Biomass transfer on autocatalytic reaction network: a delay differential equation formulation

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October 19, 2022

Abstract

For a biological system to grow, the biomass must be incorporated, transferred, and accumulated into the underlying reaction network. There are two perspectives for studying growth dynamics of reaction networks: one way is to focus on each node in the networks and study its associated influxes and effluxes. The other way is to focus on a fraction of biomass and study its trajectory along the reaction pathways. The former perspective (analogous to the "Eulerian representation" in fluid mechanics) has been studied extensively, while the latter perspective (analogous to the "Lagrangian representation" in fluid mechanics) has not been systematically explored.

In this work, I characterized the biomass transfer process for autocatalytic, growing systems with scalable reaction fluxes. Under balanced growth, the long-term growth dynamics of the systems are described by delay differential equations (DDEs). The kernel function of the DDE serves as a unique pattern for the catalytic delay for a reaction network, and in frequency domain the delay spectrum provides a geometric interpretation for long-term growth rate. The DDE formulation provides a clear intuition on how autocatalytic reaction pathways lead to system growth, it also enables us to classify and compare reaction networks with different network structures.

1. Reaction networks and biomass transfer process

Reaction network is the fundamental structure in biochemistry, cell biology and ecology. An essential feature of biological systems is their capability to expand by incorporating external resource into biomass. To achieve this, the growing reaction network must be autocatalytic and operates with sufficiently high efficiency to against dissipation and degradation.

Many basic questions for growing reaction networks remain open. In biology, reaction networks often involves thousands of nodes and typically are replaced by coarse-grained, simplified models. However, it is unclear how network dynamics changes after coarse-graining, and therefore difficult to conclude that the coarse-grained model serves as an "effective reaction network" of the original one. One interesting direction is to use biomass transfer process as a common framework for comparing reaction networks with different structures.

We start with our definition for reaction networks. A reaction network is composed by system nodes \( x = \{x_1, ..., x_n\} \), environmental nodes \( z = \{z_1, ..., z_E\} \) and reactions \( \phi = \{\phi_1, ..., \phi_m\} \). For each reaction \( \phi_a \), it can be represented by

\[
\phi_a : \sum_{i=1}^{n} c_{ia} x_i + \sum_{i=1}^{E} c'_{ia} z_i \rightarrow \sum_{i=1}^{n} d_{ia} x_i + \sum_{i=1}^{E} d'_{ia} z_i
\] (1)

where \( c_{ia}, c'_{ia}, d_{ia}, d'_{ia} \) are non-negative numbers. To describe the network topology, we use the terminology presented in [Ref1]: Given a node \( x_k \), we denote \( in(x_k) \) and \( out(x_k) \) as the inbound and outbound reactions connecting to it. Given a reaction \( \phi_a \), we denote \( up(\phi_a) \) and \( dw(\phi_a) \) as the upstream and downstream nodes of this reaction.

Figure 1A: A reaction network with five system nodes (\( x_1 \) to \( x_5 \)), four environmental nodes (\( z_1 \) to \( z_4 \)), and six reactions (\( \phi_1 \) to \( \phi_6 \)).
The structure of reaction networks mentioned in above is solely topological. To quantitatively characterize the system dynamics, we need to associate a reaction network to a dynamical system. For each node \( x_j \) in the reaction network, we denote \( X_j \) as the number of \( x_j \)-type molecules in the system. When the number of \( X_j \) are large, the system dynamics reach continuous limit and can be described by ODEs. For each reaction \( \phi_a \), we associate it with a flux function \( J_a(X) \) which is a multivariate function of \( X \) and represent the reaction rate per unit time. In this way, the system is governed by a systems ODE

\[
\frac{dX_i}{dt} = \sum_{j=1}^{m} S_{ia} J_a
\]

for \( i = 1, \ldots, n \), where \( S_{ia} \equiv d_{ia} - c_{ia} \) is a \( n \)-by-\( m \) stoichiometry matrix. Note that the reaction network \((X, \phi)\) is a discrete network structure while the dynamical system \((X, J)\) contains variables and flux functions. To study growth dynamics, we consider the reaction network as a thermodynamically open system and contact with environmental resources \((Z_j)\) under constant concentrations. We define \( N \equiv X_1 + \ldots + X_n \) as the total system size and

\[
\lambda \equiv \lim_{t \to \infty} \frac{1}{t} \log N(t)
\]

as the long-term growth rate of the system (if this limit exists). In general, for arbitrary flux functions the system may not grow exponentially and the above limit may not exist. By restricting the mathematical form of the flux functions, one could generate a large class of reaction networks with converged \( \lambda \) (see [Ref1]). In this paper, we will focus on exponentially growing systems in the regime of \( \lambda > 0 \). Since \( \lambda > 0 \) requires the total system influxes to be greater than total system effluxes, it is clear that the system is not under equilibrium nor steady-state in thermodynamics sense. The equilibrium or near-equilibrium formulation in classical thermodynamics is hence not applicable in this case, and required extension of the theory [Ref2].

In the ODE formulation, one does not distinguish between a molecule being synthesized one minute ago versus a same type of molecule already existed for several hours. Mathematically, both molecules contribute equally to the vector component they belong to. On the other hand, in biochemical network one could track a fraction of biomass a same type of molecule already existed for several hours. Mathematically, both molecules contribute equally to the vector component they belong to. In the next section, we will characterize the waiting time distribution and show how to use it to derive delay differential equation of a growing system.

Our goal is to give a quantitative description of biomass transfer process in reaction network. For each node \( x_k \) (or \( z_k \)), we associate a nonnegative number \( m(x_k) \) (or \( m(z_k) \)) as the biomass content of this type of molecule. We define a reaction pathway \( \pi(u, \omega) \) to be a collection of nodes \( u_1, \ldots, u_M \in x \cup z \) and reaction \( \omega_1, \ldots, \omega_{M-1} \in \phi \) which satisfies \( u_1 \in up(\omega_j) \) and \( u_{j+1} \in dw(\omega_j) \) for \( j = 1, \ldots, M - 1 \). Note that the set of nodes \( \{u_j\} \) and reaction \( \{\omega_j\} \) can have repeated members. Technically, we will only consider the first and last node to be the environmental nodes, which represent the environmental resources and wastes of the pathway.

![Figure 1B: A reaction pathway \( \pi(u, \omega) \), which can be represented symbolically as \( \pi : u_1u_2u_3u_4u_5 \). Other reactions \( \phi_a, \phi_b \) could share common upstream/downstream nodes of the reaction pathway, but they are not part of \( \pi \).](Image 61x277 to 277x321)

When a reaction happens, it is equivalent to think that the biomass from reaction upstream is transferred to reaction downstream. The transfer process happens instantaneously, while the time point for the biomass being transferred could be a random variable. Consider a reaction pathway \( \pi \) and its first and last reactions \( \omega_1, \omega_M \) (\( M > 1 \)). Suppose the biomass is transferred across reaction \( \omega_1 \) (i.e. move from \( u_1 \) to \( u_2 \)) at time \( T \). There is a probability \( q_a \) that the biomass eventually passes through all reactions in \( \pi \) and reaches the last node. Let \( T' > T \) be the time where the biomass is transferred across the last reaction \( \omega_M \). Conditional on the events where the biomass is transferred via \( \pi \), there is a transition probability function between \( T \) and \( T' \), which can be viewed as the waiting time distribution of \( T' - T \) (given the reaction kinetics is time-independent). In the next section, we will characterize the waiting time distribution and show how to use it to derive delay differential equation of a growing system.
2. Scalable reaction networks and gatekeepers

Up to now, our discussion about reaction networks is general for arbitrary flux functions, and hence the system may grow (or decay) without having any convergent dynamics in the long-term. In contrast, we often observe long-term dynamics such as exponential growth or periodic oscillation in natural biological systems. This motivates us to consider a class of reaction networks which display some long-term dynamics. In the following, we focus on reaction networks with flux functions $J_a(X) : [0, \infty)^n \to \mathbb{R}$, $a = 1, \ldots, m$ which satisfies the following conditions:

**Flux Conditions:**
(i) (Smoothness): $J_a \geq 0$ on $[0, \infty)^n$ and $J_a(X)$ is continuously differentiable on $(0, \infty)^n$.
(ii) (Scalability): $J_a(cX) = cJ_a(X)$ for arbitrary real number $c$.
(iii) (Maintenance): There exist at least one component $X_k$ such that $\lim_{X_k \to 0} J_a(X) = 0$ for all $X$.
(iv) (Upstream-limited): If $x_j \in \text{up}(\phi_a)$, then $\lim_{X_j \to 0} J_a(X) = 0$ for all $X$.

In addition, we will focus our discussions in the following growth regime:

**Growth Conditions:**
(i) (Positive exponential growth): The long-term growth rate of the system satisfies $\lambda > 0$.
(ii) (Balanced growth): $X(t) \to Y^* e^{\lambda t}$ for large $t$, where $Y^*$ is a constant vector in unit simplex space $\Delta^{n-1}$.

