Topology and linear response of interaction networks in molecular biology

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ABSTRACT:

We introduce here a mathematical formalism describing linear response of interaction networks occurring in molecular biology. This formalism have many similarities with the Laplace-Kirchhoff equations for electrical networks. We introduce the concept of graph boundary and we show how the linear response of the biological networks can be related to the Dirichlet or Neumann problems for the corresponding equations on the interaction graph. We use path moduli (measuring path rigidity with respect to the propagation of interaction along the graph) to express Dirichlet and Neumann solutions. Path moduli are calculated from 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.

INTRODUCTION

Network-based representations are widely used to describe gene regulation or metabolic pathways at cellular level; graph theoretical properties of these biological networks, as well as their inference from experimental data, are the subject of intensive studies. However, understanding the behavioral properties of large interaction networks remains a challenging issue. One major reason for this is that quantitative models depend on many parameters which are some of them unknown or known with poor accuracy, thus making simulation-oriented approaches less adapted.

We introduce here a mathematical framework to study the connection between the topology of an interaction network and its response to small perturbations. Theories of linear response were developed in various contexts such as condensed matter and in statistical physics [KTH98], electrical networks [CM91], metabolic networks [SAR01] and gene networks [VAR04]. The basic quantities in such theories are the susceptivities, representing derivatives (generically functional derivatives) of outputs $o(t)$ with respect to inputs $i(t)$. Dynamic susceptivity can be expressed by a relaxation kernel $K(t,t')$, such that $o(t) = \int_{-\infty}^{t} K(t,t')i(t')dt'$. Static susceptivity represents the asymptotic response to a step input $\chi_0 = \int_0^{\infty} K(t,t')dt'$. When the system has simple point attractors the static susceptivity is simply the derivative of the output asymptotic levels with respect to the amplitudes of the step inputs. For more complex attractors, a similar definition can be used after some averaging. In this paper we discuss only the static susceptivity. Static susceptivity in metabolic control theory originated from works of Kacser and Burns [KB73] and Heinrich and Rapoport [HR74]. In these works derivatives of reaction rates with respect to concentrations at steady states were called sensitivity or control coefficients; they are in fact static susceptivities. Dynamic susceptivities were introduced in metabolic control by Arkin and Ross [SAR01] who used theory of response in order to reconstruct metabolic pathways. An algorithmic approach to determining dynamical susceptivities has been discussed by Torralba [Tor03]. Vlad [VAR04] used ideas very similar to the ones of Kubo [KTH98] in order to derive dynamical susceptivities from master equations. Sontag and co-workers [Kho02] analyzed a similar problem: how to reconstruct the wiring of modules in a biological network from the linear response matrix (static susceptivity). His approach uses the Kacser-Burns connectivity property from metabolic networks [CB95], meaning that the matrix of elasticities (defining the couplings between modules) is the inverse of the matrix of control coefficients (static susceptivities). Each of these works have their own merits and treat complementary aspects of linear response applied to biological networks. Our approach restates some of their results. For instance, like Sontag we also emphasize modularity. We define response of modules as solutions of the Dirichlet or the Neumann problems for subgraphs. Nevertheless, our purpose is different from the one of the works cited above. Instead of searching for quantitative results, we wish to understand the qualitative properties of linear response in relation to topology. In consequence, we develop a new mathematical framework emphasizing the relation between graph topology and response.

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An important qualitative question in graph reasonings concerns the relation between variables. Arkin and Ross put into practice what was known since a long time in metabolic control: times series of metabolic activations can be used to reconstruct metabolic pathways [SAR01]. In other words time correlation reveals spatial connectivity. This property does not apply exclusively to dynamics, it also applies to static response. In a parallel paper [SRLB$^+$05] we used the relation between spatial connectivity and static response in order to detect errors in DNA micro-arrays or in interaction models. Here we restate this problem in a more general setting and prove its strong connections to the theory of electrical networks. In particular we show the formal analogy between the linear response of biological networks and a rather active field in applied mathematics, namely the Dirichlet-to-Neumann map [CM00]. This field originated from a problem by Calderon [Cal80] 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. Generally, we can not simply translate properties from electrical networks to biological networks. Electrical networks are reversible and correspond to undirected graphs, biological networks are irreversible and correspond to oriented graphs. Furthermore, the conductivities of electrical networks are always positive, while biological interactions can be negative or positive.

