Metabolic Network Reductions

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Abstract

Genome-scale metabolic networks are exceptionally huge and even efficient algorithms can take a while to run because of the sheer size of the problem instances. To address this problem, metabolic network reductions can substantially reduce the overwhelming size of the problem instances at hand. We begin by formulating some reasonable axioms defining what it means for a metabolic network reduction to be "canonical" which conceptually enforces reversibility without loss of any information on the feasible flux distributions. Then, we start to search for an efficient way to deduce some of the attributes of the original network from the reduced one in order to improve the performance. As the next step, we will demonstrate how to reduce a metabolic network repeatedly until no more reductions are possible. In the end, we sum up by pointing out some of the biological implications of this study apart from the computational aspects discussed earlier.

Keywords: systems biology; metabolic network analysis; canonical metabolic network reduction; quantitative flux coupling analysis; QFCA.

Author summary

Metabolic networks appear at first sight to be nothing more than an enormous body of reactions. The dynamics of each reaction obey the same fundamental laws and a metabolic network as a whole is the melange of its reactions. The oversight in this kind of reductionist thinking is that although the behavior of a metabolic network is determined by the states of its reactions in theory, nevertheless it cannot be inferred directly from them in practice. Apart from the infeasibility of this viewpoint, metabolic pathways are what explain the biological functions of the organism and thus also what we are frequently concerned about at the system level.

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Canonical metabolic network reductions decrease the number of reactions substantially despite leaving the metabolic pathways intact. In other words, the reduced metabolic networks are smaller in size while retaining the same metabolic pathways. The possibility of such operations is rooted in the fact that the total degrees of freedom of a metabolic network in the steady-state conditions are significantly lower than the number of its reactions because of some emergent redundancies. Strangely enough, these redundancies turn out to be very well-studied in the literature.

1 Introduction

About two decades ago, the first genome-scale metabolic network reconstruction of the cellular metabolism of an organism was published [EP99] shortly after the first genome was sequenced. From that time on, the ever-increasing advances in the high-throughput omics technologies have allowed for the more and more comprehensive reconstructions of exponentially growing sizes [RVSP03, FFF+03, DHP04, FSP+06, JP07, FHR+07, DBJ+07, MSM+07, HSD+08, PMSF09, dODQP+10, OCN+11, MOMM+12, KMPG+12, AHW13, TSF+13, SSH+16, BSZ+18]. These reconstructions have numerous applications in contextualization of high-throughput data, guidance of metabolic engineering, directing hypothesis-driven discovery, interrogation of multi-species relationships, and network property discovery [OPP09]. However, the vast amount of data for some organisms can be a two-edged sword which makes many essential tasks in the metabolic network analysis computationally intractable.

To overcome the demands of systems biology, even while they are outpacing Moore's law [BPS13], faster computational techniques are needed to enable the current methods to scale up to match the progress of high-throughput data generation in a prospective manner. As a natural solution, reducing the size of genome-scale metabolic networks has always been exploited to the advantage of performance for predetermined computational tasks [BVM01, JS14, VPS14, ESK15, RB17]. However, all of these mentioned studies assume that a set of protected reactions which must be retained in the reduced metabolic network is given in advance and the other reactions are dispensable. In this way, these metabolic network reductions are not agnostic to the downstream analysis and lose information on some of the reactions which are irrelevant to the task at hand.

In the field of *flux coupling analysis* (FCA), from the very beginning *Flux Coupling Finder* (FCF) [BNSM04] considers aggregating all the isozymes and removing the blocked reactions. More recently, *fast flux coupling calculator* (F2C2) [LDSB12] considers merging the enzyme subsets [PSnVN+99] too. *Flux-Analyzer* [GK04] also detects conservation relations as a preprocessing step. *MONGOOSE* [CTRB14] employs a similar loss-free network reduction to convert the input model into a canonical form.

Although these general-purpose reductions seem decent in a common sense approach, to the best of

our knowledge the formal definition of a metabolic network reduction, in general, is not clear so far. The aforementioned studies provide strong support for the idea that such general-purpose reductions preserve some interesting attributes of metabolic networks *e.g.*, the flux coupling features. In this article, we specify a broad class of metabolic network reductions with respect to which some generally desired properties of metabolic networks are invariant.

Outline. In §2, we briefly overview the FCA framework. In §3.1 and §3.2, we define and study single and multiple sequential metabolic network reductions, respectively. Afterward in §4, we argue that the reduced metabolic networks are not only interesting from the computational perspective but also they are biologically interpretable. Finally in §5, we conclude by highlighting the contributions of this work and pointing out areas for further research.

2 Background

We specify a metabolic network by an ordered quadruple $\mathcal{N} = (\mathcal{M}, \mathcal{R}, S, \mathcal{I})$ where $\mathcal{M} = \{M_i\}_{i=1}^m$ denotes the set of metabolites of size m, $\mathcal{R} = \{R_i\}_{i=1}^n$ denotes the set of reactions of size n, S denotes the $m \times n$ stoichiometric matrix, and $\mathcal{I} \subseteq \mathcal{R}$ denotes the set of irreversible reactions. Since it is often the case that $n \gg m$, we consider n as the size of $\mathcal{N}$ too.

In the constraint-based analysis of metabolic networks, the constraints $Sv = 0$ and $v_{\mathcal{I}} \geq 0$ are imposed on the metabolic network by the steady-state conditions and the definition of irreversible reactions, respectively. By a slight abuse of notation, $v_{\mathcal{I}} \geq 0$ means $v_i \geq 0$ for all the indices i for which $R_i \in \mathcal{I}$. We denote the *steady-state flux cone* [SSPH99] by

$$\mathcal{C} = \{v \in \mathbf{R}^n \mid Sv = 0,\ v_{\mathcal{I}} \geq 0\},$$

The feasible flux distributions of $\mathcal{N}$ are then defined to be the members of $\mathcal{C}$.

We call $R_k \in \mathcal{R}$ a blocked reaction if $v_k = 0$ for all the feasible flux distributions $v \in \mathcal{C}$. To the end of this paper, whenever we assume that all the blocked reactions are removed, by this, we also assume that if a reversible reaction R_k is blocked in only one direction, then this blocked direction is removed too meaning that R_k is included in $\mathcal{I}$ subsequently. In §3.1, we will review these trivial ways of reducing a metabolic network in more details.

In order to derive even more metabolic network reductions, we should exploit other redundancies of the steady-state flux cone analogous to the case of blocked reactions where the rate of a blocked reaction is always zero irrespective of what the other flux coefficients are equal to. A somewhat less obvious

situation is when the rate of one reaction is unambiguously determined by another one as is the case in the following definitions of FCA.

Definition 1 ([BNSM04])**.** For an arbitrary pair of unblocked reactions $R_i, R_j \in \mathcal{R}$:

Directional coupling: R_i is *directionally coupled* to R_j, denoted by $R_i \longrightarrow R_j$, if for all feasible flux distributions $v_i \neq 0$ implies $v_j \neq 0$.

