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Identifying network structures that can cause multistationarity

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Abstract— **In systems biology there is a growing interest in the question, whether or not a given mathematical model can admit more than one steady state. As parameter values are often unknown or subject to a high uncertainty, one is often interested in the question, whether or not a given mathematical model can exhibit multistationarity at all. And, if this is the case, one is interested in those parts of the network that are responsible for multistationarity. For certain networks with mass action kinetics, multistationarity can be established by FeinbergâĂŹs Deficiency One Algorithm. This algorithm, however, is limited to so-called regular deficiency one networks and many realistic networks have a deficiency greater than one. In a previous publication it was suggested to analyze certain well defined subnetworks that are guaranteed to be of deficiency one. If these subnetworks are regular, then one can use the Deficiency One Algorithm to establish multistationarity and, if successful, extend it to the overall network. We call those subnetworks multistationarity causing structures. Unfortunately realistic reaction networks often lead to subnetworks that are irregular. The results presented here exploit the structure of such subnetworks to derive conditions for multistationarity that are independent of the Deficiency One Algorithm. Thus, in particular, these conditions are applicable to irregular subnetworks. As a proof of principle we apply these results to a model of *E. coli*'s metabolism and establish a multistationarity causing structure.**

I. Introduction

In Systems Biology there is a growing interest in the question, whether or not a given mathematical model can admit more than one positive steady state. In cell cycle regulation, for example, one can identify different phases of the cell cycle as different (stable) steady states. The cell cycle process itself can then be considered as a switching between these steady states. As parameter values (rate constants and total concentrations) are often unknown or subject to a high uncertainty, one is often interested in the question, whether or not a given mathematical model can exhibit multistationarity.

A partial answer is given in Feinberg's chemical reaction network theory, in particular in the deficiency related theory. The network deficiency is a nonnegative integer that can be computed for every reaction network [5], [6], [7], [8]. The theory links multistationarity to the structure of the reaction network defining the dynamical system. Of particular interest here is the the Deficiency One Algorithm that can decide about multistationarity by analyzing a, potentially large, set of linear systems that are independent of parameter values. If at least one of these linear systems is feasible, then multistationarity is guaranteed and one can compute steady states and rate constants from its solutions. If all are infeasible, then multistationarity is impossible [5], [8].

The Deficiency One Algorithm, however, is limited to so-called **regular deficiency one networks** [8]. Many networks are of deficiency greater than one, thus the algorithm cannot be applied directly. In [3], [9] it was therefore suggested to partition the network uniquely into a set of subnetworks that are guaranteed to be of deficiency one. If these subnetworks are regular, then one can use the Deficiency One Algorithm to establish multistationarity. If this is successful, then [3] gives sufficient conditions (based on the implicit function theorem) that allow to extend multistationarity from the subnetwork to the overall network.

Many networks, however, can only be partitioned into irregular subnetworks, (especially metabolic networks; see [13] for an analysis of the upper part of glycolysis in *E. coli*). Consequently, the Deficiency One Algorithm cannot be applied. If the irregularity is of a special kind, then one can regularize the subnetwork and apply the Deficiency One Algorithm to the regularized subnetwork [13]. It is then again possible to extend multistationarity – so it exists – to the overall network based on [3].

Here we follow a different approach: instead of trying to regularize a subnetwork, we use the special structure of the subnetworks to derive inequality conditions for multistationarity. These conditions are independent of the Deficiency One Algorithm and thus applicable to irregular subnetworks. Again, multistationarity can be extended to the overall networks as described in [3] (provided it can be established).

This paper is organized as follows: in Section II we present the notation used to describe the dynamical systems defined by a reaction network. In Section III, we discuss positive steady states and in Section IV the partition of a reaction network into subnetworks. In Sections V and VI we present conditions for multistationarity and how to verify them. In Section VII, finally, we present the application of those results to a model describing the metabolism of *E. coli*.

II. Notation

We use the same notation as in [1] that we introduce by means of the following reaction network with 2 species A and B, 5 complexes A, 0, B, $A+B$ and $2\,A$ and 6 reactions (represented by arrows in the network below):

$$A \underset{\mathbf{k_2}}{\overset{\mathbf{k_1}}{\rightleftarrows}} 0 \underset{\mathbf{k_4}}{\overset{\mathbf{k_3}}{\rightleftarrows}} B \qquad A+B \underset{\mathbf{k_6}}{\overset{\mathbf{k_5}}{\rightleftarrows}} 2\,A \tag{1}$$

The nodes of network (1) are called complexes. Let x_1 be the concentration of A and x_2 the concentration of B. By

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associating A with e_1 and B with e_2 (the unit vectors of Euclidean 2-space), one can define 5 *complex-vectors* $y_1 = e_1$ for A, $y_2 = 0$, the 2-dimensional zero vector for the complex 0, $y_3 = e_2$ for B, $y_4 = e_1 + e_2$ for $A + B$ and $y_5 = 2\,e_1$ for $2\,A$. Collect these in a matrix $Y \in I\!R^{2\times 5}$:

$$Y = [y_1,\, y_2,\, y_3,\, y_4,\, y_5] = \begin{bmatrix} 1 & 0 & 0 & 1 & 2 \\ 0 & 0 & 1 & 1 & 0 \end{bmatrix}.$$

The complex at the tail of a reaction arrow is called *reactant complex*, the complex at the tip of a reaction arrow is called *product complex*.

Let $\mathcal{I} \in \{-1, 0-1\}^{5\times 6}$ be the incidence matrix of the graph associated to the reaction network (1) (i.e. a matrix whose columns have exactly two nonzero entries, -1 for the reactant and $+1$ for the product complex):

$$\mathcal{I} = \begin{bmatrix} -1 & 1 & 0 & 0 & 0 & 0 \\ 1 & -1 & -1 & 1 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & -1 & 1 \\ 0 & 0 & 0 & 0 & 1 & -1 \end{bmatrix}$$

A *reaction rate* is associated with every reaction. If mass action kinetics is used, each reaction rate is composed of a rate constant multiplied by the product of the concentrations of its reactant complex. With the notation

$$x^{\nu} \,=\, \Pi_{i=1}^{n}\, x_i^{\nu_i}$$

for n-dimensional vectors x and ν and the vectors y_1, …, y_5 defined above one has $v_1 = k_1\, x_1$, $v_2 = k_2$, $v_3 = k_3$, $v_4 = k_4\, x_2$, $v_5 = k_5\, x_1\, x_2$ and $v_6 = k_6\, x_1^2$.

