Sensitivity of chemical reaction networks: a structural approach. 3. Regular multimolecular systems

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Abstract

We present a systematic mathematical analysis of the qualitative steady-state response to rate perturbations in large classes of reaction networks. This includes multimolecular reactions and allows for catalysis, enzymatic reactions, multiple reaction products, nonmonotone rate functions, and non-closed autonomous systems. Our *structural sensitivity analysis* is based on the stoichiometry of the reaction network, only. It does not require numerical data on reaction rates. Instead, we impose mild and generic nondegeneracy conditions of algebraic type. From the structural data, only, we derive which steady-state concentrations are sensitive to, and hence influenced by, changes of any particular reaction rate – and which are not. We also establish transitivity properties for influences involving rate perturbations. This allows us to derive an *influence graph* which globally summarizes the influence pattern of the given network. The influence graph allows the computational, but meaningful, automatic identification of functional subunits in general networks, which hierarchically influence each other. We illustrate our results for several variants of the glycolytic citric acid cycle. Biological applications include enzyme knockout experiments, and metabolic control.

Contents

1 Introduction

For large classes of biological, chemical or metabolic reaction networks, detailed numerical data on reaction rates are neither available, nor accessible, by parameter identification. See the large, and growing, data bases on chemical and metabolic pathways like [Le+06, KG00], for thousands of examples. One standard approach to establish, and check, the validity of such networks are *knockout experiments*: some reaction is obstructed, via the knockout of its catalyzing enzyme, and the response of the network is measured, e.g., in terms of concentration changes of metabolites. The large area of *metabolic control* studies how reaction rates steer the network to desired behaviour, or switch between different tasks; see for example [HR74, Fel92, Ste84] and the references there.

In this setting it is our goal to develop a reliable, and largely automatic, mathematical tool to aid our systematic understanding of large networks. More specifically, we address the response of steady states to rate perturbations in the network. Here steady state refers to any time-independent long-term state of the system. "Long-term" refers to the relevant time-scales of the model. Time-periodic or chaotic responses are excluded, at present.

In experiments, only those steady states may be observable which are stable, or at least metastable on the relevant time scale. Our mathematical approach is not limited by any stability requirements other than some mild nondegeneracy assumption. For mathematical reasons, however, we do present our approach in a local setting of linearized steady state response to small perturbations, at first sight.

We derive a qualitative sensitivity matrix for the steady state response, which we encode as an *influence relation* "j^* influences β"; in symbols

$$j^* \rightsquigarrow \beta\,. \tag{1.1}$$

Here j^* indicates the perturbed reaction rate, and β indicates a *nonzero resulting flux change* of the reaction $\beta = j$ in the network, or a *nonzero resulting concentration change* of the metabolite $\beta = m$. We write $j^* \rightsquigarrow \beta$, if the effect of the perturbation j^* on β is a nonzero change, i.e. if the response of β is *sensitive to* j^*. It is this nonzero influence, together with a transitivity property, which will identify meaningful units in a network as presented in a *flux influence graph* $\mathcal{F}$ below.

Our main surprise, which motivates this detailed study, is the *sparsity of sensitivity*: many β are not influenced by some given j^*, at all, because that flux or metabolite β does not change. Such *zero response* is the counterpart of the flux influence graph $\mathcal{F}$. Zero response is not limited to small perturbations, because it results from only structural information on the network stoichiometry. It therefore applies to metabolic control and to knockout experiments, alike. See (1.22)–(1.24) below. In particular, any zero response is a rational and mathematically rigorous test to any purported pathway structure: *Any experimentally validated nonzero response, above error threshold, which contradicts a zero entry in the sensitivity matrix of influences,*

falsifies the underlying pathway. See section 2 for our main mathematical results, and sections 3 and 9 for examples.

We describe our mathematical setting next. Let $j \in \mathcal{E} = \{1, \dots, E\}$ be a set of isothermal biological, chemical, or metabolic reactions on metabolites or chemical species $m \in \mathcal{M} = \{X_1, \dots, X_M\}$. A *reaction network* is given by reactions

$$j : y_1^j X_1 + \ldots + y_M^j X_M \longrightarrow \bar{y}_1^j X_1 + \ldots + \bar{y}_M^j X_M \tag{1.2}$$

with nonnegative *stoichiometric coefficient* vectors $y^j, y^{-j} \in \mathbb{R}^M$, usually with integer components. Reversible reactions are not required but admitted, listed as two separate reactions. For notational convenience we will write $y^j, \bar{y}^j \in \mathcal{M}$, instead of $\mathbb{R}^M$, to indicate the supporting component set of vectors. For subsets $\mathcal{M}_0 \subset \mathcal{M}$ we will even write

$$y \in \mathcal{M}_0\,, \quad \text{for} \quad \operatorname{supp} y := \{m \,|\, y_m \neq 0\} \subseteq \mathcal{M}_0\,, \tag{1.3}$$

i.e. for the subspace of vectors $y \in \mathbb{R}^M$ which are supported on $\mathcal{M}_0$, only. We call m an *input*, or *reactant*, or *educt* of reaction j, if $y_m^j \neq 0$; in symbols we write this as

$$m \vdash j\,. \tag{1.4}$$

We use this notation even when m *catalyzes* j, i.e. $\bar{y}_m^j = y_m^j$, and in presence of further inputs of j. *Outputs*, or *products* m are given by $\bar{y}_m^j \neq 0$. *Feed reactions*, i.e. external inputs, have $y^j = 0$. *Exit reactions* have $\bar{y}^j = 0$.

The *stoichiometric matrix* S is essential to derive the differential equation (1.9) of a network (1.2). The $M \times E$-matrix S is defined by

$$\begin{aligned} S: \quad & \mathcal{E} \longrightarrow \mathcal{M} \\ & e_j \longmapsto \bar{y}^j - y^j \end{aligned} \tag{1.5}$$

where $e_j \in \mathcal{E}$, alias $\mathbb{R}^E$, denotes the j-th unit vector. In other words, the columns S^j of the stoichiometric matrix S are simply the (nonzero) differences of the stoichiometric output and input vectors $\bar{y}^j$ and y^j. The usually nonlinear *reaction rate* r_j, at which reaction j occurs per time unit, depends on the *concentrations* x_m of the metabolites X_m. Evidently $r_j = r_j(x)$ only depends on the input metabolites of reaction j, i.e.

$$r_{jm} := \partial_{x_m} r_j(x) = 0\,, \quad \text{unless} \quad m \vdash j\,, \tag{1.6}$$

holds for the partial derivatives. In other words

$$(r_{jm})_{m \in \mathcal{M}} \in \operatorname{Inputs}(j) := \{m \in \mathcal{M} \,|\, m \vdash j\}\,, \tag{1.7}$$

by our convenient abuse of notation. The isothermal time evolution of the metabolite concentrations $x_m(t)$ is given by the ODE system

$$\dot{x}_m(t) = \sum_{j \in \mathcal{E}} (\bar{y}^j - y^j)\, r_j\big(x(t)\big)\,. \tag{1.8}$$

In vector notation with $x = (x_1, \dots, x_M)$ and $r = (r_1, \dots, r_E)$ this reads

$$\dot{x} = Sr(x)\,. \tag{1.9}$$

We strongly recommend [Fei95, Pal06] for an introduction to this general setting. Throughout, we will assume

$$S \text{ has full rank } M\,. \tag{1.10}$$

This excludes cokernel of S, i.e. affine linear invariant subspaces of $x(t)$, alias the possibility of conserved linear combinations of the $x_m(t)$. Although our results extend to that case, we prefer simplicity of presentation here.

Steady states x are time-independent solutions of (1.9), i.e.

$$0 = Sr(x)\,. \tag{1.11}$$

Considerable effort has gone into the study of existence, uniqueness, and possible multiplicity of steady state solutions x of (1.11). See in particular Feinberg's pioneering work [Fei95], his rather advanced recent results [SF13], and the many references there. In the present paper we do not address this question, at all. Rather, *we assume existence of a steady state x throughout this paper*. Instead, we focus on the following *central question*:

(1.12) *How does a given steady state x respond to perturbations of any particular reaction rate r_{j^*}, qualitatively?*

To define and study this response sensitivity of steady states x with respect to small external changes of any reaction rate r_j we introduce formal reaction parameters ε_j as

$$r_j = r_j(\varepsilon_j, x)\,. \tag{1.13}$$

We also write $r = r(\varepsilon, x)$ on the right hand side of (1.11). For extreme generality, we could choose ε_j to parametrize the functions r_j, themselves. More modestly, we might think of just a rate coefficient like $r_j(\varepsilon_j, x) := (1 + \varepsilon_j) r_j(x)$.

The standard implicit function theorem in a C^1-setting immediately answers the response question – albeit rather abstractly and under mild nondegeneracy assumptions. Given a reference steady state solution $x(0)$, for $\varepsilon = 0$, we obtain a unique differentiable family of solutions $x(\varepsilon)$, for sufficiently small $|\varepsilon|$, such that

$$0 = S\,\partial_{\varepsilon_{j^*}} r + SR\,\partial_{\varepsilon_{j^*}} x(\varepsilon) \tag{1.14}$$

holds for the partial derivatives. Here $R = (r_{jm})_{j\in\mathcal{E}, m\in\mathcal{M}}$ is the Jacobian of the rate function $r(\varepsilon, x)$ with respect to x; see (1.6) for the definition of the partial derivatives r_{jm} of r_j. For the partial derivative of the rate function vector r it is sufficient to consider unit vectors

$$\partial_{\varepsilon_{j^*}} r = e_{j^*}\,; \tag{1.15}$$

all other cases can be derived from that. We call

$$\delta x_m^{j^*} := \partial_{\varepsilon_{j^*}} x_m \tag{1.16}$$

the sensitivity of metabolite m with respect to rate changes of j^*. Similarly, we call

$$\Phi_j^{j^*} := \delta_{j^*j} + (R\delta x^{j^*})_j \tag{1.17}$$

the *sensitivity of reaction flux j with respect to the rate change of j^**. The Kronecker symbol δ_{j^*j} accounts for the externally forced flux change by variation of the parameter ε_j. The reaction-inducted term $R\delta x$ may, or may not, counteract or even annihilate this forcing, depending on the effected metabolite changes δx.

In vector notation (1.14) becomes the flux balance

$$0 = S\Phi^{j^*} . \tag{1.18}$$

Our qualitative answer to the central sensitivity question (1.12) will distinguish those fluxes and metabolites j, m with nonzero response to the external rate change j^*, from those with zero response, i.e. without any response at all. Let $\beta \in \mathcal{E} \cup \mathcal{M}$ denote any flux or metabolite. We recall from (1.1) that j^* *influences* β, in symbols $j^* \rightsquigarrow \beta$, if the component β of the *response vector* $(\Phi^{j^*}, \delta x^{j^*}) \in \mathcal{E} \times \mathcal{M}$ is nonzero. Our main theoretical results, theorems 2.2–2.4 below, will investigate the influence relation (1.1) in detail. In particular transitivity theorem 2.4 will allow us to present the flux responses (1.1) of reaction $\beta = j \in \mathcal{E}$ as a directed acyclic graph $\mathcal{F}$ on certain subsets of reactions $j \in \mathcal{E}$; see theorem 2.7. The responses of metabolites $\beta = m \in \mathcal{E}$ appear as annotations of the reaction vertices in this *influence graph*. See also figs. 3.1 and 9.4 below for examples.

In [FM15] we already treated the monomolecular case, where each stoichiometric vector $y^j, \bar{y}^j$ has singleton or empty support, in a similar spirit. In that case, the original network (1.2) defines the directed edges of a reaction graph with vertices $\mathcal{M} \cup \{0\}$, and our results can be expressed, and proved, via cycles and spanning trees. For a more elegant formulation of these results see [Vas15, Vas16, MV16]. The reaction graph coincides with Feinberg's graph of reaction complexes, in that case, and exhibits Feinberg deficiency zero; see [Fei95]. We briefly comment on this case as an example, after theorem 2.3.

A theoretical example from [FM15] is detailed in section 3, and three variants of the TCAC metabolism are treated in section 9. It was such examples where we first observed hierarchies of flux influence, on a purely phenomenological level and without any mathematical justification. See also the very interesting idea of Okada in [Oka+15] and [OM16], which reappears in lemma 7.1. In sections 4–7 we provide detailed proofs of our theorems.

Well, wait a minute: didn't the "standard implicit function theorem" come with a nondegeneracy assumption? Specifically, how about nonzero Jacobian

$$\det(SR) \neq 0 , \tag{1.19}$$

in (1.14)? Nowhere did we even bother to exclude the disastrous case $R = 0$ of all constant reaction rates which leaves x undetermined. We call the reaction network (1.2) *regular* at the steady state x if (1.19) holds there. A criterion will be given in theorem 2.1. In the same spirit as in [FM15], *we consider* $\det SR$ *as a polynomial expression in the nontrivial partial derivatives* r_{jm} of the reaction rate functions r_j, evaluated at the steady state x. We call an expression *algebraically nonzero*, if it is nonzero as a polynomial in the real variables r_{jm}, taken over all metabolite inputs $m \vdash j$ of the reactions j. It is this precise sense how definition (1.1) and nondegeneracy (1.19) are supposed to hold. In particular the set of r_{jm} where our claims fail to hold is an algebraic variety of codimension at least one in the space of all real r_{jm} with $m \vdash j$. In this specific sense our results hold true for *generic rate functions* r_j.

While working on this paper we encountered the groundbreaking and monumental results by Murota on *layered matrices*, some of which precede our work by decades. See for example the monograph [Mur09] and the many references there. Very abstractly, Murota considers matrices with entries from two different fields $\mathbb{K} \subsetneqq \mathbb{F}$ and derives normal forms and order relations, very much in the same algebraic spirit which we pursue. Although his work could serve as a framework for our results, we do make use of the specific metabolite-reaction structure of chemical reaction systems, and of the specific choice $\mathbb{K} = \mathbb{R}$ or $\mathbb{F} = \mathbb{R}(r)$. It was therefore easier for us, and probably for most of our readers, to develop the relevant theory from scratch and make this paper reasonably self-contained.

As a final caveat we have to issue a warning concerning *mass action kinetics*, defined by

$$r_j(x) = k_j x^{y^j} := k_j x_1^{y_1^j} \cdot \ldots \cdot x_M^{y_M^j} \,. \tag{1.20}$$

Here y_m^j are nonnegative integers, and only a single parameter k_j is available for each reaction. The partial derivatives

$$r_{jm} = y_m^j r_j / x_m \tag{1.21}$$

are closely related to the steady state rates r_j themselves – too closely, in fact, to be considered algebraically independent. *Therefore our theory does not apply to pure mass action kinetics.* Slightly richer classes like Michaelis-Menten, Langmuir-Hinshelwood, or other kinetics where each participating species x_m, $m \vdash j$ enters with an individual kinetic coefficient, fall well within the scope of our algebraic results.

A large complementary body of work on network sensitivity, specifically in the context of mass action kinetics, has been initiated by Shinar, Feinberg and coworkers; see [Shi+09, SF10, SF11, Shi+11]. Their emphasis is on *quantitative robustness* of the steady state metabolite response to perturbations of fluxes and some "elemental" metabolite concentrations. Robustness is understood as bounded, usually small, but nonzero linear response to the external perturbation.

Large perturbations are often required, e.g. in enzyme knockout experiments of reaction j^*. They present a lesser obstacle. Indeed consider two steady states

$$0 = Sr(\varepsilon^\iota, x^\iota) \qquad \iota = 0, 1\,, \tag{1.22}$$

and consider large perturbations $\varepsilon^1 - \varepsilon^0 = \varepsilon_{j^*}$, without loss of generality. Let $\delta x^{j^*} := x^1 - x^0$. Then we may interpolate x^1 and x^0 linearly by

$$x^\vartheta := (1-\vartheta)x^0 + \vartheta x^1\,, \tag{1.23}$$

for $0 \le \vartheta \le 1$, and similarly for ε, to obtain

$$0 = S(\eta e_{j^*} + \tilde{R}\delta x^{j^*})\,. \tag{1.24}$$

Here η and $\tilde{R} = (\tilde{r}_{jm})$ abbreviate integrals

$$\eta = \int_0^1 \partial_{\varepsilon_{j^*}} r_{j^*}(\varepsilon^\vartheta_{j^*}, x^\vartheta)d\vartheta\,, \qquad \tilde{r}_{jm} = \int_0^1 r_{jm}(\varepsilon^\vartheta_j, x^\vartheta)d\vartheta\,.$$

The intermediate evaluation points $\varepsilon^\vartheta, x^\vartheta$ are not required to be steady states. We only have to assume $\eta \neq 0$ and algebraically independent $\tilde{r}_{jm}$, for $m \vdash j$, generically. Comparing (1.24) with (1.14), we obtain identical results on the influence of large perturbations.

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2 Main results

In this section we present our main results, theorems 2.1–2.7. For background notation and an outline see section 1. Throughout this paper, and in all theorems, we assume surjectivity (1.10) of the stoichiometric matrix S defined in (1.5). After our analysis of this condition in theorem 2.1 we also assume the reaction network (1.2) to be regular, i.e. the nondegeneracy condition

$$\det(SR) \neq 0 \tag{2.1}$$

holds, algebraically, for the rate Jacobian SR; see also (1.4) and (1.19). We repeat and emphasize that, here and below, any nonzero quantities in assumptions or conclusions are understood in the algebraic, polynomial sense as explained above.

