Chemical reaction network theory for *in-silico* biologists

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1 Introduction

Mathematical models of intra-cellular biological systems are often based on systems of nonlinear ordinary differential equations (ODEs). Recent studies of some signalling cascades using both computational analysis of an ODE model and experimental manipulation of the signalling pathway have revealed the presence of multiple fixed points, [3, 7, 21]. While biological systems evidently exhibit more complex behaviours, such as oscillations, [18], the significance of fixed points in biological dynamics has been a recurring theme in the literature at least as far back as Max Delbrück's observations on "cytoplasmic inheritance", [9, 24, 33].

It would be helpful if there were mathematical theorems that tell us whether a relevant system of ODEs has any fixed points. Most mathematicians would regard this as hopeless, given the nonlinearity of the equations and the complexity of the systems. The biological studies referred to above were all based on computational simulation. However, there are at least two contexts in which potentially relevant mathematical results have been established. The first is the recent proof by Christophe Soulé of René Thomas' longstanding conjecture about multiple fixed points, [31, 32]. This result gives an elegant necessary condition for multiple nondegenerate fixed points of a system of nonlinear ODEs. The second is Chemical Reaction Network Theory (CRNT), which establishes several theorems about systems of nonlinear ODEs which describe the behaviour of reactor vessels used in chemical engineering. These systems are derived from mass-action kinetics, which is probably a more realistic assumption in chemical engineering than in biology (more about this below). In these notes we discuss CRNT and leave the work of Soulé and Thomas for later consideration.

CRNT has been developed over the last 30 years, initially through the work of Horn and Jackson and subsequently by Martin Feinberg and his students. The theory introduces new concepts, such as the *deficiency* of a reaction network, and gives conditions on such networks for the existence, uniqueness, multiplicity and stability of fixed points. These conclusions sometimes hold irrespective of the values of the parameters in the system, which should be of interest to those who associate such behaviour with biological robustness, [5, 34]. The *deficiency 0 theorem* is proved in the first four lectures of [11] and draws on earlier work of Horn and Jackson, [22], and Feinberg and Horn, [16]. A discussion of this result with many examples, along with a similar discussion of the *deficiency 1 theorem*, is given in [12]. A proof of the deficiency 1 theorem is given in [14]. The *SCL graph theorem* is proved in Paul Schlosser's PhD thesis, [29], and the result is discussed in [30]. Algorithmic issues are discussed in [13] and proofs are given in [15].

CRNT appears to be as little known among *in-silico* biologists as among mathematicians; I came across it in Jim Bailey's commentary, [4]. It is still unclear to what extent CRNT is directly applicable to biological settings. Even if it is not, by understanding its mathematical basis we might hope to derive other results that are more appropriate to biology. To that end, these notes are an introduction to CRNT with proofs of all the steps leading to the deficiency 0 theorem. The results discussed here are all taken from the various papers on CRNT cited above, although the emphasis, and some of the details, are different.

The key insight of CRNT is that the apparent nonlinearity of the ODEs arising from mass-action kinetics conceals a great deal of linearity. This arises from the underlying network of chemical reactions, which define a directed graph on the *complexes* that participate in the chemical reactions. The complexes are the multisets of chemical species which occur on the left and right hand sides of each chemical reaction. The existence of this underlying structure limits the nonlinearity that can appear, which is why the strong results of CRNT are possible. Working at the level of complexes serves to disentangle the interactions between individual chemical species. Fixed points thereby fall into two categories: those that arise at the level of complexes and those that arise from the way in which different complexes contain the same chemical species. The deficiency of a network, which we define in this paper as the dimension of a certain vector space (Definition 3.2), measures the extent of the second possibility. However, one of the most interesting discoveries of CRNT has to do with fixed points of the first kind. Whenever there is at least one such fixed point, all fixed points must

be of that kind (Theorem 6.2), and, in this case, the fixed points are extremely rigid, with very nice properties of uniqueness and asymptotic stability. The usual statement of the deficiency 0 theorem, as in [12, Theorem 6.1.1], is misleading in this respect. It suggests that the nonexistence of fixed points of the second kind and the nice rigidity properties are due to the deficiency being 0. This is not the case. Deficiency 0 is only required to show the existence of some fixed point of the first kind. Accordingly, we state the main result of these notes as Theorem 6.4 while §7 contains the simple arguments needed to establish when a fixed point of the first kind exists.

Mass-action kinetics defines a labelling by positive numbers on the directed graph of complexes, corresponding to the rate constants of each reaction. There is an intimate relationship between labelled, directed graphs and matrices, which is particularly significant when the matrices are nonnegative. It should come as no surprise, therefore, that Perron-Frobenius theory plays an important role in the dynamics, at least at the level of complexes. This is the key technical result of CRNT, Theorem 4.2, and its proof occupies §4. The proof given in the CRNT literature avoids Perron-Frobenius theory and is relegated to the Appendix of [16]. Some elementary aspects of convex analysis (separating hyperplanes) and dynamical systems (Lyapunov functions) are needed in §6 but, for the most part, amazingly, linear algebra is sufficient.

I am indebted to Martin Feinberg for very kindly sending me several reprints as well as much unpublished material, on which I have relied in putting together these notes. I am also very grateful to members of the Theory Reading Group at the Bauer Center, without whose perceptive and persistent questions these notes would have been as garbled as the lectures which they endured.

2 Chemical reaction networks

A chemical reaction network is a finite set of reactions among a finite set of chemical species. The customary symbolism is used to represent reactions. The following set of reactions constitutes a network of 6 reactions among the 5 chemical species A, B, C, D, E.

$$A \rightleftharpoons 2B$$
$$A + C \rightleftharpoons D$$
$$B + E$$
(1)

The intuition behind this notation should be clear. From the first pair of reactions, one molecule of species A is converted, reversibly, into two molecules of species B. Similarly, one molecule each of A and C combine to make a single molecule of D, also reversibly, while D itself disintegrates irreversibly into one molecule each of B and E. Finally, this same combination of B and E are able to irreversibly make one molecule each of A and C.

A reaction network like (1) gives rise to a dynamical system, which describes how the state of the network changes over time. The state is given by the concentrations of all the chemical species in the network. Let $\mathbb{R}$ denote the reals, $\mathbb{P}$ the positive reals, $\mathbb{P} = \{x \in \mathbb{R} \mid x > 0\}$, and $\overline{\mathbb{P}}$ the nonnegative reals, $\overline{\mathbb{P}} = \mathbb{P} \cup \{0\}$. Let $\mathcal{S}$ denote the set of species, $\mathcal{S} = \{A, B, C, D, E\}$, and consider the vector of concentrations to lie in the vector space, $\mathbb{R}^{\mathcal{S}}$, called the *species space*. Only nonnegative concentrations are physically realistic. (It is possible for the concentration of some species to fall to zero, corresponding to it being completely consumed in the reactions.)

Constructing a vector space as a space of functions on an index set gives a standard choice of basis, corresponding to the characteristic functions of the singleton subsets. The CRNT literature uses component notation rather than functional notation for elements of $\mathbb{R}^{\mathcal{S}}$ and we do the same here: if $c \in \mathbb{R}^{\mathcal{S}}$ then c_x will denote the value of c at the species $x \in \mathcal{S}$ in preference to $c(x)$. We write vectors as column vectors.

The kinetics of the various reactions in the network determine the dynamics by specifying how dc/dt varies with c. Under the assumption of mass-action, the rate at which a reaction proceeds (ie: the rate of generation of the reaction products) is proportional to the product of the concentrations of the reaction substrates. The constant of proportionality is a specified positive *rate constant* associated to the reaction in question.

CRNT is able to draw some conclusions without assuming mass-action kinetics; see, for instance, parts (i) and (ii) of the statement of the deficiency 0 theorem in [12, Theorem 6.1.1]. In its weakest form a general kinetics associates a function to each reaction, which specifies a numerical rate for each concentration vector in $\mathbb{P}^{\mathcal{S}}$. The results for general kinetics are relatively minor in comparison to those assuming mass-action and we shall not discuss them further here.

With the assumption of mass-action, the first pair of reactions in (1) make the following contributions to the rate of formation of c_A:

$$- (\kappa_{A \to 2B})c_A + (\kappa_{2B \to A})c_B^2 \ , \tag{2}$$

where $\kappa_{A \to 2B} \in \mathbb{P}$ is the rate constant for $A \to 2B$ and $\kappa_{2B \to A} \in \mathbb{P}$ is that for the reverse reaction. The effect of these same reactions on the concentration of B must also take into account the different *stoichiometry*: two molecules of B are produced or consumed for each molecule of A. Hence, the contribution to dc_B/dt is given by

$$2(\kappa_{A \to 2B})c_A - 2(\kappa_{2B \to A})c_B^2 \ .$$

By considering all the possible ways in which a given species is produced or consumed according to (1), the dynamical evolution of the reaction network under mass-action kinetics can be described by

$$
\begin{aligned}
dc_A/dt &= -(\kappa_{A \to 2B})c_A + (\kappa_{2B \to A})c_B^2 - (\kappa_{A+C \to D})c_A c_C + (\kappa_{D \to A+C})c_D + (\kappa_{B+E \to A+C})c_B c_E \\
dc_B/dt &= 2(\kappa_{A \to 2B})c_A - 2(\kappa_{2B \to A})c_B^2 + (\kappa_{D \to B+E})c_D - (\kappa_{B+E \to A+C})c_B c_E \\
dc_C/dt &= -(\kappa_{A+C \to D})c_A c_C + (\kappa_{D \to A+C})c_D + (\kappa_{B+E \to A+C})c_B c_E \\
dc_D/dt &= (\kappa_{A+C \to D})c_A c_C - (\kappa_{D \to A+C})c_D - (\kappa_{D \to B+E})c_D \\
dc_E/dt &= (\kappa_{D \to B+E})c_D - (\kappa_{B+E \to A+C})c_B c_E \ .
\end{aligned}
\tag{3}
$$

Chemical engineers use models like (3) to describe what goes on inside a liquid or gas phase reactor vessel. Mass-action kinetics appears to be a good assumption for a well-mixed reactor of macroscopic proportions kept at a constant temperature. (Rate constants can very markedly with the ambient temperature, [8, §1.5].) However, practical reactors possess other features which are not directly represented in reaction networks like (1). Reactor vessels are usually open systems, in which there is a continuous supply of fresh substrates and outflow of products. They may also contain solid phase catalysts, which, although they participate in the reactions, are not extracted in the outflow. Refinements like this can all be accommodated within CRNT; see [11, §2E] or [12, §4]. For instance, the in-flow of a substrate can be modelled with a *pseudo-reaction* of the form $0 \to A$,

Biological systems suffer many further complexities, some of which appear to be accommodated within CRNT. Experimental studies of biological systems like metabolic pathways are often carried out by by buffering the concentration of certain substrates so that they remain approximately fixed over the duration of the experiment. With mass-action kinetics, these fixed concentrations can be incorporated into the rate constants of the reactions in which the substrates participate.

Enzymes play a catalytic role in biology. Biochemists usually describe the kinetics of enzyme catalysed reactions by formulae which look much more complex than mass-action rules like (2). The well-known Michaelis-Menten kinetics for a reaction $A \to B$, catalysed by an enzyme E,

$$\frac{dc_B}{dt} = \frac{V_{max} c_A}{K_M + c_A} \ ,$$

is a rational, and not linear, function of the concentration c_A. However, the Michaelis-Menten formula is derived from the reaction network

$$A + E \rightleftharpoons AE \to B + E \ . \tag{4}$$

under mass-action, assuming that the transitional enzyme-substrate complex, AE, is in steady state, c_B is small and $c_E \ll c_A$, [8, §2.2]. Michaelis-Menten kinetics may hence be incorporated into the CRNT framework by appropriately modifying the underlying reaction network.

It is clearly too much to hope that what works for a large, well-mixed, isothermal reactor will also work, in general, for a biological "vessel". Biological cells are small enough to be isothermal but are neither macroscopic nor well-mixed. Some chemical species, such as calcium ions, are heavily sequestered on membranes or otherwise localised, and their concentrations cannot be considered as spatially homogeneous. Measured amounts of certain proteins in E. Coli have been found to be as low as 10-100 *molecules*, [28], which should make anyone extremely uneasy about representing concentrations as smooth functions of time. At this scale stochastic variation in molecule numbers becomes important both within a single cell and from cell to cell. One of the most interesting biological questions is how cells both exploit and subvert such stochastic variation in order to carry out what appear to be very deterministic functions (such as collectively making an organism). Neither theory nor experiment has yet come to grips with this at a fundamental level, despite some pioneering efforts, [1, 10, 23, 25].

Notwithstanding these significant caveats, it appears there are situations when a chemical reaction network model is a reasonable approximation which gives some insight into the underlying biology, as in some of the studies cited in the Introduction. Hopefully, as we continue to build more models of cellular behaviour we will begin to understand better what "a reasonable approximation" means!

