COMPUTING SIGNAL TRANSDUCTION IN SIGNALING NETWORKS MODELED AS BOOLEAN NETWORKS, PETRI NETS AND HYPERGRAPHS

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Abstract. **Background** Different objects exist to describe how a signal transduces in a given intracellular signaling network, such as elementary signaling modes, T-invariants, extreme pathway analysis, elementary modes and simple paths. For modeling frameworks such as Boolean networks, Petri nets and hypergraphs, these signal transduction objects are broadly used in their respective frameworks but few studies have been done emphasizing how these signal transduction objects compare or relate to each other.

Results We provide an overview of the different methodologies for capturing signal transduction in a given model of an intracellular signaling network. 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. Furthermore, we show that computing elementary modes based on the incidence matrix of a B-hypergraph fails to capture minimal functional routes, whereas in directed graphs, it has been shown that these computations of elementary modes correspond to computations of simple paths.

Conclusions The different objects introduced in the literature to capture signal transduction are deeply related to each other, although they are based on different biological assumptions. Furthermore, methodology in metabolic networks and signaling networks are deeply related to each other.

Background

Cells must be able to respond appropriately to cues from their surrounding environment, for example by proliferating, dividing, differentiating, changing their transcriptome, or even undergoing programmed cell death. The signal transduction object in the form of cellular components 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. It is not feasible to get a complete understanding of how a cell responds to signals simply by listing the components of the pathway and thus a systems level approach must be taken. The complexity innate to these networks, both from size and connectivity, makes mathematical modeling and computational analysis a requirement to truly understand how the cell communicates with its environment [Laz04, Gar17, KL06, WF12]. It is well documented that malfunctions in signaling pathways from both epigenetic and genetic aberrations lead to cancer progression and other pathologies. For reviews of a class of signaling pathways and their relation to pathologies see *e.g.* [KC10a, KHGK15]. Moreover, mutations in components of signaling networks have also been linked to drug resistance. For example, mutations in the epidermal growth factor receptor have been associated with drug resistance in non small-cell-lung carcinoma [SGY05]. Therefore, understanding signaling networks 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 [ZOS05]. In mathematical graph models, nodes in the input layer typically have a small in-degree, and nodes in the target layer have a small out-degree. 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 can be thought as the conduits of the signal, such as second messengers and enzymes. See Figure 1 for a prototypical signaling network.

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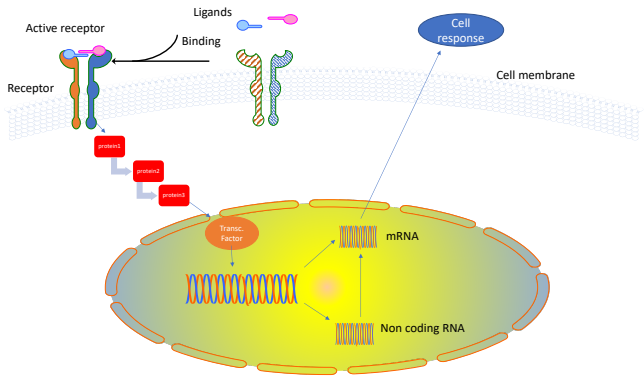

FIGURE 1. A prototypical signaling network.After a ligand binds to a receptor, the signaling process is initialized. The signal is relayed from receptor to proteins in the cytoplasm, which again relay the signal downstream. After activation of a transcription factor, transcription begins. The cell responds to the signal by producing a protein.

Several kinetic parameters, such as rates of change of biochemical reactions, required for detailed mechanistic mathematical models such as systems of ordinary differential equations, still remain difficult to obtain *in vivo* and *in vitro*. Furthermore, certain signaling components, such as GTP-binding proteins, act as molecular switches, having an "active" or "inactive" status rather than a continuum of states. 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 in [SK13]. The detailed review of [KYK12] discusses other methodologies for settings in which more kinetic information is available for modelling purposes.

In this manuscript, we provide an overview of some commonly used modelling objects and frameworks for intracellular signaling networks: graphs, hypergraphs, Boolean networks, and Petri nets. More importantly, we discuss the objects of importance that modelers using these frameworks have proposed to capture how an intracellular signal transduces or diffuses within a cell. We further adopt the definitions of a topological factory and S-factories from metabolic network analysis tools [AWK+16, AMC+12] and compare these to the elementary signaling modes methodology found in [WA11].

METHODS

We will first introduce the modeling frameworks considered in this work and the mathematical formalism necessary for the progression of the paper.

Modeling Frameworks of Interest for Signal Transduction Networks.

Graphs. Let G be a directed graph $G = (V, E)$, $V = \{v_1, \cdots, v_m\}$, $E = \{e_1, \cdots, e_n\}$ with m nodes and n edges. A node s is a *source* node if it has no incoming edges, that is, there is no node $w \in V$ such that the edge $(w, s) \in E$. A node t is a *sink* node if it has no outgoing edges, that is, there is no node $w \in V$ such that the edge $(t, w) \in E$. A *path* P_{1q} from node v_1 to node v_q is a sequence of edges $P_{1q} = ((v_1, v_2), (v_2, v_3), \cdots, (v_{q-1}, v_q))$. If all the nodes $v_1, \cdots, v_q$ are distinct, we say P_{1q} is a *simple path*. If all the nodes are distinct, except that $v_1 = v_q$, we say P_{1q} is a *directed cycle* or a *feedback loop*. Given an edge in a directed graph $e = (v_1, v_2)$, $T(e) = v_1$ and $H(e) = v_2$ are the *tail* and the *head* of e, respectively. If $T(e) = H(e)$, we will say e is a *self-loop*.

The *incidence matrix* of a directed graph $G = (V, E)$ is an m-by-n matrix $(a_{ij})_{i,j=1}^{i=m,j=n} \in \mathbb{R}^{m\times n}$

$$a_{ij} = \begin{cases} -1 & \text{if } \{v_i\} = T(e_j) - H(e_j), \\ 1 & \text{if } \{v_i\} = H(e_j) - T(e_j), \\ 0 & \text{otherwise.} \end{cases}$$

Notice that the incidence matrix does not represent self-loops.

Graph theory has been widely used in systems biology, both for modelling and analysis of biological systems [PSM+11, TAÜ09, AS06]. Its main object of study, graphs (networks) are used to represent the elements and interactions in different systems. For intracellular signaling networks, its

typical associated interaction graph is a signed directed graph where the nodes represent biological constituents (*e.g.* enzymes, metabolites, transcription factors), and edges represent interactions between the nodes. The non protein-protein or complex formations interactions are signed, and the interaction sign stands for the type of interaction, whether it is known to be activating or inhibiting. As already pointed out in [KSRL+06], sometimes a direction between nodes cannot be readily identified, such as in protein-protein interactions. Such interactions are best treated by different frameworks, or with a bidirectional edge [KSRL+06]. If the interaction type is unknown a label of 0 is used [ZOS05].

