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Acta Biotheoretica manuscript No.
(will be inserted by the editor)

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Received: date / Accepted: date

Abstract We consider metabolic networks with reversible enzymatic reactions. The model is written as a system of ordinary differential equations, possibly with inputs and outputs. We prove the global stability of the equilibrium (if it exists), using techniques of monotone systems and compartmental matrices. We show that the equilibrium does not always exist. Finally, we consider a metabolic system coupled with a genetic network, and we study the dependence of the metabolic equilibrium (if it exists) with respect to concentrations of enzymes. We give some conclusions concerning the dynamical behavior of coupled genetic/metabolic systems.

Keywords Metabolic networks, enzyme kinetics, monotone systems, compartmental systems, genetic networks, global stability.

1 Introduction

In the field of biology, metabolic systems are an important class of dynamical systems [13]. They are similar to chemical systems, but the reactions are catalyzed by enzymes. These enzymes are proteins synthesized by genes, and metabolic and genetic systems are coupled by control loops (metabolites can regulate the synthesis of an enzyme). From a biological point of view, the study of this coupled system is of first importance [25]. Its dynamical behavior can be complex and it should be studied with mathematical models [24]. These models themselves are often large and complex, and tools for reduction are necessary, as shown by some cases studies [3, 12].

One of the classical tools [16] is based on the difference between the time scales of the two subsystems. The metabolic system has a very fast dynamics compared to the genetic one. We can study the properties of stability of this metabolic system. If it is globally stable, then we can put it to its quasi-steady state (equilibrium of the fast system), and apply theorems of Tikhonov type for systems with multiple time scales

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[13], to inject the value of this quasi-steady state in the genetic slow system. The total system is then reduced to its genetic part plus some algebraic equations (see [1] for a recent application).

Of course, if there are several or no steady-states, this method cannot be applied. Moreover, the mathematical hypotheses required to apply the reduction theorem demand stability of the fast metabolic system [15]. For biologists, it seems clear that the "realistic" metabolic systems have a single stable steady-state. However, it is known that some metabolic systems can have multiple equilibria [6], or no equilibrium.

In this paper, we offer some contributions to this problem. We show that for a "pure" reversible enzyme system (all reactions are reversible enzymatic reactions), then, depending on the input, there is either no steady-state, either a single globally asymptotically stable steady-state. The mathematical tools we use are known but not so classical: they belong to the theory of monotone systems, and of compartmental systems. Our contribution lies in the mathematical global study of stability of reversible metabolic systems, with inputs and outputs.

There exist other studies of this problem, in other contexts [18], but, to our knowledge, none of them with our tools. For a work using monotone systems for chemical chains, see [9]. For a work on a similar problems of metabolic chains, with a linear approach, see [11]. We believe that this kind of tools (monotony, positive matrices) are well adapted to biological problems, as remarked by other studies [23]. These tools have strong links with the theory of stability with diagonal dominant matrices (see [21]). In spite of the complicated form of kinetics rates, we are able to study the system in a simple and global way.

The mathematical notions and theorems used in this paper are recalled in the appendix. In the first section, we describe our system, then we study three important particular structures of metabolic systems. In the last section, we make the link with genetic systems, and show how the equilibrium of the genetic part depends on concentrations of enzymes. We conclude by the study of the coupled system.

Notations: First we give some classical notations (see [19]). We are going to study the autonomous n-dimensional differential system

$$\dot{x} = f(x) \tag{1}$$

where function f is supposed to be continuously differentiable within some domain of interest, that will be in our case $X = \mathbb{R}^n_+$. We deduce the existence and uniqueness of solutions on some time interval for the differential equation (1). We define the flow $\Phi(t)$ as the set of solutions of (1) parametrized by the time t. The notation $\Phi(t, x_1)$ corresponds to the solution starting from the initial condition x_1 parametrized by time $t \geq 0$. Throughout the paper, we use the classical notions of Lyapunov stability. The term "global stability" will mean "global asymptotic stability within the domain X".

2 Reversible enzyme kinetics

In 1913, Michaelis and Menten studied the kinetics of a simple enzymatic reaction involving a single enzyme. Consider the reaction consisting of a substrate S , of an en-

zyme E_0 and a product P. Michaelis and Menten proposed the following description and equations (we refer to [16] and [10]). The enzyme forms a transitory complex E_1 before returning to its original form, giving product P from substrate S.

$$S + E_0 \rightleftarrows E_1 \rightarrow E_0 + P$$

Writing the kinetics according to the mass-action laws, and using conservations and quasi-steady state hypothesis, the following expression for the substrate velocity:
If $K_M = \frac{k_{-1}+k_2}{k_1}$, then

$$V_r = -\frac{ds}{dt} = \frac{k_2 E s(t)}{K_M + s(t)} \tag{2}$$

This equation is called the quasi-stationary equation of Michaelis-Menten: E is the total concentration of enzyme assumed to be constant ($E = E_0 + E_1$). K_2 is the maximum velocity that the reaction can reach when the substrate concentration s tends to $+\infty$ and K_M is the Michaelis constant.

