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# Topology and static response of interaction networks in molecular biology

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We introduce a mathematical framework describing static response of networks occurring in molecular biology. This formalism has many similarities with the Laplace-Kirchhoff equations for electrical networks. We introduce the concept of graph boundary and we show how the response of the biological networks to external perturbations can be related to the Dirichlet or Neumann problems for the corresponding equations on the interaction graph. Solutions to these two problems are given in terms of path moduli (measuring path rigidity with respect to the propagation of interaction along the graph). Path moduli are related to loop products in the interaction graph via generalized Mason-Coates formulae. We apply our results to two specific biological examples: the lactose operon and the genetic regulation of lipogenesis. Our applications show consistency with experimental results and in the case of lipogenesis check some hypothesis on the behaviour of hepatic fatty acids on fasting.

**Keywords:** Systems biology, gene and metabolic networks, steady state shifts, Dirichlet to Neumann map

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## Introduction

Network-based representations are widely used to describe gene regulation or metabolic pathways at cellular level; graph theoretical properties of these biological networks, dynamical properties, as well as their inference from experimental data, were the subject of intensive studies (Kaminski and Friedman, 2002; Yamanishi et al., 2004; Wagner, 2001; Kholodenko et al., 2002; Thieffry and Sanchez, 2004; de Jong, 2002; de Jong et al., 2004). However, network inference techniques need huge amounts of data which are not always available or often have poor accuracy. Similarly, dynamical studies need accurate data.

In this paper, our goal is different. Instead of using data and gene perturbations for building networks from scratch, we develop mathematical techniques in order to refine the analysis of incomplete models or to compare models and data. Our mathematical results connect network topology and the response to steady state shift experiments. Steady state shift experiments are useful tools in chemistry allowing in principle to recover the reaction mechanisms (Chevalier et al., 1993). We argue that similar approaches are well adapted to differential microarray experiments which compare gene expressions between two different states (Kaminski and Friedman, 2002).

A theory of steady state shifts is in fact a theory of static response, linear when the shifts are small and non-linear when the shifts are big. Theories of linear response were developed in various contexts such as condensed matter and in statistical physics (Kubo et al., 1998), electrical, physical and mechanical systems (MacFarlane, 1970), chemical and biochemical systems (Oster et al., 1973; Perelson and Oster, 1974), complex fluids (Larson, 1988), metabolic networks (Kacser and Burns, 1973; Heinrich and Rapoport, 1974) and gene networks (Kholodenko et al., 2002; Vlad et al., 2004). The basic quantities in such theories are the susceptivities, representing derivatives (generically functional derivatives) of outputs with respect to inputs. Susceptivities (and also response in general) are obtained from constitutive equations that must be compatible with thermodynamics (Oster et al., 1973; Grmela, 2001). Nonlinear response theories that apply to large perturbations are generally more difficult to handle (Larson, 1988; Grmela, 2001). (Vlad et al., 2004) showed that under special "neutrality" conditions the response of reaction-diffusion systems to special perturbations consisting in changing the abundance of some marked individuals is linear even at large perturbations.

In the static case, structural stability (Ruelle, 1989; Smale, 1980) suggests a simple approach to nonlinear response. Like in classical thermodynamics (Callen,

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1985), finite static response can be obtained by integrating the (state dependent) differential forms whose coefficients are the susceptivities provided that the integration path does not contain singularities (phase transitions).

Susceptivities depend on the network topology and therefore can be used in reverse-engineering. This method is complementary to already well developed correlation analysis of gene and metabolic networks (Kaminski and Friedman, 2002; Yamanishi et al., 2004) or of time series in chemical reaction systems (A.Arkin and Ross, 1995). Vlad et al. (Vlad et al., 2004) showed that under the "neutrality" condition the dynamical susceptibility of a system of chemical reactions with respect to abundance perturbations is the exponential of a connectivity matrix, which contains information about the chemical reaction mechanism. It is known (Chevalier et al., 1993) that small concentration shifts measurements allow to calculate the Jacobian of a chemical kinetics evolution equations. Very related to this is the work of Sontag (Kholodenko et al., 2002) and the older Kacser-Burns connectivity property from metabolic networks (Cornish-Bowden, 1995), meaning that the matrix of elasticities (the Jacobian) is the inverse of the matrix of control coefficients (static susceptivities). The dynamical Jacobian is also important in gene networks dynamics. It defines the interaction graph (Soulé, 2003), which is the natural mathematical candidate to represent the biologist's gene regulation graph (Thomas, 1981). Systems of chemical reactions allow for other graphical representations (the interested reader could refer to the comprehensive reviews of (Oster et al., 1973; Perelson and Oster, 1974)).

In this paper we generalize Mason-Coates type formulas from electrical circuits (Mason, 1953; Coates, 1959), providing graphical representations of static susceptivities of gene networks.

We also exploit useful connections between the static response of gene networks and the Dirichlet-to-Neumann map (Curtis et al., 2000) which is a relatively new field in graph theory. This field originated from a problem by Calderon (Calderon, 1980) who asked whether it is possible to reconstruct the conductivity of a conducting plate from measurements of injected currents and voltages on its boundary. This problem gave rise to non-trivial developments in graph theory. Our most important result is related to the Dirichlet-to-Neumann map and consists in a systematic use of the notion of boundary. Boundaries serve to delimitate subgraphs or to couple subgraphs one with another like in (Kholodenko et al., 2002). We define response of subgraphs as solutions of the Dirichlet or the Neumann problems. The novelty of our method is that boundaries delimitating analysed subgraphs are chosen according to the missing piece of information.

This paper is organized as follows. In the first section we present the interaction graph, the subgraphs and their boundaries. In the second section we define linear response and discuss an electrical network analogy. In the third section we construct Mason-Coates solutions to the linear Dirichlet and Neumann problems and we extend the applicability of the results to nonlinear

response. Finally, we give two biological applications in the last section.

## 1. Steady state shifts, interaction graph

The state of a biological network is given by a vector  $X \in \mathbb{R}^n$ , whose components are concentrations of various interacting actors, such as DNA regions coding for genes, RNA transcripts, various produced and regulating proteins, metabolites.

Here we consider a differential dynamics for  $X$ ,  $\frac{dX}{dt} = F(X, P)$ ,  $F: \mathbb{R}^n \times \mathbb{R}^k \rightarrow \mathbb{R}^n$ , where  $P \in \mathbb{R}^k$  stems for a set of parameters. This assumption is rather general, because boolean and piecewise-deterministic dynamics can be approximated by differential inclusions (Gouzé and Sari, 2003). Although not proven in full generality but used in practice, dynamical systems with delays can be approximated by differential systems either by introducing extra variables (Belych, 1998) or by centre manifold techniques (Wang and Hu, 2001). The main restriction in our theory is that we consider non-degenerate stable steady states, which are hyperbolic fixed points of the dynamics. Steady states are solutions of the nonlinear system of equations:

$$F(X, P) = 0 \quad (1)$$

Changes in the control parameters  $P$  produce steady state shifts. If  $\det[J(X_0, P_0)] \neq 0$ , where  $J_{ij} = \frac{\partial F_i}{\partial X_j}$  is the Jacobian calculated at a non-degenerate stable steady state  $X_0$ , then from the implicit function theorem Eq.1 defines  $X$  as a function of  $P$  locally, in a neighborhood of  $(X_0, P_0)$ . This local dependence represents the static linear response of the system. In section 3.4 we shall give a global existence theorem for such a function.

*Interaction graph* The Jacobian  $J$  calculated in a state  $X$  introduces in a natural way a signed oriented graph, called the interaction graph which is a triplet  $(\mathcal{I}, \mathcal{E}, \sigma)$ . The set of nodes  $\mathcal{I} = \{1, \dots, n\}$  consists of the interacting actors. The set of oriented edges  $\mathcal{A} \subset \mathcal{I} \times \mathcal{I}$  is defined by  $(j, i) \in \mathcal{A}$ , iff  $J_{ij} \neq 0$ . The edge sign is a function  $\sigma: \mathcal{A} \rightarrow \{-1, 1\}$ ,  $\sigma(j, i) = \text{sign}[J_{ij}]$ . An edge  $(j, i)$  is called positive whenever  $\sigma(j, i) > 0$  and negative whenever  $\sigma(j, i) < 0$ .

From a biological point of view there is an arc from  $j$  to  $i$  if the actor  $j$  has a direct influence on the dynamics of  $i$ . This influence can be positive (activation) or negative (repression). Many examples can be considered. For instance  $j$  may be a transcription factor regulating the expression of  $i$ , or a protein involved in the phosphorylation or methylation of  $i$ , or an enzyme involved in the production of  $i$ , etc.

When  $F$  is non-linear, the signs of its partial derivatives may change, therefore the interaction graph generally depends on the state  $X$  where it is calculated. This is a weakness of the interaction graph compared with other more complex graphical representations such as "bond graphs" (Perelson and Oster, 1974; Oster et al., 1973). Nonetheless, this weakness is largely compensated by the fact that many qualitative properties of the dynamics and of steady states depend on topological

conditions on the interaction graph (Thomas, 1981; Soulé, 2003; Gouzé, 1998; Snoussi, 1998).

