BIOINFORMATICS

Vol. 00 no. 00 2003
Pages 1–8

Topology and linear response of interaction networks in molecular biology

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ABSTRACT

Motivation: At many levels of organization, molecular biology interactions can be described as networks. These can be genetic, metabolic or mixed regulatory networks, or protein interaction networks. In absence of precise quantitative information on these networks or in the presence of overwhelming complexity we hope to find in topology hints for the understanding of functionality. Using concepts borrowed from electrical networks, this work introduces a mathematical framework for such discussions.

Results: We investigated how the steady state of an interaction network responds to a change in the external conditions. The linear response solution has a graph theoretical interpretation as path series. The coefficients of the series are path moduli that can be related to loop decomposition of the graph. This generalizes Mason-Coates graph approaches from linear electric networks. We also show the usefulness of the concept of graph boundary. We apply our findings to specific biological examples.

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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, as well as their inference from experimental data, are the subject of intense studies. However, understanding the behavioral properties of large interaction networks remains a challenging issue. One major reason for this is that quantitative models usually depend on many parameters that are hardly ever known with enough accuracy, thus making simulation-oriented approaches less adapted.

In this work, we propose a mathematical framework to study how the steady state of an interaction network responds to a change of external conditions. This network may contain genetic or metabolic regulations as well as protein interactions. Supposing that the concentrations of interacting molecules evolve according to a differential dynamics, we consider the linear approximation of the response, and study the corresponding linear system of equations. Our approach has two complementary goals: first, it focuses on how topology impacts on the response of the network. The intensity of the response is shown to be tightly related to paths and regulation loops in the graph. Second, this framework is intended to be used for qualitative reasonings: using signs of variations of the concentrations and types of interactions (activation/inhibition) is enough to get a deeper insight into regulation mechanisms.

Our work is inspired from now classical results on electrical networks. This analogy is detailed, after having introduced preliminary definitions. Then we show how the response of an interaction network can have a graph theoretical interpretations in terms of paths and loop decomposition of the associated graph. Finally, the relevance of our approach is illustrated on some examples, including lactose operon and lipid metabolism.

1 STEADY STATE 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.

Supposing a differential dynamics for X, $\frac{dX}{dt} = F(X, P)$, steady states are zeros of non-linear systems of the type:

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

where P stems for a set of parameters. The steady state shift is the result of a change of the control parameters P.

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

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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 order. 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 a directed 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 a directed 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.

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 on the interaction graph 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 entering 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 $\{i_1 = i, i_2, \dots, i_p = j\}$ 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.

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 partition of a graph is a partition of the nodes into a set of 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 Kirchhof 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 linear equations 2.

Notice that the matrix $\tilde{Y}$ is singular: $(1, \dots, 1) \in Ker(\tilde{Y})$. This comes from a special symmetry of electric networks which physically means that potentials are determined up to a constant and that the only measurable quantities are potential differences. It is possible to lift this undeterminacy by fixing the potential of a reference node to a conventional value which for convenience is chosen zero. Thus, making $V_1 = 0$ and keeping all equations in the system 2 with the exception of the first one (coming from the node 1) one can reduce the system to a compatible one eventually.

2.2 Flowgraphs, Markov chains

There are many other examples coming from different fields of science were linear relations 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 directed 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 $\sum_k \pi_k t_{kj} = (\sum_k t_{kj})\pi_k$ 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:

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

Noticing that for all $k \in I\setminus G$ and $i \in \mathring{G}$, we have $\frac{\partial F_i}{\partial X_k} = 0$ and assuming that for all $i \in G$, $\frac{\partial F_i}{\partial P} = 0$, it follows:

$$\forall i \in G, \sum_{j\in G} \frac{\partial F_i}{\partial X_j}\delta X_j = -\delta X_i^f, \tag{5}$$

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

$$\delta X_i^f = \begin{cases} \sum_{k\in I\setminus G} \frac{\partial F_i}{\partial X_k}\delta X_k & \text{if } i \in \urcorner^{in} G \\ 0 & \text{if } i \in \mathring{G}. \end{cases} \tag{6}$$

In matrix form Eq.5 reads:

$$(-J)\delta X = \delta X^f. \tag{7}$$

Eqs.7 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 $-\delta X^f$ are analogues to the injected currents I.