However, in [5], we read that in principle all reactions catalyzed by enzymes are **reversible**, and that this fact could play a prominent role in biochemistry. It turns out that it is interesting to add a reversible reaction to the last step of model (2). The new model becomes:

$$S + E_0 \rightleftarrows E_1 \rightleftarrows P + E_0$$

The corresponding equations are:

$$\begin{cases} \dfrac{ds}{dt} = -k_1 s e_0 + k_{-1} e_1 \\ \dfrac{de_0}{dt} = -k_1 s e_0 + k_{-1} e_1 + k_2 e_1 - k_{-2} p e_0 \\ \dfrac{de_1}{dt} = k_1 s e_0 - k_{-1} e_1 - k_2 e_1 + k_{-2} p e_0 \\ \dfrac{dp}{dt} = k_2 e_1 - k_{-2} p e_0 \end{cases} \tag{3}$$

By a similar approach, it is possible to reduce this system with arguments of different time scales, using Tikhonov's theorem ([13]), and conservations. The following velocity is obtained:

$$V_r = -\frac{ds}{dt} = \frac{k_1 k_2 E s(t) - k_{-1} k_{-2} E p(t)}{k_{-1} + k_2 + k_1 s(t) + k_{-2} p(t)}$$

Denoting: $k_S = k_1 k_2$, $k_P = k_{-1} k_{-2}$, $K_{SP} = k_{-1} + k_2$, $k_S^{'} = k_1$ et $k_P^{'} = k_{-2}$, we obtain:

$$V_r = E \frac{k_S s(t) - k_P p(t)}{K_{SP} + k_S^{'} s(t) + k_P^{'} p(t)} \tag{4}$$

We will use this equation in the rest of this paper for reversible enzymatic chains, without addressing the problem of knowing if it is a good approximation or not of the underlying mechanism (3). This problem is that of the quality of the quasi-steady

state approximation and is based on assumptions concerning the order of magnitude of parameters (see [20] for a case study and a review).

In our case, we wish to study the stability property of a chain made of such reversible reactions. Recall that E is the total concentration of enzyme assumed to be constant (or slowly varying) for the time being. We also observe that the function (4) is rather complex, because the two variables s and p are both in the numerator and denominator.

Our goal is to study **the global stability of enzymatic reversible networks**, with the help of mathematical tools such as monotone and compartmental systems. To simplify the exposition, we will consider enzymatic chains, and not more complex networks with loops. This will allow us to easily write the equations and describe the calculations. We will say at the end of each section what could be generalized (or not) to a network. Some extensions are straightforward, others would require more work.

To describe the inputs and outputs for this enzymatic system, and to clarify the exposition, we chose to take one single input u (at most); therefore we consider the following enzymatic chain ; vector $x \in X = \mathbb{R}^n_+$ denotes the n variables (the concentrations of the n chemicals).

$$u \rightarrow x_1 \leftrightarrows x_2 \ldots x_i \leftrightarrows x_{i+1} \ldots \leftrightarrows x_n$$

This kind of chain topology is one of the most classical for metabolic networks [24]. We have classified the cases of interest into three generic forms (see also the classification of compartmental systems in [14]):

- the system is closed: i.e. there is no input nor output and degradation terms of the variables are neglected
- the system has one single input and degradation terms are taken into account
- the system has one single input and one single output at the end of the chain; the other terms of degradation are neglected

We will study in each case the global stability. The expression of the velocity of reaction between x_i and x_{i+1} will be:

$$R_i(x_i, x_{i+1}) = E_i \frac{k_{i,i+1}x_i - k_{i+1,i}x_{i+1}}{K_{i,i+1} + k'_{i,i+1}x_i + k'_{i+1,i}x_{i+1}} \tag{5}$$

3 Closed enzymatic network

The evolution of the concentrations vector x is described in a classical form by the following system (see [13])

$$\dot{x} = AR(x) \tag{6}$$

where matrix A is the stoichiometric matrix and vector R the reaction rate vector

$$R(x) = (R_1, R_2, ..., R_{n-1})^T$$

where the R_i are given by function (5). Matrix A has (n) rows and $(n-1)$ columns, and is given by $A = \begin{pmatrix} -1 & 0 & 0 & \dots & 0 \\ 1 & -1 & 0 & \dots & 0 \\ \vdots & \ddots & \ddots & \ddots & \vdots \\ 0 & \dots & 0 & 1 & -1 \\ 0 & \dots & 0 & 0 & 1 \end{pmatrix}$

We verify that A is a matrix such that $\mathbf{1}^T A = 0$, where $\mathbf{1}$ is the n-vector $(1,1,\dots,1)^T$. This gives the conservation of mass for this system, which is necessary for a closed system. First we check (as for the other cases) that the nonnegative orthant is invariant: if the initial conditions are non negative, the variables will remain non negative for all $t \geq 0$.

