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Existence of positive steady states for mass-conserving and mass-action weakly reversible chemical reaction networks with a single linkage class

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Abstract We establish that mass-conserving weakly reversible chemical reaction networks formed by a single linkage class admit positive steady states, regardless of network deficiency and choice of reaction rate constants. This result holds for closed systems (those without material exchange across the boundary) as well as for open systems with material exchange at rates that satisfy a simple necessary and sufficient condition. Our proof uses a convex analysis formulation to define a mapping for which a positive fixed point is shown to exist. We then use the existence of the fixed point to establish the existence of a positive steady state.

The proof inspires the definition of a practical algorithm to find these steady states. The same algorithm has been consistently successful at finding positive fixed points of weakly reversible networks with *multiple* linkage classes. We report numerical experiments.

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1 Introduction

Chemical reaction network theory (CRNT) studies the behavior of ensembles of chemical reactions. One of the models considered by CRNT assumes that the rates of reaction follow mass action kinetics [7]. The behavior of these systems depends on kinetic parameters. However, measuring these parameters experimentally is difficult and error-prone. Thus, we seek properties of chemical reaction networks that are independent of them.

In this work, we address the issue of existence of positive steady states, i.e., positive concentrations of species that will stay constant under the system's dynamics. In particular, we tackle the case in which a directed graph of chemical reactions forms a *strongly connected component*, and the reactions conserve mass. We prove the existence of positive equilibria for closed networks (those without material exchange across their boundary), and extend our methods to open systems where species are exchanged across the boundary at certain rates. How best to compute such steady states remains uncertain; however, we suggest a fixed point algorithm and provide results of our numerical experiments for several cases.

We build on the work of Fleming et al. [5], where the authors address the case of ensembles of reversible reactions and find thermodynamically feasible mass-conserving fluxes by solving a linearly constrained convex optimization problem. We extend this work by tackling the case of weakly reversible networks and finding fluxes that are not only mass-conserving and thermodynamically feasible but also satisfy mass-action kinetics and are thus steady states of a dynamical system governed by mass-action kinetics.

We wish to highlight the differences between our work and that of Deng et al. [2]. Their work proves the existence of positive steady states for weakly reversible networks regardless of the number of connected components. In this sense their proof is more general. However, we also address the case when the rates of material exchange across the system's boundary are prescribed from experimental observations and show that for weakly reversible chemical reaction networks with a single linkage class, there exists a positive steady state if the exchange rate satisfies a necessary and sufficient condition. Furthermore, we define a practical algorithm to find steady states of the system.

1.1 Organization of the paper

Rather than trying to review existing results in the area, we direct the interested reader to [6,7] and the work by Feinberg et al. [3,4]. In section 2 we establish the notation and define the CRNT model that we address. In section

3 we cover our proof, and in section 4 we define the algorithm, evaluate its behavior, and make some final remarks.

2 A Model of Chemical Reaction Networks Driven by Mass-Action Kinetics

We model an ensemble of chemical reactions where the the rates of reaction are determined by mass-action kinetics. Our model differs from the traditional continuous-flow stirred tank reactor (CSTR) in that the outflow is not expected to be a mixture in the same proportions as the reactor's contents. Instead, we allow for entrapped species as in [1] and furthermore, arbitrary outflow rates of any species. In the traditional sense, this can be viewed as a network of CSTRs in series/parallel with recycle and separation. The motivation for this choice is the assumption that there can be an unknown active mechanism extracting species at a particular rate —for example active transport across a cell membrane—, and an experimenter observes both the inflow and outflow rates of the system and wishes to determine internal concentrations that would define a steady state with this observed material exchange rate. We now proceed to define the model mathematically.

A reaction network is represented by a weighted directed graph $G(V, E)$, where each node in V represents a complex, each directed edge $i \to j$ denotes a reaction using i to generate j, and the positive edge weight $k_{i\to j}$ is the reaction rate. The matrix $A \in \mathbb{R}^{n\times n}$ is the weighted adjacency matrix of the graph, where $A_{ij} = k_{i\to j}$. Define $D := \mathrm{diag}(A\mathbf{1})$, where $\mathbf{1}$ is the vector of all ones, and $K := A^T - D$. The elements of this new matrix will be $(K)_{ij} = k_{j\to i}$ for $i \neq j$, and $(K)_{ii} = -\sum_j k_{j\to i}$, so that $K^T\mathbf{1} = \mathbf{0}$. We assume that each complex is used in a reaction. Hence A will have no empty rows and D will be a positive-definite diagonal matrix.

Let $c \in \mathbb{R}^m$ be the vector of concentrations of each species, and $b \in \mathbb{R}^m$ the vector of species' exchange rates across the network boundary.