Our results can be read as pure linear algebra with an abstract underlying "network structure"; therefore no reference to any steady state x is required. To make sense in the context of a steady state response to external rate perturbations in concrete reaction networks, however, we do require existence of a steady state x as in (1.11). Since actual reaction rates may become linearly degenerate in absence of reactants, if will be safe to consider strictly positive steady state components, $x_m > 0$, in such cases, to actually provide algebraically independent partial derivatives r_{jm}. We do not require any further assumptions.

Our first three results, reminiscent of [FM15], are based on the notation of a *child selection*

$$J : \mathcal{M} \longrightarrow \mathcal{E} \tag{2.2}$$

which we define as follows. We require injectivity of J, and we require each $m \in \mathcal{M}$ to be an input to the selected reaction $J(m) \in \mathcal{E}$. In other words

$$m \vdash J(m)\,, \tag{2.3}$$

for all $m \in \mathcal{M}$. Occasionally we call m a *mother*, or *parent*, of the *child* j if $m \vdash j$, i.e. $r_{jm} \not\equiv 0$. Note that a child j may have two or more parents m, i.e. input metabolites $m \vdash j$. A *single child* j^* possesses at least one mother $m^* \vdash j^*$ which has no other children, besides j^* itself. Then any child selection J must select $j^* = J(m^*)$.

Consider any subset $\mathcal{E}'$ of $\mathcal{E}$. We say that $\mathcal{E}'$ *selects an S-basis* of the stoichiometric matrix $S\colon\ \mathcal{E} \to \mathcal{M}$, if the columns $\mathcal{E}'$ of S are a basis of range S. Since we have required full rank of S in (1.10), i.e. surjectivity $\mathcal{M} =$ range S, this means $|\mathcal{E}'| = |\mathcal{M}| = M$ and $\det S^{\mathcal{E}'} \neq 0$ for the square minor $S^{\mathcal{E}'}$ of S defined by the S-columns in $\mathcal{E}'$. In other words,

$$\ker S^{\mathcal{E}'} = \ker S \cap \mathcal{E}' = \{0\}\,. \tag{2.4}$$

According to our notation convention (1.3) this means that $S\colon \mathbb{R}^E \to \mathbb{R}^M$ does not possess any nontrivial kernel vector supported only in $\mathcal{E}' \subseteq \mathcal{E}$.

Theorem 2.1. *Let S be surjective, as required in (1.10). Then the reaction network (1.2) is regular, algebraically, i.e. the nondegeneracy condition* $\det(SR) \neq 0$ *of (2.1) holds, algebraically, if and only if there exists a child selection $J\colon \mathcal{M} \to \mathcal{E}$ such that $J(\mathcal{M})$ selects an S-basis, i.e.*

$$\ker S \cap J(\mathcal{M}) = \{0\}\,. \tag{2.5}$$

Henceforth, and throughout the rest of the paper, we assume $\det(SR) \neq 0$, i.e. the existence of such a kernel-free child selection J.

The meaning of the child selection J will become more evident in the proof; see section 4. We will in fact show that $\det(SR)$ can be written as a polynomial

$$\det(SR) = \sum_J a_J \mathbf{r}^J \,. \tag{2.6}$$

The sum runs over all child selections J, and $\mathbf{r}^J$ abbreviates the monomial

$$\mathbf{r}^J := \prod_{m\in\mathcal{M}} r_{J(m)m} \,. \tag{2.7}$$

The coefficient a_J abbreviates the determinant

$$a_J = \pm \det S^{J(\mathcal{M})} \,, \tag{2.8}$$

where $S^{J(\mathcal{M})}$ is the $M \times M$ minor of the stoichiometric S-columns $J(\mathcal{M})$. Of course condition (2.5) is equivalent to a (truly) nonzero coefficient a_J of the algebraic monomial $\mathbf{r}^J$.

Let us briefly comment on the role of *single children* $m^* \vdash j^*$, where some input metabolite m^* of the single child j^* has no other child reactions $m^* \vdash j$ besides $j = j^*$. Therefore

$$J(m^*) = j^* \tag{2.9}$$

for any child selection J. Our standing assumption $\det(SR) \neq 0$ and theorem 2.1 imply the existence of a child selection J. Because J must be injective, there cannot exist any other single-child parent $m \neq m^*$ of j^*, i.e. $m \vdash j^*$, with the same single child j^*; see (2.9) again. Therefore the mother m^*, of which j^* is a single child, is determined uniquely by the single child j^* in our setting of $\det(SR) \neq 0$.

The next theorem characterizes flux influences $j^* \rightsquigarrow j'$ in terms of swapped child selections. We recall definition (1.17) of the flux response $\Phi^{j^*}_{j'}$, and how $j^* \rightsquigarrow j'$ means $\Phi^{j^*}_{j'} \neq 0$, algebraically.

Theorem 2.2. *Assume* $\det(SR) \neq 0$ *holds, algebraically, as asserted by theorem 2.1. Then flux influence is characterized as follows.*

(i) Self-influence $j^* \rightsquigarrow j^*$ occurs, if and only if there exists a child selection $J\colon \mathcal{M} \to \mathcal{E} \smallsetminus \{j^*\}$ such that $J(\mathcal{M})$ selects an S-basis, i.e.

$$j^* \notin J(\mathcal{M}) \quad \text{and} \quad \ker S \cap J(\mathcal{M}) = \{0\} \,. \tag{2.10}$$

(ii) Influence $j^* \rightsquigarrow j' \neq j^*$ occurs, if and only if there exists a child selection $J\colon \mathcal{M} \to \mathcal{E}$ such that $j^* \notin J(\mathcal{M}) \ni j'$ and the swapped set $\{j^*\} \cup J(\mathcal{M}) \smallsetminus \{j'\}$ *selects an S-basis, i.e.*

$$j^* \notin J(\mathcal{M}) \ni j' \quad \text{and} \quad \ker S \cap \big(\{j^*\} \cup J(\mathcal{M}) \smallsetminus \{j'\}\big) = \{0\} \,. \tag{2.11}$$

Both cases may occur for the same perturbation j^*.

For an illustration we observe that *single children have no flux influence*. Indeed let $m^* \vdash j^*$ be the unique single-child mother of the single child j^*. Then (2.9) implies $J(m^*) \in j^*$. This contradicts both (2.10) and (2.11), and hence prohibits flux influence $j^* \rightsquigarrow j$ of j^* on any $j \in \mathcal{E}$. We will see many examples of this simple principle later.

To characterize metabolite influences $j^* \rightsquigarrow m' \in \mathcal{M}$ we have to define *partial child selections* $J^\vee\colon \mathcal{M} \smallsetminus \{m'\} \to \mathcal{E}$. We require injectivity and (2.3) verbatim, but just for all $m \neq m'$, of course.

Theorem 2.3. *Assume* $\det(SR) \neq 0$ *holds, algebraically, as asserted by theorem 2.1. Then metabolite influence* $j^* \rightsquigarrow m'$ *occurs, if and only if there exists a partial child selection* $J^\vee\colon \mathcal{M} \smallsetminus \{m'\} \to \mathcal{E} \smallsetminus \{j^*\}$, *such that the* augmented set $\{j^*\} \cup J^\vee(\mathcal{M} \smallsetminus \{m'\})$ *selects an* S*-basis, i.e.*

$$j^* \notin J^\vee(\mathcal{M} \smallsetminus \{m'\}) \quad \textit{and} \quad \ker S \cap \big(\{j^*\} \cup J^\vee(\mathcal{M} \smallsetminus \{m'\})\big) = \{0\}\,. \tag{2.12}$$

Again the above *single child case* $m^* \vdash j^*$ with the unique single-child mother m^* of the single child j^* is instructive. We claim that *single children only influence their mother*. Indeed consider $m' = m^*$, first. We have assumed $\det(SR) \neq 0$. By theorem 2.1 this provides a kernel-free child selection $J\colon \mathcal{M} \to \mathcal{E}$, see (2.3). Moreover $J(m^*) = j^*$, by (2.9). Define the partial child selection $J^\vee$ as the restriction of J to $\mathcal{M} \smallsetminus \{m^*\}$. Then (2.12) follows from (2.3) and hence $j^* \rightsquigarrow m^*$ influences its unique single-child mother m^*. Next consider $m' \neq m^*$. Then $m^* \in \mathcal{M} \smallsetminus \{m'\}$ implies $j^* = J \vee (m^*) \in J^\vee(\mathcal{M} \smallsetminus \{m'\})$, for any partial child selection $J^\vee$ on $\mathcal{M} \smallsetminus \{m'\}$, again by (2.9). This prevents any metabolite influence of j^* other than $j^* \rightsquigarrow m^*$.

In the *monomolecular case*, where the stoichiometric vectors $y^j, \bar{y}^j$ have singleton or empty support, we have indicated in section 1 how the reaction edges define a directed reaction graph with vertex set $\mathcal{M} \cup \{0\}$. The kernel condition (2.5) of theorem 2.1 then garantees that the child selection J does not run into oriented or nonoriented cycles. Equivalently, J defines a directed exit path γ^0 from the unique mother vertex m^* of any reaction j^* to the exit vertex 0. Theorem 2.2 then characterizes $j^* \rightsquigarrow j'$ by the existence of a second di-path γ' from the same mother m^* of j^* to j'. The paths γ^* and γ' are disjoint, except for m^*, and one of them contains j^*. These results can, and have been, derived from our more general formulation of theorems 2.1 and 2.2. In the present multimolecular case we might, of course, resort to bipartite digraphs with respective vertices $\mathcal{M} \cup \{0\}$ and $\mathcal{E}$. Directed edges $\mathcal{M} \cup \{0\} \ni m \to j \in \mathcal{E}$ then indicate inputs $m \vdash j$ or feed reactions j. The opposite edges $j \to m$ refer to outputs or exit reactions. However, quite unlike the monomolecular case, we neither succeeded with our attempts at a bipartite reaction graph proof, nor via extensive child swapping, to prove transitivity of flux or metabolite influence. Our proof of the following *transitivity theorem*, in section 6, will involve direct differentiation with respect to the intermediate variable r_{jm}, instead.

Theorem 2.4. *Assume* $\det(SR) \neq 0$ *holds, algebraically, as asserted in theorem 2.1. Then the following two transitivity properties hold.*

(i) Let $\alpha, \beta \in \mathcal{E} \cup \mathcal{M}$*, and assume* $\mathcal{M} \ni m \vdash j \in \mathcal{E}$ *is an input to reaction* j*. Furthermore assume*

$$\alpha \rightsquigarrow m \vdash j \rightsquigarrow \beta\,. \tag{2.13}$$

Then $\alpha \rightsquigarrow \beta$*.*

(ii) Let $\alpha, \beta \in \mathcal{E} \cup \mathcal{M}$*,* $j \in \mathcal{E}$ *and assume*

$$\alpha \rightsquigarrow j \rightsquigarrow \beta\,. \tag{2.14}$$

Then $\alpha \rightsquigarrow \beta$*.*

Although we have admitted metabolites $\alpha \in \mathcal{M}$ in this transitivity theorem, we have not even defined what an influence $m^* \rightsquigarrow m$ or $m^* \rightsquigarrow j$ *of* a metabolite m^* is supposed to mean. Loosely speaking this indicates nonzero sensitivity response of the steady state x to an artificial constant external feed of metabolite m^*. For a precise definition see (3.4), (3.5) and the discussion there.

Our next goal is a description of all *flux influence sets*

$$I_{\mathcal{E}}(j^*) := \{j' \in \mathcal{E} \,|\, j^* \rightsquigarrow j'\}\,, \tag{2.15}$$

and of all *metabolite influence sets*

$$I_{\mathcal{M}}(j^*) = \{m' \in \mathcal{M} \,|\, j^* \rightsquigarrow m'\}\,, \tag{2.16}$$

for rate perturbations of any single reaction j^*. Of course theorems 2.2 and 2.3 characterize these sets. For single children $m^* \vdash j^*$ we have seen $I_{\mathcal{E}}(j^*) = \emptyset$ and $I_{\mathcal{M}}(j^*) = \{m^*\}$. But transitivity theorem 2.4 suggests a hierarchy of these sets, which we begin to explore now. We begin with a useful definition.

Definition 2.5. *We call a pair* $(\mathcal{E}_0, \mathcal{M})$ *of reactions* $j_0 \in \mathcal{E}_0 \subseteq \mathcal{E}$ *and metabolites* $m_0 \in \mathcal{M}_0 \in \mathcal{M}_0 \subseteq \mathcal{M}$ *a* metabolite influence block*, if the following two conditions both hold.*

(i) $(\mathcal{E}_0, \mathcal{M}_0)$ *is* reaction complete*, i.e.*

$$\mathcal{M}_0 \ni m_0 \vdash j \quad \Longrightarrow \quad j \in \mathcal{E}_0\,. \tag{2.17}$$

In other words, $\mathcal{E}_0$ *contains all reactions with input from at least one* $m_0 \in \mathcal{M}_0$*.*

(ii) $(\mathcal{E}_0, \mathcal{M}_0)$ *is* metabolite influence complete*, i.e.*

$$\mathcal{E}_0 \ni j_0 \rightsquigarrow m \quad \Longrightarrow \quad m \in \mathcal{M}_0\,. \tag{2.18}$$

In other words, $\mathcal{M}_0$ contains the metabolite influence set $I_{\mathcal{M}}(j_0)$, for any $j_0 \in \mathcal{E}_0$.

In section 7 we will show how these influence blocks coincide with the *localized blocks* or *response blocks* introduced by Okada in [Oka+15, OM16].

By definition 2.5 it is obvious that intersections

$$(\mathcal{E}_0, \mathcal{M}_0) \cap (\mathcal{E}_1, \mathcal{M}_1) := (\mathcal{E}_0 \cap \mathcal{E}_1, \mathcal{M}_0 \cap \mathcal{M}_1) \tag{2.19}$$

of influence blocks remain influence blocks. In particular, fix any reaction $j^* \in \mathcal{E}$. We can then define the *metabolite influence block* $(\mathcal{E}^*, \mathcal{M}^*)$ *generated by* j^*, as the *minimal influence block containing* j^*, i.e.

$$(\mathcal{E}^*, \mathcal{M}^*) := \bigcap_{j^* \in \mathcal{E}_0} (\mathcal{E}_0, \mathcal{M}_0)\,. \tag{2.20}$$

Here all pairs $(\mathcal{E}_0, \mathcal{M}_0)$ in the intersection are assumed to be influence blocks. Obviously $j^* \in \mathcal{E}^*$. In particular

$$I_{\mathcal{E}}(j^*) \neq \mathcal{E}^*\,, \tag{2.21}$$

whenever j^* is without self-influence; see theorem 2.2(i).

Theorem 2.6. *Assume* $\det(SR) \neq 0$ *holds, algebraically, as asserted by theorem 2.1. Recall the above definitions* (2.15), (2.16) *of the influence sets* $I_{\mathcal{E}}(j^*)$, $I_{\mathcal{M}}(j^*)$, *and definition* (2.20) *of the (minimal) metabolite influence block* $(\mathcal{E}^*, \mathcal{M}^*)$ *generated by* j^*. *Then*

$$I_{\mathcal{E}}(j^*) \subseteq \mathcal{E}^*\,, \quad \textit{and} \tag{2.22}$$

$$I_{\mathcal{M}}(j^*) = \mathcal{M}^*\,, \tag{2.23}$$

for any reaction $j^* \in \mathcal{E}$.

Transitivity theorem 2.4 applies to fluxes $\alpha, \beta \in \mathcal{E}$, in particular. Then (2.14) states transitivity of flux influence:

$$j_1 \rightsquigarrow j_2 \rightsquigarrow j_3 \quad \Longrightarrow \quad j_1 \rightsquigarrow j_3\,. \tag{2.24}$$

See [FM15] for the monomolecular case. Verbatim as in that paper we can therefore construct the directed *pure flux influence graph* F as follows. We first define an *equivalence relation* $\approx$ on $j \in \mathcal{E}$ by

$$j \approx j\,, \quad \text{for all} \quad j \tag{2.25}$$

$$j_1 \approx j_2 \neq j_1\,, \quad \text{iff} \quad j_1 \rightsquigarrow j_2 \quad \text{and} \quad j_2 \rightsquigarrow j_1\,. \tag{2.26}$$

Note that reflexivity (2.25) may, or may not, be founded on self-influence $j \rightsquigarrow j$. Our definition generously glosses over this delicate point, now, to create some subtlety later.

The equivalence classes of $\approx$ are the vertices of F. In general, we denote an equivalence class φ by brackets

$$\varphi = \langle j_1, j_2, \ldots \rangle \tag{2.27}$$

and call it a *(mutual) flux influence class*. The subtle case of single element equivalence classes, however, comes in two flavors. We write such single element classes φ of j as

$$\varphi = \begin{cases} \langle j \rangle \,, & \text{if} \quad j \rightsquigarrow j \,, \\ j \,, & \text{otherwise} \,. \end{cases} \tag{2.28}$$

We simply omit the brackets $\langle\,\rangle$ in case j does not influence itself. This is the case where reflexivity $j \approx j$ was generously decreed by *fiat*, in (2.25), in spite of lacking self-influence.