Using the vector of rate constants $k := (k_1,\, \ldots,\, k_6)^T$ and the monomial vector

$$\Phi(x) = \left(x_1,\, 1,\, 1,\, x_2,\, x_1\, x_2,\, x_1^2\right)^T,$$

one can write the vector of reaction rates as

$$v(k,\, x) = \mathrm{diag}\,(k)\;\Phi\,(x)\,.$$

Note that $\Phi\,(x)$ is completely defined by the exponent vectors y_i. Hence we collect those in the *rate-exponent matrix* $\mathcal{Y}$, which, in case of network (1), is given by

$$\mathcal{Y} = [y_1,\, y_2,\, y_2,\, y_3,\, y_4,\, y_5] \in I\!R^{2\times 6}. \tag{2}$$

The ODEs describing the dynamics of network (1) are then given with the help of the *stoichiometric matrix* N that is defined as the product $N := Y\,\mathcal{I}$ as

$$\dot{x}(t) = N\, v(k,\, x(t))$$

We note that the matrix $\mathcal{Y}$ contains multiple copies of the vector y_2, as the corresponding complex is reactant complex in two reactions (labeled k_2 and k_3 above). Hence we can represent $\mathcal{Y}$ as the product of two matrices, the *reactant matrix* Y_r and the *doubling matrix* D: for

$$Y_r = [y_1,\, y_2,\, y_3,\, y_4,\, y_5] \text{ and } D = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix}$$

one has $\mathcal{Y} = Y_r\, D$. We use the column vectors of Y_r to define the following monomial function

$$\Psi(x) = \left(x_1,\, 1,\, x_2,\, x_1\, x_2,\, x_1^2\right)^T \tag{3}$$

and note that

$$\Phi(x) = D^T\, \Psi(x).$$

Note that in the special case of network (1) one has $Y_r = Y$ (as every complex is reactant in at least one reaction). This need not be the case in general.

In general, we assume a reaction network with mass action kinetics of n species, m complexes and r reactions and use the symbols Y, $\mathcal{I}$, $\mathcal{Y}$, and N to describe the corresponding dynamical system in an analogous way: $Y \in I\!R^{n\times m}$ denotes a matrix whose column vectors are the complexes of the network and $\mathcal{I} \in \{-1, 0, 1\}^{m\times r}$ denotes the incidence matrix of the graph.

The matrices Y and $\mathcal{I}$ define the *stoichiometric matrix*

$$N := Y\,\mathcal{I}. \tag{4}$$

From the *rate exponent matrix*

$$\mathcal{Y} := [\tilde{y}_1,\, \ldots,\, \tilde{y}_r]\,, \tag{5}$$

with columns $\tilde{y}_i$ one obtains the monomial vector

$$\Phi\,(x) := \left(x^{\tilde{y}_1},\, \ldots,\, x^{\tilde{y}_r}\right)^T =: x^{\mathcal{Y}} \tag{6a}$$

and the vector of reaction rates

$$v(k,\, x) = \mathrm{diag}(k)\,\Phi\,(x)\,, \tag{6b}$$

where $k := (k_1,\, \ldots,\, k_r)^T$ is the vector of rate constants.

Then the ODEs describing the dynamics of a mass action network are given as

$$\dot{x}(t) = N\, v(k, x(t)). \tag{7}$$

In general, the stoichiometric matrix N does not have maximal row rank. For $s := \mathrm{rank}\,(N)$ there exist $n - s$ conservation relations

$$W^T\, x = c \tag{8}$$

with $W^T\, N = 0$ for a $W \in I\!R^{n\times(n-s)}$ of rank $n - s$. Note that parallel translates of $\mathrm{im}(N)$, that is, sets $\{x \in I\!R^n \,:\, W^T x = const\}$ are invariant under the flow of (7) due to $W^T x(t) = W^T x(0)$ along solutions $x(t)$ of (7). Every biochemical reaction network with mass action kinetics defines a system of the form presented in (7), (8).

We further use $\bar{m}$ to denote the number of reactant complexes and Y_r to denote the *reactant matrix*, that is, the matrix whose column vectors are the reactant complexes of the network. As in the example above the matrices $\mathcal{Y}$ and Y_r are connected by the *doubling matrix* D of dimension $\bar{m}\times r$ that relates $\mathcal{Y}$ and Y_r via $\mathcal{Y} = Y_r\, D$. Here the i-th column vector of D is defined as the unit vector e_j of $I\!R^{\bar{m}}$ such that y_j is the reactant complex vector of the i-th reaction. The

columns of Y_r define the monomial vector $\Psi(x)$ in the same way the columns of $\mathcal{Y}$ define the monomial vector $\Phi(x)$:

$$\Psi(x) := x^{Y_r} \tag{9}$$

We will use the symbol Π to denote

$$\Pi := D^T \tag{10}$$

and later on exploit the fact that

$$\Phi(x) = \Pi\,\Psi(x) \tag{11}$$

and hence

$$v(k,\,x) = \mathrm{diag}(k)\,\Phi(x) = \mathrm{diag}(k)\,\Pi\,\Psi(x).$$

As we are interested in positive steady states only, the pointed polyhedral cone $\ker(N) \cap \mathbb{R}^r_{\geq 0}$ is of particular interest. The symbol E is used to denote the unique (up to scalar multiplication) generators of $\ker(N) \cap \mathbb{R}^r_{\geq 0}$ [10]. Let p be the number of generators; then $E \in \mathbb{R}^{r\times p}$. Define the set of all nonnegative vectors $\lambda \in \mathbb{R}^p_{\geq 0}$ such that $E\,\lambda$ is positive:

$$\Lambda\,(E) := \left\{\,\lambda \in \mathbb{R}^p_{\geq 0} \mid E\,\lambda > 0\,\right\}. \tag{12}$$

For the network (1) one obtains the generator matrix

$$E = \begin{bmatrix} 1 & 0 & 0 & 0\\ 1 & 0 & 0 & 1\\ 0 & 1 & 0 & 0\\ 0 & 1 & 0 & 1\\ 0 & 0 & 1 & 0\\ 0 & 0 & 1 & 1 \end{bmatrix} \text{ and } \Lambda\,(E) = \mathbb{R}^4_{>0}. \tag{13}$$

