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Strategies for dealing with incomplete information in the modeling of molecular interaction networks

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Abstract

Modelers of molecular interaction networks encounter the paradoxical situation that while large amounts of data are available, these are often insufficient for the formulation and analysis of mathematical models describing the network dynamics. In particular, information on the reaction mechanisms and numerical values of kinetic parameters are usually not available for all but a few well-studied model systems. In this article we review two strategies that have been proposed for dealing with incomplete information in the study of molecular interaction networks: parameter sensitivity analysis and model simplification. These strategies are based on the biologically justified intuition that essential properties of the system dynamics are robust against moderate changes in the value of kinetic parameters or even in the rate laws describing the interactions. Although advanced measurement techniques can be expected to relieve the problem of incomplete information to some extent, the strategies discussed in this article will retain their interest as tools providing an initial characterization of essential properties of the network dynamics.

Keywords: *molecular interaction networks; incomplete information; mathematical modeling and computer simulation; kinetic models; robustness; parameter sensitivity; model simplification; piecewise-linear differential equations; Boolean models*

INTRODUCTION

The concerted efforts of genetics, molecular biology, biochemistry and physiology have led to the accumulation of huge amounts of data on the molecular interaction networks controlling cellular processes. These data, heterogeneous in nature, range from the three-dimensional (3D) structure of DNA-binding proteins to genome-wide measurements of gene expression. In order to gain a better understanding of how the basic functions of the cell result from the interactions between its molecular components, the different types of data ultimately have to be integrated with mathematical modeling and computer simulation. Since most cellular interaction networks involve a large number of molecular components, whose interactions result in complex feedback loops, intuitive predictions of the system dynamics quickly become infeasible and fraught with error. Mathematical methods and computer tools may help to unambiguously describe the network structure and systematically infer predictions of the dynamical behavior of the system. This idea has been one of the driving forces behind the current interest in systems biology [1, 2].

Modeling biological systems has turned out to be a difficult job, though, especially when dealing with systems that are not already well understood by experimental biologists. In fact, modelers encounter the paradoxical situation that while large amounts of data are available, these are often insufficient for the

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formulation and analysis of mathematical models. For instance, the development of ordinary differential equation (ODE) models of biochemical reaction systems [3, 4] requires information on the rate laws describing the reactions as well as numerical values of the kinetic parameters associated with the reactions. This information is usually not available for systems at the forefront of experimental research (e.g. [5]). More generally, the breakthroughs in experimental techniques of the last decade have enabled us to obtain a coarse-grained picture of large-scale networks. However, standard modeling approaches usually focus on smaller subnetworks, but require more precise information.

In response to this mismatch between available experimental data and modeling requirements, several *strategies for dealing with incomplete information* on molecular interaction networks have been proposed. We will consider two such strategies in this review: *parameter sensitivity analysis* and *model simplification*. Both strategies are based on the same underlying intuition: essential dynamical properties of the system do not so much depend on specific rate laws or precise values of kinetic parameters, but rather on the structure of interactions of the network. Although we will focus on ODE models, the strategies also apply, appropriately modified, to other types of models that provide a detailed, quantitative view on the biochemical reaction systems, such as stochastic master equations [6, 7]. We will give an intuitive introduction to each of the strategies by means of a simple example, briefly describe the underlying mathematical and computational techniques, and illustrate the application of the strategies in the context of a few recent modeling studies.

CROSS-INHIBITION NETWORK

As a running example, we will use the simple two-gene network in Figure 1. Each of the genes encodes a regulatory protein that inhibits the expression of the other gene, by binding to a site overlapping the promoter of the gene. Simple as it is, this *cross-inhibition network* is a basic component of more complex, real networks and makes it possible to analyze some characteristic aspects of cellular differentiation [8, 9]. Moreover, its dynamical properties have been experimentally tested by Gardner *et al.* [10], who have reconstructed the network in *Escherichia coli* cells by cloning the genes on a plasmid. The genes have been chosen such that the network functions independently from the rest of the cell and the activity of the corresponding proteins can be regulated by external signals.

