Chemical Reaction Network Theory

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1 Introduction

There is a theory of the modelling of chemical reactions which was initiated at the beginning of the 1970's by Martin Feinberg, Fritz Horn und Roy Jackson. Since then it has continued to be developed further, with Feinberg playing a central role. It is known under the name 'Chemical Reaction Network Theory' and the abbreviation CRNT. In this course essential aspects of this theory will be presented. There are other interesting mathematical aspects of chemical reactions but here the focus is on CRNT. The classic text in this area is a set of lecture notes by Feinberg from 1980 [8]. These notes are very good although of course no longer up to date, since there have been many further developments since then. In my text the first sections are largely based on the text of Feinberg. Another good source are the lecture notes of Gunawardena [14].

In chemistry and biology we are often confronted with systems of chemical reactions which have certain problematic features. They are often very large, i.e. very many substances are involved. It is often the case that we do not even know all the substances involved. In addition little is known about the mechanisms of the reactions involved. In CRNT it is usual to assume that the law of mass action holds. This assumption is often not very realistic but it has the advantage of simplicity. In this course we will mainly work with this assumption. Even with this assumption the problem remains that there is a reaction constant for each reaction which is often known only very approximately or even not at all. With this background the main goal of CRNT is to obtain results which depend as little as possible, and in the best case not at all, on the precise values of the reaction constants.

What kind of systems do we want to model? First we consider chemical reactors of the type used in the chemical industry. One type is the discontinuous stirred tank reactor. Chemical substances are put into a container and stirred vigorously until the concentrations approach a stationary state. If this is done in a suitable way then at the end of the process there is a product in the container which can be used. Then the container can be emptied and the process repeated. In this context the question arises, whether a stationary state must be reached. This question will concern us later. If the external conditions (e.g. temperature, pressure) are held constant we call this a closed system. The second important

type of reactor is the continuous flow stirred tank reactor. In that case the container is connected to two pipes. A mixture with constant composition is introduced through one pipe at constant rate and material is removed through the other pipe with constant rate. In this way products can be obtained with constant rate. We call this an open system. CRNT can describe both closed and open systems. In biology many processes are described by chemical reactions whereby it must be assumed that the system is open. The container is replaced in this case by a living cell or another unit (such as a chloroplast).

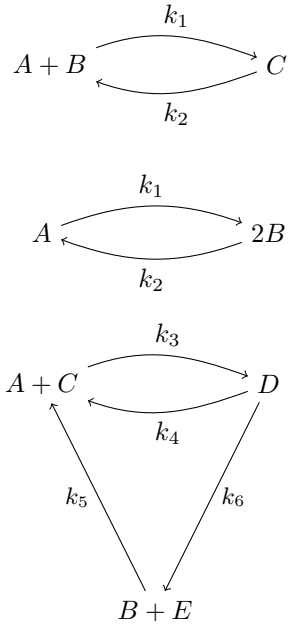
CRNT is concerned with the study of the solutions of ordinary differential equations where the unknowns are the concentrations of the substances involved as functions of time. Why are we allowed to assume that the system can be described by concentrations? In order to do this it is necessary to suppose that enough molecules of each substance are present. Otherwise it would be necessary to use a stochastic description. In an industrial reactor this condition should be comfortably satisfied. In a living cell the situation could be different. A bacterium has a volume of about 1 fl. There are not always many molecules of all substances which are to be modelled present. In concrete problems this question must be posed. If it has been assumed that the continuum description using concentrations is sufficient the question remains why it is not necessary to take the spatial variation of the concentrations into account. Otherwise it would be necessary to model diffusion and we would end up with partial differential equations (reaction diffusion equations). In a chemical reactor the stirring serves to remove possible spatial gradients. The reactor will also be built in such a way that inhomogeneous hydrodynamical effects do not play a role. In a biological cell the reactants are not stirred but a fact which we thought of as a disadvantage, the small dimensions of the cell, can be an advantage for the question under consideration here. The effects of diffusion propagate so fast in regions of such a small size that concentrations immediately become homogeneous. Spatial gradients certainly cannot always be ignored in applications to cell biology but in many cases they can. It is also the case that on these scales the fluid behaves as if it were extremely viscous, so that hydrodynamical effects can be ignored. From this point on we will only consider models given by ordinary differential equations.

Now we will start to formulate the description of the reactions mathematically. Let $\mathcal{S}$ be a finite set, the set of chemical species. It is often the case in practice that not all substances actually present can be included in the model. $\mathcal{S}$ is then the set of substances which are in the model and for clarity we call them internal species. There are often substances whose concentrations are not modelled because their concentrations are not significantly affected by those of the internal species. We call them external species. For instance it is the case that many reactions take place in an aqueous solution. Water is very important for these reactions but they neither consume nor produce water. It is possible to make the idealization that the concentrations of external species are constant. These constant concentrations are then often absorbed in the reaction constants so that they are not visible in the model.

The next element of the description consists of the reactions themselves.

Consider for instance the reaction $2H_2 + O_2 \to 2H_2O$. This formula can be thought of as a summary of the reaction which takes place in the combustion of hydrogen. This reaction is in reality much more complicated with many steps where, for instance, hydrogen radicals play a role. In this course we will not enter into these complications. We simply consider as an example a reaction of the form $2A + B \to 2C$ with substances A, B and C. Two molecules of A come together with one molecule of B and two molecules of C are produced. In reality it is very unlikely that more than two molecules come close to each other at one time as in this scenario, but this point will not be considered further here. If $\mathcal{S}$ is given a complex is a formal linear combination of elements of $\mathcal{S}$ with integer coefficients. It can also be considered as a function on $\mathcal{S}$ with values in $\mathbb{R}$. The set of complexes in the network is denoted by $\mathcal{C}$. The case we have just considered is that where the values of this function are non-negative integers but it turns out that more general cases are also of interest. We will often denote a complex by y and its value at the point with index i by y_i. In chemistry the numbers y_i are called stoichiometric coefficients. The complexes in a reaction network form a finite subset $\mathcal{C}$ of the vector space $F(\mathcal{S})$ of real-valued functions on $\mathcal{S}$ with pointwise addition. In chemistry this is not the only meaning of the word complex but when we use the word complex without further qualification then the meaning just introduced is what is intended. The space $F(\mathcal{S})$ has a natural basis which is given by the characteristic functions of the points of $\mathcal{S}$. The characteristic function of the point with index i is denoted by ω_i. In this way the vector space can be identified with $\mathbb{R}^m$, where m is the number of elements of $\mathcal{S}$. In the example introduced above $m = 3$ and the complexes are represented by the vectors $[2, 1, 0]^T$ and $[0, 0, 2]^T$. A reaction is an ordered pair of complexes. The first element is the left hand side with the substances which go into the reaction (often called educts or reactants) and the second with the products of the reaction. The reaction which transforms y into y' can also be thought of a function on $\mathcal{C}$ with the value -1 at the point y and the value $+1$ at the point y'. The space $F(\mathcal{C})$ of real-valued functions on $\mathcal{C}$ also has a natural basis given by characteristic functions and can be identified with $\mathbb{R}^n$, where n is the number of complexes. Because the reactions which occur in a model are often a combination of several elementary reactions it is useful to extend the definition of complexes to be non-negative real-valued functions on $\mathcal{S}$ which do not have to take their values in the integers. In this course we will use this extended definition. The function which is identically zero also defines a complex which is often denoted by 0. A reaction where the left hand side is 0 is a source, where certain substances are introduced into the system. One where the right hand side is 0 is a sink where certain substances are removed from the system. In a continuous flow stirred tank reactor reactions of these type always occur. They can also have other interpretations. For instance the actual reaction is $A \to B$, where A is an external species and only the internal species B is visible in the model. The space $F(\mathcal{R})$ of functions on $\mathcal{R}$ can be identified with $\mathbb{R}^r$, where r is the number of reactions.

A reaction network is a finite set $\mathcal{S}$ of species together with a finite set $\mathcal{C}$ of complexes where the species they contain belong to $\mathcal{S}$ and a finite set $\mathcal{R}$ of



reactions where the complexes they contain belong to $\mathcal{C}$. To avoid trivialities we assume that there is at least one species, that each species occurs in at least one complex, that each complex occurs in at least one reaction and that the two complexes in a reaction are always distinct. It is possible to associate a directed graph to any reaction network. The nodes of the graph are the complexes and there is a directed edge from y to y' precisely when the reaction $y \to y'$ belongs to the network.

These concepts will now be illustrated using some simple examples. Example 1 describes the combination of two substances A and B to form a substance C and the dissociation of C where A and B are released. Example 2 consists of the reactions $A \to C$, $B \to C$ and $C \to A+B$. In both cases the set of species is $\{A, B, C\}$. In the first case the set of complexes is $\{A+B, C\}$ and in the second case it is $\{A, B, C, A + B\}$. In Example 1 we have $m = 3$, $n = 2$ and $r = 2$ and in Example 2 $m = 3$, $n = 4$ and $r = 3$. In addition we consider a network which is used in [8] and [14] and which we call Example 3. In this case the set of species is $\{A, B, C, D, E\}$ and the set of complexes is $\{A, 2B, A+C, D, B+E\}$. We have $m = 5$, $n = 5$ und $r = 6$.

A path from y to y' is a sequence $y^{(i)}$, $0 \leq i \leq k$ of complexes with the properties that $y^{(0)} = y$, $y^{(k)} = y'$ and for each i either $y^{(i)} \to y^{(i+1)}$ or $y^{(i+1)} \to y^{(i)}$ belongs to $\mathcal{R}$. If $y^{(i)} \to y^{(i+1)}$ is always in $\mathcal{R}$ the path is called directed. The trivial case $k = 0$ is allowed. The condition that a path from y to y' exists defines an equivalence relation. In CRNT the corresponding equivalence classes are called linkage classes. In the standard terminology of graph theory they are called connected components. The number of linkage classes is denoted by l.

A network is called reversible if the fact that $y \to y'$ is in the network implies that $y' \to y$ is in the network. It is called weakly reversible if the existence of a directed path from y to y' implies the existence of a directed path from y' to y. In general we can define an equivalence relation by saying that y and y' are equivalent iff there is both a directed path from y to y' and one from y' to y. In CRNT the corresponding equivalence classes are called strong linkage classes. Every strong linkage class is contained in a linkage class and the strong linkage classes coincide with the linkage classes precisely when the network is weakly reversible. A strong linkage class is called terminal when there is no reaction from a complex in this class to a complex in a different strong linkage class. The number of terminal strong linkage classes is denoted by t. In each linkage class there is at least one terminal strong linkage class. If every strong linkage class is terminal then the network is weakly reversible.

With the help of the natural bases it is possible to identify linear mappings between spaces of functions on finite sets with matrices. These mappings can be fixed by specifying the images under the mapping of the characteristic functions of points. In what follows we will usually not distinguish between these matrices and the corresponding linear mappings. An example is the complex matrix Y of a reaction network which is defined by the condition that the entries of a column are the stoichiometric coefficients of a certain complex. This matrix is $m \times n$ and satisfies $Y\omega_y = y$. In Example 3 it is

$$\begin{bmatrix} 1 & 0 & 1 & 0 & 0 \\ 0 & 2 & 0 & 0 & 1 \\ 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 1 \end{bmatrix} \tag{1}$$

Another matrix I_a is defined as follows. The rows of this matrix correspond to the complexes and the columns to the reactions. The entry with indices (i, j) is -1 when the complex i is on the left hand side of the reaction j, $+1$ when the complex i is on the right hand side of the reaction j and otherwise zero. This matrix is $n \times r$. In the stoichiometric matrix N the entry with indices (i, j) is the net production of the substance i in the reaction j. This matrix is $m \times r$ and its rank is denoted by s. A column of this matrix contains the net rates of production of the different substances in a particular reaction. It can easily be seen that in Examples 1 and 2 this matrix has rank 1 and 3 respectively. In Example 3 this matrix is

$$\begin{bmatrix} -1 & 2 & -1 & 1 & 1 & 0 \\ 2 & -1 & 0 & 0 & -1 & 1 \\ 0 & 0 & -1 & 1 & 1 & 0 \\ 0 & 0 & 1 & -1 & 0 & -1 \\ 0 & 0 & 0 & 0 & -1 & 1 \end{bmatrix} \tag{2}$$

and $s = 3$. It follows from the definitions that $N = YI_a$.

A reaction network as defined here describes which substances react with which others but not how the reaction takes place. This last point is described

by the choice of a kinetics. For this a real-valued function $v_{yy'}(c)$ is fixed for each reaction $y \to y'$. This function should be non-negative and not identically zero. It describes the rate of the reaction when the concentrations are given by c. The time evolution of the system is then described by the system

$$\frac{dc_i}{dt} = \sum_{yy' \in \mathcal{R}} v_{yy'}(c)(y'_i - y_i). \tag{3}$$

The right hand side can be written in the form Nv and it follows that the sets of the form $c + \mathrm{im}N$ are invariant under the time evolution. These sets are called stoichiometric compatibility classes. In Example 2 there is only one class of this type. In Examples 1 and 3 they have codimension 2.

As has already been mentioned the kinetics which is most often used is that which follows from the law of mass action. This rule says that the reaction rate is proportional to the power of the concentration of a substance which is equal to the number of molecules of this substance which goes into the reaction. In other words

$$v_{yy'}(c) = k_{yy'} \prod_{s=1}^{m} c_i^{y_i}. \tag{4}$$

With the abbreviation $c^y = \prod_{i=1}^m c_i^{y_i}$ we get the evolution equations

$$\frac{dc_i}{dt} = \sum_{yy' \in \mathcal{R}} k_{yy'} c^y (y'_i - y_i). \tag{5}$$

Here it is assumed that the coefficients $k_{yy'}$ are all positive. An alternative is to sum over all pairs of complexes and to set $k_{yy'} = 0$ for the reactions which are not contained in the network under consideration. The result is

$$\frac{dc_i}{dt} = \sum_{(y,y') \in \mathcal{C} \times \mathcal{C}} k_{yy'} c^y (y'_i - y_i) \tag{6}$$

The right hand side of this equation consists of polynomials if the stoichiometric coefficients are integers. Otherwise it consists of generalized polynomials, i.e. linear combinations of products of positive powers of the coordinates. In Example 3 the equations with mass action are as follows

$$\frac{dc_A}{dt} = -k_1 c_A + k_2 c_B^2 - k_3 c_A c_C + k_4 c_D + k_5 c_B c_E, \tag{7}$$

$$\frac{dc_B}{dt} = 2k_1 c_A - 2k_2 c_B^2 - k_5 c_B c_E + k_6 c_D, \tag{8}$$

$$\frac{dc_C}{dt} = -k_3 c_A c_C + k_4 c_D + k_5 c_B c_E, \tag{9}$$

$$\frac{dc_D}{dt} = k_3 c_A c_C - (k_4 + k_6) c_D, \tag{10}$$

$$\frac{dc_E}{dt} = -k_5 c_B c_E + k_6 c_D. \tag{11}$$

The right hand side of the evolution equations which is now denoted by $f(c)$ can be rewritten as follows. We define a mapping Ψ from functions on $\mathcal{S}$ to functions on $\mathcal{C}$ by $\Psi(c) = \sum_{y \in \mathcal{C}} c^y \omega_y$ and a linear mapping A_k from functions on $\mathcal{C}$ to functions on $\mathcal{C}$ by $A_k(x) = \sum_{\mathcal{R}} k_{yy'} x_y (\omega_{y'} - \omega_y)$. In Example 3 the matrix A_k is

$$\begin{bmatrix} -k_1 & k_2 & 0 & 0 & 0 \\ k_1 & -k_2 & 0 & 0 & 0 \\ 0 & 0 & -k_3 & k_4 & k_5 \\ 0 & 0 & k_3 & -k_4 - k_6 & 0 \\ 0 & 0 & 0 & k_6 & -k_5 \end{bmatrix}. \tag{12}$$

Here we can observe certain properties which the matrix A_k always has. The diagonal entries are negative, the off-diagonal entries are positive and the sum of each column vanishes. The quantity f can be written as the composition $f = Y A_k \Psi$. The relation $I_a v = A_k \Psi$ also holds. We see that it is possible to obtain the mapping f as a composition of three mappings, two of them linear. It is presumably this hidden linear structure which is responsible for many of the special properties of the systems of ordinary differential equations which are defined by reaction networks.

When studying the qualitative properties of the solutions of a dynamical system stationary solutions, i.e. those which are independent of time, play an important role. Here they are the solutions of the equation $f(c) = 0$ and the number of solutions of this type is a central theme in CRNT. The solutions which are of direct interest for the applications are those which are positive, i.e. those for which $c_i > 0$ for all i. Let $g(c) = A_k \Psi(c)$. Then $f(c) = Y g(c)$ and a special class of stationary solutions are those with $g(c) = 0$. These solutions are called 'complex balanced'. Note that

$$A_k \Psi = \sum_{\mathcal{R}} k_{yy'} c^y (\omega_{y'} - \omega_y) = \sum_{\mathcal{R}} (k_{yy'} c^y - k_{y'y} c^{y'}) \omega_{y'}. \tag{13}$$

An even more special class are the solutions for which $k_{yy'} c^y - k_{y'y} c^{y'} = 0$ for all complexes y and y'. These solutions are called 'detailed balanced'. This last class of reactions used to be very popular in chemistry. It was an essential insight of CRNT that more general stationary solutions can also be very useful for chemistry. Detailed balanced solutions are only possible in reaction networks where all reactions are reversible.

A quantity of central importance in CRNT is the deficiency. Let δ' be the dimension of the vector space $\ker Y \cap \mathrm{im} A_k$. This is evidently a non-negative integer. The quantity $g(u)$ is contained in $\mathrm{im} A_k$. Hence for a system with $\delta' = 0$ every stationary solution is complex balanced. The deficiency is defined to be δ' in [14]. The usual definition in the literature, which is also the one used in this course, is $\delta = n - l - s$. The inequality $\delta \geq \delta'$ always holds but the two quantities are in general not equal. In the weakly reversible case they are equal. These statements will be proved later. The values of the deficiency in Examples 1-3 are $\delta = 2 - 1 - 1 = 4 - 1 - 3 = 5 - 2 - 3 = 0$. The best known theorem of CRNT concerns the case where the deficiency δ is zero. In this course we

will first prove this Deficiency Zero Theorem and then consider generalizations where the deficiency is allowed to be larger. Briefly, the statement is that for $\delta = 0$ in the weakly reversible case there is exactly one stationary solution in each stoichiometric compatibility class and that for systems with $\delta = 0$ which are not weakly reversible there are no positive stationary solutions. In addition, the stationary solutions in the weakly reversible case are asymptotically stable within their class.

What is the significance of such results for chemistry and, in particular, for biology? In these areas it is often implicitly assumed that a system approaches a unique equilibrium state. This is, however, in practice not always the case. The presence of several stationary solutions to which a system might converge is of importance for the description of cellular differentiation. Stem cells are the ultimate source of all cells. Cells reach their final state through many generations and in this process the nature of the cell changes. It chooses, as it were, between different states. In other cases the system does not converge at all but exhibits sustained oscillations. A striking example is that of biological clocks. The Deficiency Zero Theorem and other related results provide criteria with which it is sometimes possible to decide whether the long-time behaviour of a system shows the simplest pattern or whether it is more complicated.

A class of reaction networks with deficiency zero are the monomolecular networks. In that case each complex of the network consists of a molecule of one species. Then there is a one-to-one correspondence between complexes and particular species. The linkage classes can be identified with disjoint subsets of $\mathcal{S}$. The rank s is the sum of contributions s_i of the linkage classes, since the complexes from different linkage classes are linearly independent. Let n_i be the number of complexes in the linkage classes. Then the deficiency is given by $n - l - s = \sum_i (n_i - 1 - s_i)$. It turns out that the summands vanish so that the deficiency also vanishes. To prove this we consider a given linkage class and the corresponding set of species $\mathcal{S}_i$. If we add a new reaction between species in $\mathcal{S}_i$ then neither n_i nor the dimension s_i of the space spanned by the functions $\omega_{y'} - \omega_y$ changes. Thus $n_i - 1 - s_i$ is preserved. In order to compute this quantity we can replace the original network by the extended network where there is a reaction between each pair of elements. The values of a function in the space under consideration always have the sum zero, which implies that the dimension is at most $n_i - 1$. We can, however, find $n_i - 1$ reactions in the extended network which are linearly independent. Let the elements of $\mathcal{S}_i$ in some order be $y^{(1)}, \ldots, y^{(n_i)}$. Then the functions $\omega_{y^{(j+1)}} - \omega_{y^{(j)}}$ are linearly independent for $1 \leq i \leq n_i - 1$. The argument in which an extra reaction is added is independent of the fact that the network is monomolecular. It follows that when two networks have the same species, the same complexes and the same linkage classes they have the same deficiency. Moreover, if no species occurs in more than one linkage class then the relation $s = \sum_i s_i$ holds.

Networks of monomolecular reactions have many applications in biology. One example is that of the phosphorylation systems. We have a protein, which is a chain of amino acids. For some of these amino acids a phosphate group can be joined to them. The sites which are occupied by phosphate groups can be used to

store information. A concrete example is a protein called NFAT (nuclear factor of activated T cells) [19]. This protein occurs in certain white blood cells, for instance in T cells. In a resting cell this protein is in the cytosol, in other words it is outside the nucleus of the cell, and it is equipped with thirteen phosphate groups. When the cell is activated an enzyme called calcineurin becomes active and removes the phosphate groups. This causes a conformational change in the protein which leads to the protein being moved to the nucleus. There it can interact with the DNA and act as a transcription factor. The phosphate groups can later be attached to the protein again. These groups are removed in a certain order and attached in the reverse order. The protein exists in many different forms. The number of phosphate groups can be anything between 0 and 13. It can be in two different conformations. It can be in the cytosol or in the nucleus. The transport processes can be formally considered as reactions, where the substance in the two spatially separated regions is treated as different. This situation can be modelled by a system of ordinary differential equations with mass action kinetics. There are 56 unknowns and 134 parameters for the corresponding number of reactions. The second number is dependent on the assumptions that the rates of the reactions relating the two conformations are the same in the cytosol and in the nucleus and that the rate of transport between the two compartments is the same for all forms of the protein. Despite these large numbers it is easy, with the help of CRNT, to obtain information about the dynamics. This is a monomolecular system and thus of deficiency zero. It is reversible. Hence the Deficiency Zero Theorem can be applied to it to see that the dynamics is very simple.

A similar example is that of kinetic proofreading which was analysed in [21]. It is again concerned with T cells. On the surface of a T cell there is a molecule called the T cell receptor, which we denote by T. This receptor can bind to a certain substance, which we denote by M. (It is a protein which is bound to an MHC molecule.) The binding reaction is of the form $T + M \to C_0$. The bound receptor C_0 can be equipped with up to N phosphate groups to produce compounds C_i. Each C_i can decay, releasing T, M and the phosphate groups. Since the phosphate groups are not modelled explicitly we get the reaction $C_i \to T + M$. The network is weakly reversible. There are $N + 2$ complexes. To show that $\delta = 0$ it thus suffices to show that $s = N + 1$. To give an intuitive idea why this is the case we show the stoichiometric matrix in the case $N = 2$.

$$\begin{bmatrix} -1 & 0 & 0 & 1 & 1 & 1 \\ -1 & 0 & 0 & 1 & 1 & 1 \\ 1 & -1 & 0 & -1 & 0 & 0 \\ 0 & 1 & -1 & 0 & -1 & 0 \\ 0 & 0 & 1 & 0 & 0 & -1 \end{bmatrix} \tag{14}$$

In general the first $N+1$ columns are linearly independent and all other columns can be written as linear combinations of the first $N + 1$ columns.

2 Background from the theory of ordinary differential equations

Consider a system of the form $\dot{x} = f(x)$, where x is a mapping from an interval $I \subset \mathbb{R}$ to $\mathbb{R}^m$. A good theory of the initial value problem with statements about existence and uniqueness is obtained if f is locally Lipschitz. This holds if f is continuously differentiable. If the stoichiometric coefficients are integers this condition is always satisfied for the equations coming from reaction networks. It is also satisfied if all non-trivial stoichiometric coefficients are ≥ 1. The solutions of ordinary differential equations always depend continuously on the initial data. In applications to reaction networks we are always concerned with solutions which are positive or at least non-negative. We would like to know that these conditions follow from corresponding assumptions on the initial data. This will now be proved for a class of equations which includes the reaction equations.