Scott et al. [SIKS06] 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. Their methodology is used to find signaling pathways in yeast protein interaction networks.

Another graphical approach to represent signaling networks is to use bipartite graphs. That is, graphs with two distinct types of nodes and where no interactions occur between nodes of the same type. Such bipartite structures have been used to represent a *pathway graph*, introduced in [RNI+06]. A pathway graph is a bipartite graph $G = (M, I, E)$ where M stands for the set of nodes of the network and 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. We remark however, that there are types of interactions in signaling networks where the concept of "reactions," "products" and "reactants" is not completely clear, and thus this methodology should be used with care.

Hypergraphs. Hypergraphs are generalizations of graphs, where the edges can connect more than two nodes.

Definition 0.1. *(Directed Hypergraphs) A directed hypergraph is a pair $\mathscr{G} = (\mathscr{V}, \mathscr{E})$ where $\mathscr{V} = (v_1, \cdots, v_p)$ is a set of nodes and $\mathscr{E} = \{e_1, \cdots, e_q\}$ is a set of hyperedges. A hyperedge e_i is an ordered pair $e_i = (T(e_i), H(e_i))$ of nonempty subsets of $\mathscr{V}$.*

$H(e_i)$ is the head *of e_i and $T(e_i)$ is the* tail *of e_i.*

If $|H(e_i)| = 1$ for all i, then the directed hypergraph is a called a B-hypergraph. Notice that when $|H(e_j)| = |T(e_j)| = 1$ for $j = 1, ..., q$, the hypergraph is a standard directed graph (possibly with self loops).

We remark that one can find in the literature different definitions of directed hypergraphs. For example, [AL17] assumes hyperedges are of the form (S, v) where $v \in \mathscr{V}$ and S is a nonempty subset of $\mathscr{V}$. [GLPN93] assumes that the head and the tail of a hyperdge are disjoint. The assumption that the tail and the head of a hyperedge are disjoint makes real biological processes, such as autocatalysis and self-regulators, difficult to model. We will use definition 0.1 from now on, and explicitly state other assumptions.

Definition 0.2. *Let $\mathscr{G} = (\mathscr{V}, \mathscr{E})$ be a directed hypergraph. A* simple path *from s to t is a sequence of different nodes $\mathscr{V}_0 = (u_0, \cdots, u_a)$ and a sequence of different hyperedges $E = (f_1, \cdots, f_a)$ satisfying (1)-(3):*

(1) $u_0 = s$, $u_a = t$.
(2) $u_i \in T(f_{i+1})$ *for* $i \in \{0, \cdots, a-1\}$.
(3) $u_i \in H(f_i)$, *for* $i \in \{1, \cdots, a\}$.

We will say $E = (f_1, \cdots, f_a)$ is a cycle *from s to t if $t \in T(f_1)$.*

If a hypergraph has no cycles, we will say the hypergraph is acyclic.

Given a directed hypergraph $\mathscr{G} = (\mathscr{V}, \mathscr{E})$ where $\mathscr{V} = (v_1, \cdots, v_p)$, $\mathscr{E} = \{e_1, \cdots, e_q\}$, the *incidence matrix* of $\mathscr{G}$ is a matrix whose rows correspond to the nodes and columns correspond to the hyperedges. Namely, $A = (a_{i,j})_{i,j=1}^{i=p,j=q}$ where

$$a_{ij} = \begin{cases} -1 & \text{if } v_i \in T(e_j) - H(e_j) \\ 1 & \text{if } v_i \in H(e_j) - T(e_j) \\ 0 & \text{otherwise} \end{cases}.$$

Notice that if one assumes that $T(e_j) \cap H(e_j) = \emptyset$ for all hyperedges, there is a one-to-one correspondence between directed hypergraphs and matrices with entries $\{0, 1, -1\}$.

A subset $\mathscr{F} \subseteq \mathscr{E}$ defines a *sub-hypergraph* $\mathscr{G}_{\mathscr{F}}$ in a canonical way: $\mathscr{G}_{\mathscr{F}} = (\mathscr{V}_{\mathscr{F}}, \mathscr{F})$ where $\mathscr{V}_{\mathscr{F}} = \bigcup_{f \in \mathscr{F}} (H(f) \bigcup T(f))$.

By the source layer, we mean the set of source nodes in a graph (or hypergraph). We will assume herein that our input layer is equal to the source layer.

For reasons that will be explained later, we need a process of converting a general directed hypergraph to a B-hypergraph, which has been called the many-to-one transformation.

Definition 0.3. *(Definition 4,*[AWK+16]*) Let $\mathscr{G} = (\mathscr{V}, \mathscr{E})$ be a directed hypergraph. The many-to-one transformation of $\mathscr{G}$ is the directed hypergraph $\Phi(\mathscr{G}) = (\mathscr{V}, \Phi(\mathscr{E}))$ such that for every $e \in \mathscr{E}$ and for every $a \in H(e)$, there is a hyperedge $e_a \in \Phi(\mathscr{E})$ such that $T(e_a) = T(e)$ and $H(e_a) = \{a\}$.*

Given $e \in \mathscr{E}$, let $\Phi(e)$ be the set of hyperedges in $\Phi(\mathscr{E})$ corresponding to the many-to-one transformation of e. If $\mathscr{F} \subseteq \mathscr{E}$, let $\Phi(\mathscr{F}) = \bigcup_{f \in \mathscr{F}} \Phi(f)$.

Notice that if $|H(e)| = 1$, then $\Phi(e) = e$.

In particular, we get the following observation.

Observation 0.1. *If $\mathscr{G}$ is a B-hypergraph, then $\Phi(\mathscr{G}) = \mathscr{G}$.*

Hypergraph representations offer ways of representing cellular networks, although they remain underutilized in computational biology [KHT09]. They are, for example, a canonical topological representation of biochemical reaction networks [AWK+16, AMC+12], where the hyperedges represent reactions, the nodes in the tail are the reactants of the reaction and the nodes in the head are the products of the reaction. In particular, in the case that $T(e) \cap H(e) = \emptyset$ for all hyperedges e, notice that the incidence matrix stores the information of whether node i participates in reaction j as a product or a reactant: if a_{ij} is positive (negative), it is a product (reactant) of reaction j. Thus, the incidence matrix of a hypergraph can be seen as an unparametrized stoichiometric matrix. Transcription regulatory networks have been modeled using a matrix formalism, which can be seen as the incidence matrix of a hypergraph [GPP+06]. This modeling framework was deemed a quasi-stoichiometric matrix formalism since the coefficients do not account for mass preservation. When more information is available in the case of signaling networks, the stoichiometric coefficients can be adjusted to have arbitrary positive integers. This was done, for example, in the case of the Jax-Stat signaling network [PP04] where they compute extreme pathways, which are special cases of elementary modes [PSP+04]. The approach found in [KSRL+06, SRSL+07] is to represent Boolean networks where the logical functions are written in disjunctive form as logical hypergraphs, B-hypergraphs where the tails of the hyperedges have a sign associated to them. We note that the representation of a Boolean network as a logical hypergraph requires the update functions to be written in disjunctive form.