Partial coupling: R_i is *partially coupled* to R_j, denoted by $R_i \longleftrightarrow R_j$, if both $R_i \longrightarrow R_j$ and vice versa $R_j \longrightarrow R_i$ hold.

Full coupling: R_i is *fully coupled* to R_j, denoted by $R_i \Longleftrightarrow R_j$, if there exists a *full coupling equation* (FCE)

$$v_i = cv_j \qquad c \neq 0, \tag{1}$$

which holds for all $v \in \mathcal{C}$.

We can also apply the following proposition to get the equivalent definitions of FCA, a.k.a *quantitative flux coupling analysis* (QFCA) [TB18]. In the sequel, we will formally show that metabolic network reductions are intrinsically related to QFCA. The intuition is that redundancy in the steady-state flux cone creates flux coupling relations, and metabolic network reductions reduce redundancy by compressing this information.

Proposition 1 ([TB18])**.** *Suppose that $\mathcal{N} = (\mathcal{M}, \mathcal{R}, S, \mathcal{I})$ has no irreversible blocked reactions. Let R_j be an arbitrary unblocked reaction, and $\mathcal{D}_j \subseteq \mathcal{I}$ denote the set of all the irreversible reactions which are directionally coupled to R_j excluding itself. Then, $\mathcal{D}_j \neq \emptyset$ if and only if there exists $c_d > 0$ for each $R_d \in \mathcal{D}_j$, such that the following* directional coupling equation *(DCE)*

$$v_j = \sum_{d:R_d \in \mathcal{D}_j} c_d v_d, \tag{2}$$

holds for all $v \in \mathcal{C}$. Moreover, for any unblocked $R_i \notin \mathcal{I}$, we have $R_i \longrightarrow R_j$ if and only if there exists an extended directional coupling equation *(EDCE)*

$$v_j = \sum_{d:R_d \in \mathcal{D}_j} c'_d v_d + c'_i v_i \qquad c'_i \neq 0,$$

which holds for all $v \in \mathcal{C}$.

Prior to QFCA, the general attitude towards directional coupling was that it is a one-way relation which is, of course, true considering only one pair of reactions. However QFCA, for the first time,

revealed that this relation becomes two-sided if we consider the set of directionally coupled reactions to a single reaction which are sufficient to infer the flux coefficient of it. This redundancy motivates generalizing the previously known application of FCE in the metabolic network reductions to DCE.

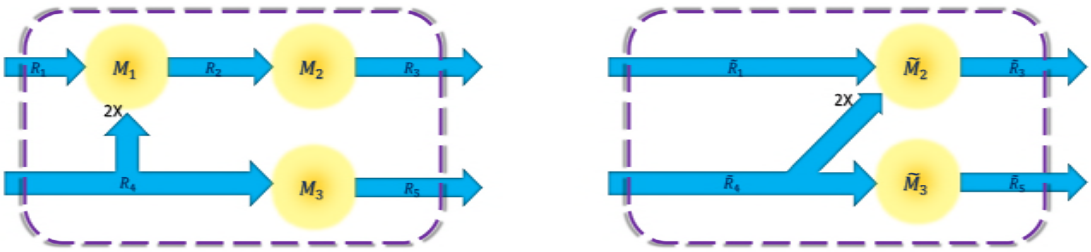
As an illustrating example, consider the following toy metabolic network in Figure 1a. We name it $\mathcal{N} = (\mathcal{M}, \mathcal{R}, S, \mathcal{I})$, where $\mathcal{M} = \{M_1, M_2, M_3\}$, $\mathcal{R} = \{R_1, R_2, R_3, R_4, R_5\}$, $\mathcal{I} = \mathcal{R}$, and

$$S = \begin{bmatrix} +1 & -1 & 0 & +2 & 0 \\ 0 & +1 & -1 & 0 & 0 \\ 0 & 0 & 0 & +1 & -1 \end{bmatrix}.$$

Note that $R_1, R_4 \longrightarrow R_2$ by the corresponding DCE $v_2 = v_1 + 2v_4$. Now if we remove M_1 and incorporate R_2 into R_1 and R_4, then we arrive at the reduced metabolic network in Figure 1b, namely $\tilde{\mathcal{N}} = (\tilde{\mathcal{M}}, \tilde{\mathcal{R}}, \tilde{S}, \tilde{\mathcal{I}})$, where $\tilde{\mathcal{M}} = \{\tilde{M}_2, \tilde{M}_3\}$, $\tilde{\mathcal{R}} = \{\tilde{R}_1, \tilde{R}_3, \tilde{R}_4, \tilde{R}_5\}$, $\tilde{\mathcal{I}} = \tilde{\mathcal{R}}$, and

$$\tilde{S} = \begin{bmatrix} +1 & -1 & +2 & 0 \\ 0 & 0 & +1 & -1 \end{bmatrix}.$$

We will come back to this example for a more careful inspection in §5.



(a) the original metabolic network

(b) the reduced metabolic network

Figure 1: a DCE-induced reduction

We continue by the following general mathematical definition, and subsequently we use this terminology to formalize the notion of a minimal feasible flux distribution. The support of a flux distribution $v \in \mathbf{R}^n$ is denoted by $\mathrm{supp}(v)$, and is defined to be the set of those reactions in $\mathcal{R}$ which are active in this flux distribution, *i.e.*,

$$\mathrm{supp}(v) = \{R_i \in \mathcal{R} \mid v_i \neq 0\}.$$

Definition 2 ([SH94])**.** We call a nonzero feasible flux distribution $0 \neq v \in \mathcal{C}$ an *elementary mode* (EM), if its support is minimal, or equivalently, if there does not exist any other nonzero feasible flux distribution $0 \neq u \in \mathcal{C}$ such that $\mathrm{supp}(u) \subset \mathrm{supp}(v)$.

Given a finite number of vectors $v_1, v_2, \ldots, v_d$, a *conic combination* of these vectors is any weighted sum $\theta_1 v_1 + \theta_2 v_2 + \cdots + \theta_d v_d$ with nonnegative weights $\theta_1, \theta_2, \ldots, \theta_d \geq 0$. The set of all such conic combinations is called the polyhedral convex cone generated by $v_1, v_2, \ldots, v_d$ [BV04].

One of the main properties of EM is that the set of all EMs in a metabolic network generates its steady-state flux cone. Another way of expressing this is to say that any arbitrary feasible flux distribution can be written as a conic combination of EMs. With QFCA in mind, this property implies that to establish a flux coupling relation instead of going through all the feasible flux distributions, it suffices to validate the corresponding flux coupling equation only for the EMs. Accordingly, reducing the size of a metabolic network so long as preserving the information on its EMs and in turn, the flux coupling relations leads to a significant speed-up during QFCA.

As we will see in the next section, the relationship between metabolic network reductions and QFCA does not end here and is surprisingly reciprocal. The uncovered connection between these two concepts sheds light on the mutual nature of both in relation to the core concept of EM. Besides presenting an axiomatic characterization to rigorously examine this connection, we will also derive metabolic network reductions inspired by QFCA which, though intuitive and straightforward, are nevertheless general enough to reduce any arbitrary metabolic network into a minimal irreducible one.

