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GRAPH THEORY AND QUALITATIVE ANALYSIS OF REACTION NETWORKS

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Abstract. Different types of macroscopic reaction kinetics can be derived from microscopic molecular interactions, with the law of mass action being the most widely used one in standard situations. After such a modeling step, where primarily the types of reactions are identified, it becomes a problem to analyse qualitative properties of complete regulatory networks. This problem has to be tackled, because chemical reaction networks play a part in some of the most fundamental cellular processes such as cell metabolism and regulation of cell signalling processes. This paper discusses how reaction networks can be described and analysed by graph theoretic means. Graph theory is a useful analysis tool for complex reaction networks, in situations where there is parameter uncertainty or modeling information is incomplete. Graphs are very robust tools, in the sense that whole classes of network topologies will show similar behaviour, independently of precise information that is available about the reaction constants. Nevertheless, one still has to take care to incorporate sufficient dynamical information in the network structure, in order to obtain meaningful results.

1. **Introduction.** Chemical reaction networks (CRNs) are among the most widely used examples of dynamical networks. They are regarded as important representatives of a whole class of networks where dynamical systems defined on the graph topology model different kinds of varying dependencies between the system components. Different CRNs have been shown to exhibit various dynamic behaviour, from multiple steady states to stable oscillations [1, 49, 60, 44, 50, 8, 30, 29, 67, 58, 59, 40]. Much of their diverse behaviour arises from the experimental or industrial set-up which they describe. For example, chemical reactions can take place in open systems, where at least one substrate can enter and/or leave the tank at a fixed volumetric rate. Open systems where all species have inflow and outflow are called continuous-flow stirred reaction tanks (CFSRT). In contrast, the other extreme possibility are closed systems, where none of the species have an inflow or outflow.
There is increasing interest in applying chemical reaction network theory to complex reaction and regulatory networks in biology. Many efforts can be found in the current systems biology literature. For example, Bailey in [6] proposed the application of methods from chemical reaction theory to the analysis of complex biochemical

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networks. One of the methods, the chemical reaction deficiency theory (c.f. Section 4) was used by Conradi et al. [13] to determine which potential mechanisms underly activation of the MAPK kinase, an important enzyme in the cellular signalling processes of eukaryotic cells. Reaction networks have also been used as models of cellular biochemistry [2, 7], genetics [66] and immunology [70]. These applications provide further motivation for the development of graph theoretic methods. Nevertheless much care has to be taken to validate the mass action hypothesis, on which we base all our further review in this article. For example, enzyme kinetics are usually derived by using a time scale analysis which transforms the non-linearities used to model the reaction velocities. In biological situations one often has to deal with large macro-molecules not changing in numbers during the time scale of measurement. Such cases cannot be modelled on basis of the mass action hypothesis. An accompanying paper in [64] gives an outline on recent methods developed for deriving alternative reaction kinetics based on microscopic stochastic processes can be found. Development of graphical methods for such approaches has not yet been undertaken.

Several qualitative studies of chemical reaction networks have been accumulated since the early work of the '70s in [10, 11, 12, 25, 26, 42, 43, 46, 47, 79]. The development of analytic tools for chemical reaction networks is driven by several experimental issues. Usually the most important one is the lack of information about the kinetic parameters in the models. In most cases, it is difficult to measure the reaction constants in chemical systems very precisely. In this situation, most traditional methods such as numerical bifurcation analysis are difficult to apply. Another important issue is the size of the network, namely the number of species involved, and the number of reactions occurring. Most known methods are very successful when applied to smaller systems with two or more variables, however they can be difficult and cumbersome to apply to large models. Therefore it is a natural idea to develop methods which combine both graph and dynamical systems theory and which can be more easily automated. There is increasing consensus in the systems biology literature that specific small subnetworks, or so-called modules, might be linked to specific biological functions and might drive the dynamics of the system [48, 54]. However, when they are embedded in large networks, as is the case with genetic and biochemical networks, they might be difficult to identify. Again, the application of chemical reaction graph theory is likely to provide a good approach. It is however a deep mathematical question which graph structures do represent and/or determine the qualitative behaviour of large dynamical systems, especially if these graph structures are combined and linked in a modular way.

Another common problem is that reaction mechanisms may not be known, but there might exist experimental evidence that the system can display a particular dynamics such as, for example, multistability. For such cases it is necessary to develop methods that will identify an appropriate mechanism that is able to reproduce the behaviour observed in experiments. Graph theoretic methods are able to exclude certain models and are therefore useful in the early stages of the model development where choices between proposed mechanisms need to be made.

For the aforementioned reasons, graph theory seems to be a natural choice as a method for fundamental analysis of CRNs. Here arises a problem, as chemical reaction networks can be interpreted by various different graphs. We will review some of the most prominent and recently discussed graphs, displaying different kinds of dynamical information. We will start the paper with some general definitions in Section 2. In Section 3 we will describe the **interaction graph** and developments by Thomas, Soulé, Kaufman, and others [74, 39, 69, 71, 75, 76, 77, 78] starting with some important conjectures on the interaction graph topology made by Thomas in the early '80s [74]. The interaction graph is the most intuitive graph, commonly applied to areas of biology, such as gene networks where formation rates other than polynomial ones are frequently chosen. The interaction graph can be easily associated with any non-linear dynamical system, and has been used in other fields, such as mathematical ecology, see [52]. Next we will cover the deficiency theory developed in the '70s by Craciun, Horn and Jackson [25, 43, 42]. This analysis relies on a graph called the **reaction diagram**, also referred to as the **species digraph** in [32]. Some of their results were verified by Gatermann and Huber via algebraic geometry methods and the aid of another graph, the **undirected bipartite graph** [32]. In the early '90s, the **SCL graph**, reviewed in Section 5, unified some ideas stemming from the interaction graphs (namely the dependence on the structure of the Jacobian matrix), and also deficiency theory. The SCL graph was developed by Feinberg and Rumschitzky [63], and by Feinberg and Schlosser [65] , to analyse particular types of systems, called open systems, which could not satisfy the conditions of deficiency theorems. Aside from the interaction graph and deficiency theory, the SCL graph is related to the **knot graph**. Knot graphs are another important class of graphs which have similarities to deficiency theory and also appeared in the '70s. We will only review them briefly in Section 6 , because they can only be applied to chemical reaction networks where all reactions are reversible.

The SCL graphs derived from nonstandard reaction diagrams are predecessors of the **SR graph**, described in Section 7. The SR graph was introduced by Craciun and Feinberg in a series of papers [15, 16, 17], as a graphical representation of injective networks. This is a class of networks described by Craciun and Feinberg in [14]. Injective networks cannot admit multiple positive steady states. In Section 7.2 we review a directed bipartite graph as described by Ivanova [46], which share a lot of their structure with the SR graph. Each graph has a section dedicated to it. We will review these graphs and throughout the paper make comparisons between them, whenever possible. It will be necessary to understand different connections between the graphs that have been defined in order to identify their information content. This will help with the further development of graph theoretic methods for more general reaction networks.

2. Background.

A chemical reaction system with m reactions and n reacting species is represented by a time-continuous dynamical system that is derived from reaction schemes. A general reaction scheme can be written as

$$\sum_{i=1}^{n} \alpha_{ji} A_i \quad \rightarrow \quad \sum_{i=1}^{n} \beta_{ji} A_i, \qquad j = 1, ..., m, \tag{1}$$

where A_i, $1 \leq i \leq n$, are the reacting species participating in the j-th **reaction** R_j. The coefficients α_{ji} and β_{ji} represent the number of A_i molecules participating in the j-th reaction at reactant and product stages, respectively. A complex is the object at the head or tail of the arrow in the reaction scheme, such as the one shown in (1). A reaction **complex** is the set of species at the tail of the arrow, labeled $y_i = \sum_{i=1}^{n} \alpha_{ji} A_i$. The product complex is the set of species at the head of the arrow, labeled $y_i' = \sum_{i=1}^{n} \beta_{ji} A_i$. Sometimes, we also write $y \to y'$ to denote a reaction whose reaction and product complexes are y and y', respectively. We label the set of all such reactions by $\mathcal{R}$. If we regard each species as an element of a basis of a formal vector field, then we can naturally associate each complex with a vector form, for example, $y_j = (\alpha_{j1}, \ldots, \alpha_{jn})^T$. We do not distinguish the label for the complex and its vector form, but from the context it will be clear to which of the two we are referring. There are two special types of reactions that we mention. A reaction is called an inflow reaction if the reaction complex vector is a zero vector, namely $0 \to y'$. It is called an outflow reaction if the product complex vector is a zero vector, $y \to 0$. A system is called open, if at least one of the species has an inflow or an outflow reaction. Feinberg and Craicun [17] call them entrapped species models, because some species can enter and leave the system, while others are "trapped". On the other hand, the system is closed if none of the species have inflow nor outflow reactions, namely all species are trapped. In the following we take a deterministic approach to modeling CRNs, but in reality, molecules are in discrete units and some cells might have a small number of species. In these cases, stochastic modeling is much more appropriate, see [64]. However, we will take the standard assumption that all species come in large numbers and hence their concentrations are continuous. CRNs of particular interest are the ones with mass-action kinetics, i.e. where the reaction scheme is interpreted to follow the **law of mass action**. They are associated with polynomial ODEs. Each reaction has only one kinetic coefficient associated with the reaction. For the reaction $y \to y'$, we denote this rate constant by $k_{y \to y'}$. If we take the concentration of each species A_i to be x_i, then the rate at which the j-th reaction takes place is modeled by

$$v_j(x) = k_{y_j \to y_j'} x^{y_j},$$

where the notation $x^{y_j} := \prod_{i=1}^{n} x_i^{\alpha_{ji}}$ has been used. The species-formation rate function takes the vector form,

$$f(x,k) \quad = \quad Nv(x,k), \tag{2}$$

where $N = [y_1' - y_1, \cdots, y_m' - y_m]$ is an $\mathbb{R}^n \times \mathbb{R}^m$ matrix, called stoichiometric matrix, with the k-th column of N composed as the vector difference of complexes $y_k' - y_k$. Moreover v is the vector of all corresponding reaction rates. The associated deterministic ODEs describing the dynamics of the reaction networks in general can be written simply as