*Boundary* Boundary is a key concept of any modular approach trying to reduce the overwhelming complexity of large networks to understandable behaviour of simpler subsystems. Furthermore, data in molecular biology can be extensive but it is rarely complete. Our modular approach to data analysis can be used to fill in missing information, to check existing data, or to correct eventual errors.

Because there is no precise criterion to fix the boundary between the actors that we consider and those that we forget, we shall allow a free choice of the boundary. We shall see that instead of becoming a handicap, this freedom of choice becomes a handy tool.

The construction by which we freely choose a boundary in an interaction graph is the following. Let  $G$  be the set of nodes that we isolate in the larger set  $\mathcal{I}$ . The entrance boundary of  $G$ , denoted by  $\mathcal{T}^{in}G$  is the set of nodes of  $G$  that have incoming arcs from the exterior, i.e. from nodes of  $\mathcal{I}$  which are not nodes of  $G$ . The other nodes of  $G$  that are not directly influenced by the exterior are in the interior of  $G$  which is denoted  $\mathring{G} = G \setminus \mathcal{T}^{in}G$ .

*Paths, loops, loop partitions* A path  $c = i \rightsquigarrow j$  is a sequence of nodes  $(i_1 = i, i_2, \dots, i_p = j)$  such that  $(i_k, i_{k+1})$  is an edge of the graph and such that all the nodes in the sequence are visited just once. Notice that such a path exists in the interaction graph if  $J_{i_2 i_1} \neq 0, \dots, J_{j i_{p-1}} \neq 0$ . A path  $i \rightsquigarrow j$  in the interaction graph is called positive if the path product of the Jacobian elements  $a_{ij} = \prod_{k=1}^{p-1} J_{i_{k+1} i_k}$  is positive (negative).

A loop  $l = i \rightsquigarrow i$  is a sequence of nodes  $\{i_1 = i, i_2, \dots, i_p = i\}$  such that  $(i_k, i_{k+1})$  is an edge of the graph and such that all the nodes in the sequence are visited just once with the exception of the two terminals that coincide. A loop is called positive (negative) if the product of the Jacobian elements along it is positive (negative). Self-interaction loops  $(i, i)$  contain only one node and correspond to diagonal elements of the Jacobian  $J_{ii}$ .

A loop partition of a graph is a partition of the nodes into disjoint loops (including self-interaction loops). The set of loop partitions of a subgraph  $G$  is denoted  $\mathcal{L}(G)$ . For  $L \in \mathcal{L}(G)$ ,  $|L|$  denotes the number of loops in  $L$ . For instance if  $G = \{1, 2, 3\}$  and the oriented edges are  $\{(1, 1), (2, 2), (3, 3), (1, 2), (2, 3)\}$ ,  $\mathcal{L}(G)$  consists of two loop partitions  $L_1 = \{(1, 1), (2, 2), (3, 3)\}$ ,  $|L_1| = 3$  and  $L_2 = \{(1, 2, 3)\}$ ,  $|L_2| = 1$ .

## 2. Linear response of a network to influences from the exterior

### 2.1. Electrical networks, flowgraphs, Markov chains

Classical examples of linear networks are the linear electrical networks. Let us consider an electrical network with  $n$  nodes. Nodes  $i$  and  $j$  are connected by wires having admittances  $Y_{ij} = Y_{ji}$ . Injecting currents  $I_i$  in some or all of the nodes we produce the steady state

potentials  $V_i$ . Applying Ohm's law and Kirchoff's first law to the node  $i$  it follows that  $\sum_{j \neq i} Y_{ji}(V_j - V_i) + I_i = 0$ . In matrix form, the linear relation between node voltages and node current sources reads:

$$\tilde{Y}V = I. \quad (2)$$

$\tilde{Y}$  is the node admittance matrix and is obtained from the edge admittances:

$$\tilde{Y}_{ij} = \begin{cases} -Y_{ij} & \text{if } i \neq j \\ \sum_{j \neq i} Y_{ij} & \text{if } i = j. \end{cases} \quad (3)$$

In order to express the voltages for a given current configuration we need to solve the system of Kirchoff-Laplace equations 2.

Notice that the matrix  $\tilde{Y}$  is singular:  $(1, \dots, 1) \in \text{Ker}(\tilde{Y})$ . This comes from a special symmetry of electrical networks meaning that voltages are determined up to a constant and that the only measurable quantities are voltage differences.

There are many other examples coming from different fields of science where linear equations are interpreted as graphs.

Markov or semi-Markov processes on multistate stochastic networks occur frequently in biology and physics and can be used for data analysis. For instance the evolution of a disease can be treated as a series of stochastic transitions between various grades of disease (Yau and Huzurbazar, 2002). The possible transitions and transition probabilities can be gathered on a flowgraph. A flowgraph is a weighted oriented graph. Each edge is labeled by a transition rate (or transmittance, or gain) which is the probability  $p_{ji}$  to perform the jump from the state  $j$  to the state  $i$  divided by the mean waiting time  $\tau_{ji}$  from a state  $j$  to  $i$ :  $t_{ji} = p_{ji}/\tau_{ji}$ . The probabilities  $\pi_i$  of being in a state  $i$  satisfy the equilibrium equation  $\sum_k \pi_k t_{ki} = (\sum_k t_{ki})\pi_i$  which is the same as Eq. 2 with zero currents  $I = 0$ .

### 2.2. Analogy between steady state shifts and electrical networks

In order to obtain an analogy between linear response of electrical and biological networks, let us consider that there is a non-degenerated stable state  $X_0$  satisfying Eq.1 for parameters  $P_0$ . Supposing that dynamics is structurally stable the theorem on persistence of hyperbolic sets (Smale, 1980; Ruelle, 1989) implies that small variations of the parameters in Eq.1 produce shifts of this state without loss of stability. Differentiating the equilibrium equations for the nodes in a subgraph  $G$  (including the boundary nodes) we obtain:

$$\forall i \in G, \sum_{j \in G} \frac{\partial F_i}{\partial X_j} \delta X_j + \sum_{k \in \mathcal{I} \setminus G} \frac{\partial F_i}{\partial X_k} \delta X_k + \frac{\partial F_i}{\partial P} \delta P = 0. \quad (4)$$

Let us also consider that all the exterior influences on  $G$  are transmitted via its boundary. This means that:

$$\frac{\partial F_i}{\partial P} = 0, \forall i \in G. \quad (5)$$

The condition in Eq.5 is a modelling assumption, that can be lifted with minor changes. From a statical

point of view the parameters  $P$  play the same role as the other variables. If the modeler judges that Eq.5 is not reasonable, it is better to consider  $P$  as a variable and to connect it to the other variables.

Noticing that for all  $k \in I \setminus G$  and  $i \in \overset{\circ}{G}$ , it follows:

$$\forall i \in G, \sum_{j \in G} \frac{\partial F_i}{\partial X_j} \delta X_j = -\delta X_i^f, \quad (6)$$

where  $\delta X_i^f$  denote the ‘‘forcing variations’’ which are non-vanishing only on the boundary, and are defined by:

$$\delta X_i^f = \begin{cases} \sum_{k \in I \setminus G} \frac{\partial F_i}{\partial X_k} \delta X_k & \text{if } i \in \mathbb{T}^{in} G \\ 0 & \text{if } i \in \overset{\circ}{G}. \end{cases} \quad (7)$$

In matrix form Eq.6 reads:

$$(-J_G)\delta X = \delta X^f. \quad (8)$$

Eqs.8 and 2 are analogous. They represent the linear response of a network to influences from the exterior:

- the opposite Jacobian  $-J_G$  restricted to  $G$  and calculated at a non-degenerated stable steady state is analogous to the node admittance matrix  $\tilde{Y}$ ,
- the concentration variations  $\delta X$  are analogous to the voltages  $V$ ,
- the forcing variations  $\delta X^f$  are analogous to the injected currents  $I$ .

Eq.5 implies that the forcing variations are vanishing in the interior and are non-zero on the boundary of the subgraph  $G$ . For electrical networks this means that the influence of the exterior on a subnetwork can be represented by current sources on its boundary.

Electrical networks are different from biological networks because they have much more symmetries, such as:

- The matrix  $-J_G$  is not necessarily symmetric, while the matrix  $\tilde{Y}$  is always symmetric. The interaction graph is intrinsically oriented, while an electrical network is not oriented (a wire conducts in both directions).
- The diagonal elements of the matrix  $\tilde{Y}$  are obtained from the non-diagonal ones:  $\tilde{Y}_{ii} = -\sum_{j \neq i} \tilde{Y}_{ij}$ . The diagonal elements of the matrix  $J_G$  are independent of the non-diagonal ones.

### 3. Topological expressions for the linear response of networks

Throughout this section we consider that the Jacobian  $J_G$  is calculated at a non-degenerate steady state (stability can be imposed but it is not compulsory). For subgraphs, steady states are constrained by imposed boundary variations or forcings. To simplify notation we drop the index  $G$  in the Jacobian; it will be supposed that the  $J$  is restricted to a subset  $G$ , unless otherwise stated. Supposing that  $J$  is invertible, solutions of Eqs.8 can be obtained from the inverse matrix  $J^{-1}$ . In this section, the elements of  $J^{-1}$  are connected to the topology of the interaction graph.