[RTK+14, RAM15] introduce the concept of a *signaling hypergraph*. Namely, given a set of nodes V and subset $\mathscr{V}$ of its power set ($\mathscr{V} \subseteq 2^V$), a *hypernode* is an element $x \in \mathscr{V}$. A hypernode stands for subsets of elements that act as a single unit. A signaling hypergraph is a triple $(V, \mathscr{V}, \mathscr{E})$, where $\mathscr{E}$ is a set of directed hyperedges e connecting hypernodes. The authors model the release of β-catenin following Wnt signaling using this framework.

Petri Nets. We now introduce Petri nets. We frame our definition from [SHK06], wherein they also provide references for more formal definitions.

Definition 0.4. *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 dynamic behavior of a Petri net is given by its marking. Given a transition $t \in T$, let $\bullet t = \{p \in P | (p,t) \in E\}$ be the set of its *preplaces* and let $t\bullet = \{p \in P | (t,p) \in E\}$ be its set of *postplaces*. Let $w(p,t)$ denote the weight of the arc (p,t). If for all $p \in \bullet t$, we have $m(p) \geq w(p,t)$ then t is *enabled*, or we say t *can fire*. If t fires, then the marking of $p \in t \bullet \bigcup \bullet t$ is updated from $m(p)$ to $m(p) - w(p,t) + w(t,p)$ (here if $e \notin E$, then $w(e) = 0$). Let the incidence matrix S of a Petri net be the matrix $(s_{ij})_{i,j=1}^{i=m,j=n}$ where $s_{i,j} = w(t_j, p_i) - w(p_i, t_j)$, *i.e.* the change of the marking in place p_i given by the firing of transition t_j. Let $\mathbf{m}_0 \in \mathbb{N}^m$ be the vector where the i entry equals the number of tokens of place p_i at an initial time. Let $v_j \in \mathbb{N}^n$ be the jth coordinate vector. Then, if t_j is enabled and t_j fires, then the marking vector can be computed via $\mathbf{m}_1 = S \cdot v_j + \mathbf{m}_0$. Let $\mathbf{m}_0$ be a given initial marking. Let $\sigma = (t_{i_1}, t_{i_2}, \cdots, t_{i_k})$ be a sequence

of transitions that can fire in order from the marking $\mathbf{m}_0$. Let $\mathbf{v}_\sigma \in \mathbb{N}^n$ be the vector where the jth entry counts how many times transition t_j occurs in σ. Then the updated marking starting from $\mathbf{t_m}$ after firing $t_{i_1}, t_{i_2}, \cdots, t_{i_k}$ is computed by $\mathbf{m}_k = S \cdot \mathbf{v}_\sigma + \mathbf{m}_0$. We say a marking $\mathbf{m}_u$ is *reachable* from $\mathbf{m}_0$ if there exists a sequence $\sigma = (t_{i_1}, t_{i_2}, \cdots, t_{i_u})$ of transitions that can fire in the given order such that $\mathbf{m}_u = S\mathbf{v}_\sigma + \mathbf{m}_0$.

Petri nets were formally introduced by Carl Petri in his PhD dissertation [Car62] and were introduced in biology to analyze metabolic networks by Reddy *et al.* [RML+93, RLM96].

In the case of modeling a metabolic network with no read arcs [SHK06], the incidence matrix corresponds to the stoichometric matrix of the metabolic system [ZOS03].

Petri nets have been used to model the pheromone response pathway in *Saccaromyces cerevisiae* in [SHK06], where they also describe a way of modeling signaling networks by Petri nets from a logical description. A Petri net model for the TNFR1-mediated NF-κB regulated signaling pathway is provided in [AAS+17]. In [LGN+07], Li *et al.* provided insight into translating molecular interactions to Petri net components, and used it to model an apoptosis network. Starting from biochemical reaction descriptions, Li *et al.* [LSG+06] model a Petri net as coupled "signal transduction components," a set of substances that make an enzyme active. Their modeling strategy sets up a correspondence between signal transduction components and subnets containing T-invariants. Coloured Petri nets, where the tokens are allowed to have different data types, are used in [ZOS05] to model signalling networks where only activations and reactions are considered.

For distinct uses of Petri nets used in biological networks, see [Cha07]. For some extensions of Petri nets and software for their computations of Petri nets see [KC10b].

As remarked in [KC10b], Petri nets are useful for representing consumption and production mechanisms, whereas logical models (*e.g.* 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). One way to possibly handle the modeling of the variety of mechanisms used by the cell is by using a hybrid/Boolean approach [BZNN13], or by the use of read-arcs [SHK06]. Allowing read-arcs in Petri nets is analogous to allowing hyperedges where the head and the tail of a hyperedge can share nodes in common.

Of particular interest is the *Signaling Petri Net* [RMT+08], a synchronized Petri net with an event generator, which was successfully used in [PUM+17] to create a signaling Petri net to model the response of Langerhans cells to interferon regulatory factors.

Boolean Networks. Boolean networks in biology are usually attributed to the work of Kauffman [Kau69b], where he used Boolean networks to model gene regulatory networks.

Definition 0.5. *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.*

We herein describe our Boolean functions in logic form, with $\neg$ standing for the logical negation, $\vee$ standing for the logical disjunction and $\wedge$ standing for the logical conjunction.

Every Boolean network has an underlying interaction graph, namely, its wiring diagram $\mathscr{W}$ [VC11]: each species x_i corresponds to a node x_i, 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. It should be remarked that in some cases, the resulting wiring diagram $\mathscr{W}$ will have edges with a 0 label. For example, consider the Boolean function $f_{x_3} = (x_1 \wedge \neg x_2) \vee (\neg x_1 \wedge x_2)$. Then the edges (x_1, x_3) and (x_2, x_3) have a zero label, since x_1 and x_2 have both an activating and inhibiting effect on x_3.

The dynamics of a Boolean network are determined by its updating functions and update schedule strategy (synchronous *vs.* asynchronous). The state of X at time t, denoted by $X(t)$, is a n-tuple $\{0, 1\}^n$ where the *ith* entry is the state of x_i at time t. The synchronous updating of a Boolean network is arguably the most computationally feasible to execute; each node updates its value simultaneously. Namely, we have $X(t + 1) = (f_1(X), \cdots, f_n(X))$.