We define $\psi(c) : \mathbb{R}^m_+ \to \mathbb{R}^n$ to be a nonlinear function that captures mass-action kinetics:

$$\psi_j(c) = \prod_i c_i^{Y_{ij}},$$

where Y_{ij} is the stoichiometric coefficient of species i in complex j. The change in concentration over time can be described by the system of ordinary differential equations

$$\dot{c} = YK\psi(c) - b.$$

Hence, steady state concentrations for a chemical reaction network are any non-negative vector $c^\star \in \mathbb{R}^m$ such that $YK\psi(c^\star) = b$. Equivalently, a pair $(c^\star, v^\star)$ with $c^\star \in \mathbb{R}^m$ and $v^\star \in \mathbb{R}^n$ will be a steady state if it satisfies the conditions

$$YKv^\star = b \tag{FB}$$

$$\psi(c^\star) = v^\star \tag{MA}$$

or *flux-balance* and *mass-action*, respectively. Thus, finding steady state concentrations is equivalent to finding a vector $v^\star$ of potentials that satisfies both (FB) and (MA) for some vector of concentrations $c^\star \in \mathbb{R}^m$.

Observe that if $(c^\star, v^\star)$ is a positive steady state, then from the definition of $\psi(c^\star)$,

$$Y^T \log(c^\star) = \log(\psi(c^\star)) = \log(v^\star). \tag{MA-log}$$

We refer to this alternative condition as the logarithmic form of the mass-action condition (MA).

Definition 1 A chemical reaction network is *mass-conserving* if and only if there exists a positive vector $e \in \mathbb{R}^m$ such that

$$e^T Y K = 0, \tag{1}$$

where e denotes the molecular weights of the species (or atomic weights if the species are elements).

Definition 2 A chemical reaction network is *weakly reversible* if for any complex i there exists a directed path in the graph G that loops from i to itself.

Definition 3 A *linkage class* is a set of complexes $\mathcal{L}$ such that for any pair of complexes $(i, j) \in \mathcal{L}$ there exists a directed path in G from i to j.

From these definitions one can deduce that the complexes of a weakly reversible reaction network must partition into linkage classes.

3 A fixed-point model

Our main result establishes that for any set of positive reaction rates $k \in \mathbb{R}^{|E|}$ and any b in the range of YK, a weakly reversible network with a single linkage class will admit a positive solution pair (c, v) that satisfies the laws (FB) and (MA).

We construct a linearly constrained convex optimization problem such that the logarithmic form of the mass-action equation (MA-log) is an optimality condition, so that any solution to this optimization problem will also satisfy (MA-log).

We use the existence and uniqueness of the solution of the convex problem to define a mapping as follows: Let $b = YK\eta$ for some $\eta \in \mathbb{R}^n$ and observe that for arbitrary $s \in \mathbb{R}^n$ we can write $b = YA^T(\eta + s) - YD(\eta + D^{-1}A^T s)$. In particular, we choose s positive and large enough so that $\eta^+ := \eta + s$ and $\eta^- := \eta + D^{-1}A^T s$ are both positive. Also, from Definition 1, $e^T b = 0$ and thus

$$e^T Y D \eta^- = e^T Y A^T \eta^+. \tag{2}$$

Define $\mu := (r, r_0) \in \mathbb{R}^{m+1}$ to be a vector parameter. Observe that if the parametric convex optimization problem

$$\begin{aligned} \underset{(v,v_0)\in\mathbb{R}^{n+1}}{\text{minimize}} \quad & v^T D(\log(v) - \mathbf{1}) + v_0(\log v_0 - 1) \\ \text{subject to} \quad & YDv + YA^T\eta^+ v_0 = YA^T r + YD\eta^- r_0 : \ y \\ & (v, v_0) \geq 0 \end{aligned} \tag{P0}$$

has a positive solution $(v^\star(\mu), v_0^\star(\mu))$, then the optimality conditions

$$YDv^\star(\mu) + YA^T\eta^+ v_0^\star = YA^T r + YD\eta^- r_0 \tag{3a}$$

$$DY^T y^\star(\mu) = D\log(v^\star(\mu)) \tag{3b}$$

$$(YA^T\eta^+)^T y^\star(\mu) = \log(v_0^\star(\mu)) \tag{3c}$$

$$(v^\star(\mu), v_0^\star(\mu)) \geq 0 \tag{3d}$$

are well defined. Since D is nonsingular, (3b) is equivalent to (MA-log) with $c^\star(\mu) := e^{y^\star(\mu)}$, where the exponential is evaluated element-wise. Hence, equation (MA-log) holds and $c^\star(\mu)$ satisfies mass-action. Note that (P0) is strictly convex, so for any feasible $\mu := (r, r_0)$ there is a unique minimizer. That is, the mapping

$$\mu = (r, r_0) \to (v^\star(\mu), v_0^\star(\mu)) \tag{4}$$

is well defined.

If $\hat{\mu} = (r, r_0)$ is a fixed point of (4), then (3a) implies

$$YDv^\star(\hat{\mu}) + YA^T\eta^+ v_0^\star(\hat{\mu}) = YA^T v^\star(\hat{\mu}) + YD\eta^- v_0^\star(\hat{\mu})$$

or equivalently

$$YKv^\star(\hat{\mu}) = Y(A^T - D)v^\star(\hat{\mu}) = v_0^\star(YA^T\eta^+ - YD\eta^-) = v_0^\star(\hat{\mu})b.$$

Therefore, if such a fixed point exists, the solution $v^\star(\hat{\mu})$ at this fixed point will satisfy

$$YKv^\star = v_0^\star b. \tag{5}$$

This implies that for $b = 0$, both (FB) and (MA-log) are satisfied, and $c^\star$ defines a positive steady state of the system. For the case where $b \neq 0$, we will show how to construct a corresponding solution so that (FB) holds.

