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Expected return time to the initial state for biochemical systems with linear cyclic chains: unidirectional and bidirectional reactions

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Abstract. Biochemical systems are robust in nature. We define robustness of a biochemical system as the property where during time evolution, a closed system returns to its initial state. In this study, we propose some mathematical formulations to analyse the robustness of a closed biochemical system. We have provided a tentative guideline towards applying the theory to a non-closed system. We know that a biochemical system evolves with time as a continuous-time Markov process. When this Markov chain is irreducible, it can be proved theoretically that the system will always return to its initial state, and also the expected time of return can be determined. This return time depends upon the stationary probability distribution, which is determined as the solution of an eigenvalue equation $xQ = 0$ where Q is the transition rate matrix. We calculate this expected return time for five different closed systems: unidirectional cyclic linear chains, bidirectional cyclic linear chains and three real biological systems, and verify the theoretical results against the average return time obtained by stochastic simulation.

Keywords. Stochastic simulation; TCA cycle; stationary probability; Markov chain; Chapman–Kolmogorov Equation.

1. Introduction

The reaction dynamics of a biochemical system is a random process that follows the Chemical Master Equation (CME) [1–3]. For large systems, it is difficult to solve the CME. Thus, it is a common practice to use stochastic simulation algorithms (SSAs) for capturing the system dynamics. Two SSAs, called direct method (DM) and first reaction method (FRM), were first introduced in [4, 5]. Later, some upgraded versions of DM were developed. They include optimized direct method (ODM), sorting direct method (SDM), partial-propensity direct method (PDM), sorting partial propensity direct method (SPDM), partial-propensity stochastic simulation algorithm with composition-rejection (PSSA-CR), rejection-based stochastic simulation algorithm (RSSA), rejection-based stochastic simulation algorithms with composition-rejection (RSSA-CR) and slow update exact stochastic simulation algorithm (SUESSA), as DM becomes computationally expensive for modelling networks with large number of reactions [6–12]. Some approximate versions of SSAs, called τ-leaping, were introduced in [13–16]; τ-leaping methods reduce computational cost of simulation by leaping over time scales. A number of deterministic, stochastic and hybrid simulation methods for modelling biochemical systems are described in [17].

Here we look at two kinds of biochemical systems having n components each. The first kind has components $X_1, \ldots, X_n$, and undergoes cyclic reactions in one direction:

$$X_1 \xrightarrow{k_1} X_2 \xrightarrow{k_2} \cdots \rightarrow X_n \xrightarrow{k_n} X_1.$$

The term X_i $(i = 1, \ldots, n)$ stands for ith substrate, and k_1, $\ldots, k_n$ are the chemical rate constants. We call this system as a unidirectional cyclic linear chain. In the second kind of biochemical systems, we allow reverse reactions as well:

$$X_1 \underset{kb_1}{\overset{kf_1}{\rightleftharpoons}} X_2 \underset{kb_2}{\overset{kf_2}{\rightleftharpoons}} X_3 \rightleftharpoons \cdots \rightleftharpoons X_n \underset{kb_n}{\overset{kf_n}{\rightleftharpoons}} X_1,$$

where kf_i are the forward rates, and kb_i are the reverse rates for the ith reaction. We call this as a bidirectional cyclic linear chain.

Let $\mathbf{X} = (\#X_1, \ldots, \#X_n) \in \mathcal{S} \subseteq (\mathbb{N} \cup 0)^n$, where $\mathcal{S}$ is the state space of the Markov chain (MC) $\mathbf{X}$, and $\#$ denotes the number of molecules of the corresponding species (substrate). The reaction propensity for a reaction R of the form $A \rightarrow B$ is given by $a = \#A \times k_{A\rightarrow B}$ where $k_{A\rightarrow B}$ is the reaction rate. For simulation of the entire process, DM of Gillespie [5] gives us the following:

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- a reaction occurs at $t = \tau$ where $\tau \sim \exp\left(\frac{1}{\sum_{\ell=1}^{r} a_\ell}\right)$, where r is the number of reactions and a_ℓ is the propensity of reaction R_ℓ;
- the reaction that occurs is one of the r reactions, selected according to the discrete probability measure $q(d) = \frac{a_d}{\sum_{\ell=1}^{r} a_\ell}$ where a_d is the propensity of reaction R_d.

In this paper, we will show that the expected time of return of a closed biochemical system to its initial molecular populations (i.e., initial state) can be calculated from the MC property, as the system follows an irreducible MC if its state space is finite. We will validate this hypothesis with a standard SSA (DM) in the case of cyclic linear chains (unidirectional and bidirectional) and three real biological systems.

In the next section, we will go through the mathematics of MCs, and show that the problem of finding the expected return times reduces to an eigenvalue equation. In the subsequent sections, we will solve the eigenvalue equation for the aforesaid biochemical systems.

2. Mathematical preliminaries

All the properties of MCs in this section are classical, and can be found in [18, 19]. For this section, we index the elements of the state space $\mathcal{S}$ using indices i, j, and consider a general system with n components $X_1, \ldots, X_n$, and r reactions $R_1, \ldots, R_r$.

2.1 *Continuous-time Markov chains (CTMCs)*

Definition 1 For every CTMC, we have the following transition-probability function:

$$\begin{aligned} P_{ij}(t) &:= P\Big(\mathbf{X}(s+t) = j \mid \mathbf{X}(s) = i, \{\mathbf{X}(u) : u \in [0,s]\}\Big) \\ &= P\Big(\mathbf{X}(s+t) = j \mid \mathbf{X}(s) = i\Big). \end{aligned}$$

The equality is a re-wording of the Markov property.

2.2 *Holding times*

Definition 2 For $\mathbf{X}(t) = i \in \mathcal{S}$, we define H_i to be the holding time for state i, i.e., the total time for which the MC remains at state i. Note that

- the past before t is the current age of H_i;
- the future after t is the remaining H_i.

Thus, H_i is a random variable with the memoryless property, and therefore, $H_i \sim \exp(\gamma_i)$ for some constant γ_i. This fact is in agreement with the derivations of Gillespie [5], and thus γ_i is related to the reaction propensities, as $\gamma_i = \sum_{l=1}^{r} a_l(i)$, where $a_l(i)$ is the propensity of reaction R_l at state i.

2.3 *Embedded discrete-time Markov chains (EDTMCs)*

Definition 3 For any CTMC $\mathbf{X}(t)$, let $\{\tau_1, \tau_2, \ldots, \tau_u, \ldots\}$ denote the transition times. Then $\mathbf{X}_u := \mathbf{X}(\tau_u)$ is a discrete-time MC that makes discrete jumps[1]. We denote P°_{ij} as the transition-probability matrix of $\mathbf{X}_u$, and assume without loss of generality that $P^\circ_{ii} = 0$. Thus

$$\begin{aligned} P^\circ_{ij} &= P(\text{next state is } j \mid \text{current state is } i) \text{for } i \neq j \\ &= 0 \text{ otherwise.} \end{aligned} \tag{1}$$

From Gillespie's results, we know that $\mathbf{X}$ selects the next reaction according to the probability measure given by $q(d) = \frac{a_d}{\sum_{\ell=1}^{r} a_\ell}$, $d = 1, 2, \ldots, r$. Thus we can compute these P°_{ij} in the following way:

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for $i \in \mathcal{S}$ do
    for $d \in \{1, \cdots, r\}$ do
        calculate $a_d(i)$, the propensities of the reactions at state $i$
    endfor
    for $d' \in \{1, \cdots, r\}$ do
        let $j$ be state reachable from $i$ via $R_{d'}$
        $P^\circ_{ij} = \frac{a_{d'}(i)}{\sum_{\ell=1}^{r} a_\ell(i)}$
    endfor
endfor
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2.4 *Chapman–Kolmogorov Equation*

The Chapman–Kolmogorov Equation tells us how to compute the transition-probability function at later instants of time.

