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A review and analysis of absolute concentration robustness in biochemical reaction networks

Bachelor thesis

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CONTENTS

1

INTRODUCTION

MOTIVATION

In 2014 Anderson et al. published a paper [3] about the stochastic analysis of biochemical reaction networks with absolute concentration robustness. The results in this paper are mainly the existence of a different equilibrium concentration in particular chemical reaction networks when described from a stochastic point of view instead of the more commonly used deterministic method, describing the change in concentration of certain compounds with differential equations.

This bachelor thesis has been established in order to further investigate the results described in [3] and its supplementary material which leads to some critique which is not mentioned in the original article and some further analysis of particular results and proofs: in particular the derivation of the so called quasi-stationary distribution, which will be described in Section 3.3.

1.1 CHEMICAL REACTIONS NETWORKS

The theory developed in [3], and reviewed in this thesis, is based on the following definitions which will be given in this section.

Definition 1.1.1. *A chemical reaction network is a set $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ in which:*

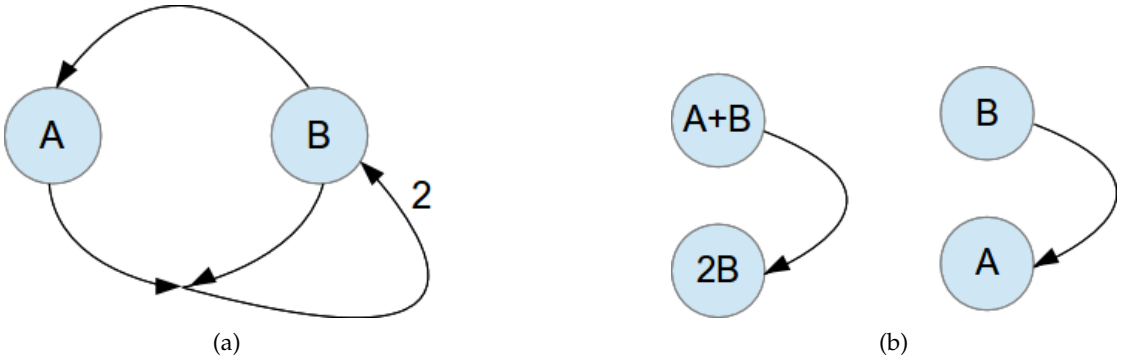
1. *A set of species $\mathcal{S} = \{X_1, ..., X_m\}$ consisting of molecules which occur in at least one reaction*
2. *A set of reactions $\mathcal{R} = \{\mathcal{R}_1, ..., \mathcal{R}_r\}$ consisting of all the possible elementary undirectional reactions in the network:*

$$\mathcal{R}_i : \sum_{j=1}^{m} y_{ij} X_j \to \sum_{j=1}^{m} y'_{ij} X_j, \qquad i = 1, ..., r,$$

in which the stoichiometrical coefficients $y_{ij}, y'_{ij} \in \mathbb{Z}_{\geq 0}$ denote the multiplicity of the molecules in the reaction. An alternative way of denoting the reactions is $y_i \to y'_i$, a reaction out of complex y_i into complex y'_i in which $y_i = (y_{i1}, ..., y_{im})$ and $y'_i = (y'_{i1}, ..., y'_{im})$.

3. *The set of complexes $\mathcal{C}$, consisting of all stoichiometric vectors $y_i = (y_{i1}, \ldots, y_{im})$ and $y_i' = (y_{i1}', \ldots, y_{im}')$ that occur in some reaction: $\mathcal{C} = \bigcup_{i=1}^{r} \{y_i, y_i'\}$. n is the number of stoichiometric complexes, such that $|\mathcal{C}| = n$.*

Each reaction yields a *reaction vector* $y_i' - y_i \in \mathbb{Z}^m$. Note that it does not fully respect the reaction $\mathcal{R}_i$. It results in a loss of information because different reactions can be represented by the same reaction vector and this reaction vector describes the net change in species. The deterministic model in Section 1.2 is defined in terms of reaction vectors, while it is not relevant for the stochastic model which is explained in Section 1.3. The *stoichiometrical subspace* of the network is defined as $S = \text{span}\{y_i' - y_i \mid i = 1, ...r\}$, also the dimension of this subspace, S, is $s = \dim(S)$. By representing each stoichiometrical complex as a vertex and the reaction arrows as edges which are directed arrows which connect y and y' if there is a reaction that turns complex y into complex y', the reaction network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ can be represented as a directed graph $G(V, E)$ in which $V = \mathcal{C}$ and $E = \mathcal{R}$.



(a) (b)

Figure 1: The network $A + B \to 2B$, $B \to A$ represented as a graph with a) the biological representation and b) the representation as defined in the previous paragraph (see Example 1.3.1 later)

Definition 1.1.2. *A complex y_i is terminal if there does not exist a reaction out of y_i. A complex y_i is non-terminal if there does exist such a reaction.*

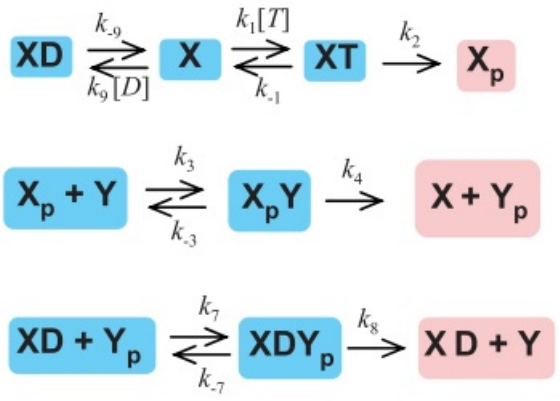


Figure 2: An EnvZ/Ompr signalling system from [1] shown as a system of chemical reactions in which the blue complexes are non-terminal and the orange complexes are terminal.