We recall in the appendix a theorem (Theorem 3) based on properties of compartmental Jacobian matrix and giving the global stability. We write the Jacobian matrix $J(x)$ with elements J_{ij} of system (6):

$$\begin{pmatrix} -\frac{\partial R_1}{\partial x_1} & -\frac{\partial R_1}{\partial x_2} & 0 & \dots & \dots \\ \frac{\partial R_1}{\partial x_1} & (\frac{\partial R_1}{\partial x_2} - \frac{\partial R_2}{\partial x_2}) & -\frac{\partial R_2}{\partial x_3} & 0 & \dots \\ \vdots & \ddots & \ddots & \ddots & \vdots \\ 0 & \dots & \frac{\partial R_{n-2}}{\partial x_{n-2}} & (\frac{\partial R_{n-2}}{\partial x_{n-1}} - \frac{\partial R_{n-1}}{\partial x_{n-1}}) & -\frac{\partial R_{n-1}}{\partial x_n} \\ 0 & \dots & \dots & \frac{\partial R_{n-1}}{\partial x_{n-1}} & \frac{\partial R_{n-1}}{\partial x_n} \end{pmatrix}$$

and we check that $J(x)$ is a compartmental matrix (see appendix Definition 2) because the elements on the main diagonal are

$$\{-\frac{\partial R_1}{\partial x_1};\ \frac{\partial R_i}{\partial x_{i+1}} - \frac{\partial R_{i+1}}{\partial x_{i+1}} \quad i = 1,...,n-2;\ \frac{\partial R_{n-1}}{\partial x_n}\}$$

on the lower diagonal:

$$\frac{\partial R_i}{\partial x_i} \qquad i = 1,...,n-1$$

on the upper diagonal:

$$-\frac{\partial R_i}{\partial x_{i+1}} \qquad i = 1,...,n-1$$

But the reaction rates $R(x)$ are such that $\frac{\partial R_i}{\partial x_i} \geq 0$ and $\frac{\partial R_i}{\partial x_{i+1}} \leq 0$, therefore the off-diagonal elements are positive. Moreover, for $j \in \{1,\dots,n\}$, $-J_{jj} = \sum_{i=1,\dots,n, i\neq j} J_{ij}$, so matrix J is compartmental.

System (6) is strongly connected (see appendix before theorem 2) because of the reversibility of the reactions. Therefore we can apply the theorem. Moreover, it is straightforward to check that this equilibrium is positive.

Proposition 1 *For a closed enzymatic chain, hyperplane* $H = \{x \in \mathbb{R}^n_+ : M(x) = \sum_{i=1}^n x_i = M_0 > 0\}$ *is invariant and contains a unique globally stable positive equilibrium in* H.

We remark that this property is wrong if the system is not reversible; in the classical Michaelis-Menten closed equation $S \to P$, substrate S tends toward zero.

Extension: If the graph is a network with loops and not a chain, all properties will be retained. The graph is strongly connected if it is connected (since all reactions are reversible).

Proposition 2 *For a closed enzymatic connected system, the hyperplane* $H = \{x \in R^n_+ : M(x) = \sum_{i=1}^n x_i = M_0 > 0\}$ *is invariant and contains a single globally stable positive equilibrium in* H.

4 Open enzymatic chain with degradation terms

In this network, all metabolites x_i are degraded with a non-zero rate γ_i. This term could also represent a dilution term due to growth of the cell. There is one input u on the first metabolite. The mathematical model of this network is given by:

$$\dot{x} = AR(x) + U - \gamma.x \tag{7}$$

A and R are the same as above, $\gamma.x$ is a vector with components $\gamma_i x_i$, U is the input vector $U = (u, 0, 0, \ldots, 0)^T$.

Now we check (S1), (S2) and (S3) of Theorem 2 (see appendix). The Jacobian matrix of system (7) is:

$$J_2(x) = \begin{pmatrix} -\frac{\partial R_1}{\partial x_1} - \gamma_1 & -\frac{\partial R_1}{\partial x_2} & 0 & \cdots & \cdots \\ \frac{\partial R_1}{\partial x_1} & (\frac{\partial R_1}{\partial x_2} - \frac{\partial R_2}{\partial x_2} - \gamma_2) & -\frac{\partial R_2}{\partial x_3} & 0 & \cdots \\ \vdots & \ddots & \ddots & \ddots & \vdots \\ 0 & \cdots & \frac{\partial R_{n-2}}{\partial x_{n-2}} & (\frac{\partial R_{n-2}}{\partial x_{n-1}} - \frac{\partial R_{n-1}}{\partial x_{n-1}} - \gamma_{n-1}) & -\frac{\partial R_{n-1}}{\partial x_n} \\ 0 & \cdots & \cdots & \frac{\partial R_{n-1}}{\partial x_{n-1}} & \frac{\partial R_{n-1}}{\partial x_n} - \gamma_n \end{pmatrix}$$

But we have, as above, $\frac{\partial R_i}{\partial x_i} \geq 0$ and $\frac{\partial R_i}{\partial x_{i+1}} \leq 0$, so condition $(S1)$ is satisfied.

