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A network dynamics approach to chemical reaction networks

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ABSTRACT

A treatment of a chemical reaction network theory is given from the perspective of nonlinear network dynamics, in particular of consensus dynamics. By starting from the complex-balanced assumption, the reaction dynamics governed by mass action kinetics can be rewritten into a form which allows for a very simple derivation of a number of key results in the chemical reaction network theory, and which directly relates to the thermodynamics and port-Hamiltonian formulation of the system. Central in this formulation is the definition of a balanced Laplacian matrix on the graph of chemical complexes together with a resulting fundamental inequality. This immediately leads to the characterisation of the set of equilibria and their stability. Furthermore, the assumption of complex balancedness is revisited from the point of view of Kirchhoff's matrix tree theorem. Both the form of the dynamics and the deduced behaviour are very similar to consensus dynamics, and provide additional perspectives to the latter. Finally, using the classical idea of extending the graph of chemical complexes by a 'zero' complex, a complete steady-state stability analysis of mass action kinetics reaction networks with constant inflows and mass action kinetics outflows is given, and a unified framework is provided for structure-preserving model reduction of this important class of open reaction networks.

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1. Introduction

Network dynamics has been the subject of intensive research in recent years due to the ubiquity of large-scale networks in various application areas. While many advances have been made in the analysis of linear network dynamics, the study of nonlinear network dynamics still poses fundamental challenges.

In this paper, we revisit the analysis of chemical reaction networks (CRNs) as a prime example of nonlinear network dynamics, playing an important role in systems biology, (bio-)chemical engineering, and the emerging field of synthetic biology. Apart from being *large-scale* (typical reaction networks in living cells involve several hundreds of chemical species and reactions), a characteristic feature of CRN dynamics is their intrinsic *nonlinearity*. In fact, mass action kinetics, the most basic way to model reaction rates, leads to polynomial differential equations. On top of this, CRNs, in particular, in a biochemical context, usually have inflows and outflows.

The foundations of the structural theory of (isothermal) CRNs were laid in a series of seminal papers by Horn, Jackson, and Feinberg in the 1970s. The basic starting point of e.g. Horn and Jackson (1972), Horn (1972), Feinberg (1972) is the identification of a graph structure for CRNs by defining the chemical complexes, i.e., the

combination of chemical species appearing on the left-hand (substrate) and right-hand (product) sides of every reaction, as the vertices of a graph and the reactions as its edges. This enables the formulation of the dynamics of the reaction network as a dynamical system on the graph of complexes. Furthermore, in these papers, the philosophy was put forward of delineating, by means of *structural* conditions on the graph, a large class of reaction networks exhibiting the same type of dynamics, irrespective of the precise values of the (often unknown or uncertain) reaction constants. This 'normal' dynamics is characterised by the property that for every initial condition of the concentrations there exists a unique positive equilibrium to which the system will converge. Other dynamics, such as multi-stability or presence of oscillations, can, therefore, only occur within reaction networks violating these conditions. For an overview of results on CRNs, and current research in this direction including the global persistence conjecture, we refer to Angeli (2009) and the references quoted therein. An important step in extending the framework of CRNs towards *feedback stabilisation* has been made in Sontag (2001); also setting the stage for *regulation* questions.

The contribution of this paper is twofold. First, the formulation and analysis of mass action kinetics CRNs

is revisited from the point of view of *consensus dynamics* and its nonlinear versions (Cherukuri & Cortés, 2015; Cortés, 2008; van der Schaft & Maschke, 2013). The consideration of concepts from the algebraic graph theory, such as the systematic use of weighted Laplacian matrices, provides a framework for (re-)proving many of the previously obtained results on CRNs in a simple and insightful manner. In particular, in our previous work (Rao, van der Schaft, & Jayawardhana, 2013a; van der Schaft, Rao, & Jayawardhana, 2013a), we have shown how under the classical assumption of existence of a detailed-balanced equilibrium, or the weaker assumption of the existence of a complex-balanced equilibrium (a concept dating back to Horn & Jackson, 1972), the weights of the graph of complexes can be redefined in such a way that the resulting Laplacian matrix becomes symmetric (detailed-balanced case) or balanced (complex-balanced case). As a result, the characterisation of the set of positive equilibria and their stability as originating in Horn and Jackson (1972), Horn (1972), Feinberg (1972) follows in a simple way. Moreover, this formulation allows for a direct port-Hamiltonian interpretation (van der Schaft, Rao, & Jayawardhana, 2013b), merging CRNs with the geometric thermodynamical theory of Oster, Perelson, and Katchalsky (1973), Oster and Perelson (1974). It also leads to new developments, such as structure-preserving model reduction of large-scale CRNs, based on Kron reduction of the Laplacian matrix (Rao et al., 2013a; Rao, van der Schaft, van Eunen, Bakker, & Jayawardhana, 2014; van der Schaft et al., 2013a).

As said, our approach is based on the assumption of existence of a complex-balanced equilibrium. Following van der Schaft, Rao, and Jayawardhana (2015), a necessary and sufficient condition is discussed for the existence of a complex-balanced equilibrium based on the matrix tree theorem (a theorem going back to the work of Kirchhoff on electrical circuits), which extends the classical Wegscheider conditions for existence of a detailed-balanced equilibrium. We also make a connection with the property of *mass conservation*. Finally, we discuss how these results can be ‘dualized’ to consensus dynamics, providing new insights.

The second main contribution of this paper is the dynamical analysis of CRNs with *inflows* and *outflows*. The extension of the stability theory of *equilibria* for reaction networks without inflows and outflows (called *closed* reaction networks in the sequel) to that of *steady states* for reaction networks *with* inflows and outflows (called *open networks*) is far from trivial, due to the intrinsic nonlinearity of the reaction dynamics. Recently, there has been a surge of interest in open CRNs; we mention Angeli, De Leenheer, and Sontag (2009), Angeli (2011), Chaves (2005), Craciun and Feinberg (2010), Flach and

Schnell (2010), Rao, van der Schaft, and Jayawardhana (2013b). In this paper, we analyse open reaction networks by revisiting¹ the classical idea of extending the graph of complexes by a ‘zero’ complex (Feinberg & Horn, 1974; Horn & Jackson, 1972). This covers the subclass of open CRNs with constant inflows and mass action kinetics outflows; a subclass which is motivated by several examples of biochemical reaction network models (see e.g. the biochemical model in van Eunen, Kiewiet, Westerhoff, and Bakker (2012) and the examples mentioned in Feinberg and Horn (1974)). This subclass also derives from the assumption that some of the complexes or species involved in the reactions are kept at a constant concentration (see e.g. van Eunen et al. (2012, 2013)). We will show how the results for closed CRNs can be fully extended to this subclass of open CRNs. Compared to Horn and Jackson (1972), Feinberg and Horn (1974), where the specific properties of the zero complex do not play any role in the analysis, this paper spells out the equivalence of steady states with equilibria of the extended network, and shows how due to the presence of inflows and outflows the set of steady states may shrink to a unique steady state. Furthermore, the presence of steady states on the boundary of the positive orthant can be precluded. Finally, it allows to extend the model reduction techniques of van der Schaft et al. (2013a), Rao et al. (2013a), Rao et al. (2014) to this important subclass of open CRNs. The obtained stability analysis of steady states of open CRNs with constant inflows and mass action kinetics outflows is one of the, up to now rare, cases of a rather complete steady-state analysis of nonlinear network dynamics with external inputs. From a control perspective, the steady-state analysis of this subclass of open CRNs opens the possibility of applying the internal model principle (see e.g. De Persis and Jayawardhana (2012)) to achieve output regulation for such systems with constant reference signals using proportional-integral controllers, for example, in the control of CSTR or gene-regulatory networks as in Uhlendorf (2012).

The structure of the paper is as follows. In Section 2, based on algebraic graph-theoretical tools explored in van der Schaft et al. (2013a), Rao et al. (2013a), we discuss the theory of closed reaction network dynamics satisfying the complex-balanced assumption, based on rewriting the dynamics in terms of a balanced Laplacian matrix directly linked to its port-Hamiltonian formulation. We provide a new perspective on the characterisation of complex-balancedness by the use of Kirchhoff’s matrix tree theorem, and establish a connection to mass conservation. Furthermore, we indicate how these ideas may be applied to consensus dynamics. Section 3 deals with the detailed modelling of reaction networks having constant inflows and mass action kinetics outflows by extending

the graph with an extra zero complex. Section 4 shows how the assumption of complex balancedness can be extended to this case, and how this allows to derive precise results on the structure and stability of steady states. Section 5 provides a brief introduction to the structure-preserving model reduction of open CRNs, based on the Kron reduction of the graph of complexes. Conclusions follow in Section 6, while the Appendix describes how the situation of detailed-balanced CRNs can be understood as a special case of the complex-balanced case.

Notation: The space of n -dimensional real vectors consisting of all strictly positive entries is denoted by \mathbb{R}_+^n and the space of n -dimensional real vectors consisting of all non-negative entries by $\bar{\mathbb{R}}_+^n$. The mapping $\text{Ln} : \mathbb{R}_+^n \rightarrow \mathbb{R}^n$, $x \mapsto \text{Ln}(x)$, is defined as the mapping whose i th component is given as $(\text{Ln}(x))_i := \ln(x_i)$. Similarly, $\text{Exp} : \mathbb{R}^n \rightarrow \mathbb{R}_+^n$ is the mapping whose i th component is given as $(\text{Exp}(x))_i := \exp(x_i)$. Furthermore, for two vectors $x, y \in \mathbb{R}_+^n$ we let $\frac{x}{y}$ denote the vector in \mathbb{R}_+^n with i th component $\frac{x_i}{y_i}$. Finally $\mathbb{1}_n$ denotes the n -dimensional vector with all entries equal to 1, and 0_n the n -dimensional vector with all entries equal to zero, while I_n is the $n \times n$ identity matrix.

