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Solutions of weakly reversible chemical reaction networks are bounded and persistent ⋆

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Abstract: We present extensions to chemical reaction network theory which are relevant to the analysis of models of biochemical systems. We show that, for positive initial conditions, solutions of a weakly reversible chemical reaction network are bounded and remain in the positive orthant. Thus, weak reversibility implies persistence as conjectured by Martin Feinberg. Our result provides a qualitative criterion to establish that a biochemical network will not diverge or converge to the boundary, where some concentration levels are zero. It relies on checking structural properties of the graph of the reaction network solely. It can also be used to characterise certain bifurcations from stationary to oscillatory behaviour. We illustrate the use of our result through applications.

Keywords: Networks, Dynamics, Nonlinear systems, Attractors, Oscillators, Biotechnology

1. INTRODUCTION

In the field of modelling biological systems very often parameter values are unattainable, can only be collected under noisy experimental conditions, or are sensitive to the laboratory environment. On the other hand, in many cases, biological systems display *robust* behaviour; that is, we observe the same qualitative behaviour of the system for a large range of most parameter values. For certain types of biological systems, and their mathematical counterparts, robustness of different system properties can be checked through analysis methods that do not require information on the quantitative values of system parameters.

Biochemical processes, but also ecological or economical, can be visualised through a *Chemical Reaction Network (CRN)*. An approach to extract important properties of the system by means of a network analysis without requiring information about the quantitative values of parameters is applying Martin Feinberg's *Chemical Reaction Network Theory (CRNT)* (Feinberg, 1979, 1987). For instance, the *Deficiency Zero Theorem* and *Deficiency One Theorem* provide means to check for the existence and stability of a unique positive equilibrium point for the system, describing the behaviour of interacting chemical concentrations, independently of parameter values as long as they are positive and non-vanishing.

An important property is *persistence*, which means that chemicals are not eliminated in the course of the process. In (Angeli et al., 2007), conditions for persistence were given for conservative systems, that is, for systems without inputs and outputs and that obey a conservation law.

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In Section 3 of this paper, we extend CRNT to provide conditions for persistence and prove that *weak reversibility* of a CRN, a graph whose *indecomposable* subgraphs are *strongly connected*, implies persistence in general (i.e., also for networks with inputs/outputs), which was conjectured in (Feinberg, 1987). Furthermore, we show that, for positive initial conditions, solutions of a weakly reversible CRN are bounded. This is another important property, since, while it is expected that for the real system some mechanisms (e.g., limit of resources) will prohibit infinite growth, mathematical models cannot take them all into account, either because the lack of detailed knowledge or because the model has to be kept relatively simple. For instance, if predators are absent in the simple Lotka-Volterra model then the prey will multiply without limit. Finally, we apply our result to important biological systems in Section 4 and draw conclusions in Section 5.

2. CHEMICAL REACTION NETWORKS OBEYING MASS ACTION KINETICS

CRNs are used to describe and understand biological processes. An illustrative example is the Michaelis-Menten reaction scheme for chemical reactions involving enzymes:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} E + P. \tag{1}$$

Here, S denotes the substrate, E the enzyme, ES the enzyme-substrate complex and P the final product. They are the so called *species* that participate in the reactions. The positive constants k_1, k_{-1}, k_2 and k_{-2} are the reaction *rate constants*. *Edges* represent reactions and *vertices* represent *complexes* (the objects that appear before and after the reaction arrows). For (1), the vectors

of concentrations of species and complexes are given by $x^{\mathrm{T}} = [[E]\ [S]\ [ES]\ [P]]$ and $\Psi(x)^{\mathrm{T}} = [[E][S]\ [ES]\ [E][P]]$, respectively. Here, $[E]$ denotes the concentration of E.

The so called *bookkeeping matrix* Y maps the space of complexes into the space of species. The elements of the ith row of matrix Y tell us in which complexes species i appears and how often; or, equivalently, the entries to the jth column tell us of how much of each species complex j is made of. For (1), $Y^{\mathrm{T}} = \begin{bmatrix} 1 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 1 & 0 & 0 & 1 \end{bmatrix}$. Matrix K is the transpose of the *weighted adjacency matrix* of the *digraph* representing the CRN; i.e., entry K_{ij} is nonnegative and corresponds to the rate constant associated with the reaction from complex j to i. The *kinetic matrix* A_κ is given by $A_\kappa = K - \mathrm{diag}(K^{\mathrm{T}}e)$, $e^{\mathrm{T}} = [1\ \cdots\ 1]$. For (1),

$$K = \begin{bmatrix} 0 & k_{-1} & 0 \\ k_1 & 0 & k_{-2} \\ 0 & k_2 & 0 \end{bmatrix} \text{ and } A_\kappa = \begin{bmatrix} -k_1 & k_{-1} & 0 \\ k_1 & -(k_{-1}+k_2) & k_{-2} \\ 0 & k_2 & -k_{-2} \end{bmatrix}$$

