On the multistationarity of chemical reaction networks

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This paper is a contribution to the theory of multistationarity of (bio)chemical networks. The mathematical theory of *injectivity* of chemical reaction networks is already well developed, thanks to the pioneering work of Feinberg [5, 6], and contributions by Craciun [2], Balaji [1], Mincheva-Roussel [10], Wiuf and Feliu [16], and others.

A *chemical reaction network* is a finite set of reactions between complexes made of *chemical species.* These reactions depend upon a *stoichiometric matrix*, and to each of them is attached a *rate function* which controls the *kinetics* of the *concentration* value of the different species. For instance, in the classical case of mass-action kinetics, the rate functions are monomials in the concentration values.

One is interested in deciding whether such a system allows for multiple positive steady states, a phenomenon also known as *multistationarity.* More precisely, from the stoichiometry and the rate functions one can define a *species formation rate function* F having n variables and n values, where n is the number of species. The dynamic of the system consists of the finite set of differential equations

$$(A) \qquad \frac{dc(t)}{dt} = F(c(t))\,,$$

where $c(t)$ is a vector of concentrations varying with time. We shall use a theorem of Wiuf and Feliu on the injectivity properties of (A) when the rate functions are *strictly monotonic* ([16], Th. 10.2), a condition fulfilled not only by mass-action kinetics, but also by Michaelis-Menten and Hill kinetics, and by others.

Our work stems from results in the biological context, namely *genetic networks* theory. This theory models the interactions (activation and repression) between genes in a given cell: if $c(t)$ denotes the vector of concentrations of proteins in this cell at time t, one assumes that $c(t)$ obeys an equation like (A). Again, one problem is to give necessary conditions for (A) to allow for multistationarity. A well known conjecture of Thomas [15] (1981), states that such a necessary condition is the fact that the *interaction graph* of the jacobian of F contains a positive circuit. Mathematical proofs of this fact were found by Plahte et al. [11], Snoussi [12], Gouzé [7], Cinquin and Demongeot [3], and Soulé [14].

In [9], another result on gene networks was proved. It says that multistationarity of (A) requires the existence of *nuclei* of given signs in the interaction graph of F, where a nucleus is a disjoint union of circuits which contains all vertices.

A major breakthrough was the paper [13] by Soliman, which proves a result "à la Thomas" for chemical reaction networks, and uses in a non-trivial way the stoichiometric matrix. In fact, as Soliman explained to us, one can recover the system (A) for any function F as the dynamic of *special* chemical reaction network (with ad hoc stoichiometry, see 1.2 below), so that the original Thomas' rule becomes a special case of [13].

In this paper we are looking for a common generalization of [9] and [13]. We state this as a conjecture about chemical reaction networks (Conjecture 1), and we prove this conjecture when the rate functions are strictly monotic (Theorem 3).

Note that an important invariant of a system like (A) is the determinant of the jacobian matrix of F. But, if the system (A) obeys *conservation laws*, this determinant is identically trivial. In [16], Wiuf and Feliu replace this invariant by the determinant of another matrix, and they give a formula for it as the sum of principal minors of the jacobian (Section 1, Prop. 1). By solving the conservation laws we derive another formula (Prop. 2).

In Section 2, following [13], we introduce the *reaction labelled influence graph* of a chemical reaction network, and we use it to formulate the main result of [13], with three important corollaries. Next, we state the main result of [9] (Theorem 2). In Section 3 we present our conjecture (Conjecture 1) and, after defining strictly monotonic kinetics, we state our main theorem (Theorem 3).

Section 4 is devoted to concrete examples which illustrate several aspects of the theory.

After that, Section 5 (resp. Section 6) gives a proof of Proposition 2 (resp. Theorem 3). Finally, in Section 7, we present some conclusions and remarks.

