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Graphical Construction of Stability Certificates for Biomolecular Interaction Networks

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***Abstract*— We study robust stability of Biological Interaction Networks (BINs) by constructing stability certificates in the form of Robust Lyapunov Functions (RLFs) using graphical methods. Previous works have mainly constructed RLFs by utilizing linear programs or iterative algorithms. Such algorithms become tedious or computationally infeasible for large networks. In addition, they do not identify motifs or graph modifications that maintain stability. In this work, we provide several graphical criteria for constructing stability certificates. We characterize a set of stability-preserving graph modifications which include, in particular, the enzymatic catalysis motif. Hence, stability of a class of arbitrarily large networks can be examined by simple visual inspection. We present applications of this technique to Post-Translational Modification (PTM) cycles, Ribosome Flow Model (RFM), and T-cell kinetic proofreading. *Index Terms*— Nonlinear Systems, Systems Biology, Petri-nets, Lyapunov Functions, Robust Stability, Reaction Networks.**

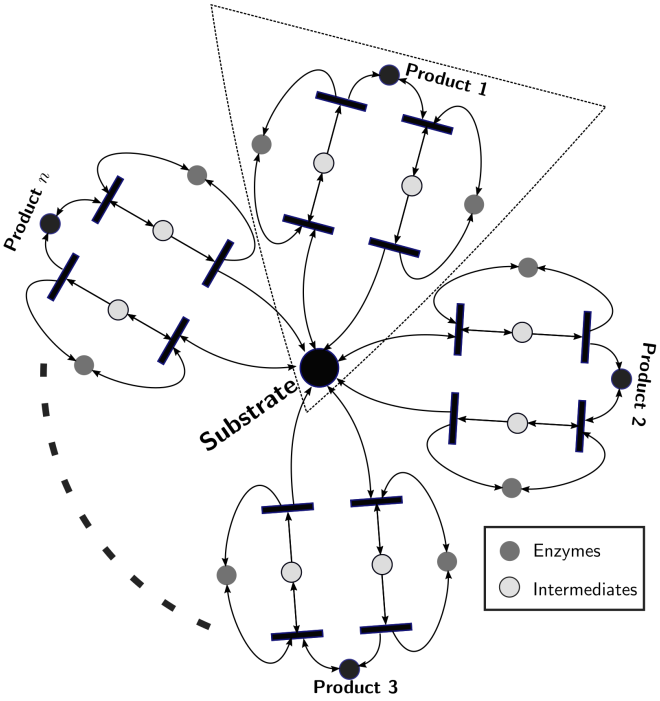

Fig. 1. **Computational RLF construction is tedious for large networks.** The figure depicts a Petri-net representation of the *PTM star*: a substrate that is a target of an arbitrary finite number of distinct competing PTM cycles (e.g, phosphorylation, methylation, ubiquitination, etc). The subnetwork inside the dotted triangle depicts a single PTM cycle. A rectangle denotes a reaction, while a circle denotes a species.

I. INTRODUCTION

Biomolecular Interaction Networks (BINs) function under severe forms of external and internal uncertainty. Nevertheless, they operate robustly and consistently to maintain *homeostasis*, which is understood as the maintenance of a desired steady-state against environmental factors, external signals, and *in-vivo* fluctuations in the concentrations of biochemical species. In fact, robustness has been proposed as a key *defining* property of biological networks [1, 2]. However, mathematical analysis of such networks has been lagging as the dynamical system descriptions of such networks suffer from *nonlinearity* and *uncertainty*. General nonlinear dynamical systems are already difficult to analyze due to their unpredictability and instability. Small fluctuations in concentrations, or tiny changes in kinetic parameters, can have radical effects causing the observable phenotype to be driven to a different region of the state space, and/or to lose stability altogether and transform into a sustained oscillation or chaotic behavior. This may make the biological network lose its function and cause key species to reach undesirable or even unsafe levels. In fact, disease can be often characterized mathematically as the loss of stability of a certain phenotype [3, 4]. A second complicating factor is the fact that the exact form of kinetics (determining the speed of interactions) are difficult to measure and are subject to environmental changes. Therefore, verifying the stability of a given nonlinear BIN without reference to its kinetics has been a challenging long-standing goal in systems biology research [5]. Nevertheless, partial success has been achieved in this endeavor. Examples include the theory of complex balance [6], [7], [8], and the theory of monotone BINs [9]. More recently, stability certificates have been constructed via Robust Lyapunov Functions (RLFs) in reaction [10–12], and concentration coordinates [12–16]. Except for a small subclass of BINs (see §III.C), such methods mainly utilize computational algorithms to construct RLFs via either iterative algorithms or linear programs. However, such algorithms act as “black-boxes” and are not interpretable in terms of the structural properties of the network’s graph. This has several drawbacks. First, computational algorithms become tedious for larger networks as the number of species and reactions grow. Consider the *PTM star* depicted in Figure 1 whose size grows considerably for large n. Second, “stability-preserving” graph modifications are not well characterized. A simple modification of the BIN graph mandates a re-run of the computational algorithm from scratch. For instance, Is the stability of the PTM star preserved if we added

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inflow/outflow reactions for the substrate ($\emptyset \rightleftharpoons$ Substrate)?. Third, fundamental "motifs" have been described as the building blocks of BINs [17]. However, a corresponding "modular" theory for RLF construction that utilizes the stability properties of its subnetworks is lacking. For example, the difference between the PTM star (Fig. 1) with n and $n+1$ products is in the addition of an extra *PTM cycle*. How does the addition of the extra motif affect stability?.

The above questions are hard to answer using computational algorithms. In this work, we identify a set of stability preserving graph modifications. In particular, we show that the stability of many large networks in systems biology such as the PTM cycle, the Ribosome Flow Model, and others can be understood modularly. For the specific network in Figure 1, we will show that it can be "reduced" to a simple linear network (See Figure 2). Hence, it admits a stability certificate for every $n \geq 1$, a result which is not readily achievable using previous results [6–9, 12, 14]. We will show that the addition of an inflow/outflow reaction to the substrate preserves stability, and that the PTM cycle is a fundamental "stable" motif in a precise manner to be defined.

The paper proceeds as follows. Section II reviews notation and definitions. Section III reviews relevant results on linear (mono-molecular) networks. The main results are stated in section IV. Applications are discussed in section V. Finally, we discuss future directions in section VI.

