

# Petri net modelling of biological networks

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## Abstract

Mathematical modelling is increasingly used to get insights into the functioning of complex biological networks. In this context, Petri nets (PNs) have recently emerged as a promising tool among the various methods employed for the modelling and analysis of molecular networks. PNs come with a series of extensions, which allow different abstraction levels, from purely qualitative to more complex quantitative models. Noteworthy, each of these models preserves the underlying graph, which depicts the interactions between the biological components. This article intends to present the basics of the approach and to foster the potential role PNs could play in the development of the computational systems biology.

**Keywords:** dynamical modelling; Petri nets; biological networks

## INTRODUCTION

Beyond the production of a huge amount of biological data, there is a real challenge in understanding how complex interaction networks control the cell behaviour. To address this challenge, one needs to resort to formal methods handling the modelling of such networks. While the study of the sole topology of the network provides some knowledge on the involved biological components, the dynamical modelling aims at understanding the dynamical properties of the system (see [1] for a review on the modelling of regulatory networks and [2] for a recent survey of computational techniques applied to model signalling networks).

One can distinguish two main classes of dynamical models: on the one hand, *quantitative* models are essentially based on systems of ordinary differential equations (ODEs); on the other hand, *qualitative* models can be defined through discrete formalisms or piecewise linear differential systems. The first models aim at representing the system in a detailed way, producing quantitative results. They require accurate kinetic data, which are often lacking. Moreover, because of the size and the preciseness of the models, most of the results are obtained by numerical integration methods. It is therefore

difficult to apprehend or prove general properties of the models under study. Quantitative approaches are thus helpfully complemented by qualitative approaches, which are more suitable to induce dynamical properties of complex systems, in particular when few data are accessible.

Petri nets (PNs) have been named after Carl Adam Petri who, in the early sixties, proposed a graphical and mathematical formalism suitable for the modelling and the analysis of concurrent, asynchronous, distributed systems [3–6]. With their various extensions, PNs allow the definition of both qualitative and quantitative models. During the past 40 years, a large amount of work has been done on theoretical developments and PNs have been successfully applied to a wide range of applications. These were mainly related to the modelling and analysis of man-made systems (manufacturing systems, communication networks, computational distributed systems, etc.). More recently, PN modelling appeared for ‘natural’ systems, in particular for molecular networks. An early attempt to apply the PN framework to biochemical reaction systems has been presented by Reddy et al. [7]. As emphasized in [8], PNs are a convenient mathematical formalism allowing an intuitive representation of

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biochemical networks. This is mainly due to their graph-based structure. Moreover, complementing a purely qualitative structural analysis, one can consider quantitative information, for instance in the form of (stochastic) reaction rates, (continuous) concentration levels, etc. The significant number of related publications reflects the growing interest for PN models in this field.

Molecular networks encompass metabolic, signalling and genetic networks. In such networks, the nature of the relations between the molecular components differs, depending on the molecular process (chemical reaction, complex formation, binding process, transcriptional regulation, etc). It also depends on the level of abstraction chosen for the modelling. For example, considering gene regulation, the activation of a gene may be modelled by taking into account the different processes from transcription to translation, or by simply considering that the gene is activated.

Recently, a number of modelling frameworks have been applied for the analysis and simulation of biological networks. Among them, process algebra (in particular  $\pi$ -calculus) can be related to Petri nets (see e.g. [9] and references therein, and [10] for a PN translation of  $\pi$ -calculus terms). However, a comparative study between the numerous modelling methods is far beyond the scope of this article. Here, complementing [11, 12], the aim is to provide gateways to PN modelling of molecular networks. This article first introduces PN basics and the most relevant extensions (further information can be found in the introductory article from Murata [3] or in the PN books [4–6]). Next, an intuitive representation of metabolic networks using PNs is presented, together with the biological questions that can be addressed. Beyond a qualitative analysis, one can use extensions of the original formalism to model metabolic networks. In particular, stochastic PNs (SPNs) and hybrid PNs (HPNs) are introduced. Then, the cases of signalling and regulatory networks are discussed. This article ends presenting assets, restrictions and prospects for the modelling and the analysis of biological networks by means of PNs.