III. Some remarks about positive steady states

The structure of (7) motivates the following result concerning positive steady states:

Lemma 1 (Existence of positive steady states): Consider a system of ODEs as in (7), with stoichiometric matrix N and let $E \in \mathbb{R}^{r\times p}_{\geq 0}$ be the generator matrix of $\ker(N)\cap\mathbb{R}^r_{\geq 0}$. Let $k \in \mathbb{R}^r_{>0}$ be given. Then a positive vector a is a solution to the polynomial equation $N\,v(k,\,a) = 0$, if and only if there exists a vector $\lambda \in \Lambda\,(E)$ such that

$$k = \mathrm{diag}\left(\Phi\left(a^{-1}\right)\right)\,E\,\lambda. \tag{14}$$

Proof: Follows from the fact that $a > 0$ and $k > 0$ implies $v(k,\,a) > 0$. Thus $N\,v(k,\,a) = 0$ holds if and only if $v(k,\,a) \in \ker(N)\cap\mathbb{R}^r_{\geq 0}$, that is, if and only if $v(k,\,a) = E\,\lambda$, for some $\lambda \in \Lambda\,(E)$. As $v(k,\,a) = \mathrm{diag}\,(k)\;\Phi\,(a)$ condition (14) follows immediately. ∎

Remark 1: If a positive steady state exists, then (14) must hold. The condition (14) can thus be used to constrain the set of rate constants that allow the existence of positive steady states.

Remark 2 (Positive steady states): Consider the system (7) with generator matrix $E \in \mathbb{R}^{r\times p}_{\geq 0}$. The system has a positive steady state, iff $\ker(N) \cap \mathbb{R}^r_{\geq 0}$ is nontrivial and E does not contain a zero row.

Remark 3: Consider the system (7) with generator matrix $E \in \mathbb{R}^{r\times p}_{\geq 0}$. Suppose that E does not contain any zero rows. Then *every positive vector* a can be a steady state of (7), by choosing k as in (14), where $\lambda \in \Lambda\,(E)$ is free and takes the role of the rate constants.

IV. Subnetworks defined by generators

First recall that r is the number of reaction and note that every vector $\nu \in \mathbb{R}^r$ defines a subnetwork: let $\mathrm{supp}\,\nu$ denote the support of the n-vector ν, that is,

$$\mathrm{supp}(\nu) = \{i \in \{1,\,\ldots,\,n\} | \nu_i \neq 0\}\,. \tag{15}$$

Then ν defines the subnetwork that consists of all reactions indexed by $\mathrm{supp}(\nu)$. If ν is a generator of $\ker(N) \cap \mathbb{R}^r_{\geq 0}$, then subnetworks are unique (as generators are unique up to scalar multiplication).

For example, the 4-th column of E from (13), the vector $(0,1,0,1,0,1)^T$ defines the subnetwork displayed below.

$$\begin{aligned} A \xleftarrow{\mathbf{k_2}} 0 \xleftarrow{\mathbf{k_4}} B\\ A + B \xleftarrow{\mathbf{k_6}} 2\,A \end{aligned} \tag{16}$$

Clearly this is a reaction network of its own, with stoichiometric matrix

$$N = \begin{bmatrix} 1 & 0 & -1\\ 0 & -1 & 1 \end{bmatrix}. \tag{17}$$

Taken with mass action kinetics one obtains for k and $\Phi(x)$:

$$k = (k_2, k_4, k_6)^T \text{ and } \Phi(x) = (1, x_2, x_1^2)^T.$$

One can obviously obtain a rate exponent matrix, a reactant complex matrix and a monomial vector $\Psi(x)$ for that network as well. Note that for this network $\ker(N) \cap \mathbb{R}^r_{\geq 0}$ is generated by $(1,1,1)^T$.

From hereon we have to distinguish between objects (e.g. the stoichiometric matrix) of a subnetwork and the corresponding objects of the overall network. For this purpose we use the symbol $\hat{}$ to denote objects of the overall network: the complex matrix $\hat{Y}$, the incidence matrix $\hat{\mathcal{I}}$, the stoichiometric matrix $\hat{N}$, the rate exponent matrix $\hat{\mathcal{Y}}$ and the reactant complex matrix $\hat{Y}_r$ all belong to the overall network, while Y, $\mathcal{I}$, N, $\mathcal{Y}$ and Y_r denote the corresponding objects of the subnetwork. Which subnetwork we are considering will be clear from the context. Likewise, we use $\hat{\Phi}$, $\hat{\Psi}$ and $\hat{v}$ to denote the monomial vectors of the overall network. The number of reactions in the overall network is called r, the number of reactions in the subnetwork ρ.

We note the following relation between objects of the subnetwork defined by $\hat{E}_i$ and the overall network:

$$N = \left(\hat{N}_i\right)_{i\in\mathrm{supp}(\hat{E}_i)}$$

with $\hat{N}_i$ columns of $\hat{N}$ and

$$k = \left(\hat{k}_i\right)_{i\in\mathrm{supp}(\hat{E}_i)} \text{ and } \Phi = \left(\hat{\Phi}_i\right)_{i\in\mathrm{supp}(\hat{E}_i)}.$$

Finally, we use $\hat{E}_i$ to denote a generator for the overall network and E_i for the corresponding generator of the

subnetwork. We note that E_i can be obtained by

$$E_i = \left(\left(\hat{E}_i \right)_j \right)_{j \in \mathrm{supp}(\hat{E}_i)}. \tag{18}$$

In the example from above we have for the generator $\hat{E}_4 = (0,1,0,1,0,1)^T$ of the overall network and for the generator $E_4 = (1,1,1)^T$ (observe that $\hat{E}_4$ is the 4-th column of the generator matrix given in (13)).

Next we recall from [3] concerning subnetworks defined by a generator $\hat{E}_i$:

Lemma 2 (Subnetworks defined by a generator $\hat{E}_i$, cf. [3]): Let N be the stoichiometric matrix of a subnetwork defined by a generator $\hat{E}_i$. Recall the corresponding generator E_i in the subnetwork (cf. equation (18)). Then $\ker(N) = \mathrm{im}(E_i)$.

The following corollary is an immediate consequence of Lemma 2 and Remark 3.