In CRNT, it is common to assume mass action kinetics. It assumes that reactions take place at constant temperature in a homogenous and well mixed solution and that then the probability of a collision between molecules is proportional to the product of their concentrations. This means that $\ln \Psi(x) = Y^{\mathrm{T}} \ln x$, and one formulates the dynamics of the CRN by the following set of nonlinear ODEs:

$$\dot{x} = Y A_\kappa \Psi(x). \tag{2}$$

In general, we assume that a CRN has n species and m complexes. Thus, in (2): $x \in \mathbb{R}^n$, $\Psi(x) \in \mathbb{R}^m$, $A_\kappa \in \mathbb{R}^{m\times m}$, $Y \in \mathbb{N}^{n\times m}$; and x_i, $\Psi_j(x)$ and Y_{ij} are nonnegative for all i, j. In the following, we will highlight certain properties of (2), which we will exploit in order to prove the main theorem of this paper (Theorem 6). Recall that $A_\kappa = K - \mathrm{diag}(K^{\mathrm{T}}e)$. Let us denote $\mathrm{diag}(K^{\mathrm{T}}e)$ by D. Then, with $\sum_{l=1}^{m} K_{lj} = D_{jj}$, this leads to:

$$\begin{aligned} \dot{x}_i &= \sum_{j=1}^{m} \Psi_j(x) \left(\sum_{l=1}^{m} Y_{il} K_{lj} - Y_{ij} D_{jj} \right) \\ &= \sum_{j=1}^{m} \sum_{l=1}^{m} K_{lj} \Psi_j(x) (Y_{il} - Y_{ij}). \end{aligned} \tag{3}$$

Let us denote the reaction rate of the reaction that transforms complex j into complex l by $k_{j\to l}$, $j, l = \{1, \ldots, m\}$. Then, $K_{lj} = k_{j\to l}$ and

$$\dot{x}_i = \sum_{\mathfrak{R}} k_{j\to l} \Psi_j(x) (Y_{il} - Y_{ij}). \tag{4}$$

'The symbol $\mathfrak{R}$ denotes the set of reactions in the underlying network, and its presence under the summation sign is intended to indicate that the sum is taken over all reactions' (Feinberg, 1987). Note also that $\Psi_j(x) = \prod_{\wp=1}^{n} x_\wp^{Y_{\wp j}}$.

An important property, which we will later use, of CRNs taken with mass action kinetics was proved in (Haddad et al., 2001; Chaves, 2003), namely, that if a dynamical system represents a CRN then $\dot{x}_i|_{x_i=0} \geq 0$ for all i. This implies that solutions remain nonnegative if the initial conditions are nonnegative, and we can restrict our analysis to the nonnegative orthant, the space of realistic solutions. The following definitions of a *linkage class* and weak reversibility are central to CRNT.

Definition 1. A linkage class is a closed set of complexes that are linked through reactions. We denote the number of linkage classes by ℓ.

Remark 2. Definition 1 implies that the linkage class of the CRN is what is called an indecomposable subgraph in graph theory. Furthermore, if $\ell > 1$ then A_κ can be block-diagonalised and each block-diagonal submatrix corresponds to a linkage class.

Definition 3. A CRN is weakly reversible if there is a directed reaction path from any complex to any other within the same linkage class.

Remark 4. Weak reversibility means that each indecomposable subgraph of the CRN is strongly connected; i.e., 'any two points are mutually reachable by means of directed paths' (Harary, 1962). Thus, in all linkage classes any individual reaction is always part of a reaction cycle. Weak reversibility also implies that every block-diagonal submatrix of A_κ associated with a linkage class is *irreducible*. A square matrix is irreducible if it is not reducible. It is reducible if and only if it can be made block upper-triangular by simultaneous row/column permutations.

2.1 Martin Feinberg's Chemical Reaction Network Theory

The Deficiency Zero Theorem and Deficiency One Theorem provide important results (Feinberg, 1979, 1987).

Definition 5. Let $q = \mathrm{rank}(Y A_\kappa)$. Then, the *deficiency* of a CRN is given by: $\delta = \dim A_\kappa - q - \ell = m - q - \ell \geq 0$.

The Deficiency Zero Theorem guarantees that, independently of parameter values, there exists a unique positive and stable equilibrium point if and only if the CRN is of deficiency zero and weakly reversible.

The Deficiency One Theorem guarantees that, independently of parameter values, there exists at most one positive equilibrium point if the deficiency of each individual linkage class is not greater than one and the deficiency of the entire network equals the sum of the deficiencies of the individual linkage classes. If the network is weakly reversible, the theorem also guarantees existence.

In the next section, we provide important results on weakly reversible CRNs, which extend Martin Feinberg's network analysis framework. Many relevant CRNs are weakly reversible (Gatermann and Huber, 2002). Importantly, our result is independent of parameter values and of the network deficiency.

3. BOUNDEDNESS OF WEAKLY REVERSIBLE CHEMICAL REACTION NETWORKS

The theorem presented in this section, is the main contribution of this paper. It proves boundedness for solutions of a weakly reversible CRN and the conjecture in (Feinberg, 1987) that weak reversibility implies persistence (and also the so called global attractor conjecture for deficiency zero networks). Note that it provides only sufficient conditions and one can construct a 'persistent' CRN that is not weakly reversible and whose solution trajectories are bounded; for instance, consider (1) and in addition a constant removal of P and a constant influx of S.