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### 3.1. Moduli and loops

We start with the following well known relation (Bloom, 1979):

$$J_{ij}^{-1} = (-1)^{i+j} \frac{\Delta_{ji}}{\Delta} \quad (9)$$

where  $\Delta_{ji}$  is the minor obtained by deleting row  $j$  and column  $i$  in  $J$ , and  $\Delta = \det(J)$ .

Next, we develop the minor  $\Delta_{ji}$  into a sum of principal minors (Bloom, 1979):

$$\Delta_{ji} = \begin{cases} (-1)^{i+j} \sum_{j \rightsquigarrow i} (-1)^{l_{j \rightsquigarrow i}} a_{j \rightsquigarrow i} \Delta_{j \rightsquigarrow i} & \text{if } i \neq j \\ \Delta_j & \text{if } i = j. \end{cases} \quad (10)$$

where  $j \rightsquigarrow i$  is any path in the subgraph  $G$  leading from  $j$  to  $i$ ,  $l_{j \rightsquigarrow i}$  is the number of edges in the path,  $a_{j \rightsquigarrow i}$  is the product of elements of  $J$  along this path,  $\Delta_{j \rightsquigarrow i}$  is the principal minor obtained by deleting all the rows and columns whose indices are included in the path,  $\Delta_j$  is the principal minor obtained by deleting the line and the column  $j$ . Finally principal minors of the Jacobian can be related to loops in the subgraph  $G$ :

$$\Delta_{j \rightsquigarrow i} = \sum_{L \in \mathcal{L}(G_{j \rightsquigarrow i})} (-1)^{\dim(\Delta_{j \rightsquigarrow i}) - |L|} lp(L) \quad (11)$$

$$\Delta_i = \sum_{L \in \mathcal{L}(G_i)} (-1)^{\dim(\Delta_i) - |L|} lp(L) \quad (12)$$

$$\Delta = \sum_{L \in \mathcal{L}(G)} (-1)^{\dim(\Delta) - |L|} lp(L). \quad (13)$$

where  $lp(L)$  is the loop product defined as the product of elements of  $J$  along the loops of  $L$ .  $G_{j \rightsquigarrow i}$  is the subgraph obtained by deleting from  $G$  all nodes in the path  $j \rightsquigarrow i$ , while  $G_i$  is obtained by deleting node  $i$ .  $\dim$  denotes the dimension of the minor.

We now introduce a quantity later referred to as *modulus* which measures rigidity of the network and can be seen as the inverse of sensibility, as shown later:

**Definition 1.** *Let  $G$  be a subgraph of  $\mathcal{I}$ , let  $\Delta$ ,  $\Delta_i$ ,  $\Delta_{j \rightsquigarrow i}$  be minors of the Jacobian restricted to  $G$  and defined as above. The **modulus** of a path  $j \rightsquigarrow i$  is:*

$$C_{j \rightsquigarrow i} = (-1)^{l_{j \rightsquigarrow i} + 1} \frac{\Delta}{\Delta_{j \rightsquigarrow i}} = \frac{\sum_{L \in \mathcal{L}(G)} (-1)^{|L|} lp(L)}{\sum_{L \in \mathcal{L}(G_{j \rightsquigarrow i})} (-1)^{|L|} lp(L)}. \quad (14)$$

The **modulus** of a node  $i$  is:

$$C_i = -\frac{\Delta}{\Delta_i} = \frac{\sum_{L \in \mathcal{L}(G)} (-1)^{|L|} lp(L)}{\sum_{L \in \mathcal{L}(G_i)} (-1)^{|L|} lp(L)}. \quad (15)$$

We shall usually consider non-degenerate steady states and  $\Delta \neq 0$ , therefore moduli never vanish; they can instead diverge when minors of  $J$  vanish.

### 3.2. Neumann and Dirichlet problems

In electrical networks, fulfillment of Kirchhoff-Laplace Eqs. 2 implies a unique set of interior voltages (up to a constant) due to imposed boundary voltages (Dirichlet problem) or a unique set of voltages (up to a constant) everywhere due to imposed boundary currents (Neumann problem). For biological networks, the Neumann and the Dirichlet problems have the following equivalents:

- Linear Dirichlet problem: determine the variations  $\delta X_i, i \in \mathring{G}$  of the interior nodes, when the values of the variations are imposed (or known) on the boundary  $\delta X_i, i \in \mathbb{T}^{in}G$ . For biological networks the Dirichlet problem shows its utility when one possesses only a partial knowledge of the system (Kuipers, 1994; Siegel et al., 2005). We can define the corresponding Dirichlet static susceptibilities:

$$\chi_{ij}^D = \frac{\partial X_i}{\partial X_j}, i \in \mathring{G}, j \in \mathbb{T}^{in}G. \quad (16)$$

- Linear Neumann problem: determine the variations  $\delta X_i, i \in G$  everywhere, when the forced variations are imposed on the boundary  $\delta X_i^f, i \in \mathbb{T}^{in}G$ . Unlike injected currents forced variations (defined by Eqs.7) can not be measured directly. In electrical networks currents can be measured as voltages across calibrated resistors of apparatuses coupled to the network. We can not see what can be a calibrated molecular apparatus for biological networks. In spite of this drawback, the Neumann problem is important in theory of control of biological networks: it describes shifts of equilibria under constraints imposed by the change of external conditions. The corresponding static susceptibilities are defined as:

$$\chi_{ij}^N = \frac{\partial X_i}{\partial X_j^f}, i \in G, j \in \mathbb{T}^{in}G. \quad (17)$$

The following theorem states the existence and gives a solution to the Neumann problem.

**Theorem 1.** *Let  $G$  be a subgraph of  $\mathcal{I}$ . Let  $J$  be the restriction of the Jacobian of  $F$  to  $G$ , calculated at a non-degenerated, Neumann constrained (given forcings) steady state:  $J_{ij} = \frac{\partial F_i}{\partial X_j}, i, j \in G$ . Let  $\mathcal{P}_G$  denote the set of paths included in  $G$ . If  $\det(J) \neq 0$  and if there is no direct influence of the parameters on the nodes of  $G$ , i.e.  $\frac{\partial X_i}{\partial P_k} = 0, \forall i \in G$ , then the response of  $i \in G$  to small changes of the exterior of  $G$  satisfies:*

$$\text{if } i \in \mathring{G}, \delta X_i = \sum_{j \in \mathbb{T}^{in}G} \sum_{j \rightsquigarrow i \in \mathcal{P}_G} \frac{a_{j \rightsquigarrow i}}{C_{j \rightsquigarrow i}} \delta X_j^f, \quad (18)$$

$$\text{if } i \in \mathbb{T}^{in}G, \delta X_i = \frac{\delta X_i^f}{C_i} + \sum_{\substack{j \in \mathbb{T}^{in}G, \\ j \neq i}} \sum_{j \rightsquigarrow i \in \mathcal{P}_G} \frac{a_{j \rightsquigarrow i}}{C_{j \rightsquigarrow i}} \delta X_j^f \quad (19)$$

**Remark 1.** We have supposed that  $\Delta = \det(J) \neq 0$ . According to Eqs.14,15, we can supplement Eqs.18,19 with the following formal rule coping with diverging moduli:  $1/\infty = 0$ .

**Remark 2.** Using a language coming half from mechanics, half from flowgraphs we can say that a “force”  $a_{j \rightsquigarrow i} \delta X_j^f$  propagates from the boundary node  $j$  along the path  $j \rightsquigarrow i$ . This force is bigger when the product of interaction coefficients  $\frac{\partial F_{i_{k+1}}}{\partial X_{i_k}}$  along the path is bigger.  $C_{j \rightsquigarrow i}$  is the ratio force/response and therefore can be called “path modulus”. A large path modulus implies a small response at the end of the path, even if

the force is big. Therefore, the modulus is the inverse of sensitivity.

Eq.18 expresses interior  $\delta X_i$  as functions of the boundary forcings  $\delta X_j^f$ . Eq.19 gives the relation between forcings and variations on the boundary. The corresponding linear mapping  $\Lambda^{ND}: \mathbb{R}^m \rightarrow \mathbb{R}^m$  ( $m = \#\mathbb{T}^{in}G$ ,  $\Lambda_{ij}^{ND} = \sum_{j \rightsquigarrow i \in \mathcal{P}_G} \frac{a_{j \rightsquigarrow i}}{C_{j \rightsquigarrow i}} + \frac{1}{C_i} \delta_{ij}$ ) is the equivalent of the Neumann-to-Dirichlet map for electrical networks (Curtis et al., 1991). The inverse (if it exists) of this mapping is the Dirichlet-to-Neumann map (Curtis et al., 1991).

