

## Chapter 1

# GRAPH-THEORETIC ANALYSIS OF MULTISTABILITY AND MONOTONICITY FOR BIOCHEMICAL REACTION NETWORKS

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**Abstract** Mathematical models of biochemical reaction networks are usually high dimensional, nonlinear, and have many unknown parameters, such as reaction rate constants, or unspecified types of chemical kinetics (such as mass-action, Michaelis-Menten, or Hill kinetics). On the other hand, important properties of these dynamical systems are often determined by the network structure, and do not depend on the unknown parameter values or kinetics. For example, some reaction networks may give rise to multiple equilibria (i.e., they may function as a biochemical switch) while other networks have unique equilibria for any parameter values. Or, some reaction networks may give rise to monotone systems, which renders their dynamics especially stable. We describe how the species-reaction graph (SR graph) can be used to analyze both multistability and monotonicity of networks.

**Keywords:** biochemical reaction networks, multistability, monotonicity, SR graph

## Introduction

There is great interest in methods that draw conclusions about the dynamical properties of a chemical reaction network based only on the network structure, i.e., with limited or absent knowledge about many kinetic details [6, 24]. Here we will concentrate on the properties of *multistability* and *monotonicity*.

Multistability refers to the capacity of a biochemical system to operate at several discrete, alternative steady-states, and plays an important role in cell signaling, division, and differentiation [28, 2, 21].

Monotone systems display well-ordered behavior that excludes the possibility for chaotic dynamics [18, 26–1]. Moreover, perturbations of such systems have unambiguous global effects and a predictability characteristic that confers robustness and adaptability [19].

In this chapter we describe some of the main results on the use of the SR graph of a reaction network to analyze its multistability and monotonicity properties, as described especially in [8] and [3], respectively. Our focus will *not* be on presenting the most powerful results in full generality (for these the reader should consult [8, 3], and also [9, 5]). Instead, we will concentrate on simpler versions of these results, and will especially focus on pointing out how these results can be formulated in an unified language based on the notion of SR graph.

## Definitions and Notation

### Dynamical systems derived from chemical reaction networks.

A chemical reaction system in which  $n$  reactants participate in  $m$  reactions has dynamics governed by the system of ordinary differential equations

$$\frac{dx}{dt} = Sv(x) \tag{1.1}$$

where  $x = (x_1, \dots, x_n)^t$  is the nonnegative  $n$ -vector of species concentrations,  $v = (v_1, \dots, v_m)^t$  is the  $m$ -vector of reaction rates, and  $S$  is the  $n \times m$  stoichiometric matrix.

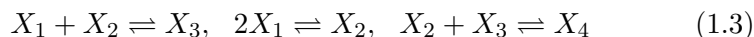
Arbitrary orderings can be chosen on the sets of substrates and reactions. Further,  $S$  is only defined up to an arbitrary re-signing of its columns, equivalent to a switching of the left and right-hand sides of a reaction. The equation (1.1) defines a dynamical system on the nonnegative orthant of  $\mathbb{R}^n$ . If we also assume that all species may have some inflow (which is allowed to be zero) and some outflow which increases

strictly with concentration, we obtain the related system

$$\frac{dx}{dt} = F + Sv(x) - Q(x) \quad (1.2)$$

Here  $F$  is a constant nonnegative vector representing the inflow, and the diagonal function  $Q(x) = (Q_1(x_1), \dots, Q_n(x_n))^t$  represents the outflow or degradation, and we assume that  $\frac{\partial Q_i}{\partial x_i} > 0$  for each  $i$ .

For example, for the reaction network



we can choose

$$S = \begin{pmatrix} -1 & -2 & 0 \\ -1 & 1 & -1 \\ 1 & 0 & -1 \\ 0 & 0 & 1 \end{pmatrix} \quad (1.4)$$

where each column of  $S$  corresponds to one reaction in the network. Sometimes the inflow and outflow terms  $F$  and  $Q(x)$  are included in the reaction network as “inflow reactions”  $X_i \rightarrow 0$  and “outflow reactions”  $0 \rightarrow X_j$ . Here we choose to associate to a reaction network (such as (1.3)) either the closed system (1.1), or the open system (1.2). Note that the dynamical properties of these two types of systems may be very different from each other, and some theorems might apply to only one or the other of them.