Lemma 2.1 Let x be a solution of the system $\dot{x}_i = f_i(x)$, where $f_i(x) = f_{i,1}(x) - x_i f_{i,2}(x)$ for each i, and the functions $f_{i,1}$ and $f_{i,2}$ are C^1 and non-negative. If $x_i(t_0) > 0$ for all i then $x_i(t) > 0$ for all i and all $t \geq t_0$. If $x_i(t_0) \geq 0$ for all i then $x_i(t) \geq 0$ for all i and all $t \geq t_0$.

Proof If the first statement were false then there would be a smallest number $t_1 > t_0$ with $\min_i x_i(t_1) = 0$. Let i be an index for which this minimum is attained. Then $x_i(t_1) = 0$. It follows that for $t_0 \leq t < t_1$ the inequality

$$\dot{x}_i(t) \geq -x_i(t) f_{i,2}(x(t)) \tag{15}$$

holds. Hence $d/dt(\log x_i) \geq -C$, where C is the supremum of $f_{i,2}$ on the image of $[t_0, t_1]$ under x. It follows that $x_i(t_1) \geq e^{-C} x_i(t_0) > 0$, a contradiction. The second statement of the Lemma then follows from the continuous dependence of the solution on the intial data.

A stationary solution of the system is a point x with $f(x) = 0$. These solutions are often significant for the behaviour of more general solutions. In this context the notion of stability plays an important role.

Definition Let x_0 be a stationary solution of the system $\dot{x} = f(x)$. It is called *stable* if for each open neighbourhood U of x_0 there is a neighbourhood V of x_0 with the property that each solution which starts in V stays in U. It is called *asymptotically stable* if it is stable and there is a neighbourhood U of x_0 with the property that each solution which starts in U converges to x_0 for $t \to \infty$.

A tool which can often be used to prove the stability of stationary solutions is the Lyapunov function. If V is a function on an open subset of $\mathbb{R}^n$ then let $\dot{V} = \frac{\partial V}{\partial x_i} f_i(x)$. By the chain rule $\dot{V} = \frac{d}{dt}(V(x(t))$. A function which satisfies $\dot{V} \leq 0$ is called a Lyapunov function.

Theorem 2.1 Let U be an open neighbourhood of a point $x_0 \in \mathbb{R}^n$. Let f be a C^1 mapping from U to $\mathbb{R}^n$ with $f(x_0) = 0$. Let V be a C^1 function with $V(x_0) = 0$ and $V(x) > 0$ for $x \neq x_0$. If $\dot{V}(x) \leq 0$ for all $x \in U$ then x_0 is stable. If $\dot{V}(x) < 0$ for all $x \in U$ except $x = x_0$ then x_0 is asymptotically stable.

This theorem will not be proved here. Let $x(t)$ be a solution of a system of ordinary differential equations. If there is a sequence of times t_n with

$\lim_{n\to\infty} t_n = \infty$ and a point x_* with the property that $\lim_{n\to\infty} x(t_n) = x_*$ then x_* is called an ω-limit point of the solution $x(t)$. The set of these points is called the ω-limit set of this solution. Let $y(t)$ be the solution with initial value x_*. Then the image of $y(t)$ lies in the ω-limit set of $x(t)$. If the image of a solution $x(t)$ is contained in a compact set then the ω-limit set of $x(t)$ is connected. If V is a Lyapunov function for the system $\dot{x} = f(x)$ then a point x_* where $\dot{V}(x_*) < 0$ can never be an ω-limit point of a solution of the system.

3 The Deficiency Zero Theorem

The theorem which is the subject of this section can be found in various forms in the literature. Here we choose the following form.

Theorem 3.1 (The Deficiency Zero Theorem) Let $\dot{x} = f(x)$ be the dynamical system which is defined by a reaction network of deficiency zero under the assumption of mass action kinetics. Then

(i) if the network is not weakly reversible then there exist no positive stationary solutions

(ii) if the network is weakly reversible then there exists precisely one positive stationary solution in each stoichiometric compatibility class. This solution is asymptotically stable in its class.

Now we develop some ideas which will be used in the proof of this theorem. First we consider the matrix A_k.

Proposition 3.1 Consider a network with terminal strong linkage classes $(\Lambda_1, \ldots, \Lambda_t)$. The kernel of A_k has a basis $\{x_1, \ldots, x_t\}$ with the property that that Λ_i is the support of x_i and x_i is positive on Λ_i for all i.

In particular the dimension of the kernel of A_k is always t and does not depend on the reaction constants. A possible choice of the basis of Proposition 3.1 in Example 3 is $([k_2, k_1, 0, 0, 0]^T, [0, 0, k_5(k_4 + k_6), k_3k_5, k_3k_6]^T)$. To prove this proposition we use the approach of [7]. There is a relation to the Perron-Frobenius theorem and another proof, which uses this theorem directly, can be found in [14].

Proposition 3.1 will now be proved in several steps, following ([7]). If χ is a function on $\mathcal{C}$ let $|\chi|$ be its modulus, calculated pointwise. For a complex y let $\mathcal{R}_{\to y}$ be the set of reactions whose right hand side is y and let $\mathcal{R}_{y\to}$ be the set of reactions whose left hand side is y.

Lemma 3.1 If $\chi \in \ker A_k$ then $|\chi| \in \ker A_k$.

Proof $A_k\chi = \sum_{y\in\mathcal{C}}\{\sum_{\mathcal{R}_{\to y}} k_{y'y}\chi(y') - (\sum_{\mathcal{R}_{y\to}} k_{yy'})\chi(y)\}\omega_y$. Since the ω_y are linearly independent χ is in the kernel of A_k precisely when the curly bracket vanishes. Hence for $\chi \in \ker A_k$ we have

$$\sum_{\mathcal{R}_{\to y}} k_{y'y}|\chi(y')| \geq (\sum_{\mathcal{R}_{y\to}} k_{yy'})|\chi(y)|. \tag{16}$$

Summing this inequality over y gives

$$\sum_{y\in\mathcal{C}}\sum_{\mathcal{R}_{\to y}} k_{y'y}|\chi(y')| \geq \sum_{y\in\mathcal{C}}\sum_{\mathcal{R}_{y\to}} k_{yy'}|\chi(y)| \tag{17}$$

Equality holds in (17) precisely when it holds in (16). Since in fact equality holds in (17) it also holds in (16). It then follows that

$$\sum_{\mathcal{R}_{\to y}} k_{y'y}|\chi(y')| = (\sum_{\mathcal{R}_{y\to}} k_{yy'})|\chi(y)| \tag{18}$$

and that $\chi \in \ker A_k$ implies $|\chi| \in \ker A_k$.

Lemma 3.2 If $\chi \in \ker A_k$, $\chi(y) = 0$ and there is a directed path from y' to y then $\chi(y') = 0$.

Proof If $y = y'$ the statement is clear. It follows from (18) that when $y'y \in \mathcal{R}$ and $\chi(y) = 0$ it is also the case that $\chi(y') = 0$. The statement of Lemma 3.2 follows by repeated application of this fact.

Lemma 3.3 Let Λ be a strong linkage class, not necessarily terminal. If $\chi \in \ker A_k$ and $\chi(y) = 0$ for some $y \in \Lambda$ then $\chi(y') = 0$ for all $y' \in \Lambda$.

Proof If y and y' are in the same strong linkage class then there exists a directed path from y' to y and the statement of Lemma 3.3 follows from that of Lemma 3.2.

Lemma 3.4 Let $\mathcal{I}$ be the set of all complexes which are contained in terminal strong linkage classes and let $\chi \in \ker A_k$. Then $\mathrm{supp}\chi \subset \mathcal{I}$.

Proof If $\mathcal{I} = \mathcal{C}$ the statement is trivial. Suppose then that $\mathcal{I} \neq \mathcal{C}$. For $y \in \mathcal{I}$ let

$$\mathcal{R}^{\mathrm{int}}_{\to y} = \{y'y \in \mathcal{R} : y' \in \mathcal{I}\}, \tag{19}$$

$$\mathcal{R}^{\mathrm{ext}}_{\to y} = \{y'y \in \mathcal{R} : y' \notin \mathcal{I}\}. \tag{20}$$

$\mathcal{R}_{\to y}$ is the union of these two sets. If we use this fact and sum (18) over $\mathcal{I}$ we get

$$\sum_{y\in\mathcal{I}} \sum_{\mathcal{R}^{\mathrm{int}}_{\to y}} k_{y'y}|\chi(y')| + \sum_{y\in\mathcal{I}} \sum_{\mathcal{R}^{\mathrm{ext}}_{\to y}} k_{y'y}|\chi(y')| = \sum_{y\in\mathcal{I}} \sum_{\mathcal{R}_{y\to}} k_{yy'}|\chi(y)|. \tag{21}$$

The first term on the left hand side in this equations is equal to the expression on the right hand side. Hence $\sum_{y\in\mathcal{I}} \sum_{\mathcal{R}^{\mathrm{ext}}_{\to y}} |\chi(y')| = 0$. Let

$$\mathcal{G} = \{y' \in \mathcal{C} \setminus \mathcal{I} : \text{ there is } y'y \in \mathcal{R} \text{ with } y \in \mathcal{I}\}. \tag{22}$$

Then χ vanishes on $\mathcal{G}$. Since $\mathcal{G}$ is a finite set it follows that for each $y'' \in \mathcal{C} \setminus \mathcal{I}$ there is $y' \in \mathcal{G}$ for which there is a directed path from y'' to y'. Hence it follows from Lemma 3.2 that $\chi(y'') = 0$ for all $y'' \in \mathcal{C} \setminus \mathcal{I}$ and that $\mathrm{supp}\chi \in \mathcal{I}$.

Let Γ_i be the space of functions on $\mathcal{C}$ whose support is contained in Λ_i. Then according to Lemma 3.4 the kernel of A_k is contained in the direct sum of of the Γ_i.

Lemma 3.5 For $1 \leq i \leq t$ the space Γ_i is invariant under A_k.

Proof Let $\mathcal{R}_i = \{yy' \in \mathcal{R} : y \in \Lambda_i\}$. If $\chi \in \Gamma_i$ then $\chi(y) = 0$ for $y \notin \Lambda_i$. Hence the sum in the definition of A_k can be restricted to $\mathcal{R}_i$ when $\chi \in \Gamma_i$. If $\mathcal{R}_i$ is empty (as it is when Λ_i only has one element) then $A_k\chi = 0$ and the statement holds. Suppose then that $\mathcal{R}_i$ is not empty. Since Λ_i is terminal the fact that

$yy' \in \mathcal{R}_i$ implies that y and y' are in Λ_i. Then the support of each summand is in Γ_i and $A_k\chi \in \Gamma_i$.

Lemma 3.6 For $1 \leq i \leq t$ there exists $\chi_i \in \Gamma_i$ with the following properties.
(a) $A_k\chi_i = 0$
(b) $\chi_i(y) > 0$ for all $y \in \Lambda_i$
(c) If $\chi_i' \in \Gamma_i$ and $A_k\chi_i' = 0$ then $\chi_i' = \alpha\chi_i$ for a number α

Proof Let $A_{k,i}$ be the restriction of A_k to Γ_i. Then

$$A_{k,i}\chi = \sum_{yy' \in \mathcal{R}_i} k_{yy'}\chi(y)(\omega_{y'} - \omega_y) \tag{23}$$

$\dim(\mathrm{im}A_{k,i})$ is smaller than $\dim\Gamma_i$, since $\sum_{y\in\Lambda_i}\omega_y$ is orthogonal to $\mathrm{im}A_{k,i}$. Hence $A_{k,i}$ has a non-trivial kernel. Let χ_i be a non-vanishing element of $\ker A_{k,i}$. Then $\chi_i \in \ker A_{k,i}$ and $\mathrm{supp}\chi_i \subset \Lambda_i$. But according to Lemma 3.3 $\Lambda_i \subset \mathrm{supp}\chi_i$. Hence $\mathrm{supp}\chi_i = \Lambda_i$. $\chi_i(y) = 0$ holds for $y \notin \Lambda_i$ and $\chi_i(y) \neq 0$ for $y \in \Lambda_i$. Thus parts (a) and (b) have been proved. Suppose there exists $\chi' \in \Gamma_i$ with $A_k\chi' = 0$. There is a number α with the property that $\chi' - \alpha\chi = 0$ for some $y \in \Lambda_i$. The function $\chi' - \alpha\chi$ is in $\ker A_k$ and according to Lemma 3.3 it vanishes on the whole of Λ_i. Since the support of $\chi' - \alpha\chi$ is in Λ_i it follows that $\chi' = \alpha\chi$ and part (c) is proved.

Now we can prove Proposition 3.1. Let $\chi_i \in \Gamma_i$ be vectors which have the properties (a) and (b) of Lemma 3.6. We will show that these vectors form a basis for the kernel of A_k. This suffices to prove the proposition. The vectors are linearly independent. It remains to show that each function χ in A_k is a linear combination of these vectors. Let $\chi \in \ker A_k$. Then

$$\chi = \chi_1' + \ldots + \chi_t' \tag{24}$$

with $\chi_i' \in \Gamma_i$ for $1 \leq i \leq t$. It follows that

$$A_k\chi = A_k\chi_1' + \ldots + A_k\chi_t' = 0 \tag{25}$$

According to Lemma 3.5 $A_k\chi_i' \in \Gamma_i$ for all i. By Lemma 3.6, part (c) there exist numbers α_i so that $A_k\chi_i' = \alpha_i\chi_i$ for all i. This completes the proof of Proposition 3.1.

It follows in particular that the dimension of the kernel of A_k is t, independent of the reaction constants.

Corollary 3.1 The kernel of A_k contains a positive vector iff the network is weakly reversible.

Proof If the network is not weakly reversible then there is a complex $\hat{y} \in \mathcal{C}$ which is not in any terminal strong linkage class. Otherwise the linkage classes would agree with the strong linkage classes. Since, according to Proposition 3.1, each vector in the kernel of A_k is a linear combination of vectors in the terminal strong linkage classes it must be the case that $x(\hat{y}) = 0$ for each function x in the kernel of A_k. Thus there is no positive vector in the kernel of A_k. Conversely, if the network is weakly reversible then each complex is in a terminal strong linkage class. Then the sum of the basis vectors in Proposition 3.1 is the desired vector.

Corollary 3.2 There exist positive constants $\alpha_{yy'}$ with

$$\sum_{\mathcal{R}} \alpha_{yy'}(\omega_y - \omega_{y'}) = 0 \tag{26}$$

precisely when the network is weakly reversible.

Proof Suppose first that the network is weakly reversible. Let k be a positive element of $\mathbb{R}^r$ and let A_k be as before. It follows from Proposition 3.1 und Corollary 3.1 that there exists a positive vector $\hat{x}$ in the kernel of A_k. We then choose $\alpha_k = k_{yy'}\hat{x}(y)$. Suppose that the network were not weakly reversible and that a positive solution α exists. Let

$$A_\alpha(x) = \sum_{\mathcal{R}} \alpha_{yy'}x(y)(\omega_y - \omega_{y'}) \tag{27}$$

and let $\bar{x}$ be the vector with $\bar{x}(y) = 1$ for all $y \in \mathcal{C}$. Then $A_\alpha(\bar{x}) = 0$ and $\bar{x}$ is a positive vector in the kernel of A_α, in contradiction to Corollary 3.1.

For a reaction network let

$$\Delta' = \{\omega_{y'} - \omega_y : yy' \in \mathcal{R}\} \tag{28}$$

and

$$\Delta = \{\omega_{y'} - \omega_y : \text{there is a path from } y \text{ to } y'\}. \tag{29}$$

It is clear that the space which is the span of Δ' is contained in the span of Δ. In fact both these spaces are equal.

Lemma 3.7 The spans of the sets Δ and Δ' are equal.

Proof We already know the one inclusion and it only remains to show the other. The cases that the length of the path is zero or one are obvious. In general there are complexes $y^{(0)}, \ldots, y^{(n)}$ which link y to y'. Then we write

$$\omega_{y'} - \omega_y = (\omega_{y^{(n)}} - \omega_{y^{(n-1)}}) + \ldots + (\omega_{y^{(1)}} - \omega_{y^{(0)}}). \tag{30}$$

Lemma 3.8 For a network with n complexes and l linkage classes the dimensions of the two spaces in the statement of Lemma 3.7 are equal to $n - l$.

Proof For the proof we consider the space which is defined by Δ. Let $\{L^1, \ldots, L^l\}$ be the linkage classes of the network and for $\theta = 1, \ldots, l$ let

$$\Delta^\theta = \{\omega_{y'} - \omega_y : y, y' \in L^\theta\} \tag{31}$$

Then Δ is the union of the Δ^θ and the span of Δ is the direct sum of the spans of the Δ^θ. Let n_θ be the number of complexes in L^θ. The dimension of the span of L^θ is $n_\theta - 1$. It follows that the dimension of the direct sum is given by $\sum_{\theta=1}^{l}(n_\theta - 1) = n - l$.

Lemma 3.9 Let L^θ be the linkage classes of a reaction network. Then the set $\{\omega_{L^\theta}\}$ is a basis for the orthogonal complement the space span(Δ).

Proof Each element of this set is orthogonal to each element of Δ and the set is linearly independent. Since the dimension of the space of functions on $\mathcal{C}$ is n

the orthogonal complement has the dimension $n-(n-l)=l$. Thus the given set is a basis of the orthogonal complement.

It follows from Lemma 3.9 that a vector g is in span(Δ) precisely when the inner product of g with ω_{L^θ} is zero for all θ.

Corollary 3.3 imA_k = span(Δ) precisely when each linkage class of the network contains exactly one terminal strong linkage class. This statement holds in particular when the network is weakly reversible.

Proof Since imA_k is contained in span(Δ) we only have to check whether these two spaces have the same dimension. From Lemma 3.8 we know that the dimension of span(Δ) is $n-l$. Since the dimension of the space of functions on $\mathcal{C}$ is n it follows from linear algebra that dim im$A_k = n-t$, where t is the number of terminal strong linkage classes. Hence the difference of the two dimensions is $t-l$ and so the desired condition holds when each linkage class contains precisely one terminal strong linkage class. This condition is evidently satisfied for weakly reversible networks.

Proposition 3.2 The deficiency δ of a network satisfies

$$\delta = \dim(\ker Y \cap \mathrm{span}(\Delta)). \tag{32}$$

When, in particular, the deficiency of the network is zero $\ker Y \cap \mathrm{span}(\Delta) = \{0\}$.

Proof Let $\bar{Y}$ be the restriction of Y to span(Δ). Then

$$\dim \mathrm{span}(\Delta) = \dim \ker \bar{Y} + \dim \mathrm{im} \bar{Y} \tag{33}$$

It follows from Lemma 3.8 that dim span(Δ) $= n-l$. In addition im$\bar{Y}$ $= Y(\mathrm{span}(\Delta)) = S$. The relation $\ker \bar{Y} = \ker Y \cap \mathrm{span}(\Delta)$ also holds. It follows that $\dim(\ker Y \cap \mathrm{span}(\Delta)) = n-l-s = \delta$.

We see that $\delta \geq 0$ and that $\delta' = \delta$ exactly when $t = l$.

Corollary 3.4 A function α on $\mathcal{R}$ satisfies the equation

$$\sum_{\mathcal{R}} \alpha_{yy'}(y'-y) = 0 \tag{34}$$

if it satisfies the equation

$$\sum_{\mathcal{R}} \alpha_{yy'}(\omega_{y'}-\omega_y) = 0 \tag{35}$$

When the deficiency of the network is zero then it satisfies the first equation only when it satisfies the second.

Proof The first equation can be rewritten as

$$Y(\sum_{\mathcal{R}} \alpha_{yy'}(\omega_{y'}-\omega_y)) = 0 \tag{36}$$

which implies the first statement of the Corollary. For the converse we use the fact that $\sum_{\mathcal{R}} \alpha_{yy'}(\omega_{y'}-\omega_y) \in \ker Y \cap \mathrm{span}(\Delta)$. When $\delta = 0$ the space on the right hand side consists only of zero.

Corollary 3.5 For a network of deficiency zero the equation (34) has a positive solution precisely when the network is weakly reversible.
Corollary 3.6 For a network of deficiency zero the condition $\ker Y A_k = \ker A_k$ holds.
Proof Since $\ker A_k$ is obviously contained in $\ker(Y A_k)$ we only have to prove the reverse inclusion. If $x \in \ker(Y A_k)$ then $A_k x$ is contained in $\ker Y$. Since A_k takes its values in $\mathrm{span}(\Delta)$ it follows that $A_k x$ is in $\ker Y \cap \mathrm{span}(\Delta)$. Hence it can be concluded using Proposition 3.2 that $A_k x = 0$ and that $x \in \ker A_k$.

4 The position of stationary solutions

In this section some properties of stationary solutions of reaction networks are derived. Later it will be shown that under certain circumstances a system of equations coming from a reaction network has exactly one stationary solution in each stoichiometric compatibility class. In order to do this it is shown that the set of stationary solutions is the set E of points of the positive orthant with $\log c - \log c^* \in S^\perp$ for a fixed positive element c^*. The logarithm is defined pointwise. We want to know that E meets each stoichiometric compatibility class in exactly one point. The uniqueness is easy. If c and c' are two points of E in the same stoichiometric compatibility class then $c - c' \in S$ and $\log c' - \log c \in S^\perp$. It follows that

$$0 = (c' - c) \cdot (\log c' - \log c) = \sum_{s \in \mathcal{S}} (c'(s) - c(s))(\log c'(s) - \log c(s)). \tag{37}$$

Since the logarithm is strictly increasing this can only hold if $c'(s) = c(s)$ for all s. It remains to prove the existence statement. We define the exponential function and the product pointwise.
Proposition 4.1 Let $\mathcal{S}$ be a finite set and $F(\mathcal{S})$ the vector space of real-valued functions on $\mathcal{S}$, let S be a linear subspace of $F(\mathcal{S})$ and let a and b be two positive elements of $F(\mathcal{S})$. Then there exists a unique vector μ in $S^\perp$ with the property that $ae^\mu - b \in S$.
Proof Let $\phi : F(\mathcal{S}) \to \mathbb{R}$ be defined by $\phi(x) = \sum_{s \in \mathcal{S}} [a(s)e^{x(s)} - b(s)x(s)]$. The gradient of ϕ at the point x is given by $\nabla\phi(x) = ae^x - b$ and its Hessian by $H(x)\gamma = (ae^x)\gamma$. $H(x)$ is positive definite. For all $\gamma \in F(\mathcal{S})$, not equal to zero, we have

$$\gamma \cdot H(x)\gamma = \gamma \cdot ae^x\gamma = \sum_{s \in \mathcal{S}} a(s)e^{x(s)}(\gamma(s))^2 > 0. \tag{38}$$

Hence the function ϕ is strictly convex. Next we want to show that $\lim_{\alpha \to \infty} \phi(\alpha x) = \infty$. Now

$$\phi(\alpha x) = \sum_{s \in \mathcal{S}} (a(s)e^{\alpha x(s)} - \alpha b(s)x(s)) \tag{39}$$

For $x(s) \neq 0$ the positivity of $a(s)$ and $b(s)$ implies

$$\lim_{\alpha \to \infty} (a(s)e^{\alpha x(s)} - \alpha b(s)x(s)) = \infty \tag{40}$$

while for $x(s) = 0$ we have

$$a(s)e^{\alpha x(s)} - \alpha b(s)x(s) = a(s) \tag{41}$$

This means that (39) has been proved. Now let $\bar{\phi}$ be the restriction of ϕ to $S^{\perp}$. Since ϕ is continuous and convex the restriction $\bar{\phi}$ also has these properties. Thus the set $C = \{x \in S^{\perp} : \bar{\phi}(x) \leq \bar{\phi}(0)\}$ is closed and convex. It does not contain any subset of the form $\{\alpha x : \alpha \in (0, \infty)\}$. Thus C is bounded [22] and compact. Hence there exists $\mu \in C$ with the property that $\bar{\phi}(\mu) \leq \bar{\phi}(x)$ for all $x \in C$. It follows that $\nabla\phi(\mu) \cdot \gamma = 0$ for all $\gamma \in S^{\perp}$ and that $\nabla\phi(\mu)$ lies in S. This completes the existence proof. To obtain uniqueness, let $\mu' \in S^{\perp}$ be a solution of $ae^{\mu'} - b = S$. Then $a(e^{\mu'} - e^{\mu}) = 0$ and, since $\mu' - \mu \in S^{\perp}$ we have

$$\sum_{s \in \mathcal{S}} a(s)(\mu'(s) - \mu(s))(e^{\mu'(s)} - e^{\mu(s)}) = 0 \tag{42}$$

Since $a(s)$ is positive and the exponential function is strictly increasing this statement can only hold if $\mu' = \mu$.

