Structural analysis in biology: a control-theoretic approach

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Abstract

Despite their complexity, biological systems are able to endure huge parameter fluctuations and survive in the most diverse environmental conditions. Mathematical tools from graph theory and systems and control theory are naturally well-suited to understand the functioning of biological systems and reveal that their tremendous robustness is often rooted in their peculiar interconnection *structure*. This survey considers a wide class of ordinary-differential-equation biological models, including chemical reaction networks, and provides an overview of structural approaches proposed in the literature to assess whether a system structure enjoys fundamental qualitative properties, yielding specific types of dynamic or steady-state behaviours, regardless of the precise parameter values.

Key words: Dynamical networks; Structural analysis; Robustness; Chemical reaction networks; Systems biology; ODE-models.

1 Why structural analysis?

How can living systems robustly preserve some fundamental properties that are crucial for survival, even though they are inherently affected by variations and fluctuations in their environment? And how can the systems and control community help unveil the secrets of nature's robustness?

Systems biology [5] is a relatively recent field that adopts an interdisciplinary, systems-level approach to study problems in biology and in the life sciences. It promotes holistic and quantitative methods that strongly rely on mathematical models of biological systems to predict possible dynamic outcomes, to understand the effect of perturbations and the sensitivity to key parameters, to help explain experimental results and formulate and test hypotheses on the underlying biological mechanisms.

The growing interest of the control community in this area – which is testified by several special issues of flagship journals, such as [137] [66] [4] [2] [32], and [211] with a particular focus on robustness – is not surprising. The concept of feedback loop was a pervasive principle of nature even before becoming a pillar of control theory and an essential enabling technology in engineering [69] [82]. The astounding complexity of biological functions at all spatial scales, from biomolecular pathways to entire organisms and to ecosystems, relies on myriads of coexisting and entangled feedback loops, which govern both dynamic and steady-state features of biological phenomena. This makes a control-theoretic approach particularly well-suited to help formulate, investigate and solve relevant problems in systems biology. In fact, methods from systems and control theory have been successfully applied to biological problems in the literature, to help gain insight into the design principles that enable complex biological phenomena, and to assess their inherent properties [188] [93] [165] [127].

The intrinsic features of biological systems pose some crucial challenges. In fact, even though systems biology aims for a *quantitative* understanding of natural phenomena (through the use of mathematical models, as opposed to the qualitative, verbal descriptions in traditional biology), biological models unavoidably suffer from huge uncertainty and variability in their parameters, whose values are hard to estimate based on very noisy measurements and are anyway subject to continuous variations and fluctuations; as an example, the circadian rhythms that affect our body functions make several vital parameters vary over a period of about 24 hours. On the other hand, biological systems keep reliably performing their specific task, notwithstanding the large environmental variations and perturbations: hence, there must be something in their "wiring" that inherently guarantees the astounding robustness so widely observed in nature. Several examples of intrinsic robustness in biological systems have been provided starting with [39] [7]. Biological robustness has been discussed from a diversity of viewpoints [140] [158] [145] [138], analysing the trade-off between robustness and fragility [183], and identifying possible paradigms and mechanisms that allow for the extraordinary robustness of living processes [207]. These observations suggest that it must be possible to make sound qualitative predictions even in the absence of quantitative information.

Only deterministic biological models based on ordinary differential equations will be considered in this survey: they are

often adopted as the natural modelling choice when the involved species are abundant and do not experience stochastic fluctuations (for biochemical reactions, this amounts to assuming a perfectly stirred, isothermal reaction environment and high species concentrations). Conversely, for biochemical networks where each species has a low copy number, stochastic models are preferred [13] [52]. However, also deterministic models inevitably include uncertain or unknown parameters that account for the complexity and variability of biological environments.

With a reasonably accurate knowledge of the parameter values, one could predict the behaviour of a biological system through extensive numerical simulations, even though such an approach would not yield any increased understanding of the underlying mechanisms and principles. The absence of quantitative information calls for theoretical approaches that are suitably tailored to draw reliable conclusions on the system properties and expected behaviours even in the presence of huge uncertainties. We wish to predict or rule out possible dynamic behaviours based on a *qualitative* model, without having a precise knowledge of the parameter values and even of the functional expressions that describe the interactions among key players in the system, or their internal dynamics.

A wide body of literature in the control community revolves around dealing with uncertainty and assessing robustness [40]: over the years, extremely powerful theoretical tools have been proposed that can now be employed to analyse the dynamic and steady-state behaviour of the systems we encounter in nature, and to mathematically explain their remarkable stability and robustness properties.

A property is robust for a family of systems with uncertain parameters, along with given bounds, if the property holds for all the elements of the family, with the parameters picked within the given bounds. Often, a stronger notion emerges in a biological context. Some pivotal properties of biological systems are more than robust: not only they hold for all the parameter choices taken within a huge set, but they hold for all possible (i.e., physically meaningful) choices of the parameters. We call structural a property that is independent of the parameter values and holds for a whole family of systems defined without resorting to any arbitrary parameter bound. Note that we will typically consider parameters to be positive; this "qualitative" bound is not an arbitrary requirement that we impose on the parameters, but a simple consequence of their physical meaning.

As an example, the linear dynamical system associated with the equation $a_2\ddot{y}(t) + a_1\dot{y}(t) + a_0 y(t) = 0$ is asymptotically stable for all possible choices of $a_2, a_1, a_0 > 0$. Hence, asymptotic stability is a *structural property* for second order linear differential equations with positive coefficients. However, for third order differential equations with positive coefficients, asymptotic stability is no longer a structural property. Assuming suitable bounds $0 < a_k^- \leq a_k \leq a_k^+$ on the coefficients, we can assess whether the system is stable for all possible choices of the constant coefficients within these bounds; if the answer is positive, asymptotic stability is a *robust property* (given the bounds). The difference between robust and structural properties is more formally discussed in Section 1.1.

Clearly, asking for a property to be structural is a very demanding requirement; nevertheless, many biological systems do enjoy some properties in view of their structure only, namely, exclusively in view of the topology of the interactions among the system components.

The goal of *structural analysis* is to provide methods and approaches to assess whether a property is structurally verified by a family of systems. The answer can only be yes or no: if it is yes, then the analysis provides a very powerful insight into the system and certifies its remarkable *parameter-free robustness*; if it is no, then this does not prevent some elements of the family from enjoying the property.

In the latter case, robustness analysis is precious to assess whether the property is verified for some parameter bounds, and the degree of robustness can be quantified. Therefore, the qualitative (parameter-free) methods stemming from a structural analysis framework are not opposed, but complementary to quantitative (numerical) approaches to establish parametric robustness, such as the early work [149] [197] [139] [181] [179] [198] [204] [64] up to the more recent [118]; see also the thorough overview in [200].

Structural approaches to the analysis of biochemical reaction networks date back to the pioneering work by Horn and Jackson in the early Seventies [122] [123] [124] and later by Reder [172] and Feinberg [95] [96] [97] [98]; along a similar timeline, qualitative approaches started to impose themselves in the field of ecology (see the discussion and the references in [77]). Interest started to arise again at the beginning of the new century. Notably, [196] points out how dynamic function is determined by metabolic network structure and [180] discusses structural sources of robustness.

This survey is specifically focused on the *structural analysis* of biological systems. The literature that explores in general the synergy between control theory and biology is too vast to be explored here; let us just recall, besides the special issues mentioned above, the surveys [188] [93] [206] [35], the books [165] [191] [127] [131] [69] [82] and the recent CDC tutorial session [51].

Also, due to space limits, we cannot deal with important topics such as synthetic biology [141] [81] [125], whose aims are the design of biomolecular controllers and the synthesis of biomolecular systems *de novo*, as well as system identification [162] [163] and model order reduction [99] [171] [178] methods specifically tailored to biological systems.

In Section 2, we introduce the considered mathematical framework, which embraces a broad class of biological models, including (bio)chemical reaction networks, activation-inhibition networks, and models for ecology and epidemics. As stressed above, we only consider ODE models and we refer the reader to the ample available literature for different types of models, such as stochastic [13] [52], piecewise affine [162], Boolean [60] [175] models, and Petri nets [17] [16].

Section 3 formally introduces the concept of structure. It discusses how a system structure can be visualised through graphs and block diagrams and how a system can be mathematically decomposed to suitably split the unknown parameters and the system structure.

Then, we survey methods to structurally assess crucial properties for biological and ecological systems. We consider positivity and boundedness in Section 4 and the existence and the number of steady states in Section 5, along with their stability properties in Section 6. Section 7 provides tools to structurally assess how a system reacts to persistent perturbations; besides input-output influences, fundamental concepts are discussed such as perfect adaptation, sensitivity analysis, ultrasensitive behaviours and scale invariance. Section 8 is devoted to the analysis of signed feedback loops in complex networks and reviews structural graph-based approaches to predict the onset of oscillations as opposed to the emergence of multiple stable equilibria, thus characterising biological oscillators, biological bistable systems and their connection with pattern formation. The structural analysis of large networks is discussed in Section 9, including the fundamental concept of recurring network motifs, methods to simplify the analysis by separating time-scales with a singular perturbation approach and by decomposing a network into an aggregate of monotone subsystems, and retroactivity phenomena arising when multiple components are interconnected. Finally, Section 10 provides some examples of biological insight that can be achieved through structural analysis and stresses the relevance of structural approaches to help validate or falsify biological models.

The concluding discussion in Section 11 points to future challenges in the field.

1.1 Structural and robust properties

The distinction between robust and structural properties is subtle, but fundamental. We can define them as follows.

Definition 1 *[43] Given a family of systems $\mathscr{F}$ and a relevant property $\mathscr{P}$, the property $\mathscr{P}$ is* robustly satisfied *(in short,* robust*) if any element of $\mathscr{F}$ enjoys the property. The property $\mathscr{P}$ is structurally satisfied (in short,* structural*) if, in addition, the family $\mathscr{F}$ is specified qualitatively by a structure, without resorting to numerical bounds.*

Arbitrary numerical bounds are ruled out, but non-negativity of some quantities (≥ 0) can still characterise a structure if it is a requirement due to the physical nature of the considered systems, as it often happens in biology.

Example 1 (Robust or structural?) *Let us exemplify the concepts of robust and structural properties. Given the positive parameters a, b, c, d, e, f and g, consider the two simple families of linear systems specified by the matrices*

$$A_1 = \begin{bmatrix} -f & a & b \\ c & -g & 0 \\ d & 0 & -e \end{bmatrix} \quad \textit{and} \quad A_2 = \begin{bmatrix} 0 & a & b \\ -c & 0 & 0 \\ -d & 0 & -e \end{bmatrix}.$$

All matrices A_1 with $0 \leq a,b,c,d \leq 1$ and $2 \leq e,f,g \leq 3$ are robustly Hurwitz (because of diagonal dominance). For other bounds, this may not be the case. Conversely, all matrices A_2 are structurally Hurwitz (cf. [88, Example 2, p. 239]). Hence, for a linear system governed by a matrix of the form A_1, stability requires that the diagonal terms, associated with the species self-dissipation, are large enough; while, for a linear system governed by a matrix of the form A_2, the presence of dissipation in the third variable ensures unconditional stability, regardless of parameter values: all the systems associated with this structure are stable.

A *structure* is the qualitative (parameter-free) description of a whole family of systems, while a specific realisation is obtained for a fixed choice of the involved quantities and parameter values. As we will discuss in Section 3, a structure can be effectively visualised as a graph formed by a set of nodes, typically representing dynamic variables or subsystems, and by arcs (or hyper-arcs), which represent the interactions among two (or more) nodes. Given a system structure, structural analysis aims at establishing whether a property of interest (typically related to an expected qualitative behaviour, such as stability, multi-stability, or sustained oscillations) is:

- inherent in the structure: the property necessarily holds, regardless of the parameter values;
- compatible but not inherent in the structure: the property holds, not structurally, but for some choice of the parameters, and then we can seek the (largest) parameter bounds for which the property holds robustly;
- incompatible with the structure: no matter how the parameters are chosen, the property cannot hold.

Assessing structural and robust properties is particularly relevant for biological systems, which are able to preserve some properties that are crucial for survival in spite of huge variations and environmental fluctuations.

2 Biological models and their representation

Many different types of models are adopted in systems biology, and including a reasonable description of all of them is far beyond the scope of this survey. Models adopted to describe natural phenomena include, beside those based on Ordinary Differential Equations (ODEs) that will be considered here, Partial Differential Equations (PDEs), Chemical Master Equations, Graphs, Boolean Networks, Petri Nets, Hybrid Systems and Statistical Models. Excellent books and surveys discuss different biological models, including [80] [88] [5] [191] [62] [69] [203] [53] [82] [142].

When studying *dynamical* biological systems, models based on ODEs are particularly popular. They can quite faithfully represent the evolution of natural systems [1] and they are supported by a solid and well-established theory and by efficient tools. Moreover, they can be effectively analysed not only via simulations, but also via theoretical investigation,

[1] For biomolecular systems and gene regulatory networks, ODE models are suitable under the assumption of high copy numbers.

and their analysis can enable the control and the optimisation of biological systems in synthetic biology.

In this survey, we consider ODE models and we focus on a general unifying framework that embraces chemical reaction networks, as well as biological models at all scales: biomolecular, ecological, epidemiological models. The broad class of models we consider can be written in the general form

$$\dot{x}(t) = Sg(x(t)) + g_0, \tag{1}$$

where $x(t) \in \mathbb{R}^n$ is the state describing e.g. (bio)chemical species concentrations, or population density; $S \in \mathbb{R}^{n\times m}$ is the (equivalent) stoichiometric matrix representing the interaction structure; $g : \mathbb{R}^n \to \mathbb{R}^m$ is a vector of reaction rate, or growth rate, functions; and $g_0 \in \mathbb{R}^n$ is a constant supply term representing the effect of the external environment.

2.1 Chemical reaction networks

In chemical reaction networks, CRNs, whose theory is thoroughly surveyed in [19] [98], the concentrations of chemical species (state variables) vary over time due to the occurring chemical reactions (flows), according to the reaction stoichiometry and mass balance rules. In these systems, $S \in \mathbb{Z}^{n\times m}$. Typical examples of reactions are

$$pA + qB \xrightarrow{g_{ab}} rC + sD \quad \text{and} \quad pA + qB \underset{g_{cd}}{\overset{g_{ab}}{\rightleftharpoons}} rC + sD,$$

meaning that p molecules of A and q of B (reagents) bind to form r molecules of C and s molecules of D (products) in an irreversible (left) or reversible (right) way; p, q, r and s are stoichiometric coefficients. In the reversible case, the products C and D can bind to form again the reagents. Species concentrations are denoted using the corresponding lowercase letters.

