# Hierarchies and modules in complex biological systems

Ovidiu Radulescu[1], Alexander N. Gorban[2], Sergei Vakulenko[3], Andrei Zinovyev[4]
[1]IRMAR, UMR CNRS 6625 and IRISA Projet Symbiose, Rennes, France,
[2]Department of Mathematics, University of Leicester, Leicester, UK,
[3] Institute for Mech. Engineering Problems, Sanct Petersburg, Russia,
[4] Institut Curie, Paris, France

**Abstract**

We review several mathematical methods allowing to identify modules and hierarchies with several levels of complexity in biological systems. These methods are based either on the properties of the input-output characteristic of the modules or on global properties of the dynamics such as the distribution of timescales or the stratification of attractors with variable dimension. We also discuss the consequences of the hierarchical structure on the robustness of biological processes. Stratified attractors lead to Waddington's type canalization effects. Successive application of the many to one mapping relating parameters of different levels in an hierarchy of models (analogue to the renormalization operation from statistical mechanics) leads to concentration and robustness of those properties that are common to many levels of complexity. Examples such as the response of the transcription factor NF$\kappa$B to signalling, and the segmentation patterns in the development of Drosophila are used as illustrations of the theoretical ideas.

## 1 Introduction

Complex systems in molecular biology have been often compared to electronic devices [HHLM99]. This picture is tightly related to the concepts of genetic and metabolic networks and circuits. Molecules inside the cell are "wired" in complex circuits as the result of mutual interactions. Modules contain several molecules and perform well defined functions. The design of a cell could be similar to the design of electronic circuits where several modules are combined to function in a well defined way. As argued by [HHLM99], modules definitely exist. The question is whether everything in molecular biology is modular. If the answer is yes, then there should be ways to identify all the modules in the cell wiring. Furthermore, we need to known which are the consequences of modularity. Several questions are important. Are the properties of a system functions of the properties of modules? Should the modelers shift from the molecular level to the modular one in the description of cell's functioning? Which are the mathematical methods to perform this shift?

In fact, it is not easy to go beyond the first enthusiastic ideas about modules. The simplest questions such as defining and identifying modules are in fact highly non-trivial. Graph representations of cellular molecular interactions suggest that one could use topological criteria such as connectivity in order to identify modules: modules could be highly connected sub-graphs. Combined with additional biochemical conditions these methods were applied to decompose metabolic networks into modules [SPM$^+$02]. Nonetheless, connectivity arguments can lead to hasty conclusions. For instance, in metabolic networks there are molecules (such as ATP) that take part into many reactions. It is nevertheless artificial to put all the ATP controlled reactions into the same module. It is more realistic to define modules as motifs with useful dynamical properties [SK04]. However, finding which properties of a motif are useful for the system in which it is contained, is a difficult

task. In practice, methods to find such motifs are based on counting repetitions and are limited to small motifs [SOMMA02]. Furthermore, the principle of a few types of frequent building units does not apply to all levels: bigger modules are more specialized and less frequent.

In biological systems, modularity is intrinsically related to hierarchical complexity [Lev70]. An hierarchical model defines several levels of complexity. Going up and down these levels of complexity implies reduction operations that lump together variables and parameters [GK05, Ga04]. This many to one relation between several levels of complexity is probably a better approach to modularity. Modules are defined by the groups of variables and parameters in one level that are lumped together and provide the atoms of the next level of complexity. Using this picture the definition of modules is more flexible. There is no unique way to lump variables: this may depend on modeler's choice and on the type of property under study. Hierarchical models could include the more or less well defined levels molecule - cell - tissue - organ - organism but also other levels such as regulation motifs and pathways, groups of interacting motifs and pathways, etc. These more flexible and more poorly defined subdivisions of biological systems could be dynamical and context dependent. It has been suggested that functioning of a cell is highly contextual [AIRRH03]. By integrating signals of various origins, cells are able to compute their response and to activate various combinations of their subsystems. These aggregates are dynamically transient and serve to lead from one stable behavior to another one. This picture is coherent to biologists' remark that living systems operate in spaces with a changing number of dimensions and that it is important to determine the correct number of dimensions [AIRRH03].

Recently, many authors reported the existence of robustness in the functioning of biochemical networks [vDMMO00, MWB$^+$02, KOK$^+$04, Kit04, Wag05, HWL02]. Historically, C.Waddington and R.Thom relate robustness to structural stability [Tho84, Wad57]. We shall argue that the modularity and the hierarchical nature of biological networks have consequences on their robust functioning. This is connected both to structural stability and to the complexity of the system.

## 2 Models

The models we shall use as examples are of the two types. One type of models is based on networks of biochemical reactions.

The dynamics of the network is described by:

$$\frac{dX}{dt} = f(X,\lambda) = \mathbf{SR}(X,\lambda) = \sum_{i=1}^{m} S^i R_i(X,\lambda) \tag{2.1}$$

where $X \in \mathbb{R}^n$ is the concentration vector, $\mathbf{R}(X,\lambda) \in \mathbb{R}^m$ gives the reaction rates depending on the concentrations and some parameters $\lambda$, $\mathbf{S}$ is the stoichiometry matrix whose vector columns are $S^i$, $f : \mathbb{R}^n \to \mathbb{R}^n$ is the non-linear function relating concentrations and concentration rates.