We assume that for each reversible reaction its reaction rate  $v_i$  can be decomposed as

$$v_i(x) = v_i^+(x) - v_i^-(x),$$

where  $v_i^+$  is the rate of the forward reaction, and  $v_i^-$  is the rate of the reverse reaction.

In biochemical applications, the most common types of reaction rates are *mass-action*, *Michaelis-Menten*, or *Hill kinetics*. For example, for the reaction  $X_1 + X_2 \rightarrow X_3$ , we could have

$$v_1^+(x) = k_1 x_1 x_2$$

or

$$v_1^+(x) = \frac{k_1 x_1 x_2}{1 + k_2 x_1 x_2}$$

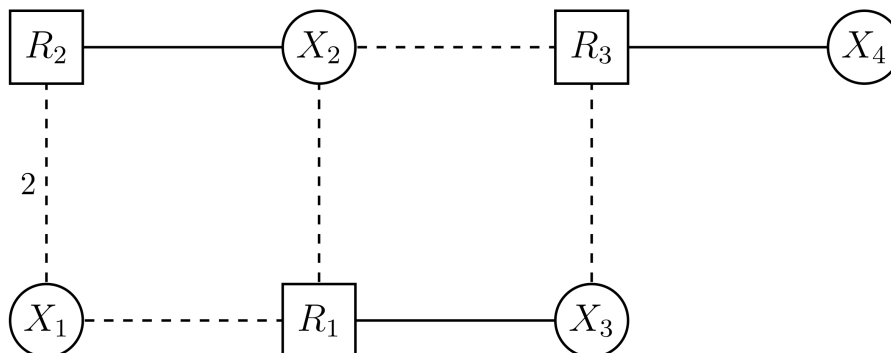
or

$$v_1^+(x) = \frac{k_1 x_1 x_2^2}{k_2 + x_2^2}$$

for some positive constants  $k_1$  and  $k_2$ .

Given a reaction network we define the its *SR graph* as follows. The SR graph is a bipartite undirected graph, where the nodes are partitioned into *species nodes* and *reaction nodes*. We draw an *edge* from a species node to a reaction node if that species appears in the reaction, i.e., we draw an edge from species node  $i$  to reaction node  $j$  if the  $s_{ij}$  entry of the stoichiometric matrix  $S$  is not zero. Moreover, if  $s_{ij} > 0$  we say that it is a *positive edge* (and will draw it with a solid line), and if  $s_{ij} < 0$  we say that it is a *negative edge* (and will draw it with a dashed line). Finally, if the stoichiometric coefficient of a species within a reaction is 2 or more, then we label the corresponding edge with this stoichiometric coefficient (so if an edge does not have a numeric label, it will follow that the corresponding stoichiometric coefficient is 1). The SR graph of reaction network (1.3) is shown in Figure 1.1.

Note that the SR graph in Figure 1.1 contains several cycles<sup>1</sup>. We will show that multistability and monotonicity of a network is strongly related to the *types* of cycles present in its SR graph. For this we need to be able to distinguish among several types of cycles.



*Figure 1.1.* The SR graph of reaction network (1.3). Positive edges are shown as solid lines, and negative edges are shown as dashed lines. Note that the graph contains three cycles, and any two of them have S-to-R intersection. Also, all cycles are *o*-cycles.

Consider a cycle that has  $p$  edges, and  $q$  of them are negative edges. We say that this cycle is an *e-cycle* if  $q \equiv \frac{p}{2} \pmod{2}$ , i.e., the number of negative edges along the cycle has the same parity as the total number of edges along the cycle divided by 2. (Note that the total number of edges along any cycle must be even, because the SR graph is a bipartite graph.) Otherwise, i.e., if the number of negative edges has different parity from the total number of edges divided by 2, we say that the cycle is an *o-cycle*. For example, the cycle  $R_2 - X_2 - R_3 - X_3 - R_1 - X_1 - R_2$  in

Figure 1.1 is an o-cycle, since it has  $p = 6$  edges and  $q = 4$  negative edges, and the numbers  $q$  and  $\frac{p}{2}$  have different parities<sup>2</sup>.

