Monomolecular reaction networks: flux-influenced sets and balloons

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ABSTRACT

In living cells we can observe a variety of complex network systems such as metabolic network. Studying their sensitivity is one of the main approaches for understanding the dynamics of these biological systems. The study of the sensitivity is done by increasing/decreasing, or knocking out separately, each enzyme mediating a reaction in the system and then observing the responses in the concentrations of chemicals or their fluxes. However, due to the complexity of the systems, it has been unclear how the network structures influence/determine the responses of the systems. In this study, we focus on monomolecular networks at steady state and establish a simple criterion for determining regions of influence when any one of the reaction rates is perturbed through sensitivity experiments of enzyme knock-out type. Specifically, we study the network response to perturbations of a reaction rate j^* and describe which other reaction rates j' respond by nonzero reaction flux, at steady state. Nonzero responses of j' to j^* are called flux-influence of j^* on j'. The main and most important aspect of this analysis lies in the reaction graph approach, in which the chemical reaction networks are modeled by a directed graph. Our whole analysis is function-free, i.e, in particular, our approach allows a graph theoretical description of sensitivity of chemical reaction networks. We emphasize that the analysis does not require numerical input but is based on the graph structure only. Our specific goal here is to address a topological characterization of the flux-influence relation in the network. In fact we characterize and describe the whole set of reactions influenced by a perturbation of any specific reaction.

1 INTRODUCTION

We follow here an approach developed by Fiedler and Mochizuki in [1] and [2]. For related, but different settings, see also the pioneering works by Feinberg [3] and [4].

A *chemical reaction* is a process that trasforms some chemical substances, called *reactants* or *reagents* of the reaction, to other substances, called *products* of the reaction. If the reactants are H, 0 and the product $H_2 0$, we write $2H + 0 \to H_2 0$ for describing the reaction which, starting from Hydrogen and Oxygen, leads to water. The coefficients in front of the chemicals are called stoichiometric coefficients of the reaction. A chemical reaction *network* is therefore a network of such processes, namely a group of connected and interacting chemical reactions.

A *monomolecular reaction network* is a chemical reaction network in which any reaction j just converts one single metabolite m into another single one m'. Namely just reactions of the kind A→B, B→C, etc., are allowed. Of course in this monomolecular case also the stoichiometric coefficients are always equal to 1. In our approach we model a *monomolecular reaction network* as a strict directed graph Γ with a vertex set $\mathbf{M} \cup \{0_F, 0_E\}$ and an arrow set $\mathbf{A}$. Here we call a directed graph *strict* if it has no self-loops and no two arrows have the same ordered endpoints. A dipath is any acyclic ordered sequence of alternatingly adjacent vertices and arrows. The vertices belonging to $\mathbf{M}$ are called *metabolites* and the arrows are called *reactions.* 0_F and 0_E are the *zero-complexes* introduced by Feinberg in [3]. According to Feinberg, we shall think the zero-complex as *'a complex in which the stoichiometric coefficient of every species is zero'* (see for more details [3], Remark 4.A). 0_F possesses just outgoing reactions, which are called *feed reactions*, while 0_E possesses just ingoing reactions, which are called *exit reactions.* Any dipath that has as ending element the vertex 0_E is called an *exit dipath.* We sometimes may omit the subscript $_E$ when no ambiguity arises.

For getting from chemical reactions structure to mathematical dynamics, let $e_m \in \mathbb{R}^{|\mathbf{M}|}$ be the m-th unit vector, for any nonzero metabolite $m \in \mathbf{M}$, and define $e_0 = 0 \in \mathbb{R}^{|\mathbf{M}|}$. The ODE for the dynamics of the vector $x{=}(x_m)_{m\in\mathbf{M}}$ of *concentrations* x_m of the metabolites $m \in \mathbf{M}$ is

$$\dot{x} = g(x) = f(\mathbf{r}, x) := \sum_{j\in\mathbf{A}} r_j(x_{m(j)})(e_{h(j)} - e_{m(j)}).$$

Here $\mathbf{r} = (r_j)_{j\in\mathbf{A}}$ are the reaction rate functions and it is crucial to underline that we consider them as smooth given parameters. With $m(j)$ we refer to the mother, i.e. input, metabolite of reaction j and with $h(j)$ to the head, i.e. product, metabolite of reaction j. Moreover, we make some mathematical assumptions, in particular we assume:

1. positivity of the reaction rate functions $r_j \in C^1$, i.e., $r_j(\xi) > 0$ if $\xi > 0$,
2. the existence of a positive steady state $x^* > 0$, i.e., $g(x^*) = 0$,
3. the regularity of the network at the steady state x^*, i.e., det $f_x(\mathbf{r}, x^*) \neq 0$.

In [1] there is an important result which states that these assumptions imply a structural feature of our monomolecular reaction network which is crucial to be underlined: from any metabolite m there exists a dipath to 0_E. For more details see [1], Lemma 2.3. Therefore, in our model, a *monomolecular reaction network* is precisely a strict directed graph with a special vertex 0_E such that from any other vertex m there exists a dipath from m to 0_E.

We skip some mathematical details of what we precisely mean by perturbation of the rate function r_j. Suffice it to say that we consider C^1 small perturbations of the kind $\mathbf{r}^\epsilon := \mathbf{r} + \epsilon\rho$. As a tool, we can apply the implicit function theorem, thanks to the regularity assumption. Suppose now that ρ perturbs the reaction j^*, only, and leaves all other reaction functions untouched. We define the infinitesimal concentration response $\partial x_m^{j^*}$ of metabolite m at steady state as

$$\partial x_m^{j^*} := \tfrac{d}{d\epsilon}|_{\epsilon=0} x_m^*(\epsilon).$$

Moreover, we say that $\partial x_m^{j^*}$ is *algebraically nonzero* if $\partial x_m^{j^*} \neq 0$ as a rational function of the derivatives $r_{jm} := r_j'(x_m^*)$ with m mother metabolite of reaction j. We define the infinitesimal change of the flux through reaction arrow j', in response to a rate perturbation of reaction j^*, as

$$\Phi_{j'j^*} := \delta_{j'j^*} + r_{j'm(j')} \partial x_{m(j')}^{j^*},$$

where $\delta_{j'j^*}$ is the Kronecker-delta. We then say that a reaction j^* *flux-influences* a reaction j', in symbols $j^* \rightsquigarrow j'$, if the flux response $\Phi_{j'j^*}$ of reaction j' to a rate perturbation as above of reaction j^* satisfies $\Phi_{j'j^*} \neq 0$, algebraically.

The crucial theorem on the flux-influence relation, according to the paper [1] by Fiedler and Mochizuki, reads as follows:

Theorem 1 (Fiedler&Mochizuki, 2015). *Let the above assumptions hold, and consider any pair of reaction arrows (j', j^*), not necessarily distinct. Then j^* flux-influences j' ($j^* \rightsquigarrow j'$) if, and only if, there exist two dipaths γ^0 and γ' for which the following four conditions all hold true:*

1. *both dipaths emanate from m^*, the mother metabolite of j^*;*
2. *one of the dipaths contains j^*;*
3. *γ^0 terminates at vertex 0_E, and γ' terminates with arrow j', omitting the head vertex of j';*
4. *except for their shared starting vertex m^*, the two dipaths γ^0 and γ' are disjoint.*

For a proof of this result, see section 3 of [1].

