1 Introduction

In this supplementary material, we will, among other things, provide the proof of the main theorem in the article text (Theorem 3.5). We will also provide a result, Theorem 3.3, which gives more general conditions under which an absorption event is guaranteed to occur. In particular, we note that Theorem 3.3 applies to models with deficiencies that are greater than one, whereas Theorem 3.5 does not. In Section 3.4.1 we provide an example of a network from the biology literature which has a deficiency of two for which Theorem 3.3 applies, whereas Theorem 3.5 stands silent. In
Section 3.4.3 we conjecture that the conclusions of Theorem 3.3 and Theorem 3.5 hold for the whole class of systems permitting absolute concentration robustness when modeled deterministically.

The outline of the remaining text is the following. We will begin in Section 2 with a synopsis of the required terminology and background material related to chemical reaction network theory, and both the deterministic and stochastic models used for the dynamics of biochemical systems. In Section 3, we state and prove our main results. These results give conditions on the associated network for when an absorption event is guaranteed to occur when the system is modeled with stochastic dynamics. The conditions of our main results overlap significantly with the conditions of Feinberg and Shinar that guarantee absolute concentration robustness for a species in the deterministic modeling context [1]. When such equilibria are stable, therefore, the results of this paper represent a distinction in the long-term behavior of a class of deterministically and stochastically modeled systems.

An immediate question that comes to mind in light of Theorems 3.3 and 3.5 is: what is the structure and long-term behavior of the post-absorption network? In Section 3.3, we answer this question in detail for the class of systems considered by Theorem 3.5. Finally, in Section 4 we consider the time until the guaranteed absorption event takes place. If the time to absorption is large relative to the relevant time-scales of the system, the processes will seem to settle down long before the resulting instability will appear. This limiting distribution is called a quasi-stationary distribution, and is also considered in Section 4 for the network models studied here.

2 Background, terminology, and notation

In this section, we will briefly introduce necessary terminology and notation. For a more complete introduction, the reader is directed to [2–4] for background on Chemical Reaction Network Theory (CRNT), [5] for background on stochastic chemical reaction systems, and [6, 7] for general information on Continuous Time Markov Chains (CTMC).

We will use the following two examples throughout this paper. We will use them to both illustrate the background concepts and as an application of our main result, Theorem 3.3.

Example 1: Consider the activation/deactivation network

\[ A + B \xrightarrow{\alpha} 2B \]
\[ B \xrightarrow{\beta} A \]

where \( A \) and \( B \) denote the active and inactive form of a protein, respectively. We imagine here that the first reaction corresponds to deactivation of an active protein facilitated by an inactive protein, and that the second reaction corresponds to spontaneous activation of the inactive protein.

We have borrowed this network and interpretation from [1]. We note that the same network is produced by reversing the roles of \( A \) and \( B \) to produce a network of facilitated activation and spontaneous deactivation. It is also considered as a chemical reaction network in [8], as a mechanism for the spread of rumours in [9], as a model of logistic population growth [10], and as a model for SIS epidemic growth in [11–15].

Example 2: Consider the hypothetical EnvZ/OmpR signal transmission network

\[ XD \xrightarrow{k_1} X \xrightarrow{k_2[D]} k_3[X] \xrightarrow{k_4} XT \xrightarrow{k_5} X_p \]
\[ X_p + Y \xrightarrow{k_6} k_7 \xrightarrow{k_8} X + Y_p \]
\[ XD + Y_p \xrightarrow{k_9} XDY_p \xrightarrow{k_{11}} XD + Y \]

where the species are \( X=\text{EnvZ}, Y=\text{OmpR}, X_p=\text{EnvZ-P}, Y_p=\text{OmpR-P}, D=\text{ADP}, \) and \( T=\text{ATP} \). The species \( D \) and \( T \), which represent ADP and ATP, respectively, are assumed to be in sufficient quantity so that binding with \( X \) to form \( XD \) and \( XT \) do not appreciably change their overall quantities. Their kinetic effects are therefore incorporated into the rate constants.

This model was proposed in [16] and [1] to underlie the EnvZ/OmpR signaling system in Escherichia coli as studied experimentally in [17]. The individual sequence of reactions are imagined to represent the phosphate (signal) transfer from the sensor molecule EnvZ to the response molecule OmpR. The first chain of reactions corresponds to the phosphorylation of EnvZ from the donor molecules ADP and ATP. Notably, only ATP is able to successfully
phosphorylate EnvZ. The second chain corresponds to the transfer of the phosphate group from EnvZ to OmpR. The third chain corresponds to the dephosphorylation of OmpR-P by the ADP-EnvZ complex. It is notable that the signaling molecule EnvZ serves a dual purpose of phosphorylating OmpR and, in the modified ADP-EnvZ form, dephosphorylating OmpR-P.

2.1 Chemical reaction networks

The following is the basic object of study in CRNT [1–4, 18].

Definition 2.1. A chemical reaction network is a triple \{S, C, R\} of finite sets:

1. A species set \(S = \{X_1, \ldots, X_m\}\) containing the basic species/molecules capable of undergoing chemical change.

2. A reaction set \(R = \{R_1, \ldots, R_r\}\) containing the elementary reactions

\[
R_i : \sum_{j=1}^{m} y_{i,j} X_j \longrightarrow \sum_{j=1}^{m} y'_{i,j} X_j, \quad i = 1, \ldots, r
\]

where the stoichiometric coefficients \(y_{i,j}, y'_{i,j} \in \mathbb{Z}_{\geq 0}\) keep track of the multiplicity of the individual species within the reactions. Using a slight abuse of notation, we allow the reactions in (3) to be represented as \(y_i \rightarrow y'_i\) where \(y_i = (y_{i,1}, \ldots, y_{i,m})\) and \(y'_i = (y'_{i,1}, \ldots, y'_{i,m})\). We say that the reaction \(y_i \rightarrow y'_i\) is a reaction out of complex \(y_i\) and into the complex \(y'_i\).

3. A complex set \(C\) containing the linear combination of the species on the left-hand and right-hand sides of the reaction arrow in (3). Using the same abuse of notation as before, we allow complexes to be represented by their support vectors so that \(C = \bigcup_{i=1}^{t} \{y_i, y'_i\}\). The number of stoichiometrically distinct complexes is denoted by \(n\) (i.e. \(|C| = n\)).

Remark 1. It is typical within CRNT to assume that (i) every species appears in at least one complex, (ii) every complex appears in at least one reaction, and (iii) there are no self-reactions (i.e. reactions of the form \(y_i \rightarrow y'_i\) where \(y_i = y'_i\)).

To each reaction \(y_i \rightarrow y'_i, i = 1, \ldots, r\), we furthermore associate the reaction vector \(y'_i - y_i \in \mathbb{Z}^m\). The components of the reaction vectors correspond to how many copies of each species are gained or lost by each instance of an individual reaction. The stoichiometric subspace of the network is defined to be \(S = \text{span} \{y'_i - y_i \mid i = 1, \ldots, r\}\) and the dimension of \(S\) is denoted \(s = \dim(S)\).

By representing each stoichiometrically distinct complex only once, the chemical reaction network \((S, C, R)\) can be interpreted as a directed graph \(G(V, E)\) where the vertex set is given by \(V = C\) and the edge set is given \(E = R\). Following [1], we introduce the following connectivity relations.

1. The complexes \(y, y' \in C\) are directly linked (denoted \(y \leftrightarrow y'\)) if \(y \rightarrow y'\) or \(y' \rightarrow y\);

2. The complexes \(y, y' \in C\) are linked (denoted \(y \sim y'\)) if either (i) \(y = y'\), or (ii) there exists a sequence of complexes such that \(y = y_{\mu(1)} \leftrightarrow y_{\mu(2)} \leftrightarrow \cdots \leftrightarrow y_{\mu(t)} = y'\);

3. There is a path from \(y \in C\) to \(y' \in C\) (denoted \(y \Rightarrow y'\)) if there exists a sequence of complexes such that \(y = y_{\mu(1)} \rightarrow y_{\mu(2)} \rightarrow \cdots \rightarrow y_{\mu(t)} = y'\); and

4. The complexes \(y, y' \in C\) are strongly linked (denoted \(y \approx y'\)) if either (i) \(y = y'\), or (ii) \(y' \Rightarrow y\). The connectivity relations “\(\sim\)” and “\(\approx\)” allow the complex set \(C\) to be partitioned into equivalence classes called linkage classes and strong linkage classes, respectively. That is to say, \(y, y' \in C\) are in the same linkage class \(L\) (respectively, strong linkage class \(\Lambda\)) if and only if \(y \sim y'\) (respectively, \(y \approx y'\)). A strong linkage class \(\Lambda\) is said to be terminal if there is no reaction \(y \rightarrow y'\) where \(y \in \Lambda\) but \(y' \notin \Lambda\). We will say that a complex is non-terminal if it is not contained in a terminal strong linkage class. We will furthermore say that two non-terminal complexes \(y, y'\) differ in the species \(X_i\) if \(y' = y + \alpha e_i\) where \(\alpha > 0\), and \(e_i \in \mathbb{R}^m\) is the canonical basis vector with a one in the \(i\)th component and zeros elsewhere.

The set of linkage classes is denoted \(L = \{L_1, \ldots, L_{\ell}\}\) where \(|L| = \ell\) and the set of terminal strong linkage classes is denoted \(\Lambda = \{\Lambda_1, \ldots, \Lambda_t\}\) where \(|\Lambda| = t\). A network is called weakly reversible if the linkage classes and strong linkage classes coincide (i.e. if \(y \Rightarrow y'\) implies \(y' \Rightarrow y\)).

Finally, we introduce the following network parameter which has been the focus of much study in the chemical reaction network literature [19–24].
**Definition 2.2.** The deficiency of a chemical reaction network \((S, C, R)\) is given by \(\delta = n - \ell - s\) where \(n\) is the number of stoichiometrically distinct complexes, \(\ell\) is the number of linkage classes, and \(s\) is the dimension of the stoichiometric subspace \(S\).

The deficiency of a chemical reaction network does not depend on the choice of kinetics or, in the case of mass-action kinetics, on the choice of rate constants, and is known to only take non-negative integer values \([3, 4]\). Nevertheless, many dynamical properties for deterministically-modeled chemical reaction systems are characterized in terms of the underlying network’s deficiency \([21, 23–26]\).

**Example 1:** The network (1) has the species set \(S = \{A, B\}\), the complex set \(C = \{A + B, 2B, B, A\}\), and the reaction set \(R = \{A + B \rightarrow 2B, B \rightarrow A\}\). The linkage classes are \(\mathcal{L}_1 = \{A + B\}\) and \(\mathcal{L}_2 = \{B, A\}\), and the strong linkage classes are \(\Lambda_1 = \{A + B\}\), \(\Lambda_2 = \{2B\}\), \(\Lambda_3 = \{B\}\) and \(\Lambda_4 = \{A\}\), of which \(\Lambda_2\) and \(\Lambda_4\) are terminal. The non-terminal complexes are \(A + B\) and \(B\), which differ in only the species \(A\). The deficiency can easily be computed: \(\delta = n - \ell - s = 4 - 2 - 1 = 1\).