There are several points of views concerning graphic models. A first one is that the graphic model stands for the holy grail in molecular biology and that every effort should concentrate on gathering the information necessary to built such a model. A different point of view which is also ours, is that the reason for a graphic model is to make biological knowledge consistent as a whole and to provide new insights into the functionality of biological systems.

1 ATTRACTOR SHIFTS, INTERACTION GRAPH

The state of a biological network is given by a vector X, 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, $\frac{dX}{dt} = F(X, P)$. This assumption is rather general, because boolean and piecewise-deterministic dynamics can be approximated by differential inclusions [GS03]. Although not proven rigorously but rather used in practice, dynamical systems with delays can be approximated by differential systems by introducing extra variables. Hence the only restriction in our theory is that we consider point attractors. Extensions of this approach to limit cycles and chaotic attractors will be given elsewhere. Point attractors are stable zeros of non-linear systems of the type:

$$F(X, P) = 0 \tag{1}$$

where P stems for a set of parameters. Changes in the control parameters P produce attractor shifts.

Very little is known on the non-linear vector functions F. There is no doubt that they possess high complexity. A part of the complexity is connected to the class of functions to which these belong. For instance chemical reaction description of gene and metabolic networks and the law of mass action lead to rational functions F whose complexity can be quantified by the degree. Another level of complexity is revealed by the Jacobian of F, i.e. the matrix of partial derivatives $\frac{\partial F_i}{\partial X_j}$. We say that j acts on i if $\frac{\partial F_i}{\partial X_j} \neq 0$.

Interaction graph The Jacobian introduces in a natural way an oriented graph, called the interaction graph. The nodes of the interaction graph are the interacting actors labelled by integers in $\{1, \dots\ n\}$ and if j acts on i there is an oriented edge from j to i.

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. Many examples can be considered. For instance j may be a transcription factor regulating the expression of i, or a protein involved in the phosporylation 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. Nevertheless it is conceivable that for certain systems and for certain changes, states do not cross zero-manifolds $\frac{\partial F_i}{\partial X_j} = 0$, meaning that the interaction graph has a relative stability. In this case, interaction graph models are useful representations of biological knowledge.

Boundary Any model is necessarily limited in complexity. It is illusory to believe that one can describe and study a network containing all the types of molecules in a cell or in an organism; by doing this one will leave the domain of validity of the locality hypothesis (well stirred reactor) allowing to consider concentration of molecules of a certain type instead of considering individual molecules or spatially varying concentrations. Thus, a model focuses on small parts of a big network.

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 is the following. Let G represent the set of nodes that we isolate. These nodes interact with other exterior nodes in a bigger network I.

Let us replace the big network I by the selected subset G to which we add a single extra node $\{\mathcal{E}\}$ representing the

exterior. The entrance boundary of G, denoted by $\urcorner^{in} G$ is the set of nodes of G that have incoming arcs from $\{\mathcal{E}\}$. The other nodes of G that are not directly influenced by the exterior are in the interior of G which is denoted $\mathring{G} = G \setminus \urcorner^{in} G$.

Paths, loops, loop partitions A path $c = i \rightsquigarrow j$ is a sequence of nodes $\{i_1 = i, i_2, \ldots, i_p = j\}$ 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.

A loop $l = i \rightsquigarrow i$ is a sequence of nodes $\{i_1 = i, i_2, \ldots, i_p = i\}$ 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 partition of a graph is a partition of the nodes into disjoint loops. The set of loop partitions of a graph G is denoted $\mathcal{L}(G)$. For $L \in \mathcal{L}(G)$, $|L|$ denotes the number of loops in L.

2 LINEAR RESPONSE OF A NETWORK TO INFLUENCES FROM THE EXTERIOR

2.1 Electrical networks

Classical examples of linear networks are the linear electric networks. The study of these is dated back to 19-th century by works of Kirchhoff and Maxwell.

Let us consider an electric 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 Kirchhoff'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:

$$\tilde{Y} V = I. \tag{2}$$

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

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

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

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

2.2 Flowgraphs, Markov chains

There are many other examples coming from different fields of science were 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 [YH02]. The possible transitions and transition probabilities can be gathered on a flowgraph. A flowgraph is a weighted oriented graph. Each edge is labelled 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 τ_{ji} from a state j to i: $t_{ji} = p_{ji}/\tau_{ji}$. The probabilities π_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 completely analogous to the electrical network equation 2.