Theorem 1 *Let* $P(t) = \big(P_{ij}(t)\big)_{i,j \in \mathcal{S}}$ *be the transition-probability function of* $\mathbf{X}$. *Then we have*

$$P(t+s) = P(t)P(s) \qquad \text{for all } s, t \geq 0,$$

where the product on the right side is matrix multiplication.

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[1] When we write $\mathbf{X}$, we refer to the CTMC. But when we write $\mathbf{X}_u$, we refer to this embedded discrete chain.

2.5 *Irreducibility and closed sets*

Irreducibility is a very special property for an MC that allows the process to retrace its path with positive probability. First we define reachability of a state from another state.

Definition 4 For any two states $i, j \in \mathcal{S}$, we write $i \rightarrow j$, i.e., j is reachable from i iff

$$P_{ij}(t) > 0 \qquad \text{for some} \quad t > 0.$$

A subset $C \subseteq \mathcal{S}$ is called closed if $i \rightarrow j$ holds for every pair $i, j \in C$. We call the MC irreducible if $\mathcal{S}$ itself is closed.

In other words, an MC is called irreducible if j is reachable from i for every pair of states $i, j \in \mathcal{S}$. Note that reachability only ensures that there is *some positive probability* of returning to the initial state. This allows us to classify states according to the probability of returning to the given state.

2.6 *Classification of states*

Consider a closed set $C \subseteq \mathcal{S}$, and any state $i \in C$. Let us start an MC $\mathbf{X}$ at state i. We can compute the probability that $\mathbf{X}$ returns to i. If this probability turns out to be 1, we call i recurrent. If the probability turns out to be less than 1, we call i transient. Formally, we define these notions via the concept of return time.

Definition 5 The return time, T_i, is the magnitude of time after which $\mathbf{X}$ returns to i, given that $\mathbf{X}(0) = i$. A state is called recurrent if $P_i(T_i < \infty) = 1$, that is, $P(T_i < \infty \mid \mathbf{X}(0) = i) = 1$. Otherwise, it is called transient [19].

Recurrent states are further classified into three types.

Definition 6 A state i is called positive recurrent if it is recurrent and $E_i(T_i) := E(T_i \mid \mathbf{X}(0) = i) < \infty$. Otherwise, it is called null recurrent [19].

Now, these properties are defined only for a single state. The following theorem asserts that they carry over to other reachable states as well.

Theorem 2 *If a state i is positive recurrent/null recurrent/transient, and $i \rightarrow j$ occurs, then j is also positive recurrent/null recurrent/transient. This means that each of these properties is inherited by the whole closed set containing a state* [19].

Therefore, the state space of $\mathbf{X}$, when finite, can be partitioned into irreducible closed sets, of which each closed set is either transient, or positive recurrent, or null recurrent.

Now we state our main theorem.

Theorem 3 *Whenever $\mathbf{X}$ is irreducible and $\mathcal{S}$ is finite, the MC is positive recurrent* [19].

This is the basis for all our conclusions in the upcoming sections.

2.7 *Stationary probability distribution*

The stationary probability distribution is a special distribution in the sense that a given MC starting with the stationary distribution remains at the same distribution at all later times.

Definition 7 Suppose P^* is a probability measure on $\mathcal{S}$ for which

$$P^* P(t) = P^*$$

for every t. Then P^* is called a stationary distribution [19]. This is because, if $X(0) \sim P^*$ then $X(t) \sim P^*$ for all subsequent times t.

The stationary probability distribution has values closely related with the expected return times, which is reflected in the following theorem.

Theorem 4 *We have, for an irreducible $\mathbf{X}(t)$, with $i \in \mathcal{S}$, $|\mathcal{S}| < \infty$ and γ_i as in Definition 2.2*

$$E_i(T_i) = \frac{1}{\gamma_i} \frac{1}{P_i^*}.$$

The stationary probabilities in this case are therefore unique.

This is described as **Theorem 3.5.3** in [18]. It tells us that the key to reach $E_i(T_i)$ is via P_i^*, which is given by their defining relation $P^* P(t) = P^*$. We need to solve for a left eigenvector for $P(t)$ with eigenvalue 1.

2.8 *The transition-rate matrix Q*

Theorem 5 *For a finite state space $\mathcal{S}$, let $Q := P'(0)$, then*

$$P'(t) = P'(0) P(t) = Q P(t).$$

Hence

$$P(t) = \exp(Qt) = \sum_{n=0}^{\infty} \frac{(Qt)^n}{n!}.$$

This Q can be determined as follows [19]:

$$Q = P'(0) = \begin{pmatrix} -\gamma_1 & \gamma_1 P^\circ_{12} & \cdots & \gamma_1 P^\circ_{1|\mathcal{S}|} \\ \gamma_2 P^\circ_{21} & -\gamma_2 & \cdots & \gamma_2 P^\circ_{2|\mathcal{S}|} \\ \vdots & & & \vdots \\ \gamma_{|\mathcal{S}|} P^\circ_{|\mathcal{S}|1} & \gamma_{|\mathcal{S}|} P^\circ_{|\mathcal{S}|2} & \cdots & -\gamma_{|\mathcal{S}|}. \end{pmatrix} \qquad (2)$$

Now, we already know that if the dth reaction R_d takes state i to state j, P°_{ij} is actually $\frac{a_d}{\gamma_i}$. For states j that are not reachable from i, $P^{\circ}_{ij}=0$. Thus, the entries of $Q=P'(0)$ in our case are

$$P'_{ij}(0)=\begin{cases}0 & \text{if } i \not\to j,\\ a_d & \text{if } i\to j \text{ via reaction } R_d.\end{cases}$$

i^{th} row sum of $P'(0)$ is

$$\begin{aligned}\sum_{j=1}^{|\mathcal{S}|}P'_{ij}(0)&=P'_{ii}(0)+\sum_{j\neq i}P'_{ij}(0)\\&=-\gamma_i+\gamma_i\sum_{j\neq i}P^{\circ}_{ij}=-\gamma_i+\gamma_i=0.\end{aligned}$$

2.9 *Left-eigenvectors of P(t)*

In this subsection, we compute the left-eigenvectors of the function $P(t)=\exp(Qt)$.

Note Observe that Q has row sums equal to zero. Therefore, Q has 0 as a right-eigenvalue. However, we know that the characteristic polynomial of Q is the same as that of Q^T. Therefore, Q has 0 also as a left-eigenvalue. Hence, $\exp(Qt)$ also has a left-eigenvalue of 1.