For simplicity, we henceforth use $(v^\star, v_0^\star)$ and $y^\star$ to denote the optimal primal and dual solution, but acknowledge their dependence on μ.

Theorem 1 *For any mass-conserving, mass-action chemical reaction network and any choice of rate constants $k > 0$, there exist nontrivial fixed points for the mapping* (4)*.*

Proof Brouwer's fixed-point theorem states that any continuous mapping from a convex and compact subset of a Euclidean space Ω to itself must have at least one fixed point.

Let $(v^\star, v_0^\star)$ be defined as in (3) and let γ be a positive fixed scalar. Define the set

$$\Omega = \left\{(v, v_0) \in \Re^{n+1} \ : \ (v, v_0) \geq 0, \quad e^T Y D v + e^T Y A^T \eta^+ v_0 = \gamma\right\},$$

where e is defined in (1). By Brouwer's fixed-point theorem, if the parameter $(r, r_0) \in \Omega$ ensures that the corresponding optimal solution $(v^\star, v_0^\star) \in \Omega$, then there is a fixed point such that the parameter and solution are equal, i.e., there exists a μ such that $\mu = (r, r_0) = (v^\star(\mu), v_0^\star(\mu))$.

The set Ω is bounded and formed by an intersection of closed convex sets, and hence is convex and compact. Moreover, the mapping $\mu \to (v^\star, v_0^\star)$ is continuous. Since problem (P0) is feasible for any $\mu \in \Omega$, the mapping $\Omega \ni \mu \to (v^\star, v_0^\star)$ is well defined.

To show that the image of Ω under the mapping $(r, r_0) \to (v^\star, v_0^\star)$ is in Ω, first observe that by the bounds in (P0), $(v^\star, v_0^\star) \geq 0$. Using (2), (3a) and Definition 1 we have

$$\begin{aligned} e^T Y D v^\star + e^T Y A^T \eta^+ v_0^\star &= e^T Y A^T r + e^T Y D \eta^- r_0 \\ &= e^T Y D r + e^T Y A^T \eta^+ r_0 = \gamma, \end{aligned}$$

and thus $(v^\star, v_0^\star) \in \Omega$. Therefore, under the mapping $(r, r_0) \to (v^\star, v_0^\star)$, $(r, r_0) \in \Omega$ implies $(v^\star, v_0^\star) \in \Omega$, and the mapping must have a fixed point. Moreover, since Ω does not contain the zero vector, the fixed point(s) are non-trivial. □

Note that the value of $YDv^\star + YA^T\eta^+ v_0^\star$ is the rate of consumption of each chemical species and $YA^T v^\star + YD\eta^- v_0^\star$ is the rate of production of each chemical species. At the fixed point, the equality $YDv^\star + YA^T\eta^+ v_0^\star = YA^T v^\star + YD\eta^- v_0^\star$ defines a steady state. The set Ω defines the parameter $\gamma = e^T(YDv^\star + YA^T\eta^+ v_0^\star)$, and since the vector e can be interpreted as an assignment of relative mass to the species, γ can be interpreted as the total amount of mass that reacts per unit time at the steady state. Therefore, looking for fixed points in Ω corresponds to looking for steady states where the amount of mass that reacts in the system is prescribed.

We have established the existence of a nontrivial fixed point μ of the mapping $\Omega \ni \mu \to (v^\star, v_0^\star) \in \Omega$. Moreover, we have shown that when the associated minimizer $(v^\star, v_0^\star)$ is positive, it is a solution to (MA) and to $YKv^\star = v_0^\star b$. However, in the case when some entries of $v^\star$ are zero, the objective function of (P0) is non-differentiable and we cannot use the optimality conditions to show that (MA) holds.

3.1 Positive fixed points in weakly reversible single linkage networks

We now consider the case when the network is weakly reversible and formed by a single linkage class and show that if $\hat{\mu}$ is a fixed point of the mapping (4), the minimizer $(v^\star(\hat{\mu}), v_0^\star(\hat{\mu}))$, and therefore $\hat{\mu}$, is positive.

Lemma 1 below shows that if problem (P0) has a feasible point with support J, the minimizer $(v^\star, v_0^\star)$ will have support at least J. Lemma 2 uses the single linkage class hypothesis to show that at a fixed point, there is a positive feasible point. These two Lemmas imply that at a fixed point $\hat{\mu}$, the minimizer will be positive. Finally, Theorem 2 shows that if $\hat{v}_0 \neq 1$ at the solution, we can construct another solution for which $\hat{v}_0 = 1$. This establishes that there is a nontrivial steady state for the network.

To complete the argument we must prove Lemmas 1, 2 and Theorem 2.

Lemma 1 *The support of any feasible point of problem* (P0) *is a subset of the support of the minimizer* $(v^\star, v_0^\star)$.