Corollary 4.1 For a positive element c^* of $F(\mathcal{S})$ the set E meets each stoichiometric compatibility class in exactly one positive point.

Proof Let p be an arbitrary positive element of $F(\mathcal{S})$. We will show that the stoichiometric compatibility class which contains p meets the set E in exactly one point. That there is at most one point of this kind was already shown. To get existence we first note that Proposition 4.1 implies the existence of a point $\mu \in S^{\perp}$ with $c^*e^{\mu} - p \in S$. Let $c = c^*e^{\mu}$. Then c is positive and lies in the stoichiometric compatibility class which contains p. Taking the logarithm of both sides shows that $\log c - \log c^* = \mu \in S^{\perp}$. Thus c lies in E.

5 Proof of the Deficiency Zero Theorem

In this section the Deficiency Zero Theorem will be proved with the help of the techniques developed up to this point. First we prove the relatively easy part (i).

Lemma 5.1 The differential equations which are obtained from a reaction network under the assumption of mass action kinetics only have a positive stationary solution when there is a positive α with $\sum_{\mathcal{R}} \alpha_{yy'}(y' - y) = 0$.

Proof Let c^* be a positive stationary solution. Then we can take $\alpha_{yy'}$ as the rate of the reaction $y \to y'$ when the concentrations are given by c^*.

Part (i) of the Deficiency Zero Theorem now follows from Corollary 3.5 and Lemma 5.1.

Next we will prove part (ii) under the assumption that a complex balanced positive stationary solution exists without using the condition $\delta = 0$. If c^* is a stationary solution then $\Psi(c^*) \in \ker(YA_k)$. If in addition $\Psi(c^*) \in \ker(A_k)$ then it is possible to draw further conclusions.

Proposition 5.1 If a positive stationary solution c^* satisfies $A_k\Psi(c^*) = 0$, i.e. c^* is complex balanced, then $f(c) \cdot (\log c - \log c^*) \leq 0$ for all positive points c of $F(\mathcal{S})$. Moreover the following conditions are equivalent:

(i) $f(c) \cdot (\log c - \log c^*) = 0$
(ii) $\log c - \log c^* \in S^\perp$
(iii) $A_k \Psi(c) = 0$
(iv) $f(c) = 0$

Remark The assumption of the Proposition implies that the network is weakly reversible.

Proof Let $\mu = \log c - \log c^*$. Then $c^y = (c^*)^y e^{y \cdot \mu(c)}$. As a consequence $f(c) = \sum_{\mathcal{R}} k_{yy'} (c^*)^y e^{y \cdot \mu(c)} (y' - y)$. It follows that

$$f(c) \cdot (\log c - \log c^*) = \sum_{\mathcal{R}} k_{yy'} (c^*)^y e^{y \cdot \mu(c)} (y' \cdot \mu(c) - y \cdot \mu(c)) \tag{43}$$

Now the exponential function has the property that $e^\alpha(\alpha' - \alpha) \leq e^{\alpha'} - e^\alpha$ for all real numbers α and α' with equality only for $\alpha = \alpha'$. Thus we can conclude that

$$f(c) \cdot (\log c - \log c^*) \leq \sum_{\mathcal{R}} k_{yy'} (c^*)^y (e^{y' \cdot \mu(c)} - e^{y \cdot \mu(c)}) \tag{44}$$

with equality only when $\mu(c) \cdot (y' - y) = 0$ for all reactions in $\mathcal{R}$. The right hand side of the last inequality can be rewritten in the form

$$\left[\sum_{\mathcal{R}} k_{yy'} (c^*)^y (\omega_{y'} - \omega_y) \right] \cdot \sum_{y'' \in \mathcal{C}} e^{y'' \cdot \mu(c)} \omega_{y''}. \tag{45}$$

The expression in the bracket is nothing other than $A_k \Psi(c^*)$, which by assumption is zero. Hence $f(c) \cdot (\log c - \log c^*) \leq 0$.

It is clear that (ii) implies (i). Conversely, when (i) holds then equality holds in (44). Since the vectors $y - y'$ span the subspace S condition (ii) follows. Now it will be shown that (ii) implies (iii). The network is weakly reversible and hence its terminal strong linkage classes coincide with its linkage classes. The latter will be denoted by $\{L^1, \ldots, L^l\}$. Moreover Proposition 3.1 implies that there is a basis $x^1, \ldots, x^l$ of $\ker A_k$ with the property that supp $x^\theta = L^\theta$ for $\theta = 1, \ldots, l$. Hence $\Psi(c^*)$ has a representation of the form

$$\Psi(c^*) = \sum_{\theta=1}^{l} \Big(\sum_{y \in L^\theta} (c^*)^y \omega_y \Big) = \sum_{\theta=1}^{l} \lambda_\theta x^\theta \tag{46}$$

for constants λ_θ. Since we know where the support of x^θ lies we can conclude that $(c^*)^y \omega_y = \lambda_\theta x^\theta$ for all θ. Hence the set $(c^*)^y \omega_y$ is also a basis for $\ker A_k$. Suppose that $\mu(c) \in S^\perp$. We know that $y' \cdot \mu(c) = y \cdot \mu(c)$ whenever there is a path joining y and y'. This means that there exist numbers $\xi_1, \ldots, \xi_l$ with the property that $y \cdot \mu(c) = \xi_\theta$ for all $y \in L^\theta$. It follows that

$$\begin{aligned} \Psi(c) &= \sum_{y \in \mathcal{C}} c^y \omega_y = \sum_{y \in \mathcal{C}} (c^*)^y e^{y \cdot \mu(c)} \\ &= \sum_{\theta=1}^{l} \Big(\sum_{y \in L^\theta} (c^*)^y e^{y \cdot \mu(c)} \omega_y \Big) = \sum_{\theta=1}^{l} e^{\xi_\theta} \Big(\sum_{y \in L^\theta} (c^*)^y \omega_y \Big) \end{aligned} \tag{47}$$

Hence $A_k\Psi(c) = 0$. That (iv) follows from (iii) is apparent from the formula for $f(c)$ and it is obvious that (i) follows from (iv).

Corollary 5.1 Under the assumptions of Proposition 5.1 the equations have precisely one stationary solution in each stoichiometric compatibility class.

Proof Due to the equivalence of (ii) und (iv) in Proposition 5.1 the positive stationary solutions coincide with the set E. According to Corollary 4.1 this set meets each stoichiometric compatibility class in precisely one point.

We have obtained a lot of information about the stationary solutions. Now we would like to have dynamical information and we will do this with the help of Lyapunov functions. For a fixed positive function c^* on $\mathcal{S}$ let h be the function

$$h(c) = \sum_{s\in\mathcal{S}}[c(s)(\log c(s) - \log c^*(s) - 1) + c^*(s)] \tag{48}$$

Evidently $h(c^*) = 0$. Furthermore, it follows from the fact that the logarithm is strictly convex that for each $s \in \mathcal{S}$ and each $c(s) > 0$ the inequality

$$\log c(s) - \log c^*(s) \geq \frac{1}{c(s)}(c(s) - c^*(s)) \tag{49}$$

holds, with equality precisely when $c(s) = c^*(s)$. Hence $h(c) > 0$ for all $c \neq c^*$. In addition we have $\nabla h(c) = \log c - \log c^*$ and the Hessian is $G(c)\gamma = \frac{\gamma}{c}$. For all c and all $\gamma \neq 0$ we have $\gamma \cdot G(c)\gamma = \sum_{s\in\mathcal{S}} \gamma(s)^2/c(s) > 0$. We see that the Hessian is positive definite and h strictly concave. Now we come to a further corollary of Proposition 5.1.

Corollary 5.2 Under the assumptions of Proposition 5.1 the inequality $\nabla h(c) \cdot f(c) < 0$ holds with equality only when $f(c) = 0$.

From these statements we see that h is monotone decreasing along solutions, $\frac{d}{dt}(h(c(t)) \leq 0$, with equality only when $f(c(t)) = 0$. It follows that the restriction of h to a stoichiometric class is a Lyapunov function. Hence the stationary solution is asymptotically stable as a consequence of Theorem 2.1.

The relation to the condition $\delta = 0$ comes through the following statement.

Proposition 5.2 Suppose that the equations for a network under the assumption of mass action have a stationary solution and that the deficiency is zero. Then the assumptions of Proposition 5.1 are satisfied.

Proof Let c^* be a stationary solution. Then $\Psi(c^*) \in \ker(YA_k)$. Since the deficiency is zero it follows from Corollary 3.6 that $\ker(YA_k) = \ker(A_k)$ and the result is proved.

To complete the proof of the deficiency zero theorem it remains to prove the existence of a positive stationary solution for a weakly reversible network with deficiency zero. Thus we are looking for c^* with $YA_k\Psi(c^*) = 0$. Since for networks with deficiency zero $\ker(YA_k) = \ker(A_k)$ it suffices to find c^* with $\Psi(c^*) \in \ker(A_k)$.

The transposed matrix Y^T satisfies $Y^T z = \sum_{y\in\mathcal{C}}(y\cdot z)\omega_y$. The characteristic functions ω_{L^θ} of the linkage classes satisfy $\omega_{L^\theta} = \sum_{y\in L^\theta}\omega_y$.

Lemma 5.2 $\dim[\text{im } Y^T + \text{span}(\omega_{L^1}, \ldots, \omega_{L^l})] = n - \delta$. In particular, when $\delta = 0$ the space $\text{im } Y^T + \text{span}(\omega_{L^1}, \ldots, \omega_{L^l})$ is the whole space of functions on $\mathcal{R}$.

Proof It follows from Proposition 3.2 that $\dim[\ker Y \cap \mathrm{span}(\Delta)] = \delta$. Since the dimension of the whole space is n we can conclude that $\dim[\ker Y \cap \mathrm{span}(\Delta)]^\perp = n - \delta$. On the other hand

$$[\ker Y \cap \mathrm{span}(\Delta)]^\perp = (\ker Y)^\perp + (\mathrm{span}(\Delta))^\perp. \tag{50}$$

At the same time $(\ker Y)^\perp = \mathrm{im} Y^T$. As a consequence of Lemma 3.9 the space $(\mathrm{span}(\Delta))^\perp$ is spanned by the ω_{L^θ}. Combining these statements gives the first part of Lemma 5.2. The second part is then clear.

Proposition 5.3 We consider a weakly reversible network and a basis $x^1, \ldots, x^l$ for $\ker A_k$ as in Proposition 3.1. Then the following statements are equivalent
(i) there exists a positive function c^* on $\mathcal{S}$ with $\Psi(c^*) \in \ker A_k$.
(ii) $\log(\sum_{\theta=1}^{l} x^\theta)$ is in $\mathrm{im} Y^T + \mathrm{span}(\omega_{L^1}, \ldots, \omega_{L^l})$.

Proof Condition (ii) is equivalent to
(iii) there exists a function z on $\mathcal{S}$ and numbers $\{-\xi_1, \ldots, -\xi_l\}$ with the property that

$$\log(\sum_{\theta=1}^{l} x^\theta) = Y^T z - \sum_{\theta=1}^{l} \xi_\theta \omega_{L^\theta}. \tag{51}$$

With z and ξ_θ as in this equation we set $c^* = e^z$ and $\lambda_\theta = e^{\xi_\theta}$ and see that (iii) is equivalent to
(iv) there exists a positive function c^* on $\mathcal{S}$ and positive constants $\{\lambda_1, \ldots, \lambda_l\}$ with the property that

$$Y^T(\log c^*) = \log(\sum_{\theta=1}^{l} x^\theta) + \sum_{\theta=1}^{l} (\log \lambda_\theta)\omega_{L^\theta} = \log(\sum_{\theta=1}^{l} \lambda_\theta x^\theta). \tag{52}$$

Now $Y^T(\log c^*) = \sum_{y \in \mathcal{C}} (y \cdot \log c^*)\omega_y$ and $y \cdot \log c^* = \log((c^*)^y)$. Together the last two equations give

$$Y^T(\log c^*) = \sum_{y \in \mathcal{C}} [\log(c^*)^y]\omega_y = \log(\sum_{y \in \mathcal{C}} (c^*)^y \omega_y) = \log \Psi(c^*). \tag{53}$$

Thus (iv) is equivalent to
(v) there exist a positive function c^* on $\mathcal{S}$ and positive constants $\{\lambda_1, \ldots, \lambda_l\}$ with the property that $\Psi(c^*) = \sum_{\theta=1}^{l} \lambda_\theta x^\theta$. Since $\{x^1, \ldots, x^l\}$ is a basis of $\ker A_k$ condition (v) implies (i). That (i) implies (v) follows from the nature of the basis and the fact that $\Psi(c^*)$ is positive for c^* positive.

Corollary 5.3 For a weakly reversible network of deficiency zero there exists a positive function c^* on $\mathcal{S}$ with $A_k \Psi(c^*) = 0$.

Proof For a network of deficiency zero Lemma 5.2 says that the linear subspace in (ii) of Proposition 5.3 is the whole space of functions on $\mathcal{C}$. Hence condition (ii) is satisfied and the equivalent condition (i) as well.

Let us summarize what has been proved. If a network is weakly reversible and the deficiency of the network is zero then there is a unique stationary solution c^* in each stoichiometric compatibility class and all solutions which start

close enough to c^* converge to it. What can be said about general positive solutions in this case? The Lyapunov function $h(c)$ provides information about this. This function is a sum of non-negative contributions from the different species, say $h(c) = \sum_{i=1}^{m} h_i(c_i)$, and each function h_i is non-negative and positive for $c \neq c^*$. Consider the subset defined by $h(c) \leq C$ for a constant C. Then $h_i(c_i) \leq C$ for all i. Now $h_i'(c_i) = \log c_i - \log c_i^*$ so that h_i is monotone increasing for $c_i > c_i^*$ and monotone decreasing for $c_i < c_i^*$. For $c_i \to 0$ the quantity $h_i(c_i)$ converges to c_i^* and for $c_i \to \infty$ we have $h_i(c_i) = c_i \log c_i + O(c_i)$. In particular $h_i(c_i)$ tends to $+\infty$ for $c_i \to \infty$. It follows that the inequality $h_i(c_i) \leq C$ implies the boundedness of c_i. For systems of this type all solutions exist globally in the future and are bounded. For systems of deficiency zero which are not weakly reversible the last statement does not hold. It suffices to consider the simple reaction $0 \to A$, which leads to the equation $\dot{x}_C = k$. The solutions are linear functions which are not bounded.

Now consider once again the case of a weakly reversible network. The Lyapunov function does not a priori prevent that a positive solution has an ω-limit point where some concentration vanishes. It does rule out positive ω-limit points other than c^*. Suppose that a positive solution $c(t)$ has no ω-limit points on the boundary. Then it converges to c^*, as the following argument shows. Consider an arbitrary sequence t_n with $t_n \to \infty$. The sequence $c(t_n)$ is bounded and thus has a convergent subsequence. The limit of this subsequence is an ω-limit point and can thus only be c^*. Hence $c(t_n)$ converges to c^*. The ω-limit set is connected and so it cannot be that one solution has both c^* and a point on the boundary as ω-limit points. Either the solution converges to c^* or its ω-limit set is contained in the boundary. In one of the first important papers on this subject, by Horn and Jackson [16], it was claimed that the second case was impossible. Later Horn [17] stated that he had no proof for this fact although he still expected it would be true. Forty years later there was still no proof known although the claim, which became known as the 'global attractor conjecture' was proved under various additional assumptions. At the beginning of 2015 a paper of Gheorghe Craciun appeared on the Internet where he proposed a proof [4].

For a network of deficiency zero that is not weakly reversible the Deficiency Zero Theorem tells us that there are no positive stationary solutions but we obtain no detailed information about the long-time behaviour of the solution. It is at least possible to show that there are no positive periodic solutions. Let $c(t)$ be a periodic solution. Then there exists a T with $c(t+T) = c(t)$. This implies that

$$0 = \int_t^{t+T} \dot{c}(t)dt = (YA_k)(\int_t^{t+T} \Psi(c(t))dt). \tag{54}$$

We see that $\int_t^{t+T} \Psi(c(t))dt$ is in the kernel of YA_k. Since the deficiency is zero this vector is according to Corollary 3.6 in the kernel of A_k. If there is a positive vector in the kernel of A_k the network is weakly reversible, contradicting the assumption that a periodic solution exists.

6 The Deficiency One Theorem

Now we formulate the Deficiency One Theorem as in [11]. First we need to define the deficiency of a linkage class. It is defined to be $\delta = n - 1 - s$ where n is the number of complexes in the class and s is the dimension of the space which is spanned by the vectors $\omega_{y'} - \omega_y$ for yy' in the class. This means that we consider the linkage class as an independent network and consider its deficiency. If the rank of the ith class is denoted by s_i then $s \leq \sum_i s_i$. Correspondingly, when δ_i is the deficiency of the ith linkage class then $\delta \geq \sum_i \delta_i$. The Deficiency One Theorem is not only concerned with networks of deficiency one. To understand this better it is useful to formulate the main assumption of the Deficiency Zero Theorem in a new way. Instead of requiring that the deficiency of the network is zero it is possible to require that the deficiency of each linkage class is no greater than zero and that $\delta = \sum_i \delta_i$. Then this assumption can be generalized by requiring that the δ_i are no greater than one.

Theorem 6.1 (The Deficiency One Theorem) Let $(\mathcal{S}, \mathcal{C}, \mathcal{R})$ be a reaction network with deficiency δ. Let δ_i, $1 \leq i \leq l$ be the deficiency of the ith linkage class. Suppose that

(i) $\delta_i \leq 1, 1 \leq i \leq l$

(ii) $\sum_{i=1}^{l} \delta_i = \delta$

(iii) each linkage class of the network contains precisely one terminal strong strong linkage class

If the system of ordinary differential equations which arises from this network by applying mass action kinetics has a positive stationary solution then there is exactly one positive stationary solution in each stoichiometric class. If the network is weakly reversible then it admits a positive stationary solution.

In networks which satisfy the assumptions of this theorem the deficiency can be arbitrarily large. On the other hand, not every network of deficiency one satisfies the assumptions of the theorem. In particular there is the case where $\delta = 1$ while $\delta_i = 0$ for all i. When there is only one linkage class assumption (ii) is automatically satisfied and the deficiency of the network is no larger than one.

Theorem 6.1 will be proved together with another statement which will now be formulated.

Theorem 6.2 When a network satisfies the assumptions of Theorem 6.1 and admits a positive stationary solution c^* the intersection of the kernel of $Df(c^*)$ with the stoichiometric class of c^* consists of the zero vector.

To prove Theorem 6.1 we use the following strategy. It is first shown that when conditions (i)-(iii) of Theorem 6.1 are satisfied and there exists a positive stationary solution c^* then the set of stationary solutions identical to the set E introduced in Section 4. Then Corollary 4.1 can be applied to show that there is exactly one positive stationary solution in each stoichiometric class. To complete the proof of Theorem 6.1 it remains to prove the existence of c^* in the weakly reversible case. For a fixed choice of c^* let κ be the function on $\mathcal{R}$ which is defined by the condition $\kappa(yy') = k_{yy'}(c^*)^y$.

Proposition 6.1 Suppose that a network is given which satisfies conditions (i)-(iii) of Theorem 6.1 and let κ be a function on $\mathcal{R}$ which satisfies the condition

$$\sum_{\mathcal{R}} \kappa(yy')(y' - y) = 0 \tag{55}$$

Suppose that $F(\mathcal{S})$ is endowed with a scalar product (not necessarily the standard one) and let $\phi : \mathbb{R} \to \mathbb{R}$ be a function which is continuous and strictly monotone. A function μ on $\mathcal{S}$ satisfies

$$\sum_{\mathcal{R}} \kappa(yy')\phi(y \cdot \mu)(y' - y) = 0 \tag{56}$$

precisely when wenn μ is orthogonal to the stoichiometric subspace.

Before we prove Proposition 6.1 we formulate a further statement.

Proposition 6.2 For a reaction network let κ be a function on $\mathcal{R}$ which satisfies the condition

$$\sum_{\mathcal{R}} \kappa(yy')(\omega_{y'} - \omega_y) = 0 \tag{57}$$

Suppose that $F(\mathcal{S})$ is equipped with an inner product (which need not be the standard one) and let $\phi : \mathbb{R} \to \mathbb{R}$ be a function which is continuous and strictly monotone. If ϕ is increasing or decreasing then we have the inequaliity

$$\sum_{\mathcal{R}} \kappa(yy')\phi(y \cdot \mu)(y' - y) \cdot \mu \leq 0 \quad \text{or} \quad \geq 0 \quad \text{respectively} \tag{58}$$

for all $\mu \in F(\mathcal{R})$. Moreover the following statements are equivalent
(i) $\sum_{\mathcal{R}} \kappa(yy')\phi(y \cdot \mu)(y' - y) \cdot \mu = 0$
(ii) μ is orthogonal to the stoichiometric subspace
(iii) $\sum_{\mathcal{R}} \kappa(yy')\phi(y \cdot \mu)(y' - y) = 0$

Proof We suppose that ϕ is monotone increasing. The proof for the monotone decreasing case is similar. Let Φ be a primitive of ϕ. It follows from the fact that ϕ is monotone increasing and with the help of the mean value theorem that $\phi(a)(b - a) \leq \Phi(b) - \Phi(a)$ for all a and b in $\mathbb{R}$ and equality holds only when $a = b$. Together with the positivity of κ this inequality implies that

$$\sum_{\mathcal{R}} \kappa(yy')\phi(y \cdot \mu)(y' - y) \cdot \mu \leq \sum_{\mathcal{R}} \kappa(yy')[\Phi(y' \cdot \mu) - \Phi(y \cdot \mu)] \tag{59}$$

with equality only when $(y' - y) \cdot \mu = 0$ for all $yy' \in \mathcal{R}$, i.e. when μ is orthogonal to the stoichiometric subspace. The right hand side of the inequlity can be written in the form

$$\left\{ \sum_{\mathcal{R}} \kappa(yy')(\omega_{y'} - \omega_y) \right\} * \left\{ \sum_{y'' \in \mathcal{C}} \Phi(y'' \cdot \mu) \right\} \tag{60}$$

where the star denotes the usual inner product in $F(\mathcal{C})$. When the assumption of the proposition holds then the right hand side of (59) vanishes for all μ.

This proves the first statement of the proposition and conditions (i) and (ii) are equivalent. It is clear that (iii) implies (i). It remains to show that (ii) implies (iii). The assumption of the proposition implies the analogous statement where the sum is only taken over a given linkage class. This then implies the corresponding statement where $\omega_{y'} - \omega_y$ is replaced by $y' - y$. That (ii) implies (iii) follows from this observation and the following lemma.

Lemma 6.1 For a reaction network let κ be a function on $\mathcal{R}$ which satisfies the condition

$$\sum_{yy' \in L^\theta} \kappa(yy')(y' - y) = 0 \tag{61}$$

for each linkage class L^θ. Suppose that $F(\mathcal{S})$ is equipped with an inner product (not necessarily the standard one). If μ is orthogonal to the stoichiometric subspace then for for each function $\phi : \mathbb{R} \to \mathbb{R}$ the equation

$$\sum_{\mathcal{R}} \phi(y \cdot \mu)\kappa(yy')(y' - y) = 0. \tag{62}$$

holds.

Proof If μ is orthogonal to the stoichiometric subspace then $y \cdot \mu = y' \cdot \mu$ always holds when y and y' are in the same stoichiometric class. Thus there exist numbers z_θ with the property that $y \cdot \mu = z_\theta$ for all $y \in L^\theta$. Hence the left hand side of the equation (62) is of the form

$$\sum_{\theta=1}^{l} \phi(z_\theta) \sum_{yy' \in L^\theta} \kappa(yy')(y' - y) \tag{63}$$

The lemma follows.

This means that Proposition 6.2 is also proved.

