

On the Realisability of Chemical Pathways

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Abstract. The exploration of pathways and alternative pathways that have a specific function is of interest in numerous chemical contexts. A framework for specifying and searching for pathways has previously been developed, but a focus on which of the many pathway solutions are realisable, or can be made realisable, is missing. Realisable here means that there actually exists some sequencing of the reactions of the pathway that will execute the pathway. We present a method for analysing the realisability of pathways based on the reachability question in Petri nets. For realisable pathways, our method also provides a certificate encoding an order of the reactions which realises the pathway. We present two extended notions of realisability of pathways, one of which is related to the concept of network catalysts. We exemplify our findings on the pentose phosphate pathway. Lastly, we discuss the relevance of our concepts for elucidating the choices often implicitly made when depicting pathways.

1 Introduction

Large *Chemical Reaction Networks* (CRN) lie at the heart of many questions and challenges in research, industry, and society. Examples include understanding metabolic networks and their regulation in health and biotechnology; planning and optimising chemical synthesis in industry and research labs; modelling the fragmentation of molecular ions inside mass spectrometers; probing hypotheses on the origins of life; and monitoring environmental pollution in air, water and soil. Subnetworks with desirable properties, often called *pathways*, such as a

synthesis plan for a given target molecule, or a metabolic subsystem, are of particular interest. The ability to specify and search for pathways in a given CRN thus is a core objective in chemical modelling, exploration, and design.

CRNs can be modelled as directed hypergraphs [25,19,4,3], where each molecule is represented by a vertex and each reaction is modelled by a directed hyperedge. Viewing pathways in CRNs as sets of reactions with integer multiplicities, [3] formally defined pathways as integer hyperflows in hypergraphs. In contrast to real-valued hyperflows, integer hyperflows account for molecules as indivisible entities and allows a more direct mechanistic interpretation. In [3], also the concept of a *chemical transformation motif* in a CRN was introduced, providing a versatile framework for querying reaction networks for pathways. A chemical transformation motif is the specification of a pathway by prescribing the input and output compounds (intermediate products may appear but must be used up again). Finding and enumerating pathways fulfilling a chemical transformation motif can be treated computationally via Integer Linear Programming (ILP) [3]. ILP is NP-hard, both in the general case and in the restricted setting of finding integer hyperflows in CRNs [1]. However, for many networks and pathways of practical interest, current ILP solvers perform well [3].

The starting point of this paper is the following observation: While integer hyperflows specify reactions and their multiplicities, they do not determine in which *order* the individual reactions take place to perform the specified overall chemical transformation. In fact, there may be *no* sequencing possible. Fig. 4 gives an example of this. Specifically, no sequencing of the reactions e_1 and e_2 in the hyperflow of Fig. 4 will make it executable—in essence, C or D must be present *before* they can be produced. We introduce the term *realisable* for hyperflows where the corresponding chemical transformation is executable by some sequence of the constituent reactions of the hyperflows. We develop a framework that converts integer hyperflows into corresponding Petri nets, which then allow us to use Petri net methodology to express and decide whether integer hyperflows are realisable. Petri nets have already been used extensively to model metabolic networks [5].

For realisable flows we introduce the concept of a *realisability certificate* that specifies an order in which the reactions can occur along the pathway. Finding an explicit order both facilitates a mechanistic understanding of pathways and is a necessity for investigations where the identity of individual atoms matter, such as computing atom traces [2]. We also study ways in which non-realisable integer hyperflows can be extended to realisable ones. One option is a scaling of the flow itself, another is borrowing additional molecules which are then returned. The latter construction is closely related to the concept of a “network catalyst” (see e.g. [8,18]). An algorithmic approach to deciding realisability by borrowing thus forms an important basis for a future formal computational treatment of higher-level chemical motifs such as autocatalysis and even hypercycles [10,11,23,24]. Finally, we utilise the non-oxidative phase of the pentose phosphate pathway (PPP) to demonstrate our approach and explore how to find potential cata-

lysts within the network. PPP is a well-known example which highlights the importance of simplicity in finding solutions [20,17].

The main thrust of our paper lies in formally defining and exploring the concept of realisability of pathways. However, we would like to point out that commonly used representations of pathways in the life science literature often fall in between the two extremes of integer hyperflows and realisability certificates. We believe that our formalisation of these concepts may help raise awareness of the choices one often subconsciously makes when creating illustrations of pathways. We elaborate on this viewpoint in Section 5.