1.2 DETERMINISTIC MODEL

When the amount of molecules reacting is large enough and the environment in which the reaction takes place is well mixed, it is possible to give a formula which relates the concentration of the reactants to the rate in which the concentration of the reactants changes, [4]. When we capture the concentrations of the molecular species in a vector: $\mathbf{c} = (c_1, c_2, \ldots, c_m) \in \mathbb{R}^m_{\geq 0}$, and use class action kinetics for the change in concentration, the rate of the reaction $y_i \to y_i'$, then the system of differential equations is given as followes:

$$\frac{d\mathbf{c}}{dt} = \sum_{i=1}^{r} k_i (y_i' - y_i)\mathbf{c}^{y_i}, \qquad \mathbf{c}^{y_i} = \prod_{j=1}^{m} c_j^{y_{ij}}$$

1.3 STOCHASTIC MODEL

In the stochastic description of the chemical reaction network,not the concentration of the substances in the network will be modelled but rather the amount of molecules of the substances. We let $X_j(t) \in \mathbb{Z}_{\geq 0}$ be the number of molecules of X_j at time t. One then defines a continuous-time Markov chain for the overall state $\mathbf{X}(t) = (X_1(t), X_2(t), \ldots, X_m(t)) \in \mathbb{Z}^m_{\geq 0}$. Note that the state space is discrete.

If reaction $\mathcal{R}_i$ occurs at time t, $y_i \to y_i'$, then the chain evolves into a new state $\mathbf{X}(t) = \mathbf{X}(t-) + (y_i - y_i')$ in which $\mathbf{X}(-t) = \lim_{h \to 0^+} \mathbf{X}(t-h)$. The state of the chain at time t is given by

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

where $N_i(t)$ is a counting process which gives the number of times reacton i has occurred at time t. $N_i(t)$ is a function of a Poisson process $Y_i(\cdot), i = 1,\dots,r$ so $N_i(t)$ can be given by

$$N_i(t) = Y_i\left(\int_0^t \lambda_i(\mathbf{X}(s))ds\right)$$

Here $\lambda_i(\mathbf{X}(t))$ are the propensity funtions and the analog of the deterministic rate functions $k_i(c)^{y_i}$ defined in Section 1.2, [3]. Reactions can only occur if there are sufficient molecules left so we have the restriction that $X_j \geq y_{ij}$ for all $j = 1,\dots,m$, should reaction $\mathcal{R}_i$ be possible. Thus $\lambda_i(\mathbf{X}) = 0$ if $X_j < y_{ij}$. If this restriction is met, the chemical reaction network is called *stoichiometrically admissible* and if the propensities are the following

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

(see [3]). Here V denotes the volume in which the reactions take place. To make the dependence on V explicit in notation we write $\mathbf{X}^V(t)$. The chain $\mathbf{X}^V(t)$ will almost surely converge on compact time intervals to the deterministic model described in the previous chapter with rate constants k_i when $V \to \infty$, as long as $\mathbf{X}_j^V(0)/V$ converges to a non-zero constant for each j [5].

This method gives insight in the behaviour of the stochastic mass-action system in a very straight-forward way in the large-scale limit as $V \to \infty$, which might give the interested reader a clearer idea of the underlying mechanism.

Now consider the following example, which serves as running example in [3]:

Example 1.3.1. *Consider the following chemical reaction network:*

$$\begin{aligned} A + B &\to 2B \\ B &\to A \end{aligned}$$

When this chemical reaction network is analyzed according to the method described above, one should note that the probability of the reaction $B \to A$ occurring repeatedly until $X_B = 0$ is non-zero. Therefore X_B will almost surely vanish as $t \to \infty$.

A proof of the statement in the previous example is given in Section 3.2.

Among other things the aim of this thesis is to give a quantitative estimation of the scale of the behaviour discussed in [3] explained in Example 1.3.1. Therefore our focus

will lie on analyzing the stochastic chemical reaction system as the propagation of a chain of molecule states in a given state as a function of time which will lead to the use of the chemical master equation, [6]. The advantage of this approach is the possibility to explicitly derive an estimation of the time until extinction of one or more species which is shown in Section 3.1.

2

ABSOLUTE CONCENTRATION ROBUSTNESS IN THE DETERMINISTIC MODEL

The study of chemical reaction networks with a deterministic model i.e. a system of ordinary differential equations that models the change in concentration of certain compounds as a function of time, can enlighten many properties of certain chemical reaction networks. One application of this approach is the possibility to predict chemical equilibrium states. Section 2.1 contains an example of the deterministic model, Example 1.3.1. Section 2.2 treats the main result of [1] and [3] which shows that structural properties of a chemical reaction network can be sufficient for giving statements about chemical equilibrium states. Finally, Section 2.3 expands the model from Section 2.1 and checks whether production and degeneration of molecules in a chemical reaction network affects the properties of the network and previously found equilibrium state.

2.1 INTRODUCTION

Recall the deterministic model for a chemical reaction network using mass action kinetics as defined in Section 1.2. We denote a steady state of the system by $\bar{\mathbf{c}}$. Following [3] we define:

Definition 2.1.1. *A chemical reaction network is absolute concentration robust in the species $X_i \in \mathcal{S}$ if $\bar{\mathbf{c}}_i$ does not depend on the initial conditions and attains the same value in every positive equilibrium concentration $\bar{\mathbf{c}} \in \mathbb{R}^m_{>0}$.*

Example 2.1.1. *Again we consider the reaction network*

$$\begin{aligned} A + B &\to 2B \\ B &\to A \end{aligned} \tag{1}$$

The mass-action system corresponding to this network is

$$\frac{d}{dt}\begin{pmatrix} c_A \\ c_B \end{pmatrix} = \alpha \begin{pmatrix} -1 \\ 1 \end{pmatrix} c_A c_B + \beta \begin{pmatrix} 1 \\ -1 \end{pmatrix} c_B,$$

which has equilibrium concentrations

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

with $M := c_A(0) + c_B(0)$. *Note that the equilibrium concentration of A does not depend on the initial concentrations of both species so the system is absolute concentration robust in the species A.*

2.2 DEFICIENCY ONE THEOREM

It turns out, that to some extent the structure of the reaction network determines whether there are species that are absolute concentration robust.

Definition 2.2.1.