Theorem 1 implies the following:

**Corollary 1.** *If there are no paths in  $\mathcal{P}_G$  connecting two different nodes on the boundary (boundary is not necessarily disconnected because connections passing through the exterior are allowed), and if for all nodes  $i \in \mathbb{T}^{in}G$ , moduli  $C_i$  are finite and non-vanishing, then the Neumann-to-Dirichlet mapping is diagonal and its inverse exists. In this case, the forcings are proportional to the variations on the boundary:*

$$\delta X_i^f = C_i \delta X_i, \quad i \in \mathbb{T}^{in}G \quad (20)$$

By using the Dirichlet-to-Neumann map we can associate to any solution of the Neumann problem, a solution of the Dirichlet problem. We can thus obtain formulae for the solutions of the Dirichlet problem. In (Siegel et al., 2005) we have used a direct method to obtain solutions to the Dirichlet problem. Briefly, we have used equilibrium equations for the interior nodes in the same way as in Theorem 1 (which uses equilibrium equations for all the nodes including the boundary). Let us state the result:

**Theorem 2.** *Let  $G$  be a subgraph of  $\mathcal{I}$ . Let  $\mathring{G} = G \setminus \mathbb{T}^{in}G$  be the interior of  $G$ . Let  $\mathring{J}$  be the restriction of the Jacobian of  $F$  to  $\mathring{G}$  calculated in a non-degenerated, Dirichlet constrained (given boundary values, free interior) steady state:  $\mathring{J}_{i,j} = \frac{\partial F_i}{\partial X_j}, i, j \in \mathring{G}$ . Let us suppose that  $\mathring{\Delta} = \det(\mathring{J}) \neq 0$  and that there is no direct influence of the parameters on the interior nodes of  $G$ , i.e.  $\frac{\partial F_i}{\partial P_k} = 0, \forall i \in \mathring{G}$ .*

*Then, the response of  $i \in \mathring{G}$  to small changes on the boundary of  $G$  is given by:*

$$\delta X_i = \sum_{j \in \mathbb{T}^{in}G} \sum_{j \rightsquigarrow i \in \mathcal{P}_G} \frac{a_{j \rightsquigarrow i}}{C_{j \rightsquigarrow i}} \delta X_j. \quad (21)$$

- $\mathcal{P}_G$  denotes the set of paths included in  $G$ , starting on the boundary and that do not return to the boundary.
- $\mathring{C}_{j \rightsquigarrow i} = (-1)^{l_{k(j) \rightsquigarrow i} + 1} \frac{\mathring{\Delta}}{\Delta_{k(j) \rightsquigarrow i}}$  denotes the path modulus of the internal path  $k(j) \rightsquigarrow i$  where  $k(j) \in \mathring{G}$  is the second node after  $j$  of the path  $j \rightsquigarrow i$ . If  $k(j) = i$ , then  $\mathring{C}_{j \rightsquigarrow i} = \mathring{C}_i = -\frac{\mathring{\Delta}}{\Delta_i}$

**Remark 3.** Theorems 1,2 make no assumption on the connectivity of the subgraph  $G$ . For disconnected internal nodes we should have  $\delta X_i = 0$ , meaning that at steady states  $X_i$  does not depend on the parameters. For such a node the stationarity equations read  $F_i(X_i) = 0$ .

Eqs.14,15 give the loop products expression of moduli. They generalize Mason and Coates gain formulae (Mason, 1953; Coates, 1959) from electrical networks. Susceptivities are related to moduli according to the following equations:

$$\chi_{ij}^N = \sum_{j \rightsquigarrow i \in \mathcal{P}_G} \frac{a_{j \rightsquigarrow i}}{C_{j \rightsquigarrow i}} + \frac{1}{C_i} \delta_{ij} \quad (22)$$

$$\chi_{ij}^D = \sum_{j \rightsquigarrow i \in \mathcal{P}_{\hat{G}}} \frac{a_{j \rightsquigarrow i}}{C_{j \rightsquigarrow i}} \quad (23)$$

Let us notice that the restriction of the Neumann susceptibility matrix to the boundary  $(\chi^N|_{\mathcal{T}^{in}G})_{ij} = \chi_{ij}^N$ ,  $i, j \in \mathcal{T}^{in}G$  is the Neumann-to-Dirichlet map.

### 3.3. Signs of moduli

The signs of moduli are important for qualitative discussions of the transmission of influences (Siegel et al., 2005). Eqs.22,23 lead to an interesting possibility. Even if all the paths from  $j$  to  $i$  correspond to globally positive regulation (path products  $a_{j \rightsquigarrow i}$  are all positive), it is possible to have negative susceptibility  $\chi_{ij}$ , which means negative correlation between  $X_i$  and  $X_j$ . This possibility occurs when the moduli  $C_{j \rightsquigarrow i}$  are negative (see Section 4.2) and is a feed-back effect. Indeed, from Eqs.14,15 it follows:

**Property 1.** *If the subgraph  $G$  contains no positive loops for a given non-degenerate constrained steady state, then all its path moduli  $C_{j \rightsquigarrow i}$  and node moduli  $C_i$  are positive for that state.*

### 3.4. Significance and limits of the approach

Eqs. 18,19,21 should be interpreted as differential constraints connecting small variations of the concentrations at steady state, when this steady state is shifted as a result of the change of parameters. The significance of these constraints is the propagation of influence along the interaction graph: for any analyzed subset of the interacting species concentration variations of internal species are obtained from concentration variations of boundary species. Mathematically, these constraints express a local functional relation between the internal variables and the boundary variables, which follows from the implicit function theorem. Let us notice that we have not addressed yet the question of the global existence of an unique implicit function expressing the internal variables as functions of the boundary variables (the non-linear Dirichlet problem). The existence of such a function depends on the non-linearities of the system and can not be proven without further additional hypothesis. Without being exhaustive, let us give here some results applying to particular cases.

Let us consider that for certain subsets  $G \subset \mathcal{I}$  we are able to specify global interiors, i.e. sets of species that are never directly influenced by external species, whatever the concentrations. The steady state equations for internal species constrained by boundary species read:

$$F_i(X, X') = 0, i \in \hat{G} \quad (24)$$

where  $X, X'$  have coordinates in  $\hat{G}$ ,  $\mathcal{T}^{in}G$ , respectively.

The implicit function theorem implies that  $X$  is a function of  $X'$  in a neighbourhood of any non-degenerate steady state satisfying  $\det(\hat{J}) \neq 0$ . The global existence of the implicit function follows from an existence result for the solution of the system 24 and from the following global univalence theorem ensuring the uniqueness of this solution:

**Theorem 3.** *Let us consider that  $F_i(X, X') = \Phi_i(X, X') - \lambda_i X_i$ ,  $i \in \hat{G}$  where  $\lambda_i > 0$  and  $\Phi_i(X, X')$  are differentiable, bounded and satisfy*

$$\Phi_i(\dots, X_i = 0, \dots, X') > 0 \quad (25)$$

*Then for any  $X'$  the system 24 has at least a solution  $X$  such that all concentrations  $X_i$ ,  $i \in \hat{G}$  are positive.*

*Let us consider that the interaction subgraph corresponding to the interior of  $G$  has no positive loop, whatever the concentrations may be. Then the solution of the system 24 is unique for any  $X'$ .*

We shall only give a brief outline of the proof. The first part of the Theorem 3 is based on the following standard mathematical lemma which is a consequence of Poincaré-Hopf formula:

**Lemma 1.** *Let  $D$  be a smooth ball in a metric space, let  $S$  be the boundary of  $D$ . Let  $F$  be a vector field defined on a neighbourhood of  $D$ . If  $F$  points inward  $D$  at any point of  $S$ , then  $F$  admits a zero in the interior of  $D$ .*

The second part of the theorem is known as the Thomas's conjecture (Thomas, 1981; Gouzé, 1998; Snoussi, 1998; Soulé, 2003) and can be proven as follows. When the subgraph  $G$  has no positive loops whatever the concentrations, Eqs.11,12,13 imply that all the minors of the opposite Jacobian  $-J$  are positive. From this and from the Gale-Nikaido-Inada theorem (Parthasarathy, 1983) it follows the uniqueness of the equilibrium. The complete proof of Thomas's conjecture can be found in (Soulé, 2003).

The conditions of the first part of the Theorem 3 are fulfilled by gene networks. Indeed, the terms  $\lambda_i X_i$  correspond to degradation or to dilution produced by cell growth. The production terms  $\Phi_i$  are bounded because they saturate at large concentrations. Eq.25 is natural, meaning that concentrations can never become negative.