Lemma *If A is a matrix having an eigenvalue of λ and eigenvector v, then* $\exp(A)$ *has an eigenvalue of* $\exp(\lambda)$ *with the same eigenvector v.*

Proof Of course, $Av=\lambda v$ implies that $A^k v=\lambda^k v$, and therefore

$$\exp(A)v=\exp(\lambda)v.$$

Thus, the eigenvalues of $P(t)$ are exponentials of the eigenvalues of Qt, and they share the same eigenspaces. By uniqueness of stationary distribution for $\mathbf{X}$, 1 is a simple eigenvalue of $P(t)$, i.e., it has multiplicity of 1. Therefore, P^* is a left-eigenvector for Qt with eigenvalue 0, which means that

$$P^*Qt=0\ \forall t\geq 0 \Rightarrow P^*Q=0. \qquad (3)$$

The equation to be solved is this final equation. This is mentioned in section 3.5 of [18, p. 117], and Theorem 1.1 of [19, p. 14]. □

3. Analysis of cyclic reaction systems

Here we describe the procedure of calculating the expected time of return to the initial states for both unidirectional and bidirectional cyclic linear chains, and also some real but closed biological systems.

Additional assumptions ensuring positive recurrence: For the rest of the article, we assume that p, q are indices varying from 1 to n, with n as the number of species in the system, and that the initial state is given as $\mathbf{X}(0)=(x_1(0),\cdots,x_n(0))$. Suppose that we have a reaction system in which we start with the state $(x_1,\cdots,x_n)$, and list down all possible states that can be reached via the reactions $R_1,\cdots,R_r$ over a number of iterations. This will form the state space $\mathcal{S}$, and on $\mathcal{S}$, the MC $\mathbf{X}$ is irreducible [4]. If we assume that the reaction system has no reaction through which the population can become unbounded, then $\mathcal{S}$ becomes finite. Reactions such as $\varnothing\to A$ (growth) and $A\to\varnothing$ (decay) are, therefore, not allowed. In the first case, $\mathcal{S}$ becomes unbounded, and in the second case, irreducibility is violated.

For systems that abide by these prototypes, it is to be mentioned that $\mathcal{S}$ becomes finite and $\mathbf{X}$ becomes irreducible. By Theorem 3, $\mathbf{X}$ is positive recurrent [2.6]. By definition, therefore, $P(\mathbf{X}(t)=(x_1(t),\ldots,x_n(t))$ for $t>0)=1$, and the expected time of return of the chain, $E_{(x_1,\cdots,x_n)}\left(T_{(x_1,\cdots,x_n)}\right)$, is finite. This time can be calculated using Theorem 4 and Eq. (3).

What happens for non-closed biological systems? Let us analyse the scenario for a general biological system. For the moment, let us allow decay reactions $A\to\varnothing$ in our system. Then, the fact that state space of the MC $\mathbf{X}$ is finite still holds. However, the chain loses its irreducibility property, which means that once the system triggers the reaction $A\to\varnothing$, it cannot retrace its path. The same analysis holds good for purely closed systems with only growth reactions $\varnothing\to B$, for example. These systems are not robust.

Since biological systems with only growth or only decay reactions are not irreducible, a robust system that is not closed must contain both of them. This ensures irreducibility of the associated MC. For simplicity, let us consider a very simple system with reactions $\varnothing\to A\rightleftharpoons B\to\varnothing$, and with initial concentrations of $A=10$, $B=0$. Clearly the state space in this scenario is infinite, as the reactions $\varnothing\to A$ could be triggered as many times as we want, meaning that $\{(x,0):x\geq 10\}\subseteq\mathcal{S}$. Therefore, we cannot conclude whether the chain $\mathbf{X}$ is positive recurrent in this case or not. In order to ensure a bound on $\mathcal{S}$, we may assume furthermore that the system does not trigger $\varnothing\to A$ indefinitely. For example, we can say that the system triggers $\varnothing\to A$ only if $[A]+[B]\leq 100$. Under this additional assumption, the state space becomes finite, $\mathbf{X}$ becomes positive recurrent and the system now becomes robust.

In this article we have actually computed the return times for the afore-mentioned simple system, just in disguise. We can think of this simple system as being equivalent to a linear bidirectional closed chain, now $\varnothing$ being interpreted as a new species. The reactions in question are therefore

$$\varnothing \underset{0}{\overset{k_1}{\rightleftharpoons}} A \underset{k_3}{\overset{k_2}{\rightleftharpoons}} B \underset{0}{\overset{k_3}{\rightleftharpoons}} \varnothing,$$

with initial concentrations $[\varnothing] = 90$, $[A] = 10$ and $[B] = 0$.

Therefore, complex biological systems are provably robust under additional assumptions for triggering growth and decay reactions, via treating $\varnothing$ as a dummy species in the system itself with a fixed concentration.

We will compute the return times only for two very specific systems under this framework, namely the unidirectional and bidirectional linear closed chains.

3.1 *Linear (unidirectional) closed chains*

First we apply the afore-mentioned theory to simple linear chains on n substances (species). The equations are of the form

$$X_1 \xrightarrow{k_1} X_2 \xrightarrow{k_2} \cdots \longrightarrow X_n \xrightarrow{k_n} X_1.$$

Suppose that the initial populations of molecules are given as $(x_1(0), \ldots, x_n(0)) := (\#X_1, \ldots, \#X_n)$. Note that in each of these reactions, the sum of populations $x_1(t) + \cdots + x_n(t) = x$, for all t, remains constant. Thus for each of the n-tuples in $\mathcal{S}$, we have

$$x_1 + x_2 + \cdots + x_n = x.$$

This has $|\mathcal{S}| = \begin{pmatrix} (x+n-1) \\ (n-1) \end{pmatrix}$ different solutions[2]. Each solution corresponds to a state, an element of $(\mathbb{N} \cup 0)^n$.

3.1a *Hunting for the stationary probabilities*: Let us consider a system of size $n = 3$ and $x = 1$. In this case, the state space has the three elements (0, 0, 1), (0, 1, 0) and (1, 0, 0). For this system, Q matrix becomes (Eq. (2))

$$Q = \begin{array}{c} \\ (0,0,1) \\ (0,1,0) \\ (1,0,0) \end{array} \begin{array}{c} \begin{array}{ccc} (0,0,1) & (0,1,0) & (1,0,0) \end{array} \\ \begin{pmatrix} -k_3 & 0 & k_3 \\ k_2 & -k_2 & 0 \\ 0 & k_1 & -k_1 \end{pmatrix} \end{array}$$

This gives us

$$P^*_{(0,0,1)} k_3 = P^*_{(0,1,0)} k_2 \text{ and } P^*_{(0,1,0)} k_2 = P^*_{(1,0,0)} k_1,$$

which, in addition to $P^*_{(0,0,1)} + P^*_{(0,1,0)} + P^*_{(1,0,0)} = 1$, leads us to

$$P^*_{(0,0,1)} = \frac{\frac{1}{k_3}}{\frac{1}{k_1} + \frac{1}{k_2} + \frac{1}{k_3}},$$

$$P^*_{(0,1,0)} = \frac{\frac{1}{k_2}}{\frac{1}{k_1} + \frac{1}{k_2} + \frac{1}{k_3}},$$

$$P^*_{(1,0,0)} = \frac{\frac{1}{k_1}}{\frac{1}{k_1} + \frac{1}{k_2} + \frac{1}{k_3}}.$$

Let us consider another system of size $n = 3$ and $x = 3$. After tedious calculations and inspection of the stationary probabilities for a system of size $n = 3$ and $x = 3$, we can observe the following pattern:

$$P^*_{(x_1,x_2,x_3)} = \begin{pmatrix} (x) \\ (x_1, x_2, x_3) \end{pmatrix} \frac{\frac{1}{k_1^{x_1}} \frac{1}{k_2^{x_2}} \frac{1}{k_3^{x_3}}}{\left(\frac{1}{k_1} + \frac{1}{k_2} + \frac{1}{k_3}\right)^x}.$$

Here

$$\begin{pmatrix} (x) \\ (x_1, x_2, x_3) \end{pmatrix} := \frac{x!}{x_1! x_2! x_3!}.$$

Note that the sum

$$\sum_{x_1+x_2+x_3=3} P^*_{(x_1,x_2,x_3)} = 1$$

has exactly $10 = \begin{pmatrix} 3+3-1 \\ 3-1 \end{pmatrix}$ terms.