Some graph-theoretic notions (see e.g. Bollobas, 1998): a directed graph² \mathcal{G} with c vertices and r edges is characterised by a $c \times r$ incidence matrix, denoted by D . Each column of D corresponds to an edge of the graph, and contains exactly one element 1 at the position of the head vertex of this edge and exactly one -1 at the position of its tail vertex; all other elements are zero. Clearly, $\mathbb{1}^T D = 0$. The graph is *connected* if any vertex can be reached from any other vertex by following a sequence of edges; direction not taken into account. It holds that $\text{rank } D = c - \ell$, where ℓ is the number of *connected components* of the graph. In particular, \mathcal{G} is connected if and only if $\ker D^T = \text{span } \mathbb{1}$. The graph is *strongly connected* if any vertex can be reached from any other vertex, following a sequence of *directed* edges. A subgraph of \mathcal{G} is a directed graph whose vertex and edge set are subsets of the vertex and edge set of \mathcal{G} . A graph is *acyclic* (does not contain cycles) if $\ker D = 0$. A *spanning tree* of a directed graph \mathcal{G} is a connected, acyclic subgraph of \mathcal{G} that spans all vertices of \mathcal{G} .

2. Closed chemical reaction networks as dynamics on graphs

In this section, we give a concise treatment of closed (i.e., no inflows or outflows) CRNs, highlight the similarities as well the differences with consensus dynamics, and provide a number of additional insights.

2.1 The complex graph formulation

Consider a CRN with m chemical species (metabolites) with concentrations $x \in \mathbb{R}_+^m$, among which r chemical reactions take place. The graph-theoretic formulation, starting with the work of Horn, Jackson and Feinberg in the 1970s, is to associate to each complex (substrate as well as product) of the reaction network a vertex of a graph, while each reaction from substrate to product complex corresponds to a directed edge (with tail vertex the substrate and head vertex the product complex).

Let c be the total number of complexes involved in the reaction network, then the resulting directed graph \mathcal{G} with c vertices and r edges is called the *graph of complexes*³, and is defined by its $c \times r$ incidence matrix D . Since each of the c complexes is a combination of the m chemical species, we define the $m \times c$ matrix Z with non-negative integer elements expressing the composition of the complexes in terms of the chemical species. The k th column of Z denotes the composition of the k th complex, and the matrix Z is called the *complex composition matrix*⁴. It can be immediately verified that ZD equals the standard $m \times r$ stoichiometric matrix S . The special case of Z being the identity matrix corresponds to *single-species* substrate and product reaction networks (*SS reaction networks*). In general, the mapping $Z : \mathbb{R}^c \rightarrow \mathbb{R}^m$ defines a *representation* (Godsil & Royle, 2001) of the graph of complexes \mathcal{G} into the space \mathbb{R}^m of chemical species (the α th vertex is mapped to the α th column of Z in \mathbb{R}^m). Compared with other forms of network dynamics, the presence of the matrix Z constitutes a major, and non-trivial, difference; especially in case Z is not injective.

The dynamics of the reaction network takes the form

$$\dot{x} = Sv(x) = ZDv(x), \quad (1)$$

where $v(x)$ is the r -dimensional vector of reaction rates depending on the m -dimensional vector of concentrations x . The most basic way to define $v(x)$ is *mass action kinetics*. For example, for the reaction $X_1 + 2X_2 \rightarrow X_3$, the mass action kinetics reaction rate is given as $v(x) = kx_1x_2^2$ with $k > 0$ a reaction constant. In general, for a single reaction with substrate complex \mathcal{S} specified by its corresponding column $Z_{\mathcal{S}} = [Z_{S_1} \cdots Z_{S_m}]^T$ of the complex composition matrix Z , the mass action kinetics reaction rate is given by

$$kx_1^{Z_{S_1}}x_2^{Z_{S_2}} \cdots x_m^{Z_{S_m}},$$

which can be rewritten as $k \exp(Z_{\mathcal{S}}^T \text{Ln } x)$. Hence, the reaction rates of the total reaction network are given by

$$v_j(x) = k_j \exp(Z_{\mathcal{S}_j}^T \text{Ln } x), \quad j = 1, \dots, r,$$

where \mathcal{S}_j is the substrate complex of the j th reaction with reaction constant $k_j > 0$. This yields the following compact description of the total mass action kinetics rate vector $v(x)$. Define the $r \times c$ matrix K as the matrix whose (j, σ) th element equals k_j , if the σ th complex is the substrate complex for the j th reaction, and zero otherwise. We will call K the *outgoing co-incidence matrix* (since the σ th column of K specifies the weighted outgoing edges from vertex σ). Then,

$$v(x) = K \text{Exp}(Z^T \text{Ln } x), \quad (2)$$

and the dynamics of the mass action kinetics reaction takes the form

$$\dot{x} = ZDK \text{Exp}(Z^T \text{Ln } x). \quad (3)$$

The same expression (in less explicit form) was already obtained in Sontag (2001).

It can be verified that the $c \times c$ matrix $L := -DK$ has non-negative diagonal elements and non-positive off-diagonal elements. Moreover, since $\mathbb{1}_m^T D = 0$ also $\mathbb{1}_m^T L = 0$, i.e., the column sums of L are all zero. Hence, L defines (a transposed version of) a weighted *Laplacian matrix*⁵. From now on, we will simply call $L = -DK$ the Laplacian matrix of the graph of complexes \mathcal{G} .

2.2 Analysis of complex-balanced reaction network dynamics

A CRN (3) is called *complex-balanced* (Horn, 1972) if there exists an $x^* \in \mathbb{R}_+^m$, called a *complex-balanced equilibrium*, satisfying⁶

$$Dv(x^*) = -L \text{Exp}(Z^T \text{Ln}(x^*)) = 0. \quad (4)$$

Chemically (4) means that at the complex-balanced equilibrium x^* , not only the chemical species but also the complexes remain constant, i.e., for each complex, the total inflow (from the other complexes) equals the total outflow (to the other complexes). Defining now the diagonal matrix

$$\Xi(x^*) := \text{diag}\left(\exp(Z_i^T \text{Ln}(x^*))\right)_{i=1,\dots,c}, \quad (5)$$

the dynamics (3) can be rewritten into the form

$$\dot{x} = -Z \mathcal{L}(x^*) \text{Exp}\left(Z^T \text{Ln}\left(\frac{x}{x^*}\right)\right), \quad \mathcal{L}(x^*) := L \Xi(x^*), \quad (6)$$

where, since $\text{Exp}(Z^T \text{Ln}(\frac{x}{x^*})) = \mathbb{1}_c$, the transformed Laplacian matrix $\mathcal{L}(x^*)$ satisfies

$$\mathcal{L}(x^*) \mathbb{1}_c = 0, \quad \mathbb{1}_c^T \mathcal{L}(x^*) = 0. \quad (7)$$

Hence, $\mathcal{L}(x^*)$ is a *balanced* Laplacian matrix (column and row sums are zero).

Remark 2.1: As shown in Rao et al. (2013a), the matrix $\mathcal{L}(x^*)$ is *independent* of the choice of the complex-balanced equilibrium x^* up to a multiplicative factor for every connected component of \mathcal{G} .

Remark 2.2: Under the stronger *detailed-balanced* assumption (Oster & Perelson, 1974; Oster et al., 1973; van der Schaft et al., 2013a), the Laplacian matrix $\mathcal{L}(x^*)$ is not only balanced, but is in fact *symmetric*. In the Appendix, it is discussed how the detailed-balanced situation can be understood as a special case of the complex-balanced one.

Remark 2.3: Note that the vector $\text{Exp}(Z^T \text{Ln}(x^*))$ corresponding to a complex-balanced equilibrium $x^* \in \mathbb{R}_+^m$ defines a vector in \mathbb{R}_+^c that is in the kernel of the Laplacian matrix L . It thus follows (Gatermann, 2002, Lemma 3.2.9) that the connected components of the graph \mathcal{G} of a complex-balanced reaction network are all *strongly connected*⁷. This follows as well from the fact that a graph with balanced Laplacian matrix is strongly connected if and only if it is connected (Godsil & Royle, 2001). The property also follows from Kirchhoff's matrix tree theorem to be discussed later on.

As shown in Rao et al. (2013a) (generalising the detailed-balanced scenario of van der Schaft et al. (2013a)) a number of key properties of the reaction network dynamics can be derived in an insightful and easy way from the following fundamental fact. It is well known (Cortés, 2008) that balancedness of $\mathcal{L}(x^*)$ is equivalent to $\mathcal{L}(x^*) + \mathcal{L}^T(x^*)$ being positive semi-definite, i.e., $\alpha^T \mathcal{L} \alpha \geq 0$ for all $\alpha \in \mathbb{R}^c$. Based on *convexity* of the exponential function, we can establish the following property (Rao et al., 2013a).

Proposition 2.4: $\gamma^T \mathcal{L}(x^*) \text{Exp}(\gamma) \geq 0$ for any $\gamma \in \mathbb{R}^r$, with equality if and only if $D^T \gamma = 0$.