In the asynchronous timing schedule (see *e.g.* [AR15, AT14]), the nodes update their values at different times. It is worth remarking that different update schedules can yield different dynamics, with only one subtlety: steady-state attractors are the same both for synchronous and asynchronous updates of a Boolean network. See *e.g.* [GJL06, LP03] for a mathematical analysis of this question.

Boolean networks have been widely used to analyze signaling networks. For example, Saez-Rodriguez *et al.* constructed and analyzed a 94-node and 123-interaction Boolean network model of T-cell activation components [SRSL+07]. In [ML09], Mai and Liu introduced a Boolean model

to analyze pro-apoptotic pathways and initial states that lead to apoptosis. Fumia and Martins [FM13] introduced a 96-node (threshold) Boolean network incorporating several of the most import signaling pathways in cancer, where they also provided hypothetical targeted therapies. A signaling pathway of 70 nodes that are known to be pertinent in the regulation of epithelial to mesenchymal transition (EMT) commonly found in a primary liver cancer can be found in [SZD+14], where they find novel hypothesis for EMT activation. Zhang et al. [ZSY+08] provide a Boolean model for survival signaling in large granular lymphocyte leukemia and hypothesize that the constant presence of IL-15 and PDGF is biologically relevant for signaling abnormalities. Calzone et al. [CTF+10] constructed a 25 node Boolean network incorporating the relationships between the NF-$\kappa\beta$ pro-survival pathway, a simplified apoptosis signaling pathway and a necrosis signaling network, regulating how the cell "chooses" its fate based on receiving signals from TNF and FASL. Probabilistic Boolean networks, where, instead of a single updating rule for each node, one has several updating rules for each node and a probability function associated to the updating of each node, have also been successfully used in signaling networks. A probabilistic Boolean network comprised of 27 nodes and 40 interactions representing deregulation of the Platelet-Derived Growth Factor (PDGF) signalling pathway in Gastrointestinal Stromal Tumour (GIST) is presented in [TWB+16]. More recently, the framework of stochastic Boolean networks has also been introduced in [PT17].

See Wynn et al. [WCMS12] for some modelling techniques and addressing of common concerns with Boolean network modeling of biological systems. A recent review on using Boolean networks in systems pharmacology [BNNM] describes how Boolean network models of biological systems are modeled and analyzed, from the perspective of drug discovery.

Several Boolean models of diverse biological systems that have been published, including intracellular signaling networks, can be found in repositories such as Cell Collective [HKM+12] and GINsim [NBF+09].

Capturing signal pathways in the different 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 graphs, hypergraphs, Petri nets and Boolean networks in the signaling network community.

Perhaps unsurprisingly, depending on the framework used to model signaling networks, different methodology has been applied to answer our motivating question.

We will now discuss the different mathematical objects that have been used in the different formalisms to capture how a signal is diffused within a cell.

In graphs. Let G be an interaction graph representation of a signaling network. Klamt et al. [KSRL+06] compute feedback loops on G by computing the (abstract) elementary modes on the incidence matrix $(a_{i,j})$ of G. Feedback loops and cycles in the representations of signaling networks have deep connections with the dynamical properties of the biological network subject to modeling [GK73, BM08]. Furthermore, given source node v_i and target node v_j, adjoining the columns 1_{v_i}, -1_{v_j} (here, 1_{v_i} is the vector with a 1 on the entry corresponding to v_i and zero everywhere else) to the incidence matrix, the simple paths from v_i to v_j are obtained by computing elementary modes. The paths and cycles are given an activating or inhibiting measure based on the parity of the sign after multiplying the weighted signs on the edges in the path. Based on whether nodes are part of activating or inhibiting paths, they are classified as activators or inhibitors, respectively. Nodes involved in both activating and inhibiting paths are classified as ambivalent nodes. Such classification of nodes as inhibitors or activators is useful for network interventions [KSRL+06, VLBBZ13].

The concept of minimal path sets (MPS) is introduced in [LLZ+06]: "An MPS in signal transduction networks can be considered as a minimal set of proteins functioning together to perform signal propagation." To compute minimal path sets the authors compute the set of all paths (not necessarily shortest) from input layer to target layer using a breadth first search algorithm [GM88] and compute the feedback loops separately, which are also counted as MPSs. They further use the concept of minimal path sets to define *Sigflux*, a measure of importance of nodes in the network based on the amount of feedback loops and paths they are part of.

In [ZOS05], Zevedei-Oancea and Schuster proposed computing the rooted trees (an acyclic subgraph with a node singled out), where the root is a given node in the input layer to compute all the nodes influenced by the input node. Similarly, by reversing the directionality of the arrows,

for a given target node O, computing trees rooted at O, one might find the nodes which affect O. Here, they also highlight the importance of cycles in the graph. Scott et al. [SIKS06] propose computing two-terminal series-parallel graphs as a way to capture parallel signaling pathways.

Nassiri et al. [NMNJM13a] weight the edges of an interaction using the normalized similarity index used in citation networks [NMNJM13b]. The use of this weight is to represent the efficiency for signal transduction between nodes of the network. A simulation of signal transduction occurs by traversing the network using a breadth first search algorithm and each node is weighted according to their previous state in the network and the activity level of their activators and repressors. The 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.

Similar approaches have been used in pathway graphs. Ruths et al. [RNI+06] find solutions to the constrained downstream problem to represent molecules that have to be targeted to inhibit signal flows while preserving the signaling capabilities of other subnetworks. Furthermore, the minimal knockout problem represents a minimal set of nodes disconnecting source nodes to target nodes. These concepts of intervening in a signaling network to disturb signal flow are similar to the concepts of minimal cut sets and minimal intervention sets [KSRL+06] and combinations of interventions [VLBBZ13].

In hypergraphs. The concept of B-connection is used in [RAM15] to describe the notion that all reactants must be present for a signaling reaction to occur. Notice we borrow our definition from [RAM15], although the concept of B-connection had previously been discussed [GLPN93].

Let $\mathscr{G} = (\mathscr{V}, \mathscr{E})$ be a directed hypergraph. For a node $u \in \mathscr{V}$, let $\mathrm{BS}(u) = \{e_i \in \mathscr{E} | u \in H(e_i)\}$. The definition of B-connection is a recursive one: given $s \in \mathscr{V}$, we say u is B-connected to $s \in \mathscr{G}$ if either $s = u$ or there exists a hyperedge $e_i \in \mathrm{BS}(u)$ such that for each $w \in T(e_i)$, w is B-connected to s. Let $\mathcal{B}_{\mathscr{G}}(s)$ be the set of hypernodes that are B-connected to $s \in \mathscr{G}$.