Reaction networks satisfied the Flux Conditions in above are called scalable reaction networks and were studied in [Ref1]. Fluxes with these properties guarantee that the long-term dynamics of the network is confined in unit simplex space $\Delta^{n-1} : \{Y \in [0, \infty)^n \mid Y_1 + \ldots + Y_n = 1\}$. This is an effective method to construct exponential-growing system for arbitrary numbers of nodes and fluxes — as long as the system converge to some ergodic measure on $T^n$, the long-term growth rate is well-defined [Ref1].

It is clear that a growing reaction network with $\lambda > 0$ must have influxes from environment, which we called the boundary influxes. In biochemical system, the boundary influx is limited by the amount of transporter, carrier or enzyme in the system, and the flux would drop to zero if the number these molecules decrease to zero. Let $J_a$ be the flux function of a boundary influx $\phi_a$. If there exist one component $X_g$ such that $\lim_{X_g \to 0} J_a(X) = 0$ for all $X$, we say the node $x_g$ is a gatekeeper of $\phi_a$. In biochemical systems, the gatekeepers can be the transporter/carrier/enzyme in the system. In ecological systems, the gatekeepers are the primary producers which fix inorganic chemical into organic biomass.

The Flux Condition (iii) guarantees that each boundary influxes has at least one gatekeeper. Therefore, there is a nonempty collection of gatekeepers nodes in the system. Since the growth of the system is limited by the boundary influxes, and these influxes are in turns limited by their gatekeepers, there must be some gatekeepers grow with the same rate of the entire system. The Flux Conditions and Growth Conditions together imply following basic property (see Appendix for proof).

**Proposition 2.1:** For a reaction network satisfying Flux Conditions and Growth Conditions, if $x_k$ is a gatekeeper node or upstream node of the flux $\phi_a$, then the flux function $J_a(X)$ in the long-term is linearly proportional to $X_k$. Namely, we have

$$\lim_{t \to \infty} J_a(X(t)) = R_a(Y^*) X_k(t)$$

where $Y^*$ is the balanced growth vector and $R_a(Y^*)$ is a constant. ■

The concept of gatekeepers is useful in analyzing autocatalytic networks. For a growing system, biomass must flow through boundary fluxes (which gated by gatekeeper nodes) and eventually reaches these gatekeeper nodes. As we will see, this enables us to write down the growth dynamics in terms of Markov process of biomass transfer.

To illustrate the essential idea, we will consider a simple reaction network where there is only one gatekeeper $x_1$ (see Figure 2A). We also assume that the biomass has 100% transfer efficiency (no loss of biomass) in these synthesis pathways. Now, consider a fraction of biomass just entered the system via boundary influx $\phi_1$ gated by $x_1$. The biomass can be transferred along the reaction pathway $\pi_1$ and flow into the gatekeeper node $x_1$. Afterward, the biomass could flow out the $x_1$ and re-entry $x_1$ for multiple times (finite or infinite) via reaction pathway $\pi_3$ before entering the reaction pathway $\pi_3$ and leaving the system. In general, based on their relation with node $x_1$, reaction pathways in this system can be classified into following categories:
\[ P_1[x_1] : \{ \pi \mid \pi \text{ starts with an environmental node and ends when it reaches } x_1 \text{ for the first time} \} \]
\[ P_2[x_1] : \{ \pi \mid \pi \text{ starts with } x_1 \text{ and ends when it meet } x_1 \text{ again} \} \]
\[ P_3[x_1] : \{ \pi \mid \pi \text{ starts with } x_1 \text{ and never return to } x_1 \} \]

By the definition of these pathway categories in above, we have \( \pi_1 \in P_1[x_1], \pi_2 \in P_1[x_2], \pi_3 \in P_3[x_1] \). Now, we start examining the flux functions in these reaction pathways. We will denote \( T \) where \( \phi \) belongs to \( \phi \). Figure 2A: A reaction network with single boundary influx \( \phi \) gated by the gatekeeper \( x_1 \).

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For the synthesis pathway \( \pi_1 \), \( J_1 \) and \( J_2 \) are the first and last flux functions in this pathway. Let \( T' \) (a random variable) be the time point where biomass go through \( \phi_1 \) and \( T > T' \) (another random variable) be the time point where the biomass go through \( \phi_2 \), then \( T - T' \) is the waiting time for the biomass to transfer along \( \pi_1 \). There is a waiting-time distribution \( f_{\tau_1}(\tau) \) for the random variable \( \tau \equiv T - T' \), which depends on the kinetic constants in fluxes belonged to \( \pi_1 \). Integrating all possible \( \tau \), we have the relation

\[
J_2(t) = \int_{t'=0}^{t} J_1(t') f_{\tau_1}(t-t') \, dt'
\]

Similarly, consider the synthesis pathway \( \pi_2 \), there is also a waiting time distribution \( f_{\tau_2}(\tau) \) with \( \tau \equiv T - T' \), where \( T', T \) now represent the time points that biomass go through reactions \( \phi_3 \) and \( \phi_4 \), respectively. This gives another relation

\[
J_4(t) = \int_{t'=0}^{t} J_3(t') f_{\tau_2}(t-t') \, dt'
\]

Now, from Proposition 2.1, in the long-term \( J_1 \) is a linear function of \( X_1 \) (since \( x_1 \) is the gatekeeper of \( \phi_1 \)). Also, in the long-term \( J_3, J_5 \) are linear functions of \( X_1 \) (since \( x_1 \) is the upstream node of \( \phi_3 \) and \( \phi_5 \)). Therefore, we have

\[
J_1(t) = R_1(Y^*)X_1, \quad J_3(t) = R_3(Y^*)X_1, \quad \text{and} \quad J_5(t) = R_5(Y^*)X_1 \quad \text{for large } t.
\]

This gives the following equation

\[
\frac{dX_1(t)}{dt} = J_2(t) - J_3(5) + J_4(t) - J_5(t)
\]

\[
= -(J_3(t) + J_5(t)) + \int_{t'=0}^{t} [J_1(t') f_{\tau_1}(t-t') + J_3(t') f_{\tau_2}(t-t')] \, dt'
\]

\[
= -\beta X_1(t) + \int_{t'=0}^{t} \alpha(t-t')X_1(t') \, dt'
\]

where \( \beta \equiv R_3(Y^*) + R_5(Y^*) \) is a constant and \( \alpha(\tau) \equiv R_1(Y^*)f_{\tau_1}(\tau) + R_3(Y^*)f_{\tau_2}(\tau) \) is a non-negative function of \( \tau \). In this way, we constructed a delay differential equation for \( X_1 \) under balanced growth.

Note that the DDE we derived in above is a long-term limit of the ODE. Intuitively, when the system reaches balanced growth its dynamics is reduced to one-dimensional exponential growth along the balanced vector \( Y^* \). The DDE formulation describes the stable biomass transfer process in the network under this long-term limit. Using the analogy from fluid mechanics, the long-term ODE formulation is similar to the Eulerian description where the system reaches “steady flow”, while the DDE formulation is similar to the Lagrangian description where the of fluid parcel travels along an “invariant streamline”.

\[ \text{Figure 2A: A reaction network with single boundary influx } \phi \text{ gated by the gatekeeper } x_1. \]
As we will see in the next section, the DDE formulation gives another way to express the long-term growth rate $\lambda$ of the system. Biologically, the long-term system growth is originate from all reaction pathways which are autocatalytic ($P_1$ pathways) or recurrent ($P_2$ pathways), and this can be quantitatively described by $\alpha(\tau)$, the decay kernel of the DDE.

3. Delayed-time formulation for scalable reaction networks

In this section we describe the general framework of the delay-time formulation. First we specify the general formula of two important concepts: the waiting time distribution and the amplification rate of a reaction pathway. We will adopt the following notations $J[\phi_a]$ represents the flux function of reaction $\phi_a$, and $S[u_k, \phi_a]$ represents the stoichiometry coefficient between reaction $\phi_a$ and node $u_k$. When $u_k = x_i$ is a system node, we define $S[u_k, \phi_a] \equiv d_{ia} - c_{ta}$; when $u_k = z_i$ is an environmental node, we define $S[u_k, \phi_a] \equiv d_{ia} - c'_{ta}$.

**Proposition 3-1**: Consider a reaction network which satisfies the Flux Conditions and Growth Conditions. Let $\pi(u, \omega)$ be a reaction pathway composed by nodes $\{u_1, ..., u_{L+1}\}$ and reactions $\{\omega_1, ..., \omega_L\}$. The time interval between the time point for biomass to pass through reaction $\omega_1$ and the time point for biomass to pass through reaction $\omega_L$, given the biomass is transferred via $\pi$, is called the *waiting time* of $\pi$. The probability distribution of this waiting time is denoted by $f_\pi(\tau)$. We have

$$f_\pi(\tau) = g_2(\tau) \ast \ldots \ast g_L(\tau)$$

$$g_j(\tau) = R_j e^{-R_j \tau} \quad \text{(} \tau \geq 0\text{)} , \quad j = 2, ..., L$$

$$R_j = \sum_{\phi_p \in \text{out}(u_j)} \left[\frac{J[\phi_p]}{U_j}\right]_{Y}$$  \hspace{1cm} (8)

Here, the $\ast$ sign represents convolution between functions, and $U_j$ represents the amount of $u_j$ objects. □

Note that $g_1(\tau)$ is not included in the waiting time distribution $f_\pi$, since at the beginning the biomass is already passed through the first reaction $\omega_1$. Next, we introduce the concept of amplification rate, which measures the rate of biomass flow through $\pi$ normalized by the biomass of the last node in $\pi$.

