bigcup_i \sigma_i$, where $\sigma_i$ is the spectrum of $N_{ii}$. Therefore the overall not–strongly–connected graph corresponds to a matrix having a real dominant eigenvalue. This implies that any simple transition to instability is an RTI.

[3] A system is monotone with respect to some orthant cone if and only if its Jacobian is a Metzler matrix (namely its non–diagonal entries are non–negative), or becomes a Metzler matrix after a proper change in the sign of some variables [50, 52].

**Sufficiency**. The idea of the proof is simple: suppose there exists a single negative cycle of order greater than two. Then we apply a $\nu_{\kappa,\epsilon}$ transformation to all remaining cycles, scaling down the $\kappa$ parameter until we virtually "eliminate" all the other cycles. For instance, in the structure shown in Figure 6a we can select the cycle formed by nodes 1–2–3, and consider the new graph achieved by considering only the orange interconnections, Figure 6b.

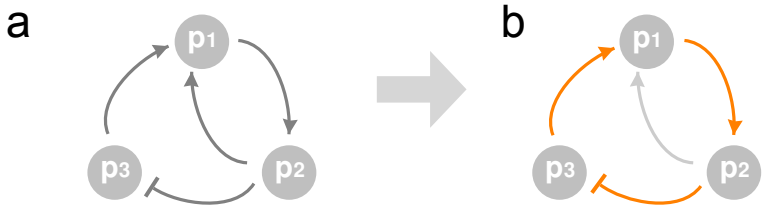


**Fig. 6** a. Example of incoherent oscillator presented in [42]. b. Emphasis on the negative cycle (orange).

Then for each arc, or Jacobian entry, we introduce an alteration with a $\nu_{\kappa,\epsilon}$ function, and we can scale the interconnections so that $\kappa_{ij} > 1$ for the arcs involved in the negative cycle, while small values $\kappa_{ij} \ll 1$ are assigned to the arcs not involved in the cycle. By reordering the nodes, we can always find a realization having a Jacobian (or a principal submatrix of the Jacobian) of the form:

$$\tilde{J} = \begin{bmatrix} -\beta_1 & \varepsilon & \varepsilon & \dots & \gamma_1 \\ \gamma_2 & -\beta_2 & \varepsilon & \dots & \varepsilon \\ \varepsilon & \gamma_3 & -\beta_3 & \dots & \varepsilon \\ \varepsilon & \varepsilon & \dots & \dots & \varepsilon \\ \varepsilon & \varepsilon & \dots & \gamma_n & -\beta_n \end{bmatrix} \tag{10}$$

where $\beta_i > 0$ and $\gamma_i \neq 0$. With an abuse of notation, the entries corresponding to interconnections scaled to arbitrarily small values are denoted as $\varepsilon \approx 0$, instead of individual terms $\varepsilon_{i,j}$. By assumption,

$$\prod_i \gamma_i < 0.$$

Since the eigenvalues of a matrix are continuous functions of the matrix entries (see for instance [62]), we can neglect the $\varepsilon$–entries, which can be arbitrarily small, so that the realization Jacobian matrix $\tilde{J}$ has characteristic polynomial

$$p(s) = \prod_i (s + \beta_i) - \prod_i \gamma_i,$$

whose coefficients are all positive. Therefore $\tilde{J}$ has no real non–negative eigenvalues, in view of Descartes' rule of signs.

The proof follows from the fact that any polynomial of order $n > 2$ of the form $\prod_i (s + \beta_i) + k$ has roots with positive real part for $k > 0$ large, as it can be seen by building a Routh–Hurwitz table or by means of standard root locus techniques (see for instance [23]). For $n \leq 2$, the two roots have negative real part. Therefore, for large $\gamma_i$, some of the (necessarily complex) eigenvalues have positive real part (while all of the eigenvalues have negative real part for suitably small $\gamma_i$), hence there is always a realization yielding an OTI. □

We now provide a characterization of strong candidate oscillators, exemplified by the well known repressilator circuit [17] and by all the negative feedback oscillator models described in [42].