We come now to the experimental significance of our approach. The type of experiment that we consider is the following: the set of parameters is changed such that we start and we end in a non-degenerate stable steady state. If the conditions of Theorem 3 are fulfilled then the nonlinear Dirichlet problem has a solution and the internal variables are constrained to be functions of the boundary variables. The finite variations of these functions between the start and the end of the experiment can be obtained by integrating the differential form in Eq.21 along any differentiable path  $\mathcal{D}$  connecting the two steady states and made of non-degenerate constrained steady states (non-degenerate

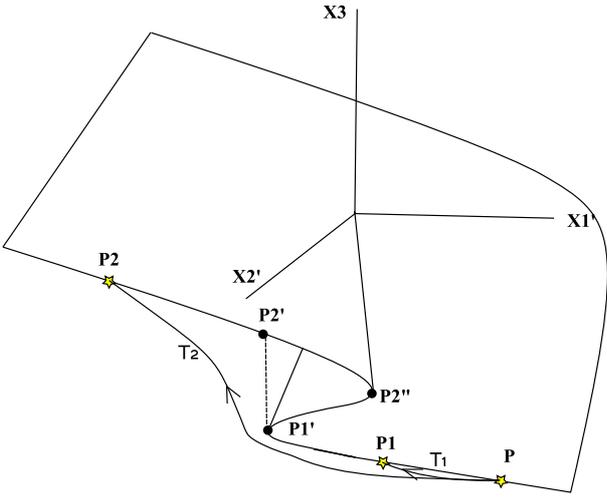


Figure 1. Cusp catastrophe. Two trajectories  $T_1$  and  $T_2$  start in the steady state  $P$  and end in the steady states  $P_1$  and  $P_2$  respectively. In order to find the variation of the internal variable  $X_3$  one could integrate the differential response along the smooth branch  $P P_1$  (for  $T_1$ ) and along the branches  $P P_1 P_1'$  and  $P_2' P_2$  (for  $T_2$ ). For  $T_2$  the finite jump  $P_1' P_2'$  should be added.

solutions of Eq.24).

$$\Delta X_i = \int_{\mathcal{D}} \sum_{j \in \mathcal{T}^{in} G} \sum_{j \rightarrow i \in \mathcal{P}_{\dot{G}}} \frac{a_{j \rightarrow i}}{C_{j \rightarrow i}} dX_j. \quad (26)$$

The uniqueness of the implicit function ensures the path independence of the result. We must emphasize that Eq.26 is not destined to provide numerical values. It serves to identify the signs of different contributions to the concentration variations and leads to qualitative equations in sign algebra (Kuipers, 1994) (details can be found in (Siegel et al., 2005)). Similar results hold for shifts of Neumann constrained steady states (nonlinear Neumann problem).

This result fails when the steady state shift meets a singular point, defined by vanishing moduli (infinite sensitivity). Then the steady state loses stability. The new attractor is either a distance apart (for instance in the cusp catastrophe), or it has a different type (for instance in the Hopf bifurcation a point attractor becomes a stable limit cycle) from the old one.

The cusp catastrophe occurs rather often in molecular biology. It occurs in the well studied lactose operon, that we shall present in the next section. This situation is represented in Fig.3.4. The boundary variables  $X_1', X_2'$  influence the internal variable  $X_3$ . Suppose that  $X_1'$  changes. Then the state of the system moves along the trajectories starting from  $P$ . For small changes ending in  $P_1$  there is a differentiable path of stable steady states connecting  $P$  and  $P_1$  and we can apply Eq.26. For large changes ending in  $P_2$  there is no such path. The steady state shift is discontinuous: the state jumps from the attractor branch  $P P_1 P_1'$  to the branch  $P_2'' P_2' P_2$ . Eq.26 has to be changed by adding a finite jump  $X_3(P_2') - X_3(P_1')$ .

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## 4. Examples

### 4.1. Lactose operon

The main enzymes for the lactose (L) metabolism in *E. coli* are LacY (lactose permease) allowing the uptake of lactose, LacZ ( $\beta$ -galactosidase) catalyzing the degradation of lactose to glucose (Mackey et al., 2004; Yildirim and Mackey, 2003).

The transcriptional regulators for the genes *lacY* and *lacZ* are an activator (CRP) and a repressor (LacI). An inducer (cAMP) binds to the activator and triggers it. Lactose, under an isomeric form named allolactose, binds to the repressor and inhibits it. The glucose inhibits the activator. The interaction graph for this system is represented in Fig.2. The exterior and interior lactose are denoted  $L^e, L^i$ , respectively.

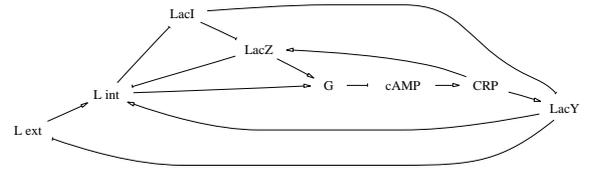


Figure 2. Operon lactose interaction network.  $L^{int}$  and  $L^{ext}$  stand for  $L^i$  and  $L^e$  respectively.

Negative self-interaction loops should be added to each node, in order to take into account degradation processes or dilution produced by cell growth. These correspond to negative diagonal elements for the Jacobian (self-interactions).

There are four loops in the interaction graph: two positive (even number of negative regulations) ones  $l_1^+ = \{L^i, LacI, LacY, L^i\}$ ,  $l_2^+ = \{L^i, G, cAMP, CRP, LacZ, L^i\}$ , and three negative (odd number of negative regulations) ones  $l_1^- = \{L^i, LacI, LacZ, L^i\}$ ,  $l_2^- = \{G, cAMP, CRP, LacZ, G\}$ ,  $l_3^- = \{L^i, G, cAMP, CRP, LacY, L^i\}$ . The existence of positive loops is a necessary condition (Soulé, 2003) for the observed bistability of the operon lactose.

Experiments show that when  $L^e$  is increased, the operon switches from a transcriptionally blocked, lactose poor state to a transcriptionally active, lactose rich state. We intend to check whether the linear response of the model given in Fig. 2 is coherent to observed variations, namely whether the Dirichlet susceptibility  $\chi_{L^i, L^e}^D$  is positive.

From Theorem 2,  $\chi_{L^i, L^e}^D = \frac{a_{L^e \rightarrow L^i}}{C_{L^e \rightarrow L^i}}$ , where  $a_{L^e \rightarrow L^i} > 0$  from the construction of the model (see Fig.2). This means that the influence of  $L^e$  on  $L^i$  is positive provided that the modulus of the path  $L^e \rightsquigarrow L^i$  is positive. The modulus is given by:

$$\begin{aligned} \dot{C}_{L^e \rightarrow L^i} &= \\ &= \chi_{L^i} \frac{1 - \sum_{i=1}^2 \tilde{l}p(l_i^+) - \sum_{i=1}^3 \tilde{l}p(l_i^-) + \tilde{l}p(l_1^+) \tilde{l}p(l_2^-)}{1 - \tilde{l}p(l_2^-)} \end{aligned} \quad (27)$$

where  $\chi_{L^i}$  is the absolute value of the self-interaction on  $L^i$ ,  $\tilde{l}p$  is the loop product divided by the product of absolute values of self-interactions for nodes in the

loop.  $\tilde{l}p$  is positive for positive loops and negative for negative loops.

The condition for a monotonic response is given by the following:

**Property 2.** *The dependence of  $L^i$  on  $L^e$  is monotonically increasing provided that the modulus  $\dot{C}_{L^e \sim L^i}$  is positive, i.e.:*

$$\tilde{l}p(l_1^+)[1 - \tilde{l}p(l_2^-)] + \tilde{l}p(l_2^+) < 1 - \sum_{i=1}^3 \tilde{l}p(l_i^-). \quad (28)$$

The monotonicity condition 28 is valid for small positive loops products. The typical scenario for strong positive loops is the cusp catastrophe: the operon becomes bistable and its response is discontinuous and hysteretic (Mackey *et al.*, 2004). Bistability requires the existence of a branch of states with negative moduli on which the condition 28 is broken, but this branch is usually unstable (see also next subsection).

The susceptibility approach can predict the effect of gene knock-outs. For instance, a knock-out of the inducer or of the activator cancels the loops  $l_2^+$ ,  $l_2^-$ ,  $l_3^-$ . According to Eq.27, the modulus increases, therefore the uptake of lactose decreases, provided that  $\tilde{l}p(l_2^+) > -\tilde{l}p(l_2^-)\tilde{l}p(l_1^-) - \tilde{l}p(l_3^-)$ . Similarly, a knock-out of the repressor cancels the loops  $l_1^+$ ,  $l_1^-$ . The uptake of lactose decreases provided that  $\tilde{l}p(l_1^+) > \tilde{l}p(l_1^+)\tilde{l}p(l_2^-) - \tilde{l}p(l_1^-)$ .

#### 4.2. Negative moduli and non-monotonic response

For the lactose operon the input/output relation relating outside and inside lactose is monotonic and the moduli for stable branches are positive. We build here an artificial example of switch with non-monotonic response.

Let us suppose that  $X_1$  regulates positively  $X_2$  and that the modulus  $\dot{C}_{12} < 0$ . Then increasing  $X_1$  produces a decrease in  $X_2$ . In order to create an example of this kind one should consider at least another component  $X_3$  of the system. Indeed, stability asks for eigenvalues of the Jacobian to be in the left half of the complex plane; in dimension 2 this is equivalent to the absence of positive loops, hence moduli are positive at steady states (Property 1). A simple example illustrating the possibility of having negative moduli is:

$$\begin{cases} \frac{dx_1}{dt} = -x_1 + 1 + \lambda, \\ \frac{dx_2}{dt} = -2x_2 - x_3 + x_1, \\ \frac{dx_3}{dt} = f(x_3) + 3x_2 \end{cases} \quad (29)$$

with  $f(x) = -ax(x-1)(x-2)$ . The steady state is shifted by changing the parameter  $\lambda$ , which at equilibrium is equivalent to changing the boundary value  $x_1 = 1 + \lambda$ .