**Proposition 3-2**: Consider a reaction network which satisfies the the Flux Conditions and Growth Conditions. Let $\pi(u, \omega)$ be a reaction pathway composed by nodes $\{u_1, ..., u_{L+1}\}$ and reactions $\{\omega_1, ..., \omega_L\}$. We define the *amplification rate* of $\pi$, denoted by $c_\pi$, as

$$c_\pi = \frac{\text{rate of biomass entering } \pi, \text{ transferred through } \pi \text{ and reached } u_{L+1}}{\text{total biomass on node } u_{L+1}}$$

The amplification rate is given by $c_\pi = c^0_\pi q_\pi \theta_\pi$, with

$$c^0_\pi = \frac{\text{rate of biomass entering } \pi \text{ on node } u_{L+1}}{\text{total biomass on node } u_{L+1}} = \frac{m(u_1)|S[u_1, \omega_1]|J[\omega_1]}{m(u_{L+1})U_{L+1}}$$

$$q_\pi = \text{probability for biomass started from } u_1, \text{ being transferred via } \pi \text{ and reached } u_{L+1}$$

$$\theta_\pi = \text{expected fraction of biomass started from } u_1, \text{ being transferred via } \pi \text{ and reached } u_{L+1}$$

The detail formula for $c_\pi$ are described in Appendix. □

Note that for the numerator of $c^0_\pi$, $|S[u_j, \omega_j]|J[\omega_1]$ is the number of molecules entering this pathway per unit time (the absolute sign is necessary since the stoichiometry coefficients for influx is negative). Multiply by $m(u_1)$, it is the rate of biomass entering $\pi$.

**Example 3A**: In this example (see Figure 3A) we assume all reactions are linear and have stoichiometry coefficients $\pm 1$, and all molecules have the same biomass $m(u_1) = 1$. 

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For reaction pathway $\pi_1$, the first and the last reaction is the same ($\omega_1$) and hence there is no time delay. The waiting time distribution is

$$f_{\pi_1}(\tau) = \delta(\tau),$$

a delta impulse located at $\tau = 0$. For reaction pathway $\pi_2$, there is a delay between first ($\omega_1$) and last ($\omega_2$) reactions, and we have

$$f_{\pi_2}(\tau) = be^{-b\tau} \ (\tau \geq 0)$$

For reaction pathway $\pi_3$, there is a delay between first ($\omega_1$) and last ($\omega_3$) reactions, and we have

$$f_{\pi_3}(\tau) = be^{-b\tau} + ce^{-c\tau} \ (\tau \geq 0)$$

Now, we consider the amplification rates $c_\pi$ of these reaction pathways. Since all pathways in this example are linear paths, 100% of the biomass transfer from the first node to the last node with no other branch pathways. Hence, $q_{\pi j} = 1$ for $j = 1, 2, 3$. Since all stoichiometry coefficients are $\pm 1$ and all nodes have the same biomass, we have no "biomass loss" during the transfer. Hence, $\theta_{\pi j} = 1$ for $j = 1, 2, 3$. Now, note that the last nodes in pathways $\pi_1, \pi_2, \pi_3$ are $u_2, u_3, u_4$, respectively and we assume $m(u_2) = m(u_3) = m(u_4) = 1$. Therefore,

$$c_{\pi_1} = \frac{J[\omega_1]}{U_2} = a, \ c_{\pi_2} = \frac{J[\omega_1]}{U_3} = a, \ c_{\pi_3} = \frac{J[\omega_1]}{U_4} = a$$

Intuitively, a linear reaction pathway with $L$ nodes is analogous to a structured population growth model with $L - 1$ developmental stages. The first node $u_1$ can be viewed as the environmental resource, and nodes $u_2, ..., u_{L-1}$ can be viewed as the "juveniles" with different ages. The last node $u_L$ can be viewed as the to "mature adult" which could reproduce and generate offspring $u_2$. Intuitively, shorter waiting time corresponds to faster maturation, and higher amplification rate correspond to more offsprings. For biochemical networks, reaction pathways may involve branches and synthesis, and the dynamics could be more complicated. The next example demonstrate how branches on reaction pathway affects the calculation of $f_\pi$ and $c_\pi$.

**Example 3B**: In this example (see Figure 3B) we assume all reactions follow linear flux functions and have stoichiometry coefficients are indicated. We assume all molecules have the same biomass $m(u_j) = m(v_j) = 1$.

$$u_1 \xrightarrow{\omega_1} u_2 \xrightarrow{\omega_2} u_3 \xrightarrow{\phi_1} v_2$$

Consider the reaction pathway $\pi : u_1 \omega_1 u_2 \omega_2 u_3$. To calculate the waiting time distribution $f_\pi$ according to Proposition 3-1, we need to consider the waiting time of reaction $\omega_2 conditional on reaction \omega_2$ happens before reaction $\phi_1$ (otherwise, if $\phi_1$ happens before $\omega_2$ the biomass will be transferred through $\phi_1$). The conditional waiting time distribution is (see Appendix)

$$f_\pi(\tau) = (b + c)e^{-(b+c)\tau} \ (\tau \geq 0)$$

Next we consider $c_\pi$. First, we have

$$c^{0}_{\pi} = \frac{m(u_1)|S[u_1, \omega_1]|J[\omega_1]}{m(u_3)U_3} = \frac{(1)(5)aU_3}{(1)U_3} = 5a$$
Since there are two competing reactions \( \omega_2 \) and \( \phi_1 \) with rate constant \( b,c \), respectively, the probability that biomass being transfer along \( \pi \) is \( q_{\pi} = \frac{b}{b+c} \). Since we assume all molecules have the same biomass content and the first reaction is \( 5u_1 \rightarrow 2u_2 + 3v_1 \), the fraction of biomass being transfer through \( \omega_1 \) along \( \pi \) is \( \theta_\pi = \frac{2}{5} \). Together, we have \( c_\pi = \frac{2b}{b+c} \).

Now we are ready to state our main result. Recall that for a fixed node \( x_i \), we can define two categories of reaction pathways:

\[
P_1[x_i] : \{ \pi \mid \pi \text{ starts with an environmental node and ends when it reaches } x_i \text{ for the first time} \}
\]

\[
P_2[x_i] : \{ \pi \mid \pi \text{ starts with } x_i \text{ and ends when it meet } x_i \text{ again} \}
\]

**Theorem 3-3:** Consider a reaction network which satisfies the Flux Conditions and Growth Conditions. Let \( x_i \) be a node in the system with positive long-term growth rate. In the long-term, \( X_i(t) \) follows the delay differential equation

\[
\frac{dX_i(t)}{dt} = -\beta_i X_i(t) + \int_{t'=0}^{t} \alpha_i(t - t') X_i(t') \, dt'
\]

Specifically, the degradation rate \( \beta_i \) is defined by

\[
\beta_i = \sum_{\phi_k \in \text{out}(x_i)} \frac{J[\phi_k]}{X_i} \bigg|_{Y^*}
\]

and the delay kernel \( \alpha_i(\tau) \) is be represented by

\[
\alpha_i(\tau) = \sum_{\pi \in P_1[x_i] \cup P_2[x_i]} c_\pi f_\pi(\tau)
\]

Note that Theorem 3-3 assume the node \( x_i \) has positive long-term growth rate. If the long-term growth rate for \( x_i \) is zero or negative, we have a similar DDE for \( x_i \) but there is an additional term in \( \alpha_i \) (see Appendix). The delay kernel \( \alpha_i(\tau) \) is a scalar function, which combines delay-time dynamics from all reaction pathways that could synthesize \( x_i \). In principle, we could write down the delay differential equation for every node \( x_i \), and each could have some different delay kernel. However, there would be many redundant information if we calculate \( \alpha_i(\tau) \) for every node.