**Proposition 2** *A non–critical system is a candidate oscillator in the strong sense if and only if its structure has only negative cycles.*

*Proof* **Necessity** Assume there is a positive cycle in the structure. We can apply the $\nu_{\kappa,\epsilon}$ transformation, scaling up the positive cycle and scaling down arbitrarily close to zero all the other cycles, achieving a realization Jacobian similar to matrix (10). Proceeding as in the sufficiency part of the proof for Proposition 1, we conclude that the characteristic polynomial of the realization is:

$$p(s) = \prod_i (s + \beta_i) - \prod_i \gamma_i,$$

where $\prod_i \gamma_i > 0$, since the cycle is positive. Therefore, a realization can be destabilized by driving one real root to cross the imaginary axis through the origin, generating an RTI. (Note the "symmetry" with respect to the sufficiency part of the proof of Proposition 1, where the presence of a negative cycle yields $\prod_i \gamma_i < 0$.)

**Sufficiency** We use algebraic tools from [37] to prove that a structure with only negative cycles can solely present complex unstable eigenvalues. Assume that the structure (sign pattern matrix associated with the Jacobian) has only negative cycles. The characteristic polynomial is

$$p(s) = \det(sI - J) = \sum_{i=0}^{n} p_k s^k. \tag{11}$$

It is well known that the characteristic polynomial is always a monic polynomial, *i.e.* $p_n = 1$. In addition

$$p_k = \sum_i \Delta_i^{(k)} (-1)^k,$$

where $\Delta_i^{(k)}$ are the determinants of all the leading minors of order $k$ of the Jacobian. We now invoke the following property, from Theorem 3.1 in [37]:

*Property 1* If all the diagonal entries of $J$ are negative and if all the cycles are non–positive, then each leading minor of order $k$ has sign $(-1)^k$.

This property implies that all the coefficients of the characteristic polynomial $p(s)$ are positive, hence no real positive roots are admitted. Therefore we conclude that if the system is destabilized, then a complex pair of eigenvalues with non–negative real part must exist, leading to an OTI. □

We can prove the following corollary, which is a consequence of degree theory. This is a classical result due to Thomas [58] and we give a proof for completeness.

**Corollary 1** *A strong candidate oscillator admits a single equilibrium.*

*Proof* We assume that the system of interest is a candidate strong oscillator, therefore its structure has only negative cycles. In view of the result proposed in [37], which we have just used in the proof of Proposition 2, we have that the determinant of a realization matrix $J(x)$ with only negative cycles, at *any* equilibrium point $\bar{x}$, satisfies:

$$\text{sign}[\det(J(\bar{x}))] = (-1)^n,$$

where $n$ is the system dimension. Moreover, $\text{sign}[\det(J(\bar{x}))]$ does not change as a function of $\bar{x}$. Now, let us denote the equilibria as $\bar{x}_i$. Degree theory also provides us with this equality, which holds for globally bounded flows (see also Lemma 2 in [27], or reference [43]):

$$\sum_i \text{sign}[\det(J(\bar{x}_i))] = (-1)^n. \tag{12}$$

Because in our case all the terms of the sum have the same sign, there must be a single equilibrium. Thus, a strong candidate oscillator structure admits a single equilibrium. □

Now we provide a characterization of candidate weak multistationary systems, examples of which include amplified negative feedback oscillators and incoherent oscillators in [42], and again the toggle–switch/oscillator circuit in [6].

**Proposition 3** *A non–critical system is a candidate multistationary system in the weak sense if and only if its structure has at least one positive cycle.*

*Proof* **Necessity** (see also [24] and [51]) If there are no positive cycles, the system is a candidate oscillator in the strong sense. We have seen that the determinant of the Jacobian is never 0, hence there cannot be 0 eigenvalues. Therefore, no simple RTI can occur.

**Sufficiency** Assume that there exists a positive cycle. Then using the $\nu_{\kappa,\epsilon}$ transformation, which allows independent scaling of Jacobian entries in a realization, we can prove the existence of a realization with a simple RTI by means of the same argument given in the necessity part of the proof of Proposition 2. □

We finally characterize strong candidate multistationary systems. Examples of these systems include the previously presented two–gene double positive feedback and the toggle–switch architectures in [3, 21].

**Proposition 4** *A non–critical system is a candidate multistationary system in the strong sense if and only if its structure has positive cycles only.*

*Proof* **Necessity** By contradiction, if not all the cycles are positive, then there exists a negative cycle. Thus, the system is a candidate oscillator in the weak sense and we can find a realization exhibiting an OTI.