The system (3) lives in 5 dimensions, has quadratic nonlinearities and multiple parameters (the rate constants). On the face of it, its behaviour could be extremely complicated. The famous Lorenz system in three variables x, y and z, [26],

$$
\begin{aligned}
dx/dt &= ay - ax \\
dy/dt &= cx - y - xz \\
dz/dt &= xy - bz
\end{aligned}
\tag{5}
$$

also has quadratic nonlinearities and is well-known to exhibit deterministic chaos for certain values of the parameters a, b and c, [35]. Nevertheless, the deficiency 0 theorem implies that, irrespective of the values of the parameters, the system (3) has a unique fixed point, in a sense that we will make precise, which is asymptotically stable. Notice that (5) cannot come from a chemical reaction network (with any kinetics), since the disintegration of y into x and z, indicated by the term $-xz$ in the second equation, is not matched by corresponding production terms for x and z. This tells us that the structure of (3) is constrained by the fact that it emerges from a chemical reaction network. We need further notation to exhibit these constraints.

A *complex* in a reaction network is any of the terms that make up the left hand or right hand side of a reaction in the network. The network (1) has five complexes: A, $2B$, $A + C$, D and $B + E$. Let $\mathcal{C}$ denote the set of complexes. Complexes can be thought of as *multisets* of species: as functions $\mathcal{S} \to \mathbb{N}$, where $\mathbb{N}$ denotes the nonnegative integers, or, equivalently, as elements of $\mathbb{N}^{\mathcal{S}}$. (In comparison, *sets* of species can be thought of as characteristic functions, $\mathcal{S} \to \{0, 1\}$, or, equivalently, as elements of $\{0, 1\}^{\mathcal{S}}$.) By the *multiplicity* of a species x in the complex y we mean simply $y(x)$, when y is thought of as a multiset: the species B has multiplicity 2 in the complex $2B$ and multiplicity 0 in the complex $A + C$. When regarded as a multiset, each species gives rise to a basis element of $\mathbb{R}^{\mathcal{S}}$, so that A corresponds to the function $\mathcal{S} \to \mathbb{R}$ which has multiplicity 1 at A and multiplicity 0 at all other species.

If y is a complex, it can be represented in species space by regarding a multiset, $y : \mathcal{S} \to \mathbb{N}$, in the obvious way as a function $y : \mathcal{S} \to \mathbb{R}$, so that

$$y_x = \text{multiplicity of } x \text{ in } y \,. \tag{6}$$

This abuse of notation, perpetrated in the CRNT literature and perpetuated here, is convenient. It should be clear from the context whether "the complex y" refers to $y \in \mathcal{C}$ or $y \in \mathbb{R}^{\mathcal{S}}$.

We also need a convenient way of representing mass-action. If $c \in \mathbb{R}^{\mathcal{S}}$ and $y \in \mathcal{C}$, define c^y by

$$c^y = \prod_{s \in \mathcal{S}} (c_s)^{y_s} \ . \tag{7}$$

Since, by assumption, $y \in \mathbb{N}^{\mathcal{S}}$ for any complex y, c^y is always well defined for all $c \in \mathbb{R}^{\mathcal{S}}$. Note that if some component of c is zero, say $c_s = 0$, then $c^y = 0$ for any $y \in \mathcal{C}$, unless it is also the case that $y_s = 0$, for then $0^0 = 1$. Finally, let $\mathcal{R}$ denote the set of reactions so that, for (1),

$$\mathcal{R} = \{A \rightarrow 2B, 2B \rightarrow A, A + C \rightarrow D, D \rightarrow A + C, D \rightarrow B + E, B + E \rightarrow A + C\} \ .$$

Definition 2.1 *A chemical reaction network is a quadruple $\{\mathcal{S}, \mathcal{C}, \mathcal{R}, \kappa\}$ where $\mathcal{S}$ is a finite set of species; $\mathcal{C}$ is a finite set of multisets of species, called complexes; $\mathcal{R}$ is a relation on $\mathcal{C}$, denoted $y \rightarrow y'$ for $y, y' \in \mathcal{C}$, which represents a reaction converting y to y'; and $\kappa : \mathcal{R} \rightarrow \mathbb{P}$ associates a positive rate constant to each reaction.*

The time evolution of a chemical reaction network is given by

$$\frac{dc}{dt} = \sum_{y \rightarrow y' \in \mathcal{R}} (\kappa_{y \rightarrow y'}) c^y (y' - y) \ . \tag{8}$$

It is easy to confirm that (8) translates into (3) for the reaction network (1). We shall use (8) as the definition of the dynamics of a chemical reaction network. It reveals the first signs of the linearity hidden in (3).

Definition 2.2 *The stoichiometric subspace, S, of a chemical reaction network is the vector subspace of $\mathbb{R}^{\mathcal{S}}$ defined by $S = \mathrm{span}\{y' - y \mid y \rightarrow y' \in \mathcal{R}\}$.*

We note from (8) that $dc/dt \in S$. It follows that any trajectory of (8) must lie in a fixed coset of S. If the initial conditions lie in a particular coset of S, then the entire subsequent time evolution of (8) from those initial conditions is constrained to lie in the same subspace. S can be easily determined for the reaction network (1). It is easiest to work with multisets. Ignoring the obvious linear dependencies coming from reverse reactions, we find that

$$S = \mathrm{span}\{2B - A, A + C - D, B + E - D, B + E - A - C\} \ .$$

Since $B + E - A - C = (B + E - D) - (A + C - D)$, this simplifies further to give

$$S = \mathrm{span}\{2B - A, A + C - D, B + E - D\} \tag{9}$$

which is clearly linearly independent in $\mathbb{R}^{\mathcal{S}}$. It follows that $\dim S = 3$, so that the stoichiometric subspace has co-dimension 2 in the species space. This represents a considerable constraint on the dynamics of (3), which can be made explicit as conservation laws. Recall that $\mathbb{R}^{\mathcal{S}}$ has a standard inner-product, denoted $\langle u, v \rangle$ for $u, v \in \mathbb{R}^{\mathcal{S}}$, under which the standard basis becomes orthonormal. Conservation laws are elements of the orthogonal complement of S, $S^{\perp}$. If $u \in S^{\perp}$ then the linear form $x \rightarrow \langle x, u \rangle$ is necessarily constant on any coset of S and hence constant on any trajectory of (8). It is not difficult to see that, for the network (1), $C + D + E$ has inner-product 0 with each basis element of S in (9), so that $C + D + E \in S^{\perp}$. It follows that the sum of the concentrations, $c_C + c_D + c_E$, is constant along any trajectory of (3). This should be intuitively clear from inspection of (1). Since $S \oplus S^{\perp} = \mathbb{R}^{\mathcal{S}}$, the dimension of $S^{\perp}$ must be 2. Hence there must be one more independent conservation law in addition to $C + D + E$. We leave it to the reader to work it out.

The right hand side of (8) is a mixture of nonlinear and linear parts. By decomposing it in a certain way, we can separate them. This forms the subject of the next section.

3 Linearity in chemical reaction networks

The dynamics of a chemical reaction network takes place in species space, $\mathbb{R}^{\mathcal{S}}$, while the network itself is a relation on the set of complexes, $\mathcal{C}$. It will turn out to be very helpful to reconstruct the dynamics in terms of complexes. Let $f : \mathbb{R}^{\mathcal{S}} \to \mathbb{R}^{\mathcal{S}}$ denote the right hand side of (8), so that $dc/dt = f(c)$.

Let $\mathbb{R}^{\mathcal{C}}$ denote *complex space*. It has a natural basis defined by the characteristic functions of the singleton subsets $\{y\}$, where $y \in \mathcal{C}$. If $U \subseteq \mathcal{C}$ let $\omega_U : \mathcal{C} \to \{0, 1\}$ denote its characteristic function:

$$\omega_U(y) = \left\{ \begin{array}{ll} 1 & \text{if } y \in U \\ 0 & \text{otherwise} \end{array} \right.$$

For a singleton subset, $\{y\}$, where $y \in \mathcal{C}$, we shall use ω_y in place of $\omega_{\{y\}}$. Complex space then has the standard basis $\{\omega_y \mid y \in \mathcal{C}\}$.

Let $Y : \mathbb{R}^{\mathcal{C}} \to \mathbb{R}^{\mathcal{S}}$ be the linear map defined by $Y(\omega_y) = y$. Let $\Psi : \mathbb{R}^{\mathcal{S}} \to \mathbb{R}^{\mathcal{C}}$ be the nonlinear map defined by $\Psi(c) = \sum_{y \in \mathcal{C}} c^y \omega_y$. These two maps enable us to move back and forth between species space and complex space. It remains only to encode the dynamics on complex space.

Let $x \in \mathbb{R}^{\mathcal{C}}$ and suppose that x has components x_y with respect to the standard basis, so that $x = \sum_{y \in \mathcal{C}} x_y \omega_y$. For a given kinetics, $\kappa : \mathcal{R} \to \mathbb{P}$, define the map $A_\kappa : \mathbb{R}^{\mathcal{C}} \to \mathbb{R}^{\mathcal{C}}$ by

$$A_\kappa(x) = \sum_{y \to y' \in \mathcal{R}} (\kappa_{y \to y'}) x_y (\omega_{y'} - \omega_y) \,. \tag{10}$$

Note that $A_\kappa(x)$ is evidently linear in x. It is now easy to see that the following diagram commutes:

$$\begin{array}{ccc} \mathbb{R}^{\mathcal{C}} & \xleftarrow{\;A_\kappa\;} & \mathbb{R}^{\mathcal{C}} \\ Y \downarrow & & \uparrow \Psi \\ \mathbb{R}^{\mathcal{S}} & \xleftarrow{\;\;f\;\;} & \mathbb{R}^{\mathcal{S}} \end{array} \tag{11}$$

In other words, $f = Y A_\kappa \Psi$. This decomposition is fundamental to what follows.

Y and Ψ are bookkeeping functions. They depend only on the set of complexes, $\mathcal{C}$. A_κ carries the weight of the dynamics and depends both on the network structure, $\mathcal{R}$, and the kinetics, κ. However, A_κ is a linear function, which gives a hint that the dynamics of f may not be as formidable as descriptions like (3) suggest. Ψ is the only nonlinearity present. As we shall see, it too conceals further linearity. We need more notation to explain this.

If $f : V \to W$ is a linear map, its transpose, $f^T : W \to V$, is defined with respect to chosen bases in V and W by the equation

$$\langle f(v), w \rangle = \langle v, f^T(w) \rangle \,,$$

for all $v \in V$ and $w \in W$. Here, the inner product on the left is the standard one taken in W, while that on the right is the standard one taken in V. Both species space, $\mathbb{R}^{\mathcal{S}}$, and complex space, $\mathbb{R}^{\mathcal{C}}$, have standard bases and inner products are taken with respect to these, so that the standard bases become orthonormal.

Let $\ln : \mathbb{P} \to \mathbb{R}$ denote the natural logarithm. If X is any finite index set, we can extend $\ln$ to $\mathbb{P}^X$ by applying it componentwise. This gives a function $\ln : \mathbb{P}^X \to \mathbb{R}^X$ where $(\ln(v))_x = \ln(v_x)$ for $v \in \mathbb{P}^X$. Note that if $c \in \mathbb{P}^{\mathcal{S}}$ then $\Psi(c) \in \mathbb{P}^{\mathcal{C}}$. The following is not stated as a separate result in CRNT but it is helpful to extract it from the proof of [11, Proposition 5.9].

Lemma 3.1 [11, 5.76]) *The following diagram commutes:*

$$
\begin{array}{ccc}
\mathbb{R}^{\mathcal{C}} & \xleftarrow{\ \ln\ } & \mathbb{P}^{\mathcal{C}} \\
\Big\uparrow{\scriptstyle Y^T} & & \Big\uparrow{\scriptstyle \Psi} \\
\mathbb{R}^{\mathcal{S}} & \xleftarrow{\ \ln\ } & \mathbb{P}^{\mathcal{S}}
\end{array}
\tag{12}
$$

In other words, if $c \in \mathbb{P}^{\mathcal{S}}$ then $\ln \Psi(c) = Y^T \ln(c)$.

Proof: If $y \in \mathcal{C}$, then it is easy to see from (7) that $\ln(c^y) = \langle y, \ln(c) \rangle$. By definition, $\Psi(c) = \sum_{y \in \mathcal{C}} c^y \omega_y$, so that $\ln \Psi(c) = \sum_{y \in \mathcal{C}} \langle y, \ln(c) \rangle \omega_y$. Hence, for any $z \in \mathcal{C}$,

$$
\langle \omega_z, \ln(\Psi(c)) \rangle = \langle z, \ln(c) \rangle = \langle Y(\omega_z), \ln(c) \rangle = \langle \omega_z, Y^T \ln(c) \rangle ,
$$

from which the result follows.

QED

We see from Lemma 3.1 that Ψ is also trying hard to be linear; one might almost say that it is "log-linear". It is also closely related to the other bookkeeping function Y, which is linear. The linearity evident in (11) and (12) is crucial to CRNT. Lemma 3.1 will turn out to be useful in §7.

The decomposition of f in (11) immediately suggests a way to study fixed points. We first need to say exactly what we mean by this.