The dynamics of the cross-inhibition network can be described by means of ordinary differential equations as shown in Figure 1B. The variables x_a and x_b represent the concentration of the proteins A and B. The time derivative of x_a equals the difference between the rate of synthesis of A, given by $\kappa_a\, h^-(x_b, \theta_b, m_b)$, and the rate of degradation, given by $\gamma_a x_a$. The use of the sigmoidal Hill function h^-, shown in Figure 1C, implies that, for low concentrations of protein B, gene a is expressed at a rate close to its maximum rate κ_a, whereas for high concentrations of B, the expression of the gene is almost completely shut off. The shape of the Hill function is in agreement with experimental evidence on gene expression regulation [11]. Additionally, we make the default assumption that the rate of degradation of A is proportional to the concentration

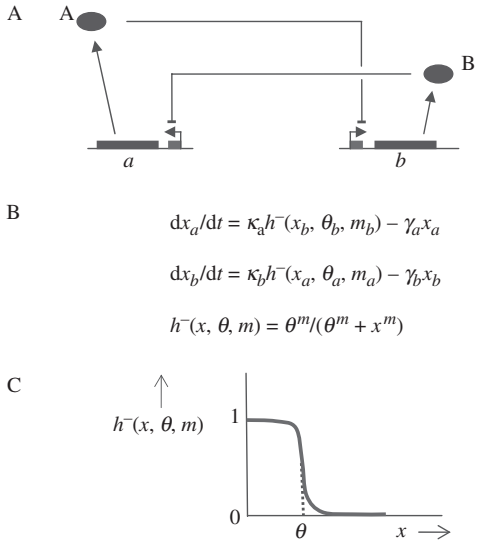

Figure 1: (A) Example of a simple genetic regulatory network, composed of two genes a and b, the proteins A and B, and their regulatory interactions. (B) Nonlinear ODE model of the cross-inhibition network in (A). The non-negative variables x_a and x_b correspond to the concentrations of proteins A and B, respectively, the parameters κ_a and κ_b to the synthesis rates of the proteins, the parameters γ_a and γ_b to the degradation constants, the parameters θ_a and θ_b to the threshold concentrations, and the parameters m_a and m_b to the degree of cooperativity of the interactions. All parameters are positive. (C) Graphical representation of the characteristic sigmoidal form, for $m > 1$, of the Hill function $h^-(x, \theta, m)$.

of the protein. The differential equation for x_b has an analogous interpretation.

Because of the nonlinearity of the Hill functions, it is not possible to analytically solve the system of differential equations. However, the dynamics of the two-gene network can be analyzed in the phase plane, using standard techniques developed in dynamical systems theory [12, 13]. The phase portrait in Figure 2A shows that the system is *bistable*, in the sense that it possesses two asymptotically stable steady states, at which either protein A or protein B is present at a high concentration. The third steady state, characterized by intermediate concentrations for proteins A and B, is unstable and has no biological significance. The phase plane analysis also reveals that the system exhibits *hysteresis*. If one strongly perturbs the system from one of its stable steady states—for instance, by provoking the degradation of the protein present at a high concentration—the other steady state can be reached (Figure 2B). From then onwards, even if the source of strong degradation has disappeared, the system will remain at the new steady state. In other words, the analysis suggests that a simple molecular mechanism may allow the system to switch from one functional state to another. Interestingly, this has been confirmed by the experiments of Gardner *et al.* [10].

The example of the two-gene network shows that dynamical systems theory provides concepts and techniques for the characterization of important properties of nonlinear dynamical systems, even if we do not have precise information on the value of the kinetic parameters. Unfortunately, their application becomes quite difficult when studying networks with a larger number of genes. While higher-order systems can sometimes be reduced to second-order systems, by time-scale abstraction or other model simplifications [3, 14], this is not always possible. More fundamentally, the insights to be obtained from dynamical systems theory are to a large extent based on visual representations that are difficult to manipulate in higher dimensions.