**Sufficiency** When all the cycles are positive, we can demonstrate, along the lines of the necessity part of the proof of Proposition 1, that, if the system admits a simple transition to instability, it must be an RTI. □

We conclude the section by proving the following corollary.

**Corollary 2** *A strong candidate multistationary system, in which a simple RTI occurs at $\mu^*$, admits additional equilibria in a right (unstable) neighborhood of $\mu^*$.*

*If these additional equilibria are non–singular (*i.e. *if the determinant of the Jacobian is non–zero), then there are at least two additional equilibria.*

*If exactly two additional non–singular equilibria appear, and the Jacobian evaluated at those points, $J(\bar{x})$, has a single dominant eigenvalue (*i.e. *the system is irreducible), then these equilibria are asymptotically stable.*

*Proof* We assume that the system is candidate multistationary in the strong sense, hence its structure has only positive cycles. Therefore any simple transition to instability must be an RTI. This means that, for any realization, the determinant of the Jacobian changes sign when parameter $\mu$ crosses the critical value $\mu^*$. In other words, for values of $\mu$ to the left of $\mu^*$ the characteristic polynomial must have a positive constant term, and thus $\text{sign}[\det(J(\bar{x}))] = (-1)^n$. For values of $\mu$ to the right of $\mu^*$, conversely, $\text{sign}[\det(J(\bar{x}))] = -(-1)^n$. This means that equality (12) cannot be true in a right neighborhood of $\mu^*$, unless additional equilibrium points appear. If the additional equilibria are non–singular, *i.e.* they have a non–singular Jacobian, then there are at least two of them.

Finally, let us assume that exactly two additional equilibria appear. Assume our original equilibrium is $\bar{x} = 0$, unstable by assumption, and let $x_U, x_L$ be additional equilibria introduced by the transition to

instability. In a right neighborhood of $\mu^*$, consider for small $\epsilon > 0$ the two regions

$$\mathcal{U} = \{x \geq 0, \quad v^\top x \geq \epsilon\}, \qquad \mathcal{L} = \{x \leq 0, \quad v^\top x \leq -\epsilon\},$$

where $v^\top$ is the left Frobenius eigenvector of $J$. In view of the monotonicity of the system, the instability of $\bar{x} = 0$ and the irreducibility of the Jacobian (hence $v^\top > 0$), both regions $\mathcal{U}$ and $\mathcal{L}$ are positively invariant. Given our assumption of boundedness of the system solution, the solutions originated in both $\mathcal{U}$ and $\mathcal{L}$ are bounded. Then, each set must include one of the equilibria: $x_U$ in $\mathcal{U}$ and $x_L$ in $\mathcal{L}$.[4]

To conclude the proof, we invoke a known result in the context of monotone systems (see, for instance [29], page 458 Theorem D): since the two equilibria are unique in the bounded sets $\mathcal{U}$ and $\mathcal{L}$, they must be stable. □

The two–gene positive feedback system and the toggle switch are clear examples of this result.

*Remark 4* Delay differential equations are often adopted to model molecular systems. For example, transcription, processing and transport of mRNA in genetic networks have been successfully modeled with explicit delays (see for instance [35]). A thorough analysis of oscillations and multistationarity in delay differential equations is out of the scope of this paper. However, we discuss this challenge in Appendix C and we provide local, cycle–based sufficient conditions for OTIs and RTIs in a wide class of systems affected by delays.

## 4 Discussion and Conclusions

We described a general, cycle–based classification of multistationary and oscillatory behaviors in dynamical systems with sign–definite Jacobian. This classification builds on the well known Thomas' conjectures, and distinguishes between strong and weak candidate oscillators and multistationary systems depending on the presence of exclusively negative or exclusively positive, or coexisting positive and negative cycles. We provide a complete characterization, where necessary and sufficient conditions rely on the ability of locally scaling the entries of the Jacobian. We say that this characterization is robust with respect to model uncertainty, because it only depends on the sign–definite Jacobian of the model, rather than on the chosen functions or parameters.

Parameter–free criteria to identify the possible dynamic phenotype of a system are particularly important in the context of molecular systems. Many biochemical network models are sign definite, and can thus be

[4] Exploiting the Jacobian irreducibility assumption, it is actually possible to prove that *at least* two additional equilibria arise.

appropriately classified according to our framework. We have mentioned a few notable examples, which include the famous Elowitz repressilator and a variety of toggle switches [6, 17, 42]. Realistic, detailed models for molecular and biochemical networks are most likely to fall into the category of weak candidate oscillator or multistationary systems. However, due to the complexity of the cellular environment, such models may not enjoy the sign definiteness property and thus be outside of the scope of this work.