2 Preliminaries

2.1 Chemical Reaction Networks and Pathways

A CRN can be modelled by a directed hypergraph $\mathcal{H} = (V, E)$, where V is the set of vertices representing the molecules. Reactions are represented as directed hyperedges E , where each edge $e = (e^+, e^-)$ is an ordered pair of multisets of vertices, i.e., $e^+, e^- \subseteq V$.⁹ We call e^+ the *tail* of the edge e , and e^- the *head*. In the interest of conciseness we will refer to directed hypergraphs simply as hypergraphs, directed hyperedges simply as edges, and CRNs as networks. For a multiset Q and an element q we use $m_q(Q)$ to denote its multiplicity, i.e., the number of occurrences of q in Q . When denoting multisets we use the notation $\{\{ \dots \}\}$, e.g., $Q = \{\{a, a, b\}\}$ is a multiset with $m_a(Q) = 2$ and $m_b(Q) = 1$. For a vertex $v \in V$ and a set of edges A we use $\delta_A^+(v)$ and $\delta_A^-(v)$ to denote respectively the set of out-edges and in-edges of v contained in A , i.e., the edges in A that have v in their tail and v in their head, respectively.

In order to later define pathways we first introduce an extension of the network for representing input and output of compounds. Given a hypergraph $\mathcal{H} = (V, E)$ we define the *extended hypergraph* $\overline{\mathcal{H}} = (V, \overline{E})$ with $\overline{E} = E \cup E^- \cup E^+$, where

$$E^- = \{e_v^- = (\emptyset, \{v\}) \mid v \in V\} \quad E^+ = \{e_v^+ = (\{v\}, \emptyset) \mid v \in V\} \quad (1)$$

The hypergraph $\overline{\mathcal{H}}$ has additional ‘‘half-edges’’ e_v^- and e_v^+ , for each $v \in V$. These explicitly represent potential input and output channels to and from \mathcal{H} , i.e., what is called exchange reactions in metabolic networks. An example of an extended hypergraph is shown in Fig. 1.

In [3] it was proposed to model a pathway in a network $\mathcal{H} = (V, E)$ as an *integer hyperflow*. This is an integer-valued function f on the extended network, $f: \overline{E} \rightarrow \mathbb{N}_0$, which satisfies the following *flow conservation constraint* on each vertex $v \in V$:

$$\sum_{e \in \delta_{\overline{E}}^+(v)} m_v(e^+)f(e) - \sum_{e \in \delta_{\overline{E}}^-(v)} m_v(e^-)f(e) = 0 \quad (2)$$

⁹ When comparing a multiset M and a set S , we view M as a set. I.e., $M \subseteq S$ holds if every element in M is an element of S .

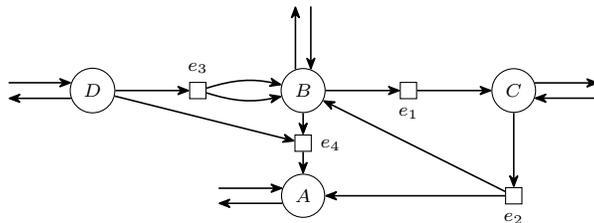


Fig. 1. Example of an extended hypergraph. It has vertices $\{A, B, C, D\}$, edges $\{e_1, e_2, e_3, e_4\}$, and a half-edge to and from each vertex. An edge e is represented by a box with arrows to (from) each element in e^- (e^+).

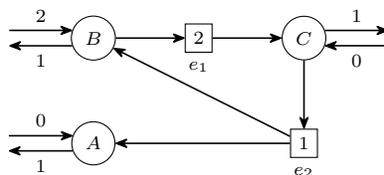


Fig. 2. Example integer hyperflow f on the extended hypergraph from Fig. 1. Vertex D has been omitted as it has no in- or out-flow. Edges leaving or entering D have also been omitted as they have no flow. The flow on an edge is represented by an integer. For example, the half edge into B has flow $f(e_B^-) = 2$, the half edge leaving B has flow $f(e_B^+) = 1$, and edge e_1 has flow $f(e_1) = 2$.

Note in particular that $f(e_v^-)$ is the input flow for vertex v and $f(e_v^+)$ is its output flow. An example of an integer hyperflow is shown in Fig. 2.

2.2 Petri Nets

Petri nets are an alternative method to analyse CRNs. Each molecule in the network forms a *place* in the Petri net and each reaction corresponds to a transition [21,16,22]. The stoichiometric matrix commonly used in chemistry has an equivalent in Petri net terminology, called the incidence matrix [16]. In Section 3 we will describe a transformation of a hyperflow to a Petri net. The following notation for Petri nets (with the exception of arc weights) follows [12].