II. BACKGROUND AND NOTATION

A. Biological Interaction Networks

Any collection of chemical reactions can be written mathematically using the formalism of of Biological Interaction Networks (BINs). Hence, we review the standard definitions and notation [7, 8, 12, 18, 19].

A BIN (also known as a Chemical Reaction Network (CRN)) is a pair $\mathscr{N} = (\mathscr{S}, \mathscr{R})$, where $\mathscr{S} = \{X_1, .., X_n\}$ is the set of species, and $\mathscr{R} = \{\mathbf{R}_1, ..., \mathbf{R}_\nu\}$ is the set of reactions. A species is the entity that partakes in or is formed in a chemical interaction. Within the realm of biomolecular networks, a species can be a substrate, a complex, an enzyme, an mRNA molecular, a gene promoter state, etc. A reaction is the transformation of reacting species into product species. Examples include complex formation, binding, unbinding, decay, production, complex formation, etc.

The mathematical structure of BINs can be described by two mathematical substructures: *the stoichiometry* and *the kinetics*.

a) The Stoichiometry: The relative gain or loss of molecules of species X_i between the sides of each reaction is the *stoichiometry of* X_i. This is represented by writing a reaction as:

$$\mathbf{R}_j : \quad \sum_{i=1}^{n} \alpha_{ij} X_i \longrightarrow \sum_{i=1}^{n} \beta_{ij} X_i, \; j = 1, .., \nu, \qquad (1)$$

where $\alpha_{ij}, \beta_{ij} \geq 0$ are integers known as the *stoichiometry coefficients*. If a transformation can happen also in the reverse direction, then $\mathbf{R}_j$ is said to be *reversible* and its reverse is denoted by $\mathbf{R}_{-j}$. A reaction can have no reactants or no products (though not simultaneously). The empty side is denoted by $\emptyset$.

If a reaction has a species both as a reactant and as a product (for example, $X+Y \to X$) then it is called *catalytic*.

The *stoichiometry matrix* Γ of a given network is an $n \times \nu$ matrix whose (i, j)th entry describes the net gain/loss of the ith species at the jth reaction. Hence, it can be written element wise as: $[\Gamma]_{ij} = \beta_{ij} - \alpha_{ij}$.

b) Kinetics.: The set of relationships that determine the speed of transformation of reactant species into product species are known as *kinetics*. In order to describe such relations, the species need to be quantified. A species X_i is quantified by assigning it a non-negative real number known as the concentration x_i. A reaction $\mathbf{R}_j$ is assigned a single-valued mapping $R_j : \bar{\mathbb{R}}_+^n \to \bar{\mathbb{R}}_+$ known as the *reaction rate*. The reaction rate vector is written as $R(x) = [R_1(x), ..., R_\nu(x)]^T$.

The most common form of kinetics is known as *Mass-Action* and it can be written as: $R_j(x) = k_j \prod_{i=1}^n x_i^{\alpha_{ij}}$, where $k_j > 0, j = 1, .., \nu$ are the *kinetic constants*. However, this form "is not based on fundamental laws" and is merely "good phenomenology" justified by imagining the reactants as colliding molecules [20]. In biological systems, in particular, other forms of kinetics usually arise when modeling networks involving multiple time-scales. This includes Michaelis-Menten, Hill kinetics, etc. Therefore, we do not assume a specific functional form of kinetics. We only assume that the kinetics are *monotone*. More precisely, the reaction rates $R_j(x), j = 1, .., \nu$ satisfy:

- **AK1.** each reaction varies smoothly with respects to its reactants, i.e $R(x)$ is C^1;
- **AK2.** a reaction requires all its reactants to occur, i.e., if $\alpha_{ij} > 0$, then $x_i = 0$ implies $R_j(x) = 0$;
- **AK3.** if a reactant increases, then the reaction rate increase, i.e $\partial R_j / \partial x_i(x) \geq 0$ if $\alpha_{ij} > 0$ and $\partial R_j / \partial x_i(x) \equiv 0$ if $\alpha_{ij} = 0$. Furthermore, the aforementioned inequality is strict whenever the reactants are strictly positive.

For a given network $\mathscr{N}$, the set of a reaction rates satisfying the assumptions above is called the *admissible kinetics* and is denoted by $\mathscr{K}_{\mathscr{N}}$.

c) Dynamics.: We view the concentrations as trajectories in time and write them as $x(t) = [x_1(t), ..., x_n(t)]^T$. The temporal evolution of the network is given by the following Ordinary Differential Equation (ODE):

$$\dot{x} = \Gamma R(x), \;\; x(0) = x_\circ. \qquad (2)$$

The positive orthant is forward-invariant for (2), i.e. if $x_\circ$ is positive, then the trajectory stays positive for all time $t \geq 0$.

In the biomolecular context, there are usually *conserved quantities* which do not get created or annihilated during the course of the reaction. This can include total amounts of DNA, enzymes, substrates, ribosomes, etc. Mathematically, a stoichiometric conservation law is a nonnegative vector $d \in \mathbb{R}^n_{\geq 0}$ satisfying $d^T \Gamma = 0$. If d is positive then the network is called *conservative*.

The existence of a conservation law implies that $d^T x(t) \equiv$

$d^T x(0)$. Hence, the positive orthant is partitioned into a foliage of subsets known as *stoichiometric classes*. For a state vector $x_\circ$, the corresponding class is written as $\mathscr{C}_{x_\circ} := (\{x_\circ\} + \mathrm{Im}(\Gamma)) \cap \bar{\mathbb{R}}_+^n$, and it is forward invariant. Therefore, all Lyapunov functions and claims of stability are relative to a stoichiometric class. For a conservative network, all stoichiometric classes are compact polyhedral sets, and hence all trajectories are bounded.

A vector v is called a *flux* if $\Gamma v = 0$. In order to simplify the treatment, we assume the following about the stoichiometry of the network:

AS1. There exists a positive flux, i.e., $\exists v \in \ker \Gamma$ such that $v \gg 0$.

AS2. The network has no catalytic reactions.

Assumption AS1 is necessary for the existence of positive steady states for the corresponding dynamical system (2).