General systems: These computations lead us to the following conjecture that will be proved next.

Theorem 6 ***(Stationary probabilities for linear (unidirectional) closed chains)***

Consider a unidirectional linear closed chain with n species, and having the following species, and having the following n reactions:

$$X_1 \xrightarrow{k_1} X_2 \xrightarrow{k_2} \cdots \rightarrow X_n \xrightarrow{k_n} X_1.$$

Then, for a state $(x_1, \ldots, x_n)$, *the stationary probability is given by*

$$P^*_{(x_1,\cdots,x_n)} = \begin{pmatrix} (x) \\ (x_1, \ldots, x_n) \end{pmatrix} \frac{\frac{1}{k_1^{x_1}} \cdots \frac{1}{k_n^{x_n}}}{\left(\frac{1}{k_1} + \cdots + \frac{1}{k_n}\right)^x},$$

where $x := x_1 + \cdots + x_n$ *is the total population of all the species, and*

$$\begin{pmatrix} (x) \\ (x_1, \ldots, x_n) \end{pmatrix} := \frac{x!}{x_1! x_2! \ldots x_n!}.$$

Proof It suffices to show, using the uniqueness of the stationary distribution (Theorem 4), that the afore-mentioned probability distribution is a left-eigenvector of the transition rate matrix Q. Note that we can explicitly write the Q matrix using the values for P°_{pq} and using the equation

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[2] This is an elementary problem in combinatorics. A solution to $x_1 + \cdots + x_n = x$ can be imagined as putting $(n-1)$ bars between x balls, and letting x_1 to be the number of balls till the first bar, x_2 to be the number of balls between the first and second bars and so on.

in [2]. For a state $(x_1, \cdots, x_n)$ with all entries nonzero (for the moment), the corresponding row can be given as

$$\begin{cases} Q_{(x_1,x_2,x_3,\cdots,x_n),(x_1-1,x_2+1,x_3,\cdots,x_n)} = a_1 = x_1k_1, \\ Q_{(x_1,x_2,x_3,\cdots,x_n),(x_1,x_2-1,x_3+1,\cdots,x_n)} = a_2 = x_2k_2, \\ \quad\vdots \\ Q_{(x_1,x_2,x_3,\cdots,x_n),(x_1+1,x_2,x_3,\cdots,x_n-1)} = a_n = x_nk_n, \\ Q_{(x_1,x_2,x_3,\cdots,x_n),(x_1,x_2,x_3,\cdots,x_n)} = -\gamma_{(x_1,x_2,x_3,\cdots,x_n)} \\ \qquad = -\sum_{p=1}^{n} x_pk_p, \\ Q_{(x_1,x_2,x_3,\cdots,x_n),j} = 0 \text{ for other } j. \end{cases}$$

Note that even if one of the x_i is zero, it only means that R_i cannot occur, and this is reflected in this representation of the matrix entries. □

Now, we will check that the P^* as shown earlier indeed satisfies

$$\sum_{(b_1,\ldots,b_n)\in\mathcal{S}} P^*_{(b_1,\ldots,b_n)} Q_{(x_1,\ldots,x_n),(b_1,\ldots,b_n)} = 0. \tag{4}$$

The states that can give rise to $(x_1, \ldots, x_n)$ are

$$\mathcal{Z} = \left\{ \begin{array}{l} (x_1-1, x_2, \ldots, x_{n-1}, x_n+1), (x_1+1, x_2-1, \ldots, \\ x_{n-1}, x_n), \ldots, \text{ and } (x_1, x_2, \ldots, x_{n-1}+1, x_n-1). \end{array} \right\},$$

so that we can write

$$\mathcal{S} = \mathcal{Z} \cup \{(x_1, \ldots, x_n)\} \cup \{ \text{ Other states } j \\ \text{where } Q_{(x_1,\ldots,x_n),j} = 0\}.$$

Now we will break up the sum in Eq. (4) over $\mathcal{S}$ into three parts. For the first state of $\mathcal{Z}$, i.e., $(x_1-1, x_2, \cdots, x_{n-1}, x_n+1)$, we get a summand of

$$\begin{aligned} &(x_n+1)k_nP^*_{(x_1-1,x_2,\cdots,x_{n-1},x_n+1)} \\ &\quad = (x_n+1)k_n \binom{(x)}{(x_1-1, x_2, \cdots, x_{n-1}, x_n+1)} \frac{\frac{1}{k_1^{x_1-1}}\frac{1}{k_2^{x_2}}\cdots\frac{1}{k_{n-1}^{x_{n-1}}}\frac{1}{k_n^{x_n+1}}}{\left(\frac{1}{k_1}+\cdots+\frac{1}{k_n}\right)^x} \\ &\quad = (x_n+1)k_n \frac{x!}{(x_1-1)!x_2!\cdots x_{n-1}!(x_n+1)!} \frac{\frac{1}{k_1^{x_1-1}}\frac{1}{k_2^{x_2}}\cdots\frac{1}{k_{n-1}^{x_{n-1}}}\frac{1}{k_n^{x_n+1}}}{\left(\frac{1}{k_1}+\cdots+\frac{1}{k_n}\right)^x} \\ &\quad = x_1k_1 \binom{(x)}{(x_1), \cdots, x_n} \frac{\frac{1}{k_1^{x_1}}\frac{1}{k_2^{x_2}}\cdots\frac{1}{k_{n-1}^{x_{n-1}}}\frac{1}{k_n^{x_n}}}{\left(\frac{1}{k_1}+\cdots+\frac{1}{k_n}\right)^x} =: x_1k_1\beta\,(\text{say}) . \end{aligned}$$

Similarly, we get the other terms from $\mathcal{Z}$ as $x_2k_2\beta, \cdots, x_nk_n\beta$. The summand $P^*_{(x_1,\cdots,x_n)}Q_{(x_1,\cdots,x_n),(x_1,\cdots,x_n)}$ will be exactly

$$-\gamma_{(x_1,\cdots,x_n)}\beta = -(x_1k_1 + \cdots + x_nk_n)\beta,$$

which gives us the desired result of $(P^*Q)_{(x_1,\cdots,x_n)} = 0$ for every state $(x_1, \cdots, x_n)$.