Corollary 1: Consider a biochemical reaction network defined by a generator $\hat{E}_i$ and the generator E_i corresponding to $\hat{E}_i$. Then a positive vector a is a steady state of (7), if and only if k is chosen as

$$k = \mathrm{diag}\left(\Phi\left(a^{-1}\right)\right) E_i \, \lambda, \; \lambda > 0. \tag{19}$$

For positive a, the vector k as in (19) is fixed up to the positive λ and as positive scalar multiplication of k corresponds to a *time scaling* of the ODEs one can – w.l.o.g. – choose $\lambda = 1$. From here on we assume

Assumption 1: The vector of rate constants is given by

$$k = \mathrm{diag}\left(\Phi\left(a^{-1}\right)\right) E_i$$

for some $a \in \mathbb{R}^n_{>0}$.

Recalling equation (11) for $\Phi(x)$ we assume in fact that k is of the form

$$k = \mathrm{diag}\left(E_i\right) \Pi \, \Psi\left(a^{-1}\right) \tag{20}$$

where we have used the fact that $\mathrm{diag}\left(\Phi\left(a^{-1}\right)\right) E_i = \mathrm{diag}\left(E_i\right) \Phi\left(a^{-1}\right)$. Thus (recall that N, k, Φ are objects of the subnetwork defined by a generator $\hat{E}_i$ with E_i as in (18))

$$\begin{aligned} N \, \mathrm{diag}(k) \, \Phi(x) &= N \, \mathrm{diag}\left(E_i\right) \Pi \, \Psi\left(\frac{x}{a}\right) \\ &= N \, \mathrm{diag}\left(E_i\right) \Pi \, \mathrm{diag}\left(\Psi\left(a^{-1}\right)\right) \Psi(x). \end{aligned}$$

We define

$$N_E := N \, \mathrm{diag}\left(E_i\right) \Pi \tag{21}$$

Obviously (recall ρ, the number of reactions in the subnetwork):

$$\ker\left(N_E\right) = \alpha \, \underline{1}_\rho, \alpha \in \mathbb{R}. \tag{22}$$

One obtains the dynamical system

$$\dot{x} = N_E \, \mathrm{diag}\left(\Psi\left(a^{-1}\right)\right) \Psi(x). \tag{23}$$

For W_E a matrix of maximal rank with $W_E \, N_E = 0$ one has $W_E^T \, x(t) = W_E^T \, x(0)$ for solutions of (23). Thus solutions of (23) are confined to parallel translates of $\mathrm{im}(N_E)$.

Recall the discussion following (8), in particular the observation that solutions of (7) are confined to parallel translates of $\mathrm{im}(N)$. Observe that solutions of (23) are solutions of (7) for the special choice of k as in (19), (20). Further note that $\mathrm{im}(N_E) \subseteq \mathrm{im}(N)$. Hence for k as in (19), (20) and for $\mathrm{im}(N_E) \subset \mathrm{im}(N)$ solutions of (7) are not only contained in parallel translates of $\mathrm{im}(N)$, but also in parallel translates of $\mathrm{im}(N_E)$, that is, in affine subspaces of smaller dimension.

To establish multistationarity we need to show the existence of a second steady state $b \in \mathbb{R}^n_{>0}$ with

$$N \, v(k, \, b) = 0,$$

for the **same vector** k. That is b must satisfy:

$$N_E \, \mathrm{diag}\left(\Psi\left(a^{-1}\right)\right) \Psi(b) = 0 \tag{24}$$

We note (24) is equivalent to (recall (22) and observe that $a, \, b > 0$ implies $\Psi\left(\frac{b}{a}\right) > 0$):

$$\Psi\left(\frac{b}{a}\right) = \alpha \, \underline{1}_\rho, \alpha > 0$$

Apply $\ln(\cdot)$ to obtain the *linear system* (where we recall that $\Psi(x)$ is defined by the matrix Y_r):

$$Y_r^T \, \mu = \ln(\alpha) \, \underline{1}_\rho, \tag{25a}$$

where

$$\mu := \ln \frac{b}{a} = \left(\ln \frac{b_1}{a_1}, \ldots, \ln \frac{b_n}{a_n} \right)^T. \tag{25b}$$

The previous discussion motivates the following Lemma (recall $\bar{m}$, the number of reactant complexes and columns of Y_r):

Lemma 3 (Parameterizing positive steady states): Recall that by Assumption 1, k is of the form (20). Let $(\mu, \, \mu_0) \in \mathbb{R}^n \times \mathbb{R}$ be a solution of

$$\left[Y_r^T - \underline{1}_{\bar{m}} \right] \begin{pmatrix} \mu \\ \mu_0 \end{pmatrix} = 0. \tag{26a}$$

And let

$$b = \mathrm{diag}\left(e^\mu\right) \, a. \tag{26b}$$

Then $N \, \mathrm{diag}(k) \Phi(a) = 0$ and $N \, \mathrm{diag}(k) \Phi(b) = 0$.

Proof: Let μ, μ_0 be a solution of (26a). Observe that $N \, \mathrm{diag}(k) \Phi(a) = N_E \, \mathrm{diag}(\Psi(a^{-1})) \Psi(a) = 0$ follows from (20) and (22). Next we have to show that $N \, \mathrm{diag}(k) \Phi(b) = 0$ holds. To this end observe that

$$\begin{aligned} N \, \mathrm{diag}(k) \Phi(b) &= N_E \, \mathrm{diag}(\Psi(a^{-1})) \Psi(b) \\ &= N_E \, \mathrm{diag}(\Psi(a^{-1})) \Psi(\mathrm{diag}(e^\mu) a) \\ &= N_E \, \mathrm{diag}(\Psi(a^{-1})) \, \mathrm{diag}(\Psi(a)) \Psi(e^\mu) \\ &= N_E e^{Y_r^T \mu} = N_E e^{\mu_0} \, \underline{1}_{\bar{m}} = 0 \end{aligned}$$

