Stability analysis of metabolic networks

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Abstract

Metabolic networks are complex systems of interconnected biochemical reactions. Mathematically, such a complex network can be conveniently represented by a set of ordinary differential equations. Mathematical models for metabolism aim to improve the understanding of metabolic regulation by quantifying essential aspects of a metabolic system. Because of the large number of species involved in these networks and the fact that many parameters are not a priori known, some aspects of the entire network (e.g., the stability) are difficult to analyse. Therefore, mechanisms for network complexity reduction are required. In this paper, we will employ a reduction mechanism that uses extreme currents, i.e. we split the whole network into smaller subnetworks, which are easier to handle and analyse. We will give here some stability conditions, which may be expressed in inequalities among the rate parameters in the network.

Introduction

As biochemical networks grow in sizes, the reduction of their complexity becomes a very important issue. The main problems we face when studying large-scale systems of biochemical reactions are: the high number of kinetic parameters, most of them unknown a priori, the high dimensionality of the system, which makes the numerical simulations very demanding, and multiple time scales in the system, leading to stiff equation systems. Due to the high number of parameters in the system and the difficulty in finding the steady states, the linear stability analysis for high dimensional reaction networks appears to be a difficult task. We would like to be able to say something about the stability of these networks without having any a priori information about the kinetic parameters of the system. There would be also nice if one can find relations among kinetic parameters, which may drive the system towards a stable steady state, or relations for which one can be sure that the steady states will not be stable.

In this paper, we present a method which can be used in the stability analysis for the steady states of a general biochemical network. The method uses the stoichiometry information of the network and it is based on the split of the whole system into sub-systems, each such sub-system being generated by an extreme current. One can thus study the stability of each sub-network in part and find which extreme current may be responsible for instabilities in the whole network. We show here some results which may lead to inequalities among kinetic parameters in the system, from which one can decide upon stability or instability of the steady state of the entire network.

Stoichiometric network analysis

Stoichiometric network analysis is a general approach to study the qualitative dynamics of chemical networks, which has the advantage that the kinetic parameters are not needed in the analysis. For a good insight into the Stoichiometric Network Analysis, one can consult [2]. The time behaviour of an arbitrary metabolic network, consisting of $m$ metabolites and $r$ reactions, can be described by a system of differential equations, written in the following form:

$$\frac{dx(t)}{dt} = N \cdot v(x(t), k), \quad \text{for } t \geq 0,$$

where $x = (x_i)_{i=1}^m$ is the vector having metabolites as components, $v = (v_j)_{j=1}^r$ is velocity (reaction rates) vector, the vector $k$ contains the kinetic parameters and $N \in \mathcal{M}_{m \times r}$ is the stoichiometric
matrix, that is the matrix which contains in its \( j \text{th} \) column the stoichiometric coefficients for the \( j \text{th} \) reaction. The reaction rates \( v_j \) are usually monomials, such as \( k_j \prod_i x_i^{\kappa_{ij}} \), where the order of kinetics \( \kappa_{ij} \) form an \( m \times r \) matrix, denoted by \( \kappa \), and called the kinetic matrix.

If a network contains conserved moieties, then these will correspond to linearly dependent rows in the stoichiometric matrix. It is then safe to remove dependent species from the stoichiometric matrix, as they will give rise to zero eigenvalues when studying linear stability of the system. Also, reducing the stoichiometry will simplify both, the computation and the analysis. We will call a \textit{reduced stoichiometric matrix}, the stoichiometric matrix which is obtained after deleting the rows corresponding to the dependent species in the network (due to conservation constraints). Let us denote by \( m_0 \) the dimension of the left null-space of \( N \). If \( m_0 = m \), then there will be no matrix reduction. If \( m_0 < m \), then after a re-ordering of the rows, such that the first \( m_0 \) rows are linearly independent, we construct the reduced stoichiometric matrix, \( N_r \), by deleting the last \( m - m_0 \) rows from \( N \). We now consider the vector of independent species, \( x_r \), which are those who correspond to the rows in the matrix \( N_r \), and write the reduced system as

\[
\frac{dx_r}{dt} = N_r \cdot v(x, k), \quad \text{for } t \geq 0. \tag{1}
\]

From now on we will be working with the reduced stoichiometric matrix \( N_r \), instead of \( N \), and the reduced kinetic matrix \( \kappa_r \), instead of \( \kappa \). The reduced matrix \( \kappa_r \) is obtained from \( \kappa \) exactly as \( N_r \) is deduced from \( N \).