Other formalisms have been developed in the past to achieve structural conclusions on the behavior of biochemical systems. The chemical reaction network theory provides many structural results. The zero deficiency theorem [18], for instance, rules out the presence of oscillations and multistationary behavior regardless of specific kinetic rates. In the broad context of chemical reaction networks, cycle conditions associated with the presence of Hopf or saddle–node/pitchfork instability have been formulated for systems governed by mass–action kinetics using species–reaction graphs [10, 11, 34, 39] and algebraic geometry [14, 15]. While chemical reaction networks are undoubtedly a very important class of models, phenomenological models are often preferred in biology when pathways are not known with sufficient mechanistic detail. Thus, we argue that Jacobian graphical or algebraic conditions may have more general applicability than species–reaction graph conditions [12], which apply for mass–action kinetics, but cannot account for Michaelis–Menten or Hill type kinetics, or for qualitative relationships. On the contrary, criteria based on the Jacobian matrix enable a qualitative analysis also when a detailed stoichiometry of the interactions is unknown [40].

Another class of very powerful robust analysis methods is given by the theory of monotone systems [50, 52], which enables the simplification of large, complex systems into interconnections of gray–box, input/output monotone subnetworks [3]. Indeed, monotonicity and existence of steady–state characteristics are properties that facilitate the detection of multistationary behaviors in systems of arbitrary size [5]. Monotonicity, accompanied by small–gain conditions, is used to provide necessary conditions for oscillations in [4].

Among the limitations of this work, we point out that our results hold for systems in which interactions between nodes are independent. Models built using mass action kinetics, for example, do not fall under this category. For instance, take the reaction $A \xrightarrow{g} B$. The corresponding concentration dynamics of $A$ will include a term $-g(A)$, representing the consumption of $A$, and the same term will appear, with opposite sign, in the concentration dynamics of $B$. This situation introduces constraints in the magnitude of the Jacobian entries, which we have not taken into account. A more detailed discussion on this point is reported in Appendix D.

Note also that we have not considered specific regions of the state space, such as the positive orthant, where biological systems are typically confined. However, the theory presented in this paper can be easily

extended to such cases, up to technical details (such as the possible presence of equilibria on the boundary, which has to be carefully handled).

We also remark that we considered only networks that are non–critical, *i.e.* such that the order of any cycle in the system structure is larger than two. If this assumption does not hold true, then our characterization of candidate oscillators is not fully valid, as discussed in Appendix B.

We believe that our classification is useful for the *design* of artificial *in vitro* biochemical networks. Recently, *in vitro* reaction environments have gained significant attention because of their reduced and controllable complexity. In particular, synthetic multistationary and oscillatory networks composed of nucleic acids and proteins have been built with systematic design methods [20, 32, 33, 41]. These networks promise to be programmable components in larger, integrated systems for nanomanufacturing, pattern formation, and artificial biomaterials. Although created with a bottom–up approach, designing reaction by reaction, these synthetic *in vitro* networks still suffer from parametric uncertainty and undesired dynamics [20], especially due to the variability in purity and activity of key components. We foresee that by following and enforcing cycle sign conditions suggested in this paper, it will be possible to increase the tunability and robustness of these artificial networks.

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# Appendices

### A Nondimensionalization and boundedness of the two–gene network

We will carry out the nondimensionalization procedure for the toggle switch network, leaving the derivation for the other cases to the reader. We follow nondimensionalization steps similar to those proposed in [17] and [20, 32]. Consider the simple (dimensional) model:

$$\tau\,\dot{R}_1 = c_1 + a_1\frac{1}{K_{M1}^n + P_2^n} - R_1, \qquad \dot{P}_1 = k_p R_1 - k_d P_1, \tag{13a}$$

$$\tau\,\dot{R}_2 = c_2 + a_2\frac{1}{K_{M2}^n + P_1^n} - R_2, \qquad \dot{P}_2 = k_p R_2 - k_d P_2. \tag{13b}$$

Here $c_i$ is the "leak" transcription of RNA. For simplicity, we assume that the translation and degradation rates for the proteins are the same. Constant $\tau$ is the mRNA half–life in the system. Constants $K_{Mi}$ represent the number of proteins necessary to half–maximally repress $R_i$. Finally, assume the translation efficiency of each RNA species is given by $\bar{p}_i$, which corresponds to the average number of proteins produced by a single RNA molecule.