**Example 2:** The network (2) has the species set \(S = \{X, Y, X_p, Y_p, X_D, XT, X_pY, XDY_p\}\), the complex set \(C = \{X_D, X, XT, X_p, Y, X_pY, X + Y_p, XD + Y_p, XDY_p, XD + Y\}\), and the reaction set
\[
R = \{X_D \rightarrow X, X \rightarrow X_D, X \rightarrow XT, XT \rightarrow X, XT \rightarrow X_p, X_p + Y \rightarrow X_pY, Y_p \rightarrow X_pY, X_pY \rightarrow X + Y_p, XD + Y_p \rightarrow XDY_p, XD + Y \rightarrow XDY_p, XDY_p \rightarrow XD + Y_p, XD + Y_p \rightarrow XD + Y\}.
\]

The linkage classes are \(\mathcal{L}_1 = \{X_D, X, XT, X_p\}\), \(\mathcal{L}_2 = \{X_p + Y, X_pY, X + Y_p\}\), and \(\mathcal{L}_3 = \{XD + Y_p, XDY_p, XD + Y\}\). These can be further decomposed into the the strong linkage classes \(\Lambda_1 = \{X_D, X, XT\}\), \(\Lambda_2 = \{X_p\}\), \(\Lambda_3 = \{X_p + Y, X_pY\}\), \(\Lambda_4 = \{X + Y_p\}\), \(\Lambda_5 = \{XD + Y_p, XDY_p\}\), and \(\Lambda_6 = \{XD + Y\}\). Of the strong linkage classes, \(\Lambda_2\), \(\Lambda_4\), and \(\Lambda_6\) are terminal. It follows that \(X_D, X, XT, X_p + Y, X_pY, XD + Y_p, XD + Y_p, XDY_p\), are non-terminal complexes. We can see that the non-terminal complexes \(X_D\) and \(XD + Y_p\) differ only in the species \(Y_p\). The network (2) has ten complexes \((n = 10)\), three linkage classes \((\ell = 3)\), and the span of the reaction vectors is six-dimensional \((s = 6)\). It follows that (1) is a deficiency one network.

### 2.2 Deterministic chemical reaction systems

It is common to model the time-evolution of chemical reaction networks via a set of deterministic differential equations over continuous state variables representing the chemical concentrations of the species \((i.e. c_j = [X_j], j = 1, \ldots, m)\). This modeling choice is suitable when the reaction vessel is well-mixed and the number of all reacting molecules is large \([27]\).

A common kinetic rate assumption is that of the law of mass-action which states that the rate of a reaction is proportional to the product of the necessary reacting species, counted for multiplicity \([28]\). Specifically, given the concentration vector \(c = (c_1, c_2, \ldots, c_m) \in \mathbb{R}^m_{\geq 0}\), the rate of the reaction \(y_i \rightarrow y_i'\) is
\[
k_i c^{y_i} = k_i c_1^{y_{i1}} c_2^{y_{i2}} \cdots c_m^{y_{im}},
\]
where \(k_i\) is the rate constant and \(y_i\) is the source complex associated with the \(i\)th reaction channel. Other kinetic rates for the reactions have been used in the biochemical literature, including Michaelis-Menten and Hill kinetics \([29, 30]\).

Given the chemical reaction network \((S, C, R)\), the associated mass-action system \((S, C, R, k)\) is given by the system of differential equations
\[
\frac{dc}{dt} = \sum_{i=1}^{r} k_i (y_i' - y_i) c^{y_i}.
\]

For any \(c_0 \in \mathbb{R}^m_{\geq 0}\), it is known that \(c(t) \in (c_0 + S) \cap \mathbb{R}^m_{\geq 0}\) for all \(t \geq 0\), where we remind the reader that \(S\) is the stoichiometric subspace of the system. Trajectories of (4) evolve in a space of dimension \(s\), which may be smaller.
than the state space $\mathbb{R}^{m}_{\geq 0}$. No trajectory of the deterministic mass-action system (4) allows the concentration of any species to hit zero in finite time [31].

**Example 1:** The mass-action system (4) corresponding to the network (1) is

$$
\left( \begin{array}{c}
\dot{c}_A \\
\dot{c}_B
\end{array} \right) = \alpha \left( \begin{array}{c}
-1 \\
1
\end{array} \right) c_A c_B + \beta \left( \begin{array}{c}
1 \\
-1
\end{array} \right) c_B.
$$

**Example 2:** The mass-action system (4) corresponding to the network (2) is

$$
\begin{align*}
\dot{c}_X &= k_1 c_X D - (k_2[D] + k_3[T]) c_X + k_4 c_X T + k_8 c_X Y \\
\dot{c}_X D &= -k_1 c_X D + k_2[D] c_X - k_9 c_X D c_Y + (k_{10} + k_{11}) c_X D Y_p \\
\dot{c}_X T &= k_3[T] c_X - (k_4 + k_5) c_X T \\
\dot{c}_X Y &= k_5 c_X T - k_6 c_X Y + k_7 c_X Y \\
\dot{c}_Y &= -k_6 c_X Y + k_7 c_X Y + k_{11} c_X D Y_p \\
\dot{c}_X Y_p &= k_6 c_X Y - (k_7 + k_8) c_X Y_p \\
\dot{c}_Y_p &= k_8 c_X Y_p - k_9 c_X D c_Y_p + k_{10} c_X D Y_p \\
\dot{c}_X D Y_p &= k_9 c_X D c_Y_p - (k_{10} + k_{11}) c_X D Y_p,
\end{align*}
$$

where we have dropped the vector notation.

### 2.2.1 Absolute concentration robustness

A concentration $\bar{c} \in \mathbb{R}^m_{\geq 0}$ of a mass-action system (4) is said to be an *equilibrium concentration* of (4) if

$$
\sum_{i=1}^r k_i(y'_i - y_i)\bar{c}^y_i = 0,
$$

and said to be a *positive equilibrium concentration* if $\bar{c} \in \mathbb{R}^m_{>0}$. Motivated by biochemical examples in [16, 17, 32], Guy Shinar and Martin Feinberg introduce the following classification of positive equilibrium concentrations in [1].

**Definition 2.3.** A mass-action system $(S, C, R, k)$ is said to possess *absolute concentration robustness* (ACR) in the species $X_i \in S$ if $\bar{c}_i$ attains the same value in every positive equilibrium concentration $\bar{c} \in \mathbb{R}^m_{>0}$ of (4).

It is worth noting that the absolutely robust equilibrium value $\bar{c}_i$ may depend upon the network’s rate constants but not on the initial conditions. This robustness with respect to overall concentrations is especially meaningful for biochemical networks since, when combined with stability, it predicts that processes requiring tight bounds in the robust species will operate similarly in the face of fluctuations in the overall concentrations of the other species.

The following result guarantees absolute concentration robustness in a particular species for deterministically modeled mass-action systems and is the main result of [1]. It is also the motivation for the current work.

**Theorem 2.4.** Consider a mass-action system $(S, C, R, k)$ governed by (4). Suppose that:

1. The system (4) admits a positive equilibrium concentration;
2. The deficiency of the underlying network $(S, C, R)$ is one (i.e. $\delta = 1$); and
3. There are non-terminal complexes which differ only in the species $X_i$.

Then the mass-action system (4) exhibits absolute concentration robustness in $X_i$.

Theorem 2.4 is surprising in that it presents *structural* conditions on the underlying reaction network which are sufficient to guarantee ACR in a particular species. That is to say, it does not depend on “fine-tuning” the network parameters [33]. More details on the theorem, and further examples of ACR networks which do not satisfy Theorem 2.4, are contained in the Supplemental Material of [1].
Example 1: We can easily compute that the only positive equilibrium concentrations permitted by (5) are

$$\bar{c}_A = \frac{\beta}{\alpha}$$

$$\bar{c}_B = M - \frac{\beta}{\alpha}$$

(8)

where $M := c_A(0) + c_B(0)$. It is readily observed that $\bar{c}_A$ has the same value for any positive equilibrium (provided $M > \beta/\alpha$). The system therefore is ACR in the species $A$. We could also have used Theorem 2.4 to find that $A$ is ACR since this network has positive equilibrium concentrations, is deficiency one, and the non-terminal complexes $A + B$ and $B$ differ only in the species $A$.

Example 2: It is shown in the supplemental material of [1] that every equilibrium concentration $\bar{c} \in \mathbb{R}_n^m$ of (6) satisfies

$$\bar{c}_{Y_p} = \frac{k_1 k_3 k_5 (k_{10} + k_{11}) [T]}{k_2 (k_4 + k_5) k_9 k_{11} [D]}.$$  

(9)

This concentration depends on the network parameters but not on the concentrations of the other species, which are related by the conservation relationships

$$X_{tot} = c_X + c_{X_p} + c_{XD} + c_{X_pY} + c_{XDY_p}$$

$$Y_{tot} = c_Y + c_{Y_p} + c_{XY} + c_{XY_p}.$$  

(10)

It follows that (6) is ACR in the species $Y_p$ (provided $Y_{tot} > \bar{c}_{Y_p}$ where $\bar{c}_{Y_p}$ is given by (9)). Again, we could also have used Theorem 2.4 to find that $Y_p$ exhibits ACR as (6) has positive equilibria, the underlying network is deficiency one, and the non-terminal complexes $XD$ and $XD + Y_p$ differ only in the species $Y_p$.

2.2.2 Reformulation of equation (4)

A key feature of CRNT has been in isolating the dependence in the mass-action system (4) on the individual complexes $y \in C$ [18]. It is often convenient therefore to work in “complex space” rather than species space. This allows us to reformulate equation (4) in a useful manner.

In order to avoid excessive enumeration, we will index elements in the complex space $\mathbb{R}^C$ explicitly by their corresponding element $y \in C$. That is to say, for $v \in \mathbb{R}^C$ we will use $v_y$ to denote the element of $v$ corresponding to the complex $y \in C$. This will allow us to maintain the indexing of complexes introduced previously, i.e. we will still allow $y_i$ and $y'_i$ to correspond to the reactant and product complex, respectively, of the $i$th reaction. We note that $|C| = n$ so that each vector $v \in \mathbb{R}^C$ has $n$ elements and that, by the support of an element $v \in \mathbb{R}^C$, we mean the set of those elements of $C$ for which $v$ takes nonzero values, that is,

$$\text{supp}(v) := \{y \in C : v_y \neq 0\}.$$  

(11)

We furthermore denote the basis vectors $\omega_y, y \in C$, which are the unit vectors with supp($\omega_y$) = $y$. The enumeration described above has a long history in chemical reaction network theory [3].

Given a choice of vectors $\kappa \in \mathbb{R}^S \geq 0$ and $\Psi \in \mathbb{R}^C \geq 0$, the kinetic or Kirchhoff mapping $A_\kappa : \mathbb{R}^C \rightarrow \mathbb{R}^C$ of a chemical reaction network $(S, C, R)$ is the linear mapping defined as

$$A_\kappa(\Psi) = \sum_{y \in C} \left[ \sum_{\substack{i=1 \\text{subject to } y' \neq y \\text{ for } y' \in y \}} \right] \kappa_i (\omega_{y'} - \omega_y) \Psi_y,$$  

(12)

where the inner sum is over the reactions with source complex $y$. The formulation (12) divides the structure of a chemical reaction network according to which complexes act as source complexes for which reactions. If a complex is a source for multiple reactions, those reactions are grouped together. We furthermore define the linear mapping $Y : \mathbb{R}^C \rightarrow \mathbb{R}^m$ by its action on the basis elements $\omega_y$ via

$$Y(\omega_y) = y.$$  

(13)

It is easy to see that the composite mapping

$$Y A_\kappa(\Psi) = \sum_{i=1}^r \kappa_i (y'_i - y_i) \Psi_{y_i}.$$  

(14)
is the right-hand-side of the mass-action form (4) with \( \kappa_i = k_i \) and \( \Psi_y = e^{uy} \) for \( i = 1, \ldots, r \).

The following two results regarding \( \ker(A_\kappa) \) and \( \ker(YA_\kappa) \) can be found in [1]. The first result is derived in the Appendix of [34] and has been restated numerous times since [3, 35]. An explicit computation of the basis of \( \ker(A_\kappa) \) appears in [20]. The second result follows from Section 6 of [34].

**Lemma 2.5.** Let \((S, C, R)\) denote a chemical reaction network with terminal strong linkage classes \( \Lambda_1, \ldots, \Lambda_t \). Then, for any \( \kappa \in \mathbb{R}_{\geq 0}^r \), \( \ker(A_\kappa) \) has a basis \( \{b_1, \ldots, b_t\} \) where \( \text{supp}(b_\theta) = \Lambda_\theta \) for all \( \theta = 1, \ldots, t \).