If the order of the matrix \( N_r \) is very high, then linear stability analysis will be a difficult task to accomplish. One needs thus to reduce the complexity of the system. The reduction method we will use in the following is via the consideration of extreme currents. By starting from the steady states set, one can the reduce the whole network into subnetworks generated by the extreme currents. One can study firstly the stability of these subnetworks and then conclude how the stability properties of them can influence the stability properties of the whole network.

\textbf{Complexity reduction via extreme currents}

For biochemical systems, the analysis of steady states is very important. The steady states are given by the system

\[
N_r \cdot v(x, k) = 0, \quad v(x, k) \geq 0, \tag{2}
\]

thus the feasible steady states are confined to the cone \( \mathcal{K}_v = \{ v \in \mathbb{R}^r; \ ker N \cap \mathbb{R}_+^r \} \). As shown in \cite{1}, \( \mathcal{K}_v \) is spanned by a finite number of generating vectors, \( E_i, \ i = 1, \ldots, q \). They are called \textit{extreme currents} and have the following properties: the set \( \{ E_i \}_i \) is unique, \( N \cdot E_i = 0, \ \forall i = 1, \ldots, q \) and any element \( v \) of \( \mathcal{K}_v \) can be generated by a non-negative linear combination of the elements of this set, that is \( \mathcal{K}_v = \{ v \in \mathbb{R}^r; \ v(j) = \sum_{i=1}^{q} j_i E_i, \ j_i \geq 0 \} \).

As shown in \cite{1} and \cite{4}, the main advantage of this method is the possibility of re-writing the Jacobian of the system (1) in the following useful form:

\[
J = N_r \cdot \text{diag} \left( \sum_{i=1}^{q} j_i E_i \right) \cdot \kappa_r^\tau \cdot \text{diag} \left( x_0^{-1} \right) \tag{3}
\]

\[
= \sum_{i=1}^{q} j_i N_r \cdot \text{diag} \left( E_i \right) \cdot \kappa_r^\tau \cdot \text{diag} \left( x_0^{-1} \right), \tag{4}
\]

where \( x_0 \) is the steady states vector. Note that in these expressions of the Jacobian the kinetic rates \( k_i \) enter in the above formulae only through \( x_0 \).

Because the mapping \( x \rightarrow v(x, k) \) is not surjective (that is \( \mathcal{K}_v \) is too wide for the steady state space), new restrictions on the \( v_i \)'s are needed. In \cite{3} and \cite{4}, the reaction rates \( v_i \), which are some monomials, are restricted further to a variety of a toric ideal generated by a family of binomials of the
form \( v_i - v_i(x, k) \).

More explicitly, the reaction rates \( v_i \) are monomials and represent basis elements for a polynomial ideal. If we consider the ideal

\[
I = \{ f; \ f = \sum_{i=1}^{r} \zeta_i [v_i - v_i(x, k)], \ v_i(x, k) \text{ reaction rates} \},
\]

then the reaction rates lie in the space

\[
V(I) = \{ x \in \mathbb{C}^m; \ f(x) = 0, \ \forall f \in I \}.
\]

We change the basis in the ideal \( I \) and use instead the Gröbner basis, based on the lexicographic order in monomials. The variety generated with the newly obtained binomials is a deformed toric ideal, which we denote by \( I_{\text{def, tor}} \). Thus, the reaction rates will finally lie in the intersection of the polyhedral cone \( K_v \) and the variety generated by the deformed toric ideal, that is

\[
v_i \in K_v \cap V(I_{\text{def, tor}}), \ \forall i.
\]

**Stability analysis**

We are now interested in studying the stability of the steady state of (5) and of the subnetworks generated by the extreme currents \( E_i \). We start with the following definitions:

**Definition 1:** We call essential eigenvalue an eigenvalue of the matrix obtained from the restricted Jacobian matrix by deleting the rows corresponding to the non-essential species, i.e. species that do not appear in the reactions corresponding to the extreme current of interest.