Definition 3.1 *A fixed point of a chemical reaction network is a state $c \in \mathbb{P}^{\mathcal{S}}$ for which $dc/dt = 0$.*

States of the network which lie on the boundary of the nonnegative cone $\overline{\mathbb{P}}^{\mathcal{S}}$, so that one or more concentrations are zero, are excluded from consideration here. It is certainly possible for a network to asymptotically tend towards such a state—the concentration of A in the network $A \to B$ decreases exponentially from its initial value—but these boundary states are peripheral to CRNT.

From the fundamental dynamical equation (8), a fixed point occurs if, and only if, $f(c) = 0$. This may come about only in certain ways along the composition $Y A_\kappa \Psi = f$. If $c \in \mathbb{P}^{\mathcal{S}}$ then $\Psi(c) \in \mathbb{P}^{\mathcal{C}}$, so that $\Psi(c) \neq 0$. The next possibility is that $A_\kappa \Psi(c) = 0$. Determining exactly when this happens will form the main part of the argument for the deficiency 0 theorem. It will clearly be important to determine $\ker A_\kappa$, which we do in Theorem 4.2. The final possibility is that $A_\kappa \Psi(c) \neq 0$ but $Y A_\kappa \Psi(c) = 0$. This directs our attention to the kernel of Y and more specifically to that part of the kernel of Y which lies in the image of A_κ.

Definition 3.2 *The deficiency of a chemical reaction network is $\dim(\ker Y \cap \mathrm{Im} A_\kappa)$.*

CRNT defines the deficiency differently, through a formula. We derive the formula in Proposition 5.1. The definition given here has the advantage that it both makes clear why the concept is important and also establishes that it is a nonnegative integer. Note that the two definitions only agree for the class of networks for which Proposition 5.1 holds.

A simple estimate for the deficiency, δ, of a network can be obtained as follows. Let $T = \mathrm{span}\{\omega_{y'} - \omega_y \mid y \to y'\}$. The map Y is evidently a surjection from T to the stoichiometric subspace, S. From the definition of A_κ in (10) we see that $\mathrm{Im} A_\kappa \subseteq T$. It follows that

$$
\ker Y \cap \mathrm{Im} A_\kappa \subseteq \ker Y|_T
\tag{13}
$$

and so $\delta \leq \dim \ker Y|_T$. Let $s = \dim S$. Since $\dim T = \dim \ker Y|_T + \dim \operatorname{Im} Y|_T$ we see that $\delta \leq \dim T - s$. It is relatively straightforward to determine s by inspection of the complexes and the reactions, as we did manually in §1 for example (1). Calculating $\dim T$ turns out to be even easier after a little linear algebra.

Let $U \subseteq \mathcal{C}$ be any subset which is closed under the reaction relation. That is, if $y \rightarrow y'$ and either y or y' is in U then so is the other. It follows that, for each reaction $y \rightarrow y'$, either it is the case that neither y nor y' are in U or it is the case that both y and y' are in U. Either way, $\langle \omega_{y'} - \omega_y, \omega_U \rangle = 0$. Since this holds for all reactions, $\omega_U \in T^{\perp}$, where $T^{\perp}$ denotes the orthogonal complement of T. By finding all subsets like U, we can determine $T^{\perp}$.

The construction of U suggests how to do this through the following equivalence relation on complexes. First, symmetrise the reaction relation, $\rightarrow$, by saying that y is *directly linked* to y', denoted $y \leftrightarrow y'$, if either $y \rightarrow y'$ or $y' \rightarrow y$. Evidently, if $y \leftrightarrow y'$ then $y' \leftrightarrow y$. Now take the reflexive, transitive closure of $\leftrightarrow$ to obtain an equivalence relation.

Definition 3.3 *If $y, y' \in \mathcal{C}$ then y is said to be linked to y', denoted $y \sim y'$, if either $y = y'$ or there are $y_1, \cdots, y_m \in \mathcal{C}$ such that $y = y_1 \leftrightarrow y_2 \leftrightarrow \cdots \leftrightarrow y_m = y'$.*

The equivalence classes of complexes under $\sim$ are termed *linkage classes*. Example (1) has 2 linkage classes: $\{A, 2B\}$ and $\{A + C, D, B + E\}$. Let l be the number of linkage classes in the network. It is easy to determine this by inspection of the reactions. Let $L_1, \cdots, L_l \subseteq \mathcal{C}$ be the linkage classes. It is clear that each linkage class is closed under the reaction relation and is hence like the subset U. It follows from what we did above that $\omega_{L_1}, \cdots, \omega_{L_l} \in T^{\perp}$. Now suppose that $x \in T^{\perp}$ and that $x = \sum_{y \in \mathcal{C}} x_y \omega_y$ in the standard basis. If $y \rightarrow y'$ then the orthogonality $\langle y' - y, x \rangle = 0$ shows that $x_y = x'_y$. Hence, if $y \sim y'$ then $x_y = x_{y'}$. We see that x_y is constant on linkage classes. It follows immediately that $T^{\perp} = \operatorname{span}\{\omega_{L_1}, \cdots, \omega_{L_l}\}$. Since equivalence classes are always distinct, it is evident that $\{\omega_{L_1}, \cdots, \omega_{L_l}\}$ is also linearly independent and gives a basis for $T^{\perp}$.

Let n denote the number of complexes in the network. Since $\dim T + \dim T^{\perp} = n$, we see that $\dim T = n - l$. We can now summarise what we have proved.

Lemma 3.2 *The following statements hold for any chemical reaction network:*

1. *([11, Lemma 4.4]) $\operatorname{span}\{\omega_{y'} - \omega_y \mid y \rightarrow y'\} = \operatorname{span}\{\omega_{y'} - \omega_y \mid y \sim y'\}$,*

2. *([11, Lemma 4.6]) $\operatorname{span}\{\omega_{y'} - \omega_y \mid y \rightarrow y'\}^{\perp} = \operatorname{span}\{\omega_{L_1}, \cdots, \omega_{L_l}\}$,*

3. *([11, Lemma 4.5]) $\dim \operatorname{span}\{\omega_{y'} - \omega_y \mid y \rightarrow y'\} = n - l$.*

We did not explicitly prove the first conclusion of Lemma 3.2 but it is implicit in the discussion above.

Proposition 3.1 *The deficiency, δ, of any chemical reaction network satisfies $0 \leq \delta \leq n - l - s$, where n is the number of complexes, l is the number of linkage classes and s is the dimension of the stoichiometric subspace.*

Example (1) has $n = 5$, $l = 2$ and $s = 3$ so that $\delta = 0$. This is our first example of a deficiency 0 network.

To calculate the deficiency for an arbitrary network is non-trivial. It depends on knowing how $\operatorname{Im} A_{\kappa}$ sits inside T. For certain networks it can be shown that $\operatorname{Im} A_{\kappa} = T$, which makes the problem particularly easy: in this case, $\delta = n - l - s$; see Proposition 5.1. The networks considered in both the deficiency 0 and deficiency 1 theorems fall into this class. We need to turn our attention from Y to A_{κ} to understand when this happens and this brings us to one of the key foundational results of CRNT.

4   The kernel of A_κ

The map $A_\kappa : \mathbb{R}^\mathcal{C} \to \mathbb{R}^\mathcal{C}$ is linear. The first thing to do is to find out what it looks like as a matrix in the standard basis. Recall that we write vectors as column vectors. Suppose that the complexes of the network are labelled $1, \cdots, n$, so that $\mathcal{C} = \{1, \cdots, n\}$. The reactions in the network then constitute the *edges* of a directed graph on the *vertices* $1, \cdots, n$. A reaction $i \to j$ corresponds to an edge from the *source* vertex i to the *target* vertex j. The kinetic specification for the network constitutes a labelling on the edges by positive numbers. Let A_{ij} denote the $n \times n$ matrix whose ij-th entry is the corresponding rate constant when that makes sense and zero otherwise:

$$A_{ij} = \left\{ \begin{array}{ll} \kappa_{i \to j} & \text{if } i \to j \\ 0 & \text{if there is no edge from } i \text{ to } j \; . \end{array} \right. \tag{14}$$

A vector $x \in \mathbb{R}^\mathcal{C}$ gives a distribution of numbers on the vertices of the directed graph. From the definition of A_κ in (10) we see that the effect of applying A_κ to x is to play the following game: on each edge $i \to j$ of the graph, the amount $x_i A_{ij}$ is taken from the source vertex, i, and given to the target vertex, j. From the point of view of a single vertex j, the net contribution of the game coming from all the edges is given by

$$\sum_{i \to j} x_i A_{ij} - x_j \Big(\sum_{j \to k} A_{jk} \Big) \; . \tag{15}$$

We can rewrite this in matrix notation as

$$A_\kappa(x) = x^T (A - \text{diag}(A1)) \; . \tag{16}$$

We commit another abuse of notation by using 1 to denote the vector all of whose components are 1. The effect of multiplying A by 1 is to sum the elements on each row, $(A1)_j = \sum_k A_{jk}$, corresponding to the right hand sum in (15). The operator $\text{diag}(x)$ converts a vector x into the diagonal matrix in which the components of x appear on the main diagonal:

$$\text{diag}(x)_{ij} = \left\{ \begin{array}{ll} 0 & \text{if } i \neq j \\ x_i & \text{otherwise} \end{array} \right. \; .$$

Note that $\text{diag}(x)1 = x$. Finally, since we have chosen to write vectors as column vectors, we need to use x^T, the transpose of x, to pre-multiply a matrix. The term $x^T A$ in (16) corresponds to the left hand sum in (15).

The matrix equation (16) immediately clarifies the nature of the problem. Suppose, for simplicity, that no row of matrix A is 0. In other words, that every vertex in the graph is the source of some edge. (This is true for the graph of (1) but false for the graph of (4).) In this case $\text{diag}(A1)$ is invertible. Hence, $x \in \ker A_\kappa$ if, and only if,

$$x^T A (\text{diag}(A1))^{-1} = x^T \; .$$

This is the eigenvector problem for the matrix $A(\text{diag}(A1))^{-1}$. Since this matrix is nonnegative, the eigenvector problem can be solved by Perron-Frobenius theory. This is a well-known branch of matrix analysis—Perron and Frobenius first proved their results around 1912—which has been widely used in applications, [6, 27]. CRNT takes a different approach in [16], although the relevance of Perron-Frobenius theory is noted on [11, page 4-11]. We take advantage of the specific properties of A_κ to gently introduce the main ideas.

The description of A_κ as a game makes it clear that there is no net effect over all vertices: whatever amount is taken from one vertex is given to another. This must reflect itself as some kind of

invariance property of A_κ. Indeed,

$$(A - \mathrm{diag}(A1))1 = A1 - \mathrm{diag}(A1)1 = A1 - A1 = 0 . \tag{17}$$

We need some further notation to exploit this. It will be helpful to extend functions and relations from numbers to vectors on a component-wise basis, while keeping the same notation. For instance, if $x \in \mathbb{R}^\mathcal{C}$, then the absolute value of x, $|x|$, is the vector such that $|x|_i = |x_i|$. Similarly, if $x, y \in \mathbb{R}^\mathcal{C}$ then $x \leq y$ if, and only if, $x_i \leq y_i$ for $1 \leq i \leq n$. By $x < y$ we mean that $x \leq y$ and, for some $1 \leq i \leq n$, $x_i < y_i$.

Lemma 4.1 *For any $x \in \mathbb{R}^\mathcal{C}$ if $x^T(A - \mathrm{diag}(A1)) \geq 0$ then $x^T(A - \mathrm{diag}(A1)) = 0$.*

Proof: Using (17), $[x^T(A - \mathrm{diag}(A1))]1 = x^T[(A - \mathrm{diag}(A1))1] = 0$. By assumption, this is also a sum of nonnegative terms, each of which must hence be zero.

QED

This Lemma is quite helpful. Suppose that we have an element in the kernel of A_κ, say $x \in \mathbb{R}^\mathcal{C}$ such that $A_\kappa(x) = 0$. Then by (16) we see that $x^T A = x^T \mathrm{diag}(A1)$. Taking absolute values on both sides and using the triangle inequality, we see that $|x|^T A \geq |x|^T \mathrm{diag}(A1)$. But then, $|x|^T(A - \mathrm{diag}(A1)) \geq 0$ and so, by Lemma 4.1, $|x|^T(A - \mathrm{diag}(A1)) = 0$. We have made the following interesting observation.

Lemma 4.2 ([16, Lemma A]) *If $x \in \ker A_\kappa$ then $|x| \in \ker A_\kappa$.*

It follows that we can always suppose, without loss of generality, that $x \in \ker A_\kappa$ satisfies $x \geq 0$. Let us consider the implications of this if some component of x is 0, say $x_j = 0$. Since $A_\kappa(x) = 0$ we see from (15) that

$$\sum_{i \to j} x_i A_{ij} = x_j \Big(\sum_{j \to k} A_{jk} \Big) = 0 .$$

But by construction, $A_{ij} > 0$ for each edge $i \to j$, while we have assumed that $x_i \geq 0$ for $1 \leq i \leq n$. It follows that, in fact, $x_i = 0$ for each vertex i with an edge to j.