2.3 Analogy between shift of equilibria and electrical networks

In order to obtain an analogy between biological and electrical networks, let us consider small variations of the parameters in Eq.1. Differentiating all the equilibrium equations for the nodes of G (including the boundary nodes) we obtain:

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

$\frac{\partial F_i}{\partial X_k} = 0$ and assuming that for all

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

$$\frac{\partial F_i}{\partial P} = 0, \forall i \in G \tag{5}$$

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

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

where δX_i^f denote the "forcing variations" which are nonvanishing only on the boundary, and are defined by:

$$\delta X_i^f = \begin{cases} \sum_{k \in I \setminus G} \frac{\partial F_i}{\partial X_k} \delta X_k & \text{if } i \in \urcorner^{in} G \\ 0 & \text{if } i \in \mathring{G}. \end{cases} \tag{7}$$

In matrix form Eq.6 reads:

$$(-J)\delta X = \delta X^f. \tag{8}$$

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

- the opposite Jacobian $-J$ is analogue to the node admittance matrix $\tilde{Y}$,
- the concentration variations δX are analogue to the voltages V,
- the forcing variations δX^f are analogues 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 an electrical networks this would mean that currents are injected into the boundary and not directly into the interior.

Electrical networks are different from biological networks because they have much more symmetries. A list of important differences is the following:

- The matrix $-J$ is not necessarily symmetric, while the matrix
  $\tilde{Y}$ is always symmetric. The interaction graph is intrinsically oriented, while a electric 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 are independent of the non-diagonal ones.

3 TOPOLOGICAL EXPRESSIONS FOR THE LINEAR RESPONSE OF NETWORKS

Supposing that J is invertible (which is true for all non-degenerated point attractors), solutions of Eqs.8 can be obtained from the inverse matrix of J. In this section, the inverse matrix is connected to the topology of the interaction graph.

3.1 Moduli and loops

The starting point is the following well known relation [Blo79] for the inverse of a matrix A:

$$A^{-1}_{ij} = (-1)^{i+j} \frac{\Delta_{ji}}{\Delta} \tag{9}$$

where Δ_{ji} is the minor obtained by deleting line j and column i in matrix A, and $\Delta = det(A)$.

Furthermore, it is a simple exercise [Blo79] to develop the minor Δ_{ji} into a sum of principal minors:

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

where $j \rightsquigarrow i$ is any path 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 A along this path, $\Delta_{j \rightsquigarrow i}$ is the principal minor obtained by deleting all the lines and columns whose indices are included in the path, Δ_j is the principal minor obtained by deleting the line and the column j. Finally principal minors can be related to loops:

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

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

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

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 graph obtained by deleting from G all nodes in the path $j \rightsquigarrow i$, while G_i is obtained by deleting node i. dim is 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 n nodes subnetwork of I. Let A be the restriction of the Jacobian of F to G: $A_{ij} = \frac{\partial F_i}{\partial X_j}, i, j \in G$, Δ_I, $I \subset \{1, \ldots, n\}$ its principal minor obtained by deleting lines and columns of indices in I.*

The **modulus** *of a path $j \rightsquigarrow i$ is* $C_{j \rightsquigarrow i} = (-1)^{l_{j \rightsquigarrow i}+1} \frac{\Delta}{\Delta_{j \rightsquigarrow i}} =$

$$= \frac{\sum_{L \in \mathcal{L}(G)} (-1)^{|L|} lp(L)}{\sum_{L \in \mathcal{L}(G_{j \rightsquigarrow i})} (-1)^{|L|} lp(L)}. \tag{14}$$

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

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

3.2 Neumann and Dirichlet problems

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

- 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 $\delta X_i, i \in \urcorner^{in} G$. For biological networks the Dirichlet problem shows its utility when one possesses only a partial knowledge of the system [SRLB$^+$05].

  We can define the corresponding Dirichlet static susceptivitities:

  $$\chi^D_{ij} = \frac{\partial X_i}{\partial X_j}, i \in \mathring{G}, j \in \urcorner^{in} G \tag{16}$$

- Neumann problem: determine the variations $\delta X_i, i \in G$ everywhere, when the forced variations are imposed on the boundary $\delta X^f_i, i \in \urcorner^{in} G$. 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 susceptivities are defined as:

$$\chi^N_{ij} = \frac{\partial X_i}{\partial X^f_j}, i \in G, j \in \urcorner^{in} G \tag{17}$$

