Sensitivity of monomolecular reaction networks: Signed flux-response to reaction rate perturbations

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***Abstract*—Sensitivity studies the network response to the perturbation of a reaction, at steady state. Ishii et al., among others, performed biological experiments, which study the response of metabolic networks to the knock-out of certain targeted reactions. The response is given as the increase, or decrease, or zero response, for each experimentally accessible concentrations of chemicals or fluxes in the system. In previous work, Brehm, Fiedler, and Mochizuki were able to present systematic criteria which distinguish zero response from nonzero response. Their results were based on the network structure, only, and neither depend on numerical models of the reactions, nor on numerical values of their parameters. With a focus on monomolecular reaction networks, our present paper extends these results to provide for the first time a criterion for predicting the sign of any nonzero response, without requiring any additional input information.**

I. Introduction

A chemical reaction network consists of metabolites m, which interact by certain reactions j. Specifically, we address *monomolecular* reactions

$$m \underset{j}{\longrightarrow} m', \tag{1}$$

where one single metabolite input m is converted into another single one metabolite educt m'.
For technical reasons, at present, we have to exclude multimolecular reactions like, for example,

$$m_1 + m_2 \underset{j}{\longrightarrow} m'. \tag{2}$$

In our approach we model a *monomolecular reaction network* as a directed graph with a vertex (or metabolite) set $\mathbf{M} \cup \{\mathbf{0}\}$ and an arrow (or reaction) set $\mathbf{A}$. We require here that there are no self-loops and that no two arrows share the same endpoints in the same parallel direction. In literature this kind of directed graph is sometimes called *strict* directed graph. A dipath (or directed path) is any acyclic ordered sequence of alternatingly adjacent vertices and arrows. The *zero-complex* $\mathbf{0}$ introduced by Feinberg in [2] is *'a complex in which the stoichiometric coefficient of every species is zero'*. The zero-complex $\mathbf{0}$ possesses here just ingoing reactions, which are called *exit reactions*.

Let e_m be the m-th unit vector, for any nonzero metabolite $m \in \mathbf{M}$, and define $e_{\mathbf{0}} = 0$. By considering the network structure, only, it is simple to derive a general differential equation for the dynamics of the *concentrations* x=$(x_m)_{m\in\mathbf{M}}$ of metabolites m:

$$\dot{x} = g(x) = f(\mathbf{r}, x) := \sum_{j\in\mathbf{A}} r_j(x_{m(j)})(e_{h(j)} - e_{m(j)}). \tag{3}$$

Here $\mathbf{r} = (r_j)_{j\in\mathbf{A}}$ are nonlinear reaction rate functions. It is crucial to underline that we consider them as smooth given parameters. With $m(j)$ we refer to the mother metabolite of reaction j and with $h(j)$ to the head metabolite of reaction j. We assume:

1) positivity of the reaction rate functions r_j;
2) the existence of a positive steady state $x^* > 0$, i.e., stationary solution of equation 3, that is:
$$0 = g(x^*); \tag{4}$$
3) the regularity of the network at the steady state x^*, i.e., nondegenerate Jacobian $\det f_x(\mathbf{r}, x^*) \neq 0$.

We skip the mathematical details of the model; see [3]. Suffice it to recall that, generically, the nondegeneracy assumption is equivalent to the existence of a dipath from any metabolite m to $\mathbf{0}$. Therefore a *monomolecular reaction network* is precisely modeled as a strict directed graph with a special vertex $\mathbf{0}$ such that from any other vertex m there exists a dipath from m to $\mathbf{0}$.

As we said, sensitivity addresses the response of the steady state x^* to rate reaction perturbation. Briefly, we consider small perturbations of any specific reaction j^*. The implicit function theorem applies, thanks to the regularity assumption. Then a first order perturbation will replace x^* by $x^* + \partial x_m^{j^*}$, where $\partial x_m^{j^*}$ is the infinitesimal concentration response, in components:

$$\partial x_m^{j^*} := \tfrac{d}{d\epsilon}|_{\epsilon=0} x_m^*(\epsilon), \tag{5}$$

which describes the perturbation of the steady state concentration of metabolite m under a slight perturbation of reaction j^*. Note that m needs not just be an input or output of reaction j^* itself. Rather, the effects of the perturbation of some reaction j^* may affect the network, far and wide, or else may remain quite limited.
Similarly and interestingly, we can discuss the resulting change $\Phi_{j^*\rightsquigarrow j'}$ in the reaction rate $r_{j'}(x_{m(j')})$ of any other reaction j', or even of j^* itself, at steady state. In formula, we specifically obtain:

$$\Phi_{j^*\rightsquigarrow j'} := \delta_{j'j^*} + r_{j'm(j')}\partial x_{m(j')}^{j^*}, \tag{6}$$

where $\delta_{j'j^*}$ is the Kronecker-delta. We then say that a reaction j^* *flux-influences* a reaction j', in symbols $j^* \rightsquigarrow j'$,