Theorem 6. If a CRN is weakly reversible then, for all positive initial conditions (i.e., $x(t_0) > 0$), its solution trajectories are bounded and remain in the positive orthant (i.e., there exist vectors $x_{\min}$ and $x_{\max}$ such that $0 < x_{\min} \leq x(t) \leq x_{\max} < \infty \ \forall t$).

Proof. Recall that solutions remain nonnegative if initial conditions are nonnegative. By Remark 4, any individual reaction is always part of a reaction cycle. Thus, (4) can be written as the sum over all S reaction cycles, where each cycle s consists of a subset of reactions $\mathfrak{R}_s$, $s = \{1, \ldots, S\}$:

$$\dot{x}_i = \sum_{s=1}^{S} \sum_{\mathfrak{R}_s} \frac{1}{\beta_{jl}} k_{j\to l} \Psi_j(x)(Y_{il} - Y_{ij}). \tag{5}$$

The constant $\beta_{jl} \in \mathbb{N}$ is the multiplicity of $k_{j\to l}$ in $\{\mathfrak{R}_1, \ldots, \mathfrak{R}_S\}$. For example, consider the network depicted in Figure 1a. It can be decomposed into two distinct cycles (Figure 1b). Moreover, reaction $1 \to 2$ is contained in both cycles, thus, the multiplicity of $k_{1\to 2}$ is two; that is, $\beta_{12} = 2$. The multiplicities of all other reactions are one.

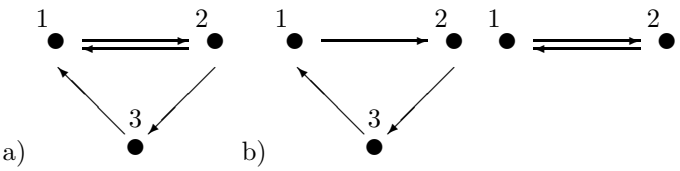

Fig. 1. The network in (a) can be decomposed into tow distinct cycles, which are shown in (b). The multiplicity of $k_{1\to 2}$ is two (i.e., $\beta_{12} = 2$). The multiplicities of all other reactions are one.

Remark 7. Note that $k_{j\to l} > 0$ for all j, l for which $k_{j\to l} \in \mathfrak{R}$ and it follows that $k_{j\to l}\Psi_j(x) > 0$ for all j, l for which $k_{j\to l} \in \mathfrak{R}$ if $x_i > 0$ for all i. (If $k_{j\to l} \notin \mathfrak{R}$ then $k_{j\to l} = 0$.)

Analogously to Kirchhoff's second law, for each reaction cycle s,

$$\sum_{\mathfrak{R}_s} \alpha^i_{jl} = 0, \ \alpha^i_{jl} = Y_{il} - Y_{ij}. \tag{6}$$

As an example, consider the hypothetical reaction cycle depicted in Figure 2. Then, $\sum_{j=1}^{4} \alpha^i_{jl} = Y_{i2} - Y_{i1} + Y_{i3} -$

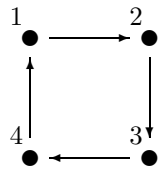

Fig. 2. The following holds for the reaction cycle: $\sum_{j=1}^{4} \alpha^i_{jl} = Y_{i2} - Y_{i1} + Y_{i3} - Y_{i2} + Y_{i4} - Y_{i3} + Y_{i1} - Y_{i4} = 0$, $l = j + 1$ and $l = 5$ corresponds to $l = 1$.

$Y_{i2} + Y_{i4} - Y_{i3} + Y_{i1} - Y_{i4} = 0$, $l = j + 1$ and $l = 5$ corresponds to $l = 1$. The equality in (6) implies:

(i) Either $\alpha^i_{jl} = 0$ for all j, l, which implies that $Y_{ij} = Y_{il}$, where j, l are such that $k_{j\to l} \in \mathfrak{R}_s$ and $j, l \in \{1, \ldots, m\}$. Moreover, if this holds for all S reaction cycles then $\dot{x}_i = 0$ for all t and $x_i(t) = x_i(t_0)$.
(ii) Or, there exists a Y_{ig} such that $k_{g\to l} \in \mathfrak{R}_s$, $Y_{ig} \geq Y_{il}$ for all l, and $Y_{ig} > Y_{il}$ for some l, $g, l \in \{1, \ldots, m\}$.

In the following, we divide the proof into three parts.