There are two graphical representation that can be associated to these networks. The first one is an oriented bipartite graph with two types of nodes (reactions and molecules). To each reaction node point arcs from reactants and from each reaction node start arcs to the reaction products. The second one is the interaction graph, which is a signed oriented graph $(\mathcal{G}, \mathcal{A}, s)$ whose set of vertices $\mathcal{G}$ include all the molecules in the model and whose arcs are defined by the Jacobian of the rate function $f$. An arc connects a pair of vertices $(i,j) \in \mathcal{A}$ iff $\frac{\partial f_j}{\partial X_i} \neq 0$. The sign function is defined as $s(i,j) = sign \frac{\partial f_j}{\partial X_i}$. The interaction graph may depend on the position in the phase space. Nevertheless, there may be domains in the phase space where the interaction graph is stable.

The second type of models has been used in morphogenesis [RS95] and is similar to the Hopfield model from neuroscience.

$$\frac{dx_i}{dt} = \sigma_\alpha(\sum_{j=1}^{n} K_{ij}x_j + \sum_{k=1}^{p} J_{ik}m_k(y) - h_i) - \lambda_i x_i, \tag{2.2}$$

where $x_j$ are zygotic genes concentrations, $K$ is a matrix describing pair interaction between zygotic genes, $J$ is a matrix describing pair interaction between zygotic genes and maternal genes, $h_i$ are thresholds, $m_i$ are functions of the spatial position $y$ which define maternal gene concentrations (morphogen gradients). Here $\sigma_\alpha(h) = \sigma(\alpha h)$, $\sigma$ is a monotone "sigmoidal" function. The function $\sigma_\alpha$ becomes a step-like function as its sharpness $\alpha$ tends to $\infty$.

An interaction graph can be defined for this type of model similarly to the previous type. The interaction graph does not depend on the point in the phase space. This graph is simply defined by the constant matrix $K$.

# 3 Modules

Given a model, we want to decompose it into sub-models (modules) that behave in a simple way and that have good compositionality properties. In this section we present two of the many possible choices.

## 3.1 Select modules by input-output response

It is rather handy to treat modules as black boxes. A black box has a certain number of input variables and a certain number of output variables. We want to relate outputs to inputs. To do that we first need some definitions.

The mathematical key concept here is the graph boundary [RLS+06]. The orientation of a graph defines a flow $\mathcal{T}$, which applies nodes onto their successors $j = \mathcal{T}i$, iff $(i, j) \in \mathcal{A}$. Any subset $S$ of an oriented graph $(\mathcal{G}, \mathcal{A})$ has an entrance boundary and an exit boundary. The entrance boundary of $S$, denoted by $\daleth^{in}S$ is the set of nodes of $S$ that are images by $\mathcal{T}$ of points from $\mathcal{G} \setminus S$. The pre-entrance boundary is the set $\mathcal{T}^{-1}\daleth^{in}S$. The exit boundary of $S$, denoted by $\daleth^{out}S$ is the set of nodes of $S$ that lead by $\mathcal{T}$ to the exterior of $S$. Notice that this definition uses only the concept of interaction graph, therefore it applies to the both types of models described in the preceding section.

Let us decompose the node variable $X = (X', X")$, where $X'$ and $X"$ are the components of $X$ on the interior of $S$ and on the entrance boundary of $S$ respectively. $S$ being a set of molecules, $X"$ represents the concentrations of molecules that receive direct influences from the exterior of $S$, and $X'$ represents the concentrations of molecules in $S$ not receiving direct influences from the exterior.

In [RLS+06] we introduced the Dirichlet nonlinear problem, which means calculating $X'$ from $X"$ at steady state. The solution of the Dirichlet nonlinear problem represents the Dirichlet static input-output response of the module. It is obtained by imposing stationarity to all the interior nodes:

$$f_i(X', X") = 0, \forall i \in \mathring{S} \tag{3.1}$$

Let us change the decomposition $X = (X', X")$, where now $(X', X")$ are the components of $X$ on $S$ and on the pre-entrance boundary of $S$, respectively. In analogy with the theory of electric circuits we can also state the Neumann nonlinear problem, that means imposing stationarity to all the nodes of $S$:

$$f_i(X', X") = 0, \forall i \in S \tag{3.2}$$

The Dirichlet static input-output response of $S$ is a function $\Phi_D : \mathbf{R}^{n_{in}} \to \mathbf{R}^{n_S - n_{in}}$ giving the values on $\mathring{S}$ as functions of values on $\daleth^{in}S$ at stationarity. Hence, $X' = \Phi_D X"$ satisfies Eq.(3.1).

Similarly, the Neumann static input-output response is a function $\Phi_N : \mathbf{R}^{n_{pre}} \to \mathbf{R}^{n_S}$ giving the values on $S$ as functions of values on $\mathcal{T}^{-1}\urcorner^{in}S$ at stationarity. Hence, $X' = \Phi_N X"$ satisfies Eq.(3.2).

### 3.1.1 Gale-Nikaido vs monotone boxes

The existence and uniqueness of the Dirichlet and of the Neumann static input-output responses are given by the following theorems [RLS$^+$06, RSPL]:

**Property 1 (Existence condition)**
*Let us consider that $f_i(X', X") = \Phi_i(X', X") - \lambda_i X'_i$ where $\lambda_i > 0$ and $\Phi_i(X', X")$ are differentiable, bounded and satisfy*

$$\Phi_i(\ldots, X'_i = 0, \ldots, X") > 0 \tag{3.3}$$

*Then for any $X"$ the system* (3.1) *(or* (3.2)*) has at least a solution $X'$ such that all the concentrations $X'_i$ are positive.*

**Property 2 (Uniqueness condition, Gale-Nikaido)**
*With the same notations as in Property 1 let us define the restricted Jacobian matrix $\tilde{J}$ such as $\tilde{J}_{ij} = \frac{\partial f_i}{\partial X'_j}$, $i, j \in \mathring{S}$ (or, for the Neumann problem $i, j \in S$). Let us consider that all the principal minors of $-\tilde{J}$ are positive for any $X$. Then, the system* (3.1) *(or* (3.2)*) has an unique solution $X'$ for any $X"$.*

Property 2 is a direct consequence of the Gale-Nikaido theorem [Par83].