Let

$$\sigma(x) = \sum_{i=1,\ldots,n} \dot{x}_i = u - \sum_{i=1,\ldots,n} \gamma_i x_i$$

$\forall i \in \{1, \ldots, n\}$, $\frac{\partial \sigma(x)}{\partial x_i} = -\gamma_i < 0$, then $(S2)$ is satisfied.
Let $S(x) = \sum_{i=1,\ldots,n} x_i$, then $\sigma(x) \leq u - mS(x)$ with $m = \min(\gamma_i)$. It is easy to choose $S(x) = k$ large enough to verify $(S3)$.

Proposition 3 *System (7) has a unique globally stable equilibrium in* $\mathbb{R}^n_+$.

Extension: If the graph is a network and not a chain, all properties will be the same to apply the theorem. Since there is only one input, we assume that the network is connected. Note that the theorem cannot apply if a degradation rate is zero, because (S2) is not verified.

Proposition 4 *The system (7) with a connected network has a unique globally stable equilibrium in* $\mathbb{R}^n_+$*, if all degradation rates are strictly positive.*

5 Open enzymatic chain without degradation terms and with one input and output

This case is the most difficult and interesting one, because it is similar to cases in the literature. There is one input on the first metabolite, and one output on the last. The diagram of the chain is as follows:

$$u \rightarrow x_1 \leftrightarrows x_2 \leftrightarrows \dots x_i \leftrightarrows x_{i+1} \dots \leftrightarrows x_n \rightarrow$$

The mathematical model is

$$\dot{x} = AR(x) + U - \Gamma(x) \tag{8}$$

A and R are the same as above, U is the input $U = (u,0,0,\dots,0)^T$ and $\Gamma(x)$ is the output vector $\Gamma(x) = (0,0,\dots,kx_n)^T$, k is a positive constant standing for the output (or degradation) of x_n.

The first point is that an equilibrium does not always exist. We are only interested by a nonnegative equilibrium. We also recall that the nonnegative orthant is positively invariant.

5.1 Existence of an equilibrium

Denote x^* the equilibrium of system (8) if it exists.

We solve $\dot{x}_i = 0 \;\; for \;\; i = 1,\dots,n$. Then $\sum_{i=1,\dots,n} \dot{x}_i = 0 \quad \Rightarrow \quad u = kx^*_n$.
Using $\sum_{j=1}^{n-1} \dot{x}_j = 0, \;\; i = 1,\dots,n-1$, we obtain :

$$u = R_{n-1}(x^*_{n-1}, x^*_n)$$

Then

$$x^*_{n-1} = \frac{E_{n-1}k_{n,n-1}x^*_n + u(K_{n-1,n} + k'_{n,n-1}x^*_n)}{E_{n-1}k_{n-1,n} - k'_{n-1,n}u}$$

if and only if $u \neq \frac{k_{n-1,n}}{k'_{n-1,n}E_{n-1}}$. Moreover x^*_{n-1} is nonnegative if and only if

$$u < \frac{k_{n-1,n}}{k'_{n-1,n}E_{n-1}}$$

Suppose that this condition is fulfilled, we proceed iteratively using the equations $\sum_{j=1}^{i} \dot{x}_j = 0, \;\; i = 1, ..., n-1$, to obtain :

$$u = R_i(x_i, x_{i+1})$$

and

$$x_i^* = \frac{E_i k_{i+1,i} x_{i+1}^* + u(K_{i,i+1} + k'_{i+1,i} x_{i+1}^*)}{E_i k_{i,i+1} - k'_{i,i+1} u}$$

if the constraints $u < \frac{k_{i,i+1}}{k'_{i,i+1}} E_i \;\; i = 1, \ldots, n$ are verified.

Proposition 5 *System (8) has a unique positive equilibrium if and only if*

$$u < \frac{k_{i,i+1}}{k'_{i,i+1}} E_i \;\; i = 1, \ldots, n \tag{9}$$

Otherwise, if there is an index i such that $u \geq \frac{k_{i,i+1}}{k_i, i+1'} E_i$*, system (8) admits no nonnegative equilibrium.*

5.2 Stability of the equilibrium when $u < \frac{k_{i,i+1}}{k'_{i,i+1}} E_i \quad i = 1, \ldots, n$

We are in the case when there is one single equilibrium x^*.

Now the Jacobian matrix J_3 of model (8) is:

$$J_3(x) = \begin{pmatrix} -\frac{\partial R_1}{\partial x_1} & -\frac{\partial R_1}{\partial x_2} & 0 & \cdots & \cdots \\ \frac{\partial R_1}{\partial x_1} & (\frac{\partial R_1}{\partial x_2} - \frac{\partial R_2}{\partial x_2}) & -\frac{\partial R_2}{\partial x_3} & 0 & \cdots \\ \vdots & \ddots & \ddots & \ddots & \vdots \\ 0 & \cdots & \frac{\partial R_{n-2}}{\partial x_{n-2}} & (\frac{\partial R_{n-2}}{\partial x_{n-1}} - \frac{\partial R_{n-1}}{\partial x_{n-1}}) & -\frac{\partial R_{n-1}}{\partial x_n} \\ 0 & \cdots & \cdots & \frac{\partial R_{n-1}}{\partial x_{n-1}} & (-k + \frac{\partial R_{n-1}}{\partial x_n}) \end{pmatrix}$$