**Lemma 2.6.** Let \((S, C, R)\) denote a chemical reaction network with deficiency \( \delta \) and \( t \) terminal strong linkage classes. Then

\[
\dim(\ker(YA_\kappa)) \leq \delta + t.
\]

Note that the condition \( \text{supp}(b_\theta) = \Lambda_\theta \) is a set equality property which is well-defined since both are subsets of \( C \) (\( \text{supp}(b_\theta) \) by (11) and \( \Lambda_\theta \) by the definitions of Section 2.1).

### 2.3 Stochastic models of chemical reaction systems

Chemical reaction systems can also be modeled with stochastic dynamics as **continuous-time Markov chains** (CTMC). The formulation here closely follows that in [5], to which we point the interested reader. In this setting, we let \( X_j(t) \in \mathbb{Z}_{\geq 0} \) denote the number of molecules of \( X_j \) at time \( t \) and consider the Markov chain \( X(t) = (X_1(t), X_2(t), \ldots, X_m(t)) \in \mathbb{Z}_{\geq 0}^m \) evolving continuously in time over the discrete state space \( \mathbb{Z}_{\geq 0}^m \). At the occurrence of a reaction \( y_i \to y_i' \) the chain instantaneously updates to the new state

\[
X(t) = X(t^-) + (y_i' - y_i),
\]

where \( X(t^-) = \lim_{h \to 0^+} X(t - h) \) is the value of the chain right before the jump. The state of the chain at time \( t \) can therefore be given by

\[
X(t) = X(0) + \sum_{i=1}^{r} N_i(t)(y_i' - y_i)
\]

where the counting process \( N_i(t) \) keeps track of the number of times the \( i \)th reaction has occurred by time \( t \). The counting processes can be further formulated as a function of the state-dependent reaction **propensities** \( \lambda_i(X(t)) \) by

\[
N_i(t) = Y_i \left( \int_0^t \lambda_i(X(s)) \, ds \right),
\]

where \( Y_i(\cdot), i = 1, \ldots, r \), are independent, unit-rate Poisson processes. The propensity functions \( \lambda_i \) are the analog of the deterministic rate functions \( k_i e^{uy} \) in equation (4), and are often termed **intensity** functions in the mathematics literature. Sample trajectories of (16) and (17) can be computed by a simulation process known as the next reaction method [36, 37], with a similar representation being simulated via the well-known **Gillespie algorithm** [38].

As with their deterministic continuous-state counterparts, reactions may only occur in the stochastic setting given sufficient multiplicity of their constituent reactant molecules. This is captured by the following definition.

**Definition 2.7.** The propensities \( \lambda_i(X), i = 1, \ldots, r \), are said to be **stochiometrically admissible** if \( \lambda_i(X) > 0 \) for all \( X \in \mathbb{Z}_{\geq 0}^m \) such that \( X_j \geq y_{ij} \) for all \( j = 1, \ldots, m \), and \( \lambda_i(X) = 0 \) otherwise.

Although stronger restrictions on the propensities are often made (e.g. monotonicity in the species counts), this definition will be sufficient for the results in this paper. The Markov chain formed by considering a chemical reaction network \((S, C, R)\) together with stochiometrically admissible propensities is called a **stochastic chemical reaction system**. We say a complex is **turned off** at a particular state if the propensity of each reaction with that complex as its source is zero at that state value; otherwise we say the complex is **turned on**.

A common choice for the propensities \( \lambda_i(X) \) is **stochastic mass-action**

\[
\lambda_i(X) = \begin{cases} 
\frac{k_i V |y_i|!}{\prod_{j=1}^{m} (X_j - y_{ij})! y_{ij}!} & \text{if } X_j - y_{ij} \geq 0 \text{ for all } j \\
0, & \text{otherwise}
\end{cases}
\]

where \( |y_i| = \sum_{j=1}^{m} y_{ij} \) and \( V \) is the volume of the reaction vessel. The stochastic chemical reaction system with mass-action propensities (18) is called a **stochastic mass-action system**.
Parametrizing the model (16) by $V$ and denoting by $X^V(t)$ the model with volume $V$, trajectories $X^V(t)/V$ of the stochastic model with mass-action propensities (18) are known to converge almost surely on compact time intervals to their deterministic mass-action counterparts (4) with rate constants $k_i$ in the large-scale limit as $V \to \infty$, so long as $X^V(0)/V$ converges to a non-zero constant for each $j$ [39].

An alternative approach to analyzing stochastic chemical reaction systems is to consider the propagation of the probability of a given chain $X(t)$ being in a given state as a function of time. We let $P_t(X) = P_t \{ X(t) = X \}$ denote the probability of the chain $X(t)$ being in the state $X$ at time $t$. If we define $\lambda_0(X) = \sum_{i=1}^r \lambda_i(X)$ then, for each state $X \in \mathbb{Z}_m^m$, we have

\[
\frac{dP_t(X)}{dt} = -\lambda_0(X)P_t(X) + \sum_{i=1}^r \lambda_i(X - (y_i' - y_i))P_t(X - (y_i' - y_i)),
\]

with an initial condition determined by an initial distribution. The equation (19) is called the chemical master equation in the biology literature, and is called Kolmogorov’s forward equations in the mathematics literature. Note that the system (19) can be formally written as

\[
\frac{d}{dt}P_t = P_t A,
\]

where $P_t$ is the row vector of probabilities of being in a particular state at time $t$ and $A$ is the generator matrix of the CTMC, which is defined via the above equations. Although (20) is a linear system of ordinary differential equations, the scale is typically large (potentially infinite-dimensional) and consequently exact probabilistic solutions to (20) are often difficult or impossible to determine explicitly. Fixed points of equation (20) are of particular interest as they correspond to stationary distributions of the system and, as a consequence, describe the long-term behavior of the chain.

### 2.4 Irreducibility, positive recurrence, and stationary distributions

The connectivity of states is a key determinant of the long-term behavior of chains $X(t)$. To that end, we now introduce the following terminology relevant to how the states of a stochastic chemical reaction system are connected.

1. A state $X \in \mathbb{Z}_m^m$ has mass on a complex $y_i \in \mathbb{C}$ if $X_j \geq y_{ij}$ for all $j = 1, \ldots, m$.
2. A state $Y \in \mathbb{Z}_m^m$ is directly accessible from $X \in \mathbb{Z}_m^m$ (denoted $X \rightarrow Y$) if $Y = X + (y_i' - y_i)$ for some $i = 1, \ldots, r$ and $X$ has mass on $y_i$.
3. A state $Y \in \mathbb{Z}_m^m$ is accessible from $X \in \mathbb{Z}_m^m$ (denoted $X \rightsquigarrow Y$) if there exists a sequence of states such that $X = X_{\mu(1)} \rightarrow X_{\mu(2)} \rightarrow \cdots \rightarrow X_{\mu(t)} = Y$; and
4. The states $X, Y \in \mathbb{Z}_m^m$ are said to communicate (denoted $X \leftrightarrow Y$) if (i) $Y = X$, or (ii) $X \rightsquigarrow Y$ and $Y \rightsquigarrow X$; and
5. A state $X \in \mathbb{Z}_m^m$ is recurrent (respectively, transient) if the Markov chain starting at $X(0) = X$ satisfies

\[
P \{ T_X < \infty \mid X(0) = X \} = 1 \quad (< 1)
\]

where $T_X$ is the first return time to $X$. A recurrent state $X \in \mathbb{R}_m^m$ is furthermore called positive recurrent (respectively, null recurrent) if

\[
\mathbb{E}(T_X \mid X(0) = X) < \infty \quad (= \infty).
\]

That is to say, a state $X \in \mathbb{Z}_m^m$ is recurrent if the chain $X(t)$ satisfying $X(0) = X$ is guaranteed to return to $X$, and positive recurrent if the expected return time is finite.

The relation $\leftrightarrow$ allows the state space $\mathbb{Z}_m^m$ to be partitioned into irreducible communicating classes or irreducible components. The states $X, Y \in \mathbb{Z}_m^m$ are in the same irreducible component $\mathcal{I} \subseteq \mathbb{Z}_m^m$ if and only if $X \leftrightarrow Y$. An irreducible component $\mathcal{I} \subseteq \mathbb{Z}_m^m$ is said to be closed if $X \not\rightarrow Y$ for all $X \in \mathcal{I}$ and $Y \not\in \mathcal{I}$, and is said to be absorbing if it is closed and $Y \rightarrow X$ for some $X \in \mathcal{I}$ and $Y \not\in \mathcal{I}$. It is a standard result from introductory texts on continuous time Markov chains that for any irreducible component $\mathcal{I} \subseteq \mathbb{Z}_m^m$, if $X \rightarrow Y$ for $X, Y \in \mathcal{I}$ and $X$ is (positive) recurrent, then $Y$ is (positive) recurrent, and that if $Y$ is transient then $X$ is transient [7]. Consequently, recurrence, positive recurrence, and transience are class properties of irreducible components [7].

In order to determine the long-term behavior of stochastic processes it is important to consider the stationary distributions $\pi = (\pi(X))_{X \in \mathbb{Z}_m^m}$, which are the non-negative fixed points of (20), normalized to sum to one. Specifically, $\pi$ is a stationary distribution if it satisfies the following
(i) \( \sum_{X \in \mathbb{Z}_{\geq 0}^m} \pi(X) = 1; \)

(ii) \( 0 \leq \pi(X) \leq 1 \) for all \( X \in \mathbb{Z}_{\geq 0}^m; \) and

(iii) \( \pi.A = 0 \) where \( A \) is the generator of the CTMC (20).

It is a standard result that the stationary distributions of continuous-time Markov chains, if they exist, are restricted to the support of the closed irreducible components and are unique on these components. That is to say, if \( I_1, \ldots, I_p, \) where \( p \) could be infinity, are closed irreducible components of a continuous time Markov chain \( X(t) \), then each component has a unique stationary distribution \( \pi_{I_j} = (\pi(X))_{X \in I_j}, j = 1, \ldots, p, \) and any stationary distribution \( \pi \) of the process can be expressed as

\[ \pi = \sum_{j=1}^p \gamma_j \pi_{I_j}, \tag{21} \]

where \( 0 \leq \gamma_j \leq 1 \) and \( \sum_{j=1}^p \gamma_j = 1 \). See, for example, [7].

3 Main results

In this section we state and prove our main results, Theorem 3.3 and Theorem 3.5.

We begin in Section 3.1 by motivating the notion that deterministic and stochastic models can exhibit fundamentally different limiting behavior through consideration of the networks (1) and (2). Next, in Section 3.2, we provide statements and proofs of our main results, which provide conditions for when an absorption event is guaranteed to occur for a stochastically modeled network. In Section 3.3 we provide a theorem detailing the deficiency, connectivity properties, and long-term behavior of the post-absorption network. In Section 3.4 we show three things: (i) that Theorem 3.3 may be successfully applied to networks more general than those covered by Theorem 3.5, which is stated in the main article text, (ii) that the results of Theorem 3.3 and Theorem 3.5 may fail for networks for which a global conservation relationship does not hold, and (iii) that the conclusions of Theorems 3.3 and 3.5 hold for more ACR models than those which fit the assumptions of the theorems, leading to a conjecture. In Section 4, we consider the time until the guaranteed absorption takes place, and the quasi-stationary distributions of the network models.

3.1 Differences in long-term behavior for ACR systems

We are interested in when the long-term behavior of deterministically modeled networks, as characterized by their stable equilibria, is qualitatively different than the long-term behavior of stochastically modeled networks, as characterized by their stationary distributions and recurrence properties. Specifically, we are interested in whether or not trajectories of the stochastic systems become concentrated around deterministically predicted stable equilibrium concentrations.