3 Methods

3.1 Canonical metabolic network reductions

Let $\mathcal{N} = (\mathcal{M}, \mathcal{R}, S, \mathcal{I})$ be an arbitrary metabolic network. We say that the metabolic network $\tilde{\mathcal{N}} = (\tilde{\mathcal{M}}, \tilde{\mathcal{R}}, \tilde{S}, \tilde{\mathcal{I}})$ is a reduction of $\mathcal{N}$ if

1. there exists a surjection $\phi : \tilde{\mathcal{C}} \to \mathcal{C}$, where $\tilde{\mathcal{C}}$ and $\mathcal{C}$ are the corresponding steady-state flux cones;
2. there exists a reduction map $r : \tilde{\mathcal{R}} \to \mathcal{P}(\mathcal{R})$ such that for any $\tilde{R}_i \in \tilde{\mathcal{R}}$ we have

$$r(\tilde{R}_i) \nsubseteq \bigcup_{k \neq i} r(\tilde{R}_k), \tag{3}$$

   where $\mathcal{P}(\mathcal{R})$ denotes the power set (the set of all the subsets) of $\mathcal{R}$;
3. and the following diagram commutes

$$\begin{tikzcd} \tilde{\mathcal{C}} \arrow[r, "\mathrm{supp}"] \arrow[d, "\phi"'] & \mathcal{P}(\tilde{\mathcal{R}}) \arrow[d, "\tilde{r}"] \\ \mathcal{C} \arrow[r, "\mathrm{supp}"] & \mathcal{P}(\mathcal{R}) \end{tikzcd}$$

where $\tilde{r} : \mathcal{P}(\tilde{\mathcal{R}}) \to \mathcal{P}(\mathcal{R})$ is defined by

$$\tilde{r}(\{\tilde{R}_i\}_{i\in I}) = \bigcup_{i\in I} r(\tilde{R}_i).$$

Let $\phi^{-1}(v)$ denote the preimage of $v \in \mathcal{C}$ under ϕ. The term $\mathrm{supp}(\phi^{-1}(v))$ is well-defined as for any $\tilde{u}, \tilde{v} \in \phi^{-1}(v)$, we have

$$\tilde{r}(\mathrm{supp}(\tilde{u})) = \mathrm{supp}(\phi(\tilde{u})) = \mathrm{supp}(v) = \mathrm{supp}(\phi(\tilde{v})) = \tilde{r}(\mathrm{supp}(\tilde{v})).$$

Therefore, $\mathrm{supp}(\tilde{u}) = \mathrm{supp}(\tilde{v})$ since $\tilde{r}$ is injective from (3).

Another observation is that for any $\tilde{u}, \tilde{v} \in \tilde{\mathcal{C}}$

$$\mathrm{supp}(\tilde{u}) \subset \mathrm{supp}(\tilde{v}) \Leftrightarrow \tilde{r}(\mathrm{supp}(\tilde{u})) \subset \tilde{r}(\mathrm{supp}(\tilde{v})) \Leftrightarrow \mathrm{supp}(\phi(\tilde{u})) \subset \mathrm{supp}(\phi(\tilde{v})),$$

which is deduced from the second and third properties of the metabolic network reductions, respectively.

We claim that if $\tilde{v} \in \tilde{\mathcal{C}}$ is an EM, then $\phi(\tilde{v})$ is also an EM. The proof is by contradiction. Let us suppose that there exists $u \in \mathcal{C}$ such that:

$$\mathrm{supp}(0) \subset \mathrm{supp}(u) \subset \mathrm{supp}(\phi(\tilde{v})) \Rightarrow \mathrm{supp}(\phi^{-1}(0)) \subset \mathrm{supp}(\phi^{-1}(u)) \subset \mathrm{supp}(\tilde{v})$$

Since $\tilde{v} \in \tilde{\mathcal{C}}$ is an EM, for any $\tilde{u} \in \tilde{\mathcal{C}}$ if $\mathrm{supp}(\tilde{u}) \subset \mathrm{supp}(\tilde{v})$, then $\mathrm{supp}(\tilde{u}) = \emptyset$. However, $\mathrm{supp}(\phi^{-1}(0)) = \mathrm{supp}(\phi^{-1}(u)) = \emptyset$ is in contradiction with $\mathrm{supp}(\phi^{-1}(0)) \subset \mathrm{supp}(\phi^{-1}(u))$ which proves the desired result.

For the converse, we claim that if $v \in \mathcal{C}$ is an EM, then any arbitrary $\tilde{v} \in \phi^{-1}(v)$ is an EM too. Assume to the contrary that for a fixed $\tilde{v} \in \phi^{-1}(v)$ there exists $\tilde{u} \in \tilde{\mathcal{C}}$ such that:

$$\mathrm{supp}(0) \subset \mathrm{supp}(\tilde{u}) \subset \mathrm{supp}(\tilde{v}) \Rightarrow \mathrm{supp}(\phi(0)) \subset \mathrm{supp}(\phi(\tilde{u})) \subset \mathrm{supp}(\phi(\tilde{v})) = \mathrm{supp}(v)$$

Since $v \in \mathcal{C}$ is an EM, for any $u \in \mathcal{C}$ if $\mathrm{supp}(u) \subset \mathrm{supp}(v)$, then $u = 0$. However, $\phi(0) = \phi(\tilde{u}) = 0$ is in contradiction with $\mathrm{supp}(\phi(0)) \subset \mathrm{supp}(\phi(\tilde{u}))$ which completes the proof.

Altogether, for any arbitrary metabolic network reduction $\tilde{v} \in \tilde{\mathcal{C}}$ is an EM if and only if $\phi(\tilde{v}) \in \mathcal{C}$ is an EM. As mentioned earlier, it is possible to infer flux coupling relations from EMs, solely. This evidence suggests that one should be able to deduce some of the flux coupling relations of the original and reduced networks from one another. The following theorem demonstrates one of the many possible ways to do so.

Theorem 3.1 (Flux coupling through reduction)**.** *Suppose that* $\tilde{\mathcal{N}} = (\tilde{\mathcal{M}}, \tilde{\mathcal{R}}, \tilde{S}, \tilde{\mathcal{I}})$ *is a metabolic network*

reduction of $\mathcal{N} = (\mathcal{M}, \mathcal{R}, S, \mathcal{I})$ *by the surjection* $\phi : \tilde{\mathcal{C}} \to \mathcal{C}$ *and the reduction map* $r : \tilde{\mathcal{R}} \to \mathcal{P}(\mathcal{R})$*. For each* $\tilde{R}_i, \tilde{R}_j \in \tilde{\mathcal{R}}$ *such that* $\tilde{R}_i \longrightarrow \tilde{R}_j$*, any reaction in* $r(\tilde{R}_i) \setminus \bigcup_{k \neq i} r(\tilde{R}_k)$ *is directionally coupled to any reaction in* $r(\tilde{R}_j)$*. Conversely, if there exists a reaction in* $r(\tilde{R}_i)$ *which is directionally coupled to some reaction in* $r(\tilde{R}_j) \setminus \bigcup_{k \neq j} r(\tilde{R}_k)$*, then* $\tilde{R}_i \longrightarrow \tilde{R}_j$*.*