We provide here a different, more extended and precise topological characterization and description of the whole set of reactions flux-influenced by the perturbation of a specific reaction j. We give a visual description of this set, describe its structure and deepen its properties. This approach simplifies some otherwise more involved problems. Transitivity of flux-influence relation becomes in this way a simpler inclusion result on the flux-influenced sets. Some specific features of flux-influence relation arise now in a crystal clear way. For instance, the fact that if a metabolite m has only two outgoing reactions, these two reactions influence exactly the same set.

For doing this, we have to introduce some new concepts which have arisen naturally in the investigation and description of the structure of the flux-influenced sets. Firstly, we say that a reaction j is in the **m0-direction** if there exists a dipath γ from metabolite m to 0_E containing j. We will mainly use this definition for outgoing reactions from m. See figure 1. We underline that this is a consistent definition due to the fact that a dipath has to be acyclic. It may happen, indeed, that the only way for reaching 0_E from m going through j is to make a dicycle around m, which is forbidden by definition of dipath, which has an injective structure. Moreover, as said before, by structure of monomolecular reaction network, from any metabolite m there exists a dipath from m to 0_E. This implies that there is always at least one outgoing reaction from m in the $m0$-direction. In particular, if j is an outgoing reaction from metabolite m *not* in the $m0$-direction, there is at least another outgoing reaction j_0 from m which is in the $m0$-direction.

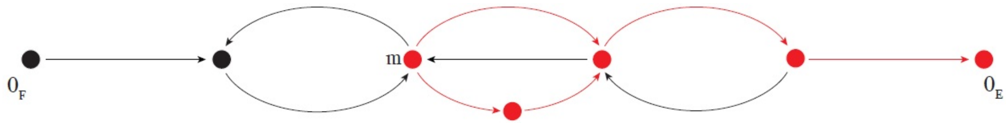


Figure 1: *In a generic monomolecular reaction netowork, we have chosen a metabolite m. In red, we have coloured the subset of the $m0$-direction.*

The second tool is the principal and leading idea of our work: the obliged elements. We provide three **equivalent** definitions, to give a viewpoint as wide as possibile. For a concrete example, see figure 2. The set O_0^m of **obliged elements from m to 0** is the intersection set of *all* dipaths from m to 0_E. In a more graph theoretic language, we may say that this set O_0^m is the set of the *cut-vertices* and *cut-edges (bridges)* of the subnetwork which consists of all dipaths from m to 0_E. Finally, plainly said, this set O_0^m contains all elements of the network whose deletion renders 0_E unreachable by any dipath from m. This set O_0^m has several interesting properties. It is crucial to mention one of those here: it possesses a total order. Of course any dipath from m to 0_E trivially induces a natural order in the set of the obliged elements from m to 0, since all the obliged elements are in particular elements of this dipath, which, being directed, possesses a natural order. By acyclicity, this order does not depend on the chosen dipath. In particular we may speak about the *first, second, ...* obliged element from m to 0. See Section 3, *Properties of obliged elements*, for further details.

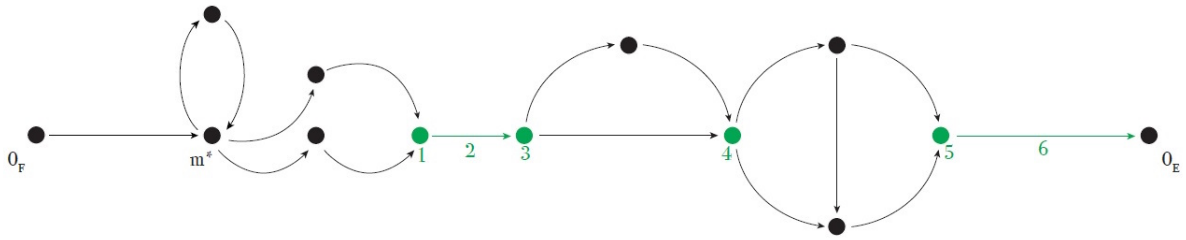


Figure 2: *In a generic example of monomolecular reaction network, we have picked a metabolite m^* and, in green, we have marked the obliged elements from m to* 0.

Lastly, we define the following subnetwork:

Definition 1 (Balloon). Let $x(m)$ be the *first* obliged element from m to 0. We define the $(m, 0)$-**Balloon** B_0^m as the subnetwork reachable from m with a dipath without passing through $x(m)$.

A Balloon consists of different components, see figure 3 for a comparison: for any outgoing reaction j' from m that is *not* in the $m0$-direction then the set reachable with a dipath from m starting along j', which we indicate with $R^{m,j'}$, is a subset of the $(m, 0)$-Balloon. Moreover, if m has just two outgoing reactions, the above j' and another reaction j'', this implies that j'' has to be in the $m0$-direction, by the structure of monomolecular reaction networks. Then j'' is clearly the first obliged element and the $(m, 0)$-Balloon is just $R^{m,j'}$, the set reachable from m with a dipath starting along j'. But, if m has more then two outgoing reactions, then, for any outgoing reaction j' from m not in the $m0$-direction, $R^{m,j'}$ is a strict subset of the $(m, 0)$-Balloon.

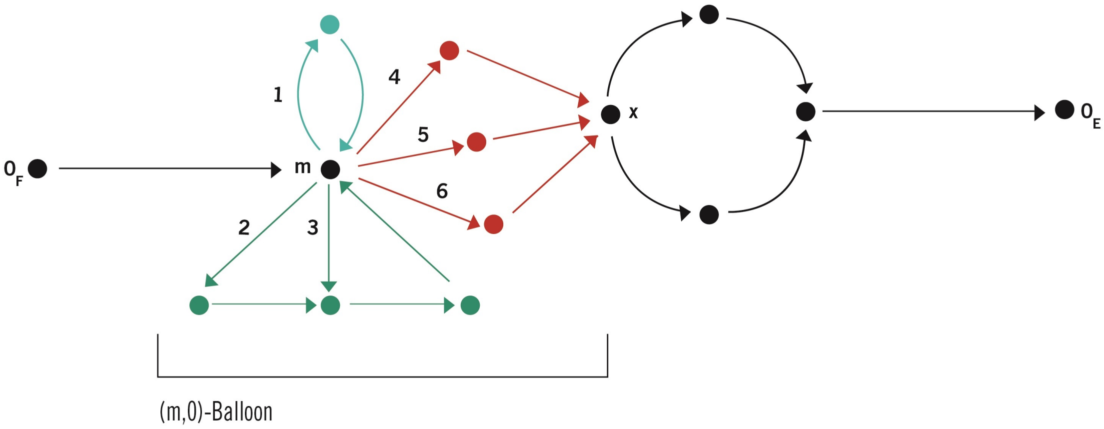


Figure 3: *The figure illustrates the structure of a* $(m, 0)$*-Balloon.* x *is the first obliged element from* m *to* 0*. From the metabolite* m *emanate six outgoing reactions. Three reactions (1, 2, 3) are* not *in the* $m0$*-direction, three reactions (4, 5, 6) are in the* $m0$*-direction. We underline that the sets reachable with dipaths starting along reaction not in the* $m0$*-direction are contained in the* $(m, 0)$*-Balloon. We have marked this part of the Balloon with greenish colours. In this case the inclusion is strict, since* m *has more than one outgoing reactions in the* $m0$*-direction.*

Now we get to our main theorem, which characterizes precisely the set flux-influenced by the perturbation of a single reaction. The only exception to this theorem is given by the artificial feed reactions. We remind that the feed reactions are the outgoing reactions from 0_F. Their influence has indeed a very specific and simple behaviour, which we are not going to deepen here, see [1]. Suffice it to say that the set flux-influenced by a feed reaction i is the entire reachable set from 0_F with a dipath starting along i. Very simple structure, indeed. We underline also the fact that these reactions are only a theoretical tool useful in some circumstances.