3.1b *Expected return time to the original state*: Finally, we have a solution for the situation when the system is expected to return to its original state. Using Theorem 4 this is given by

$$\begin{aligned} E_{(x_1,\cdots,x_n)}\left(T_{(x_1,\cdots,x_n)}\right) &= \frac{1}{x_1k_1+\cdots+x_nk_n}\frac{1}{P^*_{(x_1,\cdots,x_n)}} \\ &= \frac{1}{x_1k_1+\cdots+x_nk_n} \frac{\left(\frac{1}{k_1}+\cdots+\frac{1}{k_n}\right)^x}{\binom{x}{x_1, \cdots, x_n}\left(\frac{1}{k_1}\right)^{x_1}\cdots\left(\frac{1}{k_n}\right)^{x_n}}. \end{aligned} \tag{5}$$

3.1c *Verification using stochastic simulation*: We tested this theoretical value with the help of stochastic simulation. We used the DM to simulate the following chemical system:

$n = 5, r = 5,$ with reactions $R_1, \cdots, R_5$ in the following order:

$$X_1 \overset{0.00100}{\longrightarrow} X_2 \overset{0.00099}{\longrightarrow} X_3 \overset{0.00101}{\longrightarrow} X_4 \overset{0.00100}{\longrightarrow} X_5 \overset{0.00099}{\longrightarrow} X_1$$

initial state: $(x_1, x_2, x_3, x_4, x_5) = (1000, 1000, 1000, 1000, 1000)$.

For this system, we plugged in the values in Eq. (5) to obtain

$$\begin{aligned} &E_{(1000,\cdots,1000)}\left(T_{(1000,\cdots,1000)}\right) \\ &\quad = \frac{1}{2\cdot1.00+2\cdot0.99+1.01} \cdot \frac{1000!^5(2\cdot1.00^{-1}+2\cdot0.99^{-1}+1.01^{-1})^{5000}}{5000!(1.00^{-2}\cdot0.99^{-2}\cdot1.01^{-1})^{1000}} \\ &\quad \approx 4.07183 \times 10^6. \end{aligned}$$

We ran the simulation till time $T = 10^{10}$, and got the average return time to be (with 2500 observations) approximately equal to 3.99424×10^6. We plotted the average return time against the number of observations, and obtained the graph shown in figure 1.

3.2 *Linear (bidirectional) closed chains*

So far we have looked at the situation of linear unidirectional closed chains. We will now generalize a bit to bidirectional chains. They are systems of n species $X_1, \cdots, X_n$, and we let the reactions to be

$$X_1 \underset{kb_1}{\overset{kf_1}{\rightleftharpoons}} X_2 \underset{kb_2}{\overset{kf_2}{\rightleftharpoons}} X_3 \rightleftharpoons \cdots \rightleftharpoons X_n \underset{kb_n}{\overset{kf_n}{\rightleftharpoons}} X_1.$$

The unidirectional chain is a special case of this, as this system reduces to the unidirectional chain on setting $kb_p = 0$ for every p. Again, we shall be computing the expected return time for the initial state of $(x_1, \ldots, x_n)$, with $x_1 + \cdots + x_n = x$.

3.2a *Hunting for the stationary probabilities*: Before going on to the general form, we shall analyse the case of $x = 1$ with $n = 4$.

Let us consider a system of size $n = 4$ and $x = 1$. In this case, the state space consists of the four elements (0, 0, 0, 1), (0, 0, 1, 0), (0, 1, 0, 0) and (1, 0, 0, 0). We write down the Q matrix, as before:

$$Q = $$

$$\begin{array}{c} \\ (0,0,0,1) \\ (0,0,1,0) \\ (0,1,0,0) \\ (1,0,0,0) \end{array} \begin{array}{c} \begin{array}{cccc} (0,0,0,1) & (0,0,1,0) & (0,1,0,0) & (1,0,0,0) \end{array} \\ \begin{pmatrix} -kb_4 - kf_4 & kb_4 & 0 & kf_4 \\ kf_3 & -kf_3 - kb_3 & kb_3 & 0 \\ 0 & kf_2 & -kf_2 - kb_2 & kb_2 \\ kb_1 & 0 & kf_1 & -kf_1 - kb_1 \end{pmatrix} \end{array}$$

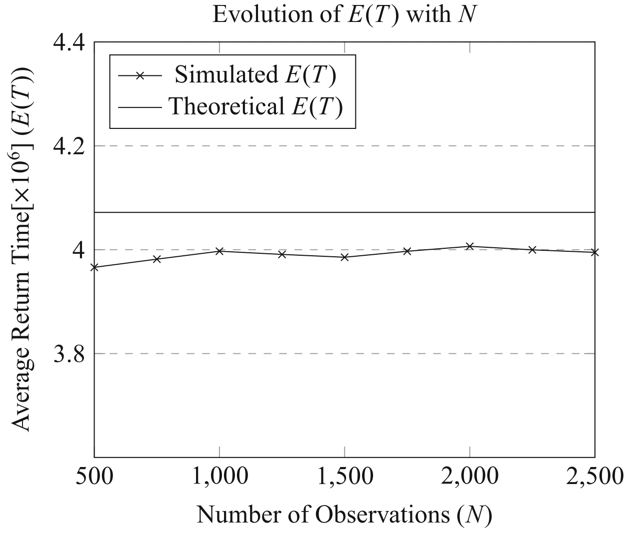

Figure 1. Average return time for test model of unidirectional linear chain.

Using Cramer's rule it can be seen that

$$\begin{aligned} &P^*_{(0,0,0,1)} \propto \\ &\left|\det\begin{pmatrix} -kf_3 - kb_3 & kb_3 & 0 \\ kf_2 & -kf_2 - kb_2 & kb_2 \\ 0 & kf_1 & -kf_1 - kb_1 \end{pmatrix}\right| \\ =&\left|\det\begin{pmatrix} -kf_3 - kb_3 & kb_3 & -kf_3 \\ kf_2 & -kf_2 - kb_2 & 0 \\ 0 & kf_1 & -kb_1 \end{pmatrix}\right| \\ =&kf_1kf_2kf_3 + kb_1kf_2kf_3 + kb_1kb_2kf_3 + kb_1kb_2kb_3, \end{aligned}$$

and

$$P^*_{(0,0,1,0)} \propto kf_4kf_1kf_2 + kb_4kf_1kf_2 + kb_4kb_1kf_2 + kb_4kb_1kb_2.$$

Writing the numerators of the stationary probabilities in this form is enlightening, as we easily notice a pattern. $P^*_{(0,0,1,0)}$ has a form in which the first term is $kf_4kf_1kf_2$, and the consecutive terms have one of the kf from the front changed to a kb.

We shall show that this indeed is the general form for the stationary distribution in the case of a bidirectional cycle.

General case: In order to write this rigorously, we shall define the following n quantities:

$$\begin{aligned} e_1 &:= (kf_2kf_3 \cdots kf_n) + (kb_2kf_3 \cdots kf_n) \\ &\quad + \cdots + (kb_2kb_3 \cdots kb_n) \\ e_2 &:= (kf_3kf_4 \cdots kf_1) + (kb_3kf_4 \cdots kf_1) \\ &\quad + \cdots + (kb_3kb_4 \cdots kb_1) \\ &\vdots \\ e_n &:= (kf_1kf_2 \cdots kf_{n-1}) + (kb_1kf_2 \cdots kf_{n-1}) \\ &\quad + \cdots + (kb_1kb_2 \cdots kb_{n-1}) \end{aligned}$$

Theorem 7 ***(Stationary probabilities for linear (bidirectional) closed chains)***

Consider a bidirectional linear closed chain with n species, and having the following 2 n reactions:

$$X_1 \underset{kb_1}{\overset{kf_1}{\rightleftharpoons}} X_2 \underset{kb_2}{\overset{kf_2}{\rightleftharpoons}} X_3 \rightleftharpoons \cdots \rightleftharpoons X_n \underset{kb_n}{\overset{kf_n}{\rightleftharpoons}} X_1.$$

Then, for a state $(x_1, \cdots, x_n)$, the stationary probability is given by

$$P^*_{(x_1,\cdots,x_n)} = \binom{x}{x_1, \cdots, x_n} \frac{e_1^{x_1} \cdots e_n^{x_n}}{(e_1 + \cdots + e_n)^x},$$

where $x := x_1 + \cdots + x_n$ is the total population of all the species, and e_p are as defined earlier.