Another relevant type of cycle is called *s-cycle*. A cycle  $\mathcal{C}$  is called an s-cycle if we have

$$\prod_{i=1}^{p/2} \sigma_{2i-1} = \prod_{i=1}^{p/2} \sigma_{2i},$$

where  $p$  is the number of edges of  $\mathcal{C}$ , and  $\sigma_1, \sigma_2, \dots, \sigma_p$  are the stoichiometric coefficients of the edges of  $\mathcal{C}$ , in the order in which they occur along  $\mathcal{C}$  (it's easy to see that it does not matter where we start along  $\mathcal{C}$ ). In other words,  $\mathcal{C}$  is an s-cycle if the two possible ways of multiplying the stoichiometric coefficients of every other edge of  $\mathcal{C}$  give rise to the same result. Obviously, if all the stoichiometric coefficients along a cycle are 1, then that cycle is an s-cycle, and if exactly one stoichiometric coefficients along a cycle is  $\neq 1$ , then that cycle is not an s-cycle. For example, for the SR graph in Figure 1.1, the cycle  $X_2 - R_3 - X_3 - R_1 - X_2$  is an s-cycle, and the cycles  $R_2 - X_2 - R_1 - X_1 - R_2$  and  $R_2 - X_2 - R_3 - X_3 - R_1 - X_1 - R_2$  are not s-cycles.

Sometimes not only the types of cycles are important, but also the way cycles intersect within the SR graph. We say that two cycles *have an S-to-R intersection* if the connected components of their intersection are paths that go from a species node to a reaction node (and not from a species node to another species node, or from a reaction node to another reaction node). For example, consider the cycles  $R_2 - X_2 - R_3 - X_3 - R_1 - X_1 - R_2$  and  $R_2 - X_2 - R_1 - X_1 - R_2$  in Figure 1.1. Their intersection has a single connected component, which is the path  $R_1 - X_1 - R_2 - X_2$ . Therefore, these two cycles have an S-to-R intersection.

Consider some closed pointed convex cone  $K \subset \mathbb{R}^n$ . We say that an autonomous dynamical system

$$\dot{x} = f(x) \tag{1.5}$$

is *monotone with respect to  $K$*  if for any two solutions  $x_1(t)$  and  $x_2(t)$  of (1.5), such that  $x_1(0) - x_2(0) \in K$ , it follows that  $x_1(t) - x_2(t) \in K$  for all  $t > 0$ . (Note that we assume that solutions  $x(t)$  exist for all times  $t > 0$ .)

A property relevant to monotonicity is *persistence*. A dynamical system defined on a domain contained within the nonnegative quadrant of  $\mathbb{R}^n$  is called *persistent* if any trajectory with positive initial condition does not have any  $\omega$ -limit points on the boundary of the nonnegative quadrant. In other words, the system is persistent if for any solution  $x(t)$  with positive initial condition such that  $x(t_n) \rightarrow L$  for some sequence  $t_n \rightarrow \infty$ , it follows that all the coordinates of  $L$  are positive.

## The main results

Throughout this chapter we assume that the following properties are satisfied by the reaction network and its reaction rate functions:

**Assumption 1.** The reaction network does not have one-step catalysis, i.e., if a species appears on one side of a reaction then it does not appear on the other side of that reaction.

**Assumption 2.** For each irreversible reaction (and also separately for the forward and reverse reactions of a reversible reaction), its reaction rate depends only on the concentrations of the reactants, which are the species that are being consumed by the reaction. Moreover, the partial derivatives of the rate function with respect to the concentrations of the reactants are nonnegative.

Neither one of these two assumptions are very restrictive; on the other hand, neither one of them is truly necessary for analyzing multistability (see [9] for details).

In this section we formulate two theorems that use the SR graph of a reaction network to analyze its multistability and monotonicity properties.

**THEOREM 1.1** (*Banaji and Craciun [8]*) *Consider a reaction network such that its SR graph satisfies the following two conditions:*

- (i) *all cycles are o-cycles or s-cycles (or both),*
- (ii) *no two e-cycles have an S-to-R intersection.*

*Then the system (1.2) does not have multiple positive equilibria, and the system (1.1) does not have multiple positive nondegenerate equilibria within any affine invariant subspace.*

Note that, in the presence of any conservation laws, the *relevant* multistability question is *not* whether there exists a unique equilibrium, but whether there exists a unique equilibrium within any affine invariant subspace, since for well-behaved systems we expect that one equilibrium should exist in every such invariant subspace (see also [13, 17]). Note that for the system (1.2) there can be no conservation law, due to the presence of nondegenerate outflow or degradation terms.