■

Remark 4: If either (i) $\underline{1}_{\bar{m}} \in \mathrm{im}(Y_r^T)$ or (ii) $\ker\left(Y_r^T\right)$ is nontrivial (i.e. $\mathrm{rank}\left(Y_r\right) < n$), or both, then every *vector* $a \in \mathbb{R}^n_{>0}$ together with k as in (20) defines an *infinite* set of positive steady states $b \in \mathbb{R}^n_{>0}$. To see this assume that (i), (ii) or both hold. Then (25a) is solvable and the solution set

$$\mathcal{M} := \left\{ \mu \in \mathbb{R}^n | \exists \mu_0 > 0, \text{ such that } Y_r^T \mu = \mu_0 \underline{1}_{\bar{m}} \right\} \tag{27}$$

defines a linear subspace. As every $\mu \in \mathcal{M}$ defines a positive b and as a linear vector space contains infinitely many elements μ, there exists, *for a fixed* $a \in \mathbb{R}^n_{>0}$ infinitely many $b \in \mathbb{R}^n_{>0}$ with

$$b_a(\mu) = \operatorname{diag}(e^{\mu})\, a,\ \mu \in \mathcal{M}. \tag{28}$$

V. Subnetwork multistationarity

If the conditions of Remark 4 hold, then there exists an infinite set of positive steady states, even for a given $a \in \mathbb{R}^n_{>0}$ (recall that $\Psi\left(a^{-1}\right)$ takes the role of the rate constants). Fix $a \in \mathbb{R}_{>0}$ and thus by (20) the vector of rate constants k. Then all $b_a(\kappa)$ as defined in (28) are steady states. However, for a given initial condition the system 'sees' only a subset of a set of positive steady states. (Recall that solutions are confined to parallel translates of $\operatorname{im}(N)$ and, in case $\operatorname{im}(N_E) \subseteq \operatorname{im}(N)$, to parallel translates of $\operatorname{im}(N_E)$.)

Thus, for a given initial condition, the system 'sees' only those positive steady states that are in the intersection of $b_a(\kappa)$ and and $\operatorname{im}(N)$ and $\operatorname{im}(N_E)$, respectively. This motivates the following definition of multistationarity with respect to a linear subspace as introduced in [9]:

Definition 2: Given a subspace $\mathcal{V} \subset \mathbb{R}^n$, the system $\dot{x} = N\, v(k,x)$ from (7) is said to exhibit $\mathcal{V}$-multistationarity if and only if there exist a positive vector $k \in \mathbb{R}^r_{>0}$ and at least two distinct positive vectors $a,\ b \in \mathbb{R}^n_{>0}$ with

$$N\, v(k,a) = 0, \quad N\, v(k,b) = 0, \tag{29a}$$

$$b - a \in \mathcal{V}. \tag{29b}$$

Remark 5: Note that if $\mathcal{V} = \operatorname{im}(N)$, then Definition 2 is equivalent to the familiar definition of multistationarity [5], [8].

Remark 6: Note that for subnetworks defined by generators two linear subspaces are of particular interest: $\operatorname{im}(N_E)$ and the image of the stoichiometric matrix of the overall network $\operatorname{im}(\hat{N})$:

- $\operatorname{im}(N_E)$-*multistationarity*, that is, multistationarity with respect to $\operatorname{im}(N_E)$ means that the subnetwork can exhibit multistationarity, if it is considered in isolation.
- $\operatorname{im}(\hat{N})$-*multistationarity*, that is, multistationarity with respect to $\operatorname{im}(\hat{N})$ means that the subnetwork as part of the larger network can exhibit multistationarity.

Note that a subnetwork that exhibits $\operatorname{im}(\hat{N})$-multistationarity but not $\operatorname{im}(N_E)$-multistationarity can give rise to multistationarity for the overall network (e.g. under the conditions stated in [3]), even though, in isolation, it does not exhibit multistationarity. Hence we consider both, subnetworks exhibiting $\operatorname{im}(N_E)$-multistationarity and subnetworks exhibiting $\operatorname{im}(\hat{N})$-multistationarity as multistationarity causing structures.

Remark 7: As an illustration consider the network in Fig.1(a). For this network the matrix N and the generator of $\ker(N) \cap \mathbb{R}^r_{\geq 0}$ are given by

$$N = \begin{bmatrix} 1 & 0 & 0 & -1 \\ 0 & 1 & 0 & -1 \\ 0 & 0 & -1 & 1 \end{bmatrix} \text{ and } E = (1,\, 1,\, 1,\, 1)^T.$$

Using x_1 for A, x_2 for B and x_3 for C, one obtains the complex vectors $y_1 = 0$, $y_2 = e_3$, $y_3 = e_1 + e_2$, $y_4 = e_1$ and $y_5 = e_2$ and one obtains for the matrices $\mathcal{Y}$ and Y_r:

$$\mathcal{Y} = [y_1,\, y_1,\, y_2,\, y_3] \text{ and } Y_r = [y_1,\, y_2,\, y_3].$$

Hence one has

$$\Phi(x) = (1,\, 1,\, x_3,\, x_1\, x_2)^T \text{ and } \Psi(x) = (1,\, x_3,\, x_1\, x_2)^T$$

and

$$\Pi = \begin{bmatrix} 1 & 0 & 0 \\ 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix} \text{ with } \Phi(x) = \Pi\, \Psi(x).$$

The vector of rate constants is given by (cf. (20))

$$k = \left(1,\, 1,\, \frac{1}{a_3},\, \frac{1}{a_1\, a_2}\right)^T \tag{30}$$

for $a > 0$. For this example one obtains the following solution to (26a): $\mu = (\alpha, -\alpha, 0)$ and $\mu_0 = 0$. Hence one has (cf. equation (26b)

$$b_a(\alpha) = (e^{\alpha}\, a_1,\, e^{-\alpha}\, a_2,\, a_3)^T.$$

As N has full row rank, the left kernel is spanned by $W = 0$, the three-dimensional zero vector. Hence one has trivially $\operatorname{im}(N)$-multistationarity (even though it is not clear whether or not this definition is at all meaningful for this example). We obtain

$$N_E = \begin{bmatrix} 1 & 0 & -1 \\ 1 & 0 & -1 \\ 0 & -1 & 1 \end{bmatrix}$$

with left kernel $W_E = (1, -1, 0)^T$. For $\operatorname{im}(N_E)$-multistationarity we need to find a scalar α and a positive vector a such that $W_E^T\,(b_a(\alpha) - a) = 0$. Observe that

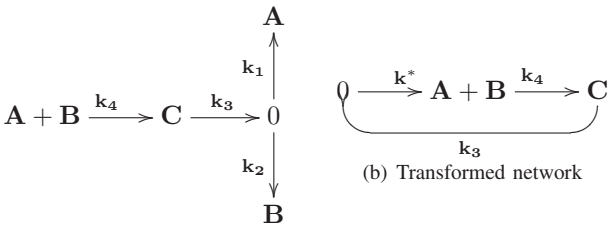
$$W_E^T\,(b_a(\alpha) - a) = (e^{\alpha} - 1)\, a_1 - (e^{-\alpha} - 1)\, a_2.$$

This equation holds for positive a_1, a_2 for $\alpha = 0$. But in this case $b_a(\alpha) = a$, hence $\operatorname{im}(N_E)$-multistationarity is excluded.