Notice that our notion of system with unique input-output response is weaker than the one in [AS03]: we do not require stability of the solution of the Dirichlet or of the Neumann problem. Generically, stability is a global property of the system that is not automatically ensured by the stability of the modules.

Boxes with unique input-output response have been used to prove the uniqueness of the steady state of a model of lipid metabolism in hepatocytes [RSPL].

An alternative decomposition has been proposed elsewhere [Ka02, AS03, AFS04, ESS06]. It consists in decomposing the system into monotone boxes. For a monotone box, the restricted Jacobian satisfies $\tilde{J}_{ij} > 0, \forall i \neq j$ or more generally, the undirected interaction graph has no negative loop.

Notice that the monotonicity and the Gale-Nikaido conditions are somehow complementary. The Gale-Nikaido condition is implied by (but largely more general than) the absence of positive loops in the interaction graph. Monotonicity excludes negative loops.

Gale-Nikaido modules and monotone modules have nice compositionality properties. For monotonic modules with stable input-output response, the stability of the global system follows from a small gain theorem [AS03]. Using Gale-Nikaido modules we can obtain conditions for uniqueness of the steady state of the global system [RSPL]. Furthermore, input-output responses of the modules can be combined in order to obtain the response of the global system [RSPL].

The possibility of separating large Gale-Nikaido boxes seems to be limited by the presence of positive loops in the interaction graph. This is not entirely true. In networks of biochemical reactions many positive reaction loops do not produce multistationarity. Therefore, it is relatively easy to find large Gale-Nikaido boxes. In order to illustrate this phenomenon let us consider a positive cycle made of two reactions whose rates $R_1, R_2$ are functions of the concentrations $X$ and $Y$. Let us consider that all the other reactions producing $X$ ($Y$) have rates $\tilde{R}_1$ ($\tilde{R}_2$) not depending on $Y$ ($X$). Furthermore by le Chatelier principle $\frac{\partial \tilde{R}_1}{\partial X}, \frac{\partial \tilde{R}_2}{\partial Y}, \frac{\partial R_1}{\partial Y}, \frac{\partial R_2}{\partial X}$ are negative and $\frac{\partial R_1}{\partial X}, \frac{\partial R_2}{\partial Y}$ are positive. Then the Jacobian restricted to $X, Y$ is:

$$J = \begin{pmatrix} \frac{\partial \tilde{R}_1}{\partial X} + \frac{\partial R_2}{\partial X} - \frac{\partial R_1}{\partial X} & \frac{\partial R_2}{\partial Y} - \frac{\partial R_1}{\partial Y} \\ \frac{\partial R_1}{\partial X} - \frac{\partial R_2}{\partial X} & \frac{\partial \tilde{R}_2}{\partial Y} + \frac{\partial R_1}{\partial Y} - \frac{\partial R_2}{\partial Y} \end{pmatrix} \tag{3.4}$$

Positive loops tend to make the determinant of $J$ negative, that would break the Gale-Nikaido condition. It can be noticed that the effect of the positive loop (terms $(\frac{\partial R_1}{\partial X} - \frac{\partial R_2}{\partial X})(\frac{\partial R_2}{\partial Y} - \frac{\partial R_1}{\partial Y})$) exactly cancels in the determinant of $J$. This determinant is always positive. A similar argument can be used to prove that modules with even larger positive reaction loops satisfy the Gale-Nikaido condition.

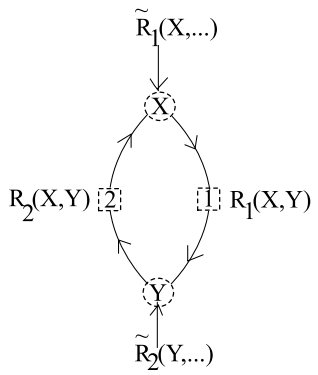



Figure 1: Positive cycle in the reaction graph does not break the Gale-Nikaido condition.

### 3.1.2 Hierarchical modules and the block triangular structure of the Jacobian

Levins [Lev70] discussed a situation that leads naturally to separation of modules. He studied the stability and the timescales of a biological system (estimated from the real parts of the eigenvalues of the Jacobian $J$).

Let us consider the situation when the nodes in the interaction graphs can be grouped in layers, such that nodes in one layer collect influences from each other and from the nodes of the superior layer. In this situation the Jacobian has a block triangular structure. Hence, its characteristic polynomial is the product of of the characteristic polynomials of the layers. This fact have several consequences: a) Asymptotic stability of the steady states of the layers implies asymptotic stability of steady states of the system. b) Relaxation timescales of the system are simply the union of relaxation timescales of the layers.

Levins' argument for this block triangular structure of the Jacobian is the absence of evolutive pressure to select long cycles: these would produce longer transition times [Lev70]. This argument seems to be supported by recent work emphasizing the relative paucity of long cycles in metabolic networks [GSWF01]. Another argument is based on the hierarchical distribution of timescales. Let us consider that nodes of the interaction graph can be grouped in such a way that relaxation times of different groups are well separated $\tau_1 << \ldots << \tau_n << \tau_{n+1} << \ldots$. We may for instance (like in [GR05]) consider that the distribution of timescales is uniform in logarithmic scale. Nodes in a group interact with nodes inside the same group, and with nodes that are in much slower or in much quicker groups. For the chosen group the values of the variables in the much slower groups can be considered to be constant and the interactions with these groups are not effective. We can then safely consider that the Jacobian has a block triangular structure.