Definition 0.6. *(B-hyperpath) Let $\mathscr{G}$ be a hypergraph and let $s, t \in \mathscr{V}$ be two distinct nodes. An s-t* B-hyperpath *$\Pi(s,t)$ is a subhypergraph of $\mathscr{G}$ such that $t \in \mathscr{B}_{\Pi(s,t)(s)}$ and $\Pi(s,t)$ is minimal with respect to deletion of hypernodes and hyperedges.*

There is another concept also usually referred to as B-hyperpaths in the literature found in [GLPN93], although these two definitions are not equivalent, even in the case of B-hypergraphs, as was shown in [NP], whose example we include in Figure 2.

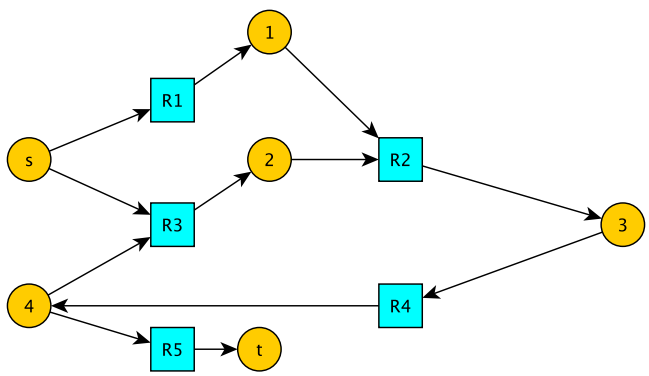

FIGURE 2. A B-hyperpath where s and t are not B-connected Adapted from [NP].

A similar definition as a B-hyperpath can be found in methodology for metabolic networks, the concept of a topological factory which we adapt here since we will compare this to an object proposed in the Boolean network formalism.

Let $\mathscr{X}$ be the source layer, and let $\mathscr{O}$ be any set of nodes disjoint from $\mathscr{X}$.

Definition 0.7. *(Topological factory, adapted from* [AWK+16, AMC+12]*) Given a hypergraph $\mathscr{G} = (\mathscr{V}, \mathscr{E})$, a* topological factory *(TF) from $X \subseteq \mathscr{X}$ to $O \subseteq \mathscr{O}$ is a subset $\mathscr{F} \subseteq \mathscr{E}$ with the property that $O \bigcup_{e \in \mathscr{F}} T(e) \subseteq \bigcup_{e \in \mathscr{F}} H(e) \bigcup X$. It is a* minimal topological factory *(MTF) if it*

contains no smaller topological factory from X to O. A set $X \subseteq \mathscr{X}$ is a topological precursor set *for $\mathscr{O}$ if there exists a topological factory from X to $\mathscr{O}$.*

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.

Definition 0.8. *(Stoichiometric factory,* [AWK+16]*) A stoichiometric factory (S-factory) from $X \subseteq \mathscr{X}$ to $O \subseteq \mathscr{O}$, is a set $F \subseteq \mathscr{E}$ such that there exists a flux vector $v \geq 0$ satisfying:*

(1) $v_i = \begin{cases} > 0 & i \in F \text{ (hyperedge } e_i \text{ is used)} \\ 0 & \text{otherwise} \end{cases}$
(2) $(Sv)_{\mathscr{V}-X} \geq 0$
(3) $(Sv)_O > 0$

An S-factory from X to O is minimal (MSF) if it does not contain any other S-factory from X to O.

To include the steady state assumption, we can replace the $\geq$ with $=$ in the second constraint for nodes that are neither in X nor O.

Analogously to a topological factory, a set $X \subseteq \mathscr{X}$ is a stoichiometric precursor set (SPS) of O if there exists an S-factory from X to O. It is minimal if it does not contain any other SPS of O.

The following observations are found in [AWK+16].

Observation 0.2. [AWK+16]

(1) *Every S-factory is a T-factory. Every SPS is a TPS.*
(2) *Not every TF is an SF. Not every TPS is an SPS.*
(3) *There exist minimal SPSs which do not consist of a union of minimal TPSs.*

The relationship between TPS and SPS was given in [AWK+16, Theorem 1].

Theorem 0.1. [AWK+16] *For any minimal S-factory $H \subseteq \mathscr{E}$ from X to O in $\mathscr{G}$ there exists a set of minimal topological factories $F_1, \cdots, F_k$ from X to O in $\Phi(\mathscr{G})$ such that:*

(1) $F_1, \cdots, F_k \subseteq \Phi(H)$;
(2) *For each hyperedge r in H, there is $i \in \{1, \cdots, k\}$ such that $\Phi(r) \cap F_i \neq \emptyset$.*

This means that S-factories are the union of minimal topological factories in the many-to-one network. In the case of a B-hypergraph, this of course means that every minimal S-factory is a union of T-factories.

In Boolean Networks. The fact that every Boolean network has an associated interaction graph, namely its wiring diagram, means we can apply techniques of analyzing signal transduction capabilities in interaction graphs. Functional cycles, *i.e.* cycles that generate attractors, in the interaction graph of a Boolean network have been shown to be deeply connected to the long term behavior of a Boolean network (see *e.g.* [CNR+13] for a mathematical perspective). Thus, the computation of these can be seen as a bridge between the dynamics and the structure.

Wang and Albert [WA11] proposed the definition of an *Elementary Signaling Mode* (ESM) based on structural analysis of a Boolean network.

Definition 0.9. *(Elementary signaling modes, adapted from* [WA11] *) An elementary signaling mode (ESM) of a signaling network represented as 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. By minimal, we mean that an Elementary Signaling Mode is not decomposable and none of its signaling components are redundant.*

The authors use an expansion of a signaling network before computing ESMs. They introduce a complementary node for nodes that are inhibited by other nodes or are inhibiting other nodes. Furthermore, they introduce a "composite" node to represent conditionally dependent relationships.

Observation 0.3. *The network expansion method requires the local update functions and their respective logical negations to be written in disjunctive form.* [WA11] *describes a way of writing the regulatory function of a node in the network in disjunctive form from the biological literature.*

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 complementary nodes are added for every node in the network is used, such as in [AR15], the update rules can be read directly from the expanded network. We remark, however, that for computations of elementary signaling modes, different signaling network expansions have been used. In [AR15], one finds an expansion where composite nodes are added for logical dependencies, and complementary nodes are added for every node in the network. In [SA16], only composite nodes are added to represent synergy, and no complementary nodes are added. Therefore, one needs to first provide a mathematical definition of ESMs to make it computationally amenable in systems biology software. Wang et al. ([WSA13]) provide a similar concept for graphs with dependent edges, namely, the concept of a minimal functional route (MFR). They again expand the network to a new network $\hat{G}$ by adding composite nodes to represent dependent edges.