B. Graphical representation: Petri-Nets

BINs can be represented graphical in several ways. We adopt the *Petri-net* formalism [21] (also known as the species-reaction graph [22]). A Petri-net is a weighted directed bipartite graph. The vertices consists of the set of the species $\mathscr{S}$ (represented by circles) and the set of reactions $\mathscr{R}$ (represented by rectangles). An edge with a weight w from $X_i \in \mathscr{S}$ to $\mathbf{R}_j \in \mathscr{R}$ means that X_i is a reactant of $\mathbf{R}_j$ with stoichiometric coefficient w, while the reverse edge means that X_i is a product of $\mathbf{R}_j$ with a stoichiometric coefficient w. For a more compact representation, if two reactions are the reverse of each other (e.g, $\mathbf{R}_j, \mathbf{R}_{-j}$) then they are represented as a single reaction in the Petri-net with reversible edges. In the formalism of Petri-nets [23], the stoichiometric matrix Γ is the *incidence matrix* of the Petri-net.

For example, the PTM star in Fig. 1 corresponds to the following network:

$$S + E_i \rightleftharpoons C_i \longrightarrow P_i + E_i, \tag{3}$$

$$P_i + F_i \rightleftharpoons D_i \longrightarrow S + F_i, \tag{4}$$

$i = 1, .., n$, where S denotes the substrate and P_i denotes the ith product.

C. Robust Lyapunov Functions

Following our previous work [11–13], a locally Lipschitz function $V : \mathbb{R}^n \to \mathbb{R}_{\geq 0}$ is a Robust Lyapunov Function (RLF) for a given network $\mathscr{N}$ with kinetics $\mathscr{K}_{\mathscr{N}}$ iff:

1) it is *positive-definite*, i.e., $V(x) \geq 0$ for all x, and $V(x) = 0$ iff $\Gamma R(x) = 0$, and
2) it is *non-increasing*, i.e., $\dot{V}(x) \leq 0$ for all x and all $R \in \mathscr{K}_{\mathscr{N}}$.

Since V is not assumed to be continuously differentiable, the derivative above is defined in the sense of Dini as $\dot{V}(x) := \limsup_{h \to 0^+} (V(x + h\Gamma R(x)) - V(x))/h$ [24]. Existence of an RLF guarantees that the steady state set is Lyapunov stable, and that all V's level sets are trapping [11, 12, 24]. Global stability can be verified by a LaSalle argument or by establishing robust non-degeneracy of the Jacobian [11, 12, 15].

III. LINEAR (MONO-MOLECULAR) NETWORKS

A. Definition

Studying general nonlinear BINs is, predictably, a difficult and open problem. In comparison, assuming linearity simplifies the analysis considerably. In order to get a linear ODE with Mass-Action kinetics, all the reactions have to be *monomolecular*. In other words, there is only a unique reactant with stoichiometry coefficient 1 for each reaction. The resulting ODE can be studied via standard analysis methods for positive linear systems [25, 26], or as a special case of complex-balanced networks [7]. Nevertheless, it has been long-observed that the linearity of the kinetics is not needed for stability analysis. Instead, similar analysis can be performed for general monomolecular networks with monotone kinetics [27]. This generalized class of networks is often known as *compartmental networks* [28]. In this paper, we call them *linear networks* since the corresponding Petri-net is linear [29], which means that each reaction has a unique reactant and a unique product with the stoichiometry coefficients equal to one. Hence, we use a graphical notion of linearity and not a kinetic one. Therefore, a linear network can have nonlinear kinetic rates. The definition is stated formally below:

Definition 1: A given BIN $(\mathscr{S}, \mathscr{R})$ is said to be *linear* if each reaction can be written as either $X_i \xrightarrow{R_{ij}} X_j$, $\emptyset \xrightarrow{u_i} X_i$, or $X_i \xrightarrow{R_i} \emptyset$ for some i, j where $R_{ij}, R_i : \mathbb{R}_{\geq 0} \to \mathbb{R}_{\geq 0}, u_i \geq 0$ are the reaction rates.

Applying the assumptions AK1-AK3, we note that R_{ij} can be any single-valued strictly increasing C^1 function that vanishes at the origin.

Remark 1: Many of the subsequent results can be generalized to networks with reactions of the form: $nX_i \xrightarrow{R_{ij}} mX_j$, where n, m are positive integers. However, we do not explicitly consider this case to simplify the notation and analysis.

B. Existence of Lyapunov functions: Sum-of-Currents (SoC) RLF

One of the advantages of studying linear networks is that their stability is well-characterized. Indeed, it has been long-known [27, 28] that linear networks can be studied using a Lyapunov function of the form:

$$V(x) = \|\dot{x}\|_1 = \sum_{i=1}^{n} \left| \sum_{j \neq i} (R_{ji}(x_j) - R_{ij}(x_i)) + u_i - R_i(x_i) \right|. \tag{5}$$

We state the following theorem:

Theorem 1: Let $\mathscr{N}$ be a linear BIN be given with any set of admissible reaction rates $\{R_{ij}(x_i), R_i(x_i), u_i\}_{i,j=1}^n$. Let (2) be the associated ODE. Let V be defined as in (5). Then, V is an RLF for $(\mathscr{N}, \mathscr{K}_{\mathscr{N}})$.

We provide a new proof of Theorem 1 in the Appendix based on the techniques used in [11, 12, 30]. The same techniques will be generalized to prove Theorem 4. In [12], we have called (5) a Sum-of-Currents (SoC) RLF, since it is a sum of the absolute values of the currents $dx_i/dt, i = 1, .., n$, which is analogous to the electric current $I = dq/dt$, where q is the electric charge.

C. Existence of Lyapunov functions: Max-Min RLF

For a subclass of linear BINs, another Lyapunov function can be used to used to establish stability, which is the Max-Min RLF [10, 11]. Define the set-valued function: $\mathcal{R}(x) = \{R_{ij}(x), R_i(x), u_i \mid i,j = 1,..,n, i \neq j\}$. Then, consider the following function:

$$V(x) = \max \mathcal{R}(x) - \min \mathcal{R}(x), \tag{6}$$

The existence of an RLF of the form (6) can be characterized graphically for general BINs [10, 11]. In order to minimize the notational inconvenience, we assume that $\mathbf{1}$ is a flux for the network $\mathscr{N}$. Hence, the result can be stated as follows:

Theorem 2: Let a BIN $\mathscr{N}$ satisfying AS1-AS2 be given. Assume that it has a *unique* positive flux equal to $\mathbf{1}$ and every species X_i is a reactant to a unique reaction. Then, V as defined in (6) is an RLF for $(\mathscr{N}, \mathscr{K}_{\mathscr{N}})$.