This suggests that timescales can be used to define modules by grouping together nodes whose variations have comparable timescales. The choice of the groups of nodes can be made experimentally

by using time series data. Also, various techniques (for instance computational singular perturbation [LG94]) were developed in chemical engineering for classifying degrees of freedom according to timescales in a given chemical reaction model. Using well chosen projectors the contributions of different nodes to these degrees of freedom and the stratification of the nodes according to the timescales can be found [MDMG99].

### 3.2 Modules and stratified epigenetic landscapes

Potentially, biological networks have huge complexity. Mammals posses $10^5$ genes and the number of interactions among these is much larger. One gene can interact with many others by many transcriptional and post-transcriptional regulation mechanisms. This tremendous complexity is used to produce no more than 300 distinct stable cellular types. In fact, in order to understand basic cell functioning it is useless to consider the entire set of genes. Dynamical complexity can be reduced and simplified descriptions are justified.

Obviously one gene does not interact with all the others all of the time. Most of the time, most of the genes are close to stable values that can be either low (silenced) or high (activated). For the Hopfield type model 2.2 let us define the following subsets of genes that are "on" and "off":

$$\begin{aligned} S_{on}(t) &= \{i | x_i(t) > x_i^{max} - \epsilon\} \\ S_{off}(t) &= \{i | x_i(t) < \epsilon\} \end{aligned} \tag{3.5}$$

where $\epsilon$ is a small positive number.

The rest of the genes have dynamically transient values, meaning that they evolve between a minimum and a maximum value.

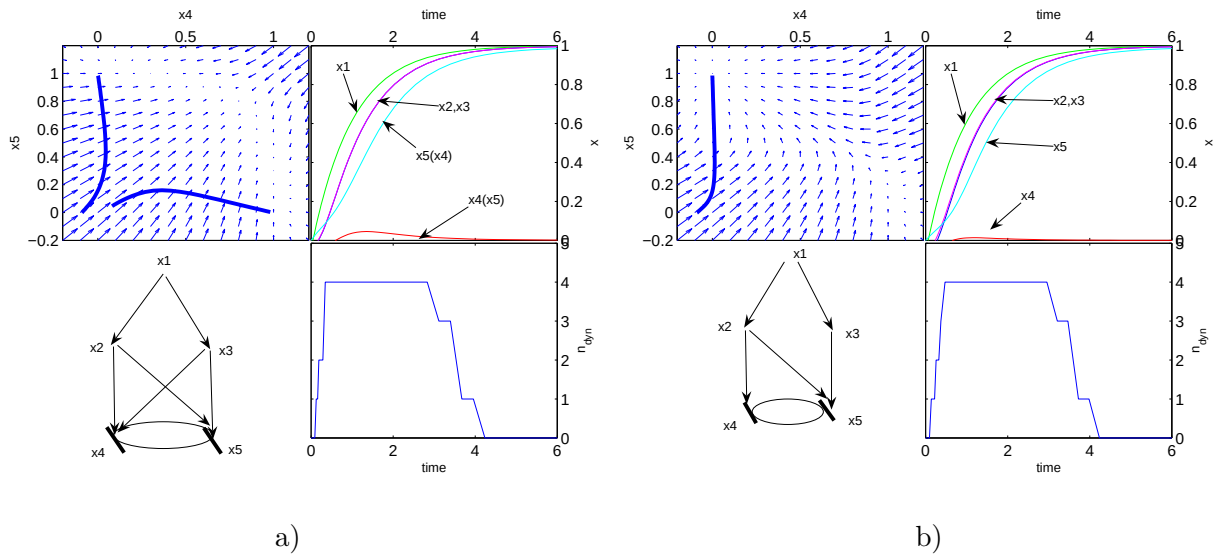
$$S_{dyn}(t) = G \setminus S_{on}(t) \cup S_{off}(t) \tag{3.6}$$

The dynamical complexity is given by the number of transient genes $n_{dyn}(t) = \#S_{dyn}(t)$.

Cell's functioning is based on interpretation of signals. Signals carry information on changes of the environment and guide important processes such as differentiation, proliferation, apoptosis. The usual behaviour of $n_{dyn}(t)$ following a signal is represented in Fig.3.2.

Typical examples when signal processing produces reliable behavior can be found in the biology of development. Canalization meaning robust development and chreod meaning "fated" developmental pathway are central concepts in Waddington's theory of development [Wad57]. René Thom [Tho84] interpreted the concept of chreod in mathematical terms as structurally stable dynamics. We say that a dynamics is structurally stable if its qualitative properties do not change when parameters have some, small variations. In Thom's theory of morphogenesis both stable regions and organizing instabilities are important, shapes and patterns being the result of the conflict of several attractors. What is no so clear in this picture is how this works, how and why one attractor wins against another one, what guarantees the reproducibility and the robustness of the result. In particular Waddington's remark that "in the development of any one organ very many genes may be involved, and in canalized epigenetic systems we are probably confronted with interactions between comparatively large numbers of genes" [Wad57] seems to be in conflict with the increase (for an "average" network) of the number of attractors when the number of genes increases (for random boolean networks the average number of attractors grows linearly with the size of the network [Ald03]).