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

THEOREM 1. *Let G be a n nodes subnetwork of I. Let A be the restriction of the Jacobian of F to G: $A_{ij} = \frac{\partial F_i}{\partial X_j}, i, j \in G$. Let $\mathcal{P}_G$ denote the set of paths included in G. If $det(A) \neq 0$ and 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:*

$$\textit{if } i \in \mathring{G},\ \delta X_i = \sum_{j \in \urcorner^{in} G} \sum_{j \rightsquigarrow i \in \mathcal{P}_G} \frac{a_{j \rightsquigarrow i}}{C_{j \rightsquigarrow i}} \delta X^f_j, \tag{18}$$

$$\textit{if } i \in \urcorner^{in} G, \delta X_i = \frac{\delta X^f_i}{C_i} + \sum_{\substack{j \in \urcorner^{in} G, \\ j \neq i}} \sum_{j \rightsquigarrow i \in \mathcal{P}_G} \frac{a_{j \rightsquigarrow i}}{C_{j \rightsquigarrow i}} \delta X^f_j \tag{19}$$

Using a language coming half from mechanics, half from flowgraphs we can say that a "force" $a_{j \rightsquigarrow i} \delta X^f_j$ 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 δX_i as functions of the boundary forcings δX^f_j. Eq.19 gives the relation between forcings and variations on the boundary. The corresponding linear application $\Lambda^{ND} : \mathbb{R}^m \rightarrow \mathbb{R}^m$ ($m = \#\urcorner^{in} G$, $\Lambda^{ND}_{ij} = \sum_{j \rightsquigarrow i \in \mathcal{P}_G} \frac{a_{j \rightsquigarrow i}}{C_{j \rightsquigarrow i}} + \frac{1}{C_i} \delta_{ij}$) is the analogue of the Neumann-to-Dirichlet map for electrical networks [CM91]. The inverse (if it exists) of this application is the Dirichlet-to-Neumann map [CM91].

Theorem 1 implies the following:

COROLLARY 1. *If there are no paths connecting two different nodes on the boundary, and if for all nodes $i \in \urcorner^{in} G$, moduli C_i are finite and non-vanishing, then the Neumann-to-Dirichlet mapping has a diagonal matrix and its inverse exists. In this case, the forcings are proportional to the variations on the boundary:*

$$\delta X^f_i = C_i \delta X_i \tag{20}$$

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 [SRLB+05] we have used a simpler method to obtain solutions to the Dirichlet problem. Briefly, this method uses only the equilibrium equations for interior nodes in the same way as the equilibrium equations for all the nodes (including the nodes on the boundary) was used here to derive Theorem 1. Let us state the result:

THEOREM 2. *Let G be a subnetwork of I. Let $\mathring{G} = G \setminus \urcorner^{in} G$ be the interior of G. Let $\mathring{A}$ be the restriction of the Jacobian of F to $\mathring{G}$: $A_{i,j} = \frac{\partial F_i}{\partial X_j}, i, j \in \mathring{G}$. Let us suppose that $det(\mathring{A}) \neq 0$ 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:

$$\delta X_i = \sum_{j \in \urcorner^{in} G} \sum_{j \rightsquigarrow i \in \mathcal{P}_{\mathring{G}}} \frac{a_{j \rightsquigarrow i}}{\mathring{C}_{j \rightsquigarrow i}} \delta X_j. \tag{21}$$

- *$\mathcal{P}_{\mathring{G}}$ denotes the set of paths included in G, starting on the boundary and that do not return to the boundary.*
- *$\mathring{C}_{j \rightsquigarrow i} = C_{k(j) \rightsquigarrow i} = (-1)^{l_{k(j) \rightsquigarrow i} + 1} \frac{det \mathring{A}_{\{k(j)\}^c}}{det \mathring{A}_{\{k(j) \rightsquigarrow i\}^c}}$ 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 $j \rightsquigarrow i$.*

Eqs.14,15 give the loop products expression of moduli. They generalize Mason and Coates gain formulae [Mas53, Coa59] from electrical networks. Susceptivities are related to modules according to the following equations:

$$\chi^N_{ij} = \sum_{j \rightsquigarrow i \in \mathcal{P}_G} \frac{a_{j \rightsquigarrow i}}{C_{j \rightsquigarrow i}} + \frac{1}{C_i} \delta_{ij} \tag{22}$$

$$\chi^D_{ij} = \sum_{j \rightsquigarrow i \in \mathcal{P}_G} \frac{a_{j \rightsquigarrow i}}{\mathring{C}_{j \rightsquigarrow i}} \tag{23}$$

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

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 susceptivity χ_{ij}, which means negative correlation between X_i and X_j. This possibility occurs when the moduli $C_{j \rightsquigarrow i}$ are negative.