Theorem 2 (Characterization of flux-influenced sets)**.** *Let* j *be a reaction with its mother metabolite* m*.*
Then the set flux-influenced by j*,* $I(j)$*, is characterized as follows:*

1. *if* j *is in the* $m0$*-direction, then* $I(j)$ *consists of all reactions in the* $(m, 0)$*-balloon* B_0^m*, and no others,*

2. *if* j *is* not *in the* $m0$*-direction, then* $I(j)$ *consists of all reactions in* $R^{m,j}$*, namely all reactions reachable from* m *with a dipath starting along* j*, and no others.*

We underline again that the set $R^{m,j}$ described in the latter case 2, namely the set flux-influenced by a perturbation on a reaction which is *not* in the $m0$-direction, is a subset of the $(m,0)$-balloon B^m_0.
In section 4 we deeply analyse in more details the structure of the flux-influenced sets. The following theorem and corollary arise straightforwardly from this analysis:

Theorem 3 (Inclusion of Flux-influenced sets)**.** *If j^* influences j', then $I(j^*) \supseteq I(j')$.*

This inclusion theorem leads automatically to the following important property of the flux-influence relation:

Corollary 4 (Transitivity of Flux-influence relation)**.** *The flux-influence relation is a transitive relation, i.e., $j^* \rightsquigarrow j' \rightsquigarrow j''$ implies $j^* \rightsquigarrow j''$.*

Recently, Brehm and Fiedler generalized this transitivity result to multimolecular networks, following a more algebraic approach (for this, see [5]). In future work, it is our goal to extend this topological and visual approach also to multimolecular networks.

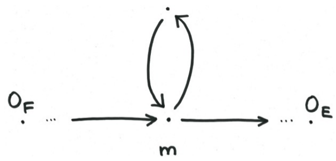
Finally, some structural features which have arisen easily as consequences of our analysis are worth to be mentioned. They give an example of the advantages of our topological approach. We have chosen some of them, which are the most significative, in our opinion:

1. For any outgoing reaction from metabolite m, no flux-influence outside the $(m,0)$-Balloon.
   Indeed, if the outgoing reaction is in the $m0$-direction, then the influenced set is precisely the $(m,0)$-Balloon. If it is *not* in the $m0$-direction, then it is a subset (which may be strict or no) of the $(m,0)$-Balloon.

2. The set flux-influenced by a reaction j is empty, namely $I(j) = \emptyset$, if and only if its mother metabolite m has just this only one outgoing reaction j.
   Indeed, if m has just one outgoing arrow j, then j is in particular the first obliged element from m to 0. Of course no reaction arrows are reachable from m without passing through j since j is the only outgoing arrow of m, then the $(m,0)$-Balloon$= \emptyset$ and in particular $I(j) = \emptyset$.
   In the other direction, if m has at least two outgoing arrows j_1 and j_2, then j_1 may be either in the $m0$-direction or not. If j_1 is in the $m0$-direction, then j_1 influences for sure j_2. In fact j_2 is not for sure the first obliged element from m to 0 and hence it lies in the $(m,0)$-Balloon. If j_1 is not in the $m0$-direction, then j_1 influences for sure j_1 itself. In both cases, $I(j_1) \neq \emptyset$.

3. If i and j are two outgoing reactions from metabolite m, both in the $m0$-direction, then $I(i) = I(j)$.
   Indeed the influenced set of both is defined by the $(m,0)$-Balloon whose definiton does not depend specifically on i or j.

4. If i and j are the two only outgoing reactions from m, then $I(i) = I(j)$.
   Indeed: if they are both in the $m0$-direction, then we have the previous observation. If one of them, wlog i, is not in the $m0$-direction, then j has to be the first obliged element from m to 0. Therefore $I(j)$, the $(m,0)$-Balloon, is just the set $R^{m,i}$ reachable by m with a dipath starting along i, namely $I(i)$. Note however that, in this last case, j flux-influences i while i does not flux-influence j.



5. In conclusion, if a metabolite m has n outgoing reactions with $p \leq n-2$ not in the $m0$-direction, then the number of different flux-influenced sets of the outgoing reactions is exactly $p+1$, one for each reaction not in the $m0$-direction, and the $(m,0)$-Balloon flux-influenced by the reactions in the $m0$-direction, which, in particular, contains all the other flux-influenced sets.

The remaining paper is organized as follows. In section 2 we define clearly some technical graph tools which are needed for giving simpler and more elegant proofs of our results. Section 3 deepens the features and structure of Obliged Elements. Our results are completely proven in section 4. The last section 5 provides interesting and important examples.

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2 PATHS: CONCATENATIONS, SHORTCUTS

Let γ be a dipath. Let p, q be elements of γ. Let

$$\tilde{\gamma} = \gamma[p,q]$$

denote the subpath of γ which starts at the element p and, following the same sequence of vertices and arrows as γ, ends with q, including p, q. We will largely use this notation also for referring generically to a dipath from p to q.
Moreover, when the situation renders impossible to use the above notation, we will use also the intuitive notations with parenthesis $\gamma(x,y)$, $\gamma(x,y]$, $\gamma[x,y)$ for referring to dipaths from x to y which do not include, respectively, x and y; x; y.

Let us, now, consider two concatenated dipaths in a graph, i.e., a dipath from x to y and then a dipath from y to z, where x, y, z are vertices. We want to define an operation which allows us to easily obtain an existing dipath from x to z. This will be very useful in our proofs. The definition, then, is as follows. See, for comparison, figure 4.