Proof Let $\tilde{v} \in \Re^{n+1}$ be any of the feasible points with the largest support and let z be any feasible direction at $\tilde{v}$. By construction, for all α in some interval $[\ell, u]$ the points $v_\alpha := \tilde{v} + \alpha z$ are non-negative and feasible. The interval can be chosen so that when $\alpha = l$ and when $\alpha = u$, one new bound constraint becomes active. This implies that $\text{supp}(v_\ell)$ and $\text{supp}(v_u)$ are strictly contained in $\text{supp}(\tilde{v})$, and $\text{supp}(v_\alpha) = \text{supp}(\tilde{v})$ for $\alpha \in (\ell, u)$.

Without loss of generality, we assume $\ell < 0 < u$, since ℓ and u will not be of the same sign; if $\ell = 0$ and $u > 0$, any point v_α can be written as a convex combination of $\tilde{v}$ and $\tilde{v} + uz$, and thus has support as large as $\tilde{v}$.

Define the univariate function

$$g(\alpha) := \phi(\tilde{v} + \alpha z), \tag{6}$$

where ϕ is the objective function of (P0). We will establish that as $\alpha \to l$ the derivative $g'(\alpha) \to -\infty$, and as $\alpha \to u$ the derivative $g'(\alpha) \to \infty$. Thus, by the mean value theorem, there must exist a zero of the function g in the interior of the interval $[l, u]$. Since this function is strictly convex, if a stationary point exists in the interior of the interval, the function value at the stationary point must be smaller than at the boundary.

Observe that if we let d_i, for $i \in [1, \ldots n]$, be the diagonal entries of D and $d_{n+1} = 1$, we can write

$$g(\alpha) = \sum_{i=1}^{n+1} (\tilde{v} + \alpha z_i) d_i \log(\tilde{v}_i + \alpha z_i).$$

An important observation is that if some entry $\tilde{v}_j = 0$ then $z_j = 0$, otherwise v_α would have a larger support for some $\alpha \neq 0$. This implies that $(v_\alpha)_j = 0$ for all entries where $\tilde{v}_j = 0$. If we let J be the set of nonzero entries of $\tilde{v}$, and L be the subset of J formed by the entries that tend to zero as $\alpha \to l$, then

$$\begin{aligned} g'(\alpha) &= \sum_{i \in J} z_i d_i (\log(\tilde{v}_i + \alpha z_i)) \\ &= \sum_{i \in (L^c \cap J)} z_i d_i (\log(\tilde{v}_i + \alpha z_i)) + \sum_{i \in L} z_i d_i (\log(\tilde{v}_i + \alpha z_i)). \end{aligned}$$

As $\alpha \to l$, the first summation will approach a finite value. Since $z_i > 0$ for all $i \in L$, the entries in the logarithm of the second sum tend to zero and the term will diverge to $-\infty$.

Similarly, let U be the subset of J formed by the entries that tend to zero as $\alpha \to u$. Observe that for these entries, $z_i < 0$ and

$$g'(\alpha) = \sum_{i \in (U^c \cap J)} z_i d_i(\log(\tilde{v}_i + \alpha z_i)) + \sum_{i \in U} z_i d_i(\log(\tilde{v}_i + \alpha z_i)).$$

The first sum will tend to a finite value and the second will diverge to ∞.

Now, assume that for some μ there is a feasible point $(\tilde{v}, \tilde{v_0})$ with larger support than the minimizer $(v^\star, v_0^\star)$ of problem (P0). Since $(v^\star, v_0^\star)$ has smaller support, we can write $(v^\star, v_0^\star) = (\tilde{v}, \tilde{v}_0) + \alpha^\star z$ where $\alpha^\star$ is on the boundary of the corresponding feasible interval. By the previous argument, there is a value of $\hat{\alpha} \neq \alpha^\star$ in the interior of the interval such that $(v^\star, v_0^\star) = (\tilde{v}, \tilde{v}_0) + \hat{\alpha} z$ has a lower function value than $(v^\star, v_0^\star)$, contradicting its optimality.

Therefore, by the mean value theorem, there must exist a stationary point of g strictly in the interior of the interval $[l, u]$ at which the function value is smaller than at the boundary. Moreover, the optimal point will have at least the support of any feasible point. □

Lemma 2 *If the network is weakly reversible and formed by a single linkage class, when problem* (P0) *is parametrized by a fixed point $\hat{\mu}$, there exists a positive feasible point $(\hat{v}, \hat{v}_0)$.*

Proof Let problem (P0) be parametrized with a fixed point $\hat{\mu}$, and let $(\hat{v}, \hat{v}_0)$ be both the minimizer and the fixed point. We prove by contradiction that no entry of the minimizer $(\hat{v}, \hat{v}_0)$ can be zero. Observe that by the definition of Ω the origin is not contained in the set, and therefore the fixed point cannot be identically zero.

First, assume that $\hat{v}_0 > 0$ and observe that $\rho := (D^{-1}A^T\hat{v} + \eta^- \hat{v}_0, 0)$ is a feasible point. Since η^- was chosen to be positive and $D^{-1}A^T$ has no zero columns, the support of ρ are the first n entries of the vector. A convex combination of $(\hat{v}, \hat{v}_0)$ and ρ will be feasible and have full support.