and it is easy to check it is a compartmental matrix, similarly to J_2. We prove that the trajectories are bounded, with the help of a norm-like function V:

$$V(x) = \sum_{i=1}^{n} |x_i - x_i^*| \tag{10}$$

This function is not differentiable when $x_i = x_i^*$, and we use the right Dini derivative with respect to time (see [21]) and define the operator:

$$\sigma_i = \begin{cases} 1 \;\; if \;\; x_i(t) > x_i^* \;\; or \;\; if \;\; x_i(t) = x_i^* \;\; and \; \dot{x}_i(t) > 0 \\ 0 \;\; if \;\; x_i(t) = x_i^* \;\; and \;\; \dot{x}_i(t) = 0 \\ -1 \; if \;\; x_i(t) < x_i^* \;\; or \;\; if \;\; x_i(t) = x_i^* \;\; and \; \dot{x}_i(t) < 0 \end{cases} \tag{11}$$

We compute the right derivative of $V(x(t)) = \sum_{i=1}^{n} \sigma_i(x_i - x_i^*)$ (R_i^* denotes $R_i(x^*)$):

$$\frac{d^+}{dt}V(x(t)) = \sum_{i=1}^{n} \sigma_i \dot{x}_i$$

$$= -\sigma_1(R_1 - R_1^*) + \sum_{i=2}^{n-1} \sigma_i(R_{i-1} - R_{i-1}^* - R_i + R_i^*) + \sigma_n((R_{n-1} - R_{n-1}^*) - k(x_n - x_n^*))$$

$$= \sum_{i=1}^{n-1} (R_i - R_i^*)(\sigma_{i+1} - \sigma_i) - \sigma_n k(x_n - x_n^*)$$

Each term $T_i = (R_i - R_i^*)(\sigma_{i+1} - \sigma_i)$ of the sum can be written

$T_i = (R_i - R_i^*)(\sigma_{i+1} - \sigma_i) = (\sigma_i A - \sigma_{i+1} B)(\sigma_{i+1} - \sigma_i)$

with $A = \frac{[k_{i,i+1}K_{i,i+1} + (k_{i,i+1}k'_{i+1,i} + k'_{i,i+1}k_{i+1,i})x_{i+1}^*]}{(K_{i,i+1} + k'_{i,i+1}x_i + k'_{i+1,i}x_{i+1})(K_{i,i+1} + k'_{i,i+1}x_i^* + k'_{i+1,i}x_{i+1}^*)}$

and $B = \frac{[k_{i+1,}K_{i,i+1} + (k_{i,i+1}k'_{i+1,i} + k'_{i,i+1}k_{i+1,i})x_i^*]}{(K_{i,i+1} + k'_{i,i+1}x_i + k'_{i+1,i}x_{i+1})(K_{i,i+1} + k'_{i,i+1}x_i^* + k'_{i+1,i}x_{i+1}^*)}$

and we check that A and B are positive, and using the facts that $\sigma_i^2 = 1$ and $|\sigma_i\sigma_{i+1}| \leq 1$, we obtain that $T_i \leq (-A - B + A + B) = 0$

Therefore all the terms (included the last one $-\sigma_n k(x_n - x_n^*)$) are non positive and

$$\frac{d^+}{dt}V(x(t)) \leq 0$$

We deduce that all trajectories are bounded, and apply Proposition 12 (see appendix). We cannot use this function as a Lyapunov function because we were not able to easily prove that the derivative only cancels at the equilibrium.

Proposition 6 *The Jacobian matrix J_3 is compartmental and all trajectories are bounded, therefore all trajectories tend to a unique equilibrium in $\mathbb{R}_+^n$ which is globally attractive.*

The network is fully outflow connected and verifies Definition 3 (see appendix), because the model has an outflow on the last variable and the network is also strongly connected. Therefore we apply Proposition 13 (see appendix) and obtain

Proposition 7 *Matrix J_3 is regular, therefore the equilibrium is locally asymptotically stable.*

Finally, the equilibrium is locally stable and globally attractive, and thus we have finally:

Proposition 8 *If $u < \frac{k_{i,i+1}}{k'_{i,i+1}}E_i$ for $i \in \{1,\ldots,n\}$, all trajectories tend to a unique globally stable equilibrium in $\mathbb{R}_+^n$.*

5.3 Behaviour when it exists $i \in \{1,\ldots,n\}$ $u \geq \frac{k_{i,i+1}}{k'_{i,i+1}}E_i$

Consider now the case $u \geq \frac{k_{i,i+1}}{k'_{i,i+1}}E_i$, then the nonnegative equilibrium does not exist. The equation for x_n still give the result: $u = kx_n^*$, but equation for another coordinate

(at least) has no solution. It is easy to see that at least one variable x_i will tend to infinity with respect to time, because $\dot{x}_i$ will always be positive.

Biologically, it means that the system is not "sustainable" or "viable": one metabolite will grow without bound. We see from the equations that this happens if the input u is too large, or the concentrations in enzymes E_i too small. We remark that an equilibrium may exist for some u and disappear if u increases.