**Definition 2:** We say that the steady state of (5) is stable if all essential eigenvalues of Jacobian matrix have negative real part. Moreover, we say that the subnetwork generated by \( E_i \) is stable if all essential eigenvalues of Jacobian matrix corresponding to \( E_i \), \( J(E_i) \), have negative real part.

**Definition 3:** We say that the subnetwork generated by \( E_i \) is mixing stable if \( J(E_i) + J(E_i)^T \) has only essential eigenvalues with negative real part.

We interpret the mixing stability as the property of an extreme current to give rise to no instabilities when mixed with other extreme currents. As one can easily check, mixing stability implies stability in the above sense.

We have seen above that the Jacobian matrix in the \( v_i \) coordinates has the form given by (3). Thus, linear stability of the system (2) is reduced to the stability of the system

\[
x' = A \cdot H \cdot x, \quad (5)
\]

where \( A = N_r \cdot \text{diag} (\sum_{j=1}^{n} j_i E_i) \cdot \kappa^r \) is a square matrix whose elements depend on the coefficients \( j_i \) and \( H = \text{diag} (x_0^{-1}) \) is a positive diagonal matrix.

We shall denote by \( \delta_k \) and \( \Delta_k \) the leading principal minors of \( A \) and, respectively, \( A + A^T \). By a leading principal minor of a matrix \( M \) we understand the determinant of a square submatrix that fits into the upper left-hand corner of \( M \). Then we can prove the following propositions:

**Proposition 1:** A necessary condition for stability of the steady state of (5) is that \((-1)^k \delta_k > 0\) holds for all \( k = 1, m_0 \).

**Proof.** The proof is straightforward. Firstly, note that the system (5) is already in the reduced form, so all its eigenvalues are essential. We know that the steady state of (5) is stable if and only if all
essential eigenvalues of $AH$ are negative, that is $AH$ is negative definite. This implies that $A$ is a negative definite matrix, which further implies $(-1)^k \delta_k > 0$, $\forall k$. This proposition will be helpful in determining when an extreme current is not stable in the sense of Definition 2, for any configuration of the kinetic parameters.

**Proposition 2:** A sufficient condition for stability of the steady state of (5) is that $(-1)^k \Delta_k > 0$ holds for all $k = 1, m_0$.

**Proof.** Remember that $\Delta_k$ are the principal leading minors of $A + A^\tau$. If $(-1)^k \Delta_k > 0$, $\forall k$, then we also have that $(-1)^k \Delta_k^{|h_k|} > 0$, $\forall k$, where $\Delta_k^{|h_k|}$ are the leading principal minors of $AH + HA^\tau$. Since $AH + HA^\tau$ is a symmetric matrix, the previous condition is equivalent to the fact that $AH + HA^\tau$ is negative definite matrix, which implies that $AH$ is also a negative definite matrix. This means that all its eigenvalues are negative. 

We also note that the condition $(-1)^k \Delta_k^{|h_k|} > 0$, $\forall k$ is equivalent to $A + A^\tau$ negative definite matrix.

If the network of interest is of a small order (usually less than 5), then the following theorem may be useful in studying the stability:

**Proposition 3** (Hurwitz): A necessary and sufficient condition for stability is that the leading principal minors of the matrix

$$
\mathcal{M} = \begin{pmatrix}
a_1 & a_3 & a_5 & a_7 & \ldots \\
1 & a_2 & a_4 & a_6 & \ldots \\
0 & a_1 & a_3 & a_5 & \ldots \\
0 & 1 & a_2 & a_4 & \ldots \\
\ldots & \ldots & \ldots & \ldots & \ldots
\end{pmatrix}
$$

are all positive.

(Here $a_i$ are the coefficients of the characteristic polynomial of $AH$, $P(\lambda) = \lambda^n + a_1 \lambda^{n-1} + a_2 \lambda^{n-2} + \cdots + a_{n-1} \lambda + a_n$)

We propose the following algorithm for the stability analysis of a general biochemical network. The algorithm may give conditions among the rate parameters $k_i$’s for which the whole network becomes stable or loses stability.