Transitivity defines a partial order on the equivalence classes, by influence. This partial order can, equivalently, be expressed by a finite directed graph F. Directed paths run in the direction of, and imply, flux influence. Since the vertices φ are equivalence classes, F does not possess any directed cycle. More precisely, a di-path from one class vertex φ^* to another class φ' implies that any single j^* in φ^* influences all j' in φ' – but not vice versa. Also, j^* influences all j' in its own class φ^* – except possibly itself; see (2.28). Therefore the pure flux influence graph F, in the above notation, directly encodes the flux influence set $I_{\mathcal{E}}(j^*)$ of a rate perturbation at any given reaction j^*.

To include the metabolite responses to j^*, i.e. the metabolite influence sets $I_{\mathcal{M}}(j^*)$ we define the *(full) flux influence graph* $\mathcal{F}$, simply by a notational modification at the vertices of F. We keep all directed edges, as defined between the equivalence classes of pure flux influence F.

Consider the metabolite influence sets $I_{\mathcal{M}}(j^*)$; see also theorem 2.6. We apply (2.14) of transitivity theorem 2.4(ii) with $\alpha \in \mathcal{E}, \beta \in \mathcal{M}$ and observe

$$j_1 \rightsquigarrow j_2 \rightsquigarrow m \quad \Longrightarrow \quad j_1 \rightsquigarrow m \,. \tag{2.29}$$

In particular

$$\begin{aligned} j_1 \rightsquigarrow j_2 \quad &\Longrightarrow \quad I_{\mathcal{M}}(j_1) \supseteq I_{\mathcal{M}}(j_2) \,, \quad \text{and} \\ j_1 \approx j_2 \quad &\Longrightarrow \quad I_{\mathcal{M}}(j_1) = I_{\mathcal{M}}(j_2) \,. \end{aligned} \tag{2.30}$$

Now fix any mutual influence class $\varphi^* := \langle j^*, \ldots \rangle$ or $\varphi^* := j^*$, i.e. any vertex of the pure flux influence graph F. By (2.30), all $j^* \in \varphi^*$ share the same metabolic influence set

$$I_{\mathcal{M}}(j^*) = I_{\mathcal{M}}(\varphi^*) \,. \tag{2.31}$$

We define the, possibly empty, *indirect metabolite influence set* $\mathcal{M}^{\neg d}(\varphi^*)$ as

$$\begin{aligned}\mathcal{M}^{\neg d}(j^*) = \mathcal{M}^{\neg d}(\varphi^*) = \\ = \{m' \in \mathcal{M} \,|\, \text{exists } j' \notin \varphi^* \text{ such that } j^* \rightsquigarrow j' \rightsquigarrow m'\}\,.\end{aligned} \tag{2.32}$$

In other words, indirect influence of j^* requires flux influence on an *intermediary agent* j' in another flux influence class φ' to exert its influence on m via j'. By transitivity (2.29) the intermediary j' mediates the (indirect) influence $j^* \rightsquigarrow m'$, and this influence does not depend on the choice of the representatives $j^* \in \varphi^*$, $j' \in \varphi'$. Conversely, the intermediary agent $j' \notin \varphi^*$ of j^* does not influence $j^* \in \varphi^*$.

Analogously we define the, possibly empty, complementary set $\mathcal{M}^d(\varphi^*)$ of *direct metabolite influence*

$$\mathcal{M}^d(j^*) = \mathcal{M}^d(\varphi^*) := I_{\mathcal{M}}(\varphi^*) \smallsetminus \mathcal{M}^{\neg d}(\varphi^*) \tag{2.33}$$

to consists of all those metabolites $j^* \rightsquigarrow m'$ which are influenced by j^*, but cannot ever be influenced by any intermediary agent $j' \notin \varphi^*$. In particular we obtain the decompositions

$$I_{\mathcal{M}}(j^*) = \mathcal{M}^d(\varphi^*) \,\dot\cup\, \mathcal{M}^{\neg d}(\varphi^*)\,, \quad \text{and} \tag{2.34}$$

$$\mathcal{M}^{\neg d}(\varphi^*) = \bigcup_{j^* \rightsquigarrow j' \notin \varphi^*} \mathcal{M}^d(j')\,. \tag{2.35}$$

By definition, the union in (2.34) is disjoint. We may replace the intermediary agents j' by a single representative in each flux influence class.

Note that the classes φ of mutual flux influence form a partition of $\mathcal{E}$. The same metabolite m, in contrast, may appear in several sets of direct metabolite influence. This is the case, if and only if there exist two distinct reactions j_1, j_2 such that neither influences the other, but each influences m. In particular, the union in (2.35) need not be disjoint.

We define the *flux influence graph* $\mathcal{F}$ as follows. The vertices of $\mathcal{F}$ are given by the pairs

$$\varphi\mathcal{M}^d(\varphi) \tag{2.36}$$

of mutual flux influence classes φ and their direct metabolite influence sets $\mathcal{M}^d(\varphi)$; see (2.27), (2.28) and (2.33). Edges are directed and coincide with the directed edges of the pure flux influence graph F on the vertices φ. Summarizing our above construction and discussion, we have proved the following theorem.

Theorem 2.7. *Assume* $\det(SR) \neq 0$*, holds, algebraically, as asserted by theorem 2.1. Define the full flux influence graph* $\mathcal{F}$ *as above, and let* φ^* *be the flux influence class of* j^**; see* (2.27), (2.28)*.*

Then a rate perturbation of reaction j^* *influences the flux of* $j' \neq j^*$*, i.e.* $j^* \rightsquigarrow j' \neq j^*$*, if and only if* $j' \in \varphi^*$*, or there exists a di-path in* $\mathcal{F}$ *from the vertex* $\varphi^*\mathcal{M}^d(\varphi^*)$

to the vertex $\varphi' \mathcal{M}^d(\varphi')$ of the flux influence class $\varphi' \neq \varphi^*$ of j'. Equivalently, the same di-path runs from φ^* to φ' in the pure flux influence graph F. Self-influence $j^* \rightsquigarrow j^*$ holds unless $\varphi^* = j^*$ with removed brackets; see (2.28).

A perturbation j^* influences the metabolite $m' \in \mathcal{M}$; i.e. $j^* \rightsquigarrow m'$, if and only if,

(i) either, $m' \in \mathcal{M}^d(\varphi^*)$ is under the direct influence of the class φ^* of j^*; see (2.33);

(ii) or else, $m' \in \mathcal{M}^d(\varphi')$ is under the direct influence of some intermediary agent j' in another class $\varphi' \neq \varphi^*$ such that $j^* \rightsquigarrow j'$; see (2.32)–(2.35).

In the flux influence graph $\mathcal{F}$ this means that we pass from $j^* \in \varphi^*$, either, to $m' \in \mathcal{M}^d(\varphi^*)$ directly in the same vertex $\varphi^* \mathcal{M}^d(\varphi^*)$, or else, to the annotation $m' \in \mathcal{M}^d(\varphi')$ of another vertex $\varphi' \mathcal{M}^d(\varphi')$, via the di-paths in $\mathcal{F}$.

We recall and emphasize that these nonzero influences are all generated, in unison and simultaneously, by flux perturbations at any single one reaction j^* of the same class φ^*.

3 Two theoretical examples

By our standing assumption (1.10), the stoichiometric matrix S: $\mathcal{E} \to \mathcal{M}$ has full rank $M = |\mathcal{M}|$. Therefore $|\mathcal{E}| \geq |\mathcal{M}|$: the number $|\mathcal{M}|$ of metabolites does not strictly exceed the number $|\mathcal{E}|$ of reactions. Our first example addresses the simplest case where $|\mathcal{E}| = |\mathcal{M}|$. Our second example studies the flux influence graph $\mathcal{F}$ for a simple hypothetical reaction network from [MF15] which is monomolecular, except for a single bimolecular reaction; here $|\mathcal{E}| = 15 > 10 = |\mathcal{M}|$. Our examples illustrate the workings of theorem 2.7 about direct and indirect flux influence.

Here, and for all our proofs in sections 4–7 below, it will be convenient to rewrite the system (1.17), (1.18) for the response vector $z^{j^*} := (\Phi^{j^*}, \delta x^{j^*}) \in \mathcal{E} \times \mathcal{M}$ in block matrix form as

$$B z^\alpha = -e_\alpha \tag{3.1}$$

with the unit vector e_α, for $\alpha = j^*$, and the $\mathcal{E} \times \mathcal{M}$ block matrix

$$B := \begin{pmatrix} -\mathrm{id}_{\mathcal{E}} & R \\ S & 0 \end{pmatrix} : \quad \mathcal{E} \cup \mathcal{M} \longrightarrow \mathcal{E} \cup \mathcal{M} \,. \tag{3.2}$$

Here we have padded the unit vector $e_\alpha = e_{j^*} \in \mathcal{E} \subseteq \mathcal{E} \cup \mathcal{M}$ for $j^* \in \mathcal{E}$ with zeros in the $\mathcal{M}$-components. By definition we have

$$\alpha \rightsquigarrow \beta \quad \Longleftrightarrow \quad z^\alpha_\beta \neq 0 \,, \quad \text{algebraically} \,, \tag{3.3}$$

for any $\alpha \in \mathcal{E}$, $\beta \in \mathcal{E} \cup \mathcal{M}$.

We digress briefly to consider metabolite "perturbations" $\alpha = m^* \in \mathcal{M}$, as well. *Define z^{m^*} as the (flux, metabolite)-response to an external perturbation of the metabolite m^*.* In other words, we define

$$m^* \rightsquigarrow \beta \quad \Longleftrightarrow \quad z_\beta^{m^*} \neq 0\,, \quad \text{algebraically}\,, \tag{3.4}$$

From an applied point of view this means that we study the (infinitesimal) steady state response δx^{m^*} of x, and the associated flux changes Φ^{m^*}, to external feeds of x_{m^*},

$$\dot{x} = S\mathbf{r}(x) + \varepsilon\, e_{m^*}\,, \tag{3.5}$$

as the rate ε of that feed varies. That said, we will not pursue perturbations by metabolic feeds further, in the present paper.

After this little digression we now present the case $|\mathcal{E}| = |\mathcal{M}|$ as our first example. Then the matrices S and R are square, and

$$\det B = (-1)^E \det S \cdot \det R = (-1)^E \det(SR)\,. \tag{3.6}$$

In particular $\det S \neq 0$, by rank condition (1.10).

The steady state equation (1.11) becomes $r(x) = 0$, for $\det S \neq 0$. If $r(x) > 0$ for $x > 0$, componentwise, this excludes the existence of positive steady states $x > 0$. However, we did not impose any such positivity restriction on $r(x)$. Even in the monomolecular case, for example, a feed reaction $0 \to X_1$ in (1.2) can be lumped with a subsequent forward reaction $X_1 \to X_2$ into a single reaction term like $r_1 = r_1(x) = k_0 - k_1 x_1$, at the expense of violating positivity $r_1 > 0$.

The child selections $J\colon\ \mathcal{M} \to \mathcal{E}$ of theorem 2.1 readily appear in the evaluation of $\det R$, and

$$a_J = \mathrm{sgn} J \cdot \det S \tag{3.7}$$

in (2.6), (2.8), if we view J as a permutation of $|\mathcal{M}| = |J|$ elements with signature $\mathrm{sgn} J$. Our nondegeneracy assumption $\det(SR) \neq 0$ for regular networks amounts to (algebraic) invertibility of R.

Theorem 2.2 informs us that there are no influences $j^* \rightsquigarrow j' \in \mathcal{E}$ at all, since $j^* \notin J(\mathcal{M}) = \mathcal{E}$ is impossible. Of course this also follows directly, because $S\Phi^{j^*} = 0$ in (1.18) and $\det S \neq 0$ imply $\Phi^{j^*} = 0$ for any $j^* \in \mathcal{E}$. Thus the flux influence sets $I_\mathcal{E}(j^*)$ of (2.15) are all empty. The pure flux influence graph F consists of the $|\mathcal{E}|$ isolated vertices $j^* \in \mathcal{E}$, sadly without any edges. The full flux influence graph $\mathcal{F}$, then, does not possess any edges either. Therefore all metabolite influences $I_\mathcal{M}(j^*)$ are direct, i.e.

$$I_\mathcal{E}(j^*) = \{\,\}\,; \qquad I_\mathcal{M}(j^*) = \mathcal{M}^d(j^*)\,. \tag{3.8}$$

Theorem 2.3 implies that $j^* \rightsquigarrow m'$, if $J(m') = j^*$, for some child selection permutation $J\colon \mathcal{M} \to \mathcal{E}$. Indeed $\ker S = \{0\}$. Hence

$$\mathcal{M}^d(j^*) \supseteq \{J^{-1}(j^*) \,|\, J \text{ is a child selection}\}\,. \tag{3.9}$$

Clearly the metabolite influence sets $\mathcal{M}^d(j^*)$ will not be disjoint, here, if the reaction network admits more than one child selection permutation J.

We now turn to the example of [MF15] with 15 reactions $\mathcal{E} = \{1, \dots, 15\}$, 10 metabolites $\mathcal{M} = \{A, \dots, J\}$, and a single bimolecular reaction

$$j = 12: \quad G + H \longrightarrow I\,, \tag{3.10}$$

see fig. 3.1(a). We do not plan to confuse metabolites F, J with the pure flux influence graph F or child selections J, in spite of this notation.

The 25×25-matrix B of (3.1), (3.2) can easily be inverted symbolically. For example

$$\det B = -r_3\, r_4\, r_5\, r_7\, r_9\, r_{11}\, r_{12H}\, r_{14}\, r_{15}\, (2r_{10} + r_{13})\,. \tag{3.11}$$

We have omitted the redundant input metabolite index m in r_{jm}, for monomolecular reactions j. In (3.11) we see explicitly what $\det B \neq 0$, algebraically, means. We would have to require nonzero prefactors $r_3, \dots, r_{15}$ and the linear nondegeneracy $2r_{10} + r_{13} \neq 0$. In this explicit sense, the bimolecular chemical reaction network of fig. 3.1(a) is regular, algebraically; see also theorem 2.1.

The feed reactions are $\{1, 2\}$ and the exit reactions are $\{14, 15\}$. We have omitted the formal metabolite entry 0 in these open system reactions. The seven single children j, as defined just after (2.3), are

$$j \in \{3, 4, 7, 9, 12, 14, 15\}\,. \tag{3.12}$$

By theorem 2.2 they have no flux influence, and therefore define terminal sinks of the pure flux influence graph F; see fig. 3.1(b).

Let us study (3.11) in a little more detail, in terms of child selections J and (2.5)–(2.9). The seven single children j in (3.12) are forced to appear in the index set of any monomial in (3.11). Indeed, their respective single-child mothers $m \vdash j$ have no choice but to select their only child $j = J(m)$; see (2.3) and (2.9). Since the bimolecular reaction $j = 12$ is the single child of metabolite H, this also forces $j = J(G) = 9$ to appear in the prefactor of (3.11). The only remaining choices are

$$J(C) \in \{5, 6\} \quad \text{and} \quad J(F) \in \{8, 10, 13\}\,. \tag{3.13}$$

The choice $J(C) = 6$ immediately leads to the indicator $1_{\{6,9,11\}}$ of the network cycle $C \xrightarrow{9} E \xrightarrow{9} G \xrightarrow{11} C$ as a kernel vector of S supported on $J(\mathcal{M})$. Indeed $J(E) = 9$ is a single child, and we just saw why $J(G) = 11$ is also forced to hold. Therefore we

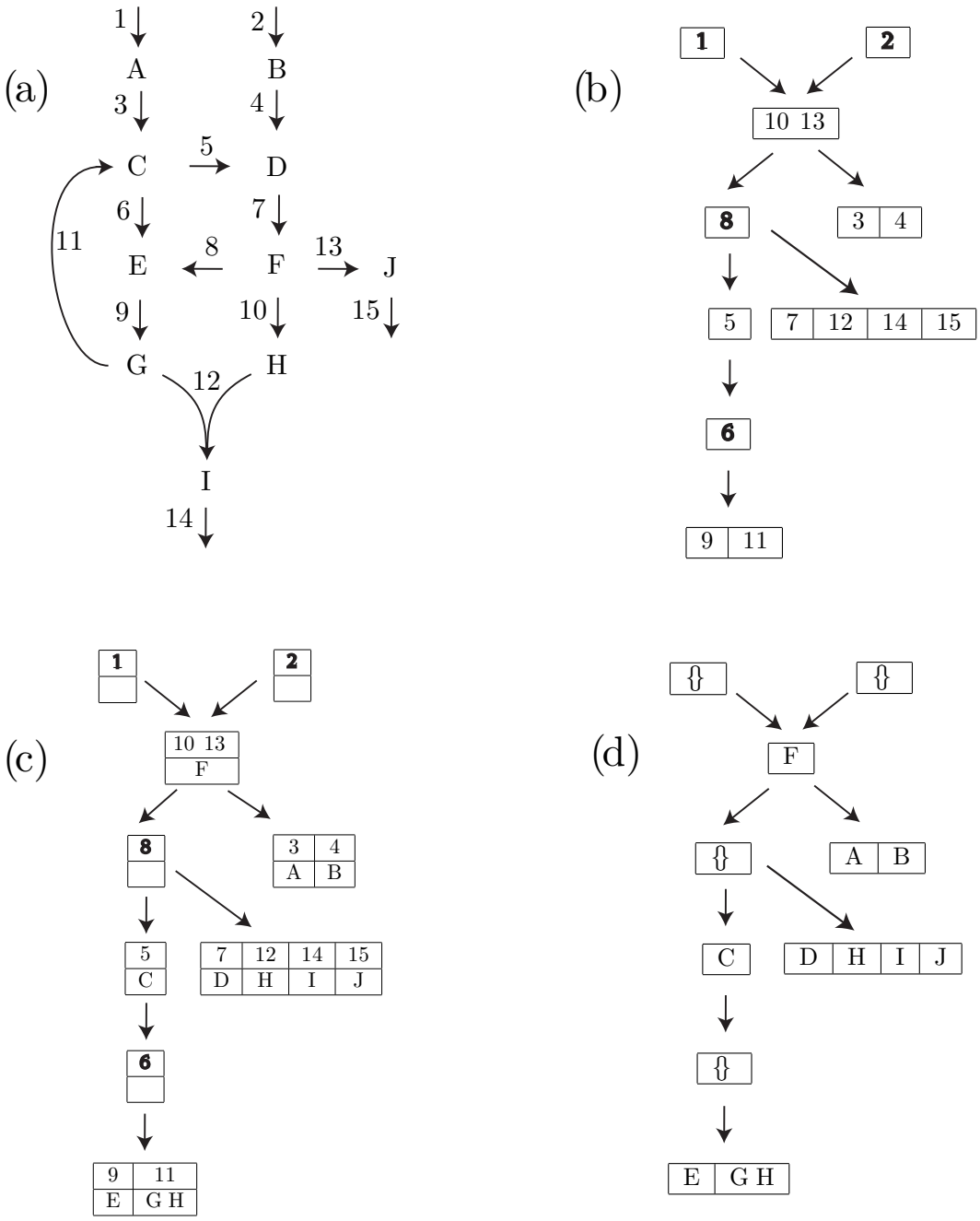

Figure 3.1: *(a) Hypothetical network with only one bimolecular reaction $j = 12$; see [MF15]. (b) The pure flux influence graph F. Equivalence classes φ^* are denoted in boxes. Self influences $1, 2, 6, 8$ are marked in boldface. Arrows to box arrays with several columns are pointing to each column, separately. (c) The full flux influence graph $\mathcal{F}$ with direct metabolite influence $\mathcal{M}^d(\varphi^*)$ attached below the vertex box φ^* of F. Omitting the flux classes φ^* from $\mathcal{F}$, we obtain the graph (d) of all possible metabolite influence sets $I_{\mathcal{M}}(\varphi^*)$. These are given by the union along all di-paths emanating from any given vertex $\mathcal{M}^d(\varphi^*)$, including empty set vertices $\{\,\}$.*