Definition 2 (Last-minute shortcut and skipped elements)**.** Consider any two dipaths $\gamma_1 = \gamma_1[x, y]$ from x to y and $\gamma_2 = \gamma_2[y, z]$ from y to z, with x, y, z vertices of a digraph. Let w be the last vertex, in the natural order given by γ_2, of the nonempty intersection $\gamma_1 \cap \gamma_2$. Then we call the concatenated dipath

$$\gamma := \gamma_1[x, w)\gamma_2[w, z]$$

the *last-minute shortcut* and we use the notation $\gamma = s(\gamma_1\gamma_2)$.
Moreover, let p be an element (either a vertex or an arrow) such that p belongs either to γ_1 or to γ_2 but not to the last-minute shortcut $\gamma = s(\gamma_1\gamma_2)$. We call p a *skipped element.*

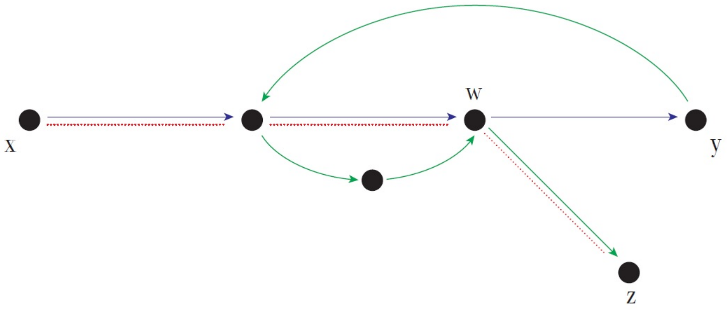


Figure 4: *In the picture, two concatenated dipaths (in blue from x to y and in green from y to z). In red we have underlined the last-minute shortcut.*

Observation 1. The last-minute shortcut is, by construction, a dipath from x to z. Indeed, since w is the last intersection between γ_1 and γ_2 in the order of γ_2, this implies that $\gamma_1 \cap \gamma_2[w, z] = \{w\}$ only, and hence in particular $\gamma_1[x, w] \cap \gamma_2[w, z] = \{w\}$ only. Therefore the sequence $\gamma = \gamma_1[x, w)\gamma_2[w, z]$ counts each elements just once and it is a dipath.

3 PROPERTIES OF OBLIGED ELEMENTS

Let $\gamma = \gamma[m, 0_E]$ be an exit dipath from m to 0_E. Trivially, γ induces a natural order in O_0^m, the obliged elements from m to 0, since all the obliged elements are in particular elements of γ, which, as a dipath, possesses a natural order. The following proposition asserts that any exit dipath from m to 0_E agrees on this order.

Proposition 5 (Order property)**.** *Any exit dipath $\gamma = \gamma[m, 0_E]$ from metabolite m to 0_E induces the same order in the set O_0^m of the obliged elements from m to 0 and therefore O_0^m admits a total order.*

Proof. Assume indirectly that exist $\bar{\gamma}, \tilde{\gamma}$ exit dipaths from m to 0_E that induce in the set O_0^m a different order, i.e., $\exists\, p, q \in O_0^m$, obliged elements from m to 0 such that:
$p < q$ in the order given by $\bar{\gamma}$;
$q < p$ in the order given by $\tilde{\gamma}$.
Consider now the last-minute shortcut $\gamma^* = s(\bar{\gamma}[m, p]\tilde{\gamma}[p, 0_E])$: γ^* is an exit dipath from m to 0_E, but γ^* does not contain q. Indeed neither $\bar{\gamma}[x, p]$ nor $\tilde{\gamma}[p, 0_E]$ contains q since $\bar{\gamma}$ reaches p before q and $\tilde{\gamma}$ reaches p after q. Hence q is not an obliged element from m to 0 against our hypothesis and we have a contradiction.

□

The following proposition characterizes the topological position of an outgoing reaction j from metabolite m in terms of obliged elements to 0.

Proposition 6 (Obliged elements and $m0$-direction)**.** *Let j be a reaction with its mother metabolite m. The following statements are equivalent:*

1. *j is not in the $m0$-direction;*
2. *for every metabolite $\tilde{m}$ in $R^{m,j}$, the metabolite m is an obliged element from $\tilde{m}$ to 0_E.*

[We recall that $R^{m,j}$ consists of all the elements of the network reachable from metabolite m with a dipath starting along j.]

Proof. 1)⇒2)
Assume indirectly that m is not an obliged element from $\tilde{m}$ to 0.
Then there exists an exit dipath $\gamma_0^{\tilde{m}}$ from $\tilde{m}$ to 0_E s.t. $m \notin \gamma_0^m$. We can therefore consider the last minute shortcut $\bar{\gamma}{=}s(\gamma_{\tilde{m}}^m \gamma_0^{\tilde{m}})$ where $\gamma_{\tilde{m}}^m$ is any dipath from m to $\tilde{m}$ containing j. $\bar{\gamma}$ is then a dipath from m to 0_E, and it contains j since $\gamma_0^{\tilde{m}}$ does not contain m, the mother of j, and hence in particular the last intersection between the two dipaths in the order of $\gamma_0^{\tilde{m}}$ (according with the definition) cannot be m. So we have reached a contradiction.
2)⇒1)
Consider any exit ordered sequence of alternatingly adjacent vertices and arrows, so called *diwalk*, even possessing cycles, $[mj...0_E]$ from m to 0_E with j as first arrow.
Assume now that it is possible to write some of these sequences as a concatenation of two dipaths $[mj...\tilde{m}][\tilde{m}...0_E]$. Otherwise trivially j is not in the $m0$-direction, since any dipath can be written as a concatenation of two dipaths.
So we have that $\tilde{m}$ is in $R^{m,j}$ and therefore, by our assumption, $m \in O_0^{\tilde{m}}$.
This holds for every $\tilde{m}$ vertex reachable from m with a dipath starting along j and therefore every sequence $[mj...0_E]$ counts m at least twice, namely no sequence is a dipath.
Hence j is not in the $m0$-direction.

□

We now describe an inclusion property of the set of obliged elements. This property is at the base of proving our more general result on the inclusion of flux-influenced sets.

Proposition 7 (Inclusion property)**.** *For any metabolite $\tilde{m}$ in the $(m, 0)$-Balloon, the following holds:*

$$O_0^m \subseteq O_0^{\tilde{m}},$$

i.e., the obliged elements from m to 0 are contained in the ones from $\tilde{m}$ to 0.

Proof. Note that if a metabolite $\tilde{m}$ is the $(m, 0)$-Balloon, by definition of Balloon, in particular there exists a directed path γ^m that emanates from metabolite m and ends at metabolite $\tilde{m}$ withouth passing through any of the obliged elements from m to 0.
Suppose now indirectly that $\exists p \in O_0^m$ s.t. $p \notin O_0^{\tilde{m}}$.
Then, by definition of obliged elements from $\tilde{m}$ to 0, there exists a dipath $\tilde{\gamma}_0 = \tilde{\gamma}_0[\tilde{m}, 0_E]$ from m to 0_E s.t. p is not in $\tilde{\gamma}_0$.
We can therefore consider the last-minute shortcut $\bar{\gamma}_0 = s(\gamma^m[m, \tilde{m})\tilde{\gamma}_0)$, by construction $\bar{\gamma}_0$ is an exit dipath from m to 0_E s.t. p is not in γ_0 against the hypothesis that p is an obliged element from m to 0. Hence we have a contradiction. □

Observation 2. In particular then, since every obliged element p from m to 0 is an obliged element from every $\tilde{m}$ in the $(m, 0)$-Balloon there exists a dipath from $\tilde{m}$ to p.

Let us conclude this section by giving a very intuitive and not mathematical explanation of the last proposition: imagine we are in a room that has a storage room reachable just with a door from the room where we are. If we go wherever without passing this door, and then want to reach the storage room, we will eventually have to pass though this single door.

4 PROOFS

This section is organized as follows: at first we state a crucial lemma, which is needed for all the proofs. Its proof relies strongly on Menger's Theorem, a classical graph theory theorem stated by Karl Menger in 1927 and then generalized in the famous Max-Flow-Min-Cut theorem in the fifties. We are going to recall this theorem in the version which perfectly fits our purposes and we derive from it a straighforward but nonetheless useful corollary. After this we are able to prove lemma 8, theorem 2 and theorem 3.