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if the *flux-response indicator* $\Phi_{j^* \rightsquigarrow j'}$ of reaction j' to a rate perturbation as above of reaction j^* satisfies $\Phi_{j^* \rightsquigarrow j'} \neq 0$, algebraically (*nonzero response*). Here, by "*algebraically*", we mean as rational function of the derivatives $r_{jm} := r'_j(x^*_m)$ with m mother metabolite of reaction j.

A small but important remark: at the fixed position x^*_m, we consider the value $r_j(x^*_m)$ and $r'_j(x^*_m)$ to be independent from each other. For example, consider n-th order mass-action kinetics $r_j(x) = k_j x^n$ with reaction rate k_j. Then $xr'_j(x) = nr_j(x)$ are related, a priori, at any given value x. For Michealis-Menten kinetics $r_j(x) = \frac{k_j x}{1+a_j x}$, in contrast, the resulting relation $(1 + a_j)r'_j(x) = r_j(x)$ still depends on the free parameter a_j even if x is fixed. Varying the parameters k_j, a_j we can therefore vary $r_j(x)$ and $r'_j(x)$ independently, even for fixed metabolite concentration x. Langmuir-Hinshelwood kinetics, likewise, satisfy our independence assumption - but mass-action fails.

Fiedler and Mochizuki were able to characterize the nonzero response, using only graph means, see [3]. The main theorem on the flux-influence relation for monomolecular reaction networks reads, in fact, as follows:

Theorem 1 (Fiedler&Mochizuki, 2015)**.** *Consider any pair of reactions (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' such that:*

i) both dipaths emanate from m^*, mother metabolite of j^*;

ii) one of the dipaths contains j^*;

iii) the exit dipath *γ^0 terminates at vertex **0**, and the* influence dipath *γ' terminates with metabolite m', the mother vertex of j';*

iv) except for their shared starting vertex m^*, the two dipaths γ^0 and γ' are disjoint.

We call (γ^0, γ') an exit-influence pair *of (j^*, j').*

Recently, Brehm and Fiedler found a more algebraic characterization of nonzero flux-influence valid also for multimolecular networks, see [4]. In that paper they also proved a general transitivity result for the flux-influence relation, i.e., ($j^* \rightsquigarrow j'$ and $j' \rightsquigarrow j''$) implies $j^* \rightsquigarrow j''$. Okada and Mochizuki, moreover, provided an interesting localization law for this kind of influence relation, see [5]. In a joint paper with Matano we have further clarified, from a network connectivity point of view, the structure of the flux-influence relation in the monomolecular case and we have described the structure of the flux-influenced sets, in details, see [6].

Here we significantly improve the Fiedler-Mochizuki result for monomolecular reaction networks, addressing for the first time the problem of the signed (+/-) response. In other words, we investigate and answer the following question:

Is the nonzero flux response positive or negative?

Moreover, we clarify precisely the relation between the choice of an exit-influence pair of dipaths of Theorem 1 and the explicit rational expression of the flux-response indicator $\Phi_{j^* \rightsquigarrow j'}$. In this sense, our present result provides a deeper interpretation of the above theorem.

First of all, we have to make a further assumption, such that it makes sense to speak about positive and negative response. Let us assume, for this purpose, that also the derivatives $r_{jm} := r'_j(x^*_m)$ are positive functions. Since $\Phi_{j^* \rightsquigarrow j'}$ is a rational function of positive variables, three nontrivial cases arise. 1) all coefficients are positive, and therefore the influence is always positive; 2) all coefficients are negative, and therefore the influence is always negative; 3) there are both positive and negative coefficients, and the influence is of undetermined sign. This is what we will analyze here.

We present only the significant case of (j^*, j') being a couple of *distinct* reactions. The theorem actually holds, as well, for the case $j^* = j'$ but the proof will be discussed elsewhere.

Theorem 2. *Consider any pair of distinct reactions (j', j^*) in a monomolecular reaction network.*
Assume that j^* flux-influences j' ($j^* \rightsquigarrow j'$), that is, there exist an exit-influence pair of two dipaths (γ^0, γ') satisfying conditions (i)-(iv) of Theorem 1.

