Computing signal transduction in signaling networks modeled as Boolean Networks, Petri Nets and hypergraphs

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Abstract. Mathematical frameworks circumventing the need of mechanistic detail exist to build models of signal transduction networks: graphs, hypergraphs, Boolean Networks, and Petri Nets. Predicting how a signal transduces in a signaling network is essential to understand cellular functions and disease. Different formalisms exist to describe how a signal transduces in a given intracellular signaling network represented in the aforementioned modeling frameworks: elementary signaling modes, T-invariants, extreme pathway analysis, elementary flux modes and simple paths. While these signal transduction formalisms are broadly used in their respective frameworks, few studies have been done emphasizing how these signal transduction methodologies compare or relate to each other.

We present an overview of how signal transduction networks have been modelled using graphs, hypergraphs, Boolean Networks, and Petri Nets in the literature. We provide a literary review of the different formalisms for capturing signal transduction in a given model of an intracellular signaling network. We also discuss the existing translations between the different modeling frameworks, and the relationships between their corresponding signal transduction representations that have been described in the literature. Furthermore, as a new formalism of signal transduction, we show how minimal functional routes proposed for signaling networks modeled as Boolean Networks can be captured by computing topological factories, a methodology found in the metabolic networks literature. We further show that in the case of an acyclic B-hypergraph, the definitions are equivalent. In directed graphs, it has been shown that computations of elementary modes via its incidence matrix correspond to computations of simple paths and feedback loops. We show that computing elementary modes based on the incidence matrix of a B-hypergraph fails to capture minimal functional routes.

1. Background

Cells must be able to receive, process and respond appropriately to cues from their surrounding environment. The signal transduction component in the form of cellular elements that produce a response to cues from the cell's environment will be referred from hereon in as a *signaling pathway*. The necessity of cells to process different signals causes several signaling pathways to interact with each other, creating signaling networks. The complexity innate to these networks, both from size and connectivity, makes computational modeling and analysis a requirement to understand how the cell communicates with its environment [1;2]. It is well documented that malfunctions in signaling pathways from both epigenetic and genetic aberrations lead to several pathologies [3;4], especially cancer. Understanding how a signal transduces is a necessity for the development of therapeutics and personalized medicine approaches.

A signaling network is usually characterized as having a three-layer structure, with an input layer, an intermediate layer, and a target layer [6]. In mathematical models of signaling networks, the input layer nodes are typically ligands, exterior signals, receptors, or events that initialize the signal transduction process. The target layer, depending on level of abstraction, may be cellular responses, transcription factors, genes, metabolites, or processes that can be considered as the result of a complete signal propagation. The intermediate layer are the conduits of the signal, such as second messengers and enzymes. See Figure 1 for a prototypical signaling network.

Several kinetic parameters required for detailed mechanistic mathematical models are difficult to obtain. Furthermore, certain signaling components, such as GTP-binding proteins, act as molecular switches, having an "active" or "inactive" status rather than a continuum. Thus, modelling frameworks circumventing the necessity of detailed kinetic parameters have been proposed in the literature, some of which, included in the present work, are reviewed by Samaga and Klamt [7] . A

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detailed review of Kholodenko et al.[8] discusses other methodologies for settings in which more kinetic information is available for modelling purposes.

We provide an overview of arguably the more commonly used coarse-grained modeling frameworks of intracellular signaling networks: graphs, hypergraphs, Boolean Networks, and Petri Nets. We discuss the formalisms that modelers using these frameworks have proposed to capture how an intracellular signal transduces within a cell. Different formalisms have been shown to relate to each other[9;10;11]. We discuss the existing translations and describe different equivalences in the formalisms for capturing signal transduction. We adopt topological stoichiometric factories from metabolic network analysis tools[12;13] and compare them to elementary signaling modes methodology[14].

To be sure, several criticisms exist against the aforementioned modeling frameworks, such as data discretization, coarseness, and time-discreteness. At no point do we wish to argue that any of the modeling frameworks mentioned are the "be all end all" of modeling. What is worth remarking, however, is that in order to understand biology, modeling is necessary; even cell lines and organoids are models of what happens *in vivo*. To this date, no mathematical *model* is able to incorporate all the diversity of biological processes and spatial heterogeneity. Furthermore, biological data is usually noisy and sparse, and thus detailed mechanistic models are often not feasible. Thus, a coarse-grained modeling approach is often necessary. A recent critical review[15] discusses in detail and provides references to the basis of using Boolean models in biology and also discusses some of the dynamics that cannot be captured by Boolean Networks.

2. Modeling frameworks for Signal Transduction Networks: an overview of their respective literature

We will first introduce the modeling frameworks considered in this work and the mathematical formalisms necessary. Definitions and details can be found in the supplementary information.

Graphs. Graph theory has been widely used in systems biology[16;17;18]. Graphs are used to represent the elements and interactions in different complex biological systems. For intracellular signaling networks, its associated graph is commonly a signed directed graph hereon deemed *interaction graphs*, as in Klamt et al.[19] . We note that the nomenclature *interaction graph*, is non-standardized. In the context of Boolean Networks, Klarner and Siebert[20] use the nomenclature *interaction graph* for an unsigned directed graph. In interaction graphs, the nodes represent biological constituents (*e.g.* enzymes, metabolites, transcription factors), and edges represent interactions between the nodes. The signed edge stands for the type of interaction, *i.e.* activating (+) or inhibitory (−). As previously remarked[19], it might not be possible to determine a direction of influence, or it might not be desirable to establish a direction of influence. Such interactions are best treated by different frameworks, or with a bidirectional edge[19]. If the interaction type is unknown, sometimes a label of 0 is used[6], although this is not a standard signed directed graph representation.

