Structural conditions for oscillations and multistationarity in aggregate monotone systems

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***Abstract*— We provide necessary and sufficient structural conditions for multistationarity and oscillations in *aggregate monotone systems*, defined as the interconnection of stable monotone components. Our classification is based on the presence of exclusively positive or exclusively negative cycles in the system *aggregate graph*, whose nodes are the monotone subsystems. The results presented here scale up the structural classification of oscillatory and multistationary behaviors in sign–definite biochemical networks previously proposed by the authors. Models of biomolecular systems are discussed to demonstrate the applicability of our classification.**

I. Introduction

Periodic and multistationary dynamics are autonomous behaviors relevant in engineering, physics and biology. These behaviors often depend on key parameters that must be carefully tuned to achieve the desired performance. However, when parameters are uncertain, even determining whether a system has *the capacity* to generate oscillations or multistationarity can be difficult. In this case, it is crucial to find *structural*, parameter–free criteria to evaluate the possible behaviors of the system.

Biological network models are almost always characterized by uncertain parameters, thus structural analysis is particularly important. In a previous paper [1] we considered dynamical systems relevant in biochemistry and biology which present a sign–definite Jacobian, and studied the corresponding Jacobian graph (nodes are associated with species concentrations and arcs with signed Jacobian entries). We called *strong (weak) candidate oscillators* the systems that can exclusively (possibly) transition to instability due to a complex pair of eigenvalues, and *strong (weak) candidate multistationary systems* those which can exclusively (possibly) transition to instability due to a real eigenvalue. Building on a vast literature (see [2], [3], [4], [5], [6], [7], [8], and the thorough discussion in [1]), we proposed a structural classification of oscillatory and multistationary networks based on the exclusive or concurrent presence of positive and negative cycles in the Jacobian graph.

In this paper, we extend our results to aggregate monotone systems, defined as the interconnection of monotone subsystems. The theory of monotone systems [9], [10] simplifies the analysis of large, complex networks which can be decomposed into interconnections of input–output monotone subsystems [11]: monotonicity facilitates the detection of multistationarity [12] and provides necessary conditions for oscillations [13]. Since a monotone subsystem within a large network can be regarded as a single element having a sign–definite input–output mapping, our classification for sign–definite biochemical systems can be successfully scaled to consider interconnections of monotone subsystems, rather than interconnections of a myriad of individual molecular species. We focus on networks comprised of stable monotone subsystems and we provide a characterization of potential multistationary and oscillatory behaviors based on the presence of exclusively positive or exclusively negative cycles. Our classification is applied to evaluate *structurally* the behavior of artificial biomolecular networks [14], [15], and reveals that their design is well suited to achieve the desired periodic or bistable dynamics.

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II. Framework and previous results

We begin by summarizing the general framework and the structural classification introduced in [1]. We consider a vector field $f(\cdot)$, continuously differentiable in all its components $f_i(\cdot)$, $i = 1, ..., n$, and the dynamical system:

$$\dot{x} = f(x), \quad x \in \mathbb{R}^n. \tag{1}$$

Assumption 1: All the solutions of (1) are globally uniformly asymptotically bounded in the compact set $\mathcal{S} \subset \mathbb{R}^n$. Hence, (1) admits an equilibrium $\bar{x}$ in $\mathcal{S}$ ([16], [17], [18]).

Assumption 2: $\partial f_i / \partial x_j$ is either always positive, always negative, or always null in the considered domain.

Assumption 3: For all i, $\partial f_i / \partial x_i < 0$, *i.e.*, the system is non–autocatalytic.

Due to the monotonicity of $f_i(\cdot)$ with respect to each argument, the Jacobian of system (1) is sign definite.

Definition 1: Given a system with a sign–definite Jacobian J, its *structure* is the sign pattern matrix $\Sigma = \text{sign}[J]$. We associate matrix Σ with a directed n–node graph, whose arcs are positive $(+1)$, negative (-1), or zero depending on the sign of the corresponding matrix entries.

Definition 2: A *realization* of a structure Σ is given by any choice of functions $f_i(\cdot)$, along with specific parameter values, which is compatible with Σ.