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

The local existence and uniqueness of the initial value problem (with the requirement that $x(0) \geq 0$) are guaranteed [81] and solutions will always stay positive, namely $x(t) \geq 0$ ($x(t) > 0$) if $x(0) \geq 0$ ($x(0) > 0$), see [80]. From now on, we will only be concerned with strictly positive solutions. We are often interested in the

behaviour of the steady states, therefore the linearized equations play a crucial role in the analysis. Such a vector field is given by the Jacobian J of the rate function $f(x,k)$ whose entries come in the form

$$J_{ik} = \sum_{j=1}^{m} (\beta_{ji} - \alpha_{ji})\alpha_{jk} \frac{v_j}{x_k}, \tag{3}$$

for $1 \leq i,k \leq n$. Note that each Jacobian entry quantifies the influence species A_k has on A_i. This can be decomposed into the influence each species has on the other via each j-th reaction. Stability of the positive steady state depends on the eigenvalues of the Jacobian matrix that are the roots of the characteristic polynomial,

$$det(\lambda I - J) = \lambda^n + a_1\lambda^{n-1} + \ldots + a_n. \tag{4}$$

Each coefficient a_k for $k = 1, \ldots, n$ is the sum of all principal minors denoted by $M(-J)(i_1, \ldots, i_k)$ of order k, where $1 \leq i_1 < \ldots < i_k \leq n$. If $rank(N) = r < n$, then the last $(n-r)$ coefficients, $a_{n-r} = \ldots = a_n = 0$. Much important dynamic behaviour is also linked to the eigenvalues of the Jacobian matrix. A necessary condition for the system to have a saddle node bifurcation (associated with the bifurcation picture of multistationarity) is that the Jacobian matrix has a zero eigenvalue. A necessary condition for oscillations is that the Jacobian matrix has a pair of purely imaginary eigenvalues.

The Jacobian matrix and its properties can also be used to deduce sufficient conditions for systems that will not to have multiple steady states. An important class of CRNs are injective networks. A network is injective if its species-rate function f are injective, namely if $f(a) \neq f(b)$ whenever $a \neq b$. This injectivity property is sufficient for a CRN not to have multiple steady states. By imposing some conditions on the rate function f and the Jacobian, the global injectivity of a network can be guaranteed. On the other hand the nonsingularity of the Jacobian alone is not sufficient to guarantee that the rate function is injective [61]. Several other conditions are needed [41, 28, 73]. In [31], Gale and Nikaidô proved that injectivity can be guaranteed if the Jacobian has some additional properties. Any matrix A is a P-matrix if all of its principal minors are strictly positive and A is a weakly P-matrix if the determinant of A is positive and all other principal minors are nonnegative. In [31] Gale and Nikaidô showed that if D is a rectangular open domain of $\mathbb{R}^n$, the function $f : D \to \mathbb{R}^n$ is a differentiable function and the Jacobian $J(x)$ of f is a weak P-matrix for all $x \in D$, then f is injective. For mass-action CRNs' rate function f is polynomial, and therefore, we would expect even stronger injectivity conditions to hold. The same should be true if other properties of f are specified. In fact, for open systems CRNs with all species having inflow and outflow reactions, the global injectivity follows if the Jacobian is nonsingular, see [14].

Any matrix property, such as being a P-matrix, are determined by the matrix structure. In fact, several of the graphs we present (interaction graph, SR graph, knot graph, directed bipartite graph) encode information entirely based on the Jacobian matrix. Their topologies are related to particular decompositions of the Jacobian matrix. First, we will review the interaction graph for which there is in fact no underlying decomposition of the Jacobian. Note that each entry of the

Jacobian J_{ik} denotes the influence species A_k exerts on A_i. From the form of the Jacobian entry in Equation 3, each j-th entry in the sum represents this influence via the j-th reaction. On the other hand, the directed bipartite graph and the SR graph are derived using a decomposition for the Jacobian entries. But the SR graph topology (cycles) relies also on additional information. Recall that the Jacobian matrix evaluated at any positive steady state x can be written in vector form as

$$J(x) = N diag(v(x,k))\kappa^T diag(1/x),$$

where *diag* denotes a diagonal matrix, $diag(1/x) = diag(1/x_1, \ldots, 1/x_n)$, and κ is the kinetic matrix $\kappa = [y_1, \ldots, y_m]$. The SR graph topology relates to the square submatrices of the stoichiometic matrix N and the kinetic matrix κ, as derived from Lemma 5.1 and 5.2 in [15]. Following from the same decomposition of the Jacobian entries, the Jacobian can also be written as a sum of m elementary matrices, where the j-th matrix contains only the entries of the Jacobian that correspond to reaction j. For example,

$$J(x) = \sum_{j=1}^{m} A_j,$$

where A_j is an elementary matrix containing only entries of the Jacobian related to the j-th reaction. Any knot graph results rely exactly on such matrix decompositions.

2.1. **Example model.** In the next few sections we will describe how graph theoretic approaches encode the information from the reaction network and hence provide easy but also diverse ways for studying dynamical CRNs. The example we consider throughout this paper is a well-known bistable model describing activity of the mitogen-activated protein kinase (MAPK) [53, 13]. MAPKs play an important part in the signalling processes of eukaryotic cells by intervening with a multitude of proteins and phosphorylating them. They themselves undergo phosphorylation by a MAPK/ERK kinase (MEK) and dephosphorylation by a phosphatase. The system has been modelled by a succession of several Michaelis-Menten type reactions. In this model we denote the MAPK kinase by S_0, and the molecules with single and double phosphorylation S_1 and S_2, respectively. The symbols E and F represent MEK and the phosphatase. The reaction scheme for the model is:

$$E + S_0 \leftrightharpoons ES_0 \to E + S_1 \leftrightharpoons ES_1 \to E + S_2$$
$$F + S_2 \leftrightharpoons FS_2 \to F + S_1 \leftrightharpoons FS_1 \to F + S_0.$$

Let us label the concentrations of species E,F, S_0, S_1, S_2, ES_0, ES_1, FS_1 and FS_2, from x_1 up to x_9, respectively. Let C_1 to C_{10} denote the complexes $E + S_0$, ES_0, $E + S_1$, ES_1, $E + S_2$, $F + S_2$, FS_2, $F + S_1$, FS_1 and $F + S_0$. With these definitions we can now associate the reaction system with the following set of ODEs:

$$
\begin{aligned}
\dot{x}_1 &= -k_{C_1\to C_2}x_1x_3 + (k_{C_2\to C_1} + k_{C_2\to C_3})x_6 - k_{C_3\to C_4}x_1x_4 \\
&\quad +(k_{C_4\to C_3} + k_{C_4\to C_5})x_7 \\
\dot{x}_2 &= -k_{C_6\to C_7}x_2x_4 + (k_{C_7\to C_6} + k_{C_7\to C_8})x_9 - k_{C_8\to C_9}x_2x_5 \\
&\quad +(k_{C_9\to C_8} + k_{C_9\to C_{10}})x_8 \\
\dot{x}_3 &= -k_{C_1\to C_2}u_1x_3 + k_{C_2\to C_1}x_6 + k_{C_9\to C_{10}}x_9 \\
\dot{x}_4 &= k_{C_2\to C_3}x_6 - k_{C_3\to C_2}x_1x_4 + k_{C_4\to C_3}x_7 + k_{C_7\to C_8}x_8 \\
&\quad -k_{C_8\to C_9}x_2x_5 + k_{C_9\to C_8}x_9 \\
\dot{x}_5 &= k_{C_4\to C_5}x_7 - k_{C_6\to C_7}x_2x_4 + k_{C_7\to C_6}x_8 \\
\dot{x}_6 &= k_{C_1\to C_2}x_1x_3 - (k_{C_2\to C_1} + k_{C_2\to C_3})x_6 \\
\dot{x}_7 &= k_{C_3\to C_4}x_1x_4 - (k_{C_4\to C_3} + k_{C_4\to C_5})x_7 \\
\dot{x}_8 &= k_{C_8\to C_9}x_2x_5 - (k_{C_9\to C_8} + k_{C_9\to C_{10}})x_8 \\
\dot{x}_9 &= k_{C_6\to C_7}x_2x_4 - (k_{C_7\to C_6} + k_{C_7\to C_8})x_9.
\end{aligned}
$$

In the next few sections we will use the different graph theoretic approaches to analyse the MAPK model.