Remark 2: In order to generalize Theorem 2 to accommodate BINs with general positive fluxes $v \gg 0$, the reactions in $\mathcal{R}(x)$ can be weighted by the corresponding entry in v [11].

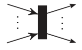
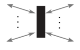
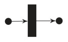
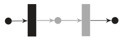
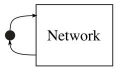
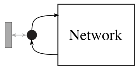
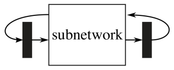
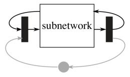
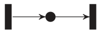
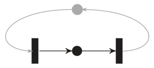
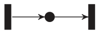
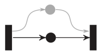
Modification	**Original motif**	**Modified motif**
Reversal of a reaction		
Adding an intermediate		
External regulation of a species		
Adding a feedback species		
Adding a catalyst		
Adding a hetero-dimer		

TABLE I

A LIST OF ELEMENTARY GRAPH MODIFICATIONS STUDIED IN THIS PAPER. FORMAL DEFINITIONS ARE PROVIDED IN DEFINITION 3.

IV. STABILITY-PRESERVING GRAPH MODIFICATIONS

A. Definitions

Consider a BIN $(\mathscr{S},\mathscr{R})$ that admits an RLF V. Assume that the network is modified to a new network $(\tilde{\mathscr{S}},\tilde{\mathscr{R}})$. We are interested in the existence of an RLF for the new network. To be more concrete, we focus on graph modifications listed in Table I. As can be noticed, some of these modification can change a linear network into a nonlinear network. First, we formalize the concept of adding an extra product or reactant to a reaction.

Definition 2: Consider a BIN $(\mathscr{S},\mathscr{R})$. We say that a reaction $\tilde{\mathbf{R}}_j$ is an *extension* of a reaction $\mathbf{R}_j \in \mathscr{R}$ if the following holds for each $X_i \in \mathscr{S}$: if $X_i \in \mathscr{S}$ is a reactant of $\mathbf{R}_j \in \mathscr{R}$, then X_i is a reactant of $\tilde{\mathbf{R}}_j \in \tilde{\mathscr{R}}$ with the same stoichiometric coefficient. Similarly, if $X_i \in \mathscr{S}$ is a product of $\mathbf{R}_j \in \mathscr{R}$, the X_i is a product of $\tilde{\mathbf{R}}_j \in \tilde{\mathscr{R}}$ with the same stoichiometric coefficient.

We next provide a formal definition of the elementary modifications in Table I.

Definition 3: Let $\mathscr{N} = (\mathscr{S},\mathscr{R})$ be a given BIN. We say that $\tilde{\mathscr{N}} := (\tilde{\mathscr{S}},\tilde{\mathscr{R}})$ is an *elementary modification* of $\mathscr{N}$ if it satisfies one of the following statements:

1) (*Reversal of a reaction*) $\tilde{\mathscr{S}} = \mathscr{S}$, and $\exists \mathbf{R}_j \in \mathscr{R}$ such that $\tilde{\mathscr{R}} = \mathscr{R} \cup \{\mathbf{R}_{-j}\}$.
2) (*Adding an intermediate*) $\tilde{\mathscr{S}} = \mathscr{S} \cup \{X^*\}$, and $\exists \mathbf{R}_j \in \mathscr{R}$ such that $\tilde{\mathscr{R}} = (\mathscr{R}/\{\mathbf{R}_j\}) \cup \{\tilde{\mathbf{R}}_j, \tilde{\mathbf{R}}^*\}$ where $\tilde{\mathbf{R}}_j$ has the reactants of $\mathbf{R}_j$ and X^* as a product, and $\tilde{\mathbf{R}}^*$ has X^* as the reactant and has the products of $\mathbf{R}_j$ as its products.
3) (*External Regulation*) $\tilde{\mathscr{S}} = \mathscr{S}$, and $\exists X_k \in \mathscr{S}$ such that $\tilde{\mathscr{R}} = \mathscr{R} \cup \{X_k \rightleftharpoons \emptyset\}$.
4) (Adding a feedback species) $\tilde{\mathscr{S}} = \mathscr{S} \cup \{X^*\}$, and $\exists \mathbf{R}_j, \mathbf{R}_k \in \mathscr{R}$ such that $\tilde{\mathscr{R}} = (\mathscr{R}/\{\mathbf{R}_j, \mathbf{R}_k\}) \cup \{\tilde{\mathbf{R}}_j, \tilde{\mathbf{R}}_k\}$ where $\tilde{\mathbf{R}}_j$ is an extension of $\mathbf{R}_j$ with X^* as an extra product, and $\tilde{\mathbf{R}}_k$ is an extension of $\mathbf{R}_k$ with X^* as an extra reactant. If $\mathbf{R}_j$ has no products and $\mathbf{R}_k$ has no reactants then we call it *adding a conserving feedback species*.
5) (*Adding a catalyst*) $\exists X_i \in \mathscr{S}$ such that $\tilde{\mathscr{S}} = \mathscr{S} \cup \{X_i^-\}$, $|\mathscr{R}| = |\tilde{\mathscr{R}}|$, and every reaction $\tilde{\mathbf{R}}_j \in \tilde{\mathscr{R}}$ is an extension of a corresponding reaction $\mathbf{R}_j \in \mathscr{R}$. Furthermore, X_i^- is a product of a reaction $\mathbf{R}_j$ iff X_i is a reactant of $\mathbf{R}_j$ with the same stoichiometry coefficient, and X_i^- is a reactant of a reaction $\mathbf{R}_j$ iff X_i is a product of $\mathbf{R}_j$ with the same stoichiometry coefficient.
6) (*Adding a hetero-dimer*) $\exists X_i \in \mathscr{S}$ such that $\tilde{\mathscr{S}} = \mathscr{S} \cup \{X_i^+\}$, $|\mathscr{R}| = |\tilde{\mathscr{R}}|$, and every reaction $\tilde{\mathbf{R}}_j \in \tilde{\mathscr{R}}$ is an extension of a corresponding reaction $\mathbf{R}_j \in \mathscr{R}$. Furthermore, $\exists X_i \in \mathscr{S}$ such that X_i^+ is a reactant of a reaction $\mathbf{R}_j$ iff X_i^+ is a reactant of $\mathbf{R}_j$ with the same stoichiometry coefficient, and X_i^+ is a product of a reaction $\mathbf{R}_j$ iff X_i is a product of $\mathbf{R}_j$ with the same stoichiometry coefficient.