Lemma 6.2 Consider a reaction network which satisfies condition (ii) in Theorem 6.1 and a function κ on $\mathcal{R}$ for which the equation

$$\sum_{\mathcal{R}} \kappa(yy')(y' - y) = 0 \tag{64}$$

holds. Suppose that $F(\mathcal{S})$ is equipped with an inner product (not necessarily the standard one). If μ is orthogonal to the stoichiometric subspace then for every function $\phi : \mathbb{R} \to \mathbb{R}$ the relation

$$\sum_{\mathcal{R}} \kappa(yy')\phi(y \cdot \mu)(y' - y) = 0. \tag{65}$$

holds.

Proof The equation in the assumption can be written in the form

$$\sum_{\theta=1}^{l} \sum_{yy' \in L^\theta} \kappa(yy')(y' - y) = 0. \tag{66}$$

The sum over L^θ lies in the subspace Δ^θ, whose dimension is s_θ. The stoichiometric subspace is the sum of the subspaces Δ^θ and the sum is direct precisely in the case that $s = \sum_{\theta=1}^{l} s_\theta$. The last equation is equivalent to condition (ii) of Theorem 6.1. Thus the vanishing of the sum in the assumption is equivalent to the vanishing of the individual summands. Hence Lemma 6.2 follows from Lemma 6.1. Now we have proved one direction in Proposition 6.1. The proof of the other direction will be carried out in several steps. First Proposition 6.1 will be formulated in a slightly different language.

Proposition 6.1 (new formulation) Let a reaction network be given which saisfies the conditions (i)-(iii) of Theorem 6.1 and let κ be a function on $\mathcal{R}$ for which the condition $\omega_{\mathcal{C}} \in \ker(YA_\kappa)$ holds. Suppose that $F(\mathcal{S})$ is equipped with an inner product (not necessarily the standard one) and let $\phi : \mathbb{R} \to \mathbb{R}$ be a function which is continuous and strictly monotone. A function μ on $\mathcal{S}$ satisfies the condition $\sum_{y\in\mathcal{C}} \phi(y \cdot \mu)\omega_y \in \ker(YA_k)$ if and only if μ is orthogonal to the stoichiometric subspace.

Here $\omega_{\mathcal{C}}$ denotes the function on $\mathcal{C}$ which is identically equal to one. The quantity A_κ is defined in the same way as the A_k we know already. It is possible to define an object A_α of this type for any function $\alpha \in F(\mathcal{R})$. The analogue of Proposition 3.1 holds for A_α.

Lemma 6.3 For a reaction network in which each linkage class contains only one terminal strong linkage class the relation $\dim(\ker(YA_\alpha)) = \delta + l$ holds for each $\alpha \in F(\mathcal{R})$.

Proof Note first that

$$\dim(\ker(YA_\alpha)) = n - \dim(\mathrm{im}(YA_\alpha)) \tag{67}$$

and $\mathrm{im}A_\alpha = \mathrm{span}(\Delta)$. The dimension of $\mathrm{im}(YA_\alpha)$ is the rank of the network. Thus $\dim(\ker(YA_\alpha)) = n - s$. The result follows from the definition of the deficiency.

For a given network and subsets P, Q of $\mathcal{C}$ we denote by $P \to Q$ the set of reactions in $\mathcal{R}$ for which the left hand side is in P and the right hand side in Q. If $P \subset \mathcal{C}$ the complement of P is denoted by P'.

Lemma 6.4 Suppose that a reaction network and a function α on $\mathcal{C}$ are given. If x and z are funktions on $\mathcal{C}$ which satisfy $z = A_\alpha x$ then for each subset P of $\mathcal{C}$ we have the relation

$$\sum_{y\in P} z(y) = \sum_{P'\to P} \alpha_{y'y}x(y') - \sum_{P\to P'} \alpha_{yy'}x(y). \tag{68}$$

Proof For each $y \in \mathcal{C}$ we have

$$z(y) = \sum_{\mathcal{C}\to\{y\}} \alpha_{y'y}x(y') - \sum_{\{y\}\to\mathcal{C}} \alpha_{yy'}x(y). \tag{69}$$

If this relation is summed over $y \in P$ we get

$$\sum_{y\in P} z(y) = \sum_{\mathcal{C}\to P} \alpha_{y'y}x(y') - \sum_{P\to\mathcal{C}} \alpha_{yy'}x(y). \tag{70}$$

It is also true that

$$\mathcal{C} \to P = \{P \to P\} \cup \{P' \to P\}, \quad P \to \mathcal{C} = \{P \to P\} \cup \{P \to P'\}. \tag{71}$$

Thus the contributions of the reactions in $P \to P$ cancel and the desired relation is obtained.

Now the remaining part of Proposition 6.1 will be proved in the special case $l = 1$. For this the new formulation will be used. In this case the condition (ii) in Theorem 6.1 is automatically satisfied. Condition (iii) becomes $t = 1$ and condition (i) means that the deficiency is zero or one. It is assumed that an inner product on $F(\mathcal{S})$ and a monotone increasing function ϕ are given. (The proof of Proposition 6.1 in the monotone decreasing case is strictly analogous.) Let us suppose that for a positive function κ on $\mathcal{R}$ the condition $\omega_{\mathcal{C}} \in \ker(YA_\kappa)$ holds. The aim is to show that $\mu \in F(\mathcal{S})$ only satisfies the condition

$$\sum_{y \in \mathcal{C}} \phi(y \cdot \mu)\omega_y \in \ker(YA_\kappa) \tag{72}$$

if μ is orthogonal to the stoichiometric subspace. For a network with $l = 1$ the condition on μ is equivalent to the condition that $y \cdot \mu = y' \cdot \mu$ for all y and y' in $\mathcal{C}$.

In the case that $\omega_{\mathcal{C}}$ not only lies in $\ker(YA_\kappa)$ but also in $\ker A_\kappa$ the condition

$$A_\kappa \omega_{\mathcal{C}} = \sum_{\mathcal{R}} \kappa_{yy'}(\omega_{y'} - \omega_y) = 0 \tag{73}$$

holds. It follows that Proposition 6.1 holds in that case. Hence it will now be supposed that $\omega_{\mathcal{C}}$ does not lie in $\ker A_\kappa$. In that case $\ker(YA_\kappa)$ and $\ker A_\kappa$ are not identical and the deficiency must be one. It follows from Lemma 6.3 that $\dim \ker(YA_\kappa) = 2$. We now choose a certain basis of $\ker(YA_\kappa)$. Let b be a vector in $\ker A_\kappa$ which is positive on Λ, the only terminal strong linkage classe, and vanishes outside Λ. The existence of a vector of this kind follows from Proposition 3.1 and it is unique according to Lemma 3.6. The vectors b and $\omega_{\mathcal{C}}$ are a basis of $\ker(YA_\kappa)$.

Now suppose that μ satisfies the condition (72). Then there exist numbers ξ and η such that $\sum_{y \in \mathcal{C}} \phi(y \cdot \mu)\omega_y = \xi\omega_{\mathcal{C}} + \eta b$. This statement is equivalent to the statement that $\phi(y \cdot \mu) = \xi + \eta b(y)$ for all $y \in \mathcal{C}$. We can conclude that $y \cdot \mu = y' \cdot \mu$ if neither y nor y' is in the terminal strong linkage class. The strict monotonicity of ϕ is used to prove this.

The aim is now to show that μ is orthogonal to the stoichiometric subspace or, in other words, that $y \cdot \mu = y' \cdot \mu$ for all y and y' in $\mathcal{C}$. Since ϕ is strictly monotone and thus, in particular, injective it suffices to show that η can only be zero. This is done by showing that it can neither be positive nor negative. First arrange the complexes in a sequence $y^{(1)}, y^{(2)}, \ldots y^{(n)}$ so that $b(y^{(1)}) \geq b(y^{(2)}) \geq \ldots b(y^{(n)})$. If the number of complexes in the terminal strong linkage class is p then clearly the first p complexes in the sequence belong to Λ. One of the inequalities must be strict since otherwise b and $\omega_{\mathcal{C}}$ would be proportional, a contradiction.

If $\eta > 0$ then $y^{(1)} \cdot \mu \geq y^{(2)} \cdot \mu \geq \ldots \geq y^{(n)} \cdot \mu$. The strict inequality holds precisely when the strict inequality holds at the corresponding point in the other chain of inequalities. When $\eta < 0$ we get a similar chain of inequalities in the other direction. Since $\omega_{\mathcal{C}}$ is not contained in $\ker A_\kappa$ the vector $g = A_\kappa \omega_{\mathcal{C}}$ is not zero. We have $g \in \ker Y \cap \mathrm{span}(\Delta)$. The relations $\sum_{y \in \mathcal{C}} g(y)y = 0$ und $\sum_{y \in \mathcal{C}} g(y) = 0$ also hold. From these equations we can also derive the following relation

$$g(y^{(1)})(y^{(1)} - y^{(2)}) + (g(y^{(1)}) + g(y^{(2)}))(y^{(2)} - y^{(3)}) + \ldots \\ + \sum_{j=1}^{n-1} g(y^{(j)})(y^{(n-1)} - y^{(n)}) = 0. \tag{74}$$

The left hand side is in fact equal to

$$(g(y^{(1)})y^{(1)} + \ldots + g(y^{(n)})y^{(n)}) - y^{(n)}(g(y^{(1)}) + \ldots + g(y^{(n)})). \tag{75}$$

Now we take the inner product of this relation with μ, with the result

$$\sum_{i=1}^{n-1} (\sum_{j=1}^{i} g(y^{(j)}))(y^{(i)} \cdot \mu - y^{(i+1)} \cdot \mu) \tag{76}$$

It will be shown that when η is positive then each summand in the outer sum is non-negative and at least one is positive. It will also be shown that when η is negative then each summand in the outer sum is non-positive and at least one is negative. In both cases a contradiction is obtained. To prove the desired statements the following lemma will be used.

Lemma 6.5 For a reaction network and a positive function κ on $\mathcal{R}$ let A_κ be defined as above. Let Λ be a terminal strong linkage class and b an element of $\ker A_\kappa$ with support Λ. If the complexes are listed in a sequence as above then for $1 \leq i \leq p$ the inequality $\sum_{i=1}^{j} g(y^{(j)}) \geq 0$ holds. For $1 \leq i < p$ this inequality is strict when $b(y^{(i)}) > b(y^{(i+1)})$. When $i = m$ the inequality is strict when Λ is smaller than the linkage class it is contained in.

Proof Let P be an arbitrary subset of $\mathcal{C}$ and P' its complement. Since $A_\kappa \omega_{\mathcal{C}} = g$ and $A_\kappa b = 0$ it follows from Lemma 6.4 that

$$\sum_{P' \to P} \kappa_{y'y} - \sum_{P \to P'} \kappa_{yy'} = \sum_{y \in P} g(y), \quad \sum_{P' \to P} \kappa_{y'y} b(y') - \sum_{P \to P'} \kappa_{yy'} b(y) = 0 \tag{77}$$

We use this statement in the case $P = \Lambda$. Since Λ is terminal there are no reactions in $\Lambda \to \Lambda'$ and we obtain $\sum_{\Lambda' \to \Lambda} \kappa_{y'y} = \sum_{j=1}^{p} g(y^{(j)})$. If Λ is smaller than the linkage class it is contained in there is at least one non-trivial on the left hand side and both sides are positive. If Λ is a whole linkage class then both sides are zero. To prove the remaining part of Lemma 6.5 we choose P as the set I consisting of the y^j with $1 \leq j \leq i < p$. Using (77) we have

$$b(y^{i+1})(\sum_{I' \to I} \kappa_{y'y}) - b(y^i)(\sum_{I \to I'} \kappa_{yy'}) \geq 0. \tag{78}$$

Since $b(y^i) > b(y^{i+1})$ it follows that $\sum_{I' \to I} \kappa_{y'y} - \sum_{I \to I'} \kappa_{yy'} \geq 0$. Equality holds in the inequality only when equality holds in the previous inequality. Thus the lemma is proved.

For a network with only one linkage class we can now prove that η must vanish.

Next Proposition 6.1 will be proved for networks with arbitrarily many linkage classes. A partition of the reaction network $(\mathcal{S}, \mathcal{C}, \mathcal{R})$ is set of subnetworks $(\mathcal{S}, \mathcal{C}_i, \mathcal{R}_i)$ mit the property that dass $\mathcal{R}$ is the disjoint union of the $\mathcal{R}_i$. The partition is called direct if the rank of the network is the sum of the ranks of the subnetworks. If S is the stoichiometric subspace of the network and S_i the stoichiometric subspace of the subnetworks then S is the sum of the S_i and this sum is direct precisely when the partition is direct. The next lemma is a generalization of Lemma 6.2.

Lemma 6.6 Suppose that a network admits a partition into subnetworks which each consist of only one linkage class. Let κ be a positive function on $\mathcal{R}$ which satisfies

$$\sum_{\mathcal{R}} \kappa_{yy'}(y - y') = 0. \tag{79}$$

Let an inner product be given on $F(\mathcal{S})$ and let $\phi : \mathbb{R} \to \mathbb{R}$ be a function. Then a function μ on $\mathcal{S}$ satisfies

$$\sum_{\mathcal{R}} \kappa_{yy'} \phi(y \cdot \mu)(y - y') = 0 \tag{80}$$

if it is orthogonal to the stoichiometric.

Proof The sums in the two conditions can be written as a sum over i of sums over $\mathcal{R}_i$. Because the partition is direct the vanishing of the sum is equivalent to the vanishing of all summands. If μ is orthogonal to S then $y \cdot \mu = y' \cdot \mu$ whenever y and y' are in the same linkage class. Since each subnetwork consists of only one linkage class $y \cdot \mu = y' \cdot \mu$ for all y and y' in $\mathcal{C}_i$. This proves the lemma.

Proposition 6.1 is a direct consequence of the next Proposition.

Proposition 6.3 Suppose a network $(\mathcal{S}, \mathcal{R}, \mathcal{R})$ is given which admits a partition into subnetworks with the properties that
(i) the deficiency of each subnetwork is either zero or one
(ii) the partition is direct
(iii) each subnetwork contains only one terminal strong linkage class
Let κ be a positive function on $\mathcal{R}$ which satisfies the condition

$$\sum_{\mathcal{R}} \kappa_{yy'}(y - y') = 0. \tag{81}$$

Let a scalar product and a monotone function be given as in previous cases. A function μ on $\mathcal{S}$ satifies

$$\sum_{\mathcal{R}} \kappa_{yy'} \phi(y \cdot \mu)(y - y') = 0 \tag{82}$$

if and only if μ is orthogonal to the stoichiometric subspace.

Proof The forward direction follows from Lemma 6.6. For the backwards direction we should first remember that the vanishing of the sums in the two conditions is equivalent with the vanishing of all summands. If μ is not orthogonal to S then there must exist an i for which μ is not orthogonal to S_i. The ith subnetwork only contains one linkage class and satisfies the conditions of Proposition 6.1. Since Proposition 6.1 was already proved in that case we have a contradiction.

We must see that Proposition 6.1 follows from Proposition 6.3. This is achieved by the following lemma

Lemma 6.7 A network which satisfies condition (ii) of Proposition 6.1 admits a partition into networks each of which has only one linkage class. If in addition the network satisfies conditions (i) und (iii) of Proposition 6.1 then it admits a partition which satisfies all conditions of Proposition 6.3.

Proof We take the linkage classes as the $\mathcal{C}_i$ and take the reactions from $\mathcal{C}_i$ to itself as $\mathcal{R}_i$. When condition (ii) of Proposition 6.1 is satisfied then this partition is direct. Each subnetwork has only one linkage class. It is then clear that when the network satisfies conditions (i) and (iii) of Proposition 6.1 this partition has the desired properties.

The results we have now obtained essentially give the proof of Theorem 6.2. It nevertheless remains to explain what these results have to do with the derivative of f at the stationary solution. The derivative is given by the formula

$$D_c f(c^*)\gamma = \sum_{\mathcal{R}} \kappa_{yy'}(y \cdot \gamma)(y' - y) \tag{83}$$

where the inner product is defined by

$$x \cdot z = \sum_{i=1}^{m} \frac{x_i z_i}{c_i^*}. \tag{84}$$

In order to prove Theorem 6.2 it is enough to show that each vector in the kernel of $D_c f$ is orthogonal to the stoichiometric subspace with respect to the given inner product. There are statements we have proved which are precisely of this type. In the present case we choose $\phi(x) = x$. The formula for the derivative of f can also be used to obtain an interesting conclusion about networks of deficiency zero. The result can be found in [11] but it does not see to have attracted much attention in the literature. We consider a network which is weakly reversible and satisfies $\delta = 0$. It will be shown that the restriction of the linear mapping $D_c f(c^*)$ to the stoichiometric subspace is negative definite with respect to the unusual inner product which has already been introduced. This means that all its eigenvalues have negative real part. The stationary solution is not only asymptotically stable but also hyperbolic. In Proposition 6.2 we set $\kappa_{yy'} = k_{yy'}(c^*)^y$ and $\phi(x) = x$ to obtain that $\gamma \cdot D_c f(c^*)\gamma \leq 0$ for all $\gamma \in F(\mathcal{S})$ with equality only when $\gamma \in S^{\perp}$.

We have also already proved most of Theorem 6.1. It remains to prove the existence of a positive stationary solution when the network is weakly reversible.

First we consider the case of a network which has only one linkage class and satisfies condition (i) of Theorem 6.1. Then the deficiency of the network is zero or one. We are looking for a positive function c on $\mathcal{S}$ which satifies

$$\sum_{\mathcal{R}} k_{yy'}(c)^y(y'-y) = 0. \tag{85}$$

This is equivalent to the condition that $\sum_{y\in\mathcal{C}}(e^{y\cdot\log c}\omega_y) \in \ker YA_k$. It follows from Proposition 3.1 that $\ker YA_k$ is non-trivial.

Lemma 6.8 The following statements are equivalent
(i) there exists a positive function c on $\mathcal{S}$ with $\sum_{y\in\mathcal{C}}(e^{y\cdot\log c}\omega_y) \in \ker YA_k$.
(ii) there exists a function z on $\mathcal{S}$, a number ξ and a positive function $a \in \ker YA_k$ so that

$$Y^T z + \xi\omega_{\mathcal{C}} = \log a \tag{86}$$

Proof First we show that (ii) implies (i). Let z, ξ and a be as in (ii), let $c = e^z$ and let $\lambda = e^{-\xi}$. Then

$$Y^T \log c = \log a + (\log\lambda)\omega_{\mathcal{C}} = \log(\lambda a). \tag{87}$$

The function λa is in $\ker YA_k$ and $Y^T(\log c) = \sum_{y\in\mathcal{C}}(y\cdot\log c)\omega_y$. It follows that (i) holds. The proof of the other direction is similar.

Thus our problem reduces to show that the set Γ of functions on $\mathcal{C}$ of the form $\log a$ with a in $\ker YA_k$ intersects the vector space $U = \mathrm{im}Y^T + \mathrm{span}(\omega_{\mathcal{C}})$. In the case with one linkage class and $\delta = 0$ the problem is already solved. Thus we can assume w.l.o.g. that $\delta = 1$. In this case U is according to Lemma 5.2 a hyperplane in $F(\mathcal{C})$. To show that the connected set Γ intersects the subspace U it suffices to show that there are elements of Γ on both sides of U.

Let g be a fixed element of the one-dimensional linear subspace $U^\perp = \ker Y \cap \mathrm{span}(\Delta)$. It will now be shown that there exist elements γ^+ and γ^- of Γ with $g\cdot\gamma^+ > 0$ and $g\cdot\gamma^+ < 0$. Because Γ is connected the existence of γ^+ and γ^- implies the existence of a γ^0 with $g\cdot\gamma^0 = 0$. Then γ^0 is in U.

We examine the convex cone consisting of the positive elements of $\ker YA_k$. It follows from Lemma 6.3 that $\dim(\ker YA_k) = 2$. We can find a basis $\{p^1, p^2\}$ of $\ker YA_k$ consisting of non-negative vectors such that the cone consists of those vectors which can be written in the form $\lambda_1 p^1 + \lambda_2 p^2$ with λ_1 und λ_2 positive. p^1 und p^2 lie in the two extreme rays of the cone. Both of them lie in the boundary of the positive orthant. Thus there are complexes y and y' so hat $p^1(y) = 0$ and $p^2(y') = 0$.

Since A_k takes values in $\mathrm{span}(\Delta)$ and p^1 und p^2 are both in $\ker YA_k$ it must be the case that $A_k p^1$ and $A_k p^2$ are in the one-dimensional subspace $(\ker Y) \cap \mathrm{span}(\Delta)$. Thus there exist numbers ξ^1 und ξ^2 with the property that $A_k p^1 = \xi^1 g$ and $A_k p^2 = \xi^2 g$.

Lemma 6.9 The numbers ξ^1 and ξ^2 are non-zero and have opposite signs.

Beweis Let x be a positive element of $\ker A_k$. Since $\ker A_k$ is contained in $\ker YA_k$ the special properties of the basis $\{p^1, p^2\}$ ensure that there exist positive numbers λ_1 and λ_2 so that $x = \lambda_1 p^1 + \lambda_2 p^2$. If we apply A_k to this equation

we get $(\lambda_1\xi^1 + \lambda_2\xi^2)g = 0$. Since g is not zero and λ_1 and λ_2 are positive it must either be the case that ξ^1 und ξ^2 are both non-zero and have opposite signs or that both are zero. The second alternative leads to a contradiction. For the case of a weakly reversible network with one linkage class considered here Proposition 3.1 implies that $\ker A_k$ is one-dimensional. If ξ^1 and ξ^2 were both zero then p^1 and p^2 would be proportional. Then they could not be a Basis of $\ker Y A_k$.

Lemma 6.10 Let p be a non-vanishing non-negative function on $\mathcal{C}$ whose support is not the whole of $\mathcal{C}$. Then $\omega_{\text{supp } p} \cdot A_k p < 0$.

Proof Let $(\text{supp } p)'$ be the complement of supp p. We have using Lemma 6.4

$$\omega_{\text{supp } p} \cdot A_k p = - \sum_{\text{supp } p \to (\text{supp } p)'} k_{yy'} p_y. \tag{88}$$

The sets supp p und $(\text{supp } p)'$ are not empty. Because the network is weakly reversible and only has one linkage class the set supp $p \to (\text{supp } p)'$ is also non-empty. This proves the lemma.

Lemma 6.11 If p^1, p^2 und g are as before then $\omega_{\text{supp } p^1} \cdot g$ and $\omega_{\text{supp } p^2} \cdot g$ are non-zero and have opposite signs.

Proof From Lemma 6.10 it follows that

$$\begin{aligned} \omega_{\text{supp } p^1} \cdot A_k p^1 &= \xi^1(\omega_{\text{supp } p^1} \cdot g) < 0 \\ \omega_{\text{supp } p^2} \cdot A_k p^2 &= \xi^2(\omega_{\text{supp } p^2} \cdot g) < 0 \end{aligned} \tag{89}$$

From Lemma 6.9 it follows that ξ^1 and ξ^2 have opposite, which gives the desired result.

Now we examine the set Γ. We are interested in the sign of $g \cdot \log(\lambda_1 p^1 + \lambda_2 p^2)$ in its dependence on λ_1 and λ_2. First we set $\lambda_2 = 1$ and let λ_1 become large.

$$\begin{aligned} \log(\lambda_1 p^1 + \lambda_2 p^2) = (\log \lambda_1)\omega_{\text{supp } p^1} + \sum_{y \in \text{supp } p^1} \log(p^1(y) + p^2(y)/\lambda_2)\omega_y \\ + \sum_{y \in (\text{supp } p^1)'} \log(p^2(y))\omega_y. \end{aligned} \tag{90}$$

We see that for λ_1 large $g \cdot \log(\lambda_1 p^1 + \lambda_2 p^2)$ has the same sign as $g \cdot \omega_{\text{supp } p^1}$. Interchanging the roles of λ_1 and λ_2 in this argument gives a similar statement in the case where λ_2 becomes large. From Lemma 6.11 we get

Lemma 6.12 The set Γ contains elements γ^+ and γ^- with $g \cdot \gamma^+ > 0$ and $g \cdot \gamma^- < 0$.

This completes the proof of the existence of a positive stationary solution in this case.

Now this result will be extended to certain networks with more than one linkage class.