The main ingredient for the proofs of the above results is to simplify the four conditions in the Fiedler-Mochizuki theorem 1 and reformulate them using the idea and properties of the obliged elements. Our reformulation reads as follows.

Lemma 8 (Reformulated influence conditions). *Given* (j', j^*, m^*) *as before, the Fiedler-Mochizuki theorem 1 on the flux-influence holds true if, and only if, there exists a dipath* γ_R *such that the following three conditions all hold true:*

1. γ_R *emanates from vertex* m^* *and terminates with reaction edge* j'*, not including the head vertex of* j'*,*
2. γ_R *has empty intersection with the obliged elements from* m^* *to* 0*,*
3. j^* *is in the* m^*0*-direction or it is in* γ_R *(both may happen simultaneously).*

Transitivity of flux-influence relation may be proven also directly from this lemma, using properties of the set of the obliged elements. This is indeed a nice example of their application. However, we will not do this way here.

Firstly, we recall briefly Menger's Theorem. The central idea of this theorem, in the version for directed graphs, is to set an equivalence between the order of cut-sets (namely, the minimum number of elements whose deletion disconnects the graph) and the number of internally disjoint dipaths. Two dipaths are called *internally disjoint* if they share starting and ending point only, namely they do intersect just in these points and they do not intersect 'internally'. There are many versions of Menger's Theorem, both for directed and undirected graphs, both for cut-sets consisting just of vertices and cut-sets consisting just of edges/arrows. We will not pursue here further explanations, which, along with more references, may be completely found in [6, 7, 8, 9, 10].
We will use the following vertex version of Menger's Theorem for directed graph.

Theorem 9 (Menger, 1927)**.** *Let x and y be vertices in a directed graph Γ, such that y is reachable from x. Then the maximum number of dipaths from x to y internally disjoint is equal to the minimum number of vertices whose deletion renders y unreachable with a dipath from x.*

We state a simple corollary, perfectly suited for our specific purposes here.

Corollary 10. *Let m be a metabolite in a monomolecular reaction network, and let $x(m)$ be the first obliged element from m to 0. If there are not any of them, let us assume $x(m) = 0_E$. Moreover assume that $x(m)$ is a vertex. Then there exist at least two dipaths internally disjoint from m to $x(m)$.*

Proof. Considering the subnetwork which consists of all dipaths from m to $x(m)$, this subnetwork has no single cut-elements. Indeed if this was the case, then this cut-element would be in particular also an obliged element from m to 0. But this is excluded by our assumption that $x(m)$ is the *first* of them. If there are no cut-elements, then in particular the minimum number of metabolites whose deletion renders $x(m)$ unreachable with a dipath from m has to be at least two. Therefore, according to Menger's theorem, the maximum number of dipaths internally disjoint from m to $x(m)$ is at least two, as well. $\square$

We can now start looking at the proof of the crucial lemma 8. Before going on to prove it, we explain the idea of the proof. The fact that the old formulation implies the new is very easy to prove. The opposite direction, however, that the new formulation implies the old, is more complicated. For doing this we will have indeed to build two dipaths as the old Fiedler-Mochizuki theorem requires. Except from the easy situations, what we generally need to show is that condition 2 of the lemma implies the existence of two internally disjoint paths. We use then corollary 10, which guarantees us that two internally disjoint dipaths exist between m^* and the first obliged element from m^* to 0. This provides us an undirected cycle structure with a source (m^*) and a sink ($x(m)$, the first obliged element from m^* to 0). Then we intersecate this structure with γ'_R for building the two desired directed paths.

Proof of lemma 8. We start with the easy part.

Firstly we prove that the conditions of theorem 1 imply the reformulated conditions 1), 2) and 3).
Indeed, assume conditions i), ii), iii), iv) of theorem 1 and let $\gamma'_R{:=}\gamma'$.
Condition 1) of lemma 8 is verified by definition of γ', which is a dipath from m to j'.
Condition 2) can be proved indirectly: if $\gamma'_R \cap O_0^{m^*} \neq \emptyset$, then there not exists an exit dipath γ_0 that intersects with γ' only in the emanating metabolite m^* as in theorem 1, since any exit dipath γ_0 contains all the obliged elements from m^* to 0.
Condition 3) of lemma 8 is directly implied by condition ii) in theorem 1. Indeed, by condition ii) of theorem 1, j^* is either in γ' or in γ_0. In the first case, j^* is in γ'_R, in the latter it is in the m^*0-direction.
This concludes the proof of the fact that conditions in theorem 1 implies conditions in lemma 8.

Assume now the reformulated conditions 1, 2 and 3 of lemma 8. Our goal is to show that there exist two dipaths γ_0 and γ' as in the Fiedler-Mochizuki theorem 1.

Let us now note two things.
Firstly, j^* cannot be the only outgoing arrow from m^*. If this would be the case, indeed, j^* would be in particular the first obliged element from m^* to 0 and therefore any dipath from m^* would contain the obliged element j^*, contradicting condition 2.
Secondly, if j^* is the only outgoing arrow from m^* which lies in the m^*0-direction, in particular j^* is the first obliged element from m^* to 0. Then the dipath γ'_R emanates from m^* along a reaction arrow $i \neq j^*$ which is *not* in the m^*0-direction. In particular, any exit dipath γ_0 from m^* to 0, belonging to the m^*0-direction, does not intersect γ'_R. Hence we have that the pair of directed paths so defined (γ_0, γ'_R) easily fullfills the conditions i), ii), iii), iv) of theorem 1.

For this reason, let us now assume that m^* has at least two outgoing reactions in the m^*0-direction. The first obliged element from m^* to 0 is therefore a vertex $x(m^*)$. It might happen also that there are no obliged elements from m^* to 0, in this case we set simply $x(m^*) = 0_E$. We can therefore apply corollary 10 and we obtain two dipaths $\gamma_{1,2}$ from m^* to x internally disjoint. Namely two dipaths that do intersect just in m^* and $x(m^*)$, i.e. $\gamma_1 \cap \gamma_2 = \{m^*, x(m^*)\}$. We now want to use the structure of this undirected cycle of $\gamma_1 \cup \gamma_2$.
We have to check all possible cases.
The case in which γ'_R intersects $\gamma_1 \cup \gamma_2$ just in m^* is easier. Indeed we take γ'_R and $\tilde{\gamma}_0 := \gamma_1[m^*, x(m^*))\gamma_x[x(m^*), 0]$, where γ_x is any exit dipath from $x(m^*)$ to 0_E. As already noticed, we have that γ'_R and $\tilde{\gamma}_0$ do intersect *only* in m^*. If they intersected in a vertex $w \neq m^*$, indeed, in particular $w \in \gamma_x$ since γ'_R intersect γ_1 just in m^*. We may consider, then, the sequence $\gamma'_R[m^*, w)\gamma_x[w, 0]$ obtaining a directed ordered sequence of vertices and arrows from m^* to 0 not containing the obliged element x, which is a contradiction. Therefore these two directed paths, γ'_R and $\tilde{\gamma}_0$ satisfy conditions i), iii), iv) of Theorem 1. Moreover, if j^* is not in the m^*0-direction, then it has to be in γ'_R and we have also condition ii).
If γ'_R has more than one intersection with $\gamma_1 \cup \gamma_2$, in particular we have that j^* is in the m^*0-direction, since we can build easily, with a last-minute shortcut, a dipath from m^* to 0 with j^* as first arrow.
Let y be the last element, in the order given by γ'_R, of $[\gamma_1 \cup \gamma_2] \cap \gamma'_R$. We know for sure that it is not $x(m^*)$ by condition 2. Without losing our generalities, we assume $y \in \gamma_1$. We can obtain a new dipath from m^* to j' by