Finally, a network $\tilde{\mathscr{N}}$ is a modification of $\mathscr{N}$ if it is a result of several elementary modifications. More formally:

Definition 4: A network $\tilde{\mathscr{N}}$ is a *modification* of $\mathscr{N}$ if there exists a finite sequence of networks $\mathscr{N}_0, \mathscr{N}_1, .., \mathscr{N}_q$, with $\mathscr{N}_0 := \mathscr{N}, \mathscr{N}_q := \tilde{\mathscr{N}}$, and for each $i \in 1,..,q$, $\mathscr{N}_i$ is an elementary modification of $\mathscr{N}_{i-1}$.

In the subsequent sections, we provide results on modifications that preserve the stability of a given BIN.

Remark 3: The standard enzymatic catalysis reaction is a combination of three elementary modifications which are adding an intermediate, reversal, and then adding a catalyst. In other words, the reaction $S \to P$ is modified into $S \to C \to P$, then to $S \rightleftharpoons C \to P$, then to $S + E \rightleftharpoons C \to P + E$.

B. Linear networks with a Sum-of-Currents RLF

It is easy to see that the first few modifications in Table I are stability preserving when applied to a linear BIN. This is stated below.

Theorem 3: Let $\mathscr{N}$ be a given linear BIN, and let $\tilde{\mathscr{N}}$ be its modification generated by a finite sequence of elementary modifications that are limited to reversal of a reaction, adding an intermediate, external regulation of a species, and adding a conserving feedback species. Then, V (5) is an RLF for $\tilde{\mathscr{N}}$.

Proof: The resulting network $\tilde{\mathscr{N}}$ after the application of the elementary modifications mentioned in the statement of the theorem is linear. Hence, the statement follows by Theorem 1. ■

The last two modifications in Table I are more interesting since they can modify a linear network into a nonlinear one. Nevertheless, we show that the resulting modified BIN continues to have an SoC RLF. The proof is provided in the appendix.

Theorem 4: Let $\mathscr{N} = (\mathscr{S}, \mathscr{R})$ be a given linear BIN, and let $\tilde{\mathscr{N}} = (\tilde{\mathscr{S}}, \tilde{\mathscr{R}})$ be its modification generated by a finite sequence of elementary modifications that are limited to adding a catalyst and adding a hetero-dimer. Then, $V = \sum_{i=1}^{|\mathscr{S}|} |\dot{x}_i|$ is an RLF for $(\tilde{\mathscr{N}}, \mathscr{K}_{\tilde{\mathscr{N}}})$.

Several modifications can be combined to yield enzymatic catalysis reactions (see Remark 3). Therefore, we can state the following corollary:

Corollary 5: Let $\mathscr{N}$ be a given linear BIN, and let $\tilde{\mathscr{N}}$ be its modification generated replacing linear reactions of the form $X_i \to X_j$, by nonlinear reactions of the form $X_i + E_{ij} \rightleftharpoons C_{ij} \to X_j + E_{ij}$. Let $\mathscr{S}_2$ be set of all the extra intermediates written as C_{ij}. Then, the function $V = \sum_{i=1}^{|\mathscr{S}|} |\dot{x}_i| + \sum_{C_{ij} \in \mathscr{S}_2} |\dot{c}_{ij}|$ is an RLF for $(\tilde{\mathscr{N}}, \mathscr{K}_{\tilde{\mathscr{N}}})$.

Proof: The proof follows by using Theorem 3 for adding an intermediate and then reversal, i.e., modifying $X_i \to X_j$ to $X_i \leftrightharpoons C \to X_j$. Then, Theorem 4 to get the reaction $X_i + E_{ij} \leftrightharpoons C_{ij} \to X_j + E_{ij}$. ■

C. Networks with a Max-Min RLF

Networks that have a Max-Min RLF admit a different set of stability-preserving modifications as we show next. Note that the original BIN does not need to be linear as is stated in the following result.

Theorem 6: Let $\mathscr{N}$ be a BIN that admits a Max-Min RLF, and let $\tilde{\mathscr{N}}$ be its modification generated by a finite sequence of elementary modifications that are limited to adding an intermediate, adding a feedback species, and adding a catalyst. Then, (6) is an RLF for $\tilde{\mathscr{N}}$.

Proof: Using the characterization in Theorem 2, any combination of the graph modifications mentioned in the statement of theorem do not create new independent vectors in the kernel of the stoichiometry matrix (i.e., it does not create new fluxes), and they do not make a single species a reactant in multiple reactions. Therefore, Theorem 2 applies to $\tilde{\mathscr{N}}$. ■

We study next the case of reversal. Since our formalism treats a reversible reaction as two reactions $\mathbf{R}_j, \mathbf{R}_{-j}$, then reversal of a reaction increases the number of fluxes, and hence violates the conditions required by Theorem 2. Nevertheless, as shown in [11], the result can be extended. We state the result here in the language of graph modifications:

Theorem 7: ([11]) Let $\mathscr{N} = (\mathscr{S}, \mathscr{R})$ be a given a network that satisfies the conditions of Theorem 2. Let $\mathscr{R}_r \subset \mathscr{R}$ be defined as follows: $\mathbf{R}^* \in \mathscr{R}_r$ iff for each $X_i \in \mathscr{S}$ that is a product of $\mathbf{R}^*$, X_i is not a product of another reaction. Then, let $\tilde{\mathscr{N}}$ be a modification of $\mathscr{N}$ generated by the reversal of the reactions in $\mathscr{R}_r$. Then, (6) is an RLF for $\tilde{\mathscr{N}} = (\tilde{\mathscr{S}}, \tilde{\mathscr{R}})$ where $\mathcal{R}(x) = \{R_j(x) - R_{-j}(x) \mid j = 1, .., |\mathscr{R}|\}$, where $R_{-j} :\equiv 0$ if $\mathbf{R}_{-j} \notin \tilde{\mathscr{R}}$.

In addition, we can strengthen Corollary 5 to include modifications by *processive* enzymatic cycles [31]:

Corollary 8: Let $\mathscr{N}$ be a given BIN satisfying the conditions of Theorem 2, and let $\tilde{\mathscr{N}}$ be its modification generated by replacing reactions of the form $\sum_i \alpha_{ij} X_i \to \sum_i \beta_{ij} X_i$, by reactions of the form $\sum_i \alpha_{ij} X_i + E^* \rightleftharpoons C_0^* \rightleftharpoons C_1^* \rightleftharpoons .... \rightleftharpoons C_m^* \to \sum_i \beta_{ij} X_i + E^*$ for some positive integer m. Then, $\tilde{\mathscr{N}}$ admits a Max-Min RLF.