(I) We pick an i, $i \in \{1, \ldots, n\}$, let $x_i(t_0) > 0$ and assume that solutions of x_w are bounded from above and below by positive constants for all $w \neq i$, $w = 1, \ldots, n$. In other words, we assume that $0 < x_{w_{\min}} \leq x_w \leq x_{w_{\max}} < \infty$. Then, it follows from (ii) that there exist a Y_{iM}, where $M \in \{1, \ldots, m\}$, such that $k_{M\to l} \in \mathfrak{R}$, $Y_{iM} \geq Y_{il}$ for all l, and $Y_{iM} > Y_{il}$ for some l. This implies that there exists a positive constant $\hat{\gamma}$ such that

$$\text{if } x_i > \hat{\gamma} \text{ then } x_i^{Y_{iM}} \geq x_i^{Y_{il}} \ \forall l \text{ and } x_i^{Y_{iM}} > x_i^{Y_{il}} \text{ for some } l.$$

Recall that $\Psi_j(x) = \prod_{\wp=1}^{n} x_\wp^{Y_{\wp j}}$ and that $0 < x_{w_{\min}} \leq x_w \leq x_{w_{\max}} < \infty$ for all t and all w, $w \neq i$. Then, there exists a positive constant $\bar{\gamma}$ such that

$$\begin{aligned} &\text{if } x_i > \bar{\gamma} \text{ then } \Psi_M(x) \geq \Psi_l(x) \text{ for all } l \\ &\qquad \text{and } \Psi_M(x) > \Psi_l(x) \text{ for some } l. \end{aligned} \tag{7}$$

Note that $Y_{iM} > Y_{il}$ means that $\alpha^i_{Ml} < 0$ and it follows from Remark 7 that in this case

$$\frac{1}{\beta_{Ml}} k_{M\to l} \Psi_M(x) \alpha^i_{Ml} < 0 \text{ if } x_i > 0 \ \forall i. \tag{8}$$

Recall that $\dot{x}_i = \sum_{s=1}^{S} \sum_{\mathfrak{R}_s} \frac{1}{\beta_{jl}} k_{j\to l} \Psi_j(x) \alpha^i_{jl}$. Then, it follows from (7) and (8) that there exists a positive constant γ_1 such that $\dot{x}_i < 0$ if $x_i > \gamma_1$. Thus, there exists a $t_1 > t_0$ such that $x_i \leq \gamma_1$ for all $t > t_1$.

Using similar arguments leads to the conclusion that there exists a $Y_{i\tilde{M}}$ such that $k_{\tilde{M}\to l} \in \mathfrak{R}$, $Y_{i\tilde{M}} \leq Y_{il}$ for all l, and $Y_{i\tilde{M}} < Y_{il}$ for some l, where $l, \tilde{M} \in \{1, \ldots, m\}$. Hence, $\frac{1}{\beta_{\tilde{M}l}} k_{\tilde{M}\to l} \Psi_{\tilde{M}}(x) \alpha^i_{\tilde{M}l} > 0$ if $x_i > 0$ for all i; and there exists a positive constant $\tilde{\gamma}$ such that $x_i^{Y_{i\tilde{M}}} > x_i^{Y_{il}}$ if $x_i < \tilde{\gamma}$ and, thus, a positive constant $\bar{\gamma}$ such that $\Psi_{\tilde{M}}(x) < \Psi_l(x)$ if $x_i < \bar{\gamma}$. This implies that there exists a positive constant γ_2 such that $\dot{x}_i > 0$ if $0 < x_i < \gamma_2$ and $x_i > 0$ for all i. It follows that if $x_i(t_0) > 0$ then there exists a $t_2 > t_0$ such that $x_i \geq \gamma_2$ for all $t > t_2$. Hence, $\gamma_2 \leq x_i \leq \gamma_1$; or in other words, if $x_i(t_0) > 0$ for all i, and solutions of x_w are bounded from above and below then solutions of x are bounded from above and below.

(II) Now, we let $x_i(t_0) > 0$ and assume that solutions of x_w are bounded only from above; that is, we assume that for all t and all w, $w \neq i$: $0 \leq x_w \leq x_{w_{\max}} < \infty$. First, let there be an x_q such that $x_q(t_0) = 0$ and $\dot{x}_q = 0$, $q \neq i$; that is, we consider the behaviour on the boundaries. Note that, for all j, if $Y_{qj} \neq 0$ then $\Psi_j(x) = \prod_{\wp=1}^{n} x_\wp^{Y_{\wp j}} = 0$. It follows that for all j: $\Psi_j(x) Y_{qj} = 0$. Therefore,

$$\begin{aligned} \dot{x}_q &= \sum_{s=1}^{S} \sum_{\mathfrak{R}_s} \frac{1}{\beta_{jl}} k_{j\to l} \Psi_j(x)(Y_{ql} - Y_{qj}) \\ &= \sum_{s=1}^{S} \sum_{\mathfrak{R}_s} \frac{1}{\beta_{jl}} k_{j\to l} \Psi_j(x) Y_{ql}. \end{aligned} \tag{9}$$

It follows that $\Psi_j(x) Y_{ql} = 0$, since $\dot{x}_q = 0$. This implies that we can 'remove' all reaction cycles in which x_q participates. That is, for all s, $s = 1, \ldots, S$, if there exists a $k_{j\to l} \in \mathfrak{R}_s$ such that $Y_{qj} \neq 0$ then

$$\sum_{\mathfrak{R}_s} \frac{1}{\beta_{jl}} k_{j\to l} \Psi_j(x)(Y_{ql} - Y_{qj}) = 0.$$