Proposition 6.4 Suppose that a network $(\mathcal{S}, \mathcal{C}, \mathcal{R})$ admits a partition into subnetworks in such a way that

(i) the deficiency of each subnetwork is either zero or one

(ii) the partition is direct
(iii) each subnetwork has only one linkage class
Then the system of equations which arises from this network by the assumption of mass action kinetics has a positive stationary solution.

A weakly reversible network which satisfies the assumptions of Theorem 6.1 admits a partition as in the assumptions of Proposition 6.4. Hence the proof of Theorem 6.1 will be complete when Proposition 6.4 has been proved. For the proof of Proposition 6.4 another terminology is necessary. The mass action system for a subnetwork $(\mathcal{S}, \mathcal{C}', \mathcal{R}')$ is obtained by restricting the reaction constants k of $\mathcal{R}$ to $\mathcal{R}'$. The results obtained up to this point show that the systems which arise from the subnetworks have positive stationary solutions.

Lemma 6.13 Suppose that a network $(\mathcal{S}, \mathcal{C}, \mathcal{R})$ admits a direct partition into subnetworks $(\mathcal{S}, \mathcal{C}', \mathcal{R}')$ and $(\mathcal{S}, \mathcal{C}'', \mathcal{R}'')$ and that there are positive functions c' and c'' on $\mathcal{S}$ with the property that the sets

$$\begin{aligned} &\{c : \log c - \log c' \in (S')^{\perp}\}, \\ &\{c : \log c - \log c'' \in (S'')^{\perp}\} \end{aligned} \tag{91}$$

consist entirely of stationary solutions of the two subnetworks. Then the network $(\mathcal{S}, \mathcal{C}, \mathcal{R})$ also has a positive stationary solution. The exists a positive function c^* on $\mathcal{S}$ with the property that the set

$$\{c : \log c - \log c^* \in S^{\perp}\} \tag{92}$$

consists entirely of stationary solutions of the network $(\mathcal{S}, \mathcal{C}, \mathcal{R})$.

Proof It is not difficult to see that each positive function which is a stationary solution for the two subnetworks is also a stationary solution for the original network. Thus we need only show that the two subsets in (91) intersect. The affine subspaces $\log c' + (S')^{\perp}$ and $\log c'' + (S'')^{\perp}$ intersect precisely when $\log c' - \log c'' \in (S')^{\perp} + (S'')^{\perp}$. Note that $(S')^{\perp} + (S'')^{\perp} = (S' \cap S'')^{\perp}$. Since the partition is direct $S' \cap S'' = \{0\}$ and thus $(S')^{\perp} + (S'')^{\perp} = F(\mathcal{S})$. Hence the condition on $\log c' - \log c''$ is obviously satisfied. Let z be a point of the intersection and $c^* = e^z$. The point c^* is a stationary solution of the original system. The intersection of the two affine subspaces is $\log c^* + [(S')^{\perp} \cap (S'')^{\perp}]$. Hence each point c with $\log c - \log c^* \in (S')^{\perp} \cap (S'')^{\perp}$ is a point of the intersection and therefore a stationary solution of the original system. On the other hand $(S')^{\perp} \cap (S'')^{\perp} = S^{\perp}$ and so the lemma is proved.

This lemma gives

Lemma 6.14 Suppose that a network $(\mathcal{S}, \mathcal{C}, \mathcal{R})$ admits a direct partition into subnetworks $(\mathcal{S}, \mathcal{C}^i, \mathcal{R}^i)$ and that there exist non-negative functions c^i such that the sets

$$\{c : \log c - \log c^i \in (S^i)^{\perp}\} \tag{93}$$

consist entirely of stationary solutions of the subnetworks. Then the network $(\mathcal{S}, \mathcal{C}, \mathcal{R})$ also a positive stationary solution.

Lemma 6.15 Suppose that a network $(\mathcal{S}, \mathcal{C}, \mathcal{R})$ admits a direct partition into subnetworks $(\mathcal{S}, \mathcal{C}^i, \mathcal{R}^i)$ and that each subnetwork consists of a single linkage

class. Then the system coming from the original network also has a positive stationary solution.

Proof Let c^i be a positive stationary solution of the ith subnetwork. When considered as an independent network the ith subnetwork admits a positive stationary solution and therefore, according to Corollary 4.1, the corresponding subset E meets each stoichiometric compatibility class in precisely one point. It follows from Lemma 6.6 that E consists of stationary solutions. Hence we know where the stationary solutions are and Lemma 6.15 follows from 6.14.

Lemma 6.16 In a direct partition of a weakly reversible network each subnetwork is weakly reversible.

Proof Let yy' be a reaction in one of the subnetworks. In the original network there is a directed path from y' to y. Hence there is a closed path through y. It follows that there is a set of vectors which are linearly dependent in the original network. Because the partition is direct the closed path must lie in the subnetwork.

Proof of Proposition 6.4 The existence of a partition as assumed in the Proposition implies that each subnetwork is weakly reversible and has deficiency zero or one and hence it has a positive stationary solution. The Proposition follows from Lemma 6.15.

Now the results so far will be made more concrete by a few examples. We first consider the equations of enzyme kinetics which are known from an influential paper of Michaelis and Menten in 1913. We begin with a simple reaction in which a substrate S is converted to a product P. If the reaction is catalysed by another substance (for instance by an enzyme E in biology) then we can extend this description. It is assumed that the enzyme and the product bind to each other to form an enzyme-substrate complex $S \cdot E$. (The word 'complex' is used here in a different sense than the one occurring up to now in this course. When we use the word in the latter sense we always write enzyme-substrate complex.) Thus we have a reaction $E + S \to S \cdot E$. The enzyme-substrate complex can dissociate and release E and S. The reaction is $S \cdot E \to E + S$. It can however also happen that within the enzyme-substrate complex a reaction takes place in which the product is formed. Then the product and the enzyme are released. This process is described by the reaction $S \cdot E \to E + P$. These three reactions, with mass action kinetics are called elementary reactions. We call the whole description the extended Michaelis-Menten description. This name is supposed to avoid confusion with another description which plays no role in this course. In the latter there is only one reaction $E \to P$ and the kinetics is different from mass action. It is often known as Michaelis-Menten kinetics. We call the second description the effective Michaelis-Menten description. It results from the extended Michaelis-Menten description through a limiting process.

The equations of the extended Michaelis-Menten description of this single reaction are

$$\frac{dc_S}{dt} = -k_1 c_S c_E + k_2 c_{S\cdot E},$$
$$\frac{dc_E}{dt} = -k_1 c_S c_E + (k_2 + k_3) c_{S\cdot E},$$

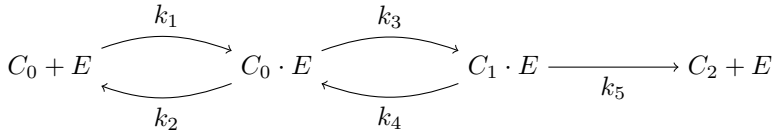
$$\frac{dc_{S\cdot E}}{dt} = k_1 c_S c_E - (k_2 + k_3) c_{S\cdot E},$$
$$\frac{dc_P}{dt} = k_3 c_{S\cdot E}. \tag{94}$$

There are four species, three complexes and three reactions. There are two linkage classes and the rank is two. The stoichiometric classes are the level surfaces of the conserved quantities $c_E + c_{S\cdot E}$ and $c_S + c_{S\cdot E} + c_P$. The deficiency is zero and the network is not weakly reversible. The Deficiency Zero Theorem shows that there are no positive stationary solutions. This is already obvious because of the fact that $-c_P$ is a Lyapunov function.

We now consider a phosphorylation system. A substance X is given which can be phosphorylated up to N times. We suppose again that the phosphate groups are attached in a particular order and removed in the reverse order. In this way there are compounds C_i with $0 \leq i \leq N$. In the first section we considered this system with mass action kinetics. Now we want to assume instead that each phosphorylation is catalysed by an enzyme E (called a kinase) and each dephosphorylation by an enzyme F (called a phosphatase). We use an extended Michaelis-Menten description for each reaction. It is assumed that only one phosphate group is changed during each binding event between substrate and enzyme (distributive phosphorylation). The species are then the substrates C_i with $0 \leq i \leq N$, the enzymes E and F, the substrate-enzyme complexes $C_i \cdot E$ with $0 \leq i \leq N-1$ and the substrat-enzyme complexes $C_i \cdot F$ with $1 \leq i \leq N$. We thus have $m = 3N + 3$. There are $4N + 2$ complexes, $6N$ reactions and two linkage classes. This system is called the multiple futile cycle [23]. It satisfies the condition $t = l$.

Now consider the simple futile cycle, i.e. the case $N = 1$. In this case we have $m = 6$, $n = 6$, $l = 2$, $s = 3$ and $\delta = 1$. The two linkage classes have deficiency zero. The Deficiency Zero Theorem cannot be useed and the Deficiency One Theorem is also not applicable because the second condition does not hold. This system has a unique positive stationary solution in each stoichiometric class and this solution is globally asymptotically stable in its class. This last statement is not easy to prove [1]. How is in the case of the dual futile cycle? In this case we have $m = 9$, $n = 10$, $l = 2$, $s = 6$ and $\delta = 2$. Both linkage classes have deficiency zero. In this case too both the Deficiency Zero Theorem and the Deficiency One Theorem fail to apply. It it known that this system exhibits multistationarity [23] - in many stoichiometric classes there exists more than one stationary solution.

A double phosphorylation does not have to be distributive. It could also be processive. In that case both phosphate groups are attached during one encounter between the substrate and the enzyme. There is more than one possibility for a mechanism of this type. Here we consider the following variant (cf. [3]). The phosphorylation has the mechanism shown in the figure and the dephosphorylation the strictly analogous one. I this case $m = 9$, $n = 8$, $l = 2$, $s = 5$ und $\delta = 1$. Both linkage classes have deficiency zero. There exist



conserved quantities $x_E + x_{C_0 \cdot E} + x_{C_1 \cdot E}$, $x_F + x_{C_1 \cdot F} + x_{C_2 \cdot F}$ and

$$x_{C_0} + x_{C_1} + x_{C_2} + x_{C_0 \cdot E} + x_{C_1 \cdot E} x_{C_1 \cdot F} + x_{C_2 \cdot F}. \tag{95}$$

Free C_1 does not participate in any reactions. Thus it can be left out of description of the system. Then the concentration of C_1 can also be removed from the last conserved quantity.

7 The Deficiency One Algorithm

The Deficiency One Theorem provides results about networks with arbitrarily large deficiency and there are networks with deficiency one for which this theorem gives no results. Another direction is given by the Deficiency One Algorithm. This algorithm can be applied to rather general networks of deficiency one. There are, however, some restrictions necessary in order to apply it. The networks which satisfy these conditions are called regular. We will now introduce these conditions. The first is

(R1) The reactions of the network are positively dependent, i.e. there exist positive numbers α_{ij} with the property that $\sum_{\mathcal{R}} \alpha_{ij}(y_j - y_i) = 0$.

This condition is necessary in order that there be a chance of a stationary solution. For weakly reversible networks it is always satisfied. In a weakly reversible network there is closed path through any complex. The sum of the vectors $y_j - y_i$ for successive reactions in this network is zero. We can then sum the quantities of this type which come from a set of closed paths whose union is the whole of $\mathcal{C}$.

(R2) Each linkage class of the network contains precisely one terminal strong linkage class.

(R2) is precisely condition (iii) of the Deficiency One Theorem. To state the third condition we need some more terminology. If there is a reaction from y to y' und removing the reactions between these complexes increases the number of linkage classes by one then this reaction is called a cut pair. (In graph theory this would be called a bridge.) We call a complex terminal when it lies in a terminal strong linkage class.

(R3) Each pair of terminal complexes which is joined by a reaction is joined by a cut pair.

A weakly reversible network which satisfies (R3) is reversible.

In [9] Feinberg suggested that networks which do not satisfy condition (R2) should perhaps not be taken too seriously. The idea is that a network of this type can be made into one with $t = l$ by complementing each irreversible reaction

with a reaction in the opposite direction with a very small reaction constant. This modification could lead to essential changes in the dynamics of the system. This means that properties which can only occur in system with $t < l$ would not be structurally stable and might therefore be irrelevant for applications. Es might also be the case that truly irreversible reactions do not occur in reality. It is, however, necessary to be careful with arguments of this type. With the same argument it would be possible to question the significance of networks which are not weakly reversible. If we consider a system which is not weakly reversible and has no positive stationary solutions and compare it with a perturbed system which is weakly reversible and has such solutions we should note that for very small vlaues of the perturbation parameter the concentrations which occur in the stationary solutions might be so large or so small that they are not biologically relevant. In any case, a perturbation of the type just mentioned does not change the deficiency of the network.

Independently of these aspects of a perturbation process of this kind it is possible to investigate the mathematical phenomena which occur in this situation. A simple example is provided by the following network with the reactions $A_1 \to A_2$, $A_1 \to A_3$, $A_2 + A_3 \to 2A_1$ and reactions constants $(1, k, 1)$. It has two linkage classes and three terminal strong linkage classes. The stoichiometric subspace is two-dimensional and $\delta = 1$. The evolution equations are

$$\begin{aligned} \dot{c}_1 &= -(k+1)c_1 + 2c_2c_3, \\ \dot{c}_2 &= kc_1 - c_2c_3, \\ \dot{c}_3 &= c_1 - c_2c_3 \end{aligned} \tag{96}$$

The quantity $c_1 + c_2 + c_3$ is conserved. We also have $\frac{d}{dt}(c_2 - c_3) = (k-1)c_1$. Thus for $k \neq 1$ there exists a Lyapunov function and no positive stationary solutions. For $k = 1$ this function is a second conserved quantity and there is a whole continuum of stationary solutions. They form the set with equation $c_1 = c_2c_3$.

In general it is possible to write the right hand side of the evolution equations in the form $\sum_{i=1}^{n} c^{y_i} \sum_{\mathcal{R}_{y_i \to}} k_{ij}(y_j - y_i)$. If we define $d_i = \sum_{\mathcal{R}_{y_i \to}} k_{ij}(y_j - y_i)$ then the right hand side is of the form $\sum_{i=1}^{n} c^{y_i} d_i$. We call the subspace of $F(\mathcal{C})$ which is spanned by the d_i the kinetic subspace S'. The vector space which is spanned by the image of Ψ is the whole of $F(\mathcal{C})$. For if the image of Ψ were smaller then there would exista generalized polynomial with non-trival coefficients which vanishes everywhere, a contradiction. Thus $S' = \mathrm{im}(YA_k) = Y(\mathrm{im}A_k)$. This relation may be compared with the relation $S = Y(\mathrm{span}(\Delta))$. If $\mathrm{im}A_k = \mathrm{span}(\Delta)$ then $S' = S$. It follows from Corollary 3.3 that $t = l$ is a sufficient condition for $S' = S$. It was shown in the proof of Proposition 3.2 that $\mathrm{dim}S = n - l - \mathrm{dim}((\mathrm{ker}Y) \cap \mathrm{span}(\Delta))$. It can be shown in a similar way that $\mathrm{dim}S' = n - t - \mathrm{dim}((\mathrm{ker}Y) \cap \mathrm{im}A_k)$. It follows that

$$\mathrm{dim}S - \mathrm{dim}S' = t - l - \delta + \delta'. \tag{97}$$

This relation allows some conclusions to be drawn. If $t - l > 0$ and $\delta < t - l$

then S and S' are always different. If $\delta = 0$ and hence $\delta' = 0$ then $S' = S$ if and only if $t = l$.

The derivative $\dot{c}$ is contained in the kinetic subspace. Der kinetic subspace is contained in the stoichiometric subspace but in general the two are not equal. The kinetic subspace is always invariant under the time evolution. While the stoichiometric subspace does not depend on the values of the reaction constants the kinetic subspace can do so. In the example the d_i are the vectors

$$k(\omega_2 - \omega_1) + (\omega_3 - \omega_1), 0, 0, 2\omega_1 - (\omega_2 + \omega_3), 0. \tag{98}$$

For $k \neq 1$ they are a basis of the stoichiometric subspace but for $k = 1$ they are linearly dependent and the kinetic subspace is one-dimensional. When the kinetic subspace is smaller than the stoichiometric subspace and then there is a stationary solution in one kinetic class which is non-degenrate in this class then there will also be similar solutions in neighbouring kinetic classes and there will be a continuum of stationary solutions within the stoichiometric compatibility class.

A non-empty set $\mathcal{A}$ is called *absorptive* if there is no reaction from a complex in $\mathcal{A}$ to a complex outside $\mathcal{A}$.

Definition 7.1 A confluence vector for a reaction network $(\mathcal{S}, \mathcal{C}, \mathcal{R})$ is a function g on $\mathcal{C}$ with the properties
(i) $\sum_{y \in \mathcal{C}} g(y)y = 0$
(ii) for each linkage class L we have $\sum_{y \in L} g(y) = 0$
(iii) for each absorptive subset $\mathcal{A}$ which is not a union of linkage classes we have $\sum_{y \in L} g(y) > 0$

When a network is weakly reversible each absorptive set is a union of linkage classes so that condition (ii) is automatically satisfied. Given a stationary solution c^* let

$$g(y) = \sum_{\mathcal{C} \to y} k_{y'y} (c^*)^{y'} - \sum_{y \to \mathcal{C}} k_{yy'} (c^*)^y. \tag{99}$$

We can consider $k_{yy'}(c^*)^y$ as a current from y to y' and then g is the net current flowing into y from all other complexes.

Lemma 7.1 Let $(\mathcal{S}, \mathcal{C}, \mathcal{R})$ be a network and $\alpha \in F(\mathcal{R})$ a solution of

$$\sum_{\mathcal{R}} \alpha_{yy'}(y' - y) = 0. \tag{100}$$

Then $g(y) = \sum_{\mathcal{C} \to y} \alpha_{y'y} - \sum_{y \to \mathcal{C}} \alpha_{yy'}$ is a confluence vector.

To prove this lemma we use

Lemma 7.2 Let $(\mathcal{S}, \mathcal{C}, \mathcal{R})$ be a network and P a subset of $\mathcal{C}$. Then

$$\sum_{y \in P} g(y) = \sum_{P' \to P} \alpha_{y'y} - \sum_{P \to P'} \alpha_{yy'} \tag{101}$$

The proof is a straightforward calculation. Now Lemma 7.1 can be proved. The condition (i) follows from the calculation

$$0 = \sum_{\mathcal{R}} \alpha_{yy'}(y' - y) = \sum_{y \in \mathcal{C}} (\sum_{\mathcal{C} \to y} \alpha_{y'y} - \sum_{y \to \mathcal{C}} \alpha_{yy'}) y = \sum_{y \in \mathcal{C}} g(y) y \tag{102}$$

The condition (ii) is obtained by setting $P = L$ in Lemma 7.2 and using the fact that in this case both sums are empty. To obtain condition (iii) use Lemma 7.2 with $P = \mathcal{A}$. In this case one sum is zero and the other positive.

In what follows we are interested in positive confluence vectors.

Lemma 7.3 For a reaction network the set of elements of $F(\mathcal{C})$ which satisfy conditions (i) and (ii) in the definition of a confluence vector is a linear subspace of dimension δ.

Proof g can be written in the form $\sum_y g(y)\omega_y$. Condition (i) says that g is in $\ker Y$. Condition (ii) says that $g \in \text{span}\Delta$. The desired subspace is then $(\ker Y) \cap (\text{span}\Delta)$ and from Proposition 3.2 we know its dimension.

When the network is weakly reversible condition (iii) is automatically satisfied. The subspace just discussed is equal to the set of confluence vectors. If in addition the deficiency is positive there are non-vanishing confluence vectors. When the network is not weakly reversible but it has the property (R1) it follows from Lemma 7.1 that a confluence vector exists which cannot be zero.

Definition 7.2 Two non-vanishing confluence vectors g and g' for network are similarly oriented if there exists a positive number λ with $g' = \lambda g$. The equivalence classes which are defined by the corresponding equivalence relation are called confluence vector orientations.

Here we are interested in regular networks of deficiency one. For networks of this kind non-zero confluence vectors always exist. In this case all confluence vectors are proportional. When the network is not weakly reversible there is only one confluence vector orientation. When the network is weakly reversible there are two.

Lemma 7.4 If g is a confluence vector then there exists a positive function α which is related to g as in Lemma 7.1.

Proof Suppose that the network is not weakly reversible. Since it is regular there exists an $\alpha^* \in F(\mathcal{R})$ with

$$\sum_{\mathcal{R}} \alpha^*_{yy'}(y' - y) = 0. \tag{103}$$

It follows from Lemma 7.1 that g^* defined by

$$g^*(y) = \sum_{\mathcal{C} \to y} \alpha^*_{yy'} - \sum_{y \to \mathcal{C}} \alpha^*_{y'y} \tag{104}$$

is a confluence vector. Neither g nor g^* is zero. Because the network satisfies $\delta = 1$ they must be proportional. At the same time there exists only one confluence vector orientation, so that $g = \rho g^*$ for a positive number ρ. $\alpha = \rho\alpha^*$ has the desired properties. Now suppose instead that the network is weakly reversible. It follows from the arguments in the proof of Lemma 7.3 that $g \in (\ker Y) \cap (\text{span}(\Delta))$. Because $g \in \text{span}(\Delta)$ there exists $\xi \in F(\mathcal{R})$ with $g = \sum_{yy' \in \mathcal{R}} \xi_{yy'}(\omega_{y'} - \omega_y)$. Hence for each number p we have the relation

$$g = \sum_{yy' \in \mathcal{R}} (\xi_{yy'} + p)(\omega_{y'} - \omega_y). \tag{105}$$

By choosing p sufficiently large it can be ensured that the coefficients in this relation are all positive. Thus there exists a positive function α with $g = \sum_{yy' \in \mathcal{R}} \alpha_{yy'}(\omega_{y'} - \omega_y)$. If Y is applied to both sides of this equation the first equation of Lemma 7.1. In addition the relation

$$g = \sum_{yy' \in \mathcal{C}} (\sum_{\mathcal{C} \to y} \alpha_{y'y} - \sum_{y \to \mathcal{C}} \alpha_{yy'})\omega_y \tag{106}$$

holds and this gives the second equation of Lemma 7.1

A complex y is called reactive if there is a another complex y' with the property that there is a reaction from y to y'. (A complex can only fail to be reactive if it is the only complex in a terminal strong linkage class.) A terminal strong linkage class is called non-trivial if it contains more than one complex.

Definition 7.3 An upper-middle-lower partition of a network is a partition of its reactive complexes into three parts U, M und L called the upper, middle and lower parts with the properties that
(i) all non-terminal complexes are in M
(ii) all complexes in the same non-trivial terminal linkage class are in the same part

It is allowed that U and L are empty. Also when all complexes are terminal (i.e. the network is weakly reversible) then M is empty.

We now consider a network with a fixed choice of $P = \{U, M, L\}$ and confluence vector orientation G. For choice of this kind there exist certain inequalities which play an important role in the Deficiency One Algorithm. The inequalities concern variables μ_s, $s \in \mathcal{S}$ or, in an equivalent formulation, a vector $\mu \in F(\mathcal{S})$. Linear forms in the set $\{y \cdot \mu : y \in \mathcal{C}, \quad y \text{ reactive}\}$ are subjected to relations $>$, $<$ or $=$. The inequalities are fixed by three rules.
Rule 1. When y and y' are in M then $\mu \in F(\mathcal{S})$ must satisfy $y \cdot \mu = y' \cdot \mu$.

A complex in U is said to be above complexes in M and L and a complex in M is said to be above one in L.
Rule 2. If y is above y' then $y \cdot \mu > y' \cdot \mu$.

If yy' is a cut pair then the network obtained by removing the reactions between y and y' from the linkage class containing y and y' is the union of two subsets $\mathcal{W}(y)$ and $\mathcal{W}(y')$ so that the reactions between y and y' are the only direct link between the two. For a confluence vector g we define $[g, yy', y]$ to be $\sum_{\bar{y} \in \mathcal{W}(y)} g_{\bar{y}}$. We have $[g, yy', y'] = -[g, yy', y]$.
Rule 3. If y and y' are adjacent terminal complexes then
(i) for $yy' \in U$, $\mu \in F(\mathcal{S})$ the sign of $y \cdot \mu - y' \cdot \mu$ is the same as that of $[g, yy', y]$
(ii) for $yy' \in L$, $\mu \in F(\mathcal{S})$ the sign of $y \cdot \mu - y' \cdot \mu$ is opposite to that of $[g, yy', y]$

Lemma 7.5 For a network $(\mathcal{S}, \mathcal{C}, \mathcal{R})$ let α be a positive function on $\mathcal{R}$ and g the corresponding confluence vector. Let yy' be a cut pair. If there is a reaction from y' to y then $\alpha_{yy'} - \alpha_{y'y} = [g, yy', y']$. If there is no reaction from y' to y then $\alpha_{yy'} = [g, yy', y']$.