Clearly, the choice of $f_i(\cdot)$ and its parameters uniquely determines the entries of the Jacobian matrix J.

Definition 3: A property is *structural* if it is satisfied by any realization of a system with a given structure Σ [19]. Hence, a property is not structural if there exists at least one realization which does not satisfy such property.

Definition 4: 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 is odd (even). The *order* of a cycle is the number of arcs involved in the cycle.

To define the concept of *transition to instability*, we consider the system

$$\dot{x}(t) = g(x(t), \mu), \qquad x \in \mathbb{R}^n, \tag{2}$$

where μ is a real–valued parameter and $g(\cdot, \cdot)$ is a sufficiently smooth function, continuous in μ, satisfying Assumptions 1, 2 and 3 for every value of μ. The structure Σ of system (2) is assumed to be invariant with respect to μ. Assumption 1 ensures that an equilibrium exists; all the following definitions refer to this equilibrium, which is, in general, a function of μ: $g(\bar{x}_\mu, \mu) = 0$. We assume that $\bar{x}_\mu$ depends continuously on μ. A suitable change of coordinates always allows us to shift the equilibrium to the origin, without affecting our analysis.

Definition 5: System (2) undergoes a *Transition to Instability (TI)* at $\mu = \mu^*$ iff its Jacobian matrix $J(\bar{x}_\mu)$ is asymptotically stable in a left neighborhood of μ^*, and unstable in a right neighborhood[1]. A TI is *simple* if at most a single real eigenvalue or a single pair of complex conjugate eigenvalues crosses the imaginary axis.

Since most systems have a *dominant* eigenvalue, non–simple TIs are unlikely to occur. We consider two types of simple TIs related to oscillations and multistationarity.

Definition 6: System (2) 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 real part:

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

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

Definition 7: System (2) 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 μ^*.

As is discussed in [1], TIs are related to bifurcation theory: typically, OTIs are related to Hopf bifurcations and RTIs are related to zero–eigenvalue bifurcations [20]; these types of bifurcations, however, occur under additional assumptions.

We now provide general definitions for candidate oscillatory and multistationary[2] systems. We consider system (1), with its given structure Σ, under Assumptions 1, 2 and 3.

[1]The definition holds as well for systems transitioning to instability from the right to the left neighborhood of μ^*: just take $\hat{\mu} = \mu^* - \mu$ as the bifurcation parameter.

[2]We speak of multistationarity, and not of multi–stability, because an RTI causes the appearance of additional equilibria, which are not necessarily stable. A strong candidate multistationary system, however, under suitable assumptions, admits two new equilibria that are asymptotically stable [1].

Definition 8: An *alteration of* (1) is a system of the form (2), such that for $\mu = \mu_0$, $g(x, \mu_0) = f(x)$, while for $\mu \neq \mu_0$ the structure Σ is preserved, yielding equilibrium $\bar{x}_\mu$.

Definition 9: A system of the form (1), with structure Σ, is structurally a candidate

i) *oscillator in the weak sense* iff there exists an alteration (2) which admits an OTI;
ii) *oscillator in the strong sense* iff, for any alteration (2), every simple TI (if any) is an OTI;
iii) *multistationary system in the weak sense* iff there exists an alteration (2) which admits an RTI;
iv) *multistationary system in the strong sense* iff, for any alteration (2), every simple TI (if any) is an RTI.

Fig. 1 summarizes necessary and sufficient conditions provided in [1] for non–critical[3] systems: the presence of negative (positive) cycles in a structure is linked to oscillatory (multistationary) system behavior.

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

Fig. 1: Structural classification provided in [1].

III. Oscillations and multistationarity in aggregate monotone systems

We now extend the structural results in [1] to aggregate systems that are composed of stable monotone components.