3. **The interaction graph.** The interaction graph (often written IG, but here we use the notation G_{int}) conveys the information about any influence one species exerts on another. An advantage of the interaction graph is that it can be constructed for all classes of ODEs and hence its application is also widespread in all of biology including ecology, where models are often not of polynomial structure. This versatility is also useful in chemical models. Since the choice of mass-action kinetics is a modeling assumption, chemical reaction networks with other kinetics (such as Michaelis-Menten) can also be analysed via the interaction graph.

Definition 3.1. The interaction graph $G_{int}(x) = (V, E)$ of a chemical reaction network is an orientated graph with vertex set given by the species A_i. A directed edge (arrow) from A_i to A_k is present if and only if $f_k(x)$ depends on x_i. Each edge is endowed with a sign, which is the sign of the associated element of the Jacobian, J_{ki}.

The interaction graph of the MAPK cascade is presented in Figure 1. Of course, the structure of the interaction graph depends on species concentrations, since edges can change sign for different species concentrations x, or can also vanish completely when there is no interaction, namely if $J_{ki} = 0$.

There are important classes of systems for which the sign pattern of the Jacobian matrix doesn't change over time. Most well known examples are quasi-monotone systems and types of monotone systems, called cooperative and competitive systems. For cooperative (competitive) systems all edges corresponding to off-diagonal entries of the Jacobian have only positive (negative) sign. Quasi-monotone systems have more general edge types. For them the signs of edges are fixed, but can be either positive or negative. Our MAPK system in Figure 1 is an example of a quasimonotone system.

A cycle in the graph $G_{int}(x)$ is a sequence of distinct vertices $A_{i_1}, \ldots, A_{i_k}$ such that there is an edge connecting A_{i_1} to A_{i_2}, A_{i_2} to A_{i_3} and so on, finishing with an edge between A_{i_k} and A_{i_1}. The length of the cycle is the number of vertices that

it contains. The special case of a cycle containing only one vertex is called a loop. A loop has obviously length one and is associated with the main diagonal entries of the Jacobian matrix. Each cycle is endowed with a sign, which is the product of the signs of its edges. In Figure 1, S_1, FS_1 and F form a negative (sign) cycle in the interaction graph of the MAPK cascade.

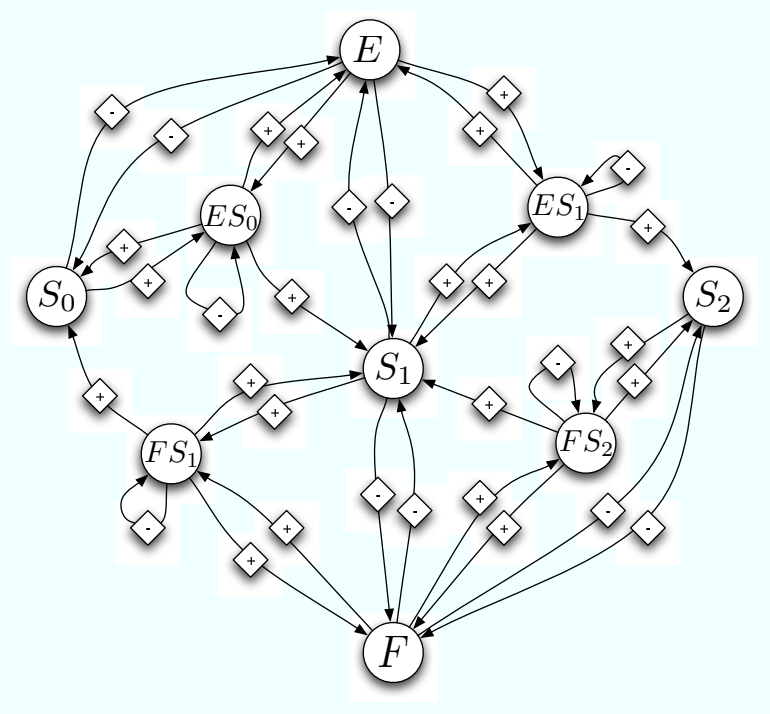

FIGURE 1. Interaction graph of the MAPK network.

Up to now we only mentioned the more intuitive cases of cooperative and competitive monotone systems, but there are some types of monotone systems can be identified via cycle signs.

A chemical reaction network is called monotone with respect to a partial order $\leq$, if the order of initial conditions $x_0 \leq y_0$ is preserved by the flow, namely $x(t) \leq y(t)$ for all time $t > 0$ where $x(t)$ and $y(t)$ are two solutions of the system with respective initial conditions. Partial order is generated by an orthant $\mathcal{O}$ of the real space $\mathbb{R}^n$ if $x \leq y \Leftrightarrow y - x \in \mathcal{O}$. Systems that are monotone with respect to an orthant partial ordering can be identified by their interaction graph topology. In fact, a system is monotone with respect to an orthant ordering if, and only if, all cycles of its associated interaction graph are positive [72]. As stated above, the interaction graph of MAPK cascade contains at least one negative cycle and hence MAPK cascade is not a monotone system with respect to any orthant order. We are interested in monotone dynamical systems, because they represent a class of systems that display restricted behaviour. They have convergent solutions and cannot admit stable periodic behaviour. A lot of theory exists for these systems, see [3, 4, 5, 72].

Two cycles are called disjoint, if they don't share any common vertices. A union of disjoint cycles containing amongst themselves all vertices of $G_{int}(x)$ is called a

nucleus. In some literature it is referred to as a Hamiltonian hooping, see [71]. The nucleus is also endowed with a sign $(-1)^{p+1}$, where p is the number of positive cycles of the nucleus. A nucleus (or a cycle) is referred to as variable if at least one of its edges is sign changeable. A nucleus (or cycle) is called ambiguous when its sign varies with species concentration x.

3.1. **Theorems related to the interaction graph.** The determinant and minors of the Jacobian matrix can be calculated via the Leibnitz formula using permutations of rows and columns of the Jacobian (or its square submatrix, in the case of minors). Since edges of the interaction graph are in one-to-one correspondence with the nonzero entries of the Jacobian matrix, a set of entries of the Jacobian whose rows and columns are in cyclic premutation are also in one-to one correspondence with the cycles of the interaction graph: an important observation. Hence, it is clear that cycles of the interaction graph are strongly linked to the system dynamics. In the early '80s René Thomas [74] conjectured that any dynamical system with multiple steady states must have a positive cycle, while a system with stable oscillations must contain at least one negative cycle (of length at least two). These conjectures are very intuitive, as we will now demonstrate on the interaction graph of the MAPK cascade. Consider the negative cycle of species S_1, F and FS_1. Increasing the concentration of S_1 will increase the concentration of FS_1 which in turn, as it unbinds, will increase the concentration of F. But with more F, the concentration of S_1 will decrease. So an initial increase in concentration of F along the negative cycle leads to a decrease in its concentration, and hence we see how negative cycles could possibly promote oscillatory behaviour, just as it has been shown for steam engines where this behaviour was first observed and analysed. Next, we consider the positive cycle between species ES_1, S_2, S_2 and S_1. An increase in S_1 leads to a further increase in its concentration when going around the cycle. Perturbation of S_1 pushes the system to a new basin of attraction and possibly to a new equilibrium. Thomas's first conjecture was proven by Soulé in [71], and here we restate it in reverse form:

Theorem 3.2 (Thomas-Soulé)**.** *If a system has no positive cycles in $G_{int}(x)$ for any x, then it cannot exhibit multi-stationarity.*

Aside from the relationship between cycles and Jacobian entries, which lead to conditions about positivity of the determinant of $-J$ and its minors, Soulé made use of Gale-Nikaidô theory [31] to show that the reaction system is injective and hence cannot exhibit multistationarity. Thomas' second conjecture has been shown to be correct for quasimonotone systems, see Snoussi [69] and Gouzé [39]. Our MAPK cascade has at least one positive cycle , Figure 1, hence it may have multiple steady states. Aside from the Thomas' conjecture, the negative cycle is linked to various dynamics. For a steady state x, a negative cycle in the graph $G_{int}(x)$ is necessary in order for a steady state to be stable, see [76, 62]. Moreover, a negative cycle of length one is necessary for the presence of a general attractor [76], a compact subset of the phase space which is invariant under the flow and which attracts a (fundamental) family of open neighbourhoods. Interestingly both of these types of cycles are present in the interaction graph for the MAPK cascade. To fully understand dynamics of the system we must understand how a particular cycle fits into the full network landscape. Let us return to the positive cycle of species ES_1, S_2, FS_2 and S_1. We concluded before that when this cycle is isolated, an increase in S_1 will lead to a further increase of S_1, if we only consider interactions along the

positive cycle. However, when we look at a full network, the influence of species outside the cycle might give a different result. Let us link it to the negative cycle S_1, FS_1, F which we reviewed before. Clearly an increasing concentration of S_1 will decrease its concentration if we follow a negative cycle. This might offset the increase from the positive cycle. We could end up with a smaller concentration of S_1 than the one we started with.