Scott et al.[21] use weighted graphs as a framework to represent protein interaction networks, where the weights stand for the reliability of the prediction of interaction of two proteins. This methodology is used to find signaling pathways in yeast protein interaction networks. Another graphical approach to represent signaling networks is to use bipartite graphs, such as *pathway graphs*[22]. A pathway graph is a bipartite graph $G = (M, I, E)$ where M stands for the set of nodes of the network, I stands for the set of interaction nodes, and E is the set of edges connecting the nodes from M to nodes from I and vice versa. This methodology was used to model the epidermal growth factor receptor signaling network. The authors developed this methodology to analyze signaling networks where only the existence of reactions and identities of products and reactants are known, *i.e.* biochemical networks.

Hypergraphs. Hypergraphs are generalizations of graphs, where the edges can connect more than two nodes. Analogously, we have directed hypergraphs.

Hypergraphs remain underutilized in computational biology[23]. They are, a canonical topological representation of biochemical reaction networks[12;13;24]. If for each hyperedge e, the tail and the head are disjoint, the incidence matrix stores the information of whether node i participates in reaction j as a product or a reactant. Thus, the incidence matrix of a hypergraph reveals the underlying structure of a metabolic network without necessarily taking into account mass-conservation. Transcription regulatory networks have been modeled using a matrix formalism[25], which can be seen as the incidence matrix of a hypergraph . When more information is available in the case of

signaling networks, the stoichiometric coefficients can be adjusted to have arbitrary positive integers, such as in a model of the JAK-STAT signaling network[26]. In Klamt et al.; Saez-Rodriguez et al.[19;27] Boolean Networks are represented as logical hypergraphs. Logical hypergraphs are B-hypergraphs where the tails of the hyperedges have a sign associated to them. However, there are hypergraphs that do not correspond to Boolean Networks where the functions are written in minimal Disjunctive normal Form. For example, the hyperedge in Figure 8 would correspond to a Boolean function $f_{x_2} = x_1 \wedge \neg x_1 = 0$.

A *signaling hypergraph*[28;29] is a triplet $(V, \mathscr{V}, \mathscr{E})$, where $\mathscr{E}$ is a set of directed hyperedges e connecting hypernodes, subsets of elements that act as a single unit, *e.g.* a protein-complex. A model of the network regulating the release of β-catenin following Wnt signaling using this framework is found in Ritz et al.[29] .

Boolean Networks. Boolean Networks in biology are usually attributed to the work of Kauffman[30] and Thomas[31].

Definition 2.1. *Consider a system X of n species (e.g. genes, proteins) to which we assign the variables $x_1, \ldots, x_n$. Each of the variables takes a value in the set $\{0, 1\}$. A* Boolean Network *is a pair (k, F) where $k = \{0, 1\}$ and $F = (f_1, \ldots, f_n) : k^n \longrightarrow k^n$, where each* local update function *$f_i : k^n \to k$ is a Boolean function in n variables.*

Every Boolean Network has an underlying directed graph, namely, its static graph or wiring diagram $\mathscr{W}$[32] where each species x_i corresponds to a node x_i, and an edge connects x_i to x_j if f_{x_j} depends on x_i with a sign corresponding to whether the effect of x_i on x_j is activating or inhibitory. In other words, the edge from x_i to x_j is positive if

$$f_{x_j}(x_2, \cdots, x_{i-1}, 0, x_{i+1}, \cdots, x_n) \leq f_{x_j}(x_2, \cdots, x_{i-1}, 1, x_{i+1}, \cdots, x_n)$$

and negative if the inequality is reversed[33]. In some cases, the resulting wiring diagram $\mathscr{W}$ will have edges where the sign of the edge is not well defined. For example, consider the Boolean function $f_{x_3} = (x_1 \wedge \neg x_2) \vee (\neg x_1 \wedge x_2)$. Then x_1 and x_2 have both an activating and inhibiting effect on x_3. In this case, the edge from x_i to x_3 can be replaced by two arrows from x_i to x_3 with a positive and negative sign for $i = 1, 2$. Alternatively, a single arrow from x_i to x_3 can be used with a zero label albeit the wiring diagram would not be a signed directed graph in the standard sense.

The dynamics are determined by its local updating functions and update schedule strategy. The synchronous updating is where each node updates its value simultaneously. In the asynchronous timing schedule[34;35], the nodes update their values at different times, either deterministically or stochastically. From the respective update schedule strategy, the *state transition graph* of a Boolean Network can be derived, a directed graph $G = (V, E)$ where the nodes of the graph correspond to elements in $\{0, 1\}^n$ and the edges are determined by both the update scheduling strategy and the update functions of the Boolean Network.

Different update strategies yield different dynamics, with subtleties: steady-state attractors are the same both for synchronous and asynchronous updates of a Boolean Network. Stable motifs[36] and trap spaces[37;38], that is, trap sets with particularly simple dynamics, are also independent of the particular updating schedule strategy.