We define an aggregate system as the interconnection of N subsystems of the form

$$\dot{z}_i(t) = F_{ii}(z_i(t)) + \sum_{j \in \mathcal{J}_i} G_{ij}(w_{ij}(t)), \tag{3}$$

$$w_{ki}(t) = H_{ki}(z_i(t)), \quad k \in \mathcal{K}_i, \tag{4}$$

where $i = 1, ..., N$, $z_i(t)$ is the state vector associated with subsystem i, $w_{ij} \in \mathbb{R}$ are the subsystem inputs and $w_{ki} \in \mathbb{R}$ are its outputs. Subsystem i receives inputs from subsystems $j \in \mathcal{J}_i$, and sends an output to subsystems $k \in \mathcal{K}_i$, where $\mathcal{J}_i$ and $\mathcal{K}_i$ are the sets that index all the subsystems having respectively an upstream or downstream connection with subsystem i (see Fig. 2). We assume that $F_{ii}(\cdot)$, $G_{ij}(\cdot)$ and $H_{ki}(\cdot)$ are sufficiently smooth functions. Function $G_{ij}(w_{ij}(t))$ models the influence of subsystem j on subsystem i through $w_{ij}(t)$, output of subsystem j.

Assumption 4: The input–to–state mappings G_{ij} are either non–decreasing or non–increasing: $w_{ij} \geq \hat{w}_{ij}$ implies either $G_{ij}(w_{ij}) \geq G_{ij}(\hat{w}_{ij})$, or $G_{ij}(w_{ij}) \leq G_{ij}(\hat{w}_{ij})$.

Assumption 5: Functions $H_{ij}(z_j)$ are non–decreasing.

Assumption 5 enables a simplified analysis without being restrictive: negative interconnection trends among subsystems can be captured by the input functions $G_{ij}(w_{ij})$. For example, consider a generic subsystem 1 and the influence of subsystem 2 on 1 given by w_{12}:

$$\dot{z}_1 = F_{11}(z_1) + G_{12}(w_{12}), \qquad w_{12} = H_{12}(z_2),$$

[3]A system is *critical* when all negative cycles (if any) are of order two.

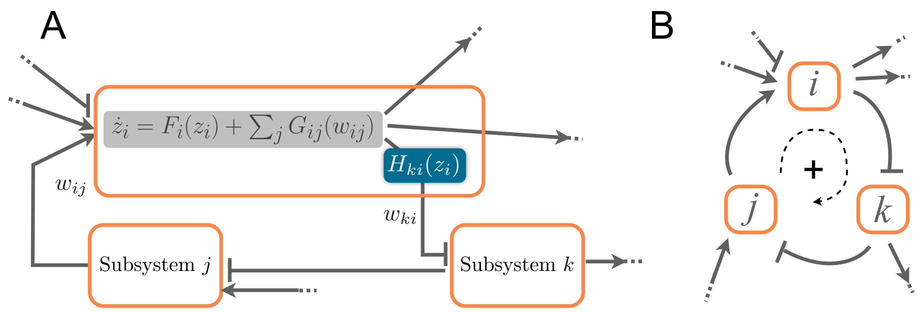

Fig. 2: (A) Sketch of an aggregate monotone system, *i.e.*, the interconnection of monotone subsystems (3)–(4). (B) Graph of the aggregate monotone system, where each monotone subsystem is collapsed in a single node. Pointed arrowheads indicate non–decreasing interconnections, while hammer–arrowheads indicate non–increasing interconnections.

with H_{12} decreasing. The overall interaction depends on the monotone compound function $G_{12} \circ H_{12}$, and we can account for the decreasing trend with a simple sign change: $\hat{w}_{12} = -w_{12}$. The net effect remains unchanged:

$$\dot{z}_1 = F_{11}(z_1) + G_{12}(\hat{w}_{12}), \qquad \hat{w}_{12} = H_{12}^-(z_2),$$

where $H_{12}^-(\omega) \doteq -H_{12}(\omega)$ is now increasing.

Definition 10: Subsystem (3)–(4) is *unconditionally stable* iff, for constant input values $\bar{w}_{ij}$, it admits a single equilibrium $\bar{z}_i$, which is the solution of

$$0 = F_{ii}(\bar{z}_i) + \sum_{j \in \mathcal{J}_i} G_{ij}(\bar{w}_{ij}), \quad \bar{w}_{ki} = H_{ki}(\bar{z}_i), \qquad (5)$$

and such an equilibrium is asymptotically stable (all of the eigenvalues of the Jacobian $J_i = \frac{\partial F_{ii}}{\partial z_i}\Big|_{\bar{z}_i}$ have a negative real part).