Now, assume that $\hat{v}_0 = 0$ and some entry of $\hat{v}$ is nonzero, and observe that $(D^{-1}A^T\hat{v}, 0)$ is feasible. A convex combination of $(D^{-1}A^T\hat{v}, 0)$ and $(\hat{v}, 0)$ is feasible and its support contains the union of the supports of the two vectors. That is, for $\beta \in [0, 1]$ the point $(\tilde{v}, \tilde{v}_0) = \beta(D^{-1}A^T\hat{v}, 0) + (1 - \beta)(\hat{v}, 0)$ is feasible, and using the fact that the support of $D^{-1}A^T\hat{v}$ is the support of $A^T\hat{v}$ along with Lemma 1,

$$\big(\mathrm{supp}(\hat{v}, 0) \cup \mathrm{supp}(A^T\hat{v}, 0)\big) \subset \mathrm{supp}(\tilde{v}, 0). \tag{7}$$

This relation can be used inductively to show that there is a feasible point with support at least as large as the union of the supports of $((A^T)^p\hat{v}, 0)$ for all positive powers of p.

The weak reversibility and single linkage class hypotheses imply that for any pair $(i, j) \in [1, \ldots, n] \times [1, \ldots, n]$, there exists a power p large enough such

that $(A^T)^p_{ij} > 0$. More importantly, if $\hat{v}_j > 0$, then there exists a p such that $[(A^T)^p \hat{v}_j]_i > 0$ for all i. Therefore, if $\hat{v}_0 = 0$ and $\hat{v} \neq 0$, there is a feasible point $(\tilde{v}, 0)$ such that $\tilde{v} > 0$.

Finally, if $\hat{v} > 0$ then there is a scalar $0 < \alpha$ mall enough such that

$$0 < \hat{v} - D^{-1} A^T \eta^+ \alpha,$$

and then the equality

$$YD(\hat{v} - D^{-1} A^T \eta^+ \alpha) + YA^T \eta^+ \alpha = YD\hat{v} = YA^T \hat{v}$$

implies that the positive point $(\hat{v} - D^{-1} A^T \eta^+ \alpha, \alpha)$ is feasible.

Therefore, if a network is weakly reversible and formed by a single linkage class, the problem (P0) has a positive feasible point $(\hat{v}, \hat{v}_0)$. □

Theorem 2 *For a mass-conserving weakly reversible network with one linkage class there exists a concentration $c > 0$ such that $YK\psi(c) = b$ if and only if b is in the range of YK.*

Proof We have shown that there exist positive vectors $c \in \mathbb{R}^m, v \in \mathbb{R}^n$ such that $Y^T \log(c) = \log(v)$ and $YKv = v_0 b$. In other words, we have proven that there is a positive vector c and a positive scalar α such that $YK\psi(c) = \alpha b$. If we can construct a new concentration vector $\tilde{c} > 0$ that satisfies $\psi(\tilde{c}) = \frac{1}{\alpha}\psi(c)$, then

$$YK\psi(\tilde{c}) = \frac{1}{\alpha} YK\psi(c) = b$$

and the steady state concentration $\tilde{c}$ satisfies (FB) and (MA).

First, we argue that the vector of all ones, $\mathbf{1} \in \mathbb{R}^n$, is in the range of Y^T when the network consists of a single linkage class and is mass-conserving. The condition of mass conservation implies that $e^T YK = 0$, or equivalently, $Y^T e \in \mathcal{N}(K^T)$. Since K^T is the Laplacian matrix of a strongly connected graph, $\mathcal{N}(K) = \{\beta \mathbf{1} : \ \beta \in \mathbb{R}\}$; thus, for some value $\hat{\beta}$, $Y^T e = \hat{\beta}\mathbf{1}$.

Now, observe that $\log\left(\frac{1}{\alpha}\psi(c)\right) = \log(\psi(c)) - \mathbf{1}\log(\alpha) = Y^T \log(c) - \mathbf{1}\log(\alpha)$, where $\log(c)$ is an entry-wise logarithm of the vector and $\log(\alpha)$ the scalar logarithm. Moreover, since $\mathbf{1}$ is in the range of Y^T, say $Y^T\delta = \mathbf{1}$ for some $\delta \in \mathbb{R}^m$, then $\log(\alpha)\mathbf{1} = Y^T(\log(\alpha)\delta)$. Thus, if we define $\tilde{c}$ as the vector that satisfies $\log(\tilde{c}) = \log(c) - \log(\alpha)\delta$, then

$$Y^T \log(\tilde{c}) = Y^T \log(c) - \log(\alpha)\mathbf{1},$$

which implies that

$$\psi(\tilde{c}) = \frac{1}{\alpha}\psi(c).$$

Therefore, the inhomogeneous system has a solution if the graph of the network is formed by a single linkage class, is weakly reversible and the network is mass-conserving, regardless of the kinetic parameters. □

4 Numerical experiments

We proceed to define the algorithm for finding positive steady states of weakly reversible networks with or without material exchange across their boundary.

4.1 Numerical method for finding fixed points

Given an initial positive point $(\hat{v}, \hat{v}_0) \in \mathbb{R}^{n+1}$ and a small tolerance τ, we use the following fixed point iteration, Algorithm 1, to find a parameter $\mu = (r, r_0)$ to the problem (P0) such that $(v^\star, v_0^\star) = (r, r_0)$.

