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STABILITY ANALYSIS FOR BACTERIAL LINEAR METABOLIC PATHWAYS WITH MONOTONE CONTROL SYSTEM THEORY

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Abstract: In this work we give technical conditions which guarantee the global attractivity of bacterial linear metabolic pathways (reversible and irreversible structures) where both genetic and enzymatic controls involve the end product through metabolic effectors. To reach this goal, we use the negative feedback theorem of the monotone control systems theory, and we represent all conditions needed to apply the negative feedback theorem to the bacterial linear metabolic pathways in convenient deduced forms.

1 INTRODUCTION

The bacterial metabolic machinery and its regulation make up a complex system involving many cellular components such as metabolites and enzymes. In this paper, we focus on the dynamical behavior of the control structures used in a large number of bacterial biosynthesis pathways where both the genetic and enzymatic controls involve the last product as metabolite effector (Goelzer et al., 2008). Stability analysis of these biological structures is recognized as an issue of great importance in order to deduce key biological properties of the bacterial metabolic pathways. In the literature, many studies focused on the analysis of the metabolic and genetic networks separately. For instance, using the stability results about cyclic dynamical systems (Tyson and Othmer, 1978), (Sanchez, 2009), (Arcak and Sontag, 2006), one can state nice stability conditions of the irreversible linear metabolic pathways with allosteric regulation. One can also use the stability results about tridiagonal systems (Angeli and Sontag, 2008), (Wang et al., 2008) to analyze the stability of the reversible metabolic pathways. However, few works have considered structures with both genetic and allosteric regulation. Thus, in this paper we investigate stability of the common structures shared by many bacteria cells and yeasts. These structures are called *end product structures*, because both genetic and enzymatic controls involve the end product of the pathway (Grundy et al., 2003), (Gollnick et al., 2005), (Goelzer et al., 2008).

We will use the monotone control system theory developed in (Angeli and Sontag, 2003) to deal with stability issue of biological systems. In particular, the negative feedback theorem has been applied to a model of Mitogen-Activated Protein Kinase (MAPK) cascades in (Angeli and Sontag, 2003), and more recently to Goldbeter's circadian model (Angeli and Sontag, 2008). The main contribution of this work consists in providing technical conditions to check all the required assumptions to apply the negative feedback theorem to *end product structures* (under irreversible and reversible forms).

This paper is structured as follows. Section 2 presents the mathematical models for the linear reversible and irreversible bacterial metabolic pathways and states the main results of this paper which consist in propositions 1 and 2. Section 3 recalls some definitions and properties of monotone control systems theory and introduces the negative feedback theorem. Section 4 addresses the stability analysis of the dynamical models introduced in section 2 and proves the two propositions.

2 LINEAR METABOLIC PATHWAYS

Consider a linear pathway with n metabolites involved in enzymatic reactions, an input flux ν_1 and an output flux ν_n as depicted in Figure 1. Each X_i and $\mathbb{E}_i$ correspond to a metabolite and an enzyme respectively. We assume that the pool X_1 of the first

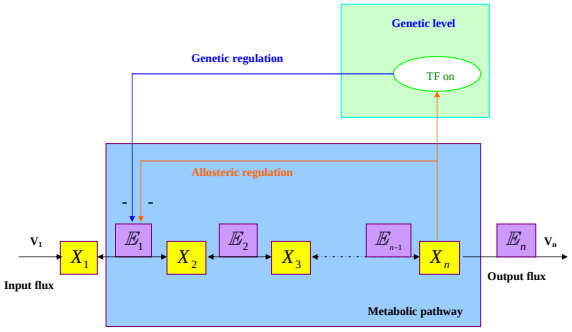

Figure 1: End product control linear structure.

metabolite is maintained by the input flux ν_1 which corresponds to a supply flux. Hence its concentration $\bar{x}_1$ is strictly positive constant. The output of the pathway is the flux ν_n which corresponds to the bacterium requirement for the metabolite X_n. Hereafter, for each $i \in \{2,\dots,n\}$ we denote by x_i the nonnegative concentration of the metabolite X_i, and by E_i the assumed constant positive concentration of the enzyme $\mathbb{E}_i$. The three phenomena, *enzymatic reactions, allosteric regulation and genetic regulation* (with respect to E_1), presented in Figure 1 can be described by a set of interconnected nonlinear differential equations. In the sequel, we analyze global stability of two types of the interconnected differential equations, namely the reversible and irreversible metabolic pathways.

2.1 Reversible Pathways

The common *end product structure* of linear reversible metabolic pathways is described by the following dynamical system:

$$\left\{\begin{array}{lcll} \dot{x}_2 & = & E_1 f_1(\bar{x}_1,x_2,x_n) - E_2 f_2(x_2,x_3) \\ \dot{x}_3 & = & E_2 f_2(x_2,x_3) - E_3 f_3(x_3,x_4) \\ \vdots & \vdots & \vdots & \vdots \\ \dot{x}_n & = & E_{n-1} f_{n-1}(x_{n-1},x_n) - E_n f_n(x_n) \\ \dot{E}_1 & = & g(x_n) - \mu E_1 \end{array}\right. \quad (1)$$

where the Lipschitz functions f_i denote the reaction rates of the enzymes $\mathbb{E}_i$. Note that, in the reversible structures all reaction rates depend on the product and substrate concentrations and have the following properties:

- *For the first enzyme*: we assume that the metabolite X_n modulates the activity of the enzyme $\mathbb{E}_1$ through, for example, an allosteric effect. The function $f_1(x_1,x_2,x_n)$ is increasing in its first argument and decreasing with respect to its second and third arguments, and we have for any $x_1 > 0$, $x_2 \geq 0$ and $x_n \geq 0$, $f_1(x_1,x_2,x_n) > 0$ and for any $x_n \geq 0$, $f_1(0,0,x_n) = 0$. In addition, there exists $M_1 > 0$ such that for any $x_1 > 0$, $x_2 \geq 0$ and $x_n \geq 0$, $f_1(x_1,x_2,x_n) \in [0,\ M_1)$. We also assume that for any $x_1 > 0$ and $x_n \geq 0$ there exists $x_2^* > 0$ such that $f_1(x_1,x_2^*,x_n) = 0$. Finally, for any $x_1 > 0$ and $x_2 > 0$ we have,
$$\lim_{x_n \to +\infty} f_1(x_1,x_2,x_n) = 0.$$
- *For the intermediate enzymes*: $f_i, i \in \{2,\dots,n-1\}$, is increasing in x_i and decreasing in x_{i+1}. For any $x_i > 0$, $f_i(x_i,0) > 0$, and for any $x_{i+1} > 0$, $f_i(0,x_{i+1}) < 0$ and $f_i(0,0) = 0$. Moreover, there exists $M_i > 0$ and $M_i' \geq 0$ such that for any $x_i > 0$ and $x_{i+1} \geq 0$, $f_i(x_i,x_{i+1}) \in (-M_i',\ M_i)$. Finally, we assume that for any $x_i > 0$ there exists $x_{i+1}^* > 0$ such that $f_i(x_i,x_{i+1}^*) = 0$.
- *For the final enzyme*: $\mathbb{E}_n$ describes the properties of the remainder part of the metabolic network and summarizes the relation between the flux supplied by the pathway and the final concentration. The properties of f_n mainly depends on the properties of the next modules, and generally f_n is a strictly increasing, positive and bounded function in x_n such that
$$f_n(0) = 0,\ \lim_{x_n \to +\infty} f_n(x_n) = M_n.$$

The dynamics of the enzyme concentrations during the exponential growth phase are mostly the result of two phenomena: (i) the *de novo* production (ii) the *dilution* effect caused by the increase of the cell volume. For this, in the last equation of (1), we have considered that the control of the concentration of the first enzyme is regulated by the concentration of the final metabolite x_n, where μ is the growth rate of the bacterium assumed to be in the exponential growth phase. The term $g(x_n)$ corresponds to the instantaneous production of the enzyme E_1 modulated by a metabolite (implicitly through a transcription factor). The continuous function $g(.)$ is positive strictly decreasing in the end product x_n with $g(0) = g_{max}$, $g_{max} > 0$ and

$$\lim_{x \to +\infty} g(x) = 0.$$

After the detailed description of the dynamical model of the linear reversible metabolic pathway, we state below the main results of this paper about its global attractivity.

Stability Results. Let us start by setting three hypotheses and then we introduce our first proposition.

- Hypothesis $\mathcal{H}_1$: The $n-1 \times n-1$ Tridiagonal matrix,

$$\mathbf{Q}=\begin{bmatrix} q_{2,2} & q_{2,3} & 0 & \dots & \dots & 0 \\ q_{3,2} & q_{3,3} & q_{3,4} & \ddots & & \vdots \\ 0 & \ddots & \ddots & \ddots & \ddots & \vdots \\ \vdots & \ddots & \ddots & \ddots & \ddots & 0 \\ \vdots & & \ddots & q_{n-1,n-2} & q_{n-1,n-1} & q_{n-1,n} \\ \vdots & \dots & \dots & 0 & q_{n,n-1} & q_{n,n} \end{bmatrix}$$

where $\forall i, j \in \{2,\dots,n\}$

$$q_{i,j} = \sup\left(\frac{\partial(E_{i-1}f_{i-1}(.) - E_i f_i(.))}{\partial x_j}\right),$$

is Hurwitz.

- Hypothesis $\mathcal{H}_2$: The inequality $E_{n-1}M_{n-1} \leq E_nM_n$ is verified.
- Hypothesis $\mathcal{H}_3$: The graph of the scalar function

$$T(u) = g \circ k_y(u)$$

and that of its reciprocal function $T^{-1}(u)$ have a unique intersection point on the open interval $u \in (0, g_{max})$.

The scalar function $k_y(.)$ is the *static input-output characteristic* associated to the monotone part of (1) (resp. (2)), see Definition 2 in subsection 3.1.

Proposition 1. *If $\mathcal{H}_1$, $\mathcal{H}_2$ and $\mathcal{H}_3$ are satisfied, then for any $\bar{x}_1$ and E_n, the reversible end product structure* (1) *has globally attractive equilibrium.*

2.2 Irreversible Pathways

The main difference between the irreversible and the reversible metabolic pathways is in the reaction rates f_i for the first and intermediate enzymes. Indeed, here we assume that the reaction rates depend only on the substrate concentration and have the following properties:

- *For the first enzyme.* We assume that the function f_1 is increasing in its first argument and decreasing in its second argument and for any $x_1 > 0$,

$$\lim_{x_n \to +\infty} f_1(x_1, x_n) = 0.$$

In addition, we have for any $x_n \geq 0$, $f_1(0, x_n) = 0$ and there exists $M_1 > 0$ such that for any $x_1 > 0$ and $x_n \geq 0$, $f_1(x_1, x_n) \in [0, M_1)$.