**Example 1:** Trajectories of the stochastic chemical reaction system corresponding to (1) have different long-term behavior than those of the deterministically modeled mass-action system (5). For all initial counts \( X_A(0) \) and \( X_B(0) \), we can see that the state \( (\bar{X}_A, \bar{X}_B) = (M, 0) \), (22)

where \( M := X_A(0) + X_B(0) \), is a trapping state since neither reaction may proceed from this state. This corresponds to an inaccessible boundary equilibrium concentration in the deterministic setting [31].

The state (22) is also, however, an accessible state for the corresponding stochastic mass-action system. From any positive state \( (X_A(t), X_B(t)) \) of the chain it is possible to transition irreversibly through the repeated occurrences of \( B \rightarrow A \) to the state (22). It follows that, for any initial counts, (22) is the unique absorbing state of the chain. Hence, for any value \( M > 0 \), the unique stationary distribution only has mass on (22) and not near the positive equilibrium \( (\bar{c}_A, \bar{c}_B) = (\beta/\alpha, M - \beta/\alpha) \) of the deterministic model.

**Example 2:** Although more challenging to see, trajectories of the stochastic chemical reaction system corresponding to the system (2) also have different long-term behavior than those of the deterministic model (6). Consider the state

\[ X_p = X_{tot} > 0, \]
\[ Y_p = Y_{tot} > 0, \]
\[ X = Y = XD = XT = X_pY = XDY_p = 0 \]
where \( X_{\text{tot}} \) and \( Y_{\text{tot}} \) are given by
\[
X_{\text{tot}} = X(0) + X_p(0) + XD(0) + X_pY(0) + XDY_p(0) \\
Y_{\text{tot}} = Y(0) + Y_p(0) + X_pY(0) + XDY_p(0).
\]
(24)

It is clear that this is an absorbing state since all reaction propensities are zero at (23). Modeled deterministically, the corresponding state is a boundary equilibrium that is not accessible from the positive orthant [31]. In the stochastic setting, however, the state can be shown to be accessible in the following manner. Start with arbitrary positive counts for every species and suppose the following reactions take place sequentially:

1. Convert all \( XDY_p \) into \( Y \) and \( XD \) through the reaction \( XDY_p \rightarrow XD + Y \).
2. Convert all \( XD, X, \) and \( XT \) to \( X_p \) through the chain of reactions \( XD \rightarrow X \rightarrow XT \rightarrow X_p \).
3. Convert as much \( Y \) into \( Y_p \) as possible through \( X_p + Y \rightarrow X_pY \rightarrow X + Y_p \).
4. Repeat 2. and 3. until all \( Y \) is converted to \( Y_p \) and all \( XD, X, \) and \( XT \) is converted to \( X_p \).

This algorithm converts all derivatives of \( X \) into \( X_p \) and all derivatives of \( Y \) into \( Y_p \). Although such a chain of reactions is a low probability event, it is irreversible and by standard probabilistic arguments the process will converge to the absorbing state with a probability of one. Note also that this conclusion holds for any choice of stoichiometric admissible propensities, not just mass-action propensities (18).

These examples demonstrate that the stability of robust equilibrium concentrations predicted for deterministic mass-action systems (4) may not be taken for granted when the underlying stochastic nature of the chemical reactions should not be ignored. In the next section we show that this difference in long-term behavior is the rule, as opposed to the exception. In Section 4 we discuss both the time until such absorption takes place and, in the case of a large expected time until absorption, the methods used to understand the behavior of these processes before absorption takes place.

### 3.2 Statements and proofs of main results

We restate two definitions presented in the main article text.

**Definition 3.1.** We say that the complex \( y \in \mathcal{C} \) is *dominated* by the complex \( y' \in \mathcal{C} \) (denoted \( y \ll y' \)) if \( y_i' \leq y_i \) for all \( i = 1, \ldots, m \).

**Definition 3.2.** A chemical reaction network \( (\mathcal{S}, \mathcal{C}, \mathcal{R}) \) is said to be conservative if there exists a vector \( w \in \mathbb{R}_{>0}^m \) such that \( w \cdot (y' - y) = 0 \) for all \( y \rightarrow y' \in \mathcal{R} \).

We again note that whenever two complexes differ in only one species, one necessarily dominates the other. For example, the complex \( X_1 + X_2 \) is dominated by the complex \( X_1 \) because we have that \( y = (1, 1) \) and \( y' = (1, 0) \) satisfy \( y_i' \leq y_i \) component-wise. Note that if \( y \ll y' \) and there are insufficient molecular counts for reactions out of \( y' \) to proceed, then there are necessarily insufficient counts for reactions out of \( y \) to proceed. We also reiterate the intuition that Definition 3.2 implies the existence of a conserved quantity \( M > 0 \) so that for the given species set \( \{X_1, X_2, \ldots, X_m\} \) we have that
\[
M := w_1c_1(t) + w_2c_2(t) + \cdots + w_mc_m(t)
\]
is invariant to the dynamics of the system.

The following is our main technical result. Note that it reduces the problem of determining the long-term dynamics of a stochastically modeled system to one of linear algebra.

**Theorem 3.3.** Let \( (\mathcal{S}, \mathcal{C}, \mathcal{R}) \) be a conservative chemical reaction network for which the following assumptions hold:

1. There are non-empty sets of non-terminal complexes \( \mathcal{C}^* \) and \( \mathcal{C}^{**} \) such that, if \( y \in \mathcal{C}^* \) then there exists a \( y' \in \mathcal{C}^{**} \) such that \( y \ll y' \).

2. For some choice of rate constants \( \{k_i\}_{i=1}^r \), the following property holds: if \( \Psi \in \ker(YA_k) \) and \( \Psi_{\bar{y}} = 0 \) for all \( y \in \mathcal{C}^* \), then \( \Psi_{\bar{y}} = 0 \) for all non-terminal \( \bar{y} \).
Then, for any choice of stoichiometrically admissible kinetics, all non-terminal complexes of the network are off at each positive recurrent state of the stochastically modeled system.

**Remark 2.** The second condition in assumption 2 above may also be formulated in the following way, which may be more intuitive to some readers.

2'. For some choice of rate constants \( \{k_i\}_{i=1}^r \), the following property holds: if \( \Psi \in \ker(YA_k) \) has support on a non-terminal complex, then \( \Psi \) has support on some \( y \in \mathcal{C}^* \).

We know that when complexes are off we have that at least one chemical species is “near” zero. This result therefore says that trajectories of the stochastic networks satisfying the required conditions will, with a probability of one, get stuck near the boundary of the positive orthant, even if the deterministic model predicts stability of a robust positive equilibrium concentration. This results therefore represents a fundamental disparity in the long-term predictions of the corresponding deterministically and stochastically modeled systems satisfying the above structural conditions.

It can be easily checked that the networks (1) and (2) satisfy the assumptions of Theorem 3.3. To motivate the proof of Theorem 3.3 we first develop some intuition based on these networks. A key observation in showing the boundary states (22) and (23) were accessible was that we never used a reaction from the dominated non-terminal complex (i.e. we never used the reaction \( A + B \rightarrow 2B \) or \( XD + Y_p \rightarrow XDY_p \), respectively). Consequently, when constructing our sequence of reactions we were actually considering a subnetwork which excluded these reactions. This suggests the following algorithm for showing the boundary is accessible:

1. Identify the sets of non-terminal complexes \( \mathcal{C}^* \) and \( \mathcal{C}^{**} \) satisfying assumption 1 of Theorem 3.3.
2. Remove all reactions out of the complexes in \( \mathcal{C}^* \).
3. Use condition 2. to show that all non-terminal complexes of the reduced network are off at each positive recurrent state; in particular, show that no positive recurrent state has mass on any \( y' \in \mathcal{C}^{**} \). Furthermore, show that the conservation property guarantees the accessibility of some set of positively recurrent states.
4. Use the condition \( y \ll y' \) to conclude the result for the original system.

The removal of the reactions will allow us to proceed with a proof by contradiction. Specifically, by consideration of the reduced network we will be able to construct a vector contradicting condition 2. in the statement of the theorem.

The idea of removing reactions from a network is captured by the following definition.

**Definition 3.4.** Let \( \mathcal{D}^+ \subset \mathcal{C} \) denote a set complexes of the chemical reaction network \( (\mathcal{S}, \mathcal{C}, \mathcal{R}) \) and \( \mathcal{R}' \subset \mathcal{R} \) denote the set of all reactions in \( \mathcal{R} \) for which some complex in \( \mathcal{D}^+ \) is the source complex. We define the \( \mathcal{D}^+\)-reduced subnetwork to be the triple \( (\mathcal{S}, \mathcal{C}, \mathcal{R}') \) where \( \mathcal{R}' = \mathcal{R} \setminus \mathcal{R}' \).

In other words, for each \( y \in \mathcal{D}^+ \), we remove from the network the set of reactions for which \( y \) is the reactant complex. Note that we have not changed the set of species or complexes, even though we may now have species and/or complexes not involved in any reaction (and so this is no longer technically a reaction network by Definition 2.1).

For example, if the original network is

\[
A \leftarrow 2B \rightarrow C, \quad B \rightarrow D,
\]

which has species \( \mathcal{S} = \{A, B, C, D\} \), complexes \( \mathcal{C} = \{A, 2B, C, B, D\} \), and reactions as given above, then the \( 2B \)-reduced network is

\[
A, \quad 2B, \quad C, \quad B \rightarrow D,
\]

with species \( \mathcal{S} = \{A, B, C, D\} \), complexes \( \mathcal{C} = \{A, 2B, C, B, D\} \), and reduced reaction set \( \{B \rightarrow D\} \). The \( 2B, B \)-reduced network is simply

\[
A, \quad 2B, \quad C, \quad B, \quad D.
\]

**Proof of Theorem 3.3.** Fix a choice of rate constants \( \{k_i\}_{i=1}^r \) for which the property of assumption 2 in the statement of the theorem holds. The proof will proceed by first showing that the conclusion of the theorem holds under the assumption of stochastic mass-action kinetics with these rate constants. We then extend to any admissible kinetics.

Let \( \mathcal{C}^* \) and \( \mathcal{C}^{**} \) be the non-empty sets satisfying assumption 1 of Theorem 3.3. Let \( (\mathcal{S}, \mathcal{C}, \mathcal{R}^*) \) denote the \( \mathcal{C}^* \)-reduced subnetwork of \( (\mathcal{S}, \mathcal{C}, \mathcal{R}) \). Let \( X^*(t) \) denote the continuous time Markov chain associated with the \( \mathcal{C}^* \)-reduced subnetwork with stochastic mass-action kinetics and rate constants \( k_i \) if \( y_i \rightarrow y'_i \in \mathcal{R}^* \).
For the time being we will only consider the chain $X^*(t)$ for the reduced network. We will show that there does not exist a positive recurrent state for the chain $X^*(t)$ which has mass on a non-terminal complex. This portion of the proof will proceed by contradiction. That is, we first suppose that there is a positive recurrent $X_0$ which has mass on a non-terminal complex, and will eventually conclude that assumption 2 is violated. After showing the result for $X^*(t)$, we will show the result holds for the original chain with any choice of stoichiometrically admissible kinetics.

Let $C_{\text{source}} \subset C$ denote the set of complexes which are source complexes for reactions in the $C^*$-reduced network and let $I$ be the irreducible component containing $X_0$. Note that, by construction,

$$C_{\text{source}} \cap C^* = \emptyset. \tag{25}$$

We define

$$C_I := \{ y \in C \mid y \in C_{\text{source}} \text{ and there is an } X \in I \text{ with mass on } y \}. \tag{26}$$

These are the relevant source complexes on the state space $I$. Note that our assumption that $X_0$ has mass on a non-terminal complex of the reduced network implies that there is at least one non-terminal complex contained in $C_I$.