Algorithm 1 Fixed point iteration to find a steady-state concentration

1: $(r, r_0) \leftarrow (\hat{v}, \hat{v}_0)$
2: **while** $\|YKv^\star - (YK\eta^+ + YD\eta^-)v_0^\star\|_\infty > \tau$ **do**
3: $\quad (v^\star, v_0^\star) \leftarrow$ unique solution of (P0)
4: $\quad (r, r_0) \leftarrow \frac{1}{2}(r, r_0) + \frac{1}{2}(v^\star, v_0^\star)$
5: **end while**.

Step 3 of Algorithm 1 requires solving the linearly constrained convex optimization problem (P0). Our implementation uses PDCO [8] to solve this problem.

Provided that at each iteration k, the unique solution of (P0) satisfies $v^\star(\mu_k) > 0$ and the minimization is solved with sufficient accuracy, the optimality conditions for (P0) will imply that for all $k = 1, 2, 3, \ldots,$

$$\|Y^T y^\star - \log(v^\star(\mu_k))\|_\infty \leq \epsilon,$$

for some small value of ϵ, where $y^\star$ is the Lagrange multiplier of the linear equality constraint at the solution that corresponds to the logarithm of the concentrations. Thus, if the iteration converges to a fixed point $(r, r_0) = (v^\star, v_0^\star)$, then (MA) will be satisfied to precision ϵ and (FB) to precision τ.

Algorithm 1 has been tested extensively on randomly generated networks with noteworthy success. The results of our experiments are shown in section 4.4.

4.2 An example network

In this section we consider the weakly reversible network shown in Figure 1. The number of complexes is $n = 7$, the number of linkage classes is $\ell = 2$. Since this network is weakly reversible, intuition suggests that a non-zero steady state exists. We use Algorithm 1 to solve for the fixed point described in section 3, obtaining a positive steady state. Figure 2 illustrates the convergence of the fixed point iterations to the steady state.

Figure 3 illustrates the change in steady state as a function of the *total mass* in the system, where total mass is defined as $\gamma = e^T(YDv^\star + YA^T\eta^+ v_0^\star)$,

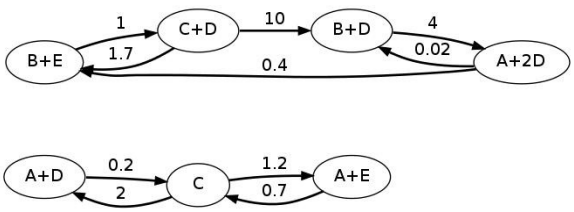

Fig. 1 Example weakly reversible network with two linkage classes

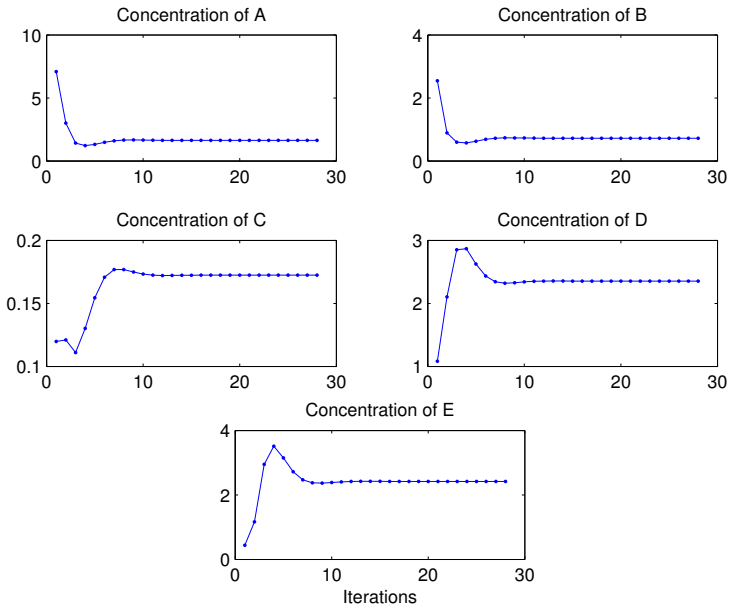

Fig. 2 Concentration convergence with iteration

as described in Theorem 1. The experiment shows that as the total mass increases, species A, B and C adjust linearly to the additional mass, while species D and E stay at the same levels. This linear growth in species A, B and C can be explained analytically by the fact that the vector $\mathbf{1}$ lies in the range of Y^T.