A more efficient way to analyze a growing reaction network is to view it as an autocatalytic system. For a growing network, all biomass must be transported from environment to the system via the gatekeeper nodes. Hence, the network growth can be viewed as a self-catalysis process of gatekeeper nodes. Let \( x_1, ..., x_G \) denote the gatekeeper nodes in the system and denote \( N_G \equiv X_1 + ... + X_G \). From Flux Condition (iii), total biomass influx is limited by \( N_G \) and hence \( N_G \) must have the same long-term growth rate as \( N \). Let \( Y^* \) be the balanced growth vector, we can use it to acquire the weighted average of the gatekeepers dynamics:

**Corollary 3-4:** Consider a reaction network which satisfies the Flux Conditions and Growth Conditions. Let \( Y^* \) be the balanced growth vector and define \( \rho(\tau), \beta \) as linear combination of \( Y^* \):

\[
\beta \equiv \sum_{g=1}^{G} Y^*_g \beta_g \bigg/ \sum_{g=1}^{G} Y^*_g
\]

\[
\alpha(\tau) \equiv \sum_{g=1}^{G} Y^*_g \alpha_g(\tau) \bigg/ \sum_{g=1}^{G} Y^*_g
\]

then we have the DDEs

\[
\frac{dN_G}{dt} = -\beta N_G + \alpha \ast N_G
\]

Note that the weighted decay kernel \( \alpha(\tau) \) and the weighted degradation constant \( \beta \) are properties of the entire network and does not depends on specific node. We can now compare different reaction network by comparing the quantities \( (\alpha(\tau), \beta) \). In the next section, we will analyze the delay kernel in the frequency domain and show its relation with long-term growth rate.
4. Decay spectrum and growth rate optimization

**Proposition 4-1**: Consider a reaction network which satisfies the same conditions in Corollary 3-4. Define the delay spectrum by \( A(s) \equiv \int_0^\infty \alpha(\tau)e^{-s\tau}d\tau \) which is the Laplace transform of the delay kernel \( \alpha(\tau) \). The function \( A(s) \) can be expressed by

\[
A(s) = \sum_{g=1}^{G} A_g(s)\tag{15}
\]

\[
A_g(s) \equiv \sum_{\pi \in P_1[x_g] \text{ or } P_2[x_g]} G_{\pi}(s)\tag{16}
\]

Let \( L_{\pi} \) represent the number of reactions in the pathways \( \pi \), we have

\[
G_{\pi}(s) = Y^* c_{\pi} \quad \text{for } L_{\pi} = 1
\]

\[
G_{\pi}(s) = Y^* c_{\pi} \frac{R_2}{s + R_2} \times \ldots \times \frac{R_L}{s + R_L} \quad \text{for } L_{\pi} > 1
\]

where \( R_2, \ldots, R_L \) are constants described in Proposition 3-1. ■

**Proof**: By direct calculation of the Laplace transform.

Under balanced growth, the delay spectrum \( A(s) \) has a particular simple form: it is product of function in form of \( R_{\pi} = s + R_{\pi} \). It is clear that for reaction pathways with two or more fluxes, \( A(s) \) monotonically decrease to zero on \([0, \infty)\).

In the next theorem, we show that the decay of \( A(s) \) has gives a geometric interpretation for long-term growth rate:

**Theorem 4-2**: Consider a reaction network which satisfies the conditions in Theorem 3-3. The delay spectrum \( A(s) \) of the reaction network (viewed as a real function) is positive at \( s = 0 \) and non-increasing. Furthermore, there is a unique \( \lambda > 0 \) satisfying

\[
\lambda + \beta = A(\lambda)\tag{17}
\]

and \( \lambda \) is the long-term growth rate of the system. ■

**Examples 4A**: We examine the long-term growth rate of the reaction networks in Example 3A. The node \( u_1 \) represents environmental resource.

![Figure 4A: Three reaction networks with different length of reaction pathways.](image)

For the first network, \( u_2 \) is the only gatekeeper and \( \pi \) is the unique element in \( P_1[u_2] \). From Example 3A we know that \( c_{\pi} = a \) and \( f_{\pi}(\tau) = \delta(\tau) \). Therefore we have \( A(\lambda) = a \) as a constant function. Since there is no degradation fluxes for node \( u_2 \), we have \( \beta = 0 \). The long-term growth rate \( \lambda \) satisfies the relation \( \lambda = A(\lambda) \), which implies \( \lambda = a \) as expected.

For the second network, \( u_3 \) is the only gatekeeper and \( \pi \) is the unique element in \( P_1[u_3] \). From Example 3A we know that \( c_{\pi} = a \) and \( f_{\pi}(\tau) = be^{-b\tau} \). Therefore, we have \( A(\lambda) = \frac{ab}{s + b} \). The long-term growth rate \( \lambda \) satisfies the relation \( \lambda = A(\lambda) \). Same result can be obtained by finding the top eigenvalue of the systems ODE

\[
x'(t) = \begin{pmatrix} -b & a \\ b & 0 \end{pmatrix} x
\]

For the third network, \( u_4 \) is the only gatekeeper \( \pi \) is the unique element in \( P_1[u_4] \). From Example 3A we know that \( c_{\pi} = a \) and \( f_{\pi}(\tau) = ce^{-c\tau} \). Therefore, we have \( A(\lambda) = \frac{abc}{s + c} \). The long-term growth rate \( \lambda \) satisfies the
relation $\lambda = A(\lambda)$. Same result can be obtained by finding the top eigenvalue of the systems ODE

\[ x'(t) = \begin{pmatrix} -b & 0 & a \\ b & -c & 0 \\ 0 & c & 0 \end{pmatrix} x \]

In Example 4A, we see that the DDE and ODE formulations give the same results of the long-term growth rates. This is expected, as the "Eulerian" or "Lagrangian" description should yield identical results for the same system. Different approaches have different advantages: the ODE approach by taking the top eigenvalue is numerically straightforward. However, it is not intuitive how these top eigenvalues arise. The DDE approach, by finding the intersects of delay spectra $A(s)$ and the reference line $s$ (see Figure 4B), give a clear interpretation of long-term growth rate in the frequency domain. It is clear that the faster decay the $A(s)$, the lower the long-term growth rate.

![Figure 4B: Geometric interpretation of $A(s)$ of different reaction networks in Example 3A. The blue, red, and yellow curves represent the Network 1,2,3, respectively. The intersect between $A(s)$ and $s$ is the long-term growth rate ($\lambda_1$ to $\lambda_3$ for Network 1,2,3, respectively).](image)

**Proposition 4-3**: Consider two reaction network, both satisfy the Flux Conditions and Growth Conditions. Let $A_a$, $A_b$ represent their decay spectra, $\beta_a$, $\beta_b$ represent their degradation constants, and $\lambda_a$, $\lambda_b$ represent their long-term growth rates. If

\[ A_a(s) - A_b(s) > \beta_a - \beta_b \] (18)

for all $s > 0$, then we have $\lambda_a > \lambda_b$. ■

We give an examples on how to use $A(s)$ for growth rate optimization. For simplicity, we consider reaction networks with a single gatekeeper.

**Examples 4B**: We consider the following reaction network (see Figure 4C). The node $u_1$ represent the environmental resource. Here, the unique gatekeeper is $x_1$ and can be synthesized through pathway $\pi_0 : z_1 \phi_1 x_2 \phi_2 x_3 \phi_3 x_1$. However, there is a "reverse reaction" $\phi_4$ and the biomass can also flow into this "wrong direction".

![Figure 4C: Examples of a reaction pathway, with a "reverse" reaction $\phi_4$.](image)

Intuitively, minimizing the reaction flux $J[\phi_4] = bx_3$ would facilitate the synthesis of gatekeeper node $x_1$ and improve the system growth. However, it is not easy to acquired the analytical formula of $\lambda$ as a function of $b$. In general, when networks are more and more complex, explicit formula of $\lambda$ is almost impossible. We use the decay spectrum $A(s)$ to analyze this question.

First, we calculate the decay kernel $\alpha(\tau)$. Since $x_1$ is the gatekeeper, we look for reaction pathways in $P_1[x_1]$. For this network, because the reversible step between $x_2$ and $x_3$, there are infinite reaction pathways in $P_1[x_1]$. Their
general form can be written as
\[ \pi_k = z_1 \phi_1 x_2 \phi_2 x_3 (\phi_4 x_2 \phi_2 x_3) k \phi_3 x_1 \]
with \( k = 0, 1, \ldots \). The segment \((\phi_3 x_1 \phi_3 x_2)\) represents the biomass go through reverse reaction and come back to node \( x_2 \), and this process can happen infinity time (although the probability decays to zero as \( k \to \infty \)). In all cases, we have \( c^0_{a_2} = a_1 \). Since there is no branch in this network, we have \( \theta_{\pi_k} = 1 \) for all \( k = 0, 1, \ldots \). Notice that when biomass is located on node \( x_3 \), it either undertake the reaction \( \phi_3 \) (with probability \( p = \frac{a_1}{a_2 + b} \)) or reaction \( \phi_4 \) (with probability \( 1 - p \)). Therefore, we have
\[ q_{\pi_k} = p(1 - p)^k \]
Next, we calculate the waiting time distribution \( f_{\pi_k} \). Observe that the waiting time of reaction \( \phi_3 \) and \( \phi_4 \) are both \( T \sim re^{-rt} \) with \( r \equiv a_3 + b \) (see Proposition 3-4). Therefore, we have
\[ f_{\pi_k}(s) = \frac{a_2}{s + a_2} (\frac{r}{s + r} \frac{a_2}{s + a_2})^k \frac{r}{s + r} = \left( \frac{r}{s + r} \frac{a_1}{a_2} \right)^{k+1} \]
Note that \( k \) is the number of times where biomass being transferred through the extra loop between \( x_2 \) and \( x_3 \). From the above equation, the waiting time function \( f_{\pi_k}(s) \) decays even faster when \( k \) increases. Now, the decay spectrum is obtained by summing up of all \( k \):
\[ A(s) = \sum_{k=0}^{\infty} c_{\pi_k} f_{\pi_k}(s) \]
Viewing \( A(s; b) \) as a function of \( b \), we can sum up the above geometric series and have
\[ A(s; b) = \frac{a_1 a_3}{b} \frac{F(s, b)}{1 - F(s, b)} \]
with \( F(s, b) = \frac{a_2}{s + a_2 s + a_3 + b} \). Direct calculation shows that \( A(s; b) \) is a decreasing function of \( b \) for any fixed \( s \geq 0 \). By Proposition 4-3, increasing \( b \) lowers the long-term growth rate. In this way, we have rigorously proved that \( \lambda \) increases as \( b \) decreases.