1 Conservation laws

1.1 Definitions

Let $\mathbb{R}_+$ be the set of positive real numbers, and $\mathbb{N}$ the set of natural integers. Let $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ be a *chemical network*. As explained in [16], Def. 3.2, $\mathcal{N}$ consists in three finite sets:

- A finite set of *species* $\mathcal{S} = \{S_1, \ldots, S_n)$;
- A set of *complexes* $\mathcal{C} \subset \mathbb{N}^n$;
- A set of *reactions* $\mathcal{R} = \{r_1, \ldots, r_m) \subset \mathcal{C} \times \mathcal{C}$.

It is assumed that if $y \in \mathcal{C}$, then $(y, y) \notin \mathcal{R}$ and there exists $y' \in \mathcal{C}$ such that $(y', y) \in \mathcal{R}$ or $(y, y') \in \mathcal{R}$. We write

$$r : y \longrightarrow y'$$

to mean that $r = (y, y') \in \mathcal{R}$.

We consider *differential kinetics* $K = (K_j)$ for the network $\mathcal{N}$ i.e. (see [16] Def. 3.3) to each reaction $r_j : y_j \to y'_j$ in $\mathcal{R}$ we attach a *rate function*

$$K_j : \mathbb{R}^n_+ \longrightarrow \mathbb{R}$$

which is differentiable (in loc.cit. K_j is supposed to be nonnegative, but we shall not need this hypothesis). Let A be the *stoichiometric matrix*, i.e. the $n \times m$ matrix with j-th column $y'_j - y_j$, where (y_j, y'_j) lies in $\mathcal{R}$. The *species formation rate function* is the map

$$F : \mathbb{R}^n_+ \longrightarrow \mathbb{R}^n$$

defined by the formula

$$F(c) = \sum_{j=1}^{m} K_j(c)(y'_j - y_j)\,. \tag{1}$$

If $c(t) \in \mathbb{R}^n_+$ is a differentiable function of $t \in \mathbb{R}_+$, and $\dot{c}(t)$ its derivative, the dynamic of $\mathcal{N}$ consists of the finite set of n ordinary differential equations

$$\dot{c} = F(c)\,. \tag{2}$$

1.2 A special case

Let

$$F : \mathbb{R}^n_+ \longrightarrow \mathbb{R}^n$$

be any differential map. One can define as follows a chemical network such that F is the corresponding species formation rate function. One takes $m = n$, and one defines y_j (resp. y'_j) to be zero (resp. the complex y'_{ij} such that $y'_{jj} = 1$, and $y'_{ij} = 0$ when $i \neq j$). The rate function K_j is defined to be the j-th component F_j of the function F. The equation (1) is then clearly satisfied.

1.3 A formula of Wiuf and Feliu

We equip $\mathbb{R}^n$ with the standard scalar product. We denote by $\mathrm{Im}(A)$ the image of A and by $\mathrm{Im}(A)^\perp$ the orthogonal complement of $\mathrm{Im}(A)$. Assume $\mathrm{Im}(A)^\perp$ has dimension d. Consider a *reduced basis* of $\mathrm{Im}(A)^\perp$ i.e. a basis $(\omega^1, \ldots, \omega^d)$ such that $\omega^i_i = 1$ if $1 \le i \le d$, and $\omega^i_j = 0$ when $j = 1, \ldots, \widehat{i}, \ldots, d$ (cf. [16], Def. 5.1). The formula

$$\widetilde{F}(c) = (\omega^1 \cdot c, \ldots, \omega^d \cdot c, F_{d+1}(c), \ldots, F_n(c))$$

defines a new map

$$\widetilde{F} : \mathbb{R}^n_+ \longrightarrow \mathbb{R}^n \,.$$

When $c \in \mathbb{R}^n_+$ we let $J_c(F)$ (resp. $J_c(\widetilde{F})$) be the jacobian matrix of F (resp. $\widetilde{F}$) at c.