Proof We consider the sets $\mathcal{W}(y)$ and $\mathcal{W}(y')$. yy' is the only reaction in $\mathcal{W}(y) \to (\mathcal{W}(y))'$. The set $(\mathcal{W}(y))' \to \mathcal{W}(y)$ contains only the reaction yy' if there is this reaction is in $\mathcal{R}$ and is empty otherwise. The result follows from Lemma 7.2.

Theorem 7.1 Let $(\mathcal{S}, \mathcal{C}, \mathcal{R})$ be a regular network of deficiency one and c^* and c^{**} distinct positive points in a stoichiometric compatibility class. Then the following statements are equivalent
(i) there exist positive reaction constants such that the corresponding network with mass action kinetics has c^* and c^{**} as stationary solutions.
(ii) there exists a confluence vector orientation G and a partition $P = (U, M, L)$ with the property that $\mu = \log \frac{c^{**}}{c^*}$ satisfies the inequalities for (P, G).

For a network with m non-trivial terminal strong linkage classes there are 3^m (in the non-weakly reversible case) or $2 \cdot 3^m$ (in the weakly reversible case) systems of linear inequalities which must be analysed during the application of this theorem.

Before continuing with the general theory and, in particular, before giving the proof of the theorem it will be shown how this theorem can be applied in an example. This is the system for processive double phosphorylation. This network satisfies condition (R1). For example, it is possible in the case of reversible reactions to choose $\alpha_{ij} = 2$ for the forward reaction and $\alpha_{ij} = 1$ for the backward reaction. For the irreversible reactions α_{ij} is chosen to be one. Condition (R2) is obviously satisfied in this example. Since in this network each terminal strong linkage class consists of one complex (R3) is also satisfied. The network is regular. Since no terminal complex is reactive in this network there is only one partition (U, M, L) where all reactive complexes are in M. In this case Rules 2. and 3. give no restrictions. We only have to examine Rule 1. According to this rule the following relations hold

$$\mu_{C_0} + \mu_E = \mu_{C_0 \cdot E} = \mu_{C_1 \cdot E} = \mu_{C_2} + \mu_F = \mu_{C_1 \cdot F} = \mu_{C_2 \cdot F}. \tag{107}$$

Call the common value of these quantities A. If we apply the exponential function to these relations we get

$$e^{\mu_{C_0}} e^{\mu_E} = e^{\mu_{C_0 \cdot E}} = e^{\mu_{C_1 \cdot E}} = e^{\mu_{C_2}} e^{\mu_F} = e^{\mu_{C_1 \cdot F}} = e^{\mu_{C_2 \cdot F}} = e^A. \tag{108}$$

For two solutions in a stoichiometric comaptibility class the conserved quantities are equal. Consider the quantity $c_E + (c_{C_0 \cdot E} + c_{C_1 \cdot E})$. If A is positive then the quantity in brackets will increase when passing from c^* to c^{**}. Hence c_E must decrease. The inequality $\mu_E < 0$ must hold and therefore $\mu_{C_0} > 0$. For similar reasons $\mu_F < 0$ and $\mu_{C_2} > 0$. Together these facts imply that the total amount of substrate increases, a contradiction. Hence A cannot be positive. The attempt to make A negative also leads to a contradiction. Hence $A = 0$. We then see that some of the μ_i are obviously zero. Looking again at the total amounts of the enzymes E and F shows that $\mu_E = 0$ and $\mu_F = 0$. It is possible to conclude from this that μ_{C_0} and μ_{C_2} vanish. Applying Theorem 7.1 we see that it is not possible for this system one stoichiometric compatibility class contains more than one stationary solution.

Theorem 7.1 does not answer the question whether the system admits even one positive stationary solution. Because of the conserved quantities all solutions are bounded and reamins in an invariant subset of its stoichiometric class which is homeomorphic to a closed ball in a Euclidean space. Hence a general

theorem [15] implies the existence of a stationary solution. It says nothing about whether this solution is positive. If the existence of a stationary solution on the boundary can be excluded then in this situation the existence of a positive stationary solution is guaranteed. We consider a stoichiometric class for which the three conservation laws are positive. We choose a fixed non-negative function $c \in F(\mathcal{S})$. Suppose that this point is either a stationary solution or an ω limit point of a positive solution. We say that a complex is switched on in this configuration if $\Phi(c)(y) > 0$. Otherwise we say that the complex is switched off. The complex y is switched off precisely when an least one of the species involved in y is not in the support of c. Now consider a reaction from y to y'. If y' is switched off then at least one of the species involved in y' must have concentration zero in the given configuration. Hence reactions for which y' is on the left hand side make no contribution to the time derivative of this concentration. But then the reaction from y to y' cannot make a contribution. For otherwise for a solution with $c_i(t_1) = 0$ the inequality $\dot{c}_i(t_1) > 0$ would hold and $c(t)$ would have been negative for t a little less than t_1, a contradiction. We conclude that y is also switched off. This statement implies conditions on the set of complexes in $\mathcal{C}$ which are switched off. In particular it can be seen that this set is a union of strong linkage classes.

We now come back to the question, whether the system for processive phosphorylation has stationary solutions on the boundary of the positive orthant for which all conserved quantities are positive. The system has only four strong linkage classes. Suppose first that the class which contains $C_0 \cdot E$ is switched off at the stationary solution. Then the complexes $C_0 + E$, $C_0 \cdot E$ and $C_1 \cdot E$ are switched off. Since the total amount of the enzyme E is positive the amount of free enzyme E must be positive. Thus the concentration of C_0 is zero. Then $C_0 + F$ is switched off and and the class which contains $C_0 + F$ is switched off. Since the total amount of substrate is positive the concentration of C_2 must be positive. This means that the concentration of all substances which contain F must be zero. It follows that the total amount of F is zero, a contradiction. It has now been shown that the class which contains $C_0 \cdot E$ is turned on. On symmetry grounds the same is true of the class which contains $C_2 \cdot F$. Thus all classes must be switched on. This proves the existence of a positive stationary solution.

Definition 7.4 In a reaction network $(\mathcal{S}, \mathcal{C}, \mathcal{R})$ a function x on $\mathcal{S}$ is sign compatible with the stoichiometric subspace S if there is an element $\sigma \in S$ with the property that $x(s)$ has the same sign as $\sigma(s)$ for all $s \in \mathcal{S}$.

An equivalent formulation of this property is that there is a positive function p on $\mathcal{S}$ with $x = p\sigma$.

Lemma 7.6 Let $(\mathcal{S}, \mathcal{C}, \mathcal{R})$ be a reaction network. If c^* and c^{**} are positive functions on $\mathcal{S}$ which are in the same stoichiometric compatibility class then $\mu = \log(c^{**}/c^*)$ is sign compatible with the stoichiometric subspace. Conversely, if μ is sign compatible with the stoichiometric subspace then there exist positive functions c^* and c^{**} in the same stoichiometric compatibility class with $\mu = \log(c^{**}/c^*)$.

Proof Suppose that c^* und c^{**} are in the same stoichiometric compatibility

class. Then $c^{**} - c^* \in S$. If we define $\mu = \log(c^{**}/c^*)$ then $\mu(s)$ has the same sign as $c^{**}(s) - c^*(s)$ for all $s \in \mathcal{S}$. Hence μ is sign compatible with the stoichiometric subspace. Conversely, suppose that μ is sign compatible with the stoichiometric subspace. Then there exist a $\sigma \in S$ and a positive function p on $\mathcal{S}$ with $\mu = p\sigma$. Let $c^* = p$ and $c^{**} = pe^{\mu}$. Then the relations $\mu = \log(c^{**}/c^*)$ and $c^{**} - c^* = p(e^{\mu} - 1)$ hold. If $c^*(s)$ and $c^{**}(s)$ are equal then $c^{**} - c^* = \sigma$ at the point s. If $c^{**}(s) - c^*(s) \neq 0$ then we can multiply $c^*(s)$ and $c^{**}(s)$ with the positive factor $[p(s)(e^{\mu}(s) - 1)]^{-1}\sigma(s)$. Then the first relation is unchanged and in the second the right hand side is replaced by σ. After the rescaling c^* and c^{**} belong to the same stoichiometric compatibility class.

Corollary 7.1 For a regular network $(\mathcal{S}, \mathcal{C}, \mathcal{R})$ with deficiency one the following conditions are equivalent

(i) there are positive reaction constants such that the corresponding system with mass action kinetics has two distinct positive stationary solutions in a stoichiometric class.

(ii) for a confluence vector orientation G and a partition $P = (U, M, L)$ the system of inequalities correspondoing to the pair (P, G) has a non-zero solution which is sign compatible with the stoichiometric subspace.

We say that a partition $P' = (U', M', L')$ is the inversion of the partition $P = (U, M, L)$ if $U' = L$, $M' = M$ and $L' = U$. In this case the system of inequalities corresponding to P' coincides with that corresponding to P up to the fact that all signs are reversed. Thus μ is a solution in the first case precisely when $-\mu$ is a solution in the second case. So it makes no sense to investigate the inversion of a partition P if P has already been analysed.

If a system is given where measurements are available for two different stationary states but where the reaction network is not known it is possible to use these ideas to test whether a certain network is consistent with the observations by comparing concentrations in the two different states.

Theorem 7.1 provides a criterion for the existence of two distinct stationary solutions. The next theorem gives a similar criterion for the existence of a degenerate stationary solution, i. e. a stationary solution where the restriction of the derivative of the right hand side of the evolution equations to the stoichiometric subspace has a non-trivial kernel.

Theorem 7.2 For a regular reaction network $(\mathcal{S}, \mathcal{C}, \mathcal{R})$ with deficiency one let c^* be a positive function on $\mathcal{S}$ and $\gamma \neq 0$ a point of the stoichiometric subspace. Then the following statements are equivalent.

(i) there exist reaction constants for which the corresponding system with mass action kinetics admits c^* as a solution for which $D_c f(c^*)\gamma = 0$.

(ii) there exists a confluence vector orientation G and a partition $P = (U, M, L)$ with the property that γ/c^* satisfies the system of inequalities corresponding to (P, G)

Lemma 7.7 Let $(\mathcal{S}, \mathcal{C}, \mathcal{R})$ be a reaction network and c^*, c^{**} positive functions on $\mathcal{S}$. Then the following conditions are equivalent.

(i) there exist reaction constants for which the mass action system has c^* and c^{**} as stationary solutions.

(i)' there exists a positive function κ on $\mathcal{R}$ with the property that

$$\sum_{yy' \in \mathcal{R}} \kappa_{yy'}(y' - y) = 0 \tag{109}$$

and

$$\sum_{yy' \in \mathcal{R}} \kappa_{yy'} e^{y \cdot \mu}(y' - y) = 0 \tag{110}$$

where $\mu = \log(c^{**}/c^*)$.

Proof Suppose that (i) holds. To say that c^* und c^{**} are stationary solutions is to say that

$$\sum_{yy' \in \mathcal{R}} k_{yy'}(c^*)^y(y' - y) = 0 \tag{111}$$

and

$$\sum_{yy' \in \mathcal{R}} k_{yy'}(c^{**})^y(y' - y) = 0. \tag{112}$$

If $\kappa_{yy'} = k_{yy'}(c^*)^y$ and μ is defined as above then the two equations in (i)' hold. Conversely, if (i)' holds then we can choose $k_{yy'}$ such that $\kappa_{yy'} = k_{yy'}(c^*)^y$ and then (i) follows.

The next lemma is a similar reformulation of (i) in Theorem 7.2.

Lemma 7.8 Let $(\mathcal{S}, \mathcal{C}, \mathcal{R})$ be a reaction network, c^* a positive function on $\mathcal{S}$ and γ a function on $\mathcal{S}$. Then the following statements are equivalent.

(i) there exist reaction constants for which the corresponding system with mass action kinetics has c^* as a stationary solution for which $D_c f(c^*)\gamma = 0$.

(i)' there exists a positive function κ on $\mathcal{R}$ with the property that

$$\sum_{yy' \in \mathcal{R}} \kappa_{yy'}(y' - y) = 0 \tag{113}$$

and

$$\sum_{yy' \in \mathcal{R}} \kappa_{yy'} \phi(y\cdot)\mu(y' - y) = 0 \tag{114}$$

where $\mu = \gamma/c^*$.

Proof Suppose that (i) holds. If c^* is a stationary solution then

$$\sum_{yy' \in \mathcal{R}} k_{yy'}(c^*)^y(y' - y) = 0. \tag{115}$$

In addition the condition on the derivative is equivalent to the condition that

$$\sum_{yy' \in \mathcal{R}} k_{yy'}(c^*)^y(y \cdot (\gamma/c^*))(y' - y) = 0. \tag{116}$$

Statement (i)' follows if we set $\kappa_{yy'} = k_{yy'}(c^*)^y$. For the converse we only need so choose $k_{yy'}$ so that this last relation holds.

Using Lemma 7.7 and Lemma 7.8 we see that Theorem 7.1 and Theorem 7.2 are consequences of

Proposition 7.1 Let $(\mathcal{S}, \mathcal{C}, \mathcal{R})$ be a regular reaction network with deficiency one, $\phi : \mathbb{R} \to \mathbb{R}$ a continuous montonically increasing function and μ a non-vanishing function on $\mathcal{R}$ which is sign compatible with the stoichiometric subspace. Then the following conditions are equivalent.

(i) there exists a positive function $\kappa_{yy'}$ on $\mathcal{R}$ with

$$\sum_{yy' \in \mathcal{R}} \kappa_{yy'}(y' - y) = 0 \tag{117}$$

and

$$\sum_{yy' \in \mathcal{R}} \kappa_{yy'} y \cdot \mu(y' - y) = 0 \tag{118}$$

(ii) there exists a confluence vector orientation G and a partition $P = (U, M, L)$ with the property that μ satisfies the system of inequalities corresponding to the pair (G, P).

To see that Theorem 7.1 follows from Proposition 7.1 choose $\mu = \log(c^{**}/c^*)$ and $\phi(x) = e^x$ and apply Lemma 7.7. To see that Theorem 7.2 follows from Proposition 7.1 choose $\mu = \gamma/c^*$ and $\phi(x) = x$ and apply Lemma 7.8.

Now Proposition 7.1 will be proved, first that (i) implies (ii). The equations in (i) are equivalent to

$$Y A_\kappa \omega_{\mathcal{C}} = 0 \tag{119}$$

and

$$Y A_\kappa (\sum_{y \in \mathcal{C}} \phi(y \cdot \mu) \omega_y) = 0. \tag{120}$$

In other words (i) is equivalent to statements $\omega_{\mathcal{C}} \in \ker(Y A_\kappa)$ and $\sum_{y \in \mathcal{C}} \phi(y \cdot \mu)\omega_y \in \ker(Y A_\kappa)$. Since the network is regular and $\delta = 1$ it follows from Lemma 6.3 that $\dim(\ker(Y A_\kappa)) = 1 + l$. We choose a basis of the kernel of A_κ as in Proposition 3.1 and call it $b^1, \ldots, b^l$. (Since the network is regular $t = l$.) Provided $\omega_{\mathcal{C}}$ does not lie in the space spanned by $b^1, \ldots, b^l$ then $(\omega_{\mathcal{C}}, b^1, \ldots, b^l)$ is a basis of the kernel of $Y A_\kappa$. If this condition does not hold then $\omega_{\mathcal{C}}$ is in $\ker A_\kappa$. According to Proposition 6.1 in this case μ must be orthogonal to the stoichiometric subspace. Since by hypothesis μ is sign compatible with the stoichiometric subspace we get a contradiction. Hence in fact we get a basis. Thus there are numbers $\lambda_0, \lambda_1, \ldots, \lambda_l$ with

$$\sum_{y \in \mathcal{C}} \phi(y \cdot \mu)\omega_y = \lambda_0 \omega_{\mathcal{C}} + \lambda_1 b^1 + \ldots + \lambda_l b^l. \tag{121}$$

Let U be the union of the non-trivial linkage classes Λ^θ for which $\lambda_\theta > 0$, L the corresponding set with $\lambda_\theta < 0$ and M the set of reactive complexes which are neither in U nor in L. M is the union of the nontrivial terminal linkage classes Λ^θ with $\lambda_\theta = 0$ and the set of non-terminal complexes. The sets U, M and L satisfy the conditions for an upper-middle-lower partition (U, M, L). Let

$g = A_\kappa \omega_{\mathcal{C}} = \sum_{\mathcal{R}} \kappa_{yy'}(\omega_{y'} - \omega_y)$. Since $\omega_{\mathcal{C}}$ is not in the kernel of A_κ the vector g is not zero. We have $g(y) = \sum_{\mathcal{C}\to y} \kappa_{yy'} - \sum_{y\to\mathcal{C}} \kappa_{y'y}$. It follows from Lemma 7.1 that g is a confluence vector. Let G be the corresponding confluence vector orientation. It will now be shown that the inequalities corresponding to the pair (G, P) are satisfied. To do this we must check the three rules.

To see that Rule 1. is satisfied we note that the properties of the basis imply that $\phi(y \cdot \mu) = \lambda_0$ for all $y \in M$. If y and y' are in M then the strict monotonicity of ϕ implies that $y \cdot \mu = y' \cdot \mu$. To check the validity of Rule 2. let y^U, y^M and y^L be complexes in the corresponding subsets. Let y^U and y^L be members of the terminal strong linkage classes Λ^1 and Λ^2. The the properties of the basis imply that

$$\begin{aligned}
&\phi(y^U \cdot \mu) = \lambda_0 + \lambda_1 b^1(y^U),\\
&\phi(y^M \cdot \mu) = \lambda_0,\\
&\phi(y^L \cdot \mu) = \lambda_0 + \lambda_2 b^2(y^L).
\end{aligned} \tag{122}$$

Since $\lambda_1 > 0$, $\lambda_2 < 0$ and ϕ is monotonically increasing it follows that $y^U \cdot \mu > y^M \cdot \mu > y^L \cdot \mu$. It remains to show that Rule 3. holds. Let y and y' be adjacent complexes in the terminal strong linkage class Λ^θ. In this case yy' and $y'y$ belong to $\mathcal{R}$ because of (R3). We have $\phi(y \cdot \mu) = \lambda_0 + \lambda_\theta b^\theta(y)$ and $\phi(y' \cdot \mu) = \lambda_0 + \lambda_\theta b^\theta(y')$. Suppose that y and y' are in U so that $\lambda_\theta > 0$. In this case it follows from the montonicity of ϕ that $y \cdot \mu - y' \cdot \mu$ and $b^\theta(y) - b^\theta(y')$ have the same sign. If on the other hand y and y' are in L then these quantities have opposite signs. Hence in order to show that μ satisfies Rule 3. it must be shown that $b^\theta(y) - b^\theta(y')$ has the same sign as $[g, yy', y]$. The reaction yy' is a cut pair and we can define sets $\mathcal{W}(y)$ and $\mathcal{W}(y')$ as above.

$$[g, yy', y] = \sum_{\bar{y}\in\mathcal{W}(y)} g(\bar{y}) = \sum_{\mathcal{W}(y)'\to\mathcal{W}(y)} \kappa_{\bar{y}'\bar{y}} - \sum_{\mathcal{W}(y)\to\mathcal{W}(y)'} \kappa_{\bar{y}\bar{y}'}. \tag{123}$$

The last equality follows from Lemma 6.4. Since yy' and $y'y$ are the only reactions in $\mathcal{W}(y)' \to \mathcal{W}(y)$ and $\mathcal{W}(y) \to \mathcal{W}(y)'$, respectively, the equation simplifies to

$$[g, yy', y] = \kappa_{y'y} - \kappa_{yy'}. \tag{124}$$

Moreover, since b^θ is in the kernel of A_κ it follows with the help of Lemma 6.4 that

$$\sum_{\mathcal{W}(y)'\to\mathcal{W}(y)} \kappa_{\bar{y}'\bar{y}} b^\theta(\bar{y}') - \sum_{\mathcal{W}(y)\to\mathcal{W}(y)'} \kappa_{\bar{y}\bar{y}'} b^\theta(\bar{y}) = 0 \tag{125}$$

and

$$\kappa_{y'y} b^\theta(y') - \kappa_{yy'} b^\theta(y) = 0. \tag{126}$$

Hence, since $\kappa_{y'y}$ and $\kappa_{yy'}$ are positive, $b^\theta(y) - b^\theta(y')$ and $[g, yy', y]$ have the same sign. This completes the proof of (ii).

Suppose conversely that (ii) holds. Then there exists a confluence vector orientation G and a partition (U, M, L) such that μ satisfies the corresponding

inequalities and we are looking for a positive function κ on $\mathcal{C}$ with certain properties. Let g be a non-vanishing element of G. It follows from Lemma 7.4 that there exists a positive functionα on $\mathcal{C}$ with the properties that

$$\sum_{\mathcal{R}} \alpha_{yy'}(y' - y) = 0, \tag{127}$$

$$g(y) = \sum_{\mathcal{C} \to y} \alpha_{y'y} - \sum_{y \to \mathcal{C}} \alpha_{yy'}. \tag{128}$$

Let $y^* y^{**}$ and $y^{**} y^*$ be a pair of reversible reactions. Then the first equation can be written in the form

$$(\alpha_{y^* y^{**}} - \alpha_{y^{**} y^*})(y^{**} - y^*) + \sum_{\mathcal{R} \setminus \{y^* y^{**}, y^{**} y^*\}} (y' - y) = 0 \tag{129}$$

It follows that if $\beta_{yy'} = \alpha_{yy'}$ for all reaktions except those between y^* and y^{**} and $\beta_{y^* y^{**}} - \beta_{y^{**} y^*} = \alpha_{y^* y^{**}} - \alpha_{y^{**} y^*}$ then $\sum_{\mathcal{R}} \beta_{yy'}(y' - y) = 0$. If $y^* y^{**}$ is a cut pair then it is also true that $\alpha_{y^* y^{**}} - \alpha_{y^{**} y^*} = [g, y^* y^{**}, y^{**}]$.

Now κ will be constructed. If in the partition (U, M, L) the set M is not empty let $\eta = y \cdot \mu$ for $y \in M$. If, on the other hand M is empty then let η be any number between the minimum of $y \cdot \mu$ on U and the maximum of $y \cdot \mu$ on L. The function κ is defined as follows. If yy' is irreversible or when yy' is reversible and $y' \cdot \mu = y \cdot \mu$ then let $\kappa_{yy'} = \alpha_{yy'}$. If yy' is reversible and $y' \cdot \mu \neq y \cdot \mu$ (which implies that yy' is a cut pair which lies in U or L) let

$$\kappa_{yy'} = [g, yy', y'] \frac{\phi(y' \cdot \mu) - \phi(\eta)}{\phi(y' \cdot \mu) - \phi(y \cdot \mu)}, \tag{130}$$

$$\kappa_{y'y} = [g, yy', y'] \frac{\phi(y \cdot \mu) - \phi(\eta)}{\phi(y' \cdot \mu) - \phi(y \cdot \mu)}. \tag{131}$$

That $\kappa_{yy'}$ and $\kappa_{y'y}$ are positive follows from the definition of μ and the inequalities corresponding to the partition (U, M, L) and G. These numbers satisfy the relations $\kappa_{yy'} - \kappa_{y'y} = [g, yy', y']$ Hence with the help of Lemma 7.5 we can replace the difference of coefficients κ by a difference of coefficients α. It follows that $\sum_{\mathcal{R}} \kappa_{yy'}(y' - y) = 0$. Thus the first equation of (i) holds.