Corollary 3.1.1. *By setting* $i = j$*, any reaction in* $r(\tilde{R}_i) \setminus \bigcup_{k \neq i} r(\tilde{R}_k)$ *is directionally coupled to any reaction in* $r(\tilde{R}_i)$*. Consequently, if* $\mathcal{N}$ *has no directionally coupled pair of reactions, then* $r(\tilde{R}_i)$ *must have exactly one distinct element for each* $\tilde{R}_i \in \tilde{\mathcal{R}}$ *and hence, the size of* $\mathcal{N}$ *cannot be reduced any further.*

As an introductory example, suppose that in $\mathcal{N} = (\mathcal{M}, \mathcal{R}, S, \mathcal{I})$, R_i is blocked. Let S^i and $S^{(i)}$ denote the ith column of S and the resulting submatrix after removing it from S, respectively. Trivially, $\tilde{\mathcal{N}} = (\mathcal{M}, \mathcal{R} \setminus \{R_i\}, S^{(i)}, \mathcal{I} \setminus \{R_i\})$ is a reduction of $\mathcal{N}$ by letting

$$\phi(\tilde{v}) = (\tilde{v}_1, \ldots, \tilde{v}_{i-1}, 0, \tilde{v}_i, \ldots, \tilde{v}_{n-1})$$

and $r(R_d) = \{R_d\}$ for all $d \neq i$. In a similar manner, if $R_i \notin \mathcal{I}$ and it is only blocked in the reverse direction, then $\tilde{\mathcal{N}} = (\mathcal{M}, \mathcal{R}, S, \mathcal{I} \cup \{R_i\})$ is a reduction of $\mathcal{N}$ by letting $\phi(\tilde{v}) = \tilde{v}$ and $r(R_d) = \{R_d\}$ for all d. On the other hand, if $R_i \notin \mathcal{I}$ and it is only blocked in the forward direction, then $\tilde{\mathcal{N}} = (\mathcal{M}, \mathcal{R}, \tilde{S}, \mathcal{I} \cup \{R_i\})$ where

$$\tilde{S}^d = \begin{cases} -S^i & \text{if } d = i \\ S^d & \text{otherwise} \end{cases}$$

is a reduction of $\mathcal{N}$ by letting

$$\phi(\tilde{v}) = (\tilde{v}_1, \ldots, \tilde{v}_{i-1}, -\tilde{v}_i, \tilde{v}_{i+1}, \ldots, \tilde{v}_n)$$

and $r(R_d) = \{R_d\}$ for all d. By applying Theorem 3.1, $\mathcal{N}$ and $\tilde{\mathcal{N}}$ have exactly the same set of flux coupling relations in all these three cases.

For another example, suppose that in $\mathcal{N} = (\mathcal{M}, \mathcal{R}, S, \mathcal{I})$, distinct R_i and R_j are fully coupled with (1) as their corresponding FCE. If we define $\phi(\tilde{v}) = (\tilde{v}_1, \ldots, \tilde{v}_{i-1}, v_i, \tilde{v}_i, \ldots, \tilde{v}_{n-1})$ where

$$v_i = \begin{cases} c\tilde{v}_j & \text{if } i > j \\ c\tilde{v}_{j-1} & \text{otherwise} \end{cases}$$

for the surjection, and

$$r(R_d) = \begin{cases} \{R_i, R_j\} & \text{if } d = j \\ \{R_d\} & \text{otherwise} \end{cases}$$

for the reduction map, then the metabolic network specified by $(\mathcal{M}, \mathcal{R} \setminus \{R_i\}, \hat{S}^{(i)}, \mathcal{I} \setminus \{R_i\})$, where

$$\hat{S}^d = \begin{cases} S^j + cS^i & \text{if } d = j \\ S^d & \text{otherwise} \end{cases}$$

is a reduction of $\mathcal{N}$.

By applying Theorem 3.1, all of the flux coupling relations of this reduced network are valid in the original network as well. Furthermore, if in the reduced network R_k is directionally, partially, or fully coupled to R_j or vice versa, then in the original network R_k is directionally, partially, or fully coupled to R_i or vice versa, and these are all the additional flux coupling relations of $\mathcal{N}$.

Arguably, fully coupled reactions are the most comprehensible ones as FCE gives an entirely straightforward relation between their activity rates. QFCA provides DCE and EDCE relating the rates of directionally coupled reactions, which naturally generalize FCE given by the full coupling definition. Later on, we intend to generalize the latter example from FCE to DCE. The point that makes DCE especially suitable for this purpose is that, just like FCE, it both maintains the steady-state conditions and respects the irreversibility constraints because of the positivity of the coefficients of (2).

Suppose that in a metabolic network $\mathcal{N} = (\mathcal{M}, \mathcal{R}, S, \mathcal{I})$, all the blocked reactions have already been removed and all the fully coupled reactions have already been merged so that there exists no more previously investigated reductions applicable to further reduce $\mathcal{N}$. For any $R_j \in \mathcal{R}$ such that $\mathcal{D}_j \neq \emptyset$ and (2) holds, we refer to $\tilde{\mathcal{N}} = (\mathcal{M}, \mathcal{R} \setminus \{R_j\}, \hat{S}^{(j)}, \mathcal{I} \setminus \{R_j\})$ where

$$\hat{S}^d = \begin{cases} S^d + c_d S^j & \text{if } R_d \in \mathcal{D}_j \\ S^d & \text{otherwise} \end{cases}$$

as a *DCE-induced reduction* of $\mathcal{N}$ if $\phi(\tilde{v}) = (\tilde{v}_1, \ldots, \tilde{v}_{j-1}, v_j, \tilde{v}_j, \ldots, \tilde{v}_{n-1})$, where

$$v_j = \sum_{d<j:R_d \in \mathcal{D}_j} c_d \tilde{v}_d + \sum_{d>j:R_d \in \mathcal{D}_j} c_d \tilde{v}_{d-1},$$

and

$$r(R_d) = \begin{cases} \{R_d, R_j\} & \text{if } R_d \in \mathcal{D}_j \\ \{R_d\} & \text{otherwise} \end{cases}$$

is its corresponding reduction map.

One can easily check to see that this is actually a legitimate metabolic network reduction. Therefore by applying Theorem 3.1, we conclude that all the flux coupling relations of the reduced network are valid in the original network as well. Moreover, we already know which reactions are directionally coupled to

R_j. But then again, how can we tell whether $R_j \longrightarrow R_i$ or not for some $R_i \in \mathcal{R} \setminus \{R_j\}$, without undoing the changes which were applied to $\mathcal{N}$? The following Lemma answers this question.

Lemma 3.2. *Let $r^{-1}(R_i)$ denote the set of reactions $\tilde{R}_d \in \tilde{\mathcal{R}}$ such that $R_i \in r(\tilde{R}_d)$. For an arbitrary pair $R_i, R_j \in \mathcal{R}$, if each of the reactions in $r^{-1}(R_i)$ is directionally coupled to some reaction in $r^{-1}(R_j)$, then $R_i \longrightarrow R_j$.*

Remark. As an immediate corollary for any arbitrary metabolic network reduction, $R_i \longrightarrow R_j$ if $r^{-1}(R_i) \subseteq r^{-1}(R_j)$.