This result leads to a direct proof of a number of key properties of (6), which are known within the CRN theory but proven by tedious derivations. The first property which directly follows from Proposition 2.4 is that all positive equilibria are in fact complex-balanced equilibria, and that given one complex-balanced equilibrium x^* the set of *all* positive equilibria is given by

$$\mathcal{E} := \{x^{**} \in \mathbb{R}_+^m \mid S^T \text{Ln}(x^{**}) = S^T \text{Ln}(x^*)\}. \quad (8)$$

In particular, the set of positive equilibria \mathcal{E} is a smooth manifold of dimension $m - \text{rank } S$. Another property of \mathcal{E} can be seen to be implied by the extra assumption of *mass conservation*, which is defined as follows.

Definition 2.5: A reaction network with complex composition matrix Z and incidence matrix D is said to satisfy *mass conservation* if there exists $\mu \in \mathbb{R}_+^m$, such that

$$Z^T \mu \in \ker D^T, \quad (9)$$

or, equivalently, $S^T \mu = D^T Z^T \mu = 0$.

Remark 2.6: In case \mathcal{G} is connected $\ker D^T = \text{span } \mathbb{1}$, and the definition of mass conservation reduces to the existence of $\mu \in \mathbb{R}_+^m$, such that $Z^T \mu = \mathbb{1}$. The vector μ specifies a vector of mass assignments (μ_i being the mass associated to the i th chemical species), and the condition $Z^T \mu = \mathbb{1}$ means that all complexes have identical mass. For the general case, this holds on any connected component of \mathcal{G} .

Proposition 2.7: *The origin 0 is on the boundary of \mathcal{E} if and only if the CRN satisfies mass conservation.*

Proof: There exists a $x^{**} \in \mathbb{R}_+^m$ with all entries arbitrarily close to 0 with $S^T \text{Ln}(x^{**}) = S^T \text{Ln}(x^*)$ if and only if there exists a vector z with all entries arbitrarily close to $-\infty$, such that $S^T z = S^T \text{Ln}(x^*)$. This, in turn, holds if and only if there exists a positive vector $\mu \in \ker S^T = \ker D^T Z^T$, or, equivalently, $Z^T \mu \in \ker D^T$. \square

It directly follows (Rao et al., 2013a; Sontag, 2001) from the structure of the Laplacian matrix L that the dynamics (3) leaves the positive orthant \mathbb{R}_+^m invariant. Hence, concentrations of chemical species remain positive for all future times. On the other hand, this does not rule out the possibility that the solution trajectories of (3) will approach the boundary of the positive orthant for $t \rightarrow \infty$. The reaction network is called *persistent*⁸ if for every $x_0 \in \mathbb{R}_+^m$ the ω -limit set $\omega(x_0)$ of the dynamics (3) does *not* intersect the boundary of \mathbb{R}_+^m .

Using a result from Feinberg (1995), there exists for any initial condition $x_0 \in \mathbb{R}_+^m$ a unique $x^{**} \in \mathcal{E}$, such that $x^{**} - x_0 \in \text{im } S$. Furthermore, Proposition 2.4 yields (Rao et al., 2013a; van der Schaft et al., 2013a) that the time derivative of the function

$$G(x) := x^T \text{Ln} \left(\frac{x}{x^{**}} \right) + (x^{**} - x)^T \mathbb{1}_m \quad (10)$$

is negative outside \mathcal{E} and thus qualifies as a Lyapunov function, implying that the vector of concentrations $x(t)$ starting from x_0 will converge to x^{**} if the reaction network is persistent.

The chemical interpretation is that G is (up to a constant) the *Gibbs' free energy* (Oster & Perelson, 1974; Oster

et al., 1973; van der Schaft et al., 2013a), with gradient vector $\frac{\partial G}{\partial x}(x) = \text{Ln} \left(\frac{x}{x^{**}} \right)$ being the vector of *chemical potentials*. Hence, (6) can be rewritten as

$$\dot{x} = -Z\mathcal{L}(x^*) \text{Exp} \left(Z^T \frac{\partial G}{\partial x}(x) \right) \quad (11)$$

and the 'driving forces' of the reactions are seen to be determined by the *complex thermodynamical affinities*⁹ $\gamma(x) := Z^T \frac{\partial G}{\partial x}(x) = Z^T \text{Ln} \left(\frac{x}{x^{**}} \right)$, and equilibrium arises whenever the components of $\gamma(x)$ reach 'consensus' on every connected component of the graph of complexes \mathcal{G} .

2.3 Port-Hamiltonian formulation

The formulation (11) admits a direct port-Hamiltonian interpretation (see e.g. van der Schaft, 2000; van der Schaft & Jeltsema, 2014; van der Schaft & Maschke, 1995 for an introduction to port-Hamiltonian systems). Indeed, consider the auxiliary port-Hamiltonian system

$$\begin{aligned} \dot{x} &= Zf \\ e &= Z^T \frac{\partial G}{\partial x}(x) \end{aligned} \quad (12)$$

with inputs $f \in \mathbb{R}^c$ and outputs $e \in \mathbb{R}^c$, and Hamiltonian given by the Gibbs' free energy G defined in (10). It follows from Proposition 2.4 that

$$f = -\mathcal{L}(x^*) \text{Exp}(e) \quad (13)$$

defines a true *energy-dissipating* relation, that is, $e^T f \leq 0$ for all $e \in \mathbb{R}^c$ and $f \in \mathbb{R}^c$ satisfying (13). By substituting (13) into (12), one recovers the chemical reaction dynamics (11).

Remark 2.8: It should be noted that the energy-dissipating relation (13) is intrinsically nonlinear, and generally (unless Z is e.g. the identity matrix; see the SS reaction networks discussed later on) cannot be integrated to a relation of the form $f = -\frac{\partial R}{\partial e}(e)$ for some (Rayleigh) dissipation function $R: \mathbb{R}^c \rightarrow \mathbb{R}$. On the other hand, in the detailed-balanced case (see the Appendix), one *can* define (generalised) conductances associated to the edges.

2.4 Characterisation of complex balancedness

Complex balancedness can be fully characterised as follows, cf. van der Schaft et al. (2015) for further details. By the definition of $\text{Ln}: \mathbb{R}_+^m \rightarrow \mathbb{R}^m$, the existence of a complex-balanced equilibrium $x^* \in \mathbb{R}_+^m$, that is, $L \text{Exp}(Z^T \text{Ln}(x^*)) = 0$, is equivalent to the existence of

a vector $\mu^* \in \mathbb{R}^m$, such that

$$L \text{Exp}(Z^T \mu^*) = 0, \quad (14)$$

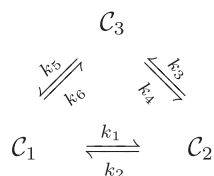
or equivalently, $\text{Exp}(Z^T \mu^*) \in \ker L$. Furthermore, note that $\text{Exp}(Z^T \mu^*) \in \mathbb{R}_+^c$.

First assume that the graph \mathcal{G} is connected. Then the kernel of L is one-dimensional, and a vector $\rho \in \mathbb{R}_+^c$ with $\rho \in \ker L$ can be computed by *Kirchhoff's matrix tree theorem*¹⁰, which can be summarised as follows. Denote the (i, j) th cofactor of L by $C_{ij} = (-1)^{i+j} M_{i,j}$, where $M_{i,j}$ is the determinant of the (i, j) th minor of L . Define the adjoint matrix $\text{adj}(L)$ as the matrix with (i, j) th element given by C_{ji} . Then $L \cdot \text{adj}(L) = (\det L) I_c$, and since $\det L = 0$, this implies $L \cdot \text{adj}(L) = 0$. Since $\mathbf{1}^T L = 0$ the sum of the rows of L is zero, and hence by the properties of the determinant function it follows that C_{ij} does not depend on i ; implying that $C_{ij} =: \rho_j, j = 1, \dots, c$. Hence, by defining $\rho := (\rho_1, \dots, \rho_c)$, it follows that $L\rho = 0$ (cf. Bollobas (1998), Theorem 14 on p.58).

Theorem 2.9: *Let \mathcal{G} be a connected graph with weighted Laplacian matrix L satisfying $\mathbf{1}^T L = 0$, implying that $L\rho = 0$, where $\rho_j = C_{ij}$ is the (i, j) th cofactor of L . Then, ρ_j is equal to the sum of the products of weights of all the spanning trees of \mathcal{G} directed towards vertex j .*

In particular, it follows that $\rho_j \geq 0, j = 1, \dots, c$. Moreover, since for every vertex j , there exists at least one spanning tree directed towards j if and only if the graph is *strongly connected*, $\rho \in \mathbb{R}_+^c$ if and only if the graph is strongly connected.

Example 2.10: Consider the cyclic reaction network



in the three (unspecified) complexes $\mathcal{C}_1, \mathcal{C}_2, \mathcal{C}_3$. The Laplacian matrix is given as

$$L = \begin{bmatrix} k_1 + k_6 & -k_2 & -k_5 \\ -k_1 & k_2 + k_3 & -k_4 \\ -k_6 & -k_3 & k_4 + k_5 \end{bmatrix}.$$

By Kirchhoff's matrix tree theorem, the corresponding vector ρ satisfying $L\rho = 0$ is given as

$$\rho = \begin{bmatrix} k_3 k_5 + k_2 k_5 + k_2 k_4 \\ k_1 k_5 + k_1 k_4 + k_4 k_6 \\ k_1 k_3 + k_3 k_6 + k_2 k_6 \end{bmatrix},$$

where each term corresponds to one of the three weighted spanning trees pointed towards the three vertices.

In case the graph \mathcal{G} is not connected, the same analysis can be performed on any of its connected components.

Remark 2.11: The existence (not the explicit construction) of $\rho \in \mathbb{R}_+^c$ already follows from the Perron-Frobenius theorem (Horn, 1972), (Sontag, 2001, Lemma V.2); exploiting the fact that the off-diagonal elements of $-L := DK$ are all non-negative.