Lemma 4.3 ([16, Lemma B]) *If $x \in \ker A_\kappa$ and $x_j = 0$ then $x_i = 0$ whenever $i \to j$.*

The effect of a zero component is propagated backwards along the graph. This suggests the following equivalence relation on complexes, which is different to the equivalence relation of linkage introduced in Definition 3.3. We first take the reflexive, transitive closure of $\to$ and then symmetrise it.

Definition 4.1 *If $y, y' \in \mathcal{C}$ then y ultimately reacts to y', denoted $y \Rightarrow y'$, if either $y = y'$ or there exists $y_1, \cdots, y_m \in \mathcal{C}$ such that $y = y_1 \to y_2 \to \cdots \to y_m = y'$. y is strongly linked to y', denoted $y \approx y'$, if both $y \Rightarrow y'$ and $y' \Rightarrow y$.*

It is evident that being strongly linked is an equivalence relation. The equivalence classes are called the *strong linkage classes* in CRNT. It is also evident that if $y \approx y'$ then $y \sim y'$ so that the strong linkage classes are contained in the linkage classes. In example (1) the strong linkage classes are $\{A, 2B\}$ and $\{A + C, D, B + E\}$ so that the strong linkage classes happen to coincide with the linkage classes. In example (4) the strong linkage classes are $\{A + E, AE\}$ and $\{B + E\}$, while $\{A + E, AE, B + E\}$ is the unique linkage class.

The point of this definition can be seen in the following observation. Recall that if $x \in \mathbb{R}^{\mathcal{C}}$ then the *support* of x, denoted $\mathrm{supp}(x) \subseteq \mathcal{C}$, is the subset of those complexes where x is non-zero: $\mathrm{supp}(x) = \{i \in \mathcal{C} \mid x_i \neq 0\}$. Suppose now that $x \in \ker A_\kappa$ and that $U \subseteq \mathcal{C}$ is a strong linkage class. Suppose further that $U \cap \mathrm{supp}(x) \neq \emptyset$ and choose $i \in U \cap \mathrm{supp}(x)$. If j is any other element of U then by Definition 4.1, $i \Rightarrow j$. It follows that $x_j \neq 0$ for if it were then Lemma 4.3 could be applied repeatedly to show that $x_i = 0$, implying that $i \notin \mathrm{supp}(x)$. It follows that $U \subseteq \mathrm{supp}(x)$. In other words, either $U \cap \mathrm{supp}(x) = \emptyset$ or $U \subseteq \mathrm{supp}(x)$. In particular, $\mathrm{supp}(x)$ is a union of strong linkage classes, [16, Lemma C].

However, not all the strong linkage classes contribute to $\mathrm{supp}(x)$. To identify the ones which do, we have to get to grips with some aspects of Perron-Frobenius theory.

It should have been evident, when we introduced the matrix A, that there is an intimate relationship between matrices and labelled, directed graphs. If A is an $n \times n$ matrix, then it gives rise to a directed graph on the vertices $\{1, \cdots, n\}$ in which there is an edge $i \to j$ if, and only if, $A_{ij} \neq 0$; if there is an edge, then its label is A_{ij}. Conversely, any directed graph whose edges are labelled with real numbers gives rise to a corresponding matrix. This relationship between matrices and graphs turns out to be particularly significant when the matrix is nonnegative or, equivalently, when the labels on the graph are positive numbers. One consequence of the graphical structure is that any matrix can be placed in block triangular form by reordering the basis vectors. This is easier to understand by drawing diagrams than by writing down all the notational intricacies. However, once we have this form, the rest of the discussion will be straightforward.

The strong linkage classes inherit a partial order from the relation $\Rightarrow$. If $i \in \mathcal{C}$, let $[i]$ denote the strong linkage class containing i: $[i] = \{j \in \mathcal{C} \mid i \Rightarrow j \text{ and } j \Rightarrow i\}$. We say that $[i] \preceq [j]$ if, and only if, $i \Rightarrow j$. This is well-defined, since if $k \in [i]$ and $m \in [j]$, then $i \Rightarrow j$ if, and only if, $k \Rightarrow m$. It is easy to see that $\preceq$ is a partial order on the set of strong linkage classes: it is reflexive, anti-symmetric and transitive. Let p be the number of strong linkage classes. Order the strong linkage classes, $L_1, \cdots, L_p$, in such a way that if $L_u \preceq L_v$ then $u \leq v$. This amounts to embedding the partial order in a linear order, which it is easy to see can always be done.

We now rename the complexes, $1, \cdots, n$, to make the ordering on them consistent with the ordering on the strong linkage classes. Let c_i be the size of the i-th linkage class, so that $c_1 + \cdots + c_p = n$. It will be convenient to let d_i denote the partial sums of this sequence, $d_i = \sum_{1 \leq j \leq i} c_i$ for $1 \leq i \leq p$, and to let $d_0 = 0$. Rename the complexes in the first linkage class, L_1, in any order, using only the numbers $1, \cdots, c_1 = d_1$. Rename the complexes in L_2, in any order, using only the numbers, $d_1 + 1, \cdots, c_1 + c_2 = d_2$. Proceed in this way until all the complexes have been renamed. The elements in the i-th linkage class, L_i, now constitute the set $\{d_{i-1} + 1, \cdots, d_i\}$, for $1 \leq i \leq p$. Such a renaming amounts to performing a permutation on the standard basis in $\mathbb{R}^{\mathcal{C}}$.

The partition of complexes into strong linkage classes under this renaming provides a convenient way to decompose vectors and matrices. If $x \in \mathbb{R}^{\mathcal{C}}$, the notation $x(i)$, will denote the sub-vector, or "block", of x corresponding to the i-th linkage class:

$$x(i) = (x_{d_{(i-1)}+1}, \cdots, x_{d_i}) \in \mathbb{R}^{L_i} \ ,$$

for $1 \leq i \leq p$. Similarly, if M is any $n \times n$ matrix, let $M(i,j)$ denote the block corresponding to the intersection of the rows of linkage class L_i and the columns of linkage class L_j. $M(i,j)$ is then the $c_i \times c_j$ sub-matrix of M whose top left entry is $M_{d_{(i-1)}+1, d_{(j-1)}+1}$ for $1 \leq i, j \leq p$.

Suppose now that $i \in L_u$ and $j \in L_v$. If $i \Rightarrow j$ then $L_u \preceq L_v$ and so $u \leq v$. If $L_u \neq L_v$ then by construction $i \leq j$. When two complexes are in different linkage classes, they can only "ultimately react" to each other in the increasing direction. It follows that the matrix of A in the new basis is

in upper-triangular block form:

$$A = \begin{pmatrix} \boxed{A(1,1)} & A(1,2) & \cdots & A(1,p) \\ 0 & \boxed{A(2,2)} & \cdots & \vdots \\ \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & \cdots & \boxed{A(p,p)} \end{pmatrix} , \tag{18}$$

where the lower off-diagonal blocks, $A(i,j)$ with $i > j$, are all zero. The diagonal blocks, indicated by boxes, are square matrices. This is the form that we need for the rest of the argument.

Choose $x \in \ker A_\kappa$. By Lemma 4.2, we may assume that $x \geq 0$. By (16), $x^T A = x^T \mathrm{diag}(A1)$. It will be convenient to let $\Delta = \mathrm{diag}(A1)$. Note that A is block upper-triangular and that Δ is block diagonal. Performing a block multiplication and isolating the first block, we see that

$$x(1)^T A(1,1) = x(1)^T \Delta(1,1) .$$

Suppose that in the first row of (18), at least one of the off-diagonal blocks, $A(1,j)$ with $1 < j$, is non-zero. It is easy to see that this means that $A(1,1)1 < A1(1)$. Hence, $\mathrm{diag}(A(1,1)1) < \Delta(1,1)$. As we saw above, $\mathrm{supp}(x)$ is a union of strong linkage classes. It follows that either $x(1) = 0$ or $x(1) \in \mathbb{P}^{L_1}$. If we suppose the latter then we may deduce that

$$x(1)^T A(1,1) > x(1)^T \mathrm{diag}(A(1,1)1)$$

so that

$$x(1)^T (A(1,1) - \mathrm{diag}(A(1,1)1)) > 0 .$$

The complexes in the first strong linkage class L_1 and all the reactions between them, form a chemical reaction network in their own right. There are c_1 complexes and the matrix corresponding to the labelled, directed graph is $A(1,1)$. Applying Lemma 4.1 to this sub-reaction network leads immediately to a contradiction, since $x(1)^T (A(1,1) - \mathrm{diag}(A(1,1)1)) = 0$. Hence, it must be the case that $x(1) = 0$. The first linkage class does not contribute to $\mathrm{supp}(x)$ if some off-diagonal block is non-zero.

This assumption is equivalent to saying that the first strong linkage class is not *terminal*. A strong linkage class L is said to be terminal if $L \preceq L'$ implies $L = L'$. A terminal class is a maximal element in the partial order. We have shown that if the first linkage class is non-terminal, then it does not contribute to $\mathrm{supp}(x)$.

What happens if L_1 is terminal? In this case there are no off-diagonal blocks, $A(1,j)$ with $1 < j$, so that $A(1,1)1 = A1(1)$ and $\mathrm{diag}(A(1,1)1) = \Delta(1,1)$. It follows that

$$x(1)^T A(1,1) = x(1)^T \mathrm{diag}(A(1,1)1) . \tag{19}$$

Suppose first that L_1 is a terminal strong linkage class such that $c_1 > 1$. If $i \in L_1$ then since $c_1 > 1$, we can always find another complex $j \in L_1$, with $i \neq j$. Since L_1 is a strong linkage class, $i \Rightarrow j$. It follows that the i-th row of $A(1,1)$ is not zero. Since this is true for all rows, $A(1,1)1 \in \mathbb{P}^{L_1}$, and so $\mathrm{diag}(A(1,1)1)$ is invertible. It follows from (19) that

$$x(1)^T A(1,1)(\mathrm{diag}(A(1,1)1))^{-1} = x(1)^T , \tag{20}$$

so that $x(1) \in \mathbb{R}^{L_1}$ is an eigenvector of the matrix $A(1,1)(\mathrm{diag}(A(1,1)1))^{-1}$. At this point we can appeal to the main result in Perron-Frobenius theory. (The Appendix of [16] sidesteps this by using the specific properties of A_κ, which simplifies the argument at this point. However, there is some value in relating the details here to a body of well-understood mathematics.)

Let M be any $m \times m$ nonnegative matrix. As described above, we can associate to it a labelled, directed graph. Nonnegative matrices whose associated graphs are strongly linked (ie: has only a

single strong linkage class) are called *irreducible*. Equivalently, an irreducible matrix is one which cannot be brought into upper triangular block form by any permutation of the basis elements. Each diagonal block $A(i,i)$ in (18) with $c_i > 1$ is irreducible. The following is the classical Perron-Frobenius theorem, [27, Theorem 4.1], stated in a way suitable for our purposes.

Theorem 4.1 (The Perron-Frobenius theorem) *Let M by any irreducible nonnegative $m \times m$ matrix. Any two eigenvectors in $\mathbb{P}^m$ have the same eigenvalue, which is the spectral radius of M, and are the same up to a positive scalar multiple. In particular, the eigenspace for the spectral radius is 1 dimensional and can always be represented as $\mathrm{span}(u)$, with $u \in \mathbb{P}^m$.*

The spectral radius of M is the maximum absolute value of any (complex) eigenvalue: the maximum absolute value of all (complex) roots of the characteristic polynomial, $\det(M - zI) = 0$. It is not usually an eigenvalue but Theorem 4.1 asserts that it is for an irreducible nonnegative matrix. Note that an irreducible nonnegative matrix can have other real eigenvalues smaller than the spectral radius but the corresponding eigenvectors can never lie in $\mathbb{P}^m$.

Since L_1 is a strong linkage class and $c_1 > 1$, it is clear that $A(1,1)$ and $A(1,1)(\mathrm{diag}(A(1,1)))^{-1}$ are irreducible. Furthermore, if $M = A(1,1)(\mathrm{diag}(A(1,1)1))^{-1}$ then it is obvious that $M1 = 1$, so that M is row-stochastic: the sum of the entries in each row is 1. Hence, if $u^T M = \lambda u^T$, and $u \in \mathbb{P}^{L_1}$, it is easy to see that $\lambda = 1$, so that the spectral radius of M is 1. We see from Theorem 4.1 and (20) that, no matter what the choice of $x \in \ker A_\kappa$, $x(1) = \lambda u$, where $\lambda \in \mathbb{R}$ and $u \in \mathbb{P}^{L_1}$ is the eigenvector guaranteed by Theorem 4.1.

