Dynamical robustness of biological networks with hierarchical distribution of time scales

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Abstract: Concepts of distributed robustness and *r*-robustness proposed by biologists to explain a variety of stability phenomena in molecular biology are analysed. Then, the robustness of the relaxation time using a chemical reaction description of genetic and signalling networks is discussed. First, the following result for linear networks is obtained: for large multiscale systems with hierarchical distribution of time scales, the variance of the inverse relaxation time (as well as the variance of the stationary rate) is much lower than the variance of the separate constants. Moreover, it can tend to 0 faster than $1/n$, where n is the number of reactions. Similar phenomena are valid in the nonlinear case as well. As a numerical illustration, a model of signalling network is used for the important transcription factor NFκB.

1 Introduction

Robustness, defined as stability against external perturbations and internal variability, represents a common feature of living systems. The fittest organisms are those that resist to diseases, to imperfections or damages of regulatory mechanisms, and that can function reliably in various conditions. There are many theories that describe, quantify and explain robustness. Waddington's canalisation [1] was formalised by Thom [2] as structural stability of attractors under perturbations. Many useful ideas on robustness have been imported from the theory of control of dynamical systems and of automata [3, 4]. The new field of systems biology places robustness in a central position among the living systems organising principles, identifying redundancy, modularity and negative feedback as sources of robustness [5–7].

In this paper, we provide some justification to a different, less understood source of robustness.

Early insights into this problem can be found in the von Neumann's discussion of robust coupling schemes of automata [8]. von Neumann noticed the intrinsic relation between randomness and robustness. Quoting him 'without randomness, situations may arise where errors tend to be amplified instead of cancelled out; for example it is possible that the machine remembers its mistakes, and thereafter perpetuates them'. To cope with this, von Neumann introduces multiplexing and random perturbations in the design of robust automata.

Related to this is Wagner's concept of distributed robustness that 'emerges from the distributed nature of many biological systems, where many (and different) parts contribute to system functions' [9, 10]. To a certain extent, distributed robustness and control are antithetical. In a robust system, any localised perturbation should have only small effects. Robust properties should not depend on only one, but on many components and parameters of the system. A weaker version of distributed robustness is the *r*-robustness, when *r* or less changes have small effect on the functioning of the system [11].

Molecular biology offers numerous examples of distributed robustness and of *r*-robustness. Single knockouts of developmental genes in the fruit fly have localised effects and do not lead to instabilities [12]. Complex diseases are the result of deregulation of many genetic pathways [13]. Transcriptional control of metazoa is based on promoter and enhancer regulating DNA regions that collect influences from many proteins [14]. Networks of regulating micro-RNA could be key players in canalising genetic developmental programmes [15]. Interestingly, computer models of gene regulation networks [16] have distributed robustness with respect to variations of their parameters. Flux balance analysis in-silico studies of the effects of multiple knockouts in *Saccharomyces cerevisiae* showed that yeast metabolism is less robust to multiple attacks than to single attacks [11].

Let us formulate the problem mathematically. A property M of the biological system is a function of several parameters of the system, $M = f(K_1, K_2, \ldots, K_n)$. Let us assume that the parameters $(K_1, K_2, \ldots, K_n)$ are independent, random variables. There are various causes of variability: mutations, across individuals variability, changes in the functional context, and so on. For different causes, the distribution of parameters may be significantly different. For example, if parameters change because of random deletion of some reactions, then the appropriate model is $K_i = K_i^0$, with probability $1 - p$, and $K_i = 0$, with probability p. On the other hand, the fluctuation of enzyme activity can be formalised as a distribution of K_i with continuous density.

Considering independent and identical distributions of K_i, we can give two basic examples of functions $M = f(K_1, K_2, \ldots, K_n)$ that have much less variability than individual K_i. The first example considers the average value of K_i, that is, $M = \sum_i K_i/n$: $\mathrm{Var}(M) = \frac{1}{n}\sum_i \mathrm{Var}(K_i)$. If all $\mathrm{Var}(K_i) = \mathrm{Var}(K)$, then $\mathrm{Var}(M) = \mathrm{Var}(K)/n$. The second example considers the order statistics [17]: $M = K_{(l)}$ or $M = K_{(n-l)}$, where K_l is the lst parameter in the order $K_{(1)} \geq K_{(2)} \geq \ldots K_{(n)}$. When l does not depend on n (or is

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uniformly bounded), $\mathrm{Var}(M)$ goes to 0 when $n \to \infty$ as $1/n^2$. This is faster than for the average.

Following these examples, for definitions of robustness we can start from the inequality: $\mathrm{Var}(M) \ll \mathrm{Var}(K)$, where $\mathrm{Var}(K_i) = \mathrm{Var}(K)$ for all $i = 1, \ldots, n$.

To avoid the problem of units and supposing that M, $K_i > 0$, we can use logarithmic scale.

Definition 1: M is robust with respect to distributed variations if the log-variance of M is much smaller than the log-variance of any of the parameters. Let $\mathrm{Var}(\log K_i) = \mathrm{Var}(\log K)$ for all $i = 1, \ldots, n$. Then

$$\mathrm{Var}(\log M) \ll \mathrm{Var}(\log K) \tag{1}$$

Let us consider r-index subsets $I_r = \{i_1, i_2, \ldots, i_r\} \subset \{1, 2, \ldots, n\}$ for given r. Let K_i^0, $i = 1, \ldots, n$, be the central values of the parameters. For given I_r, the perturbed values K_i are obtained by multiplying r-selected central values by independent random scales $s_i > 0$, $i = 1, \ldots, r$, $K_i = K_i^0 s_i$, $i \in I_r$, $\mathrm{Var}(\log s_i) = \mathrm{Var}(\log s)$ for all $i \in I_r$, and $\mathrm{Var}(K_j) = 0$ for all $j \notin I_r$.

Definition 2: M is robust with respect to r variations or r-robust if for any I_r

$$\mathrm{Var}(\log M) \ll \mathrm{Var}(\log s) \tag{2}$$

r-robustness holds if (2) is valid for any deterministic choice of r targets. If the target set I_r is randomly chosen, we shall speak of weak r-robustness. We call *robustness index* the maximal value of r such that the system is r-robust.