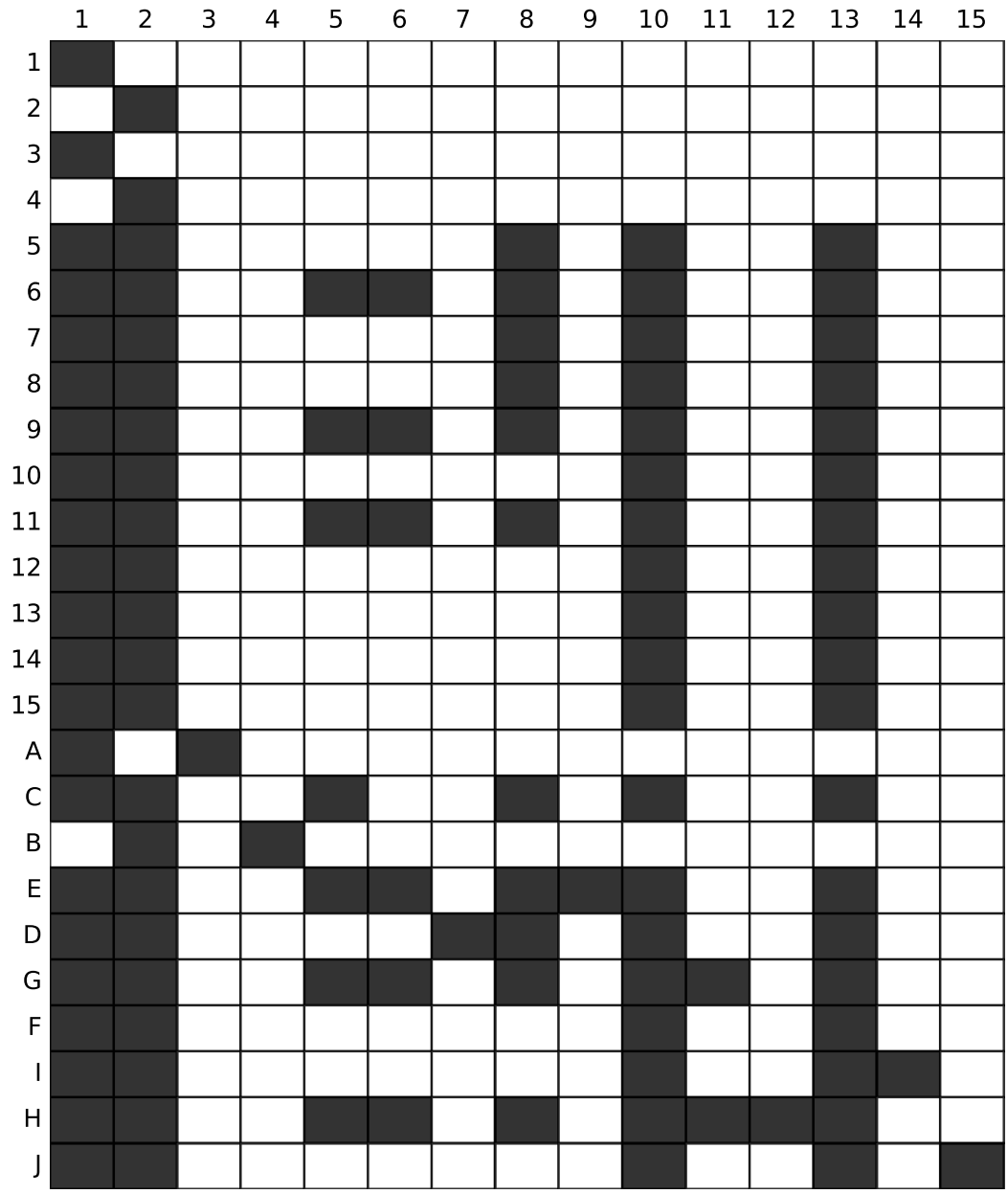

Figure 3.2: *Algebraically nonzero entries (black) of the sensitivity matrix* $B^{-1}_{\beta\alpha}$*, of example network fig. 3.1(a). A black area in row* β *and column* $\alpha \in \mathcal{E}$ *indicates nonzero influence* $\alpha \rightsquigarrow \beta$ *of the rate of reaction* α *on the flux of reaction* β*, for* $\beta \in \mathcal{E}$*, and on the concentration of metabolite* β*, for* $\beta \in \mathcal{M}$*.*

must choose $J(C) = 5$. This explains why r_5 must also appear in the prefactor of (3.11).

We could easily proceed with such elementary arguments to fully derive (3.11) by hand. Likewise, the modified child selections of theorems 2.2 and 2.3 would determine all flux and metabolite influences of rate perturbations j^*, explicitly. Instead, we present a symbolic version of B^{-1} in table 3.2. A black area entry for $B^{-1}_{\beta\alpha}$ in row β and column α of B^{-1} is equivalent to an algebraically nonzero component z^α_β of the response, i.e. to the influence $\alpha \rightsquigarrow \beta$; see (3.1), (3.2). By the construction (2.31)–(2.33), (2.36) this immediately defines the flux influence di-graphs $F, \mathcal{F}$ of fig. 3.1(b),(c). Theorem 2.7 explains how these graphs determine all flux influence sets $I_\mathcal{E}(j^*)$ and metabolite influence sets $I_\mathcal{M}(j^*)$, explicitly, by following di-paths downward in the diagrams. Note the mother-child pairs $m^* \vdash j^*$ which provide terminal sink vertices $j^*\{m^*\}$ in $\mathcal{F}$, fig. 3.1(c). The bimolecular input $\{G, T, H\}$ of reaction 12, interestingly, cannot respond to a perturbation of $j^* = 12$, because $j^* = 12$ is the single child of $m^* = H$. In fact it arises in response to the terminal sink $j^* = 11$ of the di-graph F. Indeed $j^* = 11$ cannot influence itself, or any other flux, by theorem 2.2, because we already saw that $j^* = 11 = J(G)$ must appear in any child selection. Therefore $j^* \rightsquigarrow G$, in order not to change its own flux. That metabolite change induces $j^* \rightsquigarrow H$ because the bimolecular flux of $j' = 12$ is not influenced by $j^* = 11$. This explains the "bimolecular" sink vertex $11\{G, H\}$ in the flux influence graph $\mathcal{F}$.

We have detailed in theorem 2.7 how any metabolite influence set $I_\mathcal{M}(j^*) = I_\mathcal{M}(\varphi^*)$ arises as a union of direct influence sets $\mathcal{M}^d(\varphi')$ along all downward di-paths from $\varphi^*\mathcal{M}^d(\varphi^*)$, including that starting vertex itself; see (2.34), (2.35). To obtain all metabolite influence sets we can therefore simply omit the part φ^* of the vertex labels in $\mathcal{F}$ and take unions of the remaining metabolite labels $\mathcal{M}^d(\varphi')$ along all downward di-paths; see fig. 3.1(d). Note that some empty label vertices $\{\ \}$ cannot be omitted. For example, the omission of $\langle 8\rangle\{\ \}$ would claim that $\{D, E, G, H\} = I_\mathcal{M}(8)$ is not a metabolite influence set of any single flux perturbation. Omission of $\langle 6\rangle\{\ \}$, likewise, would claim that $\{E, G, H\} = I_\mathcal{M}(6)$ does not occur.

Our simple examples indicate a wealth of sensitivity information which is extracted from structural assumptions, alone. Transitivity theorem 2.4 allows to display all flux influences in a single diagram. And the sparse, and highly structured, inverse B^{-1} of the sparse network/stoichiometry matrix B testifies against conventional "knowledge" that inverses of sparse matrices are rarely sparse.

4 Proofs of theorems 2.1–2.3

Our starting point is the B-matrix of section 3; see (3.1)–(3.3). It is easy to block-diagonalize and to invert B explicitly:

$$\begin{pmatrix} \mathrm{id}_\mathcal{E} & 0 \\ S & \mathrm{id}_\mathcal{M} \end{pmatrix} \begin{pmatrix} -\mathrm{id}_\mathcal{E} & R \\ S & 0 \end{pmatrix} \begin{pmatrix} \mathrm{id}_\mathcal{E} & R \\ 0 & \mathrm{id}_\mathcal{M} \end{pmatrix} = \begin{pmatrix} -\mathrm{id}_\mathcal{E} & 0 \\ 0 & SR \end{pmatrix} . \tag{4.1}$$

With $E := |\mathcal{E}|$ counting all reactions, this implies

$$\det B = (-1)^E \det(SR)\,. \tag{4.2}$$

Moreover B is invertible, if and only if SR is, with

$$B^{-1} = \begin{pmatrix} -\mathrm{id}_{\mathcal{E}} + R(SR)^{-1}S & R(SR)^{-1} \\ (SR)^{-1}S & (SR)^{-1} \end{pmatrix}\,. \tag{4.3}$$

Our proofs of theorems 2.1–2.3 are all based on the Cauchy-Binet formula [Gan77] for determinants like $\det(SR)$ in (4.2).

Throughout this section, and for any matrix B, let

$$B^{\sigma}_{\rho} \tag{4.4}$$

denote the submatrix of B which consists of rows ρ and columns σ, only. We frequently omit braces $\{j\}$ for single element sets; for example S_m and S^j denote row m and column j of S, respectively.

Proof of theorem 2.1. By the Cauchy-Binet formula, [Gan77],

$$\det(SR) = \sum_{\mathcal{E}' \in \mathcal{E}_M} \det S^{\mathcal{E}'} \cdot \det R_{\mathcal{E}'} \tag{4.5}$$

where the sum runs over the set $\mathcal{E}_M$ of all $\mathcal{E}' \subseteq \mathcal{E}$ with $|\mathcal{E}'| = M = |\mathcal{M}|$ elements. More explicitly,

$$\det R_{\mathcal{E}'} = \sum_{J:\mathcal{M}\to\mathcal{E}'} \mathrm{sgn} J \cdot \mathbf{r}^J \tag{4.6}$$

where $\mathrm{sgn} J$ denotes the signature, or parity, of the bijection $J\colon \mathcal{M} \to \mathcal{E}'$. We view J as a permutation of the M elements of $\mathcal{M}$, via the order preserving bijective identification of $\mathcal{M}$ and $\mathcal{E}' = J(\mathcal{M})$. The monomial $\mathbf{r}^J$ of (2.7)is nontrivial, if and only if J is a child selection; see (1.6), (1.7) and (2.1), (2.2). This proves (2.6), (2.8) with

$$a_J = \mathrm{sgn} J \cdot \det S^{J(\mathcal{M})}\,, \tag{4.7}$$

and theorem 2.1. ⋈

Henceforth we assume $\det SR \neq 0$, alias $\det B \neq 0$. By Cramer's rule and Cauchy-Binet we obtain

$$\begin{aligned} \det(SR)(SR)^{-1}_{m_1 m_2} &= (-1)^{m_1+m_2} \det(SR)^{\mathcal{M}\smallsetminus m_1}_{\mathcal{M}\smallsetminus m_2} = \\ &= (-1)^{m_1+m_2} \det(S_{\mathcal{M}\smallsetminus m_2} R^{\mathcal{M}\smallsetminus m_1}) = \\ &= (-1)^{m_1+m_2} \sum_{\mathcal{E}'\in\mathcal{E}_{M-1}} \det S^{\mathcal{E}'}_{\mathcal{M}\smallsetminus m_2} \cdot \det R^{\mathcal{M}\smallsetminus m_1}_{\mathcal{E}'}\,, \end{aligned} \tag{4.8}$$

for any $m_1, m_2 \in \mathcal{M}$. By (3.1)–(3.3) we already know how $\alpha \rightsquigarrow \beta$ is equivalent to

$$0 \neq z^{\alpha}_{\beta} = -e_{\beta}^T B^{-1} e_{\alpha} = -B^{-1}_{\beta\alpha}\,, \tag{4.9}$$

algebraically. By (4.3), (4.5), (4.8) we already know how to evaluate such terms.

Proof of theorem 2.2(i). We have to consider the special case $\alpha = \beta = j^*$. Without loss of generality, we may relabel reactions such that $j^* = 1$. Starting with (4.2), (4.9), we immediately obtain

$$
\begin{aligned}
&(-1)^E z_{j^*}^{j^*} \det B = \\
&\quad = -\det(SR) B^{-1}_{j^* j^*} = \det(SR) - \left(R \det(SR)(SR)^{-1} S\right)_{j^* j^*} = \\
&\quad = \det(SR) - \sum_{m_1, m_2 \in \mathcal{M}} R^{m_1}_{j^*} \det(SR)(SR)^{-1}_{m_1 m_2} S^{j^*}_{m_2} = \\
&\quad = \det(SR) - \\
&\quad - \sum_{m_1, m_2} \sum_{\mathcal{E}' \in \mathcal{E}_{M-1}} (-1)^{m_2 + j^*} S^{j^*}_{m_2} \det S^{\mathcal{E}'}_{\mathcal{M} \setminus m_2} \cdot (-1)^{m_1 + j^*} R^{m_1}_{j^*} \det R^{\mathcal{M} \setminus m_1}_{\mathcal{E}'} = \\
&\quad = \det(SR) - \sum_{\mathcal{E}' \in \mathcal{E}_{M-1}} \det\left(S^{j^*}, S^{\mathcal{E}'}\right) \det \begin{pmatrix} R_{j^*} \\ R_{\mathcal{E}'} \end{pmatrix} = \\
&\quad = \sum_{\mathcal{E}'' \in \mathcal{E}_M} \det\left(S^{\mathcal{E}''}\right) \det\left(R_{\mathcal{E}''}\right) - \sum_{j^* \notin \mathcal{E}' \in \mathcal{E}_{M-1}} \det\left(S^{j^*}, S^{\mathcal{E}'}\right) \det \begin{pmatrix} R_{j^*} \\ R_{\mathcal{E}'} \end{pmatrix} = \\
&\quad = \sum_{j^* \notin \mathcal{E}'' \in \mathcal{E}_{\mathcal{M}}} \det\left(S^{\mathcal{E}''}\right) \det\left(R_{\mathcal{E}''}\right) .
\end{aligned}
\tag{4.10}
$$

We have used expansions of determinants with respect to prepended S^{j^*}, R_{j^*}. We also omitted the cases $j^* \in \mathcal{E}'$ of duplicate rows and columns.