We define the nondimensional variables: $r_i = R_i/\bar{p}_i$, $p_i = P_i/K_{Mj}$, $(i,j) \in \{(1,2),(2,1)\}$. We rescale time as $\tilde{t} = t/\tau$, and

also define the nondimensional parameters:

$$\gamma_i = \frac{c_i}{\bar{p}_i}, \quad \alpha_i = \frac{a_i}{\bar{p}_i\, K_{Mi}^n}, \quad \beta_i = \frac{k_p\, \bar{p}_i}{k_d\, K_{Mj}}, \quad T = \frac{1}{\tau k_d}.$$

The resulting nondimensional equations are:

$$\dot{r}_1 = \gamma_1 + \alpha_1 \tfrac{1}{1+p_2^n} - r_1, \qquad T\dot{p}_1 = \beta_1 r_1 - p_1, \tag{14a}$$

$$\dot{r}_2 = \gamma_2 + \alpha_2 \tfrac{1}{1+p_1^n} - r_2, \qquad T\dot{p}_2 = \beta_2 r_2 - p_2, \tag{14b}$$

Finally, if we assume $T \approx 1$, we get a system in the same form as equations (1).

We also report a brief evidence of the global boundedness of the two–gene system trajectories. The system expressed by equations (1) can be rewritten as

$$\begin{bmatrix} \dot{r}_1 \\ \dot{p}_1 \\ \dot{r}_2 \\ \dot{p}_2 \end{bmatrix} = \begin{bmatrix} -1 & 0 & 0 & 0 \\ \beta_1 & -1 & 0 & 0 \\ 0 & 0 & -1 & 0 \\ 0 & 0 & \beta_2 & -1 \end{bmatrix} \begin{bmatrix} r_1 \\ p_1 \\ r_2 \\ p_2 \end{bmatrix} + \begin{bmatrix} \gamma_1 + H_1(p_2) \\ 0 \\ \gamma_2 + H_2(p_1) \\ 0 \end{bmatrix} = \tilde{M} \begin{bmatrix} r_1 \\ p_1 \\ r_2 \\ p_2 \end{bmatrix} + \tilde{H}(p_1, p_2).$$

$\tilde{M}$ is an asymptotically stable matrix and $\tilde{H}(p_1, p_2)$ is a bounded quantity ($\|\tilde{H}(p_1, p_2)\| \leq \eta$), because $\gamma_i$ are constants and $H_i(p_j)$ are Hill functions. The right–hand side of the equation is given by the sum of a linear term and a globally bounded nonlinear term. If we neglect the nonlinear part, we achieve a linear system which is asymptotically stable and admits a global quadratic Lyapunov function $V(x) = x^\top P x$, where $\tilde{M}^\top P + P\tilde{M} = -Q$, with $Q$ positive definite. Hence, all the system solutions will be globally ultimately bounded in a set of the form $x^\top P x \leq k$, for some $k > 0$ which depends on $P$, $Q$ and $\eta$ (see [30] Section 5.2 and [9] Section 4.4.1).

## B Critical systems

In the main text, we have considered non–critical systems in order to provide unified results. Here we discuss our results and examine the validity of our characterization for possibly critical systems. Note that Propositions 3 and 4, concerning candidate multistationary systems, hold true for critical systems as well. The assumption comes into play when we consider the characterization of candidate oscillatory systems.

*Validity of Proposition 1:* For critical networks, the sufficiency part of Proposition 1 does not hold: the existence of negative cycles of order two does not assure that the system can oscillate. For example, consider the following system:

$$\dot{x}_1 = -b_1 x_1 + H_{12}(x_2), \tag{15}$$

$$\dot{x}_2 = -b_2 x_2 + H_{21}(x_1),$$

where $b_1$ and $b_2$ are positive constants, and $H_{ij}$ are positive, bounded, and monotone functions. To ensure the existence of a negative cycle, we assume that (for example) $H_{12}$ is non–increasing, while $H_{21}$ is non–decreasing. (We can take $H_{ij}$ as Hill functions.) For any choice of $b_i$ and $H_{ij}$, the system has a single, asymptotically stable equilibrium point. However, oscillations are possible if we take $b_1 = b_2 = 0$, *i.e.* if we remove our assumption that negative self–loops must exist at each node.
The necessity part of Proposition 1 still holds, because if there are no negative cycles the system cannot have equilibria with oscillatory instability.