By applying Lemma 3.2 to the DCE-induced reduction corresponding to R_j, if all of the reactions in $r^{-1}(R_j)$ are directionally coupled to R_i, then $R_j \longrightarrow R_i$. Yet the converse is also true, because directional coupling is transitive, thus $\mathcal{D}_j \longrightarrow R_j \longrightarrow R_i$. Hence in order to determine if $R_j \longrightarrow R_i$ holds in the original metabolic network, it is enough to check if $r^{-1}(R_j) \longrightarrow R_i$ holds in the DCE-reduced metabolic network. This derives all the extra flux coupling relations of $\mathcal{N}$ as was promised before.

3.2 Sequential metabolic network reductions

Let $\mathcal{N} = (\mathcal{M}, \mathcal{R}, S, \mathcal{I})$ be an arbitrary metabolic network, and $\tilde{\mathcal{N}}_1 = (\tilde{\mathcal{M}}_1, \tilde{\mathcal{R}}_1, \tilde{S}_1, \tilde{\mathcal{I}}_1)$ be a reduction of $\mathcal{N}$ by ϕ_1 and r_1. Suppose that we apply several metabolic network reductions to $\mathcal{N}$ successively. For instance, suppose that we reduce $\tilde{\mathcal{N}}_1$ once more to get $\tilde{\mathcal{N}}_2 = (\tilde{\mathcal{M}}_2, \tilde{\mathcal{R}}_2, \tilde{S}_2, \tilde{\mathcal{I}}_2)$ by a different metabolic network reduction, namely the one corresponding to ϕ_2 and r_2. We will show that the composition of consecutive reductions is again a reduction itself.

1. $\phi_1 \circ \phi_2 : \tilde{\mathcal{C}}_2 \to \mathcal{C}$ is a surjection because the composition of surjective functions is surjective;

2. $\tilde{r}_1 \circ r_2 : \tilde{\mathcal{R}}_2 \to \mathcal{P}(\mathcal{R})$ is a legitimate reduction map because for any $\tilde{R}_i \in \tilde{\mathcal{R}}_2$ we have

$$\exists \tilde{R}_j \in r_2(\tilde{R}_i) \setminus \bigcup_{k \neq i} r_2(\tilde{R}_k) \Rightarrow \exists R_t \in r_1(\tilde{R}_j) \setminus \bigcup_{k \neq j} r_1(\tilde{R}_k) \Rightarrow R_t \in \tilde{r}_1 \circ r_2(\tilde{R}_i) \setminus \bigcup_{k \neq i} \tilde{r}_1 \circ r_2(\tilde{R}_k);$$

3. and the following diagram commutes

$$\begin{tikzcd}
\tilde{\mathcal{C}}_2 \arrow[r, "\mathrm{supp}"] \arrow[d, "\phi_2"'] & \mathcal{P}(\tilde{\mathcal{R}}_2) \arrow[d, "\tilde{r}_2"] \\
\tilde{\mathcal{C}}_1 \arrow[r, "\mathrm{supp}"] \arrow[d, "\phi_1"'] & \mathcal{P}(\tilde{\mathcal{R}}_1) \arrow[d, "\tilde{r}_1"] \\
\mathcal{C} \arrow[r, "\mathrm{supp}"] & \mathcal{P}(\mathcal{R})
\end{tikzcd}$$

because for any $\tilde{v} \in \tilde{\mathcal{C}}_2$

$$\mathrm{supp}(\phi_1 \circ \phi_2(\tilde{v})) = \tilde{r}_1(\mathrm{supp}(\phi_2(\tilde{v}))) = \tilde{r}_1 \circ \tilde{r}_2(\mathrm{supp}(\tilde{v})).$$

Putting it all together, $\tilde{\mathcal{N}}_2$ is a reduction of $\mathcal{N}$ by the surjection $\phi_1 \circ \phi_2$ and the reduction map $\tilde{r}_1 \circ r_2$.

In §3.1, we have covered how to reduce the metabolic network by the following three stages.

- First, we eliminate all the blocked reactions.
- Second, we merge all the fully coupled reactions.
- Third, we remove the eligible reactions by the DCE-induced reductions.

Suppose that after conducting all these three stages, the last metabolic network is $\tilde{\mathcal{N}} = (\tilde{\mathcal{M}}, \tilde{\mathcal{R}}, \tilde{S}, \tilde{\mathcal{I}})$ of size $\tilde{n}$, thus we obtain the chain of reductions

$$\mathcal{N} \xrightarrow{\phi_1, r_1} \tilde{\mathcal{N}}_1 \xrightarrow{\phi_2, r_2} \cdots \xrightarrow{\phi_{n-\tilde{n}}, r_{n-\tilde{n}}} \tilde{\mathcal{N}}_{n-\tilde{n}},$$

where $\tilde{\mathcal{N}}_{n-\tilde{n}} = \tilde{\mathcal{N}}$ since each reduction reduces exactly one reaction at a time. Throughout this process, we consider the aggregated metabolic network reduction resulting from combining the previously applied ones up to some point in time as a single reduction, *i.e.*,

$$\mathcal{N} \xrightarrow{\phi^i, r^i} \tilde{\mathcal{N}}_i,$$

where $\phi^i = \phi_1 \circ \cdots \circ \phi_i$ and $r^i = \tilde{r}_1 \circ \cdots \circ \tilde{r}_{i-1} \circ r_i$.

We claim that each ϕ^i is a one-to-one correspondence when restricted to the EMs. In §3.1, we proved that $\tilde{v} \in \tilde{\mathcal{C}}$ is an EM if and only if $\phi^i(\tilde{v}) \in \mathcal{C}$ is an EM. Furthermore, ϕ^i is surjective by definition. It only remains to show that ϕ^i is injective too. One can easily verify the injectivity of the single metabolic network reductions ϕ_t of any of these three kinds separately; hence it is also proved for ϕ^i which is the composition of i of them.

As a consequence, the overall reduction generated by compositing all the preceding three stages is a metabolic network reduction which provides a one-to-one correspondence between the EMs of $\mathcal{N}$ and $\tilde{\mathcal{N}}$. This can also be shown independently, given the straightforward form of this specific reduction which we characterize next.

For the sake of simplicity, assume that we arrange $\mathcal{R}$ in an ordering such that first come the blocked reactions removed in the first stage, $\mathcal{B} = \{R_1, \ldots, R_b\}$. After $\mathcal{B}$ come the reactions which persist to the end, and by this we mean the final set $\tilde{\mathcal{R}}$ which is a subset of $\mathcal{R}$ because $\tilde{\mathcal{R}}_t \subseteq \tilde{\mathcal{R}}_{t-1}$ for each of these

three types of reductions individually. At last come the reactions which were removed in stages two and three, respectively. Moreover, assume that $\tilde{\mathcal{R}} = \{\tilde{R}_i\}_{i=1}^{\tilde{n}}$ is sorted in such a way that $\tilde{R}_i$ is fully coupled to a_i other reactions in $\mathcal{R}$ where $a_1 \geq a_2 \geq \cdots \geq a_f > a_{f+1} = \cdots = a_{\tilde{n}} = 0$ (the descending order of the number of fully coupled reactions in $\mathcal{R}$).