The proof of theorem 2.2(i) then concludes analogously to (4.6), (4.7) and shows

$$
z_{j^*}^{j^*} \det(SR) = \sum_{J : \mathcal{M} \to \mathcal{E} \setminus j^*} a_J \, \mathbf{r}^J \, , \tag{4.11}
$$

where J are child selections and

$$
a_J = \operatorname{sgn} J \cdot \det S^{J(\mathcal{M})} \, . \tag{4.12}
$$

⋈

Proof of theorem 2.2(ii). This time we have to consider $\alpha = j^* = 1$, $\beta = j' = 2$, without loss of generality. We proceed along the lines of (4.10)–(4.12) to prove

$$
\begin{aligned}
&(-1)^{E-1} z_{j'}^{j^*} \det B = \\
&\quad = \det(SR) B^{-1}_{j' j^*} = \sum_{m_1, m_2 \in \mathcal{M}} R^{m_1}_{j'} \det(SR)(SR)^{-1}_{m_1 m_2} S^{j^*}_{m_2} = \\
&\quad = \sum_{m_1, m_2} \sum_{\mathcal{E}' \in \mathcal{E}_{M-1}} (-1)^{m_2 + j^*} S^{j^*}_{m_2} \det S^{\mathcal{E}'}_{\mathcal{M} \setminus m_2} \cdot (-1)^{m_1 + j^*} R^{m_1}_{j'} \det R^{\mathcal{M} \setminus m_1}_{\mathcal{E}'} =
\end{aligned}
$$

$$
\begin{aligned}
&= \sum_{\mathcal{E}' \in \mathcal{E}_{M-1}} \det\left(S^{j^*}, S^{\mathcal{E}'}\right) \cdot \det\begin{pmatrix} R_{j'} \\ R_{\mathcal{E}'} \end{pmatrix} = \\
&= \sum_{j^*, j' \notin \mathcal{E}' \in \mathcal{E}_{M-1}} \det\left(S^{j^*}, S^{\mathcal{E}'}\right) \cdot \det\begin{pmatrix} R_{j'} \\ R_{\mathcal{E}'} \end{pmatrix} = \\
&= \sum_{j^*, j' \notin \mathcal{E}' \in \mathcal{E}_{M-1}} \det\left(S^{\mathcal{E}' \cup j^*}\right) \det\left(R_{\mathcal{E}' \cup j'}\right) .
\end{aligned} \tag{4.13}
$$

Again we expanded determinants to prepend $S^{j^*}, R_{j'}$ and we safely omitted the zero determinants caused by duplicate columns S^{j^*} or rows $R_{j'}$. This shows

$$
z_{j'}^{j^*} \det(SR) = \sum_{j' \in J(\mathcal{M}) \not\ni j^*} a_J \, \mathbf{r}^J \tag{4.14}
$$

where J are child selections, and

$$
a_J = -\operatorname{sgn} J \cdot \det S^{J(\mathcal{M})_{\mathrm{sw}}} \tag{4.15}
$$

with the swapped columns

$$
J(\mathcal{M})_{\mathrm{sw}} = j^* \cup J(\mathcal{M}) \smallsetminus j' \,. \tag{4.16}
$$

This completes the proof of theorem 2.2. ⋈

Proof of theorem 2.3. Quite similarly to the previous cases we only have to consider $\alpha = j^* = 1$. For $\beta = E + m' = E + 1$ we pick the first element $m' = 1$ of $\mathcal{M}$. We proceed as usual:

$$
\begin{aligned}
(-1)^{E-1} z_{m'}^{j^*} \det B = \det(SR) B^{-1}_{m' j^*} &= \sum_{m \in \mathcal{M}} \det(SR) (SR)^{-1}_{m' m} S^{j^*}_m = \\
&= \sum_{m \in \mathcal{M}} \sum_{\mathcal{E}' \in \mathcal{E}_{M-1}} (-1)^{m+j^*} S^{j^*}_m \det S^{\mathcal{E}'}_{\mathcal{M} \smallsetminus m} \cdot \det R^{\mathcal{M} \smallsetminus m'}_{\mathcal{E}'} = \\
&= \sum_{\mathcal{E}' \in \mathcal{E}_{M-1}} \det\left(S^{j^*}, S^{\mathcal{E}'}\right) \cdot \det R^{\mathcal{M} \smallsetminus m'}_{\mathcal{E}'} = \\
&= \sum_{j^* \notin \mathcal{E}' \in \mathcal{E}_{M-1}} \det\left(S^{\mathcal{E}' \cup j^*}\right) \cdot \det R^{\mathcal{M} \smallsetminus m'}_{\mathcal{E}'} \,.
\end{aligned} \tag{4.17}
$$

This shows

$$
z_{m'}^{j^*} \det(SR) = \sum_{J^\vee} a_{J^\vee} \, \mathbf{r}^{J^\vee} \tag{4.18}
$$

where $J^\vee \colon \mathcal{M} \smallsetminus m' \longrightarrow \mathcal{E} \smallsetminus j^*$ is a partial child selection. We extend $J^\vee$ to a bijection

$$
J : \mathcal{M} \longrightarrow J(\mathcal{M}) := J^\vee(\mathcal{M} \smallsetminus m') \cup j^* \,,
$$

defining $J(m'){:=}\, j^*$. Note that J need not be a child selection. Then

$$\begin{aligned} a_J &= -\operatorname{sgn} J^\vee \cdot \det S^{J^\vee(\mathcal{M}\smallsetminus m')\cup j^*} = \\ &= -\operatorname{sgn} J \cdot \det S^{J(\mathcal{M})}\,. \end{aligned}$$

This completes the proof of theorem 2.3. ⋈

5 Augmenticity

As a corollary to theorems 2.1–2.3 we study how the influence relation $j^* \rightsquigarrow \alpha \in \mathcal{E} \cup \mathcal{M}$ behaves when we enlarge the network. In theorem 5.1 below we observe how existing influences $j^* \rightsquigarrow \alpha$ in the smaller network $(\mathcal{E}_0, \mathcal{M}_0)$ persist in the larger, augmented network $(\mathcal{E}_1, \mathcal{M}_1) \supseteq (\mathcal{E}_0, \mathcal{M}_0)$, possibly enriched by new, additional influences. To distinguish this "monotonicity" feature from other, more mundane and elementary features involving monotone reaction rates or comparison type theorems in a single network, we use the term *augmenticity* for such "monotonicity" under augmentation of networks.

To be more precise we call a network $(\mathcal{E}_1, \mathcal{M}_1)$ an *augmentation* of a network $(\mathcal{E}_0, \mathcal{M}_0)$, if $(\mathcal{E}_0, \mathcal{M}_0) \subseteq (\mathcal{E}_1, \mathcal{M}_1)$ and the stoichiometric vectors $y^j, \bar{y}^j$ of the networks coincide for all $j \in \mathcal{E}_0$; see (1.2). Of course we have identified $y^j, \bar{y}^j \in \mathcal{M}_0 \subseteq \mathcal{M}_1$ here; see (1.3). In particular the associated stoichiometric matrices S_0, S_1 satisfy

$$S_0 = S_{1,\mathcal{M}_0}^{\mathcal{E}_0} \quad \text{and} \quad 0 = S_{1,\mathcal{M}_1\smallsetminus\mathcal{M}_0}^{\mathcal{E}_0}\,. \tag{5.1}$$

We also call $(\mathcal{E}_0, \mathcal{M}_0)$ a *subnetwork* of $(\mathcal{E}_1, \mathcal{M}_1)$. Admittedly, new reactions or metabolites may drastically alter the values (and even the very existence) of existing steady states. The viewpoint of qualitative sensitivity, however, is determined by the collection of algebraically nontrivial response patterns, only, as derived from the stoichiometric vectors $y^j, \bar{y}^j$. Therefore the following augmenticity theorem is surprisingly simple.

Theorem 5.1. *Assume the network $(\mathcal{E}_0, \mathcal{M}_0)$ is regular, algebraically, as in theorem 2.1. Let the network $(\mathcal{E}_1, \mathcal{M}_1)$ be an augmentation of the subnetwork $(\mathcal{E}_0, \mathcal{M}_0)$. Assume there exists a partial child selection*

$$J^\vee : \quad \mathcal{M}_1 \smallsetminus \mathcal{M}_0 \longrightarrow \mathcal{E}_1 \smallsetminus \mathcal{E}_0 \tag{5.2}$$

such that the associated restriction of the stoichiometric matrix S_1 of the augmented network $(\mathcal{E}_1, \mathcal{M}_1)$ is nonsingular:

$$\det S_{\mathcal{M}_1\smallsetminus\mathcal{M}_0}^{J^\vee(\mathcal{M}_1\smallsetminus\mathcal{M}_0)} \quad \neq \quad 0\,. \tag{5.3}$$

Then the augmented network $(\mathcal{E}_1, \mathcal{M}_1)$ is also regular, algebraically. Moreover, any influence

$$\mathcal{E}_0 \ni j^* \;\rightsquigarrow\; \alpha \in \mathcal{E}_0 \cup \mathcal{M}_0 \tag{5.4}$$

in the subnetwork $(\mathcal{E}_0, \mathcal{M}_0)$ remains an influence in the augmented network $(\mathcal{E}_1, \mathcal{M}_1)$.

Proof. To prove algebraic regularity of the augmented network, we invoke theorem 2.1. Indeed, let J_0: $\mathcal{M}_0 \to \mathcal{E}_0$ be a child selection in the algebraically regular subnetwork $(\mathcal{E}_0, \mathcal{M}_0)$ such that

$$\det S_0^{J_0(\mathcal{M}_0)} \quad \neq \quad 0\,. \tag{5.5}$$

Extend J_0 by $J^\vee$ to a child selection J_1 in the augmented network $(\mathcal{E}_1, \mathcal{M}_1)$, i.e.

$$J_1(m) := \begin{cases} J_0(m) \in \mathcal{E}_0\,, & \text{for} \quad m \in \mathcal{M}_0\,; \\ J^\vee(m) \in \mathcal{E}_1 \smallsetminus \mathcal{E}_0\,, & \text{for} \quad m \in \mathcal{M}_1 \smallsetminus \mathcal{M}_0\,. \end{cases} \tag{5.6}$$

Then extension property (5.1) implies

$$\det S_1^{J_1(\mathcal{M}_1)} \quad \neq \quad 0\,, \tag{5.7}$$

by block diagonalization. This proves algebraic regularity of the augmented network $(\mathcal{E}_1, \mathcal{M}_1)$.

Next assume influence $j^* \rightsquigarrow \alpha$ in the subnetwork $(\mathcal{E}_0, \mathcal{M}_0)$; see (5.4). Choose the associated (partial) child selections $J_0, J_0^\vee$ in $(\mathcal{E}_0, \mathcal{M}_0)$ as specified in theorems 2.2, 2.3, depending on α, respectively. The same augmentation (5.6), as before, then proves $j^* \rightsquigarrow \alpha$ in the augmented network $(\mathcal{E}_1, \mathcal{M}_1)$. This proves the theorem. $\bowtie$

We comment on the special case $\mathcal{M}_1 = \mathcal{M}_0$ of the above theorem, i.e. the augmented network only adds some new reactions to the same set of metabolites. Then a partial child selection $J^\vee$ is not required in (5.6) and all influences $j^* \rightsquigarrow \alpha$ in the subnetwork $(\mathcal{E}_0, \mathcal{M}_0)$ persist under the augmentation $(\mathcal{E}_1, \mathcal{M}_1) = (\mathcal{E}_1, \mathcal{M}_0)$. Adding feed or exit reactions are particular examples. Once again, we caution our reader that such modifications actually may disrupt steady state analysis, even though our sensitivity results remain valid – on a voided example.

6 Transitivity

In this section we prove claims (i), (2.13), and (ii), (2.14), of transitivity theorem 2.4. Although theorems 2.2 and 2.3 characterize flux influence $j^* \rightsquigarrow j'$ and metabolite influence $j^* \rightsquigarrow m'$ in complete detail, we did not succeed to prove the transitivity claims (i), (2.13) and (ii), (2.14) directly. The reason was the difficulty to match the child selections given by the assumptions $\alpha \rightsquigarrow m$, or $\alpha \rightsquigarrow j$, with $j \rightsquigarrow \beta$. Instead, we will use differentiation with respect to an intermediary term $m \vdash j$.

We first show how (i), (2.13) implies (ii), (2.14). For $\alpha = j$ or $j = \beta$ there is nothing to prove. Let $\alpha \neq j \neq \beta$ and assume $\alpha \rightsquigarrow j \neq \alpha$. By (1.17),

$$0 \neq \Phi_j^\alpha := (R\delta x^\alpha)_j = \sum_{m \vdash j} r_{jm}\, \delta x_m^\alpha \tag{6.1}$$

must hold algebraically. This means that there exists at least one input $m \vdash j$ for which $\delta x^\alpha_m \neq 0$ holds algebraically. In other words

$$\alpha \rightsquigarrow m \vdash j\,; \tag{6.2}$$

see (3.3). Since we have also assumed $j \rightsquigarrow \beta$ in (2.14), assumption (i), (2.13) is satisfied. This proves $\alpha \rightsquigarrow \beta$, as claimed in (ii), (2.19).

It remains to prove (i), (2.13). In the notation of (4.9), assumption (2.13) implies that

$$z^\alpha_m = -e_m^T B^{-1} e_\alpha \neq 0 \neq -e_\beta^T B^{-1} e_j = z^j_\beta \tag{6.3}$$

both hold algebraically; see (3.3) again. We have to show

$$z^\alpha_\beta = -e_\beta^T B^{-1} e_\alpha \neq 0 \tag{6.4}$$

holds algebraically. By (4.3) and Cramer's rule (4.8), the explicit algebraic expression for z^α_β is at most fractional linear in the variable r_{jm}. To show (6.4) it is sufficient to partially differentiate the algebraic expression (6.4) with respect to r_{jm} and show

$$\partial_{r_{jm}} z^\alpha_\beta \neq 0 \tag{6.5}$$

holds algebraically. Note that $\partial_{r_{jm}} B = e_j e_m^T$, for $m \vdash j$. Therefore (6.5) implies

$$\begin{array}{rcccccc} \partial_{r_{jm}} z^\alpha_\beta & = & -e_\beta^T \partial_{r_{jm}} B^{-1} e_\alpha & = & e_\beta^T B^{-1} (\partial_{r_{jm}} B) B^{-1} e_\alpha & = \\ & = & e_\beta^T B^{-1} e_j e_m^T B^{-1} e_\alpha & = & \big(e_\beta^T B^{-1} e_j\big) \cdot \big(e_m^T B^{-1} e_\alpha\big) & = \\ & = & z^j_\beta \cdot z^\alpha_m & \neq & 0 & , \end{array} \tag{6.6}$$

by assumption (2.13) and (3.3). This little calculation shows (6.5) and completes the proof of transitivity theorem 2.4.

7 Blocks

In this section, as always, we assume $\det B \neq 0$ and full rank $M = |\mathcal{M}|$ of S. We prove theorem 2.6 about influence blocks $(\mathcal{E}_0, \mathcal{M}_0) \subseteq (\mathcal{E}, \mathcal{M})$. We also establish their equivalence to the localized blocks or response blocks introduced by Okada in [Oka+15, OM16] and thereby clarify the mathematical significance of Okada's response blocks.

From Definition 2.5 we recall that $(\mathcal{E}_0, \mathcal{M}_0)$ is a *metabolite influence block* if it is *reaction complete*, (7.1), and *metabolite influence complete*, (7.2):

$$\mathcal{M}_0 \ni m_0 \vdash j \quad \Longrightarrow \quad j \in \mathcal{E}_0\,; \tag{7.1}$$

$$\mathcal{E}_0 \ni j_0 \rightsquigarrow m \quad \Longrightarrow \quad m \in \mathcal{M}_0\,. \tag{7.2}$$

Okada, in contrast, replaces (7.2) by a dimension condition (7.3) which he calls *leakage free*:

$$|\mathcal{E}_0| = |\mathcal{M}_0| + \dim(\ker S \cap \mathcal{E}_0)\,. \tag{7.3}$$

He calls $(\mathcal{E}_0, \mathcal{M}_0)$ a *localized block* or *response block* if the pair is reaction complete, (7.1), and leakage free, (7.3). An advantage of his definition is that it replaces the metabolite influence property (7.2) by the linear algebra property (7.3). A disadvantage, at first glance, is the apparent lack of any information on influence. Or, is that so?

Lemma 7.1. *The following three equivalences hold.*

(i) A pair $(\mathcal{E}_0, \mathcal{M}_0) \subseteq (\mathcal{E}, \mathcal{M})$ is reaction complete, if and only if B restricts to a map

$$B: \quad (\ker S \cap \mathcal{E}_0) \times \mathcal{M}_0 \longrightarrow \mathcal{E}_0 \times \{0\}\,. \tag{7.4}$$

(ii) A pair $(\mathcal{E}_\prime, \mathcal{M}_0)$ is an influence block, if and only if (7.4) holds and is a linear isomorphism. Here, as always, we define vector spaces by their support; see (1.3).

(iii) The same isomorphism condition (7.4) characterizes localized, or response, blocks. Therefore these coincide with our influence blocks.

Proof. We write B in block components

$$B\begin{pmatrix}\Phi\\ \delta x\end{pmatrix} = \begin{pmatrix}\eta\\ \xi\end{pmatrix}, \quad i.e. \tag{7.5}$$

$$-\Phi + R\delta x = \eta\,, \tag{7.6}$$

$$S\Phi = \xi\,. \tag{7.7}$$

By (7.7), $\Phi \in \ker S$ always implies $\xi = 0$, i.e.

$$B: \quad (\ker S \cap \mathcal{E}_0) \times \mathcal{M}_0 \longrightarrow \mathcal{E} \tag{7.8}$$

holds automatically. Conversely, $\xi = 0$ implies $S\Phi = 0$, so that

$$B^{-1}: \quad \mathcal{E}_0 \longrightarrow \ker S \times \mathcal{M}\,. \tag{7.9}$$

We now show all equivalence claims, successively.