It remains to deal with the annoying case when $c_1 = 1$. L_1 is then a terminal strong linkage class containing only a single complex. In this case, the corresponding block matrix may not be irreducible. If the graph does not have a self-loop at this complex then $A(1,1) = 0$. In general, a directed graph may have self-loops but a chemical reaction network probably does not have reactions of the form $y \to y$ for some $y \in \mathcal{C}$. If it does in such a way that $A(1,1) \neq 0$, then we may deal with it as above in the case where $c_1 > 1$. If not, so that $A(1,1) = 0$, then $x(1) \in \mathbb{R}^1$ is unconstrained by (19), since $A(1,1) = \mathrm{diag}(A1)(1,1) = 0$. If we set $u = 1$, then we can conclude, as above, that, no matter what the choice of $x \in \ker A_\kappa$, $x(1) = \lambda u$, for some $\lambda \in \mathbb{R}$.

The behaviour of L_1 is followed by all the other linkage classes. For each $1 \leq i \leq p$ such that L_i is a terminal strong linkage class, let $u_i \in \mathbb{P}^{L_i}$ be given by $u_i = 1$ whenever $c_i = 1$ and $A(i,i) = 0$, and given by some eigenvector solution,

$$u_i^T A(1,1)(\mathrm{diag}(A(1,1)1))^{-1} = u_i^T ,$$

with $u_i \in \mathbb{P}^{L_i}$, as guaranteed by Theorem 4.1, in all other cases.

Proposition 4.1 *Suppose that $x \in \ker A_\kappa$. If L_i is not terminal then $x(i) = 0$, while if L_i is terminal then $x(i) = \lambda_i u_i$, for some $\lambda_i \in \mathbb{R}$.*

Proof: The proof is by induction on i. The case $i = 1$ was done above. Suppose now that $i > 1$ and that the claim has been established for the linkage classes $L_1, \cdots, L_{(i-1)}$. Since $x^T A = x^T \Delta$, by performing a block multiplication and isolating the i-th block, we see that

$$x(i)^T A(i,i) + \sum_{1 \leq j < i} x(j)^T A(j,i) = x(i)^T \Delta(i,i) .$$

(This should be thought of as a vector equation taking place in $\mathbb{R}^{L_i}$.) The blocks $A(j,i)$ with $j < i$ are all off-diagonal. There are two possibilities in the range $j < i$. Either L_j is terminal, in which case $A(j,i) = 0$, or L_j is not terminal, in which case the inductive hypothesis implies that $x(j) = 0$. Together these imply that $\sum_{1 \leq j < i} x(j)^T A(j,i) = 0$, and so

$$x(i)^T A(i,i) = x(i)^T \Delta(i,i) .$$

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We can now argue exactly as in the case $i = 1$ to show that $x(i) = 0$ if L_i is not terminal while $x(i) = \lambda_i u_i$, for some $\lambda_i \in \mathbb{R}$, if L_i is terminal. The result follows by induction.

<div align="right">**QED**</div>

We can now assemble what we have learned so far. We leave it to the reader to fill in the minor details.

Theorem 4.2 ([16, Appendix, Proposition]) *Let t be the number of terminal strong linkage classes in a chemical reaction network and suppose that these classes are $T_1, \cdots, T_t \subseteq \mathcal{C}$, in any order. The following statements hold.*

1. *There exist $\chi_i \in \overline{\mathbb{P}}^{\mathcal{C}}$ such that $\mathrm{supp}(\chi_i) = T_i$ for $1 \leq i \leq t$.*

2. $\ker A_\kappa = \mathrm{span}\{\chi_1, \cdots, \chi_t\}$ *and these are linearly independent.*

3. $\dim \ker A_\kappa = t$.

5   The deficiency formula and some biological examples

Theorem 4.2 is one of the key results in CRNT. Among other things, it gives a very simple condition under which the deficiency inequality of Proposition 3.1 becomes an equality. We will use this as an excuse to look at some simple biochemical examples before returning to some other important consequences of Theorem 4.2 in the next section.

It is evident from Theorem 4.2 that $\dim \mathrm{Im} A_\kappa = n - t$. Recalling the proof of Proposition 3.1, $\mathrm{Im} A_\kappa \subseteq T$, where $T = \mathrm{span}\{\omega_{y'} - \omega_y \mid y \to y' \in \mathcal{R}\}$. Lemma 3.2 tells us that $\dim T = n - l$. This tells us that $t \geq l$ but we know this for other reasons as well. Recall the notation introduced in §4. Since $i \approx j$ implies that $i \sim j$, every linkage class must be a union of strong linkage classes. Furthermore, since there are no reactions between linkage classes, if $[i] \preceq [j]$ and $[j]$ is maximal in the $\preceq$ order, then $[j]$ must be part of the same linkage class as $[i]$. Hence, each linkage class must contain at least one terminal strong linkage class and so $t \geq l$. If $t = l$ then $T = \mathrm{Im} A_\kappa$. It follows that the inclusion in (13) is an equality.

Proposition 5.1 *If each linkage class has precisely one terminal strong linkage class then the deficiency, δ, of the network is given by $\delta = n - l - s$.*

CRNT uses this formula to define the deficiency. This agrees with the one used here, Definition 3.2, when the conditions of Proposition 5.1 hold; that is, whenever, $t = l$.

We have already shown, using Proposition 3.1, that the network (1) has deficiency 0. When we discussed the Michaelis-Menten formula in §2, we considered a possible network for a single substrate enzymatic reaction in (4). In this case, $\mathcal{C} = \{A + E, AE, B + E\}$ so that $n = 3$. There is a single linkage class, given by $\mathcal{C}$ itself, but there are two strong linkage classes: $\{A + E, AE\}$, and one of those singleton classes, $\{B + E\}$, that was such a nuisance in §4. However, there is only one terminal strong linkage class, $\{B + E\}$. We see that $l = t = 1$, so that the hypothesis of Proposition 5.1 is satisfied. As for the stoichiometric subspace,

$$S = \mathrm{span}\{A + E - AE, B + E - AE\},$$

which is evidently linearly independent, so that $s = 2$. By Proposition 5.1, $\delta = 0$.

It is clear that the network (4) does not have very interesting behaviour. Because the degradation of the enzyme-substrate complex has been assumed to be irreversible, the reaction simply consumes its

substrate, A, while producing B. The concentration of A will decrease towards 0, so that, no matter what initial conditions we start from, (4) should have no fixed point in $\mathbb{P}^S$. Since its deficiency is zero, the only kind of fixed point it can have is one where $A_\kappa \Psi(c) = 0$. This can be ruled out using Proposition 6.1 because (1) is not "weakly reversible" in the sense of Definition 6.1.

There are two ways to get around the lack of fixed points. The simplest is to assume that the second reaction is reversible, so that

$$A + E \rightleftharpoons AE \rightleftharpoons B + E \ . \tag{21}$$

(This gives rise to the reversible Michaelis-Menten formula in which product inhibition also plays a part in determining the reaction rate: see [8, §2.6] for more details.) As before, $\mathcal{C} = \{A + E, AE, B + E\}$, so that $n = 3$. There is still only 1 linkage class but now there is only one strong linkage class, which must hence be terminal. It is still the case that $l = t = 1$ and s is still 2. Hence, the deficiency of (21) is also 0. We would expect, however, in contrast to the irreversible case (4), that this network does have a fixed point. Indeed, intuition suggests that no matter what rate constants and initial concentrations are chosen, (21) would reach a point of thermodynamic equilibrium, with no net flux through the network. A and B will be continually inter-converted but at the same rate. This corresponds to a fixed point albeit a rather uninteresting one from the biological viewpoint. The existence of such a fixed point can be confirmed using Theorems 6.4 and 7.1.

The other way to encourage fixed points, which is closer to what happens in biology, is to make the system open by allowing A to be continually replenished and B to be continually depleted. Pseudo-reactions allows us to do this, leading to the following network, in which we have chosen to use the reversible enzymatic reaction:

$$A + E \rightleftharpoons AE \rightleftharpoons B + E \qquad B \rightarrow 0 \rightarrow A \ . \tag{22}$$
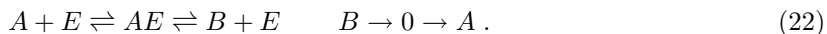
The pseudo-reaction $0 \rightarrow A$ corresponds to continual replenishment of A while $B \rightarrow 0$ corresponds to degradation of B. In this network, $\mathcal{C} = \{A + E, AE, B + E, A, B, 0\}$, so that $n = 6$. There are two linkage classes, $\{A + E, AE, B + E\}$, and $\{A, B, 0\}$, so that $l = 2$. There are 4 strong linkage classes

$$\{A + E, AE, B + E\}, \{B\}, \{0\}, \{A\} \ ,$$

so that $t = 4$, but only two of these are terminal: $\{A + E, AE, B + E\}$ and $\{A\}$. It is still the case that $l = t$, so that the hypothesis of Proposition 5.1 is still satisfied. The stoichiometric subspace is given by

$$S = \text{span}\{A + E - AE, B + E - AE, B, A, \} = \text{span}\{A, B, E - AE\} \ ,$$

so that $s = 3$. By Proposition 5.1, $\delta = 1$. Our intuition suggests that (22) will reach a fixed point in $\mathbb{P}^S$, irrespective of the initial conditions, provided that the replenishment rate of A equals the degradation rate of B, so that there is a constant flux through the reaction. However, the deficiency 0 theorem is not applicable and the conditions needed to apply the deficiency 1 theorem do not hold.

Our final example is taken from a paper of Goldbeter and Koshland, who undertook a theoretical analysis of covalent modification cycles, [17]. This example may look somewhat futile to a chemical engineer,

$$A + E \rightleftharpoons AE \rightarrow B + E \qquad B + F \rightleftharpoons BF \rightarrow A + F \ , \tag{23}$$
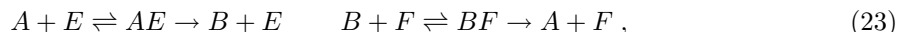
since A and B are merely mutually inter-converted but such cycles are extremely important biologically. A key regulatory mechanism at the cellular level is the covalent modification of a protein by attachment or detachment of a small molecule, such as a phosphate or methyl group. (The concentration of the molecules providing such groups, such as ATP for phosphate, is often regarded as being constant and is hence absorbed into the rate constants of (23).) Modifications of this kind serve either to activate the protein so that it can carry out further tasks, including further covalent modifications, or to deactivate it to prevent such tasks being carried out. Signals which impinge upon a cell are integrated and processed through a complex network of such regulatory interactions, [19].

Because the conversion of A to B is, in some sense, reversible, intuition suggests, as in example (21), that the system will tend to a fixed point no matter what the initial concentrations. Indeed, Goldbeter and Koshland give a formula for this fixed point, under the same approximations used to prove the Michaelis-Menten formula. However, this network is not "weakly reversible" in the sense of Proposition 6.1, nor is it of deficiency 0. It is easy to see that it has deficiency 1 (n = 6, l = t = 2, s = 3) but it falls outside the scope of the deficiency 1 theorem.

As we see from this example and the preceding one, there are situations of biological interest that are not covered by either the deficiency 0 or deficiency 1 theorems. The idea of "reversibility" alluded to in the examples above forms an interesting link between our intuitions and the mathematical statements of the deficiency theorems. It seems that some form of reversibility is important for the existence of fixed points. In this respect both the deficiency 0 and deficiency 1 theorems focus on "weak reversibility", which is defined at the level of complexes. As example (23) of Goldbeter and Koshland suggests, there are more intricate forms of reversibility in which both complexes and species play a part. Results of this nature are discussed in Paul Schlosser's PhD thesis, [29], but they lie outside the scope of these notes.

6   Fixed points for which $A_\kappa \Psi(c) = 0$

We now turn our attention back to fixed points. Recall the remarks made after Definition 3.1. It follows from (7) that $\Psi(c) \in \mathbb{P}^\mathcal{C}$ whenever $c \in \mathbb{P}^\mathcal{S}$. Hence $dc/dt = 0$ in (8) if, and only if, either $A_\kappa \Psi(c) = 0$ or, if this is not the case, if $\ker(Y A_\kappa) \neq \emptyset$. Networks of deficiency 0, for which $\ker Y \cap \mathrm{Im} A_\kappa = \emptyset$, cannot satisfy the second condition, so let us consider the first possibility. Theorem 4.2 gives a great deal of insight into this case. However, the big surprise will be that the deficiency of the network turns out to be irrelevant. If there is a fixed point for which $A_\kappa \Psi(c) = 0$ then *all* fixed points have this form, irrespective of the deficiency of the network. It will take us a while to see why.

If $\Psi(c) \in \mathbb{P}^\mathcal{C}$ then $\mathrm{supp}(\Psi(c)) = \mathcal{C}$. If $A_\kappa \Psi(c) = 0$ then from Theorem 4.2 we immediately conclude that each $i \in \mathcal{C}$ lies in a terminal strong linkage class. This leads to an important definition in CRNT.

Definition 6.1 *A chemical reaction network is said to be* weakly reversible *if it satisfies any of the conclusions of the following Lemma.*

Lemma 6.1 *For any chemical reaction network, the following statements are equivalent, where $i, j \in \mathcal{C}$.*

1. *Each complex lies in a terminal strong linkage class.*

2. *Every strong linkage class is terminal.*

3. *The terminal strong linkage classes coincide with the linkage classes.*

4. *If $[i] \preceq [j]$ then $[i] = [j]$.*

5. *If $i \Rightarrow j$ then $j \Rightarrow i$.*

Proof: (1 implies 2) Since the strong linkage classes form a partition of $\mathcal{C}$, each strong linkage class must be terminal.