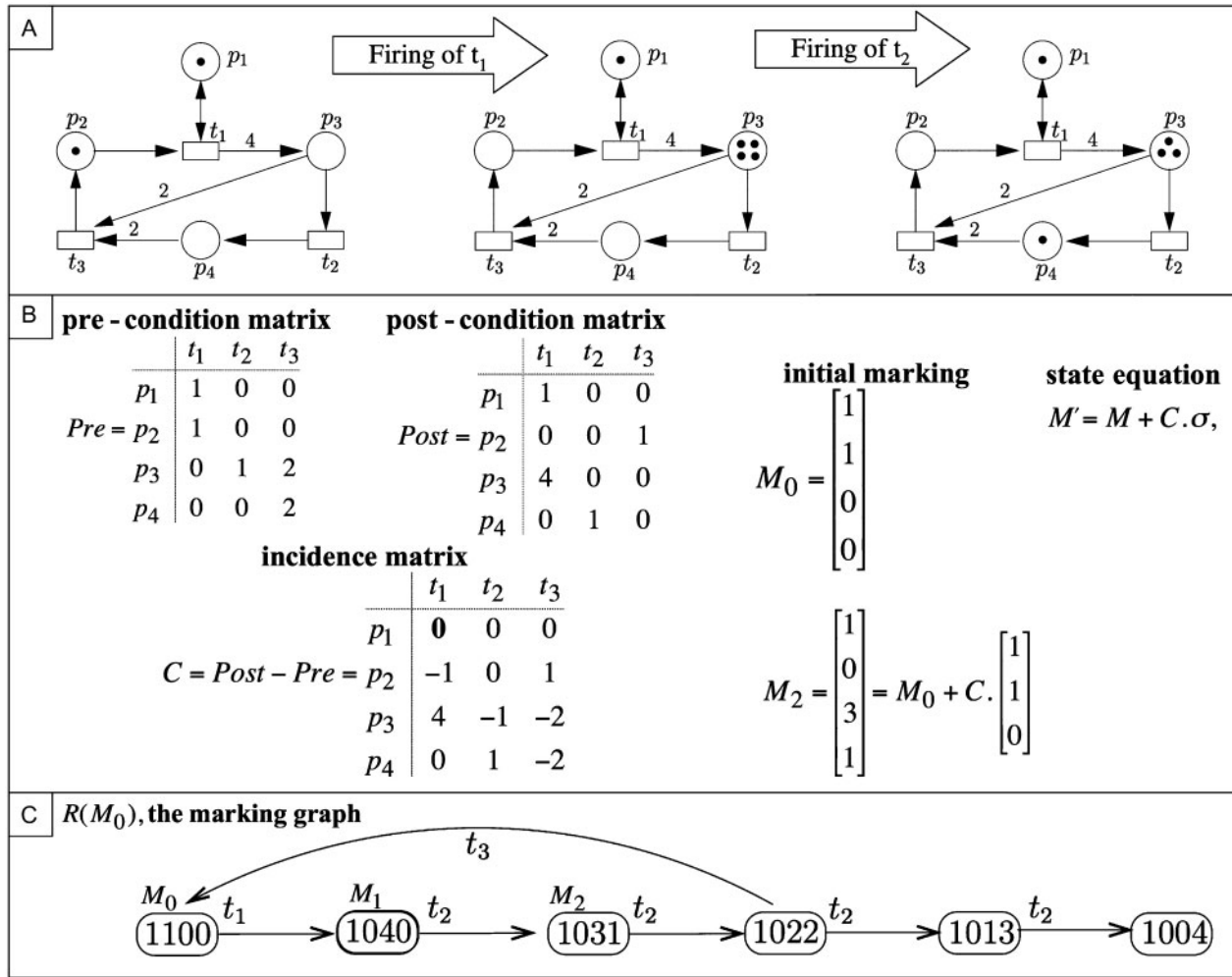
## PETRI NET BASICS

A PN is a directed-bipartite graph with two different types of nodes: places and transitions. Informally, places represent resources of the system,

while transitions correspond to events that can change the state of the resources. Weighted arcs (directed edges) connect *places* with *transitions*, depicting the relations between resources and events. A place is connected to a transition (it is said to be an *input* place of the transition) if the occurrence of the transition is conditioned by the state of the place (and subsequently changes this state). Now, a transition is connected to a place (*output* place) if the occurrence of the event has some consequence on the state of the resource.