The reaction rate functions $g_{ab}(a,b)$ and $g_{cd}(c,d)$, representing the speed at which the reactions occur, depend on the concentration of the involved reagents. They are monotonic functions of their arguments and take non-negative values. Under the semi-empirical *law of mass action*, reaction rates have the polynomial form $g_{ab}(a,b) = k_{ab}a^p b^q$ and $g_{cd} = k_{cd}c^r d^s$, where the positive constants k_{ab} and k_{cd} depend in principle on several factors, including the temperature. Homogeneity is also crucial: isotropic concentration profiles in the reaction environment must be assumed, i.e., well stirred reactors [98].

More in general, a chemical reaction network [19] [98] is a set of chemical reactions

$$\sum_{i=1}^{n} \theta_{ji} X_i \xrightarrow{g_j} \sum_{i=1}^{n} \lambda_{ji} X_i, \qquad j = 1,\dots,m,$$

each transforming the reagents, appearing on the left with stoichiometric coefficients θ_{ji}, into the products, appearing on the right with stoichiometric coefficients λ_{ji}. Each reaction speed $g_j(\cdot) \geq 0$ is a monotonic function of the reagent concentrations. The entries of the stoichiometric matrix S are defined as $S_{ij} = \lambda_{ji} - \theta_{ji}$ and the differential equation ruling the evolution of the species concentration x_i is

$$\dot{x}_i(t) = \sum_{j\in\mathbb{J}_i} S_{ij} g_j(x) + [g_0]_i, \tag{2}$$

where g_j represent incoming or outgoing flows due to chemical reactions that produce or use x_i and $\mathbb{J}_i$ is the set of reactions that either produce or consume x_i. The overall model takes the matrix-vector form (1).

All functions g_j appearing with a negative S_{ij} *must* include x_i as an argument and it must be $g_j(x_i,\dots) = 0$ if $x_i = 0$; this ensures positivity, as we will discuss in Section 4.

A function g_j can have an arbitrary number of variables; under mass-action kinetics, the reaction $pA + qB + rC \xrightarrow{g_{abc}} D$ would have the rate $g_{abc}(a,b,c) = k_{abc}a^p b^q c^r$, with $k_{abc} > 0$.

Example 2 (A metabolic network.) *The reaction network presented in [62, p. 106] involves four species A, B, C, D and the reactions*

$$\emptyset \xrightarrow{a_0} A, \quad A + C \xrightarrow{g_{ac}} B + D, \quad D \xrightarrow{g_d} C, \quad B \xrightarrow{g_b} \emptyset,$$

which correspond to the following flows: flow a_0, from the external environment, produces species A; species A interacts with C to produce species B and D, with reaction speed g_{ac}; species D produces C at rate g_d; finally, B degrades at rate g_b (which can be seen as a flow to the external environment). The corresponding differential equations are

$$\begin{aligned} \dot{a} &= -g_{ac}(a,c) + a_0, & \dot{b} &= g_{ac}(a,c) - g_b(b), \\ \dot{c} &= -g_{ac}(a,c) + g_d(d), & \dot{d} &= g_{ac}(a,c) - g_d(d). \end{aligned}$$

and can be rewritten in the form (1) *as follows*

$$\underbrace{\begin{bmatrix} \dot{a} \\ \dot{b} \\ \dot{c} \\ \dot{d} \end{bmatrix}}_{\dot{x}} = \underbrace{\begin{bmatrix} -1 & 0 & 0 \\ 1 & -1 & 0 \\ -1 & 0 & 1 \\ 1 & 0 & -1 \end{bmatrix}}_{S} \underbrace{\begin{bmatrix} g_{ac}(a,c) \\ g_b(b) \\ g_d(d) \end{bmatrix}}_{g(x)} + \underbrace{\begin{bmatrix} a_0 \\ 0 \\ 0 \\ 0 \end{bmatrix}}_{g_0}.$$

In general, the state $x(t)$ evolves in the stoichiometric compatibility class, an affine manifold depending on the initial conditions:

$$\mathscr{S}(x_0) = \left\{ x = x_0 + \mathrm{Ra}[\tilde{S}], \;\; x \geq 0 \right\}, \tag{3}$$

where $\mathrm{Ra}[\tilde{S}]$ is the range space of $\tilde{S} = [S\ g_0]$. The dimension of $\mathscr{S}(x_0)$ is the rank of $\tilde{S}$. Note that, if there exists an equilibrium point $\bar{x}$ such that $Sg(\bar{x}) + g_0 = 0$, then $\mathrm{Ra}[\tilde{S}] = \mathrm{Ra}[S]$. In Example 2, the dimension of $\mathscr{S}(x_0)$ is 3, because $[0\ 0\ 1\ 1]^\top$ belongs to $\ker[\tilde{S}^\top]$. As a consequence, $\dot{c} + \dot{d} = 0$,

hence, for any initial condition $a(0), b(0), c(0)$ and $d(0)$, the quantity $c(t)+d(t) = c(0)+d(0) = \kappa$ is constant. Taking $d = \kappa - c$ and removing the equation of $\dot{d}$, we get

$$\begin{bmatrix} \dot{a} \\ \dot{b} \\ \dot{c} \end{bmatrix} = \begin{bmatrix} -1 & 0 & 0 \\ 1 & -1 & 0 \\ -1 & 0 & 1 \end{bmatrix} \begin{bmatrix} g_{ac}(a,c) \\ g_b(b) \\ g_d(\kappa - c) \end{bmatrix} + \begin{bmatrix} a_0 \\ 0 \\ 0 \end{bmatrix}. \quad (4)$$

We consider reaction functions that satisfy general assumptions, according to the following definition.

Definition 2 *A smooth function $g : \mathbb{R}^n_+ \to \mathbb{R}_+$ is a* proper reaction function *if $g(x)$ is monotonic in each variable x_j.*

A proper reaction function is not necessarily bounded: Michaelis-Menten and Hill kinetics [5] lead to bounded functions, such as $g(x,y) = \kappa \frac{xy}{1+\alpha xy}$, while mass-action kinetics [98] lead to unbounded functions $g(x,y) = \kappa xy$.

2.2 Activation-inhibition networks

An important sub-class of models describes systems where an arbitrary number of elements or species interact by either activating or inhibiting one another. Among these we find gene regulatory networks, which describe how the activity of genes is promoted or repressed to regulate the amount of their expressed proteins.

Denoting by x_i the amount of active species i, its time evolution is described by the equation [5] [62] [43]

$$\dot{x}_i(t) = -\mu_i x_i(t) + \sum_{j \in \mathbb{J}_i} f_{ij}(x_j(t)) + \sum_{k \in \mathbb{K}_i} g_{ik}(x_k(t)) + u_i, \quad (5)$$

where $-\mu_i x_i$ represents self-degradation, $\mathbb{J}_i$ and $\mathbb{K}_i$ are the sets of species that respectively activate and inhibit x_i, the increasing positive functions $f_{ij}(x_j)$ represent the activation effect of x_j on x_i, while the decreasing positive functions $g_{ik}(x_k)$ represent the inhibition effect of x_k on x_i, and u_i is an external input. Sometimes interactions can involve multiple variables; hence, in general, interaction functions can have multiple arguments, and can be increasing in some arguments and decreasing in others, e.g. $\frac{x/\alpha}{1+y/\beta}$. To account for the (steep) nonlinearity of the phenomenon, activation and inhibition functions are typically sigmoidal functions.

Definition 3 *A smooth function $f(x)$, $x \geq 0$, is an (increasing)* sigmoid *if: $f(0) = 0$, f is strictly increasing and bounded, and its derivative f' is unimodal, namely it has a single isolated maximum point. A smooth function $g(x)$, $x \geq 0$, is a (decreasing)* complementary sigmoid *if $g(0) - g(x)$ is a sigmoid.*

Common sigmoidal functions are Hill functions [62] [5]

$$f(x) = \alpha \frac{(x/\beta)^p}{1+(x/\beta)^p}, \qquad g(x) = \gamma \frac{1}{1+(x/\delta)^p}, \quad (6)$$

and Michaelis-Menten functions, where $p = 1$. For large p, the functions in (6) tend to a threshold: if $p \to \infty$, both functions locally converge to a step function with discontinuity in β and in δ, respectively.

Example 3 (Incoherent Feedforward Loop.) *The system of equations [5]*

$$\begin{aligned} \dot{a} &= -\mu_a a + u \\ \dot{b} &= -\mu_b b + f_c(c) + f_a(a) \\ \dot{c} &= -\mu_c c + g_a(a) \end{aligned}$$

describes the regulatory interactions among genes A, B and C: A activates B and inhibits C, while C activates B, and u is an external activation signal for A. The variables a, b and c can be seen as the concentration of the proteins expressed by the corresponding gene; then, as discussed in [62], the above equations are the reduced-order model, based on a time-scale separation argument (see Section 9.2), of a more complex system involving gene-RNA-protein dynamics.

Also more sophisticated activation or inhibition functions are possible, such as the co-regulator functions $h_{OR}(x,y) = h(x^p + y^q)$ and $h_{AND}(x,y) = h(x^p y^q)$, where h is a step-like function which can be either activating (f) or inhibiting (g); for p and q sufficiently large, these functions converge to *OR* or *AND* functions. See [5, Appendix B] or [62, Section 3.3.2] for further details.

It is worth stressing that *any* linear time-invariant system (possibly uncertain) whose state matrix has sign determined entries (as well as any nonlinear system with a sign-definite Jacobian) can be seen as an activation-inhibition network.

Activation-inhibition networks representing biochemical phenomena conceptually differ from CRNs: the latter are *mechanistic* models, based on established chemical laws, while the former are *phenomenological* models, aimed at reproducing empirical observations by considering interactions that only implicitly account for the true physical mechanisms.

2.3 Population dynamics: ecosystems and epidemiology

The control-theoretic approach we consider in this survey fits very well in many other biological contexts. In fact, models representing population dynamics in ecosystems, as well as the spreading of diseases, can be typically written as an ODE system having the form (1) and often "behave as a CRN" [94]. We propose here a couple of examples.

Example 4 (Prey and predator dynamics.) *Consider the "chemical reactions" $A \xrightarrow{\kappa_a} 2A$, $A+B \xrightarrow{\kappa_{ab}} 2B$, $B \xrightarrow{\kappa_b} \emptyset$, where A produces itself (auto-catalysis); whenever A and B collide, A becomes B (conversion); B disappears (degradation). Under the law of mass action, we get the equations of the celebrated Lotka-Volterra prey-predator system:*

$$\begin{aligned} \dot{a} &= \kappa_a a - \kappa_{ab} ab, \\ \dot{b} &= -\kappa_b b + \kappa_{ba} ab, \end{aligned}$$

where $\kappa_{ab} = \kappa_{ba}$ can be ensured, without restriction, by scaling the variables as $\hat{a} = \sqrt{\kappa_{ba}/\kappa_{ab}}\, a$ and $\hat{b} = \sqrt{\kappa_{ab}/\kappa_{ba}}\, b$.

Example 5 (Epidemics.) *Another famous "chemical reaction network" is the SIR epidemiological model*

$$\begin{aligned}\dot{x}_S &= -\beta x_S x_I + \gamma x_R\\ \dot{x}_I &= \beta x_S x_I - \nu x_I\\ \dot{x}_R &= \nu x_I - \gamma x_R\end{aligned}$$

where, in a population with $n = x_S + x_R + x_I$ individuals exposed to a disease, x_S denotes the number of individuals susceptible to become ill, x_I denotes the number of ill individuals and x_R the number of recovered individuals; β, γ and ν are positive constants. Since $\dot{n} = 0$, n is a constant: no birth rate or death rate is considered. Although conceptually different, the dynamics are akin to those of the CRN

$$S + I \xrightarrow{\beta} 2I, \quad R \xrightarrow{\gamma} S, \quad I \xrightarrow{\nu} R$$

where, denoting by x_A the concentration of species A, the state evolves in the affine variety $x_S + x_R + x_I = n = $ constant (stoichiometric compatibility class). An analogous analysis applies to any compartmental model for epidemiology [53].

Although it cannot be argued that any model of natural system can be written as a chemical reaction network, or as an activation-inhibition network, models of natural phenomena often share common features that make them fit within the class of systems in (1):

- they are positive systems (the variables take non-negative values);
- they obey physical laws such as mass conservation;
- the interaction functions are typically monotone;
- the interaction pattern (structure) is effectively represented by a graph.

3 Capture the structure

The structure of a system is the topology (qualitative pattern) of the interactions among the system variables, along with qualitative information about the nature of such interactions (such as monotonicity). Powerful tools to visualise a structure are graphs and block diagrams, as well as signed matrices whose sign pattern is not affected by parameter uncertainty or variability.

3.1 Graphs and block diagrams

Graphs are a universal tool to describe a structure: they are perfectly suited to visualise the complex interplay of many entangled interactions, such as those found in biological systems, and can facilitate the analysis and the explanation of the underlying mechanisms.

Graphs are widely used across disciplines, but can have different meanings in different contexts. In this survey, we always associate the nodes of a graph either with individual state variables, which represent the amount of a species, or with subsystems (i.e., subsets of state variables). However, depending on what the arcs represent, we consider two different types of graphs:

- in *flow graphs*, arcs are associated with reactions and represent the flow of a species that moves from one node to the other, because it is transformed into a different species;
- in *signal graphs*, arcs are associated with inhibiting or activating interactions, which do not (necessarily) correspond to flows or reactions.

In our figures, arcs representing flows have open pointed arrow heads, while arcs representing signals have either closed and filled pointed arrow heads (activations) or hammer heads (inhibitions).

In a flow graph, an arc from A to B indicates the presence of a flow that decreases the amount of A and proportionally increases the amount of B (corresponding to the monomolecular reaction $A \xrightarrow{g_a} B$). Conversely, in a signal graph, an arc from A to B represents the activation of B due to A, which leads to an increase in the amount of B and leaves the amount of A unchanged. If B is a protein activated by A, the activating arc corresponds in general to a chain of chemical reactions with several intermediate steps, hence the arcs in signal graphs *do not represent single reactions*.