Now consider the process $X^*(t)$ with initial condition $X^*(0) = X_0 \in I$. We denote the $N$th return time to $X_0$ as $t_N$. It follows that we have $X^*(t_N) = X^*(0)$ for all $N$ so that (16) gives

$$X^*(t_N) = X^*(0) + \sum_{y \in C_I} N_i(t_N)(y_i - y_i) = X^*(0), \tag{27}$$

where we are only summing over those reactions that admit positive propensities for at least one state in $C_I$ (all other propensities are identically zero for all time). Since we are for the time being assuming mass-action kinetics (18), we have from (16), (17), and (26) that for each $N > 0$,

$$\sum_{y \in C_I} Y_i \left( \frac{k_i}{V|y_i| - 1} \int_0^{t_N} \prod_{j=1}^m \frac{X^*_j(s)!}{(X^*_j(s) - y_j)!y_j!} ds \right) (y_i - y_i) = 0, \tag{28}$$

where $Y_i(\cdot)$ are independent unit-rate Poisson processes.

For any complex $y \in C$, we define the function $G_y : \mathbb{R}^m \to \mathbb{R}_{\geq 0}$, via

$$G_y(X) = \begin{cases} \frac{1}{V|y| - 1} \prod_{j=1}^m \frac{X_j!}{(X_j - y_j)!y_j!}, & \text{for } y \in C_I \text{ and } X \in I \\ 0, & \text{otherwise.} \end{cases} \tag{29}$$

We note two things. First, $G_y \equiv 0$ whenever $y \in C \setminus C_I$. In particular, by (25) we have that $G_y \equiv 0$ for all $y \in C^*$. Second, note that (28) does not depend on the reaction rate constants; rather, it quantifies the intensity of the state-dependent portion of the mass-action term (18) of all the reactions with source complex $y$.

Returning to (27), by multiplying and dividing by appropriate terms, we find

$$\sum_{y \in C_I} k_i \left[ \frac{1}{k_i} \int_0^{t_N} G_{y_i}(X^*(s)) ds \right] \vee 1 \left( k_i \int_0^{t_N} G_{y_i}(X^*(s)) ds \right) \vee 1 \left( k_i \int_0^{t_N} G_{y_i}(X^*(s)) ds \right) \vee 1 \left( k_i \int_0^{t_N} G_{y_i}(X^*(s)) ds \right) = 0$, \tag{30}$$

where $a \vee b = \max\{a, b\}$. Since $I$ is a positive recurrent class, for any $y_i \in C_I$ we have that

$$\int_0^{t_N} G_{y_i}(X^*(s)) ds \to \infty, \text{ almost surely as } N \to \infty,$$

and so

$$\lim_{N \to \infty} \left[ k_i \int_0^{t_N} G_{y_i}(X^*(s)) ds \right] \vee 1 \left( k_i \int_0^{t_N} G_{y_i}(X^*(s)) ds \right) = 1, \text{ almost surely as } N \to \infty, \tag{31}$$

where we have applied the law of large numbers to the Poisson process $Y_i$. Denoting $\pi_I$ as the unique stationary distribution of $X^*$ on $I$, by basic ergodicity properties of continuous time Markov chains we have that

$$\lim_{N \to \infty} \frac{1}{t_N} \int_0^{t_N} G_{y_i}(X^*(s)) ds = \sum_{X \in I} \pi_I(X) G_{y_i}(X),$$
for all complexes \( y_i \subset C \). We now define the vector \( G_{\pi_x} \in \mathbb{R}^C \) via

\[
[G_{\pi_x}]_y := \sum_{X \in I} \pi_T(X)G_y(X),
\]

for \( y \in C \) and note that \( [G_{\pi_x}]_y > 0 \) if and only if \( y \in C_x \), and we reiterate that by our original assumptions \( C_x \) contains at least one non-terminal complex, but does not contain any \( y \in C^* \).

Combining (29) - (32), we have

\[
\sum_{y_i \in C_x} \sum_{y_i = 1}^{r} k_i(y'_i - y_i) [G_{\pi_x}]_{y_i} = 0,
\]

and so

\[
0 = \sum_{y \in C_x} \left( \sum_{y_i = 1}^{r} k_i(y'_i - y_i) \right) [G_{\pi_x}]_y = \sum_{y \in C} \left( \sum_{y_i = 1}^{r} k_i(y'_i - y_i) \right) [G_{\pi_x}]_y,
\]

where the first equality is a reordering of terms, and the second equality follows since \( [G_{\pi_x}]_y = 0 \) for \( y \notin C_x \). Hence, the vector \( G_{\pi_x} \in \mathbb{R}^C \) is contained in the kernel of \( YA_k \). By construction, however, we have

(i) \( [G_{\pi_x}]_y = 0 \) for each \( y \in C^* \).

(ii) \( [G_{\pi_x}]_{y} > 0 \) for at least one non-terminal complex,

which contradicts assumption 2 in the statement of the theorem. Consequently, we can conclude that there are no positive recurrent states of the \( C^* \)-reduced network with mass on any non-terminal complexes, including any \( y' \in C^{**} \). Note that it was by considering the reduced network, as opposed to the original network, that allowed us to conclude (i) above, which led us to the contradiction.

The above analysis shows that the conclusion of the theorem holds for the \( C^* \)-reduced subnetwork \((S, C, R^*)\) taken with mass-action propensities (18). We now argue that the result holds for the original network \((S, C, R)\) with any stoichiometrically admissible propensities. We make the following observations:

(i) \( S \) \( C^* \)-reduced subnetwork \((S, C, R^*)\) can occur in the original network \((S, C, R)\). In other words, for any two states \( X, Y \in \mathbb{Z}^m_{\geq 0} \) such that \( X \sim Y \) for \((S, C, R^*)\), we have \( X \sim Y \) for \((S, C, R)\).

(ii) \( S \) by the domination property \( y \ll y' \), any state \( X \in \mathbb{Z}^m_{\geq 0} \) which does not not have mass on any \( y' \in C^{**} \) also does not have mass on any \( y \in C^* \).

(iii) \( S \) the connectivity properties of a stochastic chemical reaction system with mass-action propensities and one with general stoichiometrically admissible propensities are identical. That is to say, for two states \( X, Y \in \mathbb{Z}^m_{\geq 0} \), \( X \sim Y \) for a stochastic mass-action system if and only if \( X \sim Y \) for the corresponding stochastic reaction system with general stoichiometrically admissible propensities.

Now consider any closed, irreducible, positive recurrent component \( I \) of the state space of the stochastic reaction system \((S, C, R^*)\), where we are for the time being still assuming mass-action kinetics with the specified rate constants. By our previous argument, we have that \( I \) does not contain a state which has mass on \( y' \in C^{**} \). Hence, it follows from (ii) that \( I \) does not contain a state that has mass on any \( y \in C^* \). Furthermore, since \( R \) and \( R^* \) differ only in reactions with source complexes \( y \in C^* \), we have exactly the same connectivity properties on \( I \) for \((S, C, R)\) as we do for the \( C^* \)-reduced subnetwork \((S, C, R^*)\). It follows that \( I \) is a closed irreducible component of the stochastic reaction system \((S, C, R)\) and, since \( I \) was chosen arbitrarily, it follows that every closed irreducible component of \((S, C, R^*)\) is a closed irreducible components of \((S, C, R)\). It furthermore follows from (i) that these are the only positive recurrent communication classes for \((S, C, R)\). Hence, the result is shown for the system \((S, C, R)\) when the kinetics are mass-action with rate constants \( k_i \). We now note that by (iii), the generalization to any choice of stoichiometrically accessible kinetics is trivial.

Thus, we conclude that all trajectories will be absorbed by states for which only the terminal complexes can be the sources (and hence the products) of reactions. An immediate corollary to Theorem 3.3 is the following, which is Theorem 2 in the main article text.
Theorem 3.5. Let \( (S, C, R) \) be a conservative chemical reaction network for which the following assumptions hold:

1. For some choice of rate constants \( \{k_i\}_{i=1}^r \), the deterministically modeled mass-action system admits a positive equilibrium;

2. The deficiency of the network is one (i.e. \( \delta = 1 \));

3. There are two non-terminal complexes, \( y \) and \( y' \) say, for which \( y \ll y' \).

Then, for any choice of stoichiometrically admissible kinetics, all non-terminal complexes of the network are off at each positive recurrent state of the stochastically modeled system.

Remark 3. Since the conditions of Theorem 2.4 are a subset of these conditions, this result says that every conservative network guaranteed to exhibit absolute concentration robustness by Theorem 2.4 in the deterministic setting has trajectories \( X(t) \) which eventually have an absorption event in the stochastic setting. Notably, this is true even when the deterministic model predicts stability of the positive ACR concentration.

Proof of Theorem 3.5. Let \( \{\Lambda_1, \ldots, \Lambda_t\} \) be the terminal strong linkage classes of the network. It is sufficient to show that for some choice of rate constants \( \{k_i\}_{i=1}^r \), the support of each element of the kernel of \(YA_k \) either contains all the non-terminal complexes, or none of them. The arguments we employ are taken from [1].

By assumption 1 there is a set of rate constants, \( \{k_i\}_{i=1}^r \), for which the deterministically modeled system (4) admits a positive equilibrium, \( \bar{c} \in \mathbb{R}_{>0}^m \). That is,

\[
\sum_{i=1}^r k_i(y'_i - y_i)\bar{c}^{y_i} = \sum_{y \in C} \left[ \sum_{i=1}^r k_i(y'_i - y_i) \right] \bar{c}^y = 0, 
\]

where the inner sum is over all reactions out of complex \( y \). It follows from (12) that \( \bar{c}^Y \in \ker(YA_k) \) where \( \bar{c}^Y \in \mathbb{R}^C \) is the vector with entries \( [\bar{c}^Y]_y = \bar{c}^y \). By assumption 2 (that the deficiency of the network is one) and Lemma 2.6 we have that \( \dim(\ker(YA_k)) \leq 1 + t \). It follows from Lemma 2.5 that \( \ker(YA_k) \) has a basis \( \{\bar{c}^Y, b_1, \ldots, b_t\} \) where the \( b_i, i = 1, \ldots, t \), have support on the \( i \)th terminal strong linkage class, \( \Lambda_i \). Thus, any \( \Psi \in \ker(YA_k) \) can be written

\[
\Psi = \lambda_0 \bar{c}^Y + \sum_{\theta=1}^t \lambda_\theta b_\theta,
\]

where \( \lambda_i \in \mathbb{R}, i = 0, \ldots, t \), are constants. It is clear that \( \bar{c}^Y \) has support on all the non-terminal complexes and the result is shown.

3.3 The post-absorption network

We now consider the structure and long-term behavior of the post-absorption network. Let \( \{S, C, R\} \) be a reaction network satisfying the assumptions of Theorem 3.5. Let \( C' \subset C \) denote those complexes that are both (i) contained in a terminal strong linkage class and (ii) the source complex for some reaction. Note that due to the definition of a terminal strong linkage class, each element of \( C' \) is also a product complex for some reaction. Let \( R' \) denote the reactions with source complexes \( C' \) and denote by \( S' \) those species in the support of some element of \( C' \). Then \( \{S', C', R'\} \) is the reaction network which could still be “on” after the absorption event guaranteed by Theorem 3.5. We note that \( \{S', C', R'\} \) could be trivial in that \( S' = C' = R' = \emptyset \), otherwise we say it is non-trivial. For example, for both networks in our running examples (1) and (2), the post-absorption network is trivial; whereas, the post-absorption network for

\[
X \rightleftharpoons Y, \quad A \to B + X, \quad A + B + X \to 2A,
\]

which satisfies the assumptions of Theorem 3.5, is \( X \rightleftharpoons Y \). Note that the network (35) does not satisfy the requirements of Theorem 2.4 and can be checked explicitly to not exhibit ACR in any species when modeled deterministically.

The following lemma, combined with results from [40], characterizes the limiting behavior of the stochastically modeled system after absorption assuming stochastic mass-action kinetics: a stationary distribution is

\[
\pi'(x) = C \prod_{i=1}^{\mid S' \mid} \frac{(c'_i)^{x_i}}{x_i!}, \quad x \in \Gamma,
\]
subject to the necessary conservation relations, where $\Gamma$ is the resulting state space, $c'$ is a positive equilibrium of the deterministically modeled mass-action system associated with the network $\{S', C', R'\}$, and $C$ is a normalizing constant.