1. *The complexes* $y, y' \in \mathcal{C}$ *are directly linked,* $y \leftrightarrow y'$, *if* $y \to y'$ *or* $y' \to y$;
2. *The complexes* $y, y' \in \mathcal{C}$ *are linked,* $y \sim y'$ *if either* $y = y'$ *or when there is a sequence of complexes such that* $y = y_{\mu(1)} \leftrightarrow y_{\mu(2)} \leftrightarrow ... \leftrightarrow y_{\mu(l)} = y'$;
3. *There exists a path of* $y \in \mathcal{C}$ *to* $y' \in \mathcal{C}$, $y \Rightarrow y'$ *if there is a sequence of complexes such that* $y = y_{\mu(1)} \to y_{\mu(2)} \to ... \to y_{\mu(l)} = y'$;

These relations make it possible to partition $\mathcal{C}$ into *linkage classes*, L. $y, y' \in \mathcal{C}$ are in the same linkage class L if and only if $y \sim y'$. $\mathcal{L}$ denotes the set of linkage classes of the chemical reaction network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$. This means that the cardinality of the linkage classes is $|\mathcal{L}| = l$ with $\mathcal{L} = \{L_1, ..., L_l\}$. Chemically, complexes are in the same linkage class if these combinations of molecules are the reactant and/or product of at least one reaction in a closed chemical system. Recall Figure 1. and note that when a chemical reaction networks is represented as a graph in the same way as Figure 1b), then every connected sub graph of the network is a linkage class. This gives the linkage classes: $L_1 = \{A + B, 2B\}$ and $L_2 = \{B, A\}$.

Definition 2.2.2. *The deficiency of a chemical reaction network* $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ *is given by* $\delta = n - l - s$ *with n the number of stoichiometrical complexes, l the number of linkage classes and s the dimension of the span of the reaction vectors.*

The concept of deficiency was introduced by Feinberg in [10].

Example 2.2.1. *consider the reaction network:*

$$\begin{aligned} A + B &\to 2B \\ B &\to A \end{aligned}$$

This network contains the set of substances $\mathcal{S} = \{A, B\}$, the set of complexes $\mathcal{C} = \{A + B, 2B, B, A\}$ and the set of reactions $\mathcal{R} = \{A + B \to 2B, B \to A\}$. The deficiency is given by $\delta = n - l - s$ in which $n = |\mathcal{C}| = 4$. The number of linkage classes is 2, $\mathcal{L}_1 = \{A + B, 2B\}$ en $\mathcal{L}_2 = \{B, A\}$ so $l = 2$. The reaction vectors are $y_1' - y_1 = (0\ 2)^\top - (1\ 1)^\top = (-1\ 1)^\top$ and $y_2' - y_2 = (1\ 0)^\top - (0\ 1)^\top = (1\ -1)^\top$, so $s = dim(S) = 1$. The deficiency is $\delta = n - l - s = 4 - 2 - 1 = 1$.

The following theorem can be found in [1], p.1390:

Theorem 2.2.1. *Consider a chemical network and suppose that:*

- *The system admits a positive equilibrium concentration;*
- *The deficiency of the network, δ, is one;*
- *There are non-terminal complexes which differ only in the species X_i;*

Then the chemical network exhibits absolute concentration robustness in X_i.

This theorem states that absolute concentration robustness can be ensured based solely on structural properties. This can be helpful in case an explicit solution cannot be determined. We do not give the proof here, as our interest is mere in understanding the stochastic dynamics. It can be found in the supplemental material of [1], Chapter S3. Also examples of networks with absolute concentration robustness which do not satisfy the conditions of Theorem 2.3.1. can be found in this article, Example S4.2

2.3 PRODUCTION AND DEGENERATION

Suppose Example 2.1.1. is a model of a certain biochemical reaction network. It has absolute concentration robustness in the species A. Many biochemical molecules can degenerate and the supply of certain molecules is also not constant. Therefore we expand Example 2.1.1. with the production and degeneration of A and B

The chemical reaction networks now leaves:

$$\begin{aligned} A + B &\to 2B, \\ B &\to A, \\ \emptyset &\to A, \to \emptyset \\ \emptyset &\to B, \to \emptyset \end{aligned}$$

which has the following system of differential equations:

$$\begin{aligned} \frac{dA}{dt} &= r_A - \lambda_A A - \alpha AB + \beta B \\ \frac{dB}{dt} &= r_B - \lambda_B B + \alpha AB - \beta B \end{aligned}$$

Here r_A, r_B and λ_A, λ_B are the production and degeneration rates of species A and B respectively.

To determine the steady state we set both equations equal to zero. We obtain:

$$\begin{aligned} 0 &= r_A - \lambda_A \bar{A} - \alpha \bar{A}\bar{B} + \beta \bar{B} \\ 0 &= r_B - \lambda_B \bar{B} + \alpha \bar{A}\bar{B} - \beta \bar{B} \end{aligned}$$

Isolating $\bar{B}$ from the first equation gives:

$$\bar{B} = \frac{\lambda_A \bar{A} - r_A}{\beta - \alpha \bar{A}}.$$

When we insert this expression into the second equation we obtain:

$$0 = \frac{\lambda_A \lambda_B \bar{A} - \alpha \lambda_A \bar{A}^2 + \lambda_A \beta \bar{A} - r_A \lambda_B + \alpha r_A \bar{A} - r_A \beta}{\beta - \alpha \bar{A}} - r_B$$

which can be rewritten into:

$$0 = \alpha \lambda_A \bar{A}^2 - (\alpha(r_A + r_B) + \lambda_A \lambda_B + \lambda_A \beta)\bar{A} + \beta(r_A + r_B) + r_A \beta \tag{1}$$

This equation can be solved with the standard quadratic formula for solving quadratic equations:

$$\bar{A} = \lambda_A \beta + \lambda_A \lambda_B + \alpha(r_A + r_B) \pm \frac{\sqrt{(\lambda_A \beta + \lambda_A \lambda_B + \alpha(r_A + r_B))^2 - 4\alpha\lambda_A(\lambda_B r_A + \beta(r_A + r_B)}}{2\alpha\lambda_A}$$

The quadratic equation (1) in the form $0 = ax^2 + bx + c$ which was solved can be rewritten as $0 = x^2 + \frac{b}{a}x + \frac{c}{a}$ with:

$$\begin{aligned} a &= \alpha \lambda_A \\ b &= -(\alpha(r_A + r_B) + \lambda_A \lambda_B + \lambda_A \beta) \\ c &= \beta(r_A + r_B) + r_A \beta \end{aligned}$$

And since we have $0 = ax^2 + bx + c \Leftrightarrow 0 = (x - \lambda_1)(x - \lambda_2)$, it is easy to see that information about the solutions can be obtained by reviewing $\lambda_1 \lambda_2 = c/a$ and $\lambda_1 + \lambda_2 = -b/a$. Because $a, c > 0$ and $b < 0$, we can see that both λ_1 and λ_2 are positive which shows that there are two positive equilibrium solutions for $\bar{A}$.