Extension 1: In the case of a complex network with one input and one output, the result of stability can be generalized with the same tools if there is an equilibrium (which is then globally stable). But conditions of existence of such an equilibrium are more difficult to compute.

Extension 2: The same results hold when the kinetics function $R(x_i,x_j)$ between x_i and x_j is defined by only qualitative properties

- $R(0,x_j) \leq 0$; $R(x_i,0) \geq 0$
- $R(x_i,x_j)$ is increasing with respect to x_i and decreasing with respect to x_j.
- $R(x,0)$ and $R(0,x)$ are bounded when x tends to infinity.

6 Enzymatic chain controlled by genes

We now couple this metabolic network with a genetic network, and apply the above results. We can assume that each step (except the input) of the following chain

$$u \rightarrow x_1 \leftrightarrows x_2 \leftrightarrows \ldots \leftrightarrows x_n \rightarrow$$

is controlled by an enzyme E_i through equation (5). Let us study the influence of the E_i on the behavior and equilibrium of the metabolic chain.

The metabolic chain corresponds to model (8), which is the more realistic and interesting. If the equilibrium exists, it verifies:

$$x_n^* = \frac{u}{k} \quad x_i^* = \frac{E_i k_{i+1,i} x_{i+1}^* + u(K_{i,i+1} + k'_{i+1,i} x_{i+1}^*)}{E_i k_{i,i+1} - k'_{i,i+1} u} \quad i = 1,\ldots,n-1.$$

In particular,

$$x_{n-1}^* = \frac{E_{n-1} k_{n,n-1} \dfrac{u}{k} + u(K_{n-1,n} + k'_{n,n-1} \dfrac{u}{k})}{E_{n-1} k_{n-1,n} - k'_{n-1,n} u} = h_{n-1}(E_{n-1})$$

$$x_{n-2}^* = \frac{E_{n-2} k_{n-1,n-2} h_{n-1}(E_{n-1}) + u(K_{n-2,n-1} + k'_{n-1,n-2} h_{n-1}(E_{n-1}))}{E_{n-2} k_{n-2,n-1} - k'_{n-2,n-1} u} = h_{n-2}(E_{n-1}, E_{n-2})$$

Because of the signs of the elements of the numerator and denominator, it is easy to check that this function is decreasing with respect to E_{n-2} and decreasing with respect to E_{n-1}, as function $h_{n-1}(E_{n-1})$.

Iteratively, we compute $x_i^* = h_i(E_i, E_{i+1}, \ldots, E_{n-1}) \quad i = 1,\ldots,n-2.$
and, for the same reasons as above, check that

$$\frac{\partial x_i^*}{\partial E_j} \leq 0 \quad i = 1,\ldots,n-1 \text{ and } \quad j = i,\ldots,n-1$$

Proposition 9 *The equilibrium x^* of the metabolic system is a decreasing function of the concentration of enzymes E_i, except for the last coordinate, which is fixed ($x_n^* = u/k$).*

Now we consider the coupled metabolic genetic system:

$$\dot{x} = f(x,E) \tag{12}$$

$$\dot{E} = g(E,x) \tag{13}$$

x is the vector of metabolite concentrations, E is the vector of enzyme concentrations. The rate of E is described by function g, and depends on E (this describes the interactions between genes) and on x (this describes the regulations of gene expression by metabolites). Classical models for (13) are sums and products of sigmoidal kinetics, like Hill functions, or piecewise-affine systems [8, 7].

The equilibrium of metabolic system (12), if it exists, is globally stable, as shown before. Therefore we can apply singular-perturbations theorems, like Tikhonov theorem (see appendix), and obtain an algebraic equations for (12) giving $x^*(E)$. We have shown above that this function is non-increasing with respect to its arguments.

The new reduced genetic systems is now:

$$\dot{E} = g(E,x^*(E)) \tag{14}$$

This system contains new negative dependence with respect to E, due to the influence of metabolites. The above study justifies the application of Tikhonov theorem for putting metabolic system to its quasi-stationary state, but it also gives some warnings: when E_i decreases, the metabolic steady-state can disappear (see the condition (9)), and the analysis with the reduced system is not valid any more. In particular, if the initial conditions are such that some enzyme E_i is too low at time $t = 0$, then condition (9) is violated and some metabolite will increase and become unbounded. We deduce that the initial conditions for enzymes concentrations have to be chosen high enough to verify the condition giving existence of an equilibrium for the metabolic system. Even in that case, it is easy to see on examples that, even if the quasi-steady state exists for the metabolic system, it can evolve (on the slow time scale) towards unbounded metabolic concentrations.

We put our conclusions into relief in this proposition:

Proposition 10 *If the metabolic equilibrium exists, it is globally stable; therefore the Tikhonov theorem can be applied to system (12, 13) giving the algebraic equations $x^*(E)$ for the metabolic equilibrium. According to the dynamical behavior of E, one coordinate of this metabolic equilibrium $x^*(E)$ may tend to infinity, and therefore disappear.*

If the initial conditions for enzymes are too low (condition (9)), then the nonnegative metabolic equilibrium does not exist; one of the metabolic concentrations tends to infinity.