Like the currents that are not necessarily injected in all nodes, the forcing variations are non-zero only on the boundary of the subgraph G.

Electrical networks are different from biological networks because they have much more symmetries. The most important differences are the following:

- The matrix $-J$ is not necessarily symmetric, while $\tilde{Y}$ is always symmetric. The interaction graph is intrinsically directed, while a electric network is not directed (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, solutions of Eqs.7 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:

$$A^{-1}_{ij} = (-1)^{i+j}\frac{\Delta_{ji}}{\Delta} \tag{8}$$

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:

$$\Delta_{ji} = \begin{cases} (-1)^{i+j}\sum_{j\rightsquigarrow i}(-1)^{l_{j\rightsquigarrow i}+1} a_{j\rightsquigarrow i}\Delta_{j\rightsquigarrow i} & \text{if } i \neq j \\ \Delta_j & \text{if } i = j. \end{cases} \tag{9}$$

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:

$$\Delta_{j\rightsquigarrow i} = \sum_{L\in\mathcal{L}(G_{j\rightsquigarrow i})}(-1)^{dim(\Delta_{j\rightsquigarrow i})-|L|} lp(L) \tag{10}$$

$$\Delta_i = \sum_{L\in\mathcal{L}(G_i)}(-1)^{dim(\Delta_i)-|L|} lp(L) \tag{11}$$

$$\Delta = \sum_{L\in\mathcal{L}(G)}(-1)^{dim(\Delta)-|L|} lp(L). \tag{12}$$

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 its associated minor.*

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

$$= (-1)^{l_{j\rightsquigarrow i}+1}\frac{\sum_{L\in\mathcal{L}(G)}(-1)^{dim(\Delta)-|L|}lp(L)}{\sum_{L\in\mathcal{L}(G_{j\rightsquigarrow i})}(-1)^{dim(\Delta_{j\rightsquigarrow i})-|L|}lp(L)}. \tag{13}$$

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

$$C_i = -\frac{\Delta}{\Delta_i} = -\frac{\sum_{L\in\mathcal{L}(G)}(-1)^{dim(\Delta)-|L|}lp(L)}{\sum_{L\in\mathcal{L}(G_i)}(-1)^{dim(\Delta_i)-|L|}lp(L)}. \tag{14}$$

3.2 Linear response and sensitivity

We can now state the following theorem that allows to express the response of an internal node to small changes on the exterior.

THEOREM 2. *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:*

$$\textit{if } i \in \mathring{G},\ \delta X_i = \sum_{j\in\urcorner^{in}G}\ \sum_{j\rightsquigarrow i\in\mathcal{P}_G}\frac{a_{j\rightsquigarrow i}}{C_{j\rightsquigarrow i}}\delta X_j^f, \tag{15}$$

$$\textit{if } i \in \urcorner^{in}G, \delta X_i = \frac{\delta X_i^f}{C_i} + \sum_{\substack{j\ \in\ \urcorner^{in}G,\ j\neq i}}\ \sum_{j\rightsquigarrow i\in\mathcal{P}_G}\frac{a_{j\rightsquigarrow i}}{C_{j\rightsquigarrow i}}\delta X_j^f \tag{16}$$

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**.

From Eq.13,14 moduli can be calculated from the loop products. This generalizes Mason and Coates gain formulae [Mas53, Coa59] from electrical networks.