The above definitions are inspired from biological ideas. Our first definition corresponds to Wagner's distributed robustness [10]. It expresses the fact that M is not sensitive to random variations of the parameters. r-robustness has been defined in [11] as resistance with respect to multiple mutations. r-robustness can also be interpreted as functional redundance (this is different from the structural redundance of Wagner [10], meaning that many genes code for the same protein) meaning that the property M is collectively controlled by more than r parameters, and cannot be considerably influenced by changing a number of parameters $\leq r$. One should also notice the introduction of a new concept. Even if there are r critical targets (for instance genes whose mutations lead to large effects), the probability of hitting these r targets randomly could be small. We have introduced the weak r-robustness to describe this situation.

Robustness with respect to distributed variations can be a consequence of the Gromov–Talagrand concentration of measure in high dimensional metric-measure spaces [18, 19]. 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 with bounded rate of change (1-Lipschitzian functions: $|f(x) - f(y)| < \|x - y\|$). This represents an important generalization of the law of large numbers and has many applications in mathematics. In this paper, we shall discuss two types of concentration effects: cube concentration that applies to sums or averages and the faster simplex concentration that applies to order statistics (see above).

In both definitions, we propose a robustness criterion. There are two difficulties in relation to this. First, it is difficult to impose an objective criterion for what '$\ll$' means in (1) and (2). In the sense of asymptotic behaviour, it is clear that $\mathrm{Var}(\log M)/\mathrm{Var}(\log K) \to 0$ when $n \to \infty$. When concentration phenomena are present, the ratio $\rho = \mathrm{Var}(\log M)/\mathrm{Var}(\log K)$ should scale like $1/n$ or even like $1/n^2$, where n is the number of independent variable parameters. In practice, we always consider finite number of parameters. In this case, ρ is finite and robustness means that the ratio is smaller than some threshold, $\rho < \theta$. Obviously, when $\mathrm{Var}(\log M)/\mathrm{Var}(\log K) \geq 1$, the system is not robust, hence $\theta < 1$. In general, we should study dependence $\mathrm{Var}(\log M)$ on $\mathrm{Var}(\log K)$ and n (or r – for r-robustness). An example of such study for nonlinear signalling network is presented below. The dependence $\mathrm{Var}(\log M)$ on $\mathrm{Var}(\log K)$ may be nonlinear, but often remains close to a piecewise linear function. In that case, the slopes $d\mathrm{Var}(\log M)/d\mathrm{Var}(\log K)$ are more informative than the ratios $\mathrm{Var}(\log M)/\mathrm{Var}(\log K)$. One can reformulate definitions of robustness and r-robustness using these slopes.

Second, some homogeneity of the parameters is implicit. For instance, in this paper, K_i are kinetic parameters. Because of the exponential Arrhenius law, log-variances of the kinetic parameters can be arbitrarily large with respect to log-variances of the activation energies. A robust property with respect to the kinetic parameters may be artificially declared non-robust with respect to activation energies. Furthermore, we want to exclude trivial cases when M does not depend on K_i. To avoid problems, we can consider only positively homogeneous functions of degree one: $f(\alpha K_1, \alpha K_2, \ldots, \alpha K_n) = \alpha f(K_1, K_2, \ldots, K_n)$ for positive α. If K_i are, for example, matrix elements of a matrix $\boldsymbol{K}$, then eigenvalues λ_i of $\boldsymbol{K}$ are homogeneous functions of K_i of degree one. If for all λ_i, the real part is non-positive, $\mathrm{Re}\lambda_i \leq 0$, and non-zero purely imaginary eigenvalues do not exist, then inverse relaxation time $1/\tau = \min\{-\mathrm{Re}\lambda_i | \lambda_i \neq 0\}$ is positively homogeneous function of K_i of degree one. If right-hand side of a system of differential equations is a homogeneous linear function of K_i, $\dot{x} = \sum K_i \phi_i(x)$, then eigenvalues of Jacobian matrices at any point, inverse periods of limit cycles, and inverse relaxation times are positively homogeneous functions of K_i of degree one. In logarithmic scale, variance of $\log M$ is the same as of $\log(M^{-1})$. Hence, we can consider in Definitions 1, 2 positively homogeneous functions of degrees 1 and -1 together. This is enough for our purposes in this paper.

In this paper, we choose a signalling module example as an illustration of the various concepts of robustness. The robust property that we study here is the relaxation time of a biological molecular system modelled as a network of chemical reactions. Relaxation time is an important issue in chemical kinetics, but there exists biological specifics. A biological system is a hierarchically structured open system. Any biological model is necessarily a submodel of a bigger one. After a change of the external conditions, a cascade of relaxations takes place and the spatial extension of a minimal model describing this cascade depends on time. Timescales are important in signalling between cells and between different parts of an organism. It is therefore important to know how the relaxation time depends on the size and the topology of a network and how robust is this time against variations of the kinetic constants.

In this paper, first, we extend the classical results on limiting steps of stationary states of one-route cyclic linear networks onto dynamic of relaxation of any linear network. This allows us to relate the relaxation time of a linear network with hierarchical distribution of time scales to low-order statistics of the network constants and to prove the distributed robustness of this relaxation time. Last, using a model of the NFκB signalling module as an example, we show that similar results apply to nonlinear networks. For this nonlinear network, the robustness of another characteristic time, the period of its oscillations is studied as well.

2 Limitation of relaxation in linear reaction networks

First, we consider a linear network of chemical reactions. In a linear network, all the reactions are of the type $A_i \to A_j$, and the reaction rates r_{ji} are proportional to the reagents A_i concentration: $r_{ji} = k_{ji}c_i$.

The dynamics of the network is described by

$$\dot{c}_i = \sum_{j,\ j\neq i} (k_{ij}c_j - k_{ji}c_i) \text{ or } \dot{c} = \boldsymbol{K}c \tag{3}$$

where $\boldsymbol{K} = (K_{ij})$, for $i \neq j$, K_{ij} is the reaction rate constant k_{ij} of the reaction producing A_i and consuming A_j (this is zero if no such reaction exists), and $K_{ii} = -\sum_{j,j\neq i} k_{ji}$.