Conjectures about the dynamics and their relation to the full network structure have also been explored. René Thomas [75] conjectured that the appearance of both negative and positive cycles is necessary for chaotic dynamics. This conjecture was discussed in detail by Toni in [78]. On the full scale of the system Kaufmann and Thomas [77] conjectured that the presence of a variable nucleus or any presence of two nuclei of opposite sign are necessary for a system to display multistationarity. Recently, together with Soulé [76], they proved their conjecture for a class of systems which encompass mass action chemical reaction systems.

In the MAPK case in Figure 1 we can identify at least two nuclei with opposite sign. The first nucleus is composed of a union of cycles $\{E, ES_0, S_0\}$, $\{F, FS_1\}$ and $\{S_1, ES_1, S_2, FS_2\}$. The second has cycles $\{FS_1, S_1, F\}$, $\{S_2, FS_2\}$, $\{ES_1, E\}$ and $\{S_0, ES_0\}$. Next, we review the chemical reaction deficiency theory, which appeared in the late '70s. It appeared in the chemical engineering literature and developed in a different direction from the interaction graph theory.

4. **Reaction diagrams and deficiency theory.** Deficiency theory was pioneered by Horn, Jackson and Feinberg [43, 42, 25] and centers around a nonnegative index, called deficiency, by which one can identify classes of networks that can or cannot have multiple positive equilibria. It was extensively developed in following decades, see [26].

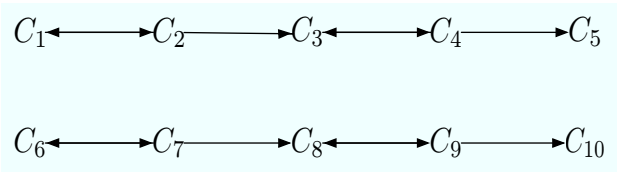

FIGURE 2. Reaction diagram of the MAPK network.

Definition 4.1. A reaction diagram of a CRN is a digraph $G_D = (V, E)$, where the vertex set V is composed of the network complexes (C_i), and a directed edge exists from C_i and C_j if there is a reaction with C_i as a reaction complex, and C_j as the product complex.

The reaction diagram of the MAPK network is shown in Figure 2. Namely $E+S_0$ is the complex C_1 etc. It is very similar to the reaction scheme presented in Section 2.1. Often the reaction diagram is called a directed graph [32]. It can be supplemented with a bipartite graph displaying the information which species are present in which complex, see Definition 4.4 and Figure 3. As will be shown the advantage is that the polynomial differential equations can be fully formulated in terms of adjancency and incidence matrices belonging to these graphs. A nonstandard version

of the reaction diagram , which we describe in Section 5, is useful in the analysis of the SCL graph [65]. We say that complexes y and y' belong to the same linkage class if there exists an undirected path in the reaction diagram connecting the two complexes. For the MAPK network given in Figure 2 it holds that the complexes C_1 to C_5 belong to the same complex linkage class. In total the MAPK network has two linkage classes. A reaction network is called weakly reversible if, whenever there exists a directed path from y to y' in the reaction diagram, there must also exist a directed path from y' to y. From Figure 2 it can be seen that the MAPK network is weakly reversible, since there exist a direct path from C_1 to C_3, but not vice versa.

4.1. **Theorems related to the deficiency index.** The deficiency of a reaction network (denoted by the symbol δ) is defined by the following formula,

$$\delta = c - l - r,$$

where c is the number of complexes, l is the number of linkage classes and r is the rank of the stoichiometric matrix N. It holds that the deficiency index is always nonnegative, see [25]. The stochiometric subspace for a reaction network is the span of the reaction vectors, namely $Im(N)$. Two vectors y and y' are stoichiometrically compatible if $y'-y \in Im(N)$. Stoichiometric compatibility is an equivalence relation that induces a partition of the space $\mathbb{R}^n_+$ into equivalence classes. Each positive stoichiometric compatibility class is a space of the form $\{x_0 + Im(N)\} \cap \mathbb{R}^N_+$, where x_0 is some positive initial concentration. We state the following version of the deficiency theorems, as given in [26]:

Theorem 4.2 (Deficiency-Zero Theorem)**.** *Consider a mass-action reaction network of deficiency zero. Then the following holds for any arbitrary parameter set:*

1. *If the network is not weakly reversible, then system admits neither a positive equilibrium, nor a positive periodic orbit.*
2. *If the network is weakly reversible, then system has the following properties: each positive stoichiometric compatibility class contains precisely one equilibrium, this equilibrium is asyptotically stable, and there is no nontrivial periodic orbit.*

The power of the deficiency-zero theorem is that by definition of the deficiency index we can identify a class of networks which cannot have multiple positive steady states. These networks can be very complicated and contain hundreds of species. Regardless of the kinetic parameter values, the deficiency zero theorem can give information about the positive steady states. However, the theory is also limited. Many open systems tend to have non-zero deficiency index [51]. The Deficiency-One Theorem relaxes the deficiency index condition, keeping the assumption that the network must be weakly reversible. The theorem now relies on the notion of the deficiency restricted to a linkage class. The deficiency of θ-th linkage class $\mathcal{L}$ (denoted δ_θ) is defined by the formula

$$\delta_\theta := c_\theta - 1 - r_\theta,$$

where c_θ is the number of complexes in linkage class $\mathcal{L}$, and r_θ is the rank of the linkage class, namely the rank of a submatrix N^T that is restricted only to columns for which $y, y' \in \mathcal{L}$. We are now able to state the theorem:

Theorem 4.3 (Deficiency-One Theorem)**.** *Assume a mass-action reaction network with l linkage classes. Let δ denote the deficiency of the network, and let δ_θ denote the deficiency of the θ-th linkage class, $\theta = 1, 2, \ldots, l$. Furthermore suppose that both of the following conditions are satisfied:*

1. *$\delta_\theta \leq 1$, $\theta = 1, 2, \ldots, l$ and,*
2. *$\delta = \sum_{\theta=1}^{l} \delta_\theta$.*

If the network is weakly reversible (in particular if the network is reversible), then the system admits precisely one equilibrium in each positive stoichiometry compatibility class.

Several versions of the deficiency theorems are given by Feinberg et al. in [25, 26]. The proofs of deficiency theorems are related directly to the structure of the species-formation rate and its stoichiometric matrix. In fact, the stoichiometric matrix is a product of two matrices, $N = YI_\alpha$. Here the incidence matrix I_α is an $c \times m$ matrix and relies on information of the directed graph. Its entry i^α_{jk} is -1 if the complex j is at the tail of a directed edge representing the k-th reaction, and is 1 if j-th complex is at the head of the edge. Otherwise it is 0, i.e. if the complex does not participate in the j-th reaction. The directed graph has also another incidence matrix, I_k, which is an $m \times c$ matrix with the i^k_{jk}th entry equal to k_k if, and only if, the complex C_j is at the tail of the directed edge responding to the k-th reaction. Otherwise, the entry is zero. The matrix Y is related to the undirected bipartite graph, a relationship first described in [32].

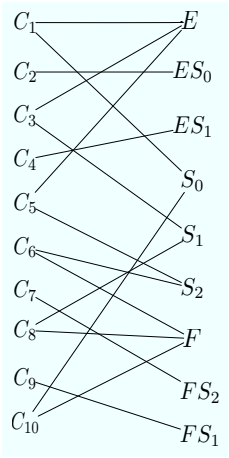

FIGURE 3. Undirected bipartite graph of the MAPK network.

Definition 4.4. The undirected bipartite graph $G_B = (V, E)$ is a graph with two sets of vertices: one for the species (V_1) and the other for the complexes (V_2). An edge exists between the vertices of two sets, say S_j and C_i, if complex C_i contains species S_j. Each edge has a weight which is the stoichiometry of the species occurring in the complex.

Weights of the edges are arranged into the adjacency matrix Y, where each entry y_{ij} is the weight of an edge between i-th complex (vertex in V_2) and j-th species (with corresponding vertex in V_1). In Figure 3, the undirected bipartite graph of

the MAPK network is shown. Now the associated ODE can be written completely in terms of an adjacency and an incidence matrix:

$$\dot{x} = Y I_\alpha I_k \psi(x),$$

where $\psi(u)$ is a vector of the reaction rate monomials (without the kinetic constant). That is one of the rare cases where a whole dynamical system can be completely defined in terms of graph theoretic concepts. Gatermann [32] showed that the deficiency index is the rank defect between I_α and N,

$$\delta = dim(I_\alpha) - dim(N). \tag{5}$$

Conditions of weak reversibility are linked to the structure of the product of matrices I_α and I_k. Gatermann [32] used algebraic geometry to prove some versions of the deficiency theorems. These algebraic geometry methods simplify the computations, however they apply only to polynomial vector fields. In fact, the deficiency theory can also be applied to non-mass action kinetic systems, namely to systems with more general vector fields [26] . In our example, the MAPK network has deficiency $\delta = 2$ and therefore deficiency theory predicts that the MAPK system could potentially have multiple steady states.