3.3 Signs of moduli and uniqueness of equilibrium

The signs of moduli are important for qualitative discussions of the transmission of influences [SRLB+05]. They are also important in discussions of existence, uniqueness and stability of equilibria.

From Eqs.14,15 it follows :

PROPERTY 1. *If the subnetwork G contains no positive loops, then all path moduli $C_{j \rightsquigarrow i}$ and node moduli C_i are positive.*

When there are no positive loops, 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

[Par83]it follows the uniqueness of the equilibrium. The same argument was used by [Sou03] to prove the conjecture of Thomas [Tho81] which says that if the interaction graph has no positive loops, then the equilibrium is unique. Reciprocally, multiple equilibria ask for at least a positive loop in the interaction graph, somewhere in the phase space.

Similarly, there seem to be some relation between the sign of moduli and the uniqueness of equilibria. At saddle-node bifurcations one positive module diverges (a susceptivity vanishes) and two equilibrium branches emerge: the first one is unstable and has a negative module, the second one is stable and every module is positive on it. We shall come back to this phenomenon in our discussion of biological exemples.

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 (β-galactosidase) catalyzing the degradation of lactose to glucose [MSY04, YM03].

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.1. The exterior and interior lactose are denoted L^e, L^i, respectively.

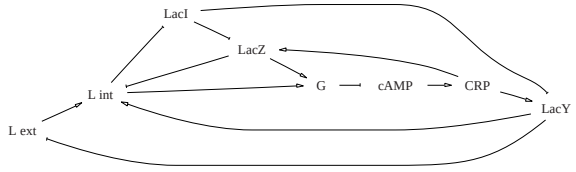

Fig. 1. Operon lactose interaction network. L int and L ext stand for L^i and L^e respectively.

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

There are four loops in the interaction graph: two positive ones $l_1^+ = \{L^i, LacI, LacY, L^i\}$, $l_2^+ = \{L^i, G, cAMP, CRP, LacZ, L^i\}$, and two negative ones $l_1^- = \{L^i, LacI, LacZ, L^i\}$, $l_2^- = \{G, cAMP, CRP, LacZ, G\}$. The existence of positive loops is a necessary condition [Sou03] 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. 1 is coherent to observed variations, namely whether the susceptivity χ_{L^i,L^e} is positive.

From Theorem 2, $\chi_{L^i,L^e} = \frac{a_{L^e \rightsquigarrow L^i}}{\mathring{C}_{L^e \rightsquigarrow L^i}}$, where $a_{L^e \rightsquigarrow L^i} > 0$ from the construction of the model (see Fig.1). 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:

$$\mathring{C}_{L^e \rightsquigarrow L^i} = \chi_{L^i} \frac{1 - \tilde{lp}(l_1^+) - \tilde{lp}(l_2^+) - \tilde{lp}(l_1^-) - \tilde{lp}(l_2^-)}{1 + \tilde{lp}(l_2^-)} \tag{24}$$

where $\tilde{lp}$ is the loop product divided by the product of absolute values of self-interactions for nodes in the loop. $\tilde{lp}$ are positive for positive loops and negative for negative loops.

Eq.24 shows that positive (negative) loops decrease (increase) the modulus thus decreasing (increasing) rigidity. For instance, a mutation of the permease $LacY$, although keeping the qualitative properties of the operon it increases the modulus $\mathring{C}_{L^e \rightsquigarrow L^i}$ decreasing the susceptivity $\chi^D_{L^i,L^e}$.

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 $\mathring{C}_{L^e \rightsquigarrow L^i}$ is positive, i.e.:*

$$\tilde{lp}(l_1^+) + \tilde{lp}(l_2^+) < 1 - \tilde{lp}(l_1^-) - \tilde{lp}(l_2^-). \tag{25}$$

The monotonicity condition 25 is valid for small positive loops products. The typical scenario for strong positive loops is the occurrence of a saddle-node bifurcation: the operon becomes bistable and its response is discontinuous and hysteretic [MSY04]. According to the remark of the preceding sequence, bistability demands the existence of a branch of states with negative moduli. Usually, this branch is unstable (see also next subsection). We have not developed here a theory of the relation between topology and stability (for some hints see [BI03]), therefore it is difficult to confirm this hypothesis without setting the details of the model.