Therefore, *numerical simulation* [15] has been the only practical approach for the analysis of dynamical properties of large molecular interaction networks. Many excellent examples illustrate the interest of this approach for gaining insight into the functioning of molecular interaction networks of biological importance, such as the control of circadian rhythms in *mammals* [16], the metabolism of the red blood cell in humans [17], the regulation of the cell cycle in yeast and higher eukaryotes [4], and the signaling pathway involved in the maturation of oocytes in *Xenopus laevis* [18]. However, as already pointed out in the introduction, the information required for specifying the models and carrying out the simulations is often not available. How can we nevertheless predict the dynamical behavior of the system?

PARAMETER SENSITIVITY ANALYSIS

The first strategy for dealing with incomplete information is motivated by the observation that varying the parameter values does not necessarily change characteristic properties of the network dynamics. Consider again the example of the cross-inhibition network. Figure 3A shows the phase

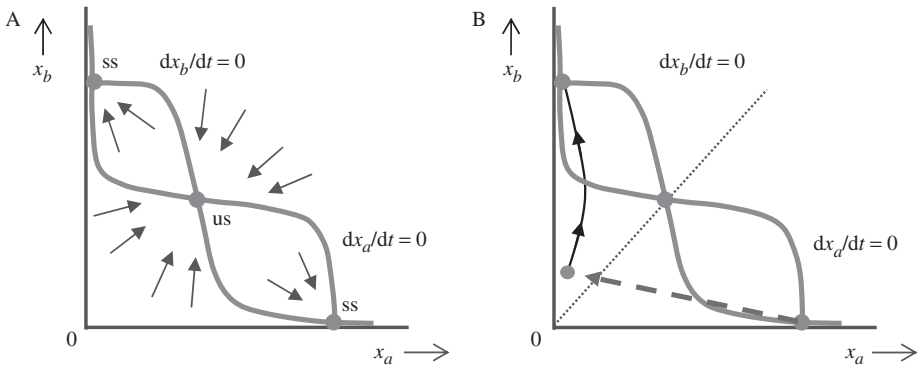

Figure 2: (A) Phase portrait of the ODE model of the cross-inhibition network (Figure 1). The system has two asymptotically stable steady states (ss) and one unstable steady state (us). The steady states lie at the intersection of the nullclines of x_a and x_b (drawn curves annotated by $dx_a/dt = 0$ and $dx_b/dt = 0$). (B) Hysteresis effect resulting from a transient perturbation of the system (dashed line with arrow).

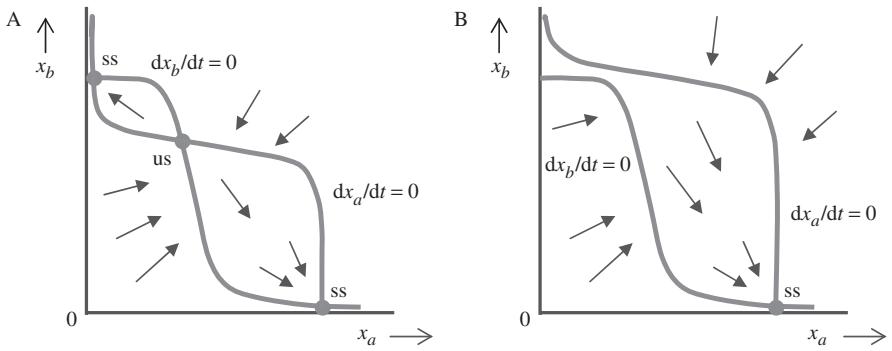

Figure 3: Changes in the phase portrait of the ODE model of the cross-inhibition network (Figure 2), following an increase in the value of the parameter θ_b. The change in (A) preserves the bistability and hysteresis properties, whereas the more important increase in (B) does not.

portrait obtained by increasing the value of the threshold parameter θ_b. As a consequence, the nullcline of x_a, defined by $x_a = (\kappa_a/\gamma_a)\ h^-(x_b, \theta_b, m_b)$, moves upwards. This deforms the phase portrait, but does not lead to the loss of the bistability and hysteresis properties. That is, these properties of the cross-inhibition network are invariant over large ranges of the parameter values. They are not invariant for all values though: when θ_b is close to or above κ_b/γ_b, one of the stable steady states and the unstable steady state approach and annihilate each other (Figure 3B). In the terminology of dynamical systems theory, a *bifurcation* has occurred. [12, 13]