Boolean Networks have been widely used to analyze signaling networks. Saez-Rodriguez et al.[27] constructed a 94-node and 123-interaction Boolean Network model of T-cell activation. Mai and Liu[39] introduced a Boolean model to analyze pro-apoptotic pathways. A signaling pathway modeling the regulation of epithelial to mesenchymal transition (EMT) in primary liver cancer can be found in Steinway et al.[40] . Zhang et al.[41] provide a Boolean model for survival signaling in large granular lymphocyte leukemia. Calzone et al.[42] constructed a 25 node Boolean Network incorporating the relationships between the NF-$\kappa\beta$ pathway, a simplified apoptosis signaling pathway and a necrosis signaling network, regulating how the cell "chooses" its fate.

Several Boolean models of diverse systems, including intracellular signaling networks, are stored in repositories such as Cell Collective[43] and BioModels[44].

Petri Nets. We briefly introduce Petri Nets and refer the reader to[45] for details or to the supplementary information.

Definition 2.2. *Petri Nets are bipartite directed multigraphs, consisting of two types of nodes,* places *$P = \{p_1, \cdots, p_m\}$ and* transitions *$T = \{t_1, \cdots, t_n\}$, and a set E of directed arcs weighted by natural numbers connecting only nodes of different types.*

A place $p \in P$ in a Petri Net may carry any non-negative number of tokens $m(p)$, called its marking.

The dynamics of a Petri Net is given by its marking, and fireable transitions. Read-arcs are bidirectional edges representing requirement of the presence of markings but do not consume tokens. The incidence matrix of a Petri Net corresponds to the topology of the network. Similar to self-loops in graphs and hypergraphs, the incidence matrix of a Petri Net does not represent read-arcs.

Chaouiya et al.; Steggles et al.[9;10] describe translations from Boolean Networks to Petri Nets. In particular, Petri Nets are able to display the dynamic properties of Boolean Networks, both under the synchronous and asyncrhronous updating strategies. Thus Petri Nets generalize Boolean Networks. Due to accumulation of the tokens, and consumption/production of tokens in the firing of transitions, Petri Nets can naturally describe types of biological processes such as biological consumption/reaction and inhibitions.

Introduced by Carl Petri[46], and in biology to analyze metabolic networks[47;48], Petri Nets have been used to model diverse systems. A model of the pheromone response pathway in *Saccaromyces cerevisiae* appears in Sackmann et al.[45] . A model for the tumor necrosis factor receptor 1-mediated NF-κB regulated signaling pathway appears in Amstein et al.[49] . Li et al.[50] translate molecular interactions to Petri Net components, and used it to model an apoptosis network. Li et al.[51] model a Petri Net as coupled "signal transduction components," a set of substances that make an enzyme active. Coloured Petri Nets, where tokens are allowed to have different data types, are used by Zevedei-Oancea and Schuster[6] to model signaling networks with only activations and reactions. The *Signaling Petri Net*[52], a synchronized Petri Net with an event generator, appeared in[53] to model the response of Langerhans cells to interferon regulatory factors. For a general overview of Petri Nets in biology, see Chaouiya; Koch and Chaouiya[54;55] .

3. Capturing signal pathways in the different modeling frameworks

Motivating question: Given a set of source nodes $\mathscr{X}$ and a set of target nodes $\mathscr{T}$ in a signaling network, what nodes and edges are involved in transducing a signal from $\mathscr{X}$ to $\mathscr{T}$?

We seek to review the different forms of answering this question using the aforementioned formalisms.

In graphs. In graph models of signaling networks, signaling pathways are often represented as simple paths or shortest paths. For example, Klamt et al.[19] compute feedback loops and simple paths from input nodes to output nodes from an interaction graph. Feedback loops in the representations of signaling networks are connected with the dynamics of the biological network[56;57]. Paths and cycles are given an activating or inhibiting measure based on the parity of the sign. Klamt et al.[19] classify nodes as activators, inhibitors or ambivalent. Minimal path sets (MPS)[58] comprises of the set of all paths from input layer to target layer and feedback. *Sigflux*[58], a measure of importance of nodes in the network based on the amount of feedback loops and paths they are part of, uses the concept of MPSs.

Lee and Cho[59] proposed an algorithm for estimating how a signal propagates through a network purely based on an interaction graph. The algorithm predicts the direction of activity of nodes change in the network (down-regulated vs up-regulated) given an input.

Zevedei-Oancea and Schuster[6] compute rooted trees to compute all the nodes influenced by an input node. Similarly, reversing the directionality of the arrows, by computing rooted trees one might find the nodes which affect output nodes. Scott et al.[21] propose computing two-terminal series-parallel graphs as a way to capture parallel signaling pathways. Nassiri et al.[60] weight the edges of the graph using the normalized similarity index[61]. Paths from input node to target nodes are weighted via a formula incorporating both node weights and edge weights, and the path with the highest weight is a likely candidate for a path from input to target node dominating the signaling process.

In hypergraphs. The concept of B-connection[29] describes the notion that all reactants must be present for a signaling reaction to occur. There is another concept also usually referred to as B-hyperpaths in the literature[64], although these two definitions are not equivalent, even in the case of B-hypergraphs[65]. Extreme pathways were computed on the JAK-STAT signaling network[26]. Extreme pathways are special cases of elementary modes[66]. A similar definition to that of a B-hyperpath can be found in methodology for metabolic networks, the concept of a topological factory (see S.I.) which we adapt here since we will compare this to an object proposed in Boolean Networks.