Chemical reaction networks of the form (1) are associated with a flow graph, which is in a one-to-one correspondence with the stoichiometric matrix S, hence it captures the system structure. Clearly some assumptions on the involved functions g_i are needed. The metabolic network in Example 2, for instance, is associated with the *flow graph* in Fig. 1, where each arc represents one of the occurring reactions.

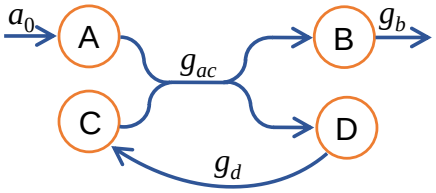


Figure 1. Flow graph corresponding to the metabolic network in Example 2. Arcs are associated with chemical reactions.

Activation-inhibition networks are instead associated with a signal graph, which is in a one-to-one correspondence with the sign pattern of the system Jacobian matrix, hence it captures again the system structure. For instance, the incoherent feedforward loop in Example 3 corresponds to the *signal graph* in Fig. 2. The arcs represent the activations f_a and f_c, and the inhibition g_a. Note that the self-degradation terms are not drawn in Fig. 2, but they would be represented as the self-loop in Fig. 3, right. In fact, self-degradation of a

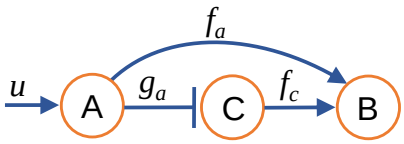


Figure 2. Signal graph corresponding to the incoherent feedforward loop in Example 3. Arcs are associated with activating (pointed head) or inhibiting (hammer head) interactions.

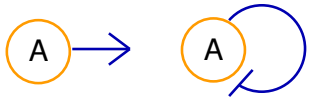


Figure 3. Self-degradation: a leak in a flow graph (left) and an inhibitory self-loop in a signal graph (right).

species corresponds to a leak towards the external environment, if seen as a flow (Fig. 3, left), and to an inhibitory self-loop, if seen as a signal (Fig. 3, right).

Block diagrams, a standard representation of interconnected systems for control engineers, are not commonly encountered in mathematical biology. In recent years they have started to appear, most probably due to the strong interactions between the two communities (see e.g. [69] [81] [165]). Block diagrams can be seen as signal graphs where the nodes are subsystems rather than individual variables.

Several additional types of graphs can be considered for the analysis of biochemical networks. Of considerable interest is the species-reaction graph [73] [24], which includes two types of nodes, one associated with species and one with reactions. The graph is bipartite: the arcs can only connect a reaction node to a species node, or vice versa.

3.2 The concept of structure

A family of systems is characterised by a structure if

- all the elements of the family are represented by the same (flow or signal) graph;
- the involved functions satisfy common qualitative assumptions.

For CRNs (1), the structure is defined by the matrix S, the sparsity pattern of vector g_0, and proper assumptions on g.

For instance, the system in Example 2, associated with the graph in Fig. 1, represents a structure if we assume that all functions are smooth, defined for nonnegative values, nondecreasing in each argument, and positive if and only if all arguments are positive (e.g., $g(a,c)$ is zero only if either a or c are zero, and positive otherwise). A sub-family is obtained if we additionally assume that the reaction rate functions are polynomial (mass action kinetics).

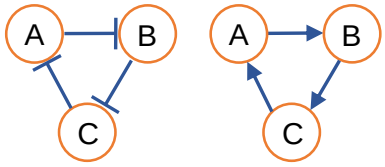


Figure 4. Signal graphs of the repressilator (left) and promotilator (right) structures in Example 6; self-loops are omitted.

Example 6 (Repressilator and Promotilator.) *Consider the system family* (5) *where each node has a self-degradation term; each interaction function is sigmoidal (e.g. Hill-type); the interaction graph is assigned. For instance, the structure visualised by the signal graph in Fig. 4, left, corresponds to a simplified version (based on time-scale separation) of the well-known* repressilator *model [90], a chain where each node inhibits the next (see [82], [5] for details). The dual* promotilator *structure [92], shown in Fig. 4, right, is a chain where each node activates the next. Denoting by g decreasing (inhibitory) functions and by f increasing (activating) functions, and including self-degradation for each species, the two structures correspond to the dynamical systems:*

$$\begin{cases} \dot{a} = -\mu_a a + g_c(c), \\ \dot{b} = -\mu_b b + g_a(a) \\ \dot{c} = -\mu_c c + g_b(b) \end{cases} \qquad \begin{cases} \dot{a} = -\mu_a a + f_c(c) \\ \dot{b} = -\mu_b b + f_a(a) \\ \dot{c} = -\mu_c c + f_b(b) \end{cases}$$

We will analyse both systems in Examples 10 and 16.

3.3 BDC and EDF decompositions

A system structure can be described resorting to the *BDC* decomposition [44] [109]. For any CRN of the form (1), or any activation-inhibition network with equations (5), the system Jacobian can be written as the positive linear combination of rank-one matrices:

$$J_x(x) = \sum_{k=1}^{q} D_k(x) J_k = BD(x)C.$$

The rank-one matrices $J_k = B_k C_k^\top$ are often marginally stable, namely $C_k^\top B_k < 0$. Matrices B and C are not square in general. For a system (1), B is a matrix formed by possibly repeated columns of S, D a diagonal matrix whose diagonal elements D_k are the absolute values of all the partial derivatives of $g(x)$, while C is a matrix whose kth row has a nonzero entry equal to 1 or -1, depending on the sign of the derivative, in the position j if D_k is a derivative with respect to x_j (see Example 7 and [109] for details). In the particular case of activation-inhibition networks, the Jacobian has a known sign pattern: then, the diagonal entries of D are the absolute values of the matrix entries, $D_k = |J_{ih}|$, and the columns of B and rows of C identify their position in the Jacobian.

The linearised system has the form

$$\dot{z}(t) = BDCz(t), \tag{7}$$

where $z(t) = x(t) - \bar{x}$ and $\bar{x}$ is any steady state such that $Sg(\bar{x}) + g_0 = 0$. The nonlinear system can be equivalently rewritten as

$$\dot{z}(t) = BD(z(t))Cz(t), \tag{8}$$

as can be proven by taking into account the formula [136]

$$f(z+\bar{x}) = f(\bar{x}) + \left[\int_0^1 J(\bar{x}+\sigma z)d\sigma\right] z,$$

where $J = \frac{\partial f}{\partial x}$. The same decomposition applies to the rate representation of CRNs [8] [9] [45]. Given the steady-state value $\bar{x}$ such that $0 = Sg(\bar{x}) + g_0$, define $\bar{r} = g(\bar{x})$ and consider as a state variable the rate vector

$$r(t) \doteq g(x(t)) - g(\bar{x}) = g(x(t)) - \bar{r}.$$

Then

$$\dot{r}(t) = \frac{\partial g}{\partial x}\dot{x} = \frac{\partial g}{\partial x}(Sg(t) + g_0) = \frac{\partial g}{\partial x}Sr(t). \tag{9}$$

Reasoning as before, we can write the system Jacobian as

$$J_r(r) = ED(x)F \tag{10}$$

and system (9) can be rewritten as $\dot{r}(t) = ED(x(t))Fr(t)$, where D is the same matrix as before, matrix F is formed by possibly repeated rows of S, and the kth column of E has a single nonzero entry, in the position j, corresponding to the sign of the derivative $D_k = \partial g_h/\partial x_i$ with $r_j = g_h(x) - g_h(\bar{x})$.

A Jacobian of the form (10) is achieved also if we consider the equations in reaction coordinates [24]. Assuming $g_0 = 0$ for brevity and denoting $w(t) = \int_0^t g(x(\sigma))d\sigma$, the reaction system is $\dot{w}(t) = g(x(t))$. Then, replacing $x(t) = x_0 + S\int_0^t g(x(\sigma))d\sigma = x_0 + Sw(t)$ yields

$$\dot{w}(t) = g(x_0 + Sw(t)) \tag{11}$$

(see [24] for details), whose Jacobian has the form (10).

Example 7 (Structural decompositions.) *For the metabolic network in Example 2 in its reduced form* (4)*, we have the decomposition* $BDC =$

$$\begin{bmatrix} -1 & -1 & 0 & 0 \\ 1 & 1 & -1 & 0 \\ -1 & -1 & 0 & 1 \end{bmatrix} \begin{bmatrix} \left|\frac{\partial g_{ac}}{\partial a}\right| & 0 & 0 & 0 \\ 0 & \left|\frac{\partial g_{ac}}{\partial c}\right| & 0 & 0 \\ 0 & 0 & \left|\frac{\partial g_b}{\partial b}\right| & 0 \\ 0 & 0 & 0 & \left|\frac{\partial g_d}{\partial c}\right| \end{bmatrix} \begin{bmatrix} 1 & 0 & 0 \\ 0 & 0 & 1 \\ 0 & 1 & 0 \\ 0 & 0 & -1 \end{bmatrix}$$

in species concentration coordinates, while in reaction rate coordinates we have $EDF =$

$$\begin{bmatrix} 1 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & -1 \end{bmatrix} \begin{bmatrix} \left|\frac{\partial g_{ac}}{\partial a}\right| & 0 & 0 & 0 \\ 0 & \left|\frac{\partial g_{ac}}{\partial c}\right| & 0 & 0 \\ 0 & 0 & \left|\frac{\partial g_b}{\partial b}\right| & 0 \\ 0 & 0 & 0 & \left|\frac{\partial g_d}{\partial c}\right| \end{bmatrix} \begin{bmatrix} -1 & 0 & 0 \\ -1 & 0 & 1 \\ 1 & -1 & 0 \\ -1 & 0 & 1 \end{bmatrix}$$

Systems admitting these decompositions are a vast class, including (bio)chemical reaction networks, gene regulatory networks, ecological and epidemiological models. As we will see later on, in Sections 6 and 7, these decompositions can nicely capture a system structure, thus enabling structural analysis results.

4 Positivity and boundedness

For biological systems, positivity is an expected feature. The concentration of biochemical species, such as mRNA or proteins, and the population density are nonnegative variables. This must be intrinsic in the model and the nonnegativity of the variables must be structurally preserved by the system evolution; otherwise, the model is unsuitable.

Definition 4 *A system with state vector $x(t)$ is* positive *if $x(t_0) \geq 0$ (componentwise) implies $x(t) \geq 0$ for all $t > t_0$.*

We can easily assess whether a system is positive thanks to a well-known property that follows by Nagumo's theorem (see Theorem 1).

Proposition 1 *The dynamical system*

$$\dot{x}(t) = f(x(t)), \tag{12}$$

with f regular enough to ensure existence and uniqueness of the solution, is positive if, when $x_i = 0$ (the ith component of x is zero), then $\dot{x}_i = f_i(x) \geq 0$.

If the condition holds for any system in a family, regardless of parameter values, then positivity is structural.

Examples of structurally positive systems are the biochemical networks in the class (1), $\dot{x} = Sg(x) + g_0$, under the following qualitative assumptions: g_0 must be a nonnegative vector and, if $S_{ij} < 0$, then the positive reaction function g_j, the jth component of vector g, must be increasing in x_i and be 0 as $x_i = 0$. Indeed, if the positive and increasing reaction function g_k appears with a minus sign in the equation of x_i, then it must be a function of x_i (in fact, X_i must be one of the reagents, and the reaction rate depends on its concentration) and the reaction must stop occurring when $x_i = 0$.

For a linear system, $\dot{x} = Ax$, positivity is equivalent to A being a Metzler matrix (namely, $A_{ij} \geq 0$ for $i \neq j$). The linearisation of a nonlinear positive system is not necessarily positive, since the Jacobian is not Metzler in general; this is not surprising, since the linearisation is referred to a steady state and is just valid locally.

A property that is related to positivity, but stronger, is persistence: a CRN is persistent if, for positive initial conditions, no solution approaches the boundary of the positive orthant [17] [18].

Biological systems are not only positive but also, often, bounded: in spite of possibly wide fluctuations, both the concentrations of biomolecular species within a living cell and the density of biological species in a natural environment cannot exceed finite thresholds, respectively due to degradation, dilution or secretion, and due to death rate and finite carrying capacity of the ecosystem. However, boundedness of the system evolution is harder to check, since no simple general conditions like the one in Proposition 1 are available.

Definition 5 *System* (12) *is* bounded *if, for all $N > 0$, there exists $M > 0$ such that $\|x(0)\| \leq N$ implies $\|x(t)\| \leq M$, for $t \geq 0$.*

For positive systems this means that, for any initial state $x(0) \geq 0$, there exists a positive constant ζ such that $0 \leq x_i(t) \leq \zeta$ for all $t \geq 0$. Even though the value of the constant ζ depends on the parameters, the existence of the bound can be a structural property.

Boundedness of the solution is clearly ensured if the stoichiometric compatibility class (3) is a bounded set for any

nonnegative initial condition x_0. This is the case of *conservative* networks [19]. A CRN (1) is conservative if $g_0 = 0$ and there exists a positive vector ν such that

$$\nu^\top S = 0.$$

This condition implies that $\nu^\top \dot{x} = 0$, hence $\nu^\top x(t) = \nu^\top x_0$ is constant. Since x is a nonnegative vector and all the entries of ν are positive, this implies boundedness. The condition is structural, since it only depends on matrix S. It is quite a strong condition, which rules out incoming flows in the network. Typically, a conservative network does not exchange mass with the external environment [19].

The condition is interestingly satisfied by the networks where all species can be present either in the active A or the inactive A^* form, so that the overall concentration $a^* + a$ is constant (see e.g. [148] [108]). Mass conservation laws are present in the *futile cycle* motif (also known as substrate or enzymatic cycle), which is widespread in cellular signalling pathways, metabolic control, cell division, apoptosis and cell-cycle control [205]. An example is given by the phosphorylation-dephosphorylation cycle reactions, $S_1 + E \rightleftharpoons C_1 \rightharpoonup S_2 + E$ and $S_2 + F \rightleftharpoons C_2 \rightharpoonup S_1 + F$, where S_1 is the base substrate, E is the enzyme kinase that adds a phosphate group to S_1, thus producing S_2 (phosphorylation), while F is a phosphatase enzyme that removes the phosphate group from S_2, thus yielding S_1 again (dephosphorylation); C_1 and C_2 are intermediate complexes. Here we can identify three conservation laws: $s_1 + c_1 + s_2 + c_2$, $e + c_1$ and $f + c_2$ are constant quantities. A double futile cycle underlies the well-studied MAPK pathway [126], $MAPK \rightleftharpoons MAPK\text{-}P \rightleftharpoons MAPK\text{-}PP$, where each *-P* suffix denotes one added phosphate group. Each reversible reaction would be more accurately described as an enzymatic reaction of the form $M_1 + E_1 \rightleftharpoons M_1 \cdot E_1 \rightharpoonup M_2 + E_1$; anyway, the total amount of MAPK in its three states (unphosphorylated, phosphorylated, and doubly phosphorylated, possibly including intermediate complexes) is constant.