For the analysis of kinetic systems, linear conservation laws and positively invariant polyhedra are important. A linear conservation law is a linear function defined on the concentrations $b(c) = \sum_{i=1}^{q} b_i c_i$ (q is the number of reagents), whose value is preserved by the dynamics (3). The conservation laws coefficient vectors b_i are left eigenvectors of the matrix $\boldsymbol{K}$ corresponding to the zero eigenvalue. For any kinetic system, $b^0 = \sum_{i=1}^{q} c_i$ is the conservation law. A set E is positively invariant with respect to kinetic equations (3), if any solution $c(t)$ that starts in E at time t_0 [$c(t_0) \in E$] belongs to E for $t > t_0$ [$c(t) \in E$ if $t > t_0$]. It is straightforward to check that the standard simplex $\Sigma = \{c | c_i \geq 0,\ \sum_i c_i = 1\}$ is positively invariant set for kinetic equation (3): just check that if $c_i = 0$ for some i, and all $c_j \geq 0$ then $\dot{c}_i \geq 0$. This simple fact immediately implies the following properties of $\boldsymbol{K}$:

- all eigenvalues λ of $\boldsymbol{K}$ have non-positive real parts, $\mathrm{Re}\lambda \leq 0$, because solutions cannot leave Σ in positive time;
- If $\mathrm{Re}\lambda = 0$, then $\lambda = 0$, because intersection of Σ with any plane is a polygon, and a polygon cannot be invariant with respect to rotations of sufficiently small angles;
- The Jordan cell of $\boldsymbol{K}$ that corresponds to zero eigenvalue is diagonal, because all solutions should be bounded in Σ for positive time.
- The shift in time operator $\exp(\boldsymbol{K}t)$ is a contraction in the l_1 norm for $t > 0$: for positive t and any two solutions of (3) $c(t)$, $c'(t) \in \Sigma$

$$\sum_i |c_i(t) - c'_i(t)| \leq \sum_i |c_i(0) - c'_i(0)|$$

Vertices of Σ correspond to components A_i (in each vertex only one $c_i \neq 0$). For any initial state, $c(0) \in \Sigma$; there exists a limit state $\lim_{t\to\infty}\exp(\boldsymbol{K}t)c(0)$. We call a linear network weakly ergodic, if these limits coincide for all $c(0) \in \Sigma$. This is equivalent to uniqueness of steady state in Σ. The steady-state $c^* \in \Sigma$ for weakly ergodic network is not obligatory strictly positive, some of c_i^* could be zero. This is the difference from ergodic networks that have strictly positive steady state.

The ergodicity of the network follows from its topological properties. A non-empty subset V of the reaction digraph vertices forms a sink, if there are no oriented edges from $A_i \in V$ to any $A_j \notin V$. For example, in the reaction digraph $A_1 \leftarrow A_2 \to A_3$, the one-vertex sets $\{A_1\}$ and $\{A_3\}$ are sinks. A sink is minimal if it does not contain a strictly smaller sink. In the previous example, $\{A_1\}$ and $\{A_3\}$ are minimal sinks. Minimal sinks are also called ergodic components.

The following properties are equivalent:

1. the network is weakly ergodic.
2. for each two vertices A_i, $A_j (i \neq j)$ we can find such a vertex A_k that an oriented paths exist from A_i to A_k and from A_j to A_k. One of these paths can be degenerated: it might be $i = k$ or $j = k$.
3. the network has only one minimal sink (one ergodic component).
4. there is an unique linear conservation law, namely $b^0(c) = \sum_{i=1}^{q} c_i$; in other words, the zero eigenvalue of the matrix $\boldsymbol{K}$ is not degenerate.

Hence, the number of independent linear conservation laws is equal to the maximal number of disjoint ergodic components.

These properties of weakly ergodic reaction networks are well known in chemical kinetics [20]. They can be also extracted from the theory of Markov chains [21].

In the proof of this statement, the following transformation plays central role. Let $b^0(c), b^1(c), \ldots, b^l(c)$ be independent linear conservation laws and $b^0(c) = \sum_i c_i$. The map $c \mapsto [b^1(c), \ldots, b^l(c)]$ projects the simplex Σ onto the l-dimensional polyhedron B. Preimage of each point of B is a positively invariant polyhedron in Σ, and preimage of a vertex is a positively invariant face of Σ. The vertices of such a face form a sink (we identify components and vertices of Σ). The number of vertices in l-dimensional polyhedron B cannot be smaller than $l + 1$. So, if there are $l + 1$ independent, linear conservation laws, then there exist $l + 1$ disjoint sinks in reaction graph. Let us assume inverse: there exist l sinks, $S_1, \ldots, S_l$. For each $c \in \Sigma$, the limit exists $c^*(c) = \lim_{t\to\infty}\exp(\boldsymbol{K}t)c$. The independent conservation laws b^j are $b^j(c) = \sum_{i\in S_j} c_i^*(c)$.

Now, let us suppose that the kinetic parameters are well separated and let us sort them in decreasing order: $k_{(1)} \gg k_{(2)} \gg \ldots \gg k_{(n)}$. Let us also suppose that the network has only one ergodic component (when there are several ergodic components, each one has its longest relaxation time that can be found independently). We say that $k_{(r)}$, $1 \leq r \leq n$ is the *ergodicity boundary* if the network of reactions with parameters $k_1, k_2, \ldots, k_r$ is weakly ergodic, but the network with parameters $k_1, k_2, \ldots, k_{r-1}$ it is not. In other words, when eliminating reactions in decreasing order of their characteristic times, starting with the slowest one, the ergodicity boundary is the constant of the first reaction whose elimination breaks the ergodicity of the reaction digraph.