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 $\mathring{C}_{12} < 0$. 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 stable steady states have positive moduli. A simple example illustrating the

possibility of having negative moduli is:

$$\begin{cases} \frac{dx_1}{dt} &= -x_1 + 1 + \lambda, \\ \frac{dx_2}{dt} &= -2x_2 - x_3 + x_1, \\ \frac{dx_3}{dt} &= f(x_3) + 3x_2 \end{cases} \tag{26}$$

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

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

$$\mathring{C}_{12} = \left[3 - 2\frac{df}{dx_3}\right] / \left[-\frac{df}{dx_3}\right] \tag{27}$$

$$\mathring{C}_{13} = 3 - 2\frac{df}{dx_3}. \tag{28}$$

The model has unique equilibrium for $a < 3/2$ and is bistable for $a > 3/2$. In the monostable regime ($a < 3/2$) $\mathring{C}_{13} > 0$ everywhere; $\mathring{C}_{12} \leq 0$ 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 $x_3 = 1 - 1/\sqrt{3}, 1 + 1/\sqrt{3}$, $\frac{1}{\mathring{C}_{12}}$ and therefore the susceptivity χ^D_{21} 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 and undergoes saddle node bifurcation. $\mathring{C}_{12} > 0$ 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 $\mathring{C}_{13} > 0$ unless when $x_3 \in I_3 = [1 - \sqrt{1/3 - 1/(2a)}, 1 + \sqrt{1/3 - 1/(2a)}]$. The region I_3 where $\mathring{C}_{13} < 0$ is in fact unstable and is never reached. When x_1 increases x_3 jumps discontinuously at the saddle-node bifurcation point from one attractor to another without taking values inside the interval I_3. The saddle-node bifurcation occurs at different values 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 $\mathring{C}_{12} < 0$; 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 synthetized 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.

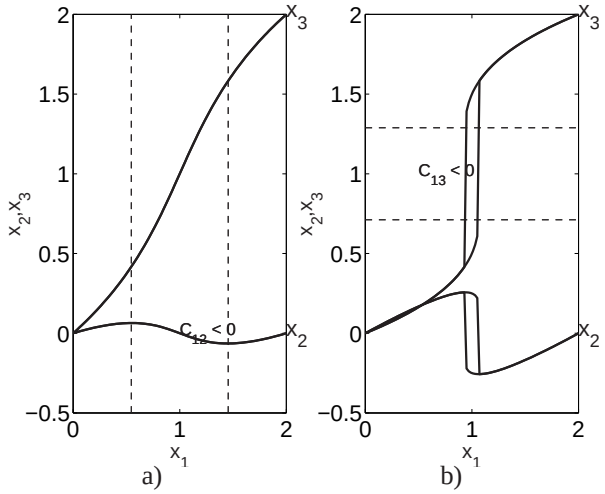

Fig. 2. Solution of the Dirichlet problem for the model in Eq.4.2: stable equilibrium values of x_2 and x_3 as a function of the boundary value x_1. a) Monostable ($a = 0.5$) with a region (betweed dotted vertical lines) of negative modulus $\mathring{C}_{12} < 0$. b) Bistable ($a = 2$) with discontinuous,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

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 (PPARα – peroxisome proliferator activated receptors, LXRα – Liver-X-Receptor α, HNF-4α) 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.) [Jum04].

We consider in our model nuclear receptors PPARα, LXRα, 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 LXRα:RXRα), PPAR-a (denoting a complex PPARα:RXRα) 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 [Jum04]. LXR-a is a direct activator of the transcription of SREBP and FAS, it also indirectly activates ACL, ACC and SCD1 [SG04]. 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) [Jum04]. 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 [MN96, TCNC03, MSYa02a].

The interaction graph for this model is shown in Fig.3. Like in the lactose operon example, for each node we have supposed the existence of negative self-interaction loops.