In the case of a metabolic network modeled as a hypergraph with stoichiometry matrix S, if $v \in \mathbb{R}^{|\mathscr{E}|}$ denotes the flux of every reaction in the network (or in the case of quasi-stoichiometry, the amount of times a hyperedge is used), then Sv specifies the net production, or net change occurring. The notation $(Sv)_A$ denotes the entries of Sv corresponding to $A \subseteq \mathscr{V}$. Notice that this is equivalent to how the net change in the marking in Petri Nets is computed. The analogous concept of elementary modes is the concept of a stoichiometric factory under the steady-state assumption (See the SI). In B-hypergraph, stoichiometric factories are unions of minimal topological factories [12].

In Boolean Networks. We now mention methodologies for analyzing signal transduction networks modeled as Boolean Networks. For a Boolean Network, we can apply techniques of analyzing signal transduction capabilities in directed graphs via its wiring diagram for computing signaling pathways. One advantage of Boolean Networks is the ability to exhibit dynamical information of the system in addition to their structural information.

Stable motifs [36;79] are a bridge between the structure and the dynamics of a Boolean Network. In particular, stable motifs are independent of the update scheduling strategy. Stable motifs are closely related to the concepts of trap spaces [38]. Using the MAPK pathway modeling cell fate decisions [80], the minimal trap spaces are computed and used to give a lower bound on the number of cyclic attractors [38]. To compute trap spaces, a graph expansion method is used; the prime implicant graph which is a *unique* hypergraph expansion of a Boolean Network not depending on any particular normal form. Maheshwari and Albert [81] label the edges of the interaction graphs with causal logic, *e.g.* if $f_{x_3} = x_1 \vee x_2$, then the arrows from x_1 to x_3 and from x_2 to x_3 are labelled as sufficient arrows (permanent activation of either one of them suffices to activate x_3 regardless of the rest of the network.) These causal edges can sometimes be chained together to give information on the causal relationships of two nodes in the network that are far apart, and can be used to compute the logic backbone of the network, a vast simplification of the Boolean structure of the network. Furthermore, it can also be used to compute some stable motifs and for network reduction purposes.

An accepted technique to analyze Boolean Networks is based on the idea that long term behavior of networks is captured by their attractors [68], often associated with cellular phenotypes and cellular responses [69;35;70]; some of the attractors in Fumia and Martins [71] are associated to a proliferative phenotype in cancer cells. Related to attractors, is the computations of the basin of attractors; the initial states that lead to an attractor. For attractor analysis, reduction techniques have been proposed in the literature; for example, using the concept of *Stable Motifs* [36], and via the polynomial dynamical system representation [72;73]. It is generally impossible however to determine the complete dynamical evolution of a Boolean Network from its structure alone [74;75]. For a survey of results connecting the dynamics and structure see Paulevé and Richard [76] . Functional cycles, *i.e.* cycles that generate attractors, in the interaction graph of a Boolean Network are connected to the long term behavior of a Boolean Network [67].

An *Elementary Signaling Mode* (ESM) [14], based on structural analysis of a Boolean Network, is a minimal set of elements of the network that can perform signal transduction from initial node to nodes in the target layer. The network is expanded by introducing complementary nodes for nodes inhibited by other nodes or are inhibiting other nodes. Wang *et al.* [14] introduce a "composite" node to represent conditionally dependent relationships. See *e.g.* Figure 2, Wang and Albert [14] for details.

The expansion of a network provides a useful compromise between the wiring diagram (structure) and the full representation of a signaling network and rids the wiring diagram of some ambiguities. In fact, if the complete network expansion where both composite nodes and complementary nodes are added for every node in the network are used [34], the update rules can be read directly from the expanded network. For computations of elementary signaling modes, different signaling network expansions have been used such as expansions where composite nodes are added for logical dependencies [34], and complementary nodes are added for every node in the network. In contrast, other expansions only add composite nodes [77] to represent synergy. Therefore, one needs to first provide a mathematical definition of ESMs to make it computationally amenable in systems biology software. Wang et al. [78] provide a similar concept for graphs with dependent edges namely, the concept of a minimal functional route (MFR). Dependent edges represent necessary and sufficient conditions for signal transduction from a set of nodes $x_1, \cdots, x_j$ to a node y. Again the network is expanded to a new network $\hat{G}$ by adding composite nodes to represent dependent edges (see Figure 3 and refer to Wang et al. [78] for details). In the special case of a graph with no

dependent edges, computations of MFRs from s to t is the same as computing simple paths from s to t.

Computing simple paths or shortest paths from source node to target node might miss key information from the signal transduction process. For example, consider the graph on the left in Figure 7. The only shortest path from s to t is the path $s, r, 2, 3$, where r is the red node. Therefore, computing simple paths would miss the fact that node 1 is necessary for a complete signal transduction process from s to t. In general, there is no relationship between the number of minimal functional routes and the number of simple (or shortest) paths from a source node s to a target node t (Figure 4.)

In Petri Nets. Similar to Boolean Networks, Petri Nets allow modeling the dynamics of a signaling network. To analyze the long term behavior of a Petri Net, one is often interested in the behavioral properties of a Petri Net, such as computing the coverability graph of the Petri Net, the boundedness of the Petri Net and whether or not the net has dead markings[55]. Unfortunately, similarly to analyzing the state graph of a Boolean Network, many of these questions are computationally limiting, and thus one often resorts to structural analysis methods.