Relaxation to equilibrium of the network is multi-exponential, but the longest relaxation time is given by

$$\tau = \frac{1}{\min\{-\mathrm{Re}\lambda_i | \lambda_i \neq 0\}} \tag{4}$$

An estimate of the longest relaxation time can be obtained by applying the perturbation theory for linear operators to the degenerated case of the zero eigenvalue of the matrix $\boldsymbol{K}$. We have $\boldsymbol{K} = \boldsymbol{K}_{<r}(k_1, k_2, \ldots, k_{r-1}) + k_r\boldsymbol{Q} + o(k_r)$, where $\boldsymbol{K}_{<r}$ is obtained from $\boldsymbol{K}$ by letting $k_r = k_{r+1} = \ldots k_n = 0$, $\boldsymbol{Q}$ is a constant matrix and $o(k_r)$ includes terms that are negligible relative to k_r. From equivalence of the properties (1)–(4), it follows that the zero eigenvalue is twice degenerate in $\boldsymbol{K}_{<r}$ and not degenerate in $\boldsymbol{K}_{<r} + k_r\boldsymbol{Q}$. One gets the following estimate

$$\overline{a}\frac{1}{k_{(r)}} \geq \tau \geq \underline{a}\frac{1}{k_{(r)}} \tag{5}$$

where $\overline{a}$, $\underline{a} > 0$ are some positive functions of $k_1, k_2, \ldots, k_{r-1}$ (and of the reaction graph topology).

Two simplest examples give us the structure of the perturbation theory terms for $\min_{\lambda\neq 0}\{-\mathrm{Re}\lambda\}$.

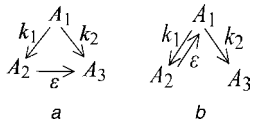

Fig. 1 *Two basic examples of ergodicity boundary reaction*

a Connection between ergodic components

b Connection from one ergodic component to element that is connected to the both ergodic components by oriented paths. In both cases, for $\varepsilon = 0$, the ergodic components are $\{A_2\}$ and $\{A_3\}$

1. For the reaction mechanism shown in Fig. 1*a*, $\min_{\lambda \neq 0}\{-\mathrm{Re}\lambda\} = \varepsilon$, if $\varepsilon < k_1 + k_2$.
2. For the reaction mechanism shown in Fig. 1*b*, $\min_{\lambda \neq 0}\{-\mathrm{Re}\lambda\} = \varepsilon k_2/(k_1 + k_2) + o(\varepsilon)$, if $\varepsilon < k_1 + k_2$. For well-separated parameters, there exists a *trigger alternative*: if $k_1 \ll k_2$, then $\min_{\lambda \neq 0}\{-\mathrm{Re}\lambda\} \simeq \varepsilon$; if, inverse, $k_1 \gg k_2$, then $\min_{\lambda \neq 0}\{-\mathrm{Re}\lambda\} = o(\varepsilon)$.

More generally

$$\tau \simeq \frac{1}{ak_{(r)}} \tag{6}$$

with $a \lesssim 1$. This means that $1/k_{(r)}$ gives the lower estimate of the relaxation time, but τ could be larger. The detailed analysis of multiscale networks [22] shows that there is a trigger alternative too: if the constants are well separated, then either $a \simeq 1$ or $a \ll 1$.

Thus, the well-known concept of stationary reaction rates limitation by 'narrow places' or 'limiting steps' (slowest reaction) should be complemented by the *ergodicity* boundary limitation of relaxation time. It should be stressed that the relaxation process is limited not by the classical limiting steps (narrow places), but by the reactions that may be absolutely different. The simplest example of this kind is an irreversible catalytic cycle: the stationary rate is limited by the slowest reaction (the smallest constant), but the relaxation time is limited by the reaction constant with the second lowest value (in order to break the weak ergodicity of a cycle two reactions must be eliminated).

3 Robustness of relaxation time in linear systems

In general, for large multiscale systems, we observe concentration effects: the log-variance of the relaxation time is much lower than that of the separate constants. For linear networks, this follows from well-known properties of the order statistics [17]. For instance, if k_i are independent, log-uniform random variables, we have $\mathrm{Var}[\log(k_{(r)})] \sim 1/n^2$. Here, we meet a "simplex-type" concentration ([19] pp. 234–236) and the log-variance of the relaxation time can tend to 0 faster than $1/n$, where n is the number of reactions.

For parameters whose logarithm is uniformly distributed in the interval [0, 1], $k_{(r)}$ has a log-beta distribution $\log(k_{(r)}) \sim \mathcal{B}(r, n+1-r)$, i.e. for any $0 \leq a \leq b \leq 1$, $\mathbb{P}[a < \log(k_{(r)}) < b] = 1/\mathrm{B}(r, n+1-r) \int_a^b x^{r-1}(1-x)^{n-r} \mathrm{d}x$, where $\mathrm{B}(r, n+1-r) = \int_0^1 x^{r-1}(1-x)^{n-r}\mathrm{d}x$.

The above estimates for the variance of the order statistics, hence of relaxation time of linear networks, are based on identical distributions of the kinetic constants. A more realistic approach is to consider non-identical distributions with different means. Let δ be the average separation between mean parameters, in logarithmic scale (this separation is zero for identical distributions) and let $\Delta = n\delta$ be the spread of the means. Let us suppose that all the parameters have the same variance $\mathrm{Var}(\log k_i) = \mathrm{Var}(\log k)$. When $\mathrm{Var}(\log k) < \delta^2$, the overlap of distributions of successive parameters is improbable and one has $\mathrm{Var}(\log k_{(r)}) = \mathrm{Var}(\log k)$. When $\delta^2 < \mathrm{Var}(\log k) < \Delta^2$, there is overlap and the variance of $\log k_{(r)}$ is limited by the distance δ, one has saturation: $\mathrm{Var}(\log k_{(r)}) = \delta^2$. Finally, when $\Delta^2 < \mathrm{Var}(\log k)$, we recover the case of identical distributions and one has simplex concentration $\mathrm{Var}(\log k_{(r)}) = \mathrm{Var}(\log k)/n^2$. The three regimes can be observed even for relaxation times of nonlinear models as will be discussed in Section 4.4.

Let us now discuss some design principles for robust networks. Suppose we have to construct a linear chemical reaction network. How to increase robustness of the largest relaxation times for this network? To be more realistic, let us take into account two types of network perturbation:

1. random noise in constants;
2. elimination of a link or of a node in reaction network.

Long routes are more robust for the perturbations of the first kind. So, the first recipe is simple: let us create long cycles! But longer cycles are destroyed by link or node elimination with higher probability. So, the second recipe is also simple: let us create a system with many alternative routes!

Finally, the resources are expensive, and we should create a network of minimal size.