Returning to the existence of $\mu^* \in \mathbb{R}^m$ satisfying $L \text{Exp}(Z^T \mu^*) = 0$ all this implies the following. Let $\mathcal{G}_j, j = 1, \dots, \ell$, be the connected components of the graph of complexes \mathcal{G} . For each connected component, define the vectors ρ^1, \dots, ρ^ℓ as above by Kirchhoff's matrix tree theorem (i.e., as cofactors of L or equivalently as sums of products of weights along spanning trees). Then, define the total vector ρ as the stacked column vector $\rho := \text{col}(\rho^1, \dots, \rho^\ell)$. Partition correspondingly the complex composition matrix Z as $Z = [Z_1 \dots Z_\ell]$. Then, there exists $\mu^* \in \mathbb{R}^m$ satisfying $L \text{Exp}(Z^T \mu^*) = 0$ if and only if each connected component is strongly connected and on each connected component

$$\text{Exp}(Z_j^T \mu^*) = \beta_j \rho^j, \quad j = 1, \dots, \ell, \quad (15)$$

for some positive constants $\beta_j, j = 1, \dots, \ell$. This in turn is equivalent to strong connectedness of each connected component of \mathcal{G} and the existence of constants $\beta_j' E$, such that

$$Z_j^T \mu^* = \text{Ln } \rho^j + \beta_j' \mathbf{1}, \quad j = 1, \dots, \ell. \quad (16)$$

Furthermore, this is equivalent to strong connectedness of each connected component, and

$$\text{Ln } \rho \in \text{im } Z^T + \ker D^T. \quad (17)$$

Finally, (17) is equivalent to

$$D^T \text{Ln } \rho \in \text{im } D^T Z^T = \text{im } S^T. \quad (18)$$

Summarising, we have obtained the following.

Theorem 2.12: *The reaction network dynamics $\dot{x} = -Z \text{Exp}(Z^T \text{Ln}(x))$ on the graph of complexes \mathcal{G} is complex balanced if and only if each connected component of \mathcal{G} is strongly connected (or, equivalently, $\rho \in \mathbb{R}_+^c$) and (18) is satisfied, where the coefficients of the sub-vectors ρ^j of ρ are obtained by Kirchhoff's matrix tree theorem for each j th connected component of \mathcal{G} .*

Clearly, if $\text{im } Z^T = \mathbb{R}^c$, or equivalently $\ker Z = 0$, then (17) is satisfied for any ρ .

Corollary 2.13: *The reaction network dynamics $\dot{x} = -Z \text{Exp}(Z^T \text{Ln}(x))$ is complex balanced if and only if*

$\rho \in \mathbb{R}_+^c$ and

$$\rho_1^{\sigma_1} \cdot \rho_2^{\sigma_2} \cdots \rho_c^{\sigma_c} = 1, \quad (19)$$

for all vectors $\sigma = \text{col}(\sigma_1, \sigma_2, \dots, \sigma_c) \in \ker Z \cap \text{im } D$.

Proof: $\text{Ln } \rho \in \text{im } Z^T + \ker D^T$ if and only if $\sigma^T \text{Ln } \rho = 0$ for all $\sigma \in (\text{im } Z^T + \ker D^T)^\perp = \ker Z \cap \text{im } D$, or equivalently

$$\begin{aligned} 0 &= \sigma_1 \ln \rho_1 + \cdots + \sigma_c \ln \rho_c = \ln \rho_1^{\sigma_1} + \cdots + \ln \rho_c^{\sigma_c} \\ &= \ln(\rho_1^{\sigma_1} \cdots \rho_c^{\sigma_c}) \end{aligned}$$

for all $\sigma \in \ker Z \cap \text{im } D$. \square

Remark 2.14: Note that the assumption of mass conservation (Definition 2.5) may interfere with condition (17). Indeed, mass conservation implies $\ker D^T \subset \text{im } Z^T$ (unless $Z = 0$), in which case (17) reduces to $\text{Ln } \rho \in \text{im } Z^T$.

In the Appendix, we will indicate how the constructive conditions for the existence of a complex-balanced equilibrium as obtained in Theorem 2.12 relate to the classical Wegscheider conditions for the existence of a *detailed-balanced* equilibrium.

2.5 SS reaction networks

Reaction networks with single-species substrate and product complexes (SS reaction networks) correspond to $c = m$ and $Z = I_m$, in which case the dynamics (3) reduces to the *linear* dynamics

$$\dot{x} = DKx. \quad (20)$$

An SS reaction network is complex-balanced, if there exists a positive equilibrium $x^* \in \mathbb{R}_+^m$, such that $DKx^* = 0$, and hence can be rewritten as

$$\begin{aligned} \dot{x} &= -\mathcal{L}(x^*) \frac{x}{x^*}, \quad \mathcal{L}(x^*) := -DK\Xi(x^*), \\ \Xi(x^*) &:= \text{diag}(x_1^*, \dots, x_m^*), \end{aligned} \quad (21)$$

where $\mathcal{L}(x^*)$ is a balanced Laplacian matrix. The set of positive equilibria of a complex-balanced SS reaction network is given as $\mathcal{E} = \{x^{**} \in \mathbb{R}_+^m \mid D^T \text{Ln}(x^{**}) = D^T \text{Ln}(x^*)\}$, and thus, in case the graph \mathcal{G} is connected, as $\mathcal{E} = \{x^{**} \mid x^{**} = px^*, p > 0\}$.

In Remark 2.3, we already mentioned that the existence of a complex-balanced equilibrium implies that the connected components of the graph are strongly connected. For SS reaction networks, also the converse holds, as follows from the above discussion, either based on Kirchhoff's matrix tree theorem or on the Perron–Frobenius theorem. Still another way is to make use of

a result of Horn (1972) stating that a mass action CRN is complex balanced if it is strongly connected and has *zero deficiency*, meaning that $\text{rank } D = \text{rank } ZD$, which obviously is the case for any SS network.

2.6 Relation with consensus dynamics

The dynamics $\dot{x} = -Lx = DKx$ with $\mathbb{1}^T L = 0$ as occurring in SS reaction networks can be regarded as ‘dual’ to the standard consensus dynamics $\dot{x} = -L_c x$, where the matrix L_c has non-negative diagonal elements and non-positive off-diagonal elements and satisfies $L_c \mathbb{1} = 0$. In a different context, this has been explored in Chapman and Mesbahi (2011), where $\dot{x} = -Lx$ with $\mathbb{1}^T L = 0$ was called *advection dynamics*. As also noted in Chapman and Mesbahi (2011), this duality originates from a duality in the interpretation of the edges of the underlying directed graph \mathcal{G} . For $\dot{x} = -Lx$ with $\mathbb{1}^T L = 0$, an edge from vertex i to j denotes ‘material flow’ from vertex i to vertex j , while for $\dot{x} = -L_c x$ with $L_c \mathbb{1} = 0$, an edge from vertex i to j denotes ‘information’ about vertex i available at vertex j . Thus, in the first case, the graph \mathcal{G} denotes a flow network, while in the latter case, \mathcal{G} is a communication graph.

It follows that the results described so far for flow networks can be ‘transposed’ to communication graphs and consensus dynamics. First of all, the Laplacian L_c with $L_c \mathbb{1} = 0$ can be expressed as $L_c = -J^T D^T$, where D is again the incidence matrix of \mathcal{G} while J (dually to the matrix K as before) can be called the *incoming co-incidence matrix*: the i th column of J specifies the weighted edges incoming to vertex i . Furthermore, the idea of transforming the ‘out-degree’ Laplacian matrix $L = -DK$ to a balanced Laplacian matrix $\mathcal{L}(x^*)$ under the assumption of complex balancedness of the graph (or equivalently, under the assumption of strong connectedness of its connected components) can be also applied to the consensus dynamics $\dot{x} = -L_c x$ with $L_c \mathbb{1} = 0$. Indeed, assume that the connected components of the graph \mathcal{G} are strongly connected. Then, Kirchhoff's matrix tree theorem provides a positive vector $\sigma \in \mathbb{R}_+^m$, such that $\sigma^T L_c = 0$. In fact, σ_j is given as the sum of the products of the weights along directed spanning trees directed *from* vertex j . It follows that $\frac{d}{dt} \sum_{j=1}^m \sigma_j x_j = 0$, implying that $\sum_{j=1}^m \sigma_j x_j$ is a conserved quantity. Defining the diagonal matrix $\Sigma := \text{diag}(\sigma_1, \dots, \sigma_m)$ the transformed Laplacian matrix $\mathcal{L}_c := \Sigma L_c$ is balanced, and hence $\mathcal{L}_c^T + \mathcal{L}_c \geq 0$. Note that this immediately yields an easy stability proof of the set of equilibria $\mathcal{E} = \{x \in \mathbb{R}_+^m \mid x = d\mathbb{1}, d > 0\}$ for the consensus dynamics $\dot{x} = -L_c x$. Indeed, the positive function $V(x) := x^T \Sigma x$ satisfies

$$\frac{d}{dt} V(x) = -x^T (L_c^T \Sigma + \Sigma L_c) x = -x^T (\mathcal{L}_c^T + \mathcal{L}_c) x \leq 0, \quad (22)$$

and thus serves as a Lyapunov function proving asymptotic stability of the set of consensus states \mathcal{E} . Furthermore, for any initial condition x_0 , the dynamics will converge to the consensus state $d^*\mathbb{1}$, where d^* is given as $d^* = \frac{1}{m} \sum_{j=1}^m \sigma_j x_{0j}$, with $\sigma_1, \dots, \sigma_m$ determined as above by Kirchhoff's matrix tree theorem.