Proof As in the previous proof, we will write down the entries of Q. We denote the forward propensities as af_p and the backward propensities as ab_p, $1 \le p \le n$, accounting for a total of $2n$ reactions. For a state $(x_1, \ldots, x_n)$ with all entries nonzero (for the moment), the corresponding row is given by

$$\begin{cases} Q_{(x_1,x_2,x_3,\ldots,x_n),(x_1-1,x_2+1,x_3,\ldots,x_n)} = af_1 = x_1kf_1, \\ Q_{(x_1,x_2,x_3,\ldots,x_n),(x_1,x_2-1,x_3+1,\ldots,x_n)} = af_2 = x_2kf_2, \\ \qquad \vdots \\ Q_{(x_1,x_2,x_3,\ldots,x_n),(x_1+1,x_2,x_3,\ldots,x_n-1)} = af_n = x_nkf_n, \end{cases}$$

and

$$\begin{cases} Q_{(x_1,x_2,x_3,\ldots,x_n),(x_1+1,x_2,\ldots,x_{n-1},x_n-1)} = ab_1 = x_1kb_1, \\ Q_{(x_1,x_2,x_3,\ldots,x_n),(x_1-1,x_2+1,\ldots,x_{n-1},x_n)} = ab_2 = x_2kb_2, \\ \qquad \vdots \\ Q_{(x_1,x_2,x_3,\ldots,x_n),(x_1,x_2,\ldots,x_{n-1}-1,x_n+1)} = ab_n = x_nkb_n, \end{cases}$$

and

$$Q_{(x_1,x_2,x_3,\ldots,x_n),(x_1,x_2,x_3,\ldots,x_n)} = -\sum_{p=1}^{n} x_p(kf_p + kb_p).$$

For other j

$$Q_{(x_1,x_2,x_3,\ldots,x_n),j} = 0.$$

It is clear that a total of $2n$ states can lead to $(x_1, \cdots, x_n)$, if all are nonzero. If one of the x_p equals zero, the corresponding entry in Q matrix is automatically set to zero, and hence we can say that these relations hold for all x_i. Now, we let, as before

$$\mathcal{Z} = \left\{ \begin{array}{r} (x_1-1, x_2\ldots, x_n+1), (x_1+1, x_2-1, \ldots, x_n), \ldots, \\ (x_1, x_2, \ldots, x_n-1), \\ (x_1+1, x_2, \ldots, x_n-1), (x_1-1, x_2+1, \ldots, x_n), \ldots, \\ (x_1, x_2, \ldots, x_n+1) \end{array} \right\},$$

so that

$$\begin{aligned} \mathcal{S} = \mathcal{Z} \cup \{(x_1, \ldots, x_n)\} \cup \\ \{\text{other states } j \text{ where } Q_{(x_1,\ldots,x_n),j} = 0\}. \end{aligned}$$

Thus, it suffices to check that

$$\begin{aligned} \sum_{(b_1,\ldots,b_n)\in\mathcal{Z}} & P^*_{(b_1,\ldots,b_n)} \cdot Q_{(x_1,\ldots,x_n),(b_1,\ldots,b_n)} \\ & + P^*_{(x_1,\ldots,x_n)} \cdot Q_{(x_1,\ldots,x_n),(x_1,\ldots,x_n)} = 0. \end{aligned} \tag{6}$$

We will divide this sum into n parts. In the first part, we will look at the contribution of $(x_1+1, x_2-1, x_3\ldots, x_n)$ and $(x_1, x_2-1, x_3+1, \ldots, x_n)$, which are the two states that can lead to an increase in the population of X_2, via the reactions $X_1 \xrightarrow{kf_1} X_2$ and $X_3 \xrightarrow{kb_3} X_2$, respectively. Now, their contributions are

$$\begin{aligned} C = & (x_1+1)kf_1 \binom{x}{x_1+1, x_2-1, x_3, \cdots, x_n} \\ & e_1^{x_1+1} e_2^{x_2-1} e_3^{x_3} \cdots e_n^{x_n} \\ & + (x_3+1)kb_3 \binom{x}{x_1, x_2-1, x_3+1, \cdots, x_n} \\ & e_1^{x_1} e_2^{x_2-1} e_3^{x_3+1} \cdots e_n^{x_n}. \end{aligned}$$

Note that

$$\begin{aligned} (x_1+1)\binom{x}{x_1+1, x_2-1, x_3, \ldots, x_n} &= x_2 \binom{x}{x_1, x_2, \ldots, x_n} \\ &= (x_3+1)\binom{x}{x_1, x_2-1, x_3+1, \ldots, x_n}. \end{aligned}$$

Therefore, we can let $\beta := \binom{x}{x_1, \ldots, x_n} e_1^{x_1} \ldots e_n^{x_n}$ to simplify C as

$$C = x_2\beta\left(\frac{kf_1e_1 + kb_3e_3}{e_2}\right).$$

Hence, it remains to check that

$$kf_1e_1 + kb_3e_3 = (kf_2 + kb_2)e_2. \tag{7}$$

Denote $kf_p =: x_p$ and $kb_p =: y_p$, $1 \le p \le n$, so that we have

$$\frac{\begin{array}{c} x_1e_1 = (x_1x_2x_3\ldots x_n) + (x_1y_2x_3\ldots x_n) + \ldots + (x_1y_2y_3\ldots y_n) \\ y_3e_3 = (y_3x_4x_5\ldots x_2) + (y_3y_4x_5\ldots x_2) + \ldots + (y_3y_4\ldots y_2) \end{array}}{(x_2+y_2)e_2 = (x_2+y_2)\Big((x_3x_4\ldots x_1) + (y_3x_4\ldots x_1) + \ldots + (y_3y_4\ldots y_1)\Big)}.$$

A general term in the expansion of $(x_2 + y_2)e_2$ is of the form

$$\begin{aligned} & (x_2+y_2)\Big((y_3\ldots y_q)(x_{q+1}\ldots x_1)\Big) \\ & \quad = (x_2+y_2)\Big(y_1y_3\ldots y_n + (x_1y_3\ldots y_q)(x_{q+1}\ldots x_n)\Big). \end{aligned}$$

Note that

- the terms $y_1x_2y_3\ldots y_n$ and $y_1y_2y_3\ldots y_n$ are contained in the expression of y_3e_3;
- the terms $x_1x_2(y_3\ldots y_q)(x_{q+1}\ldots x_n)$ $(n \ge q \ge 1)$ appear in the expression of y_3e_3;
- the term $x_1x_2(x_3\ldots x_n)$ appears in the expression of x_1e_1;
- the terms $x_1y_2(y_3\ldots y_q)(x_{q+1}\ldots x_n)$ $(n \ge q \ge 0)$ appear in the expression of x_1e_1.