Also biological models out of a biomolecular context satisfy the "mass balance" property: e.g., in the SIR model in Example 5, the total number of individuals $x_S(t) + x_I(t) + x_R(t)$ is constant, which is appropriate for a non-fatal disease.

Besides structural conditions based on the presence of conservation laws, structural algorithms have been proposed to assess the boundedness of CRNs. In [16], chemical networks are analysed as continuous-time Petri nets and, to investigate boundedness, a structural algorithm creates several compatibility scenarios, as exemplified next.

Example 8 (Boundedness analysis.) *Consider the chemical reaction network associated with the graph in Fig. 5,*

$$\begin{aligned}\dot{a} &= -g_{ab}(a,b) + a_0\\ \dot{b} &= -g_{ab}(a,b) - g_b(b) + b_0\end{aligned}$$

where a_0 and b_0 are positive constants, while $g_{ab}(a,b)$ and $g_b(b)$ are increasing and positively radially unbounded[2] *reaction functions. Denoting by $b^+ > 0$ the value such that $g_b(b^+) = b_0$, we have that, for all $b > b^+$,*

$$\dot{b} = -g_{ab}(a,b) - g_b(b) + b_0 \leq -g_b(b) + b_0 < 0,$$

which implies asymptotic boundedness of b: $b \leq b^+$. For variable a, we have two possible cases.
1) If b is bounded away from 0, namely for some $t_0 > 0$ we have $b(t) \geq b^- > 0$ for $t \in [t_0, \infty)$, then for $t > t_0$

$$\dot{a} = -g_{ab}(a,b) + a_0 < -g_{ab}(a,b^-) + a_0 < 0$$

for all $a > a^+$, where a^+ solves $g_{ab}(a^+, b^-) + a_0 = 0$, hence a is also asymptotically bounded: $a \leq a^+$.
2) If conversely b can approach 0, hence so does $g_{ab}(a,b)$, then $\dot{a}$ approaches $a_0 > 0$, therefore variable a may diverge. For this illustrative example, the analysis is actually simpler because the system is monotone, hence the theory in [119, 20] can be applied. Note that $b_0 > a_0$ is a crucial requirement for boundedness. In fact, if $a_0 > b_0$, the trajectory diverges, since $\dot{a} - \dot{b} = a_0 - b_0 + g_b(b) \geq a_0 - b_0$; then $a(t) - b(t) \to +\infty$, hence $a(t) \to +\infty$. Conversely, $a_0 < b_0$ is equivalent to the existence of a positive equilibrium (see Example 14), whose global stability can be inferred from the existence of a polyhedral Lyapunov function and the nonsingularity of the Jacobian [47].

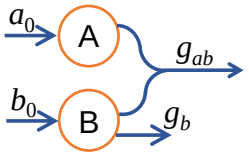


Figure 5. The flow graph representing the two-species network structure of Example 8.

Other interesting tools to establish boundedness of dynamical systems, including biological models, are based on Lyapunov or Lyapunov-like functions and positively invariant sets (see [136] and [42]).

Definition 6 *Given a system $\dot{x} = f(x)$, under the assumption of existence and uniqueness of the solution,*

- *a set $\mathscr{S}$ is* positively invariant *for the system if $x(t_0) \in \mathscr{S}$ implies $x(t) \in \mathscr{S}$ for all $t > t_0$;*
- *a locally Lipschitz function $V(x)$ is a* Lyapunov-like function *if it is non-increasing along the system trajectory;*
- *a Lyapunov-like function is a* Lyapunov function *if it is positive definite with respect to some point $\bar{x}$: $V(\bar{x}) = 0$, and $V(x) > 0$ for all $x \neq \bar{x}$.*

Function $V(x)$ needs to be locally Lipschitz to enable considering its directional derivative.

[2] Function $f(x_1, \ldots, x_n)$ is positively radially unbounded if, for each $k \in \{1, \ldots, n\}$, when all variables $x_i > 0$, $i \neq k$, are held constant, $\lim_{x_k \to \infty} f(x_1, \ldots, x_n) = +\infty$.

Lyapunov-like functions and invariant sets are strongly related: the level set $\{V(x) \leq \kappa\}$ is positively invariant as long as $V(x)$ is a Lyapunov-like function.

Sets of interest are associated with several smooth functions h_i, $i = 1,\ldots,m$. We define *practical set* a set having the form $\mathscr{S} = \{x : h_i(x) \leq 0,\ i = 1,\ldots,m\}$, such that its interior is $\text{int}\{\mathscr{S}\} = \{x : h_i(x) < 0,\ i = 1,\ldots,m\}$, its boundary is $\partial\mathscr{S} = \{x : h_i(x) = 0,\ \text{for some } i\}$ and the gradient $\nabla h_i(x)$ on the boundary satisfies: $h_i(x) = 0 \ \Rightarrow\ \nabla h_i(x) \neq 0$.

Theorem 1 (Nagumo, 1942.) *Under regularity assumptions for f, the practical set $\mathscr{S}$ is positively invariant for the system $\dot{x} = f(x)$ if and only if, for every $x \in \partial\mathscr{S}$, we have*

$$\nabla h_i(x)^\top f(x) \leq 0 \ \textit{ for all } i \ :\ h_i(x) = 0.$$

The positivity condition in Proposition 1 is the application of Nagumo's theorem to the positive orthant $x_i \geq 0$.

Activation-inhibition networks (5) are an important class of systems with structurally bounded solutions, provided that all equations $\dot{x}_i$ include a self-degradation linear term $-\mu_i x_i$ and that all interaction functions f_{ij} and g_{ik} are bounded (e.g. Michaelis-Menten and Hill functions); special cases of interest are the repressilator and the promotilator models in Example 6. In general, consider equation (5) and the simplex

$$\mathscr{S} = \{x : x_i \geq 0, \sum_{i=1}^{n} x_i \leq \kappa\}, \tag{13}$$

for some constant $\kappa > 0$. Then, Nagumo's condition applied to the points in the upper bound $\sum_{i=1}^{n} x_i = \kappa$ is

$$\begin{aligned}\frac{d}{dt}\sum_{i=1}^{n}\dot{x}_i(t) &= -\sum_{i=1}^{n}\mu_i x_i + \sum_{i,j} f_{ij}(x_j) + \sum_{i,k} g_{ik}(x_k) + \sum_{i=1}^{n} u_i \\ &\leq -\min_i\{\mu_i\}\sum_{i=1}^{n} x_i + K = -\min_i\{\mu_i\}\kappa + K \leq 0,\end{aligned}$$

where $K = \max_x \sum_{i,j} f_{ij}(x_j) + \sum_{i,k} g_{ik}(x_k) + \sum_{i=1}^{n} u_i$, and is verified for $\kappa \geq K/\min_i\{\mu_i\}$. The existence of such a bound is structural, although the value of κ is parameter-dependent. Nagumo's condition applied to the boundary $x_i = 0$ is satisfied since the system is structurally positive.

The solution of a chemical reaction network (1) is structurally bounded when each variable self-degrades and all the entries of the vector function g are bounded. In this case, we can write the system as $\dot{x} = -Mx + Sg(x) + g_0$, where M is a diagonal matrix with positive diagonal entries μ_i and $[Sg(x) + g_0]_i \leq K$. Then, $\dot{x}_i = [Sg(x) + g_0]_i - \mu_i x_i \leq K - \mu_i x_i$, hence $x_i \leq \kappa_i = K/\mu_i$. Again, albeit the value of κ_i depends on the parameters, the existence of the bound is structural. Being the system also structurally positive, its evolution is structurally bounded in the simplicial set (13), with $\kappa = \sum_{i=1}^{n} \kappa_i$.

Example 9 (Enzymatic network.) *Consider the enzymatic network model [148]*

$$\dot{x}_i = \sum_j \frac{\alpha_{ij}(1 - x_i)}{(1 - x_i) + \gamma_{ij}} x_j - \sum_k \frac{\beta_{ik} x_i}{\delta_{ik} + x_i} x_k, \tag{14}$$

where the positive terms correspond to activations and the negative terms to inhibitions, and all concentrations are normalised to 1*. Then, for any possible choice of the positive parameter values α_{ij}, β_{ik}, γ_{ij} and δ_{ik}, the solution $x(t)$ is bounded in the positive unit box $\mathscr{B} = \{x : 0 \leq x_i \leq 1\}$: the system is positive (if $x_i = 0$, $\dot{x}_i \geq 0$) and, if $x_i = 1$, $\dot{x}_i \leq 0$.*

See [1] and [43] for specific applications of set-invariance and Lyapunov approaches to biological systems.

General algorithmic methods to establish boundedness based on Lyapunov and Lyapunov-like functions have been proposed in [44] [47]; the method relies on embedding the system trajectories in a positive linear differential inclusion, an approach previously proposed in [23].

For the particular case of chemical networks endowed with mass action kinetics, the connection between weak reversibility (a key concept in chemical reaction theory, see Section 6 and [98]) and boundedness are studied in [12].

Proving boundedness of the system solutions in a compact and convex set is particularly important, because it guarantees the existence of a steady state, as we discuss next.

5 Steady-state analysis

Assessing the existence of steady states, their number and their stability is the starting point when studying all dynamical systems, including biological models. Given the dynamical system $\dot{x} = f(x)$, a steady state is a vector $\bar{x}$ such that $f(\bar{x}) = 0$. A fundamental result about the existence of steady states is proven in [195] (see also [173]).

Proposition 2 *If, for all initial conditions, the solutions of the system $\dot{x} = f(x)$ are ultimately bounded in a compact and convex set $\mathscr{X}$ (i.e., for all $x(0)$ there exists $T(x(0))$ such that $x(t) \in \mathscr{X}$ for $t \geq T(x(0))$), then the system admits a steady state in $\mathscr{X}$.*

The same result holds if we assume that $\mathscr{X}$ is positively invariant: then, the following result is a standard consequence of the Brouwer fixed point theorem (see [42] for details and references).

Proposition 3 *If the compact and convex set $\mathscr{X}$ is positively invariant for the system $\dot{x} = f(x)$, then the system admits a steady state in $\mathscr{X}$.*

Hence, whenever we can guarantee ultimate boundedness in a compact and convex set $\mathscr{X}$, or ensure its invariance, then we have at least a steady state; and, as mentioned above, boundedness can be structurally assessed. The next step is to have information about the number of the steady states and about their (local) stability.

Concerning the uniqueness of steady states, the following structural result is given in [135, 212].

Proposition 4 *Given the system $\dot{x} = f(x)$, with f smooth,*

assume that the convex and compact set $\mathscr{X}$ has a non-empty interior and is positively invariant for the system, with no steady states on the boundary. Also, assume that the Jacobian is structurally nonsingular within the set: $\det J(\bar{x}) \neq 0$ *for all* $\bar{x} \in \mathscr{X}$*. Then, if a steady state exists, it is unique.*

Example 10 (Repressilator and Promotilator, equilibria.) *Consider the systems in Example 6, for which the (compact and convex) simplex $\mathscr{S}$ in* (13) *is positively invariant. Then, a steady state exists. For the repressilator, this steady state is also unique since the Jacobian is structurally nonsingular; this is however not true for the promotilator.*

Topological degree theory [120] [147] gives interesting insight into the relation between multiple steady states.

Proposition 5 *Given the smooth function f, assume that the convex and compact set $\mathscr{X}$ has a non-empty interior and is positively invariant for the system $\dot{x} = f(x)$. If the system admits $m \geq 1$ steady states $\bar{x}_k$ that are all internal (not on the boundary of $\mathscr{X}$) and* non-degenerate *(the Jacobian at the steady state is non-singular,* $\det J(\bar{x}_k) \neq 0$*), then*

$$\text{Index} = \sum_{k=1}^{m} \text{sign}\left[\det(-J(\bar{x}_k))\right] = 1.$$

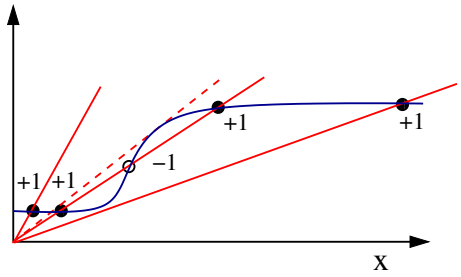


Figure 6. Intersection between $\frac{x^p}{1+x^p} + \nu$ (blue) and μx (red).

Degenerate steady states can exist, although they typically correspond to a zero-measure set in the parameter space. For instance, given $\mu, \nu \geq 0$ and $p \in \mathbb{N}$, the scalar system

$$\dot{x} = -\mu x + \frac{x^p}{1+x^p} + \nu$$

admits a degenerate steady state when the line μx is tangent to the sigmoid $\frac{x^p}{1+x^p} + \nu$ (dashed red line in Fig. 6); for a given p, the associated set of parameters is a curve in the μ-ν space. For $\nu = 0$, there is a steady state $\bar{x} = 0$; then, if we take $p = 1$, we may have two non-degenerate steady states and an Index equal to 0, which however is not in contradiction with Proposition 5, since $\bar{x}$ is on the boundary.

Also the logistic model of population growth

$$\dot{n} = rn\left(1 - \frac{n}{K}\right),$$

where n is the number of individuals, $r > 0$ is the growth rate and K is the carrying capacity of the ecosystem, admits two steady-states in any invariant interval $[0, M]$, with $M > K$: $\bar{n} = 0$ with $\det(-J_{\bar{n}}) = -r < 0$ and $\bar{n} = K$ with $\det(-J_{\bar{n}}) = r > 0$, so that the Index is 0. Yet, also in this case Proposition 5 does not apply because the steady state $\bar{n} = 0$ is on the boundary of the interval.