- *For the intermediate enzymes*: $f_i\ i \in \{2,\dots,n-1\}$ is strictly increasing in x_i and $f_i(0) = 0$. Moreover, there exists $M_i > 0$ such that

$$\lim_{x_i \to +\infty} f_i(x_i) = M_i.$$

Then, the *end product structure* of the linear irreversible metabolic pathways is described by the following dynamical system

$$\begin{cases} \dot{x}_2 &= E_1 f_1(\bar{x}_1, x_n) - E_2 f_2(x_2) \\ \dot{x}_3 &= E_2 f_2(x_2) - E_3 f_3(x_3) \\ \vdots & \vdots \quad \vdots \quad \vdots \\ \dot{x}_n &= E_{n-1} f_{n-1}(x_{n-1}) - E_n f_n(x_n) \\ \dot{E}_1 &= g(x_n) - \mu E_1. \end{cases} \tag{2}$$

Stability Results. Now, we state the contribution of this paper concerning the global attractivity of the irreversible metabolic pathway (2).

- Hypothesis $\mathcal{H}_4$: for each $i \in \{2,\dots,n\}$ the inequality is verified $\overline{E}_1 M_1 \leq E_i M_i$, where $\overline{E}_1$ is the upper bound of all solutions $E_1(t)$.

Proposition 2. *The irreversible end product structure* (2) *has globally attractive equilibrium for any $\bar{x}_1$ and E_n if hypotheses $\mathcal{H}_3$ and $\mathcal{H}_4$ are satisfied.*

To prove Proposition 1 and Proposition 2, we will use the monotone control system theory, in particular the negative feedback theorem. Thus, we present briefly this theory in the next section and then we give the proofs in section 4.

3 MONOTONE CONTROL SYSTEMS

Monotone control systems theory (Angeli and Sontag, 2003) is an extension of the autonomous monotone system theory (Smith, 1995). Briefly, monotone control system is a dynamical system on an ordered metric space which has the property that ordered initial states and ordered inputs generate ordered state trajectories and ordered outputs. In other words, a controlled dynamical system (3),

$$\begin{cases} \dot{\mathbf{x}}(t) &= \mathbf{f}(\mathbf{x}(t), \mathbf{u}(t)) \\ \mathbf{y}(t) &= \mathbf{h}(\mathbf{x}) \end{cases}, \ \mathbf{x}(t_0) = cst, \tag{3}$$

where $\mathbf{x}(t) \in \mathbb{X} \subseteq \mathbb{R}^n$ and $\mathbf{u}(t) \in \mathbb{U} \subseteq \mathbb{R}^m$, is said monotone if the following implication holds: $\forall (\mathbf{x}_1(t_0),\ \mathbf{x}_2(t_0)) \in \mathbb{X}^2$ and $\forall (\mathbf{u}_1(t),\ \mathbf{u}_2(t)) \in \mathbb{U}^2$,

$$\begin{aligned} &\mathbf{x}_1(t_0) \preceq \mathbf{x}_2(t_0), \mathbf{u}_1(t) \preceq \mathbf{u}_2(t) \Rightarrow \\ &\quad \mathbf{x}_1(t, \mathbf{x}_1(t_0), \mathbf{u}_1(t)) \preceq \mathbf{x}_2(t, \mathbf{x}_2(t_0), \mathbf{u}_2(t))\ \forall t \geqslant t_0 \end{aligned} \tag{4}$$

where $\mathbf{x}(t, \mathbf{x}(t_0), \mathbf{u}(t))$ represent the state trajectory generated by (3) with $\mathbf{x}(t_0)$ as initial state and $\mathbf{u}(t)$ as input. The dimensions of the vectors $\mathbf{x}$, $\mathbf{u}$ and $\mathbf{y}$ are respectively n, m and p.

Here, we consider that $\preceq$ is the classical lower or equal comparison operator $\leq$, applied component by

component. Systems that are monotone with respect to this order are called cooperative systems, as all state variables have a positive influence on one other and the inputs act positively on state variables.

Proposition 3. *The dynamical system* (3) *is cooperative if and only if the following properties hold:*

$$\begin{array}{ll} \frac{\partial f_i}{\partial x_j}(\mathbf{x},\mathbf{u}) \geq 0 & \forall \mathbf{x} \in \mathbb{X}, \forall \mathbf{u} \in \mathbb{U}, \forall i \neq j \\ \frac{\partial f_i}{\partial u_j}(\mathbf{x},\mathbf{u}) \geq 0 & \forall \mathbf{x} \in \mathbb{X}, \forall \mathbf{u} \in \mathbb{U}, \forall i, j \\ \frac{\partial h_i}{\partial x_j}(\mathbf{x}) \geq 0 & \forall \mathbf{x} \in \mathbb{X}, \forall i, j \end{array} \tag{5}$$

Proof. See (Angeli and Sontag, 2003; Angeli and Sontag, 2004).

After this brief recall about monotone control systems, we now introduce in the next the negative feedback theorem which states stability conditions for monotone control systems with negative feedback.