Also we can rewrite the two equations we started with in the following form:

$$\lambda_A \bar{A} + \lambda_B \bar{B} = r_a + r_B$$

and we had:

$$\bar{B} = \frac{\lambda_A \bar{A} - r_A}{\beta - \alpha \bar{A}}$$

Analyzing the intersections of these two relations give two solution $(\bar{A}_1, \bar{B}_1)$ and $(\bar{A}_2, \bar{B}_2)$ with $\bar{A}_1, \bar{A}_2 > 0$ and $\bar{B}_1 > 0, \bar{B}_2 < 0$ and therefore there is only one realistic solution to this problem. Note that the expansions for $\bar{A}$ and $\bar{B}$ do not depend on the initial conditions so we still have absolute concentration robustness when adding production and degeneration to this example, but now not only in A, but also for species B.

In fact, degradation will fade out the effects of the initial conditions, replacing it by the newly produced molecules. This van be expected in any system with production and degradation. Thus it seems that the concept of absolute concentration robustness is not so interesting in the end, as production and degradation are processes that naturally occur in most natural biochemical systems.

3

FURTHER ANALYSIS OF THE STOCHASTIC MODEL

3.1 REPRESENTATION BY CHEMICAL MASTER EQUATION

Recall the model for the evolution of the molecule states $\mathbf{X}(t) \in \mathbb{Z}_{\geq 0}^m$ as a continuous-time Markov chain (cf. Section 1.3). Here we will present a representation of the dynamics of this chain by means of the chemical master equation. The idea is to model $\mathbf{X}(t)$ as a somewhat more complex birth-death process. The different states are all feasible combinations of the different substances in the reaction network. Denote $P_t(\mathbf{X}) = \mathbb{P}\{\mathbf{X}(t) = \mathbf{X}\}$ as the probability of the chain $\mathbf{X}(t)$ being in the state $\mathbf{X}$ at time t. Denote $\lambda_i(\mathbf{X})$ as the rate at which $\mathbf{X}$ moves to another state due to reaction $\mathcal{R}_i$ and $\lambda_0(\mathbf{X}) = \sum_{i=1}^r \lambda_i(\mathbf{X})$ the sum of all reaction rates. Therefore the movement of one state to another can be modelled by an infinite system of differential equations that is called the chemical master equation:

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

This equation can also be written as

$$\frac{d}{dt}P_t = P_t\mathcal{A}$$

where P_t is a vector with the probabilities of being in each state $\mathcal{X} \in \mathbb{Z}_{\geq 0}$ at time t. If the scale is enlarged, P_t can become extremely large so it may be very hard to compute an explicit solution. In our setting of Example 1.3.1 the system is finite, because of conservation of number of molecules and therefore a finite amount of combinations (states) in which the system can be.

3.2 SCALE OF EXPECTED TIME OF ABSORPTION

The reaction network used as an example in Chapter 1 can be interpreted as an activation/deactivation system.

$$\begin{aligned} A + B &\rightarrow 2B \\ B &\rightarrow A \end{aligned}$$

We denote M as the total amount of molecules. It is clear that $X_A = M$ is an absorbing state, because then $X_B = 0$, which means that no reaction can occur any more when the chain is in this state. Also, the first reaction increases X_B by one and the second reaction increases X_A by one. Then this chemical reaction network can be seen as a birth-death process with the following propensities:

$$\lambda_i = \alpha X_B X_A = \alpha i(M - i), \tag{2}$$
$$\mu_i = \beta X_B = \beta i, \tag{3}$$

where $i \in [0, 1, \ldots, M]$ is the number of B molecules in the chain, see Figure 3.

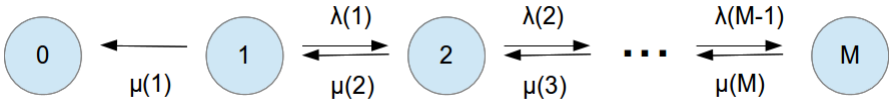


Figure 3: *Birth-death chain corresponding to the activation/deactivation system.*

Lemma 3.2.1. *The birth-death process with propensities* (2) *and* (3) *will almost surely reach* $X_B = 0$.

Proof. Recall that the waiting time for this chain in a certain state is exponential distributed. Define T_i^+ as the waiting time before going from state i to state $i+1$ and T_i^- as the waiting time going from state i to state $i+1$. Therefore we have:

$$\mathbb{P}(T_i^+ > t) = e^{-\lambda_i t}$$
$$\mathbb{P}(T_i^- > t) = e^{-\mu_i t}$$

Write $\mathbb{P}(\text{chain goes up}) = \mathbb{P}(T_i^+ < T_i^-)$. This probability gives:

$$\begin{aligned} \mathbb{P}(T_i^+ < T_i^-) &= \mathbb{P}(T_i^+ - T_i^+ < 0) \\ &= \int_0^\infty \mathbb{P}(T_i^+ < s)\mu_i e^{-\mu_i s} ds \\ &= \int_0^\infty \left(1 - e^{-\lambda_i s}\right) \mu_i e^{-\mu_i s} ds \end{aligned}$$

Evaluating this integral gives the following probability.

$$\begin{aligned}
\mathbb{P}(T_i^+ < T_i^-) &= \left[-e^{-\mu_i s}\right]_0^\infty - \mu_i \int_0^\infty e^{-(\lambda_i+\mu_i)s} ds \\
&= 1 - \mu_i \left[-\frac{1}{\lambda_i+\mu_i} e^{-(\lambda_i+\mu_i)s}\right]_0^\infty \\
&= 1 - \frac{\mu_i}{\lambda_i+\mu_i} \\
&= \frac{\lambda_i}{\lambda_i+\mu_i}
\end{aligned}$$