4.3 Generating suitable networks

This section describes the sampling scheme used to generate random mass-conserving weakly reversible chemical reaction networks with a prescribed number of linkage classes. The output of the method is a network with n

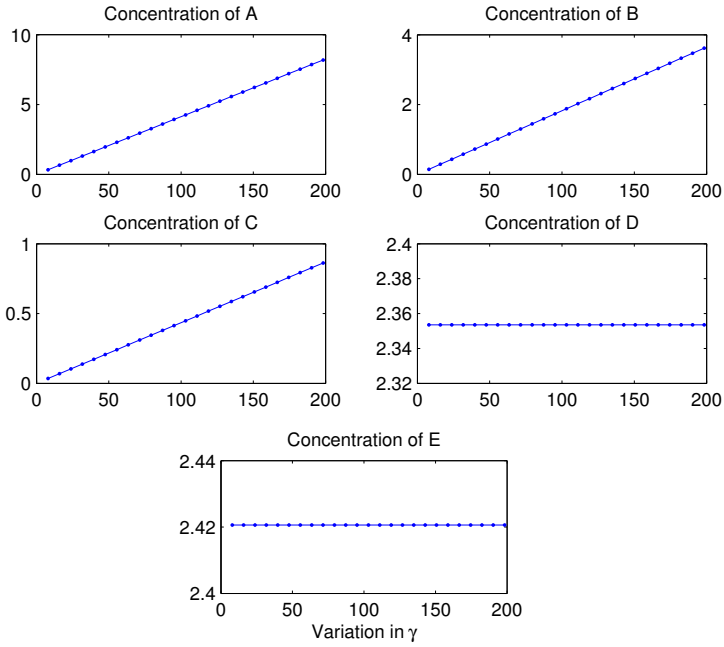

Fig. 3 Equilibrium dependence on total mass

complexes, m species, and ℓ strongly connected components, where ℓ is the desired number of linkage classes.

First, we iteratively generate Erdős-Réyni graphs[1] with m nodes until we sample a graph with ℓ strongly connected components; call this graph $\hat{G}(\hat{V}, \hat{E})$. We give each edge in $\hat{E}$ a weight of an independent and uniformly distributed value in the range $(0, 10]$. These edge weights represent the reaction rates between complexes.

To generate the stoichiometry, we define a parameter $q_{\max}$ as the maximum number of species in each complex. Each complex is constructed with a random sample of q species, where q is a random integer in $[1, q_{\max}]$. All samples are drawn uniformly and independently. Finally, we assign the multiplicity of each species in a complex with independent samples of the absolute value of a standard normal unit variance distribution. To ensure mass is conserved, we normalize the sum of the stoichiometry of the species that participate in a complex to one, so that $Y^T\mathbf{1} = \mathbf{1}$ and $K^TY^T\mathbf{1} = \mathbf{0}$.

[1] An Erdős-Réyni graph is a directed unweighted graph. Each edge is included with probability p and all edges are sampled *i.i.d.*

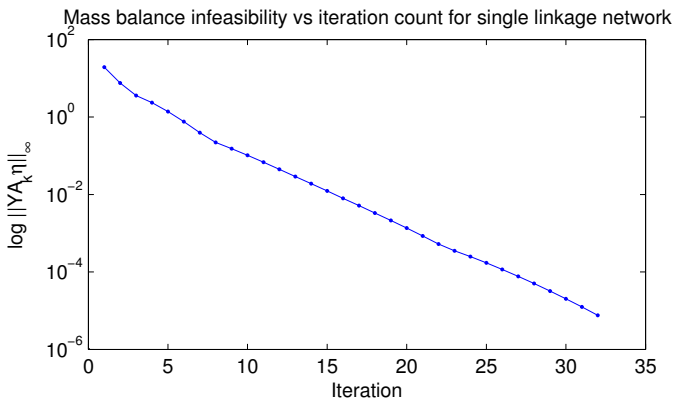

Fig. 4 Typical infeasibility of (FB) vs. Iteration for network with a single linkage class ($b = 0$)

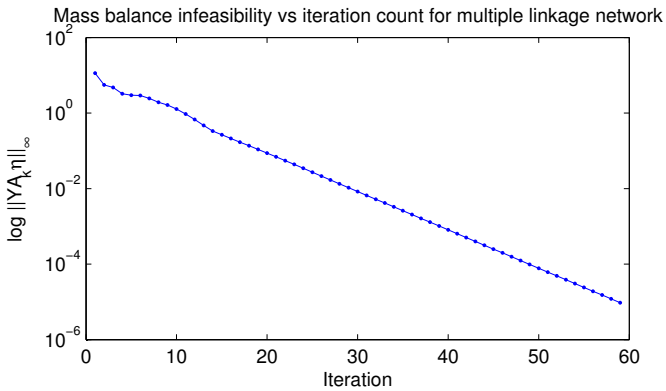

Fig. 5 Typical infeasibility of (FB) for network with two linkage classes ($b = 0$)

4.4 Convergence of the fixed point algorithm

This section illustrates the convergence of Algorithm 1 on large weakly reversible networks that consist of either a single linkage class or multiple linkage classes, for cases where there is no material exchange across the boundary ($b = 0$) and cases where there is material exchange across the boundary ($b = YK\eta$ for some $\eta \neq 0$).

Algorithm 1 produces sequences that, up to a small tolerance, satisfy (MA) at every iteration. Ideally, the infeasibility with respect to (FB) also monotonically decreases until convergence. Our extensive numerical experiments indicate that this is in fact the behavior for closed networks that are weakly reversible.

Figure 4 displays the sequence of the infeasibilities $\|YKv_k\|_\infty$ at each iteration k in Algorithm 1, for a network with a single linkage class, 50 species and 500 complexes, where at most 10 species participate in each complex.