V. APPLICATIONS

A. Post-translational Modification (PTM) cycles

The PTM cycle model is standard in systems biology [32]. The long-term dynamics of the PTM cycle have been a subject of extensive study using several methods. This includes monotonicity [9, 33], and RLFs [11, 12, 14]. In this paper, we show that the stability properties of the PTM cycle can be interpreted graphically in terms of the basic reversible reaction:

$$S \rightleftharpoons P, \tag{7}$$

where S denotes the substrate, and P denotes the product. This simple motif admits both an SoC RLF and a Max-Min RLF.

1) The single PTM: We consider the single PTM cycle:

$$S + E \leftrightharpoons C \longrightarrow P + E, P + F \leftrightharpoons D \longrightarrow S + E. \tag{8}$$

As noted in Remark 3, the reaction $S \to P$ can be modified into an enzymatic catalysis reaction. Using Corollary 5 we get that the PTM cycle above admits an SoC RLF. Furthermore, using Corollary 8 we get that it also admits a Max-Min RLF.

2) The PTM star: We can consider other modifications to (7). By adding a finite number of external regulations on S and then conserving feedback species, we get the following network which we call the linear star (depicted in Fig. 2):

$$S \rightleftharpoons P_1, S \rightleftharpoons P_2, ..., S \rightleftharpoons P_n. \tag{9}$$

Then, using Corollary 5, we get that (3)-(4) admits an SoC RLF since it is formed by enzymatic catalysis modifications. Furthermore, to answer the question posed in the introduction. We can add the external regulation $\emptyset \leftrightharpoons S$ to (9), and then apply enzymatic catalysis to all other reactions to certify the existence of an SoC RLF.

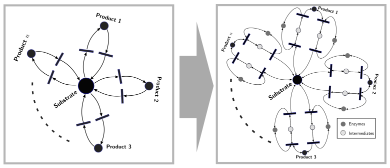


Fig. 2. **The linear star**. Using Corollary 5, existence of an RLF for the linear star implies the existence of an RLF for the PTM star depicted in Fig. 1.

3) The Processive Multi-PTM cycle.: Modifying (7) by adding intermediates gives the following network which we call the linear cycle (depicted in Fig. 3-a):

$$S_0 \to S_1 \to ... \to S_n \to S_0,$$

where $S_0 := S, S_n := P$. Theorem 2 guarantees that the modified network has a Max-Min RLF. Corollary 8 implies that the following network admits a Max-Min RLF:

$$S_{i-1} + E_i \rightleftharpoons C_{i1} \rightleftharpoons C_{i2} \rightleftharpoons .... \rightleftharpoons C_{im} \longrightarrow S_i + E_i,$$
$$S_n + E_n \rightleftharpoons C_{n1} \rightleftharpoons C_{n2} \rightleftharpoons .... \rightleftharpoons C_{nm} \longrightarrow S_1 + E_n,$$

$i = 1, .., n-1$. The above network has been called the "all-encompassing" processive cycle, and its stability has been studied in [34] using more elaborate techniques. Using our method, we show that the existence of an RLF follows by modifying the linear cycle (Fig. 3-a) using processive enzymatic reactions to get the network depicted in Fig. 3-b.

4) The PTM chain: Consider now modifying (7) by a finite number of intermediates and reversals, we get the following network:

$$S_0 \rightleftharpoons S_1 \rightleftharpoons S_2 ... \rightleftharpoons S_n, \tag{10}$$

where $S_0 := S, S_n := P$. Corollary 5 implies that the following PTM chain admits an SoC RLF:

$$S_{i-1} + E_i \rightleftharpoons C_i \longrightarrow S_i + E_i, \tag{11}$$
$$S_{i-1} + F_i \rightleftharpoons D_i \longrightarrow S_i + F_i, i = 1, .., n. \tag{12}$$

The existence of an SoC RLF of the PTM chain can be shown computationally for each given n by linear programming [12]. Nevertheless, Fig. 3-c,d shows that the existence of an SoC RLF *for each* n follows from modifying a linear chain via enzymatic catalysis reactions.

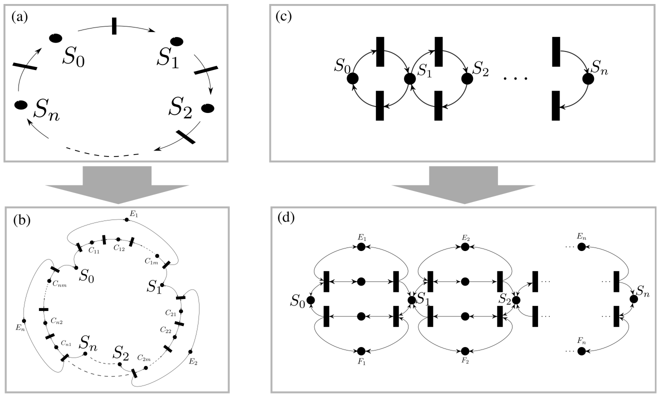


Fig. 3. **Constructing an RLF for a nonlinear network from a linear one.** (a) The linear cycle. (b) The processive multi-PTM cycle. The existence of an RLF follows from the existence of one for the linear cycle using Corollary 8. (c) The linear chain. (d) The PTM chain. The existence of an RLF follows from the existence of one for the linear chain using Corollary 5.

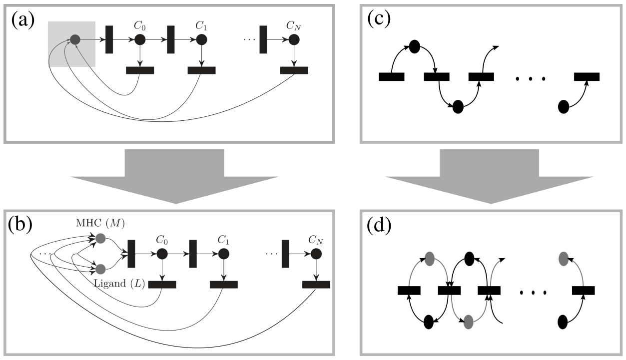


Fig. 4. **Additional examples for graphical RLF construction.** (a) A linear BIN. (b) The McKeithan network. The existence of an RLF follows from the existence of one for the linear BIN in panel (a) using Corollary 5. (c) A one-directional linear chain. (d) The RFM. The existence of an RLF follows from the existence of one for the unidirectional linear chain using Corollary 5.