By these simplifying assumptions, if P is the permutation matrix which permutes the columns of S to the ordering we have assumed, then we can give the following explicit formulas for $\tilde{S}$, $\phi^{n-\tilde{n}}$, and $r^{n-\tilde{n}}$ which is $\tilde{S} = SPA$, $\phi^{n-\tilde{n}}(\tilde{v}) = PA\tilde{v}$, and $r(\tilde{R}_i)$ is equal to the index set of the nonzero entries of the ith column of A with the following structure.

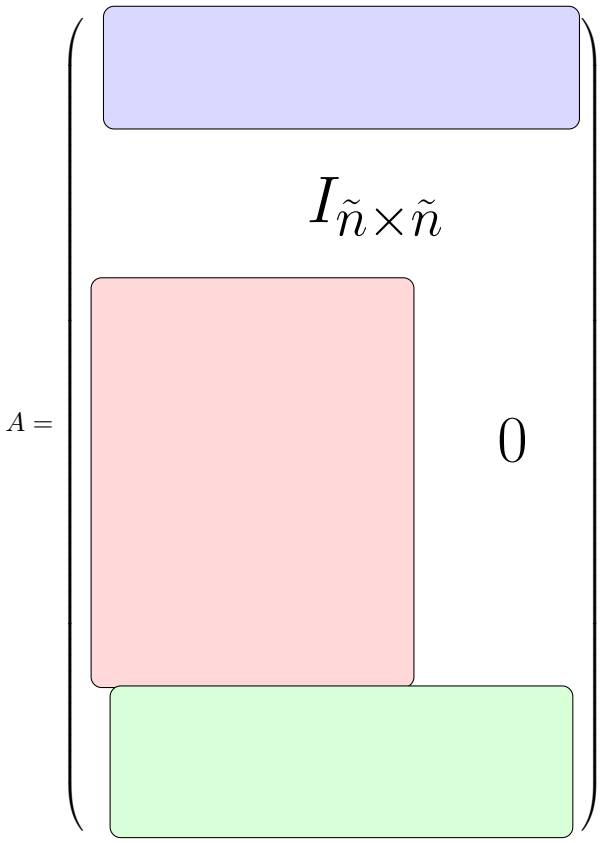


Figure 2: sparsity pattern of A

As depicted in Figure 2, the blue block has b zero rows corresponding to the blocked reactions which always have zero flux coefficients. The identity matrix corresponds to $\tilde{\mathcal{R}}$ which in turn is associated with a subset of $\mathcal{R}$. The red block corresponds to the fully coupled reactions removed in stage two, and its nonzero entries are the associated FCE coefficients *i.e.*, c in (1). So in the end, each row of the nonnegative matrix C corresponds to a reaction removed in stage three and its positive entries are the associated DCE coefficients *i.e.*, c_d in (2).

Ultimately by Corollary 3.1.1, because of the fact that this metabolic network has no more directional coupling relations (or otherwise we could continue stage three), it cannot be reduced any further without violating one of the principles we have assumed for the canonical metabolic network reductions. Even more than that, not only $\tilde{\mathcal{N}}$ is minimal in the sense that it cannot be reduced any further, but also it has the minimum size, and in this sense, this is the most compact form of an equivalent steady-state flux cone with identical EMs.

For the proof of this statement, we show that no two distinct reactions in $\tilde{\mathcal{R}}$ can be reduced to the same reaction in any possible metabolic network reduction, hence $\tilde{n}$ is the minimum size. Let $\tilde{\mathcal{N}}' = (\tilde{\mathcal{M}}', \tilde{\mathcal{R}}', \tilde{S}', \tilde{\mathcal{I}}')$ be an arbitrary reduction of $\mathcal{N}$ by ϕ' and r'. By Corollary 3.1.1, if $\tilde{R}_p, \tilde{R}_q \in \tilde{\mathcal{R}} \subseteq \mathcal{R}$ are reduced to the same reaction $\tilde{R}'_t \in \tilde{\mathcal{R}}'$, then there exists $R_s \in r'(\tilde{R}'_t) \setminus \bigcup_{k \neq t} r'(\tilde{R}'_k)$ which is directionally coupled to both $\tilde{R}_p$ and $\tilde{R}_q$, simultaneously. However, $R_s \Longleftrightarrow \tilde{R}_p$ if $R_s \longrightarrow \tilde{R}_p$, otherwise $\tilde{R}_p$ would have been removed in the third stage. Therefore $\tilde{R}_p \Longleftrightarrow R_s \Longleftrightarrow \tilde{R}_q$, which cannot hold unless $p = q$ since stage two guarantees that no two distinct reactions in $\tilde{\mathcal{R}}$ are fully coupled to each other.

4 Discussion

EM analysis, while still limited by the size of the metabolic networks it can handle, is a powerful metabolic pathway analysis tool for a wide variety of application such as characterizing cellular metabolism and reprogramming microbial metabolic pathways [TWS09, TT12, ZRHJ13]. This potential encourages conceptualizing the notion of a canonical metabolic network reduction which respects EMs as has been proposed by [GK04] long ago.

In order to realize this idea, we have proposed three axioms for characterizing a canonical metabolic network reduction. The first axiom enforces that each feasible flux distribution in either the reduced or the original metabolic network corresponds to at least one feasible flux distribution in the other one. The second axiom introduces the mapping r between reactions, which induces the injection $\tilde{r}$ between pathways essential for the third axiom to enforce that having minimal support is an invariant property under ϕ. This, in turn, implies that the EMs of these two metabolic networks are in correspondence with each other.

The only suspect property in here which seems like a strong assertion is (3) in the second axiom. Recall that by Corollary 3.1.1, all the reactions in $r(\tilde{R}_i) \setminus \bigcup_{k \neq i} r(\tilde{R}_k)$ turn out to be fully or partially coupled to each other and hence are intimately related. Consequently, instead of some abstract $\tilde{R}_i$ in an unreal metabolic network, we can think of each $\tilde{R}_i$ as a class of fully or partially coupled reactions in the current metabolic network which is very similar to the notion of enzyme subsets [PSnVN$^+$99]. Whereas, if for some $\tilde{R}_i \in \tilde{\mathcal{R}}$ we had $r(\tilde{R}_i) \subseteq \bigcup_{k \neq i} r(\tilde{R}_k)$, then no reaction in $\mathcal{R}$ was uniquely associated to $\tilde{R}_i$. To

sum up, the main reason behind this assertion is to ensure that reactions in $\tilde{\mathcal{R}}$ are meaningful from the biological point of view.