$$\tilde{\gamma}' := \gamma_1[m^*, y)\gamma'_R[y, j']$$

By construction, we have that $\gamma_2 \cap \tilde{\gamma}' = \{m^*\}$, namely our new dipath from $\tilde{\gamma}'$ still does not intersect γ_2 apart from in m^*.
Let γ_x be any exit dipath from $x(m^*)$ to 0. The two dipaths $\tilde{\gamma}_0 = \gamma_2\gamma_x$ and $\tilde{\gamma}'$ already satisfy conditions i), iii), iv).
In any unfinished situation, we have at least found a pair of directed paths $(\tilde{\gamma}_0, \gamma')$ such that the conditions i), iii), iv) of theorem 1. We need now to work on condition ii): j^* may, indeed, belong to $\tilde{\gamma}'$, $\tilde{\gamma}_0$ or to none. In the first two cases, we have for free also condition ii), hence we have finished.
In the third case: $j^* \notin \tilde{\gamma}_0 \cup \tilde{\gamma}'$, but j^* has to belong to some γ_0^* exit dipath from m^*, since we have already noticed that j^* is in the m^*0-direction.
Let z be now the *first* element after m^* of $[\tilde{\gamma}_0 \cup \tilde{\gamma}'] \cap \gamma_0^*$, in the order given by γ_0^*. (For sure the intersection is not just m^* since also x surely belongs to this intersection).

Just the followings can happen:
1) $z \in \tilde{\gamma}_0$,
2) $z \in \tilde{\gamma}'$.
In the first case we set $\gamma_0 := \gamma_0^*[m^*, z)\tilde{\gamma}_0[z, 0]$ and $\gamma' := \tilde{\gamma}'$. In the second case we set $\gamma' := \gamma_0^*[m^*, z)\tilde{\gamma}'[z, j']$ and $\gamma_0 := \tilde{\gamma}_0$. Considering the couple (γ_0, γ') as above defined we easily verify that it satisfies conditions i) ii) iii) iv). $\square$

Now we can prove also theorem 2, namely the characterization of the whole set of reaction arrows flux-influenced by a perturbation on a specific reaction j.

Proof of Theorem 2. Using the reformulated theorem:
Part 1: if j is in the $m0$-direction, condition 3 of lemma 8 is always satisfied. Therefore we just need to take care of the fact that j' is reachable with a dipath with empty intersection with the obliged elements from m to 0, corresponding to conditions 1 and 2 in the lemma 8. By the total order of the set of the obliged elements, these conditions hold if and only if j' is reachable from m with a dipath without passing through $x(m)$, the first of them. Namely if and only if j' is in the $(m, 0)$-Balloon.
Part 2: if j is not in the $m0$-direction, then, by condition 3 of lemma 8, j has to be in the influence dipath γ'_R, which leads to the influenced reaction arrow j'. Therefore to be in $R^{m,j}$ is a necessary condition for any reaction which is flux-influenced by j. By the structure of the Balloon, we have that this condition is also sufficient, since no exit dipath from m to 0 intersects with $R^{m,j}$ if j is not in the $m0$-direction, except trivially in m. In particular, no obliged element can be in $R^{m,j}$. This concludes our proof. $\square$

Now, lastly, we turn on the inclusion result.

Proof of Theorem 3. Let us observe that if m_1, m_2 are metabolites in a monomolecular reaction network such that m_2 is reachable from m_1, then the subgraph R^{m_2} reachable with a dipath from m_2 is contained in R^{m_1} reachable with a dipath from m_1, namely $R^{m_2} \subseteq R^{m_1}$ if $m_2 \in R^m_1$. Indeed, for any vertex m_2 reachable from m_1 there exists a dipath γ_1 from m_1 to m_2. For any vertex m_3 reachable from m_2 analogously there exists a dipath γ_2 from m_2 to m_3. Then it is enough to consider the last-minute shortcut $s(\gamma_1\gamma_2)$ for concluding that any metabolite reachable with a dipath from m_2 is also reachable from m_1.
Let us observe moreover that if m_2 is reachable from m_1 with a dipath starting along j, outgoing arrow from m_1, then any element reachable from m_2 with a dipath, which does not contain m_1, is again reachable from m_1 with a dipath starting along j. Indeed, it is enough to consider again a last-minute shortcut. A necessary condition for j to be a skipped part is that m_1 is in the intersection of the two directed paths, fact that we have excluded.

Let j^*, outgoing arrow from m^*, be in the m^*0-direction, and let j' be any arrow in the (m^*0)-Balloon. We have then the following conclusions:
1) j' is influenced by j^*.
2) $x(m^*)$, first obliged element from m^* to 0, is an obliged element also from $m' = m(j')$, mother of j', to 0, according to roposition 6.
Then, by using the above observation, we conclude easily that the influenced set of j' is included in the influenced set of j^*. Indeed, the whole $(m', 0)$-Balloon is contained in the

$(m^*, 0)$-Balloon.

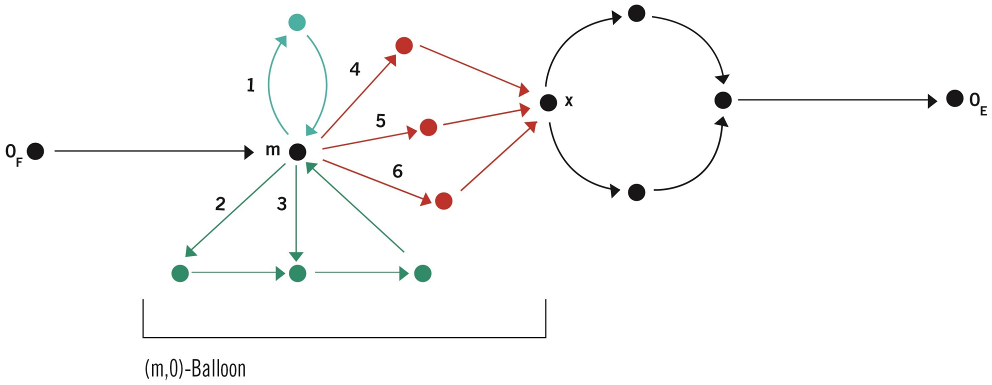
Assume now that j^{**}, outgoing reaction arrow from m^*, is *not* in the m^*0-direction, then for any $\tilde{m}$ in $R^{m^*,j^{**}}$, namely reachable from m^* with a dipath starting along j^{**}, the flux-influenced set of an outgoing arrow $\tilde{j}$ of $\tilde{m}$ is *at most* any arrow i reachable from $\tilde{m}$ without passing through m^*, since for sure m^* is an obliged element from $\tilde{m}$ to 0, again by proposition 6. Although it might not be the very first. Any reaction arrow of this kind, in particular, belongs again to $R^{m^*,j^{**}}$ according to our observation above.