The solution of the Dirichlet problem is  $\delta x_2 = \frac{\delta x_1}{\dot{C}_{12}}$ ,  $\delta x_3 = \frac{\delta x_1}{\dot{C}_{13}}$ , where

$$\dot{C}_{12} = \left[ 3 - 2 \frac{df}{dx_3} \right] / \left[ -\frac{df}{dx_3} \right] \quad (30)$$

$$\dot{C}_{13} = 3 - 2 \frac{df}{dx_3}. \quad (31)$$

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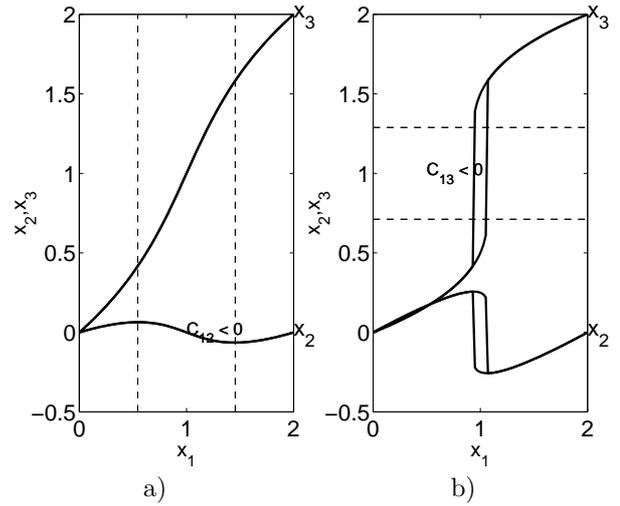


Figure 3. Solution of the Dirichlet problem for the model in Eq.4.2: stable equilibrium values of  $x_2$  and  $x_3$  as a function of the boundary value  $x_1$ . a) Monostable ( $a = 0.5$ ) with a region (between dotted vertical lines) of negative modulus  $\dot{C}_{12} < 0$ . b) Bistable ( $a = 2$ ) with discontinuous, hysteretic response; the response of  $x_2$  is non-monotonic, the response of  $x_3$  is monotonic.  $\dot{C}_{13}$  can be negative when  $x_3$  is between the two dotted horizontal line, but the corresponding states are unstable.

The model has unique equilibrium for  $a < 3/2$  and is bistable for  $a > 3/2$ . In the monostable regime ( $a < 3/2$ )  $\dot{C}_{13} > 0$  everywhere;  $\dot{C}_{12} \leq 0$  when  $x_3$  is inside the interval  $I_1 = [1 - 1/\sqrt{3}, 1 + 1/\sqrt{3}]$  and is positive outside the interval  $I_1$ . For  $x_3 = 1 - 1/\sqrt{3}, 1 + 1/\sqrt{3}$ ,  $\frac{1}{\dot{C}_{12}}$  and therefore the susceptibility  $\chi_{21}^D$  vanishes: the response curve for  $x_2$  represented in Fig.4.2 have a maximum and a minimum at these points.

For  $a > 3/2$ , the system is bistable. We have  $\dot{C}_{12} > 0$  unless when  $x_3 \in I_2 = [1 - 1/\sqrt{3}, 1 - \sqrt{1/3 - 1/(2a)}] \cup [1 + \sqrt{1/3 - 1/(2a)}, 1 + 1/\sqrt{3}]$  and  $\dot{C}_{13} > 0$  unless when  $x_3 \in I_3 = [1 - \sqrt{1/3 - 1/(2a)}, 1 + \sqrt{1/3 - 1/(2a)}]$ . The region  $I_3$  where  $\dot{C}_{13} < 0$  is in fact unstable and is never reached. When  $x_1$  increases  $x_3$  jumps discontinuously from one attractor to another without taking values inside the interval  $I_3$ . The jump occurs at different values of  $x_1$  on increase and decrease of the control parameter. Thus the response of  $x_3$  is discontinuous and hysteretic, but monotonic. The response of  $x_2$  is also discontinuous and hysteretic, but non-monotonic. There is a small region on the response curve of  $x_2$  where  $\dot{C}_{12} < 0$ ; this region corresponds to the intersection between the stability domain for  $x_3$  and  $I_2$ .

#### 4.3. Regulation of lipogenesis in hepatocytes

*Interaction model* Two ways of production of fatty acids coexist in liver. Saturated and mono-unsaturated fatty acids (MUFA) are produced from citrates thanks to a metabolic pathway composed of four enzymes, namely ACL (ATP citrate lyase), ACC (acetyl-Coenzyme A carboxylase), FAS (fatty acid synthase) and SCD1 (Stearoyl-CoA desaturase 1). Polyunsaturated fatty acids (PUFA) are synthesized from essential

fatty acids provided by nutrition; D5D (Delta-5 Desaturase) and D6D (Delta-6 Desaturase) catalyze the key steps of the synthesis of PUFA.

PUFA plays pivotal roles in many biological functions; among them, they regulate the expression of genes that impact on lipid, carbohydrate, and protein metabolism. The effects of PUFA are mediated either directly through their specific binding to various nuclear receptors (PPAR $\alpha$  – peroxisome proliferator activated receptors, LXR $\alpha$  – Liver-X-Receptor  $\alpha$ , HNF-4 $\alpha$ ) leading to changes in the trans-activating activity of these transcription factors; or indirectly as the result of changes in the abundance of regulatory transcription factors (SREBP-1c – sterol regulatory element binding-protein-, ChREBP, etc.) (Jump, 2004).

We consider in our model nuclear receptors PPAR $\alpha$ , LXR $\alpha$ , SREBP-1c (denoted by PPAR, LXR, SREBP respectively in the model), as they are synthesized from the corresponding genes and the trans-activating active forms of these transcription factors, that is, LXR-a (denoting a complex LXR $\alpha$ :RXR $\alpha$ ), PPAR-a (denoting a complex PPAR $\alpha$ :RXR $\alpha$ ) and SREBP-a (denoting the cleaved form of SREBP-1c). SCAP – (SREBP cleavage activating protein) is a key enzyme involved in the cleavage of SREBP-1c. We also include in the model “final” products, that is, enzymes ACL, ACC, FAS, SCD1 (implied in the fatty acid synthesis from citrate), D5D, D6D (implied in PUFA synthesis) as well as PUFA themselves.

Relations between the variables are the following. SREBP-a is an activator of the transcription of ACL, ACC, FAS, SCD1, D5D and D6D (Jump, 2004). LXR-a is a direct activator of the transcription of SREBP and FAS, it also indirectly activates ACL, ACC and SCD1 (Steffensen and Gustafsson, 2004). Notice that these indirect actions are kept in the model because we don't know whether they are only SREBP-mediated. PUFA activates the formation of PPAR-a from PPAR, and inhibits the formation of LXR-a from LXR as well as the formation of SREBP-a (by inducing the degradation of mRNA and inhibiting the cleavage) (Jump, 2004). SCAP represents the activators of the formation of SREBP-a from SREBP, and is inhibited by PUFA. PPAR directly activates the production of SCD1, D5D, D6D (Miller and Ntambi, 1996; Tang et al., 2003; Matsuzaka et al., 2002).

The interaction graph for this model is shown in Fig.4. Like in the lactose operon example, for each node we have supposed the existence of negative self-interaction loops.

**Fasting-refeeding protocols** The fasting-refeeding protocols are suited for studying lipogenesis regulation; during an experimentation, animals (rodents or chicken) were kept fasting during several hours and then refed. Hepatic mRNA of LXR, SREBP, PPAR, ACL, ACC and SCD1 were quantified by DNA microarray analysis. PUFA variations were determined by biochemical measurements.

A compilation of recent literature on lipogenesis regulation provides results of such protocols: SREBP, ACL, ACC, FAS and SCD1 decline in liver during

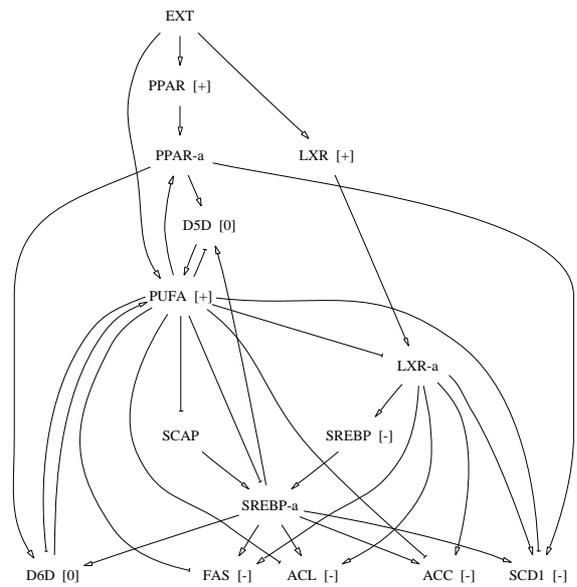


Figure 4. Interaction graph for a model of regulation of the synthesis of fatty acids. The node EXT represents the exterior world. Negative self-interaction loops on nodes are omitted for sake of clarity. Labelled loops:  $l_1^- = \{PUFA, SCAP, SREBP, SREBP-a, D6D, PUFA\}$ ,  $l_2^- = \{PUFA, SREBP, SREBP-a, D6D, PUFA\}$ ,  $l_3^- = \{PUFA, LXR, LXR-a, SREBP, SREBP-a, D6D, PUFA\}$ ,  $l_4^- = \{PUFA, D6D, PUFA\}$ ,  $l^+ = \{PUFA, PPAR, D6D, PUFA\}$ . Labelled paths  $p = \{PUFA, LXR, LXR-a, SREBP\}$ ,  $p' = \{LXR, LXR-a, SREBP\}$ ,  $p_1 = \{PUFA, D6D\}$ ,  $p_2 = \{PUFA, SCAP, SREBP, SREBP-a, D6D\}$ ,  $p_3 = \{D6D, PUFA\}$ ,  $p_4 = \{PUFA, LXR, LXR-a, SREBP, SREBP-a, D6D\}$ ,  $p_5 = \{LXR, LXR-a, SREBP, SREBP-a, D6D\}$ ,  $p_6 = \{PPAR, PPAR-a, D6D\}$ ,  $p_7 = \{PUFA, PPAR, D6D\}$ ,  $p_8 = \{PUFA, SREBP, SREBP-a, D6D\}$ ,  $p_9 = \{PPAR, PPAR-a, D6D, PUFA\}$ ,  $p_{10} = \{LXR, LXR-a, SREBP, SREBP-a, D6D, PUFA\}$ .