**Lemma 3.6.** Suppose the assumptions of Theorem 3.5 hold for the network $\{S, C, R\}$ and that the post-absorption network $\{S', C', R'\}$ is non-trivial. Then $\{S', C', R'\}$ is weakly reversible and has a deficiency of zero.

**Proof.** The network $\{S', C', R'\}$ is weakly reversible by construction. From the proofs of Theorems 3.3 and 3.5, one basis of the kernel of $Y' A_k$, which are the associated linear operators for the system $\{S', C', R'\}$, has elements, $\{b_1, \ldots, b_p\}$, where $b_y$ only has support on the $y$th terminal strong linkage class of $\{S', C', R'\}$. By Lemma 2.5 and Proposition 5.1 in [4], we can conclude that the network has a deficiency of zero. \hfill $\Box$

### 3.4 Comments on Theorems 3.3 and 3.5

In this section, we make a few notes and comments on Theorems 3.3 and 3.5. In Section 3.4.1 we demonstrate through an example that there is a difference in content between Theorems 3.3 and 3.5. In Section 3.4.2, we demonstrate through an example the importance of the conservation relation condition in our results. Finally, in Section 3.4.3 we provide motivation for a conjecture for even more general results pertaining to all ACR models.

#### 3.4.1 Importance of the kernel condition: higher deficiency models

It is clear that both the networks (1) and (2) satisfy the assumptions of Theorem 3.5. However, Theorem 3.5 stands silent on models with a deficiency greater than one. Here we provide an example of a deficiency two network taken from the literature that satisfies the assumptions of Theorem 3.3, demonstrating that there is a true difference in content between the two theorems.

**Example 3:** Consider the following generalization of Example 2, in which the phosphatase stimulation effects of both ATP and ADP are simultaneously considered:

\[
\begin{align*}
XD & \xrightleftharpoons[k_{12}]{k_1} X \xrightleftharpoons[k_3]{k_5[T]} XT \xrightarrow[k_4]{k_3} X_p \\
X_p + Y & \xrightleftharpoons[k_6]{k_7} X_pY \xrightarrow[k_8]{k_9} X + Y_p \\
XD + Y_p & \xrightleftharpoons[k_{10}]{k_6} XDY_p \xrightarrow[k_{11}]{k_9} XD + Y \\
XT + Y_p & \xrightleftharpoons[k_{13}]{k_{12}} XTY_p \xrightarrow[k_{14}]{k_9} XT + Y_p.
\end{align*}
\]  

(36)

This model was shown in [1] to have a deficiency of two, but still exhibit absolute concentration robustness in $Y_p$. They showed this through an explicit calculation of the equilibria as opposed to using theoretical results. As the deficiency of this network is two, Theorem 3.5 is inapplicable. To apply Theorem 3.3 we note that there are two pairs of complexes satisfying $y \ll y'$:

\[
XD + Y_p \ll XD, \quad \text{and} \quad XT + Y_p \ll XT.
\]

That is to say, we have $C^* = \{XD + Y_p, XT + Y_p\}$ and $C^{**} = \{XD, XT\}$ in assumption 1 of Theorem 3.3. We consider the following ordering of the non-terminal complexes, which are the only source complexes in this model:

\[
\{XD, X, XT, X_p + Y, X_pY, XD + Y_p, XDY_p, XT + Y_p, XTY_p\}.
\]  

(37)

Choosing the rate constants $k_1 = \cdots = k_{13} = 1$, there are two basis vectors of $YA_k$ with non-zero support on the complexes as ordered in (37); the components of these basis vectors restricted to the first 9 components (corresponding with the non-terminal complexes) are

\[
\{[2, 2, 1, 2, 1, 2, 1, 0, 0], [2, 2, 1, 2, 1, 0, 0, 2, 1]\}.
\]

Since $XD + Y_p$ only has support on the first basis vector above, and $XT + Y_p$ only has support on the second, we see that assumption 2 of Theorem 3.3 is satisfied, and the conclusion holds.

15
3.4.2 Importance of the conservation relation

Here we provide an example in which the conclusions of Theorem 3.5 do not hold if only the conservation assumption is dropped.

Example 4: Consider the chemical reaction network

\[ A + B \xrightarrow{\alpha} 0 \]
\[ B \xrightarrow{\beta} A + 2B. \]

The network has a deficiency of one, the non-terminal complexes \( A + B \) and \( B \) differ only in the species \( A \), and the corresponding deterministic mass-action systems have positive equilibria so long as \( c_A(0) - c_B(0) < \beta/\alpha \). It follows by Theorem 2.4 that the corresponding mass-action systems exhibit absolute concentration robustness in \( A \). It can be checked explicitly that the equilibrium value is

\[ \bar{c}_A = \frac{\beta}{\alpha}. \]

The corresponding stochastic chemical reaction system, however, does not necessarily converge to the boundary as predicted by Theorem 3.3. For any compatibility class \( M := X_B - X_A \) where \( M \geq 1 \) we can use results related to birth and death processes to conclude that the stationary distribution for the process tracking the number of \( A \) molecules has the form

\[ \pi^M(i) = \frac{K^M}{(M + i)!} \left( \frac{\beta}{\alpha} \right)^i \]

where \( K^M \) is the normalizing constant

\[ K^M = \left( \sum_{j=0}^{\infty} \frac{1}{(M + j)!} \left( \frac{\beta}{\alpha} \right)^j \right)^{-1}. \]

Interestingly, it can be seen by standard analytical methods [41] that for any \( n \geq 0 \) we have

\[ \lim_{M \to \infty} \pi^M(i) = \left[ \frac{1}{i!} \left( \frac{\beta}{\alpha} \right)^i \right] / \left[ \sum_{j=0}^{\infty} \frac{1}{j!} \left( \frac{\beta}{\alpha} \right)^j \right] \]

\[ = e^{-\frac{\beta}{\alpha}} \left( \frac{\beta}{\alpha} \right)^i. \]

In the limit as \( M \to \infty \), therefore, we have that the stationary distribution approaches a Poisson distribution with mean \( \beta/\alpha \).

It is easy to see how the argument presented in Section 3.2 breaks down. Since the network (38) is not conservative, we are not guaranteed that the \( \{A + B\}\)-reduced subnetwork obtained by removing reactions from \( A + B \) has a positively recurrent irreducible component. The relevant subnetwork for this example is \( B \to A + 2B \) which clearly grows unboundedly and therefore does not possess such a component.

3.4.3 Conclusions hold for further ACR models

We have already seen with (36) that, while the conditions of Theorem 2.4 are sufficient for ACR in deterministically-modeled mass-action systems, they are not necessary. It is also the case that, while Theorems 3.3 and 3.5 capture some ACR systems not captured by Theorem 2.4, they do not capture all such systems. Nevertheless, a sample of such systems suggests that the conclusions of Theorems 3.3 and 3.5 hold for a much wider class of conservative ACR networks. We now present one such system.
**Example 5:** Consider the following network, which is taken from [42]:

\[
\begin{align*}
A + X &\rightarrow_{k_1} F + Y \\
A &\rightarrow_{k_2} B \\
C + F &\rightarrow_{k_3} E + D + F \\
B + D &\rightarrow_{k_4} A + C \\
X &\rightarrow_{k_5} Y.
\end{align*}
\]  

(39)

The system is governed by the system of differential equations

\[
\begin{align*}
\dot{c}_A &= -k_1c_Ac_X + k_2c_Fc_Y - k_3c_A + k_6c_Bc_D \\
\dot{c}_B &= k_3c_A - k_6c_Bc_D \\
\dot{c}_C &= -k_4c_Cc_F + k_5c_Bc_D \\
\dot{c}_D &= k_5c_E - k_6c_Bc_D \\
\dot{c}_E &= k_4c_Cc_F - k_5c_E \\
\dot{c}_F &= k_1c_Ac_X - k_2c_Fc_Y - k_4c_Cc_F + k_5c_E \\
\dot{c}_X &= -k_1c_Ac_X + k_2c_Fc_Y - k_7c_X + k_8c_Y \\
\dot{c}_Y &= k_1c_Ac_X - k_2c_Fc_Y + k_7c_X - k_8c_Y.
\end{align*}
\]  

(40)

It was shown in [42] that every positive equilibrium concentration satisfies

\[
\bar{c}_C = \frac{k_2k_3k_7}{k_1k_4k_8}.
\]

That is to say, the system (40) is ACR in the concentration of \( C \).

Despite the system being demonstrably ACR in \( C \), however, it is not amenable to Theorem 2.4. The network (39) is deficiency one (\( \delta = n - \ell - s = 11 - 5 - 5 = 1 \)) but there do not exist two non-terminal complexes which differ only in the species \( C \) (or any species). Since no non-terminal complex is dominated by any other non-terminal complex, we are also unable to apply Theorem 3.3 or Theorem 3.5.

Nevertheless, we can see that there does not exist a positively recurrent state with mass on any non-terminal complex. To see this, we start by considering the following conservation laws:

\[
\begin{align*}
T_{XY} &:= X(0) + Y(0) \\
T_{ABDF} &:= A(0) + B(0) + D(0) + F(0) \\
T_{CDE} &:= C(0) + D(0) + E(0).
\end{align*}
\]  

(41)

Now consider the following chain of reactions from an arbitrary positive starting count in each species:

1. Convert all \( E \) into \( D \) and \( F \) through \( E \rightarrow D + F \).
2. Convert all \( F \) into \( A \) through \( F + Y \rightarrow A + X \) (replenishing \( Y \) with \( X \rightarrow Y \) as necessary).
3. Convert all \( D \) into \( C \) through \( B + D \rightarrow A + C \) (replenishing \( B \) with \( A \rightarrow B \) as necessary).
4. Convert all \( A \) into \( B \) through \( A \rightarrow B \).

From the conservation laws (41), the final state of this algorithm is \( \bar{X}_A = 0, \bar{X}_B = T_{ABDF}, \bar{X}_C = T_{CDE}, \bar{X}_D = 0, \bar{X}_E = 0, \bar{X}_F = 0, \bar{X}_X > 0, \bar{X}_Y > 0 \), from which no reaction from a non-terminal complex may proceed. In fact, the only reactions which may proceed are \( X \rightarrow Y \) and \( Y \rightarrow X \). It follows that, with a probability of one, there is a final time for which any chain has positive mass on any non-terminal complex. This is far removed from the deterministic prediction for the long-term value of \( X_C \).

We therefore make the following conjecture:

**Conjecture 3.7.** Consider a conservative chemical reaction network \((S,C,R)\) and associated stochastic chemical reaction system with some stoichiometrically admissible kinetics. Suppose that for some choice of rate constants \( \{k_i\}_{i=1}^n \) the deterministically modeled mass-action system exhibits ACR in some species \( X_i \). Then the conclusions of Theorems 3.3 and 3.5 hold.
4 Time until absorption and quasi-stationary distributions

If the time to absorption is large relative to the relevant time-scales of the system, the processes will seem to settle down to an equilibrium long before the resulting instability will appear. This limiting distribution is called a quasi-stationary distribution, and we refer the reader to [43, 44] for a proof of that fact that such distributions exists, and are unique, in the current setting. The resulting distribution bridges the gap between the extinction event and the transient behavior of the process.

In this section, we briefly introduce the notation and background results relevant to the study of quasi-stationary distributions, though we refer the interested reader to [44] for a recent survey on quasi-stationary distributions and population processes for a thorough introduction to the topic, including bibliography. We apply the results to the activation/deactivation network (1) and the EnvZ/OmpR signaling network (2).

4.1 Quasi-stationary probability distributions

We divide the state space $\mathbb{Z}_{\geq 0}^m$ into the set $X_T$ of transient states and $X_A$ of absorbing states. There are two related notions that capture the relevant long-term transient dynamics which we take from [44].