Definition 11: Subsystem (3)–(4), with inputs w_{ij}, is *input–to–state monotone* iff, for $w_{ij}(t) \geq \tilde{w}_{ij}(t)\ \forall\ j \in \mathcal{J}_i$, we have that either $z_i(0) \geq \tilde{z}_i(0) \implies z_i(t) \geq \tilde{z}_i(t)$, $t \geq 0$, or $z_i(0) \leq \tilde{z}_i(0) \implies z_i(t) \leq \tilde{z}_i(t)$, $t \geq 0$.
With a slight abuse of notation, we call simply *monotone* a system that is either monotone, or anti–monotone.

Assumption 6: We consider aggregate systems composed of subsystems (3)–(4), each unconditionally stable as in Definition 10 and input–to–state monotone as in Definition 11.

Given an input–output monotonicity characterization for all the subsystems, we can collapse each subsystem into an equivalent *aggregate node*. Then we can define an *aggregate graph* (cf. Fig. 2 B), whose nodes correspond to the aggregate nodes, and whose signed arcs represent the influence of subsystem j on subsystem i. The sign of each arc depends on the trend of the associated input–to–state mapping $G_{ij}(w_{ij})$: positive (resp. negative) arcs are associated with non–decreasing (resp. non–increasing) mappings.

Definition 12: A graph is *strongly connected* if an oriented path exists connecting each pair of nodes.

Based on the cycles formed by the arcs connecting aggregate nodes, we can still classify structural oscillatory and multistationary behaviors. The main result of the paper provides necessary and sufficient conditions for an aggregate monotone system to be a strong candidate oscillator (every transition to instability is an OTI) or a strong multistationary system (every transition to instability is an RTI), in terms of the cycles existing in the corresponding aggregate graph.

Theorem 1: Consider an aggregate system, formed by the interconnection of strongly connected subsystems of the form (3)–(4), satisfying Assumptions 4, 5 and 6. The aggregate system is structurally a candidate

i) oscillator in the strong sense iff all the cycles in the aggregate graph are negative;
ii) multistationary system in the strong sense iff all the cycles in the aggregate graph are positive.

□

Remark 1: Positive cycles are generally present *within the monotone subsystems*. However, if all the cycles in the aggregate graph are negative, the aggregate system is *not* a weak candidate multistationary system, due to the assumption of unconditional stability for each subsystem.

IV. Proof of the Main Result

In order to prove Theorem 1, we need to state a preliminary lemma and to introduce alterations by $\nu_{\kappa,\epsilon}$ functions.

Given an aggregate system, under the assumptions of Theorem 1, for constant input values $\bar{w}_{ij}$, each subsystem admits a single equilibrium $\bar{z}_i$, which is implicitly defined by the steady–state condition (5) and is globally asymptotically stable. Then we can define

$$A_{ii} = \frac{\partial F_{ii}}{\partial z_i}\Big|_{\bar{z}_i}, \quad B_{ij} = \frac{\partial G_{ij}}{\partial w_{ij}}\Big|_{\bar{w}_{ij}}, \quad C_{ki} = \frac{\partial H_{ki}}{\partial z_i}\Big|_{\bar{z}_i}.$$

In the corresponding aggregate graph, whose nodes are associated with the monotone subsystems, the sign of the directed arc connecting subsystems j and i depends on the trend of function $G_{ij}(w_{ij})$, *i.e.*, on the sign of B_{ij}.