This means that each of the $2n$ terms of $(x_2 + y_2)e_2$ appears either in x_1e_1 or y_3e_3, and since both the expressions have n terms each, the equality in Eq. (7) is established. □

What if only forward reactions are permitted? When $kb_p = 0$ for every p, notice that e_p becomes

$$e_p = \frac{kf_1 kf_2 \ldots kf_n}{kf_p},$$

and the result coincides with the unidirectional case [6], after cancellation of the homogeneous factor of $kf_1 kf_2 \cdots kf_n$.

3.2b *Expected return time to the original state*: Similarly as before, we have

$$\begin{aligned} E_{(x_1,\ldots,x_n)}\left(T_{(x_1,\ldots,x_n)}\right) &= \frac{1}{\sum_{p=1}^{n} x_p(kf_p + kb_p)} \frac{1}{P^*_{(x_1,\ldots,x_n)}} \\ &= \frac{1}{\sum_{p=1}^{n} x_p(kf_p + kb_p)} \frac{(e_1 + \ldots + e_n)^x}{\binom{x}{x_1,\ldots,x_n} e_1^{x_1} \ldots e_n^{x_n}}. \end{aligned} \tag{8}$$

3.2c *Verification using stochastic simulation*: We tested this theoretical value with the help of stochastic simulation, using the DM on the following chemical system:

$n = 5, r = 10,$ with reactions $R_1, \cdots, R_{10}$ in the following order:

$$X_1 \underset{0.00080}{\overset{0.00100}{\rightleftharpoons}} X_2 \underset{0.00081}{\overset{0.00099}{\rightleftharpoons}} X_3 \underset{0.00083}{\overset{0.00101}{\rightleftharpoons}} X_4 \underset{0.00079}{\overset{0.00100}{\rightleftharpoons}} X_5 \underset{0.00082}{\overset{0.00099}{\rightleftharpoons}} X_1.$$

Initial state: $(x_1, x_2, x_3, x_4, x_5) = (1000, 1000, 1000, 1000, 1000)$.

For this system, we plugged in the values in Eq. (8) to obtain

$$e_1 = 3.389 \times 10^{-12}, e_2 = 3.434 \times 10^{-12},$$
$$e_3 = 3.405 \times 10^{-12},$$
$$e_4 = 3.371 \times 10^{-12}, e_5 = 3.455 \times 10^{-12},$$

and

$$\begin{aligned} &E_{(1000,\cdots,1000)}\left(T_{(1000,\cdots,1000)}\right) \\ &= \frac{1}{(4.99 + 4.05)} \frac{1000!^5 (3.389 + 3.434 + 3.405 + 3.371 + 3.455)^{5000}}{5000!(3.389 \cdot 3.434 \cdot 3.405 \cdot 3.371 \cdot 3.455)^{1000}} \\ &\approx 2.38005 \times 10^6. \end{aligned}$$

We ran the simulation till time $T = 7.1 \times 10^9$, and got the average return time to be (with 2500 observations) approximately equal to 2.82597×10^6. Figure 2 illustrates the graph of average return time versus the number of observations.

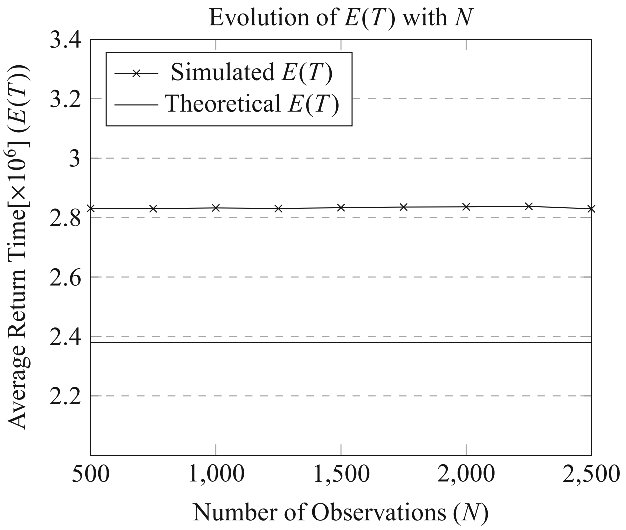

Figure 2. Average return time for test model of bidirectional linear chain.

3.3 *Biological network 1*

This network is a part of tricarboxylic acid (TCA) cycle. TCA cycle is a linear cyclic pathway in which citric acid circulates through α-ketogluterate, which is then removed from the network. The total cycle for *E. coli* growth on glucose and the reaction rates have been extracted from [20]. The reactions in the cycle are described in figure 3. However, the reactions isocitrate → succinate + glyoxylate and glyoxylate → malate have very low rates compared with the other reactions, and hence we will ignore this pathway. Besides, since no molecule can be allowed to degrade in our consideration, we have to disregard removal of α-ketogluterate from the system, in order to apply the afore-mentioned theory. Thus, we consider the modified system shown in figure 4.

This is a linear cyclic system and hence Theorem 6 works. We simulated this system for an initial population of oxaloacetate = 8, and other populations were equal to zero. The calculated return time for this is

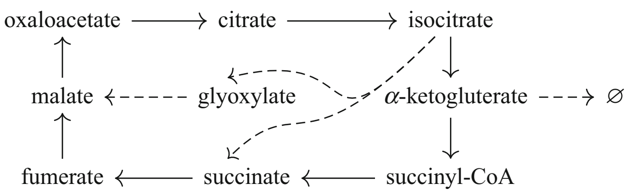

Figure 3. Reactions in the TCA cycle.

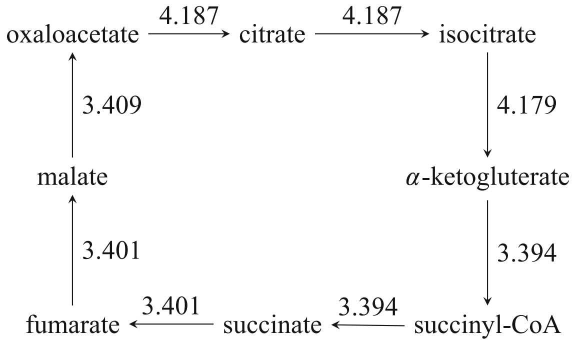

Figure 4. Reactions in biological network 1.

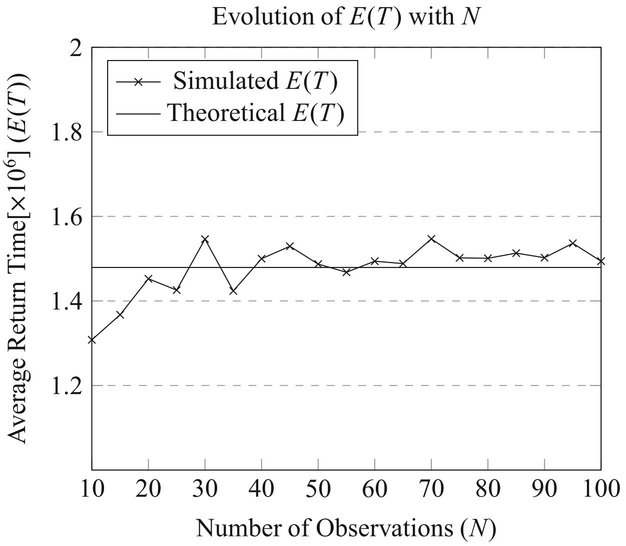

Figure 5. Average return time for biological network 1.

$$\frac{1}{8\times 4.187}\,\frac{\left(\frac{2}{4.187}+\frac{1}{4.179}+\frac{2}{3.394}+\frac{2}{3.401}+\frac{1}{3.409}\right)^{8}}{\left(\frac{1}{4.187}\right)^{8}}$$
$$\approx 1.47923\times 10^{6}.$$

This system was simulated, and for 100 observations, the value of the return time was found out to be 1.494×10^6. The evolution of the average return time is plotted in figure 5.