Example 11 (Competing populations.) *Consider a model of two populations in competition, e.g., for shared nutrient resources [53, p. 166],*

$$\dot{x} = x(\lambda - ax - by) + \sigma$$
$$\dot{y} = y(\mu - cx - dy) + \nu$$

where we have added the positive input terms σ and ν. We can structurally rule out steady states on the boundary of the positive orthant (for which $\bar{x} = 0$ or $\bar{y} = 0$). The system structurally admits at least one positive steady state, as can be inferred from the boundedness of the solutions: given the set $\mathscr{X} = \{(x,y) \geq 0 : x^2 + y^2 \leq \kappa\}$, Nagumo's condition is satisfied for $\kappa > 0$ large enough. Without restrictions, we assume that the variables are normalised so that the positive steady state corresponds to $(1,1)$*, hence* $(\lambda - a - b) + \sigma = 0$ *and* $(\mu - c - d) + \nu = 0$*. The Jacobian at this point is*

$$J = \begin{bmatrix} -(a+\sigma) & -b \\ -c & -(d+\nu) \end{bmatrix}$$

and, for large b and c, $\det(-J) < 0$*. In view of Proposition 5, this implies the existence of other two (positive) steady states at which* $\det(-J)$ *is positive.*

Several papers in the literature have considered the steady-state analysis of chemical reaction networks with mass-action kinetics, starting with Feinberg's work [95] [96] [97]. Conditions for the existence and uniqueness of steady states are given by the deficiency-one theorem [95]; the deficiency of a CRN, as discussed in the next chapter, is an integer non-negative quantity that can be computed exclusively based on the network structure. [72] and [73] investigated whether a network can admit multiple steady states, providing structural and graphical results: [72] provides conditions to structurally rule out the existence of multiple steady states, in terms of the expansion of the determinant of the network Jacobian, while [73] provides conditions for the presence of multiple steady states based on the analysis of the cycles in the species-reaction graph. Structural conditions for injectivity and existence of multiple equilibria are in [37], [38].

[168] proposed the so-called circuit breaking algorithm for steady-state analysis, which operates on the graph topology associated with the biological system. A robustness analysis of steady states was proposed in [56] within a qualitative framework. A nice survey about assessing the multistationarity of CRNs is provided in [132]; see also the book [98] for a thorough exposition.

6 Stability and instability

Listing stability as a property that is worth investigating for biological and biochemical models may be frowned upon because, in most cases, biologists know very well that the

system they are studying is stable; hence, a mathematical proof may seem of no use. However, some fundamental considerations motivate (structural) stability analysis.

- Mathematical tools are applied to the study of a model, not of the real system. Hence, checking whether some fundamental system properties do hold for its mathematical representation is crucial for model falsification.
- Identifying the mechanisms that ensure stability reveals the sources of the expected "good behaviour" and casts new light on the clever tricks adopted by nature.
- Most importantly, structural stability analysis allows us to establish that not only a specific system (with assigned parameter values) but a whole family of systems is stable/unstable.

Assessing the stability of a steady state without knowing the system parameters is very challenging, but possible in particular cases and for particular classes of systems. Here is an example of structural stability analysis, which relies on parameter-free arguments.

Example 12 (Negative and Positive Loop.) *Let us compare two simple planar systems*

$$\begin{cases} \dot{a} = -\mu_a a + g_b(b) + \nu_a \\ \dot{b} = -\mu_b b + f_a(a) + \nu_b \end{cases} \quad \begin{cases} \dot{a} = -\mu_a a + f_b(b) + \nu_a \\ \dot{b} = -\mu_b b + f_a(a) + \nu_b \end{cases}$$

where the functions f_a and f_b are increasing sigmoids and the function g_b is a decreasing complementary sigmoid. The structures of the two systems are represented in Fig. 7: the first system is a negative (activation-inhibition) loop, while the second is a positive (mutual activation) loop.

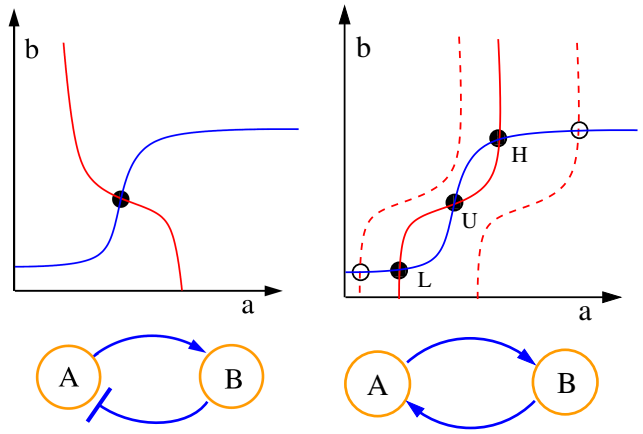


Figure 7. Negative loop (left) and positive loop (right): signal graph structure (bottom) and nullclines (top).

The first system admits a single steady state (Fig. 7 left). The Jacobian has the sign pattern $\mathrm{sign}[J_{neg}] = \left[\begin{smallmatrix} - & - \\ + & - \end{smallmatrix}\right]$, *hence J_{neg} is structurally Hurwitz. Global stability of the unique steady state can be proved based on the Poincaré-Bendixson theorem (see e.g. [136]). The second system may have one or more steady states, depending on the parameters. The Jacobian has the sign pattern* $\mathrm{sign}[J_{pos}] = \left[\begin{smallmatrix} - & + \\ + & - \end{smallmatrix}\right]$ *and is Hurwitz if and only if* $\det[-J_{pos}] = \det[J_{pos}] > 0$. *Changing the value of ν_a (as in Fig. 7 right) leads to different scenarios.*
I) For small values of ν_a, there is a single steady state L, with low values of both a and b, which is stable ($\det[-J_{pos}] > 0$*).*
II) For intermediate values of ν_a, there are three steady states, L, U and H, with small, intermediate and large values of both a and b. L is stable ($\det[-J_{pos}] > 0$*), U is unstable (*$\det[-J_{pos}] < 0$*) , H is stable (*$\det[-J_{pos}] > 0$*), hence we have bistability.*
III) For large values values of ν_a, there is a single steady state with large values of both a and b, which is stable ($\det[-J_{pos}] > 0$*).*

If, in the positive loop system, we replace functions f_b and f_a by decreasing sigmoidal functions g_b and g_a, we get a toggle-switch system (see e.g. [105] [191, Chapter 6] [104]), whose Jacobian has the sign pattern $\mathrm{sign}[J_{ts}] = \left[\begin{smallmatrix} - & - \\ - & - \end{smallmatrix}\right]$, *and can be analysed with a similar approach.*

The example highlights the following general principles:

- any system that can be modelled as a negative self-loop, or as the negative loop of two nodes (without delays) is structurally stable – however, this is not true for negative loops of more than two nodes;
- a system that can be modelled as the positive loop of two nodes may be either monostable (with a single steady state, which is stable) or multistable, typically bistable with two stable steady states and one unstable steady state (the latter is in general hard to observe).

The presence of a negative self-loop on each node (i.e., negativity of all the diagonal entries of the Jacobian) is crucial for structural stability. Consider for instance the structure of the substrate-depletion motif in [191, Chapter 6]. The system (along with its Jacobian) is

$$\begin{cases} \dot{a} = a_0 - f_{ab}(a,b) \\ \dot{b} = f_{ab}(a,b) - f_b(b) \end{cases} \qquad [J_{sd}] = \begin{bmatrix} -\alpha & -\beta \\ \alpha & \beta - \gamma \end{bmatrix},$$

where functions f_{ab} and f_b are monotonically increasing in each argument; it is BDC-decomposable, with $\alpha = \partial f_{ab}/\partial a$, $\beta = \partial f_{ab}/\partial b$ and $\gamma = \partial f_b/\partial b$. A negative loop is present, but the entry $[J_{sd}]_{2,2}$ can be positive. The system admits a unique equilibrium, computed by solving $\dot{a} + \dot{b} = a_0 - f_b(b) = 0$ to find $\bar{b}$, and then $a_0 - f_{ab}(a,\bar{b}) = 0$ to find $\bar{a}$, and might exhibit persistent oscillations, since the linearised system has complex eigenvalues with positive real part if $\beta - \gamma - \alpha \geq 0$.

A large body of literature attempts to establish the structural stability of classes of systems, in particular CRNs [19] [67] [98] [158], by assessing the *qualitative stability* of their structures. Pioneering work in the context of CRNs governed by mass action kinetics includes [122] [123] [124].

Perhaps the most famous result is the deficiency-zero theorem, which we informally summarise here. We first provide some definitions [98].

Complex: integer combination of chemical species corresponding to either reactants or products of a reaction; the network in Fig. 8 has $c = 5$ complexes: A, $2B$, $A+C$, D and $B+E$. Two complexes are connected if they are reactant and

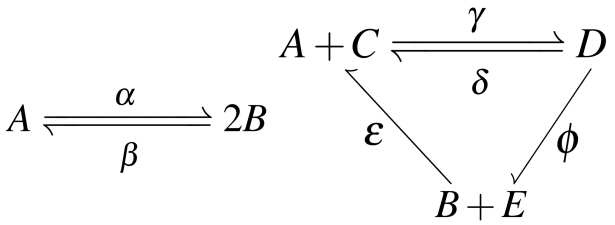


Figure 8. Example of a weakly reversible network with five complexes and two linkage classes, from [98].

product in the same reaction (directed harpoon arc in Fig. 8).

Linkage class: equivalence class of complexes that are connected by the reactions through *undirected* paths (following the reaction arrows in either direction); the network in Fig. 8 has $\ell = 2$ linkages classes: $\{A, 2B\}$, $\{A+C, D, B+E\}$.

Network rank: it is $r = \text{rank}(S)$.

A CRN is weakly reversible if all the complexes in each linkage class are connected through *directed* paths: for any pair of complexes, i and j, there is a "path" of reactions that leads from i to j, following the arrows only in the direction in which they point. For instance, in the network in Fig. 8, D is connected to $B+E$ by the reaction with rate ϕ, while $B+E$ is connected to D by the reactions with rate ε and γ; the overall network is weakly reversible. If we remove the reaction with rate ε, the network is no longer weakly reversible because no directed path leads from $B+E$ to the other complexes in the same linkage class. Conversely, the network is reversible if all reactions are reversible.

For weakly reversible networks, the deficiency is defined as

$$d = c - r - \ell.$$

The general definition requires decomposing the stoichiometric matrix as $S = NM$, where $N \in \mathbb{Z}^{n\times c}$ has in position (i,k) the stoichiometric coefficient of species i in complex k, while $M \in \{-1,0,1\}^{c\times m}$ is the complex-reaction incidence matrix, whose (k,j) entry is -1 if complex k is the reagent in reaction j, 1 if it is the product, and 0 otherwise. Then, the deficiency is $d = \dim(\ker(N) \cap \text{Ra}(M))$. See [19] [98] for details. It can be shown that $d \geq 0$. If $d = 0$, the network has zero deficiency and the following celebrated result applies [95] [96] [97] [98].

Theorem 2 (Deficiency-Zero Theorem.) *Given a weakly reversible chemical reaction network with mass-action kinetics, assume that its deficiency is* 0*. Then, there is a unique steady state in each stoichiometric compatibility class, which is (locally) asymptotically stable.*

This is a *structural* result: it holds independent of the parameter values. It is proven by adopting the entropy

$$H(x) = \sum_i \; x_i \; \log\left(\frac{x_i}{\bar{x}_i}\right) - x_i + \bar{x}_i$$

as a Lyapunov function. This Lyapunov-entropy approach works, also in the absence of the zero deficiency property, for the class of closed reversible networks as long as these admit a steady state that is also a thermodynamic equilibrium (at which each reaction has the same rate as its inverse; see [19]). Several results along these lines were proposed later [59] [11] [159] [117] [177] [134]. An extension of the deficiency-zero theorem to more general reaction kinetics of the form $\kappa\theta_a(a)^p\theta_b(b)^q$ was proposed in [186].

We stress that the deficiency-zero theorem is about *local* asymptotic stability. Although the result has been shown to hold globally, within the stoichiometric compatibility class, for significant subclasses of systems, it is still unclear whether this is true in general; this open problem is known as the *Global Attractor Conjecture* (see [98, Section 7.8] and the references therein).

A different approach to investigate *structural* local stability is based on D-stability analysis [67].

Definition 7 *A Lyapunov stable matrix M (marginal stability is admitted) is weakly D-stable if, for all diagonal matrices D with positive diagonal entries, matrix MD is also Lyapunov stable. A Hurwitz matrix M is D-stable if, for all such matrices D, MD is Hurwitz.*

As shown in [67] for CRNs, D-stability is fundamental for the analysis of the structural stability of steady states. We illustrate the results by adopting the BDC-decomposition described in Section 3.3. The Jacobian can be structurally written as BDC; now let $M = CB$. Then BDC and $MD = CBD$ have the same non-zero eigenvalues. In fact, consider two matrices P and Q, with PQ and QP square: then, if $\lambda \neq 0$ is such that $PQv = \lambda v$ for some $v \neq 0$, we can pre-multiply by Q and set $w = Qv$ ($w \neq 0$) to get $QPw = \lambda w$. Hence, assessing weak or Hurwitz structural stability of BDC can be cast as a D-stability problem. Sufficient conditions for D-stability are summarised next.

Proposition 6 *Let M be Hurwitz (or Lyapunov stable). Then M is D-stable if it satisfies any of the following conditions: i) it is symmetric; ii) it is Metzler; iii) it admits a diagonal Lyapunov matrix $\Pi \succ 0$, such that $\Pi M + M^\top \Pi \preceq 0$; iv) it is row or column diagonally dominant; v) it is triangular; vi) there exists an invertible diagonal matrix T such that $T^{-1}MT$ satisfies one of the previous conditions.*

A necessary condition is the following: matrix M is (weakly) D-stable only if all the principal minors of $-M$ are non-negative. No necessary and sufficient conditions are known. A survey on this topic can be found in [110].

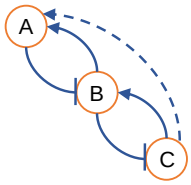
A special case of structural stability analysis is qualitative stability or sign-stability. Consider a matrix A with sign-definite entries (e.g., the matrices in Example 1), so that $\text{sign}[A_{ij}] \in \{+,-,0\}$. Then, under which conditions on the sign pattern can we conclude that A is Hurwitz, regardless of the numerical values of the entries? This problem has been deeply investigated; see e.g. [88, Section 6.5] and the references therein. First, a necessary condition is that the diagonal entries are non-positive; biologically, a positive entry can be interpreted as auto-catalysis, which can destabilise the system if it is too strong. Since the trace of a matrix is the sum of the eigenvalues, if we have a positive entry that

can be arbitrarily large, then we can have a positive trace, hence at least one eigenvalue must have a positive real part.