B. *T-cell kinetic proofreading*

McKeithan [35] proposed a nonlinear BIN to explain T-cell's ability to distinguish between different types of ligands. It is given as follows:

$$R + L \rightleftharpoons C_0 \to C_1 \to ... \to C_n$$
$$C_1 \to R + L, C_2 \to R + L, ..., C_n \to R + L.$$

Sontag [8] has studied the stability of the network using the theory of complex balance, while we have studied the network using computational RLF construction [12]. Here, we show that a stability certificate can be constructed by considering the network as a modification of a linear network. By noting that the species L is a hetero-dimer in the language of Table I, we can see that (13) is a modification of the following network by the addition of a hetero-dimer:

$$RL \rightleftharpoons C_0 \to C_1 \to ... \to C_n \tag{13}$$
$$C_1 \to RL, C_2 \to RL, ..., C_n \to RL.$$

which is in turn a modification of $RL \rightleftharpoons C_n$. Hence, existence of SoC RLF for (13) follows from Theorems 3 and 4. Fig. 4-a shows the linear network, while Fig. 4-b shows the corresponding modified network.

C. *The Ribosome Flow Model*

The Ribosome Flow Model (RFM) is a nonlinear system model of the process of translation initiation and elongation where it describes Ribosome binding to codons on an mRNA that is being translated [36]. It has been shown [12] that the corresponding ODE can be written as a BIN with species X_i, Y_i where X_i is *occupancy* of the ith codon, while Y_i is the *vacancy* of the ith codon. Hence, we get the following BIN (depicted in Fig. 4-d):

$$Y_1 \to X_1, X_n \to Y_n,$$
$$X_i + Y_{i+1} \to X_{i+1} + Y_i, \; i = 1, .., n-1.$$

The stability of the above network has been studied via monotonicity methods [37]. For a given n, the existence of

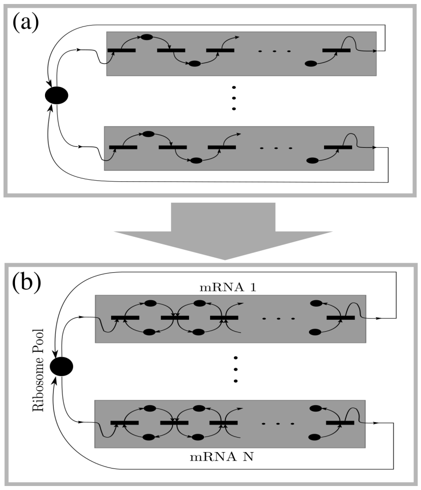


Fig. 5. **Graphical construction of an RLF for the RFM with a pool [38]**. (a) The linear network. (b) The corresponding modified nonlinear network. Stability follows via Corollary 5.

an SoC RLF can be verified via linear programming [12]. Nevertheless, Fig. 4-c,d shows that an SoC RLF can be constructed by merely noticing that the RFM is a modification generated by adding catalysts to the following unidirectional linear chain network: (depicted in Fig. 4-c)

$$\emptyset \to X_1 \to X_2 \to ... \to X_n \to \emptyset.$$

The same graphical technique can be applied to RFMs interconnected via a pool [38] (as Figure 5 shows), or via multiple pools [30].

VI. DISCUSSION

In this work, we have proposed a graphical method to certify the existence of an RLF for a given network by reducing it via a certain set of admissible modification to a network that is known to admit an RLF. Furthermore, our method can directly show that the stability of a given network is preserved under certain graph modifications. Future directions includes certifying global stability graphically. Previous works [11, 12, 15] have verified global stability via a LaSalle-like algorithm or via establishing robust non-degeneracy of the Jacobian. An extension of this manuscript is under preparation where we will provide a graphical criteria for global stability.

APPENDIX: PROOFS

Proof: [Proof of Theorem 1] The function $V(x) = \tilde{V}(R(x))$ is piecewise linear in terms of the rates, therefore there exists a positive integer m such that the space $\mathbb{R}^{\nu}_{\geq 0}$ can be partitioned into non-empty-interior regions $\{\mathcal{W}_k\}_{k=1}^{m} \subset \mathbb{R}^{\nu}_{\geq 0}$ for which $\tilde{V}$ is linear on each of them and each region corresponds to a specific sign pattern for $\dot{x}$. The geometry of such partition is discussed more thoroughly in [11].

Fix k. There exists $c_{ij}^{(k)}, c_i^{(k)}, \delta^{(k)}$ such that:

$$\begin{aligned} V(x) &= \sum_{\substack{i,j\\ i\neq j}} c_{ij}^{(k)} R_{ij}(x_i) + \sum_i c_i^{(k)} R_i(x_i) + c_0^{(k)} \qquad (14) \\ &=: {c^{(k)}}^T R(x),\ R(x) \in \mathcal{W}_k. \end{aligned}$$

Since V is defined as the ℓ_1 norm of $\dot{x}$, then the sign of $\dot{x}$ is constant and non-zero on $\mathcal{W}_k^{\circ}$. Therefore, we denote $\sigma_i := \operatorname{sgn}(\dot{x}_i) \in \{\pm 1\}$ on $\mathcal{W}_k^{\circ}$, where the superscript "$\circ$" denotes the interior of a set.

We claim that each term in the expression (14) has a nonpositive Lie derivative on $\mathcal{W}_k^{\circ}$. In order to show that, we first examine terms of the form $c_{ij}^{(k)} R_{ij}(x_i)$ where $c_{ij}^{(k)} \neq 0$ for some i, j. We will show that $c_{ij}^{(k)} \dot{R}_{ij}(x_i) \leq 0$ for $R(x) \in \mathcal{W}_k^{\circ}$. As evident by examining (5), the reaction rate R_{ij} appears only in $\dot{x}_i$ with coefficient -1 and in $\dot{x}_j$ with coefficient $+1$. W.l.o.g, assume that $c_{ij}^{(k)} > 0$. There are four possible combinations $\sigma_i^{(k)}, \sigma_j^{(k)} > 0$, $\sigma_i^{(k)}, \sigma_j^{(k)} < 0$, $\sigma_i^{(k)} > 0, \sigma_j^{(k)} < 0$, and $\sigma_i^{(k)} < 0, \sigma_j^{(k)} > 0$. The first two give $c_{ij}^{(k)} = 0$ and the third gives $c_{ij}^{(k)} = -2 < 0$. Hence, we conclude that $\sigma_i^{(k)} < 0, \sigma_j^{(k)} > 0$. Therefore, $\operatorname{sgn}(c_{ij}^{(k)} \dot{R}_{ij}(x_i)) = \operatorname{sgn}(c_{ij}^{(k)} (\partial R_{ij}(x_i)/\partial x_i)\dot{x}_i) = \operatorname{sgn}(c_{ij}^{(k)} \sigma_i^{(k)}) \leq 0$ for $R(x) \in \mathcal{W}_k^{\circ}$, where the last equality follows by the monotonicity of R_{ij}.