Theorem 1.1 does apply for reaction network (1.3) because all cycles in Figure 1.1 are o-cycles. For more examples see [8, 9].

If there exist conserved quantities, additional analysis is needed to rule out degenerate equilibria. For mass-action systems, conditions that exclude the possibility of degenerate equilibria are described in [14]. For non-mass-action systems such conditions are described in [16, 25].

Consider now the system

$$\frac{dr}{dt} = v(x_0 + Sr(t)), \quad (1.6)$$

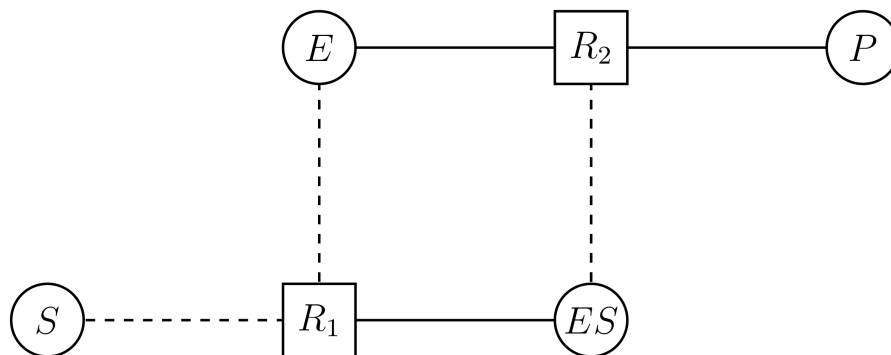
where  $r_j$  is called the *extent* of the  $j^{\text{th}}$  reaction,  $j = 1, \dots, m$ . The following theorem allows us to analyze the monotonicity of this system (i.e., monotonicity in reaction coordinates [3]), and also provides information on the dynamics of the related system (1.1).

**THEOREM 1.2** (*Angeli, DeLeenheer and Sontag [3]*) *Consider a reaction network such that its SR graph satisfies the following two conditions:*

- (i) *each species node is adjacent to at most two edges,*
- (ii) *each cycle is an e-cycle.*

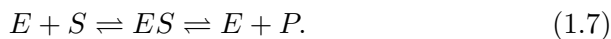
*Assume in addition that all stoichiometric compatibility classes are compact sets, that all reaction rates vanish if the concentrations of some of their reactants are zero, and that all reaction rates are strictly increasing with respect to the concentrations of their reactant species. Then the system (1.6) is monotone with respect to an order induced by some orthant cone.*

*Assume moreover that the system (1.1) is persistent, and all reactions are reversible. Then almost all positive solutions of (1.1) converge to the set of equilibria, i.e., the measure of the set of possibly non-converging initial conditions is zero <sup>3</sup>.*



*Figure 1.2.* The SR graph of reaction network (1.7). Note that the graph contains one cycle, and it is an e-cycle. Also, note that each species node is adjacent to at most two reaction nodes.

For example, consider the reaction network

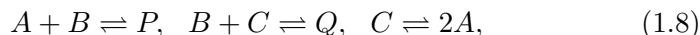


A version of this network was analyzed in detail in [3]. Theorem 1.2 does apply to this network, since its SR graph, shown in Figure 1.2, has the property that its only cycle is an e-cycle, and each species node is adjacent to at most two edges. Moreover, the network (1.7) is persistent (see [4]), and all its reactions are reversible.

In general, if there is only one cycle in an SR graph then Theorem 1.1 applies if this cycle is o- or s-cycle (or both), while Theorem 1.2 applies if the cycle is an e-cycle and no species node is adjacent to more than two edges. Therefore Theorem 1.1 also applies to network (1.7).

Note also that, if Theorem 1.2 does apply, and in particular if in the SR graph each species has at most two adjacent edges, then no two cycles can have an S-to-R intersection (because if a connected component of the intersection of two cycles is an S-to-R path, then there must be at least three adjacent edges to the species node at one end of the path). Therefore, if Theorem 1.2 does apply, and in addition all stoichiometric coefficients are 1, then the hypotheses (i) and (ii) of Theorem 1.1 also hold.