The invariance of essential dynamical properties of the network to changes in the values of kinetic parameters is consistent with the observed *robustness* of living organisms, that is, the capability to maintain their functioning in the presence of internal and external perturbations [19, 20]. This is critical for systems evolving in an unpredictable environment using unreliable components. As an example, consider a well-studied, naturally occurring bistable system, bacteriophage λ [11]. Following infection of an *E. coli* cell, bacteriophage λ follows either a lysogenic or a lytic pathway, and may switch from lysogeny to lysis after exposure to UV light. It has been shown that these properties do not depend on fine-tuning the values of the binding constants, but are a consequence of the feedback structure of the network. In fact, point mutations in the promoter region of genes coding for two key transcriptional regulators involved in the bacteriophage λ fate decision change the value of specific binding constants, but preserve the lysogenic/lytic switch [21]. Similarly, the steady-state tumbling frequency in bacterial chemotaxis has been shown to be robust over a wide range of attractant or repellent concentrations [22–24].

The robustness of network properties to changes in the values of kinetic parameters suggests a modeling strategy. Instead of worrying about exact parameter values, we could randomly choose values from a physiologically relevant range and determine important properties of the system for the different parameter combinations. Assuming that the system is functioning in a robust way, these properties should be invariant for a large fraction of the parameter combinations. If this is not the case, the model may contain structural flaws, for instance the omission of a critical feedback loop. This strategy of testing the *parameter sensitivity* of essential network properties [25] has been put to work in a number of case studies of actual biological systems, as illustrated for the establishment of segment polarity in the fruit fly *Drosophila melanogaster* [26].

Early development of the embryo of *Drosophila* involves a division of the anterio-posterior axis into segments that prefigure the body plan of the fly. The segments arise from the establishment of concentration gradients through the diffusion of transcription factors, before cellularization has occurred, as well as from the interactions between maternal genes and the gap, pair-rule, and segment-polarity genes [27]. The initial activation of the segment-polarity genes is mainly controlled by the expression level of the pair-rule genes but, once established and until the fly has reached adulthood, the spatiotemporal expression profile of the segment-polarity genes is controlled by the mutual interactions between the latter genes. This expression profile is expected to be robust, because it identifies the future function of each segment of the embryo.

The segmentation process in *Drosophila* has been studied in detail, which allows an outline to be drawn of the genetic regulatory network connecting the segment-polarity genes. Von Dassow and colleagues have proposed an ODE model consisting of five genes, their products, and the mutual interactions between the genes and gene products. In order to reproduce the observed expression profiles, in addition to temporal aspects, the model takes into account spatial aspects, by including a row of cells along the anterio-posterior axis. Each of the cells contains a copy of the genetic regulatory network and interacts with its neighboring cells. The ODE model contains almost 50 parameters, the values of which are unknown in most cases. Von Dassow and colleagues therefore searched for parameter combinations that would lead, starting from reasonable initial conditions (determined by the expression level of the pair-rule genes), to the observed expression profile of the segment-polarity genes in the embryo. Despite an extensive search, no such parameter combinations could be found. By analyzing the reasons for this negative result, the authors concluded that there must be missing interactions. In fact, when they added two additional interactions, suggested by genetic experiments and circumstantial evidence, a large number of parameter combinations led to the desired behavior. More specifically, the revised model showed a surprising robustness to variations in the parameter values, sometimes spanning several orders of magnitude. In a recent paper, this robustness has been attributed to the creation of two additional positive feedback loops by the inclusion of the suggested interactions [28].