3. Reaction networks with constant inflows and mass action kinetics outflows

In many cases of interest, including bio-chemical networks, reaction networks have inflows and outflows of chemical species. A mass action kinetics CRN with *constant inflows* and *mass action kinetics outflows* is described by the following extension of (3)

$$\dot{x} = ZDv(x) + ZD_{\text{in}}v_{\text{in}} + ZD_{\text{out}}v_{\text{out}}(x), \quad x \in \mathbb{R}_+^m. \quad (23)$$

Here the matrices D_{in} and D_{out} specify the structure of the inflows and outflows. D_{in} is a matrix whose columns consist of exactly one element equal to +1 (at the row corresponding to the complex which has inflow) while the other elements are zero. Similarly, D_{out} is a matrix whose columns consist of exactly one element equal to -1 (at the row corresponding to the complex which has outflow) while the rest are zero. As in the closed network case, $v(x)$ is the vector of (internal) mass action kinetics reaction rates given by $v(x) = K\text{Exp}(Z^T \text{Ln}(x))$. Furthermore, $v_{\text{in}} \in \mathbb{R}_+^k$ is a vector of constant positive inflows, while $v_{\text{out}}(x) \in \mathbb{R}_+^l$ is a vector of mass action kinetics outflows described by mass action kinetics as

$$D_{\text{out}}v_{\text{out}}(x) = -\Delta_{\text{out}}\text{Exp}(Z^T \text{Ln}(x)), \quad (24)$$

where Δ_{out} is a diagonal matrix with non-negative elements given by the mass action kinetics rate constants.

Remark 3.1: Note that (23) formalises a situation of *direct* in- and outflow of chemical species contained within the given chemical complexes of the reaction network. For other scenarios of open CRNs, such as continuous-stirred tank reactors with convective in- and outflows, we refer to e.g. Hangos and Cameron (2001).

A classical idea due to Horn and Jackson (1972) is that by the addition of an extra complex, the reaction network (23) can be represented as a *closed* reaction network on the extended graph. In fact, this extra complex is added in such a way that the edges from the extra complex to the ordinary complexes model the *inflows* into the network, while the edges towards the extra complex model the *outflows* of the network. The complex composition matrix z_{zero} corresponding to the extra complex is defined

to be the m -dimensional *zero column vector*, and the extra complex is therefore called the *zero complex*. Hence, the zero complex serves as a combined 'source and sink' complex, which does not contribute to the overall mass. As a consequence, the extended network cannot satisfy mass conservation, cf. Definition 9.

The resulting graph, consisting of the original graph of complexes together with the zero complex, is called the *extended graph of complexes* of the open reaction network (23), and has complex composition matrix

$$Z_e := [Z \ z_{\text{zero}}] = [Z \ 0].$$

The incidence matrix of the extended complex graph, denoted by D_e , is given as

$$D_e = \begin{bmatrix} B \\ b_{\text{zero}} \end{bmatrix},$$

with b_{zero} a row vector corresponding to the zero complex, while in the notation of (23)

$$B = [D \ D_{\text{in}} \ D_{\text{out}}]. \quad (25)$$

Now define the c -dimensional column vector l_{in} as

$$l_{\text{in}} = -D_{\text{in}}v_{\text{in}}. \quad (26)$$

Furthermore, let l_{out} be the c -dimensional row vector whose i th element is equal to minus the i th diagonal element of Δ_{out} . Then, extend the $c \times c$ Laplacian matrix L of the graph of (ordinary) complexes to an $(c+1) \times (c+1)$ Laplacian matrix L_e of the extended graph of complexes as

$$L_e := \begin{bmatrix} L + \Delta_{\text{out}} & l_{\text{in}} \\ l_{\text{out}} & \delta_{\text{in}} \end{bmatrix}, \quad (27)$$

where $\delta_{\text{in}} \geq 0$ equals minus the sum of the elements of l_{in} . By construction, L_e has non-negative diagonal elements, non-positive off-diagonal elements, while its column sums are all zero.

It follows that the dynamics (23) of the mass action reaction network with constant inflows and mass action kinetics outflows is equal to the mass action kinetics dynamics of the *extended graph of complexes* with extended stoichiometric matrix $S_e = Z_e D_e$. Indeed, since $z_{\text{zero}} = 0$

$$\begin{aligned} \dot{x} &= Z[Dv(x) + D_{\text{in}}v_{\text{in}} + D_{\text{out}}v_{\text{out}}(x)] = ZBv_e(x) \\ &= Z_e D_e v_e(x) = S_e v_e(x), \end{aligned} \quad (28)$$

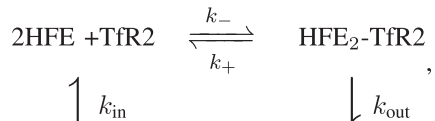
where

$$v_e(x) = \begin{bmatrix} v(x) \\ v_{in} \\ v_{out}(x) \end{bmatrix} \quad (29)$$

with $v(x) = K \text{Exp}(Z^T \text{Ln}(x))$. Furthermore, by using (24), (26), and (27),

$$\begin{aligned} \dot{x} &= Z[Dv(x) + D_{in}v_{in} + D_{out}v_{out}(x)] \\ &= -[Z \ 0]L_e \begin{bmatrix} \text{Exp}(Z^T \text{Ln}(x)) \\ 1 \end{bmatrix} \\ &= -Z_e L_e \text{Exp}(Z_e^T \text{Ln}(x)). \end{aligned} \quad (30)$$

Example 3.2: As an example, to be continued in the rest of the paper, let us consider a subnetwork of a biochemical reaction network describing liver iron metabolism as studied in Mitchell and Mendes (2013). The full CRN in Mitchell and Mendes (2013) contains 44 reactions which are mostly based on mass-action kinetics. In the present example, we focus on the subnetwork



where its interconnection with the rest of the network is represented by a constant inflow and a mass-action kinetics outflow. HFE, TfR2 and HFE₂-TfR2 denote, respectively, the plasma haeme, transferrin receptor, and a binding-complex (not to be confused with our previous definition of complexes), which are three of the species involved in the complete liver iron metabolism (Mitchell & Mendes, 2013). The constants k_+ and k_- are the forward and reverse reaction constants of the internal reversible reaction of the subnetwork, k_{in} is the constant inflow rate, and k_{out} is the outflow reaction constant. In line with the standard notation, denote $x_1 := [\text{HFE}]$, $x_2 := [\text{TfR2}]$, $x_3 := [\text{HFE}_2\text{-TfR2}]$ and $x = [x_1 \ x_2 \ x_3]^T$, where $[X]$ denotes the concentration of species X . The complex composition matrix Z for this example is given by

$$Z = \begin{bmatrix} 2 & 0 \\ 1 & 0 \\ 0 & 1 \end{bmatrix}.$$

The Laplacian matrix of the internal reversible reaction (split into a forward and reverse reaction) is

$$L = \begin{bmatrix} k_+ & -k_- \\ -k_+ & k_- \end{bmatrix}.$$

Together with the zero complex, this corresponds to the Laplacian of the extended graph of complexes

$$L_e = \begin{bmatrix} k_+ & -k_- & -k_{in} \\ -k_+ & k_- + k_{out} & 0 \\ 0 & -k_{out} & k_{in} \end{bmatrix}$$

and the following dynamics of the reaction network as in (23)

$$\dot{x} = Z \left(\begin{bmatrix} -k_+ & k_- \\ k_+ & -k_- \end{bmatrix} \begin{bmatrix} x_1^2 x_2 \\ x_3 \end{bmatrix} + \begin{bmatrix} k_{in} \\ 0 \end{bmatrix} - \begin{bmatrix} 0 \\ k_{out} x_3 \end{bmatrix} \right).$$

4. Analysis of reaction networks with constant inflows and mass action kinetics outflows

Based on the formulation of the previous section, we can extend the results concerning the stability of *closed* complex-balanced reaction networks (especially the characterisation of the set \mathcal{E} of positive equilibria in (8) and the use of the Lyapunov function G defined in (10) for proving the asymptotic stability of \mathcal{E}) to *open* reaction networks with constant inflows and mass action kinetics outflows, satisfying an adapted complex-balanced assumption. As before, we note that the representation (30) implies that the positive orthant \mathbb{R}_+^m is invariant for (23).

Definition 4.1: An $x^* \in \bar{\mathbb{R}}_+^m$ is called a *steady state* of the reaction network with constant inflows and mass action kinetics outflows given by (23) if

$$Z[Dv(x^*) + D_{in}v_{in} + D_{out}v_{out}(x^*)] = 0. \quad (31)$$

An $x^* \in \mathbb{R}_+^m$ is called a *complex-balanced* steady state if

$$Dv(x^*) + D_{in}v_{in} + D_{out}v_{out}(x^*) = 0. \quad (32)$$

If there exists a complex-balanced steady state $x^* \in \mathbb{R}_+^m$, then the open reaction network (23) is called *complex balanced*.

Note that, similar to the case of closed networks, in a complex-balanced steady state, the total inflow from every complex is equal to the total outflow from it.

The definition of a complex-balanced steady state x^* can be succinctly written as $Bv_e(x^*) = 0$, with B given by (25) and v_e given by (29). We have the following simple but crucial observation showing that complex-balanced steady states for (23) are actually complex-balanced equilibria of the extended network, and conversely.

Proposition 4.2: x^* is a complex-balanced steady state, i.e., $Bv_e(x^*) = 0$, if and only if $D_e v_e(x^*) = 0$.