Note that for $a = [1,\, 1,\, 1]$ and $p = e^{\alpha}$ one obtains the following curve of steady states:

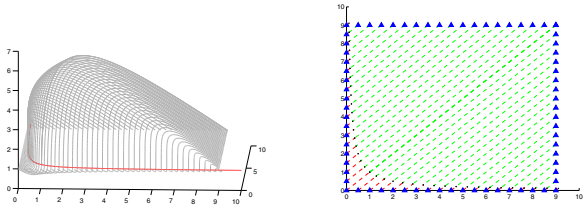
$$b_a(p) = \left(p,\, \frac{1}{p},\, 1\right). \tag{31}$$

Fig. 1(c) shows simulations for $a = 1$ and hence $k = (1,1,1,1)^T$ and different initial conditions. The curve $b_a(p)$ is depicted in red. For any initial condition, the trajectory is confined to an affine linear subspace perpendicular to W_E that contains a unique point of $b_a(p)$. And all trajectories starting in this particular affine linear subspace converge to the same steady state (cf. Fig. 1(d) for a projection to x_1-x_2-space). Note that, using k as in (30) the ODEs are equivalent to a system of ODEs derived from the network displayed in Fig.1(b), a weakly reversible deficiency zero network. From the Deficiency Zero Theorem [4] follows that this network has a unique, asymptotically stable positive steady state – relative to an affine linear subspace perpendicular to W_E. Finally we'd like to note that the network in Fig. 1(a) is irregular in the sense of CRNT [5].



(a) Example network

(b) Transformed network



(c) Simulation for selected initial conditions

(d) Projection in the x_1-x_2-plane

Fig. 1. Illustration of example network in Remark 7 with $\mathrm{im}(N)$- and $\mathrm{im}(N_E)$-multistationarity. The network in (a) admits positive steady states for k as in (30). For such a k the dynamical system with mass action kinetics is identical to the one defined by network (b), a weakly reversible deficiency zero network. (c),(d) Simulation studies: Trajectories (gray in (c) and green/red arrows in (d)) remain in affine linear subspace perpendicular to W_E. Red curve represents $b_a(p)$ from (31). For every initial condition (e.g. blue triangles in (d)) the trajectories converge to a unique point on $b_a(p)$.

VI. Establishing multistationarity for subnetworks

Consider a biochemical reaction network defined by a generator. From Section IV it is known, that for a given $a \in \mathbb{R}^n_{>0}$ all points $b_a(\mu)$ as defined in (28) are steady states. Recall the set $\mathcal{M}$ from (27) and observe that

$$\ln b_a(\mu) - \ln a \in \mathcal{M}$$

for $b_a(\mu)$ as in (26b). From Section V it is known, that $\mathcal{V}$-multistationarity requires

$$b_a(\mu) - a \in \mathcal{V} \text{ and } \ln b_a(\mu) - \ln a \in \mathcal{M}.$$

To this end the following result from [2] can be used. To state the result, let $\mathrm{sign}(u)$ denote the sign pattern of the vector $u \in \mathbb{R}^n$. Then $v = \mathrm{sign}(u)$ is a vector with entries $v_i \in \{+, -, 0\}$ depending on whether $u_i > 0$, $u_i < 0$ or $u_i = 0$, respectively.

Lemma 4 (cf. [2]): Let $M_1 \subseteq \mathbb{R}^n$ and $M_2 \subseteq \mathbb{R}^n$ be two nontrivial subsets of $\mathbb{R}^n$ and define $M_3 := \{(m_1, m_2) \in M_1 \times M_2 \mid \mathrm{sign}(m_1) = \mathrm{sign}(m_2)\}$ as the set of all ordered pairs (m_1, m_2) of elements $m_1 \in M_1$ and $m_2 \in M_2$ with the same sign pattern. Two positive vectors p and q with $\ln q - \ln p \in M_1$ and $q - p \in M_2$ exist, if and only if $M_3 \neq \emptyset$. Then p and q are given by

$$(p_i)_{i=1,\ldots,n} = \begin{cases} \frac{m_{2i}}{e^{m_{1i}}-1}, \text{ if } m_{1i} \neq 0 \\ \bar{p}_i > 0, \text{ if } m_{1i} = 0, \end{cases} \tag{32}$$

where $\bar{p}_i$ denotes an arbitrary positive number and

$$(q_i)_{i=1,\ldots,n} = e^{m_{1i}} p_i. \tag{33}$$

Thus – using $b_a(\mu)$ instead of q, a instead of p and $\mathcal{M}$ instead of M_1, $\mathcal{V}$ instead of M_2 – all one has to do is to find a vector $\mu \in \mathcal{M}$ and a vector $s \in \mathcal{V}$ with $\mathrm{sign}(\mu) = \mathrm{sign}(s)$ to establish $\mathcal{V}$-multistationarity.

VII. Multistationarity causing structures in a model of the *Escherichia coli* metabolism

As a proof of principle we apply the results of the previous sections to a mathematical model of the *E. coli* metabolism. Thereby we use an extended version of the model proposed by Kotte et al. in [12]. This model is **not** a mass action model. Most reaction rates are described by rational functions.

In our analysis we treat the model, **as if** all reaction rates were described by mass action kinetics. A subnetwork that can exhibit multistationarity, may nonetheless be regarded as a multistationarity causing structure: by using the ideas of [3], one can compute a parameter vector k^* where the mass action network admits two steady states (together with a pair of steady states a^*, b^* as witness). Then one can compute the mass action reaction rate vectors $v_{a^*} = v(k^*, a^*)$ and $v_{b^*} = v(k^*, b^*)$ at those two steady states. Now, taken with arbitrary kinetics and reaction rate vector $F(\kappa, x)$ (with parameters κ) one may use the equations

$$v_{a^*} = F(\kappa, a^*),\ v_{b^*} = F(\kappa, b^*)$$

to compute values of κ, where a^* and b^* are steady states of the reaction network with arbitrary kinetics. Provided such a positive κ exists, one has established multistationarity in the reaction network with arbitrary kinetics. Failure to establish such a κ does of course not imply that multistationarity is excluded.