3.3 Signs of moduli

The signs of moduli are important for qualitative discussions of the transmission of influences [SRLB$^+$05]. They are also important in discussions of existence, uniqueness and stability of equilibria. As noticed by Soulé [Sou03], if the interaction graph contains no positive loops, then the signs of principal minors of the Jacobian have the opposite parity of the dimension of the minors. From this and from the Eqs.13,14 it follows immediately (see also [SRLB$^+$05]):

PROPERTY 3. *If the subnetwork G contains no positive loops, then all path moduli $C_{j\rightsquigarrow i}$ and node moduli C_i in Theorem 2 are strictly positive.*

3.4 Influence of the boundary on the interior, Dirichlet problem

In many situations we would like to calculate the interior values of δX_i once the values of δX_j on the boundary are known or imposed. For partial differential equations this is called the **Dirichlet problem**. For biological networks the Dirichlet problem shows its utility when one possesses only a partial knowledge of the system [SRLB$^+$05].

Eq.15 expresses δX_i as functions of the boundary forcings δX_j^f. By using the equilibrium equations for nodes on the boundary the forcings are related to the variations on the boundary via the Eq.16. Although one can get a solution to the Dirichlet solution from Eqs.,15,16, there is a simpler procedure to obtain the same result.

Let us first notice that the denominator and numerator in the expressions of the moduli (Eqs. 13,14) are sums of terms of alternating signs corresponding to loop partitions with cardinality of different parity. In fact, the path sums are systematically redundant in the sense that many terms cancel exactly. The same redundancy phenomenon has been noticed for Mason-Coates series in electrical networks [Cha71]. When we are not interested in the equilibrium of the boundary [SRLB$^+$05] we can reduce Eqs.15,16 to the following theorem:

THEOREM 4. *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 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:

$$\delta X_i = \sum_{j\in\urcorner^{in}G}\ \sum_{j\rightsquigarrow i\in\mathcal{P}_{\mathring{G}}}\frac{a_{j\rightsquigarrow i}}{\mathring{C}_{j\rightsquigarrow i}}\delta X_j. \tag{17}$$

- *$\mathcal{P}_{\mathring{G}}$ 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 $j \rightsquigarrow i$.*

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.

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.

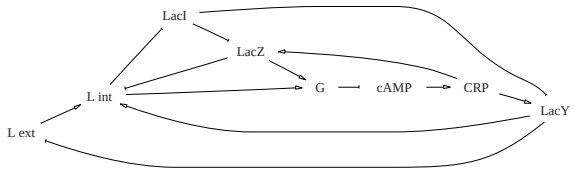

Fig. 1. Operon lactose interaction network. L int and L ext stand for L^i and L^e respectively.

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 that we denote by $-\chi_N$, where N is the node.

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.

Experimentations 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.

From Theorem 4 it 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:

$$\mathring{C}_{L^e \rightsquigarrow L^i} = \chi_{L^i} \frac{1 - \tilde{lp}(l_1^+) - \tilde{lp}(l_2^+) - \tilde{lp}(l_1^-) - \tilde{lp}(l_2^-)}{1 + \tilde{lp}(l_2^-)} \tag{18}$$

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.18 shows that positive (negative) loops decrease (increase) the modulus thus decreasing (increasing) rigidity.

PROPERTY 5. *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.:*

$$\tilde{lp}(l_1^+) + \tilde{lp}(l_2^+) < 1 - \tilde{lp}(l_1^-) - \tilde{lp}(l_2^-). \tag{19}$$

The monotonicity condition 19 is valid for small positive loops products. For strong positive loops products a saddle-node bifurcation occurs. There is a negative modulus branch of steady states, but this is unstable (see also next subsection).

4.2 Negative moduli and non-monotonic response

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

Let us suppose that X_1 regulates positively X_2 and that the modulus $C_{12} < 0$. 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, in dimension 2 stability asks for eigenvalues of the Jacobian to be in the left half of the complex plane; this is equivalent to the absence of positive loops, hence stable steady states have positive moduli . A simple example showing negative moduli is:

$$\left\{ \begin{array}{lcl} \frac{dx_1}{dt} & = & -x_1 + 1 + \lambda, \\ \frac{dx_2}{dt} & = & -2x_2 - x_3 + x_1, \\ \frac{dx_3}{dt} & = & f(x_3) + 3x_2 \end{array} \right. \tag{20}$$

with $f(x) = -ax(x-1)(x-2)$. The steady state is shifted by changing the parameter λ. Changing the parameter $a > 0$ one has unique steady state for small values of a or multiple steady states (multistationarity) for large values of a.