Then the numerator of the algebraically nonzero rational expression of the flux-response indicator $\Phi_{j^* \rightsquigarrow j'}$ possesses as many monomial summands as there are different choices of exit-influence pairs (γ^0, γ') satisfying conditions (i)-(iv) of Theorem 1.

Moreover, a summand is positive *for a specific choice (γ^0, γ') of dipaths if, and only if,*

$$j^* \in \gamma',$$

that is, j^* lies on the influence dipath.

A summand is negative *if, and only if,*

$$j^* \in \gamma^0,$$

that is, j^* lies on the exit dipath.

The concrete interpretation of the theorem, in terms of positive/negative influence or undetermined sign influence, is given by the following corollaries.

Corollary 3. *A reaction j^* flux-influences* positively *(respectively* negatively*) a reaction j' if, and only if, for* any *choice of an exit-influence pair (γ_0, γ'), j^* is in the influence dipath γ' (respectively in the exit dipath γ^0).*
There is undetermined sign influence if, and only if, there are at least two different choices of exit-influence pairs (γ^0_1, γ'_1) and (γ^0_2, γ'_2) such that $j^* \in \gamma^0_1$ is in the exit dipath of one pair, but also $j^* \in \gamma'_2$ is in the influence path of the other pair.

Corollary 4. *In particular, if i and j are the two only outgoing reactions from m, then the two reactions flux-influence exactly*

the same set (see for more details [6]), but their influences carry opposite sign.

II. JACOBIAN OF THE SYSTEM AND CHILD SELECTIONS

First of all we decompose the Jacobian matrix f_x of the system, which we have required to be nonsingular. The decomposition reads as follows: $f_x(\mathbf{r}, x^*) = SR$. Here the |**M**|x|**A**| matrix S is the *stoichiometric* matrix, defined by $Se_j = e_{h(j)} - e_{m(j)}$. The |**A**|x|**M**| matrix R is the *reactivity* matrix of the nontrivial derivatives $r_{jm} := r'_j(x^*_m)$, filled up with zeros in any entry such that m is not the corresponding mother metabolite of reaction j. In this notation equation (3) reads:

$$\dot{x} = S\mathbf{r}(x).$$

One crucial tool for our approach is the concept of *child selection* (see [3] and [4]). A child selection is a map $J : \mathbf{M} \longrightarrow \mathbf{A}$, which associates to every metabolite $m \in \mathbf{M}$ a reaction $j \in \mathbf{A}$ such that m is a mother metabolite of reaction j. The Caucht-Binet formula links the Jacobian SR with child selections. Indeed,

$$\det(SR) = \sum_J (\operatorname{sgn}(J) \det(S^{J(\mathbf{M})}) \prod_{m\in\mathbf{M}} r_{J(m)m}), \quad (7)$$

where $J : \mathbf{M} \to \mathbf{A}$ are child selections; $\operatorname{sgn}(J)$ is the signature operator applied on J if we see J as a permutation of ordered $|\mathbf{M}|$ elements, which is $+1$ if the permutation is even and -1 if it is odd. We repeat that $r_{J(m)m}$ refers to the derivative $r_{jm} := r'_j(x^*_m)$ of reaction rate r_J; $S^{J(\mathbf{M})}$ is the square minor matrix of the stoichiometric matrix chosen according to the child selection $J(\mathbf{M})$. The simple proof is omitted here. For further information and understanding, see [3] and [4].

III. PROOF OF THEOREM 2

The proof of our theorem relies on the analysis of an explicit formula for flux-influence. For brevity, we refer to the already proven formula in [4], which holds for the general multimolecular case and fits perfectly with our purposes. Given any pair of distinct reactions (j^*, j'), the formula for the flux-response indicator $\Phi_{j^*\rightsquigarrow j'}$ of j' to a perturbation of reaction j^* reads as follows: $\Phi_{j^*\rightsquigarrow j'} = \sum_{j^*\notin J\ni j'} \phi_J$. where

$$\det(SR)\phi_J = -(\operatorname{sgn}(J) \det(S^{J(\mathbf{M})_{sw}}) \prod_{m\in\mathbf{M}} r_{J(m)m}). \quad (8)$$

As above, J are child selections; $\operatorname{sgn}(J)$ is the signature operator; $r_{J(m)m}$ are the derivatives $r_{jm} := r'_j(x^*_m)$ of reaction rate r_J; $S^{J(\mathbf{M})_{sw}}$ is the square minor matrix obtained by choosing, in the stoichiometric matrix S, the swapped $|\mathbf{M}|$ columns $J(\mathbf{M})_{sw} = j^* \cup J(\mathbf{M}) \setminus j'$. The central argument argument here is that the swapping preserves the column order. That is, the new j^* column takes the same position of the old j' column. The proof consists of two preliminary steps and the core proof. The first step analyzes the signature operator $\operatorname{sgn} J$. The second step analyzes the sign of $\det(SR)$. The core proof concludes with the analysis of the sign of $\det(S^{J(\mathbf{M})_{sw}})$.