If $I \subset \{1, \ldots, n\}$ we let $J_c(F)_{I,I}$ be the submatrix of $J_c(F)$ with entries having indices in $I \times I$. The following is proved in [16], Prop. 5.3:

Proposition 1:

$$\det(J_c(\widetilde{F})) = \sum_{\substack{I \subset \{1, \ldots, n\} \\ \# I = n-d}} \det(J_c(F)_{I,I}) \,. \tag{3}$$

We simplify the notation by defining

$$d(c, K) = \det(J_c(\widetilde{F})) \,.$$

1.4 Another formula

Let $k_1, \ldots, k_d$ be d real positive constants. The *conservation laws*

$$\omega^i \cdot c = k_i \,, \qquad 1 \le i \le d \,, \tag{4}$$

reduce by d the number of variables. Namely, by solving (3), if $1 \le i \le d$, we get

$$c_i = k_i + u_i(c_{d+1}, \ldots, c_n) \,,$$

where $u_i(c_{d+1}, \ldots, c_n)$ is a linear combination of the variables $c_{d+1}, \ldots, c_n$. When $d + 1 \le i \le n$ we define

$$\varphi_i(c_{d+1}, \ldots, c_n) = F_i(k_1 + u_1(c_{d+1}, \ldots, c_n), \ldots, k_d + u_d(c_{d+1}, \ldots, c_n), c_{d+1}, \ldots, c_n) \,.$$

to be the function obtained from $F_i(c)$ by replacing $c_1, \ldots, c_d$ by their expression. If $c = (c_{d+1}, \ldots, c_n)$ we let

$$\Phi(c) = \left(\frac{\partial \varphi_i}{\partial c_j}(c) \right)_{d+1 \le i,j \le n}$$

be the corresponding Jacobian matrix, and $\Phi'(c)$ the matrix obtained from $\Phi(c)$ by replacing k_i by $\omega^i \cdot c$, $i = 1, \ldots, d$.

Proposition 2:

$$d(c, K) = \det(\phi'(c)) \,.$$

2 Previous results

2.1 The reaction labelled influence graph

We keep the definitions of 1.1 above and we fix $c \in \mathbb{R}_+^n$. The *reaction labelled infuence graph* (RLIG) $G(c) = G(c, K)$ of K at c is defined as follows (cf. [13]). The set of vertices of $G(c)$ is the set $\mathcal{S}$ of species of $\mathcal{N}$. There is a positive (resp. a negative) edge $i \to i'$ in $G(c)$, labelled by the reaction $r_j : y_j \to y'_j$, when $\frac{\partial F_{i'}}{\partial c_i}(c)$ is positive (resp. negative) and i (resp. i') is a component of y'_j (resp. y_j). In the special case considered in §1.2, the RLIG coincides with the interaction graph attached to F.

A *circuit* in $G(c)$ is a sequence of distinct vertices $i_1, \ldots, i_k$ such that there is an edge from i_α to $i_{\alpha+1}$ if $1 \leq \alpha \leq k-1$, and from i_k to i_1. The *sign* of C is the product of the signs of its edges. Several circuits are said *disjoint* when they do not share any vertex. A *hooping* is a union of one or several disjoint circuits. If $s \geq 1$ is an integer, an *s-hooping* is a hooping containing s vertices. When $s = n$, an s-hooping is called a *nucleus*. The *sign of a hooping H* is $\varepsilon(H) = (-1)^{p+1}$, where p is the number of positive circuits contained in H (see [4]). We let $\mathcal{H}_s(c, K)$ be the set of s-hoopings in $G(c)$.

Given a hooping H, the *restriction* of $\mathcal{N}$ to H is the sub-network $\mathcal{N}_H$ of $\mathcal{N}$ where reactions r_j not appearing in H are omitted. Note that, given a vertex $i \in \mathcal{S}$, there is at most one edge issued from i in the RLIG $\mathcal{N}_H$. Therefore the stoichiometric matrix of $\mathcal{N}_H$ is a square matrix. Let Λ_H be its determinant. We introduce the following

Definition 1: A hooping H of $G(c)$ is called *admissible* when the stoichiometric matrix of $\mathcal{N}_H$ is invertible (i.e. $\Lambda_H \neq 0$) .