A similar computation for the transition $i \to i-1$ gives $\mathbb{P}(T_i^- < T_i^+) = \frac{\mu_i}{\lambda_i+\mu_i}$. Define u_i as the probability of absorption when starting in i. We have the following probabilities:

$$\begin{aligned}
u_0 &= 1, && \text{since 0 is an absorbing state} \\
u_i &= \frac{\lambda_i}{\lambda_i+\mu_i} u_{i+1} + \frac{\mu_i}{\lambda_i+\mu_i} u_{i-1}, && \text{for } i = 1, 2, \ldots, M-1 \\
u_M &= u_{M-1}, && \text{since } M \text{ is a reflecting state}
\end{aligned}$$

For $M-1$, we have:

$$u_{M-1} = \frac{\lambda_{M-1}}{\lambda_{M-1}+\mu_{M-1}} u_M + \frac{\mu_{M-1}}{\lambda_{M-1}+\mu_{M-1}} u_{M-2}$$

which can be written as

$$u_{M-1} = \frac{\lambda_{M-1}}{\lambda_{M-1}+\mu_{M-1}} u_{M-1} + \frac{\mu_{M-1}}{\lambda_{M-1}+\mu_{M-1}} u_{M-2} \Leftrightarrow u_{M-1} = u_{M-2}$$

Repeatedly using this method gives $u_i = 1$ for all i. Thus the chain of states will almost surely reach $X_B = 0$.

□

We define τ_i as the random variable that represents the time needed by the chain to reach $X_B = 0$, starting from $X_B(0) = i \in [0, 1, \ldots, M]$.

$$\tau_i := \inf\{t > 0 : \ X_B(t) = 0, \text{ given } \ X_B(0) = j\}$$

In order to give an estimation for the expected time until this chain reaches the absorbing state $X_A = M$, which is the state which will be definitely visited when $t \to \infty$, we use a formula for the time until extinction from [2], equation (6.22). Which is the following:

$$\mathbb{E}(\tau_i) = \begin{cases} \frac{1}{\mu_1} + \sum_{j=2}^{N} \frac{\lambda_1 \cdots \lambda_{j-1}}{\mu_1 \cdots \mu_j}, & \text{if } i = 1 \\ \mathbb{E}[\tau_1] + \sum_{s=1}^{i-1} \left[\frac{\mu_1 \cdots \mu_s}{\lambda_1 \cdots \lambda_s} \sum_{i=s+1}^{N} \frac{\lambda_1 \cdots \lambda_{j-1}}{\mu_1 \cdots \mu_j} \right], & \text{if } i = 2, 3, \ldots, N. \end{cases}$$

In order to give an estimation, first this equation is simplified using the given propensities of which the formula is given in [3].

$$\begin{aligned} \mathbb{E}[\tau_i] &= \frac{1}{\mu_1} + \sum_{j=2}^{N} \frac{\lambda_1 \cdots \lambda_{j-1}}{\mu_1 \cdots \mu_j} + \sum_{k=1}^{i-1} \left[\frac{\mu_1 \cdots \mu_k}{\lambda_1 \cdots \lambda_k} \sum_{j=k+1}^{M} \frac{\lambda_1 \cdots \lambda_{j-1}}{\mu_1 \cdots \mu_j} \right] \\ &= \frac{1}{\beta} + \sum_{j=2}^{M} \frac{1}{\beta} \left(\frac{\alpha}{\beta} \right)^{j-1} \frac{(M-1)!}{j(M-j)!} \\ &+ \sum_{k=1}^{i-1} \left(\frac{\alpha}{\beta} \right)^{-k} \frac{(M-k-1)!}{(M-1)!} \sum_{j=k+1}^{M} \frac{1}{\beta} \left(\frac{\alpha}{\beta} \right)^{j-1} \frac{(M-1)!}{j(M-j)!} \\ &= \sum_{k=0}^{i-1} \left(\frac{\alpha}{\beta} \right)^{-k} \frac{(M-k-1)!}{(M-1)!} \sum_{j=k+1}^{M} \frac{1}{\beta} \left(\frac{\alpha}{\beta} \right)^{j-1} \frac{(M-1)!}{j(M-j)!} \\ &= \sum_{k=0}^{i-1} \sum_{j=k+1}^{M} \frac{1}{\beta} \left(\frac{\alpha}{\beta} \right)^{j-k-1} \frac{(M-k-1)!}{j(M-j)!} \end{aligned}$$

The last expansion was given in the supplemental material of [3], equation 46.

Theorem 3.2.2. *The expected time until extinction, $\mathbb{E}[\tau_i]$ can be estimated by*

$$\frac{1}{2\beta M}(1 + 2\frac{\alpha}{\beta})^{M-1} \cdot \frac{\hat{r}^i - 1}{\hat{r} - 1} \leq \mathbb{E}[\tau_i] \leq \frac{1}{\beta}(1 + M\frac{\alpha}{\beta})^{M-1} \cdot \frac{r^i - 1}{r - 1}$$

With $r = (1 + M\frac{\alpha}{\beta})^{-1}$ and $\hat{r} = (1 + 2\frac{\alpha}{\beta})^{-1}$.