An important result is the following. Consider the directed graph associated with the sign-definite matrix $A \in \mathbb{R}^{n\times n}$. The graph has n nodes and, if $a_{ij} \neq 0$, an arc goes from node j to node i: it is an activation arc (pointed head) if $a_{ij} > 0$ and an inhibition arc (hammer head) if $a_{ij} < 0$. This graph may have cycles, namely, sequences of nodes connected by arcs (which must be crossed in the direction in which they point) where the first and the last node of the sequence coincide. A cycle is positive (negative) if the number of inhibition arcs that are crossed is even (odd). The following theorem holds.

Theorem 3 ([166]) *Let A be a square matrix of size n whose diagonal entries are all negative. Then, A is sign-stable if and only if, in the associated graph, every cycle of length 2 is non-positive and there are no cycles of length 3 or more.*

Example 13 (Activation-Inhibition Chain.) *The sign pattern in Fig. 9 corresponds to a tridiagonal structure where each node inhibits the following and activates the previous one. There are no cycles of length greater than 2 and all the cycles ($A-B-A$ and $B-C-B$) are negative. This is a sign-stable system. Adding the dashed arc in the figure, thus replacing the $[0]$ in position $(1,3)$ with a $+$, destroys sign-stability: if the new entry $a_{13} > 0$ is taken large enough, the determinant $\det[-A]$ becomes negative, which means that the system is unstable.*



$$\text{sign}[A] = \begin{bmatrix} - & + & [0] \\ - & - & + \\ 0 & - & - \end{bmatrix}$$

Figure 9. Tridiagonal structure: graph and sign matrix.

Sign-stability is seldom found in practice because it requires strong conditions. Still, this type of analysis can reveal that the stability of some structures, such as arbitrarily long tridiagonal chains with forward activations and backward inhibitions, is remarkably robust.

Several techniques have been put forth to assess the structural stability of biochemical networks. Local stability can be investigated by means of parameter-dependent Lyapunov functions [67]; in fact, $A = BDC$ is Hurwitz for all diagonal $D \succ 0$ if and only if the parametric Lyapunov equation

$$P(D)BDC + [BDC]^\top P(D) + I = 0$$

has a solution $P(D) \succ 0$ for all D. For fixed D, the entries of P are the solutions of a linear equation. Then, the entries of $P(D)$ are rational functions of D, $P_{ij} = \nu_{ij}(D)/\mu(D)$, with ν_{ij} and μ polynomials. On the other hand, $P(D)$ can be scaled as $P(D) := P(D)\mu(D)$. Hence, does there exist a polynomial matrix $P(D)$, positive definite, which yields a parameter dependent Lyapunov function? As shown in [46], this problem can be solved by means of SOS programming, based on the methods proposed in [65].

Other recent approaches deal with *global* structural stability. If the network (1) has a steady state, is it globally stable? This question can be answered based on piecewise-linear [44] [47] and piecewise-linear-in-rate [8] [9] [10] Lyapunov functions. These polyhedral functions generalise those first adopted in [150] for compartmental systems [129].

A piecewise-linear Lyapunov function [155] [54] is a positive definite function of the form $V(x) = \|Gx\|_\infty$, where G is a matrix with full column rank. A piecewise-linear-in-rate Lyapunov function can be seen as a piecewise-linear Lyapunov function for the rate equations (9) or (11).

Example 14 (Stability analysis.) *Consider Example 8 and assume that $b_0 > a_0$. If g_{ab} and g_b are increasing and radially unbounded, then a steady state exists: in fact, the equality $-g_b(\bar{b}) + b_0 - a_0 = 0$ determines $\bar{b} > 0$, and then $-g_{ab}(\bar{a},\bar{b}) + g_a = 0$ defines $\bar{a} > 0$. We can then define $z = [a-\bar{a}, b-\bar{b}]^\top$ and write $\dot{z} = BD(z)Cz$ with*

$$BDC = \begin{bmatrix} -1 & -1 & 0 \\ -1 & -1 & -1 \end{bmatrix} D \begin{bmatrix} 1 & 0 \\ 0 & 1 \\ 0 & 1 \end{bmatrix} = \begin{bmatrix} -D_1 & -D_2 \\ -D_1 & -(D_2+D_3) \end{bmatrix}.$$

Since BDC is column diagonally dominant, it admits $\|x\|_1$ as a Lyapunov function, which proves global stability and convergence to the equilibrium. Considering the system in rate coordinates and piecewise-linear-in-rate Lyapunov functions [8] [9] leads to a matrix that is row-diagonally dominant, hence $\|x\|_\infty$ works as a Lyapunov function.

Example 15 (Stability analysis of a metabolic network.) *The computational procedure in [44] reveals that the metabolic network system in Example 2 admits a piecewise linear Lyapunov function with*

$$G = \begin{bmatrix} 1 & 0 & 0 & 1 & -1 & 0 \\ 0 & 1 & 0 & 1 & 0 & 1 \\ 0 & 0 & 1 & 0 & 1 & 1 \end{bmatrix}^\top$$

and a piecewise-linear-in-rate Lyapunov function with $G = I$.

There are several examples of systems that admit a piecewise-linear-in-rate Lyapunov function but not a piecewise-linear Lyapunov function, or vice versa [45]; hence, these two classes of Lyapunov functions complement each other. Lyapunov-based approaches to assess global stability have been discussed in [170]. Passivity-based criteria for stability have been put forth in [31] for biochemical systems.

Finally, if we relax our requirement of structural investigation and we perform a robust stability analysis with (arbitrarily large) bounds

$$D_i^- \leq D_i \leq D_i^+, \tag{15}$$

then a computable sufficient condition for the Hurwitz stability of *BDC* under (15) is provided by the zero exclusion and the mapping theorems (see [40] for details).

Theorem 4 (Zero Exclusion Theorem.) *Consider* $D = [D_1 \dots D_n]$*, where the parameters* D_i *are subject to* (15)*. Matrix* $A(D)$*, whose entries are continuous functions of D, is Hurwitz stable if and only if* $A(\hat{D})$ *is Hurwitz for some* $\hat{D}$*, and for all* $\omega \geq 0$ *the* value set

$$\mathscr{V}_\omega = \{z \in \mathbb{C} \colon z = \det[j\omega I - A(D)], \;\; D_i \;\; as\ in \;\; (15)\}$$

does not include the origin.

The shape of the value set can be very hard to assess, but the following theorem comes to help for matrices such that $\det[j\omega I - A(D)]$ is a multilinear function of the parameters D_i, which includes all *BDC*-decomposable matrices.

Theorem 5 (Mapping Theorem.) *Given the uncertain matrix* $A(D)$ *with* $\det[j\omega I - A(D)]$ *multilinear in the* D_k*'s, the convex hull of the value set conv*$[\mathscr{V}_\omega]$ *at the frequency* ω *is exactly the convex hull of the points* $\det[j\omega I - A(\hat{D}_k)]$*, where* $\hat{D}_k$ *are the vertices of the hyper-rectangle* (15)*.*

Hence, the set $\mathrm{conv}[\mathscr{V}_\omega]$ can be drawn very easily, and a sufficient computable condition for robust stability is that it never includes the origin.

Non-minimum phase. Natural systems may well show unstable behaviours. Can they show a non-minimum phase behaviour? A very simple example of possibly non-minimum-phase system is the incoherent feedforward loop [209] (cf. Fig. 2). If we linearise the system at a steady state, the transfer function from u to b is

$$F(s) = \frac{\mu}{s+\alpha} - \frac{\nu}{(s+\beta)(s+\gamma)}.$$

Consider the step response $f_{-1}(t)$ of this system, i.e., the inverse Laplace transform of $F(s)/s$. The initial value theorem reveals that the derivative at zero is $f'_{-1}(0) = \mu/\alpha > 0$, hence the step response is initially positive. According to the final value theorem, the steady-state value is $f_{-1}(\infty) = \mu/\alpha - \nu/(\beta\gamma) \doteq f^\infty_{-1}$. If $f^\infty_{-1} < 0$, we have the standard non-minimum-phase behaviour; as can be seen by computing the numerator of the transfer function, this condition implies the presence of a (one) positive zero. Non-minimum phase systems are observed in the presence of autocatalytic networks [156] [183] or in transcription-translation systems with resource competition [209].

7 Reaction to persistent perturbations

How a biological or ecological system reacts to external stimuli is of essential importance in the study of natural phenomena. For instance, in nature we commonly observe the phenomenon of *adaptation*, which is the ability of a stable system to compensate a *persistent* perturbation and recover, after a transient, the configuration it had before the injection of the perturbation [5] [148] [91]. The pre-stimulus steady-state values and output values can be restored either approximately or exactly (asymptotically), and in the latter case the property is denoted as *perfect adaptation*. This phenomenon has been observed and intensively studied in bacterial chemotaxis [39] [194] [7] [210] [68] and yeast osmoregulation [157]. In particular, the bacterium *E. coli* adapts its switching frequency between the running and the tumbling state, as a function of nutrient (chemoattractant) concentration. This behaviour creates a random walk aimed at maximising food intake; a specific analysis of the random walk of bacteria has been proposed in [34].

Adaptation obeys the internal model principle [187]. In terms of transfer function, perfect adaptation clearly requires the presence of a zero at the origin of the complex plane [87]; a negative zero close to the origin yields adaptation, although non-perfect. Also the presence of an integrator in a negative feedback loop ensures perfect adaptation [210] [91] [57]. Several mechanism have been studied in the literature to provide perfect adaptation, such as the recent [29] and [28], with emphasis on the robustness of the property.

A general structural condition widely applicable to nonlinear systems can be derived as follows. Assume that the stable system with scalar input u and scalar output y,

$$\dot{x}(t) = f(x(t), u(t)), \;\; y(t) = g(x(t)), \tag{16}$$

has a steady state $\bar{x}$, corresponding to the constant input $\bar{u}$, and output $\bar{y} = g(\bar{x})$. The perturbed input $\bar{u} \to \bar{u} + \delta u$ (with δu not too large, not to compromise stability) yields the new output $\bar{y} \to \bar{y} + \delta y$. We seek conditions under which the steady-state output remains unchanged, i.e. $\delta y = 0$. Equivalently, the implicitly defined function

$$y = \phi(u): \quad 0 = f(x,u), \quad y = g(x)$$

is constant, namely $\phi'(u) = 0$. By applying the theorem of the derivative of an implicit function, it must be

$$\frac{d}{du}\phi(u) = -\frac{\partial g(x)}{\partial x}\left[\frac{\partial f(x,u)}{\partial x}\right]^{-1}\frac{\partial f(x,u)}{\partial u} = 0,$$

which is equivalent to the transfer function of the linearised system having a zero at the origin. If this condition is satisfied at the steady state, then adaptation is a local property; if $d\phi(u)/du$ is identically zero, the property is global. The equality can be satisfied just for some choices of the parameter values, but for some classes of systems it is *structural*, because it exclusively relies on the structure, or even on the sign pattern, of the Jacobians of f and g.

Structural steady-state analysis can predict perfect adaptation as a special case. The more general question is: if a persistent (step) positive input δu is applied to the system, how does the steady state output δy behave? A structural answer can be

$$\frac{\delta y}{\delta u} = \frac{d}{du}\phi(u) \in \{+, -, 0, ?\},$$

meaning that δy can be either structurally positive, structurally negative or structurally zero (regardless of parameter values), or indeterminate (its sign depends on the parameters). This type of analysis has deep roots in ecology [77] [78] where the main concern is to robustly predict the effects of perturbing agents in ecosystem communities. This problem has been reconsidered later in [190] and [109]. For single-input single-output systems admitting a *BDC*-decomposition (7) of the form

$$\dot{z} = BDCz + Eu, \quad y = Hz,$$

consider the expression

$$\Delta(D) = \det \left[\begin{array}{c|c} -BDC & -E \\ \hline H & 0 \end{array} \right]$$

and let $\mathscr{D}$ be the set of all binary-valued diagonal D matrices, with $D_{ii} \in \{0,1\}$. Then:

i) $\frac{\delta y}{\delta u} = +$ iff $\Delta(I) > 0$ and $\Delta(\hat{D}) \geq 0$ for all $\hat{D} \in \mathscr{D}$;

ii) $\frac{\delta y}{\delta u} = -$ iff $\Delta(I) < 0$ and $\Delta(\hat{D}) \leq 0$ for all $\hat{D} \in \mathscr{D}$;

iii) $\frac{\delta y}{\delta u} = 0$ iff $\Delta(\hat{D}) = 0$ for all $\hat{D} \in \mathscr{D}$;

iv) $\frac{\delta y}{\delta u} = ?$ otherwise.

The outcomes with this vertex approach are fully consistent with those obtained with the approach in [190].

The *structural influence matrix* can be built by taking E and H with a single nonzero entry, say the jth and the ith respectively, and considering all possible (i,j) pairs: it shows the effect of all possible input perturbations, acting on one of the state equations, on the steady state of all possible state variables taken as outputs [109]. Non-trivial biological systems can have a surprisingly small fraction of indeterminate entries; see for instance the influence matrix of the EnvZ-OmpR osmoregulation system in *E. coli* studied in [180], which is reported in Fig. 10.

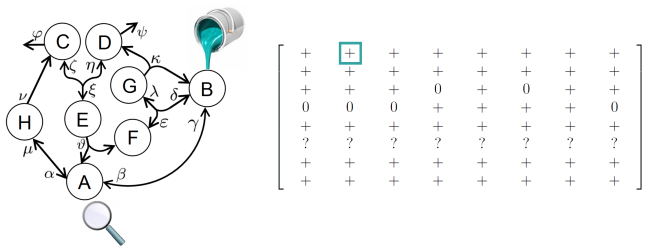


Figure 10. Structural influence matrix: entry $(1,2)$ is the structural sign of the steady-state variation in the concentration of species A ensuing a positive input perturbation acting on species B.

A step input can be seen as a special case of the frequency signal $\cos(\omega t)$ at frequency 0. So, it is natural to extend the analysis to the behaviour of a system subject to a periodic input. This problem has been considered in [128], where the frequency response is shown to offer an insight into biological processes, such as tryptophan biosynthesis and bacterial chemotaxis regulation. In [174], structural frequency analysis for nonlinear transcriptional systems is based on contraction theory. Periodic inputs have been investigated in [71] [104] and in [169], which resorts to oscillatory inputs to differentiate between adaptive topologies.