□

5 EXAMPLES

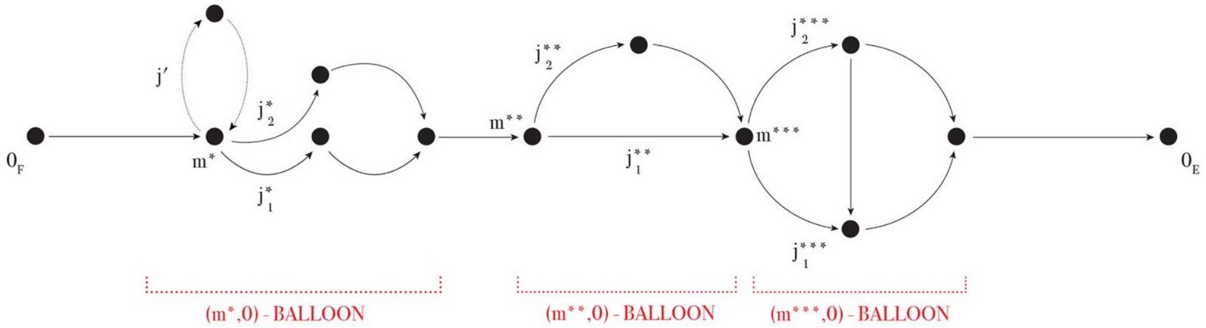
In this section we comment on some theoretical examples. Some of them are taken from [1], in order to provide a source of comparison with the approach of this reference paper. We will point out some interesting features. Again, we will not focus in our discussion on the *feed* reactions, namely the outgoing reactions from the feed zero vertex 0_F.

Example I



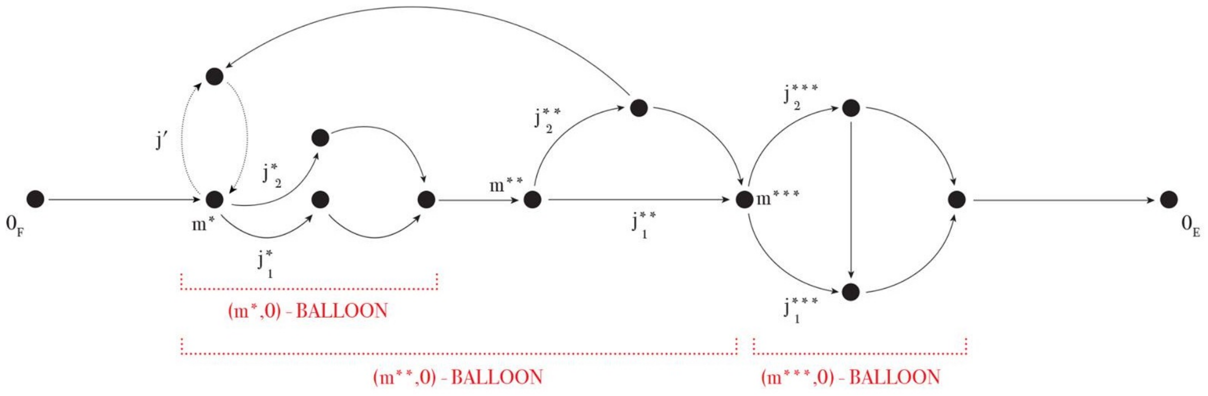
First of all we simply describe the flux-influence situation in a model Balloon. We consider again the Balloon in figure 3. Applying our results to this model example, we have that:
1) the set flux-influenced by reaction 1 consists of reactions in light blue;
2) the set flux-influenced by reaction 2 consists of reactions in green without reaction 3;
3) the set flux-influenced by reaction 3 consists of reactons in green without reaction 2 and the other only outgoing reaction from the head metabolite of reaction 2;
4) reactions 4, 5, 6 share the same set of flux-influence, which is the whole $(m, 0)$-Balloon.

Example II

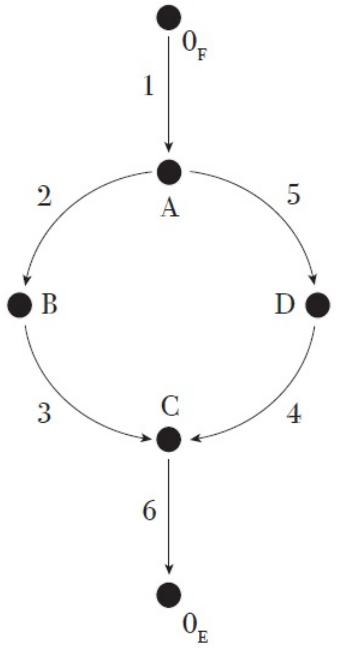


$j' \rightsquigarrow$ dashed marked reactions
$j^*_1 \rightsquigarrow (m^*, 0)$-Balloon
$j^*_2 \rightsquigarrow (m^*, 0)$-Balloon
$j^{**}_1 \rightsquigarrow (m^{**}, 0)$-Balloon
$j^{**}_2 \rightsquigarrow (m^{**}, 0)$-Balloon
$j^{***}_1 \rightsquigarrow (m^{***}, 0)$-Balloon
$j^{***}_2 \rightsquigarrow (m^{***}, 0)$-Balloon

We have labeled seven reactions: $j^*_{1,2}$, $j^{**}_{1,2}$, $j^{***}_{1,2}$ and j'. Only j' is not in the m^*0-direction, where m^* is the mother of j' and j^*. Here above we have listed the sets that they do flux-influence. In this example we want to point out specifically the connection between the obliged elements to 0 and the Balloons. j^*_1 and j^*_2 flux-influence the same set, namely the $(m^*, 0)$-Balloon. Analogously for ** and ***. We see indeed that the different balloons and hence flux-influenced sets of $j^*_{1,2}$, $j^{**}_{1,2}$, $j^{***}_{1,2}$ are precisely the 2-connected components of the graph. This holds in this precise way here because this is a particular example. This example has been chosen on purpose for showing this feature. But the idea is similar also in more complicated examples.
Note that the set flux-influenced by j', marked with dashed reactions, is a subset of the $(m^*, 0)$-Balloon, flux-influenced by $j^*_{1,2}$ since $j^*_{1,2}$ are in the m^*0-direction while j' is not.
Looking at this figure, we may be tempted to conclude that the obliged elements from 0_F to 0_E divide the networks into different flux-influenced components which do not interact between each other. This is in general not true. If we add to the network just a simple reaction arrow, the situation changes significantly. Look at the picture below.
The $(m^{**}, 0)$-Balloon now contains the $(m^*, 0)$-Balloon. This happens because the $(m^{**}, 0)$-Balloon is, we say it again, the subnetwork reachable from m^{**} withouth passing through the first obliged element from m^{**} to 0. In this case, m^{***}. Of course the $(m^*, 0)$-Balloon is then included in the $(m^{**}, 0)$-Balloon.
This suggests, correctly, that the crucial thing for a reaction to influence the largest amount of reactions is not to be 'close' to the feed 0_F, namely to be one of the first reaction which happens in the network, but rather to be possibly far away from the exit 0_E. In this last example, indeed, $j^*_{1,2}$ are the very first reactions (together with j') after the *feed* one. But j^{**} is farther from exit 0_E in the sense that j^* is reachable from j^{**} and moreover it lies in the $(m^{**}, 0)$-Balloon.