Proof: Since $\mathbb{1}^T D_e = 0$ the last row of D_e is dependent on its first c rows, i.e., the rows of B . Hence, $v_e(x^*) \in \ker D_e$ if and only if $v_e(x^*) \in \ker B$. \square

If the network with constant inflows and mass action kinetics outflows has complex-balanced steady state x^* , then similarly to (5) for closed complex-balanced reaction networks, we define the diagonal matrix

$$\begin{aligned} \Xi_e(x^*) &:= \text{diag} \left(\exp(Z_i^T \text{Ln}(x^*)) \right)_{i=1, \dots, c+1} \\ &= \begin{bmatrix} \text{diag} \left(\exp(Z_i^T \text{Ln}(x^*)) \right)_{i=1, \dots, c} & 0 \\ 0 & 1 \end{bmatrix} =: \begin{bmatrix} \Xi(x^*) & 0 \\ 0 & 1 \end{bmatrix} \end{aligned}$$

and rewrite

$$D_e v_e(x) = -\mathcal{L}_e(x^*) \text{Exp} \begin{bmatrix} Z^T \text{Ln} \left(\frac{x}{x^*} \right) \\ 0 \end{bmatrix},$$

where

$$\mathcal{L}_e(x^*) := L_e \Xi_e(x^*) = \begin{bmatrix} (L + \Delta_{\text{out}}) \Xi(x^*) & l_{\text{in}} \\ l_{\text{out}} \Xi(x^*) & \delta_{\text{in}} \end{bmatrix}. \quad (33)$$

Note that $\text{Exp} \begin{bmatrix} Z^T \text{Ln} \left(\frac{x^*}{x^*} \right) \\ 0 \end{bmatrix} = \mathbb{1}_{c+1}$. Hence, the existence of a complex-balanced steady state x^* implies, by Proposition 4.2, that $\mathcal{L}_e(x^*) \mathbb{1}_{c+1} = 0$. Thus, $\mathcal{L}_e(x^*)$ satisfies $\mathbb{1}_{c+1}^T \mathcal{L}_e(x^*) = 0$, as well as $\mathcal{L}_e(x^*) \mathbb{1}_{c+1} = 0$, and hence defines a *balanced -weighted Laplacian matrix* for the extended graph of complexes. The fact that the sum of the elements of the last row of $\mathcal{L}_e(x^*)$ is zero amounts to the equality $l_{\text{out}} \Xi(x^*) \mathbb{1}_c + \delta_{\text{in}} = 0$, or equivalently,

$$l_{\text{out}} \text{Exp} (Z^T \text{Ln}(x^*)) = \mathbb{1}_c^T l_{\text{in}}, \quad (34)$$

which can be interpreted as a total *mass-balance* condition: at steady state, the total inflow in the reaction network is equal to the total outflow.

We obtain the following refined version of Proposition 2.4.

Theorem 4.3: Define $\mathcal{L}_e(x^*)$ as above. Then,

$$\gamma_e^T \mathcal{L}_e(x^*) \text{Exp}(\gamma_e) \geq 0 \quad (35)$$

for all γ_e , while equality holds if and only if $D_e^T \gamma_e = 0$. Furthermore, if γ_e has last component zero, i.e., is of the form

$$\gamma_e = \begin{bmatrix} \gamma \\ 0 \end{bmatrix}, \quad (36)$$

then equality holds if and only if $B^T \gamma = 0$, or equivalently

$$D^T \gamma = 0, D_{\text{in}}^T \gamma = 0, D_{\text{out}}^T \gamma = 0. \quad (37)$$

Proof: Only the last statement remains to be proved. This follows by noting that if γ_e is given as in (36), then $D_e^T \gamma_e = 0$ if and only if $B^T \gamma = 0$. \square

We obtain the following basic theorem extending and refining the results for closed networks to the considered subclass of open reaction networks.

Theorem 4.4: Consider a mass action kinetics reaction network with constant inflows and mass action kinetics outflows (23), for which there exists an $x^* \in \mathbb{R}_+^m$ satisfying (32). Then

(1) The set of positive steady states is given as

$$\{x^{**} \in \mathbb{R}_+^m \mid B^T Z^T \text{Ln}(x^{**}) = B^T Z^T \text{Ln}(x^*)\}, \quad (38)$$

and all positive steady states are complex balanced.

(2) If every component of the graph of complexes is connected to the zero complex (or equivalently, if the extended graph of complexes is connected), then the set of steady states is given as

$$\{x^{**} \in \mathbb{R}_+^m \mid Z^T \text{Ln}(x^{**}) = Z^T \text{Ln}(x^*)\}.$$

In particular, if additionally Z is surjective then the steady state x^* is unique.

(3) For every $x_0 \in \mathbb{R}_+^m$, there exists a unique $x_1 \in \mathcal{E}$ with $x_1 - x_0 \in \text{im } S$. The steady state x_1 is locally asymptotically stable with respect to initial conditions x_0 with $x_1 - x_0 \in \text{im } S$. Furthermore, if the network is persistent, then x_1 is globally asymptotically stable with respect to all these initial conditions.

Proof: (1) (38) follows from the characterisation of the set of equilibria of a closed network, cf. (8), since the transpose of the stoichiometric matrix for the extended network is given as $S_e^T = D_e^T Z_e^T = B^T Z^T$. That every positive steady state is complex balanced can be proved similar to the case of the closed networks case.

(2) Let x^{**} be a positive steady state, and define $\gamma(x^{**}) = Z^T \text{Ln} \left(\frac{x^{**}}{x^*} \right)$. By the first part of the theorem, this means that $B^T Z^T \text{Ln}(x^{**}) = B^T Z^T \text{Ln}(x^*)$, which is the same as $B^T \gamma(x^{**}) = 0$, or equivalently (37). In particular, $D_{\text{in}} \gamma(x^{**}) = 0$ and $D_{\text{out}} \gamma(x^{**}) = 0$, and thus the components of $\gamma(x^{**})$ corresponding to the complexes directly linked to the zero complex are zero. Furthermore, since $B^T \gamma(x^{**}) = 0$, it follows that the components of $\gamma(x^{**})$ corresponding to each of the connected components of the extended graph are equal. Hence, if the extended graph of complexes is connected, then $\gamma(x^{**}) = 0$, which is the same as $Z^T \text{Ln}(x^{**}) = Z^T \text{Ln}(x^*)$. In particular, if Z is surjective, then this implies that the steady state x^* is unique.

(3) This follows directly from the closed network case. \square

It can be concluded from Theorem 4.4 that the presence of inflows and outflows has the tendency to ‘shrink’ the set of positive equilibria for the closed network to a smaller set of positive steady states; in fact, to a singleton if the extended graph is connected and Z is surjective. Furthermore, as shown in the following proposition, if the extended graph is connected, no steady states can occur at the boundary of the positive orthant \mathbb{R}_+^m , implying that the reaction network is automatically *persistent*.

Proposition 4.5: *Consider a reaction network with constant inflows $v_{\text{in}} \in \mathbb{R}_+^k$ and mass action outflows (23), which is complex balanced. If the extended graph of complexes is connected, then there are no steady states at the boundary of \mathbb{R}_+^m .*

Proof: Assume by contradiction that there exists a steady state $x_b \in \mathbb{R}_+^m$ with at least one component (say the i th one) equal to zero. Then, consider a complex \mathcal{C} containing this i th species. Because $x_{bi} = 0$, the outflows from complex \mathcal{C} are zero, and by complex balancedness, this means that also all inflows to it are zero. From Remark 2.3, it follows that the extended graph of complexes is strongly connected. Hence, there exists a *directed* path of reactions Π starting from the zero complex and ending at \mathcal{C} . Now consider the complex which is preceding the complex \mathcal{C} in this path. Then, its outflows are zero, and therefore by complex balancedness also its inflows. Repeating this argument, this shows that along Π , the inflow from the zero complex is zero, which yields a contradiction. \square

Example 4.6: Consider the reaction network in Example 3.2 with the Laplacian matrix of the extended graph of complexes given as

$$L_e = \begin{bmatrix} k_+ & -k_- & -k_{\text{in}} \\ -k_+ & k_- + k_{\text{out}} & 0 \\ 0 & -k_{\text{out}} & k_{\text{in}} \end{bmatrix}$$

A complex-balanced steady state $x^* = (x_1^*, x_2^*, x_3^*)$ satisfies the equations

$$\begin{bmatrix} k_+ & -k_- \\ -k_+ & k_- + k_{\text{out}} \end{bmatrix} \begin{bmatrix} (x_1^*)^2 x_2^* \\ x_3^* \end{bmatrix} = \begin{bmatrix} k_{\text{in}} \\ 0 \end{bmatrix}$$

or more explicitly

$$x_3^* = \frac{k_{\text{in}}}{k_{\text{out}}}, \quad (x_1^*)^2 x_2^* = \frac{(k_- + k_{\text{out}})k_{\text{in}}}{k_+ k_{\text{out}}}.$$

Hence, the network is complex balanced if $k_{\text{in}} \neq 0$ and $k_{\text{out}} \neq 0$ (as well as in the degenerate case $k_{\text{in}} = k_{\text{out}} = 0$). The mass-balance condition (34) in this case amounts to

$$k_{\text{out}} x_3^* = k_{\text{in}}. \quad (39)$$

If $k_{\text{in}} \neq 0$ and $k_{\text{out}} \neq 0$, then the set of steady states of the network is one-dimensional. Note, on the other hand, that the set of equilibria for the case *without* inflows and outflows ($k_{\text{in}} = k_{\text{out}} = 0$) is two-dimensional; in line with the observation that the addition of inflows and outflows has the tendency to shrink the set of steady states as compared to the set of equilibria. Finally, the resulting matrix $\mathcal{L}_e(x^*)$ is given by

$$\mathcal{L}_e(x^*) = \begin{bmatrix} k_+(x_1^*)^2 x_2^* & -k_- x_3^* & -k_{\text{in}} \\ -k_+(x_1^*)^2 x_2^* & (k_- + k_{\text{out}})x_3^* & 0 \\ 0 & -k_{\text{out}} x_3^* & k_{\text{in}} \end{bmatrix}.$$