We assume that some reaction cycles remain in which x_i participates; otherwise, $\dot{x}_i = 0$ for all t and thus, $x_i = x_i(t_0) > 0$. Without loss of generality, we denote the remaining cycles by $1, \ldots, \tilde{S} < S$. It follows that

$$\dot{x}_i = \sum_{s=1}^{\tilde{S}<S} \sum_{\mathfrak{R}_s} \frac{1}{\beta_{op}} k_{o\to p} \Psi_o(x)(Y_{ip} - Y_{io}),\ \Psi_o(x) > 0.$$

Thus, it follows from **(I)** that if $x_q = 0$ and $0 < x_w \leq x_{w_{\max}} < \infty$ for all t, w, q such that $q \neq i$ and $w \neq i$, and $0 < x_i(t_0)$ then there exist positive constants γ_1 and γ_2 and time instants $t_1 \geq t_0$ and $t_2 \geq t_0$ such that if $t > \max\{t_1, t_2\}$ then $\gamma_2 \leq x_i \leq \gamma_1$. It follows from continuity of solutions that if $0 \leq x_w \leq x_{w_{\max}} < \infty$ for all t, $w \neq i$, and $0 < x_i(t_0)$ then there exist positive constants γ_1 and γ_2 and time instants $t_1 \geq t_0$ and $t_2 \geq t_0$ such that if $t > \max\{t_1, t_2\}$ then $\gamma_2 \leq x_i \leq \gamma_1$. Since, for all nonnegative initial conditions, solutions will remain in the nonnegative orthant, this implies that there exist a positive vector $x_{\min}$ and a time instant t^* such that

$$\text{if } x(t_0) > 0 \text{ and } x \leq x_{\max} \text{ then } x \geq x_{\min}\ \forall\ t > t^*, \quad (10)$$

where $x_{\max}$ is a positive vector; that is, for positive initial conditions, if all solutions are bounded from above then they are also bounded from below. Therefore, it remains to show that all solutions are indeed bounded from above.

(III) We let $x_i(t_0) > 0$, $x_r(t_0) > 0$ and, for all w, $w \neq \{i, r\}$: $0 \leq x_w \leq x_{w_{\max}} < \infty$. It follows from (6) that for each reaction cycle s, $\sum_{\mathfrak{R}_s} \alpha^i_{jl} = 0$, $\sum_{\mathfrak{R}_s} \alpha^r_{jl} = 0$ and, hence,

$$\sum_{\mathfrak{R}_s} (\alpha^i_{jl} + \alpha^r_{jl}) = \sum_{\mathfrak{R}_s} (Y_{il} + Y_{rl} - Y_{ij} - Y_{rj}) = 0.$$

Then, either $(\alpha^i_{jl} + \alpha^r_{jl}) = 0$ for all S reaction cycles, which implies that $x_i + x_r = \gamma_2$ for all t, where γ_2 is a positive constant, or there exist Y_{iN} and Y_{rN}, where $N \in \{1, \ldots, m\}$, such that $k_{N\to l} \in \mathfrak{R}$, $Y_{iN} + Y_{rN} \geq Y_{il} + Y_{rl}$ holds for all l, and $Y_{iN} + Y_{rN} > Y_{il} + Y_{rl}$ holds for some l, $l \in \{1, \ldots, m\}$. It follows that there exists a constant $\hat{\gamma} > 0$ such that if $x_i x_r > \hat{\gamma}$ and $x_i > 0\ \forall i$ then

$$x_i^{Y_{iN}} x_r^{Y_{rN}} \geq x_i^{Y_{il}} x_r^{Y_{rl}} \text{ for all } l$$
$$\text{and } x_i^{Y_{iN}} x_r^{Y_{rN}} > x_i^{Y_{il}} x_r^{Y_{rl}} \text{ for some } l;$$

a constant $\bar{\gamma} > 0$ such that if $x_i x_r > \bar{\gamma}$ and $x_i > 0\ \forall i$ then $\Psi_N(x) \geq \Psi_l(x)$ for all l, and $\Psi_N(x) > \Psi_l(x)$ for some l; and moreover, that in the latter case for some l

$$\frac{1}{\beta_{Nl}} k_{N\to l} \Psi_N(x)(\alpha^i_{Nl} + \alpha^r_{Nl}) < 0 \text{ if } x_i > 0\ \forall i.$$

This implies that there exists a constant $\gamma^* > 0$ such that

$$\dot{x}_i + \dot{x}_r < 0 \text{ if } x_i x_r > \gamma^* \text{ and } x_i > 0\ \forall i. \quad (11)$$

As in **(II)**, it follows that the first inequality in (11) holds even if one (or more) $x_q = 0$, $\dot{x}_q = 0$, $q \neq \{i, r\}$, and thus, by continuity of solutions that

$$\dot{x}_i + \dot{x}_r \leq 0 \text{ if } x_i x_r > \gamma^* \text{ and } x_i \geq 0\ \forall i. \quad (12)$$

Without loss of generality, $\gamma^* \geq x_i(t_0) x_r(t_0)$.