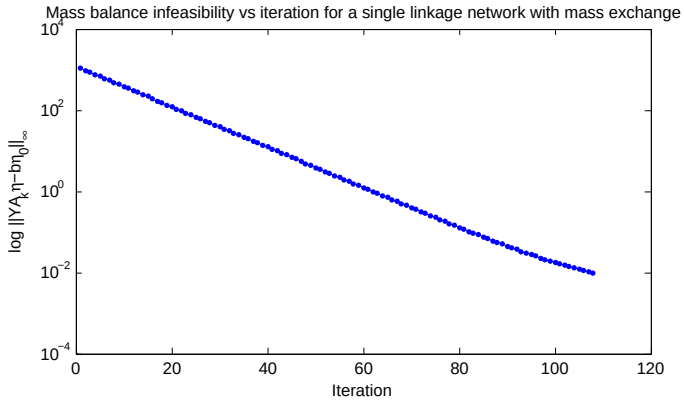

Fig. 6 Typical infeasibility of (FB) vs. Iteration for network with a single linkage class with material exchange ($b \neq 0$)

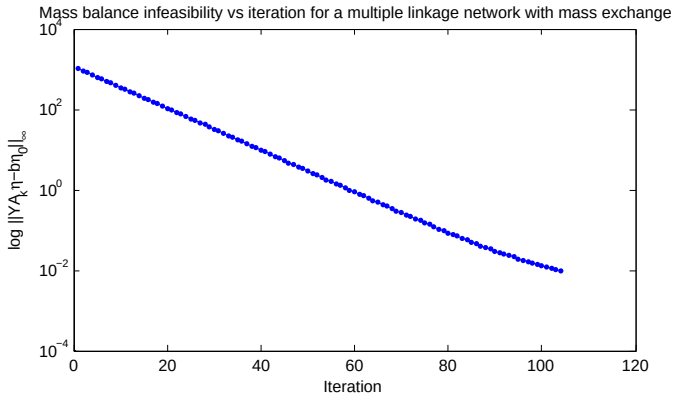

Fig. 7 Typical infeasibility of (FB) for network with two linkage classes and material exchange ($b \neq 0$)

Figure 5 displays the analogous sequence for a network of equal size and two linkage classes. We have observed this (apparently linear) convergence rate consistently over all generated networks, regardless of the number of linkage classes.

Figure 6 displays the sequence of the infeasibilities $\|YK\eta_k - b\eta_0\|_\infty$ at each iteration k, for the single linkage network with material exchange across its boundary. Figure 7 displays the analogous sequence for the multiple linkage network with material exchange across its boundary. Notably, the linear convergence rate is preserved.

We have also investigated the number of iterations necessary for Algorithm 1 to converge on closed networks of different sizes, with either one or two linkage classes. Figures 8 and 9 display the mean number of iterations necessary for convergence on networks ranging from 100 to 5000 complexes,

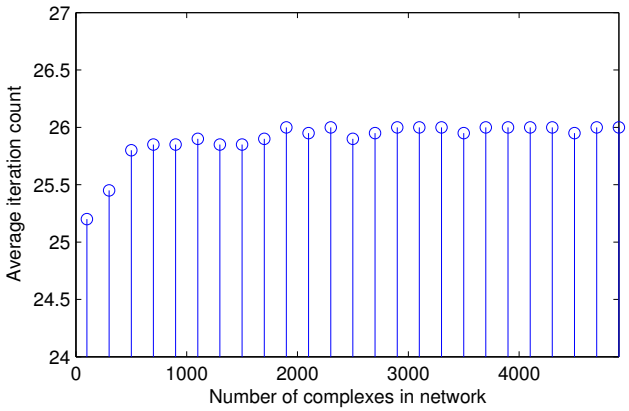

Fig. 8 Average number of iterations for single linkage class networks ($b = 0$)

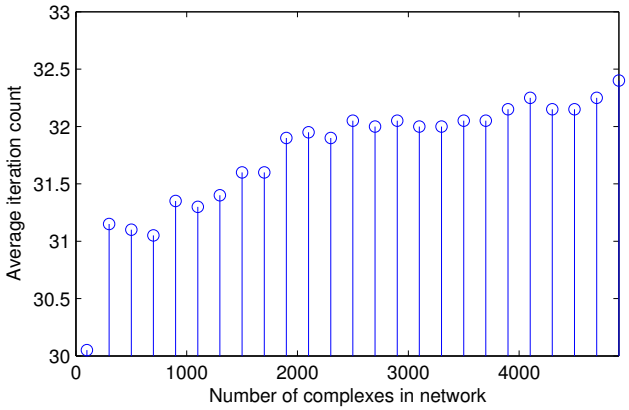

Fig. 9 Average number of iterations for networks with two linkage classes ($b = 0$)

where each average is taken over 20 instances per network size. Notably, the average number of iterations increases less than 10% as the network size grows fifty-fold.

In future work, we plan to prove theoretical results on the existence of positive equilibria for chemical reaction networks with multiple linkage classes. However, our comprehensive numerical experiments seem to indicate that even for networks with more than one linkage class, there exists at least one positive fixed point of problem (P0), and the iterates of Algorithm 1 converge to such a fixed point.

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