A similar parameter sensitivity strategy based on robustness arguments has been put to use by Stelling *et al*. [29] in their comparison of two published models of the *Drosophila* circadian clock (see also [15, 30], as well as [31] for another example). Although intuitively attractive, the practical application of the strategy raises a number of issues. First, not all properties may be robust against variations in parameter values. In the example of bacteriophage λ, it is reasonable to expect the bistability property to be robust, but this may not be true for other properties. Second, the formulation of an appropriate robustness measure is not straightforward. Von Dassow *et al*. used a simple binary criterion, indicating whether a parameter combination turns out to be able to reproduce the observed expression profiles. However, different robustness measures may need to be developed for other situations (for examples, see [29, 32, 33]). The *Drosophila* segment-polarity model also illustrates a third problem: the huge dimension of the parameter space. Although sophisticated sampling strategies have been developed [34], in practice it is possible to explore only a small fraction of the parameter space.

MODEL SIMPLIFICATION

An obvious way to deal with the high dimensionality of the parameter space is to try to simplify the models, so as to reduce the number of parameters. If appropriately chosen, these simplifications preserve the characteristic properties of the network. Figure 4 shows a modified ODE model of the cross-inhibition network in Figure 1, obtained by replacing the sigmoidal functions by step functions, thus eliminating two of the eight parameters (the cooperativity constants m_a and m_b). From a biological point of view, the use of step functions corresponds to the assumption that gene activity is switched on or off abruptly instead of progressively, when the concentration of the regulatory protein crosses a threshold concentration. The model simplification results in a strong deformation of the phase portrait, but does not affect the bistability and hysteresis properties, as shown in Figure 4C. Moreover, one can easily show that for all parameter values such that $\kappa_a/\gamma_a > \theta_a$ and $\kappa_b/\gamma_b > \theta_b$, these two properties are preserved. The dynamics of the network are not invariant to all model simplifications though. For instance, replacing the sigmoidal function by linear functions does not preserve the bistability and hysteresis properties (Figure 5; [35]).

These observations motivate a second strategy for dealing with incomplete information on molecular interaction networks: use models with a simpler mathematical form which facilitate the study of qualitative properties of the dynamics of the system. In the same way as for changes in the value of kinetic parameters, we expect these properties to be invariant under not-too-drastic transformations of the rate laws describing the reaction mechanisms. In the terminology of control theory, the models are expected to be *structurally stable*. Several approaches based on such a *model simplification strategy* have been developed in the literature (for recent reviews, see [36, 37]). In this section we will mainly discuss

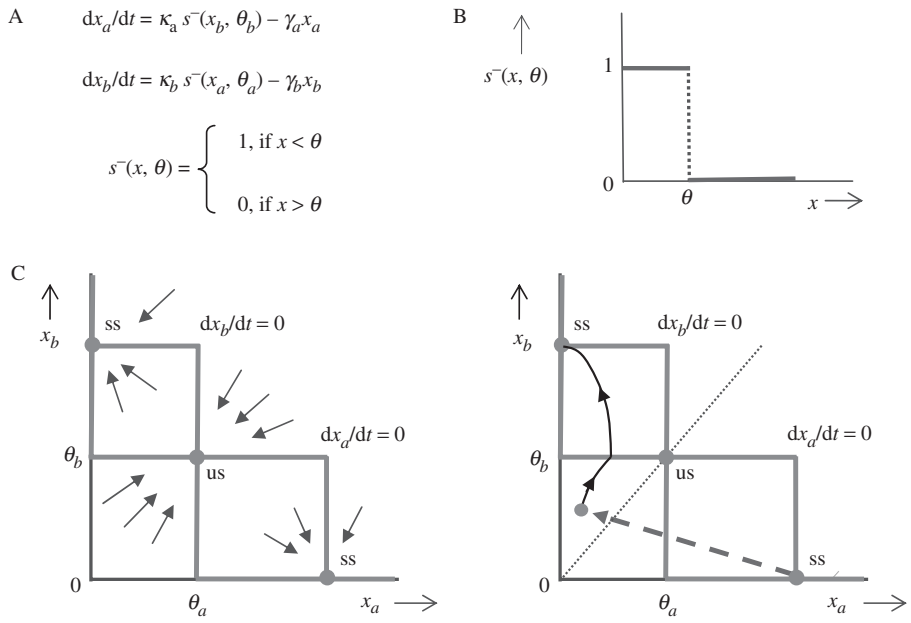

Figure 4: (A) Piecewise-linear differential equation (PLDE) model of the cross-inhibition network in Figure IA. The Hill functions $h^-(x, \theta, m)$ have been replaced by step functions $s^-(x, \theta)$. (B) Graphical representation of the step function $s^-(x, \theta)$. (C) Phase portrait of the PLDE model of the cross-inhibition network. Like in Figure 2, the system has two asymptotically stable steady states (ss) and one unstable steady state (us).