A *net* is a triple (P, T, W) with a set of places P , a set of transitions T , and an arc weight function $W: (P \times T) \cup (T \times P) \rightarrow \mathbb{N}_0$. A *marking* on a net is a function $M: P \rightarrow \mathbb{N}_0$ assigning a number of tokens to each place. With M_\emptyset we denote the empty marking, i.e., $M_\emptyset(p) = 0, \forall p \in P$. A *Petri net* is a pair (N, M_0) of a net N and an initial marking M_0 . For all $x \in P \cup T$, we define the *pre-set* as $\bullet x = \{y \in P \cup T \mid W(y, x) > 0\}$ and the *post-set* as $x^\bullet = \{y \in P \cup T \mid W(x, y) > 0\}$. We say that a transition t is enabled by the marking M if $W(p, t) \leq M(p), \forall p \in P$. When a transition t is enabled it can *fire*, resulting in a marking M' where $M'(p) = M(p) - W(p, t) + W(t, p), \forall p \in P$.

Such a firing is denoted by $M \xrightarrow{t} M'$. A *firing sequence* σ is a sequence of firing transitions $\sigma = t_1 t_2 \dots t_n$. Such a firing sequence gives rise to a sequence of markings $M_0 \xrightarrow{t_1} M_1 \xrightarrow{t_2} M_2 \xrightarrow{t_3} \dots \xrightarrow{t_n} M_n$ which is denoted by $M_0 \xrightarrow{\sigma} M_n$.

3 Realisability of Integer Hyperflows

The paper [3] gave a method (summarized in Section 2.1) for specifying pathways in CRNs and then proceeded to use ILP to enumerate pathway solutions fulfilling the specification.

In this paper, we focus on assessing the realisability of such a pathway solution and on determining a specific order of reactions that proves its realisability. To this end, we map integer hyperflows into Petri nets and rephrase the question of realisability as a particular reachability question in the resulting Petri net.

3.1 Flows as Petri Nets

We convert a hypergraph $\mathcal{H} = (V, E)$ to a net $N = (P, T, W)$ by using the vertices V as the places P and the edges E as the transitions T , and by defining the weight function from the incidence information as follows: for each vertex/place $v \in V$ and edge/transition $e = (e^+, e^-) \in E$ let $W(v, e) = m_v(e^+)$ and $W(e, v) = m_v(e^-)$. This conversion also works for extended hypergraphs, where the half-edges result in transitions with either an empty pre-set or post-set. The transitions corresponding to input reactions are thus always enabled. Denote by M_\emptyset the empty marking on N . Every firing sequence σ starting and ending in M_\emptyset , i.e., $M_\emptyset \xrightarrow{\sigma} M_\emptyset$, therefore implies a flow $f: \bar{E} \rightarrow \mathbb{N}_0$ simply by setting $f(e)$ to be the number of occurrences of the transition e in the sequence σ . The flow conservation constraint at $v \in V$ is satisfied as a consequence of the execution semantics of Petri nets.

Given a flow, we would like to constrain the Petri net to only yield firing sequences for that particular flow. We therefore further convert the extended hypergraph $\bar{\mathcal{H}}$ into an extended net $(V \cup V_E, \bar{E}, W \cup W_E)$ by adding for each edge $e \in \bar{E}$ an ‘‘external place’’ $v_e \in V_E$ with connectivity $W(v_e, e) = 1$. In the following, we will denote the extended Petri net again by N . We then proceed by translating the given flow f of $\bar{\mathcal{H}}$ into an initial marking M_0 on the extended net. To this end, we set $M_0(v) = 0$ for $v \in V$ and $M_0(v_e) = f(e)$ for places $v_e \in V_E$. Transitions in (N, M_0) therefore can fire at most the number of times specified by the flow. Furthermore, any firing sequence $M_0 \xrightarrow{\sigma} M_\emptyset$ ending in the empty marking must use each transition exactly the number of times specified by the flow. As an example, the hyperflow in Fig. 2 is converted to the Petri net in Fig. 3.