In the framework of Petri Nets, the commonly used methodology for capturing signaling pathways is T-invariants. In the case that there is a sequence of transitions realizing a vector $\mathbf{y}$, a T-invariant $\mathbf{y}$ corresponds to a sequence of transitions that does not change the given marking[45]. In the framework of metabolic networks, minimal T-invariants are counterparts to elementary flux modes[82;83], although elementary flux modes are more general due to the fact that reactions are allowed to be reversible. A place invariant is the counterpart of moiety conservation[83]. As previously mentioned, read-arcs are not reflected in the incidence matrix. Therefore, T-invariant analysis without taking into account this extra structure could yield T-invariants that are meaningless. To take this into consideration Sackmann et al.[45] introduce the concept of a feasible T-invariant to be a minimal set of transitions that can fire in sequence under the initial minimal marking, without changing the marking and discuss how to handle read-arcs for T-invariant analysis, such as considering read-arcs as unidirectional arrows or bridging T-invariants. The idea is that feasible T-invariants stand for minimal sub-entities of the Net relevant to capture signal transduction. The concept of feasible T-invariants is further developed to Manatee invariants[49], a minimal linear combination of T-invariants whose induced network is feasible under the initial marking.

4. Comparing different formalisms: A perspective

In this section we discuss some translations between the formalisms found in the literature. We also discuss what we see, from our perspective, as some of the advantages and disadvantages of the different formalisms.

An interaction graph is the easiest to construct, both from literature and data, and are easily interpretable. Signed directed graphs have been heavily studied by researchers above a broad spectrum of fields and efficient tools and algorithms have been developed to detect the signaling pathways in interactions graphs, such as shortest and simple paths, feed-back loops and cycles. Once more information becomes available, either from the literature, or from experimental work, interaction graphs can be extended into a different framework. Hypergraphs allow to express cooperativity of components and allow a useful representation of Boolean Networks in the form of a logical hypergraph[19].

Boolean Networks and Petri Nets each offer a dynamical model of a signaling network. Both Boolean Networks and Petri Nets have a large community of researchers in the biological sciences. Interestingly, every Boolean Network can be seen as a Petri Net[9;10;84]. Chaouiya et al.[9] gives a translation from the Boolean framework to a 1-safe standard Petri Net (the multistate case can be found in Chaouiya et al.[85]). Assuming one starts with a valid marking (the sum of the tokens between a gene and its complement is 1), the reachability graph of the corresponding Petri Net is equivalent to the fully asynchronous updating state graph of the corresponding Boolean Network. In particular, notice that given a valid marking, a realizable T-invariant is a multiset of transitions that does not change the given marking, and since the reachability graph and state graph of the Boolean regulatory network are equivalent, the counterparts of cycles in the state transition graph of a Boolean model are T-invariants. Stegless *et al.*[10;84] provided a translation from Boolean Networks to Petri Nets focusing on gene regulatory networks for the synchronous updating timing schedule. A related approach can be found in Sackmann et al.[45] , where insight on how to translate logical rules into the Petri Net framework is given. As previously remarked[55],

Petri Nets are useful for representing consumption and production mechanisms, whereas Boolean Networks are more appropriate to model regulatory interactions (a regulator can alter the state of a target, whereas the state of the regulator does not change itself) [55].

The graph expansion method of Wang and Albert [14] gives a hypergraph structure which can easily be translated into a Petri Net. However, the dynamics of the Boolean Network are not preserved under this translation. This expansion method is closely related to the translation from Chaouiya et al. [9], although the latter heavily relies on either read-arcs or inhibitory arcs to preserve the dynamics. Similarly, the unique prime implicant graph of a Boolean Network is a B-hypergraph [37;38], and can easily be translated into a Petri Net structure or into a graph with composite nodes. It should be further investigated how this translation preserves the dynamics.

From a modeling perspective, Boolean Networks are easier to set up than Petri Nets. Several tools exist to both model and analyze Boolean Networks [86;87;88;90;89;91;92;93].

Petri Nets are the most general, although they have a smaller track-record of being used in the modeling of intracellular signaling networks. However, Petri Nets allow the most flexibility in terms of the processes being modeled: they are natural representations of reaction networks, and due to read-arcs they allow flexibility in the signaling processes they can model. Interestingly, there are existing translations from 1-bounded Petri Nets to a family of Boolean Networks [11]. Under the given translation, the authors show that dead markings correspond to a solution of a system of polynomial equations over finite fields. Furthermore, recovering P-invariants and T-invariants of the original Petri Net is straightforward. For every place-transition Petri Net, there is an associated bipartite graph structure, and in particular, a hypergraph structure. In particular, we may apply the concepts for analyzing hypergraphs, such as hyperpaths and topological factories, to Petri Nets.

Well-known reversible construction to create bipartite graphs from hypergraphs [23] exist. Naturally, every graph can be considered as a special case of a hypergraph. The signaling hypergraphs can be converted to standard graphs in two different ways [29]. To show how closely related the methodologies across different frameworks are, we show some new results.

Some new results. We show that minimal functional routes (MFRs), and thus elementary signaling modes (ESMs), are special cases of topological factories previously described [12;13] once graphs with composite nodes are translated into a hypergraph structure. In particular, the concept of topological factories extends the concept of MFRs.