Proof. First we write $\mathbb{E}[\tau_i] = F_i(M)$ as the expected time until extinction starting in state i with a total of M states:

$$\begin{aligned} F_i(M) &= \sum_{k=0}^{i-1} \sum_{j=k+1}^{M} \frac{1}{\beta} \left(\frac{\alpha}{\beta} \right)^{j-k-1} \frac{(M-k-1)!}{j(M-j)!} \\ &= \frac{1}{\beta} \sum_{k=0}^{i-1} \sum_{j=k+1}^{M} \left(\frac{\alpha}{\beta} \right)^{j-k-1} \frac{(M-k-1)!}{(j-k-1)!(M-j)!} \cdot \frac{(j-k-1)!}{j}. \end{aligned}$$

Since $\frac{(j-k-1)!}{j} \leq M^{j-k-1}$, we get an upper bound

$$\begin{aligned} F_i(M) &\leq \frac{1}{\beta}\sum_{k=0}^{i-1}\sum_{j=k+1}^{M}\left(M\frac{\alpha}{\beta}\right)^{j-k-1}\binom{M-k-1}{j-k-1} \\ &= \sum_{k=0}^{i-1}\sum_{l=0}^{M-k-1}\left(M\frac{\alpha}{\beta}\right)^{l}\binom{M-k-1}{l}. \end{aligned}$$

This can be simplified into

$$\begin{aligned} F_i(M) &\leq \frac{1}{\beta}\sum_{k=0}^{i-1}(1+M\frac{\alpha}{\beta})^{M-k-1} \\ &= \frac{1}{\beta}(1+M\frac{\alpha}{\beta})^{M-1}\sum_{k=0}^{i-1}\frac{1}{(1+M\frac{\alpha}{\beta})^{k}} \\ &= \frac{1}{\beta}(1+M\frac{\alpha}{\beta})^{M-1}\cdot\frac{r^i-1}{r-1}, \qquad \text{with } r=(1+M\frac{\alpha}{\beta})^{-1} \end{aligned}$$

For a lower bound we state again from

$$F_i(M) = \sum_{k=0}^{i-1}\sum_{j=k+1}^{M}\frac{1}{\beta}\left(\frac{\alpha}{\beta}\right)^{j-k-1}\frac{(M-k-1)!}{j(M-j)!}.$$

Because $\frac{1}{j} \geq \frac{1}{M}$ we have a lower bound:

$$F_i(M) \geq \frac{1}{\beta M}\sum_{k=0}^{i-1}\sum_{j=k+1}^{M}\left(\frac{\alpha}{\beta}\right)^{j-k-1}\frac{(M-k-1)!}{(M-j)!}$$

$l = j-(k+1)$ so $j = l+k+1$, therefore we can change the inner summation:

$$\begin{aligned} F_i(M) &\leq \frac{1}{\beta M}\sum_{k=0}^{i-1}\sum_{l=0}^{M-k-1}\left(\frac{\alpha}{\beta}\right)^{l}\frac{(M-k-1)!}{(M-l-k-1)!} \\ &= \frac{1}{\beta M}\sum_{k=0}^{i-1}\sum_{l=0}^{M-k-1}\left(\frac{\alpha}{\beta}\right)^{l}\binom{M-k-1}{l}l! \end{aligned}$$

And since $l! \geq 2^{l-1}$ this can be bounded from below by:

$$
\begin{aligned}
F_i(M) &\geq \frac{1}{2\beta M} \sum_{k=0}^{i-1} \sum_{l=0}^{M-k-1} \left(2\frac{\alpha}{\beta}\right)^l \binom{M-k-1}{l} \\
&= \frac{1}{2\beta M} \sum_{k=0}^{i-1} \left(1+2\frac{\alpha}{\beta}\right)^{M-k-1} \\
&= \frac{(1+2\frac{\alpha}{\beta})^{M-1}}{2\beta M} \cdot \frac{\hat{r}^i - 1}{\hat{r} - 1}
\end{aligned}
$$

□

Where $\hat{r} = (1+2\frac{\alpha}{\beta})^{-1}$
Also note that:

$$
\frac{r^i - 1}{r-1} \leq \frac{1}{1-r} = \frac{1/r}{1/r - 1} = \frac{1+M\frac{\alpha}{\beta}}{M\frac{\alpha}{\beta}}
$$

so as a cruder estimation we have:

Corollary 3.2.2.1. $\mathbb{E}[\tau_i] \leq \frac{M}{\alpha}(1+M\frac{\alpha}{\beta})^M$

Consider Table 1 and note that $\mathbb{E}[\tau_i] \geq \mathbb{E}[\tau_1]$. We have captured lower bounds for $\mathbb{E}[\tau_1]$ for various values of M.

M	lower bounds for $\mathbb{E}(\tau_1)$(s)
5	0.00544
25	0.00507
50	0.01737
75	0.07931
100	0.40736
150	12.7373
250	16811.1
1000	$4.913 * 10^{28}$

Table 1: Lower bound for expected time until absorption with parameter values $i = 1$, $\alpha = 1$ and $\beta = 25$, thus starting in the state $(X_A, X_B) = (M-1, 1)$, using the lower bound presented in Theorem 3.2.1

In the case $M = 1000$ which is not even a really large amount, the expected time until absorption is still $4.913 * 10^{28}$s which is $1.55 * 10^{21}$ years. Consider the case that a more realistic amount of $M = 1\text{mol} = 6.022 * 10^{23}$ is used. This would be an unimaginable large time span so we could say the time until absorption goes to infinity rather quickly.

Chemical reactions in which such low numbers can occur and therefore this behaviour can occur are i.e. signalling cascades in biochemistry in which amounts of 60-100 are reported. [7], [8].

3.3 QUASI-STATIONARY DISTRIBUTIONS

Because the behaviour of absorption at $\mathbf{X}_B = 0$ is on such a large time-scale, it is for practical purposes more relevant to study the behaviour of this chain before it reaches this absorbing state. Associated to this is the study of the so-called quasi-stationary distribution. It does have relevance for chemical reaction networks on a practical time scale in contrast to the steady state described in the previous section.

Definition 3.3.1. *Consider a Markov process $\mathbf{X}(t)$ in continuous time and discrete state space $\mathbf{X}$. A state $i \in \mathbf{X}$ is called transient if there is a non-zero probability the chain will never return to state i if it starts in i. Also, a state i is called absorbing if it is impossible to leave this state: for all $i \neq j$: $p_{ii} = 1$ and $p_{ij} = 0$. A state i is called reflecting if it is impossible to remain in this state: $p_{ii} = 0$.*

We define the set $\mathbf{X}_T$ as the set of transient states and $\mathbf{X}_A$ as the set of absorbing states on a state space $\mathbf{X} \in \mathbb{Z}^m_{\geq 0}$.