3.2 Realisability of Integer Hyperflows

We are interested in whether a given pathway, represented by a flow f on an extended hypergraph $\bar{\mathcal{H}} = (V, \bar{E})$, is realisable in the following sense: Given the

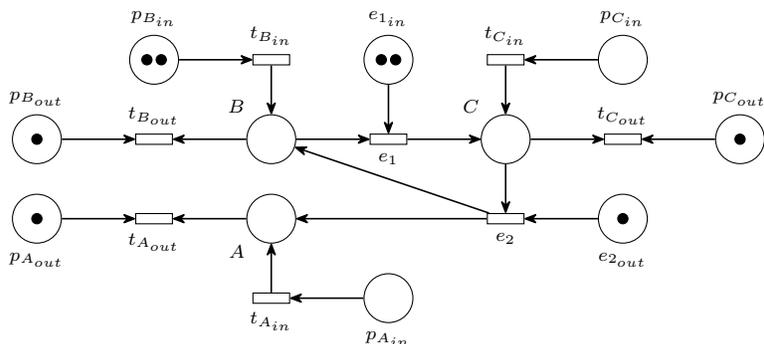


Fig. 3. The integer hyperflow from Fig. 2 converted to a Petri net. Places are circles, transitions are squares, and tokens are black dots. Arrows indicate pairs of places and transitions for which the weight function W is non-zero (in this example, all non-zero weights are equal to one). We have omitted the part of the net that corresponds to the omitted part of Fig. 2.

input molecules specified by the input flow, is there a sequence of reactions that respects the flow, which in the end produces the specified output flow? In the light of the construction of (N, M_0) from $(\overline{\mathcal{H}}, f)$, this question translates into a reachability problem on a Petri net.

Definition 1. A flow f on $\overline{\mathcal{H}}$ is realisable if there is a firing sequence $M_0 \xrightarrow{*} M_\emptyset$ on the Petri net (N, M_0) constructed from $(\overline{\mathcal{H}}, f)$.

Fig. 4 shows that not all flows f on $\overline{\mathcal{H}}$ are realisable. In this example it is impossible to realise the flow as long as there is no flow entering either C or D . For the flow in Fig. 2, on the other hand, such a firing sequence exists. The firing sequences corresponding to a realisable flow are not unique in general. For instance, the Petri net constructed from the integer hyperflow presented in Fig. 3 can reach the empty marking M_\emptyset , in essentially two different manners. Modulo the firing of input/output transitions, those two firing subsequences are $e_1e_1e_2$ and $e_1e_2e_1$.

Showing the existence of a firing sequence as specified in Def. 1 is one way of proving the realisability of an integer hyperflow. Making use of occurrence nets [13,6,15] and processes [15], a *realisability certificate* can be defined which constitute an ordered sequence of reactions together with an individual token interpretation [14]. Thus it contains the exact dependencies between reactions in the realisation of the integer hyperflow and explicitly expresses which individual molecule is used when and for which reaction. Due to space constraints, we defer a formal description of realisability certificates to the forthcoming full version of this paper. However, we do note here that a realisability certificate uniquely determines a corresponding integer hyperflow while integer hyperflows, on the other hand, do not specify the order of the reactions or which one of multiple copies of a molecules is used in which reaction. An integer hyperflow therefore

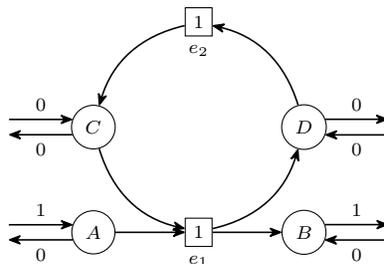


Fig. 4. Example of a flow which is not realisable. Observe that the flow is indeed viable as it fulfils the flow conservation constraint. Furthermore, notice that there is no input flow to neither C nor D , and therefore in the corresponding Petri net it will not be possible to fire either of e_1 or e_2 which is necessary for it to be realised. However, if C or D was borrowed the related flow with this borrowing would be realisable.

may correspond to multiple different realisability certificates, each representing a different mechanism. For an example of a realisability certificate see Fig. 6.

4 Extended Realisability

Although we have seen above that some integer hyperflows are not realisable, they can be turned into realisable hyperflows by means of certain modifications.

Definition 2 (Scaled-Realisable). *An integer hyperflow f on an extended hypergraph $\overline{\mathcal{H}} = (V, \overline{E})$ is scaled-realisable, if there exists an integer $k \geq 1$ such that the resulting integer hyperflow $k \cdot f$ is realisable.*

Asking if an integer hyperflow f is scaled-realisable corresponds to asking if k copies of f can be realised concurrently. This is of interest as in the real world, a pathway is often not just happening once, but multiple times. Therefore, even if the integer hyperflow is not realisable, it still has value to consider if the scaled integer hyperflow is. However, not all integer hyperflows are scaled-realisable. An example is the integer hyperflow presented in Fig. 4: no integer scaling can alleviate the fact that firing requires that C and D is present at the outlet.