*Validity of Proposition 2:* In the presence of negative cycles only, instability must be oscillatory. Thus, Proposition 2 still holds. However, some critical systems cannot be destabilized at all, as just shown in example (15).

It is legitimate to ask whether a critical system with only negative cycles (all of order two) is necessarily stable. This would extend Proposition 1 as follows:

*Conjecture*: A system is a candidate oscillator in the weak sense if and only if it has at least a negative cycle of order greater than two.

While the "if" part of this conjecture is true, unfortunately the the "'only if" part is false. This can be seen by counterexample. We modify example (15) by introducing a new variable $x_3$ and a positive cycle:

$$\begin{aligned}\dot{x}_1 &= -b_1 x_1 + H_{12}(x_2)\\ \dot{x}_2 &= -b_2 x_2 + H_{21}(x_1) + \mu x_3\\ \dot{x}_3 &= -b_3 x_3 + \nu x_2.\end{aligned}$$

The system Jacobian is:

$$J = \begin{bmatrix} -b_1 & -\delta & 0 \\ \gamma & -b_2 & \mu \\ 0 & \nu & -b_3 \end{bmatrix}$$

This matrix may well have unstable complex eigenvalues. Let us assume $b_1 = b_2 = 0$. The characteristic polynomial is:

$$s^3 + b_3 s^2 + (\gamma\delta - \mu\nu)s + \gamma\delta b_3$$

Building the Routh–Hurwitz table for this polynomial, one can check that for $\mu, \nu > 0$ two roots are complex with positive real parts. For $b_1, b_2 > 0$ small enough, the property is preserved.

A property relevant to our discussion on critical cases is the following known result (see for instance [16], Chapter 6.5):

**Proposition 5** *If all the self–loops are negative, and all the other cycles are negative and of order two, then any equilibrium of the system is stable.*

## C Systems affected by delays

Many molecular systems have been successfully modeled using delayed differential equations. A notable example is given by gene networks, where the transport of RNA and proteins across cellular membranes are well captured by explicit delays [28, 35]. Delay differential equations are infinite dimensional systems and a formal, exhaustive treatment of this case would require a more sophisticated setup. However, we can show that local, cycle–based sufficient conditions for OTIs and RTIs can be stated in a wide class of systems with delays. Consider the delay differential equation model

$$\dot{x}(t) = f(x(t), x(t-\tau_1), x(t-\tau_2), \ldots, x(t-\tau_M)). \tag{16}$$

Under standard differentiability assumptions, the corresponding linearized system around an equilibrium point is

$$\dot{\xi}(t) = A_0\xi(t) + \sum_{k=1}^{M} A_k\xi(t-\tau_k). \tag{17}$$

For simplicity assume that there are no delayed self–loops, *i.e.* that the matrices $A_k$ have zero diagonal entries for $k \geq 1$.

It is well known that the stability of the system above can be established by inspecting the roots of this equation:

$$\det\left[A_0 + \sum_{k=1}^{M} A_k e^{-\tau_k s} - sI\right] = 0. \tag{18}$$

Stability is ensured if the roots have negative real parts. We now consider the following auxiliary system, in which all delays are (fictitiously) set to 0:

$$\dot{\zeta}(t) = \left[A_0 + \sum_{k=1}^{M} A_k\right]\zeta(t) = \bar{A}\zeta(t). \tag{19}$$

Matrix $\bar{A}$ is the same Jacobian we would obtain by setting the delays $\tau_i = 0$ in system (16):

$$\dot{x}(t) = f(x(t), x(t), x(t), \ldots, x(t)).$$

Note that equilibria are delay–independent, so the delay–free system above has the same equilibria as the delayed system (16).

As previously done in this paper, we can associate graphs with systems (17) and (19), where nodes correspond to species concentrations and signed, directed arcs correspond to the dynamic interactions among species (delayed or not), defined by matrices $A_k$, $k = 0, ..., M$. Delays do not change the sign of the cycles. Therefore any positive/negative cycle of (17) corresponds to a positive/negative cycle of (19).