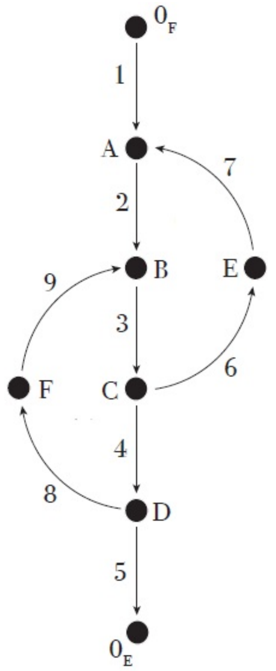
Example III



COMPLETE INFLUENCE RELATION:
1 $\rightsquigarrow$ 1, 2, 3, 4, 5, 6
2 $\rightsquigarrow$ 2, 3, 4, 5
5 $\rightsquigarrow$ 2, 3, 4, 5

This example illustrates the flux-influence structure of an undirected cycle. The obliged elements from 0_F to 0_E are in this case just *1, A, C, 6.* The only metabolite that has more than one outgoing arrow is A, namely reactions 2 and 5, both in the $A0$-direction. The first obliged element from A to 0 is C. Hence reactions 2 and 5 share the same set of influence, which is the $(A, 0)$-Balloon: the undirected cycle between the A and C, namely reactions {2,3,4,5}. The other reactions in this cycle, 3 and 4, do not flux-influence anything, as they are the only outgoing reaction from their mother metabolite.

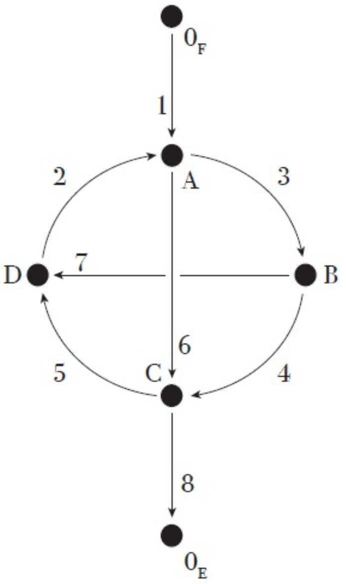
Example IV



COMPLETE INFLUENCE RELATION:
1 ⇝ 1, 2, 3, 4, 5, 6, 7, 8, 9
4 ⇝ 6, 7, 2, 3
5 ⇝ 8, 9, 3, 4, 6, 7, 2
6 ⇝ 6, 7, 2, 3
8 ⇝ 8, 9, 3, 4, 6, 7, 2

In this case we focus on metabolite D. It has exactly two outgoing reaction: reaction 5 in the $D0$-direction and reaction 8 not in the $D0$-direction. Reaction 5 is then also the first obliged element from metabolite D to 0. We know that reactions 5 and 8 do influence the same set, which in this case is the set $R^{D,8}$ of reactions reachable from D with a directed path starting along reaction 8, namely reactions: {8,9,3,4,6,7,2}. These two reactions, 5 and 8, are then of course also the peak of the flux-influence pyramid, the 'absolute influencer', if we exclude from this analysis the exceptional case of the feed reaction 1. Note that the fact that reactions 5 and 8 share the same set of flux-influence does not imply that they do flux-influence each other in an equal way. Indeed in this case reaction 5 flux-influences 8, but reaction 8 does not flux-influence reaction 5. Since reactions 4 and 6 are flux-influenced by reactions 5 and 8, their flux-influenced set (reactions 6, 7, 2, 3) is included in the one of reactions 5 and 8.

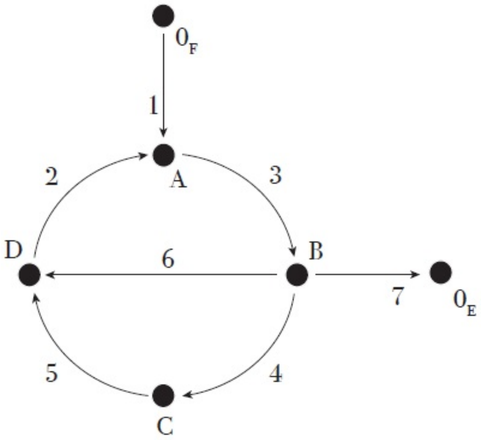
Example V



COMPLETE INFLUENCE RELATION:
1 ⇝ 1, 2, 3, 4, 5, 6, 7, 8
3 ⇝ 2, 3, 4, 6, 7
4 ⇝ 2, 3, 4, 6, 7
5 ⇝ 2, 3, 4, 5, 6, 7
6 ⇝ 2, 3, 4, 6, 7
7 ⇝ 2, 3, 4, 6, 7
8 ⇝ 2, 3, 4, 5, 6, 7

Here again we can apply an identical argument as above on metabolite C and its two outgoing reactions 5 and 8, which are the peak of the flux-influence pyramid, influencing reactions 2, 3, 4, 5, 6, 7. Reaction 2 is the only outgoing reaction from metabolite D and therefore does not influence anything and it is at the bottom of the pyramid. Apart from this, the first shared obliged element for the remaining metabolites, A and B, is C. Therefore reactions 5 and 8, outgoing from C, cannot be influenced by outgoing reactions of A and B. A has two outgoing reactions: 6 and 3, both in the $A0$-direction and B has also two outgoing reactions: 4 and 7, both also in the $B0$-direction. The $(A, 0)$-Balloon B_0^A coincides with the $(B, 0)$-Ballon B_0^B and it contains reactions $\{2, 3, 4, 6, 7\}$. Note, however, that the reactions of this shared Balloon in the $A0$-direction are reactions {6,3,4}. The ones in the $B0$-direction are instead reactions {6,4,7,2}.

Example VI



COMPLETE INFLUENCE RELATION:
1 ⇝ 1, 2, 3, 4, 5, 6, 7
4 ⇝ 4, 5, 2, 3
6 ⇝ 6, 2, 3
7 ⇝ 4, 5, 6, 2, 3

In this very interesting example we want firstly to focus on metabolite B. It has three outgoing reactions: 4, 6 and 7. Reaction 7 is in the $B0$-direction; reactions 4 and 6 are not. The first obliged element from B to 0 is of course reaction 7 itself. The flux-influenced set of reaction 4 is made by the reactions reachable from B with a directed path starting along 4, namely reactions {4,5,2,3,}. Since reaction 6 is not in the $B0$-direction, its flux-influenced is made by all the reactions reachable from B with a directed path starting along 6, namely {7,2,3,5}. Note that the two flux-influenced sets are not the same due to reactions 4 and 6 itself. In similar situations (metabolite m with two outgoing reactions not in the $m0$-direction) the behaviour is identical: the two outgoing reactions cannot share the same set of influence, due, in particular, to themselves. On the other hand, the set flux-influenced by reaction 7 are the reactions in the $(B, 0)$-Balloon which is made by the union of the sets influenced by reaction 4 and 6, namely reactions: {2,3,4,5,7}. Metabolites A, C and D have only one outgoing reaction, respectively 5 and 2. Therefore reactions 3, 5 and 2 do not influence anything.

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