First step. In this monomolecular case, we label the network in a way such that $\operatorname{sgn} J \equiv +1$ holds for all child selections J, simultaneously. The labeling is as follows. Fix any labeling $\mathbf{M} = \{m_1, m_2, ..., m_{|\mathbf{M}|}\}$ of the metabolites in the network. Then relabel the reactions following the same order. By this procedure, it is clear that $\operatorname{sgn}(J) = +1$ for *any* child selection J. Indeed $J : \mathbf{M} \to \mathbf{A}$ is order preserving.

Second step. We show that in the monomolecular case $\operatorname{sign}(\det(SR))$ follows a very simple rule, in the sense explained by the following Lemma.

Lemma 5. *For a monomolecular reaction network, it holds:*

$$\operatorname{sign}(\det(SR)) = (-1)^{|\mathbf{M}|}. \quad (9)$$

Proof. The proof follows from an application of the Cauchy-Binet equation (7) to the properly labeled network. By Gaussian elimination, we get to the conclusion. □

Now we are ready to prove Theorem 2.

Core proof of Theorem 2. We have already simplified the above formula for flux-influence up to the point:

$$\operatorname{sign}(\Phi_{j^*\rightsquigarrow j'}) = (-1)^{|\mathbf{M}|-1} \operatorname{sign}(\sum_{j^*\notin J\ni j'} \det(S^{J(\mathbf{M})_{sw}}) \prod_{m\in\mathbf{M}} r_{J(m)m}),$$

By positivity of all $r_{J(m)m}$, the sign of a summand ϕ_J in eq. (8) reads:

$$\begin{aligned}\operatorname{sign}(\phi_J) &= \operatorname{sign}((-1)^{|\mathbf{M}|-1} \det(S^{J(\mathbf{M})_{sw}}) \prod_{m\in\mathbf{M}} r_{J(m)m}) \\ &= (-1)^{|\mathbf{M}|-1} \operatorname{sign}(\det(S^{J(\mathbf{M})_{sw}})).\end{aligned}$$

It is clear now that the sign of each summand ϕ_J only depends on $\operatorname{sign}(\det(S^{J(\mathbf{M})_{sw}}))$.

This swapped determinant is nonzero if and only if the set $J(\mathbf{M})_{sw} \subseteq \mathbf{A}$ selects, jointly with all the adjacent vertices of the reaction arrows in $J(\mathbf{M})_{sw}$, a spanning tree $\mathbf{T}$ of the network. In other words, $\mathbf{T}$ contains all metabolites in $\mathbf{M}$ plus the zero-complex $\mathbf{0}$ and it does not contain cycles. Moreover, the set $J(\mathbf{M})_{sw} \subseteq \mathbf{A}$ is a child selection except for the swapped elements. Hence all metabolites $m \in \mathbf{M}$ have one single child in the spanning tree $\mathbf{T}$, with the only two exceptions of m' (mother of j') and m^* (mother of j^*). Indeed, m' has no child left and m^* has two children, due to the swapping $j^* \cup J(\mathbf{M}) \setminus j'$.

To compute the determinant, we again implement Gaussian elimination and proceed as follows. We start choosing a subset of $\mathbf{M}_0 \subset \mathbf{M}$ such that m^*, $m' \notin \mathbf{M}_0$ and any $m \in \mathbf{M}_0$ has no input reaction in $J(\mathbf{M})_{sw}$. Sometimes these vertices are called in graph theory literature *roots* of the tree $\mathbf{T}$. Of course this set might be empty, and in this case we just skip this step. For any m-row of the $S^{J(\mathbf{M})_{sw}}$ matrix with $m \in \mathbf{M}_0$, we sum the m-row to the $h(J(m))$-row. Here $h(J(m))$ simply indicates the next metabolite to m following the order given by the child selection $J(m)$. As an explanation, we are reducing tree branches. After this first step, indeed, all the columns

corresponding to $J(m)$ with $m \in \mathbf{M}_0$ possesses only a nonzero entry, which is -1 on the diagonal.