Sensitivity analysis. Biological systems often show very little changes in their (qualitative) behaviour, in spite of strong variations in relevant parameters (e.g. temperature or key species concentrations). Sensitivity analysis aims at quantifying the sensitivity to parameter variations, which explains when and why this strong resilience is possible. Low sensitivity is an essential aspect of robustness [179]; however, having low sensitivity is not always desirable: we wish to have systems that are insensitive against undesired disturbances, but also – if we need to govern them – very sensitive with respect to the control input! A structural approach to sensitivity analysis has been proposed in [154] [103] [55], while a frequency approach is suggested in [128]. Sensitivity analysis typically aims at spotting the presence/absence of sensitivity or quantifying the variations [55], rather than determining their sign as in the analysis in [190] and [109]. It is worth stressing that the approach discussed above to compute structural input-output influences can be used also to structurally analyse the sensitivity to parameter variations: it suffices to choose u as the parameter of interest.

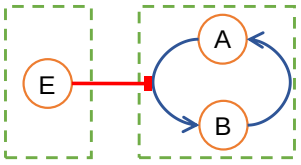


Figure 11. Simplified enzymatic reaction, typical of cell signalling (such as the MapK cascade): the variable e, with equation $\dot{e} = -\gamma e + u$, affects the flow in the a-b subsystem (with equations $\dot{a} = -\alpha e a + \beta b$, $\dot{b} = +\alpha e a - \beta b$) without being affected [108].

Ultrasensitivity. Ultrasensitive systems have a steep steady-state characteristic, so that, in certain conditions, a small variation of the system input causes a huge output variation [111] [112] [126] (see [100] [101] [102] for a recent survey). This is common in biology, especially for phenomena involving a high degree of cooperativity, which leads to systems with a Hill-type characteristic as in (6). As shown in [111] [112], this type of characteristic is typically achieved, for instance, when connecting in series several structures as in Fig. 11, where the chemical reactions are $E + A \xrightarrow{\alpha} E + B$ and $B \xrightarrow{\beta} A$. If the system in Fig. 11 is the first element of a series, then the steady-state characteristic from e to b is $b = \alpha\mu e/(\alpha e + \beta) = f_0(e)$, where $\mu = a + b$ is a constant. If we connect this first module with new ones of the same type, with inputs b_k $(k = 1,2,\ldots,n-1)$ and characteristic $b_{k+1} = \alpha_k \mu_k b_k/(\alpha_k b_k + \beta_k) = f_k(\beta_k)$, the overall characteristic, which is the composition of such functions, $b_n = f_n \circ f_{n-1} \circ \cdots \circ f_1 \circ f_0(e)$, tends to an ultrasensitive step-like function (for a suitable choice of the parameters). Ultrasensitivity can have a fundamental effect in loops and biological signalling pathways [100] [101] [102]. In fact, an ultrasensitive module can ensure very low sensitivity (hence,

very high robustness). For instance, the equation

$$\dot{x} = -\mu x + u + \psi(x),$$

where $\psi(x)$ is a decreasing Hill-type function $\psi(x) = 1/(1+x^p)$ showing an abrupt drop at $x = 1$ (it tends to a step in the limit $p \to \infty$), shows a very small sensitivity of the steady state with respect to variations of μ and of u, at least in a proper range of state values. This property can be verified by means of the implicit derivative theorem.

Scale invariance. Given a stable dynamical system of the form (16), assume that the input changes from $u = u_0 > 0$, corresponding to a steady state x_0, to $u = u_1$, corresponding to a *new* steady state x_1. Let $y(t)$ be the output during the transient. Then, perform the same experiment with scaled input values: pu_0 becomes pu_1. If the output evolution $y(t)$ is the same as in the previous case, the system is *scale invariant* [182] [184]. In simple words, the output depends on the relative change (the ratio) and not on the difference of the input values. Scale invariance entails adaptation, but not all adaptive systems are scale invariant. This phenomenon has been shown to be important in cell sensory systems, enzymatic networks and bacterial chemotaxis. The reader is referred to [184] [182] for an in-depth discussion.

8 Signed feedback loops: oscillators and switches

Natural functions heavily rely on regulatory feedback loops, which are fundamental for the robustness of biological mechanisms. However, identifying the relevant loops that give rise to a specific function, in models that typically involve a huge number of interacting species, is often challenging: graphical representations of the interactions and pathways occurring within the simplest living cell make this astounding complexity self-evident. The following dichotomy is normally encountered when tackling the loop analysis of biological systems.

- Complete models of biological phenomena are often too complex to be studied analytically. Resorting to brute-force numerical simulations does not provide any insight into the functional mechanisms underlying the observed behaviours, and requires an accurate knowledge of parameter values, which are hardly available.
- A simple model that captures the essence of a mechanism is typically amenable for analysis, yet it can be criticised for not being a faithful representation.

Considering everything means explaining nothing: therefore, in general we must select just the subset of a complex system that is relevant to give rise to a specific phenomenon. Consider for instance [3, Fig. 13-20], which visualises very effectively that glycolysis and citric acid cycle are a small fraction of the reactions occuring in a cell and the related pathway can be easily isolated. Simplification and selection of the relevant elements to explain a mechanism can be performed either based on experience and knowledge of the biological phenomenon, possibly supported by experiments, or based on mathematical tools relying on time-scale separation and aggregation, which we will discuss in Section 9.

Once the essential mechanisms involved in a phenomenon have been captured in a block diagram or interaction graph, important insight can be obtained through their structural analysis. Interactions in biological signal graphs are signed, since they can be either inhibitory or activating. Then, the presence of negative and/or positive cycles can be crucial to explain fundamental dynamic behaviours. As mentioned before Theorem 3, a directed cycle in a signed graph is positive if it includes an even number of inhibitory (negative) arcs, negative if it includes an odd number of inhibitory arcs. In Fig. 4, the repressilator has a single cycle that is odd, while the promotilator has a single cycle that is even. We focus on cycles of length ≥ 2, while self-loops are here assumed to be negative.

Negative and positive cycles are related to the capacity of a dynamical system to exhibit *multistationarity* or *oscillations*, two types of behaviours that are frequently encountered in nature. Cells must often change very rapidly the "operating point" by switching between different cellular "states": this governs cell fate (life cycle, cell division, apoptosis) and cell differentiation [208]. On the other hand, cells also show behaviours characterised by rhythms and periodicity, generally orchestrated by internal biochemical clocks [113]; many cyclic behaviours are fundamental also at the organism level (e.g. heartbeat, sleep phases, circadian rhythms, menstrual cycle, seasonal changes in coat/plumage of some animals).

The presence of positive or negative cycles in the Jacobian graph is a widely accepted method to explain multistationarity and oscillations in molecular/chemical systems [202]. Some of the first mathematical conjectures in this area were formulated by Thomas [201]: given a Jacobian graph, a negative cycle is a necessary condition for stable periodic behaviour, while a positive cycle is a necessary condition for multistationarity. These conjectures were proved in [114] [185] [27]; see also [133]. Graphic conditions for multistationarity have been given in [193]. Graph-based conditions associated with the presence of Hopf or pitchfork instability have been studied in the context of mass-action kinetics [72], and in generalised biological models [167], using species-reaction graphs [73] [85] [153] [144], multigraphs [152], and algebraic geometry [73] [84]. A structural classification of systems into (strong or weak) candidate oscillators or candidate multistable systems has been suggested in [48]. The basic principle established in the literature is:

- positive loops are necessary for multistability;
- negative loops are necessary for oscillations.

For example, given a dynamical system with an underlying graph structure, associated with the sign pattern of the Jacobian matrix, assume that its solutions are globally bounded within a compact and convex positively invariant set, with non-empty interior, and there are no equilibria on the boundary. Consider two possible sign patterns for the Jacobian:

$$\operatorname{sign}[J_o] = \begin{bmatrix} - & + & 0 & 0 \\ 0 & - & 0 & + \\ - & - & - & 0 \\ 0 & 0 & + & - \end{bmatrix}, \quad \operatorname{sign}[J_b] = \begin{bmatrix} - & + & 0 & 0 \\ 0 & - & 0 & - \\ - & - & - & 0 \\ 0 & 0 & + & - \end{bmatrix}. \tag{17}$$

The Jacobian graph associated with J_o has only negative cycles, while that associated with J_b has only positive cycles (in addition to negative self-loops). Now, consider the case in which these Jacobians, for some values of the parameters, are marginally stable with either: i) one real eigenvalue (in general associated with a pitchfork bifurcation) or ii) two imaginary eigenvalues (in general associated with a Hopf bifurcation), while all the remaining eigenvalues have negative real part. Are both of these cases possible for each of the considered systems?

The answer is no. Since J_o has only negative cycles, then $\det[-J_o] \neq 0$ [166] ($\det[-J_o] > 0$ structurally), hence case i) is ruled out. There are two imaginary eigenvalues and the linearised system exhibits sustained oscillations. Moreover, in view of Proposition 5, there cannot be other steady states. Conversely, J_b has only positive cycles, hence it is similar to a Metzler matrix through a change in the sign of some state variables (in particular, $\hat{J}_b = T^{-1}JT$ is Metzler, with $T = \mathrm{diag}\{1,1,-1,-1\}$); cf. Section 9.3. A Metzler matrix has a real dominant eigenvalue, so the marginally stable eigenvalue must be 0, which rules out case ii), and $\det[-J_b(\bar{x})] = 0$. If, for proper parameter variations, it happens that $\det[-J_b(\bar{x})] < 0$, then, in view of Proposition 5, other steady states (typically 2) must appear, which are stable under proper conditions.

Example 16 (Repressilator and Promotilator, dynamics.) *The Jacobians of the repressilator and the promotilator systems, in Examples 6 and 10, are*

$$J_R = \begin{bmatrix} -\mu_a & 0 & g_c' \\ g_a' & -\mu_b & 0 \\ 0 & g_b' & -\mu_c \end{bmatrix}, \quad J_T = \begin{bmatrix} -\mu_a & 0 & f_c' \\ f_a' & -\mu_b & 0 \\ 0 & f_b' & -\mu_c \end{bmatrix}.$$

The repressilator has a single negative loop and it admits a single steady state that can either be stable or, in case of transition to instability, exhibit imaginary eigenvalues; zero eigenvalues can be ruled out since the determinant is non-singular, because $g_*' < 0$ (cf. Propositions 4 and 5).

The promotilator has a single positive loop and can have one or more steady states. The characteristic polynomial is

$$\det[sI - J_T] = (s+\mu_a)(s+\mu_b)(s+\mu_c) - f_a' f_b' f_c'.$$

Via the standard Routh-Hurwitz table, one can prove that stability holds if and only if $\det[-J_T] = \mu_a\mu_b\mu_c - f_a' f_b' f_c' > 0$. In view of Proposition 5, in the case of a single steady state we have stability. Conversely, if there are three isolated positive steady states (the interesting case of bi-stability), $\det[-J_T]$ must be negative at one (unstable) steady state and positive at the other two (stable) steady states. Structurally, this means that the instability of an equilibrium implies the stability of other two.

The existence of positive (negative) cycles is a necessary condition for multistability (oscillations), but it is not sufficient. Paradoxically, in some cases the presence of positive cycles favours the onset of oscillations; in particular, autocatalytic reactions (positive self-loops, which we have disregarded so far) are well-known to have a destabilising effect that can lead to sustained oscillations, of course in the presence of at least one negative loop [94].

Bistability and patterns. Bistability is related to many important biological processes and governs cell fate decision [208] [89] [74] [213]. It has been structurally identified in the regulatory network of the galactose metabolic pathway in yeast [70], and it is associated with pattern formation [94] [30] [121]; structural graph-based conditions have been given in [152], while an approach based on input-to-state stability is proposed in [61].

Oscillations. Oscillatory behaviours are also fundamental in nature [94] [202]. An inner biological clock is needed, e.g., to regulate metabolism and physiological functions according to circadian rhythms [86]; the 2017 Nobel prize in physiology/medicine was awarded jointly to Jeffrey C. Hall, Michael Rosbash and Michael W. Young for discovering the molecular mechanism underlying circadian rhythms, which is (not surprisingly) based on a negative feedback loop with delay. Also pattern generation in vertebrate embryonic development is ruled by the so-called segmentation clock [63]. Oscillations have been mathematically investigated in gene networks [106] and qualitative approaches to assess the potential for periodic behaviours have been proposed in [15] and [76].

Oscillators and synchronisation. Oscillators are fundamental devices in many biological processes. In nature there are several examples of systems formed by several oscillators that need to be synchronised [36]. The research about oscillator syncronisation has an old history, starting with Kuramoto's model [143]. Given N (almost) identical oscillators that are interconnected through a network, do their outputs converge to the same, possibly periodic, function? General conditions have been given in [146] for linear uncertain systems and in [176] for nonlinear coupled systems; the reader is referred to [62, Chapter 8] for further details and references.

9 Structural analysis of large networks

Biological phenomena typically arise from the entangled interplay of myriads of interacting elements (biomolecules, cells, individuals), while the methods we have discussed so far can be more easily applied to small systems. In this section, we discuss approaches to deal with the structural analysis of large and complex systems, either by identifying recurrent structural motifs in huge networks, or by simplifying systems via singular perturbation arguments or via the decomposition into subsystems with specific structural properties. For model order reduction approaches, the reader is referred e.g. to [171] [178].

9.1 Network motifs

As nicely described in [5], a network motif is a pattern that is recurring in a network: in biological networks, some particular motifs have been shown to be significantly more fre-

quent than in randomly generated networks, which testifies the importance of their role. In particular, [5] shows that the *feedforward loop* (cf. Example 3 and Fig. 2) is a recurring three-node pattern in most biological networks: it consists of three nodes A, B and C, where A is controlled by an external input u and sends inputs $h_{ba}(a)$ and $h_{ca}(a)$ to both B and C, while B send an input $h_{cb}(b)$ to C, and C is the output.

There are eight possible structures depending on the choice of the negative or positive sign of the h functions (corresponding to inhibition or activation). These structures may have different logic functions. For instance, the configuration in Example 3 (Fig. 2) yields a system that shows adaptation: the initial activation of the third node due to the first is subsequently compensated by the delayed inhibition coming through the second node. Also the two-node negative and positive feedback loops in Fig. 7 can be regarded as pillar motifs in biological systems. Network motifs have been studied by several authors, also in combination with dynamic properties [164]. Beside the pioneering work [6] [5], the reader is referred to [82] and to the recent survey [199].