Definition 0.10. *(Minimal functional route (MFR) from* [WA11]*) Given a graph with dependent edges with source node s and sink node t, and its expanded graph $\hat{G}$, an MFR from s to t in $\hat{G}$ is a minimal set of nodes and edges satisfying: s and t are in the set; each node is connected from s by simple paths; any original node other than the source node has one direct predecessor in the MFR, and any composite node has all of its direct predecessors in the MFR.*

Observation 0.4. *In the special case of a graph with no composite nodes, computations of MFRs from s to t is the same as computing simple paths from s to t.*

Zañudo and Albert [ZA13] introduce the concept of a *stable motif* as a similar bridge between the structure and the dynamics of a Boolean network X. Using the expanded network $\mathscr{X}$ containing both composite nodes and complementary nodes, a *stable motif* $U \subseteq \mathscr{X}$ is defined to be a smallest strongly connected component (scc) satisfying the two properties that if $u \in U$ is a composite node, then all of the input nodes of u are also in U, and U does not contain both a node and its complementary node. Furthermore, U does not contain any smaller scc satisfying these two properties. These stable motifs are parts of the interaction graph relevant to the fixed states of the Boolean network.

A recent article by Maheshwari and Albert [MA17] uses the regulatory functions of the nodes of a Boolean network to 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 graph theoretically far apart *e.g.* a source and target node. This labeling of the arrows by their causal logic can also be used to compute what the authors refer to as 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. More importantly, for our purposes, in some cases, it aids to compute signalling components that once they are activated are able to transduce a signal regardless of the rest of the network.

A commonly accepted technique to analyze biological networks modeled as Boolean networks, dating all the way back to the pioneering work of Kauffman [Kau69a], is based on the idea that long term behavior of networks is captured by their attractors, the dynamically stable patterns of the system. Attractors are often associated with distinct cellular phenotypes and cellular responses [CSJ+12, AT14, GA16]. For example, some of the attractors computed in [FM13] 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 more details and references on attractor analysis, see *e.g.* [BNNM].

For a survey of results connecting the state graph of a Boolean network to its interaction graph, see [PR12].

For the purposes of attractor analysis, some reduction techniques have been proposed in the literature [VCAL15], and several algebraic techniques exist via the Boolean network's corresponding polynomial dynamical system representation [VCAHL14].

In Petri Nets. Given 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 (see [KC10b]). 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.

Given a marking M and a multiset of transitions $\mathbf{y}$ that can fire in some order, notice that the resulting marking will be given by $M' = S \cdot \mathbf{y} + M$ where S is the incidence matrix of the Petri net. A T-invariant is a non-zero, non-negative vector $\mathbf{u}$ satisfying $Su = \mathbf{0}$. 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. In the framework of metabolic networks, minimal T-invariants are counterparts to elementary flux modes [ZOS03, BdFSK12], 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: a vector $\mathbf{u} \in \mathbb{N}_{\geq 0}^{|P|}$ with $\mathbf{u}^t S = \mathbf{0}$. [SHK06] introduces 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. The idea is that feasible T-invariants stand for minimal sub-entities of the Petri net that are relevant to capture signal transduction. In a later paper [AAS+17], the authors further develop the concept of feasible T-invariants to the concept of Manatee invariants, where a Manatee invariant stands for a minimal linear combination of T-invariants whose induced network is feasible under the initial marking.

Notice that computations of T-invariants and P-invariants are computed via the incidence matrix. In the case of read-arcs [SHK06], a Petri net cannot be reconstructed from the incidence matrix.

Results

Comparing minimal functional routes and topological factories. Let G be a graph with composite nodes [WSA13] 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 interaction graphs with no self-loops where we know which edges are dependent via the Boolean function of the nodes [WA11, AR15, SA16]. Such graphs can easily be converted to a B-hypergraph $\mathscr{H}$ by collapsing incoming edges into a composite node c_1 into the tail of a hyperedge (see Figure 3). Due to the assumptions, we have a hypergraph with the property that for every edge e in the B-hypergraph, $H(e) \cap T(e) = \emptyset$.

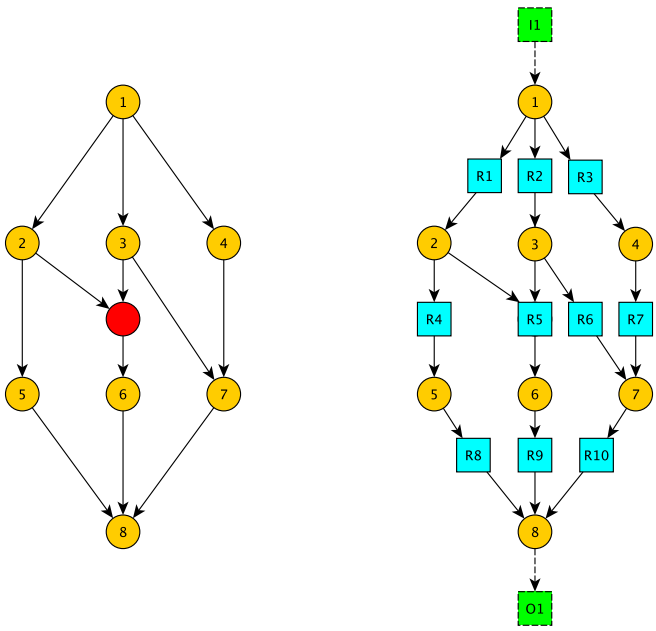

Figure 3. B-hypergraph from expanded graph Adapted from [WSA13, Figure 1(b)]. On the left, an expanded AND-OR graph. The composite node is shown in red, original nodes are shown in yellow. On the right, the corresponding hypergraph. The input node 1 has been connected to an environment transition I_1 and the target node has been connected to an environment transition O_1. for computations of elementary flux modes.

Our goal is to compare minimal functional routes and topological factories. We will assume the source layer only has one node s in it.

This can be done in a couple of ways: if the source layer is $X = \{x_1, \cdots, x_m\}$ we add an artifical node s and add an edge from s to x_i for each i, or we choose one x_i in S and connect x_j to x_i for $j \neq i$.

We see that topological factories and ESMs are deeply connected. We now formally prove this relationship.