fasting (Liang et al., 2002); this state is characterized by an inhibition of fatty acid synthesis and an activation of the fatty acid oxydation. However, Tobin et al ((Tobin et al., 2000)) showed that fasting rats for 24h increased the hepatic LXR mRNA and Matsuzaka et al (Matsuzaka et al., 2002) observe no difference in either the hepatic D5D or D6D mRNA level between fasting and refeeding in normal mouse livers.

**4.3.1. Static response and topology** One of the advantages of the approach that we present here is that we have no difficulty in focusing on subgraphs of a large network. In order to illustrate this possibility we considered the following biological questions:

**Question 1** Lee et al. studied the fatty acids profiles in triglycerides TG, which are the predominant (> 50%) hepatic lipids and also in phospholipids PL which go into cellular membranes. Moreover PL contribute much less than TG to the total lipid mass. These authors (Lee et al., 2004) show that after 72h of fasting ((Lee et al., 2004)) fatty acids profiles do not change significantly in PL, but there is a strong increase of TG and of their fatty acids constituents, in particular PUFA. Let us recall that TG are transient storage forms of fatty acids. Based on these experimental findings, we make

the hypothesis of a mass increase of regulating PUFA in the hepatic cell. Can we prove this hypothesis indirectly by using the incomplete model, that is, considering that all variations excluding PUFA are known?

**Question 2** The dual regulation of desaturases SCD1, D5D and D6D by SREBP and PPAR is paradoxical because SREBP transactivates genes for fatty acid synthesis in liver, while PPAR induces enzymes for fatty acid oxidation. Furthermore, all three desaturases have similar regulation. Nonetheless, on fasting SCD1 decreases, while D5D, D6D have non significant variations. How does the model cope with this?

In order to answer to the Question 1, let us consider the Dirichlet problem for the subgraph  $G_1 = \{ \text{PUFA, LXR, LXR-a, SREBP} \}$ . From Theorem 2 and the paths labels from Fig.4 it follows:

$$\delta \text{SREBP} = \tilde{a}_p \delta \text{PUFA} + \tilde{a}_{p'} \delta \text{LXR}. \quad (32)$$

Because  $\tilde{a}_p < 0$ ,  $\tilde{a}_{p'} > 0$ ,  $\delta \text{SREBP} < 0$ ,  $\delta \text{LXR} > 0$ , the only possible variation compatible with Eq.32 is  $\delta \text{PUFA} > 0$ . Applying a similar reasoning to the subgraph  $G_2 = \{ \text{PUFA, LXR, LXR-a, SREBP, SREBP-a, SCAP, FAS} \}$  we have:

**Property 3.** *The only possible variation compatible with  $\delta \text{SREBP} < 0$  and  $\delta \text{LXR} < 0$  is  $\delta \text{PUFA} > 0$ . The only possible variation compatible with  $\delta \text{FAS} < 0$  and  $\delta \text{LXR} < 0$ , is  $\delta \text{PUFA} > 0$ . This proves our hypothesis that regulating PUFA increase during fasting.*

In order to answer to the Question 2, let us see how the model connects the variations of PUFA and D6D. PUFA being on the boundary we need to consider the Neumann problem. Let us consider the subgraph  $G_3 = \{ X=\text{PUFA}, W=\text{PPAR}, \text{PPAR-a}, Z=\text{D6D}, \text{SREBP-a}, \text{SREBP}, \text{LXR-a}, U=\text{LXR}, \text{SCAP} \}$ . Information from literature suggests that  $G_3$  has the boundary  $\Upsilon^{in} G_3 = \{ U, W, X \}$ , meaning that all external interaction on  $G_3$  acts via LXR, PPAR, PUFA. Nevertheless, in order to understand the differential behaviour of D6D and SCD1, let us suppose that D6D has unknown extra regulation that is not represented. This can be taken into account easily by including D6D in the boundary:  $\Upsilon^{in} G_3 = \{ U, W, X, Z \}$ .

From Theorem 1 and the paths labels from Fig.4 it follows:

$$\begin{aligned} \delta X &= \frac{1}{\tilde{C}} \left[ \frac{\delta X^f}{\chi_X} + \tilde{a}_{p_3} \delta Z^f + \tilde{a}_{p_9} \delta W^f + \tilde{a}_{p_{10}} \delta U^f \right] \\ \delta Z &= \frac{1}{\tilde{C}} \left[ (\tilde{a}_{p_1} + \tilde{a}_{p_2} + \tilde{a}_{p_4} + \tilde{a}_{p_7} + \tilde{a}_{p_8}) \delta X^f + \right. \\ &\quad \left. + \tilde{a}_{p_5} \delta U^f + \tilde{a}_{p_6} \delta W^f + \frac{\delta Z^f}{\chi_Z} \right]. \end{aligned} \quad (33)$$

where  $\tilde{a}$  are path products divided by the products of the absolute values of the self-interactions  $\chi$  of nodes in the paths. The modulus is:

$$\tilde{C} = 1 - \tilde{l}_p(l^+) - \tilde{l}_p(l_1^-) - \tilde{l}_p(l_2^-) - \tilde{l}_p(l_3^-) - \tilde{l}_p(l_4^-). \quad (34)$$

Eqs.33, 34 reveal the importance of the dual regulation of D6D by PPAR and SREBP. The existence of

the positive loop  $l^+$  involving PUFA, D6D and PPAR-a decreases the modulus  $\tilde{C}$  in Eq.34 therefore, according to Eq. 33, it boosts PUFA variations. The biological significance of this has been pointed out by Nakamura and Nara (Nakamura and Nara, 2003) who refer to this phenomenon as a compensatory reaction to the increased demand of PUFA caused by oxidation at fasting.

In Eq.33 the D6D variation  $\delta Z$  is the sum of different contributions of different signs: the influences transmitted along the paths  $p_1, p_2, p_4, p_8$  are negative and those transmitted along the paths  $p_7, p_5, p_6$  are positive. The model is therefore compatible with compensated variations  $\delta Z = 0$ , even in absence of unknown interaction whose effect is the forcing  $\delta Z^f$ . Nevertheless, it is difficult to explain the difference between D6D and SCD1 without the extra forcing, because this forcing is the only difference between the two products in the model. Nakamura and Nara (Nakamura and Nara, 2003) arrive at a similar conclusion in their analysis of the regulation of D6D. To our knowledge, the nature of this extra regulation is still unknown.

## 5. Discussion

Static response provides quantitative and qualitative information on how the steady state is changed by forcings.

We have shown here how to relate static response of a biological network to the topology of its interaction graph. The key ingredient is the use of moduli that express the rigidity (opposed to sensitivity) of the network. Mason-Coates formulae from electrical networks computes moduli from loop products and were applied to biological networks. A general consequence of these formulae is that positive (negative) loops decrease (increase) moduli and rigidity. Static susceptibility was related to moduli by using path expansions. An interesting possibility arises when moduli and susceptivities have opposite signs: in this case the sign of the correlation between two nodes can be opposite to the sign of the regulation paths connecting them.

Susceptivities characterize linear response which generally applies to small variations of the concentrations. We showed that in the absence of bifurcations, local linear response can be integrated in order to obtain nonlinear response. This argument justifies qualitative analysis techniques applying to situations when the interactions have constant signs and when the constrained steady states of analysed subgraphs do not bifurcate under forcings. Our formalism is well adapted for qualitative analysis of differential transcriptional, metabolic or proteomic data. It allows several types of analysis: checking the consistency between model and data, or between different types of data, filling in missing information, and predicting the effects of gene knock-outs or of genetic deficiencies. Qualitative analysis of biological networks has been described in detail in (Siegel *et al.*, 2005).

An important ingredient in our approach is the systematic use of boundaries. This allows to separate subgraphs for independent study. The type of biological

problem dictates the choice of the subgraph and the type of boundary conditions (Neumann or Dirichlet).

Boundaries could be useful in further developments such as hierarchical connection of subgraphs. Subgraphs can be connected in series (the entrance boundary of one is included in the exit boundary of another) or in parallel (boundary subsets are common) and their equivalent response can be obtained from Eqs. 18,19. Another promising direction is the reduction of complexity of biological networks. One would like to classify all the graph contractions that preserve the response of the system. This has been done for planar electrical networks (Curtis et al., 1998) in relation to the Dirichlet-to-Neumann map. There is no similar work for biological networks.