7 Conclusion

The study of coupled metabolic genetic system in a cell is possible in concrete applications and gives interesting results (see [4, 3] and [1] for a recent work). We have shown that, for a reversible chain (or network), the hypotheses of global stability are true and justifies the application of quasi-steady state approximation for the metabolic system: of course, the kinetics of this metabolic system should be far faster than the kinetics of the enzymatic system. We have also shown that some additional dependence are created in the reduced system by the algebraic equations of the fast system: eq. (14) contains additional terms in the Jacobian matrix, due to $x^*(E)$. These new terms could add a negative or positive loop, for example; see [1] for a real application.

We also obtained that the equilibrium of the fast metabolic system does not always exist, and in that case the quasi-steady state approximation cannot be applied. It may also happen that the metabolic equilibrium exists for some time, but it disappears because the input u increased, or some enzyme E_i dynamically decreased below some value. This reminds that the behavior of a coupled slow-fast biological system can be complex, even in low dimensions (see [17]), with oscillations and other attractors. We have also shown that tools such as monotone and compartmental systems can be useful in biological models [23]: even if the nonlinear system (6) with reversible kinetics (5) is rather complex because of the denominators involving two variables, the results are obtained in a simple, global, and generic way.

Of course, the metabolic systems that we have studied are rather simple: very often, additional co-factors make the kinetics rate more complicated. If the property of monotonicity still holds, we believe that our tools could be applied, but further work is necessary.

Acknowledgments: This work was supported by the ANR Biosys METAGENOREG. We wish to thank D. Kahn (INRA) for his ideas and discussions, and the reviewers for their remarks.

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APPENDIX

1 Monotone systems

Monotone systems form an important class of dynamical systems, and are particularly well adapted to mathematical models in biology [23], because they are defined by conditions related to the signs of Jacobian matrix. Such a sign for one element traduces the fact that some variable will contribute positively to the variation of some other variables, and this kind of qualitative dependence is very frequent in biological models. The reader may consult the reference [22] for a review or an exhaustive presentation of the theory of monotone systems.

First we give some definitions. We are working with system

$$\dot{x} = f(x) \tag{15}$$

in a domain X of n-dimensional space $\mathbb{R}^n$. In this paper $X = \mathbb{R}^n_+$.

Partial order. Let the positive cone K that satisfies the properties:

- $\alpha K \subset K$ for all $\alpha \in \mathbb{R}_+$
- $K + K \subset K$
- $K \cap (-K) = \{0\}$

A partial order $\succeq$ in K is defined in the following way between two points of X: $x_1 \succeq x_2 \Leftrightarrow x_1 - x_2 \in K$ (in the following, we choose $K = \mathbb{R}^n_+$, which is the classical usual positive cone).

Definition 1 *Kamke Condition*
f is of type K for all i, if $f_i(a) \succeq f_i(b)$ for all points a and b in X such that $a \succeq b$ and $a_i = b_i$.

The following theorem is instrumental: it says that, if two initial conditions are ordered, then the solutions of (15) from these two initial conditions will remain ordered for all time t.

Theorem 1 *Let f of type K and $x_0, x_1 \in X$. If $x_0 \succeq x_1$ and $\Phi(t, x_i)$ $(i = 0, 1)$ are defined, then $\Phi(t, x_0) \succeq \Phi(t, x_1)$ for all t.*

See [22, p. 32] for the proof. The Kamke condition is easier to check by looking at the signs of the elements of the Jacobian matrix of system (1).

Proposition 11 *If f is differentiable, then Kamke condition implies*

$$\frac{\partial f_i}{\partial x_j}(x) \geq 0 \quad \forall i \neq j \tag{16}$$

Conversely, if $\frac{\partial f_i}{\partial x_j}(x)$ *is continuous and satisfies (16) in X, and if the domain X is p-convex (i.e. for all x and y in X satisfying* $x \succeq y$ *, the segment joining the two points is in X), then the Kamke condition is satisfied.*

See [22, p. 33] for a proof. System (1) is called a cooperative system. In summary, if the system is cooperative, then the flow is monotone, and preserves the partial order in $\mathbb{R}^n$. These systems have a strong tendency to converge to the set of their equilibria [22, p. 57]. It can be shown that almost any solution converges to the set of equilibria except a set of zero measure. In particular, there are no stable periodic solutions. For more precise theorems, see [22].

2 Compartmental systems

Let us now give a few reminders about compartmental systems (see [14]). This kind of models describes the dynamics of n-compartments interconnected by links with fluxes of matter. The overall equation is written by making a global mass balance between inputs and outputs of each compartment. The definition of a compartmental matrix is the following:

Definition 2 ***Compartmental Matrix***
The $n \times n$ *matrix C is a compartmental matrix if it satisfies the following three properties [14]:*

$$C_{ii} \leq 0 \;\; i = 1, \ldots, n, \tag{17}$$

$$C_{ij} \geq 0 \quad for \;\; i \neq j, \;\; i, j = 1, \ldots, n, \tag{18}$$

$$-C_{jj} \geq \sum_{i \neq j} C_{ij} \quad j = 1, \ldots, n, \tag{19}$$

Note that C_{ij} can in general depend on $x_k, \; k = 1 \ldots n$ which are the concentrations in each compartment. A common case is when C_{ij}, flow from compartment j in the compartment i, depends only on x_j (thus on the concentration of the initial compartment). This is not the case in our systems. There are also some theorems on the stability of linear and nonlinear compartmental systems (see [14]).