(2 implies 3) If i is directly linked to j, so that $i \leftrightarrow j$, and $[i] \neq [j]$, then one of $[i]$ or $[j]$ is not terminal. It follows that $[i] = [j]$. Hence if i is linked to j, so that $i \sim j$, then $[i] = [j]$. Therefore each linkage class is a strong linkage class and hence a terminal strong linkage class.

(3 implies 4) Suppose $[i] \preceq [j]$. Since there can be no reactions between elements of different linkage classes, i and j must be in the same linkage class. Hence, by assumption, they must be in the same terminal strong linkage class, so that $[i] = [j]$.

(4 implies 5) If $i \Rightarrow j$ then $[i] \preceq [j]$. Hence $[i] = [j]$, so that $j \Rightarrow i$.

(5 implies 1) If $i \in \mathcal{C}$ then, since the terminal classes are maximal in the $\preceq$ order, there must exist $j \in \mathcal{C}$ such that $[j]$ is maximal and $i \Rightarrow j$. But then $j \Rightarrow i$ and so $i \in [j]$.

$$\textbf{QED}$$

It will be helpful to record the observation that led to this definition.

Proposition 6.1 *In any chemical reaction network, if $A_\kappa \Psi(c) = 0$ then the network is weakly reversible.*

Networks (1) and (21) are weakly reversible, while (4) and (22) are not. By Proposition 6.1, the latter networks cannot have fixed points with $A_\kappa \Psi(c) = 0$. We shall show in the next section that the former networks, both of which are of deficiency 0, do have such fixed points.

Theorem 4.2 also shows that the set of fixed points $c \in \mathbb{P}^{\mathcal{S}}$, for which $A_\kappa \Psi(c) = 0$, has a simple geometric relationship to the stoichiometric subspace $S \subseteq \mathbb{R}^{\mathcal{S}}$.

Proposition 6.2 ([11, Proposition 5.3 (ii) and (iii)]) *In any chemical reaction network let $Z = \{c \in \mathbb{P}^{\mathcal{S}} \mid A_\kappa \Psi(c) = 0\}$. Suppose that $Z \neq \emptyset$. For any $c^* \in Z$, $Z = \{c \in \mathbb{P}^{\mathcal{S}} \mid \ln c - \ln c^* \in S^\perp\}$.*

Proof: Since $Z \neq \emptyset$, Proposition 6.1 tells us that the network is weakly reversible. Using Lemma 6.1 we see that the linkage classes coincide with the terminal strong linkage classes. Let $L_1, \cdots, L_l$ be the linkage classes. By Theorem 4.2, there exist vectors $\chi_1, \cdots, \chi_l \in \overline{\mathbb{P}}^{\mathcal{C}}$ with $\mathrm{supp}(\chi_i) = L_i$, which form a basis for $\ker A_k$. Accordingly, if $c^* \in Z$ then $\Psi(c^*) = \sum_{1 \leq i \leq l} \lambda(c^*)_i \chi_i$, where the coefficients $\lambda(c^*)_i \in \mathbb{P}$ are uniquely determined by the choice of $c^* \in Z$. However, by definition of Ψ, $\Psi(c^*) = \sum_{y \in \mathcal{C}} (c^*)^y \omega_y$. Since the linkage classes form a partition of the complexes, we must have that

$$\sum_{y \in L_i} (c^*)^y \omega_y = \lambda(c^*)_i \chi_i \ ,$$

for each $1 \leq i \leq l$. Any other $c \in Z$ must satisfy the same equation with $\lambda(c)_i$ in place of $\lambda(c^*)_i$. Since the ω_y form a basis, it follows that

$$c^y / \lambda(c)_i = (c^*)^y / \lambda(c^*)_i$$

for all $y \in L_i$ and all $1 \leq i \leq l$. Rearranging this, we see that $c^y / (c^*)^y$ must be constant on each linkage class. Conversely, if, for each $1 \leq i \leq l$, there exists $\mu_i \in \mathbb{P}$ such that $c^y / (c^*)^y = \mu_i$ whenever $y \in L_i$, then

$$\sum_{y \in L_i} c^y \omega_y = \mu_i \sum_{y \in L_i} (c^*)^y \omega_y = \mu_i \lambda(c^*)_i \chi_i \ .$$

Hence, $\Psi(c) = \sum_{y \in \mathcal{C}} c^y \omega_y = \sum_{1 \leq i \leq l} \mu_i \lambda(c^*)_i \chi_i \in \ker A_\kappa$. We have shown that

$$Z = \{c \in \mathbb{P}^{\mathcal{S}} \mid c^y / (c^*)^y \text{ is constant on each linkage class}\} \ .$$

If $c^y / (c^*)^y > 0$, then, recalling the proof of Lemma 3.1, we see that $\ln(c^y / (c^*)^y) = \langle y, \ln c - \ln c^* \rangle$. Suppose that this is constant on linkage classes. If $y \to y'$ is any reaction in $\mathcal{R}$, so that y and y' are

in the same linkage class, then $\langle y' - y, \ln c - \ln c^* \rangle = 0$. Conversely, if $\langle y' - y, \ln c - \ln c^* \rangle = 0$ for each reaction $y \to y' \in \mathcal{R}$, then $c^y / (c^*)^y$ must be constant on each linkage class. In other words, $c \in Z$ if, and only if, $\ln c - \ln c^* \in S^\perp$. The result follows.

<div align="right">**QED**</div>

Proposition 6.2 introduces the important vector, $\ln c - \ln c^*$, where $c, c^* \in \mathbb{P}^\mathcal{S}$ and $A_\kappa \Psi(c^*) = 0$. It arises in a natural way from the ratio $c^y / (c^*)^y$:

$$\ln(c^y / (c^*)^y) = \langle y, \ln c - \ln c^* \rangle \tag{24}$$

Let $u \in S^\perp$. If $\ln c - \ln c^* = u$, then $c = c^* e^u$, where juxtaposition of vectors means component-wise product: if $x, y \in \mathbb{R}^\mathcal{S}$, then $(xy)_i = x_i y_i$. According to Proposition 6.2, as u runs through $S^\perp$, $c^* e^u$ runs through Z, so that $S^\perp$ acts on Z as a transitive group of transformations. Another of way of looking at this is that $\ln Z$ is a coset of $S^\perp$, so that the structure of Z is remarkably rigid.

Recall from §2 that the trajectories of (8) are restricted to the cosets of S: if (8) is started from an initial condition which lies in some coset of S, then its subsequent trajectory remains with the same coset. Since $S^\perp$ meets the cosets of S in one and only one point, it is natural to ask whether something similar is true of Z.

Definition 6.2 *A (positive) stoichiometric compatibility class of the network (8) is a nonempty set of the form $\mathbb{P}^\mathcal{S} \cap (S + c)$ for some $c \in \mathbb{P}^\mathcal{S}$.*

The distinct stoichiometric compatibility classes form a partition of $\mathbb{P}^\mathcal{S}$. Note that the stoichiometric compatibility classes may sometimes be unbounded, as in (22), and sometimes bounded, as in (4). It is easy to see that Z can meet a stoichiometric compatibility class in at most one point. Suppose that $u, v \in S^\perp$ and that $c^* e^u$ and $c^* e^v$ are in the same coset of S. Then $c^* e^u - c^* e^v \in S$ and so, since $u - v \in S^\perp$,

$$\langle c^*(e^u - e^v), u - v \rangle = 0 . \tag{25}$$

Each component of this inner product looks like $c_i^* (e^{u_i} - e^{v_i})(u_i - v_i)$. It is evident that $(e^{u_i} - e^{v_i})(u_i - v_i) \geq 0$, not matter what the relative values of u_i and v_i. Since $c^* \in \mathbb{P}^\mathcal{S}$, it follows from (25) that $u = v$.

It is not so simple to show that Z actually meets each stoichiometric compatibility class. To do so we need to use some nonlinear methods from convex analysis. The basic observation is independent of chemical reaction networks—$\mathcal{S}$ could be any finite index set and S any subspace in what follows— but we will use the same notation for convenience. Choose any $a, b \in \mathbb{P}^\mathcal{S}$. We claim that there always exists some $u \in S^\perp$ such that $ae^u - b \in S$. This will be sufficient for our purposes, since ae^u will be in the same stoichiometric compatibility class as b, which was chosen arbitrarily. There is a nice idea behind the proof, which comes from [14, Proposition B1].

In one dimension, if $a, b \in \mathbb{P}$ and $x \in \mathbb{R}$, then it is easy to integrate $ae^x - b$ as a function of x. It is sufficient to take $ae^x - bx$. It follows that, as a vector, $ae^x - b$ is a gradient. For $a, b \in \mathbb{P}^\mathcal{S}$ and $x \in \mathbb{R}^\mathcal{S}$, it is sufficient to take the function $\phi : \mathbb{R}^\mathcal{S} \to \mathbb{R}$ given by

$$\phi(x) = \langle ae^x - bx, 1 \rangle . \tag{26}$$

The gradient of ϕ, denoted $\nabla \phi \in \mathbb{R}^\mathcal{S}$, is the vector of first partial derivatives:

$$\nabla \phi(x) = (\partial \phi / \partial x_1, \cdots, \partial \phi / \partial x_m)^T .$$

It is easy to see that $\nabla \phi(x) = ae^x - b$. One way to find $u \in S^\perp$ such that $ae^u - b \in S$, would be if ϕ had a minimum in the space $S^\perp$. Since ϕ is smooth, such a minimum must satisfy Fermat's principle, so that the derivative of ϕ along any direction in $S^\perp$ should vanish. Suppose that $u \in S^\perp$

is a minimum of ϕ in $S^\perp$ and let $z \in S^\perp$ be chosen arbitrarily. The derivative of ϕ at u in the direction z is given by the derivative of $t \to u + tz$ at $t = 0$. Using the chain rule,

$$\left.\frac{d}{dt}\phi(u + tz)\right|_{t=0} = \sum_{1 \leq i \leq m} \frac{\partial \phi(u)}{\partial x_i} \left.\frac{dx_i}{dt}\right|_{t=0} = \langle \nabla \phi(u), z \rangle .$$

If this is zero then, since z was chosen arbitrarily in $S^\perp$, we see that $\nabla \phi(u) \in S$. Hence $ae^u - b \in S$ and we have found the required $u \in S^\perp$.

It remains to show that ϕ has a minimum in $S^\perp$. Reverting again to one dimension, it is easy to see that the function $ae^x - bx$ goes to ∞ in both directions. If $x \to -\infty$, $ae^x \to 0$ while $-bx \to \infty$, so that $ae^x - bx \to \infty$. If $x \to \infty$ then $ae^x \to \infty$ while $-bx \to -\infty$. However, the exponential clearly outruns the linear term, so that $ae^x - bx \to \infty$. Much the same argument shows that the vector function (26) has a similar property: if $\lambda \in \mathbb{P}$, then for any $x \in \mathbb{R}^S$, $\phi(\lambda x) \to \infty$ as $\lambda \to \infty$. This suggests that ϕ has a global minimum in $\mathbb{R}^S$. Let us first find this. We can then restrict ϕ to $S^\perp$ to find the minimum in $S^\perp$.

Take any level set of ϕ, say $F = \phi^{-1}(0) = \{x \in \mathbb{R}^S \mid \phi(x) \leq 0\}$. Since ϕ is continuous, this is closed in the topology of $\mathbb{R}^S$. If F is also bounded it would then be compact and the continuous function ϕ would attain a minimum in F, say at $u \in F$. The nice thing about F is that such a minimum must also be a global minimum. If $\phi(x) \leq 0$ then by construction, $\phi(u) \leq \phi(x)$, while if $\phi(x) > 0$ then certainly $\phi(u) \leq 0 < \phi(x)$. Hence, $\phi(u) \leq \phi(x)$ for all $x \in \mathbb{R}^S$.

Unfortunately, the asymptotic behaviour of ϕ that we worked out above is not sufficient to establish that F is bounded. It only tells us that at any point $x \in F$ any half-line originating at x must eventually leave F. It is easy to find unbounded subsets of $\mathbb{R}^2$ with this property.

There is one other property of ϕ that we have not exploited. Recall that a function $g : \mathbb{R}^S \to \mathbb{R}$ is said to be convex if, given $\lambda, \mu \in [0, 1]$ such that $\lambda + \mu = 1$, and any $x, y \in \mathbb{R}^S$,

$$g(\lambda x + \mu y) \leq \lambda g(x) + \mu g(y) . \tag{27}$$

Reverting to one dimension, this means that the graph of g lies below the chord joining the points $(x, g(x))$ and $(y, g(y))$. A subset $U \subseteq \mathbb{R}^S$ is said to be convex if, given $\lambda, \mu \in [0, 1]$ such that $\lambda + \mu = 1$, and any $x, y \in U$, the point $\lambda x + \mu y \in U$. In other words, U is convex if the line segment joining any two points of U lies entirely in U. There is an intimate connection between convex functions and convex sets. For instance, it is easy to see that any level set, $g^{-1}(x)$, of a convex function is a convex set.