3.1 Stability Analysis with Monotone Control System

Recently, the negative feedback theorem of the monotone control system theory is used to analyze stability of several biological systems. Indeed, this theorem allows, under some conditions, to obtain the globally attractive stable steady state of non-monotone dynamical systems. Here we give some definitions and assumptions needed to state the negative feedback theorem.

Definition 1 (Angeli and Sontag, 2003)**.** *We say that the SISO dynamical system* (3) *($m = p = 1$) admits an input to state static characteristic $\mathbf{k}_x(.) : \mathbb{U} \to \mathbb{X}$ if, for each constant input $u \in \mathbb{U}$, there exists a unique globally asymptotically stable equilibrium noted $\mathbf{k}_x(u)$.*

Definition 2 (Angeli and Sontag, 2003)**.** *SISO system with an input-state characteristic and with a continuous output map $y = h(\mathbf{x})$ has an input to output characteristic defined as the composite function $k_y(u) = (h \circ \mathbf{k}_x)(u)$.*

Note that, if the system (3) (with $m = p = 1$) is cooperative and admits a *static input-state characteristic* $\mathbf{k}_x$ and *static input-output characteristic* k_y, then $\mathbf{k}_x$ and k_y must be increasing with respect to u, viz.

$$\forall\ (u_1,u_2) \in \mathbb{U}^2,\ u_1 \geq u_2 \quad \Leftrightarrow \quad \begin{array}{l} \mathbf{k}_x(u_1) \geq \mathbf{k}_x(u_2), \\ k_y(u_1) \geq k_y(u_2). \end{array}$$

Assumptions. Consider the non-monotone autonomous system given by (6)

$$\dot{\mathbf{x}}(t) = \mathbf{F}(\mathbf{x}), \tag{6}$$

and let us state the following assumptions,

- $\mathcal{H}_5$: Any state trajectory generated by system (6) is bounded.
- $\mathcal{H}_6$: System (6) is decomposable into an open loop SISO monotone control system (7)

$$\left\{ \begin{array}{lcl} \dot{\mathbf{x}}(t) & = & \mathbf{f}(\mathbf{x},u) \\ y(t) & = & h(\mathbf{x}), \end{array} \right. \tag{7}$$

  closed by a monotone decreasing feedback law $f_b : y \longrightarrow u$ as depicted in Figure 2.

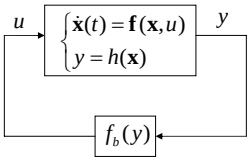

Figure 2: System (6) in closed loop configuration.

- $\mathcal{H}_7$: Open loop system (7) admits a well-defined static *input-output characteristic* $k_y(.)$.

Then, we can introduce the negative feedback theorem.

Theorem 1. *Let* (8) *be a discrete scalar dynamical system associated to the continuous non-monotone system* (6)

$$u_{j+1} = (f_b \circ k_y)(u_j). \tag{8}$$

If this iteration has a globally attractive fixed point u^* on an open interval $\mathcal{U}_x$, then the autonomous system (6)*, provided that the assumptions $\mathcal{H}_5$, $\mathcal{H}_6$ and $\mathcal{H}_7$ are satisfied, has a globally attracting steady state $\mathbf{x}^* = \mathbf{k}_x(u^*)$.*

Proof. See (Angeli and Sontag, 2003).

Hereafter, we give proofs of our main results stated in subsections 2.1 and 2.2.

4 PROOF OF THE MAIN RESULTS

In this section, we prove that propositions 1 and 2 are consequences of Theorem 1. We start with the irreversible metabolic pathways, for which the *static input-state characteristic* of its monotone part is easier to establish. Then we will focus on the reversible pathways.

4.1 Irreversible Structure

In this subsection we will show that the technical Proposition 2 is a consequence of Theorem 1.

Checking Assumption $\mathcal{H}_5$. First of all, let us prove the boundedness of the controlled enzyme E_1 which is governed by the following differential equation

$$\dot{E}_1 = g(x_n) - \mu E_1. \tag{9}$$

By definition we know that $g(.)$ is bounded, viz. $\forall x_n,\ g(x_n) \in (0, g_{max}]$. Then, for any x_n the solution $E_1(t)$ of (9) is framed by

$$\check{E}_1(t) \leq E_1(t) \leq \hat{E}_1(t),$$

where $\check{E}_1(t)$ and $\hat{E}_1(t)$ are respectively the solutions of the following stable linear differential equations

$$\dot{\check{E}}_1 = -\mu \check{E}_1 \text{ and } \dot{\hat{E}}_1 = g_{max} - \mu \hat{E}_1.$$

Thus, there exists

$$\overline{E}_1 > 0 \mid \forall t \geq 0,\ E_1(t) \leq \overline{E}_1.$$

Now, consider the first differential equation of (2)

$$\begin{array}{rcl} \dot{x}_2 & = & E_1 f_1(\overline{x}_1, x_n) - E_2 f_2(x_2) \\ & \leq & \overline{E}_1 M_1 - E_2 f_2(x_2). \end{array}$$