The genes in $S_{dyn}(t)$ define a dynamical module that changes in time. The restriction of the dynamics to $S_{dyn}(t)$ defines a epigenetic landscape that changes in time. If the function $\sigma$ is smooth, there are discrete times $t_1 < t_2 < \ldots < t_n < t_{n+1} < \ldots$ such that the set $S_{dyn}(t)$ is stable for $t_n \leq t < t_{n+1}$. Let us also define the set $S(t) = \cup_{0 \leq s < t} S_{dyn}(s)$. $S(t)$ keeps a track of all genes that have changed or are still changing.




a) b)

Figure 2: A simple example of signalling network a) non-canalized (small perturbations deviate the dynamics towards states where either $x_5$ or $x_4$ is on); b) canalized (in the end state $x_5$ is on).

The time arrow defines a stratification of the dynamical modules. When these modules change at times $t_n$, previously transient genes provide the initial data and boundary conditions for the actual transient genes. Canalization means that epigenetic landscape is simple at all times. This could mean that $n_{dyn}(t)$ stays small for all times, but it may also mean that attractor basins of variable dimensions of the genes in the increasing set $S(t)$ are embedded one into another like Russian Dolls.

In Fig.3.2 we have represented the dynamics of two networks. Initially all genes are off, then a signal is applied to $x_1$. The networks evolve to some overall attractor of the five dimensional dynamics. Nevertheless, only the network b) is canalized. In situation a) first the epigenetic landscape implying genes $x_1$,$x_2$,$x_3$ is simple: it has an unique globally stable attractor. At a certain moment genes $x_2, x_3$ are on and act as boundary conditions for the subsystem made of two genes $x_4, x_5$. The dynamics is structurally unstable: by symmetry initial data lies on the boundary between two attractors. Small perturbations can deviate the trajectory towards one or the other of the two attractors. In situation b) this is avoided by an asymmetry of the interactions. In this case the epigenetic landscape is simple at all the times. The attraction basin of the subsystem $x_1, x_2, x_3$ is embedded in a higher dimensional attraction basin of the entire network.

Notice that the sequence $t_n, n \geq 1$ and the values of $S_{dyn}(t)$ on $t_n \leq t < t_{n+1}$ may depend on the value of the small parameter $\epsilon$. Some stability properties of the dependency of these sequences on $\epsilon$ could be obtained for systems with well defined time delays. For instance in Fig.3.2 the activation of the nodes $x_2, x_3$ comes after the activation of $x_1$ and the latest active genes are $x_4, x_5$.

# 4 Hierarchies and model reduction

## 4.1 Hierarchies

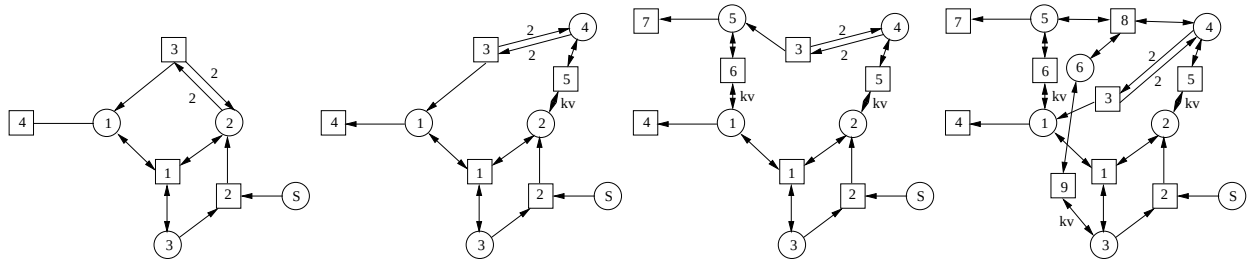
Hierarchies with various levels of complexity reproduce the organization of biological systems. However, hierarchies are also the natural result of the modeling activity itself. A model is an abstraction of reality that includes a certain number of parameters and variables. The level of complexity chosen for the model depends on the experimental needs and also on modeler's culture. Thus, a biologist will include as much variables as possible, a physicist is more inclined to produce minimal models

with small number of variables. Several questions are important here. How to compare different models in the hierarchy? Which is the minimum level of complexity one needs to consider?

### 4.2 NF$\kappa$B: a model system

We shall use as an illustration the dynamical response of the transcription factor NF$\kappa$B to a signal. This system is one of the most documented cellular phenomenon and it has been modeled by various authors [Ha02, La04, Na04, Ia04].

Under normal conditions NF$\kappa$B forms a complex with its inhibitor I$\kappa$B. This complex is trapped in the cytosol and prevents NF$\kappa$B from entering the nucleus. A signal (modeled by a kinase) frees NF$\kappa$B from I$\kappa$B (the latter is degraded). Inside the nucleus NF$\kappa$B controls the transcription of many genes. Among these, it upregulates its inhibitor I$\kappa$B. Experiments show that for a persistent signal the steady concentration of NF$\kappa$B in the nucleus is reached with more or less damped oscillations [Ha02, Na04].




$\mathcal{M}_1(3,4)$ $\mathcal{M}_2(4,5)$ $\mathcal{M}_3(5,7)$ $\mathcal{M}_4(6,9)$

Figure 3: Hierarchy of four simple models of NF$\kappa$B signaling module represented as bipartite graphs. The increasing complexity is quantified by the number of molecules and the number of reactions (the simplest model $\mathcal{M}_1(3,4)$ has 3 types of molecules and 4 reactions). Squares represent reactions, circles molecules. Numbers on arrows represent stoichiometries (by default 1). The molecular types are as follows S=signal (a kinase), 1=I$\kappa$B$_c$, 2=NF$\kappa$B$_c$, 3=I$\kappa$B-NF$\kappa$B$_c$, 4=NF$\kappa$B$_n$, 5=I$\kappa$B$_n$, 6=I$\kappa$B-NF$\kappa$B$_n$, where the indexes $c, n$ mean cytosol and nuclear compartments.