We iterate this procedure by defining a set $\mathbf{M}_1$ such that any $\tilde{m} \in \mathbf{M}_1$ is a child $\tilde{m} = h(J(m))$ of a metabolite $m \in \mathbf{M}_0$, and no $\tilde{m} \in \mathbf{M}_1$ is in $\gamma^0 \cup \gamma'$. These are the direct children metabolites of the roots $m \in \mathbf{M}_0$, which do not lie on $\gamma^0 \cup \gamma'$. Again, we sum the $\tilde{m}$-row to the $h(J(\tilde{m}))$-row. We keep on iterating by defining a $\mathbf{M}_2$ set of children metabolites of $\mathbf{M}_1$ set analogously, etc. We stop just when we have left untouched only the rows corrensponding exactly to the metabolites contained in $\gamma^0 \cup \gamma'$. This of course happens in a finite number of steps, due to finiteness of the network and acyclicity of the tree structure $\mathbf{T}$.

At this stage, the matrix $S^{J(\mathbf{M})_{sw}}$ has changed into another matrix with the same determinant (we have only added rows), such that every column corresponding to reactions $j \notin \gamma^0 \cup \gamma'$ is the opposite of the j-unitary vector $-e_j$. In other words, it has only -1 on the diagonal.

We assume now that $\boldsymbol{j^* \in \gamma'}$. We start, with the same procedure, summing the m^*-row to the $h(J(m^*))$-row. Note that $J(m) \neq j^*$, by construction, according to equation 8. The m^*-row has -1 both in the $J(m^*)$ column and in the j^* column. We iterate now this procedure on the $h(J(m^*))$-row and we keep on iterating the procedure as long as we can, namely until we have touched all the elements on γ^0 and reached the zero. We might say here *"until we get out"*. This must happen since $j^* \in \gamma'$. Also, the j^*-column has been filled with -1 in all the rows corresponding to metabolites in γ^0. We call this process a *cascade* of -1 along γ_0.

Up to now, the only rows we did not touch correspond exactly to the metabolites contained in $\gamma' \setminus m^*$.

At this second stage, the original matrix $S^{J(\mathbf{M})_{sw}}$ has changed into another matrix with the same determinant (we have just added rows), such that every column corresponding to reactions $j \notin \gamma'$ is the opposite of the j-unitary vector $-e_j$. In other words, it has only a -1 on the diagonal. Note that j^* is indeed in γ'.

Now, lastly, we start adding the $h(j^*)$-row with the same procedure of the preceding step. The $h(j^*)$-row has a -1 in the column of reaction $J(h(j^*))$ and a $+1$ in the column of reaction j^*, which is in the original position of reaction j'. Therefore, iterating the procedure along γ' we will have a cascade of $+1$ until we reach the m'-row, where m' is the mother of j'. In this way the j^* column has been filled with +1 in all the rows corresponding to metabolites in γ', including m'-row. Note that this row has been filled with +1 crucially in the diagonal entry, of course.

At the end of this third stage, the matrix is almost diagonal, with the only exception of j^* column. This transformed matrix has the same determinant of $S^{J(\mathbf{M})_{sw}}$ and it has only -1 on the diagonal except for the j^* (originally j') column, in which, due to the cascade of +1 along γ', there is now $+1$.

Therefore:

$$\operatorname{sign}(\det(S^{J(\mathbf{M})_{sw}})) = (-1)^{|\mathbf{M}|-1}.$$

and

$$\begin{aligned}\operatorname{sign}(\phi_J) &= (-1)^{|\mathbf{M}|-1} \operatorname{sign}(\det(S^{J(\mathbf{M})_{sw}})) \\ &= (-1)^{|\mathbf{M}|-1}(-1)^{|\mathbf{M}|-1} = +1.\end{aligned}$$

The case in which $\boldsymbol{j^* \in \gamma^0}$ is solved by completely analogous arguments. In this case we have a cascade of -1 getting to the diagonal of the j^* (originally j') column instead of a cascade of $+1$.

We have shown, moreover, that any different choice of couple (γ^0, γ') gives a nonzero summand to the rational expression of the flux-response indicator $\Phi_{j^* \rightsquigarrow j'}$.

This completes the proof.

□

IV. CONCLUSIONS

In the present paper we have given a first result to determine signed response to reaction rates perturbations. We have achieved it by prescriving an elementary graph theory recipe. A nonzero response is characterized by two particular directed paths: an influence one and an exit one. The influence path goes from the mother metabolite of the perturbed reaction to the influenced reaction. The exit path goes from the mother metabolite of the perturbed reaction to the zero-complex $\mathbf{0}$.

If the reaction *perturbed* lies on the influence path, then the response expression has a positive summand; if it lies on the exit path, it has a negative summand. Clearly, a case of undetermined response appears if there are both positive and negative summands.

Our results are limited to monomolecular reactions, at present. Future work will concentrate in the extension of these results to multimolecular systems.

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