To show that the second equation holds we consider the following three subsets of $\mathcal{R}$. $\mathcal{R}_1$ consists of the reactions yy' with $y \in M$. $\mathcal{R}_2$ consists of the reactions yy' with $y \neq M$ for which $y \cdot \mu = y' \cdot \mu$. $\mathcal{R}_3$ consists of the reactions yy' with $y \neq M$ for which $y \cdot \mu \neq y' \cdot \mu$. These subsets are disjoint and their union is $\mathcal{R}$. Each reaction in $\mathcal{R}_2$ or $\mathcal{R}_3$ is reversible as a consequence of (R3). Hence we can always make a choice of forward and backward reactions. The sets of reactions in $\mathcal{R}_2$ and $\mathcal{R}_3$ which were chosen to be forwards are denoted by $\mathcal{F}_2$ and $\mathcal{F}_3$. Note that

$$\sum_{\mathcal{R}} \kappa_{yy'} \phi(y \cdot \mu)(y' - y) = \phi(\eta) \sum_{\mathcal{R}_1} \kappa_{yy'}(y' - y)$$
$$+ \sum_{\mathcal{F}_2} \phi(y \cdot \mu)(\kappa_{yy'} - \kappa_{y'y})(y' - y)$$

$$+\sum_{\mathcal{F}_3}(\kappa_{yy'}\phi(y\cdot\mu)-\kappa_{y'y}\phi(y'\cdot\mu))(y'-y) \tag{132}$$

For $yy'\in\mathcal{F}_2$ we have

$$\kappa_{yy'}-\kappa_{y'y}=\alpha_{yy'}-\alpha_{y'y}=[g,yy',y']. \tag{133}$$

Moreover yy' is in$\mathcal{F}_2$ iff $[g,yy',y']=0$. Hence $\kappa_{yy'}-\kappa_{y'y}=0$ for all $yy'\in\mathcal{F}_2$. It is also the case that for $yy'\in\mathcal{F}_3$ we have

$$\kappa_{yy'}\phi(y\cdot\mu)-\kappa_{y'y}\phi(y'\cdot\mu)=\phi(\eta)(\kappa_{yy'}-\kappa_{y'y}). \tag{134}$$

Combining these relations gives

$$\begin{aligned}
&\sum_{\mathcal{R}}\kappa_{yy'}\phi(y\cdot\mu)(y'-y)\\
&=\phi(\eta)\left[\sum_{\mathcal{R}_1}\kappa_{yy'}(y'-y)+\sum_{\mathcal{F}_2}(\kappa_{yy'}-\kappa_{y'y})(y'-y)+\sum_{\mathcal{F}_3}(\kappa_{yy'}-\kappa_{y'y})(y'-y)\right]\\
&=\phi(\eta)\sum_{\mathcal{R}}\kappa_{yy'}(y'-y)=0.
\end{aligned} \tag{135}$$

This proves the second equation.

8 Elementary flux modes

The deficiency one algorithm often gives good results for networks of deficiency one but what can be done when a network is given which has a deficiency greater than one? One possibility is the method of elementary flux modes which produces smaller networks of deficiency one out of a given network. Provided these networks satisfy the condition $t=l$ they are always regular and it is possible to apply the deficiency one algorithm to them. If the smaller networks exhibit multistationarity then it is possible to conclude unter certain conditions that the original network also does so. On the other hand the converse statement is not always true.

The condition for a stationary solution can be written in the form $Nv(c)=0$. The vector $v(c)$ of reaction rates is positive. The intersection of the kernel of N with the non-negative orthant is a cone. For a positive stationary solution c the vector $v(c)$ lies in the interior of this cone. The points of such a cone can be written as linear combinations with non-negative coefficients of a finite number of vectors which lie in the boundary of the positive orthant ([20], Theorem 19.1). The points of the cone can be regarded as flux distributions. In the case of the cone we are considering here these vectors are called elementary flux modes. It can be shown that the elementary flux modes are precisely the vectors in the cone which have a maximal number of vanishing components. Thus these are the vectors which satisfy two conditions.
(i) $NE_i=0$ and E_i is non-negative

(ii) if there is another vector E_j which satisfies (i) and whose support is contained in that of E_i then either $E_j = 0$ or $E_j = \alpha E_i$
We call E_i the generators of the cone.

The generators have an interesting interpretation in terms of the reactions. The components which are non-zero correspond to active reactions and the others correspond to inactive reactions. In this sense each generator defines a subnetwork of the original network consisting of the active reactions. It is possible to distinguish between generators which are in the kernel of I_a and those which are not. The latter are called stoichiometric generators.

If we start form one network and consider a subnetwork then the quantities which have to do with the original network are denoted by symbols with a hat, for instance $\hat{I}_a$, $\hat{E}$ (for the generator). Both networks have the same complexes and Y is used for both. Let $\hat{r}$ be the number of reactions in the original network and r the number of reactions in the subnetwork. Let E be the vector which results from $\hat{E}$ by leaving out the vanishing components. Then E is positive and $YI_aE = 0$ and $I_aE \neq 0$. Let $\hat{n}$, n, $\hat{l}$ and l be the numbers of complexes and linkage classes of both networks.

It turns out that the subnetworks which arise in this way always satisfy $\delta = 1$ and that provided they satisfy the condition $t = l$ they are also regular. Hence this one condition suffices to ensure that the Deficiency One Algorithm can be applied to the subnetworks. This statement will now be proved.

Lemma 8.1 Let I_a be the matrix which is defined by a network with n complexes and l linkage classes. Then the rank of I_a is $n - l$.

Beweis This is a slightly altered form of Lemma 3.8.

Lemma 8.2 Let $\hat{E}$ be a stoichiometric generator of a network. Then the following statements hold.
(i) E is a generator of $\ker(YI_a) \cap \mathbb{R}^r_+$ and is the only such generator. This means that this space is spanned by E.
(ii) $\dim \ker(YI_a) = 1$.
(iii) I_a has maximal column rank.
(iv) I_a has $n - l$ columns.
(v) The rank of YI_a is $n - l - 1$

Proof (i) let E_0 be a non-vanishing generator. Since E is positive the support of E_0 is contained in that of E. Let $\hat{E}_0$ be the vector in $\mathbb{R}^{\hat{r}}$ which is obtained by completing E_0 with zeroes. Then $Y\hat{I}_a\hat{E}_0 = 0$ and the support of $\hat{E}_0$ is contained in that of $\hat{E}$. Since $\hat{E}$ is a generator we conclude that $\hat{E}_0 = \alpha\hat{E}$ for a positive constant α and hence that $E_0 = \alpha E$. It follows that E is a generator. This argument also shows that E is the only generator.
(ii) Let $E_0 \neq 0$ be a vector in $\mathbb{R}^r$ which is not proportional to E and which satisfies the equation $YI_aE_0 = 0$. It follows from (i) that E_0 has negative components. For this reason E_0 cannot be a generator. To prove this consider $\tilde{E} = \alpha E + E_0$. Then $\tilde{E}$ is positive for α sufficiently large and it follows from (i) that $\tilde{E} = \beta E$ for a constant β, a contradiction.
(iii) If the column rank of I_a was not maximal then there would exist a non-trivial vector in the kernel of I_a which would then also be in the kernel of

YI_a. According to (ii) this vector is proportional to E and this leads to a contradiction.
(iv) According to Lemma 8.1 the matrix I_a has rank $n-l$. But I_a has maximal column rank. Hence I_a has the claimed number of columns.
(v) For a matrix A the dimension dim ker$A$ is the difference of the dimension of the domain and the rank of A. Hence the dimension of YI_a is $n-l-1$ as a consequence of (ii).

In general the subnetwork which is defined by $\hat{E}$ contains l linkage classes. Let J_i be the set of indices which belong to the linkage class i. Each linkage class can be considered as a network in its own right with corresponding matrix $I_a^{J_i}$. This matrix consists of the columns of I_a which belong to J_i.
Corollary 8.1 Let n^{J_i} be the number of complexes in J_i. Then the rank of $YI_a^{J_i}$ is $n^{J_i}-1$.
Proof According to Lemma 8.1 the rank of $I_a^{J_i}$ is $n^{J_i}-1$. Lemma 8.2 implies that $YI_a^{J_i}$ has maximal rank and the result follows.
Corollary 8.2 For a subnetwork with l linkage classes which is defined by a stoichiometric generator $\hat{E}$ the deficiency of the network is one. The deficiency of each linkage class is zero.
Proof The first statement follows from part (v) of Lemma 8.2. The second follows from Corollary 8.1.

We have now shown that the subnetworks which are defined by stoichiometric generators have deficiency one. It will now be investigated under what circumstances the subnetworks satisfy the hypotheses of the Deficiency One Algorithm. Condition (R1) can be expressed by saying that there is a positive vector in the kernel of YI_a. According to Lemma 8.2 this condition holds. (R3) is equivalent to the condition that that the terminal strong linkage classes contain no closed loops. This statement follows from the fact that the rank of I_a is maximal. Only (R2) remains open and it must be checked in each application.

The next goal is to to obtain conditions which ensure that multistationarity in a subnetwork, which might be obtained be means of the deficiency one algorithm, implies the same condition for the original network. This is done using the implicit function theorem and it is necessary to check that certain linear mapping have full rank. We consider a network with ρ reactions and a stoichiometric generator $\hat{E}$. The reaction constants define a function k on $\mathbb{R}^\rho$. Let $\hat{k}_E$ be the restriction of k to the support of E and $\hat{k}_C = k - k_E$ the restriction to the complement of E. The evolution equations are

$$\dot{c} = Nv(\hat{k}_E, c) + Nv(\hat{k}_C, c) \tag{136}$$

where the dependence of the reaction rates on the reaction constants has been made explicit. Let $r+1$ the number of components of E which are non-zero. Let N_E be the matrix consisting of the columns of N corresponding to the non-vanishing components of E and N_C the matrix whose columns are the other columns of E. Then

$$\dot{c} = N_E v(\hat{k}_E, c) + N_C v(\hat{k}_C, c). \tag{137}$$

The ordinary differential equations for the subnetwork are $\dot{c} = N_E v(\hat{k}_E, c)$. Since E is a stoichiometric generator it follows from Lemma 8.2 that the rank of N_E is r and that the kernel of N_E consists of the functions whose support is contained in that of E. Consider an orthonormal basis for the left kernel of N. We call the matrix whose rows are the vectors of this basis W^T. It is a $p \times m$ matrix. Next consider an orthonormal basis for the left kernel of N_E and call the matrix whose rows are the vectors of the basis W_E^T. It is an $(m-r) \times m$ matrix. The lower elements of W_E^T agree with those of W^T. The upper ones are denoted by W_{add}^T. We also consider a matrix S_E whose columns are an othonormal basis of the image of N_E. Let $\mathcal{T} = (S_E, W_{\text{add}}, W)$, an orthogonal transformation. Let $\xi = S_E^T c$, $\eta = W_{\text{add}}^T c$ and $\hat{\eta} = W^T c$. Then c is the image of the vector with components $(\xi, \eta, \hat{\eta})$ by $\mathcal{T}$. In these variables the evolution equations become

$$\dot{\xi} = S_E^T N_E v_E(k_E, c) + S_E^T N_C v_C(k_C, c), \tag{138}$$

$$\dot{\eta} = W_{\text{add}}^T N_C v_C(k_E, c), \tag{139}$$

$$\dot{(\hat{\eta})} = 0. \tag{140}$$

Suppose we have stationary solutions c_1^* and c_2^* for the subnetwork with reaction constants k_E^*. This means that $N_E v_E(k_E^*, c_{1,2}^*) = 0$. Let $(\xi_{1,2}^*, \eta^*, \hat{\eta}*) = \mathcal{T}^T c_{1,2}^*$. Then

$$\dot{\xi} = S_E^T N_E v_E(k_E^*, c(\xi_{1,2}^*, \eta^*, \hat{\eta}^*)) + S_E^T N_C v_C(k_C, c(\xi_{1,2}^*, \eta^*, \hat{\eta}^*)), \tag{141}$$

$$\dot{\eta} = W_{\text{add}}^T N_C v_C(k_C, c(\xi_{1,2}^*, \eta^*, \hat{\eta}^*)), \tag{142}$$

$$\dot{(\hat{\eta})} = 0. \tag{143}$$

and the first term in the first of these three equations vanishes. For a fixed choice of k_E^* und $\hat{\eta}^*$ we define $\hat{k}_E^*$ by the conditions that $\hat{k}_E^* = k_E^*$ on the support of E and $\hat{k}_E^* = 0$ outside the support of E. Let $k = \hat{k}_E^* + \epsilon \hat{k}_C$. To determine stationary solutions of the full system we must find ξ, η und $\hat{k}_C$ with

$$S_E^T N_E v_E(k_E^*, c(\xi, \eta, \hat{\eta}^*)) + S_E^T N_C v_C(k_C, c(\xi, \eta, \hat{\eta}^*)) = 0, \tag{144}$$

$$W_{\text{add}}^T N_C v_C(k, c(\xi, \eta, \hat{\eta}^*)) = 0. \tag{145}$$

The reaction rates $v(c)$ can be written in the form $(\text{diag}\Psi(c))k$. We write $f(k, c) = Nv(k, c)$ and $f_c(k, c)$ for the Jacobian matrix $D_c f(k, c)$.

We now set up the following programme.

(1) Let

$$S_E^T f_c(\hat{k}_E^*, c_{1,2}^*)(S_E, W_{\text{add}}) = (A_{1,2}^*, B_{1,2}^*). \tag{146}$$

If $A_{1,2}^*$ is regular then we can define $X_{1,2}^* = -(A_{1,2}^*)^{-1} B_{1,2}^*$. Then $A_{1,2}^* X_{1,2}^* + B_{1,2}^* = 0$.

(2) We calculate a positive solution of $\mathcal{G}_C^* \kappa_0 = 0$ where $\mathcal{G}_C^*$ is the matrix with upper part $W_{\text{add}}^T N_c \text{diag}(\Psi_C(c_1^*))$ and lower part $W_{\text{add}}^T N_c \text{diag}(\Psi_C(c_2^*))$ and we define $\hat{k}_C^*$ to be zero on the support of E and κ_0 outside the support.

(3) Let $W_{\rm add}^T F_x(\hat{k}_C^*, c_{1,2}^*)(S_E, W_{\rm add}) = (C_{1,2}^*, D_{1,2}^*)$. We require that $\mathcal{D}_{1,2}^* = D_{1,2}^* + C_{1,2}^* X_{1,2}^*$ is regular.

The following theorem shows this programme leads to two stationary solutions $\tilde{c}_{1,2}$ close to $c_{1,2}^*$ with the reaction constants $\hat{k}_E^* + \epsilon \hat{k}_C^*$ for ϵ sufficiently small. For this purpose we choose a sufficiently small value of ϵ and solve $Nv(\hat{k}_E^* + \epsilon \hat{k}_C^*) = 0$ for $\tilde{c}_{1,2}$ close to $c_{1,2}^*$.

Theorem 8.1 Suppose that the following conditions hold.
(i) there exist $c_{1,2}^* > 0$, k_E^* with $N_E v_E(k_E^*, c_{1,2}^*) = 0$.
(ii) there exists $\kappa_0 > 0$ with $\mathcal{G}_C^* \kappa_0 = 0$.
(iii) A_1^* and A_2^* are regular
(iv) $\mathcal{D}_1^*$ and $\mathcal{D}_2^*$ are regular Then there exist $\epsilon_0 > 0$ and $\delta_0 > 0$ with the property that $0 < \epsilon < \epsilon_0$ and $|\hat{\eta} - \hat{\eta}^*| < \delta_0$ imply the existence of distinct positive hyperbolic stationary solutions

$$c_{1,2}(\hat{\eta}, \epsilon) = \left[\begin{array}{ccc} S_E & W_{\rm add} & W \end{array} \right] \left[\begin{array}{c} \Xi_{1,2}(\hat{\eta}, \epsilon) \\ H_{1,2}(\hat{\eta}, \epsilon) \\ \hat{\eta} \end{array} \right] \tag{147}$$

with $W^T c_1(\hat{\eta}, \epsilon) = W^T c_2(\hat{\eta}, \epsilon)$ for the positive reaction constants $k(\epsilon) = \hat{k}_E^* + \epsilon \hat{k}_C^*$.

Proof Applying the orthogonal transformation $\xi = S_E^T c$, $\eta = W_{\rm add}^T c$, $\hat{\eta} = W^T c$ gives

$$\dot{\xi} = S_E^T N v(\hat{k}_E^*, c) + S_E^T N v(\epsilon \hat{k}_C^*, c) \tag{148}$$

$$\dot{\eta} = W_{\rm add} N v(\hat{k}_E^* + \epsilon \hat{k}_C^*) \tag{149}$$

$$\dot{(\hat{\eta})} = 0. \tag{150}$$

For fixed values of c_j^* and k_E^* and A_j^* regular the equation $\dot{\xi} = 0$ has a locally unique solution $\xi_j = \Xi_j(\eta, \hat{\eta}, \epsilon \hat{k}_C^*)$ close to $(\eta^*, \tilde{\eta}^*, 0)$ with $A_j^* X_j^* + B_j^* = 0$, where $X_j^* = \frac{\partial}{\partial \eta} \Xi_j(\eta, \tilde{\eta}, 0)$ as a consequence of the implicit function theorem. In order to obtain solutions with common $\hat{\eta}$ components we must solve the equations

$$W_{\rm add}^T N v(\hat{k}_C^*, S_E \Xi_1(\eta, \hat{\eta}, \epsilon \hat{k}_C^*) + W_{\rm add} \eta + W \hat{\eta}) \tag{151}$$

$$W_{\rm add}^T N v(\hat{k}_C, S_E \Xi_2(\tilde{\eta}, \hat{\eta}, \epsilon \hat{k}_C^*) + W_{\rm add} \tilde{\eta} + W \hat{\eta}) \tag{152}$$

for $\eta = H_1(\hat{\eta}, \epsilon)$ and $\hat{\eta} = H_2(\hat{\eta}, \epsilon)$ close to $(\eta^*, \tilde{\eta}^*, \hat{\eta}^*, 0)$). Due to assumtion (iv) the implicit function theorem shows that functions H_1 und H_2 of this type exist. Thus locally there exist stationary solutions

$$\xi_1 = \Xi_1(H_1(\hat{\eta}, \epsilon), \hat{\eta}, \epsilon), \quad \eta = H_1(\hat{\eta}, \epsilon), \quad \hat{\eta}, \tag{153}$$

$$\xi_2 = \Xi_2(H_2(\hat{\eta}, \epsilon), \hat{\eta}, \epsilon), \quad \eta = H_2(\hat{\eta}, \epsilon), \quad \hat{\eta} \tag{154}$$

for $\epsilon > 0$ sufficiently small. The corresponding stationary solutions $c_{1,2}^*$ are obtained from the theorem as $c_{1,2}^*(\hat{\eta}, \epsilon)$.

Consider the example of the extended Michaelis-Menten description of a single reaction. We have

$$N = \begin{bmatrix} -1 & 1 & 0 \\ -1 & 1 & 1 \\ 1 & -1 & -1 \\ 0 & 0 & 1 \end{bmatrix}, \quad I_a = \begin{bmatrix} -1 & 1 & 0 \\ 1 & -1 & -1 \\ 0 & 0 & 1 \end{bmatrix}. \tag{155}$$

In addition dim ker$N$ = dim ker$I_a = 1$ and the kernel is spanned by the vector with components $(1, 1, 0)$. This vector is an elentary flux mode (the only one) and is not a stoichiometric generator. For the simple futile cycle we get

$$N = \begin{bmatrix} -1 & 1 & 0 & 0 & 0 & 1 \\ -1 & 1 & 1 & 0 & 0 & 0 \\ 1 & -1 & -1 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 1 & 0 \\ 0 & 0 & 0 & -1 & 1 & 1 \\ 0 & 0 & 0 & 1 & -1 & -1 \end{bmatrix}, \quad I_a = \begin{bmatrix} -1 & 1 & 0 & 0 & 0 & 0 \\ 1 & -1 & -1 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & -1 & 1 & 0 \\ 0 & 0 & 0 & 1 & -1 & -1 \\ 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix}. \tag{156}$$

The kernel of N is of dimension three and is spanned by the vectors with the following components: $(1, 1, 0, 0, 0, 0)$, $(1, 0, 1, 1, 0, 1)$ and $(0, 0, 0, 1, 1, 0)$. The first and third are in the kernel of I_a but the second is a stoichiometric generator. The subnetwork it defines is got by switching off the treactions in which a substrate-enzyme complex dissociates into its original constituents, According to the general theory this network has deficiency one and it is clear that it satisfies the condition $t = l$. Thus the deficiency one algorithm can be applied to it.

We now consider the dual futile cycle from this point of view. In this case

$$N = \begin{bmatrix} -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\ -1 & 1 & 1 & -1 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 1 & 0 & 0 & 0 & 1 & -1 & 1 & 0 \\ 0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & -1 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 1 & -1 & 1 & 1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 \end{bmatrix}. \tag{157}$$

$$Ru5P \xrightarrow{k_5} RuBP \xrightarrow{k_1} 2PGA$$

$$\begin{array}{ccccc} PGA & \xrightarrow{k_2} & DPGA & \xrightarrow{k_3} & GAP \\ & \searrow^{k_6} & & \swarrow_{k_7} & \\ & & 0 & & \end{array}$$

$$5GAP \xrightarrow[k_4]{} 3Ru5P$$

and

$$I_a = \begin{bmatrix} -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix}. \tag{158}$$

The kernel of I_a is the direct sum of the kernels of the two 5×6 blocks. The kernel of the first block is spanned by the vectors $(1,1,0,0,0,0)$ und $(0,0,0,1,1,0)$. The dimension of the kernel of I_a is four. The dimension of the kernel of N is four and two further elementary flux modes are $(1,0,1,0,0,0,0,0,0,1,0,1)$ and $(0,0,0,1,0,1,1,0,1,0,0,0)$. The corresponding subnetworks are the two simple futile cycles which are contained in the dual futile cycle. These two do not exhibit multistationarity so that the general method provides no useful information in this case.

Now models for another biological system will be considered. This is the Calvin cycle of photosynthesis. In the simplest model the species are Ru5P (ribulose-5-phosphate), RuBP (ribulose bisphosphate), PGA (phosphoglycerate), DPGA (diphosphoglycerate) und GAP (glyceraldehyde phosphate). In the simplest case a network for these substances is used under the assumption of mass action kinetics.

We have

$$N = \begin{bmatrix} -1 & 0 & 0 & 0 & 1 & 0 & 0 \\ 2 & -1 & 0 & 0 & 0 & -1 & 0 \\ 0 & 1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & -5 & 0 & 0 & -1 \\ 0 & 0 & 0 & 3 & -1 & 0 & 0 \end{bmatrix}, \quad I_a = \begin{bmatrix} 0 & 0 & 0 & 0 & -1 & 0 & 0 \\ -1 & 0 & 0 & 0 & 1 & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & -1 & 0 & 0 & 0 & -1 & 0 \\ 0 & 1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & -1 \\ 0 & 0 & 0 & 0 & 0 & 1 & 1 \\ 0 & 0 & 0 & -1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 \end{bmatrix}. \tag{159}$$

Some parameters of the system are $m = 5$, $n = 9$, $l = 3$, $s = 5$, $\delta = 1$, $r = 7$. The kernel of I_a is spanned by the vector $(0, 1, 1, 0, 0, -1, 1)$ and thus contains no positive vectors. The kernel of N is spanned by the vectors $(3, 6, 6, 1, 3, 0, 1)$ and $(3, 5, 5, 1, 3, 1, 0)$. In the corresponding networks one of the sinks is switched off. It can be shown using the Deficiency One Algorithm that this system does not permit bistationarity. In fact in this case the equations for stationary solutions can be solved explicitly. Depending on the reaction constants there is no or exactly one stationary solution. The first subnetwork always has exactly one stationary solution while the second has none. It is interesting that while the original network satisfies the condition $t = l$ the second subnetwork does not.

In a more complicated model an extended Michaelis-Menten description is used for all the reactions in the simple network. We call this the MM-MA model. The elementary flux modes for this larger model were calculated in [13]. There are two of them and they are analogous to the two modes for the simple system which we have presented. By means of this calculation it was possible to prove bistability for this system.

9 The Advanced Deficiency Algorithm

Another possibility to analyse networks with $\delta > 1$ is the Advanced Deficiency Algorithm, a generalization of the Deficiency One Algorithm. It produces a system of inequalities which are to be checked. In contrast to the case of the Deficiency One Algorithm it can happen that these inequalities are nonlinear. It is nevertheless often the case that the inequalities are linear and even when this is not the case useful information can often be obtained from those inequalities which are linear. There are quantities μ as in the Deficiency One Algorithm and the inequalities are linear in these quantities. There are, however, in general additional quantities M which can occur in the inequalities in a nonlinear manner. The Advanced Deficiency Algorithm produces systems of inequalities which are tests for the presence of multistationarity in a network. If one of them has a solution multistationarity occurs. A system of this kind which has a solution is called a signature of the network. If none of the systems have a solution then there is no multistationarity.