The following theorem (theorem 8 p. 56 in [14]) gives global stability results.

Theorem 2 *Let*

$$\dot{x} = f(x) \tag{20}$$

and the three conditions

- *(S1)*

$$\frac{\partial f_i}{\partial x_j} \geq 0 \quad for \;\; i \neq j, \quad i, j = 1, ..., n,$$

– *(S2)*

$$\sigma(x) = \sum_{i=1,\ldots,n} \dot{x}_i$$

is such that

$$\frac{\partial \sigma}{\partial x_i} < 0 \quad i = 1, \ldots, n$$

– *(S3) It exists* $k > 0$ *such that* $\sigma(x) \leq 0$ *when* $\sum_{i=1,\ldots,n} x_i = k$

If conditions (S1), (S2), (S3) are satisfied, then system (20) has a unique globally stable equilibrium.

The following property ([14, p. 54]) is linked to monotonicity of the flow.

Proposition 12 *If* $J(x)$ *is a compartmental matrix* $\forall x \in \mathbb{R}^n_+$*, then all bounded trajectories converge toward an equilibrium in* $\mathbb{R}^n_+$*.*

We recall ([14, p. 47]) that a graph is said strongly connected if there is a directed path from any compartment to any other compartment; equivalently, the corresponding matrix is irreducible. Now the following theorem is for closed systems, meaning, in this case, that total concentration $\sum x_i$ is conserved ($\sum_{i=1}^n f_i(x) = 0$).

Theorem 3 *Property 5 in [2]*
Suppose system (20) is closed, and let $M(x) = \sum_{i=1}^n x_i$ *the fixed total concentration. If the Jacobian matrix of the system is irreducible (the system is strongly connected) and compartmental, then for any* $M_0 > 0$*, hyperplane* $H = \{x \in \mathbb{R}^n_+ : M(x) = M_0 > 0\}$ *is invariant and contains a unique globally stable equilibrium in* H*.*

We recall some definitions and properties concerning output, see [14, p. 47] and [2].

Definition 3 *Fully outflow connected network*

A compartment x_i *is outflow (output) connected if there is a path* $x_i \to x_j \to \ldots \to x_l$ *from* x_i *until a compartment* x_l *with an outflow. The network is fully outflow connected if all compartments are outflow connected.*

The following proposition is in [14, p. 52].

Proposition 13 *Invertibility of a compartmental matrix*
A compartmental matrix is regular if and only if the associated network is fully outflow connected.

Intuitively, it means that the system has no traps where the flows accumulate (see [14]). We recall that in this case the matrix has eigenvalues with negative real parts [14, p. 51], and the associated linear system is asymptotically stable.

3 Tikhonov theorem

Consider the system

$$\Sigma^{\varepsilon} \begin{cases} \dfrac{dx}{dt} = \varepsilon f(x,z,\varepsilon) \\ \dfrac{dz}{dt} = g(x,z,\varepsilon) \end{cases} \tag{21}$$

If the following conditions are fulfilled

Assumption 1 *$z = \rho(x)$ is the solution of $g(x,z,0) = 0$, function ρ is regular, and matrix $\dfrac{\partial g}{\partial z}(x,\rho(x),0)$ has eigenvalues with negative real part.*

Assumption 2 *Reduced system*

$$\Sigma^{0} \begin{cases} \dfrac{dx}{d\tau} = f(x,\rho(x),0) \\ x_{(\tau=0)} = x_0 \end{cases} \tag{22}$$

with $\tau = \varepsilon t$ has a unique solution $x_0(\tau)$ for $\tau \in [0,T]$, $0 < T < +\infty$

Then, for ε small enough, the full system (Σ^{ε}) has a unique solution $(x_{\varepsilon}(\tau), z_{\varepsilon}(\tau))$ for $\tau \in [0,T]$, if z^0 is in the basin of attraction of equilibrium $\rho(x^0)$ of the rapid system

$$\frac{d\xi}{dt} = g(x^0,\xi,0) \tag{23}$$

and for $\tau \in [a,T]$ $(a > 0)$,

$$lim_{\varepsilon \to 0^+} x_{\varepsilon}(\tau) = x_0(\tau) \ , \ lim_{\varepsilon \to 0^+} z_{\varepsilon}(\tau) = \rho(x_0(\tau))$$

The limits are uniform with respect to time. The theorem (and more details) is in [15, p. 434]. Without further assumptions, the result is only valid for finite time T. Extensions are possible for infinite time, giving therefore asymptotic properties. For example, if the reduced system has an hyperbolic asymptotically stable equilibrium point $\bar{x}$, then, for ε small enough, the full system also admits an hyperbolic stable equilibrium, closed to $\bar{x}$. The approximation is therefore valid for infinite time (see [15, p. 439]).