At any time of the evolution of a PN, places hold zero or a positive number of *tokens*. The state of the system is represented by this allocation of tokens over the places and is called a *marking*. The definition of a PN includes the specification of an *initial marking*, which allocates a number of tokens to each place. A transition is *enabled* if its input places contain at least the required numbers of tokens (defined by the weight assigned to the arcs). The *firing* of an enabled transition will then result in the consumption of the tokens of its input places and the production of a number of tokens in its output places (this number is determined by the weights of the arcs going out of the transition). This ‘token game’ represents the dynamical evolution of the system. Figure 1 illustrates, through a simple PN, the token game, the algebraic representation, as well as the description of the behaviour by means of a marking graph. For a formal introduction to PNs, see references [3–6].

For modelling facility, PNs may encompass extended arcs (test and inhibitor arcs). In Figure 1, a *test arc* connects  $p_1$  to  $t_1$  (it is a bi-directional arc indicating that  $p_1$  marking governs the enabling of  $t_1$  but is not modified by the firing of  $t_1$ ). Test arcs are useful to model current situations in biochemical networks (such as enzymatic reactions, cf. Figure 2). *Inhibitor arcs*, from a place to a transition, notably increase the expressiveness, allowing a ‘test to zero’ (the transition is enabled provided the place is empty, Figure 2). In the presence of test or inhibitor arcs, the incidence matrix  $C$  no longer reflects the incidence relation of the net (Figure 1B). In some cases, when required, e.g. for analysis purposes, one can recover a standard PN: a test arc is replaced by a dummy couple of one place and one transition, an inhibitor arc from a bounded place (which marking is limited), is removed by adding a complementary place correctly linked to the transitions associated to the original place.