3.4 *Biological network 2*

This network is important in metabolism. It involves recycling of pyruvate in mitochondria [21]. The recycling of pyruvate cycle consists of the reactions shown in figure 6. We abbreviate 2,3-dihydroxy-3-methylbutanoate as dhmb. This is a cyclic linear chain, and therefore Theorem 6 applies. We simulated this network for an initial population of 2-oxoglutarate = pyruvate = 5, and other populations equal to zero. The calculated return time for this is

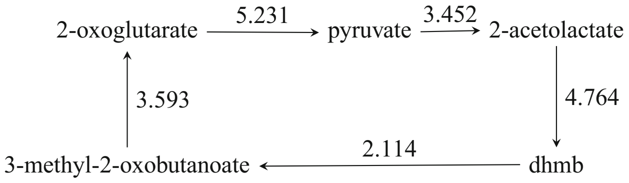

Figure 6. Reactions in biological network 2.

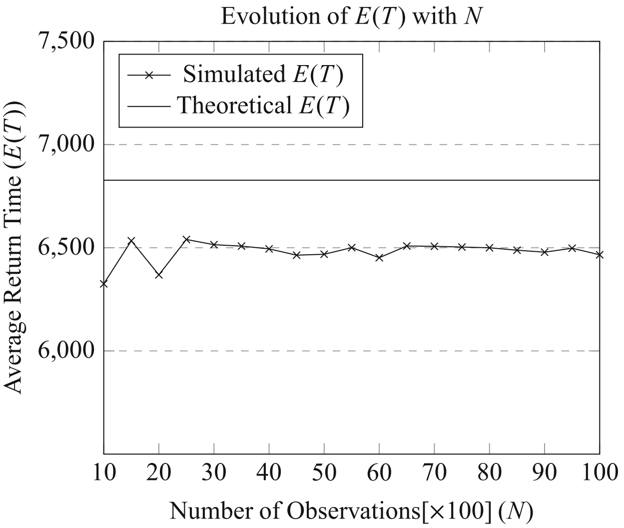

Figure 7. Average return time for the biological network 2.

$$\frac{1}{5\cdot 5.231+5\cdot 3.452}$$
$$\times\frac{5!^{2}\left(\frac{1}{5.231}+\frac{1}{3.452}+\frac{1}{4.764}+\frac{1}{2.114}+\frac{1}{3.593}\right)^{10}}{10!\left(\frac{1}{5.231}\right)^{5}\cdot\left(\frac{1}{3.452}\right)^{5}}$$
$$\approx 6827.27,$$

whereas by simulation, for 10,000 observations, the value of the return time is determined as 6465.96. The corresponding plot is in figure 7.

3.5 *Biological network 3*

Biological network 3 involves reactions of two pathways: Calvin–Benson cycle (3-phospho glycerate to ribulose 5-pho- sphate) and glycolysis (ribulose 5-phosphate to 3-phospho glycerate) [21]. This linear chain includes the reactions given in figure 8. Again, we apply Theorem 6, and this time on a system with each species having initial concentration of 10. The theoretical expected return time for this system is

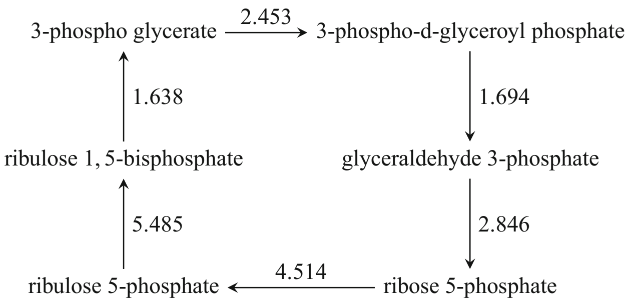

Figure 8. Reactions in biological network 3.

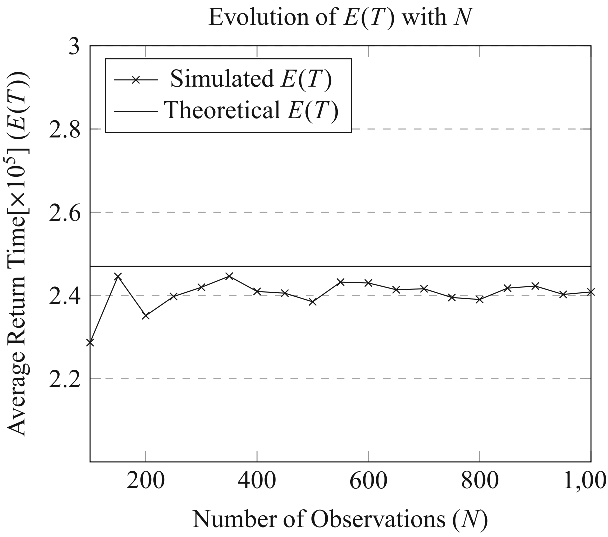

Figure 9. Average return time for biological network 3.

$$\frac{1}{10 \times 18.63} \frac{10!^6\left(\frac{1}{2.453}+\frac{1}{1.694}+\frac{1}{2.846}+\frac{1}{4.514}+\frac{1}{5.485}+\frac{1}{1.638}\right)^{60}}{60!\left(\frac{1}{2.453}\right)^{10}\left(\frac{1}{1.694}\right)^{10}\left(\frac{1}{2.846}\right)^{10}\left(\frac{1}{4.514}\right)^{10}\left(\frac{1}{5.485}\right)^{10}\left(\frac{1}{1.638}\right)^{10}} \approx 24702.2,$$

and the value obtained via simulation is 24084.7, shown in figure 9.

4. Conclusions

In this article, we have theoretically calculated the expected return time to the initial state of a biochemical system provided that it is a cyclic one and there are no signals channelling in and out of it. In other words, the system is a closed one. According to SSA, the trajectory of a biochemical system follows an MC. If it is closed and the state space is finite then its trajectory is an irreducible MC. In this case, the stationary distribution of the state space is unique and it is a left-eigenvector of the transition rate matrix Q with eigenvalue 0. We can calculate the expected return time from this eigenvalue equation.

We have tested our hypothesis in two cyclic linear chains – unidirectional and bidirectional, and in three biological systems. The first system is a part of TCA cycle. The second system involves recycling of pyruvate in mitochondria. The third one includes reactions of two different pathways, viz., Calvin–Benson cycle and glycolysis. We have validated the theoretical values of the expected return times with those obtained from simulating a standard SSA. We have considered the DM for this purpose. It is observed that the theoretical values are close enough to those of experimental ones. We have provided a tentative guideline on how the theory may be applied to a non-closed system.

Acknowledgements

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