1. Find the extreme currents, $E_i$, $i = 1, q$;
2. For each extreme current determine the number of essential eigenvalues, using
   \begin{align*}
   \text{no. essential eigenvalues} &= \text{no. metabolites in the e.c.} - \dim(Ker(N_i^r))
   \end{align*}
   Determine the reduced stoichiometric matrix, $N_i$, and the reduced kinetic matrix, $\kappa_i$, for each extreme current in part. Then write
   $$A_i = N_i \cdot \text{diag}(E_i) \cdot \kappa_i^r,$$
   $i = 1, q$.
3. Analyze stability of each extreme current by looking at the eigenvalues of $A_i$. If one of the extreme currents, say $E_\alpha$, is not stable in the sense of Definition 2, then it may induce the same feature in the whole network if the parameter $j_\alpha$ is large enough.
4. We can check under which conditions that is possible, by looking at the principal leading minors of $A =$ $N_r \cdot \text{diag} \left( \sum_{i=1}^q j_i E_i \right) \cdot \kappa_r^r$ and $A + A^\tau$;
5. Find relations among $j_i$’s for which all $(-1)^k \Delta_k > 0$, which are sufficient conditions for the stability of the network. These relations can be then transformed in relations among the $k_i$’s.
6. If there exists a $k_0$ such that $(-1)^k \delta_{k_0} < 0$, then the network may be unstable or present oscillations.
Examples

Example 1: (enzyme-catalysed reaction)

Let us consider the following enzyme-catalysed reaction, in which substrate is transformed by enzyme into product, via the formation of a enzyme-substrate complex:

$$
\begin{align*}
&k_1 & S + E & \xrightarrow{k_1} C & \xrightarrow{k_2} E + P & \xrightarrow{k_o} \\
&k_i & & & & \\
\end{align*}
$$

where $E$, $S$, $C$ and $P$ are concentrations of, respectively, the free enzyme, the free substrate, the enzyme-substrate complex and the product.

Considering mass action kinetics, the time evolution of the system is governed by the following equations:

$$
\begin{align*}
\frac{dS}{dt} &= k_i - k_1 S E + k_{-1} C, \\
\frac{dE}{dt} &= -k_1 S E + (k_{-1} + k_2) C, \\
\frac{dC}{dt} &= k_1 S E - (k_{-1} + k_2) C, \\
\frac{dP}{dt} &= k_2 C - k_o P
\end{align*}
$$

This system satisfies a single conservation relation, $E + C = \text{const.}$, and has a unique steady state,

$$
\hat{S} = \frac{k_1 k_M}{k_2 E_0 - k_i}, \quad \hat{C} = \frac{k_1}{k_2}, \quad \hat{P} = \frac{k_i}{k_o},
$$

provided $k_2 E_0 > k_i$. Due to the conservation relation, one differential equation in the system (say, the second) is redundant. To determine the linearised stability of this steady state, then one must look at the eigenvalues of the reduced Jacobian. In the this case, the reduced Jacobian matrix is

$$
J = \begin{pmatrix}
-k_1 \hat{E} & k_{-1} & 0 \\
\hat{E} & -k_{-1} - k_2 & 0 \\
0 & k_2 & -k_o
\end{pmatrix}.
$$

One can easily compute these eigenvalues,

$$
-k_o, \quad -\frac{k_1}{2} \left[ E_0 + k_M - \frac{k_i}{k_2} \pm \sqrt{(E_0 + k_M - \frac{k_i}{k_2})^2 - 4\frac{k_2 E_0 - k_i}{k_1}} \right],
$$

and conclude that the system is stable, according to the above definition.