Finally, consider the reaction network



which was also analyzed in [11] under the assumption of mass-action kinetics.

The network (1.8) is persistent because it admits a positive P-semiflow, and every minimal siphon contains the support of a P-semiflow (see [4] for details). Since its SR graph contains an e-cycle and no species node is adjacent to more than two edges, it follows that Theorem 1.2 does apply for this network (see Figure 1.3). Note that the cycle  $A - R_1 - B - R_2 - C - R_3 - A$  in Figure 1.3 is neither an o-cycle nor an s-cycle, so Theorem 1.1 does not apply. On the other hand, if the kinetics of this network is mass-action, then deficiency theory [17] guarantees that there is a unique equilibrium in each stoichiometric compatibility class, and there also exists a globally defined strict Lyapunov function. This, together with persistence, guarantees global convergence of all positive trajectories within a stoichiometric compatibility class to the unique equilibrium in that class [27].

## Discussion

The SR graph was first introduced in [10] for the analysis of mass-action systems, inspired by the SCL graph of Schlosser and Feinberg [22, 23]; see also [12, 15]. The case of networks that may contain one-step catalysis is discussed in [10, 12, 15] for mass-action kinetics, and in [9] for general kinetics.



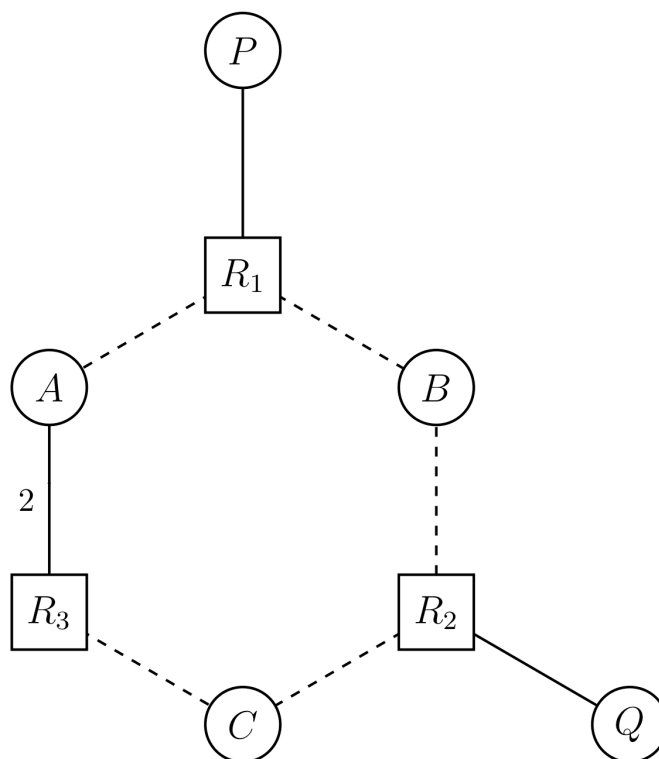


Figure 1.3. The SR graph of reaction network (1.8). The graph contains one cycle, and it is an e-cycle, and each species node is adjacent to at most two reaction nodes. Moreover, the cycle is *not* an s-cycle.

The free software package `BioNetX` provides algorithms for examining dynamical properties of biochemical reaction networks [20]. In particular, this software computes the SR graph of a network, and verifies the conditions (i) and (ii) from Theorem 1.1.

Monotonicity was also considered in [29], where it was treated in an algebraic fashion. In [7] conditions are determined in order to characterize the set of cones and associated partial orders which make a certain reaction monotone, and it is established that, under some minor assumptions, monotonicity of a network with respect to a given partial order is equivalent to asking that each individual reaction be monotone with respect to that same order. This result is also independent of reaction kinetics.

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## Notes

1. In [3] cycles are called “loops”.
2. The original definition of e-cycles and o-cycles in [10] describes these types of cycles in terms of “c-pairs”: e-cycles have an even number of c-pairs and o-cycles have an odd number of c-pairs. The two definitions are equivalent for networks that do not have one-step catalysis, which are our main focus here. Compare also with Lemma 4.4 in [3].
3. Often much more can be said if the system (1.1) is persistent; see [3] for details.

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