As above theorems show the deficiency theory can be applied to a wide range of biological systems to check whether the mechanisms in question could potentially exhibit multistationarity [13, 21]. Analysis tools based on deficiency theory have been implemented into a software tool called the 'Chemical Reaction Network Toolbox', see [27]. But as has been mentioned, deficiency theory has its limitations. Its theorems cannot be applied to many classes of systems such as the isothermal homogeneous CFSTRs, [51]. In the next section we will review theory that has been developed alongside deficiency theory and can deal with networks where deficiency theory is not applicable.

5. **The SCL graph.** Network deficiency theory provided a way of identifying a large class of networks for which some dynamical behaviour can be described without knowledge of the parameters, and without restrictions on the size of these networks. There is a class of open systems to which deficiency theory cannot be applied. These systems are of special interest from a qualitative point of view. There are reports that such networks can exhibit multistationarity [59, 58], though this behaviour is considered to be experimetally very rare [14]. CFSRTs are vessels where chemical reactions take place with the property that all species have a constant inflow and outflow. The authors in [51] argued that by including the inflow and outflow of every species, the augmented reaction network would not have deficiency index of zero nor one, and hence deficiency theory would not be applicable. The need for graphical methods that could apply to such systems lead to the development of work complementary to deficiency theory. The theory was constructed for the isothermal homogeneous CFSTRs and it was introduced in the mid '80s by Rumshitzky and Feinberg in [63]. Considerable extensions to the theory was done later by Schlosser and Feinberg in [65]. We base this review on their developments.

Schlosser and Feinberg associated a Species-Complex-Linkage (SCL) graph with each CFSTR reaction network. In terms of graph theoretic considerations, this work

was a considerable step away from deficiency theorems. The criteria for enabling or restricting specific dynamics in SCL graphs rely on the existence of cycles in the graph, hence SCL graph has many similarities to the interaction graph. Moreover, the linkage classes are also described by a subset of the graph vertices, so cycles in the SCL graph also depend on information about the linkage classes. In this way the SCl graph theory unifies deficiency theory with the interaction graph theory. The SCL theory was derived to deal with models from homogeneous isothermal CFSTRs, but its results depend only on the network of true chemical reactions. Hence it is applicable to a wider spectrum of systems. Keeping the terminology from the previous section, we can define an SCL graph as follows:

Definition 5.1. The species-complex-linkage graph $G_{SCL} = (V, G)$ is an undirected bipartite graph which has two types of vertices: The species (represented by V_1) and the complex linkage classes (represented by V_2). We draw an edge from a species vertex to a linkage vertex if the linkage class has a complex which contains this particular species.

The SCL graph of the MAPK system is shown in Figure 4. As can be seen from Figure 2, the MAPK has two linkage classes: $L_1 = \{E+S_0, ES_0, E+S_1, ES_1, E+S_2\}$ and $L_2 = \{F + S_2, FS_2, F + S_1, FS_1, F + S_0\}$. Each edge of the SCL graph has two labels associated with it: a complex label and a stoichiometric label. The first label is the complex to which the species adjacent to the edge belongs. For example, E and L_1 are connected by an edge with complex label $E + S_0$, because complex $E + S_0$ belongs to linkage class L_1, and it contains E. The second label is the stoichiometric coefficient in the complex label, associated with the species that is adjacent to the edge. In the SCL graph of the MAPK system none of the stoichiometric labels are shown, because they are simply all one. Now three types of cycles can be identified in the SCL graph: o-cycles, c-cyles and s-cycles. They are all associated to the complex labels on the edges of the cycles. A *c*-pair in a SCL graph is a pair of edges that carry the same complex label. In the MAPK SCL graph, the edges connecting S_0 to L_1 and L_1 to E form a c-pair. If a cycle contains an odd number of *c*-pairs, then it is called an *o*-cycle. If a cycle is made entirely out of *c*-pairs, then it is called a *c*-cycle. We label a cycle as a *s*-cycle if we can walk around the cycle (in either direction), alternately multiply and divide the stoichiometric coefficients of the edges, proceed until we return to the starting point, and if then the product of this calculation is 1. An alternating-1 cycle is a cycle in which there are no *c*-pairs, and in which there is an edge labeled with a stoichiometric coefficient 1 such that every second edge thereafter it is again labeled with 1 (while in-between edges can take any values). Such cycle information seems to be very specific. In the SCL graph description in [65] proofs for the theorems are actually not given, but a similar cycle terminology is applied to SR graph [15]. There these specific cycles are related to established theorems. The SCL graph of the MAPK system has two cycles, as seen in Figure 4. They are $\{S_0, L_1, S_1, L_2\}$ and $\{S_1, L_1, S_2, L_2\}$. Both cycles are *s* cycles and alternating 1 cycles.

5.1. **Qualitative theory related to the SCL graph.** In [65], Schlosser and Feinberg gave no mathematical proofs of the following statements from their paper, but it seems likely that the proofs for the graph we review next, the SR graph (also introduced by Feinberg , this time in collaboration with Craciun) can be applied. The theory based on the interaction graph is also useful in this case. In the SCL graph a lot of information from the interaction graph is simply condensed. An edge

in the interaction graph signifies that a species reacts with, or produces another species. In the SCL graph this is represented by two edges connected by the same linkage vertex. A cycle in an SCL graph will always correspond to at least one cycle in the interaction graph. For example, look at the loop with connecting E and L_1 through edges with complex labels $E + S_0$ and $E + S_2$. This is represented by two cycles in interaction graph, first cycle with species E and ES_0, and the second with species E and ES_1. An edge in the SCL graph might correspond to a set of cycles in the interaction graph. For example, look at the SCL graph of the MAPK and two edges connecting E and L_1 and L_1 with S_1. Though this is not a cycle in the SCL graph, there exists a positive cycle containing both of these species in the interaction graph, see Figure 1.

The first theorem, related to the conjectures of Thomas [74], states that a system without cycles in the SCL graph cannot exhibit interesting dynamics:

Theorem 5.2. *Consider a mass-action reaction network describing a CSFRT for which the SCL graph contains no cycles. Then the system cannot admit multiple positive steady states for any positive parameter values.*

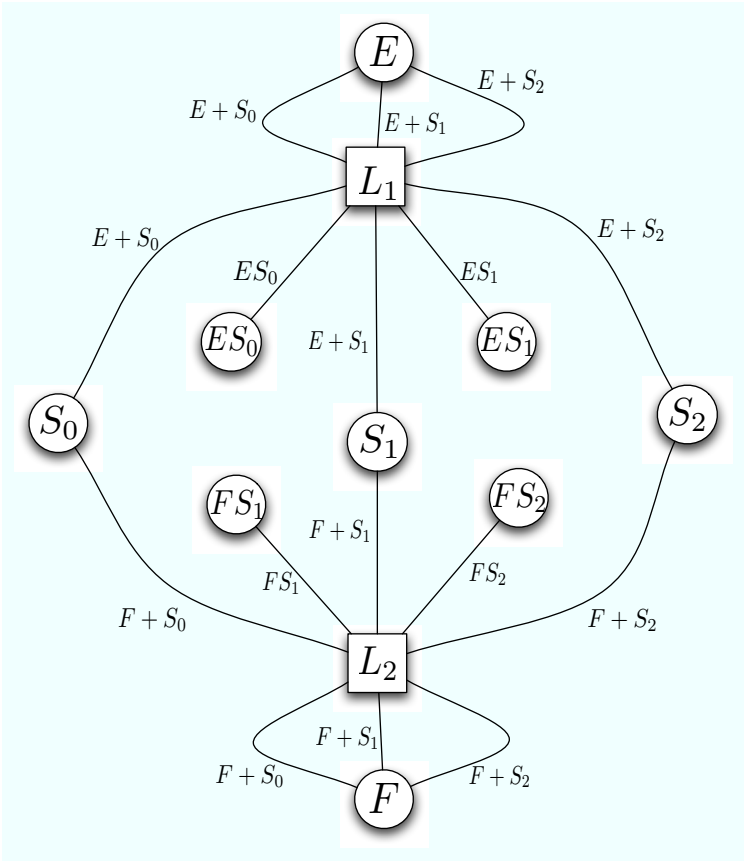

FIGURE 4. The SCL graph of the MAPK network.