**Definition 4.1.** We say that the process $X(t)$ has a Yaglom limit if there exists a probability distribution $\tilde{\pi}$ on $X_T$ such that, for any $X, Y \in X_T$,

$$
\lim_{t \to \infty} P(X(t) = Y \mid X(t) \notin X_A, X(0) = X) = \tilde{\pi}(Y).
$$

**Definition 4.2.** Let $\tilde{\pi}$ be a probability distribution on $X_T$. We say that $\tilde{\pi}$ is a quasi-stationary distribution (QSD) if, for all $t \geq 0$ and $Y \in X_T$

$$
\tilde{\pi}(Y) = P_\pi(X(t) = Y \mid X(t) \notin X_A),
$$

where $P_\pi$ is the distribution of the process given an initial distribution of $\tilde{\pi}$.

In other words, the Yaglom limit is the limiting distribution of a chain conditioned on not entering the absorbing set, while the quasi-stationary probability distribution is the distribution which remains unchanged under the condition that the associated chain does not enter a state in the absorbing set. In the current setting of a continuous time Markov chain with a finite state space, the existence, uniqueness, and equivalence of the Yaglom limit and QSD is established [43, 44].

A sometimes useful formula for the quasi-stationary distribution $\tilde{\pi}$ can be derived by first introducing the conditional probabilities

$$
Q_t(X) := P(X(t) = X \mid X(t) \notin X_A) = \frac{P_t(X)}{1 - P_t(X_A)}
$$

where $P_t(X_A) := P(X(t) \in X_A)$ is the probability the chain has entered an absorbing state by time $t$. In the present setting of a CTMC with finite state space we may differentiate (43) and collect terms appropriately to see that the evolution of the conditioned probabilities $Q_t$ satisfy a system of ordinary differential equations. Setting the resulting set of equations to zero, substituting $\tilde{\pi}$ for $Q_t$, and solving yields the system

$$
\tilde{\pi},A_Q = \theta \tilde{\pi},
$$

where $A_Q$ is the restriction of the jump rate matrix $A$ given in (20) to the transient states $X_T$. The term $\theta$ is negative and can be explicitly computed [44], but is not important for our purposes. A simple calculation using the Perron-Frobenius Theorem shows that there is a unique solution to (44) with strictly positive components, which is also the unique quasi-stationary distribution; see, for example, [13, 45].

Consideration of the quasi-stationary distribution $\tilde{\pi}$ is most appropriate when the chain $X(t)$ is expected to spend significant time in the transient region before converging to the absorbing set. In order to quantify this hitting time, it is typical to define

$$
\tau_A(X) := \inf(t \geq 0 \mid X(t) \in X_A, X(0) = X)
$$

to be the first time the chain $X(t)$ with initial condition $X(0) = X$ enters the absorbing set. The quantity $\mathbb{E}(\tau_A(X))$ is called the expected time to absorption. Explicit formulas for $\mathbb{E}(\tau_A(X))$ for continuous time Markov chains with countable state spaces may be found in Section 6.7 of [6].
respectively, from the state $X$ determining the behavior of the chain $X$. Figure 4.1: Birth-death chain corresponding to the activation/deactivation network (1). The number $i$ corresponds to the count of $X_B$ and the values $\lambda(i)$ and $\mu(i)$ correspond to the transition propensities of the first and second reaction, respectively, from the state $X_B = i$. The chain has a unique sink at $i = 0$.

We consider the stochastic model corresponding to (1) for a fixed value $M := X_A(0) + X_B(0) > 0$. Notice that determining the behavior of the chain $X_B(t)$ is sufficient to determine the chain on $A$ by the conservation relationship $X_A(t) = M - X_B(t)$. Also notice that the first reaction in (1) increases $X_B$ by one while the second reaction decreases $X_B$ by one. Consequently, the network (1) corresponds to a birth-death chain with an absorbing state at $X_B = 0$. For a graphical illustration of the state space of the chain $X_B$, see Fig. 4.1. The propensities $\lambda(i)$ and $\mu(i)$ are given by

$$\lambda(i) = \alpha X_B X_A = \alpha i (M - i),$$
$$\mu(i) = \beta X_B = \beta i.$$  \hfill (45)

The chain $X_B(t)$ with transition propensities (45) has been considered in a number of different contexts. The chemical reaction network (1) is considered explicitly in [8] where only the labeling of the species differs. A more extensive analysis is given in the papers [12, 13, 15, 46] where the chain $X_B(t)$ corresponds to chain of infected individuals $I(t)$ in the susceptible-infected-susceptible (SIS) epidemic model. The model also arises in stochastic logistic population growth models on a finite state space [10, 14, 47].

The expected time until absorption has an explicit formula for birth-death chains with a countable state space [6]. We let $\tau_i$ be shorthand for the time to absorption from the state $X_B = i$. Substituting the propensities (45) into equation (6.22) of [6] and simplifying yields

$$\mathbb{E}(\tau_i) = \sum_{k=0}^{i-1} \sum_{j=k+1}^{M} \frac{1}{\beta} \left( \frac{\alpha}{\beta} \right)^{j-k-1} \frac{(M - k - 1)!}{j(M-j)!}$$  \hfill (46)

for $i = 1, \ldots, M$. The numerical results contained in Table 1 justify the consideration of the quasi-stationary distribution $\tilde{\pi}$ for values of $M$ where $\mathbb{E}(\tau_i)$ is large.

<table>
<thead>
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<th>$M$</th>
<th>$\mathbb{E}(\tau_1)$ (s)</th>
<th>$M$</th>
<th>$\mathbb{E}(\tau_1)$ (s)</th>
<th>$M$</th>
<th>$\mathbb{E}(\tau_1)$ (s)</th>
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<td>40</td>
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<td>65</td>
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</tr>
<tr>
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<td>70</td>
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</tr>
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<td>50</td>
<td>233.051</td>
<td>75</td>
<td>$6.87 \times 10^{11}$</td>
</tr>
</tbody>
</table>

Table 1: Expected time until absorption for the activation/deactivation system (1) from the state $(X_A, X_B) = (M - 1, 1)$ with parameter values $\alpha = 1, \beta = 25$. Note that convergence to a positive equilibrium concentration is predicted in the deterministic model for $M > 25$ but that the expecting hitting time for the absorbing state (22) grows only modestly in the range Mid-scale range of $M$.

4.2 Activation/deactivation system

Reconsider the activation/deactivation network (1). We have demonstrated previously that the deterministically modeled mass-action system (4) exhibits ACR in the species $A$ while trajectories of the stochastic chemical reaction system converge almost surely to the boundary state (22) in finite time. In order to search for ACR like behavior in the stochastic setting, therefore, we consider the quasi-stationary distribution when the expected time until absorption, $\mathbb{E}(\tau_A(X))$, is high.
A numerical iteration scheme for approximating the quasi-stationary distribution \( \tilde{\pi} \) of a finite-state birth and death process is given in [48] and adapted to the stochastic SIS model in [13]. Relabeling and rearranging the relevant parameters to fit (45), we have that the quasi-stationary distribution \( \tilde{\pi} \) of the CTMC corresponding to (1) with propensities (45) can be approximated as the limit of the iterative scheme

\[
\tilde{\pi}_j(1) = \sum_{i=1}^{M} \left[ \frac{1}{i} \left( \sum_{k=1}^{i} \frac{(M-k)!}{(M-i)!} \left( \frac{\beta}{\alpha} \right)^{i-k} \left( 1 - \sum_{l=1}^{i-1} \tilde{\pi}_{j-1}(l) \right) \right) \right] \\
\tilde{\pi}_j(i) = \frac{1}{i} \sum_{k=1}^{i} (M-k)! \left( \frac{\beta}{\alpha} \right)^{i-k} \left( 1 - \sum_{l=1}^{i-1} \tilde{\pi}_{j-1}(l) \right) \tilde{\pi}_{j-1}(1), \quad i = 2, \ldots, M.
\]

(47)

That is to say, we have \( \tilde{\pi}_j(i) \to \tilde{\pi}(i) \) as \( j \to \infty \) [48]. To obtain the quasi-stationary distribution in the ACR species \( A \), we need to invert \( \tilde{\pi} \). Hence, we compute the distribution \( \tilde{\pi}_A \) which has entries \( \tilde{\pi}_A(i) = \tilde{\pi}(M-i), i = 0, \ldots, M-1 \). The results are shown in Fig. 4.2.

A striking feature of the quasi-stationary distributions shown in Fig. 4.2 is that they appear to approach the Poisson distribution

\[
\tilde{\pi}(i) = e^{-\tilde{\pi}} \left( \frac{\beta}{\alpha} \right)^{i} \frac{i^i}{i!}, \quad (48)
\]

as \( M \to \infty \). It has recently been shown that the “one permanently infected” approximation of the quasi-stationary distribution approaches the Poisson distribution (48) in this limit [49]. For completeness, we now prove that the true quasi-stationary distribution approaches a Poisson distribution.

**Lemma 4.3.** Consider the stochastically modeled activation/deactivation network (1) with mass-action propensities (45). The quasi-stationary distribution of \( X_A \) converges to the Poisson distribution (48) in the limit \( M \to \infty \).

**Proof.** We first of all reformulate the propensities (45) to correspond to the chain tracking the number of \( A \) molecules. Parameterizing the model by \( M \), and keeping the convention that \( \lambda^M \) refer to “birth” and \( \mu^M \) refers to “death” (now with respect to species \( A \)), we have

\[
\lambda^M(i) = \beta(M-i) \quad \mu^M(i) = \alpha i(M-1-i). \quad (49)
\]

Note that this chain has a reflecting state at \( X_A = 0 \) and an absorbing state at \( X_A = M \). The transient states are \( \{0, \ldots, M-2\} \) so that the generator of (20) of the Markov process conditioned on non-absorption (the \( A_Q \) in (44)) has support on these states. By (44) we have that the quasi-stationary distribution for a given \( M \) satisfies

\[
\begin{align*}
\lambda^M(i-1)\tilde{\pi}^M_{i-1} - (\lambda^M(i) + \mu^M(i))\tilde{\pi}^M_i &+ \mu^M(i+1)\tilde{\pi}^M_{i+1} = \theta^M\tilde{\pi}^M_i \\
\lambda^M(M-2)\tilde{\pi}^M_{M-2} + \mu^M(M-1)\tilde{\pi}^M_{M-1} &- \mu^M(M-1)\tilde{\pi}^M_{M-1} = \theta^M\tilde{\pi}^M_{M-1}
\end{align*}
\]

(50)

where \( i = 1, \ldots, M-2 \) and \( \theta^M \) is the eigenvalue corresponding to the eigenvector equation (44) for the given \( M \). It can be directly computed that \( \theta^M = \beta\tilde{\pi}^M_M \), which we note is uniformly bounded in \( M [13] \). Substituting \( \theta^M = \beta\tilde{\pi}^M_M \) and (49) into (50) yields

\[
\begin{align*}
-\beta M\tilde{\pi}^M_0 &+ \alpha(M-1)\tilde{\pi}^M_1 = \beta\tilde{\pi}^M_M\tilde{\pi}^M_0 \\
\beta(M(i-1))\tilde{\pi}^M_{i-1} - (\beta(M-i) + \alpha M(M-1))\tilde{\pi}^M_i &+ \alpha(i+1)(M(i+1))\tilde{\pi}^M_{i+1} = \beta\tilde{\pi}^M_M\tilde{\pi}^M_i, \\
-2\beta\tilde{\pi}^M_{M-2} &+ \alpha\tilde{\pi}^M_{M-1} = \beta\tilde{\pi}^M_M\tilde{\pi}^M_{M-1}.
\end{align*}
\]
Dividing by \( M \) yields

\[
-\beta \tilde{\pi}_i^M + \alpha \left( 1 - \frac{1}{M} \right) \tilde{\pi}_i^M = \frac{\beta \tilde{\pi}_i^M \tilde{\pi}_0^M}{M}
\]

\[
\beta \left( 1 - \frac{(i-1)}{M} \right) \tilde{\pi}_{i-1}^M - \left( \beta \left( 1 - \frac{i}{M} \right) + \alpha i \left( 1 - \frac{i}{M} \right) \right) \tilde{\pi}_i^M + \alpha (i+1) \left( 1 - \frac{(i+1)}{M} \right) \tilde{\pi}_{i+1}^M = \frac{\beta \tilde{\pi}_i^M \tilde{\pi}_1^M}{M},
\]

for \( i = 1, \ldots, M - 2 \). The use of standard limiting arguments shows that as \( M \to \infty \), the vector \( \tilde{\pi}^M \) converges to the solution of the difference equations

\[
-\beta \pi_0 + \alpha \pi_1 = 0
\]

\[
\beta \pi_{i-1} - (\beta + \alpha i) \pi_i + \alpha (i+1) \pi_{i+1} = 0,
\]

where \( i \geq 1 \), subject to the constraint \( \sum_{i=0}^{\infty} \pi_i = 1 \). This is known to be Poisson with parameter \( \beta/\alpha \) yielding (48) [7].