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### REFERENCES

- A.Arkin, A., Ross, J., 1995. Statistical construction of chemical reaction mechanisms from measured time-series. *J. Phys. Chem.* 99, 970–979.
- Belych, L., 1998. On the problem of replacing an ordinary differential equation with constant time delay by the system of ordinary differential equations. *Russian J. Numer. Anal. Math. Modelling* 13 (169–190).
- Bloom, D., 1979. *Linear algebra and geometry*. Cambridge University Press, Cambridge.
- Calderon, A., 1980. On an inverse boundary value problem. In: de Mathematica, S. B. (Ed.), *Seminar on Numerical Analysis and its applications to Continuum Physics*. Rio de Janeiro, pp. 65–73.
- Callen, H., 1985. *Thermodynamics and a Introduction to Thermostatistics*. Wiley, New York.
- Chevalier, T., Schreiber, T., Ross, J., 1993. Toward a systematic determination of complex reaction mechanisms. *J. Phys. Chem.* 97, 6776 – 6787.
- Coates, C., 1959. Flow-graph solutions of linear algebraic equations. *IRE Trans. Circuit Theory CT-6*, 170–187.
- Cornish-Bowden, A., 1995. *Fundamentals of enzyme kinetics*. Portland Press.
- Curtis, E., , Morrow, J., 1991. The dirichlet to neumann map for a resistor network. *SIAM J. Appl. Math* 51 (4), 1011–1029.
- Curtis, E., , Morrow, J., 2000. *Inverse problems for electrical networks*. World Scientific.
- Curtis, E., Ingerman, D., Morrow, J., 1998. Circular planar graphs and resistor networks. *Linear Algebra Appl.* 283, 115–150.
- de Jong, H., 2002. Modeling and simulation of genetic regulatory systems: A literature review. *Journal of Computational Biology* 9 (1), 69–105.
- de Jong, H., Gouz, J.-L., Hernandez, C., Page, M., Sari, T., Geiselmann, J., 2004. Qualitative simulation of genetic regulatory networks using piecewise-linear models. *Bulletin of Mathematical Biology* 66, 301–340.
- Gouzé, J.-L., 1998. Positive and negative circuits in dynamical systems. *J. Biol. Syst.* 6, 11–15.
- Gouzé, J.-L., Sari, T., 2003. A class of piecewise linear differential equations arising in biological models. *Dyn. Syst.* 17, 299–316.
- Grmela, M., 2001. Complex fluids subjected to external influences. *J. Non-Newtonian Fl. Mech.* 96, 221–254.
- Heinrich, R., Rapoport, T., 1974. A linear steady-state treatment of enzymatic chains. general properties, and effector strength. *Eur. J. Biochem.* 42, 89–95.
- Jump, D., 2004. Fatty acid regulation of gene transcription. *Crit. Rev. Clin. Lab. Sci.* 41 (1), 41–78.
- Kacser, H., Burns, J., 1973. The control of flux. *Symp. Soc. Exp. Biol.* 27, 65–104.
- Kaminski, N., Friedman, N., 2002. Practical approaches to analyzing results of microarray experiments. *American Journal of Respiratory and Cell Molecular Biology* 27, 125–13.
- Kholodenko, B., Kiyatkin, A., Bruggeman, F., Sontag, E., Westerhoff, H., Hoek, J., 2002. Untangling the wires: a strategy to trace functional interactions in signaling and gene networks. *PNAS* 99 (20), 12841–12846.
- Kubo, R., Toda, M., Hashitsume, N., 1998. *Statistical Physics II. Nonequilibrium Statistical Mechanics*. Springer.
- Kuipers, B., 1994. *Qualitative reasoning. Modeling and simulation with incomplete knowledge*. MIT Press.
- Larson, R., 1988. *Constitutive Equations for Polymer Melts and Solutions*. Butterworths: Boston.
- Lee, S., Chan, W., Lo, C., Wan, D., Tsang, D., Cheung, W., 2004. Requirement of pparalpha in maintaining phospholipid and triacylglycerol homeostasis during energy deprivation. *J Lipid Res.* 45 (11), 2025–37.
- Liang, G., Yang, J., Horton, J., Hammer, R., Goldstein, J., Brown, M., 2002. Diminished hepatic response to fasting/refeeding and liver x receptor agonists in mice with selective deficiency of sterol regulatory element-binding protein-1c. *J Biol Chem* 277 (15), 9520–8.
- MacFarlane, A., 1970. *Dynamical Systems Models*. Harap: London.
- Mackey, M., Santillán, M., Yildirim, N., 2004. Modeling operon dynamics : the tryptophan and lactose operons as paradigms. *C. R. Biologies* 327, 211–224.
- Mason, S., 1953. Feedback theory - some properties of signal flow graphs. *Proc. IRE* 41, 1144–1156.
- Matsuzaka, T., Shimano, H., Yahagi, N., Amemiya-Kudo, M., Yoshikawa, T., Hasty, A., Tamura, Y., Osuga, J., Okazaki, H., Iizuka, Y., Takahashi, A., Sone, H., Gotoda, T., Ishibashi, S., Yamada, N., 2002. Dual regulation of mouse delta(5)- and delta(6)-desaturase gene expression by srebp-1 and pparalpha. *J Lipid Res* 43 (1), 107–14.

- Miller, C., Ntambi, J., 1996. Peroxisome proliferators induce mouse liver stearoyl-coa desaturase 1 gene expression. *Proc Natl Acad Sci U S A.* 93 (18), 9443–8.
- Nakamura, M., Nara, T., 2003. Essential fatty acid synthesis and its regulation. *Prostaglandins, Leukotrienes and Essential Fatty Acids* 68, 145–150.
- Oster, G., Perelson, A., Katchalsky, A., 1973. Network thermodynamics: dynamic modelling of biophysical systems. *Quat. Rev. Biophys.* 6, 1–134.
- Parthasarathy, T., 1983. On Global Univalence Theorems. Vol. 977 of *Lecture Notes in Mathematics*. Springer.
- Perelson, A., Oster, G., 1974. Chemical reaction dynamics. part ii: Reaction networks. *Arch. Rat. Mech. Anal.* 57, 31–98.
- Ruelle, D., 1989. *Elements of differentiable dynamics and bifurcation theory*. Acad. Press, Boston.
- Siegel, A., Radulescu, O., Le Borgne, M., Veber, P., Ouy, J., Lagarrigue, S., 2005. Qualitative analysis of the relation between dna microarray data and behavioral models of regulation networks. *Biosystems*, in press.
- Smale, S., 1980. *Mathematics of Time*. Springer: New-York.
- Snoussi, E., 1998. Necessary conditions for multistationarity and stable periodicity. *J. Biol. Syst.* 6, 3–9.
- Soulé, C., 2003. Graphic requirements for multistationarity. *Complexus* 1 (123-133).
- Steffensen, K., Gustafsson, J., 2004. Putative metabolic effects of the liver x receptor (lrx). *Diabetes* 53 (Supp 1), 36–52.
- Tang, C., Cho, H., Nakamura, M., Clarke, S., 2003. Regulation of human delta-6 desaturase gene transcription: identification of a functional direct repeat-1 element. *J Lipid Res* 44 (4), 686–95.
- Thieffry, D., Sanchez, L., 2004. Qualitative analysis of gene networks: Towards the delineation of trans-regulatory modules. In: Schlosser, G., Wagner, G. (Eds.), *Modularity in Development and Evolution*. University of Chicago Press, pp. 222–243.
- Thomas, R., 1981. On the relation between the logical structure of systems and their ability to generate multiple steady states or sustained oscillations. *Springer Ser. Synergetics* 9, 180–193.
- Tobin, K., Steiniger, H., Alberti, S., Spydevold, O., Auwerx, J., Gustafsson, J., Nebb, H., 2000. Cross-talk between fatty acid and cholesterol metabolism mediated by liver x receptor-alpha. *Mol Endocrinol* 14 (5), 741–52.
- Vlad, M., Arkin, A., Ross, J., 2004. Response experiments for nonlinear systems with application to reaction kinetics and genetics. *PNAS* 101 (19), 7223–7228.
- Wagner, A., 2001. How to reconstruct a large genetic network from n gene perturbations in fewer than n<sup>2</sup> easy steps. *Bioinformatics* 17 (12), 1183–1197.
- Wang, Z., Hu, H., 2001. Dimensional reduction for nonlinear time-delayed systems composed of stiff and soft substructures. *Nonlinear Dynamics* 25, 317–331.
- Yamanishi, Y., Vert, J.-P., Kanehisa, M., 2004. Protein network inference from multiple genomic data: a supervised approach. *Bioinformatics* 20, i363 – i370.
- Yau, C., Huzurbazar, A., 2002. Analysis of censored and incomplete survival data using flowgraph models. *Statist. Med.* 21, 3727–3743.
- Yildirim, N., Mackey, M., 2003. Feedback regulation in the lactose operon: a mathematical modeling study and comparison with experimental data. *Biophysical J.* 84, 2841–2851.