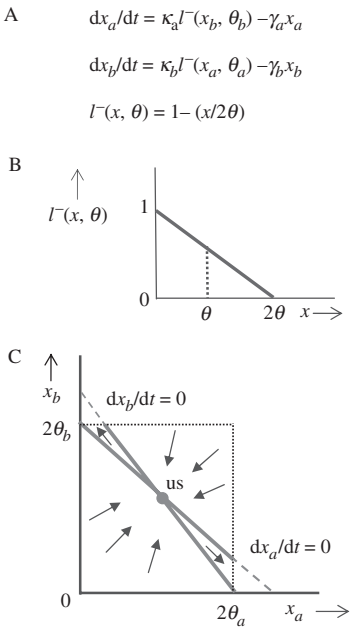

Figure 5: (A) Linear ODE model of the cross-inhibition network in Figure IA. The Hill functions $h^-(x, \theta, m)$ have been replaced by linear functions $l^-(x, \theta)$. The parameters θ_a and θ_b now represent the slope of the regulation functions. (B) Graphical representation of the function $l^-(x, \theta)$. (C) Phase portrait of the linear ODE model of the cross-inhibition network. In comparison with Figures 2 and 4, the two asymptotically stable steady states have disappeared.

an approach based on the use of a class of *piecewise-linear differential equation* (*PLDE*) models.

These PLDE models, proposed by Glass and Kauffman in the early seventies ([38]; see also [39–42]), use step functions to describe the regulation of gene expression, as exemplified in Figure 4. By means of the threshold values of the variables, the phase space can be partitioned into hyper-rectangular regions, in each of which the system behaves in a qualitatively homogeneous manner. The continuous phase–space dynamics of the system can be discretized into a state transition graph, that is, a graph composed of states corresponding to the regions in the phase space, as well as transitions between these states. The state transition graph describes the possible qualitative behaviors of the system and allows the attractors and their attainability to be determined. It has been proven that the state transition graph defined on this partition is invariant for certain inequality constraints on the parameters that can often be inferred from the experimental literature [39]. Moreover, it is possible to compute the state transition graph, by means of symbolic rules, from a PLDE model of the network supplemented by inequality constraints. This *qualitative simulation* method has been implemented in the computer tool Genetic Network Analyzer (GNA) [43].

We will illustrate its application to the analysis of the carbon starvation response in *E. coli*.

In case of nutritional stress, an *E. coli* population abandons exponential growth and enters a non-growth state called stationary phase. This growth-phase transition is accompanied by numerous physiological changes in the bacteria, concerning among other things the morphology and the metabolism of the cell, as well as gene expression [44]. On the molecular level, the transition from exponential phase to stationary phase is controlled by a complex genetic regulatory network integrating various environmental signals. The molecular basis of the adaptation of the growth of *E. coli* to the nutritional conditions has been the focus of extensive studies for decades [45]. However, notwithstanding the enormous amount of information accumulated on the genes, proteins, and other molecules known to be involved in the stress adaptation process, it is currently not understood how the response of the cell emerges from the network of molecular interactions. Moreover, with some exceptions [46], numerical values for the parameters characterizing the interactions and the molecular concentrations are absent, which makes it difficult to apply traditional methods for the dynamical modeling of genetic regulatory networks.

The aforementioned circumstances have motivated the qualitative analysis of the nutritional stress response network in *E. coli* by means of the approach outlined in the preceding text. As a first step, the study has focused on a network of six genes that are believed to play a key role in the carbon starvation response [47]. Based on the available information in the experimental literature, a PLDE model of seven variables has been constructed, one protein concentration variable for each of the six genes and one input variable representing the presence or absence of a carbon starvation signal. Seven differential equations, one for each variable, and forty parameter inequality constraints describe the dynamics of the system. Using this model, the response of *E. coli* cells to carbon starvation has been simulated. Among other things, this has led to new insights into the role of positive and negative feedback loops controlling the slowing down of bacterial growth characteristic of stationary phase. Moreover, it has suggested new experiments to test predictions on the temporal ordering of molecular events, notably the up and down regulation of the stress response genes.