Lemma 1: If the steady–state input–to–output mapping in system (3)-(4) is monotone, then the input–to–output mapping between each pair (w_{ij}, w_{ki}) is implicitly defined by (5), and

$$\frac{\partial w_{ki}}{\partial w_{ij}} = -C_{ki} A_{ii}^{-1} B_{ij}$$

is a positive or negative scalar, depending on the sign of the elements of B_{ij}. □

Proof: As a consequence of Assumption 6 (monotonicity and unconditional stability), A_{ii} is a Metzler matrix and is asymptotically stable. Therefore, all the entries of its inverse A_{ii}^{-1} are non–positive. Due to Assumption 5, C_{ki} has non–negative elements, while Assumption 4 ensures that B_{ij} has all non–negative elements or all non–positive elements,

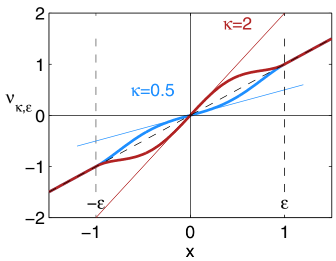

Fig. 3: Plot of an example $\nu_{\kappa,\epsilon}$ function, defined as $\nu_{\kappa,\epsilon}(x) = x + \arctan[(\kappa-1)x]\left[1-\left(\frac{x}{\epsilon}\right)^2\right]^2$ for $|x| < \epsilon$, $\nu_{\kappa,\epsilon}(x) = x$ for $|x| \geq \epsilon$, with $\epsilon = 1$; $\kappa = 2$ (red) and $\kappa = 0.5$ (blue). [1]

depending on the type of interaction. Hence, the sign of $\partial w_{ki}/\partial w_{ij}$ only depends on the sign of B_{ij}. ■

Now we define an alteration (2) of systems of the form (1) that does not alter Σ. This vector field alteration, introduced in [1], will be used 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 13: A *differential scaling map* $\nu_{\kappa,\epsilon}$ (see *e.g.* Fig. 3), where $\kappa,\ \epsilon > 0$ are real parameters, is a strictly increasing, continuously differentiable, odd function[4], such that $\nu_{\kappa,\epsilon} = x$ for $|x| \geq \epsilon$ and $\frac{d\nu_{\kappa,\epsilon}(0)}{dx} = \kappa$.

Therefore, a differential scaling map $\nu_{\kappa,\epsilon}$ has a scalable derivative at the origin and is the identity function outside the ϵ–ball.

The alteration obtained by composing the vector field $f(x)$ and a differential scaling map $\nu_{\kappa,\epsilon}$,

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

a) does not alter the equilibrium $x_i = 0$;
b) does not alter the sign of the Jacobian entries, *i.e.*, the structure Σ of the system;
c) changes the partial derivatives in $x_i = 0$ as:

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

d) does not alter $\dot{x} = f(x)$ outside the ϵ–ball, hence preserves boundedness of the system solution.

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 without changing the value of the equilibrium. Hence, $\nu_{\kappa,\epsilon}$ alterations can be used to independently scale the magnitude of desired cycles and find, given a structure, a realization that satisfies a property of interest. Even if we use different parameters κ_i to scale different arcs, we may always assume that $\kappa_i(\mu)$ are functions of a single parameter μ, consistently with (2).

Proof of Theorem 1

i): Sufficiency. We need to show that, if exclusively negative cycles are present in the aggregate graph, then the Jacobian of the system cannot have real non–negative eigenvalues (hence, only oscillatory destabilization is possible); *i.e.*, denoting by A the Jacobian of the aggregate system,

$$\det(\lambda I - A) \neq 0 \qquad \forall\ \ \lambda \in \mathbb{R}, \qquad \lambda \geq 0.$$

Ab absurdo, assume that A admits a real non–negative eigenvalue λ. Denoting the ith linearized subsystem by

$$\dot{\zeta}_i(t) = A_{ii}\zeta_i(t) + \textstyle\sum_{j\in\mathcal{J}_i} B_{ij}\omega_{ij}(t), \quad \omega_{ki}(t) = C_{ki}\zeta_i(t),$$

any eigenvalue λ must satisfy the equation

$$\lambda\zeta_i \quad = \quad A_{ii}\zeta_i + \sum_{j\in\mathcal{J}_i} B_{ij}\omega_{ij},$$

where $\zeta = [\zeta_1 \cdots \zeta_i \cdots \zeta_N]^\top$ is the associated eigenvector. We find

$$\zeta_i = -(A_{ii} - \lambda I)^{-1} \sum_{j\in\mathcal{J}_i} B_{ij}\ \omega_{ij},$$

where all the elements of $(A_{ii} - \lambda I)^{-1}$ are non–positive because of monotonicity. In fact, A_{ii} is a stable Metzler matrix; $(A_{ii} - \lambda I)$ is still a stable Metzler matrix, because $\lambda \geq 0$; thus all the elements of $(A_{ii} - \lambda I)^{-1}$ are non–positive.