Hence, we come to a new combinatorial problem. How to create a minimal network that satisfies the following restrictions

1. the length of each route is $>L$;
2. after destruction of arbitrarily chosen D_{links} and D_{nodes}, there remains at least one long route in the network.

To obtain the minimal network that fulfills the above constraints, we should include bridges between cycles, but the density of these bridges should be sufficiently low in order not to affect the length of the cycles significantly.

Additional restrictions could be involved. For example, we can discuss not all the routes, but productive routes only (that obligatory include some of the reaction steps).

For acyclic networks, we obtain similar recipes: long chains should be combined with bridges. A compromise between the chain length and number of bridges is needed.

4 Robustness of characteristic times in nonlinear systems: an example

4.1 Model

Our example is one of the most documented transcriptional regulation systems in eukaryote organisms: the signalling module of NFκB. The response of this factor to a signal has been modelled by several authors [23–26].

The transcription factor NFκB is a protein (actually a heterodimer made of two smaller molecules p50 and p65) that regulates the activity of more than one hundred genes and other transcription factors that are involved in the immune and stress response, apoptosis, and so on. NFκB is thus the principal mediator of the response to cellular agression and is activated by more than 150 different stimuli: bacteria, viral and bacterial products, mitogen agents and stress factors (radiations, ischemia, hypoxia, hepatic regeneration and drugs among which some anticancer drugs). NFκB has complex regulation, including inhibitor degradation and production, translocation between nucleus and cytoplasm,

negative and positive feed-back. Under normal conditions, NFκB is trapped in the cytoplasm where it forms a molecular complex with its inhibitor IκB. Under this form, NFκB cannot perform its regulatory function, the complex cannot penetrate the nucleus. A signal that can be modelled by a kinase (IKK) frees NFκB by degrading its inhibitor. Free NFκB enters the nucleus and regulates the transcription of many genes, among which the gene of its inhibitor IκB and the gene of a protein $A20$ that inactivates the kinase.

Here, we would like to study the robustness of the characteristic times of a nonlinear molecular system. In particular, the double negative feed-back (via $I\kappa B$ and $A20$) is responsible for oscillations of NFκB activity under persistent stimulation [23–25]. We are thus interested in three characteristic times of the NFκB model: the period and the damping time of the oscillations and the largest relaxation time (the damping of the oscillations is not necessarily the only relaxation process, therefore the damping time is not necessarily equal to the largest relaxation time).

We use the model introduced in [24] for the response of $NF\kappa B$ module to a signal. This model is represented in Fig. 2. The first reaction of the model is the activation of the kinase. In the absence of a signal, the kinetic constant of the activation reaction is zero $k_1 = 0$, meaning that the kinase IKK remains inactive. The presence of a signal is modelled by a non-zero activation constant $k_1 > 0$, meaning that the kinase is activated.

We have numerically studied the dependence of these time scales on the parameters of the model, which are the kinetic constants of the reactions. The damping time τ_d and the largest relaxation time $\tau_{\max}$ were computed by linearising the dynamical equations at steady state. The period of the oscillation has a rigorous meaning only for a limit cycle, when the oscillations are sustained. At a Hopf bifurcation and close to it, the inverted imaginary part of the conjugated eigenvalues crossing the imaginary axis provide good estimate for the period. Another method for computing the period is the direct determination of the timing between successive peaks. We have noticed that in logarithmic scale (throughout this paper, we use natural logarithm), the differences between the periods computed by the two methods were small, therefore we have decided to use the first method, which is more rapid. A criterion for the existence (observability) of the oscillations is the damping time to period ratio. This ratio is infinite for self-sustained oscillations, big for observable oscillations (when at least two peaks are visible). A low ratio means over-damped oscillations. We call the period an observable one, if the above ratio is larger than one.

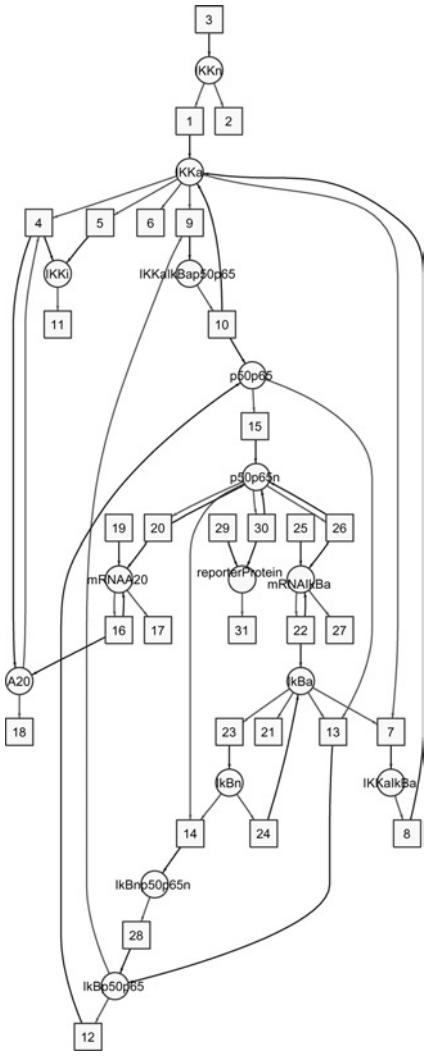

Fig. 2 *Model of NFκB signalling*

Non linear reaction mechanism is represented as bipartite graph. There are 15 chemical species and 31 reactions

4.2 r-robustness of the period

First, we have tested the 1-robustness of the characteristic times. Each parameter has been multiplied by a variable, positive scale factor (changing from 0.001 to 1000), all the other parameters being kept fixed. The result can be seen in Fig. 3.