Next, we examine $c_i^{(k)} R_i(x_i)$ for some i where $c_i^{(k)} \neq 0$. W.l.o.g, assume that $c_i^{(k)} > 0$. Since R_i appears only in $\dot{x}_i$ with coefficient -1, then $\sigma_i^{(k)} < 0$. Therefore, $\operatorname{sgn}(c_i \dot{R}_i(x_i)) = \operatorname{sgn}(c_i^{(k)} (\partial R_i(x_i)/\partial x_i)\dot{x}_i) = \operatorname{sgn}(c_i^{(k)} \sigma_i) \leq 0$ for $R(x) \in \mathcal{W}_k^{\circ}$.

Since k, i, j have been chosen arbitrarily, we conclude that $\dot{V}(x) \leq 0$ whenever $R(x) \in \mathcal{W}_k^{\circ}$ for some k. It remains to show that $\dot{V}(x) \leq 0$ when $R(x) \in \partial \mathcal{W}_k$ for some k where "∂" denotes the boundary of a set. To that end, similar to [11][Proof of Theorem 2], the Dini's derivative can be written as $\dot{V}(x) = \max_{k \in K_{x(t)}} {c^{(k)}}^T \dot{R}(x) \leq 0$ where $K_{x(t)} = \{k | R(x) \in \mathcal{W}_k\}$. ■

Proof: [Proof of Theorem 4] Let Γ be the stoichiometry matrix for $(\mathscr{S}, \mathscr{R})$. Since the modifications are limited to adding a catalysis or adding a hetero-dimer, then every reaction in $\tilde{\mathscr{R}}$ is an extension of a corresponding reaction in $\mathscr{R}$. Hence, we can write $\tilde{\Gamma} = [\Gamma^T, \Gamma_2^T]^T$ as the stoichiometry matrix for $(\tilde{\mathscr{S}}, \tilde{\mathscr{R}})$. Let $\dot{x} = \Gamma R(x), \dot{\tilde{x}} = \tilde{\Gamma} \tilde{R}(\tilde{x})$ be the corresponding ODEs. Hence, we can write $\tilde{x} = [x^T, x_2^T]^T$, where x_2 corresponds to the concentrations of the species in $\tilde{\mathscr{S}} / \mathscr{S}$.

Note that all the species in $\tilde{\mathscr{S}} / \mathscr{S}$ are either catalysts or hetero-dimers. We include an additional assumption to simplify the notation: For each species $X_i \in \mathscr{S}$, we assume that there exists at most one corresponding catalyst species in $\tilde{\mathscr{S}} / \mathscr{S}$, and it is denoted by X_i^-. Similarly, we assume that there exists at most one corresponding hetero-dimer species, and the corresponding species is denoted as X_i^+. The corresponding concentrations are x_i, x_i^-, x_i^+. The proof can be generalized easily without the last assumption .

Our construction implies that $\dot{x}_i = \dot{x}_i^+ = -\dot{x}_i^-$. Hence, $V(x) = 0$ iff $\dot{x} = 0$. Therefore, V is positive-definite. We next show that it is non-increasing.

Similar to the proof of Theorem 1, we consider a region $\mathcal{W}_k$ for which V is linear and has a fixed sign pattern for $\dot{x}$.

Fix k, There exists $c_{ij}^{(k)}, c_i^{(k)}, \delta^{(k)}$ such that:

$$\begin{aligned} V(x) &= \sum_{\substack{i,j \\ i\neq j}} c_{ij}^{(k)} R_{ij}(x_i) + \sum_i c_i^{(k)} R_i(x_i) + c_0^{(k)} \qquad (15) \\ &=: c^{(k)^T} R(x),\ R(x) \in \mathcal{W}_k. \end{aligned}$$

We claim that each term in the expression (14) has a nonpositive Lie derivative on $\mathcal{W}_k^\circ$. In order to show that, we first examine $c_{ij}^{(k)} R_{ij}(x_i)$ where $c_{ij}^{(k)} \neq 0$ for some i, j. We will show that $c_{ij}^{(k)} \dot{R}_{ij}(x_i) \leq 0$ for $R(x) \in \mathcal{W}_k^\circ$. Since the candidate RLF sums only the species in $\mathscr{S}$, the reaction rate R_{ij} appears only in $\dot{x}_i$ with coefficient -1 and in $\dot{x}_j$ with coefficient $+1$. W.l.o.g, assume $c_{ij}^{(k)} > 0$. Similar to the proof of Theorem 1, we get that $\sigma_i^{(k)} < 0, \sigma_j^{(k)} > 0$. Since $\sigma_i^{+(k)} = \sigma_i^{(k)}$, and $\sigma_j^{-(k)} = -\sigma_j^{(k)}$ we get

$$\begin{aligned} &\operatorname{sgn}(c_{ij}^{(k)} \dot{R}_{ij}(x_i, x_i^+, x_i^-)) \\ &= \operatorname{sgn}\left(c_{ij}^{(k)} \left(\frac{\partial R_{ij}}{\partial x_i} \dot{x}_i + \frac{\partial R_{ij}}{\partial x_i^+} \dot{x}_i^+ + \frac{\partial R_{ij}}{\partial x_j^-} \dot{x}_j^- \right) \right) \\ &= \operatorname{sgn}(c_{ij}^{(k)} (\sigma_i^{(k)} + \sigma_i^{+(k)} + \sigma_j^{-(k)})) \leq 0 \end{aligned}$$

for $R(x) \in \mathcal{W}_k^\circ$, where the last equality follows by the monotonicity of R_{ij}.

Since k, i, j have been chosen arbitrarily, we can use the same arguments used in the proof of Theorem 1 to conclude that $\dot{V}(x) \leq 0$ for all x. ■

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