We know that $f_2(.)$ is positive increasing and bounded. Then if

$$\overline{E}_1 M_1 \leq E_2 M_2, \tag{10}$$

there exists $x_2^\star$ such that $E_2 f_2(x_2^\star) = \overline{E}_1 M_1$, and we obtain

$$\forall x_2 > x_2^\star,\ \dot{x}_2 \leq 0,$$

namely the solution $x_2(t)$ decreases towards $x_2^\star$ and then the metabolite concentration x_2 is bounded. In addition, for any initial condition $x_2(t_0)$ there exists $t^\star \geq t_0$ such that,

$$\forall t \geq t^\star,\ E_2 f_2(x_2(t)) \leq E_2 f_2(x_2^\star) = \overline{E}_1 M_1.$$

To proof the boundedness of the remainder metabolite concentrations, we use mathematical induction. Assume that x_i is bounded, viz. the following inequality is satisfied

$$\overline{E}_1 M_1 \leq E_i M_i, \tag{11}$$

and there exists $(t^\star, x_i^\star)$ such that for all $t \geq t^\star$

$$E_i f_i(x_i(t)) \leq E_i f_i(x_i^\star) = \cdots = E_2 f_2(x_2^\star) = \overline{E}_1 M_1.$$

Then, for $t \geq t^\star$ the dynamics of the next metabolite concentration x_{i+1} is bounded by

$$\begin{array}{rcl} \dot{x}_{i+1} & = & E_i f_i(x_i) - E_{i+1} f_{i+1}(x_{i+1}) \\ & \leq & E_i f_i(x_i^\star) - E_{i+1} f_{i+1}(x_{i+1}) \\ & = & \overline{E}_1 M_1 - E_{i+1} f_{i+1}(x_{i+1}). \end{array}$$

Hence we show, with the same way used to prove the boundedness of x_2, that inequality (12) guarantees the boundedness of the metabolite concentration x_{i+1}.

$$\overline{E}_1 M_1 \leq E_{i+1} M_{i+1}. \tag{12}$$

Therefore $\mathcal{H}_4$ guarantees the boundedness of the all state trajectories generated by (2), namely $\mathcal{H}_5$.

Checking Assumption $\mathcal{H}_6$. System (2) is not monotone. However, we can regard it as a cooperative controlled system (13), which has a triangular Jacobian matrix $DF(\mathbf{x})$ with nonnegative off-diagonal entries, closed by a negative feedback (14),

- Open loop (cooperative system)

$$\left\{ \begin{array}{rcl} \dot{x}_2 & = & E_1 f_1(\overline{x}_1, g^{-1}(u)) - E_2 f_2(x_2) \\ \dot{x}_3 & = & E_2 f_2(x_2) - E_3 f_3(x_3) \\ \vdots & \vdots & \vdots \quad \vdots \\ \dot{x}_n & = & E_{n-1} f_{n-1}(x_{n-1}) - E_n f_n(x_n) \\ \dot{E}_1 & = & u - \mu E_1 \\ y & = & x_n \end{array} \right. \tag{13}$$

- Negative feedback

$$u = g(y) \tag{14}$$

where $g^{-1}(.)$ is the reciprocal function of $g(.)$ and $u \in (0, g_{max})$ since $g(.) \in (0, g_{max}]$. This verifies $\mathcal{H}_6$.

Checking Assumption $\mathcal{H}_7$. The *static input-state characteristic* $\mathbf{k}_x(u)$ of (13) is computed at steady states corresponding to constant inputs u. Thus, we vanish all the time derivatives of (13) to obtain:

$$\mathbf{k}_x^T(u) = \left[f_2^{-1}\left(\tfrac{f_1(\overline{x}_1, g^{-1}(u))u}{E_2\mu}\right), \ldots, f_n^{-1}\left(\tfrac{f_1(\overline{x}_1, g^{-1}(u))u}{E_n\mu}\right), \tfrac{u}{\mu} \right] \tag{15}$$

and for the *static input-output characteristic* we have:

$$k_y(u) = f_n^{-1}\left(\frac{f_1(\overline{x}_1, g^{-1}(u))u}{E_n\mu}\right) \tag{16}$$

Since functions $f_i(.), i = 2, \ldots, n$ are bounded, the existence of (15) is conditioned by the following inequalities:

$$\forall i,\ \forall u \in (0, g_{max}),\ \ \frac{f_1(\overline{x}_1, g^{-1}(u))u}{E_i\mu} \leq M_i$$

which are always true if assumption $\mathcal{H}_4$ is verified. Moreover, as system (13) is cooperative, both static characteristics ((15) and (16)) are increasing with respect to u.

Now, to prove that for each constant input $u \in (0, g_{max})$ there exists a unique globally asymptotically stable equilibrium point $\mathbf{k}_x(u)$ for (13), we consider separately the dynamics of the enzymatic reactions $(\dot{x}_2, \ldots, \dot{x}_n)^T$ and that of the genetic regulation $\dot{E}_1$.