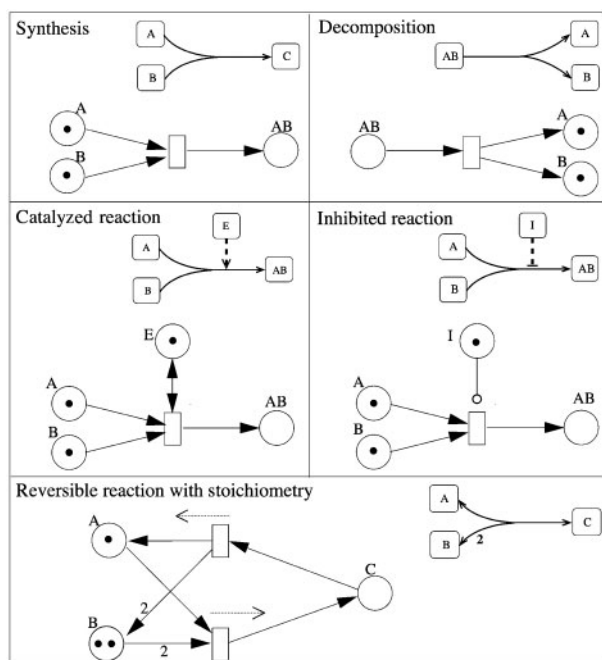


**Figure 1:** A PN example: **(A)** graphically, places are depicted by circles, transitions by rectangles and tokens by black dots. Weighted arcs connect places and transitions (the weight is omitted when it is 1). The three figures illustrate the firing process from the initial marking  $M_0$  enabling the firing of transition  $t_1$  (which leads to the new marking  $M_1$ ), which in turn enables the firing of  $t_2$  (leading to  $M_2$ ). **(B)** The algebraic description of the PN: a marking is defined as a vector giving the number of tokens allocated to each place, the *initial marking* ( $M_0$ ) defines the initial state of the net; weighted arcs define a relation between, on the one hand, places and transitions (*Pre*, denoting preconditions on the required marking of input places), on the other hand, transitions and places (*Post*, denoting the produced numbers of tokens into output places); the *incidence matrix* gives, for each transition, the balance of its firing onto each place (number of tokens produced minus number of tokens consumed); finally, the *state equation* defines the resulting marking after a firing sequence (using a *Parikh* vector  $\sigma$  encompassing, for each transition of the net, its number of occurrences). The solvability of this equation is limited (as markings must be non-negative integers and also because of the non-determinism of PNs); an application of the state equation is provided, showing that  $M_2$  is reached from  $M_0$  after the firing of both  $t_1$  and  $t_2$ . **(C)** Given the initial marking, the dynamical behaviour is described by the *marking graph* in terms of transitions between states (markings) and is denoted  $R(M_0)$ .

Standard PN models are discrete and non-temporized (time is implicit, the marking graph accounts for the possible sequences of events). One can then check qualitative properties using algebraic methods (by means of the incidence matrix), analysing the structure of the net, or investigating

the whole marking graph. Hereafter, some typical properties are described, together with their possible interpretations in the context of biological networks:

- *Boundedness* insures that, whatever the initial marking and the evolution of the net, the number of



**Figure 2:** PN modelling of different basic reactions: a synthesis, a decomposition, a catalysis, an inhibition and a reversible reaction. In the case of catalysis, the enzyme place is linked to the transition by a test arc. For an inhibition, the inhibitor place is linked to the transition by an inhibitor arc (which allows a ‘test to zero’: the transition is enabled when the place is not marked). A reversible reaction is represented by two separate transitions (one for each direction).

tokens in each place is bounded, i.e. limited. For metabolic networks, it means that no product can accumulate;

- *P-invariants* are sets of places for which the weighted sum of tokens is constant independently of the sequence of firings ( $x$ , a vector of integers defines a *P*-invariant if  $C^T \cdot x = 0$ ). In metabolic networks, these sets correspond to conservation relations;
- *T-invariants* are firing sequences, which reproduce a marking ( $y$ , a vector of integers defines a *T*-invariant if  $C \cdot y = 0$ ). In biological terms, *T*-invariants may represent cyclical behaviours and correspond to the *elementary modes* of the Metabolic Control Theory (i.e. feasible metabolic routes as defined by Schuster *et al.* [13]);
- *Reachability* of a marking  $M$  asserts that there exists an evolution (a sequence of firings) from the initial marking to the marking  $M$  (i.e.  $M$  is in the marking graph  $R(M_0)$ ). This property may be relevant for biological networks, as it ensures

the existence of a trajectory leading the system from an initial state to a desired state;

- *Liveness* insures that it is always possible to ultimately fire any transition. In other words, liveness guarantees that an event (a reaction for example) can eventually occur. Restricted definitions of liveness can also be considered (for instance, liveness of a sole transition, or even a guaranteed number of possible firings, etc.).

Extensions of the PN formalism have been defined to increase its expressiveness (in particular to allow quantitative analyses) and a series of dedicated tools have been developed to support the different PN modelling approaches (see Table 1 for a selected list of tools). However, the more expressive the formalism, the more difficult the analysis, and models featuring detailed descriptions are often checked through simulation, exhibiting specific trajectories of the system. In contrast, when available, analytical methods provide formal proofs of properties. Besides algebraic resolutions and topological analyses, *model-checking* usefully complements techniques for exact analysis [14]. Using temporal logics, one can express behavioural properties (e.g. the reachability of a given state or the fulfilment of a property along a trajectory). Model-checking methods have been used to query or validate biochemical networks in BIOCHAM (Biochemical Abstract Machine, a programming environment for biochemical networks [15]) and genetic regulatory networks in GNA (Genetic Network Analyzer [16]). Model checking has been developed also in the context of PN theory (Table 1) and should greatly facilitate the analysis of large networks.

In the sequel, the extensions that proved useful for the modelling of biological networks are shortly described.