We give the example of coupled metabolites to further demonstrate this point. *Metabolite concentration coupling analysis* (MCCA) [NBM05] is the same as FCA except it is applied to the left null space of the stoichiometric matrix [FP03]. We refer the interested reader to the results of [NBM05] for an extensive discussion of the biological relevance of the coupled metabolites. Note that the subsets $r(\tilde{R}_i) \setminus \bigcup_{k \neq i} r(\tilde{R}_k)$ are exactly the same metabolite subsets in this instance. Furthermore, the reduced metabolic network preserves the reactions among these metabolically meaningful pools in addition to all the MCCA results derived from FCA. These reactions restrain the minimal conserved pools (the analogue of elementary modes) to be in one-to-one correspondence with the original set for *minimal conserved pool identification* (MCPI) [NBM05].

5 Results

QFCA[1] is open-source and publicly available for academic and research purposes. This MATLAB© package is the first implementation of the reduction algorithm presented here. It paves the way for the development of scalable solutions for various computational tasks. This brings us to a suggestion for the future research to work out the details of integrating this universal preprocessing (or postprocessing as in *NetworkReducer* [ESK15]) step with the compatible pipelines of interest.

The reduced stoichiometric matrix introduced in §3.2 has a great potential to speed-up the downstream analyses which only rely on EMs, in particular, QFCA. The smaller the reduced $\tilde{S}$, the faster any downstream analysis on this proxy stoichiometric matrix. For a running example, iMM1415 (*Mus Musculus*, 1415 genes) [SJS+10] has 3726 reactions initially, and MONGOOSE reduces it into 1625 reactions while QFCA achieves a record 1171 reactions in the same way as we described in §3.1 and §3.2.

Even though $\tilde{S}$ has m rows in theory, it turns out that for almost every real-world metabolic network, many of its rows are identically zero and therefore, for all practical purposes they may be removed safely. For instance, consider the metabolic network in Figure 1a and suppose that we remove R_2 by the corresponding DCE-induced reduction. Although, M_1 is still there after conducting the metabolic network reduction according to the procedure of §3.1, it can be removed to arrive at the metabolic network in Figure 1b since it is no longer involved in any reactions.

Moreover, we remove the linearly dependent rows of $\tilde{S}$ as described under the title of the detection of conservation relations by [GK04]. In practice, $\tilde{S}$ may be much smaller than S both regarding the number of rows and columns. Going back to our running example, the number of metabolites can be

[1] https://mtefagh.github.io/qfca/

reduced from 2775 to 335 by QFCA while the corresponding number for MONGOOSE is 643.

We should mention that in spite of the fact that the reduced $\tilde{S}$ has a smaller size than the original S, it might be denser for some instances. As a result, if some algorithm is already exploiting the sparsity of S intricately, perhaps it does not gain much from this preprocessing step. This is a common theme in numerical analysis that sometimes sparse computation can make up for not including off-the-shelf preprocessing steps reducing the size of sparse matrices. Nonetheless, in our running example, the number of nonzero elements of S and $\tilde{S}$ are 14053 and 14385, respectively. These numbers are promising as they indicate that the reduced stoichiometric matrix is nearly as sparse as the original one.

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Appendix

Proof of Theorem 3.1. In order to show the forward direction of the theorem, assume that $R_1 \in r(\tilde{R}_i) \setminus \bigcup_{k \neq i} r(\tilde{R}_k)$ and $R_2 \in r(\tilde{R}_j)$. If $v_1 \neq 0$ for some arbitrary $v \in \mathcal{C}$, then we have

$$v_1 \neq 0 \Rightarrow R_1 \in \mathrm{supp}(v) \Rightarrow R_1 \in \tilde{r}(\mathrm{supp}(\phi^{-1}(v))) \Rightarrow R_1 \in \bigcup_{\tilde{R}_k \in \mathrm{supp}(\phi^{-1}(v))} r(\tilde{R}_k).$$

From the assumption that $R_1 \notin \bigcup_{k \neq i} r(\tilde{R}_k)$, we have

$$\tilde{R}_i \in \mathrm{supp}(\phi^{-1}(v)) \Rightarrow \tilde{R}_j \in \mathrm{supp}(\phi^{-1}(v)) \Rightarrow r(\tilde{R}_j) \subseteq \mathrm{supp}(v) \Rightarrow R_2 \in \mathrm{supp}(v) \Rightarrow v_2 \neq 0.$$

Since $v \in \mathcal{C}$ was arbitrary, this proves one direction of the theorem.

To show the other direction of the theorem, assume that $\tilde{v}_i \neq 0$ for some arbitrary $\tilde{v} \in \tilde{\mathcal{C}}$. Additionally, assume $R_1 \in r(\tilde{R}_i)$ and $R_2 \in r(\tilde{R}_j) \setminus \bigcup_{k \neq j} r(\tilde{R}_k)$ are such that $R_1 \longrightarrow R_2$. Then,

$$\tilde{v}_i \neq 0 \Rightarrow \tilde{R}_i \in \mathrm{supp}(\tilde{v}) \Rightarrow R_1 \in \mathrm{supp}(\phi(\tilde{v})) \Rightarrow R_2 \in \mathrm{supp}(\phi(\tilde{v})) \Rightarrow \tilde{R}_j \in \mathrm{supp}(\tilde{v}) \Rightarrow \tilde{v}_j \neq 0.$$