In general, increasing "reverse fluxes" or "futile cycle" on a reaction network would promote the probability to have longer \( P_1 \) synthesis pathways. Longer pathways with futile reactions make biomass transfer process less efficient (with longer waiting time and faster decay on \( A(s) \)), and therefore reduces the growth rate.

5. Discussion

Delay dynamics are prevalent in nature and in engineered systems. In biology, DDE has been used to analyze structured population [Ref4] and pandemic disease control [Ref5]. In electrical engineering, DDE has been used to analyze the feedback control of electric circuits [Ref6]. The stability of reaction networks with time-delay has been analyzed for mass action kinetics [Ref7]. In this work, we explore the delay dynamics within autocatalytic networks. The time-delay is intrinsic – between biomass entering the system via the gatekeeper nodes and the biomass being synthesized into the gatekeepers (if the synthesis pathway involves more than one reaction). The waiting time distribution of each reaction pathway are specified by the kinetic constants in the pathway and the balanced growth vector of the system.

While we focused on balanced growth regime and the long-term dynamics in this work, the delay-time formulation is generally applicable for studying transient dynamics such as periodic and chaotic behaviors. For scalable flux network under balanced growth, the results are particularly simple: all the information involves in long-term dynamics can be "encoded" in the one-dimensional delay kernel \( \alpha(\tau) \). This quantity is network-specific and hence serves as a way to compare reaction networks with different structures: If the delay kernels of two networks (for example, before and after coarse-graining) are identical or similar, then their long-term growth rates will also be identical or similar.

In practice, it is convenient to consider the decay spectrum \( A(s) \), the Laplace transform of the decay kernel. In frequency domain, we could analyze the intersection between \( A(s) \) and \( s + \beta \) and geometrically identify the long-term growth rate of the system. The decay spectrum is hence a new mathematical tool for network analysis and optimization.

We would like to point out that the delay-time formulation may not be numerically more efficient. For a scalable reaction network under balanced growth, one could use the classical ODE formulation and find the balanced vector
by considering the rescaled equation $\frac{dY}{dt} = F(Y) - \mu(Y)Y$ ($\mu(X)$ is the summation of all influx functions and efflux functions of the reaction network, see [Ref1]). Once the fixed point is found, the long-term growth rate is given from $\mu(Y^*)$. The advantage for the DDE formulation is to analyze the network topology and biomass transfer on reaction pathways. Sometimes the DDE formulation can give surprising simple statements: for example, the two reaction networks in below have different ways to allocate the reaction coefficients $a_1$ to $a_4$. From the ODE perspective, it is unclear which one has higher long-term growth rate.

The DDE formulation immediately predicts that the two networks have the same long-term growth rate, since their waiting time distributions for their gatekeeper nodes $u_6$ are identical. Hence, the two networks have the same decay kernel $\alpha(\tau)$ and the same long-term growth rate.

We envision that ODE and DDE formulation would provide complementary views on how reaction networks accumulate their biomass and grow. From thermodynamics point of view, non-equilibrium open systems such as cells and organisms are quasi-stable structures subjected to rapid metabolic turnovers. The DDE formulation, which based on biomass flow, is more nature to describe the process of metabolic turnovers and autocatalysis. Biomass transfer on reaction network also shares many similarities with fluid mechanics: instead of flowing in physical space, the biomass can be regarded as flowing in ”chemical space” which is the underlying reaction network, with chemical potential to be the driving force. We expected that there will be many interesting interaction between non-equilibrium physics and theoretical biology in the future.
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Appendix

Proposition 2.1: For a reaction network which satisfies the Flux Conditions and Growth Conditions, if \( x_k \) is a gatekeeper node or upstream node of the flux \( \phi_u \), then the flux function \( J_u(X) \) in the long-term is linearly proportional to \( X_k \). Namely, we have

\[
\lim_{t \to \infty} J_u(X(t)) = R_u(Y^*)X_k(t)
\]

where \( Y^* \) is the balanced growth vector and \( R_u(Y^*) \) is a constant.

Proof: We define \( R_u(Y) \equiv J_u(Y)/Y_k = J_u(X)/X_k \) which is well-defined and continuous for \( Y_k > 0 \) by Flux Condition (i). To extend \( R_u(Y) \) to the entire unit simplex \( T^{n-1} \), we define

\[
R_u(Y) \equiv \frac{\partial J_u(Y)}{\partial Y_k} \bigg|_{Y_k=0}
\]

where the derivative exists by the Flux Condition (i). Therefore, \( R_u(Y) \) is a continuous function defined on \( T^{n-1} \), and under balanced growth it has a well-defined value \( R_u(Y^*) \).

Proposition 3-1: Consider a reaction network which satisfies the Flux Conditions and Growth Conditions. Let \( \pi(u, \omega) \) be a reaction pathway composed by nodes \( \{u_1, ..., u_{L+1}\} \) and reactions \( \{\omega_1, ..., \omega_L\} \). The time interval between the time point for biomass to pass through reaction \( \omega_1 \) and the time point for biomass to pass through reaction \( \omega_2 \), given the biomass is transferred via \( \pi \), is called the waiting time of \( \pi \). The probability distribution of this waiting time is denoted by \( f_\pi(\tau) \). We have

\[
\begin{align*}
\pi_j(\tau) &= g_2(\tau) \ast \cdots \ast g_L(\tau) \\
g_j(\tau) &= R_j e^{-R_1\tau} \quad (\tau \geq 0), \quad j = 2, ..., L \\
R_j &= \sum_{\phi \in \text{out}(u_j)} \frac{|J(\phi)|}{U_j} |_{Y^*}
\end{align*}
\]

Here, the * sign represents convolution between functions, and \( U_j \) represents the amount of \( u_j \) objects.

Proof: First we notice that in above equation the convolution does not include the first reaction \( \omega_1 \), since we are considering the time interval after the biomass exiting the first reaction. Under balanced growth, the transition rate of \( j \)th reaction is proportional to the magnitude of \( J[\omega_j] \). However, for each step, we need to consider the conditional waiting time distribution where the biomass undergoes the pathway \( \pi \). Suppose the biomass is located on node \( u_j \) (\( j > 1 \)) and there are multiple efflux \( \kappa_1, ..., \kappa_Z \in \text{out}(u_j) \) where \( \omega_j \) in \( \pi \) is one of the efflux (denoted by \( \kappa_1 \)). The conditional waiting time means the event for the biomass to enter \( \kappa_1 \) happens earlier than the events for the biomass to enter other reactions.

Since each efflux \( \kappa_p \) has \( u_j \) as its upstream node, by Flux Condition and Proposition 2.1 the flux function can be expressed as \( J[\kappa_p] = a_p Y(U_j) \) where \( a_p(Y) \) is defined on the unit simplex space. Under balanced growth, \( a_k(Y) \rightleftharpoons a_k(Y^*) \equiv b_k \). We define

\[
W_{jp} : \text{waiting time for biomass at node } u_j \text{ to enter reaction } \kappa_p
\]

For unconditional case, we have random variables \( W_{jp} \sim b_k e^{-b_k t} \). For the conditional case where the biomass goes through reaction \( \kappa_1 \), it requires \( W_{j1} = \min\{W_{j1}, ..., W_{jZ}\} \). Define the conditional waiting time by \( T_{\pi,j} \sim h_{\pi,j} \), the conditional distribution is

\[
h_{\pi,j}(t_1) = C' \int_{t_2 = t_1}^{\infty} \ldots \int_{t_Z = t_1}^{\infty} b_1 b_2 \ldots b_Z e^{-(b_1 t_1 + b_2 t_2 + \ldots + b_Z t_Z)} dt_2 \ldots dt_Z = C''e^{-(b_1 + \ldots + b_Z)t_1}
\]

where \( C, C' \) are normalization factors. This finishes the proof of the conditional waiting time distribution.