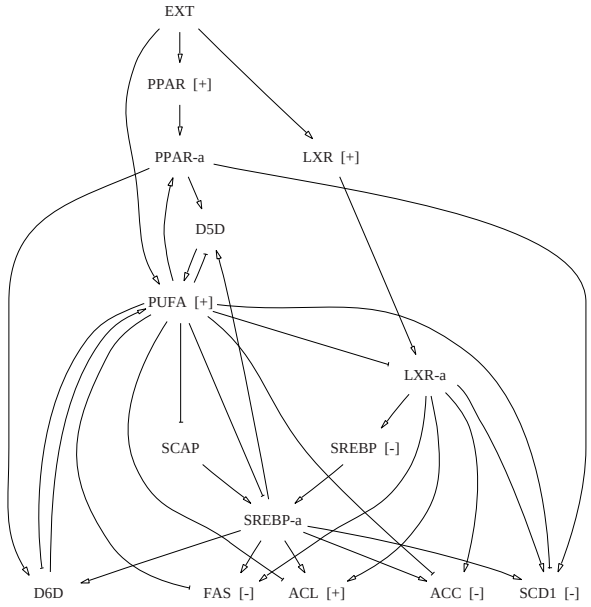

Fig. 3. Interaction graph for a model of regulation of the synthesis of fatty acids. The node EXT stands for $\mathcal{E}$ and represents the exterior world. Negative self-interaction loops on nodes are omitted for sake of clarity. Labelled loops: l_1^- = { PUFA, SCAP, SREBPa, D6D, PUFA}, l_2^- = {PUFA, SREBPa, D6D,PUFA}, l_3^- = {PUFA, LXRa, SREBP, SREBPa, D6D, PUFA}, l_4^- = {PUFA, D6D, PUFA}, l^+ = { PUFA, PPARa, D6D, PUFA}. Labelled paths $p1$ = {PUFA, D6D}, $p2$ = {PUFA, SCAP, SREBPa, D6D}, $p3$ = {D6D, PUFA}, $p4$ = {PUFA, LXRa, SREBP, SREBPa, D6D}, $p5$ = {LXR, LXRa, SREBP, SREBPa, D6D}, $p6$ = {PPAR, PPARa, D6D}.

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 fasting [LYH$^+$02]; this state is characterized by an inhibition of fatty acid synthesis and an activation of the fatty acid oxydation. However, Tobin et al ([TSA$^+$00]) showed that fasting rats for 24h increased the hepatic LXR mRNA and Matsuzaka et al ([MSYa02b]) observe no difference in either the hepatic D5D or D6D mRNA level between fasting and refeeding in normal mouse livers. Moreover, Lee et al showed that after 72h of fasting ([LCLa04]) PUFA levels increased in mice liver.

4.3.1 Linear 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 the possibilities of this we considered the following biological puzzles:

- 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.

  All three desaturases have similar regulation. Nonetheless, on fasting SCD1 decreases, while D5D, D6D have non significative variations.
- D5D, D6D are essential for PUFA synthesis. Their presence in the interaction graph close several positive and negative feed-back loops. Which are the effects of these loops?

In a first step, we try to see if our model can account for the observed null variation of D6D. Let us consider the Neumann problem for the subgraph $G = \{X$=PUFA, PPAR, PPAR-a, Z=D6D, SREBP-a, SREBP, LXR-a, LXR, SCAP$\}$. G has the boundary $\urcorner^{in}G = \{X$=PUFA, U=LXR, W =PPAR $\}$.

From Theorem 1 and the edge labels from Fig.3 it follows:

$$\delta X = \frac{\delta X^f}{\chi_X \tilde{C}_X}, \quad \delta U = \frac{\delta U^f}{\chi_U \tilde{C}_U}, \quad \delta W = \frac{\delta W^f}{\chi_W \tilde{C}_W},$$
$$\delta Z = \left(\frac{\tilde{a}_{p_1}}{\tilde{C}_{p_1}} + \frac{\tilde{a}_{p_2}}{\tilde{C}_{p_2}} + \frac{\tilde{a}_{p_4}}{\tilde{C}_{p_4}} \right) \delta X^f + \frac{\tilde{a}_{p_5}}{\tilde{C}_{p_5}} \delta U^f + \frac{\tilde{a}_{p_6}}{\tilde{C}_{p_6}} \delta W^f. \tag{29}$$

where $\tilde{a}, \tilde{C}$ are path products and moduli divided by the products of the absolute values of the self-interactions χ of nodes in the paths.

The moduli are:

$$\tilde{C}_X = \tilde{C}_{p_1} = \tilde{C}_{p_2} = \tilde{C}_{p_3} = \tilde{C}_{p_4} = \tilde{C}_{p_5} = \tilde{C}_{p_6} =$$
$$= 1 - \tilde{lp}(l^+) - \tilde{lp}(l_1^-) - \tilde{lp}(l_2^-) - \tilde{lp}(l_3^-) - \tilde{lp}(l_4^-) \tag{30}$$
$$\tilde{C}_U = \tilde{C}_W = 1 \tag{31}$$

where $\tilde{lp}$ is the loop product divided by the product of absolute values of self-interactions for nodes in the loop. If follows that the positive loops decrease and the negative loops increase the moduli. From Eq.29 this means that the

negative loops decrease and the positive loops increase the variations δX, δZ.