Step 1. In the Advanced Deficiency Algorithm an orientation $\mathcal{O}$ of the network is chosen. This means choosing a subset of the network that for each reversible reaction exactly one of the two directions belongs to the subset. Each irreversible reaction is in the subset. This choice does not influence the outcome of the algorithm.
Step 2. In the next step colinearity classes are defined. Let p be the number of elements in $\mathcal{O}$. We define a linear mapping $L_{\mathcal{O}}$ from $\mathbb{R}^p$ to $\mathbb{R}^m$ by

$$L_{\mathcal{O}}\alpha = \sum_{yy' \in \mathcal{O}} \alpha_{yy'}(y' - y). \tag{160}$$

We now consider the kernel of this mapping. Let $\{v^1, \ldots, v^d\}$ be a basis for this kernel. The dimension of the kernel is $d = p - s$ and is always at least as large as the deficiency. These two quantities are equal precisely when each reaction in the network is a cut pair. For each reaction $yy' \in \mathcal{O}$ we define a vector $w_{yy'}$ by

$$w_{yy'} = \sum_{j=1}^{d} v_{yy'}(j)\omega_j \tag{161}$$

When a reaction yy' is not in $\mathcal{O}$ then $y'y \in \mathcal{O}$ and we define $w_{yy'} = w_{y'y}$.

With vectors we define the colinearity classes of the network. Two reactions are in the same colinearity class when there is a constant $c \neq 0$ with $w_{yy'} = cw_{pp'}$. A reversible reaction and the reverse reaction are in the same class. There is a special colinearity class called the zero class in which the the w vectors are zero. A colinearity class is called reversible when each reaction in the class is reversible. If any reaction in the class is irreversible the class is called irreversible. At this point it possible to give a necessary condition for multistationarity. If the zero colinearity class is irreversible then there can be no multistationarity. It is even the case that a network of this type admits no positive stationary solutions. In this case the algorithm can be broken off at this point. It turns that the colinearity classes depend neither on the choice of $\mathcal{O}$ nor on the choice of a basis of the kernel.
Step 3. The next step is to define the colinkage sets. The network is split into subsets which are the different colinearity classes. A linkage class of a subnetwork of this type is called a colinkage set. It is possible to define strong colinkage sets and terminal strong colinkage sets in a similar way.
Step 4. For each colinearity class we define a representative vector called a colinearity class vector. If a class contains an irreversible reaction then this vector can be any positive multiple of $w_{yy'}$. If a reaction network contains two irreversible reactions yy' und pp' in the same class with $w_{yy'} = cw_{pp'}$ for a negative constant c then there is no multistationarity. There is not even a positive stationary solution. In this case the algorithm can be broken off. If a class is reversible then the colinearity class vector can be a positive multiple of any reaction yy' in the class. For the zero colinearity class zero the colinearity class vector is always zero.
Step 5. Realign the orientation. It is convenient to have each w-vector to be a positive multiple of the corresponding colinearity class vector. When attempting

to achieve this situation there is nothing to do for the irreversible reactions. In the case of the reversible reactions yy' is replaced by $y'y$ in each case where the original sign was false.
Step 6. Find coplanar sets and connected classes. Here we need two definitions. A coplanar set $\mathcal{J}$ is a set of non-zero colinearity classes with the following properties.
(i) The set $\mathcal{J}$ contains at least three colinearity classes
(ii) the colinearity class vectors in $\mathcal{J}$ lie in the same two-dimensional linear subspace. In other words each colinearity class vector in $\mathcal{J}$ can be written as a linear combination of any two other colinearity class vectors in $\mathcal{J}$
(iii) each colinearity class vector in the plane in (ii) is in $\mathcal{J}$

The coplanar sets are need not be a partition of the classes. These sets can intersect. It is also possible that a class belongs to none if these sets. It can even happen that a network has no such set.

There exists an equivalence relation on the set of non-zero colinearity classes. Two classes $\mathcal{P}_i$ und $\mathcal{P}_j$ are said to be directly connected if they belong to a common coplanar set. Two classes are said to be connected if they satisfy one of the following three properties
(i) the two classes are equal
(ii) the two classes are directly connected
(iii) there is a chain of direct connections from $\mathcal{P}_i$ to $\mathcal{P}_j$

The corresponding equivalence classes are called connected classes. If a network has more than two non-zero colinearity classes and the dimension of the kernel of $L_{\mathcal{O}}$ is two then it has only one coplanar class which contains all non-zero colinearity classes. We now define the connecting graph. The nodes are the non-zero colinearity classes and the coplanar sets. The edges connect colinearity classes with coplanar sets according to the following rule. There is an edge between a colinearity class and a coplanar set if the class is contained in the coplanar set. The connected classes are the connected components of this graph.
Step 7. Determine linearity. A system of inequalities which is used to investigate multistationarity and which has a solution is called a signature. Now two conditions will be given which together guarantee that each solution of the system of inequalities set up in this section is a signature. The conditions are
Independence linearity condition. The sum of the number of coplanar sets and the number of connected classes is d, the dimension of the kernel of $L_{\mathcal{O}}$.
Triplet independence condition. No coplanar set contains more than three colinearity classes.

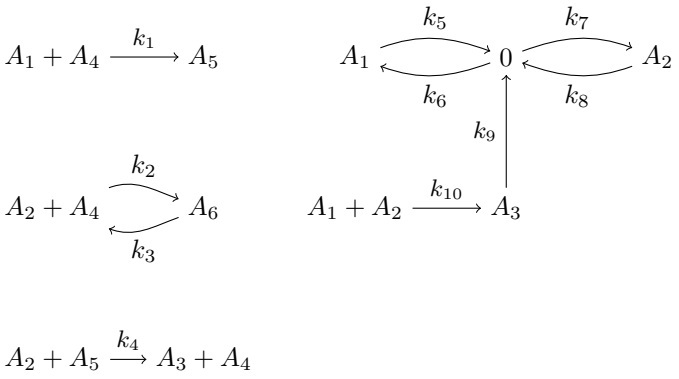
If one of these conditions is not satisfied there are possibilities to improve the situation.

Steps 1.-7. are the preliminary steps in the Advanced Deficiency Algorithm. The next steps are the heart of the algorithm. In steps 8. and 9 the colinearity classes are assigned signs and a partition of the complexes is defined. Here we adopt a terminology due to Ellison and call the subsets in this partition shelves. For each choice systems of inequalities are produced in steps 10. to 14.

Step 8. Choose signs for the colinearity classes. Each class is given a sign according to the following rules.
(i) the zero class has the sign zero
(ii) an irreversible class has a positive sign
(iii) a reversible class can have any sign which is compatible with the following conditions (iv)-(vi)
(iv) if more than one colinearity class in a coplanar set has the sign zero then each class in this set has the sign zero
(v) if there are three colinearity classes $\mathcal{P}_i$, $\mathcal{P}_j$ and $\mathcal{P}_k$ in the same coplanar set whose signs are all non-zero then there exist three numbers c_i, c_j und c_k with the signs of the corresponding classes which satisfy the condition $c_i w^i + c_j w^j + c_k w^k = 0$.
(vi) if there are three colinearity classes $\mathcal{P}_i$, $\mathcal{P}_j$ and $\mathcal{P}_k$ in the same coplanar set with the property that $\mathcal{P}_i$ has the sign zero and $\mathcal{P}_j$ and $\mathcal{P}_k$ signs which are non-zero then there exist three numbers c_i, c_j und c_k such that c_j and c_k have the same sign which satisfy the condition $c_i w^i + c_j w^j + c_k w^k = 0$.

If there is no choice of signs for which these conditions are satisfied then there is no multistationarity. There are not even any positive stationary solutions. In this case the algorithm can be broken off.
Step 9. Choose shelves for the complexes. There are two differences to the Deficiency One Algorithm. Firstly, it is the reactions and not the complexes which are put into the shelves. Secondly, there are upper, middle and lower shelves for each colinearity class with non-zero sign.

We consider a fixed colinearitry class with non-zero sign. We denote the upper, middle and lower shelves by U, M und L. The conditions are
(i) a reaction whose left hand side is in a non-terminal strong colinkage class is in M
(ii) an irreversible reaction is in M
(iii) a reversible reaction whose left hand side is in a terminal strong colinkage class can be in U, M or L provided reactions in the same colinearity class whose left hand sides are in the same strong colinkage set are put in the same shelf.
Step 10. Shelving inequalities. If a reaction yy' is in the middle shelf of a colinearity class $\mathcal{P}_i$ then $\mu \cdot y = M_i$. If yy' is in the upper shelf of the class $\mathcal{P}_i$ then $\mu \cdot y > M_i$. If yy' is in the lower shelf of the class $\mathcal{P}_i$ then $\mu \cdot y < M_i$.
Step 11. Further inequalities for the upper and lower shelves. If a reaction yy' in $\mathcal{O}$ is in the upper shelf of a class with positive sign or in the lower shelf of a class with negative sign then the inequality $\mu \cdot y' > \mu \cdot y$ holds. If a reaction yy' in $\mathcal{O}$ is in the upper shelf of a class with negative sign or in the lower shelf of a class with positive sign then the inequality $\mu \cdot y' < \mu \cdot y$ holds.
Step 12. Inequlities for classes with sign zero. For each reaction yy' in $\mathcal{O}$ which belongs to a class with sign zero the equation $\mu = y = \mu = y'$ holds.
Step 13. Inequalities for the quantities M_i. If all classes in a coplanar set have the sign zero then no further inequalities arise. If $\mathcal{P}_i$, $\mathcal{P}_j$ and $\mathcal{P}_k$ are in the same coplanar set and $\mathcal{P}_i$ has sign zero while $\mathcal{P}_j$ and $\mathcal{P}_k$ have non-zero signs then gilt $M_j = M_j$. If all classes in a coplanar set have non-zero sign then there is a choice. If there exist constants c_i with the same signs as their classes which



satisfy $c_k w^k = c_i w^i + c_j w^j$ then one of the following three conditions must hold. $M_i > M_j > M_k$, $M_i = M_j = M_k$ or $M_i < M_j < M_k$.
Step 14. Are there solutions of the system of inequalities? The inequalities which were listed up to now are the full set of linear inequalities for the network. If the system has a solution then this is called a pre-signature for the network. If it is known that linear inequalities suffice then each pre-signature is a signature.

In the search for presignatures all choices must be checked. If there are no pre-signatures then multistationarity is not possible.

The analysis of the nonlinear conditions will not be considered further here. Instead the constructions up to now will be illustrated using an example. This example does not come from a particular application but was chosen so as to allow a better understanding of the theory. The network contains six species $A_1 - A_6$.

Now the algorithm will be applied to this network. The subset $\mathcal{O}$ can be chosen as the reactions $A_1 \to 0$, $0 \to A_2$, $A_3 \to 0$, $A_1 + A_2 \to A_3$, $A_1 + A_4 \to A_5$, $A_2 + A_4 \to A_6$ and $A_2 + A_5 \to A_3 + A_4$. Thus there are seven reactions in $\mathcal{O}$. In this network there are six species. Thus in this case $L_{\mathcal{O}}$ is a mapping from $\mathbb{R}^7$ to $\mathbb{R}^6$. The corresponding matrix is made up a choice of columns of the stoichiometric matrix. The rank of this matrix is 5 and hence its kernel has dimension two. A basis of the kernel consists of the vectors $[-1,1,1,1,0,0,0]^T$ and $[-1,1,1,0,1,0,1]^T$. The w-vectors are the rows of the matrix whose columns are these vectors. We get the vectors $[-1,-1]$, $[1,1]$, $[1,0]$, $[0,1]$ und $[0,0]$. The are four colinearity classes.

$$\begin{aligned}
\mathcal{P}_0 &= \{A_2 + A_4 \to A_6, A_6 \to A_2 + A_4\},\\
\mathcal{P}_1 &= \{A_1 \to 0, 0 \to A_1, A_2 \to 0, 0 \to A_2, A_3 \to 0\},\\
\mathcal{P}_2 &= \{A_1 + A_2 \to A_3\},\\
\mathcal{P}_3 &= \{A_1 + A_4 \to A_5, A_2 + A_5 \to A_3 + A_4\}. \qquad (162)
\end{aligned}$$

$\mathcal{P}_0$ is the zero colinearity class. Only $\mathcal{P}_0$ is reversible. These classes define four subnetworks. There are five colinkage classes and nine strong colinkage sets. The strong colinkage set $\{A_3\}$ belongs to two different colinearity classes.

It is non-terminal with respect to $\mathcal{P}_1$ and terminal terminal with respect to $\mathcal{P}_2$. We choose $w^0 = [0,0]^T$, $w^1 = [1,1]^T$, $w^2 = [1,0]^T$ and $w^3 = [0,1]^T$ as representatives of the classes. In Step 5 we only have to reverse the direction of one reaction. $A_1 \to 0$ is replaced by $0 \to A_1$. In the example there is only one coplanar set $\{\mathcal{P}_1, \mathcal{P}_2, \mathcal{P}_3\}$. Up to and including Step 7 the rest of the discussion is trivial for this example. In Step 8 there is only one possible choice of signs for this network. $\mathcal{P}_0$ is given the sign zero and the other classes, which are all irreversible, are given a positive sign. All conditions are satisfied. In particular condition (v) holds. We must choose shelves for three different classes.

The left hand sides of the reactions $A_3 \to 0$, $A_1 + A_2 \to A_3$, $A_1 + A_4 \to A_5$ und $A_2 + A_5 \to A_3 + A_4$ are in non-terminal colinkage sets, so that they must be in the middle shelves of their colinkage classes. The second condition implies that the same reactions are in the middle shelves. The reactions $A_1 \to 0$, $0 \to A_1$, $A_2 \to 0$ and $0 \to A_2$ are in the class $\mathcal{P}_1$ and their left hand sides are in the same terminal strong colinkage set (with respect to $\mathcal{P}_1$) and they must be in the same shelf. Suppose they are all in the upper shelf. Then we get the distribution

$$\begin{aligned} &U_1 = \{A_1 \to 0, 0 \to A_1, A_2 \to 0, 0 \to A_2\}, M_1 = \{A_3 \to 0\}, L_1 = \emptyset \\ &U_2 = \emptyset, M_2 = \{A_1 + A_2 \to A_3\}, L_2 = \emptyset \\ &U_3 = \emptyset, M_2 = \{A_1 + A_4 \to A_5, A_2 + A_5 \to A_3 + A_4\}, L_3 = \emptyset \end{aligned} \tag{163}$$

Step 10 gives the following inequalities. $\mu_1 > M_1$, $\mu_2 > M_1$, $0 > M_1$, $\mu_3 = M_1$, $\mu_1 + \mu_2 = M_2$, $\mu_1 + \mu_4 = M_3$ and $\mu_2 + \mu_5 = M_3$. In Step 11 the inequalities $\mu_1 > 0$ and $\mu_2 > 0$ are added. In Step 12 we get $\mu_2 + \mu_4 = \mu_6$. Since the relation $w^1 = w^2 + w^3$ holds one of the relations $M_2 > M_1 > M_3$, $M_2 = M_1 = M_3$ or $M_2 < M_1 < M_3$ is obtained in Step 13. Here we concentrate on the third possibility. In this case there are no solutions with $\mu \neq 0$ which have signs compatible with the stoichiometric subspace. Thus this system is not a signature. (For this system μ has signs comptible with the stoichiometric subspace if and only if either the set $\{\mu_4, \mu_5, \mu_6\}$ contains a positive and a negative number or all three numbers are zero.)

There two other choices in Step 12 which must be examined. For the choice $M_2 = M_1 = M_3$ there is again no solution. In the case $M_2 > M_1 > M_3$ there are solutions, e.g. $\mu = [1, 4, -1, -3, -6, 1]$, $M_1 = -1$, $M_2 = 5$ and $M_3 = -2$. We obtain a signature. A choice was also made in Step 9. There is an alternative possibility. With the new choice we get three systems of inequalities. It turns out that none of these has acceptable solutions.

We will prove some statements which have to do with the linear inequalities.
Theorem 9.1 We consider a colinearity class $\mathcal{P}_i$ and shelves for the reactions in $\mathcal{P}_i$ with the properties that both reactions of a reversible pair are in the same shelf and all irreversible reactions are in the middle shelf. If a vector μ and a number M_i exist so that the inequalities from Step 10 are satisfied then all reactions in $\mathcal{P}_i$ whose left hand sides are in the same colinkage class must be in the same shelf.
Proof It is given that if yy' is a reversible reaction then yy' and $y'y$ are in the

same shelf. If two reactions in $\mathcal{P}_i$ have the same left hand side y they must be in the same shelf since if they were in different shelves the system would satisfy two of the inequalities $y\cdot > \mu$, $y\cdot = \mu$ and $y\cdot < \mu$, a contradiction.

Suppose that all reactions in $\mathcal{P}_i$ whose left hand sides are in a given strong colinkage set are reversible. Let yy' and pp' be two reactions of this kind whose left hand sides are in the given set. There must exist a path $y, y_1, \cdots, y_t, p$ in the colinearity class. All these reactions are reversible. Since yy_1 is reversible the reactions yy_1 and y_1y must be in the same shelf. At the same time y_1y and y_1y_2 must be in the same shelf. This reasoning can be continued to see that yy_1 and py_t are in the same shelf. Hence yy' and pp' are in the same shelf. This proves one case of the theorem.

Now suppose instead that there is a complex p in the given strong linkage set with the property that pp' is an irreversible reaction in $\mathcal{P}_i$. Since pp' is irreversible it must be in the middle shelf. Let yy' be another reaction in the colinearity class whose left hand side is in the given colinkage set. If yy' is irreversible it must be in the middle shelf. Suppose that yy' is reversible. Since y and p are in the same strong colinkage set there is a path $(y, y_1, \ldots, y_t, p)$ consisting of reactions in $\mathcal{P}_i$. yy' and yy_1 are in the same shelf. If yy_1 is irreversible then it must be in the middle shelf. Otherwise yy_1 would have to be in the same shelf as y_1y. It is also the case that y_1y is in the same shelf as y_1y_2. If we continue wiht this logic we see that either yy' is in the middle shelf or that yy' is in the same shelf as pp'. But pp' is in the middle shelf. Thus in either case yy' is in the middle shelf. This completes the proof.

A first useful observation when we are looking for bistability is that Lemma 7.7 also holds in the present case. Thus the task is to ask whether certain equations for quantities μ und $\kappa_{yy'}$ have a solution. We now define $g_{yy'}$ as $\kappa_{yy'} - \kappa_{y'y}$ for yy' reversible and $\kappa_{yy'}$ for yy' irreversible and $h_{yy'}$ as $e^{y\cdot\mu}\kappa_{yy'} - e^{y'\cdot\mu}\kappa_{y'y}$ for yy' reversible und $e^{y\cdot\mu}\kappa_{yy'}$ for yy' irreversible. Then the essential equations can be written in the form

$$L_{\mathcal{O}}h = \sum_{yy'\in\mathcal{O}} h_{yy'} = 0, \quad L_{\mathcal{O}}g = \sum_{yy'\in\mathcal{O}} g_{yy'} = 0. \tag{164}$$

What we need is exactly that g and h are in the kernel of $L_{\mathcal{O}}$. We choose a basis of the kernel and conclude that $g = \sum_{i=1}^{d} \lambda_i v^i$ and $h = \sum_{i=1}^{d} \eta_i v^i$ for suitable coefficients λ_i and η_i. The vectors g and h can also be expressed in terms of the w-vectors. $g_{yy'} = \lambda \cdot w_{yy'}$ and $h_{yy'} = \eta \cdot w_{yy'}$. These equations can be used to eliminate g and h from the equations in Lemma 7.7. The result is that $\lambda \cdot w_{yy'}$ is given by $\kappa_{yy'} - \kappa_{y'y}$ for yy' reversible and $\kappa_{yy'}$ for yy' irreversible and that $\eta \cdot w_{yy'}$ is given by $e^{y\cdot\mu}\kappa_{yy'} - e^{y'\cdot\mu}\kappa_{y'y}$ for yy' reversible and by $e^{y\cdot\mu}\kappa_{yy'}$ for yy' irreversible. Out task has now been reformulated as the search for the vectors λ and η. Now we would like to eliminate the coefficients $\kappa_{yy'}$. Suppose first that yy' is irreversible. Then $\kappa_{yy'} = \lambda \cdot w_{yy'}$ and $\kappa_{yy'}e^{y\cdot\mu} = \eta \cdot w_{yy'}$. In order to have $\kappa_{yy'} > 0$ we need the conditions $\lambda \cdot w_{yy'} > 0$ and $\frac{\eta\cdot w_{yy'}}{\lambda\cdot w_{yy'}} = e^{y\cdot\mu}$ when yy' is irreversible. Thus we have

Theorem 9.2 If the w-vectors of two irreversible reactions in the same colin-

earity class point in opposite directions then there exist no positive stationary solutions.
Proof Suppose that yy' and pp' are two irreversible reactions in the same colinearity class which point in opposite directions, i.e. that there exists a positive number c with $w_{yy'} = -cw_{pp'}$. Taking the inner product of this equation with λ gives a contradiction.
Theorem 9.3 If the zero colinearity class is irreversible there exist no positive stationary solutions.
Proof If the zero colinearity class is irreversible there is an irreversile reaction yy' whose representative $w_{yy'}$ is zero. It then follows that $\lambda \cdot w_{yy'} = 0$, a contradiction.

10 Summary

In this course various tools of Chemical Reaction Network Theory (CRNT) have been introduced. Various possibilities have been explained how it can be decided whether the equations with mass action kinetics defined by a reaction network have more than one positive stationary solution in a stoichiometric class. The aim is to decide whether there are reaction constants for which such solutions exist or whether there are no such reaction constants. In this context the concept of deficiency plays a central role. Now some aspects of these results will be summarized.

If a system has deficeincy zero then multistationarity is always ruled out. It is also relatively easy to decide, with the help of the Deficiency Zero Theorem, whether there are any positive stationary solutions at all. A necessary and sufficient condition is that the network is weakly reversible. If the network is weakly reversible then the stationary solution is asymptotically stable. Thus good control over the asymptotic behaviour is available in the weakly reversible case. If the system is not weakly reversible then the usual theory does not say much about the long-time behaviour.

If a network has deficiency one then the Deficiency One Algorithm is available. There is the condition of positive dependence. When this condition does not hold there exist no positive stationary solutions. It can only fail for networks which are not weakly reversible. There is also a graph-theoretic condition on the network which is needed to allow the Deficiency One Algorithm to be applied. There is also the condition $t = l$. With the help of these conditions a large class of networks are identified for which a powerful tool is available. In this case the Deficiency One Algorithm provides an equivalence between multistationarity and the existence of a solution of at least one system of linear inequalities taken from a finite set. Conditions are obtained which can be checked by hand or by computer. This provides a programme which can be applied to study networks of deficiency one. In the case that there is multistationarity no information is obtained about the number of stationary solutions. If there is no multistationarity no information is obtained about whether there is any positive solution at all. No information is obtained about the stability of the statinary solutions.

The Deficiency One Theorem gives information about multistationarity for certain networks with δ arbitrarily large but in comparison with other results of the theory this result seems to be quite isolated. The Advanced Deficiency Algorithm is an analogue of the Deficiency One Algorithm which can in principle be applied to arbitrary networks. Is some case multistationarity can be excluded. Otherwise a criterion is obtained for multistationarity which is necessary and sufficient. The perhaps greatest difference to the Deficiency One Algorithm is that the inequalities obtained are partly nonlinear. It must be asked to what extent the inequalities obtained are simpler than the equations for statoinary solutions themselves. At least there is a programme for investigating multistationarity in networks with arbitrary deficiency. There exists an extension of the Advanced Deficiency Algorithm called the Higher Deficiency Algorithm [18].

Another method of obtaining a better understanding of networks with $\delta > 0$ is the method of elementary flux modes. This gives networks of deficiency one starting from a given network. If the small networks permit multistationarity then the big one often does so too. A disadvantage of this method is it can only find multistationarity in a small part of the parameter space. An advantage is that statements about stability can be propagated from the small to the large network.

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