First let $(\mathcal{E}_0, \mathcal{M}_0)$ be reaction complete, (7.1). To show the mapping claim (7.4) it only remains to show $\eta \in \mathcal{E}_0$; see (7.8). Since $\Phi \in \mathcal{E}_0$, we only need to show

$$(R\delta x)_j = \sum_{m \vdash j} r_{jm}\,\delta x_m = 0 \tag{7.10}$$

for $j \notin \mathcal{E}_0$. But $\mathcal{M}_0 \ni m \vdash j$ implies $j \in \mathcal{E}_0$ because the influence pair $(\mathcal{E}_0, \mathcal{M}_0)$ is reaction complete, (7.1). Therefore all sums in (7.10) are empty, and $\eta \in \mathcal{E}_0$. This proves (7.4) for any reaction complete pair $(\mathcal{E}_0, \mathcal{M}_0)$.

Conversely, let us assume now that (7.4) holds for some pair $(\mathcal{E}_0, \mathcal{M}_0)$. To prove reaction completeness (7.1) consider any $m_0 \in \mathcal{M}_0$ and let $\delta x := e_{m_0}$, $\Phi := 0$ in (7.6), (7.7). Then $m_0 \vdash j$ being an input to j implies

$$\eta_j = (R\delta x)_j = r_{jm_0} \neq 0\,. \tag{7.11}$$

But (7.4) for B implies $\eta \in \mathcal{E}_0$, i.e. supp $\eta \subseteq \mathcal{E}_0$. This proves $j \in \mathcal{E}_0$. In particular this shows how (7.4) implies that the pair $(\mathcal{E}_0, \mathcal{M}_0)$ is reaction complete and establishes equivalence claim (i).

To prove equivalence claim (ii), we first assume (7.4) is a linear isomorphism. By (i), it only remains to show completeness (7.2) of metabolite influence. Consider any $j_0 \in \mathcal{E}_0$ and let $\eta := -e_{j_0}$, $\xi := 0$ in (7.6), (7.7). Then (7.4) for B^{-1} implies $\delta x \in \mathcal{M}_0$, i.e. supp $\delta x_m \subseteq \mathcal{M}_0$. In other words, $j_0 \rightsquigarrow m_0$, i.e. $\delta x_m \neq 0$, implies $m \in \mathcal{M}_0$. This proves (7.2).

Conversely, assume $(\mathcal{E}_0, \mathcal{M}_0)$ is an influence block. Since $\det B \neq 0$, and $(\mathcal{E}_0, \mathcal{M}_0)$ is reaction complete, injectivity of B in (7.4) is guaranteed. To show surjectivity, let $\eta \in \mathcal{E}_0$, $\xi = 0$ in (7.6), (7.7) be arbitrary. Since $S\Phi = \xi = 0$, it only remains to show $\Phi \in \mathcal{E}_0$ and $\delta x \in \mathcal{M}_0$. Completeness (7.2) of metabolite influence in the metabolite influence block $(\mathcal{E}_0, \mathcal{M}_0)$ asserts

$$\delta x_m = 0 \quad \text{for} \quad m \notin \mathcal{M}_0\,. \tag{7.12}$$

Therefore $\delta x \in \mathcal{M}_0$. Reaction completeness (7.1) of $(\mathcal{E}_0, \mathcal{M}_0)$ then implies $R\delta x \in \mathcal{E}_0$ by (7.10), as before. Therefore $\Phi = -\eta + R\delta x \in \mathcal{E}_0$, and (7.5) implies surjectivity in (7.4). This completes the proof of equivalence claim (ii).

To prove equivalence claim (iii), we address Okada's localized blocks or response blocks, next. By (i), reaction completeness (7.1) is equivalent to (7.4), for any pair $(\mathcal{E}_0, \mathcal{M}_0)$. It only remains to study the leakage freeness condition (7.3). First assume $(\mathcal{E}_0, \mathcal{M}_0)$ is metabolite influence complete . Then B in (ii) is a linear isomorphism between $(\ker S \cap \mathcal{E}_0) \times \mathcal{M}_0$ and $\mathcal{E}_0 \times \{0\}$. In particular the dimensions coincide, as required in (7.3), and $(\mathcal{E}_0, \mathcal{M}_0)$ is leakage free.

Conversely, assume the reaction complete pair $(\mathcal{E}_0, \mathcal{M}_0)$ is also leakage free. Again (i) implies (7.4). By $\det B \neq 0$, the map B is injective. As an aside we record here that the *leakage* λ defined as

$$\lambda(\mathcal{E}_0, \mathcal{M}_0) := |\mathcal{E}_0| - |\mathcal{M}_0| - \dim(\ker S \cap \mathcal{E}_0) \geq 0\,, \tag{7.13}$$

is therefore nonnegative, for any reaction complete pair $(\mathcal{E}_0, \mathcal{M}_0)$. For leakage free $(\mathcal{E}_0, \mathcal{M}_0)$, i.e. for $\lambda = 0$ alias (7.3), the injective map (7.4) is an isomorphism by linearity. Therefore $(\mathcal{E}_0, \mathcal{M}_0)$ is a metabolite influence block, by (ii), and the lemma is proved. ⋈

To further prepare our proof of theorem 2.6 we present a more explicit construction of the metabolite influence block $(\mathcal{E}^*, \mathcal{M}^*)$ generated by $j^* \in \mathcal{E}$. In (2.20) we have defined $(\mathcal{E}^*, \mathcal{M}^*)$ as the intersection over all influence blocks $(\mathcal{E}_0, \mathcal{M}_0)$ with $j^* \in \mathcal{E}_0$, i.e. as the minimal such influence block. We construct a more explicit candidate $(\mathcal{E}', \mathcal{M}')$ for $(\mathcal{E}^*, \mathcal{M}^*)$, from below, as follows. Given any $j^* \in \mathcal{E}$ define

$$\mathcal{M}' := I_{\mathcal{M}}(j^*) = \{m' \in \mathcal{M} \,|\, j^* \rightsquigarrow m'\} \tag{7.14}$$

as the metabolite influence set of j^*. Given $\mathcal{M}'$ define $\mathcal{E}'$ via the *reaction completion* of $\mathcal{M}'$, keeping j^*:

$$\mathcal{E}' := \{j' \in \mathcal{E} \,|\, m' \vdash j' \text{ for some } m' \in \mathcal{M}'\} \cup \{j^*\}\,. \tag{7.15}$$

Lemma 7.2.

$$(\mathcal{E}^*, \mathcal{M}^*) = (\mathcal{E}', \mathcal{M}') \tag{7.16}$$

Proof. We first show $(\mathcal{E}', \mathcal{M}') \subseteq (\mathcal{E}^*, \mathcal{M}^*)$. Afterwards we show that $(\mathcal{E}', \mathcal{M}')$ is a metabolite influence block. Since $j^* \in \mathcal{E}'$, by construction (7.15), minimality of $(\mathcal{E}^*, \mathcal{M}^*)$ then implies equality (7.16).

To show $(\mathcal{E}', \mathcal{M}') \subseteq (\mathcal{E}^*, \mathcal{M}^*)$ we first observe $j^* \in \mathcal{E}^*$, by definition (2.20). Because the metabolite influence block $(\mathcal{E}^*, \mathcal{M}^*)$ is metabolite influence complete, $j^* \rightsquigarrow m'$ implies $m' \in \mathcal{M}^*$; see (7.2). Therefore $\mathcal{M}' \subseteq \mathcal{M}^*$, by definition (7.14) of $\mathcal{M}'$. Because $(\mathcal{E}^*, \mathcal{M}^*)$ is reaction complete, (7.1) and $\mathcal{M}' \subseteq \mathcal{M}^*$ imply $\mathcal{E}' \subseteq \mathcal{E}^*$; see definition (7.15) of $\mathcal{E}'$. This proves $(\mathcal{E}', \mathcal{M}') \subseteq (\mathcal{E}^*, \mathcal{M}^*)$.

On a slightly more abstract level we can also start with the pair $(\{j^*\}, \{\ \}) \subseteq (\mathcal{E}^*, \mathcal{M}^*)$ and argue as follows. The operations of including influenced metabolites or outgoing reactions in pairs $(\mathcal{E}_\iota, \mathcal{M}_\iota)$ preserve the subset relation. They do not augment metabolite influence pairs which are invariant under both operations. The pair $(\mathcal{E}', \mathcal{M}')$ arises from $(\{j^*\}, \{0\})$ by a single step of each type. This proves $(\mathcal{E}', \mathcal{M}') \subseteq (\mathcal{E}^*, \mathcal{M}^*)$.

Evidently the above ascending process must terminate at some localized pair $(\mathcal{E}^0, \mathcal{M}^0)$, eventually, and $(\mathcal{E}^0, \mathcal{M}^0) = (\mathcal{E}^*, \mathcal{M}^*)$ by minimality of $(\mathcal{E}^*, \mathcal{M}^*)$. It is perhaps a little surprising that the ascending process terminates at $(\mathcal{E}', \mathcal{M}')$, after a single step, already.

To show that $(\mathcal{E}', \mathcal{M}')$ is a metabolite influence block, already, first observe that $(\mathcal{E}', \mathcal{M}')$ is reaction complete by definition (7.15) of $\mathcal{E}'$; see (7.1). It remains to show $(\mathcal{E}', \mathcal{M}')$ is influence complete, (7.2). Suppose $\mathcal{E}' \ni j' \rightsquigarrow m'$. We have to show $m' \in \mathcal{M}'$, i.e.

$$j^* \rightsquigarrow m'\,. \tag{7.17}$$

For $j' = j^*$ this is trivial. For $j^* \neq j' \in \mathcal{E}'$, definition (7.15) implies $m \vdash j'$ for some $m \in \mathcal{M}'$. Definition (7.14) of $\mathcal{M}'$ implies $j^* \rightsquigarrow m$. Summarizing, we have the situation

$$j^* \rightsquigarrow m \vdash j' \rightsquigarrow m' \,. \tag{7.18}$$

By transitivity theorem 2.4(i), with assumption (2.13) for $\alpha = j^*$, $j = j'$, $\beta = m'$ established in (7.18), this proves $\alpha \rightsquigarrow \beta$, as claimed in (7.17). Therefore $(\mathcal{E}', \mathcal{M}')$ is a metabolite influence block, and the lemma is proved. ⋈

Okada has pointed out that any flux or metabolite response to any perturbation $j^* \in \mathcal{E}_0$ is contained in $\mathcal{E}_0 \cup \mathcal{M}_0$, for any response block $(\mathcal{E}_0, \mathcal{M}_0)$:

$$\begin{aligned} \mathcal{E}_0 \ni j^* \rightsquigarrow j \quad &\Longrightarrow \quad j \in \mathcal{E}_0 \,, \\ \mathcal{E}_0 \ni j^* \rightsquigarrow m \quad &\Longrightarrow \quad m \in \mathcal{M}_0 \,. \end{aligned} \tag{7.19}$$

By lemma 7.2, $(\mathcal{E}_0, \mathcal{M}_0)$ is a metabolite influence block, and the linear isomorphism

$$B^{-1} : \quad \mathcal{E}_0 \longrightarrow (\ker S \cap \mathcal{E}_0) \times \mathcal{M}_0 \tag{7.20}$$

of (7.4) in lemma 7.1 recovers (7.19). In addition, we have shown that any prescribed *vector of (infinitesimal) flux responses in* $\ker S \cap \mathcal{E}_0$ *and metabolite responses in* $\mathcal{M}_0$ *can be achieved by a unique (infinitesimal) vector perturbation supported in* $\mathcal{E}_0$. However, only the minimal pair $(\mathcal{E}^*, \mathcal{M}^*)$ generated by j^* addresses the question of simultaneous sensitivity responses of fluxes and metabolites to a *single* perturbation j^*.

Proof of theorem 2.6. Let $(\mathcal{E}^*, \mathcal{M}^*)$ be the minimal metabolite influence block generated by j^*. lemma 7.2, (7.16) implies $(\mathcal{E}^*, \mathcal{M}^*) = (\mathcal{E}', \mathcal{M}')$. In particular

$$I_{\mathcal{M}}(j^*) = \mathcal{M}' = \mathcal{M}^* \tag{7.21}$$

holds by definition (7.14) of $\mathcal{M}'$. This proves claim (2.23) of theorem 2.6.

To prove the remaining claim (2.22) let $j' \in I_{\mathcal{E}}(j^*)$, i.e. $j^* \rightsquigarrow j'$. We have to show $j' \in \mathcal{E}^* = \mathcal{E}'$. We may assume $j' \neq j^*$. By definition (7.15) of $\mathcal{E}'$, it suffices to show that there exists some m such that $\mathcal{M}' \ni m \vdash j'$, i.e. such that

$$j^* \rightsquigarrow m \vdash j' \,. \tag{7.22}$$

This follows as in (6.1), (6.2) of our transitivity proof, with $\alpha := j^*$, $j := j' \neq \alpha$. This proves claim (2.22) and completes the proof of theorem 2.6. ⋈

8 Computational Aspects

We briefly discuss how to calculate the influence graph, in this section. We are aware of three schools of thought, which provide algorithms for computing the influence graph. First, we can consider the non-influence question $j^* \not\rightsquigarrow \beta$, i.e. $B^{-1}_{\beta j^*} \equiv 0$, as a question of *polynomial identity testing*. Fast probabilistic algorithms for this problem are available. Second, we could consider B as a layered mixed matrix and employ deterministic matroid algorithms, following [Mur09], to obtain a provably correct result. A third viewpoint is described in [Gio+15]. Their algorithms do not just compute generic influences $j^* \rightsquigarrow \beta$, but even compute sign $B^{-1}_{\beta j^*}$, under certain additional assumptions. However, runtime is exponential in $|\mathcal{M}| + |\mathcal{E}|$. This is impractical, already, for applications of moderate size like the TCA-cycle discussed in Section 9. We only report on the fast probabilistic approach here.

As we have seen, every entry of B^{-1} is a rational function in the rate variables r_{jm}. The numerator is of degree at most $M = |\mathcal{M}|$, but generally of exponential size in M. We therefore avoid to write down the symbolic numerator in expanded form. To check for $B^{-1}_{\beta j^*} \equiv 0$, probabilistically, we evaluate the matrix inverse for specific values of r_{jm} which are chosen at random. These values need not be related to any actual numerical values of r_{jm} in any biological application. Instead we use the following Schwartz-Zippel lemma.

Lemma 8.1. *[Sch80] Let $\mathbb{F}$ be a field, $q : \mathbb{F}^N \to \mathbb{F}$ a nonzero polynomial of degree at most M, and $T \subseteq \mathbb{F}$ any finite test set. Let $P(q = 0 \text{ in } T^N)$ denote the probability to obtain $q(x) = 0$ for random x, uniformly distributed in T^N. Then*

$$P\left(q = 0 \text{ in } T^N\right) \leq \frac{M}{|T|}\,. \tag{8.1}$$

The field $\mathbb{F}$ is chosen to computationally recognize exact zeros. This excludes floating point arithmetic. There are two obvious choices: $\mathbb{F} = \mathbb{Q}$, or the finite Galois fields $\mathbb{F} = \mathrm{GF}(p^n)$ for some prime p. We choose to work in $\mathbb{F} = GF(p) = \mathbb{Z}_p$, for simplicity and speed. We choose a moderately sized random prime $p \in [k, 2k]$. Already for $k = 2^{127}$, this makes $|T| = p$ so ludicrously large that the Schwartz-Zippel lemma 8.1 practically excludes false zero results $q(x) = 0$ for "unlucky" random choices of the components r_{jm} of x.

A slightly more subtle consideration is the possibility of choosing an unlucky prime p, i.e. a prime p which divides any of the numerators, or the denominators, of the symbolic inverse B^{-1}.

Let ℓ denote an upper bound of the greatest common divisor of all terms appearing in the numerator or denominator of a single entry of B^{-1}. Then, any number bounded by ℓ can have at most $\log \ell / \log k$ different prime factors in the range $[k, 2k]$, out of the asymptotically $k/\log k$ existing primes in the same range. Hence, if we choose the prime $p \in [k, 2k]$ uniformly at random, we need $k \gg \log \ell$ in order to avoid a

single false zero due to unlucky primes. This requires $k \gg (|\mathcal{M}|+|\mathcal{E}|)^2 \log \ell$ in order to avoid all possible false zero entries in B^{-1}, independently.

The greatest common divisor of a set of positive integers is bounded above by their minimum. The Cramer determinants of B are bounded by Hadamard's inequality, i.e. the matrix norm, and we obtain an upper bound $\ell \leq (1+\max_j |S_j|)^{|\mathcal{E}|}$. Here $|S_j| = \sum_{m\in\mathcal{M}} |S_{mj}|$, and $+1$ accounts for the $-\mathrm{id}_{\mathcal{E}}$-part of B. Hence, our extremely crude estimate requires the following lower bound on k:

$$k \gg |\mathcal{E}|(|\mathcal{E}|+|\mathcal{M}|)^2 \log(1+\max_j |S_j|). \tag{8.2}$$

The factor $(|\mathcal{E}|+|\mathcal{M}|)^2$ counts the entries of B^{-1}, independently.

A value of $k = 2^{127}$ satisfies the crude requirement (8.2), for any conceivable metabolic network. Practically, this eliminates the problem of unlucky primes p and unlucky rate entries r_{jm}. The computational overhead over floating point arithmetic turns out to be very moderate for primes of such order.

In summary, a single matrix inversion of B with random rates $r_{jm} \mod p$, for a random prime $2^{127} < p < 2^{128}$, is sufficient to compute the influence relation $\rightsquigarrow$ with an error probability far below the probability of manufacturing defects in the hardware and cosmic ray interference. This matrix inversion is a trivial task on semi-modern hardware and for realistic sizes $|\mathcal{E}|+|\mathcal{M}| \leq 500$ of the metabolic network. All remaining tasks for the construction of the full flux influence graph – computing strong connected components and a transitive reduction – are at least as fast as the matrix inversion.