From the literature δZ should be zero [MSYa02a]. This suggests that either the moduli are big (meaning that the negative loops win) or that δZ is the sum of terms of different signs that cancel each other, or both. The first hypothesis is not enough, because it would predict also a small δX and this is not the case. The second hypothesis can be checked by analyzing the signs of the terms in Eq.29. We suppose here that the moduli are positive. Investigating the graph we find $\tilde{a}_{p_1} < 0$, $\tilde{a}_{p_2} < 0$, $\tilde{a}_{p_4} < 0$, $\tilde{a}_{p_5} > 0$, $\tilde{a}_{p_6} > 0$. From Eq.29, and the observed value $\delta X > 0$, $\delta U > 0$, $\delta W > 0$ it follows that $\delta X^f > 0$, $\delta U^f > 0$, $\delta W^f > 0$. Thus δZ is indeed a sum of terms of different signs. The fact that we observe $\delta SREBP < 0$ represents an extra information on the terms adding to δZ. Indeed, by considering this information and the subgraph $G' = \{$ PUFA, LXR, LXRa, SREBP$\}$ we can prove that $\frac{\tilde{a}_{p_4}}{\tilde{C}_{p_4}}\delta X^f + \frac{\tilde{a}_{p_5}}{\tilde{C}_{p_5}}\delta U^f < 0$. This allows to draw a first conclusion:

PROPERTY 3. *There are terms of opposite signs in the expression for the variation of D6D. At least in principle the relative importance of these terms can be tuned to obtain a zero variation. This might explain the observed null variation, though it does not exclude the existence of another regulation mechanism.*

A similar discussion is valid for D5D and SCD1. In the case of SCD1, quantitative tuning can incline the balance in the negative direction, as observed.

Once the possibilities of the network proven we would like to understand the benefit of this regulation scheme. A possible explanation lies in the different roles of D5D, D6D, that are used in the synthesis of PUFA and SCD1 that is used in the synthesis of MUFA. It is known that lysis of adipose tissues results in an increase of all fatty acids in liver. However, it is plausible that at fasting the ratio PUFA/fatty acids need to be increased with respect to the same ratio under normal diet, because the cell needs PUFA to activate oxidation [Jum04]. Furthermore, PUFA is not synthesized *de novo*, and can be produced from essential fats released in the bloodstream at fasting. A simple way to improve the ratio PUFA/MUFA is to allow for some PUFA synthesis even at fasting, therefore maintaining D6D and D5D. On the opposite, there is no reason to synthesize MUFA and to maintain SCD1. Under fasting, the variation of SCD1 is negative.

A non trivial prediction of our approach is the fact that vanishing D5D and D6D variations effectively help to increase PUFA level at fasting. Applying Eq.6 to PUFA one gets $-\chi_X \delta X + a_{ZX}\delta Z + a_{VX}\delta V = -\delta X^f$, where $V = D5D$. If $\delta Z = \delta V = 0$ it follows $\delta X = \frac{\delta X^f}{\chi_X}$, and from Eq. 29 we obtain:

PROPERTY 4. *If δD5D=0, δD6D=0, then $\tilde{C}_X = 1$, $\tilde{lp}(l^+) + \tilde{lp}(l_1^-) + \tilde{lp}(l_2^-) + \tilde{lp}(l_3^-) + \tilde{lp}(l_4^-) = 0$, meaning that the effect of negative loops to decrease PUFA variation is entirely compensated by the opposite effect of positive loops.*

5 DISCUSSION

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

We have shown here how to relate linear 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 susceptivity 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 can be opposite to the sign of the regulation paths between two nodes.

Another important ingredient in our approach is the systematic use of the entrance boundary for subgraphs. This allows to separate subnetworks for independent study. The type of biological problem dictates the choice of the subgraph and the type of boundary conditions (Neumann or Dirichlet). Further developments are possible like for instance hierarchical connection of modules. For this one should also consider the exit boundary of subgraphs (modules). 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 the equivalent linear 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 [CIM] in relation to the Dirichlet-to-Neumann map. There is no similar work for biological networks.

A drawback of any linear approach is the use of small variations. Nevertheless, local linear response can be integrated in order to obtain nonlinear response. When the susceptivities have constant signs, finite variations of the outputs have the same sign as the local ones. There are several complications arising from the variations of the signs of interactions with concentration, or from balances of paths of different signs in the network. Balances can produce non-monotonic response and multi-stationarity. When multi-stationarity occurs sign analysis should be complemented by an analysis of stability. Some hints on how to connect topology and stability can be found in the work of Ivanova [BI03].

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