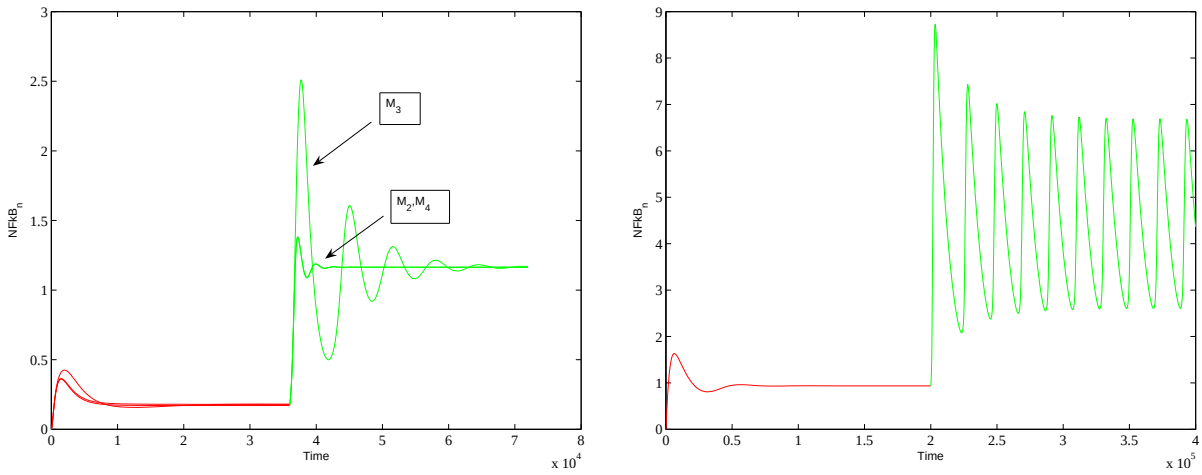
We present here (see Fig.3) four simple models of the biochemical processes described above (simpler than the models in the literature). The models differ by their complexity quantified by two integers: the number of types of molecules and the number of chemical reactions. In the models the reaction rates are given by the law of mass action. Thus, two parameters (kinetic constants) are associated to each reaction. The dynamics of the models is given by the Eq.(2.1).

Any two models have nodes and reactions in common. Some of the models can be obtained one from another by graph contractions. For instance $\mathcal{M}_1$ is a graph contraction of $\mathcal{M}_2$, which is a graph contraction of $\mathcal{M}_3$. $\mathcal{M}_1$ is also a graph contraction of $\mathcal{M}_4$.

### 4.3 Parameter renormalization

We would like to know how the sets of parameters of the models should be related one to another. First, we have to choose a property that we want to be shared by all models. A natural candidate for this property is the set of steady states, defined from Eq.(2.1) as solutions of the equation $f(X) = 0$.

We say that two models are exactly renormalizable if for each set of parameters $k$ of one model there is a set of parameters $k'$ of the other model such that at steady states the values of the concentrations in the common nodes are the same for the two models (notice that $k'$ may not be uniquely determined).




a) b)

Figure 4: Oscillations of NF$\kappa$B following a signal. a) Notice the huge amplitude and low damping predicted by $\mathcal{M}_3$ and the similarity of the dynamical responses for $\mathcal{M}_2, \mathcal{M}_4$. All models have the same statical response (attractors in the same positions). b) Sustained oscillations of $\mathcal{M}_3$.

We can show that $\mathcal{M}_1$, $\mathcal{M}_2$ are exactly renormalizable, also $\mathcal{M}_2$, $\mathcal{M}_3$. For instance, in order to pass from $\mathcal{M}_3$ to $\mathcal{M}_2$ one has to eliminate the variable $X_5$. After elimination the obtained steady state equations have exactly the same form as the equations defining steady state of $\mathcal{M}_2$, provided that $k_3' = k_v k_{-6} k_3/(k_{-6} + k_7), k_4' = k_4 + k_v k_7 k_6/(k_{-6} + k_7)$ (all other parameters being conserved). Similarly, to pass from $\mathcal{M}_2$ to $\mathcal{M}_1$ one only needs to renormalize $k_3$: $k_3' = k_3(k_5/k_{-5})^2$.

Notice that after renormalization a model keeps its description as a reaction bipartite graph and the reaction rates are still given by the mass action law. In general it is rare that two models are exactly renormalizable. Several situations may occur: a) most frequently the mass action law has to be replaced by other laws (by Michaelis-Menten law for instance) b) the equality of steady states is only approximate and the degree of approximation depends on the values of parameters $k$ (quasiequilibrium, quasistationarity situations) [GK05] c) the rates depend on all molecules of the model, not only on reactants and products [GDH04, MDMG99] d) the reaction graph representation is lost [GK05].

Let us now compare the functioning of the models in the hierarchy. We focus on the the following experiment. First, in the absence of signal we wait until all concentrations reach steady state values. Then, a signal is applied and we wait for steady state again. We renormalize the parameters such that the steady state concentrations are approximately the same for all the models.

The following behaviour is common to all the models: under signal the complex NF$\kappa$B-I$\kappa$B in the cytosol is broken and the concentration of NF$\kappa$B in the nucleus increases. Nevertheless, the steady state can be reached with more or less damped oscillations. These oscillations mean that in the presence of the signal the steady state is a focus (at steady state the eigenvalues of the Jacobian have non-zero imaginary parts).