In one dimension, if g is smooth, so that it is infinitely differentiable, it is an easy exercise in the calculus to show that g is convex if dg/dx is increasing. By increasing, we mean that if $a \leq b \in \mathbb{R}$ then $dg(a)/dx \leq dg(b)/dx$. Hence, it is sufficient for the convexity of g to show that $d^2g/dx^2 \geq 0$ everywhere. It follows that $x \to ae^x - bx$ is convex. This is sufficient to show that the vector function (26) is convex because the direct sum of two convex functions is convex. By this we mean that, if I and J are any two finite index sets and $g : \mathbb{R}^I \to \mathbb{R}$ and $h : \mathbb{R}^J \to \mathbb{R}$ are convex functions then $g + h : \mathbb{R}^I \oplus \mathbb{R}^J \to \mathbb{R}$ is also convex. It is easy to verify this. Hence, $\phi(x) = \langle ae^x - bx, 1 \rangle$ is a convex function and $F = \phi^{-1}(0)$ is a convex subset.

This supplies the missing ingredient in the argument. It is not possible to find an unbounded convex subset which contains a point such that some half-line originating from the point eventually leaves the subset. To see this, recall one of the fundamental results of convex analysis.

Theorem 6.1 (Existence of a separating hyperplane, [2, Theorem 2.4]) *Given a non-empty, closed, convex subset $F \subseteq \mathbb{R}^S$ and a point $x \in \mathbb{R}^S$ such that $x \notin F$, there exists a hyperplane containing x such that F lies entirely on one side of the hyperplane. In other words, there is some non-zero linear function $\ell : \mathbb{R}^S \to \mathbb{R}$, such that, for some $a \in \mathbb{R}$, $\ell(x) = a$ and $\ell(y) < a$ for all $y \in F$.*

The hyperplane is given by $\ell^{-1}(a)$. It follows from this result that any closed, convex subset $F \subseteq \mathbb{R}^{\mathcal{S}}$ is the intersection of a family of closed half-spaces. (The family may have uncountably infinite cardinality.) It is not difficult to see from this that there are two possibilities: either F is bounded or, given any point $x \in F$, there is some half-line originating at x which lies entirely in F.

We deduce from what was said above that the level set $F = \phi^{-1}(0)$ is a bounded subset of $\mathbb{R}^{\mathcal{S}}$ and that therefore ϕ has a global minimum in F. We can now apply exactly the same argument to ϕ restricted to $S^{\perp}$ and deduce that $\phi|_{S^{\perp}}$ has a minimum $u \in S^{\perp}$. Hence, as shown above, $ae^u - b \in S$. Using the same argument as above for $c^* e^u$ it is easy to see that u must be unique.

Proposition 6.3 ([14, Proposition B1]) *If $S \subseteq \mathbb{R}^{\mathcal{S}}$ is any subspace and $a, b \in \mathbb{P}^{\mathcal{S}}$ there exists an unique $u \in S^{\perp}$ such that $ae^u - b \in S$.*

We can now summarise what we have learnt so far about the structure of the set of fixed points for which $A_\kappa \Psi(c) = 0$.

Proposition 6.4 *In any chemical reaction network let $Z = \{c \in \mathbb{P}^{\mathcal{S}} \mid A_\kappa \Psi(c) = 0\}$. Then, either $Z = \emptyset$ or $\ln Z$ is a coset of $S^{\perp}$ and Z meets each stoichiometric compatibility class in one and only one point.*

We have still not exhausted what the function $\ln c - \ln c^*$ can teach us when $A_\kappa \Psi(c^*) = 0$. We know that at points where $A_\kappa \Psi(c) = 0$ the vector $\ln c - \ln c^*$ is orthogonal to the stoichiometric subspace, S. What happens at other points? The following is one of the most satisfying arguments in CRNT. Let $u = \ln c - \ln c^*$. Recall from §3 that we have used $f(c)$ to denote the right hand side of the fundamental dynamical equation (8). Using (24), we can rewrite f in terms of c^* and u,

$$f(c) = \sum_{y \to y' \in \mathcal{R}} (\kappa_{y \to y'})(c^*)^y e^{\langle y, u \rangle}(y' - y) \ .$$

Using the bilinearity of the inner product,

$$\langle f(c), u \rangle = \sum_{y \to y' \in \mathcal{R}} (\kappa_{y \to y'})(c^*)^y e^{\langle y, u \rangle}(\langle y', u \rangle - \langle y, u \rangle) \ .$$

The exponential function satisfies a particularly useful inequality which comes in very handy here. It is easy to see that, for all $x \in \mathbb{R}$,

$$e^x \geq 1 + x \quad \text{with equality if, and only if, } x = 0. \tag{28}$$

Substituting $x' - x$ for x in (28), we see that

$$e^x(x' - x) \leq e^{x'} - e^x \quad \text{with equality if, and only if, } x' = x \ . \tag{29}$$

Taking $x' = \langle y', u \rangle$ and $x = \langle y, u \rangle$ we see that

$$\langle f(c), u \rangle \leq \sum_{y \to y' \in \mathcal{R}} (\kappa_{y \to y'})(c^*)^y (e^{\langle y', u \rangle} - e^{\langle y, u \rangle}) \ , \tag{30}$$

with equality if, and only if, $\langle y', u \rangle = \langle y, u \rangle$, for each $y \to y' \in \mathcal{R}$. We note from the proof of Proposition 6.2 that this is precisely the condition $u \in S^{\perp}$. The right hand side of (30) has resolved the asymmetry between y' and y. This allows us to rewrite it as follows. Let $\Omega \in \mathbb{R}^{\mathcal{C}}$ be the vector given by

$$\Omega = \sum_{y \in \mathcal{C}} e^{\langle y, u \rangle} \omega_y \ .$$

Since, $\{\omega_y\}$ forms an orthonormal basis for $\mathbb{R}^{\mathcal{C}}$, $e^{\langle y, u \rangle} = \langle \omega_y, \Omega \rangle$ for all $y \in \mathcal{C}$, where now the inner-product takes place in $\mathbb{R}^{\mathcal{C}}$ rather than $\mathbb{R}^{\mathcal{S}}$. Using the bilinearity of the inner-product on $\mathbb{R}^{\mathcal{C}}$, we can rewrite the right hand side of (30) as

$$\langle \sum_{y \to y' \in \mathcal{R}} (\kappa_{y \to y'})(c^*)^y (\omega_{y'} - \omega_y), \Omega \rangle = \langle A_\kappa \Psi(c^*), \Omega \rangle = 0 \ .$$

We see that $\langle f(c), u \rangle \leq 0$ with equality if, and only if, $u \in S^\perp$.

Proposition 6.5 *If a chemical reaction network has a fixed point $c^* \in \mathbb{P}^{\mathcal{S}}$ for which $A_\kappa \Psi(c^*) = 0$ then, for any $c \in \mathbb{P}^{\mathcal{S}}$, $\langle f(c), \ln c - \ln c^* \rangle \leq 0$, with equality if, and only if, $\ln c - \ln c^* \in S^\perp$*

This result has a remarkable consequence. Suppose that $c \in \mathbb{P}^{\mathcal{S}}$ is any fixed point of (8), so that $f(c) = 0$. Then $\langle f(c), \ln c - \ln c^* \rangle = 0$ and we must conclude that $\ln c - \ln c^* \in S^\perp$. But then, by Proposition 6.2, $c \in Z$ and so $A_\kappa \Psi(c) = 0$. We have proved one of the most startling conclusions of CRNT, which deserves to be singled out.

Theorem 6.2 ([11, Proposition 5.3 (iii) and (iv)]) *In any chemical reaction network, if there exists some fixed point $c^* \in \mathbb{P}^{\mathcal{S}}$ for which $A_\kappa \Psi(c^*) = 0$ then any fixed point, $c \in \mathbb{P}^{\mathcal{S}}$ with $f(c) = 0$, must satisfy the same condition $A_\kappa \Psi(c) = 0$.*

If the deficiency of the network is 0 then we would know that there are no fixed points other than those of the form $A_\kappa \Psi(c) = 0$. Theorem 6.2 tells us this assumption about the deficiency is not required. The more that we study it, the more stringent the condition $A_\kappa \Psi(c^*) = 0$ appears.

There is still more that we can extract from it! The inequality of Proposition 6.5 is very suggestive. Suppose that we could show, for fixed $c^* \in \mathbb{P}^{\mathcal{S}}$, that the vector field $\ln c - \ln c^*$ is a gradient. In other words, suppose we could find a function $h : \mathbb{P}^{\mathcal{S}} \to \mathbb{R}$ such that $\nabla h(c) = \ln c - \ln c^*$. It then follows that h must decrease along the trajectories of (8), for suppose that $t \to c(t)$ is one such trajectory, so that $dc(t)/dt = f(c(t))$. Then, using the chain rule,

$$\frac{d}{dt} h(c(t)) = \sum_{i \in \mathcal{S}} \frac{\partial h(c)}{\partial c_i} \frac{dc_i}{dt} = \langle \nabla h(c), f(c) \rangle = \langle \ln c - \ln c^*, f(c) \rangle \ .$$

By Proposition 6.5, $dh(c(t))/dt \leq 0$ with equality if, and only if, $c(t)$ is a fixed point of (8). Hence h must strictly decrease along a trajectory until that trajectory reaches a fixed point. We have a candidate for a (strict) Lyapunov function.

Recall that the existence of a strict Lyapunov function implies that trajectories in the vicinity of a fixed point are attracted towards the fixed point and reach it asymptotically. There can be no periodic orbits or other behaviour which avoids convergence to the fixed point, at least in the immediate vicinity of the fixed point. Suppose that $dc/dt = f(c)$ is a system of nonlinear ODEs, where $f : \mathbb{R}^m \to \mathbb{R}^m$, and that the system has a fixed point at c^*, so that $f(c^*) = 0$.

Definition 6.3 *A function $h : \mathbb{R}^m \to \mathbb{R}$ is a* Lyapunov function *for the fixed point c^* if*

1. *$h(c) \geq 0$, for all $c \in \mathbb{R}^m$, with equality if, and only if, $c = c^*$*

2. *$dh(c(t))/dt \leq 0$ along any trajectory, $t \to c(t)$.*

If, furthermore, $dh(c(t))/dt = 0$ if, and only if, $c(t) = c^*$, then the Lyapunov function is strict.

Theorem 6.3 (Lyapunov's Theorem, [20, Chapter 9, §3, Theorem 1]) *If h is a Lyapunov function then c^* is stable. That is, given any neighbourhood $U \ni c^*$, there exists some neighbourhood $U_1 \ni c^*$ in U such that any trajectory $c(t)$ with $c(0) \in U_1$ satisfies $c(t) \in U$ for all $t \geq 0$. If, furthermore, the Lyapunov function is strict then c^* is asymptotically stable. That is, U_1 can always be chosen so that $u(t) \to c^*$ as $t \to \infty$.*

It is easy to construct h. In one dimension, $\ln x$ is integrable: simply take $h(x) = x \ln x - x$. For the vector function, $h : \mathbb{P}^\mathcal{S} \to \mathbb{R}$, we can use the same trick as we did in the proof of Proposition 6.3:

$$h(c) = \langle c \ln c - c - c \ln c^* + c^*, 1 \rangle . \tag{31}$$

It is easy to check that $\nabla h = \ln c - \ln c^*$ and that $h(c^*) = 0$. To see that $h(c) > 0$ unless $c = c^*$, let us go back to the handy inequality we used in the proof of Proposition 6.5. Suppose that we are in 1 dimension and set $x = \ln c$, $x' = \ln c^*$. It follows from (29) that

$$c(\ln c^* - \ln c) \leq c^* - c$$

with equality if, and only if, $c = c^*$. Rearranging, this gives $c \ln c - c - c \ln c^* + c^* \geq 0$. It follows that, in any dimension, $h(c) \geq 0$ with equality if, and only if, $c = c^*$. We see that h satisfies the first condition in Definition 6.3. However, it does not satisfy the second condition because $dh(c(t))/dt = \langle \ln c(t) - \ln c^*, f(c(t)) \rangle = 0$ if $c(t)$ is any fixed point, including a fixed point in a stoichiometric compatibility class different from the one containing c^*. We can avoid this problem by restricting h to the stoichiometric compatibility class of c^* and then applying Theorem 6.3. We leave the details to the reader.

Asymptotic stability is a local property of a fixed point. The existence of a strict Lyapunov function sometimes allows the basin of attraction of the fixed point to be estimated, as in [20, Chapter 9, §3, Problem 8], but (31) does not lend itself to this. However, a strict Lyapunov function does rule out certain global behaviours relative to the corresponding stoichiometric compatibility class. For instance, it is easy to see that there can be no periodic orbits. This assertion is usually included among the conclusions of the deficiency 0 theorem, [12, Theorem 6.1.1(2)].

We can now assemble all the things we have learned. What follows is the main result behind the deficiency 0 theorem.