Let G be a graph with composite nodes [78] and no self-loops and with the added property that if (x, c_1) is an edge where c_1 is a composite node, then there is no edge (c_1, x). Such graphs can be attained from the wiring diagram with no self-loops where we know which edges are dependent via the Boolean function of the nodes [14;34;77]. Furthermore such graphs can easily be converted to a B-hypergraph by collapsing incoming edges into a composite node c_1 into the tail of a hyperedge (see Figure 5). Due to the assumptions, we have a hypergraph with the property that for every edge e in the B-hypergraph, $H(e) \cap T(e) = \emptyset$. In the S.I., we include a proof that the set of MFRs from s to a sink node t is contained in the set of minimal topological factories from s to t. Furthermore, if the expanded graph is an acyclic connected graph with a single source and a single target node, then computing the set of MFRs is the same as computing the minimal topological factories (proofs are in the S.I.) In expanded graphs with cycles, there are minimal topological factories from s to t that are not MFRs (see Figure 6).

Given a directed graph G, node s and node t and an incidence matrix A, there is a close relationship between simple paths from s to t and elementary modes of a slightly adjusted incidence matrix [19]. It is natural to wonder if for a given directed graph with dependent edges, the analogous process as in Klamt et al. [19] can be used to compute MFRs via the incidence matrix of its respective hypergraph. In the supplementary information we show that in some hypergraphs with cycles (see *e.g.* Figure 7), there are minimal functional routes that do not correspond to elementary flux modes of the adjusted incidence matrix following the method in Klamt et al. [19]. In fact, stoichiometric factories also fail to compute minimal functional routes (Figure 7).

We remark that this is not surprising. Computations of elementary flux modes is based on a steady state assumption, that "concentrations" of internal nodes do not change. This is the same assumption for the computation of T-invariants in a Petri Net, where a T-invariant accounts for a preservation of tokens. However, conservation laws for signaling networks are difficult to define due to signal amplification motifs.

5. Conclusion

Several different methodologies exist for modeling signaling networks. The reality is that based on the particular components of the signaling network to be studied and the data available, the formalism chosen should be carefully considered, and sometimes hybrid approaches are needed [94]. Particularly useful methodologies, when we don't have enough detailed mechanistic parameters such as kinetic rates of change are graphs, Boolean Networks, hypergraphs and Petri Nets. We have discussed some of the advantages and disadvantages of the different frameworks. Many of the different methodologies for studying signaling networks, *e.g.* elementary modes, minimal T-invariants, minimal P-invariants, etc. were introduced for studying reaction networks with a steady state assumption. However, they have successfully been used to capture signaling pathways in mathematical models of signaling networks. The discussion found in Behre and Schuster [95] for applying elementary flux mode analysis to enzyme cascades show a class of signaling networks where the methodology of T-invariant analysis is directly applicable and not just a formal approach.

We discussed the different methodologies used to estimate how signals are transduced from input layer to target layer. We provided toy examples showing, that although similar, these different methodologies can be strikingly different. Therefore, it is necessary to not only consider the modelling framework, but the appropriate formalism capturing signal transduction. Using only one formalism misses the diversity of strategies the cell uses to transduce a signal.

Some work has been done showing the use of relating the frameworks to each other, which we have discussed in this review [9;11;10;82]. We also related the topological factories to minimal functional routes. It seems reasonable to adopt the concept of topological factories to signaling networks, generalizing the concept of a minimal functional route by allowing nodes to be internally activated, rather than being forced to be activated from an external source. The use of topological factories in signaling networks should be carefully assessed in future work. Relating frameworks with each other opens up the tool-box to analyze how a signal transduces within a cell. Furthermore, understanding how the different methodologies relate to each other will lead to a better understanding of what actually happens *in vivo* inside of a cell.

In graphs with dependent edges, topological factories generalize minimal functional routes. Categorizing graphs with dependent edges such that the concept of minimal functional routes and minimal topological factories are equivalent is of interest. Furthermore, due to the connection between elementary modes with the steady state assumption using the incidence matrix of a graph and T-factories in graphs with no dependent edges [19], it would be interesting to categorize graphs where the minimal S-factories and T-factories are the same.

Abbreviations

MPS: Minimal Path Set. EMT: Epithelial to Mesenchymal transition. MFR: Minimal Functional Route. ESM: Elementary Signaling Mode. S-factory: Stoichiometric factory. T-factory: Topological factory. SF (TF): Stoichiometric (Topological) factory. MSF (MTF): Minimal SF (TF). T-invariant: Transition invariant. P-invariant: Place invariant. scc: Strongly Connected Component.

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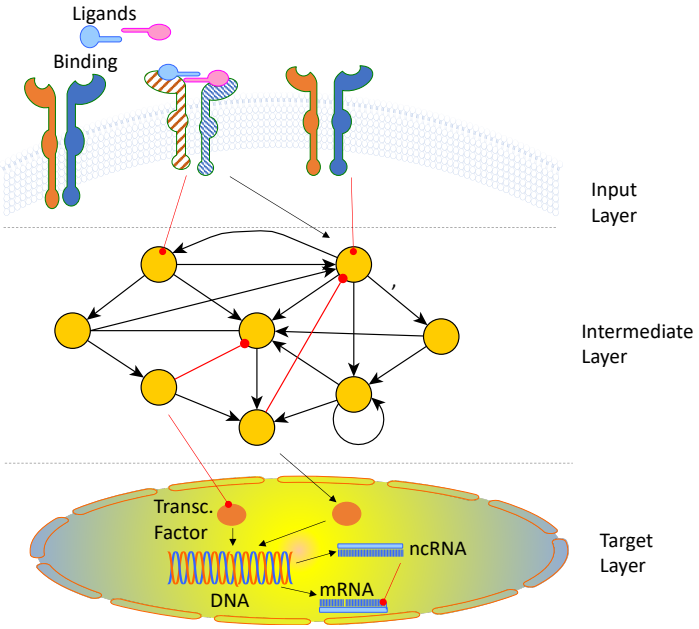