**Coloured Petri Nets** (CPNs) assign data values to the tokens (defining *colour sets*), and expressions are attached to the arcs. These define the constraints on the token values in the input places to enable the transitions, and define the token values, produced by the firings, in the output places. With CPNs, the definition and manipulation of data types increase the modelling potential of PNs thus allowing the delineation of reduced models for complex systems [17, 18]. In addition, CPNs still convey formal analysis methods. CPNs can also encompass timed transitions. *CPN Tools* is a software dedicated to hierarchical CPNs (composed of



**Table I:** Selection of existing PN tools that have been used (or could be used) for the modelling of biological molecular networks. The last row indicates the main web site of the PN community

Name	Features	Web site
INA	Analysis of standard (timed) PNs and CPNs, no graphical editor, includes a model-checker for <i>CTL</i> .	<a href="http://www2.informatik.hu-berlin.de/~starke/ina.html">http://www2.informatik.hu-berlin.de/~starke/ina.html</a>
Prod	Efficient reachability analysis tool for standard PNs.	<a href="http://www.tcs.hut.fi/Software/prod/">http://www.tcs.hut.fi/Software/prod/</a>
Maria	Extensive reachability analysis and model checking of CPNs.	<a href="http://www.tcs.hut.fi/Software/maria/">http://www.tcs.hut.fi/Software/maria/</a>
Snoopy	Edition, animation, analysis of standard PNs, simulation of continuous PNs, hierarchical modelling, export facilities to <i>INA</i> , <i>Maria</i> , <i>Prod</i> .	<a href="http://www-dssz.informatik.tu-cottbus.de/software/snoopy.html">http://www-dssz.informatik.tu-cottbus.de/software/snoopy.html</a>
CPN tools	Edition, simulation and analysis of (timed) CPNs, graphical editor, hierarchical modelling.	<a href="http://wiki.daimi.au.dk/cpntools/cpntools.wiki">http://wiki.daimi.au.dk/cpntools/cpntools.wiki</a>
GreatSPN	Modelling, validation and performance evaluation of distributed systems using Generalized SPNs and their colored extension, export facilities to <i>PRISM</i> .	<a href="http://www.di.unito.it/~greatspn/">http://www.di.unito.it/~greatspn/</a>
Möbius	Edition, analysis, simulation of stochastic models	<a href="http://www.mobius.uiuc.edu">http://www.mobius.uiuc.edu</a>
GON	Edition and simulation of biopathways by means of HFPNs. <i>GON</i> is now commercialized as <i>Cell Illustrator</i> .	<a href="http://genomicobject.net/">http://genomicobject.net/</a>
PRISM	Probabilistic model checker.	<a href="http://www.cs.bham.ac.uk/~dxp/prism/">http://www.cs.bham.ac.uk/~dxp/prism/</a>
Petri nets world	A variety of online services on Petri nets including an up-to-date database of currently used tools.	<a href="http://www.informatik.uni-hamburg.de/TGI/PetriNets/">http://www.informatik.uni-hamburg.de/TGI/PetriNets/</a>

subnets with well-defined interfaces [19]). Hierarchical modelling is convenient in specifying models of complex biological systems [12].

It may be necessary to take into account uncertainty attached to data, or to describe external noise (generated by fluctuations of the environment), or intrinsic noise (due to low molecular concentrations). Stochastic Petri Nets (SPNs) supply such random aspects: enabled transitions fire with exponentially distributed time delays. It has been demonstrated that, for a bounded SPN, the marking graph is isomorphic to a finite Markov Chain (see [20] and references therein).

Hybrid Petri Nets (HPNs) allow the coexistence of both continuous and discrete processes. They include discrete places (marked with tokens) and continuous places associated with real variables (e.g. concentration levels). Discrete transitions fire after a determined delay, while enabled continuous transitions fire continuously at a given rate. Finally, HPNs encompass both test and inhibitor arcs [21]. To further increase the modelling power of HPNs, **Hybrid Functional Petri Nets** (HFPNs) have been introduced purposely to model biological networks [22]. Additional features have been included: continuous transition firing rates can depend on the values of the input places and the weights of arcs can be defined as a function of the markings of the connected places.

## PETRI NETS APPLIED TO THE MODELLING OF BIOLOGICAL NETWORKS

This section presents a number of applications of PN theory to model a range of molecular processes, employing standard or more sophisticated PNs.