Then, for all $k \in \mathcal{K}_i$ we can write

$$\omega_{ki} = \sum_{j\in\mathcal{J}_i} -C_{ki}(A_{ii} - \lambda I)^{-1} B_{ij}\ \omega_{ij} = \sum_{j\in\mathcal{J}_i} \pi^i_{kj}\ \omega_{ij}, \quad (6)$$

where $\pi^i_{kj} \doteq -C_{ki}(A_{ii} - \lambda I)^{-1}B_{ij}$ are scalars. Equations (6) are linear in ω_{ij} and can be compactly rewritten as

$$\omega = \Pi\omega, \quad (7)$$

where ω is a vector including all the arc variables ω_{ij}, which define the interconnections in the aggregate system. In the aggregate graph, the sign of the arc from node i to node k depends on the sign of $\pi^i_{kj} = -C_{ki}(A_{ii} - \lambda I)^{-1}B_{ij}$. Therefore, matrix Π in (7) has the same cycles as the Jacobian A of the aggregate system. Let Σ_Π be the sign matrix corresponding to Π. Every cycle in the aggregate graph corresponds to a cycle in matrix Σ_Π. For example, the sign matrix Σ_Π associated with the aggregate graph in Fig. 4 is a 5×5 matrix, since there are 5 arcs. If we order the arc variables as $\omega = [\omega_{21}\ \ \omega_{32}\ \ \omega_{43}\ \ \omega_{14}\ \ \omega_{24}]^\top$, we have:

$$\Sigma_\Pi = \begin{bmatrix} 0 & 0 & 0 & - & 0 \\ + & 0 & 0 & 0 & - \\ 0 & + & 0 & 0 & 0 \\ 0 & 0 & + & 0 & 0 \\ 0 & 0 & + & 0 & 0 \end{bmatrix}. \quad (8)$$

(The sign of the interaction depends on that of the incoming arc, related to B_{ij}, as shown in Fig. 5.) Therefore, if all the cycles in the aggregate graph are negative, then all the cycles in matrix Σ_Π are negative as well. Then we can resort to the following result, from Theorem 3.1 in [21].

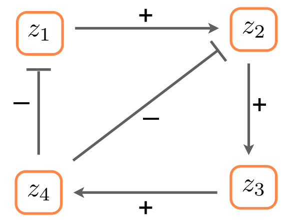

Fig. 4: Aggregate graph corresponding to the sign matrix (8).

[4]A function is odd iff $\nu_{\kappa,\epsilon}(-x) = -\nu_{\kappa,\epsilon}(x)$.

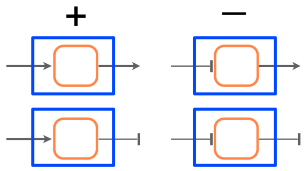

Fig. 5: Rules for determining the sign of interactions in matrix Σ_{Π}.

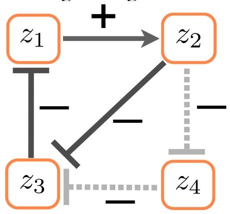

Fig. 6: Aggregate graph with emphasis on the selected positive cycle (dark gray, solid); excluded arcs are dashed, light gray.