FIGURE 1. A prototypical signaling network. After a ligand binds to a receptor, the signal transduction cascade starts. The red arrows denote inhibitory processes. The intermediate layer activates a transcription factor which bind to a portion of the DNA and transcribes messenger RNA (mRNA) and non-coding RNA (ncRNA). ncRNA degrades mRNA which ultimately gets translated to a protein.

$$x_1 = \neg\, x_3$$
$$x_2 = x_1 \wedge \neg\, x_3$$
$$x_3 = x_2$$

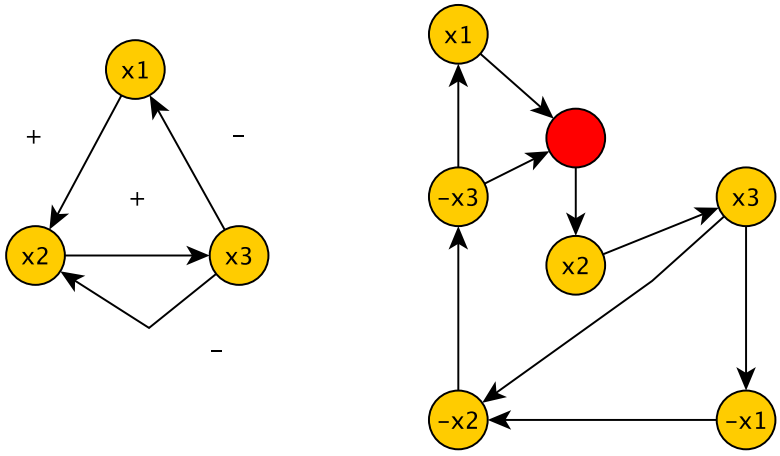

FIGURE 2. The interaction graph of the Boolean Network is shown on the left. To expand the network, we add a complimentary node for each node of the network representing the absence or inactivity of a network component. These nodes are represented by $-x_i$. The update functions for these nodes are the logical negations of the update rules of x_i. For example, the update rule for $-x_2$ is now $-x_2 = \neg x_1 \vee x_3$.

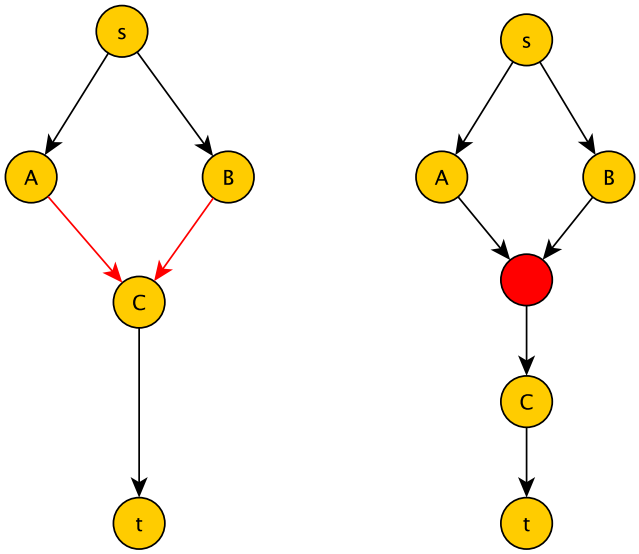

FIGURE 3. The edges in red are dependent edges. This represents that in order for node C to receive the signal, the signal from both A and B are necessary. On the right is the expanded graph, where a composite node is shown in red. In order for a signal to be transduced to C, both upstream nodes from the composite nodes must be included.

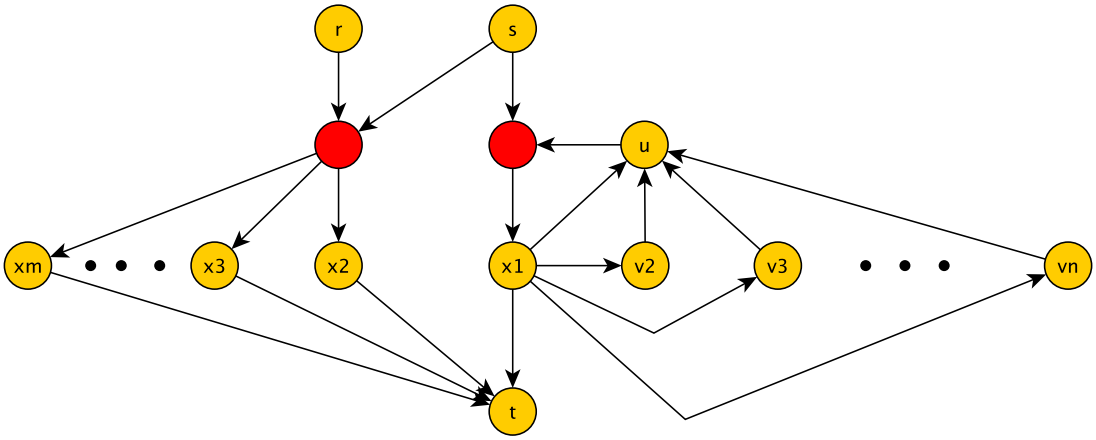

FIGURE 4. The composite nodes are shown in red. There are m simple (or shortest) paths from s to t and n minimal functional routes from s to t