The period and the damping time are the absolute vales of the inverses of the imaginary and the real parts of a pair of complex conjugate eigenvalues of the Jacobian, respectively. By changing the parameters of the model, this pair of eigenvalues eventually crosses the imaginary axis in the complex plane (Hopf bifurcation). Then, self-sustained oscillations occur (the steady state bifurcates into a limit cycle). We have noticed that three parameters are critical for the oscillatory behaviour: $\lambda = k_{-5}/k_5$ which is the ratio of the transport rates from and to the nucleus, $kv$ which is the volume

ratio between the nucleus and the cytoplasm, and $C$ which is a conserved quantity in all the models ($C = X_2 + X_3$ in $\mathcal{M}_1$, $C = X_2 + X_3 + kvX_4$ in $\mathcal{M}_2$ and $\mathcal{M}_3$, $C = X_2 + X_3 + kv(X_4 + X_6)$ in $\mathcal{M}_4$. $\mathcal{M}_1$ undergoes no oscillations (there are no transport reactions), $\mathcal{M}_3$ can easily oscillate and even produce self-sustained oscillations (see Fig. 4).

From Fig. 4 the minimal model that reproduces the experimentally observed oscillating behavior is $\mathcal{M}_2$.

Certainly, there may be some smaller dimensional models that reproduce this behaviour, that are not based on the mass action law or on reaction graphs. We would like to know how small these can be.

In order to give an approximate answer to this question, we use the following remark: the models $\mathcal{M}_4$ and $\mathcal{M}_2$ have a conservation law and by linear analysis we identify the presence of 3, and 1 rapid modes (more rapid than minutes and well separated by large spectral gaps from the other modes), respectively. This suggests that a two dimensional model should be a good approximation of the dynamics of models $\mathcal{M}_2$ and $\mathcal{M}_4$ for timescales longer than minutes. The model $\mathcal{M}_3$ that is able to produce persistent oscillations has a conservation law and only one rapid mode (more rapid than minutes); its approximate dynamics is three-dimensional.

### 4.4 Invariant manifold method

A general method to find the minimal dynamical representation of a model is the invariant manifold method.

The invariant (positively invariant) manifold is a manifold embedded in a phase space with the property that it is invariant under the flow, i.e., orbits that start out in the manifold remain in it. If a low-dimensional invariant manifold can be constructed for a dynamical system, it allows consistent model reduction by considering the low-dimensional system dynamics induced on it. A typical system trajectory goes fast towards the manifold and after slowly along the manifold, thus the system spends most of the time in its vicinity and the most interesting dynamical phenomena happen there. Existence, stability and dimensionality of the invariant manifold depend on the presence of a spectral gap in the symmetrized Jacobian in a region of the phase space (and can be different from one region to another).

An overview and many examples of application of the invariant manifolds in physics and chemical kinetics can be found in [GK05]. An application of invariant manifolds to metabolic systems was reported in [RF01].

Computation of the invariant manifold or of its approximation for an arbitrary dynamical system is a difficult task. Intrinsic low-dimensional manifold (ILDM) method based on the spectral decomposition of Jacobian fields was developed in [UP92]. Based on an iterative method for solving the invariance equation we developed recently the method of invariant grids for constructing discrete invariant manifolds approximations [GKZ04]. Currently we are working on generalization of this method and its application to biochemical dynamical models.

In [GKZ04] the idea of invariant flag was proposed in the context of the invariant grid construction, as such a hierarchical construction where invariant manifolds of lower dimension are embedded into the invariant manifolds of higher dimensions. In practice, one-dimensional invariant grid is constructed from a steady state and fixed to become the starting point for constructing the two-dimensional invariant grid, and so on. This introduces a system of internal grid coordinates which hierarchically separate the time scales: the first coordinate is the slowest, the second is the second slowest, etc.

Reducing the dynamics of a complex system by proving that this is close to the low-dimensional dynamics of a simpler system also helps to establish the correspondence between the two as well as to determine the essential complex system parameters.

## 5 Hierarchy and robustness

Let us consider that we managed to construct a function $\mathcal{K} \in \mathbb{R}^n \rightarrow f(\mathcal{K})$ expressing a certain property $P$ depending on the values of the parameters $\mathcal{K} = (K_1, K_2, \ldots, K_n)$ of a model representing the biological system. Let the parameters $K_i, i = 1, \ldots, n$ be random variables. The causes of variability of the parameters can be multiple: mutations, across individual variability, variable functional context, etc. Then, we can define two types of robustness of the property $P$:

- Robustness with respect to distributed variations, meaning that when $K_i$ are independent, the variance of $f$ is much smaller than the variance of any of the parameters: $Var(f) << Var(K_i)$.
- Robustness with respect to localized variations meaning that if all of the parameters, except a few are constant, the variance of $f$ is much smaller than the variance of any of the parameters: $Var(f) << Var(K_i), i \leq r$, $Var(K_i) = 0, i > r$, $r$ is small.

There are many causes of robustness of cellular processes. Kitano [Kit04] reviewed many of them: feedback control (responsible for buffering, important in homeostasis), redundance (alternative mechanisms), modularity (autonomy of the modules). We discuss here another possible cause of robustness that is intrinsically related to complexity and has to do with the large number of actors in biological networks.

In simple words, a property of a biological system is robust if it results from the contribution of many parameters, the contribution of each one of the parameters being small. The natural mathematical framework for the study of such effects is the concentration of measure in high dimensional metric measure spaces [Gro99].