### 4.3 EnvZ/OmpR signaling system

Reconsider the proposed EnvZ/OmpR signal transduction system (2). Since the network satisfies the assumptions of Theorem 2.4 and is furthermore conservative by (24), it follows by Theorem 3.5 that trajectories \( X(t) \) converge almost surely in finite time to an absorbing set near the boundary.

To further analyze the system, we would like to compute the expected times until absorption \( \mathbb{E}(\tau_A(X)) \) and the quasi-stationary distribution \( \tilde{\pi} \). The complexity of the network, however, prohibits explicit derivation of either of these quantities. We choose instead to approximate these quantities by numerical simulation. The parameter values we use for all simulations are given in Table 2 and are in close agreement with those contained in Table 1 of [50]. We note several differences. For instance, the authors of [50] do not consider the mechanism for ADP or ATP binding with the source kinase, EnvZ. Neither do they consider the EnvZ-ADP compound as a regulator for the phosphorylation of OmpR. We have filled in these rate constants with values of the same order as the known rates of the system.

In order to convert the deterministic rate parameters into stochastic rate parameters, we adjust the second-order reactions (\( k_6 \) and \( k_9 \)) by a factor of \( \kappa_i = k_i / (n_A V) \) where \( n_A \) is Avogadro’s number and \( V \) is the volume of the \( E. coli \) cell. The volume of an \( E. coli \) cell has been estimated at \( V = 10^{-15} \text{ L} \) [51]; for numerical simplicity, however, we choose \( V = 25/n_A \) so that \( n_A V = 25 \). After converting micromolar units \( \mu M \) to molar units \( M \), this gives a value of \( V = 4.151 \times 10^{-17} \text{ L} \). It can be easily checked in (9) that the deterministic parameter values in Table 2 give

\[
\bar{c}_p = \frac{k_1 k_3 k_5 (k_{10} + k_{11})}{k_2 (k_4 + k_5) k_9 k_{11}} = 1 \mu M.
\]

In a cell of volume \( V \) given above, this corresponds to a molecular count of

\[
Y_p = n_A V \bar{c}_p = 25
\]

for the corresponding stochastic model. We therefore expect the marginal distribution of the quasi-stationary distribution in \( Y_p \) to have a mean of roughly 25 molecules. We furthermore note that there are two conservation relationships to consider, one in the signaling protein EnvZ (\( X \)), and one in the response regulator OmpR (\( Y \)). It was found in [51] that a typical \( E. coli \) cell has roughly 100 total molecules of EnvZ and 3500 total molecules of OmpR. Therefore, for our estimations of \( \tilde{\pi} \), we chose a ratio of \( Y_{tot} : X_{tot} \) of 35 : 1, where \( X_{tot} \) and \( Y_{tot} \) are defined in (24).

We use several numerical estimators for the quantities of interest. Throughout, we denote by \( \{X_i(t)\}_{i=1}^N \) an ensemble of trajectories, where \( X_i(t) \) is the \( i \)th independent trajectory. All simulations are carried out using Gillespie’s algorithm [38]. We note that the state \( X_A \) is the absorption state given in (23).

We outline the estimators here:

<table>
<thead>
<tr>
<th>( k_i )</th>
<th>( \mu \text{ s}^{-1} )</th>
<th>( \kappa_i )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k_1 )</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>( k_2 )</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>( k_3 )</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>( k_4 )</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>( k_5 )</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>( k_6 )</td>
<td>0.5 ( \mu M \cdot s^{-1} )</td>
<td>0.02</td>
</tr>
<tr>
<td>( k_7 )</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>( k_8 )</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>( k_9 )</td>
<td>0.5 ( \mu M \cdot s^{-1} )</td>
<td>0.02</td>
</tr>
<tr>
<td>( k_{10} )</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>( k_{11} )</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>( D )</td>
<td>1 ( \mu M )</td>
<td>( n_A \times 6.022 \times 10^{23} )</td>
</tr>
<tr>
<td>( T )</td>
<td>1 ( \mu M )</td>
<td>( V = 4.151 \times 10^{-17} \text{ L} )</td>
</tr>
</tbody>
</table>

Table 2: Parameter values used for numerical simulations of (2).
\[
Y_{\text{tot}} \quad \mathbb{E}(\tau_A(X_0)) \quad Y_{\text{tot}} \quad \mathbb{E}(\tau_A(X_0))
\]

<table>
<thead>
<tr>
<th>(Y_{\text{tot}})</th>
<th>(\mathbb{E}(\tau_A(X_0))) (in s)</th>
<th>(Y_{\text{tot}})</th>
<th>(\mathbb{E}(\tau_A(X_0))) (in s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>9.3785</td>
<td>26</td>
<td>382.73</td>
</tr>
<tr>
<td>12</td>
<td>31.215</td>
<td>28</td>
<td>500.94</td>
</tr>
<tr>
<td>14</td>
<td>56.022</td>
<td>30</td>
<td>668.34</td>
</tr>
<tr>
<td>16</td>
<td>86.413</td>
<td>32</td>
<td>905.29</td>
</tr>
<tr>
<td>18</td>
<td>123.26</td>
<td>34</td>
<td>1270.7</td>
</tr>
<tr>
<td>20</td>
<td>167.19</td>
<td>36</td>
<td>1912.7</td>
</tr>
<tr>
<td>22</td>
<td>221.67</td>
<td>38</td>
<td>3158.6</td>
</tr>
<tr>
<td>24</td>
<td>284.05</td>
<td>40</td>
<td>5702.1</td>
</tr>
</tbody>
</table>

(a) Small-scale  
(b) Mid-scale  
(c) Plot of expectations

Figure 4.3: Expected time until absorption for various values of \(Y_{\text{tot}}\) when \(X_{\text{tot}} = 1\).

1. The estimator for the expected time until absorption is

\[
\mathbb{E}(\tau_A(X)) \approx \frac{1}{N} \sum_{i=1}^{N} T_i \tag{51}
\]

where \(T_i = \inf \{ t \geq 0 \mid X_{[i]}(t) = X_A, X_{[i]}(0) = X \} \) and \(X\) is the state

\[
Y_p = Y_{\text{tot}} \\
X_p = X_{\text{tot}} - 1 \\
XT = 1 \\
XD = X = X_pY = Y = XDY_p = 0. \tag{52}
\]

Note that a single occurrence of the reaction \(XT \rightarrow X_p\) takes (52) to (23). That is to say, we compute the average time to absorption for an ensemble of realizations which start one step away from the absorbing state.

2. The estimator for the probability distribution of \(Y_p\) at a fixed time \(T\) from initial distribution \(\nu\) is

\[
P_{\nu}(Y_p(t) = j) \approx \frac{1}{N} \sum_{i=1}^{N} 1(Y_{p,[i]}(T) = j) \tag{53}
\]

where \(Y_{p,[i]}(T)\) is the value of species \(Y_p\) at time \(T\) for the \(i\)th simulation, seeded according to the distribution \(\nu\).

3. The estimator for the quasi-stationary distribution of \(Y_p\) at time \(T\) is

\[
\hat{\pi}(Y_p = j) \approx \frac{1}{N_s} \sum_{i=1}^{N} 1(Y_{p,[i]}(T) = j) \cdot 1(X_{[i]}(T) \neq X_A). \tag{54}
\]

where \(X_A\) is the state (23), \(Y_{p,[i]}(T)\) is the value of \(Y_p\) at time \(T\) for the \(i\)th simulation, and \(N_s \leq N\) is the number of the \(N\) simulations which did not enter the absorbing state (23) by time \(T\). In other words, we compute the ensemble \(\{X_{[i]}(t)\}\) up to a fixed time \(T\) and record the value only if the chain has not entered the absorbing state (23). We then average over these non-absorbed trajectories.

4. In order to approximate the quasi-stationary distribution for extremely large values of \(X_{\text{tot}}\) and \(Y_{\text{tot}}\), which are required for Fig. 4.5, the ensemble estimator of (54) proved to be infeasible. Instead, we approximated the model by setting the rate of the transition to the absorbing state to zero, thereby producing an ergodic process which closely follows the dynamics of the actual process. We then used time-averaging techniques to estimate
the resulting stationary distribution. See [52] for a connection between the time averaged process and the quasi-
stationary distribution. We note that in all our simulations using this method for the values of $X_{\text{tot}}$ and $Y_{\text{tot}}$ as
given in Fig. 4.5, we never observed this change in the dynamics playing a role in the simulation. That is, the
paths never would have made the transition to the absorbing state even if we had allowed such a transition to
occur.

We note that the estimators (51) and (53) are known to converge almost surely in the limit $N \to \infty$. The estimator
(54) produces the Yaglom limit as $N \to \infty$ followed by $T \to \infty$. We know this limit to be the quasi-stationary
distribution in the present setting.

In Fig. 4.3, we provide approximations of the expected time until absorption $\mathbb{E}(\tau_{A}(X_0))$ for various
values of $Y_{\text{tot}}$ when $X_{\text{tot}} = 1$. Ten thousand data points where sampled for each value of $Y_{\text{tot}}$ using the estima-
tor (51). Note that the deterministic model predicts an attracting boundary state for the values in table (a) and
an attracting positive equilibrium for the values in table (b). The large expected times until absorption as $Y_{\text{tot}}$
grows is clear and justifies the consideration of quasi-stationary distributions.

In Figure 4.4, we estimate the distribution of the (non-conditioned) process at various times for $X_{\text{tot}} = 1$ and $Y_{\text{tot}} = 35$. The estimator (53) is used to produce the time-dependent distributions of the original process,
whereas the estimator (54) is used to produce the quasi-stationary distribution (using a time $T = 10,000$). In
(a), the initial state is $X(0) = X_{\text{tot}}, Y(0) = Y_{\text{tot}}$ while, in (b), the initial distribution is the quasi-stationary
distribution of the process. Convergence toward the absorbing state (23) is clear. It is also worth noting that
the shape of the distribution in (b) away from $Y_p = 35$ remains constant, as expected from the definition (43).
100,000 sample trajectories were computed for the es-

Figure 4.5: Approximations of the quasi-stationary distribution of $Y_p$ using the estimator (54). The ratio of $Y_{\text{tot}} : X_{\text{tot}}$
of $35 : 1$ is maintained throughout. For comparison, the Poisson distribution with mean 25 is overlain (black).

Figure 4.4: Distributions as a function of time with $X_{\text{tot}} = 1$ and $Y_{\text{tot}} = 35$. In (a), a point mass is used as the initial
distribution, whereas in (b) the initial distribution is the quasi-stationary distribution.

Figure 23
In Fig. 4.5 we provide approximations of the quasi-stationary distribution of $Y_p$ using the estimator (54). The convergence to Poisson (in black) is striking, but may only be conjectured at the current time.

References