The variations satisfy $\delta x_2 = \frac{\delta x_1}{\mathring{C}_{12}}$, $\delta x_3 = \frac{\delta x_1}{\mathring{C}_{13}}$, where $\mathring{C}_{12} = \left[3 - 2\frac{df}{dx_3}\right] / \left[-\frac{df}{dx_3}\right]$, $\mathring{C}_{13} = 3 - 2\frac{df}{dx_3}$.

The modulus C_{12} becomes negative when $\frac{df}{dx_3} > 0$, hence when x_3 has positive self-regulation. From the shape of the function $f(x)$ this occurs for $1/2 < x_3 < 1$. We have chosen the example in such a way that this domain is accessible when one changes λ. The result can be visualized in Fig.2a): for $a = 1$ when λ increases from -2 to 2, x_3 increases with x_1. x_2 has a non-monotonic behavior which is a consequence of the change of sign of the modulus $\mathring{C}_{12}$. This becomes negative when $1/2 < x_3 < 1$.

In principle $\mathring{C}_{13}$ can also become negative if a is large enough. In fact, the region $1/2 < x_3 < 1$ is no longer accessible for large a because of a saddle-node bifurcation.

Instead of shifting continuously, the system has a discontinuous jump (Fig.2b)).

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

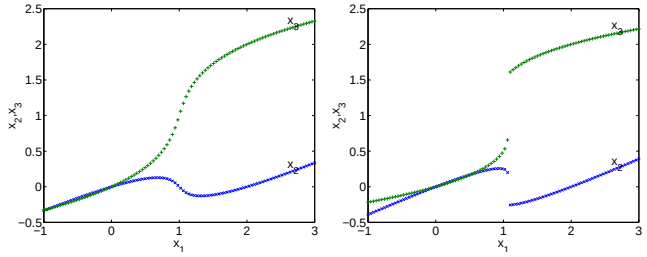

Fig. 2. Steady state values of x_2 and x_3 as a function of x_1, for the model in Eq.4.2. Left plot corresponds to $a = 1$ in the expression of f (continuous shift), right plot to a larger value (jump).

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 (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.

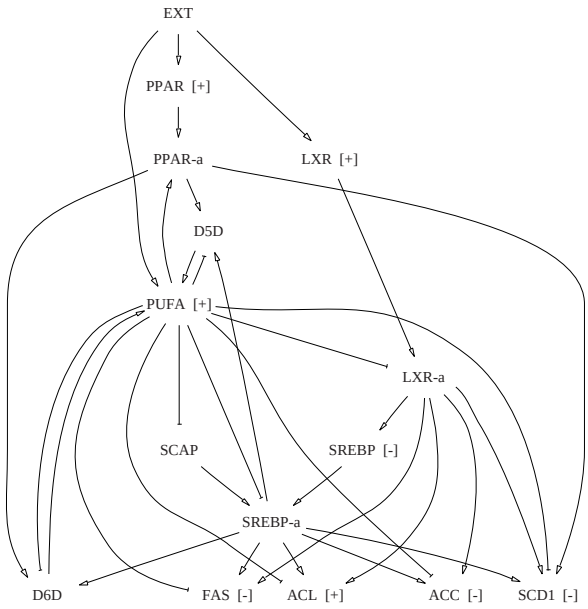

Fig. 3. Interaction graph for a model of regulation of the synthesis of fatty acids. The node EXT stands for $\mathcal{E}$ and represents the exterior world. Negative self-interaction loops on nodes are omitted for sake of clarity. Labelled loops: l_1^- = { PUFA, SCAP, SREBPa, D6D, PUFA}, l_2^- = {PUFA, 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 represent a favorable condition for studying lipogenesis regulation; we suppose that during an experimentation, animals (as rodents or chicken) were kept in a fasted state during several hours. Then, hepatic mRNA of LXR, SREBP, PPAR, ACL, ACC and SCD1 were quantified by DNA microarray analysis. Biochemical measures also provided the variation of PUFA.