Proposition 3-2: Consider a reaction network which satisfies the Flux Conditions and Growth Conditions. Let \( \pi(u, \omega) \) be a reaction pathway composed by nodes \( \{u_1, ..., u_{L+1}\} \) and reactions \( \{\omega_1, ..., \omega_L\} \). We define the amplification rate of \( \pi \), denoted by \( c_\pi \), as

\[
c_\pi = \frac{\text{rate of biomass entering } \pi, \text{ transferred through } \pi \text{ and reached } u_{L+1}}{\text{total biomass on node } u_{L+1}}
\]
The amplification rate is given by \( c_π = c_π^0 q_π \theta_π \), with
\[
c_π^0 = \frac{\text{rate of biomass entering } \pi}{\text{total biomass on node } u_{L+1}} = \frac{m(u_1)[S[u_1, \omega_1]J[\omega_1]]}{m(u_{L+1})U_{L+1}}
\]
\( q_π \) = probability for biomass started from \( u_1 \), being transferred via \( \pi \) and reached \( u_{L+1} \)
\( \theta_π \) = expected fraction of biomass started from \( u_1 \), being transferred via \( \pi \) and reached \( u_{L+1} \)

where we have
\[
q_π = q_{π,1} \times \ldots \times q_{π,L} \\
θ_π = θ_{π,1} \times \ldots \times θ_{π,L} \\
q_{π,j} = \frac{J[ω_j]}{\sum_{φ_k \in \text{out}(u_j)} J[φ_k]} \\
θ_{π,j} = \frac{m(u_{j+1}) S[u_{j+1}, ω_j]}{m(u_j) S[u_j, ω_j]}
\]

\[\text{Proposition A-1: Consider a reaction network which satisfies the Flux Conditions and Growth Conditions. Let } π(u, ω) \text{ be a reaction pathways in the network. Then every system nodes and every reaction fluxes in } π \text{ has a well-defined long-term growth rate. Furthermore, the long-term growth rate of all system nodes are equal or greater than the long-term growth rate of } J[ω_1].\]

Proof: The definitions of these quantities are straight-forward. The formula of \( q_{π,j} \) is due to the fact that there are multiple potential reactions the biomass content can undertake, and the probability is proportional to the relative flux fraction. Under balanced growth, we have \( q_π(Y^*) \) as a constant.

It is worth to mention that \( θ_π \) is a constant that purely depends on the network topology; it is independent of the form of flux function. For the \( j^{\text{th}} \) step in the reaction network, \( m(u_j)[S[u_j, ω_j]] \) is the amount of biomass come into the reaction from node \( u_j \), and \( m(u_{j+1}) S[u_{j+1}, ω_j] \) is the amount of biomass exit from the reaction to node \( u_{j+1} \) (the absolute sign is necessary since the stoichiometry coefficients for influx is negative). The ratio between them is the fraction of biomass being transported via reaction \( ω_j \).

The following Proposition is required for the proof of Theorem 3-3.

By Flux Condition, every reaction flux \( J[ω_j] \) has either a gatekeeper \( x_k \) or an upstream node \( x_k \). In either case, under balanced growth it can be expressed by \( J[ω_j](t) = G^+(Y^*)X_k(t) \) where \( G^+(Y^*) \) is a constant. Since \( X_k \) has a well-defined long-term growth rate, \( J[ω_j] \) also has a well-defined long-term growth rate. We denote \( λ_j \) as the long-term growth rate of \( J[ω_j] \), and \( γ_k \) be the long-term growth rate of node \( u_k \), \( k \geq 2 \).

First we consider the node \( u_2 \). Under balanced growth, we have
\[
\frac{dU_2}{dt} = -R_2 U_2 + \sum_{φ_ω \in \text{in}(u_2)} J[φ_ω] \geq -R_2 U_2 + J[ω_1]
\]
where \( R_2 \) is a constant defined by
\[
R_2 \equiv \frac{1}{U_2} \sum_{φ_ω \in \text{out}(u_2)} J[φ_ω] |_{Y^*}
\]

Since the long-term growth rate of \( U_2(t) \) exist, under balanced growth we have \( U_2(t) = h_2(Y^*)e^{γ_2 t} \) where \( h(Y) \) is a function defined in the unit simplex. Similarly, we can express \( J[ω_1](t) = G(Y^*)e^{λ_1 t} \). Together, we have
\[
(γ_2 + R_2)h_2(Y^*)e^{γ_2 t} \geq G(Y^*)e^{λ_1 t}
\]

Taking the logarithm of the both sides and let \( t \to \infty \), we have \( γ_2 \geq λ_1 \). This shows the long-term growth rate of node \( u_2 \) is greater or equal to the long-term growth rate of \( J[ω_1] \).
Note that since \( u_2 \) is an upstream node of \( J[\omega_2] \), the long-term growth rate of \( J[\omega_2] \) is greater or equal to \( \gamma_2 \). Therefore, we have \( \lambda_2 \geq \gamma_2 \). Using the similar argument recursively, we show that \( \gamma_{j+1} \geq \lambda_j \) for every \( j \), and \( \lambda_j \geq \gamma_j \) for every \( j \). This complete the proof of Proposition A.1.

**Theorem 3-3:** Consider a reaction network which satisfies Flux Conditions and Growth Conditions. Let \( x_i \) be a node in the system with positive growth rate. In the long-term, \( X_i(t) \) follows the delay differential equation

\[
\frac{dX_i(t)}{dt} = -\beta_i X_i(t) + \int_{t' = 0}^{t} \alpha_i(t - t') X_i(t') \, dt'
\]

(23)

Specifically, the degradation rate \( \beta_i \) is defined by

\[
\beta_i \equiv \sum_{\phi_k \in \text{out}(x_i)} \frac{J[\phi_k]}{X_1} \gamma_i
\]

(24)

and the delay kernel \( \alpha_i(\tau) \) is be represented by

\[
\alpha_i(\tau) \equiv \sum_{\pi \in \text{P}_1[x_i]} c_{\pi} f_{\pi}(\tau)
\]

(25)

Proof: The biomass transfer process can be decomposed into segments of synthesis pathways, using \( x_1 \) as a pivot node. Let \( \pi \) represent a synthesis pathway, we classify the reaction pathway based on the following categories:

\[
P_0[x_i] : \{ \pi | \pi \text{ starts with an environmental node and never reaches } x_i \} \]

\[
P_1[x_i] : \{ \pi | \pi \text{ starts with an environmental node and ends when it reaches } x_i \ \text{for the first time} \}
\]

\[
P_2[x_i] : \{ \pi | \pi \text{ starts with } x_i \ \text{and ends when it meet } x_i \ \text{again} \}
\]

\[
P_3[x_i] : \{ \pi | \pi \text{ starts with } x_i \ \text{and never return to } x_i \}
\]

\[
P_4[x_i] : \{ \pi | \pi \text{ starts with a node } x_k \neq x_i \ \text{and ends when it reaches } x_i \ \text{for the first time} \}
\]

Note that there are other types of reaction pathways, but their paths are redundant with the pathways in above. For example, suppose \( \pi' \) is a reaction pathway from one node \( x_k \neq x_1 \) to another node \( x_k' \neq x_1 \). We can extend this reaction pathway such that if it hit \( x_1 \) later, \( \pi' \) will be a subset of \( P_1 \) or \( P_4 \) pathway; otherwise, \( \pi' \) will be a subset of \( P_0 \) pathway. In our proof, it suffices to consider the categories in above.

Now, let us consider all biomass that entered \( x_i \) at a fixed time point \( t \). We can trace back the upstream pathways of the biomass, and identify all reaction pathways which contribute to the biomass influx. In this case, pathways in \( P_1[x_1], P_2[x_1], P_3[x_1] \) could contribute to this influx. Similarly, we can consider all biomass that leaving \( x_i \) at a fixed time point, and trace back the downstream pathways which contribute to the biomass efflux. In this case, pathways in \( P_2[x_1], P_4[x_1] \) contribute to this efflux. To specify the original source of the biomass influx/efflux, we use the following notation:

\[
J[\pi] \equiv \text{biomass influx/efflux contributed from reaction pathway } \pi
\]

\[
J[\text{P}_j[x_i]] \equiv \text{biomass influx/efflux contributed from all reaction pathways } \pi \in \text{P}_j[x_i]
\]

As we discussed in above, for node \( x_1 \) we can decompose its inbound and outbound biomass fluxes from different reaction pathway groups:

\[
\frac{dX_1}{dt} = \sum_{\phi_1 \in \text{in}(x_1)} S_{i\alpha} J_\alpha | P_1[x_1] + \sum_{\phi_1 \in \text{in}(x_1)} S_{i\alpha} J_\alpha | P_2[x_1] + \sum_{\phi_1 \in \text{in}(x_1)} S_{i\alpha} J_\alpha | P_3[x_1]
\]

\[ - \sum_{\phi_1 \in \text{out}(x_1)} S_{i\alpha} J_\alpha | P_2[x_1] - \sum_{\phi_1 \in \text{out}(x_1)} S_{i\alpha} J_\alpha | P_3[x_1]
\]

(26)