Feinberg and Schlosser also identified how different types of cycles and their intersections can guarantee that the system does not have multiple positive steady states:

Theorem 5.3. *Consider a mass-action CSFRT reaction network for which the corresponding SCL graph has all of the following properties:*

1. *Each cycle is an o-cycle, a c-cycle, or a s-cycle.*
2. *If both edges of a c-pair are cycle edges, then any cycle containing one of the edges contains both of them.*
3. *In an o-cycle that is neither a c-cycle nor a s-cycle, no linkage class symbol is adjacent to more than three cycle edges.*

Then the system cannot admit multiple positive steady states for any positive parameter values.

Theorems stated above have been extended to non-standard reaction diagrams. In a non-standard reaction diagram the linkage classes are separated into individual reactions. This graph will later be referred to as a SR graph. The SR graph of the MAPK is shown in Figure 6. Each complex linkage becomes separated into several reaction vertices. Some paths from standard SCl graph become cycles in the nonstandard version of the graph. For example, the path between E and S_1 in the SCL graph (Figure 4) with two edges labeled $E + S_1$ becomes a cycle in the nonstandard SCL graph (Figure 6). The SCL graphs from non-standard reaction diagrams sometimes carry information not contained in the standard SCL graphs. An example is provided in [65]. All the graph properties will carry over once the definition of a c-pair is sharpened. In an SCL graph derived from a non-standard reaction diagram, a c-pair is a pair of edges that carry the same complex label, but also share a common linkage class symbol as an end point. All the aforementioned theorems can be applied to SCL graphs taken from the non-standard reaction diagrams. With the standard reaction diagram in mind, results about the stability of the reaction network can be deduced. These results are applicable only to reversible reaction networks, i.e. networks where all reactions must be reversible. Let c be the number of complexes and l be the number of linkage classes in the network. A reversible reaction network is forest-like if the number of reactions m, is $m = c - l$.

Theorem 5.4. *Consider a (forest-like) reversible mass-action reaction network for which the SCL graph has no cycles. Then the system cannot admit any unstable positive steady states for any choice of positive parameter values.*

This theorem is a consequence of the work of Beretta [9] on knot graphs that will be described in the next section.

5.2. **General comments on the SCL graph.** The SCL graph theory has been useful to describe a large class of networks that do not have the capacity for multiple positive equilibria. On the other hand, its information is not conclusive for some reaction networks, as we will show in an example in Section 5. In some ways the SCL graph is a predecessor of the SR graph, with its results drawing on the existence of graph cycles. The SCL graph has also drawn criticism from Craciun and Feinberg (see [14]) for not being a good candidate for automation.

6. **The knot graph.** Another type of graph related to the complex linkage theory is the knot graph. It can be more widely applied than the zero-deficiency theorem.

The knot graph was introduced by Delattre [18] and Hyver [45], but here we review the latest work of Beretta and co-authors [9] which relates this graph to open chemical systems. The setting for this graph approach is very specific, since the chemical reaction network under consideration must be reversible. Due to this relatively strict condition, we only review briefly this work.

In order to define a knot we need to introduce a specific equivalence relation between the network species. Let A_i be a species in complex y_1 and let A_k be a complex in species y_l. We define an equivalence relation $A_i \sim A_k$ if, and only if, there exists a sequence of complexes from y_1 to y_l (namely $y_1, y_2, y_3, \ldots, y_l$) such that $y_j^T y_{j+1} \neq 0$ for all $j = 1, \ldots, l-1$. The classes of species defined by this equivalence relation are called knots.

As the MAPK network is not a reversible network, the knot graph theory cannot be applied. Hence, we will modify our MAPK network by assuming that all reactions are reversible. This is a perturbation of the original model, since we can assume that these added reactions have very small reaction constant. The reaction scheme for the modified MAPK network is

$$E + S_0 \leftrightharpoons ES_0 \leftrightharpoons E + S_1 \leftrightharpoons ES_1 \leftrightharpoons E + S_2$$
$$F + S_2 \leftrightharpoons FS_2 \leftrightharpoons F + S_1 \leftrightharpoons FS_1 \leftrightharpoons F + S_0.$$

The modified MAPK network has five knots $\{E, F, S_0, S_1, S_2\}$, $\{ES_0\}$, $\{ES_1\}$, $\{FS_1\}$ and $\{FS_2\}$.

Definition 6.1. The knot graph $G_K = (V, E)$ of a reversible chemical reaction network where the vertex set are the knots (equivalence classes on the set of species). We draw an undirected edge between two knots if there is a reaction for which the species set of a reactant complex is in one knot, and the species set of the product complex is in another knot.

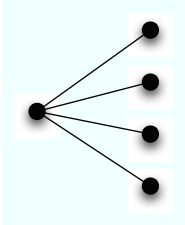

FIGURE 5. The knot graph of the modified MAPK network, with all reactions made reversible. The knot with four edges contains the species $\{E, F, S_0, S_1, S_2\}$.

The knot-graph theory as given by Beretta et al. [9] is closely tied to the additive decomposition of the Jacobian matrix into elementary matrices associated to reactions occurring inside the system (closed part of the system), and a non-positive diagonal matrix related to reactions describing the exchange of material with the environment (open part of the system). Since all reactions are reversible, the elementary matrices of each forward and backward reaction can be partitioned into blocks. These block structures identify the edges between knots. Hence certain patterns in these block matrices can represent specific graph structures. Beretta et

al. identify two such structures: tree graphs and cycle graphs. Note that the cyclomatic number is defined to be the smallest number of edges that have to be removed such that no cycles remain in the graph. Tree graphs have m knots (vertices) and $m-1$ branches (edges), therefore their cyclomatic number is zero. Cycle graphs have m knots and m branches, with $m \geq 2$. Obviously their cyclomatic number is one. In particular, block structures can give results about the eigenvalues, i.e. the spectrum of the Jacobian matrix. Hence graph structures can be related in this way to the dynamics of the system. For example, Beretta et al.[9] show that if G_K is a tree graph, then the Jacobian matrix of any positive steady state has only eigenvalues with nonpositive real part. Such a knot graph structure is creating a stable reaction network according to Beretta's definition. A knot graph of the modified MAPK network is shown in Figure 5. It is a tree graph, so any positive steady states of this system are stable.

It is important to note that the vector field of the modified MAPK network can be interpreted as a perturbation of the MAPK vector field. In perturbation theory one tries to identify classes of perturbations of the vector field such that the perturbed system has the same qualitative properties as the unperturbed system. It seems that for the 'perturbed' reversible MAPK system knot graph theory predicts all positive steady states to be stable, which would not allow multistationarity of the system where basins of attraction are separated by the unstable manifolds of some positive equilibria. A theory to test such hypothesis for letting 'reversibility tend to zero' is still missing.

7. **The SR graph.** Craciun and Feinberg identified another class of CFSTR networks that are injective [14]. Injective networks cannot admit multiple positive steady states. Injective networks contain the class of networks identified by conditions defined on the SCL graph [14]. The species-reaction graph (SR graph for short) was introduced by Craciun and Feinberg in [15] as a graphical representation of injective networks. The graph is closely related to the previous graphical methods and it is identical to the SCL graph based on the nonstandard reaction diagram. Note that the reaction complex vector of an inflow reaction, and the product complex of an outflow reaction are zero vectors, a characterisation that can be used for their definition.

Definition 7.1. The species-reaction graph $G_{SR} = (V, E)$ is a bipartite graph, i.e. its vertex set V is partitioned into two classes, with no edges inside one of the classes. The first class are vertices of species, the second are vertices of internal reactions, i.e. all inflow and outflow reactions are excluded. If A_i is participating in the reaction ($\alpha_{ji} > 0$), then there exists an edge between species vertex A_i and reaction vertex $y_j \to y'_j$. We label it with the reaction complex y_j. An edge exists between reaction node $y_j \to y'_j$ and species node A_i if $\beta_{ji} > 0$, meaning that A_i is produced in this reaction. This edge is labeled with y'_j.

Edges that are connected by the same reaction node and have identical complex label are called a c-pair. Aside from the complex label, each edge in the SR graph also has an attached stoichiometric coefficient, as in the SCL graph. The rest of the graph cycle terminology carries over from SCL graphs. Craciun and Feinberg introduce a concept for sharing a c-pair among cycles [15]. A simple path from a species vertex to a reaction vertex in G_{SR} is defined as an S-to-R chain. Two cycles

in G_{SR} are said to have an S-to-R intersection if their common edges belong to a S-to-R chain or a disjoint union of several chains. This condition implies that two cycles split a c-pair, namely if each edge of the c-pair appears in at least one of the two cycles, and if one of the edges is contained in just one of the cycles. The idea of a split c-pair was already implicitly introduced in Condition 2 of Theorem 5.3. As an extension, the SR graph has an oriented version, called the OSR graph. All theorems presented by Craciun and Feinberg are related to the properties of the SR graph, and only the proofs use the OSR graph. For the sake of brevity, we will not review their construction. Interested reader should consult [15].