2.2 A result of Soliman

A zero $c \in \mathbb{R}_+^n$ of the function F is called *non-degenerate* when the jacobian matrix $\left(\frac{\partial F_{i'}}{\partial c_i}(c)\right)_{1 \leq i, i' \leq n}$ is invertible.

Theorem 1 [13]: Assume F has several non-degenerate zeroes. Then there exists $c \in \mathbb{R}_+^n$ such that $G(c)$ has a positive circuit contained in an admissible hooping.

2.3

Soliman derived three corollaries from Theorem 1:

Corollary 1 [13]: A necessary condition for the multistationarity of a biochemical system is that there exists a positive cycle in its RLIG using at most once each reaction.

Indeed, if a cycle uses more than once some reaction, it is not admissible.

Corollary 2 [13]: A necessary condition for the multistationarity of a biochemical system is that there exists a positive cycle in its RLIG not using both forward and backward directions of any reversible reaction.

Indeed, if a cycle uses both forward and backward directions of some reversible reaction, it is not admissible.

And, by a similar argument:

Corollary 3 [13]: A necessary condition for the multistationarity of a biochemical system is that there exists a positive cycle in its RLIG not using all species involved in a conservation law.

2.4 A result of Kaufman, Soulé and Thomas

In this section we assume that we are in the special case considered in §1.2. Consider the finite set $\mathcal{F}$ of real functions on $\mathbb{R}_+^n$ of the form $\pm\prod\limits_{i\in I}(\partial F_i/\partial x_{\tau(i)})$ where $I \subset \{1,\ldots,n\}$ is any subset and $\tau : I \to \{i,\ldots,n\}$ is an injective map. We consider the following condition:

> (C) Given two functions f and g in $\mathcal{F}$ such that f is not identically zero and g is strictly positive somewhere in $\mathbb{R}_+^n$, there exists $x \in \mathbb{R}_+^n$ such that $f(x) \neq 0$ and $g(x) > 0$.

Note that (C) is very often fulfilled.

We say that the interaction graph G has a *variable nucleus* when there exist two points c and d in $\mathbb{R}_+^n$ and a nucleus of opposite signs in $G(c)$ and $G(d)$ (see [9] 3.1 for a more precise definition).

Theorem 2 [9]: Let F be such that (C) is satisfied. If F has several non-degenerate zeroes, then:

i) either there exists $c \in \mathbb{R}_+^n$ such that $G(c)$ has two nuclei of opposite signs;

ii) or G has a variable nucleus.

3 The main result

3.1 A conjecture

Assume $m \geq 1$ is any positive integer. In view of Theorems 1 and 2 it seems reasonable to make the following conjecture:

Conjecture 1: Assume that F has several non-degenerate zeroes and that (C) is satisfied. If F has d conservation laws, and $s = n - d$,

i) either there exists $c \in \mathbb{R}_+^n$ such that $G(c)$ has two admissible s-hoopings of opposite signs;

ii) or G has a variable admissible s-hooping.

3.2 Monotonic kinetics

We introduce more definitions from [16].

Let $Z = (Z_{ji})$ be a matrix of type $m \times n$ such that each entry Z_{ji} is equal to $+1, -1$ or 0. We let

$$Z_j^+ = \{i \, \text{such that} \, Z_{ji} = +1\} \,,$$

$$Z_j^- = \{i \, \text{such that} \, Z_{ji} = -1\} \,,$$

and

$$Z_j^0 = \{i \, \text{such that} \, Z_{ji} = 0\} \,.$$

If $c \in \mathbb{R}_+^n$ we let $\mathrm{Supp}(c)$ be the set of i such that $c_i \neq 0$. We assume that $K_j(c) > 0$ if and only if $i \in Z_j^+$ implies $i \in \mathrm{Supp}(c)$.

We say that K is *strictly monotonic* with respect to Z ([**?**], Def. 9.3) if, for all $j \in \{1, \ldots, m\}$, the restriction of K_j to the set of $c \in \mathbb{R}_+^n$ such that $K_j(c) \neq 0$ is

i) strictly increasing in c_i when $i \in Z_j^+$,

ii) strictly decreasing in c_i when $i \in Z_j^-$,

iii) constant in c_i when $i \in Z_j^0$.