Note that (12) implies that $x_i x_r \leq \gamma^*$ and then at any point of time either $x_i \leq \sqrt{\gamma^*}$ or $x_r \leq \sqrt{\gamma^*}$ and it follows from **(I)** and **(II)** that the value of the other specie is bounded from above by a positive constant, which we denote by $\gamma^\star$, $\gamma^\star > 0$. This implies that the sum $x_i + x_r$ is bounded from above by $\sqrt{\gamma^*} + \gamma^\star$. Thus, x_i and x_r are bounded from above and it follows from the conclusion of **(II)** that also from below. Finally, by induction we can extend above results to all x_i if $x_i(t_0) > 0$, $i = 1, \ldots, n$. Thus, there exist positive constants $\gamma_{\min}$, $\gamma_{\max}$ and a time instant t^* such that for all i, $\gamma_{\min} \leq x_i \leq \gamma_{\max}$ for all $t > t^*$ if $x(t_0) > 0$, which completes the proof. □

Remark 8. Theorem 6 together with the Brouwer Fixed Point Theorem implies that a weakly reversible CRN possesses at least one positive equilibrium point.

Remark 9. The significance of Theorem 6 and Remark 8 lies in the fact that existence of solutions and boundedness of solution trajectories are often an important requirements for the analysis of dynamical systems. Particularly, in the case of large systems checking for strong connectivity of the underlying graph can be performed automatically and efficiently as opposed to alternative means that establish boundedness of solution trajectories – for example, via Lyapunov stability theory.

Remark 10. Since Theorem 6 guarantees boundedness of solutions, instability of all equilibria of a weakly reversible CRN guarantees non-diverging oscillatory behaviour; that is, it guarantees non-diverging periodic, quasi-periodic or chaotic behaviour.

Remark 11. **('Hidden' weak reversibility)** Let $A_{\kappa,add} \in \mathcal{N}(Y)$ (that is, $YA_{\kappa,add} = 0$) and $A_{\kappa,c} = A_\kappa + A_{\kappa,add}$. Then, $\dot{x} = YA_\kappa \Psi(x) = YA_{\kappa,c}\Psi(x)$. If $-A_{\kappa,c}$ is an *M-matrix* with zero column sum and irreducible or can be block-diagonalised and each block-diagonal submatrix is irreducible (or empty) then the CRN is weakly reversible. An M-matrix is a matrix with positive diagonal entries and non-positive off-diagonal entries. Moreover, all principal minors are nonnegative (Berman and Plemmons, 1979). In other words, certain dynamical systems arising from a CRN that is not weakly reversible can be represented through a weakly reversible one. In this case, Theorem 6 guarantees persistence and boundedness of their solutions.

4. EXAMPLES FROM BIOLOGY AND CHEMISTRY

4.1 An active membrane transport model

Figure 3 shows a simple model consisting of elementary steps for an active transport system that carries molecules across the cell membrane. The corresponding CRN has the following form (Vieira and Bisch, 1994):

$$A + X' \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} B, \quad B + ATP \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} C + ADP$$

$$C \underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} D \underset{k_{-4}}{\overset{k_4}{\rightleftarrows}} E + X'', \quad E \underset{k_{-5}}{\overset{k_5}{\rightleftarrows}} F + P, \quad F \underset{k_{-6}}{\overset{k_6}{\rightleftarrows}} A$$

In (Vieira and Bisch, 1994), the concentrations of ligand molecules (X), of ATP, ADP (which drive the transport (pumping) through ATP hydrolysis) and P (inorganic phosphate involved in the phosphorylation-dephosphorylation reactions) are assumed to be externally controlled parameters. This leads to a CRN that is (weakly) reversible and to a mathematical model that consists of a set of linear ordinary differential equations. In order to simulate dynamic cooperativity, an extra autocatalytic reaction of the following form is added: $A + F \underset{k_{-7}}{\overset{k_7}{\rightleftarrows}} 2A$. This addition results in a mathematical model with a nonlinearity ([·] denotes concentration):

$$
\begin{aligned}
[\dot{A}] &= -(k_1 + k_{-6})[A] + k_{-1}[B] + k_6[F] + k_7[A][F] - k_{-7}[A]^2,\\
[\dot{B}] &= -(k_2 + k_{-1})[B] + k_{-2}[C] + k_1[A],\\
[\dot{C}] &= -(k_3 + k_{-2})[C] + k_{-3}[D] + k_2[B],\\
[\dot{D}] &= -(k_4 + k_{-3})[D] + k_{-4}[E] + k_3[C],\\
[\dot{E}] &= -(k_5 + k_{-4})[E] + k_{-5}[F] + k_4[D],\\
[\dot{F}] &= -(k_6 + k_{-5})[F] + k_{-6}[A] + k_5[E] - k_7[A][F] + k_{-7}[A]^2.
\end{aligned}
$$

The CRN is of deficiency one. This implies that the corresponding dynamical system has a unique positive equilibrium point independently of parameter values. By Remark 10, instability of the fixed point, which can be checked by evaluating the eigenvalues of the Jacobian of the system, necessarily leads to oscillatory behaviour for positive initial conditions. E.g., the Jacobian is unstable at the fixed point for the following parameter values: $k_1 = 500000$, $k_2 = \cdots = k_5 = 1000$, $k_6 = 3 \cdot 10^{-5}$, $k_7 = 5000$, $k_{-1} = 0.01$, $k_{-2} = k_{-3} = k_{-4} = 0.003$, $k_{-5} = 3 \cdot 10^{-5}$, $k_{-6} = 1.5$, $k_{-7} = 750$, and total concentrations of 104.