We now wish check stability using our method. The reduced stoichiometric matrix and the reduced kinetic matrix for the above reaction network are, respectively,

$$
N_r = \begin{pmatrix}
1 & -1 & 1 & 0 & 0 \\
0 & 1 & -1 & -1 & 0 \\
0 & 0 & 0 & 1 & -1
\end{pmatrix}, \quad \kappa_r = \begin{pmatrix}
0 & 1 & 0 & 0 & 0 \\
0 & 0 & 1 & 1 & 0 \\
0 & 0 & 0 & 0 & 1
\end{pmatrix}.
$$

We have two stable extreme currents, $E_1 = [1, 1, 0, 1, 1]$ and $E_2 = [0, 1, 1, 0, 0]$. Their reduced Jacobians are:

$$
A_1 = \begin{pmatrix}
-1 & 0 & 0 \\
1 & -1 & 0 \\
0 & 1 & -1
\end{pmatrix}, \quad A_2 = (-1)
$$
from which we can check that, indeed, all eigenvalues are negative.

The matrix $A$ is given by

$$A = N_r \cdot \text{diag}(j_1 E_1 + j_2 E_2) \cdot \kappa^r = \begin{pmatrix}
-j_1 - j_2 & j_2 & 0 \\
-j_1 + j_2 & 0 & 0 \\
0 & -j_1 - j_2 & 0
\end{pmatrix}$$

and

$$H = \begin{pmatrix}
\frac{k_3 E_3 - k_1}{k_1 k_3} & 0 & 0 \\
0 & \frac{k_2}{k_1} & 0 \\
0 & 0 & \frac{k_3}{k_1}
\end{pmatrix}$$

The necessary and the sufficient conditions in Propositions 1 and 2 are, respectively:

$$\delta_1 = j_1 + j_2 > 0, \quad \delta_2 = j_1 (j_1 + j_2) > 0, \quad -\delta_3 = j_1^2 (j_1 + j_2) > 0.$$  

and

$$-\Delta_1 = 2(j_1 + j_2) > 0, \quad \Delta_2 = j_1 (3j_1 + 4j_2) > 0, \quad -\Delta_3 = 2j_1^2 (2j_1 + 3j_2) > 0.$$  

All of them are satisfied for any configuration of the kinetic parameters, so that the network is stable. Because the network is small, one can also use the Hurwitz theorem to analyse stability. In this case, the characteristic polynomial is

$$P(\lambda) = \lambda^3 + [(h_1 + h_2 + h_3)j_1 + (h_1 + h_2)j_2] \lambda^2 + j_1 (j_1 + j_2) (h_1 h_2 + h_1 h_3 + h_2 h_3) \lambda + j_1^2 (j_1 + j_2) h_1 h_2 h_3,$$

and one can easily check that the conditions for stability in Proposition 3 are satisfied.

**Example 2** *(Oregonator)*: We consider the Belousov-Zhabotinsky set of reactions:

$$\begin{align*}
A + Y & \rightarrow X + P \\
X + Y & \rightarrow 2P \\
A + X & \rightarrow 2X + 2Z \\
2X & \rightarrow A + P \\
B + Z & \rightarrow \frac{1}{2} f Y
\end{align*}$$

Here $f$ is a positive constant (the stoichiometric factor). There are no conservation relations in the system, so that

$$N_r = \begin{pmatrix}
1 & -1 & 1 & -2 & 0 \\
-1 & -1 & 0 & 0 & f/2 \\
0 & 0 & 0 & 1 & -1
\end{pmatrix}, \quad \kappa^r = \begin{pmatrix}
0 & 1 & 1 & 2 & 0 \\
1 & 1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 1
\end{pmatrix}$$

There are two extreme currents, $E_1 = [0, 1, 1, 0, 2]$ and $E_2 = [1, 0, 1, 1, 2]$. $E_2$ is stable and $E_1$ is not. The matrix $A$ is given by:

$$A = N_r \cdot \text{diag}(j_1 E_1 + j_2 E_2) \cdot \kappa^r = \begin{pmatrix}
-3j_2 & -j_1 + j_2 & 0 \\
-j_1 & -j_1 - j_2 & f(j_1 + j_2) \\
2(j_1 + j_2) & 0 & -2(j_1 + j_2)
\end{pmatrix}$$