### Biochemical networks

In [7], Reddy *et al.* have shown that **standard PNs** allow the representation of the essential components in biochemical pathways, and that PN models can be used to perform a qualitative analysis. Metabolic pathways are generally seen as interconnected networks of enzymatic reactions, where the product of one reaction is a reactant of (or an enzyme that catalyses) a subsequent reaction. Figure 2 illustrates the PN modelling of five types of reactions; places represent reactants, products or enzymes whereas transitions represent reactions, each modifying the amounts of its products and reactants. At any time, the marking represents the distribution of molecules of each species in the network. The arc weights correspond to the stoichiometric coefficients of the reactions.

Recently, it has been shown that several concepts arising in structural pathway analysis of biochemical networks have their counterparts in PN theory [23]. In particular, it has been demonstrated that *T*-invariants correspond to elementary modes.

Indeed, the topology of the PN matches the topology of the corresponding metabolic network, and one can draw extensive relationships between the traditional biochemical models and PNs [24]. As a consequence, the stoichiometry matrix of a metabolic network corresponds to the PN incidence matrix. PN theory can be used for the structural validation of metabolic networks through qualitative analysis. For example, the sucrose breakdown metabolism in the potato tuber is considered in [25]. The authors demonstrate that qualitative analysis can be used to validate the model and that  $T$ -invariants relate to combinations of subpathways in the network.

The use of **CPNs** to simulate enzymatic reaction chains is proposed in [26]. The colour associated to a place is a pair encompassing the name and the concentration of the related substrate. Reaction transitions are associated with a kinetic function. In [27], the authors use colours to differentiate molecules of the same species (according to the paths along which they are produced and consumed) and perform a qualitative analysis of the combined glycolysis and pentose phosphate pathway in erythrocytes (a refined version of the original model proposed by Reddy *et al.* in [7]).

Deterministic simulations do not consider the intrinsic noise due to low concentrations. **SPNs** are used for the modelling and simulation of stochastic molecular interactions and facilitate the modelling process [28, 29] (these models essentially reproduce the Gillespie's algorithm [30]). In [31], Shaw and co-authors address the problem of kinetic parameter estimation by developing stochastic simulations relying on SPNs.

**Functional PNs**, primarily defined by Valk as *self-modified* PNs [32], allow the flow relations between places and transitions to depend on the marking. Hofestädt and Thelen suggested to apply this extension to quantitatively model biochemical networks [33]. Pursuing along this line, Matsuno *et al.* [22] defined the HFPNs, which provide a rich set of features for the modelling of biochemical pathways. The development of a dedicated software, Genomic Object Net (GON), surely contributed to the success of HFPNs in the field [34]. Several recent papers present HFPN-based models showing the attraction of this approach for quantitative simulation. In particular, in [35], Doi *et al.* define a detailed model of the p53 transcriptional activity, with transitions accounting for different processes in

the interactions between p53, MDM2, p19ARF (nuclear import/export, binding, transcription, translation, ubiquitination) and places representing the different substances (e.g. p53 in the nucleus, complex p53–MDM2 complex in the cytoplasm, etc). Their simulation results suggest a transcriptional activity of the complex p53–MDM2–p19ARF on genes MDM2 and BAX.

The critical problem of parameter estimation is addressed using a HFPN modelling in [36]. It exploits the topology of the network to systematically decompose it into subpathways whose parameters can be estimated independently. A detailed model of the Akt and MAPK pathways with their possible crosstalks is provided to illustrate the method.

## Genetic networks

When modelling a biological network, it is crucial to consider the relevant level of abstraction, depending on the question to be addressed, but also on available data. In the case of the regulation of gene expression, it is often sufficient to represent the fact that a particular regulatory product activates or inhibits a gene (or a set of genes) to convey the role of this product in the network. Such regulatory interactions differ semantically from metabolic reactions. Indeed, while in a chemical reaction the reactants are consumed, the expression levels of regulators do not change during the regulatory process. Figure 2 shows that chemical reactions are naturally represented in PN, but regulatory interactions are not so easily modelled (as a fundamental purpose of PNs is to represent production/consumption effects).