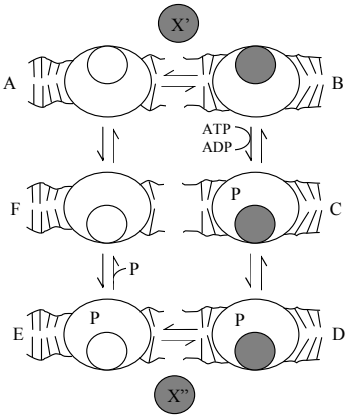

Fig. 3. **Active membrane transport model.** Molecules are actively carried across the cell membrane. An extracellular molecule, X', binds to a free transporter molecule in the membrane: $A + X' \rightleftharpoons B$. ATP hydrolysis ($B + ATP \rightleftharpoons C + ADP$) drives the transport of the molecule into the internal medium, where it is released (X''): $C \rightleftharpoons D \rightleftharpoons E + X''$. Then, the empty binding site is prepared to repeat the cycle through the de/phosphorylation reaction $E \rightleftharpoons F + P$, and the reaction $F \rightleftharpoons A$ (Vieira and Bisch, 1994).

4.2 A Lotka-Volterra system

The Lotka-Volterra equations, or the predator-prey equations, are used to describe the dynamics of biological systems in which different species interact, of which some are the prey of others. We consider the three species Lotka-Volterra system from (May and Leonard, 1975). The 'concentrations' of the three species are denoted by x_i, $i = 1, 2, 3$. The terms $k_1 x_i$ and $k_2 x_i^2$ denote birth and death rate respectively. In accordance with (May and Leonard, 1975), we set $k_1 - d = k_2 = 1$. Parameters $a > 0$ and $b > 0$ denote competition between the different species. We add to this model migration terms into and out of the habitat; the former is constant and given by $u > 0$, and the latter is proportional to the population strength and given by dx_i, $d > 0$. The mathematical model of the system has the following form:

$$
\begin{aligned}
\dot{x}_1 &= u + x_1(k_1 - d - k_2 x_1 - a x_2 - b x_3),\\
\dot{x}_2 &= u + x_2(k_1 - d - b x_1 - k_2 x_2 - a x_3),\\
\dot{x}_3 &= u + x_3(k_1 - d - a x_1 - b x_2 - k_2 x_3).
\end{aligned} \tag{13}
$$

In the following, we will construct a CRN that corresponds to (13). Note that there exists more than one valid representation. In Figure 4a, the 'biologically sensible' realisation is depicted; that is, migration of the different species is independent of each other. Although this representation is not weakly reversible, the CRN has a 'hidden' weakly reversible structure (Remark 11). A weakly reversible CRN which corresponds to (13) is shown in Figure 4b. Here, we assume that some migration occurs in pairs, where the pairs consist of members from different species.

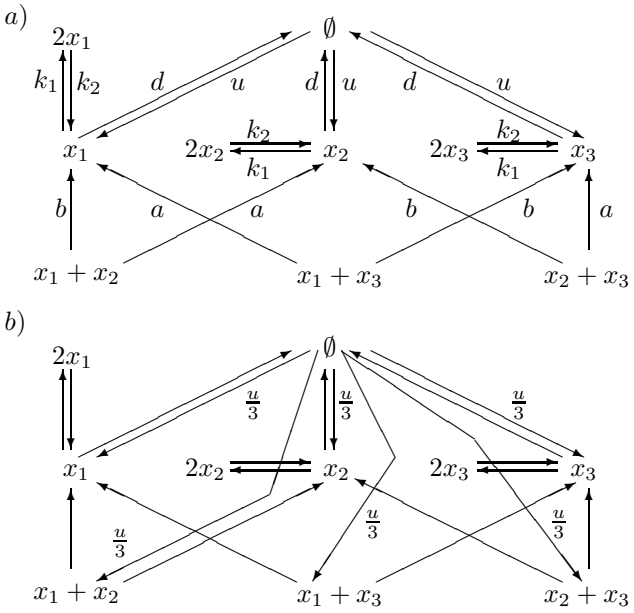

Fig. 4. **Two representations of (13).** Note that the network in (b) is weakly reversible. (The symbol $\emptyset$ represents the null-complex; it functions as a source and a sink for the system.) For clarity, we omitted the reaction rates in (b) that are identical in (a).