Our computations were done in the [Sage] framework, which internally uses the fast library [FFPack] for linear algebra over finite fields. Compared to floating point arithmetic, the matrix inversion over $\mathbb{Z}_p$ incurred a runtime overhead of less than a factor four, for random primes up to order $p \approx k \approx 2^{500}$. Runtimes for the TCA cycle (48 reactions, 29 metabolites) were on the order of 200 milliseconds, even on an obsolete 2007 laptop.

9 Example: The carbon metabolic TCA cycle

Let us illustrate our analysis with a realistic example. The previous paper [MF15] discussed a variant of the tricarboxylic citric acid cycle (TCAC) in *E. coli*. Perturbation experiments by knockout of enzymes where reported in [Ish+07, Nak+09]. The relevant reactions are listed in Table 9.1. Table 9.2 defines five variants A–E of this network which we will discuss. For a graphical representation of this metabolic network, see fig. 9.3.

We will first discuss the model variant A, consisting of the internal reactions $1-31$, the feed "reaction" `f1` and exit reactions `d1` $-$ `d6`.

Reaction	Inputs	→	Outputs
1	Glucose + PEP	→	G6P + PYR
2a	G6P	→	F6P
2b	F6P	→	G6P
3	F6P	→	F1,6P
4	F1,6P	→	G3P + DHAP
5	DHAP	→	G3P
6	G3P	→	3PG
7a	3PG	→	PEP
7b	PEP	→	3PG
8a	PEP	→	PYR
8b	PYR	→	PEP
9	PYR	→	AcCoA + CO2
10	G6P	→	6PG
11	6PG	→	Ru5P + CO2
12	Ru5P	→	X5P
13	Ru5P	→	R5P
14a	X5P + R5P	→	G3P + S7P
14b	G3P + S7P	→	X5P + R5P
15a	G3P + S7P	→	F6P + E4P
15b	F6P + E4P	→	G3P + S7P
16a	X5P + E4P	→	F6P + G3P
16b	F6P + G3P	→	X5P + E4P
17	AcCoA + OAA	→	CIT
18	CIT	→	ICT
19	ICT	→	2-KG + CO2
20	2-KG	→	SUC + CO2
21	SUC	→	FUM
22	FUM	→	MAL
23a	MAL	→	OAA
23b	OAA	→	MAL

Reaction	Inputs	→	Outputs
24a	PEP + CO2	→	OAA
24b	OAA	→	PEP + CO2
25	MAL	→	PYR + CO2
26	ICT	→	SUC + Glyoxylate
27	Glyoxylate + AcCoA	→	MAL
28	6PG	→	G3P + PYR
29	AcCoA	→	Acetate
30	PYR	→	Lactate
31	AcCoA	→	Ethanol
f1		→	Glucose
d1	Lactate	→	
d2	Ethanol	→	
d3	Acetate	→	
d4	R5P	→	
d5	OAA	→	
d6	CO2	→	
dd1	G6P	→	
dd2	F6P	→	
dd3	E4P	→	
dd4	G3P	→	
dd5	3PG	→	
dd6	PEP	→	
dd7	PYR	→	
dd8	AcCoA	→	
dd9	2-KG	→	
X1	Glucose	→	
N1	S7P	→	S1,7P
N2	S1,7P	→	E4P + DHAP

Table 9.1: *Reactions in the TCAC metabolic network; see [Ish+07, Nak+09].*

The feed reaction `f1`, by definition, does not depend on any internal metabolite. Indeed, the experiments in question normalized all their measurements by total `Glucose` uptake. This fixes `f1`, effectively. It is not necessary to include the feed `f1` in our model, at all, as long as we are only interested in the influence graph of the remaining reactions. Adding reactions which have rates independent of all metabolites, in our model, will not change any influence relations between reactions and metabolites of the smaller model. Indeed, see theorem 5.1 with $\mathcal{M}_1 = \mathcal{M}_0$.

Without the feed `f1`, on the other hand, the resulting network will not possess any nontrivial steady state at all: `Glucose` is consumed but never replenished, and the trivial zero state is globally attracting. We can still compute, visualize and discuss the influence graph for such an incomplete network, formally, with the tacit understanding that it will become meaningful when we add the necessary feed reactions. Evidently we do not even need to know, or account for, these external feed reactions, as long as we do not study their own influence on the network.

Exit reactions, in contrast, need to be included in the model. They are essential for the invertibility of B, and their presence or omission may affect the influence graph. The paper [Ish+07] does not include the exit reactions `d1-d3` explicitly. Without them, however, the products `Lactate`, `Ethanol` and `Acetate` accumulate indefinitely. Moreover, the resulting B-matrix becomes noninvertible. The common practice of omitting such "obvious" reactions from networks, in the published

Variant	Included reactions	Comment
A	`1-31,f1, d1-d6`	Reduced Network from [Ish+07], as discussed in [MF15].
B	`1-31,f1, d1-d6, dd1-dd9`	Network from [Ish+07], augmented by further exit reactions.
C	`1-31,f1, d1-d6, dd1-dd9, X1`	Artificial network to discuss `Glucose` decay.
D	`1-31,f1, d1-d6, N1, N2`	Network proposed in [Nak+09], introducing the novel metabolite `S1,7P` and reactions `N1`, `N2`, with reduced exit reactions in the spirit of A.
E	`1-31,f1, d1-d6, dd1-dd9, N1, N2`	The union of networks B and D.

Table 9.2: *Variants of the TCAC metabolic network discussed in the text.*

literature, will be put under scrutiny below.

The resulting influence graph of model A is sketched in graphical form in fig. 9.4, and in tabular form in fig. 9.6. As in fig. 3.1, we represent vertices $\varphi\mathcal{M}^d(\varphi)$ in the full influence graph as a table with one column and two rows. The first row contains the reactions φ and the second row contains the directly influenced metabolites $\mathcal{M}^d(\varphi)$. Self-influence is represented by boldface font, if the first row φ has only a single entry.

As in fig. 3.1, we coalesce fanout-vertices into a single vertex table, albeit with several columns. Fanout-vertices are vertices, which are subordinate to the same shared vertex in the full influence graph, and do not possess further influence. Chains of single children provide natural examples which get coalesced. See fig. 9.4.

Exit reactions `d1-d3` are single children, which prevent prevent perpetual increase of their mother metabolites `Lactate`, `Ethanol`, and `Acetate` by simple decay. They are all activated by the Glyoxylate citric acid cycle in the lower part of fig. 9.3. Since single children have no influence, and since mere exit reactions do not enlarge the set $\mathcal{M}$ of metabolites, their addition or (formal) omission only adds or omits their own `child{mother}` box, subject to influence by others. In fig. 9.5 we see how the boxes of `d1-d3` are influenced by the same vertex box $\varphi\mathcal{M}^d(\varphi)$, and how these boxes have been coalesced to contribute three flow columns of one larger vertex.

We discuss model variants B, C of the same TCA cycle next, to explore the effect of additional exit reactions. The experimental study [Ish+07], which we call variant B, includes nine additional exit reaction `dd1` $-$ `dd9`. For purely illustrative purposes, in variant C, we also add an artificial exit reaction `X1: Glucose` $\rightarrow$ to the model. The resulting influence graphs are included in fig. 9.5. Note how the augmentation by the additional exit reaction `X1` has a strong coarsening effect on the influence structure. We also provide the full influence graph for the three models in the gray scale heat map of fig. 9.6.

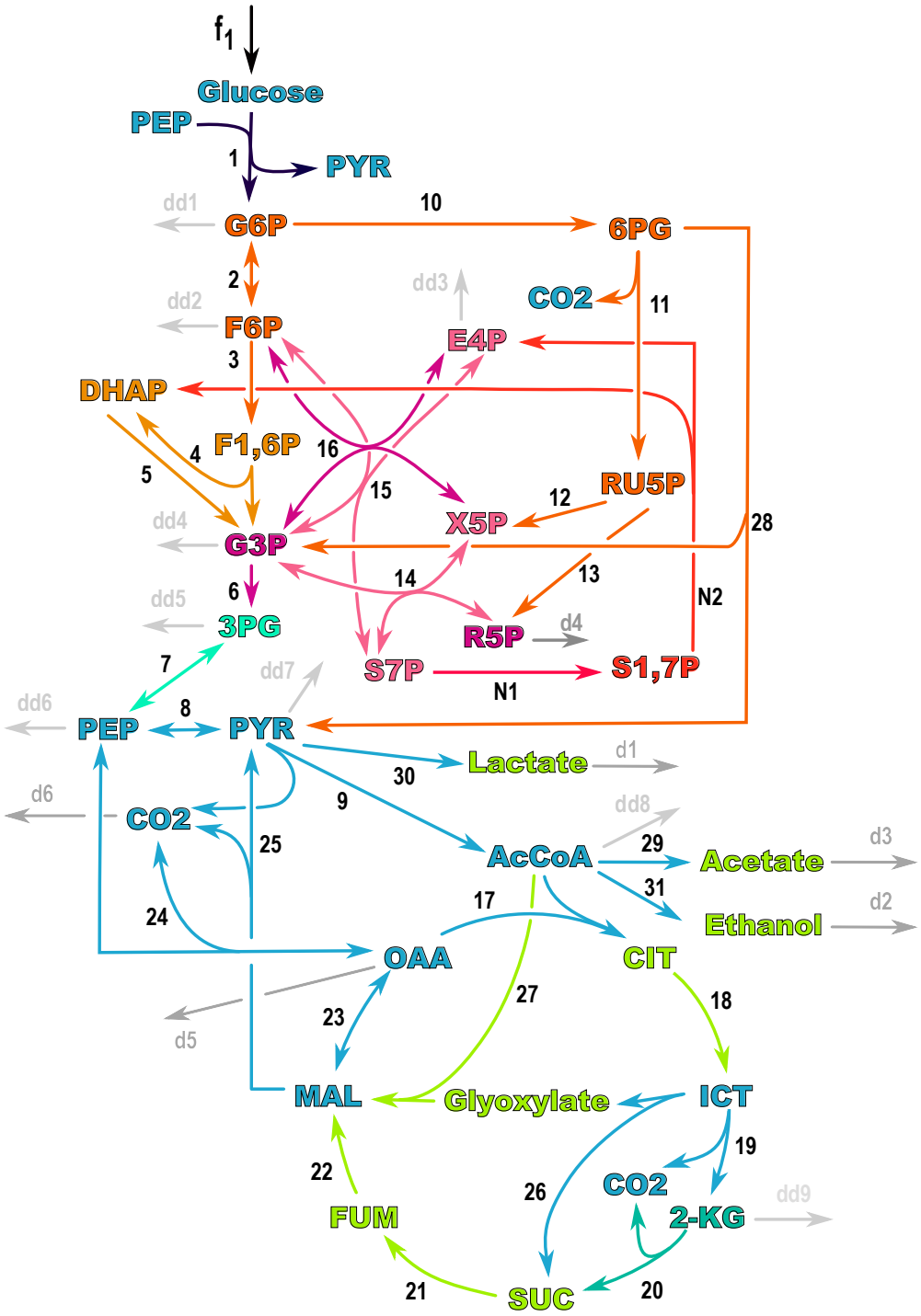

Figure 9.3: *The reaction network of the carbon metabolism TCA cycle of* E. coli. *The complete set of reactions is shown in Table 9.1, and the discussed model variants are listed in Table 9.2. Note that the metabolites* `PEP`, `CO2`, `PYR` *appear multiple times, in order to avoid excessive self-intersection of the graph. Colors (online) indicate the grouping by influence in the models A, D, E of [Ish+07] and [Nak+09]; see fig. 9.7. The graphical representation is courtesy of Anna Karnauhova.*

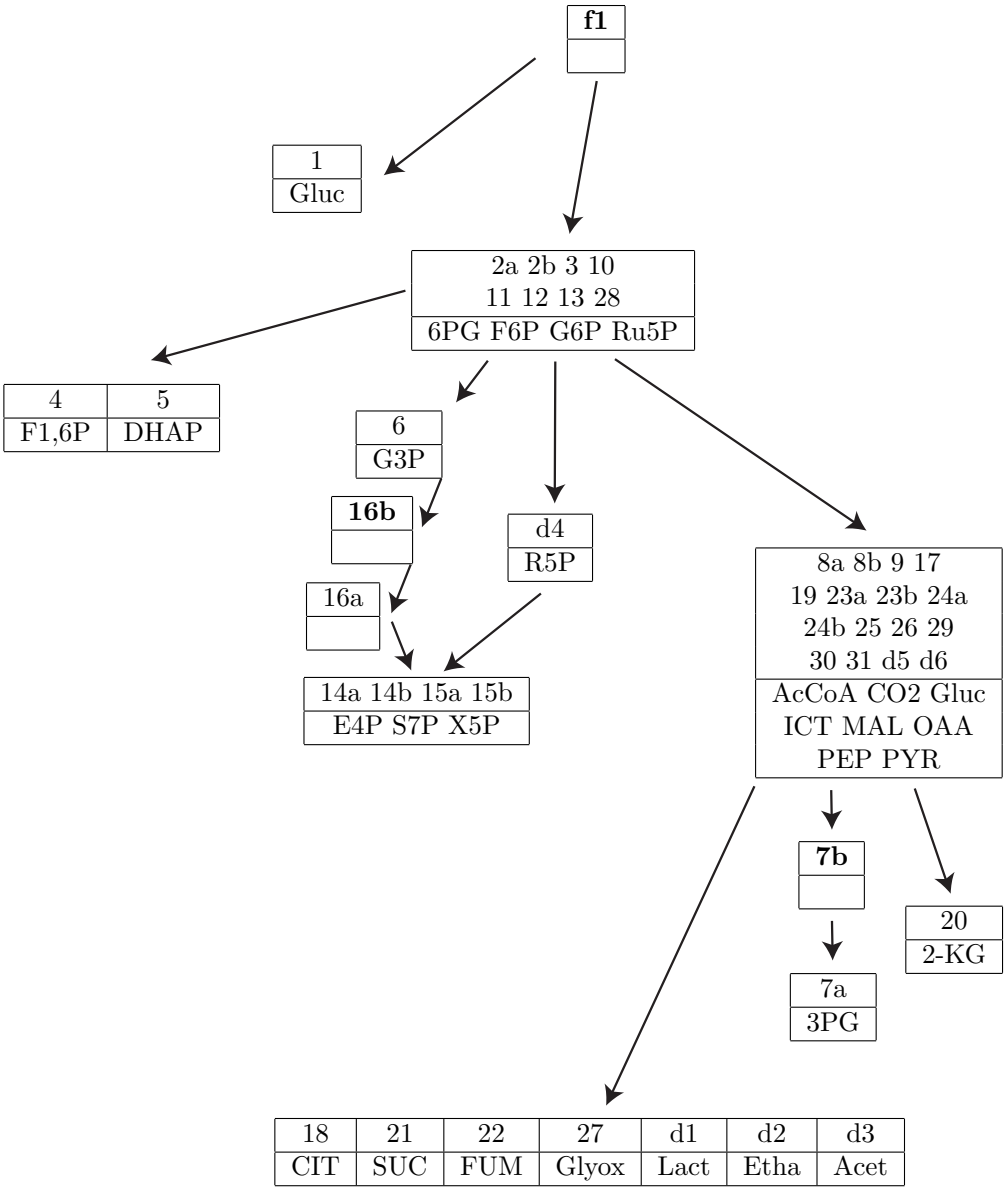

Figure 9.4: *The full flux influence graph of the TCA cycle, model variant A. Boldface reactions indicate self-influence $j^* \rightsquigarrow j^*$, for $j^* = f1, 7b, 16b$. Arrows to box arrays with multiple columns point to each column separately.*