One successful method to qualitatively model such regulatory networks is the *logical* approach initially developed by Thomas and collaborators [37]. In a *logical* regulatory graph, the nodes represent genes, which are associated with discrete levels of expression, and arcs represent interactions between genes. Each interaction is associated with an expression level threshold from which the regulator, source of the interaction, has an effect onto the targeted gene. For each gene, a discrete logical function indicates to which qualitative level the gene tends when submitted to a given combination of interactions.

It is possible to derive a **standard PN** model from a **Boolean regulatory network** (where genes are ON or OFF) [38, 39]. A systematic rewriting of multi-level models is defined in [40]. This last paper

also proposes a **CPN** representation of logical models, leading to simplified nets. This translation opens the way to the application of PN analysis tools to logical regulatory models.

Complementing the qualitative analysis tools, *model checking* can be used to check dynamical properties. As CPNs are amenable to model-checking techniques (Maria tool, see Table 1), Comet *et al.* [41] develop a translation of logical regulatory graphs into CPNs producing a compact model. The goal here, is to supply the biologist with a tool that systematically verifies the coherence of the model under various hypotheses (accounting for observed biological behaviours such as homeostasis, multistationarity, or even more specific temporal properties). For parameterized models, the CPN representation proposed in [40] will allow to check dynamical properties for large logical regulatory graphs.

In [42], Simão *et al.* propose a method to define **integrated models of regulated biochemical pathways**, considering a logical model of the regulation level (a PN representation) linked to a classical PN model of the metabolic part. This approach is illustrated with a qualitative modelling of the biosynthesis of tryptophan (Trp) in *Escherichia coli*, taking into account two regulatory feedbacks: the direct inhibition of the first enzyme of the pathway by the final product and the transcriptional inhibition of the Trp operon by the Trp repressor complex.

Matsuno *et al.* [43] have considered **HPN** modelling of gene regulatory networks. HPNs provide a convenient way to represent protein concentration dynamics being coupled with discrete switches. A detailed modelling of gene regulatory networks would require the representation of several stages including DNA modification, transcription, translation, post-transcriptional and translational modifications. Since the data and knowledge on these mechanisms are lacking, it is generally difficult to conceive kinetic models of these mechanisms, which are therefore abstracted as a single process. HPN quantitative modelling and simulation of gene-regulated metabolic networks is also illustrated through a study of the urea cycle in [44]. Several regulated metabolic pathways have been modelled and simulated using **HFPNs** (eg. [45, 46]). In [46], the Delta-Notch dependent boundary formation in the *Drosophila* large intestine is analysed, considering cell-to-cell interactions.

## Signalling networks

Signal transduction, like genetic regulation, encompasses response to signal (presence or threshold level of given molecules) rather than product transformation. Signalling pathways are generally complex, encompassing numerous mechanisms such as complex formation, translocation, etc. As in [22], which proposes a HFPN model of Fas-induced apoptosis, Heiner *et al.* [47] also consider apoptotic pathways. Using standard PNs, this signal transduction network is qualitatively modelled and validated. In the same line, the modelling of different functional forms of proteins is addressed by Sackmann *et al.*, [48] who recently applied standard PNs to signalling pathways. They further show that a structural analysis can provide a means for model validation and better understanding of the network (in particular by the determination of a meaningful decomposition of the net). A model of the mating pheromone response pathway in budding yeast is provided as a case study.

In [49], Gilbert and Heiner propose a bridge between qualitative PNs and traditional ODE modelling. Using a model of the influence of the Raf Kinase Inhibitor Protein (RKIP) on the Extracellular signal Regulated Kinase (ERK) signalling pathway, they show that a standard PN model constitutes a convenient step towards the definition of a more detailed quantitative model. More precisely, they demonstrate that analysis of a discrete PN model can then be used to derive sets of initial concentrations required by the related ODE model.

On the basis of the definition of standard PN modules for the essential components of signalling pathways, Li and co-authors propose a method to determine relevant (deterministic) transition delays to derive a **timed PN** model [50]. Simulation and token animation of the timed PN can then provide an intuitive view of the behaviour of the pathway.