First, we consider the reaction pathways \( \pi(u, \omega) \) from \( P_1[x_1] \). Consider the last reaction flux in the pathway \( \pi \) (denoted by \( J[\omega_M] \)). For this reaction, the amount of the incoming biomass contributed by pathway \( \pi \) at time \([t, t + \Delta t]\) is

\[
m(x_i) S[x_i, \omega_M] J[\omega_M] \pi(t) \, \Delta t
\]

(27)
We now try to express the same quantity in above using the waiting-time distribution $f_\pi(\tau)$ as a kernel. Consider a time points $t' < t$. By definition, the amount of biomass entered first flux $J[\omega_1]$ at time $t'$ is

$$m(u_1)|S[u_1, \omega_1]|J[\omega_1](t')$$

(note that the stoichiometry coefficient $S[u_1, \omega_1]$ is negative, and hence the absolute sign is required). Therefore, the amount of biomass being passed through the first flux $J[\omega_1]$ at time $t'$, transferred along $\pi$, and eventually reaches $x_i$ at time $[t, t + \Delta t]$ is

$$m(u_1)|S[u_1, \omega_1]|J[\omega_1](t') \times q_\pi \theta_\pi f_\pi(t - t') \Delta t$$  

(28)

Integrating $t'$ from 0 to $t$ for the above equation, we have

$$m(x_i)S[x_i, \omega_M]J[\omega_M]|_{x = t} = \int_{t'=0}^{t} m(u_1)|S[u_1, \omega_1]|J[\omega_1](t') \times q_\pi \theta_\pi f_\pi(t - t') dt'$$

Using the formula in Proposition 3-2, we get

$$S[x_i, \omega_M]J[\omega_M]|_{x = t} = \int_{t'=0}^{t} \frac{m(u_1)|S[u_1, \omega_1]|J[\omega_1](t')}{m(x_i)X_i(t')} X_i(t')q_\pi \theta_\pi f_\pi(t - t') dt'$$

(30)

Here, we need to be careful about $c_\pi$ since it could potentially blow up to infinity if the long-term growth rate of $J[\omega_1]$ is higher than $X_i$. However, this is not possible, since by definition there is a synthesis pathway $\pi$ where biomass is being transferred from $\omega_1$ to $x_i$. By Proposition A-1, the long-term growth rate of $X_i$ must be equal or greater than $J[\omega_1]$. Therefore, $c_\pi$ is always well-defined.

So far we have only considered one reaction pathway $\pi$. Now, we need to sum up all reaction pathways that contribute to the influxes of $x_i$. First, by fixing a reaction flux $\phi_a \in up(x_g)$, we sum up all reaction pathways in $P_1[x_i]$ that ends with reaction $\phi_a$. This gives we

$$S_{1a}J_a|p_1[x_i]|(t) = \sum_{\pi(u, \omega) \in P_1[x_i], \omega_M = \phi_a} \int_{t'=0}^{t} c_\pi \theta_\pi f_\pi(t - t') X_i(t') dt'$$

(31)

Next, we consider every $\phi_a$ which belongs to $up(x_i)$ and summing up all the cases. This gives

$$\sum_{\phi_a \in in(x_i)} S_{1a}J_a|p_1[x_i]|(t) = \sum_{\pi(u, \omega) \in P_1[x_i]} \int_{t'=0}^{t} c_\pi \theta_\pi f_\pi(t - t') X_i(t') dt'$$

(32)

The left had side of the above equation is the first term in Equation (26).

Next, we consider the cases where the biomass is left node $x_i$, and circulated back to node $x_i$. Let $\pi(u, \omega) \in P_2[x_i]$ be a reaction pathway. The derivation is almost identical. After some simplifications we have

$$\sum_{\phi_a \in in(x_i)} S_{1a}J_a(t)|p_2[x_i]|(t) = \sum_{\pi(u, \omega) \in P_2[x_i]} \int_{t'=0}^{t} c_\pi \theta_\pi f_\pi(t - t') X_i(t') dt'$$

(33)

This left hand side of the above equation is equal to the second term in Equation (26). Combining equations for $P_1$ and $P_2$ and use the definition of $\alpha(t)$, we have

$$\sum_{\phi_a \in in(x_i)} S_{1a}J_a(t)|p_1[x_i]|(t) + \sum_{\phi_a \in in(x_i)} S_{1a}J_a(t)|p_2[x_i]|(t) = \int_{t'=0}^{t} \alpha(t - t') X_i(t') dt'$$

(34)

Our next task is to show the influxes from $P_4[x_i]$ pathways decay exponentially and hence do not contribute to long-term growth rate. By assumption, $X_i(t)$ has positive long-term growth rate. Let $B_0$ be the total biomass at time $t = 0$. Intuitively, this fixed amount of biomass cannot support exponential growth of $X_i(t)$; more biomass must be introduced from the environment in the future. The reaction pathways in $P_4[x_i]$ therefore can be viewed as the “relaxation process” of the initial biomass $B_0$. Consider a node $x_k \neq x_i$ with at least one reaction pathway $\pi(u, \omega)$ with length $M$ such that $u_1 = x_k$ and $u_M = x_i$. As the system evolves, the amount of initial biomass remains stayed at node $x_k$ decays exponentially as $\exp(-R^{(k)}_{\min} t)$, where

$$R^{(k)}_{\min} = \min_{\phi_a \in out(x_k)} \frac{J_a}{X_k}$$

(35)
is the coefficient that correspond to the slowest efflux among all outbound fluxes of \( x_k \). Under balanced growth, \( R_{\text{min}} \) is a positive constant. Since the initial biomass remained at \( x_k \) decays exponentially, the contribution of the remaining fraction of initial biomass through \( \tau(u, \omega) \) to \( x_i \) also decay exponentially. Consider all possible \( x_k \), we have

\[
\sum_{\phi_a \in \text{in}(x_i) \text{ and } \phi_a \in \text{out}(x_i)} S_{ia} J_a |P_k| x_i \equiv \epsilon(t) \leq B_0 S_{\text{max}} e^{-R^* t}
\]

where \( S_{\text{max}} \equiv \max \{ S_{ia} \mid \phi_a \in \text{in}(x_i) \} \) and \( R^* \equiv \min \{ R_{\text{min}}^{(k)} \mid k \neq i \} \). This shows that the biomass contribution from \( P_k[x_i] \) is negligible in the long-term.

In the end, we consider the the outbound fluxes in the fourth and fifth term of equation (26). By Proposition 1-2, all outbound fluxes \( J_a \in \text{out}(x_i) \) can be represented by \( J_a(t) = R_a(Y) X_i \) where \( R_a \) is defined on the unit simplex. Therefore,

\[
\sum_{\phi_a \in \text{out}(x_i)} S_{ia} J_a |P_4[x_i]| + \sum_{\phi_a \in \text{out}(x_i)} S_{ia} J_a |P_5[x_i]| = \sum_{\phi_a \in \text{out}(x_i)} S_{ia} R_a(Y^*) X_i = \beta_i X_i(t)
\]

This concludes the proof for Theorem 3-3. ■

Note that Theorem 3-3 assume the node \( x_i \) has positive long-term growth rate. If the long-term growth rate of \( x_i \) is zero or negative, the reaction pathways in \( P_4[x_i] \) will need to be included in delay kernel \( \alpha_i(\tau) \).

**Corollary 3-4:** Consider a reaction network which satisfies the Flux Conditions and Growth Conditions. Let \( Y^* \) be the balanced growth vector and define \( \alpha(\tau), \beta \) as linear combination of \( Y^* \):

\[
\beta \equiv \sum_{g=1}^{G} Y_g^* \beta_g / \sum_{g=1}^{G} Y_g^*
\]

\[
\alpha(\tau) \equiv \sum_{g=1}^{G} Y_g^* \alpha_g(\tau) / \sum_{g=1}^{G} Y_g^*
\]

then we have the DDEs

\[
\frac{dN_G}{dt} = -\beta N_G + \alpha * N_G
\]

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\]

**Proof:** For each node \( x_i \) we have for \( i = 1, ..., n \)

\[
X_i'(t) = -\beta_i X_i + \alpha_i(\tau) * X_i
\]

Here, \( \alpha_i(\tau) \) is the decay kernel of \( x_i \). It includes \( P_1[x_i], P_2[x_i], P_4[x_i] \) pathways, and the contribution from \( P_4[x_i] \) pathways can be ignored if \( x_i \) has a positive long-term growth rate. Now, since the system is under balanced growth, we have

\[
X_i(t) = \frac{Y_i^*}{N}(t) = \frac{Y_i^*}{\sum_{g=1}^{G} Y_g^*} N_G(t)
\]

and for gatekeeper nodes \( i = 1, ..., G \) the above equation can be replaced by

\[
N_G(t) = -\beta_i N_G + \alpha_i(\tau) * N_G
\]

Summing up the above equation for \( i = 1, ..., G \) and weighted by \( Y_g^* \), we have

\[
\frac{dN_G}{dt} = -\beta N_G + \alpha * N_G
\]

Note that if a gatekeeper node \( x_i \) has long-term growth rate lower than the system, we have \( Y_i^* = 0 \) and hence this node does not contribute to the weighted average of \( \alpha(\tau) \) and \( \beta \). Therefore, all gatekeepers contributed to the weighted average has positive growth rate, and their delay kernel \( \alpha_g(\tau) \) are given by Theorem 3-3.