- The growth rate μ of the bacteria is constantly positive. Then for each constant input u all the solutions generated by the dynamics of the genetic regulation converge asymptotically to $\frac{u}{\mu}$.
- The Jacobian matrix $DF(\mathbf{x})$ of the dynamics of the enzymatic reactions is a lower triangular matrix with nonnegative off-diagonal entries and real

negative eigenvalues. Then $-DF(\mathbf{x})$ is a *M-Matrix* (Berman and Plemmons, 1994) and there exists a diagonal matrix $\mathbf{P} = diag(p_1,\dots,p_n)$ with $p_i > 0$ such that

$$\exists \varepsilon > 0,\ \forall \mathbf{x},\ \ \mathbf{P}DF(\mathbf{x}) + DF(\mathbf{x})^T\mathbf{P} < -\varepsilon \mathbf{I}_{n-1}. \tag{17}$$

Consequently, we can state that the dynamics of the enzymatic reactions have a well defined quadratic Lyapunov function:

$$V(\mathbf{z}) = \mathbf{z}^T\mathbf{P}\mathbf{z},$$

where $\mathbf{z} = \mathbf{x} - \mathbf{x}^*$, $x_i^* = (k_x(u))_i,\ i = 2,\dots,n$ and

$$\begin{aligned} \dot{V}(\mathbf{z}) &= \mathbf{z}^T\mathbf{P}[\mathbf{f}(\mathbf{x},u) - \mathbf{f}(\mathbf{x}^*,u)] \\ &= \mathbf{z}^T \textstyle\int_0^1 \mathbf{P}DF(\lambda\mathbf{z}+\mathbf{x})\mathbf{z}d\lambda \\ &= \tfrac{1}{2}\mathbf{z}^T \textstyle\int_0^1 (\mathbf{P}DF(\lambda\mathbf{z}+\mathbf{x}) + DF(\lambda\mathbf{z}+\mathbf{x})^T\mathbf{P})d\lambda\mathbf{z} \\ &\leq -\tfrac{1}{2}\varepsilon \parallel \mathbf{z} \parallel^2 \end{aligned} \tag{18}$$

Hence, for each constant input $u \in (0, g_{max})$, any solution of the open loop system (13) converges asymptotically to the unique steady state given by (15). This verifies assumption $\mathcal{H}_7$.

Now, to complete the proof that the Proposition 2 is consequence of Theorem 1, we will show that assumption $\mathcal{H}_3$ implies the global attractivity of the following scalar discrete dynamical system

$$u_{j+1} = g(f_n^{-1}(\frac{f_1(\bar{x}_1, g^{-1}(u_j))u_j}{E_n\mu})) \tag{19}$$

To do so, *(i)* we prove existence and unicity of a fixed point u^* for (19); and *(ii)* we give convenient condition which guarantee its global attractivity.

Existence and Unicity. To prove this property, it is sufficient to show that the curves of the functions $g^{-1}(u)$ and $k_y(u)$ have a unique intersection point over the interval $(0, g_{max})$. Since:

- $k_y(u)$ is is monotone increasing with respect to u and for $u = 0$, $k_y(0) \geq 0$ and $\lim_{u\to g_{max}} k_y(u) = +\infty$
- $g^{-1}(u)$ is monotone decreasing with respect to u and $\lim_{u\to 0} g^{-1}(u) = +\infty$ and for $u = g_{max}$, $g^{-1}(g_{max}) = 0$,

then the two curves have a unique intersection point u^* (see Figure 3) which present the unique fixed point of (19).

Global Attractivity. Denote by T^2 the composite function

$$T^2(u) = (T \circ T)(u),$$

where $T(u) = (g \circ k_y)(u)$. The following proposition gives the necessary and sufficient condition for the global attractivity of the unique equilibrium of (19).

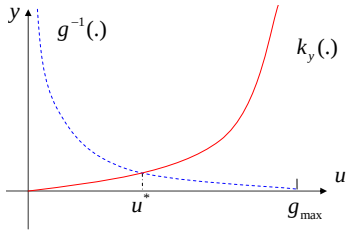

Figure 3: Graphical proof of the existence and unicity of the fixed point u^* for the discrete system (19).

Proposition 4. *If u^* is also the unique fixed point of $T^2(u)$ on $(0, g_{max})$, That is*

$$\forall u \in (0, g_{max}), T^2(u) = u \Leftrightarrow u = u^*, \tag{20}$$

then (19) *converges to its unique fixed point.*
Proof: see (Enciso and Sontag, 2006).
In practice, we can check condition (20) by graphical test ($\mathcal{H}_3$). Indeed, if the graph of $T(u)$ and that of $T^{-1}(u)$ have a unique intersection point u^* over $(0, g_{max})$, then the composite function $T^2(u)$ has unique fixed point u^*. This completes the proof.

4.2 Reversible Structure

Now, consider the reversible metabolic pathways (1) and we prove that Proposition 1 is a consequence of Theorem 1.

Checking Assumption $\mathcal{H}_5$: First, note that the enzyme E_1 is bounded (see proof given in subsection 4.1). Now, to analyze the boundedness of all the metabolite concentrations of (1), we proceed by step and we show that if any metabolite concentration x_i is bounded then the metabolite concentration x_{i-1} is also bounded. We start by x_2, and we consider the first differential equation of (1),

$$\begin{aligned} \dot{x}_2 &= E_1 f_1(\bar{x}_1, x_2, x_n) - E_2 f_2(x_2, x_3) \\ &\leq \overline{E}_1 f_1(\bar{x}_1, x_2, 0) - E_2 f_2(x_2, x_3). \end{aligned}$$

We assume that x_3 is bounded ($\forall t > 0, x_3(t) \leq \bar{x}_3$), then by definition there exists $x_2^\star$ such that:

$$f_1(\bar{x}_1, x_2^\star, 0) = 0 \ \text{ and } \ f_2(x_2^\star, \bar{x}_3) \geq 0,$$

and thus at $x_2^\star$ we obtain $\dot{x}_2 \leq 0$. Hence the threshold $x_2^\star$ is repulsive, and so we have proved that the boundedness of x_3 implies the boundedness of x_2.