5. Structure-preserving model reduction of open reaction networks

As detailed in the previous section, the dynamics of a complex-balanced CRN with constant inflows and outflows governed by mass action kinetics can be written in terms of the balanced Laplacian matrix given by (33) as follows:

$$\dot{x} = -Z_e \mathcal{L}_e(x^*) \text{Exp} \left(Z_e^T \text{Ln} \left(\frac{x}{x^*} \right) \right). \quad (40)$$

This specific form allows for the application of the model reduction method discussed in Rao et al. (2014, 2013a); see also van der Schaft et al. (2013a) for the detailed-balanced case. This method is inspired by the Kron reduction method of resistive electrical networks described in Kron (1939); see also van der Schaft (2010). The speciality of this method is that it is structure-preserving in the sense that the reduced model corresponds to a complex-balanced CRN governed by mass action kinetics just like the original model. To make the paper self-contained, we briefly describe the method below. For a detailed description of the method, the reader is referred to Rao et al. (2014, 2013a).

Let \mathcal{V} denote the set of vertices of the extended graph of complexes (i.e., including the zero complex). We perform model reduction by *deleting certain complexes*, resulting in a reduced graph of complexes. We ensure that the set of complexes that are deleted does not include the zero complex. Deletion of a complex is equivalent to imposing the complex-balancing condition on it, i.e., the condition that the net inflow into the complex is equal to the net outflow from it. Consider a subset $\mathcal{V}_o \subset \mathcal{V}$ of dimension $c + 1 - \hat{c}$ that we wish to delete in order to reduce the model. Without the loss of generality, assume that the first \hat{c} rows and columns of $\mathcal{L}_e(x^*)$ and the first \hat{c} columns of Z_e correspond to $\mathcal{V}_r := \mathcal{V} \setminus \mathcal{V}_o$. Consider the resulting

partition of $\mathcal{L}_e(x^*)$ given by

$$\mathcal{L}_e(x^*) = \begin{bmatrix} \mathcal{L}_{11}(x^*) & \mathcal{L}_{12}(x^*) \\ \mathcal{L}_{21}(x^*) & \mathcal{L}_{22}(x^*) \end{bmatrix}, \quad (41)$$

where $\mathcal{L}_{11}(x^*) \in \mathbb{R}^{\hat{c} \times \hat{c}}$, $\mathcal{L}_{12}(x^*) \in \mathbb{R}^{\hat{c} \times (c+1-\hat{c})}$, $\mathcal{L}_{21}(x^*) \in \mathbb{R}^{(c+1-\hat{c}) \times \hat{c}}$, and $\mathcal{L}_{22}(x^*) \in \mathbb{R}^{(c+1-\hat{c}) \times (c+1-\hat{c})}$, and the resulting partition of Z_e given by $Z_e = [Z_1 \ Z_2]$. Then, write out the dynamics (40) as

$$\dot{x} = -[Z_1 \ Z_2] \begin{bmatrix} \mathcal{L}_{11}(x^*) & \mathcal{L}_{12}(x^*) \\ \mathcal{L}_{21}(x^*) & \mathcal{L}_{22}(x^*) \end{bmatrix} \begin{bmatrix} \text{Exp} \left(Z_1^T \text{Ln} \left(\frac{x}{x^*} \right) \right) \\ \text{Exp} \left(Z_2^T \text{Ln} \left(\frac{x}{x^*} \right) \right) \end{bmatrix}.$$

Consider now the auxiliary dynamical system

$$\begin{bmatrix} \dot{y}_1 \\ \dot{y}_2 \end{bmatrix} = - \begin{bmatrix} \mathcal{L}_{11}(x^*) & \mathcal{L}_{12}(x^*) \\ \mathcal{L}_{21}(x^*) & \mathcal{L}_{22}(x^*) \end{bmatrix} \begin{bmatrix} w_1 \\ w_2 \end{bmatrix}.$$

Note that the complex-balancing condition on the complexes in \mathcal{V}_o can be imposed by setting the constraint $\dot{y}_2 = 0$. This results in the equation

$$w_2 = -\mathcal{L}_{22}(x^*)^{-1} \mathcal{L}_{21}(x^*) w_1,$$

leading to the reduced auxiliary dynamics defined by the Schur complement

$$\begin{aligned} \dot{y}_1 &= -(\mathcal{L}_{11}(x^*) - \mathcal{L}_{12}(x^*) \mathcal{L}_{22}(x^*)^{-1} \mathcal{L}_{21}(x^*)) w_1 \\ &=: -\hat{\mathcal{L}}_e(x^*) w_1. \end{aligned} \quad (42)$$

Substituting $w_1 = \text{Exp} \left(Z_1^T \text{Ln} \left(\frac{x}{x^*} \right) \right)$ in the above equation and making use of $\dot{x} = Z_1 \dot{y}_1 + Z_2 \dot{y}_2 = Z_1 \dot{y}_1$, we then obtain the reduced model given by

$$\dot{x} = -\hat{Z}_e \hat{\mathcal{L}}_e(x^*) \text{Exp} \left(\hat{Z}_e^T \text{Ln} \left(\frac{x}{x^*} \right) \right), \quad (43)$$

where $\hat{Z}_e := Z_1$. The following proposition ensures that $\hat{\mathcal{L}}_e(x^*)$ obeys all the properties of the weighted Laplacian matrix of a complex-balanced reaction network corresponding to a graph of complexes with vertex set \mathcal{V}_r .

Proposition 5.1: *Consider an open complex-balanced network with dynamics given by equation (40). With \mathcal{V} , \mathcal{V}_o , and $\hat{\mathcal{L}}_e$ as defined above, the following properties hold:*

- (1) *All diagonal elements of $\hat{\mathcal{L}}_e(x^*)$ are positive and off-diagonal elements are non-negative.*
- (2) *$\mathbb{1}_{\hat{c}}^T \hat{\mathcal{L}}_e(x^*) = 0$ and $\hat{\mathcal{L}}_e(x^*) \mathbb{1}_{\hat{c}} = 0$, where $\hat{c} := c + 1 - \dim(\mathcal{V}_o)$.*

If \mathcal{E} and $\hat{\mathcal{E}}$ denote the set of steady states of the original and the reduced networks described by (40) and (43), respectively, then $\mathcal{E} \subseteq \hat{\mathcal{E}}$.

Proof: See proofs of Rao et al. (2013a, Propositions 5.1, 5.2) \square

From Proposition 5.1, it follows that the reduced network (43) corresponding to a complex balanced network (40) is also complex balanced. Complexes belonging to a certain connected component remain in the same connected component if not deleted. Thus, mass conservation is preserved under our model reduction procedure.

Finally, we remark that for the application of our model reduction method, one can also start directly from the form of equations (30) given by $\dot{x} = Z_e L_e \text{Exp} \left(Z_e^T \text{Ln} \left(\frac{x}{x^*} \right) \right)$, instead of the form (40) that uses the balanced Laplacian. Let \hat{L}_e denote the Schur complement of L_e with respect to the indices corresponding to \mathcal{V}_o . Consider the reduced model given by

$$\dot{x} = \hat{Z}_e \hat{L}_e \text{Exp} \left(\hat{Z}_e^T \text{Ln} \left(\frac{x}{x^*} \right) \right)$$

and note that it is the same as the reduced model (43).

6. Conclusions and outlook

We have discussed mass action kinetics CRNs as a challenging example of large-scale and nonlinear network dynamics, and have pointed out similarities with (non-linear versions of) consensus dynamics. A fundamental difference resides in the complex composition matrix Z , which defines a representation of the graph of complexes (into the space of chemical species). Kirchhoff's matrix tree theorem has been discussed as an insightful way to compute the kernel of the Laplacian matrix, which, among others, yields an explicit characterisation of the existence of a complex-balanced equilibrium. Also, the relation to mass conservation has been pointed out.

For a particular class of open reaction networks, namely those with constant inflows and mass action kinetics outflows, a detailed stability analysis has been obtained by exploiting the notion of zero complex. By using the graph-theoretical techniques that we have used earlier to analyse closed complex-balanced reaction networks, this leads to a complete steady-state stability analysis. Our results imply the intuitively obvious fact that the presence of inflows and outflows has the tendency to shrink the set of positive equilibria to a smaller set of positive steady states, and leads to the preclusion of possible steady states at the boundary of the positive orthant. This can be related to the feedback stabilisation problem studied in Sontag (2001), as well as to internal model control.

An important extension of our results concerns the consideration of other types of kinetics, in particular, Michaelis-Menten kinetics; see already (Jayawardhana, Rao, & van der Schaft, 2012) for the closed network case. Furthermore, the framework described in this paper can serve as a starting point for the inclusion of *regulatory*

networks, thus leading to control and ‘reverse engineering’ questions.