A **(timed) CPN** model of a complex signal transduction system (the EFG-induced signalling pathways) is developed in [51]. An efficient simulation is provided by this CPN modelling approach, which is consistent with a differential approach.

Using Continuous Time Markov Chains (CTMCs), Calder *et al.* [52] propose an approach allowing a quantitative analysis of the network in terms of probabilities associated to given events. For this purpose, they have used a stochastic temporal logic and PRISM, a specific model checker [cf. Table1]. They have applied their method to

the analysis of the RKIP-inhibited ERK pathway. Interestingly, **SPNs** are isomorphic to CTMCs [20], and thus could be used for a similar approach. Indeed, SPN models are amenable to model-checking techniques since they can be translated into the input format of PRISM [53].

## DISCUSSION

This brief overview emphasizes the effectiveness of PNs for the modelling, analysis and simulation of molecular networks. Increasing use of PN-based models for biological networks can be explained by their underlying graphical representation, their suitability to model concurrent distributed systems, their well-founded mathematical theory and the availability of dedicated tools (cf. Table 1). A series of biological applications have been already developed, using purely qualitative to sophisticated HPN formalisms. These different modelling approaches led to different kinds of analyses, from structural analyses to pure simulations, from qualitative results to quantitative ones. We have seen that PN modelling of metabolic reactions is relatively intuitive. It is less natural for gene interactions (activation or inhibition) or signal transduction, which imply regulatory interactions rather than consumptions/productions. However, extended arcs (inhibitor and test arcs) and CPNs provide a convenient representation of such interactions. As for HPNs, they support the representation of a wide range of molecular mechanisms.

Biologists have in hand a large amount of interaction data, but accurate values of concentration levels or kinetic parameters are often scarce. This motivates the development of qualitative models, which further allow a formal analysis and constitute a first step towards the definition of quantitative models. Several PN extensions have been introduced to increase the expressiveness of the primary formalism (in particular, to include temporal, stochastic and continuous aspects). As a counterpart of these refined features, the analysis is more difficult and, in general, only simulation results are provided. Still, as a major advantage, the formalism opens the road to a progressive top-down modelling method, from a qualitative structural representation to a quantitative detailed description.

PNs are not meant to represent spatial properties. However, molecular transportation or diffusion can be modelled in the following way: places represent the presence of a given substance in different

compartments, whereas transitions express the displacement of this substance [35]. The size of the model can then be considerable for a large number of compartments and diffusing substances. A more convenient approach uses CPNs, where an identifier (a colour) is associated to the tokens and can serve to convey such spatial information [27]. Nevertheless, as transport mechanisms are essential in various regulatory processes, their PN representation still deserves further methodological developments. Hierarchical modelling might be a valuable means to address this issue. Hierarchical modelling can also be applied to large networks, often composed of interconnected functional modules. In order to allow model composition, PN units or modules might be defined as building blocks of biological network models. Along the same line, additional methodological work would define a systematic integration of PN models accounting for different levels of abstraction. In particular, biological processes occur at different time scales, and this question should be addressed properly.

Finally, bridges might be defined between other modelling formalisms and PNs (as in [40, 49]). In this respect, standard XML formats facilitate automatic translations, as proposed by Shaw *et al.* [54] with their mapping from SBML (Systems Biology Markup Language) to PNML (Petri Net Markup Language) (SBML web site: [sbml.org](http://sbml.org); PNML web site: [www.informatik.hu-berlin.de/top/pnml](http://www.informatik.hu-berlin.de/top/pnml)). The development of standard formats also leads up to the automatic definition and parameterization of models from data queried in dedicated databases. As a first step in this direction, standardized PN units are proposed in [55], which are based on ontology for signalling processes. This methodology could support reusing and scaling up of existing models.

### Key Points

- PNs come along with a graphical representation, a well-founded mathematical theory and a series of computational tools.
- PNs allow the analysis of qualitative structural to quantitative behavioural properties.
- PNs are effective for the modelling of molecular networks.

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