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

normal mouse livers. Moreover, PUFA levels can be considered to be increased in liver following starvation because of the important lipolysis from adipose tissue as shown by Lee et al in mice after 72h fasting ([LCLa04]).

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. The corresponding interactions introduce positive and negative loops in the interaction graph. What is the effect of these loops ?

As a first step, we try to see if our model can account for the observed null variation of D6D. Let us consider the subnetwork $G = \{X$=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 2 and the edge labels from Fig.3 it follows:

$$\delta X = \frac{\delta X^f}{\chi_X \tilde{C}_X}, \quad \delta U = \frac{\delta U^f}{\chi_U \tilde{C}_U}, \quad \delta W = \frac{\delta W^f}{\chi_W \tilde{C}_W},$$
$$\delta Z = \left(\frac{\tilde{a}_{p_1}}{\tilde{C}_{p_1}} + \frac{\tilde{a}_{p_2}}{\tilde{C}_{p_2}} + \frac{\tilde{a}_{p_4}}{\tilde{C}_{p_4}} \right) \delta X^f + \frac{\tilde{a}_{p_5}}{\tilde{C}_{p_5}} \delta U^f + \frac{\tilde{a}_{p_6}}{\tilde{C}_{p_6}} \delta W^f. \tag{21}$$

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:

$$\tilde{C}_X = \tilde{C}_{p_1} = \tilde{C}_{p_2} = \tilde{C}_{p_3} = \tilde{C}_{p_4} = \tilde{C}_{p_5} = \tilde{C}_{p_6} =$$
$$= 1 - \tilde{lp}(l^+) - \tilde{lp}(l_1^-) - \tilde{lp}(l_2^-) - \tilde{lp}(l_3^-) - \tilde{lp}(l_4^-) \tag{22}$$
$$\tilde{C}_U = \tilde{C}_W = 1 \tag{23}$$

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.4.3.1 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.4.3.1. 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.4.3.1, 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 6. *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 should 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 maintain 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.5 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 with Eq. 4.3.1 we obtain:

PROPERTY 7. *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 one can relate the linear response of a biological molecular network to the topology of the interaction graph. The key ingredient is the use of moduli, that express the rigidity (opposed to sensitivity) of the network. Moduli can be related to interaction graph loop products in relations that are analogues to the Mason-Coates formulae from electrical networks. A general feature of these relations is that positive (negative) loops decrease (increase) moduli and rigidity.

Another important ingredient in our approach is the systematic use of the entering boundary for subgraphs. This allows to separate subnetworks for independent study. The type of biological problem dictates the choice of the network. Further developments are possible like for instance model reduction and modular description. For these one should also consider the exit boundary of subgraphs (modules). A reduced subgraph could be represented as a set of direct arcs connecting points on the entering to points on the exit boundary. Subgraphs can be connected in series (the entering 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 the Eqs. 15.

A drawback of any linear approach is the use of small variations. Nevertheless, local linear response can be integrated to get nonlinear response. When linear response coefficients have constant signs the corresponding finite variations keep the same sign relation as the local ones. The signs of interactions in biological networks can depend on concentrations, therefore one should pay attention to this possibility as well. Even if the signs of interactions are constant, the topology of the network can be such that the moduli have variable sign. An interesting situation is when we are close to saddle-node bifurcations; here moduli can change sign, but this is accompanied by a loss of stability. The biological switches examples suggest the need of accompanying linear response by stability studies. This has already been done in various other contexts [BI03].

ACKNOWLEDGEMENT

This research was supported by ACI IMPBio, a French Ministry for Research program on interdisciplinarity. We thank M. Crouzeix, A. Gorban and C. Soulé for inspiring discussions.

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