A. Mathematical model of E. coli metabolism

To locate network structures in the metabolism of *E. coli* that can cause multistationarity, we obtain a model by extending the one published in [12]. This model includes the Embden-Meyerhof-Parnas (EMP) pathway, the tricarboxylicacid (TCA) cycle, the glyoxylate (GLX) shunt, isocitrate dehydrogenase catalysed carbon flux variation, the anaplerotic reaction to the TCA, glucose uptake via the phosphotransferase system (PTS), acetate (ACT) uptake and excretion, transcription factor mediated regulation of enzyme transcription and the enzymatic regulation of the aforementioned metabolic pathways.

We extend this model by including pathways for methylglyoxal (MG) production from dihydroxyacetone phosphate (DHAP) via MG synthase and MG detoxification via glyoxalase I-II, glyoxalase III and MG reductase all leading to the pyruvate (PYR) node. Furthermore, paths of the EMP are modeled in greater detail to introduce the necessary branching point for MG production (e.g. at the triangle formed by the reactions between the metabolites fructose bisphosphate (FBP), DHAP and glyceraldehyde 3-phosphate (GAP)).

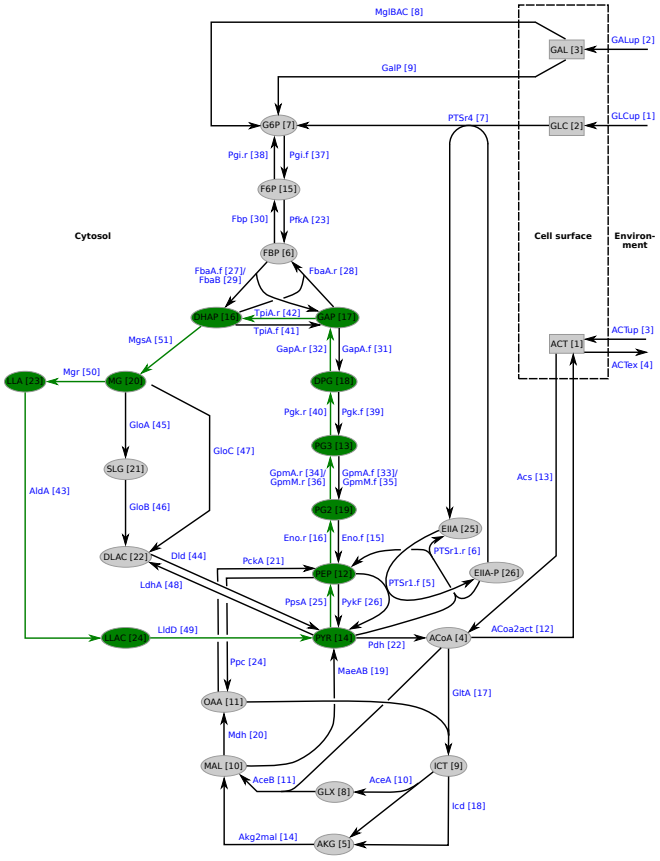


Fig. 2. Map of *E. coli* unregulated metabolism model. Arrows denote reactions. Reactions are named in blue according to the corresponding catalyzing enzymes, numbers denote the corresponding column of the stoichiometric matrix. Oval boxes represent metabolites, rectangular boxes represent external carbon sources, numbers denote the index of the corresponding variable (row of the stoichiometric matrix). Green subnetwork corresponds to a generator of $\ker(N) \cap \mathbb{R}^r_{\geq 0}$. The subnetwork contains 10 reactions, incorporating reaction 36 (catalysed by GpmM.r) and the metabolite MG and one of its detoxification pathways to PYR (via LLA). In subsection VII-B we show that for this subnetwork $\mathrm{im}(N_E)$-multistationarity is impossible and establish $\mathrm{im}(\hat{N})$-multistationarity.

Additionally galactose (GAL) uptake reactions via the beta-methylgalactoside (MglBAC) system and via galactose permease (GalP) have been added. The resulting reaction network is depicted in Fig. 2, the stoichiometric matrix $\hat{N} = \hat{N}_1 \mid \hat{N}_2$ in Fig. 3 (where $\mid$ denotes the horizontal concatenation of the two matrices).

B. Model Analysis and Results

The cone $\ker(N) \cap \mathbb{R}^r_{\geq 0}$ is generated by 597 generators that have been computed with the freely available MatLab tool CNA [11]. One such generator defines the subnetwork marked in green in Fig. 2. The corresponding matrices $N = N_1$ and $Y_r = Y_1$ are given in Fig. 4. Note that we have $E = \underline{1}$ and that every complex in the subnetwork is reactant complex of exactly one reaction Hence Π is the identity matrix in this case and $N_E = N_1$.

We examine the equation $[Y_1, -\underline{1}] \binom{\mu}{\mu_0} = 0$ with Y_1 from Fig. 4 and observe the following:

Fact 1: $\binom{\mu}{\mu_0} \in \ker\left([Y_1^T, -\underline{1}]\right)$, if and only if

$$\mu_{12} = \mu_{13} = \mu_{14} = \mu_{16} = \mu_{17} = \mu_{18} = \mu_{19} = \mu_{20} = \mu_{23} = \mu_{24} = \mu_0$$

For ease of notation we define the set

$$I = \{12, 13, 14, 16, 17, 18, 19, 20, 23, 24\} \tag{34}$$

and observe that by Fact 1 one has $\binom{\mu}{\mu_0} \in \ker([Y_1 - \underline{1}])$, if and only if

$$\mu_i = \mu_0, \ i \in I. \tag{35}$$

To show $\mathrm{im}(N_1)$-multistationarity, we nee to find a vector $s \in \mathrm{im}(N_1)$ with $\mathrm{sign}(s) = \mathrm{sign}(\mu)$ for μ as in Fact 1. Analysis of $\mathrm{im}(N_E)$ reveals:

Fact 2: $s \in \mathrm{im}(N_E)$, if and only if s satisfies

$$s_1 = s_2 = s_3 = s_4 = s_5 = s_6 = s_7 = s_8 = s_9 = s_{10} s_{11} = s_{15} = s_{21} = s_{22} = s_{25} = s_{26} = 0 \tag{36}$$

$$s_{12} + s_{13} + s_{14} + s_{16} + s_{17} + s_{18} + s_{19} + s_{20} + s_{23} + s_{24} = 0. \tag{37}$$