Definition 3 (Borrow-Realisable). *Let f be a flow on an extended hypergraph, $\overline{\mathcal{H}} = (V, \overline{E})$ and $b: V \rightarrow \mathbb{N}$. Set $f'(e_v^-) = b(v) + f(e_v^-)$ and $f'(e_v^+) = b(v) + f(e_v^+)$ for all $v \in V$, and $f'(e) = f(e)$ for all $e \in E$. Then f is borrow-realisable if there exists a borrowing function b such that f' is realisable.*

We say that f' is the flow f where $v \in V$ has been borrowed $b(v)$ times. This allows intermediary molecules required for reactions in the pathway to be available in the environment. Formally, this is modelled by having an additional input and output flow $b(v)$ for species v . Furthermore, for a borrowing function b we define $|b| = \sum_{v \in V} b(v)$, i.e., the total count of molecules borrowed. The idea of

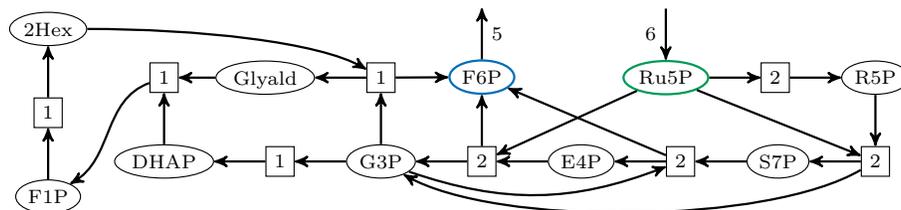


Fig. 5. Example of a hyperflow for the pentose phosphate pathway that is not scaled-realizable. The hyperflow is borrow-realizable. The input compound is marked with green and the output compound is marked with blue. The edges without flow have been omitted.

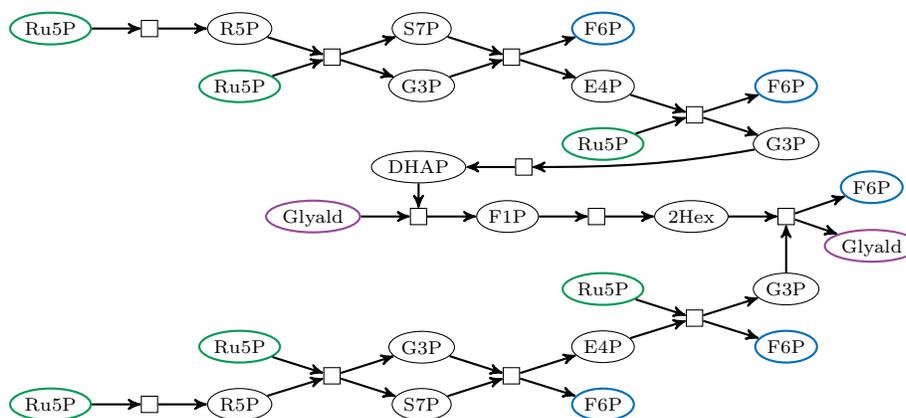


Fig. 6. A realizability certificate for the hyperflow in Fig. 5 where the molecule Glyald is borrowed in order to make it borrow-realizable. The input compounds are marked with green, the output compounds are marked with blue and the borrowed compound is marked with purple.

borrowing tokens in the corresponding Petri net setting has been proposed in [9, Proposition 10] together with a proof that f' is realizable for some b with sufficiently large $|b|$. That is, every integer hyperflow is borrow-realizable.

The combinatorics underlying the non-oxidative phase of the PPP has been analysed less formally in a series of studies focusing, e.g., on simplifying principles that explain the structure of metabolic networks, see e.g. [20,17]. An example of a simple integer hyperflow that is not scaled-realizable is shown in Fig. 5. Here, the production of glyceraldehyde (Glyald) is dependent of the presence of Hex-2-ulose (2Hex), which depends on fructose-1-phosphate (F1P), which in turn depends on Glyald. This cycle of dependencies implies that firing is impossible unless one of the molecules in this cycle is present at the outset, which cannot be achieved by scaling. As illustrated in Fig. 6 and proven by the existence of the realizability certificate, the flow is borrow-realizable with just one borrowing,

We believe that our focus on the realisability of pathways may help raise awareness of the choices one often subconsciously makes when creating pathway illustrations.

Acknowledgements This work is supported by the Novo Nordisk Foundation grant NNF19OC0057834 and by the Independent Research Fund Denmark, Natural Sciences, grant DFF-0135-00420B.

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