Now, for any metabolite concentration x_i, $i \in \{3,\dots,n-1\}$ we have x_{i-1} bounded with bound $\bar{x}_{i-1}$, and we assume that x_{i+1} is bounded with bound $\bar{x}_{i+1}$. Then the dynamics of x_i is bounded by:

$$\begin{aligned} \dot{x}_i &= E_{i-1} f_{i-1}(x_{i-1}, x_i) - E_i f_i(x_i, x_{i+1}) \\ &\leq E_{i-1} f_{i-1}(\bar{x}_{i-1}, x_i) - E_i f_i(x_i, \bar{x}_{i+1}), \end{aligned}$$

and by definition we have

$$\exists x_i^\star \mid f_{i-1}(\bar{x}_{i-1}, x_i^\star) \leq 0 \text{ and } f_i(x_i^\star, \bar{x}_{i+1}) \geq 0.$$

Hence for $x_i^\star$ we obtain $\dot{x}_i \leq 0$, and so the threshold $x_i^\star$ is repulsive. Thus, we have proved that $\forall i \in \{2,\ldots,n-1\}$ the boundedness of x_{i+1} implies the boundedness of x_i. Lastly, consider the dynamics of the concentration of the end product x_n,

$$\begin{array}{rcl} \dot{x}_n & = & E_{n-1} f_{n-1}(x_{n-1}, x_n) - E_n f_n(x_n) \\ & \leq & E_{n-1} M_{n-1} - E_n f_n(x_n). \end{array}$$

Since $f_n(.)$ is positive increasing and bounded with respect to x_n, it is clear that if $E_{n-1}M_{n-1} \leq E_n M_n$ we obtain

$$\exists \bar{x}_n \mid \forall x_n \geq \bar{x}_n \Rightarrow \dot{x}_n \leq 0$$

independently of the values of x_{n-1}. Consequently, if $\mathcal{H}_2$ is true, then all the state trajectories generated by (1) are bounded and so assumption $\mathcal{H}_5$ is verified.

Checking assumption $\mathcal{H}_6$: As in the case of the irreversible metabolic pathways, structure (1) is not monotone. Nevertheless, we can decompose it into an open loop cooperative controlled system (21), which has tridiagonal Jacobian matrix $DF(x)$ with nonnegative off-diagonal entries, closed by a negative feedback (22).

- Open loop (cooperative system)

$$\left\{ \begin{array}{rcl} \dot{x}_2 & = & E_1 f_1(\bar{x}_1, x_2, g^{-1}(u)) - E_2 f_2(x_2, x_3) \\ \dot{x}_3 & = & E_2 f_2(x_2, x_3) - E_3 f_3(x_3, x_4) \\ \vdots & \vdots & \vdots \qquad\qquad \vdots \\ \dot{x}_n & = & E_{n-1} f_{n-1}(x_{n-1}, x_n) - E_n f_n(x_n) \\ \dot{E}_1 & = & u - \mu E_1 \\ y & = & x_n \end{array} \right. \tag{21}$$

- Negative feedback

$$u = g(y) \tag{22}$$

where $g^{-1}(.)$ and $g(.)$ are the same as in the irreversible case and also $u \in (0, g_{max})$. Hence, assumption $\mathcal{H}_6$ is intrinsically satisfied.

Checking assumption $\mathcal{H}_7$: In the reversible context, build the *static input-state characteristic* is not explicit as in the irreversible case. However, to establish this characteristic we use the monotonicity property of all reaction rates $f_i(.,.),\ i \in \{1,\ldots,n\}$. First, we show that at steady state there exists a binary relation between each metabolite concentration $x_i,\ i \in \{3,\ldots,n\}$ and x_2. Second, we show that the metabolite concentration x_2 is an increasing function of the constant input u.

- Consider the dynamics corresponding to the last pool X_n. Since: *(i)* the function $f_n(x_n)$ is monotone increasing in x_n with $f_n(0) = 0$, *(ii)* the function $f_{n-1}(x_{n-1}, x_n)$ is decreasing in x_n and *(iii)* for any x_{n-1} there exists x_n^* such that $f_{n-1}(x_{n-1}, x_n^*) = 0$,

$$\forall x_{n-1},\ \exists x_n \mid E_{n-1} f_{n-1}(x_{n-1}, x_n) = E_n f_n(x_n).$$

In other words, we can say that there exists a monotone increasing function $H_n(.)$ with respect to x_{n-1} such that:

$$x_n = H_n(x_{n-1}). \tag{23}$$

- According to the previous stage, we can write

$$f_n(x_n) = f_n(H_n(x_{n-1})).$$

Thus, since $H_n(.)$ is monotone increasing in x_{n-1}, $f_n(.)$ is also monotone increasing in x_{n-1}. Now, consider the dynamics of the pool X_{n-1}. By definition $f_{n-2}(x_{n-2}, x_{n-1})$ is decreasing in x_{n-1} and for any x_{n-2} there exists x_{n-1}^* such that $f_{n-2}(x_{n-2}, x_{n-1}^*) = 0$. Hence, we deduce: $\forall x_{n-2}$,

$$\exists x_{n-1} \mid E_{n-2} f_{n-2}(x_{n-2}, x_{n-1}) = E_n f_n(H_n(x_{n-1})).$$

Therefore, there exists a monotone increasing function $H_{n-1}(.)$ with respect to x_{n-2} such that:

$$x_{n-1} = H_{n-1}(x_{n-2}) \text{ and } x_n = H_n(H_{n-1}(x_{n-2})). \tag{24}$$

- Then we repeat this reasoning to obtain at steady state the following relations between x_2 and all the metabolic concentrations $x_i,\ i \in \{3,\ldots,n\}$:

$$\begin{array}{l} x_3 = H_3(x_2) \\ x_4 = H_4(H_3(x_2)) \\ \vdots \\ x_n = H_n(H_{n-1}(\ldots H_3(x_2)\ )) \end{array} \tag{25}$$

where all H_i are increasing functions.