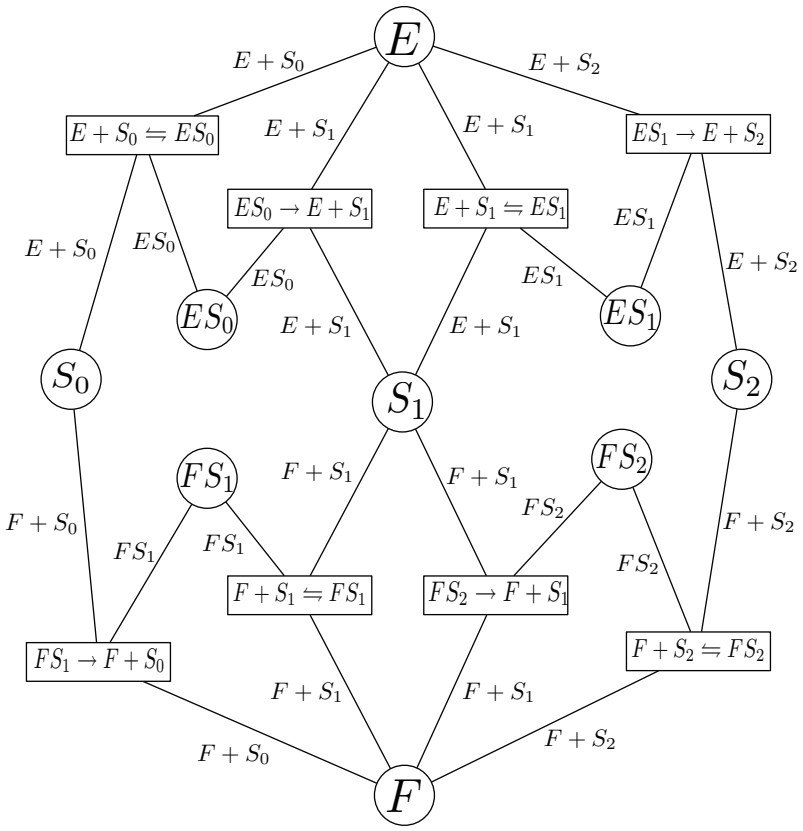

FIGURE 6. SR graph of the MAPK network.

In the interaction graph, we mentioned that every cycle presents a set of nonzero coefficients of the Jacobian whose row and column are in cyclic permutation. The addition Craciun and Feinberg make via the SR graph is that every entry J_{ik} is now split into terms related to the contribution each reaction rate generates for the entry J_{ik}. This split allows for finer results relating the graph topology to the values of the minors of the Jacobian. In contrast to the interaction graph, each path between two species vertices encodes information about the reaction that is the link for the two species. As an example we look at the cycle between species E and S_1 in the interaction graph of the MAPK network in Figure 1. The interaction between these two species here depends on two reactions.

However, there are important differences between the interaction and the SR graph. Cycles in the interaction graph are in direct correspondence with the cycles in the entries of the Jacobian matrix. Cycles in the SR graph are related to the cycles in the stoichiometric and the kinetic matrix, as described in Lemma 5.1 and 5.2 in [15]. Cycles in the interaction and the SR graph do not have a one-to-one correspondence. For example, take any one of the cycles in the interaction graph of the MAPK network in Figure 1 with species E, ES_0 and S_0. In the SR graph, Figure 6, these three species are not in a cycle. In order for a cycle to be present in the SR graph, there would have to be a third reaction vertex adjacent to the edges incident to species S_0 and ES_0.

An advantage of Craciun-Feinberg theory is that it is aimed at mass-action kinetic systems. Obviously this specific structure can lead to much stronger results, a remark that is generally true for most part of this paper.

Theorem 7.2. *Consider some CFSTR network such that its SR graph G_{SR} contains only cycles that are o-cycles or s-cycles, and such that no two e-cycles have an S-to-R intersection. Then the reaction network is injective.*

The proof presented by Craciun and Feinberg in [15] relies on two parts: that cycles conditions imply that the Jacobian matrix is nonsingular and then that this result as well as the polynomial structure of the ODEs (due to mass-action kinetics and CFSTR environment) imply that the vector field is injective.

A weaker version of the previous theorem involves a definition of a split c-pair. This version was used by Craciun in [16] to confirm that a bistable mechanism underlying the operation of the classical anti-cancer target, Dihydrofolate Reductase (DHFR), does not violate conditions of the theorem.

7.1. **Recent extensions.** The classical CFSTR models may not be appropriate inside a wider biological context. If the reaction vessel is interpreted as a cell, all chemical substrates may not be freely diffusing across the cell membrane. We might imagine that substrates and products are readily transported, but enzyme-related molecules with high molecular weight may be entrapped within the cell. Craciun and Feinberg [17] investigated variants of the CFSTR model where some species cannot diffuse. They called them the entrapped species models. In [17] it has been shown that if a CFSTR reaction network cannot have multiple steady states, then also the entrapped model network cannot create multiple non-degenerate equilibria. The success of the SR graph is that only properties of the cycles are required to determine the network dynamics. Another graph, the directed bipartite graph introduced in the next subsection, will be very similar. In addition it provides more detailed information on the relation between the coefficients of $det(-J)$ and the minors related to subgraphs. Whereas this information may not be needed in the context of searching for Turing unstable systems [20], it is useful for identifying delay-induced unstable systems [57].

7.2. **The directed bipartite graph (DB graph).** The directed bipartite graph (DB graph) was developed by Ivanova [46, 47, 79], and more rigorously analysed by Mincheva and Roussel in [55, 56]. It has been applied to some real mechanisms by Ermakov, Goldstein et al. [23, 34, 36, 37], and analysed for small networks with up to four species [22, 24, 35]. The directed bipartite graph G_{bip} has the same vertex and edge set as constructed for the SR graph, except that outflow and inflow

reactions are included in the reaction vertex set (V_2). Moreover, as the name indicates, all edges become directed. An edge is always directed away from a species vertex if it has a reaction complex label, while it is directed toward a species vertex if it has a label of a product complex . Some other nomenclature is different. A c-pair is called a negative path, and cycles are labeled positive or negative, whereas for the SR graph they were called e-cycles and o-cycles, respectively. The directed bipartite graph of the MAPK would be identical to the SR graph in Figure 6, except the edges would be directed in the way described above. In general, cycles are defined differently. A path along the directed bipartite graph is a union of two edges $(A_{i_1}, y_{i_1} \to y'_{i_1})$ and $(y_{i_1} \to y'_{i_1}, A_{i_2})$ that share a common reaction vertex. We denote the path from A_{i_1} to A_{i_2} by $(A_{i_1}, y_{i_1} \to y'_{i_1}, A_{i_2})$. A cycle C in the directed bipartite graph G_{bip} is a union of distinct paths $(A_{i_1}, y_{i_1} \to y'_{i_1}, A_{i_2})$, $(A_{i_2}, y_{i_2} \to y'_{i_2}, A_{i_3})$ etc. until $(A_{i_k}, y_{i_k} \to y'_{i_k}, A_{i_1})$. A c-pair is considered to be a cycle, because a path from one species vertex A_1 to another vertex A_2 is considered to be different from a path from A_2 to A_1, and the two paths together form a cycle. For example, in Figure 6, two c-pair edges adjacent to E, S_0 and the reaction vertex $E + ES_0 \leftrightharpoons ES_0$ would form a cycle. In the SR graph, a cycle has an equal number of species and reaction vertices, while in the directed bipartite graph a cycle might have less reaction than species vertices. Every cycle in the interaction graph corresponds to one or more cycles in the directed bipartite graph. This occurs because each edge in the interaction graph represents an interaction between two species, say S_i and S_j, so namely, $J_{ji} \neq 0$. This Jacobian entry can be decomposed by the type of reaction, which implies that this entry is represented by several paths within the directed bipartite graph, each having a different reaction vertex.

The main advantage of this graphical interpretation is the relation between each coefficient of the expansion of the determinant of $-J$ (or a minor). Each coefficient is defined in terms of a particular subgraph of the DB graph. A subgraph g of G_{bip} is a union of edges and cycles which are disjoint. The order of a subgraph is defined to be the number of V_1 vertices contained in the subgraph. The set of all subgraphs g of order k that share the same set of vertices is called a fragment of order k, and denoted by $S_k\binom{i_1,\ldots,i_k}{j_1,\ldots,j_k}$, where $i_1,\ldots,i_k$ are the indices of the species, and $j_1,\ldots,j_k$ are index subset of the reactions in the vertex sets. Note again that the DB graph G_{bip} includes information about the inflows and outflows, and hence possibly contains more information than the SR graph. With these structures the graph-theoretic formula for the coefficients a_k of the characteristic polynomial $(\lambda I - J)$ and the determinant of $-J$ (which is a_n) can be derived in terms of fragments. Using the notation in [55], we let g be a subgraph of G_{bip} of order n, and $\{g\}$ denote the set of all such graphs. Then

$$det(-J) = \sum_{g \in G_{bip}} K_g \frac{v_{j_1} \ldots v_{j_k}}{x_{i_1} \ldots x_{i_k}}, \tag{6}$$

where each coefficient K_g is a product of stoichiometric labels over all edges and all t_g cycles C of g:

$$K_g = (-1)^{t_g} \prod_{[A_k, B_j] \in g} \alpha_{jk}^2 \prod_{C \in g} K_C. \tag{7}$$