The necessary conditions for asymptotic stability become:

$$-\delta_1 = 3j_2 > 0, \quad \delta_2 = 3j_2^2 + 4j_1 j_2 - j_1^2 > 0, \quad -\delta_3 = 2(j_1 + j_2) [(f - 1)j_2^2 + 3j_1 j_2 + (3 - f)j_1^2] > 0 \quad (6)$$

and, the sufficient conditions are:

$$-\Delta_1 = 6j_2 > 0, \quad \Delta_2 = -4j_1^2 + 16j_1 j_2 + 11j_2^2 > 0 \quad \text{and} \quad (7)$$
\[-\Delta_3 = 2(j_1 + j_2)[4(f - 3)j_1^2 + (24 + 2f - 3f^2)j_1j_2 + (18 - 2f - 3f^2)j_2^2] \geq 0.\]

We can observe that, from (6) and (7) one can find conditions upon \(f\) and the \(j_i\)'s such that the steady state is stable or not. We shall discuss here in more detail only the case \(f = 1\), when the system has a unique steady state. In this particular case, the matrix \(A\) becomes

\[
A = \begin{pmatrix}
-3j_2 & -j_1 + j_2 & 0 \\
-j_1 & -j_1 - j_2 & j_1 + j_2 \\
2(j_1 + j_2) & 0 & -2(j_1 + j_2)
\end{pmatrix}
\]

The above necessary and sufficient conditions become, respectively,

\[-\delta_1 = 3j_2 > 0, \quad \delta_2 = 3j_2^2 + 4j_1j_2 - j_1^2 > 0, \quad \delta_3 = 4j_2[2j_1^2 + 3j_1j_2 + j_2^2] > 0\]

and

\[-\Delta_1 = 6j_2 > 0, \quad \Delta_2 = -4j_1^2 + 16j_1j_2 + 11j_2^2 > 0, \quad -\Delta_3 = -16j_1^3 + 30j_1^2j_2 + 72j_1j_2^2 + 26j_2^3 > 0.\]

Since \(v = \sum_i j_i E_i\), we can write the reaction rates in terms of \(j_i\)'s:

\[
v = (k_1aY, k_2XY, k_3aX, k_3X^2, k_5bZ)^T
\]

\[= (j_2, j_1, j_1 + j_2, j_2, 2j_1 + 2j_2)^T\]

We now change the basis in the steady state space from \(\{k_1aY, k_2XY, k_3aX, k_3X^2, k_5bZ\}\) to a Gröbner basis. We can do this using Mathemtica, and we obtain:

\text{Gröbner basis: } \{k_3^2v_4 - k_4v_3^2, k_2k_3v_1v_4 - k_1k_4v_2v_3, k_1k_3v_3 - k_2v_1v_3, k_2^2v_1^2v_4 - k_1^2k_4v_2^2\}

The steady states have to verify the conditions:

\[k_3^2v_4 - k_4v_3^2 = 0; \quad k_2k_3v_1v_4 - k_1k_4v_2v_3 = 0; \quad k_1k_3v_3 - k_2v_1v_3 = 0; \quad k_2^2v_1^2v_4 - k_1^2k_4v_2^2 = 0,\]

from which we get:

\[
\begin{cases}
  j_1 = k_2j, \\
  j_2 = k_1k_4^{1/3}j^{2/3}
\end{cases} \quad (j \text{ positive parameter})
\]

We can easily see that, if \(j_2\) is large enough (i.e. \(k_2\) is large enough), then \(\Delta_2, -\Delta_3 > 0\) and this implies stability in the sense of Definition 2.

On the other side, if \(j_1\) is large enough (i.e. either \(k_1\) or \(k_4\) is large enough), then \(\delta_2 < 0\) which implies the existence of an eigenvalue with \(Re(\lambda) \geq 0\), thus the system may present oscillations or instabilities

\textbf{Conclusions}

The stability of steady states in a biochemical network is a very important issue. In this paper, we have presented some conditions for the stability or instability of the steady state in a biochemical network, when that steady state exists. In the approach, we have used the method of splitting up the whole network into subnetworks generated by elements of a basis in the steady state space, called the extreme currents. As shown in the examples presented, from these conditions one may obtain relations among the kinetic parameters for which stability or instability occur.

\textbf{Acknowledgements}

I would like to thank the UniNet and the Klaus Tschira Foundation for financial support, and to Ursula Kummer and Tim Johann for many valuable discussions we have had.
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