Definition 3.3.2. *Let $\tilde{\pi}$ be a probability distribution on $\mathbf{X}_T$. $\tilde{\pi}$ is a quasi-stationary distribution if for all $t \geq 0$ and $\mathbf{Y} \in \mathbf{X}_T$*

$$\tilde{\pi}(\mathbf{Y}) = P_{\tilde{\pi}}(\mathbf{X}(t) = \mathbf{Y} | \mathbf{X}(t) \notin \mathbf{X}_A)$$

with $P_{\tilde{\pi}}$ the distribution of the process with initial distribution $\tilde{\pi}$.

The quasi stationary distribution of X_A from Example 1.3.1. converges to a Poisson distribution when $M \to \infty$ of which we will give the proof here from [3]. This quasi-stationary distribution exists in this setting, according to [11].

Theorem 3.3.1. *The quasi stationary distribution of X_A in Example 1.3.1. converges to a Poisson distribution with mean β/α when $M \to \infty$.*

Before we prove this theorem, consider the following proposition with proof from [12], Proposition 2.

Proposition 3.3.1. *Consider a Markov process $\mathbf{X}(t)$ with absorbing point 0 satisfying $\mathbb{P}(T_0 < \infty) = 1$ with T_0 the extinction time of $\mathbf{X}$. Assume that α is a quasi stationary distribution for the process. Then there exists a positive real number $\theta(\alpha)$ depending on the quasi stationary distribution such that*

$$\mathbb{P}_\alpha(T_0 > t) = e^{-\theta(\alpha)t}$$

Proof. By the Markov property

$$\begin{aligned}\mathbb{P}_\alpha(T_0 > t+s) &= \mathbb{E}_\alpha[\mathbb{P}_{\mathbf{X}_t}(T_0 > s)\mathbb{1}_{T_0>t}] \\ &= \mathbb{P}_\alpha(T_0 > t)\mathbb{E}_\alpha[\mathbb{P}_{\mathbf{X}_t}(T_0 > s)|T_0 > t]\end{aligned}$$

since $T_0 \leq t$ implies $\mathbf{X}_t = 0$, and $\mathbb{P}_0(T_0 > s) = 0$. By definition of a quasi stationary distribution, we get

$$\mathbb{E}_\alpha[\mathbb{P}_{\mathbf{X}_t}(T_0 > s)|T_0 > t) = \mathbb{P}_\alpha(T_0 > s)$$

Hence we obtain that for all $s, t > 0$, $\mathbb{P}_\alpha(T_0 > t+s) = \mathbb{P}_\alpha(T_0 > s)\mathbb{P}_\alpha(T_0 > t)$. Denote $g(t) = \mathbb{P}_\alpha(T_0 > t)$. We have $g(0) = 1$ and $g(t) \to 0$ as $t \to \infty$. Therefore this function $g(t)$ has the following form

$$\mathbb{P}_\alpha(T_0 > t) = e^{-\theta(\alpha)t}$$

with $\theta(\alpha) \in \mathbb{R}_{>0}$. □

We can now prove Theorem 3.3.1.

Proof. (Theorem 3.3.1) First the propensities will be reformulated in order to keep track of the number of A molecules. Therefore we have the propensities:

$$\begin{aligned}\lambda^M(i) &= \beta(M-i) \\ \mu^M(i) &= \alpha i(M-i)\end{aligned}$$

This chain has a reflecting state at $X_A = 0$ and an absorbing state at $X_A = M$ and transient states $0, \ldots, M-1$.

From equation (43) from the supplemental material of [3], we can calculate the derivative of $Q_t(\mathbf{X})$ in order to obtain an eigenvalue, where

$$Q_t(\mathbf{X}) = \frac{P_t(\mathbf{X})}{1 - P_t(\mathbf{X}_A)}.$$

This gives:

$$\frac{d}{dt}Q_t(\mathbf{X}) = \frac{\frac{d}{dt}P_t(\mathbf{X})(1 - P_t(\mathbf{X}_A)) + P_t(\mathbf{X})\frac{d}{dt}(1 - P_t(\mathbf{X}_A))}{(1 - P_t(\mathbf{X}_A))^2}$$

which can be simplified as:

$$\begin{aligned}\frac{d}{dt}Q_t(\mathbf{X}) &= \frac{[\mathcal{A}P_t(\mathbf{X})]}{1 - P_t(\mathbf{X}_A)} - \frac{P_t(\mathbf{X})}{1 - P_t(\mathbf{X}_A)} \cdot \frac{1}{1 - P_t(\mathbf{X}_A)} \cdot \frac{d}{dt}P_t(\mathbf{X}_A) \\ &= \mathcal{A}Q_t(\mathbf{X}) - Q_t(\mathbf{X}) \cdot \frac{1}{1 - P_t(\mathbf{X}_A)} \cdot \frac{d}{dt}P_t(\mathbf{X}_A)\end{aligned}$$

With $\frac{d}{dt}P_t(\mathbf{X}) = \mathcal{A}P_t(\mathbf{X})$ from the Chemical Master Equation. Substituting $Q_t(\mathbf{X}) = \tilde{\pi}$ and setting this equation to zero gives:

$$\mathcal{A}\tilde{\pi} = \theta\tilde{\pi}$$

With $\theta = \frac{1}{1-P_t(\mathbf{X}_A)} \cdot \frac{d}{dt}P_t(\mathbf{X}_A)$ which is the same θ as the $\theta(\alpha)$ from the proof of Proposition 3.3.1.