We claim that μ as in (35) and s as in (36) and (37) satisfy $\mathrm{sign}(\mu) = \mathrm{sign}(s)$, if and only if $\mu = 0$ and $s = 0$. To see the if part, we first note that $\mu = 0$ and $s = 0$ satisfy (35), (36), (37) and $\mathrm{sign}(\mu) = \mathrm{sign}(s)$. To see the only if part, assume $s \neq 0$ and $\mu \neq 0$ as in (35) and (36), (37) with $\mathrm{sign}(\mu) = \mathrm{sign}(s)$ exist. Then, by (35) and $\mathrm{sign}(\mu) = \mathrm{sign}(s)$ one has either $s_i > 0$ or $s_i < 0$, $\forall i \in I$. But such a vector s violates (37) and hence does not belong to $\mathrm{im}(N_E)$, a contradiction. Hence $\mu = 0$ and $s = 0$ is the only pair of vectors with $\mathrm{sign}(\mu) = \mathrm{sign}(s)$ where μ satisfies (35) and s satisfies (36), (37). Thus $\mathrm{im}(N_E)$-multistationarity is excluded.

For $\mathrm{im}(\hat{N})$-multistationarity we need to find a μ as in (35) and a vector $s \in \mathrm{im}(\hat{N})$ with $\mathrm{sign}(\mu) = \mathrm{sign}(s)$. Concerning $s \in \mathrm{im}(\hat{N})$ we observe:

Fact 3: $s \in \mathrm{im}\left(\hat{N}\right)$, if and only if $s_{25} = -s_{26}$.

From Facts 1 and 3 we conclude that all μ with

$$\mu_i = \mu_0, i \in I \text{ and } \mu_{25} = -\mu_{26} \tag{38}$$

satisfy $\left([Y_1^T, -\underline{1}]\right) \binom{\mu}{\mu_0} = 0$ and $\mu \in \mathrm{im}(\hat{N})$. Hence for every μ as in equation 38 there exists an $s \in \mathrm{im}(\hat{N})$ with $\mathrm{sign}(s) = \mathrm{sign}(\mu)$. Thus we conclude that the green subnetwork in Fig. 2 can indeed show $\mathrm{im}(\hat{N})$-multistationarity.

In a similar way we found 5 more subnetworks for which we were able to exclude $\mathrm{im}(N_E)$-multistationarity and to state $\mathrm{im}(\hat{N})$-multistationarity. The shown subnetwork as in Fig. 4 serves as example.

References

[1] C. Conradi and D. Flockerzi. Multistationarity in mass action networks with applications to ERK activation. *Journal of Mathematical Biology*, 65(1):107–156, 2012.

[2] C. Conradi, D. Flockerzi, and J. Raisch. Multistationarity in the activation of an MAPK: parametrizing the relevant region in parameter space. *Mathematical Biosciences*, 211(1):105–131, 2008.

[3] C. Conradi, D. Flockerzi, J. Raisch, and J. Stelling. Subnetwork analysis reveals dynamic features of complex (bio)chemical networks. *PNAS*, 104(49):19175–19180, 2007.

[4] M. Feinberg. Chemical reaction network structure and the stability of complex isothermal reactors–i. the deficiency zero and deficiency one theorems. *Chemical Engineering Science*, 42(10):2229 – 2268, 1987.

$$\tilde{N}_1 = \begin{bmatrix}
0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
1 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 1 & 0 & 0 & 0 & 0 & 0 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & -1 & 1 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\
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0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
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0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 1 & -1 & 0 & 0 & 1 & 0 \\
0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & -1 & 1 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & -1 & 0 & 0 & 0 & -1 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
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0 & 0 & 0 & 0 & -1 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0
\end{bmatrix}$$

$$\tilde{N}_2 = \begin{bmatrix}
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & -1 & 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
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0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
-1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & -1 & 1 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & -1 & 1 & 0 & 0 \\
0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 1 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 \\
0 & 1 & -1 & 1 & 0 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
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0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0
\end{bmatrix}$$

Fig. 3. Stoichiometric submatrices building the complete stoichiometric matrix.

$$Y_1 = \begin{bmatrix}
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
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1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\
0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\
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0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0
\end{bmatrix} \quad N_1 = \begin{bmatrix}
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
-1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 \\
0 & -1 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & -1 \\
0 & 0 & 1 & 0 & 0 & -1 & 0 & 0 & 0 & 0 \\
0 & 0 & -1 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\
1 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 1 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0
\end{bmatrix}$$

Fig. 4. Reactant complex matrix $Y_r = Y_1$ and stoichiometric matrix $N_E = N_1$ of the green subnetwork in Fig. 2.

[5] M. Feinberg. Chemical reaction network structure and the stability of complex isothermal reactors–ii. multiple steady states for networks of deficiency one. *Chemical Engineering Science*, 43(1):1 – 25, 1988.

[6] M. Feinberg. Necessary and sufficient conditions for detailed balancing in mass action systems of arbitrary complexity. *Chemical Engineering Science*, 44(9):1819 – 1827, 1989.

[7] M. Feinberg. The existence and uniqueness of steady states for a class of chemical reaction networks. *Archive for Rational Mechanics and Analysis*, 132(4):311–370, 1995.

[8] M. Feinberg. Multiple steady states for chemical reaction networks of Deficiency One. *Archive for Rational Mechanics and Analysis*, 132(4):371–406, 1995.

[9] D. Flockerzi and C. Conradi. Subnetwork analysis for multistationarity in mass action kinetics. *Journal of Physics - Conference Series*, 138:012006–, 2008.

[10] J. Gagneur and S. Klamt. Computation of elementary modes: a unifying framework and the new binary approach. *BMC Bioinformatics*, 5:175, 2004.

[11] S. Klamt, J. Saez-Rodriguez and E. D. Gilles. Structural and functional analysis of cellular networks with CellNetAnalyzer. *BMC Systems Biology*, 1:2, 2007.

[12] O. Kotte, J. B. Zaugg and M. Heinemann. Bacterial adaptation through distributed sensing of metabolic fluxes. *Molecular Systems Biology*, 6:1, 2010.

[13] M. Uhr, H.-M. Kaltenbach, C. Conradi, and J. Stelling. *Positive Systems*, volume 389 of *Lecture Notes in Control and Information Sciences*, chapter Analysis of Degenerate Chemical Reaction Networks, pages 163–182. Springer, 2009. Special Volume of the POSTA 2009.