Notes

1. Recently, also in Craciun and Feinberg (2010), the idea of adding a zero complex was followed up; however in the different context of preclusion of multi-stability.
2. Sometimes called a *multigraph*, since we allow for multiple edges between vertices.
3. In the literature sometimes also referred to as *reaction graphs*.
4. In van der Schaft et al. (2013a), Rao et al. (2013a), the matrix Z was called the ‘complex stoichiometric matrix’.
5. In Chapman and Mesbahi (2011), such a matrix L was called an *out-degree* Laplacian matrix.
6. In the special case $\text{im } D \cap \ker Z = \{0\}$ (*deficiency zero* in the terminology of Feinberg (1972)), complex-balancedness is equivalent to the existence of a positive equilibrium of (3).
7. Strong connectedness of the connected components is in the CRN literature often referred to as *weak reversibility* (Horn, 1972)
8. It is generally believed that most reaction networks are persistent. However, up to now, this *persistence conjecture* has been only proved in special cases (cf. Anderson (2011), Angeli, De Leenheer, and Sontag (2011), Siegel and MacLean (2000), and the references quoted in there).
9. See e.g. Oster et al. (1973), van der Schaft et al. (2013a), van der Schaft et al. (2013b) for further information.
10. This theorem goes back to the classical work of Kirchhoff on resistive electrical circuits (Kirchhoff, 1847); see Bollobas (1998) for a succinct treatment. Nice accounts of the matrix tree theorem in the context of CRNs can be found in Mirzaev and Gunawardena (2013), Gunawardena (2014).
11. Thermodynamically the assumption of detailed-balancedness is well-justified; it corresponds to microscopic reversibility (Oster et al., 1973).
12. It can be shown (van der Schaft et al., 2013a) that the matrix $\mathcal{K}^*(x^*)$ is *independent* of the choice of the thermodynamic equilibrium x^* up to multiplicative factor for every connected component of \mathcal{H} .
13. As shown in van der Schaft et al. (2015) the weakened Wegscheider conditions are also equivalent to the notion of *formal balancing* introduced in Dickenstein and Perez Millan (2011) as a formalization of the ‘circuit conditions’ of Feinberg (1989).

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Appendix. Detailed-balanced reaction networks

The assumption of existence of a complex-balanced equilibrium can be strengthened to the existence of a *detailed-balanced* equilibrium. In this case, we start with a directed graph of complexes \mathcal{H} with c complexes and r edges, where each edge corresponds to a *reversible* reaction, cf. van der Schaft et al. (2013a). Assuming again mass action kinetics, the reaction rate $v_j^r(x)$ of each j th reversible reaction is given as the difference

$$v_j^r(x) = k_j^+ \exp(Z_{S_j}^T \text{Ln } x) - k_j^- \exp(Z_{P_j}^T \text{Ln } x),$$

where S_j is the substrate and P_j the product complex, and where k_j^+ and k_j^- are respectively the *forward* and *reverse* reaction constants of the reversible reaction. Note that $v_j^r(x)$ may take positive and negative values, in contrast with the previously considered case of irreversible reaction rates $v_j(x) \geq 0$. A reversible reaction network can be brought into the irreversible form as discussed before by defining the directed graph \mathcal{G} as having the same vertex set as \mathcal{H} but with twice as many edges: every edge (i, j) of \mathcal{H} is split into two edges (of opposite orientation) (i, j) and (j, i) of \mathcal{G} .

A reversible mass action kinetics reaction network with graph of complexes \mathcal{H} is called *detailed balanced* if

there exists an $x^* \in \mathbb{R}_+^m$ satisfying $v^r(x^*) = 0$, i.e.

$$k_j^+ \exp\left(Z_{S_j}^T \text{Ln}(x^*)\right) = k_j^- \exp\left(Z_{P_j}^T \text{Ln}(x^*)\right), \quad j = 1, \dots, r$$

It is immediate that detailed balancedness is a special case of complex balancedness, with the reaction rates in the two opposite edges of \mathcal{G} corresponding to a single edge of \mathcal{H} being equal¹¹ (instead of having the total sum of inflows to be equal to the total sum of outflows for every complex.)

Defining the *equilibrium constants* $K_j^{\text{eq}} = \frac{k_j^+}{k_j^-}$ of each reversible reaction, and the vector $K^{\text{eq}} := (K_1^{\text{eq}}, \dots, K_r^{\text{eq}})^T$, it can be shown (Feinberg, 1989; Schuster & Schuster, 1989; van der Schaft et al., 2013a) that detailed balancedness is equivalent to the Wegscheider conditions

$$\text{Ln } K^{\text{eq}} \in \text{im } D_{\mathcal{H}}^T Z^T = \text{im } S_{\mathcal{H}}^T,$$

with $D_{\mathcal{H}}$ the incidence matrix of the graph \mathcal{H} , and $S_{\mathcal{H}}$ the stoichiometric matrix corresponding to \mathcal{H} (i.e., every column of $S_{\mathcal{H}}$ corresponds to a *reversible reaction*). The assumption of detailed balancedness implies that *all* equilibria are actually detailed balanced, and that we may define the *conductances* of the j th reversible reaction as

$$\kappa_j(x^*) := k_j^+ \exp\left(Z_{S_j}^T \text{Ln}(x^*)\right) = k_j^- \exp\left(Z_{P_j}^T \text{Ln}(x^*)\right), \quad j = 1, \dots, r \quad (44)$$

$$\begin{aligned} \mathcal{L} &= \begin{bmatrix} k_1 + k_6 & -k_2 & -k_5 \\ -k_1 & k_2 + k_3 & -k_4 \\ -k_6 & -k_3 & k_4 + k_5 \end{bmatrix} \begin{bmatrix} k_3 k_5 + k_2 k_5 + k_2 k_4 & 0 & 0 \\ 0 & k_1 k_5 + k_1 k_4 + k_4 k_6 & 0 \\ 0 & 0 & k_1 k_3 + k_3 k_6 + k_2 k_6 \end{bmatrix} \\ &= \begin{bmatrix} (k_1 + k_3)(k_3 k_5 + k_2 k_5 + k_2 k_4) & -k_2(k_1 k_5 + k_1 k_4 + k_4 k_6) & -k_5(k_1 k_3 + k_3 k_6 + k_2 k_6) \\ -k_1(k_3 k_5 + k_2 k_5 + k_2 k_4) & (k_2 + k_3)(k_1 k_5 + k_1 k_4 + k_4 k_6) & -k_4(k_1 k_3 + k_3 k_6 + k_2 k_6) \\ -k_6(k_3 k_5 + k_2 k_5 + k_2 k_4) & -k_3(k_1 k_5 + k_1 k_4 + k_4 k_6) & (k_4 + k_5)(k_1 k_3 + k_3 k_6 + k_2 k_6) \end{bmatrix}. \end{aligned}$$

(See Ederer & Gilles, 2007; van der Schaft et al., 2013b for a discussion regarding the similarities of these constants with conductances in other physical networks.) It is readily seen that the detailed-balanced assumption is equivalent to the transformed Laplacian matrix $\mathcal{L}(x^*) := L \Xi(x^*)$ being *symmetric*, with the (i, j) th $= (j, i)$ th element being equal to the conductance of the reversible reaction between the i th and the j th complex. This means that $\mathcal{L}(x^*)$ can be written as

$$\mathcal{L}(x^*) = D_{\mathcal{H}} \mathcal{K}^r(x^*) D_{\mathcal{H}}^T,$$

where¹² $\mathcal{K}^r(x^*)$ is the diagonal matrix of conductances $\kappa_j(x^*)$, $j = 1, \dots, r$. Hence (van der Schaft et al., 2013a),

the dynamics takes the form

$$\dot{x} = -Z D_{\mathcal{H}} \mathcal{K}^r(x^*) D_{\mathcal{H}}^T \text{Exp}\left(Z^T \text{Ln}\left(\frac{x}{x^*}\right)\right).$$

For $Z = I$, this amounts to *symmetric* consensus dynamics on the graph \mathcal{H} *without* its orientation.

Finally we will indicate the connections of the Wegscheider conditions mentioned above to the characterisation of complex balancedness as obtained by Kirchhoff's matrix tree theorem. For further details, we refer to van der Schaft et al. (2015). Consider a complex-balanced reaction network, with Laplacian matrix $L = -DK$. Based on Kirchhoff's matrix tree theorem, compute the vector $\rho \in \mathbb{R}_+^c$ satisfying $L\rho = 0$, leading to the transformed balanced Laplacian matrix \mathcal{L} given as $\mathcal{L} = L \text{diag}(\rho_1, \dots, \rho_c)$. In case the reaction network is *detailed balanced*, it follows that this transformed Laplacian matrix \mathcal{L} is actually *symmetric*; instead of just balanced. Symmetry of \mathcal{L} can be seen to be equivalent to the *weakened* Wegscheider conditions¹³ (only depending on the structure of the graph of complexes, and *not* on the composition of the complexes)

$$\text{Ln } K^{\text{eq}} \in \text{im } D_{\mathcal{H}}^T$$

Example A.1: Consider the network described in Example 2.10. The transformed Laplacian matrix is computed as

This matrix is symmetric if and only if

$$k_1 k_3 k_5 = k_2 k_4 k_6.$$

On the other hand, $\text{Ln } K^{\text{eq}} \in D_{\mathcal{H}}^T$ amounts to

$$\begin{bmatrix} \ln \frac{k_1}{k_2} \\ \ln \frac{k_3}{k_4} \\ \ln \frac{k_5}{k_6} \end{bmatrix} \in \text{im} \begin{bmatrix} -1 & 0 & 1 \\ 1 & -1 & 0 \\ 0 & 1 & -1 \end{bmatrix},$$

which reduces to $\ln \frac{k_1}{k_2} + \ln \frac{k_3}{k_4} + \ln \frac{k_5}{k_6} = 0$, and hence to the same condition $k_1 k_3 k_5 = k_2 k_4 k_6$.