Theorem 0.2. *The set of minimal functional routes from s to a sink node t is contained in the set of minimal topological factories from s to t.*

Proof. This follows from the fact that every node in an MFR is connected to s by a simple path. That is, for every node u in the MFR distinct from s, there exists a hyperedge e in the MFR such that $H(e) = u$. Thus, if $E = \{e_1, \cdots, e_m\}$ are the hyperedges corresponding to the MFR, we have $\{t\} \bigcup T(E) \subseteq H(E) \bigcup \{s\}$. Thus the hyperedges of the MFR form a topological factory from s to t. □

Theorem 0.3. *Let $G = (V, E)$ be an acyclic connected graph with a single source node and composite nodes. Let t be a sink node where $s \neq t$. Given a minimal topological factory U from s to t, the hypergraph induced by U is a minimal functional route from s to t.*

Proof. Since G is acyclic, there is an ordering of the nodes $w : V \to \mathbb{N}$ of the set of nodes V such that if $(v_i, v_j) \in E$, then $w(v_i) < w(v_j)$. Since s is the only source node, $w(s)$ is the minimum value w attains on V. Since U is a minimal topological factory from s to t, $t = H(e_1)$ for some $e_1 \in U$. Let u_1 be any node in the tail of e_1. Notice that $w(u_1) < w(t)$. If $u_1 = s$, then there is a simple path from s to t. Otherwise, let e_2 be the hyperedge containing u_1 as its head, and let u_2 be any node in the tail of e_2. Proceeding in this way, we get a sequence of nodes $u_m, \cdots, u_1, u_0 = t$ that are connected by a path with the property that $w(u_m) < w(u_{m-1}) < \cdots < w(t)$. Since U is a topological factory from s to t, this sequence must terminate at a node with no incoming hyperedge, *i.e.* s. Thus, we have created a simple path from s to t.

Now let u be any node different from s, t in the topological factory. Then u must be contained in the head of a hyperedge, and by minimality, a unique hyperedge. Applying the same method as above, we can create a simple path from s to u.

□

Thus, if the expanded graph is an acyclic graph with a single source and a single target node, then computing the set of minimal functional routes is the same as computing the minimal topological factories. We remark however that there are minimal topological factories from s to t that are not minimal functional routes (see Figure 4).

Comparing topological factories to S-factories in signaling networks using the incidence matrix of a hypergraph. Given a directed graph G, node s and node t and an incidence matrix A, there is a deep relationship between simple paths from s to t and elementary modes of a slightly adjusted incidence matrix. As shown in [KSRL$^+$06], adjoining the columns 1_s, -1_t (here, 1_{v_i} is the vector with a 1 on the entry corresponding to v_i and zero everywhere else) to the incidence matrix and computing the elementary modes of this adjusted matrix, one gets the simple paths from s to t. Namely, if a vector v is an elementary mode of the adjusted incidence matrix, the nonzero entries correspond to the sequence of edges visited in a path from s to t (as long as the vector is nonzero on the entries corresponding to at least one edge containing s and at least one edge containing t).

It is natural to wonder if given a directed graph with dependent edges, the analogous procedure can be done to compute MFRs via the incidence matrix of its respective hypergraph. However, it is readily verified that any vector in the nullspace of a matrix attained in this way for Figure 5 must have the coordinate corresponding to R_3 equal to zero. Furthermore, if we consider all the stoichiometric coefficients equal to 1, there is no S-factory from s to t.

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.

In the case of B-hypergraphs, Theorem 0.1 is simplified to:

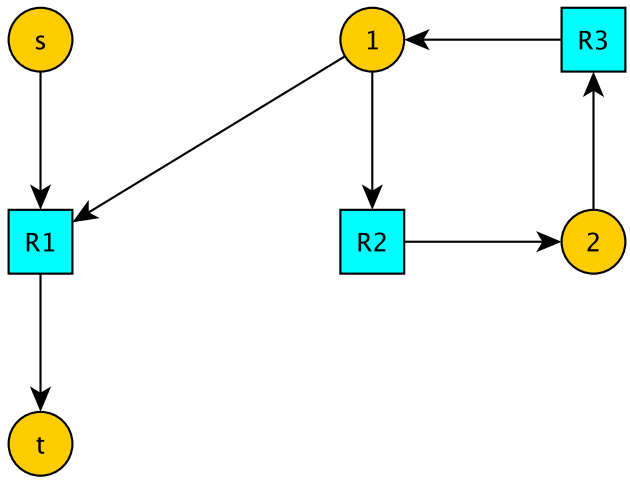

FIGURE 4. Not all topological factories are minimal functional routes from s to t. There is no MFR from s to t. However, R_1, R_2, R_3 is a topological factory from s to t. In the case the graph is acyclic, MFRs from s to t are the same as the topological factories from s to t.

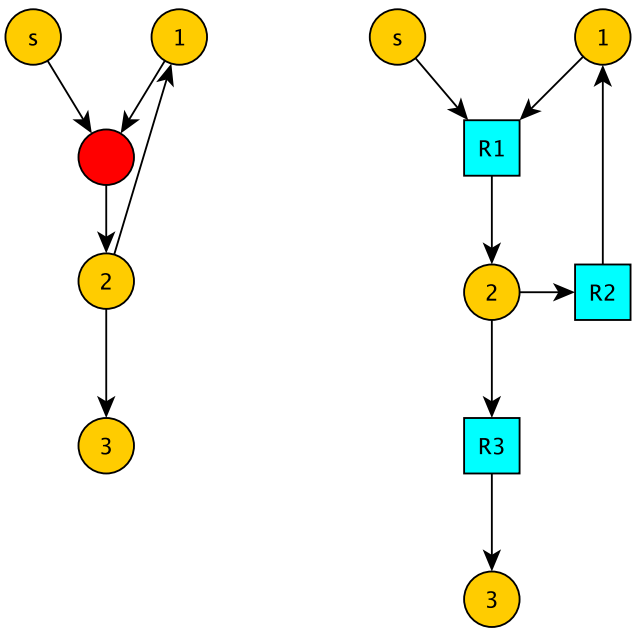

FIGURE 5. A minimal functional route (and thus topological factory) that is not an S-factory. Using the incidence matrix as the stoichiometric matrix for this hypergraph, there is no S-factory from s to t. However, it is a minimal functional route from s to t.

Theorem 0.4. [AWK+16] *For any minimal S-factory $H \subseteq \mathscr{E}$ from X to O in a B-hypergraph $\mathscr{G}$ there exists a set of minimal topological factories $F_1, \cdots, F_k$ from X to O in $\mathscr{G}$ such that:*

(1) $F_1, \cdots, F_k \subseteq H$;
(2) *For each hyperedge e in H, there is $i \in \{1, \cdots, n\}$ such that $e \cap F_i \neq \emptyset$.*

In particular, any S-factory in a B-hypergraph is the union of topological factories.

Discussion

A natural question that arises from all these different modeling techniques is how all these mathematical structures relate to each other. As already discussed, Boolean networks graphs have underlying interaction graphs, although several distinct Boolean networks could have isomorphic interaction graphs. We have also discussed how elementary modes and minimal T-invariants are counterparts of each other, and equivalent if the incidence of a Petri net corresponds to the stoichiometric matrix and no reversible reactions are allowed.

We further discussed how minimal functional routes and ESMs are special cases of the concept of a topological factory in metabolic networks and how not every minimal functional route can be computed by an elementary mode.

For the sake of completeness, we include other comparisons and translations that we found in the literature.