We denote by $K(Z)$ the set of kinetics K which are strictly monotonic with respect to Z.

Let A be the stoichiometric matrix. Given a and b in $\mathbb{R}_+^n$, such that, for all $\omega \in \mathrm{Im}(A)^+$, $\omega \cdot a = \omega \cdot b$, we say that a and b are *not overlapping* when, for every j, if Z_j^+ is not contained in $\mathrm{Supp}(a)$ then Z_j^+ is contained in $\mathrm{Supp}(b)$. The matrix A is called *not Z-injective* over $K(Z)$ when there exist $K \in K(Z)$, $a \in \mathbb{R}_+^n$ and $b \in \mathbb{R}_+^n$ such that a and b are not overlapping and $F(a) = F(b)$.

The network $\mathcal{N}$ is called *non-degenerate* when there exists $c \in \mathbb{R}_+^n$ and $K \in K(Z)$ such that $d(c, K) \neq 0$.

The following result relates the fact that F is not injective to the existence of two admissible s-hoopings of opposite signs in the RLIG. It proves Conjecture 1 when K is strictly monotonic.

Theorem 3

Assume that $\mathcal{N}$ is non-degenerate and that A is not Z-injective over $K(Z)$. Let $s = n - d$ be the rank of A. Then, for every $c \in \mathbb{R}_+^n$ and $K \in K(Z)$, the RLIG $G(c)$ contains two admissible s-hoopings of opposite signs.

4 Examples

5 Proof of Proposition 2

To prove Proposition 2 we first notice that, since $(\omega^1, \ldots, \omega^d)$ is reduced, when $k = 1, \ldots, d$, we have

$$u_k(c_{d+1}, \ldots, c_n) = -\sum_{j=d+1}^{n} \omega_j^k c_j \, .$$

Therefore, if $j = d+1, \ldots, n$ and $i = d+1, \ldots, n$, we have

$$\frac{\partial \varphi_i}{\partial c_j}(c) = -\sum_{k=1}^{d} \omega_i^k \frac{\partial F_i}{\partial c_k}(c) + \frac{\partial F_i}{\partial c_j}(c) \, . \tag{5}$$

Consider the n by n matrix $J_c(\tilde{F})$. For every $k = 1, \ldots, d$, substract from the j-th of this matrix, $j = d+1, \ldots, n$, the product of the k-th column by ω_j^k. The determinant does not change by this operation, and the matrix M obtained this way, is such that the first d lines of M are equal to δ_{ij}, $i = 1, \ldots, d$, $j = 1, \ldots, n$.

From (5) we deduce that

$$\det(M) = \det\left(\frac{\partial \varphi_i}{\partial c_j}(c)\right)_{d+1 \leq i, j \leq n} .$$

Since $\det(M) = \det(J_c(\widetilde{F}))$ Proposition 2 follows.

6 Proof of Theorem 3

6.1

According to [16], Theorem 10.2, and the line after it, since A is not injective over K, i) in [16], loc. cit., does not hold, i.e. there exist $c_1 \in \mathbb{R}_+^n$ and $K_1 \in K(Z)$ such that $d(c_1, K^1) = 0$.

On the other hand, since $\mathcal{N}$ is non degenerate, there exists $c_2 \in \mathbb{R}_+^n$ and $K^2 \in K_d(Z)$ such that $d(c_2, K^2) \neq 0$, say $d(c_2, K^2) > 0$. By (3) this implies that there exists $I \subset \{1, \ldots, n\}$ with $\# I = s$ such that $\det(J_{c_2}(F^2)_{I,I}) > 0$, where F^2 is the species formation rate function defined from K^2. Let $\mathcal{H}_2 \subset \mathcal{H}_s(c_2, K^2)$ (resp. $\mathcal{H}_1 \subset \mathcal{H}_s(c_1, K^1)$) be the collection of hoopings in $G(c_2, K^2)$ (resp. $G(c_1, K^1)$) having I as set of vertices. We also write $\mathcal{H} = \mathcal{H}_s(c, K)$.