**Theorem 4-2:** Consider a reaction network which satisfies the conditions in Corollary 3-4. The delay spectrum \( A(s) \) of the reaction network (viewed as a real function) is positive at \( s = 0 \) and non-increasing. Furthermore, there is a unique \( \lambda > 0 \) satisfying

\[
\lambda + \beta = A(\lambda)
\]
and $\lambda$ is the long-term growth rate of the system. □

Proof: We start with the delay-time equation, written in convolution form:

$$\tilde{N}_G = -\beta N_G + (\alpha \ast N_G)$$  \hspace{1cm} (43)

Apply Laplace transform to the above equation, we have

$$s\tilde{N}_G - N_G(0) = -\beta \tilde{N}_G + \tilde{\alpha} \tilde{N}_G$$  \hspace{1cm} (44)

where $\tilde{f}(s) \equiv \int_0^\infty f(t)e^{-st}dt$ represent the Laplace transform of $f$. We define complex functions $L(s) \equiv s + \beta$ and $A(s) \equiv \tilde{\alpha}$. We also define two real functions $L^R(s)$, $A^R(s)$, as the restriction of $L(s)$ and $A(s)$ on the real axis, respectively. First, we note that $A^R(s)$ is non-increasing on $[0, \infty)$. This is given by Proposition 4-1.

We claim that under the conditions of Corollary 3-4, $A(0) > \beta$. Since $A(s)$ is a weighted average of all gatekeeper nodes by $Y^*$ and the system is assumed to have $\lambda > 0$, we only need to consider the gatekeeper nodes that has long-term growth rate $\lambda$. The contribution from other gatekeeper nodes with lower long-term growth rates can be ignored.

We employ the concept of biomass autocatalysis on each gatekeeper. Suppose $x_g$ is a gatekeeper with long-term growth rate $\lambda > 0$. Let $A_g(s) \equiv \tilde{a}_g$. The degradation rate of $x_g$ molecule is $\beta_g$, and hence under balanced growth the system must generate enough $x_g$ molecules to replenish the degradation and support this positive growth. This means for system is under balanced growth with system size $N(t)$, the synthesis rate of $x_g$ must be higher than $\beta_g X_g(t) = \beta_g N(t)Y^*_g$.

Now, in this system, the number of gatekeeper molecules are $(X_1, ..., X_G) = (Y^*_1, ..., Y^*_G) \times N(t)$. By Proposition 3-2, for each reaction pathway $\pi$ the amount of biomass brings in via this pathway per unit time per unit of $x_g$ biomass is $c_\pi$. Therefore, for all gatekeeper molecules the total biomass influx by $P_1[x_g]$ to $x_g$ is

$$m(x_g)N(t)Y^*_g \sum_{\pi \in P_1[x_g]} c_\pi$$  \hspace{1cm} (45)

Similarly, the total amount of biomass influx by $P_2[x_g]$ to $x_g$ is

$$m(x_g)N(t)Y^*_g \sum_{\pi \in P_2[x_g]} c_\pi$$  \hspace{1cm} (46)

Therefore, by definition of $A_g(0) = \sum_{\pi \in P_1[x_g]} c_\pi + \sum_{\pi \in P_2[x_g]} c_\pi$, the total replenish flux for $x_g$ is

$$m(x_g)N(t)Y^*_g A_g(0)$$  \hspace{1cm} (47)

This synthesis biomass flux must be greater than the degradation biomass flux, which is $m(x_g)\beta_g N(t)Y^*_g$. Hence, we have $A_g(0) > \beta_g$. Since this inequality is valid for all gatekeepers with growth rate $\lambda$, by linear combination we also have $A(0) > \beta$.

Now we continue our analysis about $A^R(s)$, the restriction of $A(s)$ on real line. Since $A^R(s)$ is non-increasing and $A^R(0) > \delta$, there must be one unique intersection $s^* > 0$ such that $L^R(s^*) = A^R(s^*)$. Our next goal is to show that $s^*$ is equal to the long-term growth rate $\lambda$. We calculate $N_G(t)$ by inverse Laplace transform using the Bromwich integral:

$$N_G(t) = \frac{1}{2\pi i} \int_{\gamma-i\infty}^{\gamma+i\infty} e^{st} \tilde{N}_G(s)ds$$  \hspace{1cm} (48)

where $\gamma$ is a real number greater than the largest real part of all poles of $\tilde{N}_G(s)$. By the above equation, $\tilde{N}_G(s) = \frac{N_G(0)}{s + \beta - A(s)}$ and hence poles of $N_G$ are correspond to the zeros of $s + \beta - A(s) = L(s) - A(s)$. We use the Residual Theorem to evaluate the integral. To proceed, we express $L(s) - A(s)$ as polynomial fraction. From Proposition 3-4, the form of $A(s)$ can be expressed as

$$A(s) = C_0 + \sum_{k=1}^{N_p} \frac{M(k)-1}{\prod_{j=1}^{k} \frac{C_k}{s + R_k}} = \frac{B_1(s)}{B_2(s)}$$  \hspace{1cm} (49)

where $B_1, B_2$ are polynomial of $s$, $N_p$ are number of reaction pathways which have more than one reaction, $M(k)$ is the number of the reaction of $k$’th pathway, and $C_0, C_k, R_k$ are positive constants. It can be checked that the polynomial degree of $B_1, B_2$ satisfy $deg(B_1) \leq deg(B_2)$. Now, we define

$$L(s) - A(s) \equiv \frac{P_1(s)}{P_2(s)}$$  \hspace{1cm} (50)
where $P_1, P_2$ are polynomial of $s$. It can be checked that we have $\text{deg}(P_1) > \text{deg}(P_2)$ and $\text{deg}(P_1) > 0$. This allows us to use Jordan’s Lemma to evaluate the contour integral. Let $S$ denote the set of zeros of $L(s) - A(s)$. From the polynomial expression it is clear that $S$ has finite numbers of members. By contour integral, we have the formula (see Ref[8]) $X_1(t) = \sum_{s_j \in S} \rho_j(t)$. For simple poles $s_j$,

$$\rho_j(t) = \frac{P_2(s_j)}{P_1'(s_j)} e^{s_j t} \quad (51)$$

For poles with multiplicity $m$, $\rho_j(t) = Q(t)e^{s_j t}$ where $Q(t)$ is a polynomial of with degree $m - 1$ (see Ref [8]).

To calculate the long-term growth rate, we note that $N_G$ must have the same long-term growth rate as the entire system. In the above equation, the pole with largest real part dominate, therefore

$$\lambda = \lim_{t \to \infty} \frac{1}{t} \log N_G(t) = \lim_{t \to \infty} \frac{1}{t} \log \left( \sum_{s_j \in S} \rho_j(t) \right) = \max\{\text{Re}(s_j) \mid s_j \in S\} \quad (52)$$

where $\text{Re}(z)$ represents the real part of $z$.

It remains to show that the pole with the largest real part lies on the real axis. We have shown that there is a unique intersect $s^* > 0$ exist which satisfies $L_R(s^*) = A_R(s^*)$. We claim that there is no other complex number $s' \equiv p + qi$ with $p > s^*$ and $q \neq 0$ such that $L(s') = A(s')$. This is shown by noticing that any such $s'$ must satisfies $|L(s')| > |L(s^*)|$ and $|A(s')| \leq |A(s^*)|$. Therefore, the pole correspond to largest real part is the unique real number $s^* > 0$. This completes the proof of Theorem 4-2.

**Proposition 4-3:** Consider two reaction network, both satisfies the Flux Condition and Growth Conditions. Let $A_a, A_b$ represent their decay spectra, $\beta_a, \beta_b$ represent their degradation constants, and $\lambda_a, \lambda_b$ represent their long-term growth rate. If

$$A_a(s) - A_b(s) > \beta_a - \beta_b \quad (53)$$

for all $s > 0$, then we have $\lambda_a > \lambda_b$. ■

**Proof:** From Theorem 4.2, we have

$$A_a(\lambda_a) = \lambda_a + \beta_a$$
$$A_b(\lambda_a) = \lambda_b + \beta_b \quad (54)$$

Let $P_a(s) \equiv A_a(\lambda_a) - \beta_a, P_b(s) \equiv A_b(\lambda_b) - \beta_b$, and $C(s) = s$. From the equation in above, $P_a$ and line $C$ intersect at $s = \lambda_b$.

By the assumption, $P_a(s) > P_b(s)$ for all $s > 0$ we know that $P_a(\lambda_b) > P_b(\lambda_b)$, since $P_a, P_b$ are non-increasing functions, $P_a(s) > P_b(\lambda_b)$ for all $s \in [0, \lambda_b]$. Hence, $P_a$ does not intersect with $C(s)$ on $[0, \lambda_b]$. By assumption, both $\lambda_a, \lambda_b$ are positive, and hence we must have $\lambda_a > \lambda_b$. 

![Diagram](image-url)