Translation from bounded Petri Nets to a family of Boolean networks. Veliz-Cuba et al. [VCJL10] translated 1-bounded Petri nets to a family of Boolean networks. Namely, for each transition t, they find a polynomial form $f_t : \mathbb{F}_2 \to \mathbb{F}_2$ for how t changes the possible markings. Under the given translation, they show that dead markings correspond to a solution of a system of polynomial equations over finite fields. Furthermore, they show how to recover P-invariants and T-invariants of the original Petri net.

Another approach is discussed in [ENN08], where they associate Petri nets to cellular automaton and discuss the algebraic structure of the corresponding automaton.

In both cases, the translation focuses on the dynamics of the Petri net, rather than on a structural translation.

Translation from Boolean logical regulatory networks to Petri Nets. Chaouiya et al. [CRRT04] gives a translation from the Boolean framework to a 1-safe standard Petri net (the multistate case can be found in [CRT06]). 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 (see [CNT12] for software automating the translation). 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 under the proposed translation.

A related approach can be found in [SHK06], where they give insight on how to translate logical rules into the Petri net framework.

[SBSW07, SBW06] provide a translation from Boolean networks to Petri nets focusing on gene regulatory networks for the synchronous updating timing schedule. Namely, given a truth table for a Boolean network, they derive a Petri net modeling the synchronous updating scheme of the original Boolean network using logical minimization techniques, and used this process to analyze sporulation in *Bacillus subtilis*.

Translation from hypergraphs to graphs. There is a well-known reversible construction to create a bipartite graph S from a hypergraph $\mathscr{H}$ [KHT09]. Similarly, 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 [RAM15], where comparisons of graphs and hypergraph representations of signaling networks are also included.

Translations from Petri nets to Pathway graphs. As discussed in [RNI$^+$06], pathway graphs are essentially unparametrized Petri nets. Namely, a place-transition Petri net has an underlying pathway graph, $G = (P, T, E)$ where P is the set of places and T is the set of transitions. Therefore, 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, to Petri nets and topological factories.

Conclusion

Several different methodologies exist for modeling signaling networks. Particularly useful methodologies, when we don't have enough detailed mechanistic parameters such as kinetic rates of change, are Boolean networks, hypergraphs and Petri nets. Many of the different objects 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.

We related the recent concept of a topological factory to the concept of a minimal functional route. We further used the work done in metabolics showing how elementary modes in hypergraphs are related to topological factories and thus to minimal functional routes or elementary signaling modes in Boolean networks. 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. Furthermore, the concept of topological precursor sets can be used to approximate an answer to the question of "What is the minimal set of source signals necessary to transduce a signal to a target set?"

We have proved that in graphs with dependent edges, the concept of topological factories generalize functional routes. It would be interesting to categorize graphs with dependent edges such that the concept of minimal functional routes and minimal topological factories are equivalent. Furthermore, due to the connection between S-factories with the steady state assumption using the incidence matrix of a graph and T-factories in graphs with no dependent edges [KSRL$^+$06], it would be interesting to categorize graphs where the minimal S-factories and T-factories are the same.

Theoretical discussions based on biological reality discussing why the particular signaling object proposed are useful to capture signal flow are useful. For example, the discussion found in [BS09] for applying elementary flux mode analysis to enzyme cascades show us a class of signaling networks where the methodology of T-invariant analysis is directly applicable and not just a formal approach. This is not to say that formal mathematical methods are not useful, but basing them on strong biological assumptions (*e.g.* elementary flux modes) provide more useful results.

Finally, we wish to remark that at present, there is no single best modeling formalism. 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 should be used, such as the ones presented in [BZNN13].

Appendix

A remark on "disjunctive normal form". The definition of disjunctive normal form differs across fields and authors. For example, the definition of disjunctive normal form given in [Rom88] is as follows:

Definition 0.11. *A Boolean function $p(x_1, \cdots, x_n)$ is said to be in disjunctive normal form if it is the disjunction (connected by the OR operator) of a finite number of terms, each which has the form $e_1 \wedge e_2 \cdots \wedge e_n$ where $e_i = x_i$ or $e_i = \neg x_i$.*

In [AR15], disjunctive normal form simply means that AND clauses are separated by ORs. Thus, $(A \wedge B) \vee (C \wedge D)$ is in disjunctive normal form according to the definition given in [AR15], whereas it is not in disjunctive normal form according to definition given in [Rom88].

Perhaps more concerning is the following example of two update functions which have the same truth table but are written differently, which can be found in [Pur97]:

$$D = (\neg A \wedge \neg C) \vee (A \wedge \neg B) \vee (B \wedge C)$$

$$D = (\neg B \wedge \neg C) \vee (\neg A \wedge B) \vee (A \wedge C)$$

Methods that rely on disjunctive normal form, such as the graph expansion method of [WA11, WSA13], might yield different results. One possible solution to this is to write in the so called canonical sum of products form and apply *the same* deterministic logic minimization strategy for reducing the terms (see *i.e.* [SBSW07] for references to logic minimization algorithms).

Elementary Flux Modes. We follow the formalism found in [SH94] where the mathematical definition of an elementary flux mode was introduced. This has been highly successful for analyzing metabolic networks.

We take a purely abstract approach of an elementary mode.

Definition 0.12. *Let $\boldsymbol{N}$ be a matrix in $\mathbb{R}^{m \times n}$.*

An abstract flux mode $\mathbf{M}$ is the set

$$\mathbf{M} = \{\mathbf{V} \in \mathbb{R}^n_{\geq 0} | \mathbf{V} = \lambda \mathbf{V}^*, \lambda \in \mathbb{R}_{>0}\}$$

where $\mathbf{V}^*$ is a nonzero vector satisfying $\mathbf{N}\mathbf{V}^* = \mathbf{0}$.

If $\mathbf{M}$ *is a mode with representative* $\mathbf{V}^*$*, it is said to be elementary if* $\mathbf{V}^*$ *cannot be written as a nontrivial linear combination*

$$\mathbf{V}^* = \lambda_1 \mathbf{V}_1 + \lambda_2 \mathbf{V}_2, \text{ where } \lambda_1, \lambda_2 > 0,$$

of nonzero vectors where $\mathbf{V}_1, \mathbf{V}_2 \in \mathbb{R}^n_{\geq 0}$ *satisfy* $\mathbf{N}\mathbf{V}_i = \mathbf{0}$*, such that both* $\mathbf{V}_1, \mathbf{V}_2$ *contain at least the same number of zero elements as* $\mathbf{V}^*$ *and at least one of them contains more zero elements than* $\mathbf{V}^*$*.*

We will make no distinction between a mode and its representative V^*.

Notice that elementary modes are usually computed from the stoichiometry matrix of a metabolic network, and the restriction $\mathbf{V} \in \mathbb{R}^n_{\geq 0}$ is not usually placed to allow reversible reactions.

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