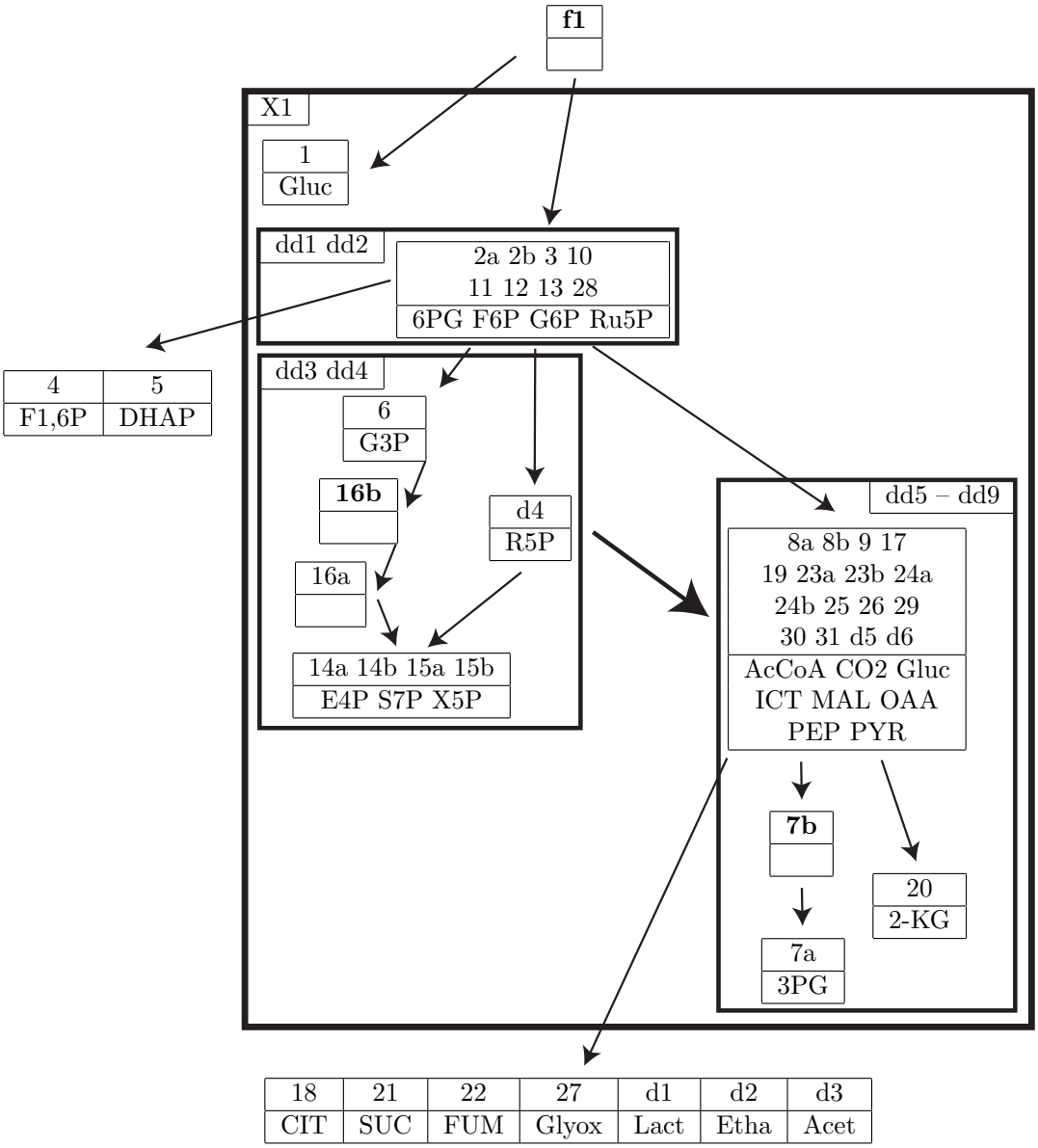

Figure 9.5: *Notation as in fig. 9.4. The lumping effect of the exit reactions* ***dd1****–* ***dd9****, added in model B, is indicated by bold boxes. Such lumbing boxes combine all reactions and metabolites inside into a new vertex box* $\phi\mathcal{M}^d(\phi)$*. This includes the labels of the added reactions in their upper corners. Note the new bold arrow which is not inherited from model A. The artificial glucose exit* ***X1*** *of model C generates the largest lump box.*

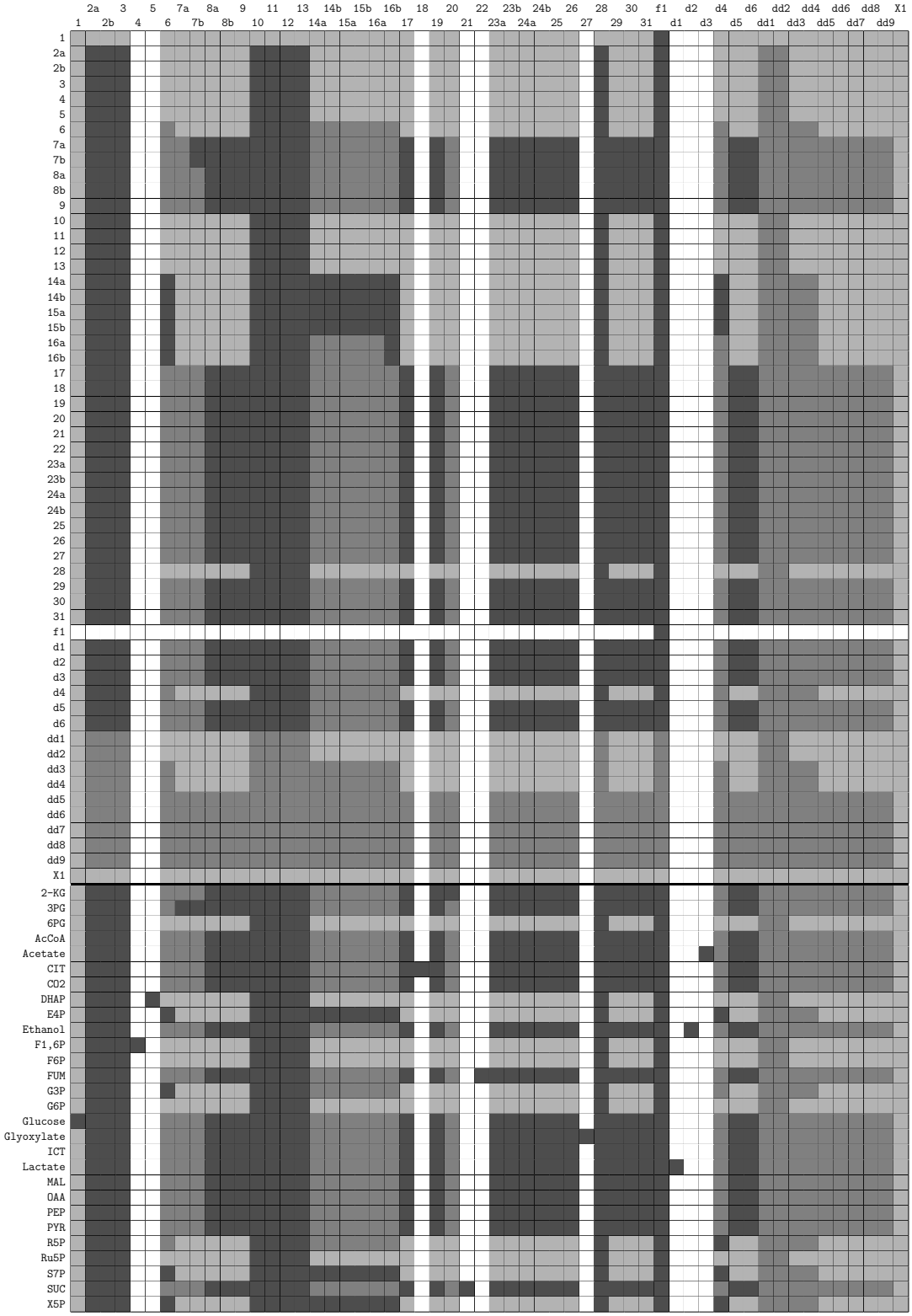

Figure 9.6: *Flux influence relations in the three variants A, B, C of the TCA cycle. As in fig. 3.2, influence $\alpha \rightsquigarrow \beta$ is indicated in column $\alpha \in \mathcal{E}$ and row $\beta \in \mathcal{E} \cup \mathcal{M}$. Gray scales encode the influence in the respective model variant.*
Here □: no influence in any variant; ▫: influence only in model C; ▪: influence in models B, C; ■: influence in all three models A, B, C.

The addition of new exit reactions dd1, ..., dd9 and X1 in model variants B and C, respectively, lumps certain vertices of model A and successively coarsens the full flux influence graphs, see fig. 9.5. Indeed, reactions ⟨dd1, dd2⟩ in B have enlarged the previous upper vertex ⟨2a, 2b, ..., 28⟩ of model A, fig. 9.4. The vertices of reactions 6, 16a, ⟨16b⟩, d4, and ⟨14a, 14b, 15a, 15b⟩ in A have been lumped into a single vertex, in model B, augmented by dd3, dd4. The remaining exit reactions dd5, ..., dd9 of B lump, and augment, the vertices ⟨8a, 8b, ..., d6⟩ and 7a, ⟨7b⟩, 20 from model A. Reactions 7a and 20, for example, lose their single child status due to exit reactions dd5 and dd9, respectively. We conclude that the lumping caused by the exit reactions of model B, in the TCAC reaction network of fig. 9.3, emphasizes the grouping into phosphorylation, by the upper two vertices, versus the large lower vertex of the citric acid cycle.

Glucose is the central driving feed metabolite of the entire network. The artificial addition, in model C, of a new exit reaction X1 of Glucose forces even stronger lumping. All vertices of model B, except for some single children, are lumped into a single large vertex of reactions ⟨1, ..., X1⟩. In fact, Glucose, the single product of the single feed reaction f1, has lost its single child mother status of reaction 1 in the initializing chain of reactions f1 and 1. Perturbations of the two Glucose children 1 and X1 therefore influence each other, and the Glucose level itself. In models A and B this could only be achieved, equivalently, by a perturbation of the driving feed rate parameter f1 – with sweeping influence on the whole network. It is therefore essential – and has been painstakingly observed by [Ish+07, Nak+09] – to carefully control the level of Glucose uptake in order to obtain meaningful results of knockout experiments.

Fig. 9.5 summarizes the results of our model comparison A – C, in view of augmenticity. Innermost boxes show the full influence graph of model A. Since no new metabolites have been added in models B, C, theorem 5.1 with $\mathcal{M}_1 = \mathcal{M}_0$ implies two possibilities. First, new influences involving the added reactions may lump existing vertices into larger vertex boxes. This is indicated by larger boldface boxes around the finer structures of model A. Second, new hierarchic arrows may appear between larger boxes. This new hierarchy in the augmented model must be compatible with the ordering in the smaller model. However, additional influence arrows may, and do, appear in the augmented model, which are not implied by the ordering in the smaller model, already. These additional influence arrows are drawn to originate or terminate outside, at the larger lumped boxes. This distinguishes the augmented influence arrows from the pre-existing ones.

Alternatively, we can visualize the augmenticity properties of the influence structures A–C in the heat map of fig. 9.6. All three models share the same metabolite set $\mathcal{M}$. The successive augmentations $\mathcal{E}_A \subset \mathcal{E}_B \subset \mathcal{E}_C$ of the respective reaction sets $\mathcal{E}$, therefore, only add influences, successively, but never remove any. See augmenticity theorem 5.1. Consider influence $\rightsquigarrow$, or non-influence $\not\rightsquigarrow$, of column $\alpha \in \mathcal{E}$ on row $\beta \in \mathcal{E} \cup \mathcal{M}$. Then only the following four triples, with their respective gray scales at position $\beta\alpha$, can appear for models (A, B, C): the white cell $\square = (\not\rightsquigarrow, \not\rightsquigarrow, \not\rightsquigarrow)$,

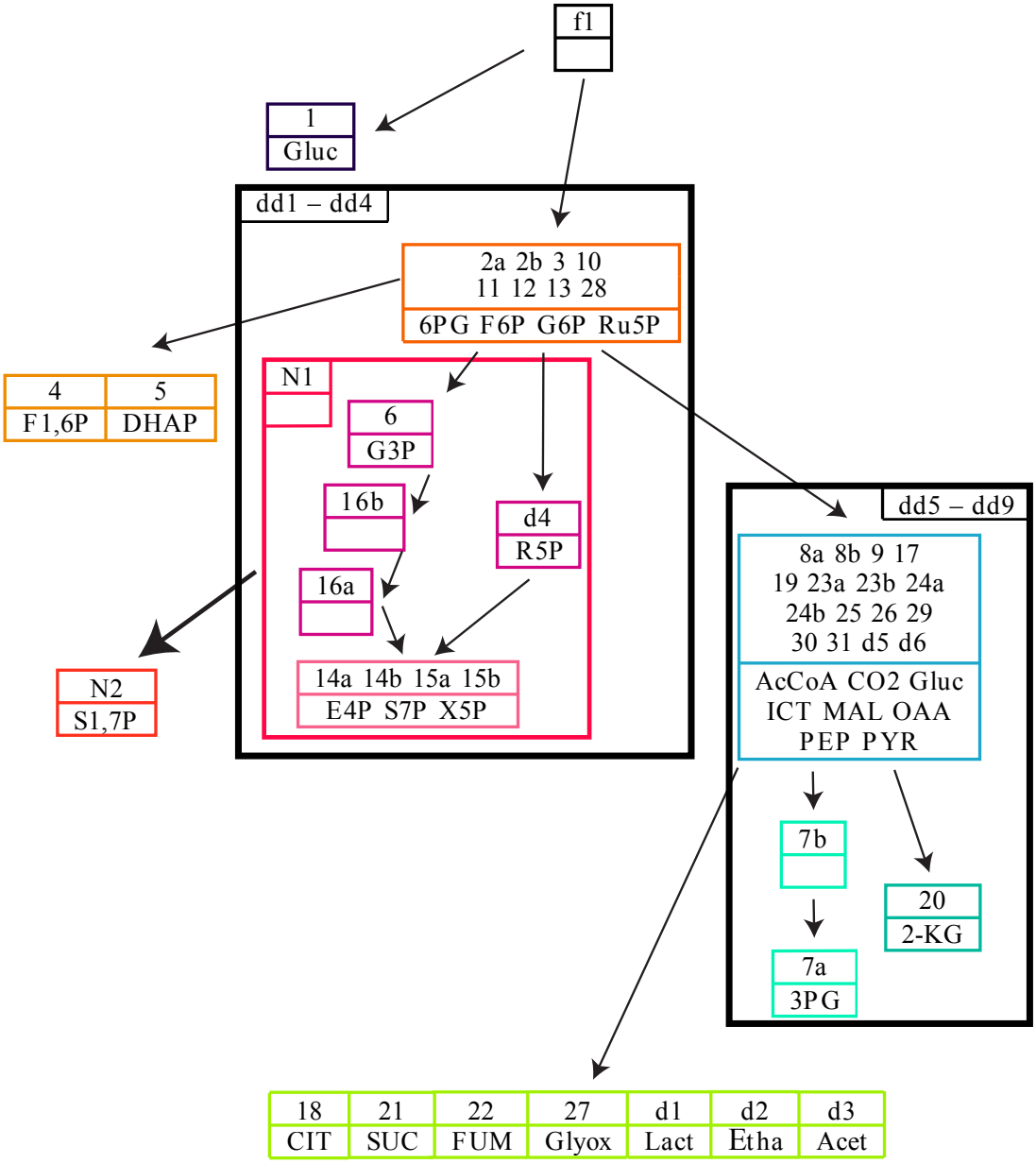

Figure 9.7: *The full flux influence graph of the TCA cycle, model invariants A, D, E. Notation as in fig. 9.4. The lumping effect of the new reactions $N1, N2$ and the new metabolite $S1, 7P$, in model D is indicated by the bold lumping box with label $N1\{\ \}$. Note the new bold arrow from that box to the new vertex $N2\{S1, 7P\}$. The lumping effect of the exit reactions* `dd1`–`dd9`*, in model E, produced two additional lumping boxes with labels* `dd1`–`dd4` *and* `dd5`–`dd9`*, respectively, but without any additional arrows. Color coding (online) emphasizes the successive lumping of these boxes. The box structure is also represented in the metabolic network of fig. 9.3, by matching colors.*

the light gray cell □ = (↛, ↛, ⇝), the medium gray cell ■ = (↛, ⇝, ⇝), and the dark cell ■ = (⇝, ⇝, ⇝). The reduced Ishii model A provides the most sparse, dark influence structure. The full Ishii model B, with all exit reactions included, provides the less sparse influence structure which adds the medium gray cells. The artificial exit `X1` of `Glucose` in model variant C, finally, adds all light gray cells. The influence matrix becomes so crowded by entries of nonzero influence ⇝ that the influence structure alone becomes visibly meaningless for the purpose of any functional understanding of the metabolite network.

In fig. 9.7 we study the augmentation of the reduced Ishii model A by the new metabolite `S1,7P` and reactions `N1`, `N2` due to Nakahigashi et al; see [Nak+09] and model D. Model E further augments D by the exit reactions `dd1`, ..., `dd9` of model B. The models D, E augment model A by the single metabolite

$$\mathcal{M}_1 \setminus \mathcal{M}_0 = \{\texttt{S1,7P}\}, \tag{9.1}$$

and at least reactions $\{\texttt{N1}, \texttt{N2}\} = \mathcal{E}_D \setminus \mathcal{E}_A$. The choice $J^\vee(\texttt{S1,7P}) = \texttt{N2}$, in theorem 5.1, makes the 1×1 determinant (5.3) nonsingular. Therefore our previous comments on the augmentation sequence of models A, B, C apply to A, D, E, verbatim.

More specifically, the augmentation of model A by model D lumps the phosphorylation branch into a single influence vertex box, augmented by the new reaction `N1`. Reaction `N1`, in turn, produces the new metabolite `S1,7P`. The flux of the onwards reaction `N2` with educt `S1,7P` is influenced by the lumped box with label `N1`.

The new exit reactions `dd1`, ..., `dd9` augment model D to model E, without new metabolites. Exit reactions `dd1`, ..., `dd4` of E simply get lumped into the phosphorylation vertex of model D. The remaining exit reactions `dd5`, ..., `dd9`, as before, lump the citric acid cycle of ⟨`8a`, `8b`, ..., `d6`⟩ with its unidirectional influences on `7a`, ⟨`7b`⟩, `20` into a new vertex of full mutual influence.

In conclusion, the flux influence graphs presented here, and their augmenticity, are designed to provide first steps towards a mathematically sound analysis of the sensitivity dependencies in metabolic networks. Our results rely on the network structure, only. They hold true, universally and in a precise generic sense, for almost all choices of rate functions and their parameters. Our approach is reliably automated, and still is able to assist in a fast and meaningful first conceptual analysis of metabolic networks – even in the hands of biological non-experts like ourselves.

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