**Proposition 6** *Assume that system* (17) *has only negative cycles. Then, the system admits solely OTIs.*

*Proof Ab absurdo*, assume that the system admits a real transition to instability, and thus one root of equation (18) crosses the imaginary axis with value $s = 0$. This is equivalent to writing equation (18) as

$$\det\left[A_0 + \sum_{k=1}^{M} A_k\right] = 0.$$

If this is true, then also the auxiliary system (19) must admit a zero eigenvalue. But this is impossible if the system has only negative cycles, according to Proposition 2. □

**Proposition 7** *Assume that system* (17) *has only positive cycles. Then, the system admits solely RTIs.*

*Proof* To prove this proposition, we need two observations:

a) In the absence of negative cycles (with the exception of self–loops), system (17) is a linear positive system with delay. Therefore, matrix $\bar{A}$ in system (19) is a Metzler matrix.

b) We invoke a well known property of *positive* linear systems with delay (with no delayed self–loops). System (18) is stable if and only if its delay–free, auxiliary counterpart (19) is stable [25, 36] (see also [61], Theorem 6.5).

If all its cycles are positive, the auxiliary system (19) can transition to instability only by means of a pole in $s = 0$. Again *ab absurdo* let us assume that the delay system (18) admits instability with a pair of dominant imaginary eigenvalues and no real eigenvalue crossing the origin. Then, the auxiliary system (19) would also be unstable. However, the dominant eigenvalue of the Metzler matrix $\bar{A}$ is real, therefore the auxiliary system (19) would transition to instability with a pole at $s = 0$. But this would also imply that equation (18) is satisfied with $s = 0$, which is a contradiction. Hence, we conclude that if all its cycles are positive, a transition to instability with a pair of imaginary eigenvalues is impossible for system (17). □

As a comment to the above proposition, we stress that equilibria are delay–independent, therefore if the auxiliary subsystem presents multiple equilibria, so does the delayed system.

We conclude this appendix by noting that Proposition 5 is not valid in the presence of delays. Indeed, the presence of a delay in a "second order negative cycle" may compromise stability, as it is well known in control theory.

## D Structural cross–constraints among functions

We have not considered models presenting cross–linked dynamic terms in several equations. Cross–linked terms appear typically in models built using the mass action kinetics formalism. For instance, consider the reactions

$$X_0 \xrightarrow{1} X, \quad X \xrightarrow{g_X} Y, \quad Y \xrightarrow{g_Y} Z, \quad pX + Z \xrightarrow{g_{XZ}} \emptyset,$$

where $\emptyset$ indicates elimination of a species from the system (degradation or out–flow). Indicating the concentration of a species with the corresponding small letter, the differential equation model is:

$$\dot{x} = x_0 - g_X(x) - p g_{XZ}(x, z) \tag{20}$$

$$\dot{y} = g_X(x) - g_Y(y) \tag{21}$$

$$\dot{z} = g_Y(y) - g_{XZ}(x, z). \tag{22}$$

Here, all the reaction rates are increasing functions. Identical dynamic terms appear in the three equations, so the Jacobian has dependent entries:

$$J = \begin{bmatrix} -(\alpha + p\mu) & 0 & -p\nu \\ \alpha & -\beta & 0 \\ -\mu & \beta & -\nu \end{bmatrix},$$

where, for a fixed equilibrium, all Greek letters represent positive constants. This structure presents both negative and positive cycles, but it may only undergo oscillatory transitions to instability. Thus, it is a candidate oscillator in the strong sense. This fact can be seen by writing the characteristic polynomial:

$$p(s) = (s + \alpha)(s + \beta)(s + \nu) + \mu p(s + \beta)s + p\nu\alpha\beta,$$

where all coefficients are positive, and thus there cannot be real positive roots.

This would seem in contradiction with the presence of the positive cycle $1 \to 3 \to 1$. However, our results are not invalidated because we do not consider cross–constraints among entries in the Jacobian, such as the fact that $J_{22} = -J_{32}$. Clearly, if we could change all entries independently (without changing sign), this system could present a real transition to instability.

For systems with cross–constrained dynamics, we believe that algorithmic/numerical methods are the best approach to discriminate admissible transitions to instability.