Theorem 2: Given a matrix M with negative diagonal entries, such that all the cycles in it are non–positive, each leading minor of M having order k has sign $(-1)^k$. $\square$

Since all the cycles in Π, hence in $(\Pi - I)$, are negative, we have that sign $[\det(\Pi - I)] = \pm 1$. Now we go back to relation (7), which is equivalent to $(\Pi - I)\omega = 0$ and thus implies $\det(\Pi - I) = 0$. Yet, if all the cycles are negative, $\det(\Pi - I) \neq 0$, hence (7) cannot be true for $\omega \neq 0$. Therefore, we conclude that the system cannot admit real non–negative eigenvalues $\lambda \geq 0$, and is thus a candidate oscillator in the strong sense.

i): Necessity. Suppose *ab absurdo* that a positive cycle exists in the aggregate graph. Then we can modify the arcs *connecting the aggregate nodes* by means of $\nu_{\epsilon,\kappa}$ alterations, without compromising stability of the monotone subsystems[5]: we apply individual differential scaling maps $\nu_{\kappa,\epsilon}$, scaling the κ parameter so as to enhance the considered positive cycle only and virtually exclude all the arcs not involved in it. For instance, in the structure in Fig. 6, we can select the cycle formed by subsystems 1–2–3 and disregard all the other arcs in the aggregate graph. Precisely, for each arc, through a differential scaling map $\nu_{\kappa,\epsilon}$ we scale the interconnections so that $\kappa_{ij} = 1$ for the arcs involved in the positive cycle, $\kappa_{ij} \ll 1$ for the arcs not involved. By reordering the nodes, we find a realization which is the positive feedback of m monotone subsystems of the form

$$\begin{aligned} \dot{z}_i(t) &= F_{ii}(z_i(t)) + \sum_{j \in \mathcal{J}_i} G_{i,i-1}(w_{i,i-1}(t)) \\ w_{i+1,i}(t) &= H_{i+1,i}(z_i(t)), \quad k \in \mathcal{K}_i, \end{aligned}$$

where m is the order of the considered positive cycle and the indices are to be intended in a circular way. The selected system is monotone and has thus a real dominant eigenvalue. Therefore a realization can be found which can be solely destabilized due to a real root which crosses the imaginary axis through the origin, yielding an RTI. This contradicts the assumption; hence we can conclude that there can be only negative cycles in the aggregate graph.

[5]We cannot scale up a single positive cycle inside a monotone subsystem in order to induce an RTI, because we assume that parameter variations preserve stability of each monotone subsystem. Hence, the only arcs which can be scaled are those connecting different subsystems.

ii). Sufficiency: if positive cycles only are present in the aggregate graph, then the overall system is monotone, hence has a dominant real eigenvalue and only real destabilization is possible. **Necessity:** if a negative cycle exists, then it can be enhanced through a differential scaling map $\nu_{\kappa,\epsilon}$, as in the necessity proof of i), and thus a realization can be found which admits an OTI, contradicting the assumption.

V. Examples

Nature provides many excellent examples of aggregate monotone systems. For instance, in the MAPK pathway, each stage of the phosphorylation cascade can be regarded as an unconditionally stable monotone module [12], [22] and, depending on the active feedback loops, the network can generate bistable or oscillatory behaviors [12], [22], [23].

Here we provide examples of *artificial* biochemical networks that turn out to be aggregate monotone systems, and candidate oscillators or multistationary systems in the strong sense. The fact that these artificial systems possess such strong properties indicates that their bottom–up design is fundamentally sound. In the following, capital letters indicate species, small letters their concentration.

Example 1: (**Biochemical oscillator**) We consider a simplified model for an artificial oscillator where transcriptional regulation is achieved with RNA aptamers, which are RNA molecules whose sequence is synthetically evolved to bind and modify the properties of a desired target [14]. In this system, two aptamers X_1 and X_3 are transcribed by RNA polymerases X_2 and X_4 respectively. Aptamer X_1 inactivates polymerase X_4, while aptamer X_3 activates polymerase X_2. The system is:

$$\begin{aligned} \dot{x}_1 &= \kappa_1 x_2 - \delta_1 x_1 - \gamma_2 x_4 x_1 \\ \dot{x}_2 &= -\beta_1 x_2 + \gamma_1 (x_2^{tot} - x_2) x_3 \\ \dot{x}_3 &= \kappa_2 x_4 - \delta_2 x_3 - \gamma_1 (x_2^{tot} - x_2) x_3 \\ \dot{x}_4 &= \beta_2 (x_4^{tot} - x_4) - \gamma_2 x_4 x_1 \end{aligned}$$