6.2

We shall need some notions and results from [8] and [13]. If $s_i \in \mathcal{S}$, $r_j \in \mathcal{R}$, $c \in \mathbb{R}_+^n$ and $K \in K_d(Z)$ we let

$$\lambda(s_i, r_j) = \frac{\partial K_j}{\partial c_i}(c)$$

and

$$\lambda(r_j, s_i) = y'_{ij} - y_{ij} \, .$$

For a path P in $G(c, K)$ we let $\lambda_{SR}(P)$ (resp. $\lambda_{RS}(P)$) be the product of all the numbers $\lambda(s_i, r_j)$ (resp. $\lambda(r_j, s_i)$) where $S_i \xrightarrow{R_j}$ (resp. $\xrightarrow{R_j} S_i$) is an edge in P. We define also

$$\lambda(P) = \lambda_{RS}(P)\, \lambda_{SR}(P) \, .$$

If $H, H' \in \mathcal{H} = \mathcal{H}_s(c, K)$ we write that $H \sim H'$ when H and H' have the same species to reactions arcs $S_i \xrightarrow{R_j}$. Note that $\lambda_{SR}(H)$ and $\Lambda(H)$ depend only on the equivalence class $[H] \in \mathcal{H}/\sim$. According to [8] Thm. 6.7 (see also [13], Thm. 2), we have

$$\det(J_c(F)) = \sum_{[H] \in \mathcal{H}/\sim} \Lambda(H)\, \lambda_{SR}(H) \, , \tag{6}$$

and

$$\Lambda(H) = \sum_{H' \in [H]} \sigma(H')\, \lambda_{RS}(H') \, , \tag{7}$$

where $\sigma(H') = \pm 1$. As a consequence ([8] Lemma 6.2 and [13], (1)) we get

$$\det(J_c(F)) = \sum_{H \in \mathcal{H}} \sigma(H)\, \lambda(H) \, . \tag{8}$$

6.3

Let us come back to the situation of §6.1. Let $J = J_{c_2}(F^2)_{I,I}$. Since $\det(J) > 0$, it follows from (6), (7) and (8) that there exists $H_2 \in \mathcal{H}_2$ such that $\Lambda(H_2) \neq 0$ and $\sigma(H_2)\, \lambda(H_2) > 0$.

We claim that there exists also $H_3 \in \mathcal{H}_2$ such that $\Lambda(H_3) \neq 0$ and $\sigma(H_3)\, \lambda(H_3) < 0$. Suppose, by contradiction, that, for any $H \in \mathcal{H}_2$ such that $\Lambda(H) \neq 0$, we have $\sigma(H)\, \lambda(H) \geq 0$. Since the kinetics are strictly monotonic, for any $H \in \mathcal{H}_s(c, K)$ the sign of $\sigma(H)\, \lambda(H)$ does not depend on c and K, but only on the arcs contained in H. Therefore, for any s-hooping H' in $\mathcal{H}_1$ such that $\Lambda(H') \neq 0$, one has $\sigma(H')\, \lambda(H') \geq 0$, and there exists $H_1 \in \mathcal{H}_1$ such that $\sigma(H_1)\, \lambda(H_1) > 0$. By (8) this implies that $\det(J_{c_1}(F^1)_{I,I}) > 0$. But $d(c_1, K_1) = 0$, so we conclude by (3) that there exists I' of cardinality s such that $\det(J_{c_1}(F^1)_{I',I'}) < 0$. Therefore, when $H \in \mathcal{H}$ and $\Lambda(H) \neq 0$, the number $\sigma(H)\, \lambda(H)$ takes both positive and negative values.

To conclude the proof of Theorem 3, it remains to note that the sign $\varepsilon(H)$ is $(-1)^{n+1}$ times the sign of $\sigma(H)\, \lambda(H)$ (cf. [4], Appendix, Lemma 2). Therefore $G(c)$ contains two admissible s-hoopings of opposite signs.

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