Before entering into details let us show how this is connected to hierarchies. Let us consider that we have an hierarchy of models, each one being obtained from an "extended" model $\mathcal{M}_0$ by renormalization operations. The extended model is the most complex one, that in systems biology is the molecular level of biochemical reactions. Let $\mathcal{K}_0$ be the set of parameters of the extended model and $\mathcal{K}$ the set of parameters of a less complex, reduced model $\mathcal{M}$. The correspondence between the set of parameters is performed by a many to one application $\mathcal{K} = \mathcal{R}\mathcal{K}_0$. The renormalization process (model reduction) is intended to preserve a property $P$ of the model $\mathcal{K}_0$ (which for the reduced model $\mathcal{M}$ is $f(\mathcal{K})$), hence we can write $P \approx f(\mathcal{K}) = f(\mathcal{R}\mathcal{K}_0)$. Thus, properties of the extended model are functions of many of its parameters, because parameters of the reduced model are functions of many parameters of the extended model. We need conditions under which such functions "concentrate", i.e. have small variability.

In Gromov's theory the concentration has a geometrical significance: objects in very high dimension look very small when they are observed via the values of real functions (1-Lipschitzian). We say that a metric measure space $E$ has a gaussian observable diameter $D$ if for any 1-Lipschitzian function $f : E \rightarrow \mathbb{R}$ and for any $t > 0$, the measure $\mu$ satisfies $\mu(\{x \in E || f(x) - m_f| > t\}) \leq 2exp(-t^2/2D^2)$. The observable diameters of spaces with positive curvature like the sphere, the cube, in high dimension $n$ converge to zero like $1/\sqrt{n}$. This means that 1-Lipschitzian functions $f$ defined on such high-dimensional spaces concentrate on some central value $m_f$. This represents an important generalization of the law of large numbers and has many applications in mathematics.

In order to apply this theory in biology the difficult part is the construction of the function $f$ giving the property $P$. The few examples that we posses are cases when the concentration of $f$ is related to concentration properties of the order statistics.

If $K_1, \ldots, K_n$ are random variables, the order statistics is the distribution of the values $K_{(1)} < K_{(2)} < \ldots < K_{(n)}$ which are the same values put in ascending order. When $K_i$ are independent, identically distributed variables with density, the distributions of $K_{(i)}$ are known [Leh75]. In particular when $K_i$ are uniform on the interval $[0, 1]$, one has $Var[K_{(s)}] = s(n-s+1)/[(n+1)^2(n+2)]$, that

converges to zero like $1/n^2$ for fixed $s$. Thus, $K_{(s)}$ are robust with respect to distributed variations of the variables $K_i$. They are also robust with respect to localized variations because variations of less than $s$ variables have limited effect on $K_{(s)}$.

These properties of order statistics have been used in [GR05] to prove the robustness of the relaxation time of a linear network of chemical reactions.

Another example, also related to order statistics comes from morphogenesis. Early stages of morphogenesis of Drosophila have been modeled by the Hopfield model (2.2). The stationary patterns satisfy:

$$x_i = \lambda_i^{-1}\sigma_\alpha(\sum_{j=1}^{n} K_{ij}x_j + \sum_{k=1}^{p} T_{ik}m_k(y) - h_i) \tag{5.1}$$

In the case $\alpha >> 1$ ("sharp" gene interactions) the local steady states (at fixed $y$) can be labelled by the subset $S \subset \{1, \ldots, n\}$ of genes that are activated. We can compute the stationary patterns as solutions of the following binary programming problem[RV]:

$$\begin{aligned} &\sum_{j\in S} \tilde{K}_{ij} + \sum_{k=1}^{p} \tilde{T}_{ik}m_k(y) > 1, \quad if\, i \in S, \\ &\sum_{j\in S} \tilde{K}_{ij} + \sum_{k=1}^{p} \tilde{T}_{ik}m_k(x) < 1, \quad if\, i \in S^c. \end{aligned} \tag{5.2}$$

where $\tilde{K}_{ij} = K_{ij}/(\lambda_i h_i)$, $\tilde{T}_{ik} = T_{ik}/h_i$, $S^c$ is the complementary set of $S$, i.e. the set of inactivated genes.

In the simple case of a single maternal gradient $m(x)$ monotonic in $x$, the pattern is made of bands. Each band is characterized by the subset $S$ of activated genes and is limited by:

$$\begin{aligned} &m_1^S(K,T) < m(y) < m_2^S(K,T) \\ &m_1^S(K,T) = max_{i\in S}[(1 - \sum_{j\in S} \tilde{K}_{ij})/\tilde{T}_i] \\ &m_2^S(K,T) = min_{i\in S^c}[(1 - \sum_{j\in S} \tilde{K}_{ij})/\tilde{T}_i] \end{aligned} \tag{5.3}$$

If the numbers of genes in $S$ and $S^c$ are large, than the domain limits $m_1^S(K,T)$,$m_2^S(K,T)$ may concentrate. This is a source of robustness of the pattern with respect to variations of the interaction parameters between genes. Nevertheless, the pattern depends not only on the interaction parameters but also is directly controlled by the maternal gradient $m(y)$. When this gradient changes, all the bands defined by Eq.(5.3) are deformed. Although their order in space is robust (this is not affected by the variations of the maternal gradient as long as there is a single monotonic gradient), their relative size can change. Other compensation mechanisms could be responsible for the stability of the pattern with respect to the variations of the maternal gradient.

# 6 Conclusion

The mathematical techniques described in this paper define a strategy for the study of modules and hierarchies in biology. The modular approach implies decomposing complex models into simpler

submodels with good compositionality properties. This will facilitate the analysis of the models by transforming extremely complex molecular biological models into readable, well structured constructs. The hierarchical approach implies creating a family of models situated at different complexity levels. Renormalization is an important technique for standardizing parameters of models with different complexities and for comparing their properties. New concepts such as variable and minimal dynamical dimension issued from model reduction could provide useful insights into functionality of the biological systems. Finally we showed how robustness can be quantified and proposed a new kind of source of robustness.

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