Therefore, $\tilde{R}_i \longrightarrow \tilde{R}_j$ as was desired. $\square$

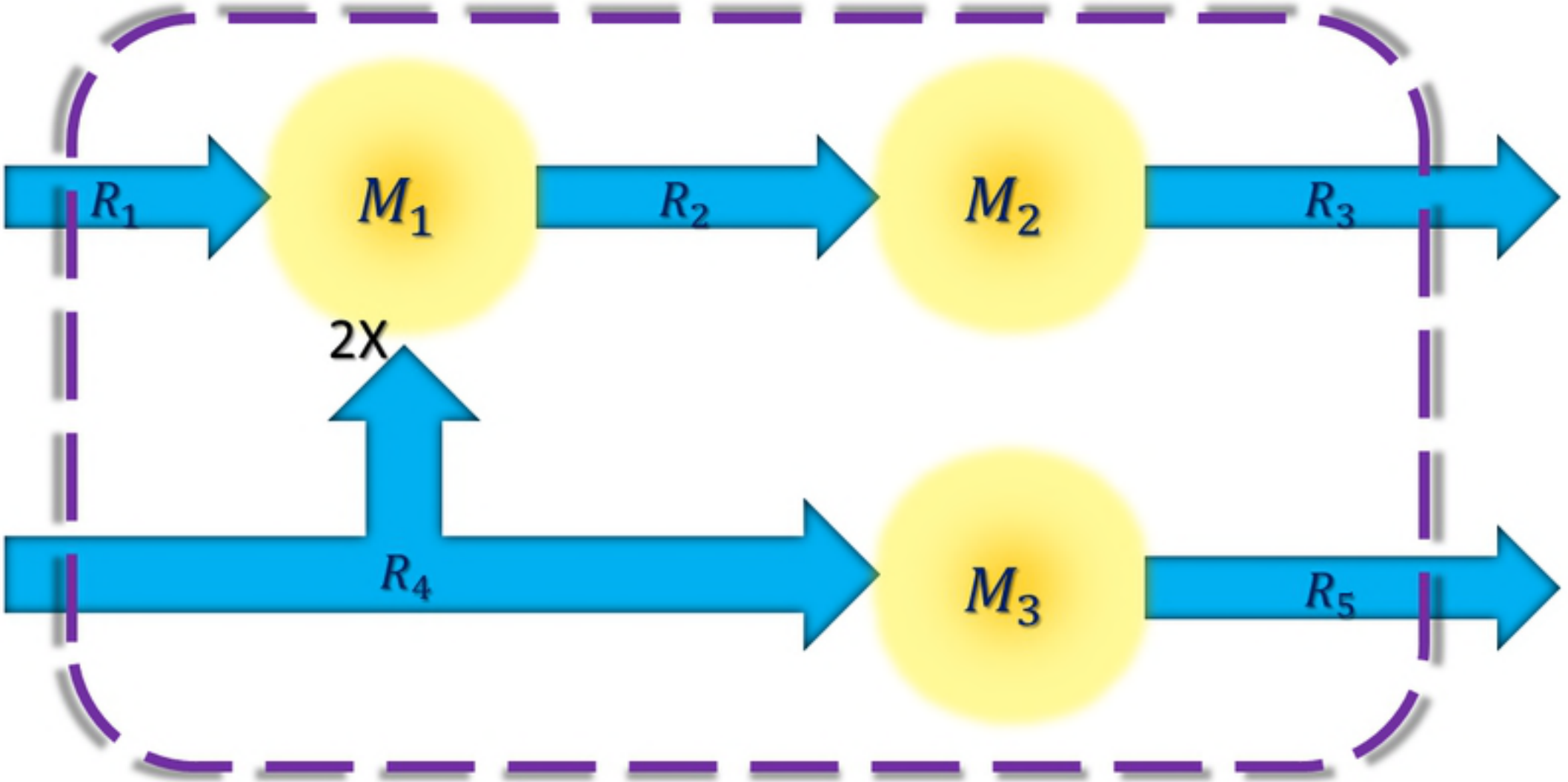
Proof of Lemma 3.2. If $v_i \neq 0$ for some fixed $v \in \mathcal{C}$, then we have

$$v_i \neq 0 \Rightarrow R_i \in \operatorname{supp}(v) \Rightarrow r^{-1}(R_i) \cap \operatorname{supp}(\phi^{-1}(v)) \neq \emptyset.$$

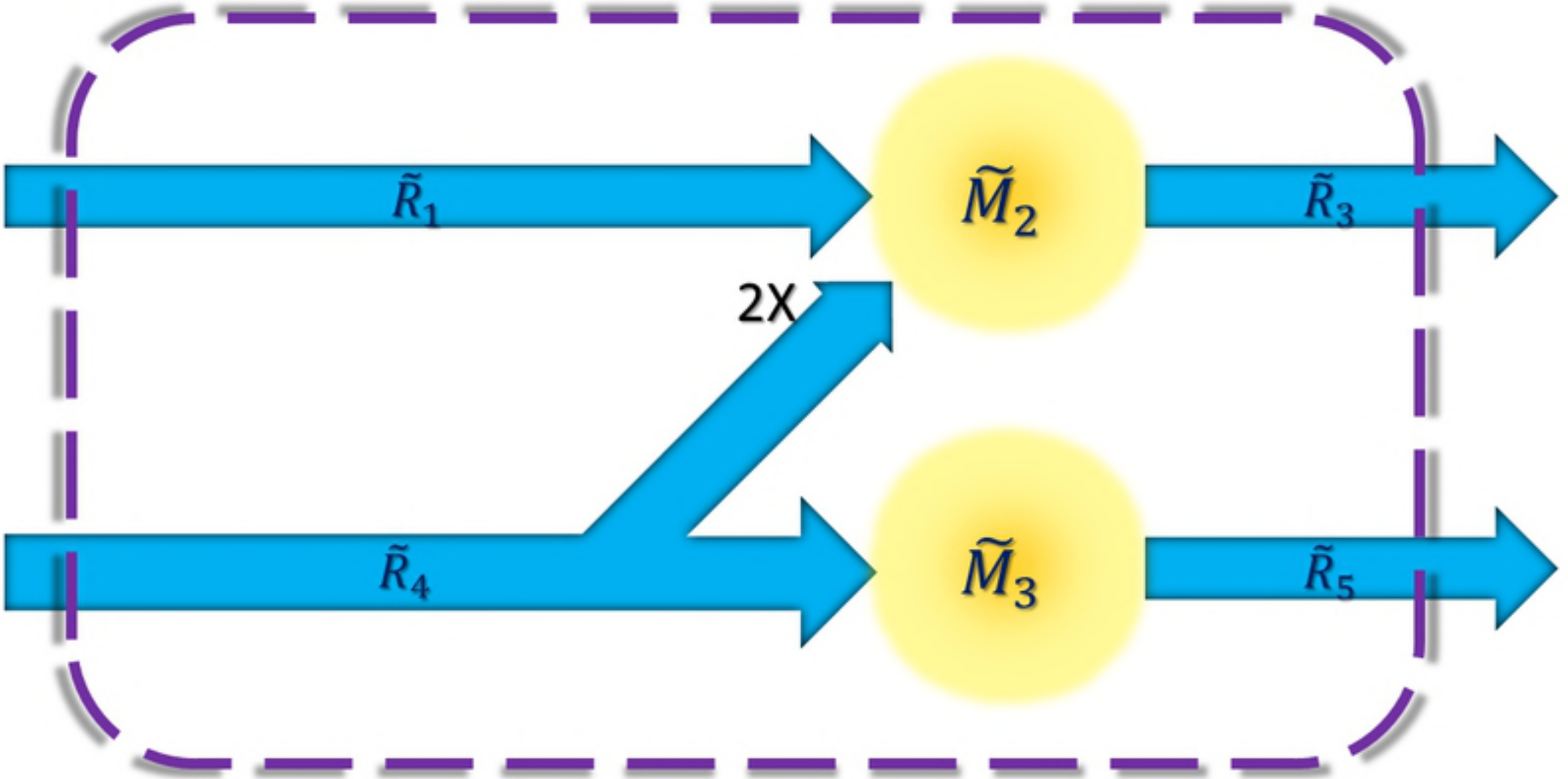
Without loss of generality, assume that $\tilde{R}_p \in r^{-1}(R_i) \cap \operatorname{supp}(\phi^{-1}(v))$. Since $\tilde{R}_p \in r^{-1}(R_i)$, there exists $\tilde{R}_q \in r^{-1}(R_j)$ such that $\tilde{R}_p \longrightarrow \tilde{R}_q$. Therefore,

$$\tilde{R}_q \in \operatorname{supp}(\phi^{-1}(v)) \Rightarrow r(\tilde{R}_q) \subseteq \operatorname{supp}(v) \Rightarrow R_j \in \operatorname{supp}(v) \Rightarrow v_j \neq 0.$$

Since $v \in \mathcal{C}$ was arbitrary, the proof of the lemma is complete. $\square$



Figure



Figure