9.2 Time-scale separation

In modelling biological systems we must take into account the time-scale on which a certain phenomenon occurs [5] [82]. Population dynamics can evolve on a scale of years (mammals) or the scale of minutes (bacteria). Chemical reactions can take fractions of seconds up to many hours.

A peculiar fact in biology is that even interacting components forming a complex system may evolve on completely different time-scales. This simple observation is of extraordinary importance in model simplification as well as in qualitative explanation of essential properties (see e.g. [60]).

Multimolecular reactions can be always expressed as a cascade of bimolecular reactions. For instance, $2A+B \rightharpoonup C$ can be decomposed as

$$A+B \underset{\beta}{\overset{\alpha}{\rightleftharpoons}} X, \quad X+A \xrightarrow{\gamma} C.$$

Such a decomposition is justified, since trimolecular reactions are considered unlikely to happen and no reactions concurrently involving more than three molecules have yet been observed; therefore an overall reaction is more plausibly modeled by a chain of bimolecular steps (see, for instance, [88, Section 7.4]). However, since the reversible reaction is much faster, then, with mass-action kinetics, $\alpha ab - \beta x \approx 0$. Under this singular perturbation [136] [82] approximation, the production of C becomes

$$\dot{c} = \gamma x a = \frac{\gamma\alpha}{\beta} a^2 b = k_{ab} a^2 b,$$

which is the accepted formula for trimolecular reactions.

Another important case is the enzymatic reaction

$$E+S \underset{\beta}{\overset{\alpha}{\rightleftharpoons}} X \xrightarrow{\gamma} E+P,$$

where $X=[ES]$ is an intermediate complex, E an enzyme, S the substrate, P the product. Again, we can apply a singular perturbation argument because the reversible reaction is much faster, hence we can consider the steady state relation $\alpha es = \beta x$. Then, since $e+x=e_0$ is constant, we have $\alpha e_0 s = \beta x + \alpha x s$ and we can derive the approximate production rate of p:

$$\dot{p} = \gamma x = \frac{\gamma e_0 s}{\beta/\alpha + s} = \frac{V_{max} s}{K_D + s},$$

with $V_{max} = \gamma e_0$ and $K_D = \beta/\alpha$, which explains the widely accepted Michaelis-Menten expression for the reaction rate.

The effect of excluding or explicitly including intermediate species in CRN models is discussed in [99].

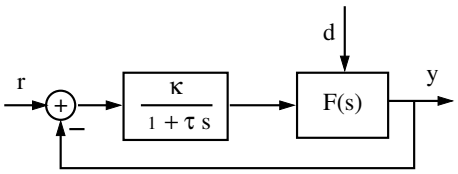


Figure 12. Reduce κ or increase τ?

Time-scale separation is fundamental to explain properties such as negative loop stability. Consider the loop in Fig. 12, where $F(s) = q(s)/p(s)$ is a stable transfer function and, without restriction, $q(0) > 0$ and $p(0) > 0$. If we can change $\kappa > 0$ and $\tau > 0$, which is the best strategy to ensure stability? If we take κ small enough, closed-loop stability is preserved. Yet, the steady-state error due to a persistent disturbance d may become very large. A different solution is achieved by augmenting τ. This has no effect on the steady-state error because, roughly, the steady-state error is achieved for $s=0$. On the other hand, the closed-loop poles are the roots of

$$\psi(s,\tau) = sp(s) + \frac{1}{\tau}\left[p(s) + \kappa q(s)\right].$$

No matter how large κ is, a large enough τ guarantees Hurwitz stability. Indeed, by continuity of the roots for $1/\tau \to 0$, the roots converge to those of $p(s)s$, namely the (stable) roots of p and 0. The root converging to 0 is a single real root, which converges to 0 from the left. In fact, $\psi(0,\tau) = [p(0) + \kappa q(0)]/\tau > 0$ and $\psi'(0,\tau) = p(0) + [p'(0) + \kappa q'(0)]/\tau > 0$, for $\tau > 0$ large enough. The root that converges to zero is therefore negative.

Hence, a dynamic element with a large time constant τ, compared to the time constants of the process F, ensures stability and good rejection of persistent disturbances, at the price of having an overall slower process. Inserting a single slow element in a negative loop has a stabilising effect: this is a well established principle [5, p. 100]. On the contrary, it has been observed in [161] [50] that homogeneous time constants, within the negative feedback loop of many dynamic elements, may destroy stability and cause oscillations.

9.3 Monotone systems and decompositions

Many biological systems have been shown to be monotone, or composed of an aggregation of monotone subsystems. A system

$$\dot{x}(t) = f(x(t), u(t)), \quad y(t) = g(x(t))$$

is *input-output monotone* if, given $x_A(0) \geq x_B(0)$ and $u_A(t) \geq u_B(t)$, the corresponding state and output solutions satisfy $x_A(t) \geq x_B(t)$ and $y_A(t) \geq y_B(t)$ (where all inequalities are intended component-wise). The definition includes systems with no input, $\dot{x}(t) = f(x(t))$, or with no output; in these cases, the system is simply said *monotone*. Monotone systems [20] [22] [119] [189] are often found in biology and chemistry, and also in thermal, fluid, electrical engineering.

A monotone system is characterised by its Jacobian matrix being Metzler. Some systems that are not monotone can become such after a change of variables in which just the sign of some components is changed [189] [24]. For instance, consider the signed Jacobian J_b in (17). A system with this (non-Metzler) Jacobian is not monotone. If we change sign to the third and fourth variables, $\hat{x}_3 = -x_3$ and $\hat{x}_4 = -x_4$, then the Jacobian becomes Metzler. As observed before, the graph associated with J_b has only positive cycles; changing the signs of the variables (associated with the nodes) does not alter the sign of the cycles. Indeed, for systems associated with strongly connected graphs, a sign-change transformation exists that renders the system monotone if and only if the system has no negative cycles [189] or, equivalently, if any pair of oriented paths connecting two nodes have the same sign. For weakly connected graphs, the situation is more tricky: the condition is now that all oriented paths connecting two nodes must have the same sign, which is no longer equivalent to the absence of negative cycles. For instance, the incoherent feedforward loop in Example 3 (Fig. 2) has no negative cycles (in fact, it is acyclic) and still it is not monotone, as one can see from its Jacobian.

Monotone systems are also denoted as *cooperative systems*, because all the variables cooperate. In the linear case, when $f = Ax + Bu$ and $g = Cx$, a system is monotone if and only if it is positive, namely A is Metzler, and input-output monotone if, moreover, B and C are nonnegative. In the nonlinear case, a positive system can be non-monotone and vice versa.

For input-output monotone systems, if we apply a step input starting from a steady state, the resulting output (as well as the corresponding state variables) is strictly monotonically increasing. Under stability assumptions, the order-preserving behaviour of (input-output) monotone systems makes their behaviour qualitatively similar to that of a first-order system. Indeed, the Jacobian, being a Metzler matrix, has a dominant real eigenvalue and the real mode associated with it dominates the long term system response. Moreover, monotone systems cannot exhibit chaotic behaviours or stable periodic orbits.

Monotone systems, besides having interest on their own, become fundamental when they are considered as components in larger systems [79] [192]: indeed, by approximating all monotone subsystems as first-order elements, a high-order model can be collapsed into the interconnection of few aggregate nodes. The negative feedback of a monotone system has been studied intensively [20] [22], to assess whether it yields a stable negative loop or sustained oscillations. In [21] [26], the positive feedback of monotone systems and the possible generation of multi-stability is investigated. See also the review in [189].

In the linear case, an input-output monotone system has a positive impulse response (which is the derivative of the step response). Hence, the family of systems having a positive impulse response generalises, in the linear case, monotone systems. Also these can be used for aggregation, as in [49], where it is shown that the same classification of [48] holds true for aggregates of positive-impulse-response systems (which includes monotone systems as a special case).

9.4 Interconnecting components: retroactivity issues

A typical attempt in systems and synthetic biology is to establish similarities between electrical circuits and biological signalling structures. The ultimate goal of synthetic biology, then, would be the bottom-up design of standard circuits, including components such as filters, amplifiers, oscillators and switches, built of engineered biomolecules such as DNA, RNA and proteins. Standard electric circuit theory enables to generate more or less any type of functions and behaviours, and electrical engineers know how to implement block diagrams, cascades, parallel, feedback of transfer functions. The biological implementation, however, is made more difficult by the presence of strong *retroactivity* [83] [130] [151]. When one chemical component affects another, also the latter typically imposes its retroaction on the former. In electrical circuits, this phenomenon is well understood. Assume that two circuits, *when disconnected*, have transfer functions $y_1 = F_1(s)u_1$ and $y_2 = F_2(s)u_2$, where u_1, u_2 are the input voltages and y_1, y_2 are the output voltages. If we physically connect them, so that $u_2 = y_1$, the transfer functions above are no longer valid. The ideal goal

$$y_2 = F_2(s)u_2 = F_2(s)F_1(s)u_1$$

is (at least approximately) achieved if the input impedance of the second circuit is very large, ideally infinite. How can infinite impedance be achieved in a biological setting? As neatly explained in [83] [151], a structural way is to consider an "insulating" device as in Fig. 11, where the dynamics of A and B do not produce any effect on E.

10 Structural biological insight and model falsification

Methods from structural analysis have effectively contributed to a deeper understanding of biological phenomena, which goes even beyond the explanation of their extraordinary robustness.

Given a reliable model of a biological phenomenon, a structural insight allows to link peculiar behaviours to topological features of the biological network, as discussed in [196]

and [160] for metabolic networks. The sharp understanding achieved by disentangling the system structure can not only reveal how a biological system works [58], but also identify suitable therapeutic targets to treat diseases [175].

Model validation, invalidation and comparison. Very importantly, qualitative information achieved through structural analysis can also be used for model invalidation. Structural approaches can predict dynamic outcomes that are an unavoidable consequence of the system structure, or rule out behaviours that are not compatible with the system structure. Then, a model can be falsified by comparing the expected qualitative behaviours with experimental results: when a behaviour is inherent in the model structure, but is not observed experimentally, or when a behaviour is incompatible with the model structure, but is nevertheless observed in experimental traces, then we know that the proposed model is *structurally unsuitable* for describing the phenomenon and needs to be radically amended (no fine tuning of the parameters will ever yield the experimentally observed behaviour). The question whether a given model is valid or not, and which are the conditions for its validity, is particularly relevant in biology, since biological systems are very hard to model due to their complexity and their seemingly haphazard variability – and, of course, different models of the same system can lead to different, often conflicting, conclusions [161]. Several authors have considered the problem of model falsification in a biological context, including the comparison between several "competing" alternative models, and suggested several techniques [14] [116] [41] [163] [25]. Qualitative "dynamic phenotypes", such as scale invariance, monotonicity and subharmonic oscillations, can be seen as signatures for biological motifs and therefore used as tools for model invalidation [51]. An example of model falsification based on structural (non-)monotonicity arguments is [33]. [169] shows that analysing the response to periodic stimuli allows to discriminate between different architectures for perfect adaptation based on incoherent feedforward loops and on integral feedback. [107] provides a structural comparison of two alternative models for protein-mediated ceramide transfer at membrane contact sites in mammalian cells. [115] discriminates between different types of cancer resistance resulting in qualitatively different responses.

Structure-based design. In synthetic biology, guidelines based on structural insight can be used to design *de novo* biological circuits that are guaranteed to always exhibit the desired qualitative behaviour (e.g., oscillatory or switching) in view of their structure [75] [35] [81] [92] [125] [141] [142]. On the other hand, looking at the structure helps us unravel the design principles that nature adopts to guarantee the extraordinary robustness and resilience of living organisms, which we can then adopt to build engineering systems that structurally share the same remarkable properties.

11 What next?

Physics, engineering, chemistry and computer science use mathematics as their main language to formulate, analyse and solve problems. It is widely believed that, in the future, biology as well will be more and more deeply characterised also by a quantitative, computational approach, so that biological laws will also be expressed in mathematical terms. Mathematical approaches in biology are precious to complement traditional biological knowledge and experimental observations, in a virtuous circle. The use of mathematics forces researchers to formulate problems with a precise and quantifiable approach and allows models to be falsified by comparing theoretical predictions with experimental data. The analysis of simple models capturing the essence of a phenomenon can provide a sharp insight into the underlying design principles [5].

In particular, structural analysis can reveal qualitative properties of natural systems, without the need of quantitative information and of precise parameter values, which are always hard to determine in a biological context. This investigation also explains why specific structures are so frequently encountered in nature, selected by evolution, and can perform their task with astounding robustness and efficiency in constantly changing environmental conditions. Establishing that a property holds for a given system even under huge parametric uncertainties is not the main achievement of structural analysis. Rather, this analysis is powerful because it allows us to state that a property is *necessarily* ensured by a certain structure, thus enabling not only the explanation of observed phenomena, but also model falsification, when structural predictions are compared to experimental results. Also, the insight achieved through structural analysis can be precious to design artificial biomolecular circuits that are guaranteed to structurally exhibit the desired qualitative function in spite of perturbations or changes in parameters.

Here, we have drawn the attention to structural approaches for the analysis of biological systems. To keep this survey within reasonable limits, we have only considered the structural analysis of some properties, including boundedness, steady state analysis, stability and response to perturbations, that are often regarded as the most important; other fundamental aspects considered in the literature have been briefly touched upon.

We have made the effort to propose a unifying perspective, to provide the reader with a general framework and tools that can be applied to an extremely vast class of systems in nature (as well as in engineering).

When adopting mathematical tools in biology, an important challenge is to focus on the simplicity of the formulation and the meaningfulness of the obtained message. This does not mean that the mathematics involved has to be trivial. Actually, proofs can be – and typically are – very complex and involved. Yet, the results need to simply provide biological insight. An excellent example is the celebrated deficiency-zero theorem, Theorem 2: it gives powerful structural stability conditions that only require computing the deficiency of the chemical reaction network, easy to calculate given the involved reactions. The theorem is insightful and easy to state and apply, but its proof is not simple at all.

Many challenges related to structural analysis are still open. In our opinion, the most important is the development of novel mathematical tools, relying on dynamical systems and

control methodologies, to expressly tackle and structurally assess properties that are highly relevant and specific to biological systems. The close collaboration with biologists and life scientists is fundamental to this aim, and we believe that our community could give a precious contribution to the understanding of biological phenomena not only through the application of systems and control approaches, but also through the creation of new biologically-driven theory.

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