Large plateaus over which characteristic times are practically constant correspond to robustness. The period of the oscillations is particularly robust. For the damping time

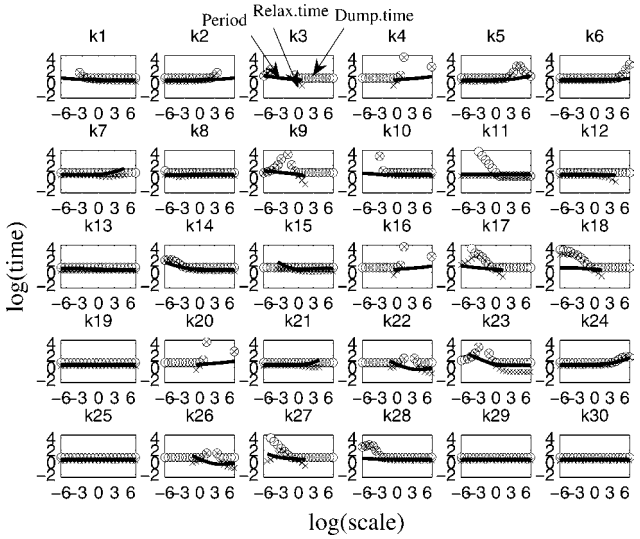

Fig. 3 *Log–log dependence of the characteristic times (circles: largest relaxation time, x marks: dumping time, solid line: period) on the scale factor that multiplies the value of one parameter, while all the other parameters are fixed*

Scale factor varies from 0.001 to 1000 (from −6.9 to 6.9 in logarithm). Oscillations have limited existence regions (outside these regions they are overdamped; our subjective criterion for overdamping is a damping time over period ratio <1.75). There are also regions where oscillations are self-sustained. The limits of these regions are Hopf bifurcation points, where the damping time and the largest relaxation time diverge. Inside these regions, the damping time and the largest relaxation time are infinite and not represented.

and the largest relaxation time, we have domains of substantial variation. There are two types of such domains:

1. domains where $\mathrm{d}\log\tau/\mathrm{d}\log k \simeq -1$.
2. domains where $|\mathrm{d}\log\tau/\mathrm{d}\log k| > 1$

where k is the variable parameter.

The first type of behaviour is the same as the one of linear networks. For linear networks, when one acts on the ergodicity boundary $k_{(r)}$, the longest relaxation time changes inversely proportional to k (this corresponds to $k = k_{(r)}$). When parameters change, they in turn become the ergodicity boundary. Acting on a parameter, which is not the ergodicity boundary, has no effect; this means a plateau in the graph.

The second type of behaviour exists only for nonlinear networks and is related to bifurcations. The variation of one parameter can bring the system close to a bifurcation (for the NFκB model, this is a Hopf bifurcation) where the relaxation time diverges.

The in silico experiment shows that the largest relaxation time is not 1-robust; this time can be significantly changed by modifying a single parameter, for instance k_9. The damping time has similar behaviour being even less robust (some plateaus of the largest relaxation time are higher than the damping time, which continues to decrease; consider for instance the effect of k_9 in Fig. 3).

As also noticed by the biologists [25], the period of the oscillations is 1-robust. We do not have a rigorous explanation of this property. An heuristic explanation is the following. Close to the Hopf bifurcation, two conjugated eigenvalues $\lambda \pm i\mu$ of the Jacobian cross the imaginary axis of the complex plane; λ vanishes that explains the divergence of the relaxation time, while μ, whose inverse is the period, does not change much. However, this is not a full explanation because it does not say what happens far from the Hopf bifurcation point.

4.3 Parameter sensitivity

Not all the parameters have the same influence on the characteristic times. This can already be seen in Fig. 3. To quantify these differences, we have computed the distributions of the characteristic times when one parameter is multiplied by a log-uniform random scale, all the other parameters being fixed. This computation, whose results are represented in Fig. 4, is also a first step towards testing weak r-robustness.

Although rather robust, the period is not constant. Several parameters induce relatively significant changes of this quantity. In the order of increasing strength of their effect on the period, these parameters are: k_7, k_9, k_{15}, k_{23}, k_{22} and k_{26}. Among these, k_{22}, k_{26}, and k_9 expressing the transcription rates of mRNA-IκB, the translation rates of IκB and the binding rate of the kinase to the NFκB–IκB complex are particularly interesting because by changing them, one can increase and also decrease the period. These results confirm and complete the findings of [26]. The parameters that have the greatest influence on the period are the kinetic constants of the production module of IκB: k_{22} and k_{26}. The strong influence of NFκB translocation constant k_{15} on the period, missed in [26], is present here. Interestingly, the delay produced in the transcription/translation module of A20 have smaller effect on the period than the delay produced by the IκB production module. Less obvious is the effect of k_7 and k_9 (binding of IKK to IκB or to the complex) on the period, detected as important both here and in [26].

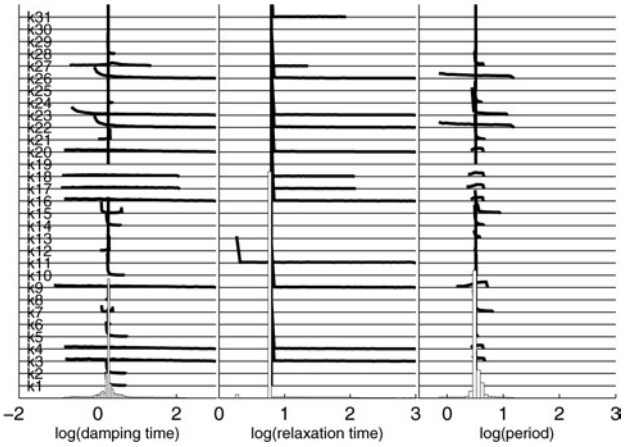

Fig. 4 *Parameter sensitivity study; distributions of the characteristic times when different parameters are, in turn, multiplied by a log-uniform (between 0.1 and 10), random scale factor, while all the other parameters are fixed*

Distributions corresponding to various parameters are spread out vertically. The lower-most, bar-plotted distribution is the average of all the distributions and corresponds to choosing randomly the parameter to be modified. 1-robustness means that all distributions are concentrated (their spread in log-scale is small). Weak 1-robustness means that only the average distribution is concentrated

The damping time to period ratio represents a criterion for observability of the oscillations. To increase the number of visible peaks, one should increase the above ratio. Because the period is robust, this is equivalent to increasing the damping time. Figs. 3 and 4 show that this is possible in many ways by changing only one parameter (decrease in k_3, k_9, k_{17}, k_{18}, k_{23} and k_{27} or increase in k_4, k_{16}, k_{20}, k_{22} and k_{26}).