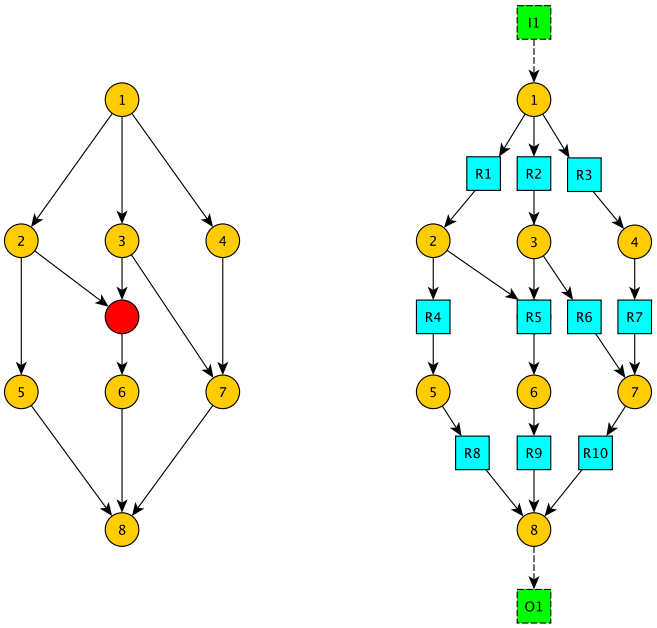

FIGURE 5. B-hypergraph from expanded graph. Adapted from[78] Figure 1(b). On the left, an expanded AND-OR graph. The composite node is shown in red, original nodes are shown in yellow. On the right, the corresponding hypergraph. The input node 1 has been connected to an environment transition I_1 and the target node has been connected to an environment transition O_1. for computations of elementary flux modes.

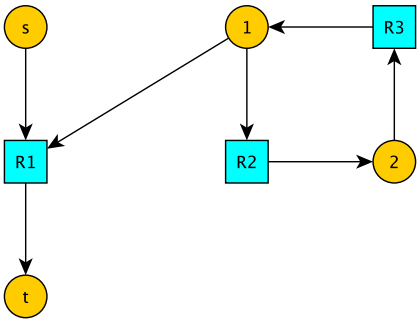

FIGURE 6. Not all topological factories are minimal functional routes from s to t. There is no MFR from s to t since transduction of a signal from s to t would require including node 1. However, there is no simple path from s to node 1. R_1, R_2, R_3 is a topological factory from s to t. In the case the graph is acyclic, MFRs from s to t are the same as the topological factories from s to t.

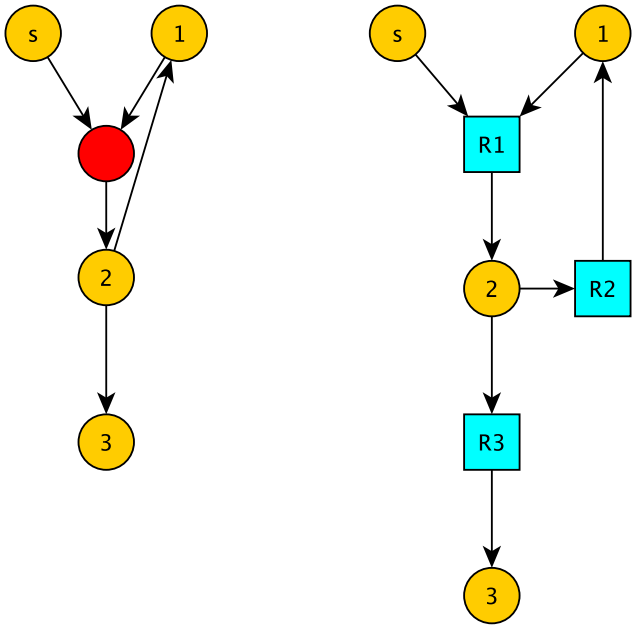

FIGURE 7. A minimal functional route (and thus topological factory) that is not an S-factory. Using the incidence matrix as the stoichiometric matrix for this hypergraph, there is no S-factory from s to t. However, it is a minimal functional route from s to 3.

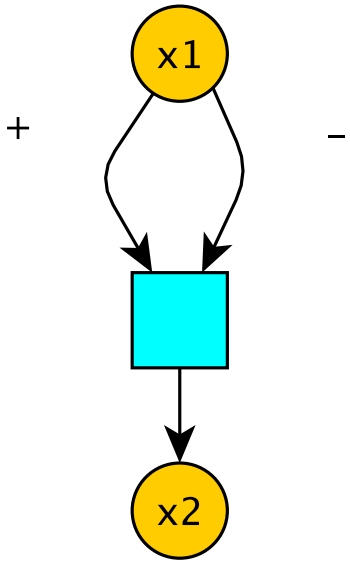

FIGURE 8. A hyperedge that does not correspond to a logical Boolean function.