Theorem 6.4 *In any chemical reaction network, suppose there exists a fixed point $c^* \in \mathbb{P}^\mathcal{S}$ for which $A_\kappa \Psi(c^*) = 0$. The following statements hold.*

1. *The network is weakly reversible.*

2. *Every fixed point, $c \in \mathbb{P}^\mathcal{S}$ with $f(c) = 0$, satisfies $A_\kappa \Psi(c) = 0$.*

3. *If Z is the set of all fixed points, $Z = \{c \in \mathbb{P}^\mathcal{S} \mid f(c) = 0\}$, then $\ln Z$ is a coset of $S^\perp$.*

4. *There is one, and only one, fixed point in each stoichiometric compatibility class.*

5. *Each fixed point has a strict Lyapunov function defined on its stoichiometric compatibility class and is asymptotically stable relative to that class.*

No assumption about the deficiency is required for Theorem 6.4. This is only needed to show the existence of some fixed point $c \in \mathbb{P}^\mathcal{S}$ for which $A_\kappa \Psi(c) = 0$. This we do in Theorem 7.1 in the next section. It is easy linear algebra and something of an anti-climax after our excursions into Perron-Frobenius theory, convex analysis and dynamical systems.

7 Existence of fixed points

We observed in Lemma 3.1 that the bookkeeping function $\Psi : \mathbb{R}^{\mathcal{S}} \to \mathbb{R}^{\mathcal{C}}$ is "log-linear", so that, for any $c \in \mathbb{P}^{\mathcal{C}}$, $\ln \Psi(c) = Y^T \ln(c)$. This suggests a method for finding $c \in \mathbb{P}^{\mathcal{C}}$ such that $\Psi(c) \in \ker A_{\kappa}$. Recall the notation used in Theorem 4.2. Suppose that there exists an element $u \in \ker A_{\kappa}$ such that $u \in \mathbb{P}^{\mathcal{C}}$ and $\ln u \in \mathrm{Im} Y^T$. Let $x \in \mathbb{R}^{\mathcal{S}}$ be such that $Y^T(x) = \ln u$ and let $c = \exp(x)$, so that $c \in \mathbb{P}^{\mathcal{S}}$. By Lemma 3.1, $\ln u = Y^T \ln(c) = \ln \Psi(c)$, so that $u = \Psi(c)$. It follows that $\Psi(c) \in \ker A_{\kappa}$, as required.

It remains only to translate this into a suitable condition. This is based on the observation that the set of logarithms of positive elements in $\ker A_{\kappa}$ has a particularly nice form. For $y \in \mathcal{C}$ and $1 \leq i \leq t$, let $p_{i,y} = \langle \chi_i, \omega_y \rangle$. By construction of the χ_i, we know that $p_{i,y} = 0$ if $y \notin T_i$, so that $\chi_i = \sum_{y \in T_i} p_{i,y} \omega_y$. Furthermore, $p_{i,y} > 0$ if $y \in T_i$. Since the T_i are pairwise mutually disjoint, it follows that $\sum_i \lambda_i \chi_i \in \mathbb{P}^{\mathcal{C}}$ if, and only if, $\lambda_i \in \mathbb{P}$ for $1 \leq i \leq t$. Let $(\ker A_{\kappa})^+ = \ker A_{\kappa} \cap \mathbb{P}^{\mathcal{C}}$ and choose any $\lambda_i \in \mathbb{P}$ for $1 \leq i \leq t$. Then

$$\ln(\sum_i \lambda_i \chi_i) = \ln(\sum_i \sum_{y \in T_i} \lambda_i p_{i,y} \omega_y) = \sum_i \ln \lambda_i (\sum_{y \in T_i} \omega_y) + \sum_i \sum_{y \in T_i} (\ln p_{i,y}) \omega_y \ .$$

The first term on the right hand side may be rewritten as $\sum_i (\ln \lambda_i) \omega_{T_i}$. Since the λ_i were chosen arbitrarily in $\mathbb{P}$, it follows that $\ln(\ker A_{\kappa})^+$ is a coset of $\mathrm{span}\{\omega_{T_1}, \cdots, \omega_{T_t}\}$. The coset is determined by the components $p_{i,y}$ or, equivalently, by the particular kinetics specified by κ.

Now consider the subspace $U = \mathrm{Im} Y^T + \mathrm{span}\{\omega_{T_1}, \cdots, \omega_{T_t}\}$. Since $\ln(\ker A_{\kappa})^+$ is a coset of $\mathrm{span}\{\omega_{T_1}, \cdots, \omega_{T_t}\}$, there are two mutually exclusive possibilities. Either $\ln(\ker A_{\kappa})^+ \cap U = \emptyset$ or $\ln(\ker A_{\kappa})^+ \subseteq U$. In the latter case, since $\ln(\ker A_{\kappa})^+$ is a coset of $\mathrm{span}\{\omega_{T_1}, \cdots, \omega_{T_t}\}$, it is evident that $\ln(\ker A_{\kappa})^+ \cap \mathrm{Im} Y^T \neq \emptyset$. Hence there is an element with the properties needed to find a fixed point, as above. Conversely, if such an element exists then the same argument as above shows that $\ln(\ker A_{\kappa})^+ \cap \mathrm{Im} Y^T \neq \emptyset$.

Lemma 7.1 $\ln(\ker A_{\kappa})^+$ *is a coset of* $\mathrm{span}\{\omega_{T_1}, \cdots, \omega_{T_t}\}$. *Furthermore, either* $\ln(\ker A_{\kappa})^+ \cap U = \emptyset$ *or* $\ln(\ker A_{\kappa})^+ \subseteq U$. *The latter case holds if, and only if, there exists* $c \in \mathbb{P}^{\mathcal{C}}$ *such that* $A_{\kappa} \Psi(c) = 0$.

Suppose now that the network in question is weakly reversible. In this case U turns out to be familiar. By Lemma 6.1 part 3, the terminal strong linkage classes coincide with the linkage classes and, in particular, $l = t$. Let $T = \mathrm{span}\{\omega_{y'} - \omega_y \mid y \to y'\}$. We know from the definition of A_{κ} that $\mathrm{Im} A_{\kappa} \subseteq T$. However, by Theorem 4.2 part 3, $\dim \mathrm{Im} A_{\kappa} = n - t$ and, by Lemma 3.2 part 3, $\dim T = n - l$. Hence, $\mathrm{Im} A_{\kappa} = T$. By Lemma 3.2 part 2, $T^{\perp} = \mathrm{span}\{\omega_{L_1}, \cdots, \omega_{L_l}\} = \mathrm{span}\{\omega_{T_1}, \cdots, \omega_{T_t}\}$. Furthermore, by elementary linear algebra, $\mathrm{Im} Y^T = (\ker Y)^{\perp}$. Hence,

$$U = (\ker Y)^{\perp} + T^{\perp} = (\ker Y \cap \mathrm{Im} A_{\kappa})^{\perp}$$

and we recognise in this the subspace of $\mathbb{R}^{\mathcal{C}}$ whose dimension is the deficiency of the network.

Theorem 7.1 *If a chemical reaction network has deficiency 0 then it has a fixed point* $c \in \mathbb{P}^{\mathcal{S}}$ *for which* $A_{\kappa} \Psi(c) = 0$ *if, and only if, it is weakly reversible.*

The usual form of the deficiency 0 theorem, as in [12, Theorem 6.1.1], can now be recovered by combining Theorem 6.4 with Theorem 7.1.

References

[1] J. Ross A. Arkin and H. McAdams. Stochastic kinetic analysis of developmental pathway bifurcation in phage λ-infected Escherichia coli cells. *Genetics*, 149:1633–48, 1998.

[2] J.-P. Aubin. *Optima and Equilibria*, volume 140 of *Graduate Texts in Mathematics*. Springer-Verlag, 1993.

[3] C. P. Bagowski and J. E. Ferrell. Bistability in the JNK cascade. *Curr. Biol.*, 11(15):1176–82, 2001.

[4] J. E. Bailey. Complex biology with no parameters. *Nature Biotechnology*, 19:503–4, 2001.

[5] N. Barkai and S. Leibler. Robustness in simple biochemical networks. *Nature*, 387:913–7, 1997.

[6] A. Berman and R. J. Plemmons. *Nonnegative Matrices in the Mathematical Sciences*. Classics in Applied Mathematics. SIAM, 1994.

[7] U. Bhalla, P. Ram, and R. Iyengar. MAP kinase phosphatase as a locus of flexibility in a mitogen-activated protein kinase signaling network. *Science*, 297:1018–23, 2002.

[8] A. Cornish-Bowden. *Fundamentals of Enzyme Kinetics*. Portland Press, 2nd edition, 1995.

[9] M. Delbrück. Discussion. In *Unités biologiques douées de continuité génétique*, pages 33–34. CNRS, 1949.

[10] M. B. Elowitz, A. J. Levine, E. D. Siggia, and P. S. Swain. Stochastic gene expression in a single cell. *Science*, 297:1183–6, 2002.

[11] M. Feinberg. Lectures on chemical reaction networks. Notes of lectures given at the Mathematics Research Centre, University of Wisconsin, in 1979.

[12] M. Feinberg. Chemical reaction network structure and the stability of complex isothermal reactors I. The deficiency zero and deficiency one theorems. *Chem. Eng. Sci*, 42(10):2229–68, 1987.

[13] M. Feinberg. Chemical reaction network structure and the stability of complex isothermal reactors II. Multiple steady states for networks of deficiency one. *Chem. Eng. Sci*, 43(1):1–25, 1988.

[14] M. Feinberg. The existence and uniqueness of steady states for a class of chemical reaction networks. *Arch. Rational Mech. Anal.*, 132:311–370, 1995.

[15] M. Feinberg. Multiple steady states for chemical reaction networks of deficiency one. *Arch. Rational Mech. Anal.*, 132:371–406, 1995.

[16] M. Feinberg and F. Horn. Chemical mechanism structure and the coincidence of the stoichiometric and kinetic subspace. *Arch. Rational Mech. Anal.*, 66:83–97, 1977.

[17] A. Goldbeter and D. E. Koshland. An amplified sensitivity arising from covalent modification in biological systems. *PNAS*, 78(11):6840–6844, 1981.

[18] A. Goldbetter. *Biochemical Oscillations and Cellular Rhythms: the molecular bases of periodic and chaotic behaviour*. Cambridge University Press, 1996.

[19] B. D. Gompaerts, L. M. Kramer, and P. E. R. Tatham. *Signal Transduction*. Academic Press, 2002.

[20] M. W. Hirsch and S. Smale. *Differential Equations, Dynamical Systems and Linear Algebra*. Pure and Applied Mathematics. Academic Press, 1974.

[21] A. Hoffman, A. Levchenko, M. L. Scott, and D. Baltimore. The IκB-NFκB signalling module: temporal control and selective gene activation. *Science*, 298:1241–5, 2002.

[22] F. Horn and R. Jackson. General mass action kinetics. *Arch. Rational Mech. Anal.*, 47:81–116, 1972.

[23] D. A. Hume. Probability in transcriptional regulation and its implications for leukocyte differentiation and inducible gene expression. *Blood*, 96(7):2323–8, 2000.

[24] M. Laurent and N. Kellershon. Multistability: a major means of differentiation and evolution in biological systems. *TIBS*, 24:418–22, 1999.

[25] J. M. Levsky and R. H. Singer. Gene expression and the myth of the average cell. *Trends in Cell Biology*, 13(1):4–6, 2003.

[26] E. N. Lorenz. Deterministic aperiodic flow. *J. Atmos. Sci.*, 20:130–41, 1963.

[27] H. Minc. *Nonnegative Matrices.* Wiley-Interscience Series in Discrete Mathematics and Optimization. John Wiley, 1988.

[28] S. Pedersen, P. L. Bloch, S. Reeh, and F. C. Neidhardt. Patterns of protein synthesis in E. coli: a catlog of the amount of 140 individual proteins at different growth rates. *Cell*, 14:179–90, 1978.

[29] P. M. Schlosser. *A graphical determination of the possibility of multiple steady states in complex isothermal CFSTRs.* PhD thesis, University of Rochester, 1988.

[30] P. M. Schlosser and M. Feinberg. A theory of multiple steady states in isothermal homogeneous CFSTRs with many reactions. *Chem. Eng. Sci.*, 49(11):1749–67, 1994.

[31] C. Soulé. Graphic requirements for multistationarity. Preprint, IHES, February 2003.

[32] R. Thomas. On the relation between the logical structure of systems and their ability to generate multiple steady states or sustained oscillations. *Springer Series in Synergetics*, 9:180–93, 1981.

[33] R. Thomas. Laws for the dynamics of regulatory networks. *Int. Jour. Dev. Biol.*, 42:479–85, 1998.

[34] G. von Dassow, E. Meir, E. M. Munro, and G. M. Odell. The segment polarity network is a robust developmental module. *Nature*, 406:188–92, 2000.

[35] J. A. Yorke and E. D. Yorke. Metastable chaos: the transition to sustained chaotic behaviour in the Lorentz model. *J. Stat. Phys*, 21:263–77, 1979.