Several other examples of the use of PLDE models can be found in the literature (e.g. [48, 49]). Alternative model simplification approaches have been based on *logical models* [50–52], which provide similar approximate descriptions of the dynamics of genetic regulatory networks. Logical models have been used, among other things, to analyze early *Drosophila* development [53–56]. The results tend to confirm the impression obtained from the cross-inhibition network: the simplified models provide a coarse-grained picture of the network dynamics which preserves essential, biologically relevant properties of the system.[1] However, few systematic studies comparing the dynamical properties of the original nonlinear ODE models and the simplified PLDE or Boolean models have been carried out thus far.

CONCLUSIONS

In this article we have discussed two strategies for dealing with a common problem in the modeling of molecular interaction networks: the absence of detailed and quantitative information on the reaction mechanisms underlying the interactions. These two strategies have been illustrated in the case of genetic regulatory networks, but there is no in-principle argument against carrying out parameter sensitivity tests and model simplifications when dealing with metabolic and signal transduction networks, or even with integrated networks combining different types of interactions. In fact, several approaches have been developed to this end, such as metabolic control theory [3, 59] and constraint-based pathway analysis methods [60].

The underlying idea of the strategies is to adapt the models, and the conclusions that can be inferred from them, to the usually imprecise and incomplete information at our disposal. Instead of making precise quantitative predictions on the dynamic properties of the system, we restrict the analysis to properties that are robust over a range of parameter values and rate laws. While the resulting predictions are weaker, they reflect the fact that the systems under study preserve certain qualitative dynamical properties in the face of variations in environmental conditions

[1] For another example of simplified models, see the powerlaw formalism developed by Savageau and colleagues [57, 58].

and in the strength of the molecular interactions. There remain many open problems though in the development and application of the aforementioned strategies.

First, one can raise the question which approximations are able to preserve the properties of interest. As illustrated by the model of the cross-inhibition network, the properties are not robust to some changes in the parameter values (Figure 3B) and in the form of the mathematical functions (Figure 5). A second issue is the relation between the model approximations and the actual computation of the properties. Can we develop methods that allow us to infer the properties from the available weak information? And how do these methods scale up to the usually large and complex networks we have to deal with? These questions find their origin in the practical problems encountered by modelers, but they also raise difficult theoretical problems. The resolution of these problems requires methodological advances in mathematics and computer science, and may be a source of inspiration for the latter fields (e.g. [61]).

The basic motivation for the strategies discussed in this article is the current absence of precise and quantitative data on most molecular interaction networks of biological interest. One could therefore argue that the rapid advance of new experimental techniques, allowing the measurement of kinetic parameters (e.g. [62, 63]), as well as the estimation of parameter values from (time series) data (e.g. [46, 64, 65]), will eventually obviate the use of the strategies for dealing with incomplete information. As mathematical modeling and computer simulation become more firmly established in molecular biology, one might expect experimental programs to be increasingly oriented towards the collection of data compatible with modeling requirements. Although this trend can indeed be observed, this does not mean that the strategies discussed here will ultimately lose their interest. Even when building detailed, quantitative models, it remains important to study the dynamics of the system by means of approximate models, either to prime the computationally expensive process of parameter estimation or to verify the robustness of the predicted properties (see [66] for a recent example, and the associated commentary in [67]). More generally, modeling is not a one-pass process, but requires the system under study to be looked at from different angles and on different levels of abstraction.

Key Points

- Information for the modeling and simulation of molecular interaction networks is often incomplete (missing parameter values, lack of details on reaction mechanisms).
- Essential properties of biological systems are robust against variations in parameter values and rate laws.
- The strategies of parameter sensitivity analysis and model simplification exploit this robustness to analyze the dynamic properties of molecular interaction networks.

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