- Lastly, the enzyme's dynamics vanished while $E_1 = \frac{u}{\mu}$. Thus, it is possible to build at the steady state a monotone relationship between the concentration of the pool X_2 and the input u. Indeed, as we have shown previously, *(i)* the monotone decreasing property of the function $\frac{u}{\mu} f_1(\bar{x}_1, x_2, g^{-1}(u))$ in x_2, *(ii)* the monotone increasing property of the function $f_n(H_n(H_{n-1}(\ldots H_3(x_2)\ ))$ in x_2, and *(iii)* the existence of x_2^* such that $f_1(\bar{x}_1, x_2^*, g^{-1}(u)) = 0$ allow to state: $\forall u,\ \exists x_2$ such that,

$$\frac{u}{\mu} f_1(\bar{x}_1, x_2, g^{-1}(u)) = f_n(H_n(H_{n-1}(\ldots H_3(x_2)\ ))$$

Then, at the steady state there exists a monotone increasing function $H_2(.)$ with respect to u such that:

$$x_2 = H_2(u). \tag{26}$$

Hence, the *static input-state characteristic* of the system (21) is given by:

$$\mathbf{k}_x^T(u) = \left[H_2(u), H_3(H_2(u)), \ldots, H_n(H_{n-1}(\ldots H_2(u)\ )), \frac{u}{\mu}\right] \tag{27}$$

and its *input-output characteristic* is obtained by the composition law between (27) and the output equation of (21),

$$k_y(u) = H_n(H_{n-1}(\ldots H_2(u)\ )). \tag{28}$$

Now, we must prove that for each constant input u the vector $[\mathbf{x}^{*T}, \frac{u}{\mu}] = \mathbf{k}_x^T(u)$ is the globally asymptotically stable equilibrium point for the open loop system (21). To do so, we use the same analysis as in the irreversible case. First, we separate the two dynamics (enzymatic reaction, genetic regulation) and we deduce that for each constant input u all the solutions generated by the dynamics of the genetic regulation ($\dot{E}_1$) converge to $\frac{u}{\mu}$. Second, hypothesis $\mathcal{H}_1$ claims the existence of Tridiagonal Hurwitz matrix $\mathbf{Q}$ with nonnegative off-diagonal entries such that for all $\mathbf{x}$ the Jacobian matrix $DF(\mathbf{x})$ of the dynamics of the enzymatic reactions $(\dot{x}_2, \ldots, \dot{x}_n)$ is bounded by, $DF(\mathbf{x}) \leq \mathbf{Q}$. Then there exists a diagonal matrix $\mathbf{N} = diag(n_1, \ldots, n_n)$ with $n_i > 0$ and a real number $\varepsilon > 0$ such that $\forall \mathbf{x}$

$$\begin{array}{rcl} \mathbf{N}DF(\mathbf{x}) + DF^T(\mathbf{x})\mathbf{N} & \leq & \mathbf{NQ} + \mathbf{Q}^T\mathbf{N} \\ & \leq & -\varepsilon \mathbf{I}_{n-1} \end{array} \tag{29}$$

because $-\mathbf{Q}$ is a *M-Matrix* (Berman and Plemmons, 1994). Thus, the dynamics of the enzymatic reactions admits as Lyapunov function the quadratic form

$$V(\mathbf{z}) = \mathbf{z}^T\mathbf{N}\mathbf{z},$$

where $\mathbf{z} = \mathbf{x} - \mathbf{x}^*$. See previous demonstration of (18). Therefore, under assumption $\mathcal{H}_1$, relation (27) gives the globally asymptotically stable steady state of the open loop system (21) for each constant input u. This verifies assumption $\mathcal{H}_7$.

Finally, as we have shown in the context of irreversible metabolic pathways (here $\mathbf{k}_x(.)$, $k_y(.)$ and $g^{-1}(.)$ have the same properties with respect to u as in the irreversible context), we can check the global convergence of the following scalar discrete time dynamical system

$$u_{j+1} = g(H_n(H_{n-1}(\ldots H_2(u_j)\ )), \tag{30}$$

to its unique fixed point $u^* \in (0, g_{max})$ by the same graphical test stated in assumption ($\mathcal{H}_3$). This completes the proof that Proposition 1 is a consequence of Theorem 1.

5 CONCLUSIONS

We have used in this paper the negative feedback theorem of monotone control SISO systems theory, to give technical propositions which prove global attractivity of linear metabolic pathways. For future works, we will consider the stability analysis for dynamical systems through monotone control MIMO systems. That will allow us to tackle the stability issue for complex bacterial metabolic networks.

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