After a state transformation, the system Jacobian is

$$\begin{bmatrix} -\gamma_1(x_2^{tot} - \bar{x}_2) - \delta_2 & \gamma_1 \bar{x}_3 & \kappa_2 & 0 \\ \gamma_1(x_2^{tot} - \bar{x}_2) & -\beta_1 - \gamma_1 \bar{x}_3 & 0 & 0 \\ 0 & 0 & -\beta_2 - \gamma_2 \bar{x}_1 & \gamma_2 \bar{x}_4 \\ 0 & -\kappa_1 & \gamma_2 \bar{x}_1 & -\gamma_2 \bar{x}_4 - \delta_1 \end{bmatrix}$$

The overall system, formed by the negative feedback interconnection of two unconditionally stable aggregate monotone subsystems, is thus a strong candidate oscillator. It is indeed the simplified model of a biochemical circuit that, if driven to instability, exhibits sustained oscillations, as shown by simulation results in [14] for some choice of the parameters.

Example 2: (**Bistable circuit**) A simplified model is proposed in [15] for an artificial bistable network where transcriptional regulation is achieved with RNA aptamers. Here aptamers X_1 and X_3 are transcribed by polymerases X_2 and X_4 respectively; X_1 represses polymerase X_4 and X_3

represses polymerase X_2. The system is:

$$\begin{aligned}\dot{x}_1 &= \kappa_1 x_2 - \delta x_1 - \gamma x_4 x_1\\ \dot{x}_2 &= \beta x_2^{tot} - \beta x_2 - \gamma x_2 x_3\\ \dot{x}_3 &= \kappa_2 x_4 - \delta x_3 - \gamma x_2 x_3\\ \dot{x}_4 &= \beta x_4^{tot} - \beta x_4 - \gamma x_4 x_1\end{aligned}$$

A state transformation yields the Jacobian

$$\begin{bmatrix} -\beta - \gamma\bar{x}_3 & \gamma\bar{x}_2 & 0 & 0\\ \gamma\bar{x}_3 & -\gamma\bar{x}_2 - \delta & \boxed{\kappa_2} & 0\\ 0 & 0 & -\beta - \gamma\bar{x}_1 & \gamma\bar{x}_4\\ \boxed{\kappa_1} & 0 & \gamma\bar{x}_1 & -\gamma\bar{x}_4 - \delta \end{bmatrix}$$

The overall system, formed by the positive feedback interconnection of two unconditionally stable monotone subsystems, is thus a strong candidate bistable network. Actual bistability of the system is shown by simulation results in [15] for some choice of the parameters.

VI. Concluding discussion

Many biochemical systems are monotone [10], or can be regarded as the interconnection of monotone subsystems: notable examples are the Cds–Wee1 network [11], the MAPK pathway [10], the Goldbeter oscillator [13] in *Drosophila*. Since monotonicity is a property that can be verified without the exact knowledge of functional expressions and system parameters, criteria relying on monotonicity can be considered robust with respect to modeling choices and parametric uncertainty. The *structural* nature of monotonicity makes it an ideal property to embed by design in artificial biochemical systems [24], [25], [26], [14], [15].

In this paper, we have provided *structural* necessary and sufficient conditions for oscillatory and multistationary behaviors in *aggregate monotone systems*. Our criteria, which are an extension of the results in [1], are based on the exclusive presence of negative or positive cycles in the system aggregate graph. No strong conclusions can be drawn for aggregate graphs where positive and negative cycles are concurrently present. Note that the proposed classification is valid only for systems in which interactions between unconditionally stable monotone components are independent, since in our proof we assume to be able to independently scale them. Although this requirement is generally satisfied, interactions between bimolecular systems may be coupled by retroactivity or competition for common cellular resources.

Our characterization applies to any system presenting a sign–definite Jacobian, but is particularly useful for the analysis of biochemical reaction networks: criteria to predict the possible dynamic behavior of a system *independent of parameter values* are especially important for biomolecular systems, due to their intrinsic uncertainty and variability. For significant biochemical examples, our classification provides a parameter–free method to assess or rule out potential dynamic behaviors. For this reason, this approach can be useful to design artificial biomolecular circuits that are structurally well suited to achieve the desired dynamics.

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