4.4 Weak r-robustness of all the characteristic times

The divergence of the relaxation time close to a bifurcation does not necessarily imply the absence of weak r-robustness or of distributed robustness. The set of bifurcation points forms a manifold in the space of parameters, of codimension equal to the codimension of the bifurcation; in general, this set has zero measure (stochastic cellular automata provide an interesting counter-example: the NEC automaton of Andrei Toom [27]). The probability of being by chance close to a bifurcation is generally small.

We have tested the weak r-robustness of the characteristic times, by using independent, log-uniform distributions of the parameters over 2 decades interval. All the three characteristic times are weakly r-robust when r is small (see Figs. 4 and 5*a* and *b*). Thus, although controlable (there are critical parameters), the system is weakly robust. Only a directed choice of the right targets has an effect, random choice of a small number of targets is inefficient.

For further study of the r-robustness, we have plotted in Fig. 6 the dependence of the log-variance of the characteristic times on the number of the perturbed parameters r ($1 \leq r \leq n$).

The dependence of the variance of the characteristic times on the number r of perturbed parameters can easily be predicted for a linear network. Let us present simple estimates for only one critical parameter, the ergodicity boundary. Suppose that perturbation of parameters is sufficiently small, $\mathrm{Var}(\log\ k_i) = \mathrm{Var}(\log\ k) < \delta^2$ (see Section 3 for the definitions and notations). If the chosen target is the ergodicity boundary, then for the log-variance of the

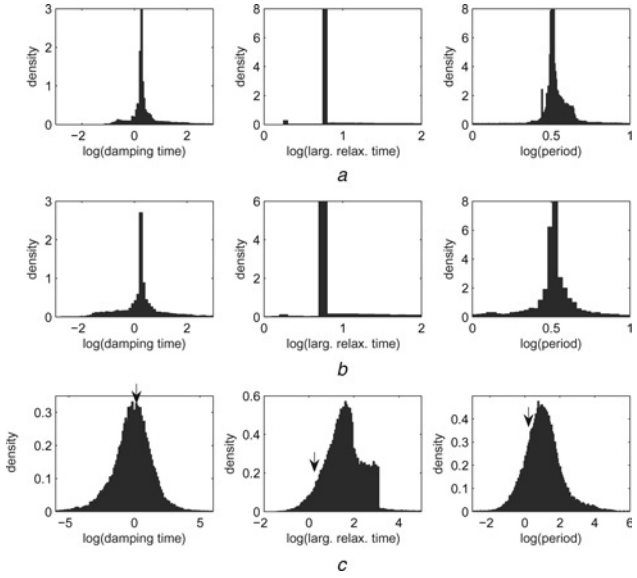

Fig. 5 *Distributions of characteristic times for log-uniform (between 0.1 and 10), independent random scales multiplying the kinetic parameters*

a one parameter, randomly chosen
b changes in two parameters, randomly chosen
c all the parameters
Unperturbed values of the characteristic times are indicated with arrows. The concentration of the distributions at *a* and *b* shows that the period and the relaxation time are weakly 1- and 2-robust. The variation in all parameters produce long tailed distributions (that can be fitted by log-generalised logistic distributions) of the period and of the damping time, slightly biased relative to the unperturbed values (the bias of the period is positive, suggesting that it is easier to increase, than to decrease the period by random perturbations). The distribution of the relaxation time can be described as a mixture of a log-generalised logistic, and of a log-beta distribution. Let us remind that order statistics for log-uniform, independent variables follow log-beta distributions

relaxation time τ we have $\mathrm{Var}(\log\tau) \sim \mathrm{Var}(\log k)$. The probability to pick the ergodicity boundary is $1-(1-1/n)^r \simeq 1-\exp(-r/n)$ (for sufficiently big r), so $\mathrm{Var}(\log\tau)/\mathrm{Var}(\log k_i) \simeq 1-(1-1/n)^r \simeq 1-\exp(-r/n)$. This result can be extended to the case when one has r_0 critical targets. In this case $\mathrm{Var}(\log\tau)/\mathrm{Var}(\log k_i) \simeq C^2[1-(1-r_0/n)^r] \simeq C^2[1-\exp(-rr_0/n)]$, where $C>0$ is a sensitivity. In our case, we know the number of critical targets from the sensitivity studies $r_0 \simeq 10$ (see Fig. 3). The theoretical curve with $C=1$, $r_0=10$ fits well with the calculated log-variance of the damping time for small values of r, see Fig. 6*a*). There are differences at larger r that should be explained by the nonlinear interference between the variations of the parameters. For quantities that follow cube concentration the log-variance is just proportional to r; it is the case of the period, see Fig. 6*a*). To conclude, the plot of $\mathrm{Var}(\log\tau)$ against r can be used to distinguish between cube concentration and presence of critical targets, and in the latter case to estimate the number of critical targets.

We have also used a protocol for testing distributed robustness. This corresponds to changing all the parameters ($r=n=31$ in Fig. 6*b*). Distributed robustness protocol can be used to distinguishing between cube concentration, simplex concentration and the cases with slightly interfering critical targets. It is then useful to plot the log-variance of the characteristic time against the log-variance of the parameters. In the case of cube concentration, one just has the proportionality. For simplex concentration, the discussion from Section 3 applies. There are three regimes: first, proportionality for log-variances up to δ^2, then saturation for log-variances up to Δ^2 and again proportionality with

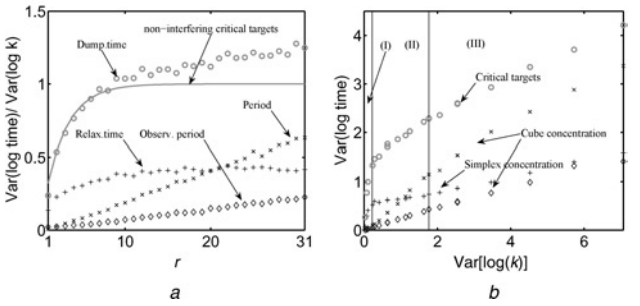

Fig. 6 *Relaxation times of nonlinear regimes*

a Log-variance of the characteristic times against r, the number of perturbed parameters. The choice of the r parameters is random (uniform) and the values of the random scales are independent, log-uniform (between 0.1 and 10). Some statistical samples correspond to overdamped oscillations (dumping time/period ratio <1); these samples were rejected when computing the log-variance of the observable period. The log-variance of the dumping time is compared with the theoretical curve for $r_0=10$ non-interfering critical targets
b Log-variance of the characteristic times against the log-variance of the parameters, for $r=31$. Relaxation time shows typically simplex concentration behaviour, with a saturation regime (II) between two proportionality regimes (I) and (III)

a smaller slope. The first regime applies with no modifications to the case with critical targets, but if there is no interference between targets, no saturation is observed. Fig. 6*b* suggests that the behaviours of the relaxation time, of the period and of the dumping time are examples of simplex concentration, of cube concentration and of weakly interfering critical targets, respectively.