Here K_C is a product of all stoichiometric labels over all paths (negative/positive) of each cycle $C \in g$:

$$K_C = \prod_{\overline{[A_k,B_j,A_i]} \in C} (-\alpha_{jk}\alpha_{ji}) \prod_{[A_k,B_j,A_i] \in C} \alpha_{jk}\beta_{ji}.$$

For all other coefficients $1 \leq k < n$, we have

$$a_k = \sum_{S_k \binom{i_1,\ldots,i_k}{j_1,\ldots,j_k}} K_{S_k} \frac{v_{j_1} \ldots v_{j_k}}{x_{i_1} \ldots x_{i_k}}, \tag{8}$$

where the contribution of fragment S_k is

$$K_{S_k} = \sum_{g \in S_k \binom{i_1,\ldots,i_k}{j_1,\ldots,j_k}} K_g.$$

The derivation of both formulas is stated in [55]. A fragment S_k with $K_{s_k} < 0$ is called a critical fragment. It contains at least one subgraph with an odd number of positive cycles, namely $K_g < 0$. In fact, from above formula, each subgraph with a disjoint union of cycles and edges must contain the same number of species and reaction vertices. Hence, the cycles formed with repeating reaction vertices do not appear in the calculation of the coefficients inside the determinant of the Jacobian and so not in any of the coefficients occurring in the characteristic polynomial. Despite the definition of the cycles, these notions are not used in the work of Mincheva and Roussel [55], and instead all attention is given to the critical fragments.

The critical fragment is a useful concept for the manipulation of the characteristic polynomial, $det(\lambda I - J)$. Since critical fragments are negative coefficients of the expansion of the determinant of $-J$ or the minors, the necessary conditions for any sort of instability reduce to a search for critical fragments [55, 56].

The DB graph has some advantages and disadvantages over the SR graph. Often the information contained in the DB graph is not needed. If we like to derive a necessary condition for the existence of a spatial instability (such as a Turing instability), it is enough to show that there exists a coefficient in the expansion of the principal minors of J that is negative. So critical fragments are not needed. This implies that it is computationally easier to use the SR graph, since it doesn't identify the coefficients explicitly, but simply gives graphical conditions for when a coefficient will be negative. Using the SR graph there are also graph-theoretic conditions allowing to derive necessary conditions for a Turing instability to occur in non-mass action kinetic systems, see [20]. Moreover, it should be noted that the search for a critical fragment in the DB graph can lead to large computations. As an example, let us consider a network with n species and m reactions. Mincheva and Roussel in [56] state that the existence of a critical fragment of order $k \leq rank(N^T)$ is a necessary condition for a Turing instability. For this network there can exist up to $\frac{n!}{(n-k)!k!}m^k$ fragments of order k. To determine whether a fragment is critical, all corresponding subgraphs have to be identified. The number of such subgraphs might also be large: there might be up to $k!$ subgraphs just composed only of edges and no cycles. This search for the critical fragments becomes increasingly

computationally expensive with the growing size of the network. However, there are indeed cases where more specific information about the coefficients via the critical fragments obtained from the DB graph is essential. For example when considering delay-induced instability, as described in [57].

8. **Conclusion.** Graph theoretic approaches applied to chemical reaction networks have attracted considerable attention in the past few decades. Each graph presented encodes different information about the system, and there is therefore a multitude of graphs which can be associated to dynamical systems defined on a network structure. Particular graphs have been developed with specific system properties in mind. For example the SR graph was developed for dealing with open systems where all species have inflows and outflows. The interaction graph, the SCL graph, the knot graph, the SR graph, and the DB graph use information encoded in the Jacobian matrix, which can then in turn be used to formulate results about the dynamics of the associated ODEs. But each graph uses very different information from the Jacobian. For example, in interaction graphs, there is no information which reactions are establishing the relation that one species can influence another. In contrast such information is available in the SR and the DB graph. The structure of the Jacobian is also influenced by the choice made in modeling, for example, the type of reactions allowed (whether the systems are open, closed, or some variant between these two extremes, whether the system has reversible reactions or not, etc.), and the kinetics (whether they are of mass-action type or not, of Michaelis-Menten type, etc.). The more specific information that exists about the network topology and the type of non-linearities defining the dynamics, the weaker are the conditions that can be applied to gather the relevant information. All the aforementioned graphs have been used in somewhat different settings, and hence the results they give are different too. For example, for reaction networks associated to a general ODE system (not necessarily polynomial) to be injective, one of the conditions is that the Jacobian matrix needs to be a weak P-matrix. For open mass-action CRNs to check injectivity, the condition is weaker: the Jacobian must have a non-vanishing determinant. Possible extension of the graphical methods would be useful so that the results based on different graphs could be better compared. We have attempted to compare some of the graphs within this paper, namely the SCL graph with the deficiency theory and the interaction graph, then the DB graph and the SR graph with the interaction graph. But we have not yet investigated or established mathematically rigorous relations between these graphs. Such a theory could help to unify some of the underlying ideas, making advances in dynamical network theory.

The historical development of the different graphs was also outlined. For the interaction graph theory, most of Thomas' conjectures have only been partially proven and hence they still remain open. The SR graph theory is useful, but it is applicable to open systems only. However, these systems may not be appropriate for modelling most biological systems. For example, if the reaction tank is treated as a cell, and some species inside the cell are enzymes, it is realistic to assume that because of the shape and sizes of their molecules, not all species can freely diffuse into and out of the cell. Hence, the cell might be better modeled by an open system where not all species have inflow and outflow. Craciun and Feinberg in [16] supplied results relating the dynamics of completely open systems with the dynamics of entrapped-species models (where some species do not have continuous inflow and

outflow). A graphical interpretation of their conditions would be useful.

A look back at the proofs would also be advantageous in identifying some further developments. The usage of contemporary mathematical theory could give an insight into chemical reaction networks dynamics. For example, Gatermann and Huber in [32] presented an algebraic geometry based version of the deficiency theorems from Horn, Feinberg and Jackson [43, 25]. In the paper of Schlosser and Feinberg [65], no mathematical proofs for the theorems on SCL graphs are supplied. Reconstructing these proofs and verifying how close they are to the results stemming from the SR graph would also be interesting. Establishing a theory whether the SR or the nonstandard SCL graph give stronger results for networks with no multiple positive equilibria is also of interest.

Most of the dynamics considered by graph theoretic approaches are related to multiple steady states. However, they do not lead to many results about other types of dynamics. Chemical reaction networks are well known to exhibit all kinds of qualitative behaviour, such as stable oscillations [67, 29, 30, 1]. Such properties are linked to numerous functions in biochemical systems [33] and hence the investigation of such phenomena is also important. For some of the graphs mentioned, there are conditions for oscillations, but such results are fewer and usually weaker. Part of the problem is that even necessary conditions for a Hopf bifurcation (a bifurcation where by a change of parameters periodic solutions are created from the change in dynamics around a steady state) can be difficult to verify. One of the necessary conditions for a Hopf bifurcation is that the Jacobian has a pair of eigenvalues that cross the imaginary axis with this change of parameter values. To check the necessary condition that a Jacobian has a unique pair of pure imaginary eigenvalues can be related to the Hurwitz criteria. Unfortunately, these are relatively cumbersome to compute. A clear relation between the graph structure and the Hurwitz matrices would need to be established, which, at the moment, does not seem straightforward. Golubitsky and Stewart [38] apply interaction graphs to networks with symmetry conditions to study synchronous behaviour, namely oscillations. The application of such theory might be useful for chemical reaction networks, but unfortunately relies on having classes of identical types of dynamics on each of the vertices.

We have not discussed 'modularity' of reaction networks. Each of the graphs discussed, or combination of graphs, can be used to derive how the 'combination of networks' is affecting the qualitative properties of the system. The usual assumption if that the overall network has to be in equilibrium. A concept already introduced by Clarke ([12], [10], [11]) are the 'extremal currents' (often called 'elementary flux modes' and widely cited in the Systems Biology literature). They are a systematic way of spanning all possible equilibria of the overall system in terms of convex coordinates (associated to sub-networks which themselves can be in equilibrium). The sub-networks that can be expressed in terms of the reaction graph in combination with the undirected bipartite graph. Such approaches are very helpful for bifurcation theory, see the work of Gatermann, cited in [32], and a more recent contribution [19].

As we mentioned earlier, graph theoretic approaches used in chemical reaction theory have also been proposed for the analysis of biochemical networks [13, 6]. However as mentioned before, in these biological settings, one very rarely can use simple mass-action kinetics alone. One argument is that there exists stochastic noise induced by the low (and finite) number of macro-molecules and whole molecular machines in the cell. This is discussed in the 'microscopic' counterpart to this paper, [64]. Moreover there is a link between the genetic and the metabolic cellular networks. For example it is possible that species and reactions are altered through genetic modifications [6]. Modeling such situations requires development of graphical tools for a wider range of relevant stochastic and deterministic dynamics.

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