We have $\tilde{\pi}\mathcal{A}_Q = \theta\tilde{\pi}$ with $\mathcal{A}$ the matrix from Section 2.1. The quasi-stationary distribution for a given M thus satisfies

$$\begin{aligned} -\lambda^M(0)\tilde{\pi}_0^M + \mu^M(1)\tilde{\pi}_1^M &= \theta^M\tilde{\pi}_0^M \\ \lambda^M(i-1)\tilde{\pi}_{i-1}^M - (\lambda^M(i) + \mu^M(i))\tilde{\pi}_i^M + \mu^M(i+1)\tilde{\pi}_{i+1}^M &= \theta^M\tilde{\pi}_i^M \\ -\lambda^M(M-2)\tilde{\pi}_{M-2}^M + \mu^M(M-1)\tilde{\pi}_{M-1}^M &= \theta^M\tilde{\pi}_{M-1}^M \end{aligned}$$

where $i = 1, \ldots, M-2$ and θ^M the corresponding eigenvalue of the eigenvector from the equation $\tilde{\pi}\mathcal{A}_Q = \theta\tilde{\pi}$. Because $\theta^M = \lambda^M(M-1)\tilde{\pi}_M^M = \beta\tilde{\pi}_M^M$ we can substitute this into the previous system of equations:

$$\begin{aligned} -\beta M\tilde{\pi}_0^M + \alpha(M-1)\tilde{\pi}_1^M &= \beta\tilde{\pi}_M^M\tilde{\pi}_0^M \\ \beta(M-(i-1))\tilde{\pi}_{i-1}^M - (\beta(M-i) + \alpha i(M-i))\tilde{\pi}_i^M &+ \\ \alpha(i+1)(M-(i+1))\tilde{\pi}_{i+1}^M &= \beta\tilde{\pi}_M^M\tilde{\pi}_i^M \\ -2\beta\tilde{\pi}_{M-2}^M + \alpha\tilde{\pi}_{M-1}^M &= \beta\tilde{\pi}_M^M\tilde{\pi}_{M-1}^M \end{aligned}$$

Divide by M to obtain

$$\begin{aligned} -\beta\tilde{\pi}_0^M + \alpha\left(1 - \frac{1}{M}\right)\tilde{\pi}_1^M &= \frac{\beta\tilde{\pi}_M^M\tilde{\pi}_0^M}{M} \\ \beta\left(1 - \frac{(i-1)}{M}\right)\tilde{\pi}_{i-1}^M - \left(\beta\left(1 - \frac{i}{M}\right) + \alpha i\left(1 - \frac{i}{M}\right)\right)\tilde{\pi}_i^M &+ \\ \alpha(i+1)\left(1 - \frac{(i+1)}{M}\right)\tilde{\pi}_{i+1}^M &= \frac{\beta\tilde{\pi}_M^M\tilde{\pi}_i^M}{M} \end{aligned}$$

Using limiting arguments as $M \to \infty$ shows that $\tilde{\pi}^M$ converges point wise to the solution of the following difference equations in the sense that $\tilde{\pi}_i^M \to \tilde{\pi}_i$ as $M \to \infty$.

$$\begin{aligned} -\beta\tilde{\pi}_0 + \alpha\tilde{\pi}_1 &= 0 \\ \beta\tilde{\pi}_{i-1} - (\beta + \alpha i)\tilde{\pi}_i + \alpha(i+1)\tilde{\pi}_{i+1} &= 0 \end{aligned}$$

which has as solution $\left(\frac{\beta}{\alpha}\right)^i / i!$ and since $\sum_{i=0}^{\infty}\tilde{\pi}_i = 1$ we scale with $e^{-\frac{\beta}{\alpha}}$ so:

$$\tilde{\pi}(i) = e^{-\frac{\beta}{\alpha}} \frac{\left(\frac{\beta}{\alpha}\right)^i}{i!},$$

which is the expression of a Poisson distribution with mean β/α.

□

Note that this Poisson distribution has mean value $\frac{\beta}{\alpha}$, which is the same as the equilibrium found with the deterministic analysis. Although the equilibrium found with the deterministic analysis was about concentration and this mean value is about amounts of molecules, we show that:

$$\bar{\mathbf{c}} = \frac{\beta_c}{\alpha_c} = \frac{X_B/V}{X_A/V} = \frac{X_B}{X_A} = \frac{\beta}{\alpha} = \mathbb{E}[\tilde{\pi}(i)],$$

With α_c and β_c the reaction rates used in the deterministic analysis in Example 2.1.1 and therefore scaled with a volume V.

4

CONCLUSIONS AND DISCUSSION

Anderson et al. describe and study in [3] a model and discuss properties that are strict theoretical. First, because the chemical reaction network used (Example 1.3.1) is rather unrealistic. Secondly, because the main point made, i.e. that the equilibrium states of the deterministic model and the stochastic model may differ happens on a time scale that is very large. However, there are biochemical systems known in which the amounts of molecules are small enough in order to have a substantial difference in the equilibrium states [7, 8]. Such realistic settings have not been considered in [3]. In Section 3.2 we gave an analysis of the time scale that only applies to the simple example used in Example 1.3.1. Therefore it is not certain whether more complex networks have the same time scale in order to show such behaviour.

A part of [3] discusses the concept of absolute concentration robustness (ACR) for deterministic models. In Section 2.3 we have shown that expanding a model which has absolute concentration robustness and therefore only one equilibrium solution that does not depend on the initial conditions is still absolute concentration robust. However, by adding production and degeneration, the notion of initial conditions seems to become irrelevant, because all species that are produced and degraded are expected to become absolute concentration robust. An example other than Example 1.3.1 that is discussed in [3][8] is the Envz/Ompr signalling system. However in this system production and degeneration plays a role [13]. Therefore it can be disputed whether ACR has practical applications or if it is interesting just for theoretic purposes. After the introduction of the notion of ACR by Shinar and Feinberg in [1] in 2010, not many articles about ACR were written. A review of several definitions of robustness in metabolic networks, including the perspective of chemical reaction networks and ACR [14] outlines the importance of robustness but states that it is hard to capture it whole in a mathematical definition: *"The robustness property does not exist independently of the other inherent properties of systems, such as: optimisation of several functions, redundancy, and fragility-and the differences between these concepts have yet to be delineated"* [14]. Finally, in [1], the authors conclude by saying that one should not expect complex networks which satisfy the conditions of Theorem 2.2.1. to have exactly ACR. This is because of the same reason as the quote above since *"(...) biochemical modules do not exist by themselves and often interact with the intracellular environment."*[1]

The deterministic model which is discussed in Section 1.2 has the most practical applications for most chemical reaction networks, however the stochastic model from Section 1.3 works for chemical reaction networks [7],[8], with small numbers of molecular species but becomes irrelevant for larger scales, see Section 3.2. We conclude by observing that the equilibrium of the deterministic model of the chemical reaction network from Example 1.3.1. is the same as the mean value of the quasi stationary distribution of the stochastic model applied to the same chemical reaction network.

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