Now, consider $\dot{x}_1 = 0$: Since $0 = u + x_1 - x_1^2 - a x_1 x_2 - b x_1 x_3$, Descartes' rule of signs implies that there exists a unique positive equilibrium point for x_1 (and analogously for x_2 and x_3). It is given by $x_{\mathrm{eq}_i} = \frac{1+\sqrt{1+4u(1+a+b)}}{2(1+a+b)}$ for $i = 1, 2, 3$. The Jacobian of (13) is a *circulant matrix*, its eigenvalues are $\lambda_1 = -\sqrt{1 + 4u(1 + a + b)}$ and $\lambda_{2,3} = 1 - (2 + 0.5a + 0.5b)x_{\mathrm{eq}_i} \pm 0.5\sqrt{3}(a - b)x_{\mathrm{eq}_i}$ (see (May and Leonard, 1975) and the references therein). After some algebraic manipulations, we obtain that the Jacobian is unstable if $9\left(\frac{a+b}{4+a+b}\right)^2 > 1 + 4u(1 + a + b)$. Since (13) has a weakly reversible CRN associated with it, instability of the unique fixed point necessarily leads to oscillatory behaviour. Using numerical methods, we find only periodic limit cycles for different parameter values even for small values of u while it was shown in (May and Leonard, 1975) that for $u = 0$, $a = 0.8$ and $b = 1.3$ nonperiodic oscillations exist. This gives rise to the question whether weakly reversibility excludes nonperiodic oscillatory behaviour.

Finally, note that boundedness of solutions for (13) can also be established via the Lyapunov function $V(x) = \sum_{i=1}^{3} x_i$, since $\dot{V}(x) < 0$ for large values of $\sum_{i=1}^{3} x_i$. (Recall that $x_i \geq 0$.) In the next example, we will provide an example of a relatively simple CRN that is weakly reversible but for which a Lyapunov function that proves boundedness of solutions cannot be readily found, which highlights the significance of Theorem 6.

4.3 A chemical oscillator

The CRN in Figure 5 is weakly reversible and of deficiency 1. Thus, the corresponding dynamical system has a unique positive equilibrium point independently of parameter values. The set of ODEs describing the system is:

$$\begin{aligned}
\dot{x}_1 &= \alpha x_1 - c x_1 - b x_1^2 + (d+\varepsilon) x_3 - g x_1 x_2, \\
\dot{x}_2 &= -h x_2 + (k+\varepsilon) x_3 - g x_1 x_2, \\
\dot{x}_3 &= -(d+k+\varepsilon) x_3 + h x_2 + c x_1 + g x_1 x_2. \qquad (14)
\end{aligned}$$

By inspection, there is only one additional nonnegative equilibrium point, which is the origin and unstable for all parameter values. This follows from applying the Routh-Hurwitz criterion (see (Murray, 1990)).

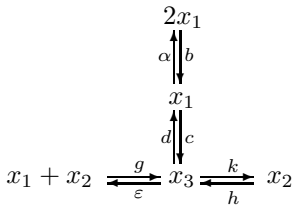

Fig. 5. **A chemical oscillator.** This CRN (from (Feinberg, 1979)) is weakly reversible and of deficiency one.

Note that a Lyapunov function that proves boundedness of solutions cannot be easily found for the dynamical system given by (14). However, it follows from Theorem 6 that solutions of (14) are bounded; thus, if the positive equilibrium is unstable then the system exhibits oscillatory behaviour. Using the Routh-Hurwitz criterion the parameter space can be easily explored. E.g., if $\alpha = 100$, $b, c, d = 0.1$, $g = 1$, $h = 1$ and $k = 100$, we observe periodic oscillatory behaviour for $\varepsilon < 354$ (see Figure 6).

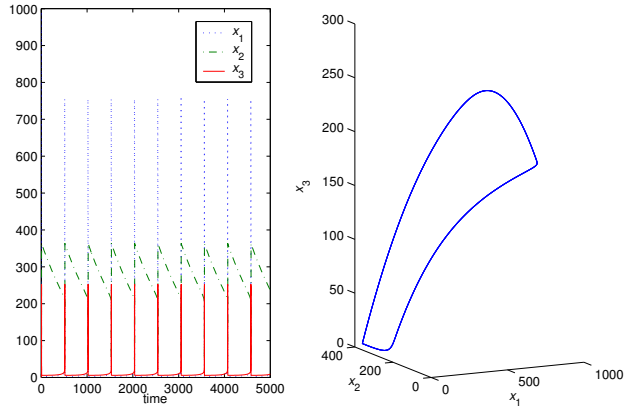

Fig. 6. **Time series and limit cycle of (14).** $\varepsilon = 100$.

5. CONCLUSION

In this paper, we showed that, for positive initial conditions, solutions of a weakly reversible CRN are bounded and remain in the positive orthant. Hence, we proved Martin Feinberg's conjecture that weak reversibility implies persistence, an important and, thus, desired property of biological systems. Moreover, when dealing with dynamical systems that have multiple equilibrium points and/or limit cycles it is often of great interest to find attracting sets or prove their existence. By checking structural properties of the graph of the CRN, our result provides a qualitative criterion, which is completely independent of parameter values, for the existence of such a set. The result can also be used to characterise certain bifurcations from stationary to oscillatory behaviour as exemplified in Section 4. Finally, since we observed only periodic behaviour for weakly reversible CRNs, an interesting question is whether weak reversibility excludes nonperiodic oscillations.

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