We may also want to know the distributions of the characteristic times for a distributed robustness protocol. When all the parameters take independent log-uniform values, the distributions of characteristic times are much broader than the ones induced by changing a small number of parameters (compare Fig. 5*a* with *c*). Neither the longest relaxation time nor the damping time has distributed robustness (quantitatively, this follows from Fig. 6*a*: for $r=31$ the variance ratios are larger than one). However, Figs. 6*a* and 5 clearly show that the period is more robust than the other characteristic times. In logarithmic scale, the distributions of the dumping time and of the period have tails with different exponential decay rates towards ∞ and $-\infty$. These distributions (a possible fit is by log-generalized logistic distributions) have longer tails in log scale (exponential, compared to gaussian) than log-normal distributions that are sometimes observed in biology [28–32]. The tails are also longer than the ones of the Tracy–Widom distribution characterising largest eigenvalues of certain classes of random matrices [33, 34]. These long tails are related to the critical retardation phenomena [35] close to the Hopf bifurcation (see also Fig. 3). The distribution of the relaxation time can be seen as a mixture between a log-beta (sharply limited by a maximal time) and a log-generalised logistic distribution (accounting for critical retardation).

5 Discussion and conclusions

We demonstrated the possibility of a new kind of robustness of biological systems. This type of robustness has geometrical origin, being related to the high dimension in which variability sources act. There are two basic types of such geometrical effects: cube-type and simplex-type concentrations.

The classical example of the cube concentration gives the central limit theorem, when the robust property is the sum of

many (n), independent contributions. For concentration of this type, the relative standard deviation decreases as $1/\sqrt{n}$. The classical example of the simplex concentration, is the situation when the robust property depends on the kth order effect (parameter) in a collection of many (n) effects (parameters), for example, the relaxation time of a system with limiting step. For concentration of this type, the relative standard deviation decreases much faster, as $1/n$.

We have also defined the concepts of distributed robustness, r-robustness that occur naturally in molecular biology. We have introduced a new notion: weak r-robustness means that the system is robust with respect to blind attacks (the targets are randomly chosen).

Both distributed and r-robustness imply low sensitivity. Thus, sensitivity studies can be useful for the analysis of robustness, but this may be not enough for proving robustness. Indeed, changing many parameters could have an effect even when there are no critical parameters (parameters with respect to which sensitivity is high). Conversely, it may be sometimes difficult to distinguish between a system with critical parameters and a system with limiting steps (simplex concentration). We showed that the log-variance of the output of the system should have a saturation plateau in the first case and not in the latter, as a function of the log-variance of the parameters. For the nonlinear model of NFκB signalling, we have distinguished among three types of phenomena: cube concentration for the period, simplex concentration for the relaxation time and critical parameters for the damping time. We have also shown that weak r-robustness protocols can be used to identify the number of critical parameters, when these exist.

For linear networks, we relate the largest relaxation time to the ergodicity boundary (a topological concept). The notion of ergodicity boundary could not be applied directly to nonlinear systems. Nevertheless, direct computation demonstrates that a nonlinear signalling network also has robust relaxation characteristics, and concentration effects for relaxation time seems similar to linear systems (with some additional long-tail effect related to critical retardation).

In our discussion of robust design of linear networks (Section 3), we considered two types of noise: random noise in constants and destruction of links. The necessity of robustness to both types leads to a new combinatorial problem. How to create a minimal network that has sufficiently long routes (the length of each route is $>L$) and, at the same time, sufficiently many routes; after destruction of D_{links} links and D_{nodes} nodes, there remains at least one long route in the network.

In a recent work, Rand *et al.* [36] introduces the flexibility dimension that quantifies the range of evolution of clocks. This notion applies to multitask evolution, simultaneously fulfilling several objectives. By using linear response theory, the authors propose a method to compute the directions in the characteristic space that are not robust to changes of the parameters: the flexibility dimension is the largest linear space of characteristics that contains non-robust directions. Our notion of robustness index is different, because it does not follow from linear response and more importantly it applies to parameters and not to characteristics. We can explain the sense of robustness index r as follows: for significant change of characteristics by random perturbation, one needs to perturbe $>r$ parameters. Nevertheless, the flexibility dimension and the robustness index have properties in common: they are both small for simple networks and tend to be increased by the loop complexity and by the unevenness of the lifetimes of various species.

Concerning the analysed example, several conclusions are important. NFκB dynamics belong to the category of ultradian oscillators. As for circadian oscillators [36], the period of the oscillations is a relatively robust property. Even if the biological role of these oscillations has not yet been proved (for some conjectures the reader can refer to [25]), it is important to know that the robustness applies to different timescales. A specificity of the NFκB system is the proximity to a Hopf bifurcation. Two nonlinear phenomena could be relevant for the behaviour of the signalling system: the critical retardation and the excitability. The first property would produce long-tail distributions of the damping time of the oscillations. Thus, there are critical parameters for the damping time, which is less robust than the period of the oscillations. The second property could raise the efficiency of the regulatory role of NFκB by increasing the amplitude of its response to signals.

The robustness of a system could be related to its complexity. To test the concentration rigorously, from high-dimension, one needs to build an hierarchy of models obtained from another model by model reduction. Parameters of simpler models in the hierarchy are functions of packages of parameters ('atoms') of more complex models. Independent perturbations of the atoms produce less variability than overall perturbation of packages. Another source of complexity is dynamics itself. It is necessary to take into account dynamical complexity as well as complexity of hierarchical organisation. These ideas have been briefly discussed in [37] and will be presented in detail in a future work.

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